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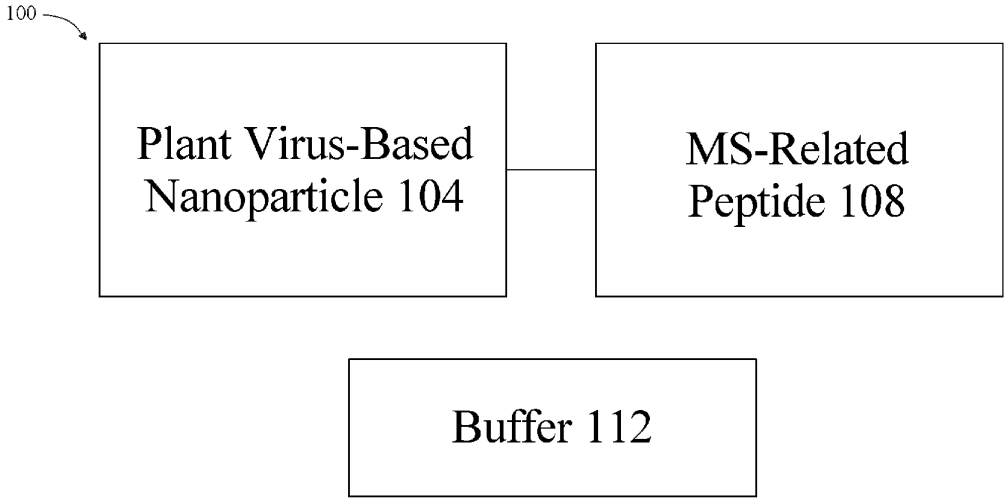

FIG. 1

(57) Abstract: A composition for treating multiple sclerosis (MS) includes a plant virus-based nanoparticle engineered to express at least an MS-related peptide and a buffer, of which a method of manufacturing includes infecting a plant with a virus to produce the plant virus-based nanoparticle, sampling symptomatic leaves from the plant, homogenizing the plant virus-based nanoparticle, incubating the plant virus-based nanoparticle, centrifuging the plant virus-based nanoparticle, and filtrating the plant virus-based nanoparticle.

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COMPOSITION AND METHODS FOR TREATING MULTIPLE SCLEROSIS

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of priority of U.S. Provisional Application Serial No. 63/530,131, filed on August 1, 2023, and entitled "COMPOSITION AND METHOD OF AN ENGINEERED VIRUS-BASED NANOPARTICLE FOR THE TREATMENT OF MULTIPLE SCLEROSIS", which is incorporated by reference herein in its entirety.

FIELD OF THE INVENTION

The present invention generally relates to the field of nanomedicine. In particular, the present invention is directed to a composition and methods of an engineered virus-based nanoparticle for the treatment of multiple sclerosis.

REFERENCE TO SEQUENCE LISTING

This specification includes a sequence listing submitted herewith, which includes the file entitled 1266-004PCT1.xml having the following size: 3,437 bytes which was created July 19, 2024, the contents of which are incorporated by reference herein.

BACKGROUND

Multiple sclerosis is a chronic inflammatory disease that affects the central nervous system (CNS), particularly the brain, spinal cord, and optic nerves. It is characterized by the immune system mistakenly attacking the protective covering of nerve fibers (myelin) in the CNS, leading to communication problems between the brain and the rest of the body. Current therapeutic strategies have several limitations that include incomplete effectiveness, side effects, and the inability to reverse the damage already done to the nervous system.

SUMMARY OF THE DISCLOSURE

In an aspect, a composition for treating multiple sclerosis (MS) is described. Composition includes a plant virus-based nanoparticle engineered to express at least a synthetic peptide associated with MS and a buffer.

In another aspect, a method of manufacturing composition for treating MS is described. Method includes infecting a plant with a virus to produce a plant virus-based nanoparticle, sampling symptomatic leaves from the plant, homogenizing the plant virus-based nanoparticle, incubating the plant virus-based nanoparticle, centrifuging the plant virus-based nanoparticle; and gel-filtrating the plant virus-based nanoparticle.

In another aspect, another method of manufacturing composition for treating MS is described. Method includes infecting a plant with a virus to produce a plant virus-based nanoparticle, sampling symptomatic leaves from the plant, homogenizing the plant virus-based nanoparticle, incubating the plant virus-based nanoparticle, and filtrating the plant virus-based nanoparticle.

In another aspect, another method of manufacturing composition for treating MS is described. Method includes agroinfiltrating a plant with a virus to produce a plant virus-based nanoparticle, sampling symptomatic leaves of the plant, homogenizing the plant virus-based nanoparticle, centrifuging the plant virus-based nanoparticle, and gel-filtrating the plant virus-based nanoparticle.

The details of one or more variations of the subject matter described herein are set forth in the accompanying drawings and the description below. Other features and advantages of the subject matter described herein will be apparent from the description and drawings, and from the claims.

DESCRIPTION OF DRAWINGS

For the purpose of illustrating the invention, the drawings show aspects of one or more embodiments of the invention. However, it should be understood that the present invention is not limited to the precise arrangements and instrumentalities shown in the drawings, wherein:

FIG. 1 is a schematic illustration of an exemplary embodiment of a composition for treating multiple sclerosis (MS).

FIGS. 2A-B are schematic illustrations of exemplary embodiments of a plant virus-based nanoparticle;

FIG. 3A is a schematic illustration of a *Tomato bushy stunt virus* (TBSV);

FIG. 3B is a schematic illustration of a *Cowpea mosaic virus* (CPMV);

FIG. 4 is a flow diagram illustrating an exemplary embodiment of a method of manufacturing the composition for treating MS;

FIG. 5 is a flow diagram illustrating an exemplary embodiment of another method of manufacturing the composition for treating MS;

FIG. 6 is an exemplary embodiment of a method of using plant virus-based nanoparticle for treating MS; and

FIG. 7 is an exemplary embodiment of an extraction process of a plant virus-based nanoparticle.

The drawings are not necessarily to scale and may be illustrated by phantom lines, diagrammatic representations, and fragmentary views. In certain instances, details that are not necessary for an understanding of the embodiments or that render other details difficult to perceive may have been omitted. Like reference symbols in the various drawings indicate like elements.

DETAILED DESCRIPTION

At a high level, aspects of the present disclosure are directed to a composition and its methods of manufacture for treating multiple sclerosis (MS). In one or more embodiments, composition may include a plant virus-based nanoparticle engineered to express at least a linear peptide associated with MS and a buffer. In one or more embodiments, method may include identifying an immunodominant peptide associated with MS, modifying a plant-virus based nanoparticle to express the identified peptide, synthesizing a plant virus-based nanoparticle containing the peptide, and/or titrating the plant-virus based nanoparticle to a set pH.

Aspects of the present disclosure can be used to prevent and treat autoimmune diseases such as MS using plant virus-based nanoparticles.

Referring now to FIG. 1, an exemplary embodiment of a composition 100 for treating MS is illustrated. For the purposes of this disclosure, multiple Sclerosis (MS) is a chronic disease that affects the central nervous system, which includes the brain and spinal cord. It is considered an autoimmune disease as it involves a body's immune system mistakenly attacking its own tissues. In the case of MS, the immune system targets the protective covering of nerve fibers, known as myelin, in the central nervous system. This attack on myelin results in communication problems between the brain and the rest of the body and may lead to a range of neurological symptoms. MS is characterized by periods of relapse and remission. Some people who suffer from MS may experience fatigue and difficulty walking, while others may have no symptoms at all for most of their lives. Depending on which nerves are affected, symptoms of MS may vary widely in severity and type. In severe cases, MS may lead to significant disability over time and cause partial or complete loss of mobility. The precise cause of MS remains unknown, yet it's speculated to be a result of an interplay of genetic and environmental factors. Moreover, certain types of immune cells, notably T cells, are believed to have a pivotal role in the onset and progression of MS.

With continued reference to FIG. 1, it is worth noting that the foundation of composition 100 lies in harnessing the immunomodulatory effect of a virus-based nanoparticle, which carries a synthetic peptide designed to recalibrate aberrant immune responses observed in MS, as described below. For the purposes of this disclosure, an "immunomodulatory effect" is an effect of modulating the immune system. As a nonlimiting example, plant virus-based nanoparticle 104 may modulate an immune system to induce tolerance and therefore prevent or treat a case of autoimmune disease, such as without limitation MS.

With continued reference to FIG. 1, composition 100 comprises a plant virus-based nanoparticle 104. For the purposes of this disclosure, a "nanoparticle" is a particle with a size that ranges between 1 and 500 nanometers. For the purposes of this disclosure, a "plant virus-based nanoparticle" is a virus nanoparticle that is capable of infecting and replicating in plants. In one or more embodiments, plant virus-based nanoparticle 104 may include at least a unit or subunit of a plant viral protein. For the purposes of this disclosure, a "plant viral protein" is a protein that constitutes or is produced by a plant virus. Since plant viruses are unable to replicate in mammals, plant virus proteins may be a safer option for medical applications in human hosts. Nanoparticles created from subunits of plant virus proteins may be genetically engineered to express, on their external surface, an antigen-specific peptide related to an autoimmune disease and then be grown in a plant host, as described in detail below in this disclosure. For the purposes of this disclosure, a "virus nanoparticle" is a proteinaceous and often infectious nanoscale structure that is capable of delivering its nucleic acid efficiently into a host plant cell, enabling production of new viruses therein. "Virus" and "virus nanoparticle" may be used interchangeably throughout this disclosure. Similarly, "plant virus" and "plant virus-based nanoparticle" may be used interchangeably as well. Virus includes a capsid. For the purposes of this disclosure, a "capsid" is a protein shell exposed at the exterior of a virus that possesses a specific geometric pattern. As a nonlimiting example, capsid may possess an icosahedral shape. For the purposes of this disclosure, an "icosahedron" is a geometric shape with 20 sides, each composed of an equilateral triangle. As another nonlimiting example, capsid may include a filamentous structure. For the purposes of this disclosure, a "filamentous structure" is an elongated, thread-like formation that makes up a capsid of certain viruses. As another nonlimiting example, capsid may include a rod-shaped structure. As another nonlimiting example, capsid may include a helical structure. For the purposes of this disclosure, a "helical

structure" is a type of structure characterized by a cylindrical, elongated shape with a helical symmetry. This structure may be formed by the regular, repeating arrangement of protein subunits around a viral nucleic acid, providing protection, structural integrity, and aiding in the infectivity of a virus. As another nonlimiting example, capsid may include a spherical structure. Additionally and/or alternatively, capsid may adopt any geometry not disclosed herein yet deemed possible by a person of ordinary skill in the art upon reviewing the entirety of this disclosure.

With continued reference to FIG. 1, in one or more embodiments, plant virus protein may be produced through a process of molecular farming. For the purposes of this disclosure, "molecular farming" is a process of producing pharmaceutically important and commercially valuable proteins in plants. Once extracted, plant virus proteins may be used to create plant virus-based nanoparticle 104. Plant virus-based nanoparticle 104 and/or plant virus protein may be sourced from a variety of plant hosts. Suitable host plants for such purpose may include *Nicotiana benthamiana* plant, a *Nicotiana tabacum* plant, a *Solanum lycopersicum* or *Lycopersicon esculentum* plant, a *Cycorium intybus* plant, a *Brassica oleracea* var. *capitata* plant, a *Beta vulgaris* var *cicla* plant, a *Ocimum basilicum* plant, a red beet plant, a spinach plant, or the like. For the purposes of this disclosure, a "*Nicotiana benthamiana* plant" is a close relative of tobacco and a species of Nicotiana indigenous to Australia. It may be used for farming monoclonal antibodies and other recombinant proteins. For the purposes of this disclosure, a "*Nicotiana tabacum plant*", commonly known as cultivated tobacco, is a plant species that belongs to the *Nicotiana* genus in the *Solanaceae* family. It is a widely grown herbaceous plant primarily used to produce tobacco products. This species is characterized by large, broad leaves and is cultivated in various climates worldwide. In some cases, a Nicotiana tabacum plant may serve as a model organism and a host for genetic engineering, enabling the expression and study of recombinant proteins, vaccines, and other biologically significant compounds. For the purposes of this disclosure, a "*Solanum lycopersicum* plant" or "*Lycopersicon esculentum* plant", commonly known as the tomato plant, is a plant species that belongs to the *Solanaceae* family and characterized by its production of edible, fleshy fruits. *Solanum lycopersicum* is noted for its agricultural significance and its utility in genetic engineering and plant breeding. This species may serve as a model organism for studying plant genetics, disease resistance, and metabolic pathways. It may also be utilized in biotechnological applications for the production of

recombinant proteins, novel traits, and improved cultivars through genetic modification techniques. For the purposes of this disclosure, a "*Cycorium intybus* plant" is a hardy plant widely used in folklore medicine to treat various ailments ranging from wounds to diabetes. It is believed to have antimicrobial, anthelmintic, antimalarial, hepatoprotective, antidiabetic, gastroprotective, anti-inflammatory, analgesic, antioxidant, tumor-inhibitory, and antiallergic activities across several different cultures. For the purposes of this disclosure, a "*Brassica oleracea* var. *capitata* plant" is a is a biennial plant grown as an annual for its dense-leaved heads and characterized by a short stem and a rosette of green, purple, or white leaves that form a tight, globular, and compact head. It is known as wild cabbage in its uncultivated form. Some cultivated forms of *Brassica oleracea* var. *capitata* plant include cabbage, broccoli, cauliflower, kale, brussels sprouts, collard greens, savoy cabbage, kohlrabi and gai lan. It is native to costal southern and western Europe. For the purposes of this disclosure, a "*Beta vulgaris* var *cicla* plant" is a plant that is more commonly known as chard or spinach beet. It originates from the Mediterranean and has some medicinal properties, mainly in boosting the immune system and lowering blood pressure. For the purposes of this disclosure, an "*Ocimum basilicum* plant" is a member of the Lamiaceae (mint) family and is more commonly referred to as basil. For the purposes of this disclosure, a "red beet plant" is a biennial plant grown as an annual for its edible root and leafy greens and characterized by its swollen root, which is typically deep red or purple in color. It is a promising candidate for some medicinal uses. As a nonlimiting example, the phytochemicals present in red beet may provide protection against diseases including cancer and cardiovascular diseases. For the purposes of this disclosure, a "spinach plant" is a leafy green that belongs to the amaranth family and is closely related to beets and quinoa.

With continued reference to FIG. 1, in one or more embodiments, plant virus-based nanoparticle 104 may contain one or more viruses, such as without limitation *Tomato bush stunt virus* (TBSV), *Cowpea mosaic virus* (CPMV), and/or the like. For the purposes of this disclosure, a "*Tomato bush stunt virus* (TBSV)" is a plant virus from the *Tombusvirus* group that primarily infects vegetable crops and causes stunting of growth, leaf mottling, and deformed or absent fruit in an infected plant. TBSV is a prototypic member of the *Tombusviridae* family. TBSV may provide a scaffold for plant virus-based nanoparticle 104 to enable a stable C-terminal display of peptides. The structure of TBSV has been resolved at an atomic resolution with a 30-nm capsid composed of 180 identical copies of a single coat protein (CP/p41) arranged

in T = 3 symmetry. CP may include an RNA binding domain (R), a shell domain (S) forming a capsid backbone, and a C-terminal protruding (P) domain that can accommodate exogenous peptides for display. The structure of TBSV may include a single-stranded RNA with a linear genome of about 4,800 nucleotides. TBSV may also possess three symmetrically distinct coat protein monomers. TBSV may replicate, without limitation, using cytoplasmic replication. This virus may penetrate a host cell by uncoating and releasing viral RNA into cytoplasm. TBSV may also spread indirectly, such as without limitation through water, soil, and/or infected seeds. When engineered to express peptides, the behavior of TBSV may experience one or more modifications, such as without limitation a decrease in virus concentration, infection percentage, and/or disease severity.

With continued reference to FIG. 1, for the purposes of this disclosure, a "*Cowpea mosaic virus* (CPMV)" or "*Sunn-hemp mosaic virus*" is a non-enveloped plant virus of the *Comovirus* group. Infection of a susceptible cowpea leaf may cause a "mosaic" pattern in the leaf and result in high virus yields. CPMV genome may include 2 strands of RNA which are separately encapsulated. CPMV *may* be approximately 30 nanometers in diameter and contain 60 copies each of a Large (L) and Small (S) coat proteins, as explained below in this disclosure. The structure of CPMV is well-characterized to atomic resolution. CPMV nanoparticles may be thermostable and readily isolated from plants. There are many stable mutants of CPMV already prepared that allow specific modification of their capsid surfaces. In some cases, CPMV may include an icosahedral capsid, as described above.

With continued reference to FIG. 1, plant virus-based nanoparticle 104 is configured to express at least a MS-related peptide 108. For the purposes of this disclosure, a "MS-related peptide" is a short chain of amino acids that may propose a disease-specific treatment for patients with MS. In one or more embodiments, MS-related peptide 108 may include PLP139-151, MOG35-55, and/or MOG1-20, among others. For the purposes of this disclosure, "PLP139-151" is a specific epitope derived from the Proteolipid Protein (PLP) with a sequence of HSLGKWLGHPDKF (SEQ ID NO:1). For the purposes of this disclosure, a "proteolipid protein (PLP)" is a membrane-bound protein integral to the structure and function of the myelin sheath in the central nervous system. PLP is crucial for the formation, maintenance, and compaction of myelin, which insulates nerve fibers and facilitates rapid neural signal transmission. Primarily expressed by oligodendrocytes (a type of glial cell found in the central nervous system (CNS)

such as the brain and spinal cord), PLPs are essential for CNS function and stability. For the purposes of this disclosure, MOG35-55 and MOG1-20 are specific epitopes derived from Myelin Oligodendrocyte Glycoprotein, which has a major role in the myelination of nerves in the central nervous system (CNS). MOG35-55 has a sequence of MEVGWYRSPFSRVVHLYRNGK (SEQ ID NO: 2), whereas MOG1-20 has a sequence of GQFRVIGPRHPIRALVGDEV (SEQ ID NO: 3). MOG35-55 and MOG1-20 are isolated from an extensive chain of complex protein amino acids and may be used for treating MS. As a nonlimiting example. an administration of PLP139-151, MOG35-55 and MOG1-20 resulted in significant changes in disease biomarkers, suggesting their potential role in the development and progression of MS. Importantly, PLP139-151, MOG35-55, and MOG1-20 were studied using a model of chronic relapsing experimental allergic encephalomyelitis (R-EAE) in mice, a well-accepted model for human MS. Significant weight loss was observed during the initial acute phase of MS, followed by weight gain correlated with disease remittance. Furthermore, numerous mononuclear cells were noted to populate the perivascular space of the anterior spinal artery, indicating initial stages of demyelinating activity. For the purposes of this disclosure, a "demyelination activity" is a process of causing damage of the myelin sheath, consistent with details described above. PLP139-151, MOG35-55, and/or MOG1-20 may therefore hold potential for reducing the symptoms and progression of MS. In addition, PLP139-151, MOG35-55 and/or MOG1-20 may specifically play a key role in reducing the incidence of clinical relapses, CNS histopathology, and T cell responses to both initiating and relapse-associated epitopes.

With continued reference to FIG. 1, in one or more embodiments, MS-related peptide 108 may include an immunodominant peptide. For the purposes of this disclosure, an "immunodominant peptide" is a peptide that is a representative epitope of a given protein antigen to an immune system in the context of a specific autoimmune disease. In one or more embodiments, immunodominant peptide may include a cytoplasmically located protein. For the purposes of this disclosure, a "cytoplasmical location" is a location inside the cytoplasm of a cell. In one or more embodiments, a coat protein of plant virus-based nanoparticle 104 may be engineered to display immunodominant peptides. For the purposes of this disclosure, a "coat protein" is a protein that is a constituent of the capsid of a virus, as described above. In one or more embodiments, at least an immunodominant peptide may be fused to a protein of CPMV and/or TBSV. In one or more embodiments, the presence of immunodominant peptide may

reduce the yield of plant virus-based nanoparticles 104 in plants. In some embodiments, the presence of the immunodominant peptide may improve the yield of plant virus-based nanoparticles 104 in plants.

With continued reference to FIG. 1, composition 100 further comprises a buffer 112. Buffer may stabilize plant virus-based nanoparticle 104 and maintain a pH for increased stability and functionality. For the purposes of this disclosure, a "buffer" is a solution or mixture that contains at least a pair of weak acid, HA, and its conjugate base, A^-, (i.e., the weak acid minus one proton) in a molar ratio between 10:1 and 1:10, wherein the solution maintains a stable pH close to the pK_a (i.e., the negative log of the acid dissociation constant, K_a) of the weak acid, against addition of acidic or basic chemical species. For simplicity, a buffer containing a pair of conjugate base and acid may be written as A^-/HA. Additional examples will be provided below. The pH of a buffer solution may be calculated using the Henderson Hasselbalch equation:

$$pH = pK_a + \log\left(\frac{[A^-]}{[HA]}\right)$$

With continued reference to FIG. 1, buffer may include any type of buffer deemed suitable by a person of ordinary skill in the art upon reviewing the entirety of this disclosure. As another nonlimiting example, buffer may include an acetate buffer 112 (i.e., CH_3COONa/CH_3COOH). As another nonlimiting example, buffer may include a borate buffer (i.e., $Na_2B_4O_7 \cdot 10H_2O/H_3BO_3$). As another nonlimiting example, buffer may include a bicarbonate buffer (i.e., $NaHCO_3/H_2CO_3$ or $Na_2CO_3/NaHCO_3$, depending on the desired pH). As another nonlimiting example, composition 100 may include a cacodylate buffer (i.e., $NaC_2H_6AsO_2/HC_2H_6AsO_2$). As another nonlimiting example, buffer may include a Good's buffer. For the purposes of this disclosure, "Good's buffers" are a group of more than 20 conjugate acid/base pairs selected and described by Norman Good and colleagues for biochemical and biological research during 1966–1980. For simplicity, only the conjugate acid may be shown for each conjugate acid/base pair. Good's buffers include MES ($C_6H_{13}NO_4S$), ACES ($C_4H_9NO_4S$), PIPES ($C_8H_{18}N_2O_6S_2$), MOPS ($C_7H_{15}NO_4S$), TES ($C_6H_{15}NO_6S$), HEPES ($C_8H_{18}N_2O_4S$), Tricine ($C_6H_{13}NO_5$), TRIS ($C_4H_{11}NO_3$), Bicine ($C_6H_{13}NO_4$), TAPS ($C_7H_{17}NO_6S$), CHES ($C_8H_{17}NO_3S$), CAPS ($C_9H_{19}NO_3S$), AMPSO ($C_9H_{19}NO_4S$), Gly-Gly ($C_4H_8N_2O_3$), ADA ($C_4H_7NO_4$), BES ($C_6H_{15}NO_5S$), MOPSO ($C_7H_{15}NO_5S$), EPPS ($C_9H_{20}N_2O_4S$), HEPPS ($C_{11}H_{24}N_2O_4S$), CAPSO ($C_9H_{19}NO_4S$), HEPPSO ($C_9H_{20}N_2O_5S$), CABS ($C_{10}H_{19}NO_3S$), ACESO

($C_4H_9NO_5S$), TES-Na ($C_6H_{14}NO_6SNa$), BICINE-Na ($C_6H_{12}NO_4Na$), TRICINE-Na ($C_6H_{12}NO_5Na$), MES-Na ($C_6H_{12}NO_4SNa$), HEPES-Na ($C_8H_{17}N_2O_4SNa$), MOPS-Na ($C_7H_{14}NO_4SNa$), and PIPES-Na ($C_8H_{17}N_2O_6S_2Na$). As a nonlimiting example, buffer may include a phosphate buffer (i.e., NaH_2PO_4/H_3PO_4, Na_2HPO_4/NaH_2PO_4, or Na_3HPO_4/ Na_2HPO_4, depending on the desired pH). As another nonlimiting example, buffer may include a phosphate-buffered saline (PBS) solution, a commonly used buffer in biological research and pharmaceutical formulations that typically contains 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4. These buffer components may help to preserve the structural integrity of plant virus-based nanoparticle 104 and its associated MS-related peptide 108, ensuring their effective delivery at a target site in a body. A person of ordinary skill in the art, upon reviewing the entirety of this disclosure, will be able to recognize suitable buffers for composition 100.

With continued reference to FIG. 1, in one or more embodiments, composition 100 may include sodium acetate. For the purposes of this disclosure, "sodium acetate" is organic sodium salt with a chemical formula of CH_3COONa. It may also be known as acetic acid, sodium salt, sodium acetate anhydrous, or acetic acid sodium salt. Sodium acetate is the anhydrous, sodium salt form of acetic acid. Sodium acetate may be a hygroscopic powder that absorbs moisture from air. Sodium acetate is a strong electrolyte that fully dissolves and disassociates in water to form sodium ions (Na^+) and acetate ions (CH_3COO^-). Na^+ is the principal cation of extracellular fluid and plays a large part in fluid and electrolyte replacement therapies. Sodium acetate may be used as an electrolyte replenisher in isosmotic solution for parenteral replacement of acute losses of extracellular fluid without disturbing normal electrolyte balance. A solution of sodium acetate and acetic acid may act as a buffer to maintain a relatively constant pH. This may be useful especially in biochemical applications where reactions are pH-dependent in a mildly acidic range. In this case, sodium acetate is used to titrate a composition to adjust its pH.

With continued reference to FIG. 1, composition 100 may be taken in any suitable form of dosage and/or delivery, including without limitation oral dosage, intravenous dosage, subcutaneous dosage, intraperitoneal dosage, or intradermal dosage. For the purposes of this disclosure, an "oral dosage" is an ingestion of a composition through the mouth. Oral dosage of composition 100 may include use of pills, syrup, tablets, thin film, liquid solution, powder, solid crystals, natural or herbal plants, seeds, or food, pastes, or the like. For the purposes of this

disclosure, an "intravenous dosage" is an administration of a composition using injection. Intravenous dosage may include without limitation intravenous injection. Composition 100 may be given intravenously in any suitable manner, including as a bolus and/or as an infusion. Alternatively, other injection methods such as intramuscular injection, intraperitoneal injection, subcutaneous injection, and/or transcutaneous injection may be used. For the purposes of this disclosure, a "subcutaneous dosage" is an administration of a pharmaceutical composition by injecting it into the subcutaneous tissue, which is the layer of tissue directly beneath the skin. This route of administration allows for the absorption of the active ingredient into the bloodstream at a controlled rate. Subcutaneous dosages are commonly used for vaccines, insulin, and other medications requiring slow, sustained release. Subcutaneous dosage may offer advantages such as ease of administration, improved patient compliance, and minimized pain compared to intramuscular or intravenous injections. For the purposes of this disclosure, an "intraperitoneal dosage" is an administration of a pharmaceutical composition by injecting it directly into the peritoneal cavity, which is the space within the abdomen that houses various organs such as the intestines, liver, and stomach. This route may allow for rapid absorption of an active ingredient into the bloodstream and is often used for delivering drugs that require swift systemic effects or for treatments targeting abdominal organs. Intraperitoneal administration may be particularly useful in research settings and certain clinical applications, providing a method for efficient and effective drug delivery. For the purposes of this disclosure, an "intradermal dosage" is an administration of a pharmaceutical composition by injecting it directly into the dermis, which is the layer of skin located between the epidermis and subcutaneous tissue. This method may be employed to deliver small volumes of medication, typically for vaccines, allergy tests, or local anesthesia. The intradermal route may allow for a controlled release and localized effect of an active ingredient, providing advantages such as enhanced immune response, reduced systemic side effects, and precise targeting of an injection site.

With continued reference to FIG. 1, in one or more embodiments, composition 100 may include one or more anti-sclerosis ingredients. For the purposes of this disclosure, an "anti-sclerosis ingredient" is a medically active chemical that may be used to treat and/or prevent MS. Anti- sclerosis ingredient may include a non-steroidal anti-inflammatory drug (NSAID), a corticosteroid, methotrexate, hydroxychloroquine, sulfasalazine, leflunomide, a tumor necrosis

factor inhibitor, a T-cell costimulatory blocking agent, a B-cell depleting agent, an Interleukin-1 receptor antagonist therapy, and/or any other immunomodulatory and/or cytotoxic agent.

Referring now to FIG. 2A, an exemplary embodiment 200a of plant virus-based nanoparticle 104 is illustrated. Embodiment 200a may be a structural representation of CPMV. In one or more embodiments, plant virus-based nanoparticle 104 may include a large coat protein subunit 204. As a nonlimiting example, for CPMV, plant virus-based nanoparticle 104 may include 60 copies of large coat protein subunit 204. In one or more embodiments, plant virus-based nanoparticle 104 may include a small coat protein subunit 208. As a nonlimiting example, plant virus-based nanoparticle 104 may include 60 copies of small coat protein subunit 208. In one or more embodiments, plant virus-based nanoparticle 104 may include at least a MS-related peptide 108. In one or more embodiments, at least a MS-related peptide 108 may include immunodominant peptide 212, such as without limitation PLP139-151, MOG35-55, and/or MOG1-20. In one or more embodiments, immunodominant peptide 212, such as without limitation PLP139-151, MOG35-55 and/or MOG1-20, may be embedded within coat protein. In one or more embodiments, immunodominant peptide 212, such as without limitation PLP139-151, MOG35-55, and/or MOG1-20, may be located outside of coat protein. In one or more embodiments, immunodominant peptide 212, such as without limitation PLP139-151, MOG35-55, and/or MOG1-20, may be exposed on the surface of large coat protein subunit 204. In one or more embodiments, immunodominant peptide 212 may be located inside of a nanoparticle. In one or more embodiments, immunodominant peptide 212 may be placed inside of coat protein. In one or more embodiments, plant virus-based nanoparticle 104 may include an icosahedral structure. In one or more embodiments, plant virus-based nanoparticle 104 may include a filamentous structure. In one or more embodiments, plant virus-based nanoparticle 104 may include a rod-shaped structure. In one or more embodiments, plant virus-based nanoparticle 104 may include a helical structure. In one or more embodiments, plant virus-based nanoparticle 104 may include a spherical structure. In one or more embodiments, plant virus-based nanoparticle 104 may be homogeneous or uniform in size. In some embodiments, plant virus-based nanoparticle 104 may be homogeneous or uniform in shape. Additionally, and/or alternatively, plant virus-based nanoparticle 104 may adopt any geometry not disclosed herein yet deemed possible by a person of ordinary skill in the art upon reviewing the entirety of this disclosure.

Referring now to FIG. 2B, an exemplary embodiment 200b of plant virus-based nanoparticle 104 is illustrated. Plant virus-based nanoparticle 104 disclosed herein may be consistent with details described with respect to FIG. 2A. Embodiment 200b may be a structural representation of TBSV. In one or more embodiments, plant virus-based nanoparticle 104 may include TBSV, as described above. In one or more embodiments, plant virus-based nanoparticle 104 may include a single protein subunit 216. As a nonlimiting example, for TBSV, plant virus-based nanoparticle 104 may include 180 copies of a single protein subunit 216. In one or more embodiments, plant virus-based nanoparticle 104 may include at least a MS-related peptide 108. In one or more embodiments, at least a MS-related peptide 108 may include immunodominant peptide 212. In one or more embodiments, immunodominant peptide 212 may be exposed on the surface of a single protein subunit 216.

Referring now to FIGS. 3A-B, exemplary structures of TBSV (300a) and CPMV (300b) are illustrated in FIG. 3A and 3B, respectively.

Referring now to FIG. 4, a flow diagram illustrating an exemplary embodiment of method 300 for preparing plant virus-based nanoparticle 104 is illustrated. Plant virus-based nanoparticle 104 may include any type of plant virus-based nanoparticle described in this disclosure without limitation. At step 405, method 400 includes infecting a plant with a virus to produce plant virus-based nanoparticle 104. For the purposes of this disclosure, "infection" is a process of delivering viral genes into a host, wherein the viral genes are capable of replication to produce new copies of the corresponding virus. Infection allows a plant to produce plant virus-based nanoparticle 104. In one or more embodiments, infection may be done by spontaneous infiltration. For the purposes of this disclosure, "spontaneous infiltration" is a type of infiltration that is associated with a negative Gibbs free energy change and occurs naturally without energy input or intervention. For the purposes of this disclosure, "infiltration" is a process through which one or more substances penetrate or permeate from the surface of a plant into its tissues. As a nonlimiting example, spontaneous infiltration may include spraying infectious viral genes onto a plant, spraying viral solution obtained from previously infected leaves, immersion of plants into a viral solution obtained from previously infected leaves and the like. As another nonlimiting example, infection may be done by infiltration of viral genes into a plant. As another nonlimiting example, infection may be done by infiltration of viral solution obtained from previously infected leaves. In one or more embodiments, infection may be done by forced

infiltration. For the purposes of this disclosure, "forced infiltration" is a type of infiltration that requires a force, a pressure, an energy input, or a similar form of intervention to be applied. As a nonlimiting example, forced infiltration may include syringe infiltration, vacuum infiltration, and the like. As another nonlimiting example, vacuum infiltration may include vacuum infiltration using a vacuum pump, vacuum infiltration using a syringe, and the like. A person of ordinary skill in the art, upon reviewing the entirety of this disclosure, will be aware of various infiltration techniques that may be applied to generate plant virus-based nanoparticle 104 as described in this disclosure.

With continued reference to FIG. 4, at step 410, method 400 includes sampling symptomatic leaves from the plant. For the purposes of this disclosure, "sampling" is an action of taking samples from the leaves of plant to inspect whether they have produced plant virus-based nanoparticles 104. This may happen after a certain time period subsequent to a successful infection step, as described above. Samples may be taken from leaves that are symptomatic. For the purposes of this disclosure, "symptomatic" is a descriptor that describes one or more characteristics of an object that appear different due to viral infection. As a nonlimiting example, it may take three to six days for a TBSV- and/or a CPMV-based nanoparticle to form and accumulate in a plant. As a result, leaves may appear symptomatic in several possible ways, such as without limitation, light and dark green patches or irregular mottling, a stunted, curled, or puckered appearance, and veins of leaves appearing lighter than normal or banded with dark green or yellow. Once plant virus-based nanoparticles 104 are detected, they may be extracted following steps described below.

With continued reference to FIG. 4, at step 415, method 400 includes homogenizing the plant virus-based nanoparticle 104. For the purposes of this disclosure, "homogenizing" is a process of blending elements into a uniform mixture with a consistent or substantially consistent composition across its entirety. In one or more embodiments, a homogenization process may include combining a tissue of plant containing plant virus-based nanoparticles 104 with an extraction buffer solution. For the purposes of this disclosure, an "extraction buffer solution" is an aqueous solution capable of breaking open cells and releasing elements therein. In one or more embodiments, extraction buffer solution may include salts to regulate its acidity. In the case of the TBSV, a sodium acetate solution may be used as extraction buffer solution.

With continued reference to FIG. 4, at step 420, method 400 includes incubating plant virus-based nanoparticle 104. A homogenous mixture of plant leaves and extraction buffer is incubated in ice for a period of time. For the purposes of this disclosure, "incubating" is a process of subjecting an item to a hot or cold temperature for a certain period of time, until a certain goal is accomplished.

With continued reference to FIG. 4, in some cases, at step 425, method 400 may include centrifuging plant virus-based nanoparticle 104. For the purposes of this disclosure, "centrifuging" is a process of separating multiple components in a mixture based on their difference in density by applying a centrifugal force to the mixture using a centrifuge device. For the purposes of this disclosure, a "centrifuge device" is a device comprising a rotating element attached to a stationary axis and configured to a spin sample under a high rotational speed in order to achieve separation between various elements therein. The amount of time to apply for this centrifuging step may vary from one type of plant virus-based nanoparticle 104 to another. In some cases, this step may be repeated multiple times to achieve an improved yield. As a nonlimiting example, for TBSV, centrifuging step may be repeated two or three times.

With continued reference to FIG. 4, in one or more embodiments, at step 430, method 400 may include filtrating plant virus-based nanoparticle 104. In some cases, filtrating plant virus-based nanoparticle 104 may include filtrating the plant virus-based nanoparticle 104 using tangential flow filtration (TFF), nanofiltration (NF), and/or gel-filtration chromatography, among other similar separation/purification techniques. For the purposes of this disclosure, tangential flow filtration (TFF), also known as cross-flow filtration, is a separation technique utilized to filter and concentrate biomolecules in solution. In TFF, a feed solution may flow tangentially across the surface of a filter membrane, while an applied pressure forces some of the fluid through the membrane as filtrate (permeate). A tangential motion may help reduce membrane fouling and allow continuous filtration. TFF is commonly employed in bioprocessing for concentrating proteins, clarifying cell lysates, and purifying biopharmaceuticals, providing an efficient method for separating components based on size and molecular weight. For the purposes of this disclosure, "nanofiltration (NF)" is a membrane filtration process that separates particles and solutes in the nanometer range, typically between 1 and 10 nanometers. NF may utilize a semi-permeable membrane to selectively allow certain molecules, such as monovalent ions and small organic molecules, to pass through while rejecting larger molecules, multivalent

ions, and contaminants. NF may operate under moderate pressure and is commonly employed in water treatment, pharmaceutical purification, and food processing, providing a means for removing impurities, softening water, and concentrating valuable substances with high efficiency. For the purposes of this disclosure, "gel-filtration chromatography" is a type of separation and purification technique based on a differing ability of chemical species to retain in pores of a gel-filtration medium. Gel-filtration chromatography is also known as size-exclusion chromatography. A column used for gel-filtration chromatography may be packed with fine, porous beads composed of dextran polymers, agarose, polyacrylamide, and/or the like. The pore sizes of these beads are used to estimate the dimensions of macromolecules and separate them accordingly. Generally, chemical species of smaller sizes tend to retain in gel-filtration medium for a longer period of time (i.e., separates from a column later), whereas chemical species of larger sizes tend to retain in gel-filtration medium for a shorter period of time (i.e., separates from a column earlier). The main application of gel-filtration chromatography is the fractionation of proteins and other water-soluble polymers. Gel-filtration chromatography may be contrasted with gel permeation chromatography, which is a similar separation and purification technique often used to analyze the molecular weight distribution of organic-soluble polymers.

Referring now to FIG. 5, a flow diagram illustrating another exemplary embodiment of method 500 for preparing plant virus-based nanoparticle 104 is illustrated. Plant virus-based nanoparticle 104 may include any type of plant virus-based nanoparticle described in this disclosure without limitation. At step 505, method 500 includes agroinfiltrating a plant with a virus to produce plant virus-based nanoparticle 104. For the purposes of this disclosure, "agroinfiltration" is a method used in plant biology to induce transient expression of genes in a plant in order to produce a desired protein. As a nonlimiting example, agroinfiltration may be used for CPMV and/or for TBSV. As a nonlimiting example, to perform agroinfiltration, *Agrobacterium tumefaciens* may be directly injected into a plant leaf or brought into association with plant cells immobilized on a porous support. Subsequently, bacteria may transfer a desired gene into plant cells via transfer of T-DNA. Agroinfiltration may be beneficial when compared to more traditional plant transformations due to its speed and convenience, although yields of the recombinant protein may generally also be higher and more consistent. Additionally, and/or alternatively, other production processes may also be used to produce plant virus-based

nanoparticle 104. Once a plant has undergone such production processes, it may produce plant virus-based nanoparticles 104 to be used in composition 100.

With continued reference to FIG. 5, at step 510, method 500 includes sampling leaves of the plant. This step may be performed utilizing any process of sampling consistent with details described above.

With continued reference to FIG. 5, at step 515, method 500 includes homogenizing the plant virus-based nanoparticle. This step may be performed utilizing any process of homogenization as explained above. As a nonlimiting example, for CPMV, a phosphate buffer or a PBS buffer may be used, consistent with details described above.

With continued reference to FIG. 5, at step 520, method 500 includes centrifuging the plant virus-based nanoparticle 104. This step may be performed utilizing any process of centrifugation as described above. It is worth noting that production of a CPMV-based nanoparticle may not necessarily involve an incubation step described above for method 400.

With continued reference to FIG. 5, at step 525, method 500 includes filtrating plant virus-based nanoparticle 104. This step may be performed utilizing any process of filtration as described above.

Referring now to FIG. 6, an exemplary embodiment of a method 600 of using plant virus-based nanoparticle 104 for treating MS is illustrated. Genetic sequences that encode MS-related peptides 108, such as PLP139-151, MOG35-55, and/or MOG1-20, among others, may be cloned into an expression vector 604 harboring a viral genome or CP gene. For the purposes of this disclosure, an "expression vector" is typically a plasmid or virus designed for gene expression in cells. Expression vector is also known as expression construct. As nonlimiting examples, PLP139-151, MOG35-55, and/or MOG1-20 genes may be isolated from TBSV, an expression vector that is infective, and incorporated into CPMV, an expression vector that is non-infective. Resulting vectors may then undergo either *in vitro* retro-transcription or an *Agrobacterium tumefaciens* transformation. For *in vitro* retro-transcription, plant may undergo infection using an infective RNA. For *Agrobacterium tumefaciens* transformation, plant may undergo agroinfiltration, as previously described.

Referring now to FIG. 7, an exemplary embodiment of a production process 700 of a plant virus-based nanoparticle 104 is presented. Once plant virus-based nanoparticle 104 (pVNP) infects plant, the plant may start to produce genetically modified plant viruses. In other words,

plant may function as a bioreactor. Upon detection of plant virus-based nanoparticles 104, samples containing such plant virus-based nanoparticles 104 may be homogenized with extraction buffer solution, as described above. Upon homogenization, samples may be centrifuged and filtrated following procedures described above to yield plant virus-based nanoparticles 104, which may be used for downstream therapeutic applications, such as treatment of MS.

The foregoing has been a detailed description of illustrative embodiments of the invention. Various modifications and additions can be made without departing from the spirit and scope of this invention. Features of each of the various embodiments described above may be combined with features of other described embodiments as appropriate in order to provide a multiplicity of feature combinations in associated new embodiments. Furthermore, while the foregoing describes a number of separate embodiments, what has been described herein is merely illustrative of the application of the principles of the present invention. Additionally, although particular methods herein may be illustrated and/or described as being performed in a specific order, the ordering is highly variable within ordinary skill to achieve embodiments as disclosed herein. Accordingly, this description is meant to be taken only by way of example, and not to otherwise limit the scope of this invention.

In the descriptions above and in the claims, phrases such as “at least one of” or “one or more of” may occur followed by a conjunctive list of elements or features. The term “and/or” may also occur in a list of two or more elements or features. Unless otherwise implicitly or explicitly contradicted by the context in which it is used, such a phrase is intended to mean any of the listed elements or features individually or any of the recited elements or features in combination with any of the other recited elements or features. For example, the phrases “at least one of A and B;” “one or more of A and B;” and “A and/or B” are each intended to mean “A alone, B alone, or A and B together.” A similar interpretation is also intended for lists including three or more items. For example, the phrases “at least one of A, B, and C;” “one or more of A, B, and C;” and “A, B, and/or C” are each intended to mean “A alone, B alone, C alone, A and B together, A and C together, B and C together, or A and B and C together.” In addition, use of the term “based on,” above and in the claims is intended to mean, “based at least in part on,” such that an unrecited feature or element is also permissible.

The subject matter described herein can be embodied in systems, apparatus, methods, and/or articles depending on the desired configuration. The implementations set forth in the foregoing description do not represent all implementations consistent with the subject matter described herein. Instead, they are merely some examples consistent with aspects related to the described subject matter. Although a few variations have been described in detail above, other modifications or additions are possible. In particular, further features and/or variations can be provided in addition to those set forth herein. For example, the implementations described above can be directed to various combinations and sub-combinations of the disclosed features and/or combinations and sub-combinations of several further features disclosed above. In addition, the logic flows depicted in the accompanying figures and/or described herein do not necessarily require the particular order shown, or sequential order, to achieve desirable results. Other implementations may be within the scope of the following claims.

WHAT IS CLAIMED IS:

1. A composition for treating multiple sclerosis (MS), the composition comprising:
a plant virus-based nanoparticle engineered to express at least an MS-related peptide; and
a buffer.
2. The composition of claim 1, wherein the buffer comprises one or more members selected from a list consisting of sodium acetate, acetate buffer, borate buffer, bicarbonate buffer, cacodylate buffer, Good's buffer, phosphate buffer, bicarbonate buffer, and PBS buffer.
3. The composition of claim 1, wherein the at least an MS-related peptide includes PLP139-151, MOG35-55, or MOG1-20.
4. The composition of claim 1, wherein the at least a synthetic peptide is engineered using a *Tomato bushy stunt virus* (TBSV).
5. The composition of claim 4, wherein the TBSV comprises 180 copies of a protein subunit.
6. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Nicotiana benthamiana* plant.
7. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Nicotiana tabacum* plant.
8. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Lycopersicon esculentum* plant.
9. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Cycorium intybus* plant.
10. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Brassica oleracea* var. *capitata* plant.
11. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a *Beta vulgaris* var *cicla* plant.
12. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from an *Ocimum basilicum* plant.
13. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a spinach plant.

14. The composition of claim 1, wherein the plant virus-based nanoparticle is sourced from a red beet plant.

15. The composition of claim 1, wherein the composition comprises an oral dosage form.

16. The composition of claim 1, wherein the composition comprises an intravenous dosage form.

17. The composition of claim 1, wherein the composition comprises a subcutaneous, intraperitoneal, or intradermal dosage form.

18. The composition of claim 1, wherein the at least a synthetic peptide is fused to a protein of a TBSV.

19. The composition of claim 1, wherein the plant virus-based nanoparticle further comprises a *Cowpea mosaic virus* (CPMV).

20. The composition of claim 19, wherein the CPMV comprises 60 copies of a protein subunit.

21. The composition of claim 20, wherein the protein subunit further comprises a large coat protein.

22. The composition of claim 20, wherein the protein subunit further comprises a small coat protein.

23. The composition of claim 19, wherein the cowpea mosaic virus includes an icosahedral capsid.

24. A method of manufacturing a composition for treating MS, wherein the method comprises:
infecting a plant with a virus to produce a plant virus-based nanoparticle;
sampling symptomatic leaves from the plant;
homogenizing the plant virus-based nanoparticle;
incubating the plant virus-based nanoparticle;
centrifuging the plant virus-based nanoparticle; and
filtrating the plant virus-based nanoparticle.

25. The method of claim 24, wherein filtrating the plant virus-based nanoparticle comprises filtrating the plant virus-based nanoparticle using tangential flow filtration (TFF), nanofiltration (NF), or gel-filtration chromatography.

26. A method of manufacturing a composition for treating MS, wherein the method comprises:
agroinfiltrating a plant with a virus to produce a plant virus-based nanoparticle;
sampling symptomatic leaves of the plant;
homogenizing the plant virus-based nanoparticle;
centrifuging the plant virus-based nanoparticle; and
filtrating the plant virus-based nanoparticle.

27. The method of claim 26, wherein filtering the plant virus-based nanoparticle comprises filtrating the plant virus-based nanoparticle using tangential flow filtration (TFF), nanofiltration (NF), or gel-filtration chromatography.

28. A method of manufacturing a composition for treating MS, wherein the method comprises:
infecting a plant with a virus to produce a plant virus-based nanoparticle;
sampling symptomatic leaves from the plant;
homogenizing the plant virus-based nanoparticle;
incubating the plant virus-based nanoparticle; and
filtrating a solution comprising the incubated plant virus-based nanoparticle.

29. The method of claim 28, wherein filtering the solution comprises filtrating the solution using tangential flow filtration (TFF), nanofiltration (NF), or gel-filtration chromatography.

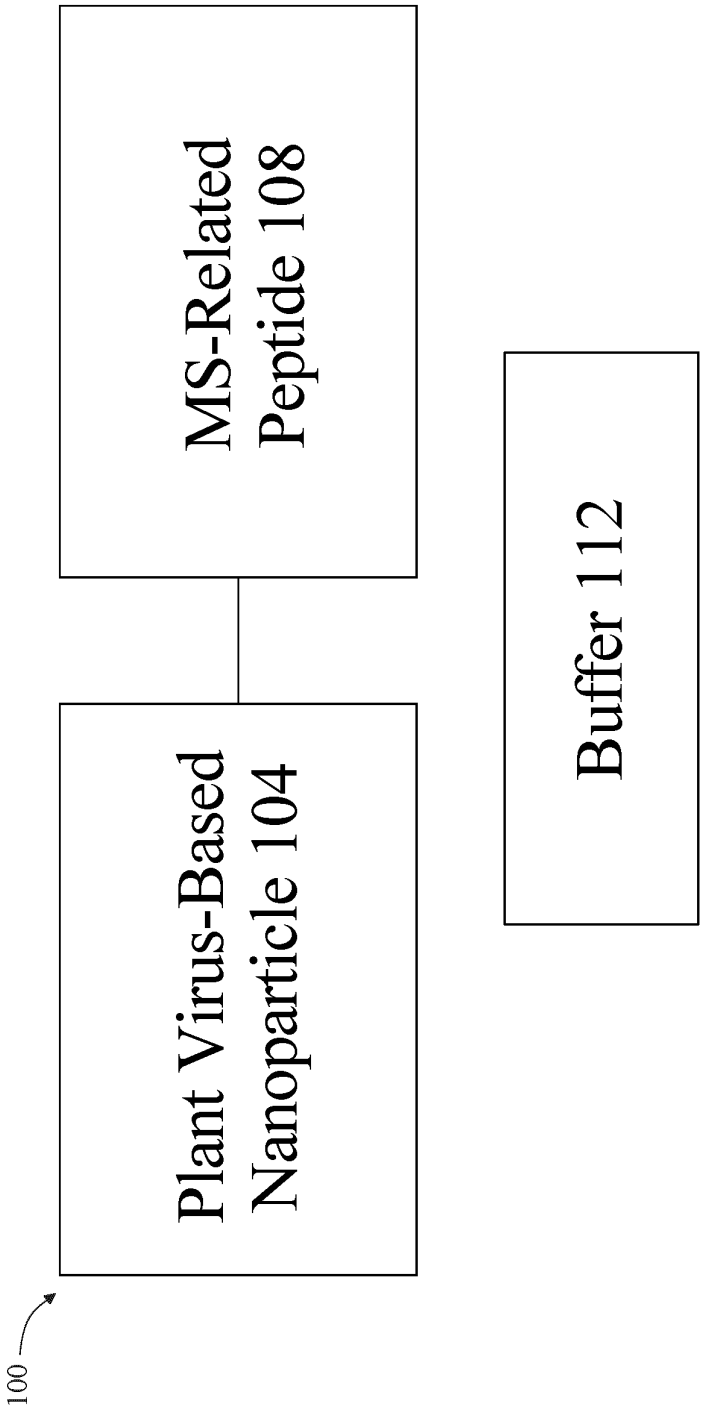
MS-Related Peptide 108
Buffer 112
Plant Virus-Based Nanoparticle 104
100

FIG. 1

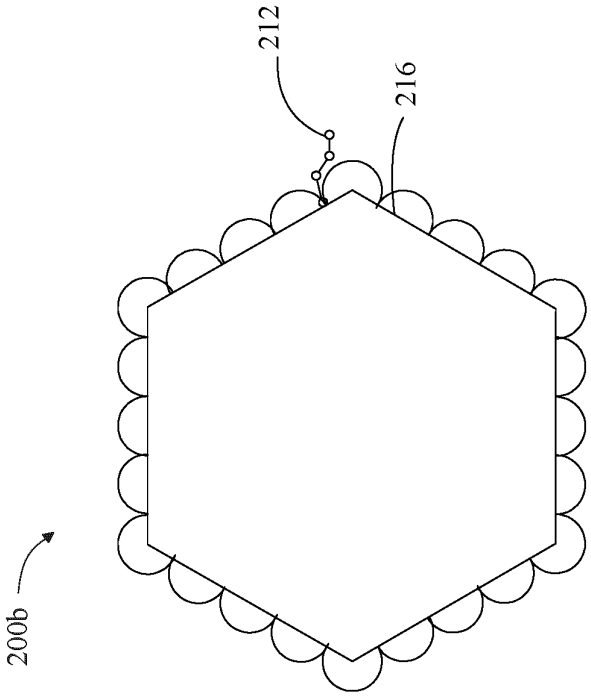

FIG. 2B

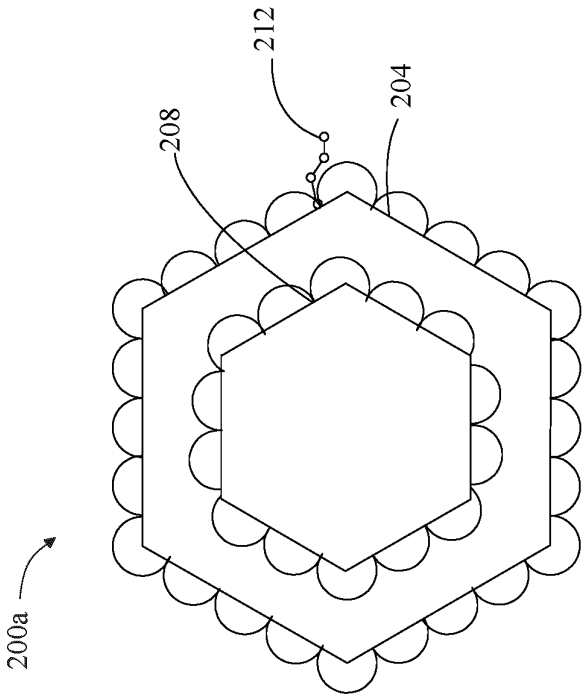

FIG. 2A

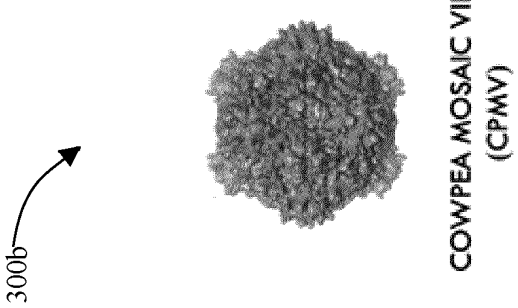
300b
COWPEA MOSAIC VIRUS
(CPMV)

FIG. 3B

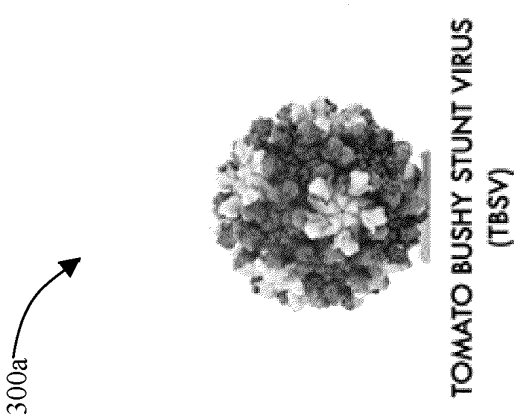
300a
TOMATO BUSHY STUNT VIRUS
(TBSV)

FIG. 3A

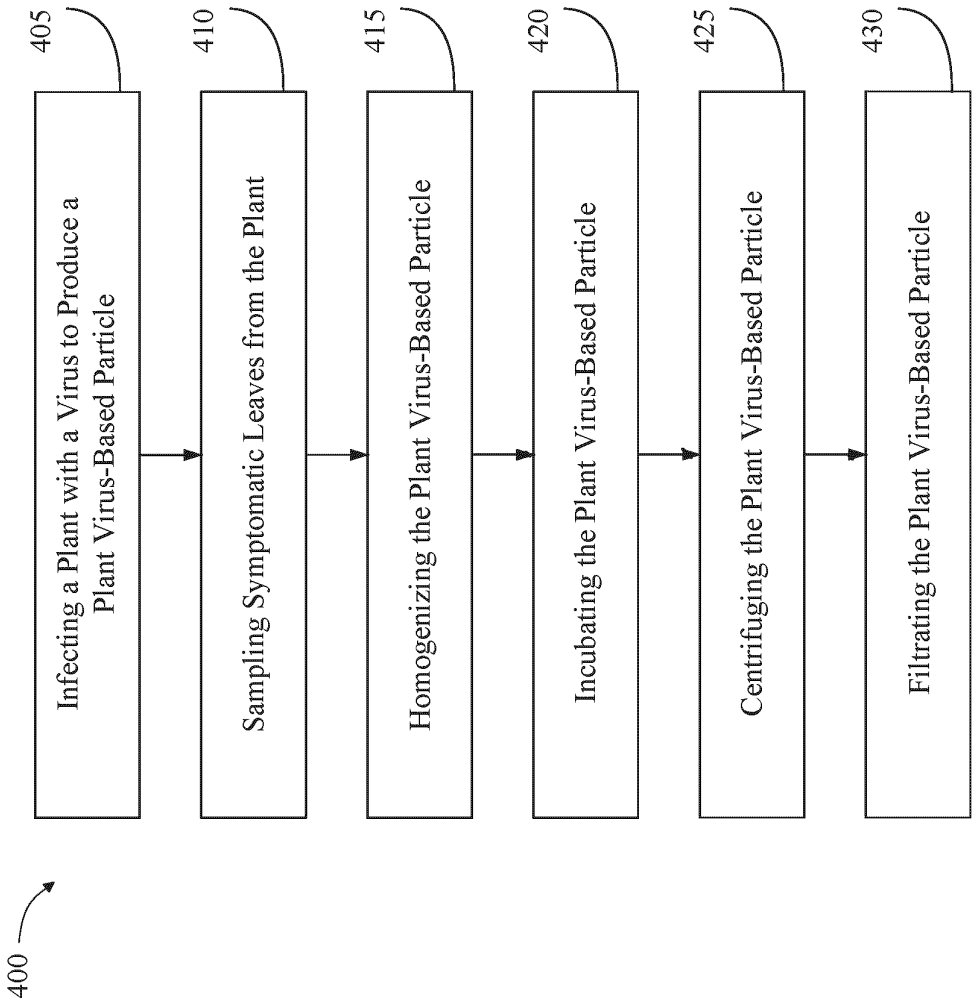
400
405
Infecting a Plant with a Virus to Produce a Plant Virus-Based Particle
410
Sampling Symptomatic Leaves from the Plant
415
Homogenizing the Plant Virus-Based Particle
420
Incubating the Plant Virus-Based Particle
425
Centrifuging the Plant Virus-Based Particle
430
Filtrating the Plant Virus-Based Particle

FIG. 4

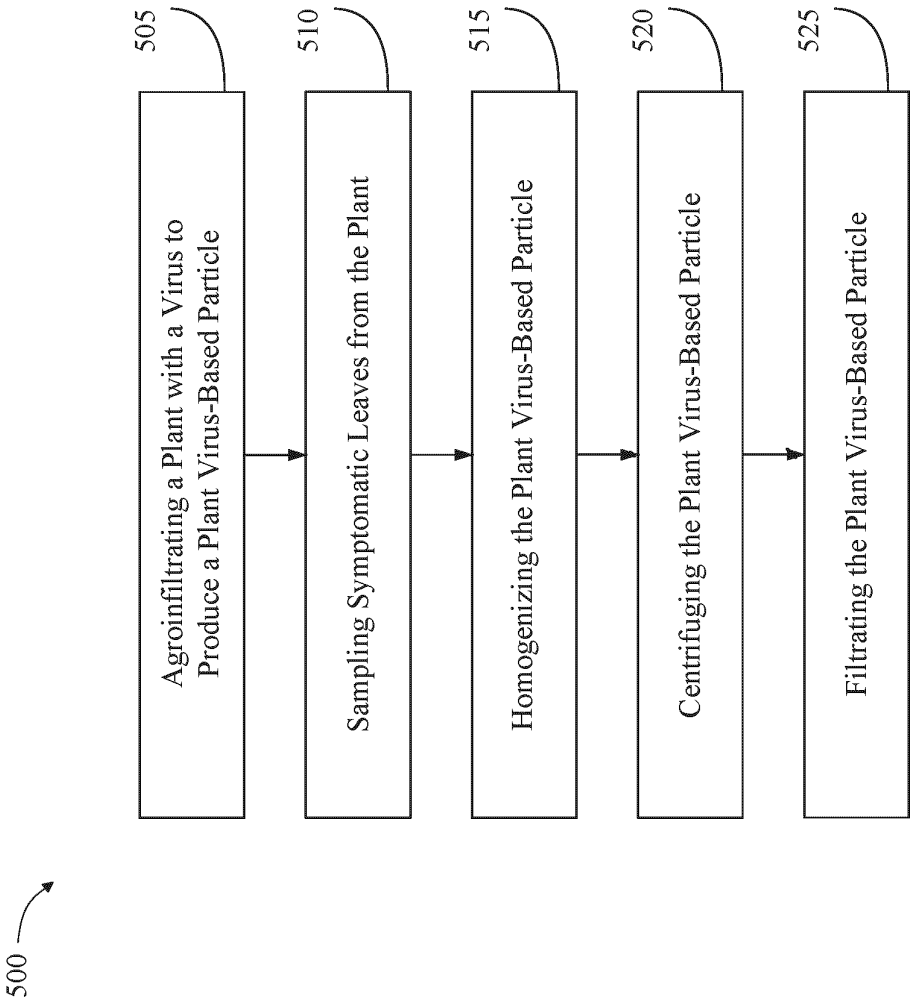
500
505
Agroinfiltrating a Plant with a Virus to Produce a Plant Virus-Based Particle
510
Sampling Symptomatic Leaves from the Plant
515
Homogenizing the Plant Virus-Based Particle
520
Centrifuging the Plant Virus-Based Particle
525
Filtrating the Plant Virus-Based Particle

FIG. 5

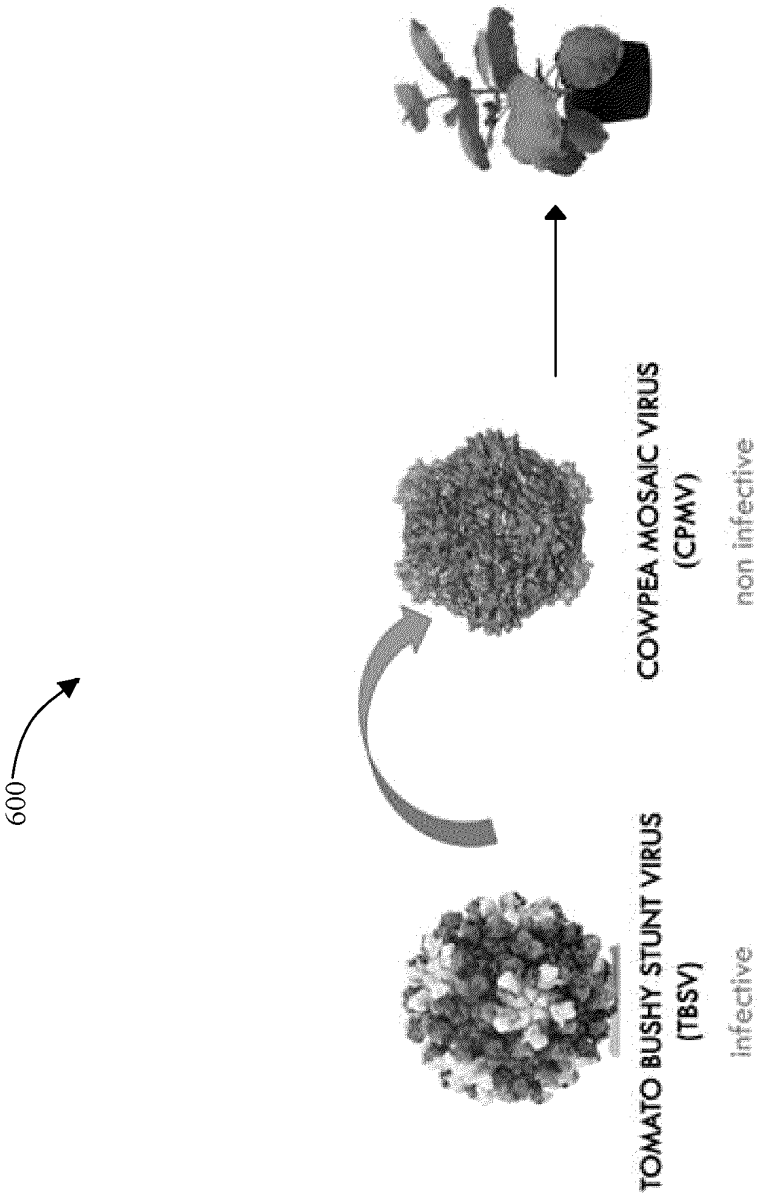
600
TOMATO BUSHY STUNT VIRUS
(TBSV)
infective
COWPEA MOSAIC VIRUS
(CPMV)
non infective

FIG. 6

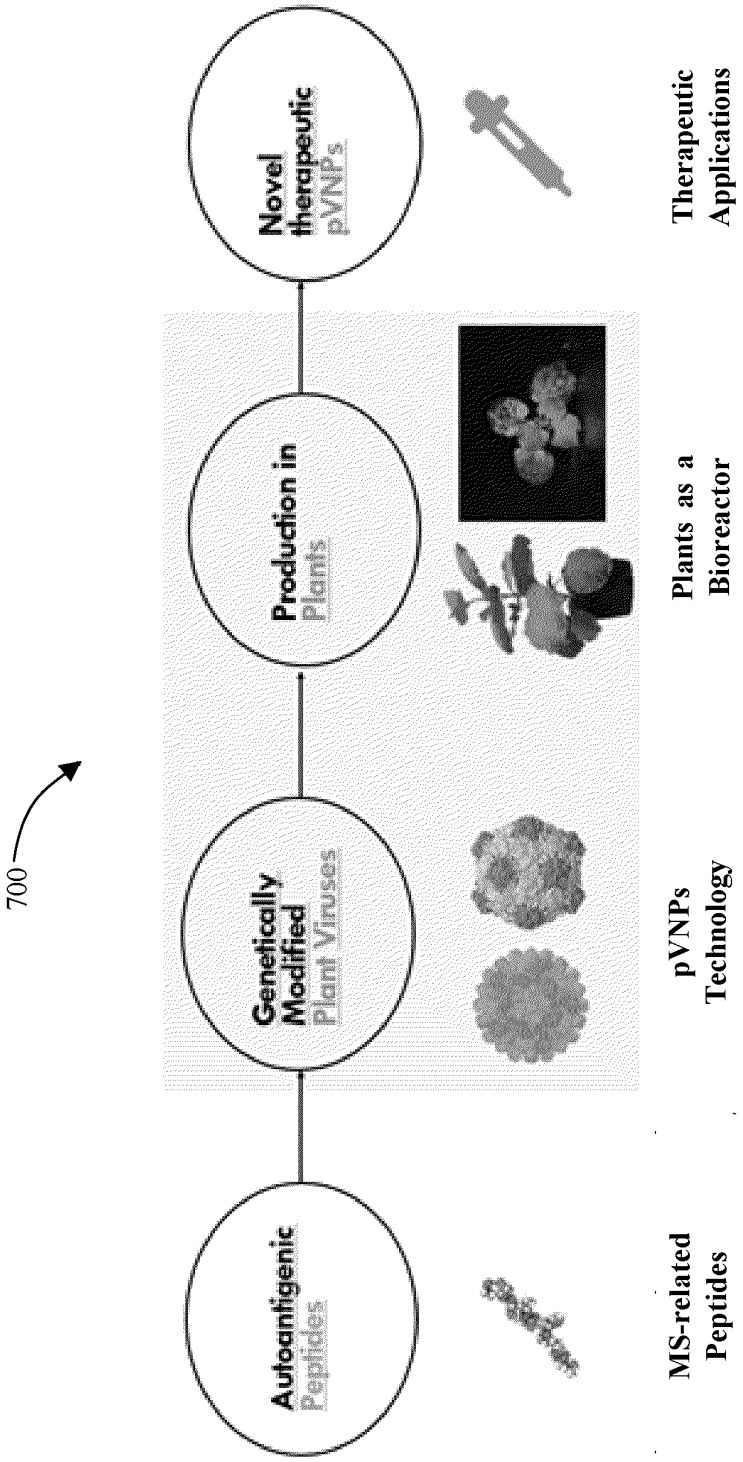
700
Autoantigenic Peptides
Genetically Modified Plant Viruses
Production in Plants
Novel therapeutic pVNPs
MS-related Peptides
pVNPs Technology
Plants as a Bioreactor
Therapeutic Applications

FIG. 7

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/071532

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/04 A61K38/10 A61K38/17 A61P25/28
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2015/125536 A1 (SANTAMARIA PEDRO [CA]) 7 May 2015 (2015-05-07) claims 1, 16-18, par 100, 123-125; -----	1-29
Y	CA 3 009 799 A1 (COUR PHARMACEUTICALS DEV COMPANY INC [US]) 13 July 2017 (2017-07-13) claims 53, 59, 64-66, 110-111; par 54, 121; ----- -/--	1-29

[x] Further documents are listed in the continuation of Box C.

[x] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 2024	02/12/2024

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Pilch, Bartosz

2

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2024/071532

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	MOORMAN CODY D. ET AL: "Emerging Therapeutics for Immune Tolerance: Tolerogenic Vaccines, T cell Therapy, and IL-2 Therapy", FRONTIERS IN IMMUNOLOGY, vol. 12, 1 January 2021 (2021-01-01), pages 657768-657768, XP055811870, Lausanne, CH ISSN: 1664-3224, DOI: 10.3389/fimmu.2021.657768 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8039385/pdf/fimmu-12-657768.pdf> page 5, left hand column, full 2nd par;, right-hand column, last par, table 1 -----	1-29
Y	US 2022/153859 A1 (KONTOS STEPHAN [US] ET AL) 19 May 2022 (2022-05-19) par 201, claims; -----	1-29

2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2024/071532

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [X] forming part of the international application as filed.

   b. [ ] furnished subsequent to the international filing date for the purposes of international search (Rule 13*ter*.1(a)).

      [ ] accompanied by a statement to the effect that the sequence listing does not go beyond the disclosure in the international application as filed.

2. [ ] With regard to any nucleotide and/or amino acid sequence disclosed in the international application, this report has been established to the extent that a meaningful search could be carried out without a WIPO Standard ST.26 compliant sequence listing.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

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