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Office de la Propriété Intellectuelle du Canada

Innovation, Science and Economic Development Canada
Canadian Intellectual Property Office

CA 3138525 A1 2020/11/05

(21) 3 138 525

(12) DEMANDE DE BREVET CANADIEN
CANADIAN PATENT APPLICATION

(13) A1

(86) Date de dépôt PCT/PCT Filing Date: 2020/04/28
(87) Date publication PCT/PCT Publication Date: 2020/11/05
(85) Entrée phase nationale/National Entry: 2021/10/28
(86) N° demande PCT/PCT Application No.: US 2020/030217
(87) N° publication PCT/PCT Publication No.: 2020/223205
(30) Priorités/Priorities: 2019/04/28 (US62/839,771); 2019/10/21 (US62/924,103); 2020/02/26 (US62/981,555)

(51) Cl.Int./Int.Cl. *A61K 48/00* (2006.01), *A61P 43/00* (2006.01), *C12N 15/864* (2006.01), *C12N 7/00* (2006.01)

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(54) Titre : METHODES DE TRAITEMENT DE SUJETS PRESENTANT UNE IMMUNITE PREEXISTANTE A DES VECTEURS DE TRANSFERT VIRAUX

(54) Title: METHODS FOR TREATMENT OF SUBJECTS WITH PREEXISTING IMMUNITY TO VIRAL TRANSFER VECTORS

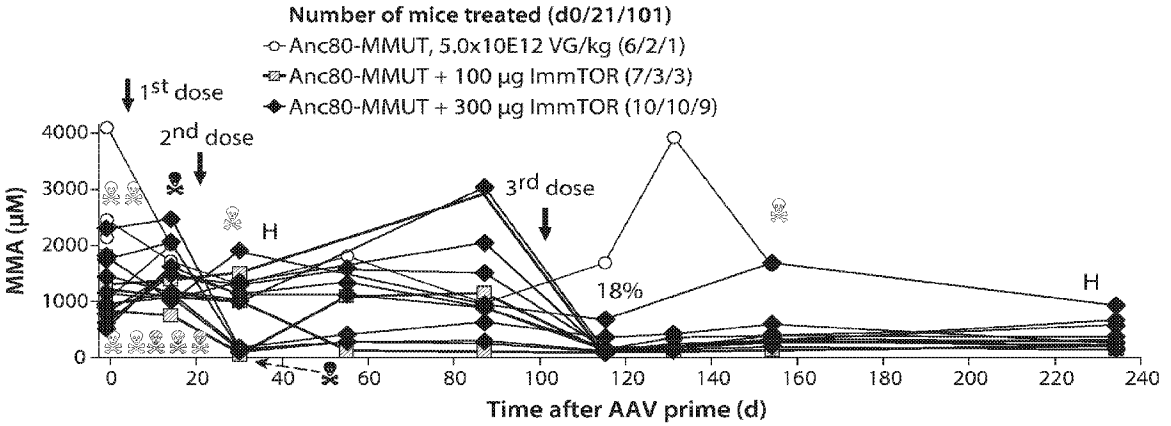


FIG. 7

(57) Abrégé/Abstract:
Provided herein are methods and related compositions for administering viral vectors with synthetic nanocarriers comprising an immunosuppressant to subjects. Such subjects may be subjects with pre-existing immunity against a viral antigen of the viral vector.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
05 November 2020 (05.11.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/223205 A1

(51) International Patent Classification:
*A61K 48/00* (2006.01) *C12N 15/864* (2006.01)
*C12N 7/00* (2006.01) *A61P 43/00* (2006.01)

(21) International Application Number:
PCT/US2020/030217

(22) International Filing Date:
28 April 2020 (28.04.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

| | | |
|---|---|---|
| 62/839,771 | 28 April 2019 (28.04.2019) | US |
| 62/924,103 | 21 October 2019 (21.10.2019) | US |
| 62/981,555 | 26 February 2020 (26.02.2020) | US |

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

**Published:**
— *with international search report (Art. 21(3))*

(54) **Title:** METHODS FOR TREATMENT OF SUBJECTS WITH PREEXISTING IMMUNITY TO VIRAL TRANSFER VECTORS

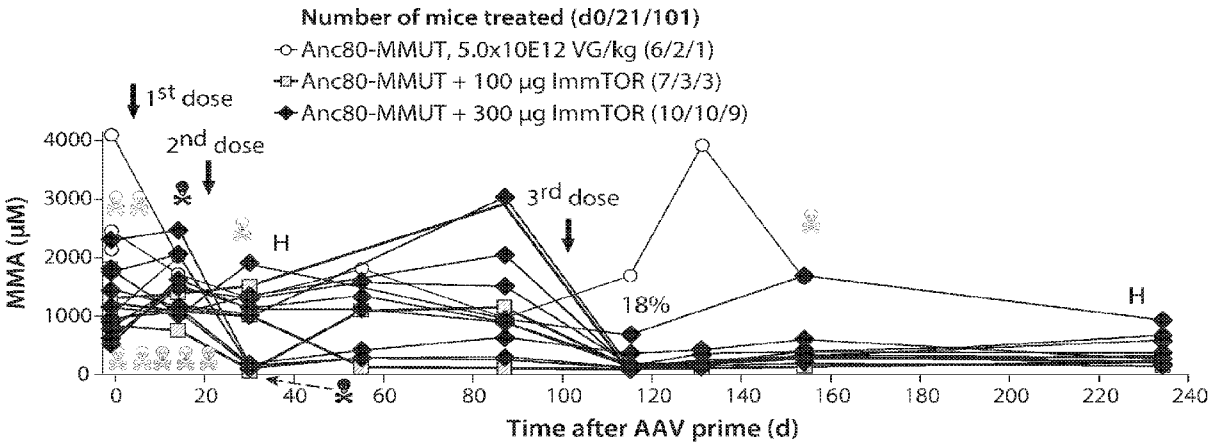


FIG. 7

(57) **Abstract:** Provided herein are methods and related compositions for administering viral vectors with synthetic nanocarriers comprising an immunosuppressant to subjects. Such subjects may be subjects with pre-existing immunity against a viral antigen of the viral vector.

WO 2020/223205 A1

# METHODS FOR TREATMENT OF SUBJECTS WITH PREEXISTING IMMUNITY TO VIRAL TRANSFER VECTORS

## RELATED APPLICATIONS

This application claims the benefit of priority under 35 U.S.C. § 119(e) of U.S. Provisional Application Serial No. 62/839,771, filed on April 28, 2019; U.S. Provisional Application Serial No. 62/924,103, filed on October 21, 2019; and U.S. Provisional Application Serial No. 62/981,555, filed February 26, 2020; the entire contents of each of which are incorporated herein by reference.

## FIELD OF THE INVENTION

This invention relates, at least in part, to methods, and related compositions, for administering viral vectors admixed with synthetic nanocarriers comprising an immunosuppressant. In some embodiments, the methods and compositions provided herein achieve increased transgene expression and/or reduced immune responses, such as downregulated immune responses against the viral vectors, such as in subjects with pre-existing immunity against the viral vector.

## SUMMARY OF THE INVENTION

In an aspect, a method comprising administering synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector to a subject is provided. In one embodiment, the subject has pre-existing immunity against a viral antigen of the viral vector.

In one embodiment of any one of the methods provided herein, the subject is one who would otherwise be excluded from treatment with the viral vector due to the level of pre-existing immunity against the viral vector in the subject. In one embodiment of any one of the methods provided herein, the subject is one with a titer or level of pre-existing immunity, such as anti-viral vector antibodies, that exceeds a threshold level for eligibility for treatment with the viral vector, such as an AAV vector, (e.g, without admixing with synthetic nanocarriers comprising an immunosuppressant). The threshold may be any one of the levels of pre-existing immunity provided herein or that would otherwise be understood by one of ordinary skill in the art, such as a clinician.

In one embodiment of any one of the methods provided herein, the subject is a pediatric or juvenile subject. In one embodiment of any one of the methods provided herein, the subject is a pediatric or juvenile subject with maternally-transferred antibodies against one

or more antigens of the viral vector. In one embodiment of any one of the methods provided herein, the subject is a newborn. In one embodiment of any one of the methods provided herein, the subject is a newborn with maternally-transferred antibodies against one or more antigens of the viral vector.

In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as neutralizing antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as combination of neutralizing antibodies and total anti-AAV capsid antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as a combination of neutralizing antibodies and anti-AAV capsid IgG antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity against a viral vector comprises pre-existing antibodies, such as a combination of neutralizing antibodies, anti-AAV IgG, and anti-AAV capsid IgM antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against the viral capsid of the viral vector.

In one embodiment of any one of the methods provided herein, the method further comprises determining the level of pre-existing immunity in the subject, such as before administration of the viral vector. In one embodiment of any one of the methods provided herein, the subject has a moderate level of pre-existing immunity against a viral antigen of the viral vector. In one embodiment of any one of the methods provided herein, the method further comprises measuring a level of pre-existing anti-viral vector antibodies, in the subject prior to administration of the viral vector to the subject. In one embodiment, the subject is one with a titer or level of anti-viral vector antibodies that exceeds a threshold level for eligibility for treatment with the viral vector, such as an AAV vector, (e.g, without admixing with synthetic nanocarriers comprising an immunosuppressant). The threshold may be any one of the levels of pre-existing immunity provided herein or that would be otherwise known to an ordinarily skilled artisan, such as a clinician. In one embodiment of any one of the methods provided herein, the method further comprises comparing the level of per-existing immunity in the subject that is determined with a threshold level.

In an aspect, a method comprising administering to a subject synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector, wherein the amount of the viral vector is less than an amount of the viral vector that, when not administered with synthetic nanocarriers comprising an immunosuppressant, increases transgene expression of

the viral vector is provided. In an aspect, a method comprising administering to a subject synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector, wherein the amount of the viral vector is less than an amount of the viral vector that, when not administered concomitantly with synthetic nanocarriers comprising an immunosuppressant, increases transgene expression of the viral vector is provided. In an aspect, a method comprising administering to a subject synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector, wherein the amount of the viral vector is less than an amount of the viral vector that, when not administered admixed with synthetic nanocarriers comprising an immunosuppressant, increases transgene expression of the viral vector is provided. In one embodiment of any one of the methods provided herein, the subject that is the subject for administration is a first subject and the comparison to another amount is based on the administration to a second subject the another amount.

In one embodiment of any one of the methods provided herein, the first subject has pre-existing immunity against a viral antigen of the viral vector and the second subject also has pre-existing immunity against a viral antigen of the viral vector.

In an aspect, a method comprising administering to a subject synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector, wherein the amount of the synthetic nanocarriers comprising an immunosuppressant is greater than an amount of the synthetic nanocarriers comprising an immunosuppressant that, when administered with a viral vector to a subject without pre-existing immunity against a viral antigen of the viral vector, increases transgene expression of the viral vector and/or results in a reduction in an immune response, such as antibodies, against a viral antigen of the viral vector. In one embodiment of any one of the methods provided herein, the subject that is the subject for administration is a first subject and the comparison to another amount is based on the administration to a second subject the another amount. In one embodiment of any one of the methods provided herein, the first subject has pre-existing immunity against a viral antigen of the viral vector and the second subject does not have pre-existing immunity against a viral antigen of the viral vector.

In one embodiment of any one of the methods provided herein, the synthetic nanocarriers comprising an immunosuppressant have not been previously administered to the first and/or second subject, or the synthetic nanocarriers comprising an immunosuppressant and the viral vector have not previously been concomitantly administered to the first and/or second subject, or the synthetic nanocarriers comprising an immunosuppressant and the viral vector have not previously been administered admixed to the first and/or second subject.

In one embodiment of any one of the methods provided, the dose of the viral vector is a lower dose than what would otherwise be expected to be necessary to result in the same or similar level of efficacy, such as the same or similar level of transgene expression. In one embodiment of any one of the methods provided, the dose of the viral vector is at least 1-fold, 2-fold, 3-fold, 4-fold , 5-fold or more less. In one embodiment of any one of the methods provided herein, the aforementioned doses are the doses for at least one or more all of the dosings of the viral vector.

In one embodiment of any one of the methods provided, the dose of the synthetic nanocarriers comprising an immunosuppressant is a greater dose than what would otherwise be expected to be necessary to result in the same or similar level of efficacy when delivered to a subject without pre-existing immunity against the viral vector. In one embodiment of any one of the methods provided, the dose of the synthetic nanocarriers comprising an immunosuppressant is at least 2-fold or 3-fold or more greater. In one embodiment of any one of the methods provided herein, the aforementioned doses are the doses for at least one or more all of the dosings of the synthetic nanocarriers comprising an immunosuppressant.

In one embodiment of any one of the methods provided herein, at least or only the first administration of the viral vector and synthetic nanocarriers comprising an immunosuppressant is an admixed composition of the viral vector and synthetic nanocarriers comprising an immunosuppressant. In one embodiment of any one of the methods provided herein every administration of the viral vector and synthetic nanocarriers comprising an immunosuppressant is an admixed composition of the viral vector and synthetic nanocarriers comprising an immunosuppressant. In one embodiment of any one of the methods provided, the viral vector and synthetic nanocarriers comprising an immunosuppressant are admixed for each coadministration.

In one embodiment of any one of the methods provided herein, the synthetic nanocarriers comprising an immunosuppressant is admixed with the viral vector and the admixture is administered as at least the first coadministration to the subject.

In one embodiment of any one of the methods provided herein, the synthetic nanocarriers comprising an immunosuppressant is admixed with the viral vector and the admixture is administered as at least the first and second coadministration to the subject.

In one embodiment of any one of the methods provided herein, the synthetic nanocarriers comprising an immunosuppressant is admixed with the viral vector and the admixture is administered as at least the first, second and third coadministration to the subject.

In one embodiment of any one of the methods provided, the administration of the synthetic nanocarriers comprising an immunosuppressant admixed with the viral vector and/or at least one repeat dose is by intravenous administration.

In one embodiment of any one of the methods provided, the viral vector comprises one or more expression control sequences. In one embodiment of any one of the methods provided, the one or more expression control sequences comprise a liver-specific promoter. In one embodiment of any one of the methods provided, the one or more expression control sequences comprise a constitutive promoter.

In one embodiment of any one of the methods provided, the method is for transgene expression in the liver.

In one embodiment of any one of the methods provided, the method further comprises assessing transgene expression, an IgM and/or IgG response, and/or a neutralizing antibody response to the viral vector in the subject at one or more time points. In one embodiment of any one of the methods provided, at least one of the time points of assessing an IgM and/or IgG response and/or a neutralizing antibody is subsequent to a coadministration.

In one embodiment of any one of the methods provided, the viral vector is a retroviral vector, an adenoviral vector, a lentiviral vector or an adeno-associated viral vector.

In one embodiment of any one of the methods provided, the viral vector is an adeno-associated viral vector. In one embodiment of any one of the methods provided, the adeno-associated viral vector is an AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV10 or AAV11 adeno-associated viral vector. In one embodiment of any one of the methods or compositions provided, the viral vector, such as an AAV vector, is a recombinant, synthetic, engineered or chimeric vector.

In one embodiment of any one of the methods provided, the immunosuppressant of the synthetic nanocarriers comprising an immunosuppressant admixed with the viral vector and/or one or more subsequent concomitant administration(s) or the one or more repeat dose(s) is an inhibitor of the NF-kB pathway. In one embodiment of any one of the methods provided, the immunosuppressant of the coadministration and/or repeat dose is an mTOR inhibitor. In one embodiment of any one of the methods provided, the mTOR inhibitor is rapamycin or a rapamycin analog.

In one embodiment of any one of the methods provided, the immunosuppressant is coupled to the synthetic nanocarriers. In one embodiment of any one of the methods provided, the immunosuppressant is encapsulated in the synthetic nanocarriers.

In one embodiment of any one of the methods provided, the synthetic nanocarriers of the synthetic nanocarriers comprising an immunosuppressant admixed with the viral vector and/or one or more subsequent concomitant administration(s) or the one or more repeat dose(s) comprise lipid nanoparticles, polymeric nanoparticles, metallic nanoparticles, surfactant-based emulsions, dendrimers, buckyballs, nanowires, virus-like particles or peptide or protein particles.

In one embodiment of any one of the methods provided, the synthetic nanocarriers comprise polymeric nanoparticles. In one embodiment of any one of the methods provided, the polymeric nanoparticles comprise a polyester, polyester attached to a polyether, polyamino acid, polycarbonate, polyacetal, polyketal, polysaccharide, polyethyloxazoline or polyethyleneimine. In one embodiment of any one of the methods provided, the polymeric nanoparticles comprise a polyester or a polyester attached to a polyether. In one embodiment of any one of the methods provided, the polyester comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone. In one embodiment of any one of the methods provided, the polymeric nanoparticles comprise a polyester and a polyester attached to a polyether. In one embodiment of any one of the methods provided, the polyether comprises polyethylene glycol or polypropylene glycol.

In one embodiment of any one of the methods provided, the mean of a particle size distribution obtained using dynamic light scattering of a population of the synthetic nanocarriers is a diameter greater than 110nm. In one embodiment of any one of the methods provided, the diameter is greater than 150nm. In one embodiment of any one of the methods provided, the diameter is greater than 200nm. In one embodiment of any one of the methods provided, the diameter is greater than 250nm. In one embodiment of any one of the methods provided, the diameter is less than 5μm. In one embodiment of any one of the methods provided, the diameter is less than 4μm. In one embodiment of any one of the methods provided, the diameter is less than 3μm. In one embodiment of any one of the methods provided, the diameter is less than 2μm. In one embodiment of any one of the methods provided, the diameter is less than 1μm. In one embodiment of any one of the methods provided, the diameter is less than 750nm. In one embodiment of any one of the methods provided, the diameter is less than 500nm. In one embodiment of any one of the methods provided, the diameter is less than 450nm. In one embodiment of any one of the methods provided, the diameter is less than 400nm. In one embodiment of any one of the methods provided, the diameter is less than 350nm. In one embodiment of any one of the methods provided, the diameter is less than 300nm.

In one embodiment of any one of the methods provided, the load of immunosuppressant comprised in the synthetic nanocarriers, on average across the synthetic nanocarriers, is between 0.1% and 50% (weight/weight). In one embodiment of any one of the methods provided, the load is between 0.1% and 40%. In one embodiment of any one of the methods provided, the load is greater than 4% but less than 40%. In one embodiment of any one of the methods provided, the load is between 2% and 25%.

In one embodiment of any one of the methods provided, an aspect ratio of a population of the synthetic nanocarriers is greater than or equal to 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7 or 1:10.

In one embodiment of any one of the methods provided, a transgene of the viral vector encodes any one of the proteins, such as enzymes, provided herein.

In one embodiment of any one of the methods provided, the subject is one in need of expression of a protein encoded by a transgene of the viral vector.

In one embodiment of any one of the methods provided, the subject is one with methylmalonic acidemia or OTC deficiency.

## BRIEF DESCRIPTION OF THE FIGURES

**Fig. 1** shows the levels of neutralizing activity and IgG anti-AAV antibodies in serum from human donors 8, 31, 35, 44, and 45. Neutralizing activity is plotted in bars as luciferase expression (normalized RLU), with high levels of luciferase expression corresponding to low neutralizing antibody activity and low levels of luciferase expression corresponding to high neutralizing antibody activity. Anti-AAV IgG neutralizing antibodies are plotted in the line.

**Fig. 2** shows treatment of naïve mice with human serum containing neutralizing antibodies, followed by injection with the AAV-SEAP vector or the AAV-SEAP vector and synthetic nanocarriers comprising rapamycin (ImmTOR in some of the figures). Serum SEAP activity was measured after injection. Numbers above the bars indicate the change in serum SEAP activity between mice not administered synthetic nanocarriers comprising rapamycin and mice administered synthetic nanocarriers comprising rapamycin. The dotted line was used to normalize serum SEAP activity levels.

**Fig. 3** shows injection of naïve mice with the AAV-SEAP vector or the AAV-SEAP vector and synthetic nanocarriers comprising rapamycin admixed with a 1:100 dilution of serum containing anti-AAV neutralizing antibodies. Numbers above the bars indicate serum SEAP activity levels relative to mice not injected with serum. The dotted line was used to normalize serum SEAP activity levels.

**FIG. 4** shows maternally transferred Anti-Anc80 IgG in offspring of Mck-MUT mice Treated with Anc80-Mut.

**FIG. 5** shows high-dose Anc80-MUT and ImmTOR in Mck-MUT mice with pre-existing maternally transferred anti-Anc80 IgG: MMA.

**FIG. 6** shows high-dose Anc80-MUT and ImmTOR in Mck-MUT mice with pre-existing maternally transferred anti-Anc80 IgG: MMA.

**FIG. 7** shows high-dose Anc80-MUT and ImmTOR in Mck-MUT mice with pre-existing maternally transferred anti-Anc80 IgG: MMA.

**FIG. 8 (FIGs. 8A-8B)** show the survival of Mck-MUT mice with pre-existing maternally transferred anti-Anc80 IgG repeatedly treated with Anc80-MUT ± ImmTOR.

## DETAILED DESCRIPTION OF THE INVENTION

Before describing the present invention in detail, it is to be understood that this invention is not limited to particularly exemplified materials or process parameters as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments of the invention only, and is not intended to be limiting of the use of alternative terminology to describe the present invention.

All publications, patents and patent applications cited herein, whether supra or infra, are hereby incorporated by reference in their entirety for all purposes. Such incorporation by reference is not intended to be an admission that any of the incorporated publications, patents and patent applications cited herein constitute prior art.

As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a polymer" includes a mixture of two or more such molecules or a mixture of differing molecular weights of a single polymer species, reference to "a synthetic nanocarrier" includes a mixture of two or more such synthetic nanocarriers or a plurality of such synthetic nanocarriers, reference to “a DNA molecule” includes a mixture of two or more such DNA molecules or a plurality of such DNA molecules, reference to "an immunosuppressant" includes a mixture of two or more such immunosuppressant molecules or a plurality of such immunosuppressant molecules, and the like.

As used herein, the term “comprise” or variations thereof such as “comprises” or “comprising” are to be read to indicate the inclusion of any recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) but not the

exclusion of any other integer or group of integers. Thus, as used herein, the term "comprising" is inclusive and does not exclude additional, unrecited integers or method/process steps.

In embodiments of any of the compositions and methods provided herein, "comprising" may be replaced with "consisting essentially of" or "consisting of". The phrase "consisting essentially of" is used herein to require the specified integer(s) or steps as well as those which do not materially affect the character or function of the claimed invention. As used herein, the term "consisting" is used to indicate the presence of the recited integer (e.g. a feature, element, characteristic, property, method/process step or limitation) or group of integers (e.g. features, elements, characteristics, properties, method/process steps or limitations) alone.

## A. INTRODUCTION

Viral vectors, such as those based on adeno-associated viruses (AAVs), have shown great potential in therapeutic applications, such as gene therapy. However, the use of viral vectors in gene therapy and other applications has been limited, such as due to pre-existing immunity as a result of viral antigen exposure. Pre-existing antibodies against AAV can form in response to a naturally occurring wild type AAV infection or via maternal transfer of antibody from an AAV-sensitized mother to her newborn baby. Indeed, pre-existing immunity to viral vectors can result in immune responses against the viral vector and reduced efficacy of the viral vector, e.g., as shown by reduced transgene expression. Both cellular and humoral immune responses against the viral vector can diminish efficacy and/or reduce the ability to use such therapeutics. These immune responses include antibody, B cell, and T cell responses and can be specific to viral antigens of the viral vector, such as viral capsid or coat proteins, or peptides thereof. The inventors have surprisingly discovered that synthetic nanocarriers comprising an immunosuppressant can be used in combination with viral vectors even in those with pre-existing immunity against viral antigen(s) of the viral vector. The synthetic nanocarriers comprising an immunosuppressant as provided herein can allow for treatment of such subjects with a viral vector.

The inventors have also surprisingly discovered synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector can achieve improved transgene expression in subjects, e.g., in subjects with pre-existing immunity to the viral vector. When the administration is the first administration of the viral vector, such improvements in immune response reduction and/or transgene expression are significant.

In addition, it was further surprisingly found that while such admixed administration of synthetic nanocarriers comprising an immunosuppressant and a viral vector achieves improved transgene expression on the first administration of the viral vector, admixing is not necessarily required for the efficacy of subsequent administrations of synthetic nanocarriers comprising an immunosuppressant and viral vectors.

Additionally, it has been surprisingly discovered that such admixed administration of synthetic nanocarriers comprising an immunosuppressant and a viral vector can be used to achieve reduced doses of viral vector without reducing transgene expression. Administering reduced doses of the viral vector can mitigate undesirable effects associated with administration of viral vector.

Further, it has been surprisingly found that higher doses of synthetic nanocarriers comprising an immunosuppressant can also be helpful in achieving efficacious administration of a viral vector in subjects with pre-existing immunity against the viral vector.

Therefore, the inventors have surprisingly and unexpectedly discovered that the problems and limitations noted above can be overcome by practicing the invention disclosed herein. Methods and compositions are provided that offer solutions to the aforementioned obstacles to effective use of viral vectors for treatment. Provided herein are methods and compositions for treating a subject with a viral vector comprising any one of the viral vector constructs provided herein, such as admixed with synthetic nanocarriers comprising an immunosuppressant. The methods and related compositions provided can allow for broader and improved use of viral vectors and can result in a reduction of undesired immune responses and/or result in improved efficacy, such as through increased transgene expression.

The invention will now be described in more detail below.

## B. DEFINITIONS

"Administering" or "administration" or "administer" means giving or dispensing a material to a subject in a manner that is pharmacologically useful. The term is intended to include "causing to be administered". "Causing to be administered" means causing, urging, encouraging, aiding, inducing or directing, directly or indirectly, another party to administer the material. When a time period between administrations are referred to, the time period is the time between the initiation of the administrations except as otherwise described.

"Admix" as used herein refers to the mixing of two or more components such that the two or more components are present together in a composition and administration of the composition provides the two or more components to a subject. Any one of the

coadministrations of any one of the methods provided herein can be administered as an admixture. In some embodiments, mixing the two components comprises dissolving, dispersing, suspending, combining, joining, or blending the two components, e.g., such as a viral transfer vector and synthetic nanocarriers comprising an immunosuppressant. Methods of admixing are known to those skilled in the art, and include, but are not limited to, standard pharmaceutical mixing methods, such as liquid-liquid mixing, powder-powder mixing, liquid-powder mixing. In some embodiments, the resulting mixture is a homogenous mixture; i.e., the viral vector may be uniformly admixed with the synthetic nanocarriers comprising an immunosuppressant. In other embodiments, the resulting mixture is a heterogeneous mixture, i.e., the viral vector is not uniformly admixed with the synthetic nanocarriers comprising an immunosuppressant.

In some embodiments of any one of the methods provided, a synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector is followed by the one or more administrations of synthetic nanocarriers comprising an immunosuppressant concomitantly with the viral vector and/or or one or more subsequent administrations of the synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector (one or more subsequent concomitant administration(s) or the one or more repeat dose(s), respectively). In some embodiments of any one of the methods provided, the subsequent concomitant administration or the repeat dose of synthetic nanocarriers comprising an immunosuppressant and viral vector is administered 1 week, 2 weeks, 3, weeks, 1 month, 2 months, or more after the administration of the synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector.

"Amount effective" in the context of a composition for administration to a subject as provided herein refers to an amount of the composition that produces one or more desired results in the subject, for example, the reduction or elimination of an immune response, such as an IgM and/or IgG immune response, against a viral vector and/or efficacious or increased transgene expression. The amount effective can be for *in vitro* or *in vivo* purposes. For *in vivo* purposes, the amount can be one that a clinician would believe may have a clinical benefit for a subject that may experience undesired immune responses as a result of administration of a viral vector. In any one of the methods provided herein, the composition(s) administered may be in any one of the amounts effective as provided herein.

Amounts effective can involve reducing the level of an undesired immune response, although in some embodiments, it involves preventing an undesired immune response altogether. Amounts effective can also involve delaying the occurrence of an undesired

immune response. An amount effective can also be an amount that results in a desired therapeutic endpoint or a desired therapeutic result. In some embodiments of any one of the compositions and methods provided, the amount effective is one in which a desired immune response, such as the reduction or elimination of an immune response against a viral vector, such as an IgM and/or IgG response, and/or the generation of efficacious or increased transgene expression. The achievement of any of the foregoing can be monitored by routine methods.

Amounts effective will depend, of course, on the particular subject being treated; the severity of a condition, disease or disorder; the individual patient parameters including age, physical condition, size and weight; the duration of the treatment; the nature of concurrent therapy (if any); the specific route of administration and like factors within the knowledge and expertise of the health practitioner. These factors are well known to those of ordinary skill in the art and can be addressed with no more than routine experimentation.

In some embodiments of any one of the methods or compositions provided, the amount effective of a viral vector administered without synthetic nanocarriers comprising an immunosuppressant is less in subjects without pre-existing immunity against a viral antigen of the viral vector than in subjects having pre-existing immunity against a viral antigen of the viral vector. In some embodiments of any one of the methods or compositions provided, the amount effective of a viral vector when administered admixed with synthetic nanocarriers comprising an immunosuppressant is less than the amount effective of the viral vector when not admixed, such as administered concomitantly but not admixed, with synthetic nanocarriers comprising an immunosuppressant or without the synthetic nanocarriers comprising an immunosuppressant in a subject, such as a subject having pre-existing immunity against a viral antigen of the viral vector. In some embodiments, the subject has not previously been administered the synthetic nanocarriers comprising an immunosuppressant and/or the viral vector.

"Assessing an immune response" refers to any measurement or determination of the level, presence or absence, reduction in, increase in, etc. of an immune response *in vitro* or *in vivo*. Such measurements or determinations may be performed on one or more samples obtained from a subject. Such assessing can be performed with any one of the methods provided herein or otherwise known in the art, including an ELISA-based assay. The assessing may be assessing the number or percentage of antibodies, such as IgM and/or IgG antibodies, such as those specific to a viral vector, such as in a sample from a subject. The assessing also may be assessing any effect related to the immune response, such as measuring

the presence or absence of a cytokine, cell phenotype, etc. Any one of the methods provided herein may comprise or further comprise a step of assessing an immune response to a viral vector or antigen thereof. The assessing may be done directly or indirectly. The term is intended to include actions that cause, urge, encourage, aid, induce or direct another party to assess an immune response.

"Average", as used herein, refers to the arithmetic mean unless otherwise noted.

"Concomitantly" means administering two or more materials/agents to a subject in a manner that is correlated in time, preferably sufficiently correlated in time so as to provide a modulation in an immune response, and even more preferably the two or more materials/agents are administered in combination. In embodiments, concomitant administration may encompass administration of two or more materials/agents within a specified period of time, preferably within 1 month, more preferably within 1 week, still more preferably within 1 day, and even more preferably within 1 hour. In embodiments, the materials/agents may be repeatedly administered concomitantly; that is concomitant administration on more than one occasion, such as provided in the Examples.

"Couple" or "Coupled" (and the like) means to chemically associate one entity (for example a moiety) with another. In some embodiments of any one of the methods or compositions provided, the coupling is covalent, meaning that the attachment occurs in the context of the presence of a covalent bond between the two entities. In non-covalent embodiments, the non-covalent coupling is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, TT stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. In embodiments of any one of the methods or compositions provided, encapsulation is a form of coupling.

"Dose" refers to a specific quantity of a pharmacologically and/or immunologically active material for administration to a subject for a given time. In general, doses of the synthetic nanocarriers comprising an immunosuppressant and/or viral vectors in the methods and compositions, which include kits, of the invention refer to the amount of immunosuppressant comprised in the synthetic nanocarriers and/or the amount of viral vectors unless otherwise provided. Alternatively, the dose can be administered based on the number of synthetic nanocarriers that provide the desired amount of an immunosuppressant, in instances when referring to a dose of synthetic nanocarriers that comprise an

immunosuppressant. When dose is used in the context of a repeated dosing, dose refers to the amount of each of the repeated doses, which may be the same or different.

"Encapsulate" means to enclose at least a portion of a substance within a synthetic nanocarrier. In some embodiments of any one of the methods or compositions provided, a substance is enclosed completely within a synthetic nanocarrier. In other embodiments of any one of the methods or compositions provided, most or all of a substance that is encapsulated is not exposed to the local environment external to the synthetic nanocarrier. In other embodiments of any one of the methods or compositions provided, no more than 50%, 40%, 30%, 20%, 10% or 5% (weight/weight) is exposed to the local environment. Encapsulation is distinct from absorption, which places most or all of a substance on a surface of a synthetic nanocarrier, and leaves the substance exposed to the local environment external to the synthetic nanocarrier.

"Expression control sequences" are any sequences that can affect expression and can include promoters, enhancers, and operators. Expression control sequences, or control elements, within vectors can facilitate proper nucleic acid transcription, translation, viral packaging, etc. Generally, control elements act in cis, but they may also work in trans. In one embodiment of any one of the methods or compositions provided, the expression control sequence is a promoter, such as a constitutive promoter or tissue-specific promoter. "Constitutive promoters," also called ubiquitous or promiscuous promoters, are those that are thought of being generally active and not exclusive or preferential to certain cells. "Tissue-specific promoters" are those that are active in a particular cell type or tissue, such activity may be exclusive to the particular cell type or tissue. In any one of the nucleic acids or viral vectors provided herein the promoter may be any one of the promoters provided herein. In any one of the nucleic acids or viral vectors provided herein the promoter may be a liver-specific promoter.

"Immune response against a viral vector" or the like refers to any undesired immune response against a viral vector, such as an antibody (e.g., IgM or IgG) or cellular response. In some embodiments, the undesired immune response is an antigen-specific immune response against the viral vector or an antigen thereof. In some embodiments, the immune response is specific to a viral antigen of the viral vector. In other embodiments, the immune response is specific to a protein or peptide encoded by a transgene of the viral vector. In some embodiments, the immune response is specific to a viral antigen of the viral vector and not to a protein or peptide that is encoded by a transgene of the viral vector.

In some embodiments, a reduced anti-viral vector response in a subject comprises a reduced anti-viral vector immune response measured using a biological sample obtained from the subject following administration as provided herein as compared to an anti-viral vector immune response measured using a biological sample obtained from another subject, such as a test subject, following administration to this other subject of the viral vector without administration as provided herein. In some embodiments, the anti-viral vector immune response is a reduced anti-viral vector immune response in a biological sample obtained from the subject following administration as provided herein upon a subsequent viral vector *in vitro* challenge performed on the subject's biological sample as compared to the anti-viral vector immune response detected upon viral vector *in vitro* challenge performed on a biological sample obtained from another subject, such as a test subject, following administration to the other subject of the viral vector without administration as provided herein. In other embodiments, an immune response can be assessed in another subject, such as in a sample from a test subject, where the results for the other subject, with or without scaling, would be expected to be indicative of what is occurring or has occurred in the subject at issue. In some embodiments, a reduced anti-viral vector response in a subject comprises a reduced anti-viral vector immune response measured using a biological sample obtained from the subject following administration as provided herein as compared to an anti-viral vector immune response measured using a biological sample obtained from the subject at a different point in time, such as at a time without administration as provided herein, for example, prior to an administration as provided herein.

"Immunosuppressant" means a compound that can cause a tolerogenic effect, preferably through its effects on APCs. A tolerogenic effect generally refers to the modulation by the APC or other immune cells systemically and/or locally, that reduces, inhibits or prevents an undesired immune response to an antigen in a durable fashion. In one embodiment of any one of the methods or compositions provided, the immunosuppressant is one that causes an APC to promote a regulatory phenotype in one or more immune effector cells. For example, the regulatory phenotype may be characterized by the inhibition of the production, induction, stimulation or recruitment of antigen-specific CD4+ T cells or B cells, the inhibition of the production of antigen-specific antibodies, the production, induction, stimulation or recruitment of Treg cells (e.g., CD4+CD25highFoxP3+ Treg cells), etc. This may be the result of the conversion of CD4+ T cells or B cells to a regulatory phenotype. This may also be the result of induction of FoxP3 in other immune cells, such as CD8+ T cells, macrophages and iNKT cells. In one embodiment of any one of the methods or compositions

provided, the immunosuppressant is one that affects the response of the APC after it processes an antigen. In another embodiment of any one of the methods or compositions provided, the immunosuppressant is not one that interferes with the processing of the antigen. In a further embodiment of any one of the methods or compositions provided, the immunosuppressant is not an apoptotic-signaling molecule. In another embodiment of any one of the methods or compositions provided, the immunosuppressant is not a phospholipid.

Immunosuppressants include, but are not limited to, statins; mTOR inhibitors, such as rapamycin or a rapamycin analog (i.e., rapalog); TGF-β signaling agents; TGF-β receptor agonists; histone deacetylase inhibitors, such as Trichostatin A; corticosteroids; inhibitors of mitochondrial function, such as rotenone; P38 inhibitors; NF-κβ inhibitors, such as 6Bio, Dexamethasone, TCPA-1, IKK VII; adenosine receptor agonists; prostaglandin E2 agonists (PGE2), such as Misoprostol; phosphodiesterase inhibitors, such as phosphodiesterase 4 inhibitor (PDE4), such as Rolipram; proteasome inhibitors; kinase inhibitors; G-protein coupled receptor agonists; G-protein coupled receptor antagonists; glucocorticoids; retinoids; cytokine inhibitors; cytokine receptor inhibitors; cytokine receptor activators; peroxisome proliferator-activated receptor antagonists; peroxisome proliferator-activated receptor agonists; histone deacetylase inhibitors; calcineurin inhibitors; phosphatase inhibitors; PI3KB inhibitors, such as TGX-221; autophagy inhibitors, such as 3-Methyladenine; aryl hydrocarbon receptor inhibitors; proteasome inhibitor I (PSI); and oxidized ATPs, such as P2X receptor blockers. Immunosuppressants also include IDO, vitamin D3, retinoic acid, cyclosporins, such as cyclosporine A, aryl hydrocarbon receptor inhibitors, resveratrol, azathiopurine (Aza), 6-mercaptopurine (6-MP), 6-thioguanine (6-TG), FK506, sanglifehrin A, salmeterol, mycophenolate mofetil (MMF), aspirin and other COX inhibitors, niflumic acid, estriol and triptolide. Other exemplary immunosuppressants include, but are not limited, small molecule drugs, natural products, antibodies (e.g., antibodies against CD20, CD3, CD4), biologics-based drugs, carbohydrate-based drugs, RNAi, antisense nucleic acids, aptamers, methotrexate, NSAIDs; fingolimod; natalizumab; alemtuzumab; anti-CD3; tacrolimus (FK506), abatacept, belatacept, etc. "Rapalog", as used herein, refers to a molecule that is structurally related to (an analog) of rapamycin (sirolimus). Examples of rapalogs include, without limitation, temsirolimus (CCI-779), everolimus (RAD001), ridaforolimus (AP-23573), and zotarolimus (ABT-578). Additional examples of rapalogs may be found, for example, in WO Publication WO 1998/002441 and U.S. Patent No. 8,455,510, the rapalogs of which are incorporated herein by reference in their entirety. Further immunosuppressants are known to those of skill in the art, and the invention is not limited in this respect. In

embodiments of any one of the methods or compositions provided, the immunosuppressant may comprise any one of the agents provided herein, such as any one of the foregoing.

"Increasing transgene expression" refers to increasing the level of transgene expression of a viral vector in a subject, a transgene being delivered by the viral vector. In some embodiments, the level of the transgene expression may be determined by measuring transgene protein concentrations in various tissues or systems of interest in the subject. Alternatively, when the transgene expression product is a nucleic acid, the level of transgene expression may be measured by transgene nucleic acid products. Increasing transgene expression can be determined, for example, by measuring the amount of the transgene expression in a sample obtained from a subject and comparing it to a prior sample. The sample may be a tissue sample. In some embodiments, the transgene expression can be measured using flow cytometry. In other embodiments, increased transgene expression can be assessed in another subject, such as in a sample from a test subject, where the results for the other subject, with or without scaling, would be expected to be indicative of what is occurring or has occurred in the subject at issue. Any one of the methods provided herein may result in increased transgene expression.

"Load", when an immunosuppressant is comprised in synthetic nanocarriers, such as when coupled thereto, is the amount of the immunosuppressant in the synthetic nanocarriers based on the total dry recipe weight of materials in an entire synthetic nanocarrier (weight/weight). Generally, such a load is calculated as an average across a population of synthetic nanocarriers. In one embodiment of any one of the methods or compositions provided the load on average across the synthetic nanocarriers is between 0.1% and 99%. In another embodiment of any one of the methods or compositions provided, the load is between 0.1% and 50%. In another embodiment of any one of the methods or compositions provided, the load is between 0.1% and 20%. In a further embodiment of any one of the methods or compositions provided, the load is between 0.1% and 10%. In still a further embodiment of any one of the methods or compositions provided, the load is between 1% and 10%. In still a further embodiment of any one of the methods or compositions provided, the load is between 7% and 20%. In yet another embodiment of any one of the methods or compositions provided, the load is at least 0.1%, at least 0.2%, at least 0.3%, at least 0.4%, at least 0.5%, at least 0.6%, at least 0.7%, at least 0.8%, at least 0.9%, at least 1%, at least 2%, at least 3%, at least 4%, at least 5%, at least 6%, at least at least 7%, at least 8%, at least 9%, at least 10%, at least 11%, at least 12%, at least 13%, at least 14%, at least 15%, at least 16%, at least 17%, at least 18%, at least 19%, at least 20%, at least 25%, at least 30%, at least 40%, at least 50%, at

least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% on average across the population of synthetic nanocarriers. In yet a further embodiment of any one of the methods or compositions provided, the load is 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.6%, 0.7%, 0.8%, 0.9%, 1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, 10%, 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19% or 20% on average across the population of synthetic nanocarriers. In some embodiments of any one of the above embodiments, the load is no more than 25% on average across a population of synthetic nanocarriers. In embodiments of any one of the methods or compositions provided, the load is calculated as known in the art.

"Maximum dimension of a synthetic nanocarrier" means the largest dimension of a nanocarrier measured along any axis of the synthetic nanocarrier. "Minimum dimension of a synthetic nanocarrier" means the smallest dimension of a synthetic nanocarrier measured along any axis of the synthetic nanocarrier. For example, for a spheroidal synthetic nanocarrier, the maximum and minimum dimension of a synthetic nanocarrier would be substantially identical, and would be the size of its diameter. Similarly, for a cuboidal synthetic nanocarrier, the minimum dimension of a synthetic nanocarrier would be the smallest of its height, width or length, while the maximum dimension of a synthetic nanocarrier would be the largest of its height, width or length. In an embodiment, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm. In an embodiment, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or less than 5 μm. Preferably, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is greater than 110 nm, more preferably greater than 120 nm, more preferably greater than 130 nm, and more preferably still greater than 150 nm. Aspects ratios of the maximum and minimum dimensions of synthetic nanocarriers may vary depending on the embodiment. For instance, aspect ratios of the maximum to minimum dimensions of the synthetic nanocarriers may vary from 1:1 to 1,000,000:1, preferably from 1:1 to 100,000:1, more preferably from 1:1 to 10,000:1, more preferably from 1:1 to 1000:1, still more preferably from 1:1 to 100:1, and yet more preferably from 1:1 to 10:1. Preferably, a maximum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total

number of synthetic nanocarriers in the sample is equal to or less than 3 μm, more preferably equal to or less than 2 μm, more preferably equal to or less than 1 μm, more preferably equal to or less than 800 nm, more preferably equal to or less than 600 nm, and more preferably still equal to or less than 500 nm. In preferred embodiments, a minimum dimension of at least 75%, preferably at least 80%, more preferably at least 90%, of the synthetic nanocarriers in a sample, based on the total number of synthetic nanocarriers in the sample, is equal to or greater than 100 nm, more preferably equal to or greater than 120 nm, more preferably equal to or greater than 130 nm, more preferably equal to or greater than 140 nm, and more preferably still equal to or greater than 150 nm. Measurement of synthetic nanocarrier dimensions (e.g., effective diameter) may be obtained, in some embodiments, by suspending the synthetic nanocarriers in a liquid (usually aqueous) media and using dynamic light scattering (DLS) (e.g. using a Brookhaven ZetaPALS instrument). For example, a suspension of synthetic nanocarriers can be diluted from an aqueous buffer into purified water to achieve a final synthetic nanocarrier suspension concentration of approximately 0.01 to 0.1 mg/mL. The diluted suspension may be prepared directly inside, or transferred to, a suitable cuvette for DLS analysis. The cuvette may then be placed in the DLS, allowed to equilibrate to the controlled temperature, and then scanned for sufficient time to acquire a stable and reproducible distribution based on appropriate inputs for viscosity of the medium and refractive indicies of the sample. The effective diameter, or mean of the distribution, is then reported. Determining the effective sizes of high aspect ratio, or non-spheroidal, synthetic nanocarriers may require augmentative techniques, such as electron microscopy, to obtain more accurate measurements. "Dimension" or "size" or "diameter" of synthetic nanocarriers means the mean of a particle size distribution, for example, obtained using dynamic light scattering.

"Not previously administered" refers to a composition that has never been administered to the subject or has not been administered within a timeframe that would result in a pharmacodynamic effect at the time when a dosing regimen for administering a viral vector for treatment would start.

"Pharmaceutically acceptable excipient" or "pharmaceutically acceptable carrier" means a pharmacologically inactive material used together with a pharmacologically active material to formulate the compositions. Pharmaceutically acceptable excipients comprise a variety of materials known in the art, including but not limited to saccharides (such as glucose, lactose, and the like), preservatives such as antimicrobial agents, reconstitution aids, colorants, saline (such as phosphate buffered saline), and buffers.

"Repeat dose" or "repeat dosing" or the like means at least one additional dose or dosing of a material or a set of materials that is administered to a subject subsequent to an earlier dose or dosing of the same material(s). While the material may be the same, the amount of the material in the repeated dose or dosing may be different.

"Pre-existing immunity against a viral antigen of the viral vector" refers to the presence of antibodies, T cells and/or B cells in a subject, which cells have been previously primed by prior exposure to antigens of the viral vector or to crossreactive antigens, including but not limited to other viruses. In one embodiment of any one of the methods provided herein, the pre-existing immunity is against the viral capsid of the viral vector. The term is also meant to include subjects with maternally-transferred antibodies against a viral antigen of a viral vector, and, thus, the subject provided herein include newborns with maternally-transferred antibodies against a viral vector. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as neutralizing antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as combination of neutralizing antibodies and total anti-AAV capsid antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity comprises pre-existing antibodies against a viral vector, such as a combination of neutralizing antibodies and anti-AAV capsid IgG antibodies. In one embodiment of any one of the methods provided herein, the pre-existing immunity against a viral vector comprises pre-existing antibodies, such as a combination of neutralizing antibodies, anti-AAV IgG, and anti-AAV capsid IgM antibodies. In one embodiment of any one of the methods provided herein, the maternally-transferred antibodies against a viral vector comprise neutralizing antibodies against the viral vector.

In some embodiments, this pre-existing immunity is at a level that is expected to result in anti-viral vector immune response(s) that interferes with the efficacy of the viral vector. In some embodiments, this pre-existing immunity is at a level that would otherwise preclude the subject from treatment with the viral vector. In an embodiment of any one of the methods provided herein, a level of pre-existing immunity against a viral antigen of the viral vector is sufficient to neutralize 25%, 30%, 40%, 50%, 60%, 70%, of viral vector, such as AAV, transduction at a titer of 1:5. In an embodiment of any one of the methods provided herein, a level of pre-existing immunity against a viral antigen of the viral vector is sufficient to neutralize 25%, 30%, 40%, 50%, 60%, 70%, of viral vector, such as AAV, transduction at a titer of 1:10. In an embodiment of any one of the methods provided herein, a level of pre-

existing immunity against a viral antigen of the viral vector is sufficient to neutralize 25%, 30%, 40%, 50%, 60%, 70%, of viral vector, such as AAV, transduction at a titer of 1:20. In an embodiment of any one of the methods provided herein, a level of pre-existing immunity against a viral antigen of the viral vector is sufficient to neutralize 25%, 30%, 40%, 50%, 60%, 70%, of viral vector, such as AAV, transduction at a titer of 1:100. In an embodiment of any one of the methods provided herein, a level of pre-existing immunity against a viral antigen of the viral vector is sufficient to neutralize 50% at a titer of 1:5, 1:10; 1:20, 1:50, 1:100. In an embodiment of any one of the methods provided herein, the subject has any one of the aforementioned levels of pre-existing immunity. In an embodiment of any one of the methods provided herein, any one of the foregoing is a threshold level.

In some embodiments, this pre-existing immunity is at a level that is expected to result in anti-viral vector immune response(s) upon subsequent exposure to the viral vector. Pre-existing immunity can be assessed by determining the level of antibodies, such as neutralizing antibodies, against a viral vector present in a sample, such as a blood sample, from the subject. Assays for assessing the level of antibodies, such as neutralizing antibodies, are described herein at least in the Examples and are also known to those of ordinary skill in the art. Such an assay can be a cell-based assay. Assays for assessing the level of antibodies, such as IgM or IgM or a neutralizing antibody. Such an assay can be an ELISA assay. Pre-existing immunity can also be assessed by determining antigen recall responses of immune cells, such as B or T cells, stimulated in vivo or in vitro with viral vector antigens presented by APCs or viral antigen epitopes presented on MHC class I or MHC class II molecules. Assays for antigen-specific recall responses include, but are not limited to, ELISpot, intracellular cytokine staining, cell proliferation, and cytokine production assays. Generally, these and other assays are known to those of ordinary skill in the art. In some embodiments, a subject that does not exhibit pre-existing immunity against a viral antigen of the viral vector is one with a level of anti-viral vector antibodies, such as neutralizing antibodies, or memory B or T cells that would be considered to be negative. In other embodiments, a subject that does not exhibit pre-existing immunity against a viral antigen of the viral vector is one with a level of an anti-viral vector response that is no more than 3 standard deviations above a mean negative control.

"Subject" means animals, including warm blooded mammals such as humans and primates; avians; domestic household or farm animals such as cats, dogs, sheep, goats, cattle, horses and pigs; laboratory animals such as mice, rats and guinea pigs; fish; reptiles; zoo and wild animals; and the like. As used herein, a subject may be in need of any one of the

methods or compositions provided herein. As used herein, a subject is a newborn with maternally-transferred antibodies or one in which the level of pre-existing immunity would otherwise preclude the subject from treatment with the viral vector. "Second subject" or "another subject" provided herein refers to another subject different from the subject to which the administrations are being provided. This subject can be any other subject, such as a test subject, which subject may be of the same or different species. In some embodiments, this second subject is one with pre-existing immunity to the viral vector. In some embodiments, this second subject is one without pre-existing immunity to the viral vector. In other embodiment, this second subject is one administered a viral vector without the synthetic nanocarriers comprising an immunosuppressant or without synthetic nanocarriers comprising an immunosuppressant administered in the same way (a different way, such as concomitantly but not admixed). In some embodiments of any one of the methods or compositions provided, when the second or other subject is of a different species the amount can be scaled as appropriate for the species of the subject to receive the administrations, which scaled amount can be used as the total as provided herein. For example, allometric scaling or other scaling methods can be used. Immune responses in second subjects or other subjects as well as transgene expression can be assessed using routine methods known to those of ordinary skill in the art or as otherwise provided herein. Any one of the methods provided herein may comprise or further comprise determining one or more of these amounts in a second or other subject as described herein.

"Synthetic nanocarrier(s)" means a discrete object that is not found in nature, and that possesses at least one dimension that is less than or equal to 5 microns in size. Albumin nanoparticles are generally included as synthetic nanocarriers, however in certain embodiments the synthetic nanocarriers do not comprise albumin nanoparticles. In embodiments, synthetic nanocarriers do not comprise chitosan. In other embodiments, synthetic nanocarriers are not lipid-based nanoparticles. In further embodiments, synthetic nanocarriers do not comprise a phospholipid.

A synthetic nanocarrier can be, but is not limited to, one or a plurality of lipid-based nanoparticles (also referred to herein as lipid nanoparticles, i.e., nanoparticles where the majority of the material that makes up their structure are lipids), polymeric nanoparticles, metallic nanoparticles, surfactant-based emulsions, dendrimers, buckyballs, nanowires, virus-like particles (i.e., particles that are primarily made up of viral structural proteins but that are not infectious or have low infectivity), peptide or protein-based particles (also referred to herein as protein particles, i.e., particles where the majority of the material that makes up their

structure are peptides or proteins) (such as albumin nanoparticles) and/or nanoparticles that are developed using a combination of nanomaterials such as lipid-polymer nanoparticles. Synthetic nanocarriers may be a variety of different shapes, including but not limited to spheroidal, cuboidal, pyramidal, oblong, cylindrical, toroidal, and the like. Synthetic nanocarriers according to the invention comprise one or more surfaces. Exemplary synthetic nanocarriers that can be adapted for use in the practice of the present invention comprise: (1) the biodegradable nanoparticles disclosed in US Patent 5,543,158 to Gref et al., (2) the polymeric nanoparticles of Published US Patent Application 20060002852 to Saltzman et al., (3) the lithographically constructed nanoparticles of Published US Patent Application 20090028910 to DeSimone et al., (4) the disclosure of WO 2009/051837 to von Andrian et al., (5) the nanoparticles disclosed in Published US Patent Application 2008/0145441 to Penades et al., (6) the protein nanoparticles disclosed in Published US Patent Application 20090226525 to de los Rios et al., (7) the virus-like particles disclosed in published US Patent Application 20060222652 to Sebbel et al., (8) the nucleic acid attached virus-like particles disclosed in published US Patent Application 20060251677 to Bachmann et al., (9) the virus-like particles disclosed in WO2010047839A1 or WO2009106999A2, (10) the nanoprecipitated nanoparticles disclosed in P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010), (11) apoptotic cells, apoptotic bodies or the synthetic or semisynthetic mimics disclosed in U.S. Publication 2002/0086049, or (12) those of Look et al., Nanogel-based delivery of mycophenolic acid ameliorates systemic lupus erythematosus in mice" J. Clinical Investigation 123(4):1741-1749(2013).

Synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface with hydroxyl groups that activate complement or alternatively comprise a surface that consists essentially of moieties that are not hydroxyl groups that activate complement in some embodiments. In a preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that substantially activates complement or alternatively comprise a surface that consists essentially of moieties that do not substantially activate complement. In a more preferred embodiment, synthetic nanocarriers according to the invention that have a minimum dimension of equal to or less than about 100 nm, preferably equal to or less than 100 nm, do not comprise a surface that activates complement or alternatively comprise a surface that consists essentially of moieties that do not activate

complement. In embodiments, synthetic nanocarriers exclude virus-like particles. In embodiments, synthetic nanocarriers may possess an aspect ratio greater than or equal to 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7, or greater than 1:10.

"Transgene of the viral vector" or "transgene" or the like refers to nucleic acid material the viral vector is used to transport into a cell and, preferably in some embodiments, once in the cell, is expressed to produce a protein or nucleic acid molecule, respectively, such as for a therapeutic application as described herein. "Expressed" or "expression" or the like refers to the synthesis of a functional (i.e., physiologically active for the desired purpose) gene product after the transgene is transduced into a cell and processed by the transduced cell. Such a gene product is also referred to herein as a "transgene expression product". The expressed products are, therefore, the resultant protein or nucleic acid, such as an antisense oligonucleotide or a therapeutic RNA, encoded by the transgene.

"Viral vector" means a viral-based delivery system that can or does deliver a payload, such as nucleic acid(s), to cells. Generally, the term refers to a viral vector construct with viral components, such as capsid and/or coat proteins, that can or does also comprise a payload (and has been so adapted). In some embodiments, the payload encodes a transgene. In some embodiments, a transgene is one that encodes a protein provided herein, such as a therapeutic protein, a DNA-binding protein or an endonuclease. In other embodiments, a transgene encodes guide RNA, an antisense nucleic acid, snRNA, an RNAi molecule (e.g., dsRNAs or ssRNAs), miRNA, or triplex-forming oligonucleotides (TFOs), etc. In other embodiments, the payload are nucleic acid(s) that themselves are the therapeutic(s) and expression of the delivered nucleic acid(s) is not required. For example, the nucleic acid(s) may be siRNA, such as synthetic siRNA.

In some embodiments, the payload may also encode other components such as inverted terminal repeats (ITRs), markers, etc. The payload may also include an expression control sequence. Expression control DNA sequences include promoters, enhancers, and operators, and are generally selected based on the expression systems in which the expression construct is to be utilized. In some embodiments, promoter and enhancer sequences are selected for the ability to increase gene expression, while operator sequences may be selected for the ability to regulate gene expression. The payload may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell, in some embodiments.

Exemplary expression control sequences include promoter sequences, e.g., cytomegalovirus promoter; Rous sarcoma virus promoter; and simian virus 40 promoter; as

well as any other types of promoters that are disclosed elsewhere herein or are otherwise known in the art. Generally, promoters are operatively linked upstream (i.e., 5') of a sequence coding for a desired expression product. Payloads also may include a suitable polyadenylation sequence (e.g., the SV40 or human growth hormone gene polyadenylation sequence) operably linked downstream (i.e., 3') of the coding sequence.

Generally, viral vectors are engineered to be capable of transducing one or more desired nucleic acids into a cell. In addition, it will be understood that for the therapeutic applications provided herein, it is preferred that the viral vectors be replication-defective. Viral vectors can be based on, without limitation, retroviruses (e.g., murine retrovirus, avian retrovirus, Moloney murine leukemia virus (MoMuLV), Harvey murine sarcoma virus (HaMuSV), murine mammary tumor virus (MuMTV), gibbon ape leukemia virus (GaLV) and Rous Sarcoma Virus (RSV)), lentiviruses, herpes viruses, adenoviruses, adeno-associated viruses, alphaviruses, etc. Other examples are provided elsewhere herein or are known in the art. The viral vectors may be based on natural variants, strains, or serotypes of viruses, such as any one of those provided herein. The viral vectors may also be based on viruses selected through molecular evolution (see, e.g., J.T. Koerber et al, Mol. Ther. 17(12):2088-2095 and U.S. Pat. No. 6,09,548). Viral vectors can be based on, without limitation, adeno-associated viruses (AAV), such as AAV8 or AAV2. Viral vectors can also be based on Anc80. Thus, an AAV vector or Anc80 vector provided herein is a viral vector based on an AAV or Anc80, respectively, and has viral components, such as a capsid and/or coat protein, therefrom that can package for delivery nucleic acid material. Other examples of AAV vectors include, but are not limited to, those based on AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10, Rh74, or AAV-2i8 or variants thereof. The viral vectors may also be engineered vectors, recombinant vectors, mutant vectors, or hybrid vectors. Methods of generating such vectors will be evident to one of ordinary skill in the art. In some embodiments, the viral vector is a "chimeric viral vector". In such embodiments, this means that the viral vector is made up of viral components that are derived from more than one virus or viral vector. See, e.g., PCT Publications WO01/091802 and WO14/168953, and U.S. Pat. No. 6,468,771. Such a viral vector may be, for example, an AAV8/Anc80 or AAV2/Anc80 viral vector.

Additional viral vector elements may function in cis or in trans. In some embodiments, the viral vector includes a vector genome that also includes one or more inverted terminal repeat (ITR) sequence(s) that flank that 5' or 3' terminus of the target (donor) sequence, an expression control element that promotes transcription (e.g., promoter or

enhancer), an intron sequence, a stuffer/filler polynucleotide sequence (generally, an inert sequence), and/or a poly(A) sequence located at the 3' end of the target (donor) sequence.

## C. COMPOSITIONS FOR USE IN THE INVENTIVE METHODS

Importantly, the methods and compositions provided herein provide for administration of viral vectors to subjects with pre-existing immunity to a viral antigen of the viral vector and/or improved effects with administration of viral vectors. Thus, the methods and compositions provided herein are useful for the treatment of subjects with viral vectors, including newborns with maternally-transferred antibodies and subjects that otherwise would be excluded from treatment with the viral vector due to the level of pre-existing immunity. Such viral vectors can be used to deliver nucleic acids for a variety of purposes, including for gene therapy, etc. As mentioned above, pre-existing immunity against a viral vector can adversely impact its efficacy and can also interfere with its readministration. Importantly, the methods and compositions provided herein have been found to overcome the aforementioned obstacles by achieving improved expression of transgenes and/or reducing immune responses to viral vectors. The inventors have surprisingly discovered that synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector can achieve improved transgene expression in subjects, e.g., in subjects with pre-existing immunity to the viral vector, such as when the viral vector has not previously been administered to the subject. In addition, it was also surprisingly found that while such admixed administration of synthetic nanocarriers comprising an immunosuppressant and viral vectors achieves improved transgene expression in subjects on the first administration of the viral vector, admixing is not necessarily required for the efficacy of subsequent administrations of synthetic nanocarriers comprising an immunosuppressant and viral vectors. Further, it has been found that higher doses of synthetic nanocarriers comprising an immunosuppressant can also allow for treatment of subjects with pre-existing immunity.

Also as mentioned above, it has been discovered that admixed administration of synthetic nanocarriers comprising an immunosuppressant and a viral vector can be used to achieve reduced doses of viral vector without reducing transgene expression.

### *Transgenes*

The payload of a viral vector may be a transgene. For example, the transgene may encode a desired expression product, such as a polypeptide, protein, protein mixture, DNA,

cDNA, functional RNA molecule (*e.g.,* RNAi, miRNA), mRNA, RNA replicon, or other product of interest.

For example, the expression product of the transgene may be a protein or portion thereof beneficial to a subject, such as one with a disease or disorder. The protein may be an extracellular, intracellular or membrane-bound protein. Transgenes, for example, may encode enzymes, blood derivatives, hormones, lymphokines, such as the interleukins and interferons, coagulants, growth factors, neurotransmitters, tumor suppressors, apolipoproteins, antigens, and antibodies. The subject may have or be suspected of having a disease or disorder whereby the subject's endogenous version of the protein is defective or produced in limited amounts or not at all. In other embodiments of any one of the methods or compositions provided, the expression product of the transgene may be a gene or portion thereof beneficial to a subject.

Examples of therapeutic proteins include, but are not limited to, infusible or injectable therapeutic proteins, enzymes, enzyme cofactors, hormones, blood or blood coagulation factors, cytokines and interferons, growth factors, adipokines, etc.

Examples of infusible or injectable therapeutic proteins include, for example, Tocilizumab (Roche/Actemra®), alpha-1 antitrypsin (Kamada/AAT), Hematide® (Affymax and Takeda, synthetic peptide), albinterferon alfa-2b (Novartis/Zalbin™), Rhucin® (Pharming Group, C1 inhibitor replacement therapy), tesamorelin (Theratechnologies/Egrifta, synthetic growth hormone-releasing factor), ocrelizumab (Genentech, Roche and Biogen), belimumab (GlaxoSmithKline/Benlysta®), pegloticase (Savient Pharmaceuticals/Krystexxa™), taliglucerase alfa (Protalix/Uplyso), agalsidase alfa (Shire/Replagal®), and velaglucerase alfa (Shire).

Examples of enzymes include lysozyme, oxidoreductases, transferases, hydrolases, lyases, isomerases, asparaginases, uricases, glycosidases, proteases, nucleases, collagenases, hyaluronidases, heparinases, heparanases, kinases, phosphatases, lysins and ligases. Other examples of enzymes include those that used for enzyme replacement therapy including, but not limited to, imiglucerase (e.g., CEREZYME™), a-galactosidase A (a-gal A) (e.g., agalsidase beta, FABRYZYME™), acid a-glucosidase (GAA) (e.g., alglucosidase alfa, LUMIZYME™, MYOZYME™), and arylsulfatase B (e.g., laronidase, ALDURAZYME™, idursulfase, ELAPRASE™, arylsulfatase B, NAGLAZYME™).

Examples of hormones include Melatonin (N-acetyl-5-methoxytryptamine), Serotonin, Thyroxine (or tetraiodothyronine) (a thyroid hormone), Triiodothyronine (a thyroid hormone), Epinephrine (or adrenaline), Norepinephrine (or noradrenaline), Dopamine (or prolactin inhibiting hormone), Antimullerian hormone (or mullerian inhibiting factor or hormone),

Adiponectin, Adrenocorticotropic hormone (or corticotropin), Angiotensinogen and angiotensin, Antidiuretic hormone (or vasopressin, arginine vasopressin), Atrial-natriuretic peptide (or atriopeptin), Calcitonin, Cholecystokinin, Corticotropin-releasing hormone, Erythropoietin, Follicle-stimulating hormone, Gastrin, Ghrelin, Glucagon, Glucagon-like peptide (GLP-1), GIP, Gonadotropin-releasing hormone, Growth hormone-releasing hormone, Human chorionic gonadotropin, Human placental lactogen, Growth hormone, Inhibin, Insulin, Insulin-like growth factor (or somatomedin), Leptin, Luteinizing hormone, Melanocyte stimulating hormone, Orexin, Oxytocin, Parathyroid hormone, Prolactin, Relaxin, Secretin, Somatostatin, Thrombopoietin, Thyroid-stimulating hormone (or thyrotropin), Thyrotropin-releasing hormone, Cortisol, Aldosterone, Testosterone, Dehydroepiandrosterone, Androstenedione, Dihydrotestosterone, Estradiol, Estrone, Estriol, Progesterone, Calcitriol (1,25-dihydroxyvitamin D3), Calcidiol (25-hydroxyvitamin D3), Prostaglandins, Leukotrienes, Prostacyclin, Thromboxane, Prolactin releasing hormone, Lipotropin, Brain natriuretic peptide, Neuropeptide Y, Histamine, Endothelin, Pancreatic polypeptide, Renin, and Enkephalin.

Examples of blood or blood coagulation factors include Factor I (fibrinogen), Factor II (prothrombin), tissue factor, Factor V (proaccelerin, labile factor), Factor VII (stable factor, proconvertin), Factor VIII (antihemophilic globulin), Factor IX (Christmas factor or plasma thromboplastin component), Factor X (Stuart-Prower factor), Factor Xa, Factor XI, Factor XII (Hageman factor), Factor XIII (fibrin-stabilizing factor), von Willebrand factor, von Heldebrant Factor, prekallikrein (Fletcher factor), high-molecular weight kininogen (HMWK) (Fitzgerald factor), fibronectin, fibrin, thrombin, antithrombin, such as antithrombin III, heparin cofactor II, protein C, protein S, protein Z, protein Z-related protease inhibitot (ZPI), plasminogen, alpha 2-antiplasmin, tissue plasminogen activator (tPA), urokinase, plasminogen activator inhibitor-1 (PAI1), plasminogen activator inhibitor-2 (PAI2), cancer procoagulant, and epoetin alfa (Epogen, Procrit).

Examples of cytokines include lymphokines, interleukins, and chemokines, type 1 cytokines, such as IFN-$\gamma$, TGF-$\beta$, and type 2 cytokines, such as IL-4, IL-10, and IL-13.

Examples of growth factors include Adrenomedullin (AM), Angiopoietin (Ang), Autocrine motility factor, Bone morphogenetic proteins (BMPs), Brain-derived neurotrophic factor (BDNF), Epidermal growth factor (EGF), Erythropoietin (EPO), Fibroblast growth factor (FGF), Glial cell line-derived neurotrophic factor (GDNF), Granulocyte colony-stimulating factor (G-CSF), Granulocyte macrophage colony-stimulating factor (GM-CSF), Growth differentiation factor-9 (GDF9), Hepatocyte growth factor (HGF), Hepatoma-derived

growth factor (HDGF), Insulin-like growth factor (IGF), Migration-stimulating factor, Myostatin (GDF-8), Nerve growth factor (NGF) and other neurotrophins, Platelet-derived growth factor (PDGF), Thrombopoietin (TPO), Transforming growth factor alpha(TGF-α), Transforming growth factor beta(TGF-β), Tumour necrosis factor-alpha(TNF-α), Vascular endothelial growth factor (VEGF), Wnt Signaling Pathway, placental growth factor (PlGF), [(Foetal Bovine Somatotrophin)] (FBS), IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, and IL-7.

Examples of adipokines, include leptin and adiponectin.

Additional examples of therapeutic proteins include, but are not limited to, receptors, signaling proteins, cytoskeletal proteins, scaffold proteins, transcription factors, structural proteins, membrane proteins, cytosolic proteins, binding proteins, nuclear proteins, secreted proteins, Golgi proteins, endoplasmic reticulum proteins, mitochondrial proteins, and vesicular proteins, etc.

The transgene may be one that encodes an enzyme to treat a metabolic liver disease, e.g., Nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH)); or an inherited metabolic disorder, e.g., Alagille Syndrome, Alpha-1 Antitrypsin deficiency, Crigler-Najjar Syndrome, Galactosemia, Gaucher disease, Gilbert Syndrome, hemochromatosis, Lysosomal acid lipase deficiency (LAL-D), organic acidemia, Reye syndrome, Type I Glycogen Storage Disease, urea cycle disorder, and Wilson's disease. In one embodiment of any one of the methods provided herein, the subject is one with any one of the foregoing. For example, the subject may be one with an organic acidemia, such as methylmalonic acidemia (MMA) or a urea cycle disorder, such as ornithine carbamylase deficiency. Thus, the transgene in some embodiments encodes methylmalonoyl-CoA mutase (MUT) or ornithine transcarbamylase (OTC).

In one embodiment of any one of the methods or compositions provided, the expression product may be used to disrupt, correct/repair, or replace a target gene, or part of a target gene. For example, the Clustered Regularly Interspaced Short Palindromic Repeat/Cas (CRISPR/Cas) system can be used for precise genome editing. In the system, single CRISPR-associated nucleases (Cas nucleases) may be programmed by a guide RNA (short RNA) to recognize a specific DNA target, which comprises DNA loci containing short repetitions of a base sequence. Each CRISPR loci is flanked by short segment of spacer DNA, which are derived from viral genomic material. In the type II CRISPR system, the most common system, spacer DNA hybridizes with trans-activating RNA (tracRNA), where it is processed into CRISPR-RNA (crRNA) and then associates with Cas nucleases, forming complexes which initiate RNAse III processing and resulting in the degradation of foreign DNA. The

target sequence preferably contains a protospacer adjacent motif (PAM) sequence on its 3′ end in order to be recognized. The system can be modified in a number of ways, for example synthetic guide RNAs may be fused to a CRISPR vector, and a variety of different guide RNA structures and elements are possible (including hairpin and scaffold sequences).

In some embodiments of any one of the methods or compositions provided, the transgene sequence may encode any one or more components of a CRISPR/Cas system, such as a reporter sequence, which produces a detectable signal when expressed. Examples of such reporters sequences include, but are not limited to, β-lactamase, β-galactosidase (LacZ), alkaline phosphatase, thymidine kinase, green fluorescent protein (GFP), chloramphenicol acetyltransferase (CAT), luciferase, membrane bound proteins including, for example, CD2, CD4, CD8, and the influenza hemagglutinin protein. Other reporters are known to those of ordinary skill in the art.

In another example of any one of the methods or compositions provided, the transgene may encode an RNA product, such as tRNA, dsRNA, ribosomal RNA, catalytic RNAs, siRNA, RNAi, miRNA, small hairpin RNA (shRNA), trans-splicing RNA, and antisense RNAs. For example, specific RNA sequences can be generated to inhibit or extinguish the expression of a targeted nucleic acid sequence in the subject. Suitable target sequences include, for example, oncologic targets and viral diseases.

In some embodiments of any one of the methods or compositions provided, the transgene sequence may encode a reporter sequence, which produces a detectable signal when expressed, or the transgene sequence may encode a protein or functional RNA that can be used to create an animal model of disease. In another example of any one of the methods or compositions provided, the transgene encodes a protein or functional RNA that is intended to be used for research purposes, *e.g.,* to create a somatic transgenic animal model harboring the transgene, *e.g.,* to study the function of the transgene product. In other embodiments of any one of the methods or compositions provided, the intent of such expression products is for treatment. Other uses of transgenes will be apparent to one of ordinary skill in the art.

The sequence of a transgene may also include an expression control sequence. Expression control sequences include promoters, enhancers, and operators, and are generally selected based on the expression systems in which the expression construct is to be utilized. In some embodiments of any one of the methods or compositions provided, promoter and enhancer sequences are selected for the ability to increase gene expression, while operator sequences may be selected for the ability to regulate gene expression. Typically, promoter sequences are located upstream (i.e., 5′) of the nucleic acid sequence encoding the desired

expression product, and are operatively linked to an adjacent sequence, thereby increasing the amount of desired product expressed as compared to an amount expressed without the promoter. Enhancer sequences, generally located upstream of promoter sequences, can further increase expression of the desired product. In some embodiments of any one of the methods or compositions provided, the enhancer sequence(s) may be located downstream of the promoter and/or within the transgene. The transgene may also include sequences that facilitate, and preferably promote, homologous recombination in a host cell and/or packaging. The transgene may also include sequences that are necessary for replication in a host cell.

Exemplary expression control sequences include liver-specific promoter sequences and constitutive promoter sequences, such as any one that may be provided herein. Other tissue-specific promoters include eye, retina, central nervous system, spinal cord, among others. Examples of ubiquitous or promiscuous promoters and enhancers include, but are not limited to the cytomegalovirus (CMV) immediate early promoter/enhancer sequences, the Rous sarcoma virus (RSV) promoter/enhancer sequences and the other viral promoters/enhancers active in various mammalian cell types, or synthetic elements that are not present in nature (see, e.g., Boshart et al, Cell, 41 :521-530 (1985)), the SV40 promoter, the dihydrofolate reductase (DHFR) promoter, the cytoplasmic β-actin promoter and the phosphoglycerol kinase (PGK) promoter.

Operators, or regulatable elements, are responsive to a signal or stimuli, which can increase or decrease the expression of the operably linked nucleic acid. Inducible elements are those that increase the expression of the operably linked nucleic acid in response to a signal or stimuli, for example, hormone inducible promoters. Repressible elements are those that decrease the expression of the operably linked nucleic acid in response to a signal or stimuli. Typically, repressible and inducible elements are proportionally responsive to the amount of signal or stimuli present. The transgene may include such sequences in any one of the methods or compositions provided.

The transgene also may include a suitable polyadenylation sequence operably linked downstream (i.e., 3') of the coding sequence.

Methods of delivering transgenes, for example, for gene therapy, are known in the art (see, e.g., Smith. Int. J. Med. Sci. 1(2): 76-91 (2004); Phillips. Methods in Enzymology: Gene Therapy Methods. Vol. 346. Academic Press (2002)). Any of the transgenes described herein may be incorporated into any of the viral vectors described herein using methods of known in the art, see, for example, U.S. Pat. No. 7,629,153.

*Viral Vectors*

Viruses have evolved specialized mechanisms to transport their genomes inside the cells that they infect; viral vectors based on such viruses can be tailored to transduce cells to specific applications. Examples of viral vectors that may be used as provided herein are known in the art or described herein. Suitable viral vectors include, for instance, retroviral vectors, lentiviral vectors, herpes simplex virus (HSV)-based vectors, adenovirus-based vectors, adeno-associated virus (AAV)-based vectors, and AAV-adenoviral chimeric vectors.

The viral vectors provided herein may be based on a retrovirus. Retrovirus is a single-stranded positive sense RNA virus. A retroviral vector can be manipulated to render the virus replication-incompetent. As such, retroviral vectors are thought to be particularly useful for stable gene transfer *in vivo*. Examples of retroviral vectors can be found, for example, in U.S. Publication Nos. 20120009161, 20090118212, and 20090017543, the viral vectors and methods of their making being incorporated by reference herein in their entirety.

Lentiviral vectors are examples of retroviral vectors that can be used for the production of a viral vector as provided herein. Examples of lentiviruses include HIV (humans), simian immunodeficiency virus (SIV), feline immunodeficiency virus (FIV), equine infectious anemia virus (EIAV) and visna virus (ovine lentivirus). Examples of lentiviral vectors can be found, for example, in U.S. Publication Nos. 20150224209, 20150203870, 20140335607, 20140248306, 20090148936, and 20080254008, the viral vectors and methods of their making being incorporated by reference herein in their entirety.

Herpes simplex virus (HSV)-based viral vectors are also suitable for use as provided herein. Many replication-deficient HSV vectors contain a deletion to remove one or more intermediate-early genes to prevent replication. For a description of HSV-based vectors, see, for example, U.S. Pat. Nos. 5,837,532, 5,846,782, 5,849,572, and 5,804,413, and International Patent Applications WO 91/02788, WO 96/04394, WO 98/15637, and WO 99/06583, the description of which viral vectors and methods of their making being incorporated by reference in its entirety.

Viral vectors can be based on adenoviruses. The adenovirus on which a viral vector may be based may be from any origin, any subgroup, any subtype, mixture of subtypes, or any serotype. For instance, an adenovirus can be of subgroup A (e.g., serotypes 12, 18, and 31), subgroup B (e.g., serotypes 3, 7, 11, 14, 16, 21, 34, 35, and 50), subgroup C (e.g., serotypes 1, 2, 5, and 6), subgroup D (e.g., serotypes 8, 9, 10, 13, 15, 17, 19, 20, 22-30, 32, 33, 36-39, and 42-48), subgroup E (e.g., serotype 4), subgroup F (e.g., serotypes 40 and 41), an unclassified

serogroup (e.g., serotypes 49 and 51), or any other adenoviral serotype. Adenoviral serotypes 1 through 51 are available from the American Type Culture Collection (ATCC, Manassas, Va.). Non-group C adenoviruses, and even non-human adenoviruses, can be used to prepare replication-deficient adenoviral vectors. Non-group C adenoviral vectors, methods of producing non-group C adenoviral vectors, and methods of using non-group C adenoviral vectors are disclosed in, for example, U.S. Pat. Nos. 5,801,030, 5,837,511, and 5,849,561, and International Patent Applications WO 97/12986 and WO 98/53087. Any adenovirus, even a chimeric adenovirus, can be used as the source of the viral genome for an adenoviral vector. For example, a human adenovirus can be used as the source of the viral genome for a replication-deficient adenoviral vector. Further examples of adenoviral vectors can be found in U.S. Publication Nos. 20150093831, 20140248305, 20120283318, 20100008889, 20090175897 and 20090088398, the description of which viral vectors and methods of their making being incorporated by reference in its entirety.

The viral vectors provided herein can also be based on adeno-associated viruses (AAVs). AAV vectors have been of particular interest for use in therapeutic applications such as those described herein. For a description of AAV-based vectors, see, for example, U.S. Pat. Nos. 8,679,837, 8,637,255, 8,409,842, 7,803,622, and 7,790,449, and U.S. Publication Nos. 20150065562, 20140155469, 20140037585, 20130096182, 20120100606, and 20070036757. The AAV vectors may be recombinant AAV vectors. The AAV vectors may also be self-complementary (sc) AAV vectors, which are described, for example, in U.S. Patent Publications 2007/01110724 and 2004/0029106, and U.S. Pat. Nos. 7,465,583 and 7,186,699, the viral vectors of which and methods or their making being incorporated herein by reference in their entirety.

The adeno-associated virus on which a viral vector may be based may be of any serotype or a mixture of serotypes. AAV serotypes include AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, and AAV11. For example, when the viral vector is based on a mixture of serotypes, the viral vector may contain the capsid signal sequences taken from one AAV serotype (for example selected from any one of AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11) and packaging sequences from a different serotype (for example selected from any one of AAV serotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, and 11). In some embodiments of any one of the methods or compositions provided herein, therefore, the AAV vector is an AAV 2/8-based vector. In other embodiments of any one of the methods or compositions provided herein, the AAV vector is an AAV 2/5-based vector.

In some embodiments of any one of the methods or compositions provided, the virus on which a viral vector is based may be synthetic, such as Anc80.

In some embodiments of any one of the methods or compositions provided, the viral vector is an AAV/Anc80 vectors, such as an AAV8/Anc80 vector or an AAV2/Anc80 vector.

Other viruses on which the vector can be based include AAV1, AAV3, AAV4, AAV5, AAV6, AAV7, AAV9, AAV10, AAV11, rhl0, rh74 or AAV-2i8, and variants thereof.

The viral vectors provided herein may also be based on an alphavirus. Alphaviruses include Sindbis (and VEEV) virus, Aura virus, Babanki virus, Barmah Forest virus, Bebaru virus, Cabassou virus, Chikungunya virus, Eastern equine encephalitis virus, Everglades virus, Fort Morgan virus, Getah virus, Highlands J virus, Kyzylagach virus, Mayaro virus, Me Tri virus, Middelburg virus, Mosso das Pedras virus, Mucambo virus, Ndumu virus, O'nyong-nyong virus, Pixuna virus, Rio Negro virus, Ross River virus, Salmon pancreas disease virus, Semliki Forest virus, Southern elephant seal virus, Tonate virus, Trocara virus, Una virus, Venezuelan equine encephalitis virus, Western equine encephalitis virus, and Whataroa virus. Examples of alphaviral vectors can be found in U.S. Publication Nos. 20150050243, 20090305344, and 20060177819; the vectors and methods of their making are incorporated herein by reference in their entirety.

Any one of the viral vectors provided herein may be for use in any one of the methods provided herein.

*Immunosuppressants*

Immunosuppressants include, but are not limited to, statins; mTOR inhibitors, such as rapamycin or a rapamycin analog; TGF-β signaling agents; TGF-β receptor agonists; histone deacetylase (HDAC) inhibitors; corticosteroids; inhibitors of mitochondrial function, such as rotenone; P38 inhibitors; NF-κβ inhibitors; adenosine receptor agonists; prostaglandin E2 agonists; phosphodiesterase inhibitors, such as phosphodiesterase 4 inhibitor; proteasome inhibitors; kinase inhibitors; G-protein coupled receptor agonists; G-protein coupled receptor antagonists; glucocorticoids; retinoids; cytokine inhibitors; cytokine receptor inhibitors; cytokine receptor activators; peroxisome proliferator-activated receptor antagonists; peroxisome proliferator-activated receptor agonists; histone deacetylase inhibitors; calcineurin inhibitors; phosphatase inhibitors and oxidized ATPs. Immunosuppressants also include IDO, vitamin D3, cyclosporine A, aryl hydrocarbon receptor inhibitors, resveratrol, azathiopurine, 6-mercaptopurine, aspirin, niflumic acid, estriol, tripolide, interleukins (e.g., IL-1, IL-10), cyclosporine A, siRNAs targeting cytokines or cytokine receptors and the like.

Examples of statins include atorvastatin (LIPITOR®, TORVAST®), cerivastatin, fluvastatin (LESCOL®, LESCOL® XL), lovastatin (MEVACOR®, ALTOCOR®, ALTOPREV®), mevastatin (COMPACTIN®), pitavastatin (LIVALO®, PIAVA®), rosuvastatin (PRAVACHOL®, SELEKTINE®, LIPOSTAT®), rosuvastatin (CRESTOR®), and simvastatin (ZOCOR®, LIPEX®).

Examples of mTOR inhibitors include rapamycin and analogs thereof (e.g., CCL-779, RAD001, AP23573, C20-methallylrapamycin (C20-Marap), C16-(S)-butylsulfonamidorapamycin (C16-BSrap), C16-(S)-3-methylindolerapamycin (C16-iRap) (Bayle et al. Chemistry & Biology 2006, 13:99-107)), AZD8055, BEZ235 (NVP-BEZ235), chrysophanic acid (chrysophanol), deforolimus (MK-8669), everolimus (RAD0001), KU-0063794, PI-103, PP242, temsirolimus, and WYE-354 (available from Selleck, Houston, TX, USA).

Examples of TGF-β signaling agents include TGF-β ligands (e.g., activin A, GDF1, GDF11, bone morphogenic proteins, nodal, TGF-βs) and their receptors (e.g., ACVR1B, ACVR1C, ACVR2A, ACVR2B, BMPR2, BMPR1A, BMPR1B, TGFβRI, TGFβRII), R-SMADS/co-SMADS (e.g., SMAD1, SMAD2, SMAD3, SMAD4, SMAD5, SMAD8), and ligand inhibitors (e.g., follistatin, noggin, chordin, DAN, lefty, LTBP1, THBS1, Decorin).

Examples of inhibitors of mitochondrial function include atractyloside (dipotassium salt), bongkrekic acid (triammonium salt), carbonyl cyanide m-chlorophenylhydrazone, carboxyatractyloside (e.g., from *Atractylis gummifera*), CGP-37157, (-)-Deguelin (e.g., from *Mundulea sericea*), F16, hexokinase II VDAC binding domain peptide, oligomycin, rotenone, Ru360, SFK1, and valinomycin (e.g., from *Streptomyces fulvissimus*) (EMD4Biosciences, USA).

Examples of P38 inhibitors include SB-203580 (4-(4-Fluorophenyl)-2-(4-methylsulfinylphenyl)-5-(4-pyridyl)1H-imidazole), SB-239063 (trans-1-(4hydroxycyclohexyl)-4-(fluorophenyl)-5-(2-methoxy-pyrimidin-4-yl) imidazole), SB-220025 (5-(2amino-4-pyrimidinyl)-4-(4-fluorophenyl)-1-(4-piperidinyl)imidazole)), and ARRY-797.

Examples of NF (e.g., NK-κβ) inhibitors include IFRD1, 2-(1,8-naphthyridin-2-yl)-Phenol, 5-aminosalicylic acid, BAY 11-7082, BAY 11-7085, CAPE (Caffeic Acid Phenethylester), diethylmaleate, IKK-2 Inhibitor IV, IMD 0354, lactacystin, MG-132 [Z-Leu-Leu-Leu-CHO], NFκB Activation Inhibitor III, NF-κB Activation Inhibitor II, JSH-23, parthenolide, Phenylarsine Oxide (PAO), PPM-18, pyrrolidinedithiocarbamic acid ammonium salt, QNZ, RO 106-9920, rocaglamide, rocaglamide AL, rocaglamide C, rocaglamide I,

rocaglamide J, rocaglaol, (R)-MG-132, sodium salicylate, triptolide (PG490), and wedelolactone.

Examples of adenosine receptor agonists include CGS-21680 and ATL-146e.

Examples of prostaglandin E2 agonists include E-Prostanoid 2 and E-Prostanoid 4.

Examples of phosphodiesterase inhibitors (non-selective and selective inhibitors) include caffeine, aminophylline, IBMX (3-isobutyl-1-methylxanthine), paraxanthine, pentoxifylline, theobromine, theophylline, methylated xanthines, vinpocetine, EHNA (erythro-9-(2-hydroxy-3-nonyl)adenine), anagrelide, enoximone (PERFAN™), milrinone, levosimendon, mesembrine, ibudilast, piclamilast, luteolin, drotaverine, roflumilast (DAXAS™, DALIRESP™), sildenafil (REVATION®, VIAGRA®), tadalafil (ADCIRCA®, CIALIS®), vardenafil (LEVITRA®, STAXYN®), udenafil, avanafil, icariin, 4-methylpiperazine, and pyrazolo pyrimidin-7-1.

Examples of proteasome inhibitors include bortezomib, disulfiram, epigallocatechin-3-gallate, and salinosporamide A.

Examples of kinase inhibitors include bevacizumab, BIBW 2992, cetuximab (ERBITUX®), imatinib (GLEEVEC®), trastuzumab (HERCEPTIN®), gefitinib (IRESSA®), ranibizumab (LUCENTIS®), pegaptanib, sorafenib, dasatinib, sunitinib, erlotinib, nilotinib, lapatinib, panitumumab, vandetanib, E7080, pazopanib, and mubritinib.

Examples of glucocorticoids include hydrocortisone (cortisol), cortisone acetate, prednisone, prednisolone, methylprednisolone, dexamethasone, betamethasone, triamcinolone, beclometasone, fludrocortisone acetate, deoxycorticosterone acetate (DOCA), and aldosterone.

Examples of retinoids include retinol, retinal, tretinoin (retinoic acid, RETIN-A®), isotretinoin (ACCUTANE®, AMNESTEEM®, CLARAVIS®, SOTRET®), alitretinoin (PANRETIN®), etretinate (TEGISON™) and its metabolite acitretin (SORIATANE®), tazarotene (TAZORAC®, AVAGE®, ZORAC®), bexarotene (TARGRETIN®), and adapalene (DIFFERIN®).

Examples of cytokine inhibitors include IL1ra, IL1 receptor antagonist, IGFBP, TNF-BF, uromodulin, Alpha-2-Macroglobulin, Cyclosporin A, Pentamidine, and Pentoxifylline (PENTOPAK®, PENTOXIL®, TRENTAL®).

Examples of peroxisome proliferator-activated receptor antagonists include GW9662, PPARγ antagonist III, G335, and T0070907 (EMD4Biosciences, USA).

Examples of peroxisome proliferator-activated receptor agonists include pioglitazone, ciglitazone, clofibrate, GW1929, GW7647, L-165,041, LY 171883, PPARγ activator, Fmoc-Leu, troglitazone, and WY-14643 (EMD4Biosciences, USA).

Examples of histone deacetylase inhibitors include hydroxamic acids (or hydroxamates) such as trichostatin A, cyclic tetrapeptides (such as trapoxin B) and depsipeptides, benzamides, electrophilic ketones, aliphatic acid compounds such as phenylbutyrate and valproic acid, hydroxamic acids such as vorinostat (SAHA), belinostat (PXD101), LAQ824, and panobinostat (LBH589), benzamides such as entinostat (MS-275), CI994, and mocetinostat (MGCD0103), nicotinamide, derivatives of NAD, dihydrocoumarin, naphthopyranone, and 2-hydroxynaphaldehydes.

Examples of calcineurin inhibitors include cyclosporine, pimecrolimus, voclosporin, and tacrolimus.

Examples of phosphatase inhibitors include BN82002 hydrochloride, CP-91149, calyculin A, cantharidic acid, cantharidin, cypermethrin, ethyl-3,4-dephostatin, fostriecin sodium salt, MAZ51, methyl-3,4-dephostatin, NSC 95397, norcantharidin, okadaic acid ammonium salt from prorocentrum concavum, okadaic acid, okadaic acid potassium salt, okadaic acid sodium salt, phenylarsine oxide, various phosphatase inhibitor cocktails, protein phosphatase 1C, protein phosphatase 2A inhibitor protein, protein phosphatase 2A1, protein phosphatase 2A2, and sodium orthovanadate.

*Synthetic Nanocarriers*

The methods provided herein include administrations of synthetic nanocarriers comprising an immunosuppressant. Generally, the immunosuppressant is an element that is in addition to the material that makes up the structure of the synthetic nanocarrier. For example, in one embodiment of any one of the methods or compositions provided, where the synthetic nanocarrier is made up of one or more polymers, the immunosuppressant is a compound that is in addition and, in some embodiments of any one of the methods or compositions provided, attached to the one or more polymers. In embodiments where the material of the synthetic nanocarrier also results in a tolerogenic effect, the immunosuppressant is an element present in addition to the material of the synthetic nanocarrier that results in a tolerogenic effect.

A wide variety of synthetic nanocarriers can be used according to the invention. In some embodiments, synthetic nanocarriers are spheres or spheroids. In some embodiments, synthetic nanocarriers are flat or plate-shaped. In some embodiments, synthetic nanocarriers

are cubes or cubic. In some embodiments, synthetic nanocarriers are ovals or ellipses. In some embodiments, synthetic nanocarriers are cylinders, cones, or pyramids.

In some embodiments, it is desirable to use a population of synthetic nanocarriers that is relatively uniform in terms of size or shape so that each synthetic nanocarrier has similar properties. For example, at least 80%, at least 90%, or at least 95% of the synthetic nanocarriers of any one of the compositions or methods provided, based on the total number of synthetic nanocarriers, may have a minimum dimension or maximum dimension that falls within 5%, 10%, or 20% of the average diameter or average dimension of the synthetic nanocarriers.

Synthetic nanocarriers can be solid or hollow and can comprise one or more layers. In some embodiments, each layer has a unique composition and unique properties relative to the other layer(s). To give but one example, synthetic nanocarriers may have a core/shell structure, wherein the core is one layer (e.g. a polymeric core) and the shell is a second layer (e.g. a lipid bilayer or monolayer). Synthetic nanocarriers may comprise a plurality of different layers.

In some embodiments, synthetic nanocarriers may optionally comprise one or more lipids. In some embodiments, a synthetic nanocarrier may comprise a liposome. In some embodiments, a synthetic nanocarrier may comprise a lipid bilayer. In some embodiments, a synthetic nanocarrier may comprise a lipid monolayer. In some embodiments, a synthetic nanocarrier may comprise a micelle. In some embodiments, a synthetic nanocarrier may comprise a core comprising a polymeric matrix surrounded by a lipid layer (e.g., lipid bilayer, lipid monolayer, etc.). In some embodiments, a synthetic nanocarrier may comprise a non-polymeric core (e.g., metal particle, quantum dot, ceramic particle, bone particle, viral particle, proteins, nucleic acids, carbohydrates, etc.) surrounded by a lipid layer (e.g., lipid bilayer, lipid monolayer, etc.).

In other embodiments, synthetic nanocarriers may comprise metal particles, quantum dots, ceramic particles, etc. In some embodiments, a non-polymeric synthetic nanocarrier is an aggregate of non-polymeric components, such as an aggregate of metal atoms (e.g., gold atoms).

In some embodiments, synthetic nanocarriers may optionally comprise one or more amphiphilic entities. In some embodiments, an amphiphilic entity can promote the production of synthetic nanocarriers with increased stability, improved uniformity, or increased viscosity. In some embodiments, amphiphilic entities can be associated with the interior surface of a lipid membrane (e.g., lipid bilayer, lipid monolayer, etc.). Many amphiphilic entities known

in the art are suitable for use in making synthetic nanocarriers in accordance with the present invention. Such amphiphilic entities include, but are not limited to, phosphoglycerides; phosphatidylcholines; dipalmitoyl phosphatidylcholine (DPPC); dioleylphosphatidyl ethanolamine (DOPE); dioleyloxypropyltriethylammonium (DOTMA); dioleoylphosphatidylcholine; cholesterol; cholesterol ester; diacylglycerol; diacylglycerolsuccinate; diphosphatidyl glycerol (DPPG); hexanedecanol; fatty alcohols such as polyethylene glycol (PEG); polyoxyethylene-9-lauryl ether; a surface active fatty acid, such as palmitic acid or oleic acid; fatty acids; fatty acid monoglycerides; fatty acid diglycerides; fatty acid amides; sorbitan trioleate (Span®85) glycocholate; sorbitan monolaurate (Span®20); polysorbate 20 (Tween®20); polysorbate 60 (Tween®60); polysorbate 65 (Tween®65); polysorbate 80 (Tween®80); polysorbate 85 (Tween®85); polyoxyethylene monostearate; surfactin; a poloxomer; a sorbitan fatty acid ester such as sorbitan trioleate; lecithin; lysolecithin; phosphatidylserine; phosphatidylinositol;sphingomyelin; phosphatidylethanolamine (cephalin); cardiolipin; phosphatidic acid; cerebrosides; dicetylphosphate; dipalmitoylphosphatidylglycerol; stearylamine; dodecylamine; hexadecyl-amine; acetyl palmitate; glycerol ricinoleate; hexadecyl sterate; isopropyl myristate; tyloxapol; poly(ethylene glycol)5000-phosphatidylethanolamine; poly(ethylene glycol)400-monostearate; phospholipids; synthetic and/or natural detergents having high surfactant properties; deoxycholates; cyclodextrins; chaotropic salts; ion pairing agents; and combinations thereof. An amphiphilic entity component may be a mixture of different amphiphilic entities. Those skilled in the art will recognize that this is an exemplary, not comprehensive, list of substances with surfactant activity. Any amphiphilic entity may be used in the production of synthetic nanocarriers to be used in accordance with the present invention.

In some embodiments, synthetic nanocarriers may optionally comprise one or more carbohydrates. Carbohydrates may be natural or synthetic. A carbohydrate may be a derivatized natural carbohydrate. In certain embodiments, a carbohydrate comprises monosaccharide or disaccharide, including but not limited to glucose, fructose, galactose, ribose, lactose, sucrose, maltose, trehalose, cellbiose, mannose, xylose, arabinose, glucoronic acid, galactoronic acid, mannuronic acid, glucosamine, galatosamine, and neuramic acid. In certain embodiments, a carbohydrate is a polysaccharide, including but not limited to pullulan, cellulose, microcrystalline cellulose, hydroxypropyl methylcellulose (HPMC), hydroxycellulose (HC), methylcellulose (MC), dextran, cyclodextran, glycogen, hydroxyethylstarch, carageenan, glycon, amylose, chitosan, N,O-carboxylmethylchitosan,

algin and alginic acid, starch, chitin, inulin, konjac, glucommannan, pustulan, heparin, hyaluronic acid, curdlan, and xanthan. In embodiments, the synthetic nanocarriers do not comprise (or specifically exclude) carbohydrates, such as a polysaccharide. In certain embodiments, the carbohydrate may comprise a carbohydrate derivative such as a sugar alcohol, including but not limited to mannitol, sorbitol, xylitol, erythritol, maltitol, and lactitol.

In some embodiments, synthetic nanocarriers can comprise one or more polymers. In some embodiments, the synthetic nanocarriers comprise one or more polymers that is a non-methoxy-terminated, pluronic polymer. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% (weight/weight) of the polymers that make up the synthetic nanocarriers are non-methoxy-terminated, pluronic polymers. In some embodiments, all of the polymers that make up the synthetic nanocarriers are non-methoxy-terminated, pluronic polymers. In some embodiments, the synthetic nanocarriers comprise one or more polymers that is a non-methoxy-terminated polymer. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% (weight/weight) of the polymers that make up the synthetic nanocarriers are non-methoxy-terminated polymers. In some embodiments, all of the polymers that make up the synthetic nanocarriers are non-methoxy-terminated polymers. In some embodiments, the synthetic nanocarriers comprise one or more polymers that do not comprise pluronic polymer. In some embodiments, at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 97%, or 99% (weight/weight) of the polymers that make up the synthetic nanocarriers do not comprise pluronic polymer. In some embodiments, all of the polymers that make up the synthetic nanocarriers do not comprise pluronic polymer. In some embodiments, such a polymer can be surrounded by a coating layer (e.g., liposome, lipid monolayer, micelle, etc.). In some embodiments, elements of the synthetic nanocarriers can be attached to the polymer.

Immunosuppressants can be coupled to the synthetic nanocarriers by any of a number of methods. Generally, the attaching can be a result of bonding between the immunosuppressants and the synthetic nanocarriers. This bonding can result in the immunosuppressants being attached to the surface of the synthetic nanocarriers and/or contained (encapsulated) within the synthetic nanocarriers. In some embodiments, however, the immunosuppressants are encapsulated by the synthetic nanocarriers as a result of the structure of the synthetic nanocarriers rather than bonding to the synthetic nanocarriers. In

preferable embodiments, the synthetic nanocarrier comprises a polymer as provided herein, and the immunosuppressants are attached to the polymer.

When attaching occurs as a result of bonding between the immunosuppressants and synthetic nanocarriers, the attaching may occur via a coupling moiety. A coupling moiety can be any moiety through which an immunosuppressant is bonded to a synthetic nanocarrier. Such moieties include covalent bonds, such as an amide bond or ester bond, as well as separate molecules that bond (covalently or non-covalently) the immunosuppressant to the synthetic nanocarrier. Such molecules include linkers or polymers or a unit thereof. For example, the coupling moiety can comprise a charged polymer to which an immunosuppressant electrostatically binds. As another example, the coupling moiety can comprise a polymer or unit thereof to which it is covalently bonded.

In preferred embodiments, the synthetic nanocarriers comprise a polymer as provided herein. These synthetic nanocarriers can be completely polymeric or they can be a mix of polymers and other materials.

In some embodiments, the polymers of a synthetic nanocarrier associate to form a polymeric matrix. In some of these embodiments, a component, such as an immunosuppressant, can be covalently associated with one or more polymers of the polymeric matrix. In some embodiments, covalent association is mediated by a linker. In some embodiments, a component can be noncovalently associated with one or more polymers of the polymeric matrix. For example, in some embodiments, a component can be encapsulated within, surrounded by, and/or dispersed throughout a polymeric matrix. Alternatively or additionally, a component can be associated with one or more polymers of a polymeric matrix by hydrophobic interactions, charge interactions, van der Waals forces, etc. A wide variety of polymers and methods for forming polymeric matrices therefrom are known conventionally.

Polymers may be natural or unnatural (synthetic) polymers. Polymers may be homopolymers or copolymers comprising two or more monomers. In terms of sequence, copolymers may be random, block, or comprise a combination of random and block sequences. Typically, polymers in accordance with the present invention are organic polymers.

In some embodiments, the polymer comprises a polyester, polycarbonate, polyamide, or polyether, or unit thereof. In other embodiments, the polymer comprises poly(ethylene glycol) (PEG), polypropylene glycol, poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid), or a polycaprolactone, or unit thereof. In some embodiments, it is preferred that the polymer is biodegradable. Therefore, in these embodiments, it is preferred that if the

polymer comprises a polyether, such as poly(ethylene glycol) or polypropylene glycol or unit thereof, the polymer comprises a block-co-polymer of a polyether and a biodegradable polymer such that the polymer is biodegradable. In other embodiments, the polymer does not solely comprise a polyether or unit thereof, such as poly(ethylene glycol) or polypropylene glycol or unit thereof.

Other examples of polymers suitable for use in the present invention include, but are not limited to polyethylenes, polycarbonates (e.g. poly(1,3-dioxan-2one)), polyanhydrides (e.g. poly(sebacic anhydride)), polypropylfumerates, polyamides (e.g. polycaprolactam), polyacetals, polyethers, polyesters (e.g., polylactide, polyglycolide, polylactide-co-glycolide, polycaprolactone, polyhydroxyacid (e.g. poly(β-hydroxyalkanoate))), poly(orthoesters), polycyanoacrylates, polyvinyl alcohols, polyurethanes, polyphosphazenes, polyacrylates, polymethacrylates, polyureas, polystyrenes, and polyamines, polylysine, polylysine-PEG copolymers, and poly(ethyleneimine), poly(ethylene imine)-PEG copolymers.

In some embodiments, polymers in accordance with the present invention include polymers which have been approved for use in humans by the U.S. Food and Drug Administration (FDA) under 21 C.F.R. § 177.2600, including but not limited to polyesters (e.g., polylactic acid, poly(lactic-co-glycolic acid), polycaprolactone, polyvalerolactone, poly(1,3-dioxan-2one)); polyanhydrides (e.g., poly(sebacic anhydride)); polyethers (e.g., polyethylene glycol); polyurethanes; polymethacrylates; polyacrylates; and polycyanoacrylates.

In some embodiments, polymers can be hydrophilic. For example, polymers may comprise anionic groups (e.g., phosphate group, sulphate group, carboxylate group); cationic groups (e.g., quaternary amine group); or polar groups (e.g., hydroxyl group, thiol group, amine group). In some embodiments, a synthetic nanocarrier comprising a hydrophilic polymeric matrix generates a hydrophilic environment within the synthetic nanocarrier. In some embodiments, polymers can be hydrophobic. In some embodiments, a synthetic nanocarrier comprising a hydrophobic polymeric matrix generates a hydrophobic environment within the synthetic nanocarrier. Selection of the hydrophilicity or hydrophobicity of the polymer may have an impact on the nature of materials that are incorporated within the synthetic nanocarrier.

In some embodiments, polymers may be modified with one or more moieties and/or functional groups. A variety of moieties or functional groups can be used in accordance with the present invention. In some embodiments, polymers may be modified with polyethylene glycol (PEG), with a carbohydrate, and/or with acyclic polyacetals derived from

polysaccharides (Papisov, 2001, ACS Symposium Series, 786:301). Certain embodiments may be made using the general teachings of US Patent No. 5543158 to Gref et al., or WO publication WO2009/051837 by Von Andrian et al.

In some embodiments, polymers may be modified with a lipid or fatty acid group. In some embodiments, a fatty acid group may be one or more of butyric, caproic, caprylic, capric, lauric, myristic, palmitic, stearic, arachidic, behenic, or lignoceric acid. In some embodiments, a fatty acid group may be one or more of palmitoleic, oleic, vaccenic, linoleic, alpha-linoleic, gamma-linoleic, arachidonic, gadoleic, arachidonic, eicosapentaenoic, docosahexaenoic, or erucic acid.

In some embodiments, polymers may be polyesters, including copolymers comprising lactic acid and glycolic acid units, such as poly(lactic acid-co-glycolic acid) and poly(lactide-co-glycolide), collectively referred to herein as "PLGA"; and homopolymers comprising glycolic acid units, referred to herein as "PGA," and lactic acid units, such as poly-L-lactic acid, poly-D-lactic acid, poly-D,L-lactic acid, poly-L-lactide, poly-D-lactide, and poly-D,L-lactide, collectively referred to herein as "PLA." In some embodiments, exemplary polyesters include, for example, polyhydroxyacids; PEG copolymers and copolymers of lactide and glycolide (e.g., PLA-PEG copolymers, PGA-PEG copolymers, PLGA-PEG copolymers, and derivatives thereof. In some embodiments, polyesters include, for example, poly(caprolactone), poly(caprolactone)-PEG copolymers, poly(L-lactide-co-L-lysine), poly(serine ester), poly(4-hydroxy-L-proline ester), poly[α-(4-aminobutyl)-L-glycolic acid], and derivatives thereof.

In some embodiments, a polymer may be PLGA. PLGA is a biocompatible and biodegradable co-polymer of lactic acid and glycolic acid, and various forms of PLGA are characterized by the ratio of lactic acid:glycolic acid. Lactic acid can be L-lactic acid, D-lactic acid, or D,L-lactic acid. The degradation rate of PLGA can be adjusted by altering the lactic acid:glycolic acid ratio. In some embodiments, PLGA to be used in accordance with the present invention is characterized by a lactic acid:glycolic acid ratio of approximately 85:15, approximately 75:25, approximately 60:40, approximately 50:50, approximately 40:60, approximately 25:75, or approximately 15:85.

In some embodiments, polymers may be one or more acrylic polymers. In certain embodiments, acrylic polymers include, for example, acrylic acid and methacrylic acid copolymers, methyl methacrylate copolymers, ethoxyethyl methacrylates, cyanoethyl methacrylate, aminoalkyl methacrylate copolymer, poly(acrylic acid), poly(methacrylic acid), methacrylic acid alkylamide copolymer, poly(methyl methacrylate), poly(methacrylic acid

anhydride), methyl methacrylate, polymethacrylate, poly(methyl methacrylate) copolymer, polyacrylamide, aminoalkyl methacrylate copolymer, glycidyl methacrylate copolymers, polycyanoacrylates, and combinations comprising one or more of the foregoing polymers. The acrylic polymer may comprise fully-polymerized copolymers of acrylic and methacrylic acid esters with a low content of quaternary ammonium groups.

In some embodiments, polymers can be cationic polymers. In general, cationic polymers are able to condense and/or protect negatively charged strands of nucleic acids. Amine-containing polymers such as poly(lysine) (Zauner et al., 1998, Adv. Drug Del. Rev., 30:97; and Kabanov et al., 1995, Bioconjugate Chem., 6:7), poly(ethylene imine) (PEI; Boussif et al., 1995, Proc. Natl. Acad. Sci., USA, 1995, 92:7297), and poly(amidoamine) dendrimers (Kukowska-Latallo et al., 1996, Proc. Natl. Acad. Sci., USA, 93:4897; Tang et al., 1996, Bioconjugate Chem., 7:703; and Haensler et al., 1993, Bioconjugate Chem., 4:372) are positively-charged at physiological pH, form ion pairs with nucleic acids. In embodiments, the synthetic nanocarriers may not comprise (or may exclude) cationic polymers.

In some embodiments, polymers can be degradable polyesters bearing cationic side chains (Putnam et al., 1999, Macromolecules, 32:3658; Barrera et al., 1993, J. Am. Chem. Soc., 115:11010; Kwon et al., 1989, Macromolecules, 22:3250; Lim et al., 1999, J. Am. Chem. Soc., 121:5633; and Zhou et al., 1990, Macromolecules, 23:3399). Examples of these polyesters include poly(L-lactide-co-L-lysine) (Barrera et al., 1993, J. Am. Chem. Soc., 115:11010), poly(serine ester) (Zhou et al., 1990, Macromolecules, 23:3399), poly(4-hydroxy-L-proline ester) (Putnam et al., 1999, Macromolecules, 32:3658; and Lim et al., 1999, J. Am. Chem. Soc., 121:5633), and poly(4-hydroxy-L-proline ester) (Putnam et al., 1999, Macromolecules, 32:3658; and Lim et al., 1999, J. Am. Chem. Soc., 121:5633).

The properties of these and other polymers and methods for preparing them are well known in the art (see, for example, U.S. Patents 6,123,727; 5,804,178; 5,770,417; 5,736,372; 5,716,404; 6,095,148; 5,837,752; 5,902,599; 5,696,175; 5,514,378; 5,512,600; 5,399,665; 5,019,379; 5,010,167; 4,806,621; 4,638,045; and 4,946,929; Wang et al., 2001, J. Am. Chem. Soc., 123:9480; Lim et al., 2001, J. Am. Chem. Soc., 123:2460; Langer, 2000, Acc. Chem. Res., 33:94; Langer, 1999, J. Control. Release, 62:7; and Uhrich et al., 1999, Chem. Rev., 99:3181). More generally, a variety of methods for synthesizing certain suitable polymers are described in Concise Encyclopedia of Polymer Science and Polymeric Amines and Ammonium Salts, Ed. by Goethals, Pergamon Press, 1980; Principles of Polymerization by Odian, John Wiley & Sons, Fourth Edition, 2004; Contemporary Polymer Chemistry by

Allcock et al., Prentice-Hall, 1981; Deming et al., 1997, Nature, 390:386; and in U.S. Patents 6,506,577, 6,632,922, 6,686,446, and 6,818,732.

In some embodiments, polymers can be linear or branched polymers. In some embodiments, polymers can be dendrimers. In some embodiments, polymers can be substantially cross-linked to one another. In some embodiments, polymers can be substantially free of cross-links. In some embodiments, polymers can be used in accordance with the present invention without undergoing a cross-linking step. It is further to be understood that the synthetic nanocarriers may comprise block copolymers, graft copolymers, blends, mixtures, and/or adducts of any of the foregoing and other polymers. Those skilled in the art will recognize that the polymers listed herein represent an exemplary, not comprehensive, list of polymers that can be of use in accordance with the present invention.

In some embodiments, synthetic nanocarriers do not comprise a polymeric component. In some embodiments, synthetic nanocarriers may comprise metal particles, quantum dots, ceramic particles, etc. In some embodiments, a non-polymeric synthetic nanocarrier is an aggregate of non-polymeric components, such as an aggregate of metal atoms (e.g., gold atoms).

Compositions according to the invention can comprise pharmaceutically acceptable excipients, such as preservatives, buffers, saline, or phosphate buffered saline. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. In an embodiment, compositions are suspended in sterile saline solution for injection together with a preservative.

## D. METHODS OF USING AND MAKING THE COMPOSITIONS

Viral vectors can be made with methods known to those of ordinary skill in the art or as otherwise described herein. For example, viral vectors can be constructed and/or purified using the methods set forth, for example, in U.S. Pat. No. 4,797,368 and Laughlin et al., Gene, 23, 65-73 (1983).

As an example, replication-deficient adenoviral vectors can be produced in complementing cell lines that provide gene functions not present in the replication-deficient adenoviral vectors, but required for viral propagation, at appropriate levels in order to generate high titers of viral vector stock. The complementing cell line can complement for a deficiency in at least one replication-essential gene function encoded by the early regions, late regions, viral packaging regions, virus-associated RNA regions, or combinations thereof, including all adenoviral functions (e.g., to enable propagation of adenoviral amplicons). Construction of

complementing cell lines involve standard molecular biology and cell culture techniques, such as those described by Sambrook et al., Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989), and Ausubel et al., Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

Complementing cell lines for producing adenoviral vectors include, but are not limited to, HEK 293 cells (described in, e.g., Graham et al., J. Gen. Virol., 36, 59-72 (1977)), PER.C6 cells (described in, e.g., International Patent Application WO 97/00326, and U.S. Pat. Nos. 5,994,128 and 6,033,908), and 293-ORF6 cells (described in, e.g., International Patent Application WO 95/34671 and Brough et al., J. Virol., 71, 9206-9213 (1997)). In some instances, the complementing cell will not complement for all required adenoviral gene functions. Helper viruses can be employed to provide the gene functions in trans that are not encoded by the cellular or adenoviral genomes to enable replication of the adenoviral vector. Adenoviral vectors can be constructed, propagated, and/or purified using the materials and methods set forth, for example, in U.S. Pat. Nos. 5,965,358, 5,994,128, 6,033,908, 6,168,941, 6,329,200, 6,383,795, 6,440,728, 6,447,995, and 6,475,757, U.S. Patent Application Publication No. 2002/0034735 A1, and International Patent Applications WO 98/53087, WO 98/56937, WO 99/15686, WO 99/54441, WO 00/12765, WO 01/77304, and WO 02/29388, as well as the other references identified herein. Non-group C adenoviral vectors, including adenoviral serotype 35 vectors, can be produced using the methods set forth in, for example, U.S. Pat. Nos. 5,837,511 and 5,849,561, and International Patent Applications WO 97/12986 and WO 98/53087.

Viral vectors, such as AAV vectors, may be produced using recombinant methods. For example, the methods can involve culturing a host cell which contains a nucleic acid sequence encoding an AAV capsid protein or fragment thereof; a functional rep gene; a recombinant AAV vector composed of AAV inverted terminal repeats (ITRs) and a transgene; and sufficient helper functions to permit packaging of the recombinant AAV vector into the AAV capsid proteins. In some embodiments, the viral vector may comprise inverted terminal repeats (ITR) of AAV serotypes selected from the group consisting of: AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV10, AAV11 and variants thereof.

The components to be cultured in the host cell to package a viral vector in a capsid may be provided to the host cell in trans. Alternatively, any one or more of the required components (e.g., recombinant AAV vector, rep sequences, cap sequences, and/or helper functions) may be provided by a stable host cell which has been engineered to contain one or

more of the required components using methods known to those of skill in the art. Most suitably, such a stable host cell can contain the required component(s) under the control of an inducible promoter. However, the required component(s) may be under the control of a constitutive promoter. The recombinant viral vector, rep sequences, cap sequences, and helper functions required for producing the viral vector may be delivered to the packaging host cell using any appropriate genetic element. The selected genetic element may be delivered by any suitable method, including those described herein. Other methods are known to those with skill in nucleic acid manipulation and include genetic engineering, recombinant engineering, and synthetic techniques. See, e.g., Sambrook et al, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. Similarly, methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, J. Virol., 70:520-532 (1993) and U.S. Pat. No. 5,478,745.

In some embodiments, recombinant AAV transfer vectors may be produced using the triple transfection method (e.g., as described in detail in U.S. Pat. No. 6,001,650, U.S. Pat. No. 6,593,123, as well as X. Xiao et al, J. Virol. 72:2224-2232 (1998), and T. Matsushita et al, Gene Ther. 5(7): 938-945 (1998), the contents of which relating to the triple transfection method are incorporated herein by reference). For example, the recombinant AAVs can be produced by transfecting a host cell with a recombinant AAV transfer vector (comprising a transgene) to be packaged into AAV particles, an AAV helper function vector, and an accessory function vector. Generally, an AAV helper function vector encodes AAV helper function sequences (rep and cap), which function in trans for productive AAV replication and encapsidation. Preferably, the AAV helper function vector supports efficient AAV vector production without generating any detectable wild-type AAV virions (i.e., AAV virions containing functional rep and cap genes). The accessory function vector can encode nucleotide sequences for non-AAV derived viral and/or cellular functions upon which AAV is dependent for replication. The accessory functions include those functions required for AAV replication, including, without limitation, those moieties involved in activation of AAV gene transcription, stage specific AAV mRNA splicing, AAV DNA replication, synthesis of cap expression products, and AAV capsid assembly. Viral-based accessory functions can be derived from any of the known helper viruses such as adenovirus, herpesvirus (other than herpes simplex virus type-1), and vaccinia virus.

Other methods for producing viral vectors are known in the art. Moreover, viral vectors are available commercially.

In regard to synthetic nanocarriers coupled to immunosuppressants, methods for attaching components to synthetic nanocarriers may be useful.

In embodiments, methods for attaching components to, for example, synthetic nanocarriers may be useful. In certain embodiments, the attaching can be a covalent linker. In embodiments, immunosuppressants according to the invention can be covalently attached to the external surface via a 1,2,3-triazole linker formed by the 1,3-dipolar cycloaddition reaction of azido groups with immunosuppressant containing an alkyne group or by the 1,3-dipolar cycloaddition reaction of alkynes with immunosuppressants containing an azido group. Such cycloaddition reactions are preferably performed in the presence of a Cu(I) catalyst along with a suitable Cu(I)-ligand and a reducing agent to reduce Cu(II) compound to catalytic active Cu(I) compound. This Cu(I)-catalyzed azide-alkyne cycloaddition (CuAAC) can also be referred as the click reaction.

Additionally, covalent coupling may comprise a covalent linker that comprises an amide linker, a disulfide linker, a thioether linker, a hydrazone linker, a hydrazide linker, an imine or oxime linker, an urea or thiourea linker, an amidine linker, an amine linker, and a sulfonamide linker.

An amide linker is formed via an amide bond between an amine on one component such as an immunosuppressant with the carboxylic acid group of a second component such as the nanocarrier. The amide bond in the linker can be made using any of the conventional amide bond forming reactions with suitably protected amino acids and activated carboxylic acid such N-hydroxysuccinimide-activated ester.

A disulfide linker is made via the formation of a disulfide (S-S) bond between two sulfur atoms of the form, for instance, of R1-S-S-R2. A disulfide bond can be formed by thiol exchange of a component containing thiol/mercaptan group(-SH) with another activated thiol group or a component containing thiol/mercaptan groups with a component containing activated thiol group.

A triazole linker, specifically a 1,2,3-triazole of the form 

, wherein R1 and R2 may be any chemical entities, is made by the 1,3-dipolar cycloaddition reaction of an azide attached to a first component with a terminal alkyne attached to a second component such as the immunosuppressant. The 1,3-dipolar cycloaddition reaction is performed with or without a catalyst, preferably with Cu(I)-catalyst, which links the two components through a 1,2,3-triazole function. This chemistry is described in detail by Sharpless et al., Angew. Chem. Int.

Ed. 41(14), 2596, (2002) and Meldal, et al, Chem. Rev., 2008, 108(8), 2952-3015 and is often referred to as a "click" reaction or CuAAC.

A thioether linker is made by the formation of a sulfur-carbon (thioether) bond in the form, for instance, of R1-S-R2. Thioether can be made by either alkylation of a thiol/mercaptan (-SH) group on one component with an alkylating group such as halide or epoxide on a second component. Thioether linkers can also be formed by Michael addition of a thiol/mercaptan group on one component to an electron-deficient alkene group on a second component containing a maleimide group or vinyl sulfone group as the Michael acceptor. In another way, thioether linkers can be prepared by the radical thiol-ene reaction of a thiol/mercaptan group on one component with an alkene group on a second component.

A hydrazone linker is made by the reaction of a hydrazide group on one component with an aldehyde/ketone group on the second component.

A hydrazide linker is formed by the reaction of a hydrazine group on one component with a carboxylic acid group on the second component. Such reaction is generally performed using chemistry similar to the formation of amide bond where the carboxylic acid is activated with an activating reagent.

An imine or oxime linker is formed by the reaction of an amine or N-alkoxyamine (or aminooxy) group on one component with an aldehyde or ketone group on the second component.

An urea or thiourea linker is prepared by the reaction of an amine group on one component with an isocyanate or thioisocyanate group on the second component.

An amidine linker is prepared by the reaction of an amine group on one component with an imidoester group on the second component.

An amine linker is made by the alkylation reaction of an amine group on one component with an alkylating group such as halide, epoxide, or sulfonate ester group on the second component. Alternatively, an amine linker can also be made by reductive amination of an amine group on one component with an aldehyde or ketone group on the second component with a suitable reducing reagent such as sodium cyanoborohydride or sodium triacetoxyborohydride.

A sulfonamide linker is made by the reaction of an amine group on one component with a sulfonyl halide (such as sulfonyl chloride) group on the second component.

A sulfone linker is made by Michael addition of a nucleophile to a vinyl sulfone. Either the vinyl sulfone or the nucleophile may be on the surface of the nanocarrier or attached to a component.

The component can also be conjugated via non-covalent conjugation methods. For example, a negative charged immunosuppressant can be conjugated to a positive charged component through electrostatic adsorption. A component containing a metal ligand can also be conjugated to a metal complex via a metal-ligand complex.

In embodiments, the component can be attached to a polymer, for example polylactic acid-block-polyethylene glycol, prior to the assembly of a synthetic nanocarrier or the synthetic nanocarrier can be formed with reactive or activatible groups on its surface. In the latter case, the component may be prepared with a group which is compatible with the attachment chemistry that is presented by the synthetic nanocarriers' surface. In other embodiments, a peptide component can be attached to VLPs or liposomes using a suitable linker. A linker is a compound or reagent that capable of coupling two molecules together. In an embodiment, the linker can be a homobifunctional or heterobifunctional reagent as described in Hermanson 2008. For example, a VLP or liposome synthetic nanocarrier containing a carboxylic group on the surface can be treated with a homobifunctional linker, adipic dihydrazide (ADH), in the presence of EDC to form the corresponding synthetic nanocarrier with the ADH linker. The resulting ADH linked synthetic nanocarrier is then conjugated with a peptide component containing an acid group via the other end of the ADH linker on nanocarrier to produce the corresponding VLP or liposome peptide conjugate.

In embodiments, a polymer containing an azide or alkyne group, terminal to the polymer chain is prepared. This polymer is then used to prepare a synthetic nanocarrier in such a manner that a plurality of the alkyne or azide groups are positioned on the surface of that nanocarrier. Alternatively, the synthetic nanocarrier can be prepared by another route, and subsequently functionalized with alkyne or azide groups. The component is prepared with the presence of either an alkyne (if the polymer contains an azide) or an azide (if the polymer contains an alkyne) group. The component is then allowed to react with the nanocarrier via the 1,3-dipolar cycloaddition reaction with or without a catalyst which covalently attaches the component to the particle through the 1,4-disubstituted 1,2,3-triazole linker.

If the component is a small molecule it may be of advantage to attach the component to a polymer prior to the assembly of synthetic nanocarriers. In embodiments, it may also be an advantage to prepare the synthetic nanocarriers with surface groups that are used to attach the component to the synthetic nanocarrier through the use of these surface groups rather than attaching the component to a polymer and then using this polymer conjugate in the construction of synthetic nanocarriers.

For detailed descriptions of available conjugation methods, see Hermanson G T "Bioconjugate Techniques", 2nd Edition Published by Academic Press, Inc., 2008. In addition to covalent attachment the component can be attached by adsorption to a pre-formed synthetic nanocarrier or it can be attached by encapsulation during the formation of the synthetic nanocarrier.

Synthetic nanocarriers may be prepared using a wide variety of methods known in the art. For example, synthetic nanocarriers can be formed by methods such as nanoprecipitation, flow focusing using fluidic channels, spray drying, single and double emulsion solvent evaporation, solvent extraction, phase separation, milling, microemulsion procedures, microfabrication, nanofabrication, sacrificial layers, simple and complex coacervation, and other methods well known to those of ordinary skill in the art. Alternatively or additionally, aqueous and organic solvent syntheses for monodisperse semiconductor, conductive, magnetic, organic, and other nanomaterials have been described (Pellegrino et al., 2005, Small, 1:48; Murray et al., 2000, Ann. Rev. Mat. Sci., 30:545; and Trindade et al., 2001, Chem. Mat., 13:3843). Additional methods have been described in the literature (see, e.g., Doubrow, Ed., "Microcapsules and Nanoparticles in Medicine and Pharmacy," CRC Press, Boca Raton, 1992; Mathiowitz et al., 1987, J. Control. Release, 5:13; Mathiowitz et al., 1987, Reactive Polymers, 6:275; and Mathiowitz et al., 1988, J. Appl. Polymer Sci., 35:755; US Patents 5578325 and 6007845; P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010)).

Materials may be encapsulated into synthetic nanocarriers as desirable using a variety of methods including but not limited to C. Astete et al., "Synthesis and characterization of PLGA nanoparticles" J. Biomater. Sci. Polymer Edn, Vol. 17, No. 3, pp. 247–289 (2006); K. Avgoustakis "Pegylated Poly(Lactide) and Poly(Lactide-Co-Glycolide) Nanoparticles: Preparation, Properties and Possible Applications in Drug Delivery" Current Drug Delivery 1:321-333 (2004); C. Reis et al., "Nanoencapsulation I. Methods for preparation of drug-loaded polymeric nanoparticles" Nanomedicine 2:8– 21 (2006); P. Paolicelli et al., "Surface-modified PLGA-based Nanoparticles that can Efficiently Associate and Deliver Virus-like Particles" Nanomedicine. 5(6):843-853 (2010). Other methods suitable for encapsulating materials into synthetic nanocarriers may be used, including without limitation methods disclosed in United States Patent 6,632,671 to Unger issued October 14, 2003.

In certain embodiments, synthetic nanocarriers are prepared by a nanoprecipitation process or spray drying. Conditions used in preparing synthetic nanocarriers may be altered to

yield particles of a desired size or property (e.g., hydrophobicity, hydrophilicity, external morphology, "stickiness," shape, etc.). The method of preparing the synthetic nanocarriers and the conditions (e.g., solvent, temperature, concentration, air flow rate, etc.) used may depend on the materials to be attached to the synthetic nanocarriers and/or the composition of the polymer matrix.

If synthetic nanocarriers prepared by any of the above methods have a size range outside of the desired range, synthetic nanocarriers can be sized, for example, using a sieve.

Elements of the synthetic nanocarriers may be attached to the overall synthetic nanocarrier, e.g., by one or more covalent bonds, or may be attached by means of one or more linkers. Additional methods of functionalizing synthetic nanocarriers may be adapted from Published US Patent Application 2006/0002852 to Saltzman et al., Published US Patent Application 2009/0028910 to DeSimone et al., or Published International Patent Application WO/2008/127532 A1 to Murthy et al.

Alternatively or additionally, synthetic nanocarriers can be attached to components directly or indirectly via non-covalent interactions. In non-covalent embodiments, the non-covalent attaching is mediated by non-covalent interactions including but not limited to charge interactions, affinity interactions, metal coordination, physical adsorption, host-guest interactions, hydrophobic interactions, TT stacking interactions, hydrogen bonding interactions, van der Waals interactions, magnetic interactions, electrostatic interactions, dipole-dipole interactions, and/or combinations thereof. Such attachments may be arranged to be on an external surface or an internal surface of a synthetic nanocarrier. In embodiments, encapsulation and/or absorption is a form of attaching.

Compositions provided herein may comprise inorganic or organic buffers (e.g., sodium or potassium salts of phosphate, carbonate, acetate, or citrate) and pH adjustment agents (e.g., hydrochloric acid, sodium or potassium hydroxide, salts of citrate or acetate, amino acids and their salts) antioxidants (e.g., ascorbic acid, alpha-tocopherol), surfactants (e.g., polysorbate 20, polysorbate 80, polyoxyethylene9-10 nonyl phenol, sodium desoxycholate), solution and/or cryo/lyo stabilizers (e.g., sucrose, lactose, mannitol, trehalose), osmotic adjustment agents (e.g., salts or sugars), antibacterial agents (e.g., benzoic acid, phenol, gentamicin), antifoaming agents (e.g., polydimethylsilozone), preservatives (e.g., thimerosal, 2-phenoxyethanol, EDTA), polymeric stabilizers and viscosity-adjustment agents (e.g., polyvinylpyrrolidone, poloxamer 488, carboxymethylcellulose) and co-solvents (e.g., glycerol, polyethylene glycol, ethanol).

Compositions according to the invention may comprise pharmaceutically acceptable excipients. The compositions may be made using conventional pharmaceutical manufacturing and compounding techniques to arrive at useful dosage forms. Techniques suitable for use in practicing the present invention may be found in Handbook of Industrial Mixing: Science and Practice, Edited by Edward L. Paul, Victor A. Atiemo-Obeng, and Suzanne M. Kresta, 2004 John Wiley & Sons, Inc.; and Pharmaceutics: The Science of Dosage Form Design, 2nd Ed. Edited by M. E. Auten, 2001, Churchill Livingstone. In an embodiment, compositions are suspended in sterile saline solution for injection with a preservative.

It is to be understood that the compositions of the invention can be made in any suitable manner, and the invention is in no way limited to compositions that can be produced using the methods described herein. Selection of an appropriate method of manufacture may require attention to the properties of the particular moieties being associated.

In some embodiments, compositions are manufactured under sterile conditions or are terminally sterilized. This can ensure that resulting compositions are sterile and non-infectious, thus improving safety when compared to non-sterile compositions. This provides a valuable safety measure, especially when subjects receiving the compositions have immune defects, are suffering from infection, and/or are susceptible to infection.

Administration according to the present invention may be by a variety of routes, including but not limited to subcutaneous, intravenous, intramuscular and intraperitoneal routes. The compositions referred to herein may be manufactured and prepared for administration, in some embodiments as an admixture, using conventional methods.

The compositions of the invention can be administered in effective amounts, such as the effective amounts described elsewhere herein. Dosage forms may be administered at a variety of frequencies. In some embodiments of any one of the methods or compositions provided, repeated administration of synthetic nanocarriers comprising an immunosuppressant with a viral vector is undertaken.

## EXAMPLES

### Example 1: Synthesis of Synthetic Nanocarriers Comprising an Immunosuppressant (Prophetic)

Synthetic nanocarriers comprising an immunosuppressant, such as rapamycin, can be produced using any method known to those of ordinary skill in the art. Preferably, in some embodiments of any one of the methods or compositions provided herein the synthetic

nanocarriers comprising an immunosuppressant are produced by any one of the methods of US Publication No. US 2016/0128986 A1 and US Publication No. US 2016/0128987 A1, the described methods of such production and the resulting synthetic nanocarriers being incorporated herein by reference in their entirety. In any one of the methods or compositions provided herein, the synthetic nanocarriers comprising an immunosuppressant are such incorporated synthetic nanocarriers. ImmTOR, biodegradable PLA + PLA-PEG nanoparticles encapsulating rapamycin, refers to an example of such synthetic nanocarriers (Kishimoto TK, Maldonado RA. Nanoparticles for the induction of antigen-specific immunological tolerance. Front Immunol. 2018;9:230. Sands E, Kivitz AJ, DeHaan W, et al. Update of SEL-212 phase 2 clinical data in symptomatic gout patients: SVP-rapamycin combined with pegadricase mitigates immunogenicity and enables sustained reduction of serum uric acid levels, low rate of gout flares and monthly dosing. Poster presentation at: 2018 American College of Rheumatology/Association of Reproductive Health Professionals (ACR/ARHP) Annual Meeting; October 19-24, 2018; Chicago, IL. Poster #2254).

**Example 2: Admixing AAV Vectors to Synthetic Nanocarriers Comprising Immunosuppressant Rescues Transgene Expression in Mice with Anti-AAV Antibodies**

The ability of admixed AAV vectors and synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) to rescue transgene expression in mice with anti-AAV antibodies was examined. First, sera from normal human donors were screened for the presence of pre-existing neutralizing and IgG antibodies against AAV in an *in vitro* assay. Briefly, Huh7 liver-derived cells were incubated with AAV8-luciferase in the presence of sera from normal human donors. Luciferase expression was assessed following transduction of Huh7 cells. Cells incubated with sera from human donor 8 showed high levels of luciferase activity, indicating the absence of significant levels of neutralizing antibodies. In contrast, cells incubated with sera from human donor 45 showed little or no luciferase expression, indicating the presence of high levels of neutralizing antibodies. Likewise, cells incubated with sera from human donor 44 also showed little luciferase expression, indicating the presence of moderately-high levels of neutralizing antibodies. Cells incubated with sera from human donors 31 and 35 showed an intermediate level of luciferase expression, indicating the presence of moderate levels of neutralizing antibodies. The predicted levels of neutralizing antibodies based on the functional neutralizing antibody assay described above were found to correlate with the observed levels of anti-AAV IgG antibodies (**FIG. 1**).

Eighty microliters (80 μL) of sera from human donors 8, 31, 35, 44, and 45 were transferred into individual mice by intravenous injection. Approximately 24 hours later, mice were injected with 5.0E11 vg/kg of the AAV8-SEAP vector or 5.0E11 vg/kg of the AAV8-SEAP vector admixed with 100 μg of synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles). Twelve days later, sera were collected from the mice and serum SEAP activity levels were measured.

The results are shown in **FIG. 2**. Mice receiving sera from human donor 8 followed by the AAV8-SEAP vector showed similar levels of SEAP activity as control mice that received only AAV8-SEAP, confirming that sera from human donor 8 had no significant levels of neutralizing anti-AAV8 antibodies (compare open donor 8 bar to open control "no serum" bar). Mice receiving sera from human donors 31 and 35 followed by the AAV8-SEAP vector showed approximately 46-47% SEAP activity relative to the no serum control mice, confirming the presence of moderately neutralizing antibodies in the sera from human donors 31 and 35 (compare open donor 31 and 35 bars to open control "no serum" bar). However, admixing synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) with the AAV8-SEAP vector in mice receiving sera from human donors 31 and 35 was found to enhance SEAP transgene expression and activity levels comparable to the no serum control mice receiving only the AAV8-SEAP vector (compare solid donor 31 and 35 bars to open control "no serum" bar). These results indicate that admixing synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) with the first dose of the AAV8-SEAP vector can rescue transgene expression and activity in mice with moderate levels of neutralizing antibodies.

## Example 3: Admixing AAV Vectors with Synthetic Nanocarriers Comprising Immunosuppressant Rescues Transgene Expression in Naïve Mice Injected with Anti-AAV Antibodies

The AAV8-SEAP vector was admixed with equal volumes of 50 μg synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) or saline for 20 minutes at room temperature and then admixed with a 1:100 dilution of normal, naïve mouse serum or mouse serum containing anti-AAV antibodies for 1 hour at room temperature. The resulting admixture was injected into naïve mice and 33 days later, serum expression of the SEAP transgene was measured. Serum expression of the SEAP transgene was compared to control mice injected with the AAV8-SEAP vector without admixing to serum.

The results are presented in **FIG. 3**. Mice injected with the AAV8-SEAP vector admixed to normal serum not containing anti-AAV antibodies showed serum SEAP activity comparable to the control mice, and mice injected with the AAV8-SEAP vector admixed to serum containing anti-AAV antibodies showed decreased SEAP serum activity compared to control mice. In contrast, admixing 50 μg synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) to the AAV8-SEAP vector prior to admixing to serum containing anti-AAV antibodies was found to rescue SEAP expression to levels comparable to the no serum control.

**Example 4: Synthetic Nanocarriers Comprising Immunosuppressant Ameliorate Maternally-Transferred Antibodies**

The ability of synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) to rescue transgene expression in mice harboring maternally-transferred anti-AAV antibodies was examined. Hypomorphic mice deficient in expression of methylmalonoyl-CoA mutase (MUT) and expressing a transgene for MUT under a muscle-specific promoter (Mck-MUT mice) were used (Manoli et al., 2018). These mice present with a severe form of methylmalonic acidemia. Male and female homozygous Mck-MUT mice received gene therapy with an AAV Anc80-hAAT-MUT vector to correct the MUT gene expression deficiency in the liver. The mice were then bred, and all the newborn pups from their progeny carried pre-existing anti-Anc80 antibodies, which were presumably transferred *in utero* from the mother (**FIG. 4**).

At approximately 26 days of age, the Mck-MUT mice still showed significant levels of maternally-transferred anti-Anc80 antibodies. The mice were randomized and treated with 5.0e12 vg/kg Anc80-Mut alone or admixed with 100 μg or 300 μg synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles). Two of the four mice treated with Anc80-MUT alone died within days after gene transfer. Three of five mice treated with Anc80-MUT admixed to100 μg synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) died shortly after gene transfer. Moreover, all seven animals treated with Anc80-MUT admixed to 300 μg synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) survived. These data indicate that admixing synthetic nanocarriers comprising rapamycin (e.g., ImmTOR nanoparticles) to a first dose of the AAV Anc80-hAAT-MUT vector enhanced survival of mice in a dose-dependent manner.

**Example 5: Effects of Synthetic Nanocarriers Comprising Immunosuppressant in a Mouse Model of Methylmalonic Acidemia with Maternally-Transferred Antibodies Treated with Anc80 AAV-Mut Vector**

Collectively, 23 Mck-MMUT mice with pre-existing IgG to Anc80 were generated. Of these, 6 mice were treated with AAV Anc80-MMUT at 5×1012 vg/kg, 7 mice were treated with the same dose of AAV Anc80-MMUT admixed with 100 μg of ImmTOR nanoparticles and 10 mice were treated with the same dose of AAV Anc80-MMUT admixed with 300 μg of ImmTOR.

No group has shown a benefit after initial treatment as measured by pMMA levels. Moreover, four out of six mice treated with Anc80-MMUT alone and four out of seven mice treated with Anc80-MUT admixed to100 μg of ImmTOR nanoparticles died shortly after gene transfer. At the same time, all ten mice in the group treated with Anc80-MUT admixed to 300 μg of ImmTOR nanoparticles survived for 21 days, at which point the second treatment was administered to all the surviving mice. This time approximately half of mice treated with Anc80-MUT admixed to 300 μg of ImmTOR and showed a substantial decrease in pMMA levels at 9 days after the 2nd gene transfer, while one out of three remaining mice in the group treated with Anc80-MUT admixed to 100 μg of ImmTOR also showed pMMA decrease. There was no benefit in the remaining two mice treated with Anc80-MMUT alone, with one of these mice succumbing to the disease within a few days.

Nine out of ten mice treated with Anc80-MUT admixed to 300 μg of ImmTOR survived for over three months at which point they exhibited highly variable levels of pMMA and were treated for the 3rd time at 101 days after the initial treatment. This intervention lead to dramatic decrease of pMMA levels in all mice in this group (to 18% vs. pre-treatment) and also in all the surviving mice treated with Anc80-MUT admixed to 100 μg of ImmTOR. No benefit was seen in a single surviving mouse treated with Anc80-MMUT alone and it soon succumbed to the disease.

Collectively, there was a statistically significant difference in survival between groups treated with Anc80-MUT admixed to 300 μg of ImmTOR and groups treated with Anc80-MUT alone ($p<0.0001$) or admixed to 100 μg of ImmTOR ($p<0.05$).

Moreover, nearly all mice (9/10) treated with Anc80-MUT admixed to 300 μg of ImmTOR showed the absence of *de novo* Anc80 IgG formation up to day 115 of the study (i.e., after three treatments). These data indicate that admixing synthetic nanocarriers

comprising rapamycin (e.g., ImmTOR nanoparticles) to a first dose of the AAV Anc80-hAAT-MMUT vector enhanced survival of mice in a dose-dependent manner and improved levels of pMMA after repeated administrations. Data are shown in **FIGs. 5-8.**

Thus, with a first dose of Anc80-MUT admixed to the synthetic nanocarriers, after retreatment, a partial reduction in serum MMA was observed (in mice treated with Anc80-MUT + 300 μg ImmTOR). Further, following the second retreatment in mice treated with Anc80-MUT + 300 μg ImmTOR, results showed a uniform reduction in serum MMA. Mck-MUT mice with pre-existing maternally transferred Anti-Anc80 IgG repeatedly treated with Anc80-MUT with admixed synthetic nanocarriers comprising an immunosuppressant had higher early survival rates. Pre-existing humoral immunity in mice with maternally-transferred anti-Anc80 IgG does not preclude treatment with a viral vector due to the administration of synthetic nanocarriers comprising an immunosuppressant, such as rapamycin. The data also show that higher doses of the synthetic nanocarriers comprising immunosuppressant can enable early survival and then provide therapeutic efficacy at repeat administrations, while delaying *de novo* IgG formation.

## CLAIMS

What is claimed is:

1. A method comprising:

administering synthetic nanocarriers comprising an immunosuppressant admixed with a viral vector to a subject, wherein the subject has pre-existing immunity against a viral antigen of the viral vector.

2. The method of claim 1, wherein the subject is one that would otherwise be excluded from treatment with the viral vector due to the pre-existing immunity.

3. The method of claim 1, wherein the subject is a newborn with maternally-transferred antibodies.

4. The method of any one of the preceding claims, wherein the viral vector has not previously been administered to the subject.

5. The method of any one of the preceding claims, wherein the viral vector has not been previously concomitantly administered with synthetic nanocarriers comprising an immunosuppressant to the subject.

6. The method of any one of the preceding claims, wherein the synthetic nanocarriers comprising an immunosuppressant has not previously been administered to the subject.

7. The method of any one of the preceding claims, wherein the method further comprises at least one subsequent concomitant administration of the synthetic nanocarriers comprising an immunosuppressant and the viral vector.

8. The method of claim 7, wherein the subsequent concomitant administration of the synthetic nanocarriers comprising an immunosuppressant and the viral vector is repeated.

9. The method of claim 7 or 8, wherein the synthetic nanocarriers comprising an immunosuppressant of the subsequent concomitant administration(s) are admixed with the viral vector.

10. The method of any one of claims 7-9, wherein the one or more subsequent concomitant administration(s) occurs within 2 month subsequent to a prior administration to the subject.

11. The method of any one of claims 7-10, wherein the one or more subsequent concomitant administration(s) occurs within 1 month subsequent to a prior administration to the subject.

12. The method of any one of claims 7-11, wherein the one or more subsequent concomitant administration(s) occurs within 1 week subsequent to a prior administration to the subject.

13. The method of any one of claims 7-9, wherein the one or more subsequent concomitant administration(s) occurs at least 1 month subsequent to a prior administration to the subject.

14. The method of any one of claims 7-9, wherein the one or more subsequent concomitant administration(s) occurs at least 1 week subsequent to a prior administration to the subject.

15. The method of any one of the preceding claims, wherein the method further comprises determining a level of pre-existing immunity to the viral vector in the subject prior to any administration of the synthetic nanocarriers comprising an immunosuppressant with the viral vector to the subject.

16. The method of claim 15, wherein the determining comprises measuring a level of anti-viral vector antibodies in the subject prior to administration to the subject.

17. The method of claim 15, wherein the determining comprises measuring a level of a T cell response against a viral antigen of the viral vector in the subject prior to administration to the subject.

18. The method of any one of the preceding claims, wherein the amount of the viral vector is less that the amount of the viral vector that, when administered concomitantly but not admixed with synthetic nanocarriers comprising an immunosuppressant, increases transgene expression of the viral vector in another subject,

optionally, wherein the another subject has pre-existing immunity against a viral antigen of the viral vector.

19. The method of any one of the preceding claims, wherein the amount of the synthetic nanocarriers comprising an immunosuppressant is greater than an amount of the synthetic nanocarriers comprising an immunosuppressant that, when administered with a viral vector to a subject without pre-existing immunity against a viral antigen of the viral vector, increases transgene expression of the viral vector and/or results in a reduction in an immune response, such as antibodies, against a viral antigen of the viral vector.

20. The method of claim 19, wherein the amount is at least two-fold greater.

21. The method of claim 19, wherein the amount is at least three-fold greater.

22. The method of any one of the preceding claims, wherein administering synthetic nanocarriers comprising an immunosuppressant with the viral vector and/or the one or more subsequent concomitant administration(s) is by intravenous administration.

23. The method of any one of the preceding claims, wherein the viral vector comprises one or more expression control sequences.

24. The method of claim 23, wherein the one or more expression control sequences comprise a liver-specific promoter.

25. The method of claim 24, wherein the one or more expression control sequences comprise a constitutive promoter.

26. The method of any one of the preceding claims, wherein the viral vector is a retroviral vector, an adenoviral vector, a lentiviral vector or an adeno-associated viral vector.

27. The method of claim 26, wherein the viral vector is an adeno-associated viral vector.

28. The method of claim 27, wherein the adeno-associated viral vector is an AAV1, AAV2, AAV5, AAV6, AAV6.2, AAV7, AAV8, AAV9, AAV10 or AAV11 adeno-associated viral vector.

29. The method of any one of the preceding claims, wherein the immunosuppressant of the synthetic nanocarriers comprising an immunosuppressants an inhibitor of the NF-kB pathway.

30. The method of any one of the preceding claims, wherein the immunosuppressants an mTOR inhibitor.

31. The method of claim 30, wherein the mTOR inhibitor is rapamycin or a rapalog.

32. The method of any one of the preceding claims, wherein the immunosuppressant is coupled to the synthetic nanocarriers.

33. The method of claim 32, wherein the immunosuppressant is encapsulated in the synthetic nanocarriers.

34. The method of any one of the preceding claims, wherein the synthetic nanocarriers comprise lipid nanoparticles, polymeric nanoparticles, metallic nanoparticles, surfactant-based emulsions, dendrimers, buckyballs, nanowires, virus-like particles or peptide or protein particles.

35. The method of claim 34, wherein the synthetic nanocarriers comprise polymeric nanoparticles.

36. The method of claim 35, wherein the polymeric nanoparticles comprise a polyester, polyester attached to a polyether, polyamino acid, polycarbonate, polyacetal, polyketal, polysaccharide, polyethyloxazoline or polyethyleneimine.

37. The method of claim 36, wherein the polymeric nanoparticles comprise a polyester or a polyester attached to a polyether.

38. The method of claim 36 or 37, wherein the polyester comprises a poly(lactic acid), poly(glycolic acid), poly(lactic-co-glycolic acid) or polycaprolactone.

39. The method of any one of claims 36-38, wherein the polymeric nanoparticles comprise a polyester and a polyester attached to a polyether.

40. The method of any one of claims 36-39, wherein the polyether comprises polyethylene glycol or polypropylene glycol.

41. The method of any one of the preceding claims, wherein the mean of a particle size distribution obtained using dynamic light scattering of a population of the synthetic nanocarriers is a diameter greater than 110nm.

42. The method of claim 41, wherein the diameter is greater than 150nm.

43. The method of claim 42, wherein the diameter is greater than 200nm.

44. The method of claim 43, wherein the diameter is greater than 250nm.

45. The method of any one of claims 41-44, wherein the diameter is less than 5μm.

46. The method of claim 45, wherein the diameter is less than 4μm.

47. The method of claim 46, wherein the diameter is less than 3μm.

48. The method of claim 47, wherein the diameter is less than 2μm.

49. The method of claim 48, wherein the diameter is less than 1μm.

50. The method of claim 49, wherein the diameter is less than 750nm.

51. The method of claim 50, wherein the diameter is less than 500nm.

52. The method of claim 51, wherein the diameter is less than 450nm.

53. The method of claim 52, wherein the diameter is less than 400nm.

54. The method of claim 53, wherein the diameter is less than 350nm.

55. The method of claim 54, wherein the diameter is less than 300nm.

56. The method of any one of the preceding claims, wherein the load of immunosuppressant comprised in the synthetic nanocarriers, on average across the synthetic nanocarriers, is between 0.1% and 50% (weight/weight).

57. The method of claim 56, wherein the load is between 0.1% and 25%.

58. The method of claim 56, wherein the load is at least 4% but less than 40%.

59. The method of claim 57, wherein the load is between 2% and 25%.

60. The method of any one of the preceding claims, wherein an aspect ratio of a population of the synthetic nanocarriers is greater than or equal to 1:1, 1:1.2, 1:1.5, 1:2, 1:3, 1:5, 1:7 or 1:10.

61. A composition comprising an immunosuppressant admixed with a viral vector described in any of claims 1-60.

62. The method of any one of the preceding claims, wherein the method further comprises assessing an IgG or IgM or neutralizing antibody response to the viral vector in the subject at one or more time points.

63. The method of claim 62, wherein at least one of the time points of assessing an IgG or IgM or neutralizing antibody response is subsequent to the administration of the synthetic nanocarriers comprising an immunosuppressant with the viral vector.

64. The method of any one of the preceding claims, wherein the method further comprises measuring the level of transgene expression in the subject at one or more time points.

65. The method of claim 64, wherein at least one of the time points of measuring the level of transgene expression is subsequent to the administration of the synthetic nanocarriers comprising an immunosuppressant with the viral vector.

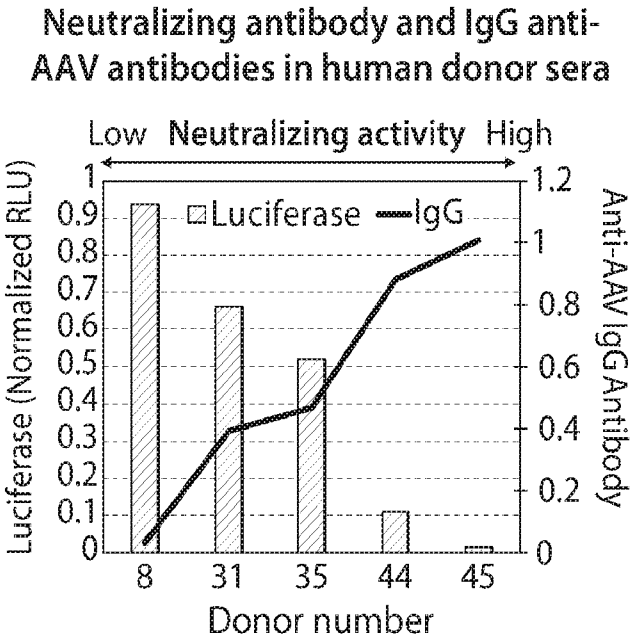
Neutralizing antibody and IgG anti-AAV antibodies in human donor sera
Low Neutralizing activity High
Luciferase (Normalized RLU)
Luciferase
IgG
Anti-AAV IgG Antibody
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
1.2
1
0.8
0.6
0.4
0.2
0
8
31
35
44
45
Donor number

FIG. 1

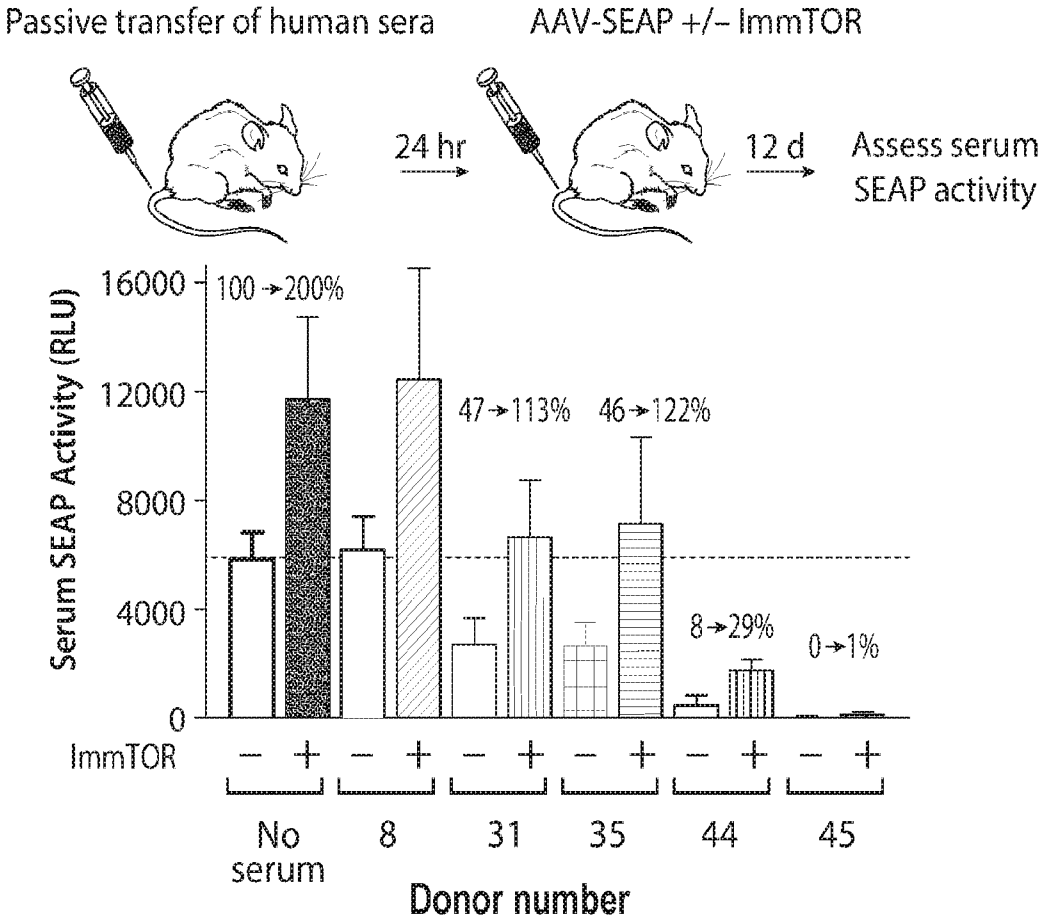
Passive transfer of human sera
AAV-SEAP +/– ImmTOR
24 hr
12 d
Assess serum SEAP activity
Serum SEAP Activity (RLU)
16000
12000
8000
4000
0
100→200%
47→113%
46→122%
8→29%
0→1%
ImmTOR
– + – + – + – + – + – +
No serum
8
31
35
44
45
Donor number

FIG. 2

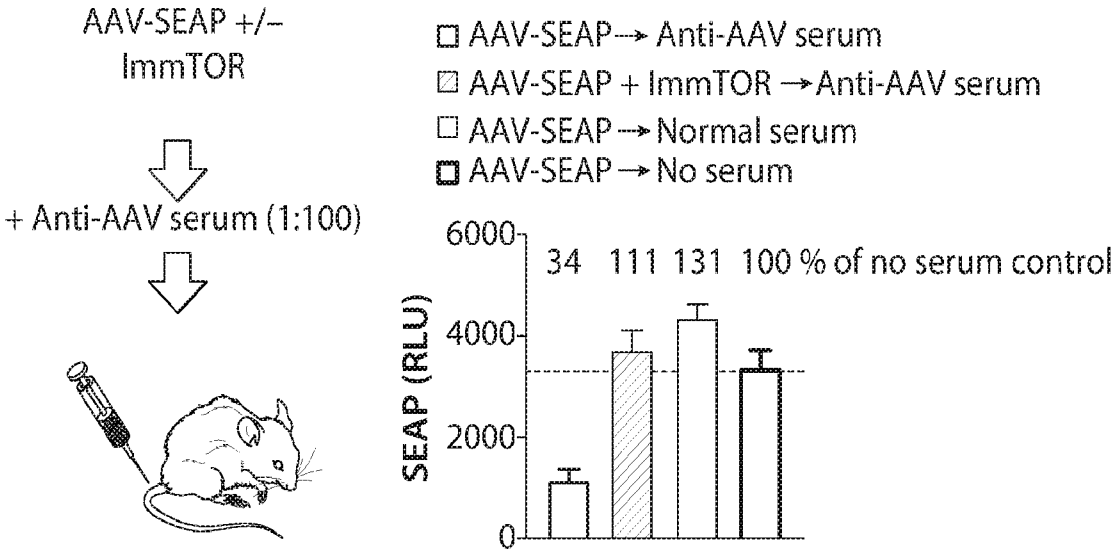
AAV-SEAP +/–
ImmTOR
+ Anti-AAV serum (1:100)
AAV-SEAP → Anti-AAV serum
AAV-SEAP + ImmTOR → Anti-AAV serum
AAV-SEAP → Normal serum
AAV-SEAP → No serum
SEAP (RLU)
6000
4000
2000
0
34
111
131
100 % of no serum control

FIG. 3

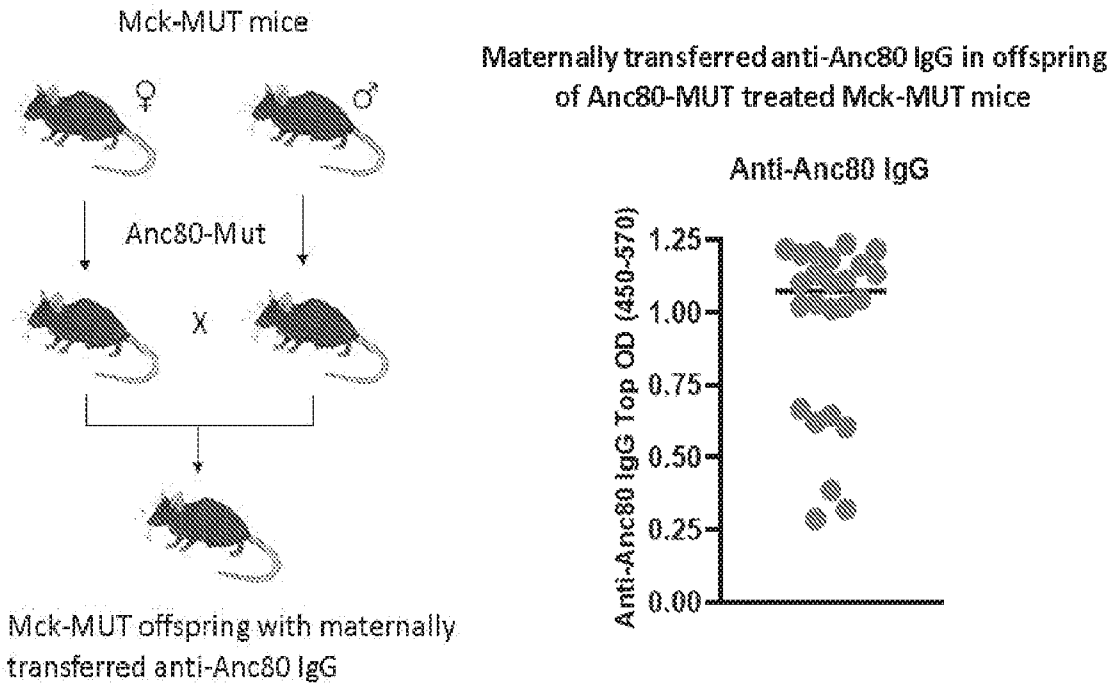
Mck-MUT mice
♀
♂
Anc80-Mut
X
Mck-MUT offspring with maternally transferred anti-Anc80 IgG
Maternally transferred anti-Anc80 IgG in offspring of Anc80-MUT treated Mck-MUT mice
Anti-Anc80 IgG
Anti-Anc80 IgG Top OD (450-570)
1.25
1.00
0.75
0.50
0.25
0.00

FIG. 4

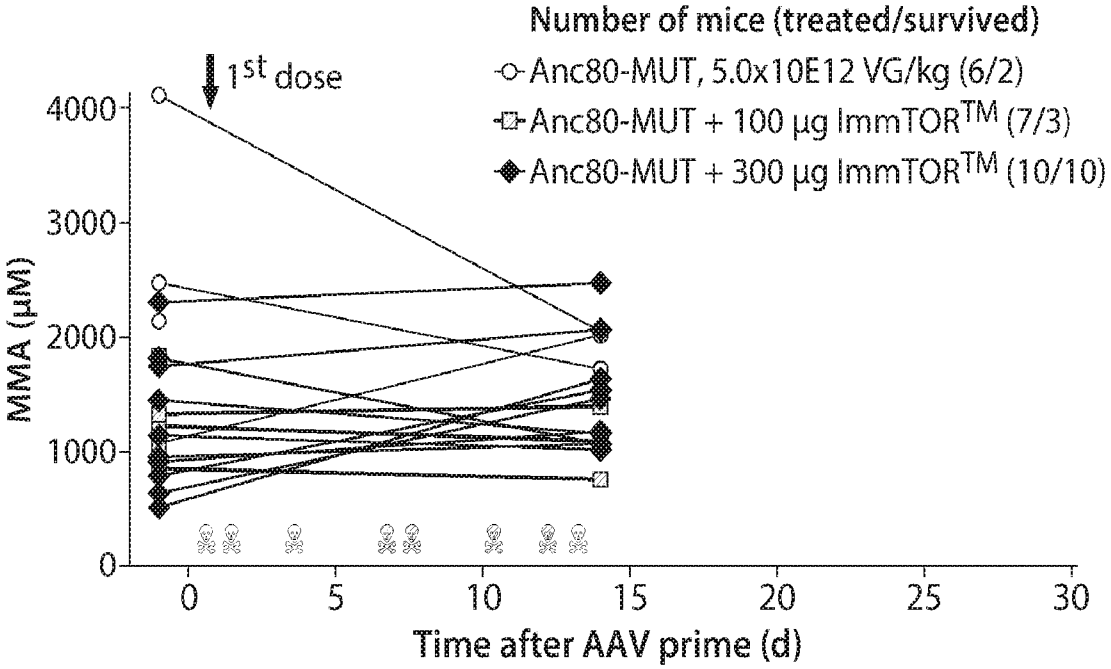
Number of mice (treated/survived)
Anc80-MUT, 5.0x10E12 VG/kg (6/2)
Anc80-MUT + 100 µg ImmTOR™ (7/3)
Anc80-MUT + 300 µg ImmTOR™ (10/10)
1st dose
MMA (µM)
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Time after AAV prime (d)

FIG. 5

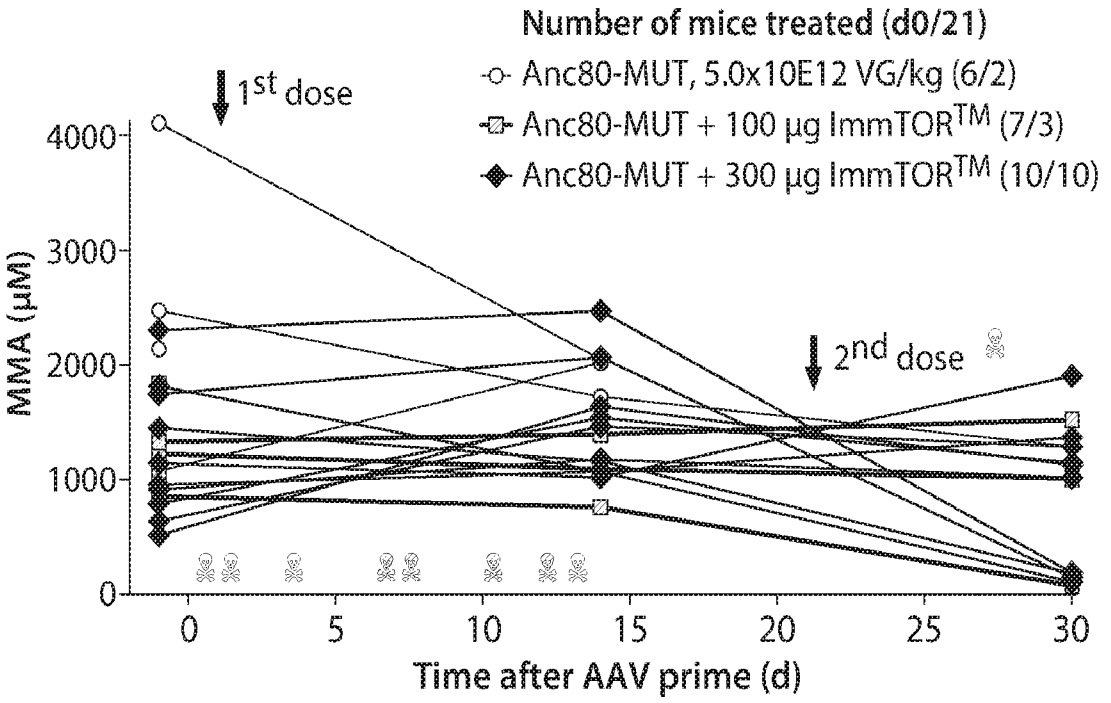
Number of mice treated (d0/21)
Anc80-MUT, 5.0x10E12 VG/kg (6/2)
Anc80-MUT + 100 µg ImmTOR™ (7/3)
Anc80-MUT + 300 µg ImmTOR™ (10/10)
1st dose
2nd dose
MMA (µM)
4000
3000
2000
1000
0
0
5
10
15
20
25
30
Time after AAV prime (d)

FIG. 6

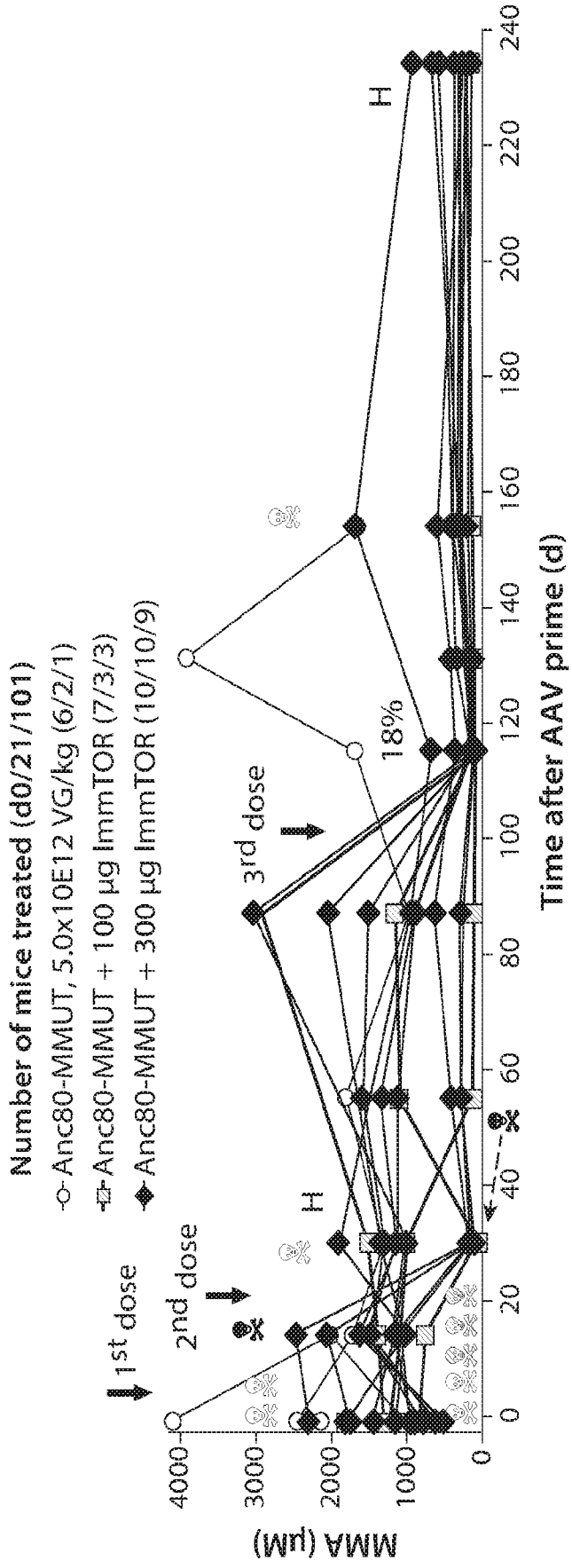
Number of mice treated (d0/21/101)
Anc80-MMUT, 5.0x10E12 VG/kg (6/2/1)
Anc80-MMUT + 100 µg ImmTOR (7/3/3)
Anc80-MMUT + 300 µg ImmTOR (10/10/9)
1st dose
2nd dose
3rd dose
H
18%
MMA (µM)
Time after AAV prime (d)
0
1000
2000
3000
4000
0
20
40
60
80
100
120
140
160
180
200
220
240

FIG. 7

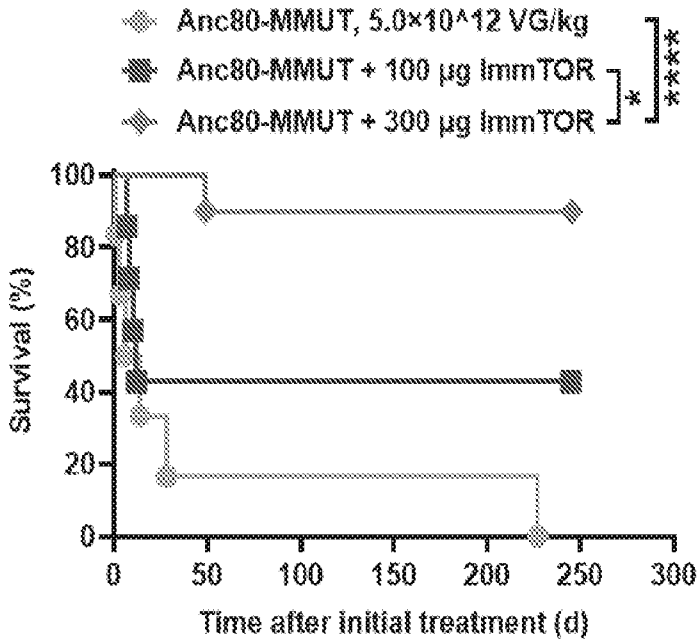
Anc80-MMUT, 5.0×10^12 VG/kg
Anc80-MMUT + 100 µg ImmTOR
Anc80-MMUT + 300 µg ImmTOR
*
****
Survival (%)
100
80
60
40
20
0
0
50
100
150
200
250
300
Time after initial treatment (d)

FIG. 8A

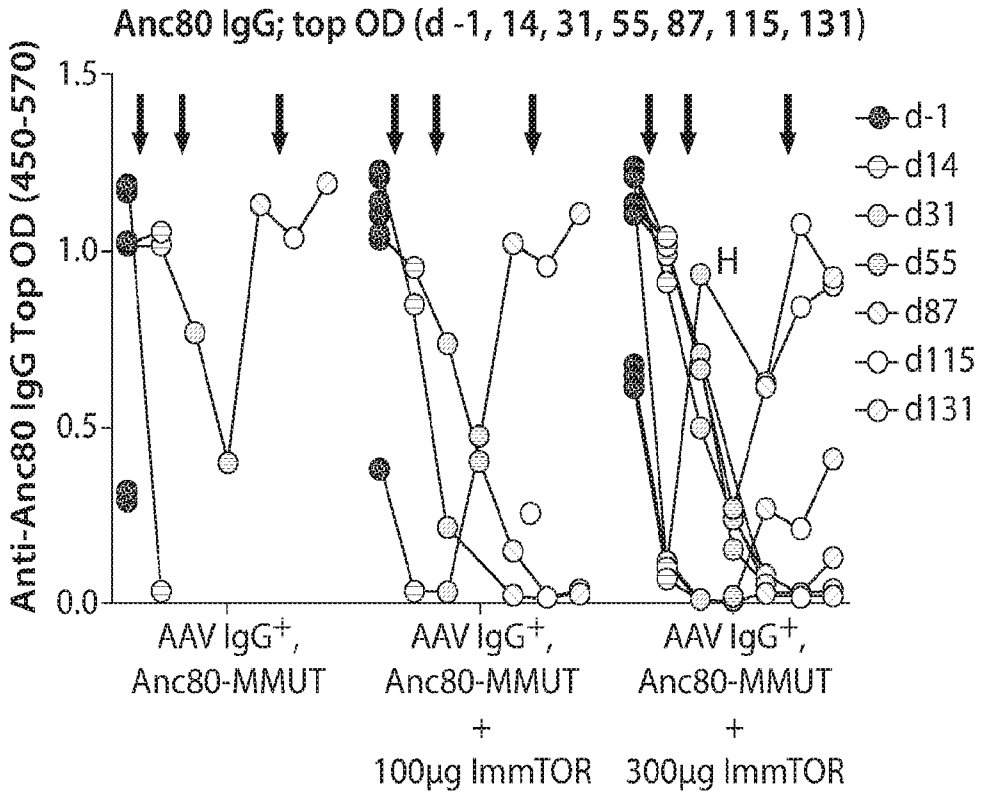
Anc80 IgG; top OD (d -1, 14, 31, 55, 87, 115, 131)
Anti-Anc80 IgG Top OD (450-570)
1.5
1.0
0.5
0.0
d-1
d14
d31
d55
d87
d115
d131
H
AAV IgG+, Anc80-MMUT
AAV IgG+, Anc80-MMUT + 100µg ImmTOR
AAV IgG+, Anc80-MMUT + 300µg ImmTOR

FIG. 8B

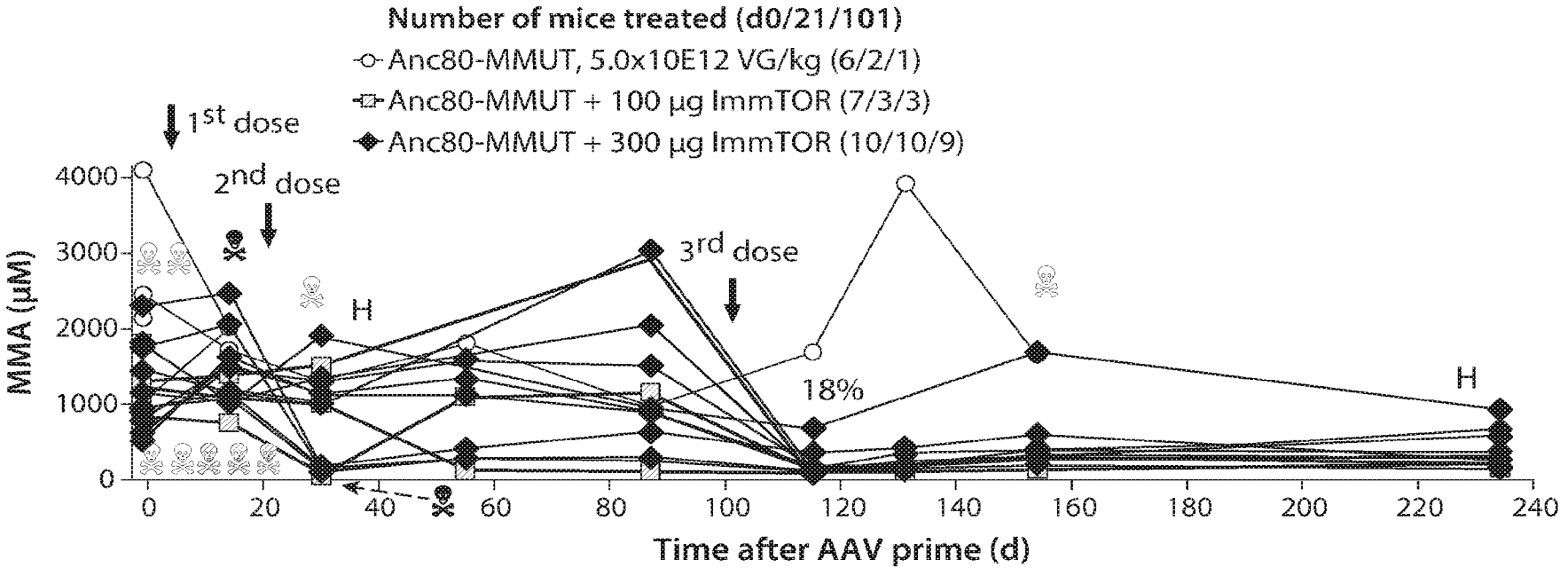
Number of mice treated (d0/21/101)
Anc80-MMUT, 5.0x10E12 VG/kg (6/2/1)
Anc80-MMUT + 100 µg ImmTOR (7/3/3)
Anc80-MMUT + 300 µg ImmTOR (10/10/9)
1st dose
2nd dose
3rd dose
H
18%
H
MMA (µM)
4000
3000
2000
1000
0
0
20
40
60
80
100
120
140
160
180
200
220
240
Time after AAV prime (d)

FIG. 7