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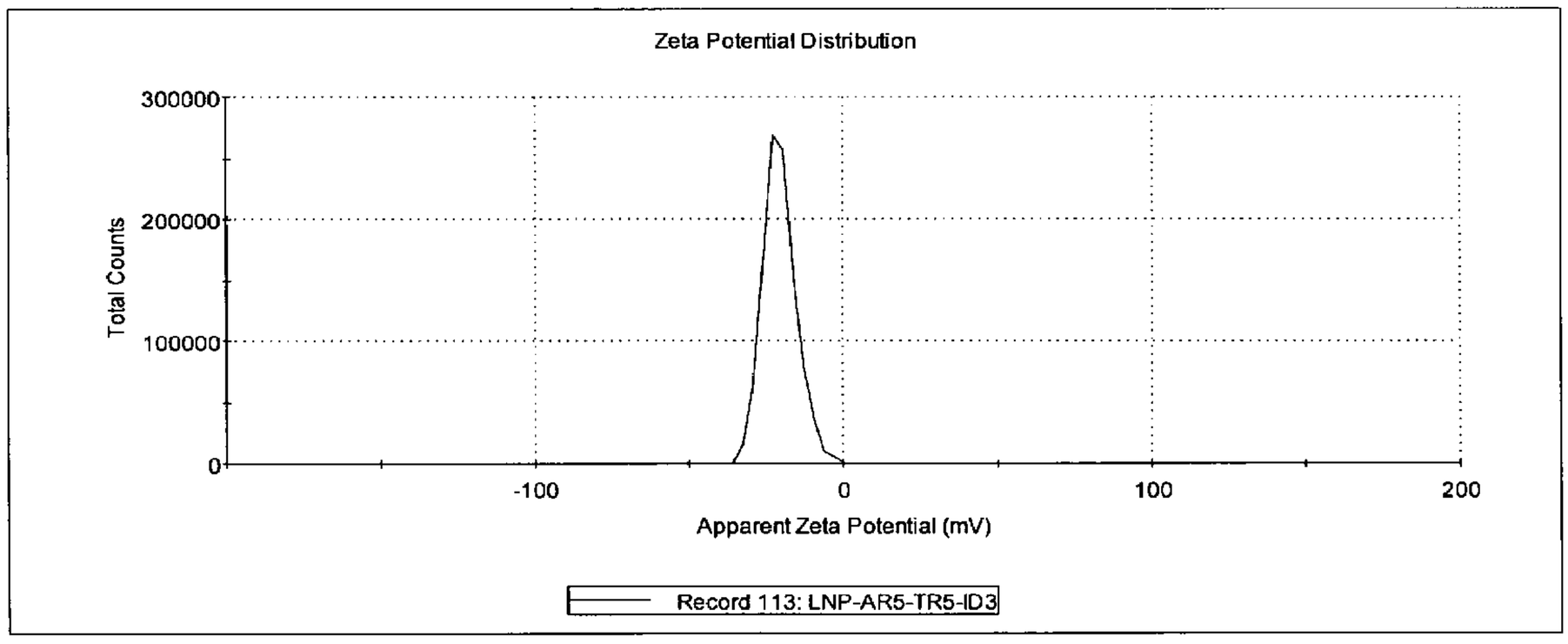
(54) Title: FORMULATED AND/OR CO-FORMULATED LIPOSOME COMPOSITIONS CONTAINING IDO ANTAGONIST PRODRUGS USEFUL IN THE TREATMENT OF CANCER AND METHODS THEREOF

Figure 14. Characterization of LNP-AR5-TR5-ID3 Liposome (Zeta Potential)



(57) Abstract: Formulated and/or co-formulated liposomes comprising IDO prodrugs and methods of making the liposomes are disclosed herein. The IDO prodrug compositions comprise a drug moiety, a lipid moiety, and linkage unit that inhibit IDO-1. The IDO prodrugs can be formulated and/or co-formulated into a liposome to provide a method of treating cancer, immunological disorders, and other disease by utilizing a targeted drug delivery vehicle.

[Continued on next page]

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Formulated and/or Co-Formulated Liposome Compositions Containing IDO Antagonist Prodrugs Useful in Treating of Cancer and Methods Thereof

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to United States Provisional Patent Application number 62/974,086 filed 12-November-2019, the contents of which are fully incorporated by reference herein.

STATEMENT OF RIGHTS TO INVENTIONS MADE UNDER FEDERALLY SPONSORED RESEARCH

Not applicable.

FIELD OF THE INVENTION

The invention described herein relates to prodrug compositions that inhibit Indoleamine-pyrrole 2,3-dioxygenase (IDO) enzyme after release of the active inhibitor from the prodrug and nano-formulations comprising such prodrugs. Specifically, the invention relates to prodrug compositions which are formulated within a nanocarrier (e.g. a liposome) and used as a vehicle for cancer therapy in humans. The invention further relates to the treatment of cancers and other immunological disorders and diseases.

BACKGROUND OF THE INVENTION

Cancer is the second leading cause of death next to coronary disease worldwide. Millions of people die from cancer every year and in the United States alone cancer kills well over a half-million people annually, with 1,688,780 new cancer cases diagnosed in 2017 (American Cancer Society). While deaths from heart disease have been declining significantly, those resulting from cancer generally are on the rise. In the early part of the next century, cancer is predicted to become the leading cause of death unless medical developments change the current trend.

Several cancers stand out as having high rates of mortality. In particular, carcinomas of the lung (18.4% of all cancer deaths), breast (6.6% of all cancer deaths), colorectal (9.2% of all cancer deaths), liver (8.2% of all cancer deaths), and stomach (8.2% of all cancer deaths) represent major causes of cancer death for both sexes in all ages worldwide (GLOBOCAN 2018). These and virtually all other carcinomas share a common lethal feature in that they metastasis to sites distant from the primary tumor and with very few exceptions, metastatic disease fatal. Moreover, even for those cancer patients who initially survive their primary cancers, common experience has shown that their lives are dramatically altered. Many cancer patients experience strong anxieties driven by the awareness of the

potential for recurrence or treatment failure. Many cancer patients also experience physical debilitations following treatment. Furthermore, many cancer patients experience a recurrence of their disease.

Although cancer therapy has improved over the past decades and survival rates have increased, the heterogeneity of cancer still demands new therapeutic strategies utilizing a plurality of treatment modalities. This is especially true in treating solid tumors at anatomical crucial sites (e.g., glioblastoma, squamous carcinoma of the head and neck and lung adenocarcinoma) which are sometimes limited to standard radiotherapy and/or chemotherapy. Nonetheless, detrimental effects of these therapies are chemo- and radio resistance, which promote loco-regional recurrences, distant metastases and second primary tumors, in addition to severe side-effects that reduce the patients' quality of life.

Indoleamine-pyrrole 2,3-dioxygenase (IDO or INDO) is a heme-containing enzyme that in humans is encoded by the *IDO1* gene. IDO is the first and rate-limiting enzyme of tryptophan catabolism through the kynurenine pathway, thus causing depletion of tryptophan, which can slow the growth of microbes as well as T cells. Additionally, IDO is an immune checkpoint molecule in the sense that it is an immunomodulatory enzyme produced by some alternatively activated macrophages and other immunoregulatory cells (also used as an immune subversion strategy by many tumors and chronic infectious viruses). IDO is known to suppress T and NK cells, generate and activate Tregs and myeloid-derived suppressor cells, and promote the growth of new blood cells to feed the tumor (angiogenesis). IDO permits tumor cells to escape the immune system by depletion of L-tryptophan in the tumor microenvironment and by production of the catabolic product kynurenine, which selectively impairs the growth and survival of T-cells. A wide range of human cancers such as prostatic, colorectal, pancreatic, cervical, gastric, ovarian, head, lung, etc. overexpress human IDO (hIDO). IDO has been implicated in immune modulation through its ability to limit T-cell function and engage mechanisms of immune tolerance. Emerging evidence suggests that IDO becomes activated during tumor development, helping malignant cells escape eradication by the immune system. *See*, MUNN, *et. al.*, Trends in Immunology, 37(3): pp. 193-207 (March 2016), and PENDERGRAST, *et. al.*, Cancer Immunol Immunother., 63(7): pp. 721-735 (July 2014).

Additionally, a prodrug is a medication or compound that, after administration, is metabolized (i.e., converted within the body) into a pharmacologically active drug. Instead of administering a drug directly, a corresponding prodrug is used instead to improve how a medicine is absorbed, distributed, metabolized, and/or excreted. Prodrugs are often designed to improve bioavailability when a drug itself is poorly absorbed from the gastrointestinal tract, for example. A prodrug may be used to improve how selectively the drug interacts with cells or processes that are not its intended target. This reduces adverse or unintended effects of a drug, especially important in treatments like chemotherapy, which can have severe unintended and undesirable side

effects. Prodrugs can thus be viewed as drugs containing specialized non-toxic protective groups used in a transient manner to alter or to eliminate undesirable properties in the parent molecule.

Finally, a nanocarrier is a nanomaterial being used as a transport for another substance, such as a drug. There are many different types of nanocarriers. For example, nanocarriers include polymer conjugates, polymeric nanoparticles, lipid-based carriers, and dendrimers to name a few. Different types of nanomaterial(s) being used in nanocarriers allows for hydrophobic and hydrophilic drugs to be delivered throughout the body. Since the human body contains mostly water, the ability to deliver hydrophobic drugs effectively in humans is a major therapeutic benefit of nanocarriers. Nanocarriers show promise in the drug delivery process because they can deliver drugs to site-specific targets, allowing drugs to be delivered in certain organs or cells but not in others. Site-specificity is a major therapeutic benefit since it prevents drugs from being delivered to the wrong places. Additionally, nanocarriers show specific promise for use in chemotherapy because they can help decrease the adverse, broader-scale toxicity of chemotherapy on healthy, fast growing cells around the body. Since chemotherapy drugs can be extremely toxic to human cells, it is important that they are delivered to the tumor without being released into other parts of the body.

From the aforementioned, it will be readily apparent to those skilled in the art that a new treatment paradigm is needed in the treatment of cancers and other immunological diseases. By using novel prodrugs in conjunction with modern nanocarrier modalities, a new disease treatment can be achieved with the overall goal of more effective treatment(s), reduced side effects, and greater therapeutic utility in the treatment of cancers, especially the treatment of cancers in solid tumors.

Given the current deficiencies associated with cancer treatment, it is an object of the present invention to provide new and improved methods of treating cancer(s), immunological disorders, and other diseases utilizing prodrugs encapsulated within a nanocarrier.

SUMMARY OF THE INVENTION

The invention provides for IDO inhibitor prodrug ("IDO Prodrug") compositions comprising an IDO inhibitor agent, a lipid, and a biologically cleavable linker. In certain embodiments, nanocarriers comprising IDO Prodrug are formulated for use as a delivery modality to treat human diseases such as cancer, including solid tumor cancers as well as other immunological disorders. In certain embodiments, the nanocarriers comprise a lipid-bilayer capable of being incorporated into a drug delivery vehicle (i.e. a liposome). In a further preferred embodiment, the liposome comprises cholesterol hemisuccinate ("CHEMS"). In a further preferred embodiment, the liposome of the invention comprises Stearic Acid.

In a further embodiment, the invention comprises methods of delivering an IDO inhibitor to a tumor comprising (i) synthesizing an IDO prodrug; (ii) formulating an IDO prodrug of the invention in a nanocarrier of the invention; and (iii) administering the nanocarrier to a patient.

In another embodiment, the invention comprises methods of delivering an IDO inhibitor with one or more additional immune modulating agent to a tumor comprising (i) synthesizing an IDO prodrug; (ii) co-formulating an IDO prodrug of the invention in a nanocarrier with one or more additional immune modulating agents of the invention; and (iii) administering the nanocarrier to a patient.

In another embodiment, the immune modulating agents comprise immunogenic-cell death inducing chemotherapeutics, PD-1 agonists, toll receptor agonists, STING agonists, CTLA4 inhibitors, and/or prodrugs thereof.

In another embodiment, the present disclosure teaches methods of synthesizing IDO prodrugs.

In another embodiment, the present disclosure teaches methods of formulating IDO prodrugs within nanocarriers, including but not limited to liposomes.

In another embodiment, the present disclosure teaches methods of treating cancer(s), immunological disorders and other diseases in humans using nanocarriers of the present disclosure.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. General Chemical Synthesis for Protected **ID3** Comprising Cholesterol Hemisuccinate ("CHEMS").

Figure 2. Chemical Synthesis for **ID3** Prodrug Intermediate.

Figure 3. Chemical Synthesis for **ID3** Prodrug Intermediate.

Figure 4. IDO Inhibitor Prodrug Synthesis Schema with Carboxylic Acid Functionality.

Figure 5. IDO Inhibitor Prodrug Synthesis Schema with Alcohol Functionality.

Figure 6. IDO Inhibitor Prodrug Synthesis Schema with Secondary Amine, Amide, or Aniline Functionality.

Figure 7. Chemical Synthesis for **ID3** Prodrug Comprising Stearic Acid.

Figure 8. Chemical Synthesis for **ID3** Prodrug Comprising Cholesterol Hemisuccinate ("CHEMS").

Figure 9. Characterization of LNP-ID3 Liposome.

Figure 10. Characterization of LNP-ID3 Liposome (Zeta Potential).

Figure 11. Characterization of LNP-AR5-ID3 Liposome.

Figure 12. Characterization of LNP-AR5-ID3 Liposome (Zeta Potential).

Figure 13. Characterization of LNP-AR5-TR5-ID3 Liposome.

Figure 14. Characterization of LNP-AR5-TR5-ID3 Liposome (Zeta Potential).

Figure 15. Characterization of LNP-ID3-NK1 Liposome.

Figure 16. Characterization of LNP-ID3-NK1 Liposome (Zeta Potential).

Figure 17. Characterization of LNP-ID3-NK1-MTO Liposome.

Figure 18. Characterization of LNP-ID3-NK1-MTO Liposome (Zeta Potential).

Figure 19. Tumor inhibition of LNP-ID3 in Combination with Other Liposome(s) Using B16F10 Cells *In Vivo*.

Figure 20. Tumor inhibition of LNP-ID3 in Combination with LNP-AR5 Using B16F10 Cells *In Vivo*.

Figure 21. Tumor inhibition of LNP-ID3-NK1 Using B16F10 Cells *In Vivo*.

Figure 22. Tumor inhibition of LNP-ID3 and LNP-ID3-NK1 Using CT26 Cells *In Vivo*.

Figure 23. Tumor inhibition of LNP-ID3 Using CT26 Cells *In Vivo*.

Figure 24. Tumor inhibition of LNP-ID3 Using MC-38 Cells *In Vivo*.

Figure 25. Tumor inhibition of LNP-ID3 in Combination with LNP-AR5 Using H22 Cells *In Vivo*

Figure 26. Measurement of IDO-1 Activity.

Figure 27. Characterization of SLNP-ID3-AR5-TR5.

Figure 28. Characterization of SLNP-ID3-AR5-TR5 (Zeta Potential).

DETAILED DESCRIPTION OF THE INVENTION

Outline of Sections

I.) Definitions

II.) Prodrugs

III.) Chemical Compounds

IV.) Lipids

V.) Linkage Unit(s) ("LU")

VI.) Nanocarriers

VII.) Liposomes

VIII.) Pharmaceutical Formulation

IX.) Combination Therapy

X.) Methods of Delivering Liposomes Comprising Prodrugs to a Cell

XI.) Methods of Treating Cancer(s) and Other Immunological Disorder(s)

XII.) KITS/Articles of Manufacture

I.) Definitions:

Unless otherwise defined, all terms of art, notations and other scientific terms or terminology used herein are intended to have the meanings commonly understood by those of skill in the art to

which this invention pertains unless the context clearly indicates otherwise. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference, and the inclusion of such definitions herein should not necessarily be construed to represent a substantial difference over what is generally understood in the art.

When a trade name is used herein, reference to the trade name also refers to the product formulation, the generic drug, and the active pharmaceutical ingredient(s) of the trade name product, unless otherwise indicated by context.

As used herein, the term "about", when referring to a value or to an amount of size (i.e., diameter), weight, concentration or percentage is meant to encompass variations of in one example ±20% or ±10%, in another example ±5%, in another example ±1 %, and in still another example ±0.1 % from the specified amount, as such variations are appropriate to perform the disclosed methods.

As used herein, the term "and/or" when used in the context of a listing of entities, refers to the entities being present singly or in combination. Thus, for example, the phrase "A, B, C, and/or D" includes A, B, C, and D individually, but also includes any and all combinations and sub combinations of A, B, C, and D.

Numerical ranges recited herein by endpoints include all numbers and fractions subsumed within that range (e.g. 1 to 5 includes, but is not limited to, 1, 1 .5, 2, 2.75, 3, 3.90, 4, and 5).

As used herein, the phrase "consisting essentially of" limits the scope of a claim to the specified materials or steps, plus those that do not materially affect the basic and novel characteristic(s) of the claimed subject matter.

The terms “advanced cancer”, “locally advanced cancer”, “advanced disease” and “locally advanced disease” mean cancers that have extended through the relevant tissue capsule, and are meant to include stage C disease under the American Urological Association (AUA) system, stage C1-C2 disease under the Whitmore-Jewett system, and stage T3-T4 and N+ disease under the TNM (tumor, node, metastasis) system. In general, surgery is not recommended for patients with locally advanced disease and these patients have substantially less favorable outcomes compared to patients having clinically localized (organ-confined) cancer.

As used herein the term "alkyl" can refer to C_1-C_{20} inclusive, linear (i.e. , "straight-chain"), branched, or cyclic, saturated or at least partially and in some cases unsaturated (i.e. , alkenyl and alkynyl) hydrocarbon chains, including for example, methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl, octyl, ethenyl, propenyl, butenyl, pentenyl, hexenyl, octenyl, butadienyl, propynyl, butynyl, pentynyl, hexynyl, heptynyl, and allenyl groups. "Branched" refers to an alkyl group in which a lower alkyl group, such as methyl, ethyl, or propyl, is attached to a linear alkyl chain. "Lower alkyl" refers to an alkyl group having 1 to about 8 carbon atoms (i.e., a C_1-C_8 alkyl), e.g., 1, 2, 3, 4, 5, 6, 7, or 8 carbon atoms. "Higher alkyl" refers to an alkyl group having about 10 to about 20 carbon atoms, e.g.,

10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. In certain embodiments, "alkyl" refers, in particular, to C_1-C_8 straight-chain alkyls. In other embodiments, "alkyl" refers, in particular, to Ci-$_8$branched-chain alkyls.

Alkyl groups can optionally be substituted (a "substituted alkyl") with one or more alkyl group substituents, which can be the same or different. The term "alkyl group substituent" includes but is not limited to alkyl, substituted alkyl, halo, arylamino, acyl, hydroxyl, aryloxyl, alkoxyl, alkylthio, arylthio, aralkyloxyl, aralkylthio, carboxyl, alkoxycarbonyl, oxo, and cycloalkyl. In some embodiments, there can be optionally inserted along the alkyl chain one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms, wherein the nitrogen substituent is hydrogen, lower alkyl (also referred to herein as "alkylaminoalkyl"), or aryl.

Thus, as used herein, the term "substituted alkyl" includes alkyl groups, as defined herein, in which one or more atoms or functional groups of the alkyl group are replaced with another atom or functional group, including for example, alkyl, substituted alkyl, halogen, aryl, substituted aryl, alkoxyl, hydroxyl, nitro, amino, alkylamino, dialkylamino, sulfate, and mercapto.

The term "aryl" is used herein to refer to an aromatic substituent that can be a single aromatic ring, or multiple aromatic rings that are fused together, linked covalently, or linked to a common group, such as, but not limited to, a methylene or ethylene moiety. The common linking group also can be a carbonyl, as in benzophenone, or oxygen, as in diphenylether, or nitrogen, as in diphenylamine. The term "aryl" specifically encompasses heterocyclic aromatic compounds. The aromatic ring(s) can comprise phenyl, naphthyl, biphenyl, diphenylether, diphenylamine and benzophenone, among others. In particular embodiments, the term "aryl" means a cyclic aromatic comprising about 5 to about 10 carbon atoms, e.g., 5, 6, 7, 8, 9, or 10 carbon atoms, and including 5- and 6-membered aromatic and heteroaromatic rings. The aryl group can be optionally substituted (a "substituted aryl") with one or more aryl group substituents, which can be the same or different, wherein "aryl group substituent" includes alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, hydroxyl, alkoxyl, aryloxyl, aralkyloxyl, carboxyl, acyl, halo, nitro, alkoxycarbonyl, aryloxycarbonyl, aralkoxycarbonyl, acyloxyl, acylamino, aroylamino, carbamoyl, alkylcarbamoyl, dialkylcarbamoyl, arylthio, alkylthio, alkylene, and -NR'R", wherein R' and R" can each be independently hydrogen, alkyl, substituted alkyl, aryl, substituted aryl, and aralkyl. Specific examples of aryl groups include, but are not limited to, cyclopentadienyl, phenyl, furan, thiophene, pyrrole, pyran, pyridine, imidazole, benzimidazole, isothiazole, isoxazole, pyrazole, pyrazine, triazine, pyrimidine, quinoline, isoquinoline, indole, carbazole, and the like.

"Heteroaryl" as used herein refers to an aryl group that contains one or more non-carbon atoms (e.g., O, N, S, Se, etc.) in the backbone of a ring structure. Nitrogen-containing heteroaryl moieties include, but are not limited to, pyridine, imidazole, benzimidazole, pyrazole, pyrazine, triazine, pyrimidine, and the like.

The terms "anticancer drug", "chemotherapeutic", and "anticancer prodrug" refer to drugs (i.e., chemical compounds) or prodrugs known to, or suspected of being able to treat a cancer (i.e., to kill cancer cells, prohibit proliferation of cancer cells, or treat a symptom related to cancer). In some embodiments, the term "chemotherapeutic" as used herein refers to a non-PS molecule that is used to treat cancer and/or that has cytotoxic ability. More traditional or conventional chemotherapeutic agents can be described by mechanism of action or by chemical compound class, and can include, but are not limited to, alkylating agents (e.g., melphalan), anthracyclines (e.g., doxorubicin), cytoskeletal disruptors (e.g., paclitaxel), epothilones, histone deacetylase inhibitors (e.g., vorinostat), inhibitors of topoisomerase I or II (e.g., irinotecan or etoposide), kinase inhibitors (e.g., bortezomib), nucleotide analogs or precursors thereof (e.g., methotrexate), peptide antibiotics (e.g., bleomycin), platinum based agents (e.g., cisplatin or oxaliplatin), retinoids (e.g., tretinoin), and vinka alkaloids (e.g., vinblastine).

"Aralkyl" refers to an -alkyl-aryl group, optionally wherein the alkyl and/or aryl moiety is substituted.

"Alkylene" refers to a straight or branched bivalent aliphatic hydrocarbon group having from 1 to about 20 carbon atoms, e.g., 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, or 20 carbon atoms. The alkylene group can be straight, branched, or cyclic. The alkylene group also can be optionally unsaturated and/or substituted with one or more "alkyl group substituents." There can be optionally inserted along the alkylene group one or more oxygen, sulfur or substituted or unsubstituted nitrogen atoms (also referred to herein as "alkylaminoalkyi"), wherein the nitrogen substituent is alkyl as previously described. Exemplary alkylene groups include methylene (-CH_2-); ethylene (-CH_2-CH_2-); propylene (-(CH_2)3-); cyclohexylene (-C_6H_{10}-); -CH=CH— CH=CH-; -CH=CH-CH_2-; -$(CH_2)_q$- N(R)-(CH_2),-, wherein each of q is an integer from 0 to about 20, e.g., 0, 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, or 20, and R is hydrogen or lower alkyl; methylenedioxyl (-0-CH_2-0-); and ethylenedioxyl (-0-$(CH_2)_2$-0-). An alkylene group can have about 2 to about 3 carbon atoms and can further have 6-20 carbons.

The term "arylene" refers to a bivalent aromatic group, e.g., a bivalent phenyl or napthyl group. The arylene group can optionally be substituted with one or more aryl group substituents and/or include one or more heteroatoms.

The term "amino" refers to the group -$N(R)_2$ wherein each R is independently H, alkyl, substituted alkyl, aryl, substituted aryl, aralkyl, or substituted aralkyl. The terms "aminoalkyl" and "alkylamino" can refer to the group -$N(R)_2$ wherein each R is H, alkyl or substituted alkyl, and wherein at least one R is alkyl or substituted alkyl. "Arylamine" and "aminoaryl" refer to the group -$N(R)_2$ wherein each R is H, aryl, or substituted aryl, and wherein at least one R is aryl or substituted aryl, e.g., aniline (i.e., -NHC_6H_5).

A "bioreactive nanomaterial" refers to an engineered biomaterial that induces or catalyzes a biological response. In certain embodiments the nanomaterial induces a response by virtue of one or more properties selected from the group consisting of composition, size, shape, aspect ratio, dissolution, electronic, redox, surface display, surface coating, hydrophobic, hydrophilic, an atomically thin nanosheet, or functionalized surface groups" to catalyze the biological response at various nano/bio interfaces. In certain embodiments the bioreactive nanomaterial has the ability to inhibit IDO-1 biological responses in cells (e.g., in tumor cells) and/or as well as activating the innate immune system through delivery of "danger signal" and adjuvant effects.

"Bulk" (a.k.a. Drug Substance) means the drug substance or the drug product which has not been filled into final containers for distribution. Final formulated bulk generally refers to drug product which is formulated and being stored or held prior to filling. Drug substance may be stored or held as "bulk" or "concentrated bulk" prior to formulation into drug product.

The terms "carboxylate" and "carboxylic acid" can refer to the groups $-C(=O)O^-$ and $-C(=O)OH$, respectively. The term "carboxyl" can also refer to the $-C(=O)OH$ group.

The terms "conjugate" and "conjugated" as used herein can refer to the attachment (e.g., the covalent attachment) of two or more components (e.g., chemical compounds, polymers, biomolecule, particles, etc.) to one another. In some embodiments, a conjugate can comprise monovalent moieties derived from two different chemical compounds covalently linked via a bivalent linker moiety (e.g., an optionally substituted alkylene or arylene). In some embodiments, the linker can contain one or more biodegradable bond, such that one or more bonds in the linker can be broken when the prodrug is exposed to a particular physiological environment or enzyme (for example, esterases).

The term "compound" refers to and encompasses the chemical compound (e.g. a prodrug) itself as well as, whether explicitly stated or not, and unless the context makes clear that the following are to be excluded: amorphous and crystalline forms of the compound, including polymorphic forms, where these forms may be part of a mixture or in isolation; free acid and free base forms of the compound, which are typically the forms shown in the structures provided herein; isomers of the compound, which refers to optical isomers, and tautomeric isomers, where optical isomers include enantiomers and diastereomers, chiral isomers and non-chiral isomers, and the optical isomers include isolated optical isomers as well as mixtures of optical isomers including racemic and non-racemic mixtures; where an isomer may be in isolated form or in a mixture with one or more other isomers; isotopes of the compound, including deuterium- and tritium-containing compounds, and including compounds containing radioisotopes, including therapeutically- and diagnostically-effective radioisotopes; multimeric forms of the compound, including dimeric, trimeric, etc. forms; salts of the compound, preferably pharmaceutically acceptable salts, including acid addition salts and base addition salts, including salts having organic counterions and inorganic counterions, and including zwitterionic forms, where if a

compound is associated with two or more counterions, the two or more counterions may be the same or different; and solvates of the compound, including hemisolvates, monosolvates, disolvates, etc., including organic solvates and inorganic solvates, said inorganic solvates including hydrates; where if a compound is associated with two or more solvent molecules, the two or more solvent molecules may be the same or different. In some instances, reference made herein to a compound of the invention will include an explicit reference to one or of the above forms, e.g., salts and/or solvates; however, this reference is for emphasis only, and is not to be construed as excluding other of the above forms as identified above.

"Drug product" means a final formulation that contains an active drug ingredient (i.e. liposomes containing IDO inhibitor prodrugs) generally, but not necessarily, in association with inactive ingredients. The term also includes a finished dosage form that does not contain an active ingredient but is intended to be used as a placebo.

The term "disulfide" can refer to the -S-S- group.

The term "empty vesicle" means an unloaded lipid vesicle by itself.

The term "ester" as used herein means a chemical compound derived from acid (organic or inorganic) in which at least one -OH hydroxyl group is replaced by an -O-alkyl (alkoxy) or O-Aryl (aryloxy) group.

The term "esterase" as used herein is a hydrolase enzyme that splits esters into an acid and an alcohol.

"Excipient" means an inactive substance used as a carrier for the active ingredients in a drug such as vaccines. Excipients are also sometimes used to bulk up formulations with very potent active ingredients, to allow for convenient and accurate dosage. Examples of excipients include but are not limited to, antiadherents, binders, coatings, disintegrants, fillers, dilutents, flavors, colors, lubricants, and preservatives.

The terms "halo", "halide", or "halogen" as used herein refer to fluoro, chloro, bromo, and iodo groups.

The terms "hydroxyl" and "hydroxy" refer to the -OH group.

The terms "inhibit" or "inhibition of" as used herein means to reduce by a measurable amount, or to prevent entirely.

The terms "individual" or "patient," as used in the context of this disclosure can be used interchangeably.

As used herein, the term "ligand" refers generally to a species, such as a molecule or ion, which interacts, e.g., binds, in some way with another species. *See* Martell, A. E., and Hancock, R. P., Metal Complexes in Aqueous Solutions, Plenum: New York (1996), which is incorporated herein by reference in its entirety.

The term “lipid” as used herein refers to a class of naturally occurring (organic) compounds that are insoluble in polar solvents. In the context of the disclosure, a lipid refers to conventional lipids, phospholipids, cholesterol, chemically functionalized lipids for attachment of PEG and ligands, etc.

The term “lipid bilayer” or "LB" refers to any double layer of oriented amphipathic lipid molecules in which the hydrocarbon tails face inward to form a continuous non-polar phase.

The term(s) "liposome" or "lipid vesicle" or "vesicle" are used interchangeably to refer to an aqueous compartment enclosed by a lipid bilayer, as being conventionally defined (*see*, Stryer (1981) Biochemistry, 2d Edition, W. H. Freeman & Co., p. 213).

The term “mammal" refers to any organism classified as a mammal, including mice, rats, rabbits, dogs, cats, cows, horses, and humans. In one embodiment of the invention, the mammal is a mouse. In another embodiment of the invention, the mammal is a human.

The terms "mercapto" or "thiol" refer to the -SH group.

The terms “metastatic cancer” and “metastatic disease” mean cancers that have spread to regional lymph nodes or to distant sites and are meant to include stage D disease under the AUA system and stage T×N×M+ under the TNM system.

The terms "nanocarrier”, “nanoparticle, and "nanoparticle drug carrier" are used interchangeably and refer to a nanostructure having an aqueous, solid, or polymeric interior core. In certain embodiments the nanocarrier comprises a lipid bilayer encasing (or surrounding or enveloping) the porous particle core. In certain embodiments the nanocarrier is a liposome, lipid nanoparticle (“LNP”) or a solid-lipid nanoparticle (“SLNP”).

The terms "nanoscale particle," "nanomaterial," “nanocarrier”, and "nanoparticle" refer to a structure having at least one region with a dimension (e.g., length, width, diameter, etc.) of less than about 1,000 nm. In some embodiments, the dimension is smaller (e.g., less than about 500 nm, less than about 250 nm, less than about 200 nm, less than about 150 nm, less than about 125 nm, less than about 100 nm, less than about 80 nm, less than about 70 nm, less than about 60 nm, less than about 50 nm, less than about 40 nm, less than about 30 nm or even less than about 20 nm). In some embodiments, the dimension is between about 20 nm and about 250 nm (e.g., about 20, 30, 40, 50, 60, 70, 80, 90, 100, 1 10, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, or 250 nm).

The term "nanovesicle" refers to a "lipid vesicle" having a diameter (or population of vesicles having a mean diameter) ranging from about 20 nm, or from about 30 nm, or from about 40 nm, or from about 50 nm up to about 500 nm, or up to about 400 nm, or up to about 300 nm, or up to about 200 nm, or up to about 150 nm, or up to about 100 nm, or up to about 80 nm. In certain embodiments a nanovesicle has a diameter ranging from about 40 nm up to about 80 nm, or from about 50 nm up to about 70 nm.

"Pharmaceutically acceptable" refers to a non-toxic, inert, and/or composition that is physiologically compatible with humans or other mammals.

"Pharmaceutical formulation" means the process in which different chemical substances are combined to a pure drug substance to produce a final drug product.

The term "phosphonate" refers to the $-P(=O)(OR)_2$ group, wherein each R can be independently H, alkyl, aralkyl, aryl, or a negative charge (i.e., wherein effectively there is no R group present to bond to the oxygen atom, resulting in the presence of an unshared pair of electrons on the oxygen atom). Thus, stated another way, each R can be present or absent, and when present is selected from H, alkyl, aralkyl, or aryl.

The term "phosphate" refers to the $-OP(=O)(OR')_2$ group, where R' is H or a negative charge.

The term "prodrug" means a medication or compound that, after administration, is metabolized into a pharmacologically active drug. For the purposes of this disclosure, a prodrug of the invention comprises three (3) components: (i) a drug moiety; (ii) a lipid moiety; and (iii) a linkage unit ("LU").

The term "IDO prodrug" means a prodrug of the inventions wherein the drug moiety comprises an IDO inhibitor.

The term "pyrolipid" refers to a conjugate of a lipid and a porphyrin, porphyrin derivative, or porphyrin analog. In some embodiments, the pyrolipid can comprise a lipid conjugate wherein a porphyrin or a derivative or analog thereof is covalently attached to a lipid side chain. See, for example U.S. Patent Application Publication No. 2014/0127763.

As used herein, the terms "specific", "specifically binds" and "binds specifically" refer to the selective binding of nanocarrier of the invention to the target IDO-1.

The term "supported lipid bilayer" means a lipid bilayer enclosing a porous particle core. This definition as set forth in the disclosure is denoted because the lipid bilayer is located on the surface and supported by a porous particle core. In certain embodiments, the lipid bilayer can have a thickness ranging from about 6 nm to about 7 nm which includes a 3-4 nm thickness of the hydrophobic core, plus the hydrated hydrophilic head group layers (each about 0.9 nm) plus two partially hydrated regions of about 0.3 nm each. In various embodiments, the lipid bilayer surrounding the liposome comprises a continuous bilayer or substantially continuous bilayer that effectively envelops and seals the IDO inhibitor.

The term "thioalkyl" can refer to the group -SR, wherein R is selected from H, alkyl, substituted alkyl, aralkyl, substituted aralkyl, aryl, and substituted aryl. Similarly, the terms "thioaralkyl" and "thioaryl" refer to -SR groups wherein R is aralkyl and aryl, respectively.

As used herein "to treat" or "therapeutic" and grammatically related terms, refer to any improvement of any consequence of disease, such as prolonged survival, less morbidity, and/or a lessening of side effects which are the byproducts of an alternative therapeutic modality; as is readily

appreciated in the art, full eradication of disease is a preferred but albeit not a requirement for a treatment act.

The term "therapeutically effective amount" refers to the amount of active prodrug, nano-encapsulated prodrug, or pharmaceutical agent that elicits the biological or medicinal response in a tissue, system, animal, individual or human.

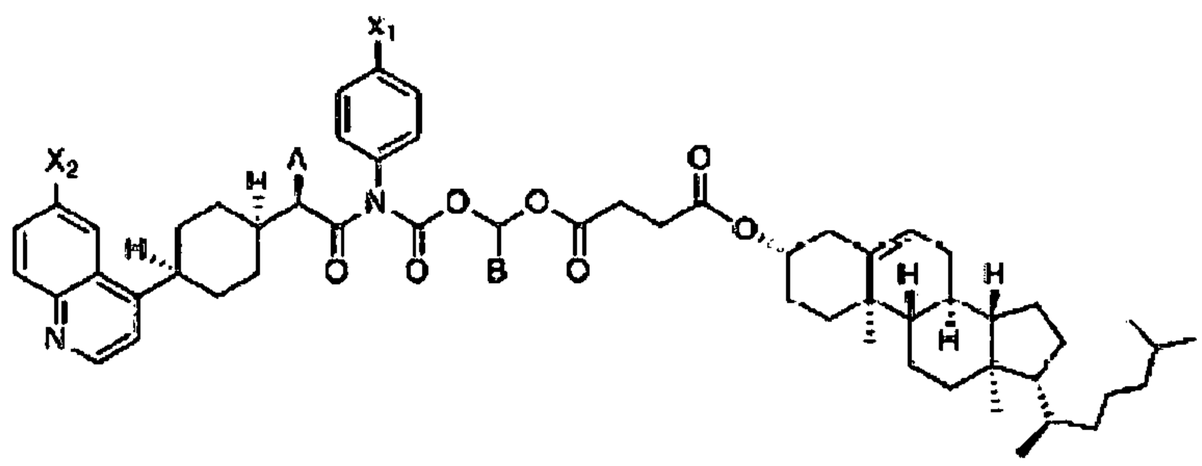
The term "unsupported lipid bilayer" means an uncoated lipid bilayer in a lipid vesicle or liposome.

II.) Prodrugs

As shown in the present disclosure and for the purposes of this invention, a suitable prodrug is formed by conjugating a drug moiety of the invention (*See*, section entitled Drug Moieties) to a lipid moiety of the invention (*See*, section entitled Lipids) via an LU (*See*, section entitled Linkage Units) of the present disclosure. For the purposes of this disclosure, formation of an IDO prodrug can utilize several strategies. (*See*, for example, **Figure 4**, **Figure 5**, and **Figure 6**).

Accordingly, in some embodiments, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the disclosure.

In one embodiment, the prodrug comprises the following chemical structure denoted Formula I:



FORMULA I

Wherein, in exemplary embodiments of FORMULA I:

X_1 = Cl, F, CN;

X_2 = H, F; and

A, B = H, CH_3;

Thus, in one embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of FORMULA I.

In one embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor set forth in **Figure 4**.

In one embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor set forth in **Figure 5**.

In one embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor set forth in **Figure 6**.

In a further embodiment, the IDO prodrug is a drug-lipid moiety comprising a lipid of the disclosure.

In a further embodiment, the IDO prodrug is a drug-lipid moiety whereby the lipid is CHEMS.

In a further embodiment, the IDO prodrug is a drug-lipid moiety comprising a LU of the disclosure.

In a further embodiment, the IDO prodrug is a drug-lipid moiety whereby the LU is a hydromethylcarbamate linker.

In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the invention, wherein the IDO inhibitor comprises the chemical composition(s) **ID3**.

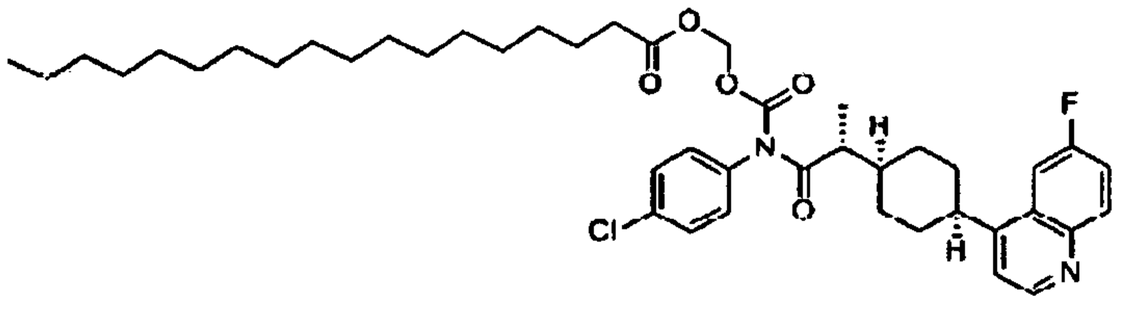
In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the invention, wherein the IDO inhibitor comprises **ID3** and further comprises CHEMS.

In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the invention, wherein the IDO inhibitor comprises **ID3** and further comprises Stearic Acid.

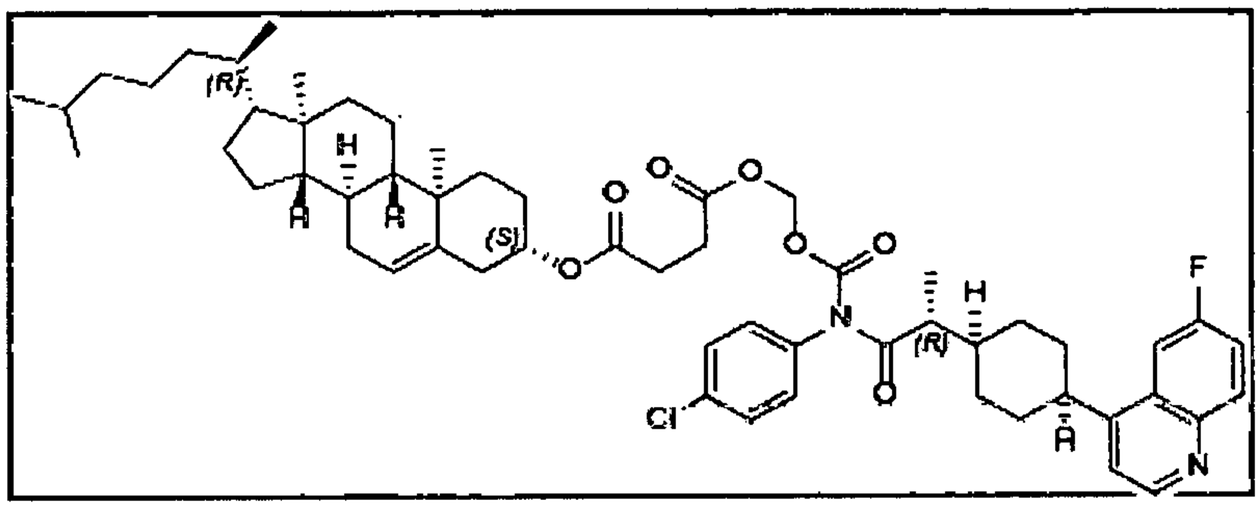
In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the invention, wherein the IDO inhibitor comprises **ID3** and further comprises CHEMS and whereby the LU is a hydromethylcarbamate linker.

In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the invention, wherein the IDO inhibitor comprises **ID3** and further comprises Stearic Acid and whereby the LU is a hydromethylcarbamate linker.

In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the inventions, wherein the IDO inhibitor comprises ID3 and further comprises Stearic Acid having the following structure:



In a further embodiment, the prodrug is a drug-lipid moiety comprising an IDO inhibitor of the inventions, wherein the IDO inhibitor comprises ID3 and further comprises cholesterol hemisuccinate having the following structure:



In additional embodiments of the disclosure the subject matter provides an IDO inhibitor prodrug comprising a lipid-conjugated therapeutic agent parent drug. In some embodiments, the prodrug comprises: (a) a monovalent drug moiety, (b) a monovalent lipid moiety, and (c) a bivalent linker moiety comprising a linkage unit that will degrade *in vivo*, such as a disulfide bond, wherein the monovalent drug moiety and the monovalent lipid moiety are linked (e.g., covalently linked) through the linker. The monovalent drug moiety and the monovalent lipid moieties can be monovalent derivatives of a chemical compound and a lipid, respectively. For instance, the monovalent derivative can be a deprotonated derivative of a chemical compound or lipid that comprises a hydroxyl, thiol, amino, or carboxylic acid group.

In further embodiments of the disclosure the subject matter provides an IDO inhibitor prodrug comprising a lipid-conjugated therapeutic agent parent drug. In some embodiments, the prodrug comprises: (a) a bivalent drug moiety, (b) a bivalent lipid moiety, and (c) a bivalent linker moiety comprising a linkage that will degrade *in vivo*, wherein the bivalent drug moiety and the bivalent lipid moiety are linked (e.g., covalently linked) through the linker. The bivalent drug moiety and the bivalent lipid moieties can be bivalent derivatives of a chemical compound and a lipid, respectively. For instance, the bivalent derivative can be a deprotonated derivative of a chemical compound or lipid that comprises a hydroxyl, thiol, amino, or carboxylic acid group.

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

III.) Drug Moieties

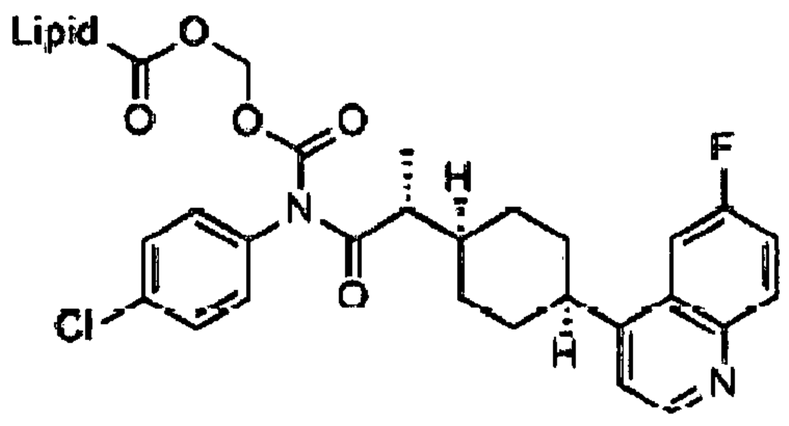
Another aspect of the invention provides for novel IDO prodrug compound(s) with the following formula(s) denoted **ID3**.

One of skill in the art will appreciate that a compound is useful as an IDO inhibitor (e.g. inhibits IDO-1). By way of brief background, IDO-1 has been implicated in immune modulation through its ability to limit T-cell function and engage mechanisms of immune tolerance. *See*, MUNN, et. al., Trends in Immuology, 34(3) pp. 137-143 (2012). Emerging evidence suggests that IDO becomes activated

during tumor development, helping malignant cells escape eradication by the immune system. In mice, IDO has a normal immune checkpoint function in immune tolerance in pregnancy, suppressing the mother's immune system. *See*, YU, et. al., Cellular Physiology and Biochemistry, 49(1): pp. 134-143 (2018). Research has shown that overexpression of IDO in some tumors, such as ovarian, colorectal, and endometrial, and esophageal cancer, correlated with swifter death, while in kidney and liver cancers it appeared to correlate with better outcomes. *Id.*

Based on the foregoing, the present disclosure describes a class of IDO inhibitors.

In one embodiment, a drug moiety of the disclosure comprises a compound with the following chemical structure (denoted **ID3**):



ID3

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

IV.) Lipids

Generally speaking, and for the purposes of this disclosure, the term "lipid" is used in its broadest sense and comprises several sub-categories of lipids, including but not limited to, phospholipids / fatty acids. As it is appreciated by one of skill in the art, a phospholipid represents a class of lipids that are a major component of all cell membranes. Phospholipids can form lipid bilayers because of their amphiphilic characteristic. The structure of the phospholipid molecule generally consists of two hydrophobic fatty acid "tails" and a hydrophilic "head" consisting of a phosphate group that can be modified with simple organic molecules such as choline, ethanolamine, or serine. These two components are usually joined together by a glycerol molecule. A representative list of phospholipids / fatty acid(s) of the invention are set forth in **Table III**.

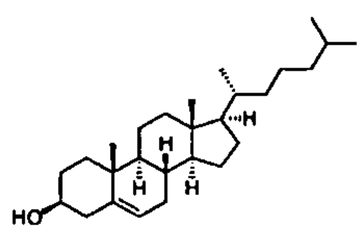
By way of brief background, at the most fundamental level, the properties of a liposome depend upon the subtle physicochemical interactions among the various lipid species in its composition. Individual lipids can be combined to form a myriad of superstructures including bilayers, and bilayer properties can be tuned to modulate drug release and membrane stability. In a simplified bilayer model

acyl chain length dictates bilayer thickness and phase transition temperature (Tm), acyl chain saturation controls bilayer fluidity, and headgroup interactions impact inter- and intra-lipid molecular forces. Liposome behavior can be adjusted by incorporating synthetic lipids such as lipid prodrugs, fusogenic lipids and functionalizable lipids into the bilayer. *See,* KOHLI, *et. al.*, J. Control Release, 0: pp. 274-287 (Sept. 28, 2014).

In one embodiment of the present disclosure, an IDO prodrug comprises a monovalent lipid moiety.

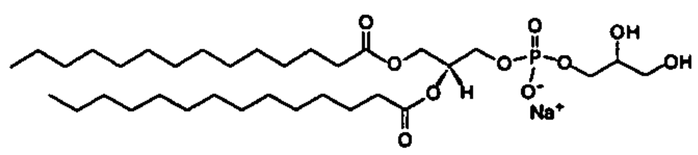
In one embodiment, an IDO prodrug comprises a bivalent lipid moiety.

In one embodiment, the lipid comprises a cholesterol with the following chemical structure:

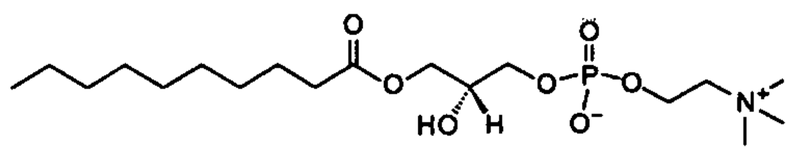


In one embodiment, the lipid comprises a DPPG with the following chemical structure:

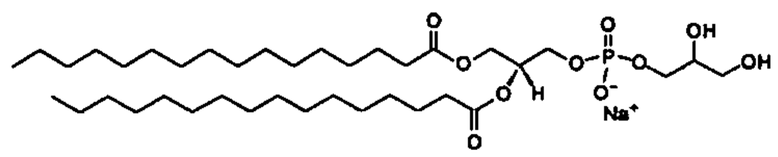
In one embodiment, the lipid comprises a DMPG with the following chemical structure:



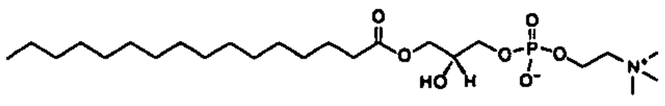
In one embodiment, the lipid comprises a Lyso PC with the following chemical structure:



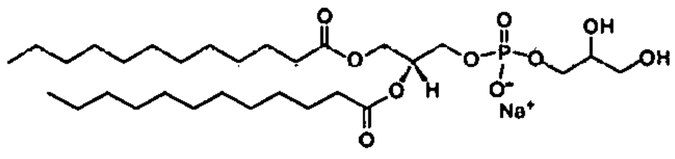
In one embodiment, the lipid comprises a (Δ9-Cis) PG with the following chemical structure:



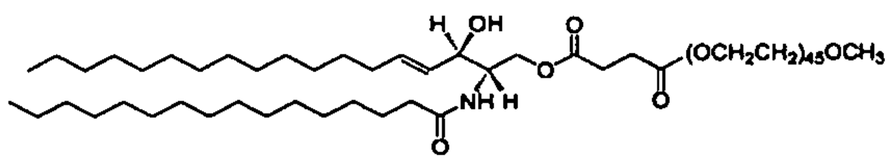
In one embodiment, the lipid comprises a Soy Lyso PC with the following chemical structure:



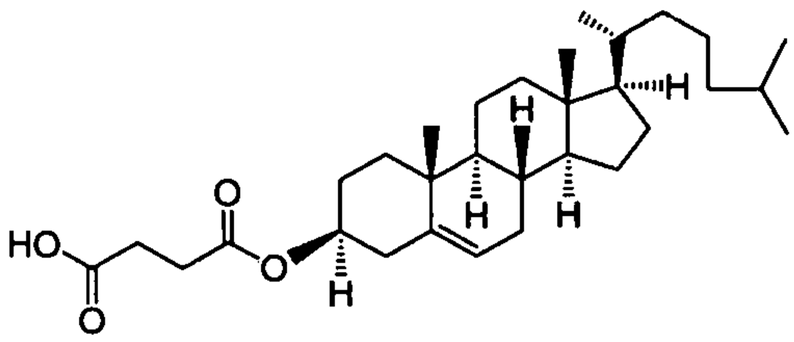
In one embodiment, the lipid comprises a PG with the following chemical structure:



In one embodiment, the lipid comprises a C16 PEG2000 Ceramde with the following chemical structure:



In one embodiment, the lipid comprises a cholesterol hemisuccinate ("CHEMS") with the following chemical structure:



In one embodiment, the lipid comprises Stearic Acid with the following chemical structure:



By way of reference, a complete list of the chemical formulas and abbreviation(s) of the lipids dlsclosed herein is set forth in **Table I**.

In an additional embodiment, the lipid comprises a phospholipid / fatty acid disclosed herein and set forth in **Table III**.

In addition, the IDO prodrugs and/or liposome(s) of the disclosure may comprise one or more helper lipids which are also referred to herein as "helper lipid components". The helper lipid components are preferably selected from the group comprising phospholipids and steroids. Phospholipids are preferably di- and monoester of the phosphoric acid. Preferred members of the phospholipids are phosphoglycerides and sphingolipids. Steroids, as used herein, are naturally

occurring and synthetic compounds based on the partially hydrogenated cyclopenta[a]phenanthrene. Preferably, the steroids contain 21 to 30 C atoms. A particularly preferred steroid is cholesterol.

It is to be noted that although not wishing to be bound by any theory, due to the particular mol percentages of the helper lipid(s) contained in the lipid compositions according to the present invention, which helper lipid can be either a PEG-free helper lipid or in particular a PEG-containing helper lipid, surprising effects can be realized, more particularly if the content of any of this kind of helper lipid is contained within the concentration range specified herein.

In a further aspect of the present invention, lipid compositions which are preferably present as lipoplexes or liposomes, preferably show a neutral or overall anionic charge. The anionic lipid is preferably any neutral or anionic lipid described herein. The lipid composition comprises in a preferred embodiment any helper lipid or helper lipid combination as well as any IDO inhibitor as described herein. In a further embodiment the composition according to the present invention containing nucleic acid(s) forms lipoplexes. In a preferred embodiment the term lipoplexes as used herein refers to a composition composed of neutral or anionic lipid, neutral helper lipid and IDO inhibitor of the invention. For reference into the usage of helper lipids in the art, see, by way of example, U.S. Patent Application Publication 2011/0178164; OJEDA, *et. al.*, Int. J. of Pharmaceutics (March 2016); DABKOWSKA, *et. al.*, J. R. Soc. Interface 9, pp. 548-561 (2012); and MOCHIZUKI, *et. al.*, Biochimica et. Biophysica Acta, 1828, pp. 412-418 (2013).

In a preferred embodiment, the helper lipids of the invention comprise the helper lipids set forth in **Table II**.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is CHEMS and wherein the drug moiety is **ID3**.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is CHEMS and wherein the drug moiety is **ID3**, further comprising a LU and wherein the LU is a hydromethylcarbamate linker.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is CHEMS and wherein the drug moiety is **ID3**, further comprising a LU and wherein the LU is a hydromethylcarbamate linker, further comprising a helper lipid component, wherein the helper lipid component comprises a helper lipid of **Table II**.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is CHEMS and wherein the drug moiety is **ID3** and wherein the CHEMS is monovalent.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is Stearic Acid and wherein the drug moiety is **ID3**.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is Stearic Acid and wherein the drug moiety is **ID3** and wherein the Stearic Acid is monovalent.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is Stearic Acid and wherein the drug moiety is I**D3**, further comprising a LU and wherein the LU is a hydromethylcarbamate linker.

In one embodiment, an IDO prodrug comprises a lipid of the invention, wherein the lipid is Stearic Acid and wherein the chemical composition is **ID3**, further comprising a LU and wherein the LU is a hydromethylcarbamate linker, further comprising a helper lipid component, wherein the helper lipid component comprises a helper lipid of **Table II**.

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

V.) Linkage Unit(s) ("LU")

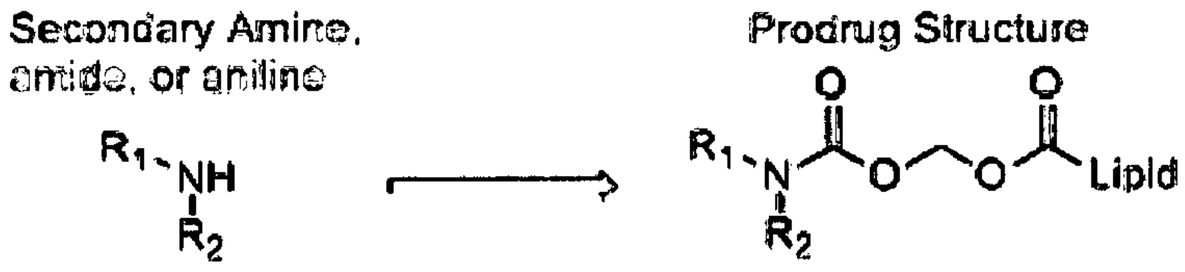
In some embodiments, the presently disclosed subject matter provides prodrugs comprising drug-lipid conjugates that include biodegradable linkages, such as esters, thioesters, and other linkers known in the art.

Exemplary embodiments of ester chemistry are set forth herein:

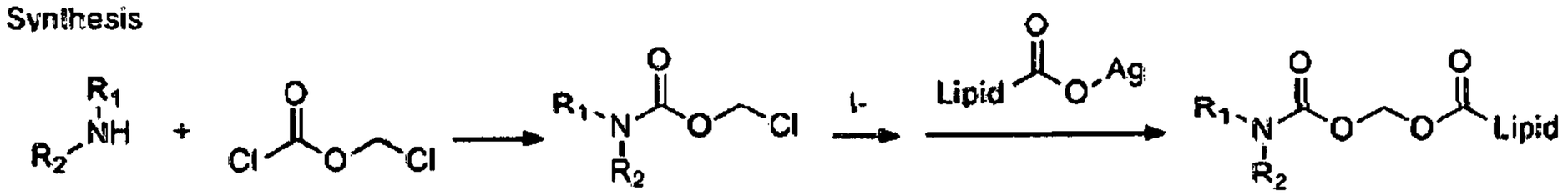


In some embodiments, the prodrug is a drug-lipid conjugate, whereby the drug-lipid conjugate is cleaved by an esterase.

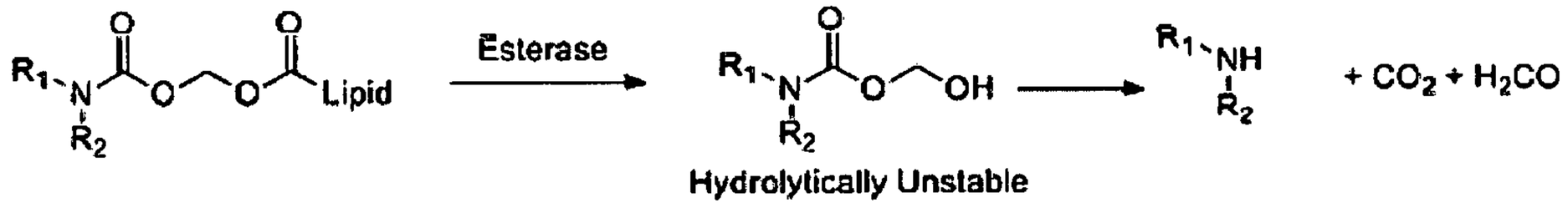
In one embodiment, a prodrug of the invention comprises a LU via a secondary amine, amide, or aniline using the following schema:



An exemplary synthesis is as follows:



Cleavage of the prodrug structure comprising a secondary amine, amide, or aniline is obtained via esterase hydrolysis of the secondary amine, amide, or aniline prodrug under the following exemplary synthesis:



Wherein:

R_1 and R_2 can be and molecule which connects a N via a C.

In one embodiment, the secondary amide nitrogen of the **ID3** drug moiety is conjugated to CHEMS via a hydromethylcarbamate linker.

In one embodiment, the secondary amide nitrogen of the **ID3** drug moiety is conjugated to Stearic Acid via a hydromethylcarbamate linker.

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

VI.) Nanocarrier(s)

Generally speaking, and for the purposes of this disclosure nanocarrier(s) are within the scope of the invention. A nanocarrier is nanomaterial being used as a transport module for another substance, such as a drug. Commonly used nanocarriers include micelles, polymers, carbon-based materials, liposomes, and other substances. Because of their small size, nanocarriers can deliver drugs to otherwise inaccessible sites around the body. Nanocarriers can include polymer conjugates, polymeric nanoparticles, lipid-based carriers, dendrimers, carbon nanotubes, and gold nanoparticles. Lipid-based carriers include both liposomes and micelles.

In addition, nanocarriers are useful in the drug delivery process because they can deliver drugs to site-specific targets, allowing drugs to be delivered in certain organs or cells but not in others. Site-specificity poses a major therapeutic benefit since it prevents drugs from being delivered to the wrong places. In addition. nanocarriers show promise for use in chemotherapy because they can help decrease the adverse, broader-scale toxicity of chemotherapy on healthy, fast growing cells around the body. Since chemotherapy drugs can be extremely toxic to human cells, it is important that they are delivered to the tumor without being released into other parts of the body.

Generally speaking, there are four (4) methods in which nanocarriers can deliver drugs and they include passive targeting, active targeting, pH specificity, and temperature specificity.

Passive targeting refers to a nanocarrier's ability to travel down a tumor's vascular system, become trapped, and accumulate in the tumor. This accumulation is caused by the enhanced

permeability and retention effect. The leaky vasculature of a tumor is the network of blood vessels that form in a tumor, which contain many small pores. These pores allow nanocarriers in, but also contain many bends that allow the nanocarriers to become trapped. As more nanocarriers become trapped, the drug accumulates at the tumor site. This accumulation causes large doses of the drug to be delivered directly to the tumor site.

Active targeting involves the incorporation of targeting modules such as ligands or antibodies on the surface of nanocarriers that are specific to certain types of cells around the body. Generally, nanocarriers have a high surface-area to volume ratio allowing for multiple ligands to be incorporated on their surfaces.

Additionally, certain nanocarriers will only release the drugs they contain in specific pH ranges. pH specificity also allows nanocarriers to deliver drugs directly to a tumor site. This is due to the fact that tumors are generally more acidic than normal human cells, with a pH around 6.8. Normal tissue has a pH of around 7.4. Thus, nanocarriers that only release drugs at certain pH ranges can therefore be used to release the drug only within acidic tumor environments. High acidic environments cause the drug to be released due to the acidic environment degrading the structure of the nanocarrier. Generally, these nanocarriers will not release drugs in neutral or basic environments, effectively targeting the acidic environments of tumors while leaving normal body cells untouched. This pH sensitivity can also be induced in micelle systems by adding copolymer chains to micelles that have been determined to act in a pH independent manor. *See*, WU, et. al., Biomaterials, 34(4): 1213-1222 (2012). These micelle-polymer complexes also help to prevent cancer cells from developing multi-drug resistance. The low pH environment triggers a quick release of the micelle polymers, causing a majority of the drug to be released at once, rather than gradually like other drug treatments.

Additionally, some nanocarriers have also been shown to deliver drugs more effectively at certain temperatures. Since tumor temperatures are generally higher than temperatures throughout the rest of the body, around 40 °C, this temperature gradient helps act as safeguard for tumor-specific site delivery. *See*, REZAEI, et. al., Polymer, 53(16): 3485-3497 (2012).

As disclosed herein, lipid-based nanocarriers, such as liposomes are within the scope of this invention. Lipid-based nanoparticles (LBNPs or LNPs) such as liposomes, solid lipid nanoparticles (SLN) and nanostructured lipid carriers (NLC) can transport hydrophobic and hydrophilic molecules, display very low or no toxicity, and increase the time of drug action by means of a prolonged half-life and a controlled release of the drug. Lipid nanoparticles can include chemical modifications to avoid the detection by the immune system (gangliosides or polyethylene glycol (PEG)) or to improve the solubility of the drug. In addition, they can be prepared in formulations sensitive to the pH in order to promote drug release in an acid environment and can also be associated with small molecules or antibodies that recognize tumor cells or their receptors (such as folic acid (FoA)). Nanodrugs can also be used in

combination with other therapeutic strategies to improve the response of patients. *See*, GARCIA-PINEL, *et. al.*, Nanomaterials 9(639) (2019).

In various embodiments silicasome drug carriers described herein comprise a porous silica (or other material) nanoparticle (e.g., a silica body having a surface and defining a plurality of pores that are suitable to receive molecules therein) coated with a lipid bilayer. The fact that the nanoparticle is referred to as a silica nanoparticle does not preclude materials other than silica from also being incorporated within the silica nanoparticle. In some embodiments, the silica nanoparticle may be substantially spherical with a plurality of pore openings through the surface providing access to the pores. However, in various embodiments the silica nanoparticle can have shapes other than substantially spherical shapes. Thus, for example, in certain embodiments the silica nanoparticle can be substantially ovoid, rod-shaped, a substantially regular polygon, an irregular polygon, and the like.

Generally, the silica nanoparticle comprises a silica body that defines an outer surface between the pore openings, as well as side walls within the pores. The pores can extend through the silica body to another pore opening, or a pore can extend only partially through the silica body such that that it has a bottom surface of defined by the silica body.

In some embodiments, the silica body is mesoporous. In other embodiments, the silica body is microporous. As used herein, "mesoporous" means having pores with a diameter between about 2 nm and about 50 nm, while "microporous" means having pores with a diameter smaller than about 2 nm. In general, the pores may be of any size, but in typical embodiments are large enough to contain one or more therapeutic compounds therein. In such embodiments, the pores allow small molecules, for example, therapeutic compounds such as anticancer compounds to adhere or bind to the inside surface of the pores, and to be released from the silica body when used for therapeutic purposes. In some embodiments, the pores are substantially cylindrical.

In certain embodiments the nanoparticles comprise pores having pore diameters between about 1 nm and about 10 nm in diameter or between about 2 nm and about 8 nm. In certain embodiments the nanoparticles comprise pores having pore diameters between about 1 nm and about 6 nm, or between about 2 nm and about 5 nm. Other embodiments include particles having pore diameters less than 2.5 nm.

In other embodiments, the pore diameters are between 1.5 and 2.5 nm. Silica nanoparticles having other pore sizes may be prepared, for example, by using different surfactants or swelling agents during the preparation of the silica nanoparticles. In various embodiments the nanoparticles can include particles as large (e.g., average, or median diameter (or another characteristic dimension) as about 1000 nm. However, in various embodiments the nanoparticles are typically less than 500 nm or less than about 300 nm as, in general, particles larger than 300 nm may be less effective in entering living cells or blood vessel fenestrations. In certain embodiments the nanoparticles range in size from about

40 nm, or from about 50 nm, or from about 60 nm up to about 100 nm, or up to about 90 nm, or up to about 80 nm, or up to about 70 nm. In certain embodiments the nanoparticles range in size from about 60 nm to about 70 nm. Some embodiments include nanoparticles having an average maximum dimension between about 50 nm and about 1000 nm. Other embodiments include nanoparticles having an average maximum dimension between about 50 nm and about 500 nm. Other embodiments include nanoparticles having an average maximum dimension between about 50 nm and about 200 nm.

In some embodiments, the average maximum dimension is greater than about 20nm, greater than about 30nm, greater than 40nm, or greater than about 50nm. Other embodiments include nanoparticles having an average maximum dimension less than about 500 nm, less than about 300nm, less than about 200nm, less than about 100 nm or less than about 75 nm. As used herein, the size of the nanoparticle refers to the average or median size of the primary particles, as measured by transmission electron microscopy (TEM) or similar visualization techniques known in the art. Further examples of mesoporous silica nanoparticles include, but are not limited to, MCM-41, MCM-48, and SBA-15. *See*, KATIYARE, *et. al.*, J. Chromotog. 1122(1-2): 13-20 (2006).

Methods of making porous silica nanoparticles are well known to those of skill in the art. In certain embodiments mesoporous silica nanoparticle are synthesized by reacting tetraethyl orthosilicate (TEOS) with a template made of micellar rods. The result is a collection of nano-sized spheres or rods that are filled with a regular arrangement of pores. The template can then be removed by washing with a solvent adjusted to the proper pH (*See*, e.g., TREWYN *et al.* (2007) Chem. Eng. J. 137(1): 23-29).

In certain embodiments mesoporous particles can also be synthesized using a simple sol-gel method (*See*, e.g., NANDIYANTO, *et al.* (2009) Microporous and Mesoporous Mat. 120(3): 447-453). In certain embodiments tetraethyl orthosilicate can also be used with an additional polymer monomer as a template. In certain embodiments 3-mercaptopropyl)trimethoxysilane (MPTMS) is used instead of TEOS.

In certain embodiments the mesoporous silica nanoparticles are cores are synthesized by a modification of the sol/gel procedure described by MENG *et. al.* (2015) ACS Nemo, 9(4): 3540-3557.

While the methods described herein have been demonstrated with respect to porous silica nanoparticles (e.g., mesoporous silica), it will be recognized by those skilled in the art that similar methods can be used with other porous nanoparticles. Numerous other mesoporous materials that can be used in drug delivery nanoparticles are known to those of skill in the art. For example, in certain embodiments mesoporous carbon nanoparticles could be utilized.

Mesoporous carbon nanoparticles are well known to those of skill in the art (*See*, e.g., HUANG *et. al.* (2016) Carbon, 101: 135-142; ZHU *et. al.* (2014) Asian J. Pharm. Sci., 9(2): 82-91; and the like).

Similarly, in certain embodiments, mesoporous polymeric particles can be utilized. The syntheses of highly ordered mesoporous polymers and carbon frameworks from organic-organic

assembly of triblock copolymers with soluble, low-molecular-weight phenolic resin precursors (resols) by an evaporation induced self-assembly strategy have been reported by MENG, *et. al.* (2006) Chem. Mat. 6(18): 4447-4464.

The nanoparticles described herein are illustrative and non-limiting. Using the teachings provided herein numerous other lipid bilayer coated nanoparticles will be available to one of skill in the art.

In one embodiment, the invention teaches nanocarriers which comprise IDO prodrugs.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises CHEMS.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises Stearic Acid.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises CHEMS and whereby the liposome further comprises an IDO prodrug.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises CHEMS and whereby the liposome further comprises **ID3**.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises Stearic Acid and whereby the liposome further comprises an IDO inhibitor.

In one embodiment, the invention teaches a nanocarrier comprising a liposome, wherein the lipid comprises Stearic Acid and whereby the liposome further comprises **ID3**.

The scope of the disclosure teaches three (3) possible treatment modalities using the formulated prodrugs of the invention. *See*, PCT Patent Publication No. WO2018/213631.

The first treatment modality involves combination of an IDO prodrug in combination with another therapeutic (e.g. another formulated prodrug which inhibits IDO-1, a chemotherapy agent (such as an ICD-inducing chemotherapy), etc.) into a single liposome that allows systemic (or local) biodistribution and drug delivery to tumor sites. The dual-delivery approach achieved synergistic enhancement of adaptive and innate immunity, leading to a significant improvement in animal survival. In certain embodiments the nanocarrier comprises a vesicle (i.e., a lipid bilayer enclosing a fluid).

A second treatment modality involves local delivery to a tumor or peri-tumoral region, of an agent that inhibits IDO-1 in combination with a lipid (e.g., a liposome) that comprises an inhibitor of IDO-1. It is demonstrated that such local delivery of an IDO-1 inhibitor in combination with an IDO prodrug induces cytotoxic tumor killing, and tumor shrinkage at the local site. These adaptive immune responses are accompanied by boosting of the innate immune system, as reflected by CRT expression, as well as the activation of a DC population, particularly well-suited for generating cytotoxic T cell responses.

A third treatment modality involves vaccination utilizing dying cancer cells {e.g., KPC cells) in which inhibition of IDO-1 is induced *ex vivo*. It is discovered that such vaccination can generate a

systemic immune response that can interfere with tumor growth at a remote site as well as allowing adoptive transfer to non-immune animals. One of skill in the art will appreciate and be enabled to perform methods the treatment modalities provided herein.

VII.) Liposomes

In one aspect, the presently disclosed subject matter is based on an approach for providing a prodrug of the disclosure (*See*, section entitled Prodrugs) suitable for incorporation into a nanocarrier comprising lipid coating layers to provide enhanced delivery of the corresponding prodrugs and for providing combination therapies including the prodrugs. The advantages for using prodrugs of the invention include the facilitation of controlled formulation into an LNP of the disclosure (e.g. a liposome). This allows the prodrug to be maintained in an inactive form during systemic circulation, which allows the liposome to release the active agent after engulfment by a cell, for example within a tumor.

In certain embodiments one or more IDO prodrugs (e.g., any one or more of the IDO prodrugs inhibitors taught in Formula I, and/or **ID3**) (*See*, section entitled prodrugs) are formulated a lipid moiety that forms a vesicle (e.g., a liposome) structure in aqueous solution or that can form a component of a lipid bilayer comprising a liposome. The liposomes can be used directly, provided as components in a combined formulation (e.g., in combination with another drug moiety or therapeutic modality as disclosed herein).

In certain embodiments, the liposome that is formulated with the IDO prodrug comprises a lipid, PHGP, vitamin E, cholesterol, and/or a fatty acid.

In one embodiment, the liposome comprises cholesterol.

In one embodiment, the liposome comprises DPPG.

In one embodiment, the liposome comprises DMPG.

In one embodiment, the liposome Lyso PC.

In one embodiment, the liposome (Δ9-Cis) PG.

In one embodiment, the liposome comprises Soy Lyso PC.

In one embodiment, the liposome comprises PG.

In one embodiment, the liposome comprises PA-PEG3-mannose.

In one embodiment, the liposome comprises C16 PEG2000 Ceramide.

In one embodiment, the liposome comprises MPLA.

In one embodiment, the liposome comprises CHEMS.

In one embodiment, the liposome comprises Stearic Acid.

In one embodiment, the liposome comprises a phospholipid set forth in **Table III**.

In one embodiment, the liposome comprises **ID3** and further comprises CHEMS and further comprises a LU wherein said LU is a hydromethylcarbamate linker.

In one embodiment, the liposome comprises I**D3** and further comprises Stearic Acid and further comprises a LU wherein said LU is a hydromethylcarbamate linker.

In one embodiment, the liposome comprises **ID3** and further comprises CHEMS and further comprises a LU wherein said LU is a hydromethylcarbamate linker and further comprises a helper lipid set forth in **Table II**.

In one embodiment, the liposome comprises **ID3** and further comprises a Stearic Acid and further comprises a LU wherein said LU is a hydromethylcarbamate linker and further comprises a helper lipid set forth in **Table II**.

In one embodiment, the liposome of the disclosure comprises a IDO prodrug co-formulated with one or more additional immune modulating agents, whereby the immune modulating agents includes, but is not limited to, immunogenic-cell death inducing chemotherapeutics, toll receptor agonists, sting agonists, CTLA4 inhibitors, PD-1 inhibitors, and/or prodrugs thereof.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with an ICD-inducing Chemotherapeutic.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with an ICD-inducing Chemotherapeutic selected from the list: doxorubicin (DOX), mitoxantrone (MTO), Oxaliplatin (OXA), Cyclophosphamide (CP), Bortezomib, Carfilzimib, or Paclitaxel.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with a Toll Receptor TLR agonist/Prodrug.

In a preferred embodiment, the liposome comprises a IDO prodrug co-formulated with Toll Receptor (TLR) agonist/Prodrug selected from the list: Resiquimod (R848), Gardiquimod, 852A, DSR 6434, Telratolimod, CU-T12-9, monophosphoryl Lipid A (MPLA), 3D(6-acyl)-PHAD®, SMU127, Pam3CSK4, or 3D-PHAD®.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with an PD-1 inhibitor/Prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with an PD-1 inhibitor/Prodrug, selected from the list: AUNP12, CA-170, or BMS-986189 or prodrugs thereof.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with doxorubicin (DOX).

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with mitoxantrone (MTO).

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with doxorubicin (DOX) and an PD-1 prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with mitoxantrone (MTO) and a PD-1 prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with doxorubicin (DOX) and a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with mitoxantrone (MTO) and a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with doxorubicin (DOX) and a PD-1 prodrug and a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with mitoxantrone (MTO) and a PD-1 prodrug and a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with a TLR agonist / prodrug and a PD-1 prodrug.

In a preferred embodiment, the liposome comprises an IDO prodrug co-formulated with a TLR agonist / prodrug and a PD-1 prodrug.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with doxorubicin (DOX).

In a preferred embodiment, the liposome comprises **ID3** co-formulated with mitoxantrone (MTO).

In a preferred embodiment, the liposome comprises **ID3** co-formulated with doxorubicin (DOX) and/or and IDO prodrug and/or a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with mitoxantrone (MTO) and/or and IDO prodrug and/or a TLR agonist / prodrug.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with AR5.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with AR5 and TR5.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with NK1.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with NK1 and MTO.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with TR3.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with TR5.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with TB4.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with PD3.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with AR5 and TR3.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with AR5 and TB4.

In a preferred embodiment, the liposome comprises **ID3** co-formulated with AR5 and PD3.

In another preferred embodiment, the liposome comprises a solid-lipid nanoparticle (SLNP) comprising a liposome which comprises an IDO prodrug.

One of skill in the art will appreciate and understand that solubility is one of most common problems faced by the artisan in the drug development process. Chemical conjugation of a drug/anti-cancer agents via lipid molecules (i.e. lipid-based prodrugs) provides a platform to solve the problem of formulating the drugs in an aqueous suspension. The major advantages of delivering drug(s) with lipid conjugation (lipid-based prodrugs) lies on its ability to improve pharmacokinetics/half-life and targeted delivery.

With suitable selection of lipid molecules, lipid-based prodrug(s) can be integrated/formulated in a liposomal formulation using techniques known in the art, which has many more advantages over conventional drug delivery system. (KOHLI, *et. al.*, J. Control Release, 0: pp 274-287 (Sept. 28, 2014); and GARCIA-PINEL, *et. al.*, Nanomaterials 9:638 (2019). The advantage of combining lipid-prodrug with liposomes is twofold: (i) liposomes containing lipid-prodrug not only increase the solubility of the drug/prodrug itself, but (ii) also have the ability to encapsulate multiple drugs (both hydrophilic and lipophilic) (*see*, section entitled nanocarriers).

For the purposes of this disclosure, the major advantage of liposome formulations are as follows:

i) biocompatibility/biodegradability and no general toxicity of the liposome's formulations;

ii) flexibility and manipulation of size and surface charge depending on the required purpose. Liposome formulation(s), for the purposes of this disclosure, can have a size range of 40-150 nm in diameter and a surface charge in the range of -40 to + 40 mV; and

iii) Liposomes of the invention have either a single or multiple lipid-prodrugs as the constituent lipid portion of the liposome(s). Additionally, multiple drugs (e.g. that work in different mechanism of action) and with different solubility profile (hydrophilic or lipophilic) can be formulated (either in the lipid bilayers or in the hydrophilic core) in these liposomes.

As one of ordinary skill in the art will appreciate, all methods of making liposomes involve four (4) basic stages:

(i) Drying down lipids from organic solvent;

(ii) Dispersing the lipid in aqueous solution;

(iii) Purifying the resultant liposome; and

(iv) Analyzing the final product.

See, AKBARZADEH, et. al., Nanoscale Research Letters, 8:102 (2013).

Another aspect of the invention discloses liposomal encapsulation technology (LET) which is a delivery technique used to transmit drugs. LET is a method of generating sub-microscopic foams called liposomes, which encapsulate numerous materials. These 'liposomes' form a barrier around their contents, which is resistant to enzymes in the mouth and stomach, alkaline solutions, digestive juices, bile salts, and intestinal flora that are generated in the human body, as well as free radicals. The contents of the liposomes are, therefore, protected from oxidation and degradation. This protective phospholipid shield or barrier remains undamaged until the contents of the liposome are delivered to the exact target gland, organ, or system where the contents will be utilized (See, section entitled nanocarriers).

In one embodiment, liposome(s) of the disclosure are synthesized using a plurality of different ratios of IDO prodrugs, lipids, and/or lipid-prodrugs. As disclosed herein, the IDO prodrugs may comprise helper lipids as disclosed herein (See, for example **Table II**).

In one embodiment, liposome(s) of the disclosure are synthesized using a plurality of different ratios of IDO prodrugs, lipids, and/or lipid-prodrugs. As disclosed herein, the IDO prodrugs may further comprise DSPE-PEGs.

In a preferred embodiment, the liposomes of the invention comprise a composition having the following ratio(s):

Constituent of the Liposome	**Amount (% w/w)**
Lipid 1 (lipid-prodrug)	5-60
Lipid 2 (lipid-prodrug)	0-60
Helper lipids	0-50
DSPEG-PEG 2000	2-5

In a further preferred embodiment, the liposomes of the invention comprise a composition having the following ratio(s):

Constituent of the Liposome	**Amount (% w/w)**
Lipid 1 (lipid-prodrug)	5-60
Helper lipids	0-50
DSPEG-PEG 2000	2-5

In a further preferred embodiment, the liposomes of the invention comprise a composition having the following ratio(s):

Constituent of the Liposome	**Amount (% w/w)**
Lipid 1 (lipid-prodrug)	5-60
Helper lipids	0-50
DSPEG-PEG 2000	2-5

Whereby Lipid 1 comprises **ID3** and CHEMS.

In a further preferred embodiment, the liposomes of the invention comprise a composition having the following ratio(s):

Constituent of the Liposome	Amount (% w/w)
Lipid 1 (lipid-prodrug)	5-60
Helper lipids	0-50
DSPEG-PEG 2000	2-5

Whereby Lipid 1 comprises **ID3** and Stearic Acid.

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

VIII.) Pharmaceutical Formulation

As used herein, the term "drug" is synonymous with "pharmaceutical". In certain embodiments, the liposome of the disclosure is fabricated to an encapsulated dosage form to and given to a patient for the treatment of disease.

Generally speaking, pharmaceutical formulation is the process in which different chemical substances are combined to a pure drug substance to produce a final drug product. Formulation studies involve developing a preparation of the drug which is both stable and acceptable to the patient. For orally taken drugs, this usually involves incorporating the drug into a tablet or a capsule. It is important to appreciate that a dosage form contains a variety of other substances apart from the drug itself, and studies have to be carried out to ensure that the drug is compatible with these other substances.

An excipient is an inactive substance used as a carrier for the active ingredients of a drug product, in this case a liposome comprising an IDO prodrug. In addition, excipients can be used to aid the process by which a drug product is manufactured. The active substance is then dissolved or mixed with an excipient. Excipients are also sometimes used to bulk up formulations with very potent active ingredients, to allow for convenient and accurate dosage. Once the active ingredient has been purified, it cannot stay in purified form for exceedingly long. In many cases it will denature, fall out of solution, or stick to the sides of the container.

To stabilize the active ingredient, excipients are added to ensure that the active ingredient stays active and is stable for a long enough period of time that the shelf-life of the product makes it competitive with other products and safe for the end-user. Examples of excipients include but are not limited to, anti-adherents, binders, coatings, disintegrants, fillers, diluents, flavors, colors, lubricants, and

preservatives. The final formulation comprises and active ingredient and excipients which are then enclosed in the pharmaceutical dosage form.

Pre-formulation involves the characterization of a drug's physical, chemical, and mechanical properties in order to choose what other ingredients should be used in the preparation. Formulation studies then consider such factors as stability, particle size, polymorphism, pH, and solubility, as all of these can influence bioavailability and hence the activity of a drug. The drug must be combined with inactive additives by a method which ensures that the quantity of drug present is consistent in each dosage unit (e.g. each vial). The dosage should have a uniform appearance.

It is unlikely that these studies will be complete by the time clinical trials commence. This means that simple preparations are developed initially for use in phase I clinical trials. These typically consist of vials, hand-filled capsules containing a small amount of the drug and a diluent. Proof of the long-term stability of these formulations is not required, as they will be used (tested) in a matter of days. However, long-term stability is critical in supply chain management since the time the final formulation is packaged until it reaches the patient can be several months or years. Consideration has to be given to what is called the drug load (i.e. the ratio of the active drug to the total contents of the dose). A low drug load may cause homogeneity problems. A high drug load may pose flow problems or require large capsules if the compound has a low bulk density. By the time phase III clinical trials are reached, the formulation of the drug should have been developed to be close to the preparation that will ultimately be used in the market.

A knowledge of stability is essential by this stage, and conditions must have been developed to ensure that the drug is stable in the preparation. If the drug proves unstable, it will invalidate the results from clinical trials since it would be impossible to know what the administered dose actually was. Stability studies are carried out to test whether temperature, humidity, oxidation, or photolysis (ultraviolet light or visible light) have any effect, and the preparation is analyzed to see if any degradation products have been formed. It is also important to check whether there are any unwanted interactions between the preparation and the container. If a plastic container is used, tests are carried out to see whether any of the ingredients become adsorbed on to the plastic, and whether any plasticizers, lubricants, pigments, or stabilizers leach out of the plastic into the preparation. Even the adhesives for the container label need to be tested, to ensure they do not leach through the plastic container into the preparation. The way a drug is formulated can avoid some of the problems associated with oral administration. Drugs are normally taken orally as tablets or capsules. The drug (active substance) itself needs to be soluble in aqueous solution at a controlled rate. Such factors as particle size and crystal form can significantly affect dissolution. Fast dissolution is not always ideal. For example, slow dissolution rates can prolong the duration of action or avoid initial high plasma levels.

In some embodiments, the nanocarrier (e.g., a liposome comprising an IDO prodrug) and/or the liposome comprising an IDO prodrug and co-formulated with an immune modulating agent are administered alone or in a mixture with a physiologically-acceptable carrier (such as physiological saline or phosphate buffer) selected in accordance with the route of administration and standard pharmaceutical practice. For example, when used as an injectable, the nanocarriers can be formulated as a sterile suspension, dispersion, or emulsion with a pharmaceutically acceptable carrier. In certain embodiments normal saline can be employed as the pharmaceutically acceptable carrier. Other suitable carriers include, e.g., water, buffered water, 0.4% saline, 0.3% glycine, 5% glucose and the like, including glycoproteins for enhanced stability, such as albumin, lipoprotein, globulin, etc. In compositions comprising saline or other salt-containing carriers, the carrier is preferably added following nanocarrier formation. Thus, after the nanocarrier is formed and loaded with suitable drug(s), the nanocarrier can be diluted into pharmaceutically acceptable carriers such as normal saline. Similarly, the IDO prodrug liposomes can be introduced into carriers that facilitate suspension of the nanomaterials (e.g., emulsions, dilutions, etc.).

The pharmaceutical compositions may be sterilized by conventional, well-known sterilization techniques. The resulting aqueous solutions, suspensions, dispersions, emulsions, etc., may be packaged for use or filtered under aseptic conditions. In certain embodiments the drug delivery nanocarriers (e.g., LB-coated nanoparticles) are lyophilized, the lyophilized preparation being combined with a sterile aqueous solution prior to administration. The compositions may also contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions, such as pH-adjusting and buffering agents, tonicity adjusting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride, calcium chloride, etc.

Additionally, in certain embodiments, the pharmaceutical formulation may include lipid-protective agents that protect lipids against free-radical and lipid-peroxidative damage on storage. Lipophilic free-radical quenchers, such as alpha-tocopherol and water-soluble iron-specific chelators, such as ferrioxamine, are suitable and contemplated herein. The concentration of nanocarrier (e.g., liposome comprising IDO prodrugs) in the pharmaceutical formulations can vary widely, e.g., from less than approximately 0.05%, usually at least approximately 2 to 5% to as much as 10 to 50%, or to 40%, or to 30% by weight and are selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. For example, the concentration may be increased to lower the fluid load associated with treatment. This may be particularly desirable in patients having atherosclerosis-associated congestive heart failure or severe hypertension. Alternatively, nanocarriers composed of irritating lipids may be diluted to low concentrations to lessen inflammation at the site of administration. The amount of nanocarriers administered will depend upon the particular drug used, the disease state being treated and the judgment of the clinician but will generally be between

approximately 0.01 and approximately 50 mg per kilogram of body weight, preferably between approximately 0.1 and approximately 5 mg per kg of body weight.

One of skill in the art will appreciate that exact dosages will vary depending upon such factors as the particular IDO prodrugs and any co-formulated immune modulating agents and the desirable medical effect, as well as patient factors such as age, sex, general condition, and the like. Those of skill in the art can readily take these factors into account and use them to establish effective therapeutic concentrations without resort to undue experimentation.

For administration to humans (or to non-human mammals) in the curative, remissive, retardive, or prophylactic treatment of diseases described herein the prescribing physician will ultimately determine the appropriate dosage of the drug for a given human (or non-human) subject, and this can be expected to vary according to the age, weight, and response of the individual as well as the nature and severity of the patient's disease. In certain embodiments the dosage of the drug provided by the nanocarrier(s) can be approximately equal to that employed for the free drug. However as noted above, the nanocarriers described herein can significantly reduce the toxicity of the drug(s) administered thereby and significantly increase a therapeutic window. Accordingly, in some cases dosages in excess of those prescribed for the free drug(s) will be utilized.

One of ordinary skill in the art will appreciate and be enabled to make variations and modifications to the disclosed embodiment without altering the function and purpose of the invention disclosed herein. Such variations and modifications are intended within the scope of the present disclosure.

IX.) Combination Therapy

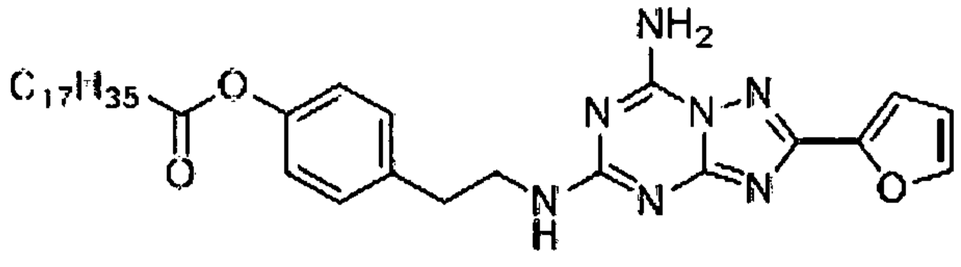
As the skilled artisan will appreciate and understand, cancer cell growth and survival can be impacted by multiple signaling pathways. Thus, it is useful to combine different enzyme/protein/receptor inhibitors, exhibiting different preferences in the targets which they modulate the activities of, to treat such conditions. Targeting more than one signaling pathway (or more than one biological molecule involved in a given signaling pathway) may reduce the likelihood of drug-resistance arising in a cell population, and/or reduce the toxicity of treatment.

Thus, the liposomes comprising IDO prodrugs of the present disclosure can be used in combination with one or more other enzyme/protein/receptor inhibitors or one or more therapies for the treatment of diseases, such as cancer or infections. Examples of diseases and indications treatable with combination therapies include those set forth in the present disclosure. Examples of cancers include, but are not limited to, solid tumors and liquid tumors, such as blood cancers. Examples of infections include viral infections, bacterial infections, fungus infections or parasite infections.

For example, the liposomes comprising IDO prodrugs of the present disclosure can be combined with one or more inhibitors of the following kinases for the treatment of cancer: Akt1, Akt2, Akt3, TGF-βR, PKA, PKG, PKC, CaM-kinase, phosphorylase kinase, MEKK, ERK, MAPK, mTOR, EGFR, HER2, HER3, HER4, INS-R, IGF-1R, IR-R, PDGFαR, PDGFβR, PI3K (*alpha*, *beta*, *gamma*, *delta*), CSFIR, KIT, FLK-II, KDR/FLK-1, FLK-4, flt-1, FGFR1, FGFR2, FGFR3, FGFR4, c-Met, Ron, Sea, TRKA, TRKB, TRKC, TAM kinases (Axl, Mer, Tyro3), FLT3, VEGFR/Flt2, Flt4, EphA1, EphA2, EphA3, EphB2, EphB4, Tie2, Src, Fyn, Lck, Fgr, Btk, Fak, SYK, FRK, JAK, ABL, ALK and B-Raf.

In further embodiments, the liposomes comprising IDO prodrugs of the present disclosure can be combined with one or more of the following inhibitors for the treatment of cancer or infections. Non-limiting examples of inhibitors that can be combined with the compounds of the present disclosure for treatment of cancer and infections include an FGFR inhibitor (FGFR1, FGFR2, FGFR3 or FGFR4, e.g., INCB54828, INCB62079 and INCB63904), a JAK inhibitor (JAK1 and/or JAK2, e.g., ruxolitinib, baricitinib or INCB39110), an IDO inhibitor (e.g., epacadostat, NLG919, or BMS-986205), an LSD1 inhibitor (e.g., INCB59872 and INCB60003), a TDO inhibitor, a PI3K-delta inhibitor (e.g., INCB50797 and INCB50465), a PI3K-gamma inhibitor such as PI3K-gamma selective inhibitor, a Pim inhibitor (e.g., INCB53914), a CSF1R inhibitor, a TAM receptor tyrosine kinases (Tyro-3, Axl, and Mer), an adenosine receptor antagonist (e.g., A2a/A2b receptor antagonist), an HPK1 inhibitor, a histone deacetylase inhibitor (HDAC) such as an HDAC8 inhibitor, an angiogenesis inhibitor, an interleukin receptor inhibitor, bromo and extra terminal family members inhibitors (for example, bromodomain inhibitors or BET inhibitors such as INCB54329 and INCB57643), a poly ADP ribose polymerase (PARP) inhibitor such as rucaparib, olaparib, niraparib, veliparib, or talazoparib, an arginase inhibitor (INCB01158), a PD-1 inhibitor, a PD-1/L-1 inhibitor, a PD-1/L-2 inhibitor, and an adenosine receptor antagonist or combinations thereof.

In one embodiment, an A2a receptor inhibitor comprises the following prodrug compound (denoted "AR5"):



In a further embodiment, a liposome comprising an IDO prodrugs further comprises α-galactosylceramide (α-GalCer) (denoted "NK1").

Additionally, the liposomes comprising IDO prodrugs of the present disclosure can further be used in combination with other methods of treating cancers, for example by chemotherapy, irradiation therapy, tumor-targeted therapy, adjuvant therapy, immunotherapy, or surgery.

Examples of immunotherapy include cytokine treatment (e.g., interferons, GM-CSF, G-CSF, IL-2), CRS-207 immunotherapy, cancer vaccine, monoclonal antibody, adoptive T cell transfer, Toll

receptor agonists, STING agonists, oncolytic virotherapy and immunomodulating small molecules, including thalidomide or JAK1/2 inhibitor and the like.

The liposomes comprising IDO prodrugs can be administered in combination with one or more anti-cancer drugs, such as a chemotherapeutics. Example chemotherapeutics include any of: abarelix, aldesleukin, alemtuzumab, alitretinoin, allopurinol, altretamine, anastrozole, arsenic trioxide, asparaginase, azacitidine, bevacizumab, bexarotene, baricitinib, bleomycin, bortezombi, bortezomib, busulfan intravenous, busulfan oral, calusterone, capecitabine, carboplatin, carmustine, cetuximab, chlorambucil, cisplatin, cladribine, clofarabine, cyclophosphamide, cytarabine, dacarbazine, dactinomycin, dalteparin sodium, dasatinib, daunorubicin, decitabine, denileukin, denileukin diftitox, dexrazoxane, docetaxel, doxorubicin, dromostanolone propionate, eculizumab, epirubicin, erlotinib, estramustine, etoposide phosphate, etoposide, exemestane, fentanyl citrate, filgrastim, floxuridine, fludarabine, fluorouracil, fulvestrant, gefitinib, gemcitabine, gemtuzumab ozogamicin, goserelin acetate, histrelin acetate, ibritumomab tiuxetan, idarubicin, ifosfamide, imatinib mesylate, interferon alfa 2a, irinotecan, lapatinib ditosylate, lenalidomide, letrozole, leucovorin, leuprolide acetate, levamisole, lomustine, meclorethamine, megestrol acetate, melphalan, mercaptopurine, methotrexate, methoxsalen, mitomycin C, mitotane, mitoxantrone, nandrolone phenpropionate, nelarabine, nofetumomab, olaparib, oxaliplatin, paclitaxel, pamidronate, panitumumab, pegaspargase, pegfilgrastim, pemetrexed disodium, pentostatin, pipobroman, plicamycin, procarbazine, quinacrine, rasburicase, rituximab, ruxolitinib, rucaparib, sorafenib, streptozocin, sunitinib, sunitinib maleate, tamoxifen, temozolomide, teniposide, testolactone, thalidomide, thioguanine, thiotepa, topotecan, toremifene, tositumomab, trastuzumab, tretinoin, uracil mustard, valrubicin, vinblastine, vincristine, vinorelbine, vorinostat, niraparib, veliparib, talazoparib and zoledronate.

Other anti-cancer agent(s) include antibody therapeutics such as trastuzumab (Herceptin), antibodies to costimulatory molecules such as CTLA-4 (e.g., ipilimumab), 4-1BB (e.g. urelumab, utomilumab), antibodies to PD-1 and PD-L1/L2, or antibodies to cytokines (IL-10, TGF-.beta., etc.).

Examples of antibodies to PD-1 and/or PD-L1/L2 that can be combined with compounds of the present disclosure for the treatment of cancer or infections such as viral, bacteria, fungus and parasite infections include, but are not limited to, nivolumab, pembrolizumab, MPDL3280A, MEDI-4736 and SHR-1210.

In addition, liposomes comprising IDO prodrugs of the present disclosure can be used in combination with one or more immune checkpoint inhibitors for the treatment of diseases, such as cancer or infections. Exemplary immune checkpoint inhibitors include inhibitors against immune checkpoint molecules such as CD27, CD28, CD40, CD122, CD96, CD73, CD47, OX40, GITR, CSF1R, JAK, PI3K delta, PI3K gamma, TAM, arginase, CD137 (also known as 4-1BB), ICOS, A2AR, B7-H3, B7-H4, BTLA, CTLA-4, LAG3, TIM3, VISTA, PD-1, PD-L1 and PD-L2.

In some embodiments, the immune checkpoint molecule is a stimulatory checkpoint molecule selected from CD27, CD28, CD40, ICOS, OX40, GITR and CD137. In further embodiments, the immune checkpoint molecule is an inhibitory checkpoint molecule selected from A2AR, B7-H3, B7-H4, BTLA, CTLA-4, IDO, KIR, LAG3, PD-1, TIM3, and VISTA. In further embodiments, the liposomes comprising IDO prodrugs provided herein can be used in combination with one or more agents selected from KIR inhibitors, TIGIT inhibitors, LAIR1 inhibitors, CD160 inhibitors, 2B4 inhibitors and TGFR beta inhibitors.

X.) Methods of Delivering Liposomes Comprising IDO Prodrugs to a Cell Expressing IDO-1

As it is known in the art, a wide variety of compositions and methods for using prodrugs and/or liposomes to kill tumor cells are known in the art. In the context of cancers, typical methods entail administering to a mammal having a tumor, a biologically effective amount of an IDO prodrug of the disclosure, and/or a liposome of the disclosure comprising an IDO prodrug.

A typical embodiment is a method of delivering a therapeutic agent to a cell expressing IDO-1, comprising forming an IDO prodrug by conjugating a drug moiety of the disclosure with a lipid of the disclosure via a Linkage Unit, and, exposing the cell to the IDO prodrug.

In one embodiment, the IDO prodrug comprises a drug moiety of Formula I and CHEMS conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the IDO prodrug comprises a drug moiety of Formula I and Stearic Acid conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the IDO prodrug comprises **ID3** and CHEMS conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the IDO prodrug comprises **ID3** and Stearic Acid conjugated via a LU comprising a hydromethylcarbamate linker.

Another illustrative embodiment is a method of treating an individual suspected of suffering from metastasized cancer, comprising a step of administering parenterally to said individual a pharmaceutical composition comprising a therapeutically effective amount of an IDO prodrug produced by conjugating a drug moiety with a lipid of the disclosure via a Linkage Unit, and, exposing the cell to the IDO prodrug.

In one embodiment, the IDO prodrug comprises a drug moiety of Formula I and CHEMS conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the IDO prodrug comprises a drug moiety of Formula I and Stearic Acid conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the IDO prodrug comprises **ID3** and CHEMS conjugated via a LU comprising a hydromethylcarbamate linker.

In one embodiment, the PD1 prodrug comprises **ID3** and Stearic Acid conjugated via a LU comprising a hydromethylcarbamate linker.

IDO prodrugs, liposomes, and co-formulated liposomes of the present disclosure inhibit the activity of IDO-1 protein/protein interaction and, thus, are useful in treating diseases and disorders associated with activity of IDO-1 and the diseases and disorders associated with kynurenine pathway including its interaction with other proteins such as IDO-2 and TDO. In further embodiments of the disclosure, the IDO prodrugs, liposomes, or pharmaceutically acceptable salts or stereoisomers thereof, are useful for therapeutic administration to enhance, stimulate and/or increase immunity in cancer, chronic infection, or sepsis, including enhancement of response to vaccination.

In further embodiments, the present disclosure provides a method for inhibiting the IDO-1 T-cell function. The method includes administering to an individual or a patient an IDO prodrug, liposomes, and/or of any of the formulas as described herein (e.g. **ID3**), or of an IDO prodrug, liposomes, and nano-encapsulated IDO inhibitor prodrugs as recited in any of the claims and described herein, or a pharmaceutically acceptable salt or a stereoisomer thereof. The IDO prodrug, liposomes, and nano-encapsulated IDO inhibitor prodrugs of the present disclosure can be used alone, in combination with other agents or therapies or as an adjuvant or neoadjuvant for the treatment of diseases or disorders, including cancer and other diseases. For the uses and methods described herein any of the IDO prodrugs, liposomes, and nano-encapsulated IDO prodrugs of the disclosure, including any of the embodiments thereof, may be used.

In addition, The IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrugs of the present disclosure inhibit the IDO-1 kynurenine pathway and/or T-cell function, resulting in an IDO pathway blockade. As is known in the art, IDO becomes activated during tumor development, helping malignant cells escape eradication by the immune system. *See,* MUNN, *et. al.*, Trends in Immunology, 37(3): 193-207 (2016).

In further embodiments, the present disclosure provides treatment of an individual or a patient *in vivo* using IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrug or a salt or stereoisomer thereof such that growth of cancerous tumors is inhibited.

IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrugs, or of any of the formulas as described herein (e.g. **ID3**), or IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrugs as recited in any of the claims and described herein, or a salt or stereoisomer thereof, can be used to inhibit the growth of cancerous tumors.

In the alternative, IDO prodrugs, liposomes, and nano-encapsulated IDO prodrugs of the disclosure, or of any of the formulas as described herein, or a compound as recited in any of the claims and described herein (e.g. **ID3**), or a salt or stereoisomer thereof, can be used in conjunction with other agents or standard cancer treatments, as described in this disclosure.

In a further embodiment, the present disclosure provides a method for inhibiting growth of tumor cells *in vitro*. The method includes contacting the tumor cells *in vitro* with IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrugs of the disclosure, or of any of the formulas as described herein (e.g. **ID3**), or of an IDO prodrug, liposomes, and nano-encapsulated IDO inhibitor prodrugs as recited in any of the claims and described herein, or of a salt or stereoisomer thereof.

In a further embodiment, the present disclosure provides a method for inhibiting growth of tumor cells in a patient. The method includes contacting the tumor cells with IDO prodrugs, liposomes, and nano-encapsulated IDO inhibitor prodrugs of the disclosure, or of any of the formulas as described herein (e.g. **ID3**), or of an IDO prodrug, liposomes, and nano-encapsulated IDO inhibitor prodrugs as recited in any of the claims and described herein, or of a salt or stereoisomer thereof.

XI.) Methods of Treating Cancer(s) and Other Immunological Disorder(s)

Another embodiment of the present disclosure is a method for treating cancer. The method comprises administering to a patient, a therapeutically effective amount of a liposome comprising an IDO prodrug (i.e. **ID3**) herein, a compound as recited in any of the claims and described herein, or a salt thereof. Examples of cancers include those whose growth may be inhibited using IDO inhibitors of the disclosure and IDO prodrugs of the disclosure and cancers typically responsive to immunotherapy.

In some embodiments, the present disclosure provides a method of enhancing, stimulating and/or increasing the immune response in a patient. The method includes administering to the patient a therapeutically effective amount of an IDO prodrug and/or a liposome comprising the same (i.e. **ID3**), a compound or composition as recited in any of the claims and described herein, or a salt thereof.

Non-limiting examples of cancers that are treatable using the liposomes comprising IDO prodrugs, IDO prodrugs and co-formulated liposomes of the present disclosure include, but are not limited to, bone cancer, pancreatic cancer, skin cancer, cancer of the head or neck, cutaneous or intraocular malignant melanoma, uterine cancer, ovarian cancer, rectal cancer, cancer of the anal region, stomach cancer, testicular cancer, uterine cancer, carcinoma of the fallopian tubes, carcinoma of the endometrium, endometrial cancer, carcinoma of the cervix, carcinoma of the vagina, carcinoma of the vulva, Hodgkin's Disease, non-Hodgkin's lymphoma, cancer of the esophagus, cancer of the small intestine, cancer of the endocrine system, cancer of the thyroid gland, cancer of the parathyroid gland, cancer of the adrenal gland, sarcoma of soft tissue, cancer of the urethra, cancer of the penis, chronic or acute leukemias including acute myeloid leukemia, chronic myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, solid tumors of childhood, lymphocytic lymphoma, cancer of the bladder, cancer of the kidney or urethra, carcinoma of the renal pelvis, neoplasm of the central nervous system (CNS), primary CNS lymphoma, tumor angiogenesis, spinal axis tumor, brain stem glioma, pituitary adenoma, Kaposi's sarcoma, epidermoid cancer, squamous cell cancer, T-cell

lymphoma, environmentally induced cancers including those induced by asbestos, and combinations of said cancers. The compounds of the present disclosure are also useful for the treatment of metastatic cancers, especially metastatic cancers that express IDO-1.

In some embodiments, cancers treatable with liposomes, or IDO prodrugs of the present disclosure include melanoma (e.g., metastatic malignant melanoma), renal cancer (e.g. clear cell carcinoma), prostate cancer (e.g. hormone refractory prostate adenocarcinoma), breast cancer, colon cancer, lung cancer (e.g. non-small cell lung cancer and small cell lung cancer), squamous cell head and neck cancer, urothelial cancer (e.g. bladder) and cancers with high microsatellite instability (MSI^{high}). Additionally, the disclosure includes refractory or recurrent malignancies whose growth may be inhibited using the liposomes, or IDO prodrugs or co-formulated liposomes of the disclosure.

In additional embodiments, cancers that are treatable using the formulated and/or co-formulated liposomes or IDO prodrugs of the present disclosure include, but are not limited to, solid tumors (e.g., prostate cancer, colon cancer, esophageal cancer, endometrial cancer, ovarian cancer, uterine cancer, renal cancer, hepatic cancer, pancreatic cancer, gastric cancer, breast cancer, lung cancer, cancers of the head and neck, thyroid cancer, glioblastoma, sarcoma, bladder cancer, etc.), hematological cancers (e.g., lymphoma, leukemia such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), DLBCL, mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma or multiple myeloma) and combinations of said cancers.

In further embodiments, cancers that are treatable using the formulated and/or co-formulated liposomes or IDO prodrugs of the present disclosure include, but are not limited to, cholangiocarcinoma, bile duct cancer, triple negative breast cancer, rhabdomyosarcoma, small cell lung cancer, leiomyosarcoma, hepatocellular carcinoma, Ewing's sarcoma, brain cancer, brain tumor, astrocytoma, neuroblastoma, neurofibroma, basal cell carcinoma, chondrosarcoma, epithelioid sarcoma, eye cancer, Fallopian tube cancer, gastrointestinal cancer, gastrointestinal stromal tumors, hairy cell leukemia, intestinal cancer, islet cell cancer, oral cancer, mouth cancer, throat cancer, laryngeal cancer, lip cancer, mesothelioma, neck cancer, nasal cavity cancer, ocular cancer, ocular melanoma, pelvic cancer, rectal cancer, renal cell carcinoma, salivary gland cancer, sinus cancer, spinal cancer, tongue cancer, tubular carcinoma, urethral cancer, and ureteral cancer.

In addition, in some embodiments, the formulated and/or co-formulated liposomes, or IDO prodrugs of the present disclosure can be used to treat sickle cell disease and sickle cell anemia.

Furthermore, in some embodiments, diseases and indications that are treatable using the formulated and/or co-formulated liposomes, or IDO prodrugs of the present disclosure include, but are not limited to hematological cancers, sarcomas, lung cancers, gastrointestinal cancers, genitourinary

tract cancers, liver cancers, bone cancers, nervous system cancers, gynecological cancers, and skin cancers.

Exemplary hematological cancers include lymphomas and leukemias such as acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), acute promyelocytic leukemia (APL), chronic lymphocytic leukemia (CLL), chronic myelogenous leukemia (CML), diffuse large B-cell lymphoma (DLBCL), mantle cell lymphoma, Non-Hodgkin lymphoma (including relapsed or refractory NHL and recurrent follicular), Hodgkin lymphoma, myeloproliferative diseases (e.g., primary myelofibrosis (PMF), polycythemia vera (PV), and essential thrombocytosis (ET)), myelodysplasia syndrome (MDS), T-cell acute lymphoblastic lymphoma (T-ALL) and multiple myeloma (MM).

Exemplary sarcomas include chondrosarcoma, Ewing's sarcoma, osteosarcoma, rhabdomyosarcoma, angiosarcoma, fibrosarcoma, liposarcoma, myxoma, rhabdomyoma, rhabdosarcoma, fibroma, lipoma, harmatoma, and teratoma.

Exemplary lung cancers include non-small cell lung cancer (NSCLC), small cell lung cancer, bronchogenic carcinoma (squamous cell, undifferentiated small cell, undifferentiated large cell, adenocarcinoma), alveolar (bronchiolar) carcinoma, bronchial adenoma, chondromatous hamartoma, and mesothelioma.

Exemplary gastrointestinal cancers include cancers of the esophagus (squamous cell carcinoma, adenocarcinoma, leiomyosarcoma, lymphoma), stomach (carcinoma, lymphoma, leiomyosarcoma), pancreas (ductal adenocarcinoma, insulinoma, glucagonoma, gastrinoma, carcinoid tumors, vipoma), small bowel (adenocarcinoma, lymphoma, carcinoid tumors, Kaposi's sarcoma, leiomyoma, hemangioma, lipoma, neurofibroma, fibroma), large bowel (adenocarcinoma, tubular adenoma, villous adenoma, hamartoma, leiomyoma), and colorectal cancer.

Exemplary genitourinary tract cancers include cancers of the kidney (adenocarcinoma, Wilm's tumor [nephroblastoma]), bladder and urethra (squamous cell carcinoma, transitional cell carcinoma, adenocarcinoma), prostate (adenocarcinoma, sarcoma), and testis (seminoma, teratoma, embryonal carcinoma, teratocarcinoma, choriocarcinoma, sarcoma, interstitial cell carcinoma, fibroma, fibroadenoma, adenomatoid tumors, lipoma).

Exemplary liver cancers include hepatoma (hepatocellular carcinoma), cholangiocarcinoma, hepatoblastoma, angiosarcoma, hepatocellular adenoma, and hemangioma.

Exemplary bone cancers include, for example, osteogenic sarcoma (osteosarcoma), fibrosarcoma, malignant fibrous histiocytoma, chondrosarcoma, Ewing's sarcoma, malignant lymphoma (reticulum cell sarcoma), multiple myeloma, malignant giant cell tumor chordoma, osteochronfroma (osteocartilaginous exostoses), benign chondroma, chondroblastoma, chondromyxofibroma, osteoid osteoma, and giant cell tumors.

Exemplary nervous system cancers include cancers of the skull (osteoma, hemangioma, granuloma, xanthoma, osteitis deformans), meninges (meningioma, meningiosarcoma, gliomatosis), brain (astrocytoma, meduoblastoma, glioma, ependymoma, germinoma (pinealoma), glioblastoma, glioblastoma multiform, oligodendroglioma, schwannoma, retinoblastoma, congenital tumors), and spinal cord (neurofibroma, meningioma, glioma, sarcoma), as well as neuroblastoma and Lhermitte-Duclos disease.

Exemplary gynecological cancers include cancers of the uterus (endometrial carcinoma), cervix (cervical carcinoma, pre-tumor cervical dysplasia), ovaries (ovarian carcinoma (serous cystadenocarcinoma, mucinous cystadenocarcinoma, unclassified carcinoma), granulosa-thecal cell tumors, Sertoli-Leydig cell tumors, dysgerminoma, malignant teratoma), vulva (squamous cell carcinoma, intraepithelial carcinoma, adenocarcinoma, fibrosarcoma, melanoma), vagina (clear cell carcinoma, squamous cell carcinoma, botryoid sarcoma (embryonal rhabdomyosarcoma), and fallopian tubes (carcinoma).

Exemplary skin cancers include melanoma, basal cell carcinoma, squamous cell carcinoma, Kaposi's sarcoma, moles dysplastic nevi, lipoma, angioma, dermatofibroma, and keloids. In some embodiments, diseases and indications that are treatable using the compounds of the present disclosure include, but are not limited to, sickle cell disease (e.g., sickle cell anemia), triple-negative breast cancer (TNBC), myelodysplastic syndromes, testicular cancer, bile duct cancer, esophageal cancer, and urothelial carcinoma.

Additionally, IDO-1 and/or kynurenine pathway blockade with formulated and/or co-formulated liposomes, or IDO prodrugs of the present disclosure can also be used for treating infections such as viral, bacteria, fungus, and parasite infections.

The present disclosure provides a method for treating infections such as viral infections. The method includes administering to a patient, a therapeutically effective amount of a formulated and/or co-formulated liposome or IDO prodrugs or any of the formulas as described herein (i.e. **ID3**) as recited in any of the claims and described herein, a salt thereof.

Examples of viruses causing infections treatable by methods of the present disclosure include, but are not limit to, human immunodeficiency virus, human papillomavirus, influenza, hepatitis A, B, C or D viruses, adenovirus, poxvirus, herpes simplex viruses, human cytomegalovirus, severe acute respiratory syndrome virus, Ebola virus, and measles virus. In some embodiments, viruses causing infections treatable by methods of the present disclosure include, but are not limit to, hepatitis (A, B, or C), herpes virus (e.g., VZV, HSV-1, HAV-6, HSV-II, and CMV, Epstein Barr virus), adenovirus, influenza virus, flaviviruses, echovirus, rhinovirus, coxsackie virus, coronavirus, respiratory syncytial virus, mumps virus, rotavirus, measles virus, rubella virus, parvovirus, vaccinia virus, HTLV virus, dengue virus, papillomavirus, molluscum virus, poliovirus, rabies virus, JC virus and arboviral encephalitis virus.

In addition, the present disclosure provides a method for treating bacterial infections. The method includes administering to a patient, a therapeutically effective amount of a formulated and/or co-formulated liposome or IDO prodrugs, or any of the formulas as described herein (i.e. **ID3**) as recited in any of the claims and described herein, or a salt thereof.

Examples of pathogenic bacteria causing infections treatable by methods of the disclosure, include but are not limited to, chlamydia, rickettsia bacteria, mycobacteria, staphylococci, streptococci, pneumonococci, meningococci and conococci, klebsiella, proteus, serratia, pseudomonas, legionella, diphtheria, salmonella, bacilli, cholera, tetanus, botulism, anthrax, plague, leptospirosis, and Lyme's disease bacteria.

In addition, the present disclosure provides a method for treating fungus infections. The method includes administering to a patient, a therapeutically effective amount of a formulated and/or co-formulated liposome or IDO prodrugs, or any of the formulas as described herein (i.e. **ID3**) as recited in any of the claims and described herein, or a salt thereof.

Examples of pathogenic fungi causing infections treatable by methods of the disclosure include, but are not limited to, Candida (albicans, krusei, glabrata, tropicalis, etc.), Cryptococcus neoformans, Aspergillus (fumigatus, Niger, etc.), Genus Mucorales (Mucor, absidia, rhizophus), Sporothrix schenkii, Blastomyces dermatitidis, Paracoccidioides brasiliensis, Coccidioides immitis and Histoplasma capsulatum.

Additionally, the present disclosure provides a method for treating parasite infections. The method includes administering to a patient, a therapeutically effective amount of a formulated and/or co-formulated liposome or IDO prodrugs, or any of the formulas as described herein (i.e. **ID3**) as recited in any of the claims and described herein, or a salt thereof.

Examples of pathogenic parasites causing infections treatable by methods of the disclosure include, but are not limited to, Entamoeba histolytica, Balantidium coli, Naegleriafowleri, Acanthamoeba sp., Giardia lambia, Cryptosporidium sp., Pneumocystis carinii, Plasmodium vivax, Babesia microti, Trypanosoma brucei, Trypanosoma cruzi, Leishmania donovani, Toxoplasma gondi, and Nippostrongylus brasiliensis.

In a further set of embodiments that are within the scope of this disclosure, the formulated and/or co-formulated liposomes, or IDO prodrugs, or any of the formulas as described herein (i.e. **ID3**) are useful in preventing or reducing the risk of developing any of the diseases referred to in this disclosure; e.g., preventing or reducing the risk of developing a disease, condition or disorder in an individual who may be predisposed to the disease, condition or disorder but does not yet experience or display the pathology or symptomatology of the disease.

XII.) Kits/Articles of Manufacture

For use in the laboratory, prognostic, prophylactic, diagnostic and therapeutic applications described herein, kits are within the scope of the invention. Such kits can comprise a carrier, package, or container that is compartmentalized to receive one or more containers such as vials, tubes, and the like, each of the container(s) comprising one of the separate elements to be used in the method, along with a label or insert comprising instructions for use, such as a use described herein. For example, the container(s) can comprise a formulated and/or co-formulated liposome that is or can be detectably labeled and/or is loaded with an IDO prodrug of the disclosure. Kits can comprise a container comprising a drug unit. The kit can include all or part of the formulated and/or co-formulated liposomes and/or an IDO prodrug.

The kit of the invention will typically comprise the container described above and one or more other containers associated therewith that comprise materials desirable from a commercial and user standpoint, including buffers, diluents, filters, needles, syringes; carrier, package, container, vial and/or tube labels listing contents and/or instructions for use, and package inserts with instructions for use.

A label can be present on or with the container to indicate that the composition is used for a specific therapy or non-therapeutic application, such as a prognostic, prophylactic, diagnostic or laboratory application, and can also indicate directions for either *in vivo* or *in vitro* use, such as those described herein. Directions and or other information can also be included on an insert(s) or label(s) which is included with or on the kit. The label can be on or associated with the container. A label can be on a container when letters, numbers or other characters forming the label are molded or etched into the container itself; a label can be associated with a container when it is present within a receptacle or carrier that also holds the container, e.g., as a package insert. The label can indicate that the composition is used for diagnosing, treating, prophylaxing or prognosing a condition, such as a cancer or other immunological disorder.

The terms "kit" and "article of manufacture" can be used as synonyms.

In another embodiment of the invention, an article(s) of manufacture containing compositions, such as formulated and/or co-formulated liposomes and/or IDO prodrugs are within the scope of this disclosure. The article of manufacture typically comprises at least one container and at least one label. Suitable containers include, for example, bottles, vials, syringes, and test tubes. The containers can be formed from a variety of materials such as glass, metal, or plastic. The container can hold formulated and/or co-formulated liposomes loaded with IDO prodrugs.

The container can alternatively hold a composition that is effective for treating, diagnosis, prognosing or prophylaxing a condition and can have a sterile access port (for example the container can be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The active agents in the composition can be formulated and/or co-formulated liposomes loaded with IDO prodrugs and/or IDO prodrugs as disclosed herein.

The article of manufacture can further comprise a second container comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and/or dextrose solution. It can further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, stirrers, needles, syringes, and/or package inserts with indications and/or instructions for use.

EXEMPLARY EMBODIMENTS

Among the provided embodiments are:

1) An IDO prodrug composition comprising,
   (i) a drug moiety;
   (ii) a lipid moiety; and
   (iii) a linkage unit ("LU"),

whereby the drug moiety comprises an IDO antagonist and whereby the LU conjugates the drug moiety with the lipid moiety.

2) The IDO prodrug of claim 1, further comprising the chemical structure set forth in **FORMULA I**.
3) The IDO prodrug of claim 1, wherein the drug moiety comprises the chemical structure set forth as **ID3**.
4) The IDO prodrug of claim 1, wherein the LU is a hydromethylcarbamate linker.
5) The IDO prodrug of claim 1, wherein the lipid moiety comprises a lipid set forth in **Table I**.
6) The IDO prodrug of claim 1, wherein the lipid moiety comprises a lipid set forth in **Table III**.
7) The IDO prodrug of claim 1, wherein the lipid moiety comprises CHEMS.
8) The IDO prodrug of claim 1, wherein the lipid moiety comprises Stearic Acid.
9) An IDO prodrug composition comprising,
   (i) a drug moiety, whereby the drug moiety comprises **ID3**;
   (ii) a lipid moiety, whereby the lipid moiety comprises CHEMS; and
   (iii) LU, whereby the LU comprises a hydromethylcarbamate linker.
10) An IDO prodrug composition comprising,
   (i) a drug moiety, whereby the drug moiety comprises **ID3**;
   (ii) a lipid moiety, whereby the lipid moiety comprises Stearic Acid; and
   (iii) a LU, whereby the LU comprises a hydromethylcarbamate linker.
11) A liposome comprising, an IDO prodrug whereby the liposome releases an active IDO inhibitor after cleavage of a LU.
12) The liposome of claim 11, wherein the LU is a hydromethylcarbamate linker.

13) The liposome of claim 11, further comprising a helper lipid, whereby the helper lipid is set forth in **Table II**.
14) The liposome of claim 11, wherein the IDO prodrug comprises **ID3**.
15) The liposome of claim 11, whereby the liposome is further co-formulated with an immune modulating agent, wherein the immune modulating agent is selected from the group consisting of immunogenic-cell death inducing chemotherapeutics, toll-receptor agonists, STING agonists, CTLA-4 inhibitors, PD-1/PD-L1 inhibitors and/or prodrugs thereof.
16) The liposome of claim 11, whereby the liposome is further co-formulated with an ICD-inducing chemotherapeutic, wherein the ICD-inducing chemotherapeutic is selected from the group consisting of DOX, MTO, OXA, CP, Bortezomib, Carfilzimib, or Paclitaxel.
17) The liposome of claim 11, further comprising DOX.
18) The liposome of claim 11, further comprising MTO.
19) The liposome of claim 11, further comprising DOX.
20) The liposome of claim 11, further comprising MTO.
21) A kit comprising a liposome of claim 11.
22) A kit comprising a liposome of claim 15.
23) A kit comprising a liposome of claim 16.
24) A method of treating a subject suffering or diagnosed with cancer comprising,
    (i) administering to a subject in need of such treatment an effective amount of a liposome, wherein the liposome comprises an IDO prodrug; and
    (ii) a pharmaceutically acceptable salt thereof.
25) The method of claim 24, wherein the IDO prodrug comprises **ID3**.
26) The method of claim 24, wherein the liposome comprises **ID3** further co-formulated with and ICD-inducing chemotherapeutic.
27) The method of claim 24, wherein the liposome comprises **ID3** further co-formulated with an immune modulating agent.
28) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with **AR5**.
29) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with **AR5** and **TR5**.
30) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with **NK1**.
31) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with **NK1** and **MTO**.
32) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with TR3.
33) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with TR5.
34) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with TB4.

35) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with PD3.
36) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with AR5 and TR3.
37) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with AR5 and TB4.
38) The liposome of claim 11, wherein the IDO prodrug comprises **ID3** co-formulated with AR5 and PD3.

EXAMPLES:

Various aspects of the invention are further described and illustrated by way of the several examples that follow, none of which is intended to limit the scope of the invention.

Example 1: General Chemical Synthesis of Protected ID3 Prodrug Comprising Cholesterol Hemisuccinate ("CHEMS").

Chemical synthesis of a protected **ID3** prodrug comprising CHEMS is synthesized using the following protocol. First, Quinoline **(1)** and Boronate **(2)** (BARLIND, J.G. *et. al*, *J. Med. Chem.*, 55:10610-10629 (2012)) were coupled with cesium carbonate and PEPPSI-IPr catalyst in dioxane/water at 100°C to yield **Intermediate 3**. Then, **Intermediate 3** was hydrogenated over palladium on carbon to yield **Intermediate 4**. Then, **Intermediate 4** was hydrolyzed with lithium hydroxide in aqueous ethanol at 50°C to yield **Intermediate 5**. Next, **Intermediate 5** was coupled with chiral auxiliary reagent **(6)** with triethylamine and pivaloyl chloride in THF, and diastereomers were separated to yield **Intermediate 7.** Then, **Intermediate 7** was alkylated with methyl iodide and NaHMDS in THF at -50°C to yield **Intermediate 8**. Then, the chiral auxiliary was cleaved from **Intermediate 8** with LiOH and hydrogen peroxide in aqueous THF at 0°C to yield **Intermediate 9.** Then, **Intermediate 9** was coupled with aniline **(10)** with polyphosphonic anhydride and pyridine in ethyl acetate to yield **Intermediate 11**. Then, **Intermediate 11** was treated with reagent **(12)** in THF, followed by treatment with sodium iodide to yield **Intermediate 13**. Finally, **Intermediate 13** was treated with the silver salt of CHEMS **(14)** at reflux in THF to yield final ID3 prodrug. (**Figure(s) 1**).

Example 2: Chemical Synthesis of ID3 Prodrug Intermediate.

Chemical synthesis if **ID3** prodrug was performed in the following manner. To a solution of **ID3** drug moiety (6.00 g, 14.6 mmol, 1.00 eq) in THF (60 mL) was added LiHMDS (1 M, 29.2 mL, 2.00 eq) at -70 °C and the reaction mixture was stirred at -70 °C for 0.5 hr. Then a solution of **compound 1** (3.24

g, 25.1 mmol, 2.23 mL, 1.72 eq) in THF (10 mL) was added to the mixture at -78 °C and stirred at -78 °C for another 1 hr. TLC (Petroleum ether : Ethyl acetate = 2:1) showed that the **ID3** drug moiety (R_f = 0.2) was consumed and one major new spot (R_f = 0.6) was formed. The reaction mixture was poured into saturated NH_4Cl aqueous solution (150 mL) and extracted with ethyl acetate (100 mL * 2). The combined organic layer was washed with brine (100 mL), dried over Na_2SO_4, filtered, and concentrated to give the crude **compound 2** (7.60 g, crude) as a yellow oil. The resulting compounds are shown in **Figure 2**.

Example 3: Alternative Chemical Synthesis of ID3 Prodrug Intermediate.

In another experiment, an alternative chemical synthesis if **ID3** prodrug was performed in the following manner. To a solution of **ID3** drug moiety (3.00 g, 7.30 mmol, 1.00 *eq*) in THF (30.0 mL) was added LiHMDS (1 M, 14.6 mL, 2.00 *eq*) at -70 °C. Then the reaction mixture was stirred at -70 °C for 0.5 hr. Then a solution of **compound 3a** (6.57 g, 51.0 mmol, 4.53 mL, 6.98 *eq*) in THF (15.0 mL) was added to the mixture at -70 °C and stirred for another 1 hr. ^1H NMR showed **the ID3** drug moiety was correct. LCMS showed the drug moiety was consumed completely and the desired MS (RT = 0.877 min) was detected. The mixture was poured into the saturated solution of NH_4Cl (200 mL) and extracted with ethyl acetate (200 mL * 3). Combined organics were washed with brine (200 mL), dried over Na_2SO_4, filtered, and concentrated to give the crude product. The **compound 3** (4.00 g, crude) was obtained as a yellow oil. The resulting compounds are shown in **Figure 3**.

Example 4: Chemical Synthesis of ID3 Prodrug Comprising Stearic Acid.

In another experiment, an **ID3** Prodrug comprising Stearic Acid was synthesized in the following manner. To a solution of Stearic acid (3.39 g, 11.9 mmol, 4.01 mL, 0.80 *eq*) in DMF (400 mL) was added Ag_2CO_3 (6.16 g, 22.3 mmol, 1.01 mL, 1.50 *eq*) at 25 °C. The reaction mixture was stirred at 25 °C for 0.5 hr. Then **compound 2 (Figure 2)** (7.50 g, 14.9 mmol, 1.00 *eq*) and NaI (3.35 g, 22.3 mmol, 1.50 *eq*) were added to the mixture. After addition, the reaction mixture was stirred at 80 °C for another 12 hrs. LCMS showed that the reaction was completed, and the desired mass (RT = 1.309 mins) was detected. The reaction mixture was cooled to 25 °C and filtered through a pad of celite and washed with DCM (300 mL). The filtrate was concentrated at 50 °C to give the crude product. The crude product was purified by reverse-MPLC (TFA condition) and then concentrated under reduced pressure to give the crude product. The crude product was purified by column chromatography (SiO_2, Petroleum ether : Ethyl acetate = 5:1 - 2:1, R_f = 0.5), which was detected by TLC (Petroleum ether : Ethyl acetate = 2:1, R_f = 0.5, PMA) to give (2.36 g, 3.09 mmol, 20.7% yield, 98.3% purity) as a yellow solid, which was confirmed by ^1H NMR, ^{19}F NMR, LCMS, HPLC and SFC. The resulting compounds are shown in **Figure 7**.

Example 5: Chemical Synthesis of ID3 Prodrug Comprising Cholesterol Hemisuccinate ("CHEMS").

In another experiment, an **ID3** Prodrug comprising cholesterol hemisuccinate ("CHEMS") was synthesized in the following manner. Briefly, a mixture of **cholesterol hemisuccinate** (5.80 g, 11.9 mmol, 1.50 *eq*) and Ag_2CO_3 (3.29 g, 11.9 mmol, 540 uL, 1.50 *eq*) in DMF (15.0 mL) was stirred at 25 °C for 0.5 hr. To the mixture was added **compound 3 (Figure 3)** (4.00 g, 7.95 mmol, 1.00 *eq*) and NaI (1.43 g, 9.54 mmol, 1.20 *eq*) in DMF (15.0 mL) at 25 °C. The mixture was stirred at 80 °C for 16 hrs. LCMS showed **compound 3** was consumed completely and the desired MS (RT = 1.286 mins) was detected. The mixture was filtered to collect the filtrate. The filtrate was concentrated to give the crude product. The crude product was purified by column chromatography (silica gel, ethyl acetate/petroleum ether = 1/10 to 1/4) to give the main spot (Petroleum ether/Ethyl acetate = 2/1, R_f = 0.4). The crude compound (**Target 2)** (3.00 g, 3.15 mmol, 39.6% yield) was obtained as a light yellow solid. The resulting compounds are shown in **Figure 8**.

Example 6: Synthesis and Characterization of LNP-ID3 Liposome.

In another experiment, a liposome comprising the ID3 prodrug (denoted LNP-ID3) was synthesized in the following manner. Briefly, a stock solution of each lipid component of the LNP-ID3 was prepared in ethanol at a concentration of 20mg/ml. Accordingly, the ethanolic stock solution Hydro Soy PC[(HSPC: L-α-phosphatidylcholine, hydrogenated (Soy)], Cholesterol and DSPE-PEG (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] was prepared at the above mentioned concentration. A stock solution of ID3 prodrug (20mg/ml) was prepared in acetonitrile. Each of the lipid components (HSPC,CHOL, DSPE-PEG and ID3 prodrug) were mixed together in a molar ratio of 51:27:17:5 to synthesize LNP-ID3. The size of the liposome LNP-ID3 depends on a variety of parameters such as (i) flow rate, (ii) temperature and (iii) concentration of the lipid mixture. The optimized ratio of the lipid mixture at the molar ratio of 51:27:17:5 was preheated at 50 C°. The aqueous phase containing 1mM PBS buffer was also preheated at 50 C° before passing through a microfluidics cartridge (Precision NanoSystems, Inc.) at the flow rate of 3:1 (aqueous: organic phase, lipid mixture). The solvent was removed using a dialysis membrane of remove 12 KDa size (Sigma Aldrich) against DI water for at least 24 hrs. The dialysis water was changed a minimum of 5 times during the period of 24 hrs. to optimize the removal of the solvent. After the removal of the solvent, the LNP-ID3 was concentrated using an Amicon centrifugal filtration device (size 10 KDa, at 3000g).

Characterization of the LNP-ID3 liposome was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of LNP-ID3 liposomes (concentration of the liposome was of 0.5 -1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed

directly at 25°C. The results shown in **Figure 9** show the Zav size of the nanoparticles were approximately 80 nm with a PDI of approximately 0.203.

Additionally, Zeta potential of the LNP-ID3 liposomes in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the liposome (concentration approximately 2mg/ml in 20mM NaCl) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25°C. The results show the Zeta potential determination of LNP-ID3 was approximately -11.6 mV (**Figure 10**).

Example 7: Synthesis and Characterization of LNP-AR5-ID3 Liposome.

In another experiment, a liposome comprising the ID3 prodrug and an A2a receptor inhibitor prodrug (AR5) (denoted LNP-AR5-ID3) was synthesized and characterized in the following manner. Briefly, Using a molar ratio of 52:27:8:8:5 of HSPC:CHOL:AR5:ID3:DSPE-PEG was synthesized where a molar ratio of AR5:ID3 was fixed at 1:1. The optimized ratio of the lipid mixture at the molar ratio of 52:27:8:8:5 was preheated at 55-60°C. The final concentration of the lipid mixture was 2.5mg/ml. The aqueous phase containing 1mM PBS buffer was also preheated at 55-60°C before passing through a microfluidics cartridge (Precision NanoSystems, Inc.) at the flow rate of 3:1 (aqueous: organic phase, lipid mixture). The solvent was removed using a dialysis membrane of remove 12 KDa size (Sigma Aldrich) against DI water for a minimum of 24 hrs. The Dialysis water was changed a minimum of 5 times during the period of 24 hrs. to optimize removal of the solvent. After the removal of the solvent, the LNP-AR5-ID3 was concentrated according using an Amicon centrifugal filtration device (cut off size 10 KDa, at 3000g).

Characterization of the LNP-AR5-ID3 liposome was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of LNP-AR5-ID3 liposomes (concentration of the liposome was of 0.5 -1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed directly at 25°C. The results shown in **Figure 11** show the Zav size of the nanoparticles were approximately 91 nm with a PDI of approximately 0.212.

Additionally, Zeta potential of the LNP-AR5-ID3 liposomes in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the liposome (concentration approximately 2mg/ml in 20mM NaCl) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25°C. The results show the Zeta potential determination of LNP-AR5-ID3 was approximately -14.1 mV (**Figure 12**).

Example 8: Synthesis and Characterization of LNP-AR5-TR5-ID3 Liposome.

In another experiment, a liposome comprising the ID3 prodrug, the A2a receptor inhibitor prodrug (AR5), and Telratolimod (TR5) (denoted LNP-AR5-TR5-ID3) was synthesized and characterized in the following manner. Briefly, a lipid stock solution of HSPC, CHOL, DSPE-PEG, AR5 prodrug. TR5 was prepared in ethanol (20mg/ml) separately. Also, ID3 prodrug stock solution was prepared in acetonitrile (20mg/ml). Using a molar ratio of 51.6:27:7:2.4:7:5 of HSPC:CHOL:AR5:TR5:ID3:DSPE-PEG was synthesized. The optimized ratio of the lipid mixture at the molar ratio of 51.6:27:7:2.4:7:5 was preheated at 55-60°C. The final concentration of the Prodrug AR5 in the lipid mixture was 0.625mg/ml. The aqueous phase containing 1mM PBS buffer was also preheated at 55-60°C before passing through a microfluidics cartridge (Precision NanoSystems, Inc.) at the flow rate of 4.5:1 (aqueous: organic phase, lipid mixture). The solvent was removed using a dialysis membrane of remove 12 KDa size (Sigma Aldrich) against DI water for a minimum of 24 hrs. The Dialysis water was changed a minimum of 5 times during the period of 24 hrs. to optimize removal of the solvent. After the removal of the solvent, the LNP-AR5-TR5-ID3 was concentrated according using an Amicon centrifugal filtration device (cut off size 10 KDa, at 3000g).

Characterization of the LNP-AR5-TR5-ID3 liposome was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of LNP-AR5-TR5-ID3 liposomes (concentration of the liposome was of 0.5 -1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed directly at 25°C. The results shown in **Figure 13** show the Zav size of the nanoparticles were approximately 96 nm with a PDI of approximately 0.117.

Additionally, Zeta potential of the LNP-AR5-TR5-ID3 liposomes in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the liposome (concentration approximately 2mg/ml in 20mM NaCl) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25°C. The results show the Zeta potential determination of LNP-AR5-TR5-ID3 was approximately -20.5 mV (**Figure 14**).

Example 9: Synthesis and Characterization of LNP-ID3-NK1 Liposome.

In another experiment, a liposome comprising the ID3 prodrug, and α-galactosylceramide (α-GalCer) (NK1) (denoted LNP-ID3-NK1) was synthesized and characterized in the following manner. Briefly, A stock solution of ID3 prodrug (20mg/ml) was prepared in acetonitrile and the stock solution of NK1 (10mg/ml) was prepared in DSMO. A lipid mixture of HSPC, CHOL, ID3, NK1 and DSPE-PEG at a molar ratio of 51:29:16:0.085:4 was prepared mixing all the lipid stock solution in appropriate amount and then further diluted with ethanol to get a lipid concentration of 10mg/ml. This lipid mixture was preheated at 50 °C using a microfluidizer. Similarly, the aqueous phase containing 1mM PBS buffer

was also preheated at 50 ºC before passing through a microfluidics cartridge (Precision NanoSystems, Inc.) at the flow rate of 3:1 (aqueous: organic phase, lipid mixture). Accordingly, the molar ratio of ID3: NK1 remain at a 12:1 ratio. The solvent was removed using a dialysis membrane of remove 12 KDa size (Sigma Aldrich) against DI water for a minimum of 24 hrs. The Dialysis water was changed a minimum of 5 times during the period of 24 hrs. to optimize removal of the solvent. After the removal of the solvent, the LNP-ID3-NK1 was concentrated according using an Amicon centrifugal filtration device (cut off size 10 KDa, at 3000g).

Characterization of the LNP-ID3-NK1 liposome was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of LNP-ID3-NK1 liposomes (concentration of the liposome was of 0.5 -1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed directly at 25ºC. The results shown in **Figure 15** show the Zav size of the nanoparticles were approximately 80 nm with a PDI of approximately 0.189.

Additionally, Zeta potential of the LNP-ID3-NK1 liposomes in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the liposome (concentration approximately 2mg/ml in 20mM NaCl) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25ºC. The results show the Zeta potential determination of LNP-ID3-NK1 was approximately -13.1 mV (**Figure 16**).

Example 10: Synthesis and Characterization of Mitoxantrone loaded LNP-ID3-NK1 Liposome.

In another experiment, a liposome comprising Mitoxantrone (MTO) was loaded with LNP-ID3-NK1 (denoted LNP-ID3-NK1-MTO) was synthesized and characterized in the following manner. Briefly, a stock solution of MTO (0.6 mg/ml) was prepared in DI water. Ten (10) ml of this stock solution was added to 20ml of LNP-ID3-NK1 formulation containing 300mM ammonium sulfate solution and incubated at 40 ºC for 2 hrs. After 2 hours of incubation time the entire liposome solution containing MTO was dialyzed (12KDa dialysis membrane, 8 hrs. dialysis time) to remove the free MTO (i.e. not incorporated in the liposomes). After dialysis LNP-ID3-NK1 loading MTO (LNP-ID3-NK1-MTO) was concentrated using Amicon centrifugal filtration device (cut off size 10 KDa, at 2500g).

Characterization of the LNP-ID3-NK1-MTO liposome was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of LNP-ID3-NK1-MTO liposomes (concentration of the liposome was of 0.5 -1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed directly at 25ºC. The results shown in **Figure 17** show the Zav size of the nanoparticles were approximately 90 nm with a PDI of approximately 0.084.

Additionally, Zeta potential of the LNP-ID3-NK1-MTO liposomes in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the liposome (concentration approximately 2mg/ml in 20mM NaCl) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25^{0}C. The results show the Zeta potential determination of LNP-ID3-NK1-MTO was approximately -11.6 mV (**Figure 18**).

Example 11: Determination of LNP-ID3-NK1-MTO Liposome Encapsulation and Loading Efficiency.

In another experiment, the LNP-ID3-NK1-MTO liposome encapsulation and loading efficiency was determined by disintegrating the liposomes and measuring MTO with ultraviolet–visible (UV-vis) spectroscopy in the following manner. Briefly, after separating out the free MTO by dialysis, the liposome was disintegrated with Dimethyl Sulfoxide. The entrapment efficiency was determined using the following formula:

$$\text{Encapsulation efficiency}(\%)=[\text{MTO}]_f/[\text{MTO}]_t\times 100$$

where $[\text{MTO}]_f$ is the concentration of MTO encapsulated in LNP-ID3-NK1 and $[\text{MTO}]_t$ is the total concentration of MTO (meaning the total amount of MTO added initially : loaded + free). The MTO concentration was then determined using a Nanodrop 2000C UV-vis spectrophotometer. It was found that entrapment efficiency of MTO in LNP-ID3-NK1-MTO is approximately 94%. Additionally, the loading efficiencies of the MTO in LNP-ID3-NK1-MTO were determined using the following formula:

$$\text{Loading efficiency}(\%)=[\text{MTO}]_{fwt}/[\text{LNP-ID3-NK1-MTO}]_{fwt}\times 100$$

$[\text{MTO}]_{fwt}$ is the total weight of MTO encapsulated in MTO and $[\text{LNP-ID3-NK1-MTO}]_{fwt}$ is the total weight of the LNP-ID3-NK1 encapsulating MTO (LNP-ID3-NK1-MTO). The loading efficiency of the MTO in LNP-ID3-NK1-MTO was found to be around 4.1%w/w.

Example 12: Tumor Inhibition of LNP-ID3 in Combination with Other Liposome(s) Using B16F10 Cells *In Vivo*.

Evaluation of LNP-ID3 in combination with a plurality of various liposomes of the disclosure was performed using the following protocols. Briefly, murine melanoma cancer B16F10 cells (cells (0.2×10^6) were inoculated subcutaneously in the right rear flank region of C57BL/6 mice. Animals were treated with vehicle control, LNP-MTO (Mitoxantrone dihydrochloride in liposome form) at 3 mg/kg, combination of LNP-ID3 and LNP-TB4 (a TGF-β inhibitor-Stearic Acid) in liposome form at 3 mg/kg, combination of

LNP-ID3 and LNP-TR6 (TLR1/2 agonist-CHEMS in liposome form) at 3 mg/kg and combination of LNP-MTO, LNP-ID3 and LNP-TB4 at 3 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension), and W is tumor width (the longest tumor dimension perpendicular to L). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day sixteen (16).

The results show that treatment of LNP-MTO as a single agent showed anti-tumor activity, the TGI was calculated at 32.56% when compared with Vehicle group ($p < 0.05$). In addition, combination treatment of LNP-TB4+LNP-MTO+LNP-ID3 had significant anti-tumor activities when compared with Vehicle group, the TGIs were 32.86% (all $p < 0.05$). (**Figure 19**).

Example 13: Tumor Inhibition of LNP-ID3 in Combination with LNP-AR5 Using B16F10 Cells *In Vivo*.

In another experiment, evaluation of LNP-ID3 in combination with LNP-AR5 was performed using the following protocols. Briefly, murine melanoma cancer B16F10 cells (cells (0.2×10^6) were inoculated subcutaneously in the right rear flank region of C57BL/6 mice. Animals were treated with vehicle control, LNP-AR5 at 3mg/kg and combination of LNP-AR5 and LNP-ID3 at 3 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day fifteen (15).

The results show that combination treatment of LNP-AR5+LNP-ID3 had significant mild anti-tumor activity when compared with Vehicle group, the TGI was 35.89% (all $p < 0.05$). (**Figure 20**).

Example 14: Tumor Inhibition of LNP-ID3-NK1 Using B16F10 Cells *In Vivo*.

In another experiment, evaluation of LNP-ID3-NK1 was performed using the following protocols. Briefly, murine melanoma cancer B16F10 cells (cells (0.2×10^6) were inoculated subcutaneously in the right rear flank region of C57BL/6 mice. Animals were treated with vehicle control, LNP-MTO (Mitoxantrone dihydrochloride in liposome form) at 2 mg/kg, LNP-ID3-NK1 (in liposome form in 1:12 ratio) at 3/0.25 mg/kg, combination of LNP-MTO at 3 mg/kg and LNP-ID3-NK1 at 3/0.25 mg/kg, and combination of LNP-ID3-NK1 at 3/0.25 mg/kg and PD3 (a PD-1 receptor inhibitor-cholesterol in liposome form) at 5 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x

W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day eighteen (18).

The results show that treatment with LNP-ID3-NK1, LNP-MTO+LNP-ID3-NK1, and LNP-ID3-NK1+LNP-PD3 caused a significant tumor growth inhibition when compared with Vehicle group, the TGIs were calculated between 42.71% and 54.31 % ($p<0.05$) respectively. . (**Figure 21**).

Example 15: Tumor Inhibition of LNP-ID3 and LNP-ID3-NK1 Using CT26 Cells *In Vivo*.

In another experiment, evaluation of LNP-ID3 and LNP-ID3-NK1 was performed using the following protocols. Briefly, murine colon carcinoma CT26 cells (cells (0.1×10^6) were inoculated subcutaneously in the right rear flank region of Balb/C mice. Animals were treated with vehicle control, LNP-DOX (Doxorubicin in liposome form) at 3 mg/kg, LNP-ID3 at 3 mg/kg, LNP-NK1 at 0.25 mg/kg, combination of LNP-DOX and LNP-ID3 at 3 mg/kg, LNP-ID3-NK1 (in 1:12 ratio) at 3/0.25 mg/kg, and combination of LNP-DOX at 3 mg/kg and LNP-ID3-NK1 at 3/0.25 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The study was terminated when the mean tumor size of the vehicle treated group reached over 2500 mm^3 (day 27 after tumor inoculation). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day twenty-seven (27).

The results show treatment with LNP-DOX, LNP-DOX+LNP-ID3, LNP-DOX+ LNP-ID3-NK1 caused a significant TGI when compared with Vehicle group ($p<0.01$). TGIs were recorded at 91%, 80%, and 89.2%, respectively. (**Figure 22**).

Example 16: Tumor Inhibition of LNP-ID3 Using CT26 Cells *In Vivo*.

In another experiment, evaluation of LNP-ID3 was performed using the following protocols. Murine colon carcinoma CT26 cells (cells (0.1×10^6) were inoculated subcutaneously in the right rear flank region of Balb/C mice. Animals were treated with vehicle control, LNP-DOX (Doxorubicin in liposome form) at 3 mg/kg, LNP-ID3 at 3 mg/kg, combination of LNP-DOX and LNP-ID3 at 3 mg/kg, combination of LNP-ID3 at 3 mg/kg and LNP-NK1 at 0.25 mg/kg, combination of LNP-ID3 and LNP-TR6 at 3 mg/kg, combination of LNP-ID3 at 3mg/kg and LNP-TR5 at 4 mg/kg, and LNP-ID3 at 3mg/kg and LNP-TR8 (3D-6-acyl-PHAD in liposome form) at 2 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated

using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L).

The results show that LNP-DOX alone or in combination with LNP-ID3 provided therapeutic benefit by significantly extending the survival of CT26 tumor-bearing mice. (**Figure 23**).

Example 17: Tumor Inhibition of LNP-ID3 Using MC-38 Cells *In Vivo.*

In another experiment, further evaluation of LNP-ID3 was performed using the following protocols. Murine colon adenocarcinoma MC-38 cells (cells (1×10^6) were inoculated subcutaneously in the right rear flank region of C57BL/6 mice. Animals were treated with vehicle control, combination, LNP-DOX (Doxorubicin in liposome form) at 4 mg/kg, anti-PD1 antibody at 10 mg/kg, combination of LNP-ID3 and LNP-AR5 at 4 mg/kg, combination LNP-DOX + LNP-ID3 + LNP-AR5 at 4 mg/kg, and combination of LNP-ID3 3.5 mg/kg, LNP-AR5 at 3.5 mg/kg, and LNP-TR5 at 4 mg/kg for the first dose and 2 mg/kg for the rest of the rest of the doses two times weekly through i.v injection. The animals received only two doses of LNP-DOX and then it was replaced with Vehicle control. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day nineteen (19).

The results show treatment with LNP-DOX, LNP-DOX+LNP-ID3+LNP-AR5, and LNP-ID3+ LNP-AR5 showed a substantial TGI when compared with Vehicle group ($p<0.05$). TGIs were recorded at 74.39%, 90.40% and 73.77%, respectively. (**Figure 24**).

Example 18: Tumor Inhibition of LNP-ID3 In Combination with LNP-AR5 Using H22 Cells *In Vivo.*

In another experiment, evaluation of LNP-ID3 in combination with LNP-AR5 was performed using the following protocols. Briefly, murine hepatocellular carcinoma H22 cells (cells (2×10^6) were inoculated subcutaneously in the right rear flank region of Balb/C mice. Animals were treated with vehicle control, and combination of LNP-ID3 and LNP-AR5 at 4 mg/kg two times weekly through i.v injection. Tumor volumes were measured 3 times in two dimensions using a caliper, and the volume was calculated using the formula: V = (L x W x W) X 0.5, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L). The tumor growth inhibition (TGI) was calculated based on the tumor size data of day fourteen (14). The results show that LNP-ID3 + LNP-AR5 caused a TGI when compared with Vehicle. TGI were recorded at 43.28%. (**Figure 25**).

Example 19: Measurement of IDO-1 Activity.

IDO-1 activity was measured indirectly using kynurenine levels using the following protocols. Briefly, hIDO1-HEK293 recombinant cells were plated overnight. The following day the cells were left untreated or treated with 10uM of ID3, ID3-SA or LNP-ID3-SA for twenty-four (24) hours. The IDO-1 activity was measured by indirect measurement of kynurenine levels by analyzing absorption at 480 nm using IDO1 Cellular Activity QuickDetectTM kit (PBSBioscience, San Diego, CA).

The results show that the untreated cells showed high level of IDO-1 activity and treatment with ID3, ID3-SA and LNP-ID3-SA inhibited IDO-1 activity. These results confirm ID3 activity in prodrug and liposome form. (**Figure 26**).

Example 20: Synthesis and Characterization of SLNP-AR5-TR5-ID3 Solid-Lipid Nanoparticle.

In another experiment, a solid-lipid nanoparticle ("SLNP") comprising ID3-AR5-TR5 (denoted SLNP-ID3-AR5-TR5 was prepared using the following protocol, using Moliwol 488 (Polyvinyl alcohol) as an emulsifier. Briefly, a lipid stock solution of HSPC, CHOL, DSPE-PEG, TR5 was prepared in ethanol (20mg/ml) separately. **ID3** prodrug stock solution was prepared in acetonitrile (20mg/ml). The of lipid mixture of HSPC, CHOL, AR5, TR5 , ID3 and DSPE-PEG at a molar ratio of 51.6:27:7:2.4:7:5 was mixed together and then was diluted with ethanol to get a lipid concentration of 5 mg/ml. This lipid mixture was heated at 50 °C using a heating block attachment in a microfluidizer. Similarly, the aqueous phase containing 2% Moliwol 488 solution was also preheated at 50 °C before passing through the microfluidics cartridge (Precision NanoSystems, Inc.) at the flow rate of 4:1 (aqueous: organic phase, lipid mixture). The solvent was removed using a dialysis membrane of cut off approximately a 12 KDa size (Sigma Aldrich) against DI water for at least 24 hrs. The Dialysis water was changed a minimum five (5) times during the period of 24 hrs. to maximize the removal of the solvent. After the removal of the solvent, SLNPs were passed through a 0.2 micron filter membrane (cellulose acetate). The SLNP-AR5-TR5-ID3 was concentrated using Amicon centrifugal filtration device (cut off size 10 KDa, at 3000g).

Characterization of the SLNP-AR5-ID3-TR5 solid-lipid nanoparticle was determined using a Malvern Zetasizer (Malvern Instrumentation Co., Westborough, MA, USA). Briefly, two (2) ml of SLNP-AR5-ID3-TR5 (concentration of 1 mg/ml) was placed in a 4-sided, clear, plastic cuvette and analyzed directly at 25°C. The results shown in **Figure 27** show the Zav size of the nanoparticles were approximately 106 nm with a PDI of approximately 0.171.

Additionally, Zeta potential of the SLNP-AR5-ID3-TR5 solid-lipid nanoparticle in aqueous dispersion was determined using a Malvern zeta seizer Instrument (Malvern Instrumentation Co, Westborough, MA, USA). Briefly, approximately one (1) ml of the nanoformulation (concentration

approximately 3mg/ml in DI water) was placed in a disposable capillary zeta potential cell available for the Zetasizer. The measurement was done at 25^{0}C. The results show the Zeta potential determination of SLNP-AR5-ID3-TR5 was approximately 9.87 mV (**Figure 28**).

Example 21: Human Clinical Trials for the Treatment of Human Carcinomas through the Use of Formilated and/or Co-Formulated Liposomes Comprising IDO Prodrugs.

Formulated and/or co-formulated liposomes containing IDO prodrugs are used in accordance with the present invention which specifically accumulate in a tumor cell and are used in the treatment of certain tumors and other immunological disorders and/or other diseases. In connection with each of these indications, two clinical approaches are successfully pursued.

I.) Adjunctive therapy: In adjunctive therapy, patients are treated with formulated and/or co-formulated liposomes containing IDO prodrugs in combination with a chemotherapeutic or pharmaceutical or biopharmaceutical agent or a combination thereof. Primary cancer targets are treated under standard protocols by the addition of formulated and/or co-formulated liposomes containing IDO prodrugs. Protocol designs address effectiveness as assessed by the following examples, including but not limited to, reduction in tumor mass of primary or metastatic lesions, increased progression free survival, overall survival, improvement of patients health, disease stabilization, as well as the ability to reduce usual doses of standard chemotherapy and other biologic agents. These dosage reductions allow additional and/or prolonged therapy by reducing dose-related toxicity of the chemotherapeutic or biologic agent.

II.) Monotherapy: In connection with the use of the formulated and/or co-formulated liposomes containing IDO prodrugs in monotherapy of tumors, the formulated and/or co-formulated liposomes containing IDO prodrugs are administered to patients without a chemotherapeutic or pharmaceutical or biological agent. In one embodiment, monotherapy is conducted clinically in end-stage cancer patients with extensive metastatic disease. Protocol designs address effectiveness as assessed by the following examples, including but not limited to, reduction in tumor mass of primary or metastatic lesions, increased progression free survival, overall survival, improvement of patients health, disease stabilization, as well as the ability to reduce usual doses of standard chemotherapy and other biologic agents.

Dosage

Dosage regimens may be adjusted to provide the optimum desired response. For example, a single formulated and/or co-formulated liposome containing IDO prodrugs may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. "Dosage Unit Form" as used herein refers to

physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the formulated and/or co-formulated liposomes containing IDO prodrugs, (b) the individual mechanics of the combination compound, if any, (c) the particular therapeutic or prophylactic effect to be achieved, and (d) the limitations inherent in the art of compounding such an compound for the treatment of sensitivity in individuals.

Clinical Development Plan (CDP)

The CDP follows and develops treatments of using formulated and/or co-formulated liposomes containing IDO prodrugs in connection with adjunctive therapy or monotherapy. Trials initially demonstrate safety and thereafter confirm efficacy in repeat doses. Trials are open label comparing standard chemotherapy and/or the current standard of therapy plus formulated and/or co-formulated liposomes containing IDO prodrugs. As will be appreciated, one non-limiting criteria that can be utilized in connection with enrollment of patients is expression of IDO-1 in a tumor as determined by standard detection methods known in the art.

It is believed that formulated and/or co-formulated liposomes, or any of the embodiments disclosed herein, may possess satisfactory pharmacological profile and promising biopharmaceutical properties, such as toxicological profile, metabolism and pharmacokinetic properties, solubility, and permeability. It will be understood that determination of appropriate biopharmaceutical properties is within the knowledge of a person skilled in the art, e.g., determination of cytotoxicity in cells or inhibition of certain targets or channels to determine potential toxicity.

The present invention is not to be limited in scope by the embodiments disclosed herein, which are intended as single illustrations of individual aspects of the invention, and any that are functionally equivalent are within the scope of the invention. Various modifications to the models, methods, and life cycle methodology of the invention, in addition to those described herein, will become apparent to those skilled in the art from the foregoing description and teachings, and are similarly intended to fall within the scope of the invention. Such modifications or other embodiments can be practiced without departing from the true scope and spirit of the invention.

Table I. Examples of Lipids.

No.	Abbreviation	Name / Chemical Formula
1	CHOL	Cholesterol
2	DPPG•Na	1,2-dipalmitoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)
3	DMPG•Na	1,2-dimyristoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)
4	Lyso PC	1-decanoyl-2-hydroxy-sn-glycero-3-phosphocholine
5	(Δ9-Cis) PG	1,2-dioleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)
6	Soy Lyso PC	L-α-lysophosphatidylcholine (Soy)
7	PG	1,2-dilauroyl-sn-glycero-3-phospho-(1'-rac-glycerol) (sodium salt)
8	PA-PEG3-mannose	1,2-dipalmitoyl-sn-glycero-3-phospho((ethyl-1',2',3'-triazole)triethyleneglycolmannose) (ammonium salt)
9	C16 PEG2000 Ceramide	N-palmitoyl-sphingosine-1-{succinyl[methoxy(polyethylene glycol)2000]}
10	MPLA	Monophosphoryl Lipid A

Table II. Examples of Helper Lipids.

No.	Abbreviation	Name
1	DOTAP	1,2-dioleoyl-3-trimethylammonium-propane (chloride salt)
2	DODMA	1,2-dioleyloxy-3-dimethylaminopropane
3	DLinDMA	1,2-dilinoleyloxy-3-dimethylaminopropane
4	DLin-KC2-DMA	2,2-dilinoleyl-4-dimethylaminoethyl-[1,3]-dioxolane
5	Δ9-Cis PE (DOPE)	1,2-Dioleoyl-sn-glycero-3-phosphoethanolamine
6	DOPC	1,2-dioleoyl-sn-glycero-3-phosphocholine
7	CHOL	Cholesterol
8	PEG-C-DMA	N-[(methoxy poly(ethylene glycol)2000)carbamyl]-1,2-dimyristyloxlpropyl-3-amine
9	CHEMS	cholesteryl hemisuccinate
10	DPPC	1,2-dipalmitoyl-sn-glycero-3-phosphocholine
11	DSPC	1,2-distearoyl-sn-glycero-3-phosphocholine
12	MO-CHOL	4-(2-aminoethyl)-morpholino-cholesterolhemisuccinate

Table III. Examples of Phospholipids / Fatty Acids.

No.	Name
1	Oleic acid
2	linolenic acid
3	arachidonic acid
4	docosahexaenoic (DHA)
5	Palmitic acid
6	Palmitoleic acid
7	Stearic acid
8	Eicosapentaenoic acid (EPA)
9	DSPE-PEG(2000) Carboxylic Acid (1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)
10	DOPE-PEG(2000) Carboxylic acid (1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-[carboxy(polyethylene glycol)-2000] (sodium salt)

CLAIMS:

1) An IDO prodrug composition comprising,
   (i) a drug moiety;
   (ii) a lipid moiety; and
   (iii) a linkage unit ("LU"),

whereby the drug moiety comprises an IDO antagonist and whereby the LU conjugates the drug moiety with the lipid moiety.

2) The IDO prodrug of claim 1, wherein the drug moiety comprises the chemical structure set forth as **ID3**.
3) The IDO prodrug of claim 1, wherein the lipid moiety comprises CHEMS.
4) The IDO prodrug of claim 1, wherein the lipid moiety comprises Stearic Acid.
5) A liposome (LPN) comprising, an IDO prodrug whereby the liposome releases an active IDO inhibitor after cleavage of a LU.
6) The liposome of claim 5, wherein the IDO prodrug comprises **ID3**.
7) The liposome of claim 5, wherein the IDO prodrug comprises **ID3** and further comprises **TR3**.
8) The liposome of claim 5, wherein the IDO prodrug comprises **ID3** and further comprises **TR5**.
9) The liposome of claim 8, wherein **TR5** comprises Telratolimod.
10) The liposome of claim 5, wherein the IDO prodrug comprises **ID3** and further comprises **AR5**.
11) The liposome of claim 10, wherein **AR5** comprises an A2a receptor inhibitor.
12) The liposome of claim 5, whereby the liposome is further co-formulated with an immune modulating agent, wherein the immune modulating agent is selected from the group consisting of immunogenic-cell death inducing chemotherapeutics, toll-receptor agonists, STING agonists, CTLA-4 inhibitors, PD-1/PD-L1 inhibitors and/or prodrugs thereof.
13) The liposome of claim 5, whereby the liposome is further co-formulated with an ICD-inducing chemotherapeutic, wherein the ICD-inducing chemotherapeutic is selected from the group consisting of DOX, MTO, OXA, CP, Bortezomib, Carfilzimib, or Paclitaxel.
14) The liposome of claim 5, further comprising DOX.
15) The liposome of claim 5, further comprising MTO.
16) The liposome of claim 13, further comprising DOX.
17) The liposome of claim 13, further comprising MTO.
18) A method of treating a subject suffering or diagnosed with cancer comprising,

(i) administering to a subject in need of such treatment an effective amount of a liposome, wherein the liposome comprises an IDO prodrug; and

(ii) a pharmaceutically acceptable salt thereof.

19) The method of claim 18, wherein the IDO prodrug comprises **ID3**.

20) The method of claim 18, wherein the cancer is colon cancer.

Figure 1. Chemical Synthesis for Protected ID3

Figure 2. Chemical Synthesis for ID3 Prodrug Intermediate

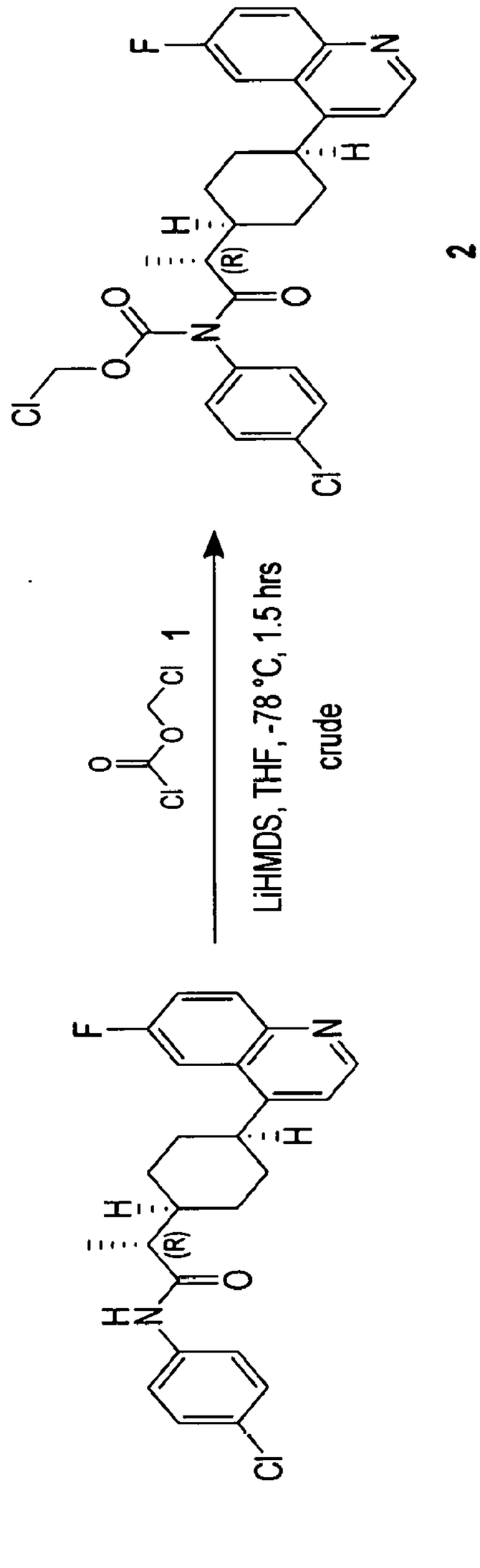
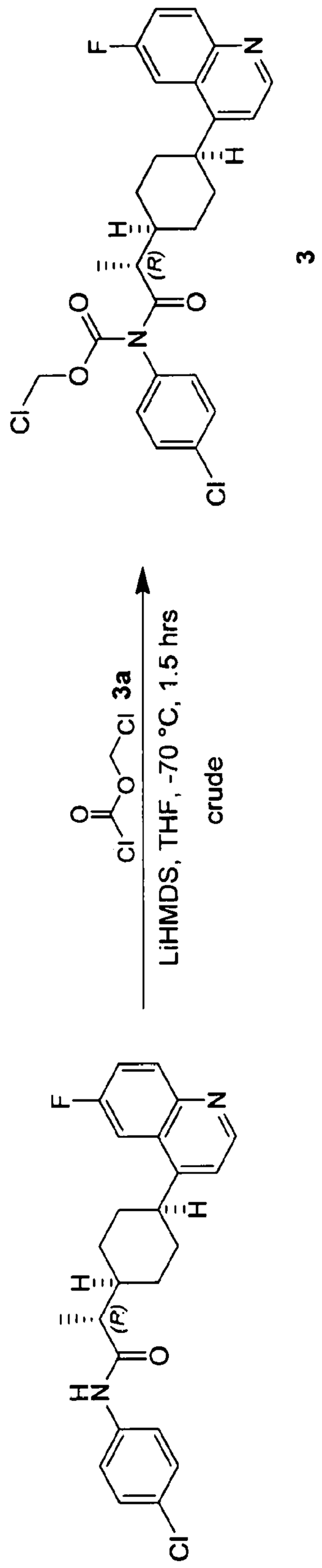


Figure 3. Chemical Synthesis for **ID3** Prodrug Intermediate



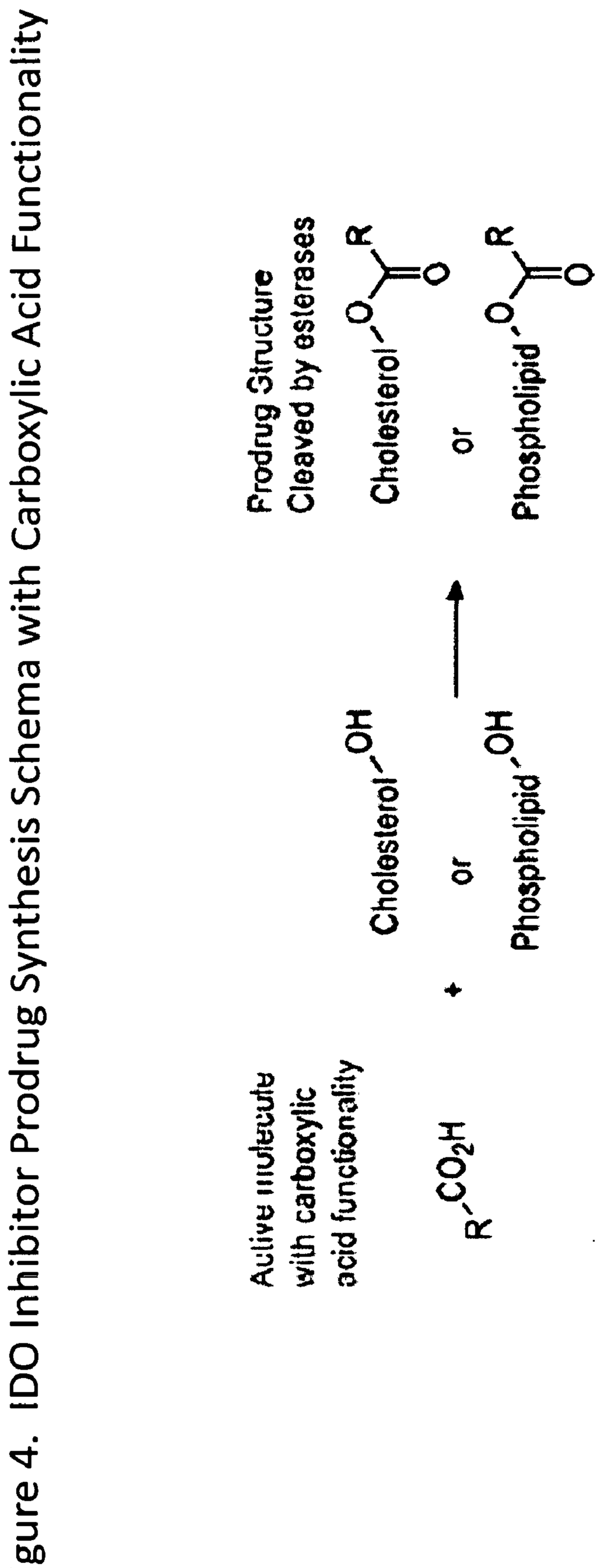


Figure 4. IDO Inhibitor Prodrug Synthesis Scheme with Carboxylic Acid Functionality

Figure 5. IDO Inhibitor Prodrug Synthesis Scheme with Alcohol Functionality

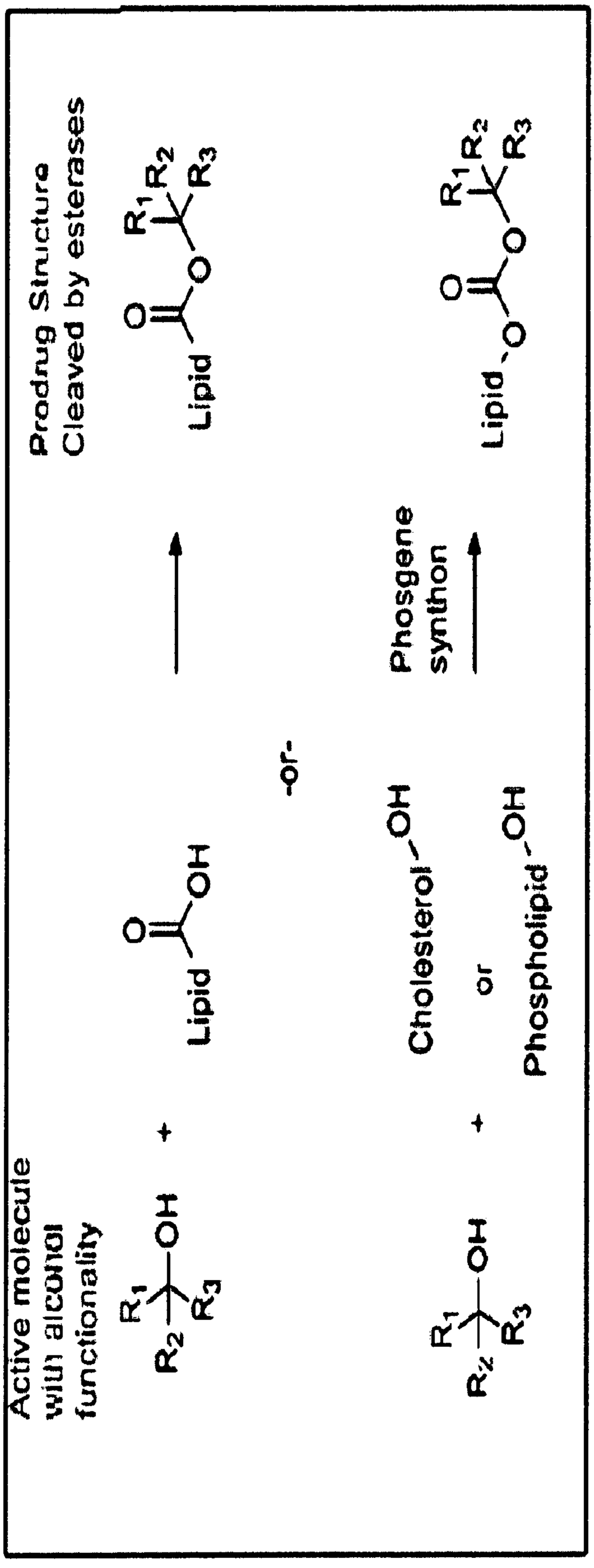


Figure 6. IDO Inhibitor Prodrug Synthesis Schema with Secondary Amine, Amide, or Aniline Functionality

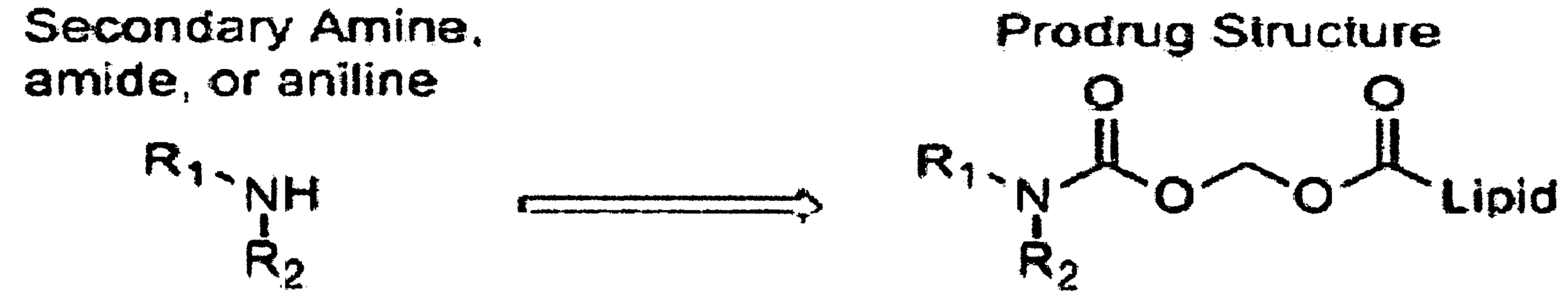


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Figure 7. Chemical Synthesis for **ID3** Prodrug Comprising Stearic Acid

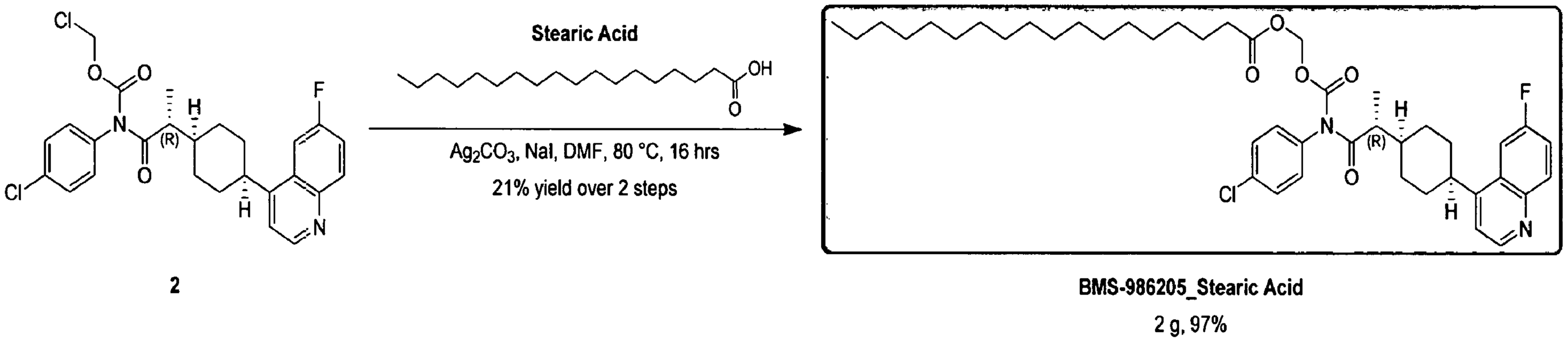

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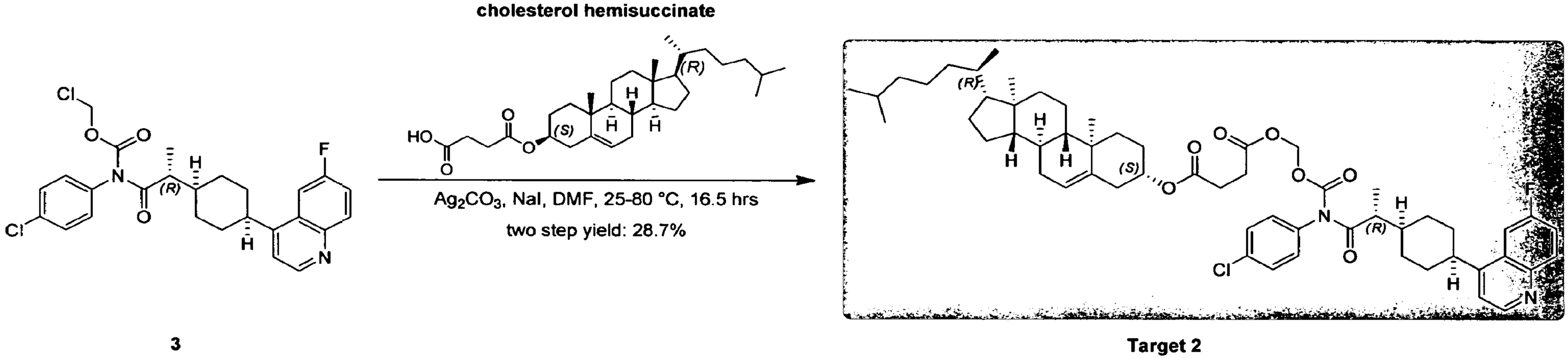
Figure 8. Chemical Synthesis for **ID3** Prodrug Comprising Cholesterol Hemisuccinate ("CHEMS")



3

Target 2

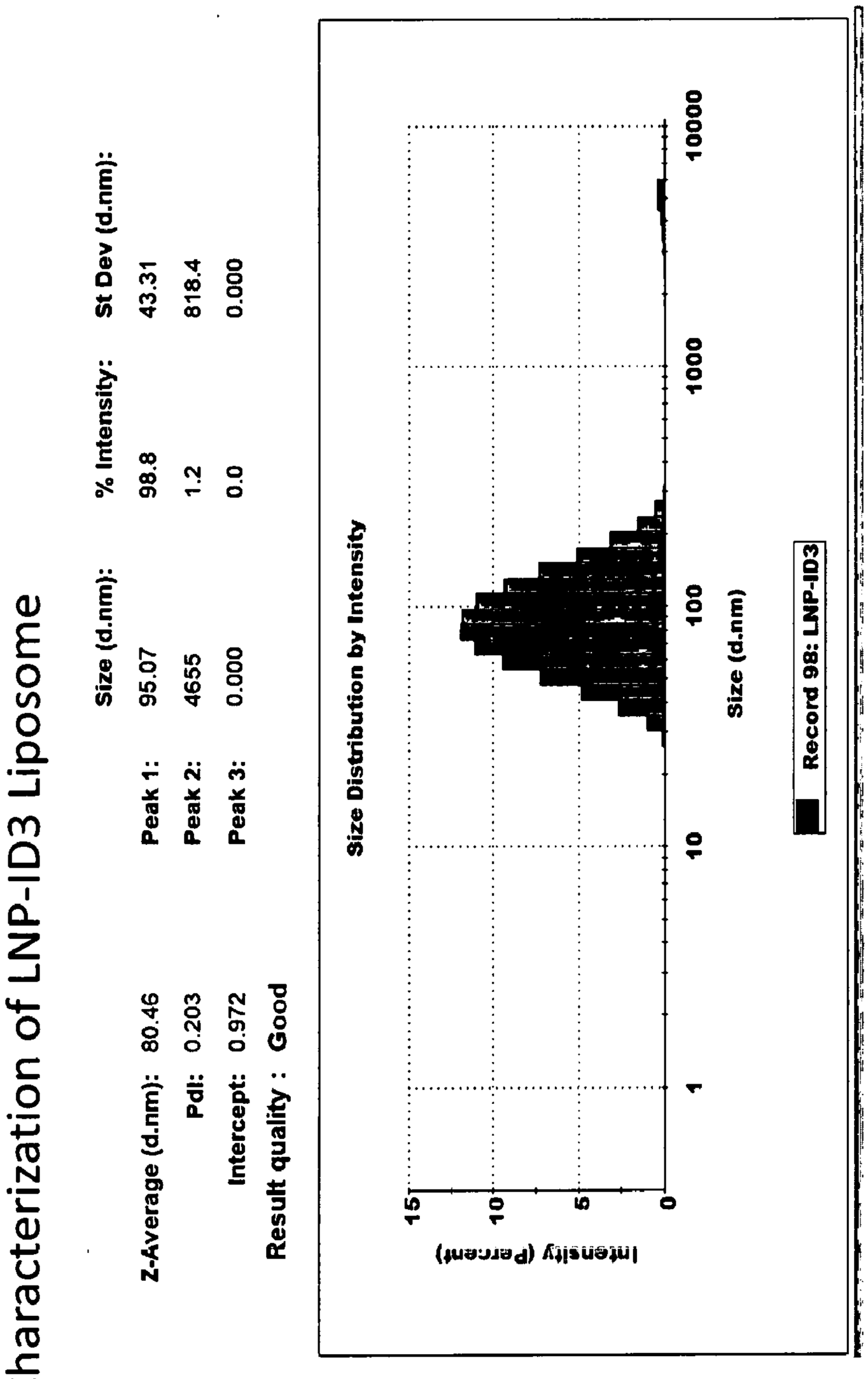

Characterization of LNP-ID3 Liposome
Size (d.nm): % Intensity: St Dev (d.nm):
95.07 98.8 43.31
4655 1.2 818.4
0.000 0.0 0.000
Z-Average (d.nm): 80.46
PdI: 0.203
Intercept: 0.972
Result quality : Good
Peak 1:
Peak 2:
Peak 3:
Size Distribution by Intensity
Intensity (Percent)
Size (d.nm)
Record 98: LNP-ID3

Figure 9.

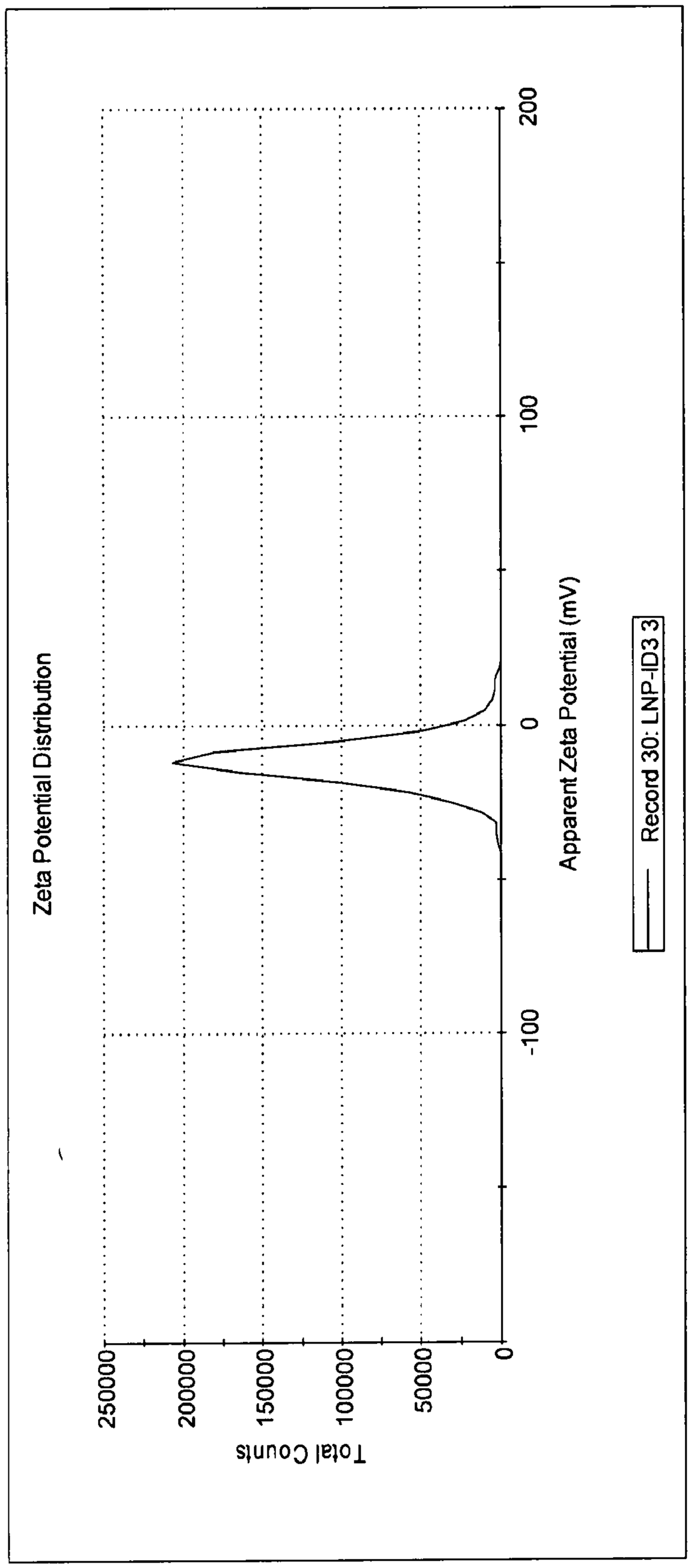

Characterization of LNP-ID3 Liposome (Zeta Potential)
Zeta Potential Distribution
Apparent Zeta Potential (mV)
200
100
0
-100
Record 30: LNP-ID3 3
Total Counts
250000
200000
150000
100000
50000
0

Figure 10.

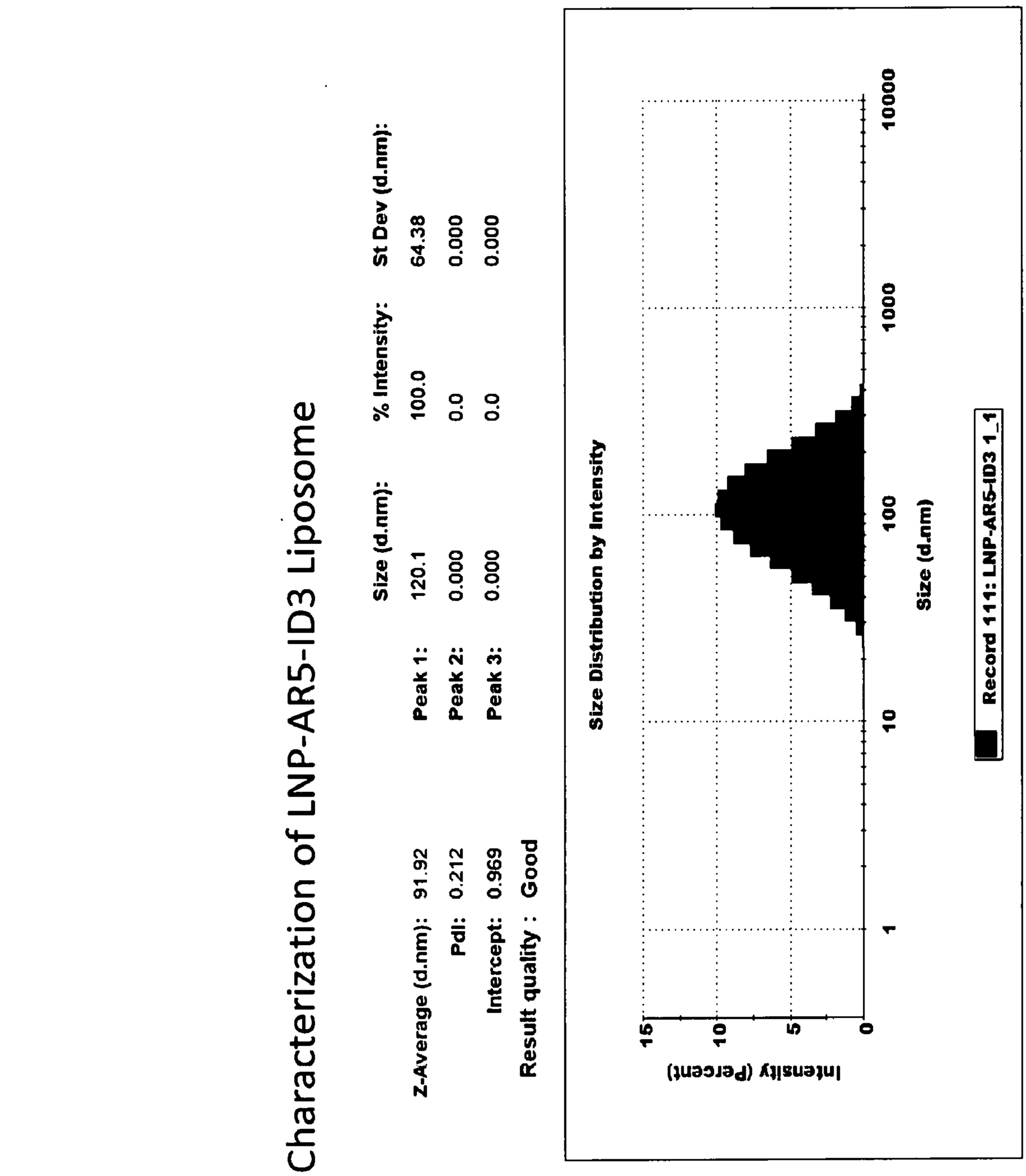

Characterization of LNP-AR5-ID3 Liposome
Size (d.nm): % Intensity: St Dev (d.nm):
Peak 1: 120.1 100.0 64.38
Peak 2: 0.000 0.0 0.000
Peak 3: 0.000 0.0 0.000
Z-Average (d.nm): 91.92
PdI: 0.212
Intercept: 0.969
Result quality : Good
Size Distribution by Intensity
Intensity (Percent)
15
10
5
0
1
10
100
1000
10000
Size (d.nm)
Record 111: LNP-AR5-ID3 1_1

Figure 11.

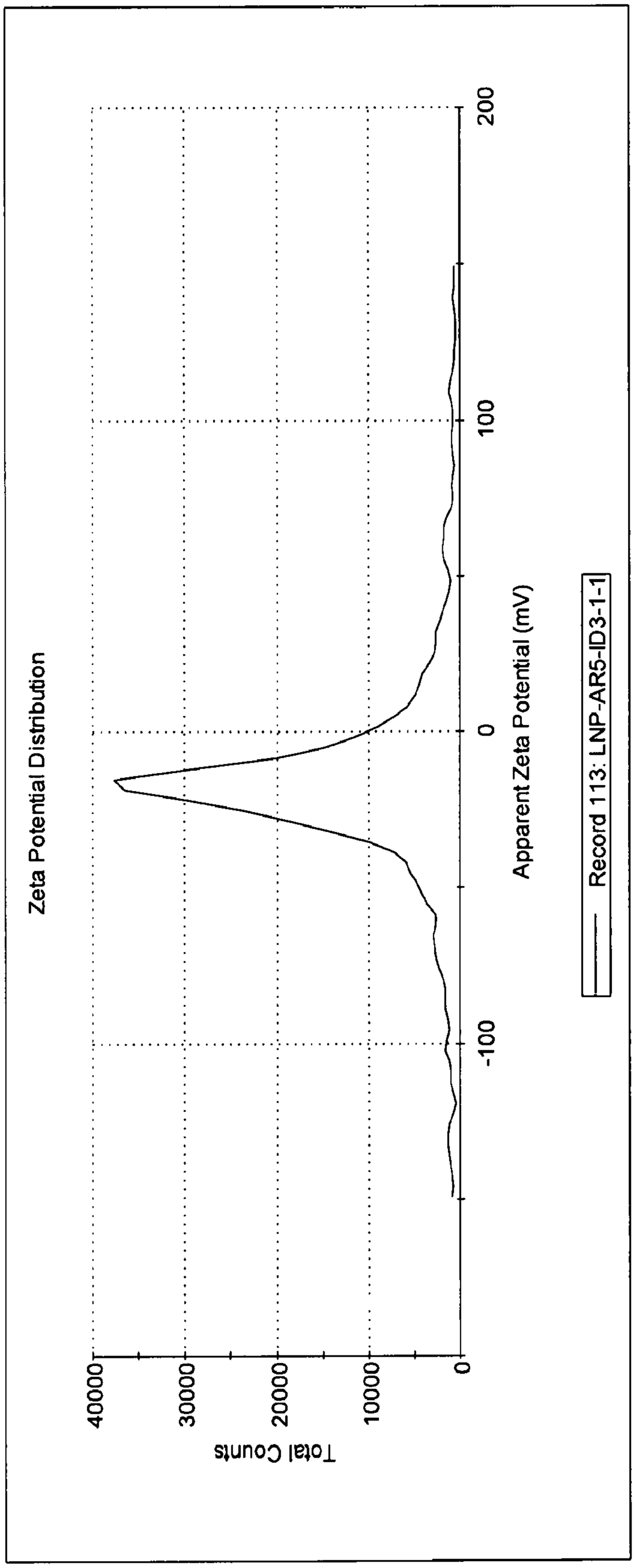

Characterization of LNP-AR5-ID3 Liposome (Zeta Potential)
Zeta Potential Distribution
Apparent Zeta Potential (mV)
Total Counts
Record 113: LNP-AR5-ID3-1-1
200
100
0
-100
40000
30000
20000
10000
0

Figure 12.

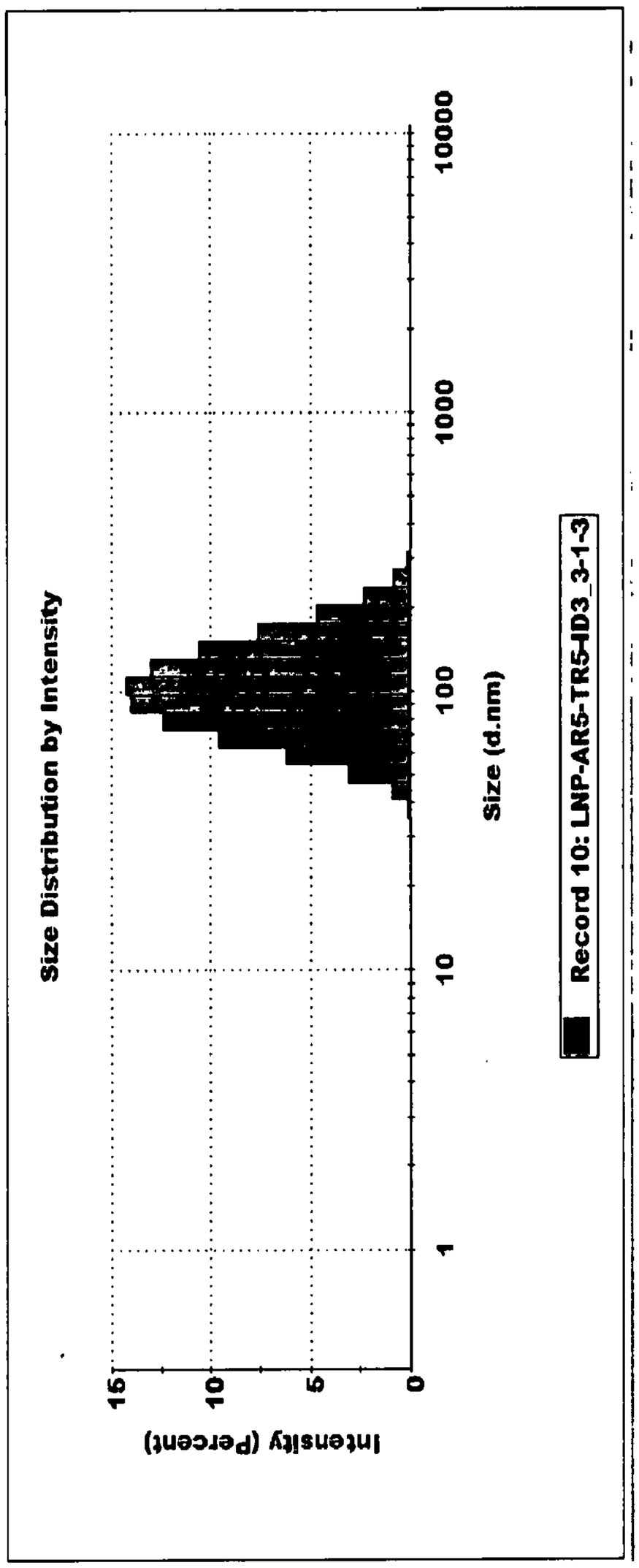

Characterization of LNP-AR5-TR5-ID3 Liposome
Size (d.nm): % Intensity: St Dev (d.nm):
110.1 100.0 42.91
0.000 0.0 0.000
0.000 0.0 0.000
Z-Average (d.nm): 96.16
PdI: 0.117
Intercept: 0.968
Result quality : Good
Peak 1:
Peak 2:
Peak 3:
Size Distribution by Intensity
Intensity (Percent)
Size (d.nm)
Record 10: LNP-AR5-TR5-ID3_3-1-3

Figure 13.

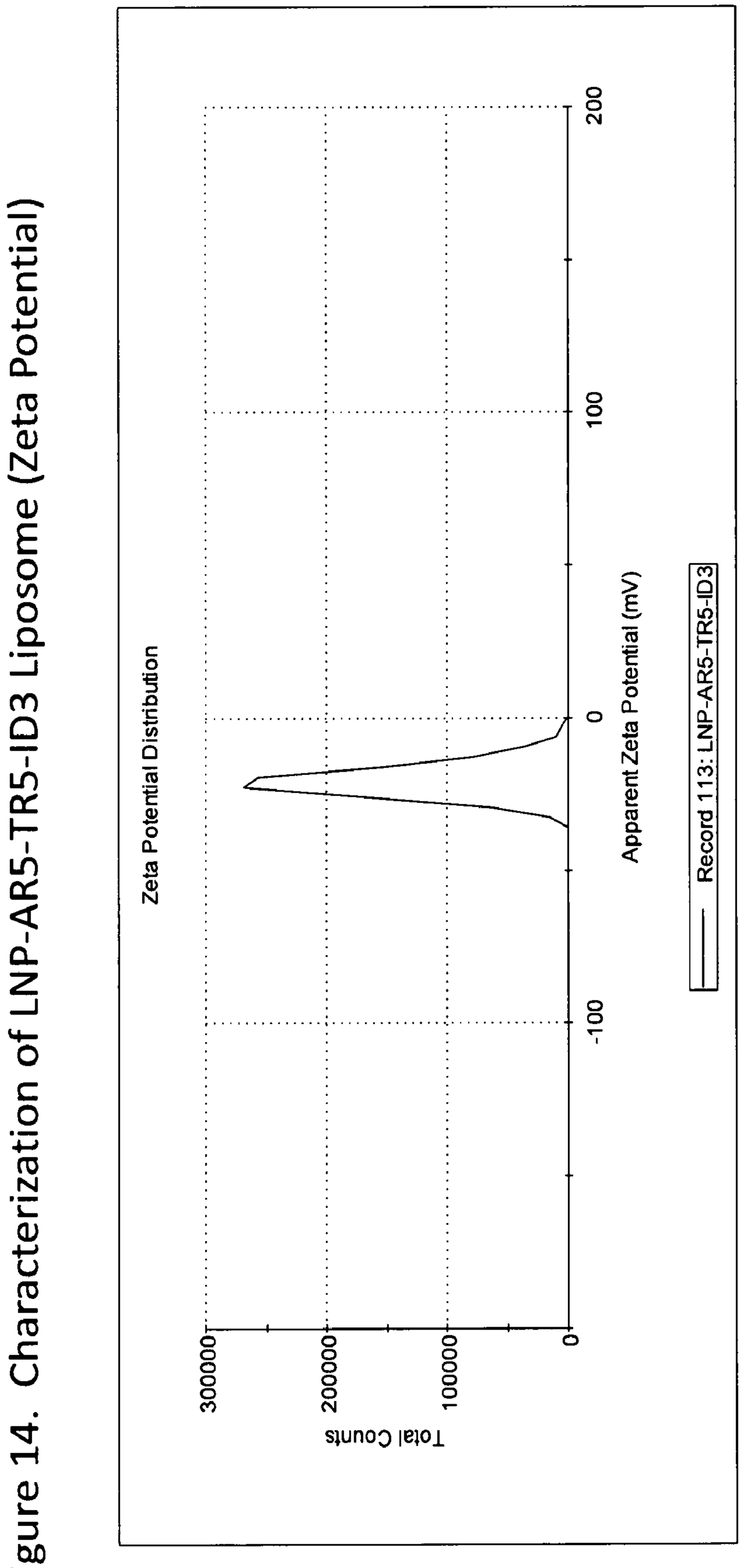
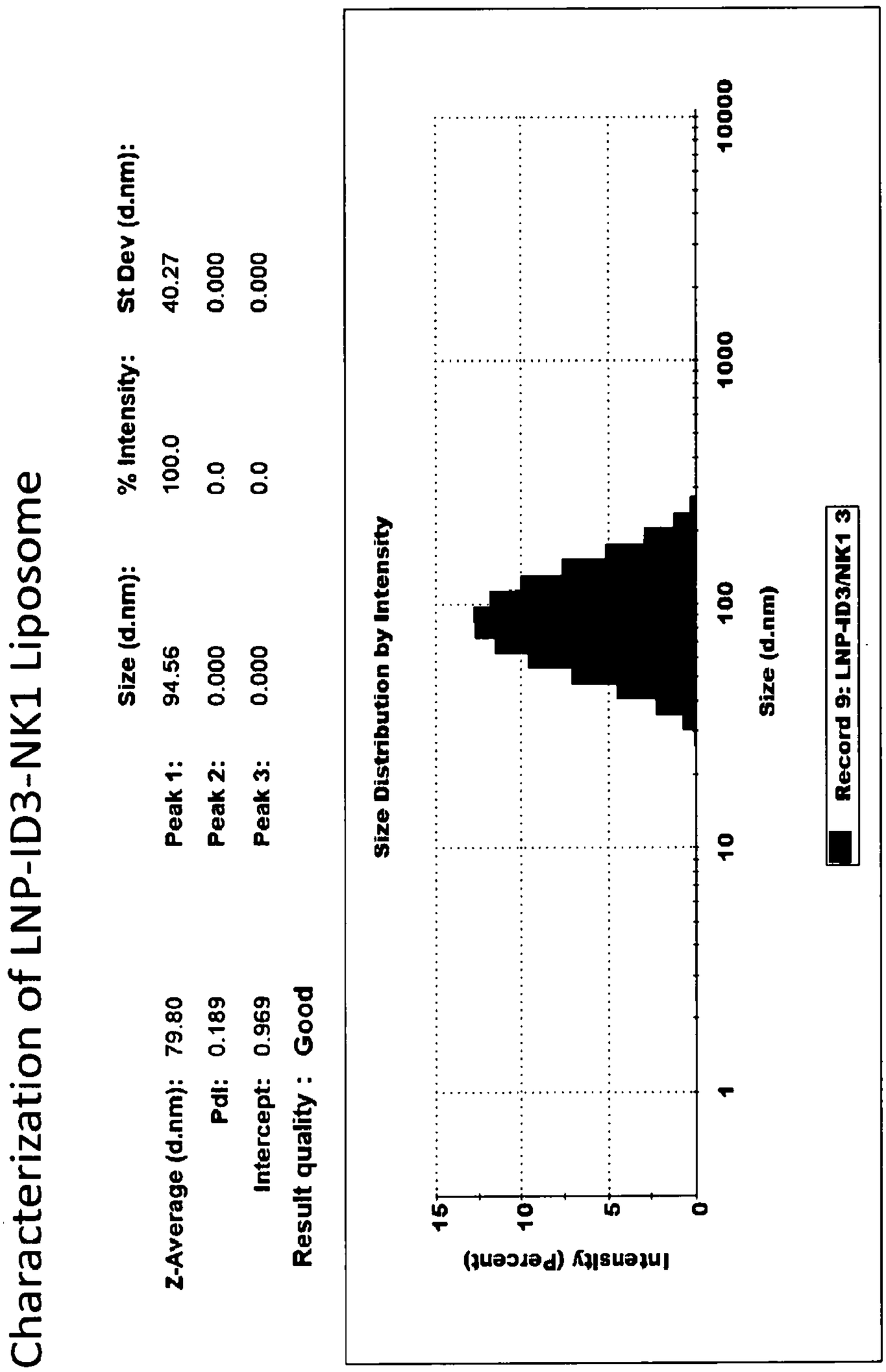

Characterization of LNP-AR5-TR5-ID3 Liposome (Zeta Potential)
Zeta Potential Distribution
Total Counts
300000
200000
100000
0
-100
0
100
200
Apparent Zeta Potential (mV)
Record 113: LNP-AR5-TR5-ID3

Figure 14.

Figure 15. Characterization of LNP-ID3-NK1 Liposome



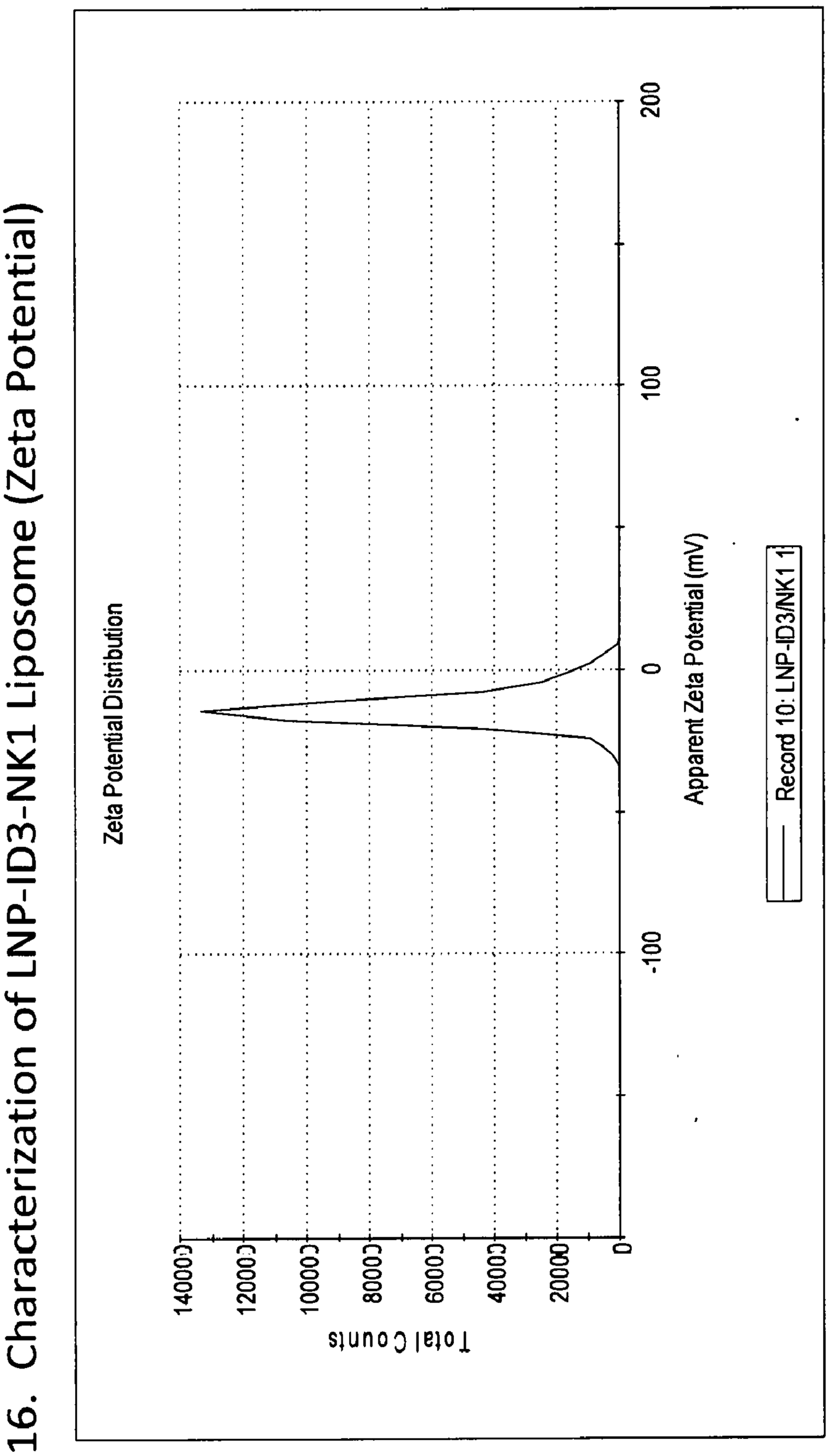


Figure 16. Characterization of LNP-ID3-NK1 Liposome (Zeta Potential)

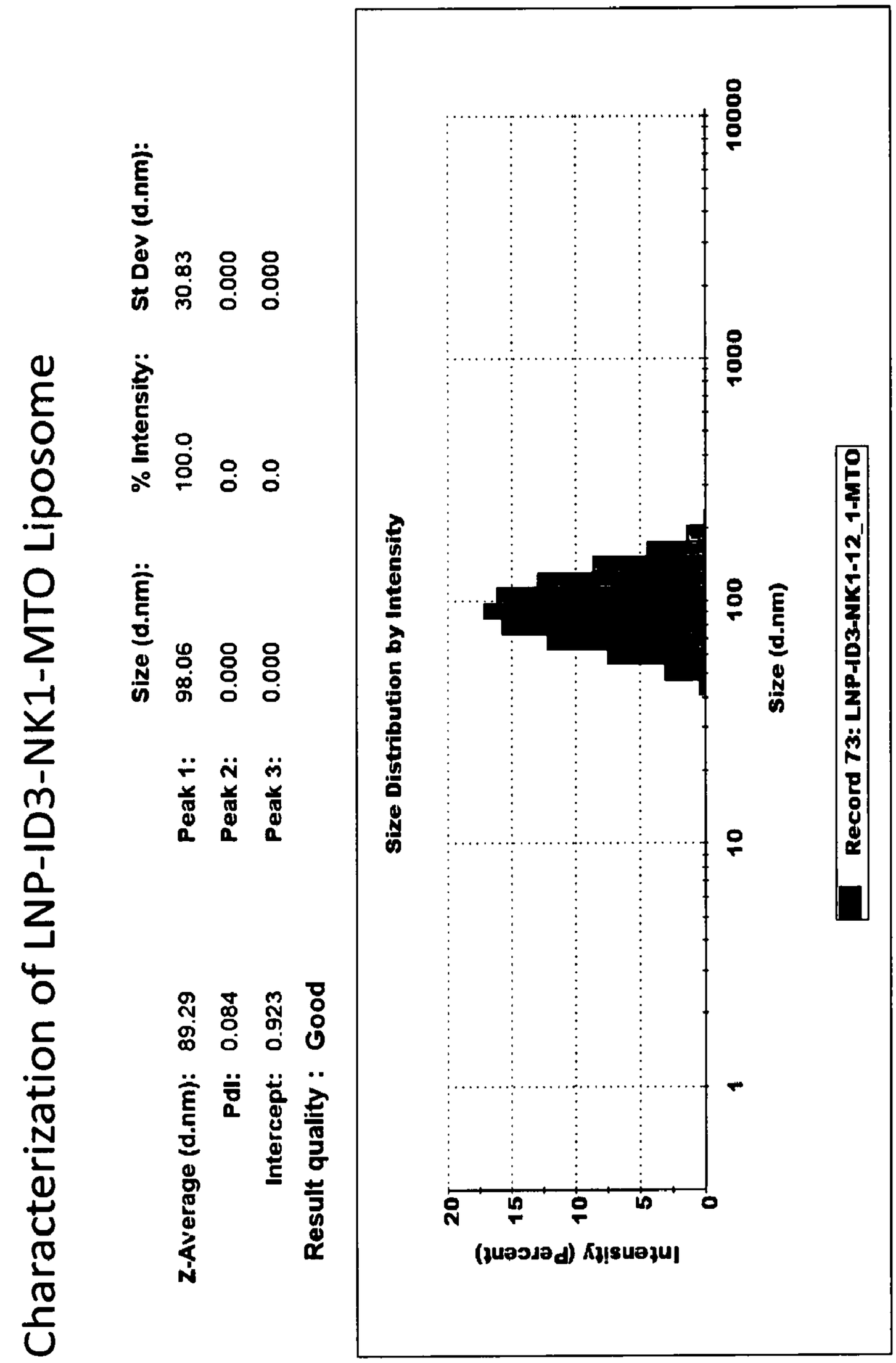


Figure 17. Characterization of LNP-ID3-NK1-MTO Liposome

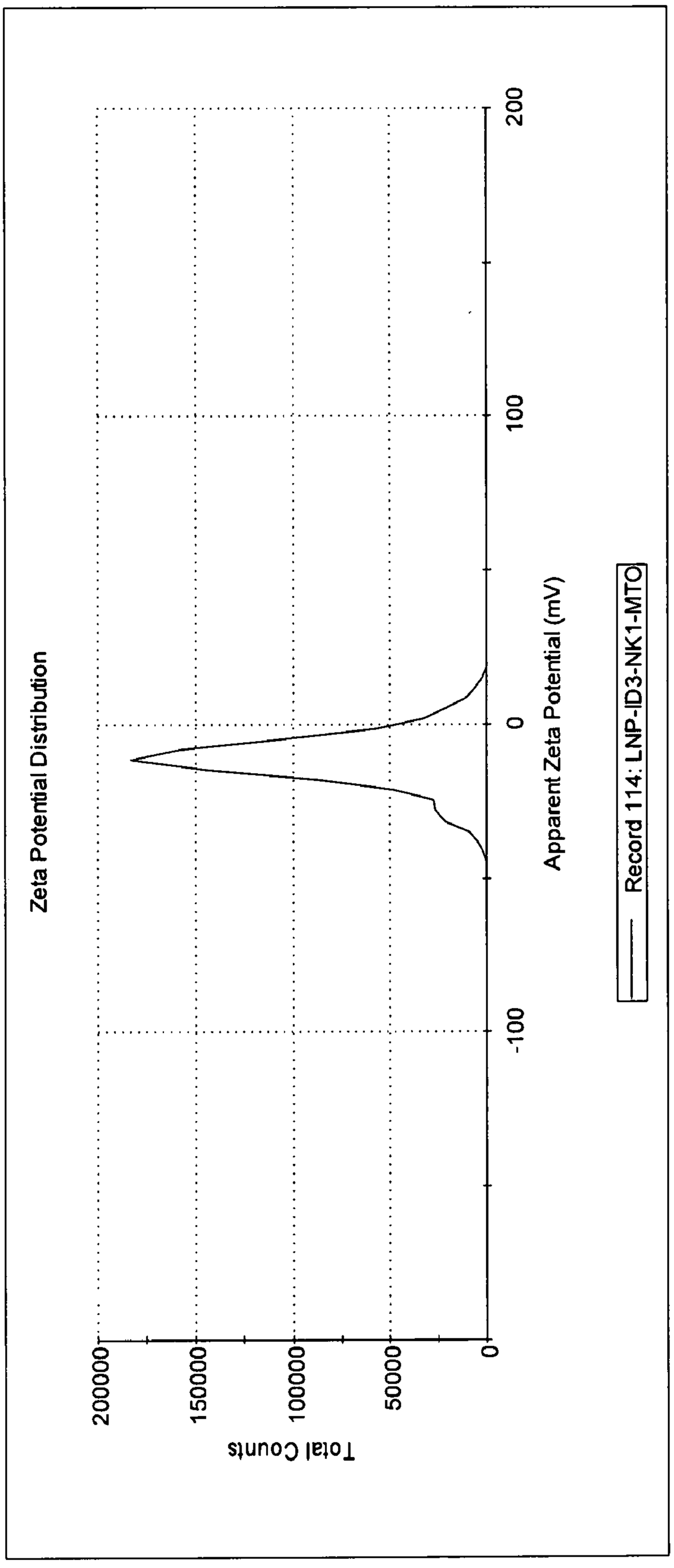


Figure 18. Characterization of LNP-ID3-NK1-MTO Liposome (Zeta Potential)

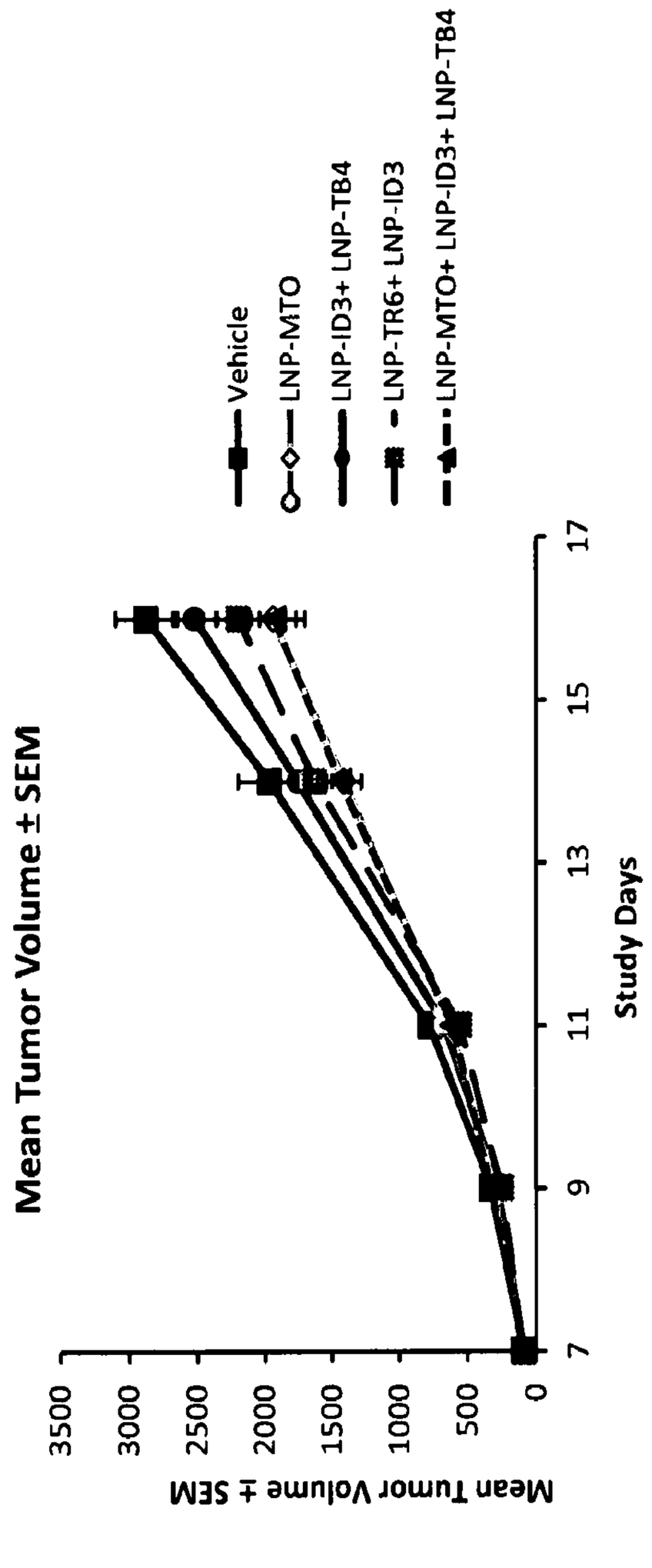


Figure 19. Tumor Inhibition of LNP-ID3 in Combination with Other Lipidoid(s) Using B16F10 Cells *In Vivo*

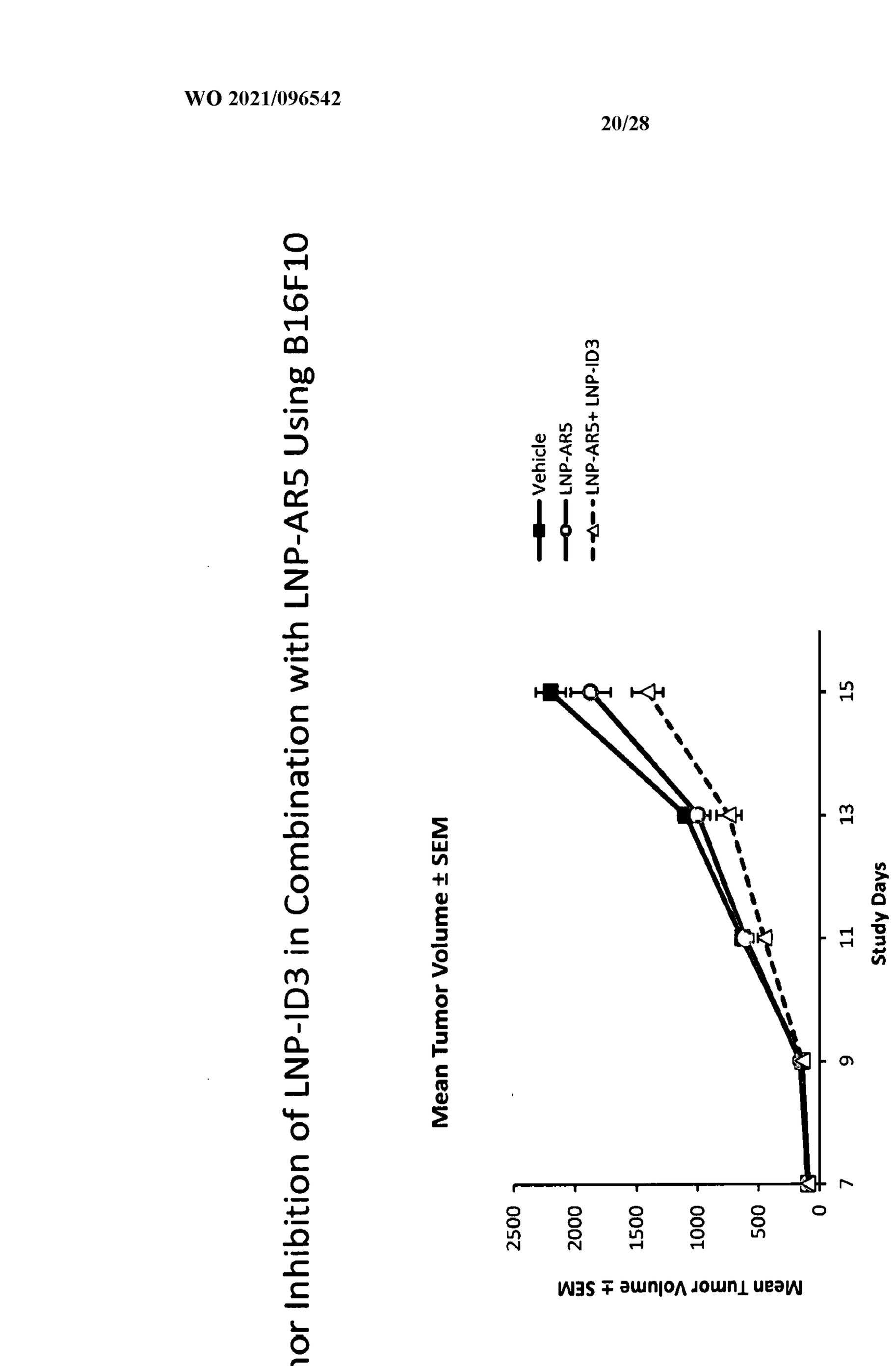


Figure 20. Tumor Inhibition of LNP-ID3 in Combination with LNP-AR5 Using B16F10 Cells *In Vivo*

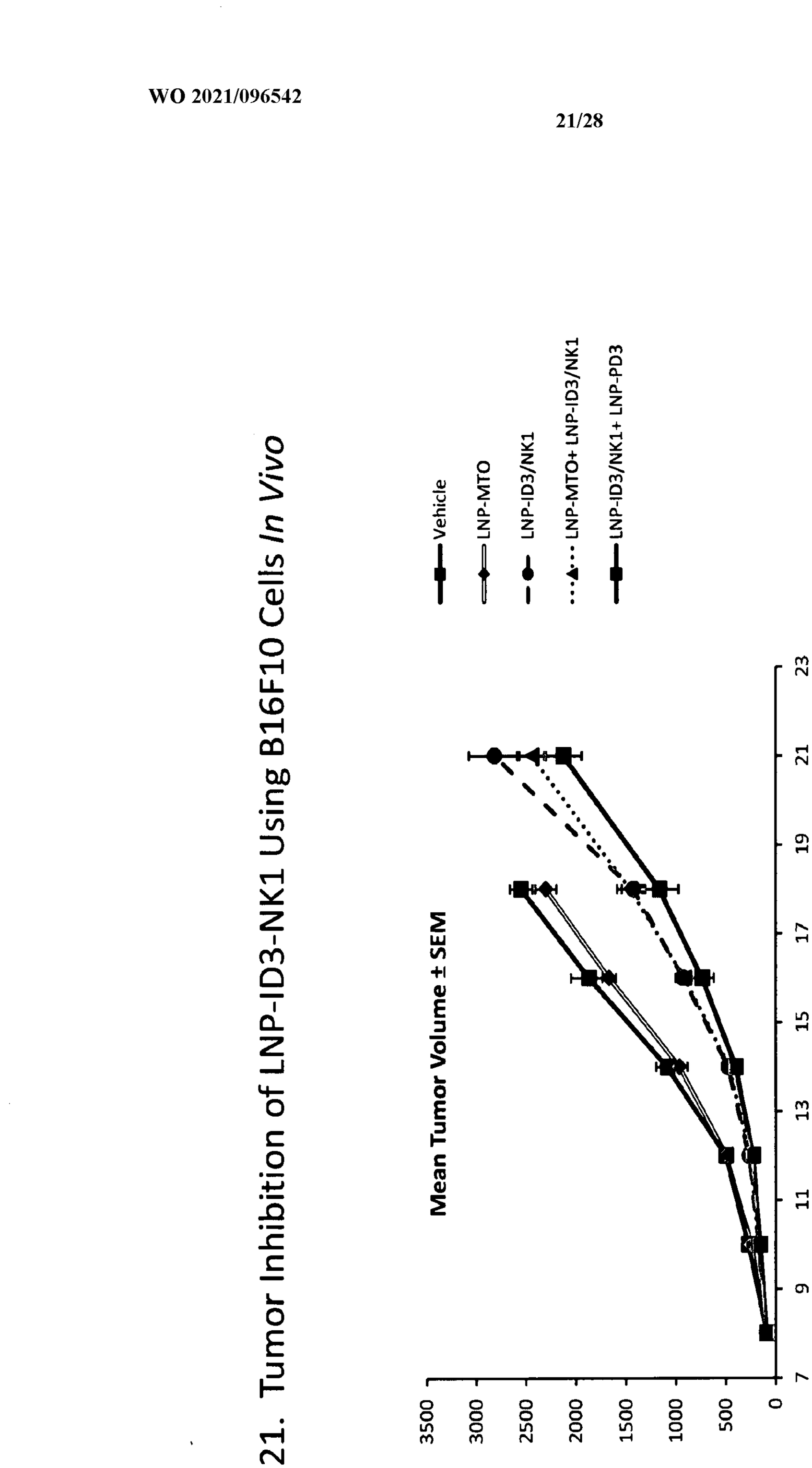


Figure 21. Tumor Inhibition of LNP-ID3-NK1 Using B16F10 Cells *In Vivo*

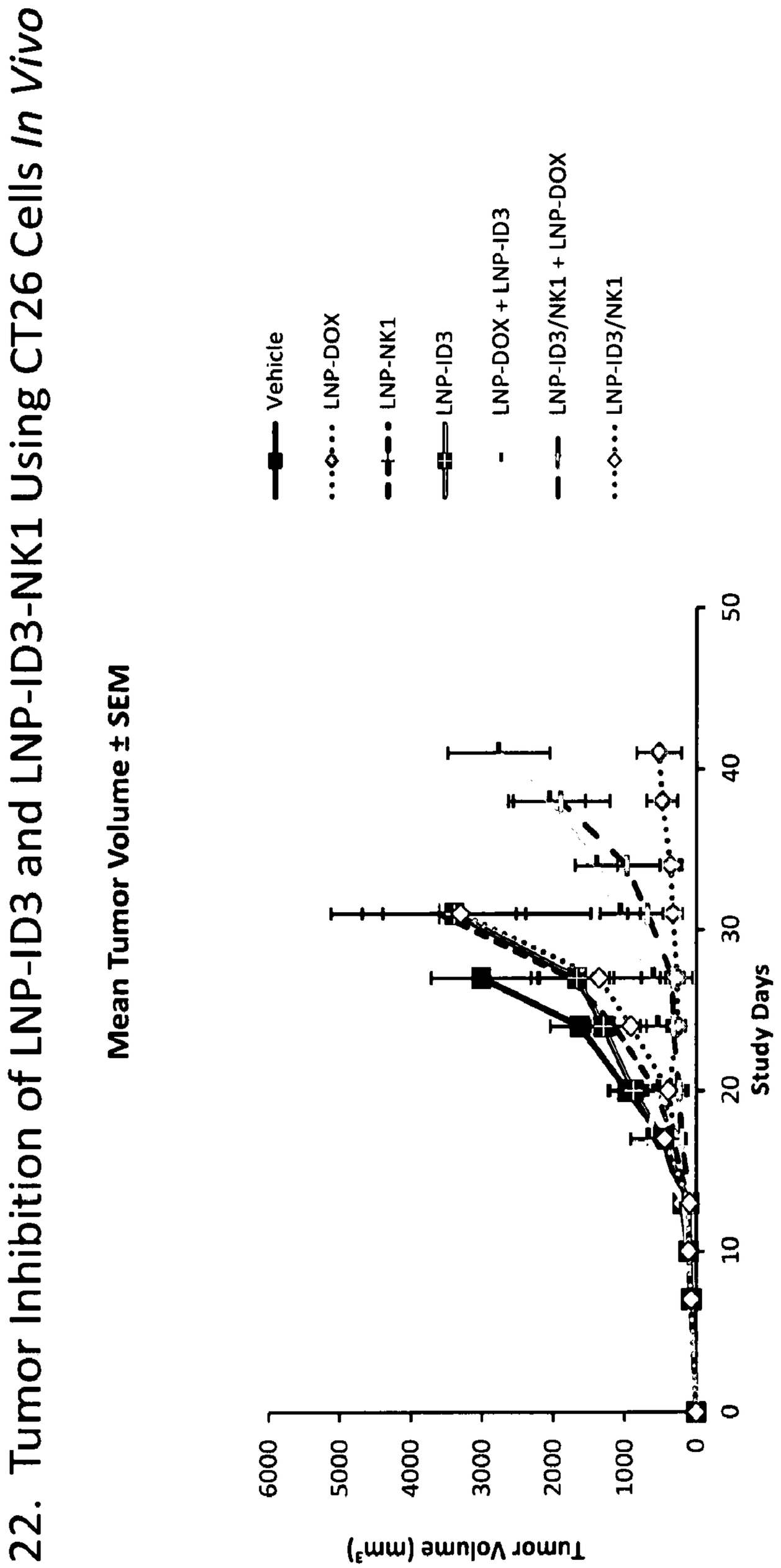


Figure 22. Tumor Inhibition of LNP-ID3 and LNP-ID3-NK1 Using CT26 Cells In Vivo

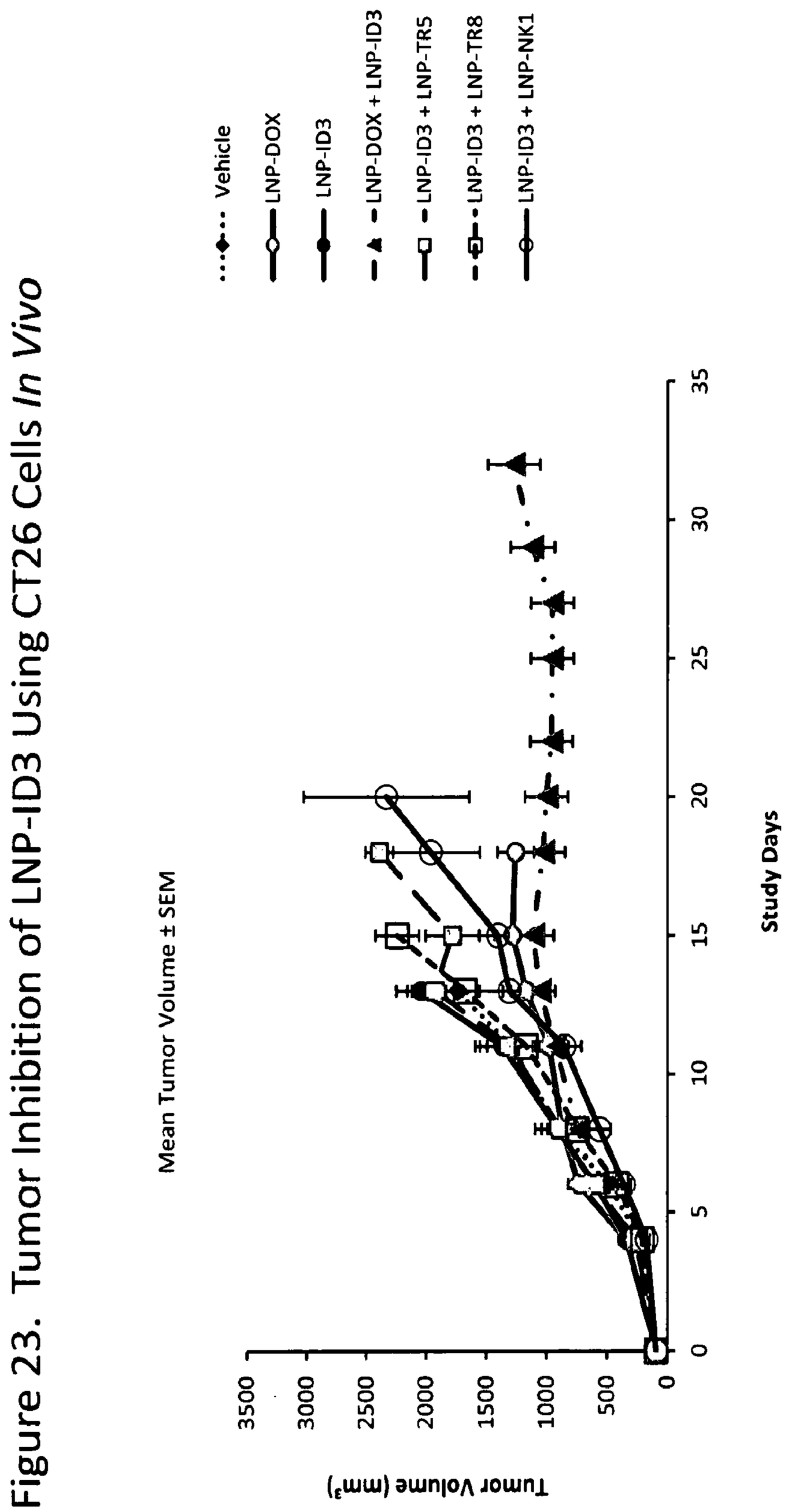


Figure 23. Tumor Inhibition of LNP-ID3 Using CT26 Cells *In Vivo*

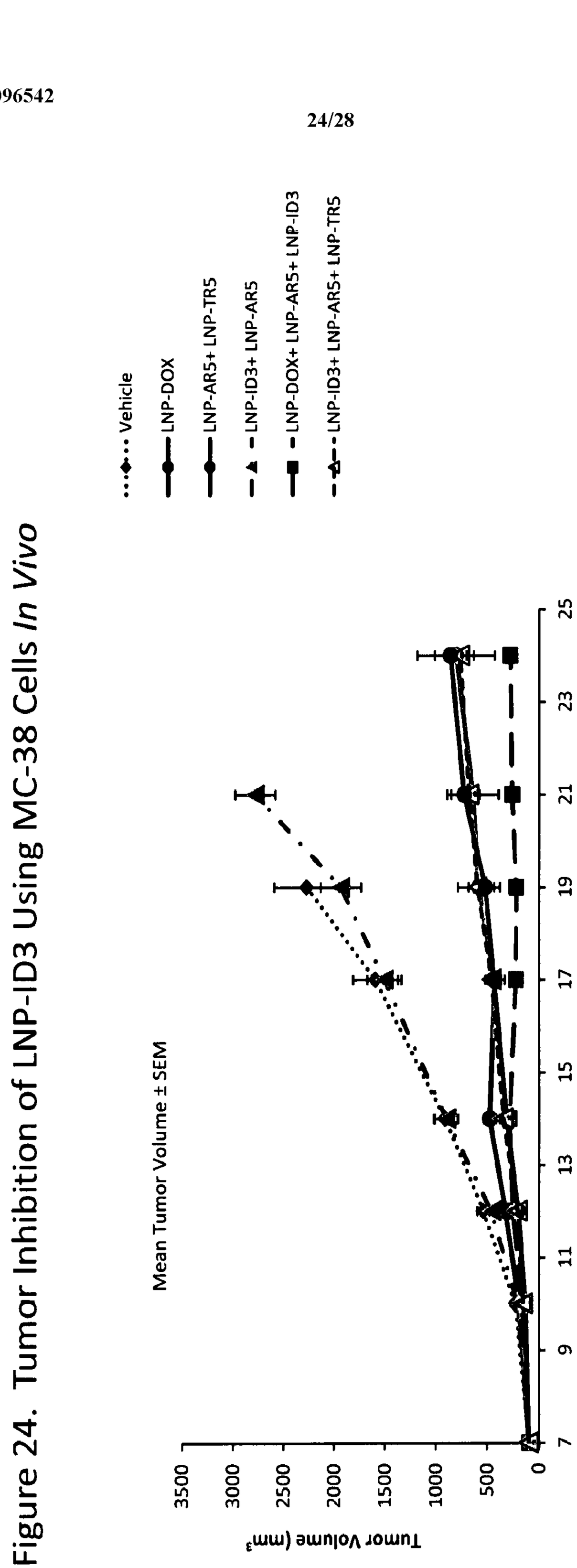


Figure 24. Tumor Inhibition of LNP-ID3 Using MC-38 Cells *In Vivo*

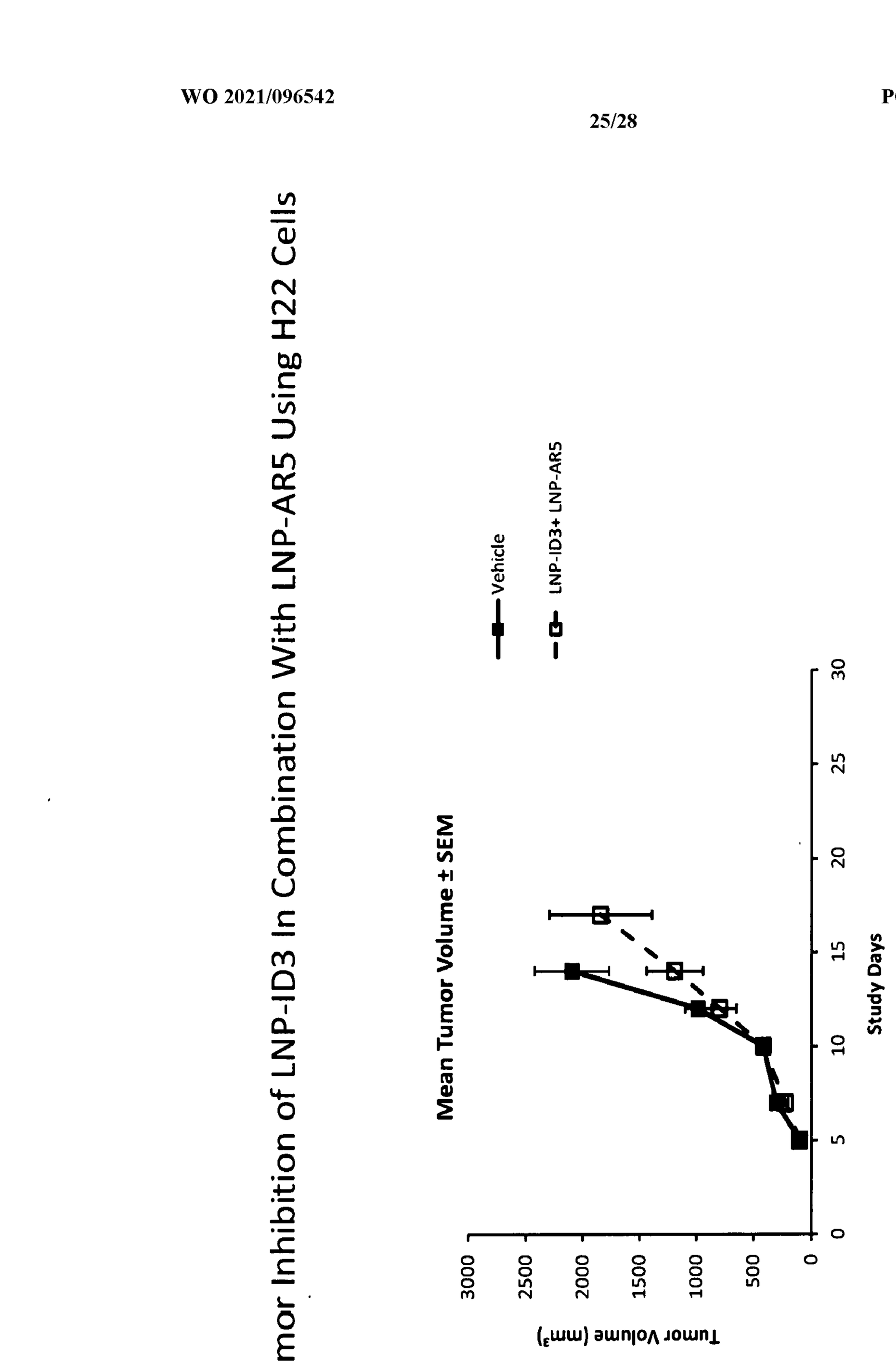


Figure 25. Tumor Inhibition of LNP-ID3 In Combination With LNP-AR5 *In Vivo* Using H22 Cells

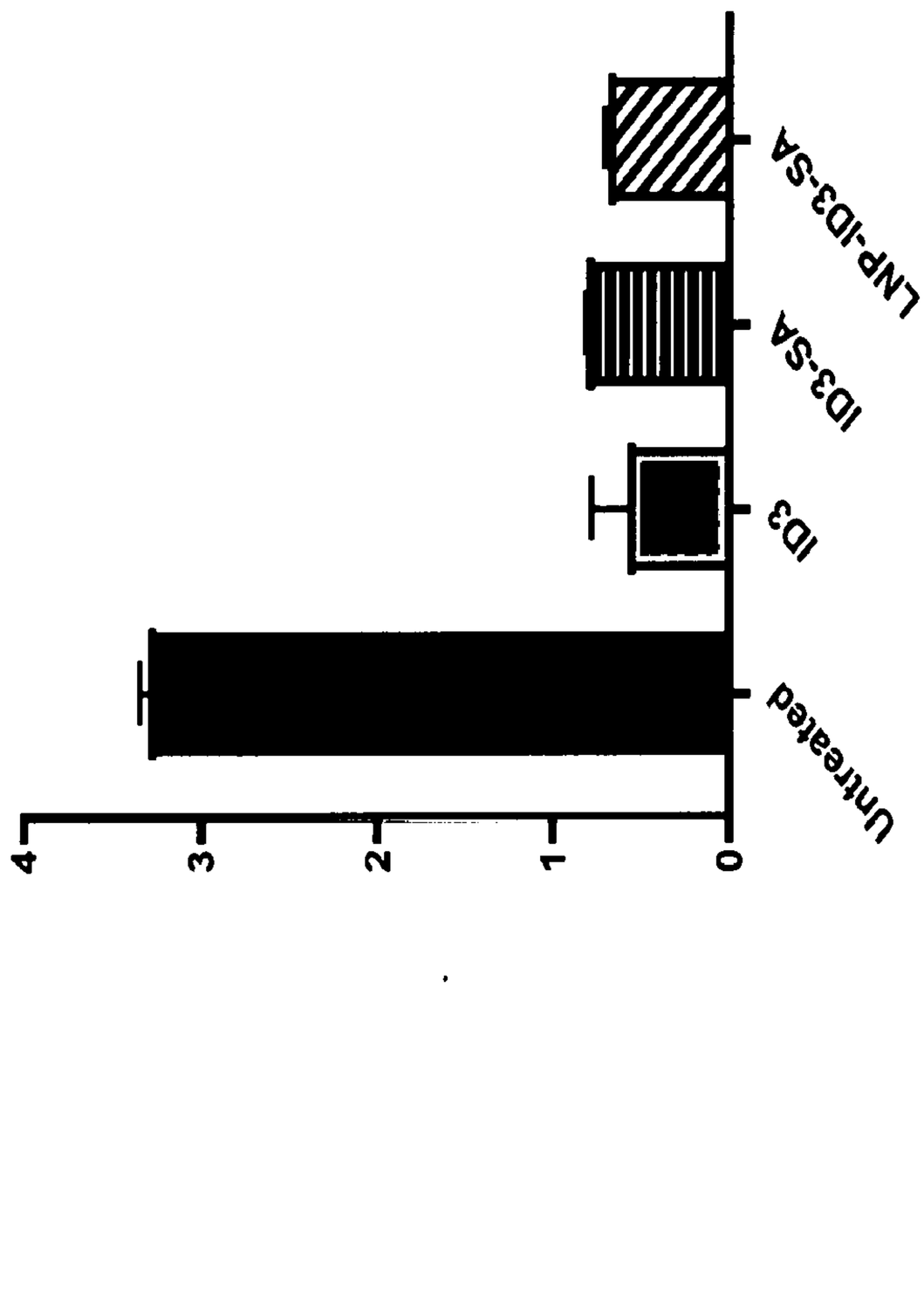


Figure 26. Measurement of IDO-1 Activity

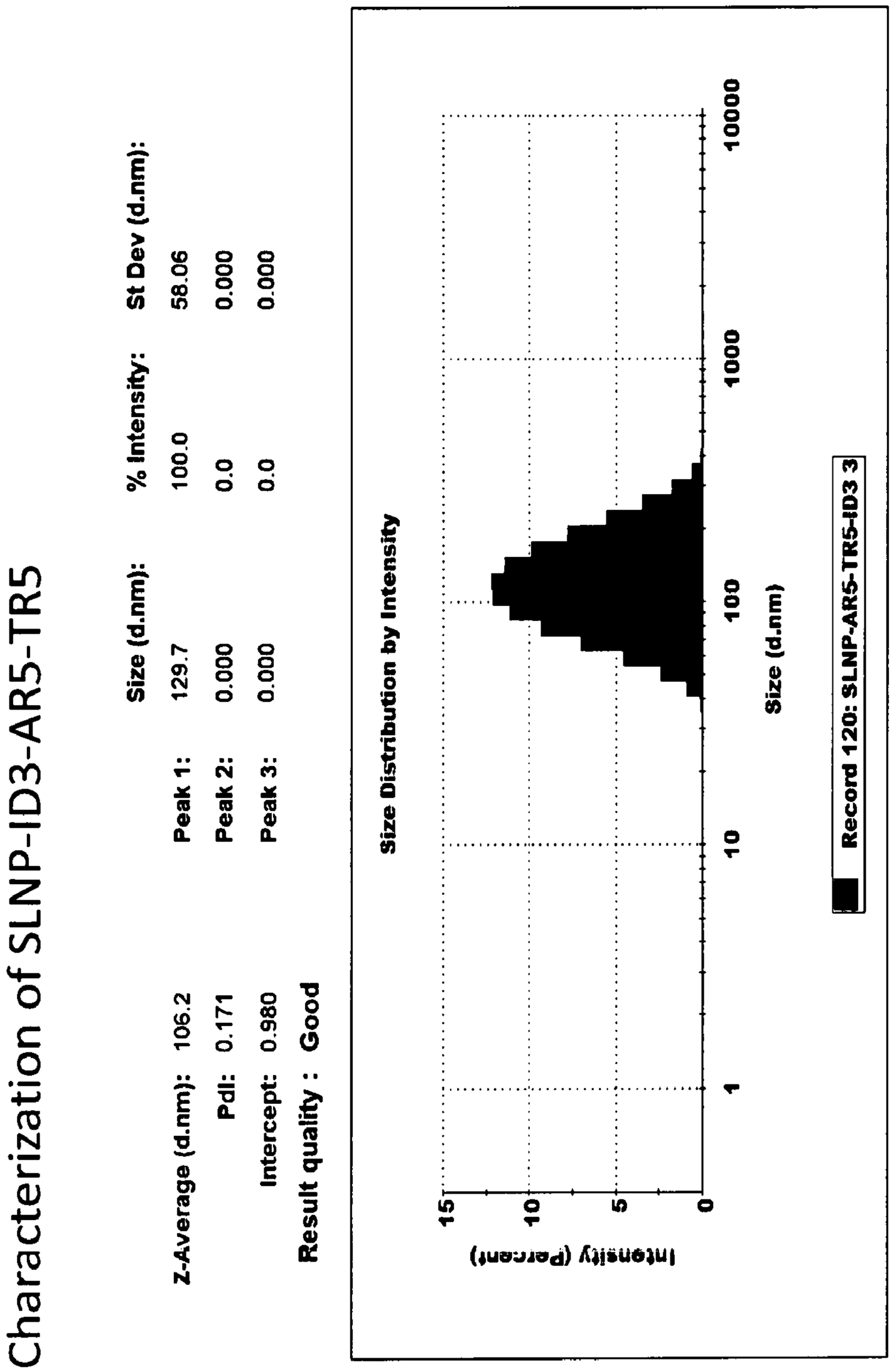


Figure 27. Characterization of SLNP-ID3-AR5-TR5

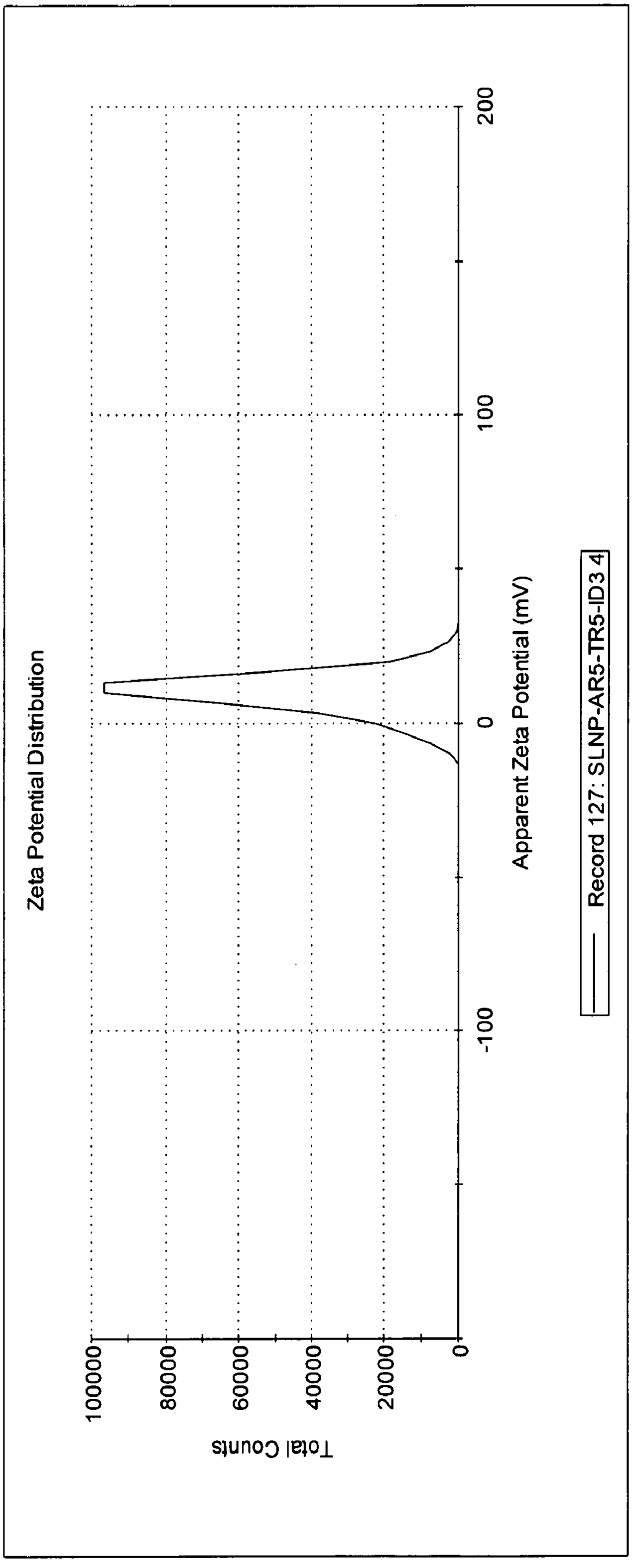


Figure 28. Characterization of SLNP-ID3-AR5-TR5 (Zeta Potential)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/000044

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61P35/00 A61K31/47 A61K47/00 C07J43/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07J

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

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

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X A	CN 108 424 434 A (UNIV SICHUAN) 21 August 2018 (2018-08-21) abstract claims 1-16 -----	1,3,5, 12-18,20 2,6-11, 19
X A	CN 105 777 770 B (UNIV PLA 2ND MILITARY MEDICAL) 25 May 2018 (2018-05-25) abstract claims 1-15 -----	1,4,5, 12-18,20 2,3, 6-11,19
X A	CN 110 025 789 A (UNIV SOUTHEAST) 19 July 2019 (2019-07-19) abstract figures 1-19 claims 1-8 -----	1,4,5, 12-18,20 2,3, 6-11,19

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

[X] See patent family annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
9 February 2021	17/02/2021

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

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2018/213631 A1 (UNIV CALIFORNIA [US]) 22 November 2018 (2018-11-22) cited in the application	1,5, 12-18,20
A	abstract claims 34-146	2-4, 6-11,19

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Information on patent family members

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		WO 2018213631 A1	22-11-2018