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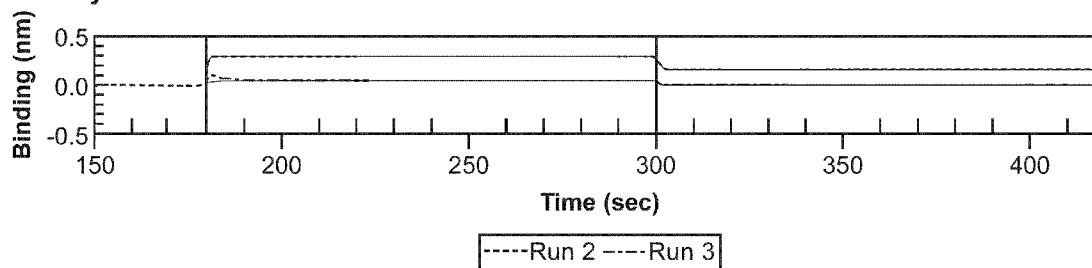


FIG. 1

(57) Abstract: Described herein are compositions of binding agents and carrier proteins, and at least one therapeutic agent, wherein the binding agents are capable of binding an antigen expressed on T-cells and methods of making and using the same, in particular, as a T-cell cancer therapeutic. Also described are lyophilized compositions of binding agents and carrier proteins, and at least one therapeutic agent, and methods of making and using the same, in particular, as a T-cell cancer therapeutic.



METHODS AND COMPOSITIONS FOR TARGETING T-CELL CANCERS

FIELD OF THE INVENTION

[0001] This application relates to novel compositions of binding agents and carrier proteins, and methods of making and using the same, in particular, as a T cell cancer therapeutic.

BACKGROUND

[0002] Chemotherapy remains a mainstay for systemic therapy for many types of cancer, including melanoma. Most chemotherapeutic agents are only slightly selective to tumor cells, and toxicity to healthy proliferating cells can be high (Allen TM. (2002) *Cancer* 2:750-763), often requiring dose reduction and even discontinuation of treatment. In theory, one way to overcome chemotherapy toxicity issues as well as improve drug efficacy is to target the chemotherapy drug to the tumor using antibodies that are specific for proteins selectively expressed (or overexpressed) by tumors cells to attract targeted drugs to the tumor, thereby altering the biodistribution of the chemotherapy and resulting in more drug going to the tumor and less affecting healthy tissue. Despite 30 years of research, however, specific targeting rarely succeeds in the therapeutic context.

[0003] Conventional antibody dependent chemotherapy (ADC) is designed with a toxic agent linked to a targeting antibody via a synthetic protease-cleavable linker. The efficacy of such ADC therapy is dependent on the ability of the target cell to bind to the antibody, the linker to be cleaved, and the uptake of the toxic agent into the target cell. Schrama, D. *et al.* (2006) *Nature reviews. Drug discovery* 5:147-159.

[0004] Antibody-targeted chemotherapy promised advantages over conventional therapy because it provides combinations of targeting ability, multiple cytotoxic agents, and improved therapeutic capacity with potentially less toxicity. Despite extensive research, clinically effective antibody-targeted chemotherapy remains elusive: major hurdles include the instability of the linkers between the antibody and chemotherapy drug, reduced tumor toxicity of the chemotherapeutic agent when bound to the antibody, and the inability of the

conjugate to bind and enter tumor cells. In addition, these therapies did not allow for control over the size of the antibody-drug conjugates.

[0005] There remains a need in the art for antibody-based cancer therapeutics that retain cytotoxic effect for targeted drug delivery to provide reliable and improved anti-tumor efficacy over prior therapeutics.

[0006] In addition, as to any therapeutic application, there also remains a need for the composition to be stable in its physical, chemical and biological properties.

[0007] T-cell lymphomas (“TCL”) are a heterogeneous group of blood cancers that account for approximately 15% of lymphomas. Every year, there are about 6,500 new cases of TCL in the United States. TCLs include peripheral T-cell lymphoma, anaplastic large cell lymphoma, cutaneous T-cell lymphoma, adult T-cell leukemia/lymphoma, lymphoblastic lymphoma, et al. Studies show that TCL can affect both children and adults, though in some subtypes of TCL, mainly teenagers and children are affected by this blood disease, e.g. lymphoblastic lymphoma. Patients with TCL present themselves with swollen lymph nodes, high-grade lesions, and systemic symptoms, e.g., severe rash, fever, and fatigue.

[0008] TCL patients were traditionally treated with the same chemotherapies (e.g., anthracyclines) as for those with B-cell lymphomas. Compared to their efficacies against B-cell lymphomas, however, the anthracycline-based regimens have not been effective in increasing the survival rates for the TCL patients. Vose J, et al., *J Clin Oncol.* 2008; 26(25):4124-30. The anthracycline-based regimens are accompanied with lower response rates and shorter times to progression for the TCL patients. Given the poor outcomes with traditional chemotherapy, non-anthracycline based therapeutic regimens are greatly needed for TCL patients.

[0009] Therefore, there is a need for a more effective composition or immunotherapy to treat the T-cell cancers with minimal or no side effects.

SUMMARY OF THE INVENTION

[0010] This disclosure is related to a nanoparticle composition that comprises nanoparticles having an outer surface, wherein each of the nanoparticles comprises: a carrier protein, a binding agent with an antigen-binding portion targeting an antigen expressed on a T-cell, and a therapeutically effective amount of a therapeutic agent. In one aspect, the binding agent is capable of binding to an antigen expressed on a T-cell or a T-cell cancer (e.g., OKT3) while, at the same time, reducing or eliminating the serious side effects associated with the immunogenic and mitogenic potential limits of the antibody (e.g., OKT3). Accordingly, the nanoparticles as described herein are thus a significant improvement compared to the conventional ADCs having serious side effects and/or toxicity.

[0011] Antibody-based therapy has emerged as a new therapeutic option for lymphoma patients. For example, a monoclonal antibody, rituximab (Rituxan®), has been used in treating B-cell lymphoma with significantly improved clinical outcomes. For the T-cell neoplasm, a number of antibodies have shown their efficacies. Among them, muromonab-CD3 (Orthoclone®, “OK T3”), a murine IgG2a monoclonal antibody against CD3 receptor on T-cells, for example, can induce complement-induced lysis of CD3+ T-cells from the peripheral circulation and lymphoid tissues. Chatenoud, L. et al, *Nat. Rev. Immunoo.* 3, 123-32 (2003). However, the serious side effects associated with the immunogenic and mitogenic potential limits of OKT3 is wide spread with its use in treating TCL patients. For example, therapeutic benefits of OKT3 are hampered by the cytokine-based inflammatory response caused by the engagement of the antibody with the CD3 receptor. Abramowicz D, et al., *Transplantation*, 1989;47:606–608. Moreover, the potent mitogenic properties of OKT3 for the T-cells may increase the proliferation of the malignant immune cells, thereby exacerbating the T-cell neoplasm in the TCL patients. Landergren U, et al., *Eur J Immunol.* 1984 Apr;14(4):325-8.

[0012] Without being bound by theory, the binding agent is believed to be bound by the carrier protein through hydrophobic interactions, which, by their nature, are weak. Yet, the activity of the individual components, as well as their relative relationship in the nanoparticle, is preserved even upon lyophilization and reconstitution of the composition as hereinafter

described. It is still further contemplated that binding to the carrier protein, e.g., complexation of the binding agent to the carrier protein, occurs through an albumin-binding motif on the binding agents and/or an antibody-binding motif on the carrier protein. In one embodiment, upon reconstitution with an aqueous solution, the antigen-binding portion of said binding agents is capable of binding to (recognizes) the antigen on a T-cell cancer. In another embodiment, fewer than about 50% of said nanoparticles are oligomeric.

[0013] Further challenges are imposed because the nanoparticles are used in therapy.

[0014] While rearrangement of the components in the nanoparticle may be mitigated through covalent bonds between the components, such covalent bonds pose challenges for the therapeutic use of nanoparticles in cancer treatment. The binding agent, carrier protein, and additional therapeutic agent typically act at different locations in a tumor and through different mechanisms. Non-covalent bonds permit the components of the nanoparticle to dissociate at the tumor. Thus, while a covalent bond may be advantageous for lyophilization, it may be disadvantageous for therapeutic use.

[0015] This disclosure is related to a nanoparticle composition that comprises nanoparticles having an outer surface, wherein each of the nanoparticles comprises: a carrier protein, a binding agent with an antigen-binding portion targeting an antigen expressed on a T cell, and optionally a therapeutically effective amount of a therapeutic agent.

[0016] The size of nanoparticles, and the distribution of the size, is also important. Nanoparticles may behave differently according to their size. At large sizes, nanoparticles or the agglomeration of the particles may block blood vessels, either of which can affect the performance and safety of the composition. In one embodiment, the average size of the nanoparticles is between 90 nm and 800 nm. In another embodiment, the average size of the nanoparticles is between 300 nm and 500 nm. In yet a further embodiment, the average size of the nanoparticles is about 90 nm to about 160 nm.

[0017] Finally, cryoprotectants and agents that assist in the lyophilization process must be safe and tolerated for therapeutic use.

[0018] In one aspect, the binding agent is capable of binding to an antigen expressed on T-cells or T-cell cancers *in vivo*. In another embodiment, the antigen-binding portion of the binding agent binds to an antigen expressed on a T-cell or a T-cell cancer. In one embodiment, the antigen is a protein expressed on the T-cell cancers, including but not limited to, CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, and CCR4. In a further embodiment, the antigen is a biomarker overexpressed in the T-cell cancers (e.g., T-cell lymphomas). The biomarkers may include PD-L1, Ly6E, HER3/EGFR DAF, ERBB-3 receptor, CSF-1R, HER2, STEAP1, CEA, OX40, Ang2-VEGF, or VEGF. In a preferred embodiment, the binding portion of the binding agent is capable of binding to CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, or CCR4. In another embodiment, the binding portion of the binding agent is capable of binding to PD-L1, Ly6E, HER3/EGFR DAF, ERBB-3 receptor, CSF-1R, HER2, STEAP1, CEA, OX40, Ang2-VEGF, or VEGF. In one embodiment, the T-cell cancer is T-cell lymphoma. In another embodiment, the T-cell cancer is peripheral T-cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma cutaneous T-cell lymphoma, Adult T-cell Leukemia/Lymphoma (ATLL), enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphoma, treatment-related T-cell lymphoma, or a combination thereof. In one aspect, the antigen may exclude one or more of the antigens recited herein. In one embodiment, the antigen is not VEGF, HER2, or EGFR.

[0019] In one aspect, provided herein are nanoparticle compositions comprising nanoparticles wherein each of the nanoparticles comprises a carrier protein, binding agents with a T-cell antigen-binding portion, and optionally at least one therapeutic agent, wherein upon reconstitution with an aqueous solution, the antigen-binding portion of said binding agents are capable of binding to an antigen expressed on a T-cell *in vivo*.

[0020] When administered intravenously, large particles (e.g. greater than 1 μm) are typically disfavored because they can become lodged in the microvasculature of the lungs. At the same time, larger particles can accumulate in the tumor or specific organs. For example, THERASPHERE® 20-60 micron glass particles that are injected into the hepatic artery feeding a tumor of the liver for the delivery of a radioactive element, also known as radioembolization, are in clinical use for liver cancer.

[0021] Therefore, for intravenous administration, particles under 1 μm are used. Particles over 1 μm are, more typically, administered directly into a tumor (“direct injection”) or into an artery feeding into the site of the tumor.

[0022] In one aspect, the nanoparticle comprises about 100 to about 1000 binding agents, preferably about 400 to about 800 binding agents. The number of binding agents in the composition also depends on the number of particles. When nanoparticles multimerize, the number of binding agents increases proportionally. For example, if a 160 nm nanoparticle contains 400 binding agents, a 320 nm dimer is expected to contain about 800 binding agents.

[0023] In other embodiments, the nanoparticles multimerize or oligomerize, e.g. dimerize. Multimerization may be observed as multiples of the weight or size of the unit molecule, e.g. 160 nm particles multimerize to about 320 nm, 480 nm, 640 nm, etc. In some embodiments, less than 40% of the nanoparticles in a population are oligomerized. In some embodiments, less than 30% of the nanoparticles are oligomerized. In a further embodiment, less than 20% of the nanoparticles are oligomerized. In another embodiment, less than 10% of the nanoparticles present are oligomerized. In a preferable embodiment, less than 5% of the nanoparticles are oligomerized.

[0024] In one embodiment, the weight ratio of carrier-bound drug to binding agent (e.g. albumin-bound paclitaxel to OKT3) is between about 5:1 to about 1:1. In one embodiment, the weight ratio of carrier-bound drug to binding agent is about 10:4. In one embodiment, the binding agents are a substantially single layer on all or part of the surface of the nanoparticle. In one embodiment, less than 0.01% of nanoparticles in the composition have a size selected from the group consisting of greater than 200 nm, greater than 300 nm, greater than 400 nm, greater than 500 nm, greater than 600 nm, greater than 700 nm and greater than 800 nm. Larger sizes are believed to be the result of multimerization (or oligomerization) of several nanoparticles.

[0025] The invention further includes lyophilized compositions, and lyophilized compositions that do not materially differ from, or are the same as, the properties of freshly-prepared nanoparticles. In particular, the lyophilized composition, upon resuspending in aqueous solution, is similar or identical to the fresh composition in terms of particle size,

particle size distribution, toxicity for cancer cells, binding agent affinity, and binding agent specificity. Surprisingly, lyophilized nanoparticles retain the properties of freshly-made nanoparticles after resuspension notwithstanding the presence of two different protein components in these particles.

[0026] In one embodiment, the average reconstituted nanoparticle size is from about 90 nm to about 1 μ m. In a preferred embodiment, the average reconstituted nanoparticle size is from about 90 nm to about 200 nm, and more preferably about 100 to about 160 nm. In one embodiment, the average reconstituted nanoparticle size is from greater than 800 nm to about 3.5 μ m, comprising multimers of smaller nanoparticles, e.g. multimers of 100-200 nm nanoparticles. In one embodiment, the weight ratio of core to binding agent is from greater than 1:1 to about 1:3. In one embodiment, the average reconstituted nanoparticle size is about 160nm to about 225nm.

[0027] In one aspect, this invention relates to a lyophilized nanoparticle composition comprising nanoparticles, wherein each of the nanoparticles comprises a carrier-bound drug core and a binding agent. In one embodiment, the binding agents retain their association with the outside surface of the nanoparticle upon reconstitution with an aqueous solution. In one embodiment, after reconstitution, the binding agent is arranged on a surface of the core such that the binding portion of the binding agent is directed outward from that surface. In one embodiment, the lyophilized composition is stable at room temperature for at least about 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10 months, 11 months, 12 months, or longer. In one embodiment, the lyophilized composition is stable at room temperature for at least 3 months. In one embodiment, the reconstituted nanoparticles retain the activity of the therapeutic agent and are capable of binding to the target *in vivo*. In another embodiment, the composition is stable at about 20°C to about 25°C for up to about 12 months or longer.

[0028] In some embodiments, the at least one therapeutic agent is located inside the nanoparticle. In other embodiments, the at least one therapeutic agent is located on the outside surface of the nanoparticle. In yet other embodiments, the at least one therapeutic

agent is located inside the nanoparticle and on the outside surface of the nanoparticle. In a further embodiment, the therapeutic agent is a therapeutic agent for cancer.

[0029] In some embodiments, the nanoparticle contains more than one type of therapeutic agent. In one embodiment, the therapeutic agent may be abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, or cyclophosphamide. In a preferred embodiment, the therapeutic agent is Paclitaxel.

[0030] In another aspect, the binding agent comprises Slipizumab, OKT3, Leu 1, Zanolimumab, Brentuximab vedotin, Mik-β1, KW-0761, or a combination thereof. In some embodiments, the binding agent is muromonab-CD3 (OKT3).

[0031] In yet another embodiment, the antigen binding portion comprises an aptamer, a receptor ligand, an Fab fragment, or a combination thereof. In a preferred embodiment, the antigen binding portion is an antibody or portion thereof.

[0032] In some embodiments, the carrier protein comprises gelatin, elastin, gliadin, legumin, zein, a soy protein, a milk protein, and a whey protein. In other embodiments, the carrier protein is albumin, for example, human serum albumin. In some embodiments, the albumin is human serum albumin (HSA). In some embodiments, the albumin is a recombinant albumin, e.g., recombinant human serum albumin.

[0033] In some embodiments, the composition is formulated for intravenous delivery.

[0034] In some embodiments, the nanoparticles have a dissociation constant between about 1×10^{-11} M and about 1×10^{-9} M.

[0035] Also provided herein are methods for killing cancer cells in a T cell cancer, which comprise contacting the cell with an effective amount of a nanoparticle composition disclosed

herein for a sufficient period of time to kill the cancerous T cells. In other embodiments, the nanoparticle composition is administered intravenously.

[0036] In some embodiments, the methods provided herein include the steps of: a) administering the nanoparticle composition once a week for three weeks; b) ceasing administration of the nanoparticle composition for one week; and c) repeating steps a) and b) as necessary to treat the tumor.

[0037] In related embodiments, the treatment comprises administration of the targeting binding agent prior to administration of the nanoparticles. In one embodiment, the targeting binding agent is administered between about 6 and 48, or 12 and 48 hours prior to administration of the nanoparticles. In another embodiment, the targeting binding agent is administered between 6 and 12 hours prior to administration of the nanoparticles. In yet another embodiment, the targeting binding agent is administered between 2 and 8 hours prior to administration of the nanoparticles. In still other embodiments, the targeting binding agent is administered a week prior to administration of the nanoparticles. For example, administration of a dose of OKT3 24 hours prior to administration of nanoparticles. In another example, OKT3 is administered prior to administering the nanoparticles. The binding agent administered prior to the nanoparticle may be administered as a dose that is subtherapeutic, such as 1/2, 1/10th or 1/20 the amount normally considered therapeutic. Thus, in humans, pretreatment with OKT3 may comprise administration of 1 mg/kg OKT3 which is 1/10th the usual dose, followed by administration of nanoparticles.

[0038] In some embodiments, the therapeutically effective amount comprises about 75 mg/m² to about 175 mg/m² of the carrier protein (i.e., milligrams carrier protein per m² of the patient). In other embodiments, the therapeutically effective amount comprises about 75 mg/m² to about 175 mg/m² of therapeutic agent (e.g., paclitaxel). In other embodiments, the therapeutically effective amount comprises about 30 mg/m² to about 70 mg/m² of the binding agent. In yet other embodiments, the therapeutically effective amount comprises about 30 mg/m² to about 70 mg/m² OKT3.

[0039] In one specific embodiment, the lyophilized composition comprises from about 75 mg/m² to about 175 mg/m² of the carrier protein which is preferably albumin; from about 30

mg/m² to about 70 mg/m² of the binding agent which is preferably OKT3; and from about 75 mg/m² to about 175 mg/m² of paclitaxel.

[0040] An embodiment of the invention includes a method for increasing the duration of cancer cell uptake of a chemotherapeutic agent by administering the chemotherapeutic agent in a nanoparticle comprising a carrier protein and the chemotherapeutic agent having surface complexation with an antibody, e.g., an antibody that specifically binds to an antigen on or shed by the cancer cell, wherein the cancer cell is a T cell.

[0041] Further provided herein are methods of making nanoparticle compositions, wherein said methods comprise contacting the carrier protein and the optionally at least one therapeutic agent with the antibodies in a solution having a pH of between 5.0 and 7.5 and a temperature between about 5°C and about 60°C, between about 23°C and about 60°C, or between about 55°C and about 60°C under conditions and ratios of components that will allow for formation of the desired nanoparticles. In one embodiment, the nanoparticle is made at 55°C to 60°C and pH 7.0. In another aspect, provided herein are methods of making the nanoparticle compositions, wherein said method comprises (a) contacting the carrier protein and optionally the at least one therapeutic agent to form a core and (b) contacting the core with the antibodies in a solution having a pH of about 5.0 to about 7.5 at a temperature between about 5°C and about 60°C, between about 23°C and about 60°C, or between about 55°C and about 60°C under conditions and ratios of components that will allow for formation of the desired nanoparticles.

[0042] The amount of components (e.g., carrier protein, antibodies, therapeutic agents, combinations thereof) is controlled in order to provide for formation of the desired nanoparticles. A composition wherein the amount of components is too dilute will not form the nanoparticles as described herein. In a preferred embodiment, weight ratio of carrier protein to binding agent is 10:4. In some embodiments, the amount of carrier protein is between about 1 mg/mL and about 100 mg/mL. In some embodiments, the amount of binding agent is between about 1 mg/mL and about 30 mg/mL. For example, in some embodiments, the ratio of carrier protein: binding agent: solution is approximately 9 mg of carrier protein (e.g., albumin) to 4 mg of binding agent (e.g., OKT3) in 1 mL of solution (e.g.,

saline). An amount of a therapeutic agent, such as, for example, paclitaxel, can also be added to the carrier protein, for example prior to contacting the antibody.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] The following figures are representative only of the invention and are not intended as a limitation. For the sake of consistency, the nanoparticles of this invention using ABRAXANE® and bevacizumab employ the acronym “AB” and the number after AB such as AB160 is meant to confer the average particle size of these nanoparticles (in nanometers). Likewise, when the binding agent is rituximab, the acronym is “AR” while the number thereafter remains the same.

[0044] FIG. 1 shows the binding affinity of Abraxane and OKT3 as determined by light absorption Bio-layer interferometry (BLItz) technology. The dissociation constant (K_d) is 2.246×10^{-9} .

DETAILED DESCRIPTION

[0045] After reading this description it will become apparent to one skilled in the art how to implement the invention in various alternative embodiments and alternative applications. However, all the various embodiments of the present invention will not be described herein. It will be understood that the embodiments presented here are presented by way of an example only, and not limitation. As such, this detailed description of various alternative embodiments should not be construed to limit the scope or breadth of the present invention as set forth below.

[0046] Before the present invention is disclosed and described, it is to be understood that the aspects described below are not limited to specific compositions, methods of preparing such compositions, or uses thereof as such may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular aspects only and is not intended to be limiting.

[0047] The detailed description of the invention is divided into various sections only for the reader's convenience and disclosure found in any section may be combined with that in

another section. Titles or subtitles may be used in the specification for the convenience of a reader, which are not intended to influence the scope of the present invention.

Definitions

[0048] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

[0049] The terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used herein, the singular forms “a”, “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise.

[0050] “Optional” or “optionally” means that the subsequently described event or circumstance can or cannot occur, and that the description includes instances where the event or circumstance occurs and instances where it does not.

[0051] The term “about” when used before a numerical designation, *e.g.*, temperature, time, amount, concentration, and such other, including a range, indicates approximations which may vary by (+) or (-) 10%, 5%, 1%, or any subrange or subvalue there between.

[0052] Preferably, the term “about” when used with regard to a dose amount means that the dose may vary by +/- 10%. For example, “about 400 to about 800 binding agents” indicates that an outside surface of a nanoparticles contain an amount of binding agent between 360 and 880 particles.

[0053] “Comprising” or “comprises” is intended to mean that the compositions and methods include the recited elements, but not excluding others. “Consisting essentially of” when used to define compositions and methods, shall mean excluding other elements of any essential significance to the combination for the stated purpose. Thus, a composition consisting essentially of the elements as defined herein would not exclude other materials or steps that do not materially affect the basic and novel characteristic(s) of the claimed

invention. “Consisting of” shall mean excluding more than trace elements of other ingredients and substantial method steps. Embodiments defined by each of these transition terms are within the scope of this invention.

[0054] The term “nanoparticle” as used herein refers to particles having at least one dimension which is less than 5 microns. In preferred embodiments, such as for intravenous administration, the nanoparticle is less than 1 micron. For direct administration, the nanoparticle is larger. Even larger particles are expressly contemplated by the invention.

[0055] In a population of particles, the sizes of individual particles are distributed about a mean. Particle sizes for the population can therefore be represented by an average, and also by percentiles. D50 is the particle size below which 50% of the particles fall. 10% of particles are smaller than the D10 value and 90% of particles are smaller than D90. Where unclear, the “average” size is equivalent to D50.

[0056] The term “nanoparticle” may also encompass discrete multimers of smaller unit nanoparticles. For example, a 320 nm particle may comprise a dimer of a unit 160 nm nanoparticle. For 160 nm nanoparticles, multimers would therefore be approximately 320 nm, 480 nm, 640 nm, 800 nm, 960 nm, 1120 nm, and so on.

[0057] The term “carrier protein” as used herein refers to proteins that function to transport binding agents and/or therapeutic agents. The binding agents of the present disclosure can reversibly bind to the carrier proteins. Examples of carrier proteins are discussed in more detail below.

[0058] The term “core” as used herein refers to central or inner portion of the nanoparticle which may be comprised of a carrier protein, a carrier protein and a therapeutic agent, or other agents or combination of agents. In some embodiments, a hydrophobic portion of the binding agent may be incorporated into the core.

[0059] The term “therapeutic agent” as used herein means an agent which is therapeutically useful, e.g., an agent for the treatment, remission or attenuation of a disease state, physiological condition, symptoms, or etiological factors, or for the evaluation or diagnosis thereof. A therapeutic agent may be a chemotherapeutic agent, for example, mitotic

inhibitors, topoisomerase inhibitors, steroids, anti-tumor antibiotics, antimetabolites, alkylating agents, enzymes, proteasome inhibitors, or any combination thereof.

[0060] As used herein, the term “binding agent”, “binding agent specific for”, or “binding agent that specifically binds” refers to an agent that binds to a target antigen and does not significantly bind to unrelated compounds. Examples of binding agents that can be effectively employed in the disclosed methods include, but are not limited to, lectins, proteins, and antibodies, such as monoclonal antibodies, e.g. humanized monoclonal antibodies, chimeric antibodies, or polyclonal antibodies, or antigen-binding fragments thereof, as well as aptamers or fusion proteins. In one embodiment, the binding agent comprises an albumin-binding motif. Non-limiting examples of albumin-binding motifs can be found in PCT Application No. PCT/US2017/045643, filed August 4, 2017, which is incorporated herein by reference in its entirety. In an embodiment, the binding agent is an exogenous antibody. An exogenous antibody is an antibody not naturally produced in a mammal, e.g. in a human, by the mammalian immune system.

[0061] The term “antibody” or “antibodies” as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules (i.e., molecules that contain an antigen binding site that immuno-specifically bind an antigen). The term also refers to antibodies comprised of two immunoglobulin heavy chains and two immunoglobulin light chains as well as a variety of forms including full length antibodies and portions thereof; including, for example, an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR- grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')₂, a Fv, a disulfide linked Fv, a scFv, a single domain antibody (dAb), a diabody, a multispecific antibody, a dual specific antibody, an anti-idiotypic antibody, a bispecific antibody, a functionally active epitope-binding fragment thereof, bifunctional hybrid antibodies (e.g., Lanzavecchia *et al.*, *Eur. J Immunol.* 17, 105 (1987)) and single chains (e.g., Huston *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 85, 5879-5883 (1988) and Bird *et al.*, *Science* 242, 423-426 (1988), which are incorporated herein by reference). (See, generally, Hood *et al.*, *Immunology*, Benjamin, N.Y., 2ND ed. (1984); Harlow and Lane, *Antibodies. A Laboratory Manual*, Cold Spring Harbor Laboratory (1988); Hunkapiller and Hood, *Nature*, 323, 15-16 (1986), which are incorporated herein by reference). The antibody may be of any

type (e.g., IgG, IgA, IgM, IgE or IgD). Preferably, the antibody is IgG. An antibody may be non-human (e.g., from mouse, goat, or any other animal), fully human, humanized, or chimeric. Antibody or antibodies include any biosimilar(s) of the antibodies disclosed herein. Biosimilars, as used herein, refers to a biopharmaceutical which is deemed to be comparable in quality, safety, and efficacy to a reference product marketed by an innovator company (Section 351(i) of the Public Health Service Act (42 U.S.C. 262(i))).

[0062] The term “dissociation constant,” also referred to as “ K_d ,” refers to a quantity expressing the extent to which a particular substance separates into individual components (e.g., the protein carrier, antibody, and therapeutic agent).

[0063] The terms “lyophilized,” “lyophilization” and the like as used herein refer to a process by which the material (e.g., nanoparticles) to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient is optionally included in pre-lyophilized formulations to enhance stability of the lyophilized product upon storage. In some embodiments, the nanoparticles can be formed from lyophilized components (carrier protein, antibody and optional therapeutic) prior to use as a therapeutic. In other embodiments, the carrier protein, binding agent, e.g., antibody, and optional therapeutic agent are first combined into nanoparticles and then lyophilized. The lyophilized sample may further contain additional excipients.

[0064] The term “bulking agents” comprise agents that provide the structure of the freeze-dried product. Common examples used for bulking agents include mannitol, glycine, lactose and sucrose. In addition to providing a pharmaceutically elegant cake, bulking agents may also impart useful qualities in regard to modifying the collapse temperature, providing freeze-thaw protection, and enhancing the protein stability over long-term storage. These agents can also serve as tonicity modifiers. In some embodiments, the lyophilized compositions comprise a bulking agent. In some embodiments, the lyophilized compositions do not comprise a bulking agent.

[0065] The term “buffer” encompasses those agents which maintain the solution pH in an acceptable range prior to lyophilization and may include succinate (sodium or potassium), histidine, phosphate (sodium or potassium), Tris(tris(hydroxymethyl)aminomethane),

diethanolamine, citrate (sodium) and the like. The buffer of this invention has a pH in the range from about 5.5 to about 6.5; and preferably has a pH of about 6.0. Examples of buffers that will control the pH in this range include succinate (such as sodium succinate), gluconate, histidine, citrate and other organic acid buffers.

[0066] The term “cryoprotectants” generally includes agents which provide stability to the protein against freezing-induced stresses, presumably by being preferentially excluded from the protein surface. They may also offer protection during primary and secondary drying, and long- term product storage. Examples are polymers such as dextran and polyethylene glycol; sugars such as sucrose, glucose, trehalose, and lactose; surfactants such as polysorbates; and amino acids such as glycine, arginine, and serine.

[0067] The term “lyoprotectant” includes agents that provide stability to the protein during the drying or ‘dehydration’ process (primary and secondary drying cycles), presumably by providing an amorphous glassy matrix and by binding with the protein through hydrogen bonding, replacing the water molecules that are removed during the drying process. This helps to maintain the protein conformation, minimize protein degradation during the lyophilization cycle and improve the long-term products. Examples include polyols or sugars such as sucrose and trehalose.

[0068] The term “pharmaceutical formulation” refers to preparations which are in such form as to permit the active ingredients to be effective, and which contains no additional components that are toxic to the subjects to which the formulation would be administered.

[0069] “Pharmaceutically acceptable” excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0070] “Reconstitution time” is the time that is required to rehydrate a lyophilized formulation into a solution.

[0071] A “stable” formulation is one in which the protein therein essentially retains its physical stability and/or chemical stability and/or biological activity upon storage. For example, various analytical techniques for measuring protein stability are available in the art

and are reviewed in Peptide and Protein Drug Delivery, 247-301, Vincent Lee Ed., Marcel Dekker, Inc., New York, N.Y., Pubs. (1991) and Jones, A. *Adv. Drug Delivery Rev.* 10:29-90 (1993). Stability can be measured at a selected temperature for a selected time period.

[0072] The term “epitope” as used herein refers to the portion of an antigen which is recognized by a binding agent, e.g., an antibody. Epitopes include, but are not limited to, a short amino acid sequence or peptide (optionally glycosylated or otherwise modified) enabling a specific interaction with a protein (e.g., an antibody) or ligand. For example, an epitope may be a part of a molecule to which the antigen-binding site of a binding agent attaches.

[0073] The term “treating” or “treatment” covers the treatment of a disease or disorder (e.g., cancer), in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, i.e., arresting its development; (ii) relieving a disease or disorder, i.e., causing regression of the disease or disorder; (iii) slowing progression of the disease or disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments “treating” or “treatment” refers to the killing of cancer cells.

[0074] The term “kill” or “killing” with respect to a cancer treatment is directed to include any type of manipulation that will lead to the death of that cancer cell or at least of portion of a population of cancer cells.

[0075] The term “aptamer” refers to a nucleic acid molecule that is capable of binding to a target molecule, such as a polypeptide. For example, an aptamer of the invention can specifically bind to the antigen expressed on a T-cell cancer, e.g., CD2, CD3, CD4, CD5, CD8, CD20, CD38, CD25, CD30, CD40, CD52, CD 122, or CCR4. The generation of antibodies with a particular binding specificity and the therapeutic use of aptamers are well established in the art. See, e.g., U.S. Pat. No. 5,475,096, U.S. Pat. Nos. 5,270,163, 5,582,981, 5,840,867, 6,011,020, 6,051,698, 6,147,204, 6,180,348 and 6,699,843, and the therapeutic efficacy of Macugen® (Eyetechnology, New York) for treating age-related macular degeneration.

[0076] The term “oligomer” or “oligomeric” or “oligomerized” as used herein refers to oligomers composed of two or more monomers.

[0077] Fusion proteins are bioengineered polypeptides that join one portion of a protein (e.g., the crystallizable fragment (Fc) domain of an antibody; or an albumin-binding motif of an antibody) with another biologically active agent, e.g., a protein domain, peptide, or nucleic acid or peptide aptamer, to generate a molecule with desired structure–function properties and significant therapeutic potential. The gamma immunoglobulin (IgG) isotype is often used as the basis for generating Fc-fusion proteins because of favorable characteristics such as recruitment of effector function and increased plasma half-life. Given the range of aptamers, both peptide and nucleic acids, that can be used as fusion partners, fusion proteins have numerous biological and pharmaceutical applications.

[0078] Additionally, some terms used in this specification are more specifically defined below.

Overview

[0079] The current invention is predicated, in part, on the surprising discovery that optionally lyophilized nanoparticles comprising a carrier protein, a binding agent, e.g., an antibody, an aptamer, or a fusion protein having an albumin-binding motif and an antigen binding domain, e.g., an albumin-binding motif fused to an aptamer or the ligand of a cellular receptor, capable of binding to an antigen expressed on a T-cell or a T-cell cancer, and a therapeutic agent provide targeted therapy to a tumor while minimizing toxicity to the patient. In one aspect, the binding agent is OKT3 capable of binding to an antigen expressed on a T-cell or a T-cell cancer while, at the same time, reducing or elimination the serious side effects associated with the immunogenic and mitogenic potential limits of OKT3. Accordingly, the nanoparticles as described herein are thus a significant improvement compared to the conventional ADCs having serious side effects and/or toxicity.

[0080] As would be understood by one skilled in the art, for conventional ADCs to be effective, it is critical that the linker be stable enough not to dissociate in the systemic circulation but allow for sufficient drug release at the tumor site. Alley, S.C., *et al.* (2008) *Bioconjug Chem* 19:759-765. This has proven to be a major hurdle in developing effective drug conjugate (Julien, D.C., *et al.* (2011) *MAbs* 3:467-478; Alley, S.C., *et al.* (2008)

Bioconjug Chem 19:759-765); therefore, an attractive feature of the nano-immune conjugate is that a biochemical linker is not required.

[0081] Another shortcoming of current ADCs is that higher drug penetration into the tumor has not been substantively proven in human tumors. Early testing of ADCs in mouse models suggested that tumor targeting with antibodies would result in a higher concentration of the active agent in the tumor (Deguchi, T. *et al.* (1986) *Cancer Res* 46: 3751-3755); however, this has not correlated in the treatment of human disease, likely because human tumors are much more heterogeneous in permeability than mouse tumors. Jain, R.K. *et al.* (2010) *Nat Rev Clin Oncol* 7:653-664. Also, the size of the nanoparticle is critical for extravasation from the vasculature into the tumor. In a mouse study using a human colon adenocarcinoma xenotransplant model, the vascular pores were permeable to liposomes up to 400 nm. Yuan, F., *et al.* (1995) *Cancer Res* 55: 3752-3756. Another study of tumor pore size and permeability demonstrated that both characteristics were dependent on tumor location and growth status, with regressing tumors and cranial tumors permeable to particles less than 200 nm. Hobbs, S.K., *et al.* (1998) *Proc Natl Acad Sci U S A* 95:4607-4612. The nano-immune conjugate described herein overcomes this issue by the fact that the large complex, which is less than 200 nm intact, is partially dissociated in systemic circulation into smaller functional units that are easily able to permeate tumor tissue. Furthermore, once the conjugate arrives to the tumor site, the smaller toxic payload can be released and only the toxic portion needs to be taken up by tumor cells, not the entire conjugate.

[0082] The advent of antibody- (i.e. AVASTIN®) coated albumin nanoparticles containing a therapeutic agent (i.e., ABRAXANE®) has led to a new paradigm of directional delivery of two or more therapeutic agents to a predetermined site *in vivo*. See PCT Patent Publication Nos. WO 2012/154861 and WO 2014/055415, each of which is incorporated herein by reference in its entirety.

[0083] When compositions of albumin and an binding agent, e.g., antibody, are admixed together in an aqueous solution at specific concentrations and ratios, the binding agents useful in this invention spontaneously self-assemble into and onto the albumin to form nanoparticles having multiple copies of the binding agent (up to 500 or more).

[0084] While protein compositions comprising a single source protein are commonly stored in lyophilized form where they exhibit significant shelf-life, such lyophilized compositions do not contain a self-assembled nanoparticle of two different proteins integrated together by hydrophobic-hydrophobic interactions. Moreover, the nanoparticle configuration wherein a majority of the binding portions of the binding agent are exposed on the surface of the nanoparticles lends itself to being susceptible to dislodgement or reconfiguration by conditions which otherwise would be considered benign. For example, during lyophilization, ionic charges on the proteins are dehydrated thereby exposing the underlying charges. Exposed charges allow for charge-charge interactions between the two proteins which can alter the binding affinity of each protein to the other. In addition, the concentration of the nanoparticles increases significantly as the solvent (e.g., water) is removed. Such increased concentrations of nanoparticles could lead to irreversible oligomerization. Oligomerization is a known property of proteins that reduces the biological properties of the oligomer as compared to the monomeric form and increases the size of the particle sometimes beyond 1 micron.

[0085] On the other hand, a stable form of a nanoparticle composition is required for clinical and/or commercial use where a shelf-life of at least 3 months is required and shelf-lives of greater than 6 months or 9 months are preferred. Such a stable composition must be readily available for intravenous injection, must retain its self-assembled form upon intravenous injection so as to direct the nanoparticle to the predetermined site *in vivo*, must have a maximum size of less than 1 micron so as to avoid any ischemic event when delivered into the blood stream, and finally must be compatible with the aqueous composition used for injection.

Compounds

[0086] As will be apparent to the skilled artisan upon reading this disclosure, the present disclosure relates to compositions of nanoparticles containing a carrier protein, binding agents, and at least one therapeutic agent, wherein said compositions are optionally lyophilized.

[0087] In some embodiments, the carrier protein can be albumin, gelatin, elastin (including topoelastin) or elastin-derived polypeptides (e.g., α -elastin and elastin-like polypeptides (ELPs)), gliadin, legumin, zein, soy protein (e.g., soy protein isolate (SPI)), milk protein (e.g., β -lactoglobulin (BLG) and casein), or whey protein (e.g., whey protein concentrates (WPC) and whey protein isolates (WPI)). In preferred embodiments, the carrier protein is albumin. In preferred embodiments, the albumin is egg white (ovalbumin), bovine serum albumin (BSA), or the like. In even more preferred embodiments, the carrier protein is human serum albumin (HSA). In some embodiments, the carrier protein (e.g., albumin) is a recombinant protein (e.g., recombinant HSA). In some embodiments, the carrier protein is a generally regarded as safe (GRAS) excipient approved by the United States Food and Drug Administration (FDA). In one embodiment, the carrier protein comprises an antibody-binding motif. Non-limiting examples of antibody-binding motifs can be found in PCT Application No. PCT/US2017/045643, filed August 4, 2017, which is incorporated herein by reference in its entirety.

[0088] In some embodiments, the binding agents are antibodies selected from the group consisting of ado-trastuzumab emtansine, alemtuzumab, bevacizumab, cetuximab, denosumab, dinutuximab, ipilimumab, nivolumab, obinutuzumab, ofatumumab, panitumumab, pembrolizumab, pertuzumab, rituximab, and trastuzumab. In some embodiment, the binding agents are antibodies comprising Slipizumab, OKT3, Leu 1, Zanolimumab, Zanolimumab, Brentuximab vedotin, Mik- β 1, KW-0761, or a combination thereof. In some embodiments, one or more of these antibodies are explicitly excluded. In some embodiments, the antibodies are a substantially single layer of antibodies on all or part of the surface of the nanoparticle.

[0089] In one aspect, the binding portion of the antibody is capable of binding to an antigen expressed on a T-cell or a T-cell cancer. In one embodiment, the antigen is a protein expressed on the T-cell cancers, including but not limited to, CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, and CCR4. In a further embodiment, the antigen is a biomarker overexpressed in the T-cell cancers (e.g., T-cell lymphomas). The biomarkers overexpressed in the T-cell cancers may include PD-L1, Ly6E, HER3/EGFR DAF, ERBB-3 receptor, CSF-1R, HER2, STEAP1, CEA, OX40, Ang2-VEGF, or VEGF. In a preferred

embodiment, the binding portion of the antibody is capable of binds CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, or CCR4. In another embodiment, the binding portion of the antibody is capable of binding PD-L1, Ly6E, HER3/EGFR DAF, ERBB-3 receptor, CSF-1R, HER2, STEAP1, CEA, OX40, Ang2-VEGF, or VEGF.

[0090] Table 1 depicts a list of non-limiting list of monoclonal antibodies for treating T-cell leukemia and lymphoma.

Table 1. Monoclonal antibodies for treating T-cell cancers.

Target Antigen	Description	Monoclonal Antibody
CD2	LFA-3 (CD58)	Slipizumab (MEDI-507)
CD3 (CD3 ζ)	TcR signaling chain	muromonab-CD3 (Orthoclone®, OKT3)
CD4	TcR co-receptor	Zanolimumab (HuMax-CD4®)
CD5	Scavenger receptor family member	Anti-Leu1/ T101
CD25	IL-2 receptor α -subunit	Daclizumab (Zenapax®)
CD30	TNF receptor family member	Brentuximab vedotin (Adcetris®)
CD52	GPI-anchored glycoprotein	Alemtuzumab (Campath®)
CD122	β -subunit of the IL-2 and IL-15 receptor	Mik- β 1
CCR4	Chemokine receptor-4	KW-0761

[0091] In some embodiments, the at least one therapeutic agent, or the at least one additional therapeutic agent, is selected from the group consisting of abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuporelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, and cyclophosphamide. In one embodiment, the therapeutic agent

comprises one or more of Adriamycin, bleomycin, vinblastine sulfate, vincristine sulfate, etoposide, prednisone, cyclophosphamide and dacarbazine. In one embodiment, the therapeutic agent comprises one or more of arranon (Nelarabine), abitrexate, adriamycin (doxorubicin hydrochloride), ambochlorin (Chlorambucil), Zydelig (Idelalisib), Vincasar PFS (Vincristine Sulfate), Velsar (Vinblastine Sulfate), Velcade (Bortezomib), Velban (Vinblastine Sulfate), Treanda (Bendamustine Hydrochloride), Romidepsin, Rheumatrex (Methotrexate), Revlimid (Lenalidomide), Procarbazine Hydrochloride, Prednisone, Pralatrexate, Plerixafor, Neosar (Cyclophosphamide), Mustargen (Mechlorethamine Hydrochloride), Methotrexate, Mechlorethamine Hydrochloride, Matulane (Procarbazine Hydrochloride), Lomustine, Linfovizin or Leukeran (Chlorambucil), Istodax (Romidepsin), Imbruvica (Ibrutinib), DTIC-Dome (Dacarbazine), Doxorubicin Hydrochloride, Denileukin Diftitox, Cytosan (Cyclophosphamide), Carmustine, Beleodaq (Belinostat), or Arranon (Nelarabine). In one embodiment, the therapeutic agent comprises one or more of a mustard derivative (e.g., Cyclophosphamide, Mechlorethamine or Ifosfamide), Doxorubicin, Vinblastine, Vincristine, Bleomycin, Etoposide, and Prednisone. One of skill in the art would understand that these are merely examples, and any chemotherapeutic agent or cancer therapeutic agent may be included.

[0092] Preferably, the nanoparticles comprise paclitaxel as a therapeutic agent.

[0093] It is to be understood that the therapeutic agent may be located inside the nanoparticle, on the outside surface of the nanoparticle, or both. The nanoparticle may contain more than one therapeutic agent, for example, two therapeutic agents, three therapeutic agents, four therapeutic agents, five therapeutic agents, or more. Furthermore, a nanoparticle may contain the same or different therapeutic agents inside and outside the nanoparticle.

[0094] In one aspect, the nanoparticle comprises at least 100 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises at least 200 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises at least 300 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises at least 400 binding agents non-

covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises at least 500 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises at least 600 binding agents non-covalently bound to the surface of the nanoparticle.

[0095] In one aspect, the nanoparticle comprises between about 100 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 200 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 300 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 400 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 500 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 600 and about 1000 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 200 and about 800 binding agents non-covalently bound to the surface of the nanoparticle. In one aspect, the nanoparticle comprises between about 300 and about 800 binding agents non-covalently bound to the surface of the nanoparticle. In preferred embodiments, the nanoparticle comprises between about 400 and about 800 binding agents non-covalently bound to the surface of the nanoparticle. Contemplated values include any value or subrange within any of the recited ranges, including endpoints.

[0096] In one aspect, the average particle size in the nanoparticle composition is less than about 1 μm . In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 1 μm . In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 900 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 800 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 700 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 600 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 500 nm. In one

aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 400 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 300 nm. In one aspect, the average particle size in the nanoparticle composition is between about 90 nm and about 200 nm. In a preferred embodiment, the average particle size in the nanoparticle composition is between about 100 nm and about 180 nm. In an especially preferred embodiment, the mean particle size in the nanoparticle composition is about 100 nm to about 160 nm. Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

[0097] In one aspect, the nanoparticle composition is formulated for intravenous injection. In order to avoid an ischemic event, the nanoparticle composition formulated for intravenous injection should comprise nanoparticles with an average particle size of less than about 1 μm .

[0098] In one aspect, the average particle size in the nanoparticle composition is greater than about 1 μm . In one aspect, the average particle size in the nanoparticle composition is between about 1 μm and about 5 μm . In one aspect, the average particle size in the nanoparticle composition is between about 1 μm and about 4 μm . In one aspect, the average particle size in the nanoparticle composition is between about 1 μm and about 3 μm . In one aspect, the average particle size in the nanoparticle composition is between about 1 μm and about 2 μm . In one aspect, the average particle size in the nanoparticle composition is between about 1 μm and about 1.5 μm . Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

[0099] In one aspect, the nanoparticle composition is formulated for direct injection into a tumor. Direct injection includes injection into or proximal to a tumor site, perfusion into a tumor, and the like. When formulated for direct injection into a tumor, the nanoparticle may comprise any average particle size. Without being bound by theory, it is believed that larger particles (e.g., greater than 500 nm, greater than 1 μm , and the like) are more likely to be immobilized within the tumor, thereby providing a beneficial effect. Larger particles can accumulate in the tumor or specific organs. *See, e.g.,* 20-60 micron glass particle that is used to inject into the hepatic artery feeding a tumor of the liver, called “TheraSphere®” (in clinical use for liver cancer). Therefore, for intravenous administration, particles under 1 μm

are typically used. Particles over 1 μm are, more typically, administered directly into a tumor (“direct injection”) or into an artery feeding into the site of the tumor.

[0100] In one aspect, less than about 0.01% of the nanoparticles within the composition have a particle size greater than 200 nm, greater than 300 nm, greater than 400 nm, greater than 500 nm, greater than 600 nm, greater than 700 nm, or greater than 800 nm. In one aspect, less than about 0.001% of the nanoparticles within the composition have a particle size greater than 200 nm, greater than 300 nm, greater than 400 nm, greater than 500 nm, greater than 600 nm, greater than 700 nm, or greater than 800 nm. In a preferred embodiment, less than about 0.01% of the nanoparticles within the composition have a particle size greater than 800 nm. In a more preferred embodiment, less than about 0.001% of the nanoparticles within the composition have a particle size greater than 800 nm.

[0101] In a preferred aspect, the sizes and size ranges recited herein relate to particle sizes of the reconstituted lyophilized nanoparticle composition. That is, after the lyophilized nanoparticles are resuspended in an aqueous solution (e.g., water, other pharmaceutically acceptable excipient, buffer, etc.), the particle size or average particle size is within the range recited herein.

[0102] In one aspect, at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, or 99.9% of the nanoparticles are present in the reconstituted composition as single nanoparticles. That is, fewer than about 50%, 40%, 30%, etc. of the nanoparticles are dimerized or multimerized (oligomerized).

[0103] In some embodiments, the nanoparticles in the composition have less than 20% by number dimerization, less than 10% by number dimerization and preferably less than 5% dimerization.

[0104] In some embodiments, the size of the nanoparticle can be controlled by the adjusting the amount (*e.g.*, ratio) of carrier protein to binding agent. The size of the nanoparticles, and the size distribution, is also important. The nanoparticles of the invention may behave differently according to their size. At large sizes, an agglomeration may block blood vessels. Therefore, agglomeration of nanoparticles can affect the performance and safety of the

composition. On the other hand, larger particles may be more therapeutic under certain conditions (e.g., when not administered intravenously).

[0105] In one aspect, the nanoparticle composition comprises at least one additional therapeutic agent. In one embodiment, the at least one additional therapeutic agent is non-covalently bound to the outside surface of the nanoparticle. In one embodiment, the at least one additional therapeutic agent is arranged on the outside surface of the nanoparticle. In one embodiment, the at least one additional therapeutic agent is selected from the group consisting of abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gemcitabine, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, and cyclophosphamide. In one embodiment, the at least one additional therapeutic agent is an anti-cancer binding agent, e.g., an anti-cancer antibody.

Methods of Making Nanoparticles

[0106] In some aspects, the current invention relates to methods of making nanoparticle compositions as described herein. Nanoparticles as described herein can be made by any method. Non-limiting examples of methods of making nanoparticles, nanoparticle compositions, and lyophilized nanoparticle compositions can be found in PCT Pub. Nos. WO2014/055415 and WO2016/057554, each of which is incorporated herein by reference in its entirety.

[0107] In one aspect, the nanoparticles of the nanoparticle composition are formed by contacting the carrier protein or carrier protein-therapeutic agent particle with the binding agent at a ratio of about 10:1 to about 10:30 carrier protein particle or carrier protein-therapeutic agent particle to binding agent. In one embodiment, the ratio is about 10:2 to about 10:25. In one embodiment, the ratio is about 10:2 to about 1:1. In a preferred embodiment, the ratio is about 10:2 to about 10:6. In an especially preferred embodiment,

the ratio is about 10:4. Contemplated ratios include any value, subrange, or range within any of the recited ranges, including endpoints.

[0108] In one embodiment, the amount of solution or other liquid medium employed to form the nanoparticles is particularly important. No nanoparticles are formed in an overly dilute solution of the carrier protein (or carrier protein-therapeutic agent) and the antibodies. An overly concentrated solution will result in unstructured aggregates. In some embodiments, the amount of solution (e.g., sterile water, saline, phosphate buffered saline) employed is between about 0.5 mL of solution to about 20 mL of solution. In some embodiments, the amount of carrier protein is between about 1 mg/mL and about 100 mg/mL. In some embodiments, the amount of binding agent is between about 1 mg/mL and about 30 mg/mL. For example, in some embodiments, the ratio of carrier protein:binding agent:solution is approximately 9 mg of carrier protein (e.g., albumin) to 4 mg of binding agent, e.g., antibody (e.g., OKT3) in 1 mL of solution (e.g., saline). An amount of a therapeutic agent (e.g., Taxol) can also be added to the carrier protein. For example, 1 mg of Taxol can be added 9 mg of carrier protein (10 mg carrier protein-therapeutic) and 4 mg of binding agent, e.g., antibody, Fc fusion molecule, or aptamer, in 1 mL of solution. When using a typical i.v. bag, for example, with the solution of approximately 1 liter one would need to use 1000x the amount of carrier protein/carrier protein-therapeutic agent and antibodies compared to that used in 1 mL. Thus, one cannot form the present nanoparticles in a standard i.v. bag. Furthermore, when the components are added to a standard i.v. bag in the therapeutic amounts of the present invention, the components do not self-assemble to form nanoparticles.

[0109] In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH between about 4 and about 8.

[0110] In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH of about 4. In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH of about 5. In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a

pH of about 6. In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH of about 7. In one embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH of about 8. In a preferred embodiment, the carrier protein or carrier protein-therapeutic agent particle is contacted with the binding agent in a solution having a pH between about 5 and about 7.

[0111] In one embodiment, the carrier protein particle or carrier protein-therapeutic agent particle is incubated with the binding agent at a temperature of about 5 °C to about 60 °C, or any range, subrange, or value within that range including endpoints. In a preferred embodiment, the carrier protein particle or carrier protein-therapeutic agent particle is incubated with the binding agent at a temperature of about 23 °C to about 60 °C.

[0112] Without being bound by theory, it is believed that the stability of the nanoparticles within the nanoparticle composition is, at least in part, dependent upon the temperature and/or pH at which the nanoparticles are formed, as well as the concentration of the components (*i.e.*, carrier protein, binding agent, and optionally therapeutic agent) in the solution. In one embodiment, the K_d of the nanoparticles is between about 1×10^{-11} M and about 2×10^{-5} M. In one embodiment, the K_d of the nanoparticles is between about 1×10^{-11} M and about 2×10^{-8} M. In one embodiment, the K_d of the nanoparticles is between about 1×10^{-11} M and about 7×10^{-9} M. In a preferred embodiment, the K_d of the nanoparticles is between about 1×10^{-11} M and about 3×10^{-8} M. Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

Lyophilization

[0113] The lyophilized compositions of this invention are prepared by standard lyophilization techniques with or without the presence of stabilizers, buffers, etc. Surprisingly, these conditions do not alter the relatively fragile structure of the nanoparticles. Moreover, at best, these nanoparticles retain their size distribution upon lyophilization and, more importantly, can be reconstituted for *in vivo* administration (e.g., intravenous delivery) in substantially the same form and ratios as if freshly made.

[0114] Lyophilization, or freeze-drying, removes water from a composition. In the process, the material to be dried is first frozen and then the ice or frozen solvent is removed by sublimation in a vacuum environment. An excipient may be included in pre-lyophilized formulations to enhance stability during the freeze-drying process and/or to improve stability of the lyophilized product upon storage. Pikal, M. Biopharm. 3(9)26-30 (1990) and Arakawa et al., Pharm. Res. 8(3):285- 291 (1991).

[0115] While proteins may be lyophilized, the process of lyophilization and reconstitution may affect the properties of the protein. Because proteins are larger and more complex than traditional organic and inorganic drugs (i.e. possessing multiple functional groups in addition to complex three-dimensional structures), the formulation of such proteins poses special problems. For a protein to remain biologically active, a formulation must preserve intact the conformational integrity of at least a core sequence of the protein's amino acids while at the same time protecting the protein's multiple functional groups from degradation. Degradation pathways for proteins can involve chemical instability (i.e. any process which involves modification of the protein by bond formation or cleavage resulting in a new chemical entity) or physical instability (i.e. changes in the higher order structure of the protein). Chemical instability can result from deamidation, racemization, hydrolysis, oxidation, beta elimination or disulfide exchange. Physical instability can result from denaturation, aggregation, precipitation or adsorption, for example. The three most common protein degradation pathways are protein aggregation, deamidation and oxidation. Cleland, et al., Critical Reviews in Therapeutic Drug Carrier Systems 10(4): 307-377 (1993).

Formulations

[0116] In one aspect, the nanoparticle composition is formulated for systemic delivery, e.g., intravenous administration.

[0117] In one aspect, the nanoparticle composition is formulated for direct injection into a tumor. Direct injection includes injection into or proximal to a tumor site, perfusion into a tumor, and the like. Because the nanoparticle composition is not administered systemically, a nanoparticle composition is formulated for direct injection into a tumor may comprise any average particle size. Without being bound by theory, it is believed that larger particles (e.g.,

greater than 500 nm, greater than 1 μm , and the like) are more likely to be immobilized within the tumor, thereby providing what is believed to be a better beneficial effect.

[0118] In another aspect, provided herein is a composition comprising a compound provided herein, and at least one pharmaceutically acceptable excipient.

[0119] In general, the compounds provided herein can be formulated for administration to a patient by any of the accepted modes of administration. Various formulations and drug delivery systems are available in the art. See, *e.g.*, Gennaro, A.R., ed. (1995) *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing Co.

[0120] In general, compounds provided herein will be administered as pharmaceutical compositions by any one of the following routes: oral, systemic (*e.g.*, transdermal, intranasal or by suppository), or parenteral (*e.g.*, intramuscular, intravenous or subcutaneous) administration.

[0121] The compositions are comprised of, in general, a compound of the present invention in combination with at least one pharmaceutically acceptable excipient. Acceptable excipients are non-toxic, aid administration, and do not adversely affect the therapeutic benefit of the claimed compounds. Such excipient may be any solid, liquid, semi-solid or, in the case of an aerosol composition, gaseous excipient that is generally available to one of skill in the art.

[0122] Solid pharmaceutical excipients include starch, cellulose, talc, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, dried skim milk and the like. Liquid and semisolid excipients may be selected from glycerol, propylene glycol, water, ethanol and various oils, including those of petroleum, animal, vegetable or synthetic origin, *e.g.*, peanut oil, soybean oil, mineral oil, sesame oil, etc. Preferred liquid carriers, particularly for injectable solutions, include water, saline, aqueous dextrose, and glycols. Other suitable pharmaceutical excipients and their formulations are described in *Remington's Pharmaceutical Sciences*, edited by E. W. Martin (Mack Publishing Company, 18th ed., 1990).

[0123] The present compositions may, if desired, be presented in a pack or dispenser device containing one or more unit dosage forms containing the active ingredient. Such a pack or device may, for example, comprise metal or plastic foil, such as a blister pack, or glass, and rubber stoppers such as in vials. The pack or dispenser device may be accompanied by instructions for administration. Compositions comprising a compound of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition.

Treatment Methods

[0124] The nanoparticle compositions as described herein are useful in treating cancer cells and/or tumors in a mammal. In a preferred embodiment, the mammal is a human (i.e., a human patient). Preferably, the lyophilized nanoparticle composition is reconstituted (suspended in an aqueous excipient) prior to administration.

[0125] In one aspect is provided a method for treating a cancer cell, the method comprising contacting the cell with an effective amount of nanoparticle composition as described herein to treat the cancer cell. Treatment of a cancer cell includes, without limitation, reduction in proliferation, killing the cell, preventing metastasis of the cell, and the like.

[0126] In one aspect is provided a method for treating T-cells or T-cell cancers in a patient in need thereof, the method comprising administering to the patient a therapeutically effective amount of a nanoparticle composition as described herein to treat the T-cell cancer, where the T-cell cancer is peripheral T-cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma cutaneous T-cell lymphoma, Adult T-cell Leukemia/Lymphoma (ATLL), enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, blastic NK-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphoma, treatment-related T-cell lymphoma, or a combination thereof.

[0127] In one aspect is provided a method for treating a tumor in a patient in need thereof, the method comprising administering to the patient a therapeutically effective amount of a nanoparticle composition as described herein to treat the tumor. In one embodiment, the size

of the tumor is reduced. In one embodiment, the tumor size does not increase (i.e. progress) for at least a period of time during and/or after treatment.

[0128] In one embodiment, the nanoparticle composition is administered intravenously. In one embodiment, the nanoparticle composition is administered directly to the tumor. In one embodiment, the nanoparticle composition is administered by direct injection or perfusion into the tumor.

[0129] In one embodiment, the method comprises: a) administering the nanoparticle composition once a week for three weeks; b) ceasing administration of the nanoparticle composition for one week; and c) optionally repeating steps a) and b) as necessary to treat the tumor.

[0130] In one embodiment, the therapeutically effective amount of the nanoparticles described herein comprises about 1 mg/m² to about 200 mg/m² antibody, about 2 mg/m² to about 150 mg/m², about 5 mg/m² to about 100 mg/m², about 10 mg/m² to about 85 mg/m², about 15 mg/m² to about 75 mg/m², about 20 mg/m² to about 65 mg/m², about 25 mg/m² to about 55 mg/m², about 30 mg/m² to about 45 mg/m², or about 35 mg/m² to about 40 mg/m² antibody. In other embodiments, the therapeutically effective amount comprises about 20 mg/m² to about 90 mg/m² antibody. In one embodiment, the therapeutically effective amount comprises 30 mg/m² to about 70 mg/m² antibody. In one embodiment, the therapeutically effective amount of the nanoparticles described herein comprises about 50 mg/m² to about 200 mg/m² carrier protein or carrier protein and therapeutic agent. In a preferred embodiment, the therapeutically effective amount comprises about 75 mg/m² to about 175 mg/m² carrier protein or carrier protein and therapeutic agent. Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

[0131] In one embodiment, the therapeutically effective amount comprises about 20 mg/m² to about 90 mg/m² binding agent, e.g., antibody, aptamer or fusion protein. In a preferred embodiment, the therapeutically effective amount comprises 30 mg/m² to about 70 mg/m² binding agent, e.g., antibody, aptamer or fusion protein. Contemplated values include any value, subrange, or range within any of the recited ranges, including endpoints.

[0132] In one aspect, the cancer is T-cell cancer. In some embodiments, the cancer is T-cell lymphoma. In another embodiment, the cancer is peripheral T-cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma cutaneous T-cell lymphoma, Adult T-cell Leukemia/Lymphoma (ATLL), enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, blastic NK-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphoma, treatment-related T-cell lymphoma, or the combination thereof.

[0133] In general, the compounds of this invention will be administered in a therapeutically effective amount by any of the accepted modes of administration for agents that serve similar utilities. The actual amount of the compound of this invention, i.e., the nanoparticles, will depend upon numerous factors such as the severity of the disease to be treated, the age and relative health of the subject, the potency of the compound used, the route and form of administration, and other factors well known to the skilled artisan.

[0134] An effective amount of such agents can readily be determined by routine experimentation, as can the most effective and convenient route of administration, and the most appropriate formulation. Various formulations and drug delivery systems are available in the art. See, e.g., Gennaro, A.R., ed. (1995) *Remington's Pharmaceutical Sciences*, 18th ed., Mack Publishing Co.

[0135] An effective amount or a therapeutically effective amount or dose of an agent, e.g., a compound of the invention, refers to that amount of the agent or compound that results in amelioration of symptoms or a prolongation of survival in a subject. Toxicity and therapeutic efficacy of such molecules can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, e.g., by determining the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population). The dose ratio of toxic to therapeutic effects is the therapeutic index, which can be expressed as the ratio LD50/ED50. Agents that exhibit high therapeutic indices are preferred.

[0136] The effective amount or therapeutically effective amount is the amount of the compound or pharmaceutical composition that will elicit the biological or medical response of a tissue, system, animal or human that is being sought by the researcher, veterinarian, medical doctor or other clinician. Dosages may vary within this range depending upon the

dosage form employed and/or the route of administration utilized. The exact formulation, route of administration, dosage, and dosage interval should be chosen according to methods known in the art, in view of the specifics of a subject's condition.

[0137] Dosage amount and interval may be adjusted individually to provide plasma levels of the active moiety that are sufficient to achieve the desired effects; i.e., the minimal effective concentration (MEC). The MEC will vary for each compound but can be estimated from, for example, *in vitro* data and animal experiments. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. In cases of local administration or selective uptake, the effective local concentration of the drug may not be related to plasma concentration.

EXAMPLES

[0138] The present disclosure is illustrated using nanoparticles composed of albumin-bound paclitaxel (i.e., ABRAXANE®) or cisplatin as core, and antibodies that recognize antigen(s) on or expressed by cancerous T cells (e.g., OKT3).

[0139] One skilled in the art would understand that making and using the nanoparticles of the Examples are for the sole purpose of illustration, and that the present disclosure is not limited by this illustration.

[0140] Any abbreviation used herein, has normal scientific meaning. All temperatures are °C unless otherwise stated. Herein, the following terms have the following meanings unless otherwise defined:

ABX	=	ABRAXANE®/(albumin-bound paclitaxel
ACN	=	acetonitrile
ADC	=	antibody dependent chemotherapy
BEV	=	bevacizumab
BSA	=	bovine serum albumin
dH ₂ O	=	distilled water
nM	=	nanomolar
EdU	=	5-ethynyl-2'-deoxyuridine

FCB	=	flow cytometry buffer
FITC	=	Fluorescein
kD	=	kilo-dalton
Kd	=	dissociation constant
kg	=	kilogram
KV	=	kilo-volts
L/hr	=	liter/hour
M	=	molar
mCi	=	millicuries
mg	=	milligram
ml or mL	=	milliliter
m ²	=	square meters
mm ³	=	cubic millimeter
OKT3	=	muromonab-CD3
μg	=	microgram
μl	=	microliter
μm	=	micrometer/micron
PBS	=	Phosphate buffered saline
pK	=	pharmacokinetics
RT	=	room temperate
rpm	=	rotations per minute
v	=	volts
x g	=	times gravity

Example 1: Nanoparticle Preparation

[0141] ABRAXANE® (ABX) is suspended in muromonab-CD3 (OKT3) with 0.9% saline. The mixture is incubated for 30 minutes at room temperature (or at the temperature indicated) to allow particle formation. For Mastersizer experiments to measure particle size of ABX:OKT3 complexes, 10 mg of ABX is suspended in OKT3 at concentrations of 0 to 25 mg/ml.

[0142] For use in humans, the ABX:OKT3 complexes may be prepared by obtaining the dose appropriate number of OKT3 and diluting each vial per the following directions to various concentrations. The dose appropriate number of 100 mg vials of ABX can be prepared by reconstituting to a final concentration containing 10 mg/mL ABX nanoparticles.

Using a sterile 3 mL syringe, the OKT3 can be withdrawn and slowly injected, over a minimum of 1 minute, onto the inside wall of each of the vials containing 100 mg of ABX. The OKT3 solution should not be injected directly onto the lyophilized cake as this will result in foaming. Then, using a sterile 12 mL sterile syringe, 8.4 mL 0.9% Sodium Chloride Injection, USP, can be withdrawn and slowly injected, over a minimum of 1 minute, 8.4 mL onto the inside wall of each vial containing ABX 100 mg and OKT from 0 to 40 mg. Once the addition of OKT3 and 0.9% Sodium Chloride Injection, USP 8.4 mL is completed, each vial can be gently swirled and/or inverted slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Generation of foam should be avoided. The vials containing the ABX and OKT3 should sit for 60 minutes. The vial(s) should be gently swirled and/or inverted every 10 minutes to continue to mix the complex. After 60 minutes has elapsed, the calculated dosing volume of ABX and OKT3 should be withdrawn from each vial and slowly added to an empty viaflex bag. An equal volume of 0.9% Sodium Chloride Injection, USP is then added to the mixture of ABX and OKT3. The bag should then be gently swirled and/or inverted slowly for 1 minute to mix. The ABX: OKT3 nanoparticles can be stored for up to 4 hours at room temperature following final dilution.

Example 2: Binding of ABX and OKT3 *in vitro*

[0143] To determine whether ABX and OKT3 interact, the nanoparticles formed in Example 1 are analyzed by flow cytometry and electron microscopy.

Methods

[0144] Flow Cytometry: The nanoparticle composition comprising ABX and OKT3 is produced as described in Example 1 above. To determine binding of OKT3 to ABX, visualization of the composition is performed on an Accuri C6 flow cytometer (BD Franklin Lakes, NJ) and data analysis is done using Accuri C6 software. Biotinylated (5 μ g) goat anti-mouse IgG (Abeam, Cambridge, MA) is labeled with 5 μ g of streptavidin PE (Abeam, Cambridge, MA). The goat anti-mouse IgG is chosen to label the composition because the Fab portion of the OKT3 is mouse derived. ABX and the composition are incubated with the PE-labeled goat anti-mouse IgG for 30 minutes at room temperature, washed and visualized by flow cytometry.

[0145] Electron Microscopy: ABX, dissolved in PBS is added to a 300-mesh parlodian-carbon coated copper grid and allowed to sit for 1 minute. A pointed piece of filter paper is touched to the drop to remove excess liquid, leaving a thin film on the grid. The grids are allowed to dry. To dissolve the buffer crystals left on the dried grid, the sample is washed three times in dH₂O. A small drop of 1% phosphotungstic acid (PTA), pH 7.2, is added to the grid. The grid is then again touched by a pointed piece of filter paper to remove excess liquid, leaving a thin film on the grid and allowed to dry. OKT3 in 0.9% sodium chloride solution is diluted with PBS at 1:10 ratio. OKT3 is loaded on nickel formvar-coated grid and allowed to air dry for 30 minutes to 1 hour. For the composition, ABX dissolved in PBS, and OKT3 in 0.9% sodium chloride solution, are mixed. The complex is further diluted with PBS at 1:5. The complex is loaded on nickel formvar-coated grid and air dried for 30 minutes to 1 hour. Both samples are incubated for 1 hour in goat anti-mouse IgG with 6 nm gold-conjugated particles (Electron Microscopy Sciences), diluted 1:30 with 10% FCB/PBS, washed 6 times with PBS (each 2 minutes), 6 times with dH₂O, then stained with the mixture of 2% methylcellulose and 4% UA (9:1) for 5 minutes. Filter paper is used to drain the stain and the grid is air dried for 1 hour. Both samples are incubated overnight in donkey anti-mouse IgG with 6 nm gold-conjugated particles (Jackson ImmunoResearch) diluted 1:25 with 10% FCB/PBS, washed 6 times with PBS (each 2 minutes), 6 times with dH₂O water, stained with 1% PTA for 5 minutes, air dried, covered with 2% methylcellulose, and air dried for 1 hour. The micrographs are taken on a JEOL1400 at operating at 80 KV.

Example 3: Function of the nanoparticle composition *in vitro*

[0146] The experiment is to confirm that the two key elements in the complexes, the antibody and the paclitaxel, retain their functions when present in the complexes.

[0147] In vitro toxicity: The HuT-78 human T-cell lymphoma cell line (ATCC Manassas, VA) are cultured in RPMI 1640 medium supplemented with 4.5g/L glucose, L-glutamine, and 10% fetal bovine serum (MG-72, CLS order number 820702). Cells are harvested and plated at 10⁶ cells per well in 24 well plates. Cells are exposed to ABX or the composition at paclitaxel concentrations from 0 to 200 µg/ml overnight at 37 °C and 5% CO₂. To measure proliferation, the Click-iT EdU (Molecular Probes, Eugene, OR) kit is utilized. Briefly, 10

mM EdU is added to the wells and incubated overnight with the cells and ABX or the nanoparticle composition. The cells are permeabilized with 1% saponin and intercalated EdU is labeled with a FITC-conjugated antibody. The proliferation index is determined by dividing the FITC positive cells from each treatment by the maximum proliferation of untreated EdU labeled cells.

[0148] VEGF ELISA: To determine whether OKT3 can still bind its ligand, CD3, when bound to ABX, a standard CD3 ELISA is used. The composition is prepared as described and CD3-Ig fusion proteins are added to the composition with complex or ABX alone. The CD3-Ig fusion protein is incubated with the nanoparticles for 2 hours at room temperature. The suspension is spun at 6000 rpm for 15 minutes, supernatants are collected and free fusion protein is measured by ELISA. Absorbance is measured by a Versamax ELISA plate reader (Molecular Devices, Sunnyvale, CA). The concentration of unbound VEGF is determined with a standard curve from 0 to 2000 pg/ml.

Example 4: Particle Size

[0149] To understand the characteristics of the nanoparticles formed when binding OKT3 to ABX, the size of the ABX:OKT3 complexes is determined relative to ABX.

[0150] Mastersizer and Nanosight: The particle size of ABX and antibody-ABX drug complexes are measured by dynamic light scattering on a Mastersizer 2000 (Malvern Instruments, Westborough, MA). To measure particle size, 2 ml (5 mg/ml) of ABRAXANE® or complex is added to the sample chamber. Data are analyzed with Malvern software and particle size distributions are displayed by volume. The particle sizes and stability are later validated using the Nanosight System (Malvern Instruments, Westborough, MA). The ABX or complex particles are diluted to the appropriate range to accurately measure particle sizes. Data is displayed by particle size distribution; however, the nanoparticle tracking analysis uses Brownian motion to determine particle size.

Example 5: Protein Affinity

[0151] To understand the characteristics of the nanoparticles formed when binding OKT3 to Abraxane, the binding affinity of the complex was determined by a Bio-layer

interferometry (BLItz) assay, which is an optical technology that uses interference of white light reflection to determine binding affinities of 2 proteins

[0152] In a BLItz assay, if the 2 proteins bind, the reflected light changes wavelength in a manner that correlates to the binding affinity of the 2 proteins. Here biotinylated OKT3 at 100ug/ml was immobilized on a streptavidin-containing probe and exposed to Abraxane at 2 concentrations—500ug/ml (Run 2) and 1000ug/ml (Run 3)

[0153] In the case of the anti-human CD3 antibody (OKT3) and Abraxane, biotinylated OKT3 at 100ug/ml was immobilized on a streptavidin-containing probe. The OKT3 bearing probe was then immersed in Abraxane at 2 concentrations, 500ug/ml and 1000ug/ml (Run 2 and Run 3). Then Abraxane was bound to OKT3 in a concentration dependent manner. The association and dissociation constants on the proteins were calculated by the BLItz software. See Fig. 18.

[0154] The results show the dissociation constant of OKT3 and Abraxane was 2.246×10^9 , which suggests a strong non-covalent bond between these two proteins.

Example 6: Efficacy of the ABX:OKT3 complex in Mice

[0155] A xenograft model of HuT-78 human T-cell lymphoma cells implanted into athymic nude mice is employed to test the efficacy of the composition with ABX:OKT3 complex *in vivo*.

[0156] *In vivo* experiments are performed at least 2 times. The number of mice required for those experiments is determined by power analysis. Mouse tumors are measured 2-3 times/week and mice are sacrificed when the tumor is 10% by weight. Mice that has complete tumor responses are monitored for 60-80 days post-treatment. The end point of the mouse studies is median survival. Kaplan-Meier curves are generated and Mantle-Cox test is performed to determine significance of median survival between treatment groups. The *in vitro* results presented are representative of at least 5 repeated experiments. Statistical analyses of *in vitro* and *in vivo* percent change from baseline experiments are done using the Student's t-test.

[0157] Mouse Model: To test tumor efficacy, 1×10^6 HuT-78 human T-cell lymphoma cells are implanted into the right flank of athymic nude mice (Harlan Sprague Dawley, Indianapolis, IN). When the tumors reach a size of about 700 mm^3 , the mice are randomized and treated with PBS, ABX, OKT3 (12 mg/kg), OKT3 followed by ABX, or the composition as described above at the above concentrations. For the mouse experiments testing bigger nanoparticles, the highest dose of OKT3 necessary to create the larger particles is used in the OKT3-only treatment group. Tumor size is monitored 3 times/week and tumor volume is calculated with the following equation: $(\text{length} \times \text{width}^2)/2$. Mice are sacrificed when the tumor size equaled 10% of the mouse body weight or about 2500 mm^3 . The day 7 percent change from baseline is calculated as follows: $[(\text{tumor size on treatment day} - \text{tumor size on day 7}) / \text{tumor size on treatment day}] \times 100$.

Example 7: Paclitaxel Pharmacokinetics in Mice

[0158] To compare the pharmacokinetics (pk) of the composition and ABX, plasma paclitaxel concentrations are measured in mice administered composition or ABX at 0, 4, 8, 12 and 24 hours.

Methods

[0159] Paclitaxel Pharmacokinetics: The liquid chromatographic separation of paclitaxel and d5 paclitaxel are accomplished using an Agilent Poroshell 120 EC-C18 precolumn (2.1 x 5 mm, 2.7 μm , Chrom Tech, Apple Valley, MN) attached to an Agilent Poroshell 120 EC-C18 analytical column (2.1 x 100 mm, 2.7 μm Chrom Tech, Apple Valley, MN) at 40 °C, eluted with a gradient mobile phase composed of water with 0.1% formic acid (A) and ACN with 0.1% formic acid (B) with a constant flow rate of 0.5 ml/minute. The elution is initiated at 60% A and 40% B for 0.5 minutes, then B is linearly increased from 40-85% for 4.5 minutes, held at 85% B for 0.2 minutes, and returned to initial conditions for 1.3 minutes. Autosampler temperature is 10 °C and sample injection volume is 2 μl . Detection of paclitaxel and the internal standard d5-paclitaxel are accomplished using the mass spectrometer in positive ESI mode with capillary voltage 1.75 kV, source temp 150 °C, desolvation temp 500 °C, cone gas flow 150 L/hr, desolvation gas flow 1000 L/hr, using multiple reaction monitoring (MRM) scan mode with a dwell time of 0.075 seconds. The

cone voltages and collision energies are determined by MassLynx-Intellistart, v4.1, software and varied between 6-16 V and 12-60 eV, respectively. The MRM precursor and product ions are monitored at m/z 854.3 > 105.2 for paclitaxel and 859.3 > 291.2 for d5 paclitaxel. The primary stock solutions of paclitaxel (1 mg/ml in EtOH) and d5 paclitaxel (1 mg/ml in EtOH) are prepared in 4 ml amber silanized glass vials and stored at -20 °C. Working standards are prepared by dilution of the stock solution with ACN in 2 ml amber silanized glass vials and stored at -20 °C. Plasma samples are extracted as follows, 100 µl plasma sample is added to a 1.7 ml microcentrifuge tube containing d5 paclitaxel (116.4 nM or 100 ng/ml) and 300 µl ACN, vortexed, incubated at room temperature for 10 minutes to precipitate proteins, and centrifuged (14,000 rpm) or 3 minutes. The supernatant is filtered on an Agilent Captiva ND^{lipids} plate (Chrom Tech, Apple Valley, MN), collected in a deep 96-well plate, and dried using nitrogen gas. The samples are reconstituted using 100 µl ACN and shaken on a plate shaker (high speed) for 5 minutes. Plasma standard curves are prepared daily containing paclitaxel (0.59-5855 nM or 0.5-5000 ng/ml) and d5 paclitaxel (116.4 nM) for paclitaxel quantitation. Mouse tumors are thawed on ice, weighed, and diluted 2 parts (weight to volume) in 1x PBS. Tumors are then homogenized using a PRO200 tissue homogenizer using the saw tooth probe (5 mm x 75 mm). Tumor homogenate is then processed the same as the human plasma samples.

[0160] Mouse Imaging: Briefly, Tris Buffer (0.125 M Tris-HCl, pH 6.8, 0.15 M NaCl) and 5 mCi Na¹²⁵ I are added directly to iodination tubes (ThermoFischer Scientific, Waltham, MA). The iodide is allowed to activate and is swirled at room temperature. Activated iodide is mixed with the protein solution. 50 µl of Scavenging Buffer (10 mg tyrosine/mL in PBS, pH 7.4) is added and incubated for five minutes. After addition of Tris/BSA buffer and mixing, samples are applied in 10K MWCO dialysis cassettes against pre-cooled PBS for 30 minutes, 1 hour, 2 hours, and overnight at 4 °C. Radioactivity is determined by Gamma counter, then disintegrations per minute (DPM) and specific activity are calculated. Mice are injected in their tail vein with OKT3, ABX-OKT3, ABX-human IgG, human IgG, and ABX only. Animals are imaged at 3, 10, 24 and 72 hours post-administration via SPECT- CT imaging using the U-SPECT-II/CT scanner (MILabs, Utrecht, The Netherlands). SPECT reconstruction is performed using a POSEM (pixelated ordered subsets by expectation

maximization) algorithm. CT data are reconstructed during the Feldkamp algorithm. Co-registered images are further rendered and visualized using PMOD software (PMOD Technologies, Zurich, Switzerland). Animals are sacrificed and dissected at 72 hours post-injection. Selected tissues and organs of interest are measured using radioisotope dose calibrator (Capintec CRC-127R, Capintec Inc.).

Example 8: Lyophilization of the nanoparticle composition

[0161] The nanoparticle composition is synthesized by adding OKT3 to ABRAXANE®. 0.9% saline is then added for a final volume of 2ml, and the mixture is allowed to incubate at room temperature for 30 minutes in a 15ml polypropylene conical tube.

[0162] After the 30 minute room temperature incubation, the mixture is diluted 1:2 in 0.9% saline, respectively. These are the concentrations of the 2 drugs when prepared by the pharmacy for administration to patients.

[0163] The nanoparticle composition is divided into twenty 200 μ l aliquots in 1.5 ml polypropylene eppendorfs and frozen at -80 °C.

[0164] Once frozen, the aliquots are lyophilized overnight with the Virtis 3L benchtop lyophilizer (SP Scientific, Warmister, PA) with the refrigeration on. A lyophilized preparation is generated.

[0165] The dried aliquots are stored at room temperature in the same 1.5ml polypropylene eppendorfs. These samples are readily reconstituted in saline at room temperature for 30 minutes, followed by centrifugation for 7 minutes at 2000x g. The resulting sample is then resuspended in the appropriate buffer, as needed.

[0166] By comparison, a sample that is dried with heat and a speed vacuum is impossible to reconstitute.

Example 9: Testing of lyophilized preparations

[0167] Samples are reconstituted at different time points after lyophilization and tested for their physical properties against ABX, and freshly made nanoparticle composition.

[0168] Particle size distribution is evaluated as described above.

[0169] CD3 binding is evaluated by incubation of the sample with CD3-IgG fusion proteins for 2 hours at room temperature, centrifuged at 2000 x g for 7 minutes. The amount of CD3 bound to the pellet (corresponding to the nanoparticles) or remaining in the supernatant is measured with ELISA.

[0170] Paclitaxel activity is assessed by cytotoxicity against HuT-78 human T-cell lymphoma cells *in vitro*.

Example 10: Follow up study to investigate whether pretreatment with OKT3 improves targeting

[0171] Following the general protocol above, athymic nude mice are injected with 1×10^6 HuT-78 cells in the right flank and then treated with PBS, OKT3, ABX, the nanoparticle composition, or pretreated with OKT3 and, 24hr later, the nanoparticle composition. Data is represented at day 7-post and day 10-post treatment as tumor volume in mm^3 . The tumor sizes are tracked over 10 days.

WHAT IS CLAIMED IS:

1. A nanoparticle composition comprising nanoparticles having an outer surface, wherein each of the nanoparticles comprises:
 - (a) a carrier protein,
 - (b) a binding agent with a T-cell antigen-binding portion, and
 - (c) a therapeutically effective amount of paclitaxel;wherein upon reconstitution with an aqueous solution, the antigen-binding portion of said binding agents are capable of binding to a T-cell antigen *in vivo*.
2. The nanoparticle composition of claim 1, wherein the nanoparticle comprises about 100 to about 1000 binding agents.
3. The nanoparticle composition of claim 1, wherein the antigen-binding portion is capable of binding to a T-cell receptor.
4. The nanoparticle composition of claim 3, wherein the antigen-binding portion binds to an antigen selected from CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, or CCR4.
5. The nanoparticle composition of claim 1, wherein the nanoparticle is lyophilized.
6. The nanoparticle composition of any one of claims 1-5, wherein the composition is stable at about 20°C to about 25°C for up to about 12 months or longer.
7. The nanoparticle composition of any one of claims 1-5, wherein the binding agent comprises Slipizumab, muromonab-CD3 (OKT3), Leu 1, Zanolimumab, Zanolimumab, Brentuximab vedotin, Mik-β1, KW-0761, or a combination thereof.
8. The nanoparticle composition of any one of claims 1-5, wherein the binding agent is muromonab-CD3 (OKT3).
9. The nanoparticle composition of any one of claims 1-5, wherein the antigen binding portion comprises an aptamer, a receptor ligand, an Fab fragment, or a combination thereof.

10. The nanoparticle composition of any one of claims 1-5, wherein the composition comprises one or more additional cancer therapeutic agents.
11. The nanoparticle composition of claim 10, wherein the additional cancer therapeutic agent comprises abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib, vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, or cyclophosphamide.
12. The nanoparticle composition of any one of claims 1-11, wherein fewer than about 50% of said nanoparticles are oligomeric.
13. The nanoparticle composition of any one of claims 1-11, wherein less than 40% of the nanoparticles present in said composition are oligomeric.
14. The nanoparticle composition of any one of claims 1-11, wherein less than 30% of the nanoparticles present in said composition are oligomeric.
15. The nanoparticle composition of any one of claims 1-11 wherein less than 20% of the nanoparticles present in said composition are oligomeric.
16. The nanoparticle composition of any one of claims 1-11 wherein less than 10% of the nanoparticles present in said composition are oligomeric.
17. The nanoparticle composition of any one of claims 1-11 wherein less than 5% of the nanoparticles present in said composition are oligomeric.
18. The nanoparticle composition of any one of claims 1-5, wherein the average size of the nanoparticles is between 90 nm and 800 nm.

19. The nanoparticle composition of claim 1, wherein the carrier protein comprises albumin, gelatin, elastin, gliadin, legumin, zein, a soy protein, a milk protein, a whey protein, or a combination thereof.
20. The nanoparticle composition of claim 19, wherein the carrier protein comprises an antibody-binding motif.
21. The nanoparticle composition of claim 20, wherein the carrier protein is albumin.
22. The nanoparticle composition of claim 21, wherein the albumin is human serum albumin.
23. The nanoparticle composition of any one of claim 21, wherein the albumin is recombinant human serum albumin.
24. The nanoparticle composition of any one of claims 1-23, wherein the composition is formulated for intravenous delivery.
25. The nanoparticle composition of any one of claims 1-5, wherein the nanoparticles have a dissociation constant between about 1×10^{-11} M and about 1×10^{-8} M.
26. A method for treating T-cell cancer cells, comprising contacting the cancer cells with an effective amount of a nanoparticle composition, said nanoparticle composition maintained in contact with said cells for a sufficient period of time to treat the cancer cells, wherein said nanoparticle composition comprises nanoparticles having an outer surface, each of said nanoparticles comprising:
 - (a) a carrier protein,
 - (b) a binding agent with a T-cell antigen-binding portion, and
 - (c) a therapeutically effective amount of paclitaxel;wherein the antigen-binding portion of said binding agents are capable of binding to a T-cell antigen *in vivo*.
27. The method of claim 26, wherein the nanoparticle comprises about 100 to about 1000 binding agents.

28. The method of claim 26, wherein the antigen-binding portion binds to T-cell receptor (TCR).
29. The method of claim 26, wherein the antigen-binding portion binds to an antigen selected from CD2, CD3, CD4, CD5, CD8, CD25, CD30, CD40, CD52, CD 122, or CCR4.
30. The method of claim 29, wherein the antigen is CD2 or CD3.
31. The method of claim 27, wherein the nanoparticle composition is a lyophilized nanoparticle composition that is reconstituted in an aqueous solution prior to administration.
32. The method of claim 27, wherein the composition is stable at about 20°C to about 25°C for up to about 12 months or longer.
33. The method of any one of claims 27-32, wherein the binding agent comprises Slipizumab, OKT3, Leu 1, Zanolimumab, Zanolimumab, Brentuximab vedotin, Mik-β1, KW-0761, or a combination thereof.
34. The method of any one of claims 27-32, wherein the binding agent is muromonab-CD3 (OKT3).
35. The method of any one of claims 27-32, wherein the antigen binding portion is an aptamer, a receptor ligand, or an Fab fragment.
36. The method of any one of claims 27-32, wherein the composition comprises one or more additional cancer therapeutic agents.
37. The method of claim 36, wherein the additional cancer therapeutic agent is selected from abiraterone, bendamustine, bortezomib, carboplatin, cabazitaxel, cisplatin, chlorambucil, dasatinib, docetaxel, doxorubicin, epirubicin, erlotinib, etoposide, everolimus, gefitinib, idarubicin, imatinib, hydroxyurea, imatinib, lapatinib, leuprorelin, melphalan, methotrexate, mitoxantrone, nedaplatin, nilotinib, oxaliplatin, paclitaxel, pazopanib, pemetrexed, picoplatin, romidepsin, satraplatin, sorafenib,

vemurafenib, sunitinib, teniposide, triplatin, vinblastine, vinorelbine, vincristine, or cyclophosphamide.

38. The method of any one of claims 27-37, wherein fewer than about 50% of said nanoparticles are oligomeric
39. The method of any one of claims 27-37, wherein less than 40% of the nanoparticles present in said composition are oligomeric.
40. The method of any one of claims 27-37, wherein less than 30% of the nanoparticles present in said composition are oligomeric.
41. The method of any one of claims 27-37, wherein less than 20% of the nanoparticles present in said composition are oligomeric.
42. The method of any one of claims 27-37, wherein less than 10% of the nanoparticles present in said composition are oligomeric.
43. The method of any one of claims 27-37, wherein less than 5% of the nanoparticles present in said composition are oligomeric.
44. The method of any one of claims 27-43, wherein the average size of the nanoparticles is between 90 nm and 800 nm.
45. The method of any one of claims 27-32, wherein the average size of the nanoparticles is between about 90 nm and about 160 nm.
46. The method of any one of claims 27-45, wherein the carrier protein comprises albumin, gelatin, elastin, gliadin, legumin, zein, a soy protein, a milk protein, a whey protein, or a combination thereof.
47. The method of claim 46, wherein the carrier protein is albumin.
48. The method of claim 47, wherein the albumin is human serum albumin.
49. The method of claim 47, the albumin is recombinant human serum albumin.

50. The method of any one of claims 27-49, wherein the composition is formulated for intravenous delivery.
51. The method of any one of claims 27-32, wherein the nanoparticles have a dissociation constant between about 1×10^{-11} M and about 1×10^{-8} M.
52. The method of any one of claims 27-32, wherein the therapeutically effective amount of the nanoparticle composition comprises about 75 mg/m^2 to about 175 mg/m^2 paclitaxel.
53. The method of any one of claims 27-52, wherein the T-cell cancer comprises peripheral T-cell lymphoma, anaplastic large cell lymphoma, angioimmunoblastic lymphoma cutaneous T-cell lymphoma, Adult T-cell Leukemia/Lymphoma (ATLL), enteropathy-type T-cell lymphoma, hematosplenic gamma-delta T-cell lymphoma, blastic NK-cell lymphoma, lymphoblastic lymphoma, nasal NK/T-cell lymphoma, treatment-related T-cell lymphoma, or a combination thereof.
54. The method of any one of claims 27-52, wherein the T-cell cancer is T-cell lymphoma.

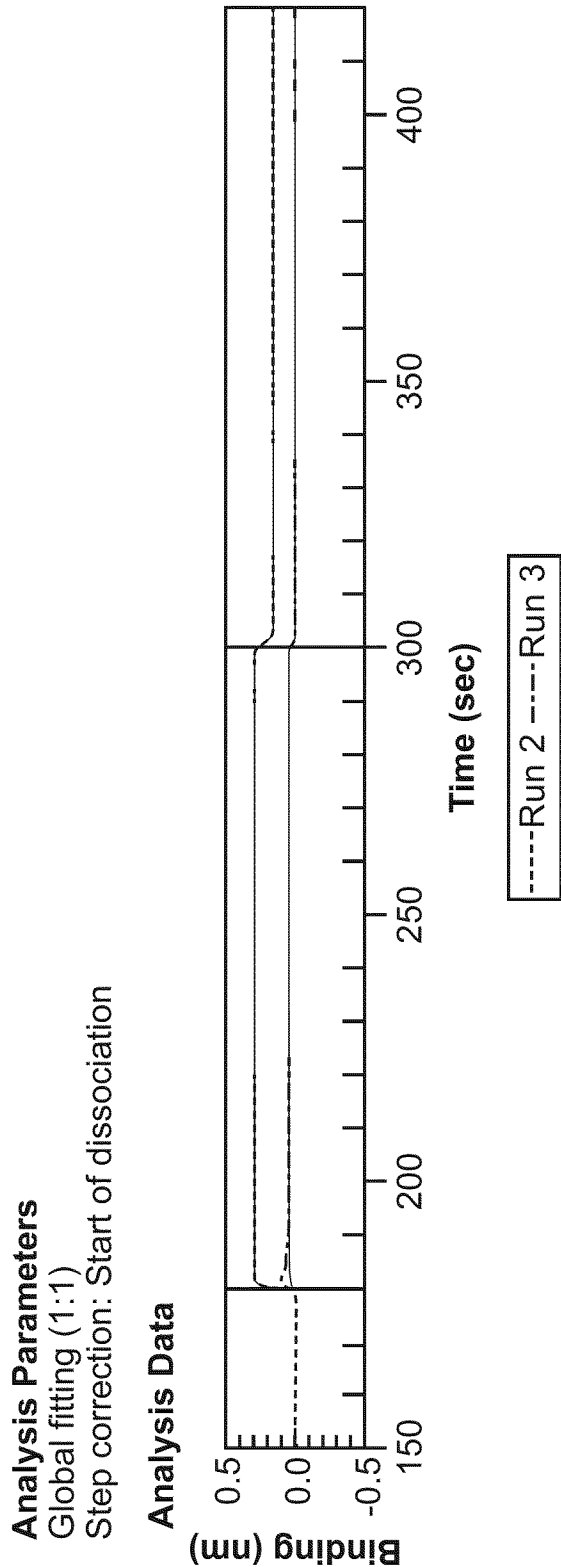


FIG. 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/049745

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K47/69 A61K47/68 A61K47/64 A61K9/51 A61K31/337
A61K39/395 A61P35/00

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)

A61K A61P

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

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

EPO-Internal, BIOSIS, CHEM ABS Data, COMPENDEX, EMBASE, INSPEC, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y	paragraphs [0010], [0019], [0020], [0024] - [0031], [0036], [0039], [0146], [0173] page 9; table 1 figure 8D	8,30,34
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Y	page 1, lines 24,26-29 page 3, lines 6-16 page 3, line 30 - page 4, line 4 claims 17,20,22	7,8,29, 30,33,34
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Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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

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

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

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

"&" document member of the same patent family

Date of the actual completion of the international search

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15/12/2017

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INTERNATIONAL SEARCH REPORT

International application No
PCT/US2017/049745

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Y,P	paragraphs [0001], [0004], [0005], [0028], [0033], [0052] -----	7,8,30, 33,34

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