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(71) Applicant: **GENISPHERE LLC** [US/US]; 2801 Sterling Drive, Hatfield, PA 19440 (US).

(72) Inventors: **GETTS, Robert, C.**; 2801 Sterling Drive, Hatfield, PA 19440 (US). **KADUSHIN, James, M.**; 2801 Sterling Drive, Hatfield, PA 19440 (US). **GETTS, Lori**; 2801 Sterling Drive, Hatfield, PA 19440 (US).

(74) Agent: **LEGAARD, Paul, K.**; Stradley Ronon Stevens & Young, LLP, 30 Valley Stream Parkway, Malvern, PA 19355 (US).

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(54) **Title**: NUCLEIC ACID CARRIERS AND THERAPEUTIC METHODS OF USE

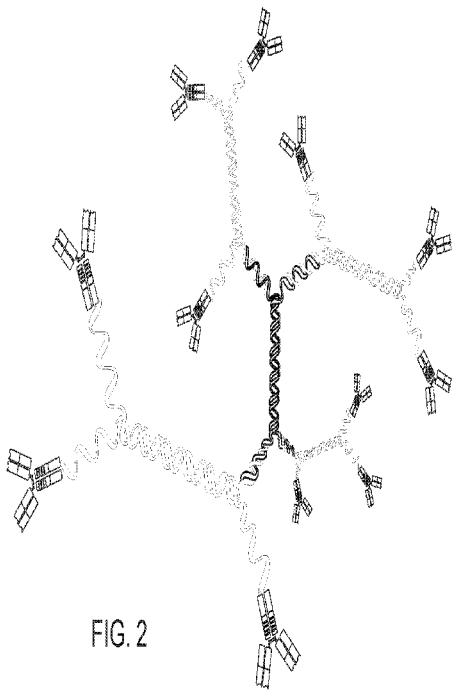

FIG. 2

(57) **Abstract**: The present disclosure provides nucleic acid carriers comprising one or more layers comprising multiple monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA. The present disclosure also provides pharmaceutical composition comprising any of the nucleic acid carriers described herein, and a pharmaceutically acceptable vehicle. The present disclosure also provides methods of inducing an immune response in an animal comprising administering to the animal any of the nucleic acid carriers described herein.

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NUCLEIC ACID CARRIERS AND THERAPEUTIC METHODS OF USE

Field

The present disclosure is directed, in part, to nucleic acid carriers comprising targeting agents, pharmaceutical compositions comprising the same, and methods of making and using the same.

Background

Multi-molecular scaffold devices, including DNA dendrimers, may be useful as cellular transfection, imaging, and drug delivery agents. DNA dendrimers may be bound with targeting devices (e.g., an antibody specific for a cell surface feature capable of eliciting an cellular endocytotic internalization event) and can bind to surface features on targeted cells to provide delivery of a cargo (e.g., a drug) intracellularly or extracellularly, respectively. Cargos may be passively associated with the targeted DNA dendrimer and enter the cell simply by spatial association with the dendrimer, or cargos may be directly bound to the dendrimer via a number of attachment strategies for release at the target destination.

It was previously found that DNA dendrimers could only be successfully made from DNA produced by "natural" means, such as DNA produced via the use of biological systems (e.g., bacterial cloning) or via the use of enzymatic polymerization of DNA sequences (as from the polymerase chain reaction (PCR) and other enzymatically dependent DNA synthesis methods). However, it is not economically feasible to produce DNA dendrimers in the quantities necessary for commercial applications such as therapeutic drug delivery applications when they are assembled from naturally produced DNA. In particular, biological replication of the sequences required for construction of a DNA dendrimer produces double-stranded molecules from which the single-strands required for construction of the dendrimer must be separated and isolated. Although chemical synthesis methods are capable of providing the quantities required for these applications, prior attempts at using synthetically manufactured DNA oligonucleotides repeatedly failed to successfully form covalent structures after cross-linking procedures described in the prior art were applied, suggesting that prior art oligonucleotide design parameters were inappropriate for chemically synthesized oligonucleotides. This disclosure presents, among other things, new compositions of matter and production methods for the use of chemically synthesized DNA as the raw materials for the production of nucleic acid carriers, which are particularly well suited for large scale synthesis.

Summary

The present disclosure provides single layer nucleic acid carriers comprising five monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to a terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to a terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to a terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to a terminal arm of the fifth monomer, forming a single layer nucleic acid carrier having twelve peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

The present disclosure also provides 1.5 layer nucleic acid carriers comprising eleven monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, and the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, forming a 1.5 layer nucleic acid carrier having 24 peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

The present disclosure also provides two layer nucleic acid carriers comprising 17 monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer, forming a two layer nucleic acid carrier having 36 peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

The present disclosure also provides 2.5 layer nucleic acid carriers comprising 35 monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first

terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer; wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer, wherein the third terminal arm of the sixth monomer is conjugated to

the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, and the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer; forming a 2.5 layer nucleic acid carrier having 72 peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

The present disclosure also provides three layer nucleic acid carriers comprising 53 monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth

monomer; wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer, wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer, the third terminal arm of the twelfth monomer is conjugated to the first terminal arm of the thirty-sixth monomer, the third terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the thirty-seventh monomer, the third terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the thirty-eighth monomer, the third terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the thirty-ninth monomer, the third terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fortieth monomer, and the third terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the forty-first monomer; wherein the fourth terminal arm of the sixth monomer is conjugated to the first terminal arm of the forty-second monomer, the fourth terminal arm of the seventh monomer is conjugated to the first terminal arm of the forty-third monomer, the fourth terminal arm of the eighth monomer is conjugated to the first terminal arm of the forty-forth monomer, the fourth terminal arm of the

ninth monomer is conjugated to the first terminal arm of the forty-fifth monomer, the fourth terminal arm of the tenth monomer is conjugated to the first terminal arm of the forty-sixth monomer, the fourth terminal arm of the eleventh monomer is conjugated to the first terminal arm of the forty-seventh monomer, the fourth terminal arm of the twelfth monomer is conjugated to the first terminal arm of the forty-eighth monomer, the fourth terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the forty-ninth monomer, the fourth terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the fiftieth monomer, the fourth terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the fifty-first monomer, the fourth terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fifty-second monomer, and the fourth terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the fifty-third monomer; forming a three layer nucleic acid carrier having 108 peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

The present disclosure also provides pharmaceutical composition comprising any of the nucleic acid carriers described herein, and a pharmaceutically acceptable vehicle.

The present disclosure also provides methods of inducing an immune response in an animal comprising administering to the animal any of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the same, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antigen, a peptide, an antibody or fragment thereof, and an adjuvant.

The present disclosure also provides methods of treating an animal having cancer comprising administering to the animal any of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the same, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

The present disclosure also provides methods of treating an animal having a disease associated with a disease-associated antigen comprising administering to the animal any of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the same, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

Brief Description Of The Drawings

Figure 1 shows representative first and second oligonucleotides (e.g., #1, #2, #3, #4, #5, #6, and #7) that form a nucleic acid carrier monomer (e.g., A, B', B'', C', and C'') when

hybridized; the oligonucleotides comprise a central core portion (e.g., "b1", "b1(-)", "b2", "b2(-1)", and "b1") that hybridize to form a double-stranded region; the oligonucleotides also comprise two terminal portions (e.g., "a", "c", "a(-)", "e", "d" "e(-)", and "d(-)") that form four single-stranded arms when the two oligonucleotides are hybridized to form the monomer.

Figure 2 shows a representative one layer nucleic acid carrier configured to provide twelve peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., antibodies as shown herein).

Figure 3 shows a representative two layer nucleic acid carrier configured to provide 36 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., antibodies conjugated to 16 terminal arms as shown herein).

Figure 4 shows a representative two layer nucleic acid carrier configured to provide 36 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., comprising an equal amount (18) of two different oligonucleotide linkers (A and B) as shown herein).

Figures 5A and 5B show representative 1.5 layer nucleic acid carriers configured to provide 24 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., comprising a ratio of 6:12:6 (or 1:2:1) of three different oligonucleotide linkers (A, B, and C) and comprising a ratio of 12:6:6 (or 2:1:1) of three different oligonucleotide linkers (A, B, and C) as shown herein, respectively).

Figure 6 shows representative serum degradation gel analysis of naked nucleic acid carrier versus phosphorothioate naked nucleic acid carrier in mouse serum.

Figure 7 shows a representative one layer nucleic acid carrier configured to provide the 1:1:1:1 ratio of clustered arms localized to distinct areas within the nucleic acid carrier.

Description Of Embodiments

Unless defined otherwise, all technical and scientific terms have the same meaning as is commonly understood by one of ordinary skill in the art to which the embodiments disclosed belongs.

As used herein, the terms "a" or "an" means that "at least one" or "one or more" unless the context clearly indicates otherwise.

As used herein, the term "about" means that the numerical value is approximate and small variations would not significantly affect the practice of the disclosed embodiments. Where a numerical limitation is used, unless indicated otherwise by the context, "about" means the numerical value can vary by ±10% and remain within the scope of the disclosed embodiments.

As used herein, the terms "comprising" (and any form of comprising, such as "comprise", "comprises", and "comprised"), "having" (and any form of having, such as "have" and "has"), "including" (and any form of including, such as "includes" and "include"), or "containing" (and any form of containing, such as "contains" and "contain"), are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

The present disclosure provides numerous nucleic acid carriers. Each of the carriers described herein comprises a plurality of monomeric core units comprising a first oligonucleotide and a second oligonucleotide. A central portion of each of the first and second oligonucleotides is complementary to each other, thus forming a double-stranded region. The two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, thus forming four single-stranded arms. Figure 1 shows representative first and second oligonucleotides (see, #1, #2, #3, #4, #5, #6, and #7 oligonucleotides) that form a nucleic acid carrier monomer (see, A, B', B'', C', and C'' monomers) when hybridized. Each oligonucleotide comprises a central core portion (see, "b1", "b1(-)", "b2", "b2(-1)", and "b1") that hybridizes with another oligonucleotide having a central core portion to form a double-stranded region. Each oligonucleotide also comprise two terminal portions (see, "a", "c", "a(-)", "e", "d" "e(-)", and "d(-)") that form four single-stranded arms when the two oligonucleotides are hybridized to form the monomer. In some embodiments, each terminal portion (i.e., the four terminal portions of a hybridized pair of oligonucleotides) comprises a unique nucleotide sequence.

In some embodiments, the oligonucleotide is 97 nucleotides in length. The 97-mer has some advantages because it is short enough for efficient synthesis and does not need to be gel-purified, but is still sufficiently large that hybridization and cross-linking are efficient. Suitable nucleotide sequences comprise little or no secondary structure such as hairpins, have a T_m greater than 50°C-55°C (suitably 65°C-75°C), at least one cross-linking site, and a central hybridization region and 3-prime and 5-prime arm regions of no less than 25 bases in length unless modified nucleotides, comprising locked nucleic acids or similar nucleotides, are used to further increase the T_m.

The nucleic acid comprising the oligonucleotides may be any type of nucleic acid or nucleic acid derivative that is amenable to chemical synthesis methods known in the art, or to chemical synthesis methods with subsequent derivatization. Suitable nucleic acids include, but are not limited to, deoxyribonucleic acid (DNA), ribonucleic acid (RNA), modified DNA or RNA, peptide nucleic acid (PNA), morpholino and locked nucleic acid (LNA), glycol nucleic

acid (GNA), threose nucleic acid (TNA), DNA containing phosphorothioate residues (S-oligos) and derivatives thereof, or any combination thereof.

In some embodiments, the nucleic acid carrier may contain oligonucleotides that comprise different nucleotide backbones. In some embodiments, in a nucleic acid carrier having two layers as described herein, the oligonucleotides making up the first inner layer may comprise a particular nucleotide backbone, whereas the oligonucleotides making up the second more outer layer may comprise a different particular nucleotide backbone. The same can be present in multiple layer nucleic acid carriers, with one or more of the layers having oligonucleotides with a different nucleotide backbone compared to an adjacent layer. For example, in some embodiments, a nucleic acid carrier can contain both phosphodiester (PO) synthetic oligonucleotide strands and phosphorothioate (PS) oligonucleotide strands. In some embodiments, the first inner layer is all PO-DNA and the oligonucleotide strands added to make the second more outer layer are PS-DNA. In some embodiments, using modified nucleotide bases can alter the serum degradation profile and the resulting clearance profile, indicating that the PK/PD profile of the carrier may be subsequently altered as well.

In some embodiments, the nucleic acid comprising the oligonucleotides is a nucleoside analog which increases the T_m of the molecule as compared to conventional nucleosides, For example Locked Nucleic Acids (LNA™) nucleosides may be used in which the ribose ring is modified with a methylene bridge between the 2′-O atom and the 4′-C atom. When the oligonucleotides comprise one or more T_m-increasing nucleotides, the overall length may be reduced compared to those consisting of all naturally occurring nucleotides without compromising hybridization efficiency. Generally, the length and composition of naturally-occurring and T_m-increasing nucleotides in the oligonucleotides can be selected such that the T_m of the segment is greater, or at least 5°C greater or at least 10°C greater, than the temperature at which the nucleic acid will be used. For example, a nucleic acid strand comprising 31 conventional DNA residues has the same T_m as a 19 nucleotide strand comprising 50% LNA residues (66°C T_m for both). That is, if the segment comprises 50% LNA, the T_m will be increased approximately 1.5-fold and the overall length of the oligonucleotide may therefore be approximately halved without compromising the efficiency of hybridization of the segment to its complementary nucleic acid molecule. In some embodiments, oligonucleotides as described herein that comprise T_m-increasing nucleotides in segments that will be used for hybridization may be from 40 to 50 nucleotides in total length, with the double-stranded region and the single-stranded arms each independently from 12 to 18 nucleotides in length. In addition, the nucleic acid may have alternate backbones, such as a phosphoramidite backbone.

In some embodiments, the oligonucleotides comprise one or more adjacent thymidine-adenine (TpA – 5' to 3' orientation) residues. A TpA site improves crosslinking of the oligonucleotide molecules when they are assembled in the branched nucleic acid carriers and psoralen or psoralen-like molecules are used as inducible cross-linking agents. TpA residues may be present in any or all of the single-stranded arms or the double-stranded region. In some embodiments, the oligonucleotides comprise from about 3 to about 7 TpA sites. In some embodiments, both of the single-stranded arms and the double-stranded region comprise at least 3 TpA residues. In some embodiments, one or more of the single-stranded arms and the double-stranded region may comprise from about 3 to about 7 TpA sites, such as 3, 4, 5, 6, or 7 TpA sites. In some embodiments, the single-stranded arms comprise from about 3 to about 7 TpA sites, or from about 3 to about 4 TpA sites. In some embodiments, the double-stranded region comprises from about 5 to about 6 TpA sites. TpA sites may also be replaced with other cross-linking sites and combined with that corresponding and most suitable cross-linking agent. For example, mitomycin C forms intrastrand cross-links at GpG, and CpG sites and may also be used as an effective alternative or additional site for cross-linking of the DNA scaffold.

It is generally desirable to select nucleotide sequences for the oligonucleotides that minimize or eliminate secondary structure capable of self-hybridizing or self-binding via Watson-Crick base pairing under physiologic conditions, and to select a suitable hybridization melting temperature (T_m) of the single-strands in monomers and of the monomers in the branched nucleic acid carriers. Typically, a T_m of about 65 to 75°C is desired, or at least 10-15°C above the temperature at which the materials will be utilized.

The double-stranded region formed by the complementarity between the first and second oligonucleotides is from about 4 to about 2000 bases in length, from about 5 to about 200 bases in length, from about 25 to about 50 bases in length, or from about 25 to about 35 bases in length. In some embodiments, the double-stranded region is 31 bases in length.

Each single-stranded arm is from about 4 to about 200 bases in length, from about 5 to about 50 bases in length, from about 25 to about 50 bases in length, from about 25 to about 35 bases in length, from about 16 to about 50 bases in length, or from about 12 to about 18 bases in length. In some embodiments, the single-stranded arm is 31 bases in length.

In some embodiments, the oligonucleotides may comprise one or two hinge segments joining one or both of the single-stranded arms to the double-stranded region. Each hinge segment may be from 1 to 4 nucleotides in length, from 2 to 4 nucleotides in length, 1 nucleotide in length, 2 nucleotides in length, 3 nucleotides in length, or 4 nucleotides in length. In some

embodiments, the nucleotide sequence of the hinge segment 5′ to the double-stranded region is CA, and the nucleotide sequence of the hinge segment 3′ to the double-stranded region is AC.

Each of the single-stranded arms has a nucleic acid sequence that can be the same as or different from any other single-stranded arm. In some embodiments, the nucleotide sequence of each of the four single-stranded arms is identical. In some embodiments, the nucleotide sequences of three of the four single-stranded arms are identical to each other and different than the nucleotide sequence of the fourth single-stranded arm. In some embodiments, the nucleotide sequences of two of the four single-stranded arms are identical to each other and different than the nucleotide sequences of the other two single-stranded arms. In some embodiments, the nucleotide sequences of two of the four single-stranded arms are identical to each other and the nucleotide sequences of the other two of the four single-stranded arms are identical to each other, wherein the nucleotide sequence of the first two single-stranded arms is different than the nucleotide sequences of the second two single-stranded arms. In some embodiments, the nucleotide sequence of each of the four single-stranded arms is different. Thus, the nucleotide sequence of any particular single-stranded arm provides flexibility in design choice and can be used to specifically bind to or be conjugated to a particular linking oligonucleotide (which may or may not be further conjugated to a targeting agent) or to another single-stranded arm of another monomeric nucleic acid carrier.

In some embodiments, one or more terminal arms may not be conjugated to a targeting agent. In these embodiments, optional oligonucleotides (which are not conjugated to any targeting agent) may be bound to such terminal arms forming double-stranded DNA structures. The double-stranded region formed by the hybridization of the optional oligonucleotide to a terminal arm can be blunt-ended, or either the optional oligonucleotide or the terminal arm can have a 1 to 5 nucleotide base overhang.

The present disclosure provides single layer nucleic acid carriers comprising five monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to a terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to a terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to a terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to a terminal arm of the fifth monomer, forming a single layer nucleic acid carrier

having twelve peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent. In some embodiments, the single layer nucleic acid carriers comprise 1, 2, 3, 4, or 5 more monomers linked thereto in the same manner as described herein. Figure 2 shows a representative one layer nucleic acid carrier configured to provide twelve peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., antibodies as shown herein).

In some embodiments, the twelve peripheral terminal arms of the single layer nucleic acid carriers comprise from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different nucleotide sequences, or a single nucleotide sequence. In some embodiments, the twelve peripheral terminal arms of the single layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 different nucleotide sequences. In some embodiments, the single layer nucleic acid carriers comprise two peripheral terminal arms having a first nucleotide sequence, two peripheral terminal arms having a second nucleotide sequence, two peripheral terminal arms having a third nucleotide sequence, two peripheral terminal arms having a fourth nucleotide sequence, two peripheral terminal arms having a fifth nucleotide sequence, and two peripheral terminal arms having a sixth nucleotide sequence (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the single layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 1:2 ratio, a 3:1 ratio, or a 5:1 ratio regarding the nucleotide sequences of the peripheral terminal arms.

In some embodiments, the ratio regarding the nucleotide sequences of the twelve peripheral terminal arms of the single layer nucleic acid carriers are randomly located within the nucleic acid carrier. In some embodiments, the ratio regarding the nucleotide sequences of the twelve peripheral terminal arms of the single layer nucleic acid carriers are localized in clusters. Figure 7 shows a representative one layer nucleic acid carrier configured to provide the 1:1:1:1 ratio of clustered arms localized to distinct areas within the nucleic acid carrier.

In some embodiments, the single layer nucleic acid carriers comprise from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms. In some embodiments, the single layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 targeting agents. In some embodiments, the nucleic acid carriers comprise from 1 to 12, from 1

to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different targeting agents, or a single targeting agent.

In some embodiments, the single layer nucleic acid carriers comprise two peripheral terminal arms conjugated to a first targeting agent, two peripheral terminal arms conjugated to a second targeting agent, two peripheral terminal arms conjugated to a third targeting agent, two peripheral terminal arms conjugated to a fourth targeting agent, two peripheral terminal arms conjugated to a fifth targeting agent, and two peripheral terminal arms conjugated to a sixth targeting agent (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the single layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the number of peripheral terminal arms conjugated to an identical targeting agent.

The present disclosure also provides 1.5 layer nucleic acid carriers comprising eleven monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, and the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, forming a 1.5 layer nucleic acid carrier having twenty-four peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent. In some embodiments, the 1.5 layer nucleic acid carriers comprise 1, 2, 3, 4, or 5 more monomers linked thereto in the same manner as described herein. Figure 3 shows a

representative two layer nucleic acid carrier configured to provide 36 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., antibodies conjugated to 16 terminal arms as shown herein). Figures 5A and 5B show representative 1.5 layer nucleic acid carriers configured to provide 24 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., comprising a ratio of 6:12:6 (or 1:2:1) of three different oligonucleotide linkers (A, B, and C) and comprising a ratio of 12:6:6 (or 2:1:1) of three different oligonucleotide linkers (A, B, and C) as shown herein, respectively).

In some embodiments, the twenty-four peripheral terminal arms of the 1.5 layer nucleic acid carriers comprise from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different nucleotide sequences, or a single nucleotide sequence. In some embodiments, the twenty-four peripheral terminal arms of the 1.5 layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 different nucleotide sequences. In some embodiments, the 1.5 layer nucleic acid carriers comprise four peripheral terminal arms having a first nucleotide sequence, four peripheral terminal arms having a second nucleotide sequence, four peripheral terminal arms having a third nucleotide sequence, four peripheral terminal arms having a fourth nucleotide sequence, four peripheral terminal arms having a fifth nucleotide sequence, and four peripheral terminal arms having a sixth nucleotide sequence (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the 1.5 layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:11:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the nucleotide sequences of the peripheral terminal arms.

In some embodiments, the ratio regarding the nucleotide sequences of the twenty-four peripheral terminal arms of the single layer nucleic acid carriers are randomly located within the nucleic acid carrier. In some embodiments, the ratio regarding the nucleotide sequences of the twenty-four peripheral terminal arms of the single layer nucleic acid carriers are localized in clusters.

In some embodiments, the 1.5 layer nucleic acid carriers comprise from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, 2, or one targeting agent conjugated to the peripheral terminal arms. In some embodiments, the 1.5 layer

nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 targeting agents. In some embodiments, the nucleic acid carriers comprise from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, 2 different targeting agents, or a single targeting agent.

In some embodiments, the 1.5 layer nucleic acid carriers comprise four peripheral terminal arms conjugated to a first targeting agent, four peripheral terminal arms conjugated to a second targeting agent, four peripheral terminal arms conjugated to a third targeting agent, four peripheral terminal arms conjugated to a fourth targeting agent, four peripheral terminal arms conjugated to a fifth targeting agent, and four peripheral terminal arms conjugated to a sixth targeting agent (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the 1.5 layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the number of peripheral terminal arms conjugated to an identical targeting agent.

The present disclosure also provides two layer nucleic acid carriers comprising seventeen monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of

the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer, forming a two layer nucleic acid carrier having thirty-six peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent. In some embodiments, the two layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 more monomers linked thereto in the same manner as described herein. Figure 4 shows a representative two layer nucleic acid carrier configured to provide 36 peripheral terminal arms capable of conjugating to any of the targeting agents described herein (e.g., comprising an equal amount (18) of two different oligonucleotide linkers (A and B) as shown herein).

In some embodiments, the thirty six peripheral terminal arms of the two layer nucleic acid carriers comprise from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different nucleotide sequences, or a single nucleotide sequence. In some embodiments, the thirty six peripheral terminal arms of the two layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 different nucleotide sequences. In some embodiments, the two layer nucleic acid carriers comprise six peripheral terminal arms having a first nucleotide sequence, six peripheral terminal arms having a second nucleotide sequence, six peripheral terminal arms having a third nucleotide sequence, six peripheral terminal arms having a fourth nucleotide sequence, six peripheral terminal arms having a fifth nucleotide sequence, and six peripheral terminal arms having a sixth nucleotide sequence (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the two layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the nucleotide sequences of the peripheral terminal arms.

In some embodiments, the ratio regarding the nucleotide sequences of the thirty six peripheral terminal arms of the single layer nucleic acid carriers are randomly located within the nucleic acid carrier. In some embodiments, the ratio regarding the nucleotide sequences of the thirty six peripheral terminal arms of the single layer nucleic acid carriers are localized in clusters.

In some embodiments, the two layer nucleic acid carriers comprise from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms. In some embodiments, the two layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, or 36 targeting agents. In some embodiments, the nucleic acid carriers comprise from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different targeting agents, or a single targeting agent.

In some embodiments, the two layer nucleic acid carriers comprise six peripheral terminal arms conjugated to a first targeting agent, six peripheral terminal arms conjugated to a second targeting agent, six peripheral terminal arms conjugated to a third targeting agent, six peripheral terminal arms conjugated to a fourth targeting agent, six peripheral terminal arms conjugated to a fifth targeting agent, and six peripheral terminal arms conjugated to a sixth targeting agent (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the two layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the number of peripheral terminal arms conjugated to an identical targeting agent.

The present disclosure also provides 2.5 layer nucleic acid carriers comprising thirty five monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide

are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer; wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and

the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer, wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, and the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer; forming a 2.5 layer nucleic acid carrier having seventy-two peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent. In some embodiments, the 2.5 layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, or 17 more monomers linked thereto in the same manner as described herein.

In some embodiments, the seventy-two peripheral terminal arms of the 2.5 layer nucleic acid carriers comprise from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1 to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different nucleotide sequences, or a single nucleotide sequence. In some embodiments, the seventy-two peripheral terminal arms of the two layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 different nucleotide sequences. In some embodiments, the 2.5 layer nucleic acid carriers comprise twelve peripheral terminal arms having a first nucleotide sequence, twelve peripheral terminal arms having a second nucleotide sequence, twelve peripheral terminal arms having a third nucleotide sequence, twelve peripheral terminal arms having a fourth nucleotide sequence, twelve peripheral terminal

arms having a fifth nucleotide sequence, and twelve peripheral terminal arms having a sixth nucleotide sequence (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the 2.5 layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the nucleotide sequences of the peripheral terminal arms.

In some embodiments, the ratio regarding the nucleotide sequences of the seventy-two peripheral terminal arms of the single layer nucleic acid carriers are randomly located within the nucleic acid carrier. In some embodiments, the ratio regarding the nucleotide sequences of the seventy-two peripheral terminal arms of the single layer nucleic acid carriers are localized in clusters.

In some embodiments, the 2.5 layer nucleic acid carriers comprise from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1 to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms. In some embodiments, the 2.5 layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, or 72 targeting agents. In some embodiments, the nucleic acid carriers comprise from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1 to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1

to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different targeting agents, or a single targeting agent.

In some embodiments, the 2.5 layer nucleic acid carriers comprise twelve peripheral terminal arms conjugated to a first targeting agent, twelve peripheral terminal arms conjugated to a second targeting agent, twelve peripheral terminal arms conjugated to a third targeting agent, twelve peripheral terminal arms conjugated to a fourth targeting agent, twelve peripheral terminal arms conjugated to a fifth targeting agent, and twelve peripheral terminal arms conjugated to a sixth targeting agent (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the 2.5 layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the number of peripheral terminal arms conjugated to an identical targeting agent.

The present disclosure also provides three layer nucleic acid carriers comprising fifty-three monomers, wherein each monomer comprises: a first and a second oligonucleotide; wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region, wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms; wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer; wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is

conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer; wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer, wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer, the third terminal arm of the twelfth monomer is conjugated to the first terminal arm of the thirty-sixth monomer, the third terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the thirty-seventh monomer, the third terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the thirty-eighth monomer, the third terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the thirty-ninth monomer, the third terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fortieth monomer, and the third terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the forty-first

monomer; wherein the fourth terminal arm of the sixth monomer is conjugated to the first terminal arm of the forty-second monomer, the fourth terminal arm of the seventh monomer is conjugated to the first terminal arm of the forty-third monomer, the fourth terminal arm of the eighth monomer is conjugated to the first terminal arm of the forty-forth monomer, the fourth terminal arm of the ninth monomer is conjugated to the first terminal arm of the forty-fifth monomer, the fourth terminal arm of the tenth monomer is conjugated to the first terminal arm of the forty-sixth monomer, the fourth terminal arm of the eleventh monomer is conjugated to the first terminal arm of the forty-seventh monomer, the fourth terminal arm of the twelfth monomer is conjugated to the first terminal arm of the forty-eighth monomer, the fourth terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the forty-ninth monomer, the fourth terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the fiftieth monomer, the fourth terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the fifty-first monomer, the fourth terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fifty-second monomer, and the fourth terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the fifty-third monomer; forming a three layer nucleic acid carrier having 108 peripheral terminal arms; and at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent. In some embodiments, the three layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, or more additional monomers linked thereto in the same manner as described herein.

In some embodiments, the 108 peripheral terminal arms of the three layer nucleic acid carriers comprise from 1 to 108, 1 to 107, from 1 to 106, from 1 to 105, from 1 to 104, from 1 to 103, from 1 to 102, from 1 to 101, from 1 to 100, from 1 to 99, from 1 to 98, from 1 to 97, from 1 to 96, from 1 to 95, from 1 to 94, from 1 to 93, from 1 to 92, from 1 to 91, from 1 to 90, from 1 to 89, from 1 to 88, from 1 to 87, from 1 to 86, from 1 to 85, from 1 to 84, from 1 to 83, from 1 to 82, from 1 to 81, from 1 to 80, from 1 to 79, from 1 to 78, from 1 to 77, from 1 to 76, from 1 to 75, from 1 to 74, from 1 to 73, from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1 to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26,

from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different nucleotide sequences, or a single nucleotide sequence. In some embodiments, the 108 peripheral terminal arms of the two layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 010, 102, 103, 104, 105, 106, 107, or 108 different nucleotide sequences. In some embodiments, the three layer nucleic acid carriers comprise 18 peripheral terminal arms having a first nucleotide sequence, 18 peripheral terminal arms having a second nucleotide sequence, 18 peripheral terminal arms having a third nucleotide sequence, 18 peripheral terminal arms having a fourth nucleotide sequence, 18 peripheral terminal arms having a fifth nucleotide sequence, and 18 peripheral terminal arms having a sixth nucleotide sequence (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the three layer nucleic acid carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the nucleotide sequences of the peripheral terminal arms.

In some embodiments, the ratio regarding the nucleotide sequences of the 108 peripheral terminal arms of the single layer nucleic acid carriers are randomly located within the nucleic acid carrier. In some embodiments, the ratio regarding the nucleotide sequences of the 108 peripheral terminal arms of the single layer nucleic acid carriers are localized in clusters.

In some embodiments, the three layer nucleic acid carriers comprise from 1 to 108, 1 to 107, from 1 to 106, from 1 to 105, from 1 to 104, from 1 to 103, from 1 to 102, from 1 to 101, from 1 to 100, from 1 to 99, from 1 to 98, from 1 to 97, from 1 to 96, from 1 to 95, from 1 to 94, from 1 to 93, from 1 to 92, from 1 to 91, from 1 to 90, from 1 to 89, from 1 to 88, from 1 to 87, from 1 to 86, from 1 to 85, from 1 to 84, from 1 to 83, from 1 to 82, from 1 to 81, from 1 to 80, from 1 to 79, from 1 to 78, from 1 to 77, from 1 to 76, from 1 to 75, from 1 to 74, from 1 to 73, from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1

to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2, or 1 targeting agent conjugated to the peripheral terminal arms. In some embodiments, the three layer nucleic acid carriers comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 010, 102, 103, 104, 105, 106, 107, or 108 targeting agents. In some embodiments, the nucleic acid carriers comprise from 1 to 108, 1 to 107, from 1 to 106, from 1 to 105, from 1 to 104, from 1 to 103, from 1 to 102, from 1 to 101, from 1 to 100, from 1 to 99, from 1 to 98, from 1 to 97, from 1 to 96, from 1 to 95, from 1 to 94, from 1 to 93, from 1 to 92, from 1 to 91, from 1 to 90, from 1 to 89, from 1 to 88, from 1 to 87, from 1 to 86, from 1 to 85, from 1 to 84, from 1 to 83, from 1 to 82, from 1 to 81, from 1 to 80, from 1 to 79, from 1 to 78, from 1 to 77, from 1 to 76, from 1 to 75, from 1 to 74, from 1 to 73, from 1 to 72, 1 to 71, from 1 to 70, from 1 to 69, from 1 to 68, from 1 to 67, from 1 to 66, from 1 to 65, from 1 to 64, from 1 to 63, from 1 to 62, from 1 to 61, from 1 to 60, from 1 to 59, from 1 to 58, from 1 to 57, from 1 to 56, from 1 to 55, from 1 to 54, from 1 to 53, from 1 to 52, from 1 to 51, from 1 to 50, from 1 to 49, from 1 to 48, from 1 to 47, from 1 to 46, from 1 to 45, from 1 to 44, from 1 to 43, from 1 to 42, from 1 to 41, from 1 to 40, from 1 to 39, from 1 to 38, from 1 to 37, from 1 to 36, from 1 to 35, from 1 to 34, from 1 to 33, from 1 to 32, from 1 to 31, from 1 to 30, from 1 to 29, from 1 to 28, from 1 to 27, from 1 to 26, from 1 to 25, from 1 to 24, from 1 to 23, from 1 to 22, from 1 to 21, from 1 to 20, from 1 to 19, from 1 to 18, from 1 to 17, from 1 to 16, from 1 to 15, from 1 to 14, from 1 to 13, from 1 to 12, from 1 to 11, from 1 to 10, from 1 to 9, from 1 to 8, from 1 to 7, from 1 to 6, from 1 to 5, from 1 to 4, from 1 to 3, or 2 different targeting agents, or a single targeting agent.

In some embodiments, the three layer nucleic acid carriers comprise 18 peripheral terminal arms conjugated to a first targeting agent, 18 peripheral terminal arms conjugated to a second targeting agent, 18 peripheral terminal arms conjugated to a third targeting agent, 18 peripheral terminal arms conjugated to a fourth targeting agent, 18 peripheral terminal arms conjugated to a fifth targeting agent, and 18 peripheral terminal arms conjugated to a sixth targeting agent (i.e., a 1:1:1:1:1:1 ratio). In some embodiments, the three layer nucleic acid

carriers likewise comprise a 3:1:2:2:2:2 ratio, a 3:1:3:1:2:2 ratio, a 3:1:3:1:3:1 ratio, a 3:1:2:2:2 ratio, a 3:1:3:1:2 ratio, a 1:1:1:1 ratio, a 2:4:3:3 ratio, a 1:2:2:1 ratio, a 1:5:5:1 ratio, a 1:1:1 ratio, a 3:4:5 ratio, a 1:1:2 ratio, a 1:2:3 ratio, a 1:1 ratio, a 7:5 ratio, a 2:1 ratio, a 3:1 ratio, or a 5:1 ratio regarding the number of peripheral terminal arms conjugated to an identical targeting agent.

In any embodiment whereby a targeting agent is conjugated to a single-stranded arm or terminal arm, such conjugation may be carried out via an oligonucleotide linker that is linked to the targeting agent, and wherein a portion of the oligonucleotide linker is complementary to a portion of the nucleotide sequence of the single-stranded arm or terminal arm. Thus, the targeting agent is conjugated to the single-stranded arm or terminal arm by an oligonucleotide linker. In some embodiments, one or more single-stranded arms or terminal arms that are not conjugated to a targeting agent are complementary base paired to an oligonucleotide linker that is not conjugated to a targeting agent. The double-stranded region formed by the hybridization of the oligonucleotide linker to a single-stranded arm or terminal arm can be blunt-ended, or either the oligonucleotide linker or the single-stranded arm or terminal arm can have a 1 to 5 nucleotide base overhang. In any of these embodiments where a single-stranded arm or terminal arm is complementary to a portion of the oligonucleotide linker, it is not necessary that the entire portion of the single-stranded arm or terminal arm or the oligonucleotide linker be complementary thereto. Thus, for example, if a particular single-stranded arm or terminal arm comprises 31 nucleotides, the oligonucleotide linker (with or without a conjugated targeting agent) need not be complementary to all 31 nucleotides. Rather, the oligonucleotide linker must be sufficiently complementary to the particular single-stranded arm or terminal arm to result in hybridization.

In some embodiments, that targeting agent may be conjugated to the nucleic acid carrier via the single-stranded arm or terminal arm by chemical modifications of the same which are incorporated into the single-stranded nucleic acid molecules during or after their synthesis. Such modification may include, for example, nucleoside amidites chemically modified with amine on a carbon chain linker. Further, sites for modification (e.g., amines, sulfhydryls, carboxyls and others) may be introduced by the use of modified phosphoramidites during chemical synthesis of the single-stranded nucleic acid molecules as is known in the art. Such modified phosphoramidites are widely and commercially available for such purposes, with such modifications suitable for further chemical derivatization and binding of biologically active molecules and compounds as described above. See, for example, the modified phosphoramidite products available from Glen Research, Sterling, VA. This type of derivatization allows internal monomers of the nucleic acid carriers or the single-stranded arms or the linker oligonucleotides

to be conjugated linked to the targeting agents. Similar chemical modifications of the chemically synthesized single-stranded nucleic acid molecules may also be made after their synthesis and prior to assembly of the nucleic acid carriers.

In some embodiments, conjugation of a targeting agent to the nucleic acid carrier may be via the unhybridized, single-stranded arms or terminal arms, such as by linkage to a capture oligonucleotide hybridized to the single-stranded monomer arms or terminal arms. In some embodiments, the capture oligonucleotide may be reversibly or irreversibly cross-linked to the monomer arm. Alternately, the targeting agent can be chemically attached via hydrophilic or hydrophobic binding characteristics (including via electrostatic attraction and hydrogen binding) to the single-stranded arm or terminal arm, or attached by binding between a hapten and its binding partner (e.g., biotin and streptavidin). Additional examples of methods of conjugating a targeting agent to a nucleic acid carrier can be found in, for example, U.S. Patent Publication No. 2005/0089890 and PCT Publications WO 2008/147526, WO 2010/017544, and WO 2011/106481, each of which is incorporated herein by reference in their entirety.

All of the nucleic acid carriers described herein may be covalently cross-linked as described herein. The cross-linked monomers provide an advantage because it maintains the structural integrity of the assembled nucleic acids and improves the stability of the nucleic acid carriers. Such cross-linking was not previously possible for branched polynucleotide molecules constructed from chemically synthesized nucleic acids. Cross-links may be reversible or irreversible. In some embodiments, a plurality of monomers is cross-linked. Alternately, all of the monomer linkages can be cross-linked. In some embodiments, the double-stranded region within a single monomer is cross-linked. In some embodiments, the single-stranded arm of one monomer is cross-linked to the oligonucleotide conjugated to the targeting agent. In some embodiments, the double-stranded region of one monomer is cross-linked to the double-stranded region of another monomer. In some embodiments, the single-stranded arm of one monomer is cross-linked to a single-stranded arm of another monomer. Alternately, the double-stranded region of one monomer is cross-linked to a single-stranded arm of another monomer. Numerous agents are known for cross-linking and include, but are not limited to, mitomycin C and daunamycin, and other anticancer drugs that cross-link DNA, ethidium diazide, cisplatin, EDC-type compounds, and psoralens. A suitable cross-linking agent can be selected by persons skilled in the art based on the particular requirements of the cross-linker and the sequence of the nucleic acids involved. In some embodiments, psoralen is used as the cross-linking agent. Psoralen intercalates into the double helix of double-stranded DNA and readily forms covalent bonds with pyrimidine nucleotides upon irradiation with UV light. In addition to psoralen, the psoralen

derivatives are useful for cross-linking the nucleic acids of the nucleic acid carriers. Psoralen derivatives include, but are not limited to, 8-methoxy psoralen, 4,5′,8-trimethylpsoralen, and 4′-adducts of trioxsalen (e.g., 4′-hydroxymethyl-4,5′,trimethyl psoralen, 4′-methoxymethyl-4,5′,8-trimethyl psoralen, 4′N-phthalimidomethyl-4,5′,8-trimethyl psoralen, and 4′-aminomethyl-4,5′-,8trimethyl psoralen hydrochloride). Procedures for cross-linking with psoralens are standard and known in the art.

In some embodiments, one or more of the targeting agents is, for example, an antigen, a peptide, an antibody or fragment thereof, an antibody-drug conjugate, a non-antibody scaffold, a label, an adjuvant, an RNA molecule, a DNA molecule, a vitamin, a protein, a fusion protein, a fusion peptide, a carbohydrate, a lipid, a polysaccharide, a lipopolysaccharide, a polymer, a virus particle, or a virus-like particle, or any combination thereof.

Any nucleic acid carrier can comprise any combination of any of these targeting agents. For example, a monomeric nucleic acid carrier can comprise up to any four of these targeting agents. Thus, a monomeric nucleic acid carrier can comprise one, two, three, or four of these targeting agents. As another example, a double nucleic acid carrier can comprise up to any six of these targeting agents. Thus, a double nucleic acid carrier can comprise one, two, three, four, five, or six of these targeting agents.

In some embodiments, the vitamin is folic acid.

In some embodiments, the carbohydrate is mannose.

In some embodiments, the polymer is hyaluronic acid, polyargenine, polylysine, polyethylenimine (PEI), polyethyleneglycol (PEG), polyglycolic acid (PGA), polylactic acid (PLA), or poly(lactic-co-glycolic acid) (PLGA).

In some embodiments, the RNA molecule is siRNA, miRNA, mRNA, snRNA, dsRNA, ncRNA, snoRNA, or an aptamer.

In some embodiments, the non-antibody scaffold is an affibody, an affilin, an anticalin, an atrimer, an avimer, a bicyclic peptide, a cys-knot, a DARPin, an FN3, a fynomer, a kunitz domain, or an O-body.

In some embodiments, the antigen is a bacterial antigen, a viral antigen, a fungal antigen, a yeast antigen, a protozoan antigen, or prion. In some embodiments, the antigen is from *Acetobacter aurantius*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Actinomyces israelii*, *Actinomyces viscosus*, *Aggregatibacter actinomycetemcomitans*, *Agrobacterium radiobacter*, *Agrobacterium tumefaciens*, *Azorhizobium caulinodans*, *Azotobacter vinelandii*, *Anaplasma phagocytophilum*, *Bacillus anthracis*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus fusiformis*, *Bacillus licheniformis*,

Bacillus megaterium, *Bacillus mycoides*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacteroides gingivalis*, *Bacteroides fragilis*, *Bacteroides melaninogenicus* (*Prevotella melaninogenica*), *Bartonella henselae*, *Bartonella quintana*, *Bordetella bronchiseptica*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia cepacia*, *Calymmatobacterium granulomatis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter pylori*, *Chlamydia trachomatis*, *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*), *Chlamydophila psittaci* (*Chlamydia psittaci*), *Citrobacter freundii*, *Citrobacter diverus*, *Citrobacter koseri*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens* (*Clostridium welchii*), *Clostridium tetani*, *Corynebacterium diphtheria*, *Corynebacterium fusiforme*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter faecalis*, *Enterococcus avium*, *Enterococcus durans*, *Enterococcus faecalis, Enterococcus faecium*, *Enterococcus galllinarum*, *Enterococcus maloratus*, *Eschericia coli, Francisella tularensis*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Haemophilus parainfluenzae*, *Haemophilus pertussis*, *Haemophilus vaginalis*, *Haemophilus influenza, Haemophilus aegyptius*, *Helicobacter pylori, Klebsiella pneumonia*, *Klebsiella oxytoca*, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactococcus lactis, Legionella pneumophila, Listeria monocytogenes, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella lacunata, Moraxella catarrhalis*, *Morganella morganii*, *Mycobacterium avium, Mycobacterium africanum*, *Mycobacterium bovis, Mycobacterium canetti*, *Mycobacterium diphtheria, Mycobacterium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacterium microti, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma penetrans, Mycoplasma pneumonia, Neisseria gonorrhoeae, Neisseria meningitides, Pasteurella multocida, Pasteurella tularensis, Porphyromonas gingivalis, Prevotella melaninogenica (Bacteroides melaninogenicus), Propionibacterium acnes*, *Proteus mirabillis, Proteus vulgaris*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Pseudomonas otitidis*, *Rhizobium radiobacter, Rickettsia prowazekii, Rickettsia psittaci, Rickettsia quintana, Rickettsia rickettsia, Rickettsia trachomae, Rochalimaea henselae, Rochalimaea Quintana, Rothia dentocariosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens*, *Shigella dysenteriae, Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus colmii*, *Staphylococcus sciuri*, *Staphylococcus warneri*, *Stenotrophomonas maltophilia, Streptococcus*

agalactiae, Streptococcus anginosus, Streptococcus avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faceium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis. Streptococcus mitior, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus, Streptococcus viridans, Treponema pallidum, Treponema denticola, Vibrio cholera, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus, Wolbachia, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Aspergillus fumigatus, Aspergillus flavus, Aspergillus clavatus, Aspergillus niger, Aspergillus terreus, Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida dubliniensis, Candida parapsilosis, Fusarium solani, Fusarium moniliforme, Fusarium proliferartum, Malessezia pachydermatis, Chrysosporium parvum, Metarhizium anisopliae, Phaeoisaria clematidis, or *Sarcopodium oculorum*. In some embodiments, the antigen is from *Mycobacterium tuberculosis*, Varicella zoster virus, *Corynebacterium diphtheria*, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, *Haemophilis influenza*, Human Papillomavirus, Influenza virus, Japanese Encephalitis virus, Measles virus, *Neisseria meningitidis*, Mumps virus, *Bordetella pertussis*, *Streptococcus pneumonia*, Poliovirus, Rabies virus, Rotavirus, Rubella virus, Herpes Zoster virus, *Clostridium tetani*, *Salmonella typhi*, Yellow Fever virus, Ebola virus, avian flu virus, *Bacillus anthracis*, Smallpox virus, or Zika virus.

In some embodiments, the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc. In some embodiments, the antibody, or fragment thereof, is directed to a disease-associated antigen. In some embodiments, the disease-associated antigen is a tumor-associated antigen. Table 1 lists exemplary antibodies (or fragments thereof) to tumor-associated antigens.

Table 1

Name	Target	Cancer
3F8	GD2	neuroblastoma
8H9	B7-H3	neuroblastoma, sarcoma, brain
Abagovomab	CA-125, MUC16	ovarian
Abituzumab	CD51	cancer
Adecatumumab	EpCAM	prostate, breast
Afutuzumab	CD20	lymphoma
Alacizumab pegol	VEGFR2	cancer
Alemtuzumab	CD52	chronic lymphocytic leukemia
Amatuximab	mesothelin	cancer
AMP-514	PD-1	advanced malignancy
AMP-224	PD-1	colorectal

Anatumomab mafenatox	TAG-72	non-small cell lung carcinoma
Anetumab ravtansine	MSLN	cancer
Apolizumab	HLA-DR	hematological
Ascrinvacumab	activin receptor -like kinase 1	cancer
Atezolizumab	CD274, PD-L1	urothelial bladder cancer, non-small cell lung carcinoma, melanoma, breast, renal cell carcinoma
Avelumab	PD-L1	solid tumors, non-small cell lung carcinoma, metastatic Merkel cell carcinoma
Bavituximab	phosphatidylserine	cancer
Belimumab	BAFF	non-Hodgkin lymphoma
Bevacizumab	VEGF-A	breast, cervical, colorectal, non-small cell lung carcinoma, renal cell carcinoma, ovarian, glioblastoma
Bivatuzumab mertansine	CD44 v6	squamous cell carcinoma
Blinatumomab	CD19	acute lymphoblastic leukemia
BMS-986016	LAG-3	breast, hematological, advanced solid tumor
BMS-936559	PD-L1	non-small cell lung carcinoma, renal cell carcinoma
Bosutinib	BCR-ABL	acute lymphoblastic leukemia
Brentuximab vedotin	CD30 (TNFRSF8)	hematologic, Hodgkin's lymphoma
Cantuzumab mertansine	mucin CanAg	colorectal
Cantuzumab ravtansine	MUC1	cancer
Carlumab	MCP-1	cancer
Catumaxomab	EpCAM, CD3	ovarian, gastric, malignant ascites
cBR96-doxorubicin immunoconjugate	Lewis-Y antigen	cancer
Cetuximab	EGFR	colorectal, head and neck cancer
Ch.14.18	unknown	neuroblastoma
CIMAvax	EGF	non-small cell lung carcinoma
Citatuzumab bogatox	EpCAM	ovarian, solid tumors
Cixutumumab	IGF-1 receptor	solid tumors
Clivatuzumab tetraxetan	MUC1	pancreatic cancer
Codrituzumab	glypican 3	cancer
Coltuximab ravtansine	CD19	cancer
Conatumumab	TRAIL-R2	cancer
Dacetuzumab	CD40	hematologic
Dalotuzumab	insulin-like growth factor I receptor	cancer
Daratumumab	CD38 (cyclic ADP ribose hydrolase)	cancer
Dasatinib	BCR-ABL	chronic myeloid leukemia
Demcizumab	DLL4	cancer
Denintuzumab mafodotin	CD19	cancer
Denosumab	RANKL	osteoporosis, bone metastases
Derlotuximab biotin	histone complex	recurrent glioblastoma multiforme
Detumomab	B-lymphoma cell	lymphoma

Dinutuximab	ganglioside GD2	neuroblastoma, retinoblastoma, melanoma
Drozitumab	DR5	cancer
Durvalumab	CD274, PD-L1	non-small cell lung carcinoma, head and neck, glioblastoma
Dusigitumab	ILGF2	cancer
Ecromeximab	GD3 ganglioside	malignant melanoma
Edrecolomab	EpCAM	colorectal
Elgemtumab	ERBB3	cancer
Elotuzumab	SLAMF7, CS1	multiple myeloma
Emactuzumab	CSF1R	cancer
EMD640744	survivin	melanoma, glioma, solid tumors
Emibetuzumab	HHGFR	cancer
Enavatuzumab	TWEAK receptor	cancer
Enfortumab vedotin	AGS-22M6	cancer expressing Nectin-4
Enoblituzumab	B7-H3	cancer
Ensituximab	5AC	cancer
Epratuzumab	CD22	acute lymphoblastic leukemia
Ertumaxomab	HER2/neu, CD3	breast
Etaracizumab	integrin αvβ3	melanoma, prostate, ovarian
Farletuzumab	folate receptor 1	ovarian
FBTA05	CD20	chronic lymphocytic leukemia
Ficlatuzumab	HGF	cancer
Figitumumab	IGF-1 receptor	adrenocortical carcinoma, non-small cell lung carcinoma
Flanvotumab	TYRP1 (glycoprotein 75)	melanoma
Fresolimumab	TGF-β	cancer
Galiximab	CD80	B-cell lymphoma
Ganitumab	IGF-I	cancer
Gemtuzumab ozogamicin	CD33	acute myelogenous leukemia
Girentuximab	carbonic anhydrase 9 (CA-IX)	clear cell renal cell carcinoma
Glembatumumab vedotin	GPNMB	melanoma, breast
gp100:209-217 (210M)	gp100	melanoma
HPV-16	HPV-16	cervical
Ibritumomab tiuxetan	CD20	non-Hodgkin's lymphoma, chronic lymphocytic leukemia, B cell non-Hodgkin's lymphoma, pre-B acute lymphoblastic leukemia
Icrucumab	VEGFR-1	cancer
IDH1(R132H)	IDH1	glioma
IMAB362	CLDN18.2	gastrointestinal adenocarcinoma and pancreatic tumor
Imalumab	MIF	cancer
Imatinib	BCR-ABL	chronic myeloid leukemia
Imgatuzumab	EGFR	cancer
Indatuximab ravtansine	SDC1	cancer
Indoximod	IDO1	breast, melanoma, non-small cell lung carcinoma

Indusatumab vedotin	GUCY2C	cancer
Intetumumab	CD51	solid tumors, prostate, melanoma
Inotuzumab ozogamicin	CD22	acute lymphoblastic leukemia
Ipilimumab	CD152, CTLA-4	melanoma
Iratumumab	CD30 (TNFRSF8)	Hodgkin's lymphoma
Isatuximab	CD38	cancer
ISCOMATRIX	NY-ESO-1	ovarian, melanoma
Labetuzumab	CEA	colorectal
Lexatumumab	TRAIL-R2	cancer
Lifastuzumab vedotin	phosphate-sodium co-transporter	cancer
Lilotomab satetraxetan	CD37	cancer
Lintuzumab	CD33	cancer
Lirilumab	KIR	lymphoma
Lorvotuzumab mertansine	CD56	cancer
Lucatumumab	CD40	multiple myeloma, non-Hodgkin's lymphoma, Hodgkin's lymphoma
Lumiliximab	CD23 (IgE receptor)	chronic lymphocytic leukemia
Lumretuzumab	ERBB3	cancer
LY6K-177	LY6K	gastric
Mapatumumab	TRAIL-R1	cancer
Margetuximab	ch4D5	cancer
MART-1(26-35, 27L)	Melan-A	melanoma
Matuzumab	EGFR	colorectal, lung, stomach
MED19447	CD73	advanced solid tumors
Milatuzumab	CD74	multiple myeloma and hematological malignancies
Mirvetuximab soravtansine	folate receptor alpha	cancer
Mitumomab	GD3 ganglioside	small cell lung carcinoma
Mogamulizumab	CCR4	cancer
Moxetumomab pasudotox	CD22	acute lymphoblastic leukemia
Nacolomab tafenatox	C242 antigen	colorectal
Naptumomab estafenatox	5T4	non-small cell lung carcinoma, renal cell carcinoma, colorectal, prostate
Narnatumab	RON	cancer
Necitumumab	EGFR	non-small cell lung carcinoma
Nesvacumab	angiopoietin 2	cancer
Nilotinib	BCR-ABL	acute lymphoblastic leukemia
Nimotuzumab	EGFR	squamous cell carcinoma, head & neck, nasopharyngeal, glioma,
Nivolumab	PD-1	metastatic melanoma, non-small cell lung carcinoma
Obinutuzumab	CD20	Chronic lymphatic leukemia
Ocaratuzumab	CD20	cancer
Ofatumumab	CD20	non-Hodgkin's lymphoma, chronic lymphocytic leukemia, B cell non-Hodgkin's lymphoma, pre-B acute lymphoblastic leukemia
Olaratumab	PDGF-R α	cancer
Onartuzumab	MET, human scatter	non-small cell lung carcinoma

	factor receptor kinase	
Ontuxizumab	TEM1	cancer
Oportuzumab monatox	EpCAM	cancer
Oregovomab	CA-125, MUC16	ovarian
Otlertuzumab	CD37	cancer
Panitumumab	EGFR	colorectal, head and neck
Pankomab	tumor specific glycosylation of MUC1	ovarian cancer
PANVAC	CEA	colorectal
Parsatuzumab	EGFL7	cancer
Pasotuxizumab	folate hydrolase	cancer
Patritumab	HER3	cancer
PBF-509	A2aR	non-small cell lung carcinoma
Pembrolizumab	PD-1	metastatic melanoma, non-small cell lung carcinoma
Pemtumomab	MUC1	cancer
Pertuzumab	HER2/neu	breast
Pidilizumab	PD-1	B cell lymphoma, metastatic melanoma, colorectal
Pinatuzumab vedotin	CD22	cancer
Polatuzumab vedotin	CD79B	cancer
Ponotinib	BCR-ABL	acute lymphoblastic leukemia
Pritumumab	vimentin	brain
PROSTVAC	PSA	prostate
Racotumomab	N-glycolylneuraminic acid	non-small cell lung carcinoma, breast, melanoma
Radretumab	fibronectin extra domain-B	cancer
Ramucirumab	VEGF-R2	gastric, non-small cell lung carcinoma
RecMAGE-A3	MAGE-A3	melanoma
Rilotumumab	HGF	solid tumors
Rituximab	CD20	non-Hodgkin's lymphoma, chronic lymphocytic leukemia, B cell non-Hodgkin's lymphoma, pre-B acute lymphoblastic leukemia
Robatumumab	IGF-1 receptor	cancer
Sacituzumab govitecan	tumor-associated calcium signal transducer 2	cancer
Samalizumab	CD200	cancer
Seribantumab	ERBB3	cancer
Sibrotuzumab	FAP	cancer
SGN-CD19A	CD19	acute lymphoblastic leukemia, B-cell non-Hodgkin lymphoma
SGN-CD33A	CD33	acute myeloid leukemia
Siltuximab	IL-6	cancer
Sipuleucel-T	PAP	prostate
Sofituzumab vedotin	CA 125	ovarian
Tabalumab	BAFF	B-cell
Tacatuzumab tetraxetan	alpha-fetoprotein	cancer

Taplitumomab paptox	CD19	cancer
Tarextumab	Notch receptor	cancer
Tecemotide	MUC1	non-small cell lung carcinoma, breast
Tenatumomab	tenascin C	cancer
Teprotumumab	CD221	hematologic
Tertomotide	telomerase	pancreatic
Tetulomab	CD37	cancer
TGN1412	CD28	chronic lymphocytic leukemia
Theratope	sialyl-Tn	breast
Ticilimumab	CTLA-4	cancer
Tigatuzumab	TRAIL-R2	cancer
TNX-650	IL-13	Hodgkin's lymphoma
Tositumomab	CD20	follicular lymphoma, non-Hodgkin's lymphoma, chronic lymphocytic leukemia, B cell non-Hodgkin's lymphoma, pre-B acute lymphoblastic leukemia
Tovetumab	CD140a	cancer
Trastuzumab	HER2/neu	breast
TRBS07	GD2	melanoma
Tremelimumab	CTLA-4	malignant mesothelioma
Tucotuzumab celmoleukin	EpCAM	cancer
Ublituximab	MS4A1	cancer
Ulocuplumab	C-X-C chemokine receptor type 4	hematologic malignancies
Urelumab	CD137, 4-1BB	advanced solid tumors
Vandortuzumab vedotin	STEAP1	cancer
Vantictumab	Frizzled receptor	cancer
Vanucizumab	angiopoietin 2	cancer
Veltuzumab	CD20	non-Hodgkin's lymphoma
Volociximab	integrin $\alpha5\beta1$	solid tumors
Vorsetuzumab mafodotin	CD70	cancer
Votumumab	tumor antigen CTAA16.88	colorectal
WT1 peptide vaccine	WT1	ovarian, uterine, acute myelogenous leukemia
Zalutumumab	EGFR	squamous cell carcinoma head and neck
Zastumotide	MAGE-A3	non-small cell lung carcinoma
Zatuximab	HER1	cancer

In some embodiments, the tumor-associated antigen is 4-1BB, 5AC, 5T4, A2aR, activin receptor-like kinase 1, AGS-22M6, AKAP4, alpha-fetoprotein, angiopoietin 2, B7-H3, BAFF, BAGE, BCR-ABL, BORIS, CA-125, CA19-9, C242 antigen, carbonic anhydrase 9 (CA-IX), CCR4, CD19, CD20, CD22, CD23 (IgE receptor), CD24, CD28, CD30 (TNFRSF8), CD33, CD37, CD38 (cyclic ADP ribose hydrolase), CD40, CD44 v6, CD51, CD56, CD70, CD71, CD73, CD74, CD79B, CD80, CD137, CD140a, CD152, CD200, CD221, CD274, CEA, ch4D5, CLDN18.2, CS1, CSF1R, CTLA-4, C-X-C chemokine receptor type 4, DLL4, DR5, EBAG9,

EGF, EGFR, EGFL7, EpCAM, ERBB2, ERBB3, FAP, fibronectin extra domain-B, folate receptor 1, folate receptor alpha, folate hydrolase, Frizzled receptor, GAGE, GD2 ganglioside, GD3 ganglioside, glioma, glypican 3, GPNMB, gp100, GUCY2C, HER1, HER2/neu, HER3, HGF, HHGFR, histone complex, HLA-DR, human scatter factor receptor kinase, HPV-16, HSP105, IDH1, IDO1, IGF-I, IGF-1 receptor, ILGF2, IL-6, IL-13, integrin αvβ3, integrin α5β1, KIR, LAG-3, Lewis-Y antigen, LY6K, MAGE-1, MAGE-A3, MAGE-C2, MAGE-D4, MAPG, MART-1, Melan-A, MET, MCP-1, mesothelin, MIF, MSLN, MS4A1, mucin CanAg, MUC1, MUC4, MUC16, NG2, N-glycolylneuraminic acid, Notch receptor PD-1, NY-ESO-1, OCAA, PAP, PDGF-R α, PDCD1, PD1, PD-L1, phosphate-sodium co-transporter, phosphatidylserine, PRAME, PSA, RANKL, RON, ROR1, SDC1, Sialyl-Tn, SLAMF7, SPAG-9, SSX1, STEAP1, survivin, TAG-72, telomerase, TEM1, tenascin C, TGF-β, TIM-3, TLR, TAM, TIM-3, TRAIL-R2, TRAIL-R1, TWEAK receptor, tumor specific glycosylation of MUC1, tumor-associated calcium signal transducer 2, tumor antigen CTAA16.88, TYRP1 (glycoprotein 75), VEGF-A, VEGFR2, VEGFR-1, vimentin, VISTA, WT1, or XAGE-1b. In some embodiments, the tumor-associated antigen is HER-2, EGFR, CD30, CD20, EpCAM, NG2, CD19, CEA, MUC-1, CA19-9, OCAA, MAPG, TAM, TLR, CD71, ERBB2, VEGF, or glioma.

Table 2 lists exemplary antibodies (or fragments thereof) to disease-associated antigens.

Table 2

Name	Target	Use
Abciximab	CD41 (integrin alpha-IIb)	platelet aggregation inhibitor
Abrilumab	integrin α4β7	inflammatory bowel disease, ulcerative colitis, Crohn's disease
Actoxumab	*Clostridium difficile*	*Clostridium difficile* infection
Adalimumab	TNF-α	rheumatoid arthritis, Crohn's Disease, plaque psoriasis, psoriatic arthritis, ankylosing spondylitis, juvenile idiopathic arthritis, hemolytic disease of the newborn
Aducanumab	beta-amyloid	Alzheimer's disease
Afelimomab	TNF-α	sepsis
ALD518	IL-6	rheumatoid arthritis
Alemtuzumab	CD52	multiple sclerosis
Alirocumab	PCSK9	hypercholesterolemia
Anifrolumab	interferon α/β receptor	systemic lupus erythematosus
Atlizumab	IL-6 receptor	rheumatoid arthritis
Atorolimumab	Rhesus factor	hemolytic disease of the newborn
Bapineuzumab	beta amyloid	Alzheimer's disease
Basiliximab	CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejections
Bavituximab	phosphatidylserine	viral infections

Benralizumab	CD125	asthma
Bertilimumab	CCL11 (eotaxin-1)	severe allergic disorders
Besilesomab	CEA-related antigen	inflammatory lesions
Bezlotoxumab	*Clostridium difficile*	*Clostridium difficile* infection
Bimagrumab	ACVR2B	myostatin inhibitor
Blosozumab	SOST	osteoporosis
Bococizumab	neural apoptosis-regulated proteinase 1	dyslipidemia
Briakinumab	IL-12, IL-23	psoriasis, rheumatoid arthritis, inflammatory bowel diseases, multiple sclerosis
Brodalumab	IL-17	inflammatory diseases
Canakinumab	IL-1?	rheumatoid arthritis
Caplacizumab	VWF	thrombotic thrombocytopenic purpura, thrombosis
Cedelizumab	CD4	prevention of organ transplant rejection, autoimmune diseases
Certolizumab pegol	TNF-α	Crohn's disease
Clazakizumab	Oryctolagus cuniculus	rheumatoid arthritis
Clenoliximab	CD4	rheumatoid arthritis
Concizumab	TFPI	bleeding
Crenezumab	1-40-β-amyloid	Alzheimer's disease
CR6261	Influenza A hemagglutinin	infectious disease/influenza A
Daclizumab	CD25 (α chain of IL-2 receptor)	prevention of organ transplant rejection
Denosumab	RANKL	osteoporosis, bone metastases etc.
Diridavumab	hemagglutinin	influenza A
Dupilumab	IL4	atopic diseases
Eculizumab	C5	paroxysmal nocturnal hemoglobinuria
Edobacomab	endotoxin	sepsis caused by Gram-negative bacteria
Efalizumab	LFA-1 (CD11a)	psoriasis (blocks T-cell migration)
Efungumab	Hsp90	invasive Candida infection
Eldelumab	interferon gamma-induced protein	Crohn's disease, ulcerative colitis
Enokizumab	IL9	asthma
Erlizumab	ITGB2 (CD18)	heart attack, stroke, traumatic shock
Etrolizumab	integrin α7 β7	inflammatory bowel disease
Evinacumab	angiopoietin 3	dyslipidemia
Evolocumab	PCSK9	hypercholesterolemia
Exbivirumab	hepatitis B surface Ag	hepatitis B
Fasinumab	HNGF	acute sciatic pain
Felvizumab	respiratory syncytial virus	respiratory syncytial virus infection
Fezakinumab	IL-22	rheumatoid arthritis, psoriasis
Fletikumab	IL 20	rheumatoid arthritis
Fontolizumab	IFN-γ	Crohn's disease
Foravirumab	rabies virus glycoprotein	rabies (prophylaxis)
Fresolimumab	TGF-β	idiopathic pulmonary fibrosis, focal

		segmental glomerulosclerosis
Gantenerumab	beta amyloid	Alzheimer's disease
Gavilimomab	CD147 (basigin)	graft versus host disease
Gevokizumab	IL-1β	diabetes
Golimumab	TNF-α	rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis
Gomiliximab	CD23 (IgE receptor)	allergic asthma
Guselkumab	IL23	psoriasis
Ibalizumab	CD4	HIV infection
Infliximab	TNF-α	rheumatoid arthritis, ankylosing spondylitis, psoriatic arthritis, psoriasis, Crohn's disease, ulcerative colitis
Inolimomab	CD25 (α chain of IL-2 receptor)	graft versus host disease
Ixekizumab	IL-17A	autoimmune diseases
Keliximab	CD4	chronic asthma
Lebrikizumab	IL-13	asthma
Lerdelimumab	TGF beta 2	reduction of scarring after glaucoma surgery
Libivirumab	hepatitis B surface Ag	hepatitis B
Ligelizumab	IGHE	severe asthma and chronic spontaneous urticaria
Lodelcizumab	PCSK9	hypercholesterolemia
Lulizumab pegol	CD28	autoimmune diseases
Mavrilimumab	GMCSF receptor α-chain	rheumatoid arthritis
Mepolizumab	IL-5	asthma and white blood cell diseases
Metelimumab	TGF beta 1	systemic scleroderma
Motavizumab	respiratory syncytial virus	respiratory syncytial virus (prevention)
Muromonab-CD3	CD3	prevention of organ transplant rejections
Natalizumab	integrin α4	multiple sclerosis, Crohn's disease
Nebacumab	endotoxin	sepsis
Obiltoxaximab	Bacillus anthracis	Bacillus anthracis spores
Ocrelizumab	CD20	rheumatoid arthritis, lupus erythematosus
Odulimomab	LFA-1 (CD11a)	prevention of organ transplant rejections, immunological diseases
Omalizumab	IgE Fc region	allergic asthma
Opicinumab	LINGO-1	multiple sclerosis
Otelixizumab	CD3	diabetes mellitus type 1
Oxelumab	OX-40	asthma
Ozanezumab	NOGO-A	ALS and multiple sclerosis
Ozoralizumab	TNF-α	inflammation
Pagibaximab	lipoteichoic acid	sepsis (Staphylococcus)
Palivizumab	F protein of respiratory syncytial virus	respiratory syncytial virus (prevention)
Panobacumab	*P. aeruginosa*	*P. aeruginosa* infection
Pascolizumab	IL-4	asthma

Perakizumab	IL17A	arthritis
Pexelizumab	C5	reduction of side effects of cardiac surgery
Ponezumab	human beta-amyloid	Alzheimer's disease
Priliximab	CD4	Crohn's disease, multiple sclerosis
PRO 140	CCR5	HIV infection
Quilizumab	IGHE	asthma
Rafivirumab	rabies virus glycoprotein	rabies (prophylaxis)
Ralpancizumab	neural apoptosis-regulated proteinase 1	dyslipidemia
Ranibizumab	VEGF-A	macular degeneration (wet form)
Raxibacumab	anthrax toxin, protective antigen	anthrax (prophylaxis and treatment)
Refanezumab	myelin-associated glycoprotein	recovery of motor function after stroke
Regavirumab	cytomegalovirus glycoprotein B	cytomegalovirus infection
Reslizumab	IL-5	inflammations of the airways, skin and gastrointestinal tract
Rinucumab	platelet-derived growth factor receptor beta	neovascular age-related macular degeneration
Romosozumab	sclerostin	osteoporosis
Rontalizumab	IFN-α	systemic lupus erythematosus
Rovelizumab	CD11, CD18	haemorrhagic shock
Ruplizumab	CD154 (CD40L)	rheumatic diseases
Sarilumab	IL6	rheumatoid arthritis, ankylosing spondylitis
Secukinumab	IL-17A	uveitis, rheumatoid arthritis psoriasis
Sevirumab	cytomegalovirus	cytomegalovirus infection
Sifalimumab	IFN-α	SLE, dermatomyositis, polymyositis
Simtuzumab	LOXL2	fibrosis
Siplizumab	CD2	psoriasis, graft-versus-host disease (prevention)
Sirukumab	IL-6	rheumatoid arthritis
Solanezumab	beta amyloid	Alzheimer's disease
Sonepcizumab	sphingosine-1-phosphate	choroidal and retinal neovascularization
Stamulumab	myostatin	muscular dystrophy
Suvizumab	HIV-1	viral infections
Tadocizumab	integrin αIIbβ3	percutaneous coronary intervention
Talizumab	IgE	allergic reaction
Tanezumab	NGF	pain
Tefibazumab	clumping factor A	*Staphylococcus aureus* infection
Teplizumab	CD3	diabetes mellitus type 1
TGN1412	CD28	rheumatoid arthritis
Tildrakizumab	IL23	inflammatory disorders
Tocilizumab	IL-6 receptor	rheumatoid arthritis
Toralizumab	CD154 (CD40L)	rheumatoid arthritis, lupus nephritis
Tralokinumab	IL-13	asthma
Trevogrumab	growth differentiation factor 8	muscle atrophy due to orthopedic disuse and sarcopenia

Tuvirumab	hepatitis B virus	chronic hepatitis B
Urtoxazumab	*Escherichia coli*	diarrhoea caused by *E. coli*
Ustekinumab	IL-12, IL-23	multiple sclerosis, psoriasis, psoriatic arthritis
Vedolizumab	integrin α4β7	Crohn's disease, ulcerative colitis
Vepalimomab	AOC3 (VAP-1)	inflammation
Visilizumab	CD3	Crohn's disease, ulcerative colitis
Zanolimumab	CD4	rheumatoid arthritis, psoriasis
Zolimomab aritox	CD5	systemic lupus erythematosus, graft-versus-host disease

In some embodiments, the disease-associated antigen is 1-40-β-amyloid , AOC3 (VAP-1), ACVR2B, angiopoietin 3, beta-amyloid, C5, CCL11 (eotaxin-1), CCR5, CD2, CD3, CD4, CD5, CD11, CD18, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD28, CD41 (integrin alpha-IIb), CD52, CD125, CD147 (basigin), CD154 (CD40L), CEA-related antigen, clumping factor A, endotoxin, GMCSF receptor α-chain, growth differentiation factor 8, hemagglutinin, HNGF, Hsp90, IGHE, IgE Fc region, IL-1β, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL-6 receptor, integrin α4β7, integrin α7β7, integrin α4, integrin αIIbβ3, interferon α/β receptor, interferon gamma-induced protein, IFN-γ, IFN-α, ITGB2 (CD18), LFA-1 (CD11a), LINGO-1, lipoteichoic acid, LOXL2, myelin-associated glycoprotein, myostatin, neural apoptosis-regulated proteinase 1, NGF, NOGO-A, Oryctolagus cuniculus, OX-40, PCSK9, phosphatidylserine, platelet-derived growth factor receptor beta, RANKL, Rhesus factor, sclerostin, SOST, sphingosine-1-phosphate, TFPI, TGF-β, TGF beta 2, TGF beta 1, TNF-α, VEGF-A, or VWF.

In some embodiments, the antibody, or fragment thereof, is directed to a cell surface marker. In some embodiments, the cell surface marker is on an immune cell. In some embodiments, the immune cell is a T cell, B cell, NK cell, macrophage, TIL, dendritic cell, neutrophil, eosinophil, basophil, or mast cell. In some embodiments, the T cell is a $CD4^+$ T cell, $CD8^+$ T cell, helper T cell, cytotoxic T cell, or natural killer T cell; and wherein the B cell is a memory B cell or a plasma cell. In some embodiments, the cell surface marker is CD36, CD68, CD83, CD180, CD206 (MRC1- mannose receptor), F4/80 (EGF-TM7 family), CD205, CD56, CD161, CD94:NKG2 heterodimer, KIR/CD158 family, CD335, CD64, CD16, CD3, CD28, CD4, CD25, CD39, Toll-like receptors (TLRs), CD281, CD283, CD284, CD286, CD289, CD282, C-type lectin receptors (CLRs), Fc Receptor, or HSG.

In some embodiments, the nucleic acid carrier comprises one or two of the following: a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64; a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent

which is an antibody, or fragment thereof, directed to CD64; ; a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1; a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX-40; a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16; a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28; a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16; a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG; a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG; a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28; a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16; a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3; a first targeting agent which is folic acid or folic acid receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16; and a first targeting agent

which is an antibody, or fragment thereof, directed to PD-1 and a second targeting agent which is an antibody, or fragment thereof, directed to PDL-1 or PDL-2.

In some embodiments, the antibody, or fragment thereof, is an antibody-drug conjugate (ADC). In some embodiments, the nucleic acid carrier comprises one, two, three, for, five, or six, or more of the ADCs listed in Table 3. In some embodiments, the nucleic acid carrier may comprise multiple copies of the same ADC, as set forth above for targeting agents.

Table 3

Name (drug) (linker)	Target	Indication
Mylotarg (Calicheamycin) (Ac-But linker)	CD33	AML
SGN-35 (MMAE) (Val-Cit)	CD30	HL/ALCL
CMC-544 (Calicheamycin) (Ac-But linker)	CD22	NHL
Kadcyla (DM1) (SMCC)	HER2	Breast cancer
IMGN901 (DM1) (SPP)	CD56	SCLC
IMGN-388 (DM4) (SPDB)	αV-integrin	Solid tumors
SAR3419 (DM4) (SPDB)	CD19	DLBCL
BIIB015 (DM4) (NA)	Cripto	Breast cancer
BT-062 (DM4) (SPDB)	CD138	Multiple myeloma
CDX-011 (MMAE) (Val-Cit)	GPNMB	Breast cancer/Melanoma
SGN-75 (MMAE) (MalC linker)	CD70	NHL/RCC
PSMA ADC (MMAE) (Val-Cit)	PSMA	Prostate cancer
MEDI-547 (MMAE) (MalC linker)	EphA2	Solid cancer
ASG-5ME (MMAE) (Val-Cit)	SLC44A4	Pancreatic cancer
ASG-15ME (MMAE) (Val-Cit)	SLITRK6	lung cancer
ASG-22ME (MMAE) (Val-Cit)	Nectin-4	Solid tumors
MDX-1203 (Duocarmycin) (Val-Cit-PABC)	CD70	NHL/RCC
BAY-94-9343 (DM4) (SPDB)	Mesothelin	Mesotheliomas/Ovarian tumor
MLN-0264 (MMAE) (NA)	Guanylyl cyclase	Gastrointestinal tumor
MLN-2704 (DM1) (NA)	PSMA	prostate cancer
SGN-75 (MMAF) (Maleimidocaproyl)	CD70	RCC
ABT-414 (NA) (NA)	EGFR	NSCLC
AMG-595 (DM1) (SMCC)	EGFRvIII	Glioma
AMG-172 (DM1) (SMCC)	CD70	RCC
RG-7596 (MMAE) (Val-Cit)	CD79b	NHL

RG-7600 (NA) (NA)	NA	Ovarian tumor
SGN-CD19A (MMAE) (Maleimidocaproyl)	CD19	AML/NHL
SGN-CD33A (PBD dimer) (MalC linker)	CD33	drug-resistant AML
SGN-CD70A (PBD dimer) (NA)	CD70	NHL/RCC
IMMU-110 (Doxorubicin) (Ac-But linker)	CD74	Multiple myeloma
IMMU-115 (Doxorubicin) (Ac-But linker)	CD74	NHL/CLL
IMMU-132 (SN38) (Phenylalaninelysine)	TACSTD2	Solid tumors
IMMU-130 (SN38) (Phenylalaninelysine)	CEACAM5	Colorectal tumor
IMGN-529 (DM1) (SPP)	CD37	Hematologic tumors
IMGN-289 (DM1) (SPP)	EGFR	Solid tumors
SAR-566658 (DM4) (SPDB)	DS6	Solid tumors
SYD985 (Duocarmycin) (NA)	HER2	Solid tumors
AGS67E (MMAE) (Val-Cit-PABC)	CD37	CLL/AML
AGS-16M8F (MMAF) (MalC linker)	ENPP3	Renal cancer
SC16LD6.5 (D6.5) (NA)	Fyn3	SCLC
DNIB0600A (MMAE) (Val-Cit)	NaPi2b	NSCLC
IMGN853 (DM4) (sulfo-SPDB)	FRα	Ovarian cancer

Abbreviations: AML: acute myeloid leukaemia; HL: Hodgkin's lymphoma; NHL: non-Hodgkin's lymphoma; ALCL: anaplasia large cell lymphoma; DLBCL: diffuse large B cell lymphoma; RCC: renal cell carcinoma; SCLC: small cell lung cancer; NSCLC: non-small cell lung cancer; CLL: chronic lymphocytic leukaemia; EGFR: epidermal growth factor receptor; GPNMB: glycoprotein NMB; PSMA: prostate-specific membrane antigen; NA: not available.

Highly potent drugs such as, for example, calicheamicins, doxorubicin, maytansinoids, auristatins, and camptothecin can be used as drug payloads for ADCs. Additional drug classes that may serve as drug payloads for ADCs include, but are not limited to, pyrrolobenzodiazepine (PDB) dimer, α-Amanitin, duocarmycin analogs, tubulysin B, and cryptophycin analogs. Additional ADCs include brentuximab vedotin and ado-trastuzumab emtansine.

In some embodiments, the label is a chemiluminescent agent, a fluorescent agent, an infrared dye, a radiolabel agent, a metal, a chelating agent, a hapten, or a gene or mRNA encoding a detectable protein. In some embodiments, the detectable protein is GFP or CFP.

In some embodiments, the adjuvant is one or more of a CpG containing oligonucleotide, a ssRNA, a dsRNA, a monophosphate lipid, aluminum, squalene, 3-deacyl-monophosphoryl lipid A, vitamin E, surfactant polysorbate 80, mannide-mono-oleate, SLAG-3, or a

galactosylceramide. In some embodiments, the CpG containing oligonucleotide is ODN 1585 (ggggtcaacgttgaggggggg; SEQ ID NO:1), ODN 1668 (tccatgacgttcctgatgct; SEQ ID NO:2), ODN 1826 (tccatgacgttcctgacgtt; SEQ ID NO:3), ODN 2006 (tcgtcgttttgtcgttttgtcgtt; SEQ ID NO:4), ODN 2007 (tcgtcgttgcgttttgtcgtt; SEQ ID NO:5), ODN 2216 (gggggacgatcgtcgggggg; SEQ ID NO:6), ODN 2336 (ggggacgacgtcgtgggggggg; SEQ ID NO:7), ODN 2395 (tcgtcgttttcggcgcgcgccg; SEQ ID NO:8), ODN M362 (tcgtcgtcgttcgaacgacgacgttgat; SEQ ID NO:9), AT-ODN-1 (tataattttaatttccaaga; SEQ ID NO:10), AT-ODN-2 (tataatttttaccaactagc; SEQ ID NO:11), ODNBW006 (tcgacgttcgtcgttcgtcgttc; SEQ ID NO:12), ODN 2088 (tcctggcggggaagt; SEQ ID NO:13), ODN 4084 (cctggatgggaa; SEQ ID NO:14), ODN INH-1 (cctggatgggaattcccatccagg; SEQ ID NO:15), ODN INH-47 (tatggattttaattaaaatccata; SEQ ID NO:16), ODN TTAGGG (tttagggttagggttagggttaggg; SEQ ID NO:17), or G-ODN (ctcctattgggggtttcctat; SEQ ID NO:18). In some embodiments, the CpG containing oligonucleotide is ODN 1668 (tccatgacgttcctgatgct; SEQ ID NO:2), ODN 1826 (tccatgacgttcctgacgtt; SEQ ID NO:3), ODN 2006 (tcgtcgttttgtcgttttgtcgtt; SEQ ID NO:4), ODN 2007 (tcgtcgttgcgttttgtcgtt; SEQ ID NO:5), ODN 2395 (tcgtcgttttcggcgcgcgccg; SEQ ID NO:8), ODN M362 (tcgtcgtcgttcgaacgacgacgttgat; SEQ ID NO:9), AT-ODN-1 (tataattttaatttccaaga; SEQ ID NO:10), AT-ODN-2 (tataatttttaccaactagc; SEQ ID NO:11), ODNBW006 (tcgacgttcgtcgttcgtcgttc; SEQ ID NO:12), ODN 4084 (cctggatgggaa; SEQ ID NO:14), ODN INH-1 (cctggatgggaattcccatccagg; SEQ ID NO:15), ODN INH-47 (tatggattttaattaaaatccata; SEQ ID NO:16), or ODN TTAGGG (tttagggttagggttagggttaggg; SEQ ID NO:17).

In some embodiments, the nucleic acid carrier comprises a targeting agent that is an antigen, an antibody, or a fragment of an antibody, and the oligonucleotide linker conjugating the targeting moiety to the single-stranded arm or terminal arm is or comprises a CpG containing oligonucleotide. In some embodiments, the nucleic acid carrier comprises at least one antigen, at least one antibody or fragment thereof directed to an immune cell, and at least one adjuvant.

The nucleic acid carriers can also comprise additional therapeutic compounds that associate therewith. Additional therapeutic compounds include, but are not limited to, chemotherapeutic agents, cytokines, and chemokines.

Suitable chemotherapeutic agents include, but are not limited to, methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, distamycin A, etidium, netropsin, auristatin, amsacrine, prodigiosin. Bortexomib, pibenzimol, tomaymycin,

Duocarmycin SA, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, and docetaxel.

Suitable cytokines include, but are not limited to, IL-1-like cytokines, common γ chain cytokines, common β chain cytokines, IL-6-like cytokines, IL-10-like cytokines, an interferon, a tumor necrosis factor, and a member of the TGF-β family. In some embodiments, the IL-1-like cytokine is IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA), or IL-18. In some embodiments, the common γ chain cytokine is IL-2, IL-4, IL-7, IL-9, IL-13, or IL-15. In some embodiments, the common β chain cytokine is IL-3, IL-5, or GM-CSF. In some embodiments, the IL-6-like cytokine is IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), granulocyte colony-stimulating factor (G-CSF), or IL-12. In some embodiments, the IL-10-like cytokine is IL-10, IL-19, or IL-20. In some embodiments, the interferon is IFN-α, IFN-β, or IFN-γ. In some embodiments, the tumor necrosis factor is TNF-α, TNF-β, lymphotoxin (LT)-β, or FasL. In some embodiments, the member of the TGF-β family is TGF-β1, TGF-β2, or TGF-β3.

Suitable chemokines include, but are not limited to, a member of the C group of chemokines, a member of the human CC chemokine group, a member of the CXC group of human chemokines, and a CX3C chemokine. In some embodiments, the member of the C group of chemokines is lymphotactin/XCL1 or SCM-1β/XCL2. In some embodiments, the member of the human CC chemokine group is monocyte chemotactic protein (MCP)-1/CCL2, macrophage inflammatory protein (MIP)-1a/CCL3, MIP-1β/CCL4, or eotaxin/CCL11. In some embodiments, the member of the CXC group of human chemokine is IL-8/CXCL8 (ELR), monokine-induced by IFN-γ (MIG)/CXCL9 (nonELR), IFN-γ inducible protein-10 (IP-10)/CXCL10 (nonELR), and stromal cell-derived factor-1 (SDF-1)/CXCL12 (nonELR). In some embodiments, the CX3C chemokine is fractalkine/CX3CL1.

The present disclosure also provides pharmaceutical compositions comprising any of the nucleic acid carriers described herein, or any combination thereof, and a pharmaceutically acceptable vehicle. In some embodiments, the pharmaceutically acceptable vehicle may be a diluent, filler, disintegrant, binder, lubricant, surfactant, hydrophobic vehicle, water soluble vehicle, emulsifier, buffer, humectant, moisturizer, solubilizer, preservative, and the like. Additional examples of vehicles include, but are not limited to, calcium carbonate, calcium phosphate, sodium phosphate, potassium phosphate, sodium chloride, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols, Cremophor EL (CrEL; polyoxyethyleneglycerol triricinoleate 35) and polysorbate 80 (Tween) 80; polyoxyethylene-sorbitan-20-monooleate).

The present disclosure also provides methods of making the monomeric nucleic acid carrier comprising: hybridizing the first oligonucleotide to the second oligonucleotide; and conjugating the at least one targeting agent to the at least one single-stranded arm. In some embodiments, the at least one targeting agent is further conjugated to an oligonucleotide linker prior to conjugating the at least one targeting agent to the at least one single-stranded arm. In some embodiments, the at least one targeting agent/oligonucleotide linker is conjugated to the at least one single-stranded arm by hybridizing the oligonucleotide linker portion of the at least one targeting agent/oligonucleotide linker to a complementary nucleotide sequence of the at least one single-stranded arm. In some embodiments, one, two, or three additional targeting agents are further conjugated to other single-stranded arms.

In general, the oligonucleotides can be synthesized using chemical techniques that do not employ synthetic enzymes (e.g., as in PCR) or *in vivo* replication (e.g., as in cloning and replicating the sequence in a microorganism). By way of example, the nucleic acids may be synthesized by solid-phase synthesis using the phosphoramidite method, with subsequent isolation of the products by HPLC or gel electrophoresis if necessary. The nucleic acids may be derived from 2′-deoxynucleosides, ribonucleosides, or chemically modified nucleosides (e.g., LNA or BNA).

In some embodiments, the single-stranded nucleic acid molecules may by synthesized using naturally-occurring or non-naturally occurring nucleosides. The chemically synthesized single-stranded nucleic acid molecules may be chemically synthesized using any of the known synthetic methods, including methods for solid-phase synthesis such as nucleoside phosphoramidite chemistry which uses cycles of deprotection, coupling, capping and stabilization to synthesize the desired nucleic acid molecule from the 3′ to the 5′ end, as is known in the art. For longer oligonucleotides it is generally recommended to purify the finished nucleic acid molecule after it is released from the solid phase, for example by polyacrylamide gel electrophoresis or high performance liquid chromatography (HPLC).

The nucleic acid carriers described herein are useful for drug delivery, diagnostics, and medical imaging, among other biotechnological applications.

The present disclosure also provides methods of inducing an immune response in an animal (suitably a mammal such as a human) comprising administering to the animal (suitably a mammal such as a human) any one or more of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the nucleic acid carrier, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antigen, a peptide, an antibody or fragment thereof, and an adjuvant.

In some embodiments, the antigen is chosen from the bacterial or viral antigens described herein. In some embodiments, the antibody fragment is any of the antibody fragments described herein. In some embodiments, the antibody, or fragment thereof, is directed to a cell surface marker described herein. In some embodiments, the adjuvant is one or more of the adjuvants described herein. In some embodiments, the oligonucleotide linker conjugating the targeting moiety to the terminal arm is a CpG containing oligonucleotide comprising one or more CpG sequences.

The present disclosure also provides methods of treating an animal (suitably a mammal such as a human) having cancer comprising administering to the animal (suitably a mammal such as a human) a nucleic acid carrier of any one or more of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the nucleic acid carrier, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

In some embodiments, the antibody fragment is any of the antibody fragments described herein. In some embodiments, a first antibody, or fragment thereof, is directed to a tumor-associated antigen. In some embodiments, the tumor-associated antigen is any of the tumor-associated antigens described herein. In some embodiments, a second antibody, or fragment thereof, is directed to a cell surface marker. In some embodiments, the cell surface marker is any of the cell surface markers described herein. In some embodiments, the nucleic acid carrier comprises any one or more of: a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is breast, ovarian, or prostrate; a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is a solid tumor, lung, or colorectal; a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1, and wherein the cancer is triple negative breast cancer; a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX-40, and wherein the cancer is breast cancer; a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is Hodgkin's disease; a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is metastatic breast cancer or prostrate; a first targeting

agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is non-Hodgkin lymphoma or multiple myeloma; a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian, gastric, colon, colorectal, breast, non-small cell lung cancer, adenocarcinoma of the lung, small cell lung cancer; a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is melanoma; a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is B-precursor acute lymphoblastic leukemia or non-Hodgkin lymphoma; a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is non-Hodgkin lymphoma; a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein the cancer is colorectal, lung carcinoma, pancreas carcinoma, stomach carcinoma, ovary carcinoma, uterus carcinoma, breast carcinoma, or melanoma; a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein the cancer is invasive pancreatic adenocarcinoma; a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a CA19-9-positive tumor; a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian; a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian; a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is metastatic melanoma; a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is a HER-2 positive tumor; a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a glioma; and a first targeting agent which is folic acid or folic acid

receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16, and wherein the cancer is small cell lung carcinoma, breast, or ovarian.

In some embodiments, the nucleic acid carrier comprises any one or more of: a first targeting agent which is an antibody, or fragment thereof, directed to a disease-associated marker, a second targeting agent which is an antibody directed to a cell surface marker, and a third targeting agent which is an antibody, or fragment thereof, directed to a second cell surface marker, and wherein the disease is a cancer.

In some embodiments, the nucleic acid carrier comprises any one or more of: a first targeting agent which is an antigen associated with a disease, a second targeting agent which is an antibody directed to a cell surface marker, and a third targeting agent which is an adjuvant, and wherein the indication is a vaccine.

In some embodiments, the label is a chemiluminescent agent, a fluorescent agent, an infrared dye, a radiolabel agent, a metal, a chelating agent, a hapten, or a gene or mRNA encoding a detectable protein. In some embodiments, the presence of the label is used to track the location of the nucleic acid carrier.

The present disclosure also provides methods of treating an animal (suitably a mammal such as a human) having a disease associated with a disease-associated antigen comprising administering to the animal (suitably a mammal such as a human) a nucleic acid carrier of any one or more of the nucleic acid carriers described herein, or a pharmaceutical composition comprising the nucleic acid carrier, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

In some embodiments, the antibody fragment is any of the antibody fragments described herein. In some embodiments, a first antibody, or fragment thereof, is directed to a disease-associated antigen. In some embodiments, the disease-associated antigen is any of the disease-associated antigens described herein. In some embodiments, a second antibody, or fragment thereof, is directed to a cell surface marker. In some embodiments, the cell surface marker is any of the cell surface markers described herein. In some embodiments, the label is a chemiluminescent agent, a fluorescent agent, an infrared dye, a radiolabel agent, a metal, a chelating agent, a hapten, or a gene or mRNA encoding a detectable protein. In some embodiments, the presence of the label is used to track the location of the nucleic acid carrier.

In any of the embodiments described herein wherein a portion of one molecule is designed to have a nucleotide sequence that is complementary to the nucleotide sequence of another molecule, the complementarity need not be 100%. In some embodiments, the two nucleotide sequences are 100% complementary. A nucleotide sequence is “complementary” to

another nucleotide sequence when it is capable of base-pairing with the oligonucleotide according to the standard Watson-Crick, Hoogsteen or reverse Hoogsteen binding complementarity rules. In some embodiments, the terms "complementary" and "complement(s)" refer to an oligonucleotide or sequence thereof comprising a sequence of consecutive nucleotides or semi-consecutive nucleotides (e.g., one or more nucleotide moieties are not present in the molecule) capable of hybridizing to another nucleic acid molecule that may be consecutive, semi-consecutive or non-consecutive nucleotides even if less than all the nucleotides base pair with a counterpart nucleotide. In some embodiments, a "complementary" nucleic acid comprises a sequence in which about 70%, about 71%, about 72%, about 73%, about 74%, about 75%, about 76%, about 77%, about 77%, about 78%, about 79%, about 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, about 89%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or about 100%, and any range derivable therein, of the nucleotide sequence is capable of base-pairing with another nucleotide sequence during hybridization. In some embodiments, the term "complementary" refers to a nucleotide sequence that may hybridize to another nucleotide sequence under stringent conditions, as would be understood by one of ordinary skill in the art. In some embodiments, the nucleotide sequence may be "partly complementary" to another nucleotide sequence, and may hybridize in low stringency conditions, or contains a sequence in which less than about 70% of the nucleotide sequence is capable of base-pairing with another nucleotide sequence.

In some embodiments, the animal is a bird, reptile, amphibian, or mammal, which can be a mouse, rat, other rodent, rabbit, dog, cat, swine, cattle, sheep, horse, or primate, such as a human. In some embodiments, the animal is a human.

In some embodiments, the animal or mammal being treated is "in need thereof," which means that the animal or mammal (suitably a human) has been identified as having a need for the particular method of treatment. In some embodiments, the identification can be by any means of diagnosis. In any of the methods and treatments described herein, the animal or mammal (suitably a human) can be in need thereof.

In some embodiments, a "therapeutically effective amount" of the nucleic acid carrier is administered to the animal or mammal (suitably a human). A "therapeutically effective amount" means the amount that elicits the biological or medicinal response that is being sought in a tissue, system, animal, mammal, individual or human by a researcher, veterinarian, medical doctor or other clinician. The therapeutic effect is dependent upon the disorder being treated or the biological effect desired. As such, the therapeutic effect can be a decrease in the severity of

symptoms associated with the disorder and/or inhibition (partial or complete) of progression of the disorder, or improved treatment, healing, prevention or elimination of a disorder, or side-effects. The amount needed to elicit the therapeutic response can be determined based on the age, health, size and sex of the animal or mammal. Optimal amounts can also be determined based on monitoring of the animal's or mammal's response to treatment. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms; diminishment of extent of condition, disorder or disease; stabilized (i.e., not worsening) state of condition, disorder or disease; delay in onset or slowing of condition, disorder or disease progression; amelioration of the condition, disorder or disease state or remission (whether partial or total), whether detectable or undetectable; an amelioration of at least one measurable physical parameter, not necessarily discernible by the patient; or enhancement or improvement of condition, disorder or disease. Treatment includes eliciting a clinically significant response without excessive levels of side effects. Treatment also includes prolonging survival as compared to expected survival if not receiving treatment.

The precise dose to be employed in the compositions will also depend on the route of administration, and the seriousness of the disorder or disease, and should be decided according to the judgment of the practitioner and each animal's or mammal's circumstances. However, a suitable dosage range for oral administration is, generally, from about 0.001 milligram to about 200 milligrams per kilogram body weight, from about 0.01 milligram to about 100 milligrams per kilogram body weight, from about 0.01 milligram to about 70 milligrams per kilogram body weight, from about 0.1 milligram to about 50 milligrams per kilogram body weight, from 0.5 milligram to about 20 milligrams per kilogram body weight, or from about 1 milligram to about 10 milligrams per kilogram body weight. In some embodiments, the oral dose is about 5 milligrams per kilogram body weight. In some embodiments, a suitable dosage range for oral administration is, generally, from about 0.1 mg to about 500 mg, from about 1 mg to about 100 mg, from about 10 mg to about 50 mg, or from about 10 mg to about 25 mg.

In some embodiments, suitable dosage ranges for intravenous (i.v.) administration are from about 0.01 mg to about 500 mg per kg body weight, from about 0.1 mg to about 100 mg per kg body weight, from about 1 mg to about 50 mg per kg body weight, or from about 10 mg to about 35 mg per kg body weight. In some embodiments, a suitable dosage range for i.v. administration is, generally, from about 0.1 mg to about 500 mg, from about 1 mg to about 100 mg, from about 10 mg to about 50 mg, or from about 10 mg to about 25 mg. Suitable dosage ranges for other modes of administration can be calculated based on the forgoing dosages as known by those skilled in the art. For example, recommended dosages for intradermal,

intramuscular, intraperitoneal, subcutaneous, epidural, sublingual, intracerebral, intravaginal, transdermal administration or administration by inhalation are in the range of from about 0.001 mg to about 200 mg per kg of body weight, from about 0.01 mg to about 100 mg per kg of body weight, from about 0.1 mg to about 50 mg per kg of body weight, or from about 1 mg to about 20 mg per kg of body weight. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems. Such animal models and systems are well known in the art.

The nucleic acid carriers described herein can be formulated for parenteral administration by injection, such as by bolus injection or continuous infusion. The nucleic acid carriers can be administered by continuous infusion subcutaneously over a period of about 15 minutes to about 24 hours. Formulations for injection can be presented in unit dosage form, such as in ampoules or in multi-dose containers, with an added preservative. The compositions can take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. In some embodiments, the injectable is in the form of short-acting, depot, or implant and pellet forms injected subcutaneously or intramuscularly. In some embodiments, the parenteral dosage form is the form of a solution, suspension, emulsion, or dry powder.

For oral administration, the nucleic acid carriers described herein can be formulated by combining the nucleic acid carriers with pharmaceutically acceptable carriers well known in the art. Such carriers enable the compounds to be formulated as tablets, pills, dragees, capsules, emulsions, liquids, gels, syrups, caches, pellets, powders, granules, slurries, lozenges, aqueous or oily suspensions, and the like, for oral ingestion by an animal or mammal to be treated. Pharmaceutical preparations for oral use can be obtained by, for example, adding a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries, if desired, to obtain tablets or dragee cores. Suitable excipients include, but are not limited to, fillers such as sugars, including, but not limited to, lactose, sucrose, mannitol, and sorbitol; cellulose preparations such as, but not limited to, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethyl-cellulose, sodium carboxymethylcellulose, and polyvinylpyrrolidone (PVP). If desired, disintegrating agents can be added, such as, but not limited to, the cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

Orally administered compositions can contain one or more optional agents, for example, sweetening agents such as fructose, aspartame or saccharin; flavoring agents such as peppermint, oil of wintergreen, or cherry; coloring agents; and preserving agents, to provide a

pharmaceutically palatable preparation. Moreover, where in tablet or pill form, the compositions may be coated to delay disintegration and absorption in the gastrointestinal tract thereby providing a sustained action over an extended period of time. Oral compositions can include standard vehicles such as mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Such vehicles are suitably of pharmaceutical grade.

Dragee cores can be provided with suitable coatings. For this purpose, concentrated sugar solutions can be used, which can optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments can be added to the tablets or dragee coatings for identification or to characterize different combinations of active compound doses.

Pharmaceutical preparations which can be used orally include, but are not limited to, push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules can contain the active ingredients in admixture with filler such as lactose, binders such as starches, and/or lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active compounds can be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers can be added.

For buccal administration, the compositions can take the form of, such as, tablets or lozenges formulated in a conventional manner.

For administration by inhalation, the nucleic acid carriers described herein can be delivered in the form of an aerosol spray presentation from pressurized packs or a nebulizer, with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas. Capsules and cartridges of, such as gelatin for use in an inhaler or insufflator can be formulated containing a powder mix of the nucleic acid carriers and a suitable powder base such as lactose or starch.

The nucleic acid carriers described herein can also be formulated in rectal compositions such as suppositories or retention enemas, such as containing conventional suppository bases such as cocoa butter or other glycerides. The nucleic acid carriers described herein can also be formulated in vaginal compositions such as vaginal creams, suppositories, pessaries, vaginal rings, and intrauterine devices.

In transdermal administration, the nucleic acid carriers can be applied to a plaster, or can be applied by transdermal, therapeutic systems that are consequently supplied to the organism. In some embodiments, the nucleic acid carriers are present in creams, solutions,

powders, fluid emulsions, fluid suspensions, semi-solids, ointments, pastes, gels, jellies, and foams, or in patches containing any of the same.

The nucleic acid carriers described herein can also be formulated as a depot preparation. Such long acting formulations can be administered by implantation (for example subcutaneously or intramuscularly) or by intramuscular injection. Depot injections can be administered at about 1 to about 6 months or longer intervals. Thus, for example, the nucleic acid carriers can be formulated with suitable polymeric or hydrophobic materials (for example as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives, for example, as a sparingly soluble salt.

In some embodiments, the nucleic acid carriers can be delivered in a controlled release system. In some embodiments, a pump may be used. In another embodiment, polymeric materials can be used. In some embodiments, a controlled-release system can be placed in proximity of the target of the nucleic acid carriers described herein, such as the liver, thus requiring only a fraction of the systemic dose.

The nucleic acid carriers can be contained in such formulations with pharmaceutically acceptable diluents, fillers, disintegrants, binders, lubricants, surfactants, hydrophobic vehicles, water soluble vehicles, emulsifiers, buffers, humectants, moisturizers, solubilizers, preservatives and the like. The pharmaceutical compositions can also comprise suitable solid or gel phase carriers or excipients. Examples of such carriers or excipients include, but are not limited to, calcium carbonate, calcium phosphate, various sugars, starches, cellulose derivatives, gelatin, and polymers such as polyethylene glycols. In some embodiments, the compounds described herein can be used with agents including, but not limited to, topical analgesics (e.g., lidocaine), barrier devices (e.g., GelClair), or rinses (e.g., Caphosol).

In some embodiments, the nucleic acid carriers described herein can be delivered in a vesicle, in particular a liposome.

In some embodiments, the formulation can be lyophilized to a solid and reconstituted with, for example, water prior to use.

When administered to a human, the nucleic acid carriers can be in a sterile vehicle. Water is a suitable carrier when the compound is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical carriers also include excipients such as starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol

and the like. The present compositions, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents.

The compositions described herein can take the form of a solution, suspension, emulsion, tablet, pill, pellet, capsule, capsule containing a liquid, powder, sustained-release formulation, suppository, aerosol, spray, or any other form suitable for use.

The pharmaceutical compositions can be in unit dosage form. In such form, the composition can be divided into unit doses containing appropriate quantities of the nucleic acid carriers. The unit dosage form can be a packaged preparation, the package containing discrete quantities of the preparations, for example, packeted tablets, capsules, and powders in vials or ampules. The unit dosage form can also be a capsule, cachet, or tablet itself, or it can be the appropriate number of any of these packaged forms.

Suitable preservatives include, but are not limited to, mercury-containing substances such as phenylmercuric salts (e.g., phenylmercuric acetate, borate and nitrate) and thimerosal; stabilized chlorine dioxide; quaternary ammonium compounds such as benzalkonium chloride, cetyltrimethylammonium bromide and cetylpyridinium chloride; imidazolidinyl urea; parabens such as methylparaben, ethylparaben, propylparaben and butylparaben, and salts thereof; phenoxyethanol; chlorophenoxyethanol; phenoxypropanol; chlorobutanol; chlorocresol; phenylethyl alcohol; disodium EDTA; and sorbic acid and salts thereof.

Optionally one or more stabilizers can be included in the compositions to enhance chemical stability where required. Suitable stabilizers include, but are not limited to, chelating agents or complexing agents, such as, for example, the calcium complexing agent ethylene diamine tetraacetic acid (EDTA). For example, an appropriate amount of EDTA or a salt thereof, e.g., the disodium salt, can be included in the composition to complex excess calcium ions and prevent gel formation during storage. EDTA or a salt thereof can suitably be included in an amount of about 0.01% to about 0.5%. In those embodiments containing a preservative other than EDTA, the EDTA or a salt thereof, more particularly disodium EDTA, can be present in an amount of about 0.025% to about 0.1% by weight.

One or more antioxidants can also be included in the compositions. Suitable antioxidants include, but are not limited to, ascorbic acid, sodium metabisulfite, sodium bisulfite, acetylcysteine, polyquaternium-1, benzalkonium chloride, thimerosal, chlorobutanol, methyl paraben, propyl paraben, phenylethyl alcohol, edetate disodium, sorbic acid, a tocopherol, or a tocotrienol, or other agents know to those of skill in the art. Such preservatives are typically employed at a level of from about 0.001% to about 1.0% by weight.

In some embodiments, the nucleic acid carriers are solubilized at least in part by an acceptable solubilizing agent. Certain acceptable nonionic surfactants, for example polysorbate 80, can be useful as solubilizing agents, as can acceptable glycols, polyglycols, e.g., polyethylene glycol 400 (PEG-400), and glycol ethers. Suitable solubilizing agents for solution and solution/suspension compositions are cyclodextrins. Suitable cyclodextrins can be chosen from α-cyclodextrin, β-cyclodextrin, γ-cyclodextrin, alkylcyclodextrins (e.g., methyl-β-cyclodextrin, dimethyl-β-cyclodextrin, diethyl-β-cyclodextrin), hydroxyalkylcyclodextrins (e.g., hydroxyethyl-β-cyclodextrin, hydroxypropyl-β-cyclodextrin), carboxy-alkylcyclodextrins (e.g., carboxymethyl-β-cyclodextrin), sulfoalkylether cyclodextrins (e.g., sulfobutylether-β-cyclodextrin), and the like. An acceptable cyclodextrin can optionally be present in a composition at a concentration from about 1 to about 200 mg/ml, from about 5 to about 100 mg/ml, or from about 10 to about 50 mg/ml.

In some embodiments, the composition optionally contains a suspending agent. For example, in those embodiments in which the composition is an aqueous suspension or solution/suspension, the composition can contain one or more polymers as suspending agents. Useful polymers include, but are not limited to, water-soluble polymers such as cellulosic polymers, for example, hydroxypropyl methylcellulose, and water-insoluble polymers such as cross-linked carboxyl-containing polymers. However, in some embodiments, compositions do not contain substantial amounts of solid particulate matter, whether of the anti-microbial polymer or oligomer active agent, an excipient, or both, as solid particulate matter, if present, can cause discomfort and/or irritation of a treated eye.

One or more acceptable pH adjusting agents and/or buffering agents can be included in the compositions, including acids such as acetic, boric, citric, lactic, phosphoric and hydrochloric acids; bases such as sodium hydroxide, sodium phosphate, sodium borate, sodium citrate, sodium acetate, sodium lactate and tris-hydroxymethylaminomethane; and buffers such as citrate/dextrose, sodium bicarbonate and ammonium chloride. Such acids, bases and buffers are included in an amount required to maintain pH of the composition in an acceptable range.

Optionally one or more acceptable surfactants, preferably nonionic surfactants, or co-solvents can be included in the compositions to enhance solubility of the components of the compositions or to impart physical stability, or for other purposes. Suitable nonionic surfactants include, but are not limited to, polyoxyethylene fatty acid glycerides and vegetable oils, e.g., polyoxyethylene (60) hydrogenated castor oil; and polyoxyethylene alkylethers and alkylphenyl ethers, e.g., octoxynol 10, octoxynol 40; polysorbate 20, 60 and 80; polyoxyethylene / polyoxypropylene surfactants (e.g., Pluronic® F-68, F84 and P-103); cyclodextrin; or other

agents known to those of skill in the art. Typically, such co-solvents or surfactants are employed in the compositions at a level of from about 0.01% to about 2% by weight.

In some embodiments, pharmaceutical packs or kits comprising one or more containers filled with one or more of the nucleic acid carriers are provided herein. Optionally associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration for treating a condition, disease, or disorder described herein. In some embodiments, the kit contains more than one nucleic acid carrier described herein. In some embodiments, the kit comprises a nucleic acid carrier described herein and optionally another therapeutic agent. In some embodiments, the kit contains an injectable device such as a syringe with a needle.

The nucleic acid carriers described herein may have numerous benefits including, but not limited to, improved flexibility, improved efficacy, enhanced immunity, less toxicity, improved solubility of hydrophobic targeting agents and drugs, and improved half-life, circulation time, clearance, and PK profile.

The nucleic acid carriers described herein are highly modular in both design and construction as well as in final formulation when combining various targeting agents to achieve a desired outcome. Individual components, nucleic acid carriers scaffold of varying design, and individual targeting agents conjugated to linker sequences that bind to the arms of the nucleic acid carriers, can be separately prepared and characterized. The individually characterized components and targets can be mixed and matched at varying desired ratios prior to testing *in vitro* or *in vivo* using the linker oligonucleotides to couple particular components to the scaffold. Combining more than one of the same targeting agent alters both the specificity and avidity of the nucleic acid carrier formulation to the disease or cellular target. This ability to fine tune a nucleic acid carrier is an advantage over bi- or tri-specific antibodies or fusion proteins because bi- or tri-specific antibodies or fusion proteins are limited to only one or sometimes two valences for a particular target. Also, many of the bi- and tri-specific constructs, especially those used in immune oncology programs, have proven to be challenging to manufacture in clinically relevant quantities and a wide variety of formats have been used, including diabodies, tandem scFv, cross-linked antigen binding fragments (Fab) and quadroma-derived constructs. It would be more advantageous to use the original whole antibody clone to produce bi-, tri-, or multi-specific combinations rather than having to generate new genetic or fusion constructs and repeat the extensive characterization process. Coupling the original antibody clone to a linker

oligonucleotide to introduce the antibody clone into a nucleic acid carrier and proceeding into formulation is a quicker and less costly process.

The nucleic acid carriers described herein may possess improved efficacy for several reasons. The nucleic acid carriers have a higher target capacity for targeting and payload (drug delivery). Control of positioning of immune cells relative to disease cells in immune oncology applications to facilitate more efficient immune cell based responses is also an advantage. The nucleic acid carrier scaffold also has a diameter that can be modulated and adjusted to maximize both the cell to cell positioning, distance between cells, and also provide for more stable binding between partner cells. For immune oncology, the nucleic acid carriers may possess more efficient binding and recruitment of immune cells and targeting to the tumor because of the multivalence to both tumor and immune cells as compare to other bi- and tri-specific constructs that only have one or two binding sites for immune and disease cells. Nanoparticle-mediated targeting of adjuvants to lymph nodes in vaccination increases both efficacy and safety, both by blocking systemic distribution of the adjuvant and by permitting significant dose-sparing (up to 250-fold). In addition, targeted delivery of TLR ligands in nanoparticles to dendritic cells has also been shown to strongly enhance the potency of the delivery adjuvant.

The nucleic acid carriers described herein may also possess enhanced adaptive (humoral) immunity for vaccine development and immune oncology applications. The multivalent structure of the nucleic acid carriers lends adaptability to include immune cell targeting molecules, one or more antigens (the same or different), and one or more adjuvants (the same or different). For example combining mannose and TLR agonists as part of one multivalent nucleic acid carriers allows for synergistic enhancement of long term humoral immune responses. This trend should continue for the foreseeable future as many classes of new materials have yet to be studied in depth and may have great potential in this field. Examples include DNA nanostructures that can present complex, three-dimensional multivalent motifs to engage and organize immune cell receptors. The nucleic acid carriers can simultaneously target specific antigen presenting cells (such as dendritic cells) while delivering one or more of the same or different adjuvants, and antigens. The nucleic acid carriers possess active targeting through conjugation of ligands for cell-surface receptors, which can often increase the uptake of nanoparticles by phagocytic cells relative to non-targeted particles, making macrophages and monocytes suitable targets for the nucleic acid carriers to deliver immunoregulatory drugs. Numerous receptors allow macrophage-specific targeting, including the Fc receptor, scavenger receptors, and mannose receptors (MMR or CD206). Targeting the macrophage Fc receptor is achieved by coating nanoparticles with IgG, which accelerates uptake of particles and enhances

their retention within macrophages. The macrophage mannose receptor (MMR) is expressed on mature macrophages and dendritic cells but not on monocytes in the blood circulation. By combining both of these targeting strategies onto one nucleic acid carrier, particle enhanced presentation of antigens and amplified immune activation properties can be achieved. The nucleic acid carriers may also possess improved delivery and presentation of MHC I antigens into the cytosol. In addition, the flexibly of the nucleic acid carrier to be easily adapted into different sizes as well as flexibly of the scaffold materials to bend, compress and change positioning allows for a more efficient uptake by the lymphatic system. This feature has been consistently linked to improved performance for immune response and reduction of system cytokine associated toxicity.

The nucleic acid carriers described herein may also possess less toxicity compared to the same targeting agents in the absence of the nucleic acid carriers. By sequestering potentially toxic materials (drugs, antibodies and other molecules) as part of the formulation until it reaches the destination, the nucleic acid carriers may prevent off target accumulation and side effects. These materials can include the targeting antibodies or portions thereof, antigens of cytotoxic drugs, or molecules to be delivered at the same time as the nanoparticle with all targeting molecules. Also, the change in biodistribution of loaded cargo (i.e., drugs) and targeting antibodies, drugs as a function of the unique properties of the nucleic acid carriers (charge, size, shape, targeting molecule density, etc.) may limit the off target exposure as well as result in a different, less toxic clearance of the cargo not reaching the disease target.

The nucleic acid carriers described herein may also possess improved solubility of hydrophobic targeting agents and drugs. Many classes of drugs, small molecules, and biologics have been linked to poor solubility when delivered as singular compounds, often reducing their circulation time or ability to reach a desired target. However, the nucleic acid carrier scaffold is highly negatively charged and once a drug is linked, it improves the ability to deliver the drug to target destination in an aqueous environment. The chemistry, composition, and negative charge of the nucleic acid carrier scaffold may reconfigure the biologically presented properties of targeting agents and drugs once attached.

The nucleic acid carriers described herein may also possess improved half-life, circulation time, clearance, and PK profile. Once attached to the nucleic acid carrier scaffold, small drugs can be redirected away from the normally observed kidney clearance, resulting in an increases in the overall circulation half-life and an increase in the PK of the drug. For example, Doxorubicin, a small drug less than 1 kDa molecular weight, is cleared through glomerular

filtration in the kidney and has a half-life measured in minutes. When attached to a nucleic acid carrier, however, the new half-life is measured in hours.

The nucleic acid carriers described herein may also possess the improved ability to transport drug cargo across the blood-brain barrier. Thus, the nucleic acid carriers described herein may be used as a delivery vehicle for active ingredients that are normally not transported across the blood-brain barrier. Accordingly, various types of diseases and disorders associated with the brain may be treated by delivery of drugs across the blood-brain barrier inlcuding, for example, neurological disorders and cancers of the brain (i.e., glioblastoma, and medulloblastoma).

The following representative embodiments are presented:

Embodiment 1. A single layer nucleic acid carrier comprising five monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to a terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to a terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to a terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to a terminal arm of the fifth monomer, forming a single layer nucleic acid carrier having twelve peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

Embodiment 2. The nucleic acid carrier of embodiment 1, wherein the twelve peripheral terminal arms comprise from 1 to 12 different nucleotide sequences.

Embodiment 3. The nucleic acid carrier of embodiment 1, wherein the twelve peripheral terminal arms comprise from 1 to 6 different nucleotide sequences.

Embodiment 4. The nucleic acid carrier of embodiment 1, wherein the twelve peripheral terminal arms comprise from 1 to 4 different nucleotide sequences.

Embodiment 5. The nucleic acid carrier of embodiment 1, wherein the twelve peripheral terminal arms comprise from 1 to 3 different nucleotide sequences.

Embodiment 6. The nucleic acid carrier of any one of embodiments 1 to 5 comprising from 1 to 12, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms.

Embodiment 7. The nucleic acid carrier of any one of embodiments 1 to 5 comprising from 1 to 4, 1 to 3, or 2 different targeting agents conjugated to the peripheral terminal arms.

Embodiment 8. A 1.5 layer nucleic acid carrier comprising eleven monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, and the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, forming a 1.5 layer nucleic acid carrier having 24 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

Embodiment 9. The nucleic acid carrier of embodiment 8, wherein the 24 peripheral terminal arms comprise from 1 to 24 different nucleotide sequences.

Embodiment 10. The nucleic acid carrier of embodiment 8, wherein the 24 peripheral terminal arms comprise from 1 to 6 different nucleotide sequences.

Embodiment 11. The nucleic acid carrier of embodiment 8, wherein the 24 peripheral terminal arms comprise from 1 to 4 different nucleotide sequences.

Embodiment 12. The nucleic acid carrier of embodiment 8, wherein the 24 peripheral terminal arms comprise from 1 to 3 different nucleotide sequences.

Embodiment 13. The nucleic acid carrier of any one of embodiments 8 to 12 comprising from 1 to 24, 1 to 18, 1 to 12, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms.

Embodiment 14. The nucleic acid carrier of any one of embodiments 8 to 12 comprising from 1 to 4, 1 to 3, or 2 different targeting agents conjugated to the peripheral terminal arms.

Embodiment 15. A two layer nucleic acid carrier comprising 17 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is

conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer, forming a two layer nucleic acid carrier having 36 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

Embodiment 16. The nucleic acid carrier of embodiment 15, wherein the 36 peripheral terminal arms comprise from 1 to 36 different nucleotide sequences.

Embodiment 17. The nucleic acid carrier of embodiment 15, wherein the 36 peripheral terminal arms comprise from 1 to 6 different nucleotide sequences.

Embodiment 18. The nucleic acid carrier of embodiment 15, wherein the 36 peripheral terminal arms comprise from 1 to 4 different nucleotide sequences.

Embodiment 19. The nucleic acid carrier of embodiment 15, wherein the 36 peripheral terminal arms comprise from 1 to 3 different nucleotide sequences.

Embodiment 20. The nucleic acid carrier of any one of embodiments 15 to 19 comprising from 1 to 36, 1 to 30, 1 to 24, 1 to 18, 1 to 12, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms.

Embodiment 21. The nucleic acid carrier of any one of embodiments 15 to 19 comprising from 1 to 4, 1 to 3, or 2 different targeting agents conjugated to the peripheral terminal arms.

Embodiment 22. A 2.5 layer nucleic acid carrier comprising 35 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is

conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer;

wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer,

wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, and the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer;

forming a 2.5 layer nucleic acid carrier having 72 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

Embodiment 23. The nucleic acid carrier of embodiment 22, wherein the 72 peripheral terminal arms comprise from 1 to 72 different nucleotide sequences.

Embodiment 24. The nucleic acid carrier of embodiment 22, wherein the 72 peripheral terminal arms comprise from 1 to 6 different nucleotide sequences.

Embodiment 25. The nucleic acid carrier of embodiment 22, wherein the 72 peripheral terminal arms comprise from 1 to 4 different nucleotide sequences.

Embodiment 26. The nucleic acid carrier of embodiment 22, wherein the 72 peripheral terminal arms comprise from 1 to 3 different nucleotide sequences.

Embodiment 27. The nucleic acid carrier of any one of embodiments 22 to 26 comprising from 1 to 72, 1 to 66, 1 to 60, 1 to 54, 1 to 48, 1 to 36, 1 to 30, 1 to 24, 1 to 18, 1 to 12, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms.

Embodiment 28. The nucleic acid carrier of any one of embodiments 22 to 26 comprising from 1 to 4, 1 to 3, or 2 different targeting agents conjugated to the peripheral terminal arms.

Embodiment 29. A three layer nucleic acid carrier comprising 53 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer;

wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and

the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer,

wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer, the third terminal arm of the twelfth monomer is conjugated to the first terminal arm of the thirty-sixth monomer, the third terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the thirty-seventh monomer, the third terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the thirty-eighth monomer, the third terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the thirty-ninth monomer, the third terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fortieth monomer, and the third terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the forty-first monomer;

wherein the fourth terminal arm of the sixth monomer is conjugated to the first terminal arm of the forty-second monomer, the fourth terminal arm of the seventh monomer is conjugated to the first terminal arm of the forty-third monomer, the fourth terminal arm of the eighth monomer is conjugated to the first terminal arm of the forty-forth monomer, the fourth terminal arm of the ninth monomer is conjugated to the first terminal arm of the forty-fifth monomer, the fourth terminal arm of the tenth monomer is conjugated to the first terminal arm of the forty-sixth monomer, the fourth terminal arm of the eleventh monomer is conjugated to the first terminal arm of the forty-seventh monomer, the fourth terminal arm of the twelfth monomer is conjugated to the first terminal arm of the forty-eighth monomer, the fourth terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the forty-ninth monomer, the fourth terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the fiftieth monomer, the fourth terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the fifty-first monomer, the fourth terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fifty-second monomer, and the fourth terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the fifty-third monomer;

forming a three layer nucleic acid carrier having 108 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

Embodiment 30. The nucleic acid carrier of embodiment 29, wherein the 108 peripheral terminal arms comprise from 1 to 108 different nucleotide sequences.

Embodiment 31. The nucleic acid carrier of embodiment 29, wherein the 108 peripheral terminal arms comprise from 1 to 6 different nucleotide sequences.

Embodiment 32. The nucleic acid carrier of embodiment 29, wherein the 108 peripheral terminal arms comprise from 1 to 4 different nucleotide sequences.

Embodiment 33. The nucleic acid carrier of embodiment 29, wherein the 108 peripheral terminal arms comprise from 1 to 3 different nucleotide sequences.

Embodiment 34. The nucleic acid carrier of any one of embodiments 29 to 33 comprising from 1 to 108, 1 to 102, 1 to 96, 1 to 90, 1 to 84, 1 to 78, 1 to 72, 1 to 66, 1 to 60, 1 to 54, 1 to 48, 1 to 36, 1 to 30, 1 to 24, 1 to 18, 1 to 12, 1 to 8, 1 to 6, 1 to 4, 1 to 3, 2, or 1 targeting agent conjugated to the peripheral terminal arms.

Embodiment 35. The nucleic acid carrier of any one of embodiments 29 to 33 comprising from 1 to 4, 1 to 3, or 2 different targeting agents conjugated to the peripheral terminal arms.

Embodiment 36. The nucleic acid carrier of any one of embodiments 1 to 35, wherein the nucleic acid is deoxyribonucleic acid (DNA), ribonucleic acid (RNA), modified DNA or RNA, or a combination thereof.

Embodiment 37. The nucleic acid carrier of any one of embodiments 1 to 36, wherein the double-stranded region is 4 to 2000 bases in length.

Embodiment 38. The nucleic acid carrier of embodiment 37, wherein the double-stranded region is 5 to 200 bases in length.

Embodiment 39. The nucleic acid carrier of embodiment 38, wherein the double-stranded region is 25 to 50 bases in length.

Embodiment 40. The nucleic acid carrier of any one of embodiments 1 to 39, wherein each terminal arm is 4 to 200 bases in length.

Embodiment 41. The nucleic acid carrier of embodiment 40, wherein each terminal arm is 5 to 50 bases in length.

Embodiment 42. The nucleic acid carrier of embodiment 41, wherein each terminal arm is 25 to 50 bases in length.

Embodiment 43. The nucleic acid carrier of embodiment 42, wherein each terminal arm is 16 to 50 bases in length.

Embodiment 44. The nucleic acid carrier of any one of embodiments 1 to 43, wherein the at least one targeting agent is conjugated to the at least one terminal arm by an oligonucleotide linker.

Embodiment 45. The nucleic acid carrier of any one of embodiments 1 to 44, wherein one or more terminal arms not conjugated to a targeting agent is complementary base paired to an oligonucleotide linker.

Embodiment 46. The nucleic acid carrier of any one of embodiments 1 to 45, wherein at least one monomer is cross-linked to another monomer by a cross-linking agent.

Embodiment 47. The nucleic acid carrier of embodiment 46, wherein the cross-linking agent is mitomycin C, daunamycin, ethidium diazide, cisplatin, an EDC-type compound, or a psoralen.

Embodiment 48. The nucleic acid carrier of embodiment 47, wherein the psoralen is 8-methoxy psoralen, 4,5′,8-trimethylpsoralen, 4′-hydroxymethyl-4,5′,trimethyl psoralen, 4′-methoxymethyl-4,5′,8-trimethyl psoralen, 4′N-phthalimidomethyl-4,5′,8-trimethyl psoralen, or 4′-aminomethyl-4,5′-8-trimethyl psoralen hydrochloride.

Embodiment 49. The nucleic acid carrier of any one of embodiments 1 to 48, wherein the at least one targeting agent is an antigen, a peptide, an antibody or fragment thereof, an antibody-drug conjugate, a non-antibody scaffold, a label, an adjuvant, an RNA molecule, a DNA molecule, a vitamin, a protein, a fusion protein, a fusion peptide, a carbohydrate, a lipid, a polysaccharide, a lipopolysaccharide, a polymer, a virus particle, or a virus-like particle, or any combination thereof.

Embodiment 50. The nucleic acid carrier of embodiment 49, wherein the polymer is hyaluronic acid, polyargenine, polylysine, polyethylenimine (PEI), polyethyleglycol (PEG), polyglycolic acid (PGA), polylactic acid (PLA), or poly(lactic-co-glycolic acid) (PLGA).

Embodiment 51. The nucleic acid carrier of embodiment 49, wherein the non-antibody scaffold is an affibody, an affilin, an anticalin, an atrimer, an avimer, a bicyclic peptide, a cys-knot, a DARPin, an FN3, a fynomer, a kunitz domain, or an O-body.

Embodiment 52. The nucleic acid carrier of embodiment 49, wherein the antigen is a bacterial antigen, a viral antigen, a fungal antigen, a yeast antigen, a protozoan antigen, or prion.

Embodiment 53. The nucleic acid carrier of embodiment 52, wherein the antigen is from *Acetobacter aurantius*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Actinomyces israelii*,

Actinomyces viscosus, *Aggregatibacter actinomycetemcomitans*, *Agrobacterium radiobacter*, *Agrobacterium tumefaciens*, *Azorhizobium caulinodans*, *Azotobacter vinelandii*, *Anaplasma phagocytophilum*, *Bacillus anthracis*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus fusiformis*, *Bacillus licheniformis*, *Bacillus megaterium*, *Bacillus mycoides*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacteroides gingivalis*, *Bacteroides fragilis*, *Bacteroides melaninogenicus* (*Prevotella melaninogenica*), *Bartonella henselae*, *Bartonella quintana*, *Bordetella bronchiseptica*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia cepacia*, *Calymmatobacterium granulomatis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter pylori*, *Chlamydia trachomatis*, *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*), *Chlamydophila psittaci* (*Chlamydia psittaci*), *Citrobacter freundii*, *Citrobacter diverus*, *Citrobacter koseri*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens* (*Clostridium welchii*), *Clostridium tetani*, *Corynebacterium diphtheria*, *Corynebacterium fusiforme*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter faecalis*, *Enterococcus avium*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecium*, *Enterococcus galllinarum*, *Enterococcus maloratus*, *Eschericia coli*, *Francisella tularensis*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Haemophilus parainfluenzae*, *Haemophilus pertussis*, *Haemophilus vaginalis*, *Haemophilus influenza*, *Haemophilus aegyptius*, *Helicobacter pylori*, *Klebsiella pneumonia*, *Klebsiella oxytoca*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactococcus lactis*, *Legionella pneumophila*, *Listeria monocytogenes*, *Methanobacterium extroquens*, *Microbacterium multiforme*, *Micrococcus luteus*, *Moraxella lacunata*, *Moraxella catarrhalis*, *Morganella morganii*, *Mycobacterium avium*, *Mycobacterium africanum*, *Mycobacterium bovis*, *Mycobacterium canetti*, *Mycobacterium diphtheria*, *Mycobacterium intracellulare*, *Mycobacterium leprae*, *Mycobacterium lepraemurium*, *Mycobacterium microti*, *Mycobacterium phlei*, *Mycobacterium smegmatis*, *Mycobacterium tuberculosis*, *Mycoplasma fermentans*, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma penetrans*, *Mycoplasma pneumonia*, *Neisseria gonorrhoeae*, *Neisseria meningitides*, *Pasteurella multocida*, *Pasteurella tularensis*, *Porphyromonas gingivalis*, *Prevotella melaninogenica* (*Bacteroides melaninogenicus*), *Propionibacterium acnes*, *Proteus mirabillis*, *Proteus vulgaris*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Pseudomonas otitidis*, *Rhizobium radiobacter*, *Rickettsia prowazekii*, *Rickettsia psittaci*, *Rickettsia quintana*, *Rickettsia rickettsia*, *Rickettsia trachomae*, *Rochalimaea henselae*, *Rochalimaea Quintana*, *Rothia dentocariosa*, *Salmonella enteritidis*, *Salmonella typhi*, *Salmonella typhimurium*, *Serratia*

marcescens, *Shigella dysenteriae, Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus colmii*, *Staphylococcus sciuri*, *Staphylococcus warneri*, *Stenotrophomonas maltophilia, Streptococcus agalactiae, Streptococcus anginosus*, *Streptococcus avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faceium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis. Streptococcus mitior, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae*, *Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus, Streptococcus viridans, Treponema pallidum, Treponema denticola, Vibrio cholera, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus, Wolbachia, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus clavatus*, *Aspergillus niger*, *Aspergillus terreus*, *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida krusei*, *Candida dubliniensis*, *Candida parapsilosis*, *Fusarium solani*, *Fusarium moniliforme*, *Fusarium proliferartum*, *Malessezia pachydermatis*, *Chrysosporium parvum*, *Metarhizium anisopliae*, *Phaeoisaria clematidis*, or *Sarcopodium oculorum.*

Embodiment 54. The nucleic acid carrier of embodiment 52, wherein the antigen is from *Mycobacterium tuberculosis*, Varicella zoster virus, *Corynebacterium diphtheria*, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, *Haemophilis influenza*, Human Papillomavirus, Influenza virus, Japanese Encephalitis virus, Measles virus, *Neisseria meningitidis*, Mumps virus, *Bordetella pertussis*, *Streptococcus pneumonia*, Poliovirus, Rabies virus, Rotavirus, Rubella virus, Herpes Zoster virus, *Clostridium tetani*, *Salmonella typhi*, Yellow Fever virus, Ebola virus, avian flu virus, *Bacillus anthracis*, Smallpox virus, or Zika virus.

Embodiment 55. The nucleic acid carrier of embodiment 49, wherein the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc.

Embodiment 56. The nucleic acid carrier of embodiment 49, wherein the antibody, or fragment thereof, is directed to a disease-associated antigen.

Embodiment 57. The nucleic acid carrier of embodiment 56, wherein the disease-associated antigen is a tumor-associated antigen.

Embodiment 58. The nucleic acid carrier of embodiment 57, wherein the tumor-associated antigen is 4-1BB, 5AC, 5T4, A2aR, activin receptor-like kinase 1, AGS-22M6, AKAP4, alpha-fetoprotein, angiopoietin 2, B7-H3, BAFF, BAGE, BCR-ABL, BORIS, CA-125, CA19-9, C242 antigen, carbonic anhydrase 9 (CA-IX), CCR4, CD19, CD20, CD22, CD23 (IgE

receptor), CD24, CD28, CD30 (TNFRSF8), CD33, CD37, CD38 (cyclic ADP ribose hydrolase), CD40, CD44 v6, CD51, CD56, CD70, CD71, CD73, CD74, CD79B, CD80, CD137, CD140a, CD152, CD200, CD221, CD274, CEA, ch4D5, CLDN18.2, CS1, CSF1R, CTLA-4, C-X-C chemokine receptor type 4, DLL4, DR5, EBAG9, EGF, EGFR, EGFL7, EpCAM, ERBB2, ERBB3, FAP, fibronectin extra domain-B, folate receptor 1, folate receptor alpha, folate hydrolase, Frizzled receptor, GAGE, GD2 ganglioside, GD3 ganglioside, glioma, glypican 3, GPNMB, gp100, GUCY2C, HER1, HER2/neu, HER3, HGF, HHGFR, histone complex, HLA-DR, human scatter factor receptor kinase, HPV-16, HSP105, IDH1, IDO1, IGF-I, IGF-1 receptor, ILGF2, IL-6, IL-13, integrin αvβ3, integrin α5β1, KIR, LAG-3, Lewis-Y antigen, LY6K, MAGE-1, MAGE-A3, MAGE-C2, MAGE-D4, MAPG, MART-1, Melan-A, MET, MCP-1, mesothelin, MIF, MSLN, MS4A1, mucin CanAg, MUC1, MUC4, MUC16, NG2, N-glycolylneuraminic acid, Notch receptor PD-1, NY-ESO-1, OCAA, PAP, PDGF-R α, PDCD1, PD1, PD-L1, phosphate-sodium co-transporter, phosphatidylserine, PRAME, PSA, RANKL, RON, ROR1, SDC1, Sialyl-Tn, SLAMF7, SPAG-9, SSX1, STEAP1, survivin, TAG-72, telomerase, TEM1, tenascin C, TGF-β, TIM-3, TLR, TAM, TIM-3, TRAIL-R2, TRAIL-R1, TWEAK receptor, tumor specific glycosylation of MUC1, tumor-associated calcium signal transducer 2, tumor antigen CTAA16.88, TYRP1 (glycoprotein 75), VEGF-A, VEGFR2, VEGFR-1, vimentin, VISTA, WT1, or XAGE-1b.

Embodiment 59. The nucleic acid carrier of embodiment 57, wherein the tumor-associated antigen is HER-2, EGFR, CD30, CD20, EpCAM, NG2, CD19, CEA, MUC-1, CA19-9, OCAA, MAPG, TAM, TLR, CD71, ERBB2, VEGF, or glioma.

Embodiment 60. The nucleic acid carrier of embodiment 56, wherein the disease-associated antigen is 1-40-β-amyloid , AOC3 (VAP-1), ACVR2B, angiopoietin 3, beta-amyloid, C5, CCL11 (eotaxin-1), CCR5, CD2, CD3, CD4, CD5, CD11, CD18, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD28, CD41 (integrin alpha-IIb), CD52, CD125, CD147 (basigin), CD154 (CD40L), CEA-related antigen, clumping factor A, endotoxin, GMCSF receptor α-chain, growth differentiation factor 8, hemagglutinin, HNGF, Hsp90, IGHE, IgE Fc region, IL-1β, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL-6 receptor, integrin α4β7, integrin α7β7, integrin α4, integrin αIIbβ3, interferon α/β receptor, interferon gamma-induced protein, IFN-γ, IFN-α, ITGB2 (CD18), LFA-1 (CD11a), LINGO-1, lipoteichoic acid, LOXL2, myelin-associated glycoprotein, myostatin, neural apoptosis-regulated proteinase 1, NGF, NOGO-A, Oryctolagus cuniculus, OX-40, PCSK9, phosphatidylserine, platelet-derived growth factor receptor beta, RANKL, Rhesus factor, sclerostin, SOST, sphingosine-1-phosphate, TFPI, TGF-β, TGF beta 2, TGF beta 1, TNF-α, VEGF-A, or VWF.

Embodiment 61. The nucleic acid carrier of embodiment 49, wherein the antibody, or fragment thereof, is directed to a cell surface marker.

Embodiment 62. The nucleic acid carrier of embodiment 61, wherein the cell surface marker is on an immune cell.

Embodiment 63. The nucleic acid carrier of embodiment 62, wherein the immune cell is a T cell, B cell, NK cell, macrophage, TIL, dendritic cell, neutrophil, eosinophil, basophil, or mast cell.

Embodiment 64. The nucleic acid carrier of embodiment 63, wherein the T cell is a $CD4^+$ T cell, $CD8^+$ T cell, helper T cell, cytotoxic T cell, or natural killer T cell; and wherein the B cell is a memory B cell or a plasma cell.

Embodiment 65. The nucleic acid carrier of embodiment 61, wherein the cell surface marker is CD36, CD68, CD83, CD180, CD206 (MRC1- mannose receptor), F4/80 (EGF-TM7 family), CD205, CD56, CD161, CD94:NKG2 heterodimer, KIR/CD158 family, CD335, CD64, CD16, CD3, CD28, CD4, CD25, CD39, Toll-like receptors (TLRs), CD281, CD283, CD284, CD286, CD289, CD282, C-type lectin receptors (CLRs), Fc Receptor, or HSG.

Embodiment 66. The nucleic acid carrier of embodiment 49, comprising one or two of the following:

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to CD64;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1;

a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX40;

a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG;

a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG;

a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is folic acid or folic acid receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16; and

a first targeting agent which is an antibody, or fragment thereof, directed to PD-1 and a second targeting agent which is an antibody, or fragment thereof, directed to PDL-1 or PDL-2.

Embodiment 67. The nucleic acid carrier of embodiment 49, wherein the label is a chemiluminescent agent, a fluorescent agent, an infrared dye, a radiolabel agent, a metal, a chelating agent, a hapten, or a gene or mRNA encoding a detectable protein.

Embodiment 68. The nucleic acid carrier of embodiment 49, wherein the adjuvant is one or more of a CpG containing oligonucleotide, a ssRNA, a dsRNA, a monophosphate lipid, aluminum, squalene, 3-deacyl-monophosphoryl lipid A, vitamin E, surfactant polysorbate 80, mannide-mono-oleate, SLAG-3, or a galactosylceramide.

Embodiment 69. The nucleic acid carrier of embodiment 68, wherein the CpG containing oligonucleotide is ODN 1585, ODN 1668, ODN 1826, ODN 2006, ODN 2007, ODN 2216, ODN 2336, ODN 2395, ODN M362, AT-ODN-1, AT-ODN-2, ODNBW006, ODN 2088, ODN 4084, ODN INH-1, ODN INH-47, ODN TTAGGG, or G-ODN.

Embodiment 70. The nucleic acid carrier of embodiment 49, wherein the RNA molecule is siRNA, miRNA, mRNA, snRNA, dsRNA, ncRNA, snoRNA, or an aptamer.

Embodiment 71. The nucleic acid carrier of any one of embodiments 1 to 48, wherein the targeting agent is an antigen, an antibody, or a fragment of an antibody, and the oligonucleotide linker conjugating the targeting moiety to the at least one single-stranded arm is a CpG containing oligonucleotide.

Embodiment 72. The nucleic acid carrier of any one of embodiments 1 to 48, wherein the nucleic acid carrier comprises at least one antigen, at least one antibody or fragment thereof directed to an immune cell, and at least one adjuvant.

Embodiment 73. The nucleic acid carrier of any one of embodiments 1 to 72, further comprising a chemotherapeutic agent, a cytokine, or a chemokine.

Embodiment 74. The nucleic acid carrier of embodiment 73, wherein the chemotherapeutic agent is methotrexate, taxol, mercaptopurine, thioguanine, hydroxyurea, cytarabine, cyclophosphamide, ifosfamide, nitrosoureas, cisplatin, carboplatin, mitomycin, dacarbazine, procarbizine, etoposides, campathecins, bleomycin, doxorubicin, idarubicin, daunorubicin, dactinomycin, plicamycin, mitoxantrone, asparaginase, vinblastine, vincristine, vinorelbine, paclitaxel, or docetaxel.

Embodiment 75. The nucleic acid carrier of embodiment 73, wherein the cytokine is an IL-1-like cytokine, a common γ chain cytokine, a common β chain cytokine, an IL-6-like cytokine, an IL-10-like cytokine, an interferon, a tumor necrosis factor, or a member of the TGF-β family.

Embodiment 76. The nucleic acid carrier of embodiment 75, wherein the IL-1-like cytokine is IL-1α, IL-1β, IL-1 receptor antagonist (IL-1RA), or IL-18.

Embodiment 77. The nucleic acid carrier of embodiment 75, wherein the common γ chain cytokine is IL-2, IL-4, IL-7, IL-9, IL-13, or IL-15.

Embodiment 78. The nucleic acid carrier of embodiment 75, wherein the common β chain cytokine is IL-3, IL-5, or GM-CSF.

Embodiment 79. The nucleic acid carrier of embodiment 75, wherein the IL-6-like cytokine is IL-6, IL-11, leukemia inhibitory factor (LIF), oncostatin M (OSM), granulocyte colony-stimulating factor (G-CSF), or IL-12.

Embodiment 80. The nucleic acid carrier of embodiment 75, wherein the IL-10-like cytokine is IL-10, IL-19, or IL-20.

Embodiment 81. The nucleic acid carrier of embodiment 75, wherein the interferon is IFN-α, IFN-β, or IFN-γ.

Embodiment 82. The nucleic acid carrier of embodiment 75, wherein the tumor necrosis factor is TNF-α, TNF-β, lymphotoxin (LT)-β, or FasL.

Embodiment 83. The nucleic acid carrier of embodiment 75, wherein the member of the TGF-β family is TGF-β1, TGF-β2, or TGF-β3.

Embodiment 84. The nucleic acid carrier of embodiment 73, wherein the chemokine is a C group chemokine, a CC group chemokine, a CXC group chemokines, or a CX3C chemokine.

Embodiment 85. The nucleic acid carrier of embodiment 84, wherein the C group chemokine is lymphotactin/XCL1 or SCM-1β/XCL2.

Embodiment 86. The nucleic acid carrier of embodiment 84, wherein the CC group chemokine is monocyte chemotactic protein (MCP)-1/CCL2, macrophage inflammatory protein (MIP)-1a/CCL3, MIP-1β/CCL4, or eotaxin/CCL11.

Embodiment 87. The nucleic acid carrier of embodiment 84, wherein the CXC group chemokine is IL-8/CXCL8 (ELR), monokine-induced by IFN-γ (MIG)/CXCL9 (nonELR), IFN-γ inducible protein-10 (IP-10)/CXCL10 (nonELR), or stromal cell-derived factor-1 (SDF-1)/CXCL12 (nonELR).

Embodiment 88. The nucleic acid carrier of embodiment 84, wherein the CX3C chemokine is fractalkine/CX3CL1.

Embodiment 89. A pharmaceutical composition comprising a nucleic acid carrier of any one of embodiments 1 to 88, and a pharmaceutically acceptable vehicle.

Embodiment 90. A method of inducing an immune response in a mammal comprising administering to the mammal a nucleic acid carrier of any one of embodiments 1 to 88, or a pharmaceutical composition comprising a nucleic acid carrier of any one of embodiments 1 to 88, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antigen, a peptide, an antibody or fragment thereof, and an adjuvant.

Embodiment 91. The method of embodiment 90, wherein the antigen is from *Acetobacter aurantius*, *Acinetobacter baumannii*, *Acinetobacter calcoaceticus*, *Acinetobacter haemolyticus*, *Acinetobacter junii*, *Acinetobacter lwoffii*, *Actinomyces israelii*, *Actinomyces viscosus*, *Aggregatibacter actinomycetemcomitans*, *Agrobacterium radiobacter*, *Agrobacterium tumefaciens*, *Azorhizobium caulinodans*, *Azotobacter vinelandii*, *Anaplasma phagocytophilum*, *Bacillus anthracis*, *Bacillus brevis*, *Bacillus cereus*, *Bacillus fusiformis*, *Bacillus licheniformis*,

Bacillus megaterium, *Bacillus mycoides*, *Bacillus stearothermophilus*, *Bacillus subtilis*, *Bacteroides gingivalis*, *Bacteroides fragilis*, *Bacteroides melaninogenicus* (*Prevotella melaninogenica*), *Bartonella henselae*, *Bartonella quintana*, *Bordetella bronchiseptica*, *Bordetella pertussis*, *Borrelia burgdorferi*, *Brucella abortus*, *Brucella melitensis*, *Brucella suis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Burkholderia cepacia*, *Calymmatobacterium granulomatis*, *Campylobacter coli*, *Campylobacter fetus*, *Campylobacter jejuni*, *Campylobacter pylori*, *Chlamydia trachomatis*, *Chlamydophila pneumoniae* (*Chlamydia pneumoniae*), *Chlamydophila psittaci* (*Chlamydia psittaci*), *Citrobacter freundii*, *Citrobacter diverus*, *Citrobacter koseri*, *Clostridium botulinum*, *Clostridium difficile*, *Clostridium perfringens* (*Clostridium welchii*), *Clostridium tetani*, *Corynebacterium diphtheria*, *Corynebacterium fusiforme*, *Coxiella burnetii*, *Ehrlichia chaffeensis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Enterobacter faecalis*, *Enterococcus avium*, *Enterococcus durans*, *Enterococcus faecalis, Enterococcus faecium*, *Enterococcus galllinarum*, *Enterococcus maloratus*, *Eschericia coli, Francisella tularensis*, *Fusobacterium nucleatum*, *Gardnerella vaginalis*, *Haemophilus ducreyi*, *Haemophilus parainfluenzae*, *Haemophilus pertussis*, *Haemophilus vaginalis*, *Haemophilus influenza, Haemophilus aegyptius*, *Helicobacter pylori, Klebsiella pneumonia*, *Klebsiella oxytoca*, *Lactobacillus acidophilus, Lactobacillus bulgaricus, Lactobacillus casei, Lactococcus lactis, Legionella pneumophila, Listeria monocytogenes, Methanobacterium extroquens, Microbacterium multiforme, Micrococcus luteus, Moraxella lacunata, Moraxella catarrhalis*, *Morganella morganii*, *Mycobacterium avium, Mycobacterium africanum*, *Mycobacterium bovis, Mycobacterium canetti*, *Mycobacterium diphtheria, Mycobacterium intracellulare, Mycobacterium leprae, Mycobacterium lepraemurium, Mycobacterium microti, Mycobacterium phlei, Mycobacterium smegmatis, Mycobacterium tuberculosis, Mycoplasma fermentans, Mycoplasma genitalium, Mycoplasma hominis, Mycoplasma penetrans, Mycoplasma pneumonia, Neisseria gonorrhoeae, Neisseria meningitides, Pasteurella multocida, Pasteurella tularensis, Porphyromonas gingivalis, Prevotella melaninogenica (Bacteroides melaninogenicus), Propionibacterium acnes*, *Proteus mirabillis, Proteus vulgaris*, *Providencia stuartii*, *Pseudomonas aeruginosa*, *Pseudomonas otitidis*, *Rhizobium radiobacter, Rickettsia prowazekii, Rickettsia psittaci, Rickettsia quintana, Rickettsia rickettsia, Rickettsia trachomae, Rochalimaea henselae, Rochalimaea Quintana, Rothia dentocariosa, Salmonella enteritidis, Salmonella typhi, Salmonella typhimurium, Serratia marcescens*, *Shigella dysenteriae, Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Staphylococcus pneumoniae*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Staphylococcus colmii*, *Staphylococcus sciuri*, *Staphylococcus warneri*, *Stenotrophomonas maltophilia, Streptococcus*

agalactiae, Streptococcus anginosus, Streptococcus avium, Streptococcus bovis, Streptococcus cricetus, Streptococcus faceium, Streptococcus faecalis, Streptococcus ferus, Streptococcus gallinarum, Streptococcus lactis. Streptococcus mitior, Streptococcus mitis, Streptococcus mutans, Streptococcus oralis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus rattus, Streptococcus salivarius, Streptococcus sanguis, Streptococcus sobrinus, Streptococcus viridans, Treponema pallidum, Treponema denticola, Vibrio cholera, Vibrio comma, Vibrio parahaemolyticus, Vibrio vulnificus, Wolbachia, Yersinia enterocolitica, Yersinia pestis, Yersinia pseudotuberculosis, Aspergillus fumigatus, Aspergillus flavus, Aspergillus clavatus, Aspergillus niger, Aspergillus terreus, Candida albicans, Candida glabrata, Candida tropicalis, Candida krusei, Candida dubliniensis, Candida parapsilosis, Fusarium solani, Fusarium moniliforme, Fusarium proliferartum, Malessezia pachydermatis, Chrysosporium parvum, Metarhizium anisopliae, Phaeoisaria clematidis, or *Sarcopodium oculorum*.

Embodiment 92. The method of embodiment 90, wherein the antigen is an antigen from *Mycobacterium tuberculosis*, Varicella zoster virus, *Corynebacterium diphtheria*, Hepatitis A virus, Hepatitis B virus, Hepatitis C virus, *Haemophilis influenza*, Human Papillomavirus, Influenza virus, Japanese Encephalitis virus, Measles virus, *Neisseria meningitidis*, Mumps virus, *Bordetella pertussis*, *Streptococcus pneumonia*, Poliovirus, Rabies virus, Rotavirus, Rubella virus, Herpes Zoster virus, *Clostridium tetani*, *Salmonella typhi*, Yellow Fever virus, Ebola virus, avian flu virus, *Bacillus anthracis*, Smallpox virus, or Zika virus.

Embodiment 93. The method of any one of embodiments 90 to 92, wherein the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc.

Embodiment 94. The method of any one of embodiments 90 to 93, wherein the antibody, or fragment thereof, is directed to a cell surface marker.

Embodiment 95. The method of embodiment 94, wherein the cell surface marker is on an immune cell.

Embodiment 96. The method of embodiment 95, wherein the immune cell is a T cell, B cell, NK cell, macrophage, TIL, dendritic cell, neutrophil, eosinophil, basophil, or mast cell.

Embodiment 97. The method of embodiment 96, wherein the T cell is a $CD4^+$ T cell, $CD8^+$ T cell, helper T cell, cytotoxic T cell, or natural killer T cell; and wherein the B cell is a memory B cell or a plasma cell.

Embodiment 98. The method of embodiment 94, wherein the cell surface marker is CD36, CD68, CD83, CD180, CD206 (MRC1- mannose receptor), F4/80 (EGF-TM7 family), CD205, CD56, CD161, CD94:NKG2 heterodimer, KIR/CD158 family, CD335, CD64, CD16,

CD3, CD28, CD4, CD25, CD39, Toll-like receptors (TLRs), CD281, CD283, CD284, CD286, CD289, CD282, C-type lectin receptors (CLRs), Fc Receptor, or HSG.

Embodiment 99. The method of any one of embodiments 90 to 98, wherein the adjuvant is one or more of a CpG containing oligonucleotide, a ssRNA, a dsRNA, a monophosphate lipid, aluminum, squalene, 3-deacyl-monophosphoryl lipid A, vitamin E, surfactant polysorbate 80, mannide-mono-oleate, SLAG-3, or a galactosylceramide.

Embodiment 100. The method of embodiment 99, wherein the CpG containing oligonucleotide is ODN 1585, ODN 1668, ODN 1826, ODN 2006, ODN 2007, ODN 2216, ODN 2336, ODN 2395, ODN M362, AT-ODN-1, AT-ODN-2, ODNBW006, ODN 2088, ODN 4084, ODN INH-1, ODN INH-47, ODN TTAGGG, and G-ODN.

Embodiment 101. The method of any one of embodiments 90 to 100, wherein the oligonucleotide linker conjugating the targeting moiety to the terminal arm is a CpG containing oligonucleotide.

Embodiment 102. A method of treating a mammal having cancer comprising administering to the mammal a nucleic acid carrier of any one of embodiments 1 to 98, or a pharmaceutical composition comprising a nucleic acid carrier of any one of embodiments 1 to 98, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

Embodiment 103. The method of embodiment 102, wherein the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc.

Embodiment 104. The method of embodiment 102 or embodiment 103, wherein a first antibody, or fragment thereof, is directed to a tumor-associated antigen.

Embodiment 105. The method of embodiment 104, wherein the tumor-associated antigen is 4-1BB, 5AC, 5T4, A2aR, activin receptor-like kinase 1, AGS-22M6, AKAP4, alpha-fetoprotein, angiopoietin 2, B7-H3, BAFF, BAGE, BCR-ABL, BORIS, CA-125, CA19-9, C242 antigen, carbonic anhydrase 9 (CA-IX), CCR4, CD19, CD20, CD22, CD23 (IgE receptor), CD24, CD28, CD30 (TNFRSF8), CD33, CD37, CD38 (cyclic ADP ribose hydrolase), CD40, CD44 v6, CD51, CD56, CD70, CD71, CD73, CD74, CD79B, CD80, CD137, CD140a, CD152, CD200, CD221, CD274, CEA, ch4D5, CLDN18.2, CS1, CSF1R, CTLA-4, C-X-C chemokine receptor type 4, DLL4, DR5, EBAG9, EGF, EGFR, EGFL7, EpCAM, ERBB2, ERBB3, FAP, fibronectin extra domain-B, folate receptor 1, folate receptor alpha, folate hydrolase, Frizzled receptor, GAGE, GD2 ganglioside, GD3 ganglioside, glioma, glypican 3, GPNMB, gp100, GUCY2C, HER1, HER2/neu, HER3, HGF, HHGFR, histone complex, HLA-DR, human scatter

factor receptor kinase, HPV-16, HSP105, IDH1, IDO1, IGF-I, IGF-1 receptor, ILGF2, IL-6, IL-13, integrin αvβ3, integrin α5β1, KIR, LAG-3, Lewis-Y antigen, LY6K, MAGE-1, MAGE-A3, MAGE-C2, MAGE-D4, MAPG, MART-1, Melan-A, MET, MCP-1, mesothelin, MIF, MSLN, MS4A1, mucin CanAg, MUC1, MUC4, MUC16, NG2, N-glycolylneuraminic acid, Notch receptor PD-1, NY-ESO-1, OCAA, PAP, PDGF-R α, PDCD1, PD1, PD-L1, phosphate-sodium co-transporter, phosphatidylserine, PRAME, PSA, RANKL, RON, ROR1, SDC1, Sialyl-Tn, SLAMF7, SPAG-9, SSX1, STEAP1, survivin, TAG-72, telomerase, TEM1, tenascin C, TGF-β, TIM-3, TLR, TAM, TIM-3, TRAIL-R2, TRAIL-R1, TWEAK receptor, tumor specific glycosylation of MUC1, tumor-associated calcium signal transducer 2, tumor antigen CTAA16.88, TYRP1 (glycoprotein 75), VEGF-A, VEGFR2, VEGFR-1, vimentin, VISTA, WT1, or XAGE-1b.

Embodiment 106. The method of embodiment 104, wherein the tumor-associated antigen is HER-2, EGFR, CD30, CD20, EpCAM, NG2, CD19, CEA, MUC-1, CA19-9, OCAA, MAPG, or glioma.

Embodiment 107. The method of any one of embodiments 102 to 106, wherein a second antibody, or fragment thereof, is directed to a cell surface marker.

Embodiment 108. The method of embodiment 107, wherein the cell surface marker is on an immune cell.

Embodiment 109. The method of embodiment 108, wherein the immune cell is a T cell, B cell, NK cell, macrophage, TIL, dendritic cell, neutrophil, eosinophil, basophil, or mast cell.

Embodiment 110. The method of embodiment 99, wherein the T cell is a $CD4^+$ T cell, $CD8^+$ T cell, helper T cell, cytotoxic T cell, or natural killer T cell; and wherein the B cell is a memory B cell or a plasma cell.

Embodiment 111. The method of embodiment 107, wherein the cell surface marker is CD36, CD68, CD83, CD180, CD206 (MRC1- mannose receptor), F4/80 (EGF-TM7 family), CD205, CD56, CD161, CD94:NKG2 heterodimer, KIR/CD158 family, CD335, CD64, CD16, CD3, CD28, CD4, CD25, CD39, Toll-like receptors (TLRs), CD281, CD283, CD284, CD286, CD289, CD282, C-type lectin receptors (CLRs), Fc Receptor, or HSG.

Embodiment 112. The method of any one of embodiments 102 to 111, wherein the nucleic acid carrier comprises:

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is breast, ovarian, or prostrate;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is a solid tumor, lung, or colorectal;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1, and wherein the cancer is triple negative breast cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX-40, and wherein the cancer is breast cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is Hodgkin's disease;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is metastatic breast cancer or prostrate;

a first targeting agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is non-Hodgkin lymphoma or multiple myeloma;

a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian, gastric, colon, colorectal, breast, non-small cell lung cancer, adenocarcinoma of the lung, small cell lung cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is B-precursor acute lymphoblastic leukemia or non-Hodgkin lymphoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is non-Hodgkin lymphoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein

the cancer is colorectal, lung carcinoma, pancreas carcinoma, stomach carcinoma, ovary carcinoma, uterus carcinoma, breast carcinoma, or melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein the cancer is invasive pancreatic adenocarcinoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a CA19-9-positive tumor;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian;

a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian;

a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is metastatic melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is a HER-2 positive tumor;

a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a glioma; and

a first targeting agent which is folic acid or folic acid receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16, and wherein the cancer is small cell lung carcinoma, breast, or ovarian.

Embodiment 113. The method of any one of embodiments 102 to 112, wherein the label is a chemiluminescent agent, a fluorescent agent, an infrared dye, a radiolabel agent, a metal, a chelating agent, a hapten, or a gene or mRNA encoding a detectable protein.

In order that the subject matter disclosed herein may be more efficiently understood, examples are provided below. It should be understood that these examples are for illustrative purposes only and are not to be construed as limiting the claimed subject matter in any manner. Throughout these examples, molecular cloning reactions, and other standard recombinant DNA

techniques, were carried out according to methods described in Maniatis et al., Molecular Cloning - A Laboratory Manual, 2nd ed., Cold Spring Harbor Press (1989), using commercially available reagents, except where otherwise noted.

Examples

Example 1: Synthesis of 1 layer nucleic acid carrier from synthetic (chemically synthesized) DNA strands

An equal mass of the following synthetic DNA strands was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; and B" Monomer: Strands 4 and 5. Appropriate quantities of saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses of each of the B' and B" Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Purification of 1 layer nucleic acid carrier:

The final 1-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Example 2: Synthesis of 1.5 layer (C') nucleic acid carrier from synthetic DNA strands

An equal mass of the following synthetic DNA strands was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; B" Monomer: Strands 4 and 5; C' Monomer: Strands 2 and 6. Appropriate quantities of saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses each of the B' and B" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Assembly of 1.5 layer (C') nucleic acid carrier:

To the 1 layer preparation, 1.2 molar masses of the C' Monomer was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was expose to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C.

Purification of 1.5 layer (C') nucleic acid carrier:

The final 1.5-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Example 3: Synthesis of 2 layer nucleic acid carrier from synthetic DNA strands

An equal mass of the following synthetic DNA strands was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; B" Monomer: Strands 4 and 5; C' Monomer: Strands 2 and 6; and C" Monomer: Strands 2 and 7. Appropriate quantities of saline were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses each of the B' and B" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was expose to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Assembly of 2 layer nucleic acid carrier:

To the 1 layer preparation, 1.2 molar masses each of the C' and C" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C.

Purification of 2 layer nucleic acid carrier:

The final 2-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Example 4: Synthesis of 2.5 layer (B') nucleic acid carrier from synthetic DNA strands

Assembly of 2.5 layer nucleic acid carrier:

To the 2 layer preparation from Example 3, 1.1 molar masses each of the B' Monomer was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30

minutes, and centrifuged at 1500xG for 10 minutes at 4°C. Purification of the 2.5 layer nucleic acid carrier was performed as indicated in Example 1.

Example 5: Synthesis of 3 layer nucleic acid carrier from synthetic DNA strands

Assembly of 3 layer nucleic acid carrier:

To the 2 layer preparation from Example 3, 1.1 molar masses each of the B' and B" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C. Purification of the 3 layer nucleic acid carrier was performed as indicated in Example 1.

Example 6: Synthesis of 4 layer nucleic acid carrier from synthetic DNA strands

Assembly of 3 layer nucleic acid carrier:

The steps set forth in Example 5 were carried out, except the final purification step.

Assembly of 4 layer nucleic acid carrier:

To the 3 layer preparation, 1.02 molar masses of each of the C' and C" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was expose to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C. Purification of the 4 layer nucleic acid carrier was performed as indicated in Example 1.

Example 7: Synthesis of 2-layer or 4-layer nucleic acid carrier with anti-EpCAM antibody and fluorescent Cy3 labe

Preparation of anti-EPCAM oligonucleotide conjugate:

Conjugation of anti-EpCAM with thiol end-labeled DNA oligonucleotide (OligoA) complementary to the two different open ssDNA sequences on the DNA monomer:

The buffer containing each of the two antibodies is exchanged into 1X PBS using standard buffer exchange spin columns.

Each of two 72-mer DNA oligonucleotides containing a 3' thiol group is separately reduced using TCEP for 1 hour at room temperature. TCEP is removed by ethanol precipitation of the oligonucleotide, and the oligonucleotide is resuspended in water.

Amines on each antibody are activated by using LC-SMCC, a heterobifunctional cross-linker with N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-labeled molecules. A 50X LC-SMCC solution is used to

treat each antibody for 1 hour at room temperature. Excess LC-SMCC is removed via desalting columns.

The two resuspended thiol DNA oligonucleotides are added to the appropriate activated antibodies at a ratio of 1.2:1 of DNA oligonucleotide molecules to antibody protein molecules in the following configuration:

DNA OligoA is added to Anti-EPCAM to form anti-EPCAM-OligoA conjugate

The conjugations are allowed to proceed for 1 hour at room temperature.

The conjugation reactions are separately purified using TAC resin. Ten wash buffer column volumes, ten elution buffer column volumes, and five water column volumes fractions are collected for each conjugate.

Purification fractions that contain conjugate (based upon running fractions on a gel) are independently pooled for each conjugate and concentrated using 10K MWCO spin concentrators.

Formualtion of anti-EPCAM antibody with 3DNA and Cy3 label:

The following are added to a 400mL polypropylene tube (or equivalent):

1.00 mg of 2-layer core dendrimer reagent as prepared above;

0.375 mg of EpCAM oligonucleotide conjugate;

0.250 mg of Cy3 labeled DNA oligonucleotide complementary to a terminal arm sequence (31-mer);

3 mL of 10X PBS, pH 7.4 (aqueous); and

water to reach a volume of 30 mL (prior to addition of next reagent).

The preparation is incubated at 37°C for 30 minutes, and allowed to cool to ambient temperature.

Example 8: *In vitro* cellular targeting of OVCAR cells using a fluorescent labeled nucleic acid carrier comprising anti-EpCAM antibody

OVCAR cells, 1.0 x 10^6 cells per well, are transferred to each of 8 wells in 8 well microscope slide and incubated at 37°C. The cells are fixed using 2% paraformaldehyde as per standard methods. The paraformaldehyde is replaced with PBS and the cells are stored at 4°C until ready to use.

When ready to use, the PBS is removed from the cells. An amount of 150 µl of 0.5% Bovine Serum Albumen (BSA) in 1X PBS is added. The cells are incubated for 30 minutes at room temperature. The solution is removed after incubation.

An amount of 150 µl of Cy3-labeled DNA nucleic acid carrier with anti-EpCAM antibody (as prepared in Example 7) is added. The DNA nucleic acid carrier reagent is diluted 1:50 in 0.5% BSA in 1X PBS to 10 ng/µL, and a sufficient quantity is added to achieve a final DNA nucleic acid carrier concentration of 20 ng/100 µl in the cell preparation.

The microscope slides are placed in a humid chamber at 37°C for 1 hour.

The microscope slides are rinsed twice with 150 µl of 1X PBS at room temperature.

An amount of 100 µl of bis-benzimide diluted 1:500 in distilled water is added, and incubated for 5 minutes at room temperature. This will stain the nuclei, which will be visible using a Dapi compatible fluorescent filter.

The cells are analyzed using a fluorescent microscope using coverslips in Fluorogel (Electron Microscopy Sciences, Hatfield, PA) or other suitable medium.

Example 9: Anti-EPCAM, Anti-CD3 1-layer nucleic acid carrier for *in vitro* and *in vivo* immune oncology assays

Step 1: Conjugation of two antibodies (anti-EPCAM and anti-CD3) with thiol end-labeled DNA oligonucleotides (OligoA and OligoB) complementary to the two different open ssDNA sequences on the DNA monomer

The buffer containing each of the two antibodies is exchanged into 1X PBS using standard buffer exchange spin columns.

Each of two 72-mer DNA oligonucleotides containing a 3' thiol group are separately reduced using TCEP for 1 hour at room temperature. TCEP is removed by ethanol precipitation of the oligonucleotide, and the oligonucleotide is resuspended in water.

Amines on each antibody are activated by using LC-SMCC, a heterobifunctional cross-linker with N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-labeled molecules. A 50X LC-SMCC solution is used to treat each antibody for 1 hour at room temperature. Excess LC-SMCC is removed via desalting columns.

The two resuspended thiol DNA oligonucleotides are added to the appropriate activated antibodies at a ratio of 1.2:1 of DNA oligonucleotide molecules to antibody protein molecules in the following configuration:

DNA OligoA is added to Anti-EPCAM to form anti-EPCAM-OligoA conjugate

DNA OligoB is added to Anti-CD3 to form anti-CD3-OligoB conjugate

The conjugations are allowed to proceed for 1 hour at room temperature.

The conjugation reactions are separately purified using TAC resin. Ten wash buffer column volumes, ten elution buffer column volumes, and five water column volumes fractions are collected for each conjugate.

Purification fractions that contain each conjugate (based upon running fractions on a gel) are independently pooled for each conjugate and concentrated using 10K MWCO spin concentrators.

Final protein and oligonucleotide concentrations for each conjugate are determined via a protein quantitation assays and spectroscopy.

Step 2: Assembly of the DNA monomers and 1-layer nucleic acid carriers

Five 97-mer DNA oligonucleotides were designed and ordered from a commercial DNA synthesizer company (e.g., IDT). The oligonucleotides were designed to share a region of sequence complementarity located in the central portion of each oligonucleotide comprising 31 nucleotides. When the two oligonucleotides anneal to form the monomer, the resulting structure can be described as having a central double-stranded region (i.e., "waist") bordered by four single-stranded arms of two different sequences. An equal mass of the following synthetic DNA oligonucleotides was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; and B" Monomer: Strands 4 and 5. Appropriate quantities of a saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses of each of the B' and B" Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Purification of 1 layer nucleic acid carrier:

The final 1-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of anti-EPCAM anti-CD3 1-layer DNA nucleic acid carrier (EPCAM/CD3 1-layer):

The 1-layer assembly has 2 outer "arms" of two different sequences complementary to the OligoA and OligoB sequences previously utilized for conjugation to anti-EPCAM and anti-CD3 antibodies. Base-pairing of the arms to the two 72-mer oligonucleotides comprising the antibody-oligonucleotide conjugate described above is accomplished by adding stoichiometric

concentrations of the antibody-oligonucleotide conjugate to the previously assembled 1-layer DNA nucleic acid carriers.

The following components were added to a polypropylene tube:

1-layer in water at 2000 ng/μL	100.0 μL	(200,000 ng)
10X PBS	30.0 μL	
Nuclease free water	30.0 μL	
Anti-EPCAM-OligoA Conjugate at 1250 ng/μL as oligo	70.0 μL	(87,500 ng as oligo)
Anti-CD3-OligoB Conjugate at 1250 ng/μL as oligo	70.0 μL	(87,500 ng as oligo)

All reactants are added together and gently heated 37°C for 10 minutes and then slowly cooled to room temperature.

In vitro cell based assays:

Confirmation of binding: OVCAR3 (EPCAM positive), A2780 (EPCAM negative) and peripheral blood mononuclear cells (PMBCs) are used to measure antibody binding. A total of $1x10^6$ cells are washed with phosphate buffered saline (PBS; 137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4 and 2 mmol/L KH_2PO_4) and incubated in 100 μl of EPCAM/CD3 1-layer (100 μg/ml as antibody in PBS) for 30 minutes at room temperature and then are washed twice with PBS. Fluorescein isothiocyanate conjugated antibody against human IgG is used for detecting the EPCAM/CD3 1-layer. The antibody is diluted at 1:200 and added to the cells for 30 minutes at room temperature. Cells are analyzed using fluorescence activated cell sorting.

Induction of T-cell activation: Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a six well flat bottom plate (Molecular Devices, Sunnyvale, CA, USA). Each well contains 2 ml of RPMI 1640 (HyClone, Logan, Utah, USA) with 10% fetal calf serum (FCS) only (control wells), or with 10% FCS and unconjugated CD3 antibody (30 ng/ml) or with 10% FCS and EPCAM/CD3 1-layer (10 ng/ml). PBMCs are incubated for 24 hours and the activation of PBMCs was measured using flow cytometric analysis. The expression levels of CD25 and CD69 on T cells are detected by flow cytometry to evaluate the T-lymphocyte activation ability of EPCAM/CD3 1-layer.

Luminex liquid chip analysis: A luminex liquid chip array is used to determine the release of inflammatory cytokines IL-2, IL-4, tumor necrosis factor (TNF) α and interferon (IFN) γ from PBMCs induced by EPCAM/CD3 1-layer. A Bio-Plex Pro™ Human Cytokine Assay kit (Bio-Rad Laboratories, Inc, Hercules, CA, USA) is used for detection. Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a 96 well flat bottom plate. Each well contains 100 μl

complete media alone (control wells), or with complete media containing 1 μg/ml CD28 Ab (TGN1412; eBioscience, San Diego, CA, USA), unconjugated CD3 Ab or EPCAM/CD3 1-layer. Each assay is performed in triplicate. The PBMCs are incubated at 37°C under 5% CO_2 for 72 hours and 50-μl aliquots of media are collected for the liquid chip array. Briefly, the diluted microparticle mixture is resuspended and 50 μl of the mixture is added to each well of the microplate. 50 μl of the standard or sample is then added to each well and incubated for 3 hours at room temperature using a vacuum manifold device designed to accommodate a microplate. Subsequently, 50 μl diluted biotin antibody cocktail is added to each well and the plate is incubated for 1 hour at room temperature, with agitation. Diluted streptavidin phycoerythrin (50 μl) is added to each well and the plate is incubated for 30 minutes at room temperature, with agitation at 500 rpm. The microparticles are resuspended by adding 100 μl wash buffer to each well and incubating for 2 minutes, with agitating at 500 rpm. The fluorescence signal is read using a Luminex 100 analyzer (Luminex Corp., Austin, TX, USA) within 90 minutes.

In vitro Cytotoxicity assay: The EPCAM-positive cell line OVCAR3 is used as positive target cells and the A2780 cells are used as negative controls. Cytotoxicity is measured using a CytoTox 96® Non-Radioactive Cytotoxicity assay kit (Promega, Madison, Wisconsin, USA) using RPMI 1640 complete medium with 5% FCS in a round bottom 96 well plates. Briefly, PBMCs are added as effector cells to each well at gradient concentrations, followed by the addition of the target cells ($1x10^4$). EPCAM/CD3 1-layer (100 ng/ml) is added to achieve final effector cell to target cell (E:T) ratios of 100:1, 50:1, 10:1 and 1:1. The cell mixtures are incubated at 37°C under 5% CO_2 for 4 hours, following which 50 μl aliquots of media are transferred to fresh 96 well flat bottom plates for the LDH release assay. The percentage of cell lysis is calculated as the specific release (%) = (experimental release - effector spontaneous release - target spontaneous release) / (target maximum release - target spontaneous release) x 100. Each assay is performed in triplicate.

In vivo Mouse Model studies: In vivo experiments are carried out in female 6- to 10-week-old C57BL/6 and immunodeficient NOD/SCID mice characterized by T cell, B cell, and natural killer cell deficiency and lack of macrophage function (The Jackson Laboratory, Bar Harbor, ME). The mice are maintained under sterile and standardized environmental conditions (20 +/- °C room temperature, 50 +/- 10% relative humidity, 12 hours light dark rhythm). For each animal, 2.5 $x10^6$ or 5 $x10^6$ human PBMC are mixed with 5 $x10^6$ OVCAR3 ovarian carcinoma cells in a final volume of 0.2 mL PBS. The PBMC effector-to-target cell mixture (E:T, 1:2 or 1:1) is s.c. injected into the right flank of each NOD/SCID mouse. Two different variants of the tumor model (early treatment and established tumor model) are used. For the

early treatment model, four to six animals per group are i.v. treated with EPCAM/CD3 1-layer, PBS control vehicle, or 1-layer only (untargeted) control starting 1 hour after OVCAR/PBMC inoculation at varying doses and treatment is repeated for 4 consecutive days. In the established tumor model, initiation of treatment is delayed until s.c. growing OVCAR3 tumors are developed (50-200 mm^3). Six animals per group are treated starting at days 4, 8, and 12, respectively, and treatment is repeated for 4 consecutive days. Tumors are measured on the indicated days with a caliper in two perpendicular dimensions and tumor volumes are calculated according to the following formula: tumor volume = [(width^2 x length) / 2].

Example 10: Anti-EPCAM, Anti-CD3 2-layer nucleic acid carrier for *in vitro* and *in vivo* immune oncology assays

Step 1: Conjugation of two antibodies (anti-EPCAM and anti-CD3) with thiol end-labeled DNA oligonucleotides (OligoA and OligoB) complementary to the two different open ssDNA sequences on the DNA monomer

The buffer containing each of the two antibodies is exchanged into 1X PBS using standard buffer exchange spin columns.

Each of two 72-mer DNA oligonucleotides containing a 3' thiol group are separately reduced using TCEP for 1 hour at room temperature. TCEP is removed by ethanol precipitation of oligonucleotide, and the oligonucleotide is resuspended in water.

Amines on each antibody are activated by using LC-SMCC, a heterobifunctional cross-linker with N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-labeled molecules. A 50X LC-SMCC solution is used to treat each antibody for 1 hour at room temperature. Excess LC-SMCC is removed via desalting columns.

The two resuspended thiol DNA oligonucleotides are added to the appropriate activated antibodies at a ratio of 1.2:1 of DNA oligonucleotide molecules to antibody protein molecules in the following configuration:

DNA OligoA is added to Anti-EPCAM to form anti-EPCAM-OligoA conjugate

DNA OligoB is added to Anti-CD3 to form anti-CD3-OligoB conjugate

The conjugations are allowed to proceed for 1 hour at room temperature.

The conjugation reactions are separately purified using TAC resin. Ten wash buffer column volumes, ten elution buffer column volumes, and five water column volumes fractions are collected for each conjugate.

Purification fractions that contain each conjugate (based upon running fractions on a gel) are independently pooled for each conjugate and concentrated using 10K MWCO spin concentrators.

Final protein and oligonucleotide concentrations for each conjugate are determined via a protein quantitation assays and spectroscopy.

Step 2: Assembly of the DNA monomers and 2-layer nucleic acid carriers

Seven 97-mer DNA oligonucleotides were designed and ordered from a commercial DNA synthesizer company (e.g., IDT). The oligonucleotides were designed to share a region of sequence complementarity located in the central portion of each oligonucleotide comprising 31 nucleotides. When the two oligonucleotides anneal to form the monomer, the resulting structure can be described as having a central double-stranded region (i.e., "waist") bordered by four single-stranded arms of two different sequences. An equal mass of the following synthetic DNA oligonucleotides was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; and B" Monomer: Strands 4 and 5, C' Monomer: Strands 6 and 2; and C" Monomer: Strands 7 and 2. Appropriate quantities of a saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 2 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses of each of the B' and B" Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

To the 1 layer preparation, 1.2 molar masses each of the C' and C" Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C. The total mass of DNA was recorded, and advanced to purification of the 2 layer synthesis.

Purification of 2 layer nucleic acid carrier:

The final 2-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of anti-EPCAM anti-CD3 2-layer DNA nucleic acid carrier (EPCAM/CD3 2-layer):

The 2-layer assembly has 2 outer arms of two different sequences complementary to the OligoA and OligoB sequences previously utilized for conjugation to anti-EPCAM and anti-CD3

antibodies. Base-pairing of the arms to the two 72-mer oligonucleotides comprising the antibody-oligonucleotide conjugate described above is accomplished by adding stoichiometric concentrations of the antibody-oligonucleotide conjugate to the previously assembled 2-layer DNA nucleic acid carriers.

The following components are added to a polypropylene tube:

2-layer in water at 2000 ng/μL	100.0 μL	(200,000 ng)
10X PBS	30.0 μL	
Nuclease free water	30.0 μL	
Anti-EPCAM-OligoA Conjugate at 1120 ng/μL as oligo	70.0 μL	(78,400 ng as oligo)
Anti-CD3-OligoB Conjugate at 1120 ng/μL as oligo	70.0 μL	(78,400 ng as oligo)

All reactants are added together and gently heated 37°C for 10 minutes and then slowly cooled to room temperature.

In vitro cell based assays:

Confirmation of binding: OVCAR3 (EPCAM positive), A2780 (EPCAM negative) and peripheral blood mononuclear cells (PMBCs) are used to measure antibody binding. A total of $1x10^6$ cells are washed with phosphate buffered saline (PBS; 137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4 and 2 mmol/L KH_2PO_4) and incubated in 100 μl EPCAM/CD3 2-layer (100 μg/ml as antibody in PBS) for 30 minutes at room temperature and then are washed twice with PBS. Fluorescein isothiocyanate conjugated antibody against human IgG is used for detecting the EPCAM/CD3 2-layer. The antibody is diluted at 1:200 and added to the cells for 30 minutes at room temperature. Cells are analyzed using fluorescence activated cell sorting.

Induction of T-cell activation: Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a six well flat bottom plate (Molecular Devices, Sunnyvale, CA, USA). Each well contains 2 ml of RPMI 1640 (HyClone, Logan, Utah, USA) with 10% fetal calf serum (FCS) only (control wells), or with 10% FCS and unconjugated CD3 antibody (30 ng/ml) or with 10% FCS and EPCAM/CD3 2-layer (10 ng/ml). PBMCs are incubated for 24 hours and the activation of PBMCs was measured using flow cytometric analysis. The expression levels of CD25 and CD69 on T cells are detected by flow cytometry to evaluate the T-lymphocyte activation ability of EPCAM/CD3 2-layer.

Luminex liquid chip analysis: A luminex liquid chip array is used to determine the release of inflammatory cytokines IL-2, IL-4, tumor necrosis factor (TNF) α and interferon (IFN) γ from PBMCs induced by EPCAM/CD3 2-layer. A Bio-Plex Pro™ Human Cytokine Assay kit

(Bio-Rad Laboratories, Inc, Hercules, CA, USA) is used for detection. Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a 96 well flat bottom plate. Each well contains 100 µl complete media alone (control wells), or with complete media containing 1 µg/ml CD28 Ab (TGN1412; eBioscience, San Diego, CA, USA), unconjugated CD3 Ab or EPCAM/CD3 2-layer. Each assay is performed in triplicate. The PBMCs are incubated at 37°C under 5% CO_2 for 72 hours and 50-µl aliquots of media are collected for the liquid chip array. Briefly, the diluted microparticle mixture is resuspended and 50 µl of the mixture is added to each well of the microplate. 50 µl of the standard or sample is then added to each well and incubated for 3 hours at room temperature using a vacuum manifold device designed to accommodate a microplate. Subsequently, 50 µl diluted biotin antibody cocktail is added to each well and the plate is incubated for 1 hour at room temperature, with agitation. Diluted streptavidin phycoerythrin (50 µl) is added to each well and the plate is incubated for 30 minutes at room temperature, with agitation at 500 rpm. The microparticles are resuspended by adding 100 µl wash buffer to each well and incubating for 2 minutes, with agitating at 500 rpm. The fluorescence signal is read using a Luminex 100 analyzer (Luminex Corp., Austin, TX, USA) within 90 minutes.

In vitro Cytotoxicity assay: The EPCAM-positive cell line OVCAR3 is used as positive target cells and the A2780 cells are used as negative controls. Cytotoxicity is measured using a CytoTox 96® Non-Radioactive Cytotoxicity assay kit (Promega, Madison, Wisconsin, USA) using RPMI 1640 complete medium with 5% FCS in a round bottom 96 well plates. Briefly, PBMCs are added as effector cells to each well at gradient concentrations, followed by the addition of the target cells ($1x10^4$). EPCAM/CD3 2-layer (100 ng/ml) is added to achieve final effector cell to target cell (E:T) ratios of 100:1, 50:1, 10:1 and 1:1. The cell mixtures are incubated at 37°C under 5% CO_2 for 4 hours, following which 50 µl aliquots of media are transferred to fresh 96 well flat bottom plates for the LDH release assay. The percentage of cell lysis is calculated as the specific release (%) = (experimental release - effector spontaneous release - target spontaneous release) / (target maximum release - target spontaneous release) x 100. Each assay is performed in triplicate

In vivo Mouse Model studies: In vivo experiments are carried out in female 6- to 10-week-old C57BL/6 and immunodeficient NOD/SCID mice characterized by T cell, B cell, and natural killer cell deficiency and lack of macrophage function (The Jackson Laboratory, Bar Harbor, ME). The mice are maintained under sterile and standardized environmental conditions (20 +/- 1°C room temperature, 50 +/- 10% relative humidity, 12 hours light dark rhythm). For each animal, 2.5 $x10^6$ or 5 $x10^6$ human PBMC are mixed with 5 $x10^6$ OVCAR3 ovarian carcinoma cells in a final volume of 0.2 mL PBS. The PBMC effector-to-target cell mixture

(E:T, 1:2 or 1:1) is s.c. injected into the right flank of each NOD/SCID mouse. Two different variants of the tumor model (early treatment and established tumor model) are used. For the early treatment model, four to six animals per group are i.v. treated with EPCAM/CD3 2-layer, PBS control vehicle, or 2-layer only (untargeted) control starting 1 hour after OVCAR/PBMC inoculation at varying doses and treatment is repeated for 4 consecutive days. In the established tumor model, initiation of treatment is delayed until s.c. growing OVCAR3 tumors are developed (50-200 mm^3). Six animals per group are treated starting at days 4, 8, and 12, respectively, and treatment is repeated for 4 consecutive days. Tumors are measured on the indicated days with a caliper in two perpendicular dimensions and tumor volumes are calculated according to the following formula: tumor volume = [(width^2 x length) / 2].

Example 11: Anti-EPCAM, Anti-CD3, Anti-PD1 1.5-layer nucleic acid carrier for *in vitro* and *in vivo* immune oncology assays

Step 1: Conjugation of three antibodies (anti-EPCAM, anti-CD3, and anti-PD1) with thiol end-labeled DNA oligonucleotides (OligoA, OligoB, and OligoC) complementary to the three different open ssDNA sequences on the DNA monomer

The buffer containing each of the two antibodies is exchanged into 1X PBS using standard buffer exchange spin columns.

Each of two 72-mer DNA oligonucleotides containing a 3' thiol group are separately reduced using TCEP for 1 hour at room temperature. TCEP is removed by ethanol precipitation of oligonucleotide, and the oligonucleotide is resuspended in water.

Amines on each antibody are activated by using LC-SMCC, a heterobifunctional cross-linker with N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl labeled molecules. A 50X LC-SMCC solution is used to treat each antibody for 1 hour at room temperature. Excess LC-SMCC is removed via desalting columns.

The two resuspended thiol DNA oligonucleotide are added to the appropriate activated antibodies at a ratio of 1.2:1 of DNA oligonucleotide molecules to antibody protein molecules in the following configuration:

DNA OligoA is added to Anti-EPCAM to form anti-EPCAM-OligoA conjugate

DNA OligoB is added to Anti-CD3 to form Anti-CD3-OligoB conjugate

DNA OligoC is added to Anti-PD-1 to form Anti-CD19-OligoC conjugate

The conjugations are allowed to proceed for 1 hour at room temperature.

The conjugation reactions are separately purified using TAC resin. Ten wash buffer column volumes, ten elution buffer column volumes, and five water column volumes fractions are collected for each conjugate.

Purification fractions that contain each conjugate (based upon running fractions on a gel) are independently pooled for each conjugate and concentrated using 10K MWCO spin concentrators.

Final protein and oligonucleotide concentrations for each conjugate are determined via a protein quantitation assays and spectroscopy.

Step 2: Assembly of the DNA monomers and 1.5-layer nucleic acid carriers

Seven 97-mer DNA oligonucleotides were designed and ordered from a commercial DNA synthesizer company (e.g., IDT). The oligonucleotides were designed to share a region of sequence complementarity located in the central portion of each oligonucleotide comprising 31 nucleotides. When the two oligonucleotides anneal to form the monomer, the resulting structure can be described as having a central double-stranded region (i.e., "waist") bordered by four single-stranded arms of two different sequences. An equal mass of the following synthetic DNA oligonucleotides was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; and B" Monomer: Strands 4 and 5, C' Monomer: Strands 6 and 2; and C" Monomer: Strands 7 and 2. Appropriate quantities of a saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1.5 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses of each of the B' and B" Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

To the 1 layer preparation, 1.2 molar mass of the C' Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C. The total mass of DNA was recorded, and advanced to purification of the 1.5 layer synthesis.

Purification of 1.5 layer nucleic acid carrier:

The final 1.5-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of anti-EPCAM anti-CD3 anti-PD-1 1.5-layer DNA nucleic acid carrier (EPCAM/CD3/PD-1 1.5-layer):

The 1.5-layer assembly has 3 outer arms of three different sequences complementary to the OligoA, OligoB, and OligoC sequences previously utilized for conjugation to anti-EPCAM, anti-CD3, and anti-PD-1 antibodies. Base-pairing of the arms to the two 72-mer oligonucleotides comprising the antibody-oligonucleotide conjugate described above is accomplished by adding stoichiometric concentrations of the antibody-oligonucleotide conjugate to the previously assembled 1.5-layer DNA nucleic acid carriers.

The following components are added to a polypropylene tube:

2-layer in water at 2000 ng/μL	100.0 μL (200,000 ng)
10X PBS	30.0 μL
Nuclease free water	20.0 μL
Anti-EPCAM-OligoA Conjugate at 800 ng/μL as oligo	50.0 μL (40,000 ng as oligo)
Anti-CD3-OligoB Conjugate at 800 ng/μL as oligo	50.0 μL (40,000 ng as oligo)
Anti-PD1-OligoC Conjugate at 800 ng/μL as oligo	50.0 μL (40,000 ng as oligo)

All reactants are added together and gently heated 37°C for 10 minutes and then slowly cooled to room temperature.

In vitro cell based assays:

Confirmation of binding: OVCAR3 (EPCAM positive), A2780 (EPCAM negative) and peripheral blood mononuclear cells (PMBCs) are used to measure antibody binding. A total of $1x10^6$ cells are washed with phosphate buffered saline (PBS; 137 mmol/L NaCl, 2.7 mmol/L KCl, 10 mmol/L Na_2HPO_4 and 2 mmol/L KH_2PO_4) and incubated in 100 μl EPCAM/CD3/PD-1 1.5-layer (100 μg/ml as antibody in PBS) for 30 minutes at room temperature and then are washed twice with PBS. Fluorescein isothiocyanate conjugated antibody against human IgG is used for detecting the EPCAM/CD3/PD-1 1.5-layer. The antibody is diluted at 1:200 and added to the cells for 30 minutes at room temperature. Cells are analyzed using fluorescence activated cell sorting.

Induction of T-cell activation: Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a six well flat bottom plate (Molecular Devices, Sunnyvale, CA, USA). Each well contains 2 ml of RPMI 1640 (HyClone, Logan, Utah, USA) with 10% fetal calf serum (FCS) only (control wells), or with 10% FCS and unconjugated CD3 antibody (30 ng/ml) or with 10%

FCS and EPCAM/CD3/PD-1 1.5-layer (10 ng/ml). PBMCs are incubated for 24 hours and the activation of PBMCs was measured using flow cytometric analysis. The expression levels of CD25 and CD69 on T cells are detected by flow cytometry to evaluate the T-lymphocyte activation ability of EPCAM/CD3/PD-1 1.5-layer.

Luminex liquid chip analysis: A luminex liquid chip array is used to determine the release of inflammatory cytokines IL-2, IL-4, tumor necrosis factor (TNF) α and interferon (IFN) γ from PBMCs induced by EPCAM/CD3/PD-1 1.5-layer. A Bio-Plex Pro™ Human Cytokine Assay kit (Bio-Rad Laboratories, Inc, Hercules, CA, USA) is used for detection. Freshly prepared PBMCs ($2x10^6$ cells/ml) are added to each well of a 96 well flat bottom plate. Each well contains 100 µl complete media alone (control wells), or with complete media containing 1 µg/ml CD28 Ab (TGN1412; eBioscience, San Diego, CA, USA), unconjugated CD3 Ab or EPCAM/CD3/PD-1 1.5-layer. Each assay is performed in triplicate. The PBMCs are incubated at 37°C under 5% CO_2 for 72 hours and 50-µl aliquots of media ware collected for the liquid chip array. Briefly, the diluted microparticle mixture is resuspended and 50 µl of the mixture is added to each well of the microplate. 50 µl of the standard or sample is then added to each well and incubated for 3 hours at room temperature using a vacuum manifold device designed to accommodate a microplate. Subsequently, 50 µl diluted biotin antibody cocktail is added to each well and the plate is incubated for 1 hour at room temperature, with agitation. Diluted streptavidin phycoerythrin (50 µl) is added to each well and the plate is incubated for 30 minutes at room temperature, with agitation at 500 rpm. The microparticles are resuspended by adding 100 µl wash buffer to each well and incubating for 2 minutes, with agitating at 500 rpm. The fluorescence signal is read using a Luminex 100 analyzer (Luminex Corp., Austin, TX, USA) within 90 minutes.

In vitro Cytotoxicity assay: The EPCAM-positive cell line OVCAR3 is used as positive target cells and the A2780 cells are used as negative controls. Cytotoxicity is measured using a CytoTox 96® Non-Radioactive Cytotoxicity assay kit (Promega, Madison, Wisconsin, USA) using RPMI 1640 complete medium with 5% FCS in a round bottom 96 well plates. Briefly, PBMCs are added as effector cells to each well at gradient concentrations, followed by the addition of the target cells ($1x10^4$). EPCAM/CD3/PD-1 1.5-layer (100 ng/ml) is added to achieve final effector cell to target cell (E:T) ratios of 100:1, 50:1, 10:1 and 1:1. The cell mixtures are incubated at 37°C under 5% CO_2 for 4 hours, following which 50 µl aliquots of media are transferred to fresh 96 well flat bottom plates for the LDH release assay. The percentage of cell lysis is calculated as the specific release (%) = (experimental release - effector spontaneous

release - target spontaneous release) / (target maximum release - target spontaneous release) x 100. Each assay is performed in triplicate

In vivo Mouse Model studies: *In vivo* experiments are carried out in female 6- to 10-week-old C57BL/6 and immunodeficient NOD/SCID mice characterized by T cell, B cell, and natural killer cell deficiency and lack of macrophage function (The Jackson Laboratory, Bar Harbor, ME). The mice are maintained under sterile and standardized environmental conditions (20 +/1 °C room temperature, 50 +/- 10% relative humidity, 12 hours light dark rhythm). For each animal, 2.5 x10^6 or 5 x10^6 human PBMC are mixed with 5 x10^6 OVCAR3 ovarian carcinoma cells in a final volume of 0.2 mL PBS. The PBMC effector-to-target cell mixture (E:T, 1:2 or 1:1) is s.c. injected into the right flank of each NOD/SCID mouse. Two different variants of the tumor model (early treatment and established tumor model) are used. For the early treatment model, four to six animals per group are i.v. treated with EPCAM/CD3/PD-1 1.5-layer, PBS control vehicle, or 1.5-layer only (untargeted) control starting 1 hour after OVCAR/PBMC inoculation at varying doses and treatment is repeated for 4 consecutive days. In the established tumor model, initiation of treatment is delayed until s.c. growing OVCAR3 tumors are developed (50-200 mm^3). Six animals per group are treated starting at days 4, 8, and 12, respectively, and treatment is repeated for 4 consecutive days. Tumors are measured on the indicated days with a caliper in two perpendicular dimensions and tumor volumes are calculated according to the following formula: tumor volume = [(width^2 x length) / 2].

Example 12: Anti-EpCAM, Ovalbumin, CpG oligonucleotide (adjuvant) 1.5-layer nucleic acid carrier for vaccine use

Step 1: Conjugation of anti-Dectin-1 antibody and ovalbumin with thiol end-labeled DNA oligonucleotides (OligoA, and OligoB) complementary to the two different open ssDNA sequences on the DNA monomer

The buffer containing each of the two antibodies is exchanged into 1X PBS using standard buffer exchange spin columns.

Each of two 72-mer DNA oligonucleotides containing a 3' thiol group are separately reduced using TCEP for 1 hour at room temperature. TCEP is removed by ethanol precipitation of oligonucleotide, and the oligonucleotide is resuspended in water.

Amines on each antibody are activated by using LC-SMCC, a heterobifunctional cross-linker with N-hydroxysuccinimide (NHS) ester and maleimide groups that allow covalent conjugation of amine- and sulfhydryl-labeled molecules. A 50X LC-SMCC solution is used to

treat each antibody for 1 hour at room temperature. Excess LC-SMCC is removed via desalting columns.

The two resuspended thiol DNA oligonucleotides are added to the appropriate activated antibodies at a ratio of 1.2:1 of DNA oligonucleotide molecules to antibody protein molecules in the following configuration:

DNA OligoA is added to Anti-Dectin-1 to form anti-Dectin-1-OligoA conjugate

DNA OligoB is added to Ovalbumin to form Ovalbumin-OligoB conjugate

The conjugations are allowed to proceed for 1 hour at room temperature.

The conjugation reactions are separately purified using TAC resin. Ten wash buffer column volumes, ten elution buffer column volumes, and five water column volumes fractions are collected for each conjugate.

Purification fractions that contain each conjugate (based upon running fractions on a gel) are independently pooled for each conjugate and concentrated using 10K MWCO spin concentrators.

Final protein and oligonucleotide concentrations for each conjugate are determined via a protein quantitation assays and spectroscopy.

Step 2: Assembly of the DNA monomers and 1.5-layer nucleic acid carriers

Seven 97-mer DNA oligonucleotides were designed and ordered from a commercial DNA synthesizer company (e.g., IDT). The oligonucleotides were designed to share a region of sequence complementarity located in the central portion of each oligonucleotides comprising 31 nucleotides. When the two oligonucleotides anneal to form the monomer, the resulting structure can be described as having a central double-stranded region (i.e., "waist") bordered by four single-stranded arms of two different sequences. An equal mass of the following synthetic DNA oligonucleotides was added to a polypropylene tube to assemble the indicated monomers: A Monomer: Strands 1 and 2; B' Monomer: Strands 3 and 4; and B" Monomer: Strands 4 and 5, C' Monomer: Strands 6 and 2; and C" Monomer: Strands 7 and 2. Appropriate quantities of a saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1.5 layer nucleic acid carrier:

To a quantity of A Monomer, 2.0 molar masses of each of the B' and B" Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

To the 1 layer preparation, 1.2 molar mass of the C' Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C. The total mass of DNA was recorded, and advanced to purification of the 1.5 layer synthesis.

Purification of 1.5 layer nucleic acid carrier:

The final 1.5-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of CpG oligonucleotide:

An oligonucleotide was designed having 31 bases of complementarity to the third available arm (oligoC) on the 1.5 layer DNA nucleic acid carrier. The 31 bases were added to the 3 prime end of the CpG containing oligonucleotide 5'-tcgtcgttttgtcgttttgtcgt -3' (SEQ ID NO:4) and was ordered from a commercial DNA synthesizer company (e.g., IDT).

Preparation of anti-Dectin-1, ovalbumin, CpG oligonucleotide 1.5-layer DNA nucleic acid carrier (Dectin-1/Ovalbumin/CpG 1.5-layer):

The 1.5-layer assembly has 3 outer arms of three different sequences complementary to the OligoA, OligoB, and OligoC sequences previously utilized for conjugation or attachment to anti-Dectin-1, ovalbumin, and CpG oligonucleotide components. Base-pairing of the arms to the two 72-mer oligonucleotides comprising the conjugates described above is accomplished by adding stoichiometric concentrations of the conjugate or CpG oligonucleotide to the previously assembled 1.5-layer DNA dendrimers.

The following components are added to a polypropylene tube:

2-layer in water at 2000 ng/μL	100.0 μL (200,000 ng)
10X PBS	30.0 μL
Nuclease free water	20.0 μL
Anti-Dectin-1-OligoA Conjugate at 800 ng/μL as oligo	50.0 μL (40,000 ng as oligo)
Ovalbumin-OligoB Conjugate at 800 ng/μL as oligo	50.0 μL (40,000 ng as oligo)
CpG-OligoC at 600 ng/μL as oligo	50.0 μL (30,000 ng as oligo)

All reactants are added together and gently heated 37°C for 10 minutes and then slowly cooled to room temperature.

Example 13: Synthesis of 2 layer nucleic acid carrier from synthetic DNA strands containing both phosphodiester (PO) and Phosphorothioate (PS)

Preparation of PO-monomers:

An equal mass of the following synthetic PO-DNA strands was added to a polypropylene tube to assemble the indicated monomers: A Monomer-PO: PO-Strands 1 and 2; B' Monomer-PO: PO-Strands 3 and 4; and B" Monomer-PO: PO-Strands 4 and 5. Appropriate quantities of saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer-PO, 2.0 molar masses of each of the B'-PO and B"-PO Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Purification of 1 layer nucleic acid carrier:

The final 1-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of PS-monomers:

An equal mass of the following synthetic PS-DNA strands was added to a polypropylene tube to assemble the indicated monomers: C' Monomer-PS: PS-Strands 2 and 6; and C" Monomer-PS: PS-Strands 2 and 7. Appropriate quantities of saline were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of PO/PS-2 layer nucleic acid carrier:

To the 1 layer-PO preparation, 1.2 molar masses each of the C'-PS and C"-PS Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C.

Purification of 2 layer nucleic acid carrier:

The final 2-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Example 14: Serum degradation study of 2 layer nucleic acid carrier from synthetic DNA strands containing all phosphodiester (PO) nucleic acid bases and strands containing both phosphodiester (PO) and Phosphorothioate (PS) nucleic acid bases

Preparation of PO-monomers:

An equal mass of the following synthetic PO-DNA strands was added to a polypropylene tube to assemble the indicated monomers: A Monomer-PO: PO-Strands 1 and 2; B' Monomer-PO: PO-Strands 3 and 4; and B" Monomer-PO: PO-Strands 4 and 5. Appropriate quantities of saline buffer were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of 1 layer nucleic acid carrier:

To a quantity of A Monomer-PO, 2.0 molar masses of each of the B'-PO and B"-PO Monomers were added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, centrifuged at 1500xG for 10 minutes at 4°C to remove precipitated 4,5,8 trimethylpsoralen.

Purification of 1 layer nucleic acid carrier:

The final 1-layer product was separated from unincorporated components and residual cross-linking reagent using differential centrifugation.

Preparation of PS-monomers:

Synthetic DNA strands are synthesized having every other base being a Phosphorothioate (PSEO). An equal mass of the following synthetic PSEO-DNA strands was added to a polypropylene tube to assemble the indicated monomers: C' Monomer-PSEO: PSEO-Strands 2 and 6; and C" Monomer-PSEO: PSEO-Strands 2 and 7. Appropriate quantities of saline were added to the monomer preparations. The monomer preparations were heated to > 70°C and allowed to slowly cool to room temperature.

Assembly of PO/PSEO-2 layer nucleic acid carrier:

To the 1 layer-PO preparation, 1.2 molar masses each of the C'-PSEO and C"-PSEO Monomers was added. 4,5,8 trimethylpsoralen was added, mixed well, and incubated at 42°C for 30 minutes. The preparation was exposed to UVA light. The preparation was chilled at 4°C for 30 minutes, and centrifuged at 1500xG for 10 minutes at 4°C.

Assembly of PO-2 layer nucleic acid carrier:

This material was prepared as described above.

Serum degradation of PO-2 layer nucleic acid carrier and PO/PSEO-2 layer nucleic acid carrier in 25% mouse serum:

5 µg of each 2 layer nucleic acid carrier (PO and PO/PSEO) were mixed with PBS and then added to a micro-centrifuge tube containing 25 µl of mouse serum bringing the total volume up to 100 µl. A 4 hour time point was started first; 2 hours later a 2 hour time point was initiated; 1 hour later the 1 hour time point was started and so on, such that all time points ended simultaneously at the appropriate time interval after 4 hours. The alkaline lysis step was immediately performed on all samples with buffers from the Qiagen miniprep kit (P/N 12381) the tubes were quickly inverted following the addition of each buffer: Buffer 1 (100 µl), Buffer 2 (200 µl) & Buffer 3 (350 µl). The buffers were added sequentially and the tubes were vortexed for 10 seconds once all 3 buffers had been added. The tubes were then incubated at 65°C for 5 minutes. The tubes were centrifuged at 12,000g for 3 minutes to remove the white cloudy material, the supernatant was transferred to a new tube. A 1X volume of formamide (~700 µl) was added to each tube. The tubes were inverted several times in order to ensure appropriate mixing. The tubes were then incubated at 65°C for an additional 5 minutes. A 1X volume phenol /chloroform /isoamyl alcohol was added to each sample. The tubes were vortexed for 30 seconds and then centrifuged for 5 minutes at 12,000g, the aqueous layer was removed and transferred to a new tube. A Sodium Acetate (NaOAc) ethanol precipitation was performed next by adding a 1/10 volume of 3M NaOAc followed by 2.5 volumes of ethanol. The samples were centrifuged at 12,000g for 30 minutes at 4°C and the liquid was carefully removed to ensure a very small faint pellet remains intact. The pellets were washed with 70% ethanol (100 µl) and centrifuged for 5 minutes at 12,000g. The 70% ethanol wash was carefully removed and the tubes were placed in a 37°C incubator with caps open to allow the samples to dry for about 10 minutes. The pellets were re-suspended in 30 µl of PBS, the DNA concentration was determined on a NanoDrop and about 2 µl of sample from each time point was loaded on a Lonza Pre-cast Fast 1.2% agarose gel.

The agarose gel images from this experiment are presented in Figure 6. PO-2 layer nucleic acid carrier starts to show degradation at 15 minutes and was completely degraded at 4 hour time point (loss of the high molecular weight band at about 3000 bp). In comparison, the PO/PSEO-2 Layer nucleic acid carrier showed no degradation even at the 4 hour time point. This indicates that by using modified bases not only can the serum degradation profile be changed but also the resulting clearance profile may be subsequently altered thereby modulating the PK/PD profile of the carrier.

Example 15: Comparison of EPCAM-MMAE antibody drug conjugate to EPCAM-MMAE antibody drug conjugate coupled to dendrimer

Preparation of EGFR-MMAE antibody drug conjugate (ADC):

Monomethyl auristatin (MMAE) is an antimitotic agent that inhibits the polymerization of tubulin. MMAE is coupled to an antibody via a maleimide linkage to cysteines on the antibody and is cleaved once delivered to the cell by protease in the cell. MMAE was linked to and antibody that recognizes epithelial cell adhesion molecule (EPCAM). EPCAM monoclonal antibody (mAb) is obtained from BioXCell and concentrated/buffer exchanged to 10 mg/ml in a PBS pH 7.4, 1 mM EDTA buffer using Amicon Spin filters (100kD MWCO, Millipore). To generate free sulfhydryls, the EGFR antibody is reduced using a 10 mM TCEP solution (ThermoFisher) with the TCEP at a 2.75-fold molar excess over antibody. TCEP is added, vortexed briefly, and incubated at 37°C for 2 hours. For example, using 0.067mM Ab, TCEP is added to a final concentration of 0.18 mM. To conjugate the drug to the prepared antibody, maleimide-MMAE (containing a val-cit cleavable linker, MC-Val-Cit-PAB-MMAE, ALB Technology Limited) is diluted to 10 mM final concentration in DMSO. The drug is then added to the prepared (reduced) EPCAM antibody at a 10-fold molar excess of drug over antibody (for example, for 0.067 mM Ab, drug is added to a final concentration of 0.67 mM). MMAE is added, vortexed briefly, and incubated at 10°C for 30 minutes. After incubation, excess drug is removed and the ADC is buffer exchanged into PBS pH 7.4 using Zeba Spin desalting columns (ThermoFisher). The EPCAM-MMAE ADC is then filter sterilized (0.22 μ filter, Millipore) and a BCA assay is performed to determine protein concentration using BGG standards (ThermoFisher).

Coupling of EPCAM-MMAE antibody to dendrimer binding oligonucleotide:

In order to attach the EGFR-MMAE to a dendrimer, an oligonucleotide complementary to one or more outer arms of the DNA dendrimer is prepared. EPCAM-MMAE-oligonucleotide conjugate is prepared by first reducing the DNA dendrimer binding oligonucleotide (DBO) that contains a 3' thiol using 50 mM TCEP for 1 hour. After reduction, the TCEP is removed via ethanol precipitation, the oligonucleotide is resuspended in nuclease-free water, and the concentration obtained via A260. To prepare the ADC for conjugation, 2.0 mg of EPCAM ADC (antibody plus drug molecules of DAR =2-4) in 1X PBS pH 7.4 is placed into a 15.0 ml conical tube. To the ADC, LC-SMCC cross-linker is added at a 12.5X molar ratio of crosslinker-to-ADC, and the mixture is reacted for 1 hour at room temperature. Excess crosslinker is removed from the ADC reaction mixture using Zeba gel filtration columns. Next, the TCEP-reduced oligo and LC-SMCC-modified ADC are combined at a 1:1.2 antibody-to-oligo ratio and allowed to react at room temperature for 1 hour then placed at 4°C overnight. Excess unconjugated oligonucleotide is removed using thiophilic adsorption chromatography. The impure ADC-oligo

conjugate is diluted 10-fold (by volume) in Buffer A (5.0 mM Sodium Phosphate pH 7.0, 500 mM Potassium Sulfate) and loaded onto a TAC column equilibrated with Buffer A. The TAC column is washed with 10 CVs Buffer A then the ADC-oligo conjugate is eluted with 10 CVs Buffer B (5.0 mM Sodium Phosphate pH 7.0). The TAC column is further washed with 5 CVs nuclease-free water. Column fractions are analyzed via 10% native-TBE gel electrophoresis and gels are stained with Syber gold. Fractions that contained ADC-conjugate free of contaminating oligo are pooled and concentrated via a Amicon spin concentrator (10000 MWCO). At the end of concentration, PBS pH 7.4 buffer is added to a final of 0.2X PBS. The amount of remaining unconjugated oligo is determined via 10% native-TBE gel electrophoresis and comparing to a standard curve of known-amounts of unmodified oligo and computed via densitometry. Finally, the protein concentration of the ADC-oligo conjugate is determined via BCA assay, with BGG used as the protein standard.

Binding of EPCAM to DNA dendrimer:

EPCAM-MMAE oligonucleotide conjugate is combined with a 2-layer DNA dendrimer to formulate a EPCAM-MMAE ADC-DNA dendrimer (12 antibodies per DNA dendrimer) in a final concentration of 1x PBS, pH 7.4 at a concentration of 1 mg/ml as EPCAM-MMAE.

EGFR-MMAE compared to EGFR-MMAE on dendrimer in vivo:

In vivo Xenograft Mouse Model studies: *In vivo* experiments are carried out in female 6- to 10-week-old immunodeficient NOD/SCID mice (The Jackson Laboratory, Bar Harbor, ME). The mice are maintained under sterile and standardized environmental conditions (20 +/- °C room temperature, 50 +/- 10% relative humidity, 12 hours light dark rhythm). For each animal, 5 $x10^6$ OVCAR3 ovarian carcinoma cells in a final volume of 0.2 mL PBS. The cells are s.c. injected into the right flank of each NOD/SCID mouse. In the established tumor model, initiation of treatment is delayed until s.c. growing OVCAR3 tumors are developed (50-200 mm^3). Once tumors develop to the appropriate size range, the animals are randomly divided into groups. Eight animals per group are treated starting at days 4, 8, and 12, respectively; using 1mg/kg as EPCAM MMAE mAb for both the DNA dendrimer bound and unbound EPCAM-MMAE ADCs. Treatment is repeated every 4 days for a total of 1-3 treatments. As a control, antibody without drug is used. Tumors are measured on the indicated days with a caliper in two perpendicular dimensions and tumor volumes are calculated according to the following formula: tumor volume = [(width^2 x length) / 2].

Various modifications of the described subject matter, in addition to those described herein, will be apparent to those skilled in the art from the foregoing description. Such

modifications are also intended to fall within the scope of the appended claims. Each reference (including, but not limited to, journal articles, U.S. and non-U.S. patents, patent application publications, international patent application publications, gene bank accession numbers, and the like) cited in the present application is incorporated herein by reference in its entirety.

What Is Claimed Is:

1. A single layer nucleic acid carrier comprising five monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to a terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to a terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to a terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to a terminal arm of the fifth monomer, forming a single layer nucleic acid carrier having twelve peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

2. A 1.5 layer nucleic acid carrier comprising eleven monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated

to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, and the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, forming a 1.5 layer nucleic acid carrier having 24 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

3. A two layer nucleic acid carrier comprising 17 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the

third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer, forming a two layer nucleic acid carrier having 36 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

4. A 2.5 layer nucleic acid carrier comprising 35 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth

terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer;

wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer,

wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, and the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer;

forming a 2.5 layer nucleic acid carrier having 72 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

5. A three layer nucleic acid carrier comprising 53 monomers, wherein each monomer comprises:

a first and a second oligonucleotide;

wherein a central portion of each of the first and second oligonucleotides is complementary to each other, forming a double-stranded region,

wherein the two terminal portions of the first oligonucleotide are not complementary to the two terminal portions of the second oligonucleotide, forming four terminal arms;

wherein the first terminal arm of the first monomer is conjugated to the first terminal arm of the second monomer, the second terminal arm of the first monomer is conjugated to the first terminal arm of the third monomer, the third terminal arm of the first monomer is conjugated to the first terminal arm of the fourth monomer, and the fourth terminal arm of the first monomer is conjugated to the first terminal arm of the fifth monomer;

wherein the second terminal arm of the second monomer is conjugated to the first terminal arm of the sixth monomer, the second terminal arm of the third monomer is conjugated to the first terminal arm of the seventh monomer, the second terminal arm of the fourth monomer is conjugated to the first terminal arm of the eighth monomer, the second terminal arm of the fifth monomer is conjugated to the first terminal arm of the ninth monomer, the third terminal arm of the second monomer is conjugated to the first terminal arm of the tenth monomer, the third terminal arm of the third monomer is conjugated to the first terminal arm of the eleventh monomer, the third terminal arm of the fourth monomer is conjugated to the first terminal arm of the twelfth monomer, the third terminal arm of the fifth monomer is conjugated to the first terminal arm of the thirteenth monomer, the fourth terminal arm of the second monomer is conjugated to the first terminal arm of the fourteenth monomer, the fourth terminal arm of the third monomer is conjugated to the first terminal arm of the fifteenth monomer, the fourth terminal arm of the fourth monomer is conjugated to the first terminal arm of the sixteenth monomer, and the fourth terminal arm of the fifth monomer is conjugated to the first terminal arm of the seventeenth monomer;

wherein the second terminal arm of the sixth monomer is conjugated to the first terminal arm of the eighteenth monomer, the second terminal arm of the seventh monomer is conjugated to the first terminal arm of the nineteenth monomer, the second terminal arm of the eighth monomer is conjugated to the first terminal arm of the twentieth monomer, the second terminal arm of the ninth monomer is conjugated to the first terminal arm of the twenty-first monomer, the second terminal arm of the tenth monomer is conjugated to the first terminal arm of the twenty-second monomer, the second terminal arm of the eleventh monomer is conjugated to the first terminal arm of the twenty-third monomer, the second terminal arm of the twelfth

monomer is conjugated to the first terminal arm of the twenty-fourth monomer, the second terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the twenty-fifth monomer, the second terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the twenty-sixth monomer, the second terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the twenty-seventh monomer, the second terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the twenty-eighth monomer, and the second terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the twenty-ninth monomer,

wherein the third terminal arm of the sixth monomer is conjugated to the first terminal arm of the thirtieth monomer, the third terminal arm of the seventh monomer is conjugated to the first terminal arm of the thirty-first monomer, the third terminal arm of the eighth monomer is conjugated to the first terminal arm of the thirty-second monomer, the third terminal arm of the ninth monomer is conjugated to the first terminal arm of the thirty-third monomer, the third terminal arm of the tenth monomer is conjugated to the first terminal arm of the thirty-fourth monomer, the third terminal arm of the eleventh monomer is conjugated to the first terminal arm of the thirty-fifth monomer, the third terminal arm of the twelfth monomer is conjugated to the first terminal arm of the thirty-sixth monomer, the third terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the thirty-seventh monomer, the third terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the thirty-eighth monomer, the third terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the thirty-ninth monomer, the third terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fortieth monomer, and the third terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the forty-first monomer;

wherein the fourth terminal arm of the sixth monomer is conjugated to the first terminal arm of the forty-second monomer, the fourth terminal arm of the seventh monomer is conjugated to the first terminal arm of the forty-third monomer, the fourth terminal arm of the eighth monomer is conjugated to the first terminal arm of the forty-forth monomer, the fourth terminal arm of the ninth monomer is conjugated to the first terminal arm of the forty-fifth monomer, the fourth terminal arm of the tenth monomer is conjugated to the first terminal arm of the forty-sixth monomer, the fourth terminal arm of the eleventh monomer is conjugated to the first terminal arm of the forty-seventh monomer, the fourth terminal arm of the twelfth monomer is conjugated to the first terminal arm of the forty-eighth monomer, the fourth terminal arm of the thirteenth monomer is conjugated to the first terminal arm of the forty-ninth monomer, the fourth terminal arm of the fourteenth monomer is conjugated to the first terminal arm of the fiftieth

monomer, the fourth terminal arm of the fifteenth monomer is conjugated to the first terminal arm of the fifty-first monomer, the fourth terminal arm of the sixteenth monomer is conjugated to the first terminal arm of the fifty-second monomer, and the fourth terminal arm of the seventeenth monomer is conjugated to the first terminal arm of the fifty-third monomer;

forming a three layer nucleic acid carrier having 108 peripheral terminal arms; and

at least one targeting agent conjugated to at least one peripheral terminal arm, wherein when the at least one targeting agent is an miRNA, the nucleic acid carrier comprises at least one different targeting agent.

6. The nucleic acid carrier of any one of claims 1 to 5, wherein the at least one targeting agent is an antigen, a peptide, an antibody or fragment thereof, an antibody-drug conjugate, a non-antibody scaffold, a label, an adjuvant, an RNA molecule, a DNA molecule, a vitamin, a protein, a fusion protein, a fusion peptide, a carbohydrate, a lipid, a polysaccharide, a lipopolysaccharide, a polymer, a virus particle, or a virus-like particle, or any combination thereof.

7. The nucleic acid carrier of claim 6, wherein:

the polymer is hyaluronic acid, polyargenine, polylysine, polyethylenimine (PEI), polyethyleglycol (PEG), polyglycolic acid (PGA), polylactic acid (PLA), or poly(lactic-co-glycolic acid) (PLGA);

the non-antibody scaffold is an affibody, an affilin, an anticalin, an atrimer, an avimer, a bicyclic peptide, a cys-knot, a DARPin, an FN3, a fynomer, a kunitz domain, or an O-body;

the antigen is a bacterial antigen, a viral antigen, a fungal antigen, a yeast antigen, a protozoan antigen, or prion;

the antibody fragment is Fab, F(ab')2, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc;

the antibody, or fragment thereof, is directed to a disease-associated antigen, which is a tumor-associated antigen chosen from 4-1BB, 5AC, 5T4, A2aR, activin receptor-like kinase 1, AGS-22M6, AKAP4, alpha-fetoprotein, angiopoietin 2, B7-H3, BAFF, BAGE, BCR-ABL, BORIS, CA-125, CA19-9, C242 antigen, carbonic anhydrase 9 (CA-IX), CCR4, CD19, CD20, CD22, CD23 (IgE receptor), CD24, CD28, CD30 (TNFRSF8), CD33, CD37, CD38 (cyclic ADP ribose hydrolase), CD40, CD44 v6, CD51, CD56, CD70, CD71, CD73, CD74, CD79B, CD80, CD137, CD140a, CD152, CD200, CD221, CD274, CEA, ch4D5, CLDN18.2, CS1, CSF1R, CTLA-4, C-X-C chemokine receptor type 4, DLL4, DR5, EBAG9, EGF, EGFR, EGFL7,

EpCAM, ERBB2, ERBB3, FAP, fibronectin extra domain-B, folate receptor 1, folate receptor alpha, folate hydrolase, Frizzled receptor, GAGE, GD2 ganglioside, GD3 ganglioside, glioma, glypican 3, GPNMB, gp100, GUCY2C, HER1, HER2/neu, HER3, HGF, HHGFR, histone complex, HLA-DR, human scatter factor receptor kinase, HPV-16, HSP105, IDH1, IDO1, IGF-I, IGF-1 receptor, ILGF2, IL-6, IL-13, integrin αvβ3, integrin α5β1, KIR, LAG-3, Lewis-Y antigen, LY6K, MAGE-1, MAGE-A3, MAGE-C2, MAGE-D4, MAPG, MART-1, Melan-A, MET, MCP-1, mesothelin, MIF, MSLN, MS4A1, mucin CanAg, MUC1, MUC4, MUC16, NG2, N-glycolylneuraminic acid, Notch receptor PD-1, NY-ESO-1, OCAA, PAP, PDGF-R α, PDCD1, PD1, PD-L1, phosphate-sodium co-transporter, phosphatidylserine, PRAME, PSA, RANKL, RON, ROR1, SDC1, Sialyl-Tn, SLAMF7, SPAG-9, SSX1, STEAP1, survivin, TAG-72, telomerase, TEM1, tenascin C, TGF-β, TIM-3, TLR, TAM, TIM-3, TRAIL-R2, TRAIL-R1, TWEAK receptor, tumor specific glycosylation of MUC1, tumor-associated calcium signal transducer 2, tumor antigen CTAA16.88, TYRP1 (glycoprotein 75), VEGF-A, VEGFR2, VEGFR-1, vimentin, VISTA, WT1, and XAGE-1b;

the antibody, or fragment thereof, is directed to a disease-associated antigen which is chosen from 1-40-β-amyloid, AOC3 (VAP-1), ACVR2B, angiopoietin 3, beta-amyloid, C5, CCL11 (eotaxin-1), CCR5, CD2, CD3, CD4, CD5, CD11, CD18, CD20, CD23 (IgE receptor), CD25 (α chain of IL-2 receptor), CD28, CD41 (integrin alpha-IIb), CD52, CD125, CD147 (basigin), CD154 (CD40L), CEA-related antigen, clumping factor A, endotoxin, GMCSF receptor α-chain, growth differentiation factor 8, hemagglutinin, HNGF, Hsp90, IGHE, IgE Fc region, IL-1β, IL-4, IL-5, IL-6, IL-9, IL-12, IL-13, IL-17, IL-17A, IL-20, IL-22, IL-23, IL-6 receptor, integrin α4β7, integrin α7β7, integrin α4, integrin αIIbβ3, interferon α/β receptor, interferon gamma-induced protein, IFN-γ, IFN-α, ITGB2 (CD18), LFA-1 (CD11a), LINGO-1, lipoteichoic acid, LOXL2, myelin-associated glycoprotein, myostatin, neural apoptosis-regulated proteinase 1, NGF, NOGO-A, Oryctolagus cuniculus, OX-40, PCSK9, phosphatidylserine, platelet-derived growth factor receptor beta, RANKL, Rhesus factor, sclerostin, SOST, sphingosine-1-phosphate, TFPI, TGF-β, TGF beta 2, TGF beta 1, TNF-α, VEGF-A, and VWF;

the antibody, or fragment thereof, is directed to a cell surface marker on an immune cell chosen from a T cell, B cell, NK cell, macrophage, TIL, dendritic cell, neutrophil, eosinophil, basophil, and mast cell; or

wherein the RNA molecule is siRNA, miRNA, mRNA, snRNA, dsRNA, ncRNA, snoRNA, or an aptamer.

8. The nucleic acid carrier of claim 6, comprising one or two of the following:

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to CD64;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1;

a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX40;

a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG;

a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG;

a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16;

a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3;

a first targeting agent which is folic acid or folic acid receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16; and

a first targeting agent which is an antibody, or fragment thereof, directed to PD-1 and a second targeting agent which is an antibody, or fragment thereof, directed to PDL-1 or PDL-2.

9. The nucleic acid carrier of any one of claims 1 to 5, wherein the targeting agent is an antigen, an antibody, or a fragment of an antibody, and the oligonucleotide linker conjugating the targeting moiety to the at least one single-stranded arm is a CpG containing oligonucleotide.

10. The nucleic acid carrier of any one of claims 1 to 5, wherein the nucleic acid carrier comprises at least one antigen, at least one antibody or fragment thereof directed to an immune cell, and at least one adjuvant.

11. The nucleic acid carrier of any one of claims 1 to 10, further comprising a chemotherapeutic agent, a cytokine, or a chemokine.

12. A pharmaceutical composition comprising a nucleic acid carrier of any one of claims 1 to 11, and a pharmaceutically acceptable vehicle.

13. A method of inducing an immune response in a mammal comprising administering to the mammal a nucleic acid carrier of any one of claims 1 to 11, or a pharmaceutical composition comprising a nucleic acid carrier of any one of claims 1 to 11, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antigen, a peptide, an antibody or fragment thereof, and an adjuvant.

14. The method of claim 13, wherein the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc.

15. A method of treating a mammal having cancer comprising administering to the mammal a nucleic acid carrier of any one of claims 1 to 11, or a pharmaceutical composition comprising a nucleic acid carrier of any one of claims 1 to 11, wherein the nucleic acid carrier comprises at least two targeting agents chosen from an antibody, or fragment thereof, and a label.

16. The method of claim 15, wherein the antibody fragment is Fab, $F(ab')_2$, scFv, tandem scFv, a BiTE, single domain (sdAb) antibody, diabody, single chain diabody, minibody, fusion protein, or scFv-Fc.

17. The method of claim 15 or claim 16, wherein the nucleic acid carrier comprises:

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is breast, ovarian, or prostrate;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to CD64, and wherein the cancer is a solid tumor, lung, or colorectal;

a first targeting agent which is an antibody, or fragment thereof, directed to EGFR and a second targeting agent which is an antibody, or fragment thereof, directed to PD-1, and wherein the cancer is triple negative breast cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to PD-L1 and a second targeting agent which is an antibody, or fragment thereof, directed to OX-40, and wherein the cancer is breast cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to CD30 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is Hodgkin's disease;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is metastatic breast cancer or prostrate;

a first targeting agent which is an antibody, or fragment thereof, directed to CD20 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is non-Hodgkin lymphoma or multiple myeloma;

a first targeting agent which is an antibody, or fragment thereof, directed to EpCAM and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and

wherein the cancer is ovarian, gastric, colon, colorectal, breast, non-small cell lung cancer, adenocarcinoma of the lung, small cell lung cancer;

a first targeting agent which is an antibody, or fragment thereof, directed to NG2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is B-precursor acute lymphoblastic leukemia or non-Hodgkin lymphoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CD19 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is non-Hodgkin lymphoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein the cancer is colorectal, lung carcinoma, pancreas carcinoma, stomach carcinoma, ovary carcinoma, uterus carcinoma, breast carcinoma, or melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to MUC-1 and a second targeting agent which is an antibody, or fragment thereof, directed to HSG, and wherein the cancer is invasive pancreatic adenocarcinoma;

a first targeting agent which is an antibody, or fragment thereof, directed to CA19-9 and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a CA19-9-positive tumor;

a first targeting agent which is an antibody, or fragment thereof, directed to CEA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian;

a first targeting agent which is an antibody, or fragment thereof, directed to OCAA and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is ovarian;

a first targeting agent which is an antibody, or fragment thereof, directed to MAPG and a second targeting agent which is an antibody, or fragment thereof, directed to CD28, and wherein the cancer is metastatic melanoma;

a first targeting agent which is an antibody, or fragment thereof, directed to HER2 and a second targeting agent which is an antibody, or fragment thereof, directed to CD16, and wherein the cancer is a HER-2 positive tumor;

a first targeting agent which is an antibody, or fragment thereof, directed to glioma and a second targeting agent which is an antibody, or fragment thereof, directed to CD3, and wherein the cancer is a glioma; and

a first targeting agent which is folic acid or folic acid receptor and a second targeting agent which is an antibody, or fragment thereof, directed to CD3 or CD16, and wherein the cancer is small cell lung carcinoma, breast, or ovarian.

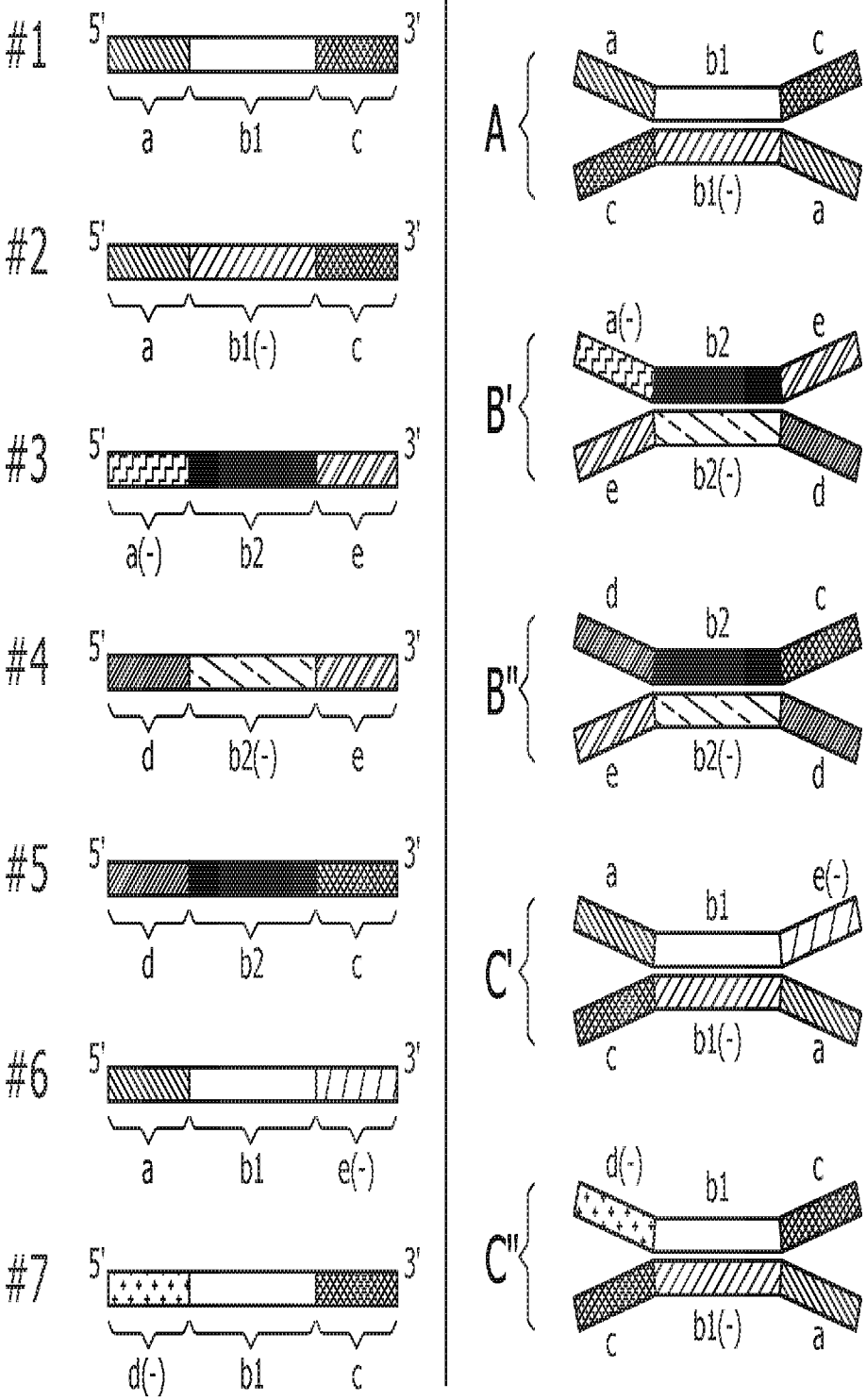
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FIG. 1

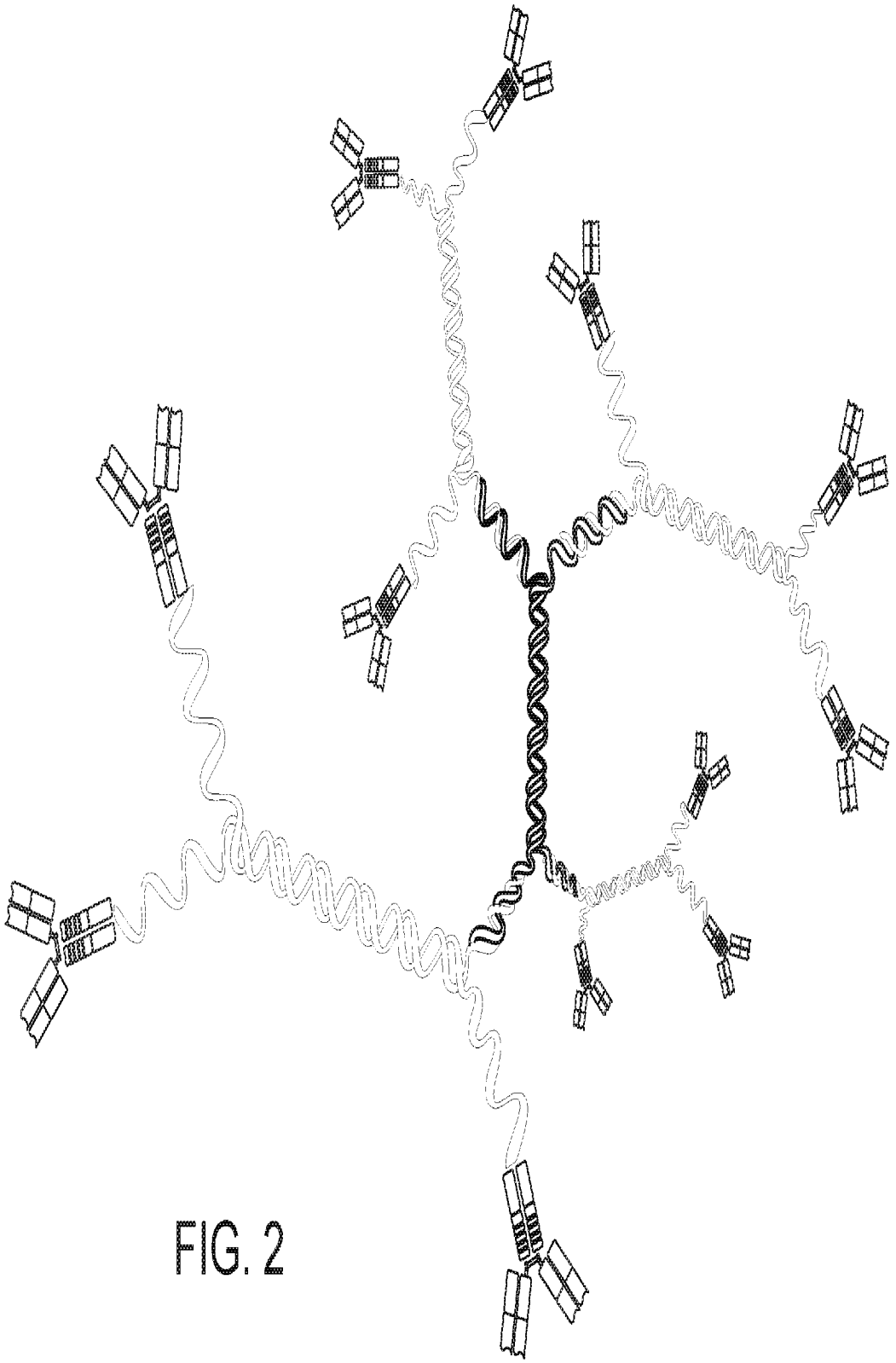
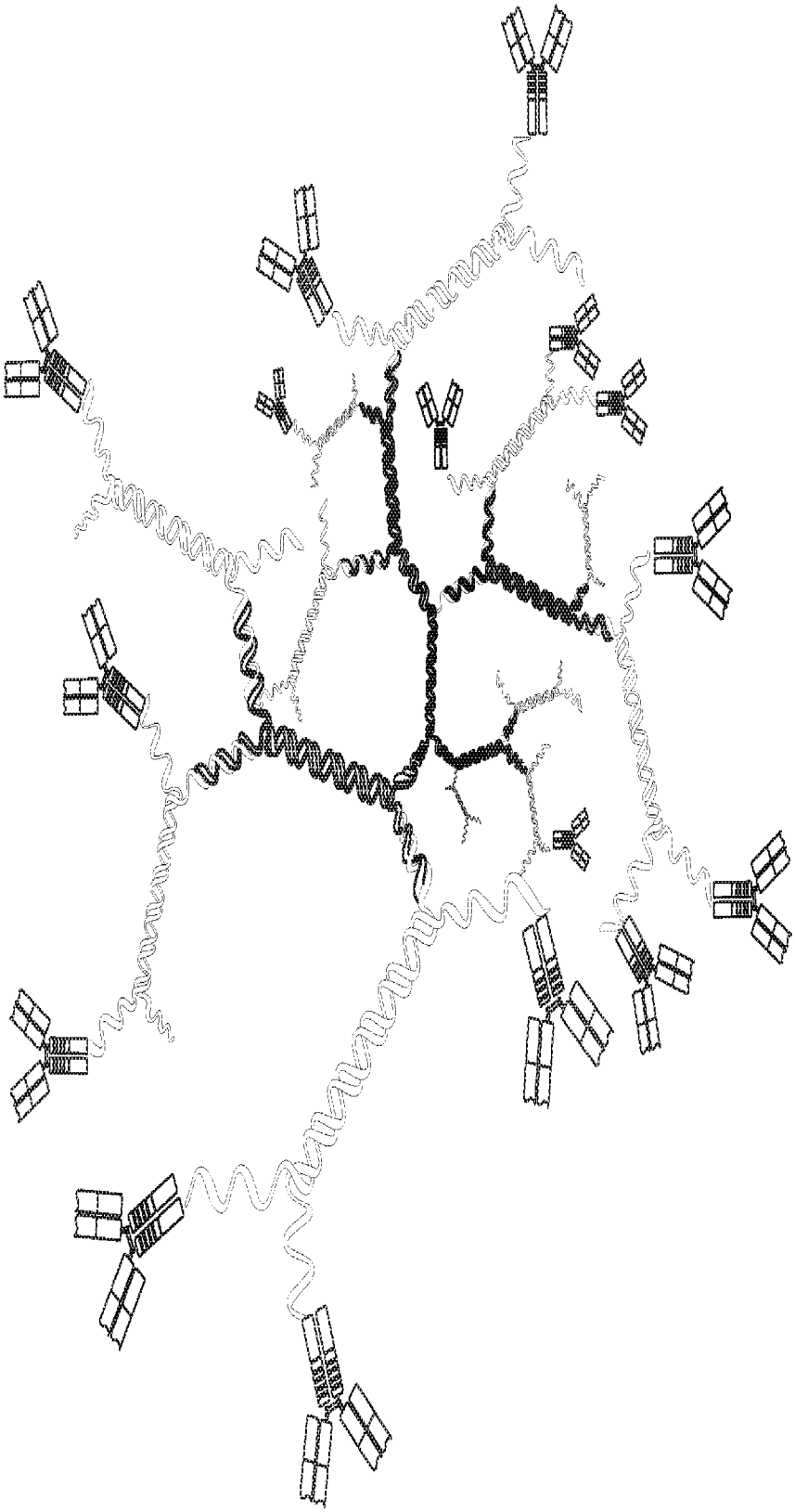


FIG. 2



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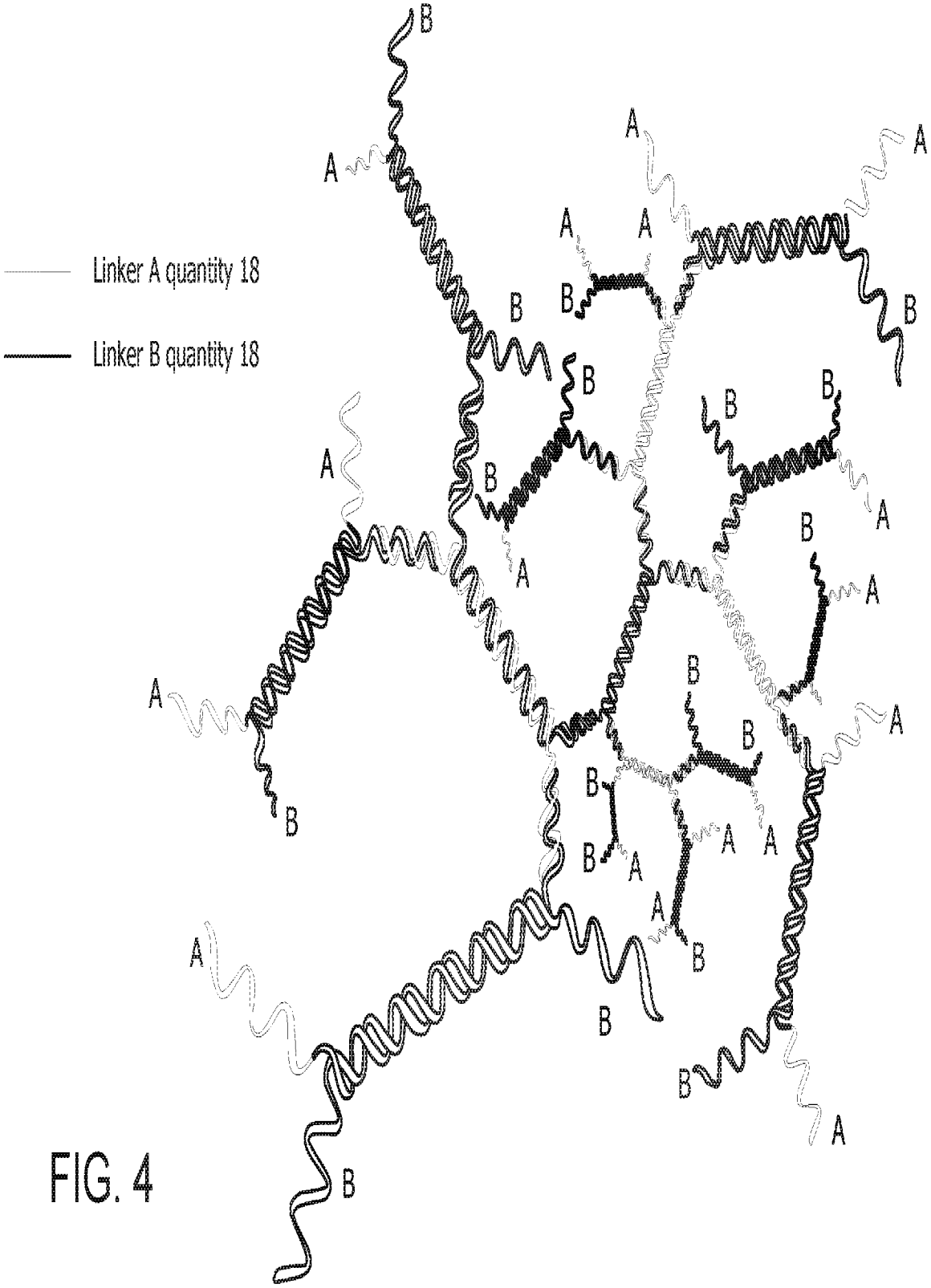

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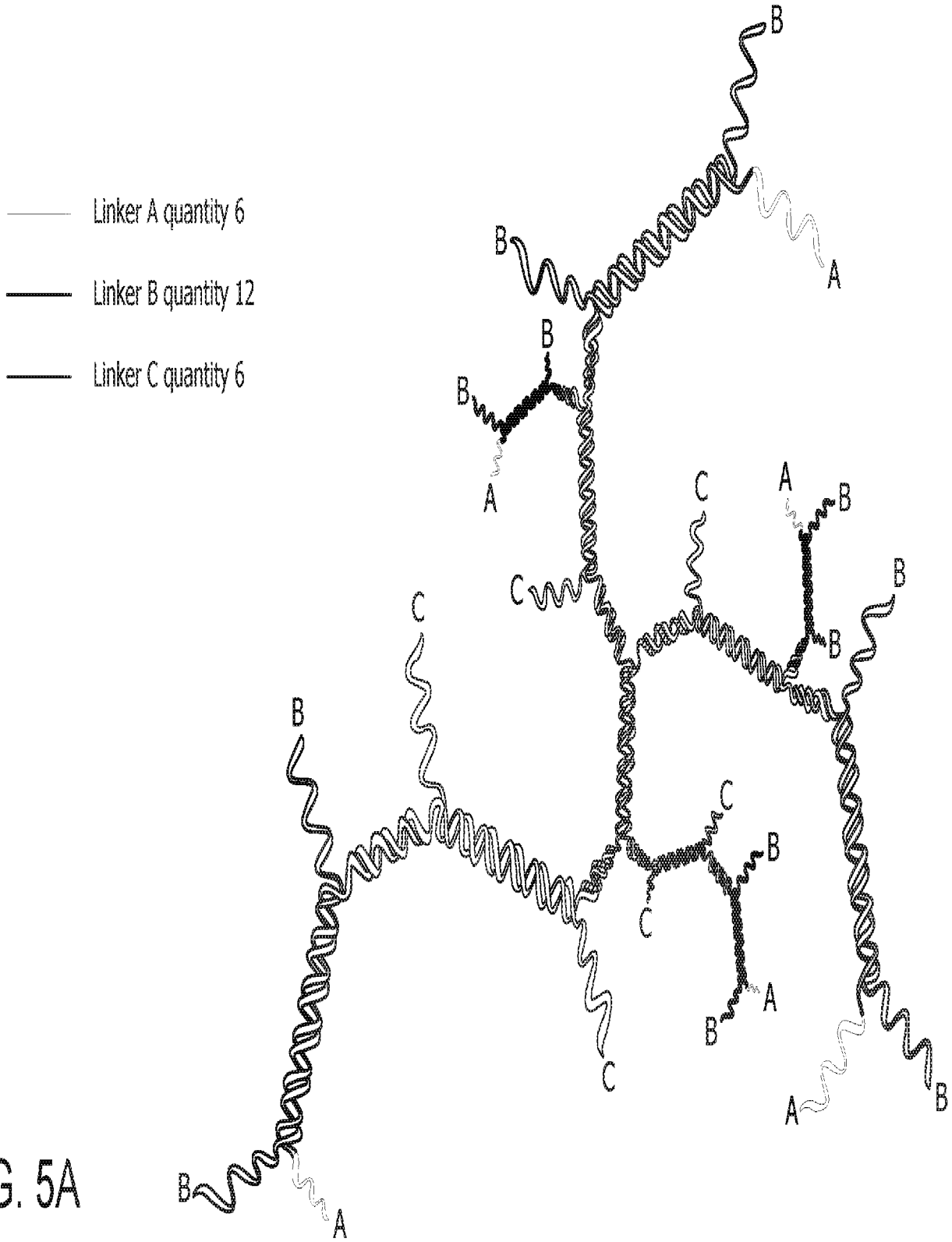
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FIG. 5A

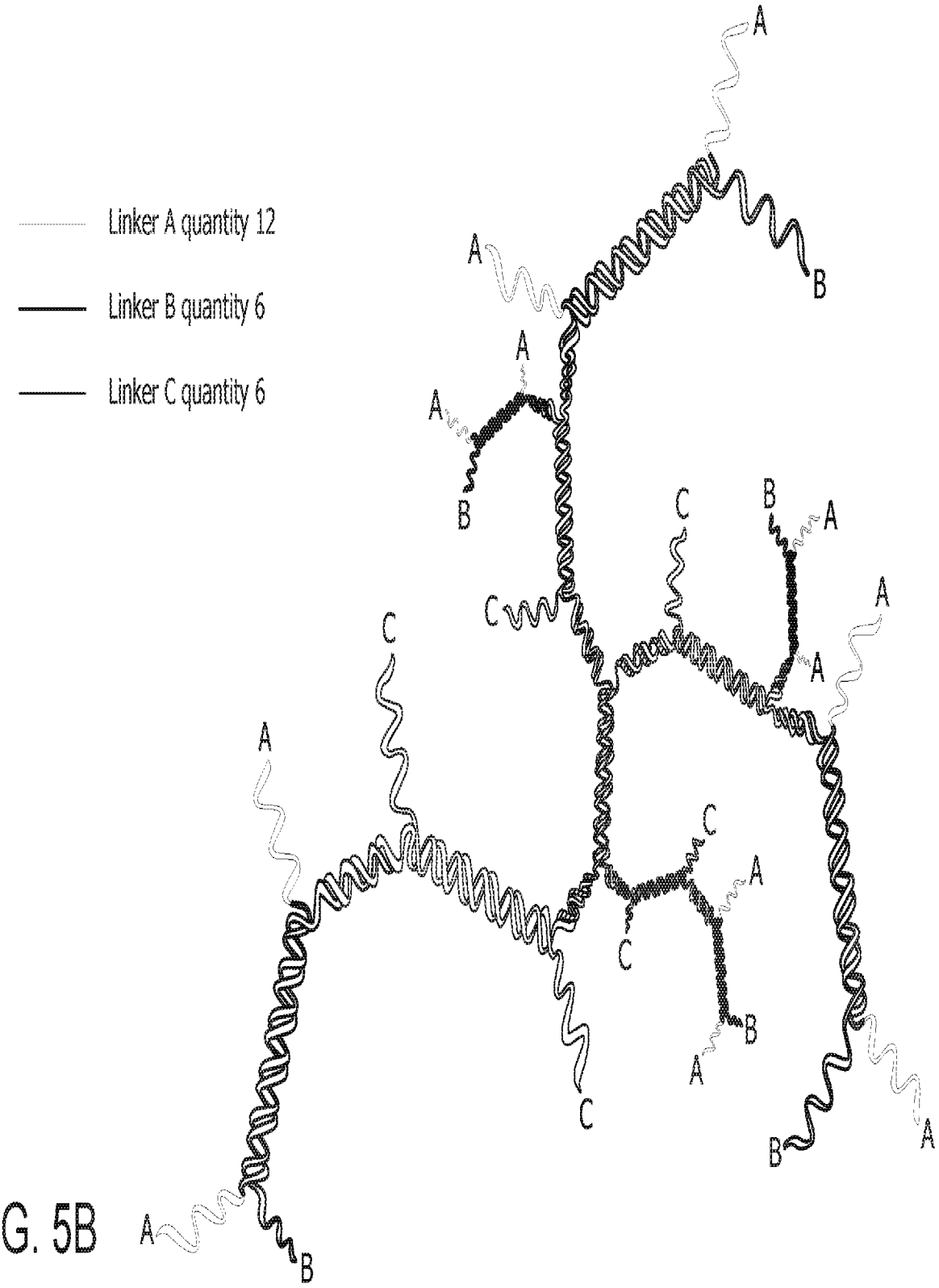
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FIG. 5B

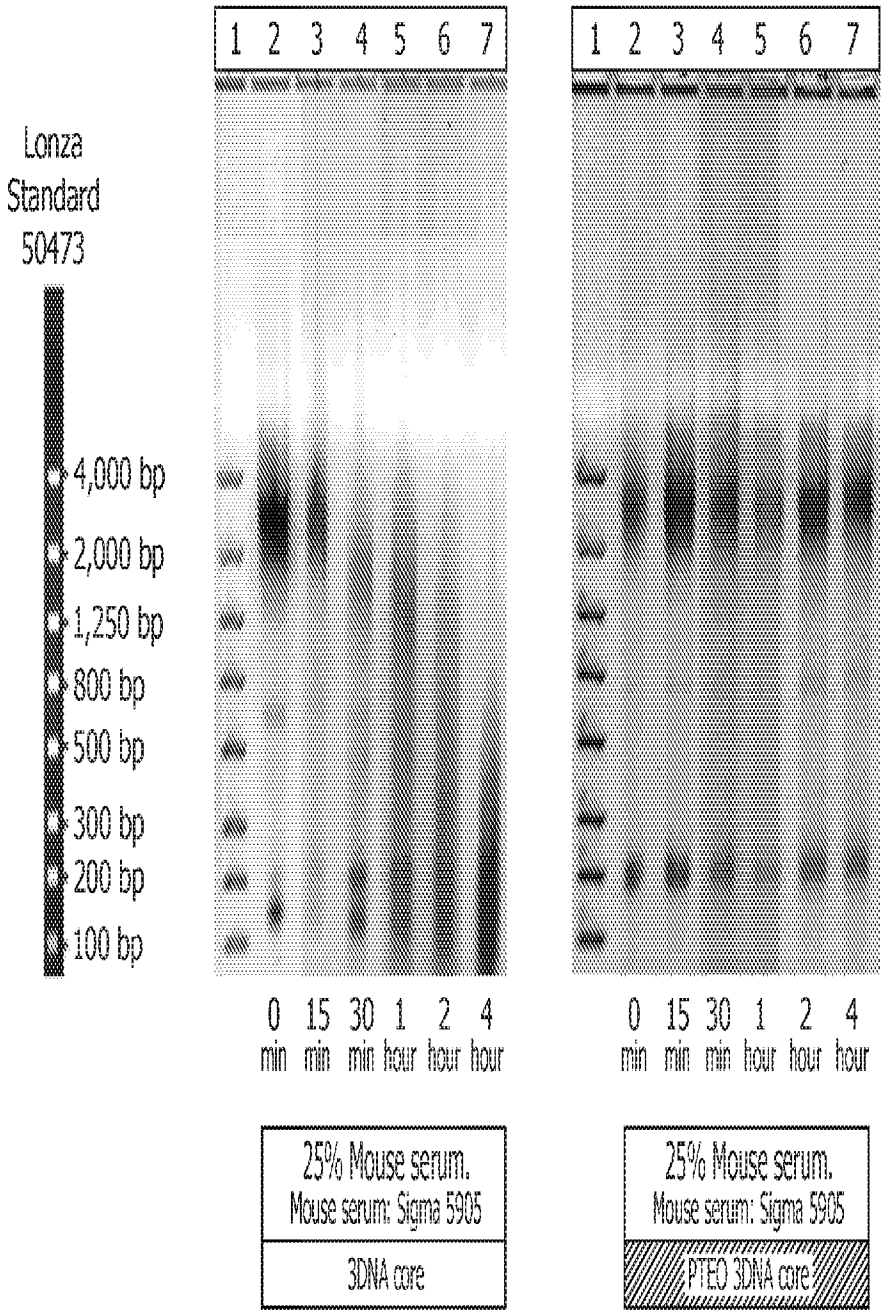
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FIG. 6

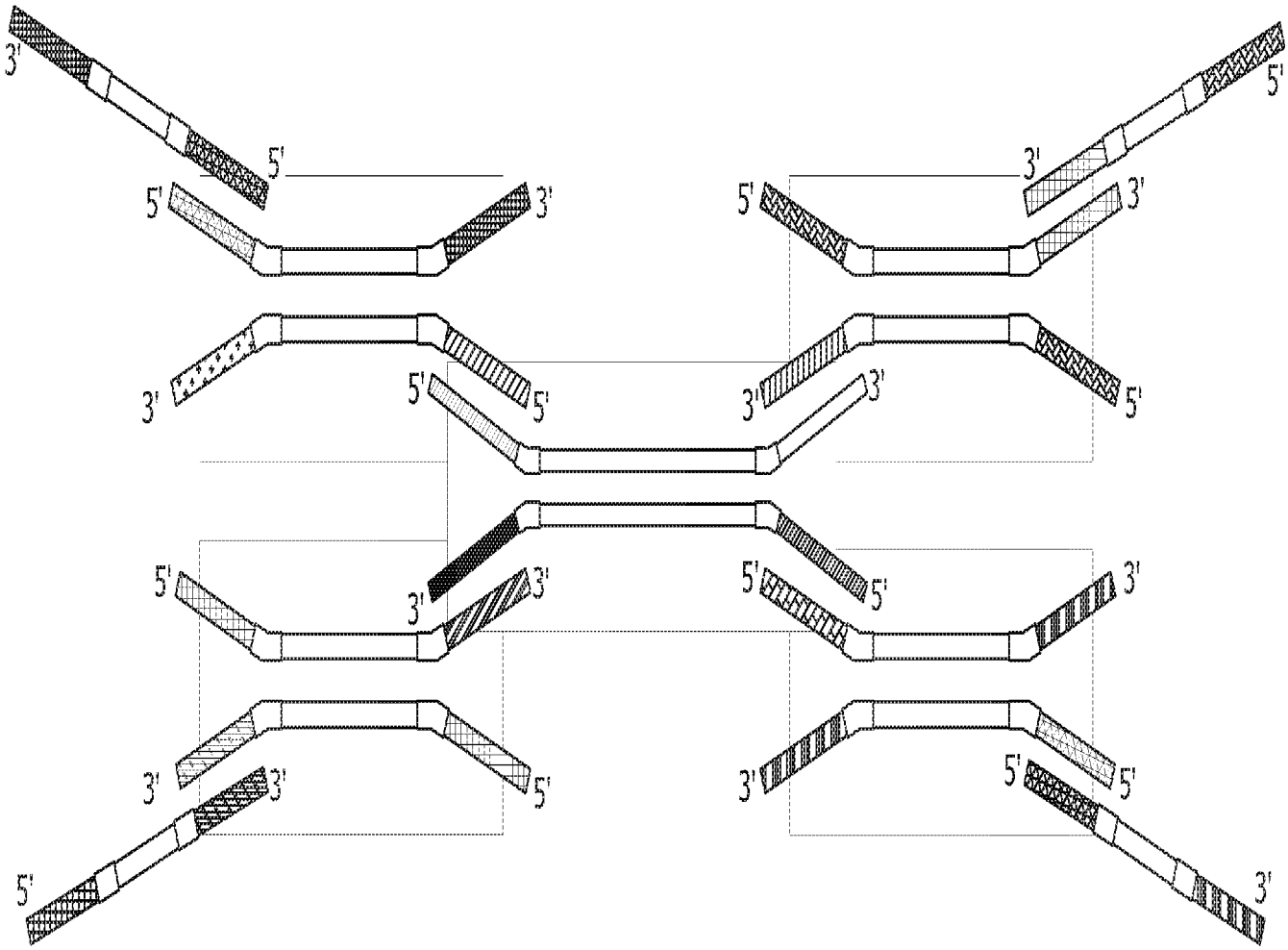

FIG. 7

SEQUENCE LISTING

<110> Getts, Robert C.
Kadushin, James M.
Getts, Lori

<120> NUCLEIC ACID CARRIERS AND THERAPEUTIC METHODS OF USE

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