Title: ANTI-CD74 IMMUNOCONJUGATES AND METHODS

Abstract: Disclosed are compositions that include anti-CD74 immunoconjugates and a therapeutic and/or diagnostic agent. Also disclosed are methods for preparing the immunoconjugates and using the immunoconjugates in diagnostic and therapeutic procedures. The compositions may be part of a kit for administering the anti-CD74 immunoconjugate compositions in therapeutic and/or diagnostic methods.
Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published: without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.
ANTI-CD74 IMMUNOCONJUGATES AND METHODS

This application claims the benefit of provisional application serial no. 60/478,830, filed on June 17, 2003, and application serial no. 10/706,852, filed November 12, 2003, the contents of which are incorporated herein by reference in its entirety.

BACKGROUND

Conventional therapeutic agents do not distinguish between normal cells and diseased cells, and therefore conventional agents can damage and kill normal proliferating cells and tissues. To reduce the toxic side effects of conventional agents, the agents can be specifically targeted to the diseased cells or tissues. One method of specifically targeting therapeutic agents to diseased cells or tissues relies on the use of specific binding molecules such as antibodies or fragments thereof, and in particular, monoclonal antibodies.

The use of targeting binding molecules (e.g., monoclonal antibodies) conjugated to therapeutic or diagnostic agents offers the possibility of delivering such agents directly to the targeted cells or tissue (e.g., a tumor), thereby limiting the exposure of normal tissues to toxic agents (Goldenberg, Semin. Nucl. Med., 19: 332 (1989)). In recent years, the potential of antibody-based therapy and its accuracy in the localization of antigens, such as tumor-associated antigens, have been demonstrated both in the laboratory and clinical studies (see, e.g., Thorpe, TIBTECH, 11: 42 (1993); Goldenberg, Scientific American, Science & Medicine, 1: 64 (1994); Baldwin et al., U.S. 4,925,922 and 4,916,213; Young, U.S. 4918163; U.S. 5,204,095; Irie et al., U.S. 5,196,337; Hellstrom et al., U.S. 5,134,075 and 5,171,665). For tumors, the use of radio-labeled antibodies or antibody fragments against tumor-
associated markers for localization has been more successful than for therapy, in part because antibody uptake by the tumor is generally low, ranging from only 0.01% to 0.001% of the total dose injected (Vaughan et al., Brit. J. Radiol., 60: 567 (1987)). Increasing the concentration of the radiolabel to increase the dosage to the tumor is counterproductive, generally, as this also increases exposure of healthy tissue to radioactivity. As such, a method of increasing uptake of therapeutic or diagnostic agents is desirable.

While therapeutic or diagnostic agents may be directed conjugated to an antibody as a targeting agent, the agents also may be indirectly associated with an antibody. For example, liposomes, nanoparticles, and polymers have been used as carriers for therapeutic agents, where antibodies may be conjugated to the carrier to provide specific targeting of the carrier/agent complex to a diseased cell or tissue. (See, e.g., Xu et al., Mol. Cancer. Ther., 1:337-346 (2002); Torchilin, et al., Proc. Nat'l. Acad. Sci., 10: 6039 (2003); U.S. 6,165,440; U.S. 5,702,727; U.S. 5,620,708; U.S. 5,565,215; U.S. 6,530,944; U.S. 6,562,318; U.S. 6,558,648; and U.S. 6,395,276). However, to facilitate uptake of the complex into the cell, it is important to select antibody/antigen partners where the antigen is rapidly cycled from the surface of the targeted cell to the interior of the targeted cell. One such antigen is CD74, which is an epitope of the major histocompatibility complex (MHC) class II antigen invariant chain, Ii, present on the cell surface and taken up in large amounts of up to 8 X 10^6 molecules per cell per day (Hansen et al., Biochem. J., 320: 293-300 (1996). CD74 is present on the cell surface of B-lymphocytes, monocytes and histocytes, human B-lymphoma cell lines, melanomas, T-cell lymphomas and a variety of other tumor cell types. (Id.)

Murine LL1 (mLL1 or murine anti-CD74 antibody) is a specific monoclonal antibody (mAb) reactive with CD74. Cell surface-bound LL1 is rapidly internalized to the lysosomal compartment and quickly catabolized, much faster than other mAbs, such as anti-CD19 and anti-CD22. (Id.) This inherent property of LL1 overcomes some of the aforementioned difficulties with immunotherapy.

Murine LL1 was developed by fusion of mouse myeloma cells with splenocytes from BALB/c mice immunized with preparations from the Raji B-
lymphoma cell line (called EPB-1 in Pawlak-Byczkowska et al., Can. Res., 49: 4568 (1989)). The clinical use of mLL1, just as with most other promising murine antibodies, has been limited by the development in humans of a human anti-mouse antibody (HAMA) response. A HAMA response is generally not observed following injection of mLL1 Fab', as evidenced in a bone marrow imaging study using a mLL1 Fab' labeled with $^{99m}$Tc. Juweid et al., Nuc. Med. Comm. 18: 142-148 (1997). However, in some therapeutic and diagnostic uses, a full-length anti-CD74 mAb may be preferred. This use of the full-length anti-CD74 mAb can limit the diagnostic and therapeutic usefulness of such antibodies and antibody conjugates, not only because of the potential anaphylactic problem, but also as a major portion of the circulating conjugate may be complexed to and sequestered by the circulating anti-mouse antibodies. Although the use of antibody fragments of mLL1 may circumvent the problems of immunogenicity, there are circumstances in which whole IgG is more desirable and the induction of cellular immunity is intended for therapy or enhanced antibody survival time. In general, HAMA responses pose a potential obstacle to realizing the full diagnostic and therapeutic potential of murine anti-CD74 mAbs. Therefore, the development of immunoconjugates that include chimeric, humanized and human anti-CD74 binding molecules, (e.g., mAbs and fragments thereof, antibody fusion proteins thereof and fragments thereof, multivalent and/or multispecific mAbs and fragments thereof), would be extremely useful for therapy and diagnosis, with reduced production of human anti-mouse antibodies.

SUMMARY

Disclosed is a composition that includes an effector molecule (e.g., a therapeutic or diagnostic agent) and an immunoconjugate, where the immunoconjugate includes an anti-CD74 binding molecule conjugated to one or more carriers. The carrier is capable of delivering the effector and may include molecules that can form a higher-ordered structure, (such as lipids or polymers), or the carrier may be a higher-ordered structure itself, (such as a micelle, liposome, or nanoparticle). The effector molecule may be covalently or non-covalently associated
with the anti-CD74 binding molecule, the carrier, or both. In one embodiment, the
composition comprises an emulsion or a liposome.

The anti-CD74 binding molecule may be conjugated or linked to the carrier by
a number of linkages including sulfide linkages, hydrazone linkages, hydrazine
linkages, ester linkages, amido linkages, amino linkages, imino linkages,
thiosemicarbazone linkages, semicarbazone linkages, oxime linkages, and carbon-
carbon linkages. A sulfide linkage may be preferred, where the binding molecule
may include disulfide linkages, which may be reduced to provide free thiol groups.

The composition may include additional binding molecules, (e.g., antibodies
or fragments thereof that bind to CD19, CD20, CD22, CD30, CD33, CD52, CD80,
HLA-DR, MUC1, TAC, IL-6, tensascin, VEGF, placental growth factor, carbonic
anhdrase IX, and mixtures thereof). The additional binding molecules may be
covaently or non-covalently associated with any component of the composition (e.g.,
the carrier).

Where the carrier is a lipid, preferably the lipid is capable of forming an
emulsion or a higher-ordered structure such as a micelle or liposome. For example,
the lipid may be amphiphilic. To facilitate conjugation to the anti-CD74 binding
molecule, the lipid may contain one or more groups capable of reacting with the anti-
CD74 binding molecule, such as nucleophilic carbons, (e.g., at a distal terminus). In
one embodiment, the lipid is polyethyleneglycol (PEG)-maleimide and the anti-CD74
binding molecule reacts via free thiol groups with the maleimide group. Maleimide
groups may also be present on other carriers as described herein for conjugating the
anti-CD74 binding molecule. For example, nanoparticles may contain maleimide
groups for conjugating the anti-CD74 binding molecule. In addition to maleimide
groups, other groups for conjugating binding molecules may include vinylsulfones.

In addition to the effector, which may include one or more therapeutic or
diagnostic agents, the composition may further include additional therapeutic or
diagnostic agents, which may be covalently, non-covalently, or otherwise associated
with any component of the composition. For example, the additional therapeutic or
diagnostic agent may be covalently linked to the anti-CD74 binding molecule.
Alternatively, the additional therapeutic or diagnostic agent may be covalently linked to the carrier or non-covalently or otherwise associated with the carrier.

The effector may include any number of therapeutic or diagnostic agents. For example, the effector may include a drug, prodrug, toxin, enzyme, radioisotope, immunomodulator, cytokine, hormone, binding molecule (e.g., an antibody), or an oligonucleotide molecule (e.g., an antisense molecule or a gene). Antisense molecules may include antisense molecules that correspond to bcl-2 or p53. The effector may include aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10- hydroxycamptothecin, carmustine, celebres, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, daunomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUDR), 3',5'-O-dioleoyl-FudR (FUDR-dO), fludarabine, flutamide, fluorouracil, fluoroxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, PSI-341, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiopeta, teniposide, toptecan, uracil mustard, velcade, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin, or combinations thereof. In certain embodiments, the effector includes FUDR, or FUDR-dO.

The composition may also include one or more hard acid chelators or soft acid chelators. For example, the chelator may include NOTA, DOTA, DTPA, TETA, Tscg-Cys, or Tsca-Cys. In certain embodiments, the chelators may form complexes with cations selected from Group II, Group III, Group IV, Group V, transition,
lanthanide or actinide metal cations, or mixtures thereof. Alternatively, the cations may be covalently, non-covalently, or otherwise associated with any component of the complex. In certain embodiments, the composition includes cations selected from Tc, Re, Bi, Cu, As, Ag, Au, At, or Pb.

5 The composition may also include a nuclide (e.g., a radionuclide). The nuclide may be selected from a number of nuclides including $^{18}$F, $^{32}$P, $^{33}$P, $^{45}$Ti, $^{47}$Sc, $^{52}$Fe, $^{59}$Fe, $^{62}$Cu, $^{64}$Cu, $^{67}$Cu, $^{68}$Ga, $^{75}$Se, $^{77}$As, $^{85}$Y, $^{89}$Sr, $^{89}$Y, $^{90}$Y, $^{94}$Tc, $^{94m}$Tc, $^{99}$Mo, $^{99m}$Tc, $^{105}$Pd, $^{106}$Rh, $^{111}$Ag, $^{111}$In, $^{123}$I, $^{124}$I, $^{125}$I, $^{131}$I, $^{142}$Pr, $^{143}$Pr, $^{149}$Pm, $^{153}$Sm, $^{154}$Gd, $^{161}$Tb, $^{166}$Dy, $^{166}$Ho, $^{169}$Er, $^{175}$Lu, $^{177}$Lu, $^{186}$Re, $^{188}$Re, $^{189}$Re, $^{194}$Ir, $^{198}$Au, $^{199}$Au, $^{211}$At, $^{211}$Pb, $^{212}$Bi, $^{212}$Pb, $^{213}$Bi, $^{223}$Ra, or $^{225}$Ac.

Where the effector is an enzyme, suitable enzymes may include carboxylesterases, glucoronidases, carboxypeptidases, beta-lactamases, phosphatases, and mixtures thereof. Where the effector is an immunomodulator, suitable immunomodulators may include IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-α, interferon-β, interferon-γ, G-CSF, GM-CSF, and mixtures thereof. The effector may also include an anti-angiogenic agent, e.g., angiostatin, endostatin, basculostatin, canstatin, maspin, anti-VEGF binding molecules, anti-placental growth factor binding molecules, or anti-vascular growth factor binding molecules.

Preferably, the anti-CD74 binding molecule may be LL1 or a fragment thereof, although any anti-CD74 binding molecule is suitable. For example, production of monoclonal antibodies is well known in the art. See Harlow & Lane (eds), Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory, NY. However, human, chimeric, or humanized derivatives of LL1 or fragments thereof may be particularly suitable. Derivatives of LL1 are described in U.S. Application Serial No. 10/377,122, and U.S. Application Serial No. 60/360,259, filed March 1, 2002, which are incorporated herein by reference in their entirety. The anti-CD74 binding molecule or fragment thereof may be monoclonal.

The anti-CD74 binding molecule may include a human, chimeric, or humanized anti-CD74 antibody or fragment thereof. For example, a binding molecule may contain CDRs of a light chain variable region of a murine anti-CD74 mAb. A humanized anti-CD74 antibody or fragment may include a heavy chain variable
region of a humanized mAb, which may include CDRs of a heavy chain variable
region of a murine anti-CD74 mAb. A humanized anti-CD74 antibody or fragment
thereof may include light and heavy chain variable regions including
complementarity-determining regions (CDRs) of murine anti-CD74 (mLL1) and a
framework (FR) regions of a human antibody, where a light chain variable region of a
humanized anti-CD74 mAb includes CDRs of a light chain variable region of a
murine anti-CD74 mAb, and where a heavy chain variable region of a humanized
mAb includes CDRs of a heavy chain variable region of a murine anti-CD74 mAb. A
humanized anti-CD74 antibody or fragment thereof may include FRs of light and
heavy chain variable regions of a humanized anti-CD74 antibody or fragment thereof,
which may be substituted with at least one amino acid from corresponding FRs of a
murine mAb. A mAb or fragment may include a humanized IgG1.

A anti-CD74 binding molecule may be a chimeric anti-CD74 antibody or
fragment thereof and may include a light chain variable region of a murine anti-CD74
mAb. For example, A chimeric anti-CD74 antibody or fragment thereof may include
a heavy chain variable region of a murine anti-CD74 mAb. A chimeric anti-CD74
antibody or fragment thereof also may include light and heavy chain variable regions
which may include complementarity-determining regions (CDRs) of a murine anti-
CD74 mAb; framework (FR) regions of a murine anti-CD 74 mAb; and light and
heavy chain constant regions of a human antibody, where light chain variable region
of the chimeric mAb may include CDRs of a light chain variable region of a murine
anti-CD74 mAb. A chimeric mAb or fragment thereof may include a chimeric IgG1
or fragment thereof.

Where the anti-CD74 binding molecule includes a human anti-CD74 antibody
or fragment thereof, the binding molecule may include a light chain variable region of
a human anti-CD74 mAb. For example, a human anti-CD74 antibody or fragment
thereof may include a heavy chain variable region of the human mAb which may
include CDRs of a heavy chain variable region of a murine anti-CD74 mAb. In
another example, a human anti-CD74 antibody or fragment thereof may include the
light and heavy chain variable and constant regions of a human antibody. A human
mAb or fragment thereof may include a human IgG1.
The anti-CD74 binding molecule may be selected such that the binding of the molecule or fragment thereof to CD-74 is blocked by an antibody of fragment thereof specific for CD74. Alternatively, the binding molecule may be selected such that the mAb or fragment thereof is internalized by Raji lymphoma cells in culture. In another embodiment, an anti-CD74 binding molecule, such as a mAb or fragment thereof, may be selected such that it induces apoptosis of Raji cells in cell culture when cross-linked with goat antisera reactive with the Fc of a murine IgG1 mAb.

The anti-CD74 binding molecule may also include a fragment which includes a F(ab')2, Fab, scFv, Fv, or a fusion protein utilizing part or all of the light and heavy chains of the F(ab')2, Fab, scFv, or Fv, such that the fragment is capable of binding to CD74. The binding molecule may be selected or designed to be multivalent, or multivalent and multispecific. The fragments may form a bispecific binding molecule or a diabody. In one embodiment, the binding molecule includes a fusion protein that includes four or more Fvs, or Fab’s of the mAbs or fragments thereof. In a further embodiment, the binding molecule includes a fusion protein that includes one or more Fvs, or Fab’s of an anti-CD74 mAb or fragment thereof, and one or more Fvs or Fab’s from an antibody specific for a tumor cell marker that is not a CD74 antigen. For example, the tumor cell marker may include a B-cell lineage antigen such as CD19, CD20, or CD22. Alternatively, the tumor cell marker may include HLA-DR, CD30, CD33, CD52, MUC1, or TAC.

The anti-CD 74 binding molecule may include constant regions of IgG1, which are replaced with human constant regions of human IgG2a, IgG3, or IgG4.

Also disclosed is a method for treating and/or diagnosing a disease or disorder that includes administering to a patient a therapeutic and/or diagnostic composition. The therapeutic and/or diagnostic composition includes any of the aforementioned compositions, generally a composition that includes: (1) an effector molecule (e.g., a therapeutic or diagnostic agent); (2) an anti-CD74 binding molecule or fragment thereof conjugated to one or more carriers; and (3) a pharmaceutically acceptable excipient. Typically, the composition is administered to the patient intravenously or intramuscularly at a dose of 20-5000 mg.
The disease or disorder is typically a CD74-expressing malignancy, which may include an immune dysregulation disease, an autoimmune disease, an organ-graft rejection, a graft-versus-host disease, a solid tumor, non-Hodgkin's lymphoma, Hodgkin's lymphoma, multiple myeloma, a B-cell malignancy, or a T-cell malignancy. A B-cell malignancy may include indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, and/or multiple myeloma. Solid tumors may include melanomas, carcinomas, sarcomas, and/or gliomas. A carcinoma may include renal carcinoma, lung carcinoma, intestinal carcinoma, stomach carcinoma, breast carcinoma, prostate cancer, ovarian cancer, and/or melanoma.

In one embodiment, the composition may comprise an agent for photodynamic therapy, e.g., a photosensitizer such as a benzoporphyrin monoacid ring A (BPDMA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AlSPc), or lutetium texaphyrin (Lutex). The method may also include administering an irradiating light source to the targeted cells or tissue. In certain embodiments, photodynamic therapy may be used diagnostically as well as therapeutically.

The method may include administering a composition that includes a diagnostic nuclide, e.g., ¹⁸F, ⁵²Fe, ⁶⁷Cu, ⁶³Cu, ⁶³Cu, ⁶⁷Ga, ⁷⁸Ga, ⁸⁶Y, ⁹⁰Zr, ⁹⁴Te, ⁹⁷⁴mTc, ⁹⁹mTc, ¹¹¹In, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I. Typically, the diagnostic nuclide will emit 25-4000 keV gamma particles and/or positrons.

The method may include administering a composition that includes a diagnostic agent, which can be used to perform positron emission tomography (PET). As such, the method may include performing positron-emission tomography (PET).

The method may include administering a composition that includes one or more image enhancing agents, e.g., gadolinium ions, lanthanum ions, manganese ions, iron, chromium, copper, cobalt, nickel, fluorine, dysprosium, rhenium, europium, terbium, holmium, neodymium, or mixtures thereof. As such, the method may include performing an imaging technique such as magnetic resonance imaging (MRI).

The method may include administering a composition that includes one or more radiopaque agents or contrast agents such as barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iocamide, iopamidone, iodoxamic acid,
iogulamide, iohexol, iopamidol, iopanoic acid, ioprocyclic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemectic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizoic acid, ipodate, meglumine, metrizamide, metrizoate, propylidone, thallous chloride, or combinations thereof. The method may include performing an X-ray or computed tomography (CT).

The method may include administering a composition that includes one or more ultrasound contrast agents, such as dextran or a liposome (e.g., a gas-filled liposome). The method may include performing an ultrasound procedure.

In addition to the aforementioned procedures, the method may also include performing an operative, intravascular, laparoscopic, or endoscopic procedure, before, simultaneously, or after the immunoconjugate or composition is administered.

The method may also include administering a second or additional composition that includes a therapeutic or diagnostic agent, where the second or additional composition is administered before, simultaneously, or after the first composition is administered. The second or additional composition may include any of the aforementioned compositions. In one embodiment the second or additional composition includes an anti-CD74 binding molecule conjugated to a therapeutic or diagnostic agent. The therapeutic or diagnostic agent may comprise any of the aforementioned drugs, prodrugs, toxins, enzymes, radioisotopes, immunomodulators, cytokines, hormones, antibodies, binding molecules, oligonucleotides, chelators, cations, therapeutic nuclides, agents for photodynamic therapy, diagnostic nuclides, image enhancing agents, radiopaque agents, and/or contrast agents. The method may also include performing a PET, MRI, X-ray, CT, ultrasound, operative, intravascular, laparoscopic, or endoscopic procedure.

Also disclosed are methods of preparing the aforementioned compositions (e.g., an anti-CD74 immunoconjugate by mixing one or more amphiphilic lipids with an effector to form a lipid drug-carrier and contacting the lipid drug-carrier with an anti-CD74 binding molecule, (e.g., an antibody or fragment thereof)). In one example, the lipid contains nucleophilic carbons (e.g., within a maleimide group), and the binding molecule contains free thiol groups (e.g., disulfides treated with a reducing agent). The method may include mixing the composition with one or more
therapeutic or diagnostic agents, which may be covalently, non-covalently, or other wise associated with any component of the composition.

Also disclosed is a kit that includes any of the aforementioned compositions or the components sufficient for preparing any of the aforementioned compositions. Typically, the kit includes instructions for administering the compositions or, where applicable, instructions for preparing the compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. DNA and amino acid sequences of the murine LL1 heavy and light chain variable regions. Figure 1A shows DNA and amino acid sequences of LL1 VH. Figure 1B shows DNA and amino acid sequences of the LL1 Vk. Amino acid sequences encoded by the corresponding DNA sequences are given as one-letter codes below the nucleotide sequence. Numbering of the nucleotide sequence is on the right side. The amino acid residues in the CDR regions are shown in bold and underlined. Kabat’s Ig molecule numbering is used for amino acid residues as shown by the numbering above the amino acid residues. The residues numbered by a letter following a particular digit indicates the insertion residues defined by Kabat numbering scheme. The insertion residues numbered with a letter have the same preceding digit. For example, residues 82A, 82B and 82C in Figure 1A are indicated as 82A, B, and C.

Figure 2. DNA and amino acid sequences of chimeric LL1 (cLL1) heavy and light chain variable region. (See, U.S. Serial No. 10/377,122.) Figure 2A shows DNA and amino acid sequences of cLL1 VH. Figure 2B shows double-stranded DNA and amino acid sequences of cLL1 Vk. Amino acid sequences encoded by the corresponding DNA sequences are given as one-letter codes. The amino acid residues in the CDR regions are shown in bold and underlined. The numbering of nucleotides and amino acids is same as that in Figure 1.

Figure 3. Alignment of amino acid sequences of light and heavy chain variable regions of a human antibody, cLL1 and hLL1. Figure 3A shows the VH amino acid sequence alignment of the human antibody RF-TS3, cLL1 and hLL1 and Figure 3B shows the Vk amino acid sequence alignment of the human antibody HF-
21/28, cLL1 and hLL1. Dots indicate the residues in cLL1 that are identical to the corresponding residues in the human antibodies. Boxed regions represent the CDR regions. Both N- and C-terminal residues (underlined) of cLL1 are fixed by the staging vectors used and not compared with the human antibodies. Kabat's Ig molecule number scheme is used as in Figure 1.

Figure 4. DNA and amino acid sequences of humanized LL1 (hLL1) heavy and light chain variable regions. Figure 4A shows the DNA and amino acid sequences of hLL1VH and Figure 4B shows the DNA and amino acid sequences of hLL1Vk. Amino acid sequences encoded by the corresponding DNA sequences are given as one letter codes. The amino acid residues in the CDR regions are shown in bold and underlined. Kabat's Ig molecule numbering scheme is used for amino acid residues as in Fig. 1A and Fig. 1B.

Figure 5. Concentration-dependent cellular association of [³H] Cholesteryl oleyl ether (COE)-labeled, targeted and untargeted, drug-carrier emulsions during a 24-h incubation at 37°C. A. Top panel. Raji cells were treated with LL1-conjugated emulsions (●) and unconjugated emulsions (○). Ramos cells were treated with LL1-conjugated emulsions (▼) and unconjugated emulsions (▼). B. Bottom panel. HL-60 cells were treated with LL1-conjugated emulsions (●) and unconjugated emulsions (○). Jurkat cells were treated with LL1-conjugated emulsions (▼) and unconjugated emulsions (▼). (Mean ± SE, n=4).

Figure 6. Cellular association of [³H]COE-labeled LL1-emulsions versus amount of LL1 per emulsion egg phosphatidylcholine (EPC) during a 24-h incubation at 37°C. (Mean ± SE, n=4).

Figure 7. Displacement curve for the association of [³H]COE-labeled LL1-emulsions in the presence of different concentrations of free LL1. (Mean ± SE, n=4).

Figure 8. Concentration-dependent association of 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS)-containing LL1-liposomes during a 6-h incubation at 37°C, measured at λ = 413 nm (●) and λ = 454 nm (○). (Mean ± SE, n=3).

Figure 9. Dose-response curves for FUDR-dO. A. Top panel. Raji lymphoma cells were treated with LL1-conjugated emulsions containing FUDR-dO
(●); unconjugated emulsions containing F UdR-dO (○); or free F UdR in PBS as a comparison (▼). B. Bottom panel. Raji lymphoma cells were treated with LL1-conjugated liposomes containing F UdR-dO (●); unconjugated liposomes containing F UdR-dO (○); or free F UdR in PBS as a comparison (▼). (Mean ± SE, n=4).

DETAILED DESCRIPTION

Definitions

In the description that follows, a number of terms are used and the following definitions are provided to facilitate understanding of the disclosure herein.

A binding molecule, as described herein, is any molecule that can specifically bind to an antigen. A binding molecule may include an antibody or a fragment thereof. An anti-CD74 binding molecule is a molecule that specifically binds to the CD74 antigen, such as an anti-CD74 antibody or fragment thereof. Other anti-CD74 binding molecules may also include multivalent molecules, multispecific molecules (e.g., diabodies), fusion molecules, or other naturally occurring or recombinantly created molecules.

An antibody, as described herein, refers to a full-length (i.e., naturally occurring or formed by normal immunoglobulin gene fragment recombinatorial processes) immunoglobulin molecule (e.g., an IgG antibody) or an immunologically active (i.e., specifically binding) portion of an immunoglobulin molecule, like an antibody fragment.

An antibody fragment is a portion of an antibody such as F(ab)₂, F(ab)₂, Fab, Fab, Fv, sFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. For example, an anti-CD74 monoclonal antibody fragment binds with an epitope of CD74. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex. For example, antibody fragments include isolated fragments consisting of the variable regions, such as the “Fv” fragments consisting of the variable regions of the heavy
and light chains, recombinant single chain polypeptide molecules in which light and heavy variable regions are connected by a peptide linker ("scFv proteins"), and minimal recognition units consisting of the amino acid residues that mimic the hypervariable region.

A chimeric antibody is a recombinant protein that contains the variable domains including the complementarity determining regions (CDRs) of an antibody derived from one species, preferably a rodent antibody, while the constant domains of the antibody molecule is derived from those of a human antibody. For veterinary applications, the constant domains of the chimeric antibody may be derived from that of other species, such as a cat or dog.

A humanized antibody is a recombinant protein in which the CDRs from an antibody from one species; e.g., a rodent antibody, is transferred from the heavy and light variable chains of the rodent antibody into human heavy and light variable domains. The constant domains of the antibody molecule is derived from those of a human antibody.

A human antibody is an antibody obtained from transgenic mice that have been "engineered" to produce specific human antibodies in response to antigenic challenge. In this technique, elements of the human heavy and light chain locus are introduced into strains of mice derived from embryonic stem cell lines that contain targeted disruptions of the endogenous heavy chain and light chain loci. The transgenic mice can synthesize human antibodies specific for human antigens, and the mice can be used to produce human antibody-secreting hybridomas. Methods for obtaining human antibodies from transgenic mice are described by Green et al., Nature Genet. 7:13 (1994), Lonberg et al., Nature 368:856 (1994), and Taylor et al., Int. Immun. 6:579 (1994). A fully human antibody also can be constructed by genetic or chromosomal transfection methods, as well as phage display technology, all of which are known in the art. (See, e.g., McCafferty et al., Nature 348:552-553 (1990) for the production of human antibodies and fragments thereof in vitro, from immunoglobulin variable domain gene repertoires from unimmunized donors). In this technique, antibody variable domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, and displayed as functional
antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. In this way, the phage mimics some of the properties of the B cell. Phage display can be performed in a variety of formats, for their review, see, e.g. Johnson and Chiswell, *Current Opinion in Structural Biology* 3:5564-571 (1993). Human antibodies may also be generated by *in vitro* activated B cells. (See, U.S. Patent Nos. 5,567,610 and 5,229,275, which are incorporated in their entirety by reference).

An *effector* is an atom, molecule, or compound that brings about a chosen result. An effector may include a therapeutic agent and/or a diagnostic agent as described herein.

A *therapeutic agent* is an atom, molecule, or compound that is useful in the treatment of a disease. Examples of therapeutic agents include antibodies, antibody fragments, drugs, toxins, enzymes, nucleases, hormones, immunomodulators, antisense oligonucleotides, chelators, boron compounds, photoactive agents or dyes and radioisotopes.

A *diagnostic agent* is an atom, molecule, or compound that is useful in diagnosing a disease. Useful diagnostic agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (e.g., paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Patent No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent and is incorporated in its entirety by reference. Preferably, the diagnostic agents are selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polyllysine, polysaccharide, or other derivatized or derivatizable chain having pendant groups to which can be bound chelating groups such as, e.g.
ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates are coupled to the peptide antigens using standard chemistries. The chelate is normally linked to the antibody by a group which enables formation of a bond to the molecule with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other, more unusual, methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Patent 4,824,659 to Hawthorne, entitled “Antibody Conjugates”, issued April 25, 1989, the disclosure of which is incorporated herein in its entirety by reference.

Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes in the general energy range of 60 to 4,000 keV. Some useful diagnostic nuclides may include, such as $^{18}$F, $^{52}$Fe, $^{62}$Cu, $^{64}$Cu, $^{67}$Cu, $^{68}$Ga, $^{86}$Ga, $^{89}$Y, $^{90}$Zr, $^{94}$Tc, $^{99m}$Tc, $^{99m}$Tc, or $^{111}$In. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the antibodies and carriers described herein. Macrocyclic chelates such as NOTA, DOTA, and TETA are of use with a variety of metals and radiometals, most particularly with radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be made very stable by tailoring the ring size to the metal of interest. Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as $^{223}$Ra for RAIT may be used.

An immunon conjugate is a conjugate of a binding molecule (e.g., an antibody component) with an atom, molecule, or a higher-ordered structure (e.g., with a carrier, a therapeutic agent, or a diagnostic agent). The diagnostic agent can comprise a radioactive or non-radioactive label, a contrast agent (such as for magnetic resonance imaging, computed tomography or ultrasound), and the radioactive label can be a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope. A naked antibody is an antibody that is not conjugated to any other agent.

A carrier is an atom, molecule, or higher-ordered structure that is capable of associating with a therapeutic or diagnostic agent to facilitate delivery of the agent to a targeted cell. Carriers may include molecules such as lipids or polymers (e.g.,
amphiphilic lipids that are capable of forming higher-ordered structures, or carbohydrates such as dextran), or higher-ordered structures themselves, such as micelles, liposomes, or nanoparticles.

As used herein, the term antibody fusion protein is a recombinantly produced antigen-binding molecule in which two or more of the same or different single-chain antibody or antibody fragment segments with the same or different specificities are linked. Valency of the fusion protein indicates how many binding arms or sites the fusion protein has to a single antigen or epitope; i.e., monovalent, bivalent, trivalent or multivalent. The multivalency of the antibody fusion protein means that it can take advantage of multiple interactions in binding to an antigen, thus increasing the avidity of binding to the antigen. Specificity indicates how many antigens or epitopes an antibody fusion protein is able to bind; i.e., monospecific, bispecific, trispecific, multispecific. Using these definitions, a natural antibody, e.g., an IgG, is bivalent because it has two binding arms but is monospecific because it binds to one epitope. Monospecific, multivalent fusion proteins have more than one binding site for an epitope but only binds with one epitope, for example a diabody with two binding site reactive with the same antigen. The fusion protein may comprise a single antibody component, a multivalent or multispecific combination of different antibody components or multiple copies of the same antibody component. The fusion protein may additionally comprise an antibody or an antibody fragment and a therapeutic agent. Examples of therapeutic agents suitable for such fusion proteins include immunomodulators ("antibody-immunomodulator fusion protein") and toxins ("antibody-toxin fusion protein"). One preferred toxin comprises a ribonuclease (RNase), preferably a recombinant RNase.

A multispecific antibody is an antibody that can bind simultaneously to at least two targets that are of different structure, e.g., two different antigens, two different epitopes on the same antigen, or a hapten and/or an antigen or epitope. One specificity would be for a B-cell, T-cell, myeloid-, plasma-, and mast-cell antigen or epitope. Another specificity could be to a different antigen on the same cell type, such as CD20, CD19, CD21, CD23, CD46, CD80, HLA-DR, CD74, and CD22 on B-cells. A multivalent antibody is an antibody that can bind simultaneously to at least
two targets that are of the same or different structure. Multispecific, multivalent antibodies are constructs that have more than one binding site of different specificity. For example, a diabody, where one binding site reacts with one antigen and the other with another antigen.

A bispecific antibody is an antibody that can bind simultaneously to two targets which are of different structure. Bispecific antibodies (bsAb) and bispecific antibody fragments (bsFab) have at least one arm that specifically binds to, for example, a B-cell, T-cell, myeloid-, plasma-, and mast-cell antigen or epitope and at least one other arm that specifically binds to a targetable conjugate that bears a therapeutic or diagnostic agent. A variety of bispecific fusion proteins can be produced using molecular engineering. In one form, the bispecific fusion protein is monovalent, consisting of, for example, a scFv with a single binding site for one antigen and a Fab fragment with a single binding site for a second antigen. In another form, the bispecific fusion protein is divalent, consisting of, for example, an IgG with a binding site for one antigen and two scFv with two binding sites for a second antigen.

A nanoparticle refers to a particle of size ranging from 1 to 1000 nm. Typically, a nanoparticle is biodegradable, biocompatible, and it functions as a carrier capable of incorporating the substance to be delivered to a targeted cell.

Preparation of Monoclonal Antibodies including Chimeric, Humanized and Human Antibodies

The immunoconjugates and compositions described herein may include monoclonal antibodies. Rodent monoclonal antibodies to specific antigens may be obtained by methods known to those skilled in the art. (See, e.g., Kohler and Milstein, Nature 256: 495 (1975), and Coligan et al. (eds.), CURRENT PROTOCOLS IN IMMUNOLOGY, VOL. 1, pages 2.5.1-2.6.7 (John Wiley & Sons 1991)).

General techniques for cloning murine immunoglobulin variable domains have been described, for example, by the publication of Orlandi et al., Proc. Nat’l Acad. Sci. USA 86: 3833 (1989), which is incorporated by reference in its entirety.

Humanized antibodies and antibody fragments are described in Provisional U.S. Application titled “Anti-CD20 Antibodies And Fusion Proteins Thereof And Methods Of Use”, U.S. Provisional No. 60/356,132 filed February 14, 2002; U.S. Provisional Application No. 60/416,232 filed October 7, 2002; and U.S. Provisional Application No. 60/366,709 filed February 14, 2003. Other humanized antibodies include hMN-14 antibodies, such as those disclosed in U.S. Application No. 5,874,540, which recognize a Class III anti-carcinoembryonic antigen antibody (anti-CEA antibody); Mu-9 antibodies, such as those described in U.S. Application No. 10/116,116; AFP antibodies, such as those described in U.S. Provisional Application No. 60/399,707 filed August 1, 2002; PAM4 antibodies, such as those described in Provisional U.S. Application 60/388/313, filed June 14, 2002; RS7 antibodies, such as those described in U.S. Provisional Application No. 60/360,229, filed March 1, 2002; and CD22 antibodies, such as those disclosed in U.S. Patent Nos. 5,789,554 and 6,187,287 and U.S. Application Nos. 09/741,843, 09/988,013, and U.S. Serial No. 10/377,122 all of which are incorporated herein by reference in their entirety.

A chimeric antibody is a recombinant protein that contains the variable domains including the CDRs derived from one species of animal, such as a rodent antibody, while the remainder of the antibody molecule; i.e., the constant domains, is derived from a human antibody. Accordingly, a chimeric monoclonal antibody can
also be humanized by replacing the sequences of the murine FR in the variable domains of the chimeric mAb with one or more different human FR. Specifically, mouse CDRs are transferred from heavy and light variable chains of the mouse immunoglobulin into the corresponding variable domains of a human antibody. As simply transferring mouse CDRs into human FRs often results in a reduction or even loss of antibody affinity, additional modification might be required in order to restore the original affinity of the murine antibody. This can be accomplished by the replacement of one or more some human residues in the FR regions with their murine counterparts to obtain an antibody that possesses good binding affinity to its epitope. (See, e.g., Tempest et al., Biotechnology 9:266 (1991) and Verhoeyen et al., Science 239: 1534 (1988)). Further, the affinity of humanized, chimeric and human MAbs to a specific epitope can be increased by mutagenesis of the CDRs, so that a lower dose of antibody may be as effective as a higher dose of a lower affinity MAb prior to mutagenesis. (See, e.g., WO0029584A1).

A fully human antibody, i.e., human anti-CD74 MAbs or other human antibodies, such as anti-CD22, anti-CD19, anti-CD23, anti-CD20 or anti-CD21 MAbs for combination therapy with humanized, chimeric or human anti-CD74 antibodies, can be obtained from a transgenic non-human animal. (See, e.g., Mendez et al., Nature Genetics, 15: 146-156 (1997)); U.S. Patent No. 5,633,425, which are incorporated in their entirety by reference. For example, a human antibody can be recovered from a transgenic mouse possessing human immunoglobulin loci.

Engineered Binding Molecules

Further recent methods for producing bispecific mAbs include engineered recombinant MAbs which have additional cysteine residues so that they crosslink more strongly than the more common immunoglobulin isotypes. (See, e.g., Fitzgerald et al., Protein Eng. 10(10):1221-1225, (1997)). Another approach is to engineer recombinant fusion proteins linking two or more different single-chain antibody or antibody fragment segments with the needed dual specificities. (See, e.g., Coloma et al., Nature Biotechnol. 15:159-163, (1997)). A variety of bispecific fusion proteins can be produced using molecular engineering. In one form, the bispecific
fusion protein is monovalent, consisting of, for example, a scFv with a single binding site for one antigen and a Fab fragment with a single binding site for a second antigen. In another form, the bispecific fusion protein is divalent, consisting of, for example, an IgG with two binding sites for one antigen and two scFv with two binding sites for a second antigen.

Production of Antibody Fragments

Antibody fragments which recognize specific epitopes can be generated by known techniques. The antibody fragments are antigen binding portions of an antibody, such as F(\(\text{ab}'\))\(_2\), Fab', Fab, Fv, sFv and the like. Other antibody fragments include, but are not limited to: the F(ab')\(_2\) fragments which can be produced by pepsin digestion of the antibody molecule and the Fab' fragments, which can be generated by reducing disulfide bridges of the F(ab')\(_2\) fragments. Alternatively, Fab' expression expression libraries can be constructed (Huse et al., 1989, Science, 246:1274-1281) to allow rapid and easy identification of monoclonal Fab' fragments with the desired specificity. The present targeted drug carriers may encompass both antibodies and antibody fragments.

A single chain Fv molecule (scFv) comprises a VL domain and a VH domain. The VL and VH domains associate to form a target binding site. These two domains are further covalently linked by a peptide linker (L). An scFv molecule is denoted as either VL-L-VH if the VL domain is the N-terminal part of the scFv molecule, or as VH-L-VL if the VH domain is the N-terminal part of the scFv molecule. Methods for making scFv molecules and designing suitable peptide linkers are described in US Patent No. 4,704,692, US Patent No. 4,946,778, R. Raag and M. Whitlow, “Single Chain Fvs.” FASEB Vol 9:73-80 (1995) and R.E. Bird and B.W. Walker, “Single Chain Antibody Variable Regions,” TIBTECH, Vol 9: 132-137 (1991). These references are incorporated herein by reference.

An antibody fragment can be prepared by known methods, for example, as disclosed by Goldenberg, U.S. Patent Nos. 4,036,945 and 4,331,647 and references contained therein, which patents are incorporated herein in their entireties by
reference. Also, see Nisonoff et al., Arch Biochem. Biophys. 89: 230 (1960); Porter, Biochem. J. 73: 119 (1959); Edelman et al., in METHODS IN ENZYMOLOGY VOL. 1, page 422 (Academic Press 1967), and Coligan at pages 2.8.1-2.8.10 and 2.10.2.10.4.

Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). A CDR is a segment of the variable region of an antibody that is complementary in structure to the epitope to which the antibody binds and is more variable than the rest of the variable region. Accordingly, a CDR is sometimes referred to as hypervariable region. A variable region comprises three CDRs. CDR peptides can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. (See, e.g., Larrick et al., Methods: A Companion to Methods in Enzymology 2: 106 (1991); Courtenay-Luck, "Genetic Manipulation of Monoclonal Antibodies," in MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION, Ritter et al. (eds.), pages 166-179 (Cambridge University Press 1995); and Ward et al., "Genetic Manipulation and Expression of Antibodies," in MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS, Birch et al., (eds.), pages 137-185 (Wiley-Liss, Inc. 1995).

Anti-CD74 Antibodies

The anti-CD74 binding molecules of the present immunoconjugates and compositions may contain specific murine CDRs that have specificity for the CD74 antigen. For example, the anti-CD74 binding molecules may be humanized, chimeric or human mAbs, and they may contain the amino acids of the CDRs of a murine anti-CD74 mAb, (e.g., the murine anti-CD74 mAb, LL1). Humanized, chimeric, and human anti-CD74 mAb or fragments thereof are described in U.S. Serial No. 10/377,122, which is incorporated herein by reference.

Where the anti-CD74 antibody is humanized, it may contain CDRs of a light chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence RSSQSLVHRNGNTYLH (SEQ ID NO:1); a CDR2 including an
amino acid sequence TVSNRFS (SEQ ID NO:2); and a CDR3 including an amino acid sequence SQSSHVPPT (SEQ ID NO:3)). The humanized anti-CD74 antibody or fragment may include the heavy chain variable region of the humanized mAb, which may include CDRs of a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDDDFKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)). The humanized anti-CD74 antibody or fragment thereof may include light and heavy chain variable regions including complementarity-determining regions (CDRs) of murine anti-CD74 (mLL1) and the framework (FR) regions of a human antibody, where the light chain variable region of the humanized anti-CD74 mAb includes CDRs of a light chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence RSSQSLVHRNGNTYLH (SEQ ID NO:1); a CDR2 including an amino acid sequence TVSNRFS (SEQ ID NO:2); and a CDR3 including an amino acid sequence SQSSHVPPT (SEQ ID NO:3)), and where the heavy chain variable region of the humanized mAb includes CDRs of a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDDDFKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)). The humanized anti-CD74 antibody or fragment thereof may include the FRs of the light and heavy chain variable regions of the humanized anti-CD74 antibody or fragment thereof, which may be substituted with at least one amino acid from the corresponding FRs of the murine mAb. In one embodiment, the substituted amino acid may be selected from amino acid residue 2, 3, 4, 46, 87 and 100 of the murine light chain variable region of the cLL1Vk sequence of Fig. 3B, and amino acid residues 5, 37, 38, 46, 68, 91 and 93 of the murine heavy chain variable region of the cLL1VH sequence of Fig. 3A. In another embodiment, the mAb or fragment thereof comprises a heavy chain variable region of Fig. 4A and a light chain variable region of Fig. 4B. In a further embodiment, the mAb or fragment thereof may comprise a light and heavy chain constant region of a human antibody or a portion thereof. The mAb or fragment may include a humanized IgG1.
Where the anti-CD74 binding molecule includes a chimeric anti-CD74 antibody, the chimeric anti-CD74 antibody or fragment thereof may include a light chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence RSSQSLVHRNGNTYLYH (SEQ ID NO:1); a CDR2 including an amino acid sequence TVSNRFS (SEQ ID NO:2); and a CDR3 including an amino acid sequence SQSSHVPPT (SEQ ID NO:3)). In another embodiment, the chimeric anti-CD74 antibody or fragment thereof may include a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDFFDKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)). In a further embodiment, The chimeric anti-CD74 antibody or fragment thereof may include light and heavy chain variable regions which may include complementarity-determining regions (CDRs) of a murine anti-CD74 mAb; the framework (FR) regions of a murine anti-CD74 mAb; and the light and heavy chain constant regions of a human antibody, where the light chain variable region of the chimeric mAb may include CDRs of a light chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence RSSQSLVHRNGNTYLYH (SEQ ID NO:1); a CDR2 including an amino acid sequence TVSNRFS (SEQ ID NO:2); and a CDR3 including an amino acid sequence SQSSHVPPT (SEQ ID NO:3)); and where the heavy chain variable region of the chimeric mAb may include CDRs of a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDFFDKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)). Alternatively, the chimeric mAb or fragment thereof may include a heavy chain variable region of Fig. 2A and a light chain variable region of Fig. 2B. The chimeric mAb or fragment thereof may include a chimeric IgG1 or fragment thereof.

Where the anti-CD74 binding molecule is a human anti-CD74 antibody, the human anti-CD74 antibody or fragment thereof may include a light chain variable region of the human anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence RSSQSLVHRNGNTYLYH (SEQ ID NO:1); a CDR2 including an amino acid sequence
sequence TVSNRFS (SEQ ID NO:2); and a CDR3 including an amino acid sequence SQSSHVPPPT (SEQ ID NO:3)). In one embodiment, the human anti-CD74 antibody or fragment thereof may include a heavy chain variable region of the human mAb which may include CDRs of a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDDDFKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)).

In another embodiment, the human anti-CD74 antibody or fragment thereof may include the light and heavy chain variable and constant regions of a human antibody, where the huCD74 CDRs of the light chain variable region of the human anti-CD74 mAb may include a CDR1 having an amino acid sequence RSSQSLVHRNGNTYLH (SEQ ID NO:1); a CDR2 having an amino acid sequence TVSNRFS (SEQ ID NO:2); and a CDR3 having an amino acid sequence SQSSHVPPPT (SEQ ID NO:3); and where the heavy chain variable region of the human mAb may include CDRs of a heavy chain variable region of a murine anti-CD74 mAb (e.g., a CDR1 including an amino acid sequence NYGVN (SEQ ID NO:4); a CDR2 including an amino acid sequence WINPNTGEPTFDDDFKG (SEQ ID NO:5); and a CDR3 including an amino acid sequence SRGKNEAWFAY (SEQ ID NO:6)). The human mAb or fragment thereof may include a human IgG1.

**Multispecific and Multivalent Antibodies**

The anti-CD74 binding molecule of the present immunoconjugates and compositions, as well as other binding molecules with different specificities for use in combination therapy, can also include multispecific antibodies (comprising at least one binding site to a CD74 epitope or antigen and at least one binding site to another epitope on CD74 or another antigen) and multivalent antibodies (comprising multiple binding sites to the same epitope or antigen), or the antibodies can be both multivalent and multispecific.

A preferred binding molecule of the present immunoconjugates or compositions is a fusion protein, which contains four or more Fvs, or Fab's of a humanized, chimeric, human or murine anti-CD74 mAb or fragment thereof as
described herein. Additionally, another preferred antibody fusion protein contains one or more Fvs, or Fab's of the mAbs or fragments thereof of a humanized, chimeric, human or murine anti-CD74 mAb or fragment thereof as described herein, and one or more Fvs or Fab's from antibodies specific for another antigen that is specific for a tumor cell marker that is not a CD74 antigen. For example, the non-CD74 antigen may be expressed by the CD74-expressing cells and may include a tumor marker selected from a B-cell lineage antigen, (e.g., CD19, CD20, or CD22 for the treatment of B-cell malignancies). The non-CD74 antigen may also be expressed on other CD74 positive cells that cause other types of malignancies, such as S100 in melanoma, etc. Further, the tumor cell marker may be a non-B-cell lineage antigen selected from the group consisting of HLA-DR, CD30, CD33, CD52 MUC1 and TAC.

Also disclosed herein are bispecific or multispecific antibodies useful for preparing the disclosed immunoconjugates and compositions, where anti-CD74 mAbs or fragments thereof or antibody fusion proteins thereof are linked to an antibody or antibody fragment specific for a cancer marker substance, an epitope on the surface of an infectious disease organism, or a noxious substance in the blood or other body fluids. The bispecific and multispecific antibodies are particularly useful in the method of inducing clearance of a variety of noxious substances, where the bispecific antibody has at least one specificity for a noxious substance, such as a pathogenic organism, and at least one specificity for CD74, the HLA class-II invariant chain (II), as described in detail in U.S. Serial No. 09/314,135, filed on May 19, 1999, entitled "Therapeutic Using a Bispecific Antibody," which is herein incorporated in its entirety by reference.

The immunoconjugates and compositions disclosed herein may also include an anti-CD74 multivalent antibody. This multivalent target binding protein may be constructed by association of a first and a second polypeptide. The first polypeptide comprises a first single chain Fv molecule covalently linked to a first immunoglobulin-like domain that preferably is an immunoglobulin light chain variable region domain. The second polypeptide comprises a second single chain Fv molecule covalently linked to a second immunoglobulin-like domain that preferably is
an immunoglobulin heavy chain variable region domain. Each of the first and second
single chain Fv molecules forms a target binding site, and the first and second
immunoglobulin-like domains associate to form a third target binding site.

Diabodies, Triabodies and Tetrabodies

The immunoconjugates and compositions disclosed herein may also include
functional bispecific single-chain antibodies (bscAb), also called diabodies. (See,
reference). For example, bscAb are produced by joining two single-chain Fv
fragments via a glycine-serine linker using recombinant methods. The V light-chain
(VL) and V heavy-chain (VH) domains of two antibodies of interest are isolated using
standard PCR methods. The VL and VH cDNA’s obtained from each hybridoma are
then joined to form a single-chain fragment in a two-step fusion PCR. The first PCR
step introduces the (Gly4-Ser1)3 linker, and the second step joins the VL and VH
amplicons. Each single chain molecule is then cloned into a bacterial expression
vector. Following amplification, one of the single-chain molecules is excised and
sub-cloned into the other vector, containing the second single-chain molecule of
interest. The resulting bscAb fragment is subcloned into a eukaryotic expression
vector. Functional protein expression can be obtained by transfecting the vector into
Chinese Hamster Ovary cells. Bispecific fusion proteins are prepared in a similar
manner. Bispecific single-chain antibodies and bispecific fusion proteins may be used
to prepare the drug carriers.

For example, a humanized, chimeric or human anti-CD74 monoclonal
antibody can be used to produce antigen specific diabodies, triabodies, and
tetrabodies. The monospecific diabodies, triabodies, and tetrabodies bind selectively
to targeted antigens and as the number of binding sites on the molecule increases, the
affinity for the target cell increases and a longer residence time is observed at the
desired location. For diabodies, the two chains comprising the VH polypeptide of the
humanized CD74 mAb connected to the VK polypeptide of the humanized CD74
mAb by a five amino acid residue linker are utilized. Each chain forms one half of
the humanized CD74 diabody. In the case of triabodies, the three chains comprising $V_H$ polypeptide of the humanized CD74 MAb connected to the $V_K$ polypeptide of the humanized CD74 MAb by no linker are utilized. Each chain forms one third of the hCD74 triabody.

More recently, a tetravalent tandem diabody (termed tandab) with dual specificity has also been reported (Cochlovius et al., Cancer Research (2000) 60: 4336-4341). The bispecific tandab is a dimer of two identical polypeptides, each containing four variable domains of two different antibodies ($V_{H1}, V_{L1}, V_{H2}, V_{L2}$) linked in an orientation to facilitate the formation of two potential binding sites for each of the two different specificities upon self-association.

Conjugated multivalent and multispecific anti-CD74 antibodies

In another embodiment, a conjugated multivalent anti-CD74 antibody may be used to prepare the immunoconjugate or composition. Additional amino acid residues may be added to either the N- or C-terminus of the first or the second polypeptide. The additional amino acid residues may comprise a peptide tag, a signal peptide, a cytokine, an enzyme (for example, a pro-drug activating enzyme), a hormone, a peptide toxin, such as pseudomonas exotoxin, a peptide drug, a cytotoxic protein or other functional proteins. As used herein, a functional protein is a protein that has a biological function.

In one embodiment, drugs, toxins, radioactive compounds, enzymes, hormones, cytotoxic proteins, chelates, cytokines and other functional agents may be conjugated to the multivalent target binding protein, preferably through covalent attachments to the side chains of the amino acid residues of the multivalent target binding protein, for example amine, carboxyl, phenyl, thiol or hydroxyl groups. Various conventional linkers may be used for this purpose, for example, diisocyanates, diisothiocyanates, bis(hydroxysuccinimide) esters, carbodiimides, maleimide-hydroxysuccinimide esters, glutaraldehyde and the like. Conjugation of agents to the multivalent protein preferably does not significantly affect the protein’s binding specificity or affinity to its target. As used herein, a functional agent is an
agent which has a biological function. A preferred functional agent is a cytotoxic agent.

In still other embodiments, bispecific antibody-directed delivery of therapeutics or prodrug polymers to in vivo targets can be combined with bispecific antibody delivery of radionuclides, such that combination chemotherapy and radioimmunotherapy is achieved. Each therapeutic agent can be conjugated to the targetable conjugate and administered simultaneously, or the nuclide can be given as part of a first targetable conjugate and the drug given in a later step as part of a second targetable conjugate.

In another embodiment, cytotoxic agents may be conjugated to a polymeric carrier, and the polymeric carrier may subsequently be conjugated to the multivalent target binding protein. For this method, see Ryser et al., Proc. Natl. Acad. Sci. USA, 75:3867-3870, 1978, US Patent No. 4,699,784 and US Patent No. 4,046,722, which are incorporated herein by reference. Conjugation preferably does not significantly affect the binding specificity or affinity of the multivalent binding protein.

Humanized, Chimeric and Human Antibodies Use for Treatment and Diagnosis

Humanized, chimeric and human monoclonal antibodies, i.e., anti-CD74 mAbs and other MAbs described herein, are suitable for use in the therapeutic methods and diagnostic methods which utilize immunoconjugates and compositions as described herein. Accordingly, the immunoconjugates or compositions may include naked humanized, chimeric and human antibodies or antibodies, which have been conjugated to a carrier, a therapeutic agent, or a diagnostic agent. The immunoconjugates may be administered as a multimodal therapy. For example, additional therapeutic or diagnostic agents may be administered before, simultaneously, or after administration of the immunoconjugate or composition.

The efficacy of the immunoconjugates may be enhanced by supplementing the anti-CD74 immunoconjugates with one or more other binding molecules, (i.e., mAbs to specific antigens, such as CD4, CD5, CD8, CD14, CD15, CD19, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54,
CD80, CD126, B7, MUC1, Ia, tenascin, HM1.24, or HLA-DR, preferably mature
HLA-DR dimer, with one or more immun conjugates of anti-CD74, or antibodies to
theses recited antigens). Preferred B-cell-associated antigens include those equivalent
to human CD19, CD20, CD21, CD22, CD23, CD46, CD52, CD74, CD80, and CD5
antigens. Preferred T-cell antigens include those equivalent to human CD4, CD8 and
CD25 (the IL-2 receptor) antigens. An equivalent to HLA-DR antigen can be used in
treatment of both B-cell and T-cell disorders. Particularly preferred B-cell antigens
are those equivalent to human CD19, CD22, CD21, CD23, CD74, CD80, and HLA-
DR antigens. Particularly preferred T-cell antigens are those equivalent to human
CD4, CD8 and CD25 antigens. CD46 is an antigen on the surface of cancer cells that
block complement-dependent lysis (CDC). Preferred malignant melanoma associated
antigens are those equivalent to MART-1, TRP-1, TRP-2 and gp100. Further,
preferred multiple myeloma-associated antigens are those equivalent to MUC1 and
CD38.

The supplemental binding molecule may be naked or conjugated with a
carrier, a therapeutic agent, or a diagnostic agent, including lipids, polymers, drugs,
toxins, immunomodulators, hormones, enzymes, and therapeutic radionuclides, etc.
The supplement binding molecule may be administered concurrently, sequentially, or
according to a prescribed dosing regimen, with the anti-CD74 immun conjugate.

Further, contemplating herein is the administration of an immun conjugate for
diagnostic and therapeutic uses in B cell lymphomas and other disease or disorders.
An immun conjugate, as described herein, is a molecule comprising a binding
molecule conjugated to a carrier. The immun conjugate may be used to form a
composition that further includes a therapeutic or diagnostic agent, which may include
a peptide that may bear the diagnostic or therapeutic agent. An immun conjugate
retains the immunoreactivity of the binding molecule, (i.e., the antibody moiety has
about the same or slightly reduced ability to bind the cognate antigen after
conjugation as before conjugation). Immun conjugates may include binding
molecule conjugated to any suitable second molecule, (e.g., lipids, proteins,
carbohydrates, (which may form higher-ordered structures), or higher-ordered
structures themselves, such as liposomes, micelles, and/or nanoparticles). To
facilitate delivery of certain effectors, it may be desirable to conjugate an anti-CD74 antibody to one or more molecules that are capable of forming higher-ordered structures (e.g., amphiphilic lipids). Amphiphilic molecules may also be desirable to facilitate delivery of effectors that demonstrate limited solubility in aqueous solution.

A wide variety of diagnostic and therapeutic reagents can be advantageously used to form the immunoconjugates and compositions as described herein. Therapeutic agents include, for example, chemotherapeutic drugs such as vinca alkaloids, anthracyclines, epidophyllotoxins, taxanes, antimetabolites, alkylating agents, antibiotics, Cox-2 inhibitors, antimitotics, angiogenic and apoptotic agents, particularly doxorubicin, methotrexate, taxol, CPT-11, camptothecans, and others from these and other classes of anticancer agents, and the like. Other useful cancer chemotherapeutic drugs for the preparation of immunoconjugates and antibody fusion proteins include nitrogen mustards, alkyl sulfonates, nitrosoureas, triazenes, folic acid analogs, COX-2 inhibitors, pyrimidine analogs, purine analogs, platinum coordination complexes, hormones, and the like. Suitable chemotherapeutic agents are described in REMINGTON’S PHARMACEUTICAL SCIENCES, 19th Ed. (Mack Publishing Co. 1995), and in GOODMAN AND GILMAN’S THE PHARMACOLOGICAL BASIS OF THERAPEUTICS, 7th Ed. (MacMillan Publishing Co. 1985), as well as revised editions of these publications. Other suitable chemotherapeutic agents, such as experimental drugs, are known to those of skill in the art.

Additionally, a chelator such as DTPA, DOTA, TETA, or NOTA can be conjugated to one or more components of the compositions as described herein. Alternatively, a suitable peptide including a detectable label, (e.g., a fluorescent molecule), or a cytotoxic agent, (e.g., a heavy metal or radionuclide), can be covalently, non-covalently, or other associated with more components of the compositions as described herein. For example, a therapeutically useful immunoconjugate can be obtained by incorporating a photoactive agent or dye in the composition as described herein. Fluorescent compositions, such as fluochrome, and other chromogens, or dyes, such as porphyrins sensitive to visible light, have been used to detect and to treat lesions by directing the suitable light to the lesion. In
therapy, this has been termed photoradiation, phototherapy, or photodynamic therapy (Jori et al. (eds.), PHOTODYNAMIC THERAPY OF TUMORS AND OTHER DISEASES (Libreria Progetto 1985); van den Bergh, Chem. Britain 22:430 (1986)). Moreover, monoclonal antibodies have been coupled with photoactivated dyes for achieving phototherapy. Mew et al., J. Immunol. 130:1473 (1983); idem., Cancer Res. 45:4380 (1985); Oseroff et al., Proc. Natl. Acad. Sci. USA 83:8744 (1986); idem., Photochem. Photobiol. 46:83 (1987); Hasan et al., Prog. Clin. Biol. Res. 288:471 (1989); Tatsuta et al., Lasers Surg. Med. 9:422 (1989); Pelegrin et al., Cancer 67:2529 (1991). Endoscopic applications are also contemplated. Endoscopic methods of detection and therapy are described in U.S. patent numbers 4,932,412; 5,525,338; 5,716,595; 5,736,119; 5,922,302; 6,096,289; and 6,387,350, which are incorporated herein by reference in their entirety. Thus, contemplated herein is the therapeutic use of anti-CD74 immunoconjugate compositions comprising photoactive agents or dyes, and the present diagnostic/therapeutic methods may include the diagnostic or therapeutic use of anti-CD74 immunoconjugate compositions comprising photoactive agents or dyes.

Also contemplated is the use of radioactive and non-radioactive agents as diagnostic agents in the anti-CD74 immunoconjugate compositions as described herein. A suitable non-radioactive diagnostic agent is a contrast agent suitable for magnetic resonance imaging, computed tomography or ultrasound. Magnetic imaging agents include, for example, non-radioactive metals, such as manganese, iron and gadolinium, complexed with metal-chelate combinations that include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, when used along with the antibodies described herein. (See U.S. Serial No. 09/921,290 filed on October 10, 2001, which is incorporated in its entirety by reference).

Furthermore, the anti-CD74 immunoconjugate compositions may include a radioisotope or a positron-emitter useful for diagnostic imaging. Suitable radioisotopes may include those in the energy range of 60 to 4,000 keV. Suitable radioisotopes may include $^{18}$F, $^{52}$Fe, $^{62}$Cu, $^{64}$Cu, $^{67}$Cu, $^{67}$Ga, $^{68}$Ga, $^{86}$Y, $^{89}$Zr, $^{94}$Tc, $^{94m}$Tc, $^{99m}$Tc, $^{111}$In, $^{123}$I, $^{124}$I, $^{125}$I, $^{131}$I, and the like. (See, e.g., U.S. Patent Application entitled “Labeling Targeting Agents with Gallium-68”- Inventors G.L.Griffiths and
W.J. McBride, (U.S. Provisional Application No. 60/342,104), which discloses positron emitters, such as \( ^{18}F \), \( ^{68}Ga \), \( ^{94m}Tc \) and the like, for imaging purposes and which is incorporated in its entirety by reference).

A toxin, such as *Pseudomonas* exotoxin, may also be present in the anti-CD74 immunoconjugate compositions as described herein. For example, the toxin may be complexed to or form the therapeutic agent portion of an antibody fusion protein of an anti-CD74 antibody described herein. Other toxins include ricin, abrin, ribonuclease (RNase), DNase I, *Staphylococcal* enterotoxin-A, pokeweed antiviral protein, gelonin, diphtherin toxin, *Pseudomonas* exotoxin, and *Pseudomonas* endotoxin. (See, e.g., Pastan et al., *Cell* 47:641 (1986), and Goldenberg, *CA - A Cancer Journal for Clinicians* 44:43 (1994). Additional toxins suitable for use herein are known to those of skill in the art and are disclosed in U.S. Patent 6,077,499, which is incorporated in its entirety by reference).

An immunomodulator, such as a cytokine may also be present in the administered anti-CD74 immunoconjugate compositions as described herein. For example, an immunomodulator may be conjugated to, or form the therapeutic agent portion of an antibody fusion protein or be administered as part of the anti-CD74 immunoconjugate compositions as described herein. Suitable cytokines include, but are not limited to, interferons and interleukins, as described below.

**Preparation of Immunoconjugates**

The immunoconjugates described herein can be prepared by known methods of linking antibodies with lipids, carbohydrates, protein, or other atoms and molecules. For example, the binding molecules described herein can be conjugated with one or more of the carriers described herein (e.g., lipids, polymers, liposomes, micelles, or nanoparticles) to form an immunoconjugate, and the immunoconjugate can incorporate a therapeutic or diagnostic agent either covalently, non-covalently, or otherwise. Further, any of the binding molecules described herein can be further conjugated with one or more therapeutic or diagnostic agents described herein, or additional carriers. Generally, one therapeutic or diagnostic agent may be attached to each binding molecule but more than one therapeutic agent or diagnostic agent can be attached to the same binding molecule. The antibody fusion proteins contemplated...
herein comprise two or more antibodies or fragments thereof and each of the antibodies that comprises this fusion protein may be conjugated with one or more of the carriers described herein. Additionally, one or more of the antibodies of the antibody fusion protein may have one or more therapeutic or diagnostic agent attached. Further, the therapeutic do not need to be the same but can be different therapeutic agents. For example, the compositions described herein may include a drug and a radioisotope.

For example, an IgG can be radiolabeled with $^{131}I$ and conjugated to a lipid, such that the IgG-lipid conjugate can form a liposome. The liposome may incorporate one or more therapeutic or diagnostic agents, (e.g., a drug such as FUdR-dO).

Alternatively, in addition to the carrier, the IgG may be conjugated to $^{131}I$ (e.g., at a tyrosine residue) and a drug (e.g., at the epsilon amino group of a lysine residue), and the carrier may incorporate an additional therapeutic or diagnostic agent. Therapeutic and diagnostic agents may be covalently associated with the binding molecule, (e.g., conjugated to reduced disulfide groups, carbohydrate side chains, or any other reactive group on the binding molecule.

A carrier, therapeutic agent, or diagnostic agent can be attached at the hinge region of a reduced antibody component via disulfide bond formation. As an alternative, peptides can be attached to an antibody component using a heterobifunctional cross-linker, such as $N$-succinyl 3-(2-pyridyldithio)propionate (SPDP). Yu et al., Int. J. Cancer 56: 244 (1994). General techniques for such conjugation are well known in the art. (See, e.g., Wong, CHEMISTRY OF PROTEIN CONJUGATION AND CROSS-LINKING (CRC Press 1991); Upeslacis et al., “Modification of Antibodies by Chemical Methods,” in MONOCLONAL ANTIBODIES: PRINCIPLES AND APPLICATIONS, Birch et al. (eds.), pages 187-230 (Wiley-Liss, Inc. 1995); Price, “Production and Characterization of Synthetic Peptide-Derived Antibodies,” in MONOCLONAL ANTIBODIES: PRODUCTION, ENGINEERING AND CLINICAL APPLICATION, Ritter et al. (eds.), pages 60-84 (Cambridge University Press 1995)). Alternatively, the carrier, therapeutic agent, or diagnostic agent can be conjugated via a carbohydrate moiety in the Fc region of an antibody. The carbohydrate group can be used to increase the
loading of the same peptide that is bound to a thiol group, or the carbohydrate moiety can be used to bind a different peptide.

Methods for conjugating peptides to antibody components via an antibody carbohydrate moiety are well known to those of skill in the art. (See, e.g., Shih et al., Int. J. Cancer 41: 832 (1988); Shih et al., Int. J. Cancer 46: 1101 (1990); and Shih et al., U.S. Patent No. 5,057,313, all of which are incorporated in their entirety by reference). Similar chemistry can be used to conjugate one or more anti-CD74 binding molecules to one or more carriers, therapeutic agents, or diagnostic agents. The general method involves reacting an antibody component having an oxidized carbohydrate portion with a carrier polymer that has at least one free amine function and that is loaded with a plurality of peptide. This reaction results in an initial Schiff base (imine) linkage, which can be stabilized by reduction to a secondary amine to form the final conjugate.

The Fc region may be absent if the anti-CD74 binding molecule is an antibody fragment. However, it is possible to introduce a carbohydrate moiety into the light chain variable region of a full-length antibody or antibody fragment. (See, e.g., Leung et al., J. Immunol. 154: 5919 (1995); Hansen et al., U.S. Patent No. 5,443,953 (1995), Leung et al., U.S. patent No. 6,254,868, all of which are incorporated in their entirety by reference). The engineered carbohydrate moiety may be used to attach a carrier or a therapeutic or diagnostic agent.

Carriers (Lipids, Liposomes, Micelles, Polymers, and Nanoparticles)

The formation of liposomes and micelles is known in the art. (See, e.g., Wrobel et al., Biochimica et Biophysica Acta, 1235:296 (1995); Lundberg et al., J. Pharm. Pharmacol., 51:1099-1105 (1999); Lundberg et al., Int. J. Pharm., 205:101-108 (2000); Lundberg, J. Pharm. Sci., 83:72-75 (1994); Xu et al., Molec. Cancer Ther., 1:337-346 (2002); Torchilin et al., Proc. Nat’l. Acad. Sci., 100:6039-6044 (2003); U.S. 5,565,215; U.S. 6,379,698; and U.S. 2003/0082154). Nanoparticles or nanocapsules formed from polymers, silica, or metals, which are useful for drug delivery or imaging, have been described as well. (See, e.g., West et al., Applications
5,702,727; and U.S. 6,330,944).

Immunoliposomes

The conjugation of antibodies or binding molecules to liposomes to form a
targeted carrier for therapeutic or diagnostic agents has been described. (See, e.g.,
Lundberg, J. Pharm. Pharmacol., 49:16-21 (1997); Lundberg, Anti-cancer Drug
U.S. Serial No. 09/590,284, and U.S. Serial No. 60/138,284, filed June 9, 1999. All
these references are incorporated herein by reference.

Pharmacologically Acceptable Excipients

The immunoconjugates or compositions may include one or more
pharmacologically suitable excipients, one or more additional ingredients, or some
combination of these.

The immunoconjugate or compositions disclosed herein can be formulated
according to known methods to prepare pharmaceutically useful compositions,
whereby the immunoconjugate or compositions are combined in a mixture with a
pharmacologically suitable excipient. Sterile phosphate-buffered saline is one example
of a pharmacologically suitable excipient. Other suitable excipients are well known to
those in the art. (See, e.g., Ansel et al., PHARMACEUTICAL DOSAGE FORMS
AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and
Gennaro (ed.), REMINGTON'S PHARMACEUTICAL SCIENCES, 18th Edition
(Mack Publishing Company 1990), and revised editions thereof.

The immunoconjugate or compositions disclosed herein can be formulated for
intravenous administration via, for example, bolus injection or continuous infusion.
Formulations for injection can be presented in unit dosage form, e.g., in ampules or in
multi-dose containers, with an added preservative. The compositions can take such

-36-
forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient can be in powder form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

Additional pharmaceutical methods may be employed to control the duration of action of the therapeutic or diagnostic conjugate or naked antibody. Control release preparations can be prepared through the use of polymers to complex or absorb the immunoconjugate or naked antibody. For example, biocompatible polymers include matrices of poly(ethylene-co-vinyl acetate) and matrices of a polyanhydride copolymer of stearic acid dimer and sebacic acid. Sherwood et al., *Bio/Technology* 10: 1446 (1992). The rate of release of an immunoconjugate or antibody from such a matrix depends upon the molecular weight of the immunoconjugate or antibody, the amount of immunoconjugate, antibody within the matrix, and the size of dispersed particles. Saltzman et al., *Biophys. J.* 55: 163 (1989); Sherwood et al., *supra*. Other solid dosage forms are described in Ansel et al., PHARMACEUTICAL DOSAGE FORMS AND DRUG DELIVERY SYSTEMS, 5th Edition (Lea & Febiger 1990), and Gennaro (ed.), REMINGTON’S PHARMACEUTICAL SCIENCES, 18th Edition (Mack Publishing Company 1990), and revised editions thereof.

The immunoconjugate or compositions may also be administered to a mammal subcutaneously or even by other parenteral routes. Moreover, the administration may be by continuous infusion or by single or multiple boluses. In general, the dosage of an administered immunoconjugate, fusion protein or naked antibody for humans will vary depending upon such factors as the patient’s age, weight, height, sex, general medical condition and previous medical history. Typically, it is desirable to provide the recipient with a dosage of immunoconjugate or composition including the immunoconjugate that is in the range of from about 1 mg/kg to 20 mg/kg as a single intravenous infusion, although a lower or higher dosage also may be administered as circumstances dictate. This dosage may be repeated as needed, for example, once per week for 4-10 weeks, preferably once per week for 8 weeks, and more preferably, once per week for 4 weeks. It may also be given less frequently, such as every other
week for several months. The dosage may be given through various parenteral routes, with appropriate adjustment of the dose and schedule.

For purposes of therapy, the immunoconjugate, or composition including the immunoconjugate, is administered to a mammal in a therapeutically effective amount. A suitable subject for the therapeutic and diagnostic methods disclosed herein is usually a human, although a non-human animal subject is also contemplated. An antibody preparation is said to be administered in a “therapeutically effective amount” if the amount administered is physiologically significant. An agent is physiologically significant if its presence results in a detectable change in the physiology of a recipient mammal. In particular, an antibody preparation is physiologically significant if its presence invokes an antitumor response or mitigates the signs and symptoms of an autoimmune disease state. A physiologically significant effect could also be the evocation of a humoral and/or cellular immune response in the recipient mammal.

Methods of Treatment

Contemplated herein is the use of immunoconjugates or compositions including immunoconjugates as the primary composition for treatment of a CD74 expressing malignancy, where the disease or disorder is selected from the group consisting of an immune dysregulation disease, an autoimmune disease, organ graft rejection, and graft versus host disease. The CD74 expressing malignancy is selected from the group consisting of a solid tumor, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, multiple myeloma, a B-cell malignancy and/or a T-cell malignancy. The solid tumor is selected from the group consisting of a melanoma, carcinoma and sarcoma and the carcinoma is selected from the group consisting of a renal carcinoma, lung carcinoma, intestinal carcinoma, stomach carcinoma and melanoma. The B-cell malignancy is selected from the group consisting of indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, and multiple myeloma, B-cell disorders and other diseases. In particular, the compositions described herein are particularly useful for treatment of various autoimmune as well as indolent forms of B-cell lymphomas,
aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, multiple myeloma, and Waldenström’s macroglobulinemia. For example, humanized anti-CD74 antibody components and immunoconjugates can be used to treat both indolent and aggressive forms of non-Hodgkin’s lymphoma.

More specifically, the method for treating a B-cell malignancy may include administering to a subject with a B-cell related malignancy, a therapeutic composition comprising a pharmaceutically acceptable carrier, a therapeutic agent, and an immunoconjugate including an anti-CD74 binding molecule, (e.g., a humanized, chimeric, or human anti-CD74 mAb or fragment thereof or antibody fusion protein thereof), wherein the B-cell malignancy is a lymphoma or leukemia. More specifically, the B-cell malignancy is indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, multiple myeloma, chronic lymphatic leukemias, or acute lymphatic leukemias. The immunoconjugate or composition comprising the immunoconjugate is administered intravenously or intramuscularly at a dose of 20-2000 mg. The present method further comprises administering the immunoconjugate or composition before, simultaneously, or after the administration of at least one additional therapeutic agent or diagnostic agent used to treat the B-cell malignancy. The additional agent may include an additional immunoconjugate as described herein, including a therapeutic or diagnostic agent. A therapeutic agent may include a naked antibody, an immunomodulator, a hormone, a cytotoxic agent, an enzyme, and/or an antibody conjugated to at least one immunomodulator, radioactive label, hormone, enzyme, or cytotoxic agent, or a combination thereof. The immunomodulator preferably is a cytokine and the cytotoxic agent preferably is a drug or toxin. The antibody that is administered in combination as a naked antibody or as a supplemental immunoconjugate preferably is reactive with CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD80, CD126, B7, MUC2, MUC3, MUC4, Ia, HM1.24, tenascin, and HLA-DR, (preferably a mature HLA-DR dimer), VEGF, EGFR, CEA, CSAp, ILGF, placental growth factor, carbonic anhydrase IX, IL-6 or combinations thereof.
Also contemplated herein is the treatment of a malignancy comprising administering to a subject with a CD74 antigen-positive malignancy other than lymphoma or leukemia, a therapeutic composition that includes: (1) an immunoconjugate of an anti-CD74 binding molecule and a carrier; (2) an effector; and (3) a pharmaceutically acceptable excipient. The immunoconjugate or composition is administered intravenously or intramuscularly at a dose of 20-5000 mg. Further, the immunoconjugate may be administered before, simultaneously, or after the administration of at least one additional therapeutic agent or diagnostic agent. Therapeutic agents, as described above and throughout the specification, may include an immunomodulator, a hormone, a cytotoxic agent, or a binding molecule (either naked or conjugated to at least one immunomodulator, radioactive label, enzyme, hormone, cytotoxic agent, antisense oligonucleotide, or a combination thereof, where the immunomodulator preferably is a cytokine and the cytotoxic agent preferably is a drug or toxin). A therapeutic agent or diagnostic agent may include the compositions or immunoconjugates as disclosed herein. When an antibody is administered in combination with the therapeutic and/or diagnostic composition to treat a malignancy that is not a B-cell malignancy, it should be reactive with a tumor marker other than CD74, which is expressed by the cells that comprise the malignancy that is treated, and the antibody should be formulated in a pharmaceutically acceptable vehicle. Examples of antibodies that can be administered for malignant melanoma associated antigens are those antibodies reactive with MART-1, TRP-1, TRP-2 and gp100. Further, preferred antibodies to multiple myeloma-associated antigens are those reactive with MUC1 and CD38.

The compositions for treatment contain at least one immunoconjugate, which typically includes a humanized, chimeric or human monoclonal anti-CD74 antibody alone or in combination with other antibodies, such as other humanized, chimeric, or human antibodies. In particular, combination therapy wherein the immunoconjugate includes a fully human antibody is also contemplated.

The compositions also may include an immunomodulator as an effector. As used herein, the term "immunomodulator" includes cytokines, stem cell growth factors, lymphotoxins, such as tumor necrosis factor (TNF), and hematopoietic
factors, such as interleukins (e.g., interleukin-1 (IL-1), IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, and IL-21), colony stimulating factors (e.g., granulocyte-colony stimulating factor (G-CSF) and granulocyte macrophage-colony stimulating factor (GM-CSF)), interferons (e.g., interferons-α, -β, and -γ), the stem cell growth factor designated "S1 factor," erythropoietin, thrombopoietin or a combination thereof. Examples of suitable immunomodulator moieties include IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, and a combination thereof, and interferon-α, TNF-α, and the like. The immunomodulator may be present in the composition, or alternatively, the immunomodulator can be administered before, simultaneously, or after administration of the therapeutic and/or diagnostic compositions. As discussed supra, the anti-CD74 antibody may also be conjugated to the immunomodulator. The immunomodulator may also be conjugated to a hybrid antibody consisting of one or more antibodies binding to different antigens.

Multimodal therapies contemplated herein further include immunotherapy with immunoconjugates that include anti-CD74 binding molecules supplemented with administration of additional binding molecules, (e.g., anti-CD22, anti-CD19, anti-CD21, anti-CD20, anti-CD23, anti-CD46 or HLA-DR, preferably the mature HLA-DR dimer antibodies in the form of naked antibodies, fusion proteins, or as immunoconjugates). Further, a micelle, liposome, or nanoparticle, as described herein, may include binding molecules in addition to anti-CD74 binding molecules. Useful antibodies may be polyclonal, monoclonal, chimeric, human or humanized antibodies that recognize at least one epitope on the above-noted antigenic determinants. For example, anti-CD19 and anti-CD22 antibodies are known to those of skill in the art. (See, e.g., Ghetie et al., Cancer Res. 48:2610 (1988); Hekman et al., Cancer Immunol. Immunother. 32:364 (1991); Longo, Curr. Opin. Oncol. 8:353 (1996) and U.S. Patent Nos. 5,798,554 and 6,187,287, incorporated in their entirety by reference.)

In another form of multimodal therapy, subjects receive anti-CD74 immunoconjugates, in conjunction with standard cancer chemotherapy. For example, "CVB" (1.5 g/m² cyclophosphamide, 200-400 mg/m² etoposide, and 150-200 mg/m² carmustine) is a regimen used to treat non-Hodgkin's lymphoma. Patti et al., Eur. J.
Haematol. 51: 18 (1993). Other suitable combination chemotherapeutic regimens are well known to those of skill in the art. (See, e.g., Freedman et al., "Non-Hodgkin's Lymphomas," in CANCER MEDICINE, VOLUME 2, 3rd Edition, Holland et al. (eds.), pages 2028-2068 (Lea & Febiger 1993)). As an illustration, first generation chemotherapeutic regimens for treatment of intermediate-grade non-Hodgkin's lymphoma (NHL) include C-MOPP (cyclophosphamide, vincristine, procarbazine and prednisone) and CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone). A useful second-generation chemotherapeutic regimen is m-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine, dexamethasone and leucovorin), while a suitable third generation regimen is MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine, prednisone, bleomycin and leucovorin). Additional useful drugs include phenyl butyrate and bryostatin-1. In a preferred multimodal therapy, both chemotherapeutic drugs and cytokines are co-administered with an antibody, immunoconjugate or fusion protein.

The cytokines, chemotherapeutic drugs and antibody or immunoconjugate can be administered in any order, or together.

In a preferred embodiment, NHL is treated with 4 weekly infusions of the humanized anti-CD74 immunoconjugate (e.g., a therapeutic emulsion) at a dose of 200-400 mg/m² weekly for 4 consecutive weeks or every-other week (iv over 2-8 hours), repeated as needed over next months/yrs. Also preferred, NHL is treated with 4 semi-monthly infusions as above, but combined with epratuzumAb (anti-CD22 humanized antibody) on the same days, at a dose of 360 mg/m², given as an iv infusion over 1 hour, either before, during or after the anti-CD74 immunoconjugate infusion. Still preferred, NHL is treated with 4 weekly infusions of the anti-CD74 immunoconjugate as above, combined with one or more injections of CD22 mAb radiolabeled with a therapeutic isotope such as yttrium-90 (at dose of ⁹⁰Y between 5 and 35 mCi/meter-square as one or more injections over a period of weeks or months.

In addition, a therapeutic composition as contemplated herein can contain a mixture or hybrid molecules of monoclonal anti-CD74 immunoconjugates directed to different, non-blocking CD74 epitopes. Accordingly, contemplated herein are therapeutic compositions comprising a mixture of monoclonal anti-CD74
immunoconjugates that bind at least two CD74 epitopes. Additionally, the immunoconjugates described herein may contain a mixture of anti-CD74 antibodies with varying CDR sequences.

As discussed supra, the immunoconjugates can be used for treating B cell lymphoma and leukemia, and other B cell diseases or disorders as well as other malignancies in which affected or associated malignant cells are reactive with CD74. For example, anti-CD74 immunoconjugates can be used to treat immune dysregulation disease and related autoimmune diseases, including Class-III autoimmune diseases such as immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpura and chronic idiopathic thrombocytopenic purpura, dermatomyositis, Sjögren’s syndrome, multiple sclerosis, Sydenham’s chorea, myasthenia gravis, systemic lupus erythematosus, lupus nephritis, rheumatic fever, polyglandular syndromes, bullous pemphigoid, diabetes mellitus, Henoch-Schonlein purpura, post-streptococcal nephritis, erythema nodosum, Takayasu’s arteritis, Addison’s disease, rheumatoid arthritis, sarcoidosis, ulcerative colitis, erythema multiforme, IgA nephropathy, polyarteritis nodosa, ankylosing spondylitis, Goodpasture’s syndrome, thromboangiitis obliterans, primary biliary cirrhosis, Hashimoto’s thyroiditis, thyrotoxicosis, scleroderma, chronic active hepatitis, polymyositis/dermatomyositis, polychondritis, panniculitis vulgaris, Wegener’s granulomatosis, membranous nephropathy, amyotrophic lateral sclerosis, tabes dorsalis, giant cell arteritis/polymyalgia, pernicious anemia, rapidly progressive glomerulonephritis and fibrosing alveolitis.

In particular, immunoconjugates including humanized, chimeric or human anti-CD74 mAbs or fragments thereof or antibody fusion proteins thereof are administered to a subject with one or more of these autoimmune diseases. The anti-CD74 immunoconjugates disclosed herein are particularly useful in the method of treating autoimmune disorders, disclosed in pending U.S. Serial No. 09/590,284 filed on June 9, 2000 entitled “Immunotherapy of Autoimmune Disorders using Antibodies that Target B-Cells,” which is incorporated in its entirety by reference. Preferably the anti-CD74 immunoconjugate is administered intravenously or intramuscularly at a dose of 20-5000 mg. Further, the anti-CD74 immunoconjugate is administered
before, during or after the administration of at least one therapeutic agent or
diagnostic agent. The therapeutic agent, as described above and throughout the
specification, may include an antibody, an immunomodulator, a hormone, an enzyme,
a cytotoxic agent, an antibody conjugated to at least one immunomodulator,
radioactive label, hormone, enzyme, or cytotoxic agent, antisense oligonucleotide or
a combination thereof, where the immunomodulator is a cytokine and said cytotoxic
agent is a drug or toxin. The therapeutic agent may include an immunoconjugate as
described herein. Antibodies that may be administered in combination as a naked
antibody or as a supplemental immunoconjugate include antibodies that react with
CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30,
CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD80, CD126, B7,
MUC1, Ia, HM1.24, tenascin, and mature HLA-DR, preferably a mature HLA-DR
dimer, formulated in a pharmaceutically acceptable vehicle.

Method of Diagnosis

Also provided is a method of diagnosing a disease in a subject, diagnosed with
or suspected of having at least one of the diseases selected from the groups consisting
of lymphoma, leukemia, myeloma, other CD-74-expressing malignancies, immune
dysregulation disease, autoimmune disease and a combination thereof, comprising
administering to said subject a diagnostically effective amount of a composition that
includes (1) an immunoconjugate including at least one anti-CD74 binding molecule
conjugated to a carrier, (2) a diagnostic agent, and (3) a pharmaceutically acceptable
excipient. The diagnostic agent may be covalently, non-covalently, or otherwise
associated with one or more components of the composition. A useful diagnostic
agent may include a radioisotope, wherein the photons of the radioisotope are
detected by radioscintigraphy or PET, or a metal that can be detected by MRI, or a
liposome or gas filled liposome, and wherein the liposome can be detected by an
ultrasound scanning device. As such, the immunoconjugate may form a liposome,
and/or the diagnostic agent may comprise a second liposome.
The internalization of the immunoconjugate into target cells can be followed by fluorescence labeling, essentially according to the procedure of Pirker et al., J. Clin. Invest., 76: 1261 (1985), which is incorporated by reference.

In a related vein, a method for screening/diagnosing bone cancers is described in Juweid et al., 1999, could benefit from the immunoconjugates disclosed herein. Accordingly, a method comprising $^{99m}$Tc-labeled humanized or chimeric anti-CD74 mAb immunoconjugates is contemplated.

EXAMPLES

**Example 1 Preparation of Anti-CD74 Immunoliposomes Carrying FUdR-dO**

Triolein (TO), egg phosphatidylcholine (EPC), dipalmitoyl phosphatidylethanolamine (DPPE), cholesterol (CHOL), 8-hydroxy-1,3,6-pyrenetrisulfonate (HPTS), polyoxyethylene sorbitan monooleate (sorbitan 80), methoxypolyethylene glycol (mean mol. wt 2000), oleoyl chloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and D,L-dithiothreitol (DTT) were obtained from Sigma Chemical Co. (St. Louis, MO). Poly(ethylene glycol)-maleimide-N-hydroxy-succinimidy ester (MAL-PEG$_{2000}$-NHS) was purchased from Shearwater Polymers Europe (Enschede, The Netherlands). [$^3$H]Cholesterol oleoyl ether (COE) and [$^{14}$C]dipalmitoyl phosphatidylcholine were obtained from Amersham International plc (Amersham, UK). A PEG$_{2000}$ derivative of DPPE with a maleimide group at the distal terminus of the PEG chain (DPPE-PEG-MAL) was synthesized by reacting 25 μmol NHS-PEG-MAL with 23 μmol DPPE and 50 μmol triethylamine in chloroform for 6 h at 40°C. The product was purified by preparative silica gel TLC. 3',5'-O-dioleoyl-FUdR (FUdR-dO) was synthesized by adding 20 μmol oleoyl chloride and 50 μl N,N-diisopropylethylamine to 10 μmol FUdR in dimethylacetamide. The mixture was incubated overnight at 40°C and then water was added to the mixture and the fatty acid derivative of FUdR was extracted with chloroform. The prodrug was purified by preparative silica gel TLC with chloroform/methanol (95:5) as eluent.
The Burkitt’s lymphoma cell lines, Raji and Ramos, Jurkat acute lymphoblastic leukemia T-cells and HL-60 myelomonocytic leukemia cells, obtained from American Type Culture Collection (Rockville, MD), were grown in RPMI 1640 medium with 10% heat-inactivated fetal calf serum. Cells were maintained at 37° C and gassed with 5% CO₂ in air.

The anti-CD74 Ab, LL1, was obtained from Immunomedics, Inc. (Morris Plains, NJ). It was labeled with fluorescein (FITC) for quantitation.

Submicron lipid emulsions were prepared as described in detail elsewhere. (See, Lundberg, J. Pharm. Sci., 83:72-75 (1994); Lundberg et al., Int. J. Pharm., 134:119-127 (1996)). The composition of the drug-loaded emulsions was TO, EPC, polysorbate 80, DPPE-PEG₂₀₀₀-MAL, F UdR-dO 2:2:0.8:0.6:0.3 (w/w). The components were dispensed into vials from stock solutions and the solvent was evaporated to dryness under reduced pressure. Phosphate-buffered saline (PBS) was added and the mixture was heated to 50°C, vortex mixed for 30 s, and sonicated with a Branson probe sonicator for 2 min.

Drug loaded liposomes were composed of EPC, DPPE-PEG₂₀₀₀-MAL, F UdR-dO 1:0:2:0.1 (w/w). In experiments involving HPTS-encapsulated liposomes the composition was EPC, CHOL, DPPE-PEG₂₀₀₀-MAL 2:0.5:0.4. When required, the lipid drug-carriers were labeled with trace amounts of [³H]COE. Dried lipid films were hydrated in 25 mM HEPES and 140 mM NaCl buffer (pH 7.4), (containing 35 mM HPTS when appropriate) subjected to five freezing-thawing cycles and subsequent sonication for 2 min with a Branson probe sonicator. The phospholipid concentration was quantitated by [¹⁴C]DPPC.

Coupling of LL1 to lipid drug-carriers was performed by reaction between the maleimide (MAL) groups at the distal PEG termini on the surface of the carriers and free thiol groups on the Ab. Before the coupling reaction LL1 was reduced with 50 mM dithiothreitol for 1 h at 4°C in 0.2 M tris buffer (pH 6.5). The reduced Ab was separated from excess dithiothreitol by use of Sephadex G-25 spin-columns, equilibrated with 50 mM sodium acetate buffered 0.9 % saline (pH 5.3). The conjugation was performed in HEPES-buffered saline (pH 7.4) for 16 h at room
temperature under argon. Excess maleimide groups were blocked with 2 mM 2-
mercaptoethanol for 30 min, whereafter excess Ab and 2-mercaptoethanol were
removed on a Sepharose CL-4B column. The immunoliposomes were collected near
the void volume of the column, passed through a 0.22 μm sterile filter and stored at
4°C. The coupling efficiency was estimated by use of fluorescein labeled LL1.

Example 2 Cellular Uptake and Metabolism of the Anti-CD74

Immunoliposomes

Lipid drug-carriers containing the non-exchangeable marker [3H]COE were
used to study the cellular uptake of drug carrier. After completed incubation, the cells
were thoroughly washed three times with cold PBS and the radioactivity measured by
liquid scintillation counting. The pH-sensitive probe HPTS was used to study the
internalization of liposomes to low pH compartments. HPTS exhibits two major
fluorescence excitation maxima: a peak at 403 maximal at low pH values and a peak
at 454 maximal at high pH values, while the fluorescence is independent of pH at 413
nm (isobestic point). (See, Straubinger, et al., Biochemistry, 29:4929-4939 (1990)).
The ratio between the fluorescence at 454 nm and 413 nm can be used to study the
internalization of the HPTS-liposomes to intracellular acidic compartments. HPTS-
liposomes were diluted to 80 μM phospholipid in HEPES buffer and added to culture
dishes (4 x 10⁶ cells) at 37°C. After incubation for 6 h the cells were washed twice
with cold PBS and the fluorescence was measured in a stirred cuvette at 20°C. Peak
heights were measured at 510 nm emission at the two excitation wavelengths (413
and 454 nm) and corrected for appropriate background fluorescence.

Figure 5 demonstrates the concentration-dependent cellular association of lipid
drug-carriers with coupled LL1 and lipid drug-carriers without coupled LL1.
Association of lipid drug-carriers with and without coupled LL1 was concentration
dependent. Figure 5 shows that the Burkitt’s lymphoma cells, Raji, show a massive
interaction with LL1-lipid drug-carrier complexes as compared to untargeted
preparations. LL1-emulsion conjugates, labeled with the nontransferable compound
[3H]COE, were taken up about 50 times faster than unconjugated emulsions by Raji
cells in culture. The fast and massive uptake of immunoemulsions is demonstrated by the fact that under standard incubation conditions about 30% of the added preparation was associated with cells after 24 h. The corresponding association of emulsions without coupled Ab was about 0.6%. The uptake values for Ramos cells were considerably lower but still about 30 times higher for LL1-complexes than for uncomplexed emulsions. The time-dependent association of targeted carriers was fairly linear up to 24-h, but at prolonged incubation times the curve declined.

**Example 3 Specificity of Immunoconjugates**

The cell specificity of the preparations was tested on HL-60 and Jurkat cells. See Figure 5, B. Bottom panel. The cellular association obtained after 24 h was between 1 and 2% for both cell types with no evident difference between conjugates and plain emulsions. This extent of cellular association clearly represent unspecific uptake. The specificity of the interaction was further studied by measuring the cellular association of [³H]COE-labeled LL1-emulsions versus the amount of LL1 per emulsion EPC. See Figure 6. These experiments demonstrated that association of the LL1-emulsions was dependent on the concentration of LL1. The specificity of the interaction of immunoemulsions with cells was also studied by displacement experiments. Figure 7 shows that free LL1 competes effectively with the LL1-emulsion complexes and at high concentrations the cellular association is practically abolished. These findings strongly indicates that LL1 preserves its immunoreactivity after binding to lipid drug-carriers.

**Example 4 Endocytosis of HPTS-containing immunoliposomes**

The intracellular fate of LL1-liposomal complexes was studied by use of the pH-sensitive probe HPTS. The spectral shifts of the probe with changes in pH make it a useful marker of the uptake and fate of the encapsulated dye. Figure 8 shows the concentration-dependent cellular association of HPTS loaded LL1-liposomes. Internalization of LL1-liposomes to low-pH compartments was demonstrated by the fluorescence ratio λex 454/413. Values near 0.6 were obtained which corresponds to a pH value of 6.5. This value is near those obtained by other authors with HPTS-

**Example 5 Cytotoxicity Assays**

Comparison of the in vitro cytotoxicity of free FUdR and FUdR-dO-loaded emulsions and liposomes with and without coupled LL1 was performed on Raji human B-cell lymphoma lines with a proliferation assay utilizing tetrazolium dye, MTT. (See, Mosmann, *J. Immun. Meth.*, 65:55-63 (1983)). To begin, 4 x 10⁶ cells were plated in 24-well plates and incubated with drug containing preparations. Control experiments included free LL1 and drug free emulsion and liposomes. Cells were incubated for 24 h at 37°C in an atmosphere of 95% humidity and 5% CO₂. At the 24 h time point, the cells were washed twice before replacing with fresh media and incubated for an additional 48 h. At the end of the incubation time, tetrazolium dye was added, the formed reduction product was spun down, dissolved in EtOH:DMSO 1:1 and read at 570 nm.

The cytotoxic activity of FUdR-dO in LL1 conjugated emulsions and liposomes was tested and compared with the activity of unconjugated drug-carriers on Raji lymphoma cells. See Figure 9. The effect of free FUdR (in PBS) was also recorded. The cells were incubated with the various preparations for 24-h, followed by an additional 48-h in fresh medium. From the dose-response curves it could be seen that FUdR-dO is somewhat more efficacious in emulsions than in liposomes.

However, the activity of FUdR-dO administered in both LL1-emulsions and LL1-liposomes exceeded that of FUdR. The IC₇₀ values obtained were 0.45, 1.25, 5.3 and 7.3 µM for FUdR-dO loaded LL1-emulsions, LL1-liposomes, emulsions and liposomes, respectively. The corresponding value for FUdR was calculated to 4.35 µM. The IC₅₀ values were 2.5, 5.3 and 7.0 µM for LL1-emulsions, LL1-liposomes
and FudR, respectively (F UdR-dO in plain emulsions and liposomes did not reach that level).

The prodrug F UdR-dO employed in this study shows several advantageous features for administration in lipid drug-carriers. It is amphiphilic and will be situated in the phospholipid monolayer and bilayer of lipid emulsions and liposomes, respectively. This makes the preparation of drug-carrier very convenient; the components are just mixed together and sonicated. An alternative method, which is more suited for large scale production, would be the use of high pressure homogenization.

A prerequisite for site-specific delivery of the prodrug to the target cells is the stable entrapment of the prodrug in the drug-carrier. The unspecific transfer of F UdR-dO from carrier to cells was not actually measured but the much higher cytotoxic activity of the LL1-conjugated preparations indicates that the unspecific transfer of prodrug to cells is relatively low. That some degree of surface transfer probably occurs find support by a study of Koning et al., Biochim. Biophys. Acta 1420 (1999) 153-167. They found that dipalmitoyl-F UdR immunoliposomes, without internalization, could deliver the prodrug to target cells more efficient than liposomes without antibody.

The prodrug concept comprises a pharmacologically inactive compound that is activated when exposed into the target cells. In this respect F UdR-dO may fulfill the criteria of a good prodrug. It has been shown that F UdR fatty acid esters are hydrolyzed fast in cells, apparently in lysosomes. See id. An efficient intracellular liberation of the parent drug F UdR is also indirectly supported by the high cytotoxic efficacy of the F UdR-dO preparations.

An explanation for such an favorable effect appears to be that the half-life of the drug increases and the tolerability is improved so that high doses can be administered. A recent in vivo study with nude mice demonstrated specific Ab localization of LL1 to Ramos xenografts. See Shih et al., Cancer Immunol. Immunother. 49 (2000) 208-216. The present study shows an improved cytotoxic activity of the targeted prodrug compared to the parent drug. Immunoliposomes generally show lower or similar activity compared to the untargeted drug, but still demonstrate improved efficacy in in vivo experiments. See Moase et al., Biochim. Biophys. Acta 1510 (2001) 43-55; Lopes de Menezes et al., Cancer Res. 58 (1998) 3320-3330.

All patents and other references cited in the specification are indicative of the level of skill of those skilled in the art to which the invention pertains, and are incorporated by reference in their entireties, including any tables and figures, to the same extent as if each reference had been incorporated by reference in its entirety individually.

One skilled in the art would readily appreciate that the present invention is well adapted to obtain the ends and advantages mentioned, as well as those inherent therein. The methods, variances, and compositions described herein as presently representative of preferred embodiments are exemplary and are not intended as limitations on the scope of the invention. Changes therein and other uses will occur to those skilled in the art, which are encompassed within the invention.

It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. For example, a variety of different binding pairs can be utilized, as well as a variety of different therapeutic and diagnostic agents. Thus, such additional embodiments are within the scope of the present invention.

The invention illustratively described herein suitably may be practiced in the absence of any element or elements, limitation or limitations which is not specifically disclosed herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with
either of the other two terms. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention. Thus, it should be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

Also, unless indicated to the contrary, where various numerical values are provided for embodiments, additional embodiments are described by taking any 2 different values as the endpoints of a range. Such ranges are also within the scope of the described invention.
CLAIMS

1. A composition comprising:

an immunoconjugate which comprises one or more anti-CD74 binding molecules conjugated to one or more lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof; and

one or more effectors.

2. The composition of claim 1 further comprising an emulsion.

3. The composition of claim 1 further comprising a liposome.

4. The composition of claim 1 wherein one or more of the anti-CD74 binding molecules are conjugated to one or more lipids.

5. The composition of claim 1 wherein one or more of the anti-CD74 binding molecules are conjugated to one or more polymeric carriers.

6. The composition of claim 1 wherein one or more of the anti-CD74 binding molecules are conjugated to one or more micelles.

7. The composition of claim 1 wherein one or more of the anti-CD74 binding molecules are conjugated to one or more nanoparticles.

8. The composition of claim 1 wherein the one or more anti-CD74 binding molecules comprise LL1 or fragments thereof.

9. The composition of claim 1 wherein the one or more anti-CD74 binding molecules are conjugated to the one or more lipids by one or more of a sulfide linkage, a hydrazine linkage, a hydrazine linkage, an ester linkage, an amido linkage, an amino linkage, an imino linkage, a thiosemicarbazone linkage, a semicarbazone linkage, an oxime linkage, a carbon-carbon linkage, or combinations thereof.

10. The composition of claim 9 wherein the binding molecule is conjugated by a sulfide linkage.

11. The composition of claim 1 further comprising one or more additional binding molecules which specifically bind to one or more antigens selected from the group consisting of CD4, CD5, CD8, CD14, CD15, CD19, CD20, CD21, CD22, CD23, CD25, CD30, CD33, CD37, CD38, CD40, CD40L, CD46, CD52, CD54, CD80, CD126, B7, MUC1, MUC2, MUC3, MUC4, Ia, HM1.24, tensascin, VEGF,
EGFR, CEA, CSAP, ILGF, placental growth factor, carbonic anhydrase IX, IL-6 and combinations thereof.

12. The composition of claim 11 wherein the additional binding molecule is conjugated to the one or more lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof.

13. The composition of claim 1 wherein one or more of the lipids are amphiphilic.

14. The composition of claim 1 wherein one or more of the lipids comprise one or more nucleophilic carbons at a distal terminus.

15. The composition of claim 1 wherein one or more of the lipids comprise one or more maleimide groups at a distal terminus.

16. The composition of claim 15 wherein one or more the lipids comprise PEG-maleimide.

17. The composition of claim 15 wherein one or more of the anti-CD74 binding molecules are linked to one or more of the maleimide groups.

18. The composition of claim 15 wherein one or more of the anti-CD74 binding molecules are linked by one or more free thiol groups to one or more of the maleimide groups.

19. The composition of claim 1 wherein one or more of the lipids comprise polyethylene glycol (PEG).

20. The composition of claim 1 wherein the effector comprises a therapeutic agent or a diagnostic agent.

21. The composition of claim 1 wherein the effector comprises a drug, a prodrug, a toxin, an enzyme, a radioisotope, an immunomodulator, a cytokine, a hormone, a binding molecule, an oligonucleotide, a photodynamic agent, or mixtures thereof.

22. The composition of claim 1 wherein the effector comprises aplidin, azaridine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin
glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethynyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUDR), 3',5'-O-dioleoyl-FudR (FUDR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, PSI-341, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiotepa, teniposide, topotecan, uracil mustard, velcade, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, onconase, rapL1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin, or combinations thereof.

23. The composition of claim 22 wherein the effector comprises FUDR, FUDR-dO, or mixtures thereof.

24. The composition of claim 1 further comprising one or more hard acid chelators or soft acid chelators.

25. The composition of claim 1 further comprising cations selected from Group II, Group III, Group IV, Group V, transition, lanthanide or actinide metal cations, or mixtures thereof.

26. The composition of claim 1 further comprising cations selected from Tc, Re, Bi, Cu, As, Ag, Au, At, Pb, or mixtures thereof.

27. The composition of claim 1 further comprising NOTA, DOTA, DTPA, TETA, Tscg-Cys, Tsca-Cys, or mixtures thereof.

28. The composition of claim 1 wherein the effector comprises a nuclide.

29. The composition of claim 28 wherein the nuclide comprises 18F, 32P, 33P, 45Ti, 47Sc, 52Fe, 59Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 75Se, 77As, 86Y, 89Sr, 89Zr, 90Y, 94Tc, 94mTc, 99Mo, 99mTc, 105Pd, 105Rh, 111Ag, 111In, 123I, 124I, 125I, 131I, 142Pr, 143Pr, 149Pm, 153Sm, 154-158Gd, 161Tb, 166Dy, 166Ho, 169Er, 175Lu, 177Lu, 186Re, 188Re, 189Re, 194Ir, 198Au, 199Au, 211At, 211Pb, 212Bi, 212Pb, 213Bi, 223Ra, 225Ac, or mixtures thereof.
30. The composition of claim 1 wherein the effector comprises an enzyme.

31. The composition of claim 30 wherein the enzyme is selected from the group consisting of carboxylesterases, glucuronidases, carboxypeptidases, beta-lactamases, phosphatases, and mixtures thereof.

32. The composition of claim 1 wherein the effector comprises an immunomodulator.

33. The composition of claim 32 wherein the immunomodulator is selected from the group consisting of IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-α, interferon-β, interferon-γ, G-CSF, GM-CSF, and mixtures thereof.

34. The composition of claim 1 wherein the effector comprises an anti-angiogenic agent selected from the group consisting of angiostatin, endostatin, basculostatin, canstatin, maspin, anti-VEGF binding molecules, anti-placental growth factor binding molecules, anti-vascular growth factor binding molecules, and mixtures thereof.

35. The composition of claim 1 wherein the anti-CD74 binding molecule is conjugated to one or more therapeutic agents, diagnostic agents, or mixtures thereof.

36. The composition of claim 1 wherein the anti-CD74 binding molecule comprises a humanized, human or chimeric anti-CD74 antibody or fragment thereof.

37. The composition of claim 36 wherein the humanized, human or chimeric anti-CD74 antibody or fragment thereof comprises a monoclonal antibody (mAb) or fragment thereof.

38. The composition of claim 36 wherein the human, chimeric, or humanized anti-CD 74 antibody or fragment thereof comprises:

   a fragment which comprises F(ab')2, Fab, scFv, Fv, or a fusion protein utilizing part or all the light and heavy chains of the F(ab')2, Fab, scFv, or Fv; and wherein the fragment binds to CD74.

39. The composition of claim 38 wherein the fusion protein is multivalent, or multivalent and multispecific.

40. The composition of claim 36 wherein the human, chimeric, or humanized anti-CD 74 antibody or fragment thereof further comprises constant
regions of IgG1, which are replaced with human constant regions of human IgG2a, IgG3, or IgG4.

41. The composition of claim 1 wherein the anti-CD 74 binding molecule is an anti-CD 74 diabody, a triabody, or a tetrabody.

42. A method for treating and/or diagnosing a disease or disorder comprising administering to a patient a therapeutic and/or diagnostic composition comprising the composition of claim 1 and a pharmaceutically acceptable excipient.

43. The method of claim 42 wherein the disease or disorder is a CD74-expressing malignancy.

44. The method of claim 42 wherein the disease or disorder is selected from the group consisting of an immune dysregulation disease, an autoimmune disease, an organ-graft rejection, and a graft-versus-host disease.

45. The method of claim 43 wherein the CD74-expressing malignancy is selected from the group consisting of a solid tumor, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, multiple myeloma, a B-cell malignancy, and a T-cell malignancy.

46. The method of claim 42 wherein the disease or disorder is a CD74-expressing malignancy other than lymphoma or leukemia.

47. The method of claim 43 wherein the CD74-expressing malignancy is a solid tumor.

48. The method of claim 47 wherein the solid tumor is selected from the group consisting of a melanoma, carcinoma, sarcoma, and glioma.

49. The method of claim 48 wherein the carcinoma is selected from the group consisting of a renal carcinoma, lung carcinoma, intestinal carcinoma, stomach carcinoma, breast carcinoma, prostate cancer, ovarian cancer, and melanoma.

50. The method of claim 43 wherein the CD74-expressing malignancy is a B-cell malignancy selected from the group consisting of indolent forms of B-cell lymphomas, aggressive forms of B-cell lymphomas, chronic lymphatic leukemias, acute lymphatic leukemias, and multiple myeloma.

51. The method of claim 42 wherein the composition is administered intravenously or intramuscularly at a dose of 20-5000 mg.
52. The method of claim 42 wherein the composition comprises LL1 or a fragment thereof.

53. The method of claim 42 wherein the composition further comprises one or more additional antibodies or fragments thereof selected from the group consisting of anti-CD19, anti-CD20, anti-CD22, anti-CD30, anti-CD33, anti-CD52, anti-HLA-DR, anti-MUC1, anti-TAC, and mixtures thereof.

54. The method of claim 43 wherein one or more of the additional antibodies are conjugated to one or more of the lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof.

55. The method of claim 42 wherein the effector molecule comprises one or more drugs, prodrugs, toxins, enzymes, radioisotopes, immunomodulators, cytokines, hormones, antibodies, oligonucleotides, or combinations thereof.

56. The method of claim 42 wherein the effector molecule comprises aplidin, azarbine, anastrozole, acyclovir, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboptatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethinyl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (FUDR), 3',5'-O-dioleoyl-FUDR (FUDR-dO), fludarabine, flutamide, fluorouracil, fluoxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroprogesterone acetate, megestrol acetate, melphalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, PSI-341, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiopeta, teniposide, topotecan, uracil mustard, velcade, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, onconase, rapLR1, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxin, Pseudomonas exotoxin, and Pseudomonas endotoxin, or combinations thereof.
57. The method of claim 56 wherein the effector comprises FUDR, FUDR-dO, or mixtures thereof.

58. The method of claim 42 wherein the composition further comprises one or more hard acid chelators or soft acid chelators.

59. The method of claim 42 wherein the composition further comprises cations selected from Group II, Group III, Group IV, Group V, transition, lanthanide or actinide metal cations, or mixtures thereof.

60. The method of claim 42 wherein the composition further comprises cations selected from Tc, Re, Bi, Cu, As, Ag, Au, At, Pb, or mixtures thereof.

61. The method of claim 42 wherein the composition further comprises NOTA, DOTA, DTPA, TETA, Tscg-Cys, Tsca-Cys, or mixtures thereof.

62. The method of claim 42 wherein the composition comprises a nuclide.

63. The method of claim 62 wherein the nuclide comprises 18F, 32P, 33P, 45Ti, 47Sc, 52Fe, 59Fe, 62Cu, 64Cu, 67Cu, 67Ga, 68Ga, 75Se, 77As, 86Y, 89Sr, 89Zr, 90Y, 94Tc, 94Mo, 99mTc, 105Pd, 105Rh, 111In, 123I, 124I, 125I, 131I, 142Pr, 143Pm, 149Pm, 153Sm, 154-158Gd, 161Tb, 166Dy, 166Ho, 169Er, 175Lu, 177Lu, 186Re, 188Re, 189Re, 194Ir, 198Au, 199Au, 211At, 212Bi, 212Pb, 213Bi, 223Ra, 225Ac, or mixtures thereof.

64. The method of claim 62 wherein the composition comprises an enzyme.

65. The method of claim 64 wherein the enzyme comprises carboxylesterases, glucoronidases, carboxypeptidases, beta-lactamases, phosphatases, or mixtures thereof.

66. The method of claim 62 wherein the composition comprises an immunomodulator.

67. The method of claim 66 wherein the immunomodulator comprises IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-α, interferon-β, interferon-γ, G-CSF, GM-CSF, or mixtures thereof.

68. The method of claim 42 wherein the composition comprises one or more agents for photodynamic therapy.

69. The method of claim 68 wherein the agent for photodynamic therapy is a photosensitizer.
70. The method of claim 69 wherein the photosensitizer comprises a benzoporphyrin monoacid ring A (BDP-MA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AISPe) and lutetium texaphyrin (Lutex).

71. The method of claim 42 wherein the composition comprises one or more diagnostic agents.

72. The method of claim 42 wherein the composition comprises a diagnostic nuclide.

73. The method of claim 72 wherein the diagnostic nuclide comprises $^{18}$F, $^{52}$Fe, $^{62}$Cu, $^{64}$Cu, $^{67}$Cu, $^{67}$Ga, $^{68}$Ga, $^{86}$Y, $^{89}$Zr, $^{94}$Tc, $^{94m}$Tc, $^{99m}$Tc, $^{111}$In, $^{123}$I, $^{124}$I, $^{125}$I, $^{131}$I, or mixtures thereof.

74. The method of claim 73 wherein the diagnostic nuclide emits 25-4000 keV gamma particles and/or positrons.

75. The method of claim 71 wherein the diagnostic agent is used for performing positron emission tomograph (PET).

76. The method of claim 42 further comprising performing positron-emission tomography (PET).

77. The method of claim 71 wherein the diagnostic agent comprises one or more image enhancing agents and the method further comprises performing magnetic resonance imaging (MRI).

78. The method of claim 77 wherein the image enhancing agent comprises gadolinium ions, lanthanum ions, manganese ions, iron, chromium, copper, cobalt, nickel, fluorine, dysprosium, rhenium, europium, terbium, holmium, neodymium, or mixtures thereof.

79. The method of claim 42 wherein the composition comprises one or more radiopaque agents or contrast agents for X-ray or computed tomography (CT).

80. The method of claim 42 wherein said radiopaque or contrast agents include barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iopamidol, iodoxamic acid, iogumil, iohexol, iopromide, iopanoic acid, ioprocemic acid, iosefamic acid, ioserolic acid, iosulamide meglumine, iosemestic acid, iotasul, iotetric acid, iothalamic acid, iotroxic acid, ioxaglic acid, ioxotrizonic
acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, thallous chloride, or combinations thereof.

81. The method of claim 42 wherein the composition comprises one or more ultrasound contrast agents.

82. The method of claim 81 wherein said ultrasound contrast agent includes a liposome or dextran.

83. The method of claim 82 wherein the liposome is gas-filled.

84. The method of claim 42 further comprising performing an operative, intravascular, laparoscopic, or endoscopic procedure.

85. The method of claim 42 further comprising administering an additional composition which comprises a therapeutic agent, a diagnostic agent, or mixtures thereof.

86. The method claim 42 wherein the additional composition comprises: an immunoconjugate which comprises one or more anti-CD74 binding molecules conjugated to one or more lipids, polymeric carriers, micelles, nanoparticles, or combinations thereof; and one or more effectors.

87. The method of claim 86 wherein the anti-CD 74 antibody or fragment thereof is conjugated to the therapeutic agent, the diagnostic agent, or mixtures thereof by chemical conjugation or genetic fusion.

88. The method of claim 85 wherein the composition is administered before, during, simultaneously, or after the administration of the additional composition.

89. The method of claim 85 wherein the additional composition comprises one or more drugs, prodrugs, toxins, enzymes, radioisotopes, immunomodulators, cytokines, hormones, antibodies, oligonucleotides, or combinations thereof.

90. The method of claim 85 wherein the additional composition comprises: aplidin, azaribine, anastrozole, azacytidine, bleomycin, bortezomib, bryostatin-1, busulfan, calicheamycin, camptothecin, 10-hydroxycamptothecin, carmustine, celebrex, chlorambucil, cisplatin, irinotecan (CPT-11), SN-38, carboplatin, cladribine, cyclophosphamide, cytarabine, dacarbazine, docetaxel, dactinomycin, daunomycin
glucuronide, daunorubicin, dexamethasone, diethylstilbestrol, doxorubicin, doxorubicin glucuronide, epirubicin glucuronide, ethynl estradiol, estramustine, etoposide, etoposide glucuronide, etoposide phosphate, floxuridine (F UdR), 3',5'-O-dioleoyl-FudR (F UdR-dO), fludarabine, flutamide, fluorouracil, fluoroxymesterone, gemcitabine, hydroxyprogesterone caproate, hydroxyurea, idarubicin, ifosfamide, L-asparaginase, leucovorin, lomustine, mechlorethamine, medroxyprogesterone acetate, megestrol acetate, melfalan, mercaptopurine, 6-mercaptopurine, methotrexate, mitoxantrone, mithramycin, mitomycin, mitotane, phenyl butyrate, prednisone, procarbazine, paclitaxel, pentostatin, PSI-341, semustine streptozocin, tamoxifen, taxanes, taxol, testosterone propionate, thalidomide, thioguanine, thiopeta, teniposide, topotecan, uracil mustard, velcade, vinblastine, vinorelbine, vincristine, ricin, abrin, ribonuclease, onconase, rapLrI, DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, gelonin, diphtheria toxoid, Pseudomonas exotoxin, and Pseudomonas endotoxin, or combinations thereof.

91. The method of claim 90 wherein the additional composition comprises F UdR, F UdR-dO, or mixtures thereof.

92. The method of claim 85 wherein the additional composition comprises one or more hard acid chelators or soft acid chelators.

93. The method of claim 85 wherein the additional composition comprises cations selected from Group II, Group III, Group IV, Group V, transition, lanthanide or actinide metal cations, or mixtures thereof.

94. The method of claim 85 wherein the additional composition comprises cations selected from Tc, Re, Bi, Cu, As, Ag, Au, At, Pb, or mixtures thereof.

95. The method of claim 85 wherein the additional composition comprises NOTA, DOTA, DTPA, TETA, Tscg-Cys, Tsca-Cys, or mixtures thereof.

96. The method of claim 85 wherein the additional composition comprises a nuclide.

97. The method of claim 96 wherein the nuclide comprises ¹⁸F, ⁴⁵P, ¹³¹P, ⁴⁵Ti, ⁴⁷Sc, ⁵²Fe, ⁵⁹Fe, ⁶²Cu, ⁶⁴Cu, ⁶⁷Cu, ⁶⁸Ga, ⁷⁵Se, ⁷⁷As, ⁸⁶Y, ⁸⁹Sr, ⁹⁰Y, ⁹⁴Tc, ⁹⁴mTc, ⁹⁹mTc, ¹⁰⁵Pd, ¹⁰⁵Rh, ¹¹⁰Ag, ¹¹¹In, ¹²³I, ¹²⁴I, ¹²⁵I, ¹³¹I, ¹⁴²Pr, ¹⁴³Pr, ¹⁴⁹Pm,
153Sm, 154,155Gd, 161Tb, 166Dy, 166Ho, 169Er, 175Lu, 177Lu, 186Re, 188Re, 189Re, 194Ir, 198Au, 199Au, 211At, 211Pb, 212Bi, 213Bi, 223Ra, 225Ac, or mixtures thereof.

98. The method of claim 85 wherein the additional composition comprises an enzyme.

99. The method of claim 85 wherein the enzyme comprises carboxylesterases, glucoronidases, carboxypeptidases, beta-lactamases, phosphatases, or mixtures thereof.

100. The method of claim 85 wherein the additional composition comprises an immunomodulator.

101. The method of claim 100 wherein the immunomodulator comprises IL-1, IL-2, IL-3, IL-6, IL-10, IL-12, IL-18, IL-21, interferon-α, interferon-β, interferon-γ, G-CSF, GM-CSF, or mixtures thereof.

102. The method of claim 85 wherein the additional composition comprises one or more diagnostic agents.

103. The method of claim 85 wherein the additional composition comprises one or more agents for photodynamic therapy.

104. The method of claim 103 wherein the agent for photodynamic therapy is a photosensitizer.

105. The method of claim 104 wherein the photosensitizer comprises a benzoporphyrin monoacid ring A (BDP-MA), tin etiopurpurin (SnET2), sulfonated aluminum phthalocyanine (AISPe) and lutetium texaphyrin (Lutex).

106. The method of claim 85 wherein the additional composition comprises a diagnostic nuclide.

107. The method of claim 106 wherein the diagnostic nuclide comprises 18F, 52Fe, 62Cu, 64Cu, 67Cu, 67Ga, 86Y, 89Zr, 94Tc, 94mTc, 99mTc, 111In, 123I, 124I, 125I, 123I, or mixtures thereof.

108. The method of claim 106 wherein the diagnostic nuclide emits 25-4000 keV gamma particles and/or positrons.

109. The method of claim 102 wherein the diagnostic agent is used for performing positron emission tomograph (PET).
110. The method of claim 85 further comprising performing positron-emission tomography (PET).

111. The method of claim 102 wherein the diagnostic agent comprises one or more image enhancing agents and the method further comprises performing magnetic resonance imaging (MRI).

112. The method of claim 111 wherein the image enhancing agent comprises gadolinium ions, lanthanum ions, manganese ions, iron, chromium, copper, cobalt, nickel, fluorine, dysprosium, rhenium, europium, terbium, holmium, neodymium, or mixtures thereof.

113. The method of claim 85 wherein the additional composition comprises one or more radiopaque agents or contrast agents for X-ray or computed tomography (CT).

114. The method of claim 85 wherein said radiopaque or contrast agents include barium, diatrizoate, ethiodized oil, gallium citrate, iocarmic acid, iocetamic acid, iodamide, iopamipam, iodoxamic acid, iogulamide, iohexol, iopamidol, iopanoic acid, ioprocemic acid, iosefamic acid, ioseric acid, iosulamide meglumine, iosemamic acid, iotasul, iotetric acid, iothalamic acid, iotrophic acid, ioxaglic acid, ioxorizioic acid, ipodate, meglumine, metrizamide, metrizoate, propyliodone, thallous chloride, or combinations thereof.

115. The method of claim 85 wherein the additional composition comprises one or more ultrasound contrast agents.

116. The method of claim 115 wherein said ultrasound contrast agent includes a liposome or dextran.

117. The method of claim 116 wherein the liposome is gas-filled.

118. The method of claim 85 further comprising performing an operative, intravascular, laparoscopic, or endoscopic procedure.

119. A method of preparing a carrier comprising: mixing one or more amphiphilic lipids with an effector to form a carrier; and contacting the carrier with an anti-CD74 antibody.

120. The method of claim 119 wherein one or more of the lipids comprise a maleimide group.
121. The method of claim 119 further comprising reducing the antibody.

122. The method of claim 120 further comprising reacting one or more of the maleimide groups with a free thiol group on the anti-CD74 antibody.

123. The method of claim 119 wherein the effector comprises one or more drugs, prodrugs, toxins, enzymes, radioisotopes, immunomodulators, cytokines, hormones, antibodies, oligonucleotides, or mixtures thereof.

124. The method of claim 119 further comprising mixing the carrier with one or more therapeutic or diagnostic agents.

125. A kit comprising the composition of claim 1.
Figure 6

Cellular association (cpm x 10^3 / 2 x 10^9 cells) vs. LL1/EPC (ng/pmol)
Figure 9

![Graph showing cytotoxicity (% of control) against FUrR-dO (µM)]