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(54) **MORPHOLINO IMAGING AND THERAPY**

MORPHOLINOBILDGEBUNG UND THERAPIE

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(56) References cited:

US-A- 5 645 985

US-A- 5 698 685

US-A- 5 958 408

- **LIU GUOZHENG ET AL: "Tumor pretargeting in mice using (99m)Tc-labeled morpholino, a DNA analog." JOURNAL OF NUCLEAR MEDICINE : OFFICIAL PUBLICATION, SOCIETY OF NUCLEAR MEDICINE. MAR 2002, vol. 43, no. 3, March 2002 (2002-03), pages 384-391, XP002277164 ISSN: 0161-5505**
- **MANG'ERA K O ET AL: "Initial investigations of 99mTc-labeled morpholinos for radiopharmaceutical applications." EUROPEAN JOURNAL OF NUCLEAR MEDICINE. NOV 2001, vol. 28, no. 11, November 2001 (2001-11), pages 1682-1689, XP002277165 ISSN: 0340-6997**

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Description

[0001] This application is based on U.S. provisional patent application serial Nos. 60/279,809, filed March 30, 2001, and 60/341,794, filed December 21, 2001.

FIELD OF THE INVENTION

[0002] The present invention is directed to a kit for targeting of a diagnostic or therapeutic agent to a target site in a mammal, as well as to a use of said kit for the preparation of a medicament for diagnosing or treating a pathological condition in a multistep process using a kit containing a complementary pair of single-stranded Morpholino oligomers.

BACKGROUND OF THE INVENTION

[0003] The objective of drug targeting research is to improve the effectiveness of therapeutic drugs by delivering them directly to the targeted tumor sites and allowing a more effective dosing at these sites, thereby reducing non-tumor-related side effects. Another objective is to achieve an absolute accretion of the therapeutic agent at the target site thereby increasing the target/non-target ratio.

[0004] Different targeting vectors comprising diagnostic or therapeutic agents conjugated to a targeting moiety for selective localization have long been known. Examples of targeting vectors include diagnostic agent or therapeutic agent conjugates of targeting moieties such as antibodies or antibody fragments, cell- or tissue-specific peptides, hormones and other receptor binding molecules. For examples, antibodies against different determinants associated with pathological and normal cells, as well as associated with pathogenic microorganisms, have been used for the detection and treatment of a wide variety of pathological conditions or lesions. In these methods, the targeting antibody is directly conjugated to an appropriate detecting or therapeutic agent as described, for example in, Hansen *et al.*, U.S. Pat. No. 3,927,193 and Goldenberg, U.S. Pat. Nos. 4,331,647, 4,348,376, 4,361,544, 4,468,457, 4,444,744, 4,460,459, 4,460,561, 4,624,846 and 4,818,709.

[0005] One of the problems encountered in direct targeting methods is that a relatively small fraction of the conjugate actually binds to the target site, while the majority of the conjugate remains in circulation and compromises in one way or another the function of the targeted conjugate. Other problems include high background and low resolution when a diagnostic agent is administered and marrow toxicity or systemic side effects when a therapeutic agent is attached to a long circulating targeting moiety.

[0006] Pretargeting methods have been developed to increase the target:background ratios of the detection or therapeutic agents. Examples of pretargeting and biotin/avidin approaches are described, for example, in Goodwin *et al.*, U.S. Pat. No. 4,863,713; Goodwin *et al.*, *J. Nucl. Med.* **29**:226 (1988); Hnatowich *et al.*, *J. Nucl. Med.* **28**:1294 (1987); Oehr *et al.*, *J. Nucl. Med.* **29**:728 (1988); Klibanov *et al.*, *J. Nucl. Med.* **29**:1951 (1988); Sinitsyn *et al.*, *J. Nucl. Med.* **30**:66 (1989); Kalofonos *et al.*, *J. Nucl. Med.* **31**:1791 (1990); Schechter *et al.*, *Int. J. Cancer.* **48**:167 (1991); Paganelli *et al.*, *Cancer Res.* **51**:5960 (1991); Paganelli *et al.*, *Nucl. Med. Commun.* **12**:211 (1991); Stickney *et al.*, *Cancer Res.* **51**:6650 (1991); and Yuan *et al.*, *Cancer Res.* **51**:3119 (1991).

[0007] In pretargeting methods, a primary targeting species (which is not bound to a diagnostic agent or therapeutic agent) comprising a first targeting moiety which binds to the targeting site and a binding site that is available for binding by a subsequently administered second targeting species is targeted to an *in vivo* target site. Once sufficient accretion of the primary targeting species is accomplished, a second targeting species comprising a diagnostic or therapeutic agent and a second targeting moiety, which recognizes the available binding site of the primary targeting species, is administered.

[0008] An illustrative example of pretargeting methodology is the use of a biotin-(strept)avidin system to administer a cytotoxic radioantibody to a tumor. In the first step, a monoclonal antibody targeted against a tumor-associated antigen is conjugated to avidin (or biotin) and administered to a patient who has a tumor recognized by the antibody. In the second step, the therapeutic agent, via its attached biotin (or avidin), is taken up by the antibody-avidin (or -biotin) conjugate pretargeted to the tumor.

[0009] However, difficulties have arisen in the applications of biotin-avidin or (strept)avidin system during pretargeting. First of all, unless properly constructed, radiolabeled biotins may be subject to plasma biotinidase degradation. Furthermore, when conjugated to antibodies, strept/avidin and avidin can generate anti-strept/avidin antibodies in a patient. Finally, the potential effects of endogenous biotin during *in vivo* pretargeting can lead to the disappearance of biotin binding expression because of saturation by biotin. This happened, for example, when one strept/avidin-conjugated antibody localized in a nude mouse xenograft became saturated with biotin. Rusckowski *et al.*, *Cancer* **80**:2699-705 (1997). A three-step strategy involving administration of biotinylated monoclonal antibody, avidin, followed by radiolabeled biotin alleviates some of the drawbacks; however, this procedure is considered complex for imaging and does not address immunogenicity.

[0010] Another recognized example of pretargeting method involves the use of the bispecific antibody-hapten recognition system which uses a radiolabeled hapten and a bispecific antibody in place of (strept)avidin and biotin. Barbet, J. *et al. Cancer Biother. Radiopharm.* **14**:153-166 (1999); Karacay, H. *et al., Bioconj. Chem.* **11**: 842-854 (2000); Gautherot, E. *et al., J. Nucl. Med.* **41**:480-487 (2000); Lubic, S.P. *et al., J. Nucl. Med.* **42**:670-678 (2001); Gestin, J.F. *et al., J. Nucl. Med.* **42**:146-153 (2001). The hapten is often a coordination complex, for example, indium-DTPA. The bispecific antibody is the product of linking two antibodies or antibody fragments against separate determinants, the hapten and a tumor marker such as carcinoembryonic antigen. In addition to the need to prepare bispecific antibodies, this approach may suffer from lower affinities. The affinity of an antibody for its hapten, particularly for a monovalent one, is orders of magnitude lower than that of (strept)avidin for biotin. Mathematical modeling has shown that a high affinity between an antibody and its hapten is an important determinant of successful pretargeting. Zhu, H. *et al., J. Nucl. Med.* **39**: 65-76-(1998).

[0011] As an alternative to the biotin-avidin and bispecific antibody-hapten systems for pretargeting, single-stranded oligomers, such as peptide nucleic acid (PNA), have been used. Single-stranded oligomers bind specifically to their complementary single-stranded oligomers by *in vivo* hybridization. A single-stranded PNA bound to a targeting moiety is first administered to a patient, followed by the single-stranded complementary PNA radiolabeled with a diagnostic agent. An example of this methodology is described in Rusckowski *et al., Cancer* **80**:2699-705 (1997). An optional intermediate step can be added to the two-step method by administration of a clearing agent. The purpose of the clearing agent is to remove circulating primary conjugate which is not bound at the target site. This is disclosed by Griffiths *et al.*, in U.S. Pat. No. 5,958,408.

[0012] Chemical modifications to the backbone of these single-stranded oligomers for attachment to radionucleotides are usually required to improve nuclease stability and decrease protein binding affinities. The influence of three distinct chemical modifications to one 18 mer phosphorothioate DNA to permit labeling with ^{99m}Tc have been compared *in vitro* and *in vivo* in mice. Zhang, Y.M. *et al., Eur. J. Nucl. Med.* **27**:1700-1707 (2000). While the association rate constant for hybridization was found to be independent of labeling method, both cellular accumulations in culture and the pharmacokinetic behavior of the radiolabel in normal mice was strongly influenced by the labeling method.

[0013] These *in vivo* properties of oligomers may possibly be influenced by changes in their chain length and/or base sequences. Conceivably, the pharmacokinetics of an oligomer may thereby be modified in a useful manner if the influences of chain length and base sequence were to be understood. Despite this possibility (and as in the case of the chemical modifications), these additional influences have almost entirely gone uninvestigated thus far. In part, this may be attributed to constraints placed on these parameters by the application. For example, antisense chemotherapy is thought to achieve efficacy usually by the hybridization of a short, single-chain oligomer with a base sequence complementary to that of its mRNA target. Hnatowich, D.J., *J. Nucl. Med.* **40**:693-703 (1999). The base sequence, and to an extent the chain length as well, are thus restricted to those providing the desired hybridization. Nevertheless, there are combinations of bases that have received attention. One example is the presence of a G-quartet (i.e. four guanine bases in a row) in either phosphodiester or phosphorothioate DNAs. Shafter, R.H. *et al., Biopoly (Nucleic Acid Sci.)* **56**:209-227 (2001). In the case of these chemical forms of DNAs at least, the stacking of the guanine bases provides the oligonucleotides with a particular three dimensional quadruplex structure. This structure is apparently responsible for a variety of sequence-specific effects with significance to various biological processes. Shafter, R.H. *et al., Biopoly. (Nucleic Acid Sci.)* **56**:209-227 (2001). Another example is the CpG motif, a cytosine base followed immediately by a guanine, that has been shown to be immunostimulatory. Zhao, Q. *et al., Antisense Nucleic Acid Drug Dev.* **7**:495-502 (1997). The influences of these sequences, if any, on pharmacokinetics has yet to be established.

[0014] A variety of other published reports have appeared concerning the *in vitro* influences of oligomer chain length and sequence. Cytotoxicity in one cell line of phosphodiester DNAs composed entirely of guanine and thymidine bases was found to require at least a chain length of 20 bases and the cytotoxicity disappeared with the introduction of adenines or cytosines at either end. Morassutti, C. *et al., Nucleosides & Nucleotides* **18**:1711-1716 (1999). The efficiency with which PNAs initiated transcription and gene expression in cells was found to be optimum at chain lengths of 16 to 18 bases. Wang, G. *et al., J. Mol. Biol.* **313**:933-940 (2001). Rat liver homogenates have been used *ex vivo* to investigate the metabolism of a series of phosphorothioate DNAs differing in chain length and base sequence. Crooke, R.M. *et al., J. Pharm. Exp. Therapeutics* **292**:140-149 (2000). All oligomers were degraded primarily by 3'exonucleases with the rate of metabolism increasing with increasing chain length. The rate and extent of nuclease metabolism was also related to base sequence in that pyrimidine-rich oligonucleotides were more labile. This particular investigation was unusual in that the influence of stereoisomerism was also studied. The metabolism rate was found to be more rapid for one of the diastereoisomers than the other with mixtures being digested at rates in between. Finally, a recent report described the influence of base sequence on reactivity of the phosphodiester bond in RNAs. Kaukinen, U. *et al., Nucl. Acids Res.* **30**: 468-474 (2002).

[0015] Despite the several advantages over the strept/avidin-biotin and bispecific antibody-hapten systems, a few limitations exist in the use of these oligomers in pretargeting. These limitations include poor specificity, possible insolubility in aqueous solutions, and high costs.

[0016] A need continues to exist for an improved kit and method for *in vivo* targeting to deliver a therapeutic or diagnostic agent to a target site in a mammal, that is more specific, affordable and inexpensive and provides higher target uptake and lower uptake in normal tissues.

SUMMARY OF THE INVENTION

[0017] Accordingly, an object of the present invention is to provide a kit and a use of said kit for the preparation of a medicament for targeting of a diagnostic or therapeutic agent in a mammal which can be prepared from relatively inexpensive starting materials but yet provides better specificity, stability, predictable targeting and/or more desirable antigen-antibody effects than conventional and other known kits and methods.

[0018] It is another object to provide an alternative use for tumor localization/imaging by stepwise targeting using a pair of single-stranded Morpholino oligomers (MORFs, as defined below) instead of strep/avidin-biotin, peptide nucleic acids and other oligomers, wherein a radiolabeled targeting moiety is highly accreted to the primary target-specific binding site within the target thereby providing a good tumor to non-tumor ratio for imaging purposes.

[0019] These and other objects are achieved, in accordance with one embodiment of the present invention by the provision of a kit for targeting of a diagnostic or therapeutic agent in a mammal comprising: (A) a first conjugate comprising a targeting moiety and a Morpholino oligomer, wherein said targeting moiety selectively binds to a primary, target-specific binding site of the target site or to a substance produced by or associated with the target site; (B) optionally, a clearing agent; and (C) a second conjugate comprising a complementary Morpholino oligomer and a diagnostic agent or therapeutic agent.

[0020] The targeting moiety of step (a) preferably comprises an antibody, especially a humanized antibody or an antigen-binding fragment of a humanized antibody. One such humanized antibody is an anti-carcinoembryonic antigen (CEA) antibody. The targeting moiety is selected from the group consisting of proteins, small peptides, polypeptides, enzymes, hormones, steroids, cytokines, neurotransmitters, oligomers, vitamins and receptor binding molecules.

[0021] In accordance with another aspect of the present invention, a kit is provided, as described above, wherein the length of the Morpholino oligomer and its complementary Morpholino oligomer is at least about 6 bases to about 100 bases. In addition, the Morpholino and its complementary Morpholino oligomer can be a 15-mer, an 18-mer or a 25-mer. The target moiety is bound to a 15-mer, an 18-mer or a 25-mer Morpholino oligomer.

[0022] In a preferred embodiment, the clearing agent is an anti-idiotypic antibody or antigen-binding antibody fragment.

[0023] In another preferred embodiment, the therapeutic agent is selected from the group consisting of antibodies, antibody fragments, drugs, toxins, nucleases, hormones, immunomodulators, chelators, boron compounds, photoactive agents or dyes and radioisotopes.

[0024] In yet another preferred embodiment, the diagnostic agent is selected from the group consisting of radionuclides, dyes, contrast agents, fluorescent compounds or molecules and enhancing agents useful for magnetic resonance imaging (MRI).

[0025] The present invention contemplates a targeting method for delivering a diagnostic or therapeutic agent to a target site in a mammal, comprising: (a) administering to said mammal a first conjugate comprising a targeting moiety and a Morpholino oligomer, wherein said targeting moiety selectively binds to a primary, target-specific binding site of the target site or to a substance produced by or associated with the target site; (b) optionally, administering to said mammal a clearing agent, and allowing said clearing agent to clear non-localized first conjugate from circulation; and (c) administering to said mammal a second conjugate comprising a complementary Morpholino oligomer and a diagnostic agent or therapeutic agent, wherein said complementary Morpholino oligomer binds its Morpholino oligomer complement on the first conjugate thereby targeting the diagnostic or therapeutic agent to the target site.

[0026] Other objects, features and advantages of the present invention will become apparent from the following detailed description and appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027]

Figure 1. A. UV absorption spectra of MORF15 (0.025 $\mu\text{g}/\mu\text{L}$) and native MN14 (0.5 $\mu\text{g}/\mu\text{L}$). B. Size exclusion HPLC UV chromatograms of MN14-MORF15 (left panel) and MN14-MORF18 (right panel) at 280 nm. C-D. Absorptions at 280 and 265 nm vs. weight of native MN14 (C) and native MORF 15 (D).

Figure 2. UV (260 nm) size exclusion HPLC profiles of uncoupled, native cMORF18 (A), MAG_3 -coupled cMORF18 (B), and radioactivity profile of $^{99\text{m}}\text{Tc}$ -labeled MAG_3 -cMORF18 (C).

Figure 3. UV (260 nm) size exclusion HPLC profiles of MN14-MORF18 (A) and radioactivity profiles of labeled-

cMORF18 added to MN14-MORF18 at weight ratios of 0.04 $\mu\text{g}/25\mu\text{g}$ (B) and 0.25 $\mu\text{g}/25\mu\text{g}$ (C), respectively. Also shown is the radioactivity profile of labeled-cMORF18 added to native MN14 as control. (D). The extent of MN14 binding decreases as the weight of labeled-cMORF18 increases.

Figure 4. Size exclusion radioactivity HPLC profiles of urine obtained at 3 h from a control mouse receiving only labeled-cMORF18 (A) and a study mouse receiving both MN14-MORF18 and labeled-cMORF18 (B). Also presented are radiochromatograms on the same axes of plasma obtained at 3 h from a control mouse (C) and from a study mouse (D). The results show that radioactivity in urine is present primarily as labeled-cMORF18, while in plasma, significant levels of radioactivity are present only in study mice and then only as the labeled-cMORF18-MN14-MORF18 complex.

Figure 5. Whole body anterior gamma camera images of LS174T tumor bearing nude mice obtained at 3 h (A) and 24 h (C) and the image obtained in a repeat study at 3 h (B) with prior removal of urine. In each image, the study animal is on the left and the control animal is on the right.

Figure 6. Size exclusion HPLC radiochromatograms of $^{99\text{m}}\text{Tc}$ -labeled-cMORF15, cMORF18, and cMORF25 and MORF15, MORF18, and MORF25.

Figure 7. Biodistribution of $^{99\text{m}}\text{Tc}$ -labeled-cMORF15, cMORF18, and cMORF25 and MORF15, MORF18 and MORF25 at 0.5, 1, and 3 hrs. *Body = whole body radioactivity minus urine and kidney.

Figure 8. Whole body scintigraphic images obtained simultaneously of LS 174T tumored nude mice at 3 hrs post injection. Control animal received $^{99\text{m}}\text{Tc}$ -cMORF15 only (right image), 15 mer pretargeting animal received MN14-MORF15 followed by $^{99\text{m}}\text{Tc}$ -cMORF15 (middle image) and 18 mer pretargeting animals received MN14-MORF18 followed by $^{99\text{m}}\text{Tc}$ -cMORF18 (left image). Tumors are in the right thigh in each case.

DETAILED DESCRIPTION OF THE INVENTION

[0028] Unless otherwise specified, "a" or "an" means "one or more".

[0029] The present invention provides a kit and a use of said kit for the preparation of a medicament for *in vivo* targeting of a diagnostic or therapeutic agent in a mammal (preferably human) comprising: (A) a first conjugate comprising a targeting moiety and a Morpholino oligomer, wherein said targeting moiety selectively binds to a primary, target-specific binding site of the target site or to a substance produced by or associated with the target site; (B) optionally, a clearing agent; and (C) a second conjugate comprising a complementary Morpholino oligomer and a diagnostic agent or therapeutic agent.

[0030] The targeting moiety may be, for example, an antibody or an antigen binding antibody fragment. Preferred are the monoclonal antibodies (Mabs) due to their high specificities. They are readily prepared by what are now considered conventional procedures of immunization of mammals with immunogenic antigen preparation, fusion of immune lymph or spleen cells with an immortal myeloma cell line, and isolation of specific hybridoma clones. More unconventional methods of preparing monoclonal antibodies are also contemplated, such as interspecies fusions and genetic engineering manipulations of hypervariable regions, since it is primarily the antigen specificity of the antibodies that affects their utility in the present invention. It will be appreciated that newer techniques for production of monoclonals can also be used, e.g., human monoclonals, interspecies monoclonals, chimeric (e.g., human/mouse) monoclonals, genetically engineered antibodies and the like.

[0031] Antibody fragments useful in the invention include F(ab')_2 , F(ab)_2 , Fab' , Fab , Fv and the like including hybrid fragments. Preferred fragments are Fab' , F(ab')_2 , Fab , and F(ab)_2 . Also useful are any subfragments retaining the hypervariable, antigen-binding region of an immunoglobulin and having a size similar to or smaller than a Fab' fragment. This will include genetically-engineered or recombinant antibodies and proteins, whether single-chain or multiple-chain, which incorporate an antigen-binding site and otherwise function *in vivo* as targeting vehicles in substantially the same way as natural immunoglobulin fragments. Such single-chain binding molecules are disclosed in U.S. Pat. No. 4,946,778.

[0032] Fab' fragments may be conveniently made by reductive cleavage of F(ab')_2 fragments, which themselves may be made by pepsin digestion of intact immunoglobulin, under reducing conditions, or by cleavage of F(ab')_2 fragments which result from careful papain digestion of whole immunoglobulin. The fragments may also be produced by genetic engineering.

[0033] It should be noted that mixtures of antibodies and immunoglobulin classes can be used, as can hybrid antibodies. Multispecific, including bispecific and hybrid, antibodies and antigen binding antibody fragments are useful in the present invention. Bispecific and hybrid antibodies are capable of specifically binding to at least one epitope on the marker substances, or on a component of the second conjugate. These antibodies preferably are comprised of at least two

different substantially monospecific antibodies or antibody fragments, which at least bind to at least one epitope on the marker substance produced by or associated with the cancer cells and with at least one epitope of a component of the second conjugate. Multispecific antibodies and antibody fragments with dual specificities can be prepared analogously to the anti-tumor marker hybrids disclosed in U.S. Pat. No. 4,361,544, the contents of which are incorporated by reference herein in their entirety. Other techniques for preparing hybrid antibodies are disclosed in, for example, U. S. Pat. Nos. 4,474,893 and 4,479,895, and in Milstein *et al.*, *Immunol. Today* 5:299, (1984).

[0034] Also preferred are antibodies having a specific immunoreactivity to a marker substance produced by or associated with the cancer cells of at least 60% and a cross-reactivity to other antigens or non-targeted substances of less than 35%. A monoclonal antibody that specifically targets tumor sites by binding to antigens produced by or associated with the tumors is particularly preferred.

[0035] Antibodies against tumor antigens are known. For example, antibodies and antibody fragments which specifically bind markers produced by or associated with tumors have been disclosed, *inter alia*, in Hansen *et al.*, U. S. Pat. No. 3,927,193, and Goldenberg's U. S. Pat. Nos. 4,331,647, 4,348,376, 4,361,544, 4,468,457, 4,444,744, 4,818,709 and 4,624,846. In particular, antibodies against an antigen, e.g., a gastrointestinal, lung, breast, prostate, ovarian, testicular, brain or lymphatic tumor, a sarcoma or a melanoma, are advantageously used.

[0036] The antibodies and antigen-binding antibody fragments useful in the methods of the present invention may be conjugated to the member of the binding pair by a variety of methods of chemical conjugation known in the art. Many of these methods are disclosed in the above-referenced U.S. patents and patent applications. See also Childs *et al.*, *J. Nuc. Med.* 26:293 (1985).

[0037] One monoclonal antibody useful in the present invention is MN-14, a second generation CEA-antibody that has ten times more affinity for CEA than the first generation version, NP-4. Hansen *et al.*, *Cancer* 71:3478-85, (1993). MN-14 internalizes slowly, making it suitable for targeting approach, and has been chimerized and humanized. Leung *et al.*, U.S. Pat. No. 5,874,540.

[0038] Other targeting moieties useful in the present invention can also be non-antibody species selecting from the group consisting of proteins, small peptides, polypeptides, enzymes, hormones, steroids, cytokines, neurotransmitters, oligomers, vitamins, and receptor binding molecules, which preferentially bind marker substances that are produced by or associated with the target site.

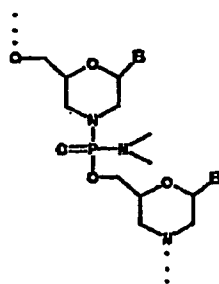
[0039] Morpholino oligomers (herein "Morpholinos" or "MORFs") bind and inactivate selected RNA sequences. These oligomers are assembled from four different Morpholino subunits, each of which contains one of the four genetic bases (A, G, C, T or U), linked to a six-membered morpholine ring. These subunits, as 15 - 25 mers, are joined together in a specific order by non-ionic phosphorodiamidate intersubunit linkages to produce a Morpholino oligomer. They may offer better antisense properties than do DNA, RNA, and their analogs having five-membered ribose or deoxyribose backbone moieties joined by ionic linkages. Summerton's work on Morpholinos is disclosed in U.S. Pat. Nos. 5,142,047 and 5,185,444. Morpholinos are commercially available from Gene Tools, LLC., Corvallis, Oregon.

[0040] Because they are readily delivered to the target, Morpholinos are effective tools for genetic studies and drug target validation programs. They are completely resistant to nucleases. A more rigid MORF backbone may offer better access during duplex formation when compared with a peptide backbone or with the more common sugar backbone. When compared to PNAs, Morpholinos are less expensive and more soluble in aqueous solutions, and provide better predictable targeting and higher efficacy in RNA binding affinities.

[0041] In the present invention, a Morpholino oligomer (herein MORF) bound to a targeting antibody is *in vivo* hybridized to the complementary MORF (herein cMORF) bound to a diagnostic or therapeutic agent. In a preferred embodiment, the length of the MORF and its complementary Morpholino (cMORF) is from 6 bases to about 100 bases, for example, MORF15 and cMORF15 (15-mer), MORF18 and cMORF18 (18-mer) or MORF25 and cMORF25 (25-mer).

[0042] The MORFs used in the present invention include a 15-mer (5' equivalent TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF15), and TAG-TTG-TGA-CGT-ACA-linker-amine (herein complementary MORF15 or cMORF15)), an 18-mer (5' equivalent CGG-TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF18) and TAG-TTG-TGA-CGT-ACA-CCC-linker-amine (herein complementary MORF18 or cMORF18)), and a 25-mer (5' equivalent T-GGT-GGT-GGG-TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF25), and TAG-TTG-TGA-CGT-ACA-CCC-ACC-ACC-A-linker-amine (herein complementary MORF25 or cMORF25)).

[0043] The Morpholino oligo structure (Summerton and Weller, *Antisense Nucl. Acid Drug Dev.* 7:187-95, 1997) used in the present invention, is shown below as:



B = adenine, cytosine, guanine, thymine/uracil

[0044] Clearing agents known in the art may be used in accordance with the present invention. For example, if the first conjugate comprises avidin or streptavidin, biotin may be used as a clearing agent. Alternatively, if the first conjugate comprises biotin, avidin or streptavidin may be used as a clearing agent.

[0045] In a preferred embodiment, the clearing agent is an antibody which binds the binding site of the targeting moiety, wherein the targeting moiety can be an antibody, an antigen-binding antibody fragment or a non-antibody targeting moiety. In a more preferred method, the clearing agent is a monoclonal antibody that is an anti-idiotypic to the monoclonal antibody of the conjugate used in the first step, as described in U.S. application Ser. No. 08/486,166. In another preferred embodiment, the clearing agent is substituted with multiple residues of carbohydrate, such as galactose, which allow the clearing agent to be cleared quickly from circulation by asialoglycoprotein receptors in the liver.

[0046] A physiological solution of the targeting species is advantageously metered into sterile vials, e.g., at a unit dosage of about 1.0-500 mg targeting species/vial, and the vials are either stoppered, sealed and stored at low temperature or lyophilized, stoppered, sealed and stored.

[0047] Variations and modifications of these formulations will be readily apparent to the ordinary skilled artisan, as a function of the individual needs of the mammal or treatment regiment, as well as of variations in the form in which radioisotopes may be provided or may become available.

[0048] Routes of administration include intravenous, intraarterial, intrapleural, intraperitoneal, intrathecal, subcutaneous or by perfusion.

[0049] Methods useful for internal detection or treatment of tumors or other lesions, such as cardiovascular lesions (clots, emboli, infarcts, etc.), infectious diseases, inflammatory diseases, and autoimmune diseases are disclosed in U.S. Pat. Nos. 4,782,840 4,932,412 and 5,716,595. The methods of the present invention can be used to enhance the methods disclosed in these references. The present invention also may be practiced in conjunction with intraoperative probes, endoscopic and laparoscopic uses, and in methods for imaging normal organs. The methods of the present invention can be used in other methods that will be apparent to those skilled in the art.

[0050] Examples of therapeutic agents include antibodies, antibody fragments, drugs, toxins, nucleases, hormones, immunomodulators, chelators, boron compounds, photoactive agents or dyes and radioinucleides.

[0051] In a further preferred embodiment, the cMORF is conjugated to a bifunctional chelator which in turn, is radiolabeled with an isotope. A chelator is radiolabeled first prior to conjugation (preconjugation labeling) to a protein, a polypeptide or an oligonucleotide which cannot withstand harsh conditions. Examples of chelators may include hydrazino nicotinamide (HYNIC), diethylenetriaminepentaacetic acid (DTPA), 1, 4, 7, 10-tetraazacyclododecane N, N', N'', N'''-tetraacetic acid (DOTA), and mercaptoacetylglycylglycylglycine (MAG₃). A preferred bifunctional chelator used in the present invention is N-hydroxysuccinimidyl derivative of acetyl-S-protected mercaptoacetyl triglycine (NHS-MAG₃). NHS-MAG₃ is synthesized according to the method of Winnard, P. *et al.*, *Nucl. Med. Biol.* **24**:425-32 (1997). The conjugation of single-stranded morpholino oligomers with NHS-MAG₃ was accomplished as described in Mardirosian, G. *et al.*, *J. Nucl. Med.* **38**:907-13 (1997).

[0052] Radionuclides useful as therapeutic agents, which substantially decay by beta-particle emission include, but are not limited to P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213. Maximum decay energies of useful beta-particle-emitting nuclides are preferably 20-5,000 keV, more preferably 100-4,000 keV, and most preferably 500-2,500 keV.

[0053] Radionuclides useful as therapeutic agents, which substantially decay with Auger-emitting particles include, but are not limited to Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m and Ir-192. Maximum decay energy of these radionuclides is preferably less than 1,000 keV, more preferably less than 100 keV, and most preferably less than 70 keV.

[0054] Radionuclides useful as therapeutic agents, which substantially decay with generation of alpha-particles include,

but are not limited to Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 and Fm-255. Decay energies of useful alpha-particle-emitting radionuclides are preferably 2,000-9,000 keV, more preferably 3,000-8,000 keV, and most preferably 4,000-7,000 keV.

[0055] Metals useful, as complexes, as part of a photodynamic therapy procedure include, but are not limited to zinc, aluminum, gallium, lutetium and palladium.

[0056] Radionuclides useful in therapies based on neutron capture procedures include, but are not limited to B-10, Gd-157 and U-235.

[0057] Useful diagnostic agents include, but are not limited to radionuclides, 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. Preferably, the diagnostic agents are selected from the group consisting of radionuclides, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds.

[0058] Radionuclides useful as diagnostic agents that are used in positron emission tomography include, but are not limited to F-18, Mn-51, Mn-52m, Fe-52, Co-55, Cu-62, Cu-64, Ga-68, As-72, Br-75, Br-76, Rb-82m, Sr-83, Y-86, Zr-89; Tc-94m, In-110, I-120, and I-124. Total decay energies of useful positron-emitting radionuclides are preferably less than 2,000 keV, more preferably under 1,000 keV, and most preferably less than 700 keV.

[0059] Metals useful in diagnostic agents utilizing magnetic resonance imaging techniques include, but are not limited to gadolinium, manganese, iron, chromium, copper, cobalt, nickel, dysprosium, rhenium, europium, terbium, holmium and neodymium.

[0060] Radionuclides useful as diagnostic agents utilizing gamma-ray detection include, but are not limited to Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, I-123, I-125, I-131, Yb-169, Hg-197, and Tl-201. Decay energies of useful gamma-ray emitting radionuclides are preferably 20-2000 keV, more preferably 60-600 keV, and most preferably 100-300 keV.

[0061] The embodiments of the invention may be further illustrated through examples which show aspects of the invention in detail. These examples illustrate specific elements of the invention and are not to be construed as limiting the scope thereof.

Examples

Materials and Methods

[0062] The MORFs used in the present invention are obtained from Gene Tools, LLC. (Corvallis, Oregon). They include a 15-mer (5' equivalent TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF15), and TAG-TTG-TGA-CGT-ACA-linker-amine (herein complementary MORF15 or cMORF15)), an 18-mer (5' equivalent CGG-TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF18) and TAG-TTG-TGA-CGT-ACA-CCC-linker-amine (herein complementary MORF18 or cMORF18)), and a 25-mer (5' equivalent T-GGT-GGT-GGG-TGT-ACG-TCA-CAA-CTA-linker-amine (herein MORF25), and TAG-TTG-TGA-CGT-ACA-CCC-ACC-ACC-A-linker-amine (herein complementary MORF25 or cMORF25)). The linker used in the present invention is H₂N-CH₂-CH(CH₂CH₂)₂-N-COCH₂CH₂-CO-. The molecular weights ranged from 5090 to 8568 Daltons. The cMORFs were occasionally purchased with a biotin group on the 3' end in place of the amine.

[0063] Streptavidin-coated magnetic beads (BioMag, Streptavidin Ultra-Load, Polysciences Inc Warrington, PA); 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (Pierce, Rockford, IL); the gel (Bio-Gel P-4 Gel, Medium) for separation (Bio-Rad Laboratories, Hercules, CA) and the Sephadex G100 resin (Pharmacia Biotech, Uppsala, Sweden) were purchased and used as received. All other chemicals were reagent grade and were used without purification. The ^{99m}Tc-pertechnetate was eluted from a ⁹⁹Mo-^{99m}Tc generator (Dupont, Billerica, MA).

[0064] The bifunctional chelator, N-hydroxysuccinimidyl derivative of acetyl-S-protected mercaptoacetyl-triglycine (NHS-MAG₃), was synthesized according to the method of Winnard, P. *et al.*, *Nucl. Med. Biol.* **24**:425-32 (1997). The structure was confirmed by elemental analysis, proton NMR, and mass spectroscopy. To 0.97 ml of a 0.225 M sodium hydroxide was added 50 mg of triglycine (264 μmol) and 10 μl of a freshly-prepared 50 mM disodium ethylenetriamine-tetracetic acid (EDTA). This solution was passed through a 0.2 μm filter to remove amine-containing particulates. A solution of 90 mg (390 μmol) of S-acetylthioglycolic acid N-hydroxysuccinimide ester (SATA) in 340 μl of dimethylformamide (DMF; dried over molecular sieve) was prepared and was added dropwise to the stirred triglycine solution. After 15 min of stirring at room temperature, the non-aqueous solution was adjusted from an apparent pH of 8.9 to an apparent pH of approximately 2.7 (measured with a glass electrode-pH meter) by the addition of 37.6 μl of 6 M hydrochloric acid. An initial pH of about 8.9 was selected to deprotonate the amine on triglycine (pK 7.9; Fasman, G.D. [ed.] 1976, CRC Handbook of Biochemistry and Molecular Biology, Third Edition, Vol. I, p 321, CRC Press, Boca Raton, FL) but without reaching extreme basic pH values in which the acetyl group on SATA may hydrolyze. The pH was lowered as soon as possible to minimize hydrolysis of the acetyl group.

[0065] A solution of 60 mg (290 μmol) of dicyclohexylcarbodiimide (DCC) in 3.6 ml of dry DMF was added rapidly to

the stirred triglycine/SATA solution (apparent pH of about 5.0). The solution became cloudy within 2 min. as dicyclohexylurea began to precipitate. The reaction was stirred at room temperature in the dark for 2-4 hrs and was then cooled to -20°C for an additional hour to encourage complete precipitation. After centrifugation at 4°C, 2500 g for 15 min., the clear supernatant was removed.

[0066] Due to the presence of water in the DMF solution, the NHS-MAG₃ preparation in this form was always used within 24 hours of preparation. For long-term storage, the NHS-MAG₃ water/DMF solution was evaporated to near-dryness in 15-30 min. on a rotary flash evaporator (Rotavapur-R, Buchi, Switzerland) and was then lyophilized to dryness within 1 hr on a lyophilizer (Virtis, Gardenier, NY). After drying in this fashion, the NHS-MAG₃ can be stored indefinitely at room temperatures in a dessicator. When using the dry, powdered NHS-MAG₃ for conjugation, an arbitrary value of 50% by weight was assumed for its purity.

[0067] Size exclusion (SE) HPLC analysis was performed on a Superose-12 column (HR10/30, Amersham Pharmacia Biotech) with 0.10 mol/L phosphate buffer pH 7.0 as eluant at a flow rate of 0.6 mL/min. In-line UV absorbance at 260 nm and radioactivity detectors were used to identify and quantitate peak fractions. Recovery of radioactivity was determined routinely.

Example 1 Coupling of MN-14 with MORF15 AND MORF18

[0068] A solution of murine anti-CEA IgG antibody MN-14 (IgG₁ subtype, MW 150-kDa) was prepared at a concentration of 1000 µg/83 µL in phosphate buffered saline (pH 7.0-7.2) and was conjugated with either MORF15 (500 µg/250 µL) or MORF18 (600 µg/250 µL) in 2-(N-morpholino)ethane sulfonic acid (MES), pH 5.0, in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) in water (1000 µg/50 µL). The above mixture was incubated at room temperature for at least 1 hour.

[0069] Purification was achieved on a 0.7 × 20 cm Sephadex G-100 column with 0.05 mol/L, pH 7.0 phosphate buffer as eluant. The concentration of the recovered fraction(s) was estimated with respect to MN14 by UV absorbance at 280 nm using the absorbance coefficient of 1.40 µL/µg.

[0070] The antibody-MORF conjugates were characterized in terms of antibody concentration and MORF group per molecule. Previously, groups per molecule were estimated by adding increasing amounts of radiolabeled-cMORF to the conjugated antibody followed by size exclusion HPLC analysis to establish the saturation point by a shift in the radioactivity profile to that of free cMORF. Liu, G. *et al.*, *J. Nucl. Med.* **43**:384-391 (2002). In this present investigation, an alternative and more accurate method using UV absorbance was employed. The separate absorbance profiles of native MN14, free MORF15 and free MORF18 were first measured in the range of 200 to 300 nm using a scanning UV spectrophotometer (U-2000, Hitachi Instruments, Inc, Danbury, CT). It was therefore determined that the maximum absorbance of MN14 is at 280 nm while that of both MORFs is at 265 nm. Because the MORF-conjugated MN14 contained traces of free MORF even after purification, the measurement was performed during HPLC separation. Thus standard curves were constructed by HPLC-dual UV analysis in which the absorbance of native MN14 and native MORF15 at both 280 nm and 265 nm were measured. Under the assumption that the absorbance coefficients of MN14 and MORF does not change after conjugation, the concentration of MN14 and the MORF groups per molecule was calculated by measuring the absorbance at both wave lengths during HPLC analysis of the conjugated antibody. The concentration of MN14 and the MORF groups per MN14 in each MN14-MORF sample was calculated according to the following equations:

$$H^{280} = H^{280}_{\text{MN14}} + H^{280}_{\text{MORF}}$$

$$H^{265} = H^{265}_{\text{MN14}} + H^{265}_{\text{MORF}}$$

$$H^{280}_{\text{MN14}} = k^{280}_{\text{MN14}} W_{\text{MN14}}$$

$$H^{280}_{\text{MORF}} = k^{280}_{\text{MORF}} W_{\text{MORF}}$$

$$H^{265}_{\text{MN14}} = k^{265}_{\text{MN14}} W_{\text{MN14}}$$

$$W_{\text{MORF}} = \left[\frac{H^{265}}{k^{265}_{\text{MN14}}} - \frac{H^{280}}{k^{280}_{\text{MN14}}} \right] / \left[\frac{k^{265}_{\text{MORF}}}{k^{265}_{\text{MN14}}} - \frac{k^{280}_{\text{MORF}}}{k^{280}_{\text{MN14}}} \right] \quad \text{Equation 1}$$

$$W_{\text{MN14}} = \left[\frac{H^{265}}{k^{265}_{\text{MORF}}} - \frac{H^{280}}{k^{280}_{\text{MORF}}} \right] / \left[\frac{k^{265}_{\text{MN14}}}{k^{265}_{\text{MORF}}} - \frac{k^{280}_{\text{MN14}}}{k^{280}_{\text{MORF}}} \right] \quad \text{Equation 2}$$

$$\text{Concentration of MN14} = W_{\text{MN14}} / \text{volume applied} \quad \text{Equation 3}$$

$$\text{MORF number per MN14} = (W_{\text{MORF}}/M_{\text{MORF}}) / (W_{\text{MN14}}/M_{\text{MN14}}) \quad \text{Equation 4}$$

where H represents peak height in absorbance units (AU), W is weight (μg), M is molecular weight (Da), and k is the slope of standard curve (AU/ μg).

[0071] Figure 1A presents profiles at 280 nm obtained by HPLC analysis of the conjugated and purified MN14-MORF15 (left panel) and MN14-MORF18 (right panel) used in this investigation. The chromatograms at 265 nm are basically the same except for minor differences in peak heights (data not shown). In both panels, several small peaks are present. Those to the left of the main peak are higher molecular weight impurities possibly due to cross linking while that to the right is due to free MORF.

[0072] The UV spectra of both MN14 and MORF15 are shown in Figure 1B. Overlap of the 265 nm peak of MORF 15 and the 280 nm peak of MN14 is clearly demonstrated. Nevertheless, it is possible to determine accurately the weight of MORF and the weight of MN14 in a sample of MORF-conjugated MN14 by measuring absorption at both wave lengths and by calculating the weights of both MORF and MN14 in the protein peak using Equations 1 and 2. Figures 1C and 1D present standard absorption curves for native MN14 and native cMORF15. Both curves are linear at least within the range studied (i.e. 0-100 μg for MN14 and 0-10 μg for MORF) with slopes of 0.00104 AU/ μg and 0.00073 AU/ μg for MN14 and 0.01022 AU/ μg and 0.01816 AU/ μg for MORF15 at 280 nm and 265 nm, respectively. The standard curves of MORF18 and MORF25 (not shown) are quite similar with slopes of 0.00738 and 0.00749 AU/ μg and 0.01287 and 0.01314 AU/ μg for MN14 and MORF15 at 280 nm and 265 nm, respectively. From Equations 1, 2, and 4, the MORF-groups per MN14 for the MN14-MORF15 and MN14-MORF18 conjugates used in the present invention were calculated to be identical at 0.28 ± 0.1 and 0.27 ± 0.1 groups per molecule, respectively. The concentrations of conjugated antibody was also calculated using Equations 1, 2 and 3.

Example 2 Coupling of cMORF15 and cMORF18 to NHS-MAG₃

[0073] The conjugation of cMORF15 and cMORF18 with NHS-MAG₃ was accomplished as described in Mardirossian, G. *et al.*, *J. Nucl. Med.* **38**:907-13 (1997) and Wang, Y. *et al. Bioconj. Chem.* **12**:807-816 (2001). NHS-MAG₃ powder (410 μg) was conjugated to both cMORF15 and cMORF18 (880 μg /200 μL) in 0.2M HEPES, pH 8. Fifty microliters of HEPES was added to the resulting mixture and incubated for 4 hours. CMORFs (880 μg) were dissolved in 250 μL of 0.2 M HEPES buffer (pH 8.0) and were added to a vial containing 1150 μg of solid NHS-MAG₃. After vortexing, the mixture was incubated at room temperature for at least 1 hr. The molar ratio of MORF18 to NHS-MAG₃ was about 1:20. The incubated solution was separated on a 0.7 x 20 cm column of P4 with an eluant of 0.25 M ammonium acetate buffer, pH 5.2. The concentration with respect to cMORF of the recovered fractions were estimated by UV absorbance at 265 nm using an absorbance coefficient of 31 $\mu\text{L}/\mu\text{g}$. The peak fractions were identified by UV spectrophotometry at 265 nm and were stored in a freezer at 20°C.

[0074] The coupling mixture was purified on a P4 column (0.7 x 20 cm; BioRad, Melville, NY) using ammonium acetate (0.25 M, pH 5.2) as the eluent. The cMORF15-MAG₃ and cMORF18-MAG₃ peaks were determined by UV absorption at 260 nm using an extinction coefficient of 31.1 $\mu\text{L}/\mu\text{g}$.

Example 3 Radiolabeling of cMORF15-MAG₃ and cMORF18-MAG₃

[0075] Twenty-five microliters of cMORF15-MAG₃ and cMORF18-MAG₃ (0.25 M ammonium acetate, pH 5.2) was mixed with 6 μ L sodium tartrate solution (50 mg/ml of fresh sodium tartrate in 0.5 M sodium bicarbonate, 0.25M ammonium acetate, 0.18 ammonium hydroxide, pH 9.2). This was followed by adding 20 μ L of about 5 mCi of ^{99m}Tc-pertechnetate generator eluant. Finally, 2 μ L tin(II) chloride (1 μ g / μ L in 10mM HCl; Sigma) was quickly added by agitation. The conjugates were capped and placed in a boiling water bath for 20-30 min.

[0076] After 5-15 minutes at room temperature, the labeled-cMORFs-MAG₃ was purified on a P4 column (0.7 x 20 cm) with 0.05 M phosphate buffer, pH 7 as the eluant. The peak was identified by counting fractions in a dose calibrator. The purified product was analyzed by size-exclusion HPLC using a Superose-12 (Pharmacia, Piscataway, NJ). Figure 1 represents a size exclusion HPLC radiochromatogram showing that the peak-times for both cMORF18-MAG₃-^{99m}Tc and cMORF15-MAG₃-^{99m}Tc were 30.8 min and 31.2 min, respectively.

Quality Assurance of Labeled-cMORF18

[0077] Figure 2 presents UV chromatograms of native cMORF18 (Fig. 2A), MAG₃-coupled cMORF18 (Fig. 1B), and radiochromatogram of ^{99m}Tc-MAG₃-cMORF18 (Fig. 2C). The labeling efficiency of labeled-cMORF18 was between 40%-60%. The radioactivity recovery of labeled-cMORF 18 off the HPLC was always over 90%. The retention time of both native and coupled-cMORF18 is 29.5 min, however the latter shows a small shoulder with a retention time at about 28 min (possibly a result of MAG₃-cMORF18 dimer formation through disulfide bonds). The radiolabeled-cMORF18 shows a slightly longer retention time of 31.1 min.

Hybridization Ability of ^{99m}Tc Labeled-cMORF18

[0078] Binding to beads is a convenient method to evaluate hybridization between an oligomer and its complement. Mardirossian, G. *et al.*, *J. Nucl. Med.* **38**:907-13 (1997) and Wang, Y. *et al. Bioconj. Chem.* **12**:807-816 (2001). After optimization of hybridization conditions, the hybridization ability of radiolabeled-cMORF18 was evaluated on streptavidin-coated magnetic beads.

[0079] Beads (300 μ L) were washed three times with 200 μ L of washing buffer (20 mmol/L Tris buffer-0.5 mol/L sodium chloride, pH 8.2). The beads were retained in the tube during washing by using a magnetic separator (MPC, Dynal, A.S., Lake Success, NY). After the last wash, the beads were suspended in 200 μ L of the washing buffer, 1.0 μ g biotin-MORF18 was added and, 15 min later, the beads were washed three times again each with 200 μ L of the washing buffer. After resuspension in 200 μ L washing buffer, 2 μ L radiolabeled-ORF18 (0.02 μ g cMORF18) was added. After 1 h of incubation with constant agitation, the beads were separated, washed three times and counted in a NaI(Tl) well counter. The supernatant and washing solutions were combined and counted. Two control groups, one without biotin-MORF18 and one in which biotin-cMORF18 substituted for biotin-MORF18, were simultaneously carried through the identical procedure.

[0080] Under the conditions of the bead study, 90.2 ± 0.9 % (n=3) of the labeled-cMORF18 bound to MORF18 beads compared to only 0.4 ± 0.1 % and 0.3 ± 0.1 % (n = 3) of the labeled-cMORF18 in the case of the blank control and the biotin-cMORF18 control, respectively. The radioactivity on beads in the study group therefore is due to hybridization of labeled-cMORF18 to MORF18 on the beads. These hybridization results of ^{99m}Tc-MAG₃-cMORF (18 mer) to MORF (18 mer) on beads agree with earlier findings on hybridization on beads of a ^{99m}Tc-MAG₃-MORF(15 mer) to cMORF (15 mer) (Mang'era, K.O. *et al. Eur. J. Nucl. Med.* **28**:1682-1689 (2001).

[0081] The single peak observed for labeled-cMORF18 by HPLC (Fig. 2C) and the fact that at least 90 % of the radioactivity hybridized to its complement in the bead study indicate that coupling with NHS-MAG₃, purification on the P4 column, labeling with ^{99m}Tc, and purification again on the P4 column provided a satisfactory radiolabeled-cMORF18.

Estimation of MORF18 Groups Per Molecule on Coupled-MN14

[0082] After coupling, the MORF18 on MN14 could not be detected by its UV absorbance. To estimate the number of MORF18 groups on MN14, a series of aliquots of purified radiolabeled-cMORF18 (4, 25, 50, 150 μ L at 0.01 μ g/ μ L) were added to 50 μ L of MN14-MORF18 (0.5 μ g/ μ L), incubated at room temperature for 1 h and analyzed by SE HPLC. A control study was performed identically with 4 μ L (0.01 μ g/ μ L) of radiolabeled-cMORF18 but added to native MN14. Using SE HPLC, radioactivity bound to MN14 is detected readily by the shift in radioactivity profile to higher molecular weight (earlier retention times) from labeled-cMORF to labeled-cMORF18-MN14-MORF18.

[0083] Figure 3 presents HPLC profiles obtained by the hybridization of MN14-conjugated MORF18 with labeled-cMORF18. Recovery of radioactivity was over 90%. The UV chromatogram of MN14-MORF18 is presented for reference (Fig. 3A) along with the radioactivity profile of a mixture of 50 μ L (25 μ g) of native MN14 and 4 μ L (0.04 μ g) of labeled-

cMORF18 (Fig. 3D). When the same weight of labeled-cMORF18 is now added to the same weight of MN14-MORF18 instead of native MN14, a shift of at least 80% to higher molecular weight is evident (Fig. 3B). The 20 % remaining is composed of two peaks, one with a retention time that corresponds to labeled-cMORF18 and another with the same retention time as a hybridized duplex of MORF18 and labeled-cMORF18 (data not presented). Thus, the two peaks are probably due respectively to traces of free labeled-cMORF18 and labeled-cMORF18 hybridized to free MORF18 not removed during the purification of the MN14 preparation. That 80% of labeled-cMORF18 binds to MN14 demonstrates that MORF18 has been coupled successfully to the antibody and is still capable of hybridizing. Native MN14 does not bind ^{99m}Tc -MAG₃-cMORF18 (Fig. 3D).

[0084] Figure 3 also presents radiochromatograms of a mixture of 25 μL (0.25 μg) labeled-cMORF18 and 50 μL (25 μg) MN14-MORF18 (Fig. 3C). More than half of the radioactivity is not hybridized under these conditions. Increasing the dosage of labeled-cMORF18 to 50 μL or 150 μL increases the relative size of the peak of free ^{99m}Tc -MAG₃-cMORF18 as the MORF18 molecules on MN14 become saturated.

Example 4 Biodistribution of Labeled-cMORF in Normal CD-1 Mice

[0085] Twelve normal CD-1 mice (30-35 g) each received 1.5 μg (3.7 MBq) of labeled-cMORF18 by tail vein injection. Four mice were sacrificed at each of 0.5, 1.0, and 3.0 h. The mice were dissected, urine was carefully drawn and organs were removed and weighed. The radioactivity in each organ was counted in the NaI(Tl) well counter along with blood samples of known volume and an aliquot of the injectate. The radioactivity remaining in the carcass was measured in a dose calibrator.

[0086] Table 1 lists the biodistribution results of ^{99m}Tc in normal CD-1 mice at three time-points 0.5, 1.0, and 3.0 h after injection of ^{99m}Tc -cMORF18. The whole body radioactivity remaining (not including urine) was 23 %, 12 % and 7 %ID respectively at these points. There was no significant uptake in liver, small intestines, and large intestines, indicating that the labeled-cMORF18 is almost solely excreted through the kidneys. Highest radioactivity levels were in kidneys at 6-7 %ID/g throughout. At 0.5 h, the blood level (0.7% ID/g) was relatively high, but at 1 h, blood and all other organs except kidneys were < 0.2 %ID/g.

Table 1 Biodistribution of ^{99m}Tc -cMORF18 in normal mice (%ID/g) (n=4)

Organ	0.5h		1.0 h		3.0h	
	Mean	SD	Mean	SD	Mean	SD
Liver	0.23	0.06	0.15	0.02	0.15	0.04
Heart	0.22	0.07	0.07	0.01	0.04	0.01
Kidneys	6.1	0.75	7.1	0.57	6.4	1.02
Lungs	0.42	0.09	0.15	0.02	0.07	0.01
Spleen	0.16	0.02	0.10	0.02	0.09	0.02
Muscle	0.17	0.04	0.09	0.07	0.02	0.01
Blood	0.74	0.18	0.18	0.05	0.04	0.01
Whole body in ID%	22.5	4.4	11.7	2.3	7.0	1.0

Example 4 Biodistribution of Labeled-cMORF18 in Tumored Nude Mice

[0087] Biodistribution data in Table 2 show that, with the exception of kidneys, radioactivity accumulated primarily in tumor with values of 1.7-1.8 %ID/g and that the radiolabel was retained in tumor over this period. Whole body radioactivity levels for the study animals were 14 % and 11 %ID at 3 h and 24 h while these values for the control animals were 7 % and 5 %ID (in all cases with radioactive urine removed).

**Table 2
Biodistribution of ^{99m}Tc -cMORF18 in LS174T Tumor Bearing Nude Mice (%ID/g) (n=4)**

Organ	3 h				24 h			
	Control		Study		Control		Study	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Liver	0.23	0.01	0.41	0.05	0.10	0.01	0.60	0.05

(continued)

Biodistribution of ^{99m}Tc-cMORF18 in LS174T Tumor Bearing Nude Mice (%ID/g) (n=4)

Organ	3 h				24 h			
	Control		Study		Control		Study	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Heart	0.04	0.01	0.18	0.01	0.02	0.00	0.13	0.02
Kidneys	6.4	1.95	6.9	1.96	3.2	0.68	4.0	1.00
Lungs	0.07	0.01	0.34	0.06	0.04	0.01	0.24	0.04
Spleen	0.11	0.01	0.29	0.08	0.07	0.01	0.31	0.04
Muscle	0.03	0.03	0.09	0.01	0.02	0.00	0.07	0.01
Tumor	0.09	0.01	1.8	0.24	0.05	0.00	1.7	0.14
Blood	0.04	0.01	1.02	0.13	0.00	0.01	0.50	0.12
Whole body in ID%	6.9	3.3	14.4	0.9	5.0	2.0	11.4	0.7

[0088] Figure 4 presents HPLC radiochromatograms of urine at 3 h obtained from a control animal (Fig. 4 A) and from a study animal (Fig. 4B). These results suggest that radioactivity in urine was present as intact labeled-cMORF18 (Fig. 2C) and, therefore, that labeled-cMORF is stable *in vivo*.

[0089] Figure 4 also presents radio chromatograms of plasma from a control animal (Fig. 4C) and a study animal (Fig. 4D) at 3 h. Because radioactivity levels were too low for measurement by the in-line radioactivity detector, fractions off the HPLC were collected for counting in a well NaI(Tl) detector. In the figure, the axes have been adjusted so that retention times for all panels may be compared. There is no radioactive peak in plasma from the control animal (in Fig. 4C) whereas the radiochromatogram of plasma on the same scale from the study animal (Fig. 4D) shows only one radioactive peak corresponding in retention time to labeled-cMORF18 hybridized to MN14-MORF18 (Fig. 3B). The higher blood radioactivity in the study animals compared to the control (Table 2) indicates that a considerable amount of the radiolabeled antibody was still in circulation in the study animals after 48 h at the time when the labeled-cMORF18 was injected (as expected for an IgG antibody). In the absence of the antibody, the labeled-cMORF18 cleared rapidly as shown in the control animals.

Example 5 Pretargeting of Labeled-cMORF18 in LS174T Tumored Nude Mice

[0090] The LS 174T tumor was obtained from the American Type Culture Collection and was grown in minimal essential medium (Gibco, Grand Island, NY). The cells were removed from the culture flask by trypsinization and then washed in the culture media. Nude mice bearing LS174T tumor were prepared according to the protocol as disclosed by Rusckowski *et al.*, *Cancer* **80**:2699-705 (1997).

[0091] Eight nude mice (25-30 g) were each injected with 10⁶ LS174T colon tumor cells into one thigh. After 14 days when the tumors were no more than 1 cm in any dimension, half the animals each received 50 µg of MN14-MORF18. After 48 h, the mice in both groups received 1.0 µg of labeled-cMORF18 (7.4-8.9 MBq) via a tail vein. At 3 and 24 h post injection of labeled-cMORF18, animals were anesthetized with ketamine plus xylazine and imaged anteriorly with a large field of view scintillation camera (Elscent, Hackensack, NJ). Immediately after imaging at 24 h, the mice were sacrificed and the biodistribution of the radiolabel was determined as described.

[0092] In an identical repeat study, the imaging was performed after sacrifice at 3 h and after carefully removing most of the urine with a syringe. Urine and plasma samples from mice sacrificed at 3 h were analyzed by SE HPLC, in the former case using in-line radioactivity detection and in the latter case by collecting fractions for counting in a NaI(Tl) well counter.

[0093] Figure 5 presents whole body images at 3 h (Fig. 5A) and 24 h (Fig. 5C) after injection of labeled-cMORF18 to the tumored mice previously injected with MN14-MORF18 48 h earlier. The tumor in the study animal (left in each panel) is clearly seen in contrast to the control animal not receiving the MN14-MORF18 (right in each panel). In the early images (Fig. 5A), radioactivity in bladder is pronounced and at interfering levels. At 24 h however (Fig. 5C), urine activity has diminished so that the image now clearly shows only the tumor along with kidneys. A repeat study at 3 h (Fig. 5B) with imaging was performed with removal of the urine. Only the kidneys and tumor are prominent at this time. In all three images, tumor is not visible in the control animals.

Example 6 The Influence of Chain Length and Base Sequence on the Pharmacokinetic Behavior of ^{99m}Tc-Morpholinos in Mice

Pharmacokinetics of Labeled cMORF15, cMORF 18, cMORF 25 and MORF15, MORF 18, MORF 25

[0094] Twelve normal CD-1 mice weighing 30-40 g (Charles River, Wilmington, MA) each received 0.3 µg (50-70 µCi) of the labeled-cMORF by tail vein injection. Four mice were sacrificed at each of 0.5, 1, and 3 hrs. The radioactivity in each organ and blood was counted in a NaI(Tl) well counter along with an aliquot of the injectate. The radioactivity remaining in the carcass was measured in a dose calibrator.

[0095] Quality assurance of all the six labeled cMORFs was achieved by HPLC and by using beads. Figure 6 present HPLC radiochromatograms of all six labeled MORFs and cMORFs showing in each case a single peak and with a relative retention time reflecting the slight differences in molecular weight (33.5, 32.3, and 29.9 min for MORF25, MORF18, and MORF15, respectively, and 31.3, 31.0, and 30.5 min for cMORF25, cMORF18, and cMORF15, respectively). Recovery in each case was greater than 90%. The hybridization of each ^{99m}Tc labeled (c)MORF to its biotinylated complement immobilized on streptavidin beads was in all cases essentially quantitative (data not presented).

[0096] Tables 4 and 5 present the biodistributions in ID%/gm of ^{99m}Tc at 0.5, 1.0 and 3.0 hrs post administration of labeled-cMORFs and MORFs to normal mice. Figure 7 presents in histographic form the same biodistribution results but in ID% per organ. For all six cMORFs at 0.5 hrs, the radiolabel is still present in circulation as indicated by the relatively high values in blood and muscle. Rapid clearance from circulation is also indicated in all cases by the decrease in these radioactivity levels at 1 hr. From 1 hr on, the only organs with high radioactivity levels are the kidneys, for all six cMORFs. Interestingly, the influence of chain length is pronounced only in the case of cMORF where kidney levels increase dramatically with increasing length at all times. For example, at 3 hrs, kidney values increase from 1.7 to 13.5 ID% for cMORF15 to cMORF25 respectively but remain fairly constant at 4.5 and 5.6 ID% for MORF15 to MORF25. In the case of cMORF25, kidney levels account for more than 80% of the whole body radioactivity at this time. The presence of high levels of radioactivity only in kidneys is in agreement with earlier studies of ^{99m}Tc-labeled MORFs that showed rapid and almost exclusive clearance through the kidneys. Mang'era, K., *et al.*, *Eur. J. Nucl. Med.* **28**:1682-1689 (2001) and Liu G. *et al.*, *J. Nucl. Med.* **43**:384-391 (2002).

Table 4 Biodistribution of ^{99m}Tc-cMORFs in normal mice (ID%/g, n=4)

Organ	cMORF15- ^{99m} Tc			cMORF18- ^{99m} Tc*			cMORF25- ^{99m} Tc		
	0.5 h	1 h	3 h	0.5 h	1 h	3 h	0.5 h	1 h	3 h
Liver	0.43 (0.17)	0.23 (0.07)	0.15 (0.02)	0.23 (0.06)	0.15 (0.02)	0.15 (0.04)	0.23 (0.03)	0.14 (0.02)	0.10 (0.02)
Heart	0.60 (0.43)	0.10 (0.04)	0.04 (0.01)	0.22 (0.07)	0.07 (0.01)	0.04 (0.01)	0.25 (0.05)	0.10 (0.01)	0.05 (0.01)
Kidney	7.76 (2.15)	4.90 (1.83)	4.13 (0.29)	6.10 (0.75)	7.10 (0.57)	6.40 (1.02)	23.84 (9.53)	21.86 (4.04)	20.94 (4.38)
Lung	0.60 (0.21)	0.17 (0.05)	0.09 (0.01)	0.42 (0.09)	0.15 (0.02)	0.07 (0.01)	0.54 (0.03)	0.2 (0.02)	0.10 (0.00)
Spleen	0.29 (0.11)	0.11 (0.03)	0.08 (0.03)	0.16 (0.02)	0.10 (0.02)	0.09 (0.02)	0.17 (0.02)	0.10 (0.01)	0.06 (0.01)
Muscle	0.40 (0.17)	0.08 (0.02)	0.09 (0.07)	0.17 (0.04)	0.09 (0.07)	0.02 (0.01)	0.22 (0.02)	0.06 (0.01)	0.02 (0.00)
Blood	1.79 (1.03)	0.24 (0.10)	0.05 (0.01)	0.74 (0.18)	0.18 (0.05)	0.04 (0.01)	0.77 (0.06)	0.23 (0.01)	0.04 (0.01)

*Taken from Liu, G. *et al.*, *J. Nucl. Med.* **43**:384-391 (2002)..

Table 5 Biodistribution of ^{99m}Tc-MORFs in normal mice (ID%/g, n=4)

Organ	MORF15- ^{99m} Tc			MORF18- ^{99m} Tc			MORF25- ^{99m} Tc		
	0.5 h	1 h	3 h	0.5 h	1 h	3 h	0.5 h	1 h	3 h
Liver	0.27 (0.01)	0.17 (0.03)	0.11 (0.01)	0.35 (0.12)	0.14 (0.02)	0.12 (0.05)	0.22 (0.04)	0.14 (0.02)	0.11 (0.01)
Heart	0.28 (0.04)	0.08 (0.01)	0.03 (0.00)	0.33 (0.05)	0.10 (0.01)	0.04 (0.00)	0.24 (0.09)	0.10 (0.02)	0.04 (0.00)
Kidney	9.83 (2.85)	7.28 (1.57)	7.00 (0.55)	10.75 (2.95)	8.73 (0.98)	7.76 (1.33)	11.91 (5.27)	11.33 (1.86)	9.22 (1.60)
Lung	0.58 (0.05)	0.20 (0.04)	0.07 (0.01)	0.73 (0.02)	0.21 (0.05)	0.10 (0.03)	0.52 (0.16)	0.22 (0.07)	0.08 (0.02)
Spleen	0.29 (0.12)	0.10 (0.01)	0.09 (0.06)	0.21 (0.03)	0.10 (0.02)	0.08 (0.05)	0.21 (0.08)	0.11 (0.01)	0.07 (0.01)
Muscle	0.20 (0.04)	0.05 (0.00)	0.03 (0.01)	0.29 (0.06)	0.07 (0.01)	0.03 (0.03)	0.21 (0.05)	0.06 (0.02)	0.02 (0.00)
Blood	0.91 (0.17)	0.20 (0.01)	0.04 (0.00)	1.04 (0.08)	0.25 (0.04)	0.04 (0.01)	0.71 (0.22)	0.22 (0.04)	0.03 (0.00)

Pretargeting Using MN14-MORF15 and Labeled-cMORF15

[0097] The biodistributions in ID% per organ and target to non-target ratios at 3 hrs post administration of radiolabeled-cMORF15 or cMORF18 to tumored animals receiving MN14-MORF15 or MN14-MORF18 respectively 48 hrs earlier are presented in Table 6. Also presented are the results of administering radiolabeled-cMORF15 to control animals not having received the antibody. These data show the superiority of cMORF15-^{99m}Tc pretargeting over cMORF18-^{99m}Tc pretargeting. At 3 hrs, the biodistribution of cMORF15-^{99m}Tc in tumored nude mice by pretargeting (Table 6) is almost the same as that in normal CD-1 mice (Table 4) in that there is little uptake in all other organs except kidneys and intestines. There is also no large difference in radioactivity between 15 mer pretargeting and 18 mer pretargeting in all organs other than kidneys. Kidney radioactivity uptake of MN14-MORF18/cMORF18-^{99m}Tc pretargeting is almost twice that of MN14-MORF15/cMORF15-^{99m}Tc pretargeting. Because the tumor uptake is also higher for the 15 mer pretargeting, the tumor/normal tissue ratios are improved in all cases for 15 mer pretargeting. Slight differences in the MORF groups per antibody (0.28 vs. 0.27) and dosage administered (14 µg vs. 13 µg) between these two pretargeting studies performed simultaneously should not have influenced these observations.

**Table 6
Biodistributions of Radiolabeled-cMORF15 or cMORF18 at 3 Hr in Tumor-Bearing Mice**

Organ	15 mer control			15 mer pretargeting			18 mer pretargeting		
	ID%	SD	T/NT	ID%	SD	T/NT	ID%	SD	T/NT
Liver	0.18	0.08	0.86	0.44	0.02	3.1	0.51	0.12	2.4
Heart	0.00	0.00	3	0.01	0.00	9.2	0.02	0.00	5.6
Kidney	1.52	0.43	0.04	1.84	0.17	0.22	3.12	0.56	0.12
Lung	0.01	0.00	1.5	0.03	0.00	4.8	0.05	0.01	3.3
Spleen	0.01	0.01	1.5	0.02	0.00	5.8	0.03	0.00	3.0
Stomach	0.09	0.04	---	0.23	0.06	---	0.27	0.04	---
Sm.Int.	0.76	0.42	---	0.30	0.06	---	0.23	0.04	---
Lg. Int.	1.30	0.43	---	0.55	0.06	---	0.36	0.03	---
Muscle	0.01	0.00	3	0.02	0.00	12	0.02	0.01	8.8
Tumor	0.16	0.03	1.0	1.35	0.30	1.0	1.24	0.16	1.0
Blood	0.08	0.02	2	0.91	0.15	1.5	1.13	0.33	1.4

(continued)

Biodistributions of Radiolabeled-cMORFIS or cMORF18 at 3 Hr in Tumor-Bearing Mice

Organ	15 mer control			15 mer pretargeting			18 mer pretargeting		
	ID%	SD	T/NT	ID%	SD	T/NT	ID%	SD	T/NT
Whole body	9.4	1.3	---	10.1	1.6	---	11.5	2.1	---

Control animals received ^{99m}Tc-cMORF15 only, 15 mer pretargeting animals received MN14-MORF15 followed by ^{99m}Tc-cMORF15, 18 mer pretargeting animals received MN14-MORF18 followed by ^{99m}Tc-cMORF18 ((ID%/organ, n=4)

Imaging Study of LS174T Tumor-Bearing Nude Mice

[0098] Figure 8 presents representative whole body images obtained of both a MN14-MORF15 and a MN14-MORF18 pretargeted tumored mouse at 3 hrs post-injection of ^{99m}Tc-cMORF15 and ^{99m}Tc-cMORF18 respectively. Both animals and the control were imaged simultaneously. The lower kidney radioactivity levels in the case of the cMORF15-^{99m}Tc pretargeted animal compared to the cMORF18-^{99m}Tc pretargeted animal provides an obvious improvement to the image.

Claims

1. A kit for *in vivo* targeting of a diagnostic or therapeutic agent to a target site comprising:

- (a) a first conjugate comprising a targeting moiety and a Morpholino oligomer, wherein said targeting moiety binds selectively to a primary, target-specific binding site of the target site or to a substance produced by or associated with the target site;
- (b) optionally, a clearing agent; and
- (c), a second conjugate comprising a complementary Morpholino oligomer and a diagnostic agent or therapeutic agent.

2. The kit of claim 1, wherein said targeting moiety comprises a humanized antibody or an antigen-binding fragment of a humanized antibody.

3. The kit of claim 2, wherein said humanized antibody is an anti-carcinoembryonic antigen (CEA) antibody.

4. The kit of claim 1, wherein said targeting moiety is selected from the group consisting of proteins, small peptides, polypeptides, enzymes, hormones, steroids, cytokines, neurotransmitters, oligomers, vitamins and receptor binding molecules.

5. The kit of claim 1, wherein the length of said Morpholino oligomer and/or said complementary Morpholino oligomer is at least about 6 bases to about 100 bases.

6. The kit of claim 1, wherein said Morpholino oligomer and/or said complementary Morpholino oligomer is 15-mer, 18-mer or 25-mer.

7. The kit of claim 1, wherein said clearing agent is an anti-idiotypic antibody or antigen-binding antibody fragment.

8. The kit of claim 1, wherein said therapeutic agent is selected from the group consisting of antibodies, antibody fragments, drugs, toxins, nucleases, hormones, immunomodulators, radiometal chelates, boron compounds, photoactive agents or dyes and radionuclides.

9. The kit of claim 8, wherein

- (a) said radionuclide substantially decays by beta-particle emission and is selected from the group consisting of P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212, and Bi-213; or
- (b) said radionuclide substantially decays by Auger particle emission and is selected from the group consisting

of Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m and Ir-192; or
(c) said radionuclide substantially decays by alpha-particle emission and is selected from the group consisting of Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 and Fm-255.

10. The kit of claim 1, wherein said therapeutic agent is used in photodynamic therapy or neutron capture procedures.

11. The kit of claim 10, wherein said photodynamic therapy uses metal complexes, and said metal complexes are selected from the group consisting of zinc, aluminium, gallium, lutetium and palladium complexes.

12. The kit of claim 10, wherein said neutron capture procedures use a radionuclide selected from the group consisting of B-10, Gd-157 and U-235.

13. The kit of claim 1, wherein said diagnostic agent is selected from the group consisting of radionuclides, dyes, contrast agents, fluorescent compounds or molecules and enhancing agents useful for magnetic resonance imaging (MRI).

14. The kit of claim 13, wherein said diagnostic agent is

(a) a radionuclide useful in positron emission and said radionuclide is selected from the group consisting of F-18, Mn-51, Mn-52m, Fe-52, Co-55, Cu-62, Cu-64, Ga-68, As-72, Br-75, Br-76, Rb-82m, Sr-83, Y-86, Zr-89, Tc-94m; In-110, I-120, and I-124; or

(b) a metal ion useful in magnetic resonance imaging techniques and wherein said metal ion is selected from the group consisting of gadolinium, manganese, iron, chromium, copper, cobalt, nickel, dysprosium, rhenium, europium, terbium, holmium and neodymium ions; or

(c) a radionuclide useful in gamma-ray detection and wherein said radionuclide is selected from the group consisting of Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, I-123, I-125, I-131, Yb-169, Hg-197, and Tl-201.

15. Use of:

(a) a first conjugate comprising a targeting moiety and a Morpholino oligomer, wherein said targeting moiety binds selectively to a primary, target-specific binding site of a mammalian target site or to a substance produced by or associated with said target site;

(b) optionally a clearing agent; and

(c) a second conjugate comprising a complementary Morpholino oligomer and a diagnostic agent or therapeutic agent,

in the preparation of a medicament for use in an *in vivo* targeting method for delivering a diagnostic or therapeutic agent to said target site.

16. The use of claim 15, wherein said medicament is suitable for intravenous, intraarterial, intrapleural, intraperitoneal, intrathecal, subcutaneous administration or administration by perfusion.

17. The use of claim 15, wherein said target site is a tumor or other lesions, an infectious disease site, an inflammatory disease site, or an autoimmune disease site.

18. The use of claim 15, wherein said medicament is in a form suitable for administration in conjunction with endoscopy, laparoscopy, normal organ imaging or with an intraoperative probe.

19. The kit of claim 3, wherein said anti-carcinoembryogenic antigen antibody is an anti-MN14 antibody.

Patentansprüche

1. Ein Kit zum *in vivo* Ansteuern eines diagnostischen oder therapeutischen Mittels auf einen Zielort umfassend:

(a) ein erstes Konjugat umfassend eine Ansteuerungseinheit und ein Morpholino-Oligomer, wobei die Ansteuerungseinheit selektiv an eine primäre, zielspezifische Bindungsstelle des Zielortes oder an eine vom Zielort gebildete oder mit dem Zielort assoziierte Substanz bindet;

(b) gegebenenfalls ein Aufreinigungsmittel; und

(c) ein zweites Konjugat umfassend ein komplementäres Morpholino-Oligomer und ein diagnostisches Mittel oder therapeutisches Mittel.

- 5 **2.** Kit nach Anspruch 1, wobei die Ansteuerungseinheit einen humanisierten Antikörper oder ein antigen-bindendes Fragment eines humanisierten Antikörpers umfasst.
- 3.** Kit nach Anspruch 2, wobei der humanisierte Antikörper ein antikarcinoembryonischer Antigen-Antikörper (CEA) ist.
- 10 **4.** Kit nach Anspruch 1, wobei die Ansteuerungseinheit ausgewählt ist aus der Gruppe bestehend aus Proteinen, kleinen Peptiden, Polypeptiden, Enzymen, Hormonen, Steroiden, Cytokinen, Neurotransmittern, Oligomeren, Vitaminen und rezeptorbindenden Molekülen.
- 5.** Kit nach Anspruch 1, wobei die Länge des Morpholino-Oligomers und/oder des komplementären Morpholino-Oligomers mindestens etwa 6 Basen bis etwa 100 Basen ist.
- 15 **6.** Kit nach Anspruch 1, wobei das Morpholino-Oligomer und/oder das komplementäre Morpholino-Oligomer ein 15-mer, 18-mer oder 25-mer ist.
- 20 **7.** Kit nach Anspruch 1, wobei das Aufreinigungsmittel ein anti-idiotypischer Antikörper oder ein antigenbindendes Antikörperfragment ist.
- 8.** Kit nach Anspruch 1, wobei das therapeutische Mittel ausgewählt ist aus der Gruppe bestehend aus Antikörpern, Antikörperfragmenten, Wirkstoffen, Toxinen, Nukleasen, Hormonen, Immunomodulatoren, Radiometallchelaten, Borverbindungen, photoaktiven Mitteln oder Farbstoffen und Radionukliden.
- 25 **9.** Kit nach Anspruch 8, wobei
 - 30 (a) das Radionuklid im Wesentlichen durch Betateilchenemission zerfällt und ausgewählt ist aus der Gruppe bestehend aus P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77, Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212 und Bi-213; oder
 - 35 (b) das Radionuklid im Wesentlichen durch Augerteilchenemission zerfällt und ausgewählt ist aus der Gruppe bestehend aus Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m und Ir-192; oder
 - (c) das Radionuklid im Wesentlichen durch Alphateilchenemission zerfällt und ausgewählt ist aus der Gruppe bestehend aus Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 und Fm-255.
- 40 **10.** Kit nach Anspruch 1, wobei das therapeutische Mittel in der photodynamischen Therapie oder in Neutronen-Einfangreaktionsverfahren verwendet wird.
- 11.** Kit nach Anspruch 10, wobei die photodynamische Therapie Metallkomplexe verwendet und die Metallkomplexe ausgewählt sind aus der Gruppe bestehend aus Zink-, Aluminium-, Gallium-, Lutetium- und Palladiumkomplexen.
- 45 **12.** Kit nach Anspruch 10, wobei die Neutronen-Einfangreaktionsverfahren ein Radionuklid verwenden, dass ausgewählt ist aus der Gruppe bestehend aus B-10, Gd-157 und U-235.
- 13.** Kit nach Anspruch 1, wobei das diagnostische Mittel ausgewählt ist aus der Gruppe bestehend aus Radionukliden, Farbstoffen, Kontrastmitteln, fluoreszierenden Verbindungen oder Molekülen und verstärkenden Mitteln verwendbar in magnetischem Resonanz-Abbilden (MRI).
- 50 **14.** Kit nach Anspruch 13, wobei das diagnostische Mittel
 - 55 (a) ein radioaktives Nuklid ist, dass in Positronenemission verwendbar ist, und das Radionuklid ausgewählt ist aus der Gruppe bestehend aus F-18, Mn-51, Mn-52m, Fe-52, Co-55, Cu-62, Cu-64, Ga-68, As-72, Br-75, Br-76, Rb-82m, Sr-83, Y-86, Zr-89, Tc-94m, In-110, I-120 und I-124; oder
 - (b) ein Metallion ist, dass in magnetischen Resonanzabbildungstechniken verwendbar ist, und wobei das Me-

tallion ausgewählt ist aus der Gruppe bestehend aus Gadolinium-, Mangan-, Eisen-, Chrom-, Kupfer-, Kobalt-, Nickel-, Dysprosium-, Rhenium-, Europium-, Terbium-, Holmium- und Neodymium-Ionen; oder
(c) ein radioaktives Nuklid ist, dass in Gammastrahlendetektion verwendbar ist, und wobei das radioaktive Nuklid ausgewählt ist aus der Gruppe bestehend aus Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, I-123, I-125, I-131, Yb-169, Hg-197 und Tl-201.

15. Verwendung von:

- (a) einem ersten Konjugat umfassend eine Ansteuerungseinheit und ein Morpholino-Oligomer, wobei besagte Ansteuerungseinheit selektiv an eine primäre zielspezifische Bindungsstelle eines Zielortes von einem Säuger oder an eine Substanz gebildet von oder assoziiert mit besagtem Zielort bindet;
- (b) gegebenenfalls einem Aufreinigungsmittel; und
- (c) einem zweiten Konjugat umfassend ein komplementäres Morpholino-Oligomer und ein diagnostisches Mittel oder therapeutisches Mittel,

bei der Herstellung eines Medikaments zur Anwendung in einer *in vivo* zielgerichteten Methode zum Transportieren eines diagnostischen oder therapeutischen Mittels zu besagtem Zielort.

16. Verwendung nach Anspruch 15, wobei das Medikament geeignet ist für intravenöse, intraarterielle, intrapleurale, intraperitoneale, intrathecale, subkutane Verabreichung oder Verabreichung durch Perfusion.

17. Verwendung nach Anspruch 15, wobei der Zielort ein Tumor oder eine andere Schädigung, ein infektiöser Krankheitsort, ein entzündlicher Krankheitsort, oder ein autoimmuner Krankheitsort ist.

18. Verwendung nach Anspruch 15, wobei das Medikament in einer Form geeignet für Verabreichung in Verbindung mit Endoskopie, Laparoskopie, normalen Organabbildungen oder mit einer intraoperativen Probe ist.

19. Kit nach Anspruch 3, wobei der antikarcinoembryonische Antigen-Antikörper ein anti-MN14-Antikörper ist.

Revendications

1. Kit pour le ciblage *in vivo* d'un agent diagnostique ou thérapeutique sur un site cible comprenant :

- (a) un premier conjugué comprenant un groupement de ciblage et un oligomère morpholino, ledit groupement de ciblage se liant sélectivement à un site de liaison primaire spécifique de cible du site cible ou à une substance produite par le site cible ou associée au site cible;
- (b) facultativement, un agent d'élimination ; et
- (c) un second conjugué comprenant un oligomère morpholino complémentaire et un agent diagnostique ou un agent thérapeutique.

2. Kit selon la revendication 1, dans lequel ledit groupement de ciblage comprend un anticorps humanisé ou un fragment de liaison à un antigène d'un anticorps humanisé.

3. Kit selon la revendication 2, dans lequel ledit anticorps humanisé est un anticorps anti-antigène carcino-embryonnaire (ACE).

4. Kit selon la revendication 1, dans lequel ledit groupement de ciblage est choisi dans le groupe composé des protéines, des petits peptides, des polypeptides, des enzymes, des hormones, des stéroïdes, des cytokines, des neurotransmetteurs, des oligomères, des vitamines et des molécules de liaison à un récepteur.

5. Kit selon la revendication 1, dans lequel la longueur dudit oligomère morpholino et/ou dudit oligomère morpholino complémentaire est d'au moins environ 6 bases à environ 100 bases.

6. Kit selon la revendication 1, dans lequel ledit oligomère morpholino et/ou ledit oligomère morpholino complémentaire est un 15-mère, un 18-mère ou un 25-mère.

7. Kit selon la revendication 1, dans lequel ledit agent d'élimination est un anticorps anti-idiotypique ou un fragment

d'anticorps de liaison à un antigène.

8. Kit selon la revendication 1, dans lequel ledit agent thérapeutique est sélectionné dans le groupe composé des anticorps, des fragments d'anticorps, des médicaments, des toxines, des nucléases, des hormones, des immuno-modulateurs, des chélates de radiométal, des composés du bore, des agents photo-actifs ou des colorants et des radionucléides.

9. Kit selon la revendication 8, dans lequel

(a) ledit radionucléide présente sensiblement une décroissance radioactive par émission de particule bêta et est sélectionné dans le groupe composé de P-32, P-33, Sc-47, Fe-59, Cu-64, Cu-67, Se-75, As-77 ; Sr-89, Y-90, Mo-99, Rh-105, Pd-109, Ag-111, I-125, I-131, Pr-142, Pr-143, Pm-149, Sm-153, Tb-161, Ho-166, Er-169, Lu-177, Re-186, Re-188, Re-189, Ir-194, Au-198, Au-199, Pb-211, Pb-212 et Bi-213 ; ou

(b) ledit radionucléide présente sensiblement une décroissance radioactive par émission de particule Auger et est sélectionné dans le groupe composé de Co-58, Ga-67, Br-80m, Tc-99m, Rh-103m, Pt-109, In-111, Sb-119, I-125, Ho-161, Os-189m et Ir-192 ; ou

(c) ledit radionucléide présente sensiblement une décroissance radioactive par émission de particule alpha et est sélectionné dans le groupe composé de Dy-152, At-211, Bi-212, Ra-223, Rn-219, Po-215, Bi-211, Ac-225, Fr-221, At-217, Bi-213 et Fm-255.

10. Kit selon la revendication 1, dans lequel ledit agent thérapeutique est utilisé dans des procédures de thérapie photodynamique ou de capture de neutron.

11. Kit selon la revendication 10, dans lequel ladite thérapie photodynamique utilise des complexes métalliques et lesdits complexes métalliques sont sélectionnés dans le groupe composé des complexes de zinc, d'aluminium, de gallium, de lutétium et de palladium.

12. Kit selon la revendication 10, dans lequel lesdites procédures de capture de neutron utilisent un radionucléide sélectionné dans le groupe composé de B-10, Gd-157 et U-235.

13. Kit selon la revendication 1, dans lequel ledit agent diagnostique est sélectionné dans le groupe composé des radionucléides, des colorants, des agents de contraste, des composés ou des molécules fluorescents et des agents de renforcement utiles pour l'imagerie par résonance magnétique (IRM).

14. Kit selon la revendication 13, dans lequel l'agent de diagnostic est

(a) un radionucléide utile dans l'émission de positron et ledit radionucléide est sélectionné dans le groupe composé de F-18, Mn-51, Mn-52m, Fe-52, Co-55, Cu-62, Cu-64, Ga-68, As-72, Br-75, Br-76, Rb-82m, Sr-83, Y-86, Zr-89, Tc-94m, In-110, I-120 et I-124 ; ou

(b) un ion métallique utile dans des techniques d'imagerie par résonance magnétique et dans lequel ledit ion métallique est sélectionné dans le groupe composé des ions gadolinium, manganèse, fer, chrome, cuivre, cobalt, nickel, dysprosium, rhénium, europium, terbium, holmium et néodyme ; ou

(c) un radionucléide utile dans la détection des rayons gamma et dans lequel ledit radionucléide est sélectionné dans le groupe composé de Cr-51, Co-57, Co-58, Fe-59, Cu-67, Ga-67, Se-75, Ru-97, Tc-99m, In-111, In-114m, I-123, I-125, I-131, Yb-169, Hg-197 et Tl-201.

15. Utilisation :

(a) d'un premier conjugué comprenant un groupement de ciblage et un oligomère morpholino, ledit groupement de ciblage se liant sélectivement à un site de liaison primaire spécifique de cible d'un site cible de mammifère ou à une substance produite par ledit site cible ou associée audit site cible ;

(b) facultativement, d'un agent d'élimination ; et

(c) d'un second conjugué comprenant un oligomère morpholino complémentaire et un agent diagnostique ou un agent thérapeutique,

pour la préparation d'un médicament à utiliser dans un procédé de ciblage *in vivo* pour administrer un agent diagnostique ou thérapeutique audit site cible.

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16. Utilisation selon la revendication 15, dans laquelle ledit médicament est adapté pour une administration intraveineuse, intra-artérielle, intrapleurale, intra-péritonéale, intrathécale, sous-cutanée ou par perfusion.
- 5 17. Utilisation selon la revendication 15, dans laquelle ledit site cible est une tumeur ou d'autres lésions, un site de maladie infectieuse, un site de maladie inflammatoire ou un site de maladie auto-immune.
- 10 18. Utilisation selon la revendication 15, dans laquelle ledit médicament est sous une forme adaptée pour une administration en association avec une endoscopie, une laparoscopie, une imagerie d'organe normal ou avec une sonde per-opératoire.
19. Kit selon la revendication 3, dans lequel ledit anticorps anti-antigène carcino-embryonnaire est un anticorps anti-MN14.

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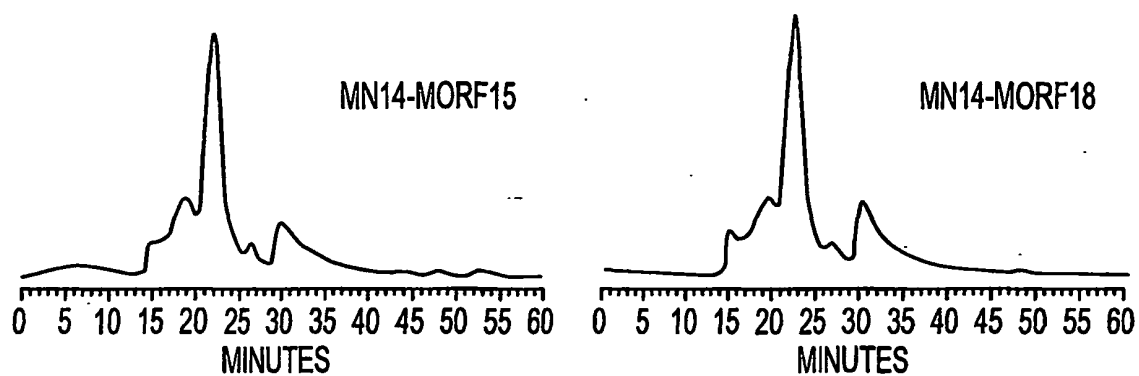


FIG. 1A

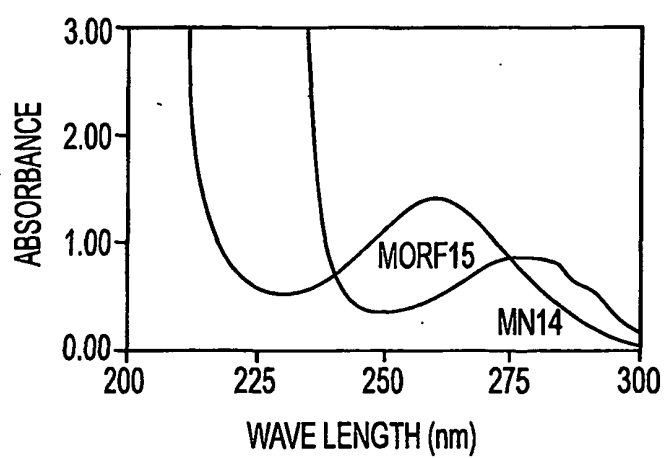


FIG. 1B

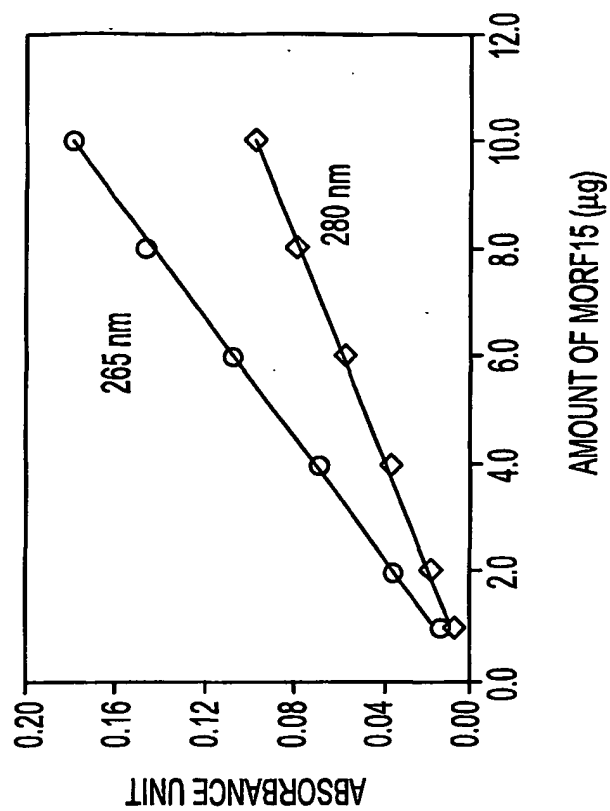


FIG. 1D

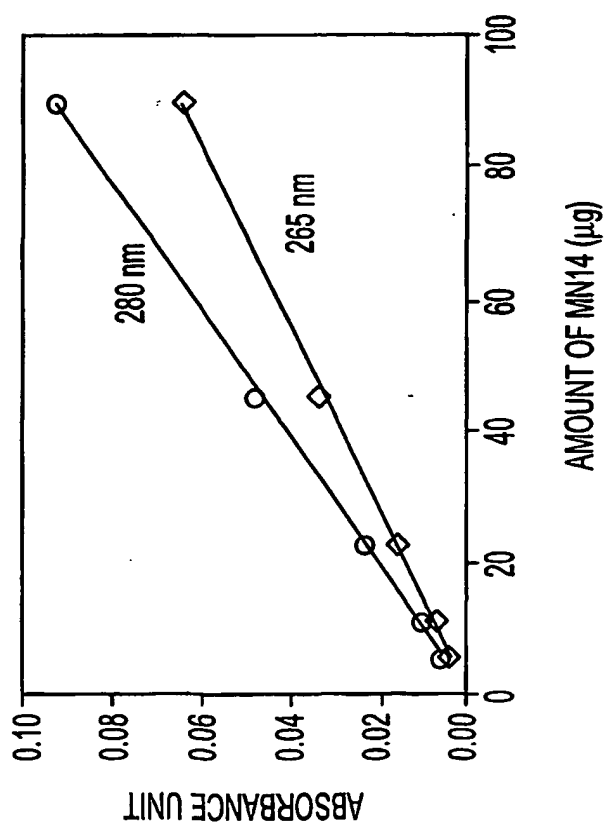


FIG. 1C

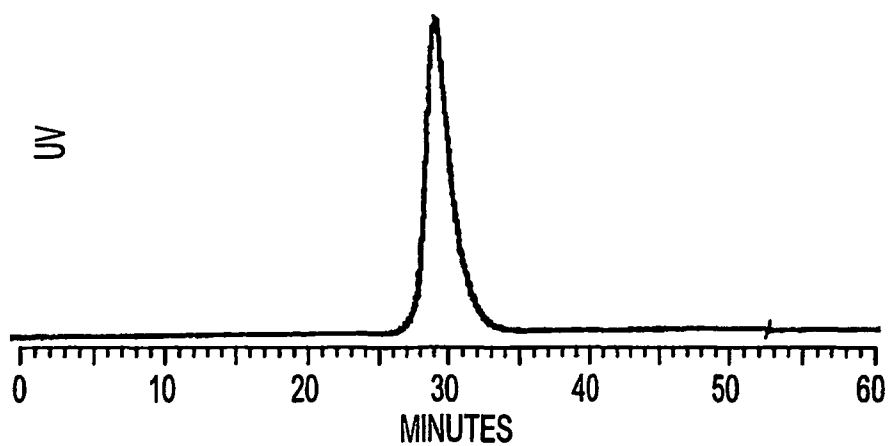


FIG. 2A

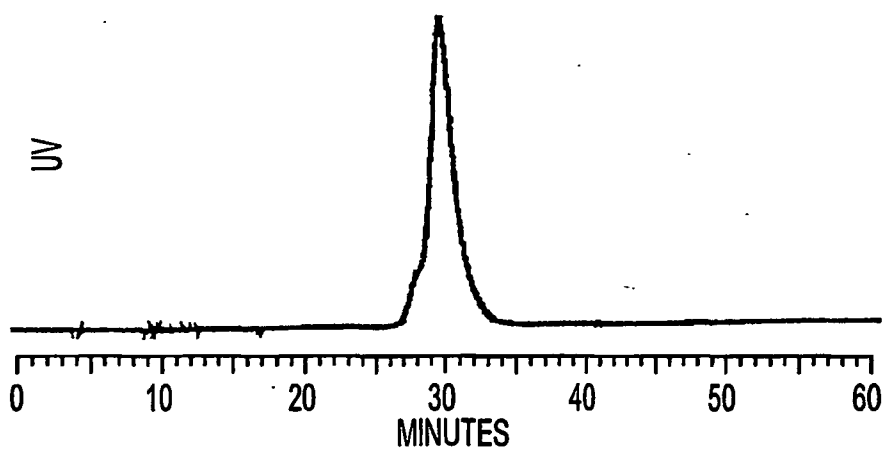


FIG. 2B

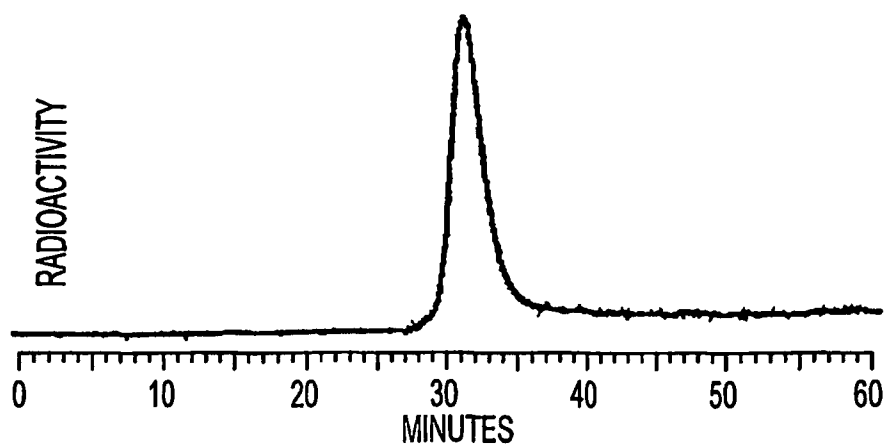
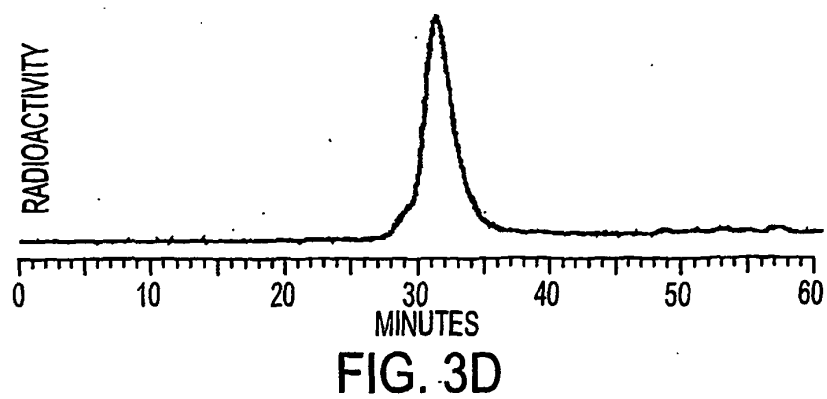
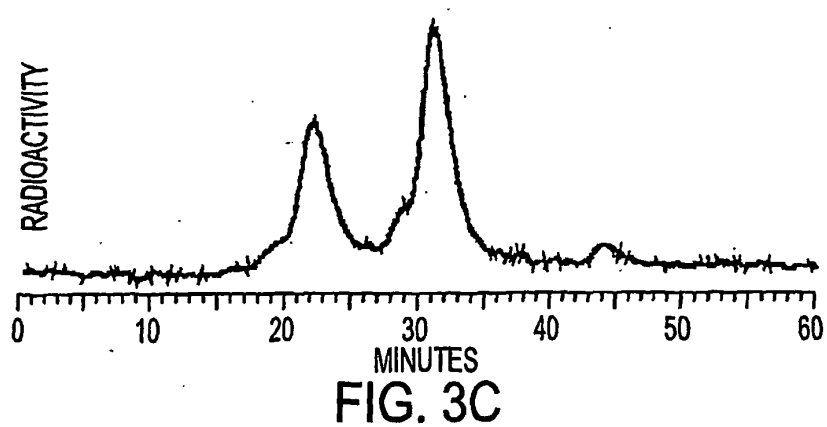
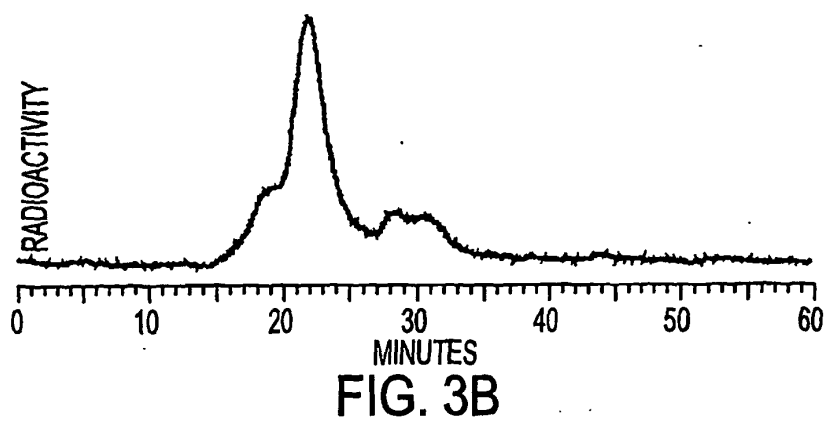
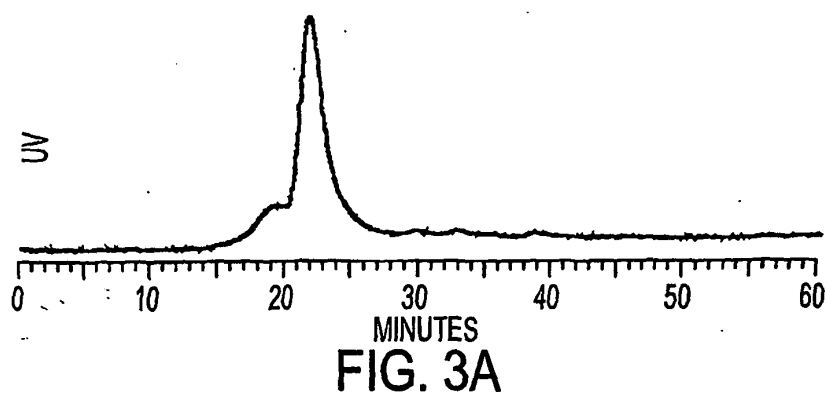


FIG. 2C



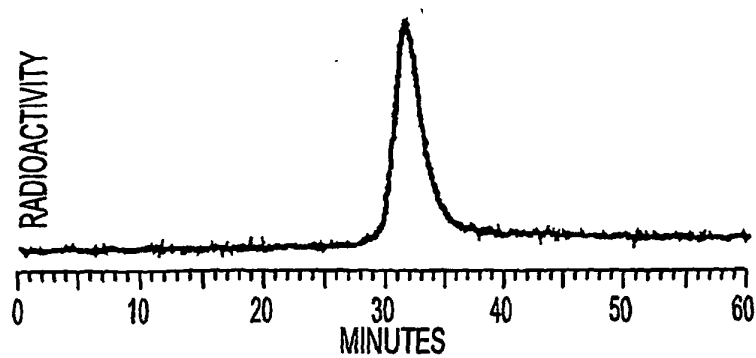


FIG. 4A

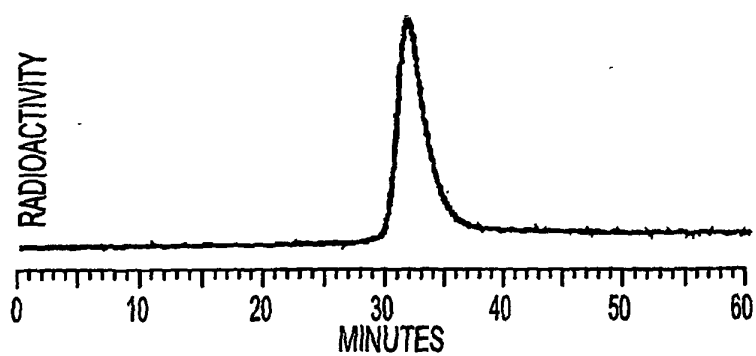


FIG. 4B

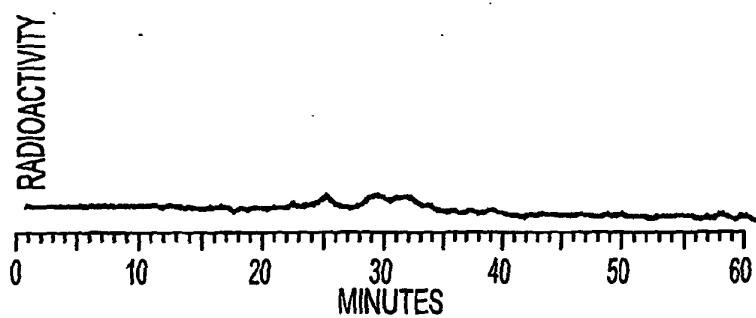


FIG. 4C

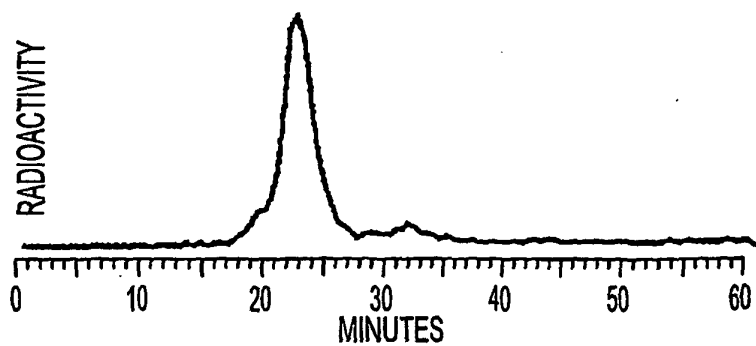


FIG. 4D

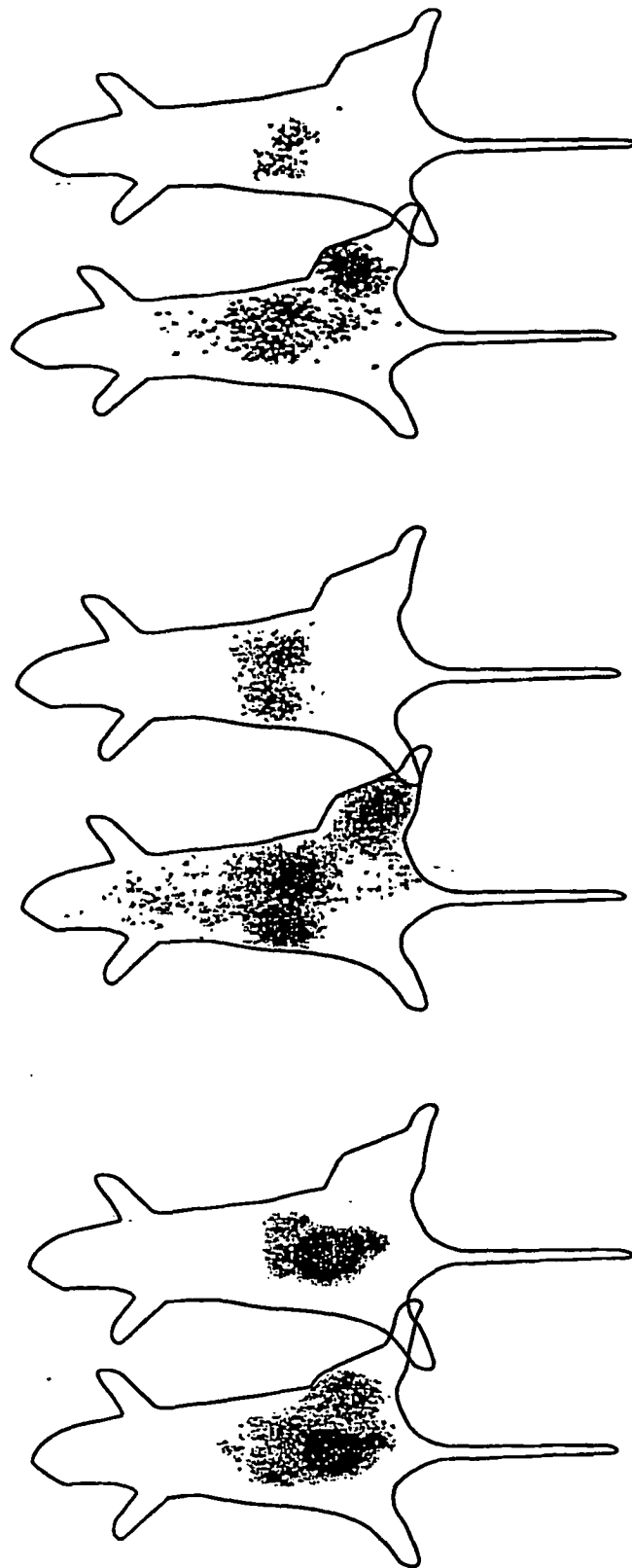


FIG. 5

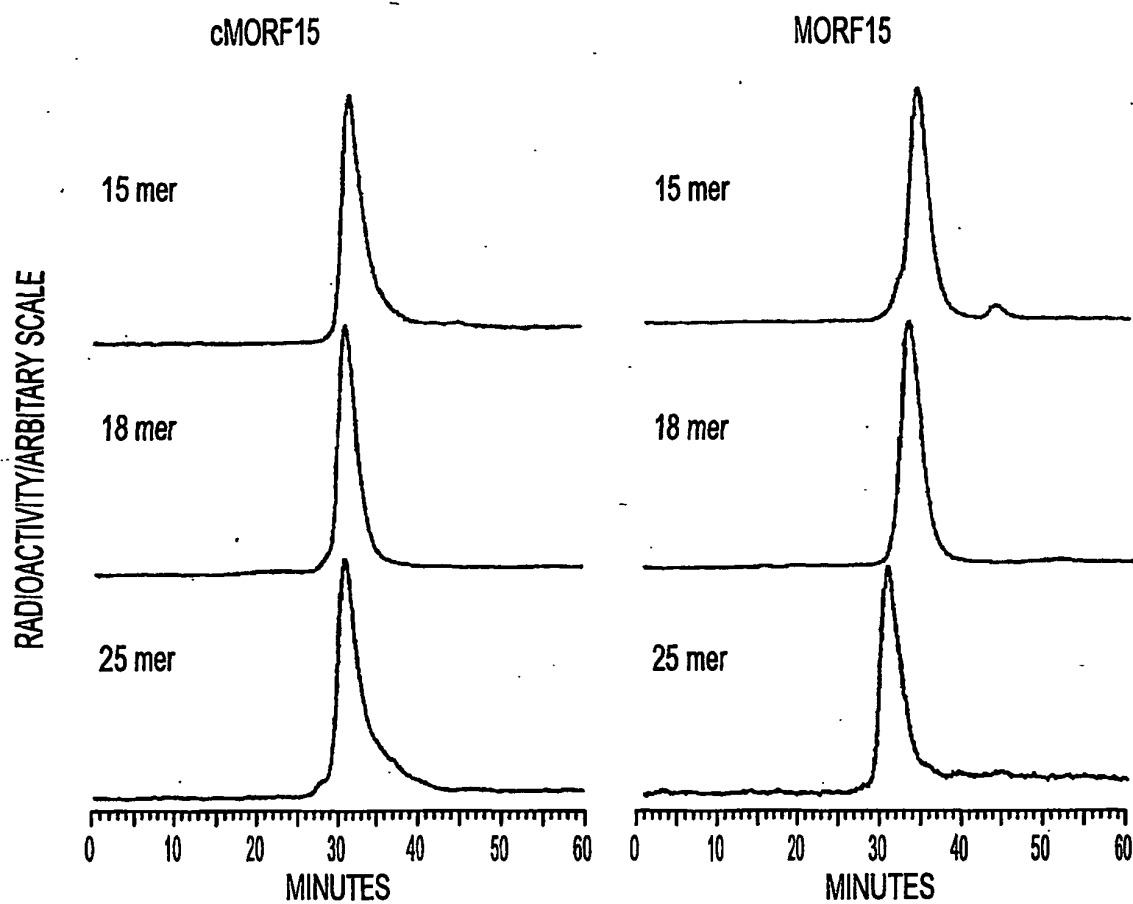


FIG. 6

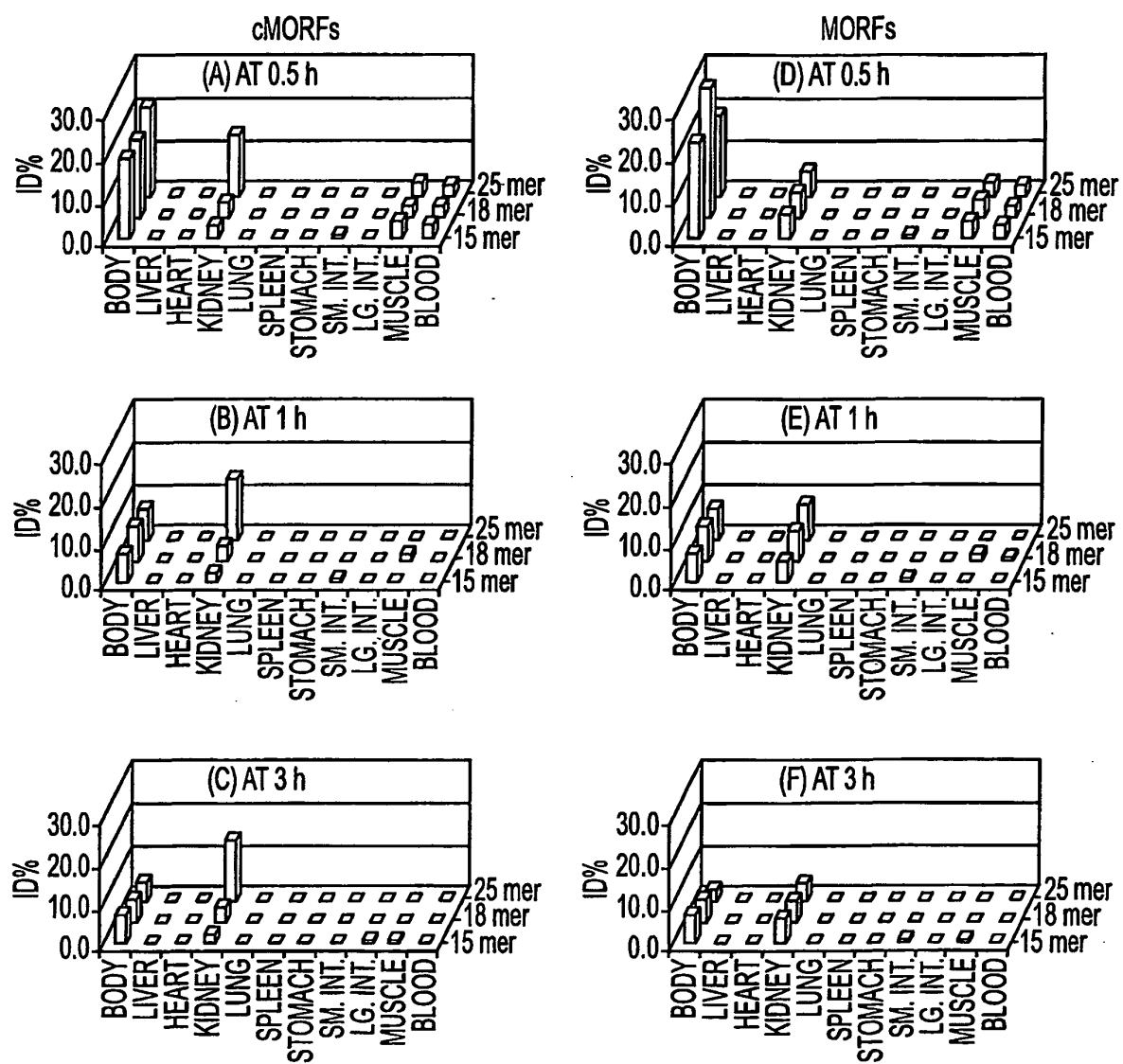


FIG. 7

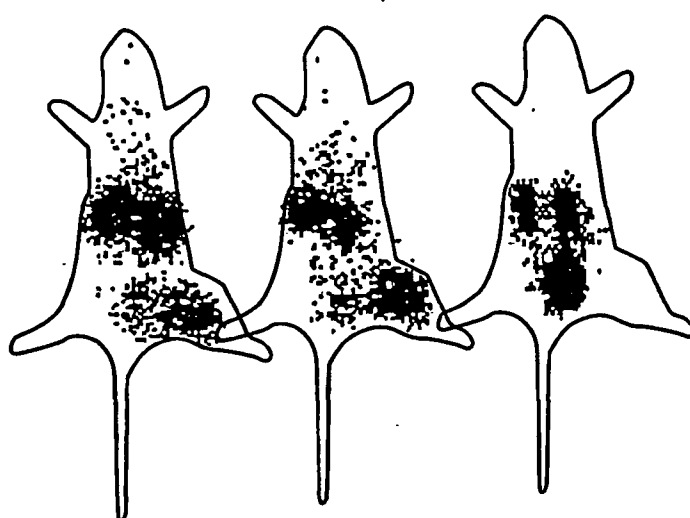
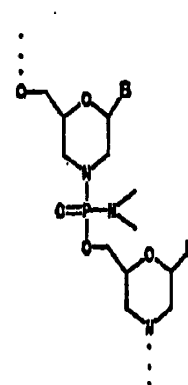


FIG. 8

专利名称(译)	Morpholino成像和治疗		
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当前申请(专利权)人(译)	马萨诸塞大学		
[标]发明人	HNATOWICH DONALD J RUSCKOWSKI MARY LIU GUOZHENG		
发明人	HNATOWICH, DONALD, J. RUSCKOWSKI, MARY LIU, GUOZHENG		
IPC分类号	A61K51/04 A61K31/535 A61K39/395 A61K45/00 A61K51/10 A61K47/48 A61K41/00 A61K49/08 A61B5/055 A61K A61K51/00 A61P35/04 C07K16/30 C12P21/08 C12Q1/68		
CPC分类号	C07K16/3007 A61K47/6897 A61K51/0495 B82Y5/00		
优先权	60/279809 2001-03-30 US 60/341794 2001-12-21 US		
其他公开文献	EP1372741A2 EP1372741A4		
外部链接	Espacenet		

摘要(译)

本发明提供了用于将诊断剂或治疗剂靶向具有病理状况的哺乳动物的靶位点的试剂盒和方法。所述试剂盒在单独的容器中包含 (a) 包含靶向部分和吗啉代寡聚体的第一缀合物，其中所述靶向部分选择性结合靶位点的主要靶标特异性结合位点或由所述靶标部分产生或与之相关的物质。与目标网站; (b) 可选的清算代理人; (c) 第二种缀合物，其包含互补的吗啉代寡聚物和诊断剂或治疗剂。该方法包括给哺乳动物施用 (a)，任选地 (b) 和 (c)。



B = adenine, cytosine, guanine, thymine/uracil