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(54) Title: KIT FOR PRETARGETING AND NOVEL PRETARGETING CONJUGATES

(57) Abstract

The invention relates to a method of pretargeting therapeutic or diagnostic compounds to target cells in the body and a kit for such a method. The targeting is preferably through a specific antibody. The binding between the preinjected targeting moiety and the effector moiety is established by a zinc finger and its cognate nucleotide sequence, conjugated to the targeting or effector moiety. Also part of the invention are conjugates of zinc fingers and therapeutic or diagnostic compounds.

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KIT FOR PRETARGETING AND NOVEL PRETARGETING CONJUGATES.

The invention relates to a novel kit for the delivery of pharmaceutical compounds or diagnostics to target cells in the body. This kit comprises a targeting conjugate and an effector conjugate, these conjugates being able to form a specifically interacting binding pair by means of a zinc finger and its cognate nucleotide sequence. Specifically the invention relates to targeting cytotoxic drugs or radioisotopes to tumor cells, preferably with a human antibody.

The in vivo use of antibodies for the selective delivery of diagnostic or therapeutic agents to target tissues is known in the art.
Since the advent of methodology for the production of monoclonal antibodies, antibodies of predefined specificities (Köhler and Milstein, 1975), numerous studies aiming at specific targeting of radionuclides, plant or bacterial toxins, biological agents and cytostatic agents (drugs) to target cells or tissues with the help of said antibodies have been performed, as reviewed by Bator and Reading (in: Therapeutic Monoclonal Antibodies, eds: C.A.K. Borrebaeck and J.W. Larrick, Stockton Press, pp. 35-56, 1990). In in vitro studies the great potential of these immunoconjugates has been amply demonstrated. Application of such immunoconjugates in vivo in cancer patients has met with less success, in particular with solid tumors. A major problem has been the inability to attain a sufficient difference in uptake of the immunoconjugates between the target tissues as compared to the non-target tissues. As a result it has been difficult for instance to effect an increase in therapeutic index of clinically used anti-cancer drugs by administration in the form of a drug-immunoconjugate. Similarly, due to the lack of operational selectivity of therapeutic radio-immunoconjugates

when administered in vivo, normal tissues are unduly exposed to destructive radiation. The therapeutic and diagnostic limitations of immunoconjugates, apart from a number of factors adversely affecting antibody delivery, are amongst others due to changes in antibody affinity, specificity and immunogenicity upon chemically linking a diagnostic or therapeutic agent to an antibody.

In order to overcome part of the problems mentioned, newer approaches to delivery of therapeutic or diagnostic agents have recently been suggested, that are characterised in that the localisation phase of the antibody component is separated in time from the phase in which the therapeutic or diagnostic agent is administered. In these so-called pretargeting schemes sufficient time is permitted for accumulation of non-labelled antibody at the target site and for elimination of the antibody from other non-target tissues. The latter process, the clearing phase, may be aided by, for example, plasmaphoresis or use of a second antibody reactive with the first. At the time of optimum antibody accumulation at the target site as compared to the non-target tissue, the diagnostic or therapeutic agent to be recognised by the antibody already localised, is administered.

This approach has the advantage that the non-toxic targeting moiety can be administered in large quantities without the risk of serious toxic side effects. Furthermore, increased uptake ratios and faster localization can be expected, since the toxic or diagnostic moiety is attached to a low molecular weight compound which is capable of fast distribution through the body tissues and rapid clearance through the kidneys.

The pretargeting approaches which have been so far described in literature require antibodies or antibody conjugates with a dual specificity: a specific site that allows binding to a target tissue associated antigen and a specificity that enables specifically binding of the diagnostic or therapeutic agent.

Early on, specific antibodies were constructed by chemically linking univalent fragments of antibodies with different specificities. Raso and Griffin (Cancer Res. 41, 207, 1981) for instance constructed a bifunctional antibody that was able to bind human IgG and to ricin, a toxic lectin. Later on, technology has been developed to produce hybrid hybridomas that produce monoclonal antibodies of dual specificity (Reading, US Patent 4,474,893). A biologically produced bifunctional antibody recognising both carcinoembryonic antigen (CEA) and vinca alkaloids was described by Corvalen et al. (Cancer Immunol. Immunother. 24, 133, 1987). Studies on xenografts in nude mice revealed the combined use of the bifunctional antibody and vinblastin, given 7-11 days following administration of the antibody, to be more effective than drug alone in suppressing tumor growth.

Recently pretargeting techniques have been proposed in which the specific antibody recognises a haptenic group, i.e. an EDTA derivative chelating indium (Stickney et al., Cancer Res. 51, 6650, 1991).

The bifunctional antibody approach, however, suffers from the fact that the antibody molecule is composed of two monovalent antibody fragments with different specificities. The avidity of monovalent antibody fragments such as Fab fragments is orders of magnitude lower than that of bivalent antibody molecules. The efficacy of the two step bifunctional antibody approach, however, is dependent on high avidity binding of the bifunctional antibody to the toxin-conjugated hapten and to extracellular or cell surface antigens at the target site. Moreover, to allow for efficient clearance of non-bound bifunctional antibody from circulation before injection of the conjugated hapten, a period of 4 to 6 days is required. Using monovalent antibody fragments, complete dissociation of bound antibody molecules from the target site is expected in this period of time. A study of the kinetics of antibody binding to surface-immobilized antigen demonstrated that the

intact antibody, bound to the surface-immobilized antigen, did not dissociate significantly over a period of almost 3 days, whereas a monovalent Fab' fragment prepared from the same antibody dissociated from the surface-bound antigen with a half-life of 16 hours (Nygre, N. et al., J. Immunol. Meth. 85, 87-95, 1985).

Bifunctional antibodies have also been prepared with specificity for both tumor antigens and (radiolabelled) chelates (Goodwin, D.A. et al., J. Nucl. Med. 29, 226-234, 1988; Stickney, D.R. et al., Cancer Res. 51, 6650-6655, 1991; Le Doussal, J.-M., J. Cancer Suppl. 7, 58-62, 1992). Major drawback in this approach is the low affinity of the bispecific antibodies for the chelating moiety.

In a closely related domain of the prior art monovalent, target site selective antibodies have been endowed with a second specificity by linkage, through either chemical or biological methods, to a member of a specifically interacting binding pair. The high affinity system avidin-biotin has been adopted in such an approach (European Patent Appl. No. 0 251 494). The pretargeting system used in said patent application employs an antibody that has been linked to the protein avidin or streptavidin and a biotinylated metal chelate.

A major problem with the pretargeting system mentioned above is the immunogenicity of the antibody-conjugate. First of all immunogenic activity is caused by the murine antibodies, which are often used in this kind of conjugates. These, like all other foreign proteins, are highly immunogenic in man. The phenomenon of HAMA, human anti mouse antibodies, is well known in the field and severely limits the use of mouse derived antibodies in diagnostic and especially in therapeutic applications in human beings. A single application of a murine antibody is usually sufficient to mount an immune response that will prevent subsequent applications to be effective. However, in the avidin-biotin pretargeting scheme a much larger immunogenicity problem is

caused by the avidin-component, which is very highly immunogenic in man.
A second problem is caused by the use of biotin, which is an endogenous factor (vitamin H) in the human body, especially in the liver. Injection with a conjugate with avidin, which has a very high affinity to biotin will lead to accumulation of the avidin-conjugate in the liver and other organs where biotin can be found.

Zinc fingers are parts of transcription factors and were firstly discovered in 1985 (Miller, J. et al., EMBO Journal, 4(6), 1609-1614, 1985). Zinc fingers are amino acid sequences of about 25-30 amino acids in a protein which form a tertiary structure able to chelate a zinc ion in a loop protruding from the rest of the protein. These zinc fingers are able to bind to a specific ds (double strand) DNA nucleotide sequence. One large group of molecules which naturally contain zinc fingers are steroid receptors. In each DNA binding domain of a steroid receptor two zinc fingers can be found which are able to bind to a genomic DNA sequence, generally consisting of about 15 nucleotides, which sequence is also known as hormone responsive element (HRE).

Zinc fingers of steroid receptors are relatively highly conserved sequences (Fuller, P.J., FASEB Journal, 5, 3092-3099, 1991): for all receptors across species, 18 of 68 amino acid residues are invariant and for human receptors this figure rises to 36 of 68. This conservation of structure extends to function; the DNA-binding domain interacts with hormone responsive elements which are enhancer elements in the regulatory (usually 5' flanking) region of the specific steroid-induced gene.
The conservation of the consensus sequence of the various HREs appears from the fact that in the mouse mammary tumor virus long-terminal repeat (MMTV-LTR) the same HRE is recognized by androgen, progesterone, glucocorticoid and

mineralocorticoid receptors. Several classes of HRE have been characterized; the GRE (glucocorticoid receptor responsive element) is, next to the glucocorticoid receptor DNA binding domain, also bound by the androgen, progesterone and mineralocorticoid receptor DNA binding domains. The estrogen responsive element (ERE) with the consensus sequence AGGTCAnnnTGACCT, can be converted to a functional HRE with minor base changes. The tyrosine response element, which also binds to the vitamin D receptor and the retinoic acid receptor, is the same as the ERE, only lacking the three intervening variable bases.

Variations from the above given consensus sequences can either increase or decrease the affinity of a response element for the receptor.

Next to the response elements which exert in vivo gene-activating effects also negative response elements have been found. They also bind to the DNA binding domain of the receptor albeit with a lower affinity than the positive HREs.

Our invention now is a kit, comprising a targeting conjugate comprising a targeting moiety that can bind to a target cell, and an effector conjugate which can bind to said targeting conjugate and which also comprises an effector moiety, characterised in that either the targeting moiety of the targeting conjugate or the effector moiety of the effector conjugate is conjugated to a zinc finger, while the other conjugate comprises a nucleotide sequence which can bind with the zinc finger.

The present invention proposes to administer to a patient a targeting conjugate in a dose, or in successive doses, sufficient to maximise target site accumulation of the targeting moiety. At some optimum time later, when most of the non-bound targeting moiety has been cleared, a therapeutic or diagnostic effector molecule carried by the

effector conjugate or a cross-reactive analogue thereof, is administered.

It is the property of a strong and specific binding of the zinc finger with the hormone responsive element that enables the use of this binding in the above-mentioned method of treatment. For the purpose of pretargeting it is irrelevant if the zinc finger is conjugated to the targeting moiety and the HRE to the effector moiety or just the other way round.

In the context of the present invention a zinc finger is defined as a peptide whose amino acid sequence comprises one or more than one amino acid sequence corresponding with a zinc binding domain, or a functional derivative of the zinc binding domain. As a functional derivative is considered any modified zinc finger sequence wherein the ability to bind to the hormone responsive element is retained.

Zinc fingers and their HRE are thus very useful for this kind of pretargeting methods on the basis of their high affinity, but they have also the disadvantage that both zinc finger and HRE are already endogenously present in the human body. However, this presence is only intracellularly in case of the zinc fingers or intranuclearly for the HRE. Interaction of conjugates with circulating, endogenous zinc fingers or HRE's will be minimal and only limited to cases of circulating cell components from degenerating cells.

Preferably, the zinc finger is derived from a steroid receptor. From most steroid receptors the amino acid sequence and the underlying DNA sequence for the transcription domain, which harbours the zinc fingers, is known (Fuller 1991, *supra*).

Conjugates of zinc finger with either targeting moiety or with effector moiety can be produced by normal chemical means, but it is also possible to make fusion proteins.

Chemical conjugation can, for example, be obtained through conjugation of the zinc finger to an antibody through a normal peptide bond or via disulphide or thiether bonds. Such conjugation techniques (and others) have been described in a review article of J. Goodchild, Bioconjug. Chem. 1, 165-187, 1990), which is encorporated herein by reference.

Fusion proteins can be made by standard biotechnological techniques. In short, DNA coding for the light and heavy chains of an antibody is cloned into a plasmid. DNA encoding the zinc finger is cloned in a position that enables it to be expressed as an extension of the Fc part of the heavy chain. Next, the plasmid is brought into a host capable of expressing and joining the light and heavy chains from this plasmid.

Although any pair of zinc finger and HRE is equally suited in the pretargeting scheme, preferably the glucocorticoid receptor and the corresponding GRE are used.

Preferably the nucleotide sequences constituting the HRE are chemically modified for protection against enzymatic degradation by nucleases. The main nuclease activity in the blood origins from 3'-exonucleases. Therefore, modification of the phosphodiester at the 3'-end of the oligonucleotide is indicated. This also enhances the stability of the nucleotide sequence.

When the oligonucleotide is bound to the targeting moiety it should be stable for quite a long period (2-3 days). Most commonly used for this are nucleotide analogues which are backbone-modified such as phosphorothioate, methylphosphonate and alkyl phosphotriester (inter alia phosphate-methylated DNA) oligonucleotides. In all these cases, however, introduction of modified phosphate groups should not lead to poorer solubility of the oligonucleotide in water or to a decrease of the stability of the complex with the zinc finger. Methylphosphonate linkage is most preferred because this can be easily introduced into DNA fragments using

standard (phosphoroamidite) DNA chemistry. Furthermore, this modification is stable towards the chemical conditions necessary for conjugations, and the introduction of neutral internucleoside linkages diminishes the non-specific binding of the highly charged oligonucleotides to positively charged blood proteins.
Partially phosphate-methylated DNA fragments can as well be applied.
These modified oligonucleotides and methods of preparing them are known in the art (Akzo Nobel, European Patent Appl. No. 0 490 434).
For diagnostic use unmodified double stranded DNA can be used as well.

Linking of these oligonucleotides to a targeting moiety or to an effector moiety is facilitated by introducing a 5'-5' linked uridine nucleotide. This uridine moiety offers, after activation of the cis diol, the possibility to incorporate various amino-containing linkers via reductive amination. Preparation of an antibody-oligonucleotide conjugate according to this method has also been described in EP-A 0 490 434.

Alternatively, at least one of the nucleotide strands is equipped at either the 3' or 5' end, with a functional group that allows for coupling to a targeting moiety or effector moiety. Such functional groups, like a thiol- group, a maleimido-group, a pyridyldithio-group or an amino-group, are well known in the art of conjugation chemistry. Preferably, an amino group is introduced at either end, or both ends, of a nucleotide strand, during or after the nucleotide synthesis process. This can, for instance, be done by esterification of the terminal phosphate groups with an amino-group containing alcohol, such as, for example 3-amino-2-hydroxy-propanol. The amino group can subsequently be derivatized with one of a number of bifunctional reagents that are known in the art for

introducing a functional group that is suitable for conjugation. Preferred bifunctional reagents are SPDP (3-(2-pyridyldithio)propionic acid N-hydroxy-succinimide ester), SMCC (N-succinimidyl-4-maleimidomethyl-cyclohexane-1-carboxylate), iminothiolane (Traut's reagent), 2-(acetyl-thio)succinic anhydride or SATA (S-acetylthioglycolic acid N-hydroxysuccinimide ester).
In a preferred embodiment the amino-group is derivatized with SPDP to introduce a pyridyldithio-group, which can subsequently be reduced, for instance with the use of dithioerythritol (DTE), dithiothreitol (DTT) or tributyl-phosphine, to a thiol group. The thiol-group containing nucleotide strand is then conjugated to a targeting or effector moiety that contains maleimido-groups. This method and further examples have been described in Kuijpers, W.H.A., Ph.D. dissertation, University of Leiden, 1993, which is incorporated herein by reference.

Targeting moieties which can be used in the present invention are compounds which after administration are predominantly found to be present at or in the neighbourhood of the target cells. For this purpose compounds which adhere to a structure present at the site of the target cells are especially useful. Such compounds can be specific antibodies, ligands or enzymes. Preferably an antibody is used as targeting moiety. Under the definition of antibody as used herein also parts of antibodies, and/or analogues of these antibodies or parts thereof should be comprised.
For tumor therapy antibodies directed to a tumor related epitope are preferred.

Monospecific antibodies to a tumor related epitope can be obtained by affinity purification from polyspecific antisera by a modification of the method of Hall et al. (Nature 311, 379-387 1984). Polyspecific antisera can be obtained by immunizing rabbits according to standard immunization

schemes. Monospecific antibody as used herein is defined as a single antibody species or multiple antibody species with homogeneous binding characteristics for the relevant antigen. Homogeneous binding as used herein refers to the ability of the antibody species to bind to a specific antigen or epitope.

The antibody is preferably a monoclonal antibody, more preferably a humanised or human monoclonal antibody.

Monoclonal antibodies can be prepared by immunizing inbred mice, preferably Balb/c with the appropriate protein by techniques known in the art (Köhler and Milstein, Nature 256; 495-497, 1975). Hybridoma cells are subsequently selected by growth in hypoxanthine, thymidine and aminopterin in an appropriate cell culture medium such as Dulbecco's modified Eagle's medium (DMEM). Antibody producing hybridomas are cloned, preferably using the soft agar technique of MacPherson, (Soft Agar Techniques, Tissue Culture Methods and Applications, Kruse and Paterson, eds., Academic Press, 276, 1973). Discrete colonies are transferred into individual wells of culture plates for cultivation in an appropriate culture medium. Antibody producing cells are identified by screening with the appropriate immunogen. Immunogen positive hybridoma cells are maintained by techniques known in the art. Specific anti-monoclonal antibodies are produced by cultivating the hybridomas in vitro or preparing ascites fluid in mice following hybridoma injection by procedures known in the art.

It is preferred to use human or humanized antibodies. Methods for humanizing antibodies, such as CDR-grafting, are known (Jones et al., Nature 321, 522-525, 1986). Another possibility to avoid antigenic response to antibodies reactive with polypeptides according to the invention is the use of human antibodies or fragments or derivatives thereof. Human antibodies can be produced by in vitro stimulation of isolated B-lymphocytes, or they can be isolated from (immortalized) B-lymphocytes which have been harvested from a

human being immunized with an autologous tumor cell vaccine (Haspel, M.V. et. al., Cancer Res. 45, 3951-3961, 1985).

A preferred human monoclonal antibody is MoAb 16-88 produced by a hybridoma cell line which is deposited under number HB 8495 with the American Type Culture Collection on January 30, 1984. Reactivity of this monoclonal antibody has been specified in US Patent No. 5,106,738.

The effector moiety can be either a therapeutic drug or a diagnostic compound. As a diagnostic compound preferably a gamma-emitter is used, such as ^{111}In or ^{131}I.
When using a therapeutic drug as effector moiety preferably a compound selected from the group consisting of α- and ß-emitting radioisotopes, boron addends and radiosensitizers is used. As radioisotopes preferably ^{90}Y, ^{131}I, ^{153}Sm, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi or ^{225}Ac are used.
It is also possible that a diagnostic compound is linked to the antibody of the targeting conjugate. Use of a diagnostic moiety linked to the targeting moiety enables the detection of the amount of targeting conjugate localized at the target site and detection of the amount of circulating or eliminated material. As a diagnostic compound preferably a γ-emitter is used, such as ^{99m}Tc, ^{111}In or ^{123}I.

Also part of the invention are effector conjugates for therapeutical use comprising a zinc finger. These zinc finger-conjugates can be used for bringing a therapeutic or diagnostic effector moiety to the cognate hormone responsive element of the zinc finger.
The effector moiety can be either a therapeutic drug or a diagnostic compound. As a diagnostic compound preferably a γ-emitter is used, such as ^{99m}Tc, ^{111}In or ^{123}I.
When using a therapeutic drug as effector moiety preferably a compound selected from the group consisting of α- and ß-emitting radioisotopes, boron addends and radiosensitizers

is used. As radioisotopes preferably ^{90}Y, ^{131}I, ^{153}Sm, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi or ^{225}Ac are used.

For pharmaceutical use the targeting conjugate and the effector conjugate should be in a pharmaceutically acceptable form, which may be administered parentally.
The useful dosage to be administered will vary depending on the age and the weight of the patient and on the mode of administration.
The conjugates are mixed with pharmaceutically suitable auxiliaries, pharmaceutically suitable liquids, and one or more pharmaceutically acceptable carriers, e.g. as described in the standard reference Gennaro et.al., Remington's Pharmaceutical Sciences (18th ed., Mack Publishing Co., 1990, see especially Part 8: pharmaceutical Preparations and Their Manufacture). The conjugates may be processed to an injection preparation in the form of a solution, or the conjugates may be processed to a preparation in lyophilized form. Examples of pharmaceutically acceptable carriers or diluents useful in the present invention include stabilizers such as carbohydrates (e.g. sorbitol, mannitol, starch, sucrose, glucose, trehalose, dextran), proteins such as albumin, preferably human serum albumin, scavengers for radiolysis, and buffers (e.g. phosphate buffer). In general any pharmaceutically acceptable additive which does not interfere with the function of the active compounds can be used.

Kits and conjugates according to the present invention can be used for the treatment and detection of tissue specific cell disorders such as cancer, auto-immune diseases, antiviral diseases and inflammatory diseases.

The present invention is further described by way of example with reference to the accompanying figures, in which:

Fig. 1 shows chemical linkage possibilities to 3'- and 5'-end modified synthetic ologonucleotide sequences for introduction of (radio)labels or -SH-groups for conjugation purposes.

Fig. 2 shows the pretargeting of a zinc finger. Microtiter plate wells were coated with recombinant DNA zinc finger DBD-93. Specific reaction of the zinc finger with increasing concentrations of a conjugate of double-stranded DNA and monoclonal IgM antibody 16-88 is shown.

In Fig. 2a detection of antibody 16-88 is obtained with the cognate antigen of MoAb 16-88, designated CTA1, coupled to horse radish peroxidase (HRP) which is detected.

In Fig. 2b detection of the antibody is obtained by binding to goat-antihuman-IgM which is labelled with HRP.

Fig. 3 shows a dot-blot of the same reactions.

EXAMPLE 1

A: Hormone responsive element (HRE).

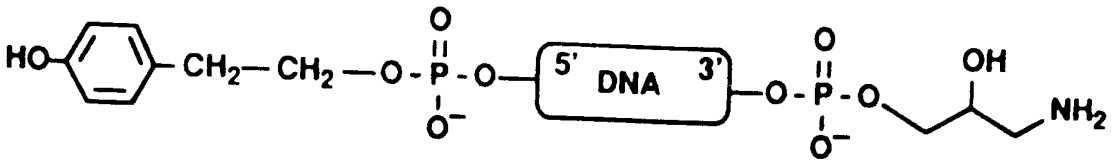
The nucleotide sequence and chemical structure of the ds DNA strands representing the hormone-responsive element (HRE) of the human glucocorticoid receptor (which is also presented in SEQ ID NO:1) are as follows:

Nucleotide sequences:

DNA 5' CAAAGTCAGAACACAGTCTTCTGATCAAGA 3' (A)

DNA 5' TCTTGATCAGAACACTGTGTTCTGACTTTG 3' (B)

Chemical structure:



Both complementary strands A and B contain natural phosphodiester linkages, and were protected at the 3'-end against nuclease degradation by the introduction of a phosphopropylamino (-O-PO_2-O-CH_2-CHOH-CH_2-NH_2) group. According to literature (Zendegui, J.G, et. al., Nucleic Acids Research, 20, 307-314, 1992) this group offers sufficient protection against exonucleases. Moreover, the terminal amino-group serves as a selective site for labeling (attachment of a diagnostic or therapeutic effector molecule), and a site through which the oligonucleotide can be conjugated to a targeting moiety, such as an antibody.

The oligonucleotides were prepared by automated synthesis on a CPG (controlled glass support) support (P.N. Nelson et.al. Nucleic Acid Research 17, 7189, 1989) using a trifluoroacetyl amino protecting group instead of the described fluorenyl-

methyloxycarbonyl (Fmoc) group, and using otherwise standard DNA-synthesizing conditions. The p-hydroxyphenylethyl moiety at the 5'-end of both strands A and B, was introduced in the last synthetic cycle using the appropriate phosphoramidite. Removal of the synthetic oligonucleotides from the support was achieved by treatment with liquid ammonia.

A second set of complementary oligonucleotides, which do not correspond with a hormone responsive element and which is referred to as irrelevant ds-DNA in the binding assays described under D, were prepared and derivatized at the 3' and 5' ends as described above for the HRE. The sequences of the complementary strands of irrelevant ds-DNA are:

DNA 5' GCGCCTTGCGCCGGC 3' (SEQ. ID. NO. 3) and
DNA 5' GCCGGCGCAAGGCGC 3'

B: HRE-antibody conjugate

Conjugates of the HRE under A and the human monoclonal antibody 16.88 were prepared as follows:
Strand A was biotinylated at the 3'-end with NHS-biotin (N-hydroxysuccinimido-biotin) at a molar DNA/biotin ratio of 1:30 in sodium phosphate buffer pH 8.5. Excess NHS-biotin was removed by ion-exchange chromatography on Q-Sepharose. The fraction eluted at 0.6 mol/l NaCl in phosphate buffer represented the biotinylated oligonucleotide A. To this solution, an equal amount of strand B was added and the mixture was heated for 5 min at 100° au bain marie and allowed to cool down to ambient temperature. The dsDNA thus formed was incubated with SPDP at a 30 molar excess for 30 min. By this derivatization a dithiopyridyl-group is introduced at the 3'-end of the B strand of the doublestrand oligonucleotide. Subsequently, dithiothreitol was added to a final concentration of 25 mmol/l and incubation was continued for 10 min. The reaction mixture was diluted tenfold with

phosphate buffer pH 8.5 and applied on a Q-Sepharose column equilibrated in phosphate buffer. The absorbance of the eluate was monitored at 254 nm. The column was washed with buffer till the absorbance reached baseline level and the bound ds-DNA, derivatized at the 3'-end of the B strand with a 3-mercaptopropionyl group, was eluted with 0.6 mol/l NaCl in phosphate buffer. This eluate was added to a solution of MoAb 16.88 that had been reacted with a 1:16 molar excess of GMBS (γ-maleimidobutyric acid N-hydroxy succinimide ester) which resulted in the introduction of 6 moles of maleimido groups per mole antibody. Maleimido-derivatized antibody and mercaptopropionyl-derivatized dsDNA were mixed at a 1:10 molar ratio and incubation was performed overnight at 4°C. The MoAb-DNA conjugate was purified by precipitation with ammonium sulphate at 50% saturation and washed three times with 50% ammonium sulphate and was dissolved in PBS. Residual $(NH_4)_2SO_4$ was removed by gel filtration on a PD10 column equilibrated in PBS (phosphate buffered saline).

The same procedure was used for the preparation of the irrelevant ds-DNA and the antibody 16-88.

C: Zincfinger.

A recombinant (expressed in Escherichia Coli) protein fragment, a peptide of 93 residues the sequence of which is represented in SEQ. ID. NO. 2, containing the DNA-binding domain (DBD) of the human glucocorticoid receptor (GR) was purified and checked for DNA-binding activity as described by Freedman et al. (Nature, 334, 543-546, 1988). The peptide is referred to as DBD-93 by Härd et.al. (Biochemistry, 29, 9015-9023, 1990; see Fig.1) and contains an 80 amino acid segment - the sequence 6-85 of SEQ. ID. NO. 2 - of the human GR (the rat GR contains an identical sequence). The peptide further contains an N-terminal linker sequence (1-5 of SEQ. ID. NO. 2) and a C-terminal linker sequence (92-93 of SEQ. ID. NO. 2)

which do not belong to the native GR, while the sequence 86-91 (SEQ. ID. NO. 2) of the peptide corresponds to the the unique rat sequence. This zinc-finger peptide contains two zinc-finger domains, corresponding with the segments 6-26 and 42-61 of SEQ. ID. NO. 2, respectively.

D: Binding assays.

The zinc finger (ZnF) peptide DBD-93, described under C, was adsorbed overnight to the wells of a microtitre plate at an initial concentration of 1 µg/ml in PBS.
Excess binding sites were blocked with a 50 g/l skim milk suspension (Difco) in PBS for 1 h at 37°C. Subsequently a dilution series of a 16.88 HRE conjugate, of a 16.88 irrelevant dsDNA conjugate and of the unmodified MoAb 16.88 were pipetted into the wells of the Zn-finger coated microtitre plates and incubated for 1h at 37°C.
After washing the plates three times with PBS.containing Tween-20, binding of MoAb 16.88 was assessed by incubation with a goat-anti-human IgM-HRP or antigen-HRP conjugate, followed by end point determination with TMB (tetra methyl benzidine)/H_2O_2.

The results are presented in Figure 2a and 2b. A positive reaction is observed only when the MoAb 16.88-HRE conjugate was employed indicating a highly specific interaction between ZnF and HRE with high affinity.

Similar results were obtained in a dot-blot ELISA where zinc finger DBD-93 was bound to a PVDF membrane and the same reaction steps were carried out as described for the microtitre plate ELISA. End point determination was carried out with a DAB(diamino benzidine)/$CaCl_2$/H_2O_2 substrate solution. The results are shown in Figure 3.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

(A) NAME: Akzo Nobel N.V.

(B) STREET: Velperweg 76

(C) CITY: Arnhem

(E) COUNTRY: The Netherlands

(F) POSTAL CODE (ZIP): 6824 BM

(G) TELEPHONE: 04120-66380

(H) TELEFAX: 04120-50592

(I) TELEX: 37503 akpha nl

(ii) TITLE OF INVENTION: Kit for pretargeting and novel pretargeting conjugates

(iii) NUMBER OF SEQUENCES: 3

(iv) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 30 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: protein_bind
(B) LOCATION:9..22
(C) IDENTIFICATION METHOD: experimental
(D) OTHER INFORMATION:/bound_moiety= "zinc finger"
/evidence= EXPERIMENTAL
/standard_name= "hormone responsive element"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

CAAAGTCAGA ACACAGTCTT CTGATCAAGA

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 93 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(ix) FEATURE:
(A) NAME/KEY: Binding-site
(B) LOCATION:6..26
(D) OTHER INFORMATION:/label= Znfinger-domain
(ix) FEATURE:

(A) NAME/KEY: Binding-site
(B) LOCATION:42..61
(D) OTHER INFORMATION:/label= Znfinger-domain

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION:1..5
(D) OTHER INFORMATION:/label= linker
/note= "non-human sequence"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION:92..93
(D) OTHER INFORMATION:/label= linker
/note= "non-human sequence"

(ix) FEATURE:
(A) NAME/KEY: Region
(B) LOCATION:86..92
(D) OTHER INFORMATION:/note= "unique rat GR sequence"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Lys Pro Ala Arg Pro Cys Leu Val Cys Ser Asp Glu Ala Ser Gly Cys
1 5 10 15
His Tyr Gly Val Leu Thr Cys Gly Ser Cys Lys Val Phe Phe Lys Arg
20 25 30
Ala Val Glu Gly Gln His Asn Tyr Leu Cys Ala Gly Arg Asn Asp Cys
35 40 45
Ile Ile Asp Lys Ile Arg Arg Lys Asn Cys Pro Ala Cys Arg Tyr Arg
50 55 60
Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys Lys
65 70 75 80
Lys Ile Lys Gly Ile Gln Gln Ala Thr Ala Gly Arg Leu
85 90

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid
(A) DESCRIPTION: /desc = "synthetic DNA"

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

GCGCCTTGCG CCGGC

CLAIMS

1. A kit, comprising
   a) a targeting conjugate comprising a targeting moiety that can bind to a target cell, and
   b) an effector conjugate which can bind to said targeting conjugate and which also comprises an effector moiety,

   characterised in that either the targeting moiety of the targeting conjugate or the effector moiety of the effector conjugate is conjugated to a zinc finger, while the other conjugate comprises a nucleotide sequence which can bind the zinc finger.

2. A kit according to claim 1, characterised in that the zinc finger is derived from a steroid receptor.

3. A kit according to claim 2, characterized in that the zinc finger comprises a peptide having the sequence of SEQ. ID.NO. 2.

4. A kit according to claim 2 or 3, characterized in that the nucleotide sequence comprises a glucocorticoid receptor responsive element.

5. A kit according to claim 4, characterized in that the nucleic acid sequence comprises the sequence shown in SEQ. ID. No. 1.

6. A kit according to any of claims 1 to 5, characterised in that the nucleotide sequence comprises a chemically modified nucleotide structure.

7. A kit according to any of the claims 1 to 6, characterised in that the targeting moiety is an antibody, preferably a human antibody, more preferably a human monoclonal antibody, most preferably the human monoclonal antibody 16-88.

8. A kit according to any of the claims 1 to 7, characterised in that the effector moiety is a diagnostic agent, preferably a γ-emitting radioisotope, more preferably selected from the group consisting of ^{99m}Tc, ^{111}In and ^{123}I

9. A kit according to any of the claims 1 to 7, characterised in that the effector moiety is a therapeutic agent, preferably. selected from the group consisting of ß- and α-emitting radioisotopes, boron addends and radiosensitizers, most preferably selected from the group consisting of ^{90}Y, ^{131}I, ^{153}Sm, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi and ^{225}Ac

10. A kit comprising an antibody and an effector moiety according to claim 9, characterized in that a diagnostic agent is attached to said antibody.

11. A kit according to claim 10, characterised in that said diagnostic agent is a γ-emitting radioisotope, preferably selected from the group consisting of ^{111}In and ^{131}I.

12. A conjugate of an antibody and a zinc finger.

13. A conjugate of an effector moiety and a zinc finger.

14. A conjugate according to claim 13, characterised in that said effector moiety is a therapeutic agent, preferably selected from the group consisting of ß- or α-emitting radioisotopes, boron addends and radiosensitizers, more preferably selected from the group consisting of ^{90}Y, ^{131}I, ^{153}Sm, ^{186}Re, ^{188}Re, ^{211}At, ^{212}Bi, ^{213}Bi and ^{225}Ac.

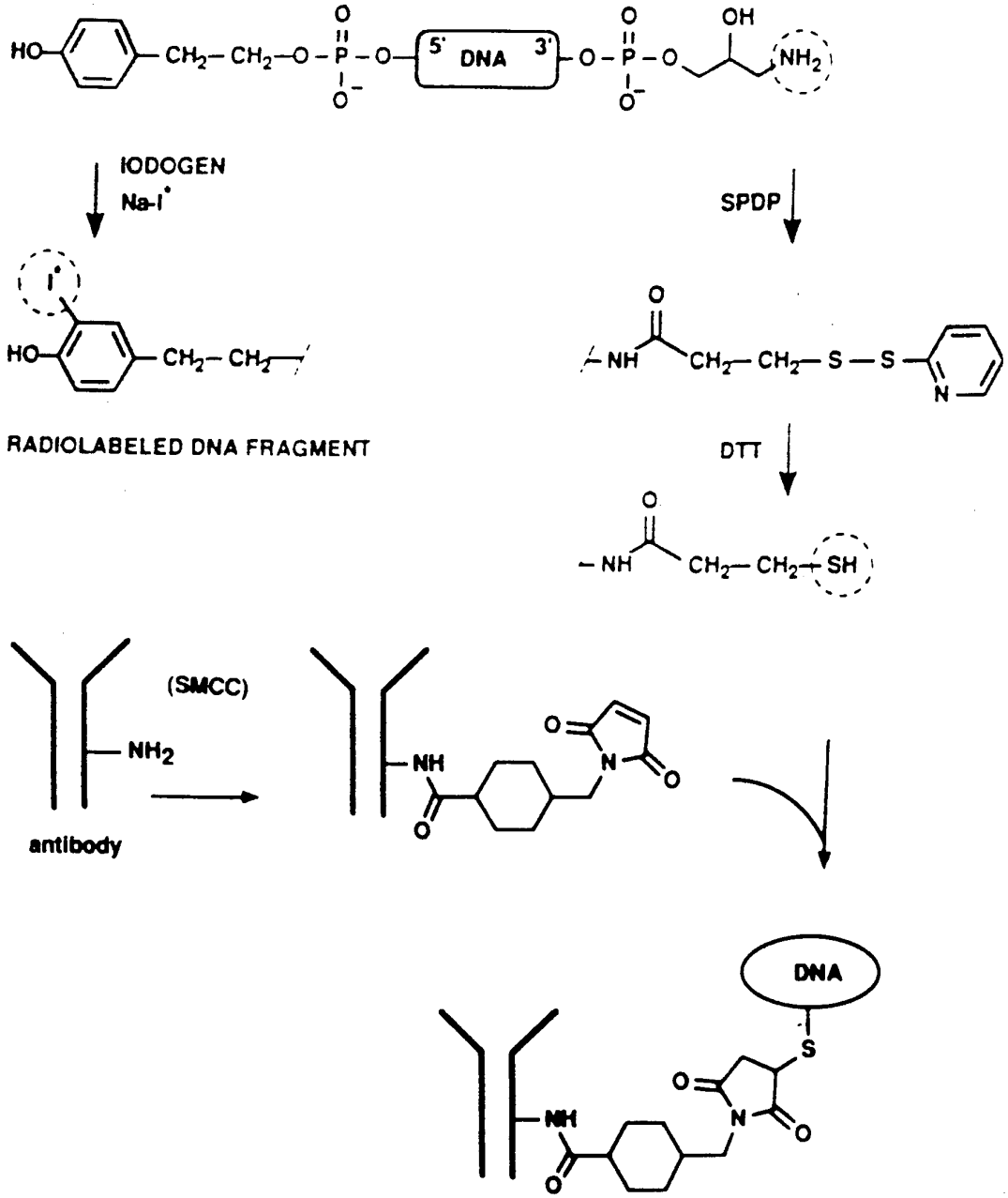
15. A conjugate according to claim 13, characterised in that the effector moiety is a diagnostic agent, preferably a γ-emitting radioisotope, more preferably selected from the group consisting of ^{99m}Tc, ^{111}In and ^{123}I

16. Pharmaceutical preparation comprising a conjugate according to any of the claims 12-15 and a pharmaceutical acceptable carrier.

17. Use of a conjugate accordig to any of the claims 13-15 for the manufacture of a pharmaceutical preparation for treatment of tissue specific cell disorders, preferably selected from the group consisting of cancer and inflammatory diseases.

FIGURE 1

5'- AND 3'-END MODIFICATIONS OF SYNTHETIC OLIGONUCLEOTIDES



CONJUGATION WITH ANTIBODY

FIG. 2A

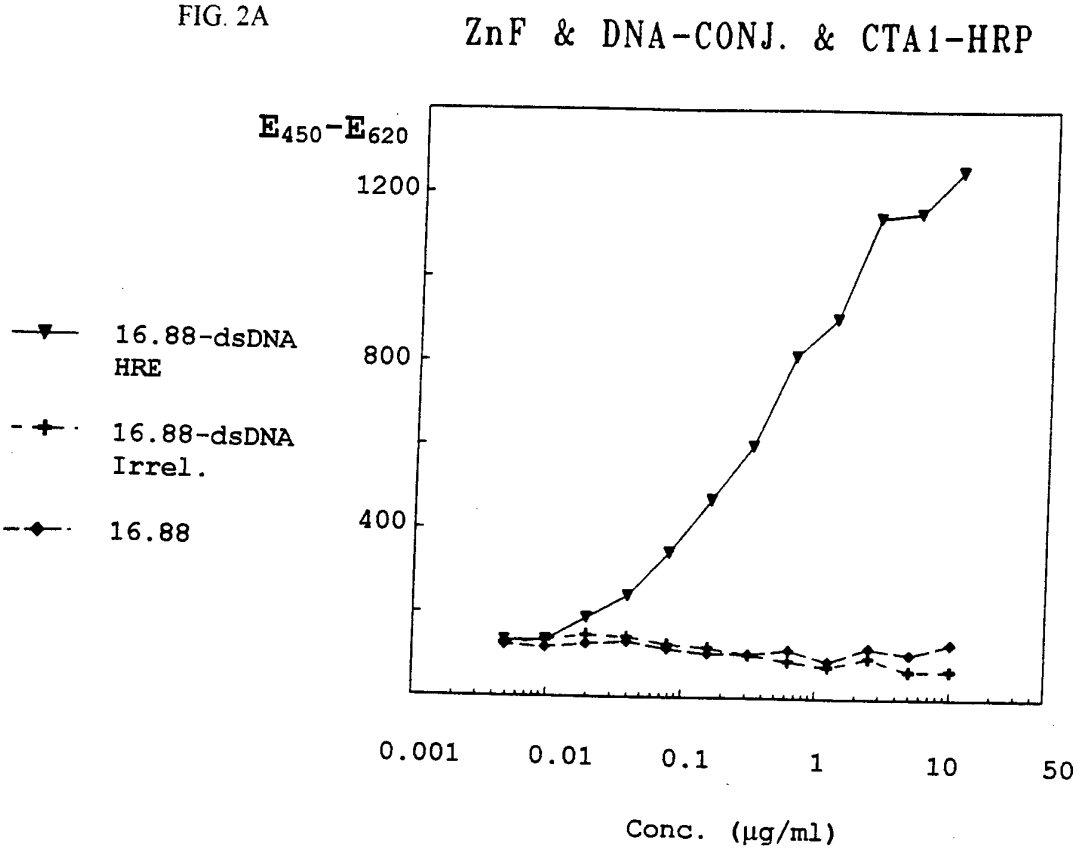

ZnF & DNA-CONJ. & CTA1-HRP
E450-E620
1200
800
400
16.88-dsDNA HRE
16.88-dsDNA Irrel.
16.88
0.001
0.01
0.1
1
10
50
Conc. (µg/ml)

FIG. 2B

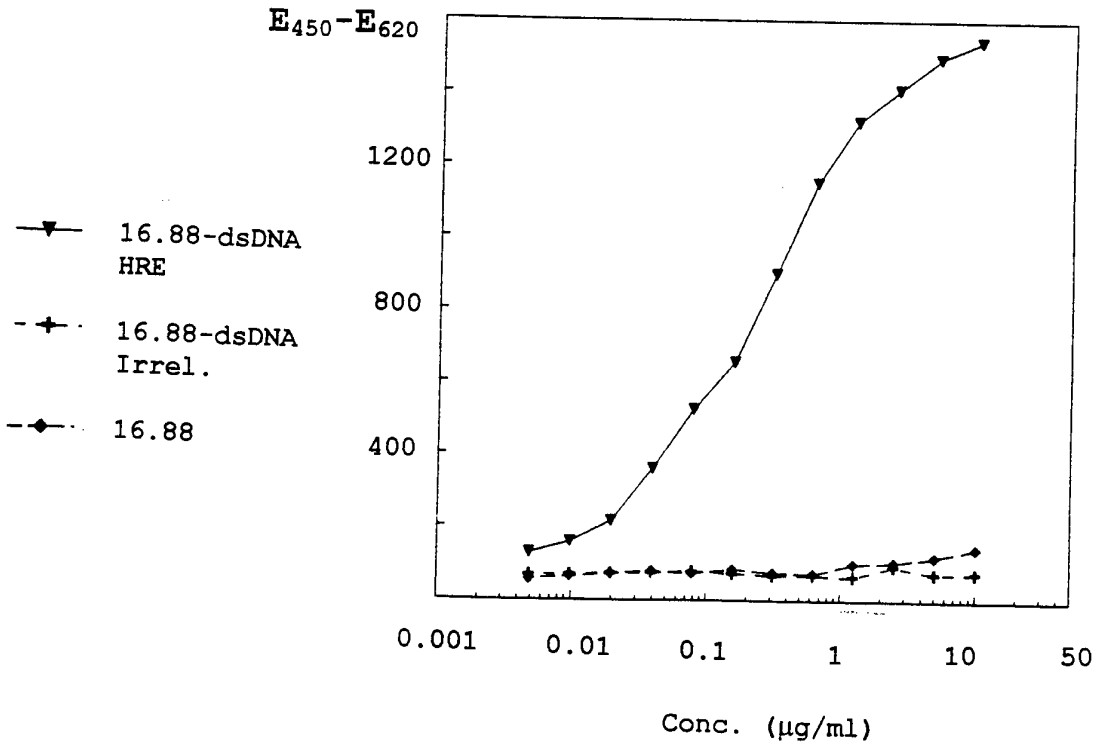
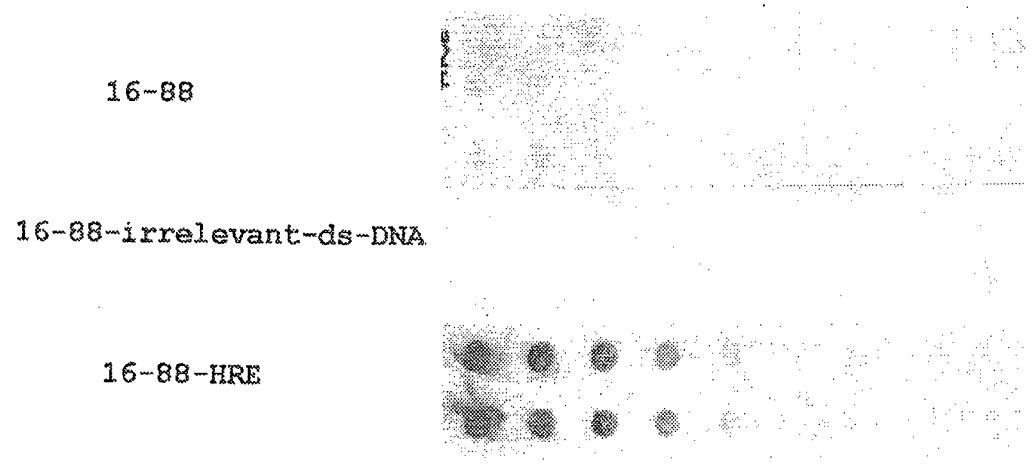
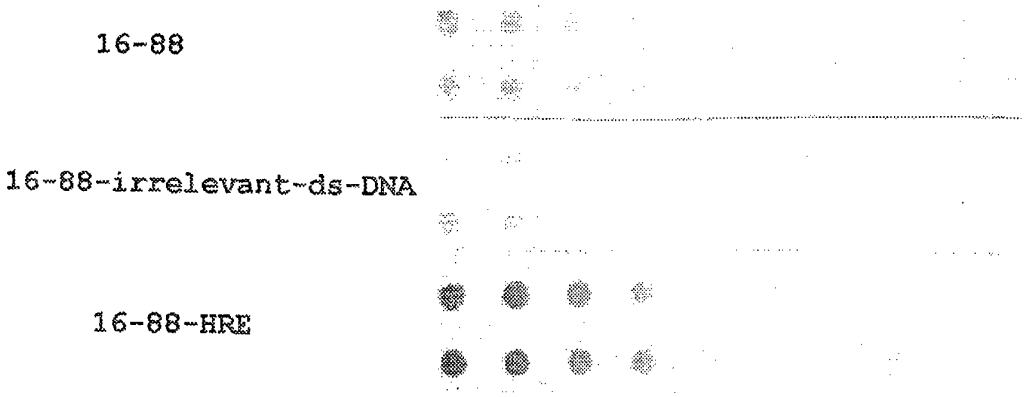

ZnF & DNA-CONJ. & GaHuIgM-HRP
E450-E620
1200
800
400
16.88-dsDNA HRE
16.88-dsDNA Irrel.
16.88
0.001
0.01
0.1
1
10
50
Conc. (µg/ml)

FIGURE 3



Detection: antigen-HRP conjugate



Detection: GαHuIgM-HRP conjugate