# ABERRANT CELL-RESTRICTED IMMUNOGLOBULINS PROVIDED WITH A TOXIC MOIETY

## TECHNICAL FIELD

The invention relates to the field of biotherapeutics. More specifically, the invention relates to immunoglobulins provided with a toxic moiety. Even more specifically, the invention relates to human antibodies. The invention also relates to the use of these biotherapeutics in the treatment of a host suffering from a disease associated with aberrant cells, such as cancers and autoimmune diseases.

## BACKGROUND

The development of immunoglobulin-drug conjugates is one of the drug development fields that receives high attention nowadays. Humanized or human antibodies are the largest and most important class of immunoglobulins under investigation for use in antibody-drug conjugates (ADCs) and in immunotoxins and antibody-radionuclide conjugates. These antibodies target binding sites (over)expressed at aberrant cells, such as those exposed in cancers and (auto)immune diseases, and during infections. Many of the conjugates have a limited degree of efficacy. For example, the maximum tolerated dose of immunotoxins is relatively low due to their toxicity towards healthy tissue. Lowering the dose is one way of protecting healthy cells for the non-specific toxic activity of the toxin or the drug in ADCs. Lowering the dose, however, hampers the delivery of an efficacious amount of conjugate at the site of for example a tumor. The unwanted side reactions are mainly due to the targeting of the antibodies to binding sites that are not exclusively exposed by aberrant cells but also to some extent by healthy cells. Thus, insufficient specificity for aberrant cells over healthy cells hampers desired efficacy and hampers obtaining the desired safety profiles of the nowadays immunoglobulin-drug conjugates.

Toxic moieties currently in the clinic or under investigation are numerous and diverse [6]. Amongst the first toxins that were chemically linked to murine antibodies are plant derived protein toxins and bacterial toxins such as saporin, Diphtheria toxin,

Pseudomonas exotoxin, gelonin, ricin, ricin A chain, abrin and pokeweed antiviral protein. Other immunoglobulins provided with a toxin moiety comprise single chain Fv fused at the DNA level with toxins. An example is the recombinant protein BL22 consisting of the Fv portion of an anti-human CD22 antibody fused to a fragment of Pseudomonas exotoxin-A, that targets B-cell malignancies such as hairy cell leukemia and non-Hodgkin's lymphoma. Other examples of immunoglobulins conjugated to toxins are the antibody-radionuclide conjugates. Human CD20 has been chosen by drug developers as the target for two monoclonal antibodies, conjugated with 90-Yttrium or with 131-Iodine, for treatment of non-Hodgkin's lymphomas. In attempts to improve the tumor selectivity of certain drugs, murine monoclonal antibodies were conjugated to compounds such as doxorubicin, vinblastine, methotrexate, providing so-called antibody-drug conjugates. Insufficient tumor cell specificity however still limited the therapeutic usefulness. Even when selecting tumor cell surface antigens that are (highly) over-expressed at aberrant cells, still the low expression levels at healthy cells gives rise to insufficient selectivity of the antibody-drug conjugates. Current cytotoxic anti-tumor drugs under investigation are for example maytansinoids and dolastatin analogs, that both target intracellular tubulin, and duocarmycins and calicheamicins, that target DNA structure. These compounds are potent in their cytotoxic activtiy, though not selective for aberrant cells. Antibiotic calicheamicin conjugated to an anti-human CD33 monoclonal antibody was approved and used in the clinic, but was withdrawn due to serious side effects. Additional examples of drugs currently under investigation for their potential beneficial use in antibody-drug conjugates meant for the treatment of cellular aberrancies are ozogamicin, hydrazone-calicheamicin, vedotin, emtansine, mertansine. These toxic moieties are conjugated to immunoglobulins targeting cell surface markers expressed at tumor cells, though also expressed to some extent at healthy cells. Typical examples of immunoglobulin-drug conjugate-targeted cell surface markers present at both tumor cells and healthy cells are CD19, CD20, CD22, CD25, CD30, CD33, CD56, CD70, HER2/neu. All these immunoglobulin-drug conjugate development programs thus inherently bear the risk for unacceptable safety profiles and consequent poor efficacy due to low maximum tolerated doses. Conjugating drugs, radionuclides or toxins to immunoglobulins specifically and selectively targeting aberrant cells and not targeting healthy cells would thus provide

for therapies with improved specificity and selectivity for aberrant cells and with an improved safety profile.

## DISCLOSURE OF THE INVENTION

Specific and selective delivery of a toxic moiety in target aberrant cells demands for binding molecules specific for binding sites preferentially associated with aberrant cells. These binding molecules then are used as carriers and transporters of the toxic moieties, specifically and selectively delivering the toxic moieties at and in the aberrant cells. We here disclose immunoglobulin-drug conjugates comprising these preferred features. The immunoglobulins in the immunoglobulin-drug conjugates of the invention comprise immunoglobulin binding regions with improved selectivity for aberrant cells by specifically binding to binding sites preferentially associated with these aberrant cells. We disclose as preferred targets for the antibody of the invention, intracellular proteins that are associated with aberrant cells. These proteins are available as peptides presented by MHC on the surface of aberrant cells. The use of MHC-peptide complexes as targets opens us a new field of tumor targets, because so far typically targets associated with the surface of aberrant cells have been envisaged. Although it is preferred that the target is specific for aberrant cells (tumor cells) in many cases upregulated intracellular proteins are also suitable for at least improving the therapeutic window of immunotoxins. Our most preferred targets are peptides derived from MAGE presented in the context of MHC-1. In particular MAGE peptides that are present in more than one MAGE protein (multi-MAGE epitope; see WO2012/091564 incorporated herein by reference). The toxic moiety according to the invention is preferably a drug compound, a radionuclide or a toxin. The toxic moiety according to the invention is a non-proteinaceous molecule or a proteinaceous molecule. In the immunoglobulin-drug conjugates of the invention, the toxic moiety is preferably conjugated by chemical conjugation. Also preferred are immunoglobulins of the invention fused at the DNA level to a proteinaceous toxic moiety.

The immunoglobulins in the immunoglobulin-drug conjugates of the invention are suitable for the specific and selective localization of a toxic effect inside targeted aberrant cells, leaving healthy cells essentially unaffected. Immunoglobulins comprise

immunoglobulin binding domains, referred to as immunoglobulin *variable domains*, comprising immunoglobulin variable regions. Maturation of immunoglobulin variable regions results in variable domains adapted for specific binding to a target binding site. Immunoglobulins are therefore particularly suitable for providing the immunoglobulin-drug conjugates of the invention with the ability to specifically and selectively target aberrant cells. At their surface, aberrant cells present aberrant cell-associated antigen peptides in the context of major histocompatibility complex (MHC). Therefore, for the immunoglobulins in the immunoglobulin-drug conjugates of the invention, aberrant cell-associated MHC-1 peptide complexes are a preferred target on aberrant cells. In addition, aberrant cell-associated MHC-2 peptide complexes are valuable targets on e.g. tumors of hematopoietic origin, for the immunoglobulins in the immunoglobulin-drug conjugates of the invention. The present invention therefore provides immunoglobulins in immunoglobulin-drug conjugates, with improved specificity and selectivity for aberrant cells by targeting MHC-peptide complexes which are preferentially associated with aberrant cells. This improved specificity and selectivity for aberrant cells is accompanied with a reduced level of unintentional targeting of healthy cells by the immunoglobulins in the immunoglobulin-drug conjugates of the invention. Most preferably, healthy cells are not targeted by the immunoglobulin-drug conjugates of the invention. Thus, in a first embodiment the invention provides an immunoglobulin provided with a toxic moiety, comprising at least an immunoglobulin variable region that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells. Preferred immunoglobulins of the invention are antibodies, but fragments and/or derivatives such as Fab and/or ScFv can also be used. Even more preferred immunoglobulins of the invention are antibodies of the immunoglobulin G (IgG) type. Other immunoglobulins of the invention are for example heavy-chain (only) antibodies comprising Vh or Vhh and IgA, and their fragments such as Fab fragments, and Fab fragments of IgG's. Immunoglobulins bind via their immunoglobulin variable regions to binding sites on molecules, such as epitopes, with a higher binding affinity than background interactions between molecules. In the context of the invention, background interactions are typically interactions with an affinity lower than a $K_D$ of 10E-4 M. Immunoglobulin variable domains in light chains (Vl) and immunoglobulin variable domains in heavy chains (Vh) of antibodies typically comprise the aberrant-cell specific immunoglobulin

variable regions of the invention. Thus, in one embodiment the invention provides an immunoglobulin provided with a toxic moiety, comprising at least an immunoglobulin variable region, wherein said immunoglobulin variable region is a Vh(h) that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells. Thus, in yet another embodiment the invention also provides an immunoglobulin provided with a toxic moiety, comprising at least an immunoglobulin variable region, wherein said immunoglobulin variable region is a Vh that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells, and wherein said immunoglobulin variable region further comprises a Vl.

As said, immunoglobulins G are particularly suitable binding molecules for use in therapies specifically and selectively targeting aberrant cells, for site-specific delivery of a toxic moiety according to the invention. Because the anticipated predominant use of the antibodies of the invention is in therapeutic treatment regimes meant for the human body, in a particular embodiment of the invention, the immunoglobulins provided with a toxic moiety have an amino-acid sequence of human origin. Thus, in one embodiment the invention provides a human IgG provided with a toxic moiety, comprising at least an immunoglobulin variable region, wherein said immunoglobulin variable region is a Vh that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells, and wherein said immunoglobulin variable region further comprises a Vl. Of course, humanized antibodies, with the precursor antibodies encompassing amino acid sequences originating from other species than human, are also part of the invention. Also part of the invention are chimeric antibodies, comprising (parts of) an immunoglobulin variable region according to the invention originating from a species other than human, and grafted onto a human antibody.

An *aberrant cell* is defined as a cell that deviates from its healthy normal counterparts. Aberrant cells are for example tumor cells, cells invaded by a pathogen such as a virus, and autoimmune cells.

Thus, in one embodiment the invention provides an immunoglobulin according to any of the aforementioned embodiments wherein the MHC-peptide complex is specific for aberrant cells.

In the molecules of the invention the toxic moieties are preferably chemically linked to the immunoglobulins via any linker chemistry know in the art, and optionally via an additional spacer. According to the invention, one or several, preferably two to six toxic moiety molecules are chemically linked to an immunoglobulin molecule of the invention. The number of conjugated toxic moiety molecules per single immunoglobulin molecule is restricted by boundaries such as the number of available sites for conjugation on the immunoglobulin, the stability of the conjugate, the preservation of the ability of the immunoglobulin to specifically bind to an aberrant cell, etc. Of course, also two, three, etc. different toxic moieties can be linked to an immunoglobulin, depending amongst others on available binding sites and the applied linker chemistry. Chemical linking of the toxic moieties has several advantages when working with immunoglobulins. This way, toxic moieties cannot interfere with expression, folding, assembly and secretion of the immunoglobulin molecules. Thus, in one embodiment the invention provides an immunoglobulin according to any of the aforementioned embodiments wherein the toxic moiety is chemically linked to the immunoglobulin. It is then also part of the current invention that toxic moieties are covalently bound via peptide bonds, and preferably via a peptide linker, to the immunoglobulins of the invention. The toxic moiety and the immunoglobulin are then fused at the DNA level. Thus, in one embodiment the invention provides an immunoglobulin according to any of the aforementioned embodiments wherein the toxic moiety is a protein, preferably fused to the immunoglobulin at the DNA level, preferably through a linker sequence. In many instances a simple Gly-Ser linker of 4-15 amino-acid residues may suffice, but if greater flexibility between the immunoglobulin and the toxic moiety is desired longer or more complex linkers may be used. Preferred linkers are $(Gly_4Ser)_n$, $(GlySerThrSerGlySer)_n$, GlySerThrSerGlySerGlyLysProGlySerGlyGluGlySerThrLysGly, GlyPheAlaLysThrThrAlaProSerValTyrProLeuAlaProValLeuGluSerSerGlySerGly or any other linker that provides flexibility allowing protein folding, stability against

undesired proteolytic activity and flexibility for the immunoglobulins of the invention to exert their activity. Another group of preferred linkers are linkers based on hinge regions of immunoglobulins. These linkers tend to be quite flexible and quite resistant to proteases. The most preferred linkers based on hinge regions are GluProLysSerCysAspLysThrHisThr (linking Ch1 and Ch2 in IgG1), GluLeuLysThrProLeuGlyAspThrThrHisThr (IgG3), and GluSerLysTyrGlyProPro (IgG4). Thus, the role of any applied chemical linker in conjugates according to the invention or the role of any applied peptide linker in fused molecules according to the invention is aiding the dual activity of the antibodies of the invention, i.e. specific and selective binding of the immunoglobulin to aberrant cells, and subsequent delivery of at least the toxic moiety in the targeted aberrant cells. Thus, in one embodiment the invention provides the use of an immunoglobulin provided with a toxic moiety according to any of the aforementioned embodiments, for the treatment of a host suffering from a disease associated with aberrant cells. In a further embodiment the invention provides the use of an immunoglobulin provided with a toxic moiety according to any of the aforementioned embodiments, for the treatment of a host suffering from a disease associated with aberrant cells wherein at least the toxic moiety is internalized into the aberrant cell. According to the invention, the immunoglobulins provided with a toxic moiety are for example used for the treatment of cancer. Thus, in a preferred embodiment the invention provides an immunoglobulin provided with a toxic moiety according to any of the aforementioned embodiments for use in the treatment of cancer.

Preferred toxic moieties according to the invention are numerous. Several examples of preferred toxic moieties according to the invention are drugs such as doxorubicin, cisplatin, carboplatin, vinblastine, methotrexate, chelated radioactive metal ions, (synthetic) antineoplastic agents such as monomethyl auristatin E, radioactive iodine, radionuclides such as 90-Yttrium, 131-Iodine, to name a few, which are chemically conjugated to the immunoglobulins of the invention. Also preferred toxic moieties according to the invention are proteinaceous toxins such as a fragment of Pseudomonas exotoxin-A, statins, ricin A, gelonin, saporin, interleukin-2, interleukin-12, viral proteins E4orf4, apoptin and NS1, and non-viral proteins HAMLET, TRAIL and mda-7. Thus, in one embodiment of the invention, antibodies are provided for the specific

targeting of aberrant cells, wherein the toxic moiety is selected from the list of available toxic moieties comprising toxins such as a fragment of Pseudomonas exotoxin-A, statins, chelated radioactive metal ions, radioactive iodine, ricin A, gelonin, saporin, interleukin-2, interleukin-12, radionuclides such as 90-Yttrium, 131-Iodine, drugs such as doxorubicin, taxol or derivatives, 5-FU, anthracyclines, vinca alkaloids, calicheamicins, cisplatin, carboplatin, vinblastine, methotrexate, (synthetic) antineoplastic agents such as monomethyl auristatin E, apoptin, parvovirus-H1 NS1 protein, E4orf4, TRAIL, mda-7, HAMLET.

According to the invention *proteinaceous* molecules are molecules comprising at least a string of amino acid residues. In addition, according to the invention the proteinaceous molecules may comprise carbohydrates, disulphide bonds, phosphorylations, sulphatations, etc.

When antibodies of the invention are designed to first bind to a target aberrant cell, followed by internalization, the toxic moiety can then subsequently have its intracellular (cytotoxic) function, i.e. inducing apoptosis.

For administration to subjects the antibodies of the invention must be formulated. Typically these antibodies will be given parenterally. For formulation simply water (saline) for injection may suffice. For stability reasons more complex formulations may be necessary. The invention contemplates lyophilized compositions as well as liquid compositions, provided with the usual additives. Thus, in one embodiment the invention provides a pharmaceutical composition comprising an immunoglobulin provided with a toxic moiety according to any of the aforementioned embodiments and suitable diluents and/or excipients.

The dosage of the antibodies of the invention must be established through animal studies, (cell-based) *in vitro* studies and clinical studies in so-called rising-dose experiments. Typically, the doses will be comparable with present day antibody dosages (at the molar level). Typically, such dosages are 3-15 mg/kg body weight, or 25-1000 mg per dose.

In addition, especially in the more difficult to treat cellular aberrancies the first applications of the antibodies of the invention will (at least initially) probably take

place in combination with other treatments (standard care). Of course, the invention also provides antibodies for use in novel or first treatments of any malignancy accompanied by the occurrence of aberrant cells, for which current treatments are not efficient enough or for which currently no treatment options are available. Thus, for example the invention also provides a pharmaceutical composition comprising an invented immunoglobulin provided with a toxic moiety and a conventional cytostatic and/or tumoricidal agent. Moreover, the current invention also provides a pharmaceutical composition comprising an invented immunoglobulin provided with a toxic moiety for use in an adjuvant treatment of cancer. Thus, in one embodiment of the invention, an invented immunoglobulin provided with a toxic moiety for use in an adjuvant treatment of cancer is provided. Additionally, the current invention also provides a pharmaceutical composition comprising an invented invented immunoglobulin provided with a toxic moiety for use in a combination chemotherapy treatment of cancer. Examples of chemotherapeutical treatments that are combined with the pharmaceutical composition of the current invention are etoposide, paclitaxel, cisplatin, doxorubicin and methotrexate.

The pharmaceutical compositions according to the invention will typically find their use in the treatment of cancer, particularly in forms of cancer where the targets of the preferred antibodies of the invention (complexes of MHC and tumor-specific antigen peptides) are presented by the tumors. Table 1, for example, gives a list of tumors on which complexes of MHC and MAGE-A peptides have been found. It is easy using an antibody of the invention to identify tumors that present these target MHC-peptide complexes. This can be done *in vitro* or *in vivo* (imaging).

It is preferred that the cell-surface molecules comprising the binding sites for the antibodies of the invention are internalized into the targeted aberrant cell, together with the antibodies of the invention, or together with at least the toxic moiety of the antibodies of the invention. In a particularly preferred embodiment of the invention the targeted aberrant cells go into apoptosis as a result of said internalization. Thus, in one embodiment the invention provides the use of an immunoglobulin provided with a toxic moiety according to any of the aforementioned embodiments, for the treatment of a host suffering from cancer, wherein at least the toxic moiety is internalized into the aberrant cell.

The invention of course also comprises a nucleic acid molecule encoding the immunoglobulin part of an antibody according to any of the embodiments of the invention, when the toxic moiety is chemically linked to the immunoglobulin in the antibody of the invention. Thus, the invention also comprises a nucleic acid molecule encoding an immunoglobulin and a toxic moiety according to any of the embodiments of the invention, when the toxic moiety is fused to the immunoglobulin at the DNA level. These molecules according to the invention can be produced in prokaryotes or eukaryotes. The codon usage of prokaryotes may be different from that in eukaryotes. The nucleic acids according to the invention can be adapted in these respects. Also, elements that are necessary for secretion may be added, as well as promoters, terminators, enhancers etc. Also, elements that are necessary and/or beneficial for the isolation and/or purification of the immunoglobulins of the invention or of the antibodies of the invention may be added. Typically, the nucleic acids according to the invention are provided in an expression vector suitable for the host in which they are to be produced. Choice of a production platform will depend on the size of the molecule, the expected issues around protein folding, whether amino-acid sequences are present in the immunoglobulin or in the antibody that require glycosylation, expected issues around isolation and/or purification, etc. For example, the presence of disulfide bonds in immunoglobulins or proteinaceous toxins of the invention will typically guide the selection of the preferred production platform. Thus, typically nucleic acids according to the invention are adapted to the production and purification platform in which the immunoglobulins optionally with their fused proteinaceous toxins of the invention are to be produced. Thus, the invention provides a vector comprising a nucleic acid molecule encoding an immunoglobulin or an antibody of the invention. For stable expression in an eukaryote it is preferred that the nucleic acid encoding the immunoglobulin or the antibody of the invention is integrated in the host cell genome (at a suitable site that is not silenced). In one embodiment the invention therefore comprises: a vector comprising means for integrating the nucleic acid in the genome of a host cell. The invention further comprises the host cell or the organism in which the nucleic acid molecule encoding for the immunoglobulin of the invention optionally with their fused proteinaceous toxins, is present and which is thus capable of producing the immunoglobulin optionally with their fused proteinaceous toxins of the invention.

Thus, in a preferred embodiment the invention comprises a cell comprising a nucleic acid molecule according to the invention, preferably integrated in its genome and/or a vector according to the invention, comprising a nucleic acid molecule encoding an immunoglobulin optionally with their fused proteinaceous toxins of the invention.

Included in the present invention is also a method for producing an immunoglobulin optionally with their fused proteinaceous toxins of the invention, comprising culturing a cell according to the invention, comprising a nucleic acid molecule encoding an immunoglobulin optionally with their fused proteinaceous toxins of the invention, preferably integrated in the cell's genome and/or a vector according to the invention, comprising a nucleic acid molecule encoding an immunoglobulin optionally with their fused proteinaceous toxins of the invention, allowing for expression of the immunoglobulin optionally with their fused proteinaceous toxins and separating the immunoglobulin optionally with their fused proteinaceous toxins from the culture.

In one embodiment of the invention the immunoglobulin variable domains in the molecules of the invention target one binding site. Also according to the invention, bi-specific immunoglobulins provided with a toxic moiety are provided that are specifically binding to two different binding sites associated with the cell surface of aberrant cells. By targeting with a single antibody of the invention two different binding sites on an aberrant cell such as a tumor cell, the risk that both targets are also jointly present on a healthy cell is significantly further diminished. The affinity of the antibodies of the invention for the two different target binding sites separately, preferably is designed such that $K_{on}$ and $K_{off}$ are very much skewed towards binding to both different binding sites simultaneously. Thus, the specificity of the bi-specific antibodies of the invention is increased by increasing their specificity for binding to two different binding sites associated with aberrant cells. Thus, in one embodiment of the invention, the antibody according to any of the previous embodiments is a hetero-dimeric bi-specific immunoglobulin G or heavy-chain only antibody comprising two different but complementary heavy chains. The two different but complementary heavy chains may then be dimerized through their respective Fc regions. Upon applying preferred pairing biochemistry, hetero-dimers are preferentially formed over homo-dimers. For example, two different but complementary heavy chains are subject to

forced pairing upon applying the "knobs-into-holes" CH3 domain engineering technology as described [Ridgway *et al.*, *Protein Engineering*, 1996 (ref. 14)]. In a preferred embodiment of the invention the two different immunoglobulin variable regions in the bi-specific immunoglobulins of the invention specifically bind to an MHC-peptide complex preferentially associated with aberrant cells.

Typical preferred antibodies of the invention are exemplified by the antibodies outlined in this section, in Figure 5B, and by the examples provided below and in the Examples section. Thus the invention provides an immunoglobulin provided with a toxic moiety according to Figure 5B.

## DETAILED DESCRIPTION OF THE INVENTION

One aspect of the invention relates to a method for providing the antibodies of the invention. As described herein above, it typically involves providing a nucleic acid construct encoding the desired immunoglobulin part of antibodies of the invention, or encoding the desired immunoglobulin fused to a proteinaceous toxic moiety. Said nucleic acid construct can be introduced, preferably via a plasmid or expression vector, into a prokaryotic host cell and/or in a plant cell and/or in a eukaryotic host cell capable of expressing the construct. In one embodiment, a method of the invention to provide an immunoglobulin or to provide an immunoglobulin fused to a proteinaceous toxic moiety comprises the steps of providing a host cell with the nucleic acid(s) encoding said immunoglobulin or said immunoglobulin fused to a proteinaceous toxic moiety, and allowing the expression of said nucleic acid(s) by said host cell.

It is part of the invention that nucleic acids coding for selected (human) immunoglobulin Vh(h) domains according to any of the above embodiments are combined with nucleic acids coding for human immunoglobulin heavy chain constant domains, providing nucleic acid molecules of the invention encoding for a heavy chain of a human antibody. The human antibody heavy chain protein product of such a nucleic acid molecule of the invention, then may be hetero-dimerized with a universal human antibody light chain. It is also part of the invention that nucleic acids coding for

(jointly) selected human immunoglobulin Vl domains and Vh domains according to any of the above embodiments are combined with nucleic acids coding for a human immunoglobulin light chain constant domain and are combined with nucleic acids coding for human immunoglobulin heavy chain constant domains, respectively, providing nucleic acid molecules of the invention encoding for a light chain and for a heavy chain of a human antibody. In yet another embodiment of the invention, the nucleic acids coding for the complementarity determining regions 1, 2 and 3 (CDR1, CDR2, CDR3), forming together the immunoglobulin variable region of a selected immunoglobulin Vh domain and/or a selected immunoglobulin Vl domain according to any of the above embodiments are combined with nucleic acids coding for human immunoglobulin Vh domain frame work regions and/or human immunoglobulin Vl domain frame work regions, respectively, providing nucleic acid molecules of the invention encoding for a heavy chain variable domain (Vh) of a human antibody and/or encoding for a light chain variable domain (Vl) of a human antibody (A method known in the art as 'grafting'). These nucleic acid molecules encoding for variable domains Vh and/or Vl are, as part of the invention, then combined with nucleic acids coding for human immunoglobulin constant domains, providing a nucleic acid molecule encoding for a human antibody heavy chain and/or providing a nucleic acid molecule encoding for a human antibody light chain.

According to the invention, immunoglobulins or immunoglobulins fused to a proteinaceous toxic moiety are for example expressed in plant cells, eukaryotic cells or in prokaryotic cells. Non-limited examples of suitable expression systems are tobacco plants, *Pichia pastoris*, *Saccharomyces cerevisiae*. Also cell-free recombinant protein production platforms are suitable. Preferred host cells are bacteria, like for example bacterial strain BL21 or strain SE1, or mammalian host cells, more preferably human host cells. Suitable mammalian host cells include human embryonic kidney (HEK-293) cells, PerC6 cells or preferably Chinese hamster ovary (CHO) cells, which can be commercially obtained. Insect cells, such as S2 or S9 cells, may also be used using baculovirus or insect cell expression vectors, although they are less suitable when the immunoglobulins or the fused immunoglobulins-toxic moiety molecules according to the invention include elements that involve glycosylation. The produced immunoglobulins or fused immunoglobulin-toxic moiety molecules according to the

invention can be extracted or isolated from the host cell or, if they are secreted, from the culture medium of the host cell. Thus, in one embodiment a method of the invention comprises providing a host cell with one or more nucleic acid(s) encoding said immunoglobulin or said fused immunoglobulin-toxic moiety molecule, allowing the expression of said nucleic acids by said host cell. In another preferred embodiment a method of the invention comprises providing a host cell with one or more nucleic acid(s) encoding two or more different immunoglobulins or two or more different fused immunoglobulin-toxic moiety molecules, allowing the expression of said nucleic acids by said host cell. For example, in one embodiment, nucleic acids encoding for a so-called universal immunoglobulin light chain and nucleic acids encoding for two or more different immunoglobulin heavy chains are provided, enabling isolation of mono-specific immunoglobulins or mono-specific fused immunoglobulin-toxic moiety molecules comprising homo-dimers of heavy chains and/or enabling isolation of bi-specific immunoglobulins or bi-specific fused immunoglobulin-toxic moiety molecules comprising hetero-dimers of heavy chains, with all different heavy chains complexed with a universal light chain. Methods for the recombinant expression of (mammalian) proteins in a (mammalian) host cell are well known in the art.

As said, it is preferred that the immunoglobulins of the invention are linked with the toxic moieties via bonds and/or binding interactions other than peptide bonds. Methods for linking proteinaceous molecules such as immunoglobulins to other proteinaceous molecules or non-proteinaceous molecules are numerous and well known to those skilled in the art of protein linkage chemistry. Protein linkage chemistry not based on peptide bonds can be based on covalent interactions and/or on non-covalent interactions. A typical example of linkage chemistries applicable for linking toxic moieties to immunoglobulins of the invention are the various applications of the *Universal Linkage System* disclosed in patent applications WO92/01699, WO96/35696, WO98/45304, WO03040722.

As will be clear, an antibody of the invention finds its use in many therapeutic applications and non-therapeutic applications, e.g. diagnostics, or scientific applications. Antibodies of the invention, or more preferably the immunoglobulin part of the antibodies of the invention, suitable for diagnostic purposes are of particular use

for monitoring the expression levels of molecules exposing binding sites on aberrant cells that are targeted by antibodies of the invention. In this way, it is monitored whether the therapy remains efficacious or whether other antibodies of the invention targeting one or two different binding sites on the aberrant cells should be applied instead. This is beneficial when the expression levels of the first or the first two targeted binding site(s) are below a certain threshold, whereas another or new binding sites (still) can serve as newly targeted binding sites for antibodies of the invention comprising the appropriate specific immunoglobulin variable regions for these alternative binding site(s). Antibodies of the invention may also be used for the detection of (circulating) tumor cells, and for the target-cell specific delivery of immune-stimulatory molecules. For these later two uses, the sole immunoglobulins of the invention without the fused or conjugated toxic moiety may also be used.

Provided herein is a method for inducing *ex vivo* or *in vivo* a modulating effect on a biological process in a target cell, comprising contacting said cell with an antibody of the invention in an amount that is effective to induce the modulating effect. Preferably, the antibody of the invention is used for a modulating effect on a biological process of aberrant cells in a subject, more preferably a human subject. For therapeutic applications in humans it is of course preferred that an antibody of the invention does not contain amino acid sequences of non-human origin. More preferred are antibodies of the invention, which only contain human amino acid sequences. Therefore, a therapeutically effective amount of an antibody of the invention capable of recognizing and binding to one or two disease-specific binding sites and subsequently inducing a modulating effect on a biological process in the cell, can be administered to a patient to stimulate eradication of aberrant cells expressing the binding site(s) without affecting the viability of (normal) cells not expressing said disease-specific binding site(s). The specific killing of aberrant cells while minimizing or even avoiding the deterioration or even death of healthy cells will generally improve the therapeutic outcome of a patient after administration of the antibodies of the invention.

Accordingly, also provided is the use of an antibody of the invention as medicament. In another aspect, the invention provides the use of an antibody of the invention for the manufacture of a medicament for the treatment of cancer, autoimmune disease, infection or any other disease of which the symptoms are reduced upon targeting

aberrant cells expressing disease-specific binding sites with antibodies of the invention. For example, an antibody of the invention is advantageously used for the manufacture of a medicament for the treatment of various cancers (e.g. solid tumors, hematologic malignancies).

An example of a preferred antibody of the invention is an antibody comprising at least an immunoglobulin variable region specifically binding to the complex between MHC-1 HLA-0201 and a multi-MAGE-A epitope, conjugated with a toxic moiety, using for example Universal Linkage System linker chemistry for conjugation. A second example of a preferred antibody of the invention is an antibody comprising at least an immunoglobulin variable region specifically binding to the complex between MHC-1 HLA-CW7 and a multi-MAGE-A epitope, conjugated with a toxic moiety, using for example Universal Linkage System linker chemistry for conjugation. With the bi-specific antibodies of the invention, difficult to target and/or difficult to reach aberrant cells have a higher chance of being 'hit' by at least one of the two different immunoglobulin variable regions in the bi-specific antibodies of the invention, thereby providing at least in part the therapeutic activity. An example of a preferred bi-specific antibody of the invention is an immunoglobulin comprising an immunoglobulin variable region specific for the complex between MHC-1 HLA-0201 and a multi-MAGE-A epitope and comprising a second immunoglobulin variable region specific for the complex between MHC-1 HLA-CW7 and a second multi-MAGE-A epitope, conjugated with a toxic moiety.

Antibody fragments of human origin can be isolated from large antibody repertoires displayed by phages. One aspect of the invention, known by the art, is the use of human antibody phage display libraries for the selection of human antibody fragments specific for a selected binding site, e.g. an epitope. Examples of such libraries are phage libraries comprising human Vh repertoires, human Vh-Vl repertoires, human Vh-Ch1 or human antibody Fab fragment repertoires.

Although the invention contemplates many different combinations of MHC and antigenic peptides the most preferred is the combination of MHC-1 and an antigenic peptide from a tumor related antigen presented by said MHC-1, exclusively expressed

by aberrant cells and not by healthy cells. Because of HLA restrictions, there are many combinations of MHC-1 – peptide complexes as well as of MHC-2 – peptide complexes that can be designed based on the rules for presentation of peptides in MHC. These rules include size limits on peptides that can be presented in the context of MHC, restriction sites that need to be present for processing of the antigen in the cell, anchor sites that need to be present on the peptide to be presented, etc. The exact rules differ for the different HLA classes and for the different MHC classes. We have found that MAGE derived peptides are very suitable for presentation in an MHC context. An MHC-1 presentable antigenic peptide with the sequence Y-L-E-Y-R-Q-V-P-G in MAGE-A [SEQ-ID 3] was identified, that is present in almost every MAGE-A variant (*multi MAGE* peptide) and that will be presented by one of the most prevalent MHC-1 alleles in the Caucasian population (namely *HLA-A0201*). A second MAGE peptide that is presented by another MHC-1 allele (namely *HLA-CW7*) and that is present in many MAGE variants, like for example MAGE-A2, -A3, -A6 and -A12, is E-G-D-C-A-P-E-E-K [SEQ-ID 4]. These two combinations of MHC-1 and MAGE peptides together would cover 80% of the Caucasian population. The same approach can be followed for other MHC molecules, other HLA restrictions and other antigenic peptides derived from tumor-associated antigens. Relevant is that the chosen antigenic peptide to elicit the response to must be presented in the context of an MHC molecule and recognized in that context only. Furthermore, the antigenic peptide must be derived from a sufficiently tumor specific antigen and the HLA restriction must occur in a relevant part of the population. One of the important advantages of the present invention is that tumors that down regulate their targeted MHC-peptide complex, can be treated with a second immunoglobulin comprising at least one variable region binding to a different MHC-peptide complex based on the same antigen. If this one is down regulated a third one will be available. For heterozygotes six different targets on MHC-1 may be available. Since cells need to be “inspected” by the immune system from time to time, escape through down regulation of all MHC molecules does not seem a viable escape route. In the case that MAGE is the antigen from which the peptide is derived escape through down regulation of the antigen is also not possible, because MAGE seems important for survival of the tumor [8]. Thus the present invention, in an important aspect reduces or even prevents escape of the tumor from the therapy. Thus the invention provides in a preferred embodiment an antibody of the

invention whereby the immunoglobulin variable region is capable of binding to an MHC-I – peptide complex. In a further preferred embodiment the invention provides an immunoglobulin whereby the immunoglobulin variable region is capable of binding to MHC-I – peptide complexes comprising an antigenic peptide derived from a tumor related antigen, in particular MHC-I – peptide complexes comprising an antigenic peptide present in a variety of MAGE antigens, whereby the immunoglobulin is provided with a toxic moiety.

Because in one embodiment the invention uses MHC molecules as a target, and individuals differ in the availability of MHC targets, the invention also provides a so-called companion diagnostic to determine the HLA composition of an individual. Although the invention preferably uses a more or less universal (MAGE) peptide, the invention also provides a diagnostic for determining the expression of the particular antigen by the tumor. In this manner the therapy can be geared to the patient (*personalized medicine*, *patient stratification*), particularly also in the set-up to prevent escape as described herein before. It is known that the HLA restriction patterns of the Asian population and the black population are different from the Caucasian population. For different populations different MHC-peptide complexes can be targeted.

Although the present specification presents more specific disclosure on tumors, it must be understood that other aberrant cells can also be targeted by the antibodies of the present invention. These other aberrant cells are typically cells that also proliferate without sufficient control. This occurs in autoimmune diseases. It is typical that these cells start to show expression of tumor antigens. In particular MAGE polypeptides have been identified in rheumatoid arthritis [7].

In literature it is shown that a single nine amino-acid (A.A.) peptide present in MAGE-A2, -A3, -A4, -A6, -A10, and -A12 is presented by HLA-A0201 on tumor cells, and can be recognized by cytotoxic T-lymphocytes [1]. This nine amino acid residues peptide with sequence Y-L-E-Y-R-Q-V-P-G [SEQ-ID 3] is almost identical to the HLA-A0201 presented MAGE-A1 peptide Y-L-E-Y-R-Q-V-P-D [SEQ-ID 5], except for the anchor residue at position 9. Replacement of the anchor residue with Valine results in a 9 amino acid residues peptide with enhanced binding capacity to HLA-A0201 molecules [1]. Human and mouse T-lymphocytes recognizing the Y-L-E-Y-R-

Q-V-P-V [SEQ-ID 6] peptide presented by HLA-0201 also recognize the original MAGE-A Y-L-E-Y-R-Q-V-P-G and Y-L-E-Y-R-Q-V-P-D peptides presented on tumors of distinct origin. As diverse tumors may each express at least one MAGE-A gene, targeting of this so-called multi-MAGE-A epitope includes the vast majority of tumors. As an example, MAGE-A expression in human prostate tumor cell lines and in human xenographs was analyzed and shown to be highly diverse, but in each individual sample tested at least one MAGE-A gene was expressed (Table 2), confirming that targeting this multi-MAGE-A epitope serves as a universal HLA-A0201 restricted target for therapy.

Of course several other multi-MAGE or multi-target epitopes may be designed. In principle the invention contemplates combinations of tumor specific antigen derived MHC presented epitopes in different HLA restrictions of both MHC-I and MHC-II, targeted by immunoglobulins linked to a toxic moiety, to induce apoptosis in aberrant cells. Examples of MHC - MAGE peptide combinations that can be targeted by antibodies of the invention are peptide IMPKAGLLI (MAGE-A3) [SEQ-ID 8] and HLA-DP4 or peptide 243-KKLLTQHFVQENYLEY-258 (MAGE-A3) [SEQ-ID 9] and HLA-DQ6. Other non-limiting examples of tumor specific complexes of HLA and antigen peptide are: HLA A1 – MAGE-A1 peptide EADPTGHSY [SEQ-ID 10], HLA A3 – MAGE-A1 SLFRAVITK [SEQ-ID 11], HLA A24 – MAGE-A1 NYKHCFPEI [SEQ-ID 12], HLA A28 – MAGE-A1 EVYDGREHSA [SEQ-ID 13], HLA B37 – MAGE-A1/A2/A3/A6 REPVTKAEML [SEQ-ID 14], expressed at aberrant cells related to melanoma, breast carcinoma, SCLC, sarcoma, NSCLC, colon carcinoma (Renkvist, N. *et al.*, Cancer Immunol. Immunother. (2001) V50:3-15 (ref. 13)). Further examples are HLA B53 – MAGE-A1 DPARYEFLW [SEQ-ID 15], HLA Cw2 – MAGE-A1 SAFPTTINF [SEQ-ID 16], HLA Cw3 – MAGE-A1 SAYGEPRKL [SEQ-ID 17], HLA Cw16 – MAGE-A1 SAYGEPRKL [SEQ-ID 18], HLA A2 – MAGE A2 KMVELVHFL [SEQ-ID 19], HLA A2 – MAGE-A2 YLQLVFGIEV [SEQ-ID 20], HLA A24 – MAGE-A2 EYLQLVFGI [SEQ-ID 21], HLA-A1 – MAGE-A3 EADPIGHLY [SEQ-ID 22], HLA A2 – MAGE-A3 FLWGPRALV [SEQ-ID 23], HLA B44 – MAGE-A3 MEVDPIGHLY [SEQ-ID 24], HLA B52 – MAGE-A3 WQYFFPVIF [SEQ-ID 25], HLA A2 – MAGE-A4 GVYDGREHTV [SEQ-ID 26], HLA A34 – MAGE-A6 MVKISGGPR [SEQ-ID 27], HLA A2 – MAGE-A10 GLYDGMEHL [SEQ-ID 28], HLA Cw7 – MAGE-A12 VRIGHLYIL [SEQ-ID 29],

HLA Cw16 – BAGE AARAVFLAL [SEQ-ID 30], expressed by for example melanoma, bladder carcinoma, NSCLC, sarcoma, HLA A2 – DAM-6/-10 FLWGPRAYA [SEQ-ID 31], expressed by for example skin tumors, lung carcinoma, ovarian carcinoma, mammary carcinoma, HLA Cw6 – GAGE-1/-2/-8 YRPRPRRY [SEQ-ID 32], HLA A29 – GAGE-3/-4/-5/-6/-7B YYWPRPRRY [SEQ-ID 33], both expressed by for example melanoma, leukemia cells, bladder carcinoma, HLA B13 – NA88-A MTQGQHFLQKV [SEQ-ID 34], expressed by melanoma, HLA A2 – NY-ESO-1 SLLMWITQCFL [SEQ-ID 35], HLA A2 – NY-ESO-1a SLLMWITQC [SEQ-ID 36], HLA A2 - NY-ESO-1a QLSLLMWIT [SEQ-ID 37], HLA A31 – NY-ESO-1a ASGPGGGAPR [SEQ-ID 38], the latter four expressed by for example melanoma, sarcoma, B-lymphomas, prostate carcinoma, ovarian carcinoma, bladder carcinoma.

The invention is further exemplified by the non-limiting Examples provided below.

**Abbreviations used**

A.A., amino acid; Ab, antibody; β2-M, CDR, complementarity determining region; CHO, Chinese hamster ovary; CT, cancer testis antigens; CTL, cytotoxic T-lymphocyte; E4orf4, adenovirus early region 4 open reading frame; EBV, Epstein-Barr virus; ELISA, enzyme linked immunosorbent assay; HAMLET, human α-lactalbumin made lethal to tumor cells; HEK, human embryonic kidney; HLA, human leukocyte antigen; Ig, immunoglobulin; i.v., intravenously; kDa, kilo Dalton; MAGE, melanoma-associated antigen; Mda-7, melanoma differentiation-associated gene-7; MHC, major histocompatibility complex; MHC-p, MHC-peptide; NS1, parvovirus-H1 derived non-structural protein 1; PBSM, PBS containing 2% non-fat dry milk; TCR, T-cell receptor; VH, Vh or $V_H$, amino-acid sequence of an immunoglobulin variable heavy domain; Vl, amino-acid sequence of an immunoglobulin variable light domain; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand.

**Examples**.

**EXAMPLE 1**

Non-exhaustive examples of immunoglobulins of the invention comprising at least an immunoglobulin variable region that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells or to an aberrant cell surface marker preferentially associated with aberrant cells, with domain topologies as outlined for example in Figure 5B, are:

Antibodies of the invention comprising immunoglobulin variable regions that specifically bind to

a. a complex comprising a T-cell epitope selected from 146-KLQCVDLHV-154 [SEQ-ID 74], 141-FLTPKKLQCV-150 [SEQ-ID 75], 154-VISNDVCAQV-163 [SEQ-ID 76], 154-YISNDVCAQV-163 [SEQ-ID 77] of PSA, presented by HLA-A2 and/or 162-QVHPQKVTK-170 [SEQ-ID 78] of PSA, presented by HLA-A3, and/or 152-CYASGWGSI-160 [SEQ-ID 79], 248-HYRKWIKDTI-257 [SEQ-ID 80] of PSA, presented by HLA-A24, and/or 4-LLHETDSAV-12 [SEQ-ID 81], 711-ALFDIESKV-719 [SEQ-ID 82], 27-VLAGGFFLL-35 [SEQ-ID 83] of PSMA, presented by HLA-A2, and/or 178-NYARTEDFF-186 [SEQ-ID 84], 227-LYSDPADYF-235 [SEQ-ID 85], 624-TYSVSFDSL-632 [SEQ-ID 86] of PSMA, presented by HLA-A24, and/or 299-ALDVYNGLL-307 [SEQ-ID 87] of PAP, presented by HLA-A2 and/or 213-LYCESVHNF-221 [SEQ-ID 88] of PAP, presented by HLA-A24 and/or 199-GQDLFGIWSKVYDPL-213 [SEQ-ID 89], 228-TEDTMTKLRELSELS-242 [SEQ-ID 90] of PAP, presented by MHC-2 and/or 14-ALQPGTALL-22 [SEQ-ID 91], 105-AILALLPAL-113 [SEQ-ID 92], 7-ALLMAGLAL-15 [SEQ-ID 93], 21-LLCYSCKAQV-30 [SEQ-ID 94] of PSCA, presented by HLA-A2 and/or 155-LLANGRMPTVLQCVN-169 [SEQ-ID 95] of Kallikrein 4, presented by DRB1*0404 and/or 160-RMPTVLQCVNVSVVS-174 [SEQ-ID 96] of Kallikrein 4, presented by DRB1*0701 and/or 125-SVSESDTIRSISIAS-139

[SEQ-ID 97] of Kallikerein 4, presented by DPB1*0401, for the treatment of prostate cancer;

b. the HLA B8 restricted epitope from EBV nuclear antigen 3, FLRGRAYGL [SEQ-ID 98], complexed with MHC I, for the clearance of EBV infected cells;

c. the MAGE-A peptide YLEYRQVPG presented by MHC 1 HLA-A0201, for treatment of cancers accompanied by tumor cells expressing these MHC-peptide complexes (see Table 1);

d. the MAGE-A peptide EGDCAPEEK presented by MHC-1 HLA-CW7, for treatment of cancers accompanied by tumor cells expressing these MHC-peptide complexes (see Table 1);

e. complexes of HLA-A2 and HLA-A2 restricted $CD8^+$ T-cell epitopes, e.g. nonamer peptides FLFLLFFWL [SEQ-ID 99] (from prostatic acid phosphatase (PAP, also prostatic specific acid phosphatase (PSAP))), TLMSAMTNL [SEQ-ID 100] (from PAP), ALDVYNGLL [SEQ-ID 101] (from PAP), human HLA-A2.1-restricted CTL epitope ILLWQPIPV [SEQ-ID 102] (from PAP-3), six-transmembrane epithelial antigen of prostate (STEAP), or complexes of HLA-A2.1 and HLA-A2.1-restricted CTL epitope LLLGTIHAL [SEQ-ID 103] (from STEAP-3), epitopes from mucin (MUC-1 and MUC-2), MUC-1-32mer (CHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPA [SEQ-ID 104]), epitopes from Globo H, $Lewis^y$, Tn(c), TF(c) clusters, GM2, prostate-specific membrane antigen (PSMA), kallikrein 4, prostein, or complexes of HLA-A2.1 and HLA-A2.1-restricted epitopes from BA46, PTH-rP, HER-2/neu, hTERT, and MAGE-A8, for the treatment of prostate cancer;

f. an aberrant cell specific epitope in aberrant cell-specific altered MUC-1 complexed with MHC, or to an aberrant cell specific epitope in aberrant cell-specific altered MUC-1 for, the targeting of aberrant cells in for example breast cancer or for the treatment of colorectal cancer;

g. an aberrant cell specific epitope of the aberrant-cell specific epidermal growth factor receptor mutant form vIII complexed with MHC, or to an aberrant cell specific epitope of the epidermal growth factor receptor mutant form vIII, for the treatment of the brain neoplasm glioblastoma multiforme;

h. the complex of MHC with T-cell epitope peptide 369–376 from human Her-2/neu, for the treatment of malignancies related to Her-2 and/or Her-1 over-expression;

i. an epitope of the aberrant-cell specific surface marker CD44 splice variants known as CD44-v6, CD44-v9, CD44-v10, complexed with MHC, or to an aberrant cell specific epitope of an aberrant-cell specific CD44 splice variant, for the treatment of multiple myeloma;

Target binding sites suitable for specific and selective targeting of infected aberrant cells by antibodies of the invention are pathogen-derived antigen peptides complexed with MHC molecules. Examples of T-cell epitopes of the E6 and E7 protein of human papilloma virus, complexed with indicated HLA molecules, are provided below. Any combination of an HLA molecule complexed with a pathogen-derived T-cell epitope provides a specific target on infected aberrant cells for antibodies of the invention. An example of an infected aberrant cell is a keratinocyte in the cervix infected by human papilloma virus (HPV), presenting T-cell epitopes derived from for example E6 or E7 protein, in the context of MHC. Examples of suitable target HPV 16 E6 T-cell epitopes are peptides FQDPQERPR [SEQ-ID 39], TTLEQQYNK [SEQ-ID 40], ISEYRHYCYS [SEQ-ID 41] and GTTLEQQYNK [SEQ-ID 42] binding to HLA A1, KISEYRHYC [SEQ-ID 43] and YCYSIYGTTL [SEQ-ID 44] binding to HLA A2, LLRREVYDF [SEQ-ID 45] and IVYRDGNPY [SEQ-ID 46] binding to HLA A3, TTLEQQYNK [SEQ-ID 47] binding to HLA A11, CYSLYGTTL [SEQ-ID 48], KLPQLCTEL [SEQ-ID 49], HYCYSLYGT [SEQ-ID 50], LYGTTLEQQY [SEQ-ID 51], EVYDFAFRDL [SEQ-ID 52] and VYDFAFRDLC [SEQ-ID 53] binding to HLA A24, 29-TIHDIILECV-38 [SEQ-ID 54] binding to HLA A*0201. Equally suitable are HPV 16 E7 T-cell epitopes such as 86-TLGIVCPI-93 [SEQ-ID 55], 82-LLMGTLGIV-90 [SEQ-ID 56], 85-GTLGIVCPI-93 [SEQ-ID 57] and 86-TLGIVCPIC-94 [SEQ-ID 58] binding to HLA A*0201, HPV 18 E6 T-cell epitopes and HPV 18 E7 T-cell epitopes, binding to HLA A1, A2, A3, A11 or A24. Yet additional examples of T-cell epitopes related to HPV infected cells are HPV E7 derived peptides 1-MHGDTPTLHEYD-12 [SEQ-ID 59], 48-DRAHYNIVTFCCKCD-62 [SEQ-ID 60] and 62-DSTLRLCVQSTHVD-75 [SEQ-ID 61] binding to HLA DR, 7-TLHEYMLDL-15 [SEQ-ID 62], 11-YMLDLQPETT-20 [SEQ-ID 63], 11-YMLDLQPET-19 [SEQ-ID 64]

and 12-MLDLQPETT-20 [SEQ-ID 65] binding to HLA A*201, 16-QPETTDLYCY-25 [SEQ-ID 66], 44-QAEPDRAHY-52 [SEQ-ID 67] and 46-EPDRAHYNIV-55 [SEQ-ID 68] binding to HLA B18, 35-EDEIDGPAGQAEPDRA-50 [SEQ-ID 69] binding to HLA DQ2, 43-GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR-77 [SEQ-ID 70] binding to HLA DR3, 50-AHYNIVTFCCKCD-62 [SEQ-ID 71] binding to HLA DR15, 58-CCKCDSTLRLC-68 [SEQ-ID 72] binding to HLA DR17 and 61-CDSTLRLCVQSTHVDIRTLE-80 [SEQ-ID 73] binding to HLA-DRB1*0901.

A good source for selecting binding sites suitable for specific and selective targeting of aberrant cells by antibodies of the invention, is the *Peptide Database* listing T-cell defined tumor antigens and the HLA's binding the T-cell epitopes [9-12; www.cancerimmunity.org/peptidedatabase/Tcellepitopes.htm]. The database provides combinations of antigen peptides complexed with MHC molecules comprising the indicated class of HLA, unique to tumor cells or over-expressed by tumor cells.

**EXAMPLE 2: Selection of human antibody fragments specific for HLA-A0201/multi-MAGE-A.**

To obtain human antibody fragments comprising immunoglobulin variable regions specific for the HLA-A0201 presented multi-MAGE-A epitope Y-L-E-Y-R-Q-V-P-V [SEQ-ID 6] and FLWGPRALV [SEQ-ID 23] a Human Fab phage display library was constructed according to the procedure previously described by de Haard *et al* (2) and used for selections 1) essentially as described by Chames *et al* using biotinilated MHC/p complexes (3), or 2) on cells expressing the relevant antigen.

2.1: selection of human antibody fragments specific for HLA-A0201/YLEYRQVPV using biotinilated MHC-peptide complexes:

Human Fab phages ($10^{13}$ colony forming units) were first pre-incubated for 1 h at room temperature in PBS containing 2% non-fat dry milk (PBSM). In parallel, 200 µl Streptavidin-coated beads (Dynal™) were equilibrated for 1 h in PBSM. For

subsequent rounds, 100 µl beads were used. To deplete for pan-MHC binders, each selection round, 200 nM of biotinylated MHC class I-peptide (MHC-p) complexes containing an irrelevant peptide (Sanquin, the Netherlands) were added to the phages and incubated for 30 minutes under rotation. Equilibrated beads were added, and the mixture was incubated for 15 minutes under rotation. Beads were drawn to the side of the tube using magnetic force. To the depleted phage fraction, subsequently decreasing amounts of biotinylated MHC-p complexes (200 nM for the first round, and 20 nM for the second and third round) were added and incubated for 1 h at room temperature, with continuous rotation. Simultaneously, a pan-MHC class I binding soluble Fab (D3) was added to the phage-MHC-p complex mixture (50, 10, and 5 µg for rounds 1-3 respectively). Equilibrated streptavidin-coated beads were added, and the mixture was incubated for 15 minutes under rotation. Phages were selected by magnetic force. Non-bound phages were removed by 5 washing steps with PBSM, 5 steps with PBS containing 0.1% Tween, and 5 steps with PBS. Phages were eluted from the beads by 10 minutes incubation with 500 µl freshly prepared tri-ethylamine (100 mM). The pH of the solution was neutralized by the addition of 500 µl 1 M Tris (pH 7.5). The eluted phages were incubated with logarithmic growing *E. Coli* TG1 cells ($OD_{600nm}$ of 0.5) for 30 minutes at 37°C. Bacteria were grown overnight on 2x TYAG plates. Next day, colonies were harvested, and a 10 µl inoculum was used in 50 ml 2x TYAG. Cells were grown until an $OD_{600nm}$ of 0.5, and 5 ml of this suspension was infected with M13k07 helper phage ($5x10^{11}$ colony forming units). After 30 minutes incubation at 37°C, the cells were centrifuged, resuspended in 25 ml 2x TYAK, and grown overnight at 30°C. Phages were collected from the culture supernatant as described previously, and were used for the next round panning. After three selection rounds a 261-fold enrichment was obtained, and 46 out of 282 analyzed clones were shown to be specific for the HLA-A2-multi-MAGE-A complex (Figure 1). ELISA using the HLA-A0201/multi-MAGE-A complexes as well as HLA-A0201 complexes with a peptide derived from JC virus was used to determine the specificity of the selected Fab.

2.2: Selection of human Fab specific for HLA-A0201/FLWGPRALV using cells.

Selections of Fab-phages specifically binding to HLA-A0201/FLWGPRALV were performed using mouse CMT64 lung tumor cells. To obtain CMT64 cells stably expressing HLA-A0201/FLWGPRALV (A2/FLW) complexes, the CMT64 cells were retroviral infected with a vector encoding a single chain peptide-□2M-HLA-A0201 heavy chain construct [SEQ ID 105]. Human Fab phages ($10^{13}$ colony forming units) were first pre-incubated for 1 h at room temperature in PBS containing 2% FCS (PBSF). In parallel, $1.0x10^6$ CMT64-A2/FLW cells were equilibrated for 1 h in PBSF. The phages were first incubated for one hour with $10x10^6$ CMT 64 cells expressing HLA-A0210/ YLEYRQVPG to deplete non-specifically binding phages. The non-bound fraction was then incubated (1 hr at 4°C) with HLA-A0201/FLWGPRALV expressing CMT64 cells. After extensive washing, bound phages were eluted by adding 500 µl freshly prepared tri-ethylamine (100 mM). The pH of the solution was neutralized by the addition of 500 µl 1 M Tris (pH 7.5). The eluted phages were incubated with logarithmic growing *E. Coli* TG1 cells ($OD_{600nm}$ of 0.5) for 30 minutes at 37°C. Bacteria were grown overnight on 2x TYAG plates. Next day, colonies were harvested. After four rounds of selection individual clones were selected and tested for specificity of binding.

**2.3: Human Fab specific for HLA-A0201/multi-MAGE-A epitopes bind antigen positive cells.**

**Multi-MAGE-A;** Y-L-E-Y-R-Q-V-P-V

Fab phages were analyzed for their capacity to bind HLA-A0201 positive EBV-transformed B-LCL loaded with the multi-MAGE-A peptide Y-L-E-Y-R-Q-V-P-V. The B-LCL line BSM ($0.5x10^6$) was loaded with multi-MAGE-A peptide (10 µg in 100 µl PBS) for 30 minutes at 37°C, followed by incubation with the Fab phages AH5, CB1, CG1, BD5 and BC7 and analyzed by flow-cytometry. As shown in Figure 2, Fab AH5, CB1 and CG1, specifically bound to the peptide loaded cells only, whereas Fab BD5 and BC7 displayed non-specific binding to BSM that was not loaded with the multi-MAGE-A peptide. No binding was observed by AH5, CB1 and CG1 to non-peptide loaded cells.

Phages presenting AH5, CB1 and CG1, as well as the HLA-A0101/MAGE-A1 specific Fab phage G8 (4) were then used to stain tumor cell lines of distinct histologic origin.

To this end prostate cancer cells (LNCaP), multiple myeloma cells (MDN), melanoma cells (MZ2-MEL43 and G43), and breast cancer cells (MDA-MB157) were stained and analyzed by flow cytometry (Figure 3). The Fab AH5 specifically bound multiple myeloma cells MDN, and not the HLA-A0201 negative melanoma and breast cancer cells. Both CB1 and CG1 displayed non-specific binding on the melanoma cell line G43. The positive control Fab G8 demonstrated binding to all cell lines tested.

Multi-MAGE-A: F-L-W-G-P-R-A-L-V

To determine the cell-binding capacity of the HLA-A0201/ FLWGPRALV selected Fab clone F9 soluble Fab fragments were made by induction of TG-1 bacteria. TG-1 containing pCes-F9 were grown until OD=0,8 and Fab production was induced by addition of 1 mM IPTG. After 13 hours induction the bacterial periplasmic fraction was isolated and dialyzed overnight. Next day soluble Fab F9 fragments were purified by IMAC.

Purified Fab F9 was added to $0{,}5x10^6$ CMT 64 cells expressing either HLA-A0210/ YLEYRQVPG, HLA-A0201/ FLWGPRALV, or CMT 64 cells that do not express human HLA. As shown in Figure 6 the Fab clone F9 specifically binds HLA-A0201/ FLWGPRALV expressing CMT64 cells and not CMT 64 cells that do not express human HLA or that do express the irrelevant HLA-A0201/ YLEYRQVPG molecules.

**2.4: Fab AH5 binds HLA-A0201/multi-MAGE-A complexes only.**

ELISA using multiple peptide/MHC complexes then confirmed the specificity of Fab-AH5. To this end HLA-A0201 complexes presenting peptides multi-MAGE-A, gp100, JCV and MAGE-C2, as well as a HLA-A1/MAGE-A1 complex were immobilized on 96 well plates and incubated with phages displaying Fab AH5 and control Fab G8. As shown in Figure 4, AH5 only binds HLA-A0201/multi-MAGE-A and not the irrelevant complexes HLA-A0201/gp100, HLA-A0201/MAGE-C2, HLA-A0201/JCV and HLA-A0101/MAGE-A1. The positive control Fab G8 only binds to its relevant target HLA-A0101/MAGE-A1.

The nucleic acids encoding for the HLA-A0201 – multi-MAGE-A complex binding Fab AH5 will be combined with nucleic acids encoding for human antibody Ch2-Ch3

domains, providing nucleic acid molecules encoding for a human antibody light chain encompassing the selected Cl-Vl encoding nucleic acids and encoding for a human antibody heavy chain encompassing the selected Ch-Vh encoding nucleic acids. These nucleic acid molecules encoding the desired immunoglobulin will be introduced, via a plasmid or via an expression vector, into a eukaryotic host cell such as a CHO cell. After expression of the immunoglobulin, it will be isolated from the cell culture and purified. Then, a selected toxic moiety will be linked to the immunoglobulin, for example using Universal Linkage System linker chemistry.

**Example 3. Cell binding and internalization of an immunoglobulin provided with a toxic moiety.**

Binding capacity of an antibody of the invention is analyzed by flow-cytometry. For example, an antibody comprising immunoglobulin variable regions specific for complexes of HLA-A0201 and the multi-MAGE-A peptide is analysed. HLA-A0201/multi-MAGE-A positive tumor cells (Daju, MDN and mel 624) and HLA-A0201/multi-MAGE-A negative cells (BSM, G43 and 293) are incubated on ice with purified antibody and detected by addition of fluorescently labeled antibodies. Cells bound by the antibody are quantified and visualized by flow-cytometry. Internalisation of antibody is analysed by confocal microscopy. To this end cells are incubated with the antibody, kept on ice for 30 minutes to allow binding but no internalization. Next, fluorescently labeled antibodies specific for the antibody are added. To induce internalization cells are transferred to 37°C and fixed with 1% PFA after 5, 10 and 15 minutes.

**Example 4: Apoptosis induction by antibodies of the invention in diverse tumor cells.**

**4.1: killing of diverse tumor cells by immunoglobulin provided with a toxic moiety.**

Antibodies of the invention are analyzed for their capacity to induce apoptosis by incubation with diverse tumor cells, known to express the antigens comprising the

binding sites for the immunoglobulin variable regions. For example, an antibody comprising immunoglobulin variable region VH specific for complexes of HLA-A0201 and the multi-MAGE-A peptide, AH5-BTX, is coupled to a synthetic HPMA polymer containing the BTX peptide and Doxorubicin (as we described in WO2009131435) and analyzed. To this end antibodies of the invention coupled to doxorubicin are analyzed for their capacity to induce apoptosis by incubation with diverse tumor cells known to express both HLA-A0201 and MAGE-A genes. The cell-lines Daju, Mel 624 (melanoma), PC346C (prostate cancer), and MDN (multiple myeloma) as well as MAGE-A negative cells (911 and HEK293T) are incubated with different concentrations of the antibodies of the invention (in DMEM medium, supplemented with pen/strep, Glutamine and non-essential amino acids). Several hours later, cells are visually inspected for classical signs of apoptosis such as detachment of the cells from tissue culture plates and membrane blebbing. In addition, cells are stained for active caspase-3 to demonstrate apoptosis. It is excepted that the antibodies of the invention induce apoptosis in the Daju Mel 624, PC346C and MDN cells. Cells that are not treated with the antibodies of the invention are not affected, as well as cells that do not express HLA-A0201 (HEK293T) and MAGE-A genes (911 and HEK293T).

Another antibody, comprising Vh and Vl domains (scFv) with specificity for complexes of HLA-A01, presenting a MAGE-A1 peptide was also analyzed. The scFv-BTX construct was coupled to the HPMA polymer containing doxorubicin and incubated with MAGE-A1 positive and MAGE-A1 negative cells. Apoptosis is shown by staining for active caspase-3.

**4.2: Detection of active caspase-3.**

A classical intra-cellular hallmark for apoptosis is the presence of active caspase-3. To determine whether or not the antibodies of the invention induce active caspase-3, Daju, Mel624 and MDN cells are incubated with various concentrations of antibodies of the invention. After four and 13 hours FAM –DEVD-FMK, a fluorescently caspase-3/7 inhibitor, is added and positively stained cells are visualized by fluorescent microscopy and flow-cytometry. Caspase-3 activity is shown in antigen positive cells and not in antigen negative cells, with the (fragment of the) antigen providing the specific target-binding site for the antibodies of the invention.

**4.3 Treatment of tumor bearing mice with immunoglobulins provided with a toxic moiety.**

Nude mice (NOD-scid, 8 per group) with a palpable subcutaneous transplantable human tumor (Daju or MDN) are injected with different doses of immunoglobulins provided with a toxic moiety. As a control mice are treated with standard chemotherapy or receive an injection with PBS. Mice receiving an optimal dose of the immunoglobulins provided with a toxic moiety survive significantly longer that those mice receiving chemotherapy or PBS, when the aberrant cells expose the target binding sites for the antibodies of the invention.

**Example 5: selection of llama VHH with specificity for HLA-A0201/ FLWGPRALV and HLA-A0201/ YLEYRQVPG.**

Selection of Llama VHH fragments with specificity for HLA-A0201/ FLWGPRALV (A2/FLW) and HLA-A0201/YLEYRQVPG (A2/YLE) were performed on CMT64 cells stably expressing these HLA/peptide complexes. Llama VHH phages ($10^{11}$ colony forming units) were first pre-incubated for 1 h at room temperature in PBS containing 2% FCS (PBSF). In parallel, $1.0x10^6$ CMT64-A2/FLW and $1.0x10^6$ CMT64 A2/YLE cells were equilibrated for 1 h in PBSF. To deplete for non-specific binding phages $10x10^6$ CMT 64 cells expressing either A2/FLW or A2/YLE were incubated for one hour with the llama VHH. The non-bound fractions were then incubated (1 hr at 4°C) with A2/FLW or A2/YLE expressing CMT64 cells. After extensive washing, bound phages were eluted by adding 500 µl freshly prepared tri-ethylamine (100 mM). The pH of the solution was neutralized by the addition of 500 µl 1 M Tris (pH 7.5). The eluted phages were incubated with logarithmic growing *E. Coli* TG1 cells ($OD_{600nm}$ of 0.5) for 30 minutes at 37°C. Bacteria were grown overnight on 2x TYAG plates. Next day, colonies were harvested. After four rounds of selection individual clones were selected and tested for specificity of binding.

**5.2: Llama VHH specific for HLA-A0201/multi-MAGE-A epitopes bind antigen positive cells.**

To determine the cell-binding capacity of the A2/FLW and A2/YLE selected VHH soluble VHH fragments were made by induction of TG-1 bacteria. TG-1 containing pHen-VHH were grown until OD=0,8 and Fab production was induced by addition of 1 mM IPTG. After 13

hours induction the bacterial periplasmic fraction was isolated and dialyzed overnight. Next day soluble VHH fragments were purified by IMAC.

CMT 64 cells ($0,5x10^6$) expressing either HLA-A0210/ YLEYRQVPG, HLA-A0201/ FLWGPRALV, or CMT 64 cells that do not express human HLA were incubated with purified VHH fragments for one hour at 4°C. As shown in Figure 7 the A2/FLW specific VHH bind HLA-A0201/ FLWGPRALV expressing CMT64 cells and not CMT 64 cells that do not express human HLA or that do express the irrelevant HLA-A0201/ YLEYRQVPG molecules. The A2/YLE specific VHH only bind HLA-A2/YLEYRQVPG expressing CMT64 cells and not A2/FLW positive CMT64 cells and CMT64 cells that do not express human HLA.

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**Table 1: Examples of the frequency of MAGE-A expression by human cancers.**

| | Frequency of expression (%) | | | | | | |
|---|---|---|---|---|---|---|---|
| **Cancer** | **MAGE -A1** | **MAGE -A2** | **MAGE -A3** | **MAGE -A4** | **MAGE -A6** | **MAGE -A10** | **MAGE -A11** |
| **Melanoma** | 16 | E | 36 | E | 64 | E | 74 |
| **Head and neck** | 25 | 42 | 33 | 8 | N | N | N |
| **Bladder** | 21 | 30 | 35 | 33 | 15 | N | 9 |
| **Breast** | 6 | 19 | 10 | 13 | 5 | N | N |
| **Colorectal** | N | 5 | 5 | N | 5 | N | N |
| **Lung** | 21 | 30 | 46 | 11 | 8 | N | N |
| **Gastric** | 30 | 22 | 57 | N | N | N | N |
| **Ovarian** | 55 | 32 | 20 | E | 20 | N | N |
| **Osteosarcoma** | 62 | 75 | 62 | 12 | 62 | N | N |
| **hepatocarcinoma** | 68 | 30 | 68 | N | 30 | 30 | 30 |
| **Renal cell carcinoma** | 22 | 16 | 76 | 30 | N | N | N |

**E**, expressed but the frequency is not known; **N**, expression by tumors has never been observed

**Table 2: MAGE-A expression in human prostate cancer cell lines and prostate cancer xenografts.**

| | MAGE- | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Cell line / Xenograft** | **A1** | **A2** | **A3** | **A4** | **A5** | **A6** | **A7** | **A8** | **A9** | **A10** | **A11** | **A12** |
| LNCaP | + | ++ | ++ | | | ++ | + | | | | | |
| PC346C | + | ++ | ++ | + | | ++ | + | | | + | ++ | |

| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| OVCAR | | | + | + | | + | | | | + | | |
| JON | | | ++ | ++ | | ++ | | | | + | + | |
| PNT 2 C2 | | | + | + | | + | | | | + | + | |
| SD48 | | | | + | | + | | | | + | + | |
| PC-3 | | | | | | + | | | | + | + | |
| PC 374 | | + | | | | | | | | | | |
| PC 346p | + | ++ | ++ | | | ++ | | | + | | ++ | + |
| PC 82 | | | + | + | | | | | | | | |
| PC 133 | ++ | + | | | | | | + | | | | |
| PC 135 | + | | | | | | | | | | | |
| PC 295 | + | | | | | | | | | | | |
| PC 324 | | | + | | | + | | | + | | | |
| PC 310 | + | ++ | | + | | ++ | | | | | | + |
| PC 339 | | ++ | ++ | | + | ++ | | + | + | | | + |

Expression of the MAGE-A1, A2, A3, A4, A5, A6 ,A7, A8, A9, A10, A11 and A12 genes in diverse prostate tumor cell lines and prostate xenografts was analysed by RT-PCR. Shown are expression levels in individual samples tested. Blank= no expression, + = low expression, ++ = high expression.

All cell lines / xenografts express at least one MAGE-A gene.

**Figure legends**

**Figure 1: Specific binding of HLA-A0201/multi-MAGE-A specific phage clones isolated from a large human non-immune antibody Fab phage library.** Individual antibody Fab expressing phages that were selected against biotinylated HLA-A0201/multi-MAGE-A were analysed by ELISA for their capacity to bind the relevant peptide/MHC complex only. Streptavidin coated 96 well plates were incubated with soluble HLA-A0201/multi-MAGE-A (A2/multiMage) or HLA-A0201/JCV (A2/JC) peptide/MHC complexes (10 μg/ml), washed to remove non-bound complexes and incubated with individual phage clones. Non-binding phages were first removed by three washes with PBS/Tween, followed by incubation with anti-M13 antibody (1 μg/ml, Amersham) for one hour by room temperature. Finally the wells were incubated with an HRP-labeled secondary antibody and bound phages detected.

**Figure 2: Phages AH5, CB1 and CG1 specifically bind cells presenting the multi-MAGE-A peptide.** Phages AH5, CB1, CG1, BD5 and BC7 that had shown specific binding in ELISA using the relevant HLA-A201/multi-MAGE-A complex and an irrelevant HLA-A201 complex loaded with a JCV peptide were analysed for their capacity to bind cells presenting the multi-MAGE-A peptide in HLA-A0201 molecules. To this end, human B-LCL (BSM) were loaded with multi-MAGE-A peptide (10 μg in 100 μl PBS) for 30 minutes at 37°C, followed by incubation with the Fab phages AH5, CB1, CG1, BD5 and BC7 and analyzed by flow-cytometry using anti-phage antibodies and a fluorescently labeled secondary antibody.

**Figure 3: Phages expressing HLA-A2/multi-MAGE-A specific Fab bind tumor cells of distinct histologic origin.** Phages AH5, CB1 and CG1 specific for HLA-A0201/multi-MAGE-A and a positive control phage specific for HA-0101/MAGE-A1 were used for staining of distinct tumor cell lines. To this end the prostate cancer cell line LNCaP, the multiple myeloma cell line MDN, the melanoma cell lines MZ2-MEL43 and G43, and the breast cancer cell line MDA-MD157 were incubated with the different phages (30 minutes at 4°C), bound phages were then detected by flow cytometry using anti-phage antibodies and fluorescently labeled secondary antibodies.

**Figure 4: Phage AH5 specifically binds HLA-A0201/multi-MAGE-A complexes only.** To determine specificity of the phage AH5 an ELISA was performed using relevant and irrelevant peptide/MHC complexes. HLA-A0201 with multi-MAGE-A, gp100, JCV and MAGE-C2 peptides, as well as HLA-A1 with MAGE-A1 peptide were coated on streptavidin 96 well plates and incubated with phage AH5.

**Figure 5: Cartoon displaying examples of preferred immunoglobulins provided with a toxic moiety, according to the invention.**

**A.** Cartoon displaying the topology of the twelve immunoglobulin domains assembled in an immunoglobulin G. **B.** Examples are provided of preferred immunoglobulins provided with a toxic moiety, according to the invention. Shown are immunoglobulins provided with a single toxic moiety such as for example a cytostatic agent, linked to the immunoglobulin with a chemical linker (exemplified by I. and II.; immunoglobulin-toxic moiety conjugates), or immunoglobulins provided with a single toxic moiety, linked to the immunoglobulin with a peptide linker (exemplified by III.; fused immunoglobulin-toxic moiety molecule). In IV., an immunoglobulin provided with a toxic moiety, according to the invention, is shown, comprising one immunoglobulin heavy chain comprising a fused proteinaceous toxic moiety, comprising immunoglobulin variable regions specific for a certain binding site, and comprising a second immunoglobulin heavy chain comprising immunoglobulin variable regions specific for a different binding site. Of course, also part of the invention are bi-specific immunoglobulins provided with a toxic moiety, according to the invention, comprising two heavy chains comprising different immunoglobulin variable regions specific for different binding sites and further comprising the same or different proteinaceous toxic moieties fused two the heavy chains. Of course, as part of the invention, more than one, and typically two to six toxic moiety molecules can be fused or conjugated to an immunoglobulin molecule.

**Figure 6: Human Fab phage F9 specifically binds HLA-A2/ FLWGPRALV positive CMT64 mouse lung tumor cells.**

Human Fab clone F9 was analysed for its capacity to bind mouse lung tumor cells (CMT64) stably expressing the HLA-A2/ FLWGPRALV complex. Purified Clone F9 Fab fragments (3

μg total) were incubated with $0,5x10^6$ CMT64 cells that do not express human HLA, that express HLA-A2/YLEYRQVPG or that express HLA-A2/ FLWGPRALV. After one hour incubation on ice CMT64 cells were incubated with a fluorescently labeled secondary antibody and analysed by flow cytometry.

**Figure 7: Llama VHH specifically binds CMT64 mouse lung tumor cells expressing human HLA-A2/multi-MAGE-A.**

Llama VHH specific for A2/FLW or A2/YLE were analysed by flow cytometry for their binding capacity to CMT64 cells expressing these human HLA-A0201/multi-MAGE-A complexes. Purified VHH fragments (3 μg total) were incubated with $0,5x10^6$ CMT64 cells, that do not express human HLA, that express HLA-A2/YLEYRQVPG or that express HLA-A2/ FLWGPRALV. After one hour incubation on ice CMT64 cells were incubated with a fluorescently labeled secondary antibody and analysed by flow cytometry.

**Sequence identifiers**

SEQ-ID 1. Amino acid sequence Vh AH5
QLQLQESGGG VVQPGRSLRL SCAASGFTFS SYGMHWVRQA PGKEREGVAV
ISYDGSNKYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAGGS
YYVPDYWGQG TLVTVSSGST SGS

SEQ-ID 3. Amino acid sequence MHC-1 *HLA-A0201* presentable peptide in MAGE-A
YLEYRQVPG

SEQ-ID 4. Amino acid sequence MHC-1 *HLA-CW7* presentable peptide in MAGE-A
EGDCAPEEK

SEQ-ID 5. Amino acid sequence MHC-1 *HLA-A0201* presentable peptide in MAGE-A1
YLEYRQVPD

SEQ-ID 6. Amino acid sequence MHC-1 *HLA-A0201* presentable peptide in MAGE-A1, with enhanced binding capacity for *HLA-A0201*
YLEYRQVPV

SEQ-ID 7. Amino acid sequence Vh binding domain 11H
EVQLVQSGGG LVKPGGSLRL SCAASGFTFS DYYMSWIRQA PGKGLEWLSY
ISSDGSTIYY ADSVKGRFTV SRDNAKNSLS LQMNSLRADD TAVYYCAVSP
RGYYYYGLDL WGQGTTVTVS S

SEQ-ID 8, amino acid sequence of MAGE-A3 peptide epitope binding to HLA
IMPKAGLLI

SEQ-ID 9, amino acid sequence of MAGE-A3 peptide epitope binding to HLA
KKLLTQHFVQENYLEY

SEQ-ID 10, amino acid sequence of MAGE peptide epitope binding to HLA

EADPTGHSY

SEQ-ID 11, amino acid sequence of MAGE peptide epitope binding to HLA
SLFRAVITK

SEQ-ID 12, amino acid sequence of MAGE peptide epitope binding to HLA
NYKHCFPEI

SEQ-ID 13, amino acid sequence of MAGE peptide epitope binding to HLA
EVYDGREHSA

SEQ-ID 14, amino acid sequence of MAGE peptide epitope binding to HLA
REPVTKAEML

SEQ-ID 15, amino acid sequence of MAGE peptide epitope binding to HLA
DPARYEFLW

SEQ-ID 16 amino acid sequence of MAGE peptide epitope binding to HLA
SAFPTTINF

SEQ-ID 17, amino acid sequence of MAGE peptide epitope binding to HLA
SAYGEPRKL

SEQ-ID 18, amino acid sequence of MAGE peptide epitope binding to HLA
SAYGEPRKL

SEQ-ID 19, amino acid sequence of MAGE peptide epitope binding to HLA
KMVELVHFL

SEQ-ID 20, amino acid sequence of MAGE peptide epitope binding to HLA
YLQLVFGIEV

SEQ-ID 21, amino acid sequence of MAGE peptide epitope binding to HLA

EYLQLVFGI

SEQ-ID 22, amino acid sequence of MAGE peptide epitope binding to HLA
EADPIGHLY

SEQ-ID 23, amino acid sequence of MAGE peptide epitope binding to HLA
FLWGPRALV

SEQ-ID 24, amino acid sequence of MAGE peptide epitope binding to HLA
MEVDPIGHLY

SEQ-ID 25, amino acid sequence of MAGE peptide epitope binding to HLA
WQYFFPVIF

SEQ-ID 26, amino acid sequence of MAGE peptide epitope binding to HLA
GVYDGREHTV

SEQ-ID 27, amino acid sequence of MAGE peptide epitope binding to HLA
MVKISGGPR

SEQ-ID 28, amino acid sequence of MAGE peptide epitope binding to HLA
GLYDGMEHL

SEQ-ID 29, amino acid sequence of MAGE peptide epitope binding to HLA
VRIGHLYIL

SEQ-ID 30, amino acid sequence of BAGE peptide epitope binding to HLA
AARAVFLAL

SEQ-ID 31, amino acid sequence of DAM-6 and DAM-10 peptide epitope binding to HLA
FLWGPRAYA

SEQ-ID 32, amino acid sequence of GAGE-1/-2/-8 peptide epitope binding to HLA
YRPRPRRY

SEQ-ID 33, amino acid sequence of GAGE-3/-4/-5/-6/-7B peptide epitope binding to HLA
YYWPRPRRY

SEQ-ID 34, amino acid sequence of NA88-A peptide epitope binding to HLA
MTQGQHFLQKV

SEQ-ID 35, amino acid sequence of NY-ESO-1 peptide epitope binding to HLA
SLLMWITQCFL

SEQ-ID 36, amino acid sequence of NY-ESO-1a peptide epitope binding to HLA
SLLMWITQC

SEQ-ID 37, amino acid sequence of NY-ESO-1a peptide epitope binding to HLA
QLSLLMWIT

SEQ-ID 38, amino acid sequence of NY-ESO-1a peptide epitope binding to HLA
ASGPGGGAPR

SEQ-ID 39, HPV 16 E6 T-cell epitope binding to HLA A1
FQDPQERPR

SEQ-ID 40, HPV 16 E6 T-cell epitope binding to HLA A1
TTLEQQYNK

SEQ-ID 41, HPV 16 E6 T-cell epitope binding to HLA A1
ISEYRHYCYS

SEQ-ID 42, HPV 16 E6 T-cell epitope binding to HLA A1
GTTLEQQYNK

SEQ-ID 43, HPV 16 E6 T-cell epitope binding to HLA A2
KISEYRHYC

SEQ-ID 44, HPV 16 E6 T-cell epitope binding to HLA A2
YCYSIYGTTL

SEQ-ID 45, HPV 16 E6 T-cell epitope binding to HLA A3
LLRREVYDF

SEQ-ID 46, HPV 16 E6 T-cell epitope binding to HLA A3
IVYRDGNPY

SEQ-ID 47, HPV 16 E6 T-cell epitope binding to HLA A11
TTLEQQYNK

SEQ-ID 48, HPV 16 E6 T-cell epitope binding to HLA A24
CYSLYGTTL

SEQ-ID 49, HPV 16 E6 T-cell epitope binding to HLA A24
KLPQLCTEL

SEQ-ID 50, HPV 16 E6 T-cell epitope binding to HLA A24
HYCYSLYGT

SEQ-ID 51, HPV 16 E6 T-cell epitope binding to HLA A24
LYGTTLEQQY

SEQ-ID 52, HPV 16 E6 T-cell epitope binding to HLA A24
EVYDFAFRDL

SEQ-ID 53, HPV 16 E6 T-cell epitope binding to HLA A24
VYDFAFRDLC

SEQ-ID 54, HPV 16 E6 T-cell epitope binding to HLA A*0201
29-TIHDIILECV-38

SEQ-ID 55, HPV 16 E7 T-cell epitope binding to HLA A*0201
86-TLGIVCPI-93

SEQ-ID 56, HPV 16 E7 T-cell epitope binding to HLA A*0201
82-LLMGTLGIV-90

SEQ-ID 57, HPV 16 E7 T-cell epitope binding to HLA A*0201
85-GTLGIVCPI-93

SEQ-ID 58, HPV 16 E7 T-cell epitope binding to HLA A*0201
86-TLGIVCPIC-94

SEQ-ID 59, HPV E7 T-cell epitope binding to HLA DR
1-MHGDTPTLHEYD-12

SEQ-ID 60, HPV E7 T-cell epitope binding to HLA DR
48-DRAHYNIVTFCCKCD-62

SEQ-ID 61, HPV E7 T-cell epitope binding to HLA DR
62-DSTLRLCVQSTHVD-75

SEQ-ID 62, HPV E7 T-cell epitope binding to HLA A*201
7-TLHEYMLDL-15

SEQ-ID 63, HPV E7 T-cell epitope binding to HLA A*201
11-YMLDLQPETT-20

SEQ-ID 64, HPV E7 T-cell epitope binding to HLA A*201
11-YMLDLQPET-19

SEQ-ID 65, HPV E7 T-cell epitope binding to HLA A*201
12-MLDLQPETT-20

SEQ-ID 66, HPV E7 T-cell epitope binding to HLA B18
16-QPETTDLYCY-25

SEQ-ID 67, HPV E7 T-cell epitope binding to HLA B18
44-QAEPDRAHY-52

SEQ-ID 68, HPV E7 T-cell epitope binding to HLA B18
46-EPDRAHYNIV-55

SEQ-ID 69, HPV E7 T-cell epitope binding to HLA DQ2
35-EDEIDGPAGQAEPDRA-50

SEQ-ID 70, HPV E7 T-cell epitope binding to HLA DR3
43-GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR-77

SEQ-ID 71, HPV E7 T-cell epitope binding to HLA DR15
50-AHYNIVTFCCKCD-62

SEQ-ID 72, HPV E7 T-cell epitope binding to HLA DR17
58-CCKCDSTLRLC-68

SEQ-ID 73, HPV E7 T-cell epitope binding to HLA-DRB1*0901
61-CDSTLRLCVQSTHVDIRTLE-80

SEQ-ID 74, PSA T-cell epitope binding to HLA-A2
146-KLQCVDLHV-154

SEQ-ID 75, PSA T-cell epitope binding to HLA-A2
141-FLTPKKLQCV-150

SEQ-ID 76, PSA T-cell epitope binding to HLA-A2
154-VISNDVCAQV-163

SEQ-ID 77, PSA T-cell epitope binding to HLA-A2
154-YISNDVCAQV-163

SEQ-ID 78, PSA T-cell epitope binding to HLA-A3
162-QVHPQKVTK-170

SEQ-ID 79, PSA T-cell epitope binding to HLA-A24
152-CYASGWGSI-160

SEQ-ID 80, PSA T-cell epitope binding to HLA-A24
248-HYRKWIKDTI-257

SEQ-ID 81, PSMA T-cell epitope binding to HLA-A2
4-LLHETDSAV-12

SEQ-ID 82, PSMA T-cell epitope binding to HLA-A2
711-ALFDIESKV-719

SEQ-ID 83, PSMA T-cell epitope binding to HLA-A2
27-VLAGGFFLL-35

SEQ-ID 84, PSMA T-cell epitope binding to HLA-A24
178-NYARTEDFF-186

SEQ-ID 85, PSMA T-cell epitope binding to HLA-A24
227-LYSDPADYF-235

SEQ-ID 86, PSMA T-cell epitope binding to HLA-A24
624-TYSVSFDSL-632

SEQ-ID 87, PAP T-cell epitope binding to HLA-A2
299-ALDVYNGLL-307

SEQ-ID 88, PAP T-cell epitope binding to HLA-A24
213-LYCESVHNF-221

SEQ-ID 89, PAP T-cell epitope binding to MHC-2
199-GQDLFGIWSKVYDPL-213

SEQ-ID 90, PAP T-cell epitope binding to MHC-2
228-TEDTMTKLRELSELS-242

SEQ-ID 91, PSCA T-cell epitope binding to HLA-A2
14-ALQPGTALL-22

SEQ-ID 92, PSCA T-cell epitope binding to HLA-A2
105-AILALLPAL-113

SEQ-ID 93, PSCA T-cell epitope binding to HLA-A2
7-ALLMAGLAL-15

SEQ-ID 94, PSCA T-cell epitope binding to HLA-A2
21-LLCYSCKAQV-30

SEQ-ID 95, Kallikrein 4 T-cell epitope binding to DRB1*0404
155-LLANGRMPTVLQCVN-169

SEQ-ID 96, Kallikrein 4 T-cell epitope binding to DRB1*0701
160-RMPTVLQCVNVSVVS-174

SEQ-ID 97, Kallikrein 4 T-cell epitope binding to DPB1*0401
125-SVSESDTIRSISIAS-139

SEQ-ID 98, EBV nuclear antigen 3 T-cell epitope binding to MHC I HLA B8
FLRGRAYGL

SEQ-ID 99, HLA-A2 restricted $CD8^+$ T-cell epitope of PAP binding to HLA-A2
FLFLLFFWL

SEQ-ID 100, HLA-A2 restricted $CD8^+$ T-cell epitope of PAP binding to HLA-A2
TLMSAMTNL

SEQ-ID 101, HLA-A2 restricted $CD8^+$ T-cell epitope of PAP binding to HLA-A2
ALDVYNGLL

SEQ-ID 102, human HLA-A2.1-restricted CTL epitope of PAP-3 binding to HLA A2.1
ILLWQPIPV

SEQ-ID 103, HLA-A2.1-restricted CTL epitope of STEAP-3 binding to HLA-A2.1
LLLGTIHAL

SEQ-ID 104, HLA-A2.1-restricted CTL epitope of MUC-1 and MUC-2 binding to HLA-A2.1
CHGVTSAPDTRPAPGSTAPPAHGVTSAPDTRPA

SEQ-ID 105, single chain HLA-A0201/FLWGPRALV construct.

MAVMAPRTLVLLLSGALALTQTWAFLWGPRALVGGGGSGGGGSGGGGSGGGGSGIQRTPKIQV
YSRHP
AENGKSNFLNCYVSGFHPSDIEVDLLKNGERIEKVEHSDLSFSKDWSFYLLYYTEFTPTEKDEYA
CRVNH
VTLSQPKIVKWDRDMGGGGSGGGGSGGGGSGSHSMRYFFTSVSRPGRGEPRFIAVGYVDDTQF
VRFDSDA
ASQRMEPRAPWIEQEGPEYWDGETRKVKAHSQTHRVDLGTLRGYYNQSESHTVQRMYGCDV
GSDWRFLRG
YHQYAYDGKDYIALKEDLRSWTAADMAAQTTKHKWEAAHVAEQLRAYLEGTCVEWLRRYL
ENGKETLQRT

DSPKAHVTHHPRSKGEVTLRCWALGFYPADITLTWQLNGEELTQDMELVETRPAGDGTFQKW
ASVVVPLG
KEQNYTCRVYHEGLPEPLTLRWEPPPSTDSYMVIVAVLGVLGAMAIIGAVVAFVMKRRRNTGG
GDYALAP GSQSSEMSLRDCKA

5

**Claims.**

1. An immunoglobulin provided with a toxic moiety, comprising at least an immunoglobulin variable region that specifically binds to an MHC-peptide complex preferentially associated with aberrant cells.
2. The immunoglobulin according to claim 1 wherein said immunoglobulin variable region is a Vh or Vhh.
3. The immunoglobulin according to claim 2 wherein said immunoglobulin variable region further comprises a Vl.
4. The immunoglobulin according to claim 3 being a human IgG.
5. The immunoglobulin according to any one of claims 1 to 4 wherein the MHC-peptide complex is specific for aberrant cells, preferably through a peptide derived from MAGE, preferably from MAGE-A.
6. The immunoglobulin according to any one of claims 1 to 5 wherein the toxic moiety is chemically linked to the immunoglobulin.
7. The immunoglobulin according to any one of claims 1 to 5 wherein the toxic moiety is a fusion protein, fused to the immunoglobulin at the DNA level, preferably through a linking sequence
8. A pharmaceutical composition comprising an immunoglobulin provided with a toxic moiety according to any one of claims 1 to 7 and suitable diluents and/or excipients.
9. Use of an immunoglobulin provided with a toxic moiety according to any of claims 1 to 7, for the treatment of a host suffering from a disease associated with aberrant cells.
10. Use of an immunoglobulin provided with a toxic moiety according to any of claims 1 to 7, for the treatment of a host suffering from a disease associated with aberrant cells wherein the toxic moiety is internalized into the aberrant cell.
11. An immunoglobulin provided with a toxic moiety according to any one of claims 1 to 7 for use in the treatment of cancer.
12. Use of an immunoglobulin provided with a toxic moiety according to any of claims 1 to 7, for the treatment of a host suffering from cancer, wherein at least the toxic moiety is internalized into the aberrant cell.

13. An immunoglobulin provided with a toxic moiety according to Figure 5B.