Antibodies specific for CD44v6

Technical Field of the Invention

The present invention belongs to the field of oncology. The invention relates to antibodies with specified sequence which are specific for an epitope which is coded by the variant exon v6 of the CD44 gene and to derivatives of said antibody. The invention also provides nucleic acid molecules encoding said antibody proteins. The invention furthermore pertains to methods for producing said antibody proteins. The invention also provides pharmaceutical compositions comprising said antibody proteins. The invention furthermore is concerned with the use in the manufacture of a medicament for the treatment of cancer.

Background Art

Recently it has been shown that the expression of variants of the surface glycoprotein CD44 is necessary and sufficient for causing so-called spontaneous metastatic behaviour of a non-metastasizing rat pancreatic adenocarcinoma cell line as well as a non-metastasizing rat fibrosarcoma cell line (Günthert *et al.*, 1991). While the smallest CD44 isoform, the standard form CD44s (or CD44std), is ubiquitary expressed in different tissues including epithelial cells, certain CD44 splice variants (CD44v, CD44var) are expressed only on a subset of epithelial cells. The CD44 variants are generated by alternative splicing in a way that the sequences of ten exons (v1-v10) are completely excised in CD44s but can appear in the bigger variants in different combinations (Screaton *et al.*, 1992; Tölg *et al.*, 1993; Hofmann *et al.*, 1991). The variants differ in that different amino acid sequences are inserted at a certain site of the extracellular part of the protein. Such variants can be detected in various human tumor cells as well as in human tumor tissue. So, the expression of CD44 variants in the course of colorectal carcinogenesis has recently been investigated (Heider *et al.*, 1993a). The expression of CD44 variants is absent in normal human colon epithelium, and only a weak expression is detectable in the proliferating cells of the crypts. In later stages of the tumor progression, e.g. in adenocarcinomas, all malignancies express variants of CD44. Tissue expression of variant

CD44 on a high level has also been shown in aggressive Non-Hodgkin lymphomas (Koopman *et al.*, 1993).

Exon v6 appears to play a special role especially in the course of metastatic spread (Rudy *et al.*, 1993). In an animal model, antibodies against v6 specific epitopes could prevent the settlement of metastatic cells and the growth of metastases (Seiter *et al.*, 1993). In colon carcinomas, v6 expression correlates with tumor progression (Wielenga *et al.*, 1993). In gastric carcinomas, v6 expression is an important diagnostic marker to distinguish tumors of the intestinal type from those of the diffuse type (Heider *et al.*, 1993b). In the latter two publications, v6 expression has been determined using antibodies against v6 specific epitopes.

As CD44v6 has been shown to be a tumor-associated antigen with a favorable expression pattern in human tumors and normal tissues (Heider et al., 1995; Heider et al., 1996), it has been subject to antibody-based diagnostic and therapeutic approaches, (Heider et al., 1996; WO 95/33771; WO 97/21104).

One serious problem that arises when using non-human antibodies for applications in humans is that they quickly raise a human anti-non-human response that reduces the efficacy of the antibody in patients and impairs continued administration. To overcome that probem, concepts of "humanising" non-human antibodies have been developed in the art. In the first approach, humanization of non-human antibodies has been tried to achieve by constructing non-human/human chimeric antibodies, wherein the non-human variable regions are joined to human constant regions (Boulianne G. L., Hozumi N. and Shulman, M .J. (1984) Production of functional chimeric mouse/human antibody *Nature* **312**: 643) The chimeric antibodies thus generated retain the binding specificity and affinity of the original non-human antibody. However, chimeric antibodies, although significantly better than mouse antibodies, can still elicit an anti-chimeric response in humans (LoBuglio A. F., Wheeler R. H., Trang J., Haynes A., Rogers K., Harvey E. B., Sun L., Ghrayeb J. and Khazaeli M. B. (1989) Mouse/human chimeric monoclonal antibody in man: Kinetics and immune response. *Proc. Natl. Acad. Sci.* **86**: 4220). This approach was later refined by further reducing the amount of non-human

sequences by grafting the complementarity determining regions (CDRs) from the non-human variable regions to human variable regions and then joining these "reshaped human" variable regions to human constant regions (Riechmann L., Clark M., Waldmann H. and Winter G. (1988) Reshaping human antibodies for therapy. *Nature* **332**: 323). CDR-grafted or reshaped human antibodies contain little or no protein sequences that can be identified as being derived from mouse antibodies. Although an antibody humanised by CDR-grafting may still be able to elicit some immune reactions, such as an anti-allotype or an anti-idiotypic response, as seen even with natural human antibodies, the CDR-grafted antibody will be significantly less immunogenic than a mouse antibody thus enabling a more prolonged treatment of patients.

However, it soon turned out that CDR-grafting alone did not result in antibodies with sufficient binding affinity. CDR-grafted antibodies have relatively poor binding characteristics as compared to their parent non-human antibodies because more amino acids than those within the CDR's are involved in antigen binding. In consequence, CDR-grafted antibodies with poor binding affinity are not regarded to be useful in therapy. Therefore, attempts have been made to create antibodies which combine the low immunogenicity of CDR-grafted antibodies with the good binding characteristics of the non-human parent antibodies. The concept was developed that, in addition to CDR-grafting, one to several amino acids in the humanized framework region have to be retained as residues of rodent donor origin for retaining binding affinity (Queen et al, (1989)*Proc. Natl. Acad. Sci.* **86:** 10029-10033).

Because of the high potential utility such antibodies could have in diagnosis and therapy, there is a need of antibodies with improved properties which are suitable for treatment of human cancer.

The problem underlying the present invention was to provide an antibody with significantly better properties as compared to the known CD44v6 specific antibodies.

Summary of the invention

The above-captioned technical problem is solved by the embodiments characterized in the claims and the description. The before-mentioned disadvantages in the art are overcome by the claims and the description of the present invention.

In order to solve the problems mentioned above, the present inventors have designed and generated a CD44v6 specific humanised antibody called BIWA8, which was both CDR-grafted and framework-mutated and had low immunogenicity combined with high affinity.

However, the inventors were able to create an antibody with even superior therapeutic utility, called BIWA4. Albeit this one has less binding affinity as compared to BIWA8, it surprisingly shows a much more favorable biodistribution and tumor uptake when administered in vivo.

The present invention belongs to the field of oncology. The invention relates to antibodies with specified sequence which are specific for an epitope which is coded by the variant exon v6 of the CD44 gene and to derivatives of said antibody. The invention also provides nucleic acid molecules encoding said antibody proteins. The invention furthermore pertains to methods for producing said antibody proteins. The invention also provides pharmaceutical compositions comprising said antibody proteins. The invention furthermore is concerned with the use in the manufacture of a medicament for the treatment of cancer.

Brief description of the figures
(see also example 1)

Figure 1: Evaluation of relative binding affinities tested in a competitive cell ELISA.
IC50: concentrations of cMAb and hMAbs at which binding of mMAb BIWA 1 to attached A431 cells is reduced by 50%. IC50 values relative to BIWA 2 are indicated.

Figure 2. Biodistributions of co-injected ^{125}I- and ^{131}I-labeled CD44v6-specific MAbs (10 µCi, 50 µg) in HNX-OE xenograft-bearing mice at 3 or 4 days p.i. Three groups of mice received either (A) ^{131}I- U36 (black bars) and ^{125}I-BIWA 1 (hatched bars) (n=5), (B) ^{131}I-BIWA 4 (black

bars) and ^{125}I-BIWA 2 (hatched bars) (n=6) or (C) ^{131}I-BIWA 4 (black bars) and ^{125}I-BIWA 8 (hatched bars) (n=6). At 3 (A) or 4 days (B,C) after injection mice were bled, sacrificed, dissected and the radioactivity levels (%ID/g ± s.e.m.) of tumor, blood and several organs were assessed. (Bld: blood, Tum: tumor, Liv: liver, Spl: spleen, Kid: kidney, Hrt: heart, Stm: stomach, Ilm: ileum, Cln: colon, Blr: bladder, Str: sternum, Msc: muscle, Lng: lung, Skn: skin, Tng: tongue).

Figure 3. Therapeutic efficacy of ^{186} Re-labeled CD44v6-specific MAbs in HNX-OE xenograft-bearing nude mice. Mice received 300 µCi ^{186}Re-U36 (- ❁ -, Fig A), 300 µCi ^{186} Re-BIWA 1 (-✍-, Fig A), 300 µCi ^{186}Re-BIWA 4 (- ✠-, Fig. B), 300 µCi ^{186}Re-BIWA 2 (- ☝ -, Fig. B), 400 µCi ^{186}Re-BIWA 4 (- ✠ -, Fig. C), 400 µCi ^{186}Re-BIWA 8 (- ☞ -, Fig. C), or saline (- ✌-, Fig. A, B, C) as control. Control groups in Fig A and B are the same. The tumor size is expressed as the average tumor volume (± s.e.m.) during treatment relative to the average tumor volume at the start of therapy.

Figure 4. Relationship between MAb dose administered and the AUC observed following BIWA 4 intravenous infusion to 10 patients in Part A of the study.

Figure 5. Relationship between MAb dose administered and the maximum plasma BIWA 4 concentration observed following BIWA 4 intravenous infusion to 10 patients in Part A of the study.

Disclosure of the preferred embodiments of the invention

Before the embodiments of the present invention it must be noted that as used herein and in the appended claims, the singular forms "a", "an", and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, reference to "an antibody" includes a plurality of such antibodies, reference to the "cell" is a reference to one or more cells and equivalents thereof known to those skilled in the art, and so forth. Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are incorporated herein by reference for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The terms “antibody molecule” or “antibody protein” or “antibody” as used herein shall be considered equivalent.

"Complementarity determining regions of a monoclonal antibody" are understood to be those amino acid sequences involved in specific antigen binding according to Kabat (Kabat E. A., Wu T. T., Perry H. M., Gottesman K. S. and Foeller C. (1991) *Sequences of Proteins of Immunological Interest* (5th Ed.). NIH Publication No. 91-3242. U.S. Department of Health and Human Services, Public Health Service, National Institutes of Health, Bethesda, MD.) in connection with Chothia and Lesk (Chothia and Lesk (1987) *J. Mol. Biol.* 196:901-917).

As used herein, the term "framework modifications" refers to the exchange, deletion or addition of single or multiple amino acids in the variable regions surrounding the individual complementarity determining regions. Framework modifications may have an impact on the immunogenicity, producibility or binding specificity of an antibody protein.

The present invention provides an antibody molecule comprising a variable region of the heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Both antibodies BIWA4 and BIWA 8 comprise the variable region of the heavy chain as characterized in amino acid sequence SEQ ID No. 1.

A „fragment" according to the invention is a shorter antibody molecule, i.e. any polypeptide subset, characterized in that it is encoded by a shorter nucleic acid molecule than disclosed below, however still retains its antibody binding activity.

A „functional variant" of the antibody molecule according to the invention is an antibody molecule which possesses a biological activity (either functional or structural) that is substantially similar to the antibody molecule according to the invention, i.e. a substantially similar substrate specificity or cleavage of the substrate. The term „functional variant" also includes „a fragment", „an allelic variant" „a functional variant", „variant based on the degenerative nucleic acid code" or „chemical derivatives". Such a „functional variant" e.g. may carry one or several point mutations, one or several nucleic acid exchanges, deletions or insertions or one or several amino acid exchanges, deletions or insertions. Said functional variant is still retaining its biological activity such as antibody binding activity, at least in part or even going along with an improvement said biological activity.

A „functional variant" of the antibody molecule according to the invention is a antibody molecule which possesses a biological activity (either functional or structural) that is substantially similar to the antibody molecule according to the invention, i.e. a substantially similar target molecule binding activity. The term „functional variant" also includes „a fragment", „an allelic variant" „a functional variant", „variant based on the degenerative nucleic acid code" or „chemical derivatives".

An „allelic variant" is a variant due to the allelic variation, e.g. differences in the two alleles in humans. Said variant is still retaining its biological activity such as antibody target binding activity, at least in part or even going along with an improvement said biological activity.

A „variant based on the degenerative of the genetic code" is a variant due to the fact that a certain amino acid may be encoded by several different nucleotide triplets. Said variant is still

retaining its biological activity such as antibody binding activity, at least in part or even going along with an improvement said biological activity.

A „fusion molecule" may be the antibody molecule according to the invention fused to e.g. a reporter such as a radiolabel, a chemical molecule such as a toxin or a fluorescent label or any other molecule known in the art.

As used herein, a „chemical derivative" according to the invention is an antibody molecule according to the invention chemically modified or containing additional chemical moieties not normally being part of the molecule. Such moieties may improve the molecule's activity such as target destruction (e.g. killing of tumor cells) or may improve its solubility, absorption, biological half life etc.

A molecule is „substantially similar" to another molecule if both molecules have substantially similar structures or biological activity. Thus, provided that two molecules possess a similar activity, they are considered variants as that term is used herein even if the structure of one of the molecules is not found in the other, or if the sequence of amino acid residues is not identical.

For many uses of the antibodies according to the invention it is desirable to have the smallest possible antigen-binding, i.e. CD44v6-binding units. Therefore in another preferred embodiment an antibody protein according to the invention is a Fab fragment (Fragment antigen-binding = Fab). These CD44v6-specific antibody proteins according to the invention consist of the variable regions of both chains which are held together by the adjacent constant region. These may be formed by protease digestion, e.g. with papain, from conventional antibodies, but similar Fab fragments may also be produced in the mean time by genetic engineering. In another preferred embodiment an antibody protein according to the invention is an F(ab')2 fragment, which may be prepared by proteolytic cleaving with pepsin.

Using genetic engineering methods it is possible to produce shortened antibody fragments which consist only of the variable regions of the heavy (VH) and of the light chain (VL). These are referred to as Fv fragments (Fragment variable = fragment of the variable part). In another preferred embodiment an CD44v6-specific antibody molecule according to the invention is such an Fv fragment. Since these Fv-fragments lack the covalent bonding of the two chains by the cysteines of the constant chains, the Fv fragments are often stabilised. It is advantageous to

link the variable regions of the heavy and of the light chain by a short peptide fragment, e.g. of 10 to 30 amino acids, preferably 15 amino acids. In this way a single peptide strand is obtained consisting of VH and VL, linked by a peptide linker. An antibody protein of this kind is known as a single-chain-Fv (scFv). Examples of scFv-antibody proteins of this kind known from the prior art are described in Huston et al. (1988, PNAS 16: 5879-5883). Therefore, in another preferred embodiment an CD44v6-specific antibody protein according to the invention is a single-chain-Fv protein (scFv).

In recent years, various strategies have been developed for preparing scFv as a multimeric derivative. This is intended to lead, in particular, to recombinant antibodies with improved pharmacokinetic and biodistribution properties as well as with increased binding avidity. In order to achieve multimerisation of the scFv, scFv were prepared as fusion proteins with multimerisation domains. The multimerisation domains may be, e.g. the CH3 region of an IgG or *coiled coil* structure (helix structures) such as *Leucin-zipper* domains. However, there are also strategies in which the interaction between the VH/VL regions of the scFv are used for the multimerisation (e.g. di-, tri- and pentabodies). Therefore in another preferred embodiment an antibody protein according to the invention is an CD44v6-specific diabody antibody fragment. By diabody the skilled person means a bivalent homodimeric scFv derivative (Hu et al., 1996, PNAS 16: 5879-5883). The shortening of the *Linker* in an scFv molecule to 5- 10 amino acids leads to the formation of homodimers in which an inter-chain VH/VL-superimposition takes place. Diabodies may additionally be stabilised by the incorporation of disulphide bridges. Examples of diabody-antibody proteins from the prior art can be found in Perisic et al. (1994, Structure 2: 1217-1226).

By minibody the skilled person means a bivalent, homodimeric scFv derivative. It consists of a fusion protein which contains the CH3 region of an immunoglobulin, preferably IgG, most preferably IgG1 as the dimerisation region which is connected to the scFv via a *Hinge region* (e.g. also from IgG1) and a *Linker* region. The disulphide bridges in the *Hinge* region are mostly formed in higher cells and not in prokaryotes. In another preferred embodiment an antibody protein according to the invention is an CD44v6-specific minibody antibody fragment. Examples of minibody-antibody proteins from the prior art can be found in Hu et al. (1996, Cancer Res. 56: 3055-61).

By triabody the skilled person means a: trivalent homotrimeric scFv derivative (Kortt et al. 1997 Protein Engineering 10: 423-433). ScFv derivatives wherein VH-VL are fused directly without a linker sequence lead to the formation of trimers.

The skilled person will also be familiar with so-called miniantibodies which have a bi-, tri- or tetravalent structure and are derived from scFv. The multimerisation is carried out by di-, tri- or tetrameric coiled coil structures (Pack et al., 1993 Biotechnology 11:, 1271-1277; Lovejoy et al. 1993 Science 259: 1288-1293; Pack et al., 1995 J. Mol. Biol. 246: 28-34).

Therefore in a preferred embodiment an antibody protein according to the invention is an CD44v6-specific multimerised molecule based on the abovementioned antibody fragments and may be, for example, a triabody, a tetravalent miniantibody or a pentabody.

In a more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1.

In another preferred embodiment, the invention relates to an antibody molecule comprising a variable region of the light chain as characterized by the amino acid sequence as defined in SEQ ID No. 2 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA4 as used herein comprises the variable region of the light chain as defined in amino acid sequence SEQ ID No. 2.

In another more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 2.

In another preferred embodiment, the invention relates to an antibody molecule comprising a variable region of the light chain as characterized by the amino acid sequence as defined in SEQ ID No. 3 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA 8 comprises the variable region of the light chain as characterized in amino acid sequence SEQ ID No. 3.

In another more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 3.

In another more preferred embodiment, the invention relates to an antibody molecule according to the invention comprising a variable region of the heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 and comprising a variable region of light chain as characterized by the amino acid sequence as defined in SEQ ID No. 2 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the variable region of the heavy chain as characterized in amino acid sequence SEQ ID No. 1 and variable region of the light chain as defined in amino acid sequence SEQ ID No. 2.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the variable region of the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1 and wherein the variable region of the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 2.

In another more preferred embodiment, the invention relates to an antibody molecule according to the invention comprising a variable region of the heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 and comprising a variable region of the light chain as characterized by the amino acid sequence as defined in SEQ ID No. 3 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA8 comprises the variable region of the heavy chain as characterized in amino acid sequence SEQ ID No. 1 and variable region of the light chain as defined in amino acid sequence SEQ ID No. 3.

In another most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the variable region of the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1 and wherein the variable region of the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 3.

In another preferred embodiment, the invention relates to an antibody molecule comprising a variable region of the heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Both antibodies BIWA4

and BIWA 8 comprise the variable region of the heavy chain as characterized in nucleic acid sequence SEQ ID No. 4.

In another more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4.

In another preferred embodiment, the invention relates to an antibody molecule comprising a variable region of the light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA4 as used herein comprises the variable region of the light chain as defined in nucleic acid sequence SEQ ID No. 5.

In another more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 5.

In another preferred embodiment, the invention relates to an antibody molecule comprising a variable region of the light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 6 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA 8 comprises the variable region of the light chain as characterized in nucleic acid sequence SEQ ID No. 6.

In another more preferred embodiment, the invention relates to an antibody molecule wherein the variable region of the light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 6.

In another more preferred embodiment, the invention relates to an antibody molecule according to the invention comprising a variable region of the heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and comprising a variable region of the light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the variable region of the heavy

chain as characterized in nucleic acid sequence SEQ ID No. 4 and variable region of the light chain as defined in nucleic acid sequence SEQ ID No. 5.

In another most preferred embodiment, the invention relates to an antibody molecule according to to the invention wherein the variable region of the heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and wherein the variable region of the light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 5.

In another more preferred embodiment, the invention relates to an antibody molecule according to the invention comprising a variable region of the heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and comprising a variable region of the light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 6 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA8 comprises the variable region of the heavy chain as characterized in nucleic acid sequence SEQ ID No. 4 and variable region of the light chain as defined in nucleic acid sequence SEQ ID No. 6.

In another most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the variable region of the heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and wherein the variable region of the light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 6.

To generate humanised CD44v6-specific antibody proteins the disclosed nucleic acid sequences were expressed (see *infra* and examples) by molecular biology methods known in the art.

The variable regions of the antibody proteins of the present invention are typically linked to at least a portion of the immunoglobulin constant region (F_C), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well-known procedures from a variety of human cells, but preferably immortalized B cells (see Kabat et al., *supra*, and WO 87/02671). Hence the antibody proteins of the invention may contain all or only a portion of the constant region as long as they exhibit specific binding to the CD44v6 antigen. The choice of the type and extent of the constant region depends on whether effector functions like complement fixation or antibody dependent cellular toxicity are desired, and on the desired pharmacological properties of the antibody protein. The antibody

protein of the invention will typically be a tetramer consisting of two light chain/heavy chain pairs, but may also be dimeric, i.e. consisting of a light chain/heavy chain pair, e.g. a Fab or Fv fragment.

Therefore, in a further embodiment the invention relates to antibody proteins according to the invention, characterised in that they have a variable light chain region and a variable heavy chain region, each joined to a human constant region. In particular, the variable region of the light chain was joined to a human kappa constant region and the variable region of the heavy chain was joined to a human gamma-1 constant region. Other human constant regions for chimerizing light and heavy chains are also available to the expert.

Humanization of the variable region of a murine antibody may be achieved employing methods known in the art. EP 0239400 discloses grafting of the CDRs of a murine variable region into the framework of a human variable region. WO 90/07861 discloses methods of reshaping a CDR-grafted variable region by introducing additional framework modifications. WO 92/11018 discloses methods of producing humanized Ig combining donor CDRs with an acceptor framework that has a high homology to the donor framework. WO 92/05274 discloses the preparation of framework mutated antibodies starting from a murine antibody. Further prior art references related to humanization of murine monoclonal antibodies are EP 0368684; EP 0438310; WO 92/07075, or WO 92/22653.

In another preferred embodiment, the invention relates to an antibody molecule according to the invention characterised in that each of said variable region of the light chain and said variable region of the heavy chain region is separately joined to a human constant region.

In another more preferred embodiment, the invention relates to an antibody molecule according to the invention, wherein said human constant region of the light chain is a human kappa constant region.

In another more preferred embodiment, the invention relates to an antibody protein according to the invention, wherein said human constant region of the heavy chain is a human IgG1 constant region.

Preferred are also antibodies comprising the heavy chain as characterized by the amino acid sequence of SEQ ID No. 7 and/or the light chain as characterized by the amino acid sequence of SEQ ID No. 8 or as characterized by the amino acid sequence of SEQ ID No. 9.

Thus, another important embodiment is an antibody molecule according to the invention comprising a heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 7 and comprising a light chain as characterized by the amino acid sequence as defined in SEQ ID No. 8 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the heavy chain as characterized in amino acid sequence SEQ ID No. 7 and variable region of the light chain as defined in amino acid sequence SEQ ID No. 8.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 7 and wherein the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 8. Antibody BIWA4 consists of the sequences as disclosed in amino acid sequence SEQ ID No. 7 (heavy chain) and amino acid sequence SEQ ID No. 8 (light chain). BIWA4 is a CDR-grafted antibody without framework modifications. Surprisingly, this antibody has, despite lower binding affinity, superior therapeutic efficacy, better biodistribution and tumor uptake over the framework-mutated antibody BIWA8 (see example). It is a humanised version of antibody VFF-18 (=BIWA1) mentioned above, having the complementary determining regions of the murine monoclonal antibody VFF-18 in a completely human framework, and human constant regions. It is therefore an antibody of very low immunogenicity in man, which is a favorable trait. However, as it has no murine framework residues to optimise antigen binding, it has a significanty lower antigen binding affinity as its parent antibody VFF-18, and therefore would not have been regarded as a good candidate for a therapeutic drug. Unexpectedly, it has been found that BIWA4, despite its poor binding affinity, has a very favorable biodistribution and tumor uptake in vivo, making it superior to other humanised versions of VFF-18 with higher binding affinitity.

Another important embodiment is an antibody molecule according to the invention comprising a heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 7 and

comprising a light chain as characterized by the amino acid sequence as defined in SEQ ID No. 9 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof. Antibody BIWA8 comprises the heavy chain as characterized in amino acid sequence SEQ ID No. 7 and variable region of the light chain as defined in amino acid sequence SEQ ID No. 9.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 7 and wherein the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 9. Antibody BIWA8 consists of the sequences as disclosed in amino acid sequence SEQ ID No. 7 (heavy chain) and amino acid sequence SEQ ID No. 9 (light chain). BIWA8 is a CDR-grafted antibody with framework modifications. This antibody has significant higher binding affinity than BIWA4 (see example).

Preferred are also antibodies comprising the heavy chain as encoded by the nucleic acid sequence of SEQ ID No. 10 and/or the light chain as characterized by the nucleic acid sequence of SEQ ID No. 11 or as characterized by the nucleic acid sequence of SEQ ID No. 12. Said sequences include non-translated sequences and the leader sequence as cloned in vector pAD-CMV1/pAD-CMV19.

Therefore, another important embodiment is an antibody molecule according to the invention comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 10 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 11 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the heavy chain as encoded by nucleic acid sequence SEQ ID No. 10 and variable region of the light chain as encoded by nucleic acid sequence SEQ ID No. 11.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 10 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 11.

Another important embodiment is an antibody molecule according to the invention comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 10 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID

No. 12 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA8 comprises the heavy chain as encoded by nucleic acid sequence SEQ ID No. 10 and variable region of the light chain as encoded by nucleic acid sequence SEQ ID No. 12.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 10 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 12.

Preferred are also antibodies comprising the heavy chain as encoded by the nucleic acid sequence of SEQ ID No. 13 and/or the light chain as characterized by the nucleic acid sequence of SEQ ID No. 14 or as characterized by the nucleic acid sequence of SEQ ID No. 15. Said sequences include the leader sequence as cloned in vector N5KG1val.

Therefore, another important embodiment is an antibody molecule according to the invention comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 13 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 14 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the heavy chain as encoded by nucleic acid sequence SEQ ID No. 13 and variable region of the light chain as encoded by nucleic acid sequence SEQ ID No. 14.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 13 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 14.

Another important embodiment is an antibody molecule according to the invention comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 13 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 15 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA8 comprises the heavy chain as encoded by nucleic acid sequence SEQ ID No. 13 and variable region of the light chain as encoded by nucleic acid sequence SEQ ID No. 15.

In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 13 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 15.

Most preferred is the antibody protein comprising the heavy and light chain as encoded by the nucleic acid sequence of SEQ ID No. 16. Said sequence includes the leader sequence as cloned in vector N5KG1val.

Therefore, another highly important embodiment is an antibody molecule according to the invention comprising a heavy and light chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 16 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof. Antibody BIWA4 comprises the heavy and light chain as encoded by nucleic acid sequence SEQ ID No. 16.

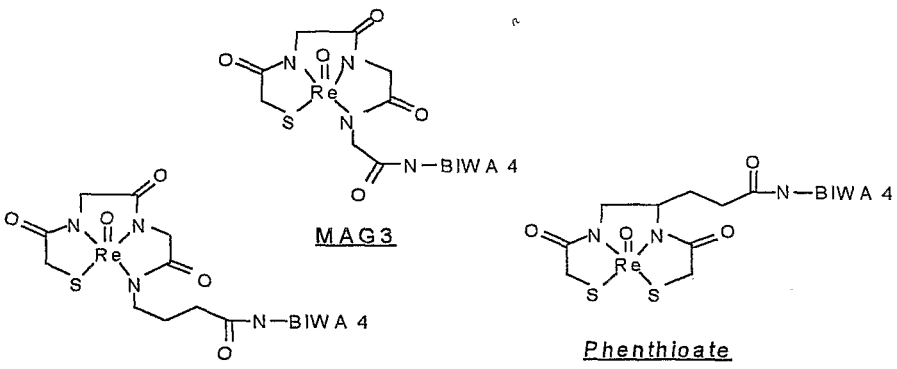
In a most preferred embodiment, the invention relates to an antibody molecule according to the invention wherein the heavy and light chain is encoded by the nucleic acid sequence of SEQ ID No. 16. This sequence is encoding the entire antibody BIWA4.

The antibody proteins of the invention provide a highly specific tool for targeting therapeutic agents to the CD44v6 antigen. Therefore, in a further aspect, the invention relates to antibody proteins according to the invention, wherein said antibody protein is conjugated to a therapeutic agent. Of the many therapeutic agents known in the art, therapeutic agents selected from the group consisting of radioisotopes, toxins, toxoids, inflammatogenic agents, enzymes, antisense molecules, peptides, cytokines, and chemotherapeutic agents are preferred. Among the radioisotopes, gamma, beta and alpha-emitting radioisotopes may be used as a therapeutic agent. ß-emitting radioisotopes are preferred as therapeutic radioisotopes. ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium have been proven to be particularly useful ß-emitting isotopes to achieve localized irradiation and destruction of malignant tumor cells. Therefore, radioisotopes selected from the group consisting of ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium are particularly preferred as therapeutic agents conjugated to the antibody proteins of the invention. For example, for the radioiodination of an antibody of the invention, a method as disclosed in WO 93/05804 may be employed.

Thus, a more preferred aspect of the present invention is an antibody protein according to the invention, wherein said therapeutic agent is a therapeutic agent selected from the group consisting of radioisotopes, toxins, toxoids, pro-drugs and chemotherapeutic agents.

A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said therapeutic agent is linked to the antibody protein via a linker selected from the group of MAG-3 (US 5082930 A, EP 0247866 B1 (page 2 lines 55-56 – page 3 lines 1-23)); MAG-2 GABA (US 5681927 A, EP 0284071 B1 (page 6 lines 9-29)); and N2S2 ((=phenthioate) US 4897255 A, US 5242679 A, EP 0188256 B1 (page 2, lines 38- page 3, lines 18)), all herein incorporated by reference.

The formulae of said linkers are as follows:



A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said therapeutic agent is linked to the antibody protein via MAG-2 GABA.

A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said radioisotope is a ß-emitting radioisotope.

A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said radioisotope is selected from the group consisting of ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium.

A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said radioisotope is ^{186}Rhenium.

A further aspect of the present invention pertains to antibody proteins according to the invention, characterised in that they are labelled. Such an CD44v6-specific labelled antibody allows for the localisation and/or detection of the CD44v6 antigen *in vitro* and/or *in vivo*. A label is defined as a marker that may be directly or indirectly detectable. An indirect marker is defined as a marker that cannot be detected by itself but needs a further directly detectable marker specific for the indirect marker. Preferred labels for practicing the invention are detectable markers. From the large variety of detectable markers, a detectable marker selected from the group consisting of enzymes, dyes, radioisotopes, digoxygenin, and biotin is most preferred.

Thus, a more preferred aspect of the present invention is an antibody protein according to the invention, characterised in that it is labelled. More preferred is the antibody protein according to the invention, wherein said label is a detectable marker. Also more preferred is the antibody protein according to the invention, wherein the detectable marker is a detectable marker selected from the group consisting of enzymes, dyes, radioisotopes, digoxygenin, and biotin.

A further aspect of the present invention relates to antibody proteins according to the invention, characterised in that they are conjugated to an imageable agent. A large variety of imageable agents, especially radioisotopes, are available from the state of the art. For practising the invention gamma-emitting isotopes are more preferred. Most preferred is ^{125}Iodine.

Therefore, a more preferred aspect of the present invention is an antibody protein to the invention conjugated to an imageable agent. A more preferred aspect of the present invention is an antibody protein according to the invention, wherein the imageable agent is a radioisotope. A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said radioisotope is a γ-emitting radioisotope. A more preferred aspect of the present invention is an antibody protein according to the invention, wherein said radioisotope is ^{125}I.

Therefore, a more preferred aspect of the present invention is an antibody protein conjugated to a radioisotope as described above, wherein the antibody protein has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to

about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

Another preferred embodiment of the present invention is a pharmaceutical composition containing an antibody according to the invention and a pharmaceutically acceptable carrier or excipient.

A pharmaceutically acceptable carrier can contain physiologically acceptable compounds that act, for example, to stabilize or to increase the absorption of an AMPA glutamate receptor agonist, antagonist or modulator. Such physiologically acceptable compounds include, for example, carbohydrates, such as glucose, sucrose or dextrans, antioxidants, such as ascorbic acid or glutathione, chelating agents, low molecular weight proteins or other stabilizers or excipients (see also e.g. Remington's Pharmaceutical Sciences (1990), 18th ed. Mack Publ., Easton). One skilled in the art would know that the choice of a pharmaceutically acceptable carrier, including a physiologically acceptable compound, depends, for example, on the route of administration of the composition.

In an animal or human body, it can prove advantageous to apply the pharmaceutical compositions as described above via an intravenous or other route, e.g. systemically, locally or topically to the tissue or organ of interest, depending on the type and origin of the disease or problem treated, e.g. a tumor. For example, a systemic mode of action is desired when different organs or organ systems are in need of treatment as in e.g. systemic autoimmune diseases, or allergies, or transplantations of foreign organs or tissues, or tumors that are diffuse or difficult to localise. A local mode of action would be considered when only local manifestations of neoplastic or immunologic action are expected, such as, for example local tumors.

The pharmaceutical compositions comprising antibody proteins of the present invention may be applied by different routes of application known to the expert, notably intravenous injection or direct injection into target tissues. For systemic application, the intravenous, intravascular, intramuscular, intraarterial, intraperitoneal, oral, or intrathecal routes are preferred. A more local application can be effected subcutaneously, intracutaneously, intracardially, intralobally, intramedullarly, intrapulmonarily or directly in or near the tissue to be treated (connective-, bone-, muscle-, nerve-, epithelial tissue). Depending on the desired duration and effectiveness

of the treatment, pharmaceutical antibody compositions may be administered once or several times, also intermittently, for instance on a daily basis for several days, weeks or months and in different dosages.

For preparing suitable pharmaceutical compositions comprising antibody preparations for the applications described above, the expert may use known injectable, physiologically acceptable sterile solutions. For preparing a ready-to-use solution for parenteral injection or infusion, aqueous isotonic solutions, such as e.g. saline or corresponding plasma protein solutions are readily available. The pharmaceutical compositions may be present as lyophylisates or dry preparations, which can be reconstituted with a known injectable solution directly before use under sterile conditions, e.g. as a kit of parts. The final preparation of the antibody compositions of the present invention are prepared for injection, infusion or perfusion by mixing purified antibodies according to the invention with a sterile physiologically acceptable solution, that may be supplemented with known carrier substances or/and additives (e.g. serum albumin, dextrose, sodium bisulfite, EDTA).

The amount of the antibody applied depends on the nature of the disease. In cancer patients, the applied dose of a 'naked' antibody which is comprised in the pharmaceutical composition according to the invention may be between 0.1 and 100 mg/m^2, preferably between 5 and 50 mg/m^2 per application, preferably 10 mg/m^2 to about 40 mg/m^2, preferably 10 mg/m^2 to about 30 mg/m^2, also preferably 20 mg/m^2 to about 30 mg/m^2, and most preferably about 25 mg/m^2 body surface area. Also most preferred is an antibody protein dose of about 50 mg/m^2 body surface area.

The dose of radioactivity applied to the patient per administration has to be high enough to be effective, but must be below the dose limiting toxicity (DLT). For pharmaceutical compositions comprising radiolabeled antibodies, e.g. with ^{186}Rhenium, the maximally tolerated dose (MTD) has to be determined which must not be exceeded in therapeutic settings. Application of radiolabeled antibody to cancer patients may then be carried out by repeated (monthly or weekly) intravenous infusion of a dose which is below the MTD (See e.g. Welt et al. (1994) *J. Clin. Oncol.* **12**: 1193-1203). Multiple administrations are preferred, generally at weekly intervals; however, radiolabelled materials should be administered at longer intervals,

i.e., 4-24 weeks apart, preferable 12-20 weeks apart. The artisan may choose, however, to divide the administration into two or more applications, which may be applied shortly after each other, or at some other predetermined interval ranging, e.g. from 1 day to 1 week.

Furthermore, the applied radioactivity dose will be in accordance with the guidelines outlined below. In general, the radioactivity dose per administration will be between 30 and 75 mCi/m^2 body surface area (BSA). Thus, the amount of radiolabelled antibody in the pharmaceutical composition according to the invention, preferably labelled with ^{186}Rhenium, ^{188}Rhenium, ^{99m}Technetium, ^{131}Iodine, or ^{90}Yttrium, most preferably labelled with ^{186}Rhenium, to be applied to a patient is 10, 20, 30, 40, 50 or 60 mCi/m^2, preferably 50 mCi/m^2. In a preferred embodiment, the invention relates to a pharmaceutical composition, wherein the dose of said radiolabelled antibody according to the invention is MTD, preferably 50 mCi/m^2. This is extensively exemplified in clinical studies as set out in examples 3 to 6.

Preferred also is a pharmaceutical composition according to the invention comprising an antibody protein conjugated to a radioisotope according to the invention as defined *supra*, wherein the antibody protein has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

Preferred also is a pharmaceutical composition according to the invention comprising an antibody protein conjugated to a radioisotope according to the invention as defined *supra*, wherein said antibody or antibody derivative is in an aqueous solution at pH of from about 7 to about 8, and at a concentration of from about 0.5 to about 2.0 mg/ml.

A preferred embodiment is a pharmaceutical composition according to the invention, further comprising one or more radioprotectants selected from the group of ascorbic acid, gentisic acid, reductic acid, erythrorbic acid, p-aminobenzoic acid, 4-hydroxybenzoic acid, nicotinic acid, nicotinamide, 2-5-dihydroxy-1,4-benzenedisulfonic acid, povidone, inositol, and/or citrate.

Preferred is a pharmaceutical composition according to the invention, wherein the radioprotectant is ascorbic acid.

Another preferred embodiment is a pharmaceutical composition according to the invention, wherein said antibody protein comprises an antibody molecule selected from the group of

antibody molecules BIWA4 or BIWA8 as described *supra* linked to ^{186}Rhenium via MAG-2 GABA further comprising the radioprotectant ascorbic acid.

Another preferred embodiment of the present invention is the use of an antibody protein according to the invention in the manufacture of a medicament for treatment of cancer. In a preferred embodiment the present invention relates to the use of antibody proteins according to the invention conjugated to a therapeutic agent as described above for the treatment of cancer. Cancer includes any disease associated with malignant growth such as solid tumors, sarcomas and leukemias. A necessary precondition for such diseases is the expression of CD44v6. Cancer according to the invention includes, but is not limited to:

1) The treatment of epithelial carcinomas including breast, lung, colorectal, head and neck, pancreatic, ovarian, bladder, gastric, skin, endometrial, ovarian, testicular, esophageal, prostatic and renal origin;
2) Bone and soft-tissue sarcomas: Osteosarcoma, chondrosarcoma, fibrosarcoma, malignant fibrous histiocytoma (MFH), leiomyosarcoma;
3) Hematopoietic malignancies: Hodgkin's and non-Hodgkin's lymphomas, leukemias;
4) Neuroectodermal tumors: Peripheral nerve tumors, astrocytomas, melanomas;
5) Mesotheliomas.

Examples for cancerous disease states associated with solid tumors include, but are not limited to: colorectal cancer, non-small cell lung cancer, breast cancer, head and neck cancer, ovarian cancer, lung cancer, bladder cancer, pancreatic cancer and metastatic cancers of the brain.

Thus, a preferred embodiment is the use of an antibody protein according to the invention wherein said cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer, breast cancer, head and neck cancer, ovarian cancer, lung cancer, bladder cancer, pancreatic cancer and metastatic cancers of the brain.

Preferably also is the use of an antibody protein according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein the amount of antibody protein per application is between 0.1 and 100 mg/m^2, preferably between 5 and 50 mg/m^2, preferably 10 mg/m^2 to about 40 mg/m^2, preferably 10 mg/m^2 to about 30 mg/m^2, also

preferably 20 mg/m^2 to about 30 mg/m^2, and most preferably about 25 mg/m^2 body surface area. Also most preferred is an antibody protein dose of about 50 mg/m^2 body surface area.

Preferred also is the use of an antibody protein conjugated to a radioisotope according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein the radioactivity dose per administration is between 30 and 75 mCi/m^2 body surface area (BSA). Preferred is the use of an antibody protein conjugated to a radioisotope according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein the antibody protein according to the invention is radiolabelled with ^{186}Rhenium, ^{188}Rhenium, ^{99m}Technetium, ^{131}Iodine, or ^{90}Yttrium, and most preferably is labelled with ^{186}Rhenium. In yet another preferred embodiment the invention relates to the use of an antibody protein conjugated to a radioisotope according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein to antibody dose is 10, 20, 30, 40, 50 or 60 mCi/m^2, most preferably 50 mCi/m^2. This is extensively exemplified in clinical studies as set out in examples 3 to 6.

Preferred also is the use of an antibody protein conjugated to a radioisotope according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein the antibody protein has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

Preferred also is the use of an antibody protein conjugated to a radioisotope according to the invention as defined *supra* in the manufacture of a medicament for treatment of cancer, wherein said antibody or antibody derivative is in an aqueous solution at pH of from about 7 to about 8, and at a concentration of from about 0.5 to about 2.0 mg/ml.

The invention further relates to a method of cancer treatment, wherein an antibody protein according to the invention is administered once to several times to an individual in need thereof, said antibody protein selectively binds to CD44v6, destroys tumor cells via the therapeutic agent linked to the antibody protein and the therapeutic success is monitored. Said antibody protein may be present as naked/unmodified antibody protein, modified antibody protein, such as e.g. fusion protein, or antibody protein conjugated to a therapeutic agent, which comprises contacting the tumor with an effective amount of said antibodies. The method

of treating tumors as described above may be effective *in vitro or in vivo*. Cancer is any cancer as described above.

The amount of the antibody applied depends on the nature of the disease. In cancer patients, the applied dose of a 'naked' antibody may be between 0.1 and 100 mg/m^2, preferably between 5 and 50 mg/m^2 per application, preferably 10 mg/m^2 to about 40 mg/m^2, preferably 10 mg/m^2 to about 30 mg/m^2, also preferably 20 mg/m^2 to about 30 mg/m^2, and most preferably about 25 mg/m^2 body surface area. Also most preferred is an antibody protein dose of about 50 mg/m^2 body surface area.

The dose of radioactivity applied to the patient per administration has be high enough to be effective, but must be below the dose limiting toxicity (DLT). For radiolabeled antibodies, e.g. with ^{186}Rhenium, the maximally tolerated dose (MTD) has to be determined which must not be exceeded in therapeutic settings. Application of radiolabeled antibody to cancer patients may then be carried out by repeated (monthly or weekly) intravenous infusion of a dose which is below the MTD (See e.g. Welt et al. (1994) *J. Clin. Oncol.* **12**: 1193-1203). Multiple administrations are preferred, generally at weekly intervals; however, radiolabelled materials should be administered at longer intervals, i.e., 4-24 weeks apart, preferable 12-20 weeks apart. The artisan may choose, however, to divide the administration into two or more applications, which may be applied shortly after each other, or at some other predetermined interval ranging, e.g. from 1 day to 1 week.

Furthermore, the applied radioactivity dose will be in accordance with the guidelines outlined below. In general, the radioactivity dose per administration will be between 30 and 75 mCi/m^2 body surface area (BSA). Thus, the amount of radiolabelled antibody, preferably labelled with ^{186}Rhenium, ^{188}Rhenium, ^{99m}Technetium, ^{131}Iodine, or ^{90}Yttrium, most preferably labelled with ^{186}Rhenium, to be applied to a patient is 10, 20, 30, 40, 50 or 60 mCi/m^2, preferably 50 mCi/m^2. In a preferred embodiment, the invention relates to a method of treatment, wherein the radiolabelled antibody as described above is administered to a patient suffering from cancer, wherein the dose of said radiolabelled antibody is MTD, preferably 50 mCi/m^2, whereby said cancer is prevented or treated. This is extensively exemplified in clinical studies as set out in examples 3 to 6.

Preferred also is a method of cancer treatment according to the invention (see above), wherein the antibody protein conjugated to a radioisotope according to the invention as defined *supra* has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

Preferred also is a method of cancer treatment according to the invention (see above), wherein the antibody protein conjugated to a radioisotope according to the invention as defined *supra* is in an aqueous solution at pH of from about 7 to about 8, and at a concentration of from about 0.5 to about 2.0 mg/ml.

Preferably, the invention relates to a method according to the invention, wherein the tumor is a tumor selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

A further aspect of the present invention is a nucleic acid, characterised in that it codes for an antibody protein according to the invention. Said nucleic acid may be RNA or preferably DNA. Said DNA molecule may be chemically synthesized. First, suitable oligonucleotides can be synthesized with methods known in the art (e.g. Gait,M.J., 1984, *Oligonucleotide Synthesis. A Practical Approach.*IRL Press, Oxford, UK), which can be used to produce a synthetic gene. Methods to generate synthetic genes are known in the art (e.g. Stemmer et al. 1995, *Single-step assembly of a gene and entire plasmid from large numbers of oligodeoxyribonucleotides,* Gene 164(1): 49-53; Ye et al. 1992, *Gene synthesis and expression in E. coli for pump, a human matrix metalloproteinase,* Biochem Biophys Res Commun 186(1):143-9; Hayden et Mandecki 1988, *Gene synthesis by serial cloning of oligonucleotides,* DNA 7(8): 571-7). These methods can be used to synthesize any DNA molecule disclosed in the present application, e.g. the DNA encoding BIWA4.

Preferably, too, a nucleic acid according to the invention is characterised in that it contains 5‘ or 3‘ or 5‘ and 3‘ untranslated regions. The nucleic acid according to the invention may contain other untranslated regions upstream and/or downstream. The untranslated region may contain a regulatory element, such as e.g. a transcription initiation unit (promoter) or enhancer. Said promoter may, for example, be a constitutive, inducible or development-controlled

promoter. Preferably, without ruling out other known promoters, the constitutive promoters of the human Cytomegalovirus (CMV) and Rous sarcoma virus (RSV), as well as the Simian virus 40 (SV40) and Herpes simplex promoter. Inducible promoters according to the invention comprise antibiotic-resistance promoters, heat-shock promoters, hormone-inducible „Mammary tumour virus promoter" and the metallothioneine promoter. Preferably, too, a nucleic acid according to the invention is characterised in that it codes for a fragment of the antibody protein according to the invention. This refers to part of the polypeptide according to the invention.

Preferably, a nucleic acid according to the invention is a nucleic acid as disclosed in SEQ ID SEQ ID No. 4, 5, 6, 10, 11, 12, 13, 14, 15, and/or 16. Most preferred, said nucleic acid is a nucleic acid of SEQ ID No. 16.

Another important aspect of the present invention is a recombinant DNA vector, characterised in that it contains a nucleic acid according to the invention. Preferably, said vector contains a nucleic acid as characterized in SEQ ID No. 4, 5, 6, 10, 11, 12, 13, 14, 15, and/or 16. Most preferred, said vector contains the nucleic acid as characterized in SEQ ID No. 16.

Examples are viral vectors such as e.g. Vaccinia, Semliki-Forest-Virus and Adenovirus. Vectors for use in COS-cells have the SV40 origin of replication and make it possible to achieve high copy numbers of the plasmids. Vectors for use in insect cells are, for example, *E. coli* transfer vectors and contain e.g. the DNA coding for polyhedrin as promoter.

Another preferred aspect of the present invention is a recombinant DNA vector according to the invention, characterized in that it is an expression vector.

Another preferred aspect of the present invention is a recombinant DNA vector according to the invention, characterized in that it is vector pAD-CMV or a functional derivative thereof. Such derivatives are e.g. pAD-CMV1, pAD-CMV19 or pAD-CMV25.

Another preferred aspect of the present invention is a recombinant DNA vector according to the invention, characterized in that it is the of SEQ ID No. 17 or a functional derivative thereof.

Another preferred aspect of the present invention is a recombinant DNA vector according to the invention, characterized in that it is the of SEQ ID No. 18 or a functional derivative thereof.

Preferably also, said vectors comprise one or several of the nucleic acid molecules as characterized in SEQ ID No. 4, 5, 6, 10, 11, 12, 13, 14, 15, and/or 16.

Preferred is also a vector as disclosed in US 5648267 A or US US 5733779 A comprising a nucleotide sequence according to the invention. Preferably also, said vector comprises one or several of the nucleic acid molecules as characterized in SEQ ID No. 4, 5, 6, 10, 11, 12, 13, 14, 15, and/or 16. Another preferred aspect of the present invention is a recombinant DNA vector according to the invention, characterized in that it is vector N5KG1Val or a derivative thereof.

Another important aspect is a host, characterised in that it contains a vector according to the invention.

Another important aspect is a host according to the invention, characterised in that it is a eukaryotic host cell. The eukaryotic host cells according to the invention include fungi, such as e.g. *Pichia pastoris, Saccharomyces cerevisiae, Schizosaccharomyces, Trichoderma,* insect cells (e.g. from *Spodoptera frugiperda* Sf-9, with a Baculovirus expression system), plant cells, e.g. from *Nicotiana tabacum,* mammalian cells, e.g. COS cells, BHK, CHO or myeloma cells.

In descendants of the cells of the immune system in which antibody proteins are also formed in our body, the antibody proteins according to the invention are particularly well folded and glycosylated. Mammalian host cells, preferably CHO or COS cells are preferred, e.g. a CHO DG44 (Urlaub and Chasin, Proc. Natl. Acad. Sci. U.S.A. 77(7): 4216-20 (1980)), or CHO-K1 (ATCC CCL-61) cells. Thus, another preferred aspect is a host according to the invention according to the invention, characterised in that it is a BHK, CHO or COS cell, most preferred CHO DG44 or CHO-K1 (ATCC CCL-61) cells.

Another preferred aspect is a host according to the invention, characterised in that it is a bacteriophage.

Another preferred aspect is a host according to the invention, characterised in that it is a prokaryotic host cell. Examples of prokaryotic host cells are *Escherichia coli, Bacillus subtilis, Streptomyces* or *Proteus mirabilis.*

The invention further relates to a process for preparing an antibody protein according to the invention, characterized in that it comprises the following steps: a host according to the invention is cultivated under conditions in which said antibody protein is expressed by said host

cell and said antibody protein is isolated. The antibody according to the invention may be produced as follows. Nucleic acid molecules coding for the light chain and the heavy chain may be synthesised chemically and enzymatically by standard methods. First, suitable oligonucleotides can be synthesized with methods known in the art (details *supra*). Methods to generate synthetic genes from oligonucleotides are known in the art (details *supra*). These nucleic acid molecules encoding the antibody heavy and light chains may be cloned into an expression vector (either both chains in one vector molecule, or each chain into a separate vector molecule), which then is introduced into a host cell. The host cell preferably is a mamalian host cell (details *supra*), e.g. a COS, CHO, or BHK cell, more preferably a chinese hamster ovary (CHO) cell, The host cell then is cultured in a suitable culture medium under conditions where the antibody is produced, and the antibody is then isolated from the culture according to standard procedures. Procedures for production of antibodies from recombinant DNA in host cells and respective expression vectors are well-known in the art (see e.g. WO 94/11523, WO 97/9351, EP 0481790.)

The invention preferably relates to a process according to the invention, characterised in that said host is a mammalian cell, preferably a CHO or COS cell.

The invention preferably relates to a process according to the invention, characterised in that said host cell is co-transfected with two plasmids which carry the expression units for the light or the heavy chain.

The following examples serve to further illustrate the present invention; but the same should not be construed as limiting the scope of the invention disclosed herein.

Examples

Example 1 Radioimmunotherapy

MATERIALS AND METHODS.

Monoclonal antibodies. mMAb BIWA 1=VFF 18 (which is secreted by a hybridoma cell line which has been deposited on 7 June 1994 with the accession number DSM ACC2174 with the DSM-Deutsche Sammlung für Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg

1b, D-38124 Braunschweig, Deutschland; see also WO 95/33771) was generated by immunizing BALB/c mice with glutathione S-transferase fusion protein containing the human CD44 domains v3-v10 (Heider et al., 1996). The epitope recognized by BIWA 1 has been mapped to amino acids 360-370 in domain v6 of CD44 (numbering according to Kugelman *et al.* (1992)). The batch used for the present studies was obtained after purification on protein-G-Sepharose and dialysis against PBS.

MAb U36 (IgG1) was derived after immunization of mice with the HNSCC cell line UM-SCC-22B and recognised a different epitope within CD44v6 as BIWA 1. U36 was purified from a concentrated tissue culture supernatant by affinity chromatography on protein-A-Sepharose and further purified on Q-Sepharose.

Generation of chimeric and humanized MAbs. mRNA was isolated from the BIWA 1 hybridoma cell line by use of the QuickPrep mRNA Purification Kit (Pharmacia, Uppsala, Sweden). cDNA from the variable heavy (V_H) and variable light (V_L) chain was generated by RT-PCR.

The fragments were cloned into the TA cloning vector pCR II (Invitrogen, Groningen, The Netherlands) and sequenced. Two expression vectors derived from the plasmid pAD CMV1 (Himmler et al., 1990) were constructed carrying the constant region of human gamma-1 and the constant region of the human kappa light chain, respectively. Subsequently, the V_H and V_L fragments of BIWA 1 were cloned into the corresponding expression vectors in front of the constant regions. The chimeric antibody was named cMAb BIWA 2. Humanized versions of the BIWA 1 heavy and light chain variable regions (generated by CDR grafting) were cloned in front of the immunoglobulin constant regions of the above mentioned expression vectors. For the construction of humanized antibodies, the human variable regions used were derived for the heavy chain from the human immunoglobulin fragment accession number S31669 of databank GenPept and for the light chain from the human immunoglobulin HUMIGKAX (rearranged anti-myelin kappa chain), Genbank accession number M29469. The resulting MAbs were named hMAb BIWA 4 and BIWA 8, respectively. BIWA 8 contained two amino acids of the murine parent antibody within the light chain framework 2 while BIWA 4 did not contain murine residues in the framework.

Recombinant MAbs were stably expressed in dihydrofolate reductase deficient Chinese hamster ovary cells by electroporation with heavy and light chain expression plasmids. Cells were seeded into 96 well microtiter plates at densities of 500 and 100 cells/well in selection medium (α-MEM with 10% dialyzed fetal calf serum). When colonies became visible (after ~ 14 days), culture supernatants were tested for their IgG content by ELISA, and the best producers were expanded. Gene amplification was performed by culturing in the presence of increasing concentrations of methotrexate (20-500 nM).

Laboratory scale production of chimeric and humanized MAbs was performed in a standard culture medium containing 1% fetal calf serum. IgG fractions were purified from tissue culture supernatants by affinity chromatography on protein A sepharose. Purity was tested by SDS-PAGE and high performance size exclusion chromatography.

Evaluation of antibody affinity. Measurement of kinetic and affinity constants using recombinant antigen was performed on a BIAcore 2000 system (BIAcore AB, Uppsala, Sweden). A glutathione-S-transferase fusion protein containing domains v3-v10 of human CD44 (GST/CD44v3-v10; 20 µg/ml) was immobilized on a CM5 sensor chip by the amine coupling method according to the manufacturer's instructions, using 10 mM sodium acetate pH 5.0 as coupling buffer. 35 µl of MAb at various concentrations (8-67 nM) in HBS (10 mM HEPES, pH 7.4, 150 mM NaCl, 3.4 mM EDTA, 0.05% BIAcore surfactant P20) were injected over the antigen-coated surface at a flow rate of 5 µL/min. Dissociation of the MAb was assessed for 5 minutes in buffer flow (HBS). Between two analyses, the surface of the chip was regenerated with a single pulse of 15 µl 30 mM HCl. Analysis of the data and calculation of the kinetic constants were performed with BIAcore's BIAevaluation software, version 2.1. Association rates (k_a), dissociation rates (k_d), and dissociation constants (K_d) were assessed for all antibodies.

Relative binding affinities were also evaluated by competitive cell ELISA. Human A431 cells, originating from an epidermoid carcinoma of the vulva and known to express high levels of CD44v6, were seeded in 96 well tissue culture plates in 200 µl per well RPMI 1640 with 10% fetal calf serum at a density of 2.5 – $5x10^5$ cells/ml. The plates were incubated overnight at 37°C in a humidified incubator with 5% CO_2 in air. After removal of the medium the cells were

washed once with PBS, fixed with 96% ethanol for 1 min, and washed again with PBS. cMAb BIWA 2, hMAb BIWA 4 and hMAb BIWA 8 (prediluted to 10 µg/ml) were applied in 1:2 serial dilutions (8 steps) in 100 µl/well in PBS/0.5%BSA/0.05% Tween 20 (assay buffer) and incubated for 30 min at room temperature. 100 µl prediluted mMAb BIWA 1 (20 ng/ml) was added and the plates were incubated for 2 h at room temperature on an orbital shaker. Control samples contained prediluted samples only, without BIWA 1 (0% control) or BIWA 1 only without any competing antibodies (100% control). After washing three times with PBS/0.05% Tween 20 (washing buffer), 100 µl of the secondary antibody (peroxidase-conjugated goat anti-mouse Fc, diluted 1:15,000 in assay buffer, DAKO Copenhagen, Denmark) was added for detection of mMAb BIWA 1, and plates were incubated for 1 h at room temperature on an orbital shaker. After washing three times with washing buffer, the plates were developed with 100 µl/well tetramethylbenzidine substrate solution (Kierkegaard and Perry Laboratories, Gaithersburg, USA). The reaction was stopped after 15 min with 50 µl/well 1 M phosphoric acid. Absorbance was measured in an ELISA plate reader at 450 nm (reference 610-690 nm).

Radioiodination of Antibodies. Iodination of MAbs was performed essentially as described by Haisma *et al.* (1986), using either ^{125}I (100 mCi/mL) or ^{131}I (200 mCi/mL), both purchased from Amersham, Aylesbury, England. One mg MAb IgG dissolved in 500 µl PBS, pH=7.4, and 1 mCi ^{125}I or ^{131}I were mixed in a vial coated with 75 µg Iodogen (Pierce, Oud Bijerland, The Netherlands). After 5 minutes of incubation at room temperature, free iodine was removed by gelfiltration on a PD10-column (Pharmacia-LKB, Woerden, The Netherlands). After removal of unbound ^{125}I or ^{131}I the radiochemical purity always exceeded 97% as determined by TLC and HPLC procedures which have been described before (Van Gog et al., 1997a). No aggregates or fragments were formed as assessed by HPLC analysis.

Preparation of Rhenium-186-labeled MAbs. ^{186}Re-labeled MAbs were prepared according to a multistep procedure using the chelate S-benzoylmercaptoacyltriglycine (S-benzoyl-MAG3) as previously described (Van Gog et al., 1997a). In this procedure a solid-state synthesis for the preparation of ^{186}Re-MAG3 is followed by esterification with 2,3,5,6-tetrafluorophenol (TFP) and conjugation of the reactive ^{186}Re-MAG3-TFP ester to the MAb.

After conjugation the ^{186}Re-labeled MAb was purified on a PD10-column. After removal of unbound ^{186}Re the radiochemical purity always exceeded 98%.

Binding-assay for radiolabeled antibodies. *In vitro* binding characteristics of the labeled MAbs used in the biodistribution and therapy studies were determined in an immunoreactivity assay essentially as described previously (Van Gog et al., 1997a). To test the binding of iodinated or ^{186}Re-labeled MAbs, UM-SCC-11B cells fixed in 0.1% glutaraldehyde were used. UM-SCC-11B cells were kindly provided by Dr. T.E. Carey, University of Michigan, Ann Arbor, MI. Five serial dilutions (ranging from 5 x 10^6 cells per tube to 3.1 x 10^5 cells per tube) were prepared with 1% BSA in PBS. Excess of unlabeled MAb IgG was added to a second tube with the lowest concentration of cells to determine non-specific binding. IgG labeled with 10,000 cpm of ^{125}I, ^{131}I or ^{186}Re was added to each tube and the samples were incubated overnight at 4°C. Cells were spun down, radioactivity in the pellet and supernatant was measured in a gamma counter (LKB-Wallace 1282 CompuGamma, Kabi Pharmacia, Woerden, The Netherlands), and the percentage of bound and free radioactivity was calculated. Data were graphically analyzed in a modified Lineweaver Burk plot and the immunoreactivity was determined by linear extrapolation to conditions representing infinite antigen excess.

Biodistribution studies in HNSCC-bearing nude mice. For the biodistribution experiments nude mice bearing subcutaneously implanted human HNSCC xenografts (HNX-OE) were used as described previously (Van Gog et al., 1997a). Female mice (Hsd: Athymic nu/nu, 25-32 g, Harlan CPB, Zeist, The Netherlands) were 8-10 weeks old at the time of the experiments. Three biodistribution experiments were conducted with mice bearing 1 or 2 tumors ranging from 30 to 470 mm^3. In the first experiment, 10 μCi (50 μg) ^{131}I-labeled mMAb U36 were injected simultaneously with 10 μCi (50 μg) ^{125}I-labeled mMAb BIWA 1 in mice bearing tumors of 133 ± 28 mm^3 (n=20 mice, 37 tumors). In the second experiment, 10 μCi (50 μg) ^{131}I-labeled hMAb BIWA 4 and 10 μCi (50 μg) ^{125}I-labeled cMAb BIWA 2 were co-injected in mice bearing tumors of 167 ± 31 mm^3 (n=21 mice, 32 tumors). In the third experiment, 10 μCi (50 μg) ^{131}I-labeled hMAb BIWA 4 and 10 μCi (50 μg) ^{125}I-labeled hMAb BIWA 8 were co-injected in mice with tumors of 130 ± 21 mm^3 (n=23 mice, 40 tumors). Conjugates were

intravenously (i.v.) injected in a volume of 100 µl after dilution in 0.9% NaCl. To obtain a comparable blood/body clearance of the co-injected MAbs, only MAbs with an identical murine or human isotype were combined. The antibody dose (total dose 100 µg per mouse) was chosen high enough to prevent rapid isotype-related elimination of the MAb from the blood (Sharkey et al., 1991, Van Gog et al., 1997b), and low enough to prevent antigen saturation in the tumor.

At indicated time points after injection, mice were anaesthetized, bled, killed and dissected. Besides the tumors, the following organs were removed: liver, spleen, kidney, heart, stomach, ileum, colon, bladder, sternum, muscle, lung, skin and tongue. After weighing, radioactivity in tumors, blood and organs was counted in a dual-isotope gamma counter (LKB-Wallace 1282 CompuGamma), with automatic correction for the ^{131}I-comptons in the ^{125}I window setting. Radioactivity uptake in these tissues was calculated as the percentage of the injected dose per gram of tissue (%ID/g).

Until the day of MAb administration mice were routinely housed under specific-pathogen-free conditions, in sterile cages in a humidity- and temperature controlled clean room, classification 2000 according to the Federal Standard 209d. On the day of injection, mice were transported to a Radio Nuclide Center, and sterile radioimmunoconjugates were administered under aseptic conditions in a laminar flow hood.

Radioimmunotherapy studies in nude mice. Animal RIT studies were performed to compare the therapeutic efficacy of the different MAbs labeled with ^{186}Re. The immunoreactive fractions of the conjugates always exceeded 75 %. Three therapy experiments were conducted with mice bearing 1 or 2 HNX-OE tumors ranging from 45 to 195 mm^3. The ^{186}Re doses were chosen at the maximum tolerated dose (MTD) level (i.e. 400 µCi) or lower (300 µCi). The MTD level is defined as the dose resulting in 5-15% body weight loss. In the first experiment mice were given a single i.v. injection with either 300 µCi (100 µg) ^{186}Re-labeled mMAb U36 or 300 µCi (100 µg) ^{186}Re-labeled mMAb BIWA 1. In the second experiment either 300 µCi (100 µg) ^{186}Re-labeled hMAb BIWA 4 or 300 µCi (100 µg) ^{186}Re-labeled cMAb BIWA 2 were administered, and in the third experiment either 400 µCi (100 µg) ^{186}Re-labeled hMAb BIWA 4 or 400 µCi (100 µg) ^{186}Re-labeled hMAb BIWA 8. Average tumor volumes were

similar for all experimental groups. Experiment 1: 95 mm^3 ± 34 mm^3 (n = 7 mice, 12 tumors) for the ^{186}Re-mMAb U36 treated group, 91 mm^3 ± 15 mm^3 (n = 7 mice, 12 tumors) for the ^{186}Re-mMAb BIWA 1 treated group, and 99 mm^3 ± 54 mm^3 (n = 6 mice, 11 tumors) for the control group. Experiment 2: 101 mm^3 ± 35 mm^3 (n = 7 mice, 12 tumors) for the ^{186}Re-hMAb BIWA 4 treated group, 92 mm^3 ± 43 mm^3 (n = 7 mice, 12 tumors) for the ^{186}Re-cMAb BIWA 2 treated group, while the control group was the same as in experiment 1. Experiment 3: 105 mm^3 ± 43 mm^3 (n = 8 mice, 13 tumors) for the ^{186}Re-hMAb BIWA 4 treated group, 100 mm^3 ± 42 mm^3 (n = 8 mice, 13 tumors) for the ^{186}Re-hMAb BIWA 8 treated group, and 110 mm^3 ± 46 mm^3 (n = 7 mice, 11 tumors) for the control group. During treatment tumors were measured twice weekly and tumor volumes relative to the volume at the start of treatment were calculated. Toxicity was monitored by measurement of the body weight twice weekly. Mice were sacrificed when one of the tumors exceeded 1000 mm^3.

Statistics. Differences in tissue uptake between co-injected MAbs were statistically analyzed for each time point with the Student's *t*-test for paired data. Differences in average tumor volume between the various RIT treatment groups were statistically analyzed for each time point with the Student's *t*-test for independent samples.

RESULTS

***In vitro* binding characteristics of the CD44v6-specific MAbs.** The binding affinities of the five MAbs were analyzed using recombinant antigen as well as human tumor cell lines. Kinetic and affinity constants were evaluated by surface plasmon resonance using GST/CD44v3-v10 as immobilized antigen. Table 1 shows the association rates (k_a), dissociation rates (k_d) and dissociation constants (K_d). mMAb BIWA 1 and cMAb BIWA 2, containing identical variable regions, have similar k_a, k_d, and K_d and show the highest affinity. In contrast, mMAb U36 and hMAb BIWA 4 have lower k_a and higher k_d, resulting in markedly lower dissociation constants (factors 35.0 and 10.5, respectively). hMAb BIWA 8, containing murine residues in the light chain framework region 2, shows a marked decrease of k_d resulting in increased affinity.

Table 1 Kinetics and affinity constants of MAbs directed against CD44v6.

Antibody	k_a ($M^{-1}s^{-1}$)	k_d(s^{-1})	K_d(M)	K_d relative to murine BIWA 1
Murine BIWA 1	1.3×10^5	4.2×10^{-5}	3.2×10^{-10}	1.0
Murine U36	1.5×10^4	1.7×10^{-4}	1.1×10^{-8}	35.0
Chimeric BIWA 2	1.7×10^5	4.1×10^{-5}	2.4×10^{-10}	0.7
Humanized BIWA 4	6.5×10^4	2.2×10^{-4}	3.4×10^{-9}	10.5
Humanized BIWA 8	7.5×10^4	6.3×10^{-5}	8.4×10^{-10}	2.6

The relative binding affinities of the cMAb and the hMAbs were also evaluated in a competitive cell ELISA using human A431 tumor cells (Fig. 1). In accordance with the affinity measurements on recombinant antigen, cMAb BIWA 2 was the most effective competitor, followed by hMAb BIWA 8 and hMAb BIWA 4. Similar results (not shown) were obtained with two other human HNSCC cell lines (FaDu and LICR-LON-HN5).

Biodistribution in HNSCC-bearing nude mice. Biodistribution studies were performed in HNX-OE xenograft bearing nude mice. Two MAbs with identical murine or human isotype were labeled with either ^{125}I or ^{131}I and injected simultaneously (50 μg, 10 μCi each). Each pair of MAbs was selected to provide a stepwise decrease in the difference in affinities: mMAb U36 has a 35.0 fold lower affinity than mMAb BIWA 1 (experiment 1), hMAb BIWA 4 has a 14.0-fold lower affinity than cMAb BIWA 2 (experiment 2) and hMAb BIWA 4 has a 4.0-fold lower affinity than hMAb BIWA 8 (experiment 3). The immunoreactive fractions of all iodinated MAbs were at least 74% after extrapolation (Table 2).

Table 2 Immunoreactive fraction of iodinated MAbs determined by binding to UM-SCC-11B cells.

Experiment no.	Antibody	Label	Binding to $5x10^6$ cells (%)	Binding Extrapolated[a] (%)
1	Murine U36	^{131}I	59.7	87.4
	Murine BIWA 1	^{125}I	91.1	91.1
2	Humanized BIWA 4	^{131}I	77.4	82.3
	Chimeric BIWA 2	^{125}I	80.5	79.9
3	Humanized BIWA 4	^{131}I	77.3	74.5
	Humanized BIWA 8	^{125}I	91.8	92.1

[a] Immunoreactivity was determined by linear extrapolation to conditions representing infinite antigen excess: see Materials and Methods

The biodistributions in experiment 1 were determined at day 1, 2, 3 and 7 after injection; biodistributions in experiments 2 and 3 were determined at day 1, 2, 4 and 7 days after injection. The calculated average %ID/g of tumor and blood of all three experiments are given in Table 3.

Table 3 Tumor and blood levels of iodinated CD44v6-specific MAbs with different affinity after co-injection to HNX-OE bearing mice

			Conjugate A (%ID/g)		Conjugate B (%ID/g)		Conj. A:B ratio	
Exp. No		Time after injection	Tumor	Blood	Tumor	Blood	Tumor	Blood
1	Conj. A: ^{131}I-mMAb U36	1 d	15.7	17.9	13.0	17.8	1.2	1.0
	Conj. B: ^{125}I-mMAb BIWA 1	2 d	18.4	15.0	13.4	14.9	1.4	1.0
		3 d	20.6	11.8	13.7	11.0	1.5	1.1
		7 d	16.5	7.1	7.8	4.8	2.1	1.5

2. Conj. A:^{131}I-hMAb BIWA 4	1 d	10.8	10.2	9.1	10.6	1.2	
1.0							
Conj. B: ^{125}I-cMAb BIWA 2	2 d	12.4	10.2	9.8	10.1	1.3	1.0
	4 d	12.9	7.2	8.9	7.2	1.5	1.0
	7 d	7.6	3.3	4.8	2.7	1.6	1.2
3. .Conj. A:^{131}I-hMAb BIWA 4	1 d	10.5	11.1	9.5	11.4	1.1	
1.0							
Conj. B: ^{125}I-hMAb BIWA 8	2 d	10.9	10.0	9.6	9.8	1.1	1.0
	4 d	11.7	6.2	9.6	5.9	1.2	1.0
	7 d	10.1	4.2	7.8	3.9	1.3	1.1

For each pair of co-injected MAbs the uptake ratios for tumor and blood are provided. The average %ID/g and s.e.m. of tumor, blood and various organs at 3 (experiment 1) or 4 days p.i. (experiments 2 and 3) are shown in Figure 2.

In a direct comparison of the two murine MAbs, tumor uptake of low affinity U36 was significantly higher than uptake of high affinity BIWA 1 at all time points ($p < 0.001$) (Table 3). In contrast, no significant differences were found between the uptake values of these MAbs in blood and normal tissues at 1, 2 and 3 days p.i.. At day 7 p.i., BIWA 1 levels in blood and most of the organs were significantly lower ($p < 0.05$) than U36 levels, indicating more rapid clearance of BIWA 1 from the blood/body. A 50% higher tumor uptake of U36 in comparison with BIWA 1 at day 3 p.i. is illustrated by Figure 2A.

Similar relationships were found in the evaluation of the two other MAb pairs. hMAb BIWA 4, while having the lower affinity, showed a significantly higher tumor uptake ($p < 0.001$) than cMAb BIWA 2 and hMAb BIWA 8 at all time points (Table 3). In contrast, MAb levels in blood and normal tissues were similar for these pairs of MAbs at 1, 2, and 4 days p.i.. At 7 days p.i., BIWA 2 and BIWA 8 levels in blood and most of the organs were significantly lower ($p < 0.05$) than BIWA 4 levels, indicating more rapid clearance of these MAbs from the blood/body. A 45% higher tumor uptake of BIWA 4 in comparison with BIWA 2 is illustrated by Figure 2B, while a 20% higher tumor uptake of BIWA 4 in comparison with BIWA 8 is illustrated by Figure 2C, for the 4 days post injection time points.

Consistent results were obtained from an additional experiment (data not shown) in which the radiolabels were exchanged: ^{125}I-BIWA 4 *versus* ^{131}I-BIWA 8 instead of ^{131}I-BIWA 4 *versus*

^{125}I-BIWA 8. Data from this latter experiment rule out the possibility that the type of radiolabel had influenced the pharmacokinetic behavior of the labeled MAb.

Radioimmunotherapy in HNSCC-bearing nude mice. From the three biodistribution experiments it appeared that the low affinity MAbs showed a higher and more selective tumor uptake than the high affinity MAbs, and thus might be better suited for RIT. To test this possibility the following treatment groups were compared in RIT studies with HNX-OE xenograft bearing mice:

Experiment 1: 300 µCi ^{186}Re-U36 or 300 µCi ^{186}Re-BIWA 1 or saline as control. Experiment 2: 300 µCi ^{186}Re-BIWA 4 or 300 µCi ^{186}Re-BIWA 2 or saline as control. Experiment 3: 400 µCi ^{186}Re- BIWA 4 or 400 µCi ^{186}Re-BIWA 8 or saline as control.

In Figure 3, the mean relative tumor volume (as a percentage of the tumor volume at day 0) for the control and treatment groups is plotted against time. Tumors of mice in the control group in all three experiments showed exponential growth with a tumor volume doubling time of about 7 days. In the groups treated with the ^{186}Re-labeled MAbs, tumors stopped growing, in some cases accompanied by tumor regression, shortly after injection of the conjugates. However, all tumors ultimately regrew.

In experiment 1, administration of 300 µCi ^{186}Re-BIWA 1 resulted in a decrease of the tumor growth rate, but not in a reduction of the mean tumor size. Administration of 300 µCi ^{186}Re-U36, however, caused a reduction of the mean tumor volume from 185 mm^3 to 120 mm^3 between day 7 and day 17 post injection, after which tumors started growing again. The mean relative tumor volume in the ^{186}Re-U36-treated group was significantly smaller ($p < 0.001$) than that of the ^{186}Re-BIWA 1-treated group from day 14 on.

In experiment 2, administration of either 300 µCi ^{186}Re-BIWA 4 or 300 µCi ^{186}Re-BIWA 2 resulted in tumor growth arrest at day 7 with start of regrowth at day 17 p.i.. BIWA 4 was more effective in RIT than BIWA 2 from day 14 on, but a significant difference between the mean relative tumor volumes was only found at day 14 p.i. ($p < 0.05$).

In experiment 3, mice were treated with either 400 µCi ^{186}Re-BIWA 4 or BIWA 8, which resulted in a decrease of the relative tumor volume to a minimum of 80 ± 62 % and 98 ± 81%, respectively, at day 19. Thereafter, tumors started regrowth.

These data indicate that the low affinity MAb BIWA 4 is more effective in RIT than the high affinity Mabs cBIWA 2 and BIWA 8.

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Example 2 Details of sequences

This example shows the details of sequences, e.g. the position of cloning sites, leaders and untranslated regions.

Abbreviations:
aa = amino acids
nt = nucleotide sequence

SEQ ID No. 1 VH BIWA 4/8 aa
EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSTISSGGSY
TYYLDSIKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGLDYWGRGTLVTVSS

SEQ ID No. 2 VL BIWA 4 aa
EIVLTQSPATLSLSPGERATLSCSASSSINYIYWYQQKPGQAPRLLIYLTSNLASGVPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCLQWSSNPLTFGGGTKVEIK

SEQ ID No. 3 VL BIWA 8 aa
EIVLTQSPATLSLSPGERATLSCSASSSINYIYWLQQKPGQAPRILIYLTSNLASGVPARF
SGSGSGTDFTLTISSLEPEDFAVYYCLQWSSNPLTFGGGTKVEIK

SEQ ID No. 4 VH BIWA 4/8 nt
GAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTAAGACTCTCC
TGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGACATGTCTTGGGTTCGCCAGGCTCCGGGG
AAGGGGCTGGAGTGGGTCTCAACCATTAGTAGTGGTGGTAGTTACACCTACTATCTAGACAGT
ATAAAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCCCTGTACCTGCAAATGAAC
AGTCTGAGGGCTGAGGACACGGCCGTGTATTACTGTGCAAGACAGGGGTTGGACTACTGGGGT
CGAGGAACCTTAGTCACCGTCTCCTCA

SEQ ID No. 5 VL BIWA 4 nt

GAAATTGTTCTCACCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTG
TCCTGCAGTGCCAGCTCAAGTATAAATTACATATACTGGTACCAGCAGAAGCCAGGACAGGCT
CCTAGACTCTTGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCTGCGCGCTTCAGTGGC
AGTGGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTGAAGATTTTGCCGTT
TATTACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGATT
AAA

SEQ ID No. 6 VL BIWA 8 nt

GAAATTGTTCTCACCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTG
TCCTGCAGTGCCAGCTCAAGTATAAATTACATATACTGGCTCCAGCAGAAGCCAGGACAGGCT
CCTAGAATCTTGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCTGCGCGCTTCAGTGGC
AGTGGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTGAAGATTTTGCCGTT
TATTACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGATT
AAA

SEQ ID No. 7 heavy chain (variable + constant) BIWA 4/8 aa

EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYDMSWVRQAPGKGLEWVSTISSGGSY
TYYLDSIKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGLDYWGRGTLVTVSS
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID No. 8 light chain (variable + constant) BIWA 4 aa

EIVLTQSPATLSLSPGERATLSCSASSSINYIYWYQQKPGQAPRLLIYLTSNLASGVPAR
FSGSGSGTDFTLTISSLEPEDFAVYYCLQWSSNPLTFGGGTKVEIKRTVAAPSVFIFPPS
DEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSST
LTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No. 9 light chain (variable + constant) BIWA 8 aa

EIVLTQSPATLSLSPGERATLSCSASSSINYIYWLQQKPGQAPRILIYLTSNLASGVPARF
SGSGSGTDFTLTISSLEPEDFAVYYCLQWSSNPLTFGGGTKVEIKRTVAAPSVFIFPPSD
EQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTL
TLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID No. 10 heavy chain (variable + constant) BIWA 4/8 nt ; insert in pAD-CMV1/pAD-CMV19, contains introns (lower case) between CH1-hinge, hinge-CH2, CH2-CH3, leader sequence underlined, non-translated sequences in italic, cloning sites bold

aagctt*tgacagacgcacaaccctggactcccaagtctttctcttcagtgacaaacacagacataggatatcaca*
*tttgcttctgacacaactgtgttcactagcagcctcaaacagacacc*ATGAACTTTGGGCTCAGCTTGATTTTCC
TTGTCCTAATTTTAAAAGGTGTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAG
GGTCCCTAAGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGACATGTCTTGGGTTCGCCAGGCTC
CGGGGAAGGGGCTGGAGTGGGTCTCAACCATTAGTAGTGGTGGTAGTTACACCTACTATCTAGACAGTATAAAGG
GCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCCCTGTACCTGCAAATGAACAGTCTGAGGGCTGAGGACA
CGGCCGTGTATTACTGTGCAAGACAGGGGTTGGACTACTGGGGTCGAGGAACCTTAGTCACCGTCTCCTCAGCTA
GCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT
GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACA
CCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGG
GCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTggtgagaggc
cagcacagggagggagggtgtctgctggaagcaggctcagcgctcctgcctggacgcatcccggctatgcagccc
cagtccagggcagcaaggcaggccccgtctgcctcttcacccggagcctctgcccgccccactcatgctcaggga
gagggtcttctggctttttcccaggctctgggcaggcacaggctaggtgcccctaacccaggccctgcacacaaa
ggggcaggtgctgggctcagacctgccaagagccatatccgggaggaccctgcccctgacctaagcccaccccaa
aggccaaactctccactccctcagctcggacaccttctctcctcccagattccagtaactcccaatcttctctct
gcaGAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAggtaagccagcccaggcctcgccctcc
agctcaaggcgggacaggtgccctagagtagcctgcatccagggacaggccccagccgggtgctgacacgtccac
ctccatctcttcctcaGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACAC
CCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTT
CAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTA

CCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAA
CAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAggtgggacccgtggggtgcgagggccaca
tggacagaggccggctcggcccaccctctgccctgagagtgaccgctgtaccaacctctgtcctacaGGGCAGCC
CCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCT
GGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAACTACAAGAC
CACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCA
GCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCT
GTCTCCGGGTAAATGA*gtgcgacggccgc**gaattc***

SEQ ID No. 11 light chain (variable + constant) BIWA 4 nt ; sequence in pAD-CMV1/pAD-CMV19, leader sequence underlined, non-translated sequences in italic, cloning sites bold

aagctt*gatcttcaggatatcacatttgcttctgacacaactgtgttcactagcaacctcaaacagacacc*ATGG
ATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATGAGTGCCTCAGTCATAATGTCCAGGGGAGAAATTGTTCTCA
CCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTGTCCTGCAGTGCCAGCTCAAGTATAA
ATTACATATACTGGTACCAGCAGAAGCCAGGACAGGCTCCTAGACTCTTGATTTATCTCACATCCAACCTGGCTT
CTGGAGTCCCTGCGCGCTTCAGTGGCAGTGGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTG
AAGATTTTGCCGTTTATTACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGA
TTAAACGGACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCTA
GCGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAAT
CGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGC
TGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG***gaattc***

SEQ ID No. 12 light chain (variable + constant) BIWA 8 nt ; sequence in pAD-CMV1/pAD-CMV19, leader sequence underlined, non-translated sequences in italic, cloning sites bold

aagctt*gatcttcaggatatcacatttgcttctgacacaactgtgttcactagcaacctcaaacagacacc*ATGG
ATTTTCAGGTGCAGATTTTCAGCTTCCTGCTAATGAGTGCCTCAGTCATAATGTCCAGGGGAGAAATTGTTCTCA
CCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTGTCCTGCAGTGCCAGCTCAAGTATAA
ATTACATATACTGGCTCCAGCAGAAGCCAGGACAGGCTCCTAGAATCTTGATTTATCTCACATCCAACCTGGCTT
CTGGAGTCCCTGCGCGCTTCAGTGGCAGTGGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTG
AAGATTTTGCCGTTTATTACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGA
TTAAACGGACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAACTGCTA
GCGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAAT
CGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGC
TGAGCAAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCA
CAAAGAGCTTCAACAGGGGAGAGTGTTAG***gaattc***

SEQ ID No. 13 heavy chain (variable + constant) BIWA 4/8 nt ; sequence in N5KG1val, no introns contained, leader sequence underlined

ATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGTGAAGTG
CAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTAAGACTCTCCTGTGCA
GCCTCTGGATTCACTTTCAGTAGCTATGACATGTCTTGGGTTCGCCAGGCTCCGGGGAAGGGG
CTGGAGTGGGTCTCAACCATTAGTAGTGGTGGTAGTTACACCTACTATCTAGACAGTATAAAG
GGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCCCTGTACCTGCAAATGAACAGTCTG
AGGGCTGAGGACACGGCCGTGTATTACTGTGCAAGACAGGGGTTGGACTACTGGGGTCGAGGA
ACCTTAGTCACCGTCTCCTCAGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCC
TCCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAA
CCGGTGACGGTGTCGTGGAACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTC
CTACAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGC
ACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTT

GAGCCCAAATCTTGTGACAAAACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGG
GGACCGTCAGTCTTCCTCTTCCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCT
GAGGTCACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTAC
GTGGACGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACG
TACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAG
TGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGG
CAGCCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAG
GTCAGCCTGACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGC
AATGGGCAGCCGGAGAACAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTC
TTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGC
TCCGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGT
AAATGA

SEQ ID No. 14 light chain (variable + constant) BIWA 4 nt ; sequence in N5KG1val, leader sequence underlined

ATGGAAGCCCCAGCTCAGCTTCTCTTCCTCCTGCTGCTCTGGCTCCCAGATACCACCGGAGAA
ATTGTTCTCACCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTGTCC
TGCAGTGCCAGCTCAAGTATAAATTACATATACTGGTACCAGCAGAAGCCAGGACAGGCTCCT
AGACTCTTGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCTGCGCGCTTCAGTGGCAGT
GGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTGAAGATTTTGCCGTTTAT
TACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGATTAAA
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG
GTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC
AGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA
GAGTGTTGA

SEQ ID No. 15 light chain (variable + constant) BIWA 8 nt ; sequence in N5KG1val, leader sequence underlined

ATGGAAGCCCCAGCTCAGCTTCTCTTCCTCCTGCTGCTCTGGCTCCCAGATACCACCGGAGAA
ATTGTTCTCACCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACCCTGTCC
TGCAGTGCCAGCTCAAGTATAAATTACATATACTGGCTCCAGCAGAAGCCAGGACAGGCTCCT
AGAATCTTGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCTGCGCGCTTCAGTGGCAGT
GGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTGAAGATTTTGCCGTTTAT
TACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAGATTAAA
CGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGA
ACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAG
GTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGCAAGGAC
AGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTC
TACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA
GAGTGTTGA

SEQ ID No. 16 BIWA 4 in N5KG1val

CTGACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGCGTGACCG
CTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTCGCTTTCTTCCCTTCCTTTCTCGCCACGT

TCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTAGGGTTCCGATTTAGTGCTT
TACGGCACCTCGACCCCAAAAAACTTGATTAGGGTGATGGTTCACGTAGGGTCGCGACGTACC
GGGCCCCCCCTCGATTAATTAATCGAGCTACTAGCTTTGCTTCTCAATTTCTTATTTGCATAA
TGAGAAAAAAAGGAAAATTAATTTTAACACCAATTCAGTAGTTGATTGAGCAAATGCGTTGCC
AAAAAGGATGCTTTAGAGACAGTGTTCTCTGCACAGATAAGGACAAACATTATTCAGAGGGAG
TACCCAGAGCTGAGACTCCTAAGCCAGTGAGTGGCACAGCATTCTAGGGAGAAATATGCTTGT
CATCACCGAAGCCTGATTCCGTAGAGCCACACCTTGGTAAGGGCCAATCTGCTCACACAGGAT
AGAGAGGGCAGGAGCCAGGGCAGAGCATATAAGGTGAGGTAGGATCAGTTGCTCCTCACATTT
GCTTCTGACATAGTTGTGCCAGCATGGAGGAATCGATCCTCCATGCTTGAACAAGATGGATTG
CACGCAGGTTCTCCGGCCGCTTGGGTGGAGAGGCTATTCGGCTATGACTGGGCACAACAGACA
ATCGGCTGCTCTGATGCCGCCGTGTTCCGGCTGTCAGCGCAGGGGCGCCCGGTTCTTTTTGTC
AAGACCGACCTGTCCGGTGCCCTGAATGAACTGCAGGTAAGTGCGGCCGCTCTAGGCCTCCAA
AAAAGCCTCCTCACTACTTCTGGAATAGCTCAGAGGCCGAGGCGGCCTCGGCCTCTGCATAAA
TAAAAAAAATTAGTCAGCCATGCATGGGGCGGAGAATGGGCGGAACTGGGCGGAGTTAGGGGC
GGGATGGGCGGAGTTAGGGGCGGGACTATGGTTGCTGACTAATTGAGATGCATGCTTTGCATA
CTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCTGGTTGCTGACTAATTGAGATGCATG
CTTTGCATACTTCTGCCTGCTGGGGAGCCTGGGGACTTTCCACACCCTAACTGACACACATTC
CACAGAATTAATTCCCCTAGTTATTAATAGTAATCAATTACGGGGTCATTAGTTCATAGCCCA
TATATGGAGTTCCGCGTTACATAACTTACGGTAAATGGCCCGCCTGGCTGACCGCCCAACGAC
CCCCGCCCATTGACGTCAATAATGACGTATGTTCCCATAGTAACGCCAATAGGGACTTTCCAT
TGACGTCAATGGGTGGACTATTTACGGTAAACTGCCCACTTGGCAGTACATCAAGTGTATCAT
ATGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAG
TACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACC
ATGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTT
CCAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTT
CCAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAG
GTCTATATAAGCAGAGCTGGGTACGTGAACCGTCAGATCGCCTGGAGACGCCATCACAGATCT
CTCACCATGGAAGCCCCAGCTCAGCTTCTCTTCCTCCTGCTGCTCTGGCTCCCAGATACCACC
GGAGAAATTGTTCTCACCCAGTCTCCAGCAACCCTGTCTCTGTCTCCAGGGGAGAGGGCCACC
CTGTCCTGCAGTGCCAGCTCAAGTATAAATTACATATACTGGTACCAGCAGAAGCCAGGACAG
GCTCCTAGACTCTTGATTTATCTCACATCCAACCTGGCTTCTGGAGTCCCTGCGCGCTTCAGT
GGCAGTGGGTCTGGAACCGACTTCACTCTCACAATCAGCAGCCTGGAGCCTGAAGATTTTGCC
GTTTATTACTGCCTGCAGTGGAGTAGTAACCCGCTCACATTCGGTGGTGGGACCAAGGTGGAG
ATTAAACGTACGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAA
TCTGGAACTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAG
TGGAAGGTGGATAACGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGACAGC
AAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGCAAAGCAGACTACGAGAAACAC
AAAGTCTACGCCTGCGAAGTCAC
CCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGAGAGTGTTGAATTCAGAT
CCGTTAACGGTTACCAACTACCTAGACTGGATTCGTGACAACATGCGGCCGTGATATCTACGT
ATGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCC
TTCCTTGACCCTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATC
GCATTGTCTGAGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGA
GGATTGGGAAGACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGAC
TAGTAGCTTTGCTTCTCAATTTCTTATTTGCATAATGAGAAAAAAAGGAAAATTAATTTTAAC
ACCAATTCAGTAGTTGATTGAGCAAATGCGTTGCCAAAAAGGATGCTTTAGAGACAGTGTTCT
CTGCACAGATAAGGACAAACATTATTCAGAGGGAGTACCCAGAGCTGAGACTCCTAAGCCAGT
GAGTGGCACAGCATTCTAGGGAGAAATATGCTTGTCATCACCGAAGCCTGATTCCGTAGAGCC
ACACCTTGGTAAGGGCCAATCTGCTCACACAGGATAGAGAGGGCAGGAGCCAGGGCAGAGCAT
ATAAGGTGAGGTAGGATCAGTTGCTCCTCACATTTGCTTCTGACATAGTTGTGTTGGGAGCTT

GGATAGCTTGGACAGCTCAGGGCTGCGATTTCGCGCCAAACTTGACGGCAATCCTAGCGTGAA
GGCTGGTAGGATTTTATCCCCGCTGCCATCATGGTTCGACCATTGAACTGCATCGTCGCCGTG
TCCCAAAATATGGGGATTGGCAAGAACGGAGACCTACCCTGGCCTCCGCTCAGGAACGAGTTC
AAGTACTTCCAAAGAATGACCACAACCTCTTCAGTGGAAGGTAAACAGAATCTGGTGATTATG
GGTAGGAAAACCTGGTTCTCCATTCCTGAGAAGAATCGACCTTTAAAGGACAGAATTAATATA
GTTCTCAGTAGAGAACTCAAAGAACCACCACGAGGAGCTCATTTTCTTGCCAAAAGTTTGGAT
GATGCCTTAAGACTTATTGAACAACCGGAATTGGCAAGTAAAGTAGACATGGTTTGGATAGTC
GGAGGCAGTTCTGTTTACCAGGAAGCCATGAATCAACCAGGCCACCTTAGACTCTTTGTGACA
AGGATCATGCAGGAATTTGAAAGTGACACGTTTTTCCCAGAAATTGATTTGGGGAAATATAAA
CTTCTCCCAGAATACCCAGGCGTCCTCTCTGAGGTCCAGGAGGAAAAAGGCATCAAGTATAAG
TTTGAAGTCTACGAGAAGAAAGACTAACAGGAAGATGCTTTCAAGTTCTCTGCTCCCCTCCTA
AAGCTATGCATTTTTATAAGACCATGGGACTTTTGCTGGCTTTAGATCAGCCTCGACTGTGCC
TTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACCCTGGAAGGTGC
CACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTGAGTAGGTGTCA
TTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAAGACAATAGCAG
GCATGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGGCTCGAAGCGGCCGCTCCGGATA
TGCCAAGTACGCCCCCTATTGACGTCAATGACGGTAAATGGCCCGCCTGGCATTATGCCCAGT
ACATGACCTTATGGGACTTTCCTACTTGGCAGTACATCTACGTATTAGTCATCGCTATTACCA
TGGTGATGCGGTTTTGGCAGTACATCAATGGGCGTGGATAGCGGTTTGACTCACGGGGATTTC
CAAGTCTCCACCCCATTGACGTCAATGGGAGTTTGTTTTGGCACCAAAATCAACGGGACTTTC
CAAAATGTCGTAACAACTCCGCCCCATTGACGCAAATGGGCGGTAGGCGTGTACGGTGGGAGG
TCTATATAAGCAGAGCTGGGTACGTCCTCACATTCAGTGATCAGCACTGAACACAGACCCGTC
GACATGGAGTTTGGGCTGAGCTGGCTTTTTCTTGTGGCTATTTTAAAAGGTGTCCAGTGTGAA
GTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGGGTCCCTAAGACTCTCCTGT
GCAGCCTCTGGATTCACTTTCAGTAGCTATGACATGTCTTGGGTTCGCCAGGCTCCGGGGAAG
GGGCTGGAGTGGGTCTCAACCATTAGTAGTGGTGGTAGTTACACCTACTATCTAGACAGTATA
AAGGGCCGATTCACCATCTCCAGAGACAATGCCAAGAACTCCCTGTACCTGCAAATGAACAGT
CTGAGGGCTGAGGACACGGCCGT
GTATTACTGTGCAAGACAGGGGTTGGACTACTGGGGTCGAGGAACCTTAGTCACCGTCTCCTC
AGCTAGCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTGGGGG
CACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCGTGGAA
CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTA
CTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAA
CGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGAAAGTTGAGCCCAAATCTTGTGACAA
AACTCACACATGCCCACCGTGCCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCCTCTT
CCCCCCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGT
GGACGTGAGCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCA
TAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGTGTGGTCAGCGTCCT
CACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC
CCTCCCAGCCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGT
GTACACCCTGCCCCCATCCCGGGATGAGCTGACCAAGAACCAGGTCAGCCTGACCTGCCTGGT
CAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAA
CTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTCCTCTACAGCAAGCTCAC
CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCT
GCACAACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAATGAGGATCCGTTAACGG
TTACCAACTACCTAGACTGGATTCGTGACAACATGCGGCCGTGATATCTACGTATGATCAGCC
TCGACTGTGCCTTCTAGTTGCCAGCCATCTGTTGTTTGCCCCTCCCCCGTGCCTTCCTTGACC
CTGGAAGGTGCCACTCCCACTGTCCTTTCCTAATAAAATGAGGAAATTGCATCGCATTGTCTG
AGTAGGTGTCATTCTATTCTGGGGGGTGGGGTGGGGCAGGACAGCAAGGGGGAGGATTGGGAA
GACAATAGCAGGCATGCTGGGGATGCGGTGGGCTCTATGGAACCAGCTGGGGCTCGACAGCGC

TGCGATCGCCTCGAGGCCGCTACTAACTCTCTCCTCCCTCCTTTTTCCTGCAGGACGAGGCAG
CGCGGCTATCGTGGCTGGCCACGACGGGCGTTCCTTGCGCAGCTGTGCTCGACGTTGTCACTG
AAGCGGGAAGGGACTGGCTGCTATTGGGCGAAGTGCCGGGGCAGGATCTCCTGTCATCTCACC
TTGCTCCTGCCGAGAAAGTATCCATCATGGCTGATGCAATGCGGCGGCTGCATACGCTTGATC
CGGCTACCTGCCCATTCGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTACTCGGATGG
AAGCCGGTCTTGTCGATCAGGATGATCTGGACGAAGAGCATCAGGGGCTCGCGCCAGCCGAAC
TGTTCGCCAGGCTCAAGGCGCGCATGCCCGACGGCGAGGATCTCGTCGTGACCCATGGCGATG
CCTGCTTGCCGAATATCATGGTGGAAAATGGCCGCTTTTCTGGATTCATCGACTGTGGCCGGC
TGGGTGTGGCGGACCGCTATCAGGACATAGCGTTGGCTACCCGTGATATTGCTGAAGAGCTTG
GCGGCGAATGGGCTGACCGCTTCCTCGTGCTTTACGGTATCGCCGCTCCCGATTCGCAGCGCA
TCGCCTTCTATCGCCTTCTTGACGAGTTCTTCTGAGCGGGACTCTGGGGTTCGAAATGACCGA
CCAAGCGACGCCCAACCTGCCATCACGAGATTTCGATTCCACCGCCGCCTTCTATGAAAGGTT
GGGCTTCGGAATCGTTTTCCGGGACGCCGGCTGGATGATCCTCCAGCGCGGGGATCTCATGCT
GGAGTTCTTCGCCCACCCCAACTTGTTTATTGCAGCTTATAATGGTTACAAATAAAGCAATAG
CATCACAAATTTCACAAATAAAGCATTTTTTTCACTGCATTCTAGTTGTGGTTTGTCCAAACT
CATCAATCTATCTTATCATGTCTGGATCGCGGCCGGCCGCACCGCGGTGGAGCTTTAATTAAG
GCGCGCCAGCTCCAGCTTTTGTTCCCTTTAGTGAGGGTTAATTTCGAGCTTGGCGTAATCATG
GTCATAGCTGTTTCCTGTGTGAA
ATTGTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAAAGTGTAAAGCCTGGG
GTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCACTGCCCGCTTTCCAGTCGG
GAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGTA
TTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAG
CGGTATCAGCTCACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAA
AGAACATGTGAGCAAAAGGCCAGCAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGT
TTTTCCATAGGCTCCGCCCCCCTGACGAGCATCACAAAAATCGACGCTCAAGTCAGAGGTGGC
GAAACCCGACAGGACTATAAAGATACCAGGCGTTTCCCCCTGGAAGCTCCCTCGTGCGCTCTC
CTGTTCCGACCCTGCCGCTTACCGGATACCTGTCCGCCTTTCTCCCTTCGGGAAGCGTGGCGC
TTTCTCATAGCTCACGCTGTAGGTATCTCAGTTCGGTGTAGGTCGTTCGCTCCAAGCTGGGCT
GTGTGCACGAACCCCCCGTTCAGCCCGACCGCTGCGCCTTATCCGGTAACTATCGTCTTGAGT
CCAACCCGGTAAGACACGACTTATCGCCACTGGCAGCAGCCACTGGTAACAGGATTAGCAGAG
CGAGGTATGTAGGCGGTGCTACAGAGTTCTTGAAGTGGTGGCCTAACTACGGCTACACTAGAA
GGACAGTATTTGGTATCTGCGCTCTGCTGAAGCCAGTTACCTTCGGAAAAAGAGTTGGTAGCT
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SEQ ID No. 18 pAD-CMV19, cloning sites in bold

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Example 3 Clinical study 1170.1

1. LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

ADCC	Antibody Dependent Cell-mediated Cytotoxicity
AE	Adverse Event
ALT	Alanine Amino Transferase
a.p.	Anterior posterior
AP	Alkaline phosphatase
AST	Aspartate Amino Transferase
AUC	Area Under the concentration-time Curve
Bq	Becquerel, SI unit for radioactivity Becquerel (1Bq = one decay /s)
BSA	Bovine Serum Albumin
CD44v6	CD44 variant isoform v6
CDS	Corporate Drug Safety
cGy	Centi Gray measure for radioactivity
CHO	Chinese Hamster Ovary cells
Ci	Curie; unit for radioactivity; 1 Ci = 37 x 10^9 decays / s = 37 GBq
CL	Total body clearance
cMAb	Chimeric Monoclonal Antibody
cm	Centimetre
C_{max}	Maximum drug concentration observed
cpm	Count per minute
CRF	Case Report/Record Form
CT (scan)	Computed Tomography
CTC	Common Toxicity Criteria
CV	Coefficient of Variation
DLT	Dose Limiting Toxicity
ECG	Electrocardiogram
ELISA	Enzyme-Linked Immuno-Sorbent Assay
ENT	Ear Nose Throat
f	Female
FDA	Food and Drug Administration
g	Gram
GBq	SI unit for radioactivity Giga Becquerel (1GBq = 10^9 decays /s)
GCP	Good Clinical Practice

GGT	Gamma Glutaryl Transpeptidase
GMP	Good Manufacturing Practice
Gy	Gray
HAHA	Human-Anti-Human-Antibody
Hb	Haemoglobin
HER2	Human Epidermal growth factor Receptor 2
hMAb	Humanised Monoclonal Antibody
HNSCC	Head and Neck Squamous Cell Carcinoma
HPLC	High Performance Liquid Chromatography
hr / h	Hour
hrs	Hours
Ht	Haematocrit
^{131}I	Iodine-131 (half-life 8.05 days)
ICH	International committee on harmonisation
ID	Injected Dose
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
INN	International Non proprietary Name
IRB	Institutional Review Board
ITT	Intent-To-Treat
i.v.	Intravenous(ly)
KeV	Kilo electron volt
l / L	Litre
m	Male
m^2	Square metres
MAb	Monoclonal Antibody
MAG2GABA-TFP	Mercaptoacetylglycylglycyl-gamma-aminobutyrate-tetrafluorophenol ester (MAG2GABA-TFP), chelate used for coupling ^{186}Re to monoclonal antibodi (future method)
MAG3	Mercaptoacetyltriglycine. Chelate used for coupling of ^{99m}Tc and ^{186}Re to monoclonal antibodies
MBq	SI unit for radioactivity; Mega Becquerel (1 MBq = 10^6 decays/s)
mCi	Milli Curie; unit for radioactivity; 1 mCi = 37 million decays / s = 37 MBq
MCV	Mean Corpuscular Volume
mGy	Milli Gray
µg	Microgram
mg	Milligram
min	Minute

ml / mL	Millilitre
mMAb	Murine Monoclonal Antibody
mmHg	Millimetre mercury
µmol	Micromol
mmol	Millimol
MRI	Magnetic Resonance Imaging
MRT	Mean Residence Time
mSv	Milli Sievert
MTD	Maximum Tolerated Dose
NA / n.a.	Not Applicable
NCI	US National Cancer Institute
ND	Not Done
ng	Nanogram
No / N	Number
nos	Not other specified
NSCLC	Non Small Cell Lung Cancer
n.y.r.	Not yet recovered
p.a.	Posterior anterior
PBS	Phosphate Buffered Saline
p.i.	Post infusion
p.o.	Per os
PP	Per Protocol
Pt. / Pat	Patient
Pts.	Patients
^{186}Re	Rhenium-186, radionuclide; half-life of 3.7 days (tissue penetration of ß-particles of about 1.2 mm)
Recov.	Recovered
RES	Reticulo Endothelial System
RIS	Radioimmunoscintigraphy
RIT	Radioimmunotherapy
ROI	Region of Interest
SAE	Serious Adverse Event
SCC	Squamous Cell Carcinoma
sCD44v6	Soluble CD44v6
SD	Stable Disease
sGOT	Serum Glutamic Oxalacetic Transaminase
sGPT	Serum Glutamic Pyruvic Transaminase

SOC	System Organ Class
SOP	Standard Operating Procedure
SPECT	Single Photon Emission Computed Tomography
$t_{½}$	Elimination half life
^{99m}Tc	Technetium 99m (half-life 6 hours)
TLC	Thin Layer Chromatography
T_{max}	Time point at which the maximum drug concentration is observed
TNM	Tumour Node Metastasis system for staging tumours
TSH	Thyroid Stimulating Hormone
UICC	Union Internationale Contre le Cancer (international union against cancer)
unk	Unknown
V_{ss}	Apparent volume of distribution under steady-state conditions
V_z	Apparent volume of distribution during the terminal phase
WBC	White Blood Count
WHO	World Health Organisation

2. Study Objectives

2.1 GENERAL AIM / CLINICAL OBJECTIVE

The general aim of the present study was to assess the safety and tolerability of intravenously administered ^{99m}Tc and ^{186}Re-labelled hMAb BIWA 4, to confirm preferential accumulation in the tumour of ^{99m}Tc-labelled hMAb BIWA 4, to determine the maximum tolerated radiation dose of ^{186}Re-labelled hMAb BIWA 4 and to propose a safe dose for phase II development. In order to reach this goal the study was divided into two parts:

Part A:

Objectives:

- To determine the safety and tolerability of a single infusion of intravenously administered ^{99m}Tc-labelled hMAb BIWA 4 in patients with advanced squamous cell carcinoma of the head and neck.

- To determine the biodistribution of a single infusion of ^{99m}Tc-labelled hMAb BIWA 4 at different BIWA 4 dose levels in patients with advanced squamous cell carcinoma of the head and neck.

- To study the pharmacokinetics of a single infusion of ^{99m}Tc-labelled hMAb BIWA 4.

Part B:

Objectives

- To determine the qualitative and quantitative toxic effects of ^{186}Re-labelled hMAb BIWA 4 and to study the predictability, onset, duration, intensity, reversibility and dose-relationship of the toxic side effects.

- To determine the maximum tolerated radiation dose of intravenously administered ^{186}Re-labelled hMAb BIWA 4 in head and neck cancer patients.

- To study the pharmacokinetics of intravenously administered ^{186}Re-labelled hMAb BIWA 4 in patients with squamous cell carcinoma of the head and neck.

- A secondary objective was to determine the preliminary therapeutic effects of ^{186}Re-labelled hMAb BIWA 4.

The following objectives outlined in the protocol could not be addressed during the performance of the trial. The development of the programme with the linker chelate mercaptoacetyltriglycine (MAG3), used for coupling of ^{99m}Tc and ^{186}Re to monoclonal antibodies was discontinued and thus the trial was finished. The further development with BIWA 4 will continue by using the linker mercaptoacetylglycylglycl-gamma-aminobutyrate-tetrafluorophenol ester (MAG2GABA-TFP).

- The MTD for single dose treatment was to be identified first before more patients were entered to define the MTD for a second treatment with ^{186}Re-labelled hMAb BIWA 4.

- To propose a safe dose for first and consecutive infusions with ^{186}Re-labelled hMAb BIWA 4 for further studies.

- To obtain initial results on a dose schedule for repeated dosing.

2.2 PRIMARY VARIABLES

- Safety: clinical laboratory tests, human-anti-human-antibody (HAHA) assessments, vital signs measurements and adverse events.

- Efficacy: biopsy biodistribution data (Part A only) and radioimmunoscintigraphic images (Part A and B including dosimetry for Part B of the trial).

- Pharmacokinetic results.

2.3 SECONDARY VARIABLES

A secondary variable for the study was tumour response (Part B only).

3. INVESTIGATIONAL PLAN

3.1 OVERALL STUDY DESIGN AND PLAN – DESCRIPTION

The trial was performed in two parts. Part A evaluated the optimal dose of cold BIWA 4 and quantified tumour uptake while Part B investigated the maximum tolerated dose of ^{186}Re-BIWA 4.

3.1.1 Part A:

This part of the clinical trial was an uncontrolled, rising dose sequential group study. It was designed to provide initial data on the safety and tolerability of a single infusion of ^{99m}Tc-labelled hMAb BIWA 4, to investigate the pattern and level of biodistribution and to establish the pharmacokinetic profile of hMAb BIWA 4 in patients with head and neck cancer. Three protein doses of hMAb BIWA 4 were used in this Part A of the study with three patients planned to be treated at each dose level.

Patients were routinely investigated by the Department of Otolaryngology to determine the extent of the tumour. This included physical examination, computed tomography (CT) or MRI scans of the head and neck and panendoscopy (optional). During these procedures samples of tissues, suspect of tumour, were taken and investigated for the presence of squamous cell carcinoma. Based on the results of these investigations, the patients were destined to undergo surgery including neck dissection.

Radiolabelled antibody was injected and the patient was observed for occurrence of adverse events. Radioimmunoscintigraphic scans were performed 21 hours post infusion (p.i.) prior to surgery. Patients underwent surgery 48 hours (hrs) after the infusion of the radioactively labelled hMAb BIWA 4. The pathologist investigated the neck dissection specimen to determine the exact tumour load. Moreover, the amount of ^{99m}Tc in biopsies from tumour site(s) and normal tissues in the surgical specimen was measured. Tumour sites and tumour infiltrated nodes were examined for the presence of CD44v6 antigen by immunohistochemical techniques. The first three patients were administered 2 mg hMAb BIWA 4 labelled with 20 mCi ^{99m}Tc combined with 23 mg unlabelled hMAb BIWA 4. The second group of three evaluable patients was administered 2 mg hMAb BIWA 4 labelled with 20 mCi ^{99m}Tc combined with 48 mg unlabelled hMAb BIWA 4 and the third group was administered 2 mg of the labelled antibody combined with 98 mg of unlabelled antibody.

Pharmacokinetic assessments were done at the specified timepoints.

3.1.2 Part B:

This part of the clinical trial was an open uncontrolled, dose escalation study. It was designed to assess the safety and tolerability of ^{186}Re-labelled hMAb BIWA 4, to determine the maximum tolerated dose (MTD) of intravenously administered ^{186}Re-labelled hMAb BIWA 4 and to determine the preliminary therapeutic effects of ^{186}Re-labelled hMAb BIWA 4 in head

and neck cancer patients for whom no curative options were available. In addition the pharmacokinetic profile of ^{186}Re-labelled hMAb BIWA 4 was assessed.

All patients entering into this part of the trial received the dose of hMAb BIWA 4, which was selected basing on the results of Part A of the study. The hMAb BIWA 4 was labelled with escalating doses of ^{186}Re. At the lower dose levels (toxicity observed did not exceed grade 1) two patients were entered per dose group and at the higher levels (≥ grade 2 toxicity) a minimum of three patients were entered per dose group. All patients were evaluated to determine the safety of the administered hMAb BIWA 4.

Patients were routinely investigated by the Department of Otolaryngology to determine the extent of the tumour. This included physical examination and CT or MRI scanning of tumour locations.

^{186}Re-labelled antibody was injected with escalating radiation doses: patients in the first dose group received a radiation dose of 20 mCi/m^2, after which the dose for patients in the subsequent dose groups was escalated by 10 mCi/m^2 increments until the MTD was reached. Patients were observed for occurrence of adverse events. Radioimmunoscintigraphic scans were performed.

3.1.3 STUDY PROCEDURES AT EACH VISIT

3.1.3.1 Visit Schedule

a) Screening visit

Before entry into the study each patient was screened for eligibility. Demographics and medically relevant history were recorded, concomitant therapy was recorded and a general physical examination was done and the body weight was measured. The Karnofsky performance score was determined. Written informed consent had to be obtained. A pregnancy test was required for women with childbearing potential. A 12-lead electrocardiogram (ECG) was made and blood as well as urine safety laboratory assessments were done. A blood sample for HAHA-testing was also taken. A Chest X-ray was made and a full disease assessment (CT/MRI, ear-nose-throat [ENT] examination) was performed.

At the end of this visit the results of the required investigations were evaluated, the inclusion and exclusion criteria verified and arrangements for surgery (Part A only) were made.

b) Visit 2: Study days 1-7

<u>Parts A and B:</u>

On the first study day, which was not more than 3 weeks after the screening visit, the patient was admitted to the hospital. All required baseline laboratory assessments not obtained at the screening visit had to be done and evaluated prior to the infusion and the eligibility criteria had to be met. The body weight was measured. All concomitant therapy had to be recorded. Every

adverse event starting after the signing of the written informed consent had to be recorded in the patient file and the case report form (CRF).

On the day of treatment, before antibody administration a blood sample was collected for pharmacokinetics (including the assessment of soluble CD44v6) and the vital signs were recorded. Urine was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs and 12-24 hrs during the first 24 hrs and then in 24 hrs intervals up to 48 and 96 hrs post infusion for Part A and B, respectively.

The antibody was administered at the department of Nuclear Medicine or in a designated room in the hospital. The antibody had to be administered through a peripheral upper extremity vein as proximally as possible. In all cases, the infusion was given over 5 minutes, after a 10-mL saline flush, through a freely flowing line. In order to obtain reproducible pharmacokinetic results a syringe pump had to be used. Therefore, dilution of the volume with NaCl 0.9 % was required to 20 mL. This antibody infusion was followed by a 10 mL saline flush. Any suspected extravasation was documented in the CRF and the patient was imaged.

An emergency unit available with resuscitation equipment, anti-histamines, corticosteroids and epinephrine was within reach to counteract possible anaphylaxis. Adverse events and changes in the concomitant therapy were recorded daily.

Part A:

Vital signs were recorded at 10, 60 and 120 minutes p.i. Blood samples for safety and soluble CD44v6 were collected 21, 48 and 144 hrs post infusion.

Blood samples for pharmacokinetics were drawn at the end of the infusion and at 5, 10, 30 minutes and 1, 2, 4, 16, 21, 48, 72 hrs after the end of the infusion, and on day 7 (144-hrs p.i. including a serum sample for HAHA and soluble CD44v6 assessment).

Urine for pharmacokinetics was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs and 12-24 hrs during the first 24 hrs and a 24-hr sample until 48 hrs p.i.

Whole body scintigraphic images were made directly after the infusion and 21 hours after the infusion. Twenty-one hours after infusion, in addition to the whole body image, single photon emission computed tomography (SPECT) and planar images of the head and neck region were made.

The patient was operated at 48 hrs p.i. and stayed in the hospital for post-operative care.

On day 7 (144 hrs p.i.) safety urine samples were collected.

Adverse events and changes in the concomitant therapy were recorded daily.

Part B:

Vital signs were recorded at 10, 60, 120 and 240 minutes post infusion. Blood samples for safety and for soluble CD44v6 were collected 21, 48 and 144 hrs post infusion. A blood sample for HAHA assessment was collected 144 hrs after the infusion. A urine sample for safety was also collected 144 hrs p.i.

Blood samples for pharmacokinetics were drawn at the end of the infusion and at 5 and 30 minutes and 1, 2, 4, 16, 21, 48, 72 hours after the end of the infusion and on day 7 (144-hrs p.i.) after infusion. The originally planned sample at 10 minutes was omitted as per amendment 1.

Urine for pharmacokinetics was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs and 12-24 hrs during the first 24 hrs and in 24-hr samples until 96 hrs p.i.

Whole body scintigraphic images were made directly after, at 21, 48, 72 and 144 hrs p.i. and optionally at two weeks p.i. if counting statistics permitted.

Planar images of the head and neck region were made at 21, 48 (optional), 72 and 144 hrs p.i. and optionally at two weeks p.i., if counting statistics permitted.

SPECT imaging was only performed seventy-two hours after the infusion.

Adverse events and changes in the concomitant therapy were recorded daily.

Patients were allowed to leave the hospital after three days.

c) Visit 3: Follow-up visits

Part A:

Patients visited the outpatient clinic six weeks after the infusion. A physical examination was performed, safety blood and urine samples collected, body weight and vital signs measured and adverse events recorded. Blood samples for pharmacokinetics, HAHA assessment and soluble CD44v6 were also obtained. A pregnancy test was required for women with childbearing potential. Follow-up of adverse events was recorded. Any changes in the concomitant therapy were recorded.

Part B:

Patients visited the outpatient clinic weekly for at least six weeks for recording of adverse events and collection of safety blood samples. Blood samples for pharmacokinetics were drawn at 240 hours and 336 hours after infusion. The originally planned sample at 6 weeks p.i. was omitted as per amendment 1. Any changes in the concomitant therapy were also recorded. The disease assessment performed at baseline was repeated six weeks after the infusion and thereafter if indicated (the assessment was done after six weeks rather than after four weeks as mentioned once in the protocol). Six weeks after the infusion blood samples for safety, HAHA assessment and soluble CD44v6 were collected, vital signs recorded and body weight measured. A physical examination was done during this visit. A urine sample for safety was collected. A pregnancy test was required for women with childbearing potential.

d) Visits 4 and 5: second treatment (Part B)

A second treatment as outlined in the original protocol was not performed due to the premature termination of the trial because of the linker change. Instead patients who responded to the first dose of ^{186}Re-BIWA 4 were eligible for a second administration. They underwent the same visit schedule as for the first administration.

3.2 DISCUSSION OF STUDY DESIGN, INCLUDING THE CHOICE OF CONTROL GROUPS

The aim of the present study was to assess the preferential accumulation of ^{99m}Tc-labelled BIWA 4 in the tumour (Part A) and to evaluate the maximum tolerated dose of ^{186}Re-BIWA 4 (Part B) as well as the pharmacokinetics of BIWA 4 in patients suffering from advanced head and neck cancer (Part A and B).

An open design was used as is general practice in these types of Phase I trials in oncology. A minimum of two patients in the lower radiation dose tiers and three patients in the higher radiation dose tiers were included in Part B of the trial. In case of occurrence of drug-related Common Toxicity Criteria (CTC) grade 4 haematology and grade 3 non-haematology toxicity a further three patients were treated with the respective dose tier (for further dosing details refer to section 3.4.4. and 3.4.5).

The dose of BIWA 4 administered was based on previous results with mMAb BIWA 1 indicating that a dose of 50 mg yielded a high and selective uptake in tumour tissue with a low uptake in non-tumour tissues and the results from Part A of the trial (see also section 3.4.4.1).

The starting dose level for radioactivity chosen was based on previous data suggesting that a dose of 20 mCi/m^2 may be a safe dose (see also section 3.4.4.2).

The criteria for efficacy which applied as well as the criteria for assessing tolerability are well-established for this patient population and can also be evaluated in an open design.

3.3 SELECTION OF STUDY POPULATION

3.3.1 Inclusion criteria

- Patients with histological confirmation of squamous cell carcinoma in the head and neck.
- Patients destined for surgery by means of a neck dissection (Part A)

or:

- Patients with either local and/or regional recurrent disease for which curative treatment options were not available, or distant metastases. The tumour deposits had to be measurable either clinically or by one or more radiological technique(s) (CT, MRI, bone scintigraphy).

Because RIT was expected to be more effective in smaller size tumour deposits, patients with lesions measuring < 3 cm in greatest dimension were preferred (Part B).

- Patients over 18 years of age.
- Patients younger than 80 years of age.
- Patients who had given "written informed consent".
- Patients with a life expectancy of at least 3 months.
- Patients with a good performance status: Karnofsky > 60.

3.3.2 Exclusion criteria

- Life-threatening infection, allergic diathesis, organ failure (bilirubin > 30 µmol/l and/or creatinine > 150 µmol/l) or evidence of a recent myocardial infarction on ECG or unstable angina pectoris.
- Pre-menopausal women (last menstruation ≤ 1 year prior to study start):
  - Not surgically sterile (hysterectomy, tubal ligation) and
  - Not practising acceptable means of birth control, (or not planned to be continued throughout the study). Acceptable methods of birth control include oral, implantable or injectable contraceptives.
- Women with a positive serum pregnancy test at baseline.
- Chemotherapy or radiotherapy within 4 weeks before inclusion in the study.
- White blood cell count < 3000/mm^3, granulocyte count < 1500/mm^3 or platelet count < 100,000 /mm^3.
- Haematological disorders, congestive heart failure, bronchial asthma, alimentary or contact allergy, severe atopy or allergy.

3.3.3 Removal of subjects from therapy or assessment

3.3.3.1 Criteria for Stopping Subject Treatment

The infusion had to be terminated immediately if the patient developed tachycardia (pulse rate greater than 120 per minute), hypotension (blood pressure less than 100 mm Hg systolic), respiratory distress, chest pain, or any symptoms intolerable to the patient.

3.3.3.2 Dropouts and Withdrawals

The subjects were free to discontinue their participation in this study at any time.

Evaluation of radiolabelled hMAb BIWA 4 was considered not to be feasible if the patient was prematurely removed from the study because of voluntary withdrawal. A case was considered not evaluable if adequate follow-up information was not available. Any unevaluable patients were planned to be replaced.

If the patient discontinued early from the study, the reason had to be documented on the CRF. If a patient did not return for the post-infusion blood samples for HAHA determination or pharmacokinetics, the reason had to be documented. If a patient developed a serious adverse event the study schedule had to be followed as closely as possible depending on the serious adverse events (SAE).

3.4 TREATMENTS

3.4.1 Treatments administered

Patients in Part A were administered ^{99m}Tc-BIWA 4 at a radioactivity dose of 20 mCi. The dose of BIWA 4 administered was 25 mg, 50 mg or 100 mg for three patients each. The drug was administered intravenously as a single dose.

Patients in Part B received 50 mg BIWA 4 labelled with Rhenium 186. The lowest radioactivity dose was 20 mCi/m^2 which was increased in dose tiers of 10 mCi/m^2. The trial drug was administered intravenously as a single dose.

3.4.2 Identity of investigational product

Part A:

Substance (INN):	BIWA 4 (bivatuzumab)
Pharmaceutical form:	solution for injection
Chiffre number:	BIWA 4 LOI 99 1D 1A
Batch number:	B981101
Source:	Boehringer Ingelheim Pharma KG
Unit strength:	5 mg / mL
Daily dose:	25 mg
Duration of use:	single dose
Route of administration:	intravenous
Posology:	infusion over five minutes

Substance (INN):	BIWA 4 (bivatuzumab)
Pharmaceutical form:	solution for injection
Chiffre number:	BIWA 4 LOI 99 1D 1A
Batch number:	B981101
Source:	Boehringer Ingelheim Pharma KG
Unit strength:	5 mg / mL

Daily dose:	50 mg
Duration of use:	single dose
Route of administration:	intravenous
Posology:	infusion over five minutes

Substance (INN):	BIWA 4 (bivatuzumab)
Pharmaceutical form:	solution for injection
Chiffre number:	BIWA 4 LOI 99 1D 1A
Batch number:	B981101
Source:	Boehringer Ingelheim Pharma KG
Unit strength:	5 mg / mL
Daily dose:	100 mg
Duration of use:	single dose
Route of administration:	intravenous
Posology:	infusion over five minutes

BIWA 4 was administered as radioconjugate linked with ^{99m}Tc. Linker molecule was MAG3. MAG3 was purchased from Mallinckrodt, Petten, The Netherlands.

^{99m}Tc was ordered locally via the laboratory where the radioimmunoconjugate was prepared (laboratory of Prof. Dr. van Dongen, Section Tumor Biology, Department of Otorhinolaryngology / Head and Neck Surgery, *Vrije Universiteit* University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands).

Part B:

Substance (INN):	BIWA 4 (bivatuzumab)
Pharmaceutical form:	solution for injection
Chiffre number:	BIWA 4 LOI 99 1D 1A
Batch number:	B981101
Source:	Boehringer Ingelheim Pharma KG
Unit strength:	5 mg / mL
Daily dose:	50 mg
Duration of use:	single dose
Route of administration:	intravenous
Posology:	infusion over five minutes

BIWA 4 was administered as radioconjugate linked with ^{186}Re. Linker molecule was MAG3. MAG3 was purchased from Mallinckrodt, Petten, The Netherlands.

^{186}Re was ordered locally via the laboratory where the radioimmunoconjugate was prepared (laboratory of Prof. Dr. van Dongen, Section Tumor Biology, Department of Otorhinolaryngology / Head and Neck Surgery, *Vrije Universiteit* University Medical Center, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands).

3.4.2.1 Characteristics and quality of the trial drug

Antibody characteristics

The antigen recognised by hMAb BIWA 4 is a transmembrane glycoprotein located on the outer cell surface and is only to a small extent internalised (< 20 %). Further analysis revealed that hMAb BIWA 4 recognises an epitope encoded by variant exon v6 of CD44. The antigen was shown to be expressed by all primary head and neck tumours (n=54) and by the majority of cells within these tumours. A comparable expression was observed for sixty-eight tumour infiltrated lymph nodes from neck dissection specimens (R97-2054). The reactivity pattern of hMAb BIWA 4 in human normal tissues is provided in Appendix III of the protocol. Reactivity of hMAb BIWA 4 was found to be essentially restricted to squamous epithelia. As demonstrated by the previous RIS study with murine monoclonal antibody (mMAb) BIWA 1, reactivity with normal squamous epithelium was not a limiting factor for utility in tumour targeting with respect to tumour uptake.

Quality control of ^{99m}Tc- or ^{186}Re-labelled hMAb BIWA 4

The antibody was labelled with ^{99m}Tc or ^{186}Re according to a method described by Fritzberg *et al.* (R96-2106: See protocol Appendix IV) which was modified according to Visser *et al.* (R96-2094) and Van Gog *et al.* (R96-2111).

The procedures for radiolabelling hMAb BIWA 4 with ^{99m}Tc and ^{186}Re had been validated with respect to the final quality of the prepared conjugate. In five independent labelling experiments, performed according to the procedure described in appendix IV of the protocol, the percentage of label bound to the antibody was found to be 96-99%.

In this clinical investigation the radiochemical purity of each ^{99m}Tc- or ^{186}Re-labelled antibody batch prepared was assessed by thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) and passage through a PD-10 gel filtration column, and had to be more than 90% to allow administration to a patient.

The immunoreactive fraction of each ^{99m}Tc/^{186}Re -labelled antibody batch was checked after administration by use of validated methods and had to be above 60%. In brief, analyses were essentially performed according to a procedure as described by Lindmo *et al.* (R96-2104). UM-SCC 11B cells (human larynx carcinoma), containing the CD44v6 antigen, were fixed in 0.1% glutaraldehyde. Six dilutions, ranging from 5 x 10^6 cells per tube to 3.1 x 10^5 cells per tube, were made with 1% bovine serum albumine (BSA) in phosphate buffered saline (PBS).

To the tubes, 80,000 counts per minute (cpm) of the ^{99m}Tc- or 10,000 of the ^{186}Re-labelled hMAb BIWA 4 were added and incubated overnight at room temperature.

To the last sample, excess unlabelled hMAb BIWA 4 was added to determine non-specific binding. Cells were spun down and the radioactivity in pellet and supernatant were determined in a gamma counter and the percentage bound and free radiolabelled MAb calculated (LKB-Wallac 1218 CompuGamma).

Data were graphically analysed in a modified Lineweaver Burk plot and the immunoreactive fraction was determined by linear extrapolation to conditions representing infinite antigen excess.

In case the discharge level of 60% was not reached in this binding assay the preparation for infusion was re-evaluated in a second binding assay. For this purpose the antibody preparation was labelled with ^{131}I. The immunoreactive fraction in at least one of both assays, had to be larger than 60% for each patient to be evaluable and for the study to continue with the next patient.

Antibody Safety

BIWA 4 used for radiolabelling with ^{99m}Tc and ^{186}Re in this study is a well-characterised monoclonal antibody product which was produced by chinese hamster ovary (CHO)-cell culture fermentation. A master cell bank was established under Good Manufacturing Practice (GMP) conditions and thoroughly examined for microbiological status (bacteria, fungi, mycoplasma) as well as viral status (adventitious viruses, retroviruses). With the exception of endogenous retroviruses, which were known to be present in most CHO cells, no contaminants were detected.

In accordance with the Food and Drug Administration (FDA) 1994 "Points to consider" document on MAbs used in clinical phase I studies in cancer patients, a standardised downstream purification process, which has been validated for efficient virus removal, was applied to the purification of BIWA 4 material. Four model viruses were included in the validation (MuLV, PsRV, REO-3, SV 40). The concentrated bulk harvest was examined for the retrovirus titer to ensure that the subsequent downstream purification process was capable of adequately removing the retrovirus. Data on removal of endotoxins were within the acceptance limits ($\leq$ 0.01 EU/mg) and were available for the bulk product. Furthermore pyrogenicity testing according to European Pharmacopoea guidelines had been successfully performed.

The final hMAb BIWA 4 to be used for further radiolabelling was analysed in great detail and was proven to be a highly pure (SDS page, isoelectric focusing), sterile solution containing minimal endotoxin levels ($\leq$ 0.01 EU/mg). Results of testing for pyrogenicity were conform to European Pharmacopoea standard. The equality of the BIWA 4 product from the manufacture for pre-clinical and clinical supplies was demonstrated by analytical results.

Radiolabelling of hMAb BIWA 4 with ^{99m}Tc or ^{186}Re was performed by the department of Otolaryngology and Head & Neck Surgery of the *Vrije Universiteit* University Medical Center according to Standard Operating Procedures (SOPs). Sterility of the final product was guaranteed. Absence of endotoxins was tested during validation runs. For drug administration to a patient in the University Hospital Nijmegen the proper amount of radiolabelled hMAb BIWA 4 was transported in a special container directly after preparation to the study centre in Nijmegen. Twenty-four hours were allowed between labelling of the compound and administration to the patient.

See also appendix 16.1.6 for an allocation of the individual Rhenium batches to the patients.

3.4.2.2 Packaging, Labelling and Supply

BIWA 4 was supplied by Boehringer Ingelheim The Netherlands. It was produced by Boehringer Ingelheim, Germany using a GMP manufacturing and purification process and filled in vials as a sterile, non-pyrogenic solution containing 25 mg hMAb BIWA 4 in 5 mL isotonic PBS, pH 7.2. Examples of the vial labels of the native and also of the labelled antibody were included in the Clinical Trial Manual.

One batch with a total amount of 27 g of hMAb BIWA 4 had been prepared and filled into vials. Labelling of hMAb BIWA 4 with ^{99m}Tc or ^{186}Re was performed in a class B certified nuclear laboratory of the *Vrije Universiteit* University Medical Center, Amsterdam.

3.4.2.3 Storage Conditions

The unlabelled hMAb BIWA 4 had to be stored in the hospital pharmacy in a limited access area for study materials at a monitored temperature between +2 and +8°C.

3.4.3 Method of assigning subjects to treatment groups

No randomisation was used. Patients were assigned to the different dose groups according to the sequence of inclusion.

3.4.4 Selection of doses in the study

3.4.4.1 Selection of the BIWA 4 doses

Part A:

Since hMAb BIWA 4 had never been administered to patients before, it was essential to be informed about its safety and biodistribution before starting RIT trials. Its biodistribution might strongly depend on the MAb dose used for tumour targeting and would need careful consideration. On the basis of MAb protein dose escalation studies with the low affinity anti-CD44v6 mMAb U36 and the high affinity anti-CD44v6 mMAb BIWA 1, it was anticipated that the optimal dose was in the range of 25-100 mg, with 50 mg being optimal for mMAb U36.

To confirm that this dose also was adequate for the intermediate affinity of hMAb BIWA 4 and to obtain initial information on the variability of the tumour uptake the biodistribution was assessed at 25, 50 and 100 mg total hMAb BIWA 4 and three evaluable patients were planned to be treated at each of these dose levels.

All evaluable patients received a single intravenous infusion of 2 mg hMAb BIWA 4 labelled with 20 mCi ^{99m}Tc, measured by a radiation calibration system just prior to administration. Patients scheduled to receive 25 mg, 50 mg or 100 mg received 23 mg, 48 mg or 98 mg,

respectively of unlabelled hMAb BIWA 4 administered together with this 2 mg hMAb BIWA 4 labelled with 20 mCi ^{99m}Tc.

Selection of the BIWA 4 dose for Part B:

On theoretical grounds a hMAb BIWA 4 dose of 50 mg was calculated as most suited for development. Part A of this study was performed to confirm the tumour preferential uptake of hMAb BIWA 4 at the three dose levels tested (25 mg, 50 mg and 100 mg). It was expected that the tumour uptake (expressed as percent injected dose per kilogram, % ID/kg) and tumour to non-tumour uptake ratio for these three dose levels would not differ much. If this was the case the hMAb BIWA 4 dose to be used for Part B of the study was 50 mg. However, if there was a clinically relevant difference between these dose levels, which favoured one over the other levels, the dose level with the best pattern of biodistribution would have been selected.

In this case a clinically relevant difference was defined as a difference of the tumour (mean of the tumour uptake) to bone marrow uptake ratio (mean of the bone marrow uptake = the cellular fraction and the supernatant) of more than 50 %.

Finally the dose selected was 50 mg hMAb BIWA 4 basing on the results of Part A.

3.4.4.2 Selection of the radiation starting dose

Part B:

The hMAb BIWA 4 dose selected in Part A of the study was labelled with escalating doses of ^{186}Re.

It has been reported that the maximum tolerated dose of ^{186}Re-labelled murine MAb NR-LU-10 was 90 mCi/m^2 for heavily pre-treated patients, while dose-limiting myelosuppression was observed at 120 mCi/m^2. For chimeric MAb NR-LU-13, recognising the same antigen as NR-LU-10, reversible myelosuppression occurred at 60 mCi/m^2. In a study with cMAb U36, performed at the *Vrije Universiteit* University Medical Center dose limiting myelosuppression was observed at 41 mCi/m^2.

In the light of the above-mentioned results a radiation dose of 20 mCi/m^2 administered as ^{186}Re-labelled hMAb BIWA 4 was considered a safe starting dose.

The dose was escalated with 10 mCi/m^2 increments. Two evaluable patients were entered at the lower dose levels. When ≥ grade 2 drug-related toxicity according to the CTC was observed a minimum of three patients were treated per dose level.

Before entering patients at a next higher dose level it had to be sure that the patients at the ongoing dose level did not experience dose limiting toxicity (DLT) defined as: drug-related CTC grade 3 non-haematologic toxicity or drug-related CTC grade 4 haematologic toxicity excluding nausea and vomiting without adequate antiemetic treatment. For this purpose all patients at such an ongoing dose level had to be observed long enough to ensure that possibly induced toxicity was reversible.

When at the ongoing dose level one patient experienced DLT the number of patients treated at that dose level was increased to a total of six patients maximum. When 1 out of 6 patients experienced DLT the dose was escalated to the next level. When two or more of the patients experienced DLT, the next lower dose level was expanded (if not previously done) to a total of six patients in order to establish MTD and a safe recommended dose for phase II.

It was originally planned that in case of acceptable toxicity (less than two patients with DLT) at that dose level additional patients could be entered who received this dose and an escalating, lower second dose. This plan was not pursued due to the change of the linker. MAG3 will be replaced by MAG2GABA-TFP, a comparable linker, but with a more convenient linkage procedure. The present development programme with MAG3 has been finished.

Alternatively patients who responded to and tolerated the first administration of ^{186}Re-BIWA 4 were eligible for a second administration with a dose of 50 mCi/m^2 ^{186}Re-BIWA 4.

3.4.5 Selection and timing of dose for each subject

3.4.5.1 Dosage and Treatment Schedule

The investigators were allowed to administer the trial drug at any time. The time elapsed between radiolabelling and administration, however, should not exceed 24 hours.

3.4.6 Blinding

This was an open uncontrolled trial. No blinding was done.

3.4.7 Prior and concomitant therapy or procedures

3.4.7.1 Rescue Medication and Additional Treatment(s)

Anaphylaxis was considered to be the most serious potential side effect and would have mandated immediate cessation of antibody infusion and the institution of appropriate resuscitative measures. Precautions to be taken were: Resuscitation equipment, within reach: anti-histaminics, corticosteroids and epinephrine. Any patient experiencing this type of adverse reaction was not allowed to receive additional monoclonal antibody.

Patients were allowed to receive other treatment(s) either for the study indication or for unrelated illnesses providing the inclusion and exclusion criteria were met. All concomitant therapy had to be recorded in the CRF.

In case of serious haematologic toxicity (CTC grade 4) cytokine intervention or other methods for alleviating bone marrow toxicity were allowed.

3.4.7.2 Restrictions

Additional chemotherapy or radiotherapy was not allowed and the last chemotherapy or radiotherapy should have stopped more than 4 weeks before inclusion in this study. All prior chemotherapy and radiotherapy was to be recorded in the CRF.

3.4.8 Treatment compliance

The study medication was given as a single intravenous infusion. The compliance was verified with pharmacokinetic assessments and with radioimmunoscintigraphic images.

3.5 EFFICACY / CLINICAL PHARMACOLOGY AND SAFETY VARIABLES

3.5.1 Efficacy / pharmacodynamics and safety measurements assessed and flow chart

Flow Charts:

Part A

The investigations performed in Part A and the respective times are detailed in the flow chart given below. A description of the investigations is given in the following sections:

TABLE 3.5.1: 1 FLOW CHART: Part A: Biodistribution study with ^{99m}Tc-labelled hMAb BIWA 4

	Visit 1	Visit 2						Visit 3
	screening visit*	Day 1*		Day 2	Day 3	Day 4	Day 7	Follow-Up Week 6
		pre-inj.	Post-inj.					
Signed informed consent	x							
Demographics Conc. Therapy Medical History Physical Examination	x x x x	x	x	x	x	x	x	X x
ECG Chest X-ray Blood safety analysis Urine safety analysis Pregnancy test HAHA assessment	x x x[1] x x x[2]	x[1]		x[1]	x[1]		x[1] x x[2]	x[1] x x x[2]
ENT examination CT or MRI	x x							
In-/exclusion criteria	x							
Adverse events Vital signs body weight	x[3] x	x x[3] x	x x[3]	x	x	x	x	X x[3] x
Immunoscintigraphy: - Whole Body Scan - Planar Scan - SPECT Scan			x[4]	x[4] x[5] x[5]				
Pharmacokinetics: Blood samples Urine collection Serum soluble CD44v6		x[6] x[6] x[7]	x[6] x[6]	x[6] x[6] x[7]	x[6] x[6] x[7]	x[6]	x[6] x[7]	6 6
Surgery					x			

*: The pre-infusion assessments should occur no more than 3 weeks prior to the actual infusion.

x[1]: Blood samples were tested for: Glucose, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, serum glutamic oxalacetic transaminase (sGOT), serum glutamic pyruvic transaminase (sGPT), Alkaline Phosphatase, Gamma Glutaryl Transpeptidase (GGT), bilirubin, urea, uric-acid, thyroid stimulating hormone (TSH), haemoglobin (Hb), haematocrit (Ht), mean corpuscular volume (MCV), reticulocytes, leucocytes, neutrophils, bands, lymphocytes, basophils, eosinophils, monocytes and platelets at the screening visit or on day 1 pre-infusion at 21, 48 and 144 hrs p.i. and at six weeks post infusion (p.i.).

x[2]: HAHA was assessed in a serum sample obtained at the screening visit, after one week (144 hrs) and after six weeks p.i.

x[3]: Vital signs were assessed at the screening visit, pre-infusion, at 10, 60 and 120 minutes post infusion and after 6 weeks.

x[4]: Whole body scan was done immediately after infusion and 21 hrs p.i.

x[5]: The SPECT scan and planar scan was done once 21 hrs p.i.

x[6]: Blood samples for pharmacokinetics were obtained pre-infusion, at the end of the infusion, at 5, 10, 30 minutes; 1, 2, 4, 16, 21, 48, 72 and 144 hrs post end of infusion and six weeks p.i. Blood samples were drawn for enzyme-linked

immuno-sorbent assay (ELISA) and for measurement of radioactivity (for details see section 9.5.4.1). Urine was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs, and 12-24 hrs during the first 24 hrs and in a 24-hr sample for the remaining time until 48 hrs p.i.

x^7: Soluble CD44v6 was measured from a serum sample obtained pre-infusion, at 21, 48 and 144 hrs p.i and six weeks p.i.

6 week six

Part B

The investigations performed and the respective times are given in the flow chart below. The individual investigations are described in more detail in the following sections.

TABLE 3.5.1: 2 FLOW CHART Part B: Dose escalation study with ^{186}Re-labelled hMAb BIWA 4

	Visit 1	Visit 2						Visit 3
	Screening visit*	Day 1*		Day 2	Day 3	Day 4	Day 7	Follow-Up Week No.
		pre-inj.	Post-inj.					
Signed informed consent	x							
Demographics Conc. Therapy Medical History Physical Examination	x x x x	x	x	x	x	x	x	2, 3, 4, 5, 6 6
ECG Chest X-ray Blood safety analysis Urine safety analysis Pregnancy test HAHA assessment	x x x^1 x x x^2	x^1		x^1	x^1		x^1 x x^2	2, 3, 4, 5, 6 6 6 6
ENT examination CT or MRI CT thorax	x x x^8							6 x^8 6 x^8
In-/exclusion criteria	x							
Adverse events Vital signs Body weight	 x^3 x	x x^3 x	x x^3	x	x	x	x	2, 3, 4, 5, 6 6 6
Immunoscintigraphy: - Whole Body Scan - Planar Scan - SPECT Scan			x^4	x^4 x^5	x^4 (x^5)	x^4 x^5 x^5	x^4 x^5	

Pharmacokinetics:								
Blood samples		x^6	x^6	x^6	x^6	x^6	x^6	6
Urine collection		x^6	x^6	x^6	x^6	x^6		
Serum soluble CD44v6		x^7		x^7	x^7		x^7	6

*: The pre-infusion assessment had to occur no more than 3 weeks prior to the actual infusion.

x^1: Blood samples were tested for: Glucose, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, sGOT, sGPT, Alkaline Phosphatase, GGT, bilirubin, urea, uric-acid, TSH, Hb, Ht, MCV, reticulocytes, leucocytes, neutrophils, bands, lymphocytes, basophils, eosinophils, monocytes and platelets at the screening visit or on day 1 pre-infusion, at 21 hrs p.i., at 48 hrs p.i. and at 144 hrs p.i.. During weeks 2-6 p.i. safety blood samples were obtained at least weekly.

x^2: HAHA was assessed in a serum sample obtained at the screening visit, at 144 hrs and six weeks post infusion.

x^3: Vital signs were assessed once at the screening visit, pre-infusion, at 10, 60, 120 and 240 minutes post infusion and after 6 weeks.

x^4: Whole body scan was done immediately p.i., at 21, 48, 72, 144 hrs p.i, and optionally at two weeks p.i.

x^5: The planar scan was done at 21, 48 (optional), 72, 144 hrs p.i. and optionally at two weeks p.i. The SPECT scan was done at 72 hrs p.i.

x^6: Blood samples for pharmacokinetics were obtained pre-infusion, at the end of the infusion, at 5, 30 minutes; 1, 2, 4, 16, 21, 48, 72, 144 , 240 and 336 hrs post end of infusion. Blood samples were drawn for ELISA and for measurement of radioactivity. Urine was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs, and 12-24 hrs during the first 24 hrs and in 24-hr samples for the remaining time until 96 hrs p.i.

x^7: Soluble CD44v6 was measured from a serum sample obtained pre-infusion, at 21, 48 and 144 hrs p.i and six weeks p.i.

x^8: Disease assessment was to be repeated every 6 weeks until disease progression or loss to follow-up. CT thorax was done at baseline and repeated at follow-up if there were abnormalities.

3.5.1.1 Radioimmunoscintigraphy and dosimetry

The data obtained from radioimmunoscintigraphy (RIS) were presented qualitatively for both Part A and B (i.e. uptake in tumour, bone marrow, liver, lung, intestine, kidney and additional organs expressed as low, medium or high) and quantitatively for Part B.

For qualitative assessment the rating scale was transformed into numbers (0 being no uptake, 1 being low uptake, 2 being medium uptake and 3 being high uptake). Mean values were calculated and presented.

For Part B the quantitative presentation of RIS results used dosimetric calculations as outlined in section 5.1.4 of the protocol by including the following parameters:

- Actual organ activity at a given time point expressed in MBq.
- The residence time of radioactivity in organs expressed in hours.
- The absorbed (radiation) dose expressed in mGy/MBq.
- The effective (radiation) dose expressed in mSv.

More details concerning the analysis of dosimetry can be found in the dosimetry report (dated November 21, 2001).

e) Radioimmunoscintigraphic Procedures

A description of the methods used for Part A and B and the time points are given in the following section:

Part A

With a large field of view dual headed gamma camera equipped with a low energy collimator, digital whole body images (anterior posterior (a.p.) and posterior anterior (p.a.)) were obtained directly after and 21 hrs after infusion. At 21 hrs p.i. planar and SPECT images of the head and neck were acquired. A calibration source also was acquired. Data was stored to enable quantitative analysis.

Part B

With a large field of view dual headed gamma camera equipped with a low energy collimator, digital whole body images (a.p. and p.a.) were obtained directly after, at 21, 48, 72 and 144 hrs after administration of the radioimmunoconjugate. Additional planar images of the head and neck region were made at 21, 48 (optional), 72, and 144 hrs p.i. Optionally additional imaging (whole body and planar) were performed two weeks p.i. A calibration source (i.e. an aliquot with a known fraction of the injected dose in a 10 millilitre (ml) vial), inserted in an Adams phantom, had to be placed between the lower legs of the patient during the whole body scan. The initial activity of the calibration source had to be 100-200 MBq ^{186}Re and this source had to be used during all whole body imaging studies. The energy-window and peak settings, the scanspeed, the scanlength, the scanning date, the time of starting the scan and the scan duration had to be reported. The anterior-posterior thickness of the neck and of the abdomen had to be measured, while the patient was in supine position on the scanning table.

At each imaging session, anterior and planar static images had to be taken and just before or just after this image a static image had to be acquired from the calibration source in the Adams-phantom.

At 72 hr. p.i. a SPECT study of the neck had to be performed. The methods used for reconstruction and the filter functions with cut off frequencies had to be reported.

Single Photon Emission Computed Tomography (SPECT)

SPECT images were obtained using a double headed rotating gamma camera equipped with a low energy collimator. Acquisition required at least thirty minutes. Twenty percent symmetric windows were centred at the 137 keV photon peaks.

Planar and SPECT data acquisition parameters

Planar imaging included the following minimal requirements: matrix 128x128 (detail) or 256 x 256 (whole body) and a minimum of 400000 counts with a maximum acquisition time of 10 minutes for detail and 60 minutes for whole body.

SPECT imaging included the following minimal requirements: 64 images, matrix size 64 x 64, 360 degree circular orbit, 60 second acquisitions per angle.

Analysis of the data

At the 21 hr p.i. anterior whole body images, rectangular regions of interest (ROI's) had to be drawn around the whole body and the calibration source. Also irregular ROI's around the organs which accumulated ^{186}Re (e.g. liver, spleen and left kidney) and around the tumour had to be drawn. One or more representative background regions had to be drawn. These regions had to be mirrored to the posterior images. Originally it was planned to draw a ROI around the sacrum on the posterior image. This plan was not pursued during the trial. All regions had to be saved on the computer, in order to project these ROI's on images at other time points. The

number of pixels and the counts per pixel in each region had to be reported. The number of counts in the regions for all imaging time points had to be recorded digitally in a spreadsheet.

Calculation of the absorbed dose in the organs

The amount of activity in the organs, tumour and the total body was estimated from the geometric mean counts in the ROI's of the anterior and posterior views. Background and attenuation correction were applied when indicated. The activity in the urine was not used to estimate the absorbed dose in the bladder as originally planned. Instead the dynamic bladder model was used. The residence times in the organs and the rest of the body were calculated and imported in the MIRDOSE3 program.

Required quality control tests

Planar Imaging
Routine quality controls were performed at the department of nuclear medicine weekly:
1) 100M flood table.
2) Extrinsic ^{57}Co flood with the low energy collimator was obtained each week.

SPECT imaging
Quality control of the SPECT imaging system had to include center of rotation determination.

Tomographic processing

A filtered back projection algorithm was used for tomographic image reconstruction using a ramp filter.

Data storage

All planar images and tomographic data had to be stored permanently on magnetic tape or optical disc. For tomographic studies, original projecting images and reconstructed studies had to be written to a back up tape.

Copies of images and reports

Copies of all relevant images, pathology and surgery reports were required for all patients. Also copies of MRI and CT reports were required.

3.5.1.2 Biopsy biodistribution of ^{99m}Tc-labelled hMAb BIWA 4 (Part A)

Patients entered in Part A of the study underwent surgery 48 hrs after infusion of the radiolabelled hMAb BIWA 4. Biopsies from tumour site(s) and from normal tissues (as many as possible) in the surgical specimen were taken. Then also under general anaesthesia a bone biopsy and a bone marrow aspirate was taken. The bone marrow aspirates were centrifuged to assess the radioactivity in the supernatant (plasma) and in the sediment (cellular fraction).

All biopsies were weighed and the amount of ^{99m}Tc was measured. Specific uptake of radioactivity into tumour was evaluated by comparing %ID/kg tumour with %ID/kg normal tissue.

CD44v6 antigen expression was assessed by immunohistochemistry using cryostate sections, which were first incubated with mMAb BIWA 1, followed by anti-mouse Immunoglobulin G (IgG) secondary reagent. The surgical specimen and the biopsies were investigated histo/cytopathologically.

Evaluation of surgical specimen and biodistribution

After receiving the surgical specimen it was processed in the following chronological order.

1. Pictures were taken of the specimen. A Polaroid from the front and slides from the front and back.

2. The size of the surgical specimen was assessed.

3. Biopsies of primary tumour, suspect lymph node, and if possible from normal tissues in the surgical specimen like normal mucosa, normal lymph node, fat and muscle were taken. All biopsies were weighed and the amount of ^{99m}Tc was measured. All data were converted to percentages injected dose/kilogram tissue. Specific uptake of radioactivity into tumour was evaluated by comparing %ID/kg tumour with %ID/kg normal tissue.

4. Next the specimen was nailed to a board and fixed in formaldehyde 4% for at least 36 hours.

5. After dissecting the sternocleidomastoid muscle (a structure with a high radiodensity), a specimen radiograph was made to show the exact size and location of the lymph nodes involved. This radiograph was made while the specimen was being immersed in ethanol 96%, which has the same X-ray absorption as fat.

6. All the nodes visualised with the X-ray were indicated on the Polaroid and specimen radiograph.

7. All the nodes found by examining the surgical specimen and by X-ray were dissected from the specimen.

8. All macroscopically negative nodes were entirely processed for microscopy and one single section was evaluated. Of all macroscopically positive nodes two or more slices were made. Macroscopical evidence for the presence or absence of tumour necrosis were recorded.

9. Furthermore, the number of nodes enclosed and the number, localisation and lymph node level (according to the Memorial Sloan Kettering Cancer Center Classification) of tumour containing nodes were recorded.

3.5.1.3 Tumour Response (Part B)

The efficacy parameter for the radioimmunotherapy treatment was tumour response. Tumour response was assessed with tumour measurements as assessed clinically and/or with CT, MRI or bone scintigraphy investigations. Evaluation was done according to response criteria of the World Health Organisation (WHO). See Appendix VI of the protocol, (R96-0941).

3.5.1.4 Physical Examination

Before inclusion in the study the disease status of each patient was evaluated by ENT-examination (palpation included) and by CT or MRI of the tumour site(s).

All head and neck lesions were described per neck side.

Palpation

All patients were examined at baseline by an otolaryngologist/head and neck surgeon. This clinical investigator assessed the number, size, location and mobility of all palpable lymph nodes in the head and neck area. The character of the lymph nodes was described as: not suspected, suspected or tumour infiltrated. The status of the neck lymph nodes was classified according to the Tumour Node Metastasis system for staging tumours (TNM) of the Union Internationale Contre le Cancer (UICC) at diagnosis.

Radiological examination

Depending on the localisation of the tumour site(s), one or more of the following examinations were required: CT, MRI, bone scintigraphy. For tumour involvement of the head and neck region MRI was generally preferred. In case there were bone lesions, CT was preferred. For Part B all radiological disease assessment parameters obtained at baseline were repeated six weeks after the infusion and every six weeks until progression or lost to follow-up. For patients entered in Part B of the study a CT thorax was obtained at baseline, and was repeated at follow-up if there were tumour lesions in the thorax. Ultrasound might have been employed as additional technique of tumour imaging. Guidelines for the investigations are given below:

Primary tumour / loco-regional recurrence

For patients participating in Part A of the study and patients with a loco-regional recurrence (Part B) CT scan and/or MRI of the head and neck region had to be performed.

Computed Tomography

Computed tomography of the Head and Neck region: Dynamic CT was preferred over spiral CT. However, spiral CT might have been helpful in patients who were unable to cooperate.

The patient had to be examined in supine position, the neck slightly hyperextended, the head immobilised and the shoulders relaxed and pushed downwards. The patient had to breath quietly with use of abdominal rather than chest muscles. The area to be scanned was

determined from the initial overview made in the lateral projection. The plane of the scan had to be parallel to that of the vocal cords. Contiguous three millimetre-slices had to be used routinely. Imaging had to be performed from skull base to upper mediastinum.

Magnetic resonance imaging

The patients had to be examined in supine position, the neck slightly hyperextended and the head immobilised. Quiet breathing was mandatory during the examination with the use of abdominal rather than the chest muscles. The appropriate images to demonstrate the neck anatomy and to assess the extent of the primary lesion and the presence of lymph node spread was obtained. They had to be obtained from the skull base to the upper mediastinum.

Distant metastases

For all patients participating in Part B of the study CT scanning of the thorax was done at baseline. Additional investigations to visualise metastases, like bone scintigraphy or CT abdomen, were optional.

CT thorax

Spiral CT-scans were obtained. Patients were examined in supine position and had to raise their arms above their heads. Patients had to breath quietly with use of abdominal rather than chest muscles. The patient was scanned from just above the lungs to the level of the adrenal glands. Images had to be photographed in mediastinal and lung setting.

CT abdomen

Spiral scans were obtained. Patients were examined in supine position and had to raise their arms above their heads. Patients had to breath quietly. Oral contrast was used in all patients. The area to be scanned ran from just above the diaphragm to the symphysis. Images had to be photographed in abdominal and liver setting.

Bone scintigraphy

After intravenous infusion of maximally 600 MBq of ^{99m}Tc-labelled HDP/MDP, the patient was asked to consume enough fluids and to void frequently. Static views were obtained 3 hours after infusion: one whole body view and if necessary 3 more detailed views. Suspect lesions might have needed closer analysis by CT and/or MRI.

Reporting of response of bone metastases were planned to be conducted according to a separate set of response criteria. No data was provided.

3.5.1.5 Soluble CD44v6

Soluble CD44v6 (sCD44v6) had to be measured in serum. Concentrations were determined by means of a validated enzyme-linked immuno-sorbent assay (ELISA) that was based on a commercially available test kit and conducted in accordance with current international

guidelines at the Boehringer Ingelheim Department of Pharmacokinetics and Drug Metabolism, Biberach, Germany. Blood samples (to be processed to serum) of 5 mL were obtained pre-infusion, at 21, 48 and 144 hrs p.i. and six weeks after the infusion. Samples were allowed to clot and centrifuged to prepare serum. Storage and shipment conditions: The sample for ELISA measurement was put in cryotubes, labelled carefully to enable unique identification, stored at –20°C until radioactivity has decreased (^{186}Re: 4 weeks, ^{99m}Tc: 3 days) and sent to BI Department of Pharmacokinetics and Drug Metabolism, Biberach, Germany in batches every four weeks. The serum samples were sent on dry ice.

3.5.1.6 Safety

Protection of subjects

All patients were monitored carefully during and after administration of the radiolabelled monoclonal antibody. For this purpose at least one of the investigators was present.

Laboratory Assessments

Blood samples were collected for glucose, sodium, potassium, calcium, chloride, creatinine, total protein, albumin, serum glutamic oxalacetic transaminase (sGOT), serum glutamic pyruvic transaminase (sGPT), alkaline phosphatase (AP), gamma glutaryl transpeptidase (GGT), bilirubin, urea, uric-acid, thyroid stimulating hormone (TSH), haemoglobin (Hb), haematocrit (Ht), median corpuscular volume (MCV), reticulocytes, leucocytes, neutrophils, bands, lymphocytes, basophils, eosinophils, monocytes and platelets at the following time points:

- At the screening visit or on day one pre-infusion and
- At 21, 48 and 144 hrs post infusion.
- For Part A at 6 weeks post infusion. For Part B: During weeks 2, 3, 4, 5 and 6 of the study at least once weekly (more often in case of toxicity).

Baseline laboratory assessments pre-infusion could have been obtained either at the screening visit or on day one of the study, provided the samples were obtained less then 21 days before the infusion of radiolabelled hMAb BIWA 4 and all required assessments were done. All required laboratory test results had to be reviewed and checked for eligibility by the responsible physician prior to the antibody infusion. The same also applied to a second hMAb BIWA 4 administration in Part B of the study.

Urine samples were collected for standard hospital screening (protein, blood, and glucose) at the time points:

- Screening visit
- One week post infusion (144 hrs p.i.)

- Six weeks post infusion

A pregnancy test was required for women with childbearing potential at the screening visit and at the study end.

Human-Anti-Human-Antibody Assessment

The presence and/or the development of HAHA was evaluated in serum samples. Therefore blood samples (to be processed to serum) of 5 mL were taken at the screening visit, at one week (144 hrs p.i.) and at six weeks after infusion of the antibody. For patients receiving two BIWA 4 administrations in Part B of the study an additional HAHA sample was collected before the second administration. Serum level was evaluated by means of validated ELISA methods.

Samples were allowed to clot and centrifuged to prepare serum.

Storage and shipment conditions: The samples for ELISA measurement were to be put in cryotubes, labelled carefully to enable unique identification, stored at –20°C until radioactivity had decreased (^{186}Re: 4 weeks, ^{99m}Tc: 3 days) and sent toBI Department of Pharmacokinetics and Drug Metabolism, Biberach, Germany in batches every four weeks. The serum samples had to be sent on dry ice.

Any elevations in human anti-human antibody levels were compared casewise carefully with the patient's possible adverse events. This data was collected and analysed retrospectively during the course of the study.

Adverse Events

All adverse events were recorded in the CRF. The events were graded according to the US National Cancer Institute CTC (NCI-CTC) version 2.0 (a version of this document had to be downloaded from the Internet at the site: http://ctep.info.nih.gov/CTC3/ctc.htm. All SAEs had to be reported.

Immunogenicity and toxicity

Before performance of this trial no human data existed with respect to safety and immunogenicity of hMAb BIWA 4. In the previous study performed with the parental murine antibody BIWA 1 no clinically significant toxicity was encountered. No toxicity was expected from the naked hMAb BIWA 4 antibody alone (no antibody dependent cell-mediated cytotoxicity (ADCC) effector functions in vitro; no interference with proliferation of antigen-expressing cells expected). It was demonstrated that hMAb BIWA 4 could be safely administered to mice (xenograft studies) and monkeys (toxicity studies). Good local tolerability was demonstrated in three local toxicity studies.

Murine and chimeric MAb U36 (anti-CD44v6-epitope with defined overlapping epitope specificity) was so far safe in HNSCC patients; no toxicities except for myelotoxicity (caused

by ^{186}Re-labels) were seen in a Phase I dose-escalation RIT study with cMAb U36. It was theoretically possible that hypersensitivity reactions to radiolabelled hMAb BIWA 4 might occur. Monoclonal antibodies have been administered to several thousands of patients for diagnostic applications.

Although unlikely, potential reactions to intravenously administered BIWA 4 might include hypotension, transient fever and chills, skin rashes, dyspnoea, itching, nausea, and anaphylaxis.

Vital signs (blood pressure, temperature, pulse rate and respiratory rate) were therefore recorded at the screening visit and at the following time points: pre-infusion and 10, 60, 120 minutes post-infusion and after 6 weeks. Additionally vital signs were recorded at 240 minutes post-infusion for patients participating in Part B only.

In this study radionuclides were used. The radiation burden associated with the gamma emitting radionuclide ^{99m}Tc (in the current study 20 mCi) was similar to that encountered in many routine nuclear medicine procedures, and was known to be small. To minimise radiation exposure to the bladder and kidneys the patients were well hydrated and asked to void at frequent intervals during the first 48 hours (96 hours after infusion with ^{186}Re-labelled antibody).

For the beta- and gamma-emitting radionuclide ^{186}Re, Breitz *et al.* (R98-2459) found a maximum tolerated dose of ^{186}Re-labelled MAb IgG in heavily pretreated patients of 90 mCi/m^2. One of the aims of this study was to investigate the toxicity of ^{186}Re-labelled hMAb BIWA 4 in patients who either had local and/or regional recurrent disease for which curative treatment options were not available, or distant metastases. Because bone marrow, thyroid, kidney and liver toxicity might occur, blood samples for organ function testing were taken as described in section 3.5.1.6.

To monitor the occurrence of weight loss or weight gain the body weight was recorded at the screening visit, before the infusion on day 1 and after 6 weeks.

Patients were monitored for the occurrence of local toxicity. Any sign of local toxicity was recorded as adverse event.

3.5.2 Appropriateness of measurements

The measurements used are well-accepted for this type of trial.

3.5.3 Primary efficacy / pharmacodynamic variables(s)

3.5.3.1 Part A of the trial

The primary efficacy variables determined in Part A of the trial were the biopsy distribution and the radioimmunoscintigraphy. Description of the methods how to obtain the data is given in section 3.5.1.1 and 3.5.1.2.

The uptake in the normal tissue and tumour was measured in biopsies of the surgical specimen and the uptake expressed as percent of the injected dose per kg (% ID/kg).The time course of uptake in the tumour and other tissues was evaluated and compared concerning the different doses of BIWA 4.

3.5.3.2 Part B of the trial

The primary parameters for efficacy were the analysis of radioimmunoscintigraphy and dosimetry.

3.5.4 Drug concentration measurements / pharmacokinetics

Blood concentrations of radiolabelled BIWA 4 were determined in both Part A and Part B of the trial.

3.5.4.1 Methods and timing of sample collection

Patient blood samples were collected and handled as follows: 7 mL of blood (3.5 mL in potassium EDTA containing tubes and 3.5 mL in coagulation tubes: amount of millilitres requested are given as per amendment 1) were sampled from a peripheral vein of the arm opposite to the infusion site at the designated time points (i.e. just prior to antibody administration, at the end of the infusion and at 5, 10, 30 minutes; 1, 2, 4, 16, 21, 48, 72 hrs post-end of infusion and on day 7 p.i. (144 hrs) and 6 weeks after the infusion). End of infusion was regarded as t = 0.

The planned samples at 10 minutes and 6 week p.i. were omitted for Part B of the trial. Instead samples were collected at 240 and 366 hour p.i. as per amendment 1.

Urine was collected during 48 hrs after the ^{99m}Tc-labelled hMAb BIWA 4 infusion and during 96 hrs after the ^{186}Re-labelled hMAb BIWA 4 infusion. Urine was collected from 0-4 hrs, 4-8 hrs, 8-12 hrs, and 12-24 hrs during the first 24 hrs and in 24-hr samples for the remaining time. Radioactivity of urine samples was counted to determine the excretion of radioactivity.

Blood samples were centrifuged to prepare serum. Before processing, the radioactivity in whole blood was measured. Radioactivity was also measured in serum. Refrigerated storage of blood samples was obligatory, though samples had not to be frozen down. Using the aliquot retained from the conjugate preparation, a weighted dilution of the injected patient dose was prepared as standard. The counting of the patient samples and standards were performed according to local SOPs. Results were recorded in the appropriate sections of the CRF. The radionuclide content was reported as a percentage of the injected doses (expressed as % ID/kg blood or serum). Apart from radioactivity counting all samples were analysed for presence of immune complexes by means of HPLC analysis.

All sample tubes were labelled with the following information: Trial number, patient number, sample identification (i.e., serum, plasma, or blood), time relative to infusion, actual time, actual date and isotope (i.e., ^{99m}Tc or ^{186}Re). The volume of each urine sample was measured

and recorded and all urine samples were labelled with the following information: Trial number, patient number, total sample volume, collection interval, actual time, date and isotope (i.e., ^{99m}Tc or ^{186}Re).

Dates, times and radioactivity measurement results of all pharmacokinetic samples were recorded in the CRF.

The plasma samples for ELISA measurement were transferred in cryotubes, labelled carefully to enable unique identification, stored at –20°C until radioactivity had decreased (^{186}Re: 4 weeks, ^{99m}Tc: 3 days) and sent to Boehringer Ingelheim Department of Pharmacokinetics and Drug Metabolism, Biberach, Germany in batches every four weeks. The plasma samples were sent on dry ice.

3.5.4.2 Analytical determinations

Plasma samples were measured by validated ELISA methods at the Boehringer Ingelheim Department of Pharmacokinetics and Drug Metabolism, Biberach, Germany. Radioactivity counting of the samples were performed in full blood, serum and urine by the investigator.

3.5.4.3 Parameters and evaluation

The following pharmacokinetic parameters using WinNonlin 3.1 Professional (Pharsight Corporation, Mountain View, CA) were determined from full blood, plasma, serum levels and imaging data as described below:

The primary pharmacokinetic parameters are the time point at which the maximum drug concentration is observed (T_{max}), maximum drug concentration observed (C_{max}), area under the concentration-time curve $(AUC)_{0\rightarrow\infty}$, terminal elimination half-life ($t_{1/2}$), volume of distribution (terminal phase and predicted steady state), total body clearance (CL), and mean residence time $(MRT)_{0\rightarrow\infty}$.

The primary objectives of pharmacokinetic analysis were:

To determine and compare the disposition of total radioactivity and immunoreactive hMAb BIWA 4 following administration of single doses of ^{99m}Tc-labelled (Part A) and ^{186}Re-labelled hMAb (Part B) BIWA 4.

To calculate, from imaging data and blood disposition data, the fraction of the injected radioactivity reaching the liver and spleen during the studies. To achieve this objective, the WinNonlin® software package was used to perform non-compartmental pharmacokinetic analysis on the plasma concentration vs. time profiles of immunoreactive hMAb BIWA 4 and of total blood radioactivity levels, as a function of time, following intravenous administration.

The results are reported as summary statistics.

3.5.5 Pharmacokinetic / pharmacodynamic relationship

A compartmental pharmacokinetic model was initially intended to describe the distribution and metabolism of ^{186}Re-labelled BIWA 4 in humans. It was intended to combine data from BIWA 4 plasma levels, full blood and serum radioactivity measurements, radioactivity from whole body images and specific regions of interest, the administered dose ^{186}Re-labelled BIWA 4, the dose of unlabelled BIWA 4, soluble CD44v6 levels and the assessment of the radiolabelled antibody prior to infusion.

The results were to be reported as summary statistics. However, variability was too high to be used for a reasonable model.

3.5.6 Primary Safety variable

Determination of maximum tolerated dose and assessment of safety were the primary endpoints in Part B of the trial. The measurements done are described in section 3.5.1.6.

3.5.6.1 Dose Limiting Toxicity and Maximum Tolerated Dose

DLT was defined as drug-related CTC grade 3 non-haematologic toxicity or drug-related CTC grade 4 haematologic toxicity, excluding nausea and vomiting without adequate antiemetic treatment.

The MTD was defined as the dose level at which less than two out of six patients developed drug-related DLT.

3.6 DATA QUALITY ASSURANCE

The trial was in general conducted according to the principles of Good Clinical Practice as specified in the appropriate regulations and in the company standard operating procedures reflecting these regulations.

Throughout the course of the study, a representative from Boehringer Ingelheim The Netherlands was contacting and/or visiting the study site to monitor the progress of the study. There were frequent contacts with the investigator and onsite visits for the purpose of data audits, including the comparison of source documents with Case Report Forms and drug accountability checks. The investigator or his/her designee had to be available to the Boehringer Ingelheim The Netherlands representative during these onsite visits.

Duly filled in CRFs were collected on a regular basis. Data were double-data entered in-house. Review of the data was done and implausibilities questioned to the investigator.

Serious adverse events were reported according to the guidelines defined in the protocol and CRF and according to Boehringer Ingelheim SOPs.

3.7 STATISTICAL METHODS PLANNED IN THE PROTOCOL AND DETERMINATION OF SAMPLE SIZE

3.7.1 Statistical and analytical plans

3.7.1.1 Statistical design / model

The designs used in this trial are commonly used in Phase I oncology trials.

Part A

Part A of this phase I trial was an uncontrolled, rising dose sequential group study to determine the safety, tolerability, biodistribution and pharmacokinetics of a single infusion of ^{99m}Tc-labelled hMAb BIWA 4 in patients with advanced squamous cell carcinoma of the head and neck.

Three hMAb BIWA 4 dose levels were used in this part of the study with three patients planned at each dose level. All patients had a proven tumour of the head and neck and were destined for surgery.

Part B

Part B of this phase I trial was an open uncontrolled dose escalation study to determine the safety, tolerability, MTD, pharmacokinetics and preliminary therapeutic effects of a single infusion of ^{186}Re-labelled hMAb BIWA 4 in patients with advanced squamous cell carcinoma of the head and neck for whom no curative treatment options were available.

In this part of the study the radiation dose was escalated. Two patients were treated at dose levels where < grade 2 CTC toxicity was seen. If ≥ grade 2 toxicity was seen at least three patients were treated per dose group.

Safety and tolerability evaluation (Parts A + B)

Safety and tolerability of the study were assessed in terms of changes in laboratory parameters, vital signs, development of HAHA and the incidence of adverse experiences. The results were reported for each dose level separately as well as in terms of overall means.

Biodistribution evaluation (Part A)

The biodistribution of ^{99m}Tc-labelled hMAb BIWA 4 in solid tissue was assessed by radioimmunoscintigraphy (see sections 3.5.1.1) and by measurements of radioactivity of biopsy specimens (see sections 3.5.1.1 and 3.5.1.2). Because only a maximum of 3 patients were entered per dose level only descriptive statistics were possible.

Immunoscintigraphic imaging evaluation (Parts A+B)

At given time-points after the administration of radio-labelled antibody immunoscintigraphic images were obtained (see section 9.5.1.1). Again only descriptive statistics were applicable.

Pharmacokinetic evaluation (Parts A + B)

The following pharmacokinetic parameters were determined from plasma or serum, urine levels and imaging data as described below:

Primary parameters (non-compartmental)

T_{max}, C_{max}, AUC (0-infinite time), terminal elimination half-life, volume of distribution (terminal phase V_z and predicted steady state V_{ss}), CL, and MRT (0-infinite time). Cumulative urinary excretion of radioactivity over time was determined from total urine output.

Secondary parameters (compartmental)

A compartmental pharmacokinetic model was planned to be developed to define the distribution and metabolism of ^{186}Re-labelled hMAb BIWA 4 in humans. The model collated data from serum and urine radioactivity measurements, radioactivity from whole body images and specific regions of interest, the administered dose ^{186}Re-labelled hMAb BIWA 4, the cold-loading dose of hMAb BIWA 4, soluble CD44v6 levels and the assessment of the radiolabelled antibody prior to infusion.

The results were planned to be reported for each dose level separately and by means of summary statistics.

Therapeutic efficacy evaluation (Part B)

Tumour response was the parameter for therapeutic efficacy. Tumour response was assessed according to the WHO guidelines. The sum of the products of the largest perpendicular diameters of all measured tumour lesions were the primary parameter for tumour response. There were separate criteria for the assessment of response of bone lesions.

3.7.1.2 Null and alternative hypotheses

All analyses in this trial were descriptive and exploratory by nature. Any statistical tests were performed only to provide a statistical framework from which to view the results and providing aid for planning further studies. No formal statistical inferences were foreseen and, accordingly, no statistical tests were performed.

3.7.1.3 Planned analyses

Two populations were distinguished:

- The intention to treat subset consists of all patients who received an infusion of radiolabelled hMAb BIWA 4 and for whom data after baseline were available.
- The per protocol subset consists of evaluable patients. A patient was evaluable if the following criteria were met:

Part A:

1. Fulfilling the criteria for the intention to treat subset.

2. The follow-up in terms of adverse events, vital signs and safety blood and urine samples was adequate.

3. The immunoreactive fraction in at least one of both performed assays was larger than 60%.

4. Adequate biopsies and scintigraphic images were available to assess the biodistribution.

Part B:

1. Fulfilling the criteria for the intention to treat subset.

2. The follow-up in terms of adverse events, vital signs and safety blood and urine samples was adequate.

3. The immunoreactive fraction in at least one of both performed assays was larger than 60%.

4. Response: Patients (Pts.) were evaluable for response if adequate tumour measurements were obtained at baseline and if patients were followed over at least a six week period. If disease progression was observed within this six weeks period, patients were also evaluable for response.

Primary Analyses

The primary analysis was performed for the per protocol subset.

Non evaluable patients were replaced. For Part A therefore the evaluable subset was planned to consist of 9 patients and the intent to treat subset consisted of at least 9 patients. For Part B the number of patients depended on the toxicity encountered. Patients who were evaluable for toxicity but not for response were not replaced.

Pharmacokinetic data:

The analysis of pharmacokinetic data was performed by Dr. Thomas R. MacGregor, Boehringer Ingelheim Pharmaceuticals Inc. Ridgefield CT, USA.

The primary objectives of pharmacokinetic analysis were:

1. To determine and compare the disposition of total radioactivity and immunoreactive hMAb BIWA 4 following administration of single doses of ^{99m}Tc-labelled hMAb BIWA 4 and ^{186}Re-labelled hMAb BIWA 4.

2. To identify an appropriate cold-loading dose of hMAb BIWA 4.

3. To assess the disposition of a second dose of ^{186}Re-labelled hMAb BIWA 4.

4. To calculate, from imaging data and blood disposition data, the fraction of the injected radioactivity reaching the liver and spleen during the studies. To achieve this objective, the WinNonLin® software package was used to perform noncompartmental pharmacokinetic analysis on the plasma concentration vs. time profiles of immunoreactive hMAb BIWA 4 and of total blood radioactivity levels, as a function of time, following intravenous administration. Any measurements involving radioactivity were normalised to the fraction of the radiolabel that was protein-bound and immunoreactive prior to infusion. The specific pharmacokinetic parameters that were generated include: T_{max}, C_{max}, AUC (0- infinite time), terminal elimination half-life, volume of distribution (terminal phase and predicted steady state), total body clearance, and mean residence time (0- infinite time). Cumulative urinary excretion of radioactivity over time were determined from total urine output.

The secondary objective of the pharmacokinetic analysis to develop a pharmacokinetic model to describe the distribution and metabolism of ^{186}Re-labelled hMAb BIWA 4 in humans was not achieved due to the high variability of the data (especially the radioscintigraphic data).

Secondary Analyses

The secondary analyses were performed for the intention to treat subset of patients participating in Part B of the study.

The secondary analyses were restricted to key endpoints: e.g.: Response rate, duration of response, time to progression.

Interim Analysis

No interim analyses were made.

3.7.1.4 Handling of missing data

In this phase I study it was anticipated that most data were available for analysis. In case of missing data it was most likely that the reason for missing was not outcome related. No missing data were imputed.

3.7.2 Determination of sample size

In Part A three patients per dose level provide some information on safety of the hMAb BIWA 4 and on the anticipated correct dose of 50 mg unlabelled hMAb BIWA 4.

In Part B six patients were regarded sufficient to determine DLT. TABLE 3.7.2:1 exhibits the probabilities of 2 or more out of 6 patients to be observed with DLT for some assumed underlying rates of DLT in the population of all patients.

TABLE 3.7.2: 1 Assumed Population Rates of DLT

	0.40	0.42	0.45
Probability of observing DLT in two or more out of six patients	0.77	0.80	0.84

Source: Probabilities calculated from the cumulative distribution function of the binomial distribution with n=6 and varying p's.

With the escalation scheme in this trial, there was a probability of at least 80% for two or more patients to exhibit DLT, if the underlying individual probability for a patient to reach DLT is 42 % or larger.

3.8 CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

The following changes from protocol were implemented via an amendment.

Time points for collection of blood samples were adjusted by implementation of amendment No 1 dated 1 September 1999. The samples at 10 minutes and 6 weeks after infusion were dropped while additional samples were collected after 240 and 336 hours post end of infusion in Part B of the trial. It had turned out that the original blood sampling schedule was not sufficient to adequately cover an assumed half-life of 50 hours which was observed in Part A of the trial. A total of 3.5 mL blood was collected in potassium EDTA tubes and 3.5 mL in coagulation tubes.

The protocol stated that patients could be administered a second dose of ^{186}Re-BIWA 4 after having determined the MTD. This procedure was modified by amendment 2. Patients were considered eligible for a second treatment cycle in case they responded to the first administration of ^{186}Re-BIWA 4, were free of or had recovered from adverse events and had no HAHA antibodies. Response was defined as stable disease (i.e. no change), partial remission or complete remission. The second dose administered was always 50 mCi/m^2. Due to the change in the linker the trial was concluded before having investigated a second dose with the dosing regimen described in the protocol. To allow patients who responded to be treated anyhow, amendment 2 was issued.

The trial was completed and terminated after having reached the MTD due to the change of the linker. MAG3 will be replaced by MAG2GABA-TFP in the future development of the BIWA 4 project.

Samples were available for sCD44v6 determination for more time points than originally planned.

The following changes were made after having evaluated the data and after discussion within the trial team.

The ratio of %ID/kg tumour versus %ID/kg non-tumour tissue was performed for bone marrow only, due to the variability of the results.

The same holds true for the evaluation of the tumour size. Measurements were incomplete thus jeopardising a reasonable evaluation. Therefore, no formal analysis was done.

4. STUDY SUBJECTS

4.1 DISPOSITION OF SUBJECTS

Of the 33 patients screened three patients discontinued the trial due to adverse events before administration of the trial drug (TABLE 6.1: 1). These three patients are not included in the analyses. A total of 30 patients were entered and treated in this trial.

Part A

Ten patients of the 30 patients treated were included in Part A with 3 each receiving 25 mg and 100 mg ^{99m}Tc-BIWA 4, respectively, while four patients received 50 mg ^{99m}Tc-BIWA 4. All patients in Part A completed the trial.

Part B

Two out of the 20 patients treated in Part B discontinued due to adverse events (two patients died). Three patients were administered a second dose of 50 mCi/m^2.

TABLE 6.1: 1 Disposition of Patients

	Part A			Part B					Total
	^{99m}Tc-BIWA 4			^{186} Re-BIWA 4 [mCi/m^2]					
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	20	30	40	50	60	
Screened									33
Entered	3	4	3	2	4	3	6	5	30
Treated	3	4	3	2	4	3	6	5	30
Completed	3	4	3	2	3	2	6	5	28
Discontinued	0	0	0	0	1	1	0	0	2
due to AE	0	0	0	0	1	1	0	0	2
due to lack of efficacy	0	0	0	0	0	0	0	0	0
due to other reason	0	0	0	0	0	0	0	0	0

Source Appendix 16.1.9.2 TABLE 1.1

4.2 PROTOCOL DEVIATIONS

The majority of protocol violations were deviations (> ± 10%) from the time windows provided in the protocol concerning blood and/or urine sampling. The patients affected were not excluded from the analyses but the actual times of collecting blood or urine were used for determination of blood, plasma, serum and urine concentrations.

5. EFFICACY / CLINICAL PHARMACOLOGY EVALUATION

Results from Part A confirmed a dose of 50 mg BIWA 4 as the optimal dose for treatment. Data from Part B indicate that patients may clinically benefit from ^{186}Re-BIWA 4 therapy at a radiation dose below that dose at which DLT was observed.

Concentrations of BIWA 4 measured were dose-proportional and a moderate amount of the dose administered was excreted via the kidneys.

5.1 DATA SETS ANALYSED

All patients who had received at least one dose of the trial drug were included in the intent-to-treat analysis. Seven patients from Part A were included in the per protocol analysis. No per protocol analysis was performed for Part B.

5.2 DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

The mean age of all patients included was 56 years (range 37 to 78). Nineteen patients were males and eleven were females (TABLE 5.2: 1).

Three patients treated in Part A never smoked while seven were current smokers (pack years range 19 - 47). Frequent alcohol consumption was reported in eight patients while two were non drinkers (TABLE 5.2: 1).

All patients in Part B were either ex-smokers or current smokers (TABLE 5.2: 1) with a mean pack-year of 35 (range 10 to 86). Six patients were non-drinkers while the remaining patients were frequent consumers of alcohol (TABLE 5.2: 1).

Stage of the disease was local operable for all patients included in Part A while patients from Part B had recurrent disease (15 patients) and/or metastases (3 patients). One patient had local inoperable disease. In one patient the information is missing.

Concomitant diseases or relevant medical history were reported in 28 patients (TABLE 5.2: 1). Concomitant therapy was required by all patients. The most often used medications were analgesics, sedatives, lactulose, H_2-blockers, anti-emetics and antimicrobial agents.

TABLE 5.2: 1 Demographics and baseline characteristics. Given are means for age. All other figures denote numbers of patients

	Part A			Part B					Total
	^{99m}Tc-BIWA 4			^{186}Re-BIWA 4 [mCi/m^2]					
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	20	30	40	50	60	
n	3	4	3	2	4	3	6	5	30
age [mean]	45.7	58.5	58.7	57.0	61.0	53.0	57.0	60.4	56.4
male/female	2/1	2/2	1/2	1/1	3/1	2/1	5/1	3/2	19/11
conc.disease	3	3	2	2	4	3	6	5	28
concom. therapy	3	4	3	2	4	3	6	5	30
smoker	2	4	1	0	1	0	3	1	12
ex-smoker	0	0	0	2	3	3	3	4	15
Alcohol yes	3	3	2	0	3	3	6	2	22

Initial diagnosis of disease was known in patients from Part A about one month before inclusion while patients included in Part B were ill since two years on average (range 0.1 to 17.5 years; TABLE 5.2: 2). The Karnofsky score ranged from 70 to 100 in patients from Part A. Patients from Part B had Karnofsky scores of 70 to 90 (TABLE 5.2: 2).

Metastases at diagnosis were reported for one patient in Part B of the trial.

All patients in Part B had undergone previous radio- and / or chemotherapy while patients in Part A had neither previous chemo- nor radiotherapy reported (TABLE 5.2: 2). Cisplatin, methotrexate and fluorouracil were the most often systemic anticancer drugs used).

Previous surgery was radical in eleven of the 13 patients in Part B who had a previous surgery. Only one patient from Part A, had undergone previous surgery (TABLE 5.2: 2).

Lesion sizes (including metastases) of the tumours ranged from 14 mm^2 to 10304 mm^2. The primary tumour site was moderately differentiated in the majority of the patients. Lymph nodes were affected in 13 patients (TABLE 5.2: 2).

TABLE 5.2: 2 Demographics and baseline characteristics. Given are means for years of known disease and Karnofsky score. All other figures denote numbers of patients

	Part A			Part B				
	^{99m}Tc-BIWA 4			^{186} Re-BIWA 4 [mCi/m^2]				
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	20	30	40	50	60
n	3	4	3	2	4	3	6	5
years of known disease	0.11	0.08	0.07	9.30	2.13	1.17	0.80	1.16
Karnofsky score	90	90	100	80	75	76.7	85	80
radical surgery	0	0	0	2	2	1	2	4
lymph nodes affected	2	2	0	2	3	2	1	1
previous chemotherapy	0	0	0	1	3	1	4	1
previous radiotherapy	0	0	0	2	4	3	6	5

5.3 MEASUREMENTS OF TREATMENT COMPLIANCE

All medication was administered under the supervision of the investigator or his/her delegate. Blood was collected to determine the pharmacokinetics of BIWA 4. All patients had detectable blood levels of BIWA 4.

5.4 EFFICACY / CLINICAL PHARMACOLOGY RESULTS

Three out of six patients experienced stable disease at the maximum tolerated dose of 50 mCi/m^2. The dose of 50 mg BIWA 4 was confirmed in Part A of the trial to be the optimal dose concerning blood concentrations and selective tumour uptake . The plasma concentrations of BIWA 4 were dose-proportional in the range of 25 mg to 100 mg BIWA 4 in Part A and peaked at 0.9 hours with a terminal elimination half-life of 54 – 74 hours and 94 hours for Part A and Part B, respectively.

5.4.1 Analysis of efficacy / pharmacodynamics

5.4.1.1 Primary endpoints

Primary endpoint for efficacy was the biodistribution of ^{99m}Tc-BIWA 4 in Part A. Further primary endpoints were the analyses of the radioimmunoscintigraphic images and the

pharmacokinetics (described in section 5.4.2) for Part A and B of the trial, respectively. Dosimetry was done for Part B only.

Biodistribution of ^{99m}Tc-BIWA 4 in Part A

Tissue samples were obtained during surgery and the uptake of ^{99m}Tc-BIWA 4 expressed as % injected dose (ID)/kg tissue.

Intent-to-treat (ITT) subset: The relative biodistribution of ^{99m}Tc-BIWA 4 was highest in the tumour in all three dosing groups except for patient 1 (25 mg BIWA 4), patient 5 (50 mg BIWA 4, no tumour cells) and patient 9 (100 mg BIWA 4), respectively. The uptake in tumour ranged from 6 to 17 % ID/kg, 5 to 28 % ID/kg and 13 to 17 % ID/kg for the dose group 25 mg, 50 mg and 100 mg, respectively. The calculated mean ratio of tumour uptake versus uptake in bone marrow was 1.7, 2.6 and 2 in the 25 mg, 50 mg and 100 mg dose group, respectively, (TABLES 7.2: 1 and 7.2: 2).

Per-protocol (PP) subset: The relative biodistribution of ^{99m}Tc-BIWA 4 was highest in the tumour in all three dosing groups except for patient 1 (25 mg BIWA 4). The uptake in tumour ranged from 6 to 17 % ID/kg, 23 to 28 % ID/kg and 16 % ID/kg for the dose group 25 mg, 50 mg and 100 mg, respectively. The calculated mean ratio of tumour uptake versus uptake in bone marrow was 1.7, 3.2 and 2.5 in the 25 mg, 50 mg and 100 mg dose group, respectively, (TABLES 7.2: 1 and 7.2: 3).

A trend to greater uptake with smaller tumour size appeared to be present for the 25 mg BIWA 4 dose group. A similar evaluation was not possible for the 50 mg and 100 mg BIWA 4 dose groups due to the limited number of patients (TABLE 7.2: 4).

High %ID/kg uptake was observed in bone marrow supernatant (up to 17 %ID/kg) and plasma or blood (up to 13 %ID/kg) which was always below the uptake in the tumour in the per protocol population except for patient 1 (25 mg BIWA 4). Uptake in skin and mucosa was always below the uptake in primary tumour in the per protocol subset.

Radioimmunoscintigraphic images of ^{99m}Tc-BIWA 4 in Part A

While no radioactivity uptake was found in the tumour directly after infusion, a medium uptake of 1.9 was recorded after 21 hours. Uptake of radioimmunoconjugate appeared to be low in bone marrow and kidney both directly after infusion and 21 hours after infusion. Uptake was low to medium in lung while liver revealed a higher uptake.

A similar pattern was observed for the per protocol population. The only difference appeared to be a higher uptake in kidney while the uptake was lower in lung as compared to the intent-to-treat population.

Radioimmunoscintigraphic images of ^{186}Re-BIWA 4 in Part B

Radioiummunoscintigraphic examinations were done immediately after infusion and 21, 48, 72, 144 and 336 hours after infusion. Hardly any uptake in tumour was observed directly after the infusion. Relative uptake in tumour appeared to be dose-dependent and increased over time reaching medium and high uptake after 72 to 144 hours with a decline after 336 hours.

Biodistribution of radioactivity was similar in bone marrow, lung, liver, kidney and intestine and did not reveal the same dose dependent effect (except for the intestine). Moreover, relative uptake of radioactivity appeared to be constant or modestly decreased over time and was similar for all doses of radioactivity. Uptake in bone marrow was lowest for most treatment groups.

5.4.1.2 Secondary endpoint(s)

Secondary endpoints of efficacy were tumour response to therapy in Part B and the level of CD44v6 expression in tumour in Part A, respectively. Soluble CD44v6 expression was determined for both Part A and Part B of the trial.

CD44v6 expression in tumour in Part A

Data of determination of CD44v6 antigen expression is available for seven patients. CD44v6 antigen expression was observed in more than 90% and 80% of the tumour cells in five and two patients, respectively, while CD44v6 antigen expression was detected in more than 90% of the cells of lymph node metastases in the four patients for which lymph node was available for evaluation. Homogenous CD44v6 antigen expression was observed in all patients in the mucosa.

Tumour response in Part B

Patients treated with doses of up to 40 mCi/m^2 ^{186}Re-BIWA 4 did not experience a clinical tumour response. All patients experienced progressive disease. One patient was not evaluable due to intercurrent death.

Three of the six patients treated with 50 mCi/m^2 ^{186}Re-BIWA 4 developed stable disease or no change after the first cycle. Two of these three patients who were treated with another cycle with 50 mCi/m^2 ^{186}Re-BIWA 4 had progressive disease after the second cycle. Progressive disease was observed after a total of 148 days (patient 109) and 127 days (patient 116) after first administration of the trial drug, respectively.

One patient treated with 60 mCi/m^2 ^{186}Re-BIWA 4 experienced stable disease after the first cycle. This patient went into progressive disease after a second cycle with 50 mCi/m^2 ^{186}Re-BIWA 4 (173 days after the first administration of the trial drug).

The overall time to progression for patients who did not respond to therapy ranged from 0 to 55 days with a mean of about five to six weeks irrespective of the treatment group.

Soluble CD44v6 in Part A and B

Soluble CD44v6 was determined in all patients treated with BIWA 4 in the present trial.

The amount of soluble CD44v6 detected tended to increase for the ^{186}Re-BIWA 4 treated patients (TABLE 7.2: 6) during the trial in Part B while for patients treated with ^{99m}Tc in Part A no such trend could be observed.

5.4.2 Drug dose, drug concentration, and relationship to response

The pharmacokinetics of BIWA 4 will be presented separately for Parts A and B because the radiolabel was different (Part A: ^{99m}Tc; Part B: ^{186}Re).

5.4.2.1 Analytical performance with regard to BIWA 4, HAHAs and CD44v6

Validated assays were used for analysis of plasma samples. Analysis of quality control samples produced results of high accuracy and precision.

Analytical performance with regard to gamma counting of samples

The signal linearity of the scintillation crystal in the gamma counter was not tested. It can be assumed based on the type of instrument at least in the energy range that was used in the current study. Calibration samples showed typically 40000-200000 counts per minute *vs* less than 50-100 counts per minute in background samples. The precision of the seven measurements per calibration standard was regularly within 2%. The same applies for the precision of the individual triplicate measurements in blood, serum and urine. Blood and serum samples showed typically activities between 2000 and 100000 counts per minute.

There were no quality control samples for radioactivity counting. The precision of repeated measurements of calibration standards and samples derived from the current study indicate that the precision of triplicate measurements was at least better than 2 %.

5.4.2.1 Pharmacokinetics

Part A.

Part A consisted of 10 patients receiving a single BIWA 4 dose ranging from 25 to 100 mg with a constant radiolabelled dose of 20 mCi ^{99m}Tc. The pharmacokinetic parameters in plasma for BIWA 4 measured by ELISA following a short intravenous infusion are given in TABLE 5.4.2.2: 1.

TABLE 5.4.2.2: 1 Pharmacokinetic parameters for BIWA 4 in plasma from Part A of the study

Patient	BIWA 4 Dose (mg)	tmax (min)	Cmax (ng/mL)	Half-Life (h)	AUC_{inf} (µg•h/mL)	Vz (L)	CL (mL/min)	MRT (h)
1	25	0	6634	44.1	387.4	4.1	1.076	58.0
2	25	30	6640	67.0	534.7	4.5	0.779	94.8
3	25	10	9590	53.3	542.9	3.5	0.768	71.0
4	50	29	14900	64.9	1112.3	4.2	0.749	84.8
5	50	121	15500	102.8	1580.2	4.7	0.527	135.2
8	50	5	13900	53.0	831.0	4.6	1.003	71.7
10	50	41	16700	83.8	1461.6	4.1	0.570	110.0
6	100	4	30500	85.0	2548.9	4.8	0.654	112.0
7	100	17	30600	76.0	2709.6	4.0	0.615	110.4
9	100	30	24500	44.6	1708.1	3.8	0.976	61.1
Geometric mean								
	25 mg	.	7503	54.0	482.7	4.0	0.863	73.1
	50 mg	29	15216	73.8	1208.8	4.4	0.689	97.5
	100 mg	13	28383	66.0	2276.4	4.2	0.732	91.1

The increase in C_{max} BIWA 4 plasma concentrations observed and the area under the curve were proportional to the dose administered (FIGURES 4 and 5).

Serum concentrations of the radiolabel (^{99m}Tc) were also determined and the pharmacokinetic parameters are presented in TABLE 5.4.2.2: 2.

TABLE 5.4.2.2: 2 Pharmacokinetic parameters for ^{99m}Tc-BIWA 4 in serum from Part A of the study

Patient	^{99m}Tc-BIWA 4 Dose (mCi)	Tmax (min)	Cmax (%ID/kg)	Half-Life (h)	AUC_{inf} (%ID•h/kg)	Vz (kg)	CL (kg/min)	MRT (h)
1	19.0 mCi	117	52.66	44.6	3376.9	1.9	0.030	63.8
2	20.0 mCi	10	28.08	55.6	2151.7	3.7	0.048	79.1
3	21.4 mCi	10	39.11	27.5	1445.3	2.7	0.072	41.5
4	19.5 mCi	29	31.84	31.3	1432.8	3.2	0.072	43.8
5	19.1 mCi	121	32.29	39.6	1934.8	3.0	0.054	56.3
8	18.8 mCi	114	28.41	42.8	1620.1	3.8	0.060	60.8
10	19.3 mCi	41	40.31	43.1	2247.1	2.8	0.042	62.3
6	19.3 mCi	120	30.45	45.3	1946.5	3.4	0.054	65.2
7	19.8 mCi	77	35.79	36.0	1628.6	3.2	0.060	51.1
9	18.3 mCi	238	31.94	35.5	1805.6	2.8	0.054	50.6
Harmonic mean				38.7				
Geometric mean		57.9	34.5	39.4	1898.0	3.0	0.053	56.5

The geometric mean half-life for BIWA 4 measured by ELISA in plasma following intravenous infusion ranged from 54 to 73.8 hours for the three treatment groups studied in Part A. The geometric mean half-life for the ^{99m}Tc-radiolabeled BIWA 4 for the same samples was shorter at 39.4 hours in serum and 46.4 hours in blood. The discrepancy between the two mean estimates was attributed to longer sampling times possible with BIWA 4 due to radioactivity assay restrictions.

Radioactivity was excreted into the urine in the amounts collected in TABLE 5.4.2.2: 3. Due to incomplete collections over the 48 hours (i.e. cumulative data may not always be comparable because of varying duration of collection), no further assessment was made of this urinary excretion data. For six patients with complete 48-hour collections, the mean amount of radioactivity collected was 10% of the initial dose.

TABLE 5.4.2.2: 3 Amount of radioactivity (^{99m}Tc) excreted (as percent of initial dose) into urine in Part A

Patient	Collection Interval (h)					Cumulative Amount (%ID)
	0-4	4-8	8-12	12-24	24-48	
1	2.80	10.13	5.08	n.a.	n.a.	18.01
2	1.94	0.97	0.52	3.09	3.23	9.75
3	0.29	1.44	1.19	n.a.	n.a.	2.92
4	1.68	1.42	1.95	1.14	2.21	8.40
5	2.20	1.60	1.02	3.96	3.98	12.76
8	1.29	2.45	0.88	3.67	3.00	11.29
10	n.a.	n.a.	n.a.	n.a.	n.a.	
6	2.55	1.59	1.80	1.89	2.00	9.83
7	n.a.	3.14	n.a.	1.79	3.13	8.06
9	1.54	3.34	0.00	1.05	2.31	8.24

n.a. = not applicable, no data available

Part B:

Part B consisted of 20 patients receiving BIWA 4 doses of 50 mg with varying radiolabelled doses of ^{186}Re-BIWA 4 ranging from 20-60 mCi/m^2. Three patients received a second intravenous infusion while the other 17 patients received only a single course of therapy.

Graphically, there was consistency between plasma BIWA 4 concentrations and serum radioactivity concentrations for each patient and within the three patients that received ^{186}Re-BIWA 4 on two occasions. This consistency observed graphically contrasts with the perception of a longer half-life for radioactivity when the data was modelled (noncompartmental evaluation). The geometric mean half-life of 122 hours for the radioactive portion of the ^{186}Re-BIWA 4 was longer than the ELISA-determined plasma half-life of 94 hours for BIWA 4.

Grouping (TABLES 5.4.2.2: 6 and 5.4.2.2: 7) the pharmacokinetic parameters by amount of radioactivity administered showed some trends of longer exposure with increased radioactivity dosed, but the numbers of individuals in each dose group are too small to make any consistent conclusions.

TABLE 5.4.2.2: 6 Geometric means for plasma BIWA 4 pharmacokinetic parameters grouped by amount of radioactivity given.

^{186}Re Dose (mCi/m^2)	n	Cmax (ng/mL)	Half-Life (h)	AUC_{inf} (µg•h/mL)	Vz (L)	CL (mL/h)	MRT (h)
20	2	12160	106.5	1217	6.3	41.1	130.3
30	4	15239	86.2	1399	4.4	35.7	116.2
40	3	11121	90.6	1201	5.4	41.7	125.1
50	9*	12982	92.4	1055	6.3	47.4	117.4
60	5	11927	99.8	939	7.7	53.2	115.9

* patients who received two doses of 50 mCi/m^2 are included

TABLE 5.4.2.2: 7 Geometric means for serum ^{186}Re-BIWA 4 pharmacokinetic parameters grouped by amount of radioactivity given.

^{186}Re Dose (mCi/m^2)	n	Cmax (%ID/kg)	Half-Life (h)	AUC_{inf} (%ID•h/kg)	Vz (kg)	CL (kg/h)	MRT (h)
20	2	30.88	114.4	3296	5.01	0.030	154.6
30	4	40.09	98.8	4365	3.27	0.023	134.5
40	3	28.82	126.4	3856	4.73	0.026	172.4
50	9*	40.22	123.3	4182	4.25	0.024	162.5
60	4	32.10	145.2	3493	6.00	0.029	185.5

* patients who received two doses of 50 mCi/m^2 are included

5.4.2.2 Pharmacokinetic / pharmacodynamic analysis

A pharmacokinetic/pharmacodynamic analysis was not performed.

5.4.3 Statistical / analytical issues

The analysis was performed as originally planned. Details are described in section 3.7.1.3.

5.4.3.1 Primary endpoint(s)

Not applicable.

5.4.3.2 Secondary endpoint(s)

Not applicable.

5.4.4 Drug-drug and drug-disease interactions

No tests concerning drug-drug or drug-disease interactions were done.

5.4.5 By-subject displays

For details see section 6.4.2.2 and 6.4.2.3.

5.5 EFFICACY / CLINICAL PHARMACOLOGY CONCLUSIONS

Part A:

Results from Part A confirmed a dose of 50 mg BIWA 4 as the optimal dose for treatment based on blood concentrations and tissue uptake level. The distribution as assessed by radioscintigraphy and biopsy measurements was in almost all cases highest in the tumour as compared to other tissues. Uptake of radioactivity increased in the tumour over time. CD44v6 expression was present in more than 80 % and 90% of the cells of primary tumour and lymph node metastases, respectively, and in all mucosa specimens obtained.

The amount of soluble CD44v6 appeared to be constant before and after ^{99m}Tc-BIWA 4 administration.

Concentrations of BIWA 4 measured were dose-proportional in the range of 25 mg to 100 mg BIWA 4. A moderate amount of the dose administered was excreted via the kidneys. The % ID excreted in urine was similar for all dosing groups.

Part B:

Data from Part B indicate that patients may clinically benefit from ^{186}Re-BIWA 4 therapy at MTD. One out of five patients treated with 60 mCi/m^2 had stable disease. Three out of six patients experienced stable disease at the maximum tolerated dose of 50 mCi/m^2. Time until progression ranged between 127 and 173 days in those patients who received a second dose of 50 mCi/m^2. Radioimmunoscintigraphy indicates uptake of radioactivity in tumour tissue. Biodistribution was comparable in other tissues irrespective of dose. Dosimetric analysis did not reveal unexpected high absorbed doses in tissues other than the tumour except for the

testes. However, the relevance of the observed testes dose for the impairment of fertility is currently unclear.

The amount of soluble CD44v6 detected tended to increase for the ^{186}Re-BIWA 4 treated patients.

The plasma concentrations of BIWA 4 peaked at 0.92 hours and the antibody was eliminated with a geometric mean half-life of 94 hours for BIWA 4 determined by ELISA. C_{max} and AUC values (ELISA measurement) were similar to those obtained in Part A of the trial for the 50 mg BIWA 4 dose group.

Dose limiting toxicity occurred at a dose of 60 mCi/m^{2}while a dose of 50 mCi/m^2 ^{186}Re-BIWA 4 turned out to be the maximum tolerated dose in the present trial. The dose limiting toxicity were adverse events from the bone marrow i.e. thrombocytopenia and leucopenia.

6.1 EXTENT OF EXPOSURE

All ten patients treated in Part A of the trial were administered one single dose of ^{99m}Tc-BIWA 4. Doses of BIWA 4 were either 25 mg, 50 mg or 100 mg while ^{99m}Tc was 20 mCi.

In Part B, 20 patients were administered one single dose of ^{186}Re-BIWA 4. Three patients received a second dose of 50 mCi/m^2 due to stable disease (no change). The dose of BIWA 4 was kept stable at 50 mg while the radioactivity of ^{186}Re was increased in 10 mCi/m^2 body surface increments.

The dose was calculated according to body surface area (TABLE 6.1: 1).

TABLE 6.1: 1 Mean dose of radioactivity administered

	Part A			Part B				
	^{99m}Tc-BIWA 4			^{186} Re-BIWA 4 [mCi/m^2]				
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	20	30	40	50	60
N	3	4	3	2	4	3	6	5
mCi ^{99m}Tc	20.1±1.2	19.2±0.3	19.1±0.8	n.a	n.a	n.a	n.a	n.a
mCi ^{186}Re	n.a.	n.a	n.a	33.1±1.7	52.4±5.6	70.6±5.2	87.2±8.5	97.2±8.3

n.a. = not applicable, mean values and standard deviation are given

The radiochemical purity of the drug was more than 95 % and the immunoreactive fraction was higher than 80 %.

An observation period of at least 6 weeks followed the administration of the trial drug.

6.2 ADVERSE EVENTS (AEs)

Adverse events were reported in all patients treated. Two patients discontinued the observation period in Part B due to an AE. No action was taken with the trial drug since treatment had been completed.

Part A:

The majority of patients in Part A experienced adverse events from body as a whole (50 %) which may be also due to the underlying surgery and the associated pain. No special pattern of adverse events was reported and none of the patients developed CTC criteria 3 or 4 which were judged to be drug-related (TABLES 6.2.2: 1 and 2).

Three patients experienced serious adverse events which are described in section 6.3.1.

Part B:

Adverse events of the system organ class (SOC) 'body as a whole' were reported in 65 % of the patients, followed by adverse events from the system organ class 'platelet, bleeding and clotting disorders' (60 % of the patients) and 'white cell and reticulo endothelial system disorders' (50 % of the patients). Drug-related thrombocytopenias and leucopenias occurred in 11 (55 %) and 10 (50 %) patients on average 21 days after start of radioimmunotherapy and were generally reversible after 6 weeks. A dose-response was observed for these events (TABLE 6.2.2: 3).

Haematological dose-limiting toxicity defined as drug-related CTC grade 4 was reported in three patients.

Non-haematological dose-limiting toxicity defined as drug-related CTC grade 3 or 4 non-haematological toxicity occurred in 4 patients (at 30 mCi/m^{2}one patient experienced rash and Quincke's oedema, two patients experienced fever at 60 mCi/m^{2}and one patient treated with 60 mCi/m^2 experienced fatigue.

Six of the nine patients who experienced serious adverse events died. Deaths and serious adverse events are described in section 12.3.1.

6.2.2 Display of adverse events

Part A:

The following adverse events sorted by SOC, preferred term and the three dose groups of BIWA 4 were reported in more than one patient in Part A of the trial (TABLE 6.2.2: 1). More details as well as the preferred terms can be found in section 7.3.1.

TABLE 6.2.2: 1: Patients with adverse events in Part A (pre-surgical treatment with a single dose of 20 mCi ^{99m}Tc-BIWA 4)

	^{99m}Tc-BIWA 4			
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	total
number of patients (% of patients treated)	3 (100)	4 (100)	3 (100)	10 (100)
number of patients with any adverse event	3 (100)	4 (100)	3 (100)	10 (100)
application site disorders	2 (66.7)	0 (0)	0 (0)	2 (20.0)
body as a whole	2 (66.7)	2 (50.0)	1 (33.3)	5 (50.0)
Pain	1 (33.3)	1 (25.0)	1 (33.3)	3 (30.0)

metabolic and nutritional disorders	1 (33.3)	2 (50.0)	1 (33.3)	4 (40.0)
weight decrease	1 (33.3)	1 (25.0)	0 (0)	2 (20.0)
resistance mechanism disorder	0 (0)	4 (100)	0 (0)	4 (40.0)
Infection	0 (0)	3 (75.0)	0 (0)	3 (30.0)
vascular disorders	1 (33.3)	1 (25.0)	1 (33.3)	3 (30.0)
Haemorrhage (not specified)	1 (33.3)	1 (25.0)	0 (0)	2 (20.0)

given are SOCs and preferred terms in case they were reported in more than one patient; figure in brackets denote percentage of patients treated

The events which were considered drug-related by the investigator graded according to CTC criteria are listed in TABLE 6.2.2: 2. The events were grade 1 according to CTC.

TABLE 6.2.2: 2 Severity of drug-related (as judged by the investigator) adverse events according to CTC criteria reported in Part A (pre-surgical treatment with a single dose of 20 mCi ^{99m}Tc-BIWA 4).

	^{99m}Tc-BIWA 4			
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	total
number of patients	3	4	3	10
Number of adverse events considered drug-related	0	2	0	2
Liver and biliary system disorder	0	2	0	2
sGOT increased	0	1	0	1
sGPT increased	0	1	0	1

sGOT = serum glutamic oxalacetic transaminase ; sGPT = serum glutamic pyruvic transaminase

Part B:

TABLE 6.2.2: 3 presents the number of patients with adverse events sorted by SOC, preferred term and the radiation dose groups of ^{186}Re- BIWA 4.

^{186}Re-BIWA 4 first and second doses considered)

	^{186}Re-BIWA 4 [mCi/m^2]					
	20	30	40	50	60	total
number of patients treated	2 (100)	4 (100)	3 (100)	6 (100)	5 (100)	20 (100)
number of patients with adverse events	2 (100)	4 (100)	3 (100)	6 (100)	5 (100)	20 (100)
body as a whole	2 (100)	3 (75.0)	2 (66.7)	2 (33.3)	4 (80.0)	13 (65.0)
allergic reaction	0 (0)	1 (25.0)	0 (0)	0 (0)	1 (20.0)	2 (10.0)
Fatigue	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (20.0)	2 (10.0)
Fever	0 (0)	0 (0)	2 (66.7)	0 (0)	2 (40.0)	4 (20.0)
oedema mouth	1 (50.0)	0 (0)	0 (0)	1 (16.7)	0 (0)	2 (10.0)
Pain	0 (0)	2 (50.0)	0 (0)	1 (16.7)	1 (20.0)	4 (20.0)
central and peripheral nervous system disorder	0 (0)	1 (25.0)	2 (66.7)	1 (16.7)	0 (0)	4 (20.0)
gastro-intestinal system disorder	0 (0)	1 (25.0)	1 (33.3)	2 (33.3)	3 (60.0)	7 (35.0)
Mucositis	0 (0)	0 (0)	0 (0)	1 (16.7)	3 (60.0)	4 (20.0)
Stomatitis	0 (0)	0 (0)	0 (0)	1 (16.7)	1 (20.0)	2 (10.0)
liver and biliary system disorders	1 (50.0)	0 (0)	0 (0)	0 (0)	1 (20.0)	2 (10.0)
sGPT increased	1 (50.0)	0 (0)	0 (0)	0 (0)	1 (20.0)	2 (10.0)
metabolic and nutritional disorders	1 (50.0)	0 (0)	1 (33.3)	1 (16.7)	1 (20.0)	4 (20.0)
Neoplasm	0 (0)	0 (0)	2 (66.7)	0 (0)	2 (40.0)	4 (20.0)
neoplasm malignant aggravated	0 (0)	0 (0)	1 (33.3)	0 (0)	2 (40.0)	3 (15.0)
platelet, bleeding and clotting disorders	0 (0.0)	1 (25.0)	1 (33.3)	5 (83.3)	5 (100)	12 (60.0)
Thrombocytopenia	0 (0.0)	1 (25.0)	1 (33.3)	4 (66.7)	5 (100)	11 (55.0)
red blood cell disorder	0 (0)	0 (0)	0 (0)	2 (33.3)	3 (60.0)	5 (25.0)
Anaemia	0 (0)	0 (0)	0 (0)	2 (33.3)	3 (60.0)	5 (25.0)
respiratory system disorder	0 (0)	1 (25.0)	1 (33.3)	0 (0)	2 (40.0)	4 (20.0)
Bronchitis	0 (0)	1 (25.0)	0 (0)	0 (0)	1 (20.0)	2 (10.0)
Dyspnoea	0 (0)	0 (0)	0 (0)	0 (0)	2 (40.0)	2 (10.0)
Rhinitis	0 (0)	0 (0)	1 (33.3)	0 (0)	1 (20.0)	2 (10.0)
skin and appendages disorder	1 (50.0)	1 (25.0)	1 (33.3)	0 (0)	1 (20.0)	4 (20.0)
Rash	0 (0)	1 (25.0)	0 (0)	0 (0)	1 (20.0)	2 (10.0)
white cell and RES disorder	0 (0)	0 (0)	1 (33.3)	4 (66.7)	5 (100)	10 (50.0)
Granulocytopenia	0 (0)	0 (0)	0 (0)	1 (16.7)	2 (40.0)	3 (15.0)
Leucopenia	0 (0)	0 (0)	1 (33.3)	4 (66.7)	5 (100)	10 (50.0)

given are SOCs and preferred terms in case they were reported in more than one patient; sGPT = serum glutamic pyruvic transaminase ; RES = reticulo endothelial system, figure in brackets denote per centage of patients treated

Drug-related adverse events were reported in 75 % of the patients. Drug-related leucopenia was reported in 50 % and thrombocytopenia in 55 % of the patients, respectively.

Adverse events considered drug-related are provided in TABLE 6.2.2: 4 graded according to the CTC.

CTC criteria reported in Part B (first and second dose considered)

	adverse event	^{186}Re-BIWA 4 [mCi/m^2]					
		20	30	40	50	60	total
CTC grade 1		1	0	2	6	5	14
	face oedema	1	n.a.	0	0	0	1
	leucopenia	0	n.a.	1	2	0	3
	moniliasis	0	n.a.	0	0	1	1
	mucositis	0	n.a.	0	0	1	1
	platelets abnormal	0	n.a.	0	1	0	1
	purpura	0	n.a.	0	0	1	1
	stomatitis	0	n.a.	0	1	0	1
	thrombocytopenia	0	n.a.	1	2	2	5
CTC grade 2		0	1	0	7	7	15
	anaemia	n.a.	0	n.a.	1	1	2
	gout	n.a.	0	n.a.	1	0	1
	leucopenia	n.a.	0	n.a.	1	2	3
	mucositis	n.a.	0	n.a.	2	3	5
	stomatitis	n.a.	0	n.a.	0	1	1
	taste loss	n.a.	0	n.a.	1	0	1
	thrombocytopenia	n.a.	1	n.a.	1	0	2
CTC grade 3		0	2	0	2	8	12
	allergic reaction	n.a.	1	n.a.	0	0	1
	fatigue	n.a.	0	n.a.	0	1	1
	fever	n.a.	0	n.a.	0	2	2
	leucopenia	n.a.	0	n.a.	1	1	2
	rash	n.a.	1	n.a.	0	0	1
	thrombocytopenia	n.a.	0	n.a.	1	4	5
CTC grade 4		0	0	0	3	5	8
	anaemia	n.a.	n.a.	n.a.	0	1	1
	granulocytopenia	n.a.	n.a.	n.a.	1	2	3
	leucopenia	n.a.	n.a.	n.a.	1	2	3
	thrombocytopenia	n.a.	n.a.	n.a.	1	0	1

given is the number of adverse events; n.a. = not applicable

6.2.3 Analysis of adverse events

Part A:

None of the adverse events occurring in Part A of the trial were considered drug-related except for one patient experiencing mild and reversible CTC grade 1 elevation of AST and ALT.

No difference in AE profile was observed when comparing the different BIWA 4 doses.

Part B:

Thrombocytopenia and leucopenia were dose-dependent (TABLES 6.2.2: 3 and 6.2.2: 4) and dose-limiting. Time course is provided in section 6.4.2.1.

causing dose-limiting toxicity (TABLES 6.2.2: 3 and 6.2.2: 4).

An allergic reaction was reported in two patients, one experiencing drug-related Quincke's oedema and rash (30 mCi/m^2) and one patient (60 mCi/m^2) had an allergic reaction after a thrombocyte concentrate transfusion, which was not considered drug-related. One patient 20 mCi/m^2) had urticaria which also was not considered drug-related and one patient (20 mCi/m^2) experienced drug-related face oedema.

HAHAs were detected in two patients (see section 12.4.3).

Discontinuations due to adverse events occurred in two patients. Both patients died.

Adverse events required initiation of concomitant therapy in 17 patients. These patients are shortly described in section 6.3.1.

6.3 DEATHS, OTHER SERIOUS ADVERSE EVENTS, AND OTHER SIGNIFICANT ADVERSE EVENTS

Serious adverse events were reported in 12 patients. Of those six patients died in the course of the trial or follow-up. All patients who died were treated in Part B of the trial. None of the patients treated in Part A died. All fatalities were due to disease progression.

Two patients in Part B discontinued the trial prematurely due to adverse events (death in both patient 112 and 105).

Adverse events requiring therapy were most often thrombocytopenia, leucopenia and fever.

INCLUDED IN THE TEXT

7.1 DEMOGRAPHIC DATA

Details of demographics are not included.

7.2 EFFICACY / PHARMACODYNAMIC DATA

TABLE 7.2: 1	Tumour to bone marrow uptake ratio – Part A of the trial
TABLE 7.2: 2	Tumour to bone marrow uptake ratio (ITT subset) – Part A of the trial
TABLE 7.2: 3	Tumour to bone marrow uptake ratio (PP subset) – Part A of the trial
TABLE 7.2: 4	Tumour size and % uptake of antibody – Part A of the trial (per protocol population)
TABLE 7.2: 5	CD44v6 antigen expression in tumour and other tissue – Part A of the trial
TABLE 7.2: 6	Soluble CD44v6 in serum. Mean values are given in ng/mL

		Part A ^{99m}Tc-BIWA 4		
	patient No	uptake tumour (%ID/kg; calculated)	uptake bone marrow (%ID/kg; calculated)	ratio tumour / bone marrow (calculated)
25 mg BIWA 4	1*	6.14	5.58	1.10
	2*	15.78	7.49	2.11
	3*	16.78	8.63	1.95
50 mg BIWA 4	4*	28.10	6.98	4.03
	8*	27.76	7.57	3.67
	10*	22.63	11.54	1.96
100 mg BIWA 4	6*	15.89	6.47	2.46
	7	16.99	7.70	2.21
	9	13.31	10.11	1.32

No = number; * = included in per protocol analysis; % ID = % injected dose; calculations were done by taking different weights of samples into account

TABLE 7.2: 2 Tumour to bone marrow uptake ratio (ITT subset) – Part A of the trial

	Part A ^{99m}Tc-BIWA 4 ratio tumour / bone marrow (calculated)
25 mg BIWA 4	1.72
50 mg BIWA 4	2.57
100 mg BIWA 4	1.99

mean values are given; ITT = intent-to-treat; calculations were done by taking different weights of samples into account

TABLE 7.2: 3 Tumour to bone marrow uptake ratio (PP subset) – Part A of the trial

	Part A ^{99m}Tc-BIWA 4 ratio tumour / bone marrow (calculated)
25 mg BIWA 4	1.72
50 mg BIWA 4	3.22
100 mg BIWA 4	2.46

mean values are given; PP = per protocol; calculations were done by taking different weights of samples into account

population)

		Part A ^{99m}Tc-BIWA 4	
	patient No	tumour size [mm^2]	uptake tumour (%ID/kg; calculated)
25 mg BIWA 4	1	1350	6.14
	2	900	15.78
	3	225	16.78
50 mg BIWA 4	4	n.a.	28.10
	8	1504	27.76
	10	1400	22.63
100 mg BIWA 4	6	800	15.89

No = patient number, n.a. = not applicable, only length or width given but not length and width; calculations were done by taking different weights of samples into account

TABLE 7.2: 5 CD44v6 antigen expression in tumour and other tissue – Part A of the trial

		Part A ^{99m}Tc-BIWA 4		
	patient No	CD44v6 antigen expression in tumour	CD44v6 expression in mucosa	CD44v6 expression in lymph node metastases
25 mg BIWA 4	1*	n.a.	n.a.	n.a.
	2*	++/+++; > 80 %	+++	++/+++; > 90 %
	3*	+++; > 90 %	+++	+++; > 90 %
50 mg BIWA 4	4*	+++; > 90 %	+++	+++; > 90 %
	8*	n.a.	n.a.	n.a.
	10*	++; > 90 %	+++	+++; > 90 %
100 mg BIWA 4	6*	+++; > 80 %	+++	n.a.
	7	+++; > 90 %	+++	n.a.
	9	+++; > 90 %	+++	n.a.

No = number; * = included in per protocol analysis, n.a. = not applicable

	Part A			Part B				
	^{99m}Tc-BIWA 4			^{186} Re-BIWA 4 [mCi/m^2]				
	25 mg BIWA 4	50 mg BIWA 4	100 mg BIWA 4	20	30	40	50	60
N	3	4	3	2	4	3	6	5
pre-dose	203	337	174	408	262	346	171	160
21 hours	223	301	179	396	177	403	199	170
48 hours	200	275	157	384	198	423	199	174
72 hours	140	386	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.
144 hours	188	282	182	444	284	516	233	195
240/336 hours	n.a.	n.a.	n.a.	445	337	n.a.	n.a.	n.a.
week 6	203	310	210	289	220	487	226	162

n.a. = not applicable, data include the second dosing

Efficacy results:	Part A: Results from Part A confirmed a dose of 50 mg BIWA 4 as the optimal dose for treatment based on blood concentrations and tissue uptake levels. The distribution as assessed by radioscintigraphy and biopsy measurements was in almost all cases highest in the tumour as compared to other tissues. Uptake of radioactivity increased in the tumour over time. CD44v6 expression was present in more than 80 % and 90% of the cells of primary tumour and lymph node metastases, respectively, and in all mucosa specimens obtained. No correlation was observed between tumour size and uptake of radioactivity in the 50 mg BIWA 4 dose group. The amount of soluble CD44v6 appeared to be constant before and after ^{99m}Tc-BIWA 4 administration. Concentrations of BIWA 4 measured were dose-proportional in the range of 25 mg to 100 mg BIWA 4. A moderate amount of the dose administered was excreted via the kidneys. The percent injected dose (% ID) excreted in urine was similar for all dosing groups. Part B: Data from Part B indicate that patients may clinically benefit from ^{186}Re-BIWA 4 therapy at maximum tolerated dose (MTD). One out of five patients treated with 60 mCi/m^2 had stable disease. Three out of six patients experienced stable disease at the maximum tolerated dose of 50 mCi/m^2. Time until progression ranged between 127 and 173 days in those patients who received a second dose of 50 mCi/m^2. Radioimmunoscintigraphy indicates uptake of radioactivity in tumour tissue. Biodistribution was comparable in other tissues irrespective of dose. Dosimetric analysis did not reveal unexpected high absorbed doses in tissues other than the tumour except for the testes. The amount of soluble CD44v6 detected tended to increase for the ^{186}Re-BIWA 4 treated patients. The plasma concentrations of BIWA 4 peaked at 0.92 hours and the antibody was eliminated with a geometric mean half-life of 94 hours for BIWA 4 determined by enzyme-linked immuno-sorbent assay (ELISA) measurement. C_{max} and AUC values (ELISA) were similar to those obtained in Part A of the trial for the 50 mg BIWA 4 dose group.

	Tolerability of single dose BIWA 4 coupled to low radiation dose of Technetium-99 was acceptable. Two of the three serious adverse events were due to complications as a result of surgery. Part B: The maximum tolerated dose (MTD) was 50 mCi/m^2 ^{186}Re-BIWA 4. Dose-limiting adverse events were dose dependent reversible reductions in thrombocyte and leucocyte count with subsequent fever in individual patients. Clinical symptoms of thrombocytopenia were mild petechiae. Mucositis was observed in patients treated with higher radiation doses but was not dose-limiting. No relevant changes in thyroid stimulating hormone (TSH) values were observed during the course of the trial. Twelve patients experienced a serious adverse event. Of those, six patients died during the course of Part B of the trial mainly due to progression of the underlying disease. Allergic reactions were observed rarely with one serious drug-related Quincke's oedema. No allergic reactions occurred during or shortly after infusion of the drug. Two patients developed HAHAs. Repeated dosing did not induce HAHA development.
Conclusions:	Results indicate uptake of ^{186}Re-BIWA 4 in tumour tissue and clinical benefit in patients with advanced squamous cell carcinoma of the head and neck. The safety profile appears to be acceptable. BIWA 4 showed dose-proportional pharmacokinetics and tumour uptake did not change relevantly between doses of 50 mg and 100 mg BIWA4.

Example 4

Introduction. Patients with an advanced stage of head and neck squamous cell carcinoma (HNSCC) have an increased risk for development of locoregional recurrent tumors and/or distant metastases. For these patients, development of an effective adjuvant systemic treatment is needed. Knowing that HNSCC are intrinsically radiosensitive, targeting of radionuclides selectively to HNSCC by use of monoclonal antibodies as a form of radioimmunotherapy, might contribute to a more effective therapy.

Objective. To determine the safety, maximum tolerated dose (MTD), immunogenicity and efficacy of radioimmunotherapy with Rhenium-186 (^{186}Re)-labelled humanised monoclonal antibody BIWA4 in patients with HNSCC.

Patients and methods. A phase I dose escalation study was conducted in HNSCC patients for whom no curative therapeutic options were available. In a total of 20 patients ^{186}Re-labelled BIWA4 was administered intravenously in doses of 20, 30, 40, 50 or 60 mCi/m^2. Three patients received, at least 3 months after a dose of 50 or 60 mCi/m^2, a second dose of 50 mCi/m^2.

Results. All single as well as repeated administrations were well tolerated and no signs of acute adverse events were observed. The only significant manifestations of toxicity at the higher doses were oral mucositis and dose-limiting myelotoxicity consisting of thrombo- and leucocytopenia. The MTD was established at 50 mCi/m^2. One patient developed a human-anti-human response after one single administration. Stable disease, lasting 4 to 19 weeks, was observed in 5 patients treated at the highest dose levels.

Conclusion. Radioimmunotherapy with ^{186}Re-labelled BIWA4 in HNSCC patients seems to be safe and tumoricidal doses can be reached. Moreover, due to the low rate of immunogenicity, repeated administrations appear possible. The results of this phase I study encourage the further development of radioimmunotherapy with Rhenium-186 (^{186}Re)-labelled humanised monoclonal antibody BIWA4 towards an adjuvant therapy for head and neck cancer patients.

claims

1. Antibody molecule comprising a variable region of the heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.
2. Antibody molecule wherein the variable region heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1.
3. Antibody molecule comprising a variable region light chain as characterized by the amino acid sequence as defined in SEQ ID No. 2 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.
4. Antibody molecule wherein the variable region light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 2.
5. Antibody molecule comprising a variable region light chain as characterized by the amino acid sequence as defined in SEQ ID No. 3 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.
6. Antibody molecule wherein the variable region light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 3.
7. Antibody molecule according to claim 1 and 3 comprising a variable region heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 and comprising a variable region light chain as characterized by the amino acid sequence as defined in SEQ ID No. 2 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.
8. Antibody molecule according to claim 2 and 4 wherein the variable region heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1 and wherein the variable region light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 2.
9. Antibody molecule according to claim 1 and 5 comprising a variable region heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 1 and comprising a variable region light chain as characterized by the amino acid sequence as defined in SEQ ID No. 3 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.
10. Antibody molecule according to claim 2 and 6 wherein the variable region heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 1 and

wherein the variable region light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 3.

11. Antibody molecule comprising a variable region heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.
12. Antibody molecule wherein the variable region heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4.
13. Antibody molecule comprising a variable region light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.
14. Antibody molecule wherein the variable region light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 5.
15. Antibody molecule comprising a variable region light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 6 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.
16. Antibody molecule wherein the variable region light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 6.
17. Antibody molecule according to claim 11 and 13 comprising a variable region heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and comprising a variable region light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 5 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.
18. Antibody molecule according to claim 12 and 14 wherein the variable region heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and wherein the variable region light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 5.
19. Antibody molecule according to claim 11 and 15 comprising a variable region heavy chain encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and comprising a variable region light chain encoded by the nucleic acid sequence as defined in SEQ ID No. 6 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.
20. Antibody molecule according to claim 12 and 16 wherein the variable region heavy chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 4 and wherein the variable region light chain is encoded by the nucleic acid sequence as defined in SEQ ID No. 6.

21. An antibody molecule according to any one of claims 1 to 20 characterised in that each of said variable light chain and said variable heavy chain region is separately joined to a human constant region.

22. The antibody molecule of claim 21, wherein said human constant region of the light chain is a human kappa constant region.

23. The antibody molecule of claim 21 or 22, wherein said human constant region of the heavy chain is a human IgG1 constant region.

24. Antibody molecule according to any one of claims 1 to 23 comprising a heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 7 and comprising a light chain as characterized by the amino acid sequence as defined in SEQ ID No. 8 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

25. The antibody molecule according to any one of claims 1 to 24 wherein the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 7 and wherein the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 8.

26. The antibody molecule according to any one of claims 1 to 25 comprising a heavy chain as characterized by the amino acid sequence as defined in SEQ ID No. 7 and comprising a light chain as characterized by the amino acid sequence as defined in SEQ ID No. 9 or a fragment, allelic variant, functional variant, glycosylation variant, fusion molecule or a chemical derivative thereof.

27. The antibody molecule according to any one of claims 1 to 26 wherein the heavy chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 7 and wherein the light chain consists of the amino acids as characterized by the amino acid sequence of SEQ ID No. 9.

28. The antibody molecule according to any one of claims 1 to 27 comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 10 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 11 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

29. The antibody molecule according to any one of claims 1 to 28 wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 10 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 11.

30. The antibody molecule according to any one of claims 1 to 29 comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 10 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 12 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

31. The antibody molecule according to any one of claims 1 to 30 wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 10 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 12.

32. The antibody molecule according to any one of claims 1 to 31 comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 13 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 14 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

33. The antibody molecule according to any one of claims 1 to 32 wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 13 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 14.

34. The antibody molecule according to any one of claims 1 to 33 comprising a heavy chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 13 and comprising a light chain as characterized by the nucleic acid sequence as defined in SEQ ID No. 15 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

35. The antibody molecule according to any one of claims 1 to 34 wherein the heavy chain is encoded by the nucleic acid sequence of SEQ ID No. 13 and wherein the light chain is encoded by the nucleic acid sequence of SEQ ID No. 15.

36. The antibody molecule according to any one of claims 1 to 35 comprising a heavy and light chain as encoded by the nucleic acid sequence as defined in SEQ ID No. 16 or a fragment, allelic variant, functional variant, variant based on the degenerative nucleic acid code, fusion molecule or a chemical derivative thereof.

37. The antibody molecule according to any one of claims 1 to 36 wherein the heavy and light chain is encoded by the nucleic acid sequence of SEQ ID No. 16.

38. An antibody protein according to any one of claims 1 to 37, wherein said antibody protein is conjugated to a therapeutic agent.

39. The antibody protein of claim 38, wherein said therapeutic agent is a therapeutic agent selected from the group consisting of radioisotopes, toxins, toxoids, inflammatory agents and chemotherapeutic agents.

40. The antibody protein of claim 39, wherein said therapeutic agent is linked to the antibody protein via a linker selected from the group of MAG-3 GABA, MAG-2 GABA and N2S2.

41. The antibody protein of claim 40, wherein said therapeutic agent is linked to the antibody protein via MAG-2 GABA.

42. The antibody protein of any one of claims 38 to 41, wherein said radioisotope is a ß-emitting radioisotope.

43. The antibody protein of claim 42, wherein said radioisotope is selected from the group consisting of ^{186}Rhenium, ^{188}Rhenium, ^{131}Iodine and ^{90}Yttrium.

44. The antibody protein of claim 43, wherein said radioisotope is ^{186}Rhenium.

45. The antibody protein according to any one of claims 39 to 44, wherein the antibody protein has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

46. The antibody protein according to any one of claims 1 to 37, characterised in that it is labelled.

47. The antibody protein of claim 46, wherein said label is a detectable marker.

48. The antibody protein of claim 47, wherein the detectable marker is a detectable marker selected from the group consisting of enzymes, dyes, radioisotopes, digoxygenin, and biotin.

49. An antibody protein according to any one of claims 1 to 37 conjugated to an imageable agent.

50. The antibody protein of claim 49, wherein the imageable agent is a radioisotope.

51. The antibody protein of claim 50, wherein said radioisotope is a γ-emitting radioisotope.

52. The antibody protein of claim 51, wherein said radioisotope is ^{125}I.

53. A pharmaceutical composition containing an antibody protein according to any one of claims 1 to 45 and a pharmaceutically acceptable carrier or excipient.

54. Pharmaceutical composition according to claim 53, wherein the antibody protein is conjugated to a radioisotope according to any one of claims 39 to 44 and has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

55. Pharmaceutical composition according to claim 53 or 54, wherein the amount of radiolabelled antibody in the pharmaceutical composition to be applied to a patient is 10, 20, 30, 40, 50 or 60 mCi/m^2.

56. Pharmaceutical composition according to any one of claims 53 to 55, wherein the amount of radiolabelled antibody in the pharmaceutical composition to be applied to a patient is 50 mCi/m^2.

57. Pharmaceutical composition according to any one of claims 53 to 56, further comprising one or more radioprotectant selected from the group of ascorbic acid, gentisic acid, reductic acid, erythrorbic acid, p-aminobenzoic acid, 4-hydroxybenzoic acid, nicotinic acid, nicotinamide, 2-5-dihydroxy-1,4-benzenedisulfonic acid, povidone, inositol, and/or citrate.

58. Pharmaceutical composition according to any one of claims 53 to 57, wherein the radioprotectant is ascorbic acid.

59. Pharmaceutical composition according to any one of claims 53 to 58, wherein said antibody protein comprises an antibody molecule selected from the group of antibody molecules of claims 8, 10, 18, 20, 24, 25, 28, 29, 32, 33, 36, 37 linked to ^{186}Rhenium via MAG-2 GABA further comprising the radioprotectant ascorbic acid.

60. Use of an antibody protein according to any one of claims 1 to 45 in the manufacture of a medicament for treatment of cancer.

61. Use of an antibody protein according to claim 60 wherein said cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer, breast cancer, head and neck cancer, ovarian cancer, lung cancer, bladder cancer, pancreatic cancer and metastatic cancers of the brain.

62. Use of an antibody protein according to any one of claims 60 or 61, wherein the antibody protein is conjugated to a radioisotope according to any one of claims 39 to 44 and has specific activity of from about 0.5 to about 15 mCi/mg, or from about 0.5 to about 14 mCi/mg, preferably about 1 to about 10 mCi/mg, preferably about 1 to about 5 mCi/mg, and most preferably 2 to 6 mCi/mg or 1 to 3 mCi/mg.

63. Use of an antibody protein according to claim 62, wherein the amount of radiolabelled antibody in the pharmaceutical composition to be applied to a patient is 10, 20, 30, 40, 50 or 60 mCi/m^2.

64. Use of an antibody protein according to claim 62, wherein the amount of radiolabelled antibody in the pharmaceutical composition to be applied to a patient is 50 mCi/m^2.

65. Method of cancer treatment, wherein an antibody protein according to any one of claims 1 to 45 is administered once to several times to an individual in need thereof, said antibody protein selectively binds to CD44v6, destroys tumor cells via the therapeutic agent linked to the antibody protein and the therapeutic success is monitored.

66. The method of claim 65, wherein the tumor is a tumor selected from the cancer group consisting of colorectal cancers, non-small cell lung cancers, breast cancers, head and neck cancer, ovarian cancers, lung cancers, bladder cancers, pancreatic cancers and metastatic cancers of the brain.

67. Nucleic acid, characterised in that it codes for an antibody protein according to one of claims 1 to 37.

68. Recombinant DNA vector, characterised in that it contains a nucleic acid according to claim 67.

69. Recombinant DNA vector according to claim 68, characterized in that it is an expression vector.

70. Recombinant DNA vector according to claim 68 or 69, characterized in that it is vector pAD-CMV or a functional derivative thereof.

71. Recombinant DNA vector according to any one of claims 68 to 70, characterized in that it is vector N5KG1Val or a derivative thereof.

72. Host, characterised in that it contains a vector according to any one of claims 68 to 71.

73. Host according to claim 72, characterised in that it is a eukaryotic host cell.

74. Host according to claim 72 or 73, characterised in that it is a mammalian cell.

75. Host according to any one of claims 72 to 74, characterised in that it is a BHK, CHO or COS cell.

76. Host according to claim 72, characterised in that it is a bacteriophage.

77. Host according to claim 72, characterised in that it is a prokaryotic host cell.

78. Process for preparing an antibody protein according to any one of claims 1 to 37, characterized in that it comprises the following steps: a host according to one of claims 72 to 77 is cultivated under conditions in which said antibody protein is expressed by said host cell and said antibody protein is isolated.

79. Process according to claim 78, characterised in that said host is a mammalian cell, preferably a CHO or COS cell.

80. Process according to claim 78 or 79, characterised in that said host cell is co-transfected with two plasmids which carry the expression units for the light or the heavy chain.