S016704J.200

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Case 12/178,181

Process for the diagnosis and treatment of squamous cell carcinomas

The invention relates to processes for the diagnosis and therapy of squamous cell carcinomas which are based on the expression of the variable exon v6 of the CD44 gene, agents for these processes and the use thereof.

It has recently been shown that the expression of variants of the surface glycoprotein CD44 is necessary and sufficient to trigger so-called spontaneous metastatic behaviour both in a non-metastasising pancreas-adenocarcinoma cell line of the rat and also in a non-metastasising fibrosarcoma cell line of the rat (Günthert *et al.*, 1991). Whereas the smallest CD44-isoform, the standard form CD44s, are expressed ubiquitously in a series of different tissues, including epithelial cells, certain splice variants of CD44 (CD44v) are expressed only in a subgroup of epithelial cells. The CD44-isoforms are produced by alternative splicing in such a way that the sequences of 10 exons (v1-v10) in CD44s are excised completely, but may occur in different combinations in the larger variants (Screaton *et al.*, 1992; Heider *et al.*, 1993; Hofmann *et al.*, 1991). The variants differ in that different amino acid sequences are inserted at a specific site of the extracellular part of the protein. Such variants can be detected in different human tumour cells and in human tumour tissue. Thus, the expression of CD44-variants in the course of colorectal carcinogenesis has recently been investigated (Heider *et al.*, 1993). There is no expression of CD44-variants in normal human colon epithelium and only slight expression can be detected in the proliferating cells of the cavities. At later stages of the tumour progression, e.g. in adenocarcinomas, all malignant degenerations express variants of CD44. Moreover, the expression of CD44-splice variants has recently been demonstrated in activated lymphocytes and in non-Hodgkin's lymphomas (Koopman *et al.*, 1993).

Various approaches have been adopted for making use of the differential expression of variant exons of the CD44-gene in tumours and normal tissue for diagnostic and therapeutic purposes (WO 94/02633, WO 94/12631, WO 95/00658, WO 95/00851, EP 0531300).

The expression of variant CD44-molecules in squamous cell carcinomas has also

been investigated. Salmi *et al.* (1993) found, with the v6-specific antibody Var3.1, that there was a reduction in the v6-expression in tumour cells compared with normal cells. With the v6-specific antibody 11.9, Brooks *et al.* (1995) obtained a heterogeneous staining of nasopharyngeal carcinomas. Strong staining was achieved in only 2/12 cases, whilst in the majority of cases only a slight focal v6-expression could be detected by immunohistology.

The aim of the present invention was to develop new methods for the diagnosis and therapy of squamous cell carcinomas and to provide agents for such methods.

This objective has been achieved by means of the present invention. It relates to processes for the diagnosis and therapy of squamous cell carcinomas which are based on the expression of the variant exon v6 of the CD44 gene as a molecular marker or target. In particular, the present invention relates to processes based on the powerful homogeneous expression of v6 in squamous cell carcinomas, which was surprisingly detected, in contradiction to the teaching known from the prior art. Antibody molecules of corresponding specificity are particularly suitable as vehicles for selectively reaching squamous cell carcinomas *in vivo*.

The preferred processes are those characterised in that an antibody molecule is used which recognises the amino acid sequence QWFGNRWHEGYRQT, more preferably the amino acid sequence WFGNRWHEGYR. The monoclonal antibody BIWA-1 (clone VFF-18) secreted by a hybridoma cell line which was deposited on 7.6.1994 under Accession No. DSM ACC2174 at the DSM-Deutsche Sammlung fhr Mikroorganismen und Zellkulturen GmbH, Mascheroder Weg 1b, D-38124 Braunschweig, Germany (WO 95/33771), and derivatives of this antibody, are particularly preferred.

Other aspects of the present invention are the use of antibody molecules of this kind in the processes according to the invention and agents for carrying out these processes.

The nucleic and amino acid sequence of the variant exon v6 of the CD44-gene is known (Screaton *et al.*, 1992, Tölg *et al.*, 1993). The existence of degenerate or allelic variants is of no significance to the implementation of the invention; such variants are therefore expressly included.

The sequence of exon v6 of the human CD44-gene is:

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     Q   A   T   P   S   S   T   T   E   E   T   A   T   Q
TC CAG GCA ACT CCT AGT AGT ACA ACG GAA GAA ACA GCT ACC CAG

 K   E   Q   W   F   G   N   R   W   H   E   G   Y   R   Q
AAG GAA CAG TGG TTT GGC AAC AGA TGG CAT GAG GGA TAT CGC CAA

 T   P   R   E   D   S   H   S   T   T   G   T   A
ACA CCC AGA GAA GAC TCC CAT TCG ACA ACA GGG ACA GCT G.
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The invention may be carried out with polyclonal or monoclonal antibodies which are specific to an epitope coded by exon v6, particularly an epitope within the amino acid sequence QWFGNRWHEGYRQT, most preferably within the amino acid sequence WFGNRWHEGYR. The preparation of antibodies against known amino acid sequences may be carried out according to methods known *per se* (Catty, 1989). For example, a peptide of this sequence may be prepared synthetically and used as an antigen in an immunisation procedure. Another method is to prepare a fusion protein which contains the desired amino acid sequence, by integrating a nucleic acid (which may be prepared synthetically or, for example, by polymerase chain reaction (PCR) from a suitable probe) which codes for this sequence into an expression vector and expressing the fusion protein in a host organism. The fusion protein, optionally purified, can then be used as an antigen in an immunisation procedure and insert-specific antibodies or, in the case of monoclonal antibodies, hybridomas which express insert-specific antibodies can then be selected by suitable methods. Processes of this kind are known in the art. Heider *et al.* (1993, 1996a) and Koopman *et al.* (1993) describe the preparation of antibodies against variant epitopes of CD44.

However, for the process according to the invention, it is also possible to use other antibody molecules, which are derived from poly- or monoclonal antibodies, e.g. Fab- or $F(ab')_2$-fragments of immunoglobulins, single-chain antibodies (scFv) prepared by the recombinant method, chimeric or humanised antibodies and other molecules which bind specifically to epitopes coded by exon v6. Fab- or $F(ab')_2$-fragments or other fragments may be produced, for example, from the complete immunoglobulin of the antibody BIWA-1 (VFF-18) or other antibodies (Kreitman *et al.*, 1993). The skilled person is also in a position to produce recombinant v6-specific antibody molecules. In particular, after analysing the amino acid sequence of the antibody BIWA-1 (VFF-18) and/or using the hybridoma cell line which produces this antibody, particularly the genetic information contained therein, he can produce recombinant antibody molecules having the same idiotype as BIWA-1 (VFF-18), i.e. antibody molecules which have the same amino acid

sequence in the region of the antigen binding site (*complementarity-determining regions*, CDR) as the antibody BIWA-1 (VFF-18). Such processes are known in the art. Recombinant antibody molecules of this kind may be, for example, humanised antibodies (Shin *et al.*, 1989; Güssow and Seemann, 1991), bispecific or bifunctional antibodies (Weiner *et al.*, 1993; Goodwin, 1989, Featherstone, 1996), *single-chain* antibodies (scFv, Johnson and Bird, 1991), complete or fragmentary immunoglobulins (Coloma *et al.*, 1992; Nesbit *et al.*, 1992; Barbas *et al.*, 1992) or antibodies produced by *chain shuffling* (Winter *et al.*, 1994). Humanised antibodies may be prepared, for example, by CDR-grafting (EP 0239400). Framework regions can also be modified (EP 0519596; WO 9007861). For humanising antibodies, methods such as PCR (see for example EP 0368684; EP 0438310; WO 9207075) or computer-modelling (see for example WO 9222653) may be used nowadays. It is also possible to prepare and use fusion proteins, e.g. *single-chain* antibody/toxin fusion proteins (Chaudhary *et al.*, 1990; Friedman *et al.*, 1993). The terms "antibodies" and "antibody molecules" are intended to cover not only polyclonal and monoclonal antibodies but all the compounds discussed in this paragraph which can be structurally derived from immunoglobulins and may be prepared by methods known *per se*.

It is also within the capabilities of the average skilled person with a knowledge of the epitope (cf. Fig. 1, Fig. 4) of BIWA-1 (VFF-18), to produce equivalent antibodies with the same binding specificity. Such antibodies are therefore also included in the invention.

For diagnostic purposes, antibody molecules, preferably BIWA-1 antibody molecules, fragments thereof or recombinant antibody molecules with the same idiotype, may be linked for example with radioactive isotopes such as ^{125}I, ^{131}I, ^{111}In, ^{99m}Tc or radioactive compounds (Larson *et al.*, 1991; Thomas *et al.*, 1989; Srivastava, 1988), enzymes such as peroxidase or alkaline phosphatase (Catty and Raykundalia, 1989), with fluorescent dyes (Johnson, 1989) or biotin molecules (Guesdon *et al.*, 1979). For therapeutic purposes v6-specific antibody molecules, preferably BIWA-1 (VFF-18)-antibody molecules or VFF-18-derived antibody molecules, e.g. fragments thereof or recombinant antibody molecules with the same idiotype, may be linked to radioisotopes such as ^{90}Y, ^{131}I, ^{186}Re, ^{188}Re, ^{153}Sm, ^{67}Cu, ^{212}Bi, ^{213}Bi, ^{177}Lu (Quadri *et al.*, 1993; Lenhard *et al.*, 1985, Vriesendorp *et al.*, 1991; Wilbur *et al.*, 1989, Maraveyas *et al.*, 1995a, Jurcic *et* Scheinberg, 1994), toxins (Vitetta *et al.*, 1991; Vitetta *et* Thorpe, 1991; Kreitman *et al.*, 1993; Theuer *et al.*, 1993), cytostatics (Schrappe *et al.*, 1992), prodrugs (Wang *et al.*, 1992; Senter *et al.*, 1989), photoactivatable substances (Hemming *et al.*, 1993), an antibody molecule with a different specificity or radioactive compounds. The antibody molecule may also be linked to a cytokine or some other immunomodulatory polypeptide, e.g. with tumour necrosis factor, lymphotoxin (Reisfeld *et al.*, 1996) or

interleukin-2 (Becker *et al.*, 1996). The antibody molecules may also be modified for use in a pretargetting system, e.g. with streptavidin or biotin (Goodwin, 1995).

Advantageously, the diagnostic process according to the invention may be used to investigate samples from patients, e.g. from biopsies, where there is a suspicion of squamous cell carcinoma or the diagnosis has already been made but the tumour needs to be further characterised. The detection of variant CD44-molecules which contain an amino acid sequence coded by the variable exon v6 may be carried out at the protein level using antibodies or at the nucleic acid level using specific nucleic acid probes or primers for polymerase chain reaction (PCR). The invention consequently also relates to antibody molecules and nucleic acids which are suitable as probes or primers for such processes, and the use of these antibodies and nucleic acids for diagnosing and analysing squamous cell carcinomas. For example, tissue sections can be examined immunohistochemically using antibodies by methods known *per se*. Extracts taken from tissue samples or body fluids may also be investigated by other immunological methods using antibodies, e.g. in Western blots, enzyme-linked immunosorbent assays (ELISA, Catty and Raykundalia, 1989), radioimmunoassays (RIA, Catty and Murphy, 1989) or related immunoassays. The investigations may be qualitative, semi-quantitative or quantitative.

Apart from *in vitro* diagnosis, antibody molecules with specificity according to the invention are also suitable for the *in vivo* diagnosis of squamous cell carcinomas. If the antibody molecule carries a detectable label, the label can be detected for diagnostic purposes, e.g. for imaging the tumour *in vivo* or for radioguided surgery, for example. For using antibodies conjugated with radioactive isotopes for immunoscintigraphy (imaging), for example, there are numerous procedures which can be used by the skilled person as the basis for performing the invention (Siccardi *et al.*, 1989; Keenan *et al.*, 1987; Perkins and Pimm, 1992; Colcher *et al.*, 1987; Thompson *et al.*, 1984).

Data obtained by the detection and/or quantifying of the expression of the variant CD44-epitope v6 can thus be used for diagnosis and prognosis. It may be advantageous to combine these data with other prognostic parameters, e.g. the grade of tumour.

Antibody molecules having the specificity according to the invention, optionally combined with a cytotoxic agent, may advantageously be used for the treatment of squamous cell carcinomas. They may be administered systemically or topically, e.g. by intravenous route (as a bolus or continuous infusion) or by intraperitoneal, intramuscular, subcutaneous or other injection/infusion. Procedures for administering conjugated or non-conjugated antibodies (be they in the form of complete immunoglobulins, fragments,

recombinant humanised molecules or the like) are known in the art (Mulshine *et al.*, 1991; Larson *et al.*, 1991; Vitetta and Thorpe, 1991; Vitetta *et al.*, 1991; Breitz *et al.*, 1992, 1995; Press *et al.*, 1989; Weiner *et al.*, 1989; Chatal *et al.*, 1989; Sears *et al.*, 1982). They may be used therapeutically, for example, in the same way as the antibody 1.1ASML (Seiter *et al.*, 1993). Unmodified monoclonal antibodies can be used directly for therapeutic purposes if they have the intrinsic effector function suitable for a cytotoxic effect, e.g. for complement-induced or antibody-induced cell cytotoxicity (Riethmüller *et al.* 1994). Suitable monoclonal antibodies for this application are murine antibodies of isotype IgG2a or antibodies of the human IgG1-type. Unmodified antibodies may also be administered in order to induce the patient's own antitumoral reaction through an anti-idiotypic mechanism (Baum *et al.*, 1993; Khazaeli *et al.*, 1994).

According to a preferred embodiment of a therapeutic application, a humanised v6-specific immunoglobulin or a $F(ab')_2$ fragment thereof is linked with ^{90}Y (Quadri *et al.*, 1993; Vriesendorp *et al.*, 1995), ^{131}I (Maraveyas *et al.*, 1995a, 1995b; Juweid *et al.*, 1995; Press *et al.*, 1995; Thomas *et al.*, in: Catty 1985, p. 230-239) ^{186}Re (Breitz *et al.*, 1992, 1995) or another suitable radioisotope and used for radioimmunotherapy of squamous cell carcinomas. For example, the antibody BIWA-1, a humanised version of BIWA-1 or a $F(ab')_2$ fragment of BIWA-1 or the humanised antibody may be linked to ^{90}Y using a chelating linker such as ITCB-DTPA (isothiocyanatobenzyl-diethylenetriaminepentaacetate), achieving a specific activity of 5-20 mCi/mg, preferably 10 mCi/mg. This agent can then be administered to a patient with an antigen-positive tumour in a dose of 0.1 to 1 mCi/kg of body weight, preferably 0.3 to 0.5 mCi/kg of body weight. If the antibody molecule is linked to ^{131}I, a possible dosage plan might be, for example, 2 x 150 mCi at 6 week intervals for a specific activity of 2 mCi/mg. The skilled person can determine the maximum possible dosages using methods known *per se* (Maraveyas *et al.*, 1995a, 1995b). When the total quantity of protein to be administered is 2 to 5 mg it may be given in the form of a fast intravenous bolus injection. In the case of larger amounts of protein an infusion might be a more appropriate method of administration. With monoclonal antibodies it may be necessary to mix the agent with an excess (e.g. a tenfold molar excess) of the non-radioactive antibody before administration; in this case, it is better to administer the agent in the form of an intravenous infusion, e.g. over 15 minutes. This can be repeated. The therapy may be combined with external radiation therapy. It may also be backed up by bone marrow transplantation; this is particularly necessary when a dosage of more than 1.6 Gy is reached in the bone marrow during therapy.

Antibody molecules according to the invention may also be used *ex vivo* to purify CD34-positive stem and precursor cell preparations (immunopurging). Radiation or chemotherapy of squamous cell carcinomas may also be backed up with autologous bone marrow transplantation. The preparation of haematopoietic stem and precursor cells thus administered must be free from tumour cells. This can be achieved by incubation with antibody molecules according to the invention, e.g. antibody-toxin conjugates (Myklebust *et al.*, 1994; DE P 196 48 209.7).

Antibody molecules according to the invention may also be introduced into the T-cell receptor of T-lymphocytes in the form of recombinant constructs. Reprogrammed T-lymphocytes of this kind bind selectively to the antigen-expressing tumour cells and develop a cytotoxic activity, with the result that they can be used for the treatment of squamous cell carcinomas (PCT/EP9604631; Altenschmidt *et al.*, 1996).

Figures

Fig. 1: *Determining the epitope specificity of BIWA-1 by binding to synthetic peptides derived from the human CD44v6 sequence.* The corresponding peptide from rat CD44v6 was tested with the antibody 1.1ASML. Binding was determined in an ELISA in which the peptides were immobilised on microtitre plates (cf. Heider *et al.*, 1996b, Fig. 2). -: no binding, +/-: slight binding, +: strong binding.

Fig. 2: *Immunohistochemical analysis of a squamous cell carcinoma of the larynx (a) and a liver metastasis of a carcinoma of the oesophagus (b) with the CD44v6-specific monoclonal antibody BIWA-1.* In both cases the reactivity of the antibody with the membrane of the tumour cells can be observed. Original magnification 40x, counterstaining haematoxylin.

Fig. 3: *Comparison of antigen binding of various CD44v6-specific mAbs.* The binding of four different CD44v6-specific mAbs to human SCC A-431-cells was measured in a cell ELISA. MAb BIWA-1 shows a higher affinity for the tumour cells than the other mAbs.

Fig. 4: *Refined epitope mapping of the mAb BIWA-1.* The binding of BIWA-1 to various overlapping synthetic peptides which span the amino acids 18-32 of the CD44v6-coded region was measured by competitive ELISA. The minimum binding sequence (peptide v6 (19-29)) is underlined.

Fig. 5: *Biodistribution of ^{125}I-BIWA-1 in A-431 xenotransplanted nude mice.* The accumulation of the antibody is given as % ID/g (mean ± SEM) at 4, 24, 48, 120 and 168 hours post injection.

Examples

Example 1: Expression of CD44v6 in squamous cell carcinoma

Tissue

A total of 126 cases of paraffin-embedded tumour samples were analysed immunohistochemically with the mAb BIWA-1 (clone VFF-18) for expression of CD44v6. The samples included 31 cases of primary squamous cell carcinomas (15 cases larynx, 16 cases skin), 91 cases of lymph node metastases (larynx, n=38; lung, n=27; oesophagus, n=11; oral cavity, n=11; tonsils, n=4) and 4 cases of liver metastases (oesophagus).

Antibodies

The total variant region of the HPKII type of CD44v (Hofmann *et al.*, 1991) was amplified from human keratinocyte-cDNA by polymerase chain reaction (PCR). The two PCR primers 5'-CAGGCTGGGAGCCAAATGAAGAAAATG-3', positions 25-52, and 5'-TGATAAGGAACGATTGACATTAGAGTTGGA-3', positions 1013-984 of the LCLC97-variant region as described by Hofmann *et al.*, contained an EcoRI recognition site which was used in order to clone the PCR product directly into the vector pGEX-2T (Smith *et al.*, 1988). The resulting construct (pGEX CD44v HPKII, v3-v10) codes for a fusion protein of ~70 kD, consisting of glutathione-S-transferase from *Schistosoma japonicum* and the exons v3-v10 of human CD44 (Fig. 1; Heider *et al.*, 1993). The fusion protein was expressed in *E. coli* and then subjected to affinity purification over glutathione-agarose (Smith *et al.*, 1988).

Female Balb/c mice were intraperitoneally immunised with the affinity-purified fusion protein according to the following plan:

1st immunisation:	90 µg of fusion protein in complete Freund's adjuvant
2nd and 3rd immunisations:	50 µg of fusion protein in incomplete Freund's adjuvant.

The immunisations were given at intervals of 4 weeks. 14 days after the last immunisation the animals were immunised on three successive days with 10 µg of fusion protein in PBS. On the following day, the spleen cells from an animal with a high antibody titre were fused with P3.X63-Ag8.653 mouse myeloma cells using polyethyleneglycol

4000. The hybridoma cells were then selected in microtitre plates in HAT-medium (Köhler and Milstein, 1975; Kearney *et al.*, 1979).

Measurement of the antibody titre in the serum or screening of the hybridoma supernatants were carried out using an ELISA. In this test, first of all microtitre plates were covered with fusion protein (GST-CD44v3-10) or only with glutathione-S-transferase. They were then incubated with serial dilutions of serum samples or hybridoma supernatants and the specific antibodies were detected using peroxidase-conjugated antibodies against mouse immunoglobulin. Any hybridomas which reacted only with glutathione-S-transferase were discarded. The remaining antibodies were first characterised in an ELISA with domain-specific fusion proteins (exon v3, exon v5 + v6, exon v6 + v7, exon v8 - v10) (Koopman *et al.*, 1993). Their immunohistochemical reactivity was tested on human skin sections.

BIWA-1 (VFF-18; for preparation and properties see also WO 95/33771) bound only to fusion proteins which contained a domain coded by the exon v6. In order to further restrict the epitope of the antibody, various synthetic peptides which represented parts of the v6 domain were used in ELISA binding assays (Fig. 1). The 14 amino acid peptide v6D showed the strongest binding. Consequently, the epitope of BIWA-1 is wholly or partly within the sequence QWFGNRWHEGYRQT of the domain coded by exon v6. This sequence is homologous to the binding epitope of the antibody 1.1ASML, which was used in a therapeutic rat model and which is specific to rat CD44v6 (Fig. 1).

Immunohistochemistry

Before incubation with the primary antibody, paraffin sections (4 µm) in Rotihistol (Roth, Germany) were deparaffinated 3 times for 10 minutes each time and then rehydrated in a rising alcohol series. The sections were briefly washed with distilled water and then cooked in a microwave oven (Sharp Model R-6270) 3 times for 10 minutes each time at 600 Watts in 0.01 M Na-citrate buffer. After each microwave incubation the sections were cooled for 20 minutes. After the final cooling stage the carriers were washed in PBS and pre-incubated with normal goat serum (10% in PBS). After 3 washes in PBS the sections were incubated with primary antibody (BIWA-1: 5 µg/ml; murine IgG (isotype-corresponding negative control) 5 µg/ml in PBS/1% BSA) for 1 hour. The positive control used for the staining reaction consisted of normal human skin sections, as keratinocytes express a CD44-isoform which contains v3-v10. Endogenous peroxidases were blocked with 0.3% H_2O_2 in PBS, and the sections were incubated for 30 minutes with the biotinylated secondary antibody (anti-mouse IgG-F(ab')$_2$, DAKO Corp.). In order to

develop the stain, the sections were incubated for 30 minutes with horseradish peroxidase which was coupled to biotin as a streptavidin-biotin-peroxidase complex (DAKO Corp.). The sections were then incubated for 5-10 minutes in 3,3-amino-9-ethyl-carbazole substrate (Sigma Immunochemicals), the reaction was stopped with H_2O and the sections were counterstained with haematoxylin. The stains were evaluated using a Zeiss Axioskop Light Microscope and the colour intensities were quantified as follows: +++, strong expression; ++, moderate expression; +, weak expression; -, unclear or no expression detected. Only tumour cells with a clear membrane stain were evaluated as positive. The percentage of positive tumour cells in each section was roughly estimated and two groups were formed: focally positive tumours (less than 10% of the tumour cells reacted with the antibody) and positive tumours (10 or more % of the tumour cells positive). If fewer than 80% of the tumour cells in the positive cells reacted with the antibody, the corresponding percentage was indicated.

126 cases of squamous cell carcinomas of various origins were analysed using the CD44v6-specific monoclonal antibody BIWA-1. The expression of isoforms containing CD44v6 was observed in all but one tumour sample. The majority of the samples exhibited expression of the antigen on 80-100% of the tumour cells and the staining was restricted to the membrane of the tumour cells. No reaction was observed with stroma tissue, lymphocytes, muscle cells or endothelium.

In order to quantify the expression of CD44v6-molecules on these tumour cells, sections of normal human skin were stained parallel to the tumour sections. Normal skin keratinocytes express high levels of CD44-isoforms and are among the strongest expressors of CD44v6 of the normal cells which have been described hitherto. Consequently, keratinocyte staining was used as the reference and classified as "strong" (+++) in our system of evaluation. In the majority of the tumour samples examined, the staining of the tumour cells was comparable with or even greater than the staining of the skin keratinocytes, with only a few cases showing weak tumour staining (3 cases of lymph node metastasis) or moderate tumour staining (2 primary carcinomas, 10 metastases). The staining reaction was very homogeneous within a given tumour section, with the majority of tumour cells in the section having the same stain intensity. No significant differences were observed in the CD44v6-expression pattern between the primary tumours and metastases. A detailed summary of the results is shown in Table 1 with the Examples shown in Fig. 2.

Table 1: Expression of CD44v6 in squamous cell carcinomas

Sample			**Type of tumour**		**BIWA-1 Reactivity**
46937	86		Primary	Larynx	+++*
4687	90		Primary	Larynx	+++
8372	90		Primary	Larynx	+++
17427	90		Primary	Larynx	+++
27298	90		Primary	Larynx	+++
46908	90		Primary	Larynx	+++
51334	90		Primary	Larynx	+++
51402	91		Primary	Larynx	+++
60414	91		Primary	Larynx	+++
61733	91		Primary	Larynx	+++
12280	92		Primary	Larynx	+++
23140	92		Primary	Larynx	+++
31792	92		Primary	Larynx	+++
32214	92		Primary	Larynx	+++
10209	95		Primary	Larynx	+++
2366	86		Primary	Skin	+++
2574	86		Primary	Skin	+++
9916	86		Primary	Skin	++/+++
2696	87		Primary	Skin	+++
8906	87		Primary	Skin	+++
8191	88		Primary	Skin	+++
8354	88		Primary	Skin	++50%
11963	88		Primary	Skin	++
5590	90		Primary	Skin	++/+++
530	92		Primary	Skin	+++
2583	94		Primary	Skin	+++
11337	94		Primary	Skin	+++
10901	95		Primary	Skin	+++

11557	95		Primary	Skin	+++
11744	95		Primary	Skin	+++
11917	95		Primary	Skin	+++
4688	90	I	Lymph node metastasis	Larynx	++/+++
4688	90	II	Lymph node metastasis	Larynx	-
8374	90		Lymph node metastasis	Larynx	+++
17428	90		Lymph node metastasis	Larynx	+++
27300	90		Lymph node metastasis	Larynx	+++
36942	90		Lymph node metastasis	Larynx	+++
46909	90		Lymph node metastasis	Larynx	++
51336	90		Lymph node metastasis	Larynx	+++
41108	91		Lymph node metastasis	Larynx	+++
51398	91		Lymph node metastasis	Larynx	+++
60416	91		Lymph node metastasis	Larynx	+++
61734	91		Lymph node metastasis	Larynx	+++
1318	92	I	Lymph node metastasis	Larynx	+++
1318	92	II	Lymph node metastasis	Larynx	+++
1318	92	III	Lymph node metastasis	Larynx	+++
1318	92	IV	Lymph node metastasis	Larynx	+++
2863	92	I	Lymph node metastasis	Larynx	+++
2863	92	II	Lymph node metastasis	Larynx	+++
5745	92	I	Lymph node metastasis	Larynx	+++
5745	92	II	Lymph node metastasis	Larynx	+++
8969	92	I	Lymph node metastasis	Larynx	+++
8969	92	II	Lymph node metastasis	Larynx	+++
8969	92	III	Lymph node metastasis	Larynx	++
8969	92	IV	Lymph node metastasis	Larynx	+++
8969	92	2/I	Lymph node metastasis	Larynx	+++
8969	92	2/II	Lymph node metastasis	Larynx	+++
8969	92	2/III	Lymph node metastasis	Larynx	++
8969	92	2/IV	Lymph node metastasis	Larynx	+/++
9366	92		Lymph node metastasis	Larynx	+++

9509	92		Lymph node metastasis	Larynx	+++
9566	92		Lymph node metastasis	Larynx	+++
12283	92		Lymph node metastasis	Larynx	+++
14046	92		Lymph node metastasis	Larynx	+++
31787	92		Lymph node metastasis	Larynx	+++
49228	92		Lymph node metastasis	Larynx	+++ 50%
29228	93		Lymph node metastasis	Larynx	+++
29829	93		Lymph node metastasis	Larynx	++
29804	95		Lymph node metastasis	Larynx	++/+++
15293	91		Lymph node metastasis	Lung	+ 25% -
1667	92		Lymph node metastasis	Lung	+ 20%
2757	92	I	Lymph node metastasis	Lung	+++
2757	92	II	Lymph node metastasis	Lung	+++
2757	92	III	Lymph node metastasis	Lung	+++
2757	92	IV	Lymph node metastasis	Lung	+++
4790	92		Lymph node metastasis	Lung	+++
6168	92	I	Lymph node metastasis	Lung	++ 50%
6168	92	II	Lymph node metastasis	Lung	+++ -
6168	92	III	Lymph node metastasis	Lung	+++
6168	92	IV	Lymph node metastasis	Lung	+++
7206	92		Lymph node metastasis	Lung	+++
7531	92	I	Lymph node metastasis	Lung	+++
7531	92	II	Lymph node metastasis	Lung	+++
7531	92	III	Lymph node metastasis	Lung	++/+++
7531	92	IV	Lymph node metastasis	Lung	+++
10324	92		Lymph node metastasis	Lung	+++
10519	92	II	Lymph node metastasis	Lung	+++
10519	92	RM II	Lymph node metastasis	Lung	+++
10958	92		Lymph node metastasis	Lung	+++
11425	92	I	Lymph node metastasis	Lung	+++
11425	92	II	Lymph node metastasis	Lung	+++

13055	92		Lymph node metastasis	Lung	++/+++
13055	92	II	Lymph node metastasis	Lung	focal +++
13055	92	III	Lymph node metastasis	Lung	+++
15663	92		Lymph node metastasis	Lung	+++
16713	92		Lymph node metastasis	Lung	+++
14980	91	I	Lymph node metastasis	Oesophagus	+++
14980	91	II	Lymph node metastasis	Oesophagus	+++
16641	91	I	Lymph node metastasis	Oesophagus	+++
16641	91	II	Lymph node metastasis	Oesophagus	+++
16641	91	III	Lymph node metastasis	Oesophagus	+++ -
1059	92		Lymph node metastasis	Oesophagus	+
1710	92	I	Lymph node metastasis	Oesophagus	+++
1710	92	II	Lymph node metastasis	Oesophagus	+++
1710	92	III	Lymph node metastasis	Oesophagus	+++
11502	92	I	Lymph node metastasis	Oesophagus	+++
11502	92	II	Lymph node metastasis	Oesophagus	++
202	92		Lymph node metastasis	Oral cavity	++ 60%
6030	92		Lymph node metastasis	Oral cavity	+/++/+++ 25%
7335	92	I	Lymph node metastasis	Oral cavity	+++
7335	92	II	Lymph node metastasis	Oral cavity	+++
15324	92	II	Lymph node metastasis	Oral cavity	+++ 70%
16164	92	I	Lymph node metastasis	Oral cavity	+++
16164	92	II	Lymph node metastasis	Oral cavity	+++ 50%
16412	92		Lymph node metastasis	Oral cavity	++/+++
16836	92	I	Lymph node metastasis	Oral cavity	+++
16836	92	II	Lymph node metastasis	Oral cavity	+++
16836	92	III	Lymph node metastasis	Oral cavity	+++
6228	92	I	Lymph node metastasis	Tonsil	+++
6228	92	II	Lymph node metastasis	Tonsil	+++
6618	92		Lymph node metastasis	Tonsil	+++
11840	92		Lymph node metastasis	Tonsil	++

14172	91	4	Liver metastasis	Oesophagus	+++
14172	91	5	Liver metastasis	Oesophagus	+++
4131	94	1	Liver metastasis	Oesophagus	+/++
8438	94		Liver metastasis	Oesophagus	focal ++/+++

80-100% of the tumour cells reacted positively with BIWA-1. In cases where fewer tumour cells reacted with the antibody, the percentage obtained is given.

Example 2: Expression of CD44v6 in kidney cell carcinomas, prostate carcinomas and liver metastases of colon carcinomas

Tissue

19 cases of kidney cell carcinomas (12 cases of clear cell, 5 cases of chromophilic, 1 case of chromophobic, 1 oncocytoma), 16 primary adenocarcinomas of the prostate and 19 cases of lymph node metastases of prostate carcinoma, and 30 cases of liver metastases of colon carcinomas were analysed.

Antibody

BIWA-1 (see Example 1).

Immunohistochemistry

For method see Example 1.

In contrast to the squamous cell carcinomas, no or only focal expression of CD44v6-isoforms could be detected in the majority of the kidney cell and prostate carcinomas investigated. In the case of a more than focal expression in the prostate carcinomas the staining was predominantly diffusely cytoplasmic and weak or heterogeneous, compared with the staining of normal prostate epithelium. In 50% of the liver metastases of colon carcinomas investigated, a more than focal expression of CD44v6 isoforms was detected. The staining in the majority of cases was faint to moderate, but generally fewer than 100% of the tumour cells in a sample showed any staining with BIWA-1. The results are summarised in Table 2.

Table 2: Expression of CD44v6 in prostate adenocarcinomas, kidney cell carcinomas and liver metastases of colorectal carcinomas

Type of tumour		n	BIWA-1 Reactivity		
			negative	focal pos.	positive
Prostate adenocarcinoma	Primary	16	8	3	5
Prostate adenocarcinoma	Lymph node metastases	19	15	2	2
Kidney cell carcinoma	Primary	19	17	0	2
Colorectal carcinoma	Liver metastases	30	7	8	15

Example 3: Characterisation of CD44v6-specific antibodies

Cell line

The human SCC cell line A-431 (spontaneous epidermoid carcinoma of the vulva) was obtained from the American Type Culture Collection (Rockwell MD) and cultured in accordance with the manufacturer's instructions. The surface expression of CD44v6-containing isoforms was determined by FACS analysis, using an FITC-linked mAb BIWA-1.

Analysis of the kinetic constants

The affinity and kinetics of the monoclonal antibody CD44v6-interaction was determined by Surface Plasmon Resonance (SPR), using a BIAcore 2000 system (Pharmacia Biocensor). A glutathione-S-transferase-CD44-fusion protein which contained the region coded by the exons v3-v10 (GST/CD44 v3-v10) was immobilised on a CM5 Sensor Chip, the amine coupling method being carried out in accordance with the manufacturer's instructions. Antibodies in various concentrations (8-132 nM) in HBS (10 mM HEPES pH 7.4, 150 mM sodium chloride, 3.4 mM EDTA, 0.05% BIA core surfactant P20) was injected over the antigen-specific surface at a flow rate of 5 µl/min.

The interaction was recorded as a change in the SPR signal. Dissociation of the antibody was observed for 5 minutes in the buffer flow (HBS). The surface of the chip was regenerated with a single pulse of 15 µl 30 mM HCl. The data analysis and calculation of the kinetic constants were carried out using the Pharmacia Biocensors BIA Evaluation Software, Version 2.1.

In this way, the antigen affinity of BIWA-1 was compared with other CD44v6-specific mAbs (VFF4, VFF7, BBA-13 (IgG1, R&D Systems, Abingdon, U.K.)). Kinetic and affinity constants of the various antibodies were determined in two independent experiments. Table 3 shows the values of the association rates (k_a), dissociation rates (k_d) and dissociation constants (K_d) for the 4 mAbs. All the mAbs showed similar k_a and k_d, with the exception of BBA-13, which has a 3-times lower k_a and VFF7, which has a significantly higher dissociation rate (factor 5) compared with the other mAbs. This results in a lower binding affinity for VFF7 and BBA-13 compared with VFF4 and BIWA-1. BIWA-1 shows the lowest K_d of all the antibodies investigated.

Table 3: Kinetic and affinity constants of various CD44v6-specific mAbs

Antibody	**k_a ($M^{-1}s^{-1}$)**	**k_d(s^{-1})**	**K_d (M)**
VFF4	1.1×10^5	2.6×10^{-5}	2.4×10^{-10}
VFF7	1.1×10^5	1.2×10^{-4}	1.1×10^{-9}
BIWA-1	1.3×10^5	2.2×10^{-5}	1.7×10^{-10}
BBA-13	3.7×10^4	2.3×10^{-5}	6.2×10^{-10}

Analysis of the antibody-protein interaction using ELISA.

CD44v6 expressing A-431 cells were cultivated in 96-well plates (Falcon Microtest III, Becton Dickinson, Lincoln Park, NJ) in numbers of 5×10^4 per well in RPMI 1640 with 10% foetal calf serum overnight at 37°C. After washing with PBS/0.05% Tween 20 the cells were fixed for 1 minute with ice cold ethanol, followed by a washing step. Incubation with the primary antibodies (VFF4, VFF7, BIWA-1, BBA-13, 1 ng/ml to 600 ng/ml, in each case in assay buffer: PBS/0.5% BSA/0.05% Tween 20) was carried out for 1 hour at ambient temperature and followed by 3 washing steps. The secondary antibody used was a rabbit-antimouse-IgG horseradish peroxidase-conjugated antibody (DAKO Corporation,

Copenhagen, Denmark; dilution 1:6000 in assay buffer) (1 hour at room temperature). After 3 washing steps the colour was developed using TMB solution (Kirkegaard and Perry, Gaithersburg, USA). The extinction was measured using a Hewlett-Packard ELISA Reader.

Figure 3 shows that the relative affinities of the antibodies as determined by BIAcore analysis are reflected in their interaction with the tumour cells, with BIWA-1 clearly showing the highest binding affinity.

The protein domain which is coded by the CD44-exon v6 consists of 45 amino acids (Figure 4). In order to define more accurately the epitope which is recognised by BIWA-1, a series of synthetic peptides were used in ELISA assays. Preliminary experiments showed binding to a centrally located 14-mer (amino acid groups 18-31; Figure 4; cf. also Figure 1) but not to peptides outside this region. A second series of peptides were therefore synthesised and tested in competitive ELISAs (Figure 4). The results show that the peptide 19-29 (WFGNRWHEGYR) represents the minimum structure required for high affinity binding. Elimination of the C-terminal arginine groups resulted in a more than 100 times weaker binding.

Example 4: Biodistribution of radio-iodinated CD44v6-antibodies in xenotransplant-carrying nude mice

A-431-Xenotransplant model

8 week old female BALB/c nu/nu nude mice (B & K Universal, Renton, WA) were subcutaneously injected in the left-hand median line with 5 x 10^6 cultivated A-431 cells (human epidermoid carcinoma of the vulva). Xenotransplanted animals carrying A-431 tumours were used for biodistribution experiments within two weeks (weight of tumours: 40-50 mg).

Radio-iodination of BIWA-1

Protein G-purified mAb BIWA-1 (murine IgG1) was coupled to streptavidin, using the heterobifunctional crosslinker succinimidyl 4-(N-maleimido-methyl)cyclohexane-1-carboxylate. Streptavidin-lysyl groups were linked to reduced antibody-cysteinyl groups produced by preliminary treatment of the antibody with dithiothreitol. The 1:1 conjugates obtained (> 90%) were further purified by ion exchange chromatography. For biodistribution experiments, BIWA-1/SA was labelled via primary amines of lysine with

^{125}I, using p-iodophenyl labelling reagent (PIP; NEN Dupont, Wilmington, DE), followed by the process of Willbur *et al.* (1989). Labelling BIWA-1 with SA or ^{125}I did not affect the immunoreactivity or the pharmacokinetics of the antibody in mice.

Biodistribution experiments

Nude mice which were xenotransplanted with human A-431 tumours were intravenously injected through the lateral caudal vein with 5-7 µCi ^{125}I on 50 µg of mAb BIWA-1 (specific activity 0.1-0.14 mCi/mg). Time-biodistribution studies were carried out in groups of n=3 animals for each point of time at 4, 24, 48, 120 and 168 hours post-injection. At selected times, mice were weighed, blood was extracted from the retro-orbital plexus and they were killed by cervical dislocation. Nine organs and tissues were collected and weighed: blood, tail, lung, liver, spleen, stomach, kidney, intestine and tumour. The radioactivity in the tissues was counted in a gamma-scintillation counter (Packard Instrument Company, Meriden, CT) by comparison with standards of the injected antibody preparation, the energy window being set at 25-80 keV for ^{125}I. The percentage of injected dose per gram of tissue was calculated (% ID/g).

Preliminary experiments had shown that BIWA-1 did not cross-react with murine CD44v6-antigen. Table 4 and Figure 5 show the absorption of radioactivity in tumours and normal tissue. Iodinated BIWA-1 showed a rapid tumour absorption (7.6% injected dose/g at 4 hours post-injection) which increased to more than 18% ID/g after 48 hours and then remained constant for up to 120 hours. Seven days post-injection (168 hours) the tumour still contained 15.3% ID/g of tissue. Tumour:tissue ratios were calculated for individual times and these are shown in Table 4. At 24 hours post-injection the tumour:blood ratio was 0.48 and increased to 3.16 on day 7. The uptake in normal tissue was low and most probably caused by blood-pool background in the tissue biopsies. Selective *in vivo* targeting of human SCC-xenotransplants in nude mice with ^{125}I-labelled BIWA-1 shows that this monoclonal antibody has a high potential as a targeting vehicle for diagnostic and therapeutic use in SCC patients.

Table 4: Tumour:tissue ratios of ^{125}I-BIWA-1 in A-431-tumour-carrying nude mice at various times post-injection

Ratio of tumour to	4h	24h	48h	120h	168h
Blood	0.22[a]	0.48	1.31	2.60	3.16
Tail	1.18	2.62	7.70	12.28	13.06
Lung	0.40	1.03	2.65	7.04	4.82
Liver	0.94	1.18	2.28	3.57	3.24
Spleen	1.40	1.84	4.00	4.86	4.42
Stomach	3.89	7.37	19.40	25.56	33.96
Kidney	0.82	1.31	2.72	2.79	2.53
Intestine	3.54	6.24	11.94	19.24	27.78

[a] mean values (n=3); SD are <7%

Example 5: Different expression of CD44v6 in a large number of human tumours

In a wider investigation, a total of 544 tumour samples were examined immunohistochemically with the monoclonal antibody BIWA 1 (clone VFF-18) for the expression of CD44v6. The samples were either embedded in paraffin or frozen in liquid nitrogen immediately after surgical removal and stored at -70°C until required. The following tumours were analysed: basalioma (n=16), adenocarcinoma (AC) of the breast (n=55), AC of the colon (n=83), squamous cell carcinomas (SCC) of the head and neck (n=125), lung carcinomas (n=120), prostate AC (n=34), kidney cell carcinomas (n=27), SCC of the skin (n=15) and AC of the stomach (n=69). The tissues were obtained by routine surgery or biopsy and normal tissue was obtained to accompany the tumour samples. The immunohistochemical investigations were carried out as in Example 1.

Table 5 shows a summary of the immunohistochemical analysis of 397 different types of tumour with the mAb BIWA 1.

Table 5: Expression of CD44v6 in human tumours

Type		Total number	Positive cases*	
			n	%
Basalioma	Primary tumour	16	10	62
Breast AC	Primary tumour	17	15	88
	Lymph node metastases	34	31	91
	Liver metastases	4	4	100
Colon AC	Lymph node metastases	51	21	41
	Liver metastases	26	13	50
	Brain metastases	6	6	100
Larynx SCC	Lymph node metastases	18	18	100
Lung AC	Primary tumour	35	15	43
Lung SCC	Primary tumour	9	9	100
Oesphagus SCC	Primary tumour	20	20	100
Prostate AC	Primary tumour	16	5	31
	Lymph node metastases	18	0	0
RCC	Primary tumour	27	5	18
SCLC	Primary tumour	31	7	23
Stomach AC	Primary tumour	22	15	68
	Lymph node metastases	43	16	37
	Liver metastases	4	4	100
	Total number	397		
*: ≥ 10% of tumour cells positive				
AC: adenocarcinoma; RCC: renal cell carcinoma				
SCLC: small cell lung cancer; SCC: squamous cell carcinoma				

In small cell lung cancers, renal cell carcinomas and AC of the prostate, no or only little reactivity was observed. All the other types of tumour investigated expressed CD44v6-containing isoforms to varying degrees. The majority of the AC of the breast investigated showed reactivity with BIWA 1, and the SCC tested (larynx, lung and oesophagus) expressed CD44b6 in 100% of all cases.

185 additional cases of SCC of various types and classification were investigated for their reactivity with BIWA 1. These included 67 cases of primary SCC (larynx, n=15; oral cavity, n=16; oropharynx, n=3; skin, n=15), 77 samples of lymph node metastases

(larynx, n=12; lung, n=27; oesphagus, n=11; oral cavity, n=6; oropharynx, n=7; hypopharynx, n=10; tonsil, n=4), and 3 samples of liver metastases (oesphagus). Table 6 summarises the immunohistochemical analysis of all the SCC samples investigated.

Table 6: Expression of CD44v6 in squamous cell carcinomas

Type		Total number	Negative		Focal pos.		Positive	
			n	%	n	%	n	%
Hypopharynx	LNM	10	0	0	0	0	10	100
Oropharynx	PT	3	0	0	0	0	3	100
	LNM	7	0	0	0	0	7	100
Larynx	PT	15	0	0	0	0	15	100
	LNM	30	1	3	0	0	29	97
Lung	PT	18	2	11	0	0	16	89
	LNM	27	0	0	1	4	26	96
Oesphagus	PT	20	0	0	1	5	19	95
	LNM	11	0	0	0	0	11	100
	LM	3	0	0	0	0	3	100
Oral cavity	PT	16	0	0	0	0	16	100
	LM	6	0	0	0	0	6	100
Skin	PT	15	0	0	0	0	15	100
Tonsil	LNM	4	0	0	0	0	4	100
Total number		185						

Focal pos.: <10% of tumour cells positive; LNM: lymph node metastases; PT: primary tumour; LM: liver metastases

Expression of CD44v6 containing isoforms was found in all but three tumour samples (one case of larynx, 2 cases of lung). The majority of the samples showed expression of the antigen on 80 to 100% of the tumour cells within a single section, the staining being concentrated chiefly on the membrane of the tumour cells. The most homogeneous staining pattern was found in carcinomas of the larynx, oesphagus and hypopharynx, with the majority of tumour cells in the section having the same intensity of staining.

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SEQUENCE LISTING

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(A) NAME: Adolf, Guenther
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(F) POSTCODE: 3002

(A) NAME: Sproll, Marlies
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(E) COUNTRY: Austria
(F) POSTCODE: 1120

(ii) TITLE OF THE INVENTION: Process for the diagnosis and treatment of squamous cell carcinomas

(iii) NUMBER OF SEQUENCES: 16

(iv) COMPUTER-READABLE VERSION:
(A) DATA CARRIER: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) DATA RELATING TO SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 129 base pairs
(B) NATURE: Nucleotide
(C) STRAND FORM: both
(D) TOPOLOGY: both

(ii) NATURE OF THE MOLECULE: Genome DNA

(ix) FEATURE:
(A) NAME/KEY: exon
(B) POSITION:1..129
(D) OTHER DATA:/product= "CD44"
/label= v6
/note= "GenBank data base accession No. L05411"
/citation= ([1])

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) POSITION:3..128

(x) PUBLICATION INFORMATION:
(A) AUTHORS: Screaton, GR
Bell, MV
Jackson, DG
Cornelis, FB
Gerth, U
Bell, JI
(B) TITLE: Genomic structure of DNA encoding the lymphocyte homing receptor CD44 reveals at least 12 alternatively spliced exons
(C) JOURNAL: Proc. Natl. Acad. Sci. U.S.A.
(D) VOLUME: 89
(F) PAGES: 12160-12164
(G) DATE: December-1992
(K) IMPORTANT GROUPS IN SEQ ID NO: 1: FROM 1 TO 129

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

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

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TC CAG GCA ACT CCT AGT AGT ACA ACG GAA GAA ACA GCT ACC CAG AAG  47
   Gln Ala Thr Pro Ser Ser Thr Thr Glu Glu Thr Ala Thr Gln Lys
    1               5                  10                  15

GAA CAG TGG TTT GGC AAC AGA TGG CAT GAG GGA TAT CGC CAA ACA CCC 95
Glu Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr Pro
                20                  25                  30

AGA GAA GAC TCC CAT TCG ACA ACA GGG ACA GCT G                   129
Arg Glu Asp Ser His Ser Thr Thr Gly Thr Ala
            35                  40
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(2) DATA RELATING TO SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) NATURE: amino acid
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: Protein

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

```
Gln Ala Thr Pro Ser Ser Thr Thr Glu Glu Thr Ala Thr Gln Lys Glu
 1               5                  10                  15

Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr Pro Arg
            20                  25                  30

Glu Asp Ser His Ser Thr Thr Gly Thr Ala
        35                  40
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(2) DATA RELATING TO SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

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

Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr
1 5 10

(2) DATA RELATING TO SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) NATURE: Nucleotide
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

CAGGCTGGGA GCCAAATGAA GAAAATG 27

(2) DATA RELATING TO SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 base pairs
(B) NATURE: Nucleotide
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: other nucleic acid
(A) DESCRIPTION: /desc = "PCR primer"

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

TGATAAGGAA CGATTGACAT TAGAGTTGGA 30

(2) DATA RELATING TO SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg
1 5 10

(2) DATA RELATING TO SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 43 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3

(I) FILING DATE: 06-DEC-1995

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: DE 196 15 074.4

(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Gln Ala Thr Pro Ser Ser Thr Thr Glu Glu Thr Ala Thr Gln Lys Glu
1 5 10 15

Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr Pro Arg
20 25 30

Glu Asp Ser His Ser Thr Thr Gly Thr Ala Ala
35 40

(2) DATA RELATING TO SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11 amino acids

(B) NATURE: amino acid

(C) STRAND FORM: single strand

(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: DE 195 45 472.3

(I) FILING DATE: 06-DEC-1995

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: DE 196 15 074.4

(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Ser Ser Thr Thr Glu Glu Thr Ala Thr Gln Lys
1 5 10

(2) DATA RELATING TO SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Glu Glu Thr Ala Thr Gln Lys Glu Gln Trp
1 5 10

(2) DATA RELATING TO SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand

(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Thr Ala Thr Gln Lys Glu Gln Trp Phe Gly Asn
1 5 10

(2) DATA RELATING TO SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Gln Trp Phe Gly Asn Arg Trp His Glu Gly Tyr Arg Gln Thr
1 5 10

(2) DATA RELATING TO SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asn Arg Trp His Glu Gly Tyr Arg Gln Thr Pro
1 5 10

(2) DATA RELATING TO SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Glu Gly Tyr Arg Gln Thr Pro Arg Glu Asp Ser
1 5 10

(2) DATA RELATING TO SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Thr Pro Arg Glu Asp Ser His Ser Thr Gly
1 5 10

(2) DATA RELATING TO SEQ ID NO: 15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 42 amino acids
(B) NATURE: amino acid
(C) STRAND FORM: single strand
(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 195 45 472.3
(I) FILING DATE: 06-DEC-1995
(x) PUBLICATION INFORMATION:
(H) DOCUMENT NUMBER: DE 196 15 074.4
(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Trp Ala Asp Pro Asn Ser Thr Thr Glu Glu Ala Ala Thr Gln Lys Glu
1 5 10 15

Lys Trp Phe Glu Asn Glu Trp Gln Gly Lys Asn Pro Pro Thr Pro Ser
20 25 30

Glu Asp Ser His Val Thr Glu Gly Thr Thr
35 40

(2) DATA RELATING TO SEQ ID NO: 16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) NATURE: amino acid

(C) STRAND FORM: single strand

(D) TOPOLOGY: linear

(ii) NATURE OF THE MOLECULE: peptide

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: DE 195 45 472.3

(I) FILING DATE: 06-DEC-1995

(x) PUBLICATION INFORMATION:

(H) DOCUMENT NUMBER: DE 196 15 074.4

(I) FILING DATE: 17-APR-1996

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Lys Trp Phe Glu Asn Glu Trp Gln Gly Lys Asn Pro Pro Thr
1 5 10

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Claims

1. Process for diagnosing or treating squamous cell carcinomas, characterised in that this process is based on the expression of the variable exon v6 of the gene CD44 as the molecular marker.

2. Process according to claim 1, characterised in that it is based on the binding of an antibody molecule to an epitope which is coded by the variable exon v6 of the gene CD44.

3. Process according to claim 2, characterised in that an antibody molecule is used which recognises the amino acid sequence WFGNRWHEGYR.

4. Process according to claim 3, characterised in that the monoclonal antibody BIWA-1 (VFF-18), which is formed by the hybridoma cell line with the Accession Number DSM ACC2174, or a derivative of this antibody is used.

5. Process according to one of claims 1 to 4, characterised in that the antibody molecule is a monoclonal antibody, a Fab- or $F(ab')_2$-fragment of an immunoglobulin, an antibody prepared by recombinant methods, a chimeric or humanised antibody prepared by recombinant methods, a bifunctional or a single-chain antibody (scFv).

6. Use of an antibody molecule which is specific to an epitope which is coded by the variant exon v6 of the CD44-gene, in a process according to one of claims 1 to 4.

7. Use according to claim 6, characterised in that the antibody molecule binds to the amino acid sequence WFGNRWHEGYR.

8. Use according to claim 6 or 7, characterised in that the antibody molecule is the monoclonal antibody BIWA-1 (VFF-18), which is formed by the hybridoma cell line with the Accession Number DSM ACC2174, or a derivative of this antibody.

9. Use according to one of claims 6 to 8, characterised in that the antibody molecule is a monoclonal antibody, a Fab- or $F(ab')_2$-fragment of an immunoglobulin, an antibody prepared by recombinant methods, a chimeric or

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humanised antibody prepared by recombinant methods, a bifunctional or a single-chain antibody (scFv).

10. Use of an antibody molecule which is specific to an epitope within the amino acid sequence which is coded by the variable exon v6 of the CD44-gene, for the treatment of squamous cell carcinomas.

11. Use according to claim 10, characterised in that the antibody molecule binds to the amino acid sequence WFGNRWHEGYR.

12. Use according to claim 10 or 11, characterised in that the antibody molecule is the monoclonal antibody BIWA-1 (VFF-18), which is formed by the hybridoma cell line with the Accession Number DSM ACC2174, or a derivative of this antibody.

13. Use according to one of claims 10 to 12, characterised in that the antibody molecule is a monoclonal antibody, a Fab- or $F(ab')_2$-fragment of an immunoglobulin, an antibody prepared by recombinant methods, a chimeric or humanised antibody prepared by recombinant methods, a bifunctional or a single-chain antibody (scFv).

14. Use according to one of claims 10 to 13, characterised in that the antibody molecule is linked to a radioactive isotope, a photoactivatable compound, a radioactive compound, an enzyme, a fluorescent dye, a biotin molecule, a toxin, a cytostatic, a prodrug, an antibody molecule with a different specificity, a cytokine or another immunomodulatory polypeptide.

15. Agent for performing a process according to one of claims 1 to 5, characterised in that it is an antibody or antibody molecule which is specific to an epitope coded by the exon v6 of the CD44-gene.

16. Use of an antibody molecule which is specific to an epitope within the amino acid sequence which is coded by the variable exon v6 of the CD44 gene, for the preparation of a pharmaceutical composition for the diagnosis and/or treatment of squamous cell carcinomas.

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