Antibodies for the detection of integrin complexes in FFPE material

The invention relates to antibodies that are capable to bind the extracellular domain of integrin. Another object of the invention concerns the use of said antibodies for detecting integrins in archival formalin fixed paraffin embedded (FFPE) tissue. The invention also relates to methods for preparing monoclonal rabbit antibodies, wherein the immunogen is an insect expression culture-derived recombinant extracellular integrin domain, and another method for screening anti-integrin antibodies that discriminate between closest integrin homologues and that are especially suited for immunohistochemistry in FFPE material.

Integrins are a family of cell adhesion molecules composed of two non-covalently associated chains. The complex multi-domain structure of integrins is sensitive to subtle modulation. Integrins are regulated at many levels, including translation and transcription, post-translational glycosylation, cell surface delivery, cell surface activation by intracellular prompts and cell surface activation by extracellular prompts. Both alpha and beta chains are class I transmembrane proteins, which transverse the membrane and integrate extracellular matrix with intracellular compartment, thus providing a pathway for the signals that ultimately lead to control of adhesion, proliferation, survival, migration and invasion.

Integrins are therapeutic targets in much human pathology. For example in cancer, alpha-v series integrins (αvß1, αvß3 ,αvß5, αvß6 and αvß8) are variously implicated in angiogenesis, protecting tumor cells from chemo- and radiotherapy, tumor survival and local immune suppression. α5ß1 and α4ß1 are also implicated in angiogenesis, while α2ß1 and α6ß4 have been implicated in tumor proliferation. αvβ3 over-expression correlates with the invasive phase of human melanoma, and both αvβ3 and αvβ5 are specifically up-regulated in tumor-invasive endothelium, where they appear to regulate the functions of angiogenic growth factors on the endothelial surface. The precise expression pattern of the integrins is highly variable both between and within a given class of tumors and reflects the functional biology. Hence, they are also biomarkers of tumor status, and the expression pattern is prognostic for outcome and can define therapeutic opportunities.

The monoclonal antibody DI-17E6 directed against the αv-integrin chain, and cilengitide, a cyclized RGD-containing pentapeptide, that inhibits integrins αvβ3 and αvβ5 are in clinical development. However, the full therapeutic potential of therapies targeting integrins has yet

to be attained, in part because there is a remarkably incomplete picture of the integrin expression patterns in pathological conditions. Pathological characterization of integrin distribution has relied on studies on fresh frozen tissues. The live cell-to-cryostaining linkage is well established, and frozen tissues are excellent substrates for integrin staining, but their level of preservation and the ultra-structural fealty are much lower than that routine in FFPE material. This can critically affect the interpretations of staining in complex tissue. Furthermore, routine clinical practice, and generally and commercially available tissue banks, provides FFPE material: obtaining frozen clinical material is a logistic and often a clinical-cultural challenge, or simply an impossibility when dealing with certain tumors and with rare and precious clinical samples.

It is due to the conflicting needs of classical histology and of the integrins' structure that unequivocal integrin detection in FFPE material is prevented in prior art. Histology needs excellent and robust morphological preservation of tissue structures, involving an extensive cross linking, infiltration and stabilization of soft hydrophilic tissues by hydrophobic insolubilizing reagents, such as formaldehyde solution, graded alcohols and paraffin wax, optionally along with heat impact. It is known that fixation and embedding, especially as practiced in clinical histology laboratories can conceal or even destroy epitopes. The non-native conditions result in integrins that are rather not extracted or degraded, but mainly occluded. The conformationally active obligate integrin heterodimers are sensitive to such conformational change, and they cannot readily be recovered from occlusion as it occurs during FFPE procedures.

Since the chemistries involved in tissue fixation and embedding affect integrin structure seriously, the defining available monoclonal antibodies used by skilled artisans in the field do not reliably recognize integrins after FFPE processing. Antibodies that recognize integrin cytoplasmic domains are necessarily restricted to single integrin chains, leading to ambiguous staining patterns in FFPE material since they do not report the distribution of intact integrin heterodimers. Furthermore, such antibodies, being directed against short peptide epitopes, tend to be conformation independent, which leads to the detection of single chains or degradation products, and a lower specificity and affinity than antibodies which would detect intact integrin complexes.

Several mouse monoclonal antibodies, such as mouse monoclonal anti-integrin avß3 antibody LM609, detect αvβ3 and αvβ5 integrins using FACS or frozen tissue, however, they do not show significant or reproducible labeling of their epitopes in FFPE material. The deficiencies of murine monoclonals in their restricted epitope recognition and low affinity

are widely recognized. The distribution patterns seen when such antibodies are used on FFPE material diverge from the patterns observed in fresh-frozen cryo-sectioned material; while these latter expression profiles closely match those of viable cells isolated from such tissues. FFPE staining with such antibodies must be viewed as of dubious provenance, and a technology on antigen retrieval has to grow up to recover such determinants from FFPE material.

At present, no monoclonal antibody is available that robustly recognizes the αvβ3 or αvβ5 extracellular epitopes in FFPE tissue, allowing the characterization of integrins in the FFPE patient tumor tissue. The end result of this situation is that decades of pathological specimens cannot be analyzed for the integrin expression profiles that might reveal patient populations who could benefit from therapies that target integrins. In the emerging therapeutic landscape, such a deficit can mean that effective therapeutics may tragically never reach the needy.

Therefore, the technical problem forming the basis of the present invention is to provide antibodies, which allow the reliable and unequivocal detection of integrin complexes in FFPE material, especially in routine FFPE tumor biopsies. It is another problem to provide a method for screening anti-integrin antibodies, which exhibit an effective discriminatory behavior between integrin homologues during immunohistochemistry in FFPE material.

The present invention solves the first problem by providing an antibody comprising one or more light chains and/or heavy chains, each of the chains comprising one or more complementarity determining regions (CDRs) of rabbit origin and optionally framework regions (FRs) in variable regions of the light (V_L) and/or heavy (V_H) chains, wherein the antibody has the capacity to bind an extracellular or intracellular domain of integrin. In other words, the antibody comprises at least one light chain variable region (V_L) and/or at least one heavy chain variable region (V_H), each of the regions comprising at least one complementarity determining region (CDR) of rabbit origin and optionally one or more framework regions (FRs), wherein the antibody has the capacity to bind an extracellular or intracellular domain of integrin.

In more detail, the present invention solves the first problem by providing a monoclonal rabbit antibody, or a fragment thereof, against both integrin with insect-derived glycosylation pattern and integrin with any other eukaryotic glycosylation pattern, wherein the antibody or the fragment thereof comprises at least a light chain variable region (V_L) and a heavy chain variable region (V_H), wherein the antibody has antigen binding specificity

for a non-occluded epitope of an extracellular integrin domain, extracellular integrin chain domain or intracellular integrin chain domain, and wherein the antibody is able to bind to intact heterodimers of integrin in formalin fixed paraffin embedded (FFPE) material and in an isolated form in ELISA and/or in a native state on viable cells with the substantially same specificity.

It has been surprisingly demonstrated by the inventors that FFPE-capable antibodies can readily be generated by using the extracellular or intracellular domain of integrins or integrin chains as immunogen in rabbits. Best results are obtained with the intact domain, which can be favorably recombinantly expressed. In particular, the extracellular heterodimeric integrin domains have been proven to be effective immunogens if prepared in insect cells. The provision of the truncated integrin immunogens according to the invention significantly enhances the accessibility of epitopes and results in antibodies of exquisite sensitivity and specificity to the antigen. The monoclonal rabbit antibodies bind the antigen selectively, but independently from the glycosylation pattern. Even though the active antibodies of the invention are raised against insect-derived recombinant proteins, they are multi-functional in terms of antigenic glycosylation pattern and hence, they are considered as suitable for the recognition of an insect-derived recombinant antigen, but without being limited to this pattern. The antibodies of the invention are well suited to recognize the extracellular domain of a specific integrin or parts thereof of any eukaryotic glycosylation pattern. It shall be understood that the glycosylation patterns are not mixed up, but derived from a distinct eukaryotic cell or organism, respectively. In doing so, the generated antibodies are especially capable of recognizing the target structure within a complex FFPE matrix. The inventors have shown the unexpected suitability of these antibodies for integrin detection in FFPE tissue. The suitability is demonstrated in so far as the resulting antibodies are intensively specific and active on FFPE material. It is an overwhelming effect that integrin complexes in FFPE material can be easily detected by the antibody of the invention. While classical monoclonal antibodies do not work in FFPE material, the antibodies of the invention substantially bind their antigens in FFPE material and on viable cells with the same specificity; the latter is proven without limitations in live cell flow cytometry (e.g. fluorescence-activated cell sorting, briefly FACS). The antibodies of the invention can also substantially bind their antigens in FFPE material and in an isolated form in ELISA with the same specificity; the latter is proven without limitations in standard ELISAs as described in the course of the present specification and detailed in example 3.3. The staining pattern in FFPE tissue achieved herewith is of clear advantage over ambiguous results necessarily obtained from antibodies of prior art.

To date, the compositions of at least 24 integrin complexes have been described. Integrins are a family of cell adhesion molecules composed of two non-covalently associated chains. Both subunits, alpha (α) and beta (ß), traverse the membrane and integrate extracellular matrix with intracellular compartment, to deliver those extracellular signals which control cell adhesion, proliferation, migration and invasion. Based on the respective composition, the extracellular and intracellular integrin domains are assigned and known and they can be prepared by conventional processes. Either a domain of natural origin is isolated from a biological sample or the domain is recombinantly expressed and purified thereafter. Particularly, the sample is taken in-vivo from a mammal to be analyzed for integrin distribution pattern. The withdrawal of the sample shall follow good medical practice. Biological samples may be taken from any kind of biological species having an integrin of interest, but the sample is especially taken from a laboratory animal or a human, more preferably a rat, mouse, rabbit or human. The downstream processing of integrin is conducted by any process known in the art and followed by domain splitting and separating the extracellular or intracellular domain. Cell lysis can be performed in suitable, well-known lysis buffers, which may cause an osmotic shock and perforate the cell membrane. The stability of the cell structure can also be destroyed by mechanical forces, such as ball mill, French press, ultrasonic, etc., by enzymatic degradation of cell wall and cell membrane, respectively, and/or by the action of tensides. The integrins may be further purified to remove disturbing substances, or the integrins can be concentrated in the sample. Downstream-processing and/or concentrating are preferably performed by the method of precipitation, dialysis, gel filtration, gel elution or chromatography, such as HPLC or ion exchange chromatography. It is recommended to combine several methods for better yields.

Preferably, the extracellular integrin domain is recombinantly expressed and purified. The DNA encoding the protein sequence can be obtained, amplified, optionally altered or synthesized with techniques known to the skilled artisan. The DNA can be introduced into a vector and transcribed and translated in cells. The domain can be fused with a tag for affinity chromatography, such as Strep-tag, His-tag, GST-tag, Arg-tag or the calmodulin binding protein, or purified using established antibody-affinity purification techniques. A column is loaded with the protein suspension and all components lacking the tag are immediately eluted. After removal of unspecific binders by washing steps, the tag-fused construct is removed from the column. If the tag affects the induction of antibodies, it is cleaved off before immunization.

Several expression systems are state of the art. Interestingly, the titer against the protein elements of the immunogen can be beneficially increased if insect-derived recombinant integrin domains are applied. Insect-derived, recombinant mammalian glycoproteins are incompletely glycosylated, and lack terminal sugar processing and extension, which means that the protein epitopes are highly exposed in comparison to non-recombinant proteins or recombinant proteins of conventional eukaryotic expression. It is preferred, therefore, that the immunogenic integrin domain has an insect-derived glycosylation pattern, preferably the extracellular domain. Moreover, the antigenic properties to elicit or rather increase an immune response can be affected when attaching the antigen to a large carrier, such as a protein or polysaccharide; the carrier may be one which does not elicit an immune response by itself.

It is a preferred embodiment that the integrin domain has a human primary structure, i.e. the amino acid sequence aligns with an human entry in matching databases, such as the accession number of the sequence database Swiss-Prot. The skilled artisan knows such databases of molecular biology in order to extract sequences to be applied herein. In a more preferred embodiment of the present invention, the extracellular integrin domain has a human primary structure and an insect glycosylation pattern.

The inventive antibody denotes a polypeptide encoded by an immunoglobulin gene, or fragments thereof. The antibody comprises at least one light chain and/or at least one heavy chain, preferably at least one light chain and at least one heavy chain, more preferably two light chains and two heavy chains, each of them as defined hereunder. That means, the light chain comprises at least a single CDR, particularly of rabbit origin, in the variable region of said light (V_L) chain and optionally at least a single FR in the variable region of said light (V_L) chain, preferably at least said CDR and at least said FR. The heavy chain comprises at least a single CDR, particularly of rabbit origin, in the variable region of said heavy (V_H) chain and/or at least a single FR in the variable region of said heavy (V_H) chain, preferably at least said CDR and at least said FR. Within the antigen-binding portion of an antibody, the CDRs directly interact with the epitope of the antigen while the FRs maintain the tertiary structure of the paratope. In both the light chain and the heavy chain of immunoglobulins, there are three to four framework regions (FR-1 through FR-4) separated respectively by three complementarity determining regions (CDR-1 through CDR-3). The CDRs or hyper-variable regions, in particular the CDR-3 regions, more particularly the heavy chain CDR-3, are largely responsible for antibody affinity and specificity.

In another preferred embodiment of the invention, the light chain variable region (V_L) comprises two CDRs, more preferably three CDRs, most preferably together with the same number of FRs or even one FR more. In still another preferred embodiment of the invention, the heavy chain variable region (V_H) comprises two CDRs, more preferably three CDRs, most preferably together with the same number of FRs or even one FR more. In another more preferred embodiment, the antibody of the invention comprises the light chain variable region (V_L) and the heavy chain variable region (V_H), each of the regions comprises two CDRs, most preferably three CDRs, highly preferably together with the same number of FRs or even one FR more.

In other words, the antibody of the invention shall comprise at least that minimum scaffold from a variable region of a single chain, which confers binding capacity to any integrin domain or the extracellular domain in particular, respectively. According to the invention, the antibody can also be present as a number of other well-characterized fragments of an immunoglobulin or even as an intact immunoglobulin provided that the aforementioned minimum scaffold is given. Fragments are preferably selected from the group comprising heavy chain (H), light chain (L), variable regions (V), single chain variable fragment (scFv), F_{ab} fragments consisting of a covalently bound antibody light chain and a portion of the antibody heavy chain (F_d), and the like.

The light chain of the antibody can additionally comprise a constant region of the light (C_L) chain. Similarly, the heavy chain of the antibody can additionally comprise a constant region of the heavy (C_H) chain, or a portion thereof, wherein the portion especially refers to the constant region within the F_d region. The F_d fragment is the major determinant of antibody specificity and retain epitope-binding ability in isolation. The antibody of the invention can also be completed by F_c fragment as effector of the complement cascade, which is not involved in antigen binding. Fragments, such as F_{ab} and F_c fragments, can be produced by cleavage using various peptidases. Furthermore, fragments can be engineered and recombinantly expressed, preferably scFv.

In the scope of the invention, the antibody can be of polyclonal or monoclonal origin. Polyclonal antibodies are usually produced in mammal organisms when an immune response is caused by antigens being strange to the organism and having a molecular weight that exceeds 3.000 g/mol. Preferably, the antibodies of the invention are monoclonals. The great advantages of monoclonal antibodies include an immortal source of reagents, stable antibody properties and precise specificity. Popular techniques for

producing monoclonal antibodies, such as the hybridoma technology, are also well-known to the skilled artisan.

Favorable host species for polyclonal and/or monoclonal antibody production comprise rat, goat, rabbit and mouse, more preferably rabbit. The rabbit antibodies, more preferably rabbit monoclonal antibodies (RabMabs), exhibit higher affinity along with a wider range of epitope recognition than mouse monoclonals, while due to divergence in the immune systems, and extended CDRs, stronger responses to epitopes, preferably human epitopes, can be produced compared to murine responses. It shall be understood that chimeric antibodies can be genetically engineered, which CDRs, FRs and/or constant regions are derived from different mammalian sources provided that one or more CDRs have a rabbit source. Accordingly, chimeric antibodies can be obtained by replacing not only the CDR but the whole variable regions of the light and heavy chains of non-rabbit origin. The affinity of the antigen-binding sites can be alternatively influenced by selective exchange of some amino acids within the variable regions.

The basic principal for making monoclonal rabbit antibodies were as for mouse monoclonals. Following the immunization of rabbits, the spleen is taken from those rabbits producing polyclonal serum. The isolated rabbit B cells of the immunized rabbits are fused with a rabbit plasmocytoma cell line to produce stable hybridomas. The hybridoma cells are tested for secretion of antibodies, which are specific for the immunogen, and they can be subsequently cloned. The original establishment of the rabbit hybridomas fusion partner cell line is described by Spieker-Polet et al., PNAS USA 1995, 92(20): 9348-9352. Further developments of the fusion partner cell line are disclosed in US 7,429,487 B2. Still further methods are published in the US Appl. Nos. 10/705,109; 10/266,387; 10/313,881; 10/350,841 and 11/476,277. The cDNA of inserts encoding the antibody is preferably cloned, sequenced and inserted in an expression vector to allow production of wholly defined antibodies. The skilled artisan knows suitable techniques for the recombinant production of antibodies, such as in the EBNA cell expression system according to Pham et al., Biotech Bioeng 2003, 84(3): 332-342. Said publications are incorporated by reference as a whole in the disclosure of the invention.

The antibody or a fragment thereof is particularly directed against the extracellular domain of integrin αvß3, αvß5, αvß6 or αvß8.

In a preferred special embodiment of the present invention, the antibody or a fragment thereof is directed against the extracellular domain of the integrin αvß3. Suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 81 (CDR-1-V_L-αvß3), SEQ ID NO: 82 (CDR-2-V_L-αvß3) and/or SEQ ID NO: 83 (CDR-3-V_L-αvß3), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 84 (CDR-1-V_H-αvß3), SEQ ID NO: 85 (CDR-2-V_H-αvß3) and/or SEQ ID NO: 86 (CDR-3-V_H-αvß3). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 81 (CDR-1-V_L-αvß3), SEQ ID NO: 82 (CDR-2-V_L-αvß3) and SEQ ID NO: 83 (CDR-3-V_L-αvß3), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 84 (CDR-1-V_H-αvß3), SEQ ID NO: 85 (CDR-2-V_H-αvß3) and SEQ ID NO: 86 (CDR-3-V_H-αvß3). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 81 (CDR-1-V_L-αvß3), SEQ ID NO: 82 (CDR-2-V_L-αvß3) and SEQ ID NO: 83 (CDR-3-V_L-αvß3), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 84 (CDR-1-V_H-αvß3), SEQ ID NO: 85 (CDR-2-V_H-αvß3) and SEQ ID NO: 86 (CDR-3-V_H-αvß3).

Yet referring to the context of the anti-αvß3 antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 87 (FR-1-V_L-αvß3), SEQ ID NO: 88 (FR-2-V_L-αvß3) and/or SEQ ID NO: 89 (FR-3-V_L-αvß3), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 91 (FR-1-V_H-αvß3), SEQ ID NO: 92 (FR-2-V_H-αvß3), SEQ ID NO: 93 (FR-3-V_H-αvß3) and/or SEQ ID NO: 94 (FR-4-V_H-αvß3). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 87 (FR-1-V_L-αvß3), SEQ ID NO: 88 (FR-2-V_L-αvß3) and SEQ ID NO: 89 (FR-3-V_L-αvß3), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 91 (FR-1-V_H-αvß3), SEQ ID NO: 92 (FR-2-V_H-αvß3), SEQ ID NO: 93 (FR-3-V_H-αvß3) and SEQ ID NO: 94 (FR-4-V_H-αvß3). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 87 (FR-1-V_L-αvß3), SEQ ID NO: 88 (FR-2-V_L-αvß3) and SEQ ID NO: 89 (FR-3-V_L-αvß3), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 91 (FR-1-V_H-αvß3), SEQ ID NO: 92 (FR-2-V_H-αvß3), SEQ ID NO: 93 (FR-3-V_H-αvß3) and SEQ ID NO: 94 (FR-4-V_H-αvß3).

It is another combinatorial embodiment in the anti-αvß3 antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 81 (CDR-1-V_L-αvß3), SEQ ID NO: 82 (CDR-2-V_L-αvß3) and/or SEQ ID NO: 83 (CDR-3-V_L-αvß3), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 87 (FR-1-V_L-αvß3), SEQ ID NO: 88 (FR-2-V_L-αvß3) and/or SEQ ID NO: 89 (FR-3-V_L-αvß3). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 81 (CDR-1-V_L-αvß3), SEQ ID NO: 82 (CDR-2-V_L-

αvß3) and SEQ ID NO: 83 (CDR-3-V_L-αvß3), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 87 (FR-1-V_L-αvß3), SEQ ID NO: 88 (FR-2-V_L-αvß3) and SEQ ID NO: 89 (FR-3-V_L-αvß3).

It is still another combinatorial embodiment in the anti-αvß3 antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 84 (CDR-1-V_H-αvß3), SEQ ID NO: 85 (CDR-2-V_H-αvß3) and/or SEQ ID NO: 86 (CDR-3-V_H-αvß3), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 91 (FR-1-V_H-αvß3), SEQ ID NO: 92 (FR-2-V_H-αvß3), SEQ ID NO: 93 (FR-3-V_H-αvß3) and/or SEQ ID NO: 94 (FR-4-V_H-αvß3). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 84 (CDR-1-V_H-αvß3), SEQ ID NO: 85 (CDR-2-V_H-αvß3) and SEQ ID NO: 86 (CDR-3-V_H-αvß3), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 91 (FR-1-V_H-αvß3), SEQ ID NO: 92 (FR-2-V_H-αvß3), SEQ ID NO: 93 (FR-3-V_H-αvß3) and SEQ ID NO: 94 (FR-4-V_H-αvß3).

In another preferred embodiment in the anti-αvß3 antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 95 (V_L-αvß3) and/or V_H comprises an amino acid sequence of SEQ ID NO: 96 (V_H-αvß3), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 95 (V_L-αvß3) and/or V_H consists of an amino acid sequence of SEQ ID NO: 96 (V_H-αvß3), most preferably the antibody is shaped as anti-αvß3 scFv.

The anti-αvß3 antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 97 (C_L-αvß3) and/or C_H comprises an amino acid sequence of SEQ ID NO: 98 (C_H-αvß3).

Accordingly, the anti-αvß3 antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 99 (L-αvß3) and/or the heavy chain comprises an amino acid sequence of SEQ ID NO: 100 (H-αvß3). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 99 (L-αvß3) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 100 (H-αvß3). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 99 (L-αvß3) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 100 (H-αvß3).

In another preferred special embodiment of the present invention, the antibody or a fragment thereof is directed against the extracellular domain of the integrin αvß5. Suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 1 (CDR-1-V_L-αvß5), SEQ ID NO: 2 (CDR-2-V_L-αvß5) and/or SEQ ID NO: 3 (CDR-3-V_L-αvß5), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 4 (CDR-1-V_H-αvß5), SEQ ID NO: 5 (CDR-2-V_H-αvß5) and/or SEQ ID NO: 6 (CDR-3-V_H-αvß5). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 1 (CDR-1-V_L-αvß5), SEQ ID NO: 2 (CDR-2-V_L-αvß5) and SEQ ID NO: 3 (CDR-3-V_L-αvß5), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 4 (CDR-1-V_H-αvß5), SEQ ID NO: 5 (CDR-2-V_H-αvß5) and SEQ ID NO: 6 (CDR-3-V_H-αvß5). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 1 (CDR-1-V_L-αvß5), SEQ ID NO: 2 (CDR-2-V_L-αvß5) and SEQ ID NO: 3 (CDR-3-V_L-αvß5), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 4 (CDR-1-V_H-αvß5), SEQ ID NO: 5 (CDR-2-V_H-αvß5) and SEQ ID NO: 6 (CDR-3-V_H-αvß5).

Yet referring to the context of the anti-αvß5 antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 7 (FR-1-V_L-αvß5), SEQ ID NO: 8 (FR-2-V_L-αvß5) and/or SEQ ID NO: 9 (FR-3-V_L-αvß5), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 11 (FR-1-V_H-αvß5), SEQ ID NO: 12 (FR-2-V_H-αvß5), SEQ ID NO: 13 (FR-3-V_H-αvß5) and/or SEQ ID NO: 14 (FR-4-V_H-αvß5). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 7 (FR-1-V_L-αvß5), SEQ ID NO: 8 (FR-2-V_L-αvß5) and SEQ ID NO: 9 (FR-3-V_L-αvß5), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 11 (FR-1-V_H-αvß5), SEQ ID NO: 12 (FR-2-V_H-αvß5), SEQ ID NO: 13 (FR-3-V_H-αvß5) and SEQ ID NO: 14 (FR-4-V_H-αvß5). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 7 (FR-1-V_L-αvß5), SEQ ID NO: 8 (FR-2-V_L-αvß5) and SEQ ID NO: 9 (FR-3-V_L-αvß5), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 11 (FR-1-V_H-αvß5), SEQ ID NO: 12 (FR-2-V_H-αvß5), SEQ ID NO: 13 (FR-3-V_H-αvß5) and SEQ ID NO: 14 (FR-4-V_H-αvß5).

It is another combinatorial embodiment in the anti-αvß5 antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 1 (CDR-1-V_L-αvß5), SEQ ID NO: 2 (CDR-2-V_L-αvß5) and/or SEQ ID NO: 3 (CDR-3-V_L-αvß5), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 7 (FR-1-V_L-αvß5), SEQ ID NO: 8 (FR-2-V_L-αvß5) and/or SEQ ID NO: 9 (FR-3-V_L-αvß5). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 1 (CDR-1-V_L-αvß5), SEQ ID NO: 2 (CDR-2-V_L-αvß5) and

SEQ ID NO: 3 (CDR-3-V_L-αvß5), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 7 (FR-1-V_L-αvß5), SEQ ID NO: 8 (FR-2-V_L-αvß5) and SEQ ID NO: 9 (FR-3-V_L-αvß5).

It is still another combinatorial embodiment in the anti-αvß5 antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 4 (CDR-1-V_H-αvß5), SEQ ID NO: 5 (CDR-2-V_H-αvß5) and/or SEQ ID NO: 6 (CDR-3-V_H-αvß5), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 11 (FR-1-V_H-αvß5), SEQ ID NO: 12 (FR-2-V_H-αvß5), SEQ ID NO: 13 (FR-3-V_H-αvß5) and/or SEQ ID NO: 14 (FR-4-V_H-αvß5). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 4 (CDR-1-V_H-αvß5), SEQ ID NO: 5 (CDR-2-V_H-αvß5) and SEQ ID NO: 6 (CDR-3-V_H-αvß5), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 11 (FR-1-V_H-αvß5), SEQ ID NO: 12 (FR-2-V_H-αvß5), SEQ ID NO: 13 (FR-3-V_H-αvß5) and SEQ ID NO: 14 (FR-4-V_H-αvß5).

In another preferred embodiment in the anti-αvß5 antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 15 (V_L-αvß5) and/or V_H comprises an amino acid sequence of SEQ ID NO: 16 (V_H-αvß5), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 15 (V_L-αvß5) and/or V_H consists of an amino acid sequence of SEQ ID NO: 16 (V_H-αvß5), most preferably the antibody is shaped as anti-αvß5 scFv.

The anti-αvß5 antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 17 (C_L-αvß5) and/or C_H comprises an amino acid sequence of SEQ ID NO: 18 (C_H-αvß5).

Accordingly, the anti-αvß5 antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 19 (L-αvß5) and/or the heavy chain comprises an amino acid sequence of SEQ ID NO: 20 (H-αvß5). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 19 (L-αvß5) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 20 (H-αvß5). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 19 (L-αvß5) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 20 (H-αvß5).

In still another preferred special embodiment of the present invention, the antibody or a fragment thereof is directed against the extracellular domain of the integrin αvß6. Suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 121 (CDR-1-V_L-αvß6), SEQ ID NO: 122 (CDR-2-V_L-αvß6) and/or SEQ ID NO: 123 (CDR-3-V_L-αvß6), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 124 (CDR-1-V_H-αvß6), SEQ ID NO: 125 (CDR-2-V_H-αvß6) and/or SEQ ID NO: 126 (CDR-3-V_H-αvß6). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 121 (CDR-1-V_L-αvß6), SEQ ID NO: 122 (CDR-2-V_L-αvß6) and SEQ ID NO: 123 (CDR-3-V_L-αvß6), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 124 (CDR-1-V_H-αvß6), SEQ ID NO: 125 (CDR-2-V_H-αvß6) and SEQ ID NO: 126 (CDR-3-V_H-αvß6). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 121 (CDR-1-V_L-αvß6), SEQ ID NO: 122 (CDR-2-V_L-αvß6) and SEQ ID NO: 123 (CDR-3-V_L-αvß6), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 124 (CDR-1-V_H-αvß6), SEQ ID NO: 125 (CDR-2-V_H-αvß6) and SEQ ID NO: 126 (CDR-3-V_H-αvß6).

Yet referring to the context of the anti-αvß6 antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 127 (FR-1-V_L-αvß6), SEQ ID NO: 128 (FR-2-V_L-αvß6) and/or SEQ ID NO: 129 (FR-3-V_L-αvß6), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 131 (FR-1-V_H-αvß6), SEQ ID NO: 132 (FR-2-V_H-αvß6), SEQ ID NO: 133 (FR-3-V_H-αvß6) and/or SEQ ID NO: 134 (FR-4-V_H-αvß6). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 127 (FR-1-V_L-αvß6), SEQ ID NO: 128 (FR-2-V_L-αvß6) and SEQ ID NO: 129 (FR-3-V_L-αvß6), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 131 (FR-1-V_H-αvß6), SEQ ID NO: 132 (FR-2-V_H-αvß6), SEQ ID NO: 133 (FR-3-V_H-αvß6) and SEQ ID NO: 134 (FR-4-V_H-αvß6). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 127 (FR-1-V_L-αvß6), SEQ ID NO: 128 (FR-2-V_L-αvß6) and SEQ ID NO: 129 (FR-3-V_L-αvß6), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 131 (FR-1-V_H-αvß6), SEQ ID NO: 132 (FR-2-V_H-αvß6), SEQ ID NO: 133 (FR-3-V_H-αvß6) and SEQ ID NO: 134 (FR-4-V_H-αvß6).

It is another combinatorial embodiment in the anti-αvß6 antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 121 (CDR-1-V_L-αvß6), SEQ ID NO: 122 (CDR-2-V_L-αvß6) and/or SEQ ID NO: 123 (CDR-3-V_L-αvß6), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 127 (FR-1-V_L-αvß6), SEQ ID NO: 128 (FR-2-V_L-αvß6) and/or SEQ ID NO: 129 (FR-3-V_L-αvß6). Preferably, the CDRs in V_L

comprise amino acid sequences of SEQ ID NO: 121 (CDR-1-V_L-αvß6), SEQ ID NO: 122 (CDR-2-V_L-αvß6) and SEQ ID NO: 123 (CDR-3-V_L-αvß6), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 127 (FR-1-V_L-αvß6), SEQ ID NO: 128 (FR-2-V_L-αvß6) and SEQ ID NO: 129 (FR-3-V_L-αvß6).

It is still another combinatorial embodiment in the anti-αvß6 antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 124 (CDR-1-V_H-αvß6), SEQ ID NO: 125 (CDR-2-V_H-αvß6) and/or SEQ ID NO: 126 (CDR-3-V_H-αvß6), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 131 (FR-1-V_H-αvß6), SEQ ID NO: 132 (FR-2-V_H-αvß6), SEQ ID NO: 133 (FR-3-V_H-αvß6) and/or SEQ ID NO: 134 (FR-4-V_H-αvß6). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 124 (CDR-1-V_H-αvß6), SEQ ID NO: 125 (CDR-2-V_H-αvß6) and SEQ ID NO: 126 (CDR-3-V_H-αvß6), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 131 (FR-1-V_H-αvß6), SEQ ID NO: 132 (FR-2-V_H-αvß6), SEQ ID NO: 133 (FR-3-V_H-αvß6) and SEQ ID NO: 134 (FR-4-V_H-αvß6).

In another preferred embodiment in the anti-αvß6 antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 135 (V_L-αvß6) and/or V_H comprises an amino acid sequence of SEQ ID NO: 136 (V_H-αvß6), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 135 (V_L-αvß6) and/or V_H consists of an amino acid sequence of SEQ ID NO: 136 (V_H-αvß6), most preferably the antibody is shaped as anti-αvß6 scFv.

The anti-αvß6 antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 137 (C_L-αvß6) and/or C_H comprises an amino acid sequence of SEQ ID NO: 138 (C_H-αvß6).

Accordingly, the anti-αvß6 antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 139 (L-αvß6) and/or the heavy chain comprises an amino acid sequence of SEQ ID NO: 140 (H-αvß6). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 139 (L-αvß6) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 140 (H-αvß6). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 139 (L-αvß6) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 140 (H-αvß6).

In still another preferred special embodiment of the present invention, the antibody or a fragment thereof is directed against the extracellular domain of the integrin αvß8. Suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 161 (CDR-1-V_L-αvß8), SEQ ID NO: 162 (CDR-2-V_L-αvß8) and/or SEQ ID NO: 163 (CDR-3-V_L-αvß8), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 164 (CDR-1-V_H-αvß8), SEQ ID NO: 165 (CDR-2-V_H-αvß8) and/or SEQ ID NO: 166 (CDR-3-V_H-αvß8). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 161 (CDR-1-V_L-αvß8), SEQ ID NO: 162 (CDR-2-V_L-αvß8) and SEQ ID NO: 163 (CDR-3-V_L-αvß8), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 164 (CDR-1-V_H-αvß8), SEQ ID NO: 165 (CDR-2-V_H-αvß8) and SEQ ID NO: 166 (CDR-3-V_H-αvß8). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 161 (CDR-1-V_L-αvß8), SEQ ID NO: 162 (CDR-2-V_L-αvß8) and SEQ ID NO: 163 (CDR-3-V_L-αvß8), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 164 (CDR-1-V_H-αvß8), SEQ ID NO: 165 (CDR-2-V_H-αvß8) and SEQ ID NO: 166 (CDR-3-V_H-αvß8).

Yet referring to the context of the anti-αvß8 antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 167 (FR-1-V_L-αvß8), SEQ ID NO: 168 (FR-2-V_L-αvß8) and/or SEQ ID NO: 169 (FR-3-V_L-αvß8), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 171 (FR-1-V_H-αvß8), SEQ ID NO: 172 (FR-2-V_H-αvß8), SEQ ID NO: 173 (FR-3-V_H-αvß8) and/or SEQ ID NO: 174 (FR-4-V_H-αvß8). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 167 (FR-1-V_L-αvß8), SEQ ID NO: 168 (FR-2-V_L-αvß8) and SEQ ID NO: 169 (FR-3-V_L-αvß8), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 171 (FR-1-V_H-αvß8), SEQ ID NO: 172 (FR-2-V_H-αvß8), SEQ ID NO: 173 (FR-3-V_H-αvß8) and SEQ ID NO: 174 (FR-4-V_H-αvß8). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 167 (FR-1-V_L-αvß8), SEQ ID NO: 168 (FR-2-V_L-αvß8) and SEQ ID NO: 169 (FR-3-V_L-αvß8), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 171 (FR-1-V_H-αvß8), SEQ ID NO: 172 (FR-2-V_H-αvß8), SEQ ID NO: 173 (FR-3-V_H-αvß8) and SEQ ID NO: 174 (FR-4-V_H-αvß8).

It is another combinatorial embodiment in the anti-αvß8 antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 161 (CDR-1-V_L-αvß8), SEQ ID NO: 162 (CDR-2-V_L-αvß8) and/or SEQ ID NO: 163 (CDR-3-V_L-αvß8), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 167 (FR-1-V_L-αvß8), SEQ ID NO: 168 (FR-2-V_L-αvß8) and/or SEQ ID NO: 169 (FR-3-V_L-αvß8). Preferably, the CDRs in V_L

comprise amino acid sequences of SEQ ID NO: 161 (CDR-1-V_L-αvß8), SEQ ID NO: 162 (CDR-2-V_L-αvß8) and SEQ ID NO: 163 (CDR-3-V_L-αvß8), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 167 (FR-1-V_L-αvß8), SEQ ID NO: 168 (FR-2-V_L-αvß8) and SEQ ID NO: 169 (FR-3-V_L-αvß8).

It is still another combinatorial embodiment in the anti-αvß8 antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 164 (CDR-1-V_H-αvß8), SEQ ID NO: 165 (CDR-2-V_H-αvß8) and/or SEQ ID NO: 166 (CDR-3-V_H-αvß8), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 171 (FR-1-V_H-αvß8), SEQ ID NO: 172 (FR-2-V_H-αvß8), SEQ ID NO: 173 (FR-3-V_H-αvß8) and/or SEQ ID NO: 174 (FR-4-V_H-αvß8). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 164 (CDR-1-V_H-αvß8), SEQ ID NO: 165 (CDR-2-V_H-αvß8) and SEQ ID NO: 166 (CDR-3-V_H-αvß8), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 171 (FR-1-V_H-αvß8), SEQ ID NO: 172 (FR-2-V_H-αvß8), SEQ ID NO: 173 (FR-3-V_H-αvß8) and SEQ ID NO: 174 (FR-4-V_H-αvß8).

In another preferred embodiment in the anti-αvß8 antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 175 (V_L-αvß8) and/or V_H comprises an amino acid sequence of SEQ ID NO: 176 (V_H-αvß8), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 175 (V_L-αvß8) and/or V_H consists of an amino acid sequence of SEQ ID NO: 176 (V_H-αvß8), most preferably the antibody is shaped as anti-αvß8 scFv.

The anti-αvß8 antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 177 (C_L-αvß8) and/or C_H comprises an amino acid sequence of SEQ ID NO: 178 (C_H-αvß8).

Accordingly, the anti-αvß8 antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 179 (L-αvß8) and/or the heavy chain comprises an amino acid sequence of SEQ ID NO: 180 (H-αvß8). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 179 (L-αvß8) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 180 (H-αvß8). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 179 (L-αvß8) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 180 (H-αvß8).

In still another preferred special embodiment of the present invention, the antibody or a fragment thereof is directed against the extracellular domain of the integrin αv. Suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 201 (CDR-1-V_L-αv), SEQ ID NO: 202 (CDR-2-V_L-αv) and/or SEQ ID NO: 203 (CDR-3-V_L-αv), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 204 (CDR-1-V_H-αv), SEQ ID NO: 205 (CDR-2-V_H-αv) and/or SEQ ID NO: 206 (CDR-3-V_H-αv). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 201 (CDR-1-V_L-αv), SEQ ID NO: 202 (CDR-2-V_L-αv) and SEQ ID NO: 203 (CDR-3-V_L-αv), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 204 (CDR-1-V_H-αv), SEQ ID NO: 205 (CDR-2-V_H-αv) and SEQ ID NO: 206 (CDR-3-V_H-αv). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 201 (CDR-1-V_L-αv), SEQ ID NO: 202 (CDR-2-V_L-αv) and SEQ ID NO: 203 (CDR-3-V_L-αv), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 204 (CDR-1-V_H-αv), SEQ ID NO: 205 (CDR-2-V_H-αv) and SEQ ID NO: 206 (CDR-3-V_H-αv).

Yet referring to the context of the anti-αv antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 207 (FR-1-V_L-αv), SEQ ID NO: 208 (FR-2-V_L-αv) and/or SEQ ID NO: 209 (FR-3-V_L-αv), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 211 (FR-1-V_H-αv), SEQ ID NO: 212 (FR-2-V_H-αv), SEQ ID NO: 213 (FR-3-V_H-αv) and/or SEQ ID NO: 214 (FR-4-V_H-αv). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 207 (FR-1-V_L-αv), SEQ ID NO: 208 (FR-2-V_L-αv) and SEQ ID NO: 209 (FR-3-V_L-αv), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 211 (FR-1-V_H-αv), SEQ ID NO: 212 (FR-2-V_H-αv), SEQ ID NO: 213 (FR-3-V_H-αv) and SEQ ID NO: 214 (FR-4-V_H-αv). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 207 (FR-1-V_L-αv), SEQ ID NO: 208 (FR-2-V_L-αv) and SEQ ID NO: 209 (FR-3-V_L-αv), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 211 (FR-1-V_H-αv), SEQ ID NO: 212 (FR-2-V_H-αv), SEQ ID NO: 213 (FR-3-V_H-αv) and SEQ ID NO: 214 (FR-4-V_H-αv).

It is another combinatorial embodiment in the anti-αv antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 201 (CDR-1-V_L-αv), SEQ ID NO: 202 (CDR-2-V_L-αv) and/or SEQ ID NO: 203 (CDR-3-V_L-αv), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 207 (FR-1-V_L-αv), SEQ ID NO: 208 (FR-2-V_L-αv) and/or SEQ ID NO: 209 (FR-3-V_L-αv). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 201 (CDR-1-V_L-αv), SEQ ID NO: 202 (CDR-2-V_L-αv) and

SEQ ID NO: 203 (CDR-3-V_L-αv), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 207 (FR-1-V_L-αv), SEQ ID NO: 208 (FR-2-V_L-αv) and SEQ ID NO: 209 (FR-3-V_L-αv).

It is still another combinatorial embodiment in the anti-αv antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 204 (CDR-1-V_H-αv), SEQ ID NO: 205 (CDR-2-V_H-αv) and/or SEQ ID NO: 206 (CDR-3-V_H-αv), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 211 (FR-1-V_H-αv), SEQ ID NO: 212 (FR-2-V_H-αv), SEQ ID NO: 213 (FR-3-V_H-αv) and/or SEQ ID NO: 214 (FR-4-V_H-αv). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 204 (CDR-1-V_H-αv), SEQ ID NO: 205 (CDR-2-V_H-αv) and SEQ ID NO: 206 (CDR-3-V_H-αv), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 211 (FR-1-V_H-αv), SEQ ID NO: 212 (FR-2-V_H-αv), SEQ ID NO: 213 (FR-3-V_H-αv) and SEQ ID NO: 214 (FR-4-V_H-αv).

In another preferred embodiment in the anti-αv antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 215 (V_L-αv) and/or V_H comprises an amino acid sequence of SEQ ID NO: 216 (V_H-αv), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 215 (V_L-αv) and/or V_H consists of an amino acid sequence of SEQ ID NO: 216 (V_H-αv), most preferably the antibody is shaped as anti-αv scFv.

The anti-αv antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 217 (C_L-αv) and/or C_H comprises an amino acid sequence of SEQ ID NO: 218 (C_H-αv).

Accordingly, the anti-αv antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 219 (L-αv) and/or the heavy chain comprises an amino acid sequence of SEQ ID NO: 220 (H-αv). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 219 (L-αv) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 220 (H-αv). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 219 (L-αv) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 220 (H-αv).

In another embodiment of the present invention, integrin cytoplasmic domains are used as primary immunogen. Said cytoplasmic domains are also referred to as intracellular domains. They are especially expressed as N-terminal fusion proteins. The fusion partner can be varied (e.g. GST, MBP, KLH, etc.) to allow the differential screening described below, or the primary and secondary screens can be excluded, going straight to the tertiary screen on cell line arrays. The conformation of the cytoplasmic domains is less defined than that of the extracellular domains, and it is relatively independent of the paired chain, i.e. an antibody directed against ß3, e.g., will recognize ß3 associated both with αvß3 and with αiibß3. This is effectively a reduction in specificity over antibodies directed against the DTM-αvß3 complex, which can be screened to obtain antibodies that recognize ß3 only when it is in association with αv. Similar considerations apply for antibodies generated against αvß5. The advantage is, however, that the integrin cytoplasmic domains are entirely conserved across mammalian and hence, broad species cross reactivity can be made.

In particular, the antibody or a fragment thereof is directed against the cytoplasmic domain of the integrin ß3 chain. It is an special embodiment of such an anti-ß3 antibody, that suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 41 (CDR-1-V_L-ß3), SEQ ID NO: 42 (CDR-2-V_L-ß3) and/or SEQ ID NO: 43 (CDR-3-V_L-ß3), and/or suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 44 (CDR-1-V_H-ß3), SEQ ID NO: 45 (CDR-2-V_H-ß3) and/or SEQ ID NO: 46 (CDR-3-V_H-ß3). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 41 (CDR-1-V_L-ß3), SEQ ID NO: 42 (CDR-2-V_L-ß3) and SEQ ID NO: 43 (CDR-3-V_L-ß3), and/or the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 44 (CDR-1-V_H-ß3), SEQ ID NO: 45 (CDR-2-V_H-ß3) and SEQ ID NO: 46 (CDR-3-V_H-ß3). More preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 41 (CDR-1-V_L-ß3), SEQ ID NO: 42 (CDR-2-V_L-ß3) and SEQ ID NO: 43 (CDR-3-V_L-ß3), and the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 44 (CDR-1-V_H-ß3), SEQ ID NO: 45 (CDR-2-V_H-ß3) and SEQ ID NO: 46 (CDR-3-V_H-ß3).

Yet referring to the context of the anti-ß3 antibody, suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 47 (FR-1-V_L-ß3), SEQ ID NO: 48 (FR-2-V_L-ß3) and/or SEQ ID NO: 49 (FR-3-V_L-ß3), and/or suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 51 (FR-1-V_H-ß3), SEQ ID NO: 52 (FR-2-V_H-ß3), SEQ ID NO: 53 (FR-3-V_H-ß3) and/or SEQ ID NO: 54 (FR-4-V_H-ß3). Preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 47 (FR-1-V_L-ß3), SEQ ID NO: 48 (FR-2-V_L-ß3) and SEQ ID NO: 49 (FR-3-V_L-ß3), and/or the FRs in V_H comprise amino acid sequence of SEQ ID NO: 51 (FR-1-V_H-ß3),

SEQ ID NO: 52 (FR-2-V_H-ß3), SEQ ID NO: 53 (FR-3-V_H-ß3) and SEQ ID NO: 54 (FR-4-V_H-ß3). More preferably, the FRs in V_L comprise amino acid sequences of SEQ ID NO: 47 (FR-1-V_L-ß3), SEQ ID NO: 48 (FR-2-V_L-ß3) and SEQ ID NO: 49 (FR-3-V_L-ß3), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 51 (FR-1-V_H-ß3), SEQ ID NO: 52 (FR-2-V_H-ß3), SEQ ID NO: 53 (FR-3-V_H-ß3) and SEQ ID NO: 54 (FR-4-V_H-ß3).

It is another combinatorial embodiment in the anti-ß3 antibody context, in which suitable CDRs in V_L comprise amino acid sequences of SEQ ID NO: 41 (CDR-1-V_L-ß3), SEQ ID NO: 42 (CDR-2-V_L-ß3) and/or SEQ ID NO: 43 (CDR-3-V_L-ß3), and suitable FRs in V_L comprise amino acid sequences of SEQ ID NO: 47 (FR-1-V_L-ß3), SEQ ID NO: 48 (FR-2-V_L-ß3) and/or SEQ ID NO: 49 (FR-3-V_L-ß3). Preferably, the CDRs in V_L comprise amino acid sequences of SEQ ID NO: 41 (CDR-1-V_L-ß3), SEQ ID NO: 42 (CDR-2-V_L-ß3) and SEQ ID NO: 43 (CDR-3-V_L-ß3), and the FRs in V_L comprise amino acid sequences of SEQ ID NO: 47 (FR-1-V_L-ß3), SEQ ID NO: 48 (FR-2-V_L-ß3) and SEQ ID NO: 49 (FR-3-V_L-ß3).

It is still another combinatorial embodiment in the anti-ß3 antibody context, in which suitable CDRs in V_H comprise amino acid sequences of SEQ ID NO: 44 (CDR-1-V_H-ß3), SEQ ID NO: 45 (CDR-2-V_H-ß3) and/or SEQ ID NO: 46 (CDR-3-V_H-ß3), and suitable FRs in V_H comprise amino acid sequence of SEQ ID NO: 51 (FR-1-V_H-ß3), SEQ ID NO: 52 (FR-2-V_H-ß3), SEQ ID NO: 53 (FR-3-V_H-ß3) and/or SEQ ID NO: 54 (FR-4-V_H-ß3). Preferably, the CDRs in V_H comprise amino acid sequences of SEQ ID NO: 44 (CDR-1-V_H-ß3), SEQ ID NO: 45 (CDR-2-V_H-ß3) and SEQ ID NO: 46 (CDR-3-V_H-ß3), and the FRs in V_H comprise amino acid sequence of SEQ ID NO: 51 (FR-1-V_H-ß3), SEQ ID NO: 52 (FR-2-V_H-ß3), SEQ ID NO: 53 (FR-3-V_H-ß3) and SEQ ID NO: 54 (FR-4-V_H-ß3).

In another preferred embodiment in the anti-ß3 antibody context, V_L comprises an amino acid sequence of SEQ ID NO: 55 (V_L-ß3) and/or V_H comprises an amino acid sequence of SEQ ID NO: 56 (V_H-ß3), more preferably V_L consists of an amino acid sequence of SEQ ID NO: 55 (V_L-ß3) and/or V_H consists of an amino acid sequence of SEQ ID NO: 56 (V_H-ß3), most preferably the antibody is shaped as anti-ß3 scFv.

The anti-ß3 antibody can be completed by constant regions of the light (C_L) and/or heavy (C_H) chain. Preferably, C_L comprises an amino acid sequence of SEQ ID NO: 57 (C_L-ß3) and/or C_H comprises an amino acid sequence of SEQ ID NO: 58 (C_H-ß3).

Accordingly, the anti-ß3 antibody comprises more preferably light and/or heavy chains, wherein the light chain comprises an amino acid sequence of SEQ ID NO: 59 (L-ß3) and/or

the heavy chain comprises an amino acid sequence of SEQ ID NO: 60 (H-ß3). Most preferably, the light chain consists of an amino acid sequence of SEQ ID NO: 59 (L-ß3) and/or the heavy chain consists of an amino acid sequence of SEQ ID NO: 60 (H-ß3). In a highly preferred embodiment of the present invention, the light chain consists of an amino acid sequence of SEQ ID NO: 59 (L-ß3) and the heavy chain consists of an amino acid sequence of SEQ ID NO: 60 (H-ß3).

It shall be understood that the combinations of CDRs, FRs, V_L, V_H, C, L and/or H are not exhausted as detailed hereunder, but said components can be combined in any other manner. Each combination shall be regarded to read upon the scope of the present invention provided that the resulting antibody or fragment thereof recognizes an extracellular domain of integrin.

It shall also be understood that variants, mutants, parts of said amino acid sequences or homologous sequences having the same function are included in the scope of definition as well as protection. The degree of alteration between the original sequence and its derivatives is inevitably limited by the requirement of antigen recognition within the structural context, particularly in FFPE material. A couple of methods are known to the skilled artisan to generate equivalent peptides and proteins, i.e. amino acid sequences that are analogous in function to those of the inventive teaching by realizing the benefits of the invention to a large extent. Therefore, the invention also contains the alterations as listed herein. Variants of the amino acid sequences underlying the antibody of the invention can arise from modifications (e.g. alkylation, arylation or acetylation of at least a single amino acid), incorporation of enantiomers, addition of at least a single amino acid and/or fusion with another peptide or a protein. Possible mutations comprise deletion, insertion, substitution, translocation and/or inversion. Parts of the amino acid sequences and antibodies, respectively, relate to a restriction to those regions that are sufficient for the expression of a specific function. The parts of the antibody can be very small due to the characterization of the paratope, for instance, which also binds to an antigen as to the extracellular integrin domain. In the meaning of the invention, it is to be clearly distinguished between parts of any size and homologous sequences; the homology of the latter is related to the entire sequence. Preferably, the homology between an original sequence and its derivatives having the same features amounts to at least 80 %, more preferably at least 95 %, most preferably at least 98 %. Similarly, the homology is to be considered if the aforementioned part of any size is altered to a variant or mutant. The present teaching if solving the problem of the invention covers all peptide derivatives, which are developed on the basis of the present ingredients by such procedures.

Moreover, several techniques are described in prior art to generate non-homologous peptides with the same function. Herein, non-homologous peptides denote amino acid sequences having less homology compared to the preferred amounts of homology above. For example, it is possible to replace a single amino acid or multiple amino acids without adversely affecting the activity with respect to accomplishing the object of the present invention. For replacement of such amino acids, reference is made to appropriate standard textbooks of biochemistry and genetics. As well-known to those skilled in the art, some amino acids have analogous physicochemical properties and hence, these amino acids can be advantageously replaced by each other. These include the amino acid groups (a) glycine, alanine, valine, leucine and isoleucine, (b) serine and threonine, (c) asparagine and glutamine, (d) aspartic acid and glutamic acid, (e) lysine and arginine, and (f) phenylalanine, tyrosine and tryptophan. Amino acids within one and the same group (a) to (f) can be replaced among one another. Further alterations are possible in accordance with the teaching of Schneider et al., PNAS 1998, 95: 12179-12184; WO 1999/62933 and/or WO 2002/38592, describing one way of generating functionally analogous amino acid sequences. The references are hereby incorporated in the disclosure of the invention. All amino acid sequences, sequence parts or structures comprising sequences, which are designed by using the cited methods and starting from any amino acid sequence of the invention, are considered as sequences in the meaning of the invention, and they shall be included in the teaching according to the invention, provided they accomplish the object of the invention.

Object of the invention is also a polynucleotide encoding the antibody according to the invention, or a fragment thereof. The term “polynucleotide” refers to a natural or synthetic polymer of single or double-stranded DNA or RNA alternatively including synthetic, non-natural or modified nucleotides, which can be incorporated in DNA or RNA polymers. Each nucleotide consists of a sugar moiety, a phosphate moiety, and either a purine or pyrimidine residue. The nucleic acids can be optionally modified as phosphorothioate DNA, locked nucleic acid (LNA), peptide nucleic acid (PNA) or spiegelmer. The term "polynucleotide encoding" refers to that part of a gene which enciphers a protein, a polypeptide or a part thereof. The regulatory sequences and/or elements controlling the initiation or termination of transcription are excluded. The coding sequence and/or the regulatory element can normally be found in cells, in which case it is referred to as autologous one or endogenic one, or it cannot be located in cells, in which case it is referred to as heterologous one. The term “gene” denotes a DNA sequence encoding a specific protein and regulatory elements controlling the expression of said DNA sequence.

A heterologous gene may also be composed of autologous elements arranged in an order and/or orientation, which is normally not found in that cell, the gene is transferred into. A heterologous gene can be derived completely or partially from any source known in the art, including a bacterial or viral genome or episome, eukaryotic nuclear or plasmid DNA, cDNA, or chemically synthesized DNA. The structural gene may form a continuous coding region, or it may comprise one or more introns bordered by suitable splice junctions. The structural gene can consist of segments derived from various naturally occurring or synthetic sources.

In a preferred embodiment of the present invention, the polynucleotide encoding the antibodies of the invention comprises one or more nucleic acid sequences selected from the group of SEQ ID NOs: 21 to 29 and 31 to 40, SEQ ID NOs: 61 to 69 and 71 to 80, SEQ ID NOs: 101 to 109 and 111 to 120, SEQ ID NOs: 141 to 149 and 151 to 160, SEQ ID NOs: 181 to 189 and 191 to 200, and SEQ ID NOs: 221 to 229 and 231 to 240. The prior teaching of the present specification concerning the antibody and specific amino acid sequences thereof is considered as valid and applicable without restrictions to the polynucleotide and specific nucleic acid sequences if expedient.

Another object of the invention concerns a vector comprising the antibody-encoding polynucleotide according to the invention as described above. The term "vector" denotes a recombinant DNA construct which can be a plasmid, a virus, an autonomously replicating sequence, a phage, or a nucleotide sequence, which is linear or circular, consisting of single or double-stranded DNA or RNA, wherein a number of nucleotide sequences are linked or recombined to form a unique construction, and which is capable of introducing a promoter fragment and a DNA sequence of a selected gene product in sense or antisense orientation into a cell, together with suitable non-translated 3' sequences.

It is preferred that a plasmid comprises the antibody-encoding polynucleotide of the invention, particularly to clone and express recombinant genes of the inventive antibody or a fragment thereof. In the meaning of the invention, plasmids are genetic elements which are stable inherited without being part of the chromosome of their host cell. They may comprise DNA or RNA, and they can be both linear and circular. Plasmids encode molecules ensuring their replication and stable inheritance during cell replication. The starting plasmids disclosed in the present specification are either commercially available, accessible to the public, or can be constructed from available plasmids by routine use of well-known, published methods. Many plasmids and other cloning and expression vectors, which can be used according to the invention, are well-known and easily available to the

skilled artisan. Furthermore, a person skilled in the art can easily construct any number of other plasmids suitable for the use in this invention.

The vector shall be suitable for introduction into host cells. Accordingly, a host cell comprising the vector with the antibody-encoding polynucleotide is still another object of the invention. The present invention preferably relates to isolated prokaryotic or eukaryotic cells, but it shall also cover cell cultures, tissues, organs, and the like, and even organisms, which comprise the host cell of the invention, including an above-described vector. The term "host cell" denotes a cell that has been genetically modified by the transfer of a chimeric, heterologous or autologous nucleic acid sequence or derivatives thereof still including said sequence. These cells are also referred to as transgenic cells. Where an autologous nucleic acid sequence is transferred, the number of copies of this sequence in the host cell is higher than that of the naturally occurring sequences.

The invention also relates to a recombinant immunogen consisting of an extracellular integrin domain with insect-derived glycosylation pattern. The extracellular domain is preferably coupled as delta-trans membrane (DTM) form. Said immunogen of the invention is able to provoke an adaptive immune response if injected on its own in a mammalian species of choice, including rabbit. More preferably, the immunogen of the invention has an amino acid sequence of SEQ ID NOs: 10, 90, 130, 170 or 210, or variants, mutants, parts of the amino acid sequence or at least 95 % homologous sequences having the same function. Object of the invention is also a polynucleotide encoding said immunogens of the invention. In a preferred embodiment, the immunogen-encoding polynucleotide has a nucleotide sequence of SEQ ID NOs: 30, 110, 150, 190 or 230, or variants, mutants, parts of the amino acid sequence or at least 95 % homologous sequences having the same function. Another object of the invention concerns a vector comprising the immunogen-encoding polynucleotide according to the invention. Still another object is a host cell comprising the vector with the immunogen-encoding polynucleotide according to the invention. It shall be understood that the host species is included in the present scope of protection according to the present invention. The prior teaching of the present specification concerning the antibodies, or variants, mutants, parts of sequences or homologous sequences thereof, antibody-encoding polynucleotides, or vectors, host cells and the like, is valid and applicable without restrictions to the immunogen for raising said or other antibodies, if appropriate.

The invention also relates to a method for preparing rabbit antibodies comprising the steps of: (a) recombinantly expressing an extracellular integrin domain or a fragment thereof in insect cells; (b) purifying the expressed extracellular domain; (c) immunizing a rabbit with the purified extracellular domain; (d) taking polyclonal antiserum comprising polyclonal antibodies from the rabbit; and optionally (e) preparing monoclonal antibodies.

Preferably, the method for preparing monoclonal antibodies comprises the following steps: (a) recombinantly expressing an extracellular integrin domain in insect cells; (b) purifying the expressed extracellular integrin domain; (c) immunizing a rabbit with the purified extracellular integrin domain; (d) taking polyclonal antiserum comprising polyclonal antibodies from the rabbit; and (e) preparing the monoclonal antibodies. More preferably, the method refers to the preparation of said monoclonal antibodies of the invention as described in detail above.

The protein expression of step (a) is a matter of routine for the skilled artisan who has access to several appropriate insect cells, insect cell lines and ways for transfecting them. For example, the BTI-Tn5B1-4 (High Five) insect cell line infected with a recombinant baculovirus has gained widespread use within baculovirus/insect cell expression system because many secreted recombinant proteins are produced at considerably higher rates than in Spodoptera frugiperda derived cell lines, such as Sf9. To optimize the yield of the extracellular integrin domain from the baculovirus/insect cell expression system, experiments can be easily performed with suspension adapted cultures of High Five cells to investigate the effects of the state of the host cell, multiplicity of infection, cell density at the time of infection and supplementation of the medium with nutrients and oxygen. Such procedures are state of the art and published, e.g. by Vallazza & Petri, Cytotechnology 1999, 29: 85–92, or Mehta et al., Biochem J 1998, 330: 861–869.

The prior teaching concerning antibody or immunogen alterations is considered to be valid and applicable without restrictions to altered immunogens of step (a) if expedient. As obvious to the skilled artisan, the present invention shall not be construed to be limited to the full-length extracellular domains of integrin. Physiological or artificial fragments of the extracellular domains, secondary modifications of the extracellular domains, species-dependent alterations as well as allelic variants of the extracellular domains are also encompassed by the present invention. In this regard, an "allelic variant" is understood to represent the gene product of one of two or more different forms of a gene or DNA sequence that can exist at a genetic single locus. Artificial fragments preferably encompass

a peptide produced synthetically or by recombinant techniques, which at least comprises the epitopes of diagnostic interest.

If expressed in insect cells according to step (e), the extracellular integrin domain has a defective abherent glycosylation pattern that differs from the glycosylation pattern on mature mammalian cells. As the integrins are extensively glycosylated, i.e. more than 10 % by mass, this means that the insect protein is more divergent from rabbit native integrins produced in mammalian systems. The insect-derived immunogen leads to a greatly enhanced immunogenicity and stronger antibody response to the protein elements of said immunogen.

The protein purification, mammal immunization and serum extraction of steps (b) to (d) follow well known techniques and good laboratory practice, such as described in the course of the specification and examples. Sera of step (d) are subsequently tested for the presence of polyclonals, and the detected antibodies are screened for antigen recognition. Suitable tests and screens are available to those skilled in the art.

Optionally, the antibody preparation is continued to the species of mono-specific, identical antibodies, i.e. monoclonals of step (e). Monoclonal antibodies are typically made by fusing myeloma cells with the spleen cells from the mammal that has been immunized according to step (c). A selective HAT medium containing hypoxanthine, aminopterin and thymidine is particularly used in which only fused cells can grown. The so-called hybridomas are then diluted and clones are grown from single parent cells on microtiter wells. The antibodies secreted by the different clones are tested for their ability to bind to the antigen of the extracellular integrin domain. Accordingly, the antibodies of the invention are especially prepared by the method hereunder.

It goes without saying that antibodies may be similarly prepared by said method of the invention by using an intracellular integrin domain. The method shall apply mutatis mutandis.

Object of the invention are also the antibodies obtained by immunization of a rabbit with an extracellular or cytoplasmic domain of integrin recombinantly expressed in insect cells. As the immunogen of the invention can be used for raising antibodies, the invention particularly relates to monoclonal antibodies obtained by immunizing a rabbit with the immunogen and/or polynucleotide, each according to the invention, taking polyclonal antiserum with polyclonal antibodies and preparing the monoclonal antibodies. The prior

teaching of the present specification concerning the immunogen and the method for preparing rabbit antibodies shall be considered as valid and applicable without restrictions to the antibody product as produced by this process, as appropriate.

Although the most productive and stable clone can be grown in culture medium to a high volume, the monoclonal of choice is preferably expressed in a recombinant fashion. It requires cDNA cloning of the antibody encoding inserts, sequencing and inserting in expression vectors to allow production of wholly defined antibodies. Subsequently, the invention also relates to a method for manufacturing a recombinant monoclonal antibody or a fragment thereof comprising the steps of (a) introducing vector(s), which comprises nucleic acid sequence(s) of SEQ ID NOs: 21 to 29 and 31 to 40, SEQ ID NOs: 61 to 69 and 71 to 80, SEQ ID NOs: 101 to 109 and 111 to 120, SEQ ID NOs: 141 to 149 and 151 to 160, SEQ ID NOs: 181 to 189 and 191 to 200, and/or SEQ ID NOs: 221 to 229 and 231 to 240 into a host cell, (b) cultivating the host cell in a culture medium, thereby expressing the encoded antibody or fragment thereof, and (c) purifying the expressed antibody or fragment thereof.

The vector can be introduced by any method of the art, such as transformation, transfection or transduction. It shall be understood that prokaryotic cells, including bacteria and archaea, are particularly transformed, such as Escherichia species or Bacillus species, whereas eukaryotic cells are particularly transfected, such as CHO, HeLa, and the like. The three domain systems can also be transducted by viral vehicles. The vector can comprise either one or more nucleic acid sequences encoding the monoclonal antibody or a fragment thereof.

In still another preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 115 (V_L-αvß3) and/or SEQ ID NO: 116 (V_H-αvß3). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 119 (L-αvß3) and/or SEQ ID NO: 120 (H-αvß3).

In a preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 35 (V_L-αvß5) and/or SEQ ID NO: 36 (V_H-αvß5). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 39 (L-αvß5) and/or SEQ ID NO: 40 (H-αvß5).

In a preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 155 (V_L-αvß6) and/or SEQ ID NO: 156 (V_H-αvß6). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 159 (L-αvß6) and/or SEQ ID NO: 160 (H-αvß6).

In a preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 195 (V_L-αvß8) and/or SEQ ID NO: 196 (V_H-αvß8). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 199 (L-αvß8) and/or SEQ ID NO: 200 (H-αvß8).

In a preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 235 (V_L-αv) and/or SEQ ID NO: 236 (V_H-αv). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 239 (L-αv) and/or SEQ ID NO: 240 (H-αv).

In another preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 75 (V_L-ß3) and/or SEQ ID NO: 76 (V_H-ß3). In a more preferred embodiment of step (a), the vector(s) to be introduced comprise(s) the nucleic acid sequences of SEQ ID NO: 79 (L-ß3) and/or SEQ ID NO: 80 (H-ß3).

In a preferred aspect that the invention relates a method for manufacturing a recombinant monoclonal antibody comprising a light chain variable region (V_L) and a heavy chain variable region (V_H) with the steps of: (a) introducing one or more vectors comprising nucleic acid sequences of (i) SEQ ID NO: 115 (V_L-αvß3) and SEQ ID NO: 116 (V_H-αvß3), (ii) SEQ ID NO: 35 (V_L-αvß5) and SEQ ID NO: 36 (V_H-αvß5), (iii) SEQ ID NO: 155 (V_L-αvß6) and SEQ ID NO: 156 (V_H-αvß6), (iv) SEQ ID NO: 195 (V_L-αvß8) and SEQ ID NO: 196 (V_H-αvß8), or (v) SEQ ID NO: 235 (V_L-αv) and SEQ ID NO: 236 (V_H-αv) into a host cell, (b) cultivating the host cell in a culture medium, thereby expressing the encoded antibody, and (c) purifying the expressed antibody. It shall be understood that several vectors are favorably different by bearing only a single sequence of said SEQ ID NOs above. It is preferred in step (a) to introduce two vectors, each of them bearing one sequence of said SEQ ID NOs above.

Moreover, the prior teaching of the present specification concerning the antibody, amino acid sequences and alterations thereof, polynucleotides encoding the same as well as the

preparation of rabbit antibodies is considered as valid and applicable without restrictions to the manufacture of recombinant monoclonals if expedient.

It is still another object to use the antibody of the invention, or a fragment thereof, for the detection of integrins in formalin fixed paraffin embedded (FFPE) material. To date, there are no classical monoclonal antibodies directed to integrins and specifically and reliably reacting with the complexes in FFPE material. Only the antibodies of the invention, particularly rabbit monoclonals, have such a high affinity and specificity, which allows the detection of non-occluded epitopes of integrins. The terms "non-occluded" and "exposed", which are interchangeably used herein, are taken to mean the molecular confirmation of an antigen in which the epitopes can be recognized by an antibody. Hence, the same staining pattern is observed if comparing the antibodies of the invention on FFPE material with murine monoclonals on frozen material. Moreover the substantially same staining pattern is observed if comparing the antibodies of the invention on FFPE material and isolated integrin forms in ELISA and/or the native integrin state on viable cells, preferably if comparing the antibodies of the invention on FFPE material and on viable cells.

In a preferred embodiment of the invention, the FFPE material is a tissue. FFPE tissue is a piece of tissue which is first separated from a specimen animal by dissection or biopsy. Then, this tissue is fixed in order to prevent it from decaying or degeneration and to examine it clearly under a microscope for histological, pathological or cytological studies. Fixation is the process by which the tissue is immobilized, killed and preserved for the purpose of staining and viewing it under a microscope. Post-fixation processing makes tissue permeable to staining reagents and cross-links its macromolecules so that they are stabilized and locked in position. Many fixatives are used for this purpose for example, Bouine solution, formalin or liquid nitrogen. This fixed tissue is then embedded in the wax to allow it to be cut into thin sections and be stained with hematoxylin and eosin stain. After that, microtoming is done by cutting fine sections to study stain with antibodies under microscope.

In a more preferred embodiment of the invention, the FFPE tissue is a tumor tissue, most preferably human tumor tissue. The tumor is particularly selected from the group of tumors of the squamous epithelium, bladder, stomach, kidneys, head, neck, oesophagus, cervix, thyroid, intestine, liver, brain, prostate, urogenital tract, lymphatic system, stomach, larynx and/or lung. The tumor is furthermore particularly selected from the group of lung adenocarcinoma, small-cell lung carcinomas, pancreatic cancer, glioblastomas, colon carcinoma and breast carcinoma. In addition, preference is given to a tumor of the blood

and immune system, more particularly for a tumor selected from the group of acute myeloid leukemia, chronic myeloid leukemia, acute lymphatic leukemia and/or chronic lymphatic leukemia. Such tumors can also be designated as cancers in the meaning of the invention.

The antibody of the invention is incubated with the FFPE material for integrin detection. The term "incubation" denotes the contacting of the FFPE material with the antibody of the invention for a distinct period, which depends on the kind of material, antibody and/or antigen. The incubation process also depends on various other parameters, e.g. the sensitivity of detection, which optimization follows routine procedures known to those skilled in the art. Adding chemical solutions and/or applying physical procedures, e.g. impact of heat, can improve the accessibility of the target structures in the sample. Specific incubation products are formed as result of the incubation.

Suitable tests for the detection of formed antibody/antigen complexes are known to those skilled in the art or can be easily designed as a matter of routine. Many different types of assays are known, examples of which are set forth below. Although the assay according to the invention may be any assay suitable to detect and/or quantify integrin expression, the latter is preferably determined by means of substances specifically interacting with the primary antibody of the invention.

The term "specific substances" as used herein comprises molecules with high affinity to the anti-integrin antibody of the invention in order to ensure a reliable binding. The substances are preferably specific to parts of the antibody, e.g. constant regions, particularly rabbit constant regions, more particularly an F_c fragment, if any. There are a distinct number of specific antibodies against rabbit antibodies existing. Parts represent a restriction to those regions which are sufficient for the expression of a specific function, i.e. the provision of a structural determinant for recognition. In the context of the present invention, the term "recognition" - without being limited thereto - relates to any type of interaction between the specific substances and the target antibody, particularly covalent or non-covalent binding or association, such as a covalent bond, hydrophobic/ hydrophilic interactions, van der Waals forces, ion pairs, hydrogen bonds, ligand-receptor interactions, interactions between epitope and antibody binding site, nucleotide base pairing, and the like. Such association may also encompass the presence of other molecules such as peptides, proteins or other nucleotide sequences.

The specific substances are composed of biological and/or chemical structures capable to interact with the target molecule in such a manner that makes a recognition, binding and

interaction possible. In particular, the substances are selected from the group of proteins, peptides, nucleic acids, carbohydrates, polymers and small molecules having a molecular weight between 50 and 1.000 Da, preferably proteins and nucleic acids. The specific substances express a sufficient sensitivity and specificity in order to ensure a reliable detection. A specific substance has at least an affinity of 10^{-7} M for the anti-integrin antibody. The specific substance has preferably an affinity of 10^{-8} M or even more preferred of 10^{-9} M for its target molecule. As the skilled artisan will appreciate, the term specific is used to indicate that other biomolecules present in the sample do not significantly bind to the substance specific for anti-integrin antibody. Preferably, the level of binding to a biomolecule other than the target molecule results in a binding affinity of only 10 % of the affinity of the target molecule, more preferably only 5 % or less. Most preferably, the substances are mono-specific in order to guarantee an exclusive and directed interaction with the chosen primary anti-integrin antibody of the invention. A highly preferred specific substance will fulfill both the above minimum criteria for affinity as well as for specificity.

The proteins or peptides are preferably selected from the group consisting of antibodies, cytokines, lipocalins, receptors, lectins, avidins, lipoproteins, glycoproteins, oligopeptides, peptide ligands and peptide hormones. More preferably, antibodies are used as specific substance. The nucleic acids are preferably single or double stranded DNA or RNA, primers, antisense oligonucleotides, ribozymes, DNA enzymes, aptamers and/or siRNA, or parts thereof. More preferred nucleic acid probes are aptamers, most preferably RNA aptamers since the 2'-hydroxyl group available in RNA promotes a couple of intra- and intermolecular contacts. Aptamers can be synthesized using standard phosphoramidite chemistry. In addition, RNA aptamers having more than approximately 30 nucleotides can be favorably synthesized in large amounts by in-vitro transcription. Selection, synthesis, and purification of aptamers are well-known to those skilled in the art.

The specific substances can be labeled; in doing so the labeling depends on the inherent features of specific substances and specific incubation products to be monitored, as well as the detection method to be applied, i.e. the required sensitivity, ease of conjugation, stability requirements, and available instrumentation and disposal provisions. A labeling method is not particularly limited as long as a label is easily detected. A "labeled specific substance" is one that is bound, either covalently through a linker or a chemical bond, or non-covalently through ionic, van der Waals, electrostatic, hydrophobic interactions or hydrogen bonds, to a label such that the presence of the anti-integrin antibody of the invention may be detected by detecting the presence of the label.

Specific immunological binding of an antibody to a protein can be detected directly or indirectly. Hereunder, the antibody-to-protein pair shall be understood to include either the primary antibody of the invention directed to integrin or a secondary antibody directed to the primary anti-integrin antibody. Preferred examples of suitable detection methods according to the present invention are luminescence, particularly fluorescence, furthermore VIS coloring and/or radioactive emission.

Luminescence concerns the emission of light as a result of chemiluminescence, bioluminescence or photoluminescence. Chemiluminescence involves the emission of visible light as a result of a chemical reaction, whereas bioluminescence requires the activity of luciferase. The presently preferred photoluminescence, which is also known as fluorescence stimulation, is caused by the absorption of photons, preferably provided by radiation, which is released again as photon with a shift in wavelength of 30 to 50 nm and within a period of approximately 10^{-8} seconds. The instruments for fluorescence detection include, but are not limited to typical benchtop fluorometers, fluorescence multi-well plate readers, fiber optic fluorometers, fluorescence microscopes and microchips/microfluidics systems coupled with fluorescence detection.

VIS coloring denotes the visualization of any achromatic substance in order to be visible to the naked eye. Preferably, the intensity of coloring is measured by a photometer.

Radioactive radiation of isotopes is measured by scintillation. The process of liquid scintillation involves the detection of beta decay within a sample via capture of beta emissions in a system of organic solvents and solutes referred to as the scintillation cocktail. The beta decay electron emitted by radioactive isotopes such as ^{3}H, ^{14}C, ^{32}P, ^{33}P and ^{35}S in the sample excites the solvent molecule, which in turn transfers the energy to the solute. The energy emission of the solute (the light photon) is converted into an electrical signal by a photo-multiplier tube within a scintillation counter. The cocktail must also act as a solubilizing agent keeping a uniform suspension of the sample. Gamma ray photons often arise as a result of other decay processes (series decay) to rid the newly formed nucleus of excess energy. They have no mass and produce little if any direct ionization by collision along their path. Gamma photons are absorbed for detection and quantization by one or more of three mechanisms: the Compton effect, the photoelectric effect and pair production. A favorable gamma decay isotope of the present invention is ^{125}I.

Direct labels include fluorescent or luminescent tags, metals, dyes, radionuclides, and the like, attached to the antibody. An antibody labeled with iodine-125 (^{125}I) can be used. A

chemiluminescence assay using a chemiluminescent antibody specific for the protein is suitable for sensitive, non-radioactive detection of protein levels. An antibody labeled with fluorochrome is also suitable. Examples of fluorochromes include, without limitation, DAPI, fluorescein, Hoechst 33258, R-phycocyanin, B-phycoerythrin, R-phycoerythrin, rhodamine, Texas red, and lissamine.

Indirect labels include various enzymes well known in the art, such as horseradish peroxidase (HRP), alkaline phosphatase (AP), β-galactosidase, urease and the like. The covalent linkage of an anti-integrin antibody to an enzyme may be performed by different methods, such as the coupling with glutaraldehyde. Both, the enzyme and the antibody are interlinked with glutaraldehyde via free amino groups, and the by-products of networked enzymes and antibodies are removed. In another method, the enzyme is coupled to the antibody via sugar residues if it is a glycoprotein, such as peroxidase. The enzyme is oxidized by sodium periodate and directly interlinked with amino groups of the antibody. Other enzyme containing carbohydrates can also be coupled to the antibody in this manner. Enzyme coupling may also be performed by interlinking the amino groups of the antibody with free thiol groups of an enzyme, such as ß-galactosidase, using a heterobifunctional linker, such as succinimidyl 6-(N-maleimido) hexanoate. The horseradish-peroxidase detection system can be used, for example, with the chromogenic substrate tetramethylbenzidine (TMB), which yields a soluble product in the presence of hydrogen peroxide that is detectable at 450 nm. The alkaline phosphatase detection system can be used with the chromogenic substrate p-nitrophenyl phosphate, for example, which yields a soluble product readily detectable at 405 nm. Similarly, the ß-galactosidase detection system can be used with the chromogenic substrate o-nitrophenyl-ß-D-galactopyranoxide (ONPG), which yields a soluble product detectable at 410 nm. A urease detection system can be used with a substrate, such as urea-bromocresol purple.

In a preferred embodiment of the present invention, the antibodies are labeled with detectable moieties, which include, but are not limited to, radionuclides, fluorescent dyes, e.g. fluorescein, fluorescein isothiocyanate (FITC), Oregon Green™, rhodamine, Texas red, tetrarhodimine isothiocynate (TRITC), Cy3, Cy5, etc., fluorescent markers, e.g. green fluorescent protein (GFP), phycoerythrin, etc., auto-quenched fluorescent compounds that are activated by tumor-associated proteases, enzymes, e.g. luciferase, HRP, AP, etc., nanoparticles, biotin, digoxigenin, and the like.

In another preferred embodiment of the present invention, the nucleic acids are labeled with digoxigenin, biotin, chemiluminescence substances, fluorescence dyes, magnetic

beads, metallic beads, colloidal particles, electron-dense reagents, enzymes; all of them are well-known in the art, or radioactive isotopes. Preferred isotopes for labeling nucleic acids in the scope of the invention are ^{3}H, ^{14}C, ^{32}P, ^{33}P, ^{35}S or ^{125}I, more preferred ^{32}P, ^{33}P or ^{125}I.

A variety of immunoassay techniques, including competitive and non-competitive immunoassays, can be used. The term "immunoassay" encompasses techniques including, without limitation, flow cytometry, FACS, enzyme immunoassays (EIA), such as enzyme multiplied immunoassay technique (EMIT), enzyme-linked immunosorbent assay (ELISA), IgM antibody capture ELISA (MAC ELISA) and microparticle enzyme immunoassay (MEIA), furthermore capillary electrophoresis immunoassays (CEIA), radio-immunoassays (RIA), immunoradiometric assays (IRMA), fluorescence polarization immunoassays (FPIA) and chemiluminescence assays (CL). If desired, such immunoassays can be automated. Immunoassays can also be used in conjunction with laser induced fluorescence. Liposome immunoassays, such as flow-injection liposome immunoassays and liposome immunosensors, are also suitable for use in the present invention. In addition, nephelometry assays, in which the formation of protein/antibody complexes results in increased light scatter that is converted to a peak rate signal as a function of the marker concentration, are suitable for use in the methods of the present invention. In a preferred embodiment of the present invention, the incubation products are detected by ELISA, RIA, fluoro immunoassay (FIA) or soluble particle immune assay (SPIA).

Component of ELISAs are enzymes which are bound to one partner of the immunological reaction. The tracer antigen (analyte derivative) of integrin is preferably labeled in the competitive ELISA using a single capture antibody (herein after referred to as primary), whereas the antibody is preferably labeled in the non-competitive ELISA, preferably comprising the precipitation of the antigen-antibody complex by a second antibody (herein after referred to as secondary). Complexes consisting of antigen and two antibodies are also called sandwich complexes. The detection comprises the subsequent enzymatic conversion of a substrate to a product, preferably a colored product, which is recognized by visual coloring, bioluminescence, fluorescence or the measurement of electrical signals (enzyme electrode). Favorable enzymes for labeling in the present invention are known to the skilled artisan, such as peroxidase (e.g. HRP), chloramphenicol acetyl transferase (CAT), green fluorescent protein (GFP), glutathione S-transferase (GST), luciferase, ß-galactosidase and AP.

Additionally preferred are radioactive immunoassays utilizing radioactive isotopes which are either incorporated into an immune reagent during synthesis or subsequently coupled to an immune reagent of the assay, preferably to an antibody.

Antibodies, which are favorably labeled with fluorophores, are used in FIAs.

SPIA utilizes the color change of silver particle as result of agglutination. Neither a secondary antibody nor an indicator reaction are required making it particularly useful in the scope of the present invention. Similarly favorably is the latex agglutination test using antibodies which are bound to colored latex particles. However, it requires a strong immobilization of integrin to remove unbound and/or non-specifically bound antigens in previous washing steps.

In general, all methods for detection include intensive washing steps to separate unbound antibodies from the integrin/antibody complex. Furthermore, the experimental procedure of any detection method is well-known to those skilled in the art.

A signal from the direct or indirect label can be analyzed, for example, using a spectrophotometer to detect color from a chromogenic substrate, using a radiation counter to detect radiation, such as a gamma counter for detection of ^{125}I, or using a fluorometer to detect fluorescence in the presence of light of a certain wavelength. For detection of enzyme-linked antibodies, a quantitative analysis can be made using a spectrophotometer, such as an EMAX Microplate Reader (Molecular Devices; Menlo Park, CA) in accordance with the manufacturer's instructions. If desired, the assays of the present invention can be automated or performed robotically, and the signal from multiple samples can be detected simultaneously.

Optical images viewed and optionally recorded by a camera or other recording device (e.g. a photodiode and data storage device) are optionally further processed in any of the embodiments herein, e.g. by digitizing the image and storing and analyzing the image on a computer. A variety of commercially available peripheral equipment and software is available for digitizing, storing and analyzing a digitized video or digitized optical image. One conventional system carries light from the specimen field to a cooled charge-coupled device (CCD) camera, in common use in the art. A CCD camera includes an array of picture elements (pixels). The light from the specimen is imaged on the CCD. Particular pixels corresponding to regions of the specimen are sampled to obtain light intensity readings for each position. Multiple pixels are processed in parallel to increase speed. The

apparatus and methods of the invention are easily used for viewing any sample, e.g. by fluorescent or dark field microscopic techniques.

In a preferred embodiment of the invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 99 (L-αvß3) and SEQ ID NO: 100 (H-αvß3), which are generated against DTM-αvß3 of SEQ ID NO: 90, produce antibodies suitable for FFPE tissue. They bind αvß3 selectively. In another preferred embodiment of the present invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 19 (L-αvß5) and SEQ ID NO: 20 (H-αvß5), which are generated against DTM-αvß5 of SEQ ID NO: 10, produce antibodies suitable for FFPE tissue. They bind αvß5 selectively. In still another preferred embodiment of the present invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 139 (L-αvß6) and SEQ ID NO: 140 (H-αvß6), which are generated against DTM-αvß6 of SEQ ID NO: 130, produce antibodies suitable for FFPE tissue. They bind αvß6 selectively. In still another preferred embodiment of the present invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 179 (L-αvß8) and SEQ ID NO: 180 (H-αvß8), which are generated against DTM-αvß8 of SEQ ID NO: 170, produce antibodies suitable for FFPE tissue. They bind αvß8 selectively. In still another preferred embodiment of the present invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 219 (L-αv) and SEQ ID NO: 220 (H-αv), which are generated against DTM-αv of SEQ ID NO: 210, produce antibodies suitable for FFPE tissue. They bind αv selectively. In still another preferred embodiment of the invention, the rabbit hybridoma clones consisting of the amino acid sequences of SEQ ID NO: 59 (L-ß3) and SEQ ID NO: 60 (H-ß3), which are generated against the ß3 immunogen of SEQ ID NO: 50, produce antibodies suitable for FFPE tissue. They bind ß3 selectively. It shall be understood, however, that any alternative sequence or combinations thereof as described in the present specification may be applied for the inventive use. The prior teaching of the present specification concerning the antibodies and amino sequences thereof is considered as valid and applicable without restrictions to the use if expedient.

Further, the invention may be practiced as a kit comprising the antibody, polynucleotide, vector or host cell, each of them according to the present invention, in order to perform the inventive use of detecting integrins in FFPE material. Particularly, the antibodies can be incorporated into a diagnostic detection kit for characterizing the integrin profile, e.g. the αv integrin or other integrin expression profiles of tumors or other human pathologies, and especially in archival FFPE material. The kit of the invention may include an article that

comprises written instructions or directs the user to written instructions for how to practice the method of the invention. In an embodiment, the kit further comprises a reporter moiety or a reporter apparatus. The prior teaching of the present specification concerning the kit ingredients and the use thereof is considered as valid and applicable without restrictions to the kit if expedient.

The present invention solves the second problem by teaching a method for screening anti-integrin antibodies, which are capable of discriminating between the respective closest homologues of integrin α-subunit and/or ß-subunit and suitable for immunohistochemistry in FFPE material, comprising the steps of: (a) providing a sample of antibodies being capable of binding a selected integrin; (b) aligning integrin sequences to identify the closest homologue of the α-subunit and/or ß-subunit of the selected integrin; (c) performing an differential ELISA on native forms of the selected integrin and the closest homologue(s) thereof with the antibody sample, thereby accumulating antibodies against the selected integrin (primary screen); (d) performing another differential ELISA on native forms of the selected integrin and another integrin with the accumulated antibodies of step c), thereby further accumulating antibodies against the selected integrin (secondary screen); (e) performing immunohistochemistry of FFPE cell lines with the accumulated antibodies of step d), wherein at least one cell line is capable of expressing the selected integrin and optionally another cell line is not capable of expressing the selected integrin, thereby further accumulating antibodies against the selected integrin (tertiary screen); (f) performing immunohistochemistry of FFPE cell lines of step e) with the accumulated antibodies of step e), wherein the cell line is grown as xenograft tumor in a mammal, thereby further accumulating antibodies against the selected integrin (quaternary screen); and (g) performing immunohistochemistry of archival FFPE tumors with the accumulated antibodies of step f), thereby further accumulating antibodies against the selected integrin (quintenary screen).

Primary screening is performed by differential ELISA on native, biologically active, un-denatured forms of the immunogens (Mehta et al., 1998, Biochem J 330: 861–869). If the target immunogen is αvß3, for example, the primary screen is αvß3 versus αvß5. That means the primary screen uses a counter-screen on integrin with the closest sequence homology to the primary target. Closest homologue to the ß3 chain is ß5, while αv is identical in both complexes. In this way the most discriminatory antibodies can be obtained. Similarly, αvß5 can be screened versus αvß8. Screens for alpha-chain specific antibodies can follow the same procedure, i.e. αvß1 could be used as counter screen for a α5ß1

specific antibody. The secondary screen looks at a wider set of recombinant integrins in ELISA to confirm further the specificity, e.g. αiibß3 can be used to confirm specificity for αv complexes rather than ß3-chain alone of αvß3 antibodies, preferably αvß3 monoclonals. It is preferred in step (d) that the differential screen is performed on native forms of the selected integrin and another closely related integrin with the accumulated antibodies of step (c). The tertiary screen looks at antibody staining in IHC of FFPE cell lines that are biochemically characterized for their integrin expression profiles. The quaternary screen uses FFPE-IHC on the same cell lines grown as xenograft tumors in nude mice. The quintenary screen looks at archival FFPE human tumors. For example, tertiary and quaternary screens are on M21, U87MG and M24 melanomas as positive screen targets. All these lines are known from in house and literature profiling to express αvß3, while A549 NSCLC, Raji and HT29 are negative screen targets. All these lines are known from in house and literature profiling to not express αvß3. The quintenary screens are preferably on malignant melanoma and glioblastoma as αvß3 positive, and NSCLC and CRC as αvß3 negative human tumors.

In an embodiment of the screening method, any of steps (c) to (g) comprises the further step of detecting the discriminatory capacity and/or specificity of the accumulated antibodies.

In the scope of the present invention, antibodies have been provided for the first time, which allow the validated detection of integrins in FFPE archival patient material, such as tumor biopsies, and also by live cell flow cytometry (FACS). The staining patterns in FACS correspond to the patterns obtained with the relevant monoclonal antibodies know to those skilled in the art (e.g. LM609 for αvß3; P1F6 for αvß5). It shows that the antibodies of the invention detect the respective integrins not only in FFPE material, but also in their native state on viable cells. Integrins, particularly αvß3, αvß5, αvß6 or αvß8 are primary therapeutic targets that could not be reliably visualized in routine FFPE biopsy material before filing this application. The robust antibodies of the invention have the potential to recognize their integrin targets in archival FFPE material in identical staining pattern to the distribution seen by known αvß3-, αvß5- or αvß6-specific monoclonal antibodies on cryo-preserved material, but with the well-known, much higher spatial resolution and quality of morphological preservation typical of FFPE vs. cryo-histology material. Very suitable antibodies are rabbit monoclonals that are not simply originated from another species, but these RabMabs are favorably proven to possess specificity, reproducibility and eternality (i.e. the same reagent and same specificity for ever). RabMabs, which are generated by

using αvß3 or αvß5 clones, recognize archival αvß3 or αvß5 in human tumors in identical staining patterns to cryo-fixed material stained with the classical anti-αvß3 antibody LM609 or the anti-avß5 antibody PIF6. RabMabs, which are generated by using ß3-cytoplasmic domains, stain xenograft arrays in pattern corresponding to known αvß3 expression profile of target cells. Although antibodies produced, and optionally selected by screening in the way revealed hereunder, mainly function on FFPE integrins, they can also be used in ELISA on isolated integrins, for flow cytometry on live cell populations, or even have other standard biochemical applications. The antibodies provide an unusual and valuable validation bridge between the observed human pathologies and the biochemistry of the receptors.

The invention teaches the generation of anti-integrin antibodies by using purified integrin domains, particularly purified integrin extracellular domains, more particularly of human origin. The immunogen of the invention causes high titers of antibodies within short periods of immunization. The high antibody titers are reflected by a high dilution of serum which is obtained after immunization and used in assays. Simultaneously, adverse effects which could be caused by other serum components are largely reduced due to their diluted presence. The titer could be advantageously increased further by insect recombinant immunogen production that generates a divergence in the glycosylation from the endogenous and highly homologous rabbit integrins. The antibodies and derivatives thereof are characterized by a high specificity stability and expression in mammalian expression systems in an industrial production scale, low manufacturing costs and convenient handling. These features form the basis for a reproducible action, wherein the lack of cross-reactivity and adverse effects is included, and for a reliable and safe interaction with their matching integrin structures. As the antibodies can be cloned into expression vectors, they provide an absolutely stable and reproducible source of material for basic research and diagnosis. In addition, the appropriate kit is cost-efficiently produced.

All the references cited herein are incorporated by reference in the disclosure of the invention hereby.

It is to be understood that this invention is not limited to the specific antibodies, particular methods, uses and kits described herein, as such matter may, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to limit the scope of the present invention, which is only defined by the appended claims. As used herein, including the appended claims,

singular forms of words such as "a," "an," and "the" include their corresponding plural referents unless the context clearly dictates otherwise. Thus, e.g., reference to "an antibody" includes a single or several different antibodies, whereas reference to "antibodies" shall be applicable mutatis mutandis, and reference to "a method" includes reference to equivalent steps and methods known to a person of ordinary skill in the art, and so forth. Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by a person of ordinary skill in the art to which this invention belongs.

The techniques that are essential according to the invention are described in detail in the specification. Other techniques which are not described in detail correspond to known standard methods that are well known to a person skilled in the art, or the techniques are described in more detail in cited references, patent applications or standard literature. Other microorganisms, cell lines, plasmids, promoters, resistance markers, replication origins, and the like, which are not mentioned in the application, are commercially available. Provided that no other hints in the application are given, they are used as examples only, they are not considered to be essential according to the invention, but they can be replaced by other suitable tools and biological materials. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable examples are described below. The following examples are provided by way of illustration and not by way of limitation. Within the examples, standard reagents and buffers that are free from contaminating activities (whenever practical) are used. The examples are particularly to be construed such that they are not limited to the explicitly demonstrated combinations of features, but the exemplified features may be unrestrictedly combined again if the technical problem of the invention is solved.

Figure 1 shows the immunohistochemical staining of FFPE cancer cell lines (left) and xenografts (right) with supernatants of the subclone E3528-2-12 generated against the external domain of αvβ3.

Figure 2 shows the plasma membrane staining of M21 cells in xenografts with the purified anti-αvβ3 integrin antibody clone E3528-2-7.

Figure 3 shows the immunohistochemical staining of the cancer cell line M21 (left) and the M21 xenograft (right) with the purified anti-αvβ3 integrin antibodies E3528-2-7, E3528-2-11 and E3528-2-12.

Figure 4 shows the analysis of immunohistochemical staining with the anti-αvβ3 antibodies E3528-2-7, E3528-2-11 and E3528-2-12 with the help of image analysis (Ariol SL-50) and graphical representation with Spotfire.

Figure 5 shows the analysis of immunohistochemical staining with the antibody αvβ3_E3528-2-7 and the mouse monoclonal antibody 20H9 with the help of image analysis (Ariol SL-50). Clone 20H9 is directed against the β3-integrin chain. The "Expression (%max)" is normalized to the expression of M21.

Figure 6 shows the plasma membrane staining of M21 cells in xenografts with the purified anti-αvβ3 integrin antibodies E3531-227-3 and E3531-229-3.

Figure 7 shows the immunohistochemical staining of the cancer cell line M21 (left) and the M21 xenograft (right) with the purified anti-αvβ3 integrin antibodies of multiclone 227 (E3531-227-3, E3531-227-3 and E3531-227-6).

Figure 8 shows the analysis of immunohistochemical staining with the anti-αvβ3 antibodies of the clones E3531-227 (above) and in comparison to the mouse monoclonal anti-ß3 antibody 20H9 (below), calculated as % of the expression in M21 cells. Expression was analyzed with the help of image analysis (Ariol SL-50).

Figure 9 shows the immunohistochemical staining of FFPE cancer cell lines (left) and xenografts (right) with supernatants of the subclone E3536-99-3 generated against the external domain of αvβ5.

Figure 10 shows the ELISA profile of purified monoclonal hybridoma antibodies E3531-227-3, E3531-229-3 and E3536-99-2 against recombinant human extracellular domains of integrin αvß3 and αvß5 and full length purified platelet gpiibiiia.

Figure 11 shows the plasma membrane staining of A431 and HCT116 cells in xenografts with the purified anti-αvβ5 integrin antibody clone E3536-99-3.

Figure 12 shows the immunohistochemical staining of the cancer cell line U87MG (left) and the U87MG and A431 xenografts with the purified anti-αvβ5 integrin antibodies E3536-99-1, E3536-99-2 and E3536-99-3.

Figure 13 shows the analysis of immunohistochemical staining with the anti-αvβ5 antibodies E3536-99-1, E3536-99-2 and E3536-99-3 with the help of image analysis (Ariol SL-50) and graphical representation with Spotfire.

Figure 14 shows the immunohistochemical staining of FFPE cancer cell lines (left) and xenografts (right) with supernatants of the subclone E3866-52-1.

Figure 15 shows the immunohistochemical staining of FFPE cancer cell lines with the purified antibody of subclone E3866-52-1.

Figure 16 shows the immunohistochemical staining of cancer cell lines and xenografts with the recombinant anti-αvβ6 integrin antibody.

Figure 17 shows the plasma membrane staining of prostata carcinoma cells (above) and HT29 colon carcinoma cells in xenografts with the recombinant anti-αvβ6 integrin antibody.

Figure 18 shows the analysis of immunohistochemical staining (run 3421) with the anti-αvβ6 antibody with the help of image analysis (Ariol SL-50).

Figure 19 shows the slide-to-slide and run-to-run reproducibility with the anti-avβ6 recombinant antibody.

Figure 20 shows the immunohistochemical staining of FFPE cancer cell lines and xenografts with supernatants of the anti-αvβ8 subclone 133-9.

Figure 21 shows the immunohistochemical staining of FFPE cancer cell lines with the purified antibody of anti-αvβ8 subclone E3875-133-9.

Figure 22 shows the immunohistochemical staining of cancer cell lines and xenografts with the recombinant anti-αvβ8 integrin antibody EM13309.

Figure 23 shows the immunohistochemical staining of human tissue with the recombinant anti-αvβ8 integrin antibody EM13309.

Figure 24 shows the plasma membrane staining of prostata carcinoma cells (above) and H1975 lung carcinoma cells in xenografts with the recombinant anti-αvβ8 integrin antibody.

Figure 25 shows the analysis of immunohistochemical staining (run 3422) with the anti-αvβ8 antibody EM13309 with the help of image analysis (Ariol SL-50).

Figure 26 shows the slide-to-slide and run-to-run reproducibility with the anti-avβ8 recombinant antibody EM13309.

Figure 27 shows the immunohistochemical staining of FFPE cancer cell lines and xenografts with supernatants of the anti-αv subclone E3875-13-9.

Figure 28 shows the immunohistochemical staining of FFPE cancer cell lines with the purified anti-αv antibody of subclone E3875-13-9.

Figure 29 shows the immunohistochemical staining of cancer cell lines and xenografts with the recombinant anti-αv antibody EM01309.

Figure 30 shows the plasma membrane staining of DU-145 (above) and HT29 cells in xenografts with the recombinant anti-αv antibody EM01309.

Figure 31 shows the analysis of immunohistochemical staining with the recombinant anti-αv antibody EM01309 with the help of image analysis (Ariol SL-50).

Figure 32 shows the slide-to-slide and run-to-run reproducibility with the anti-αv recombinant antibody EM01309.

Figure 33 shows the plasma membrane staining of M21 cells in xenografts with the purified anti-β3 integrin antibody clone E3592-2-12.

Figure 34 shows the immunohistochemical staining of the cancer cell line M21 (left) and the M21 xenograft (right) with the purified anti-β3 integrin antibodies E3592-2-4, E3592-2-10 and E3592-2-12.

Figure 35 shows the analysis of immunohistochemical staining with the anti-β3 antibodies E3592-2-4, E3592-2-10 and E3592-2-12 with the help of image analysis (Ariol SL-50) and graphical representation with Spotfire.

Figure 36 shows the analysis of immunohistochemical staining with the antibody β3_E3592-2-12 and the mouse monoclonal antibody 20H9 with the help of image analysis (Ariol SL-50). Clone 20H9 is directed against the β3-integrin chain. The "Expression (%max)" is normalized to the expression of M21.

Figure 37 shows the ELISA profile of purified monoclonal hybridoma antibodies E3875-133-9, E3866-052-1 and E3875-013-9 from rabbit anti-integrin against recombinant human αv-integrin extracellular domains and full length purified platelet gpiibiiia.

Figure 38 shows the ELISA profile of EBNA-recombinant rabbit anti-integrin monoclonal antibodies EM22703, EM09902, EM00212, EM05201, EM13309 and EM01309 against recombinant human αv-integrin extracellular domains and full length purified platelet gpiibiiia.

Figure 39 shows the receptor inhibition assay for RabMab antibodies EM22703, EM09902, EM00212 using biotin vitronectin as ligand.

Figure 40 shows the FACS titration of EM022703 on M21.

Figure 41 shows the FACS titration of EM009902 on A549.

Figure 42 shows the FACS titration of EM05202 on HT29.

Figure 43 shows the FACS titration of EM13309 on M24-met cells.

EXAMPLE 1: Generation of immunogens

Example 1.1: Generation of extracellular domains αvβ3, αvβ5, αvβ6 and αvβ8v

Recombinant human integrin extracellular domains, αvβ3, αvβ5, αvβ6 and αvβ8, were raised in insect cell lines (Hive Five) using baculovirus infection. The use of the insect line as negative control was apposite. After fermentation, downstream processing comprised the following elements: chromatography on <Mab 14D9> Toyopearl affinity column, dialysis with Spectra/POR dialyze tubing (6-8 kDa for DTM-αvβ3 and DTM-αvβ5; 25 kDa for DTM-αvβ8), concentration with Millipore TFF Labscale having cut off 30 kDa (DTM-αvβ5 and DTM-αvβ8 only), concentration with Amicon Ultra-15 centrifugal filter units having cut off 30 kDa and 0.2 µm filtration with Millex GV (DTM-αvβ3, DTM-αvβ6, DTM-αvβ8 only).

22 mg DTM-αvβ3 were dissolved in buffer of 50 mM $Na(CH_3COO)$, 0.2 mM $MnCl_2$, pH 7.4, to give a protein concentration of 2.0 mg/ml. The stock solution was subsequently aliquoted into 22 vials of 500 µl. 10.6 mg DTM-αvβ5 were dissolved in buffer of 50 mM $Na(CH_3COO)$, 0.2 mM $MnCl_2$, pH 7.4, to give a protein concentration of 2.36 mg/ml. The stock solution was subsequently aliquoted into 9 vials of 500 µl. 15 mg DTM-αvβ6 were dissolved in buffer of 50 mM $Na(CH_3COO)$, 0.2 mM $MnCl_2$, pH 7.4, to give a protein concentration of 2.36 mg/ml. 16.6 mg DTM-αvβ8 were dissolved in buffer of 50 mM $Na(CH_3COO)$, 0.2 mM $MnCl_2$, pH 7.4, to give a protein concentration of 2.78 mg/ml.

The aliquots were frozen in liquid nitrogen and stored at -80°C. Analytics was performed by BCA assay and SDS page with Coomassie staining or western blotting pursuant to routine experimental praxis. The following antibodies were used for DTM-αvβ3 detection by western blotting: primary Mab AP3 EMD 330515/CH000, 5 µg/ml, 2 h RT, and secondary gout anti mouse IgG (H+L) x AP, Dianova, 115-055-062, 1:1000, 1 h RT, followed by Precision Step Tractin x AP, BioRad, 161-0382, 1:5000. The following antibodies were used for DTM-αvβ5 detection by western blotting: primary Mab <11D1> -CH004, 2.5 µg/ml, 1 h RT, and secondary goat anti mouse IgG (H+L) x AP, Dianova, 115-055-062, 1:1000, 1 h RT, followed by Precision Step Tractin x AP, BioRad, 161-0382, 1:5000. The following antibodies were used for DTM-αvβ6 detection by western blotting: primary Mab 442-5C4 x Biotin <hu-Integrin ß6> 330510/CH001, 2 µg/ml, 2 h RT, and secondary anti Biotin x AP, Sigma A-7064, 1:2500, 2 h RT, followed by Precision Step Tractin x AP, BioRad, 161-0380, 1:5000. The following antibodies were used for DTM-αvβ8 detection by western blotting: primary Mab LM 142 x Biotin Pool A 269A07H1.G01, 5 µg/ml, 2 h RT, and secondary goat anti mouse IgG (H+L) x AP, Dianova, 115-055-062, 1:1000, 1 h RT, followed by Precision Step Tractin x AP, BioRad, 161-0382, 1:5000, 1 h RT.

The immunogens were characterized as biologically active and specific by their ability to bind their cognate substrates, e.g. vitronectin (αvβ3 and αvβ5) and fibronectin (αvβ3). These preparations were acknowledged as a gold-standard for integrin structural fealty (Mehta et al., Biochem J 1998, 330: 861–869; Xiong et al., Science 2001, 294: 339–345). The recombinant human integrin extracellular domains DTM-αvβ3, DTM-αvβ5, DTM-αvβ6 and DTM-αvβ8 were used as immunogens.

Example 1.2: Generation of cytoplasmic domain β3

The human β3 integrin cytoplasmic domain, fused to GST was produced in E.coli BL21 and purified as a recombinant fusion protein as immunogen. After fermentation, downstream

processing comprised the following elements: cell lysis, French press, preparation of inclusion bodies, refolding by dialysis and concentration. 55 mg protein were dissolved in buffer of 0.1 M sodium carbonate, 5 mM DTT, pH 9.5, to give a protein concentration of 1.27 mg/ml. The stock solution was subsequently aliquoted into 2 vials of 10 ml, 4 vials of 5 ml and 4 vials of 1 ml. The aliquots were filtrated (0.2 µm), frozen in liquid nitrogen and stored at -80°C. Analytics was performed by Bradford assay and SDS page with Coomassie staining or western blotting pursuant to routine experimental praxis. The following antibodies were used for β3 detection by western blotting: primary Goat-Anti – GST, Amersham, No. 27-4577-01, 1:5000, 1h RT, and secondary $F(ab')_2$ Fragment Rabbit-Anti-Goat IgG (H+L) x AP, Dianova, 305-056-045, 1:1000, 1h RT, followed by Precision Strep Tactin-AP Conjugate, BioRad, Nr. 161-0382, 1:5000.

Example 1.3: Generation of gpiibiiia

Full length human gpiibiiia was extracted from outdated human platelets using the octylglucoside as previously detailed (Mitjans et al., J Cell Sci 1995, 108(Pt 8): 2825-38).

EXAMPLE 2: Generation of antibodies

The generation of rabbit monoclonal antibodies followed a four-step procedure: (A) immunization of rabbits and screening of polyclonal sera, (B) fusion to generate hybridoma cells and screening of supernatants of multiclones, (C) subcloning and screening of supernatants of subclones, and (D) cDNA cloning of the antibody encoding inserts, sequencing and insertion in EBNA expression vectors to allow production of wholly defined antibodies. Rabbit bleeds, hybridoma supernatants and purified antibodies were analyzed in ELISA against immobilized purified immunogens, following standard protocols. Positive clones were retested by differential screen against recombinant extracellular domains of αvβ3, αvβ5 αvβ6 and αvβ8 on delivery, to confirm specificity and activity.

In step (A), several rabbits per immunogen were immunized and the antisera titers were monitored. Prebleeds of all the rabbits gave no signal even at low dilution (1:50) on the FFPE materials, while the primary bleeds (polyclonal sera) prior to fusion already gave clear and unequivocal signals, with strong indications of specificity for cell surface proteins. Three bleeds of each rabbit were delivered, and a single positive rabbit per immunogen was selected for fusion after 8 to 12 weeks.

In step (B), the B cells from the serum positive rabbits were isolated, and the rabbit fusion partner cell line 240E-W were fused to the isolated rabbit B-cells to create rabbit hybridoma cells. 96-well plates were screened for fusion by ELISA. The supernatant for 10 to 100 positive clones were delivered, and 3 multiclones per immunogen were selected after 5 to 6 weeks.

In step (C), hybridomas were cloned and screened to select clones secreting antibodies with appropriate specific antigen recognition, and the antibodies are characterized using a variety of methods (western blotting, IHC, ICC, flow cytometry, etc.). Supernatants of subclones were particularly screened with ELISA for specific antigen recognition. Supernatants of positive tested subclones were frozen and stored at -80°C until use. Subsequently, the subclone supernatants were screened in the two-step process of Example 3, first on the cancer cell line array and in the second step on xenograft tissue with a cancer cell line array in parallel to verify the first screen.

In step (D), the DNA sequences of the selected antibody clones were excised, cloned into EBNA expression vectors, and sequenced by automated cDNA Sanger dye sequencing. The recombinant antibodies were produced in the EBNA cell expression system according to Pham et al., Biotech Bioeng 2003, 84(3): 332-342, but with the minor modification of using HEK293-6E cells with the pTT5 vector for the transient transfection system. Antibody production was verified by ELISA and IHC. mRNA from hybridoma cells was isolated using TuboCapture Kit (Qiagen) following the manufacturer's suggestion and then reverse transcribed into cDNA using oligo-dT primer. The variable region of heavy chain (V_H) was PCR amplified using proprietary primers OYZ64-2 and OYZvh3. The entire light chain (L) was PCR amplified using proprietary primers OYZ62 and OYZ71. The V_H region of PCR fragments was digested using restriction enzyme HindIII and KpnI. The L PCR fragments were digested using HindIII and NotI. All digested product was purified using Qiagen PCR cleaning up kit. After purification, the V_H or L fragment was ligated into the corresponding heavy or light chain proprietary expression vector and transformed into competent cells DH5α (MC Lab). The transformed colonies were picked and inserts were confirmed using the corresponding restriction enzymes (by expected size: approximately 440 bp for V_H and 740 bp for L). Plasmids with inserts of the expected size were sequenced using TT5 for primer. The entire light chain or heavy chain fragment was excised from the corresponding vector with HindIII and NotI and subsequently purified using Qiagen PCR cleaning up kit. Approximately 50 to 100 ng of cDNA inserts were banked.

EXAMPLE 3: Methods for screening and characterizing antibodies

EXAMPLE 3.1 Array compositions

Twenty seven cancer cell lines and one insect cell line were fixed in phosphate buffered 4 % paraformaldehyde, pH 7, over 16 to 24 hours at room temperature, embedded in paraffin and arranged into a 28 cell line paraffin block (CAX05). The integrin cell surface expression profile of several of the cell lines used in the array was previously characterized by flow cytometry, using defined mouse monoclonal antibodies, such as LM609 (Cheresh & Spiro, JBC 1987, 262: 17703-17712) and P1F6 (Varner & Cheresh, Important Adv Oncol 1996, 87: 69) directed against the αvβ3 and αvβ5 integrin complexes, respectively (Mitjans et al., J Cell Sci 1995, 108(Pt 8): 2825-38).

CAX05:

A 431	squams cancer oes
A 549	lung cancer
A2780 ADR	ovarian cancer
C 8161	melanoma
Calu 6	lung adeno
Colo 205	colon cancer
DU145	prostate cancer
HCT 116	colon cancer
HT 29	colon cancer
Igrov 1	ovarian cancer
Kyse 30	squamous cancer
Lox	melanoma
M21	melanoma
M24-met	melanoma
MCF 7	breast cancer
MDA-MB 23	breast cancer
MDA-MB468	breast cancer
MiaPaCa2	pancreas cancer
NCI-H460LC	lung cancer
Ovcar-3	ovarian cancer
PC 3	prostate cancer
Raji	BuBVLtt´s Lym
Sf9	Insect cell
SKOV 3	ovarian cancer

Suit 7	pancreas cancer
SW707	colon cancer
U87MG	glioblastoma
WM 164	melanoma

Arrays out of different experimental studies (Xeno-08-A; Xeno-08-Mu1) were composed by using xenografts from vehicle treated mice.

Xeno-08-A:

M21	mouse
U87MG	mouse
HCT116	CD1 nu/nu mouse
A549 (human lung carcinoma)	CD1 nu/nu mice
Calu 6	CD1 nu/nu mice

Xeno-08-Mu1:
A549, HCT116, U87MG, M21, Calu 6, A431, BT474, Colo205, H1975, MDA MB-231, Mes-Sa/Dx5, PC3, SW707, A2780, A2780ADR

Sections of 3 µm of the cancer cell line array and the xenograft arrays were mounted on positively charged SuperFrost®Plus slides (Menzel-Glaeser, Braunschweig, Germany) and stored at -80°C with desiccant.

Example 3.2: IHC procedure

The immunohistochemical staining procedure starting with the deparaffinization of sections was done with the staining instruments Discovery™ or the Discovery® XT (Ventana Medical Systems, Inc., Tucson, USA). After deparaffinization sections were heated for epitope retrieval in Tris-EDTA buffer pH 8 or incubated with protease at 37°C during 8 (protease 1) or 12 min (protease 2). Endogenous peroxidase was blocked by incubation in 3 % hydrogen peroxide (part of OmniMap™ or UltraMap™ Kits, Ventana Medical Systems). After warming the supernatants at room temperature at the day of the first immunohistochemical run, sodium azide was added to a final concentration of 0.01 % (w/v), and supernatants were stored at 4°C. One series of supernatants was always stained with the same instrument. Sections were incubated with the supernatants of multiclones and subclones, or recombinantly expressed antibodies (2-10 µg/ml; 100 µl per slide), and then with the appropriated secondary antibody, as are the HRP conjugated

polymers of the OmniMap or UltraMap Kit, for 16 min at 37°C. Horseradish peroxidase (HRP) catalyzes the 3,3'-diaminobenzidine tetrahydrochloride (DAB)/H_2O_2 reaction to produce an insoluble dark brown precipitate that can be visualized. Sections were counterstained with hematoxylin. Slides were washed in tap water, dehydrated, and mounted with glass coverslips in permanent mounting media Entellan® Neu (VWR, Germany). Slides were stored at room temperature, and paraffin blocks were stored at 6°C

Diagram of immunohistochemical staining procedure:

A. Pre-treatment

- Deparaffinization (temperature: 75°C during 8 min, then EZ Prep Buffer at 75°C during 8 min)
- Cell conditioning (Tris EDTA buffer pH 8, time: 48 min; temperature: 95°C)

or

- Protease conditioning (protease 1: 0.5 U/ml, or protease 2: 0.1 U/ml; time: 8 or 12 min; temperature: 37°C)

B. Detection

- Primary antibody (volume: 100 µl; time: 32 min; temperature: 37°C)
- Secondary antibody (OmniMap or UltraMap conjugated with HRP; volume: 100 µl; time: 16 min; temperature: 37°C)
- Detection (ChromoMap DAB)
- Counterstain (Hematoxylin II; time: 8 min)
- Post-counterstain (Bluing Reagent)
- Slide cleaning

Cell line arrays were scanned with the automated microscope Ariol SL-50 at X20 (scale x/y: 1 pixel = 0.38 x 0.38 μm^2). A circular region (input region area) of 0.1 mm^2 was set in each tissue spot. The brown color of the positive immunohistochemical labeling was quantified with the help of the image analysis software of the Ariol SL-50 by setting thresholds for "color", "hue", and "saturation". The positive area in the input region area was the fraction of brown labeled tissue. The intensity of positive area was the mean grey value of brown color measured in 3 black and white images photographed with a red, a blue and a green filter. Grey values range from 0 (black) to white (255). Expression was calculated according to positive area fraction*(255-intensity). Data were displayed with Spotfire®DecisionSite™ (version 9.0, Spotfire Inc.).

Example 3.3: ELISA protocol

Recombinant integrins (1 µg/ml) were coated on microtiter plates by adsorption (4°C; 16 h) from coating buffer (150 mM NaCl; 1 mM $CaCl_2$; 1 mM $MgCl_2$; 10 µM $MnCl_2$; 50 mM Tris-Cl; pH 7.5). The plates were washed (wash buffer: 0.5% BSA; 0.05% Tween 20 in PBS), blocked (1 h; 4°C; 5 % BSA in PBS), and incubated with primary antibodies serially diluted in wash buffer (1 h; 37°C). After washing, secondary detection antibody (goat-anti-rabbit HRP; 1:5000) was added (1 h; 37°C), followed by washing and detection using tetramethyl-benzidine (100 µg/ml) in citrate-phosphate buffer (pH 5.0), development with sulphuric acid, and reading against a reagent blank at 450 nm. Results were expressed following subtraction of the blank values which were typically < 5 % of positive control values.

Example 3.4: FACS analysis

Cells in log growth were harvested using trypsin (0.5 µg/ml)/EDTA (0.2 µg/ml), washed in FACS buffer (PBS plus 0.9 mM $CaCl_2$; 0.5 mM $MgCl_2$; 0.5% w/v BSA), and incubated with anti-integrin antibodies (60 min; 4°C; 10 µg/ml in FACS buffer). After washing, the cells were stained with Alexa-488 labeled antirabbit IgG (Invitrogen), or goat anti-mouse IgG FITC (Becton-Dikinson) (30 min; 4°C), washed and re-suspended in FACS buffer (500 µl/tube). Cells were analyzed on a FACScan (Becton-Dickinson) and the mean intensity fluorescence (MIF) was normalized to the MIF of the negative control (cells stained with PI and secondary labeled antibody, without primary antibody).

Example 3.5: Evaluation and statistics

The IC_{50} for antibody binding in ELISA was determined from triplicate data points by nonlinear curve fitting in the graphic software package Graphpad Prism (Ver 5.0: GraphPad Software, Inc. LaJolla Ca). Flow cytometry was analyzed using the BD Facs-scan program (CellQuest MacOS 8.6).

EXAMPLE 4: Characterization of anti-αvβ3 clones and anti-αvβ3-antibodies

Example 4.1: Characterization of E3528-2-7, E3528-2-11 and E3528-2-12

The supernatants from 24 subclones obtained from multiclones 2 and 63 of rabbit E3528 were screened undiluted on the FFPE cell line array of cancer cell lines CAX05. Cytoplasmic signals without clear membrane profile were excluded as non-integrin specific. Subclones of the multiclone 2 exhibit a plasma membrane staining (Figure 1). The selectivity of the subclones regarding certain cell types was compared with the mouse

monoclonal IgG, clone 20H9. Clone 20H9 is an anti-β3 chain antibody (Mitjans et al., J Cell Sci 1995, 108(Pt 8): 2825-38), that cross reacts in FFPE, however with a low binding affinity. The positive subclones were tested in a second run on the xenograft array Xeno-08-A to confirm cross-reactivity on tumor tissue (Table 1).

Table 1: Clones to extracellular αvβ3 domain. The staining intensity was graded from - (negative) to +++ (strong).

Clone ID	Cancer cell line array (CAX05)	Xenograft array (Xeno-08-A)
MRK-1a-E3528-2 multiclone	M21++, U87MG++, HCT116-, Calu 6-, A549-, SUIT 7-, WM164+, HT29-, MDA-MB231-	M21+-, U87MG-, HCT116-, Calu 6-, A549-
MRK-1a-E3528-2 subclones	clones 2-2 to 2-12 were positive	M21+, U87MG-, HCT116-, Calu 6-, A549-

Three subclones, 2-7, 2-11 and 2-12, were selected as final clones, based on staining intensity, selectivity regarding known αvβ3 integrin positive cells and quality of plasma membrane staining. The three anti-αvβ3 clones exhibited similar staining characteristics, showing distinct plasma membrane staining (Figure 2). In the xenograft array Xeno-08-Mu1, M21 xenografts were the only positive ones (Figure 3). The subclones were negative in a range of carcinomas including A549 and HCT116, known not to express αvβ3 (Table 2), and in the anchorage independent Raji-T-cell lymphoma. These data were in agreement with a αvβ3-integrin epitope of the antibodies. The selectivity and intensity of staining with the three antibodies on the cancer cell line array was nearly identical (Figure 4). The selectivity of staining of the three antibodies was compared with the monoclonal anti-β3 integrin antibody clone 20H9 (Figure 5, shown for clone E3528-2-7). Regarding cell selectivity the three clones showed similar characteristics to the clone 20H9, indicating that the epitope of the three antibodies was a αvβ3 epitope. High expression of αvβ3 in M21 cell lines was shown previously by FACS analysis with clone LM609 (Table 2; Mitjans et al., Int J Cancer 2000, 87(5): 716-723).

Table 2: FACS analysis and anti-αvβ3 immunohistochemistry of several cancer cell lines.

Cancer cell line	FACS αvβ3 (MIF/mean background)	FACS % cells	FACS αvβ3 x % cells	IHC αvβ3_E3528-2-7 on CAX08 (Expression)
HCT116	0.96	0.2	0.2	0.9
KYSE-30	0.98	0.52	0.5	0.3
M21	1.55	91.8	142.3	75.6
A549	0.63	1.2	0.8	6.7
NCI-H460	0.79	0.0	0.0	6.5
Calu-6	1.5	3.6	5.4	1.1

Example 4.2: Characterization of E3531-227 and E3531-229

Similarly to Example 4.1, the subclone 227-3 was obtained following a second fusion run of B-lymphocytes of rabbit E3531. The supernatants from 18 subclones obtained from multiclones 227 and 229 were screened undiluted on the FFPE cell line array of cancer cell lines CAX05. Cytoplasmic signals without clear membrane profile were excluded as non-integrin specific. Subclones of both multiclones exhibited a good plasma membrane staining. The selectivity of the subclones regarding certain cell types was compared with the mouse monoclonal IgG, clone 20H9. The positive subclones were tested in a second run on the xenograft array Xeno-08-A to confirm cross-reactivity on tumor tissue. Six subclones, E3531-227-2, -227-3, 227-6, -229-3, 229-9 and -229-11 were selected as final clones, based on staining intensity, selectivity regarding known αvβ3 integrin positive cells, and quality of plasma membrane staining.

The selected final clones were cultured and the antibodies purified. The six anti-αvβ3 clones exhibited similar staining characteristics, showing distinct plasma membrane staining (Figure 6). In the xenograft array Xeno-08-Mu1, M21 xenografts were the only positive ones (Figure 7). U87MG were negative. The selectivity of staining with the three antibodies E3531-227-2, E3531-227-3 and E3531-227-6 on the cancer cell line array CAX08 was nearly identical (Figure 8). The selectivity of staining of the anti-αvb3 antibodies was compared with the monoclonal anti-β3 integrin antibody clone 20H9, shown for clone E3531-227-3 (Figure 8). Regarding cell selectivity, the clones showed similar characteristics to the clone 20H9, indicating that the epitope of the six antibodies was an αvβ3 epitope. High expression of αvβ3 in M21 cell lines was shown previously by FACS analysis with clone LM609 (Table 3; Mitjans et al., Int J Cancer 2000, 87(5): 716-723). The clones E3531-227-3 and E3531-229-3 producing the highest IgG amount were sequenced and showed identical sequences (cf. below).

Table 3: FACS analysis and anti-β3 immunohistochemistry of several cancer cell lines.

Cancer cell line	**FACS αvβ3 (MIF/mean background)**	**FACS % cells**	**FACS αvβ3 x % cells**	**IHC αvβ3_E3531-227-3 on CAX08 (Expression)**
HCT116	0.96	0.2	0.2	1.17
KYSE-30	0.98	0.52	0.5	0.00
M21	1.55	91.8	142.3	97.4
A549	0.63	1.2	0.8	0.02
NCI-H460	0.79	0.0	0.0	0.01
Calu-6	1.5	3.6	5.4	0.12

The staining characteristics of the six clones E3531-227-2, -227-3, 227-6, -229-3, 229-9 and -229-11, as are "plasma membrane staining" and high signal in M21 cells, were in agreement with an αvβ3-integrin epitope of the antibodies. The antibodies detected αvb3-integrin in formaldehyde-fixed paraffin-embedded tissue. EM22703 was further developed. It reacts equally well on intact αiibβ3 (IC_{50} was the same in ELISA), indicating that it was detecting the β3 chain in complex with both partners. This reflected the power of the monoclonal antibody to detect exactly what it was screened against. In practice, the cross reactivity should not prove a serious disadvantage to detecting αvβ3 in situ: αiibβ3 is expressed solely on the macrophage / megakaryocytic blood borne lineages, and rarely expected to be seen in the intra-tissue locations characteristic of αvβ3.

EXAMPLE 5: Characterization of anti-αvβ5 clones and anti-αvβ5-antibodies

The supernatants from 27 subclones obtained from multiclones 13, 40 and 99 of rabbit E3536 were screened undiluted on the FFPE cell line array of cancer cell lines CAX05. Three subclones, 99-1, 99-2 and 99-3, exhibited a plasma membrane staining (Figure 9). They hybridoma supernatants were highly specific for αvβ5 over αvβ3 (a factor of > 100 in apparent Kd), with EC_{50} on the immunogen of 50 pM (Figure 10). The positive subclones were tested on the xenograft array to confirm cross-reactivity on tumor tissue (Figure 9, right column). Cell lines showed different degrees of αvβ5 expression if grown in culture compared to xenograft tissue.

Table 4: Clones to extracellular αvβ5 domain. The staining intensity was graded from - (negative) to +++ (strong).

Clone ID	**Cancer cell line array (CAX05)**	**Xenograft array (Xeno-08-A)**
MRK-1c-E3536-99 multiclone	HT29+++, WM164-, M21++, U87MG++, HCT116+++, Calu 6++, A549+++, Suit 7+++, MDA-MB231++, Kyse30++, NCI-H460+++	M21++, U87MG++, HCT116++, Calu 6++, A549+
MRK-1c-E3536-99 subclones	clones 99-1, 99-2 and 99-3 were positive; plasma membrane was labeled	M21++, U87MG++, HCT116+, Calu 6+, A549+

Three subclones, 99-1, 99-2, and 99-3, were selected as final clones based on staining intensity, selectivity regarding known αvβ5 integrin positive cells and quality of plasma membrane staining (Table 4). The three anti-αvβ5 clones labeled the plasma membrane (Figure 11). In the xenograft array Xeno-08-Mu1, several xenografts were positive, especially A431 (Figure 12). The three anti-αvβ5 clones, E3536-99-1, -99-2, and -99-3, exhibited very similar staining characteristics regarding cell selectivity and staining intensity

measured with image analysis (Figure 13). Cell lines that showed a high αvβ5_E3536-99-1 (or -99-2, or -99-3) signal (i.e. HT-29, HCT116, Kyse 30, A549 and NCI-H460) exhibited high αvβ5 expression analyzed by FACS with the clone P1F6 (Kemperman et al., Exp Cell Res 1997, 234(1): 156-164; Mitjans et al., Int J Cancer 2000, 87(5): 716-723). The M21 cell line exhibited a low signal with immunohistochemistry and a corresponding low signal by FACS analysis (Table 5). Raji lymphoma cells, that were αv negative, showed no signal on the cancer cell line array with immunohistochemistry.

Table 5: FACS analysis and anti-αvβ5 immunohistochemistry of several cancer cell lines.

Cancer cell line	**FACS αvβ5 (MIF/mean background)**	**FACS % cells**	**FACS αvβ5 x % cells**	**IHC αvβ5_E3536-99-1 on CAX08 (Expression)**
HCT116	6.21	72.5	450.2	103.0
KYSE-30	7.16	80.39	575.6	136.0
M21	0.84	39.1	32.8	50.0
A549	1.69	97.0	163.9	142.0
NCI-H460	1.67	78.0	130.3	128.0
Calu-6	5.2	74.4	386.9	75.0

The subclone characteristics matched FACS and biochemical data for the distribution of αvβ5 integrin and supported subclones 99 as reacting with a αvβ5-integrin epitope. Clones 99 were derived from a unique hybridoma cell, as revealed by cDNA sequencing (cf. below).

EXAMPLE 6: Characterization of anti-αvβ6 clones and anti-αvβ6-antibodies

The supernatants from 33 subclones obtained from these multiclones were screened undiluted on the FFPE cell line array of cancer cell lines CAX08. Cytoplasmic signals without clear membrane profile were excluded as non-integrin specific. Many subclone supernatants tested on the cancer cell lines were positive after heat as well as after protease pretreatment. Subclones of the multiclones 52 (Figure 14), 106 and 118 showed a good plasma membrane staining. The positive subclones were tested in a second run on the xenograft array Xeno-08-Mu1 to confirm cross-reactivity on tumor tissue. Protease pre-treatment resulted in a higher signal for the subclones of 52 and 106. Therefore, these subclone supernatants were tested on protease pretreated xenografts only. The different subclones of the multiclone 52 were identical in their staining selectivity and specificity. The clone 106-1 was negative in SW707 in contrast to the subclones of the multiclones 52 and 118 (Table 6).

Table 6: Subclone supernatants to extracellular αvβ6 domain. The staining intensity was graded from - (negative) to +++ (strong) as well as from 0 (negative) to 3 (strong).

Tissue Pretreatment	Cancer cell lines (CAX08) Protease 0.1 units/ml 12 min		
Clone ID	Plasma-membrane	Cytoplasm	Comment
52-1	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-2	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-3	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-4	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-5	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-6	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-7	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-8	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-9	3	0	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707+
52-10	2	0	Kyse30++, A431++, MDA-MB468++, Colo205+, HT29+, SW707+
52-11	2	0	Kyse30++, A431++, MDA-MB468++, Colo205+, HT29+, SW707+
52-12	2	0	Kyse30++, A431++, MDA-MB468++, Colo205+, HT29+, SW707+
106-1	3	1	Kyse30+++, A431+++, MDA-MB468+++, Colo205++, HT29++, SW707-
118-1	3	0	Kyse30++, A431++, MDA-MB468++, Colo205++, HT29++, SW707-
	Xenografts (Xeno-08-Mu1) Protease 0.1 units/ml 12 min		
52-1	3	1	H1975+++, A431+++, Colo205++, SW707-
52-2	3	1	H1975+++, A431+++, Colo205++, SW707-
52-3	3	1	H1975+++, A431+++, Colo205++, SW707-
52-4	3	1	H1975+++, A431+++, Colo205++, SW707-
52-6	3	1	H1975+++, A431+++, Colo205++, SW707-
52-8	3	1	H1975+++, A431+++, Colo205++, SW707-
52-9	3	1	H1975+++, A431+++, Colo205++, SW707-
106-1	1	0-1	H1975+, A431+
118-1	3	0	A431+++, H1975+++, Col205+, protease pre-treatment was better

In xenograft tissue, the non small cell lung cancer cell line (NSCLC) H1975 showed the highest staining intensity (Figure 14H). On the cancer cell line array, the two squamous cell carcinomas Kyse30 (Figure 14I) and A431 (Figures 14A), and on the xenograft array the A431 xenografts (Figures 14B) showed a high signal. The cell lines with high staining intensity on the cancer cell line array were HT29 (Figure 14G), MDA-MB468, Colo205 and A431. This corresponded to high β6 integrin mRNA of these cell lines. The selectivity and specificity of the subclone supernatants of the multiclones 52, 106 and 118 were in agreement with a αvβ6 epitope recognized by the antibodies. Nine subclones, 52-1, 52-2, 52-3, 52-4. 52-6, 52-8, 52-9, 106-1 and 118-1 were selected as final clones, based on

staining intensity, selectivity regarding known αvβ6 integrin positive cells and quality of plasma membrane staining (Table 6). Of subclones with identical stainings, the ones with the highest IgG concentration were selected as final clones.

The clone with the highest IgG concentration, clone E3866-52-1, was cultured and the antibody purified according to standard protocols. Activity of the antibody was shown by IHC on the cancer cell line array (Figure 15). With the recombinant antibody, several slides of the cancer cell line array and xenografts were stained (Figure 16). In xenografts of HT29 colon cancer, H1975 lung carcinoma and a patient prostata tumor explant PRXF MRIH (Oncotest GmbH, Freiburg) the anti-αvß6 recombinant antibody showed a pronounced signal, whereas a M21 melanoma xenograft with no β6 mRNA expression, was negative (Figure 16). The anti-αvβ6 recombinant antibody showed a clear staining of the plasma membrane (Figure 17). The signal on the cancer cell line array was quantified with the help of image analysis (Figure 18). The cell lines with high antibody staining signal, as were HT29, Colo205 or MDA-MB468, corresponded to the cell lines with high mRNA levels of the β6 integrin mRNA. The recombinant anti-αvβ6 antibody showed high slide-to-slide (r=0.996) and run-to-run reproducibility (r=0.991, Figure 19) using automatized staining procedures.

The rabbit IgG recombinant antibody αvβ6 (EM05201) generated against an αvβ6-integrin peptide was suitable for FFPE tissue. The ELISA specificities and staining characteristics of the recombinant antibody αvβ6 (EM05201), as were "plasma membrane staining" and high signal in cell lines expressing high β6 integrin mRNA, were in agreement with an αvβ6-integrin epitope of the antibody.

EXAMPLE 7: Characterization of anti-αvβ8 clones and anti-αvβ8-antibodies

The supernatants from 36 subclones obtained from these multiclones were screened undiluted on the FFPE cell line array of cancer cell lines CAX08. All 36 subclones showed a membrane signal, none was excluded due to non-integrin specific cytoplasmic staining. Many subclone supernatants tested on the cancer cell lines were positive after heat as well as after protease pre-treatment. For each multiclone, the four subclones with the highest IgG concentrations were selected for further testing on the xenograft array Xeno-08-Mu1 to confirm cross-reactivity on tumor tissue (Figure 20). Protease pre-treatment resulted in a higher signal for the subclones. The subclones of the multiclone 6 were negative on the xenografts. The cell lines with high staining intensity on the cancer cell line array, as were

Ovcar-3, M24met, MDA-MB 468 and A431 showed the highest mRNA expression of the ß8 integrin (Table 7). The subclones 40-4, 40-10, 40-11, 133-5, 133-8 and 133-9 were selected as final clones, based on selectivity regarding known β8 mRNA expression and quality of plasma membrane staining. Of subclones with identical stainings, the ones with the highest IgG concentration were selected as final clones.

Table 7: Subclone supernatants to extracellular αvβ8 domain. The staining intensity was graded from - (negative) to +++ (strong) as well as from 0 (negative) to 4 (very strong).

Tissue Pre-treatment	**Cancer cell lines (CAX08)** Protease 0.1 units/ml 12 min		
Clone ID	**Plasma-membrane**	**Cytoplasm**	**Comment**
6-1	3	0	Ovcar3+++ and Scov3++ at contact zones
6-5	3	0	Ovcar3+++ and Scov3++ at contact zones
6-8	3	0	Ovcar3+++ and Scov3++ at contact zones
6-12	3	0	Ovcar3+++ and Scov3++ at contact zones
40-4	3	0	Ovcar3+++ and Scov3++ at contact zones
40-9	3	0	Ovcar3+++ and Scov3++ at contact zones
40-10	3	0	Ovcar3+++ and Scov3++ at contact zones
40-11	3	0	Ovcar3+++ and Scov3++ at contact zones
133-5	4	0	Ovcar3+++ and Scov3++ at contact zones, A431++, MDA-MB468+++, M24-met+++, Scov3++, Igrov1+++, PC3++, Kyse30++
133-8	4	0	Ovcar3+++ and Scov3++ at contact zones, A431++, MDA-MB468+++, M24-met+++, Scov3++, Igrov1+++, PC3++, Kyse30++
133-9	4	0	Ovcar3+++ and Scov3++ at contact zones, A431++, MDA-MB468+++, M24-met+++, Scov3++, Igrov1+++, PC3++, Kyse30++
133-12	4	0	Ovcar3+++ and Scov3++ at contact zones, A431++, MDA-MB468+++, M24-met+++, Scov3++, Igrov1+++, PC3++, Kyse30++
	Xenografts (Xeno-08-Mu1) Protease 0.1 units/ml 12 min		
6-1	0	0	strong on Ovcar3 in vitro, negative on xenografts
6-5	0	0	strong on Ovcar3 in vitro, negative on xenografts
6-8	0	0	strong on Ovcar3 in vitro, negative on xenografts
6-12	0	0	strong on Ovcar3 in vitro, negative on xenografts
40-4	0	0	strong on Ovcar3 in vitro, negative on xenografts
40-9	0	0	strong on Ovcar3 in vitro, negative on xenografts
40-10	0	0	strong on Ovcar3 in vitro, negative on xenografts
40-11	0	0	strong on Ovcar3 in vitro, negative on xenografts
133-5	3	0	U87MG++, A431++
133-8	3	0	U87MG++, A431++
133-9	3	0	U87MG++, A431++
133-12	3	0	U87MG++, A431++

The clone with the highest IgG concentration, clone E3875-133-9, was cultured and the antibody purified according to standard protocols. Activity of the antibody was shown by IHC on the cancer cell line array (Figure 21).

With the recombinant antibody, several slides of the cancer cell line arrays, xenografts, and an array out of normal human tissue were stained. The cancer cell lines Ovcar-3 (ovarian carcinoma), M24-met (melanoma) and MDA-MB468 (breast carcinoma), all expressing β8 mRNA, were positive, whereas MCF-7 cells (breast carcinoma) without β8 mRNA, were negative (Figure 22). From these cell lines, no xenografts were available. In H1975 lung carcinoma xenografts and stronger in the prostata tumor explant PRXF MRIH (Oncotest GmbH, Freiburg) xenografts the anti-αvβ8 recombinant antibody showed some signal (Figure 22). The strongest signal was observed in human peripheral nerves (Figure 23). The anti-αvβ8 recombinant antibody showed a clear staining of the plasma membrane (Figure 24). The signal on the cancer cell line array was quantified with the help of image analysis (Figure 25). The cell lines with high antibody staining signal, as were Ovcar-3, M24-met, and MDA-MB468, corresponded to the cell lines with high mRNA levels of the β8 integrin mRNA. The recombinant anti–αvβ8 antibody showed high slide-to-slide (r=0.982) and run-to-run reproducibility (r=0.986, Figure 26).

The rabbit IgG recombinant antibody αvβ8 (EM13309) generated against a αvβ8-integrin peptide was suitable for FFPE tissue. The ELISA specificities and staining characteristics of the recombinant antibody αvβ8 (EM13309) as are "plasma membrane staining", high signal in cell lines expressing high ß8 integrin mRNA, and strong labeling of myelinated peripheral nerves were in agreement with a αvß8-integrin epitope of the antibody.

EXAMPLE 8: Characterization of anti-αv clones and anti-αv-antibodies

The previously selected multiclones that bind to αvß6 as well as to αvß8 were the multiclones E3866-68 and E3875-13. The supernatants from 24 subclones obtained from these multiclones were screened undiluted on the FFPE cell line array of cancer cell lines CAX08. All 24 subclones showed a high plasma membrane signal, however also some cytoplasmic signal (Figure 27). Nine subclones, 5 of the multiclone E3875-13 and 4 of the multiclone E3866-68, were selected for testing on xenograft tissue to confirm cross-reactivity on tumor tissue. Because of a very high signal, supernatants of clones 13-3, 13-9 and 68-7 were diluted 1:5 and 1:10. The diluted supernatants 13-3- and 13-9 stained all cells on the cancer cell line array except Raji lymphoma cells and the Sf9 insect cell. The xenografts show high plasma membrane signal, and also some cytoplasmic staining (Figure 27). After 1:5 dilution the subclone 68-7 did not stain MiaPaca2, a cell line that was positive with the subclone 13-3. The epitope of the subclones of the multiclone E3688-68

might be different from E3875-13. The subclones E3875-13-3 and -13-9 were selected as final clones, based on their highest IgG concentration (Table 8).

Table 8: Subclone supernatants to extracellular αv domain. The staining intensity was graded from - (negative) to +++ (strong) as well as from 0 (negative) to 3 (strong).

Tissue Pre-treatment	**Cancer cell lines (CAX08)** Heat in Tris EDTA pH 8		
Clone ID	**Plasma-membrane**	**Cytoplasm**	**Comment**
2b-E3875-13-3	3	1	high plasma membrane and Golgi, some cytoplasmic
2b-E3875-13-5	3	1	high plasma membrane and Golgi, some cytoplasmic
2b-E3875-13-6	3	1	high plasma membrane and Golgi, some cytoplasmic
2b-E3875-13-7	3	1	high plasma membrane and Golgi, some cytoplasmic
2b-E3875-13-9	3	1	high plasma membrane and Golgi, some cytoplasmic
2a-E3866-68-4	3	2	high plasma membrane, Golgi also positive, not clean, some cytoplasmic
2a-E3866-68-5	3	2	high plasma membrane, Golgi also positive, not clean, some cytoplasmic
2a-E3866-68-7	3	2	high plasma membrane, Golgi also positive, not clean, some cytoplasmic
2a-E3866-68-9	3	2	high plasma membrane, Golgi also positive, not clean, some cytoplasmic
	Xenografts (Xeno-08-Mu1) Heat in Tris EDTA pH 8		
2b-E3875-13-3	3	2	1:5 and 1:10 dilution tested, high plasma membrane and Golgi, also still some cytoplasm, better staining of A431 than 68-clone
2b-E3875-13-5	3	2	high plasma membrane and Golgi, also cytoplasm probably due to very high signal, better staining of A431 than 68-clone
2b-E3875-13-6	3	2	high plasma membrane and Golgi, also cytoplasm probably due to very high signal, better staining of A431 than 68-clone
2b-E3875-13-7	3	2	high plasma membrane and Golgi, also cytoplasm probably due to very high signal, better staining of A431 than 68-clone
2b-E3875-13-9			1:5 and 1:10 dilution tested, high plasma membrane and Golgi, also still some cytoplasm, better staining of A431 than 68-clone
2a-E3866-68-4	3	1	high plasma membrane staining and Golgi, several cells also cytoplasm, might be due to high concentration
2a-E3866-68-5	3	1	high plasma membrane staining and Golgi, several cells also cytoplasm, might be due to high concentration
2a-E3866-68-7	3	1	high plasma membrane staining and Golgi, several cells also cytoplasm, might be due to high concentration, with 1:5 and 1:10 dilution MiaPaCa2 negative
2a-E3866-68-9	3	1	high plasma membrane staining and Golgi, several cells also cytoplasm, might be due to high concentration

The clone with the highest IgG concentration, clone E3875-13-9, was cultured and the antibody purified according to standard protocols (Protein G Sepharose, HiLoad Superdex

200 pg). Activity of the antibody was shown by IHC on the cancer cell line array (Figure 28).

With the recombinant antibody, several slides of the cancer cell line arrays and xenograft arrays were stained (Figure 29). In cancer cell lines as well as in xenografts the anti-αv recombinant antibody showed a pronounced signal. Negative are lymphoma cell lines, like Raji and Pfeiffer lymphoma that do not express αv-integrin mRNA. The anti-αv recombinant antibody showed a clear staining of the plasma membrane (Figure 30). The signal on the cancer cell line array was quantified with the help of image analysis (Figure 31). The recombinant anti-αv antibody showed slide-to-slide (r=0.947) and run-to-run reproducibility (r=0.924, Figure 32).

The rabbit IgG recombinant antibody αv (EM01309) generated against the αvß8-integrin peptide was suitable for FFPE tissue. The ELISA specificities and staining characteristics of the recombinant antibody αv (EM01309), as were "plasma membrane staining", high signal in cell lines expressing αv-integrin mRNA, and no signal in lymphoma cell lines not expressing αv-integrin were in agreement with the αv-chain epitope of the antibody.

EXAMPLE 9: Characterization of anti-β3 cytoplasmic domain integrin clones and anti-β3-cytoplasmic domain integrin antibodies

The supernatants from 24 subclones obtained from multiclones 2 and 67 were screened undiluted on the FFPE cell line array of cancer cell lines CAX05. Cytoplasmic signals without clear membrane profile were excluded as non-integrin specific. Subclones of the multiclone 2 exhibited a good plasma membrane staining. The selectivity of the subclones regarding certain cell types was compared with the mouse monoclonal IgG, clone 20H9. The positive subclones were tested in a second run on the xenograft array Xeno-08-A to confirm cross-reactivity on tumor tissue. Three subclones, 2-4, 2-10 and 2-12, were selected as final clones, based on staining intensity, selectivity regarding known αvβ3 integrin positive cells, and quality of plasma membrane staining (Table 9).

Table 9: Subclones to intracellular β3 domain. The staining intensity was graded from - (negative) to +++ (strong) as well as 1 (low), 2(medium), 3 (high).

Tissue	CAX05			Xeno-08-A		
Clone ID	Plasma-mem-brane	Cytoplasm	Comment	Plasma-mem-brane	Cytoplasm	Comment
2-1	3	0	M21 +Golgi, avb3 specific			
2-2	3	0	M21 +Golgi, avb3 specific			
2-3	1	0	M21 +Golgi, avb3 specific			
2-4	3	0	M21 +Golgi, avb3 specific	3	0	+ Golgi, M21 +++, U87MG+
2-5	1	0	M21 +Golgi, avb3 specific			
2-6	1	0	M21 +Golgi, avb3 specific			
2-7	1	0	M21 +Golgi, avb3 specific			
2-8	3	0	M21 +Golgi, avb3 specific			
2-9	1	0	M21 +Golgi, avb3 specific			
2-10	3	0	M21 +Golgi, avb3 specific	3	0	+ Golgi, M21 +++, U87MG+
2-11	1	0	M21 +Golgi, avb3 specific			
2-12	3	0	M21 +Golgi, avb3 specific	3	0	+ Golgi, M21 +++, U87MG+
67-1	0	0				
67-2	0	0				
67-3	0	0				
67-4						
67-5	1	2	similar to 67-7			
67-6						
67-7	1	2	WM164+++, M21++, U87MG+, however predominantly cytoplasmic diffuse and precipitation in many other cells			
67-8	0	0				
67-9	1	2	similar to 67-7			
67-10	1	2	similar to 67-7			
67-11	0	0				
67-12	0	0				

The selected final clones were cultured and the antibodies purified. The three anti-β3 clones, E3592-2-4, -2-10, and -2-12, exhibited similar staining characteristics, showing distinct plasma membrane staining (Figure 33). In the xenograft array Xeno-08-Mu1, M21 xenografts were positive (Figure 34). U87MG were negative. The selectivity of staining with the three antibodies on the cancer cell line array CAX08 was nearly identical (Figure 35). The intensity of staining varied and was strongest for clone E3592-2-12. The selectivity of staining of the three antibodies was compared with the monoclonal anti-β3 exo-domain integrin antibody clone 20H9, shown for clone E3592-2-12 (Figure 36). Regarding cell selectivity, the three clones showed similar characteristics to the clone 20H9, indicating that

the epitope of the three antibodies was a β3 epitope. High expression of αvβ3 in M21 cell lines was shown previously by FACS analysis with clone LM609 (Table 10; Mitjans et al., Int J Cancer 2000, 87(5): 716-723).

Table 10: FACS analysis and anti-β3 immunohistochemistry of several cancer cell lines.

Cancer cell line	FACS αvβ3 (MIF/mean background)	FACS % cells	FACS αvβ3 x % cells	IHC β3_E3592-2-12 on CAX08 (Expression)
HCT116	0.96	0.2	0.2	10.1
KYSE-30	0.98	0.52	0.5	9.2
M21	1.55	91.8	142.3	119.6
A549	0.63	1.2	0.8	6.7
NCI-H460	0.79	0.0	0.0	1.5
Calu-6	1.5	3.6	5.4	2.4

The staining characteristics of the three clones E3592-2-4, -2-10, and -2-12, as were "plasma membrane staining" and high signal in M21, were in agreement with an β3-integrin epitope of the antibodies. The rabbit hybridoma clones E3592-2-4 2-10, and -2-12 generated against β3-integrin peptide produced antibodies suitable for FFPE tissue. Their epitope recognition was in agreement with their binding β3 cytoplasmic domain epitope. The antibody chains from the clone producing the most strongly staining antibody, E3592-2-12, was cDNA cloned and the antibody encoding regions were multiply sequenced (cf. below).

EXAMPLE 10: Sequencing and sequence listing

Several clones were assessed by cDNA sequencing (Table 11). The information recorded in computer readable form is identical to the written sequence listing.

Table 11: Sequenced clones.

Clone ID	Recombinant antibody identifier	Specificity	Example	SEQ ID NOs
E3531-227-3	EM22703	anti-αvβ3	4.2	81-120 except 90, 110
E3531-229-3	EM22903	anti-αvβ3	4.2	81-120 except 90, 110
E3536-99-1	-	anti-αvβ5	5	1- 40 except 10, 30
E3536-99-2	EM09902	anti-αvβ5	5	1- 40 except 10, 30
E3536-99-3	-	anti-αvβ5	5	1- 40 except 10, 30
E3592-2-12	EM00212	anti-ß3	9	41- 80 except 50, 70
E3866-052-1	EM05201	anti-αvβ6	6	121-160 except 130, 150
E3875-0133-9	EM13309	anti-αvβ8	7	161-200 except 170, 190
E3875-013-9	EM01309	anti-αv	8	201-240 except 210, 230

Three primary sequencing runs on each heavy and light chain from the clones were assembled into contigs using Lasergene software (DNAstar Inc.) and analyzed using ClustalW multiple alignment tools. Clones E3531-227-3 and -229-3 had identical heavy and light chain sequences, confirming monoclonality of the antibody population. Clones E3536-99-1, -99-2 and -99-3 had identical heavy and light chain sequences, confirming monoclonality of the antibody population.

EXAMPLE 11: Recombinant RabMabs are specific for their ligands by ELISA.

In standard ELISA conditions wits 1 µg/ml coated integrin on the plate, the antibodies were essentially mono-specific for their immunogens, as defined to better than 4 logs of concentrations, and did not cross react significantly with the most closely related integrin chains (Figures 10, 37, 38), with the exception of EM09902 that showed a cross-reactivity (IC_{50} ~ 100 fold lower) with both gpiibiiia and αvβ3, so apparently recognized a related epitope on both the β5 and β3 chains of the complexes. It should not seriously affect FFPE usage, as the expression of αvβ5 is more prevalent than αvβ3 and the signal from EM09902 is extremely strong in IHC. The specificities of the other recombinant antibodies were indistinguishable in ELISA and in IHC staining from the hybridoma supernatants and antibodies derived from them (Figures 10, 37, 38). Indeed, on cDNA sequencing, two of the anti-αvβ3 antibodies, EM22703 and EM22903, were found to be derived from a single clone. The specificities in ELISA and the apparent binding affinities expressed as IC_{50} in ELISA were shown in Table 12.

Table 12: Recombinant RabMab IC_{50} in ELISA on isolated integrins.

Recombinant antibody identifier	Immunogen	αvβ3 (ng/ml)	αvβ5 (ng/ml)	αvβ6 (ng/ml)	αvβ8 (ng/ml)	Gpiibiiia (ng/ml)
EM22703	Human αvβ3 ECD	1.4	>10000	>>10000	>>10000	0.85
EM09902	Human αvβ5 ECD	~450	5.6	>>10000	>>10000	~400
EM00212	Human β3 ICD	>>10000	>>10000	>>10000	>>10000	>>10000
EM005201	Human αvβ6 ECD	>>10000	>>10000	3.8	>>10000	>>10000
EM013309	Human αvβ8 ECD	>>10000	>>10000	>>10000	4.0	>>10000
EM001309	Human αvβ3 ECD	4.7	5.6	4.9	4.1	>>10000

EXAMPLE 12: Recombinant RabMabs do not affect ligand binding to their receptors.

Both antibodies and small molecules can inhibit or enhance integrin activity, however the RabMabs selected here had no effect on ligand binding (Figure 39). Inhibitors of αvb3 and αvb5, reacted as predicted, positive (cilengitide) and negative (c(RßA-DfV)).

EXAMPLE 13: Recombinant RabMabs in live cell flow cytometry ("FACS")

In FACS native integrins present their native glycosylation pattern in situ, so this represents a specificity "gold standard". The RabMabs were assessed in FACS compared to standard well characterized murine monoclonal antibodies. For αvβ8 no antibody is commercially available. The antibodies reacted in FACS in a cell type dependent fashion and the FACS profiles closely matched the ELISA profiles of the antibodies. The results were summarized as mean intensity of fluorescence normalized to second layer control antibodies (Table 13). Differences in the absolute levels of expression between RabMabs and mouse Mabs were likely due to the varying affinity of the second layer antibodies.

Table 13: RabMab and comparator antibody activity in live cell flow cytometry using Alexa-488 labeled 2nd layer antibody vs. rabbit Ig or FITC labeled anti-mouse. rMIF is peak mean intensity of fluorescence, relative to second layer alone.

Antibody type	Antibody Identifier	Immunogen / target	HUVEC	HT-29	A549	M24Met	M21	M21-L	M21-Gpiib
MoMab	17E6	Human αv	9.9	7.3	6.8	7.8	9.3	1.0	1.1
MoMab	LM609	αvβ3	10.7	1.0	1.3	4.9	9.1	1.1	1.2
MoMab	P1F6	αvβ5	2.7	3.2	3.6	3.8	3.8	1.1	1.0
MoMab	P4C10	β1	38.9	14.5	7.5	14.0	7.2	12.1	10.8
RabMab	EM22703	Human αvβ3 / ECD β3	12.4	1.7	1.7	8.1	17.6	1.7	56.0
RabMab	EM09902	Human αvβ5 / ECD αvβ5	33.9	98.9	77.8	40.1	7.2	2.0	1.9
RabMab	EM00212	Human β3 cytoplasmic	1.1	1.1	1.1	1.2	1.2	1.2	1.2
RabMab	EM05201	Human αvβ6 / ECD αvβ6	1.6	8.0	1.3	1.0	1.1	1.1	1.0
RabMab	EM013309	Human αvβ8 / ECD αvβ8	1.1	19.9	1.8	15.5	22.1	1.2	1.0
RabMab	EM01309	Human αvβ3 / *ECD αv	28.8	1.8	1.3	2.5	1.3	1.3	1.0

Murine antibodies showed the HUVEC cells to express high levels of αv, αvβ3 and αvβ5 and no αvβ6 or αvβ8. In these cells, the RabMab EM01309 reacted strongly, and at levels comparable to 17E6 the murine anti-αv comparator. Murine antibodies showed high levels of αv, no αvβ3, high levels of αvβ5 and some αvβ6 on the HT-29 CRC cells. The RabMabs confirmed this and also showed high expression of αvβ8 integrin. RabMab EM01309

reacted weakly. Murine antibodies showed high levels of αv, no αvβ3, high levels of αvβ5 and no αvβ6 on the A549 NSCLC cells. The RabMabs binding confirmed this, and also showed no expression of αvβ8 integrin. RabMab EM01309 did not react. Murine antibodies showed high levels of αv, αvβ3 and αvβ5 and no αvβ6, and strong expression of β1 on the M24 Met melanoma cells. The RabMabs confirmed this and also showed strong binding of EM13309, revealing expression of αvβ8 integrin. RabMab EM01309 did not react. Murine antibodies showed high levels of αv, αvβ3 and αvβ5, no αvβ6, and strong expression of β1 on the M21 melanoma cells. The RabMabs confirmed this and also showed high levels of EM13309 binding, showing αvβ8 expression. RabMab EM01309 did not react. Murine antibodies showed no αv, αvβ3, αvβ5 or αvβ6 on the M21-L melanoma cells, and strong expression of β1. None of the RabMabs bound significantly above background. Murine antibodies showed no αv, αvβ3, αvβ5 or αvβ6 on the M21-gpiib melanoma cells, but strong expression of β1 and β3. The RabMabs EM05201, EM13309 and EM01309 did not bind. However, both EM22703 and EM09902 reacted, EM22703 strongly. This supported the ELISA data (cf. Example 11) that EM22703 could cross react with αIIbβ3, and that EM09902 could weakly cross react with both αvβ3 and αIIbβ3.

Live cell flow cytometry was unequivocal. The antibodies did not react above background with the αv-deficient M21-L cell line. As the normalized MIF attained with EM22703 and EM09902 approach 100, and with EM05201 and EM13309, this indicated the basic routinely attainable signal-to-noise of the antibodies, which was considerably above that attained with the standard LM609 and P1F6 reagents. It was not yet clear whether this was a result of higher affinity second layer fluorescinated reagents, rather than the properties of the primary RabMabs themselves, whatever the reason, the RabMabs were excellent reagents for FACS.

EM22703 gave a parallel staining in FACS to LM609, confirming that it was recognizing the αvβ3 complex, but also reacted strongly with M21-gpiib, showing that it was the β3 chain in the integrin complex that was being recognized by EM22703.

EM09902 staining generally paralleled the P1F6 staining, but reacted weakly with β3 as well as β5 chains. This was visible in the FACS of M21-gpiib cells, which did not express αvβ5, the supposed target of EM09902. By titering the antibody, the optimal concentration of reagent could be selected to minimize αvβ3 cross reactivity, while retaining a potent αvβ5 signal, as predicted from the ELISA data, and for FACS this was 0.3-1 µg/ml.

EM00212, directed against the b3 cytoplasmic domain was negative in FACS and ELISA.

As this is a species, isotype and target control, it is an excellent indicator of specificity, and suggests that an excellent signal to noise ratio of 100:1 is being achieved in FACS.

EM05201 was intensely specific for αvβ6 and revealed this protein only on HT29 cells, where it is known to be expressed.

EM13309 is the first reagent capable of live cell FACS of αvβ8 integrin, and provided the surprising information that αvβ8 is more widely expressed than αvβ6, on HT29 carcinoma, and M21 and M24 met melanomas. The staining of the neuroectodermal lineage was perhaps not surprising as αvβ8 was reported in the astrocyte neuronal lineage, however, staining of the carcinoma was unexpected, and may reflect biology: recent analysis of the αvβ8 showed that its expression in gut APCs controlled inflammatory response in this site. Conceivably, the CRC line HT29 also reflected such a mechanism.

EM01309, against the αv extracellular domain, was uniformly negative with the exception of HUVEC.

In summary, the RabMab antibodies were shown to function in live cell flow cytometry. This provides a valuable bridge between the biochemistry and tissue IHC for tumor validation and characterization. Especially the αvβ6 and αvβ8 reagents are an important resource for integrin studies, and the ability to make such antibodies with these reactivity profiles in RabMabs opens a door, finally on a rigorous analysis of integrin expression patterns in archival tissues.

EXAMPLE 14: Titration experiments

Titration experiments in FACS were performed to investigate appropriate staining concentrations (Figures 40-43). The curve forms did not indicate saturation, but begun to flatten above 1 mg/ml. The rabbit monoclonal antibodies are strong binders in FACS. Especially EM09902 had high affinity, and strong binding was seen to 0.1 µg/ml antibody concentration and hence, it could be successfully used at < 1 ug/ml staining concentration.

CLAIMS

1. Monoclonal rabbit antibody, or a fragment thereof, against integrin with insect-derived glycosylation pattern and integrin with another eukaryotic glycosylation pattern, each comprising at least a light chain variable region (V_L) and a heavy chain variable region (V_H), wherein the antibody has antigen binding specificity for a non-occluded epitope of an extracellular integrin domain or extracellular integrin chain domain, and wherein the antibody is able to bind to intact heterodimers of integrin in formalin fixed paraffin embedded (FFPE) material and on viable cells with the substantially same specificity.

2. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß3 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 95 (V_L-αvß3) and V_H comprises an amino acid sequence of SEQ ID NO: 96 (V_H-αvß3).

3. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß5 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 15 (V_L-αvß5) and V_H comprises an amino acid sequence of SEQ ID NO: 16 (V_H-αvß5).

4. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß6 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 135 (V_L-αvß6) and V_H comprises an amino acid sequence of SEQ ID NO: 136 (V_H-αvß6).

5. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin αvß8 and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 175 (V_L-αvß8) and V_H comprises an amino acid sequence of SEQ ID NO: 176 (V_H-αvß8).

6. Antibody according to claim 1, wherein the antibody binds to the extracellular domain of integrin chain αv and preferably, V_L comprises an amino acid sequence of SEQ ID NO: 215 (V_L-αv) and V_H comprises an amino acid sequence of SEQ ID NO: 216 (V_H-αv).

7. Polynucleotide encoding the antibody, or a fragment thereof, of any of claims 1 to 6.

8. Recombinant immunogen consisting of an extracellular integrin domain with insect-derived glycosylation pattern, optionally coupled as delta-trans membrane form.

9. Immunogen according to claim 9 having an amino acid sequence of SEQ ID NOs: 10, 90, 130, 170 or 210, or variants, mutants, parts of the amino acid sequence or at least 95 % homologous sequences having the same function.

10. Polynucleotide encoding the immunogen of claim 9.

11. Monoclonal antibodies obtained by immunizing a rabbit with the immunogen according to claim 8 or 9 and/or the polynucleotide according to claim 10, taking polyclonal antiserum with polyclonal antibodies and preparing the monoclonal antibodies.

12. Method for preparing monoclonal rabbit antibodies comprising the steps of:
(a) recombinantly expressing an extracellular integrin domain in insect cells,
(b) purifying the expressed extracellular integrin domain,
(c) immunizing a rabbit with the purified extracellular integrin domain,
(d) taking polyclonal antiserum comprising polyclonal antibodies from the rabbit, and
(e) preparing the monoclonal antibodies.

13. Method for manufacturing a recombinant monoclonal antibody comprising a light chain variable region (V_L) and a heavy chain variable region (V_H) with the steps of:
(a) introducing at least one vector comprising antibody-encoding nucleic acid sequences of
(i) SEQ ID NO: 115 (V_L-αvß3) and SEQ ID NO: 116 (V_H-αvß3),
(ii) SEQ ID NO: 35 (V_L-αvß5) and SEQ ID NO: 36 (V_H-αvß5),
(iii) SEQ ID NO: 155 (V_L-αvß6) and SEQ ID NO: 156 (V_H-αvß6),
(iv) SEQ ID NO: 195 (V_L-αvß8) and SEQ ID NO: 196 (V_H-αvß8), or
(v) SEQ ID NO: 235 (V_L-αv) and SEQ ID NO: 236 (V_H-αv)
into a host cell,
(b) cultivating the host cell in a culture medium, thereby expressing the encoded antibody, and
(c) purifying the expressed antibody.

14. Use of the antibody, or a fragment thereof, of any of claims 1 to 6 or 11 for the detection of integrins in formalin fixed paraffin embedded (FFPE) material.

15. Method for screening anti-integrin antibodies, which are capable of discriminating between the respective closest homologues of integrin α-subunit and/or ß-subunit and suitable for immunohistochemistry in formalin fixed paraffin embedded (FFPE) material, comprising the steps of:

(a) providing a sample of antibodies being capable of binding a selected integrin,

(b) aligning integrin sequences to identify the closest homologue of the α-subunit and/or ß-subunit of the selected integrin,

(c) performing a differential ELISA on native forms of the selected integrin and the closest homologue(s) thereof with the antibody sample, thereby accumulating antibodies against the selected integrin (primary screen),

(d) performing another differential ELISA on native forms of the selected integrin and another integrin with the accumulated antibodies of step (c), thereby further accumulating antibodies against the selected integrin (secondary screen),

(e) performing immunohistochemistry of FFPE cell lines with the accumulated antibodies of step (d), wherein at least one cell line is capable of expressing the selected integrin and optionally another cell line is not capable of expressing the selected integrin, thereby further accumulating antibodies against the selected integrin (tertiary screen),

(f) performing immunohistochemistry of FFPE cell lines of step (e) with the accumulated antibodies of step (e), wherein the cell line is grown as xenograft tumor in a mammal, thereby further accumulating antibodies against the selected integrin (quaternary screen), and

(g) performing immunohistochemistry of archival FFPE tumors with the accumulated antibodies of step (f), thereby further accumulating antibodies against the selected integrin (quintenary screen).