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(54) Title: BINDING AGENTS TO THE INTEGRIN ALPHA-11 SUBUNIT, AND USES THEREOF

Joint Swelling in arthritic mice



(57) Abstract: The present invention relates to an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, for use in medicine, wherein the antibody or antigen-binding fragment, variant, fusion or derivative thereof has binding specificity for an integrin α 11 subunit, or a heterodimer thereof. In particular, the invention relates to the use of such antibodies or antigen-binding fragments, variants, fusions or derivatives thereof, in the treatment of inflammatory disorders such as arthritic diseases (e.g. RA and OA).

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BINDING AGENTS TO THE INTEGRIN ALPHA-11 SUBUNIT , AND USES THEREOF

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Field of the Invention

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The present invention relates to binding agents with binding specificity for an integrin $\alpha 11$ subunit, or heterodimer comprising the same (for example, an $\alpha 11\beta 1$ heterodimer), and the use thereof in the treatment of an inflammatory condition. Specifically, the invention provides antibodies and antigen-binding
15 fragments, variants, fusions and derivatives thereof, for use in the treatment of inflammatory conditions, such as arthritic diseases, and methods of using the same.

20 Background

Integrins regulate diverse events by cell-cell and cell-matrix interactions

Integrins were originally identified as intermediary cell surface structures that
25 linked the internal cytoskeleton with the immediate environment or extracellular cell matrix, and were considered functionally “dead” molecules. This reasoning was partially based on the observation that most integrins contain only a very small cytoplasmic tail lacking any signalling motifs. Today, integrins are known as highly complex structures that via interaction with other
30 cell surface receptors and recruitment of intracellular adapter proteins participate in cell signalling from the inside and out (Phillips *et al.*, 1988, *Blood* 71:831-43.), from outside and in (Law *et al.*, 1999, *Nature* 401:808-811.), and have been shown to transduce signals laterally across the cell membrane

(Hynes, 2002, *Cell* 110:673-87; for review see Miranti & Brugge, 2002, *Nat Cell Biol* 4:E83-90.).

5 Blocking of some of the best studied integrins using monoclonal antibodies or small molecule inhibitors has been shown to abrogate cell-cell and cell-matrix contacts resulting in intervention of diverse biological processes including development, tissue repair, angiogenesis, inflammation and haemostasis. Some of these antibodies are the subject of clinical phase trials (Shimaoka & Springer, 2003, *Nat Rev Drug Discov* 2:703-16).

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Integrin-mediated immunomodulatory and extracellular matrix modulatory effects have thus far largely been restricted to stimulation of integrins via their native ECM ligands or anti- β 1 integrin antibodies (Loeser, 2002, *Biorheology* 39:119-24). Such studies have, in analogy with other integrin receptors, 15 demonstrated inside-out, outside-in, and lateral signalling participation of chondrocyte integrins.

Rheumatoid arthritis

20 Rheumatoid arthritis (RA) is an inflammatory, autoimmune disease that affects synovial joints, causing pain, swelling, and reduced mobility for the patient. All peripheral joints can be affected in RA, but the most commonly affected are those of the hands, feet and knees. In RA, the immune system attacks the synovium (tissue lining the joint capsule) for unknown reasons, causing local 25 inflammation. The inflammation ultimately results in destruction of cartilage and bone within the joint, as well as the destruction of ligaments, tendons, and muscles that support the joint. In the rheumatoid synovium, activated T cells, B cells, macrophages, fibroblasts, endothelial cells and plasma cells can be identified. Many of the treatments in the pipeline focus on the more targeted 30 inhibition of these specific cells. Although the actual cause of RA is unknown, there is a strong inheritance component.

RA occurs in 0.5-2.0% of the adult population worldwide with one in three patients becoming severely disabled within 20 years. The prevalence of RA is

three times greater in women than in men, with a peak age of onset usually between 20 to 45 years. Mortality rates are 27% higher in RA sufferers than in age- and gender-matched controls and even higher in the subset of women. This translates to a reduced life expectancy of somewhere between 5 to 18 years depending on the study. Radiographically evident joint disease is seen in >67% of patients with the first 2 years and >72% of patients within the first 5 years.

RA is a chronic, systemic inflammatory disease that targets synovial joints and is often accompanied by an array of extra-articular manifestations. The arthritis is characterized by a multicellular inflammatory process of infiltration of lymphocytes and granulocytes into the articular cartilage, proliferation of synovial fibroblast and macrophages and neovascularization of the synovial lining surrounding the joint. This proliferative process induces swelling, erythema and pain of the joints, but also progress into destruction of the joint and loss of bone density and architecture.

The pathological mechanisms of RA are not fully understood. It is believed, however, that many cell types, such as macrophages, dendritic cells, fibroblast like synoviocytes, mast cells, T cells and B cells are involved. The release of cytokines such as TNF- α , IL-1, IL-6, IL-8, IL-15 and various chemokines is substantial in the RA synovium. The levels of TNF- α and IL-1 strongly correlate with disease symptoms and joint damage. Levels of auto-antibodies, such as rheumatoid factor (RF) and anti-citrulline antibodies in blood is characteristic for RA and provide diagnostic and prognostic markers for the disease.

Rheumatoid arthritis is initially characterised by an inflammatory response of the synovial membrane conveyed by an influx of a number of different cell types. The lining becomes hyperplastic and expands. In addition, bone destruction is seen.

Disease-modifying drugs are available in RA, but they are of limited use due to side-effects, and many patients do not respond to existing therapy. During the past 20 years a more aggressive approach to the treatment of RA has led to the

development of disease-modifying antirheumatic drugs (DMARDs) that attempt to halt disease progression. The most prescribed DMARD is methotrexate, originally used in the treatment of cancer. Others include gold salts, antimalarials, sulphasalazine, tetracyclines and cyclosporine. Physicians usually prescribe DMARDs at disease onset while moderate-to-severe RA suffers are given NSAIDs, corticosteroids and DMARDs concurrently.

Although methotrexate is effective in the majority of RA patients, many cannot tolerate the side effects of the drug, and roughly 50% of patients eventually fail treatment, subsequently becoming eligible for more recent treatment innovations, such as biologic response modifiers. Leflunomide (Arava; Aventis) which was launched in 1998, is similar in mechanism and side effects to methotrexate and is used as a second-line agent in patients that have failed methotrexate therapy. The most successful of the novel RA therapies are the tumour-necrosis factor- α (TNF α) inhibitors, such as etanercept (Enbrel; Amgen), infliximab (Remicade; J&J/Centocor) and adalimumab (Humira; Abbott). The TNF α -inhibitors, however, also have potential side effects such as increased infections and cancer in long term use.

20 *Osteoarthritis*

Osteoarthritis (OA) is a progressive, degenerative joint disease and is the most common form of arthritis. It strongly associates with aging and is a major cause of pain and disability in the elderly. A variety of mechanical, metabolic or constitutional insults may trigger OA. Often the insults remain unclear ('primary' OA) but sometimes a clear cause such as trauma may be apparent ('secondary' OA). All the joint tissues (cartilage, bone, synovium, capsule, ligament, muscle) depend on each other for health and function. Insult to one impacts on the others resulting in a common OA phenotype affecting the whole joint. The OA process involves new tissue production, most notably bone ('osteophyte'), and remodelling of joint shape. Often OA compensates for the insults, resulting in an anatomically altered but pain-free functioning joint ('compensated' OA). Sometimes, however, it fails, resulting in progressive damage, associated symptoms and presentation as an OA patient with 'joint

failure'. Such a perspective explains the clinical heterogeneity of OA and the variable clinical outcomes.

Osteoarthritis is the leading cause of physical disability over the age of 65 years affecting an estimated 10% of the population. The prevalence of OA-related physical disability is greater in women than men and rises steadily to 25% in women over the age of 85 years. Predictions suggest that there will be a 66% increase in the number of people with OA-related disability by the year 2020. A similar increase in the number of people with severe symptomatic OA of the hip and knee requiring joint replacement surgery in the next 30 years is predicted if disease-modifying strategies for the medical treatment and prevention of OA cannot be found.

Osteoarthritis (OA) is not a single disease or process but rather the clinical and pathological outcome of a range of processes and disorders that lead to structural, and eventually symptomatic, failure of one or more synovial joints. OA involves the entire joint including the subchondral bone, ligaments, capsule, synovial membrane and periarticular muscles as well as the articular cartilage. Ultimately the articular cartilage degenerates with fibrillation, fissures, ulceration and full thickness loss of the joint surface.

Treatment of osteoarthritis includes a wide spectrum of approaches where most treatments are palliative with the exception of surgery. This means that most treatments relieve pain and thereby increase joint function of the patient, but the treatments do not change the course of the disease. Surgical interventions include joint replacement and osteotomy, which may reverse the progress of osteoarthritis and provide long-term improved function and pain relief for a specific joint.

There is thus also an urgent need to improve the options to treat OA. Existing drug therapies for OA reduce the symptoms (mainly pain), and are only moderately effective and often leave patients with a substantial pain burden. Thus, to date, no drugs are available with proven disease-modifying efficacy in OA.

Accordingly, the present invention seeks to provide novel treatments for inflammatory conditions such as RA and OA, and novel binding agents for use in the same.

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Summary of the Invention

A first aspect of the present invention provides the use of an antibody or an antigen-binding fragment thereof with binding specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, or of a variant, fusion or derivative of said
 10 antibody or antigen binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity of said antibody or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer thereof, in the preparation of a medicament for treating an inflammatory condition,
 15 wherein the antibody or fragment comprises the following amino acid sequences:

	FSRYMHWRQVPG	[SEQ ID NO: 1];
	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
20	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
	CAAWDDSLSGHV	[SEQ ID NO: 6].

25 It will be appreciated by persons skilled in the art that the above amino acid sequences of SEQ ID NOS:1 to 6 represent complementarity determining regions (CDRs).

A second aspect of the invention provides the use of an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above in
 30 the preparation of a diagnostic or prognostic agent for an inflammatory condition.

A third aspect of the invention provides the use of an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above in the preparation of an agent for detecting and/or imaging cells expressing an integrin $\alpha 11$ subunit, or heterodimer thereof.

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Related aspects of the invention provide:

- (a) the use of an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above for treating an inflammatory condition;
- (b) the use of an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, for diagnosing or prognosing an inflammatory condition; and
- (c) the use of an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, for detecting and/or imaging cells expressing an integrin $\alpha 11$ subunit, or heterodimer thereof.

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By “binding specificity” for an integrin $\alpha 11$ subunit, or a heterodimer thereof, we mean an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, which is capable of binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof such as an $\alpha 11\beta 1$ heterodimer.

In one embodiment, the integrin $\alpha 11$ subunit, or the heterodimer thereof, is localised on the surface of a cell, such as a chondrocyte, a fibroblast, a mesenchymal cell (*e.g.* a mesenchymal stem cell) or a dendritic cell.

25

In a further embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof *in vivo*, *i.e.* under the physiological conditions in which an integrin $\alpha 11$ subunit exists inside the body. Such binding specificity may be determined by methods well known in the art, such as *e.g.* ELISA, immunohistochemistry, immunoprecipitation, Western blots, chromatography and flow cytometry using transfected cells expressing the $\alpha 11$ subunit or a heterodimer thereof (see Examples). Examples of how to measure specificity of

30

an antibody is given in *e.g.* Harlow & Lane, "Antibodies: A Laboratory", Cold Spring Harbor Laboratory Press, New York, which is incorporated herein by reference.

- 5 It will be appreciated by persons skilled in the art that the integrin $\alpha 11$ subunit may be a human or animal integrin $\alpha 11$ subunit. In one embodiment, the integrin $\alpha 11$ subunit is human. Examples of known integrin $\alpha 11$ subunits are disclosed in International Patent Application (Publication) No. WO 00/75187 (to Cartela AB), which is incorporated herein by reference.

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- In another embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, selectively. By "capable of binding selectively" we include such antibody-derived binding moieties which bind at least 10-fold
15 more strongly to integrin $\alpha 11$ subunit or a heterodimer thereof than to another proteins (in particular other integrins, such as $\alpha 10$, $\alpha 1$ and $\alpha 2$ having most identity with $\alpha 11$); for example at least 50-fold more strongly or at least 100-fold more strongly. The binding moiety may be capable of binding selectively to integrin $\alpha 11$ subunit or a heterodimer thereof under physiological conditions,
20 *e.g. in vivo*. Suitable methods for measuring relative binding strengths include immunoassays, for example where the binding moiety is an antibody (see Harlow & Lane, "Antibodies: A Laboratory", Cold Spring Harbor Laboratory Press, New York, which is incorporated herein by reference). Alternatively, binding may be assessed using competitive assays or using Biacore® analysis
25 (Biacore International AB, Sweden).

In a further embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, binds exclusively to an integrin $\alpha 11$ subunit or a heterodimer thereof.

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It will be appreciated by persons skilled in the art that the binding specificity of an antibody or antigen binding fragment thereof is conferred by the presence of complementarity determining regions (CDRs) within the variable regions of the constituent heavy and light chains. In the uses of the present invention, binding

specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, is conferred by the presence of the CDRs identified as SEQ ID NOS: 1 to 6 above, or variant sequences thereof which retain the same binding specificity.

- 5 By “retain the binding specificity” we mean that the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of competing for binding to an integrin $\alpha 11$ subunit with antibody ‘A03’ (see SEQ ID NO: 11 and Examples below).
- 10 For example, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may bind to the same epitope on the integrin $\alpha 11$ subunit as an antibody comprising the CDRs identified as SEQ ID NOS: 1 to 6.

By “epitope” it is herein intended to mean a site of a molecule to which an
 15 antibody binds, *i.e.* a molecular region of an antigen. An epitope may be a linear epitope, which is determined by *e.g.* the amino acid sequence, *i.e.* the primary structure, or a three-dimensional epitope, defined by the secondary structure, *e.g.* folding of a peptide chain into beta sheet or alpha helical, or by the tertiary structure, *e.g.* way which helices or sheets are folded or arranged to give a
 20 three-dimensional structure, of an antigen.

An example of a suitable epitope (or part thereof) of the integrin $\alpha 11$ subunit is as follows:

25 WNGCNEDEHCVPDLVLDARSDLPTAMEYCQRVLRKPAQDCSAYTLSE
 DTTVFIIESTRQRVAVEATLENRGENAYSTVLNISQSANLQFASLIQKED
 SDGSIECVNEERLQKQVCNVSYPPFRAKAKVAFRLDFEFSKSIFLHHLE
 IELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSSLSHYEVKLN
 SSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIPIATRSGNRLLKLRDF
 30 LTDEANTSCNIWGNSTEYRPTPVEEDLRRAPQLNHSNSDVVSINCNIRLV
 PNQEIFHLLGNLWLRSLKALKYKSMKIMVNAALQRQFHSPFIFREEDP
 SRQIVFEISKQEDWQVP

[SEQ ID NO: 7]

Further suitable epitopes of integrin alpha11 may include fragments, fusions or natural variants of SEQ ID NO: 7.

For example, suitable fragments comprise or consist of from about amino acid
5 number 778 to amino acid number 1142 of the human alpha 11 integrin
sequence, as shown in SEQ ID NO: 7, or the extracellular extension region,
such as a peptide comprising essentially the amino acid sequence from about
amino acid number 804 to about amino acid number 826 of the human alpha 11
integrin sequence, especially a peptide comprising essentially the amino acid
10 sequence EYCQRVLRKPAQDCSAYTLSFDT [SEQ ID NO: 8], or any poly
peptide fragment that comprises, or consists of, about amino acid amino acid
(aa) numbers: aa778 - to aa800, about aa no 800-820, about aa no 820-840,
about aa no 840-860, about aa no 860-880, about aa no 880-900, about aa no
900-920, about aa no 920-940, about aa no 940-960, about aa no 960-980, about
15 aa no 980-1000, about aa no 1020-1040, about aa no 1040-1060, about aa no
1080-1100, about aa no 1100-1120, about aa no 1120-1142 of the human
alpha11 sequence.

All amino acid numbers above are calculated from the human alpha11 sequence
20 amino acid numbers where amino acid no 1 is M, aa no 2 is D, aa no 3 is L, aa
no 4 is P, aa no 5 is R, aa no 6 is G and aa no 7 is L, *etc.* of the human integrin
alpha11 as disclosed in WO 00/75187 (which is incorporated herein by
reference).

25 Suitable epitope fusions include a fusion of the above peptide to a StrepTag tail.

Epitope variants, such as natural variants, of the above epitope polypeptide
sequence include polypeptides comprising a sequence with at least 60% identity
to the amino acid sequence of SEQ ID NO: 7, preferably at least 70% or 80% or
30 85% or 90% identity to said sequences, and more preferably at least 95%, 96%,
97%, 98% or 99% identity to said amino acid sequences.

Percent identity can be determined by methods well known in the art, for example using the LALIGN program (Huang and Miller, *Adv. Appl. Math.* (1991) 12:337-357) at the Expasy facility site:

5 (http://www.ch.embnet.org/software/LALIGN_form.html)

using as parameters the global alignment option, scoring matrix BLOSUM62, opening gap penalty -14; extending gap penalty -4.

10 Alternatively, the percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequence has been aligned optimally.

15

The antibody or antigen-binding fragment, or variant, fusion or derivative thereof, which retains the binding specificity of antibody 'A03' may also retain one or more of the same biological properties as antibody 'A03' (see below).

20 Methods for determining whether a test antibody is capable of competing for binding with second antibody are well known in the art.

One suitable method is a sandwich ELISA for evaluation of the $\alpha 11$ -binding antibody, A03. A suitable amount of, *e.g.* a polyclonal antibody, such as
25 10 $\mu\text{g/ml}$ of a polyclonal rabbit antibody, directed against the cytoplasmatic tail of human $\alpha 11$. The antibody is then used as a capture antibody when coated in a 96-well plate, such as *e.g.* Maxisorp NuncTM. Coating is done according to standard procedure known in the art, *e.g.* by incubation the plate at 4°C over night, followed by three times washing with *e.g.* TBS-T (20 mM Tris-HCl pH
30 7.5, 150 mM NaCl and 0.05% Tween-20). Wells may be blocked for 1h with 3% BSA in TBS-T at *e.g.* room temperature. Cell extract from HEK-cells stably transfected with human $\alpha 11$ expression vector, is then diluted in assay buffer (*e.g.* TBS-T supplemented with 0.1% BSA, 1 mM MgCl_2 and 10 μM CaCl_2). Suitable amount of diluted cell extract, such as *e.g.* 50 μl is then added

per well and incubated to allow binding to the coated antibody, *e.g.* for 1h at room temperature. Plates are then washed three times with TBS-T. Primary antibody (A03 or control antibody) conjugated with biotin is then added in a suitable amount, such as *e.g.* at 2 µg/ml in assay buffer. Plates are then
5 incubated for a sufficient time to allow binding of A03 and control antibody where possible, *e.g.* for 1h at room temperature followed by three washes in TBS-T. In case where biotinylated primary antibodies are used, Streptavidin-HRP antibody (DAKO) may be used and diluted accordingly in assay buffer (1:5000), added to the wells. Plates are then incubated enough for the
10 streptavidin-biotin complex to form, *e.g.* for 1h at room temperature. Following washing, *e.g.* three times with TBS-T and the plates are developed with peroxidase substrate (*e.g.* OPD SigmaFast, Sigma). The absorbance of the colorimetric change is determined at suitable wavelength, in this case 490 nm.

15 If primary antibodies are not conjugated, the same ELISA can instead be incubated with a secondary antibody against the human IgG4 directly conjugated with HRP, *e.g.* mouse anti-human IgG4-HRP, from *e.g.* Serotec, or, if not conjugated, followed by a HRP-conjugated anti-mouse antibody from *e.g.* DAKO. Plates are then washed and developed as outlined above.

20 Further methods include reversing the sandwich ELISA outlined above, and instead use the α11 antibody A03 as a capture antibody. In this case, cell extracts containing α11 is added and the ELISA is preceded as outlined above with the following modifications:

25 As primary antibody the polyclonal rabbit antibody directed against the cytoplasmic tail of human alpha11 is diluted in assay buffer (1 µg/ml). As a secondary antibody a HRP-conjugated goat anti-rabbit antibody may be used (*e.g.* DAKO, diluted 1:5000). Plates are then washed and developed as outlined
30 above.

In a further ELISA assay, it is possible to evaluate epitope-modifying or blocking antibodies. This can be done in several ways, for instance using the sandwich ELISA detailed above with the following modifications:

Capture antibody as well as alpha11 is added to the 96-well plate as detailed above. Before applying primary antibody, plates are incubated with a test antibody, *e.g.* epitope-modifying or blocking antibodies at 10 µg/ml in assay
5 buffer. Plates are incubated for 15 min at room temperature after which primary antibody (A03 or control antibody) conjugated with biotin is added and plates are incubated for 1h at room temperature. Plates are washed and developed as outlined above.

10 The reverse sandwich ELISA can also be used to evaluate a test antibody, *e.g.* an epitope-modifying or blocking antibody. In this ELISA, the alpha11 antibody A03 can be used as a capture antibody. Cell extract containing alpha11 is then added as outlined above and different conjugated, potentially modifying antibodies, can be used as primary antibodies.

15 A further method to evaluate if a test antibody is capable of competing for binding with second antibody is by flow cytometry, such as Fluorescence Activated Cell Sorting (FACS®) as described in Example IV below.

20 Additional methods suitable for identifying competing antibodies are disclosed in *Antibodies: A Laboratory Manual*, Harlow & Lane, Cold Spring Harbor Laboratory Press, New York, ISBN 0-87969-314-2 which is incorporated herein by reference (for example, see pages 567 to 569, 574 to 576, 583 and 590 to 612).

25 By "antibody" we include substantially intact antibody molecules, as well as chimaeric antibodies, humanised antibodies, human antibodies (wherein at least one amino acid is mutated relative to the naturally occurring human antibodies), single chain antibodies, bispecific antibodies, antibody heavy chains, antibody
30 light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen binding fragments and derivatives of the same. For example, the antibody may be a monoclonal antibody.

Thus, in one embodiment the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of an intact antibody.

For example, the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, may consist essentially of an intact antibody. By “consist essentially of” we mean that the antibody or antigen-binding fragment, variant, fusion or derivative thereof consists of a portion of an intact antibody sufficient to retain binding specificity for an integrin $\alpha 11$ subunit.

The term ‘antibody’ also includes all classes of antibodies, including IgG, IgA, IgM, IgD and IgE. In one embodiment, however, the antibody is an IgG molecule, such as an IgG1, IgG2, IgG3, or IgG4 molecule.

In one embodiment, the antibody is an IgG4 molecule.

In a further embodiment, the antibody is a non-naturally occurring antibody. Of course, where the antibody is a naturally occurring antibody, it is provided in an isolated form (*i.e.* distinct from that in which it is found in nature).

The variable heavy (V_H) and variable light (V_L) domains of the antibody are involved in antigen recognition, a fact first recognised by early protease digestion experiments. Further confirmation was found by “humanisation” of rodent antibodies. Variable domains of rodent origin may be fused to constant domains of human origin such that the resultant antibody retains the antigenic specificity of the rodent-parented antibody (Morrison *et al* (1984) *Proc. Natl. Acad. Sci. USA* **81**, 6851-6855).

Antigenic specificity is conferred by variable domains and is independent of the constant domains, as known from experiments involving the bacterial expression of antibody fragments, all containing one or more variable domains. These molecules include Fab-like molecules (Better *et al* (1988) *Science* **240**, 1041); Fv molecules (Skerra *et al* (1988) *Science* **240**, 1038); single-chain Fv (ScFv) molecules where the V_H and V_L partner domains are linked via a flexible oligopeptide (Bird *et al* (1988) *Science* **242**, 423; Huston *et al* (1988) *Proc. Natl.*

Acad. Sci. USA **85**, 5879) and single domain antibodies (dAbs) comprising isolated V domains (Ward *et al* (1989) *Nature* **341**, 544). A general review of the techniques involved in the synthesis of antibody fragments which retain their specific binding sites is to be found in Winter & Milstein (1991) *Nature* **349**, 293-
5 299.

Thus, by “antigen-binding fragment” we mean a functional fragment of an antibody that is capable of binding to an integrin α 11 subunit, or a heterodimer thereof.

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Exemplary antigen-binding fragments may be selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments), single antibody chains (*e.g.* heavy or light chains), single variable domains
15 (*e.g.* V_H and V_L domains) and domain antibodies (dAbs, including single and dual formats [*i.e.* dAb-linker-dAb]).

In one embodiment, the antigen-binding fragment is an scFv.

20 The advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli*, thus allowing the facile production of
25 large amounts of the said fragments.

Also included within the scope of the invention are modified versions of antibodies and an antigen-binding fragments thereof, *e.g.* modified by the covalent attachment of polyethylene glycol or other suitable polymer, and uses
30 of the same.

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening

of immunoglobulin libraries (Orlandi. *et al.*, 1989. *Proc. Natl. Acad. Sci. U.S.A.* 86:3833-3837; Winter *et al.*, 1991, *Nature* 349:293-299, which are incorporated herein by reference) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (see Kohler *et al.*, 1975. *Nature* 256:495-497; Kozbor *et al.*, 1985. *J. Immunol. Methods* 81:31-42; Cote *et al.*, 1983. *Proc. Natl. Acad. Sci. USA* 80:2026-2030; Cole *et al.*, 1984. *Mol. Cell. Biol.* 62:109-120, which are incorporated herein by reference).

The antibody or antigen-binding fragment or derivative thereof may be produced by recombinant means.

Suitable monoclonal antibodies to selected antigens may be prepared by known techniques, for example those disclosed in "*Monoclonal Antibodies: A manual of techniques*", H Zola (CRC Press, 1988) and in "*Monoclonal Hybridoma Antibodies: Techniques and Applications*", J G R Hurrell (CRC Press, 1982), which are incorporated herein by reference.

Antibody fragments can also be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, "*Antibodies: A Laboratory Manual*", Cold Spring Harbor Laboratory, New York, which is incorporated herein by reference). For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (*e.g.* Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

It will be appreciated by persons skilled in the art that for human therapy or diagnostics, humanised antibodies may be used. Humanised forms of non-human (*e.g.* murine) antibodies are genetically engineered chimaeric antibodies or antibody fragments having preferably minimal-portions derived from non-human antibodies. Humanised antibodies include antibodies in which

complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non human species (donor antibody) such as mouse, rat or rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported complementarity determining region or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non-human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Riechmann *et al.*, 1988, *Nature* 332:323-329; Presta, 1992, *Curr. Op. Struct. Biol.* 2:593-596, which are incorporated herein by reference).

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, *Nature* 321:522-525; Reichmann *et al.*, 1988, *Nature* 332:323-327; Verhoeyen *et al.*, 1988, *Science* 239:1534-1536; US 4,816,567, which are incorporated herein by reference) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimaeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, *J. Mol. Biol.* **227**:381; Marks *et al.*, 1991, *J. Mol. Biol.* **222**:581; Cole *et al.*, 1985, In: *Monoclonal antibodies and Cancer Therapy*, Alan R. Liss, pp. 77; 5 Boerner *et al.*, 1991. *J. Immunol.* **147**:86-95, Soderlind *et al.*, 2000, *Nat Biotechnol* **18**:852-6 and WO 98/32845 which are incorporated herein by reference).

10 Once suitable antibodies are obtained, they may be tested for activity, such as binding specificity or a biological activity of the antibody, for example by ELISA, immunohistochemistry, flow cytometry, immunoprecipitation, western blots, *etc.* The biological activity may be tested in different assays with readouts for that particular feature. Examples of one or more biological activity of the 15 antibodies according to the invention are:

- a) protection of cartilage from degradation (*e.g.* reduction in proteoglycan content);
- b) reduction in inflammation of the joints (*e.g.* swelling); and/or
- 20 c) reduction in infiltration of inflammatory cells.

Examples of suitable assays for testing said biological activity is known in the art and also given in the examples herein.

In one embodiment of the first aspect of the invention, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises a heavy chain variable region comprising the CDRs identified by SEQ ID NOS: 1 to 3. For example, the heavy chain variable region may comprise, consist essentially of or consist of the amino acid sequence of SEQ ID NO: 9:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYMHVVRQVPGKGLEW
VSGVSWNGSRTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY
YCARVSGDGYNFGAWGQGTLTVSS [SEQ ID NO:9]

In a further embodiment of the first aspect of the invention, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises a light chain variable region comprising the CDRs identified by SEQ ID NOS: 4 to 6. For example, the light chain variable region may comprise, consist essentially of or consist of the amino acid sequence of SEQ ID NO: 10:

QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI
YGYNERPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSG
HVVFGGGTKLTVLG [SEQ ID NO:10]

Thus, in one embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises a heavy chain variable region comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 9 and a light chain variable region comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 8.

For example, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may comprise a heavy chain comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 11 and/or a light chain comprising, consisting essentially of or consisting of the amino acid sequence of SEQ ID NO: 12:

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYMHWRQVPGKGLEW
VSGVSWNGSRTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY
YCARVSGDGYNFGAWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAA
10 LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
SSLGKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFL
FPPKPKDTLMISRTPEVTCVVDVVSQEDPEVQFNWYVDGVEVHNAKTK
PREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKA
KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
15 NNYKTTTPVLDSGDFLYSRLTVDKSRWQEGNVFSCSV MHEALHNHY
TQKSLSLSLGK

[SEQ ID NO:11]

QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI
20 YGYNERPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSG
HVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPG
AVTVAWKADSSPVKAGVETTTTPSKQSNKYAASSYLSLTPEQWKSHRS
YSCQVTHEGSTVEKTVAPTECS

[SEQ ID NO:12]

25

In a further exemplary embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of the amino acid sequence of SEQ ID NO:13.

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYMHWRQVPGKGLEW
VSGVSWNGSRTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY
YCARVSGDGYNFGAWGQGTLVTVSSGGGGSGGGGSGGGGSQSVLTQP
30 PSASGTPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGYNER
PSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGHVVFGG
35 GTKLTVLG

[SEQ ID NO:13]

This sequence corresponds to the exemplary 'A03' scFv molecule for use in the methods of the invention (the linker sequence is underlined). Optionally, the sequence may also comprise a c-myc and/or histidine tags at the C-terminus, for example:

5

EQKLISEEDLSGSAAAHHHHHH

[SEQ ID NO:14]

The term 'amino acid' as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids other naturally-occurring amino acids, unconventional amino acids (*e.g.* α,α -disubstituted amino acids, N-alkyl amino acids, *etc.*) and chemically derivatised amino acids (see below).

When an amino acid is being specifically enumerated, such as 'alanine' or 'Ala' or 'A', the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present invention, as long as the desired functional property is retained by the polypeptide. For the peptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

In one embodiment, the polypeptide binding agents described herein comprise or consist of L-amino acids.

It will be appreciated by persons skilled in the art that invention encompasses variants, fusions and derivatives of the defined polypeptides, and fusions of said variants or derivatives, as well as uses thereof, provided that such variants, fusions and derivatives retain binding specificity for an integrin $\alpha 11$ subunit or heterodimer thereof.

30

Variants may be made using the methods of protein engineering and site-directed mutagenesis well known in the art using the recombinant polynucleotides (see example, see *Molecular Cloning: a Laboratory Manual*,

3rd edition, Sambrook & Russell, 2001, Cold Spring Harbor Laboratory Press, which is incorporated herein by reference).

By 'fusion' of said polypeptide we include a polypeptide fused to any other
5 polypeptide. For example, the said polypeptide may be fused to a polypeptide
such as glutathione-S-transferase (GST) or protein A in order to facilitate
purification of said polypeptide. Examples of such fusions are well known to
those skilled in the art. Similarly, the said polypeptide may be fused to an
oligo-histidine tag such as His6 or to an epitope recognised by an antibody such
10 as the well-known Myc tag epitope. Fusions to any variant or derivative of said
polypeptide are also included in the scope of the invention. It will be
appreciated that fusions (or variants or derivatives thereof) which retain
desirable properties, such as retain binding specificity for an integrin $\alpha 11$
subunit or heterodimer thereof, are preferred.

15 The fusion may comprise a further portion which confers a desirable feature on
the said polypeptide of the invention; for example, the portion may be useful in
detecting or isolating the polypeptide, or promoting cellular uptake of the
polypeptide. The portion may be, for example, a biotin moiety, a radioactive
20 moiety, a fluorescent moiety, for example a small fluorophore or a green
fluorescent protein (GFP) fluorophore, as well known to those skilled in the art.
The moiety may be an immunogenic tag, for example a Myc tag, as known to
those skilled in the art or may be a lipophilic molecule or polypeptide domain
that is capable of promoting cellular uptake of the polypeptide, as known to
25 those skilled in the art.

By 'variants' of the polypeptide we include insertions, deletions and
substitutions, either conservative or non-conservative. In particular we include
variants of the polypeptide where such changes do not substantially alter the
30 activity of the said polypeptide. For example, we include variants of the
polypeptide where such changes do not substantially alter the binding
specificity for an integrin $\alpha 11$ subunit or heterodimer thereof.

The polypeptide variant may have an amino acid sequence which has at least 75% identity with one or more of the amino acid sequences given above, for example at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% identity with one or more of the amino acid sequences specified above.

The percent sequence identity between two polypeptides may be determined using suitable computer programs, for example the GAP program of the University of Wisconsin Genetic Computing Group and it will be appreciated that percent identity is calculated in relation to polypeptides whose sequences have been aligned optimally.

The alignment may alternatively be carried out using the Clustal W program (as described in Thompson *et al.*, 1994, *Nuc. Acid Res.* **22**:4673-4680).

The parameters used may be as follows:

Fast pairwise alignment parameters: K-tuple(word) size; 1, window size; 5, gap penalty; 3, number of top diagonals; 5. Scoring method: x percent.

Multiple alignment parameters: gap open penalty; 10, gap extension penalty; 0.05.

Scoring matrix: BLOSUM.

Alternatively, the BESTFIT program may be used to determine local sequence alignments.

The polypeptide, variant, fusion or derivative of the invention may comprise one or more amino acids which have been modified or derivatised.

Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatised molecules include, for example,

those molecules in which free amino groups have been derivatised to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine and ornithine for lysine. Derivatives also include peptides containing one or more additions or deletions as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (*e.g.* acetylation or thioglycolic acid amidation), terminal carboxylamidation (*e.g.* with ammonia or methylamine), and the like terminal modifications.

It will be further appreciated by persons skilled in the art that peptidomimetic compounds may also be useful. Thus, by 'polypeptide' we include peptidomimetic compounds which are capable of binding an integrin α 11 subunit. The term 'peptidomimetic' refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent.

For example, the polypeptides of the invention include not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al.* (1997) *J. Immunol.* **159**, 3230-3237, which is incorporated herein by reference. This approach involves making pseudopeptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis. Alternatively, the polypeptide of the invention may be a peptidomimetic

compound wherein one or more of the amino acid residues are linked by a -
y(CH₂NH)- bond in place of the conventional amide linkage.

5 In a further alternative, the peptide bond may be dispensed with altogether
provided that an appropriate linker moiety which retains the spacing between
the carbon atoms of the amino acid residues is used; it is particularly preferred
if the linker moiety has substantially the same charge distribution and
substantially the same planarity as a peptide bond.

10 It will be appreciated that the polypeptide may conveniently be blocked at its N-
or C-terminus so as to help reduce susceptibility to exoproteolytic digestion.

A variety of uncoded or modified amino acids such as D-amino acids and N-
methyl amino acids have also been used to modify mammalian peptides. In
15 addition, a presumed bioactive conformation may be stabilised by a covalent
modification, such as cyclisation or by incorporation of lactam or other types of
bridges, for example see Veber *et al.*, 1978, *Proc. Natl. Acad. Sci. USA* **75**:2636
and Thursell *et al.*, 1983, *Biochem. Biophys. Res. Comm.* **111**:166, which are
incorporated herein by reference.

20

A common theme among many of the synthetic strategies has been the
introduction of some cyclic moiety into a peptide-based framework. The cyclic
moiety restricts the conformational space of the peptide structure and this
frequently results in an increased specificity of the peptide for a particular
25 biological receptor. An added advantage of this strategy is that the introduction
of a cyclic moiety into a peptide may also result in the peptide having a
diminished sensitivity to cellular peptidases.

Thus, exemplary polypeptides comprise terminal cysteine amino acids. Such a
30 polypeptide may exist in a heterodetic cyclic form by disulphide bond
formation of the mercaptide groups in the terminal cysteine amino acids or in a
homodetic form by amide peptide bond formation between the terminal amino
acids. As indicated above, cyclising small peptides through disulphide or amide
bonds between the N- and C-terminus cysteines may circumvent problems of

specificity and half-life sometime observed with linear peptides, by decreasing proteolysis and also increasing the rigidity of the structure, which may yield higher specificity compounds. Polypeptides cyclised by disulphide bonds have free amino and carboxy-termini which still may be susceptible to proteolytic degradation, while peptides cyclised by formation of an amide bond between the N-terminal amine and C-terminal carboxyl and hence no longer contain free amino or carboxy termini. For example, the peptides of the present invention can be linked either by a C-N linkage or a disulphide linkage.

The present invention is not limited in any way by the method of cyclisation of peptides, but encompasses peptides whose cyclic structure may be achieved by any suitable method of synthesis. Thus, heterodetic linkages may include, but are not limited to formation via disulphide, alkylene or sulphide bridges. Methods of synthesis of cyclic homodetic peptides and cyclic heterodetic peptides, including disulphide, sulphide and alkylene bridges, are disclosed in US 5,643,872, which is incorporated herein by reference. Other examples of cyclisation methods are discussed and disclosed in US 6,008,058, which is incorporated herein by reference.

A further approach to the synthesis of cyclic stabilised peptidomimetic compounds is ring-closing metathesis (RCM). This method involves steps of synthesising a peptide precursor and contacting it with an RCM catalyst to yield a conformationally restricted peptide. Suitable peptide precursors may contain two or more unsaturated C-C bonds. The method may be carried out using solid-phase-peptide-synthesis techniques. In this embodiment, the precursor, which is anchored to a solid support, is contacted with a RCM catalyst and the product is then cleaved from the solid support to yield a conformationally restricted peptide.

Another approach, disclosed by D. H. Rich in *Protease Inhibitors*, Barrett and Selveson, eds., Elsevier (1986), which is incorporated herein by reference, has been to design peptide mimics through the application of the transition state analogue concept in enzyme inhibitor design. For example, it is known that the

secondary alcohol of staline mimics the tetrahedral transition state of the scissile amide bond of the pepsin substrate.

5 In summary, terminal modifications are useful, as is well known, to reduce susceptibility by proteinase digestion and therefore to prolong the half-life of the peptides in solutions, particularly in biological fluids where proteases may be present. Polypeptide cyclisation is also a useful modification and is preferred because of the stable structures formed by cyclisation and in view of the biological activities observed for cyclic peptides.

10

Thus, in one embodiment the polypeptide binding moiety is cyclic. However, in an alternative embodiment, the polypeptide is linear.

15 The present invention also includes compositions comprising pharmaceutically acceptable acid or base addition salts of the polypeptide binding moieties of the present invention. The acids which are used to prepare the pharmaceutically acceptable acid addition salts of the aforementioned base compounds useful in this invention are those which form non-toxic acid addition salts, *i.e.* salts containing pharmacologically acceptable anions, such as the hydrochloride, 20 hydrobromide, hydroiodide, nitrate, sulphate, bisulphate, phosphate, acid phosphate, acetate, lactate, citrate, acid citrate, tartrate, bitartrate, succinate, maleate, fumarate, gluconate, saccharate, benzoate, methanesulphonate, ethanesulphonate, benzenesulphonate, *p*-toluenesulphonate and pamoate [*i.e.* 1,1'-methylene-bis-(2-hydroxy-3 naphthoate)] salts, among others.

25

Pharmaceutically acceptable base addition salts may also be used to produce pharmaceutically acceptable salt forms of the compounds according to the present invention.

30 The chemical bases that may be used as reagents to prepare pharmaceutically acceptable base salts of the present compounds that are acidic in nature are those that form non-toxic base salts with such compounds. Such non-toxic base salts include, but are not limited to those derived from such pharmacologically acceptable cations such as alkali metal cations (*e.g.* potassium and sodium) and

alkaline earth metal cations (*e.g.* calcium and magnesium), ammonium or water-soluble amine addition salts such as N-methylglucamine-(meglumine), and the lower alkanolammonium and other base salts of pharmaceutically acceptable organic amines, among others.

5

The polypeptides described herein may be lyophilised for storage and reconstituted in a suitable carrier prior to use. Any suitable lyophilisation method (*e.g.* spray drying, cake drying) and/or reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilisation and reconstitution can lead to varying degrees of antibody activity loss (*e.g.* with conventional immunoglobulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted upward to compensate. In one embodiment, the lyophilised (freeze dried) polypeptide loses no more than about 20%, or no more than about 25%, or no more than about 30%, or no more than about 35%, or no more than about 40%, or no more than about 45%, or no more than about 50% of its activity (prior to lyophilisation) when rehydrated.

It will be further appreciated by persons skilled in the art that the antibodies and antigen-binding fragments, variants, fusions and derivatives thereof, described herein may exist in monomeric form or in the form of a homo- or hetero-multimer thereof (*e.g.* dimer, trimer, tetramer, pentamer, *etc.*).

In a further embodiment of the invention, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises a therapeutic and/or detectable moiety.

By a "detectable moiety" we include the meaning that the moiety is one which, when located at the target site following administration of the compound of the invention into a patient, may be detected, typically non-invasively from outside the body and the site of the target located. The detectable moiety may be a single atom or molecule which is either directly or indirectly involved in the production of a detectable species. Thus, the binding agents of this embodiment of the invention are useful in imaging and diagnosis.

- Suitable detectable moieties are well known in medicinal chemistry and the linking of these moieties to polypeptides and proteins is well known in the art. Examples of detectable moieties include, but are not limited to, the following:
- 5 radioisotopes (*e.g.* ^3H , ^{14}C , ^{35}S , ^{123}I , ^{125}I , ^{131}I , ^{99}Tc , ^{111}In , ^{90}Y , ^{188}Re),
 radionuclides (*e.g.* ^{11}C , ^{18}F , ^{64}Cu), fluorescent labels (*e.g.* FITC, rhodamine, lanthanide phosphors, carbocyanine), enzymatic labels (*e.g.* horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase),
 chemiluminescent, biotinyl groups and predetermined polypeptide epitopes
 10 recognised by a secondary reporter (*e.g.* leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags). In some embodiments, labels are attached by spacer arms of various lengths to reduce potential steric hindrance.
 - 15 The radio- or other labels may be incorporated in the polypeptides of the invention in known ways. For example, if the binding moiety is a polypeptide it may be biosynthesised or may be synthesised by chemical amino acid synthesis using suitable amino acid precursors involving, for example, fluorine-19 in place of hydrogen. Labels such as $^{99\text{m}}\text{Tc}$, ^{123}I , ^{186}Rh , ^{188}Rh and ^{111}In can, for
 20 example, be attached *via* cysteine residues in the binding moiety. Yttrium-90 can be attached *via* a lysine residue. The IODOGEN method (Fraker *et al* (1978) *Biochem. Biophys. Res. Comm.* **80**, 49-57, which is incorporated herein by reference) can be used to incorporate ^{123}I . Reference ("Monoclonal Antibodies in Immunoscintigraphy", J-F Chatal, CRC Press, 1989, which is
 25 incorporated herein by reference) describes other methods in detail.

The antibodies and antigen-binding fragments, or variants, fusions or derivatives thereof, as described above have efficacy in the treatment of an inflammatory condition.

30

By 'treatment' we include both therapeutic and prophylactic treatment of a subject/patient. The term 'prophylactic' is used to encompass the use of a polypeptide or formulation described herein which either prevents or reduces the likelihood of an inflammatory condition in a patient or subject.

For example, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may have efficacy in the treatment of an inflammatory condition selected from the group consisting of arthritic diseases (such as
5 rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, periodontitis, psoriasis, asthma, systemic lupus erythematosus, multiple sclerosis, ankylosing spondylitis, psoriatic arthritis and autoimmune chronic inflammatory diseases.

10

In one embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, has efficacy in the treatment an arthritic disease, such as rheumatoid arthritis or osteoarthritis. Such efficacy may be determined in suitable animal models, such as arthritis models in mice (see Examples).

15

For example, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may be capable of modulating (e.g. inhibiting) the degradation of collagen, *in vitro* and/or *in vivo*.

20 By "modulate" we include increasing or decreasing the rate of degradation of collagen. It will be appreciated that inhibition of the degradation of collagen may be in whole or in part. For example, collagen degradation may be inhibited by 10% or more, e.g. 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, compared to the degradation of collagen in the absence of the antibody or
25 antigen-binding fragment, or variant, fusion or derivative thereof.

The antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may exhibit one or more of the following medically useful properties:

- 30 a) protection of cartilage from degradation (e.g. reduction in proteoglycan content);
b) reduction in inflammation of the joints (e.g. swelling); and/or
c) reduction in infiltration of inflammatory cells.

Methods of testing for the above properties are described in the Examples below.

Hence, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may have advantageous properties over established therapies for arthritic disease, which reduce swelling (NSAIDS) or swelling and cell infiltration (anti-TNF antibodies) in the same animal model, but are not able to affect all three parameters.

Thus, the invention provides the following methods:

- a) a method for protecting cartilage from degradation comprising administering to the site of the cartilage an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above;
- b) a method for reducing joint inflammation comprising administering to the joint an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above; and
- c) a method for reducing the infiltration of inflammatory cells into a joint comprising administering to the joint an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, as described above.

In particular, a fourth aspect of the invention provides a method for treating an individual with an inflammatory condition, the method comprising administering to the individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined above.

A fifth aspect of the invention provides a method for diagnosing or prognosing an inflammatory condition in an individual, the method comprising administering to the individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined above.

A sixth aspect describes a method for imaging cells expressing an integrin $\alpha 11$ subunit associated with an inflammatory condition, or heterodimer thereof, in the body of an individual, the method comprising administering to the

individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined above.

5 In one embodiment of the above aspects of the invention, the method further comprises the step of detecting the location of the compound in the individual.

A seventh aspect of the invention provides a method for monitoring the progression of an inflammatory condition in an individual, the method comprising:

10

(a) providing a sample of cells collected from the individual at a first time point and measuring the amount of integrin $\alpha 11$ subunit protein therein using an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as described herein;

15

(b) providing a sample of cells collected from the individual at a second time point and measuring the amount of integrin $\alpha 11$ subunit protein therein using an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as described herein; and

20

(c) comparing the amount of integrin $\alpha 11$ subunit protein measured in steps (a) and (b)

wherein an increased amount of integrin $\alpha 11$ subunit protein measured in step
25 (b) compared to step (a) is indicative of a progression in the inflammatory condition.

An eighth aspect of the invention provides a method for identifying cells associated with an inflammatory condition, the method comprising measuring
30 the amount of integrin $\alpha 11$ subunit protein in a sample of cells to be tested using an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as described herein and comparing it to the amount of integrin $\alpha 11$ subunit protein to a positive and/or negative control. The positive control may comprise cells from a subject who is suffering from the inflammatory

condition and the negative control may comprise cells from a healthy subject who is not suffering from the inflammatory condition.

In one embodiment of the invention, the cells are selected from the group
5 consisting of chondrocyte cells, fibroblast cells and mesenchymal cells (e.g. mesenchymal stem cells).

The amount of integrin $\alpha 11$ subunit in a sample may be determined using methods well known in the art. Suitable methods for assaying integrin $\alpha 11$
10 protein levels in a biological sample include antibody-based techniques. For example, integrin $\alpha 11$ protein expression in tissues can be studied with classical immunohistological methods. In these, the specific recognition is provided by the primary antibody (polyclonal or monoclonal) but the secondary detection
15 system can utilize fluorescent, enzyme, or other conjugated secondary antibodies. As a result, an immunohistological staining of tissue section for pathological examination is obtained. Tissues can also be extracted, e.g. with urea and neutral detergent, for the liberation of integrin $\alpha 11$ protein for western
20 blot or dot/slot assay (Jalkanen *et al.*, 1985, *J. Cell. Biol.* **101**:976-985; Jalkanen *et al.*, 1987, *J. Cell. Biol.* **105**:3087-3096, which are incorporated herein by reference). In this technique, which is based on the use of cationic solid phases, quantitation of integrin $\alpha 11$ protein can be accomplished using isolated integrin $\alpha 11$ protein as a standard. This technique can also be applied to body fluids.

25 In one embodiment, the cells to be tested are identified as cells associated with an inflammatory condition by the upregulation of integrin $\alpha 11$ subunit protein levels compared to corresponding normal healthy cells. By "upregulated" we mean that the integrin $\alpha 11$ subunit protein is increased by at least 10% compared to expression of the integrin in normal (healthy) cells. For example,
30 the level of the integrin $\alpha 11$ subunit protein may be increased by at least 20%, 30%, 40%, 50%, or even 100% or more.

In a further embodiment, the above methods further comprise the step of detecting the location of the compound in the individual.

Detecting the compound or antibody can be achieved using methods well known in the art of clinical imaging and diagnostics. The specific method required will depend on the type of detectable label attached to the compound
5 or antibody. For example, radioactive atoms may be detected using autoradiography or in some cases by magnetic resonance imaging (MRI) as described above.

In a further embodiment of the above methods of the invention, the
10 inflammatory condition is selected from the group consisting of arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, peridontitis, psoriasis, asthma, systemic lupus erythematosus, multiple sclerosis, ankylosing spondylitis,
15 psoriatic arthritis and autoimmune chronic inflammatory diseases.

For example, the inflammatory condition may be an arthritic disease, such as rheumatoid arthritis or osteoarthritis.

20 A ninth aspect of the invention provides an antibody or antigen-binding fragment thereof capable of competing for binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, with an scFv molecule having the amino acid sequence of SEQ ID NO: 11, or a variant, fusion or derivative of said antibody or antigen-binding fragment, or a fusion of a said variant or derivative thereof,
25 which retains the binding specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, with the proviso that the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, does not comprise all of the amino acid sequences of SEQ ID NOS: 1 to 6.

30 Suitable antibodies, antigen-binding fragments, variants, fusions and derivatives are described above in relation to the uses and methods of the invention.

Such antibodies, antigen-binding fragments, variants, fusions and derivatives capable of competing for binding to an integrin $\alpha 11$ subunit with an scFv molecule having the amino acid sequence of SEQ ID NO: 11 may be identified using techniques well known in the art. For example, phage display antibody libraries may first be screened to identify antibodies which bind to an integrin $\alpha 11$ subunit, which antibodies may then be further tested to identify those which compete with an scFv molecule having the amino acid sequence of SEQ ID NO: 11.

10 In one embodiment of the antibodies, antigen-binding fragments, variants, fusions and derivatives of the ninth aspect of the invention, the integrin $\alpha 11$ subunit, or a heterodimer thereof, is localised on the surface of a cell (such as a chondrocyte or fibroblast).

15 It will be appreciated by persons skilled in the art that the antibodies, antigen-binding fragments, variants, fusions and derivatives of the ninth aspect of the invention have utility in the above-described uses and methods of the invention.

By “capable of competing” for binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, with an scFv molecule having the amino acid sequence of SEQ ID NO: 11, we mean that the antibody or antigen-binding fragment, variant, fusion or derivative thereof, or fusion of a said variant or derivative thereof, is capable of inhibiting or otherwise interfering, at least in part, with the binding of the scFv molecule of SEQ ID NO: 11 to the integrin $\alpha 11$ subunit, or heterodimer thereof. Competitive binding may be determined by methods well known to those skilled in the art, such as ELISA (as described above).

For example, the antibody or antigen-binding fragment, variant, fusion or derivative thereof, or fusion of a said variant or derivative thereof, may be capable of inhibiting the binding of the scFv molecule of SEQ ID NO: 11 to the integrin $\alpha 11$ subunit, or heterodimer thereof by at least 10%, for example at least 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 35% or even by 100%.

In one embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of binding to same epitope as an scFv molecule having the amino acid sequence of SEQ ID NO: 11.

- 5 In an alternative embodiment, the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of binding to an epitope distinct from that to which an scFv molecule having the amino acid sequence of SEQ ID NO: 11 binds.
- 10 By "epitope" it is herein intended to mean a site of a molecule to which an antibody binds, *i.e.* a molecular region of an antigen. An epitope may be a linear epitope, which is determined by *e.g.* the amino acid sequence, *i.e.* the primary structure, or a three-dimensional epitope, defined by the secondary structure, *e.g.* folding of a peptide chain into beta sheet or alpha helical, or by the tertiary
- 15 structure, *e.g.* way which helices or sheets are folded or arranged to give a three-dimensional structure, of an antigen.

- In one embodiment of the ninth aspect of the invention, the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, comprises or
- 20 consists of an intact antibody.

- In an alternative embodiment of the ninth aspect of the invention, the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, comprises or consists of an antigen-binding fragment selected from the group
- 25 consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), and Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments).

- It will be appreciated that the antibody may be a recombinant antibody, such as a monoclonal antibody. For example, the antibody or antigen-binding fragment
- 30 thereof may be human or humanised.

In a further embodiment of the ninth aspect of the invention, the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof is capable of modulating (*e.g.* inhibiting) the degradation of collagen, *in vitro* and/or *in vivo*

The antibodies, antigen-binding fragments, variants, fusions and derivatives of the ninth aspect of the invention may have utility in the treatment of an inflammatory condition, for example selected from the group consisting of
5 arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, peridontitis, psoriasis, asthma, systemic lupus erythematosus, multiple sclerosis and autoimmune chronic inflammatory diseases.

10

In an additional embodiment of the ninth aspect of the invention, the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof further comprises a therapeutic and/or detectable moiety (as described above).

15 A tenth aspect of the invention provides a nucleic acid molecule encoding an antibody or antigen-binding fragment, or variant, fusion or derivative thereof according to the ninth aspect of the invention, or a component polypeptide chain thereof.

20 The isolated nucleic acid molecule is suitable for expressing antibody or antigen-binding fragment, or variant, fusion or derivative thereof, according to the ninth aspect of the invention. By 'suitable for expressing' is meant that the nucleic acid molecule is a polynucleotide that may be translated to form the polypeptide, for example RNA, or that the polynucleotide (which is preferably
25 DNA) encoding the polypeptide of the invention is inserted into an expression vector, such as a plasmid, in proper orientation and correct reading frame for expression. The polynucleotide may be linked to the appropriate transcriptional and translational regulatory control nucleotide sequences recognised by any desired host; such controls may be incorporated in the expression vector.

30

Thus, the nucleic acid molecule may be DNA or RNA.

The nucleic acid molecule (or polynucleotide) may be expressed in a suitable host to produce the polypeptide product of the invention. Thus, the

polynucleotide encoding the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, may be used in accordance with known techniques, appropriately modified in view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell
5 for the expression and production of the polypeptide of the invention (for example, see Sambrook & Russell, 2000, *Molecular Cloning, A Laboratory Manual*, Third Edition, Cold Spring Harbor, New York, which is incorporated herein by reference).

10 The nucleic acid molecule encoding the polypeptide may be joined to a wide variety of other polynucleotide sequences for introduction into an appropriate host. The companion polynucleotide will depend upon the nature of the host, the manner of the introduction of the polynucleotide into the host, and whether episomal maintenance or integration is desired.

15

In brief, expression vectors may be constructed comprising a nucleic acid molecule which is capable, in an appropriate host, of expressing the polypeptide binding moiety or compound encoded by the nucleic acid molecule.

20 A variety of methods have been developed to operably link nucleic acid molecules, especially DNA, to vectors, for example, via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted into the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the
25 complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, e.g. generated by endonuclease restriction digestion, is treated with
30 bacteriophage T4 DNA polymerase or *E. coli* DNA polymerase I, enzymes that remove protruding, 3'-single-stranded termini with their 3'-5'-exonucleolytic activities, and fill in recessed 3'-ends with their polymerising activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a larger molar excess of linker molecules in the presence of an enzyme that is able to catalyse the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA
5 ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

10

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies Inc., New Haven, CN, USA.

15 A desirable way to modify the DNA encoding the polypeptide of the invention is to use PCR. This method may be used for introducing the DNA into a suitable vector, for example by engineering in suitable restriction sites, or it may be used to modify the DNA in other useful ways as is known in the art.

20 In this method the DNA to be enzymatically amplified is flanked by two specific primers which themselves become incorporated into the amplified DNA. The said specific primers may contain restriction endonuclease recognition sites which can be used for cloning into expression vectors using methods known in the art.

25

The DNA (or in the case of retroviral vectors, RNA) is then expressed in a suitable host to produce a polypeptide comprising the compound of the invention or binding moiety thereof. Thus, the DNA encoding the polypeptide may be used in accordance with known techniques, appropriately modified in
30 view of the teachings contained herein, to construct an expression vector, which is then used to transform an appropriate host cell for the expression and production of the compound of the invention or binding moiety thereof. Such techniques include those disclosed in US Patent Nos. 4,440,859 issued 3 April 1984 to Rutter *et al*, 4,530,901 issued 23 July 1985 to Weissman, 4,582,800

issued 15 April 1986 to Crowl, 4,677,063 issued 30 June 1987 to Mark *et al*,
4,678,751 issued 7 July 1987 to Goeddel, 4,704,362 issued 3 November 1987 to
Itakura *et al*, 4,710,463 issued 1 December 1987 to Murray, 4,757,006 issued
12 July 1988 to Toole, Jr. *et al*, 4,766,075 issued 23 August 1988 to Goeddel *et*
5 *al* and 4,810,648 issued 7 March 1989 to Stalker, all of which are incorporated
herein by reference.

The DNA (or in the case of retroviral vectors, RNA) encoding the polypeptide
binding moiety for use in the methods of the invention may be joined to a wide
10 variety of other DNA sequences for introduction into an appropriate host. The
companion DNA will depend upon the nature of the host, the manner of the
introduction of the DNA into the host, and whether episomal maintenance or
integration is desired.

15 Generally, the DNA is inserted into an expression vector, such as a plasmid, in
proper orientation and correct reading frame for expression. If necessary, the
DNA may be linked to the appropriate transcriptional and translational
regulatory control nucleotide sequences recognised by the desired host,
although such controls are generally available in the expression vector. The
20 vector is then introduced into the host through standard techniques. Generally,
not all of the hosts will be transformed by the vector. Therefore, it will be
necessary to select for transformed host cells. One selection technique involves
incorporating into the expression vector a DNA sequence, with any necessary
control elements, that codes for a selectable trait in the transformed cell, such as
25 antibiotic resistance. Alternatively, the gene for such selectable trait can be on
another vector, which is used to co-transform the desired host cell.

Host cells that have been transformed by the expression vector of the invention
are then cultured for a sufficient time and under appropriate conditions known
30 to those skilled in the art in view of the teachings disclosed herein to permit the
expression of the polypeptide, which can then be recovered.

Many expression systems are known, including bacteria (for example, *E. coli*
and *Bacillus subtilis*), yeasts (for example *Saccharomyces cerevisiae*),

filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells.

5 The vectors typically include a prokaryotic replicon, such as the ColE1 *ori*, for propagation in a prokaryote, even if the vector is to be used for expression in other, non-prokaryotic, cell types. The vectors can also include an appropriate promoter such as a prokaryotic promoter capable of directing the expression (transcription and translation) of the genes in a bacterial host cell, such as *E. coli*, transformed therewith.

10

A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and transcription to occur. Promoter sequences compatible with exemplary bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA
15 segment of the present invention.

20

Typical prokaryotic vector plasmids are pUC18, pUC19, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA, USA) and pTrec99A and pKK223-3 available from Pharmacia, Piscataway, NJ, USA.

25

A typical mammalian cell vector plasmid is pSVL available from Pharmacia, Piscataway, NJ, USA. This vector uses the SV40 late promoter to drive expression of cloned genes, the highest level of expression being found in T antigen-producing cells, such as COS-1 cells.

30

An example of an inducible mammalian expression vector is pMSG, also available from Pharmacia. This vector uses the glucocorticoid-inducible promoter of the mouse mammary tumour virus long terminal repeat to drive expression of the cloned gene.

Useful yeast plasmid vectors are pRS403-406 and pRS413-416 and are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (YIps) and incorporate the yeast selectable markers *HIS3*, *TRP1*,

LEU2 and *URA3*. Plasmids pRS413-416 are Yeast Centromere plasmids (Ycps).

Other vectors and expression systems are well known in the art for use with a
5 variety of host cells.

The host cell can be either prokaryotic or eukaryotic. Bacterial cells are preferred prokaryotic host cells and typically are a strain of *E. coli* such as, for example, the *E. coli* strains DH5 available from Bethesda Research
10 Laboratories Inc., Bethesda, MD, USA, and RR1 available from the American Type Culture Collection (ATCC) of Rockville, MD, USA (No. ATCC 31343). Exemplary eukaryotic host cells include yeast, insect and mammalian cells, preferably vertebrate cells such as those from a mouse, rat, monkey or human fibroblastic and kidney cell lines. Yeast host cells include YPH499, YPH500
15 and YPH501 which are generally available from Stratagene Cloning Systems, La Jolla, CA 92037, USA. Exemplary mammalian host cells include Chinese hamster ovary (CHO) cells available from the ATCC as CRL 1658 and 293 cells which are human embryonic kidney cells. Exemplary insect cells are Sf9 cells which can be transfected with baculovirus expression vectors.

20 Transformation of appropriate cell hosts with a DNA construct of the present invention is accomplished by well-known methods that typically depend on the type of vector used. With regard to transformation of prokaryotic host cells, see, for example, Cohen *et al* (1972) *Proc. Natl. Acad. Sci. USA* **69**, 2110 and
25 Sambrook *et al* (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY. Transformation of yeast cells is described in Sherman *et al* (1986) *Methods In Yeast Genetics, A Laboratory Manual*, Cold Spring Harbor, NY, all of which are incorporated herein by reference. The method of Beggs (1978) *Nature* **275**, 104-109, which is
30 incorporated herein by reference, is also useful. With regard to vertebrate cells, reagents useful in transfecting such cells, for example calcium phosphate and DEAE-dextran or liposome formulations, are available from Stratagene Cloning Systems, or Life Technologies Inc., Gaithersburg, MD 20877, USA.

Electroporation is also useful for transforming and/or transfecting cells and is well known in the art for transforming yeast cells, bacterial cells, insect cells and vertebrate cells.

- 5 For example, many bacterial species may be transformed by the methods described in Luchansky *et al* (1988) *Mol. Microbiol.* **2**, 637-646, which is incorporated herein by reference. The greatest number of transformants is consistently recovered following electroporation of the DNA-cell mixture suspended in 2.5 PEB using 6250V per cm at 25 μ FD.

10

Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente (1990) *Methods Enzymol.* **194**, 182, which is incorporated herein by reference.

- 15 Successfully transformed cells, *i.e.* cells that contain a DNA construct of the present invention, can be identified by well-known techniques. For example, cells resulting from the introduction of an expression construct of the present invention can be grown to produce the polypeptide of the invention. Cells can be harvested and lysed and their DNA content examined for the presence of the
- 20 DNA using a method such as that described by Southern (1975) *J. Mol. Biol.* **98**, 503 or Berent *et al* (1985) *Biotech.* **3**, 208, which is incorporated herein by reference. Alternatively, the presence of the protein in the supernatant can be detected using antibodies as described below.

- 25 In addition to directly assaying for the presence of recombinant DNA, successful transformation can be confirmed by well-known immunological methods when the recombinant DNA is capable of directing the expression of the protein. For example, cells successfully transformed with an expression vector produce proteins displaying appropriate antigenicity.

30

Samples of cells suspected of being transformed are harvested and assayed for the protein using suitable antibodies.

The host cell may be a host cell within a non-human animal body. Thus, transgenic non-human animals which express a polypeptide according to the invention by virtue of the presence of the transgene are included. In one embodiment, the transgenic non-human animal is a rodent such as a mouse.

- 5 Transgenic non-human animals can be made using methods well known in the art.

Methods of cultivating host cells and isolating recombinant proteins are well known in the art. It will be appreciated that, depending on the host cell, the
10 compounds of the invention (or binding moieties thereof) produced may differ. For example, certain host cells, such as yeast or bacterial cells, either do not have, or have different, post-translational modification systems which may result in the production of forms of compounds of the invention (or binding moieties thereof) which may be post-translationally modified in a different way.

15

In one embodiment, the host cell is a bacterial cell. Alternatively, the host cell may be a mammalian cell, for example a human cell, for example a cell from the order of primates, such as a human cell or an ape cell, or a cell from the order of rodentia, such as a rat cell, a mouse cell, a hamster cell, a rabbit cell, a
20 squirrel cell, a guinea pig cell, a marmot cell, or a beaver cell.

Further embodiments are where the host cell is a CHO (Chinese hamster ovary) cell, or a HEK (human embryonic kidney) cell.

- 25 Thus, an eleventh aspect of the invention provides a vector comprising a nucleic acid molecule according to the tenth aspect of the invention.

Likewise, twelfth aspect of the invention provides recombinant host cell comprising a nucleic acid molecule according to the tenth aspect of the
30 invention or a vector according to the eleventh aspect of the invention.

A thirteenth aspect of the invention provides a method for producing an antibody or antigen-binding fragment, or variant, fusion or derivative thereof,

according to the ninth aspect of the invention, the method comprising culturing a host cell according to the twelfth aspect of the invention under conditions which permit expression of the encoded antibody or antigen-binding fragment thereof. It will be appreciated that such methods may also be used to produce
5 an antibody or antigen-binding fragment, or variant, fusion or derivative thereof for use in the methods of the invention.

Such polypeptides can be produced *in vitro* using a commercially available *in vitro* translation system, such as rabbit reticulocyte lysate or wheatgerm lysate
10 (available from Promega). For example, the translation system may be rabbit reticulocyte lysate. Conveniently, the translation system may be coupled to a transcription system, such as the TNT transcription-translation system (Promega). This system allows the production of a suitable mRNA transcript from an encoding DNA polynucleotide in the same reaction as the translation.

15

Persons skilled in the art will further appreciate that the medicaments and agents described above have utility in both the medical and veterinary fields. Thus, the medicaments and agents may be used in the treatment of both human and non-human animals (such as horses, dogs, mice, rats, apes, monkeys, pigs,
20 and cats). Preferably, however, the patient is human.

A fourteenth aspect of the invention provides a pharmaceutical composition comprising an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, according to the ninth aspect of the invention, and a
25 pharmaceutically acceptable excipient, diluent or carrier.

As used herein, 'pharmaceutical composition' means a therapeutically effective formulation.

30 A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen (for example, an amount sufficient to inhibit the degradation of collagen). This is a predetermined quantity of active material calculated to produce a desired

therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce or prevent a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent.

10 In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in

15 the art.

It will be appreciated by persons skilled in the art that such an effective amount of the antibody or antigen-binding fragment, or a variant, fusion or derivative therefor formulation thereof may be delivered as a single bolus dose (*i.e.* acute administration) or, more preferably, as a series of doses over time (*i.e.* chronic administration).

20

It will be further appreciated by persons skilled in the art that the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, for use in to the methods of the invention may be administered in combination with one or more other conventional agents for the treatment of inflammatory conditions.

25

For example, in the case of rheumatoid arthritis, suitable conventional agents include, but are not limited to, disease-modifying antirheumatic drugs (DMARDs, *e.g.* methotrexate), gold salts, antimalarials, sulphasalazine, tetracyclines, cyclosporine, NSAIDs, corticosteroids, Leflunomide (Arava; Aventis), tumour-necrosis factor- α (TNF α) inhibitors, such as etanercept (Enbrel; Amgen), infliximab (Remicade; J&J/Centocor) and adalimumab (Humira; Abbott).

30

In the case of OA, suitable conventional agents include, but are not limited to analgesics or anti-inflammatory agents, such as acetaminophen (also known as paracetamol), or non-steroidal anti-inflammatory drugs (NSAIDs) inhibiting
5 cyklooxygenase (COX), such as COX2 inhibitors, Disease-Modifying Osteoarthritis Drugs (DMOADs), such as MMP inhibitors, or interleukin 1 (IL-1) inhibitor.

The antibody or antigen-binding fragment, or variant, fusion or derivative
10 thereof, according to the ninth aspect of the invention may be formulated at various concentrations, depending on the efficacy/toxicity of the compound being used. In one embodiment, the formulation comprises the agent of the invention at a concentration of between 0.1 μ M and 1 mM, for example between 1 μ M and 100 μ M, between 5 μ M and 50 μ M, between 10 μ M and
15 50 μ M, between 20 μ M and 40 μ M or about 30 μ M. For *in vitro* applications, formulations may comprise a lower concentration of a compound of the invention, for example between 0.0025 μ M and 1 μ M.

Thus, there is provided a pharmaceutical formulation comprising an amount of
20 an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, effective to treat an inflammatory condition (as described above).

It will be appreciated by persons skilled in the art that the medicaments and agents will generally be administered in admixture with a suitable
25 pharmaceutical excipient diluent or carrier selected with regard to the intended route of administration and standard pharmaceutical practice (for example, see *Remington: The Science and Practice of Pharmacy*, 19th edition, 1995, Ed. Alfonso Gennaro, Mack Publishing Company, Pennsylvania, USA, which is incorporated herein by reference).

30

For example, the medicaments and agents can be administered orally, buccally or sublingually in the form of tablets, capsules, ovules, elixirs, solutions or suspensions, which may contain flavouring or colouring agents, for immediate-,

delayed- or controlled-release applications. The medicaments and agents may also be administered via intracavernosal injection.

Such tablets may contain excipients such as microcrystalline cellulose, lactose,
5 sodium citrate, calcium carbonate, dibasic calcium phosphate and glycine, disintegrants such as starch (for example, corn, potato or tapioca starch), sodium starch glycollate, croscarmellose sodium and certain complex silicates, and granulation binders such as polyvinylpyrrolidone, hydroxypropylmethylcellulose (HPMC), hydroxy-propylcellulose (HPC),
10 sucrose, gelatin and acacia. Additionally, lubricating agents such as magnesium stearate, stearic acid, glyceryl behenate and talc may be included.

Solid compositions of a similar type may also be employed as fillers in gelatin capsules. Exemplary excipients in this regard include lactose, starch, cellulose,
15 milk sugar or high molecular weight polyethylene glycols. For aqueous suspensions and/or elixirs, the compounds of the invention may be combined with various sweetening or flavouring agents, colouring matter or dyes, with emulsifying and/or suspending agents and with diluents such as water, ethanol, propylene glycol and glycerin, and combinations thereof.

20 The medicaments and agents can also be administered parenterally, for example, intravenously, intra-articularly, intra-arterially, intraperitoneally, intra-theccally, intraventricularly, intrasternally, intracranially, intra-muscularly or subcutaneously, or they may be administered by infusion techniques. They are
25 best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (for example, to a pH of from 3 to 9), if necessary. The preparation of suitable parenteral formulations under sterile conditions is readily accomplished by standard
30 pharmaceutical techniques well known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood

of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

For oral and parenteral administration to human patients, the daily dosage level of the medicaments and agents will usually be from 1 to 1000 mg per adult (*i.e.* from about 0.015 to 15 mg/kg), administered in single or divided doses.

It is appreciated that for the prevention or treatment of disease, the appropriate dosage of an antibody will depend on the type of disease to be treated, the severity and of course of the disease, whether the antibody or a fragment thereof is administered for preventative or therapeutic purposes, the course of previous therapy and the patient's clinical history and response to the antibody or a fragment thereof. The antibody, antigen-binding fragment, variant, fusion or derivative thereof according to the present invention is suitably administered to the patient at one time or over a series of treatments. Depending on the type and severity of the disease, about 0.015 to 15mg of antibody or a fragment thereof /kg of patient weight is an initial candidate dosage for administration to the patient. Administration may be, for example, by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is repeated until a desired suppression or alleviation of the disease symptoms occurs. However, other dosage regimens may be useful and are not excluded.

The effectiveness of antibody, antigen binding fragment, variant, fusion or derivative thereof in alleviating the symptoms, preventing or treating disease may be improved by serial administering or administration in combination with another agent that is effective for the same clinical indication, such as another antibody or a fragment thereof directed against a different epitope than that of

the antibody according to the invention, or one or more conventional therapeutic agents known for the intended therapeutic indication,

Suitable pharmaceutically acceptable agents affecting such indications may be
5 anti-inflammatory drugs such as non steroidal anti-inflammatory drugs (NSAIDS) for the treatment of inflammatory diseases such as arthritic diseases e.g. osteoarthritis, rheumatoid arthritis; anti-cytokine agents e.g. anti-TNF antibodies, interleukin receptor antagonist, matrix metalloproteinase (MMP) inhibitors or bone morphogenic proteins (BMP); local anaesthetics for use post-
10 operatively following orthopaedic surgery for the treatment of pain management or hypolipidemic drugs for treatment of atherosclerotic plaque, matrix metalloproteinase (MMPs) inhibitors or bone morphogenic proteins (BMPs).

15 The medicaments and agents can also be administered intranasally or by inhalation and are conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, e.g. dichlorodifluoromethane, trichlorofluoro-methane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such
20 as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas. In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active compound, e.g. using a
25 mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

30

Aerosol or dry powder formulations may be arranged so that each metered dose or 'puff' contains at least 1 mg of a compound of the invention for delivery to the patient. It will be appreciated that the overall daily dose with an aerosol will

vary from patient to patient, and may be administered in a single dose or, more usually, in divided doses throughout the day.

Alternatively, the medicaments and agents can be administered in the form of a suppository or pessary, or they may be applied topically in the form of a lotion, solution, cream, ointment or dusting powder. The compounds of the invention may also be transdermally administered, for example, by the use of a skin patch. They may also be administered by the ocular route.

For application topically to the skin, the medicaments and agents can be formulated as a suitable ointment containing the active compound suspended or dissolved in, for example, a mixture with one or more of the following: mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene polyoxypropylene compound, emulsifying wax and water. Alternatively, they can be formulated as a suitable lotion or cream, suspended or dissolved in, for example, a mixture of one or more of the following: mineral oil, sorbitan monostearate, a polyethylene glycol, liquid paraffin, polysorbate 60, cetyl esters wax, cetearyl alcohol, 2-octyldodecanol, benzyl alcohol and water.

Formulations suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatin and glycerin, or sucrose and acacia; and mouthwashes comprising the active ingredient in a suitable liquid carrier.

Where the medicament or agent is a polypeptide, it may be preferable to use a sustained-release drug delivery system, such as a microsphere. These are designed specifically to reduce the frequency of injections. An example of such a system is Nutropin Depot which encapsulates recombinant human growth hormone (rhGH) in biodegradable microspheres that, once injected, release rhGH slowly over a sustained period.

Sustained-release immunoglobulin compositions also include liposomally entrapped immunoglobulin. Liposomes containing the immunoglobulin are

prepared by methods known *per se*. See, for example Epstein *et al.*, *Proc. Natl. Acad. Sci. USA* **82**: 3688-92 (1985); Hwang *et al.*, *Proc. Natl. Acad. Sci. USA* **77**: 4030-4 (1980); U.S. Patent Nos. 4,485,045; 4,544, 545; 6,139,869; and 6,027,726, which are incorporated herein by reference. Ordinarily, the
5 liposomes are of the small (about 200 to about 800 Angstroms), unilamellar type in which the lipid content is greater than about 30 mole percent (mol. %) cholesterol; the selected proportion being adjusted for the optimal immunoglobulin therapy.

10 Alternatively, polypeptide medicaments and agents can be administered by a surgically implanted device that releases the drug directly to the required site.

Electroporation therapy (EPT) systems can also be employed for the administration of proteins and polypeptides. A device which delivers a pulsed
15 electric field to cells increases the permeability of the cell membranes to the drug, resulting in a significant enhancement of intracellular drug delivery.

Proteins and polypeptides can also be delivered by electroincorporation (EI). EI occurs when small particles of up to 30 microns in diameter on the surface of
20 the skin experience electrical pulses identical or similar to those used in electroporation. In EI, these particles are driven through the stratum corneum and into deeper layers of the skin. The particles can be loaded or coated with drugs or genes or can simply act as "bullets" that generate pores in the skin through which the drugs can enter.

25

An alternative method of protein and polypeptide delivery is the thermo-sensitive ReGel injectable. Below body temperature, ReGel is an injectable liquid while at body temperature it immediately forms a gel reservoir that slowly erodes and dissolves into known, safe, biodegradable polymers. The
30 active drug is delivered over time as the biopolymers dissolve.

Protein and polypeptide pharmaceuticals can also be delivered orally. One such system employs a natural process for oral uptake of vitamin B12 in the body to co-deliver proteins and polypeptides. By riding the vitamin B12 uptake system,

the protein or polypeptide can move through the intestinal wall. Complexes are produced between vitamin B12 analogues and the drug that retain both significant specificity for intrinsic factor (IF) in the vitamin B12 portion of the complex and significant bioactivity of the drug portion of the complex.

5

The antibody and antibody-derived binding agents according to the ninth aspect of the invention may be provided in the form of a kit comprising a pharmaceutical composition as described above. Thus, a kit may be provided for use in the treatment of an inflammatory condition

10

Alternatively, the kit may comprise a detectable antibody or antigen-binding fragment or derivative thereof according to the invention, suitable for use in diagnosis. Such a diagnostic kit may comprise, in an amount sufficient for at least one assay, the diagnostic agent as a separately packaged reagent.

15 Instructions for use of the packaged reagent are also typically included. Such instructions typically include a tangible expression describing reagent concentrations and/or at least one assay method parameter such as the relative amounts of reagent and sample to be mixed, maintenance time periods for reagent/sample admixtures, temperature, buffer conditions and the like.

20

A further aspect of the present invention provides a method for identifying a candidate compound for treating an inflammatory condition, the method comprising the following steps:

- 25 a) providing a compound to be tested;
- b) testing the ability of the compound to compete for binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, with an antibody or antigen-binding fragment comprising the following CDR sequences:

30

FSRYMHWRQVPG	[SEQ ID NO: 1];
SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
ARVSGDGYNFGA	[SEQ ID NO: 3];
CTGSSSNIGAGYDVH	[SEQ ID NO: 4];

GYNERPS

[SEQ ID NO: 5]; and

CAAWDDSLSGHV

[SEQ ID NO: 6].

5 or a variant, fusion or derivative thereof, or a fusion of a said variant or derivative thereof, which retains the binding specificity for the integrin $\alpha 11$ subunit, or heterodimer thereof,

wherein the test compound is identified as a candidate compound for treating an inflammatory condition if it is able to compete for binding to the integrin $\alpha 11$ subunit, or heterodimer thereof.

Competitive binding may be assayed using methods well known in the art, such as ELISA (see above).

15 In one embodiment, the method further comprises the step of testing the ability of the compound to modulate (*e.g.* inhibit) the degradation of collagen.

By “modulate” we include increasing or decreasing the rate of degradation of collagen. It will be appreciated that inhibition of the degradation of collagen by be in whole or in part. For example, collagen degradation may be inhibited by 20 10% or more, *e.g.* 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or 100%, compared to the degradation of collagen in the absence of the test compound.

Conveniently, some or all of the testing steps are performed *in vivo*.

25 Alternatively, some or all of the testing steps may be performed *in vitro*, for example using immunohistochemical methods (*e.g.* staining with Safranin) (see Examples below).

30 Exemplary test compounds include polypeptides, or a fusion or derivative thereof, or a fusion of a said derivative thereof. For example, the test compound may be an antibody or antigen-binding fragment thereof, or a variant, fusion or derivative thereof, or a fusion of a said variant or derivative thereof.

In one embodiment, the test compound is an intact antibody.

Alternatively, the test compound may be an antigen-binding fragment selected
 5 from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-
 bonded Fv), Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂
 fragments), single antibody chains (*e.g.* heavy or light chains), single variable
 domains (*e.g.* V_H and V_L domains) and domain antibodies (dAbs, including
 single and dual formats [*i.e.* dAb-linker-dAb]).

10

A further aspect of the invention provides an epitope defined by an antibody or
 an antigen-binding fragment thereof with binding specificity for an integrin $\alpha 11$
 subunit, or a heterodimer thereof, or of a variant, fusion or derivative of said
 antibody or an antigen-binding fragment, or a fusion of a said variant or
 15 derivative thereof, which retains the binding specificity of said antibody or an
 antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer
 thereof, wherein the antibody or fragment comprises the following amino acid
 sequences:

20	FSRYMHWRQVPG	[SEQ ID NO: 1];
	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
25	CAAWDDSLSGHV	[SEQ ID NO: 6].

In one embodiment, the epitope comprises or consists of the following amino acid sequence:

SDGSIECVNEERRLQKQVCNVSYPPFRAKAKVAFRLDFEFSKSIFLHHLE
5 IELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSSLSHYEVKLN
SSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIPIATRSGNRLLKLRDF
LTDEANTSCNIWGNSTEYRPTPVEEDLRRAPQLNHSNSDVVSINCNIRLV
PNQEINFHLLGNLWLRSLKALKYKSMKIMVNAALQRQFHSPFIFREEDP
SRQIVFEISKQEDWQVP

10

[SEQ ID NO: 15]

or a fragments, fusions or variants thereof.

Exemplary variants, fusions and fragments thereof are disclosed above.

15

Thus, as an additional aspect, the invention also provides an isolated polypeptide comprising or consisting of the following amino acid sequence:

SDGSIECVNEERRLQKQVCNVSYPPFRAKAKVAFRLDFEFSKSIFLHHLE
20 IELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSSLSHYEVKLN
SSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIPIATRSGNRLLKLRDF
LTDEANTSCNIWGNSTEYRPTPVEEDLRRAPQLNHSNSDVVSINCNIRLV
PNQEINFHLLGNLWLRSLKALKYKSMKIMVNAALQRQFHSPFIFREEDP
SRQIVFEISKQEDWQVP

25

[SEQ ID NO: 15]

or a fragments, fusions or variants thereof.

Said polypeptide may stimulate an immune response and thus work as an immunogenic epitope.

30

In one embodiment, the polypeptide, fragment, fusion or variant thereof is capable of binding to an antibody or an antigen-binding fragment thereof with binding specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, or of a

variant, fusion or derivative of said antibody or an antigen-binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity of said antibody or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer thereof,

5

wherein the antibody or fragment comprises the following amino acid sequences:

	FSRYMHWRQVPG	[SEQ ID NO: 1];
10	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
	CAAWDDSLSGHVV	[SEQ ID NO: 6].

15

An additional aspect of the invention provides the use of the above-described epitopes and isolated polypeptides to identify or produce an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, according to the ninth aspect of the invention.

20

The present invention also encompasses isolated nucleic acid molecules encoding the above-described epitopes and isolated polypeptides, as well as vectors, host cells and methods of making the same.

25 As used herein, the singular forms “a”, “and”, and “the” include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to “an antibody” includes a plurality of such antibodies and reference to “the dosage” includes reference to one or more dosages and equivalents thereof known to those skilled in the art, and so forth.

Non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

5 **Brief Description of the Figures**

Figure 1: Screening the *n-CoDeR*® antibody library for anti- α 11 integrin binders. One hundred and ninety two 192 screened α 11 integrin active scFv clones were confirmed for binding to α 11 integrin transfected 293 cells, but no
10 binding to α 10 integrin transfected 293 cells, by co-incubation of *E. coli* expressed His-tagged scFv clones with integrin transfected cells (as indicated in figure), mouse anti-His mAb, Cy5-conjugated anti-mouse mAb reagent, and subsequent screening in an FMAT macroconfocal screening instrument. Screened scFv were retrieved following panning of the *n-CoDeR*® antibody
15 library for α 11 integrin specific binders. One hundred and seventy two 172 scFv clones were verified as selective α 11 integrin binders using this approach.

Figure 2: Target integrin specificity of anti-integrin scFv. Hek 293 cells transfected with human α 10 integrin (left graph) or human α 11 integrin (right
20 graph) were incubated with anti- α 10 integrin scFv (control, grey peak with label), anti- α 11 integrin scFv (grey peak with label) or irrelevant control scFv (solid black peak). Binding of scFv to cells was analysed by flow-cytometry following detection with secondary Phycoerythrin conjugated anti-His mAb.

Figure 3: The ability of anti- α 11 integrin scFv to selectively inhibit
25 adhesion of α 11 integrin transfected C2C12 cells to collagen II coated plates. Alpha 10 integrin transfected C2C12 mouse fibroblast cells (A) or α 11 integrin transfected C2C12 mouse fibroblast cells (B) were pre-incubated with 10 μ g/ml of anti- α 11 integrin scFv, FITC-8 control scFv, anti- α 10 integrin
30 monoclonal antibody, or IgG2a isotype control antibody. Cells were then added to collagen II coated microtiter plates and were allowed to adhere. Unbound cells were washed away and the number of bound cells determined by an enzymatic assay. Graph represent combined data from three independent

experiments and data from each experiment was normalized to the control without antibody. (Top) ScFvs effect on cell adhesion of $\alpha 10$ transfected cells. (Bottom) ScFvs effect on cell adhesion of $\alpha 11$ transfected cells. Mean values with s.e.m. Significance calculated using one-way Anova and Dunnett's multiple comparison test. White bars: $\alpha 11$ -scFvs. Striped bars: controls.

Figure 4: Lack of binding of *n-CoDeR*® anti- $\alpha 11$ integrin scFv to peripheral blood leukocyte subpopulations. Purified human PBLs were incubated with anti- $\alpha 11$ integrin scFv $\alpha 11_A_001$ -A03-MH (grey line), positive control scFv C11 (black dotted line) or negative control scFv FITC-8 (solid black peak) in the presence of differently fluorochrome conjugated cell subpopulation specific monoclonal antibodies. Cells were washed and incubated with 1/20 PE-conjugated anti-His antibodies (R&D Systems). Positive binding of anti- $\alpha 11 \beta 1$ integrin specific scFv was recorded as FL2 fluorescence intensities greater than those observed for 97-99 % of cells stained with negative control CT17 scFv. Prior to incubation with mouse anti-His antibodies, cells were pre-blocked and kept in the presence of 200 μ g/ml of mouse IgG per 2×10^6 total leukocytes T cells, B cells, and NK cells were defined as CD3+, CD19+, CD3-CD56+ cells (gates 5, 4 and 3 respectively in left hand graphs). Monocytes (R2) and granulocytes (R1) were defined by unique forward scatter and side scatter properties and additionally as being CD3-CD19-CD56-.

Figure 5: Ratio of joint swelling, measured by Tc-99m uptake, in induced and non-induced knee at day 3 after i.a. injection of mBSA. Key: 'CT17' = Control Ab (to cholera toxin), 'A03' = exemplary anti-alpha11 whole IgG antibody.

Figure 6: *Histological score of inflammation as infiltrating cells in synovial space in sections of whole knee joint.* Arbitrary scale from 0 to 3: 0 – no inflammation, 1 – minor inflammation, 2 – moderate inflammation, 3 – severe inflammation. Key: 'CT17' = Control Ab, 'A03' = exemplary anti-alpha11 antibody.

Figure 7: *Histological score of exudate, infiltrating of cells the joint space, in sections of whole knee joint.* Arbitrary scale from 0 to 3: 0 – no inflammation, 1 – minor inflammation, 2 – moderate inflammation, 3 – severe inflammation. Key: ‘CT17’ = Control Ab, ‘A03’ = exemplary anti-alpha11 antibody.

5

Figure 8: *Proteoglycan (PG) depletion on day 15 after arthritis induction in patella cartilage measured by image analysis.* Key: ‘CT17’ = Control Ab, ‘A03’ = exemplary anti-alpha11 antibody.

10 **Figure 9:** *PG depletion – A03 reduces PG depletion on day 15 after arthritis induction in first experiment; Figure 11A shows histological scoring from patella (shown as scoring by image analysis in figure 10), Figure 11B shows histological scoring of the lateral tibia, and figure 11C shows an overview of the knee.*

15

Figure 10: *PG depletion – A03 reduces PG depletion on day 15 after arthritis induction in second experiment. Figure 12A shows histological scoring from patella, Figure 12B shows histological scoring of the lateral tibia, (p=0.02 for lateral tibia)*

20

Figure 11: *A03 reduces osteophyte size as measured on day 15 after arthritis induction.*

Figure 12: *Incidence (frequency) of arthritis in G6PI-induced arthritis.*

25 Treatment was administrated day 7, 10, 13 and 16 after G6PI immunization.

Figure 13: *Mean clinical score of arthritis in G6PI-induced arthritis.* Treatment was administrated day 7, 10, 13 and 16 after G6PI immunization.

30 **Figure 14:** *Weight change (in %) at day 13 after G6PI immunization.* Mice treated with control antibody lost mean weight of 10%. A03 treated mice lost at mean 5% in weight, clear reduction in weight loss compared to control group (p=0.08).

Example I - Production of exemplary antibody of the invention (designated 'A03')

5

Identification of *n-CoDeR*®-derived human antibodies specific for $\alpha 11\beta 1$ integrin

10 The fully human antibody library *n-CoDeR*® (see Soderlind *et al.*, 2000, *Nat Biotechnol* 18:852-6 and WO 98/32845, which are incorporated herein by reference) was screened for antibody fragments (scFv) with specificity for the $\alpha 10\beta 1$ integrin or the $\alpha 11\beta 1$ integrin. A series of combined positive and subtractive panning methodologies was devised in order to maximise retrieval of highly target integrin specific antibodies (see Appendix below). Following
15 conversion to scFv format, antibody fragments were expressed in *E. coli* and were positively or negatively screened for binding to Hek-293 cells expressing $\alpha 10\beta 1$ integrin or $\alpha 11\beta 1$ integrin, as appropriate, using FMAT technology (Fig. 1, FMAT scatter plot). Thus, hundreds of genotype unique antibody fragments specific for $\alpha 10\beta 1$ integrin or $\alpha 11\beta 1$ integrin were identified (Table 1).

20

Antibody panning procedure

A phage stock of *n-CoDeR*® scFv library in a buffer of 3% BSA, 0.02% sodium azide, 0.1% NP40, 10 mM MgCl₂ and 0.01 mM CaCl₂ in PBS was pre-
25 selected over night at 4°C using BSA coated in an immunotube and tosyl activated Dynabeads M-280 (DynaL Cat. # 142.04) coupled with rabbit-anti- $\alpha 10$ integrin and rabbit-anti- $\alpha 11$ integrin polyclonal antibodies according to Dynal's instructions. The first panning was performed over night at 4°C on lysate from 15×10^6 HEK293 cells expressing $\alpha 11\beta 1$ loaded on magnetic beads
30 coupled with rabbit-anti- $\alpha 11$ polyclonal antibody (as above). Beads were washed with 9×1ml buffer and bound phage were eluted with trypsin and amplified as described elsewhere (Hallborn & Carlsson, 2002, *Biotechniques* Dec;Suppl:30-37).

Amplified phage from the 1st panning, buffered as above, were pre-selected on polyclonal coupled Dynabeads as in 1st panning followed by a selection analogous to the first panning with lysate from 10×10^6 HEK293 $\alpha_{11}\beta_1$ cells loaded on the Dynabeads with rabbit-anti- α_{11} polyclonal antibody. After washing (9×1ml buffer) bound phage were eluted with trypsin and amplified.

Amplified phage from the 2nd panning, diluted in DMEM cell medium containing 10% FCS, 10 mM $MgCl_2$ and 10 μM $CaCl_2$, were pre-selected over night at 4°C against 45×10^6 C2C12 cells expressing $\alpha_{10}\beta_1$ and 13×10^6 HEK cells expressing $\alpha_{10}\beta_1$. Phages were then incubated for 4h at 4°C with $\alpha_{11}\beta_1$ displayed on recombinant C2C12 cells (5×10^6 cells used). After density centrifugation through 40% Ficoll (containing 2% FCS, 10 mM $MgCl_2$ and 10 μM $CaCl_2$) to remove unbound phages, remaining binders were eluted with both 76 mM citric acid pH 2.5 and 200 mM triethanolamine. The pool of eluted phages were amplified in *Escherichia coli* HB101F' for conversion to scFv format.

Conversion to scFv format

Conversion to c-myc/6xhis scFv format (*EagI* conversion)
Protein III is the functional link between the scFv and the phage particle and removal of gene *III* results in production of soluble scFv. Phagemid DNA from the n-CoDeR Lib2000 selection is digested with *EagI* to remove gene *III* and bring the 6xhis tag next to the scFv fragment and c-myc tag. The plasmid is ligated and a "killer-cut" with *EcoRI*, which has a restriction site within gene *III*, is done to eliminate re-ligated phagemids. The plasmid DNA is transformed into chemically competent *Escherichia coli* TOP10.

Table 1

Number of $\alpha 11$ integrin specific antibody fragments with unique genotype isolated from the n-CoDeR® library

5

Specificity	Type of antibody fragment	Number of clones
Anti $\alpha 11$ integrin	scFv	60
Anti $\alpha 11$ integrin	Fab	11

Specificity of antibodies for their targeted integrin α -chain

To date four different collagen binding integrin heterodimers have been identified, namely $\alpha 1\beta 1$, $\alpha 2\beta 1$, $\alpha 10\beta 1$ and $\alpha 11\beta 1$. Their inter-individual preference for binding to different types of collagen is incompletely characterised. Among the collagen binding integrins $\alpha 10\beta 1$ $\alpha 11\beta 1$ integrin and $\alpha 11\beta 1$ integrin show high homology (60% identity at amino acid level of ligand binding I-domains) (see Gullberg *et al.*, 2003, *Structure and function of $\alpha 11\beta 1$ integrin*. In: D. Gullberg (Ed) *I Domains in Integrins*). Consequently, some antibodies raised against either of these integrin heterodimers are likely to cross-react with the other.

In order to be able to attribute putative observed effects of the isolated anti- $\alpha 11\beta 1$ integrin antibodies on extracellular matrix turnover to exclusive interactions with $\alpha 11\beta 1$ integrin heterodimers, the fine specificity of antibodies for this complex was determined. Thus, anti- $\alpha 11\beta 1$ integrin scFv were examined for binding to $\alpha 10\beta 1$ integrin transfected or $\alpha 11\beta 1$ integrin transfected Hek-293 cells using flow-cytometry. By this approach, twenty-nine anti- $\alpha 11\beta 1$ integrin scFv (out of 38 examined) that bound strongly to $\alpha 11\beta 1$ integrin transfected cells, but not to $\alpha 10\beta 1$ integrin transfected cells were identified (Fig. 2).

The fine-specificity of anti-integrin antibodies is further illustrated by the ability of anti- $\alpha 11\beta 1$ integrin scFv to selectively inhibit adhesion of $\alpha 11\beta 1$ integrin transfected (but not $\alpha 10\beta 1$ integrin transfected) C2C12 cells to collagen II

coated plates (Figure 3). Considering the integrin structure and the structural changes taking place during integrin activation and binding an agent that interferes with cell adhesion (*i.e.* integrin binding) is potentially interesting. Antibodies or scFv's that bind at or close to the ligand-binding site (*i.e.* MIDAS) could physically interfere with ligand interaction. Antibodies or scFv's that do not bind close to MIDAS (*e.g.* not I-domain binders) but still have an effect on cell adhesion are likely to interfere with integrin alpha chain structure, integrin activation or alpha/beta chain interactions.

Finally, prior to examination in functional *in vitro* and *in vivo* test systems anti- $\alpha 11\beta 1$ integrin antibody clones were shown not to cross-react with defined peripheral blood leukocyte subpopulations including Granulocytes, Monocytes, T lymphocytes, B lymphocytes, and Natural Killer cells (Figure 4).

Sequence information on exemplary anti- $\alpha 11$ antibodies

Heavy chain of anti- $\alpha 11$ whole IgG antibody (A03) – amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYYMHWVRQVPGKGLEW
 VSGVSWNGSRTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY
 YCARVSGDGYNFGAWGQGT LVT VSSASTKGPSVFPLAPCSRSTSESTAA
 LGCLVKDYFPEPVT VSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS
 SSLGTKTYTCNV D HKPSNTKVDKR VESKYGPPCPSCP APEFLGGPSVFL
 FPPKPKDTLMISRTPEVTCVVDVSQEDPEVQFNWYVDGVEVHNAKTK
 PREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKA
 KGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE
 NNYKTTTPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
 TQKSLSLSLGK

[SEQ ID NO:11]

30

Light chain of anti- $\alpha 11$ whole IgG antibody (A03) – amino acid sequence

QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLI
 YGYNERPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSG
 HVVFGGGTKLTVLGQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPG
 AVTVAWKADSSPVKAGVETTPSKQSNKYAASSYLSLTPEQWKSHRS
 YSCQVTHEGSTVEKTVAPTECS

[SEQ ID NO:12]

ScFv format of anti- α 11 antibody (A03) – amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYMHVVRQVPGKGLEW
VSGVSWNGSRTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY
5 YCARVSGDGYNFGAWGQGT LVT VSSGGGGSGGGGSGGGGSQSVLTQP
PSASGTPGQRVTISCTGSSSNIGAGYDVHWYQQLPGTAPKLLIYGNER
PSGV PDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGHVVF GG
GTKLTVLG

[SEQ ID NO:13]

10

Heavy chain of anti- α 11 whole IgG antibody (A03) – nucleotide sequence

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGG
15 GGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACCTTCAGTCGTT
ACTACATGCACTGGGTCCGCCAAGTTCAGGGAAGGGGCTGGAGTG
GGTATCGGGTGTTAGTTGGAATGGCAGTAGGACGCACTATGCAGAC
TCTGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACAC
GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACTGCCGTG
20 TATTACTGTGCGAGAGTAAGTGGAGATGGCTACAATTTTGGCGCCTG
GGGCCAGGGTACACTGGTCACCGTGAGCTCAGCTTCCACCAAGGGC
CCATCCGTCTTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAG
CACAGCCGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGG
TGACGGTGTCGTGGA ACTCAGGCGCCCTGACCAGCGGCGTGACAC
25 CTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGCAGCG
TGGTGACCGTGCCCTCCAGCAGCTTGGGCACGAAGACCTACACCTGC
AACGTAGATCACAAGCCCAGCAACACCAAGGTGGACAAGAGAGTTG
AGTCCAAATATGGTCCCCCATGCCCATCATGCCCAGCACCTGAGTTC
CTGGGGGGGACCATCAGTCTTCTGTTCCCCC AAAACCCAAGGACAC
30 TCTCATGATCTCCCGGACCCCTGAGGTCACGTGCGTGGTGGTGGACG
TGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTGGATGG
CGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTTC
AACAGCACGTACCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGA
CTGGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGC
35 CTCCCGTCTCTCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGC
CCCGAGAGCCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGAT
GACCAAGAACCAGGTACGCTGACCTGCCTGGTCAAAGGCTTCTACC
CCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAA
CAACTACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCT
40 TCCTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGG
GAATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCACT
ACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAA

[SEQ ID NO: 16]

Light chain of anti- $\alpha 11$ whole IgG antibody (A03) – nucleotide sequence

CAGTCTGTGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCA
 GAGGGTCACCATCTCCTGCACTGGGAGCAGCTCCAACATCGGGGCA
 5 GGTTATGATGTACACTGGTATCAGCAGCTCCCAGGAACGGCCCCCAA
 ACTCCTCATCTATGGTTATAATGAGCGGCCCTCAGGGGTCCCTGACC
 GATTCTCTGGCTCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGT
 GGGCTCCGGTCCGAGGATGAGGCTGATTATTACTGTGCAGCATGGG
 ATGACAGCCTGAGTGGTCATGTGGTATTCGGCGGAGGAACCAAGCT
 10 GACGGTCCTAGGTCAGCCCAAGGCTGCCCCCTCGGTCACTCTGTTCC
 CGCCCTCCTCTGAGGAGCTTCAAGCCAACAAGGCCACACTGGTGTGT
 CTCATAAGTGACTTCTACCCGGGAGCCGTGACAGTGGCCTGGAAGG
 CAGATAGCAGCCCCGTCAAGGCGGGAGTGGAGACCACCACACCCTC
 CAAACAAAGCAACAACAAGTACGCGGCCAGCAGCTATCTGAGCCTG
 15 ACGCCTGAGCAGTGGAAAGTCCCACAGAAGCTACAGCTGCCAGGTCA
 CGCATGAAGGGAGCACCGTGGAGAAGACAGTGGCCCCCTACAGAATG
 TTCA

[SEQ ID NO: 17]

20

ScFv format of anti- $\alpha 11$ antibody (A03) – nucleotide sequence

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGG
 GTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTCAGTCGTT
 25 ACTACATGCACTGGGTCCGCCAAGTTCCAGGGAAGGGGCTGGAGTG
 GGTATCGGGTGTTAGTTGGAATGGCAGTAGGACGCACTATGCAGAC
 TCTGTGAAGGGCCGATTACCATCTCCAGAGACAATTCCAAGAACAC
 GCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACTGCCGTG
 TATTACTGTGCAAGAGTAAGTGGAGATGGCTACAATTTTGGCGCCTG
 30 GGGCCAGGGTACACTGGTCAACCGTGAGCAGCGGTGGAGGCGGTTCA
 GGCGGAGGTGGATCCGGCGGTGGCGGATCGCAGTCTGTGCTGACTC
 AGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCAACATCTCC
 TGCACTGGGAGCAGCTCCAACATCGGGGCAGGTTATGATGTACACT
 GGTATCAGCAGCTCCCAGGAACGGCCCCCAAACCTCCTCATCTATGGT
 35 TATAATGAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGCTCCAA
 GTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCGGTCCGAGG
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[SEQ ID NO: 18]

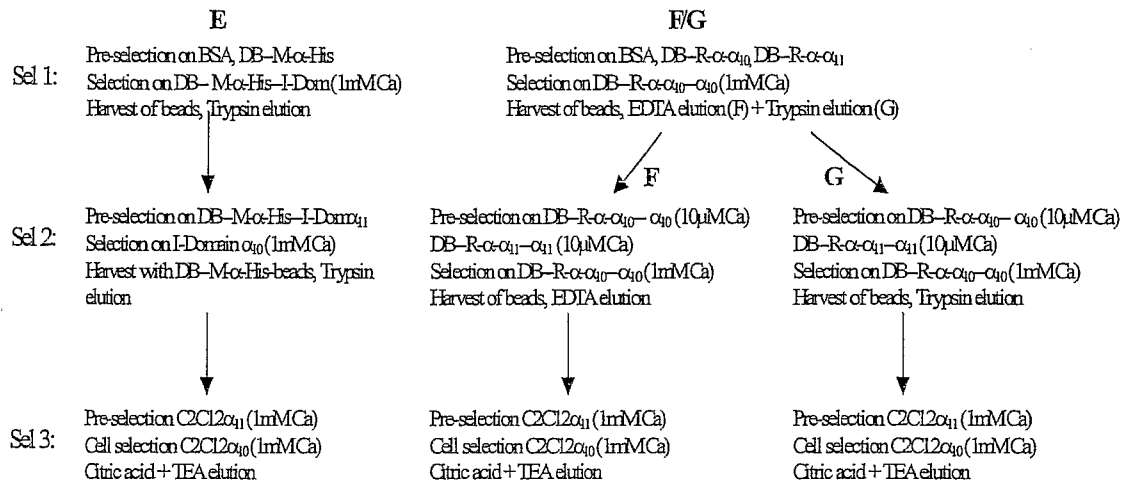
40

Initial screening experiments were performed using the scFv format of exemplary antibody 'A03'.

45 *In vivo* studies were performed using the whole IgG format of exemplary antibody 'A03'.

Appendix I. - Selection strategies utilized to isolate anti- $\alpha 11\beta 1$ integrin scFv

Selection strategies E to G (lower graph) are exemplified by selections
 5 performed with the aim to retrieve $\alpha 11\beta 1$ integrin specific antibodies .



Example II - Effect of exemplary antibody of the invention on antigen-induced arthritis

The purpose of the study was to test the effect of an exemplary whole IgG
 5 antibody of the invention, designated 'A03', in mBSA antigen-induced arthritis (AIA) in C57Bl/6 mice.

Materials and Methods

10 *Testing facility*

Experimental Rheumatology & Advanced Therapeutics, University Medical
 Center Nijmegen, Geert Grooteplein 26-28, 6525 GA Nijmegen, The
 Netherlands.

15

Experimental Design

The experimental protocol is shown in Tables 2 and 3 below.

20

Table 2

Day	Procedure
- 21	Immunization of mice with mBSA
-14	Booster with mBSA
0	Intra-articular injection with mBSA (60 µg/knee)
1, 5, 9, 13	Administration of anti-integrin antibodies
3, 10	Measurement of Technetium Uptake
15	Sacrifice of animals, taking tissues for histology

Table 3

Group	Test drug	Target integrin	Dose total (µg/mouse)
<i>Experiment 1</i>			
1 (control antibody)	IgG4 CT17	-	900
2	IgG4 A03 Batch 1	alpha 11	900
3	IgG4 A04	alpha 11	900
<i>Experiment 2</i>			
1 (control antibody)	IgG4 CT17	-	900
2 (control)	PBS	-	N/A
3	IgG4 A03 Batch 1	alpha 11	900
4	IgG4 A03 Batch 2	alpha 11	900

5 Animals

Male C57Bl/6 mice (Jackson Laboratories, Bar Harbor, ME, USA) of the age of 10 weeks (23-25 g) were used for the study. Mice are acclimatised after arrival for at least one week. Food and acidified water *available ad libitum*. The mice were weighed weekly.

Induction of antigen-induced arthritis

Antigen-induced arthritis was induced using a similar protocol as described (1). Shortly, mice were immunized with 100 µg (microgram) of methylated BSA (mBSA, Sigma) emulsified in Freund's complete adjuvant (CFA) (100 µl mg/ml) on day -21. Injection was divided over both flanks and footpath of the forelegs. Heat-killed *Bordetella pertussis* (RIVM, Bilthoven, The Netherlands), 1 ml, was administrated i.p. as an additional adjuvant. Seven days after the initial immunization animals were boosted with 100 µg (100 µl mg/ml) mBSA in CFA in the neck and 1 ml *Bordetella pertussis* (i.p.). Two weeks after the booster injection arthritis was induced by intra-articular injection of 60 µg mBSA (mBSA) in the right knee joint of the mice.

Antibodies

Antibodies were produced by BioInvent International AB (Lund, Sweden).

5 *Administration of test antibodies*

Antibodies were stored at 4°C after arrival from Cartela AB (Lund, Sweden) to the test facility. Antibodies were dissolved to obtain the correct concentration in sterile PBS (phosphate-buffered saline) and stored under sterile conditions at 10 4°C. Administration of the antibodies drugs was started on day 1 and ended on day 13. Antibody administration was performed on day 1, 5, 9 and 13. The antibodies were given i.p. 200 µl/mouse. Administration of the antibodies was always performed at the same time of the day -/+ 1hr. A total of 900 µg antibody was give per mouse in the following scheme:

15

Table 4

Day	Date	Microgram antibody per mouse
0	i.a. injection mBSA	
1		300
5		200
9		200
13		200

20 *Scoring inflammation as measurement of isotope uptake*

Joint swelling was measured using ⁹⁹Tc pertechnetate uptake in the knee joint on day 3 and 10. Briefly, mice were injected i.p. with 12 µCi of ⁹⁹Tc and subsequently sedated with chloradēhydrate. Thirty minutes thereafter, gamma- 25 radiation was assessed by use of a collimated Na-I-scintillation crystal with the knee in a fixed position. Arthritis was scored as the ration of the ⁹⁹Tc uptake in the right (injected) knee and the left (non-injected) knee. Ratios >1.1 were taken

to indicate inflammation of the right knee joint. This method has previously been shown to correlate well with histological findings (2).

Histology

5 Both right and left total knee joints were dissected and processed for histology as previously described (1). Shortly, the knees were fixed in phosphate buffered formalin (pH 7.4) for 3 days, decalcified in 5% buffered formic acid, and subsequently embedded in paraffin wax. Semi serial coronal, 7 μ m sections
10 were cut on a microtome. The sections were stained by haematoxylin and eosin (H&E) or by Safranin-O (Safranin O: Merck #1.15948) and Fastgreen (Fast Green FCF: Merck # 1.04022). Histological parameters (joint inflammations, proteoglycan depletion, osteophyte formation) were scored by light microscopy. Proteoglycan depletion in patella was also scored using digital image analysis.

15

Scoring of inflammation

Frontal sections of whole knee were stained by H&E. Histological scoring of inflammation was by light microscopy. The degree of inflammation was scored
20 as the amount of infiltrating cells in the synovial space and as the amount of exudate, cells in the joint space. Both exudate and infiltrate were scored using an arbitrary scale from 0 to 3: 0 – no inflammation, 1 – minor inflammation, 2 – moderate inflammation, 3 – severe inflammation (1).

25 *Scoring of cartilage damage*

Total knee joint sections stained with Safranin-O and Fastgreen. Loss of red staining from 6 positions in various cartilage layers (patella, patella femoris, lateral tibia, lateral femur, medial tibia, medial femur), as a measurement of loss
30 of proteoglycan, was determined using an arbitrary scale from 0 to 3: 0 – no proteoglycan depletion, 1 – minor proteoglycan depletion, 2 – moderate proteoglycan depletion, 3 – severe proteoglycan depletion (1). Proteoglycan depletion in patella was also scored using digital image analysis.

Scoring of osteophytes

The size of the osteophyte was scored on sections from medial femur stained by H&E using an arbitrary scale from 0 to 3: 0 - no osteophytes, 1 - Signs of chondrogenesis, 2 - small osteophytes, 3 - large osteophytes.

Statistics

Cartilage proteoglycan depletion damage was analysed using non-parametric Kruskal-Wallis test and Dunn's post test. Differences between groups were tested with the Mann-Whitney test. All locations were tested separately. The technetium uptake was tested using the Student t-test. To perform the statistical calculations the Graphpad Prism 4 software was used.

Results*Treatment with antibody against $\alpha 11$ -integrin reduced inflammation in arthritic mice*

Mono-arthritis was induced by mBSA injection i.a. into the knee joint. The disease has a fast onset where the joint swelling reached its maximum on day 1 after i.a. injection and gradually declining until day 7. The mice were sacrificed after additional 8 days (day 15 after i.a. injection) in the more chronic phase of the disease. The treatment of arthritic mice with anti- $\alpha 11$ integrin antibody (A03) resulted in a significant reduction in inflammation, measure as the degree of swelling of knee joints in the mice as well as degree of infiltrating inflammatory cells into the joint. There was a significant reduction in swelling of the induced knee joint at day 3 after i.a. injection of mBSA in the mice treated with anti- $\alpha 11$ integrin antibody (A03) compared to mice treated with control antibody (CT17) ($P = 0.035$, T test). (Figure 5). Similar results were shown in two subsequent experiments.

The anti- $\alpha 11$ integrin treated mice had lower amount of infiltrating cells in the synovial space (Figure 6) and in the joint space (exudate) (Figure 8) at the end

of the experiment (day 15 after i.a. injection) compared to mice treated with control antibody, although the difference was not statistically different.

Treatment with antibody against $\alpha 11$ protects the cartilage from destruction

5 *in arthritic mice*

In the mice treated with the anti- $\alpha 11$ integrin antibody, less destruction of the cartilage was seen compared to the mice treated with control antibody ($P = 0.015$). (Figure 8). The amount of cartilage destruction was scored as loss of
10 proteoglycans in histological section using an arbitrary scale from 0 to 3 as well as using image analysis. The protective effect of the anti- $\alpha 11$ integrin treatment on proteoglycan depletion was seen at all six locations scored in the knee.

Figures 9 and 10 further show the effect of treatment with the anti- $\alpha 11$ integrin
15 antibody on patella and lateral tibia, measured as proteoglycan (PG-depletion and reduction thereof using an arbitrary scale measurement. As could be seen in the two figures, which represent two different experiments, treatment with A03 reduces the PG-depletion in both patella and lateral tibia. The reduction in PG-depletion for A03 in lateral tibia in experiment 2 is significant ($p=0.02$). The
20 two figures 9 and 10 should be compared to the image analysis of patella (corresponds to figure 9, patella analysed with arbitrary scale) where the result is shown in figure 8 ($P=0.015$).

Figure 11 demonstrates the effect of treatment with the anti- $\alpha 11$ integrin
25 antibody on osteophyte size. As could be seen in the figure, a reduction of the osteocyte size is seen after treatment with the A03-antibody compared to treatment with control antibody (CT17).

Conclusion

30

Treatment with the exemplary anti- $\alpha 11$ integrin antibody of the invention (A03) reduces inflammation of the joints of arthritic mice induced with Antigen-Induced Arthritis (AIA), measured as joint swelling and infiltration of inflammatory cells. In addition, the anti- $\alpha 11$ integrin treatment protects the

cartilage from degradation, shown as reduction of proteoglycan degradation, in the AIA mice. Our results show a clear correlation between inflammation on day 3 (TC up take) and cartilage degradation (PG depletion) on day 15.

- 5 Current established treatments for RA reduces swelling (NSAIDS) or swelling and amount of infiltrating cells (anti-TNF α) in the same AIA model, but none have a protective effect on the cartilage (6-9). This suggests that anti- α 11 integrin treatment has a great potential in RA therapy either as stand-alone therapy or in combination with other therapies.

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Example III - Effect of exemplary antibody of the invention on Glucose-6-Phosphate-induced arthritis

The purpose of the study was to test the effect of an exemplary whole IgG
5 antibody, designated 'A03', in DBA/1 mouse.

Materials and methods

Testing facility

10

Cartela AB, Scheelvagen 22, 223 63 Lund, Sweden.

Experimental Design

15 The experimental protocol is shown in Tables 5 and 6 below.

Table 5

Day	Procedure
0	Immunization of mice with hG6PI
7, 10, 13, 16	Administration of anti-integrin antibodies
7 to 18	Scoring of inflammation
18	Sacrifice of animals, bleeding, taking tissues for histology

20

Table 6

Group	Test drug	Batch	Dose total (µg/mouse)
1 (control antibody)	IgG4 CT17	CAR 141, Peak 2 run 4+5	880
2	TNFR-p75- IgFc	Enbrel, Vnr 566661	880
3	IgG4 A03	PID 3640	880

25

Animals

Female DBA1 mice were obtained from Taconic M&B, Denmark. The mice are fed with RMI Expanded (special Diet Services Ltd, UK) and water *ad libitum*. The mice are allowed an acclimatization period to the new environment for at least one week. Water and cages are changed 2 times per week. Food is completed when needed. The mice are stored 7 mice / Mac3 cage with enhanced lid.

Antigen

Recombinant human glucose-6-phosphate isomerase (G6PI) was purchased from Kamradt (Deutsches Rheumaforschungszentrum Berlin, Berlin, Germany), produced as previously described (3).

15

Induction of glucose-6-phosphate isomerase-induced arthritis

Mice were immunised with 200µg recombinant human G6PI emulsified in a ratio of 1:1 in CFA (Difco, Detroit, MI, USA). Injection was given s.c. at the base of the tail with a volume of 100 µl (3).

20

Administration of test drug

Antibodies were stored at 4°C. Antibodies were dissolved in sterile PBS and stored under sterile conditions at 4°C. Antibody administration was performed on day 7, 10, 13, 16 after injection of hG6PI. The antibodies were given i.p. 200 µl/mouse. Administration of the antibodies was always performed at the same time of the day $\pm$ 1hr. A total of 880 µg antibody was given per mouse in the following scheme:

30

Table 7

Day	microgram antibody per mouse
0	injection of hG6PI
7	280
10	200
13	200
16	200

5 *Scoring inflammation*

Mice were visually scored for using an extended scoring protocol ranging from 1 to 15 for each paw, allowing a maximum score of 60 per mouse, arthritis as previously described (4). Each arthritic (red and swollen) toe and knuckle was
 10 scored as 1, whereas an affected ankle was scored as 5.

Histology

Back paws and ankles were fixed in phosphate buffered formalin (pH 7.4) for 3
 15 days, decalcified in EDTA and subsequently embedded in paraffin wax. Semi serial 5µm sections were cut on a microtome. Front paws were decalcified in EDTA and then embedded in OCT and frozen. Semi serial 5µm sections were cut on cryostat.

20 *Scoring of cartilage damage and bone destruction*

Paraffin sections of back paws and ankles were stained with haematoxylin and eosin (H&E) or by Safranin-O. Sections were graded for proteoglycan depletion using the Mankin grading system (5) and in addition also for
 25 osteophyte formation and bone destruction by light microscopy. Proteoglycan depletion was also scored as loss of red staining using digital image analysis.

Immunostaining

30 Cryosections were allowed to warm up to room temperature then fixed in acetone for 10 minutes at -20°C. Slides were washed in PBS for 3x5 minutes,

and were then incubated with hyaluronidase (2mg/ml in PBS; Sigma), at 37°C for 30 minutes. A second 3x5 minutes wash in PBS was performed before incubating sections with 4% goat serum (Sigma, G-9023) diluted in PBS for 30 minutes to block. Sections were incubated with biotinylated IgG4-anti- α 11
5 integrin Ab diluted 1:100 (=20ug/ml) in 4% goat serum in PBS, for 60 minutes at room temperature, and then washed in PBS for 3x5 minutes. Sections were then incubated for another 60 minutes with fluorescence (Alexa Fluor 448 or Cy3) conjugated Streptavidin. After additional wash in PBS for 3x5 minutes, slides were mounted with Vectashield, and observed under a fluorescence
10 microscope.

Results

Treatment with antibody against α 11-integrin reduced incidence and severity of
15 *G6PI-induced arthritis in mice*

Immunization with recombinant human glucose-6-phosphate isomerase (rhG6PI) induces a poly-arthritis with severe swelling of both front and hind paws. Clinical signs of arthritis first appeared at day 7 after immunization,
20 rapidly progressed and reached its maximum at day 9 (Figure 12). The mice were sacrificed at day 18 after immunization.

Treatment started at day 7 and was then given at day 10, 13, 16 after injection with a total of 880 μ g of the test-antibody per mouse. The TNFR-p75-IgFc
25 (Enbrel) was used as positive control. Treatment with Enbrel reduced the arthritis incidence to 50%, compared to 100% in the group treated with control antibody (CT17). The A03 treatment resulted in clear reduction of arthritis incidence and severity (Figures 12 and 13). The mice treated with A03 all developed arthritis (incidence 100% day 9) after which 50% of the mice
30 recovered ($p < 0.05$) (Figure 13) and the incidence was reduced to the same level as that of the Enbrel treated group ($p = 0.051$) (Figure 12). Figure 13 demonstrates the severity of arthritis measured as mean of arthritis scores of the individuals in each group.

During the arthritis experiment the mice was weighted as a measurement of their general disease status. The A03 treated mice showed a clear, although not significant, less reduction of weight compared to the control treated mice
 5 (p=0.08) (Figure 14).

Table 8 below demonstrates the incidence (frequency) of arthritis developed in each group tested in the experiments.

10

Table 8

Treatment	Healthy	Sick	Totals
A03	0	10	10
CT17	0	10	10
Enbrel	5	5	10
<i>Totals</i>	<i>5</i>	<i>25</i>	<i>30</i>

Conclusion

15

Treatment with the anti- α 11 integrin antibody (A03) reduces inflammation of the joints of arthritic mice in the poly-arthritic model glucose-6-phosphate isomerase (rhG6PI) induced arthritis. Using a treatment scheme where the test antibody was administrated at the day of onset and the subsequently every third
 20 day until termination of the experiment, one may demonstrate that the A03 treatment resulted in recovery of 50% of the arthritic mice. Incidence of arthritis was reduced from 100 to 50% in the test group, down to the level of incidence with Enbrel treatment.

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Example IV - Generation of monoclonal antibodies competing to exemplary "A03" antibody.

The purpose of this example is to generate mouse monoclonal antibodies competing to the exemplary "A03" antibody using hybridoma technology.

Materials and methods

Immunization protocol

HEK cell produced recombinant protein is used for immunization. The protein contains the upper-lower calf domain of human $\alpha 11$ (E-ULCD) to direct the antibody response against the stalk region of $\alpha 11$ integrin.

Sequence of E-ULCD is

SDGSIECVNEERRLQKQVCNVSYFFRAKAKVAFRLDFEFSKSIFLHHLE
IELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSSLSHYEVKLN
SSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIPATRSNGNRLCLKLRDF
LTDEANTSCNIWGNSTEYRPTPVEEDLRRAPQLNHSNSDVVSINCNIQLV
PNQEINFHLLGNLWLRSLKALKYKSMKIMVNAALQRQFHSPFIFREEDP
SRQIVFEISKQEDWQVP.

[SEQ ID NO: 19]

C57bl/6- $\alpha 11$ knock-out mice (as described in detail in WO 03/101497, incorporated herein by reference) were immunized.

Blood was drawn at indicated time points to be able to follow the immune response via FACS analysis. The following immunization scheme was applied:

30

Day 0	Day 0 blood for FACS analysis was drawn
	$\alpha 11$ Stalk protein (species of the $\alpha 11$ sequence is human, described in Velling et al. <i>J.Biol.Chem.</i> 1999, 274 :25735-25742.) (Cartela AB, batch#53; 070509; pooled peak 1+2, E-ULCD) (80 μ g/mouse) was emulsified in CFA (Complete Freuds Adjuvant, Sigma) by sonication and injected at the base of the tail of four C57bl/6- $\alpha 11$ ko mice.

35

- Day 14 Blood was drawn for FACS analysis
 Booster immunization with alpha11 stalk (E-ULCD)
 (50µg/mouse) in IFA (incomplete Freuds Adjuvant, Sigma).
- 5 Day 22 Blood was drawn for FACS analysis
- Day 24 NS0 fusion partners were expanded for fusion
- Day 31 Pre-fusion i.p. boost with α11 stalk (batch #53 E-ULCD; 20mM
10 Tris, 1mM Ca²⁺, Batch I) (50µg/mouse) in PBS.
- Day 34 B-cell fusion with the myeloma cell line NS0 (ECACC)

15 *Fusion with NS0 cells Day 34 (070625)*

- Spleens of three C57bl/6-α11ko mice were used for fusions.
- Single cell suspensions were made and erythrocytes were lysed using 9ml
20 sterile ddH₂O (10sec) before adding 1ml 10x PBS to stop the lysis.
- Cells were washed with serum-free D-MEM (2 times) and mixed with aliquots
of washed NS0 cells in a 50ml Falcon tube (to remove free proteins which can
be toxic during fusion with PEG). The pellets were kept at 37C in a water bath.
1ml 50% PEG (1500MW) in D-MEM at 37C was added drop by drop under
25 stirring, using glass pipettes over a 1min time period.
- 2ml pre-warmed serum free D-MEM was added under a period of 2min.
- 7ml pre-warmed serum free D-MEM was added drop by drop over a period of
3min.
- Cells were centrifuged 5min at 500x g.
- 30 Discharged supernatant and added pre-warmed complete D-MEM (Pyruvat,
10% FCS, PEST). Nr1 40ml in 3plates), Nr3 50ml in 4plates), Nr10 50ml in
4 plates. Cells rested at RT for 10min before adding approx. 100µl of cells
(1 drop per well) with 10ml pipette to 96-well cell culture plates.

The fusion was performed according to the protocol in 'Current Protocols in Immunology, Unit 2.5, "Production of monoclonal antibodies" (ISBN ISSN: 1934-3671, Wiley, incorporated here in by reference.)

5 Day 35 Added 100µl 2x HAT (hypoxantin-aminopterin-tymidin, Apoteket, Sweden) medium to wells for selection

Day 38 Remove toxic supernatant from dying cells, added fresh HT medium (hypoxantin-tymidin, Apoteket, Sweden)

10

Day 44 Remove 100µl medium for FACS screening and added HT medium to the wells.

Identification of stalk specific hybridoma through FACS screens

15

Supernatants from wells containing the candidate hybridomas are tested for reactivity to the stalk region using flow cytometry (FACS). The clones are further characterized for their stalk reactivity and unwanted possible cross reactivity of the anti- α 11 integrin stalk candidate hybridomas

20

Characterization of Stalk specific hybridomas:

Unwanted cross reactivity of the anti α 11-integrin stalk candidate hybridoma to the related α 10-integrin is tested using α 10-integrin transfected HEK and α 10-integrin transfected C2C12 cells. Cross reactivity to the corresponding stalk from other species, i.e. mouse is tested using mouse α 11-integrin transfected C2C12 cells.

25

Subcloning of α 11-stalk hybridomas

30

FACS-positive cells are subcloned using a limiting dilution method (approx. 0.3 cells/well). Rat thymocytes are used as feeder cells to ensure hybridoma survival. Hybridoma cells are frozen down during different subcloning stages.

35

Generation of antibodies to the full length human alpha11 integrin.

Generation of antibodies to the full length human alpha11 integrin. Said full
 5 length alpha 11 integrin protein is affinity purified from human alpha11-
 transfected HEK cell lysates, using a polyclonal antiserum specific for the
 cytoplasmatic tail (as described in WO00/75187).

10 *Immunization protocol: full-length $\alpha 11$ -integrin project*

Day 0 4 female C57bl/6-alpha11ko mice were used for immunization
 with 40 μ g per mouse of full-length alpha11 protein (batch#
 070717) in CFA at the base of the tail.
 Blood was taken for FACS analysis.

15

Day13 Day 13 blood was taken for FACS analysis

Day16 Boost with full-length alpha11 protein (batch# 070717)
 10 μ g/mouse in IFA

20

Day26 Boost with a mix of 10 μ g/mouse full-length alpha11 integrin and
 40 μ g stalk alpha11 protein (batch #53 E-ULCD) /mouse in PBS
 i.p.

25

Day29 Fusion of spleen and lymph node cells with NS0 cells
 Tail tips for DNA and serum was saved

Fusion with NS0 cells at day 29

30 Spleens and cells from draining lymph nodes (LN) of three immunized
 alpha11ko mice were used for fusion.
 Single cell suspension was made and erythrocytes of the spleen cells were lysed
 using 0.84% ammonium chloride.

Cells were washed with serum-free D-MEM (2 times) and mixed with aliquots of washed NS0 cells in a 50ml Falcon tube. The pellet was kept at 37C in a water bath.

1ml 50% PEG 1500 MW in DMEM at 37C was added under stirring drop by drop over a 1min time period. 2ml pre-warmed serum free DMEM was added dropwise under a period of 2min. 7ml pre-warmed serum free DMEM was added drop by drop over a period of 3min.

Cells were centrifuged 5min at 500x g. Supernatants were discharged before adding pre-warmed complete DMEM (Pyruvat, 10% FCS, PS) to LN and spleen preparations; for Nr.1 in 50ml, for Nr.3 in 50ml, for Nr.10 in 50ml. One drop (approx. 100µl) per well were added to 4.5 plates per fusion. 100µl 2x HAT medium was added 4 hours later.

The fusion was performed according to the protocol in 'Current Protocols in Immunology, Unit 2.5, "Production of monoclonal antibodies" (ISBN ISSN: 1934-3671, Wiley, incorporated here in by reference.)

Day 100µl medium was removed and HT medium added to the wells.

Supernatants are taken at day 11 after fusion for FACS screening of positive well as described above. After identifying positive wells, supernatants were tested for cross reactivity between human and mouse alpha11 integrins (hybridoma screen using C2C12 wt cells and cells transfected with human alpha11, and alpha10-integrin.

Hybridoma cells were subcloned (0,3 cells/well) in BM-condimed H1 supplemented medium (Roche, DE). Wells with one colony only growing were selected for specificity screening.

Screening for competition with exemplary antibody "AO3"

Further, newly generated clones are tested for its ability to block the binding of the exemplary antibody "AO3". A FACS based method is used. Human
5 alpha11-integrin transfected C2C12 cells are incubated with purified newly generated antibodies in a dilution series of about 1.6 up to 1000 ng/ml to occupy their epitope on the alpha11-integrin expressed on the cell surface. In a second step biotinylated exemplary antibody AO3 is added. If these antibodies share an epitope region, the binding of the human IgGs will be reduced.

CLAIMS

1. Use of an antibody or an antigen-binding fragment thereof with binding specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, or of a variant, fusion or derivative of said antibody or an antigen-binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity of said antibody or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer thereof, in the preparation of a medicament for treating an inflammatory condition,
- wherein the antibody or fragment comprises the following amino acid sequences:

	FSRYMHWRQVPG	[SEQ ID NO: 1];
15	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
	CAAWDDSLSGHV	[SEQ ID NO: 6].

2. Use of an antibody or an antigen-binding fragment thereof with binding specificity for an integrin $\alpha 11$ subunit, or a heterodimer thereof, or of a variant, fusion or derivative of said antibody or an antigen-binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity of said antibody or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer thereof, in the preparation of a medicament for treating an inflammatory condition,
- wherein the antibody or antigen-binding fragment thereof is capable of competing for binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, with an scFv molecule having the amino acid sequence of SEQ ID NO: 13

with the proviso that the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, does not comprise all of the amino acid sequences of SEQ ID NOS: 1 to 6.

- 5 3. Use of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in Claim 1 or 2 in the preparation of a diagnostic or prognostic agent for an inflammatory condition.
- 10 4. Use of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in Claim 1 or 2 in the preparation of an agent for detecting and/or imaging cells expressing an integrin $\alpha 11$ subunit, or heterodimer thereof.
- 15 5. The use according to any one of the preceding claims wherein the integrin $\alpha 11$ subunit, or the heterodimer thereof, is localised on the surface of a cell.
- 20 6. The use according to Claim 5 wherein the cell is a chondrocyte or a fibroblast.
- 25 7. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of an intact antibody.
- 30 8. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of an antigen-binding fragment selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments), single antibody chains (*e.g.* heavy or light chains), single variable domains (*e.g.* V_H and V_L domains) and domain antibodies (dAbs, including single and dual formats [*i.e.* dAb-linker-dAb]).

9. The use according to Claim 8 wherein the antigen-binding fragment is an scFv.
10. The use according to any one of the preceding claims wherein the antibody is a recombinant antibody.
11. The use according to any one of the preceding claims wherein the antibody is a monoclonal antibody.
12. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment thereof is human or humanised.
13. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a heavy chain variable region comprising the following CDRs:
- a) SEQ ID NO: 1;
 - b) SEQ ID NO: 2; and
 - c) SEQ ID NO: 3.
14. The use according to Claim 12 wherein the heavy chain variable region comprises or consists of the amino acid sequence of SEQ ID NO: 9.
15. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a light chain variable region comprising the following CDRs:
- a) SEQ ID NO: 4;
 - b) SEQ ID NO: 5; and
 - c) SEQ ID NO: 6.

16. The use according to Claim 14 wherein the light chain variable region comprises or consists of the amino acid sequence of SEQ ID NO: 10.
17. The use according to any one of the Claims 1 or 3 to 16 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a heavy chain variable region as defined in Claim 12 or 13 and a light chain variable region as defined in Claim 14 or 15.
18. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a heavy chain which comprises or consists of the amino acid sequence of SEQ ID NO: 11.
19. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a light chain which comprises or consists of the amino acid sequence of SEQ ID NO: 12.
20. The use according to any one of Claims 1 or 3 to 19 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of a heavy chain as defined in Claim 17 and a light chain as defined in Claim 18.
21. The use according to any one of Claims 1 or 3 to 20 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, comprises or consists of the amino acid sequence of SEQ ID NO:13.
22. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, variant, fusion or derivative thereof is capable of binding to the same epitope as an scFv molecule having the amino acid sequence of SEQ ID NO: 13.

23. The use according to any one of Claims 1 to 21 wherein the antibody or antigen-binding fragment, variant, fusion or derivative thereof is capable of binding to an epitope distinct from that to which an scFv molecule having the amino acid sequence of SEQ ID NO: 13 binds.
- 5
24. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, further comprises a therapeutic and/or detectable moiety.
- 10 25. The use according to Claim 24 wherein the detectable moiety is selected from the group consisting of radioisotopes (*e.g.* ^3H , ^{14}C , ^{35}S , ^{123}I , ^{125}I , ^{131}I , ^{99}Tc , ^{111}In , ^{90}Y , ^{188}Re), radionuclides (*e.g.* ^{11}C , ^{18}F , ^{64}Cu), fluorescent labels (*e.g.* FITC, rhodamine, lanthanide phosphors, carbocyanine), enzymatic labels (*e.g.* horseradish peroxidase, β -galactosidase, luciferase, alkaline phosphatase), chemiluminescent, biotinyl groups and predetermined polypeptide epitopes recognised by a secondary reporter (*e.g.* leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags).
- 15
26. The use according to Claim 24 or 25 in the preparation of a diagnostic agent for the diagnosis or imaging of an inflammatory condition.
- 20
27. The use according to any one of the preceding claims wherein the inflammatory condition is selected from the group consisting of arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, peridontitis, psoriasis, asthma, systemic lupus erythematosus, multiple sclerosis, ankylosing spondylitis, psoriatic arthritis and autoimmune chronic inflammatory diseases.
- 25
28. The use according to Claim 27 wherein the inflammatory condition is an arthritic disease.
- 30

29. The use according to Claim 28 wherein the arthritic disease is rheumatoid arthritis or osteoarthritis.
30. The use according to any one of the preceding claims wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of modulating the degradation of collagen.
31. The use according to Claim 30 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of inhibiting the degradation of collagen.
32. The use according to Claim 30 or 31 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of modulating the degradation of collagen *in vivo*.
33. A method for treating an individual with an inflammatory condition, the method comprising administering to the individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32.
34. A method for diagnosing or prognosing an inflammatory condition in an individual, the method comprising administering to the individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32.
35. A method for imaging cells expressing an integrin $\alpha 11$ subunit associated with an inflammatory condition, or heterodimer thereof, in the body of an individual, the method comprising administering to the individual an effective amount of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32.

36. A method according to Claim 34 or 35 further comprising the step of detecting the location of the compound in the individual.
37. A method according to any one of Claims 33 to 36 wherein the inflammatory condition is selected from the group consisting of arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, peridontitis, psoriasis, asthma, systemic lupus erythematosus, multiple sclerosis, ankylosing spondylitis, psoriatic arthritis and autoimmune chronic inflammatory diseases.
38. A method according to Claim 37 wherein the inflammatory condition is an arthritic disease.
39. A method according to Claim 38 wherein the arthritic disease is rheumatoid arthritis or osteoarthritis.
40. A method for monitoring the progression of an inflammatory condition in an individual, the method comprising:
- (a) providing a sample of cells collected from the individual at a first time point and measuring the amount of integrin $\alpha 11$ subunit protein therein using an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32;
 - (b) providing a sample of cells collected from the individual at a second time point and measuring the amount of integrin $\alpha 11$ subunit protein therein an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32; and
 - (c) comparing the amount of integrin $\alpha 11$ subunit protein measured in steps (a) and (b)

wherein an increased amount of integrin $\alpha 11$ subunit protein measured in step (b) compared to step (a) is indicative of a progression in the inflammatory condition.

- 5 41. A method for identifying cells associated with an inflammatory condition, the method comprising measuring the amount of integrin $\alpha 11$ subunit protein in a sample of cells to be tested using an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, as defined in any one of Claims 1 to 32 and comparing it to the amount of
10 integrin $\alpha 11$ subunit protein to a positive and/or negative control.
42. A method according to Claim 41 wherein the positive control comprises cells from a subject who is suffering from the inflammatory condition.
- 15 43. A method according to Claim 41 wherein the negative control comprises cells from a healthy subject who is not suffering from the inflammatory condition.
44. A method according to any one of Claims 40 to 43 wherein the
20 inflammatory condition is selected from the group consisting of arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, periodontitis, psoriasis, asthma, systemic lupus erythematosus, multiple
25 sclerosis, ankylosing spondylitis, psoriatic arthritis and autoimmune chronic inflammatory diseases.
45. A method according to Claim 44 wherein the inflammatory condition is an arthritic disease.
- 30 46. A method according to Claim 45 wherein the arthritic disease is rheumatoid arthritis or osteoarthritis.

47. A method for identifying a candidate compound for treating an inflammatory condition, the method comprising the following steps:

a) providing a compound to be tested;

5

b) testing the ability of the compound to compete for binding to an integrin $\alpha 11$ subunit, or a heterodimer thereof, with an antibody or antigen-binding fragment comprising the following CDR sequences:

10	FSRYMHVWRQVPG	[SEQ ID NO: 1];
	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
15	CAAWDDSLSGHV	[SEQ ID NO: 6].

or a variant, fusion or derivative thereof, or a fusion of a said variant or derivative thereof, which retains the binding specificity for the integrin $\alpha 11$ subunit, or heterodimer thereof,

20

wherein the test compound is identified as a candidate compound for treating an inflammatory condition if it is able to compete for binding to the integrin $\alpha 11$ subunit, or heterodimer thereof.

25 48. A method according to Claim 47 further comprising the step of testing the ability of the compound to modulate the degradation of collagen.

49. A method according to Claim 48 comprising the step of testing the ability of the compound to inhibit the degradation of collagen.

30

50. A method according to any one of Claim 47 to 49 wherein the testing steps are performed *in vivo*.

51. A method according to any one of Claim 47 to 50 wherein the test compound is a polypeptide, or a fusion or derivative thereof, or a fusion of a said derivative thereof.
- 5 52. A method according to Claim 51 wherein the test compound is an antibody or antigen-binding fragment thereof, or a variant, fusion or derivative thereof, or a fusion of a said variant or derivative thereof.
- 10 53. A method according to Claim 52 wherein the test compound is an intact antibody.
54. A method according to Claim 52 wherein the test compound is an antigen-binding fragment selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), Fab-like
15 fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments), single antibody chains (*e.g.* heavy or light chains), single variable domains (*e.g.* V_H and V_L domains) and domain antibodies (dAbs, including single and dual formats [*i.e.* dAb-linker-dAb]).
- 20 55. A method according to Claim 54 wherein the test compound is an scFv.
56. An antibody or antigen-binding fragment thereof capable of competing for binding to an integrin α 11 subunit, or a heterodimer thereof, with an scFv molecule having the amino acid sequence of SEQ ID NO: 13
25
- or a variant, fusion or derivative of said antibody or antigen-binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity for an integrin α 11 subunit, or a heterodimer thereof,
- 30
- with the proviso that the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, does not comprise all of the amino acid sequences of SEQ ID NOS: 1 to 6.

57. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 56 capable of binding to the same epitope as an scFv molecule having the amino acid sequence of SEQ ID NO: 13.
58. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 56 capable of binding to an epitope distinct from that to which an scFv molecule having the amino acid sequence of SEQ ID NO: 13 binds.
59. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 58 wherein the integrin $\alpha 11$ subunit, or a heterodimer thereof, is localised on the surface of a cell.
60. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 60 wherein the cell is a chondrocyte or a fibroblast.
61. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 60 wherein the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, comprises or consists of an intact antibody.
62. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 60 wherein the antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, comprises or consists of an antigen-binding fragment selected from the group consisting of Fv fragments (*e.g.* single chain Fv and disulphide-bonded Fv), and Fab-like fragments (*e.g.* Fab fragments, Fab' fragments and F(ab)₂ fragments).

63. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 62 wherein the antigen-binding fragment is an scFv.
- 5 64. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 63 wherein the antibody is a recombinant antibody.
- 10 65. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 64 wherein the antibody is a monoclonal antibody.
- 15 66. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 65 wherein the antibody or antigen-binding fragment thereof is human or humanised.
- 20 67. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 66 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of modulating the degradation of collagen
- 25 68. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 67 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of inhibiting the degradation of collagen.
- 30 69. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 67 or 68 wherein the antibody or antigen-binding fragment, or variant, fusion or derivative thereof, is capable of modulating the degradation of collagen *in vivo*.
70. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 69 wherein the

antibody or antigen-binding fragment has efficacy in the treatment of an inflammatory condition.

- 5 71. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 70 wherein the inflammatory condition is selected from the group consisting of arthritic diseases (such as rheumatoid arthritis and osteoarthritis), joint inflammation, inflammation-induced cartilage destruction, chronic inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, peridontitis, psoriasis, 10 asthma, systemic lupus erythematosus, multiple sclerosis and autoimmune chronic inflammatory diseases.
- 15 72. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 71 wherein the inflammatory condition is rheumatoid arthritis.
- 20 73. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to Claim 71 wherein the inflammatory condition is osteoarthritis
74. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 74 further comprising a therapeutic and/or detectable moiety.
- 25 75. A nucleic acid molecule encoding an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, according to any one of Claims 56 to 74, or component polypeptide chain thereof.
- 30 76. A vector comprising a nucleic acid molecule according to Claim 75.
77. A vector according to Claim 76 wherein the vector is an expression vector.

78. A recombinant host cell comprising a nucleic acid molecule according to Claim 75 or a vector according to Claim 76 or 77.
79. A host cell according to Claim 78 wherein the host cell is a bacterial cell.
80. A host cell according to Claim 78 wherein the host cell is a mammalian cell.
81. A host cell according to Claim 80 wherein the host cell is a human cell.
82. A method for producing an antibody or antigen-binding fragment according to any one of the Claims 56 to 74, the method comprising culturing a host cell as defined in any of Claims 78 to 81 under conditions which permit expression of the encoded antibody or antigen-binding fragment thereof.
83. A pharmaceutical composition comprising an antibody or antigen-binding fragment according to any one of the Claims 56 to 74 and a pharmaceutically acceptable excipient, diluent or carrier.
84. A pharmaceutical composition according to Claim 83 suitable for parenteral administration.
85. An antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, according to any one of Claims 56 to 74 for use in medicine.
86. A kit comprising a pharmaceutical composition according to Claim 83 or 84.
87. An epitope defined by an antibody or an antigen-binding fragment thereof with binding specificity for an integrin α 11 subunit, or a heterodimer thereof, or of a variant, fusion or derivative of said antibody

or an antigen-binding fragment, or a fusion of a said variant or derivative thereof, which retains the binding specificity of said antibody or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a heterodimer thereof, wherein the antibody or fragment comprises the following amino acid sequences:

5
10
FSRYMHWVRQVPG [SEQ ID NO: 1];
SGVSWNGSRTHYADSVKGR [SEQ ID NO: 2];
ARVSGDGYNFGA [SEQ ID NO: 3];
CTGSSSNIGAGYDVH [SEQ ID NO: 4];
GYNERPS [SEQ ID NO: 5]; and
CAAWDDSLSGHVV [SEQ ID NO: 6].

88. An epitope according to Claim 87 comprising or consisting of the following amino acid sequence:

15
20
SDGSIECVNEERRLQKQVCNVSYPPFRAKAKVAFRLDFEFSKSIF
LHHLEIELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSS
LSHYEVKLNSSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIP
ATRSGNRLLKLRDFLTDEANTSCNIWGNSTEYRPTPVEEDLRRA
PQLNHSNSDVVSINCNIQLVPNQEIFHLLGNLWLRSLKALKYKS
MKIMVNAALQRQFHSPFIFREEDPSRQIVFEISKQEDWQVP
[SEQ ID NO: 15]

25 or a fragments, fusions or variants thereof.

89. An isolated polypeptide comprising or consisting of the following amino acid sequence:

SDGSIECVNEERRLQKQVCNVSYPFFRAKAKVAFRLDFEFSKSIF
 5 LHHLEIELAAGSDSNERDSTKEDNVAPLRFHLKYEADVLFTRSSS
 LSHYEVKLNSSLERYDGIGPPFSCIFRIQNLGLFPIHGMMMKITIPI
 ATRSGNRLLKLRDFLTDEANTSCNIWGNSTEYRPTPVEEDLRRA
 PQLNHSNSDVVSINCNIRLVPNQEINFHLLGNLWLRLSKALKYKS
 MKIMVNAALQRQFHSPFIFREEDPSRQIVFEISKQEDWQVP
 10 [SEQ ID NO: 15]

or a fragments, fusions or variants thereof.

90. An isolated polypeptide according to Claim 89 wherein polypeptide is
 15 capable of stimulating an immune response.

91. An isolated polypeptide according to Claim 89 or 90 wherein
 polypeptide is capable of binding to an antibody or an antigen-binding
 fragment thereof with binding specificity for an integrin $\alpha 11$ subunit, or
 20 a heterodimer thereof, or of a variant, fusion or derivative of said
 antibody or an antigen-binding fragment, or a fusion of a said variant or
 derivative thereof, which retains the binding specificity of said antibody
 or an antigen-binding fragment thereof for an integrin $\alpha 11$ subunit, or a
 heterodimer thereof,

25

wherein the antibody or fragment comprises the following amino acid sequences:

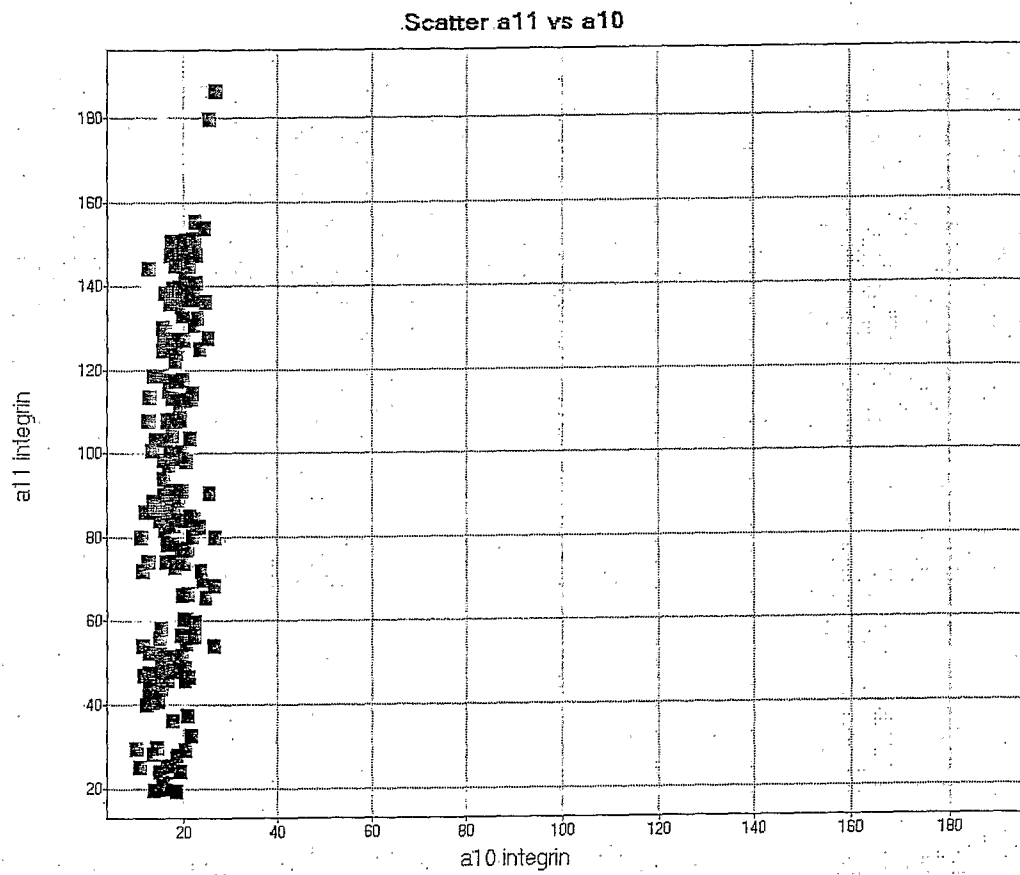
30	FSRYMHWVRQVPG	[SEQ ID NO: 1];
	SGVSWNGSRTHYADSVKGR	[SEQ ID NO: 2];
	ARVSGDGYNFGA	[SEQ ID NO: 3];
	CTGSSSNIGAGYDVH	[SEQ ID NO: 4];
	GYNERPS	[SEQ ID NO: 5]; and
	CAAWDDSLSGHVV	[SEQ ID NO: 6].

92. Use of an epitope according to Claim 87 or 88 or an isolated polypeptide according to any one of Claims 89 to 91 to identify or produce an antibody or antigen-binding fragment, or variant, fusion or derivative thereof, according to any one of Claims 56 to 74.
93. The use of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, in the preparation of a medicament substantially as described herein with reference to the description.
94. The use of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, in the preparation of a diagnostic or prognostic agent substantially as described herein with reference to the description.
95. The use of an antibody or antigen-binding fragment, or a variant, fusion or derivative thereof, in the preparation of an agent for detecting and/or imaging cells substantially as described herein with reference to the description.
96. A method of treating an individual with an inflammatory condition substantially as described herein with reference to the description.
97. A method of diagnosing or prognosing an inflammatory condition substantially as described herein with reference to the description.
98. A method of imaging cells expressing an integrin $\alpha 11$ subunit substantially as described herein with reference to the description.
99. A method for identifying a candidate compound substantially as described herein with reference to the description.
100. An antibody or antigen-binding fragment thereof substantially as described herein with reference to the description.

101. A pharmaceutical composition substantially as described herein with
reference to the description.
102. A nucleic acid molecule substantially as defined herein with reference to
the description.
103. A vector substantially as defined herein with reference to the
description.
104. A host cell substantially as defined herein with reference to the
description.
105. A method of producing an antibody or antigen-binding fragment thereof
substantially as defined herein with reference to the description.
106. A kit substantially as defined herein with reference to the description.
107. An epitope as defined herein with reference to the description.
108. An isolated polypeptide as defined herein with reference to the
description.
109. Use of an epitope or isolated polypeptide to identify or produce an
antibody or antigen-binding fragment, or variant, fusion or derivative
thereof, as defined herein with reference to the description.

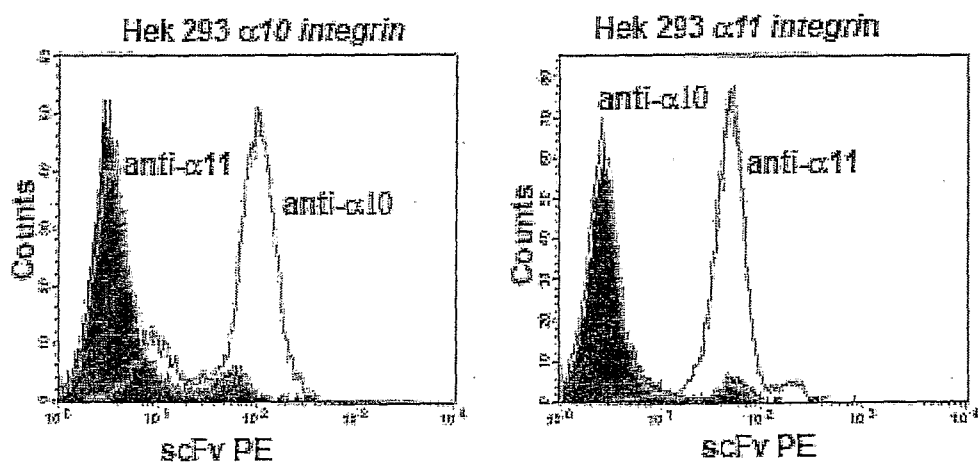
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Figure 1



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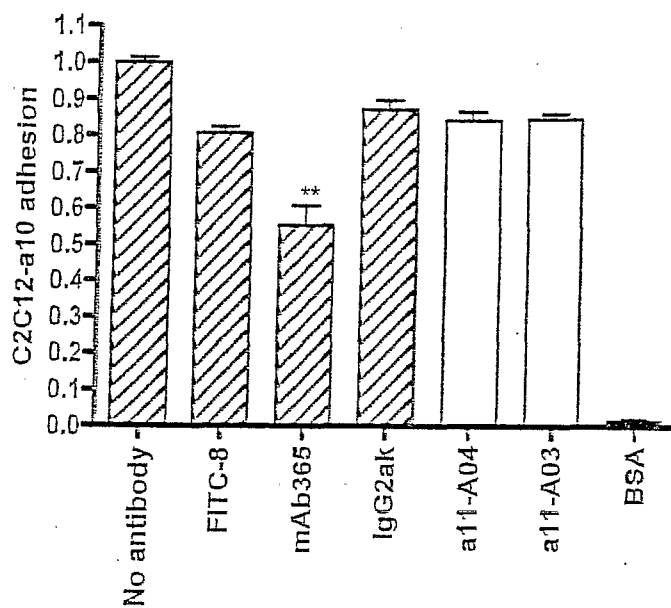
Figure 2



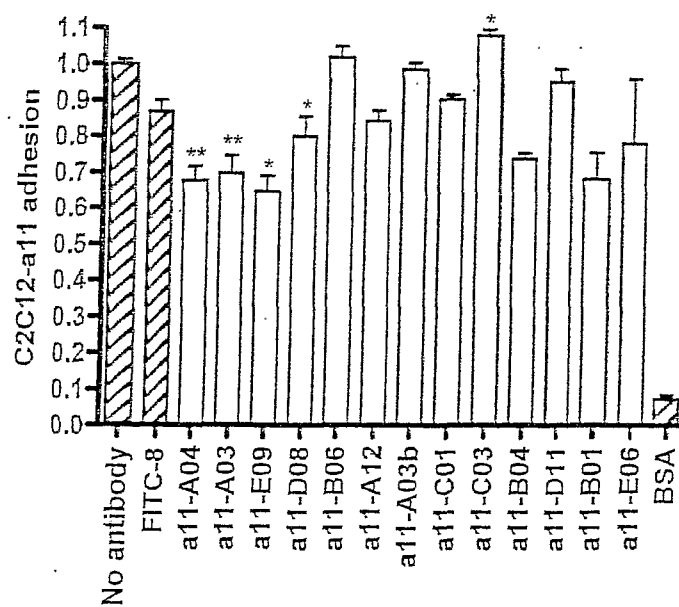
3/14

Figure 3

(A)

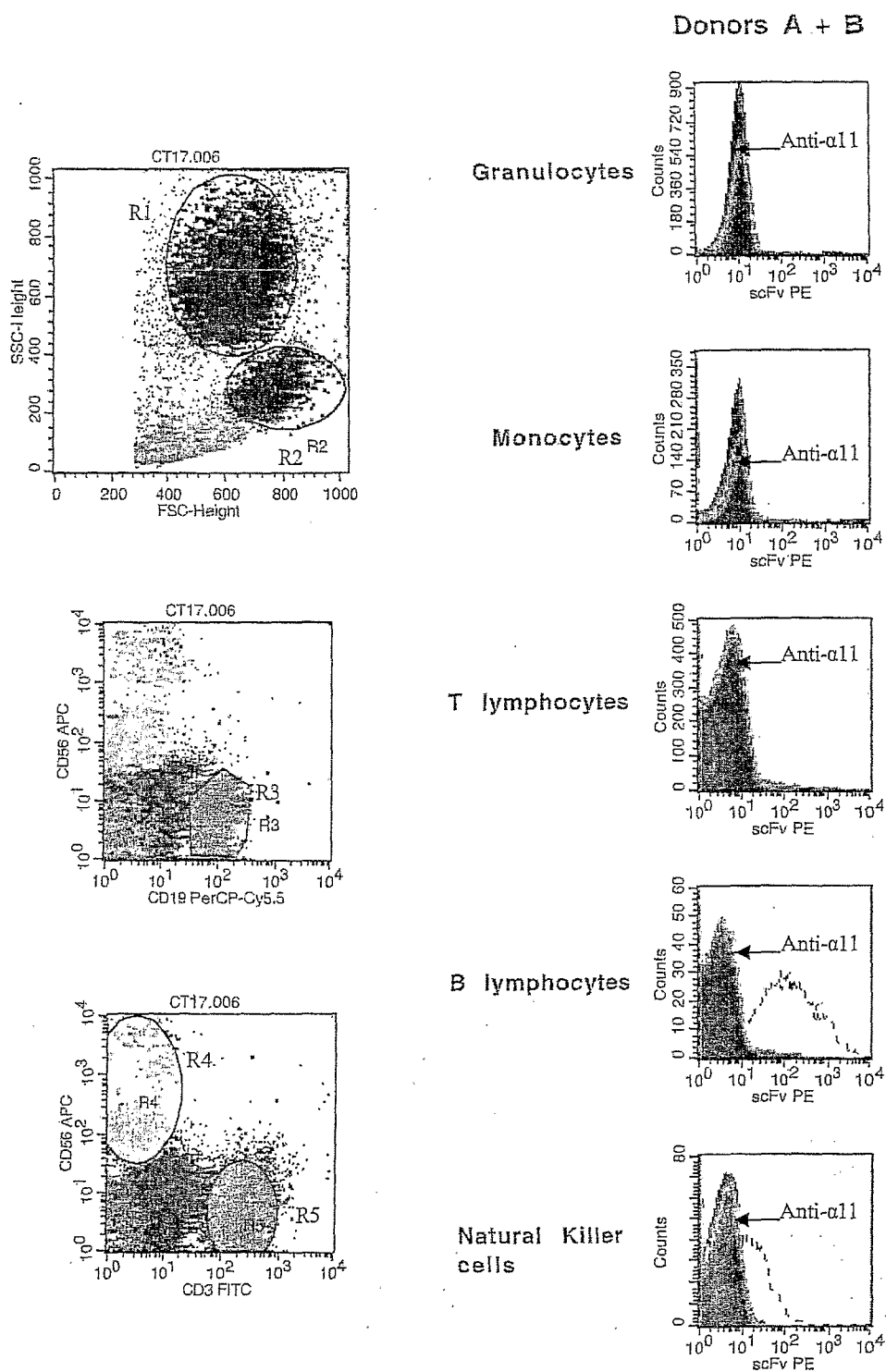


(B)



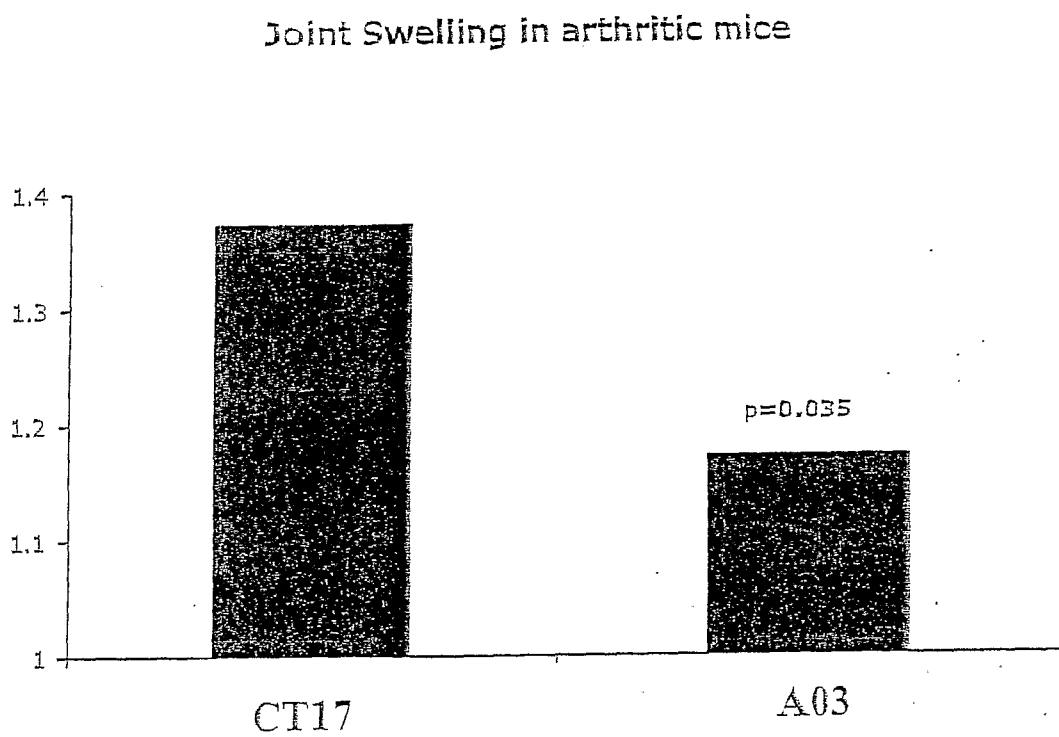
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Figure 4



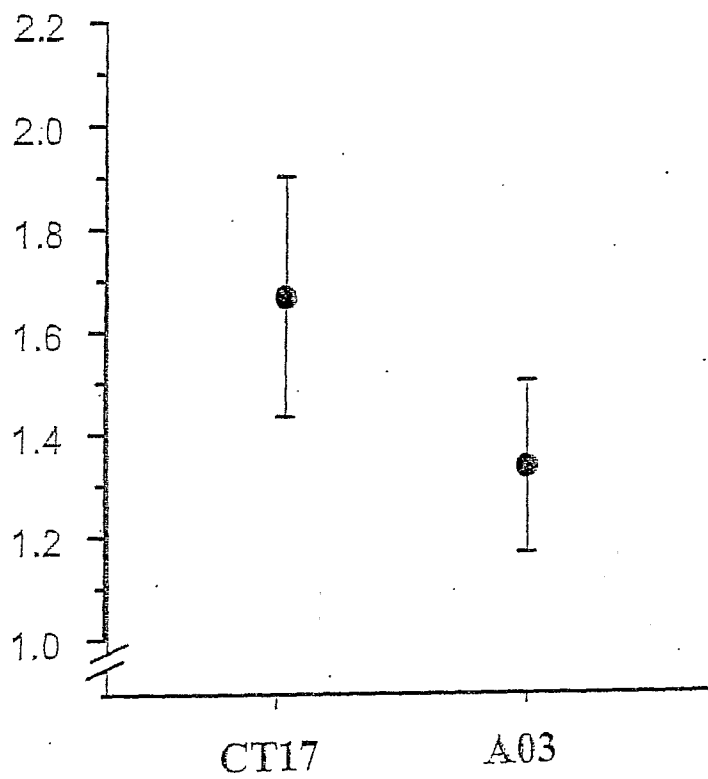
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Figure 5



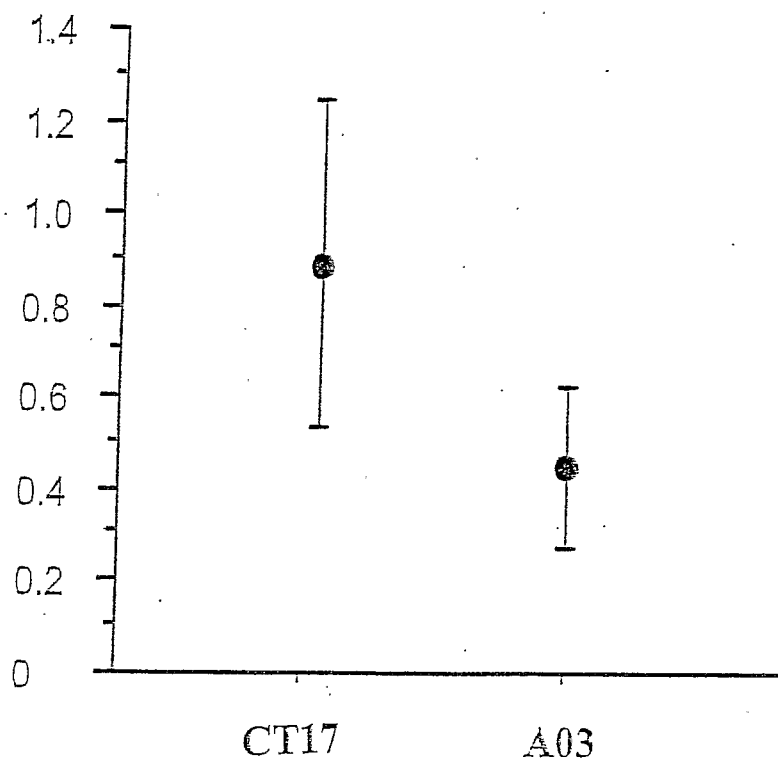
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Figure 6



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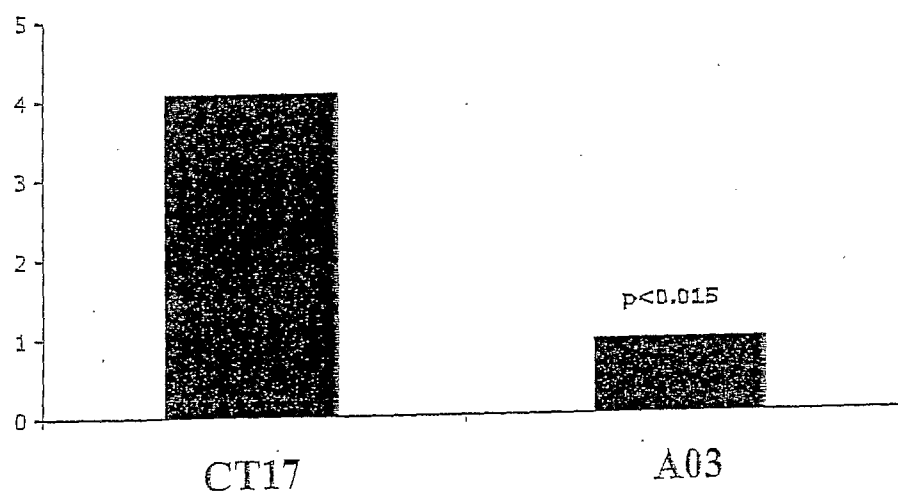
Figure 7



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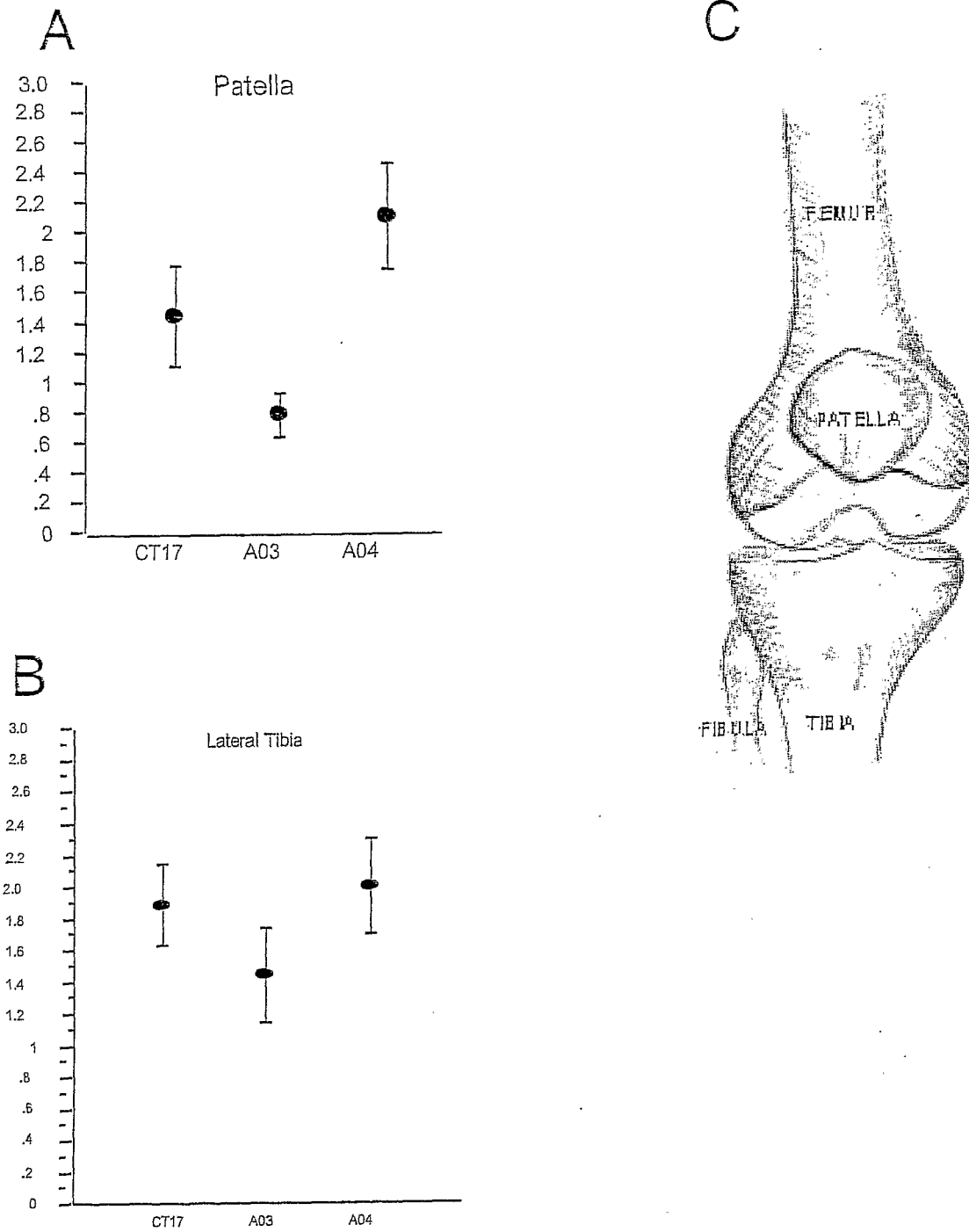
Figure 8

PG degradation in arthritic mice



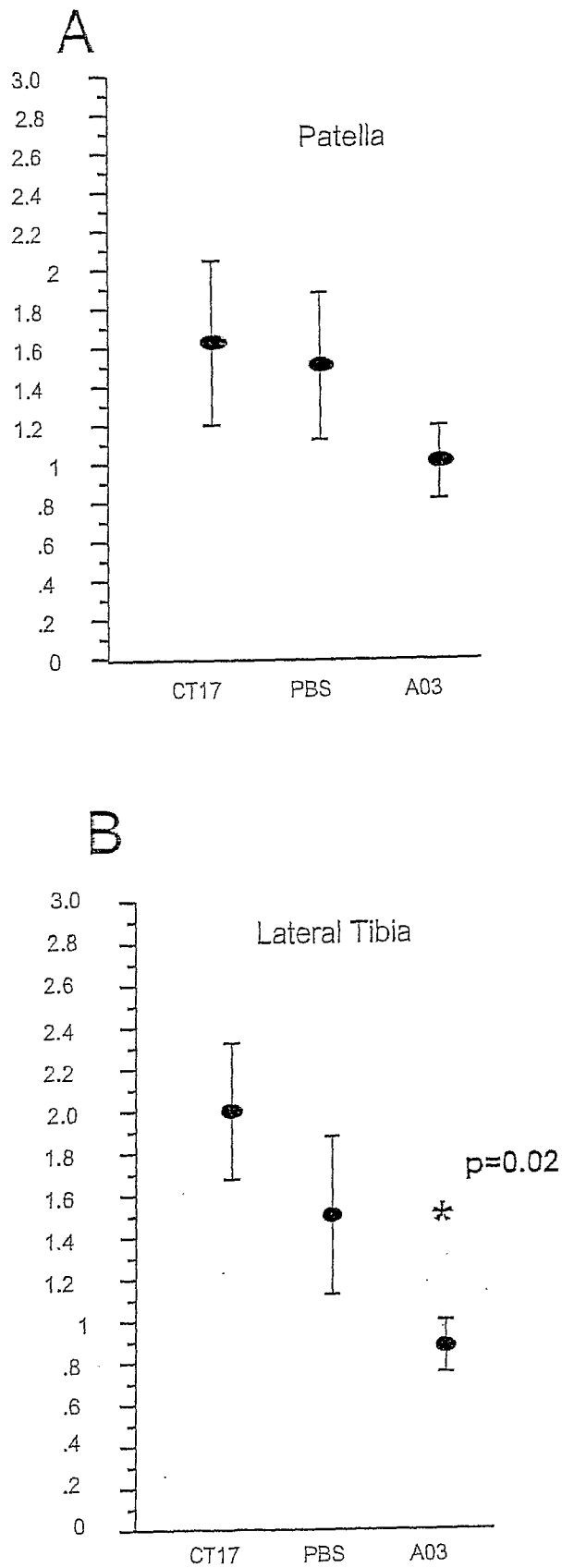
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Figure 9



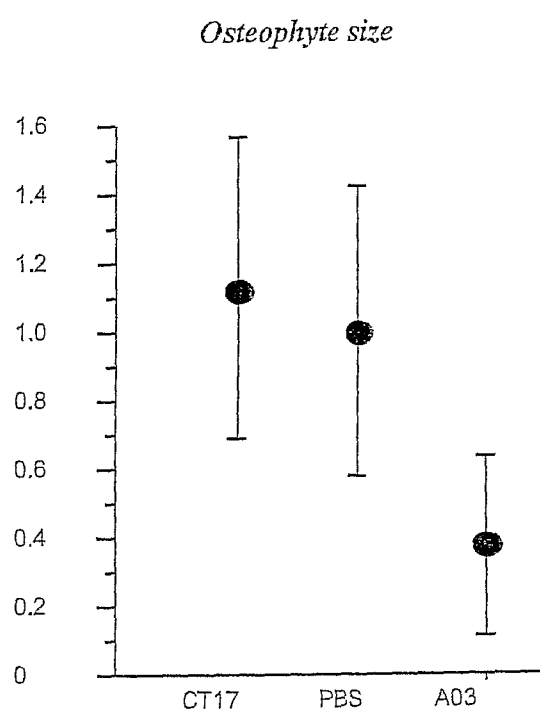
10/14

Figure 10



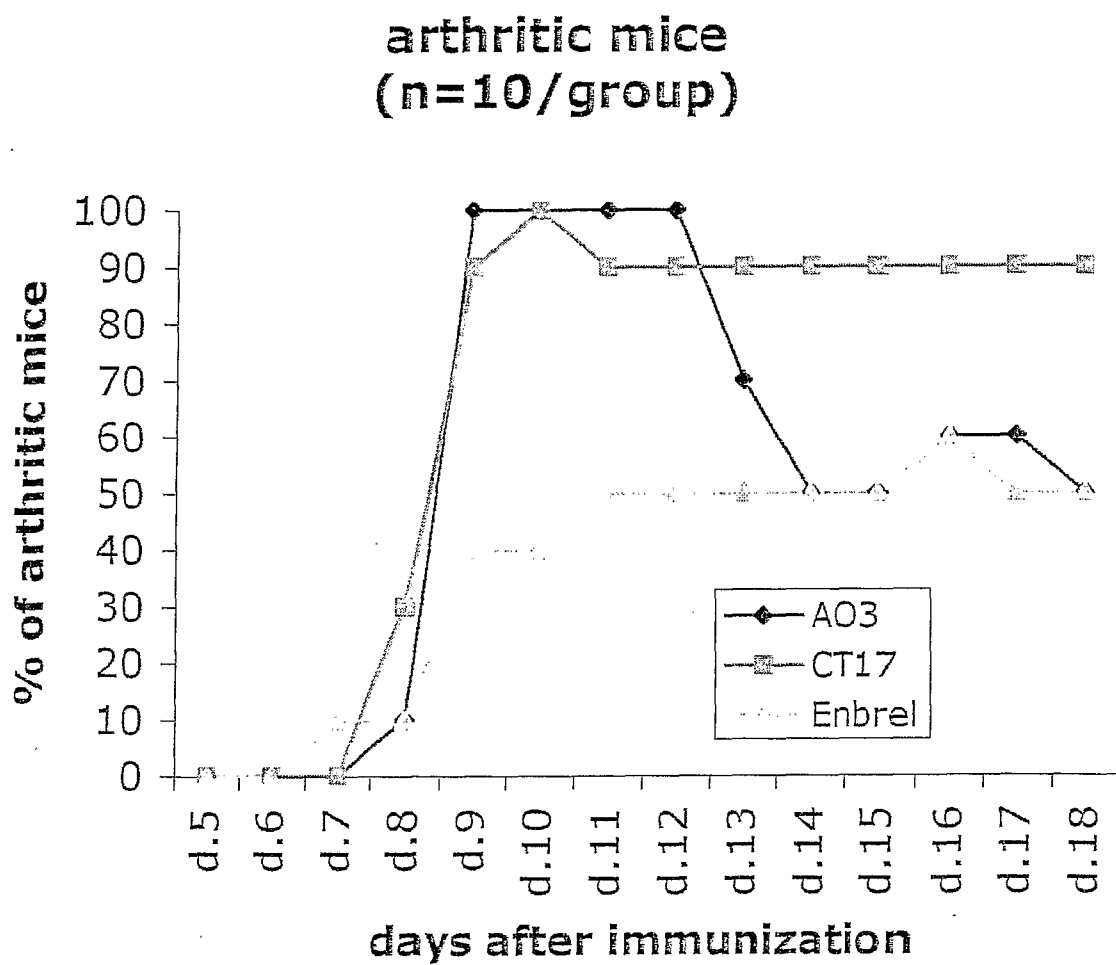
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Figure 11



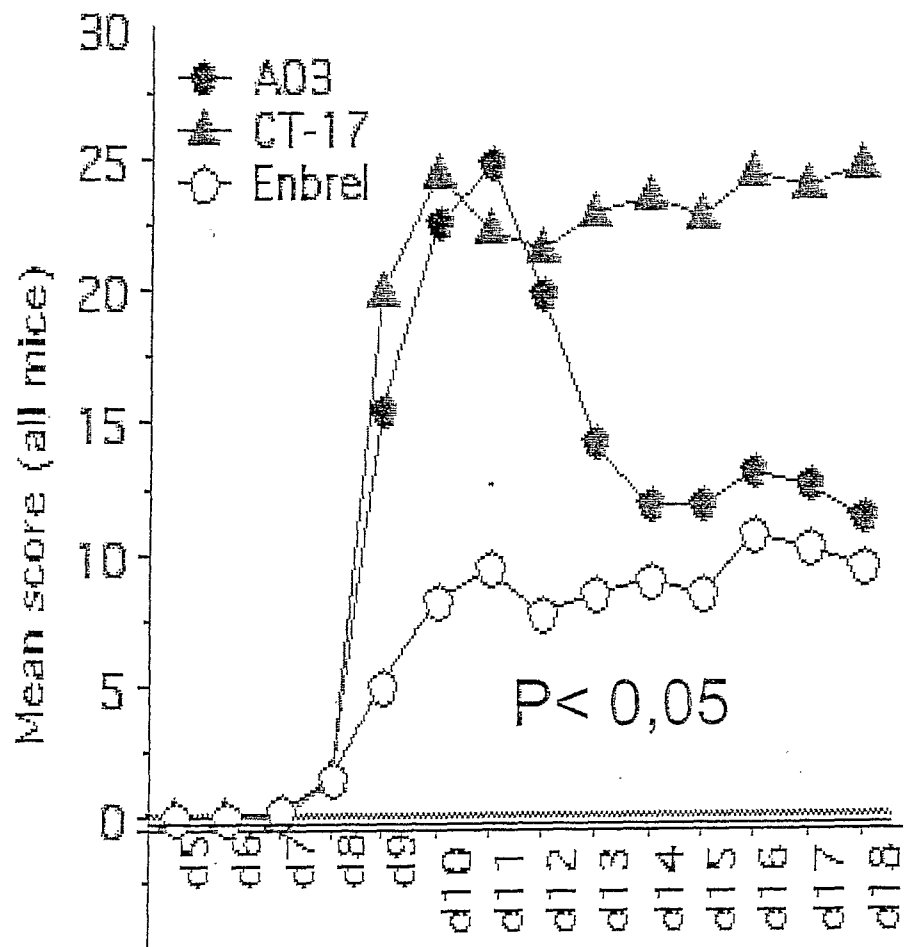
12/14

Figure 12



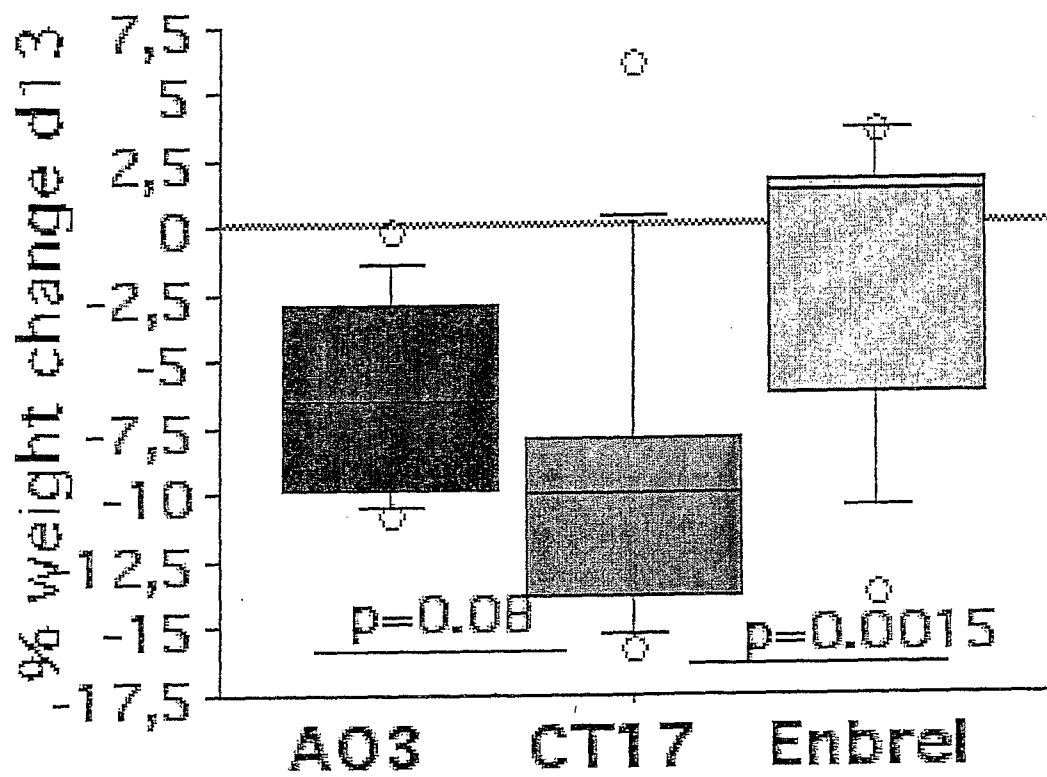
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Figure 13



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Figure 14



INTERNATIONAL SEARCH REPORT

International application No

PCT/GB2007/004886

A. CLASSIFICATION OF SUBJECT MATTER

INV. A61K39/395 C07K16/28 A61K47/48 A61K51/10 A61P19/02
A61P37/00 C07K14/705

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, CHEM ABS Data, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A ₁	WO 00/75187 A (ACTIVE BIOTECH AB) 14 December 2000 (2000-12-14) cited in the application claims 92,93,105 page 12, line 1 - line 6 page 14, line 14 - line 21 page 17, line 14 - line 19 ----- -/--	1-92

☒ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

22 April 2008

Date of mailing of the international search report

07/05/2008

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INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2007/004886

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	T. VELLING ET AL.: "cDNA cloning and chromosomal localization of human alpha11 integrin." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 36, 3 September 1999 (1999-09-03), pages 25735-25742, XP001061984 U.S.A. cited in the application abstract page 25736, right-hand column, line 62 - line 66	1-92
A	T. MIRTITI ET AL.: "Regulation of prostate cell collagen receptors by malignant transformation." INTERNATIONAL JOURNAL OF CANCER, vol. 118, no. 4, 15 February 2006 (2006-02-15), pages 889-898, XP008077317 figure 2	1-92
A	W. ZHANG ET AL.: "Alpha11beta1 integrin recognizes the GFOGER sequence in interstitial collagens." THE JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 278, no. 9, 28 February 2003 (2003-02-28), pages 7270-7277, XP002434866 U.S.A. * material and methods - antibodies *	1-92
A	K. LEHNERT ET AL.: "Cloning, sequence analysis, and chromosomal localization of the novel human integrin alpha 11 subunit (ITGA11)." GENOMICS, vol. 60, no. 2, 1 September 1999 (1999-09-01), pages 179-187, XP004444819 San Diego, CA, USA figure 2	1-92
A	D. GULLBERG & E. LUNDGREN-AKERLUND: "Collagen-binding I domain integrins - What do they do?" PROGRESS IN HISTOCHEMISTRY AND CYTOCHEMISTRY, vol. 37, no. 1, 2002, pages 3-54, XP005032609 Germany Chapter 5.3 - alpha11beta1 integrin Chapter 6 - Cell-collagen interactions in disease.	1-92

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB2007/004886

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
see FURTHER INFORMATION sheet PCT/ISA/210
2. ☒ Claims Nos.: 93-109
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.1

Although claims 33-39 and 50 (all completely) and 47-49 and 51-55 (all partially, as far as an in vivo method is concerned) are directed to a method of treatment of, or a diagnostic method practised on the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box II.2

Claims Nos.: 93-109

Present claims 93-109 are unclear to such an extent that no search for these claims is possible

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.2), should the problems which led to the Article 17(2)PCT declaration be overcome.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/GB2007/004886

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 0075187	A	14-12-2000	AU 770652 B2	26-02-2004
			AU 5435500 A	28-12-2000
			CA 2375876 A1	14-12-2000
			EP 1181317 A1	27-02-2002
			JP 2003501077 T	14-01-2003
			JP 2006246894 A	21-09-2006