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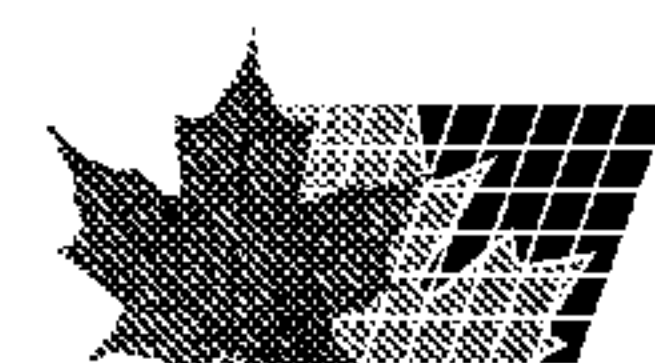
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Antibodies which bind an antigen of the bone marrow neovasculature in leukaemia patients, for use in treatment and diagnosis of leukaemia, in particular the treatment and diagnosis of acute myeloid leukaemia (AML).



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(54) Title: TARGETING OF BONE MARROW NEOVASCULATURE

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Targeting of Bone Marrow Neovasculature

The present invention relates to the use of antibodies which target an antigen expressed in bone marrow neovasculature, in particular the use of such antibodies for treating or diagnosing leukaemia.

5

Bone marrow neovascular structures are a characteristic feature of a number of diseases, including leukaemias, myelodysplastic syndromes (also sometimes referred to as preleukaemias) and multiple myeloma.

10 Leukaemia is a cancer of the blood and bone marrow which is characterized by an abnormal proliferation of blood cells. Blood cells are produced in the bone marrow where they develop from stem cells. The first stage in the development of blood cells is the differentiation of stem cells into myeloid stem cells or lymphoid stem cells. In healthy individuals, the myeloid stem cells then continue to differentiate into one of three types of mature blood cells: red blood
15 cells, white blood cells and platelets, while the lymphoid stem cells differentiate into another type of white blood cells, referred to as lymphocytes. Either of these two cell lineages can be affected by leukaemia. Depending on the cell lineage affected, the leukaemias is referred to either as a myeloid (or alternatively as myelocytic, myelogenous, myeloblastic or non-lymphocytic) leukaemia, or a lymphocytic (or alternatively as lymphoblastic or
20 lymphogenous) leukaemia.

In addition, leukaemias are also differentiated on the basis of whether the disease is acute or chronic. As the name implies, acute leukaemias progress rapidly while chronic leukaemia progress slowly and develop over many years. In acute forms of the disease, the affected
25 bone marrow releases large numbers of immature white blood cells, called blasts or blast cells, which cannot carry out the normal white blood cell functions. If left untreated, acute leukaemias lead to death within a matter of weeks.

The most common form of acute leukaemia in adults, and the second most common
30 leukaemia in children, is acute myeloid leukaemia (AML). AML, as the name implies, affects the myeloid rather than lymphocytic white blood cells and is therefore also sometimes referred to as non-lymphocytic leukaemia (ANLL).

Leukaemias differ from most other cancers in that they do not normally form static tumours.
35 Rare exceptions include solid tumours composed of blast cells occurring outside the bone marrow in AML patients. These tumours are referred to as extramedullary myeloid tumours

(or alternatively as chloroma, granulocytic sarcoma or myeloid sarcoma) and the disease is then called extramedullary AML.

Acute forms of leukaemia are usually treated using chemotherapy. For example, common
5 treatment regimens for AML include cytarabine administered either alone or, more
commonly, in combination with an anthracycline such as daunorubicin or idarubicin.
However, despite the availability of aggressive multi-agent chemotherapy regimens, only 20-
30% of AML patients are currently cured. The reason for this low success rate is the
emergence of dominant, multidrug and radiation resistant subclones of leukaemia cells. The
10 insidious nature of AML also relates to the fact that, while all circulating blasts in the blood
and most blasts in readily accessible bone marrow regions are rapidly killed by cytarabine-
based chemotherapeutic regimens, some blasts in bone marrow sanctuaries survive
chemotherapy and grow again at the end of treatment, causing a relapse. Any treatments
which would allow these resistant blasts to be eradicated, preferably without causing major
15 additional toxicity to the bone marrow, would represent a major advance in the treatment of
leukaemia.

The present inventors have discovered that certain antigens are expressed in bone marrow
neovasculature, such as the neovasculature found in the bone marrow of leukaemia
20 patients.

Specifically, the present inventors have shown that tenascin-C, and the Extra Domain-A
(ED-A) isoform of fibronectin, are expressed in neovascular structures present in bone
marrow biopsies obtained from AML patients.

25 That an increase in angiogenesis takes place in the bone marrow of patients with AML has
been previously reported (Padro et al., 2000). However, it was not known that antigens exist
which are specifically expressed in the neovascular structures in the bone marrow these
patients.

30 The discovery of these antigens opens up new avenues for treating and diagnosing diseases
characterized by the presence of bone marrow neovasculature, including all those
mentioned herein, such as leukaemia, myelodysplastic syndromes, and multiple myeloma.

35 For example, conventional chemotherapeutic treatments for leukaemia do not discriminate
between diseased and healthy tissues. Consequently, large drug doses have to be
administered to the patient to reach therapeutically relevant concentrations, leading to side

effects such as toxicities to healthy tissues. In contrast, antibodies which bind the bone marrow neovasculature in leukaemia patients allow therapeutic agents to be delivered directly to the affected tissues, thus avoiding or reducing the disadvantages associated with conventional chemotherapeutic treatments. In addition, favourable toxicity profiles of site-specific therapeutics may also open new avenues in the therapy of diseases characterized by the presence of bone marrow neovasculature by allowing the systemic administration of highly potent and promising agents, which are currently either given at suboptimal doses or whose clinical application has to date been impeded by unacceptable toxicities when applied in an unmodified form.

Thus an aspect of the invention provides an antibody for use in a method of treatment of a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, wherein the antibody binds an antigen of the bone marrow neovasculature in patients suffering from said disease. Individuals or patients referred to herein are preferably human.

Another aspect of the present invention provides use of an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterized by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in the manufacture of a medicament for use in a method of treating said disease.

Another aspect of the present invention provides a method of treating a disease characterized by bone marrow neovasculature, comprising administering a therapeutically effective amount of an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from said disease to an individual in need thereof.

Another aspect of the present invention provides an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, for use in a method of treating said disease, the method comprising administering the antibody and an anti-cancer compound to an individual in need thereof.

Another aspect of the present invention provides an anti-cancer compound for use in a method of treating a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, the method comprising administering the anti-cancer compound and an antibody that binds an antigen of the bone

marrow neovasculature in patients suffering from said disease to an individual in need thereof.

Another aspect of the present invention provides use of an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in the manufacture of a medicament for use in a method of treating said disease, the method comprising administering the antibody and an anti-cancer compound.

Another aspect of the present invention provides use of an anti-cancer compound in the manufacture of a medicament for use in a method of treating a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, the method comprising administering the anti-cancer compound and an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from said disease.

Another aspect of the present invention provides a method of treating a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, comprising administering a therapeutically effective amount of an antibody and an anti-cancer compound to an individual in need thereof, wherein the antibody binds an antigen of the bone marrow neovasculature in patients suffering from said disease.

Another aspect of the present invention provides an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, for use in a method of diagnosis of said disease.

Another aspect of the present invention provides use of an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in the manufacture of a diagnostic agent for diagnosing said disease.

Another aspect of the present invention provides use of an antibody that binds an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, for *in vitro* detection or diagnosis of said disease.

Another aspect of the present invention provides a method of detecting or diagnosing a disease characterized by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in an individual, wherein the method comprises:

- 5 administering an antibody that binds an antigen of the bone marrow neovasculature in said patients to the individual; and
 detecting binding of the antibody to bone marrow neovasculature in the individual.

Another aspect of the present invention provides a method of detecting or diagnosing a disease characterized by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in an individual, wherein the method comprises:

- 10 administering an antibody that binds an antigen of the bone marrow neovasculature in these patients to the individual; and
 determining the presence or absence of the antibody in the bone marrow of the
15 individual,
 wherein the presence of the antibody in the bone marrow neovasculature of the individual indicates that the individual has said disease.

Another aspect of the present invention provides an *in vitro* method of detecting or diagnosing a disease characterized by bone marrow neovasculature, such as leukaemia, myelodysplastic syndromes, or multiple myeloma, in an individual, wherein the method comprises:

- 20 applying an antibody that binds an antigen of the bone marrow neovasculature in said patients to a bone marrow sample obtained from the individual; and
25 detecting binding of the antibody in the sample,
 wherein binding of the antibody to bone marrow neovasculature in the sample indicates that the individual has said disease.

Another aspect of the present invention provides a method of targeting bone marrow neovasculature, e.g. *in vitro*, comprising the use of an antibody that binds tenascin-C or the Extra Domain-A (ED-A) isoform of fibronectin.

Another aspect of the present invention provides use of an antibody that binds tenascin-C or the Extra Domain-A (ED-A) isoform of fibronectin for targeting bone marrow neovasculature, e.g. *in vitro*.

Another aspect of the present invention provides use of an antibody that binds tenascin-C or the Extra Domain-A (ED-A) isoform of fibronectin for the manufacture of a medicament for use in targeting bone marrow neovasculature.

- 5 Another aspect of the present invention provides an antibody that binds tenascin-C or the Extra Domain-A (ED-A) isoform of fibronectin for use in a method of treatment or diagnosis comprising targeting bone marrow neovasculature.

10 Diseases characterized by the presence of neovascular structures in the bone marrow include leukaemia, myelodysplastic syndromes (also referred to as preleukaemias), and multiple myeloma. Exemplary leukaemias include acute and chronic leukaemias. For example, a leukaemia as referred to herein may be a myeloid or a lymphocytic leukaemia. Preferably, a leukaemia as referred to herein is acute myeloid leukaemia (AML).

- 15 Myelodysplastic syndromes are bone marrow stem cell disorders characterized by ineffective production (or dysplasia) of myeloid blood cells and risk of transformation to acute myelogenous leukemia (AML).

20 Multiple myeloma is also known as myeloma, plasma cell myeloma, or Kahler's disease and is a cancer which affects the plasma cells in the bone marrow. All of the above diseases are known to be characterized by neovessels, or angiogenesis, in the bone marrow.

Bone marrow neovasculature as referred to herein may be vascular structures found in the bone marrow of patients suffering from a disease characterized by bone marrow
25 angiogenesis such as leukaemia, myelodysplastic syndromes, or multiple myeloma. These vascular structures may not be found in the bone marrow of healthy individuals, or may be found in the bone marrow of healthy individuals but to a lesser extent than in individuals suffering from such a disease. Thus, the disease may be a disease characterized by increased bone marrow neovasculature.

30

Antibodies for use in the present invention may bind an antigen expressed in bone marrow neovasculature. The bone marrow neovasculature may be the neovasculature present in the bone marrow of a patient suffering from a disease characterised by bone marrow angiogenesis, e.g. leukaemia, myelodysplastic syndromes or multiple myeloma. Preferably
35 an antibody for use in the present invention binds an antigen of the bone marrow neovasculature in leukaemia patients. Most preferred for use in the present invention are

antibodies that bind an antigen of the bone marrow neovasculature in acute myeloid leukaemia (AML) patients.

The antigen may be an antigen which is differentially expressed in bone marrow neovasculature compared with normal tissue. For example, the antigen may be an isoform of a protein, wherein the isoform is differentially expressed in bone marrow neovasculature compared with normal tissue. Normal tissue in this context may be healthy tissues, i.e. tissues not affected by disease. Where the antigen is an antigen of the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow angiogenesis, e.g. leukaemia patients, such as e.g. acute myeloid leukaemia patients, patients with myelodysplastic syndromes or multiple myeloma, the antigen may be differentially expressed in the bone marrow neovasculature of these patients compared with other tissues of these patients. For example, the antigen may be differentially expressed in the bone marrow neovasculature of these patients compared with other bone marrow tissues of these patients, such as other bone marrow blood vessels.

The antigen may be an antigen (e.g. an isoform of a protein) that is differentially expressed in the bone marrow neovasculature of patients suffering from a disease characterised by bone marrow angiogenesis, e.g. leukaemia patients, such as e.g. acute myeloid leukaemia patients, patients with myelodysplastic syndromes or multiple myeloma, compared with normal tissues, e.g. bone marrow tissues, of healthy individuals. Normal tissue in this context are healthy tissues, i.e. tissues not affected by disease. For example, the antigen may be an antigen that is differentially expressed in the bone marrow neovasculature of these patients compared with the bone marrow blood vessels found in healthy individuals.

Differential expression in this context may mean that the antigen is expressed in bone marrow neovasculature and not expressed, or not significantly expressed, in normal tissue. Alternatively, differential expression may mean that expression of the antigen in bone marrow neovasculature is higher, e.g. significantly higher, than in normal tissue. The level of expression of an antigen in a relevant tissue may be measured using, for example, ELISA, Western Blotting, or Mass Spectrometry. All of these methods are well established in the art. "Significantly" in the context of antigen expression may mean statistically significantly, e.g. when measured using a Student T-test. Where a Student T-test is used, a p-value below e.g. 0.1, 0.05, or 0.01 (depending on the threshold chosen for statistical significance), indicates that the level of expression of the antigen in question is significantly different in the tissues that are being compared. Thus, where the level of expression of an antigen in bone marrow neovasculature and normal tissue is compared using a Student T-test, a p-value

below e.g. 0.1, 0.05, or 0.01 indicates that the level of expression of the antigen differs significantly between the two tissues. Similarly, an antigen is not significantly expressed in a tissue if the level of expression of the antigen in said tissue is not statistically different from a negative control. Where a Student T-test is used to compare the level of expression in a tissue with a negative control, a p-value of 0.1 or above, 0.05 or above, or 0.01 or above (again depending on the threshold chosen for statistical significance), indicates that the level of expression of the antigen in the tissue question does not differ significantly from the negative control, and hence is not significantly expressed in said tissue.

The antigen may be an antigen of the extracellular matrix, e.g. the subendothelial extracellular matrix, of bone marrow neovasculature. The antigen may be expressed on cells of the bone marrow neovasculature.

Examples of suitable antigens include tenascin-C and the Extra Domain-A (ED-A) isoform of fibronectin. Preferred antigens are the Extra Domain-A (ED-A) of fibronectin and the tenascin-C large isoform, in particular the A1 domain of the tenascin-C large isoform.

Thus, in one example, an antibody for use in the invention may bind to an isoform of fibronectin that is differentially expressed in the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow angiogenesis, e.g. leukaemia patients, such as e.g. acute myeloid leukaemia patients, patients with myelodysplastic syndromes or multiple myeloma as described above. For example, an antibody for use in the invention may bind the Extra Domain-A (ED-A) isoform of fibronectin. The antibody may bind preferentially to the Extra Domain-A (ED-A) isoform of fibronectin compared with other isoforms of fibronectin. Preferred antibodies for use in the invention include antibodies which bind to the Extra Domain-A (ED-A) of fibronectin.

Alternatively, an antibody for use in the invention may bind to an isoform of tenascin-C that is differentially expressed in the bone marrow neovasculature in patients suffering from a disease characterised by bone marrow angiogenesis, e.g. leukaemia patients, such as e.g. acute myeloid leukaemia patients, patients with myelodysplastic syndromes or multiple myeloma as described above. For example, an antibody for use in the invention may bind to the tenascin-C large isoform. The antibody may bind preferentially to tenascin-C large isoform relative to tenascin-C small isoform. Antibodies for use in the invention may bind a domain of tenascin-C which is subject to alternative splicing and is expressed only in the large isoform, e.g. any of domains A1 to D (see Figure 1). Preferred antibodies for use in the invention include antibodies which bind the A1 domain of the tenascin-C large isoform.

Human monoclonal antibody fragments specific to tenascin-C are described, for example, in WO2006/050834, while human monoclonal antibodies specific for the ED-A isoform of fibronectin are described in WO2008/120101 and also in Villa *et al.* (2008).

5

In some embodiments, an antibody for use in the present invention competes for binding to tenascin-C with an antibody comprising the 4A1-F16 VH domain of SEQ ID NO: 2 and the 4A1-F16 VL domain SEQ ID NO: 4. Alternatively, an antibody for use in the present invention may compete for binding to the ED-A isoform of fibronectin with an antibody comprising the F8 (V5L) VH domain of SEQ ID NO. 13 and the F8 (K18R) VL domain of SEQ ID NO. 15.

10

Competition between antibodies may be assayed easily *in vitro*, for example using ELISA and/or by tagging a specific reporter molecule to one antibody which can be detected in the presence of other untagged antibody(s), to enable identification of antibodies which bind the same epitope or an overlapping epitope.

15

In one example, antibody for use in the invention may bind tenascin C (e.g. the A1 domain of the tenascin-C large isoform), or the Extra Domain-A isoform of fibronectin (e.g. the ED-A of fibronectin) with a K_D of at least 1 μ M, 100nM, 50nM, or 25nM, when measured using surface plasmon resonance, e.g. using a BIAcore3000TM instrument. When measuring affinity, the antibody may be in any convenient format: including small immunoprotein (SIP), scFv, or whole IgG format. A suitable method for determining the affinity of an antibody is described, for example, in Brack *et al.* (2006).

20

For example, an antibody for use in the present invention may bind the A1 domain of the tenascin-C large isoform with the same affinity as antibody 4A1-F16-SIP when measured using a BIAcore3000 instrument or with an affinity that is better. Alternatively, an antibody for use in the invention may bind A-FN and/or the ED-A of fibronectin with the same affinity as antibody F8-SIP (V5L/K18R) when measured using surface plasmon resonance, e.g. using a BIAcore3000TM instrument, or with an affinity that is better.

25

The antibody 4A1-F16 has VH and VL domain amino acid sequences and CDRs as shown in the appended sequence listing.

30

A suitable antibody for use in the present invention may comprise an antibody antigen binding site comprising a VH domain and a VL domain,

35

the VH domain comprising a VH CDR1 of SEQ ID NO. 5, a VH CDR2 of SEQ ID NO. 6 and a VH CDR3 of SEQ ID NO. 7; and

the VL domain comprising a VL CDR1 of SEQ ID NO. 8, a VL CDR2 of SEQ ID NO. 9 and a VL CDR3 of SEQ ID NO. 10.

5

In some preferred embodiments, an antibody for use in the present invention may comprise an antibody antigen binding site comprising the 4A1-F16 VH domain of SEQ ID NO. 2 and the 4A1-F16 VL domain of SEQ ID NO. 4.

10 Antibody F8 (V5L/K18R) has VH and VL domain amino acid sequences and CDRs as shown in the appended sequence listing.

A suitable antibody for use in the present invention may comprise an antibody antigen binding site comprising a VH domain and a VL domain,

15 the VH domain comprising a VH CDR1 of SEQ ID NO:16, a VH CDR2 of SEQ ID NO:17, and a VH CDR3 of SEQ ID NO:18;

the VL domain comprising a VL CDR1 of SEQ ID NO: 19, a VL CDR2 of SEQ ID NO:20, and a VL CDR3 of SEQ ID NO:21.

20 In some preferred embodiments, the antibody for use in the present invention may comprise an antibody antigen binding site comprising the F8 (V5L) VH domain of SEQ ID NO:13 and the F8 (K18R) VL domain of SEQ ID NO:15.

25 A number of antibody molecule formats are known and any suitable format may be used for an antibody for use in the invention.

In some embodiments, an antibody for use in the invention may be or comprise a single chain Fv (scFv), comprising a VH domain and a VL domain joined via a peptide linker. The skilled person may select an appropriate length and sequence of linker, e.g. at least 5 or at
30 least 10 amino acids in length, up to about 15, up to about 20 or up to about 25 amino acids in length. For example, the linker may have the amino acid sequence shown in SEQ ID NO:11 or SEQ ID NO:22.

In some embodiments, an antibody for use in the present invention may be a mini-immunoglobulin or small immunoprotein (SIP) comprising a single chain Fv (scFv), e.g. as
35 described in (Li *et al.*, 1997). An SIP may comprise an scFv molecule fused to the CH4 domain of the human IgE secretory isoform IgE-S2 (ϵ_{S2} -CH4; Batista *et al.*, 1996) forming an

homo-dimeric mini-immunoglobulin antibody molecule. The CH4 domain may have the amino acid sequence shown in SEQ ID NO:24 and may be linked to the VL domain via a peptide linker. A suitable peptide linker is shown in SEQ ID NO:23.

- 5 In some embodiments, an antibody for use in the present invention may be a whole IgG antibody molecule, e.g. a whole IgG1 antibody molecule.

10 Variants of the VH and VL domains and CDRs described herein may also be employed in antibodies for use in the present invention. Suitable variants can be obtained by means of methods of sequence alteration or mutation and screening.

Particular variants for use as described herein may include one or more amino acid sequence alterations (addition, deletion, substitution and/or insertion of an amino acid residue), maybe less than about 20 alterations, less than about 15 alterations, less than
15 about 10 alterations or less than about 5 alterations, 4, 3, 2 or 1. Alterations may be made in one or more framework regions and/or one or more CDRs. In particular, alterations may be made in VH CDR1, VH CDR2 and/or VH CDR3, especially VH CDR3.

20 In some embodiments, the 4A1-F16 VL domain of SEQ ID NO: 4 may lack the Serine at position 1.

In some preferred embodiments an antibody for use in the present invention is conjugated to a bioactive molecule, such as a cytokine (e.g. IL2), cytotoxic agent, photosensitizer, or therapeutic radioisotope.

25

IL2-containing immunocytokines have previously been shown to be capable of eradicating tumours and lymphomas in mouse models of cancer when used alone or in combination with other therapeutic agents such as chemotherapy or intact antibodies (Schliemann *et al.*, 2009; Marlind, *et al.*, 2008; Menrad *et al.*, 2005; and Carnemolla *et al.*, 2002).

30

Thus, in some embodiments an antibody for use in the present invention may be conjugated to a cytokine, e.g. interleukin 2 (IL2), to form an antibody-cytokine conjugate. The main attraction of using such immunocytokines is the activation of immune cells (e.g., natural killer [NK] cells) which may allow the last surviving blast cells to be eradicated, thereby making the
35 difference between a patient suffering relapses after treatment and a cure.

As NK cells are mainly responsible for the therapeutic action of antibody-IL2 conjugates, the activity of such molecules can be studied in tumour-bearing immunocompromised mice. For example, mouse models of human leukaemia can be used to study the *in vivo* targeting potential and the therapeutic activity of antibody-IL2 conjugates in the treatment of
5 leukaemia. A suitable mouse model for human leukaemia employs the HL-60 leukaemia cell line in nude mice, as disclosed in Potter *et al.* (1984).

Interleukin-2 (IL2) is a secreted cytokine which is involved in immunoregulation and the proliferation of T and B lymphocytes. IL2 has been shown to have a cytotoxic effect on
10 tumour cells and recombinant human IL2 (aldesleukin: ProleukinTM) has FDA approval for treatment of metastatic renal carcinoma and metastatic melanoma. The sequence of human IL2 is set out in SEQ ID NO: 27 and publicly available under sequence database reference NP_000577.2 GI: 28178861.

15 In some preferred embodiments, the IL2 moiety of the antibody-IL2 conjugate comprises a sequence which has at least 90% sequence identity, at least 95% sequence identity or at least 98% sequence identity to the mature human IL2 sequence set out in SEQ ID NO: 27.

Sequence identity is commonly defined with reference to the algorithm GAP (Wisconsin GCG package, Accelrys Inc, San Diego USA). GAP uses the Needleman and Wunsch
20 algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul *et al.* (1990) *J. Mol.*
25 *Biol.* 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448), or the Smith-Waterman algorithm (Smith and Waterman (1981) *J. Mol Biol.* 147: 195-197), or the TBLASTN program, of Altschul *et al.* (1990) *supra*, generally employing default parameters. In particular, the psi-Blast algorithm (Nucl. Acids Res. (1997) 25 3389-3402) may be used.

30 In some especially preferred embodiments, the IL2 moiety of the antibody-IL2 conjugate comprises the sequence of mature human IL2 set out in SEQ ID NO: 27.

The IL2 moiety may be fused upstream (N-terminal) or downstream (C-terminal) of the
35 antibody or polypeptide component thereof.

The IL2 moiety may be connected or attached to the antibody moiety of the antibody-IL2 conjugate by any suitable covalent or non-covalent means. In preferred embodiments, the antibody-IL2 conjugate may be a fusion protein comprising IL2 and the antibody or a polypeptide component thereof (e.g. a heavy chain or a light chain of an antibody or multi-chain antibody fragment, such as a Fab. Thus, for example, the IL2 moiety may be fused to a VH domain or VL domain of the antibody. Typically the antibody, or component thereof, and IL2 moiety are joined via a peptide linker, e.g. a peptide of about 5-25 residues, e.g. 10-20 residues, preferably about 15 residues. Suitable examples of peptide linkers are well known in the art. In some embodiments, a linker may have an amino acid sequence as set out in SEQ ID NO: 28. Normally, the linker has an amino acid sequence comprising one or more tandem repeats of a motif. Typically the motif is a five residue sequence, and preferably at least 4 of the residues are Gly or Ser. Where four of the five residues is Gly or Ser, the other residue may be Ala. More preferably each of the five residues is Gly or Ser. Preferred motifs are GGGGS (SEQ ID NO: 33), SSSSG (SEQ ID NO: 34), GSGSA (SEQ ID NO: 35) and GSGGG (SEQ ID NO: 36). Preferably, the motifs are adjacent in the sequence, with no intervening nucleotides between the repeats. The linker sequence may comprise or consist of between one and five, preferably three or four, repeats of the motif. For example, a linker with three tandem repeats may have one of the amino acid sequences shown in SEQ ID NOs. 29 to 32.

Antibody-drug conjugates are known to be useful for selectively delivering a cytotoxic agent to a target such as a tumour-associated antigen (Carter *et al.*, 2008). Such conjugates allow the delivery of cytotoxic agents directly to the affected tissues, thereby avoiding the disadvantages associated with conventional chemotherapy. For example, it has previously been shown that antibodies such as F16 or F8 can be coupled to cytotoxic drugs and can localize with extraordinary efficiency and selectivity around tumour blood vessels. Thus, in some embodiments an antibody for use in the invention may be conjugated to a cytotoxic agent. Exemplary cytotoxic agents include cytotoxic agents which are suitable for treating cancer. For example a cytotoxic agent may be suitable for treating a disease characterized by bone marrow neovasculation, such as leukaemia myelodysplastic syndromes, or multiple myeloma, e.g. AML.

Preferred cytotoxic agent include potent cytotoxic agent of relatively simple chemical structure to facilitate manufacture. The use of potent cytotoxic agents is preferred because of the difference in molecular weight between antibodies and cytotoxic agents (Carter *et al.*, 2008). A potent cytotoxic agent may be a cytotoxic agent capable of killing tumour cells at

sub-nanomolar concentrations. Suitable cytotoxic agents which may be conjugated to an antibody for use in the present invention include dolastatins, vinblastines, epothilones, tubulysins, and derivatives and analogues thereof.

5 Dolastatins are a family of antiproliferative peptides which inhibit the growth and reproduction of target cells and induce apoptosis in a variety of malignant cell types. Exemplary dolastatins include dolastatin-10 and dolastatin-15, and their derivatives, which have been shown to have particularly strong antiproliferative bioactivity (de Arruda *et al.*, 1995). One preferred dolastatin derivative is cemadotin which is a dolastatin-15 analogue. In
10 preferred embodiments, the antibody-dolastatin conjugate may be a fusion protein comprising the dolastatin and the antibody or a polypeptide component thereof (e.g. a heavy chain or a light chain of an antibody or multi-chain antibody fragment, such as a Fab. Thus, for example, the dolastatin moiety may be fused to a VH domain or VL domain of the antibody.

15

Vinblastine is a chemical analogue of vincristine which is used in a number of chemotherapy regimens including treatment for Hodgkin lymphoma. Potent analogues of vinblastine are described in Barnett *et al.* (1978) and include 4-desacetyl-3-vinblastine monohydrazide.

20 Both 4-desacetyl-3-vinblastine monohydrazide and cemadotin act on microtubuli with a similar mechanism of action and are capable of killing target tumour cells and endothelial cells in the picomolar concentration range (de Arruda *et al.*, 1995; Barnett *et al.*, 1978; Reddy *et al.*, 2007; Ray *et al.*, 2007; and Leamon *et al.*, 2007).

25 Epothilones are a class of cytotoxic molecules which have been shown to have antitumour activity. Exemplary epothilones include ixabepilone, epothilone B, and epothilone D.

Tubulysins are another family of antiproliferative agents which are leading candidates for the development of anticancer agents. Exemplary tubulysins include tubulysin A and tubulysin
30 D. Exemplary tubulysin derivatives are described in Neri *et al.* (2006), Sani *et al.* (2007) and Patterson *et al.* (2007).

In some embodiments the antibody for use in the invention may be conjugated to a cytotoxic agent comprising a terminal maleimido group. Maleimido groups can be used for the site-
35 specific drug conjugation to unique reactive cysteine residues present in the antibodies described herein (Borsi *et al.*, 2002; Berndorff *et al.*, 2006). Most preferably, a cleavable linker is present between the cytotoxic agent and the maleimido moiety.

It has previously demonstrated how the intraluminal blood coagulation in tumour neo-vasculature, caused by the antibody-mediated delivery of pro-coagulant factors such as a truncated version of tissue factor, can lead to rapid tumour cell death. Thus, in some
5 embodiments an antibody for use in the invention may be conjugated to a pro-coagulant factor such as a truncated version of tissue factor. Such conjugates have been previously described in Nilsson *et al.* (2001).

Vascular targeting antibodies have also previously been shown to be suitable for depositing
10 photosensitizers around neo-vasculature of tumours *in vivo*, thus mediating endothelial cell damage and intraluminal blood coagulation upon irradiation, followed by tumour cell death (Birchler *et al.*, 1999; Fabbrini *et al.*, 2006). Specifically, it has been shown that photosensitizers can efficiently generate singlet oxygen outside endothelial cells and kill tumour cells indirectly. Prior to these experiments, it was generally believed that antibody-
15 photosensitizer conjugates needed to be internalized by the target cells, in order to mediate a toxic effect upon irradiation.

Thus, in some embodiments an antibody for use in the invention may be conjugated to a photosensitizer. Exemplary photosensitizers which may be conjugated to an antibody for use
20 in the present invention are described in detail in WO01/62800 and include tin (IV) chlorine e6 and derivatives thereof.

Antibodies conjugated to therapeutic radionuclides have also previously been shown to be effective in the treatment of cancer (Tijink *et al.*, J Nucl Med. 47(7):1070-4, 2006). Thus in
25 some embodiments an antibody for use in the present invention may be conjugated to a therapeutic radionuclide. Exemplary therapeutic radionuclides include ^{131}I , ^{90}Y , ^{124}I , ^{211}At , ^{77}Br , and ^{76}Br . Preferably, the therapeutic radionuclide is ^{131}I or ^{90}Y .

The bioactive molecule may be connected or attached to the antibody moiety by any suitable
30 covalent or non-covalent means. In preferred embodiments the bioactive molecule is conjugated to the antibody by a cleavable linker, thereby allowing the bioactive molecule to be released. For example, the linker may allow release of the bioactive molecule into the sub-endothelial extracellular matrix present in the bone marrow of a patient suffering from a disease characterized by bone marrow neovasculature thereby allowing the drug to diffuse
35 to the bone marrow neovasculature and, where the disease is leukaemia, potentially also to neighbouring blasts.

Suitable cleavable linkers include Schiff bases, peptide linkers cleavable by proteases and stabilized esters. All of these linkers are well known in the art. Exemplary Schiff base linkers are described, for example, in US Patent No. 5633351. Preferred cleavable linkers exhibit reaction half-lives in the 5-20 hour range.

5

An antibody or antibody conjugate for use in the present invention may be administered to an individual in need thereof together with an anti-cancer compound, e.g. an anti-leukaemia compound.

10 Anti-cancer compounds are cytotoxic compounds which inhibit the growth, division and/or proliferation of cancer cells. Anti-cancer compounds may, in some circumstances, have an effect on normal non-cancer cells in a patient. An anti-cancer compound may, for example, be a compound suitable for treating leukaemia. Where the patient is an acute myeloid leukaemia patient, the compound may be a compound suitable for treating acute myeloid
15 leukaemia.

In some embodiments of the invention, the anti-cancer compound may be selected from the group of: alkylating agents, anti-metabolites, plant alkaloids and terpenoids, topoisomerase inhibitors, antitumour antibiotics, monoclonal antibodies, and corticosteroids. Examples of
20 alkylating agents include cyclophosphamide, cisplatin, chlorambucil, carboplatin, and oxaliplatin. Examples of anti-metabolites include methotrexate, purine analogues such as cladribine, fludarabine, tioguanine and pentostatin, and pyrimidine analogues such as cytarabine, 5-fluorouracil, and floxuridine. Examples of plant alkaloids and terpenoids include vinca alkaloids, such as vincristine, vinblastine, vinorelbine, and vindesine;
25 chemotherapeutic agents derived from podophyllotoxin such as etoposide phosphate and teniposide taxanes; and taxanes, which include paclitaxel and docetaxel. Examples of topoisomerase inhibitors include type I topoisomerase inhibitors such as camptothecins and type II topoisomerase inhibitors such as amsacrine, etoposide, etoposide phosphate, and teniposide. Examples of antitumour antibiotics include anthracyclines, such as doxorubicin
30 and epirubicin, actinomycins, and bleomycin. Examples of monoclonal antibodies include rituximab, and examples of corticosteroids include prednisone and prednisolone.

Exemplary anti-cancer compounds suitable for treating leukaemia include: anthracyclines, cytarabine, vincristine, L-asparaginase, cyclophosphamide, methotrexate and 6-
35 mercaptopurine, chlorambucil, cyclophosphamide, corticosteroids, such as prednisone and prednisolone, imatinib, cladribine, pentostatin, rituximab, chlorambucil, and doxorubicin.

Preferred anti-cancer compounds include anthracyclines and cytarabine. These anti-cancer compounds are suitable for treating AML.

For example, in some embodiments of the invention, an antibody or antibody conjugate (e.g. an antibody-cytokine conjugate) may be administered to an individual in need thereof in combination with chemotherapy or IgG-based immunotherapy. For example, anti-CD33 antibodies are currently being investigated for the treatment of AML in Phase IIb clinical trials. Suitable anti-CD33 antibodies are described, for example in Feldman et al. (2003), Feldman et al. (2005) and Kobayashi et al. (2009). In addition, IgG based anti-CD123 antibodies are also being investigated in the treatment of AML (Jin et al., 2009). Thus, in one example, IgG-based immunotherapy may involve treatment with an anti-CD33 or anti-CD123 antibody.

In some embodiments an antibody for use in the invention may be labelled with a detectable or functional label. Antibodies labelled with a detectable label, may be used diagnostically *in vivo*, *ex vivo* or *in vitro*, and/or therapeutically.

A detectable label may be any molecule that produces or can be induced to produce a signal, including but not limited to fluorescers, radiolabels, enzymes, chemiluminescers or photosensitizers. Thus, binding may be detected and/or measured by detecting fluorescence or luminescence, radioactivity, enzyme activity or light absorbance. Detectable labels may be attached to antibodies for use in the invention using conventional chemistry known in the art.

There are numerous methods by which the label can produce a signal detectable by external means, for example, by visual examination, electromagnetic radiation, heat, and chemical reagents. The label can also be bound to another specific binding member that binds the antibody for use in the invention, or to a support.

Administration of an antibody, antibody conjugate, anti-cancer compound and compositions comprising one or more of these molecules is preferably in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated.

Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

The precise dose will depend upon a number of factors, the size and location of the area to be treated, the precise nature of the antibody (e.g. whole antibody, fragment or diabody). A typical antibody, or antibody conjugate, dose will be in the range 0.5mg to 100g for systemic applications, and 10µg to 1mg for local applications. The antibody, or antibody moiety of the conjugate, may be an scFv, SIP or whole antibody. Where the antibody or antibody moiety is a whole antibody, it is preferably the IgG isotype, e.g. IgG1. This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Appropriate doses and regimens for anti-cancer compounds are well known in the art.

Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician.

Where an antibody (or antibody conjugate) and an anti-cancer compound are administered to a patient, these may be administered sequentially or simultaneously in accordance with any suitable regimen.

An antibody, antibody conjugate or anti-cancer compound may be administered to an individual in the form of a pharmaceutical composition, which may comprise at least one component in addition to the active compound. Where both an antibody (or antibody conjugate) and an anti-cancer compound are administered to a patient, these may be formulated in separate pharmaceutical compositions or, where appropriate, in the same pharmaceutical composition.

Suitable components include a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. intravenous.

Apart from antibodies, there are also other types of binding members which are also suitable for use in the present invention. Thus, in one example, a non-antibody binding member may be used in an embodiment of the present invention instead of an antibody. Suitable non-antibody binding members for use in the present invention may comprise an antigen-binding site, normally provided by one or more CDRs, e.g. a set of CDRs, in a non-antibody protein scaffold, as described in more detail below.

Terminology

Antibody

This describes an immunoglobulin whether natural or partly or wholly synthetically produced. The term also covers any polypeptide or protein comprising an antibody antigen-binding site.

5 Antibody fragments that comprise an antibody antigen-binding site include, but are not limited to, molecules such as Fab, Fab', Fab'-SH, scFv, Fv, dAb and Fd. Various other antibody molecules including one or more antibody antigen-binding sites have been engineered, including for example Fab₂, Fab₃, diabodies, triabodies, tetrabodies, minibodies and small immunoproteins (SIPs). Antibody molecules and methods for their construction
10 and use are described in Holliger & Hudson, *Nature Biotechnology* 23(9):1126-1136 2005.

It is possible to take monoclonal and other antibodies and use techniques of recombinant DNA technology to produce other antibodies or chimeric molecules that bind the target antigen. Such techniques may involve introducing DNA encoding the immunoglobulin
15 variable region, or the CDRs, of an antibody to the constant regions, or constant regions plus framework regions, of a different immunoglobulin. See, for instance, EP-A-184187, GB 2188638A or EP-A-239400, and a large body of subsequent literature. A hybridoma or other cell producing an antibody may be subject to genetic mutation or other changes, which may or may not alter the binding specificity of antibodies produced.

20

As antibodies can be modified in a number of ways, the term "antibody molecule" should be construed as covering any binding member or substance having an antibody antigen-binding site with the required specificity and/or binding to antigen. Thus, this term covers antibody fragments and derivatives, including any polypeptide comprising an antibody antigen-binding
25 site, whether natural or wholly or partially synthetic. Chimeric molecules comprising an antibody antigen-binding site, or equivalent, fused to another polypeptide (e.g. derived from another species or belonging to another antibody class or subclass) are therefore included. Cloning and expression of chimeric antibodies are described in EP-A-0120694 and EP-A-0125023, and a large body of subsequent literature.

30

Preferably, the antibody molecules used in the invention are human or humanised antibody molecules.

It has been shown that fragments of a whole antibody can perform the function of binding
35 antigens. Examples of binding fragments are (i) the Fab fragment consisting of VL, VH, CL and CH1 domains; (ii) the Fd fragment consisting of the VH and CH1 domains; (iii) the Fv

fragment consisting of the VL and VH domains of a single antibody; (iv) the dAb fragment (Ward, E.S. et al., Nature 341, 544-546 (1989); McCafferty et al (1990) Nature, 348, 552-554; Holt et al (2003) Trends in Biotechnology 21, 484-490), which consists of a VH or a VL domain; (v) isolated CDR regions; (vi) F(ab')₂ fragments, a bivalent fragment comprising two
 5 linked Fab fragments (vii) single chain Fv molecules (scFv), wherein a VH domain and a VL domain are linked by a peptide linker which allows the two domains to associate to form an antigen binding site (Bird et al, Science, 242, 423-426, 1988; Huston et al, PNAS USA, 85, 5879-5883, 1988); (viii) bispecific single chain Fv dimers (WO1993/011161) and (ix) "diabodies", multivalent or multispecific fragments constructed by gene fusion (WO94/13804;
 10 Holliger, P. et al, Proc. Natl. Acad. Sci. USA 90 6444-6448, 1993). Fv, scFv or diabody molecules may be stabilized by the incorporation of disulphide bridges linking the VH and VL domains (Reiter, Y. et al, Nature Biotech, 14, 1239-1245, 1996). A single chain Fv (scFv) may be comprised within a mini-immunoglobulin or small immunoprotein (SIP), e.g. as described in (Li et al., 1997). An SIP may comprise an scFv molecule fused to the CH₄
 15 domain of the human IgE secretory isoform IgE-S2 (ϵ_{S2} -CH₄; Batista et al., 1996) forming an homo-dimeric mini-immunoglobulin antibody molecule. Further, minibodies comprising a scFv joined to a CH₃ domain may also be made (Hu, S. et al, Cancer Res., 56, 3055-3061, 1996). Other examples of binding fragments are Fab', which differs from Fab fragments by the addition of a few residues at the carboxyl terminus of the heavy chain CH₁ domain,
 20 including one or more cysteines from the antibody hinge region, and Fab'-SH, which is a Fab' fragment in which the cysteine residue(s) of the constant domains bear a free thiol group.

Qui et al., *Nat. Biotechnol.* 25:921-929 (2007) described antibody molecules containing just
 25 two CDRs linked by a framework region. CDR3 from the VH or VL domain was linked to the CDR1 or CDR2 loop of the other domain. Linkage was through the C terminus of the selected CDR1 or CDR2 to the N terminus of the CDR3, via a FR region. Qui et al. selected the FR region having the fewest hydrophobic patches. The best combination for the antibody tested was found to be VL CDR1 linked by VH FR2 to VH CDR3 (VHCDR1-VHFR2-VLCDR3). At a molecular weight of around 3 kDa, these antibody molecules offer
 30 advantages in terms of improved tissue penetration as compared with full immunoglobulins (approx. 150 kDa) or scFv (approx. 28 kDa).

Antibody fragments of the invention can be obtained starting from a parent antibody
 35 molecule by methods such as digestion by enzymes e.g. pepsin or papain and/or by cleavage of the disulfide bridges by chemical reduction. In another manner, the antibody

fragments comprised in the present invention can be obtained by techniques of genetic recombination likewise well known to the person skilled in the art or else by peptide synthesis by means of, for example, automatic peptide synthesizers, such as those supplied by the company Applied Biosystems, etc., or by nucleic acid synthesis and expression.

- 5 Functional antibody fragments according to the present invention include any functional fragment whose half-life is increased by a chemical modification, especially by PEGylation, or by incorporation in a liposome.

A dAb (domain antibody) is a small monomeric antigen-binding fragment of an antibody, namely the variable region of an antibody heavy or light chain (Holt et al (2003) Trends in Biotechnology 21, 484-490). VH dAbs occur naturally in camelids (e.g. camel, llama) and may be produced by immunizing a camelid with a target antigen, isolating antigen-specific B cells and directly cloning dAb genes from individual B cells. dAbs are also producible in cell culture. Their small size, good solubility and temperature stability makes them particularly physiologically useful and suitable for selection and affinity maturation. Camelid VH dAbs are being developed for therapeutic use under the name "nanobodiesTM". An antibody molecule of the present invention may be a dAb. The antibody molecule comprise a VH or VL domain substantially as set out herein, or a VH or VL domain comprising a set of CDRs substantially as set out herein.

20

Bispecific or bifunctional antibodies form a second generation of monoclonal antibodies in which two different variable regions are combined in the same molecule (Holliger and Bohlen, Cancer and metastasis rev. 18: 411-419, 1999). Their use has been demonstrated both in the diagnostic field and in the therapy field from their capacity to recruit new effector functions or to target several molecules on the surface of tumour cells. Where bispecific antibodies are to be used, these may be conventional bispecific antibodies, which can be manufactured in a variety of ways (Holliger, P. and Winter G. Current Opinion Biotechnol 4, 446-449, 1993), e.g. prepared chemically or from hybrid hybridomas, or may be any of the bispecific antibody fragments mentioned above. These antibodies can be obtained by chemical methods (Glennie M J et al., 1987 J. Immunol. 139, 2367-2375; Repp R. et al., 1995 J. Hemat. 377-382) or somatic methods (Staerz U. D. and Bevan M. J. 1986 PNAS 83; Suresh M. R. et al., 1986 Method Enzymol. 121: 210-228) but likewise and preferentially by genetic engineering techniques which allow the heterodimerization to be forced and thus facilitate the process of purification of the antibody sought (Merchand et al., 1998 Nature Biotech. 16:677-681). Examples of bispecific antibodies include those of the BiTETM technology in which the binding domains of two antibodies with different specificity can be

used and directly linked via short flexible peptides. This combines two antibodies on a short single polypeptide chain. Diabodies and scFv can be constructed without an Fc region, using only variable domains, potentially reducing the effects of anti-idiotypic reaction.

- 5 Bispecific antibodies can be constructed as entire IgG, as bispecific Fab'2, as Fab'PEG, as diabodies or else as bispecific scFv. Further, two bispecific antibodies can be linked using routine methods known in the art to form tetravalent antibodies.

- 10 Bispecific diabodies, as opposed to bispecific whole antibodies, may also be particularly useful because they can be readily constructed and expressed in E.coli. Diabodies (and many other polypeptides, such as antibody fragments) of appropriate binding specificities can be readily selected using phage display (WO94/13804) from libraries. If one arm of the diabody is to be kept constant, for instance, with a specificity directed against the antigen of the tumour neovasculature, then a library can be made where the other arm is varied and an
15 antibody of appropriate specificity selected. Bispecific whole antibodies may be made by alternative engineering methods as described in Ridgeway et al., 1996 (Ridgeway, J. B. B. et al, Protein Eng., 9, 616-621, 1996).

- Various methods are available in the art for obtaining antibodies against a target antigen.
20 The antibodies may be monoclonal antibodies, especially of human, murine, chimeric or humanized origin, which can be obtained according to the standard methods well known to the person skilled in the art.

- In general, for the preparation of monoclonal antibodies or their functional fragments,
25 especially of murine origin, it is possible to refer to techniques which are described in particular in the manual "Antibodies" (Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor) or to the technique of preparation from hybridomas described by Köhler and Milstein (Köhler and Milstein, Nature, 256:495-497, 1975).

- 30 Monoclonal antibodies can be obtained, for example, from an animal cell immunised against the target antigen or one of its fragments containing the epitope recognised by the monoclonal antibodies. Suitable fragments and peptides or polypeptides comprising them are described herein, and may be used to immunise animals to generate antibodies against a target antigen. Said antigen, or one of its fragments, can especially be produced according
35 to the usual working methods, by genetic recombination starting with a nucleic acid sequence contained in the cDNA sequence coding for the antigen or fragment thereof, by

peptide synthesis starting from a sequence of amino acids comprised in the peptide sequence of the antigen and/or fragment thereof.

5 The monoclonal antibodies can, for example, be purified on an affinity column on which the antigen or one of its fragments containing the epitope recognised by said monoclonal antibodies, has previously been immobilised. More particularly, the monoclonal antibodies can be purified by chromatography on protein A and/or G, followed or not followed by ion-exchange chromatography aimed at eliminating the residual protein contaminants as well as the DNA and the LPS, in itself, followed or not followed by exclusion chromatography on
10 Sepharose gel in order to eliminate the potential aggregates due to the presence of dimers or of other multimers. In one embodiment, the whole of these techniques can be used simultaneously or successively.

15 In addition to antibody sequences and/or an antigen-binding site, an antibody for use in the present invention may comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Antibodies for use in the invention may carry a detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker). For example, an antibody may comprise a catalytic site (e.g. in an enzyme
20 domain) as well as an antigen binding site, wherein the antigen binding site binds to the antigen and thus targets the catalytic site to the antigen. The catalytic site may inhibit biological function of the antigen, e.g. by cleavage.

Non-antibody binding member

25 This describes one member of a pair of non-antibody molecules that bind one another. The members of a binding pair may be naturally derived or wholly or partially synthetically produced. One member of the pair of molecules has an area on its surface, or a cavity, which binds to and is therefore complementary to a particular spatial and polar organization of the other member of the pair of molecules. Examples of types of non-antibody binding pairs are biotin-avidin, hormone-hormone receptor, receptor-ligand, enzyme-substrate.
30

A non-antibody binding member normally comprises a molecule having an antigen-binding site. For example, a non-antibody binding member may be a non-antibody protein that comprises an antigen-binding site.

35 An antigen binding site may be provided by means of arrangement of complementarity determining regions (CDRs) on non-antibody protein scaffolds such as fibronectin or

cytochrome B etc. (Haan & Maggos, 2004; Koide 1998; Nygren 1997), or by randomising or mutating amino acid residues of a loop within a protein scaffold to confer binding specificity for a desired target. Scaffolds for engineering novel binding sites in proteins have been reviewed in detail by Nygren et al. (1997). Protein scaffolds for antibody mimics are disclosed in WO/0034784, in which the inventors describe proteins (antibody mimics) that include a fibronectin type III domain having at least one randomised loop. The scaffold may be a human or non-human protein. An advantage of a non-antibody protein scaffold is that it may provide an antigen-binding site in a scaffold molecule that is smaller and/or easier to manufacture than at least some antibody molecules. Small size of a binding member may confer useful physiological properties such as an ability to enter cells, penetrate deep into tissues or reach targets within other structures, or to bind within protein cavities of the target antigen. Use of antigen binding sites in non-antibody protein scaffolds is reviewed in Wess, 2004. Typical are proteins having a stable backbone and one or more variable loops, in which the amino acid sequence of the loop or loops is specifically or randomly mutated to create an antigen-binding site that binds the target antigen. Such proteins include the IgG-binding domains of protein A from *S. aureus*, transferrin, tetranectin, fibronectin (e.g. 10th fibronectin type III domain) and lipocalins as well as gamma-crystalline and other Affilin™ scaffolds (Scil Proteins). Examples of other approaches include synthetic "Microbodies" based on cyclotides - small proteins having intra-molecular disulphide bonds, Microproteins (Versabodies™, Amunix) and ankyrin repeat proteins (DARPin, Molecular Partners).

Similarly to antibodies, a non-antibody binding member for use in the present invention may, in addition to antibody sequences and/or an antigen-binding site, comprise other amino acids, e.g. forming a peptide or polypeptide, such as a folded domain, or to impart to the molecule another functional characteristic in addition to ability to bind antigen. Non-antibody binding members for use in the invention may carry a detectable label, or may be conjugated to a toxin or a targeting moiety or enzyme (e.g. via a peptidyl bond or linker). For example, a non-antibody binding member may comprise a catalytic site (e.g. in an enzyme domain) as well as an antigen binding site, wherein the antigen binding site binds to the antigen and thus targets the catalytic site to the antigen. The catalytic site may inhibit biological function of the antigen, e.g. by cleavage.

Complementary Determining Regions

As noted, CDRs can be carried by antibody and non-antibody scaffolds. The structures and locations of immunoglobulin variable domains may be determined by reference to Kabat 1987, and updates thereof. A number of academic and commercial on-line resources are

available to query this database. For example, see Martin (1996) and the associated on-line resource, currently at the web address of <http://www.bioinf.org.uk/abs/simkab.html>.

By CDR region or CDR, it is intended to indicate the hypervariable regions of the heavy and light chains of the immunoglobulin as defined by Kabat et al. (1987), (Kabat 1991a, and later editions). An antibody typically contains 3 heavy chain CDRs and 3 light chain CDRs. The term CDR or CDRs is used here in order to indicate, according to the case, one of these regions or several, or even the whole, of these regions which contain the majority of the amino acid residues responsible for the binding by affinity of the antibody for the antigen or the epitope which it recognizes.

Among the six short CDR sequences, the third CDR of the heavy chain (HCDR3) has a greater size variability (greater diversity essentially due to the mechanisms of arrangement of the genes which give rise to it). It can be as short as 2 amino acids although the longest size known is 26. CDR length may also vary according to the length that can be accommodated by the particular underlying framework. Functionally, HCDR3 plays a role in part in the determination of the specificity of the antibody (Segal 1974; Amit 1986; Chothia 1987; Chothia 1989; Caton 1990; Sharon 1990a; Sharon 1990b; Kabat et al., 1991b).

Antigen binding domain

This describes the part of a molecule that binds to and is complementary to all or part of the target antigen. In an antibody molecule it is referred to as the antibody antigen-binding site, and comprises the part of the antibody that binds to and is complementary to all or part of the target antigen. Where an antigen is large, an antibody may only bind to a particular part of the antigen, which part is termed an epitope. An antibody antigen-binding site may be provided by one or more antibody variable domains. An antibody antigen-binding site may comprise an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH).

WO2006/072620 describes engineering of antigen binding sites in structural (non-CDR) loops extending between beta strands of immunoglobulin domains. An antigen binding site may be engineered in a region of an antibody molecule separate from the natural location of the CDRs, e.g. in a framework region of a VH or VL domain, or in an antibody constant domain e.g. CH1 and/or CH3. An antigen binding site engineered in a structural region may be additional to, or instead of, an antigen binding site formed by sets of CDRs of a VH and VL domain. Where multiple antigen binding sites are present in an antibody molecule, they

may bind the same antigen (target antigen), thereby increasing valency of the antibody molecule. Alternatively, multiple antigen binding sites may bind different antigens (the target antigen and one or more another antigen), and this may be used to add effector functions, prolong half-life or improve *in vivo* delivery of the antibody molecule.

5 *Specific*

This may be used to refer to the situation in which one member of a specific binding pair will not show any significant binding to molecules other than its specific binding partner(s). For example, an antibody specific for the ED-A isoform of fibronectin may show little or no binding to other isoforms of fibronectin. An antibody specific for the ED-A domain of
10 fibronectin may show little or no binding to other domains of fibronectin. Similarly, an antibody specific for the tenascin C large isoform may show little or no binding to other isoforms of tenascin C. The term is also applicable where e.g. an antigen binding domain is specific for a particular epitope which is carried by a number of antigens, in which case the specific binding member carrying the antigen binding domain will be able to bind to the
15 various antigens carrying the epitope.

Comprise

This is generally used in the sense of include, that is to say permitting the presence of one or more features or components.

20 By "substantially as set out" it is meant that the relevant CDR or VH or VL domain of the invention will be either identical or highly similar to the specified regions of which the sequence is set out herein. By "highly similar" it is contemplated that from 1 to 5, preferably from 1 to 4 such as 1 to 3 or 1 or 2, or 3 or 4, substitutions may be made in the CDR and/or VH and/or VL domain.

25

The structure for carrying a CDR of the invention will generally be that of an antibody heavy or light chain sequence or substantial portion thereof in which the CDR is located at a location corresponding to the CDR of naturally occurring VH and VL antibody variable domains encoded by rearranged immunoglobulin genes. The structures and locations of
30 immunoglobulin variable domains and CDRs may be determined by reference to (Kabat, E.A. et al, Sequences of Proteins of Immunological Interest. 4th Edition. US Department of Health and Human Services. 1987, and updates thereof, now available on the Internet (<http://immuno.bme.nwu.edu>)).

Fibronectin

Fibronectin (FN) is a glycoprotein and is widely expressed in a variety of normal tissues and body fluids. It is a component of the extracellular matrix (ECM), and plays a role in many biological processes, including cellular adhesion, cellular migration, haemostasis,
5 thrombosis, wound healing, tissue differentiation and oncogenic transformation.

Fibronectin is subject to alternative splicing, and a number of alternative isoforms of fibronectin are known. Extra Domain-A (EDA or ED-A) is also known as ED, extra type III repeat A (EIIIA) or EDI. The sequence of human ED-A has been published by Kornblihtt *et al.* (1984), Nucleic Acids Res. 12, 5853-5868 and Paoletta *et al.* (1988), Nucleic Acids Res. 16, 3545-3557. The sequence of human ED-A (SEQ ID NO: 39) is also available on the SwissProt database as amino acids 1631-1720 (Fibronectin type-III 12; extra domain 2) of the amino acid sequence deposited under accession number P02751. The sequence of mouse ED-A is available on the SwissProt database as amino acids 1721-1810 (Fibronectin
15 type-III 13; extra domain 2) of the amino acid sequence deposited under accession number P11276.

The ED-A isoform of fibronectin (A-FN) contains the Extra Domain-A (ED-A). The sequence of the human A-FN can be deduced from the corresponding human fibronectin precursor
20 sequence which is available on the SwissProt database under accession number P02751. The sequence of the mouse A-FN can be deduced from the corresponding mouse fibronectin precursor sequence which is available on the SwissProt database under accession number P11276. The A-FN may be the human ED-A isoform of fibronectin. The ED-A may be the Extra Domain-A of human fibronectin.

25 ED-A is a 90 amino acid sequence which is inserted into fibronectin (FN) by alternative splicing and is located between domain 11 and 12 of FN (Borsi *et al.*, 1987, J. Cell Biol., 104, 595-600). ED-A is mainly absent in the plasma form of FN but is abundant during embryogenesis, tissue remodelling, fibrosis, cardiac transplantation and solid tumour growth.

Tenascin-C

30 Tenascin-C is a large hexameric glycoprotein of the extracellular matrix which modulates cellular adhesion. It is involved in processes such as cell proliferation and cell migration and is associated with changes in tissue architecture as occurring during morphogenesis and embryogenesis as well as under tumourigenesis or angiogenesis. A schematic
35 representation of the small (A) and large (B) tenascin-C isoform is shown in Figure 1.

Further information on the structure of tenascin-C is available in the UniProt database under accession number P24821, including amino acid sequence SEQ ID NO: 40 for tenascin C large isoform. An amino acid sequence of the tenascin-C domain A1 is shown as SEQ ID
5 NO: 42, encoded by SEQ ID NO: 41.

A strong over-expression of the large isoform of tenascin-C has been reported for a number of tumours, and monoclonal antibodies specific for domains A1 and D, respectively, have been extensively characterised in the clinic (Riva *et al.*, 1992; Riva *et al.*, 1995; Paganelli *et al.*, 1994; Reardon *et al.*, 2002; Bigner *et al.*, 1998).
10

Various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

15 "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

20 Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

25 Certain aspects and embodiments of the invention will now be illustrated by way of example.

Brief Description of the Drawings

Figure 1 shows a schematic representation of the small (A) and large (B) tenascin-C isoform. Several fibronectin type III like domains are subject to alternative splicing, either being included (B) or omitted (A) in the molecule. The amino acid sequence and encoding nucleotide sequence of tenascin C are publically available under sequence database references NP_002151.1 GI:4504549 (SEQ ID NO: 37) and NM_002160.1 GI:4504548 (SEQ ID NO: 38), respectively.
30

35

Figure 2 A and B show the results of immunohistochemical analyses of bone marrow biopsies from a patient with AML, stained with either the F8-SIP (V5L/K18R) (F8) or 4A1-F16-SIP (F16) antibody, as indicated. The negative control in each case is a bone marrow biopsy from the same patient stained with streptavidin-biotinylated alkaline phosphatase complex only. 4A1-F16-SIP strongly stained the blood vessels present in the bone marrow biopsy. Staining with F8-SIP (V5L/K18R) was also visible, although the level of staining was weaker than that observed with 4A1-F16-SIP. No staining was observed in the negative control. The size of the scale bar shown in Figure 1A is 100 μ m.

Figure 3 A and B show immunohistochemical analyses of bone marrow biopsies from two patients with extramedullar AML, stained with either the F8-SIP (V5L/K18R) (F8) or 4A1-F16-SIP (F16) antibody, as indicated. The negative control in each case is a bone marrow biopsy from the same patient stained with streptavidin-biotinylated alkaline phosphatase complex only. 4A1-F16-SIP strongly stained the blood vessels present in the bone marrow biopsies. Staining with F8-SIP (V5L/K18R) was also visible and was either similar or slightly weaker than the level of staining observed with 4A1-F16-SIP. No staining was observed in the negative control.

Figure 4 A shows immunohistochemical analyses of HL-60 tumours obtained from a human leukaemia mouse model stained with either the F8-SIP (V5L/K18R) (F8) or 4A1-F16-SIP (F16) antibody, as indicated. **B** shows immunohistochemical analyses of bone marrow biopsies obtained from the same mice as in A and also stained with either the F8-SIP (V5L/K18R) (F8) or 4A1-F16-SIP (F16) antibody, as indicated. Both antibodies strongly stained the vessels of the HL-60 tumour sections, while no staining was visible with either antibody in the sections of healthy bone marrow. The size of the scale bar shown in Figure 4 A and B is 100 μ m.

Experiments

The below experiments show that specific antigens, such as tenascin-C and the ED-A isoform of fibronectin, are expressed in bone marrow neovasculature, e.g. the neovasculature present in the bone marrow of leukaemia patients, in particular those with AML.

Materials and Methods*Antibodies*

4A1-F16-SIP is a human monoclonal mini-immunoglobulin specific to the A1 domain of tenascin-C. The sequence of the 4A1-F16-SIP antibody is shown in SEQ ID NO:25.

5

F8-SIP (V5L/K18R) is a human monoclonal mini-immunoglobulin specific to the alternatively spliced EDA domain of fibronectin. The sequence of the F8-SIP (V5L/K18R) antibody is shown in SEQ ID NO:26.

Bone Marrow Biopsies

- 10 Immunohistochemistry and immunofluorescence analysis were performed on freshly frozen bone marrow biopsies of patients with acute myeloid leukaemia.

Immunohistochemistry

- For immunohistochemistry, biotinylated antibodies were used in small immunoprotein format (SIP) under identical conditions (2 µg/ml). Aliquots were prepared from a single batch of antibodies, frozen and used only once to ensure reproducibility of immunohistochemical stainings. Frozen tissue samples were stored at -80°C. Sections of 10 µm thickness were fixed in chilled acetone, rehydrated in TBS buffer (50 mM Tris, 100 mM NaCl, 0.001% Aprotinin, pH 7.4) and blocked with 20% fetal calf serum in TBS. The antibodies were added onto the sections in a final concentration of 2 µg/mL in 3% bovine serum albumin (BSA)/TBS solution and incubated for one hour. After washing in TBS, bound antibodies were detected with streptavidin-biotinylated alkaline phosphatase complex (Biospa, Milan, Italy) in TBS 3% BSA + 2 mM MgCl₂. The Fast Red substrate (Sigma) was used for detection of phosphatase activity. Sections were counterstained with Gill's hematoxylin No.2 (Sigma) and mounted with Glycergel mounting medium (Dako, Glostrup, Denmark).

25 *Multicolour Immunofluorescence Studies*

Biotinylated antibodies were used in small immunoprotein format (SIP) under identical conditions (2 µg/ml). Aliquots were prepared from a single batch of antibodies, frozen and used only once to ensure reproducibility of immunohistochemical stainings.

Sections of 10 µm thickness were fixed in chilled acetone and blocked with 20% fetal calf serum in PBS. Biotinylated F8-SIP (V5L/K18R) and 4A1-F16-SIP were added onto the sections in a final concentration of 2 µg/mL in 3% bovine serum albumin (BSA)/PBS solution and incubated for one hour. Mouse anti-human vWF (von Willebrandt factor) was used to
5 outline endothelial cells. After washing in PBS, bound primary antibodies were detected with Streptavidin-Alexa Fluor 488 and anti-mouse IgG Alexa Fluor 594 (Invitrogen) were used as secondary antibodies. Nuclei were counterstained with DAPI and images were captured on an Axioskop 2 Mot plus microscope equipped with an AxioCam MRc camera (Zeiss). Human Leukaemia Mouse Model

10 The mouse model for human leukaemia used here was previously described in Potter *et al.* (1984). Specifically, nude mice were xenografted with cells from the HL-60 leukaemia cell line and, after development of HL-60 tumours (granulocytic sarcomas), samples were obtained both from said tumours and from the bone marrow of the mice. Immunohistochemistry studies were then performed as described under
15 "immunohistochemistry" above.

Results

Immunohistochemistry analysis showed that antibody 4A1-F16-SIP was capable of staining the vast majority of blood vessels in the bone marrow of AML patients. Antibody F8-SIP (V5L/K18R) also stained a large proportion of these blood vessels but fewer than observed
20 with the 4A1-F16-SIP antibody. These results are shown in Figure 2 A and B.

Similar results were obtained when bone marrow biopsies from two patients with extramedullary AML were subjected to immunohistochemistry analysis. Antibody 4A1-F16-SIP strongly stained the blood vessels present in the bone marrow biopsies in both cases.
25 The level of staining observed with antibody F8-SIP (V5L/K18R) was similar to that observed using antibody 4A1-F16-SIP in one biopsy (Figure 3B) but weaker in the other (Figure 3A).

The differences in the level of staining observed with antibodies F8-SIP (V5L/K18R) and 4A1-F16-SIP may be due to differences in the level of expression of domain A1 of tenascin-
30 C relative to the ED-A isoform of fibronectin in the bone marrow blood vessels of AML patients.

Multicolour immunofluorescence studies of areas of bone marrow from AML patients with high blast densities further showed an excellent co-localization of antibody 4A1-F16-SIP with
35 antibodies specific for von Willebrand Factor (vWF).

Antibodies 4A1-F16 SIP and the F8-SIP (V5L/K18R) also stained sections HL-60 tumours obtained from a mouse model of human leukaemia. Specifically, both of antibodies strongly stained the vessels present in the HL-60 tumour (granulocytic sarcoma) sections, while no
5 staining was visible in the sections of healthy bone marrow obtained from the same mice (Figure 4).

These results show for the first time that antigens exist which are differentially expressed in the bone marrow neovasculature, in particular the bone marrow neovasculature of
10 leukaemia patients, compared to normal tissues. The results also show that the same antigens are also differentially expressed in the neovasculature of tumours formed by leukaemic cells, such as granulocytic sarcomas, compared to normal tissues. These antigens therefore represent attractive targets for the development of selective and efficient pharmacodelivery strategies in the treatment of diseases characterised by bone marrow
15 neovasculature, such as leukaemia. In particular, as targets present in vasculature are often more easily accessible from the bloodstream to systemically administered agents, overcoming the problem of access and allowing an efficient delivery of the compound to the site of disease.

20 For example, the antigens expressed in bone marrow neovasculature, such as the bone marrow neovasculature of leukaemia patients, can be targeted using antibodies capable of binding to said antigens. By conjugating bioactive agents to said antibodies, the bioactive agents can be delivered directly to the bone marrow neovasculature. Selective targeting of the bioactive agent to the site of disease will ultimately result in an increased local
25 concentration at its site of action, thus reducing or eliminating the exposure of normal tissues to any toxic effects of the bioactive agent used. Such a targeted delivery can improve the therapeutic index of the delivered bioactive agent by providing a higher efficacy with minimized side effects. In addition, the favourable toxicity profile of site-specific therapeutics may open new avenues in the therapy of diseases characterized by bone marrow
30 neovasculature, such as leukaemia, by allowing the systemic administration of highly potent and promising agents, which are currently either given at suboptimal doses or whose clinical application has to date been impeded by unacceptable toxicities when applied in an unmodified form.

Sequences – 4A1-F16 Antibody

SEQ ID NO: 1. 4A1-F16 VH domain nucleotide sequence

GAG GTG CAG CTG TTG GAG TCT GGG GGA GGC TTG GTA CAG CCT GGG GGG
TCC CTG AGA CTC TCC TGT GCA GCC TCT GGA TTC ACC TTT AGC CGG TAT GGT
5 ATG AGC TGG GTC CGC CAG GCT CCA GGG AAG GGG CTG GAG TGG GTC TCA
GCT ATT AGT GGT AGT GGT GGT AGC ACA TAC TAC GCA GAC TCC GTG AAG GGC
CGG TTC ACC ATC TCC AGA GAC AAT TCC AAG AAC ACG CTG TAT CTG CAA ATG
AAC AGC CTG AGA GCC GAG GAC ACG GCC GTA TAT TAC TGT GCG AAA GCG CAT
AAT GCT TTT GAC TAC TGG GGC CAG GGA ACC CTG GTC ACC GTG TCG AGA

10 SEQ ID NO: 2 4A1-F16 VH domain amino acid sequence

EVQLLES GGG LVQPGGSLRL SCAASGFTFS RYGMSWVRQA PGKGLEWWSA
ISGSGGSTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYYCAKAH
NAFDYWGQGT LVTVSR

SEQ ID NO: 3 4A1-F16 VL domain nucleotide sequence

15 TCT TCT GAG CTG ACT CAG GAC CCT GCT GTG TCT GTG GCC TTG GGA CAG ACA
GTC AGG ATC ACA TGC CAA GGA GAC AGC CTC AGA AGC TAT TAT GCA AGC TGG
TAC CAG CAG AAG CCA GGA CAG GCC CCT GTA CTT GTC ATC TAT GGT AAA AAC
AAC CGG CCC TCA GGG ATC CCA GAC CGA TTC TCT GGC TCC AGC TCA GGA AAC
ACA GCT TCC TTG ACC ATC ACT GGG GCT CAG GCG GAA GAT GAG GCT GAC TAT
20 TAC TGT AAC TCC TCT GTT TAT ACT ATG CCG CCC GTG GTA TTC GGC GGA GGG
ACC AAG CTG ACC GTC CTA

SEQ ID NO: 4 4A1-F16 VL domain amino acid sequence

SSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNRPSGIPDR
FSGSSSGNTASLTITGAQAEDEADYYCNSSVYTMPPVVFGGGTKLTVL

25 SEQ ID NO: 5 4A1-F16 VH CDR1 amino acid sequence

RYGMS

SEQ ID NO: 6 4A1-F16 VH CDR2 amino acid sequence

AISGSGGSTYYADSVKG

SEQ ID NO: 7 4A1-F16 VH CDR3 amino acid sequence

30 AHNAFDY

SEQ ID NO: 8 4A1-F16 VL CDR1 amino acid sequence

QGDSLRSYYAS

SEQ ID NO: 9 4A1-F16 VL CDR2 amino acid sequence

GKNNRPS

5 SEQ ID NO: 10 4A1-F16 VL CDR3 amino acid sequence

NSSVYTMPPVV

SEQ ID NO: 11 4A1-F16 VH and VL domain peptide linker amino acid sequence

GGSGGGSGG

Sequences – F8 (V5L/K18R) Antibody

10 SEQ ID NO:12 F8 (V5L) VH domain nucleotide sequence

GAGGTGCAGCTGTTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGAGA
CTCTCCTGTGCAGCCTCTGGATTACCTTTAGCCTGTTTACGATGAGCTGGGTCCGCCA
GGCTCCAGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCAC
ATACTACGCAGACTCCGTGAAGGGCCGGTTCACCATCTCCAGAGACAATTCCAAGAAC
15 ACGCTGTATCTGCAAATGAACAGCCTGAGAGCCGAGGACACGGC

SEQ ID NO:13 F8 (V5L) VH domain amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWWSAISGSGGSTYY
ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSS

SEQ ID NO:14 F8 (K18R) VL domain nucleotide sequence

20 GAAATTGTGTTGACGCAGTCTCCAGGCACCCTGTCTTTGTCTCCAGGGGAAAGAGCCA
CCCTCTCCTGCAGGGCCAGTCAGAGTGTTAGCATGCCGTTTTTAGCCTGGTACCAGCA
GAAACCTGGCCAGGCTCCCAGGCTCCTCATCTATGGTGCATCCAGCAGGGCCACTGG
CATCCCAGACAGGTTTCAGTGGCAGTGGGTCTGGGACAGACTTCACTCTCACCATCAGC
AGACTGGAGCCTGAAGATTTTGCAGTGTATTACTGTCAGCAGATGCGTGGTCGGCCGC
25 CGACGTTCGGCCAAGGGACCAAGGTGGAAATCAAA

SEQ ID NO:15 F8 (K18R) VL domain amino acid sequence

EIVLTQSPGTLSPGERATLSCRASQSVSMPLAWYQQKPGQAPRLLIYGASSRATGIPD
RFSGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIK

SEQ ID NO:16 F8 (V5L) VH CDR1 amino acid sequence

LFT

SEQ ID NO:17 F8 (V5L) VH CDR2 amino acid sequence

SGSGGS

5 *SEQ ID NO:18 F8 (V5L) VH CDR3 amino acid sequence*

STHLYL

SEQ ID NO:19 F8 (K18R) VL CDR1 amino acid sequence

MPF

SEQ ID NO:20 F8 (K18R) VL CDR2 amino acid sequence

10 GASSRAT

SEQ ID NO:21 F8 (K18R) VL CDR3 amino acid sequence

MRGRPP

SEQ ID NO: 22 F8 (V5L/K18R) VH and VL domain peptide linker amino acid sequence

GGGSGGGSGGGGG

15 *SEQ ID NO: 23 4A1-F16-SIP and F8-SIP (V5L/K18R) VL and CH4 domain peptide linker amino acid sequence*

SG

SEQ ID NO: 24 amino acid sequence of CH4 dimerization domain of 4A1-F16-SIP and F8-SIP (V5L/K18R)

20 GSGGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQ
PRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPRESSRRGGC

SEQ ID NO: 25 4A1-F16-SIP amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSRYGMSWVRQAPGKGLEWWSAISGSGGSTY
YADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKAHNAFDYWGQGTLVTVSRGGG
25 SGGGSGGSSELTQDPAVSVALGQTVRITCQGDSLRSYYASWYQQKPGQAPVLVIYGKNNR
PSGIPDRFSGSSSGNTASLTITGAQAEDEADYYCNSSVYTMPPVVFSGGGTKLTVLSGGSGG
PRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTTQPRKT
KGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPRESSRRGGC

SEQ ID NO: 26 F8-SIP (V5L/K18R) amino acid sequence

EVQLLESGGGLVQPGGSLRLSCAASGFTFSLFTMSWVRQAPGKGLEWVSAISGSGGSTYY
 ADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKSTHLYLFDYWGQGTLVTVSSGG
 GGSGGGSGGGGGGEIVLTQSPGTL SLSPGERATLSCRASQSVSMPFLAWYQQKPGQAPRL
 5 LIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQMRGRPPTFGQGTKVEIKS
 GGSGGPRAAPEVYAFATPEWPGSRDKRTLACLIQNFMPEDISVQWLHNEVQLPDARHSTT
 QPRKTKGSGFFVFSRLEVTRAWEQKDEFICRAVHEAASPSQTVQRAVSVNPESRRGGC

Sequences - Interleukin 2

SEQ ID NO: 27 hIL2 precursor sequence (mature hIL2: residues 7-150)

10 MYRMQLLSCI ALSLALVTNS APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML
 TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN VIVLELKGSE
 TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT

SEQ ID NO: 28 IL-2 linker sequence

GGGGSGGGGGSGGGG

15 *SEQ ID NO: 29 IL-2 linker sequence*

GGGGSGGGGGSGGGGS

SEQ ID NO: 30 IL-2 linker sequence

SSSSGSSSSGSSSSG

SEQ ID NO: 31 IL-2 linker sequence

20 GSGSAGSGSAGSGSA

SEQ ID NO: 32 IL-2 linker sequence

GGSGGGGGSGGGGGSGG

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CLAIMS:

1. A use of an antibody for targeting an antigen of the bone marrow
5 neovasculature in acute myeloid leukemia patients, wherein the antigen is the A1 domain of tenascin-C large isoform and the antibody competes for binding to tenascin-C with an antibody comprising the 4A1-F16 VH domain of SEQ ID NO:2 and the 4A1-F16 VL domain of SEQ ID NO:4.
- 10 2. The use according to claim 1, wherein said antibody for targeting an antigen of the bone marrow neovasculature in acute myeloid leukemia patients is conjugated to a bioactive molecule selected from the group consisting of a cytokine, cytotoxic agent, photosensitizer, and a therapeutic radioisotope.
- 15 3. The use according to claim 2, wherein said antibody for targeting an antigen of the bone marrow neovasculature in acute myeloid leukemia patients is conjugated to the bioactive molecule via a cleavable linker.
4. The use according to claim 1 wherein said antibody for targeting an antigen
20 of the bone marrow neovasculature in acute myeloid leukemia patients is for use with an anti-cancer compound.
5. The use according to claim 2, wherein said antibody for targeting an antigen
25 of the bone marrow neovasculature in acute myeloid leukemia patients is for use with an anti-cancer compound.
6. The use according to claim 1, wherein said antibody for targeting an antigen
of the bone marrow neovasculature in acute myeloid leukemia patients comprises
an antigen binding site comprising a VH domain and a VL domain, the VH domain
30 comprising a VH CDR1 of SEQ ID NO:5, a VH CDR2 of SEQ ID NO:6 and a VH

CDR3 of SEQ ID NO:7, and the VL domain comprising a VL CDR1 of SEQ ID NO:8, a VL CDR2 of SEQ ID NO:9 and a VL CDR3 of SEQ ID NO:10.

7. The use according to claim 1, wherein said antibody for targeting an antigen of the bone marrow neovasculature in acute myeloid leukemia patients comprises an antigen binding site comprising a VH domain and a VL domain, wherein the VH domain is the 4A1-F16 VH domain of SEQ ID NO: 2 and the VL domain is the 4A1-F16 VL domain of SEQ ID NO:4.

8. The use according to claim 1, wherein said antibody for targeting an antigen of the bone marrow neovasculature in acute myeloid leukemia patients is a small immunoprotein (SIP), scFv, or whole IgG molecule, said antibody binding the A1 domain of tenascin C large isoform.

9. The use as claimed in claim 5, wherein said bioactive molecule is IL-2 and said anti-cancer compound is selected from the group consisting of anthracyclines, cytarabine, vincristine, L-asparaginase, cyclophosphamide, methotrexate, 6-mercaptopurine, chlorambucil, cyclophosphamide, a corticosteroid, prednisone, prednisolone, imatinib, cladribine, pentostatin, rituximab, and doxorubicin.

10. The use as claimed in claim 9, wherein said anticancer agent is cytarabine.

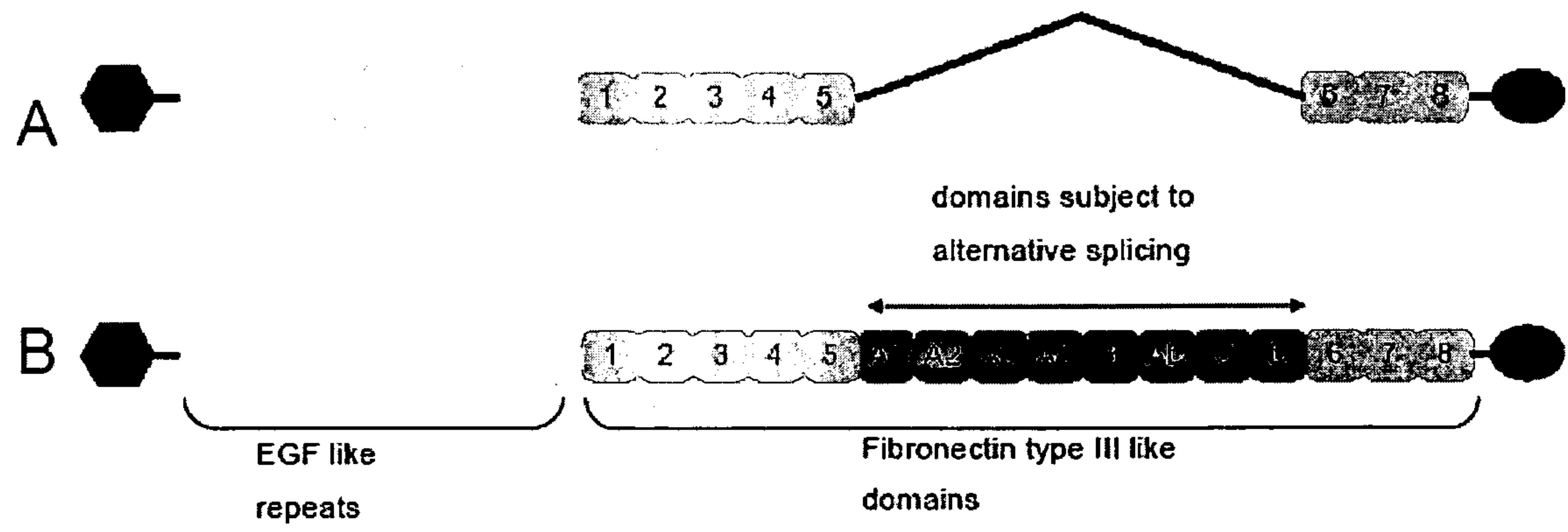
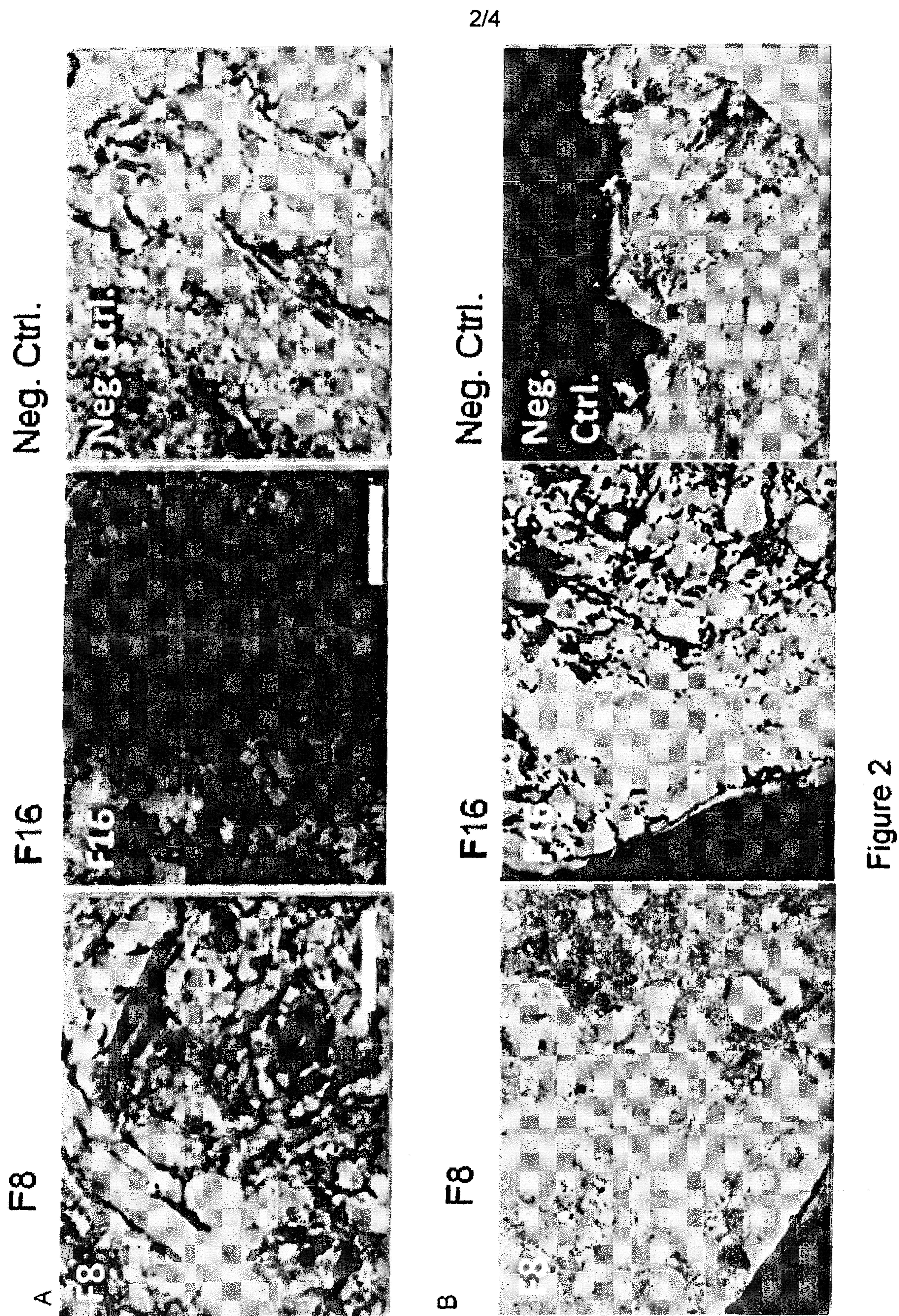


Figure 1



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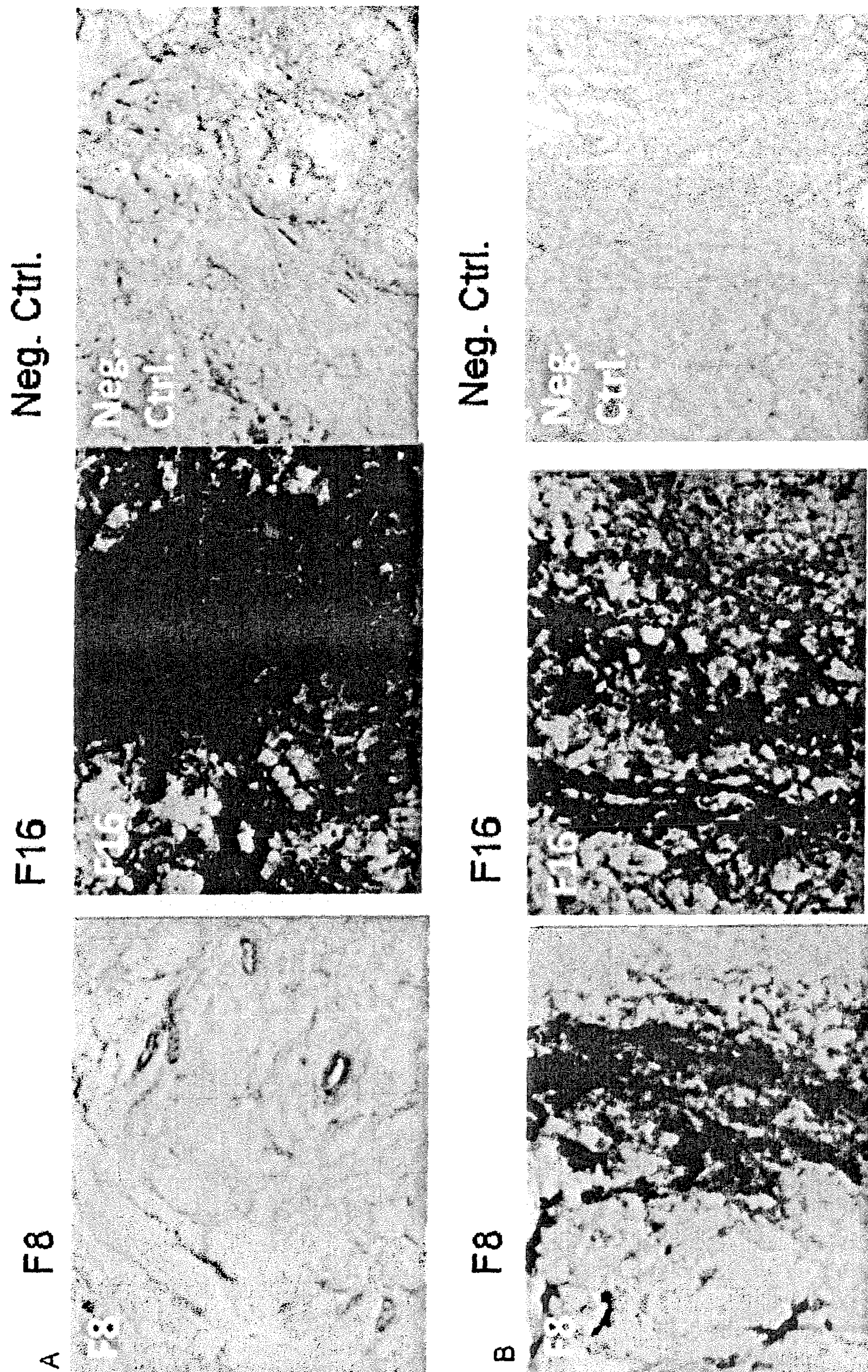


Figure 3

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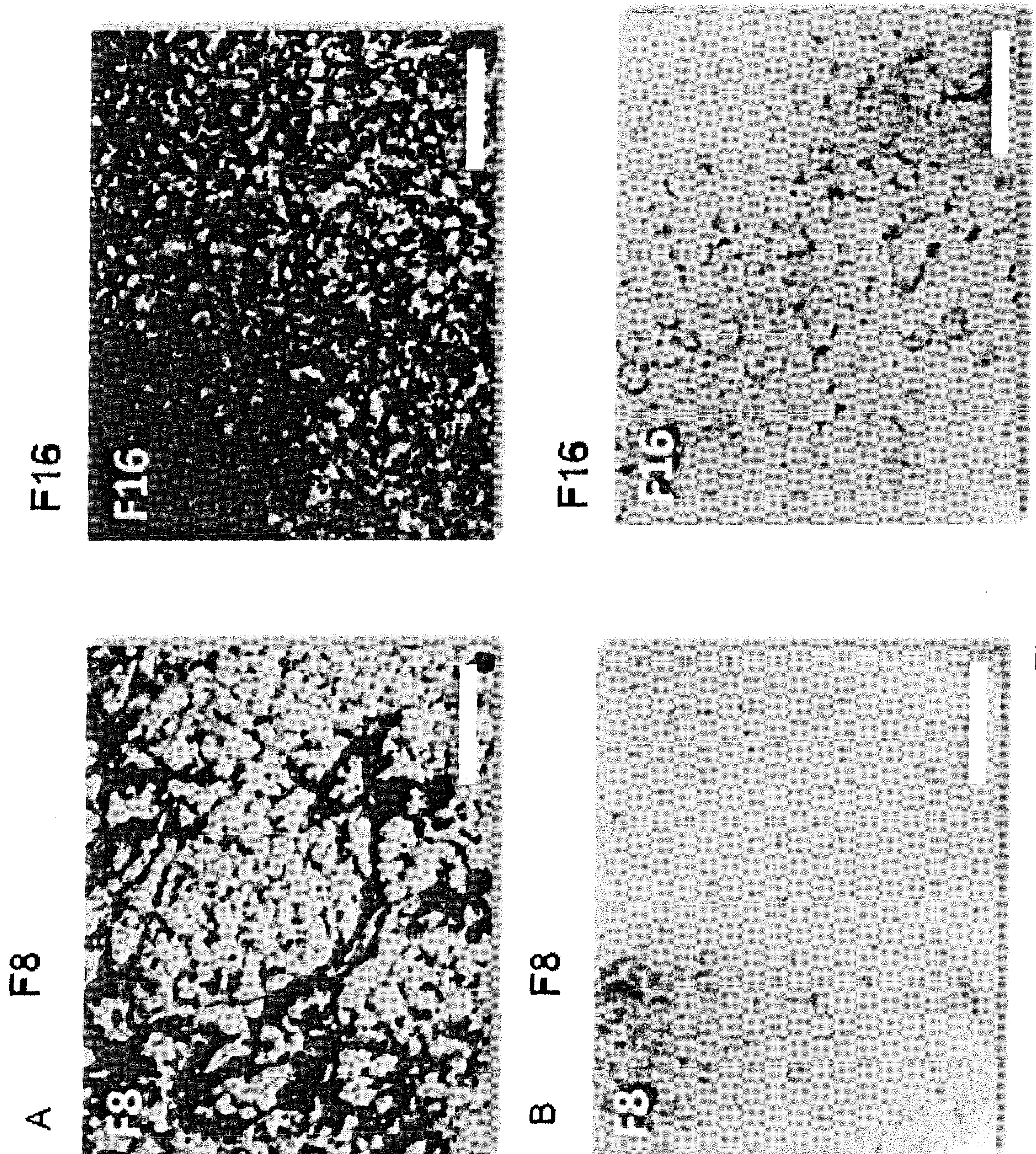


Figure 4