Title Immunoglobulin-like molecules directed against fibronectin-EDA

Field of the invention

The present invention is in the field of medicine, in particular in the field of cardiology and angiogenesis. The invention provides immunoglobulin (Ig)-like molecules, such as antibodies, and antigen-binding fragments thereof that bind specifically to the EDA-domain of fibronectin-EDA. These Ig-like molecules are particularly suited for treating, preventing or preventing the progression of adverse cardiac or vascular remodelling and myocardial infarction-related complications and for improving or stimulating angiogenesis. The invention also relates to pharmaceutical compositions comprising the Ig-like molecules, e.g., antibodies, or antigen-binding or fragments thereof and methods using the Ig-like molecules, e.g., antibodies, or antigen-binding or fragments thereof for treating, preventing or preventing the progression of myocardial infarction-related complications and adverse cardiac or vascular remodelling and for improving or stimulating angiogenesis.

Background of the invention

Ischemic heart disease is the largest socio-economic burden to Western societies. It becomes an even bigger problem in this era of rapid modernization of developing countries like China and India. The most severe and acute complication of ischemic heart disease is a heart attack, also known as myocardial infarction. In the USA, EU and Japan, 2.4 million patients suffer from a myocardial infarction each year. The amount of money spent in the USA and the EU only for the treatment of ischemic heart disease exceeds €150 billion every year. Unfortunately, myocardial infarction-related complications or conditions are increasing because more patients survive the initial life-threatening infarction, but have progressively worse cardiac function afterwards. Complications after myocardial infarction such as heart failure, fibrosis and arrhythmia result in high mortality rates and morbidity. The most important determinant of these complications is an improper cardiac repair response, referred to as adverse (cardiac) remodelling or adverse ventricular remodelling.

Heart failure (HF) has gained much attention, as it is the most severe and most frequent consequence of adverse remodelling after myocardial infarction. The European Society of Cardiology (ESC) stated that "HF is the epidemic of the 21st century in Western societies". In the USA, EU and Japan alone, at least 1.8 million patients are hospitalized with newly diagnosed infarction-related HF each year. The mortality rate is 20% within a year from diagnosis, while 50% die within 5 years. Quality of life of those that survive is

severely affected as they suffer from progressively decreasing exercise tolerance and reduced capacity to conduct normal daily activities. The socio-economic burden is nearly €60 billion annually for the USA and EU only, as a consequence of 1) the reduced exercise tolerance and subsequent reduced productivity, 2) expensive medical treatments that are not preventive or curative but decrease symptoms and 3) rehospitalisation. Current therapy for myocardial infarction aims at restoring blood flow through the occluded coronary artery. Anti-thrombotics (i.e., agents preventing blood clot formation) together with stents are the most important drug and device classes to optimize blood flow restoration after myocardial infarction. Despite these advances in blood flow optimization, infarction-related complications still occur and are increasing. The main reason is the fact that adverse remodelling is a completely different pathophysiological process than is blood flow restoration.

The healing of the infarcted heart is a very complex process involving many types of cells. Myocardial infarction is an acute event in which part of the heart muscle dies resulting in loss of pump function. Immediately after this acute event, repair processes are induced in the blood and the heart muscle, characterized by enhanced inflammation. However, the type of inflammation determines whether the infarcted heart is repaired and remodelled properly. The key factor that drives improper healing and deleterious inflammation is the activation of innate immunity by molecules related to cardiac death and matrix degradation. In many patients, the immune system becomes activated in a detrimental way, resulting in inappropriate healing of the heart after myocardial infarction. In those cases, the heart will enter a process called adverse remodelling. Adverse remodelling has several deleterious consequences: heart failure, dilatation and fibrosis of the heart, disturbed contractility and relaxation, and disturbed electrical activation are known complications. The increasing incidence of infarction-related morbidity, like heart failure, emphasizes the need for novel therapeutics to enhance cardiac repair after infarction. Another factor contributing to healing of the infarcted heart, in particular in the early stage following myocardial infarction, is angiogenesis. De novo formation of microvessels has the potential to recover ischemic myocardium at early stages after myocardial information, contributes to prevent the transition to heart failure.

The main determinant for leukocytes to cause a deleterious inflammatory reaction is the deposition of fibronectin-EDA. After myocardial infarction, fibronectin-EDA is newly synthesized and transiently upregulated in the infarcted myocardium. Fibronectin-EDA can activate the immune system and other cells involved in matrix turnover, thereby inducing the migration and differentiation of cells involved in cardiac repair (e.g.

leukocytes, lymphocytes and fibroblasts). Subsequently, cells activated by fibronectin-EDA induce detrimental inflammatory reactions in the healing heart.

Cellular fibronectin is a multifunctional adhesive glycoprotein present in the ECM and is produced by cells in response to tissue injury as occurs with MI. It contains an alternatively spliced exon encoding type III repeat extra domain A (EIIIA; EDA), that acts as an endogenous ligand for both TLR2 and TLR4 and integrin α4β1, α4β7 and α9β1. *In vitro*, fibronectin-EDA induces pro-inflammatory gene expression and activates monocytes. *In vivo* injection of fibronectin-EDA in murine joints results in enhanced inflammation. Fibronectin-EDA is not normally expressed in healthy human tissue, but is highly upregulated in newly developing vasculature during embryogenesis and in several (pathological) conditions such as (cardiac) ischemic tissue, atherosclerotic lesions, fibrotic tissue, tumors, transplant rejection and wounds. Overexpression of EDA results in enhanced inflammation and injury after brain ischemia. Thus, fibronectin-EDA is capable of activating leukocytes and cause an upregulation of cytokines and chemokines. It was recently shown that fibronectin-EDA knockout mice exhibited reduced fibrosis, preserved cardiac function and reduced ventricular dilatation compared to wild-type mice after myocardial infarction (Arslan F. et al. Circ. Res., Mar 2011; 108: 582 – 592). WO2012/057613 describes that treatment of mice with antibodies directed to the EDA domain of fibronectin-EDA prevents left ventricular dilatation in said mice and improves survival after myocardial infarction.

There remains a need in the art for antibodies that are capable of treating, preventing, or preventing the progression of myocardial infarction-related conditions in a subject and that increase the chance of survival of a subject after myocardial infarction. It is an object of the present invention to provide for such antibodies.

Summary

The present disclosure describes an isolated immunoglobulin (Ig)-like molecule or antigen-binding fragment thereof specifically binding to an amino acid sequence GIXXXF (SEQ ID NO:1), wherein X may be any amino acid. In one aspect, the present invention refers to an antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising a heavy chain variable region comprising: a CDRl having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A; a CDR2 having the amino acid sequence YIHX$_2$SGX3ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I; and a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y; and a light chain variable region

comprising: a CDRl having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T; a CDR2 having the amino acid sequence KVSNRFS; and a CDR3 having the amino acid sequence X$_{10}$QX$_{11}$X$_{12}$HVPPT, wherein X$_{10}$ is selected from S and F, X$_{11}$ is selected from S and G, and X$_{12}$ is selected from A and S. In another aspect, the present invention refers to an antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising a heavy chain variable region comprising: a CDRl having the amino acid sequence shown in SEQ ID NO:9; a CDR2 having the amino acid sequence shown in SEQ ID NO:10; and a CDR3 having the amino acid sequence shown in SEQ ID NO:11; and a light chain variable region comprising: a CDRl having the amino acid sequence shown in SEQ ID NO:12; a CDR2 having the amino acid sequence shown in SEQ ID N0:13; and a CDR3 having the amino acid sequence shown in SEQ ID NO:14. In another aspect, the present invention refers to an antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising a heavy chain variable region comprising: a CDR1 having the amino acid sequence shown in SEQ ID NO:3; a CDR2 having the amino acid sequence shown in SEQ ID NO:4; and a CDR3 having the amino acid sequence shown in SEQ ID NO:5; and a light chain variable region comprising: a CDRl having the amino acid sequence shown in SEQ ID NO:6; a CDR2 having the amino acid sequence shown in SEQ ID NO:7; and a CDR3 having the amino acid sequence shown in SEQ ID NO:8. In yet another aspect, the present invention refers to an antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising a heavy chain variable region comprising: a CDR1 having the amino acid sequence shown in SEQ ID NO:15; a CDR2 having the amino acid sequence shown in SEQ ID NO:16; and a CDR3 having the amino acid sequence shown in SEQ ID NO:17; and a light chain variable region comprising: a CDRl having the amino acid sequence shown in SEQ ID NO: 18; a CDR2 having the amino acid sequence shown in SEQ ID NO: 19; and a CDR3 having the amino acid sequence shown in SEQ ID NO:20.

In an example, the amino acid at position 3 of SEQ ID NO:1 is selected from histidine, arginine, lysine and alanine, and preferably is histidine.

In an example, the amino acid at position 4 of SEQ ID NO: 1 is selected from glutamic acid and alanine, and preferably is glutamic acid.

In an example, the amino acid at position 5 of SEQ ID NO:1 is selected from leucine and alanine, and preferably is leucine.

In an example, the Ig-like molecule or fragment thereof specifically binds to an amino acid sequence GIHELF (SEQ ID NO:2).

The present disclosure further disclosesan isolated immunoglobulin (Ig)-like molecule or antigen-binding fragment thereof specifically binding to an amino acid sequence LFPAP (SEQ ID NO:28).

The Ig-like molecule or antigen-binding fragment thereof may be an antibody, e.g., a monoclonal antibody. The Ig-like molecule or antigen-binding fragment thereof may be of murine origin, e.g., may be derived from a mouse. The Ig-like molecule or antigen-binding fragment thereof may be chimeric, humanized or human.

The disclosure also pertains to an Ig-like molecule or antigen-binding fragment thereof comprising a heavy chain variable region comprising a complementarity determining region (CDR)1 having the amino acid sequence shown in SEQ ID NO:3 or an amino acid sequence as shown in SEQ ID NO:3, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:4 or an amino acid sequence as shown in SEQ ID NO:4, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence as shown in SEQ ID NO:5, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted.

In an example, the present disclosure describes an Ig-like molecule or antigen-binding fragment thereof comprising a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A;
- a CDR2 having the amino acid sequence YIHX$_2$SGX$_3$ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I;
- a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y.

The present disclosure also describes an Ig-like molecule or antigen-binding fragment thereof comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:6 or an amino acid sequence as shown in SEQ ID NO:6, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:7 or an amino acid sequence as shown in SEQ ID NO:7, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:8 or an amino acid sequence as shown in SEQ ID NO:8, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted.

In an example, there is disclosed an Ig-like molecule or antigen-binding fragment thereof comprising a light chain variable region comprising:

- a CDR1 having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T;
- a CDR2 having the amino acid sequence KVSNRFS;
- a CDR3 having the amino acid sequence X$_{10}$QX$_{11}$X$_{12}$HVPPT, wherein X$_{10}$ is selected from S and F, X$_{11}$ is selected from S and G, and X$_{12}$ is selected from A and S.

The present disclosure further describes an Ig-like molecule or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:3 or an amino acid sequence as shown in SEQ ID NO:3, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:4 or an amino acid sequence as shown in SEQ ID NO:4, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence as shown in SEQ ID NO:5, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted; and a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:6 or an amino acid sequence as shown in SEQ ID NO:6, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:7 or an amino acid sequence as shown in SEQ ID NO:7, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:8 or an amino acid sequence as shown in SEQ ID NO:8, wherein at most 3, preferably at most 2, more preferably at most 1 amino acid is substituted.

In an example, such Ig-like molecule or antigen-binding fragment comprises a heavy chain variable region comprising

- a CDR1 having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A;
- a CDR2 having the amino acid sequence YIHX$_2$SGX$_3$ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I;
- a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T;
- a CDR2 having the amino acid sequence KVSNRFS;

- a CDR3 having the amino acid sequence $X_{10}QX_{11}X_{12}HVPPT$, wherein X_{10} is selected from S and F, X_{11} is selected from S and G, and X_{12} is selected from A and S.

In one example, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:9;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:10; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:11;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:12;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:13; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:14.

Preferably, said Ig-like molecule, antibody or antigen-binding fragment thereof comprises a humanized heavy chain variable region of the heavy chain variable region having the amino acid sequence of SEQ ID NO:40 and a humanized light chain variable region of the light chain variable region having the amino acid sequence of SEQ ID NO:41.

In another example, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:3;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:4; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:5;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:6;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:7; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:8.

Preferably, said Ig-like molecule, antibody or antigen-binding fragment thereof comprises a humanized heavy chain variable region of the heavy chain variable region having the amino acid sequence of SEQ ID NO:38 and a humanized light chain variable region of the light chain variable region having the amino acid sequence of SEQ ID NO:39.

In yet another example, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:15;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:16; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:17;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:18;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:19; and

- a CDR3 having the amino acid sequence shown in SEQ ID NO:20.

Preferably, said Ig-like molecule, antibody or antigen-binding fragment thereof comprises a humanized heavy chain variable region of the heavy chain variable region having the amino acid sequence of SEQ ID NO:42 and a humanized light chain variable region of the light chain variable region having the amino acid sequence of SEQ ID NO:43.

The present disclosure further describes an Ig-like molecule or antigen-binding fragment thereof comprising a heavy chain variable region comprising a complementarity determining region (CDR)1 having the amino acid sequence shown in SEQ ID NO:29 or an amino acid sequence as shown in SEQ ID NO:29 wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:30 or an amino acid sequence as shown in SEQ ID NO:30, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence SHY.

The disclosure also describes an Ig-like molecule or antigen-binding fragment thereof comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31 or an amino acid sequence as shown in SEQ ID NO:31, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:32 or an amino acid sequence as shown in SEQ ID NO:32, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:33 or an amino acid sequence as shown in SEQ ID NO:33, wherein at most 2, preferably at most 1 amino acid is substituted.

In an example, Ig-like molecule or antigen-binding fragment thereof comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:29 or an amino acid sequence as shown in SEQ ID NO:29, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:30 or an amino acid sequence as shown in SEQ ID NO:30, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence SHY, and further comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31 or an amino acid sequence as shown in SEQ ID NO:31, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:32 or an amino acid sequence as shown in SEQ ID NO:32, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:33 or an amino acid sequence as shown in SEQ ID NO:33, wherein at most 2,

preferably at most 1 amino acid is substituted. In one embodiment, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:29;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:30; and
- a CDR3 having the amino acid sequence SHY;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:31;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:32; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:33.

Preferably, said Ig-like molecule, antibody or antigen-binding fragment thereof comprises a humanized heavy chain variable region of the heavy chain variable region having the amino acid sequence of SEQ ID NO:44 and a humanized light chain variable region of the light chain variable region having the amino acid sequence of SEQ ID NO:45.

In an example, the CDRs of the light chain and/or the heavy chain are integrated into human-derived framework regions.

The Ig-like molecule or antigen-binding fragment thereof may be an antibody, e.g., a chimeric antibody or a humanized antibody.

The disclosure also describes a nucleic acid molecule encoding the Ig-like molecule or antigen-binding fragment thereof taught herein, and a vector comprising such nucleic acid molecule. Such vector may be a gene therapy vector. In one aspect, the present invention refers to a nucleic acid molecule encoding the antibody or antigen-binding fragment thereof according as disclosed herein. In another aspect, the present invention refers to a vector comprising the nucleic acid molecule as disclosed herein.

Additionally, the disclosure describes a host cell comprising the nucleic acid molecule taught herein or the vector taught herein. The host cell may be a mammalian host cell. The host cell may be a hybridoma. In one aspect, the present invention refers to a host cell comprising the nucleic acid molecule as disclosed herein or the vector as disclosed herein.

The disclosure also describes a pharmaceutical composition comprising an agent selected from the group consisting of (a) the Ig-like molecule or antigen-binding fragment thereof as taught herein, (b) a nucleic acid molecule encoding the Ig-like molecule or antigen-binding fragment thereof, (c) a vector comprising such nucleic acid molecule and (d) a host cell comprising such nucleic acid molecule or such vector, and a pharmaceutically acceptable carrier. In one aspect, the present invention refers to a pharmaceutical composition comprising an agent selected from the group consisting of (a) the antibody or antigen-binding fragment thereof as disclosed herein, (b) a nucleic acid molecule as disclosed herein, (c) a vector as disclosed herein and (d) a host cell as disclosed herein, and a pharmaceutical acceptable carrier.

The disclosure further describes an Ig-like molecule or antigen-binding fragment thereof or pharmaceutical composition as taught herein for use as a medicament, particularly for use in the treatment, prevention or prevention of the progression of adverse cardiac remodelling and conditions resulting from or relating to myocardial infarction and/or

pressure overload, or for use in the treatment, prevention or prevention of the progression of adverse tissue remodelling, in particular fibronectin-EDA mediated adverse tissue remodelling. In one example, there is disclosed an antibody or antigen-binding fragment as disclosed herein for use in improving angiogenesis, preferably after tissue injury. In another example, there is disclosed use of an antibody or antigen-binding fragment thereof as disclosed herein in the manufacture of a medicament for stimulating angiogenesis in a subject.

The present disclosure further describes a method of treating, preventing or preventing progression of adverse cardiac remodelling and conditions resulting from or relating to myocardial infarction and/or pressure overload, which comprises administering to a subject in need thereof, a therapeutically effective amount of an agent selected from the group consisting of (a) the Ig-like molecule or antigen-binding fragment thereof as taught herein, a nucleic acid molecule encoding the Ig-like molecule or antigen-binding fragment thereof, (c) a vector comprising such nucleic acid molecule and (d) a host cell comprising such nucleic acid molecule or such vector. The subject may be human. In one example, there is disclosed use of an antibody or antigen-binding fragment thereof as disclosed herein or pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment, prevention or prevention of the progression of adverse cardiac remodelling and/or conditions resulting from or relating to myocardial infarction and/or pressure-overload. In another example, there is disclosed use of an antibody or antigen-binding fragment thereof disclosed herein or pharmaceutical composition as disclosed herein in the manufacture of a medicament for the treatment, prevention or prevention of the progression of adverse tissue remodelling.In yet another example, there is disclosed use of an agent selected from the group consisting of (a) the antibody or antigen-binding fragment as disclosed herein, (b) a nucleic acid molecule as disclosed herein, (c) a vector as disclosed herein and (d) a host cell as disclosed herein in the manufacture of a medicament for treating, preventing or preventing progression adverse cardiac remodelling and/or conditions resulting from or relating to myocardial infarction and/or pressure-overload.

The present disclosure further describes an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for use in improving angiogenesis and a method for improving angiogenesis comprising administering to a

subject in need thereof a therapeutically effective amount of an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof. The subject may be human.

Detailed description of the invention

Immunoglobulin-like molecules, antibodies and antigen-binding fragments thereof

In a first aspect, the invention relates to an isolated Ig-like molecule or antigen-binding fragment thereof that specifically binds to the amino acid sequence GIXXXF (SEQ ID NO:1), wherein X can be any amino acid or to amino acid sequence LFPAP (SEQ ID NO:28).

The term "isolated" as used herein refer to material which is substantially or essentially free from components which normally accompany it in nature.

The term “immunoglobulin” (abbreviated as “Ig”) as used herein is well-known in the art and equals the term “antibody”. The term “Ig-like molecule” as used herein refers to any polypeptide comprising an antigen-binding site with at least one complementarity determining region (CDR). The term includes, but is not limited to polyclonal antibodies, monoclonal antibodies, monospecific antibodies, multispecific antibodies, humanized antibodies, chimeric antibodies, human antibodies, and single-chain antibodies (e.g., VHH). The term “Ig-like molecule” also includes antibody fragments such Fab, $F(ab')_2$, Fv, scFv, Fd, dAb, and other antibody fragments or other constructs comprising CDRs

that retain antigen-binding function. Typically, such fragments would comprise an antigen-binding domain. The Ig-like molecules or antigen-binding fragments thereof may be any of the known antibody isotypes and their conformations, for example, IgA, such as IgA1 or IgA2, IgD, IgE, IgG, such as IgG1, IgG2a, IgG2b, IgG3, IgG4, or IgM class, or may constitute mixtures thereof in any combination, such as a mixture of antibodies from the IgG1 and IgG2a class. In a preferred embodiment the Ig-like molecules, antibodies or antigen-binding fragment are of the IgG4 isotype, more preferably a stabilized IgG4 with reduced dissociation of intrachain bonds resulting in the relatively high levels of IgG4 half-molecules.

Immunoglobulins or antibodies are immune system-related proteins. Each antibody consists of four polypeptides– two heavy chains and two light chains joined to form a "Y"-shaped molecule. The amino acid sequence in the tips of the "Y" varies greatly among different antibodies. This variable region, composed of 110-130 amino acids, gives the antibody its specificity for binding antigen. The variable region includes the ends of the light and heavy chains. The constant region determines the mechanism used to destroy antigen. Antibodies are divided into five major classes, IgM, IgG, IgA, IgD, and IgE, based on their heavy chain constant region structure and immune function. Also subclasses of the heavy chain are known. For example, IgG heavy chains in humans can be any of the IgG1, IgG2, IgG3 and IgG4 subclasses.

The variable region is subdivided into hypervariable (HV) and framework (FR) regions. HV regions have a high ratio of different amino acids in a given position, relative to the most common amino acid in that position. Within light and heavy chains, three HV regions exist – HV 1, 2 and 3. Four FR regions, which have more stable amino acids sequences, separate the HV regions. The HV regions directly contact a portion of the antigen's surface. For this reason, HV regions are also sometimes referred to as complementarity determining regions, or CDRs. The FR regions form a beta-sheet structure which serves as a scaffold to hold the HV regions in position to contact antigen. The term “antigen” as used herein refers to the target molecule that binds specifically to the respective antibody. Antigens usually present several surface features that can act as points of interaction for specific antibodies. Any such surface feature may constitute an epitope. Therefore, most antigens have the potential to be bound by several distinct antibodies, each of which may bind to a different epitope.

The Ig-like molecules, antibodies, or antigen-binding fragments as taught herein are capable of binding to fibronectin-EDA or solely the EDA domain of fibronectin-EDA or a part of the EDA domain of fibronectin-EDA such as a specific amino acid region,

particularly the amino acid sequence GIXXXF, wherein X can be any amino acid, as set forth in SEQ ID NO:1, or the amino acid sequence LFPAP (SEQ ID NO:28).

In the present invention, the term "antigen-binding fragment" is understood as a part or portion of an Ig-like molecule, e.g., antibody, as taught herein, which at least comprises a domain specifically binding to the amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 and/or SEQ ID NO:28. Antigen-binding fragments may be obtained via chemical or enzymatic treatment of an intact or complete Ig-like molecule, e.g., antibody. Alternatively, antigen-binding fragments may be obtained using standard molecular biology techniques and protocols. Non-limiting examples of antigen-binding fragments of Ig-like molecules include Fab, Fab', F(ab')2, and Fv fragments; diabodies; linear antibodies and single-chain antibody molecules.

In an embodiment, the antigen-binding fragment comprises at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguous amino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, or at least 150 contiguous amino acid residues of the amino acid sequence of an Ig-like molecule that specifically binds to the EDA domain of fibronectin-EDA as taught herein, preferably the N-terminal portion of an antibody specifically binding to the EDA domain of fibronectin-EDA, more preferably to the amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 and/or 28. The antigen-binding fragment preferably retains the antigen-binding specificity of the Ig-like molecule.

In a preferred embodiment, the antigen-binding fragment comprises all three CDRs, i.e. CDR1, CDR2, and CDR3, of both the heavy and light chains. In an even more preferred embodiment, the antigen-binding fragment comprises the entire variable domain of both the heavy and light chains.

Preferably, the Ig-like molecule, e.g. an antibody or antigen-binding fragment thereof binds specifically to the EDA domain of fibronectin-EDA. In a preferred embodiment, the Ig-like molecule, e.g. an antibody or antigen-binding fragment thereof specifically binds to a part of the EDA domain of fibronectin-EDA, wherein said part has the amino acid sequence as set forth in SEQ ID NO 1. “An antibody or antigen-binding fragment thereof that binds fibronectin-EDA” as used herein refers to an antibody or antigen-binding fragment thereof that specifically bind to the EDA domain of fibronectin. Such antibody

or fragment does not bind fibronectin lacking the type III repeat extra domain A (EIIIA; EDA).

The skilled person is aware of the meaning of the term 'specifically binding'. The term "specifically binding" as used herein means that an Ig-like molecule or antibody or a fragment thereof as taught herein exhibits appreciable binding affinity for an antigen or a particular epitope and, preferably, does not exhibit significant cross-reactivity. "Appreciable" binding affinity includes binding with an affinity of at least 10^6 M^{-1}, preferably at least 10^7 M^{-1}, more preferably at least 10^8 M^{-1}, yet more preferably at least 10^9 M^{-1}, or even yet more preferably at least 10^{10} M^{-1}. An antibody that "does not exhibit significant cross-reactivity" is one that will not appreciably bind to an undesirable entity or tissue where fibronectin-EDA expression is absent. Specific binding can be determined according to any art-recognized means for determining such binding. For example, specific binding may be determined according to Scatchard analysis and/or competitive binding assays or other assays accepted in the field.

In an embodiment of the invention, the Ig-like molecules as taught herein are antibodies, preferably monoclonal antibodies. The term "monoclonal antibody" is well known in the art. It is understood to refer to an antibody that is the product of a single cloned antibody-producing cell. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that is capable of producing the antibody. Monoclonal antibodies can be produced by a variety of routine techniques.

The Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein may be of any immunoglobulin isotype, such as IgG, IgM, IgA, IgD, and IgE. In a preferred embodiment, the Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein are IgG, such as IgG1, IgG2, IgG3 or IgG4, preferably IgG4.

In an embodiment, the monoclonal antibodies or antigen-binding fragments thereof as taught herein are from mammalian origin such as from human, non-human primates, sheep, rabbits, pigs, dogs, horses, cow, chicken, and murine and other mammals.

The antibody or antigen-binding fragment thereof as taught herein may also be a hybrid antibody, e.g., a chimeric, or humanized monoclonal antibody. In the present invention, the term "hybrid antibody" refers to an antibody in which one or more regions of the antibody are derived from an antibody derived from a first species (for instance a mouse) and one or more regions of the antibody are from an antibody derived from a second, different species (for instance a human). In chimeric antibodies typically non-human (e.g., mouse) constant regions of the antibody are substituted by human constant regions.

A humanized antibody is understood to refer to an antibody where at least one of the heavy and light chain is humanized, i.e. at least one of the heavy and light chain comprises a variable region wherein one or more, preferably all, of the framework regions are primarily human. The hypervariable (CDR) regions of the humanized antibody may be derived from a non-human source, typically of a rodent (for instance a mouse). The humanized antibody may optionally comprise at least a portion of an Ig constant region (Fc), preferably that of a human Ig molecule.

Chimeric and humanized antibodies may be prepared by methods well known in the art including CDR grafting approaches (see, e.g., U.S. Pat. Nos. 5,843,708; 6,180,370; 5,693,762; 5,585,089; 5,530,101), chain shuffling strategies (see e.g., U.S. Pat. No. 5,565,332; Rader et al., Proc. Natl. Acad. Sci. USA (1998) 95:8910-8915), molecular modelling strategies (U.S. Pat. No. 5,639,641), and the like. For instance, a best-fit human heavy and light chain is selected based on 3D models and CDR lengths of a non-human antibody. Subsequently, amino acids in the framework regions that differ between the human and non-human antibody and that may influence antigen binding are identified. The use of chimeric or humanized antibodies or antigen-binding fragments thereof may minimize or eliminate the occurrence or risk of immune-rejections or other adverse immune responses in humans. In addition generally a longer half-life in the circulation is achieved when chimeric, humanized or human antibodies are used because of reduced clearance when compared to non-human antibodies.

In a particularly preferred embodiment, an antibody of the invention is a humanized antibody. A humanized antibody according in accordance with the invention preferably comprises a human heavy chain and a light chain constant region, and more preferably further comprises human heavy chain and light chain framework regions. As examples, the present invention provides humanized antibodies of murine antibodies 27A12.70 and 33E3.10. Germline genes VK2-29 and JK4 are used as acceptor sequences for the light chain of humanized antibody 27A12.70 and germline genes VH4-31 and JH4 are used as acceptor sequence for the heavy chain of humanized antibody 27A12.70. SEQ ID NO:34 and SEQ ID NO:35 provide the sequences of the heavy chain and light chain, respectively, of humanized antibody 27A12.70. Germline genes VK1-12 and JK4 are used as acceptor sequences for the light chain of humanized antibody 33E3.10and germline genes VH3-23 and JH4 are used as acceptor sequence for the heavy chain of humanized antibody 33E3.10. SEQ ID NO:36 and SEQ ID NO:37 provide the sequences of the heavy chain and light chain, respectively, of humanized antibody 33E3.10.

A particularly preferred antibody according to the invention is a humanized antibody of the IgG4 subtype. It is known in the art that IgG4 antibodies behave differently in vivo

than other IgG subtype antibodies. IgG4 molecules occurs both in a form in which the inter-heavy chain disulphide bonds have been formed and in form in which one or both of the bonds have not been formed. The IgG4 form in which the inter-heavy chain disulphide bonds are lacking consists of one heavy chain and one light chain, which is also referred to as a half-molecule. It is believed the cause of this flexibility of IgG4 lies in the core sequence of the IgG4 hinge region. This region consists of Cys-Pro-Ser-Cys, whereas the corresponding sequence in IgG1 and IgG2 is Cys-Pro-Pro-Cys. The result of the IgG4 flexibility is that in vivo IgG4 is present in several forms, including half-molecules, monospecific antibodies and bispecific antibodies which have formed as a result of the association of two IgG4 molecules with different antigen specificity. For therapeutic use of an antibody formation of half-molecules and bispecific antibodies is unfavourable because in vivo stability of the antibody is desired. Therefore, stabilized IgG4 molecules have been designed which have reduced half-molecule formation and exchange in vivo. In a preferred embodiment, a humanized IgG4 antibody according to the invention is a stabilized IgG4 antibody. The term "stabilized IgG4 antibody" as used herein refers to an IgG4 antibody which has been modified to reduce half-molecule formation and exchange. Examples of suitable stabilized IgG4 antibodies are antibodies, wherein arginine at position 409 in the heavy chain constant region of human IgG4, (Kabat numbering, Kabat et al. Sequences of Proteins of Immunological interest, 5th Ed. Public Health Service, National Institute of Health, Bethesda, MD, 1991) is substituted with lysine, threonine, methionine, or leucine and/or wherein the hinge region of the IgG4 comprises a Cys-Pro-Pro-Cys sequence. The amino acid sequences of the heavy chain and light chain of preferred stabilized humanized IgG4 having the heavy and light chain CDRs of antibody 27A12.70 are shown in SEQ ID NO:34 and SEQ ID NO:35, respectively, and that of a preferred humanized IgG4 having the heavy and light chain CDRs of antibody 33E3.10 in SEQ ID NO:36 and SEQ ID NO:37, respectively. The amino acid sequence of the constant regions and framework regions of one or more of these preferred stabilized IgG4 heavy chain and light chain are in a preferred embodiment used to humanized the other antibodies disclosed herein.

Provided is therefore a humanized antibody or antigen-binding fragment thereof specifically binding to an amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:2, wherein the antibody or fragment thereof comprises a heavy chain and a light chain, wherein said heavy chain has an amino acid sequence as shown in SEQ ID NO:34 and said light chain has an amino acid sequence as shown in SEQ ID NO:35. Further provided is a humanized antibody or antigen-binding fragment thereof specifically binding to an amino acid sequence as set forth in SEQ ID NO:28, wherein the antibody or

fragment thereof comprises a heavy chain and a light chain, wherein said heavy chain has an amino acid sequence as shown in SEQ ID NO:36 and said light chain has an amino acid sequence as shown in SEQ ID NO:37.

Further provided is a humanized antibody or antigen-binding fragment thereof specifically binding to an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:28, wherein the antibody or fragment thereof comprises a heavy chain and a light chain, wherein said heavy chain comprises a humanized variable region comprising one or more, preferably all, of the framework regions of an IgG4 heavy chain as set forth in SEQ ID NO:34 or SEQ ID NO:36 and wherein said light chain comprises a humanized variable region comprising one or more, preferably all, of the framework regions of an IgG4 light chain as set forth in SEQ ID NO:35 or SEQ ID NO:37. Further provided is a humanized antibody or antigen-binding fragment thereof specifically binding to an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:28, wherein the antibody or fragment thereof comprises a heavy chain and a light chain, wherein said heavy chain comprises a constant region of an IgG4 heavy chain as set forth in SEQ ID NO:34 or SEQ ID NO:36 (preferably amino acids 118-444 of SEQ ID NO:34 or amino acids 112-438 of SEQ ID NO:36) and said light chain comprises a constant region of an IgG4 light chain as set forth in SEQ ID NO:35 or SEQ ID NO:37 (preferably amino acids 114-219 of SEQ ID NO:35 or amino acids 109-214 of SEQ ID NO:37). Further, said heavy chain and/or light chain may comprise a variable region comprising one or more, preferably all, of the framework regions of an IgG4 heavy chain as set forth in SEQ ID NO:34 or SEQ ID NO:36 and/or a variable region comprising one or more, preferably all, of the framework regions of an IgG4 light chain as set forth in SEQ ID NO:35 or SEQ ID NO:37. Said antibody further preferably comprise heavy chain and light chain CDRs of antibody 27A12.70, 29E7.35, 17G8.72 or 33E3.10. The CDR sequences are shown in SEQ ID NO:3-8 (27A12.70), SEQ ID NO:9-14 (29E7.35), SEQ ID NO:15-20 (17G8.72) and SEQ ID NO:29-33 (33E3.10).

Further provided is therefore a humanized antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for use in improving angiogenesis, preferably for use in improving angiogenesis in ischemic tissue, in fibrotic tissue, in pressure-overload conditions, in wound tissue and/or upon organ or tissue transplantation, wherein said antibody or fragment specifically bind to an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:28, wherein the antibody or fragment thereof comprising a heavy chain and a light chain, wherein said heavy chain comprises a constant region of an IgG4 heavy chain as set forth in SEQ ID NO:34 or SEQ ID NO:36 and said light chain comprises a constant region of an IgG4 light chain as set forth in

SEQ ID NO:35 or SEQ ID NO:37. Further, said heavy chain and/or light chain, preferably both, may comprise a variable region comprising one or more, preferably all, of the framework regions of an IgG4 heavy chain as set forth in SEQ ID NO:34 or SEQ ID NO:36 and/or a variable region comprising one or more, preferably all, of the framework regions of an IgG4 light chain as set forth in SEQ ID NO:35 or SEQ ID NO:37. Said antibody further preferably comprise heavy chain and light chain CDRs of antibody 27A12.70, 29E7.35, 17G8.72 or 33E3.10.

In one aspect, an Ig-like molecule or antigen-binding fragment taught herein specifically binds to the amino acid sequence GIXXXF (SEQ ID NO:1), wherein the X may be any amino acid.

In one embodiment, the amino acid at position 3 of SEQ ID NO:1 may be any amino acid. Alternatively, the amino acid at position 3 of SEQ ID NO:1 may be selected from histidine, arginine, lysine, and alanine. More preferably, the amino acid at position 3 of SEQ ID NO:1 is histidine.

Similarly, the amino acid at position 4 of SEQ ID NO:1 may be any amino acid. The amino acid at position 4 of SEQ ID NO:1 is preferably selected from glutamic acid and alanine. In a preferred embodiment, the amino acid at position 4 of SEQ ID NO:1 is glutamic acid.

The amino acid at position 5 of SEQ ID NO:1 may be any amino acid. Preferably, the amino acid at position 5 of SEQ ID NO:1 is a small amino acid selected from the group consisting of leucine and alanine. In a preferred embodiment, the amino acid at position 5 of SEQ ID NO:1 is leucine.

In a more preferred embodiment, the Ig-like molecules or antigen-binding fragments thereof specifically bind to the amino acid sequence GIHELF (SEQ ID NO:2). The present inventor found that Ig-like molecules or fragments thereof as taught herein bind with high affinity to the amino acid sequence as set forth in SEQ ID NO:2. The present inventor also found that Ig-like molecules, antibodies and fragments thereof as taught herein resulted in increased survival and improved cardiac function after myocardial infarction. It was further found that the Ig-like molecules, antibodies and fragments thereof as taught herein are further preferred because they do not influence the amount myofibroblasts in the heart tissue and doe not interfere with collagen production. This is advantageous because it is important to have a firm collagen based scar in this tissue to prevent rupture of the infarcted area. As demonstrated in Example 5, treatment with an antibody that binds fibronectin-EDA of the invention did not affect proper scar formation. In addition, it was found that anti-fibronectin EDA treatment delays clearance of

acellular matrix. The formation of such provisional acellular matrix is crucial for hemodynamic compensation for non-viable tissue and scar formation after MI. During wound healing the provisional matrix is slowly degraded and replaced by a firm collagen-based scar.

Under non-ischemic conditions, the absence of EDA also protected against adverse remodeling after pressure-overload of the heart in mice. Both cardiac function and heart/body weight (marker for degree of heart failure and subsequent congestion) was significantly improved in EDA deficient mice. These data demonstrate that anti-EDA treatment in pressure-overload conditions (e.g. hypertension, valvular disease and non-ischemic cardiomyopathy) is of therapeutic value.

The present invention also provides an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a complementarity determining region (CDR)1 having the amino acid sequence shown in SEQ ID NO:3 or an amino acid sequence as shown in SEQ ID NO:3, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:4 or an amino acid sequence as shown in SEQ ID NO:4, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence as shown in SEQ ID NO:5, wherein at most 3, such as at most 2, preferably at most 1 amino acid is substituted.
For example, it was found that the threonine at position 5 of SEQ ID NO:3 may also be an alanine. Additionally, it was found that tyrosine at position 4 of SEQ ID NO:4 may also be phenylalanine, and that isoleucine at position 7 of SEQ ID NO:4 may also be serine. Finally, it was found that lysine at position 2 of SEQ ID NO:5 may also be alanine, that threonine at position 3 of SEQ ID NO:5 may also be arginine, and that phenylalanine at position 5 of SEQ ID NO:5 may also be tyrosine.
Also provided is an Ig-like molecule, antibody or antigen-binding fragment thereof comprising:
a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A (SEQ ID NO:21);
- a CDR2 having the amino acid sequence YIHX$_2$SGX$_3$ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I (SEQ ID NO:22);
- a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y (SEQ ID NO:23).

The invention also provides an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:6 or an amino acid sequence as shown in SEQ ID NO:6, wherein at most 3, such as at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:7 or an amino acid sequence as shown in SEQ ID NO:7, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:8 or an amino acid sequence as shown in SEQ ID NO:8, wherein at most 3, such as at most 2, preferably at most 1 amino acid is substituted.

For example, it was found that the leucine at position 6 of SEQ ID NO:6 may also be an isoleucine, that the histidine at position 8 of SEQ ID NO:6 may also be an arginine, and that the histidine at position 16 of SEQ ID NO:6 may also be a threonine. Additionally, it was found that the serine at position 1 of SEQ ID NO:8 may also be a phenylalanine, that the serine at position 3 of SEQ ID NO:8 may also be a glycine, and that the alanine at position 4 of SEQ ID NO:8 may also be a serine.

Also provided is an Ig-like molecule, antibody or antigen-binding fragment thereof comprising:

a light chain variable region comprising:

- a CDR1 having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T (SEQ ID NO: 24);
- a CDR2 having the amino acid sequence KVSNRFS (SEQ ID NO: 25);
- a CDR3 having the amino acid sequence X$_{10}$QX$_{11}$X$_{12}$HVPPT, wherein X$_{10}$ is selected from S and F, X$_{11}$ is selected from S and G, and X$_{12}$ is selected from A and S (SEQ ID NO:26).

In a preferred embodiment, such Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:3 or an amino acid sequence as shown in SEQ ID NO:3, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:4 or an amino acid sequence as shown in SEQ ID NO:4, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:5 or an amino acid sequence as shown in SEQ ID NO:5, wherein at most 3, such as at most 2, preferably at most 1 amino acid is substituted; and a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:6 or an amino acid sequence as shown in SEQ ID NO:6, wherein at most 3, such as at most 2, preferably at most 1 amino

acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:7 or an amino acid sequence as shown in SEQ ID NO:7, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:8 or an amino acid sequence as shown in SEQ ID NO:8, wherein at most 3, such as at most 2, preferably at most 1 amino acid is substituted. The present inventor found that the Ig-like molecules, antibodies or antigen-binding fragments thereof of the present embodiment are particularly suitable for treating, preventing or preventing the progression of cardiac and vascular remodelling, myocardial infarction- and pressure-overload-related complications. Specifically, the Ig-like molecules, antibodies or antigen-binding fragments thereof of the present embodiment were found to be particularly effective in increasing survival and improving cardiac function after myocardial infarction. Further, the Ig-like molecules, antibodies or antigen-binding fragments thereof of the invention are particularly preferred because they delay clearance of acellular matrix and do not affect proper scar formation, which is important to prevent dilatation and rupture of the heart after myocardial infarction. In addition, the Ig-like molecules, antibodies or antigen-binding fragments thereof of the invention are preferred because they also improve angiogenesis in the infarct and borderzone after myocardial infarction thus contributing to improved wound healing and preventing adverse remodeling.

In another preferred embodiment, such Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A (SEQ ID NO:21);
- a CDR2 having the amino acid sequence YIHX$_2$SGX$_3$ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I (SEQ ID NO:22); and
- a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y (SEQ ID NO:23);

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T (SEQ ID NO:24);
- a CDR2 having the amino acid sequence KVSNRFS (SEQ ID NO: 25); and

- a CDR3 having the amino acid sequence $X_{10}QX_{11}X_{12}HVPPT$, wherein X_{10} is selected from S and F, X_{11} is selected from S and G, and X_{12} is selected from A and S (SEQ ID NO:26).

In an embodiment such Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:3;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:4; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:5;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:6;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:7; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:8.

In another embodiment, such Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:9;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:10; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:11;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:12;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:13; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:14.

In yet another embodiment, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:15;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:16; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:17;

and a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:18;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:19; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:20.

In another aspect, an Ig-like molecule, antibodies or antigen-binding fragments thereof as taught herein specifically bind to the amino acid sequence LFPAP (SEQ ID NO:28). The present inventor found that Ig-like molecules or fragments thereof as taught herein bind with high affinity to the amino acid sequence as set forth in SEQ ID NO:28. The

present inventor also found that Ig-like molecules taught herein resulted in increased survival after myocardial infarction.

The present invention also provides an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:29 or an amino acid sequence as shown in SEQ ID NO:29, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:30 or an amino acid sequence as shown in SEQ ID NO:30, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence SHY.

Additionally, the present invention provides an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31 or an amino acid sequence as shown in SEQ ID NO:31, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:32 or an amino acid sequence as shown in SEQ ID NO:32, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:33 or an amino acid sequence as shown in SEQ ID NO:33, wherein at most 2, preferably at most 1 amino acid is substituted.

In an embodiment, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:29 or an amino acid sequence as shown in SEQ ID NO:29, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:30 or an amino acid sequence as shown in SEQ ID NO:30, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence SHY, and further comprises a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31 or an amino acid sequence as shown in SEQ ID NO:31, wherein at most 2, preferably at most 1 amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:32 or an amino acid sequence as shown in SEQ ID NO:32, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:33 or an amino acid sequence as shown in SEQ ID NO:33, wherein at most 2, preferably at most 1 amino acid is substituted.

In a suitable embodiment, additionally, the present invention provides an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31 or an amino acid sequence as shown in SEQ ID NO:31, wherein at most 2, preferably at most 1

amino acid is substituted, a CDR2 having the amino acid sequence shown in SEQ ID NO:32 or an amino acid sequence as shown in SEQ ID NO:32, wherein at most 2, preferably at most 1 amino acid is substituted, and a CDR3 having the amino acid sequence shown in SEQ ID NO:33 or an amino acid sequence as shown in SEQ ID NO:33, wherein at most 2, preferably at most 1 amino acid is substituted.

In an embodiment, the Ig-like molecule, antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:29;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:30; and
- a CDR3 having the amino acid sequence SHY;

and/or a light chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:31;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:32; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:33.

The present inventor found that the Ig-like molecules, antibodies or antigen-binding fragments as taught herein were all particularly effective in increasing survival and improving cardiac function after myocardial infarction. Further, it was found that the Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein delay clearance of acellular matrix and do not affect proper scar formation, which is important to prevent dilatation and rupture of the heart after myocardial infarction.

Nucleic Acid Molecules, Vectors and Host Cells of the Invention

The present invention also relates to a (isolated) nucleic acid molecule encoding the Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein. In the present invention, the terms "nucleic acid molecule," or "polynucleotide molecule" are understood to refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogues, or any substrate that can be incorporated into a polymer by DNA or RNA polymerase, or by a synthetic reaction. Methods and standard protocols for the preparation, synthesis, and production of nucleic acid molecules which are capable of encoding the Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein are well-known in the art through conventional molecular biology teachings.

In case the Ig-like molecule taught herein is an antibody comprising a light chain and a heavy chain, two nucleic acid molecules may be introduced into a host cell, i.e., one

nucleic acid molecule encoding the amino acid sequence of the light chain and a second nucleic acid molecule encoding the amino acid sequence of the heavy chain. The invention may provide a set of nucleic acid molecules, said set comprising a first nucleic acid molecule encoding a light chain of an antibody, said light chain comprising a variable region and a constant region, and a second nucleic acid molecule encoding a heavy chain of an antibody, said heavy chain comprising a variable region and a constant region.

In one embodiment, the invention relates to a vector comprising the nucleic acid molecule(s) as taught herein, which is capable of encoding the Ig-like molecules, antibodies and antigen-binding fragments thereof as taught herein. The term "vector" is well-known in the art and is understood to refer to a nucleic acid molecule capable of artificially carrying or transporting foreign genetic material (i.e. nucleic acid molecule) to which it has been linked, into another cell, where it can be replicated and/or expressed. In an embodiment of the invention, certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, vectors may comprise promoters that are capable of directing the expression of genes to which they are operatively linked. Vectors may be “expression vectors". Other types of vectors include cosmids and artificial chromosomes. Methods and standard protocols for the preparation of suitable vectors comprising nucleic acid molecules which are capable of encoding the Ig-like molecules or fragments thereof as taught herein are also well known to the skilled person.

In an embodiment, the vector(s) is/are preferably (a) gene therapy vector(s).

The invention also relates to a host cell comprising and optionally expressing the nucleic acid molecule(s) or the vector(s) of the invention. Preferably, the host cell is a mammalian host cell. Mammalian host cells are well-known in the art and are commercially available. Non-limiting examples of mammalian host cells are Chinese hamster ovary (CHO) cells, NS0 murine myeloma cells, and PER.C6® human cells.

In a preferred embodiment, the mammalian host cell is a hybridoma. Methods and protocols to produce and maintain immortalized B cells in culture medium for the production of antibodies or Ig-like molecules or fragments thereof as taught herein are well-known in the art.

Pharmaceutical compositions

The present invention also provides a pharmaceutical composition comprising an agent selected from the group consisting of (a) an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (b) a nucleic acid comprising a nucleic acid molecule encoding an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (c) a vector comprising the nucleic acid molecule encoding an Ig-like molecule, antibody, or antigen-binding fragment thereof as taught herein, and (d) a host cell expressing the nucleic acid of (b) or the vector of (c) as taught herein; and a pharmaceutically acceptable diluent or carrier.

In the present invention, the term “pharmaceutically acceptable" refers to those compositions or combinations of agents, materials, or compositions, and/or their dosage forms, which are within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. Furthermore, the term "pharmaceutically acceptable diluent or carrier" refers to a pharmaceutically acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject chemical from one organ, or portion of the body, to another organ, or portion of the body. Other examples of materials widely used in medicine are stents, included but not limited to polymer-based or absorbable (i.e. biodegradable) stents. In the art, these stents are called drug-eluting stents. In the present invention, the stents are covered with or include the pharmaceutical composition in order to have the pharmaceutical composition released to the site of interest (e.g. coronary arteries in case of myocardial infarction, carotid artery or its distal branches in case of ischemic brain injury/stroke). Other non-limiting examples of materials which can serve as pharmaceutically-acceptable carriers include: (1) sugars, such as lactose, glucose and sucrose; (2) starches, such as corn starch and potato starch; (3) cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; (4) powdered tragacanth; (5) malt; (6) gelatin; (7) talc; (8) excipients, such as cocoa butter and suppository waxes; (9) oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; (10) glycols, such as propylene glycol; (11) polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; (12) esters, such as ethyl oleate and ethyl laurate; (13) agar; (14) buffering agents, such as magnesium hydroxide and aluminum hydroxide; (15) alginic acid; (16) pyrogen-free water; (17) isotonic saline; (18) Ringer's solution; (19) ethyl alcohol; (20) phosphate buffer solutions; and (21) other non-toxic compatible substances employed in pharmaceutical formulations.

The pharmaceutical composition may be administered by any suitable routes and mode. As will be appreciated by the person skilled in the art, the route and/or mode of administration will vary depending upon the desired results.

The pharmaceutical compositions according to the invention may be formulated in accordance with routine procedures for administration by any routes, such as parenteral, topical, oral, sublingual, transdermal, or by inhalation or via drug-eluting stents. The compositions may be in the form of tablets, capsules, powders, drug-eluting stents, granules, lozenges, creams or liquid preparations, such as sterile parenteral solutions or suspensions or in the form of a spray, aerosol or other conventional method for inhalation.

The pharmaceutical compositions of the present invention include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration.

In an embodiment, the pharmaceutical composition is administered parenterally.

The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intramuscular, intraarterial, intracoronary, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

In an embodiment the pharmaceutical composition is administered by intravenous injection or infusion.

Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical composition of the invention is contemplated. Preferably, the carrier is suitable for parenteral administration, e.g. intravenous injection or infusion.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl

oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

Sterile injectable solutions can be prepared by incorporating the Ig-like molecules, or antibodies, or antigen-binding fragments thereof as taught herein in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilisation) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Dosage regimens may be adjusted to provide the optimum desired therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals, and (c) duration and level of expression of fibronectin-EDA in the related disease entity. For example, fibronectin-EDA expression reaches a peak at 2 to 3 weeks and reduces to baseline levels 5 to 6 weeks after acute myocardial infarction. Depending on the half-life of the anti-fibronectin-EDA compound (e.g. the Ig-like molecules, antibodies or antigen-binding fragment thereof as taught herein), the compound will be administered once, twice, three times or more frequent if desired to cover the entire expression duration of fibronectin-EDA.

Actual dosage levels of the Ig-like molecules or antibodies or antigen-binding fragments thereof as taught herein in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of Ig-like molecules or antibodies or antigen-binding fragments thereof which is effective ("effective amount") to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, the route of administration, the time of administration, the rate of excretion, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

Methods and uses of the invention

The present invention also relates to a method for improving the intrinsic angiogenic response in fibronectin-EDA-mediated wound healing after ischemic injury. Such method comprises administering to a subject in need thereof a therapeutically effective amount of antibody that binds fibronectin-EDA or an antigen-binding fragment thereof. Further provided is an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for use in stimulating angiogenesis. As demonstrated in the Example 5, the present inventor found that treatment with anti-fibronectin-EDA antibodies after myocardial infarction increases blood vessel formation in the heart, in particular in both the infarcted area of the heart and in the border zone of infarcted and unaffected area of the heart. Further, an in vitro spouting assay shows that fibronectin-EDA is capable of inhibiting sprouting of endothelial cells. These findings collectively indicate that fibronectin-EDA inhibits angiogenesis in the infarcted heart. Without wishing to be bound by theory, it is believed that fibronectin-EDA inhibits endothelial cell sprouting by binding to α1 integrins on these cells and that the increase in vessel formation caused by anti-fibronectin-EDA antibodies is due to the prevention of this inhibition. Hence, the present inventor found that anti-fibronectin-EDA antibodies can be used to prevent the inhibitory effects of fibronectin-EDA on angiogenesis after tissue injury. This is in particular surprising since the use of anti-fibronectin-EDA antibodies as anti-tumour agents is currently considered. Fibronectin-EDA is found to be highly upregulated around tumour vasculature and in contrast to the findings of the present inventor, fibronectin-EDA is thought to associate with angiogenesis in tumours.

In addition to the heart following myocardial infarction, fibronectin-EDA can be upregulated in other ischemic tissue, where improvement of the angiogenic response generally has a beneficial effect after ischemic injury.

Provided is therefore a method for improving angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof. Also provided is an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for use in improving angiogenesis. Angiogenesis is preferably improved in fibronectin-EDA-mediated wound healing after ischemic injury.

As used herein the term "improving angiogenesis" indicates that there is an increase in the level of angiogenesis in tissue, preferably after injury, following administration of an antibody that binds fibronectin-EDA according to the invention as compared the level of angiogenesis in said tissue, preferably after injury, wherein fibronectin-EDA is expressed but wherein an antibody that binds fibronectin-EDA according to the invention is absent. Hence, such antibody according to the invention induces angiogenesis as compared to a situation wherein said antibody is absent, but preferably wherein fibronectin-EDA is expressed. Preferably, angiogenesis is improved in tissue following injury, such as in ischemic tissue, wounds, fibrotic tissue and/or upon organ or tissue transplantation. Preferably, angiogenesis is improved in a subject suffering from ischemic disease, in particular from cardiac ischemia, peripheral ischemia and/or peripheral arterial disease, in fibrotic disease, in pressure-overload conditions, in wounds and/or upon organ or tissue transplantation, most preferably in a subject suffering from ischemic disease. Angiogenesis is preferably improved in ischemic tissue, such as cardiac ischemic tissue or peripheral ischemic tissue, in fibrotic tissue, in pressure-overload conditions, in wound tissue and/or upon organ or tissue transplantation. Most preferably angiogenesis is improved in ischemic tissue. The term "ischemic disease" as used herein refers to diseases whereby one or more organs or tissues are affected by ischemia. Examples of ischemic diseases are cardiac ischemia, peripheral ischemia, and peripheral arterial disease. Examples of cardiac ischemia or causes thereof include, but are not limited to, myocardial infarction, mitral valve disease, chronic atrial fibrillation and cardiomyopathies in which thrombi are prone to develop. "Peripheral ischemia" as used herein refers to a condition of decreased blood supply to one or more limbs, which may be connected to peripheral arterial disease. Examples of causes of peripheral ischemia include embolism, thrombosis, dissection, venous occlusion, atherosclerosis, aneurysm and trauma. "Pressure-overload conditions" is a term well known in the art and relates to

Preferred but non-limiting examples of pressure-overload conditions are hypertension, valvular disease and non-ischemic cardiomyopathy.

In a preferred embodiment, an Ig-like molecule or antigen-binding fragment thereof according to the present invention is used for stimulating angiogenesis as described herein. Hence, provided is an isolated immunoglobulin (Ig)-like molecule or antigen-binding fragment thereof specifically binding to an amino acid sequence as set forth in SEQ ID NO:1, SEQ ID NO:2 or SEQ ID NO:28 for use in stimulating angiogenesis. Preferably an Ig-like molecule, antibody or antigen-binding fragment thereof selected from the group consisting of:

- Ig-like molecule, antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:3; a CDR2 having the amino acid sequence shown in SEQ ID NO:4; and a CDR3 having the amino acid sequence shown in SEQ ID NO:5; and a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:6; a CDR2 having the amino acid sequence shown in SEQ ID NO:7; and a CDR3 having the amino acid sequence shown in SEQ ID NO:8;
- an Ig-like molecule, antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:9; a CDR2 having the amino acid sequence shown in SEQ ID NO:10; and a CDR3 having the amino acid sequence shown in SEQ ID NO:11; and a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:12; a CDR2 having the amino acid sequence shown in SEQ ID NO:13; and a CDR3 having the amino acid sequence shown in SEQ ID NO:14;
- Ig-like molecule, antibody or antigen-binding fragment thereof comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:15; a CDR2 having the amino acid sequence shown in SEQ ID NO:16; and a CDR3 having the amino acid sequence shown in SEQ ID NO:17; and a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:18; a CDR2 having the amino acid sequence shown in SEQ ID NO:19; and a CDR3 having the amino acid sequence shown in SEQ ID NO:20;
- Ig-like molecule or antigen-binding fragment thereof according to claim 10 comprising a heavy chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:29; a CDR2 having the amino acid sequence shown in SEQ ID NO:30; and a CDR3 having the amino acid sequence SHY; and/or a light chain variable region comprising a CDR1 having the amino acid sequence shown in SEQ ID NO:31; a CDR2

having the amino acid sequence shown in SEQ ID NO:32; and a CDR3 having the amino acid sequence shown in SEQ ID NO:33;
is used for improving angiogenesis, preferably for improving angiogenesis is a subject suffering from ischemic disease, in fibrotic disease, in pressure-overload conditions, in wounds and/or upon organ or tissue transplantation. More preferably such Ig-like molecule, antibody or antigen-binding fragment thereof is used from improving angiogenesis in ischemic tissue, such as cardiac ischemic tissue or peripheral ischemic tissue, in fibrotic tissue, in pressure-overload conditions, in wound tissue and/or upon organ or tissue transplantation, most preferably in ischemic tissue. Preferably such antibody is a humanized antibody, preferably a humanized stabilized IgG4 antibody as described herein.

Further provided is a method for improving angiogenesis in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of a therapeutically effective amount of an agent selected from the group consisting of (a) an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (b) a nucleic acid comprising (a) nucleic acid molecule(s) encoding an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (c) one or more vector(s) comprising the nucleic acid molecule(s) as taught herein and (d) a host cell expressing the nucleic acid molecule(s) as taught herein.

Also provided is an agent selected from the group consisting of (a) an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (b) a nucleic acid comprising (a) nucleic acid molecule(s) encoding an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (c) one or more vector(s) comprising the nucleic acid molecule(s) as taught herein and (d) a host cell expressing the nucleic acid molecule(s) as taught herein for use in improving angiogenesis in a subject in need thereof.

The present invention further provides an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for use in stimulating angiogenesis as described herein and a method for stimulating angiogenesis comprising administering to a subject in need thereof a therapeutically effective amount of an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof.

The present invention also relates to a method of treating, preventing or preventing progression of adverse cardiac and vascular remodelling and myocardial infarction- and pressure-overload-related complications. Such method as taught herein comprises administering to a subject in need thereof, a therapeutically effective amount of an agent

selected from the group consisting of (a) an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (b) a nucleic acid comprising (a) nucleic acid molecule(s) encoding an Ig-like molecule, antibody or antigen-binding fragment thereof as taught herein, (c) one or more vector(s) comprising the nucleic acid molecule(s) as taught herein and (d) a host cell expressing the nucleic acid molecule(s) as taught herein.

As used herein, the term "effective amount" refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, e.g., an amount which results in the treatment, prevention of, or prevention of progression of adverse cardiac remodelling and/or a disease or condition associated with, related to or resulting from myocardial infarction and/or pressure-overload, such as heart failure or one or more symptoms associated with heart failure.

The Ig-like molecules, antibodies or antigen-binding fragments as taught herein may be administered to a subject having one or more signs or symptoms of myocardial infarction and/or heart failure, such as chest pain, dyspnea, edema and cardiomegaly. For example, a "therapeutically effective amount" of the Ig-like molecules, antibodies or antigen-binding fragments as taught herein refers to levels in which the physiological effects of a disease or condition associated with, related to or resulting from myocardial infarction, pressure-overload and/or adverse cardiac remodelling, such as heart failure are, at a minimum, ameliorated. The skilled person will be capable of determining when such disease or condition has been treated, prevented, or when its progression has been prevented.

In an embodiment, an effective amount of the Ig-like molecule, antibody, or antigen-binding fragment thereof as taught herein, such as a monoclonal antibody, may be in the range of about 0.1 µg/kg to about 10 g/kg, such as about 1 µg/kg to about 1 g/kg, about 10 µg/kg to about 100 mg/kg, or about 0.1 mg/kg to about 50 mg/kg.

The Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein may be administered once in a single dosage, or may be administered several times after myocardial infarction. For example, Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein may be administered intravenously, once immediately after the myocardial infarction (i.e., within 48 hours of the myocardial infarction), followed by one or more intravenous administrations on the days following the first administration of the binding member of the invention. The Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein may be administered with an interval ranging from about 2 hours to about 14 days, such as about 4 hours to about 10 days, about 6 hours to about 8 days, about 8 hours to about 6 days, about 12

hours to about 4 days, or about 24 hours to about 2 days to achieve optimal therapeutic or preventive effect.

In one embodiment, therapeutic benefits are observed following treatment of subjects according to the method as taught herein. For instance, therapeutic benefits may be exemplified by observing: 1) prevention of the progression of myocardial infarct- and pressure-overload-related conditions and adverse cardiac remodelling in a subject, and/or 2) decreased or lower levels of complications relating to or resulting from myocardial infarction, pressure-overload and adverse cardiac remodelling in a subject, and/or 3) lower risk of developing or suffering from complications relating to or resulting from myocardial infarction, pressure-overload and adverse cardiac remodelling in a subject, and/or 4) halted the progression of myocardial infarct- and pressure-overload-related conditions and adverse cardiac remodelling in a subject, and/or 5) increased survival following myocardial infarction, pressure-overload and adverse cardiac remodelling in a subject.

In the present invention, disease conditions relating to or resulting from myocardial infarction, pressure-overload and adverse cardiac remodelling include conditions or diseases such as heart failure; remote myocardial fibrosis; aneurysm or rupture of the ventricle; mitral regurgitation, particularly if the infarction is large and likely to cause severe ventricular dilatation; and arrhythmias, such as ventricular fibrillation and ventricular tachycardia due to the ventricular enlargement, reactive and/or replacement (scar) fibrosis.

In one embodiment of the invention, the Ig-like molecules, antibodies or fragments thereof and method using them as taught herein are particularly suitable for the treatment, prevention or prevention of the progression of myocardial infarct- and pressure-overload-related conditions (e.g. heart failure; remote myocardial fibrosis; aneurysm or rupture of the ventricle; mitral regurgitation, particularly if the infarction is large and likely to cause severe ventricular dilatation; and arrhythmias, such as ventricular fibrillation and ventricular tachycardia due to the ventricular enlargement, reactive and replacement (scar) fibrosis).

The Ig-like molecules, antibodies or fragments thereof may also be suitable for the treatment, prevention or prevention of the progression of adverse tissue remodelling, such as fibrosis and reduced cardiac function that result from hypertension and/or aortic valve stenosis; allograft rejection that results after heart transplantation; pulmonary hypertension and lung dysfunction that result from lung fibrosis, joint dysfunction that results from rheumatoid arthritis and/or osteoarthritis, seizures, paralysis and/or paresis, and infarction of the brain that result from an ischemic stroke.

In one embodiment of the invention, other therapeutic benefits may be exemplified by observing: 1) reduced heart failure, or reduced fibrosis or reduced risk for arrhythmias in a subject suffering from hypertension and/or aortic stenosis, and/or 2) improved cardiac function, or reduced fibrosis, or reduced risk for arrhythmias in a subject suffering from allograft rejection after heart transplantation, and/or 3) reduced infarct size, or less severe paralysis and/or paresis, or improved neurological condition in a subject suffering from ischemic stroke, and/or 4) reduced risk for developing lung fibrosis in a subject exposed to certain drugs (amiodarone, bleomycin, methotrexate, nitrofurantoin, busulfan), or radiation, or sarcoidosis or other connective tissue diseases, and/or 5) reduced pain or improved joint function in a subject suffering from osteo- or rheumatoid arthritis, and/or 6) reduced metastasis, or improved local function, or improved survival in a subject suffering from cancer.

In the present invention, the term "heart failure" is understood to refer to any condition characterized by decreased cardiac output and/or abnormal filling pressures in the ventricles. In these situation, the hearth is unable to pump blood at an adequate rate or in an adequate volume and/or in adequate force (i.e. systolic heart failure) or exhibits increased ventricular stiffness and/or disturbed ventricular relaxation (i.e. diastolic heart failure). In heart failure, blood perfusion of organs is hampered thereby deteriorating organ function (e.g. kidneys or liver failure). In addition, blood can back up into the lungs, causing the lungs to become congested with fluid. Typical symptoms of heart failure include shortness of breath (dyspnea), fatigue, weakness, difficulties breathing when lying flat, and swelling of the legs, ankles or abdomen (edema).

In the present invention, the terms "treating", "preventing", or "preventing progression of" are understood to not only encompasses the onset of adverse cardiac remodelling, but also encompasses the situation in which adverse cardiac remodelling has commenced but is halted from continuing further. Thus, it encompasses the situation in which full-blown development of myocardial infarction- and/or pressure-overload-related complications is prevented, even if such myocardial infarction- and/or pressure-overload-related complications have already started to develop. For example, cardiac dilatation that has already commenced can be stopped by the therapeutic intervention using the Ig-like molecules, or antibodies or antigen-binding fragment thereof as taught herein. Such method is encompassed by the present invention. As adverse remodelling is reversible, adverse remodelling-related complications, herein also referred to as "myocardial infarction and/or pressure-overload-related complications", can also be treated using the method of the present invention.

In the present invention, the term "subject" is understood to refer to any vertebrate animal, but will typically pertain to a mammal, for example a human patient, a domesticated animal (such as dog or cat), a farm animal (such as horse, cow, or sheep) or a laboratory animal (such as rat, mouse, non-human primate or guinea pig). In certain examples, the subject is human.

In one embodiment, the subject is selected from the group consisting of horse, dog and human. In another embodiment of the invention, the Ig-like molecules, or antibodies or fragment thereof as taught herein, the pharmaceutical composition as taught herein, and the method of the invention as taught herein may be used to prevent or treat complications related to myocardial infarction, pressure-overload and adverse cardiac remodelling and/or to increase survival or decrease mortality following myocardial infarction, pressure-overload and adverse cardiac remodelling in dogs and horses, particularly racing dogs and horses.

In a preferred embodiment, the subject is human, particularly a human subject at risk of developing adverse cardiac remodelling and/or a human subject having suffered a myocardial infarction, pressure-overload and adverse cardiac remodelling and/or a human subject suffering from complications related to myocardial infarction, pressure-overload and adverse cardiac remodelling. In another embodiment, the human subject is at risk of suffering or developing fibronectin-EDA-mediated adverse tissue remodelling in the lungs (e.g. dyspnea, pulmonary hypertension and lung dysfunction that result from lung fibrosis), in joints (e.g. joint dysfunction that results from rheumatoid and/or osteoarthritis), in the brain (e.g. seizures, paralysis and/or paresis, and infarction of the brain that result from an ischemic stroke). The present inventor found that the Ig-like molecules, antibodies or antigen-binding fragments thereof as taught herein increased survival and improved cardiac function following myocardial infarction and pressure overload, presumably by preventing, treating and/or preventing progression of adverse cardiac remodelling in a subject.

Methods of carrying out the conventional techniques used in the present invention will be evident to the skilled person. The practice of conventional techniques in molecular biology, biochemistry, computational chemistry, cell culture, recombinant DNA, bioinformatics, genomics, sequencing and related fields are well-known to those of skill in the art and are discussed, for example, in the following literature references: Sambrook et al., Molecular Cloning. A Laboratory Manual, 2nd Edition, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N. Y., 1989; Ausubel et al., Current Protocols in Molecular Biology, John Wiley & Sons, New York, 1987 and periodic updates; and the series Methods in Enzymology, Academic Press, San Diego.

In this document and in its claims, the verb "to comprise" and its conjugations is used in its non-limiting sense to mean that items following the word are included, but items not specifically mentioned are not excluded. In addition, the verb "to consist" may be replaced by "to consist essentially of" meaning that a composition of the invention may comprise additional component(s) than the ones specifically identified, said additional component(s) not altering the unique characteristics of the invention.

The term "and/or" as used herein refers to a situation wherein one or more of the stated cases may occur, alone or in combination with at least one of the stated cases, up to with all of the stated cases.

The term "at least" refers to a situation wherein a particular value is the same as said particular value or more. For example, "at least 2" is understood to be the same as "2 or more" i.e., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, ... , etc.

The indefinite article "a" or "an" thus usually means "at least one". It is further understood that, when referring to "sequences" herein, generally the actual physical molecules with a certain sequence of subunits (e.g. amino acids) are referred to.

Figures

Figure 1 depicts the percentage of survival following myocardial infarction observed over a period of 30 days for mice treated with various antibodies directed against the EDA domain of the fibronectin-EDA (i.e. 17G8.72, 27A12.70, 29E7.35, and 42H11.51) relative to mice treated with saline.

Figure 2 depicts the ejection fraction (EF) following myocardial infarction for mice treated with various antibodies directed against the EDA domain of the fibronectin-EDA (i.e. 17G8.72, 27A12.70, 29E7.35, and 42H11.51).

Figure 3 depicts the percentage of survival following myocardial infarction observed over a period of 30 days for mice treated with antibody 33E3.10, which is directed against the EDA domain of fibronectin-EDA, relative to mice treated with saline.

Figure 4 depicts the ejection fraction (EF) following myocardial infarction for mice treated with antibody 33E3.10, which is directed against the EDA domain of the fibronectin-EDA.

Figure 5: Improved cardiac function after MI with anti-EDA treatment. MRI images of A) a saline treated and B) an anti-EDA treated mouse heart. C) End-diastolic volume (EDV) increase after MI. Anti-EDA treatment shows attenuated increase in EDV compared to saline *p=0.011 and #p=0.014 and isotype control treated animals. D) End-systolic volume (ESV) increase after MI. compared to saline *p=0.011 and #p=0.014 isotype

treated animals; n=14 in saline, n=9 in anti-EDA treated animals and n=5 in isotype control treated animals.

Figure 6: Anti-EDA treatment reduces blood leukocyte levels after 7 days of MI. A) Ly-6G+ neutrophils were significantly reduced in the anti-EDA treated group compared to control treated animals. B) CD3+ T-cells were non-significantly reduced after anti-EDA treatment. C) Ly-6C+/Ly-6G- monocytes and D) Ly-6C-/Ly-6G- monocytes were non-significantly reduced after anti-EDA treatment. E) CD49d expression was significantly increased on Ly-6C-/Ly-6G- monocytes after anti-EDA treatment. N=8 in control group, N=5 in anti-EDA group. Data is expressed as mean ± SEM.

Figure 7: Inflammatory cytokines are decreased in the infarct area; however this does not affect leukocyte numbers. A) The pro-inflammatory cytokines IL-1b (p=0.03), GM-CSF (p=0.04), TNF-a (p=ns), MIP-1b (p=ns), RANTES (p=0.01), IL-4 (p=0.02) are decreased in the infarcted area of anti-EDA treated animals. The anti-inflammatory cytokine IL-10 is decreased (p=ns) and IL-17 is increased (p=0.03) in the infarcted area of anti-EDA treated animals. P values are not corrected for multiple testing. B) Example of Ly-6G neutrophil staining in the infarcted area. C) Quantification of neutrophil staining. D) Example of Mac-3 macrophage staining in the infarcted area. E) Quantification of neutrophil staining. N=8 in control group, N=5 in anti-EDA group. Data is expressed as mean ± SEM.

Figure 8: Scar formation is not affected by anti-EDA treatment. A) Example of polarized light picture of picrosirius red staining in the infarcted area. B) Quantification of picrosirius red staining. C) Quantification of mRNA levels of Col1 in the infarcted area. D) Quantification of mRNA levels of Col3 in the infarcted area. N=8 in control group, N=5 in anti-EDA group. Data is expressed as mean ± SEM.

Figure 9: Anti-EDA treatment increases small vessel formation in border and infarct zone. A) CD31 vessel staining in the border zone of a control heart. B) CD31 vessel staining in the border zone of an anti-EDA treated heart. C) Quantification of total vessels in the border zone. There is an increase in total vessel number in the anti-EDA treated group ($p<0.0001$) D) Vessels are subdivided in classes based on diameter. The increase in total vessel number in the anti-EDA treated group is mainly due to an increase in small vessels 5-10 µm ($p<0.0001$) E) Quantification of total vessels in the infarct zone. There is an increase in total vessel number in the anti-EDA treated group ($p=0.05$). F) Vessels are subdivided in classes based on diameter. The increase in total vessel number in the anti-EDA treated group is mainly due to an increase in small vessels 10-16 um ($p=0.01$) G) Example of a positive control of sprouting assay H) Example of EDA inhibition in spouting assay. I) Quantification of relative sprouting. Negative

control is set to 1. N=8 in control group, N=5 in anti-EDA group. Data is expressed as mean ± SEM.

Figure 10: There is a delayed clearance of acellular matrix upon anti-EDA treatment. A) Representative image of acellular matrix in control animal after 7 days of MI. B) Representative image of acellular matrix in anti-EDA treatment animal after 7 days of MI. C) Quantification of acellular matrix. There is more acellular matrix present in the anti-EDA treated group. ($p<0.05$) D) Zymogram showing MMP2 activity in heart tissue from control and anti-EDA treated animals. E) Quantification of total MMP2 activity. There is a decrease in MMP2 activity in the anti-EDA treated animals. F) Quantification of different MMP2 forms. There is a decrease in all different MMP2 forms in the anti-EDA treated animals. G) Quantification of periostin staining in the infarct area of control and anti-EDA treated animals. There is a decrease in periostin staining in the anti-EDA treated group. H) Quantification of fibroblast adhesion assay. Fibroblast adhere more to EDA-his fragment coating compared to III4-his fragment coating or no coating. This adhesion to EDA-his fragments can be inhibited by the anti-EDA antibody and not by isotype control. N=1. N=8 in control group, N=5 in anti-EDA group. Data is expressed as mean ± SEM.

Figure 11: EDA immunohistochemical staining in infarcted human myocardium. A) Myocardial infarction with coagulation necrosis of cardiomyocytes and infiltration of some neutrophil granulocytes. B) EDA immunostain of necrotic myocardium showing weak red staining in the infarcted area. C) EDA immunostain at the border of the infarcted area showing cytoplasmatic and nuclear staining (in red) in cardiomyocytes surrounding the infarct. D) Young granulation tissue with numerous fibroblasts. E) EDA immunostain showing strong cellular staining of fibroblasts. Inlay higher magnification of the EDA positive fibroblasts. F) EDA immunostain of myocardium surrounding the granulation tissue with weak cytoplasmatic and strong nuclear staining of part of the surrounding cardiomyocytes. G) Scar tissue with collagenous connective tissue with some fibroblasts. H) and I) Absent EDA immunostaining of the scar and surrounding myocardium. All bars are 100 µm. Representative pictures of 3 patients per time point; H&E= Hematoxylin and eosin staining.

Figure 12. A) Ejection fraction (EF) and B) heart/body weight ratio 6 weeks after pressure-overload induced by transaortic constriction (TAC) in mice. Differences between EDA-deficient (KO; EDA-/-) and wild-type (WT) mice are at $p<0.05$ level. (EDA-deficient mice are described in Arslan F. et al. Circ. Res., Mar 2011; 108: 582 – 592 and WO2012/057613, Transaortic constriction (TAC) in the mouse is a commonly used

experimental model for pressure overload-induced cardiac hypertrophy and heart failure).

Sequences

SEQ ID NO: 1: Amino acid sequence of the epitope GIXXXF

SEQ ID NO: 2: Amino acid sequence of the epitope GIHELF

SEQ ID NO: 3: CDR1 of heavy chain variable region (antibody 27A12.70)
GYSITSGYSWH

SEQ ID NO: 4: CDR2 of heavy chain variable region (antibody 27A12.70)
YIHYSGIANYNPSLKS

SEQ ID NO: 5: CDR3 of heavy chain variable region (antibody 27A12.70)
EKTGFFDY

SEQ ID NO: 6: CDR1 of light chain variable region (antibody 27A12.70)
RSSQSLVHSNGNTYLH

SEQ ID NO: 7: CDR2 of light chain variable region (antibody 27A12.70)
KVSNRFS

SEQ ID NO: 8: CDR3 of light chain variable region (antibody 27A12.70)
SQSAHVPPT

SEQ ID NO: 9: CDR1 of heavy chain variable region (antibody 29E7.35)
GYSITSGYSWH

SEQ ID NO: 10: CDR2 of heavy chain variable region (antibody 29E7.35)
YIHYSGSANYNPSLKS

SEQ ID NO: 11: CDR3 of heavy chain variable region (antibody 29E7.35)
EKTGFFDY

SEQ ID NO: 12: CDR1 of light chain variable region (antibody 29E7.35)

RSSQSLVHSNGNTYLH

SEQ ID NO: 13: CDR2 of light chain variable region (antibody 29E7.35)
KVSNRFS

SEQ ID NO: 14: CDR3 of light chain variable region (antibody 29E7.35)
SQSAHVPPT

SEQ ID NO: 15: CDR1 of heavy chain variable region (antibody 17G8.7VL)
GYSIASGYSWH

SEQ ID NO: 16: CDR2 of heavy chain variable region (antibody 17G8.7VL)
YIHFSGSANYNPSLKS

SEQ ID NO: 17: CDR3 of heavy chain variable region (antibody 17G8.7VL)
EARGYFDY

SEQ ID NO: 18: CDR1 of light chain variable region (antibody 17G8.7VL)
RSSQSIVRSNGNTYLT

SEQ ID NO: 19: CDR2 of light chain variable region (antibody 17G8.7VL)
KVSNRFS

SEQ ID NO: 20: CDR3 of light chain variable region (antibody 17G8.7VL)
FQGSHVPPT

SEQ ID NO: 21: CDR1 of the heavy chain variable region (consensus)
GYSIX$_1$SGYSWH
X$_1$=T or A

SEQ ID NO: 22: CDR2 of the heavy chain variable region (consensus)
YIHX$_2$SGX$_3$ANYNPSLKS
X$_2$= Y or F; and X$_3$= S or I

SEQ ID NO: 23: CDR3 of the heavy chain variable region (consensus)
EX$_4$X$_5$GX$_6$FDY

X_4= K or A; and X_5= T or R; and X_6= F or Y

SEQ ID NO: 24: CDR1 of the light chain variable region (consensus)
RSSQSX$_7$VX$_8$SNGNTYLX$_9$
X_7= L or I; and X_8= H or R; and X_9= H or T

SEQ ID NO: 25: CDR2 of the light chain variable region (consensus)
KVSNRFS

SEQ ID NO: 26: CDR3 of the light chain variable region (consensus)
X$_{10}$QX$_{11}$X$_{12}$HVPPT
X_{10}= S or F; and X_{11}= S or G; and X_{12}= A or S

SEQ ID NO: 27 (immunizing peptide)
TYSSPEDGIHELFPAPDGEEDTAELQGGC

SEQ ID NO: 28: Amino acid sequence of the epitope on the EDA domain of fibronectin-EDA for antibody 33E3.10)
LFPAP

SEQ ID NO: 29: CDR1 of heavy chain variable region (antibody 33E3.10)
GFTFSNSAMT

SEQ ID NO: 30: CDR2 of heavy chain variable region (antibody 33E3.10)
SISGGGTTYYPDSVKG

CDR3 of heavy chain variable region (antibody 33E3.10)
SHY

SEQ ID NO: 31: CDR1 of light chain variable region (antibody 33E3.10)
KASQNVVTNVA

SEQ ID NO: 32 CDR2 of light chain variable region (antibody 33E3.10)
SASYRYS

SEQ ID NO: 33: CDR3 of light chain variable region (antibody 33E3.10)

QQYNSYPYT

SEQ ID NO: 34: Humanized heavy chain of antibody 27A12.70
QVQLQESGPGLVKPSQTLSLTCTVSGYSITSGYSWHWIRQHPGKKLEWMGYIHYSGIA
NYNPSLKSRITISRDTSKNQFSLKLSSVTAADTAVYYCATEKTGFFDYWGQGTLVTVSS
ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS
SGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP
PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRL
TVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 35: Humanized light chain of antibody 27A12.70
DVVMTQTPLSLSVTPGQPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQLLIYKVSN
RFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSAHVPPTFGGGTKVEIKRTVA
APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 36: Humanized heavy chain of antibody 33E3.10
EVQLLESGGGLVQPGGSLRLSCAASGFTFSNSAMTWVRQAPGKRLEWVASISGGGTT
YYPDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSHYWGQGTLVTVSSAST
KGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG
LYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEAAGGPS
VFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQF
NSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPS
QEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLT
VDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

SEQ ID NO: 37: Humanized light chain of antibody 33E3.10
DIQMTQSPSSVSASVGDRVTITCKASQNVVTNVAWYQQKPGKSPKALIYSASYRYSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYFCQQYNSYPYTFGGGTKVEIKRTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL
SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 38: heavy chain variable region (antibody 27A12.70)

DVQLQESGPDLVKPSQSLSLTCTVT**GYSITSGYSWH**WIRQFPGNKLEWMG**YIHYSGI**
ANYNPSLKSRISITRDTSKNHFFLQLNSVTTEDTATYYCAT**EKTGFFDY**WGQGTTLT
VSS

SEQ ID NO: 39: light chain variable region (antibody 27A12.70)
DVVMTQTPLSLPVSLGDQASISC**RSSQSLVHSNGNTYLH**WYLQKPGQSPKLLIY**KVS**
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**SQSAHVPPT**FGGGTKLEIKR

SEQ ID NO: 40: heavy chain variable region (antibody 29E7.35)
AVQLQESGPDLVKPSHSLSLTCTVT**GYSITSGYSWH**WIRQFPGNKLEWMG**YIHYSGS**
ANYNPSLKSRFSITRDTSKNQFFLQLNSVTTEDTATYYCAT**EKTGFFDY**WGQGTTLT
VSS

SEQ ID NO: 41: light chain variable region (antibody 29E7.35)
DVVMTQTPLSLPVSLGDQASISC**RSSQSLVHSNGNTYLH**WYLQKPGQSPKLLIY**KVS**
NRFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**SQSAHVPPT**FGGGTKLEIKR

SEQ ID NO: 42: heavy chain variable region (antibody 17G8.7VL)
DVQLQESGPDLVKPSQSLSLTCTVT**GYSIASGYSWH**WIRQFPGNKLEWMG**YIHFSGS**
ANYNPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCAS**EARGYFDY**WGQGTTLT
VSS

SEQ ID NO: 43: light chain variable region (antibody 17G8.7VL)
DVLMTQTPLSLPVSLGDQASISC**RSSQSIVRSNGNTYLT**WYLQKPGQSPKLLIY**KVSN**
RFSGVPDRFSGSGSGTDFTLKISRVEAEDLGVYFC**FQGSHVPPT**FGSGTKLEIKR

SEQ ID NO: 44: heavy chain variable region (antibody 33E3.10)
EVKLVESGGGLVKPGGSLKLSCAAS**GFTFSNSAMT**WVRQTPEKRLEWVA**SISGGGT**
TYYPDSVKGRFTISRDNARNILYLQMSSLRSEDTAIYYCAR**SHY**WGQGTTLTVSS

SEQ ID NO: 45: light chain variable region (antibody 33E3.10)
DIVMTQSQKFMSTSIGDRVSVTC**KASQNVVTNVA**WYQQKPGQSPKALIY**SASYRYS**G
VPDRFTGSGSGTDFTLTISNVQSEDLAEYFC**QQYNSYPYT**FGGGTKLEIKR

Examples

Example 1

Methods

Peptide synthesis and screening assays

Linear and CLIPS peptides were synthesized based on the amino acid sequence of the target peptide (i.e. TYSSPEDGIHELFPAPDGEEDTAELQGGC (SEQ ID NO: 27)) using standard Fmoc-chemistry and deprotected using trifluoric acid with scavengers. The linear and CLIPS peptides were short fragments of various length, which may possibly contain the epitope. The constrained CLIPS peptides were synthesized on chemical scaffold in order to reconstruct conformational epitopes, using Chemically Linked Peptides on Scaffolds (CLIPS) technology. For example, the single looped peptides were synthesized containing a dicysteine, which was cyclized by treating with alpha, alpha'-dibromoxylene. The size of the loop was varied by introducing cysteine residues at variable spacing. If other cysteines besides the newly introduced cysteines were present, they were replaced by alanine. The side-chains of the multiple cysteines in the peptides were coupled to CLIPS templates by reacting onto credit-card format polypropylene PEPSCAN cards (455 peptide formats/card) with a 0.5 mM solution of CLIPS template such as 1,3-bis (bromomethyl) benzene in ammonium bicarbonate (20 mM, pH 7.9)/acetonitrile (1:1 (v/v)). The cards were gently shaken in the solution for 30 to 60 minutes while completely covered in solution. Finally, the cards were washed extensively with excess of H_2O and sonicated in disrupt-buffer containing 1 percent SDS/0.1 percent beta-mercaptoethanol in PBS (pH 7.2) at 70 °C for 30 minutes, followed by sonication in H_2O for another 45 minutes.

The binding of antibody to each peptide was tested in a PEPSCAN-based ELISA. The 455-well credit card format polypropylene cars containing the covalently linked peptides were incubated with primary antibody solution consisting of 0.05 micrograms antibody/mL diluted in blocking solution, i.e., 4 % horse serum, 5% ovalbumin (w/v) in PBS/0.1% Tween. After washing, the peptides were incubated with a 1/1000 dilution of antibody peroxidase conjugate for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ehtylbenzthiazoline sulfonate (ABTS) and 2 microliters of 3 percent H_2O_2 were added. After one hour, the colour development was measured. The colour development was quantified with a charge coupled device (CCD)-camera and an image processing system (as firstly described in Slootstra et al. Mol Divers. 1996 Feb;1(2):87-96).

Data calculation

Raw Data: Optical density (Arbitrary OD units)

The raw data were optical values obtained by a CCD-camera. The values mostly ranged from 0 to 3000, a log scale similar to 1 to 3 of a standard 96-well plate ELISA-reader. First, the CCD camera made a picture of the card prior to peroxidase coloring and then again after the peroxidase colouring. These two pictures were subtracted from each other providing raw data. The raw data were copied into the Peplab^{tm} database. The cards were manually inspected to correct for false-positives (e.g., presence of an air bubble).

Results

The epitopes for antibodies 27A12.70, 29E7.35, 17G8.72, 42H11.51 and 33E3.10 were mapped using the CLIPS™ technology (Timmerman et al. J Mol Recognit. 2007 Sep-Oct;20(5):283-99.) with overlapping 7 to 14–mer linear and CLIPS peptides of which the sequence was based on the peptide used for immunization (SEQ ID NO:27). The epitope for antibodies 27A12.70, 29E7.35, 17G8.72, and 42H11.51 was found to consist of amino acids GIHELF. The epitope for antibody 33E3.10 was found to consist of amino acids LFPAP.

Additionally, alanine-scanning mutagenesis was performed to determine the key amino acids within the epitope for the antibodies 27A12.70, 29E7.35, 17G8.72, and 42H11.51 using a 13-mer peptide ranging from Tyr^{36} through Pro^{48}. Alanine-scanning mutagenesis revealed that Gly^{42}, Ile^{43} and Phe^{47} are the crucial amino acids within the epitope GIHELF.

Example 2

Animals and experimental design

Male Balb/C wild-type mice (10-12 weeks, 25-30 g) received standard diet and water *ad libitum*. Myocardial infarction was induced by left coronary artery ligation, just below the left atrial appendage. All animal experiments were performed in accordance with the national guidelines on animal care and with prior approval by the Animal Experimentation Committee of Utrecht University.

Myocardial infarction in vivo

Mice (Balb/C) were anesthetized with a mixture of Fentanyl (Jansen-Cilag) 0.05 mg/kg, Dormicum (Roche) 5 mg/kg and medetomidine 0.5 mg/kg through an intraperitoneal injection. Core body temperature was maintained around 37°C during surgery by continuous monitoring with a rectal thermometer and automatic heating blanket. Mice were intubated and ventilated (Harvard Apparatus Inc.) with 100% oxygen. The left coronary artery (LCA) was permanently ligated using an 8-0 vicryl suture. Ischemia was

confirmed by bleaching of the myocardium and ventricular tachyarrhythmia. In sham operated animals the suture was placed beneath the LCA without ligating. The chest wall was closed and the animals received subcutaneously Antisedan (Pfizer) 2.5 mg/kg, Anexate (Roche) 0.5 mg/kg and Temgesic (Schering-Plough) 0.1 mg/kg.

Mice underwent cardiac function and geometry assessment 28 days after myocardial infarction. Mice were given intravenously 250 microliters (µl) of saline (control) or antibody solution via the tail vein. The animals were randomized to receive saline, 27A12.70 (10 mg/kg), 29E7.35 (10 mg/kg), 17G8.72 (10 mg/kg), 42H11.51 (10 mg/kg) or 33E3.10 (10 mg/kg) monoclonal antibody 2 days after infarction. The bolus injections were repeated at day 4 and day 5 post-infarction. The 2nd and 3rd antibody injections were given at a dosage of 10 mg/kg.

Results

Percentage of Survival

Antibodies 27A12.70, 29E7.35, 17G8.72, 42H11.51

No differences were observed between the different groups of mice at baseline (t=0). However, a differential survival profile emerged depending on the antibody tested. The antibodies tested increased survival of mice following myocardial infarction relative to the control situation (saline). Mice treated with the antibodies 27A12.70 and 29E7.35 displayed the highest percentage of survival after myocardial infarction over 30 days follow-up after myocardial infarct (Fig. 1). Specifically, it was observed that the percentage of survival for mice treated with antibodies 27A12.70 and 29E7.35 was significantly improved over the percentage of survival for mice in the control group (saline), where only 50% of mice survived after a period of 30 days post myocardial infarction. Additionally, survival of mice treated with antibodies 17G8.72 and 42H11.51 was improved compared to mice in the control group (saline).

Antibody 33E3.10

No differences were observed between the two groups of mice at baseline (t=0). However, a differential survival profile emerged depending on the antibody tested. The antibodies tested increased survival of mice following myocardial infarction relative to the control situation (saline). Mice treated with the antibody 33E3.10 displayed increased survival after myocardial infarction over 30 days follow-up after myocardial infarct (Fig. 3). Specifically, it was observed that the percentage of survival for mice treated with antibody 33E3.10 was significantly improved over the percentage of survival for mice in the control group (saline), where only 50% of mice survived after a period of 30 days post myocardial infarction.

Example 3

Myocardial infarction in vivo

Male Balb/C wild-type mice (10-12 weeks, 25-30 g) were subjected to the procedure for induction of myocardial infarction as taught herein in example 2. They were also treated with the same antibodies.

Echocardiography

Mice underwent serial assessments of cardiac dimensions and function by high resolution echocardiography (Vevo 2100, FUJIFILM VisualSonics, Inc., Toronto, Canada) under isoflurane anaesthesia 28 days after myocardial infarction. Long axis and short axis images with 1.0 mm interval between the slices were obtained and used to compute end-diastolic volume (EDV, largest volume) and end-systolic volume (ESV, smallest volume). The ejection fraction (EF) was calculated as 100*(EDV-ESV)/EDV.

Results

Antibodies 27A12.70, 29E7.35, 17G8.72, 42H11.51

The results showed that relative to mice treated with saline, mice treated with antibodies 27A12.70, 29E7.35, 17G8.72 and 42H11.51 displayed improved heart function following myocardial infarction, as indicated by an increase in ejection fraction (EF), with the highest improvement as shown by the higher percentage of EF measurement displayed upon treatment with antibodies 27A12.70 and 29E7.35 (Figure 2).

Antibody 33E3.10

The results showed that relative to mice treated with saline, mice treated with antibody 33E3.10 displayed improved heart function following myocardial infarction, as indicated by an increase in ejection fraction (EF), with the highest improvement as shown by the higher percentage of EF measurement displayed upon treatment with antibody 33E3.10 (Figure 4).

Example 4

Methods

Peptide synthesis, screening assays and data calculation for alanine-scan of fibronectin-EDA epitope are performed as described in Example 1. Peptides based on TYSSPEDGIHELFP were synthesized wherein in each peptide one amino acid is replaced by an alanine. The peptides were tested for binding by antibodies 27A12.70, 29E7.35, 17G8.72 and 42H11.51.

Results

Results are displayed in the table below.

		Antibody binding (arbitrary optical density units)			
Peptide	**Replaced amino acid**	**17G8.72**	**27A12.70**	**29E7.35**	**42H11.51**
TYSSPEDGIHELFP	none	2087	2681	2754	2845
AYSSPEDGIHELFP	T	2146	2709	2779	2859
TASSPEDGIHELFP	Y	2668	2786	2841	2865
TYASPEDGIHELFP	S	2501	2719	2735	2867
TYSAPEDGIHELFP	S	2098	2680	2631	2853
TYSSAEDGIHELFP	P	2091	2712	2656	2866
TYSSPADGIHELFP	E	2481	2693	2627	2835
TYSSPEAGIHELFP	D	2646	2751	2724	2834
TYSSPEDAIHELFP	G	89	122	81	433
TYSSPEDGAHELFP	I	66	91	74	120
TYSSPEDGIAELFP	H	2563	2405	1798	2869
TYSSPEDGIHALFP	E	2101	2204	302	2842
TYSSPEDGIHEAFP	L	1552	1783	194	2819
TYSSPEDGIHELAP	F	106	249	124	2642
TYSSPEDGIHELFA	P	2164	2601	2596	2869
Epitope		GIHELF	GIHELF	GIHELF	GIHEL
Crucial amino acids		G, I, F	G, I, F	G, I, E, L, F	G, I

Example 5

Methods

Animals and experimental design

Male Balb/C wild-type (WT) (10-12 wks, 25-30 g) received standard diet and water ad libitum. Myocardial infarction was induced by left coronary artery ligation, just below the left atrial appendage as described in Example 2. Animals were randomized to anti-EDA IgG1 antibody (20 mg/kg; 250 µL) directed against the epitope GIXXXF or saline control.

Another group with isotype control (20 mg/kg; 250 µL) was also included at a different time point. Treatment or placebo was initiated intravenously 3 days after MI. A researcher blinded to treatment assessed heart function and geometry. All animal experiments are performed in accordance with the national guidelines on animal care and with prior approval by the Animal Experimentation Committee of Utrecht University.

Anti-EDA IgG1 monoclonal antibody

IgG1 and IgG2b isotypes were separately purified from conditioned hybridoma medium by differential pH elution from Protein A Sepharose, and purified IgG1 was used in experiments described in this study.

Infarct Size & Cardiac Magnetic Resonance Imaging

Infarct size was assessed in a subset of mice prior to compound infusion using late Gadolinium enhancement magnetic resonance imaging (LGE-MRI), 3 days after MI. Local and global cardiac function as well as left ventricular geometry was assessed by high resolution MRI (9.4T, Bruker, Rheinstetten, Germany).

Flow cytometry

Blood was collected in EDTA tubes 7 days after MI. Fifty (50) µL of blood was added to 100 µL of antibody mixture and incubated for 30 min in the dark at RT. After red blood cell lysis using Optilyse buffer (Beckman Coulter) for 10 min the samples were measured on the Gallios (Beckman Coulter).

Quantitative PCR

Isolated total RNA from mouse hearts was reverse transcribed to cDNA using the iScript™ cDNA sythesis kit (Bio-Rad) following the manufacturer's guidelines. Quantitative PCR was performed using the SYBR green (Bio-Rad) method on the iQ™5 Real-Time PCR Detection System (Bio-Rad). The following sets of primers were used in the study: collagen I pro-alpha I chain (forward), (reverse); collagen III pro-alpha I chain (forward), (reverse); TIMP-1 (forward), (reverse);TIMP-2 (forward), (reverse), MMP-2 (forward), (reverse); P0 (forward), (reverse), RPL27 (forward), (reverse), EDA-FN (forward), (reverse), total FN (forward), (reverse). Each sample was run in duplicate. Gene expression levels were normalized to P0 and RPL27 using the protocol described in (Willems, Leyns, & Vandesompele, Anal Biochem. 2008 Aug 1;379(1):127-9). To assess qPCR efficiency, a standard curve with 5 different cDNA dilutions was included.

Zymography

MMP activity in the infarcted myocardium and in collagen pads was examined by gelatin zymography. Running gel (2.68 ml 30% (bis)acrylamide (Bio-Rad), 4.82 ml 2 mg/ml porcine skin gelatin solution (Sigma-Aldrich), 2.5 ml Tris-HCl 1.5 M pH 8,8 (Roche), 100 µl 10% SDS (Sigma-Aldrich) 50 µl APS and 17.8 µl of TEMED (GE Life Sciences, Pittsburgh, US)) and stacking gel (0.67 ml 30% (bis)acrylamide, 3.04 ml aqua dest, 1.25 ml Tris-HCl 0.5 M pH 6.8, 50 µl 10% SDS, 25 µl APS, and 8.8 µl of TEMED)) were poured. Myocardial samples were homogenized in Roche lysis buffer. The protein concentration was measured using BCA Protein Assay Kit (Thermo Scientific).The myocardial extracts at 5 µg were mixed in a ratio 1:4 with Laemlli buffer and loaded on the gels. The gels were run at 30 mA through stacking phase and at 60 mA for running phase. After running the gels were washed 2x15 min in 2.5% Triton X-100 to remove SDS. Then, gels were incubated overnight in Brij-solution (50 mM Tris-HCl pH 7.4; 10 mM CaCl2 (Merck, Whitehouse Station, USA); 0.05% Brij35 (Sigma-Aldrich)). After incubation, the gels were stained with Coomassie blue (0.1% Coomassie Brilliant blue R-250 (Bio-Rad), 25% methanol and 15% acetic acid (both from Sigma-Aldrich)) and subsequently destained (in 25% methanol and 15% acetic acid) until clear bands appeared against blue background. Pictures were taken on a Chemidoc XRS+. Images were analyzed using ImageLab (Bio-Rad).

Cell culture

Human mammary endothelial cells (HMECs) were cultured in MCDB131 medium (10372-019,Gibco) supplemented with 10% FBS, pen/strep, 50 mM Hydrocortison, 50 mM epidermal growth factor and L-glutamine. Cells were subcultured every three days. The NIH3T3 fibroblast cell line was used for adhesion studies. Cells were cultured in DMEM medium (41965, Gibco) supplemented with 10% FBS and pen/strep. Cells were subcultured every three days.

Sprouting assay

Cytex beads were coated with HMECs and placed in matrigel (Corning). Bare MCDB131 medium was used as a negative control, full MCDB131 as positive control. EDA fragments and III4 fragments were added in a concentration of 1 µM. Pictures were taken at 48 hours and quantified using photoshop and ImageJ.

Cell adhesion assay

Ninety-six (96) wells plate was coated with 1 µM of EDA-his or III4-his fragments for 1 hour at 37°C, then blocked with PBS 1%BSA. NIH3T3 0.5x10^5 cells were added per well in bare DMEM and incubated for 1 hour at 37°C. After washing of unattached cells, the attached cells were fixed and stained using 0.5% crystal violet 1% formaldehyde, 20% methanol. The plate was read with a microplate reader at 540 nm.

Protein purification

BL21 pLysS E.coli were transformed with 1 µl of the DNA constructs and plated on Yeast tryptone ampicillin/chloramphenicol plates overnight. The next day all the colonies were scraped off and grown in LB medium with Amp/Chlor till an OD of 0.6-0.8 was reached. IPTG was added to start the production of the protein. After 24 hours, bacteria were pelleted and lysed, using lysis buffer and sonication steps. Debris was pelleted and supernatant was used for further processing. Ni-NTA beads were used for his purification. Briefly, Ni-NTA beads were incubated ON with supernatant at 4°C. Beads were poured into columns on the ACTA system. After a couple of washes, bound EDA-his or III4-his was eluted using 300 mM imidazole. Fractions containing EDA-his or III4-his were collected and injected on a gel filtration column. Proteins were separated based on size and eluted in HEPES buffer. Fractions containing EDA-his or III4-his were pooled and concentrated using Vivaspin 5 kD concentration columns. Fragment concentration was assed using BCA kit. LAL assays were performed to check for endotoxins. Fragments were used with a concentration lower than <0.1 EU per uM .

Histology

Upon termination, hearts were excised and fixated in 4% formaldehyde and embedded in paraffin. Paraffin sections were stained for Ly-6G (for neutrophils; rat anti-mouse Ly-6G, Abcam, Cambridge, United Kingdom), MAC-3 (for macrophages; rat anti-mouse MAC-3, BD Pharmingen, Breda, the Netherlands) and CD31 (for vessels, Santa Cruz, Heidelberg, Germany).

Before staining, sections were deparaffinized and endogenous peroxidase was blocked by 30 minutes incubation in methanol containing 1.5% H2O2. Antigen retrieval was performed by 20 minutes boiling in citrate buffer (MAC-3 and CD31) or by 15 minutes incubation at 37°C in a 0.08% pepsin solution (Ly-6G).

For MAC-3 staining, sections were pre-incubated with normal goat serum and incubated overnight at 4°C (MAC-3, 1:30; CD163, 1:500). For CD31 staining, sections were

incubated overnight at RT with the primary antibody (1:1500). Sections were then incubated for 1 hour at RT with a biotin labeled secondary goat antibody and 1 hour incubation with streptavidin-horseradish peroxidase at RT. All sections were developed with AEC.

For Ly-6G, sections were incubated for 1 hour at RT with the primary antibody (1:100). Sections were then incubated for 30 minutes with Powervision poly-HRP anti-rabbit IgG (ImmunoVision Technologies, Daily City, USA). The staining was immediately visualized with Vector NovaREDTM substrate kit following the manufacturer's instructions (Vector Laboratories Inc., Burlingame, USA).

All sections were counterstained with Mayer's hematoxylin stain.

Quantification of collagen density was performed using Picrosirius Red staining of 4% formalin fixated and paraffin embedded heart sections. Collagen density analysis was done with circularly polarized light after conversion into grey values and digital image microscopy. Grey values below 30 were considered as background signal of the image

Human EDA staining after acute MI

Myocardial tissue was obtained during autopsy of patients who died due to a myocardial infarction, and was retrieved from the Pathology archive. The study met the criteria of the code of proper use human tissue that is used in the Netherlands. To visualize EDA, sections were incubated with our monoclonal mouse-anti EDA antibody (dilution 1/800) after boiling in citrate buffer (pH 6.0). Poly AP-Anti-Mouse IgG (Immunologic, Duiven, the Netherlands) was used as secondary antibody and the signal was visualized using Liquid permanent red (Dako, Glostrup, Denmark).

Statistics

Data are represented as Mean±SEM. One-way ANOVA with post-hoc 2-sided Dunnett t-test adjustment (saline was set as control) was used for multiple comparisons between groups. Mann-Whitney U test was used to compare the difference in survival between anti-EDA and saline treated animals. All statistical analyses were performed using SPSS 15.1.1. and $p<0.05$ was considered significant.

Results

Anti-EDA treatment improves survival and prevents adverse remodeling after myocardial infarction

Baseline MRI assessment of cardiac function and dimensions revealed no differences between the treatment groups. Infarct size was 42±3% prior to compound infusion (Figure 5A). During the study period of 28 days, 3/9 of anti-EDA treated mice were found dead, while 9/14 saline treated animals died during follow-up (p=0.06). Pathological analysis revealed only 2 ruptures (both in saline group), while in the remaining cadavers excessive lung congestion was noticeable. Serial MRI assessments of cardiac function and geometry showed a significantly preserved left ventricular function as well as dimension in anti-EDA treated animals compared to both saline and isotype control (Figure 5; Table 1) .

Table 1. Cardiac function and geometry after acute MI

	Saline			Anti-EDA				
	Baseline	7 days MI	28 days MI	Baseline	7 days MI	†*p*	28 days MI	†*p*
EDV, µL	58.7±3.8	101.9±6.9*	151.2±13.4**	58.2±1.0	82.8±6.7*†	0.052	106.5±10.2**†	0.021
ESV, µL	25.1±2.2	86.3±4.3*	128.3±12.9**	26.4±.7	63.8±7.2*†	0.011	81.6±10.4**†	0.014
EF, %	57.1±3.2	14.7±2.8*	15.7±2.0**	54.7±1.3	23.6±3.0*	NS	24.4±2.5**†	0.067
WT septum (remote, mm)	0.90±0.02	0.70±0.03*	0.91±0.04	0.99±0.01	0.97±0.02*†	0.01	0.94±0.03	NS
WT free wall (infarct, mm)	0.93±0.02	0.57±0.04*	0.56±0.03**	0.91±0.01	0.65±0.06*	NS	0.60±0.04**	NS
SWT septum (remote, %)	49.2±2.0	49.7±5.0	42.4±3.7	50.9±2.9	32.0±2.4*†	0.006	27.8±8.6**	NS
SWT free wall (infarct, %)	54.8±2.3	-23.7±4.7*	-26.5±3.5**	51.1±5.0	-8.2±4.6*†	0.047	-7.8±3.1**†	0.002

Data are represented as Mean±SEM. *p-values are compared to baseline and below 0.05 level; †p-value compared to saline treatment. BPM=beats per minute, EDV=end-diastolic volume, ESV=end-systolic volume, EF=ejection fraction, WT=wall thickness, SWT=systolic wall thickening, NS=not significant.

Circulating Leukocyte numbers are decreased in anti-EDA treated mice/Anti-EDA treatment reduces leukocyte numbers in peripheral blood after MI

Leukocyte subset numbers in the blood were assessed using flow cytometry 7 days after MI. Anti-EDA treated mice showed a significant reduction in neutrophils and a trend towards reduced T-cell number after anti-EDA treatment (Figure 6a and b). The inflammatory $Ly6C^{pos}$ monocyte subset and the anti-inflammatory $Ly6C^{neg}$ monocyte subset were both not significantly decreased after anti-EDA treatment (Figure 6c and d).

Anti-EDA treatment reduces cytokine burst but not leukocyte numbers in the infarcted heart

In the infarcted area, the inflammatory cytokines IL-1β, TNFα, GM-CSF, IL-4, RANTES, IL-10 and MIP-1α were decreased in the anti-EDA treated animals (Figure 7a). No difference was observed in cytokine levels in the remote area of the heart (data not show). Neutrophil, T-cell and macrophage numbers were not affected by anti-EDA treatment 7 days after MI (Figure 7b to e).

Anti-EDA treatment does not affect proper scar formation

A proper scar formation is of utmost importance to prevent expansive remodeling, thinning of the scar and/or rupture of the infarcted wall after MI. In the present study, There was no difference in collagen content between control and anti-EDA treated animals 28 days after MI (Figure 8 a and b). In line with this observation, we did not observe any difference in the total amount of myofibroblasts, the main collagen producing cells after MI (data not shown) or mRNA levels of Col1 and Col3 (Figure 8c and d).

Anti-EDA treatment increases vessel formation in the heart

Upon anti-EDA treatment there were more vessels formed in both the infarct and the border zone (Figure 9). When we subdivide the vessels in different size classes, we can clearly see that this difference is in the smallest vessels of 5-10 µm in the border zone (Figure 9d) and in 10-16 µm vessels in the infarct area (Figure 9f). These classes correspond to capillaries in the myocardium. In vitro 3D sprouting assays show that EDA, and not control fragment III4, inhibits sprouting of endothelial cells (Figure 9g to i).

Anti-EDA treatment delays clearance of acellular matrix

A provisional acellular matrix formation is crucial for hemodynamic compensation for non-viable tissue and scar formation after MI. During wound healing the provisional matrix is slowly degraded and replaced by a firm collagen-based scar. We observed a delayed clearance of acellular matrix in anti-EDA treated mice (Figure 10a to c). To find an explanation for the delayed clearance of the provisional matrix we studied intrinsic MMP2 and -9 activity in the treatment groups. We found that anti-EDA treatment reduced MMP2 and -9 activity, quantified by zymography (Figure 10d to f). No change in mRNA levels of MMP2 and -9 was observed between the groups (data not shown). Fibroblasts are the main MMP producing cells in the heart. Periostin staining can estimate the amount of mature fibroblasts in the heart. Upon anti-EDA treatment, periostin expression is reduced (Figure 10g). Furthermore, in vitro cell adhesion assay show that fibroblast can adhere to EDA and that the anti-EDA antibody can prevent this cell adhesion (Figure 10h).

EDA is transiently expressed in the infarcted human heart

To date, there is no evidence of EDA expression in patients who suffered from an acute MI. We used post-mortem human heart specimens to study the timeline of EDA expression in human after MI. EDA was observed in the first 2-3 weeks after an infarction in the infarcted area and in the infarct border zone (Figure 11). EDA expression was observed in fibroblasts residing in young granulation tissue and in border zone cardiomyocytes. These findings are in line with our previous murine data, in which we show that EDA-FN is indeed temporarily expressed in the ischemic myocardium.

Claims

1. Antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising

   a heavy chain variable region comprising:

   - a CDRl having the amino acid sequence GYSIX$_1$SGYSWH, wherein X$_1$ is selected from T and A;
   - a CDR2 having the amino acid sequence YIHX$_2$SGX3ANYNPSLKS, wherein X$_2$ is selected from Y and F, and wherein X$_3$ is selected from S and I; and
   - a CDR3 having the amino acid sequence EX$_4$X$_5$GX$_6$FDY, wherein X$_4$ is selected from K and A, X$_5$ is selected from T and R, and X$_6$ is selected from F and Y;

   and a light chain variable region comprising:

   - a CDRl having the amino acid sequence RSSQSX$_7$VX$_8$SNGNTYLX$_9$, wherein X$_7$ is selected from L and I, X$_8$ is selected from H and R, and X$_9$ is selected from H and T;
   - a CDR2 having the amino acid sequence KVSNRFS; and
   - a CDR3 having the amino acid sequence X$_{10}$QX$_{11}$X$_{12}$HVPPT, wherein X$_{10}$ is selected from S and F, X$_{11}$ is selected from S and G, and X$_{12}$ is selected from A and S.

2. Antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising

   a heavy chain variable region comprising:

   - a CDRl having the amino acid sequence shown in SEQ ID NO:9;
   - a CDR2 having the amino acid sequence shown in SEQ ID NO:10; and
   - a CDR3 having the amino acid sequence shown in SEQ ID NO:11;

   and a light chain variable region comprising:

   - a CDRl having the amino acid sequence shown in SEQ ID NO:12;
   - a CDR2 having the amino acid sequence shown in SEQ ID N0:13; and
   - a CDR3 having the amino acid sequence shown in SEQ ID NO:14.

3. Antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising

   a heavy chain variable region comprising:

   - a CDR1 having the amino acid sequence shown in SEQ ID NO:3;
   - a CDR2 having the amino acid sequence shown in SEQ ID NO:4; and

- a CDR3 having the amino acid sequence shown in SEQ ID NO:5;

and a light chain variable region comprising:

- a CDRl having the amino acid sequence shown in SEQ ID NO:6;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:7; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:8.

4. Antibody that binds the EDA domain of fibronectin-EDA or antigen-binding fragment thereof comprising

a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:15;
- a CDR2 having the amino acid sequence shown in SEQ ID NO:16; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:17;

and a light chain variable region comprising:

- a CDRl having the amino acid sequence shown in SEQ ID NO: 18;
- a CDR2 having the amino acid sequence shown in SEQ ID NO: 19; and
- a CDR3 having the amino acid sequence shown in SEQ ID NO:20.

5. Antibody or antigen-binding fragment thereof according to any one of claims 1-4, wherein the CDRs of the light chain and/or the heavy chain are integrated into human-derived framework regions.

6. Antibody or antigen-binding fragment thereof according to anyone of claims 1-5, which is an antibody.

7. Antibody or antigen-binding fragment thereof according to claim 6, which is a chimeric antibody.

8. Antibody or antigen-binding fragment thereof according to claim 6 which is a humanized antibody.

9. Antibody or antigen-binding fragment thereof according to claim 8 comprising a heavy chain and a light chain, wherein said heavy chain has an amino acid sequence as shown in SEQ ID NO:34 and said light chain has an amino acid sequence as shown in SEQ ID NO:35.

10. Antibody or antigen-binding fragment thereof according to claim 8,comprising a heavy chain and a light chain, wherein said heavy chain comprises a constant region of an IgG4 heavy chain as set forth in SEQ ID NO:34 and said light chain comprises a constant region of an IgG4 light chain as set forth in SEQ ID NO:35.

11. Antibody or antigen-binding fragment thereof according to claim 10, wherein said heavy chain and/or light chain further comprise a variable region comprising one or more, preferably all, framework regions of an IgG4 heavy chain as set forth in SEQ ID NO:34 and/or of an IgG4 light chain as set forth in SEQ ID NO:35.

12. Antibody or antigen-binding fragment thereof according to any one of the claims 1-11 wherein the antibody or fragment thereof specifically binds to an amino acid sequence as set forth in SEQ ID NO: 2.

13. A nucleic acid molecule encoding the antibody or antigen-binding fragment thereof according to any one of claims 1-12.

14. A vector comprising the nucleic acid molecule of claim 13.

15. Vector according to claim 14, which is a gene therapy vector.

16. A host cell comprising the nucleic acid molecule of claim 13 or the vector of claims 14 or 15.

17. Host cell according to claim 16, which is a mammalian host cell.

18. Host cell according to any one of claims 16 or 17, which is a hybridoma.

19. A pharmaceutical composition comprising an agent selected from the group consisting of (a) the antibody or antigen-binding fragment thereof according to any one of claims 1-12, (b) a nucleic acid molecule according to claim 13, (c) a vector according to any one of claims 14-15 and (d) a host cell according to any one of claims 16-18, and a pharmaceutical acceptable carrier.

20. Antibody or antigen-binding fragment thereof according to any one of claims 1-11 or pharmaceutical composition according to claim 19 for use as a medicament.