

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-

5 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  $\alpha 4\beta 1$ ,  $\alpha 4\beta 7$  and  $\alpha 9\beta 1$ .

10 *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,

15 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 causes 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

20 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

25 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 of the invention

30 In one aspect, the present invention provides use of an antibody that binds fibronectin-

EDA or an antigen-binding fragment thereof for the preparation of a medicament for

improving angiogenesis, wherein the use is not for treating, preventing or preventing

progression of adverse cardiac remodelling and conditions resulting from or relating to

myocardial infarction.

Disclosed is use of an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for the preparation of a medicament for use in improving angiogenesis, preferably after tissue injury.

The disclosure provides an antibody that binds fibronectin-EDA or an antigen-binding

5 fragment thereof for use in improving angiogenesis, preferably after tissue injury.

Preferably wherein angiogenesis is improved in a subject suffering from ischemic disease, preferably in ischemic tissue of said subject. The disclosure further provides a method for stimulating angiogenesis in a subject in need thereof comprising administering to said subject a therapeutically effective amount of an antibody that binds fibronectin-EDA or

10 an antigen-binding fragment thereof. Preferably wherein said subject is suffering fro

Claims

1. Use of an antibody that binds fibronectin-EDA or an antigen-binding fragment thereof for the preparation of a medicament for improving angiogenesis, wherein the use  
5 is not for treating, preventing or preventing progression of adverse cardiac remodelling and conditions resulting from or relating to myocardial infarction.
2. The use according to claim 1, where in angiogenesis is improved after tissue injury, wherein the injury is selected from wounds, fibrotic tissue, and/or organ or tissue  
10 transplantation.
3. The use according to claim 1 or 2, wherein the antibody or antigen-binding fragment thereof binds an amino acid sequence as set forth in SEQ ID NO:1 or SEQ ID NO:28.  
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4. The use according to claim 3, wherein the antibody or antigen-binding fragment thereof binds to an amino acid sequence as set forth in SEQ ID NO:2.
5. The use according to any one of claims 3-4 wherein the antibody comprising a  
20 heavy chain variable region comprising:
  - a CDR1 having the amino acid sequence GYSIX<sub>1</sub>SGYSWH, wherein X<sub>1</sub> is selected from T and A;
  - a CDR2 having the amino acid sequence YIHX<sub>2</sub>SGX<sub>3</sub>ANYNPSLKS, wherein X<sub>2</sub> is selected from Y and F, and wherein X<sub>3</sub> is selected from S and I;
  - 25 – a CDR3 having the amino acid sequence EX<sub>4</sub>X<sub>5</sub>GX<sub>6</sub>FDY, wherein X<sub>4</sub> is selected from K and A, X<sub>5</sub> is selected from T and R, and X<sub>6</sub> is selected from F and Y.
6. The use according to claim 5 wherein the antibody comprising a light chain variable region comprising:  
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  - a CDR1 having the amino acid sequence RSSQSX<sub>7</sub>VX<sub>8</sub>SNGNTYLX<sub>9</sub>, wherein X<sub>7</sub> is selected from L and I, X<sub>8</sub> is selected from H and R, and X<sub>9</sub> 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.

5     7.     The use according to claim 3 wherein the antibody comprises  
a heavy chain variable region comprising

- a CDR1 having the amino acid sequence  $GYSIX_1SGYSWH$ , wherein  $X_1$  is selected from T and A;
- a CDR2 having the amino acid sequence  $YIHX_2SGX_3ANYNPSLKS$ , wherein  $X_2$  is  
10     selected from Y and F, and wherein  $X_3$  is selected from S and I; and
- a CDR3 having the amino acid sequence  $EX_4X_5GX_6FDY$ , 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  $RSSQSX_7VX_8SNGNTYLX_9$ , wherein  $X_7$  is  
15     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  
20     S.

8.     The use according to claim 3 wherein the antibody comprises a heavy chain  
variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:9;
  - 25     – 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
- 30     – a CDR3 having the amino acid sequence shown in SEQ ID NO:14.

9.     The use according to claim 3 wherein the antibody comprises  
a heavy chain variable region comprising:

- a CDR1 having the amino acid sequence shown in SEQ ID NO:3;
- 35     – 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.

10. The use according to claim 3 wherein the antibody 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.

11. The use according to claim 3 wherein the antibody 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.

12. The use according to any one of claims 3-4, wherein the CDRs of the light chain and/or the heavy chain are integrated into human-derived framework regions.

13. The use according to any one of claims 3-4 wherein the antibody 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 or 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.

14. The use according to any one of claims 3-4 wherein the antibody 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 and said light chain comprises a constant region of an IgG4 light chain as set forth in SEQ ID NO:35 or wherein said heavy chain comprises a constant region of an IgG4 heavy chain as set forth in SEQ ID NO:36 and said light chain comprises a constant region of an IgG4 light chain as set forth in SEQ ID NO:37.

15. The use according to claim 14, 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 or 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:36 and/or of an IgG4 light chain as set forth in SEQ ID NO:37.