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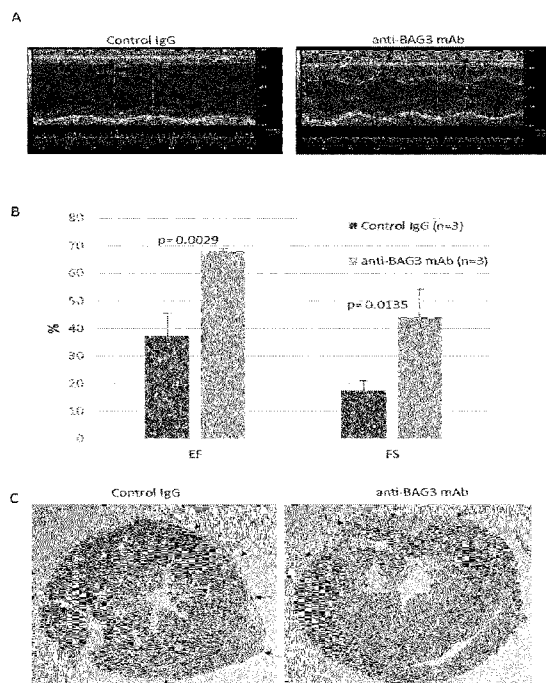
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(54) Title: ANTI-BAG3 ANTIBODIES AS THERAPEUTIC REAGENT IN CARDIOVASCULAR DISEASES

Figure 1



(57) Abstract: The present invention relates to the use of anti-BAG3 antibodies and its pharmaceutical formulation in the treatment of cardiovascular diseases.

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TITLE

“Anti-BAG3 antibodies as therapeutic reagent in cardiovascular diseases”

The present invention relates to the use of anti-BAG3 antibodies and its pharmaceutical formulation in the treatment of cardiovascular diseases.

Background of the invention

BAG3 protein is a 74 kDa cytoplasmic protein which belongs to the family of co-chaperons that interact with the ATPase domain of the protein HSP70 (Heat Shock Protein) through the structural domain known as the BAG domain (amino acids 110-124). Furthermore, BAG3 protein contains a WW domain (Trp-Trp), a proline-rich region (PXXP), and two conserved motifs IPV (Ile-Pro-Val), which can mediate binding to other proteins. Thanks to the nature of BAG3 protein as an adapter, attributable to the presence of many functional domains, such protein can therefore interact with different proteins.

In humans, bag3 gene expression is constitutive for a few kinds of normal cells, including myocytes, while mutations thereof are associated with diseases of the skeletal and cardiac muscles. Furthermore, BAG3 protein is expressed in many types of primary tumours or tumour cell lines (lymphoid or myeloid leukemias, neuroblastoma, pancreatic cancer, thyroid cancer, breast cancer and prostate cancer, melanoma, osteosarcoma, glioblastoma and tumours of the kidney, colon, ovary, etc.) (Rosati A. et al Cell Death Dis. 2011 Apr 7;2:e141).

In normal cell types, such as leukocytes, epithelial cells and glial cells and cells of the retina, bag3 gene expression can be induced by stressors, such

as oxidants, high temperatures, lack of serum, heavy metals, HIV-1 infections, etc. These findings indicate that bag3 gene expression regulation is an important component in the cellular response to stress and is correlated with the presence of elements that respond to the transcription factor HSF1 (Heat Shock Transcription Factor), which is activated in various forms of cellular stress in bag3 gene promoter. Moreover, due to the presence of many protein-protein interaction domains in the structure thereof, BAG3 protein influences cell survival in different types of cells, interacting with different molecular partners (Rosati A. et al Cell Death Dis. 2011 Apr 7;2:e141). The first mechanism reported in relation to BAG3 anti-apoptotic activity was identified in osteosarcoma and melanoma cells, where it was observed that BAG3 protein modulates the activation of transcription factor NF- κ B and cell survival (Ammirante M. et al. Proc Natl Acad Sci U S A. 2010;107(16):7497-502.). A different molecular mechanism has been described in glioblastoma cells, where BAG3 protein cooperates in a positive way with HSP70 protein to maintain BAX protein in the cytosol and prevent the translocation thereof into the mitochondria (Festa M. et al. Am J Pathol. 2011;178(6):2504-12). Finally, in some tumours, BAG3 has been shown to regulate proteins that modulate cell adhesion.

The presence of cytoplasmic BAG3 protein has also been described in many different cellular systems and has been associated, not only with various tumours, but also in pathologies in general related to cell survival.

Furthermore, patent application n. WO2011/067377 describes extracellular BAG3 protein, secreted by some cell types, as a biochemical marker in

serum, which is highly specific for the diagnosis of certain pathological conditions, such as cardiac pathologies and pancreatic tumour.

It has recently been reported that BAG3 protein is expressed in 346/346 patients with pancreatic ductal adenocarcinoma (PDAC) and is released by the cells of the pancreatic tumour, as a soluble protein, but such protein is not expressed in either the surrounding non-neoplastic tissues or in a normal pancreas; likewise, it has been reported that the levels of BAG3 expression are related to patient survival. The results of the study demonstrate that the use of specific siRNA molecules for BAG3 mRNA can silence bag3 gene expression and induce cell death, confirming that BAG3 protein is an important survival factor for pancreatic tumour cells and that the down-regulation thereof, when combined with gemcitabine, may contribute to the eradication of the tumour cells (Rosati A. et al. Am J Pathol. 2012 Nov;181(5):1524-9).

Moreover, in a recent paper (Rosati A. et al. Nat Commun. 2015 Nov 2;6:8695), we have reported that PDAC-released BAG3 binds macrophages inducing their activation and the secretion of PDAC supporting factors. We have also identified IFITM-2 as a BAG3 receptor and showed that it signals through PI3K and the p38 MAPK pathways. Finally, we have showed that the use of a mouse monoclonal anti-BAG3 antibody results in reduced tumour growth and prevents metastasis formation in three different mouse models. We have therefore identified a paracrine loop involved in PDAC growth and metastatic spreading, and showed that an anti-BAG3 antibody has therapeutic potential (Rosati A. et al. Nat Commun. 2015 Nov 2;6:8695).

Indeed, we showed that an anti-BAG3 antibody blocked BAG3 activity on macrophages.

In vivo, we showed the ability of this antibody to block tumour growth in different animal models, including a model of patient-derived xenograft and a syngeneic model. This last model is of great importance since mice have an intact immune system.

Intracellular BAG3 protein is known to maintain cardiomyocyte homeostasis and myofibrillar integrity during mechanical, proteotoxic and other types of stress; such property is related to BAG3 anti-apoptotic activity, participation in macroautophagy, and structural role in myofibrils. Therefore BAG3 defects can result in impairing cardiomyocyte survival or contractility and producing arrhythmias, dilated cardiomyopathy, or Takotsubo cardiomyopathy (C. Behl. Breaking BAG: The Co-Chaperone BAG3 in Health and Disease. Trends Pharmacol. Sci. 2016;37:672-688).

Carizzo et al. (Cell Death and Disease, 2016, 7; e2431) discloses that soluble BAG3, released by stressed cardiomyocytes, has a role in regulating blood pressure levels and in modulating the vascular tone and investigate the possible hemodynamic effect of BAG3.

Furthermore WO2015/117010A3 reports the use of composition comprising molecules that increase the intracellular expression of BAG3 and its use in the treatment of heart failure, providing evidences that the BAG3 levels are decreased in the failing heart.

However, all the information reported above relates to the intracellular BAG3 protein and to its effect in maintaining the normal functional heart.

We have now discovered a new and different aspect of BAG3 extracellular

(soluble) activity that can contribute to its role in inflammatory diseases, in particular in heart diseases, such as AVM (Acute Viral myocarditis) (S. Belkaya, A.R. Kontorovich, M. Byun, et al. J Am Coll Cardiol. 2017;69:1653-1665). Indeed, in different systems, we demonstrated that BAG3 is secreted by stressed cardiomyocytes (M. De Marco, R. D'Auria, A. Rosati, et al. BAG3 protein in advanced-stage heart failure. JACC Heart Fail. 2014;2:673-675) and that it binds to specific receptors on macrophages inducing their activation (Rosati A. et al. Nat Commun. 2015 Nov 2;6:8695).

Therefore, if some BAG3 variation(s) facilitate its release, this might expectedly result in macrophages-driven cardiac inflammation. Furthermore, since activated macrophages produce fibrogenic cytokines, it might be predicted that BAG3 release, by activating macrophages, can stimulate the fibrotic process, resulting in reducing the Left Ventricular Ejection Fraction (LVEF), that is a measure of the efficiency of pumping into the systemic circulation and serves as a general measure of a person's cardiac function. In particular, a low ejection fraction is always associated with an heart disease.

In this view, BAG3 neutralization should be able to improve LVEF and therefore to preserve normal cardiac functions. This prediction appears supported by data from our laboratory.

It has also been reported that fibrotic and inflammatory processes appear to be some of the integral components that causes most of the cardiac pathologies, such as angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart

failure, chronic heart failure and iatrogenic heart disease (Ruparella N. et al., Nature Reviews, Online 1, 2016, pp. 1-12; Gourdie RG et al., Nature, Vol. 15, 2016, pp.620-638). Furthermore, nearly all etiologies of heart disease involve pathological myocardial remodeling characterized by excessive deposition

of extracellular matrix (ECM) proteins by cardiac fibroblasts (CFs), which reduces tissue compliance and accelerates the progression to heart failure (Joshua G. et al., Circ. Res. 2016 Mar 18;118(6):1021-40.)

Conventional treatments for cardiovascular diseases inhibiting or reversing fibrosis and its adverse consequences, such as ACE-inhibitors, aldosterone antagonism statins and β -blockers, pose numerous drawbacks linked to side effects and are not, at present, definitive means of treating such pathologies.

There is therefore an evident need for a new and improved therapeutic treatment that the target cardiac diseases processes linked to fibroblast function, which has the advantage of being highly specific and having few or no side effects, as compared with the conventional, commonly known therapies used for the treatment of cardiovascular diseases, such as angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease, that are caused by an inflammatory and fibrotic process.

Definitions

Unless otherwise defined, all terms of art, notations and other scientific terminology used herein are intended to have the meanings commonly

understood by those persons skill in the art to which this disclosure pertains. In some cases, terms with commonly understood meanings are defined herein for clarity and/or for ready reference; thus, the inclusion of such definitions herein should not be construed to represent a substantial difference over what is generally understood in the art.

The term “soluble BAG3 protein” is understood as extra-cellular BAG3 protein, i.e. the protein secreted externally to the cell.

The term “pharmaceutically acceptable excipient” herein refers to a substance devoid of any pharmacological effect of its own and which does not produce adverse reactions when administered to a mammal, preferably a human. Physiologically acceptable excipients are well known in the art and are disclosed, for instance in the Handbook of Pharmaceutical Excipients, sixth edition 2009, herein incorporated by reference.

The term “simultaneous, separate or sequential administration” herein refers to administration of the first and second compound at the same time or in such a manner that the two compound act in the patient's body at the same time or administration of one compound after the other compound in such a manner to provide a therapeutic effect. In some embodiments the compounds are taken with a meal. In other embodiments, the compounds are taken after a meal, such as 30 minutes or 60 minutes after a meal. In some embodiments, one compound is administered to a patient for a time period followed by administration of the other compound.

The terms “approximately” and “about” herein refers to the range of the experimental error, which may occur in a measurement.

The terms “comprising”, “having”, “including” and “containing” are to be

construed open-ended terms (i.e. meaning “including, but not limited to”) and are to be considered as providing support also for terms as “consist essentially of”, “consisting essentially of”, “consist of” or “consisting of”.

The terms “consist essentially of”, “consisting essentially of” are to be construed as semi-closed terms, meaning that no other ingredients which materially affects the basic and novel characteristics of the invention are included (optional excipients may thus included).

The terms “consists of”, “consisting of” are to be construed as closed terms.

The term "antibody" as used herein includes "fragments" or "derivatives", which have at least one antigen binding site of the antibody and/or show the same biological activity.

An antibody preferably comprises at least one heavy immunoglobulin chain and at least one light immunoglobulin chain. An immunoglobulin chain comprises a variable domain and optionally a constant domain. A variable domain may comprise complementarity determining regions (CDRs), e.g. a CDR1, CDR2 and/or CDR3 region, and framework regions.

The term “humanized antibody” refers to an antibody of human origin, whose hypervariable region has been replaced by the homologous region of non-human monoclonal antibodies.

The term “chimeric antibody” refers to an antibody containing portions derived from different antibodies.

The term “recombinant antibody” refers to an antibody obtained using recombinant DNA methods.

The term “scFv fragment” (single chain variable fragment) refers to immunoglobulin fragments only capable of binding with the antigen

concerned. ScFv fragments can also be synthesised into dimers (diabodies), trimers (triabodies) and tetramers (tetrabodies) using peptide linkers.

The terms "Fab fragment" (antigen-binding fragment) and "Fab2 fragment" refer to immunoglobulin fragments consisting of a light chain linked to the Fc fragment of the adjacent heavy chain, and such fragments are monovalent antibodies. When the Fab portions are in pairs, the fragment is called Fab2.

The term "hybridoma" refers to a cell producing monoclonal antibodies.

The term "monospecific antibodies" refers to antibodies that all have affinity for the same antigen.

The term "multispecific antibodies" refers to antibodies that have affinity for several antigens.

The term "bispecific antibody" refers to an antibody that has affinity for two different antigens.

The term "sequence identity" between two polypeptide sequences, indicates the percentage of amino acids that are identical between the sequences.

Description of the figures

Figure 1. A. M-mode hearts ultrasound imaging recordings in end diastole and end systole.

B. Graph depicting calculation of ejection fraction (EF) and fractional shortening (FS).

C. Masson's trichrome staining for collagen fibers.

Disclosure of the invention

Surprisingly, it has been demonstrated, for the first time, that the inhibition of soluble (i.e. extra-cellular) BAG3 protein through the use of anti-BAG3 monoclonal antibodies, impairs development of heart failure.

In particular, we found that in mice subjected to heart cryoinjury (B.D. Polizzotti, B. Ganapathy, B.J. Haubner, J.M. Penninger, B. Kühn. A cryoinjury model in neonatal mice for cardiac translational and regeneration research. Nat Protoc. 2016;11:542-552), LVEF was reduced after five weeks, while was significantly higher in animals treated with a BAG3-neutralizing monoclonal antibody (Fig. 1). Furthermore fibrosis, measured by Masson's trichrome staining of collagen fibers, increased in mice treated with PBS, while was markedly reduced in mice that received the murine anti-BAG3 mAb under the same conditions.

The results obtained in the experimental data therefore demonstrates that reducing the level of BAG3 leads to a reduced inflammation and fibrotic process with an improvement of LVEF, that contribute to preserve the normal cardiac functions.

Anti-BAG3 antibodies therefore represent a new and improved therapeutic tool for the treatment of heart diseases.

Therefore, the treatment with any of the anti-BAG3 antibodies described in the patent application n. WO03/055908 and with any of the humanized anti-BAG3 antibodies discloses in WO2017/076878, whose content is herein entirely incorporated by reference, that is able to inhibit, specifically, the activity of soluble BAG3 protein (i.e. extra-cellular) on macrophages and fibroblasts, that are considered the target cells, proves particularly effective

in the treatment of those pathologies characterised by the activation of macrophages, such as heart diseases.

In particular, the use of anti-BAG3 antibodies in this process has the surprising advantage of being more specific for the selected pathological states characterised by the over-expression and release of BAG3 protein, and also less damaging in terms of side effects.

One aim of the present invention is therefore the use of anti-BAG3 antibodies in the treatment of cardiovascular diseases.

Preferably said cardiovascular diseases are selected from angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease.

The antibodies useable in accordance with the present invention may be either monoclonal or polyclonal antibodies, and are preferably monoclonal antibodies.

Still more preferably, said monoclonal antibodies may be chosen from the following: murine antibodies, humanized antibodies, chimeric antibodies, recombinant antibodies, conjugated antibodies, scFv fragments (diabody, triabody and tetrabody), Fab fragments, and fragments F (ab')₂.

The monoclonal antibodies used in the examples were obtained by immunizing mice against four distinct BAG3 protein peptides using any method known to a person skilled in the art. Such peptides were chosen because they are BAG3 protein-specific and are not shared with any other protein, including BAG proteins.

The sequences of the four peptides are included in the BAG3 amino acid

sequence (RefSeq: NP_004272; Gene ID 9531) and are selected from the following:

SEQ ID NO 1 DRDPLPPGWEIKIDPQ; (includes BAG3 protein amino acids 18-33);

SEQ ID NO 2: SSPKSVATEERAAPS; (includes BAG3 protein amino acids 385-399);

SEQ ID NO 3: DKGKKNAGNAEDPHT; (includes BAG3 protein amino acids 533-547);

SEQ ID NO 4: NPSSMTDTPGNPAAP; (includes BAG3 protein amino acids 561-575).

Preferably, said antibodies may be obtained by means of the Multiple Antigen Peptide approach (MAP) (Keah HH et al., J Pept Res (1988); 51: 2. Tam JP et al; Proc Natl acad Sci USA (1988), 85: 5409. Ota S, et al., Cancer Res (2002), 62: 1471), using the following map constructs:

- MAP-BAG3-1: nh2- DRDPLPPGWEIKIDPQ- MAP (which contains sequence SEQ ID NO: 1);
- MAP-BAG3-2: nh2- SSPKSVATEERAAPS - MAP (which contains sequence SEQ ID NO: 2);
- MAP-BAG3-3: nh2- DKGKKNAGNAEDPHT - MAP (which contains sequence SEQ ID NO: 3);
- MAP-BAG3-4: nh2- NPSSMTDTPGNPAAP - MAP (which contains sequence SEQ ID NO: 4);

According to a preferred embodiment of the present invention, said polyclonal anti-BAG3 antibodies are obtained by immunizing the animals against one of the four peptides of the sequences SEQ ID NO. 1 - 4 stated

above.

According to a preferred embodiment, the monoclonal anti-BAG3 antibodies of the present invention are obtained by means of a standard procedure (Tassone P., et al., Tissue Antigens 51: 671 (1998)) using the four MAP-BAG3 peptides described above and are produced by at least one of the nine mother clones chosen from the following: AC-1, AC-2, AC-3, AC-4, AC-5, AC-6, AC-7, AC-8, or AC-9 (described in WO03/055908), which contain specific hybridomas for each of the four MAP-BAG3 constructs used.

Said antibodies recognize the sequence of the four peptides of SEQ ID NO. 1-4.

According to a further embodiment, the antibodies used are monoclonal anti-BAG3 antibodies obtained from at least one of the aforesaid mother clones, and preferably at least one chosen from the following: AC-1, AC-2, AC-3, AC-4, or AC-5. More preferably, said monoclonal antibodies are obtained from at least one mother clone chosen from the following: AC-1, AC-2, and AC-3.

According to a further preferred embodiment, with the standard procedure (Geran C, Cokol M, Cingöz S, Tasan I, Ozturk M, Yagci T. Novel anti-HER2 monoclonal antibodies: synergy and antagonism with tumor necrosis factor- α . BMC Cancer. 2012 Oct 4;12:450) and the immunization of mice with a BAG3 recombinant protein, the monoclonal anti-BAG3 antibodies envisaged in the present invention are obtained from at least one of the following clones: AC-rb1, AC-rb2, AC-rb3 and AC-rb4, and/or at least one of the following subclones: AC-rb1a, AC-rb1b, AC-rb2a, AC-rb2b, AC-rb3a,

AC-rb3b, AC-rb4a, and AC-rb4b.

The monoclonal antibodies produced by all these clones and subclones recognize the BAG3 recombinant protein in an ELISA test.

Preferably, said monoclonal anti-BAG3 antibodies are those that recognize epitopes in the BAG3 protein amino acid sequence, which include at least one of the following fragments: 18-33, 385-399, 533-547 or 562-575.

More preferably said antibodies recognize the sequence of the four peptides of SEQ ID NO. 1-4.

In a preferred embodiment of the present invention the humanized anti-BAG3 antibodies are the anti-BAG3 antibodies or fragments thereof disclosed in WO2017/076878.

Preferably the anti-BAG3 antibodies or fragments thereof usable according to the present invention are humanized antibodies which comprises:

- a) a heavy chain amino acid sequence as encoded by SEQ ID NO: 12 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof, and
- b) a light chain amino acid sequence as encoded by SEQ ID NO: 20 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof.

As used herein, sequence identity between two polypeptide sequences, indicates the percentage of amino acids that are identical between the sequences, preferably over the entire length of the amino acid sequences as encoded by SEQ ID NO: 12 and SEQ ID NO: 20.

Preferred polypeptide sequences of the invention have a sequence identity of at least 85%, more preferably 90%, even more preferably 93%, 95%,

96%, 97%, 98% or 99%.

In a preferred embodiment of the present invention said amino acid sequence having a sequence identity of at least 80% with respect to SEQ ID N. 12 is selected from SEQ ID N. 14, SEQ ID N: 16 or SEQ ID N. 18.

In a further preferred embodiment said amino acid sequence having a sequence identity of at least 80% with respect to SEQ ID N. 20 is selected from SEQ ID N. 22, SEQ ID N: 24 or SEQ ID N. 26.

In a preferred embodiment the antibody of the present invention is the antibody wherein the heavy chain amino acid sequence is encoded by SEQ ID NO. 18 and the light chain amino acid sequence is encoded by SEQ ID NO 22 or SEQ ID N. 26.

In a preferred embodiment, the heavy chain amino acid sequence or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof, comprises the CDRs regions having the following amino acid composition: H-CDR1 comprises the amino acids GFNIKDTYMY (SEQ ID N. 5), H-CDR2 comprises the amino acids GVDPANGNTRYDPKFQG (SEQ ID N. 6), H-CDR3 comprises the amino acids DGAMDY (SEQ ID N. 7) and the light chain amino acid sequence or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof, comprises the CDRs regions having the following amino acid composition: L-CDR1 comprises the amino acids KSSQSLLYSSNQKNYLA (SEQ ID N. 8), L-CDR2 comprises the amino acids WASTRES (SEQ ID N. 9) and L-CDR3 comprises the amino acids QQYYTYPLT (SEQ ID N. 10).

In a more preferred embodiment, the heavy chain amino acid sequence or

at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereof, comprises the CDRs regions having the following amino acid composition: H-CDR1 comprises the amino acids GFNIKDTYMY (SEQ ID N. 5), H-CDR2 comprises the amino acids GVDPANGNTRYDPKFQG (SEQ ID N. 6), H-CDR3 comprises the amino acids DGAMDY (SEQ ID N. 7) and the light chain amino acid sequence or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 90% thereof, comprises the CDRs regions having the following amino acid composition: L-CDR1 comprises the amino acids KSSQSLLYSSNQKNYLA (SEQ ID N. 8), L-CDR2 comprises the amino acids WASTRES (SEQ ID N. 9) and L-CDR3 comprises the amino acids QQYYTYPLT (SEQ ID N. 10).

In a further preferred embodiment, the heavy chain amino acid sequence or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95% thereof, comprises the CDRs regions having the following amino acid composition: H-CDR1 comprises the amino acids GFNIKDTYMY (SEQ ID N. 5), H-CDR2 comprises the amino acids GVDPANGNTRYDPKFQG (SEQ ID N. 6), H-CDR3 comprises the amino acids DGAMDY (SEQ ID N. 7) and the light chain amino acid sequence or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95% thereof, comprises the CDRs regions having the following amino acid composition: L-CDR1 comprises the amino acids KSSQSLLYSSNQKNYLA (SEQ ID N. 8), L-CDR2 comprises the amino acids WASTRES (SEQ ID N. 9) and L-CDR3 comprises the amino acids QQYYTYPLT (SEQ ID N. 10).

A further embodiment of the present invention, is an antibody or a fragment thereof which binds to the BAG3 protein and which comprises:

- a) a heavy chain nucleotide sequence as encoded by SEQ ID NO: 11 or at least the variable domain thereof or a nucleotide sequence having a sequence identity of at least 80% thereof, and
- b) a light chain nucleotide sequence as encoded by SEQ ID NO: 19 or at least the variable domain thereof or a nucleotide sequence having a sequence identity of at least 80% thereof.

As used herein, "sequence identity" between two nucleotide sequences, indicates the percentage of nucleotides that are identical between the sequences, preferably over the entire length of the nucleotide sequences as encoded by SEQ ID NO: 11 and SEQ ID NO: 19.

Preferred nucleotide sequences of the invention have a sequence identity of at least 85%, more preferably 90%, even more preferably 93%, 95%, 96%, 97%, 98% or 99%.

In a preferred embodiment of the present invention said nucleotide sequence having a sequence identity of at least 80% with respect to SEQ ID N. 11 is selected from SEQ ID N. 13, SEQ ID N: 15 or SEQ ID N. 17.

In a further preferred embodiment said amino acid sequence having a sequence identity of at least 80% with respect to SEQ ID N. 19 is selected from SEQ ID N. 21, SEQ ID N: 23 or SEQ ID N. 25.

In a preferred embodiment the antibody of the present invention is the antibody wherein the heavy chain amino acid sequence is encoded by SEQ ID NO. 17 and the light chain amino acid sequence is encoded by SEQ ID NO 21 or SEQ ID N. 25.

Monoclonal antibodies may be produced by any suitable method such as that of Köhler and Milstein (1975) or by recombinant DNA methods. Monoclonal antibodies may also be isolated from phage antibody libraries using techniques described in Clackson et al. (1991).

Humanized forms of the antibodies may be generated according to the methods known in the art, (Kettleborough C.A. et al., 1991), such as chimerization or CDR grafting. Alternative methods for the production of humanized antibodies are well known in the art and are described in, e.g., EP 0239400 and WO 90/07861. Human antibodies can also be derived by in vitro methods. Suitable examples include but are not limited to phage display, yeast display, and the like.

The humanized anti-BAG3 antibodies or fragments thereof according to the present invention are obtained according to the method disclosed in WO2017/076878. A further aim of the present invention is the use of the aforesaid anti-BAG3 antibodies in the treatment of a particular pathological state which involves the activation of macrophages and fibroblasts.

Such pathological states is a heart disease, wherein said heart disease is selected from angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease.

A further aim of the present invention is the use of a pharmaceutical composition comprising the aforesaid anti-BAG3 antibody in association with at least one pharmaceutically acceptable excipient in the treatment of cardiovascular diseases.

Preferably, said cardiovascular diseases are selected from angina pectoris,

pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease.

The composition according to the present invention can be formulated in a form suitable for oral administration or in a form suitable for parenteral or topical administration.

In a preferred embodiment of the present invention, said oral form can be chosen from the following: tablets, capsules, solutions, suspensions, granules, and oily capsules.

In a further preferred embodiment of the present invention, said topical form can be chosen from the following: cream, ointment, ointment, solution, suspension, eye drops, pessary, nebuliser solution, spray, powder, or gel.

In a further preferred embodiment of this invention, said parenteral form can be either an aqueous buffer solution or an oily suspension.

Said parenteral administration include administration by intramuscular, intravenous, intradermal, subcutaneous, intraperitoneal, intranodal, or intrasplenic means.

EXAMPLE

Example 1

Myocardial infarct (MI) was induced using a model of cryoinfarction that produces a highly reproducible loss of myocardium. Briefly, 6 week-old mice (C75BL/6) were anesthetized by 2% isoflurane (v/v) oxygen mixture. The heart was exposed through a median sternotomy and the pericardium was opened. A 6-0 suture was placed at the apex of the LV (Left Ventricle). An 8 mm diameter cylindrical stamp was cooled in liquid

nitrogen and then pressed on the LV free wall. Cryothermia was applied for 5 seconds.

Post-MI mice were randomized into two groups: the control group received intra-peritoneal injection of control IgG1 (20 mg/Kg) while the experimental group received 20 mg/Kg of the murine anti-BAG3 mAb in PBS. Mice were treated 3 times a week for 5 weeks.

To measure global cardiac function, echocardiography was performed 5 weeks post-MI by use of the VisualSONICS VeVo 770 imaging system with a 710 scanhead in anesthetized animals (2% isoflurane, v/v). A) The internal diameter of the LV was measured in the short-axis view from M-mode recordings in end diastole and end systole. B) analysis software was used to calculate ejection fraction (EF) and fractional shortening (FS).

At the end of the experiment hearts were paraffin embedded. Sections (5 μ m), mounted on glass slides, were processed and stained with Masson's trichrome staining kit (04- 010802, Bio- Optica, Milano- Italy) accordly the manufacture instructions. Images were acquired using a microscopy Olympus BX53 (2X objective).

As is showed in figure 1C, fibrosis increased in mice treated with PBS, while was markedly reduced in mice that received the murine anti-BAG3 mAb under the same conditions.

The obtained results demonstrated that in mice subjected to heart cryoinjury, LVEF was reduced after five weeks, but significantly higher in animals treated with a BAG-3 neutralizing monoclonal antibody (Figure 1).

Therefore the treatment with anti-BAG3 antibodies is able to reduce the inflammation and the fibrotic process in the cardiac tissue and to preserve

the normal cardiac functions.

CLAIMS

1. Anti-BAG3 antibody for use in the treatment of cardiovascular diseases.
2. Anti-BAG3 antibody for use according to claim 1, characterised in that said cardiovascular diseases are selected from angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease.
3. Anti-BAG3 antibody for use according to any one of the previous claims, characterised in that such antibody is a polyclonal or a monoclonal antibody.
4. Anti-BAG3 antibody for use according to claim 3, characterised in that said monoclonal antibody is a murine antibody, a humanised antibody, a chimeric antibody, a recombinant antibody, a conjugated antibody, an scFv fragment (diabody, triabody and tetrabody), a Fab fragment or a F(ab')₂ fragment.
5. Humanized anti-BAG3 antibody for use according to claim 4, characterised in that it comprises:
 - a) a heavy chain amino acid sequence as encoded by SEQ ID N. 12 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof, and
 - b) a light chain amino acid sequence as encoded by SEQ ID N. 20 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 80% thereof.
6. Humanized anti-BAG3 antibody for use according to claim 5,

characterized in that the heavy chain amino acid sequence as encoded by SEQ ID N. 12 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95% thereof, comprises the CDRs regions having the following amino acid composition: H-CDR1 comprises the amino acids GFNIKDTYMY (SEQ ID N. 5), H-CDR2 comprises the amino acids GVDPANGNTRYDPKFQG (SEQ ID N. 6), H-CDR3 comprises the amino acids DGAMDY (SEQ ID N. 7) and the light chain amino acid sequence as encoded by SEQ ID N. 20 or at least the variable domain thereof or an amino acid sequence having a sequence identity of at least 95% thereof, comprises the CDRs regions having the following amino acid composition: L-CDR1 comprises the amino acids KSSQSLLYSSNQKNYLA (SEQ ID N. 8), L-CDR2 comprises the amino acids WASTRES (SEQ ID N. 9) and L-CDR3 comprises the amino acids QQYYTYPLT (SEQ ID N. 10).

7. Humanized anti-BAG3 antibody for use according claim 5, characterized in that said amino acid sequence having a sequence identity of at least 80% with respect to SEQ ID N. 12 is selected from SEQ ID N. 14, SEQ ID N. 16 or SEQ ID N. 18.

8. Humanized anti-BAG3 antibody for use according claim 5, characterized in that said amino acid sequence having a sequence identity of at least 80% with respect to SEQ ID N. 20 is selected from SEQ ID N. 22, SEQ ID N. 24 or SEQ ID N. 26.

9. Pharmaceutical composition comprising at least one anti-BAG3 antibody according to any one of the previous claims and at least one pharmaceutically acceptable excipient for use in the treatment of

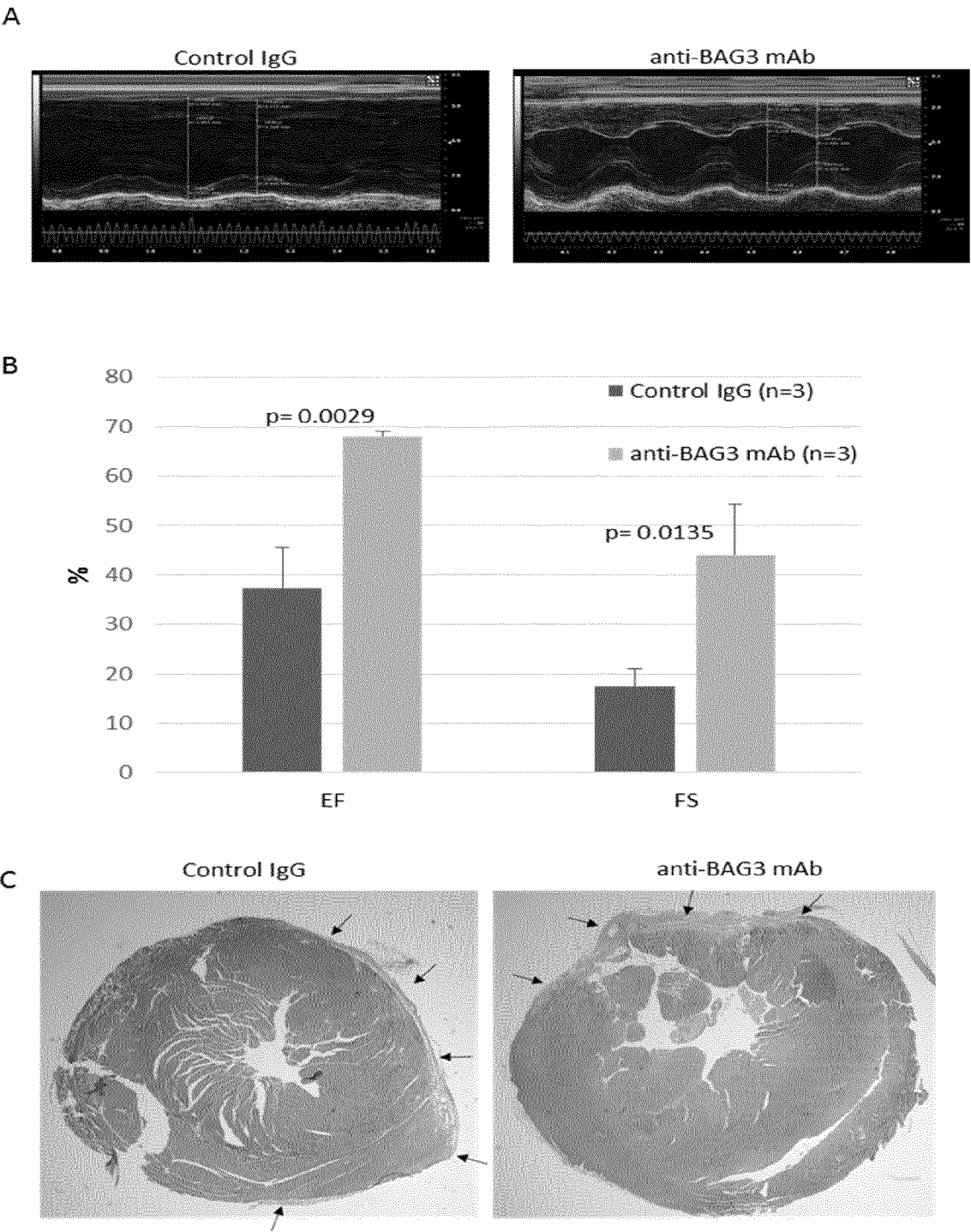
cardiovascular diseases, selected from angina pectoris, pre-infarction angina, myocardial infarction, heart failure, ischemia, acute coronary disease, acute heart failure, chronic heart failure and iatrogenic heart disease.

10. Pharmaceutical composition for use according to claim 9, characterised in that said composition can be formulated in a form suitable for oral administration or in a form suitable for parenteral or topical administration.

11. Pharmaceutical composition for use according to claim 9, characterised in that said form of oral administration can be selected from tablets, capsules, solutions, suspensions, granules, and oily capsules.

12. Pharmaceutical composition for use according to claim 9, characterised in that said form of parenteral administration can be selected from intramuscular, intravenous, intradermal, subcutaneous, intraperitoneal, intranodal, or intrasplenic administration.

Figure 1



INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2018/070259

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61P9/00
ADD.

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

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

EPO-Internal, BIOSIS, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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X	ALBINO CARRIZZO ET AL: "The prosurvival protein BAG3: a new participant in vascular homeostasis", CELL DEATH AND DISEASE, vol. 7, no. 10, 20 October 2016 (2016-10-20), page e2431, XP055366731, DOI: 10.1038/cddis.2016.321 the whole document	1-12
A	----- WO 2017/031182 A2 (UNIV TEMPLE [US]) 23 February 2017 (2017-02-23) paragraphs [0002], [0003], [0019], [0020] examples 3,7 ----- -/-	1-12



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International application No

PCT/EP2018/070259

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
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Information on patent family members

International application No

PCT/EP2018/070259

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