(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
23 June 2022 (23.06.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/132986 A3

(51) International Patent Classification:
G01N 33/68 (2006.01) *A61K 35/28* (2015.01)
A61P 9/00 (2006.01) *A61K 35/12* (2015.01)
C12N 5/0775 (2010.01)

(21) International Application Number:
PCT/US2021/063645

(22) International Filing Date:
15 December 2021 (15.12.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

2020904675	15 December 2020 (15.12.2020)	AU
2021900059	12 January 2021 (12.01.2021)	AU
2021902941	10 September 2021 (10.09.2021)	AU
2021903365	20 October 2021 (20.10.2021)	AU
63/289,868	15 December 2021 (15.12.2021)	US

(71) Applicant: **MESOBLAST INTERNATIONAL SARL** [CH/CH]; 20, Route de Pre-Bois, c/o Accounting & Management Service SA, 1217 Meyrin (CH).

(72) Inventors: **ITESCU, Silviu**; c/o Level 38, 55 Collins Street, Melbourne, Victoria 3000 (AU). **BOROW, Kenneth**; c/o 505 Fifth Avenue, Third Floor, New York, New York 10017 (US). **HAYES, Jack**; c/o 505 Fifth Avenue, Third Floor, New York, New York 10017 (US).

(74) Agent: **STEFFE, Eric K.** et al.; Sterne, Kessler, Goldstein and Fox P.L.L.C., 1100 New York Avenue, NW, Washington, District of Columbia 20005 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

(88) Date of publication of the international search report:
28 July 2022 (28.07.2022)

WO 2022/132986 A3

(54) Title: METHOD OF TREATING PROGRESSIVE HEART FAILURE IN SUBJECTS WITH CLASS II HEART FAILURE

(57) Abstract: The present disclosure relates to methods for treating and/or preventing progressive heart failure in subjects with earlier stages of heart failure. Such method may be used for treating or preventing progressive heart failure in subjects with Class II heart failure according to the New York Heart Association (NYHA) classification scale.

METHOD OF TREATING PROGRESSIVE HEART FAILURE IN SUBJECTS WITH CLASS II HEART FAILURE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the priority benefit of Australian Application No. 2020904675, filed December 15, 2020, Australian Application No. 2021900059, filed January 12, 2021, Australian Application No. 2021902941, filed September 10, 2021, Australian Application No. 2021903365, filed October 20, 2021, and U.S. Application No. 63/289,868, filed December 15, 2021, each of which is herein incorporated by reference in its entirety.

Technical Field

[0002] The present disclosure relates to methods for treating and/or preventing progressive heart failure in subjects with earlier stages of heart failure. Such methods may be used for treating or preventing progressive heart failure in subjects with Class II heart failure according to the New York Heart Association (NYHA) classification scale.

Background

[0003] Myocardial infarction (MI) is still one of the main causes of mortality and morbidity in developed countries. An update of US Medicare records was published that evaluated data involving 350,509 acute MI hospitalization in patients >65 years who were discharged alive after their event (Schuster et al. (2004) Physiol Heart Circa Physiol., 287(2):525-32). Within the first year post the index event, 25.9% of the MI patients died with 50.5% re-hospitalized. In the month after a MI, the likelihood of death was 21 times higher and the likelihood of hospitalization and was 12 times higher than among the general Medicare-age population.

[0004] During the past decade, numerous clinical trials evaluating novel drug therapies have been conducted in patients with advanced heart failure (HF). Despite progress made in reducing morbidity and mortality in patients with HF, those with advanced disease continue to experience an unfavourable clinical course characterized by frequent hospitalizations and premature death.

[0005] Clearly, there is a need in the art for treating or preventing progressive heart failure.

SUMMARY

[0006] The present inventors have surprisingly found that cell therapy is particularly effective in subjects with earlier stages of progressive heart failure. Accordingly, in an example, the present disclosure relates to a method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising cells. In an example, the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale. In an example, the subject may have less than Class III heart failure according to the New York Heart Association (NYHA) classification scale. In an example, the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale. Accordingly, in an example, the present disclosure relates to a method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising cells, wherein the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale.

[0007] In another example, the present disclosure relates to a method of reducing progression of heart failure in a subject, the method comprising administering to the subject a composition comprising cells, wherein the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale.

[0008] In another example, the present disclosure relates to a method of reducing cardiac death in a subject with Class II heart failure according to the New York Heart Association (NYHA) classification scale, the method comprising administering to the subject a composition comprising cells.

[0009] In another example, the present disclosure relates to a method of selecting heart failure patients for treatment with cell therapy, the method comprising i) assessing heart failure according to the New York Heart Association (NYHA) classification scale, and ii) selecting a subject having Class II heart failure according to NYHA. In an example, the method further comprises administering a composition comprising cells.

[0010] In an example, the cells induce new blood vessel formation in target tissue. In an example, the cells promote arteriogenesis. In an example, the cells secrete factors that

protect at risk myocardium. Accordingly, in an example, the present disclosure relates to a method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising cells, wherein the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale, and wherein the cells induce new blood vessel formation in target tissue and/or secrete factors that protect at risk myocardium.

[0011] In an example, the cells are mesenchymal lineage precursor or stem cells (MLPSCs). In an example, the MLPSCs are STRO-1+. In an example, the MLPSCs are mesenchymal stem cells (MSCs). In an example, the MLPSCs are allogeneic. In an example, the cells are culture expanded. In this example, the cells may be TNAP+ before they are culture expanded. In an example, the cells have been cryopreserved.

[0012] In another example, the methods of the disclosure comprise the steps of: i) selecting a subject having Class II heart failure according to the New York Heart Association (NYHA) classification scale, and ii) administering to the subject a composition comprising cells which induce new blood vessel formation in target tissue.

[0013] In another example, administering the composition inhibits the subject's progression to NYHA class III progressive heart failure.

[0014] In an example, the subject's level of N-terminal pro–B-type natriuretic peptide (NT-proBNP) is less than 2200 pg/ ml. In another example, the subject's NT-proBNP is less than 2000 pg/ml, prior to administering the cells. In another example, the subject's level of NT-proBNP is between 1000 pg/ml and 2000 pg/ ml prior to administering the cells.

[0015] The present inventors have also surprisingly found that cell therapy is particularly effective in subjects with earlier stages of progressive heart failure that have elevated C-reactive protein (CRP) levels. Accordingly, in an example, the subject's CRP level is elevated. In an example, the subject's CRP level is >1 mg/L. In an example, the subject's CRP level is >1.5 mg/L. In an example, the subject's CRP level is ≥2 mg/L. In an example, the subject's CRP level is >2 mg/L. In another example, the subject's CRP level is between 1.5 and 5 mg/L. In another example, the subject's C-reactive protein (CRP) level is <5 mg/L, preferably <4 mg/L. In another example, the subject's CRP level is between 1 and 5 mg/L. In another example, the subject's CRP level is between 1.5 and 5 mg/L.

[0016] In another example, the subject has had a heart failure hospitalisation event over the previous 9 months.

[0017] In another example, the subject has a LVEF of less than about 45%, preferably less than 40%. In another example, the subject has persistent left ventricular dysfunction.

[0018] In another example, the subject's heart failure results from an ischemic event.

[0019] In another example, the subject's heart failure results from a non-ischemic event.

[0020] In an example, the subject has a reduced risk of cardiac death after treatment. In an example, the reduced risk is relative to risk of cardiac death in a subject with NYHA class III progressive heart failure. In another example, the subject has a reduced risk of ischemic MACE (MI or stroke) after treatment.

[0021] In an example, a class III heart failure subject has a reduced risk of ischemic MACE (non-fatal MI or non-fatal stroke). In another example, a class III heart failure subject has a reduced risk of cardiac death after treatment. In another example, a class III heart failure subject has a reduced risk of ischemic MACE and cardiac death after treatment. In an example, the class III heart failure subjects CRP level is $\geq$2 mg/L.

[0022] In an example, the composition is administered transendocardially and/or intravenously. In an example, the composition is administered transendocardially.

[0023] The present inventors also surprisingly identified that cell therapy reduced the risk of ischemic events in subjects with cardiomyopathy. Accordingly, in an example, the present disclosure also encompasses a method of reducing risk of an ischemic event in a subject, the method comprising administering to the subject a composition comprising cells. In an example, the subject has a cardiomyopathy. In an example, the ischemic event is formation of a cerebrovascular or cardiac occlusion. In an example, the ischemic event is a stroke or myocardial infarction. In an example, the subject has non-ischemic cardiomyopathy. In an example, the cells are administered transendocardially. In an example, the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale. In an example, the subject has active inflammation. Surprisingly, in relation to this example, the present inventors identified that administering a composition of the disclosure can treat a wider range of patients, such as patients with Class II or Class III heart failure.

[0024] In an example, the present disclosure relates to a method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells, wherein the

subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale and active inflammation. In another example, the present disclosure relates to a method of reducing progression of heart failure in a subject, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells, wherein the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale and active inflammation. In another example, the present disclosure relates to a method of reducing cardiac death in a subject with Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale active inflammation, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells. In another example, the present disclosure relates to a method of selecting heart failure patients for treatment with cell therapy, the method comprising i) assessing CRP levels and heart failure according to the New York Heart Association (NYHA) classification scale, and ii) selecting a subject having Class II or Class III heart failure according to NYHA and active inflammation, preferably, wherein the method comprises administering a composition comprising mesenchymal precursor lineage or stem cells. In an example, active inflammation is determined based on the subject's CRP level. In an example, the subject has Class II heart failure and "active inflammation". In an example, the active inflammation is characterised by a level of CRP >1.5 mg/L. In another example, active inflammation is characterised by a level of CRP >2 mg/L. Exemplary cells are discussed above and throughout the present disclosure. In an example, the cells induce new blood vessel formation in target tissue. In an example, the cells promote arteriogenesis. In an example, the cells secrete factors that protect at risk myocardium. In an example, the cells are MLPSCs. In an example, the MLPSCs are STRO-1+. In an example, the MLPSCs are mesenchymal stem cells (MSCs). In an example, the MLPSCs are allogeneic. In an example, the cells are culture expanded. In this example, the cells may be TNAP+ before they are culture expanded. In an example, the cells have been cryopreserved.

[0025] In an example, the subject's level of N-terminal pro–B-type natriuretic peptide (NT-proBNP) is between 1000 pg/ml and 2000 pg/ ml prior to administering the cells. In another example, the subject's C-reactive protein (CRP) level is elevated. In another example, the subject's CRP level is ≥1 mg/L. In another example, the subject's CRP

level is ≥2 mg/L. In another example, the subject's CRP level is between 2 and 5 mg/L. In another example, the subject's CRP level is between 3 and 5 mg/L.

[0026] In an embodiment of the above examples, the methods of the disclosure comprise administering between 1 x 10^7 and 2 x 10^8 cells.

[0027] In another example, the administered composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO), human serum albumin (HSA). In an example, the administered composition further comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer. In an example, the composition comprises greater than 6.68x10^6 viable cells/mL.

[0028] In another example, the composition comprises human bone marrow-derived allogeneic mesenchymal precursor cells (MPCs) isolated from bone mononuclear cells with anti-STRO-3 antibodies, expanded *ex vivo*, and cryopreserved.

[0029] In an example, the ischemic events are one or more of myocardial infarction, stroke or cardiac death. In an example, the method reduces risk of 3-point MACE.

[0030] The present inventors have also surprisingly identified that elevated CRP levels is associated with increased cardiac death risk, myocardial infarction or stroke. Accordingly, in an example, the present disclosure relates to a method for determining elevated risk of one or more of cardiac death, myocardial infarction or stroke in a subject, the method comprising measuring the level of CRP in a sample obtained from a subject, wherein elevated CRP indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, the subject has progressive heart failure. In an example, the subject's heart failure is NYHA Class II heart failure. In another example, a level of CRP >1 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In another example, a level of CRP >1.5 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In another example, a level of CRP ≥2 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, the method determines elevated risk of cardiac death.

BRIEF DESCRIPTION OF DRAWINGS

[0031] **Figure 1:** Reduced Incidence of Ischemic MACE (MI, Stroke).

[0032] **Figure 2:** Reduced Incidence of Ischemic MACE (MI, Stroke); NYHA Class II and Class III.

[0033] **Figure 3:** Reduced Incidence of Ischemic MACE (MI, Stroke); ischemic and non-ischemic.

[0034] **Figure 4:** Cardiac death in all treated (n=537); Class II (n=206); Class III patients (n=331).

[0035] **Figure 5:** Cardiac death in NYHA Class II patients; ischemic and non-ischemic.

[0036] **Figure 6:** Cardiac death in trial patients.

[0037] **Figure 7:** A) TTFE composite IMM MACE; B) Rate normalised composite IMM MACE; C) Curves for all treated patients with baseline CRP ≥2mg/L vs CRP ≤2 mg/ml. Curves shown for Non-fatal MI or Non-fatal stoke; 3-point TTFE composite IMM MACE for CV death or non-fatal MI or non-fatal stroke.

[0038] **Figure 8:** A) TTFE Irreversible morbidity; B) Rate normalised irreversible morbidity; C) Curves for irreversible morbidity TTFE MACE (non-fatal MI or non-fatal stroke) for All treated (n=537); Class II (n=206); Class III patients (n=331).

[0039] **Figure 9:** Reduced Incidence of composite cardiac death or Ischemic MACE (MI, Stroke); All treated (n=537); Class II (n=206); Class III patients (n=331).

[0040] **Figure 10.** Cardiac Death in NYHA Class II patients - Multiple Years of Follow-up.

[0041] **Figure 11.** NYHA Class II patients with baseline hsCRP ≥2 mg/L are at significantly greater risk of progression to cardiac death.

[0042] **Figure 12.** NYHA Class II patients with baseline hsCRP ≥2 mg/L are at significantly greater risk of 3-point MACE (Cardiac death/MI/stroke).

DETAILED DESCRIPTION

General Techniques and Definitions

[0043] Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (e.g., in cell culture, molecular biology, stem cell culture, immunology, clinical trials, medicine, and biochemistry).

[0044] Unless otherwise indicated, cell culture techniques and assays utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, A Practical Guide to Molecular Cloning, John Wiley and Sons (1984), J.

Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), Essential Molecular Biology: A Practical Approach, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), and F.M. Ausubel et al. (editors), Current Protocols in Molecular Biology, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) Antibodies: A Laboratory Manual, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan et al. (editors) Current Protocols in Immunology, John Wiley & Sons (including all updates until present).

[0045] The term "and/or", e.g., "X and/or Y" shall be understood to mean either "X and Y" or "X or Y" and shall be taken to provide explicit support for both meanings or for either meaning.

[0046] As used herein, the term "about", unless stated to the contrary, refers to +/- 10%, more preferably +/- 5%, of the designated value.

[0047] The terms "level" and "amount" are used to define the amount of a particular substance in a sample from a subject or in a cell culture media (or sample therefrom). For example, a particular concentration, weight, percentage (e.g. v/v%) or ratio can be used to define the level of a particular substance in a sample. In an example, the level is expressed in terms of how much of a particular marker is expressed by cells of the disclosure under culture conditions. In an example, expression represents cell surface expression. In another example, the level is expressed in terms of how much of a particular marker is release from cells described herein under culture conditions. In an example, the sample is obtained from a patient or subject (e.g. a blood sample) and the level of a substance is measured in the sample to determine the level of the substance in the sample.

[0048] In an example, the level is expressed in pg/ml. For example, the level of NT-proBNP can be expressed in pg/ml. In an example, the level is expressed in mg/L. For example, the level of CRP can be expressed in mg/L. In another example, the level is expressed in pg per 10^6 cells.

[0049] In an example, the level of a particular marker in a cell culture medium is determined under culture conditions. The term "culture conditions" is used to refer to cells growing in culture. In an example, culture conditions refers to an actively dividing population of cells. Such cells may, in an example, in exponential growth phase. For example, the level of a particular marker can be determined by taking a sample of cell

culture media and measuring the level of marker in the sample. In another example, the level of a particular marker can be determined by taking a sample of cells and measuring the level of the marker in the cell lysate. Those of skill in the art that secreted markers will be measured by sampling the culture media while markers expressed on the surface of the cell may be measured by assessing a sample of cell lysate. In an example, the sample is taken when the cells are in exponential growth phase. In an example, the sample is taken after at least two days in culture.

[0050] Culture expanding cells from a cryopreserved intermediate means thawing cells subject to cryogenic freezing and *in vitro* culturing under conditions suitable for growth of the cells.

[0051] In an example, the "level" or "amount" of a particular marker is determined after cells have been cryopreserved and then seeded back into culture. For example, the level is determined after a first cryopreservation of cells. In another example, the level is determined after a second cryopreservation of cells. For example, cells may be culture expanded from a cryopreserved intermediate, cryopreserved a second time before being re-seeded in culture so that the level of a particular marker can be determined under culture conditions.

[0052] As used herein, the terms "treating", "treat", "treatment", "reducing progression" include administering a population of mesenchymal lineage stem or precursor cells and/or progeny thereof and/or soluble factors derived therefrom and/or extracellular vesicles derived therefrom to thereby reduce or eliminate at least one symptom of progressive heart failure or, in the context of reducing progression, delay development of the same.

[0053] The term "subject" as used herein refers to a human subject. For example, the subject can be an adult. In another example, the subject can be a child. In another example, the subject can be an adolescent. Terms such as "subject", "patient" or "individual" are terms that can, in context, be used interchangeably in the present disclosure. Subjects in need of treatment include those already having progressive heart failure as well as those in which progressive heart failure is to be prevented, delayed or halted.

[0054] In an example, compositions of the disclosure comprise genetically unmodified mesenchymal precursor lineage or stem cells. As used herein, the term "genetically unmodified" refers to cells that have not been modified by transfection with a nucleic acid. For the avoidance of doubt, in the context of the present disclosure a mesenchymal

lineage precursor or stem cell transfected with a nucleic acid encoding a protein would be considered genetically modified.

[0055] As used herein, the term "sample" refers to an extract from a subject in which CRP levels can be measured. The "sample" includes extracts and/or derivatives and/or fractions of the sample. In the present disclosure, any biological material can be used as the above-mentioned sample so long as it can be collected from the subject and assayed to determine the level of CRP in the subject. In an example, the sample is a blood sample. In an example, the blood sample is obtained from a subject with NYHA Class II heart failure.

[0056] Throughout this specification the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

[0057] Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or group of compositions of matter.

[0058] Those skilled in the art will appreciate that the disclosure described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

[0059] The present disclosure is not to be limited in scope by the specific embodiments described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the disclosure, as described herein.

[0060] Any example disclosed herein shall be taken to apply mutatis mutandis to any other example unless specifically stated otherwise.

Progressive heart failure

[0061] Cardiomyopathy is a disease of the heart muscle that makes it harder for the heart to pump blood to the rest of the body. When the heart is unable to pump sufficiently to maintain blood flow to meet the needs of the body heart failure can occur. Cardiomyopathy can occur after an ischemic or non-ischemic event. One cause of ischemic heart failure is systolic dysfunction following a myocardial infarction (MI) (e.g. heart attack). A MI occurs when blood stops flowing properly to a part of the heart. The lack of blood supply results in a localized area of myocardial necrosis referred to as an infarct or infarction. The infarcted heart is unable to pump sufficiently to maintain blood flow to meet the needs of the body leading to multiple pathophysiologic responses and ultimately heart failure. Non-ischemic cardiomyopathy is not related to known coronary artery disease. One example, is dilated cardiomyopathy (DCM) where the heart's ability to pump blood is decreased because the heart's main pumping chamber, the left ventricle, becomes enlarged, dilated and weak.

[0062] Once the heart is unable to pump sufficiently to maintain blood flow to meet the needs of the body, a series of compensatory mechanisms are initiated, serving to buffer the fall in cardiac output and assisting to maintain sufficient blood pressure to perfuse the vital organs. As a result, patients with heart failure may not progress for extended periods of time. However, the compensatory mechanisms eventually fail to compensate for the damaged heart, resulting in a progressive decline in cardiac output, termed “progressive heart failure”. In the context of the present disclosure, the terms chronic heart failure, congestive heart failure, congestive cardiac failure, systolic dysfunction and advanced heart failure can be used interchangeably with “progressive heart failure”.

[0063] The methods of the present disclosure are effective in a subset of patients with progressive heart failure. In an example, these subjects are defined based on the New York Heart Association (NYHA) classification scale. In an example, the subject’s progressive heart failure is less than Class III. In an example, the subject has Class II heart failure. In an example, the NYHA classification is assigned based on the subject’s symptoms. For example, the NYHA classification can be assigned based on the following Table:

Class	Patient Symptoms
I	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea (shortness of breath).
II	Slight limitation of physical activity. Comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea (shortness of breath).
III	Marked limitation of physical activity. Comfortable at rest. Less than ordinary activity causes fatigue, palpitation, or dyspnea.
IV	Unable to carry on any physical activity without discomfort. Symptoms of heart failure at rest. If any physical activity is undertaken, discomfort increases.

[0064] In an example, the subject's heart failure results from an ischemic event. In an example, the subject's heart failure results from a myocardial infarction (MI). For example, the subject can be a MI subject. The term "myocardial infarction (MI) subject" is used to define subjects who have had a myocardial infarction. In an example, the subject's heart failure results from a non-ischemic cardiomyopathy.

[0065] The methods of the present disclosure can be used to treat progressive heart failure in a specific population of MI subjects. Subjects in need of treatment include those already having progressive heart failure as well as those in which progressive heart failure is to be prevented, delayed or halted. In these examples, the subject has Class II or Class III progressive heart failure based on the NYHA. For example, the subject can have Class II progressive heart failure.

[0066] In an example, subjects treating according to the present disclosure have "active inflammation" as defined by elevated C-reactive protein levels. In an example, active inflammation is characterised by CRP levels $\geq$2 mg/L.

[0067] "C-reactive protein" or "CRP" is an inflammatory mediator whose levels are raised under conditions of acute inflammatory recurrence and rapidly normalize once the inflammation subsides. In an example, subjects treated according to the present disclosure have elevated risk of cardiac death. Cardiac death is death due to loss of cardiac function. In an example, subjects treated according to the present disclosure have elevated CRP. The term "elevated CRP" is used in the context of the present disclosure to refer to CRP levels that are increased relative to baseline CRP levels. In an example, CRP levels $\geq$1 mg/L are elevated. In another example, CRP levels $\geq$1.5 mg/L are elevated. In another example, CRP levels $\geq$2 mg/L are elevated.

[0068] In an example, subjects treated according to the present disclosure have an initial CRP level ≥2 mg/L. For example, the subject can have Class II or Class III heart failure and an initial CRP level ≥2 mg/L. In another example, the subject can have Class II heart failure and an initial CRP level ≥2 mg/L. In an example, subjects treated according to the present disclosure have an initial CRP level <5 mg/L. In another example, subjects have an initial CRP level <4 mg/L. In another example, subjects have an initial CRP level between 2 and 6 mg/L. In another example, subjects have an initial CRP level between 3 and 6 mg/L. In another example, subjects have an initial CRP level between 4 and 5 mg/L.

[0069] There are various assays available for measuring CRP levels such as antibody based immunoassays. For example, CRP levels can be measured in blood samples using an Enzyme-Linked Immunosorbent (ELISA) assay. In an example, a blood sample is obtained from a patient and then purified before being contacted with anti-CRP antibody. Extent of antibody binding is used to quantify the level of CRP in the blood sample (e.g. mg/L). In an example, CRP is measured by a plasma high sensitivity CRP (hsCRP) ELISA assay.

[0070] B-type natriuretic peptide (BNP) is a hormone produced by the heart. N-terminal (NT)-pro hormone BNP (NT-proBNP) is a non-active prohormone that is released from the same molecule that produces BNP. Both BNP and NT-proBNP are released in response to changes in pressure inside the heart. These changes can be related to heart failure and other cardiac problems. Levels goes up when heart failure develops or gets worse, and levels goes down when heart failure is stable. Accordingly, BNP is an effective marker of heart failure progression. In an example, the subject's level of NT-proBNP is less than 2200 pg/ ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is less than 2100 pg/ ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is less than 2000 pg/ ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is less than 1900 pg/ ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is between 2200 pg/ ml and 1000 pg/ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is between 2200 pg/ ml and 1100 pg/ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is between 2100

pg/ ml and 1200 pg/ml prior to administering a composition of the disclosure. In another example, the subject's level of NT-proBNP is between 2000 pg/ ml and 1500 pg/ml prior to administering a composition of the disclosure. There are various assays available for measuring NT-proBNP levels such as antibody based immunoassays, for example, an ELISA assay. In an example, a blood sample is obtained from a patient and then purified before being contacted with anti- NT-proBNP antibody. Extent of antibody binding is used to quantify the level of NT-proBNP in the blood sample (e.g. pg/L).

[0071] In another example, the subject has had a heart failure hospitalisation event over the previous 12 months prior to administration of a composition disclosed herein. In another example, the subject has had a heart failure hospitalisation event over the previous 9 months prior to administration of a composition disclosed herein. In another example, the subject has had a heart failure hospitalisation event over the previous 6 to 12 months prior to administration of a composition disclosed herein. In an example, the heart failure hospitalisation event is worsening signs and symptoms of heart failure. In another example, the heart failure hospitalisation event is an ischemic event. In another example, the heart failure hospitalisation is non-ischemic event.

[0072] In another example, the subject is able to walk at least 320 meters in 6 minutes prior to administering a composition of the disclosure. In another example, the subject is able to walk at least 330 meters in 6 minutes prior to administering a composition of the disclosure. In another example, the subject is able to walk at least 340 meters in 6 minutes prior to administering a composition of the disclosure. In another example, the subject is able to walk at least 350 meters in 6 minutes prior to administering a composition of the disclosure.

[0073] In an example, subjects can have persistent left ventricular dysfunction. Left ventricular dysfunction is characterised by a decrease in myocardial contractility. A reduction in the left ventricular ejection fraction (LVEF) results when myocardial contractility is decreased within the left ventricle. Thus, LVEF provides one way of determining left ventricular dysfunction.

[0074] LVEF and LVESV can be measured by a number of methods known in the art such as echocardiogram, Single Photon Emission Computed Tomography (SPECT) or cardio magnetic resonance imaging (cMRI).

[0075] In an example, a subject having an LVEF of less than 45% has left ventricular dysfunction. In other examples, a subject with a LVEF of less than about 44%, 43%,

42%, 41% has left ventricular dysfunction. In another example, a subject with a LVEF of less than about 40% has left ventricular dysfunction. In other examples, a subject with a LVEF of less than about 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30% has left ventricular dysfunction.

[0076] In the context of the present disclosure the term "persistent left ventricular dysfunction" is used to define left ventricular dysfunction that persists over a period of time or series of measurements. For example, "persistent left ventricular dysfunction" can include left ventricular dysfunction that persists for between about 1 to about 14 days or longer.

[0077] In an example, the subject has a LVEF of less than 45%. In another example, the subject has a LVEF of less than 40%. In other examples, the subject has a LVEF of less than 39%, 38%, 37%, 36%, 35%, 34%, 33%, 32%, 31%, 30%.

[0078] The methods of the present disclosure relate to the treatment of the progressive decline in cardiac output characteristic of progressive heart failure. Accordingly, "treat" and "treatment", in the context of the present disclosure refers to both therapeutic treatment and prophylactic or preventative measures.

[0079] In an example, treatment includes administering a composition of the disclosure. In an example, methods of the present disclosure reduce or inhibit progression of progressive heart failure. In an example, treatment inhibits the subject's progression to NYHA class III progressive heart failure. In another example, treatment reduces the risk of cardiac death. In an example, the reduced risk of cardiac death is relative to risk of cardiac death in a subject with NYHA class III progressive heart failure. In another example, the risk of ischemic MACE (MI or stroke) is reduced after treatment. In an example, risk of ischemic MACE (MI or stroke) is reduced by at least 50% relative to baseline. In another example, risk of ischemic MACE (MI or stroke) is reduced by at least 55% relative to baseline. In another example, risk of ischemic MACE (MI or stroke) is reduced by at least 60% relative to baseline. In another example, risk of ischemic MACE (MI or stroke) is reduced by at least 65% relative to baseline. In another example, risk of ischemic MACE (MI or stroke) is reduced by at least 70% relative to baseline. In another example, risk of ischemic MACE (MI or stroke) is reduced by at least 50% to 70% relative to baseline.

[0080] In another example, the risk of 3-Point MACE (Cardiac death/MI/stroke) is reduced after treatment. In the context of the present disclosure, "3-point MACE" is used

to refer to is defined as a composite of cardiovascular death, nonfatal myocardial infarction and nonfatal stroke (Cardiac death/MI/stroke). In an example, risk of 3 point MACE is reduced by at least 30% relative to baseline. In another example, risk of 3 point MACE is reduced by at least 40% relative to baseline. In another example, risk of 3 point MACE is reduced by at least 45% relative to baseline. In another example, risk of 3 point MACE is reduced by at least 50% relative to baseline. In another example, risk of 3 point MACE is reduced by at least 30% to 50% relative to baseline.

[0081] In an example, treatment increases patient survival. In an example, treatment increases the probability of a subject surviving for at least 1000 days after initiation of treatment. In another example, treatment increases the probability of a subject surviving for at least 2000 days after initiation of treatment. In an example, the increased probability is determined relative to a subject that is not treated with a composition of the disclosure. In an example, the increased probability is determined relative to a subject that has Class III heart failure.

[0082] In an example, treatment reduces the chance or risk of heart failure-related Major Adverse Cardiac Events (HF-MACE) defined as a composite of cardiac related death or resuscitated cardiac death, or non-fatal decompensated heart failure events. In an example, the chance or risk of HF-MACE is reduced over at least 6 months, at least 12 months, at least 24 months, at least 36 months after administration of a composition disclosed herein. In an example, treatment reduces the chance or risk of all-cause mortality.

Ischemic events

[0083] In an example, the present disclosure relates to a method of reducing the risk or incidence of ischemic events in subjects, in particular subjects with cardiomyopathy. In an example, the present disclosure relates to a method of reducing the risk or incidence of ischemic events in subjects with cardiomyopathy and elevated CRP. In an example, risk or incidence is reduced relative to a subject that does not receive a composition of the disclosure. For example, risk or incidence can be reduced relative to an untreated subject. In an example, the ischemic event is caused by the formation of an occlusion. In an example, the occlusion is an arterial occlusion. In an example, the ischemic event is formation of a cerebrovascular occlusion. In another example, the ischemic event is a

formation of a cardiac occlusion. For example, the occlusion can form in the coronary artery.

[0084] Examples of ischemic events caused by formation of an occlusion include myocardial infarction and stroke. Accordingly, in an example, the present disclosure relates to methods of reducing the risk or incidence of myocardial infarction or stroke in a subject with cardiomyopathy.

[0085] The risk or incidence of ischemic events in subjects with cardiomyopathy is reduced by administering a cell therapy such as a composition of the disclosure.

[0086] In an example, the subject has non-ischemic cardiomyopathy. For example, the subject's cardiomyopathy may be caused by an enlarged left ventricle (dilated cardiomyopathy. In another example, the cardiomyopathy is caused by a viral infection.

[0087] In another example, the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale.

[0088] In another example, the subject's level of N-terminal pro–B-type natriuretic peptide (NT-proBNP) is between 1000 pg/ml and 2000 pg/ ml prior to administering the cells. In another example, the subject's C-reactive protein (CRP) level is elevated. In another example, the subject's CRP level is ≥1.5 mg/L. In another example, the subject's CRP level is ≥2 mg/L. In another example, the subject's CRP level is between 1 and 5 mg/L. In another example, the subject's CRP level is between 3 and 5 mg/L.

[0089] In an example, the cells are administered transendocardially.

[0090] In an example, reduced risk is reduced 3 year risk. In another example, the reduced risk is reduced 5 year risk. In these examples, the risk of ischemic event is reduced over a defined period of time.

Mesenchymal lineage precursor cells

[0091] As used herein, the term "mesenchymal lineage precursor or stem cell (MLPSC)" refers to undifferentiated multipotent cells that have the capacity to self-renew while maintaining multipotency and the capacity to differentiate into a number of cell types either of mesenchymal origin, for example, osteoblasts, chondrocytes, adipocytes, stromal cells, fibroblasts and tendons, or non-mesodermal origin, for example, hepatocytes, neural cells and epithelial cells. For the avoidance of doubt, a "mesenchymal lineage precursor cell" refers to a cell which can differentiate into a mesenchymal cell such as bone, cartilage, muscle and fat cells, and fibrous connective tissue.

[0092] The term "mesenchymal lineage precursor or stem cells" includes both parent cells and their undifferentiated progeny. The term also includes mesenchymal precursor cells, multipotent stromal cells, mesenchymal stem cells (MSCs), perivascular mesenchymal precursor cells, and their undifferentiated progeny.

[0093] Mesenchymal lineage precursor or stem cells can be autologous, allogeneic, xenogenic, syngenic or isogenic. Autologous cells are isolated from the same individual to which they will be reimplanted. Allogeneic cells are isolated from a donor of the same species. Xenogenic cells are isolated from a donor of another species. Syngenic or isogenic cells are isolated from genetically identical organisms, such as twins, clones, or highly inbred research animal models.

[0094] In an example, the mesenchymal lineage precursor or stem cells are allogeneic. In an example, the allogeneic mesenchymal lineage precursor or stem cells are culture expanded and cryopreserved.

[0095] Mesenchymal lineage precursor or stem cells reside primarily in the bone marrow, but have also shown to be present in diverse host tissues including, for example, cord blood and umbilical cord, adult peripheral blood, adipose tissue, trabecular bone and dental pulp. They are also found in skin, spleen, pancreas, brain, kidney, liver, heart, retina, brain, hair follicles, intestine, lung, lymph node, thymus, ligament, tendon, skeletal muscle, dermis, and periosteum; and are capable of differentiating into germ lines such as mesoderm and/or endoderm and/or ectoderm. Thus, mesenchymal lineage precursor or stem cells are capable of differentiating into a large number of cell types including, but not limited to, adipose, osseous, cartilaginous, elastic, muscular, and fibrous connective tissues. The specific lineage-commitment and differentiation pathway which these cells enter depends upon various influences from mechanical influences and/or endogenous bioactive factors, such as growth factors, cytokines, and/or local microenvironmental conditions established by host tissues.

[0096] The terms “enriched”, “enrichment” or variations thereof are used herein to describe a population of cells in which the proportion of one particular cell type or the proportion of a number of particular cell types is increased when compared with an untreated population of the cells (e.g., cells in their native environment). In one example, a population enriched for mesenchymal lineage precursor or stem cells comprises at least about 0.1% or 0.5% or 1% or 2% or 5% or 10% or 15% or 20% or 25% or 30% or 50% or 75% mesenchymal lineage precursor or stem cells. In this regard, the term “population of

cells enriched for mesenchymal lineage precursor or stem cells" will be taken to provide explicit support for the term "population of cells comprising X% mesenchymal lineage precursor or stem cells", wherein X% is a percentage as recited herein. The mesenchymal lineage precursor or stem cells can, in some examples, form clonogenic colonies, e.g. CFU-F (fibroblasts) or a subset thereof (e.g., 50% or 60% or 70% or 70% or 90% or 95%) can have this activity.

[0097] In an example of the present disclosure, the mesenchymal lineage precursor or stem cells are mesenchymal stem cells (MSCs). The MSCs may be a homogeneous composition or may be a mixed cell population enriched in MSCs. Homogeneous MSC compositions may be obtained by culturing adherent marrow or periosteal cells, and the MSCs may be identified by specific cell surface markers which are identified with unique monoclonal antibodies. A method for obtaining a cell population enriched in MSCs is described, for example, in U.S. Patent No. 5,486,359. Alternative sources for MSCs include, but are not limited to, blood, skin, cord blood, muscle, fat, bone, and perichondrium. In an example, the MSCs are allogeneic. In an example, the MSCs are cryopreserved. In an example, the MSCs are culture expanded and cryopreserved.

[0098] In another example, the mesenchymal lineage precursor or stem cells are CD29+, CD54+, CD73+, CD90+, CD102+, CD105+, CD106+, CD166+, MHC1+ MSCs.

[0099] Isolated or enriched mesenchymal lineage precursor or stem cells can be expanded *in vitro* by culture. Isolated or enriched mesenchymal lineage precursor or stem cells can be cryopreserved, thawed and subsequently expanded *in vitro* by culture.

[0100] In one example, isolated or enriched mesenchymal lineage precursor or stem cells are seeded at 50,000 viable cells/cm^2 in culture medium (serum free or serum-supplemented), for example, alpha minimum essential media (αMEM) supplemented with 5% fetal bovine serum (FBS) and glutamine, and allowed to adhere to the culture vessel overnight at 37°C, 20% O_2. The culture medium is subsequently replaced and/or altered as required and the cells cultured for a further 68 to 72 hours at 37°C, 5% O_2.

[0101] As will be appreciated by those of skill in the art, cultured mesenchymal lineage precursor or stem cells are phenotypically different to cells *in vivo*. For example, in one embodiment they express one or more of the following markers, CD44, NG2, DC146 and CD140b. Cultured mesenchymal lineage precursor or stem cells are also biologically different to cells *in vivo*, having a higher rate of proliferation compared to the largely non-cycling (quiescent) cells *in vivo*.

[0102] In one example, the population of cells is enriched from a cell preparation comprising STRO-1+ cells in a selectable form. In this regard, the term "selectable form" will be understood to mean that the cells express a marker (e.g., a cell surface marker) permitting selection of the STRO-1+ cells. The marker can be STRO-1, but need not be. For example, as described and/or exemplified herein, cells (e.g., mesenchymal precursor cells) expressing STRO-2 and/or STRO-3 (TNAP) and/or STRO-4 and/or VCAM-1 and/or CD146 and/or 3G5 also express STRO-1 (and can be STRO-1bright). Accordingly, an indication that cells are STRO-1+ does not mean that the cells are selected solely by STRO-1 expression. In one example, the cells are selected based on at least STRO-3 expression, e.g., they are STRO-3+ (TNAP+).

[0103] Reference to selection of a cell or population thereof does not necessarily require selection from a specific tissue source. As described herein STRO-1+ cells can be selected from or isolated from or enriched from a large variety of sources. That said, in some examples, these terms provide support for selection from any tissue comprising STRO-1+ cells (e.g., mesenchymal precursor cells) or vascularized tissue or tissue comprising pericytes (e.g., STRO-1+ pericytes) or any one or more of the tissues recited herein.

[0104] In one example, the cells used in the present disclosure express one or more markers individually or collectively selected from the group consisting of TNAP+, VCAM-1+, THY-1+, STRO-2+, STRO-4+ (HSP-90β), CD45+, CD146+, 3G5+ or any combination thereof.

[0105] By "individually" is meant that the disclosure encompasses the recited markers or groups of markers separately, and that, notwithstanding that individual markers or groups of markers may not be separately listed herein the accompanying claims may define such marker or groups of markers separately and divisibly from each other.

[0106] By "collectively" is meant that the disclosure encompasses any number or combination of the recited markers or groups of markers, and that, notwithstanding that such numbers or combinations of markers or groups of markers may not be specifically listed herein the accompanying claims may define such combinations or sub-combinations separately and divisibly from any other combination of markers or groups of markers.

[0107] As used herein the term "TNAP" is intended to encompass all isoforms of tissue non-specific alkaline phosphatase. For example, the term encompasses the liver isoform

(LAP), the bone isoform (BAP) and the kidney isoform (KAP). In one example, the TNAP is BAP. In one example, TNAP as used herein refers to a molecule which can bind the STRO-3 antibody produced by the hybridoma cell line deposited with ATCC on 19 December 2005 under the provisions of the Budapest Treaty under deposit accession number PTA-7282.

[0108] Furthermore, in one example, the STRO-1+ cells are capable of giving rise to clonogenic CFU-F.

[0109] In one example, a significant proportion of the STRO-1+ cells are capable of differentiation into at least two different germ lines. Non-limiting examples of the lineages to which the STRO-1+ cells may be committed include bone precursor cells; hepatocyte progenitors, which are multipotent for bile duct epithelial cells and hepatocytes; neural restricted cells, which can generate glial cell precursors that progress to oligodendrocytes and astrocytes; neuronal precursors that progress to neurons; precursors for cardiac muscle and cardiomyocytes, glucose-responsive insulin secreting pancreatic beta cell lines. Other lineages include, but are not limited to, odontoblasts, dentin-producing cells and chondrocytes, and precursor cells of the following: retinal pigment epithelial cells, fibroblasts, skin cells such as keratinocytes, dendritic cells, hair follicle cells, renal duct epithelial cells, smooth and skeletal muscle cells, testicular progenitors, vascular endothelial cells, tendon, ligament, cartilage, adipocyte, fibroblast, marrow stroma, cardiac muscle, smooth muscle, skeletal muscle, pericyte, vascular, epithelial, glial, neuronal, astrocyte and oligodendrocyte cells.

[0110] In an example, mesenchymal lineage precursor or stem cells are obtained from a single donor, or multiple donors where the donor samples or mesenchymal lineage precursor or stem cells are subsequently pooled and then culture expanded.

[0111] Mesenchymal lineage precursor or stem cells encompassed by the present disclosure may also be cryopreserved prior to administration to a subject. In an example, mesenchymal lineage precursor or stem cells are culture expanded and cryopreserved prior to administration to a subject.

[0112] In an example, the present disclosure encompasses mesenchymal lineage precursor or stem cells as well as progeny thereof, soluble factors derived therefrom, and/or extracellular vesicles isolated therefrom. In another example, the present disclosure encompasses mesenchymal lineage precursor or stem cells as well as extracellular vesicles isolated therefrom. For example, it is possible to culture expand

mesenchymal precursor lineage or stem cells of the disclosure for a period of time and under conditions suitable for secretion of extracellular vesicles into the cell culture medium. Secreted extracellular vesicles can subsequently be obtained from the culture medium for use in therapy.

[0113] The term "extracellular vesicles" as used herein, refers to lipid particles naturally released from cells and ranging in size from about 30 nm to as a large as 10 microns, although typically they are less than 200 nm in size. They can contain proteins, nucleic acids, lipids, metabolites, or organelles from the releasing cells (e.g., mesenchymal stem cells; STRO-1^+ cells).

[0114] The term "exosomes" as used herein, refers to a type of extracellular vesicle generally ranging in size from about 30 nm to about 150 nm and originating in the endosomal compartment of mammalian cells from which they are trafficked to the cell membrane and released. They may contain nucleic acids (*e.g.*, RNA; microRNAs), proteins, lipids, and metabolites and function in intercellular communication by being secreted from one cell and taken up by other cells to deliver their cargo.

[0115] In an example, compositions of the disclosure comprise cells that induce new blood vessel formation in target tissue. In an example, the target tissue is the heart. In another example, the cells secrete factors that protect at risk or damaged myocardium. In an example, at risk or damaged myocardium has been subject to a lack of blood flow resulting from an ischemic event. In an example, the cells secrete factors that reduce apoptosis in cardiomyocytes.

Culture expansion of the cells

[0116] In an example, mesenchymal lineage precursor or stem cells are culture expanded. "Culture expanded" mesenchymal lineage precursor or stem cells media are distinguished from freshly isolated cells in that they have been cultured in cell culture medium and passaged (i.e. sub-cultured). In an example, culture expanded mesenchymal lineage precursor or stem cells are culture expanded for about 4 – 10 passages. In an example, mesenchymal lineage precursor or stem cells are culture expanded for at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 passages. For example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 – 10 passages. In an example, mesenchymal lineage precursor or stem cells can be culture

expanded for at least 5 – 8 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 – 7 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for more than 10 passages. In another example, mesenchymal lineage precursor or stem cells can be culture expanded for more than 7 passages. In these examples, stem cells may be culture expanded before being cryopreserved to provide an intermediate cryopreserved MLPSC population. In an example, compositions of the present disclosure are produced by culturing cells from an intermediate cryopreserved MLPSC population or, put another way, a cryopreserved intermediate.

[0117] In an example, compositions of the disclosure comprise mesenchymal lineage precursor or stem cells that are culture expanded from a cryopreserved intermediate. In an example, the cells culture expanded from a cryopreserved intermediate are culture expanded for at least 5, at least 6, at least 7, at least 8, at least 9, at least 10 passages. For example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 – 10 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 – 8 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for at least 5 – 7 passages. In an example, mesenchymal lineage precursor or stem cells can be culture expanded for more than 10 passages. In another example, mesenchymal lineage precursor or stem cells can be culture expanded for more than 7 passages.

[0118] In an example, mesenchymal lineage precursor or stem cells culture expanded from a cryopreserved intermediate can be culture expanded in medium free of animal proteins. In an example, mesenchymal lineage precursor or stem cells culture expanded from a cryopreserved intermediate can be culture expanded in xeno-free medium. In an example, mesenchymal lineage precursor or stem cells culture expanded from a cryopreserved intermediate can be culture expanded in medium that is fetal bovine serum free.

[0119] In an embodiment, mesenchymal lineage precursor or stem cells can be obtained from a single donor, or multiple donors where the donor samples or mesenchymal lineage precursor or stem cells are subsequently pooled and then culture expanded. In an example, the culture expansion process comprises:

i. expanding by passage expansion the number of viable cells to provide a preparation of at least about 1 billion of the viable cells, wherein the passage expansion comprises establishing a primary culture of isolated mesenchymal lineage precursor or stem cells and then serially establishing a first non-primary (P1) culture of isolated mesenchymal lineage precursor or stem cells from the previous culture;

ii. expanding by passage expansion the P1 culture of isolated mesenchymal lineage precursor or stem cells to a second non-primary (P2) culture of mesenchymal lineage precursor or stem cells; and,

iii. preparing and cryopreserving an in-process intermediate mesenchymal lineage precursor or stem cells preparation obtained from the P2 culture of mesenchymal lineage precursor or stem cells; and,

iv. thawing the cryopreserved in-process intermediate mesenchymal lineage precursor or stem cells preparation and expanding by passage expansion the in-process intermediate mesenchymal lineage precursor or stem cells preparation.

[0120] In an example, the expanded mesenchymal lineage precursor or stem cell preparation has an antigen profile and an activity profile comprising:

i. less than about 0.75% CD45+ cells;

ii. at least about 95% CD105+ cells;

iii. at least about 95% CD166+ cells.

[0121] In an example, the expanded mesenchymal lineage precursor or stem cell preparation is capable of inhibiting IL2-Rα expression by CD3/CD28-activated PBMCs by at least about 30% relative to a control.

[0122] In an example, culture expanded mesenchymal lineage precursor or stem cells are culture expanded for about 4 – 10 passages, wherein the mesenchymal lineage precursor or stem cells have been cryopreserved after at least 2 or 3 passages before being further culture expanded. In an example, mesenchymal lineage precursor or stem cells are culture expanded for at least 1, at least 2, at least 3, at least 4, at least 5 passages, cryopreserved and then further culture expanded for at least 1, at least 2, at least 3, at least 4, at least 5 passages before being cultured according to the methods of the disclosure.

[0123] The process of mesenchymal lineage precursor or stem cell isolation and *ex vivo* expansion can be performed using any equipment and cell handing methods known in the art. Various culture expansion embodiments of the present disclosure employ steps that require manipulation of cells, for example, steps of seeding, feeding, dissociating an

adherent culture, or washing. Any step of manipulating cells has the potential to insult the cells. Although mesenchymal lineage precursor or stem cells can generally withstand a certain amount of insult during preparation, cells are preferably manipulated by handling procedures and/or equipment that adequately performs the given step(s) while minimizing insult to the cells.

[0124] In an example, mesenchymal lineage precursor or stem cells are washed in an apparatus that includes a cell source bag, a wash solution bag, a recirculation wash bag, a spinning membrane filter having inlet and outlet ports, a filtrate bag, a mixing zone, an end product bag for the washed cells, and appropriate tubing, for example, as described in US 6,251,295, which is hereby incorporated by reference.

[0125] In an example, a mesenchymal lineage precursor or stem cell composition cultured according to the present disclosure is 95% homogeneous with respect to being CD105 positive and CD166 positive and being CD45 negative. In an example, this homogeneity persists through *ex vivo* expansion; i.e. though multiple population doublings.

[0126] In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded in 3D culture. For example, mesenchymal lineage precursor or stem cells of the disclosure can be culture expanded in a bioreactor. In an example, mesenchymal lineage precursor or stem cells of the disclosure are initially culture expanded in 2D culture prior to being further expanded in 3D culture. In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded from a master cell bank. In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded from a master cell bank in 2D culture before seeding in 3D culture. In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded from a master cell bank in 2D culture for at least 3 days before seeding in 3D culture in a bioreactor. In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded from a master cell bank in 2D culture for at least 4 days before seeding in 3D culture in a bioreactor. In an example, mesenchymal lineage precursor or stem cells of the disclosure are culture expanded from a master cell bank in 2D culture for between 3 and 5 days before seeding in 3D culture in a bioreactor. In these examples, 2D culture can be performed in a cell factory. Various cell factory products are available commercially (e.g. Thermofisher, Sigma).

Cell Culture Medium

[0127] Mesenchymal lineage precursor or stem cells disclosed herein can be culture expanded in various suitable growth mediums.

[0128] The term "medium" or "media" as used in the context of the present disclosure, includes the components of the environment surrounding the cells. The media contributes to and/or provides the conditions suitable to allow cells to grow. Media may be solid, liquid, gaseous or a mixture of phases and materials. Media can include liquid growth media as well as liquid media that do not sustain cell growth. Media also include gelatinous media such as agar, agarose, gelatin and collagen matrices. Exemplary gaseous media include the gaseous phase that cells growing on a petri dish or other solid or semisolid support are exposed to.

[0129] The cell culture media used for culture expansion contains all essential amino acids and may also contain non-essential amino acids. In general, amino acids are classified into essential amino acids (Thr, Met, Val, Leu, Ile, Phe, Trp, Lys, His) and non-essential amino acids (Gly, Ala, Ser, Cys, Gln, Asn, Asp, Tyr, Arg, Pro).

[0130] Those of skill in the art will appreciate that for optimal results, the basal medium must be appropriate for the cell line of interest. For example, it may be necessary to increase the level of glucose (or other energy source) in the basal medium, or to add glucose (or other energy source) during the course of culture, if this energy source is found to be depleted and to thus limit growth. In an example, dissolved oxygen (DO) levels can also be controlled.

[0131] In an example, the cell culture medium contains human derived additives. For example, human serum and human platelet cell lysate can be added to the cell culture media.

[0132] In an example, the cell culture medium contains only human derived additives. Thus, in an example, the cell culture media is xeno-free. For avoidance of doubt, in these examples, the culture medium is free of animal proteins. In an example, cell culture medium used in the methods of the disclosure is free of animal components.

[0133] In an example, the culture medium comprises serum. In other examples the culture medium is fetal bovine serum free culture medium comprising growth factors that promote mesenchymal lineage precursor or stem cell proliferation. In an embodiment,

the culture medium is serum free stem cell culture medium. In an example, the cell culture medium comprises:

a basal medium;

platelet derived growth factor (PDGF);

fibroblast growth factor 2 (FGF2).

[0134] In an example, the culture medium comprises platelet derived growth factor (PDGF) and fibroblast growth factor 2 (FGF2), wherein the level of FGF2 is less than about 6 ng/ml. For example, the FGF2 level may be less than about 5 ng/ml, less than about 4 ng/ml, less than about 3 ng/ml, less than about 2 ng/ml, less than about 1 ng/ml. In other examples, the FGF2 level is less than about 0.9 ng/ml, less than about 0.8 ng/ml, less than about 0.7 ng/ml, less than about 0.6 ng/ml, less than about 0.5 ng/ml, less than about 0.4 ng/ml, less than about 0.3 ng/ml, less than about 0.2 ng/ml.

[0135] In another example, the level of FGF2 is between about 1 pg/ml and 100 pg/ml. In another example, the level of FGF2 is between about 5 pg/ml and 80 pg/ml.

[0136] In an example, the PDGF is PDGF-BB. In an example, the level of PDGF-BB is between about 1 ng/ml and 150 ng/ml. In another example, the level of PDGF-BB is between about 7.5 ng/ml and 120 ng/ml. In another example, the level of PDGF-BB is between about 15 ng/ml and 60 ng/ml. In another example, the level of PDGF-BB is at least about 10 ng/ml. In another example, the level of PDGF-BB is at least about 15 ng/ml. In another example, the level of PDGF-BB is at least about 20 ng/ml. In another example, the level of PDGF-BB is at least about 21 ng/ml. In another example, the level of PDGF-BB is at least about 22 ng/ml. In another example, the level of PDGF-BB is at least about 23 ng/ml. In another example, the level of PDGF-BB is at least about 24 ng/ml. In another example, the level of PDGF-BB is at least about 25 ng/ml.

[0137] In another example, the PDGF is PDGF-AB. In an example, the level of PDGF-AB is between about 1 ng/ml and 150 ng/ml. In another example, the level of PDGF-AB is between about 7.5 ng/ml and 120 ng/ml. In another example, the level of PDGF-AB is between about 15 ng/ml and 60 ng/ml. In another example, the level of PDGF-AB is at least about 10 ng/ml. In another example, the level of PDGF-AB is at least about 15 ng/ml. In another example, the level of PDGF-AB is at least about 20 ng/ml. In another example, the level of PDGF-AB is at least about 21 ng/ml. In another example, the level of PDGF-AB is at least about 22 ng/ml. In another example, the level of PDGF-AB is at

least about 23 ng/ml. In another example, the level of PDGF-AB is at least about 24 ng/ml. In another example, the level of PDGF-AB is at least about 25 ng/ml.

[0138] In other examples, additional factors can be added to the cell culture medium. In an example, the culture medium further comprising EGF. EGF is a growth factor that stimulates cell proliferation by binding to its receptor EGFR. In an example, the method of the present disclosure comprises culturing a population of stem cells in a fetal bovine serum free cell culture medium further comprising EGF. In an example, the level of EGF is between about 0.1 and 7 ng/ml. For example, the level of EGF can be at least about 5 ng/ml.

[0139] In another example, the level of EGF is between about 0.2 ng/ml and 3.2 ng/ml. In another example, the level of EGF is between about 0.4 ng/ml and 1.6 ng/ml. In another example, the level of EGF is between about 0.2 ng/ml. In another example, the level of EGF is at least about 0.3 ng/ml. In another example, the level of EGF is at least about 0.4 ng/ml. In another example, the level of EGF is at least about 0.5 ng/ml. In another example, the level of EGF is at least about 0.6 ng/ml. In another example, the level of EGF is at least about 0.7 ng/ml. In another example, the level of EGF is at least about 0.8 ng/ml. In another example, the level of EGF is at least about 0.9 ng/ml. In another example, the level of EGF is at least about 1.0 ng/ml.

[0140] In the above examples, basal medium such as Alpha MEM or StemSpan™ can be supplemented with the referenced quantity of growth factor. In an example, the culture medium comprises Alpha MEM or StemSpan™ supplemented with 32 ng/ml PDGF-BB, 0.8 ng/ml EGF and 0.02 ng/ml FGF.

[0141] In other examples, additional factors can be added to the cell culture medium. For example, the cell culture media can be supplemented with one or more stimulatory factors selected from the group consisting of epidermal growth factor (EGF), lα,25-dihydroxyvitamin D3 (1,25D), tumor necrosis factor α (TNF- α), interleukin -lβ (IL-lβ) and stromal derived factor lα (SDF-lα). In another embodiment, cells may also be cultured in the presence of at least one cytokine in an amount adequate to support growth of the cells. In another embodiment, cells can be cultured in the presence of heparin or a derivative thereof. For example, the cell culture medium may contain about 50ng/ml of heparin. In other examples, the cell culture medium contains about 60ng/ml of heparin, about 70ng/ml of heparin, about 80ng/ml of heparin, about 90ng/ml of heparin, about 100ng/ml of heparin, about 110ng/ml of heparin, about 110ng/ml of heparin, about

120ng/ml of heparin, about 130ng/ml of heparin, about 140ng/ml of heparin, about 150ng/ml of heparin or a derivative thereof. In an example, the heparin derivative is a sulphate). Various forms of heparin sulphate are known in the art and include heparin sulphate 2 (HS2). HS2 can be derived from various sources including for example, the liver of male and/or female mammals. Thus, an exemplary heparin sulphate includes male liver heparin sulphate (MML HS) and female liver heparin sulphate (FML HS).

[0142] In another example, the cell culture medium of the present disclosure promotes stem cell proliferation while maintaining stem cells in an undifferentiated state. Stem cells are considered to be undifferentiated when they have not committed to a specific differentiation lineage. As discussed above, stem cells display morphological characteristics that distinguish them from differentiated cells. Furthermore, undifferentiated stem cells express genes that may be used as markers to detect differentiation status. The polypeptide products may also be used as markers to detect differentiation status. Accordingly, one of skill in the art could readily determine whether the methods of the present disclosure maintain stem cells in an undifferentiated state using routine morphological, genetic and/or proteomic analysis.

Modification of the cells

[0143] The mesenchymal lineage precursor or stem cells disclosed herein may be altered in such a way that upon administration, lysis of the cell is inhibited. Alteration of an antigen can induce immunological non-responsiveness or tolerance, thereby preventing the induction of the effector phases of an immune response (e.g., cytotoxic T cell generation, antibody production etc.) which are ultimately responsible for rejection of foreign cells in a normal immune response. Antigens that can be altered to achieve this goal include, for example, MHC class I antigens, MHC class II antigens, LFA-3 and ICAM-1.

[0144] The mesenchymal lineage precursor or stem cells may also be genetically modified to express proteins of importance for the differentiation and/or maintenance of striated skeletal muscle cells. Exemplary proteins include growth factors (TGF-β, insulin-like growth factor 1 (IGF-1), FGF), myogenic factors (e.g. myoD, myogenin, myogenic factor 5 (Myf5), myogenic regulatory factor (MRF)), transcription factors (e.g. GATA-4), cytokines (e.g. cardiotropin-1), members of the neuregulin family (e.g. neuregulin 1, 2 and 3) and homeobox genes (e.g. Csx, tinman and NKx family).

Compositions

[0145] Mesenchymal lineage or stem cells disclosed herein can be culture expanded from a cryopreserved intermediate to produce a preparation containing at least one therapeutic dose.

[0146] In an example, compositions of the disclosure comprise between 10 x 10^6 cells and 35 x 10^6 cells. In another example, the composition comprises between 20 x 10^6 cells and 30 x 10^6 cells. In other examples, the composition comprises at least 100 x 10^6 cells. In another example, the composition comprises between 50 x 10^6 cells and 500 x 10^6 cells. In other examples, compositions of the disclosure comprise 150 million cells.

[0147] In one example, compositions of the disclose comprise a pharmaceutically acceptable carrier and/or excipient. The terms "carrier" and "excipient" refer to compositions of matter that are conventionally used in the art to facilitate the storage, administration, and/or the biological activity of an active compound (see, e.g., Remington's Pharmaceutical Sciences, 16th Ed., Mac Publishing Company (1980). A carrier may also reduce any undesirable side effects of the active compound. A suitable carrier is, for example, stable, e.g., incapable of reacting with other ingredients in the carrier. In one example, the carrier does not produce significant local or systemic adverse effect in recipients at the dosages and concentrations employed for treatment.

[0148] Suitable carriers for the present disclosure include those conventionally used, e.g., water, saline, aqueous dextrose, lactose, Ringer's solution, a buffered solution, hyaluronan and glycols are exemplary liquid carriers, particularly (when isotonic) for solutions. Suitable pharmaceutical carriers and excipients include starch, cellulose, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, magnesium stearate, sodium stearate, glycerol monostearate, sodium chloride, glycerol, propylene glycol, water, ethanol, and the like.

[0149] In another example, a carrier is a media composition, e.g., in which a cell is grown or suspended. Such a media composition does not induce any adverse effects in a subject to whom it is administered. Exemplary carriers and excipients do not adversely affect the viability of a cell and/or the ability of a cell to treat or prevent disease.

[0150] In one example, the carrier or excipient provides a buffering activity to maintain the cells and/or soluble factors at a suitable pH to thereby exert a biological activity, e.g., the carrier or excipient is phosphate buffered saline (PBS). PBS represents an attractive

carrier or excipient because it interacts with cells and factors minimally and permits rapid release of the cells and factors, in such a case, the composition of the disclosure may be produced as a liquid for direct application to the blood stream or into a tissue or a region surrounding or adjacent to a tissue, e.g., by injection.

[0151] Compositions of the disclosure may be cryopreserved. Cryopreservation of mesenchymal lineage precursor or stem cells can be carried out using slow-rate cooling methods or 'fast' freezing protocols known in the art. Preferably, the method of cryopreservation maintains similar phenotypes, cell surface markers and growth rates of cryopreserved cells in comparison with unfrozen cells.

[0152] The cryopreserved composition may comprise a cryopreservation solution. The pH of the cryopreservation solution is typically 6.5 to 8, preferably 7.4.

[0153] The cyropreservation solution may comprise a sterile, non-pyrogenic isotonic solution such as, for example, PlasmaLyte ATM. 100 mL of PlasmaLyte ATM contains 526 mg of sodium chloride, USP (NaCl); 502 mg of sodium gluconate (C6H11NaO7); 368 mg of sodium acetate trihydrate, USP (C2H3NaO2•3H2O); 37 mg of potassium chloride, USP (KCl); and 30 mg of magnesium chloride, USP (MgCl2•6H2O). It contains no antimicrobial agents. The pH is adjusted with sodium hydroxide. The pH is 7.4 (6.5 to 8.0).

[0154] The cryopreservation solution may comprise Profreeze^{TM}. The cryopreservation solution may additionally or alternatively comprise culture medium, for example, αMEM.

[0155] To facilitate freezing, a cryoprotectant such as, for example, dimethylsulfoxide (DMSO), is usually added to the cryopreservation solution. Ideally, the cryoprotectant should be nontoxic for cells and patients, nonantigenic, chemically inert, provide high survival rate after thawing and allow transplantation without washing. However, the most commonly used cryoprotector, DMSO, shows some cytotoxicity . Hydroxylethyl starch (HES) may be used as a substitute or in combination with DMSO to reduce cytotoxicity of the cryopreservation solution.

[0156] The cryopreservation solution may comprise one or more of DMSO, hydroxyethyl starch, human serum components and other protein bulking agents. In one example, the cryopreserved solution comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

[0157] In an example, the cryopreservation solution may further comprise one or more of methycellulose, polyvinyl pyrrolidone (PVP) and trehalose.

[0158] The cryopreserved composition may be thawed and administered directly to the subject or added to another solution, for example, comprising hyaluronic acid. Alternatively, the cryopreserved composition may be thawed and the mesenchymal lineage precursor or stem cells resuspended in an alternate carrier prior to administration.

[0159] The compositions described herein may be administered alone or as admixtures with other cells. The cells of different types may be admixed with a composition of the disclosure immediately or shortly prior to administration, or they may be co-cultured together for a period of time prior to administration.

[0160] In one example, the composition comprises an effective amount or a therapeutically or prophylactically effective amount of mesenchymal lineage precursor or stem cells and/or progeny thereof and/or soluble factor derived therefrom. For example, the composition comprises about $1x10^5$ stem cells to about $1x10^9$ stem cells or about $1.25x10^3$ stem cells to about $1.25x10^7$ stem cells/kg (80 kg subject). The exact amount of cells to be administered is dependent upon a variety of factors, including the age, weight, and sex of the subject, and the extent and severity of the disorder being treated.

[0161] Despite the number of cells provided in the composition, in an example, 50×10^6 to 200×10^7 cells are administered. In other examples, 60×10^6 to 200×10^6 cells or 75×10^6 to 150×10^6 cells are administered. In an example, 75×10^6 cells are administered. In another example, 150×10^6 cells are administered.

[0162] In an example, the composition comprises greater than $5.00x10^6$ viable cells/mL. In another example, the composition comprises greater than $5.50x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.00x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.50x10^6$ viable cells/mL. In another example, the composition comprises greater than $6.68x10^6$ viable cells/mL.

[0163] In an example, the mesenchymal lineage precursor or stem cells comprise at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 99% of the cell population of the composition.

[0164] In an example, the composition may optionally be packaged in a suitable container with written instructions for a desired purpose.

[0165] Compositions of the disclosure may be administered systemically, such as, for example, by intravenous administration. In an example, compositions are administered transendocardially.

Risk of cardiac death, myocardial infarction or stroke

[0166] In an example, the methods of the present disclosure relate to methods of assessing risk of one or more of cardiac death, myocardial infarction or stroke based on the level of CRP in a subject. For example, the methods of the present disclosure can relate to methods of assessing risk of cardiac death based on the level of CRP in a subject. In an example, the present disclosure encompasses a method for determining elevated risk of one or more of cardiac death, myocardial infarction or stroke in a subject, the method comprising measuring the level of CRP in a sample obtained from a subject, wherein elevated CRP indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, the subject has progressive heart failure. For example, the subject can have NYHA Class II progressive heart failure. Accordingly, in an example, the sample is obtained from a subject with NYHA Class II progressive heart failure. In an example, the sample is a blood sample.

[0167] In an example, a level of CRP >1 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, a level of CRP >1.5 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, a level of CRP ≥2 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, a level of CRP between ≥2 and 5 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke. In an example, the level of CRP is measured after an ischemic event. In an example, the ischemic event is a myocardial infarction.

[0168] In an example, a composition comprising cells which induce new blood vessel formation in target tissue is administered to subjects assessed as having elevated risk of cardiac death, myocardial infarction or stroke. Accordingly, in an example, the present disclosure relates to a method of treating progressive heart failure, the method comprising i) selecting a subject having Class II heart failure according to the New York Heart Association (NYHA) classification scale and elevated CRP level, and ii) administering to the subject a composition according to the present disclosure. In an example, the subject's CRP level is ≥2 mg/ml.

[0169] It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

[0170] This application claims priority from AU2020904675 filed on 15 December 2020, 2021900059 filed on 12 January 2021, AU2021902941 filed on 10 September 2021 and AU2021903365 filed on 20 October 2021 the disclosures of which are incorporated herein in their entirety.

[0171] All publications discussed and/or referenced herein are incorporated herein in their entirety.

[0172] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this application.

EXAMPLES

Composition

[0173] The composition consists of human bone marrow-derived allogeneic MPCs isolated from bone mononuclear cells with anti-STRO-3 antibodies, expanded ex vivo, and cryopreserved.

Patients

Baseline data

Parameter	Units	Total (n=537)		NYHA II		NYHA III	
NYHA Functional Class	Number, %			206	38.4%	331	61.6%
II	Number, %	206	38.4%				
III	Number, %	331	61.6%				
Age (537 unique subjects)	Yrs (mean)	62.7	64.0	62.2	63.0	63.1	65.0
Sex	(537 unique subjects)*						
Males	Number, %	428	79.7%	168	81.6%	260	78.5%
Females	Number, %	109	20.3%	38	18.4%	71	21.5%
Race	(537 unique subjects)*						
White	Number, %	414	77.1%	166	80.6%	248	74.9%
Black	Number, %	99	18.4%	34	16.5%	65	19.6%
Asian	Number, %	7	1.3%	2	1.0%	5	1.5%
Other	Number, %	17	3.2%	4	1.9%	13	3.9%
Ethnic Group	(537 unique subjects)*						
Hispanic	Number, %	34	6.3%	11	5.3%	23	6.9%
Blood pressure							
Systolic	mmHg (mean)	117.1	114.0	117.2	116	117	113
Diastolic	mmHg (mean)	70.2	70.0	71.2	71	69.5	68
Heart rate	Beats/min (mean)	72.3	71.0	70.9	70	73.1	71
Body weight	kg (mean, median)	92.8	90.9	91.8	89.2	93.4	92.5

Parameter	Units	Total (n=537)		NYHA II		NYHA III	
Non-Ischemic vs Ischemic Cardiomyopathy (per baseline CRF)							
Ischemic	Number, %	303	56.4%	111	53.9%	192	58.0%
Non-Ischemic	Number, %	234	43.6%	95	46.1%	139	42.0%
Past Myocardial infarction	Number, %	280	52.1%	106	51.5%	174	52.6%
Coronary revascularization							
Previous PCI and/or CABG (unique subjects)	Number, %	307	57.2%	119	57.8%	188	56.8%
Echocardiographic Imaging (530 unique subjects)							
Left ventricular ejection fraction	% (mean, median)	28.6	28.6	28.6	29.0	28.6	28.5
Left ventricular end-systolic volume	mL (mean, median)	149.8	135.9	155.4	137.4	146.2	135.0
<=100	number, %	101	18.8%	41	19.9%	60	18.1%
> 100	number, %	429	79.9%	163	79.1%	266	80.4%
missing	number, %	7	1.3%	2	1.0%	5	1.5%
Left ventricular end-diastolic volume	mL (mean, median)	206.5	194.0	213.1	193.7	202.3	194.3
6MWT distance (537 unique subjects)	m (mean, median)	331.5	340.5	356.8	367	311.7	329.2
Biomarkers							
NT-proBNP	pg/mL (mean, median)	2166	1400	1809	1322	2390	1458
hsCRP	mg/L (mean, median)	5.3	2.5	3.6	2	6.4	3.3
AICD without CRT	Number, %	245	84.0%	99	81.6%	146	85.5%
CRT-D	Number, %	206		69		137	
Laboratory measurements							
Sodium	mequiv/L (mean)	139.5	140.0	139.5	140.0	139.6	140.0
Potassium	mequiv/L (mean)	4.5	4.5	4.5	4.5	4.5	4.5
Creatinine	mg/dL (mean)	1.2	1.1	1.1	1.1	1.3	1.2
BUN	mg/dL (mean)	24.2	21.0	22.7	20.5	25.2	22.0
HbA1c	% (mean)	6.4	6.1	6.3	6.0	6.4	6.2
Hemoglobin	g/dL (mean)	13.6	13.7	13.8	13.9	13.5	13.5
Hematocrit	% (mean)	41.8	41.8	42.3	42.4	41.5	41.1

[0174] Primary and secondary outcome measurements were as follows:

1) Non-Fatal Major Adverse Cardiac Events (MACE):

Heart Failure MACE (recurrent decompensated heart failure events or high-grade arrhythmias);

Ischemic MACE (heart attacks or strokes)

2) Death from cardiac causes

[0175] Cell therapy unexpectedly reduced incidence of ischemic MACE (MI, Stroke) by 60% relative to Controls (N = 537 Patients; p=0.002; Figure 1). Figure 2 shows the significantly reduced incidence of ischemic MACE (MI, Stroke) observed relative to controls in NYHA Class II & III. These data suggest that cell therapy can reduce risk of ischemic events in patients with cardiomyopathy. Figure 3 shows that the significantly reduced incidence of ischemic MACE (MI, Stroke) was observed in both ischemic and non-ischemic patients. Cardiomyopathy patients are at risk of occlusive plaque development due to ongoing inflammatory processes in these patients. These problematic processes appear to be inhibited by cell therapy in view of the general reduction of ischemic MACE observed in both ischemic and non-ischemic cardiomyopathy patients. Accordingly, the present data appears to underpin a general concept whereby cell therapy can be administered to reduce the risk of ischemic event(s) in patients suffering cardiomyopathy.

[0176] Surprisingly, cell therapy reduced cardiac death in NYHA Class II patients but did not reduce cardiac death in NYHA Class III patients (Figure 4 and 6). This result was surprising because it suggested a threshold level of viable myocardium was required to reduce cardiac death with cell therapy. In other words, patients with Class III heart failure may have progressed too far along the disease continuum for cell therapy to improve their survival. These findings suggest that cell therapy would be particularly effective in NYHA Class II patients. Given the capacity of the administered cells to induce new blood vessel formation in target tissue, the findings of the present inventors suggest a general concept for reducing cardiac death in patients graded lower than NYHA class III by administering cell therapy. As further support for this concept, the inventors noted that the observed reduction in cardiac death for NYHA Class II patients was maintained despite the cause of cardiomyopathy, with reductions in cardiac death being observed in ischemic and non-ischemic NYHA Class II patients (Figure 5).

3) Improved outcomes

3 Point MACE

[0177] Subsequent analysis revealed that a single injection of cell therapy significantly decreased risk of the composite outcome of 3-point Irreversible Morbidity or Mortality MACE compared to controls across all 537 treated patients. Decreased risk was even more apparent in subject with CRP ≥2 mg/ml. Risk of 3-point MACE was reduced by 33% using a time to first event analysis [HR 0.667; 95% CI (0.472, 0.941); P=0.021; Figure 7A] and by 35% in a recurrent event rate analysis normalized for time of follow-up (i.e., events per 100 patient-years) [HR 0.646; 95% CI (0.466, 0.895); P=0.009; Figure 7B]. Kaplan-Meier curves for this composite outcome in patients with plasma hsCRP levels ≥2 mg/L or < 2 mg/L are depicted in Figure 7C. As shown in Figure 7C, in all treated patients with CRP ≥2 mg/L, cell therapy significantly reduced the risk of:

- Non-fatal MI and non-fatal stroke (Figure 7C1); and,
- Composite of cardiac death or ischemic MACE (MI or Stroke) (Figure 7C2).

Ischemic MACE

[0178] Across all 537 treated patients, a single injection of cell therapy decreased risk for occurrence of Irreversible Morbidity (non-fatal MI or non-fatal stroke) by 65% compared to controls using time to first event analysis (TTFE) [HR 0.346; 95% CI (0.180, 0.664); P=0.001; Figure 8A] and by 69% using recurrent event rate normalization [HR 0.306; 95% CI (0.162, 0.579); P<0.001; Figure 8B].

[0179] Pre-specified sub-group analyses were performed for all treated patients based on presence or absence of inflammation at the time of treatment. A single injection of cell therapy in the 301 patients with inflammation (CRP ≥2 mg/L) decreased risk of non-fatal MI or non-fatal stroke by 79% using TTFE [HR 0.206; 95% CI (0.070, 0.611); P=0.004; Figure 8A] and by 83% using recurrent event rate normalization [HR 0.170; 95% CI (0.059, 0.492); P=0.001; Figure 8B]. Taken together with the above referenced 3 point-MACE analysis, these data underpin a rationale for selecting and treating patients heart failure an active inflammation, preferably as defined by CRP ≥2 mg/L.

[0180] Cell therapy significantly reduced composite of cardiac death or ischemic MACE (MI or Stroke) in all patients by 33% (Figure 9). Further analysis of patient groups then revealed that cell therapy significantly reduced by 60% composite of cardiac death or

ischemic MACE (MI or Stroke) in NYHA Class II patients compared to controls, further supporting the rationale of selecting and treating patients with NYHA Class II heart failure (Figure 9). It was also noted that cell therapy prevented progression of cardiac death in NYHA Class II patients over multiple years of follow-up (Figure 10).

[0181] Cell therapy also unexpectedly reduced risk of cardiac death (Figure 11) and 3-point MACE (Cardiac death/MI/Stroke; Figure 12) in NYHA Class II patients with elevated CRP levels, in particular, CRP levels ≥2 mg/L. These beneficial effects were not evident in patients with baseline CRP <2 mg/, suggesting that cell therapy may be particularly beneficial in the presence of active inflammation.

[0182] Further data analysis revealed that CRP was an important marker of cardiac death. As shown in Figure 11, patients having elevated CRP levels (≥2 mg/L) had significantly increased risk of cardiac death. These data further support the treatment of NYHA class II patients with cell therapy, in particular when those patients have elevated CRP levels, for example CRP ≥2 mg/L. These data also indicate the utility of CRP level as an indicator or patients at risk of cardiac death.

CLAIMS

1. A method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising cells, wherein the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale.

2. A method of reducing progression of heart failure in a subject, the method comprising administering to the subject a composition comprising cells, wherein the subject has Class II heart failure according to the New York Heart Association (NYHA) classification scale.

3. A method of reducing cardiac death in a subject with Class II heart failure according to the New York Heart Association (NYHA) classification scale, the method comprising administering to the subject a composition comprising cells.

4. A method of selecting heart failure patients for treatment with cell therapy, the method comprising i) assessing heart failure according to the New York Heart Association (NYHA) classification scale, and ii) selecting a subject having Class II heart failure according to NYHA, preferably, wherein the method comprises administering a composition comprising cells.

5. The method according to anyone of claims 1 to 4, wherein the subject's CRP level is elevated prior to administering cells.

6. The method of claim 5, wherein the subject's CRP level is ≥2 mg/L.

7. The method according to any one of claims 1 to 6, wherein the cells:

induce new blood vessel formation in target tissue, preferably wherein the cells promote arteriogenesis; and/or

secrete factors that protect at risk myocardium.

8. The method according to claim 1 or claim 2 which comprises the steps of: i) selecting a subject having Class II heart failure according to the New York Heart Association (NYHA) classification scale, and ii) administering to the subject a composition comprising cells which induce new blood vessel formation in target tissue.

9. The method according to any one of claims 1 to 8, wherein administering the composition inhibits the subject's progression to NYHA class III progressive heart failure.

10. The method according to any one of claims 1 to 9, wherein the subject's level of N-terminal pro–B-type natriuretic peptide (NT-proBNP) is:

less than 2200 pg/ ml, preferably less than 2000 pg/ml, prior to administering the cells; or,

between 1000 pg/ml and 2000 pg/ ml prior to administering the cells.

11. The method according to any one of claims 1 to 10, wherein the subject's C-reactive protein (CRP) level is <5 mg/L, preferably <4 mg/L.

12. The method according to any one of claims 1 to 11, wherein the subject's CRP level is between 1.5 and 5 mg/L.

13. The method according to any one of claims 1 to 12, wherein the subject has had a heart failure hospitalisation event over the previous 9 months.

14. The method according to any one of claims 1 to 13, wherein the subject has a LVEF of less than about 45%, preferably less than 40%.

15. The method according to any one of claims 1 to 14, wherein the subject has persistent left ventricular dysfunction.

16. The method according to any one of claims 1 to 15, wherein the subject's heart failure results from an ischemic event or from a non-ischemic event.

17. The method according to any one of claims 1, 2 or 5 to 16, wherein the subject has a reduced risk of cardiac death after treatment.

18. The method of claim 17, wherein the reduced risk is relative to risk of cardiac death in a subject with NYHA class III progressive heart failure.

19. The method according to any one of claims 1 to 18, wherein the subject has a reduced risk of ischemic MACE (MI or stroke) after treatment.

20. The method according to any one of claims 1 to 19, wherein the composition is administered transendocardially and/or intravenously.

21. The method according to any one of claims 1 to 20, wherein the cells are mesenchymal lineage precursor or stem cells (MLPSCs).

22. The method of claim 21, wherein the MLPSCs are STRO-1+.

23. The method according to claim 21, wherein the MLPSCs are mesenchymal stem cells (MSCs).

24. The method according to claim 21 or claim 22, wherein the MLPSCs are allogeneic.

25. The method according to any one of claims 21 to 24, wherein the cells are culture expanded.

26. The method according to claim 25, wherein the cells are TNAP+ before they are culture expanded.

27. The method according to any one of claims 21 to 26, wherein the cells have been cryopreserved.

28. The method according to any one of claims 1 to 27 which comprises administering between 1 x 10^7 and 2 x 10^8 cells.

29. The method according to any one of claims 1 to 28, wherein the composition further comprises Plasma-Lyte A, dimethyl sulfoxide (DMSO), human serum albumin (HSA).

30. The method according to any one of claims 1 to 29, wherein the composition further comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

31. The method according to any one of claims 1 to 30, wherein the composition comprises greater than 6.68x10^6 viable cells/mL.

32. The method according to any one of claims 1 to 25 or 27 to 31, wherein the composition comprises human bone marrow-derived allogeneic mesenchymal precursor cells (MPCs) isolated from bone mononuclear cells with anti-STRO-3 antibodies, expanded *ex vivo*, and cryopreserved.

33. A method of reducing risk of an ischemic event in a subject, the method comprising administering to the subject a composition comprising cells.

34. The method of claim 33, wherein the subject's CRP level is ≥2 mg/L

35. The method of claim 33 or 34, wherein the ischemic event is formation of an arterial occlusion.

36. The method of claim 33 or 34, wherein the ischemic event is formation of a cerebrovascular or cardiac occlusion.

37. The method of claim 33 or 34, wherein the ischemic event is a stroke or myocardial infarction.

38. The method according to any one of claims 33 to 37, wherein the subject has non-ischemic cardiomyopathy.

39. The method according to any one of claims 33 to 38, wherein the cells are administered transendocardially.

40. The method according to any one of claims 33 to 39, wherein the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale.

41. The method according to any one of claims 33 to 40, wherein the cells:

induce new blood vessel formation in target tissue, preferably wherein the cells promote arteriogenesis; and/or

secrete factors that protect at risk myocardium.

42. The method according to any one of claims 33 to 41, wherein the cells are mesenchymal lineage precursor or stem cells (MLPSCs).

43. The method according to any one of claims 33 to 42, wherein the subject's level of N-terminal pro–B-type natriuretic peptide (NT-proBNP) is between 1000 pg/ml and 2000 pg/ ml prior to administering the cells.

44. The method according to any one of claims 33 or 35 to 44, wherein the subject's C-reactive protein (CRP) level is between 1.5 and 5 mg/L.

45. The method of claim 42, wherein the MLPSCs are one or more of STRO-1+, allogeneic, culture expanded, subject to cryopreservation.

46. The method according to claim 45, wherein the cells are culture expanded and express TNAP+ before they are culture expanded.

47. The method according to any one of claims 33 to 46 which comprises administering between 1 x 10^7 and 2 x 10^8 cells.

48. The method according to any one of claims 33 to 47, wherein the composition comprises Plasma-Lyte A (70%), DMSO (10%), HSA (25%) solution, the HSA solution comprising 5% HSA and 15% buffer.

49. The method according to any one of claims 33 to 48, wherein the composition comprises human bone marrow-derived allogeneic mesenchymal precursor cells (MPCs) isolated from bone mononuclear cells with anti-STRO-3 antibodies, expanded *ex vivo*, and cryopreserved.

50. A method for determining elevated risk of one or more of cardiac death, myocardial infarction or stroke in a subject, the method comprising measuring the level of CRP in a sample obtained from a subject, wherein elevated CRP indicates elevated risk of cardiac death, myocardial infarction or stroke.

51. The method of claim 50, wherein the subject has progressive heart failure.

52. The method of claim 51, wherein the subject has Class II progressive heart failure.

53. The method according to any one of claims 50 to 52, wherein a level of CRP ≥2 mg/L indicates elevated risk of cardiac death, myocardial infarction or stroke.

54. The method according to any one of claims 50 to 53, wherein the method determines elevated risk of cardiac death.

55. A method for treating or preventing progressive heart failure in a subject, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells, wherein the subject has Class II or Class III heart failure

according to the New York Heart Association (NYHA) classification scale and active inflammation.

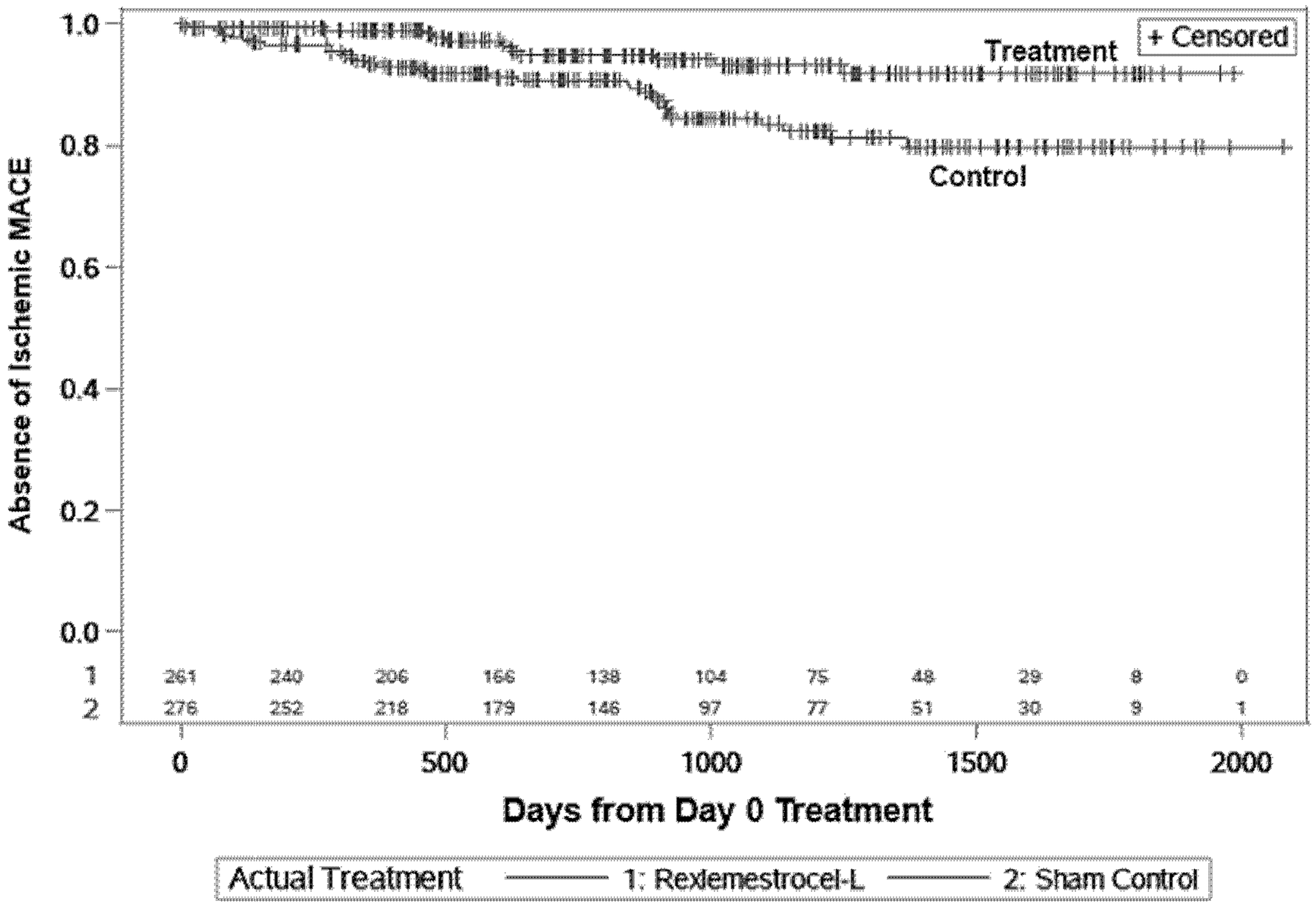
56. A method of reducing progression of heart failure in a subject, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells, wherein the subject has Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale and active inflammation.

57. A method of reducing cardiac death in a subject with Class II or Class III heart failure according to the New York Heart Association (NYHA) classification scale active inflammation, the method comprising administering to the subject a composition comprising mesenchymal precursor lineage or stem cells.

58. A method of selecting heart failure patients for treatment with cell therapy, the method comprising i) assessing CRP levels and heart failure according to the New York Heart Association (NYHA) classification scale, and ii) selecting a subject having Class II or Class III heart failure according to NYHA and active inflammation, preferably, wherein the method comprises administering a composition comprising mesenchymal precursor lineage or stem cells.

59. The method according to anyone of claims 55 to 58, wherein active inflammation is determined based on the subject's CRP level.

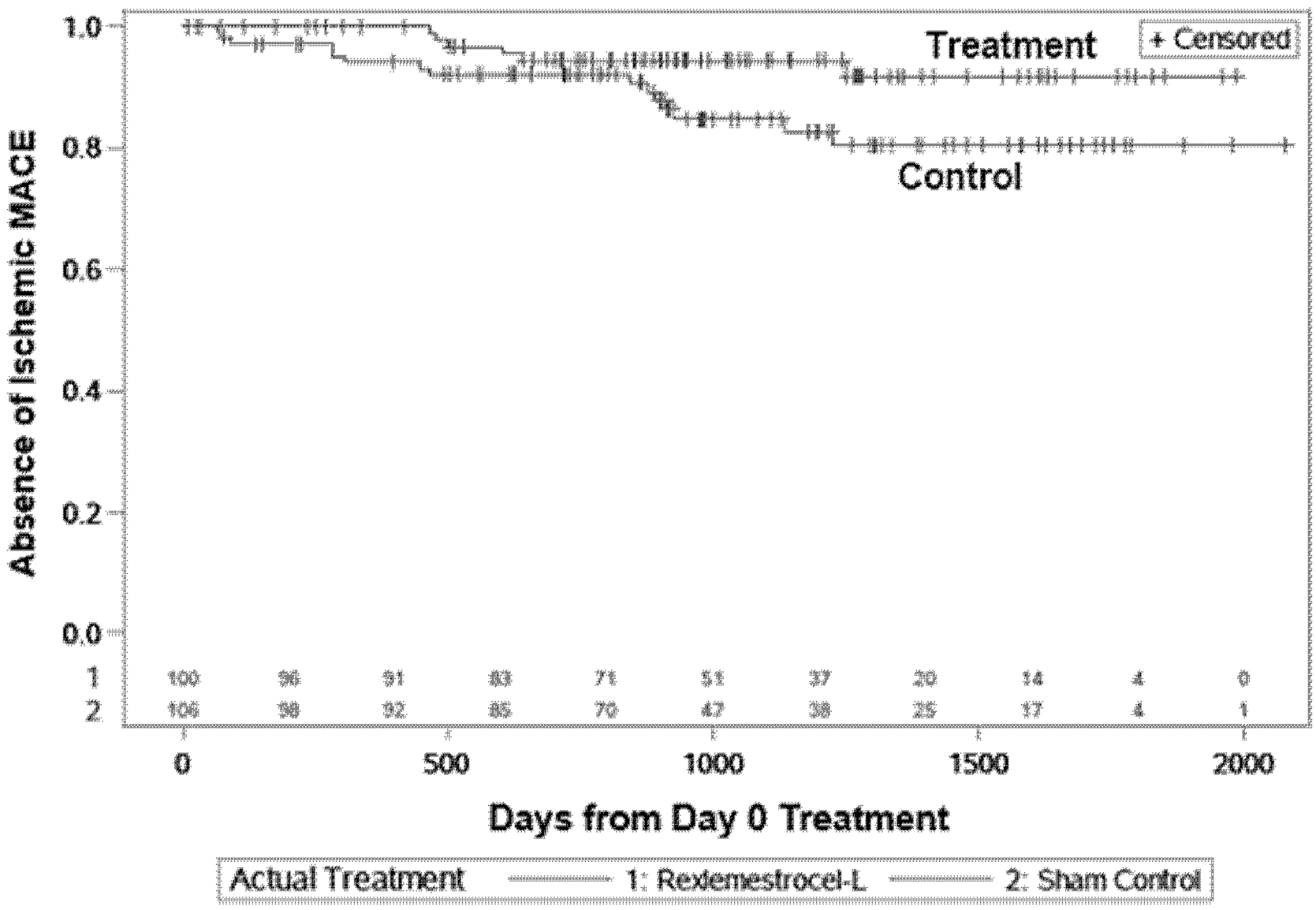
60. The method of claim 59, wherein active inflammation is characterised by a CRP level ≥2 mg/L.



All Patients (n=537)
MPC: 5%; Control: 13%
Rexlemestrocel Effect Size: 60%
p=0.002

FIGURE 1

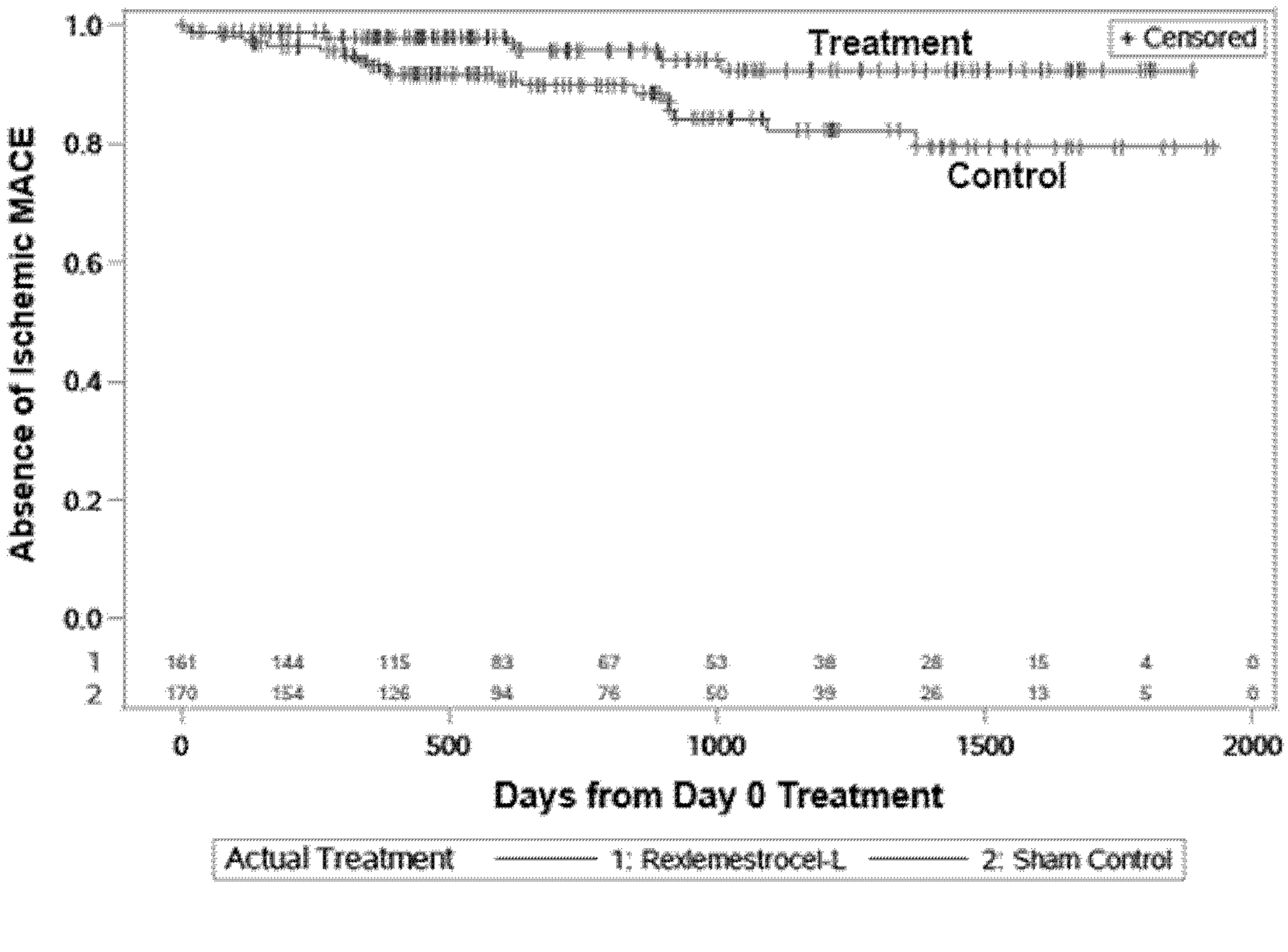
A



NYHA II
MPC: 6%; Control: 14%
Rexlemestrocel Effect Size: 57%
p=0.05

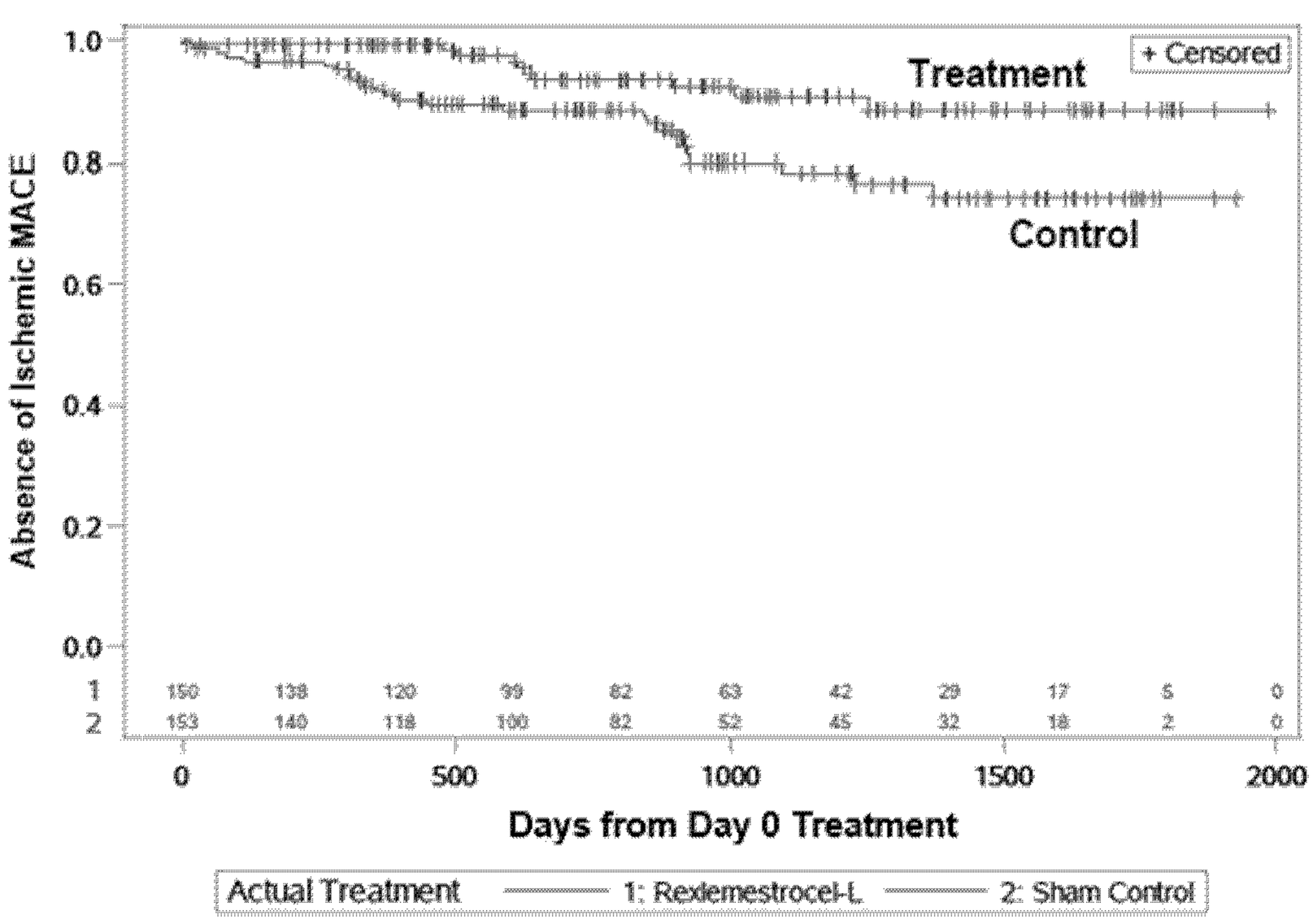
FIGURE 2-1

B



NYHA III
MPC: 4%; Control: 12%
Rexlemestrocel Effect Size: 67%
p=0.014

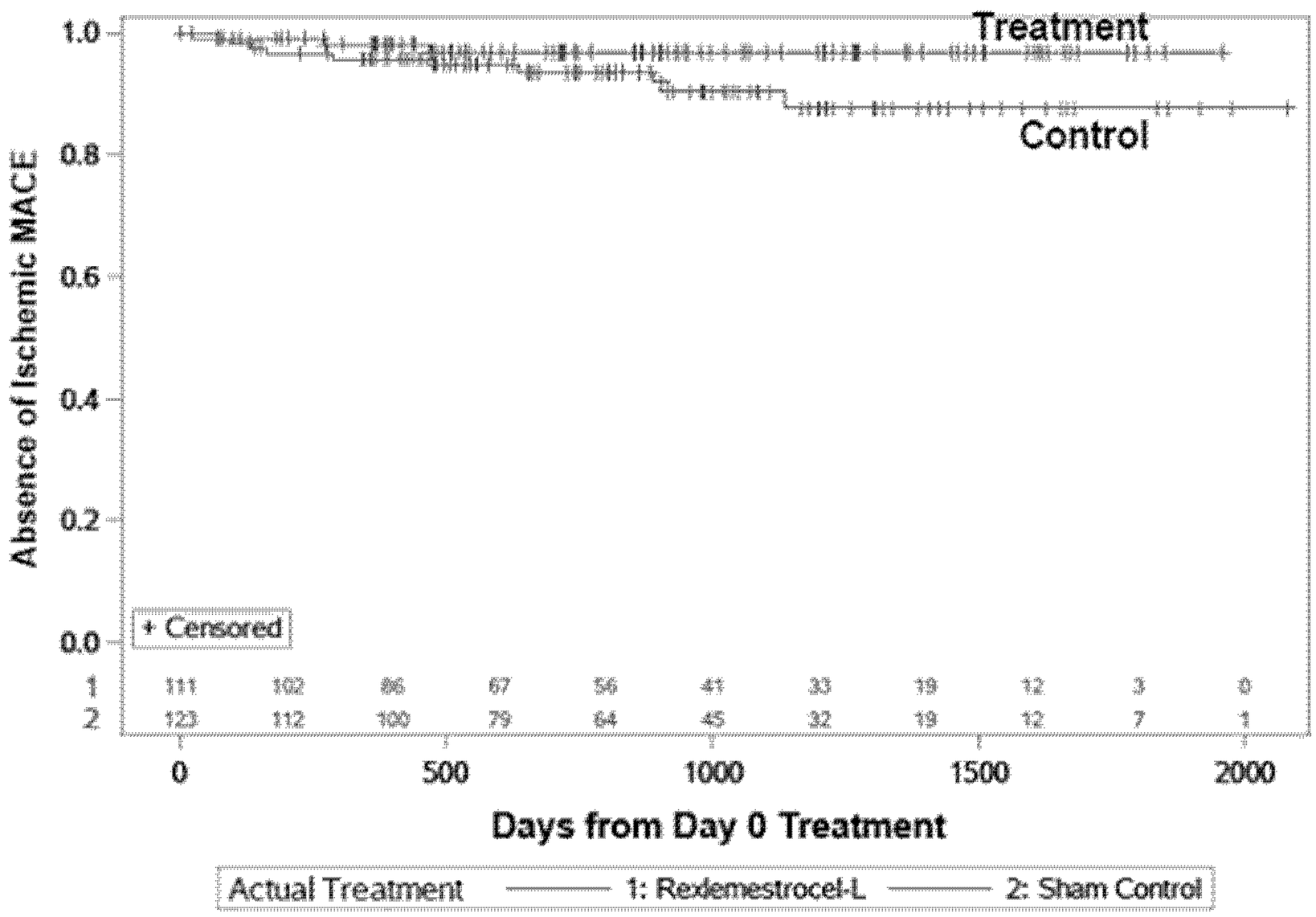
FIGURE 2-2



Ischemic Patients (n=303)
MPC: 6.6%; Control: 17%
Rexlemestrocel Effect Size: 61%
p=0.007

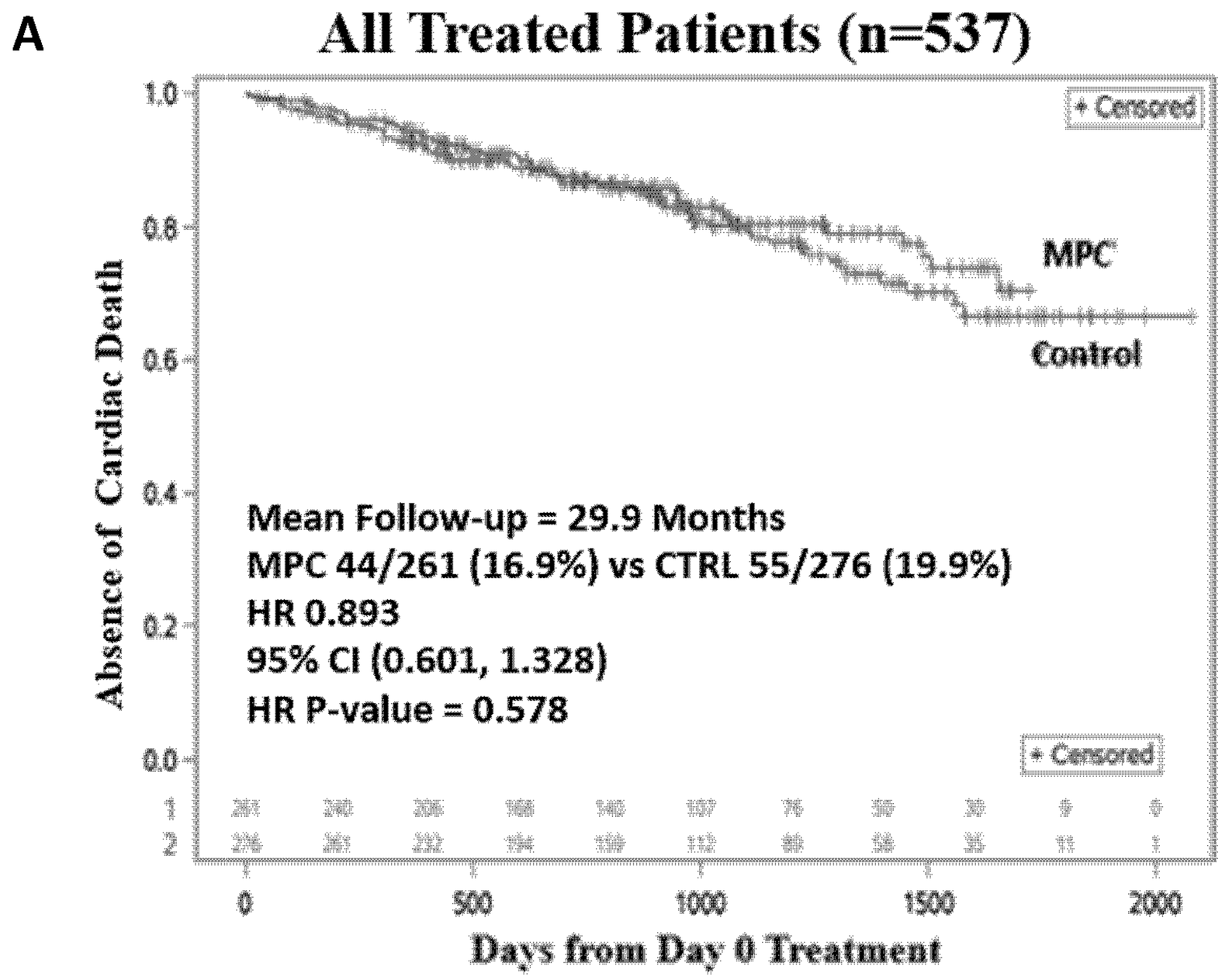
FIGURE 3-1

B



Non-Ischemic Patients (n=234)
MPC: 2.7%; Control: 8.1%
Rexlemestrocel Effect Size: 66%
p=0.08

FIGURE 3-2


A
All Treated Patients (n=537)
Absence of Cardiac Death
1.0
0.8
0.6
0.4
0.2
0.0
+ Censored
MPC
Control
Mean Follow-up = 29.9 Months
MPC 44/261 (16.9%) vs CTRL 55/276 (19.9%)
HR 0.893
95% CI (0.601, 1.328)
HR P-value = 0.578
+ Censored
1 261 240 206 168 140 107 76 59 30 9 0
2 276 261 232 194 159 112 89 58 36 11 1
0
500
1000
1500
2000
Days from Day 0 Treatment

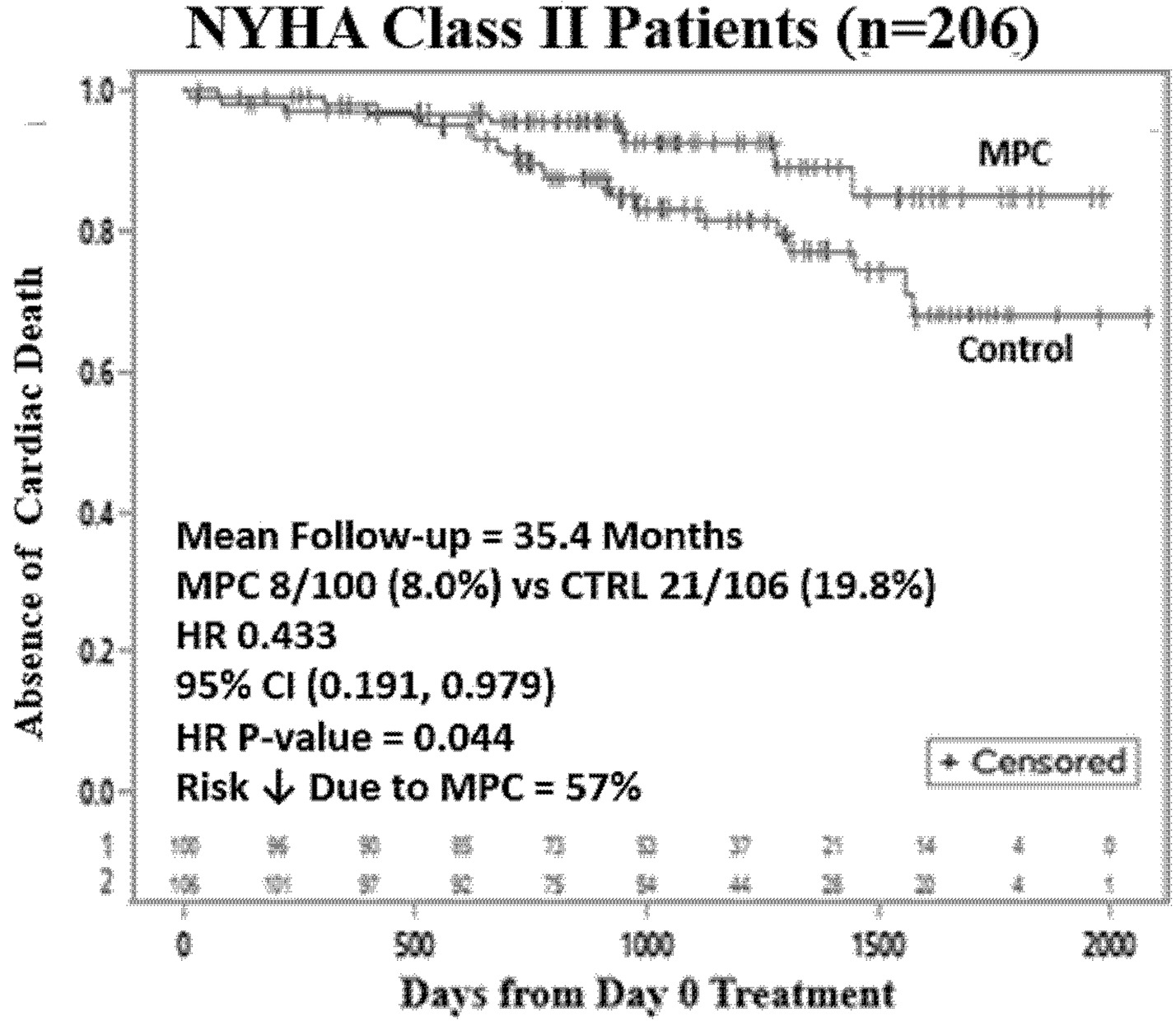

B
NYHA Class II Patients (n=206)
Absence of Cardiac Death
1.0
0.8
0.6
0.4
0.2
0.0
MPC
Control
Mean Follow-up = 35.4 Months
MPC 8/100 (8.0%) vs CTRL 21/106 (19.8%)
HR 0.433
95% CI (0.191, 0.979)
HR P-value = 0.044
Risk ↓ Due to MPC = 57%
+ Censored
1 100 96 90 85 73 53 37 21 14 4 0
2 106 101 97 92 75 54 44 28 20 4 1
0
500
1000
1500
2000
Days from Day 0 Treatment

FIGURE 4-1

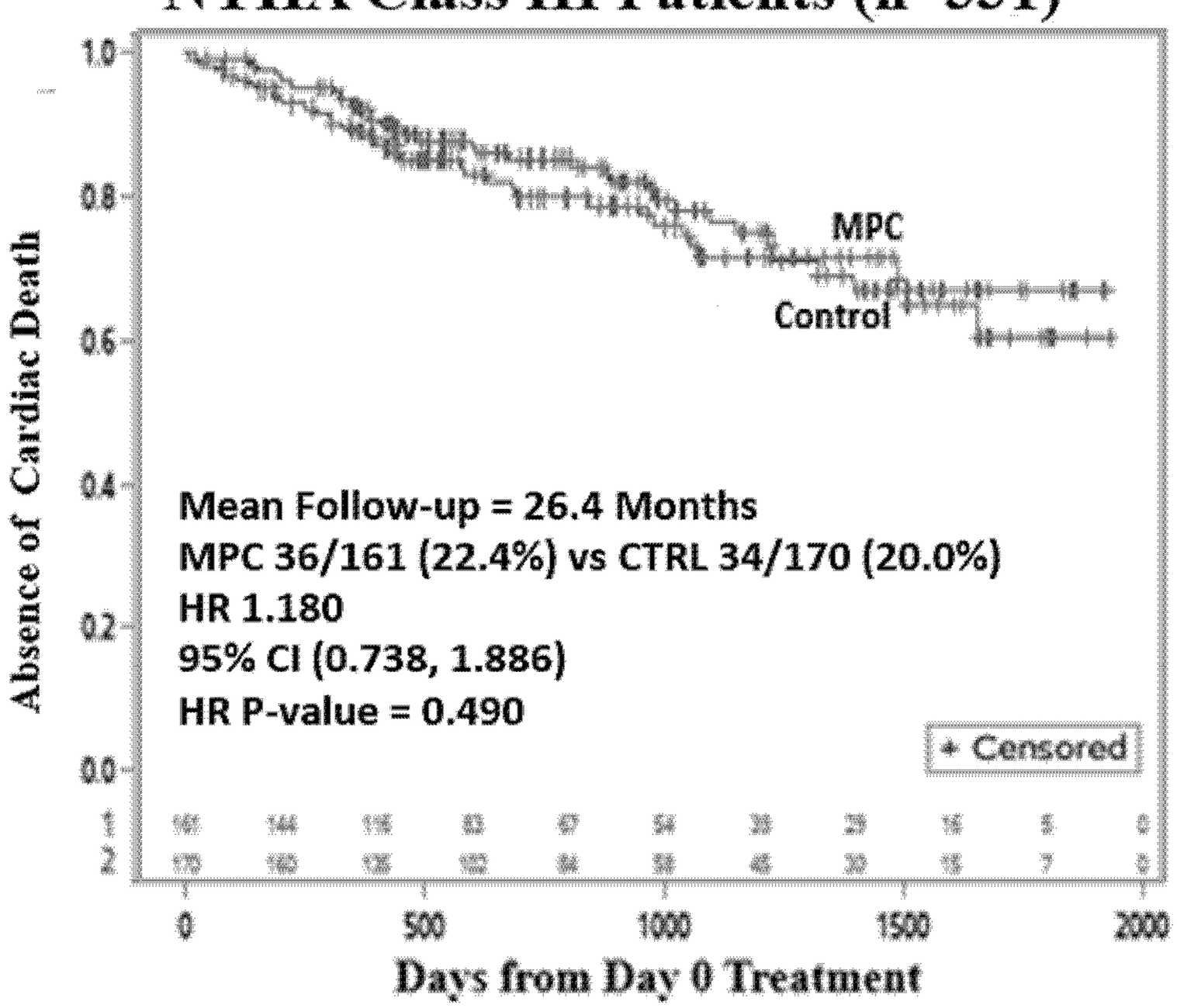

C
NYHA Class III Patients (n=331)
Absence of Cardiac Death
1.0
0.8
0.6
0.4
0.2
0.0
MPC
Control
Mean Follow-up = 26.4 Months
MPC 36/161 (22.4%) vs CTRL 34/170 (20.0%)
HR 1.180
95% CI (0.738, 1.886)
HR P-value = 0.490
Censored
1 161 144 116 83 67 54 38 29 16 5 0
2 170 160 135 102 84 58 46 30 15 7 0
0 500 1000 1500 2000
Days from Day 0 Treatment

FIGURE 4-2

D

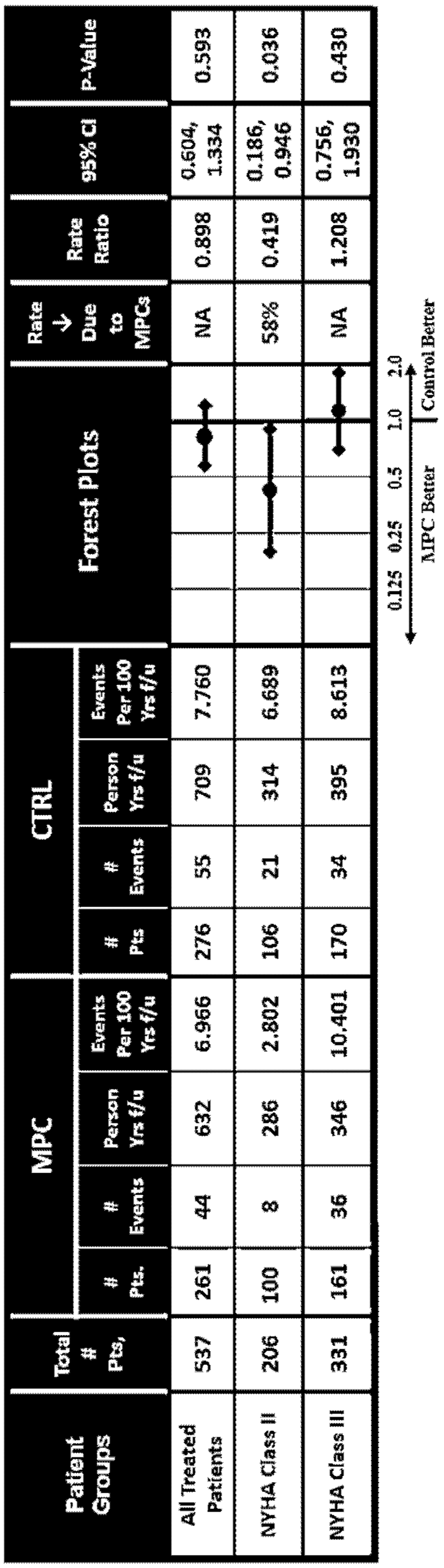
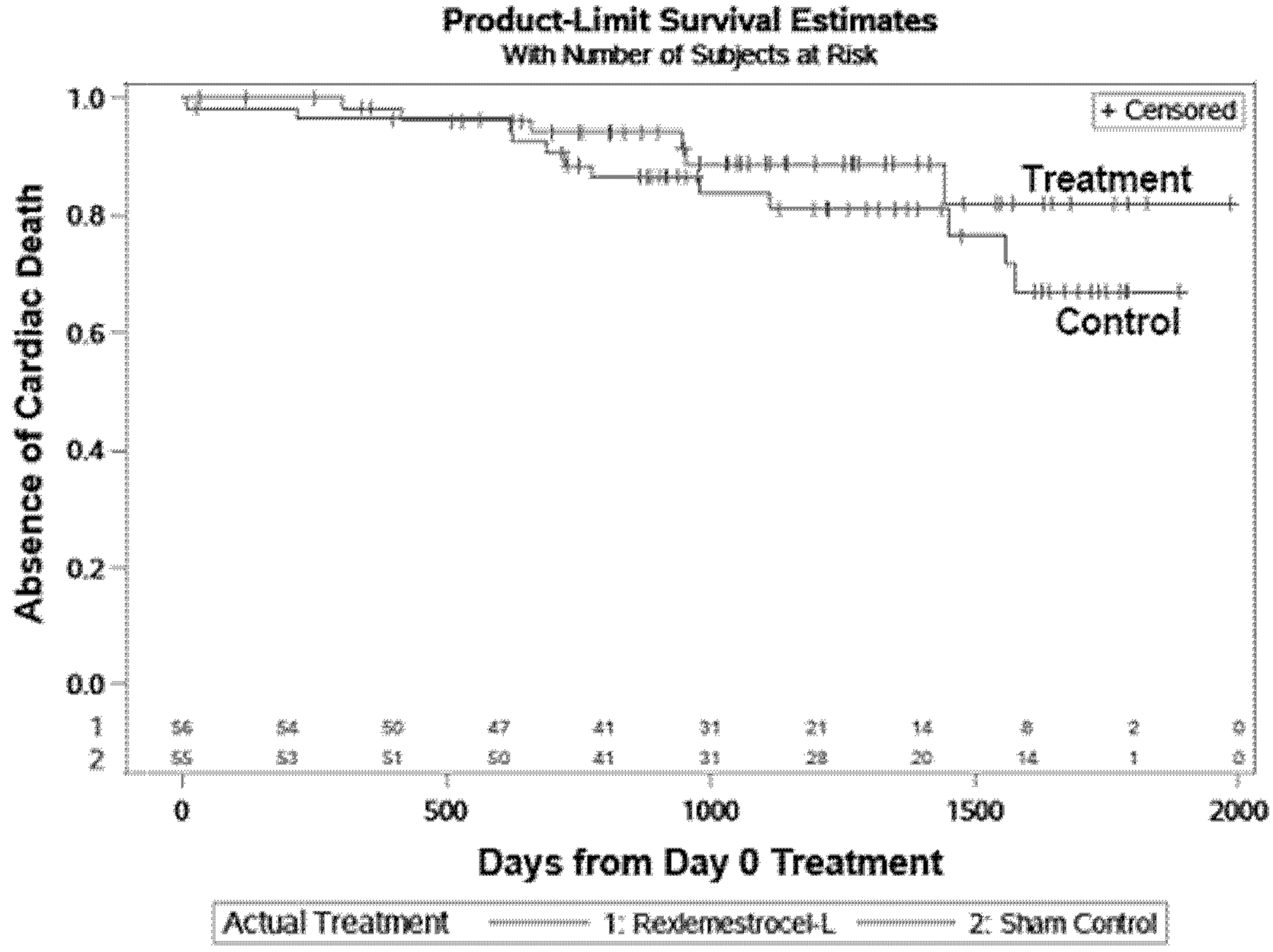

Patient Groups
Total # Pts,
MPC
CTRL
Pts.
Events
Person Yrs f/u
Events Per 100 Yrs f/u
Pts
Events
Person Yrs f/u
Events Per 100 Yrs f/u
Forest Plots
Rate Ratio
95% CI
P-Value
Rate → Due to MPCs
All Treated Patients
537
261
44
632
6.966
276
55
709
7.760
0.898
0.604, 1.334
0.593
NA
NYHA Class II
206
100
8
286
2.802
106
21
314
6.689
0.419
0.186, 0.946
0.036
58%
NYHA Class III
331
161
36
346
10.401
170
34
395
8.613
1.208
0.756, 1.930
0.430
NA
0.125
0.25
0.5
1.0
2.0
MPC Better
Control Better

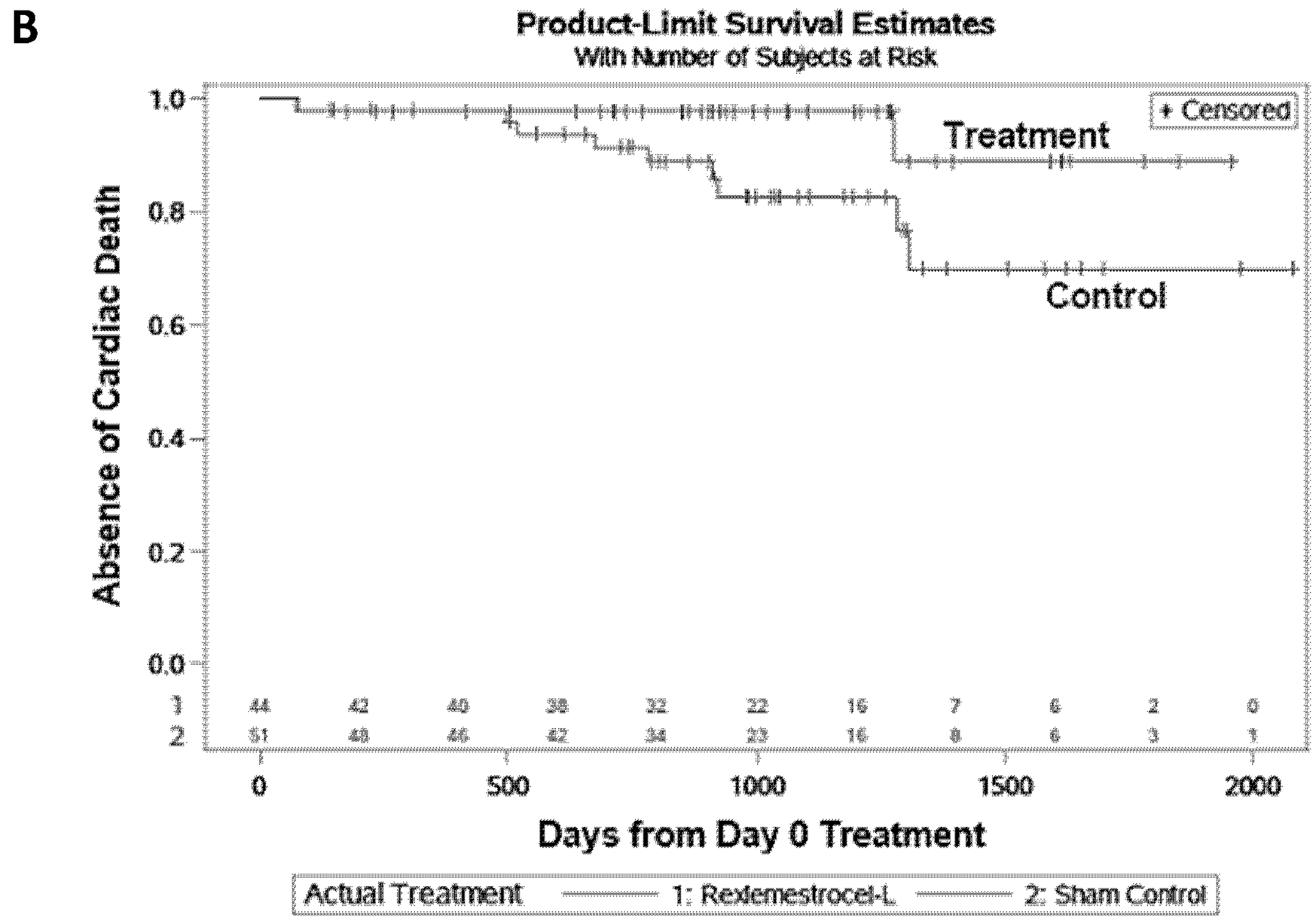
FIGURE 4-3

A



NYHA II Ischemic (n=111)
MPC: 10.7%; Control: 21.8%
Rexlemestrocel Effect Size: 51%
p=0.251

FIGURE 5-1



NYHA II Non-Ischemic (n=95)
MPC: 4.5%; Control: 17.6%
Rexlemestrocel Effect Size: 74%
p=0.06

FIGURE 5-2

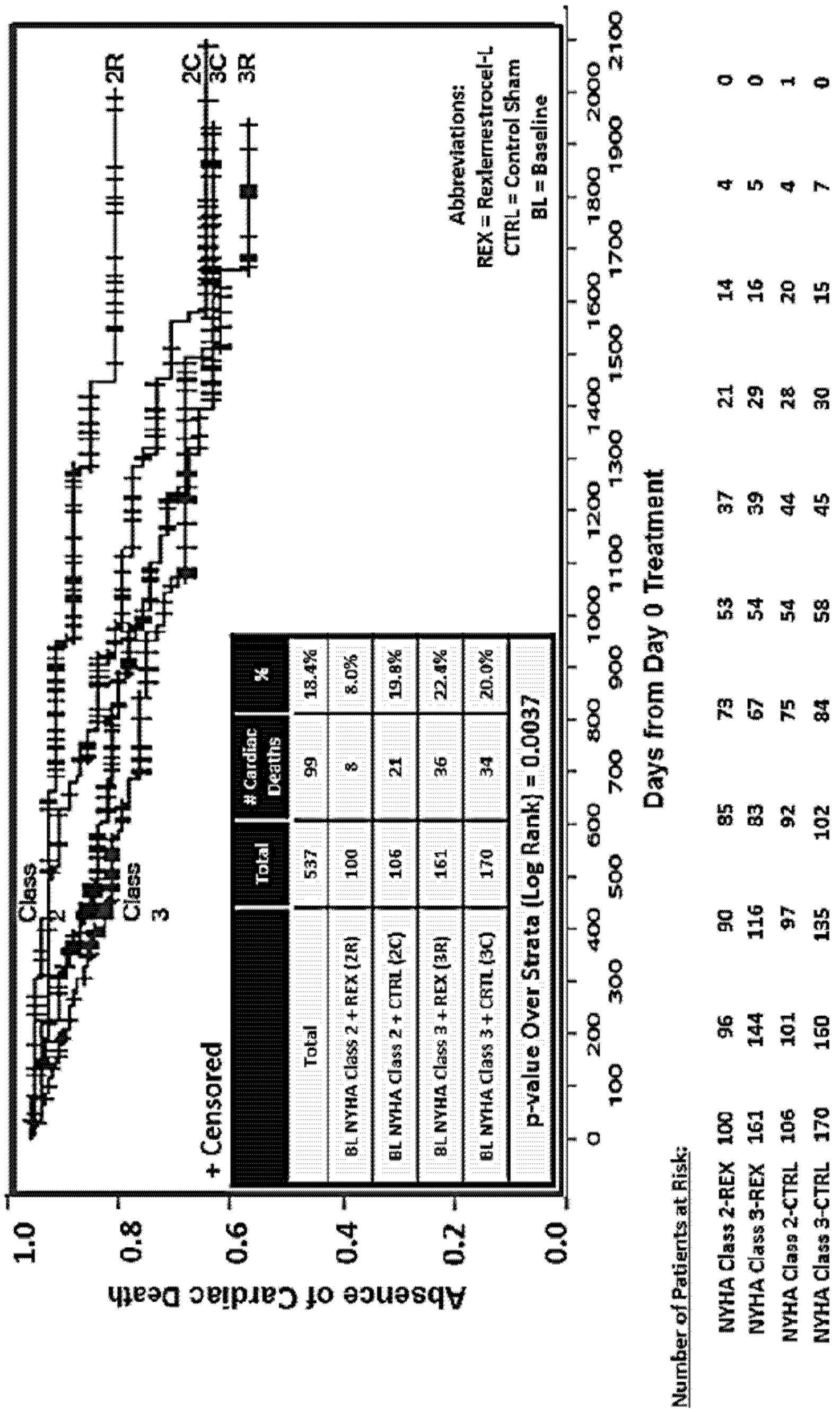

Absence of Cardiac Death
1.0
0.8
0.6
0.4
0.2
0.0
0
100
200
300
400
500
600
700
800
900
1000
1100
1200
1300
1400
1500
1600
1700
1800
1900
2000
2100
Days from Day 0 Treatment
+ Censored
Class 2
Class 3
Abbreviations:
REX = Rexlemestrocel-L
CTRL = Control Sham
BL = Baseline
Total # Cardiac Deaths %
Total 537 99 18.4%
BL NYHA Class 2 + REX (2R) 100 8 8.0%
BL NYHA Class 2 + CTRL (2C) 106 21 19.8%
BL NYHA Class 3 + REX (3R) 161 36 22.4%
BL NYHA Class 3 + CRTL (3C) 170 34 20.0%
p-value Over Strata (Log Rank) = 0.0037
2R
2C
3C
3R
Number of Patients at Risk:
NYHA Class 2-REX 100 96 90 85 73 67 53 37 21 14 4 0
NYHA Class 3-REX 161 144 116 83 67 54 39 29 16 5 0 0
NYHA Class 2-CTRL 106 101 97 92 75 54 44 28 20 4 1 0
NYHA Class 3-CTRL 170 160 135 102 84 58 45 30 15 7 0 0

FIGURE 6

A

TTFE Analysis	Total # Pts.	MPC Events/ Group	CTRL Events/ Group	Risk ↓ Due to MPCs	HR	95% Confidence Interval	P-Value
Forest Plots							
TTFE Composite IMM MACE: Cardiovascular Death or Non-fatal MI or Non-fatal Stroke							
All Treated Patients	537	53/261	83/276	33%	0.667	0.472, 0.941	0.021
BL hsCRP ≥2 mg/L	301	29/153	48/148	45%	0.551	0.346, 0.876	0.012

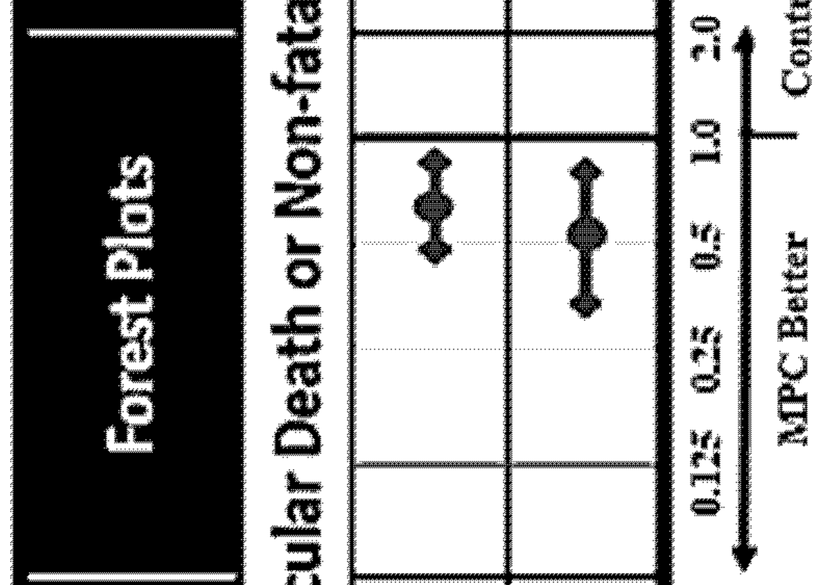


FIGURE 7-1

B

Rate Normalized for Follow-up Time (Events Per 100 PtYears)		CTRL		MPC					
		# Pts	# Events	Person Yrs f/u	Events Per 100 Yrs f/u	Total # Pts,	Risk for Same Time (Years)	Forest Plots	Risk ↓ due to MPCs	HR	95% CI	P-Value
Composite		276	99	709	13.968	537			35%	0.646	0.466, 0.895	0.009
All Treated Patients		148	59	352	16,767	301			50%	0.497	0.320, 0.772	0.002
BL hsCRP >2 mg/L												

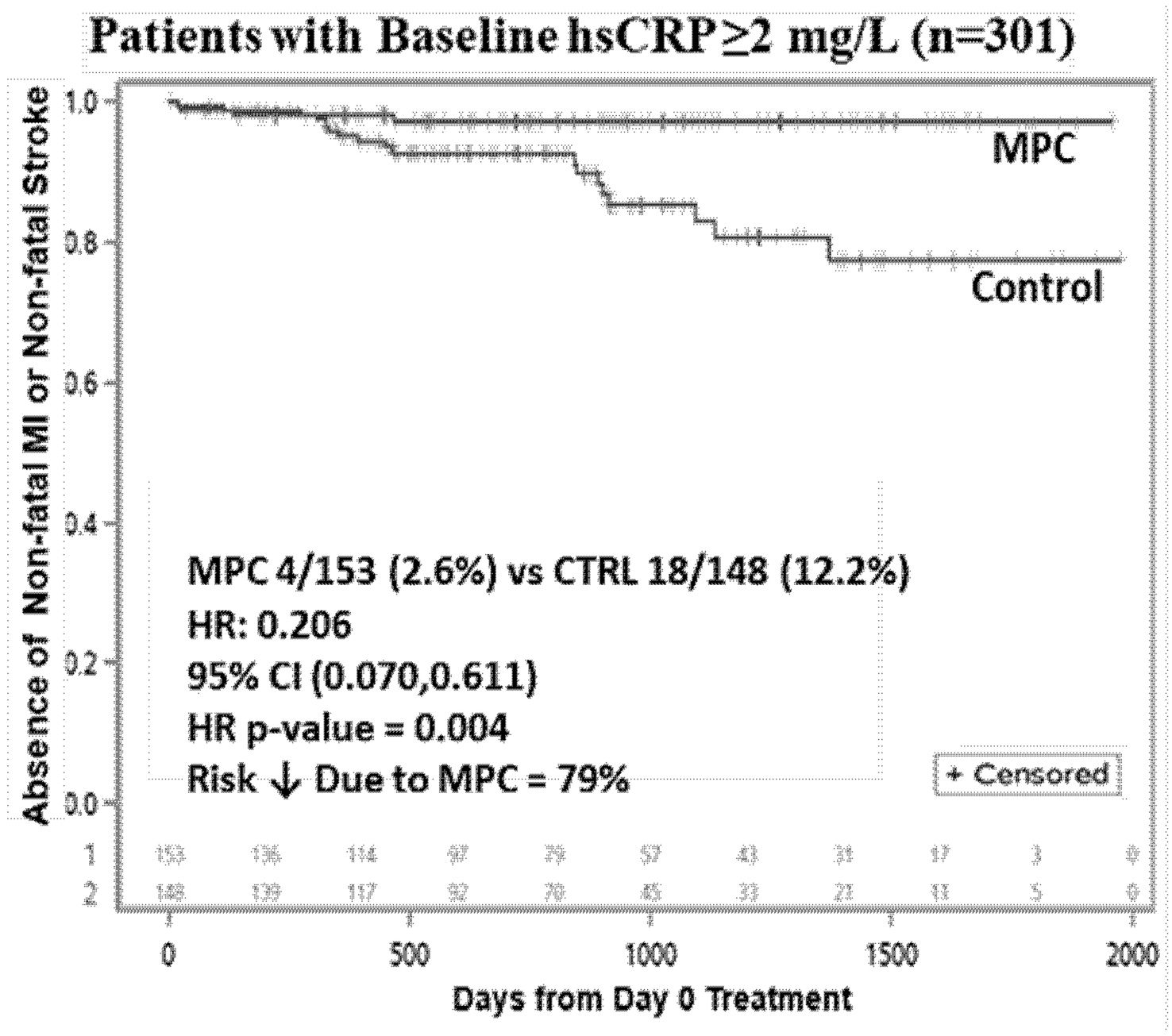
Composite IMM MACE: Recurrent Non-fatal Stroke or Cardiovascular Death

0.125 0.25 0.5 1.0 2.0

MPC Better | Control Better

FIGURE 7-2

C1



C2

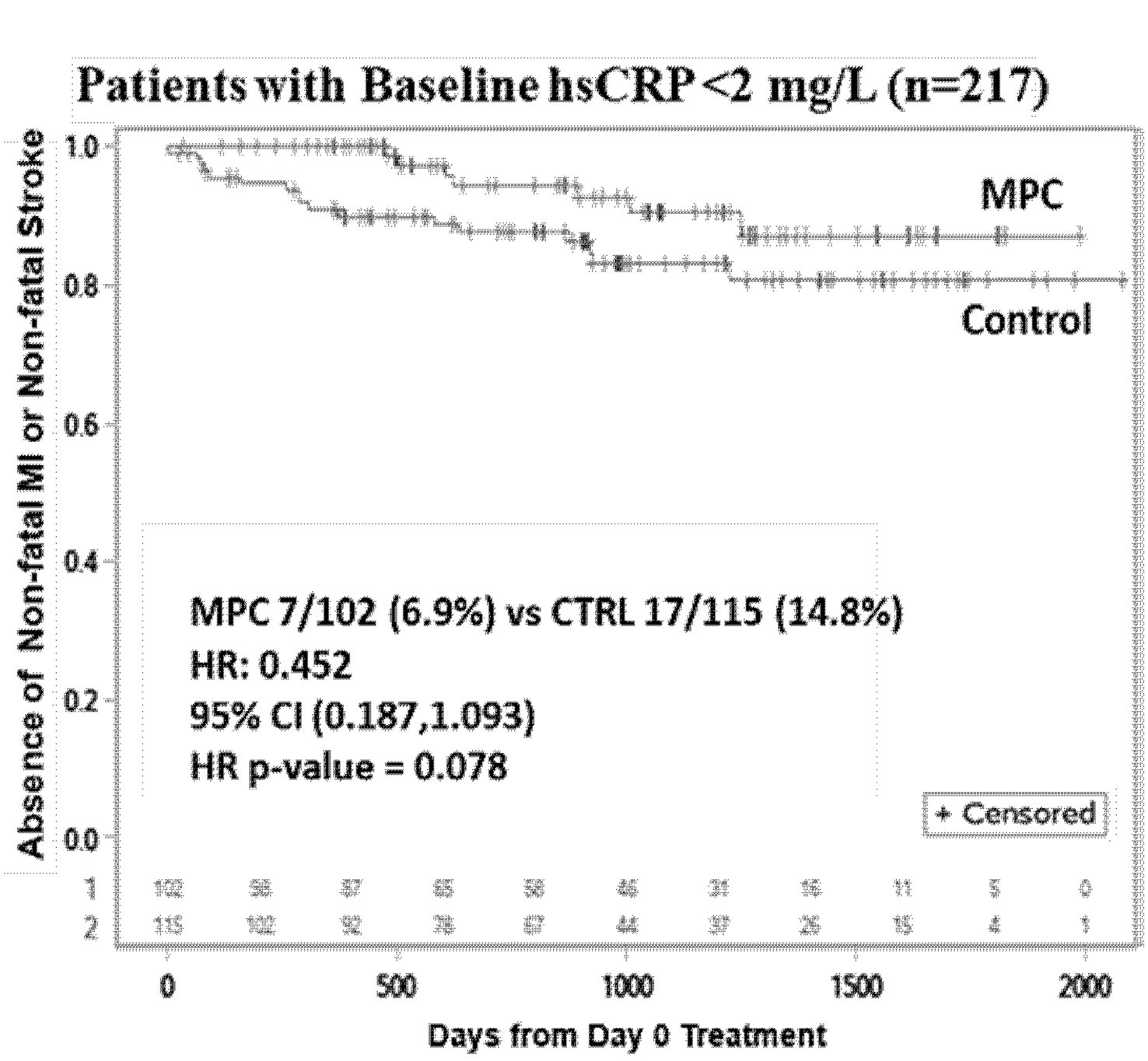
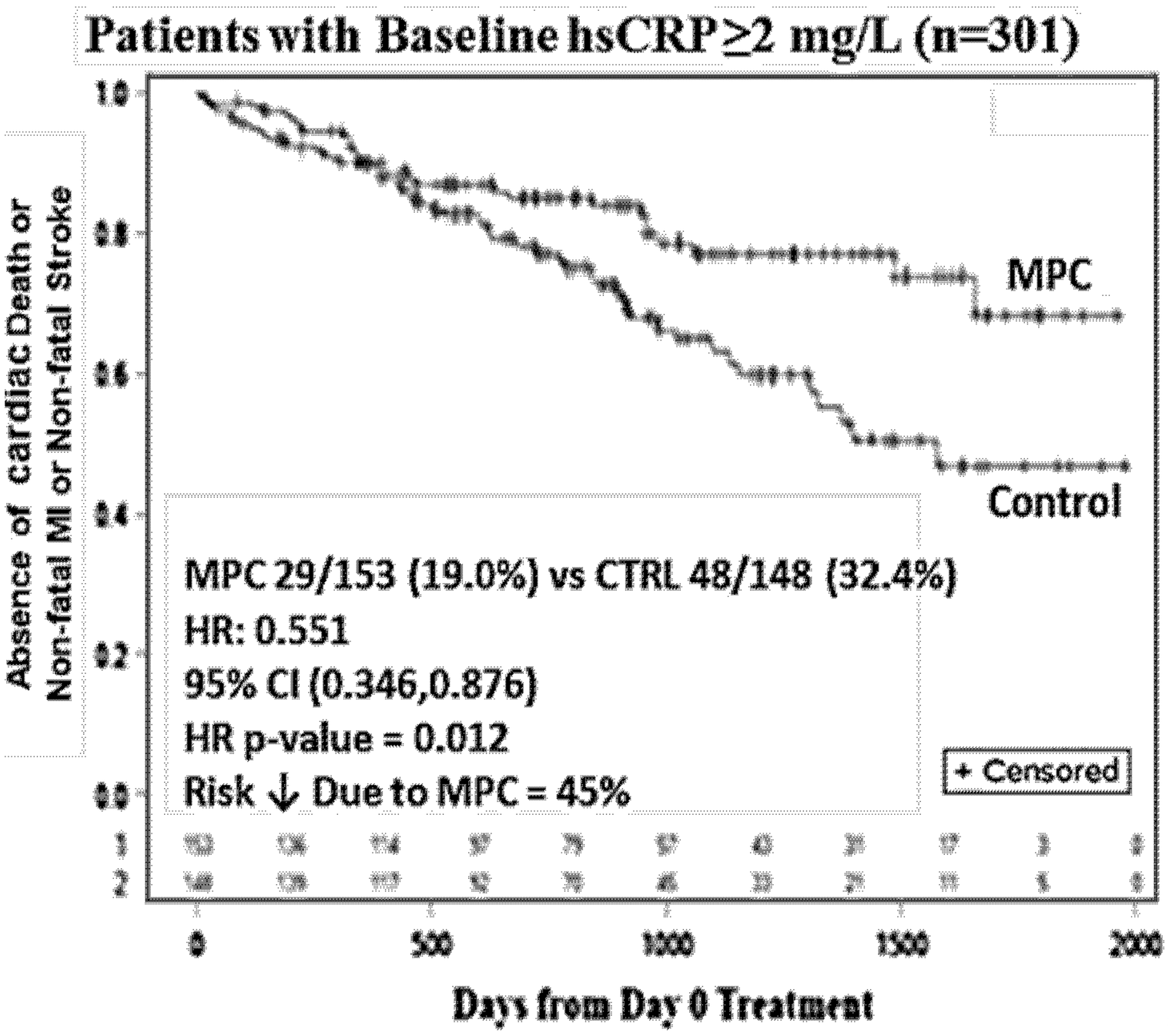


FIGURE 7-3

C3



C4

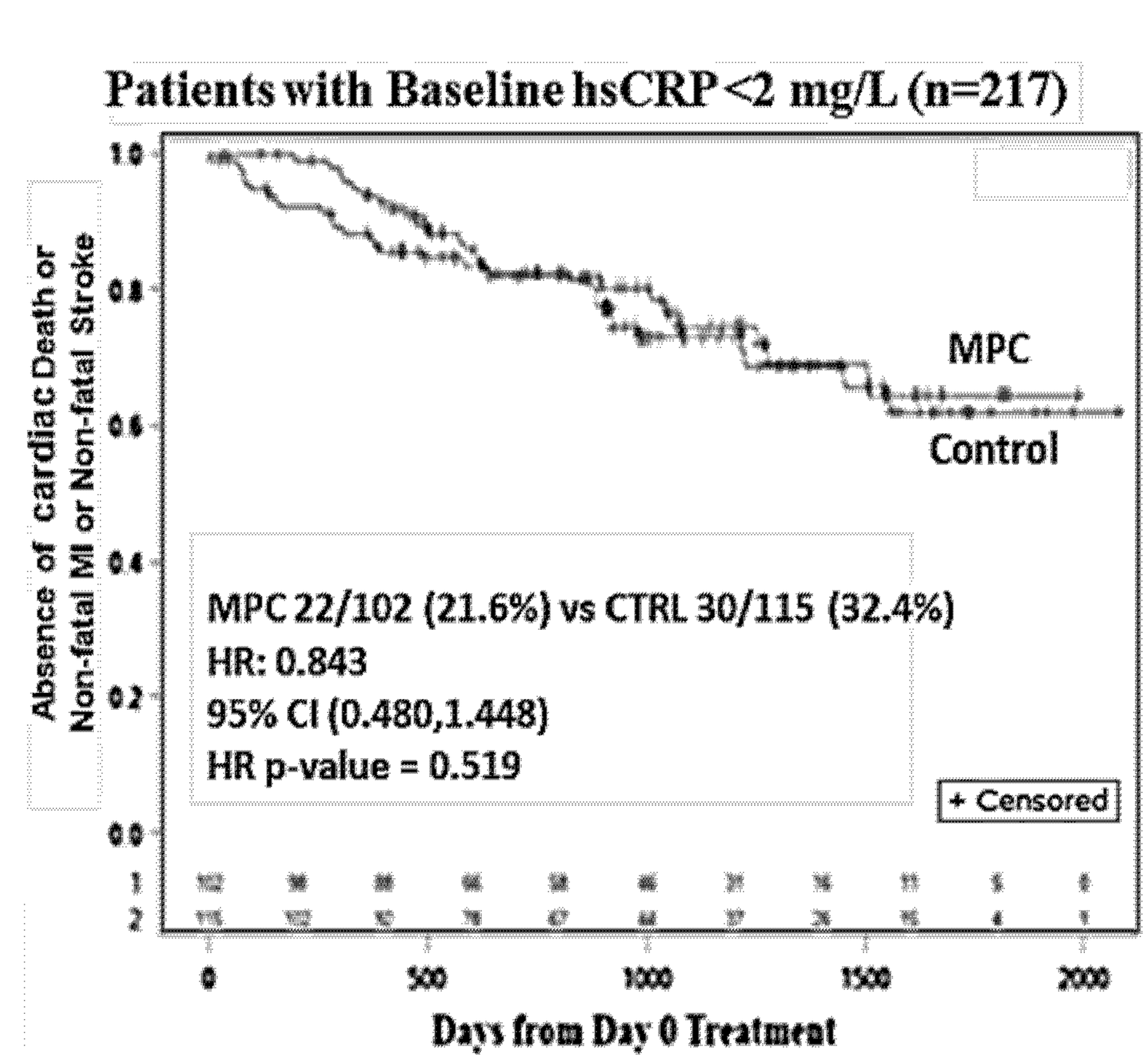


FIGURE 7-4

A

TTFE Analysis	Total # Pts.	MPC Events/ Group	CTRL Events/ Group	Forest Plots	Risk ↓ Due to MPCs	HR	95% Confidence Interval	P-Value
Irreversible Morbidity (IM): TTFE Non-fatal MI or Non-fatal Stroke								
All Treated Patients	537	12/261	36/276		65%	0.346	0.180, 0.664	0.001
BL hsCRP ≥2 mg/L	301	4/153	18/148		79%	0.206	0.070, 0.611	0.004
NYHA Class II	206	5/100	15/106		66%	0.336	0.122, 0.923	0.035
Class II + BL hsCRP ≥2 mg/L	97	1/49	6/48		86%	0.141	0.017, 1.174	0.070
NYHA Class III	331	7/161	21/170		65%	0.350	0.149, 0.827	0.017
Class III + BL hsCRP ≥2 mg/L	204	3/104	12/100		74%	0.257	0.072, 0.901	0.035

0.125 0.25 0.5 1.0 2.0
MPC Better | Control Better

FIGURE 8-1

B

Irreversible Morbidity (IM): Recurrent Non-fatal MI or Recurrent Non-fatal Stroke	MPC				CTRL				Rate Normalized for Follow-up Time (Events Per 100 Pt-Years)	Forest Plots	Risk ↓ due to MPCs	HR	95% CI	P-Value
	Total # Pts.	# Pts.	# Events	Person Yrs f/u	Events Per 100 Yrs f/u	# Pts	# Events	Person Yrs f/u						
All Treated Patients	537	261	12	632	1.900	276	44	709	6.208		69%	0.306	0.162, 0.579	<0.001
BL hsCRP ≥2 mg/L	301	153	4	360	1.112	148	23	352	6.536		83%	0.170	0.059, 0.492	0.001
NYHA Class II	206	100	5	286	1.751	106	19	314	6.052		71%	0.289	0.108, 0.775	0.014
Class II + BL hsCRP ≥2 mg/L	97	49	1	145	0.690	48	8	125	6.419		89%	0.107	0.013, 0.859	0.035
NYHA Class III	331	161	7	346	2.022	170	25	395	6.333		68%	0.319	0.138, 0.738	0.008
Class III + BL hsCRP ≥2 mg/L	204	104	3	215	1.397	100	15	227	6.601		79%	0.212	0.061, 0.731	0.014

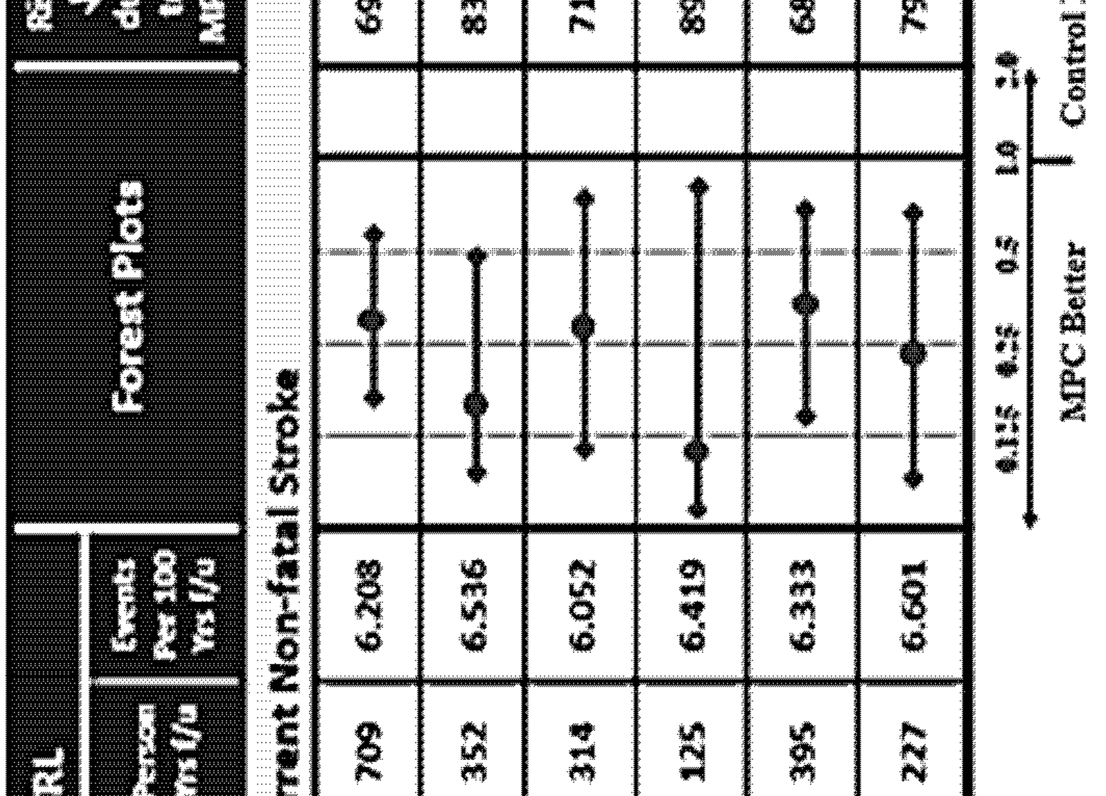
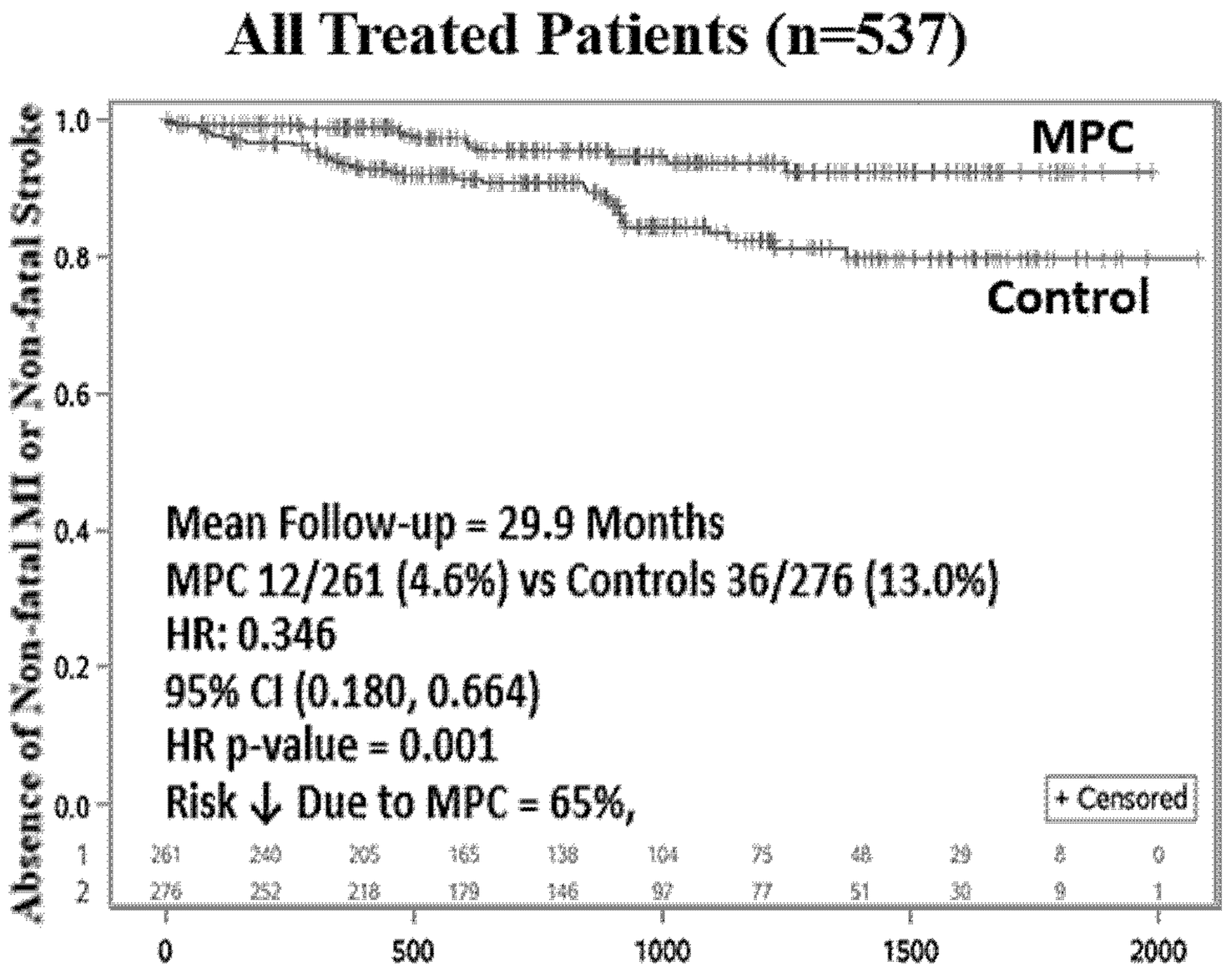


FIGURE 8-2

C1



C2

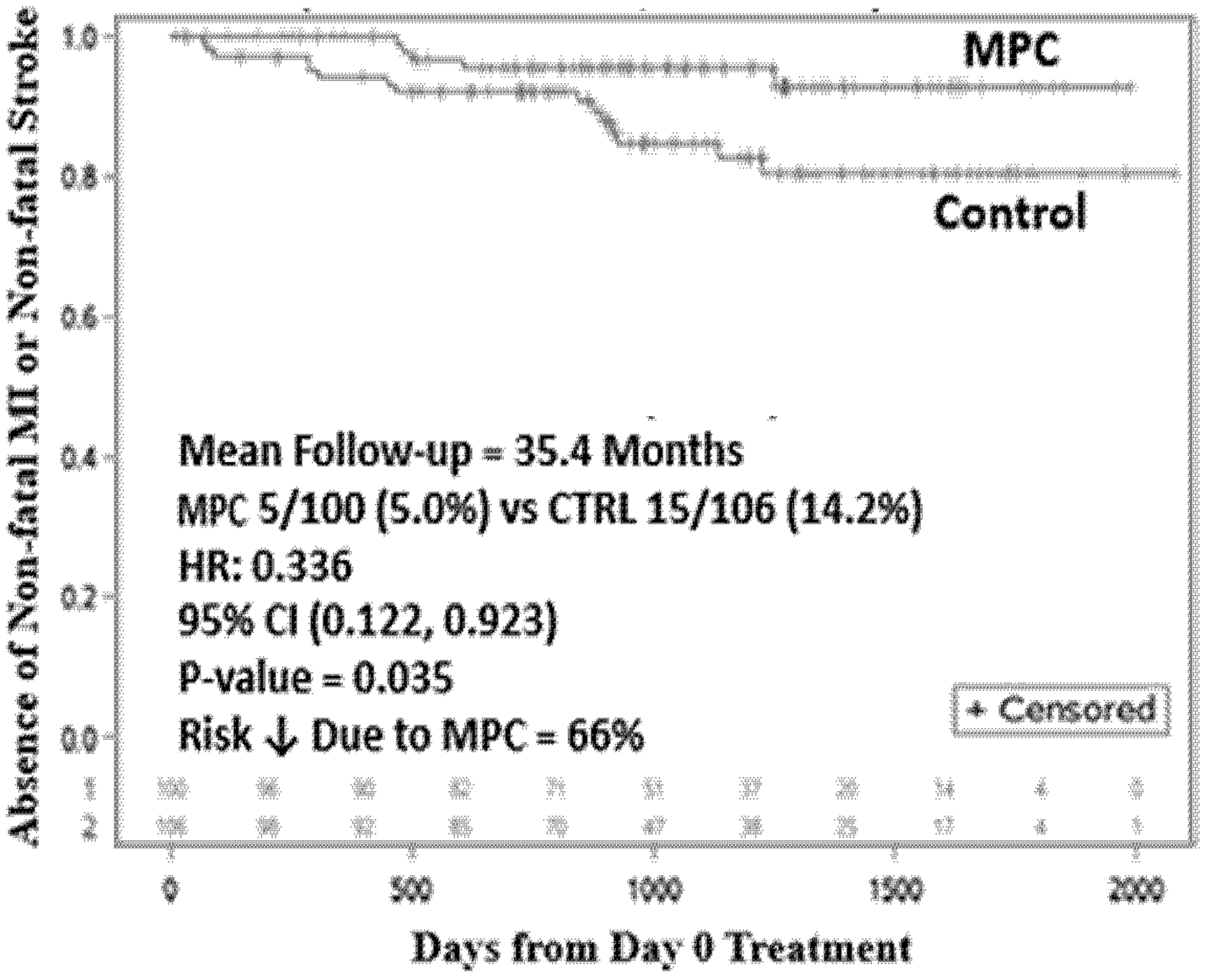


FIGURE 8-3

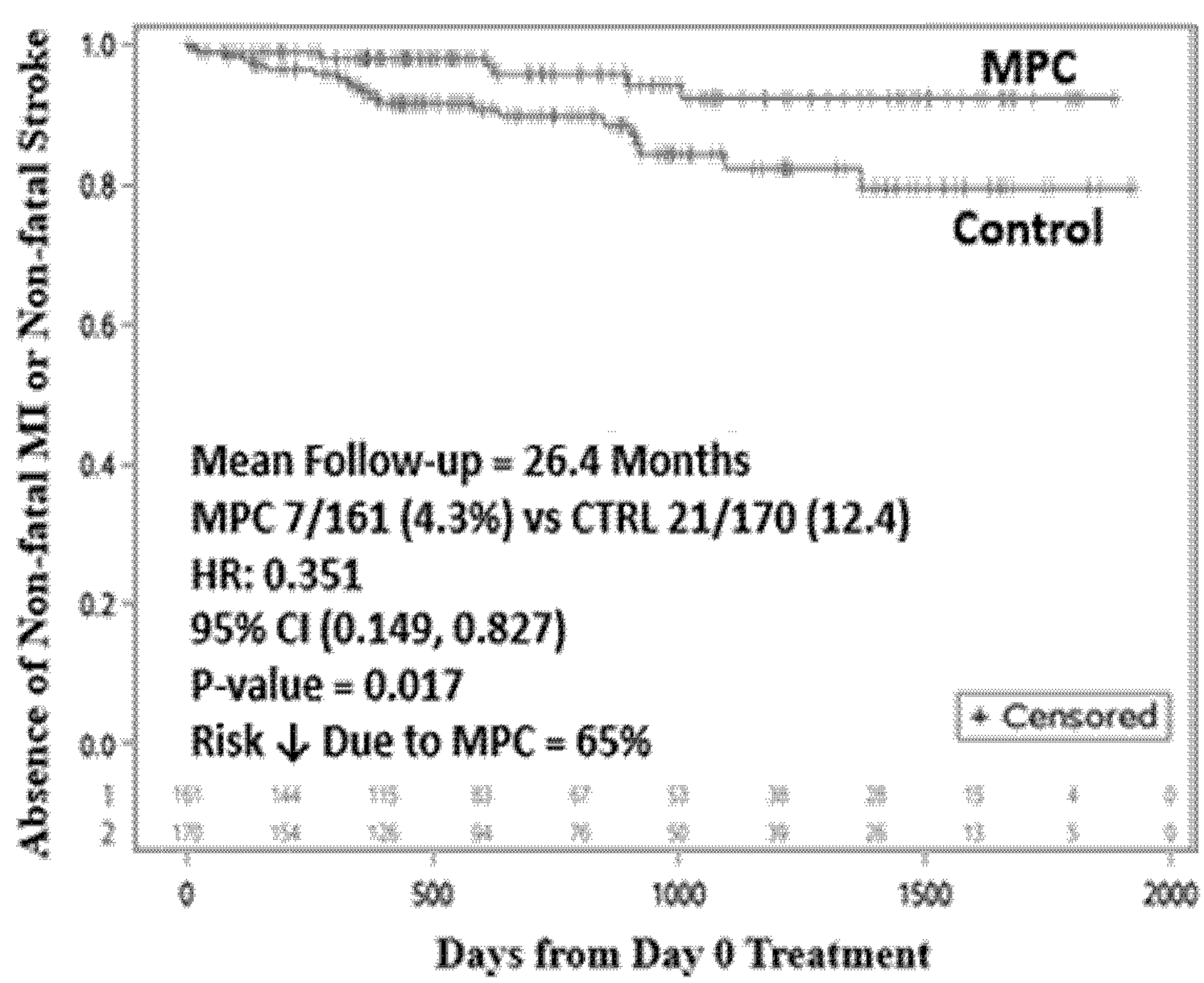

C3
NYHA Class III Patients (n=331)
Absence of Non-fatal MI or Non-fatal Stroke
1.0
0.8
0.6
0.4
0.2
0.0
MPC
Control
Mean Follow-up = 26.4 Months
MPC 7/161 (4.3%) vs CTRL 21/170 (12.4)
HR: 0.351
95% CI (0.149, 0.827)
P-value = 0.017
Risk ↓ Due to MPC = 65%
Censored
1 161 144 115 83 47 33 30 28 15 4 0
2 170 154 126 94 70 42 36 26 13 3 0
0 500 1000 1500 2000
Days from Day 0 Treatment

FIGURE 8-4

C4

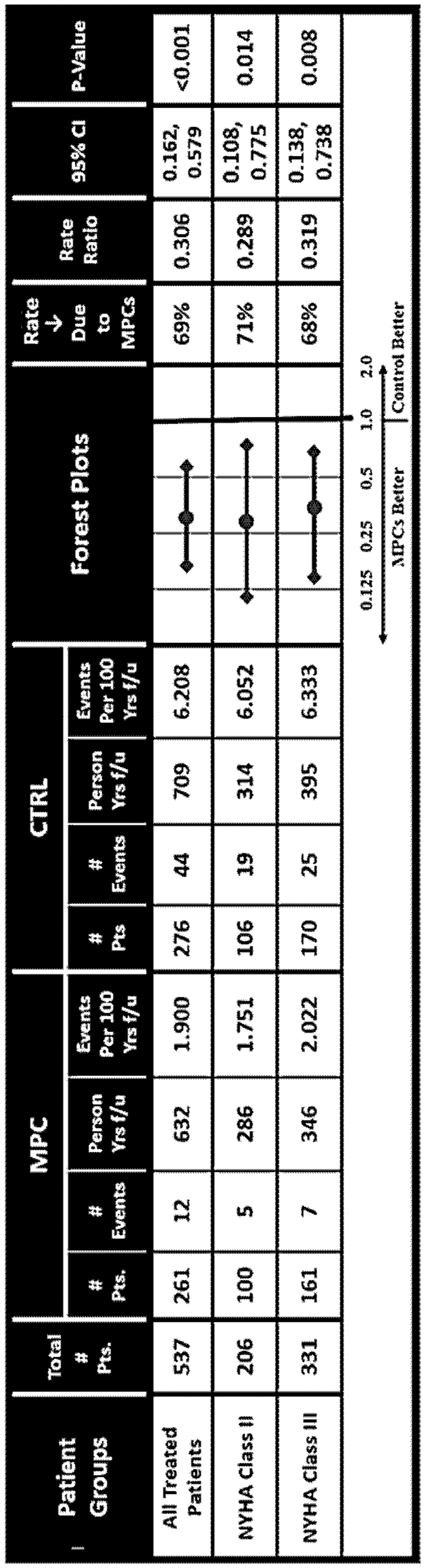
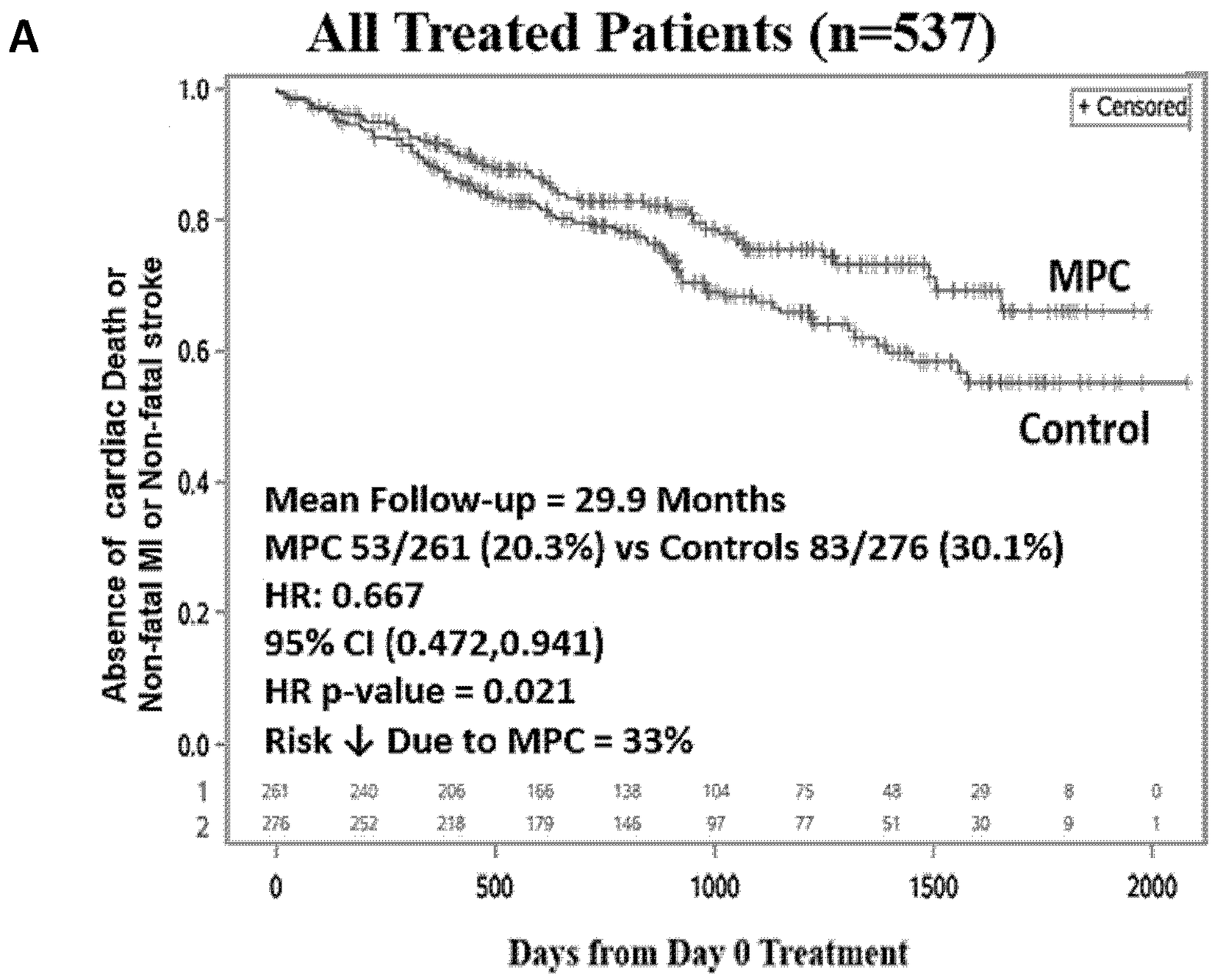

Patient Groups
Total # Pts.
All Treated Patients
NYHA Class II
NYHA Class III
537
206
331
MPC
Pts.
Events
Person Yrs f/u
Events Per 100 Yrs f/u
261
12
632
1.900
100
5
286
1.751
161
7
346
2.022
CTRL
Pts
Events
Person Yrs f/u
Events Per 100 Yrs f/u
276
44
709
6.208
106
19
314
6.052
170
25
395
6.333
Forest Plots
0.125
0.25
0.5
1.0
2.0
MPCs Better
Control Better
Rate Ratio
95% CI
P-Value
0.306
0.162, 0.579
<0.001
0.289
0.108, 0.775
0.014
0.319
0.138, 0.738
0.008
Rate ↓ Due to MPCs
69%
71%
68%

FIGURE 8-5


A
All Treated Patients (n=537)
+ Censored
MPC
Control
Mean Follow-up = 29.9 Months
MPC 53/261 (20.3%) vs Controls 83/276 (30.1%)
HR: 0.667
95% CI (0.472,0.941)
HR p-value = 0.021
Risk ↓ Due to MPC = 33%
Absence of cardiac Death or Non-fatal MI or Non-fatal stroke
1.0
0.8
0.6
0.4
0.2
0.0
1 261 240 206 166 138 104 75 48 29 8 0
2 276 252 218 179 146 97 77 51 30 9 1
0 500 1000 1500 2000
Days from Day 0 Treatment

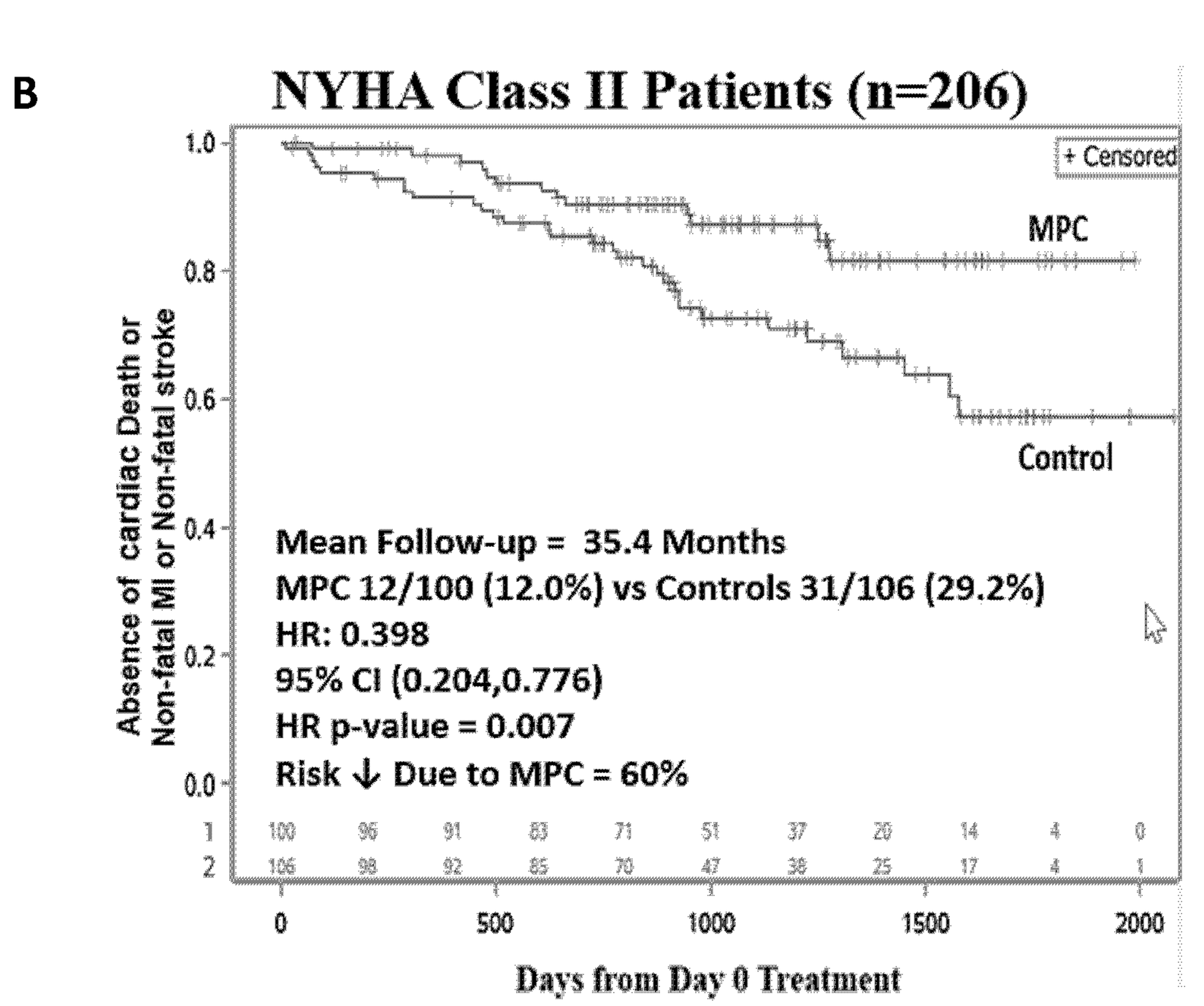

B
NYHA Class II Patients (n=206)
+ Censored
MPC
Control
Mean Follow-up = 35.4 Months
MPC 12/100 (12.0%) vs Controls 31/106 (29.2%)
HR: 0.398
95% CI (0.204,0.776)
HR p-value = 0.007
Risk ↓ Due to MPC = 60%
Absence of cardiac Death or Non-fatal MI or Non-fatal stroke
1.0
0.8
0.6
0.4
0.2
0.0
1 100 96 91 83 71 51 37 20 14 4 0
2 106 98 92 85 70 47 38 25 17 4 1
0 500 1000 1500 2000
Days from Day 0 Treatment

FIGURE 9-1

C

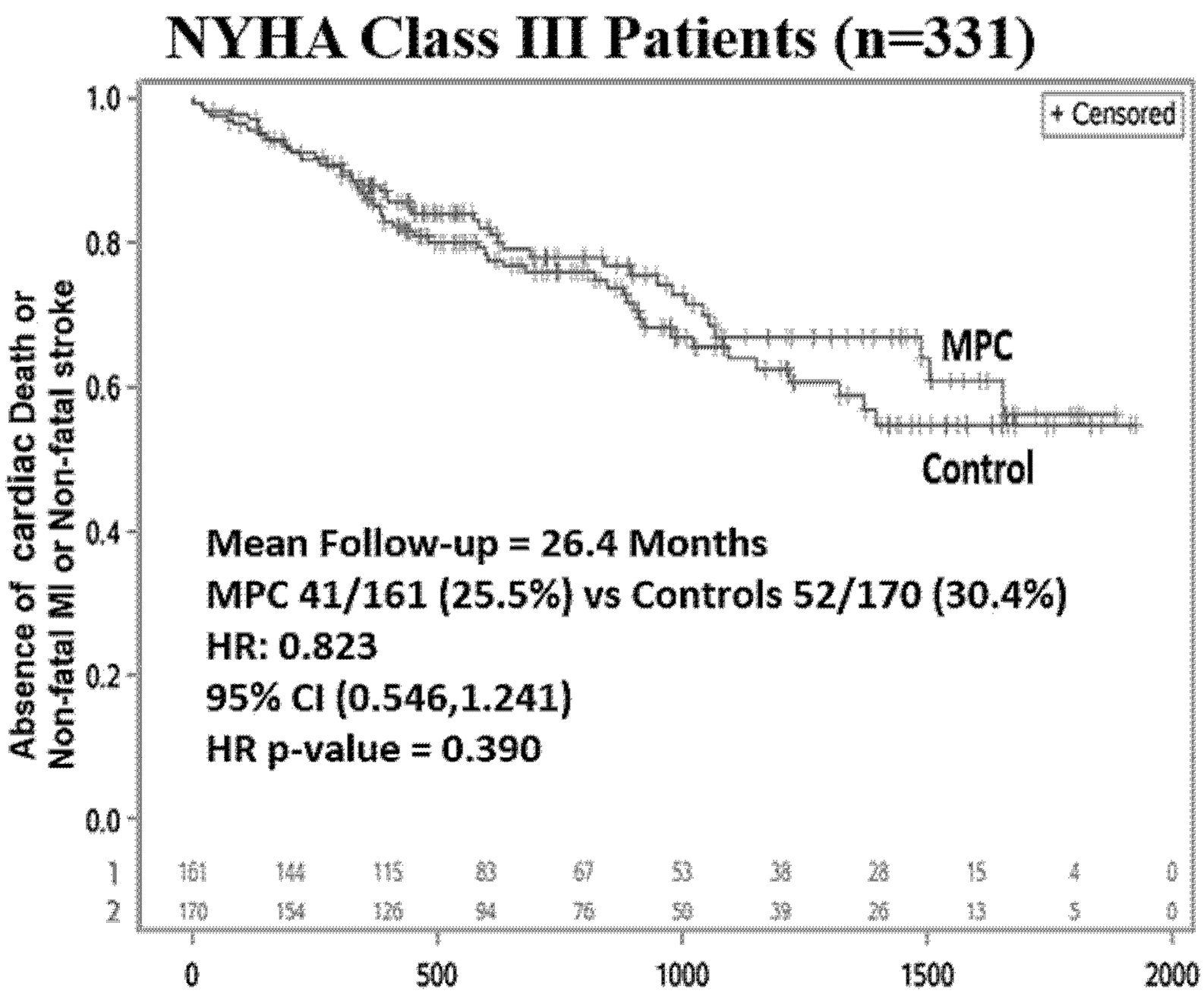

NYHA Class III Patients (n=331)
+ Censored
MPC
Control
Mean Follow-up = 26.4 Months
MPC 41/161 (25.5%) vs Controls 52/170 (30.4%)
HR: 0.823
95% CI (0.546,1.241)
HR p-value = 0.390
Absence of cardiac Death or Non-fatal MI or Non-fatal stroke
1.0
0.8
0.6
0.4
0.2
0.0
1 161 144 115 83 67 53 38 28 15 4 0
2 170 154 126 94 76 50 39 26 13 5 0
0 500 1000 1500 2000
Days from Day 0 Treatment

FIGURE 9-2

D

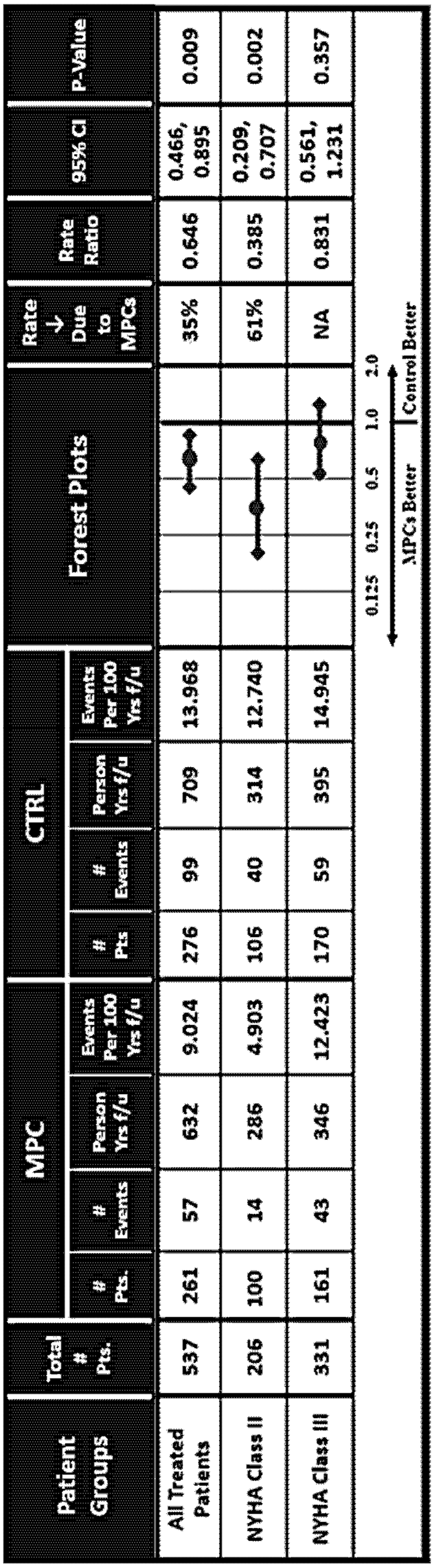
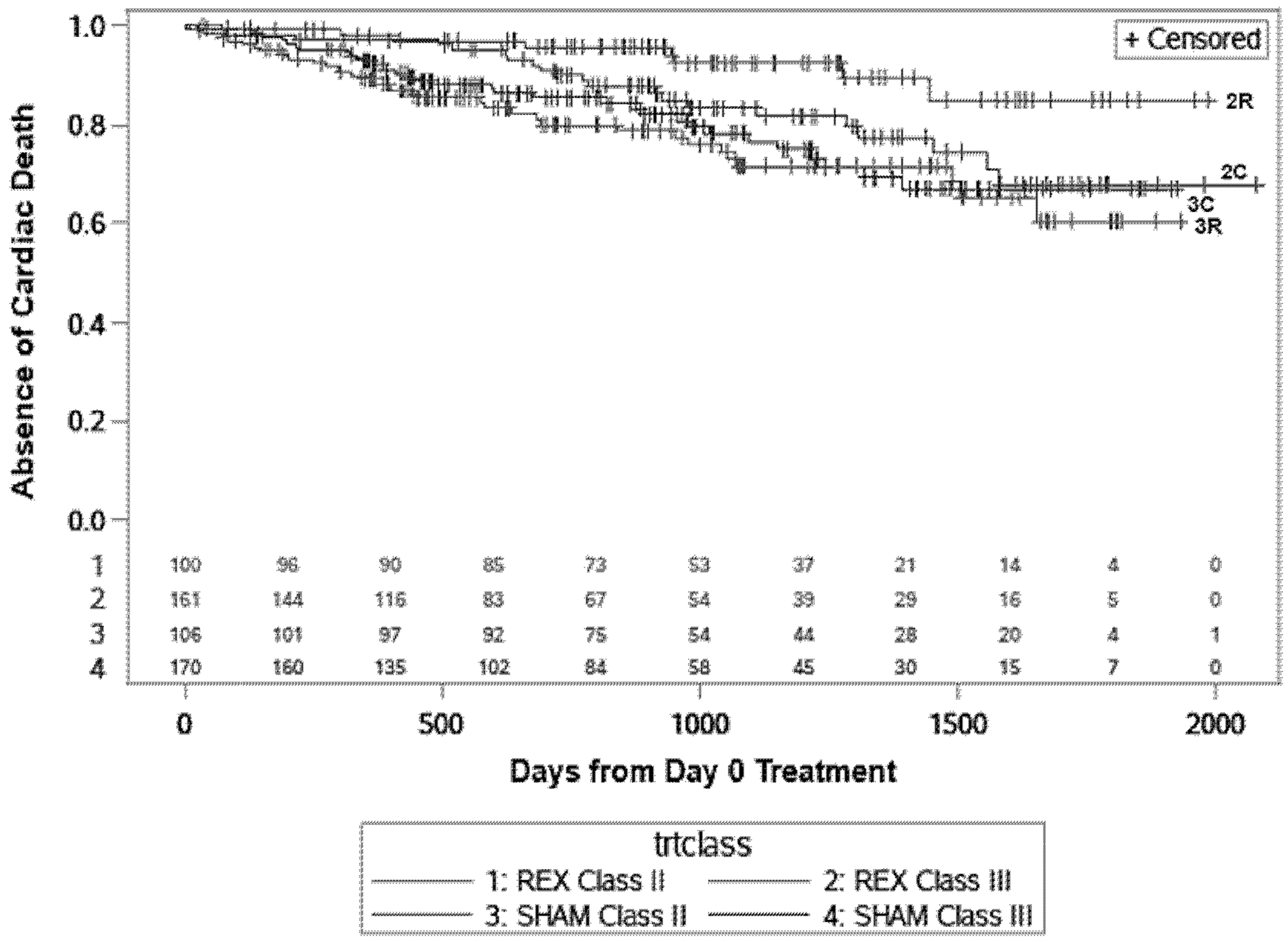
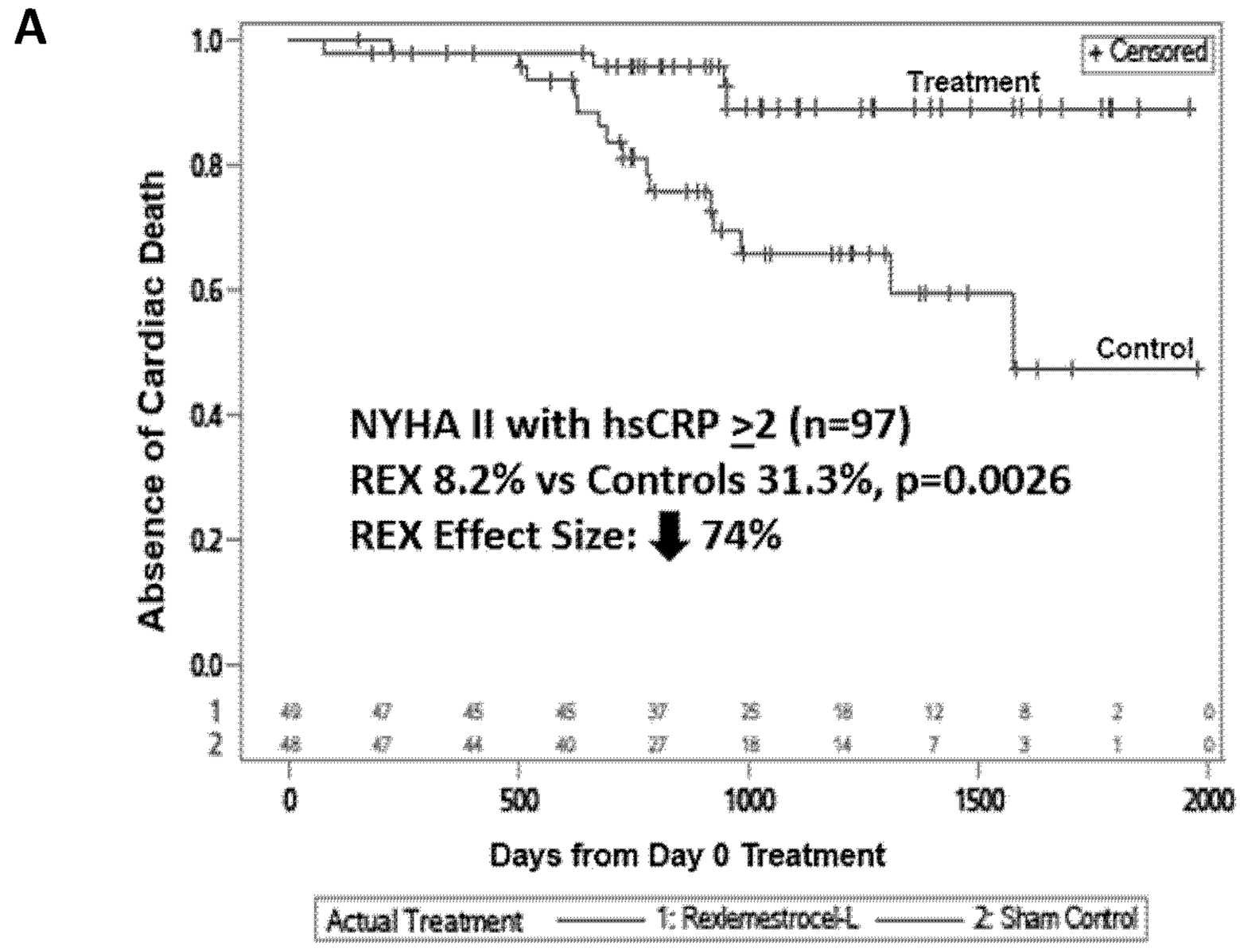

Patient Groups
Total # Pts.
MPC
CTRL
Pts.
Events
Person Yrs f/u
Events Per 100 Yrs f/u
Pts
Events
Person Yrs f/u
Events Per 100 Yrs f/u
Forest Plots
Rate ↓ Due to MPCs
Rate Ratio
95% CI
P-Value
All Treated Patients
537
261
57
632
9.024
276
99
709
13.968
35%
0.646
0.466, 0.895
0.009
NYHA Class II
206
100
14
286
4.903
106
40
314
12.740
61%
0.385
0.209, 0.707
0.002
NYHA Class III
331
161
43
346
12.423
170
59
395
14.945
NA
0.831
0.561, 1.231
0.357
0.125
0.25
0.5
1.0
2.0
MPCs Better
Control Better

FIGURE 9-3



MPC NYHA II: 8%
MPC NYHA III: 22.3%
Saline NYHA II: 20%
Saline NYHA III: 20%
P=0.004

FIGURE 10


A
1.0
0.8
0.6
0.4
0.2
0.0
Absence of Cardiac Death
+ Censored
Treatment
Control
NYHA II with hsCRP ≥2 (n=97)
REX 8.2% vs Controls 31.3%, p=0.0026
REX Effect Size: ⬇ 74%
1 49 47 45 45 37 25 16 12 8 2 0
2 48 47 44 40 27 18 14 7 3 1 0
0 500 1000 1500 2000
Days from Day 0 Treatment
Actual Treatment 1: Rexlemestrocel-L 2: Sham Control

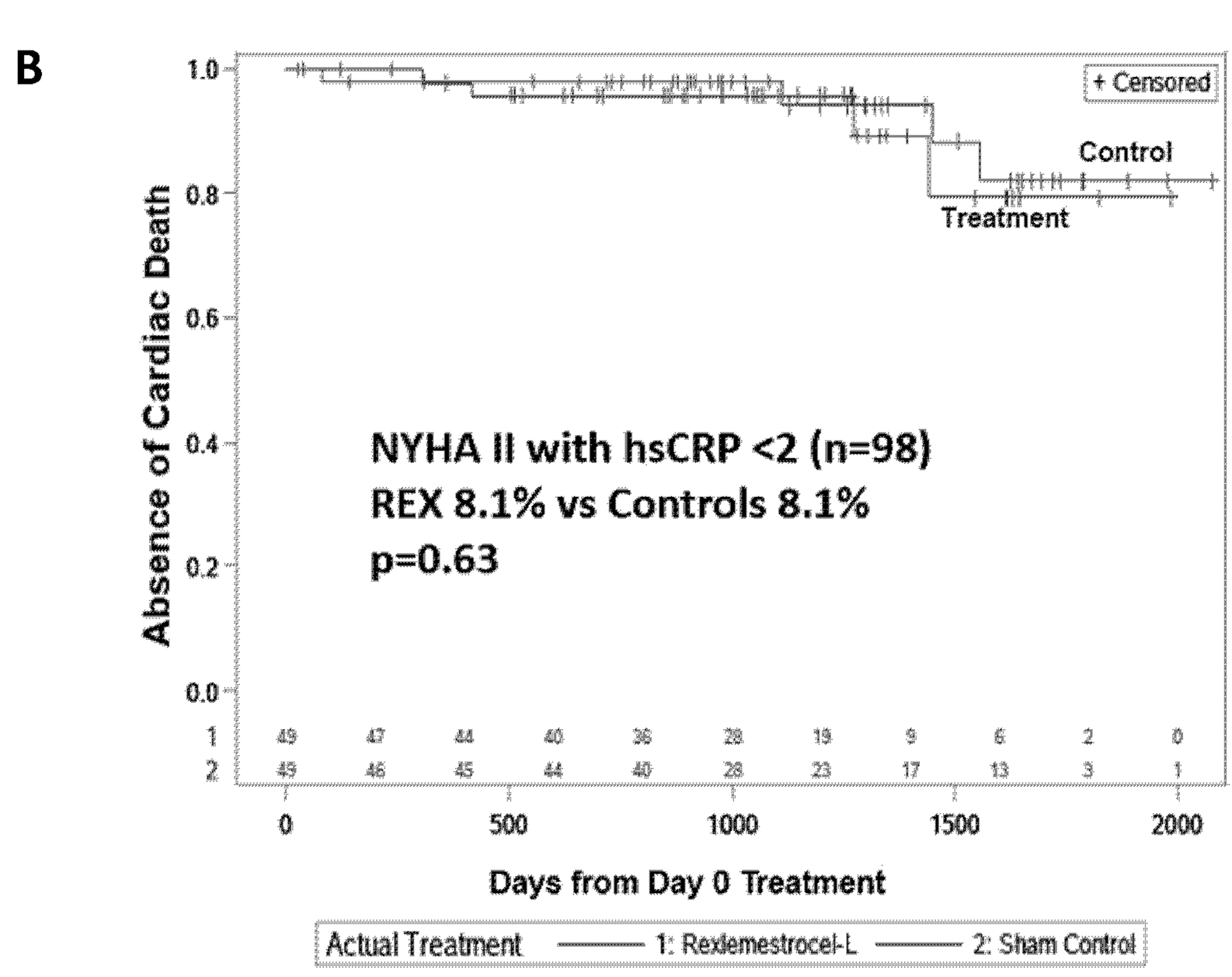
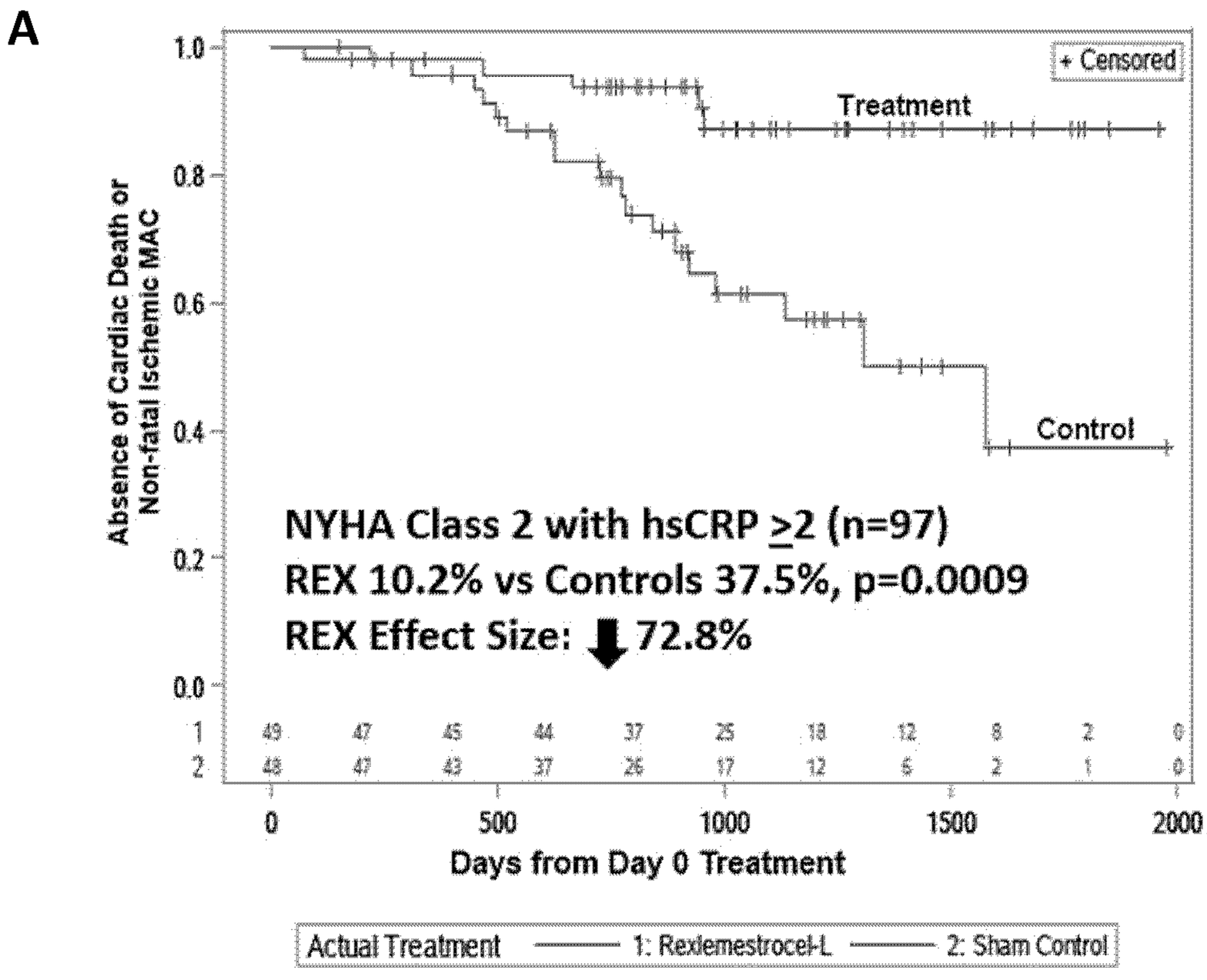

B
1.0
0.8
0.6
0.4
0.2
0.0
Absence of Cardiac Death
+ Censored
Control
Treatment
NYHA II with hsCRP <2 (n=98)
REX 8.1% vs Controls 8.1%
p=0.63
1 49 47 44 40 36 28 19 9 6 2 0
2 49 46 45 44 40 28 23 17 13 3 1
0 500 1000 1500 2000
Days from Day 0 Treatment
Actual Treatment 1: Rexlemestrocel-L 2: Sham Control

FIGURE 11


A
Absence of Cardiac Death or Non-fatal Ischemic MAC
1.0
0.8
0.6
0.4
0.2
0.0
+ Censored
Treatment
Control
NYHA Class 2 with hsCRP ≥2 (n=97)
REX 10.2% vs Controls 37.5%, p=0.0009
REX Effect Size: ⬇ 72.8%
1 49 47 45 44 37 25 18 12 8 2 0
2 48 47 43 37 26 17 12 6 2 1 0
0 500 1000 1500 2000
Days from Day 0 Treatment
Actual Treatment 1: Rexlemestrocel-L 2: Sham Control

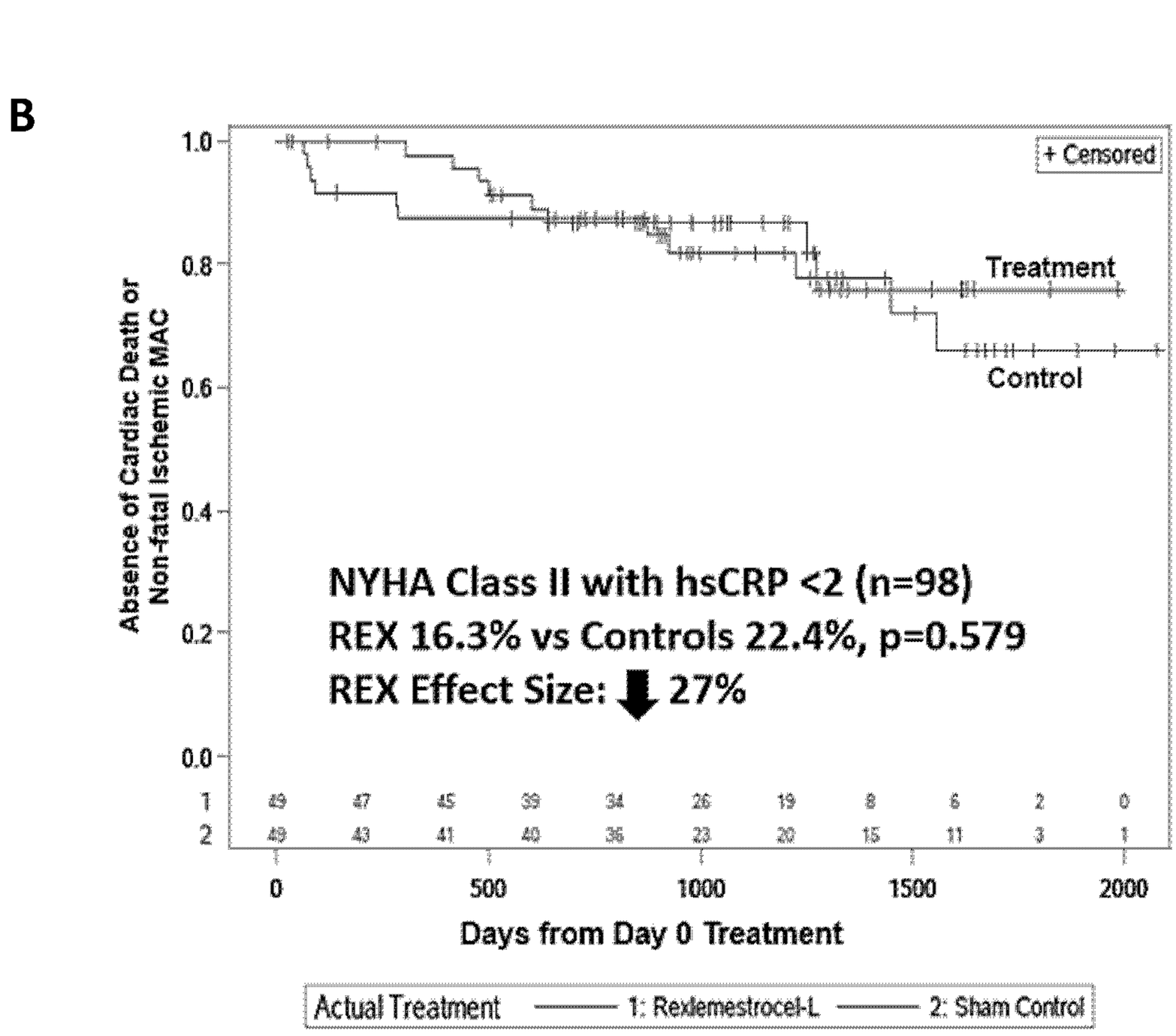

B
Absence of Cardiac Death or Non-fatal Ischemic MAC
1.0
0.8
0.6
0.4
0.2
0.0
+ Censored
Treatment
Control
NYHA Class II with hsCRP <2 (n=98)
REX 16.3% vs Controls 22.4%, p=0.579
REX Effect Size: ⬇ 27%
1 49 47 45 39 34 26 19 8 6 2 0
2 49 43 41 40 36 23 20 15 11 3 1
0 500 1000 1500 2000
Days from Day 0 Treatment
Actual Treatment 1: Rexlemestrocel-L 2: Sham Control

FIGURE 12