

MOLECULAR IDENTIFICATION OF CARDIOMYOCYTES FOR CARDIAC CELL THERAPY

RELATED APPLICATIONS

This application claims the benefit under 35 U.S.C. § 119(e) of U.S. Provisional Application No. 63/614,475, filed December 22, 2023, entitled “MOLECULAR IDENTIFICATION OF CARDIOMYOCYTES FOR CARDIAC CELL THERAPY,” the entire disclosure of which is hereby incorporated by reference herein in its entirety.

FIELD

This disclosure relates to compositions and methods related to cardiomyocytes assessed for risk of graft-induced arrhythmia.

BACKGROUND

The ability of stem cells to regenerate injured tissue holds great promise for the treatment of cardiac disease and injury; however, several obstacles to their adoption persist. Grafts of pluripotent stem cell-derived cardiomyocytes have been shown to induce arrhythmias (*e.g.*, sustained ventricular tachycardia) in large animal models. These graft-induced arrhythmias may occur rapidly after administration (*e.g.*, within 48 hours) and are sometimes resistant to antiarrhythmic drugs and cardioversion. Despite being highly desired, methods and compositions that mitigate graft-induced arrhythmia have been difficult to identify thus far, posing a significant impediment to the treatment of cardiac disease and injury.

SUMMARY

Cardiac cell therapies, such as cardiomyocyte grafts, can potentially treat subjects having injured cardiac tissue (*e.g.*, as the result of heart disease or failure). However, large animal model studies have shown that administration of cardiomyocyte grafts can sometimes induce arrhythmia with severe consequences. Surprisingly, the inventors of the present disclosure have identified methods and compositions which reduce the risk of graft-induced arrhythmias; these methods and compositions are described herein.

Aspects of this disclosure provide a method of preparing a cellular composition for cardiac cell therapy, the method comprising: (a) determining that a plurality of cardiomyocytes do not have a molecular profile associated with a high risk of graft-induced arrhythmia (GIA); and (b) preparing a cellular composition for cardiac cell therapy from the plurality of

cardiomyocytes. In some embodiments, the plurality of cardiomyocytes represents greater than 80% of all cardiomyocytes used to prepare the cardiac cell therapy. In some embodiments, the plurality of cardiomyocytes is obtained by: (i) determining a molecular profile of a batch of cells; and (ii) selecting the plurality of cardiomyocytes from the batch of cells, wherein the plurality of cardiomyocytes does not have the molecular profile associated with high risk of GIA.

In some embodiments, the molecular profile of the batch of cells is obtained from a transcriptome of the batch of cells. In some embodiments, the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq). In some embodiments, the molecular profile of the batch of cells is obtained via flow cytometry.

In some embodiments, the molecular profile associated with a high risk of GIA comprises negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2. In some embodiments, the molecular profile associated with a high risk of GIA comprises negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A. In some embodiments, the molecular profile associated with a high risk of GIA comprises negative/low expression of SCN5A. In some embodiments, the molecular profile associated with a high risk of GIA comprises positive/high expression of one or more genes selected from: AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91. In some embodiments, the molecular profile associated with a high risk of GIA comprises positive/high expression of any one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A. In some embodiments, the molecular profile associated with a high risk of GIA comprises positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A.

In some embodiments, the plurality of cardiomyocytes comprises mature cardiomyocytes. In some embodiments, the mature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SSXA3/4, TRA-160, OCT3/4, NANOG, and SOX2. In some embodiments, the mature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the

mature cardiomyocytes comprise positive/high expression of MLC2v and CTNT2; and negative/low expression of MLC2a.

In some embodiments, preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a physiologically acceptable medium.

In some embodiments, the plurality of cardiomyocytes comprises immature cardiomyocytes. In some embodiments, the immature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SS CA3/4, TRA-160, OCT3/4, NANOG, SOX2. In some embodiments, the immature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the immature cardiomyocytes comprise positive/high expression of cTNT, MYL2, MYL7, KCNJ2, CACNA1C, CACNA1H, SCN5A, HCN4 and negative/low expression of MKI67, CD90.

In some embodiments, preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.

In some aspects, this disclosure provides a method of preparing a cellular composition for cardiac cell therapy, the method comprising: (a) determining that a plurality of cardiomyocytes have a molecular profile associated with a low risk of graft-induced arrhythmia (GIA); and (b) preparing a cellular composition for cardiac cell therapy from the plurality of cardiomyocytes. In some embodiments, the plurality of cardiomyocytes represents greater than 80% of all cardiomyocytes used to prepare the cardiac cell therapy. In some embodiments, the plurality of cardiomyocytes is obtained by: (i) determining a molecular profile of a batch of cells; (ii) selecting the plurality of cardiomyocytes from the batch of cells, wherein the plurality of cardiomyocytes does not have the molecular profile associated with low risk of GIA.

In some embodiments, the molecular profile of the batch of cells is obtained from a transcriptome of the batch of cells. In some embodiments, the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq). In some embodiments, the molecular profile of the batch of cells is obtained via flow cytometry.

In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: ACOX1, CACNB1C, CAMK2A, CAMK2B, COX6B2, CPT1A, DSG2, FGF12, GJB1, ITGB7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2. In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high

expression of one or more genes selected from: CACNB1C, COX6B2, GJB1, KCNH2, MYH7, MYL2, PPARA, and SCN5A. In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high expression of SCN5A. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from: AEBP1, B3GALT2, BCHE, BRINP3, COL19B1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of any one or more genes selected from: BCHE, BRINP3, EPHB4, ID4, IRX2, and SCN3A. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from BCHE, BRINP3, EPHB4, ID4, and IRX2 and positive/high expression of SCN5A.

In some embodiments, the plurality of cardiomyocytes comprises mature cardiomyocytes. In some embodiments, the mature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SSCB3/4, TRA-160, OCT3/4, NANOG, and SOX2. In some embodiments, the mature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the mature cardiomyocytes comprise negative/low expression of MLC2v and CTNT2; and positive/high expression of MLC2a.

In some embodiments, preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a physiologically acceptable medium.

In some embodiments, the plurality of cardiomyocytes comprises immature cardiomyocytes. In some embodiments, the immature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SSCB3/4, TRA-160, OCT3/4, NANOG, SOX2. In some embodiments, the immature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the immature cardiomyocytes comprise negative/low expression of cTNT, MYL2, MYL7, KCNJ2, CACNB1C, CACNB1H, SCN5A, HCN4 and positive/high expression of MKI67, CD90.

In some embodiments, preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.

In some aspects, this disclosure provides a method of preparing a batch of cells for cardiac cell therapy, the method comprising: (a) determining that an at least first batch of cells

among a plurality of batches comprises a molecular profile associated with a low risk of graft-induced arrhythmia (GIA) and; (b) preparing the at least first batch of cells for cardiac cell therapy.

In some embodiments, the molecular profile of the at least first batch of cells is obtained from a transcriptome of the batch of cells. In some embodiments, the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq). In some embodiments, the molecular profile of the at least first batch of cells is obtained via flow cytometry.

In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: ACOX1, CACNC1C, CAMK2A, CAMK2B, COX6C2, CPT1A, DSG2, FGF12, GJC1, ITGC7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2. In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: CACNC1C, COX6C2, GJC1, KCNH2, MYH7, MYL2, PPARA, and SCN5A. In some embodiments, the molecular profile associated with a low risk of GIA comprises positive/high expression of SCN5A. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from: AEBP1, C3GALT2, BCHE, BRINP3, COL19C1, CPNE5, CXXC4, GUCY1A, HSPC2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of any one or more genes selected from: BCHE, BRINP3, EPHC4, ID4, IRX2, and SCN3A. In some embodiments, the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from BCHE, BRINP3, EPHC4, ID4, and IRX2 and positive/high expression of SCN5A.

In some embodiments, the at least first batch of cells comprises mature cardiomyocytes. In some embodiments, the mature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SSCC3/4, TRA-160, OCT3/4, NANOG, SOX2. In some embodiments, the mature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the mature cardiomyocytes comprise positive/high expression of MLC2v, CTNT2; and negative/low expression of MLC2a.

In some embodiments, preparing the mature cardiomyocytes for cardiac cell therapy comprises contacting the mature cardiomyocytes with a physiologically acceptable medium suitable for administration to a subject.

In some embodiments, the at least first batch of cells comprises immature cardiomyocytes. In some embodiments, the immature cardiomyocytes are derived from pluripotent stem cells. In some embodiments, the pluripotent stem cells express one or more of the following markers: SSCC3/4, TRA-160, OCT3/4, NANOG, SOX2. In some embodiments, the immature cardiomyocytes are derived from embryonic stem cells. In some embodiments, the immature cardiomyocytes comprise positive/high expression of cTNT, MYL2, MYL7, KCNJ2, CACNC1C, CACNC1H, SCN5A, HCN4 and negative/low expression of MKI67, CD90.

In some embodiments, preparing the immature cardiomyocytes for cardiac cell therapy comprises contacting the immature cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.

In some aspects, the present disclosure provides a method, comprising preparing a batch of cells for cardiac cell therapy, wherein the cells are characterized as having a molecular profile comprising one of the following: (i) negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91; (ii) positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2; (iii) positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A; (iv) negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A; (v) negative/low expression of BCHE; (vi) negative/low expression of BRINP3; (vii) negative/low expression of EPHA4; (viii) negative/low expression of ID4; (ix) negative/low expression of IRX2; and (x) positive/high expression of SCN5A.

In some aspects, the present disclosure provides a method of differentiating a plurality of cardiomyocyte precursor cells, the method comprising contacting a plurality of cardiomyocyte precursor cells that have been determined to have a molecular profile associated with a low risk of graft-induced arrhythmia with a medium comprising a differentiating agent under conditions that promote cardiomyocyte differentiation.

In some aspects, the present disclosure provides a method of maturing a plurality of immature cardiomyocytes, the method comprising contacting a plurality of immature cardiomyocytes that been determined to have a molecular profile associated with a low risk of

graft-induced arrhythmia with a maturation cocktail under conditions that promote cardiomyocyte maturation.

In some aspects, the present disclosure provides a method of preparing a plurality of mature cardiomyocytes for cardiac cell therapy, the method comprising contacting a plurality of mature cardiomyocytes that been determined to have a molecular profile associated with a low risk of graft-induced arrhythmia with a physiologically acceptable medium suitable for administration to a subject in need of cardiac cell therapy.

In some aspects, the present disclosure provides a composition for cardiac cell therapy comprising: a plurality of cells having molecular profile associated with low risk of ventricular tachycardia (VT); and a physiologically acceptable medium. In some embodiments, the molecular profile associated with a low risk of GIA comprises one of the following: (i) negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91; (ii) positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2; (iii) positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A; (iv) negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A; (v) negative/low expression of BCHE; (vi) negative/low expression of BRINP3; (vii) negative/low expression of EPHA4; (viii) negative/low expression of ID4; (ix) negative/low expression of IRX2; and (x) positive/high expression of SCN5A.

In some aspects, the present disclosure provides a composition for cardiac cell therapy comprising: a plurality of cells in a physiologically acceptable medium suitable for administration to a patient in need thereof; wherein the plurality of cells comprises mature cardiomyocytes; and wherein no more than 20% of the mature cardiomyocytes comprise a molecular profile associated with a high risk of graft-induced arrhythmia (GIA). In some aspects, the molecular profile associated with a high risk of GIA comprises one of the following: (i) positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A and SNAP91; (ii) negative/low expression

of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2; (iii) negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A; (iv) positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A; (v) positive/high expression of BCHE; (vi) positive/high expression of BRINP3; (vii) positive/high expression of EPHA4; (viii) positive/high expression of ID4; (ix) positive/high expression of IRX2; and (x) negative/low expression of SCN5A.

In some aspects, this disclosure provides a composition comprising a plurality of cells; wherein the plurality of cells comprises immature cardiomyocytes; and wherein no more than 20% of the immature cardiomyocytes have a molecular profile associated with a high risk of graft-induced arrhythmia (GIA). In some embodiments, the molecular profile associated with a high risk of GIA comprises one of the following: (i) positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A and SNAP91; (ii) negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2; (iii) negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A; (iv) positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A; (v) positive/high expression of BCHE; (vi) positive/high expression of BRINP3; (vii) positive/high expression of EPHA4; (viii) positive/high expression of ID4; (ix) positive/high expression of IRX2; and (x) negative/low expression of SCN5A.

In some aspects, this disclosure provides a method of treating a subject in need of cardiac cell therapy, the method comprising administering to the subject a cardiac cell therapy comprising a cellular composition prepared according to a method provided herein, a batch of cells prepared according to a method provided herein, or a composition provided herein.

In some aspects, this disclosure provides a method of treating a subject in need of cardiac cell therapy, the method comprising: (a) determining that no more than 20% of cardiomyocytes in a batch of cells comprise a molecular profile associated with a high risk of graft-induced arrhythmia (GIA); and (b) administering the batch of cells or a portion thereof to the subject.

In some aspects, this disclosure provides a method of treating a subject in need of cardiac cell therapy, the method comprising administering a plurality of cells to a subject, wherein the plurality of cells has been determined to have a molecular profile associated with low risk of graft-induced arrhythmia (GIA). In some aspects, this disclosure provides a method of treating a subject in need of cardiac cell therapy, the method comprising administering a plurality of cells to a subject, wherein the plurality of cells has a molecular profile associated with low risk of graft-induced arrhythmia (GIA).

BRIEF DESCRIPTION OF DRAWINGS

FIGs. 1A-1C show how treatment of myocardial infarction (MI) by transplantation of cardiomyocyte cells can cause sustained graft-induced arrhythmia (GIA) post-cell delivery. **FIG. 1A** shows an administration protocol for induction of MI in a Yorkshire pig and subsequent cell transplantation. **FIG. 1B** shows an electrocardiogram (EKG) recording of an exemplary MI-model pig having GIA after receiving cell transplantation. Neither administration of an anti-arrhythmic cocktail nor cardioversion reversed the sustained GIA. VAP = vascular access port. **FIG. 1C** shows post-necropsy scoring of the implanted cardiomyocytes in two media preparations by severity of induced GIA and categorization as low GIA (scores under 3) or high GIA (scores above 3).

FIGs. 2A-2J show single-cell RNA-seq (scRNA-seq) data from 19 samples of transplanted cardiomyocytes. **FIG. 2A** shows uniform manifold approximation and projection (UMAP) clustering of scRNA-seq data by percent mitochondrial transcripts. **FIG. 2B** shows UMAP clustering of scRNA-seq data with varying intensity by number of genes. **FIG. 2C** shows UMAP clustering of scRNA-seq data with varying intensity by GIA status. OOS indicates out of spec; UK indicates unknown. **FIG. 2D** shows UMAP clustering of scRNA-seq data by protocol. **FIG. 2E** shows UMAP clustering of scRNA-seq data by cluster. **FIG. 2F** shows gene expression of TNNT2, a cardiomyocyte cell marker, projected onto UMAP space. **FIG. 2G** shows gene expression of THY1, a fibroblast cell marker, projected onto UMAP space. **FIG. 2H** shows gene expression of MKI67, a proliferation cell marker, projected onto UMAP space. **FIG. 2I** shows gene expression of MYL7, a marker of immature cardiomyocytes,

projected onto UMAP space. **FIG. 2J** shows gene expression of MYL2, a marker of mature cardiomyocytes, projected onto UMAP space.

FIGs. 3A-3I show gene expression of fibroblast (FB), endothelial cell (EC), and cardiomyocyte (CM) cell type-specific markers. In the dotplots, the size of the dot represents the percentage of cells in the clusters expressing the marker and the intensity represents the average expression of the marker in that cluster. **FIG. 3A** shows gene expression of the top 5 differentially expressed genes for each cluster. **FIG. 3B** shows gene expression of cardiomyocyte markers across the cardiomyocyte clusters. **FIG. 3C** shows gene expression of cardiomyocytes prepared with a first bioprocess (Medium 1). **FIG. 3D** shows gene expression of cardiomyocytes prepared using a second bioprocess (Medium 2). **FIG. 3E** shows gene expression of cardiomyocytes originating from Low GIA or High GIA samples processed using either bioprocess. **FIG. 3F** shows cluster distribution of High GIA (“High”) and Low GIA (“Low”) batches by sample type. **FIG. 3G** shows cluster distribution of High GIA and Low GIA batches by bioprocess (Medium 1 or Medium 2). **FIG. 3H** shows cluster distribution of all batches by sample type (“Low” = Low GIA; “High” = High GIA; “UK” = Unknown; “OOS” = Out of Spec). **FIG. 3I** shows the cluster distribution of all batches colored by bioprocess (Medium; Medium 2; Medium 3).

FIGs. 4A-4E show expression of maturity markers across all clusters identified via UMAP analysis. **FIG. 4A** shows gene expression of the top 5 differentially expressed genes for each cluster. The size of the dot represents the percentage of cells in the clusters expressing the marker and intensity represents the average expression of the marker in that cluster. **FIG. 4B** shows gene expression of SCN5A, a marker of electrophysiological maturation, projected onto UMAP space. **FIG. 4C** shows gene expression of CACNA1C, a marker of electrophysiological maturation, projected onto UMAP space. **FIG. 4D** shows gene expression of GAPJ1, a marker of myofibril structure maturation, projected onto UMAP space. **FIG. 4E** shows gene expression of COX6A2, a marker of metabolism maturation, projected onto UMAP space.

FIG. 5 shows gene expression of the top 20 highly expressed genes in the CM_5 cluster projected onto UMAP space.

FIGs. 6A-6G shows expression of genes involved in the myofibrillar structure maturation axis. **FIG. 6A** shows expression of MYBPC3 projected onto UMAP space. **FIG. 6B** shows expression of ACTN2 projected onto UMAP space. **FIG. 6C** shows expression of TTN projected onto UMAP space. **FIG. 6D** shows expression of DES projected onto UMAP space. **FIG. 6E** shows expression of MYH6 (left) and MYH7 (right) projected onto UMAP space. **FIG.**

6F shows expression of MYL7 (left) and MYL2 (right) projected onto UMAP space. **FIG. 6G** shows expression of TNNI1 (left) and TNNI3 (right) projected onto UMAP space.

FIGs. 7A-7H show expression of genes involved in the ion channel maturation axis. **FIG. 7A** shows expression of CACNA1C projected onto UMAP space. **FIG. 7B** shows expression of CACNA1H projected onto UMAP space. **FIG. 7C** shows expression of *HCN4* projected onto UMAP space. **FIG. 7D** shows expression of KCND3 projected onto UMAP space. **FIG. 7E** shows expression of KCNH2 projected onto UMAP space. **FIG. 7F** shows expression of SCN5A (left), SCN9A (middle), and SCN3A (right) projected onto UMAP space. **FIG. 7G** shows expression of KCNJ2 (first from left), KCNJ3 (second from left), KCNJ5 (second from right), and KCNJ8 (first from right) projected onto UMAP space. **FIG. 7H** shows expression of KCNQ1 (left), KCNQ3 (middle), and KCNQ5 (right) projected onto UMAP space.

FIGs. 8A-8F show expression of genes involved in calcium handling. **FIG. 8A** shows expression of ATPase Sarcoplasmic/Endoplasmic Reticulum Ca^{2+} Transporting 2 (ATP2A2) projected onto UMAP space. **FIG. 8B** shows expression of Ryanodine Receptor 2 (RYR2) projected onto UMAP space. **FIG. 8C** shows expression of Solute Carrier family 8 Member A1 (SLC8A1) projected onto UMAP space. **FIG. 8D** shows expression of Protein Kinase CAMP-Activated Catalytic Subunit Alpha (PRKACA) projected onto UMAP space. **FIG. 8E** shows expression of CAMK-related genes projected onto UMAP space. **FIG. 8F** shows expression of T-tubule-related genes projected onto UMAP space.

FIGs. 9A-9B show expression of genes involved in the metabolism maturation axis. **FIG. 9A** shows expression of genes in the PPARA signaling pathway. **FIG. 9B** shows expression of genes involved in mitochondrial metabolism.

FIGs. 10A-10D show expression of genes involved in the cardiomyocyte ultrastructure maturation axis. **FIG. 10A** shows expression of genes related to desmosome formation. **FIG. 10B** shows expression of genes related to integrin formation. **FIG. 10C** shows expression of genes related to costamere formation. **FIG. 10D** shows expression of genes related to gap junction formation.

DETAILED DESCRIPTION

Described herein, in some aspects, are methods and compositions useful for preparing cardiac cell therapies. Cardiac cell therapies generally refer to stem-cell-derived exogenous cells that are administered to the heart of a subject (*e.g.*, in the form of a graft) in order to repair cardiac tissue in need thereof. Though cardiac cell therapies hold great promise for treating

cardiac injuries, their use in large animal (*e.g.*, pigs) or human subjects has faced a number of obstacles, including the observed emergence of graft-induced arrhythmias (GIAs). Large animal test subjects to which cardiac grafts are administered often experience arrhythmias of varying frequency and severity, some of which cannot be treated with antiarrhythmic drugs or cardioversion. Though many attempts have been made to identify cells having a risk of contributing to arrhythmias, these attempts have been unsuccessful. However, using clustering analysis of high dimensional genetic and molecular data, the inventors have developed methods for identifying cells suitable for preparation into cardiac cell therapies and having a low risk of causing GIAs.

Described herein, in some embodiments, are compositions and methods related to identifying and/or preparing suitable cells and/or suitable batches of cells for cardiac cell therapies. In some aspects, compositions disclosed herein comprise isolated cardiomyocytes (*e.g.*, precursors, immature, mature) having a molecular profile (*e.g.*, expressing one or more gene markers) associated with low risk of causing GIA (low GIA) wherein the molecular profile is selected from one or more of the following:

- a1. negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, and SNAP91;
- a2. positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- a3. positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- a4. negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A;
- a5. negative/low expression of BCHE;
- a6. negative/low expression of BRINP3;
- a7. negative/low expression of EPHA4;
- a8. negative/low expression of ID4
- a9. negative/low expression of IRX2;

- a10. positive/high expression of SCN5A;
- a11. negative/low expression of BCHE and BRINP3;
- a12. negative/low expression of BCHE and EPHA4;
- a13. negative/low expression of BCHE and ID4;
- a14. negative/low expression of BCHE and IRX2;
- a15. negative/low expression of BCHE and positive/high expression of SCN5A;
- a16. negative/low expression of BRINP3 and EPHA4;
- a17. negative/low expression of BRINP3 and ID4;
- a18. negative/low expression of BRINP3 and IRX2;
- a19. negative/low expression of BRINP3 and positive/high expression of SCN5A;
- a20. negative/low expression of EPHA4 and ID4;
- a21. negative/low expression of EPHA4 and IRX2;
- a22. negative/low expression of EPHA4 and positive/high expression of SCN5A;
- a23. negative/low expression of ID4 and IRX2;
- a24. negative/low expression of ID4 and positive/high expression of SCN5A;
- a25. negative/low expression of IRX2 and positive/high expression of SCN5A;
- a26. negative/low expression of BCHE, BRINP3, and EPHA4;
- a27. negative/low expression of BCHE, BRINP3, and ID4;
- a28. negative/low expression of BCHE, BRINP3, and IRX2;
- a29. negative/low expression of BCHE and BRINP3, and positive/high expression of SCN5A;
- a30. negative/low expression of BCHE, EPHA4, and ID4;
- a31. negative/low expression of BCHE, EPHA4, and IRX2;
- a32. negative/low expression of BCHE, EPHA4, and positive/high expression of SCN5A;
- a33. negative/low expression of BCHE, ID4, and IRX2;
- a34. negative/low expression of BCHE and ID4, and positive/high expression of SCN5A;
- a35. negative/low expression of BCHE and IRX2, and positive/high expression of SCN5A;
- a36. negative/low expression of BRINP3, EPHA4, and ID4;
- a37. negative/low expression of BRINP3, EPHA4, and IRX2;
- a38. negative/low expression of BRINP3, ID4, and IRX2;

- a39. negative/low expression of BRINP3 and ID4, and positive/high expression of SCN5A;
- a40. negative/low expression of BRINP3 and IRX2, and positive/high expression of SCN5A;
- a41. negative/low expression of EPHA4, ID4, and IRX2;
- a42. negative/low expression of EPHA4 and ID4, and positive/high expression of SCN5A;
- a43. negative/low expression of EPHA4 and IRX2, and positive/high expression of SCN5A;
- a44. negative/low expression of ID4 and IRX2, and positive/high expression of SCN5A;
- a45. negative/low expression of BCHE, BRINP3, EPHA4, and ID4;
- a46. negative/low expression of BCHE, BRINP3, EPHA4, and IRX2;
- a47. negative/low expression of BCHE, BRINP3, and EPHA4, and positive/high expression of SCN5A;
- a48. negative/low expression of BCHE, BRINP3, and ID4, and positive/high expression of SCN5A;
- a49. negative/low expression of BCHE, BRINP3, and IRX2, and positive/high expression of SCN5A;
- a50. negative/low expression of BCHE, BRINP3, EPHA4, ID4, and IRX2;
- a51. negative/low expression of BCHE, BRINP3, EPHA4, ID4, and IRX2, and positive/high expression of SCN5A;

In some embodiments, a mature cardiomyocyte comprises the molecular profile of any one of a1-a51. In some embodiments, a mature cardiomyocyte having the molecular profile of any one of a1-a51 also exhibits positive/high expression of CTNT2 and MLC2V and negative/low expression of MLC2a.

In some embodiments, an immature cardiomyocyte comprises the molecular profile of any one of a1-a51. In some embodiments, an immature cardiomyocyte having the molecular profile of any one of a1-a51 also exhibits positive/high expression of MLC2a, NKX2, NKX3, NKX4, NKX5, and HCN4, and negative/low expression of CD36 and MLC2v.

In some embodiments, a cardiomyocyte precursor comprises the molecular profile of any one of a1-a51. In some embodiments, a cardiomyocyte precursor having the molecular profile of

any one of a1-a51 also exhibits positive/high expression of any one of SSEA3/4, TRA-160, OCT3/4, NANOG, and SOX2.

In some aspects, compositions disclosed herein comprise cardiomyocytes having the molecular profile of any one of a1-a51 (*e.g.*, “low GIA” cardiomyocytes). In some aspects, methods disclosed herein comprise contacting cardiomyocytes having the molecular profile of any one of a1-a51 (*e.g.*, “low GIA” cardiomyocytes) with a differentiating agent under conditions that promote cardiomyocyte differentiation. In some aspects, methods disclosed herein comprise contacting cardiomyocytes having the molecular profile of any one of a1-a51 (*e.g.*, “low GIA” cardiomyocytes) with a maturation cocktail under conditions that promote cardiomyocyte maturation. In some aspects, methods disclosed herein comprise contacting cardiomyocytes having the molecular profile of any one of a1-a51 (*e.g.*, “low GIA” cardiomyocytes) with a physiologically acceptable medium suitable for administration to a subject in need of cardiac cell therapy.

In some embodiments, isolated cardiomyocytes (*e.g.*, precursors, immature, mature) are identified as having a molecular profile (*e.g.*, expressing one or more gene markers) associated with high risk of causing graft-induced arrhythmia (high GIA) wherein the molecular profile is selected from one or more of the following:

- b1. positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, and SNAP91;
- b2. negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- b3. negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- b4. positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A;
- b5. positive/high expression of BCHE;
- b6. positive/high expression of BRINP3;
- b7. positive/high expression of EPHA4;

- b8. positive/high expression of ID4
- b9. positive/high expression of IRX2;
- b10. negative/low expression of SCN5A;
- b11. positive/high expression of BCHE and BRINP3;
- b12. positive/high expression of BCHE and EPHA4;
- b13. positive/high expression of BCHE and ID4;
- b14. positive/high expression of BCHE and IRX2;
- b15. positive/high expression of BCHE and negative/low expression of SCN5A;
- b16. positive/high expression of BRINP3 and EPHA4;
- b17. positive/high expression of BRINP3 and ID4;
- b18. positive/high expression of BRINP3 and IRX2;
- b19. positive/high expression of BRINP3 and negative/low expression of SCN5A;
- b20. positive/high expression of EPHA4 and ID4;
- b21. positive/high expression of EPHA4 and IRX2;
- b22. positive/high expression of EPHA4 and negative/low expression of SCN5A;
- b23. positive/high expression of ID4 and IRX2;
- b24. positive/high expression of ID4 and negative/low expression of SCN5A;
- b25. positive/high expression of IRX2 and negative/low expression of SCN5A;
- b26. positive/high expression of BCHE, BRINP3, and EPHA4;
- b27. positive/high expression of BCHE, BRINP3, and ID4;
- b28. positive/high expression of BCHE, BRINP3, and IRX2;
- b29. positive/high expression of BCHE and BRINP3, and negative/low expression of SCN5A;
- b30. positive/high expression of BCHE, EPHA4, and ID4;
- b31. positive/high expression of BCHE, EPHA4, and IRX2;
- b32. positive/high expression of BCHE, EPHA4, and negative/low expression of SCN5A;
- b33. positive/high expression of BCHE, ID4, and IRX2;
- b34. positive/high expression of BCHE and ID4, and negative/low expression of SCN5A;
- b35. positive/high expression of BCHE and IRX2, and negative/low expression of SCN5A;
- b36. positive/high expression of BRINP3, EPHA4, and ID4;

- b37. positive/high expression of BRINP3, EPHA4, and IRX2;
- b38. positive/high expression of BRINP3, ID4, and IRX2;
- b39. positive/high expression of BRINP3 and ID4, and negative/low expression of SCN5A;
- b40. positive/high expression of BRINP3 and IRX2, and negative/low expression of SCN5A;
- b41. positive/high expression of EPHA4, ID4, and IRX2;
- b42. positive/high expression of EPHA4 and ID4, and negative/low expression of SCN5A;
- b43. positive/high expression of EPHA4 and IRX2, and negative/low expression of SCN5A;
- b44. positive/high expression of ID4 and IRX2, and negative/low expression of SCN5A;
- b45. positive/high expression of BCHE, BRINP3, EPHA4, and ID4;
- b46. positive/high expression of BCHE, BRINP3, EPHA4, and IRX2;
- b47. positive/high expression of BCHE, BRINP3, and EPHA4, and negative/low expression of SCN5A;
- b48. positive/high expression of BCHE, BRINP3, and ID4, and negative/low expression of SCN5A;
- b49. positive/high expression of BCHE, BRINP3, and IRX2, and negative/low expression of SCN5A;
- b50. positive/high expression of BCHE, BRINP3, EPHA4, ID4, and IRX2;
- b51. positive/high expression of BCHE, BRINP3, EPHA4, ID4, and IRX2, and negative/low expression of SCN5A;

In some aspects, compositions disclosed herein are cellular compositions (*e.g.*, batches, cardiac cell therapies, cardiac grafts) comprising a plurality of cardiomyocytes, wherein fewer than 30% of the plurality of cardiomyocytes are high GIA cardiomyocytes (*e.g.*, comprise the molecular profile of any one of b1-b51). In some aspects, cellular compositions disclosed herein comprise a plurality of cardiomyocytes, wherein fewer than 30%, fewer than 25%, fewer than 20%, fewer than 15%, fewer than 10%, fewer than 9%, fewer than 8%, fewer than 7%, fewer than 6%, fewer than 5%, fewer than 4%, fewer than 3%, fewer than 2%, fewer than 1%, fewer than 0.5%, fewer than 0.4%, fewer than 0.3%, fewer than 0.2%, fewer than 0.1%, fewer than

0.01% of the cardiomyocytes are high GIA cardiomyocytes (*e.g.*, comprise the molecular profile of any one of b1-b51).

In some aspects, methods disclosed herein comprise excluding (*e.g.*, removing) cardiomyocytes having the molecular profile of any one of b1-b51 (*e.g.*, “low GIA” cardiomyocytes) from further preparation and/or from compositions for cardiac cell therapy.

These embodiments and others are further described below.

Cardiomyocytes

The present disclosure relates, in some aspects, to cardiac cell therapies (*e.g.*, cardiac grafts) comprising cardiomyocytes having one or more properties associated with low risk causing graft-induced arrhythmia when administered to a subject as part of a cardiac cell therapy. In some embodiments, cardiomyocytes are cells having cardiomyocyte lineage, including, but not limited to ventricular cardiomyocytes, atrial cardiomyocytes, and/or cardiac smooth muscle cells. Cardiomyocytes can be understood to be cells at any stage of cardiomyocyte development without restriction, unless stated otherwise.

In some embodiments, cardiomyocytes are cardiomyocyte precursors. In some embodiments, a cardiomyocyte precursor is any precursor (*e.g.*, progenitor) cell with the capacity to differentiate into a cardiomyocyte cell. In some embodiments, a cardiomyocyte precursor is or is derived from a stem cell. In some embodiments, a cardiomyocyte precursor is or is derived from an induced pluripotent stem cell (iPSC). In some embodiments, a cardiomyocyte precursor is or is derived from a human pluripotent stem cell (hPSC). In some embodiments, a cardiomyocyte precursor is or is derived from an embryonic stem cell (ESC). As described herein, cardiomyocyte precursors may have the following characteristics: (1) positive/high expression of one or more of the following markers: stage-specific embryonic antigen 3 and/or 4 (SSEA3/4), podocalyxin (TRA-160), octamer-binding transcription 3 and/or 4 (OCT3/4), homeobox protein NANOG (NANOG), and sex-determining region Y-box 2 (SOX2) (*e.g.*, as determined using flowcytometric analysis); (2) capable of self-replication. In some embodiments, cardiomyocyte precursors are capable of differentiation into all three germ layers (*e.g.*, endoderm, mesoderm, ectoderm) or derivatives thereof. In some embodiments, cardiomyocyte precursors are mesoderm cells induced from stem cells which have been contacted by induction media (*e.g.*, a Wnt agonist). In some embodiments, cardiomyocyte precursors are cardiac progenitors differentiated from mesoderm cells which have been

contacted by cardiomyocyte differentiation media (*e.g.*, contacted by a Wnt antagonist). In some embodiments, cardiomyocyte precursor cells are not excitable.

In some embodiments, cardiomyocytes are immature cardiomyocytes. In some embodiments, an immature cardiomyocyte is any cell which is in any stage of differentiation to become a mature cardiomyocyte and comparable to cardiac muscle cells in early stages of fetal development. In some embodiments, an immature cardiomyocyte has been contacted with one or more differentiation agents (*e.g.*, RPMI-1640 supplemented with B27). In some embodiments, an immature cardiomyocyte has been contacted with a maturation media (*e.g.*, RPMI-1640 supplemented with B27 without insulin]). Immature cardiomyocytes are generally not quiescent (*e.g.*, have not entered cell cycle arrest). In some embodiments, cardiomyocytes are immature ventricular cardiomyocytes. In some embodiments, cardiomyocytes are immature atrial cardiomyocytes. In some embodiments, cardiomyocytes are immature cardiac smooth muscle cells. As described herein, immature cardiomyocytes may exhibit the following characteristics within about 14 days from mesoderm induction: (1) cluster of differentiation 36 (CD36) negative/low (relative to a mature cardiomyocyte), myosin regulatory light chain 2v (MLC2v) negative/low (relative to a mature cardiomyocyte), and myosin regulatory light chain 2a (MLC2a) positive/high (relative to a mature cardiomyocyte), *e.g.*, using flow cytometric analysis; and (2) capable of spontaneous action potentials and contraction. In some embodiments, an immature cardiomyocyte expresses (*e.g.*, is positive/high for) NK2 homeobox 5 (NKX2-5). In some embodiments, an immature cardiomyocyte has positive/high expression of one or more “Positive/High” genetic or molecular markers provided in Table 1; and has negative/low expression of one or more “Negative/Low” genetic or molecular markers provided in Table 1. In some embodiments, an immature cardiomyocyte has one or more electrophysiological properties according to Table 1.

In some embodiments, cardiomyocytes are mature cardiomyocytes. In some embodiments, a mature cardiomyocyte is any terminally differentiated (*e.g.*, quiescent) cardiac muscle cell. In some embodiments, a mature cardiomyocyte is derived from an immature cardiomyocyte which has been contacted by a maturation cocktail (*e.g.*, RPMI-1640 supplemented with B27 minus insulin). In some embodiments, the cardiomyocytes are matured *in vivo* (*e.g.*, in a subject). In some embodiments, the cardiomyocytes are matured *in vitro*. In some embodiments, cardiomyocytes are mature ventricular cardiomyocytes. In some embodiments, cardiomyocytes are mature atrial cardiomyocytes. In some embodiments, cardiomyocytes are mature cardiac smooth muscle cells. In some embodiments, mature

cardiomyocytes exhibit the following characteristics within about 51 days from mesoderm induction: (1) CD36 positive/high, MLC2v positive/high, and downregulated (*e.g.*, reduced expression of) MLC2a (relative to immature cardiomyocytes), *e.g.*, using flow cytometric analysis; and (2) capable of contraction with application of an external stimulus (*e.g.*, electrical stimulus). In some embodiments, mature cardiomyocytes are TNI positive/high. In some embodiments, a mature cardiomyocyte has positive/high expression of one or more positive/high genetic or molecular markers provided in Table 1 and has negative/low expression of one or more negative genetic or molecular markers provided in Table 1. In some embodiments, a mature cardiomyocyte has one or more electrophysiological properties according to Table 1.

Table 1. Non-limiting characteristics of cardiomyocytes throughout development

		Positive/High	Negative/Low
Cardiomyocyte Precursors	Genetic	SSEA3/4, TRA-160, OCT3/4, NANOG, SOX2	--
	Molecular	SSEA3/4, TRA-160, OCT3/4, NANOG, SOX2	--
	Electrophysiology	--	--
Immature Cardiomyocytes (Day 14)	Genetic	NKX2-5, HCN4, KCNJ2, MYL2, ACTC1, ACTN2, ALPK3, AQP1, CAV1, CSRP3, DES, GSN, ITGA7, LMOD1, LMOD2, MYBPC3, MYH10, MYH6, MYH7, MYL2, MYL3, MYL7, MYOZ2, PDLIM1, PKP2, POSTN, SYNPO2L, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, ACSL1, ALDOC, CKMT2, FABP3, FHL2, G0S2, HIF1A, IGFBP7, MAN1C1, MT-ND2, PGAM2, PGC1A, PPP1R3A, SLC25A4, SLC27A6, SLC2A1, TGM2, CCNB1, CDK1, CDKN2A, MKI67, GATA4, HAND1, HEY2, HOPX, IRX4, LEF1, MEF2C, TBX5, TBX2	CD36, MLC2v, CACNA1D, KCNJ3, NPPA, NR2F2, SHOX2, MYL4, CD90, COL1A2, COL3A1, IGF2, VCAN, ADAMTS19, ESAM, FOXA2, KCTD12, MYH11, PECAM1, PLVAP, VCAM1, MYL9, POU5F1, L1TD1, LINC00678, FOXD3-AS1, GAL,
	Molecular	MLC2a, NKX2-5, cTNT, CD172a	MLC2v
	Electrophysiology	capable of spontaneous action potentials and contraction	--
Mature Cardiomyocytes (Day 51)	Genetic	CD36, KCNJ2, MYL2, ACTC1, ACTN2, ALPK3, AQP1, CAV1, CSRP3, DES, GSN, ITGA7, LMOD1, LMOD2, MYBPC3, MYH10, MYH6, MYH7, MYL2, MYL3, MYL7, MYOZ2, PDLIM1, PKP2, POSTN, SYNPO2L, TCAP, TNNC1, TNNI3, TNNT2, TPM1, TTN, ACSL1, ALDOC, CKMT2, FABP3, FHL2, G0S2, HIF1A, IGFBP7, MAN1C1, MT-ND2, PGAM2, PGC1A, PPP1R3A, SLC25A4, SLC27A6, SLC2A1, TGM2, CCNB1, CDK1, CDKN2A, MKI67,	CACNA1D, KCNJ3, NPPA, NR2F2, SHOX2, MYL4, CD90, COL1A2, COL3A1, IGF2, VCAN, ADAMTS19, ESAM, FOXA2, KCTD12, MYH11, PECAM1, PLVAP, VCAM1, MYL9, POU5F1, L1TD1, LINC00678, FOXD3-AS1, GAL

		GATA4, HAND1, HEY2, HOPX, IRX4, LEF1, MEF2C, NKX2-5, TBX5, TBX2	
	Molecular	MLC2v, CD36, NKX2-5, TNI, cTNT, CD172a	MLC2a
	Electrophysiology	capable of contraction with external stimulus	--

Non-limiting examples of cardiomyocyte differentiation and maturation procedures can be found in PCT Publication No.: WO 2014/200339, PCT Publication No.: WO 2017/039445, PCT Publication No.: WO 2020/227232, U.S. Publication No.: US 2020/0407687, each of which are incorporated herein by reference.

Methods of Identifying Cells Suitable for Cardiac Cell Therapy

The present disclosure relates, in some aspects, to methods of identifying cells that are suitable for cardiac cell therapy. In some embodiments, the method comprises identifying cells as having one or more properties associated with low risk or high risk of causing graft-induced arrhythmia (GIA) when administered to a subject as part of a cardiac cell therapy (*e.g.*, cardiac graft). Generally, the methods disclosed herein can be practiced on any cardiomyocyte (*e.g.*, mature, immature, precursor). Cells, such as cardiomyocytes, can be understood to comprise a molecular profile. A cell's molecular profile encompasses a signature that is based on the expression level of one or more markers (*e.g.*, nucleic acids, peptide/protein).

The term "marker" is used widely in the art and can be commonly understood to refer to a biological molecule and/or a detectable fragments thereof. In some embodiments, markers comprise nucleic acids, peptides/proteins, and detectable fragments thereof. Nucleic acid markers may comprise nuclear RNA, pre-mRNA, mRNA, copy DNA, and detectable fragments thereof, *e.g.*, nucleic acids transcribed by a cell from an endogenous gene. Peptide/protein markers may comprise any polymer comprising amino acid residues linked by peptide bonds, including, but not limited to, native proteins, recombinant proteins, synthetic proteins, proteins having one or more co- or post-expression modifications (*e.g.*, glycosylation, acetylation, phosphorylation, sulfonation, methylation, ubiquitination, signal peptide removal, etc.), and any detectable fragments thereof, *e.g.*, peptides/proteins translated by a cell from an endogenous gene. In some embodiments, a molecular profile is based on the expression level of one or more nucleic acid markers. In some embodiments, a molecular profile is based on the expression level of one or more peptide/protein markers. In some embodiments, a molecular profile is based on the expression level of one or more nucleic acid markers and one or more peptide/protein markers.

Expression of a marker by a cell can be measured any number of ways, but generally refers to quantification of the presence (or absence) of a distinct signal corresponding to the marker in or on the cell, compared to a control and/or baseline. In some instances, expression of a marker is measured as an absolute quantity (*e.g.*, weight, molar amount, concentration). In some instances, expression of a marker is expressed as a relative quantity (*e.g.*, fold-increase, fold-decrease, percent). In some instances, expression of a marker in a cell is measured in a semi-quantitative manner (*e.g.*, “high” or “low” fluorescence as used in flow cytometry).

A cell may be understood to be positive (+) for a marker which is present in the cell at a sufficient level to be detected in the cell. A cell may be understood to have “high” expression for a marker when the quantity (*e.g.*, absolute, relative, semi-quantitative) of the marker in the cell is above some baseline or threshold (*e.g.*, above a population average, at a higher quantity than a control cell). A cell may be understood to be negative (-) for a marker which is not present in the cell in a sufficient amount to be detected in the cell. A cell may be understood to have “low” expression for a marker when the quantity (*e.g.*, absolute, relative, semi-quantitative) of the marker in the cell is below some baseline or threshold (*e.g.*, below a population average, at a lower quantity relative to a control cell).

In some instances, quantification of a signal for a marker in a test cell is compared to quantification of a signal for the same marker in a negative control cell (*e.g.*, a cell known to not highly express the marker) or a positive control cell (*e.g.*, a cell known to highly express the marker). In some embodiments, a test cell determined to be “positive” for the marker or have “high” expression of the marker can be understood to express the marker at an increased average level compared to the level of the marker expressed by a negative control cell, for example, at a 1.5-fold increase, 2-fold increase, 3-fold increase, 4-fold increase, 5-fold increase, 10-fold increase, 20-fold increase, 30-fold increase, 40-fold increase, 50-fold increase, or higher than the level of the marker expressed by the negative control cell. When used in the context of expression, a cell which is deemed to have “positive/high” expression of one or more markers can be understood to have “positive or high” expression of the one or more markers.

In some embodiments, a test cell determined to be “negative” for the marker or have “low” expression of the marker can be understood to express the marker at a reduced (*e.g.*, decreased) average level compared to the level of the marker expressed by a positive control cell, for example, at a 1.5-fold decrease, 2-fold decrease, 3-fold decrease, 4-fold decrease, 5-fold decrease, 10-fold decrease, 20-fold decrease, 30-fold decrease, 40-fold decrease, 50-fold decrease, or less relative to the level of the marker expressed by the positive control cell. A cell

which is deemed to have “negative/low” expression of a marker can be understood to have “negative or low” expression of the one or more markers.

In some instances, quantification of a signal for a marker in a first test cell is compared to quantification of a signal for the same marker in a second test cell, such that expression of the marker in the first cell is described relative to the second cell. In some embodiments, a first test cell is deemed to comprise positive/high expression of one or more markers relative to expression of the same marker by a second cell. In some embodiments, a first test cell is identified as comprising a high GIA molecular profile because of positive/high expression of one or more markers (*e.g.*, markers in Table 2) relative to a second cell. In some embodiments, a first test cell is identified as comprising a low GIA molecular profile because of positive/high expression of one or more markers (*e.g.*, markers in Table 2) relative to a second cell. In some embodiments, a first test cell is deemed to comprise negative/low expression of one or more markers relative to expression of the same marker by a second cell. In some embodiments, a first test cell is identified as comprising a high GIA molecular profile because of negative/low expression of one or more markers (*e.g.*, markers in Table 2) relative to a second cell. In some embodiments, a first test cell is identified as comprising a low GIA molecular profile because of negative/low expression of one or more markers (*e.g.*, markers in Table 2) relative to a second cell.

In some embodiments, methods of identifying cells (*e.g.*, cardiomyocytes) comprise identifying expression of one or more cell markers. In some embodiments, one or more cell markers is a cell type-specific marker. Methods of identifying cell type-specific markers are known to those of skill in the art and are dependent on the marker of interest. In a non-limiting example, cell surface proteins or cell type-specific genes may be used to identify or define specific cell populations or subsets using flow cytometry, immunohistochemistry, immunofluorescence, polymerase chain reaction (*e.g.*, reverse transcription PCR), or fluorescence-activated cell sorting (FACS). The markers of interest may be determined by a number of techniques, including microarrays and ribonucleic acid (RNA) sequencing.

In some embodiments, cells are identified as being cardiomyocytes (*e.g.*, mature, immature, precursor) using cell-type labeling. Cell-type labeling, as it relates to RNA sequencing, refers to the process of identifying cell-type-specific canonical gene markers that are differentially expressed and labeling the cells based on the canonical marker genes. In some embodiments, cells are identified as being cardiac cells using cell-type labeling. In some

embodiments, cells are identified as being cardiomyocytes, endothelial cells, and/or fibroblasts using cell-type labeling.

In some embodiments, cells of a first type are differentiated from cells of a second type using differential gene analysis. Differential gene expression analysis, as it relates to RNA sequencing, identifies genes that are differentially expressed (*e.g.*, more highly or lowly expressed) between two or more states (*e.g.*, cell types, bioprocess) that may be a molecular marker that differentiates the two or more states. Methods of differential gene expression analysis are known to those of skill in the art and may employ Bayesian statistics, linear models, negative binomial distributions, t-tests, or machine learning algorithms (*e.g.*, random forest, support vector machine). In some embodiments, cells in a first developmental stage are differentiated from cells in a second developmental stage using differential gene analysis. In some embodiments, a batch of low GIA cells is differentiated from a batch of high GIA cells using differential gene analysis.

In some embodiments, one or more cell markers may be identified using RNA sequencing (RNA-seq). Non-limiting examples of RNA-seq include bulk RNA sequencing, single-cell RNA sequencing, spatial RNA sequencing, mRNA sequencing, total RNA-seq, strand-specific RNA-seq, and long read RNA-seq. Those of skill in the art will recognize that methods of RNA-seq may vary depending on the RNA of interest, sequencing platform, and the intended application, but generally require 1) reverse transcription of RNA into DNA; 2) preparation of a sequencing library from the DNA; 3) sequencing; and, 4) data processing, which may include batch correction, clustering, cell-type labeling, and differential gene expression analysis.

In some embodiments, cells having molecular profiles associated with low GIA or high GIA are identified using clustering (*e.g.*, RNA-seq clustering). Clustering, as it relates to RNA-seq, refers to the process of grouping individual cells based on their gene expression profiles to identify cell populations within a heterogeneous sample. Since gene expression profiles are typically high-dimensional, clustering usually requires dimensionality reduction prior to clustering to facilitate analysis. Methods of dimensionality reduction are known to those of skill in the art and can include principal component analysis (PCA), non-negative matrix factorization (NMF), linear discriminant analysis (LDA), T-distributed Stochastic Neighbor Embedding (t-SNE), and uniform manifold approximation and projection (UMAP). Each method of dimensionality reduction is known to those of skill in the art to differ in their ability to preserve global and local structure. Other algorithms for dimensionality reduction are contemplated.

Clustering algorithms can then be applied to the reduced gene expression profile to group and visualize cells that have similar gene expression profiles. Non-limiting clustering algorithms include k-means clustering, hierarchical clustering, and expectation-maximization. Visualized clusters (*e.g.*, on a t-SNE or UMAP plot) can be used by those of skill in the art to explore heterogeneity within cell populations and relationships between cell populations. In some embodiments, clustering analysis is used to identify molecular profiles associated with one or more desirable characteristics (*e.g.*, cells at a particular developmental timepoint having a low risk of causing GIA).

In some embodiments, cells are identified as having a molecular profile associated with one or more desired characteristics. Desired characteristics may be any characteristic which is deemed to be useful for a composition or method related to cardiac cell therapy. In some embodiments, desired characteristics comprise cells of a particular cell type (*e.g.*, cardiac cells). In some embodiments, desired characteristics comprise cells of particular subtypes of a cell type (*e.g.*, cardiomyocytes, endothelial cells, fibroblasts).

In some embodiments, cardiomyocytes are identified as being “low GIA,” such that the cardiomyocytes have a molecular profile associated with low risk of causing graft-induced arrhythmias (*e.g.*, drug graft-induced arrhythmia) when administered to a subject as part of a graft (*e.g.*, cellular composition). In some embodiments, cardiomyocytes are identified as being “high GIA,” such that the cardiomyocytes have a molecular profile associated with high risk of causing graft-induced arrhythmias (*e.g.*, drug graft-induced arrhythmia) when administered to a subject as part of a graft.

In some embodiments, cardiomyocytes identified as low GIA are mature cardiomyocytes. In some embodiments, cardiomyocytes identified as low GIA are immature cardiomyocytes. In some embodiments, cardiomyocytes identified as low GIA are cardiomyocyte precursors.

In some embodiments, cardiomyocytes identified as high GIA are mature cardiomyocytes. In some embodiments, cardiomyocytes identified as high GIA are immature cardiomyocytes. In some embodiments, cardiomyocytes identified as high GIA are cardiomyocyte precursors.

As used herein, a “batch” of cells (*e.g.*, cardiomyocytes) comprises a plurality of cells belonging to a same treatment group. In some embodiments, a batch comprises cells having about the same chronological age (*e.g.*, days since contact with a differentiation or maturation agent). In some embodiments, a batch comprises cells having about the same developmental age

(*e.g.*, expressing cell markers within a particular range). In some embodiments, a batch comprises cells derived from the same ancestral cells. In some embodiments, a batch comprises cells derived from the same cell line. In some embodiments, a batch comprises cells from two or more different cell lines. In some embodiments, a batch of cells comprises at least 1 billion cells. In some embodiments, a batch of cells comprises about 2 billion cells. In some embodiments, a batch of cells comprises about 3 billion cells. In some embodiments, a batch of cells comprises about 4 billion cells. In some embodiments, a batch of cells comprises about 5 billion cells. In some embodiments, a batch of cells comprises about 10 billion cells. In some embodiments, a batch of cells comprises about 20 billion cells. In some embodiments, a batch of cells comprises about 1 billion cells to about 25 billion cells. In some embodiments, a batch of cells comprises about 10 billion to about 20 billion cells. In some embodiments, a batch of cells comprises about 1 billion, about 2 billion, about 3 billion, about 4 billion, about 5 billion, about 6 billion, about 7 billion, about 8 billion, about 9 billion, about 10 billion, about 11 billion, about 12 billion, about 13 billion, about 14 billion, about 15 billion, about 16 billion, about 17 billion, about 18 billion, about 19 billion, about 20 billion cells.

A sample is a portion of a batch and comprises one or more cells of the batch. In some embodiments, a sample comprises $1 \times 10^{-9}\%$ to 1% of a batch. In some embodiments, properties of a batch are determined from three or more samples of the batch. Preferably, a sample is representative of a batch.

In some embodiments, a batch is heterogenous and comprises cells having two or more different characteristics, including, but not limited to, cell types (*e.g.*, cardiomyocytes, endothelial cells, fibroblasts), ages, and/or molecular profiles. In some embodiments, a batch is homogenous and consists of cells having shared characteristics, including, but not limited to cell type (*e.g.*, cardiomyocytes), age (*e.g.*, chronological, developmental), and/or molecular profile (*e.g.*, low GIA or high GIA).

In some embodiments, a heterogenous batch is made homogenous by isolating cells having one or more desired characteristics (*e.g.*, molecular profile identified as being associated with high GIA cardiomyocytes) for further preparation. In some embodiments, a heterogenous batch is made homogenous by isolating cells having one or more undesired characteristics (*e.g.*, molecular profile identified as being associated with high GIA cardiomyocytes) for removal from the batch. For example, a heterogenous batch may comprise a first subset of cells having a molecular profile identified as being associated with low GIA cardiomyocytes and a second subset of cells having a molecular profile identified as being associated with high GIA

cardiomyocytes. Either subset of cells may be removed from the batch by using any number of methods, for example, immunofluorescent or immunomagnetic labeling of select surface markers (*e.g.*, markers from molecular profiles associated with low GIA), coupled with corresponding FACS gating strategy or immunomagnetic separation, respectively.

Markers Related to Expression of Myofibrillar Structure

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more markers related to expression (*e.g.*, transcript, protein) of myofibrillar structure (*e.g.*, myosin binding proteins, actin, filaments).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin binding protein C, cardiac type (MYBPC3). MYBPC3 is a gene encoding myosin binding protein C, cardiac type protein (also known as cMYBP-C, CMD1MM, CMH4, FHC, LVNC10, and MYBP-C), which is involved in the regulation of positioning of myosin and actin in cardiomyocytes. In some embodiments, expression of MYBPC3 by a cell is determined by measuring a level of an MYBPC3 RNA (*e.g.*, mRNA transcribed from MYBPC3). Exemplary nucleic acid sequences of human MYBPC3 can be found, for example, at GenBank Accession Nos. NG_007667.1, NM_000256.3, UniProt Accession No. Q14896; and OMIM Accession No. 600958. In some embodiments, expression of MYBPC3 by a cell is determined by a measuring level of an MYBPC3 protein or fragment thereof (*e.g.*, a protein encoded by MYBPC3). An exemplary amino acid sequence of MYBPC3 can be found, for example, at GenBank Accession No. NP_000247.2. Further information on MYBPC3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYBPC3. “MYBPC3,” as used herein, also refers to variations of the MYBPC3 gene including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYBPC3).

Non-limiting examples of markers related to expression of myofibrillar structures include alpha-actinin-2 (ACTN2). ACTN2 is a gene encoding alpha-actinin-2 protein (also known as MPD6, CMH23, CMYO8, CMYP8, CMD1AA, and MYOCOZ), which is involved in crosslinking filamentous actin molecules and titin molecules to Z-discs. In some embodiments, expression of ACTN2 by a cell is determined by measuring a level of an ACTN2 RNA (*e.g.*, mRNA transcribed from ACTN2). Exemplary nucleic acid sequences of human ACTN2 can be found, for example, at GenBank Accession Nos. NG_009081.2, NM_001103.4; UniProt Accession No. P35609; and OMIM Accession No. 102573. In some embodiments, expression of ACTN2 by a cell is determined by a measuring level of an ACTN2 protein or fragment thereof (*e.g.*, a protein encoded by ACTN2). An exemplary amino acid sequence of ACTN2 can be

found, for example, at GenBank Accession No. NP_001094.1. Further information on ACTN2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ACTN2. "ACTN2," as used herein, also refers to variations of the ACTN2 gene including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ACTN2).

Non-limiting examples of markers related to expression of myofibrillar structures include Titin (TTN). TTN is a gene encoding Titin protein (also known as connectin, TMD; CMH9; CMD1G; CMPD4; CMYO5; CMYP5; EOMFC; HMERF; MYLK5; SALMY; LGMD2J; LGMDR10), which is involved in sarcomere elasticity. In some embodiments, expression of TTN by a cell is determined by measuring a level of an TTN RNA (*e.g.*, mRNA transcribed from TTN). Exemplary nucleic acid sequences of human TTN can be found, for example, at GenBank Accession Nos. NG_011618.3, NM_001256850.1; UniProt Accession No. Q8WZ42; and OMIM Accession No. 188840. In some embodiments, expression of TTN by a cell is determined by a measuring level of an TTN protein or fragment thereof (*e.g.*, a protein encoded by MYBPC3). An exemplary amino acid sequence of TTN can be found, for example, at GenBank Accession No. NP_001243779.1. Further information on TTN can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=TTN. "TTN" as used herein, also refers to variations of TTN including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=TTN).

Non-limiting examples of markers related to expression of myofibrillar structures include desmin (DES). DES is a gene encoding desmin (also known as CDCD3, CSM1, CSM2, LGMD1D, LGMD1E, LGMD2R), which is involved in sarcomere architecture. In some embodiments, expression of DES by a cell is determined by measuring a level of an DES RNA (*e.g.*, mRNA transcribed from DES). Exemplary nucleic acid sequences of human DES can be found, for example, at GenBank Accession Nos. NG_008043.1, NM_001382708.1; UniProt Accession No. P17661 and OMIM Accession No. 125660. In some embodiments, expression of DES by a cell is determined by a measuring level of DES protein or fragment thereof (*e.g.*, a protein encoded by DES). An exemplary amino acid sequence of DES can be found, for example, at GenBank Accession No. NP_001369637.1. Further information on DES can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=DES. "DES" as used herein, also refers to variations of DES including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=DES).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin heavy chain 6 (MYH6). MYH6 is a gene encoding myosin heavy chain α

(MHC- α ; also known as myosin heavy chain 6, ASD, MYHC, SSS3, CMH14, MYHCA, CMD1EE, alpha-MHC), which is involved in active force generation. In some embodiments, expression of MYH6 by a cell is determined by measuring a level of an MYH6 RNA (*e.g.*, mRNA transcribed from MYH6). Exemplary nucleic acid sequences of human MYH6 can be found, for example, at GenBank Accession Nos. NG_023444.1, NM_002471.4; UniProt Accession No. P13533; and OMIM Accession No. 160710. In some embodiments, expression of MYH6 by a cell is determined by a measuring level of MYH6 protein or fragment thereof (*e.g.*, a protein encoded by MYH6). An exemplary amino acid sequence of MYH6 can be found, for example, at GenBank Accession No. NP_002462.2. Further information on MYH6 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYH6. "MYH6" as used herein, also refers to variations of MYH6 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYH6).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin heavy chain 7 (MYH7). MYH7 is a gene encoding myosin heavy chain 7 (MHC- β ; also known as myosin heavy chain β , CMH1, MPD1, SPMD, SPMM, CMD1S, MYHCB, CMYO7A, CMYO7B, CMYP7A, CMYP7B), which is involved in contractile velocity of cardiac muscle. In some embodiments, expression of MYH7 by a cell is determined by measuring a level of an MYH7 RNA (*e.g.*, mRNA transcribed from MYH7). Exemplary nucleic acid sequences of human MYH7 can be found, for example, at GenBank Accession Nos. NG_007884.1, NM_000257.4; UniProt Accession No. P12883; and OMIM Accession No. 160760. In some embodiments, expression of MYH7 by a cell is determined by a measuring level of a MYH7 protein or fragment thereof (*e.g.*, a protein encoded by MYH7). An exemplary amino acid sequence of MYH7 can be found, for example, at GenBank Accession No. NP_000248.2. Further information on MYH7 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYH7. "MYH7" as used herein, also refers to variations of MYH7 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYH7).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin light polypeptide 6 (MYL6). MYL6 is a gene encoding myosin light polypeptide 6 (also known as myosin light chain 6, LC17, ESMLC, LC17A, LC17B, MLC-3, MLC1SM, MLC3NM, MLC3SM, LC17-GI, LC17-NM), which is involved in stabilizing the structure of myosin. In some embodiments, expression of MYL6 by a cell is determined by measuring a level of an MYL6 RNA (*e.g.*, mRNA transcribed from MYL6). Exemplary nucleic acid

sequences of human MYL6 can be found, for example, at GenBank Accession Nos. NG_029223.1, NM_021019.5; UniProt Accession No. P60660; and OMIM Accession No. 609931. In some embodiments, expression of MYL6 by a cell is determined by a measuring level of MYL6 protein or fragment thereof (*e.g.*, a protein encoded by MYL6). An exemplary amino acid sequence of MYL6 can be found, for example, at GenBank Accession No. NP_066299.2. Further information on MYL6 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYL6. "MYL6" as used herein, also refers to variations of MYL6 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYL6).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin light chain 7 (MYL7). MYL7 is a gene encoding myosin light chain 7 (also known as myosin regulatory light chain 2, atrial isoform, MYL2A, MYLC2A), which is involved in enabling calcium ion binding activity related to heart contraction. In some embodiments, expression of MYL7 by a cell is determined by measuring a level of an MYL7 RNA (*e.g.*, mRNA transcribed from MYL7). Exemplary nucleic acid sequences of human MYL7 can be found, for example, at GenBank Accession No. NM_021223.3; UniProt Accession No. Q01449; and OMIM Accession No. 613993. In some embodiments, expression of MYL7 by a cell is determined by a measuring level of MYL7 protein or fragment thereof (*e.g.*, a protein encoded by MYL7). An exemplary amino acid sequence of MYL7 can be found, for example, at GenBank Accession No. NP_067046.1. Further information on MYL7 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYL7. "MYL7" as used herein, also refers to variations of MYL7 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYL7).

Non-limiting examples of markers related to expression of myofibrillar structures include myosin light chain 2 (MYL2). MYL2 is a gene encoding myosin light chain 2 (also known as MLC2, CMH10, MFM12, MLC-2, MLC-2v, MLC-2s/v), which is involved in cardiac myosin cycling kinetics, torsion, and function. In some embodiments, expression of MYL2 by a cell is determined by measuring a level of an MYL2 RNA (*e.g.*, mRNA transcribed from MYL2). Exemplary nucleic acid sequences of human MYL2 can be found, for example, at GenBank Accession Nos. NG_007554.1, NM_000432.4; UniProt Accession No. P10916; and OMIM Accession No. 160781. In some embodiments, expression of MYL2 by a cell is determined by a measuring level of MYL2 protein or fragment thereof (*e.g.*, a protein encoded by MYL2). An exemplary amino acid sequence of MYL2 can be found, for example, at

GenBank Accession No. NP_000423.2. Further information on MYL2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MYL2. "MYL2" as used herein, also refers to variations of MYL2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MYL2).

Non-limiting examples of markers related to expression of myofibrillar structures include troponin I (TNNI1). TNNI1 is a gene encoding troponin I (also known as troponin I1, slow skeletal type, slow-twitch isoform, TNN1; SSTNI), which is involved in regulating calcium sensitivity of the myofibril contractile apparatus. In some embodiments, expression of TNNI1 by a cell is determined by measuring a level of an TNNI1 RNA (*e.g.*, mRNA transcribed from TNNI1). Exemplary nucleic acid sequences of human TNNI1 can be found, for example, at GenBank Accession Nos. NG_016649.2, NM_003281.4; UniProt Accession Nos. P19237; and OMIM Accession No. 191042. In some embodiments, expression of TNNI1 by a cell is determined by a measuring level of TNNI1 protein or fragment thereof (*e.g.*, a protein encoded by TNNI1). An exemplary amino acid sequence of TNNI1 can be found, for example, at GenBank Accession No. NP_003272.3. Further information on TNNI1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=TNNI1. "TNNI1" as used herein, also refers to variations of TNNI1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=TNNI1).

Non-limiting examples of markers related to expression of myofibrillar structures include troponin I3 (TNNI3). TNNI3 is a gene encoding troponin I3 (also known as troponin I3, cardiac type; CMH7; RCM1; cTnI; CMD2A; TNNC1; CMD1FF), which is involved in regulating calcium sensitivity of the myofibril contractile apparatus. In some embodiments, expression of TNNI3 by a cell is determined by measuring a level of an TNNI3 RNA (*e.g.*, mRNA transcribed from TNNI3). Exemplary nucleic acid sequences of human TNNI3 can be found, for example, at GenBank Accession Nos. NG_007866.2, NM_000363.5; UniProt Accession Nos. P19429; and OMIM Accession No. 191044. In some embodiments, expression of TNNI3 by a cell is determined by a measuring level of an TNNI3 protein or fragment thereof (*e.g.*, a protein encoded by TNNI3). An exemplary amino acid sequence of TNNI3 can be found, for example, at GenBank Accession No. NP_000354.4. Further information on TNNI3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=TNNI3. "TNNI3" as used herein, also refers to variations of TNNI3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=TNNI3).

In some embodiments, a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected from myosin binding protein C, cardiac type (MYBPC3); alpha-actinin-2 (ACTN2); Titin (TTN); desmin (DES); myosin heavy chain, α isoform (MHC- α); myosin heavy chain beta (MHC- β); myosin light polypeptide 6 (MYL6); myosin light chain 7 (MYL7); myosin light chain 2 (MYL2); troponin I (TNNI1); troponin I3 (TNNI3); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or all 11).

Markers Related to Expression of Cardiac Ion Channels

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more markers related to expression (*e.g.*, transcript, protein) of cardiac ion channels (*e.g.*, calcium channels, potassium channels, sodium channels, and mixed potassium and sodium channels).

Non-limiting examples of markers related to expression of cardiac ion channels include calcium voltage-gated channel subunit alpha 1 C (CACNA1C). CACNA1C is a gene encoding calcium voltage-gated channel subunit alpha 1 C (also known as TS; LQT8; CACH2; CACN2; CaV1.2; CCHL1A1; NEDHLSS; CACNL1A1; TS. LQT8; CACNA1C-IT2), which is a subunit of a voltage-dependent calcium channel and has numerous isoforms. In some embodiments, expression of CACNA1C by a cell is determined by measuring a level of a CACNA1C RNA (*e.g.*, mRNA transcribed from CACNA1C). Exemplary nucleic acid sequences of human CACNA1C can be found, for example, at GenBank Accession Nos. NG_008801.2, NM_000719.7; UniProt Accession No. Q13936; and OMIM Accession No. 114205. In some embodiments, expression of CACNA1C can be found, for example, at GenBank Accession No. NP_000710.5. Further information on CACNA1C can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CACNA1C. "CACNA1C" as used herein, also refers to variations of CACNA1C including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CACNA1C).

Non-limiting examples of markers related to expression of cardiac ion channels include calcium voltage-gated channel subunit alpha1 H (CACNA1H). CACNA1H is a gene encoding calcium voltage-gated channel subunit alpha1 H (also known as ECA6, EIG6, HALD4, Cav3.2, CACNA1HB), which is a subunit of a voltage-dependent calcium channel complex. In some embodiments, expression of CACNA1H by a cell is determined by measuring a level of an CACNA1H RNA (*e.g.*, mRNA transcribed from CACNA1H). Exemplary nucleic acid

sequences of human CACNA1H can be found, for example, at GenBank Accession Nos. NG_012647.1, NM_001005407.2; UniProt Accession No. O95180; and OMIM Accession No. 607904. In some embodiments, expression of CACNA1H by a cell is determined by measuring a level of a CACNA1H protein or fragment thereof (*e.g.*, a protein encoded by CACNA1H). An exemplary amino acid sequence of CACNA1H can be found, for example, at GenBank Accession No. NP_001005407.1. Further information on CACNA1H can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CACNA1H. "CACNA1H" as used herein, also refers to variations of CACNA1H including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CACNA1H).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4). HCN4 is a gene encoding hyperpolarization activated cyclic nucleotide-gated potassium channel 4 (also known as SSS2; EIG18; BRGDA8), which is a potassium channel necessary for cardiac pacemaking. In some embodiments, expression of HCN4 by a cell is determined by measuring a level of an HCN4 RNA (*e.g.*, mRNA transcribed from HCN4). Exemplary nucleic acid sequences of human HCN4 can be found, for example, at GenBank Accession Nos. NG_009063.1, NM_005477.3; UniProt Accession No. Q9Y3Q4; and OMIM Accession No. 605206. In some embodiments, expression of HCN4 by a cell is determined by measuring a level of a HCN4 protein or fragment thereof (*e.g.*, a protein encoded by HCN4). An exemplary amino acid sequence of HCN4 can be found, for example, at GenBank Accession No. NP_005468.1. Further information on HCN4 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=HCN4. "HCN4" as used herein, also refers to variations of HCN4 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=HCN4).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium voltage-gated channel subfamily D member 3 (KCND3). KCND3 is a gene encoding potassium voltage-gated channel subfamily D member 3 (also known as KV4.3; SCA19; SCA22; BRGDA9; KCND3L; KCND3S; KSHIVB), which is a voltage gated potassium channel important for repolarization during cardiac action potentials. In some embodiments, expression of KCND3 by a cell is determined by measuring a level of an KCND3 RNA (*e.g.*, mRNA transcribed from KCND3). Exemplary nucleic acid sequences of human KCND3 can be found, for example, at GenBank Accession Nos. NG_032011.2, NM_001378969.1; UniProt Accession No. Q9UK17; and OMIM Accession No. 605411. In some embodiments, expression of KCND3

by a cell is determined by measuring a level of a KCND3 protein or fragment thereof (*e.g.*, a protein encoded by KCND3). An exemplary amino acid sequence of KCND3 can be found, for example, at GenBank Accession No. NP_001365898.1. Further information on KCND3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCND3. "KCND3" as used herein, also refers to variations of KCND3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCND3).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium voltage-gated channel subfamily D member 2 (KCND2). KCND2 is a gene encoding potassium voltage-gated channel subfamily D member 2 (also known as RK5; KV4.2), which is a voltage-gated potassium channel. In some embodiments, expression of KCND2 by a cell is determined by measuring a level of an KCND2 RNA (*e.g.*, mRNA transcribed from KCND2). Exemplary nucleic acid sequences of human KCND2 can be found, for example, at GenBank Accession Nos. NG_034230.1, NM_012281.3; UniProt Accession No. Q9NZV8; and OMIM Accession No. 605410. In some embodiments, expression of KCND2 by a cell is determined by measuring a level of a KCND2 protein or fragment thereof (*e.g.*, a protein encoded by KCND2). An exemplary amino acid sequence of KCND2 can be found, for example, at GenBank Accession No. NP_036413.1. Further information on KCND2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCND2. "KCND2" as used herein, also refers to variations of KCND2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCND2).

Non-limiting examples of markers related to expression of cardiac ion channels include sodium voltage-gated channel alpha subunit 5 (SCN5A). SCN5A is a gene encoding sodium voltage-gated channel alpha subunit 5 (also known as HB1; HB2; HH1; IVF; VF1; HBBD; ICCD; LQT3; SSS1; CDCD2; CMD1E; CMPD2; PFHB1; Nav1.5), which is a tetrodotoxin-resistant voltage-gated sodium channel subunit. In some embodiments, expression of SCN5A by a cell is determined by measuring a level of an SCN5A RNA (*e.g.*, mRNA transcribed from SCN5A). Exemplary nucleic acid sequences of human SCN5A can be found, for example, at GenBank Accession Nos. NG_008934.1, NM_000335.5; UniProt Accession No. Q14524; and OMIM Accession No. 600163. In some embodiments, expression of SCN5A by a cell is determined by measuring a level of a SCN5A protein or fragment thereof (*e.g.*, a protein encoded by SCN5A). An exemplary amino acid sequence of SCN5A can be found, for example, at GenBank Accession No. NP_000326.2. Further information on SCN5A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=SCN5A. "SCN5A" as used

herein, also refers to variations of SCN5A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=SCN5A).

Non-limiting examples of markers related to expression of cardiac ion channels include sodium voltage-gated channel alpha subunit 9 (SCN9A). SCN9A is a gene encoding sodium voltage-gated channel alpha subunit 9 (also known as PN1, ETHA, NENA, SFNP, FEB3B, NENNA, GEFSP7, HSN2D, Nav1.7), which is a subunit of a voltage-gated sodium channel. In some embodiments, expression of SCN9A by a cell is determined by measuring a level of an SCN9A RNA (*e.g.*, mRNA transcribed from SCN9A). Exemplary nucleic acid sequences of human SCN9A can be found, for example, at GenBank Accession Nos. NG_012798.1, NM_001365536.1; UniProt Accession No. Q15858; and OMIM Accession No. 603415. In some embodiments, expression of SCN9A by a cell is determined by measuring a level of a SCN9A protein or fragment thereof (*e.g.*, a protein encoded by SCN9A). An exemplary amino acid sequence of SCN9A can be found, for example, at GenBank Accession No. NP_001352465.1. Further information on SCN9A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=SCN9A. "SCN9A" as used herein, also refers to variations of SCN9A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=SCN9A).

Non-limiting examples of markers related to expression of cardiac ion channels include sodium voltage-gated channel alpha subunit 3 (SCN3A). SCN3A is a gene encoding sodium voltage-gated channel alpha subunit 3 (also known as NAC3; DEE62; EIEE62; FFEVF4; Nav1.3), which is a subunit of a voltage-gated sodium channel. In some embodiments, expression of SCN3A by a cell is determined by measuring a level of an SCN3A RNA (*e.g.*, mRNA transcribed from SCN3A). Exemplary nucleic acid sequences of human SCN3A can be found, for example, at GenBank Accession Nos. NG_042289.1, NM_001081676.2; UniProt Accession No. Q9NY46; and OMIM Accession No. 182391. In some embodiments, expression of SCN3A by a cell is determined by measuring a level of a SCN3A protein or fragment thereof (*e.g.*, a protein encoded by SCN3A). An exemplary amino acid sequence of SCN3A can be found, for example, at GenBank Accession No. NP_001075145.1. Further information on SCN3A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=SCN3A. "SCN3A" as used herein, also refers to variations of SCN3A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=SCN3A).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium inwardly rectifying channel subfamily J member 2 (KCNJ2). KCNJ2 is a gene encoding potassium inwardly rectifying channel subfamily J member 2 (also known as IRK1; LQT7; SQT3; ATFB9; HHIRK1; KIR2.1; HHBIRK1), which is an inward-rectifying potassium channel implicated in cardiac arrhythmia. In some embodiments, expression of KCNJ2 by a cell is determined by measuring a level of an KCNJ2 RNA (*e.g.*, mRNA transcribed from KCNJ2). Exemplary nucleic acid sequences of human KCNJ2 can be found, for example, at GenBank Accession Nos. NG_008798.1, NM_000891.3; UniProt Accession No. P63252; and OMIM Accession No. 600681. In some embodiments, expression of KCNJ2 by a cell is determined by measuring a level of a KCNJ2 protein or fragment thereof (*e.g.*, a protein encoded by KCNJ2). An exemplary amino acid sequence of KCNJ2 can be found, for example, at GenBank Accession No. NP_000882.1. Further information on KCNJ2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNJ2. "KCNJ2" as used herein, also refers to variations of KCNJ2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNJ2).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium inwardly rectifying channel subfamily J member 3 (KCNJ3). KCNJ3 is a gene encoding potassium inwardly rectifying channel subfamily J member 3 (also known as KGA; GIRK1; KIR3.1), which is an inward-rectifying potassium channel. In some embodiments, expression of KCNJ3 by a cell is determined by measuring a level of an KCNJ3 RNA (*e.g.*, mRNA transcribed from KCNJ3). Exemplary nucleic acid sequences of human KCNJ3 can be found, for example, at GenBank Accession Nos. , NM_001260508.2; UniProt Accession No. P48549; and OMIM Accession No. 601534. In some embodiments, expression of KCNJ3 by a cell is determined by measuring a level of a KCNJ3 protein or fragment thereof (*e.g.*, a protein encoded by KCNJ3). An exemplary amino acid sequence of KCNJ3 can be found, for example, at GenBank Accession No. NP_001247437.1. Further information on KCNJ3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNJ3. "KCNJ3" as used herein, also refers to variations of KCNJ3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNJ3).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium inwardly rectifying channel subfamily J member 5 (KCNJ5). KCNJ5 is a gene encoding potassium inwardly rectifying channel subfamily J member 5 (also known as CIR; GIRK4; KATP1; LQT13; KIR3.4), which is an inward-rectifier potassium channel. In some

embodiments, expression of KCNJ5 by a cell is determined by measuring a level of an KCNJ5 RNA (*e.g.*, mRNA transcribed from KCNJ5). Exemplary nucleic acid sequences of human KCNJ5 can be found, for example, at GenBank Accession Nos. NG_023406.2, NM_000890.5; UniProt Accession No. P48544; and OMIM Accession No. 600734. In some embodiments, expression of KCNJ5 by a cell is determined by measuring a level of a KCNJ5 protein or fragment thereof (*e.g.*, a protein encoded by KCNJ5). An exemplary amino acid sequence of KCNJ5 can be found, for example, at GenBank Accession No. NP_000881.3. Further information on KCNJ5 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNJ5. "KCNJ5" as used herein, also refers to variations of KCNJ5 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNJ5).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium inwardly rectifying channel subfamily J member 8 (KCNJ8). KCNJ8 is a gene encoding potassium inwardly rectifying channel subfamily J member 8 (also known as KIR6.1; uKATP-1), which is an inward-rectifier potassium channel. In some embodiments, expression of KCNJ8 by a cell is determined by measuring a level of an KCNJ8 RNA (*e.g.*, mRNA transcribed from KCNJ8). Exemplary nucleic acid sequences of human KCNJ8 can be found, for example, at GenBank Accession Nos. NG_041794.1, NM_004982.4; UniProt Accession No. Q15842; and OMIM Accession No. 600935. In some embodiments, expression of KCNJ8 by a cell is determined by measuring a level of a KCNJ8 protein or fragment thereof (*e.g.*, a protein encoded by KCNJ8). An exemplary amino acid sequence of KCNJ8 can be found, for example, at GenBank Accession No. NP_004973.1. Further information on KCNJ8 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNJ8. "KCNJ8" as used herein, also refers to variations of KCNJ8 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNJ8).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium voltage-gated channel subfamily Q member 1 (KCNQ1). KCNQ1 is a gene encoding potassium voltage-gated channel subfamily Q member 1 (also known as LQT; RWS; WRS; LQT1; SQT2; ATFB1; ATFB3; JLNS1; KCNA8; KCNA9; Kv1.9; Kv7.1; KVLQT1), which is a voltage-gated potassium channel. In some embodiments, expression of KCNQ1 by a cell is determined by measuring a level of an KCNQ1 RNA (*e.g.*, mRNA transcribed from KCNQ1). Exemplary nucleic acid sequences of human KCNQ1 can be found, for example, at GenBank Accession Nos. NG_008935.1, NM_000218.3; UniProt Accession No. P51787; and OMIM

Accession No. 604115. In some embodiments, expression of KCNQ1 by a cell is determined by measuring a level of a KCNQ1 protein or fragment thereof (*e.g.*, a protein encoded by KCNQ1). An exemplary amino acid sequence of KCNQ1 can be found, for example, at GenBank Accession No. NP_000209.2. Further information on KCNQ1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNQ1. "KCNQ1" as used herein, also refers to variations of KCNQ1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNQ1).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium voltage-gated channel subfamily Q member 3 (KCNQ3). KCNQ3 is a gene encoding potassium voltage-gated channel subfamily Q member 3 (also known as EBN2; BFNC2; KV7.3), which is part of an M-channel associated with KCNQ2 or KCNQ5. In some embodiments, expression of KCNQ3 by a cell is determined by measuring a level of an KCNQ3 RNA (*e.g.*, mRNA transcribed from KCNQ3). Exemplary nucleic acid sequences of human KCNQ3 can be found, for example, at GenBank Accession Nos. NG_008854.2, NM_001204824.2; UniProt Accession No. Q8K3F6; and OMIM Accession No. 602232. In some embodiments, expression of KCNQ3 by a cell is determined by measuring a level of a KCNQ3 protein or fragment thereof (*e.g.*, a protein encoded by KCNQ3). An exemplary amino acid sequence of KCNQ3 can be found, for example, at GenBank Accession No. NP_001191753.1. Further information on KCNQ3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNQ3. "KCNQ3" as used herein, also refers to variations of KCNQ3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNQ3).

Non-limiting examples of markers related to expression of cardiac ion channels include potassium voltage-gated channel subfamily Q member 5 (KCNQ5). KCNQ5 is a gene encoding potassium voltage-gated channel subfamily Q member 5 (also known as Kv7.5; MRD46), which is a voltage-gated potassium channel. In some embodiments, expression of KCNQ5 by a cell is determined by measuring a level of an KCNQ5 RNA (*e.g.*, mRNA transcribed from KCNQ5). Exemplary nucleic acid sequences of human KCNQ5 can be found, for example, at GenBank Accession Nos. NG_047170.1, NM_001160130.2; UniProt Accession No. Q9NR82; and OMIM Accession No. 607357. In some embodiments, expression of KCNQ5 by a cell is determined by measuring a level of a KCNQ5 protein or fragment thereof (*e.g.*, a protein encoded by KCNQ5). An exemplary amino acid sequence of KCNQ5 can be found, for example, at GenBank Accession No. NP_001153602.1. Further information on KCNQ5 can be found, for example, on

the world wide web at ncbi.nlm.nih.gov/gene/?term=KCNQ5. "KCNQ5" as used herein, also refers to variations of KCNQ5 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KCNQ5).

In some embodiments a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected from calcium voltage-gated channel subunit alpha 1 c (CACNA1C); calcium voltage-gated channel subunit alpha 1 h (CACNA1H); potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4); potassium voltage-gated channel subfamily D member 3 (KCND3); potassium voltage-gated channel subfamily D member 2 (KCND2); sodium voltage-gated channel alpha subunit 5 (SCN5A); sodium voltage-gated channel alpha subunit 9 (SCN9A); sodium voltage-gated channel alpha subunit 3 (SCN3A); potassium inwardly rectifying channel subfamily J member 2 (KCNJ2); potassium inwardly rectifying channel subfamily J member 3 (KCNJ3); potassium inwardly rectifying channel subfamily J member 5 (KCNJ5); potassium inwardly rectifying channel subfamily J member 8 (KCNJ8); potassium voltage-gated channel subfamily Q member 1 (KCNQ1); potassium voltage-gated channel subfamily Q member 3 (KCNQ3); potassium voltage-gated channel subfamily Q member 5 (KCNQ5); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, or all 15).

Markers Related to Expression of Calcium Handling Molecules

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more markers related to expression (*e.g.*, transcript, protein) of calcium handling molecules (*e.g.*, calcium release/storage proteins, calmodulin-associated proteins, T-tubule function).

Non-limiting examples of markers related to expression of calcium handling molecules include sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (ATP2A2). ATP2A2 is a gene encoding ATPase sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (also known as DD; DAR; ATP2B; SERCA2), which is an intracellular calcium pump involved in catalysis of ATP. In some embodiments, expression of ATP2A2 by a cell is determined by measuring a level of an ATP2A2 RNA (*e.g.*, mRNA transcribed from ATP2A2). Exemplary nucleic acid sequences of human ATP2A2 can be found, for example, at GenBank Accession Nos. NG_007097.2, NM_001413013.1; UniProt Accession No. P16615; and OMIM Accession No. 108740. In some embodiments, expression of ATP2A2 by a cell is determined by measuring a level of a ATP2A2

protein or fragment thereof (*e.g.*, a protein encoded by ATP2A2). An exemplary amino acid sequence of ATP2A2 can be found, for example, at GenBank Accession No. NP_001399942.1. Further information on ATP2A2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ATP2A2. "ATP2A2" as used herein, also refers to variations of ATP2A2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ATP2A2).

Non-limiting examples of markers related to expression of calcium handling molecules include ryanodine receptor 2 (RYR2). RYR2 is a gene encoding ryanodine receptor 2 (also known as RyR; ARVC2; ARVD2; RYR-2; VTSIP; VACRDS), which is a ryanodine receptor and a component of a calcium channel. In some embodiments, expression of RYR2 by a cell is determined by measuring a level of an RYR2 RNA (*e.g.*, mRNA transcribed from RYR2). Exemplary nucleic acid sequences of human RYR2 can be found, for example, at GenBank Accession Nos. NG_008799.3, NM_001035.3; UniProt Accession No. Q92736; and OMIM Accession No. 180902. In some embodiments, expression of RYR2 by a cell is determined by measuring a level of a RYR2 protein or fragment thereof (*e.g.*, a protein encoded by RYR2). An exemplary amino acid sequence of RYR2 can be found, for example, at GenBank Accession No. NP_001026.2. Further information on RYR2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=RYR2. "RYR2" as used herein, also refers to variations of RYR2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=RYR2).

Non-limiting examples of markers related to expression of calcium handling molecules include solute carrier family 8 member A1 (SLC8A1). SLC8A1 is a gene encoding solute carrier family 8 member A1 (also known as NCX1), which is a sodium/calcium exchanger. In some embodiments, expression of SLC8A1 by a cell is determined by measuring a level of an SLC8A1 RNA (*e.g.*, mRNA transcribed from SLC8A1). Exemplary nucleic acid sequences of human SLC8A1 can be found, for example, at GenBank Accession No. NM_001112800.4; UniProt Accession No. P32418; and OMIM Accession No. 182305. In some embodiments, expression of SLC8A1 by a cell is determined by measuring a level of a SLC8A1 protein or fragment thereof (*e.g.*, a protein encoded by SLC8A1). An exemplary amino acid sequence of SLC8A1 can be found, for example, at GenBank Accession No. NP_001106271.1. Further information on SLC8A1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=SLC8A1. "SLC8A1" as used herein, also refers to variations of

SLC8A1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=SLC8A1).

Non-limiting examples of markers related to expression of calcium handling molecules include protein kinase A catalytic subunit α (PRKACA). PRKACA is a gene encoding protein kinase A catalytic subunit α (also known protein kinase cAMP-activated catalytic subunit alpha; CAFD1; PKACA; PPNAD4), which is a catalytic subunit of protein kinase A. In some embodiments, expression of PRKACA by a cell is determined by measuring a level of an PRKACA RNA (*e.g.*, mRNA transcribed from PRKACA). Exemplary nucleic acid sequences of human PRKACA can be found, for example, at GenBank Accession Nos. NG_029699.1, NM_001304349.2; UniProt Accession No. P17612; and OMIM Accession No. 601639. In some embodiments, expression of PRKACA by a cell is determined by measuring a level of a PRKACA protein or fragment thereof (*e.g.*, a protein encoded by PRKACA). An exemplary amino acid sequence of PRKACA can be found, for example, at GenBank Accession No. NP_001291278.1. Further information on PRKACA can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PRKACA. "PRKACA" as used herein, also refers to variations of PRKACA including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PRKACA).

Non-limiting examples of markers related to expression of calcium handling molecules include calmodulin-dependent protein kinase type II subunit alpha (CAMK2a). CAMK2a is a gene encoding calmodulin dependent protein kinase type II subunit alpha (also known as calcium/calmodulin dependent protein kinase II alpha; CAMKA; MRD53; MRT63; CaMKIIalpha; CaMKIINalpha), which is a component of calcium calmodulin dependent protein kinase. In some embodiments, expression of CAMK2a by a cell is determined by measuring a level of an CAMK2a RNA (*e.g.*, mRNA transcribed from CAMK2a). Exemplary nucleic acid sequences of human CAMK2a can be found, for example, at GenBank Accession Nos. NG_047040.1, NM_001363989.1; UniProt Accession No. Q9UQM7; and OMIM Accession No. 114078. In some embodiments, expression of CAMK2a by a cell is determined by measuring a level of a CAMK2a protein or fragment thereof (*e.g.*, a protein encoded by CAMK2a). An exemplary amino acid sequence of CAMK2a can be found, for example, at GenBank Accession No. NP_001350918.1. Further information on CAMK2a can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CAMK2a. "CAMK2a" as used herein, also refers to variations of CAMK2a including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CAMK2a).

Non-limiting examples of markers related to expression of calcium handling molecules include calmodulin-dependent protein kinase type II subunit beta (CAMK2b). CAMK2B is a gene encoding calmodulin-dependent protein kinase type II subunit beta (also known as calcium/calmodulin dependent protein kinase II beta; CAM2; CAMK2; CAMKB; MRD54; CaMKIIbeta), which is a component of calcium calmodulin dependent protein kinase. In some embodiments, expression of CAMK2B by a cell is determined by measuring a level of an CAMK2B RNA (*e.g.*, mRNA transcribed from CAMK2B). Exemplary nucleic acid sequences of human CAMK2B can be found, for example, at GenBank Accession Nos. NG_029407.1, NM_001220.5; UniProt Accession No. Q13554; and OMIM Accession No. 607707. In some embodiments, expression of CAMK2B by a cell is determined by measuring a level of a CAMK2B protein or fragment thereof (*e.g.*, a protein encoded by CAMK2B). An exemplary amino acid sequence of CAMK2B can be found, for example, at GenBank Accession No. NP_001211.3. Further information on CAMK2B can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CAMK2B. "CAMK2B" as used herein, also refers to variations of CAMK2B including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CAMK2B).

Non-limiting examples of markers related to expression of calcium handling molecules include calmodulin-dependent protein kinase type II inhibitor 1 (CAMK2N1). CAMK2N1 is a gene encoding calmodulin-dependent protein kinase type II inhibitor 1 (also known as calcium/calmodulin dependent protein kinase II inhibitor 1; PRO1489), which is involved in calcium-dependent protein kinase inhibitor activity and protein kinase binding activity. In some embodiments, expression of CAMK2N1 by a cell is determined by measuring a level of an CAMK2N1 RNA (*e.g.*, mRNA transcribed from CAMK2N1). Exemplary nucleic acid sequences of human CAMK2N1 can be found, for example, at GenBank Accession Nos. , NM_018584.6; UniProt Accession No. Q7Z7J9; and OMIM Accession No. 614986. In some embodiments, expression of CAMK2N1 by a cell is determined by measuring a level of a CAMK2N1 protein or fragment thereof (*e.g.*, a protein encoded by CAMK2N1). An exemplary amino acid sequence of CAMK2N1 can be found, for example, at GenBank Accession No. NP_061054.2. Further information on CAMK2N1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CAMK2N1. "CAMK2N1" as used herein, also refers to variations of CAMK2N1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CAMK2N1).

Non-limiting examples of markers related to expression of calcium handling molecules include calmodulin-dependent protein kinase type II inhibitor 2 (CAMK2N2). CAMK2N2 is a gene encoding calmodulin-dependent protein kinase type II inhibitor 2 (also known as calcium/calmodulin dependent protein kinase II inhibitor 2; CAMKIIN; CAM-KIIN), which is involved in calcium-dependent protein kinase inhibitor activity and protein kinase binding activity. In some embodiments, expression of CAMK2N2 by a cell is determined by measuring a level of an CAMK2N2 RNA (*e.g.*, mRNA transcribed from CAMK2N2). Exemplary nucleic acid sequences of human CAMK2N2 can be found, for example, at GenBank Accession No. NM_033259.3; UniProt Accession No. Q96S95; and OMIM Accession No. 608721. In some embodiments, expression of CAMK2N2 by a cell is determined by measuring a level of a CAMK2N2 protein or fragment thereof (*e.g.*, a protein encoded by CAMK2N2). An exemplary amino acid sequence of CAMK2N2 can be found, for example, at GenBank Accession No. NP_150284.1. Further information on CAMK2N2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CAMK2N2. "CAMK2N2" as used herein, also refers to variations of CAMK2N2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CAMK2N2).

Non-limiting examples of markers related to expression of calcium handling molecules include myc box-dependent-interacting protein 1 (BIN1). BIN1 is a gene encoding myc box-dependent-interacting protein 1 (also known as CNM2; AMPH2; AMPHL; SH3P9), which is a nucleocytoplasmic adaptor protein and caspase-independent apoptosis. In some embodiments, expression of BIN1 by a cell is determined by measuring a level of an BIN1 RNA (*e.g.*, mRNA transcribed from BIN1). Exemplary nucleic acid sequences of human BIN1 can be found, for example, at GenBank Accession Nos. NG_012042.1, NM_001320632.2; UniProt Accession No. O00499; and OMIM Accession No. 601248. In some embodiments, expression of BIN1 by a cell is determined by measuring a level of a BIN1 protein or fragment thereof (*e.g.*, a protein encoded by BIN1). An exemplary amino acid sequence of BIN1 can be found, for example, at GenBank Accession No. NP_001307561.1. Further information on BIN1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=BIN1. "BIN1" as used herein, also refers to variations of BIN1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=BIN1).

Non-limiting examples of markers related to expression of calcium handling molecules include junctophilin 2 (JPH2). JPH2 is a gene encoding junctophilin 2 (also known as JP2; JP-2; CMD2E; CMH17), which is a component of junctional complexes. In some embodiments,

expression of JPH2 by a cell is determined by measuring a level of an JPH2 RNA (*e.g.*, mRNA transcribed from JPH2). Exemplary nucleic acid sequences of human JPH2 can be found, for example, at GenBank Accession Nos. NG_031867.1, NM_020433.5; UniProt Accession No.; and OMIM Accession No. 605267. In some embodiments, expression of JPH2 by a cell is determined by measuring a level of a JPH2 protein or fragment thereof (*e.g.*, a protein encoded by JPH2). An exemplary amino acid sequence of JPH2 can be found, for example, at GenBank Accession No. NP_065166.2. Further information on JPH2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=JPH2. "JPH2" as used herein, also refers to variations of JPH2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=JPH2).

Non-limiting examples of markers related to expression of calcium handling molecules include nexilin F-actin binding protein (NEXN). NEXN is a gene encoding nexilin F-actin binding protein (also known as CMH20; NELIN), which is a filamentous actin-binding protein. In some embodiments, expression of NEXN by a cell is determined by measuring a level of an NEXN RNA (*e.g.*, mRNA transcribed from NEXN). Exemplary nucleic acid sequences of human NEXN can be found, for example, at GenBank Accession Nos. NG_016625.1, NM_001172309.2; UniProt Accession No. Q0ZGT2; and OMIM Accession No. 613121. In some embodiments, expression of NEXN by a cell is determined by measuring a level of a NEXN protein or fragment thereof (*e.g.*, a protein encoded by NEXN). An exemplary amino acid sequence of NEXN can be found, for example, at GenBank Accession No. NP_001165780.1. Further information on NEXN can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=NEXN. "NEXN" as used herein, also refers to variations of NEXN including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=NEXN).

In some embodiments, a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected from sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (ATP2A2); ryanodine receptor 2 (RYR2); solute carrier family 8 member A1 (SLC8A1); protein kinase A catalytic subunit α (PRKACA); calmodulin-dependent protein kinase type II subunit alpha (CAMK2a); calmodulin-dependent protein kinase type II subunit beta (CAMK2b); calmodulin-dependent protein kinase type II inhibitor 1 (CAMK2N1); calmodulin-dependent protein kinase type II inhibitor 2 (CAMK2N2); myc box-dependent-interacting protein 1 (BIN1); junctophilin 2 (JPH2); nexilin F-actin binding protein (NEXN); and any combination thereof (*e.g.*, any

combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, or all 11).

Markers Related to Expression of Metabolism-Related Molecules

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more markers related to expression (*e.g.*, transcript, protein) of metabolism-related molecules (*e.g.*, signaling, mitochondrial metabolism, fatty acid oxidation).

Non-limiting examples of markers related to metabolism-related molecules include peroxisome proliferator-activated receptor alpha (PPARA). PPARA is a gene encoding peroxisome proliferator activated receptor alpha (also known as PPAR; NR1C1; hPPAR; PPARalpha; PPAR-alpha), which is involved in modulating size and number of peroxisomes. In some embodiments, expression of PPARA by a cell is determined by measuring a level of an PPARA RNA (*e.g.*, mRNA transcribed from PPARA). Exemplary nucleic acid sequences of human PPARA can be found, for example, at GenBank Accession Nos. NG_012204.2, NM_001001928.4; UniProt Accession No. Q07869; and OMIM Accession No. 170998. In some embodiments, expression of PPARA by a cell is determined by measuring a level of a PPARA protein or fragment thereof (*e.g.*, a protein encoded by PPARA). An exemplary amino acid sequence of PPARA can be found, for example, at GenBank Accession No. NP_001001928.1. Further information on PPARA can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PPARA. "PPARA" as used herein, also refers to variations of PPARA including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PPARA).

Non-limiting examples of markers related to metabolism-related molecules include peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A). PPARGC1A is a gene encoding peroxisome proliferator-activated receptor gamma coactivator 1-alpha (also known as LEM6; PGC1; PGC1A; PGC-1v; PPARGC1; PGC-1alpha; PGC-1(alpha)), which is a transcriptional coactivator of PPARG. In some embodiments, expression of PPARGC1A by a cell is determined by measuring a level of an PPARGC1A RNA (*e.g.*, mRNA transcribed from PPARGC1A). Exemplary nucleic acid sequences of human PPARGC1A can be found, for example, at GenBank Accession Nos. NG_028250.2, NM_001330751.2; UniProt Accession No. Q9UBK2; and OMIM Accession No. 604517. In some embodiments, expression of PPARGC1A by a cell is determined by measuring a level of a PPARGC1A protein or fragment thereof (*e.g.*, a protein encoded by PPARGC1A). An exemplary amino acid sequence of PPARGC1A can be found, for example, at GenBank Accession No. NP_001317680.1.

Further information on PPARGC1A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PPARGC1A. "PPARGC1A" as used herein, also refers to variations of PPARGC1A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PPARGC1A).

Non-limiting examples of markers related to metabolism-related molecules include retinoid X receptor alpha (RXRA). RXRA is a gene encoding retinoid X receptor alpha (also known as NR2B1; RXRalpha; RXR-alpha), which mediates the biological effects of retinoids. In some embodiments, expression of RXRA by a cell is determined by measuring a level of an RXRA RNA (*e.g.*, mRNA transcribed from RXRA). Exemplary nucleic acid sequences of human RXRA can be found, for example, at GenBank Accession Nos. , NM_001291920.2; UniProt Accession No. P19793; and OMIM Accession No. 180245. In some embodiments, expression of RXRA by a cell is determined by measuring a level of a RXRA protein or fragment thereof (*e.g.*, a protein encoded by RXRA). An exemplary amino acid sequence of RXRA can be found, for example, at GenBank Accession No. NP_001278849.1. Further information on RXRA can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=RXRA. "RXRA" as used herein, also refers to variations of RXRA including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=RXRA).

Non-limiting examples of markers related to metabolism-related molecules include acyl-coenzyme A oxidase 1 (ACOX1). ACOX1 is a gene encoding acyl-coenzyme A oxidase 1 (also known as AOX; ACOX; SCOX; MITCH; PALMCOX), which is an enzyme of the fatty acid beta-oxidation pathway. In some embodiments, expression of ACOX1 by a cell is determined by measuring a level of an ACOX1 RNA (*e.g.*, mRNA transcribed from ACOX1). Exemplary nucleic acid sequences of human ACOX1 can be found, for example, at GenBank Accession Nos. NG_008190.1, NM_001185039.2 ; UniProt Accession No. Q15067; and OMIM Accession No. 609751. In some embodiments, expression of ACOX1 by a cell is determined by measuring a level of a ACOX1 protein or fragment thereof (*e.g.*, a protein encoded by ACOX1). An exemplary amino acid sequence of ACOX1 can be found, for example, at GenBank Accession No. NP_001171968.1. Further information on ACOX1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ACOX1. "ACOX1" as used herein, also refers to variations of ACOX1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ACOX1).

Non-limiting examples of markers related to metabolism-related molecules include cytochrome P450 oxidoreductase (POR). POR is a gene encoding cytochrome P450 oxidoreductase (also known as CPR; CYPOR; P450R), which is an endoplasmic reticulum membrane oxidoreductase involved in metabolism of hormones, drugs, and xenobiotics. In some embodiments, expression of POR by a cell is determined by measuring a level of an POR RNA (*e.g.*, mRNA transcribed from POR). Exemplary nucleic acid sequences of human POR can be found, for example, at GenBank Accession Nos. NG_008930.1, NM_001367562.3 ; UniProt Accession No. P16435; and OMIM Accession No. 124015. In some embodiments, expression of POR by a cell is determined by measuring a level of a POR protein or fragment thereof (*e.g.*, a protein encoded by POR). An exemplary amino acid sequence of POR can be found, for example, at GenBank Accession No. NP_001354491.2. Further information on POR can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=POR. "POR" as used herein, also refers to variations of POR including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=POR).

Non-limiting examples of markers related to metabolism-related molecules include cytochrome C oxidase subunit 5a (COX5A). COX5A is a gene encoding cytochrome C oxidase subunit 5a (also known as VA; COX; COX-VA; MC4DN20), which is a subunit of COX, the terminal enzyme of the mitochondrial respiratory chain. In some embodiments, expression of COX5A by a cell is determined by measuring a level of an COX5A RNA (*e.g.*, mRNA transcribed from COX5A). Exemplary nucleic acid sequences of human COX5A can be found, for example, at GenBank Accession Nos. , NM_004255.4; UniProt Accession No. P20674; and OMIM Accession No. 603773. In some embodiments, expression of COX5A by a cell is determined by measuring a level of a COX5A protein or fragment thereof (*e.g.*, a protein encoded by COX5A). An exemplary amino acid sequence of COX5A can be found, for example, at GenBank Accession No. NP_004246.2. Further information on COX5A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=COX5A. "COX5A" as used herein, also refers to variations of COX5A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=COX5A).

Non-limiting examples of markers related to metabolism-related molecules include cytochrome C oxidase subunit 6a (COX6A2). COX6A2 is a gene encoding cytochrome C oxidase subunit 6A (also known as cytochrome C oxidase subunit 6A2; COX6AH; COXVIAH; MC4DN18; COXVIa-M), which is a subunit of COX, the terminal enzyme of the mitochondrial respiratory chain. In some embodiments, expression of COX6A2 by a cell is determined by

measuring a level of an COX6A2 RNA (*e.g.*, mRNA transcribed from COX6A2). Exemplary nucleic acid sequences of human COX6A2 can be found, for example, at GenBank Accession Nos. , NM_005205.4; UniProt Accession No. Q02221; and OMIM Accession No. 602009. In some embodiments, expression of COX6A2 by a cell is determined by measuring a level of a COX6A2 protein or fragment thereof (*e.g.*, a protein encoded by COX6A2). An exemplary amino acid sequence of COX6A2 can be found, for example, at GenBank Accession No. NP_005196.1. Further information on COX6A2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=COX6A2. "COX6A2" as used herein, also refers to variations of COX6A2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=COX6A2).

Non-limiting examples of markers related to metabolism-related molecules include OPA1 mitochondrial dynamin like GTPase (OPA1). OPA1 is a gene encoding OPA1 mitochondrial dynamin like GTPase (also known as Dynamin-like GTPase OPA1, mitochondrial; NPG; NTG; MGM1; BERHS; largeG; MTDPS14), which mediates fusion of mitochondrial inner membranes, regulating cristae morphology and maintaining respiratory chain function. In some embodiments, expression of OPA1 by a cell is determined by measuring a level of an OPA1 RNA (*e.g.*, mRNA transcribed from OPA1). Exemplary nucleic acid sequences of human OPA1 can be found, for example, at GenBank Accession Nos. NG_011605.1 , NM_001354663.2; UniProt Accession No. O60313; and OMIM Accession No. 605290. In some embodiments, expression of OPA1 by a cell is determined by measuring a level of a OPA1 protein or fragment thereof (*e.g.*, a protein encoded by OPA1). An exemplary amino acid sequence of OPA1 can be found, for example, at GenBank Accession No. NP_001341592.1. Further information on OPA1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=OPA1. "OPA1" as used herein, also refers to variations of OPA1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=OPA1).

Non-limiting examples of markers related to metabolism-related molecules include mitofusin-1 (MFN1). MFN1 is a gene encoding mitofusin-1 (also known as hfo1; hfo2), which is a mitochondrial outer membrane GTPase that mediates mitochondrial clustering and fusion. In some embodiments, expression of MFN1 by a cell is determined by measuring a level of an MFN1 RNA (*e.g.*, mRNA transcribed from MFN1). Exemplary nucleic acid sequences of human MFN1 can be found, for example, at GenBank Accession Nos. , NM_033540.3 ; UniProt Accession No. Q8IWA4; and OMIM Accession No. 608506. In some embodiments, expression

of MFN1 by a cell is determined by measuring a level of a MFN1 protein or fragment thereof (*e.g.*, a protein encoded by MFN1). An exemplary amino acid sequence of MFN1 can be found, for example, at GenBank Accession No. NP_284941.2. Further information on MFN1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MFN1. "MFN1" as used herein, also refers to variations of MFN1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MFN1).

Non-limiting examples of markers related to metabolism-related molecules include mitofusin-2 (MFN2). MFN2 is a gene encoding mitofusin-2 (also known as HSG; MSL; MARF; CMT2A; CPRP1; CMT2A2; HMSN6A; CMT2A2A; CMT2A2B), which is a mitochondrial outer membrane GTPase that mediates mitochondrial clustering and fusion. In some embodiments, expression of MFN2 by a cell is determined by measuring a level of an MFN2 RNA (*e.g.*, mRNA transcribed from MFN2). Exemplary nucleic acid sequences of human MFN2 can be found, for example, at GenBank Accession Nos. NG_007945.1, NM_001127660.2; UniProt Accession No. O95140; and OMIM Accession No. 608507. In some embodiments, expression of MFN2 by a cell is determined by measuring a level of a MFN2 protein or fragment thereof (*e.g.*, a protein encoded by MFN2). An exemplary amino acid sequence of MFN2 can be found, for example, at GenBank Accession No. NP_001121132.1. Further information on MFN2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=MFN2. "MFN2" as used herein, also refers to variations of MFN2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=MFN2).

Non-limiting examples of markers related to metabolism-related molecules include carnitine palmitoyltransferase I (CPT1A). CPT1A is a gene encoding carnitine palmitoyltransferase I (also known as carnitine palmitoyltransferase 1A; CPT1; CPT1-L; L-CPT1), which is involved in mitochondrial oxidation of long-chain fatty acids. In some embodiments, expression of CPT1A by a cell is determined by measuring a level of an CPT1A RNA (*e.g.*, mRNA transcribed from CPT1A). Exemplary nucleic acid sequences of human CPT1A can be found, for example, at GenBank Accession Nos. NG_011801.2, NM_001031847.3; UniProt Accession No. P50416; and OMIM Accession No. 600528. In some embodiments, expression of CPT1A by a cell is determined by measuring a level of a CPT1A protein or fragment thereof (*e.g.*, a protein encoded by CPT1A). An exemplary amino acid sequence of CPT1A can be found, for example, at GenBank Accession No. NP_001027017.1. Further information on CPT1A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CPT1A. "CPT1A" as used herein, also refers to variations of

CPT1A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CPT1A).

Non-limiting examples of markers related to metabolism-related molecules include butyrylcholinesterase (BCHE). BCHE is a gene encoding butyrylcholinesterase (also known as E1; CHE1; CHE2; BCHED), which is an esterase that contributes to detoxification and drug metabolism. In some embodiments, expression of BCHE by a cell is determined by measuring a level of an BCHE RNA (*e.g.*, mRNA transcribed from BCHE). Exemplary nucleic acid sequences of human BCHE can be found, for example, at GenBank Accession Nos. NG_009031.1, NM_000055.4 ; UniProt Accession No. P06276; and OMIM Accession No. 177400. In some embodiments, expression of BCHE by a cell is determined by measuring a level of a BCHE protein or fragment thereof (*e.g.*, a protein encoded by BCHE). An exemplary amino acid sequence of BCHE can be found, for example, at GenBank Accession No. NP_000046.1. Further information on BCHE can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=BCHE. "BCHE" as used herein, also refers to variations of BCHE including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=BCHE).

Non-limiting examples of markers related to metabolism-related molecules include copine 5 (CPNE5). CPNE5 is a gene encoding copine 5 (also known as CPN5; COPN5), which calcium-dependent phospholipid-binding protein that may play a role in calcium-mediated intracellular processes. In some embodiments, expression of CPNE5 by a cell is determined by measuring a level of an CPNE5 RNA (*e.g.*, mRNA transcribed from CPNE5). Exemplary nucleic acid sequences of human CPNE5 can be found, for example, at GenBank Accession Nos. , NM_001314017.1; UniProt Accession No. Q9HCH3; and OMIM Accession No. 604209. In some embodiments, expression of CPNE5 by a cell is determined by measuring a level of a CPNE5 protein or fragment thereof (*e.g.*, a protein encoded by CPNE5). An exemplary amino acid sequence of CPNE5 can be found, for example, at GenBank Accession No. NP_001300946.1. Further information on CPNE5 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CPNE5. "CPNE5" as used herein, also refers to variations of CPNE5 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CPNE5).

In some embodiments, a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected from peroxisome proliferator-activated receptor alpha (PPARA); peroxisome

proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A); retinoid X receptors (RXRA); acyl-coenzyme A oxidase 1 (ACOX1); cytochrome P450 oxidoreductase (POR); cytochrome C oxidase subunit 5a (COX5A2); cytochrome C oxidase subunit 6a (COX6A2); OPA1 mitochondrial dynamin like GTPase (OPA1); mitofusin-1 (MFN1); mitofusin-2 (MFN2); carnitine palmitoyltransferase I (CPT1A); butyrylcholinesterase (BCHE); copine 5 (CPNE5); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, or all 13).

Markers Related to Expression of Ultrastructure Related Molecules

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more markers related to expression (*e.g.*, transcript, protein) of ultrastructure (*e.g.*, desmosomes, integrins, costamere, gap junctions) related molecules.

Non-limiting examples of markers related to expression of ultrastructure related molecules include plakophilin-2 (PKP2). PKP2 is a gene encoding plakophilin-2 (also known as ARVD9), which is involved in linking cadherins to intermediate filaments in the cytoskeleton. In some embodiments, expression of PKP2 by a cell is determined by measuring a level of an PKP2 RNA (*e.g.*, mRNA transcribed from PKP2). Exemplary nucleic acid sequences of human PKP2 can be found, for example, at GenBank Accession Nos. NG_009000.1, NM_001005242.3; UniProt Accession No. Q99959; and OMIM Accession No. 602861. In some embodiments, expression of PKP2 by a cell is determined by measuring a level of a PKP2 protein or fragment thereof (*e.g.*, a protein encoded by PKP2). An exemplary amino acid sequence of PKP2 can be found, for example, at GenBank Accession No. NP_001005242.2. Further information on PKP2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PKP2. "PKP2" as used herein, also refers to variations of PKP2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PKP2).

Non-limiting examples of markers related to expression of ultrastructure related molecules include desmoplakin (DSP). DSP is a gene encoding desmoplakin (also known as DP; DCWHKTA), which is involved in regulation of profibrotic gene expression in cardiomyocytes. In some embodiments, expression of DSP by a cell is determined by measuring a level of an DSP RNA (*e.g.*, mRNA transcribed from DSP). Exemplary nucleic acid sequences of human DSP can be found, for example, at GenBank Accession Nos. NG_008803.1, NM_001008844.3; UniProt Accession No. P15924; and OMIM Accession No. 125647. In some embodiments, expression of DSP by a cell is determined by measuring a level of a DSP protein or fragment thereof (*e.g.*, a protein encoded by DSP). An exemplary amino acid sequence of DSP can be

found, for example, at GenBank Accession No. NP_001008844.1. Further information on DSP can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=DSP. "DSP" as used herein, also refers to variations of DSP including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=DSP).

Non-limiting examples of markers related to expression of ultrastructure related molecules include junction plakoglobin (JUP). JUP is a gene encoding junction plakoglobin (also known as PG; DP3; PDGB; PKGB; CTNNG; DPMI), which is a junctional plaque protein that influences the structure and function of submembranous plaques. In some embodiments, expression of JUP by a cell is determined by measuring a level of an JUP RNA (*e.g.*, mRNA transcribed from JUP). Exemplary nucleic acid sequences of human JUP can be found, for example, at GenBank Accession Nos. NG_009090.2, NM_001352773.2; UniProt Accession No. P14923; and OMIM Accession No. 173325. In some embodiments, expression of JUP by a cell is determined by measuring a level of a JUP protein or fragment thereof (*e.g.*, a protein encoded by JUP). An exemplary amino acid sequence of JUP can be found, for example, at GenBank Accession No. NP_001339702.1. Further information on JUP can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=JUP. "JUP" as used herein, also refers to variations of JUP including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=JUP).

Non-limiting examples of markers related to expression of ultrastructure related molecules include desmoglein-2 (DSG2). DSG2 is a gene encoding desmoglein-2 (also known as HDGC; CDHF5), which is involved in the interaction of plaque proteins and intermediate filaments mediating cell-cell adhesion. In some embodiments, expression of DSG2 by a cell is determined by measuring a level of an DSG2 RNA (*e.g.*, mRNA transcribed from DSG2). Exemplary nucleic acid sequences of human DSG2 can be found, for example, at GenBank Accession Nos. NG_007072.3, NM_001943.5; UniProt Accession No. Q14126; and OMIM Accession No. 125671. In some embodiments, expression of DSG2 by a cell is determined by measuring a level of a DSG2 protein or fragment thereof (*e.g.*, a protein encoded by DSG2). An exemplary amino acid sequence of DSG2 can be found, for example, at GenBank Accession No. NP_001934.2. Further information on DSG2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=DSG2. "DSG2" as used herein, also refers to variations of DSG2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=DSG2).

Non-limiting examples of markers related to expression of ultrastructure related molecules include desmocollin-2 (DSC2). DSC2 is a gene encoding desmocollin-2 (also known as DG2; DSC3; CDHF2; ARVD11; DGII/III), which is involved in the interaction of plaque proteins and intermediate filaments mediating cell-cell adhesion. In some embodiments, expression of DSC2 by a cell is determined by measuring a level of an DSC2 RNA (*e.g.*, mRNA transcribed from DSC2). Exemplary nucleic acid sequences of human DSC2 can be found, for example, at GenBank Accession Nos. NG_008208.2, NM_001406506.1; UniProt Accession No. Q02487; and OMIM Accession No. 125645. In some embodiments, expression of DSC2 by a cell is determined by measuring a level of a DSC2 protein or fragment thereof (*e.g.*, a protein encoded by DSC2). An exemplary amino acid sequence of DSC2 can be found, for example, at GenBank Accession No. NP_001393435.1. Further information on DSC2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=DSC2. "DSC2" as used herein, also refers to variations of DSC2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=DSC2).

Non-limiting examples of markers related to expression of ultrastructure related molecules include integrin beta-1 (ITGB1). ITGB1 is a gene encoding integrin beta-1 (also known as integrin subunit beta-1; CD29; FN1B; MDFA; VLAB; GPIIA; MSK12; VLA-BETA), which is a subunit of integrins, and is involved in cell adhesion and recognition. In some embodiments, expression of ITGB1 by a cell is determined by measuring a level of an ITGB1 RNA (*e.g.*, mRNA transcribed from ITGB1). Exemplary nucleic acid sequences of human ITGB1 can be found, for example, at GenBank Accession Nos. NG_029012.1, NM_002211.4; UniProt Accession No. P05556; and OMIM Accession No. 135630. In some embodiments, expression of ITGB1 by a cell is determined by measuring a level of a ITGB1 protein or fragment thereof (*e.g.*, a protein encoded by ITGB1). An exemplary amino acid sequence of ITGB1 can be found, for example, at GenBank Accession No. NP_002202.2. Further information on ITGB1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITGB1. "ITGB1" as used herein, also refers to variations of ITGB1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITGB1).

Non-limiting examples of markers related to expression of ultrastructure related molecules include integrin alpha chain V (ITGAV). ITGAV is a gene encoding integrin alpha chain V (also known as integrin subunit alpha V; CD51; MSK8; VNRA; VTNR), which is a subunit of integrins, and is involved in cell adhesion and recognition. In some embodiments,

expression of ITGAV by a cell is determined by measuring a level of an ITGAV RNA (*e.g.*, mRNA transcribed from ITGAV). Exemplary nucleic acid sequences of human ITGAV can be found, for example, at GenBank Accession No. NM_001144999.3; UniProt Accession No. P06756; and OMIM Accession No. 193210. In some embodiments, expression of ITGAV by a cell is determined by measuring a level of a ITGAV protein or fragment thereof (*e.g.*, a protein encoded by ITGAV). An exemplary amino acid sequence of ITGAV can be found, for example, at GenBank Accession No. NP_001138471.2. Further information on ITGAV can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITGAV. "ITGAV" as used herein, also refers to variations of ITGAV including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITGAV).

Non-limiting examples of markers related to expression of ultrastructure related molecules include integrin alpha 7 (ITGA7). ITGA7 is a gene encoding integrin alpha 7 (also known as integrin subunit alpha 7), which is the primary laminin receptor on skeletal myoblasts and adult myofibers. In some embodiments, expression of ITGA7 by a cell is determined by measuring a level of an ITGA7 RNA (*e.g.*, mRNA transcribed from ITGA7). Exemplary nucleic acid sequences of human ITGA7 can be found, for example, at GenBank Accession Nos. NG_012343.1, NM_001144996.2; UniProt Accession No. Q13683; and OMIM Accession No. 600536. In some embodiments, expression of ITGA7 by a cell is determined by measuring a level of a ITGA7 protein or fragment thereof (*e.g.*, a protein encoded by ITGA7). An exemplary amino acid sequence of ITGA7 can be found, for example, at GenBank Accession No. NP_001138468.1. Further information on ITGA7 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITGA7. "ITGA7" as used herein, also refers to variations of ITGA7 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITGA7).

Non-limiting examples of markers related to expression of ultrastructure related molecules include integrin alpha 6 (ITGA6). ITGA6 is a gene encoding integrin alpha 6 (also known as integrin subunit alpha 6; JEB6; CD49f; VLA-6; ITGA6A; ITGA6B), which is a receptor for laminin on platelets. In some embodiments, expression of ITGA6 by a cell is determined by measuring a level of an ITGA6 RNA (*e.g.*, mRNA transcribed from ITGA6). Exemplary nucleic acid sequences of human ITGA6 can be found, for example, at GenBank Accession Nos. NG_008853.1, NM_000210.4; UniProt Accession No. P23229; and OMIM Accession No. 147556. In some embodiments, expression of ITGA6 by a cell is determined by measuring a level of a ITGA6 protein or fragment thereof (*e.g.*, a protein encoded by ITGA6).

An exemplary amino acid sequence of ITGA6 can be found, for example, at GenBank Accession No. NP_000201.2. Further information on ITGA6 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITGA6. "ITGA6" as used herein, also refers to variations of ITGA6 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITGA6).

Non-limiting examples of markers related to expression of ultrastructure related molecules include integrin alpha 2 (ITGA2). ITGA2 is a gene encoding integrin alpha 2 (also known as integrin subunit alpha 2; BR; GPIa; CD49B; HPA-5; VLA-2; VLAA2), which is a receptor for laminin, collagen, collagen C-propeptides, fibronectin and E-cadherin. In some embodiments, expression of ITGA2 by a cell is determined by measuring a level of an ITGA2 RNA (*e.g.*, mRNA transcribed from ITGA2). Exemplary nucleic acid sequences of human ITGA2 can be found, for example, at GenBank Accession Nos. NG_008330.2, NM_002203.4; UniProt Accession No. P17301; and OMIM Accession No. 192974. In some embodiments, expression of ITGA2 by a cell is determined by measuring a level of a ITGA2 protein or fragment thereof (*e.g.*, a protein encoded by ITGA2). An exemplary amino acid sequence of ITGA2 can be found, for example, at GenBank Accession No. NP_002194.2. Further information on ITGA2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITGA2. "ITGA2" as used herein, also refers to variations of ITGA2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITGA2).

Non-limiting examples of markers related to expression of ultrastructure related molecules include LIM zinc finger domain containing 1 (LIMS1). LIMS1 is a gene encoding LIM zinc finger domain containing 1 (also known as PINCH; PINCH1; PINCH-1), which is thought to be involved in integrin signaling. In some embodiments, expression of LIMS1 by a cell is determined by measuring a level of an LIMS1 RNA (*e.g.*, mRNA transcribed from LIMS1). Exemplary nucleic acid sequences of human LIMS1 can be found, for example, at GenBank Accession No. NM_001193482.2 ; UniProt Accession No. P48059; and OMIM Accession No. 602567. In some embodiments, expression of LIMS1 by a cell is determined by measuring a level of a LIMS1 protein or fragment thereof (*e.g.*, a protein encoded by LIMS1). An exemplary amino acid sequence of LIMS1 can be found, for example, at GenBank Accession No. NP_001180411.1. Further information on LIMS1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=LIMS1. "LIMS1" as used herein, also refers to

variations of LIMS1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=LIMS1).

Non-limiting examples of markers related to expression of ultrastructure related molecules include talin2 (TLN2). TLN2 is a gene encoding talin 2 (also known as ILWEQ), which is thought to be involved in the assembly of actin filaments. In some embodiments, expression of TLN2 by a cell is determined by measuring a level of an TLN2 RNA (*e.g.*, mRNA transcribed from TLN2). Exemplary nucleic acid sequences of human TLN2 can be found, for example, at GenBank Accession Nos. NG_033932.2, NM_001394547.1; UniProt Accession No. Q9Y4G6; and OMIM Accession No. 607349. In some embodiments, expression of TLN2 by a cell is determined by measuring a level of a TLN2 protein or fragment thereof (*e.g.*, a protein encoded by TLN2). An exemplary amino acid sequence of TLN2 can be found, for example, at GenBank Accession No. NP_001381476.1. Further information on TLN2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=TLN2. "TLN2" as used herein, also refers to variations of TLN2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=TLN2).

Non-limiting examples of markers related to expression of ultrastructure related molecules include parvin alpha (PARVA). PARVA is a gene encoding parvin alpha (also known as MXRA2; CH-ILKBP), which plays a role in sarcomere organization. In some embodiments, expression of PARVA by a cell is determined by measuring a level of an PARVA RNA (*e.g.*, mRNA transcribed from PARVA). Exemplary nucleic acid sequences of human PARVA can be found, for example, at GenBank Accession No. NM_018222.5; UniProt Accession No. Q9NVD7; and OMIM Accession No. 608120. In some embodiments, expression of PARVA by a cell is determined by measuring a level of a PARVA protein or fragment thereof (*e.g.*, a protein encoded by PARVA). An exemplary amino acid sequence of PARVA can be found, for example, at GenBank Accession No. NP_060692.3. Further information on PARVA can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PARVA. "PARVA" as used herein, also refers to variations of PARVA including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PARVA).

Non-limiting examples of markers related to expression of ultrastructure related molecules include fermitin family homolog 1 (FERMT1). FERMT1 is a gene encoding fermitin family homolog 1 (also known as FERM domain containing kindlin 1; URP1; KIND1; DTGCU2; UNC112A; C20orf42), which is involved in cell adhesion and thought to contribute to integrin activation. In some embodiments, expression of FERMT1 by a cell is determined by

measuring a level of an FERMT1 RNA (*e.g.*, mRNA transcribed from FERMT1). Exemplary nucleic acid sequences of human FERMT1 can be found, for example, at GenBank Accession Nos. NG_016213.1, NM_017671.5; UniProt Accession No. Q9BQL6; and OMIM Accession No. 607900. In some embodiments, expression of FERMT1 by a cell is determined by measuring a level of a FERMT1 protein or fragment thereof (*e.g.*, a protein encoded by FERMT1). An exemplary amino acid sequence of FERMT1 can be found, for example, at GenBank Accession No. NP_060141.3. Further information on FERMT1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=FERMT1. "FERMT1" as used herein, also refers to variations of FERMT1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=FERMT1).

Non-limiting examples of markers related to expression of ultrastructure related molecules include beta-parvin (PARVB). PARVB is a gene encoding beta-parvin (also known as CGI-56), which is an adapter protein that plays a role in integrin signaling. In some embodiments, expression of PARVB by a cell is determined by measuring a level of an PARVB RNA (*e.g.*, mRNA transcribed from PARVB). Exemplary nucleic acid sequences of human PARVB can be found, for example, at GenBank Accession Nos. NG_029743.3, NM_001003828.3 ; UniProt Accession No. Q9HBI1; and OMIM Accession No. 608121. In some embodiments, expression of PARVB by a cell is determined by measuring a level of a PARVB protein or fragment thereof (*e.g.*, a protein encoded by PARVB). An exemplary amino acid sequence of PARVB can be found, for example, at GenBank Accession No. NP_001003828.1. Further information on PARVB can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=PARVB. "PARVB" as used herein, also refers to variations of PARVB including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=PARVB).

Non-limiting examples of markers related to expression of ultrastructure related molecules include gap junction alpha-1 (GJA1). GJA1 is a gene encoding gap junction alpha-1 (also known as HSS; CMDR; CX43; EKVP; GJAL; ODDD; AVSD3; EKVP3; HLHS1; PPKCA), which is a component of gap junctions in the heart. In some embodiments, expression of GJA1 by a cell is determined by measuring a level of an GJA1 RNA (*e.g.*, mRNA transcribed from GJA1). Exemplary nucleic acid sequences of human GJA1 can be found, for example, at GenBank Accession Nos. NG_008308.1, NM_000165.5; UniProt Accession No. P17302; and OMIM Accession No. 121014. In some embodiments, expression of GJA1 by a cell is determined by measuring a level of a GJA1 protein or fragment thereof (*e.g.*, a protein encoded

by GJA1). An exemplary amino acid sequence of GJA1 can be found, for example, at GenBank Accession No. NP_000156.1. Further information on GJA1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=GJA1. "GJA1" as used herein, also refers to variations of GJA1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=GJA1).

Non-limiting examples of markers related to expression of ultrastructure related molecules include gap junction alpha-3 (GJA3). GJA3 is a gene encoding gap junction alpha-3 (also known as CX46; CZP3; CTRCT14), which is a connexin and is a component of lens fiber gap junctions. In some embodiments, expression of GJA3 by a cell is determined by measuring a level of an GJA3 RNA (*e.g.*, mRNA transcribed from GJA3). Exemplary nucleic acid sequences of human GJA3 can be found, for example, at GenBank Accession Nos. NG_016399.1, NM_021954.4; UniProt Accession No. Q9Y6H8; and OMIM Accession No. 121015. In some embodiments, expression of GJA3 by a cell is determined by measuring a level of a GJA3 protein or fragment thereof (*e.g.*, a protein encoded by GJA3). An exemplary amino acid sequence of GJA3 can be found, for example, at GenBank Accession No. NP_068773.2. Further information on GJA3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=GJA3. "GJA3" as used herein, also refers to variations of GJA3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=GJA3).

Non-limiting examples of markers related to expression of ultrastructure related molecules include gap junction gamma-1 protein (GJC1). GJC1 is a gene encoding gap junction gamma-1 (also known as CX45; GJA7), which is a component of gap junctions. In some embodiments, expression of GJC1 by a cell is determined by measuring a level of an GJC1 RNA (*e.g.*, mRNA transcribed from GJC1). Exemplary nucleic acid sequences of human GJC1 can be found, for example, at GenBank Accession No. NM_001080383.2 ; UniProt Accession No. P36383; and OMIM Accession No. 608655. In some embodiments, expression of GJC1 by a cell is determined by measuring a level of a GJC1 protein or fragment thereof (*e.g.*, a protein encoded by GJC1). An exemplary amino acid sequence of GJC1 can be found, for example, at GenBank Accession No. NP_001073852.1. Further information on GJC1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=GJC1. "GJC1" as used herein, also refers to variations of GJC1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=GJC1).

Non-limiting examples of markers related to expression of ultrastructure related molecules include gap junction alpha-5 protein (GJA5). GJA5 is a gene encoding gap junction

alpha-5 (also known as CX40; ATFB11), which is a component of gap junctions. In some embodiments, expression of GJA5 by a cell is determined by measuring a level of an GJA5 RNA (*e.g.*, mRNA transcribed from GJA5). Exemplary nucleic acid sequences of human GJA5 can be found, for example, at GenBank Accession Nos. NG_009369.2, NM_005266.7; UniProt Accession No. P36382; and OMIM Accession No. 121013. In some embodiments, expression of GJA5 by a cell is determined by measuring a level of a GJA5 protein or fragment thereof (*e.g.*, a protein encoded by GJA5). An exemplary amino acid sequence of GJA5 can be found, for example, at GenBank Accession No. NP_005257.2. Further information on GJA5 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=GJA5. "GJA5" as used herein, also refers to variations of GJA5 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=GJA5).

In some embodiments, a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected from plakophilin-2 (PKP2); desmoplakin (DSP); junction plakoglobin (JUP); desmoglein-2 (DSG2); desmocollin-2 (DSC2); integrin beta-1 (ITGB1); integrin alpha chain V (ITGAV); integrin alpha 7 (ITGA7); integrin alpha 6 (ITGA6); integrin alpha 2 (ITGA2); LIM zinc finger domain containing 1 (LIMS1); talin2 (TLN2); parvin alpha (PARVA); fermitin family homolog 1 (FERMT1); beta-parvin (PARVB); gap junction alpha-1 (GJA1); gap junction alpha-3 (GJA3); gap junction gamma-1 protein (GJC1); gap junction alpha-5 protein (GJA5); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, or all 19).

Developmental Markers

In some embodiments, a low GIA or high GIA molecular profile is based on the expression level of one or more developmental (*e.g.*, embryonic, organ or tissue organization, aging) markers.

Non-limiting examples of developmental markers include synaptosome associated protein 91 (*SNAP91*). *SNAP91* is a gene encoding synaptosome associated protein 91 (also known as CALM; AP180), which is a component of the adapter complexes that link clathrin to receptors in coated vesicles. In some embodiments, expression of *SNAP91* by a cell is determined by measuring a level of an *SNAP91* RNA (*e.g.*, mRNA transcribed from *SNAP91*). Exemplary nucleic acid sequences of human *SNAP91* can be found, for example, at GenBank Accession No. NM_001242792.2; UniProt Accession No. O60641; and OMIM Accession No.

607923. In some embodiments, expression of SNAP91 by a cell is determined by measuring a level of a SNAP91 protein or fragment thereof (*e.g.*, a protein encoded by SNAP91). An exemplary amino acid sequence of SNAP91 can be found, for example, at GenBank Accession No. NP_001229721.1. Further information on SNAP91 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=SNAP91. "SNAP91" as used herein, also refers to variations of SNAP91 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=SNAP91).

Non-limiting examples of developmental markers include Iroquois homeobox 2 (*IRX2*). *IRX2* is a gene encoding Iroquois-class homeobox 2 (also known as Iroquois-class homeodomain protein IRX-2; IRXA2), which is a member of the Iroquois homeobox gene family. In some embodiments, expression of *IRX2* by a cell is determined by measuring a level of an *IRX2* RNA (*e.g.*, mRNA transcribed from *IRX2*). Exemplary nucleic acid sequences of human *IRX2* can be found, for example, at GenBank Accession No. NM_001134222.2; UniProt Accession No. Q9BZI1; and OMIM Accession No. 606198. In some embodiments, expression of *IRX2* by a cell is determined by measuring a level of a *IRX2* protein or fragment thereof (*e.g.*, a protein encoded by *IRX2*). An exemplary amino acid sequence of *IRX2* can be found, for example, at GenBank Accession No. NP_001127694.1. Further information on *IRX2* can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=IRX2. "*IRX2*" as used herein, also refers to variations of *IRX2* including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=IRX2).

Non-limiting examples of developmental markers include roundabout guidance receptor 2 (*ROBO2*). *ROBO2* is a gene encoding roundabout guidance receptor 2 (also known as SAX3), which functions in axon guidance and cell migration. In some embodiments, expression of *ROBO2* by a cell is determined by measuring a level of an *ROBO2* RNA (*e.g.*, mRNA transcribed from *ROBO2*). Exemplary nucleic acid sequences of human *ROBO2* can be found, for example, at GenBank Accession Nos. NG_027734.2, NM_001128929.3; UniProt Accession No. Q9HCK4; and OMIM Accession No. 602431. In some embodiments, expression of *ROBO2* by a cell is determined by measuring a level of a *ROBO2* protein or fragment thereof (*e.g.*, a protein encoded by *ROBO2*). An exemplary amino acid sequence of *ROBO2* can be found, for example, at GenBank Accession No. NP_001122401.1. Further information on *ROBO2* can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ROBO2. "*ROBO2*" as used herein, also refers to variations of *ROBO2* including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ROBO2).

Non-limiting examples of developmental markers include bone morphogenetic protein (BMP)/retinoic acid inducible neural specific 3 (BRINP3). BRINP3 is a gene encoding bone morphogenetic protein/retinoic acid inducible neural specific 3 (also known as FAM5C; DBCCR1L; DBCCR1L1), which inhibits neuronal cell proliferation by negative regulation of the cell cycle transition and promotes pituitary gonadotrope cell proliferation, migration, and invasion. In some embodiments, expression of BRINP3 by a cell is determined by measuring a level of an BRINP3 RNA (*e.g.*, mRNA transcribed from BRINP3). Exemplary nucleic acid sequences of human BRINP3 can be found, for example, at GenBank Accession No. NM_001317188.2; UniProt Accession No. Q76B58; and OMIM Accession No. 618390. In some embodiments, expression of BRINP3 by a cell is determined by measuring a level of a BRINP3 protein or fragment thereof (*e.g.*, a protein encoded by BRINP3). An exemplary amino acid sequence of BRINP3 can be found, for example, at GenBank Accession No. NP_001304117.1. Further information on BRINP3 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=BRINP3. "BRINP3" as used herein, also refers to variations of BRINP3 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=BRINP3).

Non-limiting examples of developmental markers include AE binding protein 1 (AEBP1). AEBP1 is a gene encoding AE binding protein 1 (also known as ACLP), which functions as a transcriptional repressor and plays a role in adipogenesis and smooth muscle cell differentiation. In some embodiments, expression of AEBP1 by a cell is determined by measuring a level of an AEBP1 RNA (*e.g.*, mRNA transcribed from AEBP1). Exemplary nucleic acid sequences of human AEBP1 can be found, for example, at GenBank Accession Nos. NG_056775.1, NM_001129.5; UniProt Accession No. Q8IUX7; and OMIM Accession No. 602981. In some embodiments, expression of AEBP1 by a cell is determined by measuring a level of a AEBP1 protein or fragment thereof (*e.g.*, a protein encoded by AEBP1). An exemplary amino acid sequence of AEBP1 can be found, for example, at GenBank Accession No. NP_001120.3. Further information on AEBP1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=AEBP1. "AEBP1" as used herein, also refers to variations of AEBP1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=AEBP1).

Non-limiting examples of developmental markers include galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 2 (B3GAT2). B3GAT2 is a gene encoding galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 2 (also known

as beta-1,3-glucuronyltransferase 2; GLCATS), which is implicated in cellular migration and adhesion in the nervous system. In some embodiments, expression of B3GAT2 by a cell is determined by measuring a level of an B3GAT2 RNA (*e.g.*, mRNA transcribed from B3GAT2). Exemplary nucleic acid sequences of human B3GAT2 can be found, for example, at GenBank Accession No. NM_080742.3; UniProt Accession No. Q9NPZ5; and OMIM Accession No. 607497. In some embodiments, expression of B3GAT2 by a cell is determined by measuring a level of a B3GAT2 protein or fragment thereof (*e.g.*, a protein encoded by B3GAT2). An exemplary amino acid sequence of B3GAT2 can be found, for example, at GenBank Accession No. NP_542780.1. Further information on B3GAT2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=B3GAT2. "B3GAT2" as used herein, also refers to variations of B3GAT2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=B3GAT2).

Non-limiting examples of developmental markers include collagen type XIX alpha 1 chain (COL19A1). COL19A1 is a gene encoding collagen type XIX alpha 1 chain (also known as COL9A1L; D6S228E), which may play a role in the developing esophagus and organization of the pericellular matrix or the sphincteric smooth muscle. In some embodiments, expression of COL19A1 by a cell is determined by measuring a level of an COL19A1 RNA (*e.g.*, mRNA transcribed from COL19A1). Exemplary nucleic acid sequences of human COL19A1 can be found, for example, at GenBank Accession No. NM_001858.6; UniProt Accession No. Q14993; and OMIM Accession No. 120165. In some embodiments, expression of COL19A1 by a cell is determined by measuring a level of a COL19A1 protein or fragment thereof (*e.g.*, a protein encoded by COL19A1). An exemplary amino acid sequence of COL19A1 can be found, for example, at GenBank Accession No. NP_001849.2. Further information on COL19A1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=COL19A1. "COL19A1" as used herein, also refers to variations of COL19A1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=COL19A1).

Non-limiting examples of developmental markers include CXXC finger protein 4 (CXXC4). CXXC4 is a gene encoding CXXC finger protein 4 (also known as IDAX), which acts as a negative regulator of the Wnt signaling pathway. In some embodiments, expression of CXXC4 by a cell is determined by measuring a level of an CXXC4 RNA (*e.g.*, mRNA transcribed from CXXC4). Exemplary nucleic acid sequences of human CXXC4 can be found, for example, at GenBank Accession No. NM_025212.4; UniProt Accession No. Q9H2H0; and OMIM Accession No. 611645. In some embodiments, expression of CXXC4 by a cell is

determined by measuring a level of a CXXC4 protein or fragment thereof (*e.g.*, a protein encoded by CXXC4). An exemplary amino acid sequence of CXXC4 can be found, for example, at GenBank Accession No. NP_079488.2. Further information on CXXC4 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=CXXC4. "CXXC4" as used herein, also refers to variations of CXXC4 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=CXXC4).

Non-limiting examples of developmental markers include ephrin type-A receptor 4 (EPHA4). EPHA4 is a gene encoding ephrin type-A receptor 4 (also known as EPH receptor A; EK8; SEK; HEK8; TYRO1), which is implicated in mediating developmental events. In some embodiments, expression of EPHA4 by a cell is determined by measuring a level of an EPHA4 RNA (*e.g.*, mRNA transcribed from EPHA4). Exemplary nucleic acid sequences of human EPHA4 can be found, for example, at GenBank Accession No. NM_001304536.2; UniProt Accession No. P54764; and OMIM Accession No. 602188. In some embodiments, expression of EPHA4 by a cell is determined by measuring a level of a EPHA4 protein or fragment thereof (*e.g.*, a protein encoded by EPHA4). An exemplary amino acid sequence of EPHA4 can be found, for example, at GenBank Accession No. NP_001291465.1. Further information on EPHA4 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=EPHA4. "EPHA4" as used herein, also refers to variations of EPHA4 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=EPHA4).

Non-limiting examples of developmental markers include guanylate cyclase 1 soluble subunit alpha 1 (GUCY1A1). GUCY1A1 is a gene encoding guanylate cyclase 1 soluble subunit alpha 1 (also known as GUCA3; MYMY6; GC-SA3; GUC1A3; GUCSA3; GUCY1A3; GCS-alpha-3; GC-S-alpha-1), which is a subunit of a soluble guanylate cyclase that catalyzes the conversion of GTP to 3',5'-cyclic GMP and pyrophosphate. In some embodiments, expression of GUCY1A1 by a cell is determined by measuring a level of an GUCY1A1 RNA (*e.g.*, mRNA transcribed from GUCY1A1). Exemplary nucleic acid sequences of human GUCY1A1 can be found, for example, at GenBank Accession Nos. NG_034128.1, NM_000856.6; UniProt Accession No. Q02108; and OMIM Accession No. 139396. In some embodiments, expression of GUCY1A1 by a cell is determined by measuring a level of a GUCY1A1 protein or fragment thereof (*e.g.*, a protein encoded by GUCY1A1). An exemplary amino acid sequence of GUCY1A1 can be found, for example, at GenBank Accession No. NP_000847.2. Further information on GUCY1A1 can be found, for example, on the world wide web at

ncbi.nlm.nih.gov/gene/?term=GUCY1A1. "GUCY1A1" as used herein, also refers to variations of GUCY1A1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=GUCY1A1).

Non-limiting examples of developmental markers include integral membrane protein 2A (ITM2A). ITM2A is a gene encoding integral membrane protein 2A (also known as E25A; BRICD2A), which is involved in osteo- and chondrogenic differentiation. In some embodiments, expression of ITM2A by a cell is determined by measuring a level of an ITM2A RNA (*e.g.*, mRNA transcribed from ITM2A). Exemplary nucleic acid sequences of human ITM2A can be found, for example, at GenBank Accession Nos. NG_016412.1, NM_001171581.2; UniProt Accession No. O43736; and OMIM Accession No. 300222. In some embodiments, expression of ITM2A by a cell is determined by measuring a level of a ITM2A protein or fragment thereof (*e.g.*, a protein encoded by ITM2A). An exemplary amino acid sequence of ITM2A can be found, for example, at GenBank Accession No. NP_001165052.1. Further information on ITM2A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=ITM2A. "ITM2A" as used herein, also refers to variations of ITM2A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=ITM2A).

Non-limiting examples of developmental markers include heat shock protein beta-2 (HSPB2). HSPB2 is a gene encoding heat shock protein beta-2 (also known as heat shock protein family B (small) member ; MKBP; HSP27; Hs.78846; LOH11CR1K), which is involved in maintenance of muscle structure and function, *e.g.*, in the heart. In some embodiments, expression of HSPB2 by a cell is determined by measuring a level of an HSPB2 RNA (*e.g.*, mRNA transcribed from HSPB2). Exemplary nucleic acid sequences of human HSPB2 can be found, for example, at GenBank Accession Nos. NG_033080.2, NM_001541.4 ; UniProt Accession No. Q16082; and OMIM Accession No. 602179. In some embodiments, expression of HSPB2 by a cell is determined by measuring a level of a HSPB2 protein or fragment thereof (*e.g.*, a protein encoded by HSPB2). An exemplary amino acid sequence of HSPB2 can be found, for example, at GenBank Accession No. NP_001532.1. Further information on HSPB2 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=HSPB2. "HSPB2" as used herein, also refers to variations of HSPB2 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=HSPB2).

Non-limiting examples of developmental markers include kinesin-like protein (KIF1A). KIF1A is a gene encoding kinesin-like protein (also known as kinesin family member 1A;

ATSV; MRD9; HSN2C; SPG30; SPG30A; SPG30B; UNC104; C2orf20; NESCAVS), which is a motor protein involved in anterograde transport of synaptic vesicle precursors along axons. In some embodiments, expression of KIF1A by a cell is determined by measuring a level of an KIF1A RNA (*e.g.*, mRNA transcribed from KIF1A). Exemplary nucleic acid sequences of human KIF1A can be found, for example, at GenBank Accession Nos. NG_029724.1, NM_001244008.2; UniProt Accession No. Q12756; and OMIM Accession No. 601255. In some embodiments, expression of KIF1A by a cell is determined by measuring a level of a KIF1A protein or fragment thereof (*e.g.*, a protein encoded by KIF1A). An exemplary amino acid sequence of KIF1A can be found, for example, at GenBank Accession No. NP_001230937.1. Further information on KIF1A can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=KIF1A. "KIF1A" as used herein, also refers to variations of KIF1A including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=KIF1A).

Non-limiting examples of developmental markers include neuronal growth regulator 1 (NEGR1). NEGR1 is a gene encoding neuronal growth regulator 1 (also known as Ntra; KILON; IGLON4; DMML2433), which is predicted to be involved in cell adhesion and development of the nervous system. In some embodiments, expression of NEGR1 by a cell is determined by measuring a level of an NEGR1 RNA (*e.g.*, mRNA transcribed from NEGR1). Exemplary nucleic acid sequences of human NEGR1 can be found, for example, at GenBank Accession No. NM_173808.3; UniProt Accession No. Q7Z3B1; and OMIM Accession No. 613173. In some embodiments, expression of NEGR1 by a cell is determined by measuring a level of a NEGR1 protein or fragment thereof (*e.g.*, a protein encoded by NEGR1). An exemplary amino acid sequence of NEGR1 can be found, for example, at GenBank Accession No. NP_776169.2. Further information on NEGR1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=NEGR1. "NEGR1" as used herein, also refers to variations of NEGR1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=NEGR1).

Non-limiting examples of developmental markers include neuroligin-1 (NLGN1). NLGN1 is a gene encoding neuroligin-1 (also known as NL1; NLG1), which is thought to be involved in the formation and remodeling of central nervous system synapses. In some embodiments, expression of NLGN1 by a cell is determined by measuring a level of an NLGN1 RNA (*e.g.*, mRNA transcribed from NLGN1). Exemplary nucleic acid sequences of human NLGN1 can be found, for example, at GenBank Accession Nos. NG_046919.2,

NM_001365923.2; UniProt Accession No. Q8N2Q7; and OMIM Accession No. 600568. In some embodiments, expression of NLGN1 by a cell is determined by measuring a level of a NLGN1 protein or fragment thereof (*e.g.*, a protein encoded by NLGN1). An exemplary amino acid sequence of NLGN1 can be found, for example, at GenBank Accession No. NP_001352852.1. Further information on NLGN1 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=NLGN1. "NLGN1" as used herein, also refers to variations of NLGN1 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=NLGN1).

Non-limiting examples of developmental markers include periostin (POSTN). POSTN is a gene encoding periostin (also known as PN; OSF2; OSF-2; PDLPOSTN), which is a secreted extracellular matrix protein involved in tissue development and regeneration. In some embodiments, expression of POSTN by a cell is determined by measuring a level of an POSTN RNA (*e.g.*, mRNA transcribed from POSTN). Exemplary nucleic acid sequences of human POSTN can be found, for example, at GenBank Accession No. NM_001135934.2; UniProt Accession No. Q15063; and OMIM Accession No. 608777. In some embodiments, expression of POSTN by a cell is determined by measuring a level of a POSTN protein or fragment thereof (*e.g.*, a protein encoded by POSTN). An exemplary amino acid sequence of POSTN can be found, for example, at GenBank Accession No. NP_001129406.1. Further information on POSTN can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=POSTN. "POSTN" as used herein, also refers to variations of POSTN including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=POSTN).

Non-limiting examples of developmental markers include regucalcin (RGN). RGN is a gene encoding regucalcin (also known as RC; GNL; SMP30; HEL-S-41), which is thought to be involved in calcium homeostasis and play a role in aging. In some embodiments, expression of RGN by a cell is determined by measuring a level of an RGN RNA (*e.g.*, mRNA transcribed from RGN). Exemplary nucleic acid sequences of human RGN can be found, for example, at GenBank Accession No. NM_001282848.2; UniProt Accession No. Q15493; and OMIM Accession No. 300212. In some embodiments, expression of RGN by a cell is determined by measuring a level of a RGN protein or fragment thereof (*e.g.*, a protein encoded by RGN). An exemplary amino acid sequence of RGN can be found, for example, at GenBank Accession No. NP_001269777.1. Further information on RGN can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=RGN. "RGN" as used herein, also refers to variations of

RGN including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=RGN).

Non-limiting examples of developmental markers include fibroblast growth factor 12 (FGF12). FGF12 is a gene encoding fibroblast growth factor 12 (also known as FHF1; DEE47; EIEE47; FGF12B), which is thought to be involved in various processes, including embryonic development, cell growth, morphogenesis, tissue repair, tumor growth, and invasion. In some embodiments, expression of FGF12 by a cell is determined by measuring a level of an FGF12 RNA (*e.g.*, mRNA transcribed from FGF12). Exemplary nucleic acid sequences of human FGF12 can be found, for example, at GenBank Accession Nos. NG_051966.1, NM_001377292.1; UniProt Accession No. P61328; and OMIM Accession No. 601513. In some embodiments, expression of FGF12 by a cell is determined by measuring a level of a FGF12 protein or fragment thereof (*e.g.*, a protein encoded by FGF12). An exemplary amino acid sequence of FGF12 can be found, for example, at GenBank Accession No. NP_001364221.1. Further information on FGF12 can be found, for example, on the world wide web at ncbi.nlm.nih.gov/gene/?term=FGF12. "FGF12" as used herein, also refers to variations of FGF12 including variants provided in the SNP database (see, *e.g.*, ncbi.nlm.nih.gov/snp/?term=FGF12).

In some embodiments, a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers from synaptosome associated protein 91 (SNAP91); Iroquois Homeobox 2 (IRX2); Roundabout Guidance Receptor 2 (ROBO2); BMP/retinoic acid inducible neural specific 3 (BRINP3); AE binding protein 1 (AEBP1); galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 2 (B3GAT2); collagen type XIX alpha 1 chain (COL19A1); CXXC finger protein 4 (CXXC4); ephrin type-A receptor 4 (EPHA4); guanylate cyclase 1 soluble subunit alpha 1 (GUCY1A1); integral membrane protein 2A (ITM2A); heat shock protein beta-2 (HSPB2); kinesin-like protein (KIF1A); neuronal growth regulator 1 (NEGR1); neuroligin-1 (NLGN1); periostin (POSTN); regucalcin (RGN); fibroblast growth factor 12 (FGF12); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, or all 18).

Exemplary Molecular Profiles

In some embodiments a cell (*e.g.*, a cardiomyocyte) is characterized as having a low GIA or high GIA molecular profile based on an expression level of one or more markers selected

from the group consisting of myosin binding protein C, cardiac type (MYBPC3); alpha-actinin-2 (ACTN2); Titin (TTN); desmin (DES); myosin heavy chain, α isoform (MHC- α); myosin heavy chain beta (MHC- β); myosin light polypeptide 6 (MYL6); myosin light chain 7 (MYL7); myosin light chain 2 (MYL2); troponin I (TNNI1); troponin I3 (TNNI3); Calcium Voltage-Gated Channel subunit Alpha 1 C (CACNA1C); Calcium Voltage-Gated Channel subunit Alpha 1 H (CACNA1H); potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 4 (HCN4); potassium voltage-gated channel subfamily D member 3 (KCND3); potassium voltage-gated channel subfamily D member 2 (KCND2); Sodium Voltage-Gated Channel Alpha Subunit 5 (SCN5A); Sodium Voltage-Gated Channel Alpha Subunit 9 (SCN9A); Sodium Voltage-Gated Channel Alpha Subunit 3 (SCN3A); potassium inwardly rectifying channel subfamily J member 2 (KCNJ2); potassium inwardly rectifying channel subfamily J member 3 (KCNJ3); potassium inwardly rectifying channel subfamily J member 5 (KCNJ5); potassium inwardly rectifying channel subfamily J member 8 (KCNJ8); potassium voltage-gated channel subfamily Q member 1 (KCNQ1); potassium voltage-gated channel subfamily Q member 3 (KCNQ3); potassium voltage-gated channel subfamily Q member 5 (KCNQ5); sarcoplasmic/endoplasmic reticulum calcium ATPase 2 (ATP2A2); ryanodine receptor 2 (RYR2); solute carrier family 8 member A1 (SLC8A1); protein kinase A catalytic subunit α (PRKACA); calmodulin-dependent protein kinase type II subunit alpha (CAMK2a); calmodulin-dependent protein kinase type II subunit beta (CAMK2b); calmodulin-dependent protein kinase type II inhibitor 1 (CAMK2N1); calmodulin-dependent protein kinase type II inhibitor 2 (CAMK2N2); myc box-dependent-interacting protein 1 (BIN1); junctophilin 2 (JPH2); nexilin F-actin binding protein (NEXN); peroxisome proliferator-activated receptor alpha (PPARA); peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PPARGC1A); retinoid X receptors (RXRA); acyl-coenzyme A oxidase 1 (ACOX1); cytochrome P450 oxidoreductase (POR); cytochrome C oxidase subunit 5a (COX5A2); cytochrome C oxidase subunit 6a (COX6A2); OPA1 mitochondrial dynamin like GTPase (OPA1); mitofusin-1 (MFN1); mitofusin-2 (MFN2); carnitine palmitoyltransferase I (CPT1A); butyrylcholinesterase (BCHE); Copine 5 (CPNE5); plakophilin-2 (PKP2); desmoplakin (DSP); junction plakoglobin (JUP); desmoglein-2 (DSG2); desmocollin-2 (DSC2); integrin beta-1 (ITGB1); integrin alpha chain V (ITGAV); integrin alpha 7 (ITGA7); integrin alpha 6 (ITGA6); integrin alpha 2 (ITGA2); LIM zinc finger domain containing 1 (LIMS1); talin2 (TLN2); parvin alpha (PARVA); fermitin family homolog 1 (FERMT1); beta-parvin (PARVB); gap junction alpha-1 (GJA1); gap junction alpha-3 (GJA3); gap junction gamma-1 protein (GJC1); gap junction alpha-5 protein (GJA5);

synaptosome associated protein 91 (SNAP91); Iroquois Homeobox 2 (IRX2); Roundabout Guidance Receptor 2 (ROBO2); BMP/retinoic acid inducible neural specific 3 (BRINP3); AE binding protein 1 (AEBP1); galactosylgalactosylxylosylprotein 3-beta-glucuronosyltransferase 2 (B3GAT2); collagen type XIX alpha 1 chain (COL19A1); CXXC finger protein 4 (CXXC4); ephrin type-A receptor 4 (EPHA4); guanylate cyclase 1 soluble subunit alpha 1 (GUCY1A1); integral membrane protein 2A (ITM2A); heat shock protein beta-2 (HSPB2); kinesin-like protein (KIF1A); neuronal growth regulator 1 (NEGR1); neuroligin-1 (NLGN1); periostin (POSTN); regucalcin (RGN); fibroblast growth factor 12 (FGF12); and any combination thereof (*e.g.*, any combination of 2 or more, 3 or more, 4 or more, 5 or more, 6 or more, 7 or more, 8 or more, 9 or more, 10 or more, 11 or more, 12 or more, 13 or more, 14 or more, 15 or more, 16 or more, 17 or more, 18 or more, 19 or more, 20 or more, 21 or more, 22 or more, 23 or more, 24 or more, 25 or more, 26 or more, 27 or more, 28 or more, 29 or more, 30 or more, 31 or more, 32 or more, 33 or more, 34 or more, 35 or more, 36 or more, 37 or more, 38 or more, 39 or more, 40 or more, 41 or more, 42 or more, 43 or more, 44 or more, 45 or more, 46 or more, 47 or more, 48 or more, 49 or more, 50 or more, 51 or more, 52 or more, 53 or more, 54 or more, 55 or more, 56 or more, 57 or more, 58 or more, 59 or more, 60 or more, 61 or more, 62 or more, 63 or more, 64 or more, 65 or more, 66 or more, 67 or more, 68 or more, 69 or more, 70 or more, 71 or more, 72 or more, 73 or more, 74 or more, 75 or more, 76 or more, 77 or more, 78 or more, 79 or more, 80 or more, 81 or more, 82 or more, 83 or more, 84 or more, 85 or more, 86 or more, or all 87).

In some embodiments, a cardiomyocyte is characterized as “high GIA” or “low GIA” based on differential expression levels of markers in Table 2. In some embodiments, a cell comprising a high GIA molecular profile has positive/high expression of one or more high GIA markers in Table 2. In some embodiments, a cell comprising a high GIA molecular profile has negative/low expression of one or more high GIA markers in Table 2. In some embodiments, a cell comprising a low GIA molecular profile has positive/high expression of one or more low GIA markers in Table 2. In some embodiments, a cell comprising a low GIA molecular profile has negative/low expression of one or more low GIA markers in Table 2. Certain molecular profiles are described below.

Table 2. Non-limiting molecular markers associated with risk of causing graft-induced arrhythmias.

	High GIA	Low GIA
Positive/high expression	AEBP1, BCHE, B3GALT2, BRINP3, COL19A1, CPNE5, CXXC4, EPHA4, GUCY1A, ID4, ITM2A, HSPB2, IRX2, KCNJ3, KIF1A, NEGR1, NLGN1, POSTN, SCN3A, SNAP91, RGN, ROBO2, SCN9A	ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, TLN2
Negative/low expression	ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, TLN2	AEBP1, BCHE, B3GALT2, BRINP3, COL19A1, CPNE5, CXXC4, EPHA4, GUCY1A, ID4, ITM2A, HSPB2, IRX2, KCNJ3, KIF1A, NEGR1, NLGN1, POSTN, SCN3A, SNAP91, RGN, ROBO2, SCN9A

In some embodiments, low GIA cardiomyocytes comprise a molecular profile selected from one or more of the following:

- a1. negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91;
- a2. positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- a3. positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- a4. negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A;
- a5. negative/low expression of BCHE;
- a6. negative/low expression of BRINP3;
- a7. negative/low expression of EPHA4;
- a8. negative/low expression of ID4
- a9. negative/low expression of IRX2;
- a10. positive/high expression of SCN5A;
- a11. negative/low expression of BCHE and BRINP3;

- a12. negative/low expression of BCHE and EPHA4;
- a13. negative/low expression of BCHE and ID4;
- a14. negative/low expression of BCHE and IRX2;
- a15. negative/low expression of BCHE and positive/high expression of SCN5A;
- a16. negative/low expression of BRINP3 and EPHA4;
- a17. negative/low expression of BRINP3 and ID4;
- a18. negative/low expression of BRINP3 and IRX2;
- a19. negative/low expression of BRINP3 and positive/high expression of SCN5A;
- a20. negative/low expression of EPHA4 and ID4;
- a21. negative/low expression of EPHA4 and IRX2;
- a22. negative/low expression of EPHA4 and positive/high expression of SCN5A;
- a23. negative/low expression of ID4 and IRX2;
- a24. negative/low expression of ID4 and positive/high expression of SCN5A;
- a25. negative/low expression of IRX2 and positive/high expression of SCN5A;
- a26. negative/low expression of BCHE, BRINP3, and EPHA4;
- a27. negative/low expression of BCHE, BRINP3, and ID4;
- a28. negative/low expression of BCHE, BRINP3, and IRX2;
- a29. negative/low expression of BCHE and BRINP3, and positive/high expression of SCN5A;
- a30. negative/low expression of BCHE, EPHA4, and ID4;
- a31. negative/low expression of BCHE, EPHA4, and IRX2;
- a32. negative/low expression of BCHE, EPHA4, and positive/high expression of SCN5A;
- a33. negative/low expression of BCHE, ID4, and IRX2;
- a34. negative/low expression of BCHE and ID4, and positive/high expression of SCN5A;
- a35. negative/low expression of BCHE and IRX2, and positive/high expression of SCN5A;
- a36. negative/low expression of BRINP3, EPHA4, and ID4;
- a37. negative/low expression of BRINP3, EPHA4, and IRX2;
- a38. negative/low expression of BRINP3, ID4, and IRX2;
- a39. negative/low expression of BRINP3 and ID4, and positive/high expression of SCN5A;

- a40. negative/low expression of BRINP3 and IRX2, and positive/high expression of SCN5A;
- a41. negative/low expression of EPHA4, ID4, and IRX2;
- a42. negative/low expression of EPHA4 and ID4, and positive/high expression of SCN5A;
- a43. negative/low expression of EPHA4 and IRX2, and positive/high expression of SCN5A;
- a44. negative/low expression of ID4 and IRX2, and positive/high expression of SCN5A;
- a45. negative/low expression of BCHE, BRINP3, EPHA4, and ID4;
- a46. negative/low expression of BCHE, BRINP3, EPHA4, and IRX2;
- a47. negative/low expression of BCHE, BRINP3, and EPHA4, and positive/high expression of SCN5A;
- a48. negative/low expression of BCHE, BRINP3, and ID4, and positive/high expression of SCN5A;
- a49. negative/low expression of BCHE, BRINP3, and IRX2, and positive/high expression of SCN5A;
- a50. negative/low expression of BCHE, BRINP3, EPHA4, ID4, and IRX2;
- a51. negative/low expression of BCHE, BRINP3, EPHA4, ID4, and IRX2, and positive/high expression of SCN5A;

In some embodiments, a cardiomyocyte having the molecular profile of any one of a1-a51 is a mature cardiomyocyte. In some embodiments, a mature cardiomyocyte having the molecular profile of any one of a1-a51 also exhibits positive/high expression of CTNT2 and MLC2V and negative/low expression of MLC2a.

In some embodiments, a cardiomyocyte having the molecular profile of any one of a1-a51 is an immature cardiomyocyte. In some embodiments, an immature cardiomyocyte having the molecular profile of any one of a1-a51 also exhibits positive/high expression of MLC2a, NKX2, NKX3, NKX4, NKX5, and HCN4, and negative/low expression of CD36 and MLC2v.

In some embodiments, a cardiomyocyte having the molecular profile of any one of a1-a51 is a cardiomyocyte precursor. In some embodiments, a cardiomyocyte precursor having the molecular profile of any one of a1-a51 also exhibits positive/high expression of any one of SSEA3/4, TRA-160, OCT3/4, NANOG, and SOX2.

In some embodiments, high GIA cardiomyocytes comprise a molecular profile selected from one or more of the following:

- b1. positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A and SNAP91;
- b2. negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- b3. negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- b4. positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A;
- b5. positive/high expression of BCHE;
- b6. positive/high expression of BRINP3;
- b7. positive/high expression of EPHA4;
- b8. positive/high expression of ID4
- b9. positive/high expression of IRX2;
- b10. negative/low expression of SCN5A;
- b11. positive/high expression of BCHE and BRINP3;
- b12. positive/high expression of BCHE and EPHA4;
- b13. positive/high expression of BCHE and ID4;
- b14. positive/high expression of BCHE and IRX2;
- b15. positive/high expression of BCHE and negative/low expression of SCN5A;
- b16. positive/high expression of BRINP3 and EPHA4;
- b17. positive/high expression of BRINP3 and ID4;
- b18. positive/high expression of BRINP3 and IRX2;
- b19. positive/high expression of BRINP3 and negative/low expression of SCN5A;
- b20. positive/high expression of EPHA4 and ID4;
- b21. positive/high expression of EPHA4 and IRX2;

- b22. positive/high expression of EPHA4 and negative/low expression of SCN5A;
- b23. positive/high expression of ID4 and IRX2;
- b24. positive/high expression of ID4 and negative/low expression of SCN5A;
- b25. positive/high expression of IRX2 and negative/low expression of SCN5A;
- b26. positive/high expression of BCHE, BRINP3, and EPHA4;
- b27. positive/high expression of BCHE, BRINP3, and ID4;
- b28. positive/high expression of BCHE, BRINP3, and IRX2;
- b29. positive/high expression of BCHE and BRINP3, and negative/low expression of SCN5A;
- b30. positive/high expression of BCHE, EPHA4, and ID4;
- b31. positive/high expression of BCHE, EPHA4, and IRX2;
- b32. positive/high expression of BCHE, EPHA4, and negative/low expression of SCN5A;
- b33. positive/high expression of BCHE, ID4, and IRX2;
- b34. positive/high expression of BCHE and ID4, and negative/low expression of SCN5A;
- b35. positive/high expression of BCHE and IRX2, and negative/low expression of SCN5A;
- b36. positive/high expression of BRINP3, EPHA4, and ID4;
- b37. positive/high expression of BRINP3, EPHA4, and IRX2;
- b38. positive/high expression of BRINP3, ID4, and IRX2;
- b39. positive/high expression of BRINP3 and ID4, and negative/low expression of SCN5A;
- b40. positive/high expression of BRINP3 and IRX2, and negative/low expression of SCN5A;
- b41. positive/high expression of EPHA4, ID4, and IRX2;
- b42. positive/high expression of EPHA4 and ID4, and negative/low expression of SCN5A;
- b43. positive/high expression of EPHA4 and IRX2, and negative/low expression of SCN5A;
- b44. positive/high expression of ID4 and IRX2, and negative/low expression of SCN5A;
- b45. positive/high expression of BCHE, BRINP3, EPHA4, and ID4;

- b46. positive/high expression of BCHE, BRINP3, EPHA4, and IRX2;
- b47. positive/high expression of BCHE, BRINP3, and EPHA4, and negative/low expression of SCN5A;
- b48. positive/high expression of BCHE, BRINP3, and ID4, and negative/low expression of SCN5A;
- b49. positive/high expression of BCHE, BRINP3, and IRX2, and negative/low expression of SCN5A;
- b50. positive/high expression of BCHE, BRINP3, EPHA4, ID4, and IRX2;
- b51. positive/high expression of BCHE, BRINP3, EPHA4, ID4, and IRX2, and negative/low expression of SCN5A.

Cardiac Cell Therapies

Cells identified by a method disclosed herein may be used for cardiac cell therapy. Cardiac cell therapies (*e.g.*, cardiac grafts) of the disclosure include, in some embodiments, cellular compositions comprising a plurality of cardiomyocytes and physiologically acceptable medium. Cardiac cell therapies may comprise one or more types of cardiomyocytes (*e.g.*, ventricular cardiomyocytes, atrial cardiomyocytes, and/or smooth muscle cells) at one or more stages of development (*e.g.*, mature cardiomyocytes and/or immature cardiomyocytes). In some embodiments, the cardiomyocytes are ventricular cardiomyocytes. In some embodiments, cardiomyocytes are immune evading or hypimmune. In some embodiments, cardiac cell therapies (*e.g.*, cardiac grafts) further comprise vascular cells and/or cardiac cells. In some embodiments, cardiac cell therapies (*e.g.*, cardiac grafts) further comprise endothelial cells, conduction cells, pacemaker cells, and/or fibroblasts. Cardiac cell therapies may comprise any physiologically acceptable medium helpful for administering, adhering, growing, and/or maintaining the cardiac cell therapy (*e.g.*, cardiac graft) in a subject. Physiologically acceptable media for administration of tissues are known in the art.

In embodiments, cardiac cell therapies are administered to a subject having experienced cardiac injury, including, but not limited to, damage to cells and tissue of the heart, including cardiomyocytes. Cardiac injury may be caused by a number of factors, including, but not limited to, heart disease (*e.g.*, coronary heart disease, cardiomyopathy, endocarditis, congenital cardiovascular defects, congestive heart failure), medications, and non-cardiac diseases (*e.g.*, high blood pressure, diabetes, viruses). In some embodiments, cardiac injury is caused by heart failure (*e.g.*, HFrEF). In some embodiments, cardiac injury is caused by myocardial infarction.

In some embodiments, cardiac injury is caused by ischemia. In some embodiments, cardiac injury comprises an injured ventricle (*e.g.*, left ventricle). In some embodiments, a cardiac cell therapy (*e.g.*, a cardiac graft) is administered to a subject in need thereof, such as a subject having experienced cardiac injury. A subject may be any mammal, including, but not limited to, mice, rats, guinea pigs, hamsters, pigs, cows, sheep, goats, horses, and primates, including humans.

In some embodiments, a cardiac cell therapy (*e.g.*, a cardiac graft) disclosed herein, when administered to a subject, is associated with a reduced risk of graft-induced arrhythmia compared to other cardiac cell therapies. Graft-induced arrhythmias (GIAs) are arrhythmias in the heart of a subject to whom cardiac cell therapy (*e.g.*, a cardiac graft) has been administered, and which are presumed to be caused, wholly or in part, by the cardiac cell therapy. Arrhythmia, also known as dysrhythmia, can refer to an irregularity in cardiac activity (*e.g.*, rate, rhythm). Non-limiting examples of arrhythmias include: extrasystole, supraventricular arrhythmia (*e.g.*, paroxysmal supraventricular tachycardia (PSVT), accessory pathway tachycardia, AV nodal re-entrant tachycardia (AVNRT), Atrial tachycardia, Atrial fibrillation, Atrial flutter), ventricular arrhythmia (*e.g.*, Premature ventricular contractions (PVCs), ventricular tachycardia (V-tach), ventricular fibrillation (V-fib), or long QT), and bradyarrhythmia (*e.g.*, Sinus node dysfunction or heart block). In some embodiments, a GIA is tachycardia. In some embodiments, a GIA is ventricular tachycardia, such as sustained ventricular tachycardia. In some embodiments, a cardiac cell therapy disclosed herein reduces the risk of GIA (*e.g.*, sustained ventricular tachycardia).

A GIA may occur after a cardiac graft. GIAs (*e.g.*, ventricular tachycardia) may be caused at least in part by a subpopulation of cells (*e.g.*, atrial cells, pacemaker cells, conduction system cells, and/or other cardiac or non-cardiac subpopulation of cells responsible or contributing to graft associated arrhythmia). A GIA may be caused, for example, by a subpopulation of stem cell-derived cardiomyocytes. GIAs may be transient or sustained (*e.g.*, persistent, or chronic). A GIA may be associated without adverse symptoms. In some embodiments, a graft-induced arrhythmia is associated with adverse symptoms of which non-limiting examples include palpitations, lightheadedness, shortness of breath, chest pain, decreased levels of consciousness, stroke, heart failure, or death.

In some embodiments, a graft-induced arrhythmia can be treated or managed such that adverse symptoms are reduced or eliminated. In some embodiments, a graft-induced arrhythmia can be treated such that normal cardiac activity is restored. In some embodiments, a graft-

induced arrhythmia is a graft-induced arrhythmia. Graft-induced arrhythmias (GIA) are arrhythmias which are not fully eliminated by treatment (*e.g.*, administration of antiarrhythmic drugs and/or cardioversion). In some embodiments, GIAs are temporarily eliminated or reduced, such that the arrhythmia recurs even with treatment. In some embodiments, GIAs are not eliminated or reduced by administration of antiarrhythmic drugs. In some embodiments, GIAs are not eliminated or reduced by cardioversion (*e.g.*, pharmacological, electrical). Table 3 below summarizes graft-induced arrhythmias observed in Yorkshire pig models of myocardial infarction, as described in Example 1.

Table 3. Arrhythmia Scores in Yorkshire Pig Models of Myocardial Infarction

Score	Definition	Risk
1	Pig is BAR; no/minimal PVCs and/or non-sustained arrhythmia	Low GIA
2	Pig is BAR; PVCs with infrequent runs of no sustained arrhythmia or sustained arrhythmia; HR <150bpm at rest; increase in antiarrhythmic drug dosage reduces score to 1	Low GIA
3	Pig is BAR; non-sustained arrhythmia and/or sustained arrhythmia lasting <1hr; HR fluctuates but remains <150 bpm at rest; increase in antiarrhythmic drug dosage reduces score to 2	Low GIA
4	Sustained arrhythmia for ≥12hrs; HR >150bpm at rest; cardioversion can revert pig back to normal sinus rhythm	High GIA
5	Severe sustained arrhythmia >12hrs; cannot be corrected by cardioversion or an increase in antiarrhythmic drug dosage	High GIA

GIA = graft-induced arrhythmia; BAR = bright, alert, and responsive; PVC = premature ventricular contractions; non-sustained GIA = GIA lasting < 30s; sustained GIA = GIA lasting ≥30s;

In some embodiments, a cardiac graft is administered to a subject which may have or may receive mechanical circulatory support before, after, or at the time of receiving the cardiac graft. In some embodiments the mechanical circulatory support may be a Left Ventricular Assist Device (LVAD), a Right Ventricular Assist Device (RVAD), a Bi Ventricular Assist Device (BiVAD), Extra Corporeal Membrane Oxygenation (ECMO), or Implantable Cardiac Defibrillator (ICD), or a combination thereof. In certain embodiments, the ICD is linked by a biventricular pacer. In some embodiments, mechanical circulatory support may be an intravascular, microaxial blood pump (such as Impella 5.0). In some embodiments, this mechanical circulatory support is ceased after the subject receives a cardiac graft. In some embodiments, the mechanical circulatory support is used or implanted on or to the subject prior to the administration of the cardiac graft.

Cardiac cell therapies (*e.g.*, cardiac grafts) are typically administered directly to the heart of a subject and may be administered to a subject through any suitable method; non-limiting examples include open surgical approaches (*e.g.*, direct administration to the heart during open

heart surgery), minimally invasive approaches (*e.g.*, administration via a cardiac catheter or needle), or percutaneous/intravascular approaches.

In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) is a cellular composition comprising a plurality of mature cardiomyocytes. In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises a plurality of mature cardiomyocytes wherein no more than 50% of the mature cardiomyocytes are high GIA mature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises mature cardiomyocytes wherein no more than 40% of the mature cardiomyocytes are high GIA mature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises mature cardiomyocytes wherein no more than 30% of the mature cardiomyocytes are high GIA mature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises mature cardiomyocytes wherein no more than 20% of the mature cardiomyocytes are high GIA mature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises mature cardiomyocytes wherein no more than 10% of the mature cardiomyocytes are high GIA mature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA).

In some embodiments, a cardiac graft comprises mature cardiomyocytes produced from a batch of immature cardiomyocytes identified as being low GIA. In some embodiments, a batch of low GIA immature cardiomyocytes is contacted with a maturation cocktail under conditions that promote cardiomyocyte maturation. Standard methods for maturing cardiomyocytes are described in Guo *et al.*, *Circulation Research*, **2020**, 126, 1086-1106, incorporated herein by reference in its entirety. In some embodiments, mature cardiomyocytes are made by culturing low GIA immature cardiomyocyte on a substrate (*e.g.*, adherent culture) for at least 2 weeks (*e.g.*, between 2 and 3, between 3 and 4, between 4 and 5, between 5 and 6, between 6 and 8, between 8 and 10, or between 10 and 12 weeks, inclusive, or more than 12 weeks) in culture media comprising a maturation cocktail. In some embodiments, low GIA mature cardiomyocytes are made by culturing low GIA immature cardiomyocyte on a substrate (*e.g.*, adherent culture) for at least 2 weeks (*e.g.*, between 2 and 3, between 3 and 4, between 4 and 5, between 5 and 6, between 6 and 8, between 8 and 10, or between 10 and 12 weeks, inclusive, or more than 12 weeks) in culture media that promotes oxidative phosphorylation.

In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises a plurality of cells comprising immature cardiomyocytes. In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises immature cardiomyocytes wherein no more than 50% of the immature cardiomyocytes are high GIA immature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises immature cardiomyocytes wherein no more than 40% of the immature cardiomyocytes are high GIA immature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises immature cardiomyocytes wherein no more than 30% of the immature cardiomyocytes are high GIA immature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises immature cardiomyocytes wherein no more than 20% of the immature cardiomyocytes are high GIA immature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA). In some embodiments, a cardiac cell therapy (*e.g.*, cardiac graft) comprises immature cardiomyocytes wherein no more than 10% of the immature cardiomyocytes are high GIA immature cardiomyocytes (*e.g.*, have a molecular profile associated with a high risk of GIA).

In some embodiments, a cardiac graft comprises immature cardiomyocytes produced from a batch of low GIA cardiomyocyte precursors. In some embodiments, a batch of low GIA cardiomyocyte precursors is contacted with a differentiating agent under conditions that promote cardiomyocyte differentiation. Differentiating agents for cardiac cells are known by those of skill in the art. In some embodiments, immature cardiomyocytes are made by culturing low GIA cardiomyocyte precursors on a substrate (*e.g.*, adherent culture) for at least 2 weeks (*e.g.*, between 2 and 3, between 3 and 4, between 4 and 5, between 5 and 6, between 6 and 8, between 8 and 10, or between 10 and 12 weeks, inclusive, or more than 12 weeks) in culture media comprising a differentiating agent.

In some embodiments, a cardiac cell therapy is a cardiac graft. In some embodiments, a cardiac graft comprises about 100 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 200 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 300 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 400 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 500 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 600

million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 700 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 800 million to about 1 billion cardiomyocytes. In some embodiments, a cardiac graft comprises about 900 million to about 1 billion cardiomyocytes.

EXAMPLES

Example 1. Identification of molecular markers linked to graft-induced arrhythmias.

Graft-induced arrhythmia observed in myocardial infarction model pigs.

Graft-induced arrhythmia (GIA) poses a significant barrier to administration of cardiac stem cell therapies to human subjects. To identify structural and functional differences between high and low GIA cardiomyocyte (CM) batches, human pluripotent stem cells (hPSCs) were differentiated and matured into mature CMs, then transplanted into pig models of myocardial infarction (MI). FIG 1A shows a 2-3-month protocol for cardiomyocyte implantation and recovery. MI was induced in Yorkshire pigs between 25-40kg through balloon occlusion of the mid left anterior descending coronary artery for 90-120 minutes, followed by reperfusion. CM were delivered 3-5 weeks post-MI. Vascular access ports (VAP) were implanted during MRI-guided surgery 7 days prior to CM graft transplant. Prepared hPSC-derived cardiomyocytes were implanted along with a telemetry device, after which electrocardiographic activity was recorded until the end of the protocol; pigs were also administered immunosuppressants through the end of the protocol. Typically, pigs were monitored for 28-56 days, after which they were sacrificed and underwent necropsy; however, several pigs displayed sustained graft-induced arrhythmia (GIA). Table 3 above shows criterion for determining sustained GIA in MI pigs.

FIG. 1B shows an EKG recording of an exemplary MI pig displaying anti-arrhythmic drug-resistant and cardioversion-resistant sustained GIA as early as 48 hours post transplantation. As shown in FIG. 1C, after necropsy, CMs associated with GIA scores of 4 or 5 were deemed “high GIA” and CMs associated with GIA scores of 1-3 were designated “low GIA”.

scRNA-seq analysis identifies markers associated with high GIA.

Nineteen samples of post-implantation CMs taken at day 51 were evaluated using single-cell RNA sequencing (Table 4). Quality control of scRNA-seq data was performed by evaluating the percent mitochondrial transcripts and the number of genes obtained (FIGs. 2A-2B). Batch corrected single-cell transcriptomic data was visualized using Uniform Manifold Approximation

and Projection (UMAP) to ensure the removal of batch effects due to GIA status or protocol used (FIGs. 2C-2D). Clustering of gene expression data revealed 10 clusters, which were classified as a cardiomyocyte (CM) population, a fibroblast (FB) population, or an endothelial cell (EC) population based on differential expression of cell-type-specific canonical gene markers (*e.g.*, cardiomyocytes were identified by expression of *TNNT2*, *MYL7*, *MYL2*; fibroblasts were identified by expression of *COL1A1*, *THY1*, *SPARC*, *COL2*; endothelial cells were identified by expression of *PEACAM1*, *PODXL*, *SPARC*) (FIG. 2E). Differential gene expression analysis revealed that the majority of cells obtained were cardiomyocytes (FIG. 2F); a smaller subset of cells were fibroblasts (FIG. 2G). Cell maturity was also evaluated using a global proliferation cell marker *MKI67* (FIG. 2H) as well as two cardiomyocyte-specific markers *MYL7* and *MYL2*, which are markers of immature and mature cardiomyocytes, respectively (FIGs. 2I-2J). A subset of cells (cycle_CM) was identified that had high expression of *MKI67*, *MYL7*, and *MYL2*, suggesting that a portion of cardiomyocytes had entered the cell cycle.

Table 4. Sample overview.

#	Sample	Process	GIA status
1	109388-Run4	Medium 1	Low GIA
2	109388-Run5	Medium 1	High GIA
3	109388-Run6	Medium 1	High GIA
4	109388-Run10	Medium 1	Low GIA
5	109388-Run11	Medium 1	High GIA
6	109388-Run12	Medium 2	Low GIA
7	109388-Run13	Medium 2	Low GIA
8	111106-Run1	Medium 2	Unknown
9	111106-Run2	Medium 2	Unknown
10	111106-Run3	Medium 2	OOS
11	111106-Run4	Medium 2	OOS
12	111106-RUN5	Medium 2	UK
13	111106-RUN6d	Medium 2	UK
14	111106-RUN6m	Medium 2	UK
15	111482-TT3-MANUAL	Medium 2	UK
16	111482-TT3-AUTO	Medium 2	UK
17	112039-PD1	Medium 2	UK
18	112039-PD2	Medium 2	High GIA
19	112039-PD3	Medium 2	High GIA

OOS = out of spec; indicates <85% of cells expressed *TNNT2*, a cardiac cell marker, as determined by flow cytometry; unknown indicates that a pig study was not performed in the sample.

Evaluation of cell-specific markers revealed phenotypic heterogeneity across the ten clusters (FIG. 3A). Within the cardiomyocyte populations, only 5 of the 8 clusters showed high expression of genes involved in the ultrastructure maturity axis, metabolism maturity axis, calcium handling maturity axis, and electrophysiology maturation axis (FIG. 3B). There were also subclusters within the cardiomyocyte populations that showed a lag in phenotypic maturity (*e.g.*, CM_5, CM_7, and CM_3, as evidenced by the absence of expression of genes involved in the ultrastructure, metabolism, calcium, and electrophysiology maturation axes). Additionally, gene expression across cardiomyocytes was found to be influenced by the bioprocess (FIG. 3C-3E). This suggested that bulk predictors of GIA were not predictive across bioprocesses. However, evaluation of the cardiomyocyte clusters revealed that a significant percent of cells belonging to the CM_5 cluster came from High GIA samples (FIG. 3F). Further, evaluation of the High and Low GIA cells revealed that a majority of the CM_5 cells were prepared using a particular maturation bioprocess (Medium 2) (FIG. 3G). The cluster distribution by batch is provided in Table 5 and in FIGs. 3H-3I.

Table 5. Cluster distribution by batch.

#	Sample	Medium	GIA status	CM_1	CM_2	CM_3	CM_4	CM_5	CM_6	CM_7	EC	FB	Cycle_CM
1	109388-Run4	Med. 1	Low GIA	74	7.39	1.06	1.61	4.16	0.65	0	0.32	2.87	7.94
2	109388-Run5	Med. 1	High GIA	74.98	5.42	1.19	1.23	8.11	0.7	0	0.18	2.86	5.33
3	109388-Run6	Med. 1	High GIA	76.05	2.83	1.01	1.23	9.51	0.17	0.04	0.25	1.82	7.1
4	109388-Run10	Med. 1	Low GIA	77.19	6.57	0.46	0.56	6.67	0.4	0	0.1	5.25	2.81
5	109388-Run11	Med. 1	High GIA	48.67	9.04	3.11	4.37	29.19	0.04	0	0	0.19	5.41
6	109388-Run12	Med. 2	Low GIA	50.35	13.24	1.85	9.92	11.77	0.04	0.57	0.16	0.7	11.4
7	109388-Run13	Med. 2	Low GIA	45.28	21.82	2.71	9.21	8.55	0.23	0.26	0.07	0.96	10.92
8	111106-Run1	Med. 2	UK	33.21	1.68	1.59	6.03	28.48	1.3	0.75	0.84	12.02	14.11
9	111106-Run2	Med. 2	UK	30.2	0.31	0.35	4.12	41.27	1.89	0.7	1.54	6.08	13.55
10	111106-Run3	Med. 2	OOS	29.31	1.42	1.65	6.02	32.76	1.88	0.23	1.92	15.84	9.98
11	111106-Run4	Med. 2	OOS	30.7	2.56	1.74	10.86	18.77	1.34	0.12	0.98	22.03	10.89
12	111106-RUN5	Med. 2	UK	28.54	3.83	3.12	9.76	18.84	7.45	0.12	1.24	15.72	11.37
13	111106-RUN6d	Med. 2	UK	22.18	1.84	1.37	5.7	12.29	1.84	0.19	0.66	42.51	11.44

14	111106-RUN6m	Med. 2	UK	20.02	2.69	1.01	4.91	11.68	3.15	1.05	1.09	44.51	9.89
15	111482-TT3-MANUAL	Med. 2	UK	29.18	2.55	0.73	4.67	23.02	0.89	0.79	20.34	8.98	8.84
16	111482-TT3-AUTO	Med. 2	UK	26.25	5.01	1.77	8.09	29.84	3.34	0.05	8.35	4.81	12.49
17	112039-PD1	Med. 2	UK	20.35	18.33	1.34	8.33	38.61	0.14	0.14	1.09	1.05	10.61
18	112039-PD2	Med. 2	High GIA	18.17	15.6	1.36	6.73	44.54	0.4	0.12	2.11	1.7	9.27
19	112039-PD3	Med. 2	High GIA	22.61	18.53	1.26	6.04	37.73	0.97	0	1.85	1.72	9.29

OOS indicates <85% of cells expressed *TNNT2*, a cardiac cell marker, as determined by flow cytometry; UK (unknown) indicates that a pig study was not performed in the sample.

Gene expression analysis of the CM_5 cluster showed that reduced expression of Calcium Voltage-Gated Channel subunit Alpha 1 C (*CACNA1C*) and Sodium Voltage-Gated Channel Alpha Subunit 5 (*SCN5A*) and increased expression of BMP/Retinoic Acid Inducible Neural Specific 3 (*BRINP3*), Butyrylcholinesterase (*BCHE*), Synaptosome Associated Protein 91 (*SNAP91*), Roundabout Guidance Receptor 2 (*ROBO2*), and Copine 5 (*CPNE5*), relative to all other cardiomyocyte clusters (FIG. 4A). The expression of *SCN5A* and *CACNA1C*, two key electrophysiological genes, were shown to be most highly expressed in the CM_1 cluster and existed as a gradient within the cluster (FIGs. 4B-4C). For example, cells within the CM_1 cluster near the CM_4 cluster had higher expression of *SCN5A* and *CACNA1C* than cells in the CM_1 cluster near the CM_2 cluster. Since cardiac maturation is known to be a complex, multi-dimensional phenotypic trajectory through various states, genes involved in these axes were also evaluated. For example, Gap Junction Protein Alpha 1 (*GAPJ1*), which is involved myofibril structure maturation, and Cytochrome C Oxidase Subunit 6A2 (*COX6A2*), which is involved in metabolism maturation, were also found to be expressed as a gradient across the clusters, suggesting that the cardiomyocyte clusters may represent different states of maturity (FIGs. 4D-4E). Twenty genes were identified as being highly expressed ($|\text{fold change}| > 1.5$) in the CM_5 cluster, with varying degrees of specificity (FIG. 5). For example, the Iroquois Homeobox 2 (*IRX2*) gene was found to be highly specific to the cells belonging to the CM_5 cluster. In contrast, Proprotein Convertase subtilisin/Kexin Type 1 (*PCSK1N*) was found to be expressed in many cardiomyocyte clusters but most highly expressed in cells belonging to the CM_5 cluster. The average expression of each of the genes in the CM_5 cluster and, as well as their expression in non-CM_5 cardiomyocyte clusters, non-cardiomyocyte clusters, all cardiomyocytes, and the difference between the expression in the CM_5 cluster and non-CM_5 cardiomyocyte clusters is

shown in Table 6. These results suggest that CM_5 could represent atypically maturing cardiomyocytes.

Table 6. Average gene expression of top 20 CM_5 gene markers.

Gene	CM_i	Rest_CMs	OT	CM	CMi-rCM
<i>BRINP3</i>	5.61	1.67	0.23	2.67	3.94
<i>BCHE</i>	4.69	1.61	2.25	2.40	3.08
<i>POSTN</i>	4.43	1.60	4.18	2.32	2.83
<i>ID4</i>	4.84	2.02	7.61	2.74	2.82
<i>SNAP91</i>	5.20	2.67	0.33	3.32	2.52
<i>ROBO2</i>	5.10	2.62	0.90	3.25	2.48
<i>CPNE5</i>	4.14	1.67	0.31	2.30	2.47
<i>NEGR1</i>	3.67	1.62	0.55	2.14	2.06
<i>IRX2</i>	3.82	1.78	0.62	2.30	2.04
<i>AEBP1</i>	3.73	1.84	6.62	2.32	1.89
<i>ITM2A</i>	3.75	2.03	2.25	2.47	1.73
<i>PCSKIN</i>	5.31	3.59	2.26	4.03	1.72
<i>KIF1A</i>	4.62	3.09	1.10	3.48	1.53
<i>GUCY1A</i>	4.82	3.51	3.06	3.84	1.32
<i>COL19A1</i>	3.97	2.82	0.52	3.11	1.16
<i>B3GALT2</i>	4.35	3.35	1.41	3.60	1.01
<i>NLGN1</i>	4.24	3.25	0.35	3.50	0.99
<i>HSPB2</i>	4.68	3.79	0.34	4.02	0.88
<i>RGN</i>	3.56	2.68	0.32	2.90	0.87
<i>CXXC4</i>	3.55	2.80	0.30	2.99	0.75

CM_i = CM_5 cluster; rest_CMs = non-CM_5 cardiomyocyte clusters; OT = non-cardiomyocyte clusters; CM = all cardiomyocyte clusters; CMi-rCM = difference between CM_5 and no-CM_5 cardiomyocyte clusters.

scRNA-seq analysis identifies markers associated with axes of cardiac maturation.

Typical cardiac maturation comprises a complex, multi-dimensional phenotypic trajectory through canonical maturation states. Although these states are highly interrelated, typical maturation can be measured along many axes, such as myofibril structure, electrophysiology, calcium handling, metabolism, and ultrastructure. Maturation of each of the

axes was evaluated in the gene expression data obtained from the 19 day 51 samples described above.

Mature cardiomyocytes typically express myofibrillar markers including Myosin Binding Protein C3 (*MYBPC3*), Actinin Alpha 2 (*ACTN2*), Titin (*TTN*), and Desmin (*DES*) (FIGs. 6A-6D), all of which were uniquely expressed in all of the cardiomyocyte clusters. Some gradients of myofibrillar genes could also be detected, indicating that several cardiomyocyte cluster may represent a different stage of myofibrillar structure development. Three gene pairs in particular were evaluated: Myosin Heavy Chain 6 (*MYH6*) and Myosin Heavy Chain 7 (*MYH7*); Myosin Light Chain 7 (*MYL7*) and Myosin Light Chain 2 (*MYL2*); and Troponin I1, Slow Skeletal Type (*TNNI1*) and Troponin I3, Cardiac Type (*TNNI3*). *MYH6*, *MYL7*, and *TNNI1* are three myofibrillar genes not associated with typical mature cardiomyocytes, while *MYH7*, *MYL2*, and *TNNI3* are three myofibrillar genes associated with typical mature cardiomyocyte. Analysis of gene expression of each of these genes revealed that of the cardiomyocytes, CM_5 highly expresses the three markers of atypical myofibrillar structure development (FIGs. 6E-6G). The CM_5 cluster exhibited a gradient of *MYH7* and *MYL2*, further supporting the interpretation that cells in the CM_5 cluster lack typical development markers.

Most canonical cardiomyocyte ion channels showed a clear pattern of increased expression consistent with the myofibrillar structure axis (FIGs. 7A-7E). However, some ion channels which are not typically expressed in mature cardiomyocytes showed elevated expression in certain cardiomyocyte clusters. For example, Sodium Voltage-Gated Channel Alpha Subunit 5 (*SCN5A*) is the main voltage-gated sodium channel expressed in cardiomyocytes, while other voltage-gated sodium channels such as those encoded by *SCN9A* and *SCN3A* are not typically expressed in mature cardiomyocytes. Projection of gene expression values of *SCN5A* onto UMAP space revealed low expression in the CM_5 cluster, while the expression of *SCN9A* and *SCN3A* was more highly expressed in the CM_5 cluster (FIG. 7F). Evaluation of genes encoding potassium voltage-gated channels showed a similar pattern (FIGs. 7G-7H).

In contrast, calcium handling appeared consistent across all clusters, as evidenced by similar expression levels of ATPase Sarcoplasmic/Endoplasmic Reticulum Ca²⁺ Transporting 2 (*ATP2A2*), Ryanodine Receptor 2 (*RYR2*), Solute Carrier Family 8 Member A1 (*SLC8A1*), and Protein Kinase CAMP-Activated Catalytic Subunit Alpha (*PRKACA*) across all cardiomyocyte clusters (FIGs. 8A-8D). However, a clear gradient of expression was found for CAMK-related genes (FIG. 8E) and Junctophilin 2 (*JPH2*), an essential component of T-tubule function (FIG.

8F). Gene expression gradients corresponding to genes involved in metabolism maturity, such as PPARA signaling, mitochondrial metabolism, and fatty acid oxidation, as well as genes involved in ultrastructure maturity were also found (FIGs. 9A-10D).

Taken together, these results support characterization of the CM_5 cluster as lacking typical development across electrophysiology, structure (myofibrillar and ultrastructure), and metabolism axes.

EQUIVALENTS AND SCOPE

In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes aspects in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes aspects in which more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process.

Furthermore, the invention encompasses all variations, combinations, and permutations in which one or more limitations, elements, clauses, and descriptive terms from one or more of the listed claims is introduced into another claim. For example, any claim that is dependent on another claim can be modified to include one or more limitations found in any other claim that is dependent on the same base claim. Where elements are presented as lists, *e.g.*, in Markush group format, each subgroup of the elements is also disclosed, and any element(s) can be removed from the group. It should be understood that, in general, where the invention, or aspects of the invention, is/are referred to as comprising particular elements and/or features, certain aspects of the invention or aspects of the invention consist, or consist essentially of, such elements and/or features. For purposes of simplicity, those aspects have not been specifically set forth in haec verba herein.

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, *e.g.*, elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, *e.g.*, “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically

identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one aspect, to A only (optionally including elements other than B); in another aspect, to B only (optionally including elements other than A); in yet another aspect, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, *e.g.*, the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (*e.g.*, “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one aspect, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another aspect, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another aspect, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, *e.g.*, to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United States Patent Office Manual of Patent Examining Procedures, Section 2111.03. It should be appreciated that aspects described in this document using an open-ended transitional phrase (*e.g.*, “comprising”) are also contemplated, in alternative aspects, as “consisting of” and “consisting essentially of” the feature described by the open-ended transitional phrase. For example, if the application describes “a composition comprising A and B,” the application also contemplates the alternative aspects “a composition consisting of A and B” and “a composition consisting essentially of A and B.”

Where ranges are given, endpoints are included. Furthermore, unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or sub-range within the stated ranges in different aspects of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

This application refers to various issued patents, published patent applications, journal articles, and other publications, all of which are incorporated herein by reference. If there is a conflict between any of the incorporated references and the instant specification, the specification shall control. In addition, any particular aspect of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Because such aspects are deemed to be known to one of ordinary skill in the art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular aspect of the invention can be excluded from any claim, for any reason, whether or not related to the existence of prior art.

Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation many equivalents to the specific aspects described herein. The scope of the present aspects described herein is not intended to be limited to the above Description, but rather is as set forth in the appended claims. Those of ordinary skill in the art will appreciate that

various changes and modifications to this description may be made without departing from the spirit or scope of the present invention, as defined in the following claims.

The recitation of a listing of chemical groups in any definition of a variable herein includes definitions of that variable as any single group or combination of listed groups. The recitation of an aspect for a variable herein includes that aspect as any single aspect or in combination with any other aspects or portions thereof. The recitation of an aspect herein includes that aspect as any single aspect or in combination with any other aspects or portions thereof.

CLAIMS

What is claimed is:

1. A method of preparing a cellular composition for cardiac cell therapy, the method comprising:
 - (a) determining that a plurality of cardiomyocytes do not have a molecular profile associated with a high risk of graft-induced arrhythmia (GIA); and
 - (b) preparing a cellular composition for cardiac cell therapy from the plurality of cardiomyocytes.
2. The method of claim 1, wherein the plurality of cardiomyocytes represents greater than 80% of all cardiomyocytes used to prepare the cardiac cell therapy.
3. The method of claim 1 or 2, wherein the plurality of cardiomyocytes is obtained by:
 - (i) determining a molecular profile of a batch of cells;
 - (ii) selecting the plurality of cardiomyocytes from the batch of cells, wherein the plurality of cardiomyocytes does not have the molecular profile associated with high risk of GIA.
4. The method of claim 3, wherein the molecular profile of the batch of cells is obtained from a transcriptome of the batch of cells.
5. The method of claim 4, wherein the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq).
6. The method of claim 3, wherein the molecular profile of the batch of cells is obtained via flow cytometry.
7. The method of any one of claims 1 to 6, wherein the molecular profile associated with a high risk of GIA comprises negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2.

8. The method of claim 7, wherein the molecular profile associated with a high risk of GIA comprises negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A.
9. The method of claim 8, wherein the molecular profile associated with a high risk of GIA comprises negative/low expression of SCN5A.
10. The method of any one of claims 1 to 9, wherein the molecular profile associated with a high risk of GIA comprises positive/high expression of one or more genes selected from: AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91.
11. The method of claim 10, wherein the molecular profile associated with a high risk of GIA comprises positive/high expression of any one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A.
12. The method of any one of claims 1 to 11, wherein the molecular profile associated with a high risk of GIA comprises positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A.
13. The method of any one of claims 1 to 12, wherein the plurality of cardiomyocytes comprises mature cardiomyocytes.
14. The method of claim 13, wherein the mature cardiomyocytes are derived from pluripotent stem cells.
15. The method of claim 14, wherein the pluripotent stem cells express one or more of the following markers: SSCA3/4, TRA-160, OCT3/4, NANOG, and SOX2.
16. The method of claim 14, wherein the mature cardiomyocytes are derived from embryonic stem cells.
17. The method of any one of claims 13 to 16, wherein the mature cardiomyocytes comprise positive/high expression of MLC2v and CTNT2; and negative/low expression of MLC2a.

18. The method of any one of claims 13 to 17, wherein preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a physiologically acceptable medium.
19. The method of any one of claims 1 to 12, wherein the plurality of cardiomyocytes comprises immature cardiomyocytes.
20. The method of claim 19, wherein the immature cardiomyocytes are derived from pluripotent stem cells.
21. The method of claim 20, wherein the pluripotent stem cells express one or more of the following markers: SSCA3/4, TRA-160, OCT3/4, NANOG, SOX2.
22. The method of claim 19, wherein the immature cardiomyocytes are derived from embryonic stem cells.
23. The method of any one of claims 19 to 22, wherein the immature cardiomyocytes comprise positive/high expression of cTNT, MYL2, MYL7, KCNJ2, CACNA1C, CACNA1H, SCN5A, HCN4 and negative/low expression of MKI67, CD90.
24. The method of any one of claims 19 to 23, wherein preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.
25. A method of preparing a cellular composition for cardiac cell therapy, the method comprising:
- (a) determining that a plurality of cardiomyocytes have a molecular profile associated with a low risk of graft-induced arrhythmia (GIA); and
 - (b) preparing a cellular composition for cardiac cell therapy from the plurality of cardiomyocytes.
26. The method of claim 25, wherein the plurality of cardiomyocytes represents greater than 80% of all cardiomyocytes used to prepare the cardiac cell therapy.
27. The method of claim 25 or 26, wherein the plurality of cardiomyocytes is obtained by:
- (i) determining a molecular profile of a batch of cells;

(ii) selecting the plurality of cardiomyocytes from the batch of cells, wherein the plurality of cardiomyocytes does not have the molecular profile associated with low risk of GIA.

28. The method of claim 27, wherein the molecular profile of the batch of cells is obtained from a transcriptome of the batch of cells.

29. The method of claim 28, wherein the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq).

30. The method of claim 27, wherein the molecular profile of the batch of cells is obtained via flow cytometry.

31. The method of any one of claims 25 to 30, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: ACOX1, CACNB1C, CAMK2A, CAMK2B, COX6B2, CPT1A, DSG2, FGF12, GJB1, ITGB7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2.

32. The method of claim 31, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: CACNB1C, COX6B2, GJB1, KCNH2, MYH7, MYL2, PPARA, and SCN5A.

33. The method of claim 32, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of SCN5A.

34. The method of any one of claims 25 to 33, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from: AEBP1, B3GALT2, BCHE, BRINP3, COL19B1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91.

35. The method of claim 34, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of any one or more genes selected from: BCHE, BRINP3, EPHB4, ID4, IRX2, and SCN3A.

36. The method of any one of claims 25 to 35, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from BCHE, BRINP3, EPHB4, ID4, and IRX2 and positive/high expression of SCN5A.

37. The method of any one of claims 25 to 36, wherein the plurality of cardiomyocytes comprises mature cardiomyocytes.
38. The method of claim 37, wherein the mature cardiomyocytes are derived from pluripotent stem cells.
39. The method of claim 38, wherein the pluripotent stem cells express one or more of the following markers: SSCB3/4, TRA-160, OCT3/4, NANOG, and SOX2.
40. The method of claim 38, wherein the mature cardiomyocytes are derived from embryonic stem cells.
41. The method of any one of claims 37 to 40, wherein the mature cardiomyocytes comprise negative/low expression of MLC2v and CTNT2; and positive/high expression of MLC2a.
42. The method of any one of claims 37 to 41, wherein preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality of cardiomyocytes with a physiologically acceptable medium.
43. The method of any one of claims 25 to 36, wherein the plurality of cardiomyocytes comprises immature cardiomyocytes.
44. The method of claim 43, wherein the immature cardiomyocytes are derived from pluripotent stem cells.
45. The method of claim 44, wherein the pluripotent stem cells express one or more of the following markers: SSCB3/4, TRA-160, OCT3/4, NANOG, SOX2.
46. The method of claim 43, wherein the immature cardiomyocytes are derived from embryonic stem cells.
47. The method of any one of claims 43 to 46, wherein the immature cardiomyocytes comprise negative/low expression of cTNT, MYL2, MYL7, KCNJ2, CACNB1C, CACNB1H, SCN5A, HCN4 and positive/high expression of MKI67, CD90.
48. The method of any one of claims 43 to 47, wherein preparing the cellular composition for cardiac cell therapy from the plurality of cardiomyocytes comprises contacting the plurality

of cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.

49. A method of preparing a batch of cells for cardiac cell therapy, the method comprising:
- (a) determining that an at least first batch of cells among a plurality of batches comprises a molecular profile associated with a low risk of graft-induced arrhythmia (GIA) and;
 - (b) preparing the at least first batch of cells for cardiac cell therapy.
50. The method of claim 49, wherein the molecular profile of the at least first batch of cells is obtained from a transcriptome of the batch of cells.
51. The method of claim 50, wherein the transcriptome is obtained via single cell ribonucleic acid sequencing (scRNA-Seq).
52. The method of claim 50, wherein the molecular profile of the at least first batch of cells is obtained via flow cytometry.
53. The method of any one of claims 49 to 52, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: ACOX1, CACNC1C, CAMK2A, CAMK2B, COX6C2, CPT1A, DSG2, FGF12, GJC1, ITGC7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2.
54. The method of claim 53, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of one or more genes selected from: CACNC1C, COX6C2, GJC1, KCNH2, MYH7, MYL2, PPARA, and SCN5A.
55. The method of claim 54, wherein the molecular profile associated with a low risk of GIA comprises positive/high expression of SCN5A.
56. The method of any one of claims 49 to 52, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from: AEBP1, C3GALT2, BCHE, BRINP3, COL19C1, CPNE5, CXXC4, GUCY1A, HSPC2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91.

57. The method of claim 56, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of any one or more genes selected from: BCHE, BRINP3, EPHC4, ID4, IRX2, and SCN3A.

58. The method of any one of claims 49 to 57, wherein the molecular profile associated with a low risk of GIA comprises negative/low expression of one or more genes selected from BCHE, BRINP3, EPHC4, ID4, and IRX2 and positive/high expression of SCN5A.

59. The method of any one of claims 49 to 58, wherein the at least first batch of cells comprises mature cardiomyocytes.

60. The method of claim 59, wherein the mature cardiomyocytes are derived from pluripotent stem cells.

61. The method of claim 60, wherein the pluripotent stem cells express one or more of the following markers: SSCC3/4, TRA-160, OCT3/4, NANOG, SOX2.

62. The method of claim 59, wherein the mature cardiomyocytes are derived from embryonic stem cells.

63. The method of any one of claims 59 to 62, wherein the mature cardiomyocytes comprise positive/high expression of MLC2v, CTNT2; and negative/low expression of MLC2a.

64. The method of any one of claims 59 to 63, wherein preparing the mature cardiomyocytes for cardiac cell therapy comprises contacting the mature cardiomyocytes with a physiologically acceptable medium suitable for administration to a subject.

65. The method of any one of claims 49 to 58, wherein the at least first batch of cells comprises immature cardiomyocytes.

66. The method of claim 65, wherein the immature cardiomyocytes are derived from pluripotent stem cells.

67. The method of claim 66, wherein the pluripotent stem cells express one or more of the following markers: SSCC3/4, TRA-160, OCT3/4, NANOG, SOX2.

68. The method of claim 65, wherein the immature cardiomyocytes are derived from embryonic stem cells.

69. The method of any one of claims 65 to 68, wherein the immature cardiomyocytes comprise positive/high expression of cTNT, MYL2, MYL7, KCNJ2, CACNC1C, CACNC1H, SCN5A, HCN4 and negative/low expression of MKI67, CD90.
70. The method of any one of claims 65 to 69, wherein preparing the immature cardiomyocytes for cardiac cell therapy comprises contacting the immature cardiomyocytes with a maturation cocktail under conditions that promote cardiomyocyte maturation.
71. A method, comprising preparing a batch of cells for cardiac cell therapy, wherein the cells are characterized as having a molecular profile comprising one of the following:
- i) negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91;
 - ii) positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
 - iii) positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
 - iv) negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A;
 - v) negative/low expression of BCHE;
 - vi) negative/low expression of BRINP3;
 - vii) negative/low expression of EPHA4;
 - viii) negative/low expression of ID4;
 - ix) negative/low expression of IRX2; and
 - x) positive/high expression of SCN5A.
72. A method of differentiating a plurality of cardiomyocyte precursor cells, the method comprising contacting a plurality of cardiomyocyte precursor cells that have been determined to

have a molecular profile associated with a low risk of graft-induced arrhythmia with a medium comprising a differentiating agent under conditions that promote cardiomyocyte differentiation.

73. A method of maturing a plurality of immature cardiomyocytes, the method comprising contacting a plurality of immature cardiomyocytes that been determined to have a molecular profile associated with a low risk of graft-induced arrhythmia with a maturation cocktail under conditions that promote cardiomyocyte maturation.

74. A method of preparing a plurality of mature cardiomyocytes for cardiac cell therapy, the method comprising contacting a plurality of mature cardiomyocytes that been determined to have a molecular profile associated with a low risk of graft-induced arrhythmia with a physiologically acceptable medium suitable for administration to a subject in need of cardiac cell therapy.

75. A composition for cardiac cell therapy comprising:
a plurality of cells having molecular profile associated with low risk of ventricular tachycardia (VT); and
a physiologically acceptable medium.

76. The composition of claim 75, wherein the molecular profile associated with a low risk of GIA comprises one of the following:

- i) negative/low expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A, and SNAP91;
- ii) positive/high expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- iii) positive/high expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and negative/low expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- iv) negative/low expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and positive/high expression of SCN5A;
- v) negative/low expression of BCHE;
- vi) negative/low expression of BRINP3;

- vii) negative/low expression of EPHA4;
- viii) negative/low expression of ID4;
- ix) negative/low expression of IRX2; and
- x) positive/high expression of SCN5A.

77. A composition for cardiac cell therapy comprising: a plurality of cells in a physiologically acceptable medium suitable for administration to a patient in need thereof; wherein the plurality of cells comprises mature cardiomyocytes; and wherein no more than 20% of the mature cardiomyocytes comprise a molecular profile associated with a high risk of graft-induced arrhythmia (GIA).

78. The composition of claim 77, wherein the molecular profile associated with a high risk of GIA comprises one of the following:

- i) positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A and SNAP91;
- ii) negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
- iii) negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
- iv) positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A;
- v) positive/high expression of BCHE;
- vi) positive/high expression of BRINP3;
- vii) positive/high expression of EPHA4;
- viii) positive/high expression of ID4;
- ix) positive/high expression of IRX2; and
- x) negative/low expression of SCN5A.

79. A composition comprising a plurality of cells;
wherein the plurality of cells comprises immature cardiomyocytes; and
wherein no more than 20% of the immature cardiomyocytes have a molecular profile associated with a high risk of graft-induced arrhythmia (GIA).
80. The composition of claim 79, wherein the molecular profile associated with a high risk of GIA comprises one of the following:
- i) positive/high expression of one or more genes selected from AEBP1, B3GALT2, BCHE, BRINP3, COL19A1, CPNE5, CXXC4, GUCY1A, HSPB2, ID4, IRX2, ITM2A, KIF1A, NEGR1, NLGN1, POSTN, RGN, ROBO2, SCN3A and SNAP91;
 - ii) negative/low expression of one or more genes selected from: ACOX1, CACNA1C, CAMK2A, CAMK2B, COX6A2, CPT1A, DSG2, FGF12, GJA1, ITGA7, JPH2, KCNH2, KCNJ2, KCNJ5, KCNJ8, KCNQ1, MFN2, MYH7, MYL2, PPARA, RXRA, SCN5A, and TLN2;
 - iii) negative/low expression of one or more genes selected from: CACNA1C, COX6A2, GJA1, KCNH2, MYH7, MYL2, PPARA, and SCN5A; and positive/high expression of one or more genes selected from: BCHE, BRINP3, EPHA4, ID4, IRX2, and SCN3A;
 - iv) positive/high expression of one or more genes selected from BCHE, BRINP3, EPHA4, ID4, and IRX2 and negative/low expression of SCN5A;
 - v) positive/high expression of BCHE;
 - vi) positive/high expression of BRINP3;
 - vii) positive/high expression of EPHA4;
 - viii) positive/high expression of ID4;
 - ix) positive/high expression of IRX2; and
 - x) negative/low expression of SCN5A.

81. A method of treating a subject in need of cardiac cell therapy, the method comprising administering to the subject a cardiac cell therapy comprising a cellular composition prepared according to the method of any one of claims 1-48, a batch of cells prepared according to the method of any one of claims 49 to 71, or the composition of any one of claims 75-80.

82. A method of treating a subject in need of cardiac cell therapy, the method comprising:

- (a) determining that no more than 20% of cardiomyocytes in a batch of cells comprise a molecular profile associated with a high risk of graft-induced arrhythmia (GIA); and
- (b) administering the batch of cells or a portion thereof to the subject.

83. A method of treating a subject in need of cardiac cell therapy, the method comprising administering a plurality of cells to a subject, wherein the plurality of cells has been determined to have a molecular profile associated with low risk of graft-induced arrhythmia (GIA).

84. A method of treating a subject in need of cardiac cell therapy, the method comprising administering a plurality of cells to a subject, wherein the plurality of cells has a molecular profile associated with low risk of graft-induced arrhythmia (GIA).