Adult Human Ex-Vivo Models for Preclinical Cardiac Safety Assessment of Drugs

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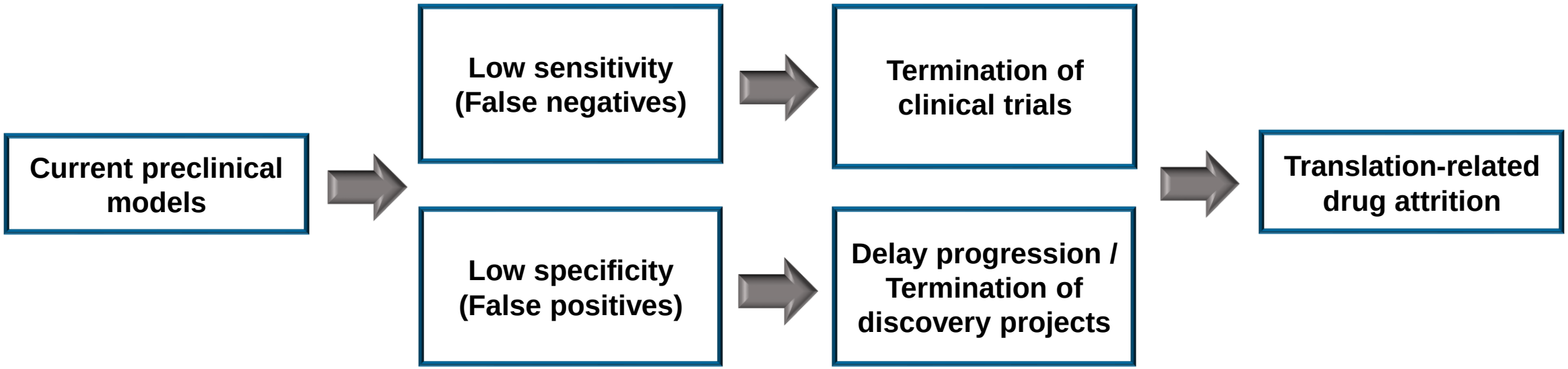
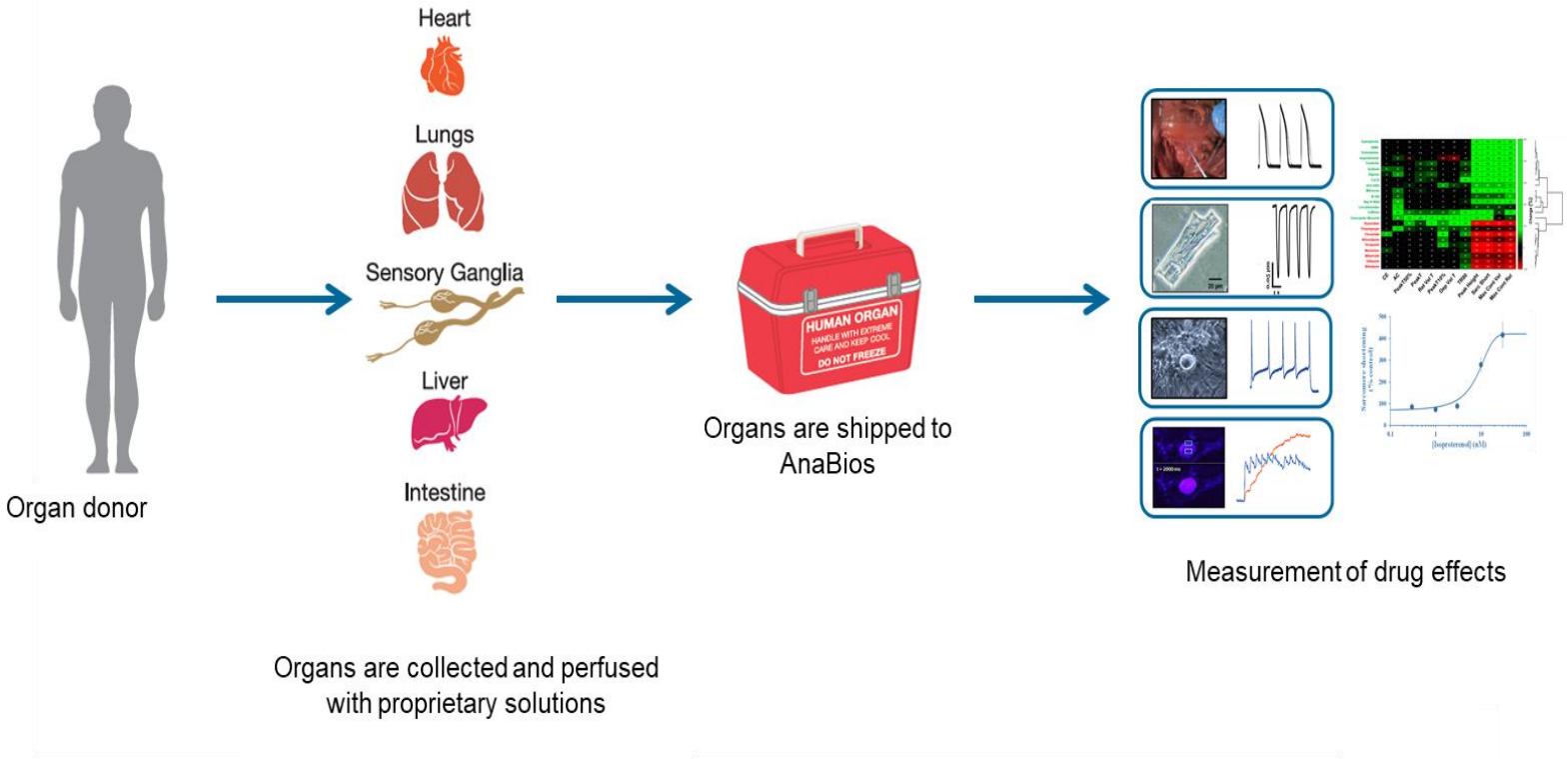
Current Preclinical Cardiac Safety Approaches
Have Significant Limitations



Human cardiac models are urgently needed

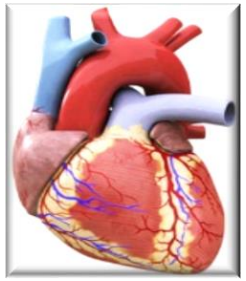
for detection of drug-induced cardiotoxicity

Enabling Drug Discovery
in Human Healthy and Diseased Tissues



- Tissue harvesting methods and solutions are designed to avoid ischemic damage and reperfusion injury
- Complete chain of custody, processing methods and rigorous QC ensure excellent tissue quality
- Large U.S.A.-based network ensures the availability of samples
- Excellent heart quality permits integrated human cardiac drug discovery at the preclinical stages

Human Ex-Vivo
Cardiac Safety Assessment at AnaBios



>1000 **ex vivo**
human hearts tested
2-4 hearts / week

CELL-BASED ASSAYS
(Optimization of drugs)

Arrhythmia & Inotropy
Ventricular or atrial myocytes contractility

Intracellular Ca^{2+} Dynamics
Ventricular Myocytes

Ion Channel Block
Ventricular or atrial myocytes V-clamp

Action Potential
Ventricular & Atrial Myocytes I-clamp

Cardiac Fibrosis
Cardiac Fibroblasts

TISSUE-BASED ASSAYS
(Nomination of drugs)

Pro-arrhythmia
Action Potential Ventricular Trabeculae

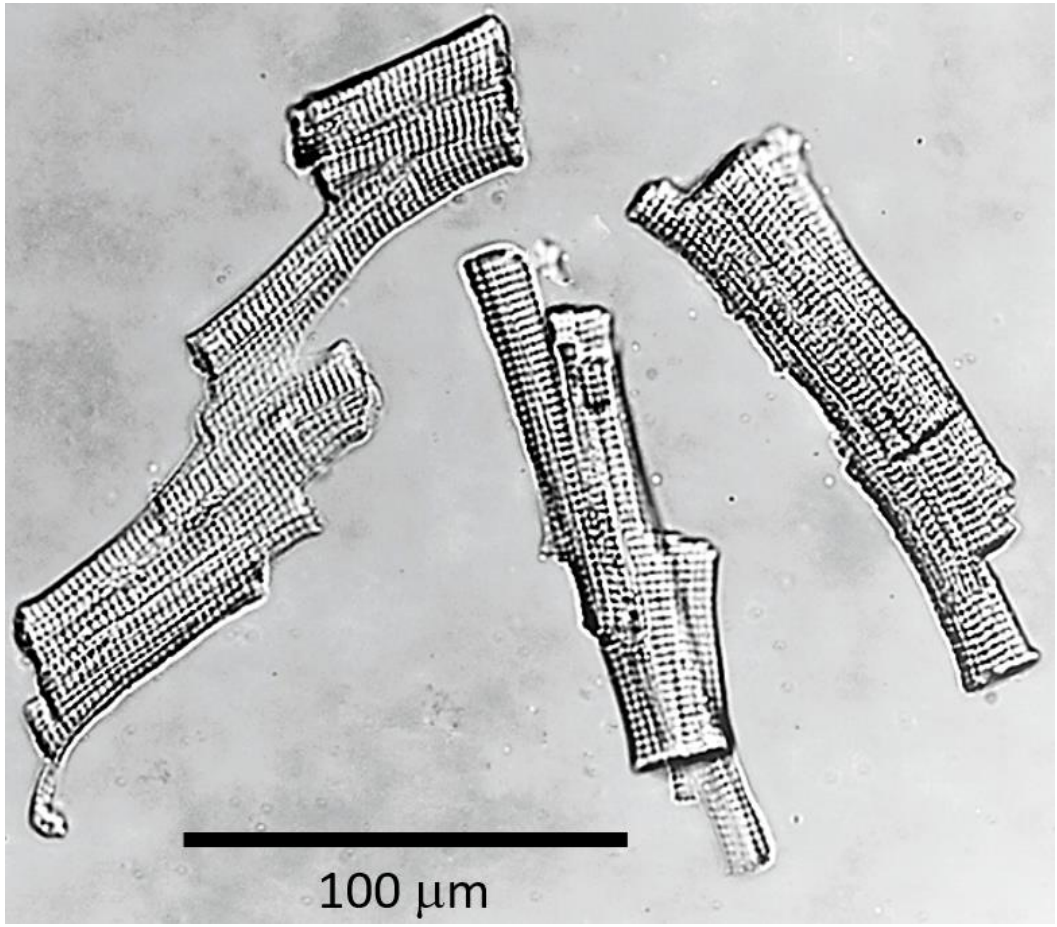
Inotropy
Contractility
Ventricular & Atrial Trabeculae

Chronotropy
Spontaneous Action Potential *Sinoatrial Node*

Vaso-constriction Dilation
Coronary Rings

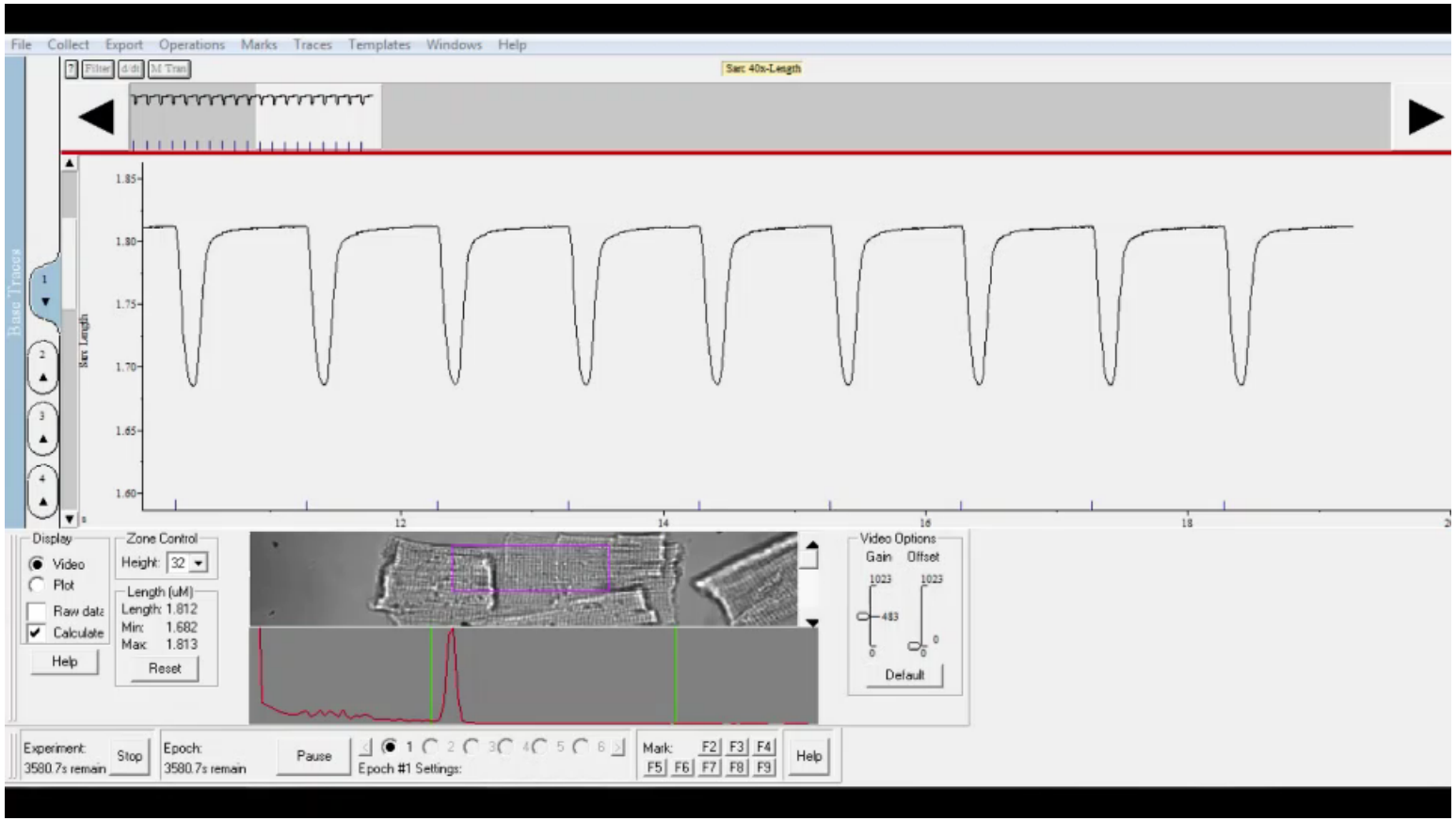
New Isolation Method Provides High Yield of Cardiomyocytes

1000 **ex vivo**
human hearts
tested
2-4 hearts / week



Non-Invasive Measurement of Contraction

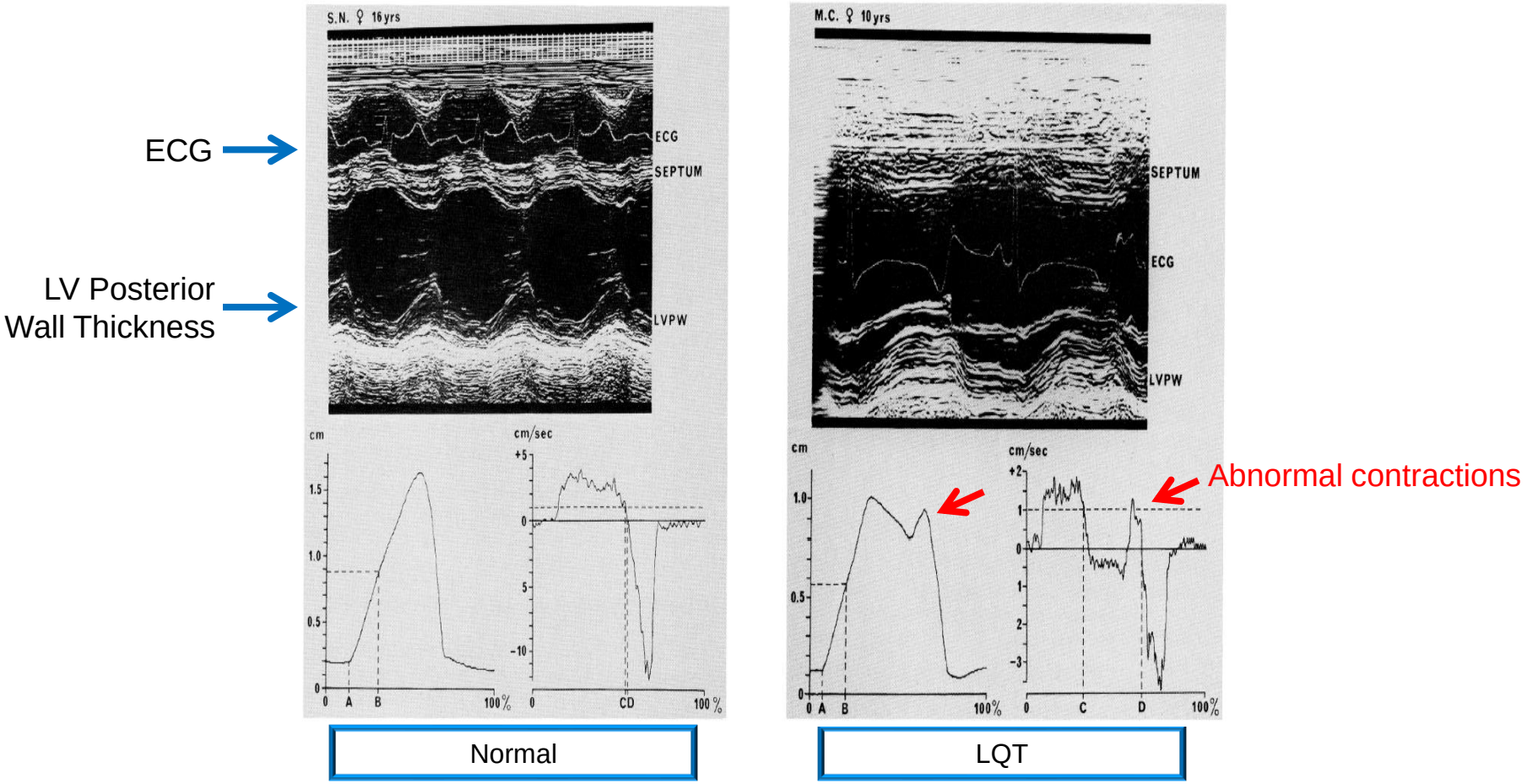
Full Retention of Cardiomyocyte Functionality



- Bright-field imaging
- Low technical complexity
- No cytotoxic fluorescent reagents
- High information content

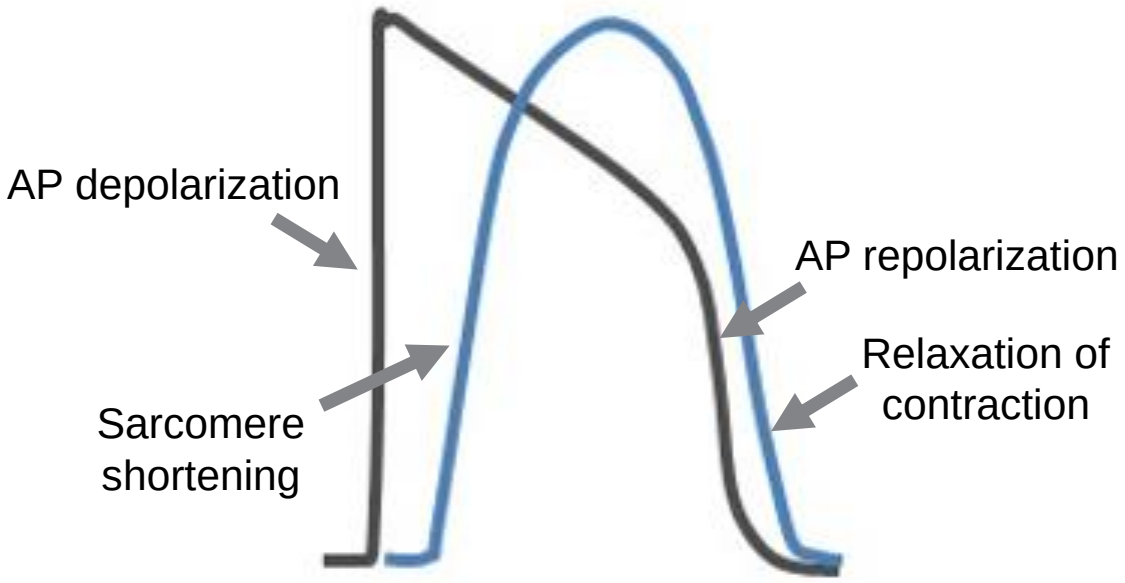
IonOptix: Sarcomere shortening measured by digital cell geometry tracking; stimulation frequency 1Hz

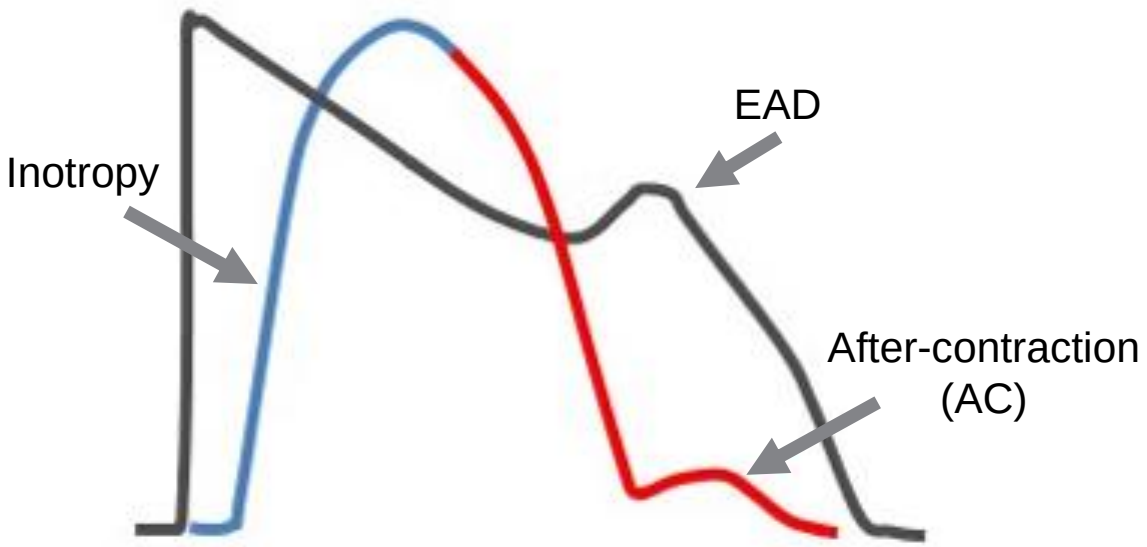
Strong Correlation Between Electrical and Mechanical Abnormalities in the Human Heart



Nador *et al.*, (1991) Circulation 84:1530-1542 and other papers (De Ferrari *et al.*, 1994; Nakayama *et al.*, 1998; Haugaa *et al.*, 2009; Ferrari & Schwartz, 2009; Belardinelli *et al.*, 2009)

Markers of Pro-arrhythmia and Contractility Risk





Normal condition

Drug treatment

- After-contraction represents the mechanical manifestation of triggered EAD
- TdP arrhythmia arises from PVCs due to triggered EADs (Kaumann et al. 1968; Noda et al. 2014)

Validating Clinical Relevance

- Validated 33 clinical well characterized controls (24 CiPA / 32 JiCSA):

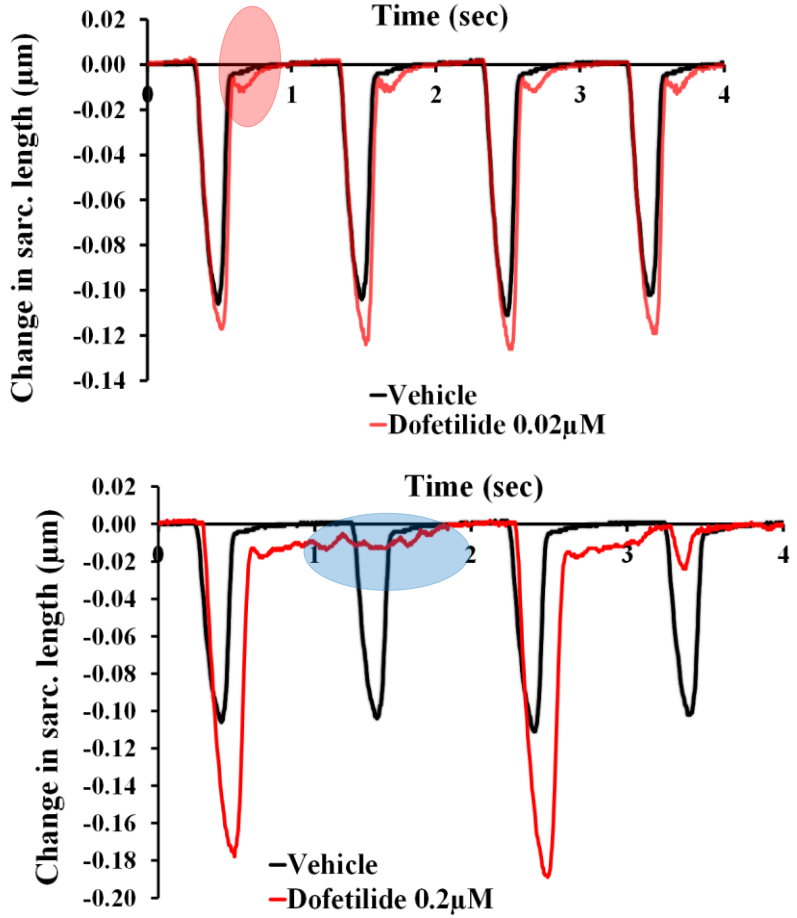
  1) 23 pro-arrhythmic drugs

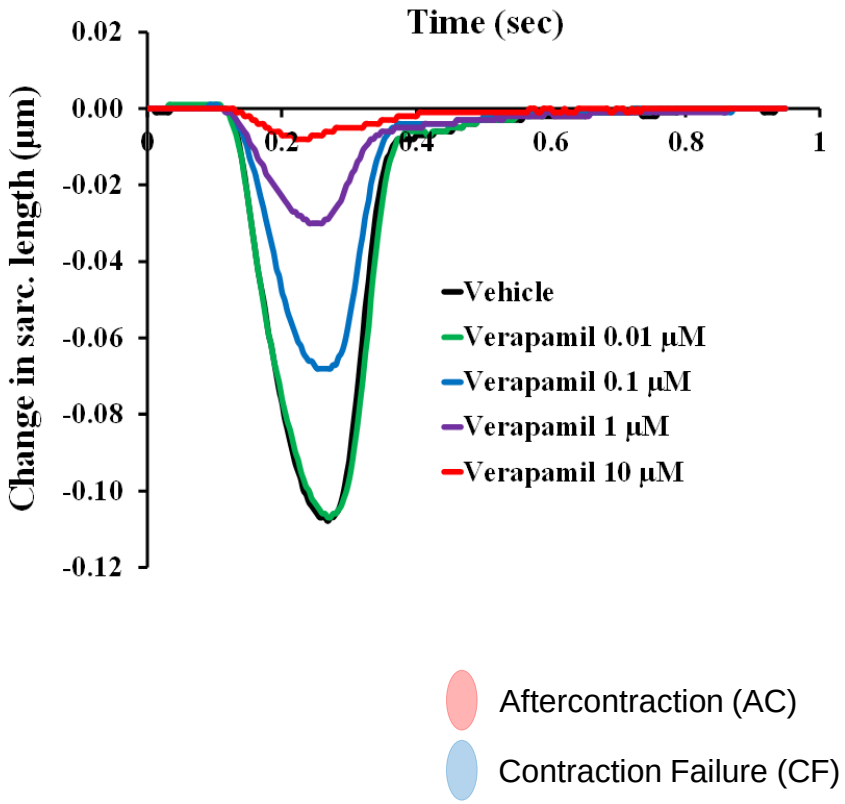
  2) 10 non-pro-arrhythmic drugs

  3) Each drug was tested at multiples of the free Effective Therapeutic Plasma Concentration (fETPC, mimic pharmacokinetic aspect)

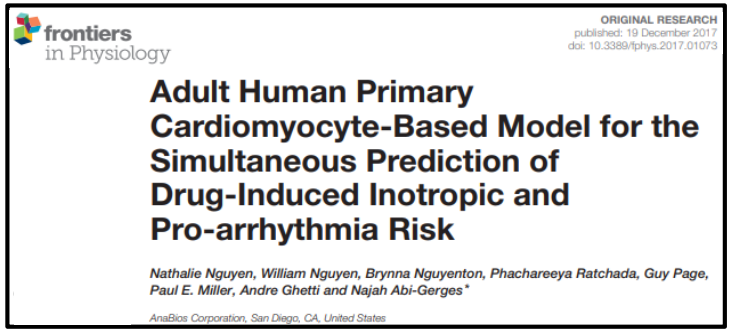
  4) Establish pharmacodynamic exposure response

AC for Predicting Pro-arrhythmic Risk





Prediction of Pro-arrhythmic Risk of Drugs
with 96% sensitivity



frontiers in Physiology

ORIGINAL RESEARCH
published: 19 December 2017
doi: 10.3389/fphys.2017.01073

Adult Human Primary Cardiomyocyte-Based Model for the Simultaneous Prediction of Drug-Induced Inotropic and Pro-arrhythmia Risk

*Nathalie Nguyen, William Nguyen, Brynna Nguyenton, Phachareeya Ratchada, Guy Page, Paul E. Miller, Andre Ghetti and Najah Abi-Gerges**

AnaBios Corporation, San Diego, CA, United States

Table 1. Pro-arrhythmia prediction of the adult human primary cardiomyocyte-based model

		Pro-arrhythmia risk at 10-fold fETPC					
Drug name	Clinical TdP risk	ANABIOS Adult human primary ventricular (Sarc. Short., AC) Nguyen et al., 2017	AMGEN hiPSC-derived cardiomyocytes (iCell®, MEA FPD) Qu et al., 2015	AMGEN hiPSC-derived cardiomyocytes (iCell®, MEA EAD) Qu et al., 2016	JiCSA hiPSC-derived cardiomyocytes (iCell®, MEA Score) Ando et al., 2017	FDA hiPSC-derived cardiomyocytes (iCell®, MEA Arrhythmia) Blinova et al., 2017	FDA hiPSC-derived cardiomyocytes (Cor.4U, MEA Arrhythmia) Blinova et al., 2017
Ajmaline			Not tested	Not tested		Not tested	Not tested
Astemizole[a]		False negative	Not tested	Not tested		Not tested	Not tested
Azimilide[a]			Not tested	Not tested	Not tested	Not tested	Not tested
Bepridil[a]			Not tested	Not tested	False negative	False negative	False negative
Chlorpromazine[a]			Not tested	Not tested	False negative	False negative	False negative
Cisapride[a]				False negative		False negative	False negative
Clarithromycin[a]			Not tested	Not tested		Not tested	Not tested
Clozapine[a]			Not tested	Not tested	False negative	Not tested	Not tested
D, L-Sotalol[a]						Not tested	Not tested
Disopyramide[a]			Not tested	Not tested		Not tested	Not tested
Dofetilide[a]							
Domperidone[a]			Not tested	Not tested		Not tested	Not tested
Droperidol[a]			Not tested	Not tested		Not tested	Not tested
Erthromycin			Not tested	Not tested		Not tested	Not tested
Flecainide						Not tested	Not tested
Ibutilide[a]			Not tested	Not tested		Not tested	Not tested
Moxifloxacin				Not tested		False negative	False negative
Ondansetron[a]			Not tested	Not tested		Not tested	Not tested
Procanaimide			Not tested	Not tested		Not tested	Not tested
Quinidine[a]			Not tested	Not tested			
Sematilide			Not tested	Not tested		Not tested	Not tested
Terodiline			False negative	False negative		Not tested	Not tested
Vandetanib[a]			Not tested	Not tested		Not tested	Not tested

[a]: CiPA-selected drug; Red: positive pro-arrhythmia risk; Green: negative pro-arrhythmia risk; hiPSC: human induced pluripotent stem cell (hiPSC); iCell® hiPSC-derived cardiomyocytes from Cellular Dynamics; MEA: micro-electrode array; FPD: Field Potential Duration; JiCSA: Japan iPS Cardiac Safety Assessment; FDA: Food and Drug Administration; Cor.4U: hiPSC-derived cardiomyocytes from Axiogenesis AG; EAD: Early afterdepolarization.

Prediction of Non-Pro-arrhythmic Drugs
with 100% Specificity

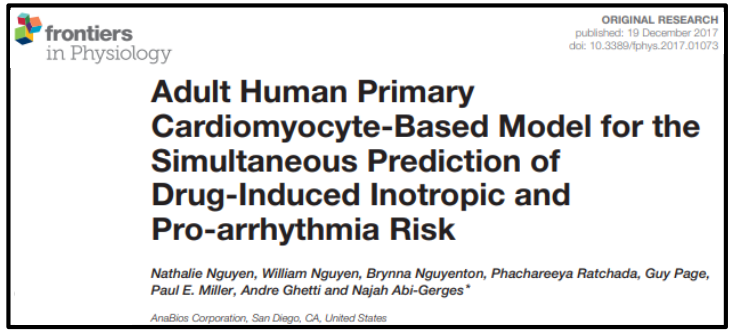


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Table 3. Pro-arrhythmia prediction of the adult human primary cardiomyocyte-based model

		Pro-arrhythmia risk at 10-fold fETPC					
Drug name	**Clinical TdP risk**	**ANABIOS primary ventricular (Sarc. short AC) Nguyen et al., 2017**	**AMGEN hiPSC-derived cardiomyocytes (iCell®, MEA FPD) Qu et al., 2015**	**AMGEN hiPSC-derived cardiomyocytes (iCell®, MEA EAD) Qu et al., 2016**	**JiCSA hiPSC-derived cardiomyocytes (iCell®, MEA Score) Ando et al., 2017**	**FDA hiPSC-derived cardiomyocytes (iCell®, MEA Arrhythmia) Blinova et al., 2017**	**FDA hiPSC-derived cardiomyocytes (Cor.4U, MEA Arrhythmia) Blinova et al., 2017**
Diltiazem[a]			Not tested	Not tested			
Diphenhydramine			Not tested	Not tested	False positive	Not tested	Not tested
Loratadine[a]			Not tested	Not tested		Not tested	Not tested
Mexiletine[a]			False positive	Not tested	False positive	Quiescent	
Mibefradil			Not tested	Not tested			
Nifedipine[a]			Not tested	Not tested		Not tested	Not tested
Nitrendipine[a]			Not tested	Not tested		Not tested	Not tested
Ranolazine[a]			False positive		False positive	False positive	
Tamoxifen[a]			Not tested	Not tested		Not tested	Not tested
Verapamil[a]			Not tested	Not tested			Quiescent

[a]: CiPA-selected drug; Red: positive pro-arrhythmia risk; Green: negative pro-arrhythmia risk; hiPSC: human induced pluripotent stem cell (hiPSC); iCell® hiPSC-derived cardiomyocytes from Cellular Dynamics; MEA: micro-electrode array; FPD: Field Potential Duration; JiCSA: Japan iPS Cardiac Safety Assessment; FDA: Food and Drug Administration; Cor.4U: hiPSC-derived cardiomyocytes from Axiogenesis AG; EAD: Early afterdepolarization.

ICH S7B IWG Recognizes the Value of Human Primary Cardiomyocytes for Pro-arrhythmia Assessment

ICH
harmonisation for better health

Final Concept Paper

ICH S7B and E14 Q&A

Endorsed by the ICH Assembly on 15 November 2018

ICH S7B recommends Follow-up Studies (Sec. 2.3.5) to inform the integrated risk assessment if a test articles blocks the $hK_V11.1$ IKr current (hERG) or prolongs the QT interval. These could include the test article effects on additional ionic currents, and the use of *in vitro* and *in vivo* assays. Newer assays and technologies such as *in silico* ventricular models, and human primary and induced pluripotent stem cell-derived cardiomyocytes, can provide insights into the relative proarrhythmic liability of test articles. Guidance is needed on when and how these novel approaches play a role in determining the proarrhythmic risk to inform clinical development.

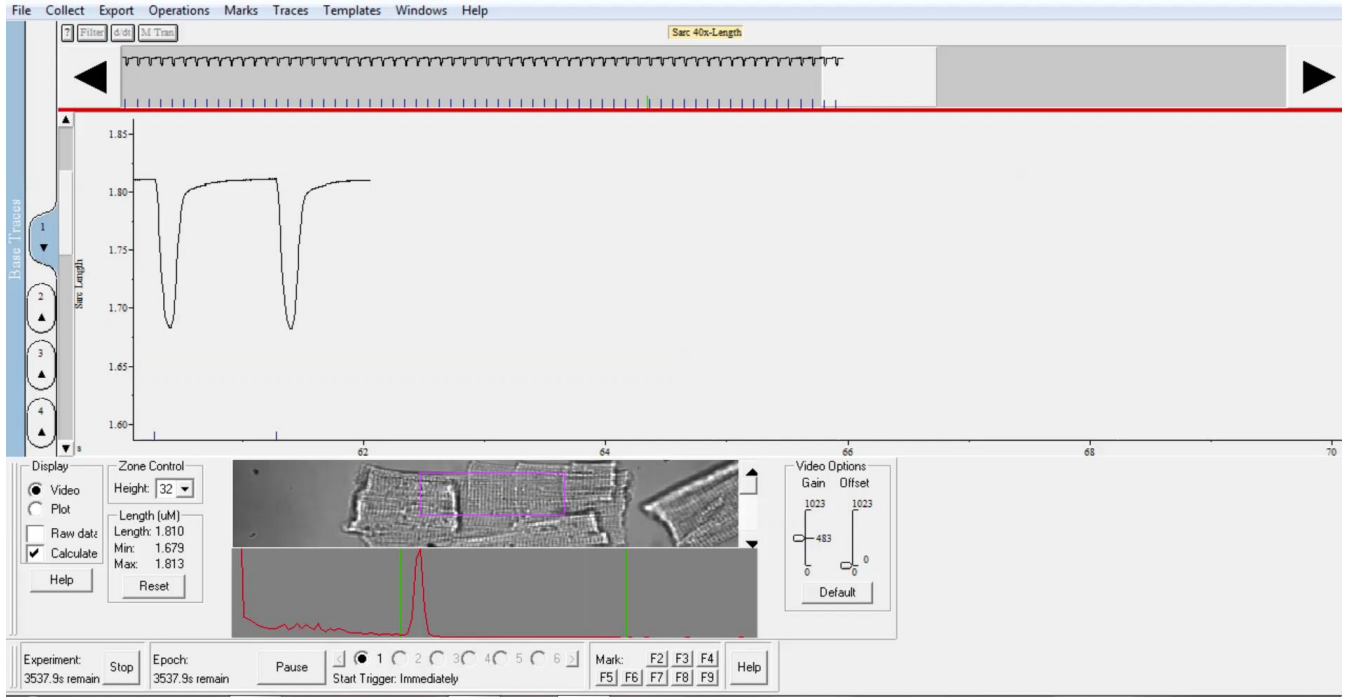
Validating Clinical Relevance of Negative Inotropes

- Validated 33 clinical well characterized controls:

  1) 27 multichannel blockers (mainly K^+, Na^+ and Ca^{2+} channels) as positive controls

  2) 6 selective hERG blockers as negative controls

Verapamil Induces Negative Inotropic Effect



Identification of Negative Inotropic Effects
and Determination of Exposure Responses

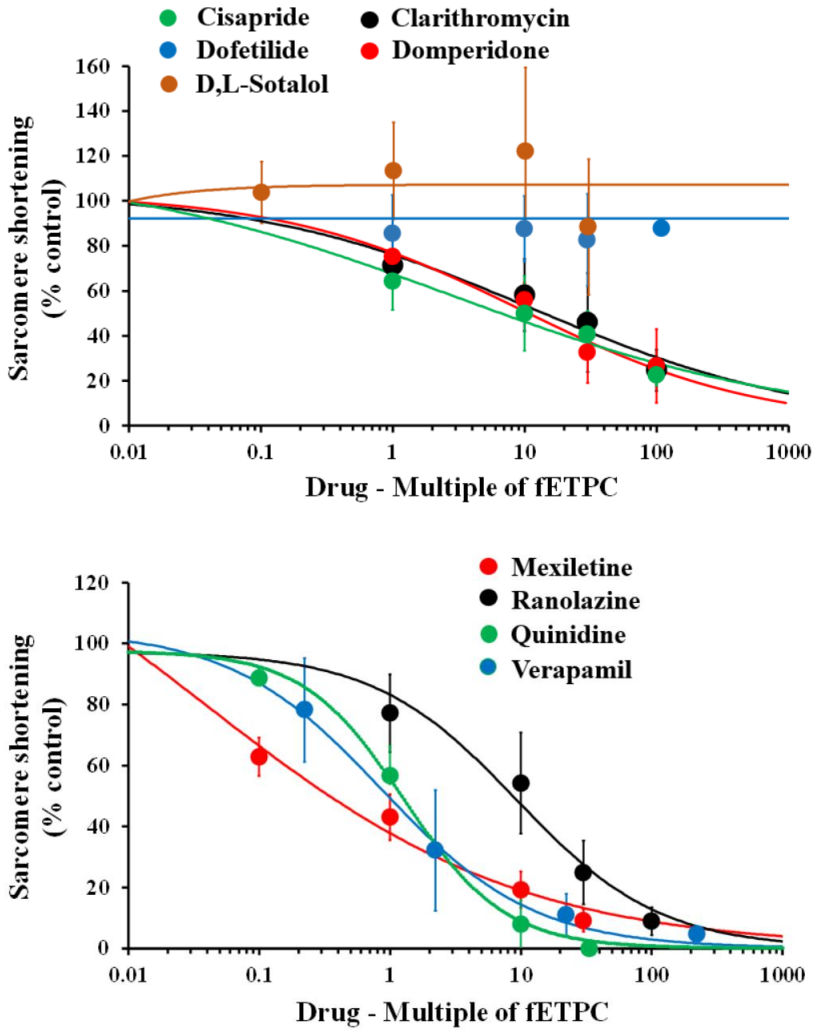


Table 5. Sarcomere shortening effects for reference drugs measured in adult human primary cardiomyocytes

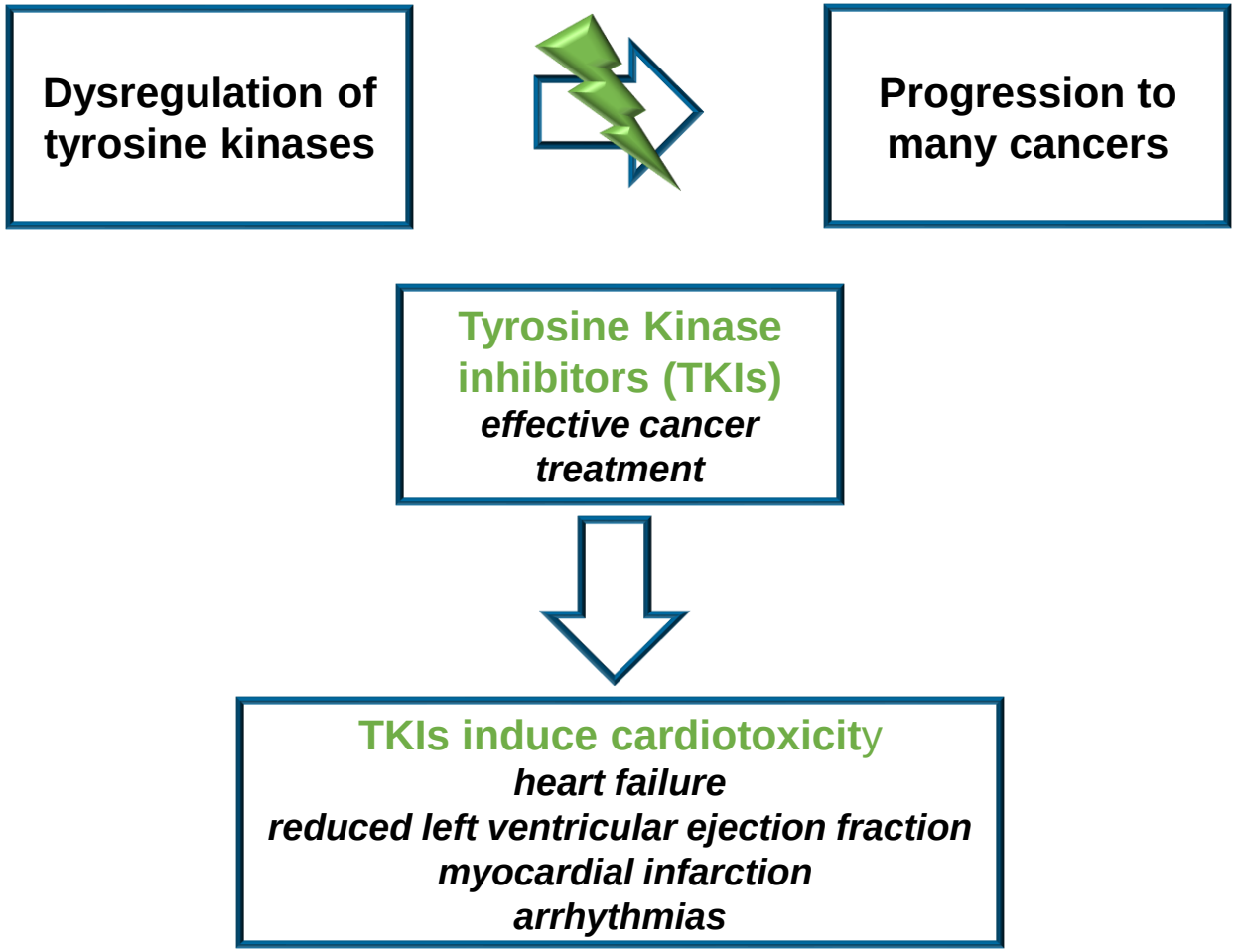
Drug name	Top test concentration (μM)	Human myocyte effect	IC_{50} (μM)	Ratio (IC_{50}/fETPC)
Ajmaline	1.95	-ve inotrope	2	31
Astemizole[a]	0.009	No effect	>0.009	30
Azimilide[a]	2.1	-ve inotrope	1.07	15
Bepridil[a]	0.96	-ve inotrope	0.7	22
Chlorpromazine[a]	1.04	-ve inotrope	1.02	28
Cisapride[a]	0.26	-ve inotrope	0.02	8
Clarithromycin[a]	120	-ve inotrope	16	13
Clozapine[a]	2.13	-ve inotrope	1.5	21
D, L-Sotalol[a]	450	No effect	>450	>30
Disopyramide[a]	21	-ve inotrope	9.3	13
Dofetilide[a]	0.2	No effect	>0.2	>100
Domperidone[a]	2	-ve inotrope	0.2	10
Droperidol[a]	0.48	-ve inotrope	0.18	11
Erythromycin	5.1	No effect	>5.1	>30
Flecainide	22.6	-ve inotrope	1.1	2
Ibutilide[a]	3	-ve inotrope	2	20
Moxifloxacin	329	No effect	>329	>30
Ondansetron[a]	11.2	-ve inotrope	14	34
Procainamide	1625	-ve inotrope	2215	38
Quinidine[a]	100	-ve inotrope	3.6	1
Sematilide	133	No effect	>133	>30
Terodiline	4.35	-ve inotrope	0.7	5
Vandetanib[a]	9	-ve inotrope	2.7	9
Diltiazem[a]	3.84	-ve inotrope	1	8
Diphenhydramine	1.02	-ve inotrope	0.6	17
Loratadine[a]	0.0135	-ve inotrope	0.0175	35
Mexiletine[a]	75	-ve inotrope	0.9	0.4
Mibefradil	0.36	-ve inotrope	0.18	13
Nifedipine[a]	0.23	-ve inotrope	0.04	5
Nitrendipine[a]	0.091	-ve inotrope	0.06	18
Ranolazine[a]	200	-ve inotrope	17	9
Tamoxifen[a]	0.663	-ve inotrope	0.99	36
Verapamil[a]	10	-ve inotrope	0.04	2

IC_{50}; Concentration inducing 50% decrease in sarcomere shortening; Hill equation using SigmaPlot v13 was fitted to sarcomere shortening concentration-effect curves, assuming drugs would eventually cause complete inhibition of the contractility when they decreased sarcomere shortening by ≥25%. [a]: CiPA-selected drug; fETPC, free effective therapeutic plasma concentration.

Nguyen et al., 2017 FiP

Inhibition of Kinase Activity to Control Tumor Growth

Can Lead to Cardiotoxicity

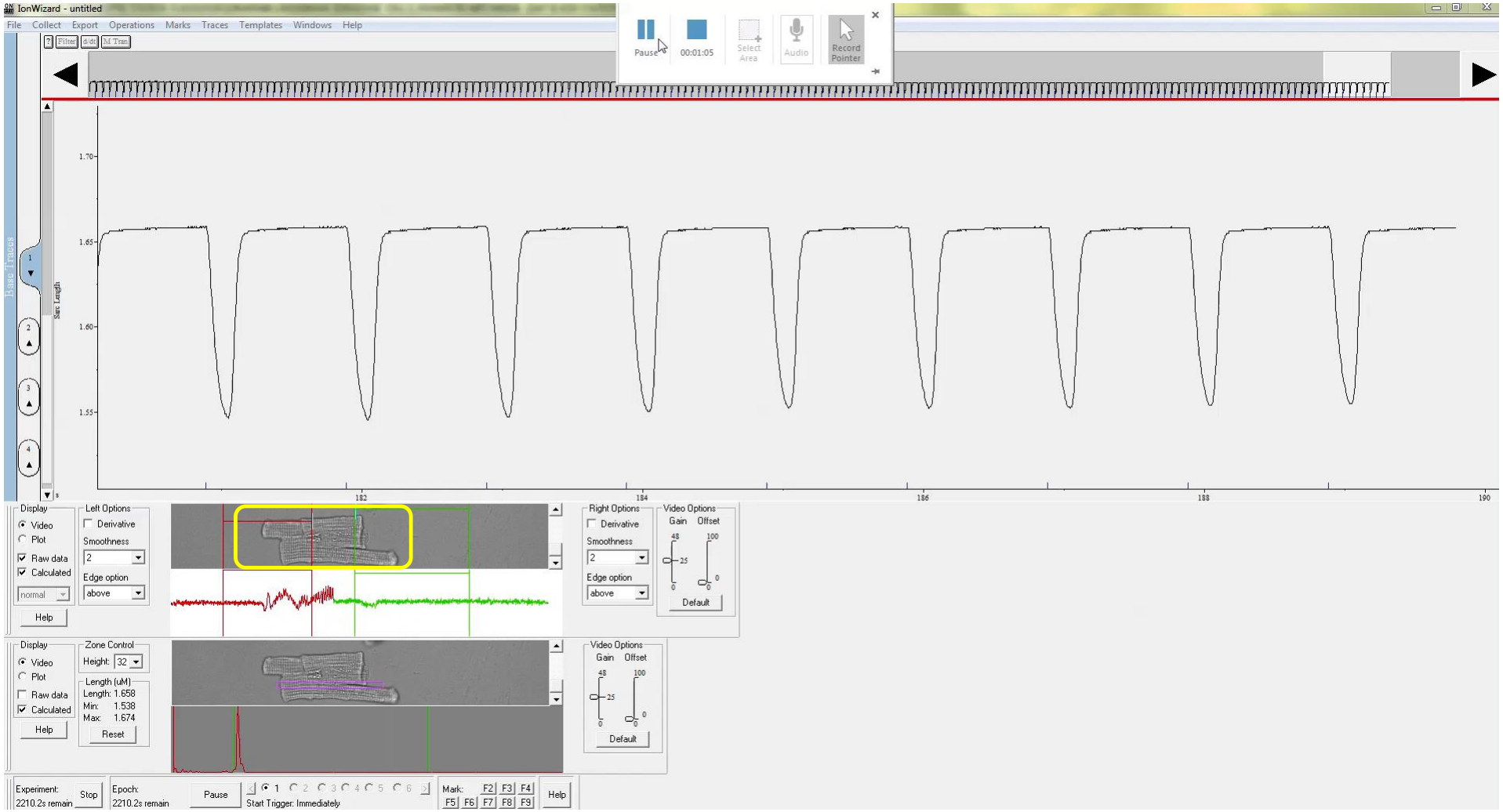


Validating Clinical Relevance of Cancer Agents

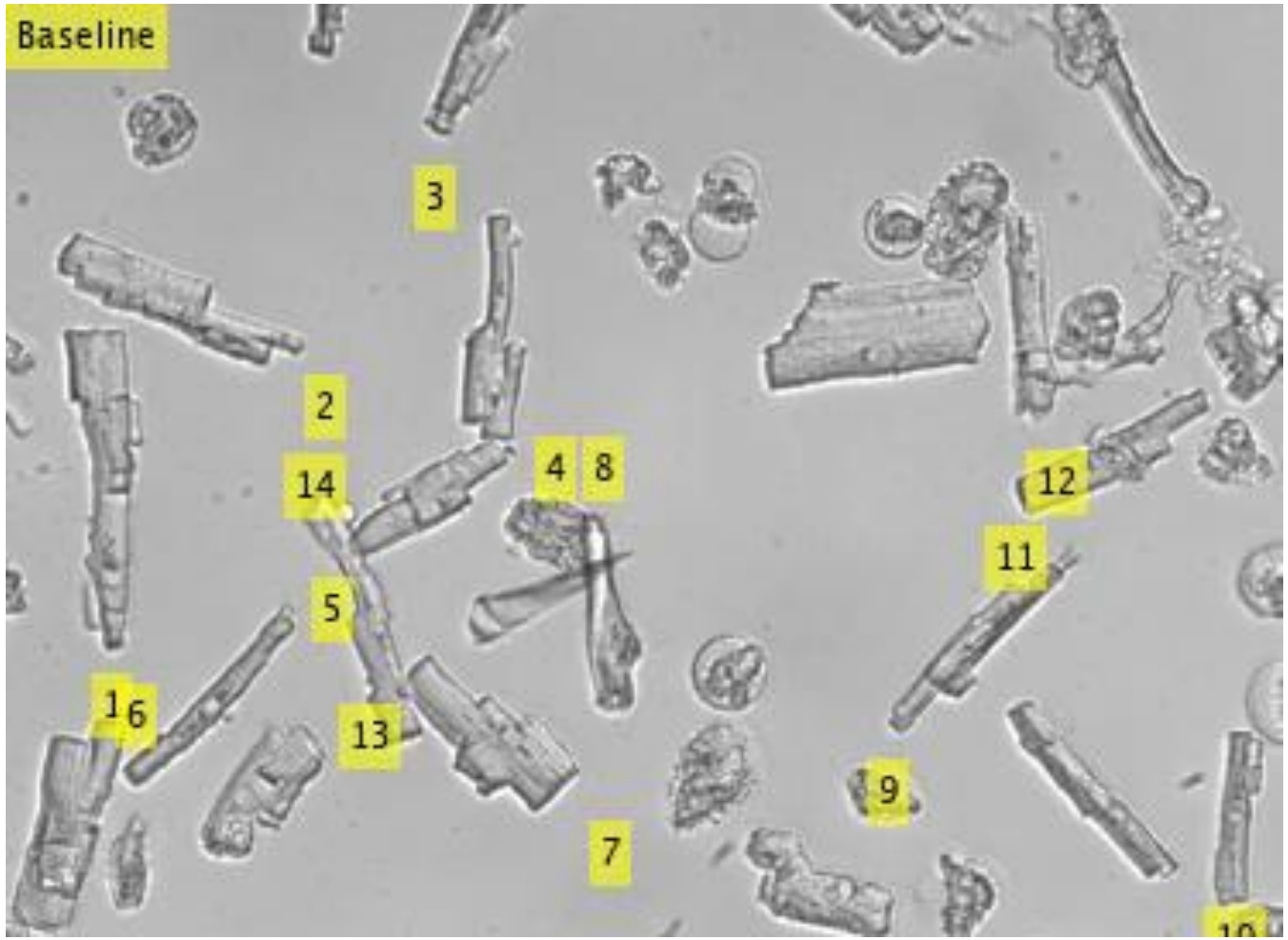
- Validated 9 clinical well characterized controls :

1) 4 toxic TKIs (Sorafenib, Vandetanib, AZD7762, Imatinib)
2) 4 non-toxic TKIs (Erlotinib, Dasatinib, Afatinib, Gefitinib)
3) One toxic anthracycline (Doxorubicin)
4) Each drug was tested at multiples of the Cmax
5) Each concentration was perfused for 5 mins

Sorafenib Induces Functional Cardiotoxicity

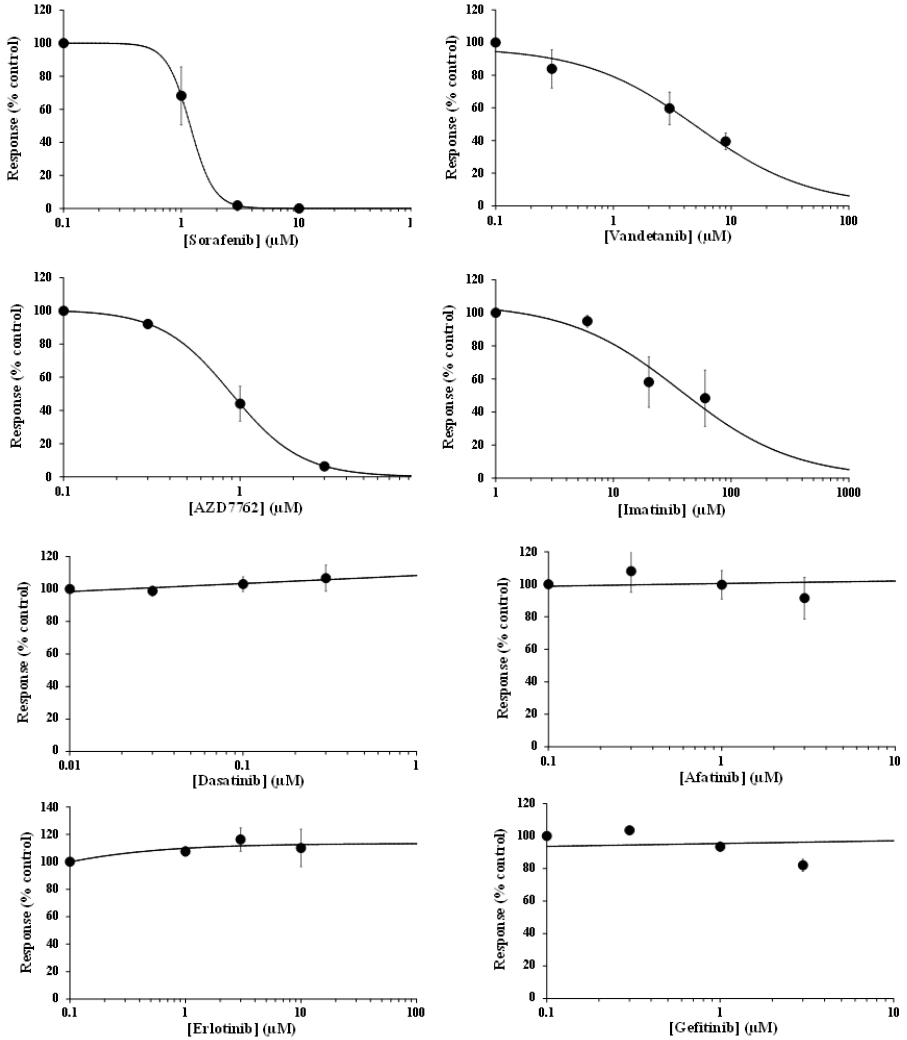


Afatinib Induces No Functional or Structural Cardiotoxicity



0.3μM = 3-fold Cmax
1μM = 10-fold Cmax
3μM = 30-fold Cmax

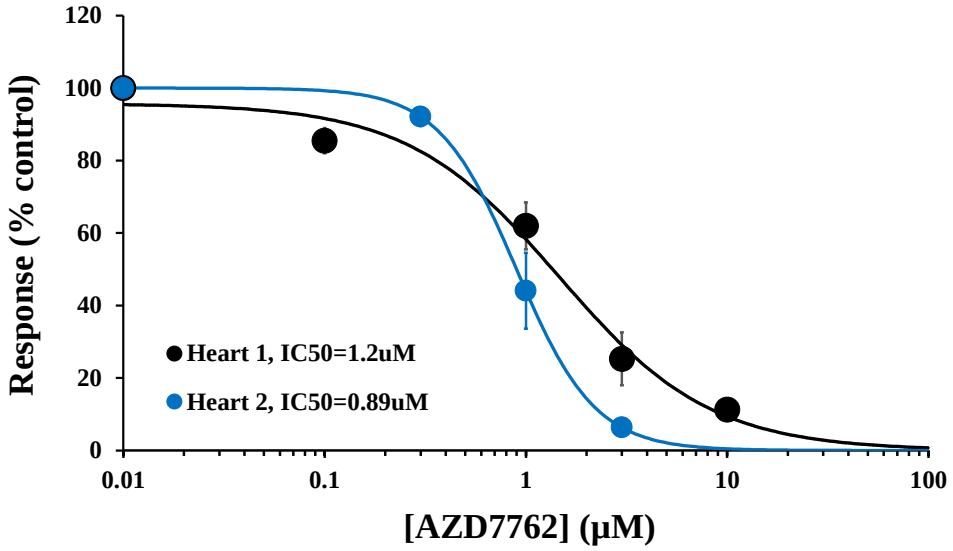
Tyrosine Kinase Inhibitors Affect Human Cardiomyocyte Contractility

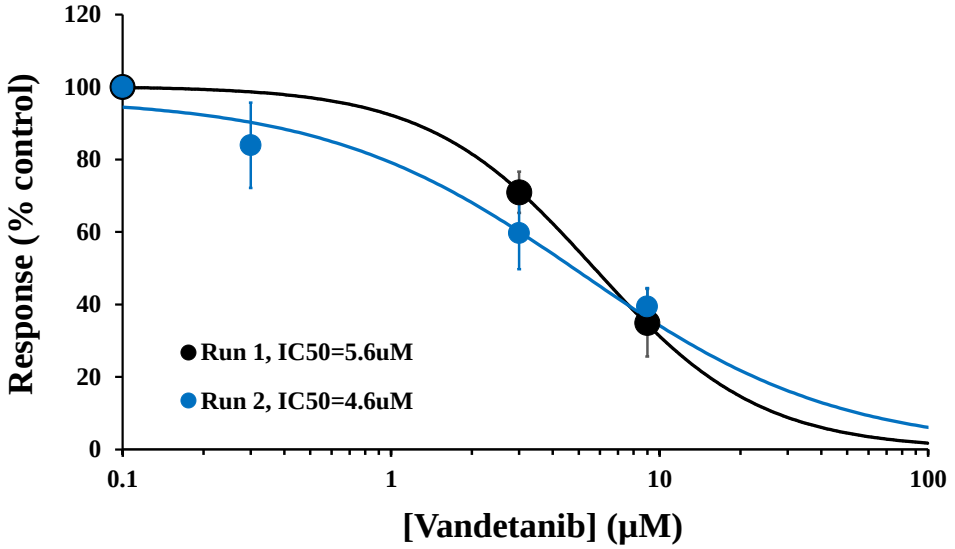


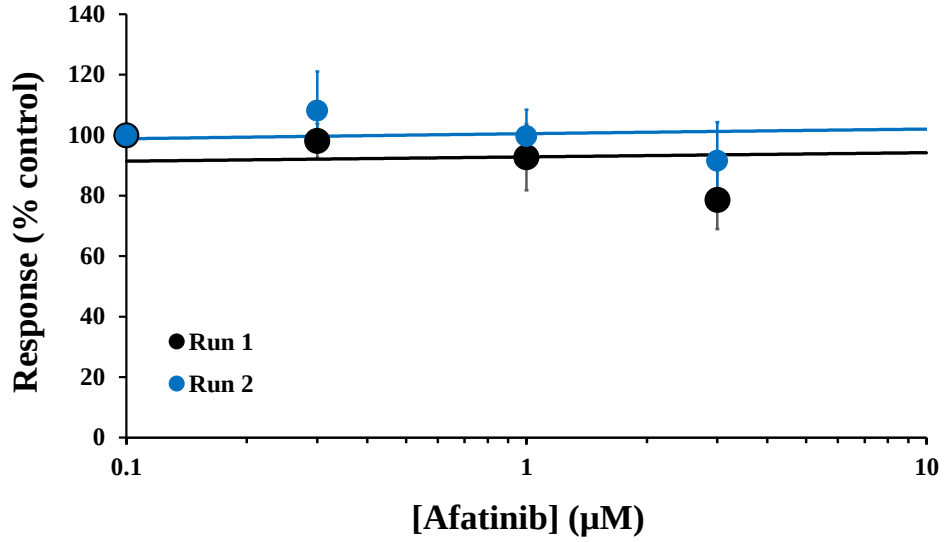
TKI	Clinical contractility risk	Human cardiomyocyte contractility	Cmax (µM)	IC_{50} (µM)	Ratio (IC_{50}/C_{max})
Sorafenib			3.4	1.2	0.35
Vandetanib			1.8	4.6	2.55
AZD7762			0.12	0.8	6
Imatinib			5	44	8
Erlotinib			2.5	>10*	>4
Dasatinib			0.01	>0.3	>30
Afatinib			0.1	>3	>30
Gefitinib			0.1	>3	>30

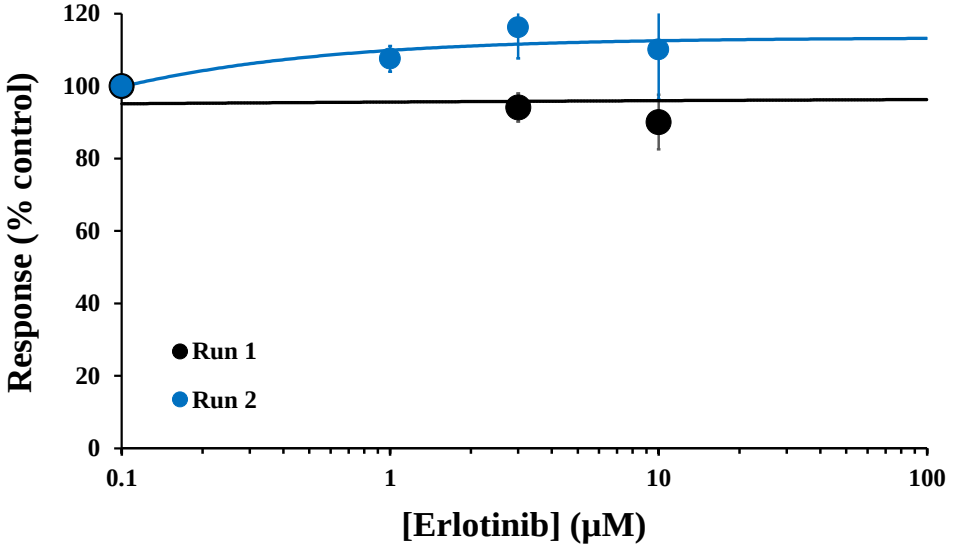
Similar human cardiac tissue data recently published by Schneider C et al., 2018 Nature Scientific Reports
**: Limit of solubility*

Low Inter- and Intra-Heart Variability

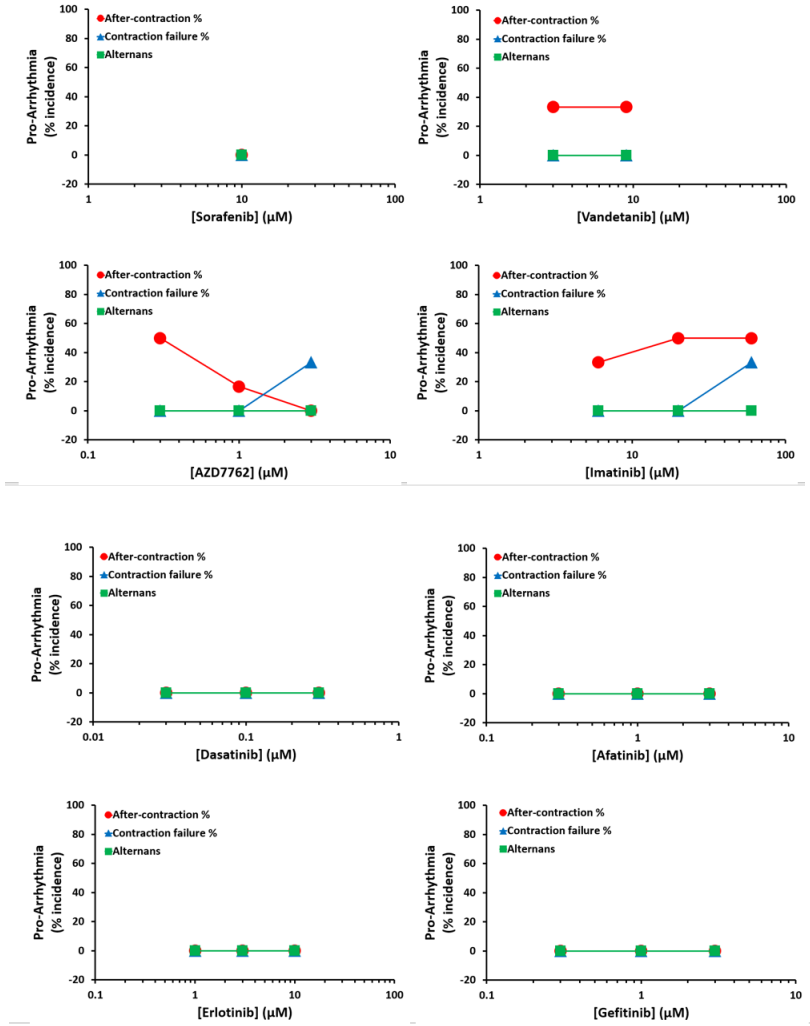








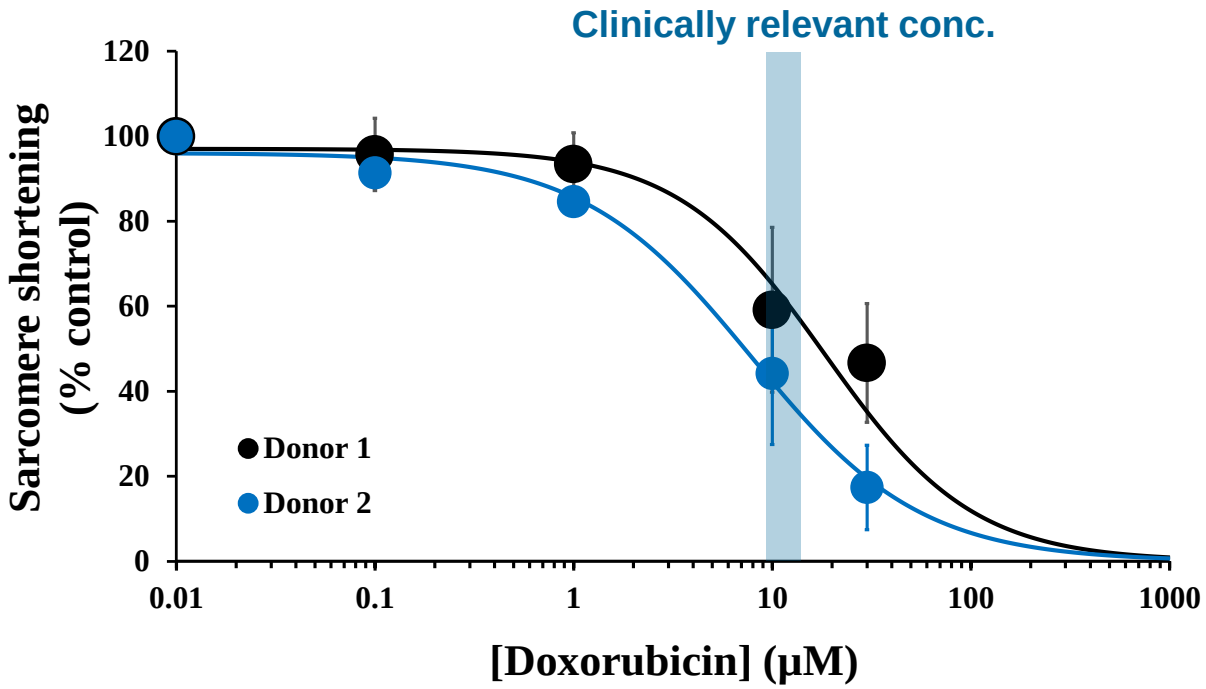
Human cardiomyocyte Contractility Model Differentiates Pro-arrhythmic from Non-Pro-arrhythmic TKIs

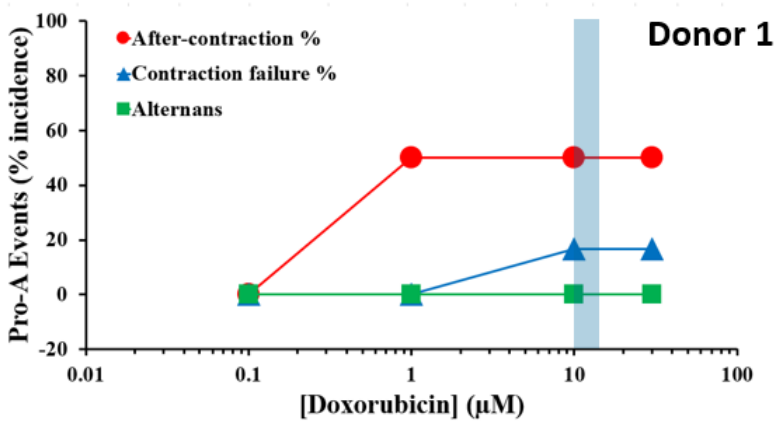


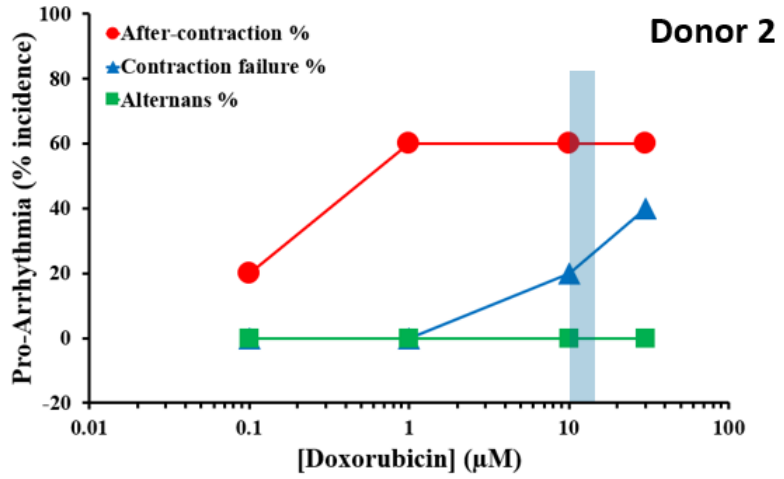
TKI	Clinical contractility risk	Human cardiomyocyte pro-A	Cmax (μM)	Pro-A conc. (μM)	Ratio (Pro-A conc./C_{max})
Vandetanib			1.8	3	1.6
AZD7762			0.12	0.3	2.5
Imatinib			5	6	1.2
Sorafenib			3.4	>10	>3
Erlotinib			2.5	>10*	>4
Dasatinib			0.01	>0.3	>30
Afatinib			0.1	>3	>30
Gefitinib			0.1	>3	>30

**: Limit of solubility*

Doxorubicin, Anthracycline Agent, Affects Human Cardiomyocyte Contractility and Induces Pro-arrhythmia







Adult Human Heart Kinome Profiling

Cancer-driving kinases

Expression and role of kinases in cancer are well understood

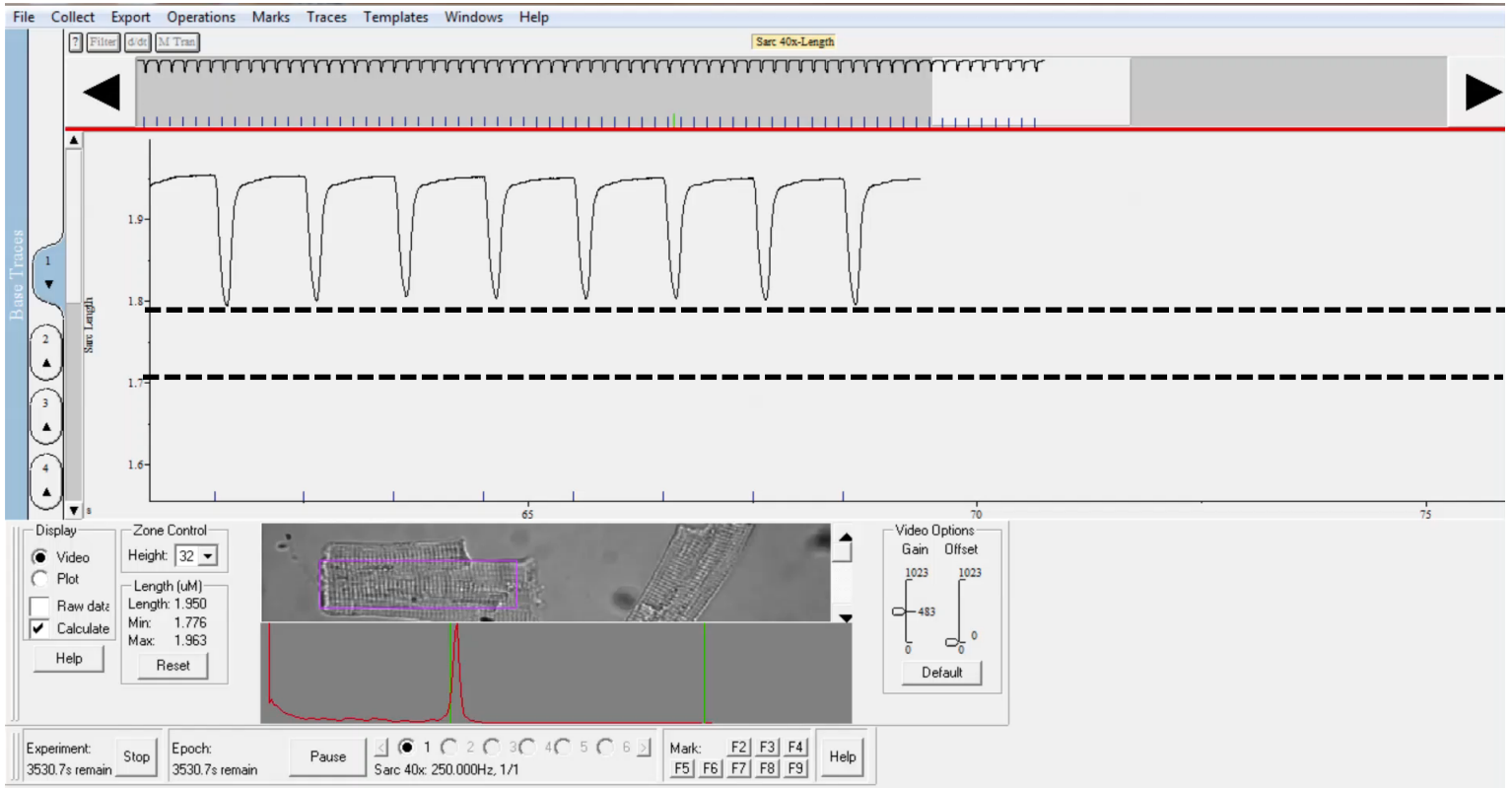
Heart kinases

- **Expression & function of kinases in cardiac tissue are poorly characterized**
- **Mechanisms of KI-induced cardiotoxicity are not fully understood**

Positive Inotropy Assessment - Validation Set Targets 12 Different Mechanisms of Action

Mechanism of Action	Drug
Na^+/K^+ pump inhibition	Digoxin
Na^+/K^+ pump inhibition	Ouabain
Na^+/Ca^{2+} exchanger inhibition	SEA-0400
Myosin activation	Omecamtiv Mecarbil
Myosin activation	EMD-57003
Ca^{2+} sensitization	Levosimendan
Non-selective b-adrenoceptor activation	Isoproterenol
Non-selective b-adrenoceptor activation	Epinephrine
b1-adrenoceptor activation	Dobutamine
PDE3 inhibition	Milrinone
PDE inhibition	IBMX
Ca^{2+} channel activation	Bay-K 8644
Adenylyl cyclase activation	Forskolin
Adenylyl cyclase activation	NKH-477
SERCA activation	N106
RyR activation	Caffeine

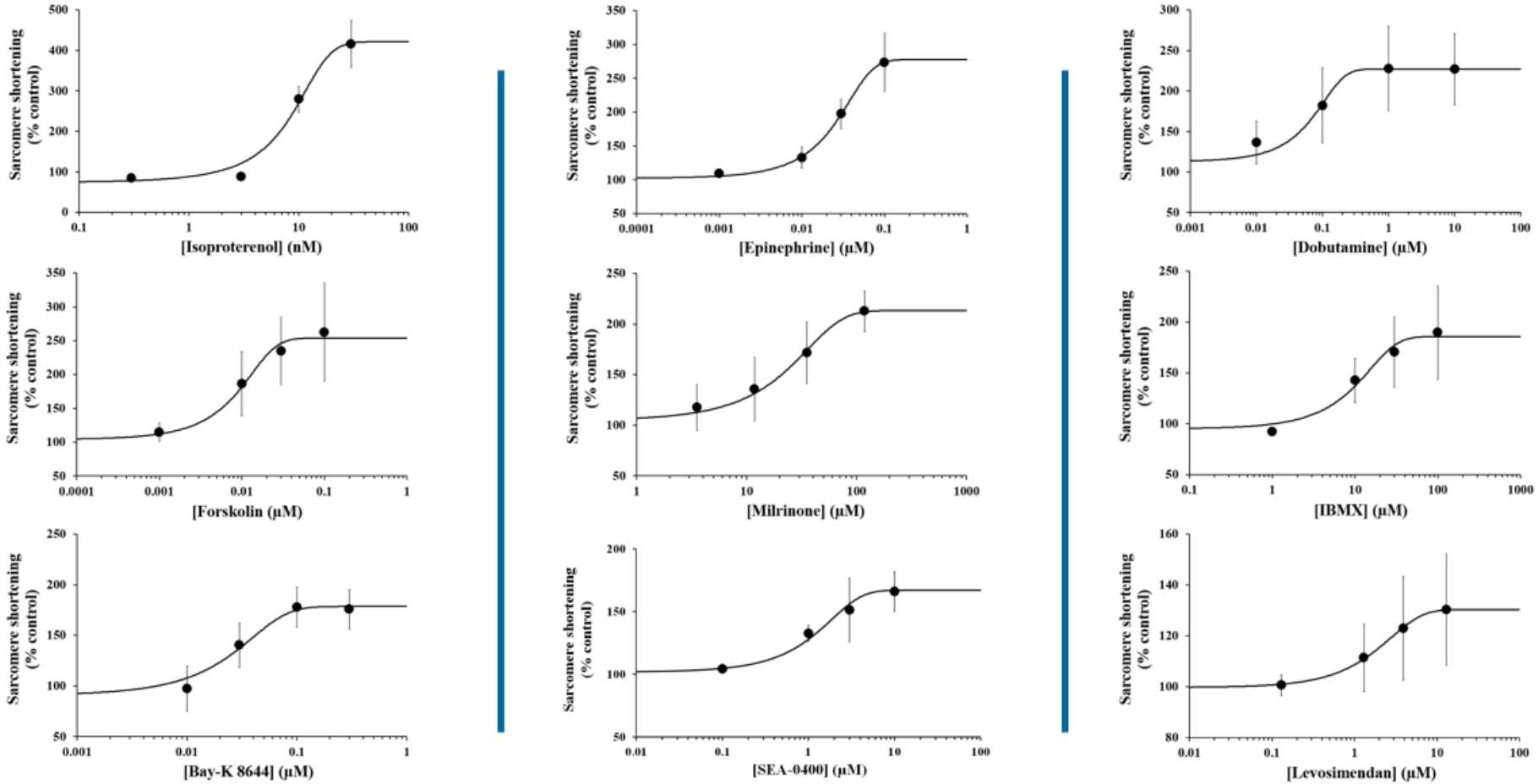
Isoproterenol Induces Positive Inotropic Effect



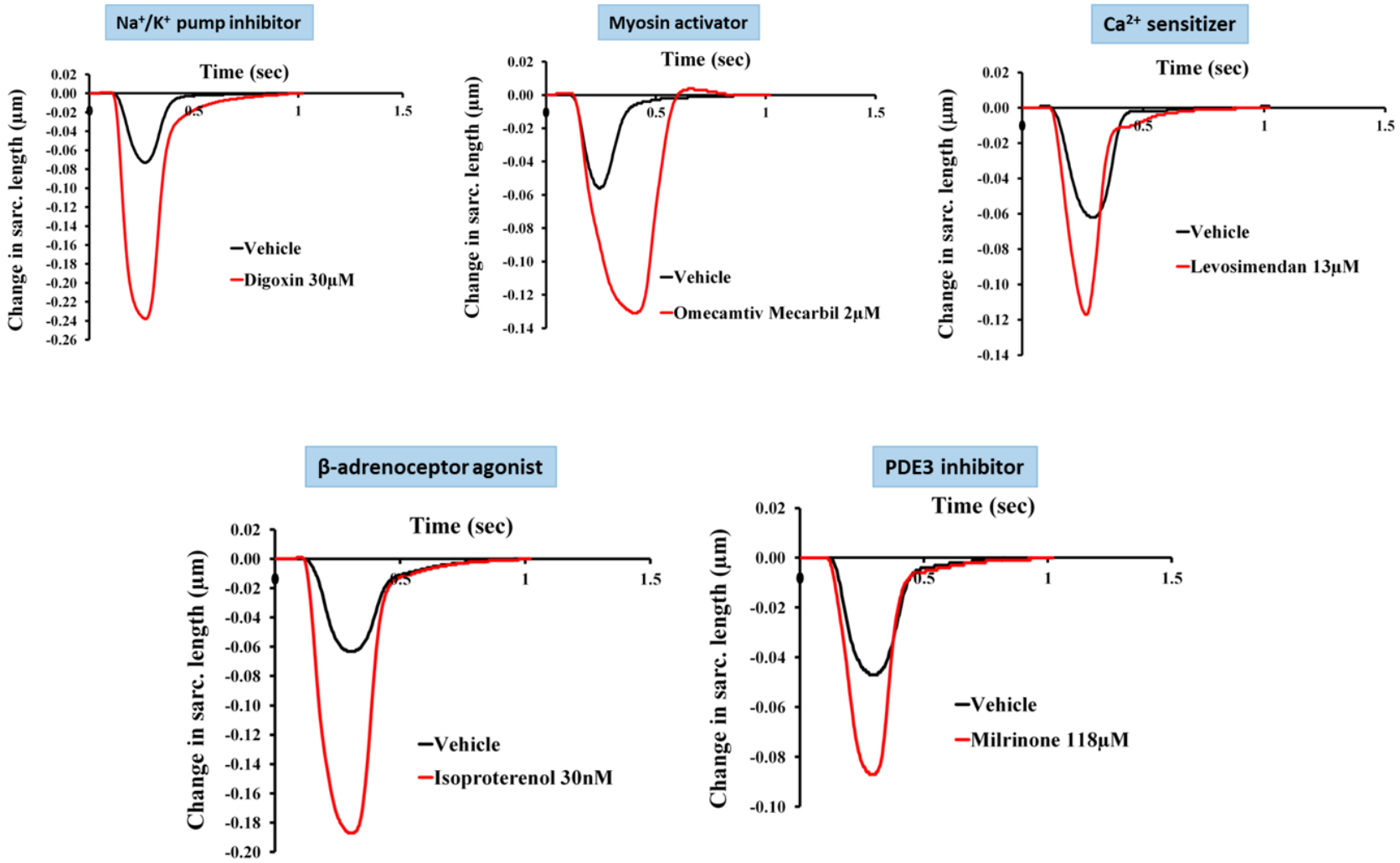
Baseline level

Iso level

Identification of Positive Inotropic Effects
and Determination of Exposure Responses

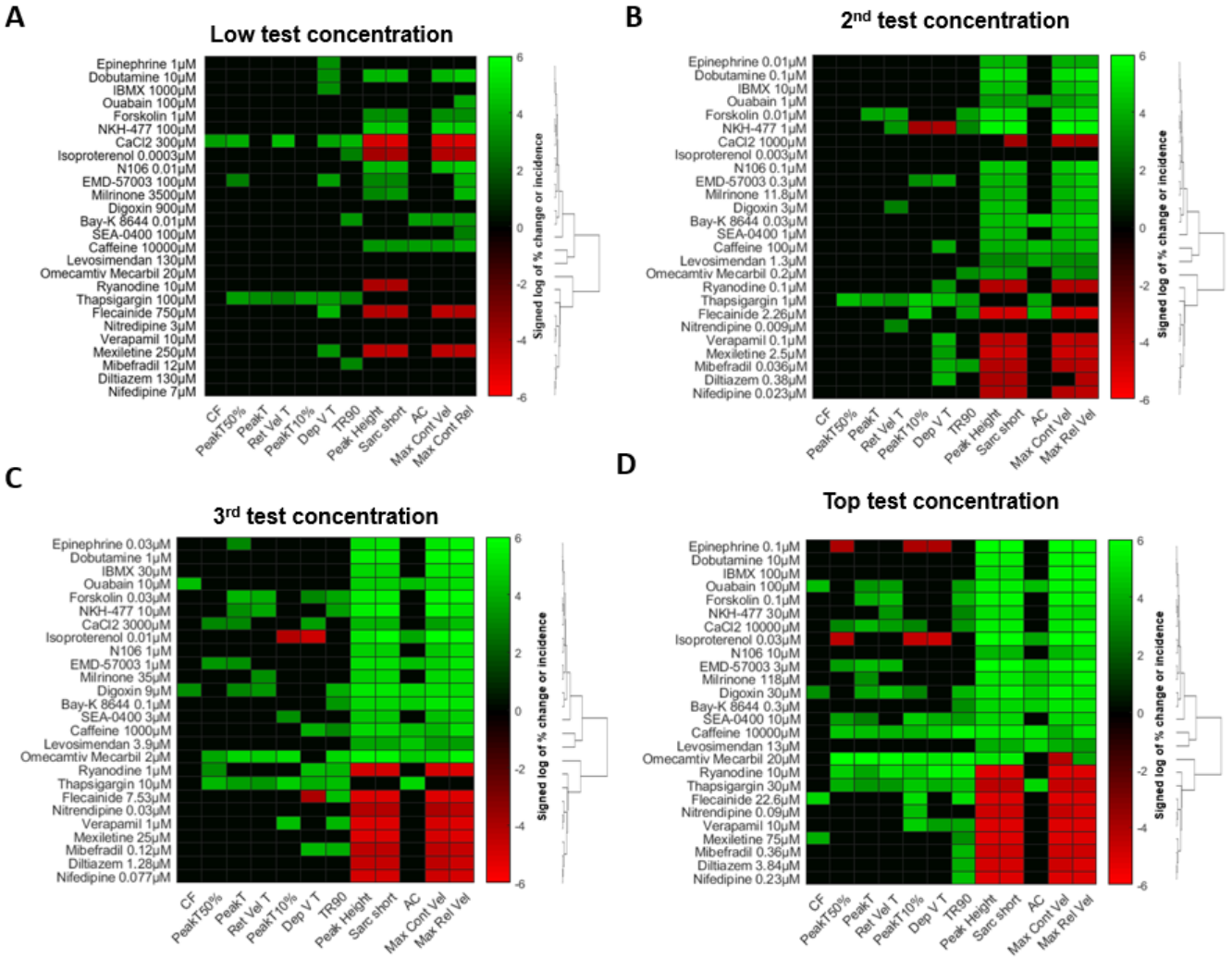


Differential Effects of Positive Inotropes



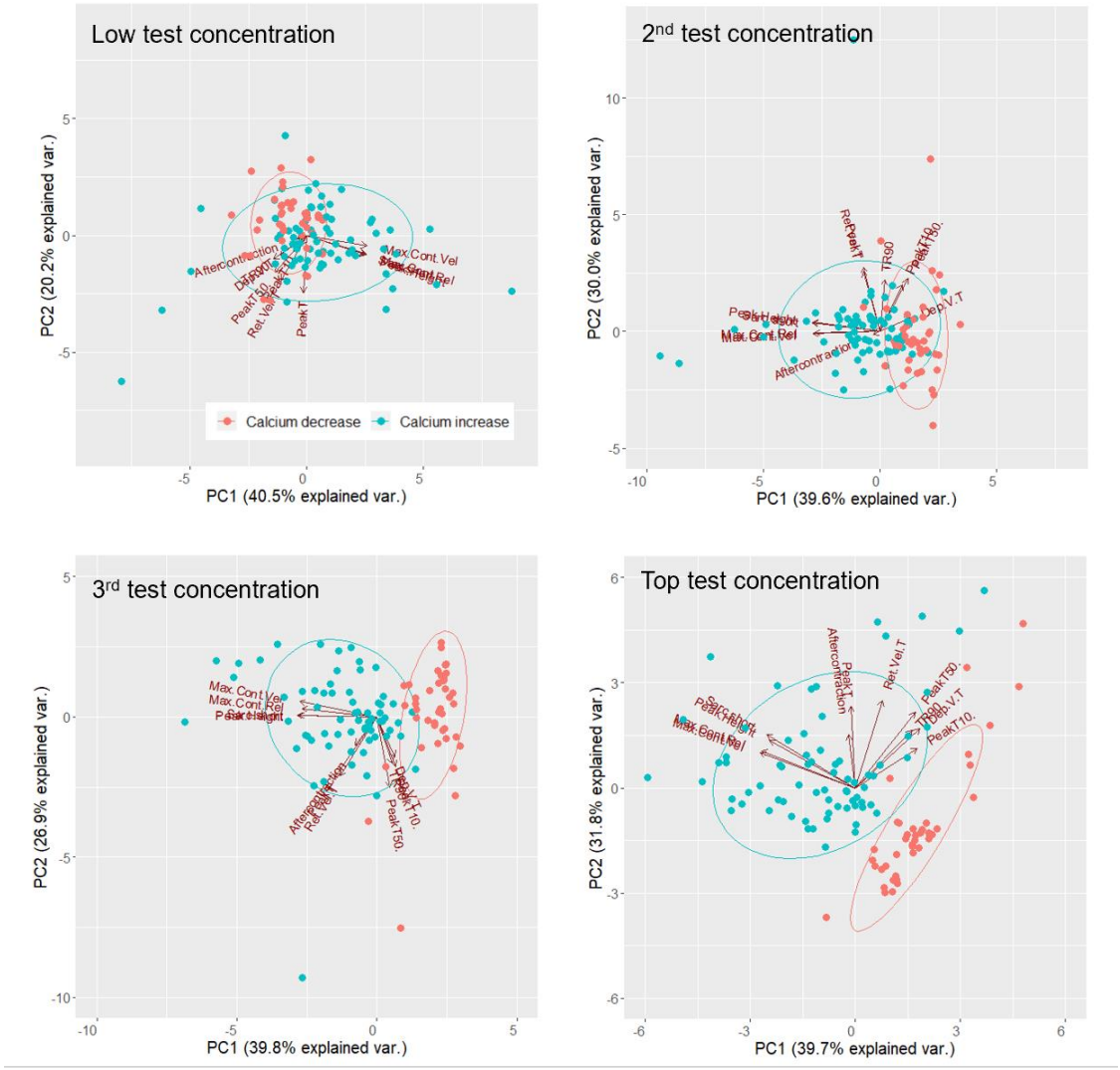
Cluster Analysis is Used to Mechanistically Fingerprint

Compounds with Inotropic Effects



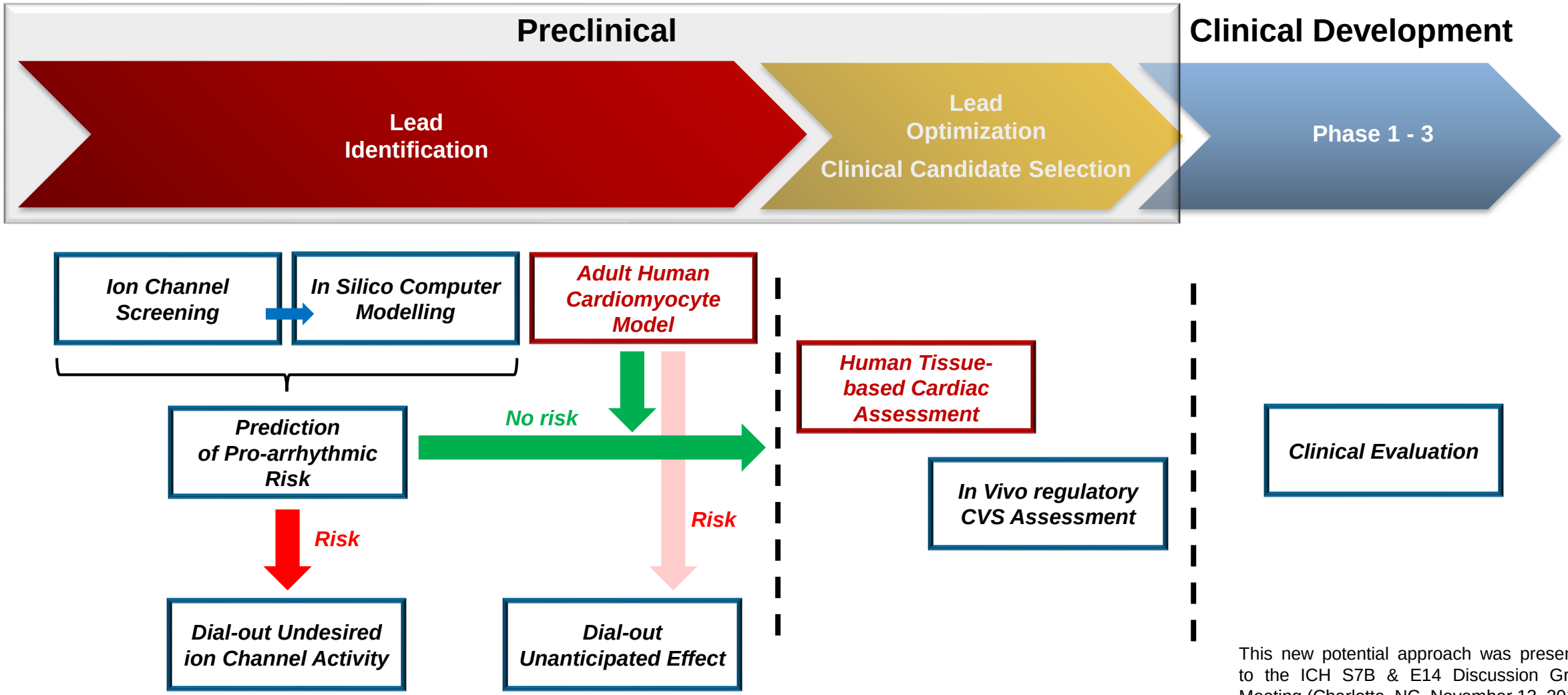
Heatmap data generated from 4th concentration data. Red and green colors iindicate decrease and increase of >25% and 10% change, respectively. Black colors iindicate no effect (<-25% < % change < 10%). Numbers in boxes indicate means % change relative to vehicle.

Segregation of Ca^{2+}-Dependent Mechanisms



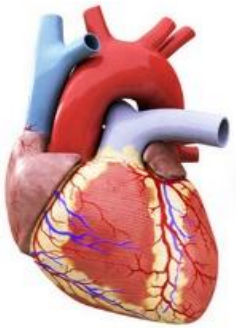
2D PCA generated from top test concentration data. Blue and red colors indicate increase and decrease in Ca^{2+}, respectively. Ellipses show confidence intervals of 0.75.

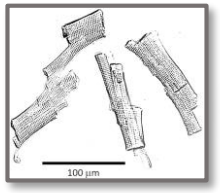
Human Adult Cardiomyocyte Model
Integrated Into the CiPA Paradigm



This new potential approach was presented to the ICH S7B & E14 Discussion Group Meeting (Charlotte, NC, November 13, 2018).

MyoBLAZER™: Proprietary Technology Used to Measure the Biomarker Currently Undergoing Qualification with FDA





Isolation of human cardiac cells

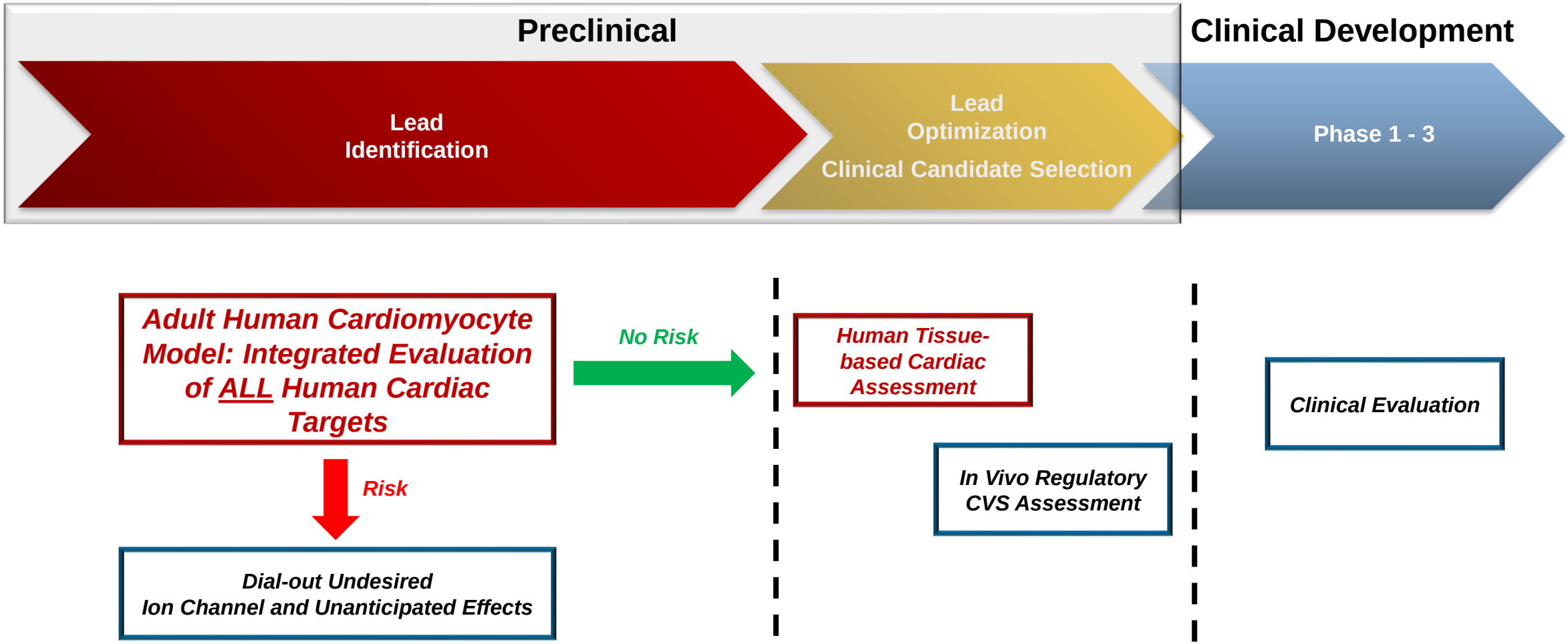
MyoBLAZER

Proprietary cell contractility testing station

Proprietary acquisition and analysis algorithms allow to establish drug effects with high level of predictivity of the human clinical response

Adult Human Cardiomyocyte Model

Early Primary Screening Tool



Adult Human Cardiomyocyte Model
Early Primary Cardiotoxicity Screening Tool

- Permit rigorous and integrated human cardiac drug discovery at the preclinical stages
- Differentiate safe from cardiotoxic drugs
- Can enable mechanistic assessment
- Predictive of clinical outcomes

Thank You!