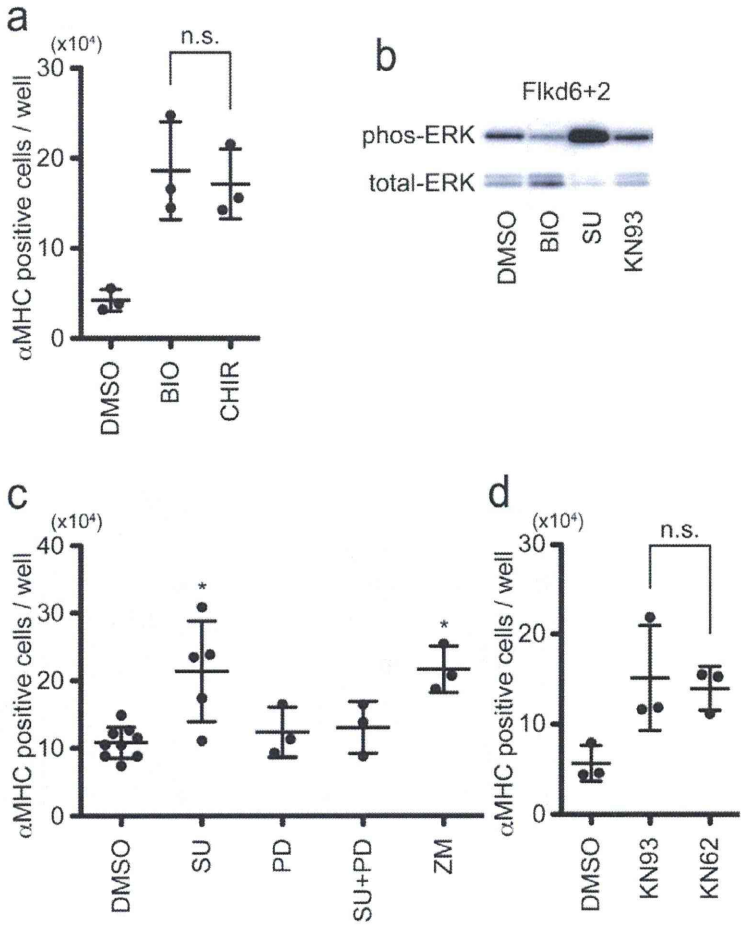

Figure 3.

Identified Chemicals were GSK3 Inhibitor, ERK Activator and CaMKII Inhibitor. (a) Effects of GSK3 inhibitors, BIO and CHIR99021 (CHIR) (Mann-Whitney test, $n = 3$) on mESCM numbers. (b) Western blotting for Phosphorylated ERK (phos-ERK) and total ERK at Flkd6+2. SU1498 treatment increased phos-ERK. (c) mESCM cell number at Flkd6+5. SU1498 (SU)-elicited mESCM proliferation was attenuated by a MEK inhibitor (PD98059, PD) treatment. A Raf activator (ZM336372, ZM) treatment also increased mESCM number. *, $p < 0.05$ *vs.* control group (Dunn's test, n = 3–9). (d) Effects of CaMKII inhibitors, KN93 and KN62, on mESCM numbers at Flkd6+5 (Mann-Whitney test, $n = 3$).

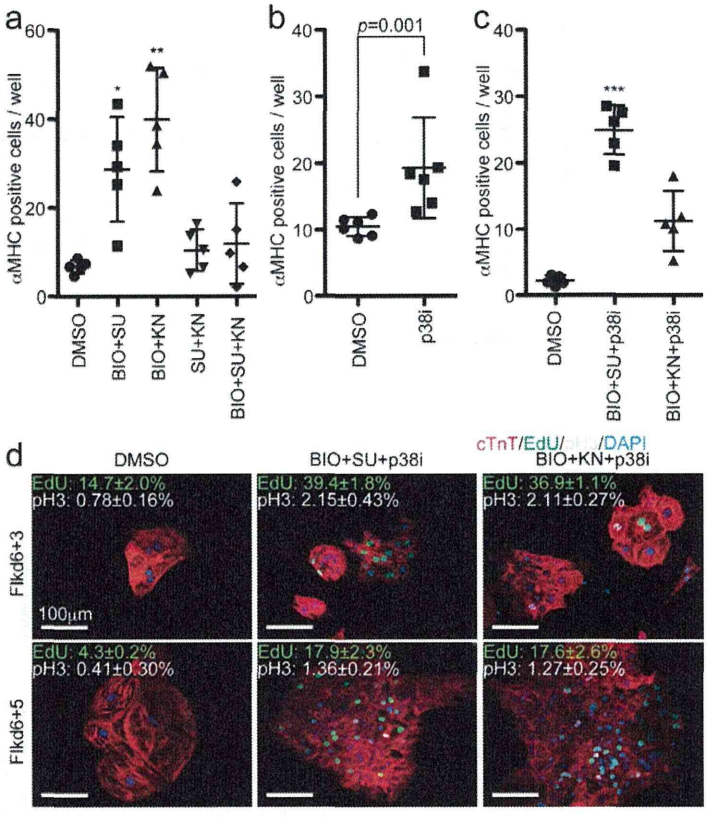

Figure 4.

Combination of Chemicals Synergistically Enhanced mESCM Proliferation. (a) Combinatory effects of chemicals on mESCM cell number (Dunn's test: *, $p < 0.05$ and **, $p < 0.01$, *vs.* DMSO treated group, n = 5) (b) Effects of a p38 inhibitor (p38i, SB203580). p value: Mann-Whitney test (n = 6) (c) Combinatory effects of chemicals on mESCM cell number in low cell density culture (Dunn's test: ***, $p < 0.001$, $n = 5$, *vs.* DMSO control). Note that BIO+SU+p38i treatment increased mESCM number 14-fold compared to DMSO treatment. (d) Immunostaining and quantification of cell cycle markers. Cardiac troponin T (cTnT, red), EdU (Green), phospho-histone H3 (pH3: white), and DAPI (Blue). Scale bar = 100 μm.

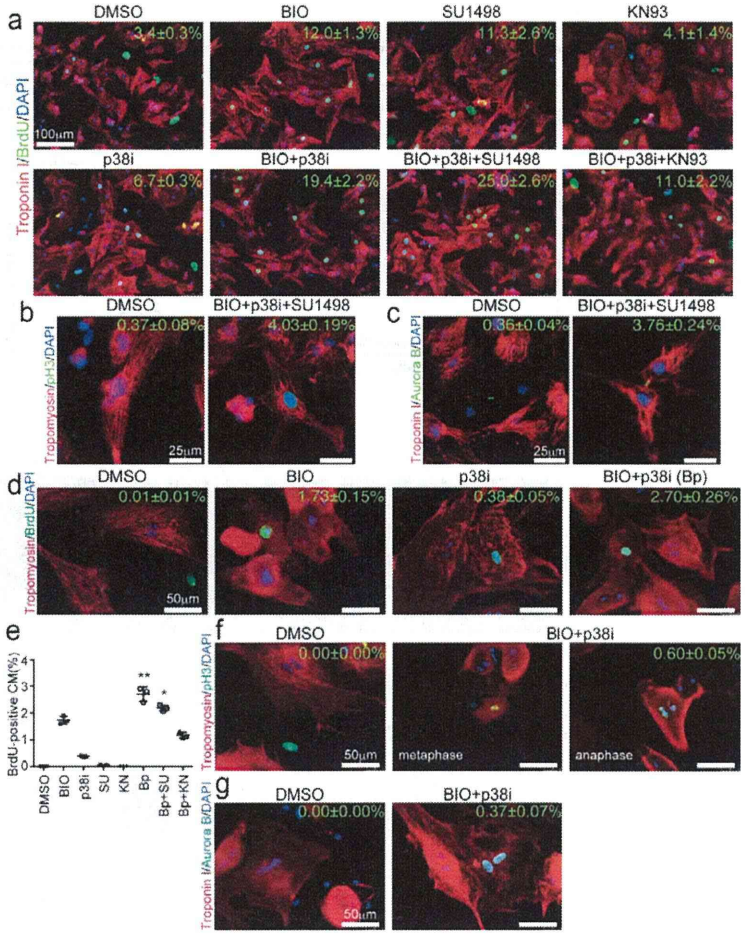

Figure 5.

Identified Chemicals Induced Cardiomyocytes Proliferation with Developmental Stage Specific Manner. (a–c) Immunostaining of neonatal rat cardiomyocytes for cell cycle analysis at day 2 after chemical treatment. (a) Troponin I (red), BrdU (green) and DAPI (blue). Scale bar = 100 μm (b) Tropomyosin (red), pH3 (green) and DAPI (blue). Scale bar = 25 μm (c) Troponin I (red), Arurora B (green) and DAPI (blue). Scale bar = 25 μm (d–g) Immunostaining of adult rat cardiomyocytes for cell cycle markers at day 6 after chemical treatment. (d) Tropomyosin (red), BrdU (green) and DAPI (blue). (e) Quantification of BrdU-positive cardiomyocytes. (Dunn's test: *, $p < 0.05$ and **, $p < 0.01$, *vs.* DMSO treated group, n = 3.) (f) Tropomyosin (red), pH3 (green) and DAPI (blue). (g) Troponin I (red), Aurora B (green) and DAPI (blue).

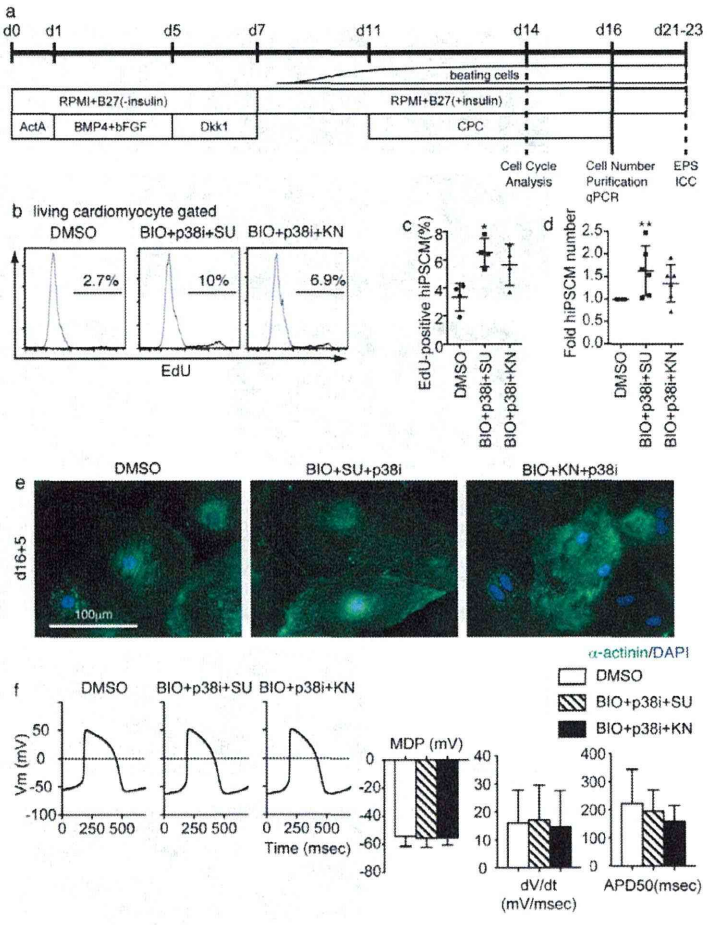

Figure 6.

Effects of Chemicals on Human iPSCs-Derived Cardiomyocytes. (a) Differentiation and experimental scheme for human iPSC study. Differentiation of human iPSCs was induced for 11 days. Cells were then treated by CPCs. (b) Representative plot of EdU in cardiomyocytes at d14. (c) EdU-positive ratio in cTnT-positive cell at d14 after induction. Dunn's test: *, $p < 0.05$, *vs.* DMSO treated group, n = 3.) (d) cTnT-positive cell number at d16. Dunn's test: *, $p < 0.05$, *vs.* DMSO treated group, n = 6.) (e) Immunostaining for α-actinin at d16+5-7. Scale bar = 100μm. (f) Representative action potential of purified VCAM1-positive cells and graphs for action potential parameters at d16+5. MDP, mean diastolic potential. dV/dt, maximum rate of depolarization. APD50, action potential duration. (i) Immuno staining for α-actinin at d16+5-7. No significant difference *vs.* control (n = 17).

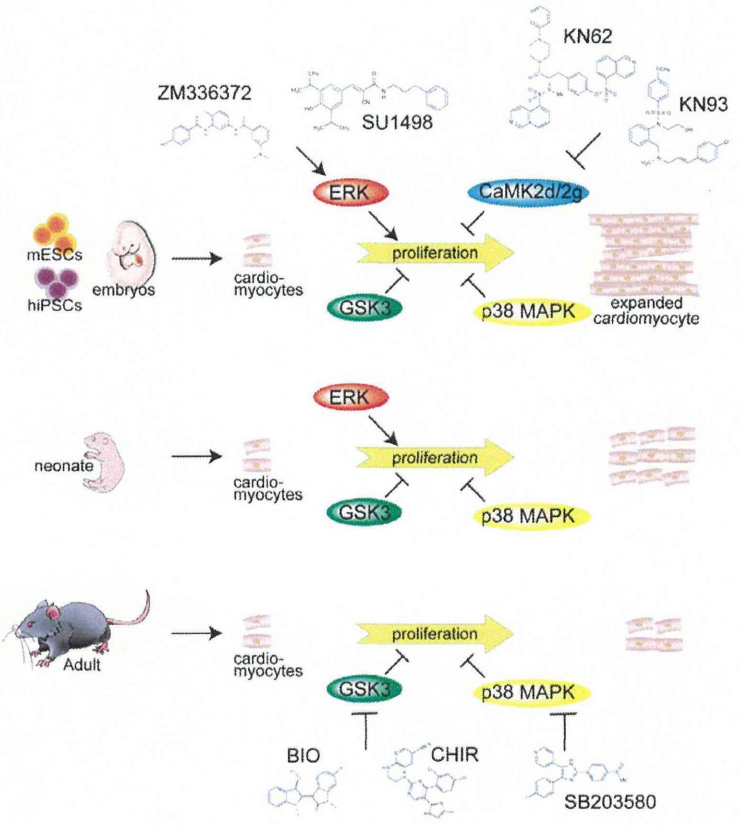

Figure 7.

Scheme of Regulatory Machinery of Cardiomyocyte Proliferation. Four distinct signaling pathways, GSK3, p38 MAPK, and CaMKII signaling negatively, and ERK signaling positively, regulate cardiomyocyte proliferation. Inhibitors for GSK3 (BIO and CHIR), p38 MAPK (SB203580), and CaMKII (KN93 and KN62), and activators for ERK (SU1498 and ZM336372) can be used to induce cardiomyocyte proliferation. A combinatory regulation of these signaling efficiently enhances each effect. We named these chemicals as cardiomyocyte proliferating chemicals (CPCs). CPCs show developmental stage-specific effects. CaMKII inhibitors act only on embryonic and PSC-derived cardiomyocytes. ERK activators still work on neonatal cardiomyocytes but not in adult cardiomyocytes. GSK3 and p38 MAPK inhibitors synergistically enhance cardiomyocyte proliferation broadly even in adults.

Ⅴ. 公開シンポジウム 抄録・シンポジウム発表内容

1st Think Tank Meeting on Cardiac Safety 2014 in Kirishima <Kirishima Meeting>

-From Follower to Player Against Galapagosization-

Date : Jan 11 (Sat) – 12 (Sun), 2014

Venue: Kirishima Iwasaki Hotel

PRESIDENT

Atsushi Sugiyama, MD, PhD
Toho University

VICE PRESIDENT

Kaoru Sugi, MD, PhD
Toho University Ohashi Medical Center

Kohei Sawada, PhD
Eisai Co., Ltd

Yuko Sekino, PhD
National Institute of Health Sciences

Hiroyuki Fukase, MD, PhD
CPC Clinical Trial Hospital, Medipolis Medical Research Institute

THE ORGANIZER

- The Japanese Safety Pharmacology Society

IN ASSOCIATION

- The Japanese Society of Clinical Pharmacology and Therapeutics

UNDER THE AUSPICES

- Ministry of Health Labour and Welfare
- Pharmaceuticals and Medical Devices Agency
- National Institute of Biomedical Innovation
- The Japanese Society of Electrocardiology
- The Japanese Pharmacological Society
- The Japanese Society of Toxicology

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Ryoichi Nagata, MD, PhD (SHIN NIPPON BIOMEDICAL LABORATORIES, LTD.)

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Koki Nakamura, MD, PhD (Takeda Pharmaceutical Company Limited)

Haruaki Nakaya, MD, PhD (Chiba University)

Ikuo Horii, PhD (Pfizer Japan Inc. / Showa University)

Junko Matsuo, MS (Toho University / SHIN NIPPON BIOMEDICAL LABORATORIES, LTD.)

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Shinichi Yoshihara (Mitsubishi Chemical Medience Corporation)

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OVERVIEW

At a FDA/CSRC/HESI-sponsored Think Tank Meeting held at FDA Headquarters on July 23rd, 2013, one of FDA speakers suggested new cardiac safety paradigm to propose "Abandon ICH E14 by July 2015" and "Revise ICH S7B by July 2016". The current paradigm based on S7B and E14 has largely eliminated new drugs with torsadogenic potential entering the market. Since the current paradigm cannot necessarily provide precise information enough to predict proarrhythmic effects of new compounds, pharmaceutical company may have inappropriately discontinued the development of efficacious drugs solely due to their QT–interval prolonging property. Proposed new cardiac safety paradigm involves the Comprehensive *In Vitro* Proarrhythmia Assay (multiple ionic current measurement, *in silico* proarrhythmia prediction model, and usage of human ventricular myocytes) and careful Phase 1 ECG assessment.

Unfortunately, the Japanese regulatory, industry and academia were not directly represented in this discussion. There is a clear risk that Japan will again remain behind in this important debate on the new cardiac safety paradigm. To take the initiative and grow beyond Galapagosization, what goals should we set and how can we achieve them? We have to discuss and develop a strategy. It is critically important in the process to share information on our priorities and be conscious of the same problem in common to develop and propose revised/new ICH Guidelines based on the strategy.

iPS cell related technologies have been originated from Japan. Using iPS-cell derived cardiomyocytes, it may be possible to evaluate not only modulatory effects of a compound on ion channels but also its proarrhythmic potential, especially torsadogenic risk in a quantitative manner. We have to work in research and development in this important area from a leading position to establish de facto standard which will be introduced in future ICH Guidelines.

The objectives of Kirishima Meeting are as follows

To provide an opportunity for the Japanese regulatory, industry and academia to discuss about the latest cardiac safety issues

To show Japan's strategic direction on future technology development

To produce and distribute worldwide a consensus report "Kirishima Declaration"

DAY 1 Saturday, January 11, 2014

OPENING

■ **13:30 - 13:45 Welcome, Agenda Overview and Raising Issues**

Atsushi Sugiyama, MD, PhD

Department of Pharmacology, School of Medicine, Faculty of Medicine, Toho University

■ **13:45 - 14:00 The mission of Kirishima Meeting**

Yuko Sekino, PhD

Division of Pharmacology, Biological Safety Research Center, National Institute of Health Sciences

SYMPOSIUM I

SESSION 1 (14:00 - 15:30)
Clinical Cardiac Safety Evaluation
- ICH E14 and Beyond -

Moderators

Maki Ito, RN, MD, PhD

MSD K.K.

Kaori Shinagawa, MD, PhD

Pharmaceuticals and Medical Devices Agency

■ **14:00 - 14:15 The Thorough QT/QTc Studies - Current Status in Japan -**

Yuji Kumagai, MD, PhD

Clinical Trial Center, Kitasato University East Hospital

■ **14:15 - 14:30 The Current Trends and Issues on Integrated Cardiac Safety**

Hiroyuki Fukase, MD, PhD

CPC Clinical Trial Hospital, Medipolis Medical Research Institute

■ **14:30 - 15:00 The New Paradigm for Proarrhythmia Assessment Without the TQT Study**

Philip T. Sager, MD, FACC, FAHA

Chair, Scientific Oversight Committee, FDA-Sponsored Cardiac Safety Research Consortium, USA

■ **15:00 - 15:30 PANEL DISCUSSION**

All speakers from this session

■ **15:30 - 15:45 BREAK**

SESSION 2-1 (15:45 - 17:15)

Future Perspectives on ICH S7B

- Key Directions for the New Paradigm -

Moderators

Kohei Sawada, PhD

Global CV Assessment, Eisai Co., Ltd.

Junko Kurokawa, PhD

Department of Bio-Informational Pharmacology, Medical Research Institute,

Tokyo Medical and Dental University

■ **15:45 - 16:15 The Current Status of Non-clinical Cardiac Safety in Japan**

- Focusing on Non-clinical Proarrhythima Models -

Atsushi Sugiyama, MD, PhD

Department of Pharmacology, School of Medicine, Faculty of Medicine, Toho University

■ **16:15 - 16:35 Assessment of drug effects on multiple ionic currents *in vitro* & *in vivo***

Haruaki Nakaya, MD, PhD

Department of Pharmacology, Chiba University Graduate School of Medicine

■ **16:35 - 16:55 *In silico* Predictive Modeling in Cardiology**

Takashi Ashihara, MD, PhD

Department of Cardiovascular Medicine, Shiga University of Medical Science

■ **16:55 - 17:15 Regarding UT Heart Simulator**

Seiryo Sugiura, MD, PhD

Graduate School of Frontier Sciences, the University of Tokyo

■ **19:00 - 21:00 NETWORKING RECEPTION**

DAY 2 Sunday, January 12, 2014

SYMPOSIUM II

SESSION 2-2 (8:30 - 9:30)

Future Perspectives on ICH S7B

- Key Directions for the New Paradigm (iPS-cell Derived Cardiomyocytes for Cardiac Safety Pharmacology Studies) -

Moderators

Yuko Sekino, PhD

Division of Pharmacology, Biological Safety Research Center, National Institute of Health Sciences

Yasunari Kanda, PhD

Division of Pharmacology, Biological Safety Research Center, National Institute of Health Sciences