

1-3-2014

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### Recommended Citation

Pleger, Sven T; Raake, Philip; Katus, Hugo A; and Most, Patrick, "Cardiac calcium handling on trial: targeting the failing cardiomyocyte signalosome." (2014). *Center for Translational Medicine Faculty Papers*. Paper 27.

<http://jdc.jefferson.edu/transmedfp/27>

# Circulation Research

JOURNAL OF THE AMERICAN HEART ASSOCIATION



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*Circ Res.* 2014;114:12-14

doi: 10.1161/CIRCRESAHA.113.302748

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231  
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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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## Cardiac Calcium Handling on Trial Targeting the Failing Cardiomyocyte Signalosome

Sven T. Pleger, Philip Raake, Hugo A. Katus, Patrick Most

Targeting abnormal calcium ( $\text{Ca}^{2+}$ ) handling in ventricular cardiomyocytes emerged as a new paradigm for human heart failure (HF) therapy.<sup>1</sup> Cardiomyocytes come with an extensive  $\text{Ca}^{2+}$  signaling toolkit consisting of various  $\text{Ca}^{2+}$  transporters,  $\text{Ca}^{2+}$  channels,  $\text{Ca}^{2+}$  buffer, and sensor proteins. Organized into self-contained signaling modules in which  $\text{Ca}^{2+}$  signaling functions within highly localized environments, the cardiac  $\text{Ca}^{2+}$  signalosome delivers dynamic signals with different spatial and temporal properties that relay compartmentalized  $\text{Ca}^{2+}$  oscillations into specific cellular functions.<sup>2</sup> As a result,  $\text{Ca}^{2+}$  governs not only the cardiomyocyte contractile cycle, but also concurrently control transcription and muscle growth, electric excitability, cell survival, and energy metabolism.<sup>3</sup>

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Key components of the cardiac  $\text{Ca}^{2+}$  signalosome remodel as a molecular hallmark in HF: loss of sarco(endo)plasmic reticulum ATPase 2a (SERCA2a) expression surfaced as 1 critical abnormality in experimental HF and human failing myocardium. The defect alone is sufficient to disable various  $\text{Ca}^{2+}$ -dependent homeostatic mechanisms that drive further deterioration of cardiac function and structure after a primary insult, such as myocardial infarction.<sup>1</sup> Translational studies, particularly in human-relevant large animal models, have successfully used recombinant adeno-associated viral (rAAV) vectors for cardiac-targeted delivery of therapeutically formulated synthetic SERCA2a DNA that resulted in safe and long-term restoration of cardiac function and reversal of structural, electric, and metabolic remodeling in experimental HF.<sup>4</sup> Hence, targeting defective components of the cardiomyocyte  $\text{Ca}^{2+}$  signalosome in HF might bear therapeutic benefits beyond sole improvement of cardiac contractile performance (Figure).

The Calcium Up-regulation by Percutaneous Administration of Gene Therapy In Cardiac Disease (CUPID) trial is the first

The opinions expressed in this editorial are not necessarily those of the editors or of the American Heart Association.

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(*Circ Res.* 2014;114:12-14.)

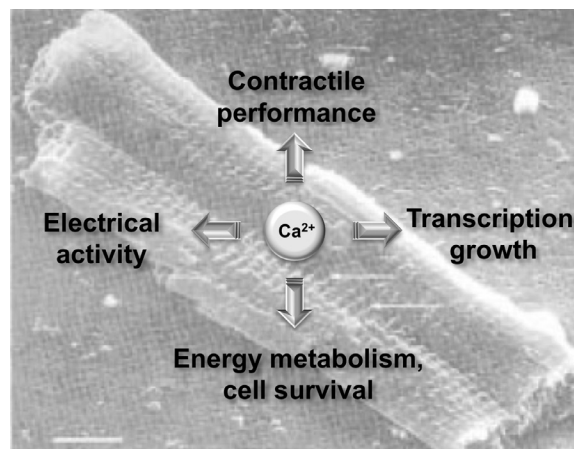
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*Circulation Research* is available at <http://circres.ahajournals.org>  
DOI: 10.1161/CIRCRESAHA.113.302748

translation of this concept into clinically reality. Intracoronary infusion of an rAAV serotype 1 (rAAV1) carrying the human SERCA2a gene under control of a ubiquitous cytomegalovirus promoter (AAV1-cytomegalovirus-SERCA2a) is meant to replenish diminished SERCA2a protein levels in hearts of patients with New York Heart Association (NYHA) stage II/III HF (<http://clinicaltrials.gov>; NCT00454818). To date, this trial epitomizes the quest for novel molecular-targeted HF treatments that could improve conventional clinical regimes, which cannot target underlying molecular defects in failing cardiomyocytes. At the same time, it blazes new regulatory trails for advanced molecular cardiovascular therapies; an experience that is expected to greatly benefit future DNA-based therapeutic developments against human HF.<sup>1</sup>

In this issue of *Circulation Research*, Zsebo et al<sup>5</sup> report on the 36-month follow-up of 39 patients enrolled in CUPID trial phase IIa, which is a randomized, double-blind, placebo-controlled, dose-ranging study. This report advances previously published data on a 12-month active observation period<sup>6</sup> by additional 24-month follow-up, this time using nonadjudicated patient self-reported history. During the second and third year of follow-up, patients were contacted every 6 months by the healthcare provider for a structured questionnaire on health status.

Most importantly, the study reports no apparent adverse events in patients with HF potentially related to the long-term treatment with increasing dosages of rAAV1-cytomegalovirus-SERCA2a.



**Figure. Calcium ( $\text{Ca}^{2+}$ ) regulation of cardiomyocyte function is not limited to contractile performance but extends to control over nuclear transcription, electric activity, cell survival, and energy metabolism.** Targeting key components of the defective  $\text{Ca}^{2+}$  signalosome inside failing cardiomyocytes using recombinant adeno-associated viral-based therapeutic formulations of DNA bears the promise to achieve sustained therapeutic efficacy beyond improvement of cardiac performance.

From an immunologic point of view, this is noteworthy, because studies using rAAV-based therapeutic formulations have progressed from rodent models to clinical trials (see <http://www.abedia.com/wiley/vectors.php> for continuous update). From this, we have learned that immune responses after rAAV gene delivery occur more readily in larger animal models and in humans.<sup>7</sup> Potential immune responses are transgene specific and influenced by ways of administration, choice of rAAV serotypes, as well as dosage, transgene expression levels, and expression control elements. Hence, long-term safety of rAAV1-cytomegalovirus-SERCA2a in humans cannot be taken for granted, particularly, because initial attempts at viral vector-based human gene therapy using retro- or adenoviruses in other fields have been met with issues of toxicity, either through activation of immunity or genomic integration and tumor formation.<sup>8</sup>

Unlike adenoviruses, *in vivo* use of rAAVs entails only transient induction of cytokines in target cells and, in addition, shows inefficient transduction of antigen-presenting cells. Subsequent lack of major histocompatibility complex I-mediated direct transgene presentation may allow rAAVs to evade the generation of a cytotoxic T-cell response; a mechanism likely contributing to rAAV-mediated long-term transgene expression.<sup>7,8</sup> Importantly, the use of ubiquitously active promoters, which can result in high off-target transgene expression in tissues other than the targeted organ, has been reported to drive transgene expression in antigen-presenting cells enabling major histocompatibility complex I-mediated direct transgene presentation and development of transgene-specific immunity over time.<sup>9</sup> In addition, combined use of an rAAV serotype that readily transduces tissues other than the targeted organ can enhance the risk of triggering cellular immunity.<sup>7</sup> In this regard, the encouraging 3-year safety profile of the rAAV1-cytomegalovirus-SERCA2a formulation is an important finding at this early stage of clinical testing where safety is embedded to serve as stopping rule. This result paves the way to phase IIb of the CUPID trial aimed at enrolling 200 patients with HF.

With respect to efficacy and statistical power, a phase IIa clinical trial generally presents special difficulties because it involves the use of a therapeutic agent in a small patient population whose likelihood of benefit and effect size is poorly understood. To address this issue, a statistical method (joint frailty model) of concordant changes in clinical end points for group- and individual-level comparisons and outcome at 6 months for rAAV1-cytomegalovirus-SERCA2a treatment was used in a previous report to determine predefined meaningful changes in the CUPID phase IIa patient population.<sup>6</sup> Now, Zsebo et al<sup>5</sup> report a promising lower 3-year cumulative and cardiovascular clinical event rate, including decreased hospitalization and improved survival in the high-dose rAAV1-cytomegalovirus-SERCA2a group. Because these positive results are based on nonadjudicated patient self-reported history and small patient numbers, more conventional statistical analysis and larger patient populations at the end are actually needed to assess clinical efficacy. Because of the promising trends seen in phase IIa,<sup>6</sup> phase IIb is expected to establish evidence of therapeutic efficacy of rAAV1-cytomegalovirus-SERCA2 in human HF on top of standard

therapy eventually. As part of the study protocol, it will deliver relevant functional and clinical end points such as 6-month changes in left ventricular ejection fraction and peak oxygen consumption, as well as all-cause mortality and hospitalization rate adjudicated by a clinical end point committee.

The authors further provide first information on rAAV1-cytomegalovirus-SERCA2a transgene persistence in explanted hearts of patients with HF<sup>5</sup> who eventually underwent transplantation, required placement of a ventricular assist device, or died. Quantitative polymerase chain reaction analysis was used to detect SERCA2a DNA copies with positive results in the high-dosage group up to month 31 after treatment but not in the placebo, low-dose, or in mid-dose rAAV1-cytomegalovirus-SERCA2a-treated patients. This is another encouraging finding indicating successful transduction of failing myocardium by intracoronary infusion of the therapeutic vector in combination with nitro-glycerine to enhance delivery efficacy.<sup>5</sup> As previous studies have shown that rAAV1 can transduce both cardiomyocytes and noncardiomyocytes,<sup>10</sup> it would be desirable to determine whether myocardial transgene persistence at this point has actually resulted in elevated SERCA2a expression levels in the high-dose rAAV1-cytomegalovirus-SERCA2a treatment group. In this regard, phase IIb of the CUPID trial might provide access to more patient samples potentially enabling assessment of myocardial SERCA2a mRNA expression levels in placebo and treatment groups.

Overall, the authors are to be congratulated for their outstanding achievement. It is the first gene-based therapy against HF being developed in a basic science laboratory that eventually entered clinical testing. Hajjar et al<sup>4-6</sup> came a long way and mastered numerous regulatory hurdles not to mention other challenges, including scalability and establishing clinical feasibility of the therapeutic approach. Now, CUPID phase IIb is both expected and needed to bring the necessary breakthrough to establish clinical efficacy of a novel therapeutic principle targeting the defective cardiac Ca<sup>2+</sup> signalosome.

### Sources of Funding

This work is funded by National Institutes of Health grants RO1 HL 092130-01 (to P. Most), grants from the DZHK (German Center for Cardiovascular Research (to P. Most and H.A. Katus), and the DFG (562 1/1 to S.T. Pleger and P. Most).

### Disclosures

Drs Most and Katus hold patents on the therapeutic use of S100A1 in cardiovascular diseases. The other authors report no conflicts.

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KEY WORDS: Editorials ■ calcium signaling ■ genetic therapy ■ heart failure