

ARTICLE TYPE: SUBJECT CLASSIFICATION (Example: RNOMICS)- [Bell MT, 11]

# CLOCKWORK HEARTS: CRISPR-ENGINEERED HIPSC CARDIOMYOCYTES FOR IMMUNE-INVISIBLE, CIRCADIAN SMART CARDIAC REPAIR

Saidulu Mattapally<sup>1</sup>, and Christine E. Seidman<sup>2</sup> (Font: Times New Roman; bold, size: 12; Color: Black, Full name of authors, no initial is allowed)

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\*Corresponding author: Email: [xxx@upm.edu](mailto:xxx@upm.edu) Tel: +40-9001010010 ; Fax: +40-9001010012

## ABSTRACT

Maximum limit for abstract is 250-400 words. It should be in Arial, 9 font size, Italics. Induced pluripotent stem cells (hiPSCs) and CRISPR/Cas9 technology are being used to study circadian rhythm regulation and develop therapies for cardiac repair, including creating "immune-invisible" cardiomyocytes and improving cell engraftment. Preclinical studies using hiPSC-derived cardiomyocyte spheroids transplanted into animal models show potential for cardiac repair by promoting healing, vascularization, and immune modulation, although long-term effects and immune rejection remain challenges for clinical translation. Furthermore, the role of circadian rhythm—a fundamental biological regulator—in hiPSC and hiPSC-CM function, and its potential impact on cardiac repair outcomes, is not fully understood. This study addresses these challenges by developing a "Clockwork Hearts" platform, which utilizes CRISPR/Cas9 technology to engineer hiPSC-CMs for enhanced immune compatibility and circadian regulation. We generated cardiomyocytes with genetic knockouts of immune-related genes to investigate their regulatory role. Additionally, CRISPR/Cas9 was used to overexpress Connexin43, enhancing gap junction formation and electrical coupling. Three-dimensional hiPSC-CM spheroids were produced from these engineered cells for preclinical transplantation studies in a myocardial infarction model. Our findings demonstrate that these engineered spheroids promote robust cardiac healing, evidenced by reduced inflammation (e.g., downregulated TNF- $\alpha$ , IL-1 $\beta$ , IL-6), enhanced angiogenesis, and improved cardiac remodeling. The circadian-engineered cells further elucidate the link between circadian gene regulation and cardiomyocyte function, while immune-invisible spheroids show reduced immune cell infiltration. This work establishes a novel approach for developing next-generation cell therapies that are both "immune-invisible" and "circadian-smart," overcoming key barriers to successful clinical translation for cardiac repair.

**Keywords:** 5-6 keywords separated by semicolon (;), Trebuchet MS, 9 font size

## [I] NTRODUCTION (TREBUCHET MS, 12; ALL CAP BOLD)

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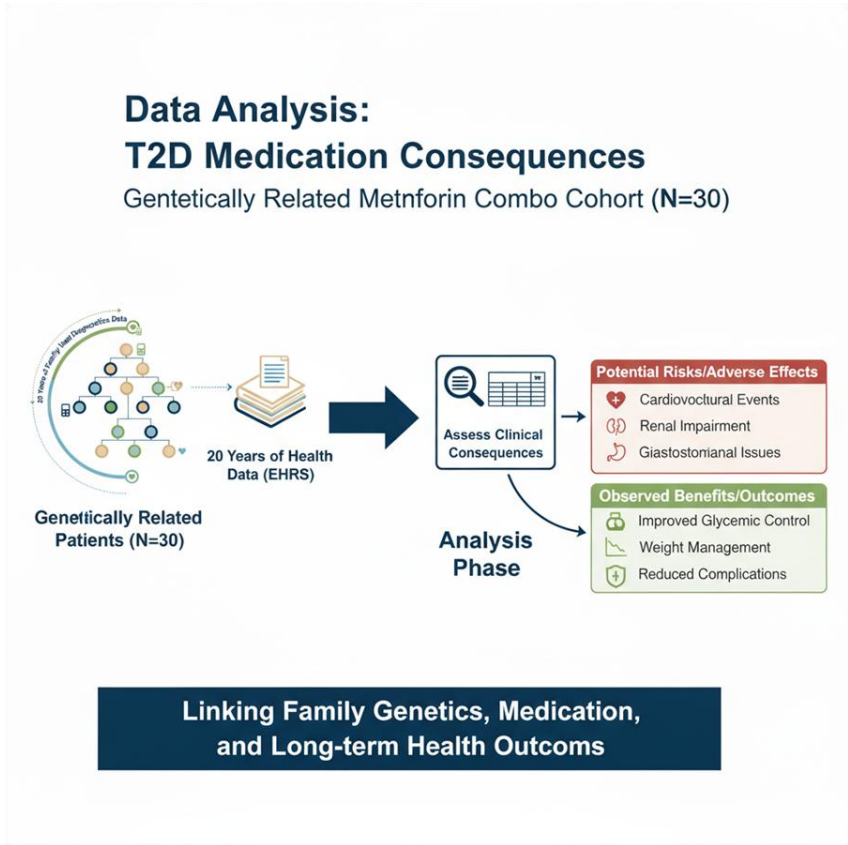


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**[V] CONCLUSION (TREBUCHET MS, 12; ALL CAP BOLD)**

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**FINANCIAL DISCLOSURE (Trebuchet MS, 10; all cap; Dark red, Bold)**

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**ACKNOWLEDGEMENT (Trebuchet MS, 10; all cap; Dark red, Bold)**

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**REFERENCES (TREBUCHET MS, 12; ALL CAP BOLD)**

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ABOUT AUTHORS



Prof. ABC is.....



Prof. XYZ is.....