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Rapid and scalable preclinical evaluation of personalized antisense oligonucleotide therapeutics using organoids derived from rare disease patients

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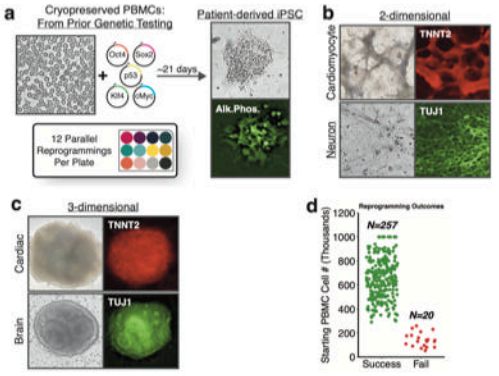
Abstract

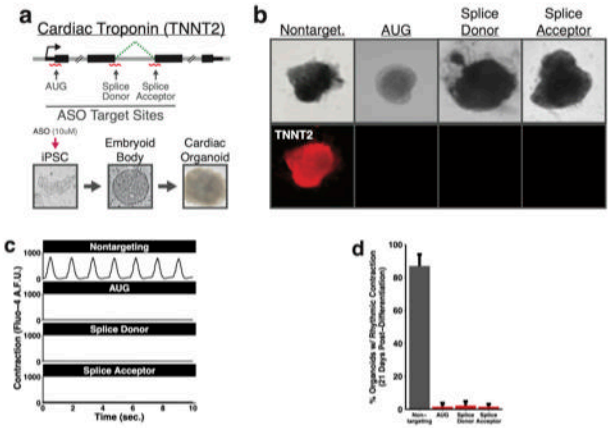
Personalized antisense oligonucleotides (ASOs) have achieved positive results in the treatment of rare genetic disease. As clinical sequencing technologies continue to advance, the ability to identify rare disease patients harboring pathogenic genetic variants amenable to this therapeutic strategy will likely improve. Here, we describe a scalable platform for generating patient-derived cellular models and demonstrate that these personalized models can be used for preclinical evaluation of patient-specific ASOs. We establish robust protocols for delivery of ASOs to patient-derived organoid models and confirm reversal of disease-associated phenotypes in cardiac organoids derived from a Duchenne muscular dystrophy (DMD) patient harboring a structural deletion in the dystrophin gene amenable to treatment with existing ASO therapeutics. Furthermore, we design novel patient-specific ASOs for two additional DMD patients (siblings) harboring a deep intronic variant in the dystrophin gene that gives rise to a novel splice acceptor site, incorporation of a cryptic exon, and premature transcript termination. We show that treatment of patient-derived cardiac organoids with patient-specific ASOs results in restoration of DMD expression and reversal of disease-associated phenotypes. The approach outlined here provides the foundation for an expedited path towards the design and preclinical evaluation of personalized ASO therapeutics for a broad range of rare diseases.

Figure 1. A rapid and scalable platform for the generation of patient-derived cellular models.



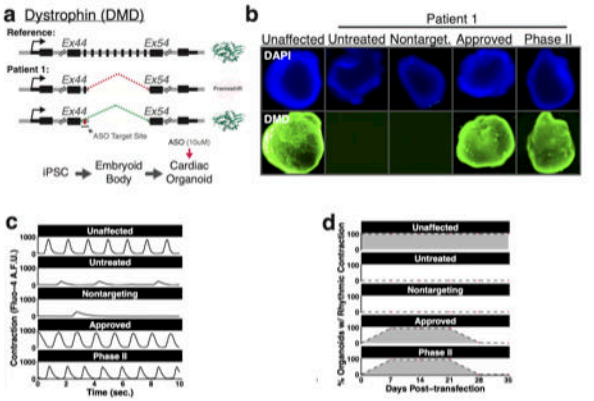
a) Schematic of iPSC reprogramming workflow. **b)** Differentiation of patient-derived iPSCs into 2-dimensional cardiomyocytes and 3-dimensional cardiac organoids. **c)** Differentiation of patient-derived iPSCs into 2-dimensional neurons and 3-dimensional brain organoids. **d)** Reprogramming outcomes relative to PBMC input cell counts.

Figure 2. Robust genetic perturbation in iPSC systems using ASOs.



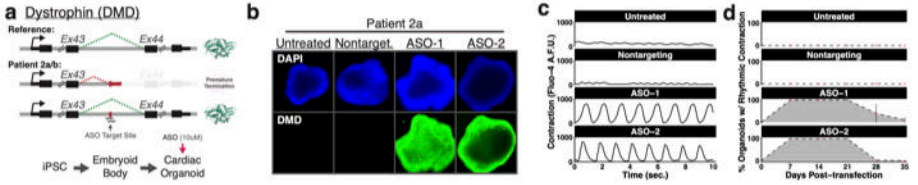
a) Schematic of design and delivery strategy for ASOs targeting cardiac troponin T. **b)** Cardiac troponin T expression in cardiac organoids derived from ASO-treated iPSCs. **c)** Contraction of individual cardiac organoids derived from ASO-treated iPSCs as determined by intracellular calcium levels. **d)** Summarized contraction of cardiac organoids derived from ASO-treated iPSCs.

Figure 3. Profiling activity of existing ASO therapeutics in a patient-derived organoid model of disease.



a) Schematic of design and delivery strategy to profile existing ASO therapeutics for the treatment of DMD. **b)** Dystrophin expression in DMD patient-derived cardiac organoids (patient 1) treated with ASOs matching the sequence of existing ASO therapeutics. **c)** Contraction of individual DMD patient-derived cardiac organoids (patient 1) treated with ASOs matching the sequence of existing ASO therapeutics. **d)** Time course analysis of restored contraction in DMD patient-derived cardiac organoids (patient 1) treated with ASOs matching the sequence of existing ASO therapeutics.

Figure 4. Design and preclinical evaluation of patient-specific ASOs in patient-derived organoids.



a) Schematic of design and delivery strategy for preclinical evaluation of novel patient-specific ASOs. **b)** Dystrophin expression in DMD patient-derived cardiac organoids (patient 2a) treated with patient-specific ASOs. **c)** Contraction of individual DMD patient-derived cardiac organoids (patient 2a) treated with patient-specific ASOs. **d)** Time course analysis of restored contraction in DMD patient-derived cardiac organoids (patient 2a) treated with patient-specific ASOs.

Conclusion

In conclusion, the data described here demonstrate reduction to practice for the use of patient-derived organoid models in the evaluation of personalized therapeutics. The methods and protocols generated in this study are accessible and can be implemented in any standard research laboratory without the need for specialized equipment or high-cost reagents. The widespread ability to generate patient-derived cellular systems will have a significant impact on the understanding of disease mechanisms as well as potential therapeutic avenues for the treatment of many rare diseases.

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