ACCSCIENCE PUBLISHING

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COMMENTARY

Organoids as a platform for personalized antisense oligonucleotide screening: Advancing precision medicine

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Citation: Wan X, Zhou Z, Wang D. Organoids as a platform for personalized antisense oligonucleotide screening: Advancing precision medicine. *Organoid Res*. 2025;1(3):025120012. doi: 10.36922/OR025120012

Received: March 21, 2025

Revised: April 29, 2025

Accepted: May 16, 2025

Published online: June 6, 2025

Abstract

Organoid technology has transformed precision medicine by enabling patient-specific 3D models that replicate tissue complexity, facilitating high-throughput antisense oligonucleotide (ASO) therapeutic screening. Patient-derived organoids retain donor-specific genetic and phenotypic profiles, offering physiologically relevant platforms for modeling diseases, such as Duchenne muscular dystrophy (DMD). For example, DMD cardiac organoids rapidly identify dystrophin-restoring ASOs through a 6-week validation pipeline, overcoming limitations of 2D cultures by preserving multicellular interactions. Challenges include expanding tissue representation (e.g., skeletal muscle in DMD), enhancing ASO pharmacokinetic modeling in avascular organoids, and standardizing protocols to minimize variability. Future integration of vascularized or organ-on-chip models, multi-tissue assembloids, and artificial intelligence-driven screening could improve predictive accuracy. Chemically optimized ASOs with reduced off-target effects, combined with clustered regularly interspaced short palindromic repeats-based editing, may synergistically enhance therapeutic precision. As regulatory frameworks adapt to incorporate organoid-based validation, this technology accelerates personalized drug discovery for genetic disorders. Addressing present limitations through bioengineering and standardization will solidify organoids as critical tools for tailoring precision therapies to individual patient needs.

Keywords: Organoids; Antisense oligonucleotides; Personalized drug screening

The advent of organoid technology has fundamentally transformed biomedical research by providing three-dimensional (3D) models that closely replicate the intricate architecture and function of human tissues.[1] Conventional two-dimensional (2D) cell cultures, though widely utilized, fail to capture the complex cellular interactions inherent to native tissue environments, while animal models, despite their physiological relevance, often exhibit species-specific differences that limit their translational value. Organoids bridge this gap by self-organizing into miniature, organ-like structures, allowing for precise investigation into tissue development, disease pathogenesis, and therapeutic interventions within a controlled, patient-specific framework.[2] Among the most impactful applications of organoid technology is its role in disease modeling, particularly in genetic disorders where patient-derived organoids (PDOs) serve as high-fidelity pre-clinical platforms for evaluating individualized therapeutic strategies.[3] These organoids retain the genetic, molecular, and phenotypic characteristics of their donor tissue, making them highly relevant for studying pathogenic mutations, transcriptomic alterations, and drug responses. Antisense oligonucleotide (ASO) therapies hold transformative potential for treating genetic disorders by directly targeting disease-causing RNA, yet their development is hindered by the lack of pre-clinical models that accurately reflect patient-specific genetic diversity and tissue complexity. Conventional screening platforms

often fail to predict clinical outcomes due to oversimplified cellular environments or interspecies discrepancies, leading to high attrition rates in clinical trials.[4]

Means *et al.*[5] introduced an innovative approach that integrates PDOs with high-throughput ASO screening, establishing a methodology that significantly accelerates the identification and validation of patient-specific ASO candidates. Published in *Nature* under the title *Rapid and Scalable Personalized ASO Screening in* PDOs, this study showcases a streamlined workflow whereby cardiac organoids derived from Duchenne muscular dystrophy (DMD) patient-specific induced pluripotent stem cells (iPSCs) effectively recapitulate the cardiac dysfunction characteristic of the disease. The study evaluates ASO-mediated dystrophin restoration, demonstrating that PDO-based ASO screening constitutes a scalable and efficient platform for precision medicine. This study establishes a rapid and scalable platform for generating patient-derived cellular models (Figure 1A-H). The findings open new avenues for organoid-based genetic therapy testing, extending beyond DMD to a broad spectrum of hereditary disorders.

Organoids are increasingly recognized as powerful tools in personalized medicine, revolutionizing pre-clinical drug development by offering a patient-specific testbed for ASO therapeutics. Unlike conventional models, organoids faithfully preserve patient-specific genetic mutations, ensuring that *in vitro* therapeutic responses closely mirror those observed *in vivo*. Furthermore, they provide a complex, multicellular microenvironment, which is superior to traditional 2D cultures that often fail to recapitulate the tissue-level effects of gene-targeting therapies. An additional advantage of this approach is its efficiency and scalability. The study's methodology reduces the ASO validation timeline to merely 6 weeks, a significant acceleration compared to conventional drug development pipelines. Such a rapid and adaptable workflow facilitates the customization of gene therapies at the individual level, expediting their transition into clinical applications. Beyond ASO therapeutics, organoids hold immense promise for screening an array of personalized treatments, including small molecules, RNA-based drugs, and clustered regularly interspaced short palindromic repeats (*CRISPR*)-*Cas9* gene-editing therapies. Given their high fidelity in replicating disease phenotypes, organoids are poised to serve as next-generation pre-clinical models for a diverse range of conditions, encompassing neuromuscular diseases, metabolic disorders, and oncological pathologies.

Despite these advantages, several key challenges must be addressed before PDO-based ASO screening can be seamlessly integrated into clinical practice. A primary limitation is tissue representation.[6] The study

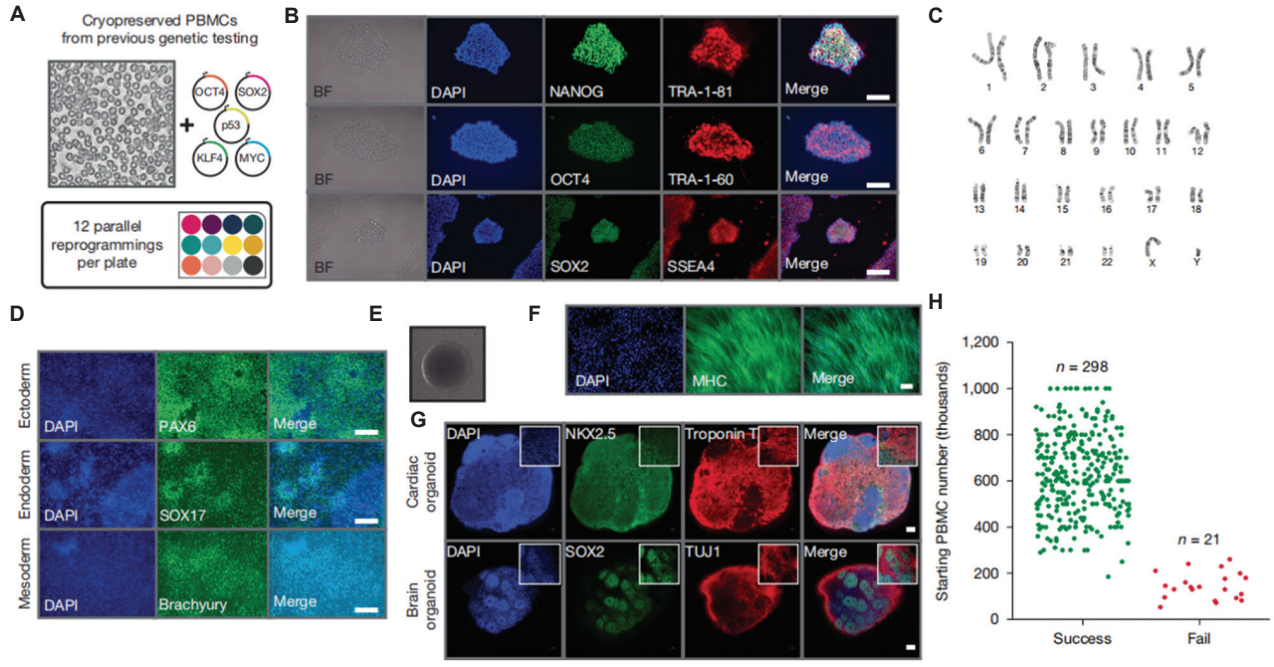

Figure 1. A rapid and scalable platform for the generation of patient-derived cellular models. (A) Schematic of the iPSC reprogramming workflow. (B) iPSC marker expression in patient-derived iPSCs. (C) Representative karyotype of patient-derived iPSs. (D) Differentiation of patient-derived iPSCs into ectoderm, endoderm and mesoderm lineages. (E) Embryoid body formation using patient-derived iPSs (patient 1). (F) Differentiation of patient-derived iPSs into two-dimensional skeletal muscle. (G) Differentiation of patient-derived iPSs into three-dimensional cardiac and brain organoids. (H) Reprogramming outcomes relative to PBMC input cell counts.[5]
Abbreviations: BF: Bright field; iPSC: Induced pluripotent stem cell; PBMC: Peripheral blood mononuclear cell.

predominantly focuses on cardiac organoids, whereas DMD primarily manifests in skeletal muscle. Although cardiac complications contribute to disease morbidity, skeletal muscle degeneration is the defining pathological hallmark of DMD.[7] This underscores the necessity for developing skeletal muscle organoids and neuromuscular junction models, as well as expanding the organoid repertoire to encompass multi-tissue co-culture systems that can enhance the translational fidelity of ASO screening. To resolve this, future work should prioritize developing multi-lineage organoid systems that replicate tissue crosstalk, such as neuromuscular junction co-cultures for muscular dystrophies. Another challenge lies in ASO delivery and pharmacokinetics within organoid models. While ASOs efficiently modulate gene expression *in vitro*, their therapeutic efficacy in patients depends on factors such as cellular uptake, tissue penetration, and metabolic stability. The absence of a vascularized system in present organoid models restricts their ability to recapitulate systemic ASO absorption, distribution, and clearance, limiting their predictive accuracy for clinical applications.[8] The development of vascularized, perfusable organoids or microfluidic-based organ-on-a-chip models represents a promising avenue to overcome these limitations, enabling a more physiologically relevant assessment of ASO pharmacodynamics. In addition, genetic and epigenetic variability among PDOs poses a challenge for reproducibility. iPSC-derived organoids may exhibit batch-to-batch variability, which can affect experimental consistency and ASO responsiveness. Ensuring genetic stability through standardized differentiation protocols, whole-genome sequencing, and epigenetic profiling is paramount to improving reproducibility and ensuring therapeutic reliability. The final barrier to widespread clinical adoption is regulatory approval and integration into existing drug development pipelines. While the study establishes a robust pre-clinical platform, regulatory agencies have yet to fully incorporate organoid-based drug screening into standardized approval frameworks.[9] Furthermore, ethical considerations surrounding patient-derived iPSC biobanking, data privacy, and long-term storage of PDOs require careful oversight. Developing comprehensive regulatory guidelines and quality control measures will be instrumental in facilitating the transition of PDO-based drug screening into routine clinical use.

To fully harness the therapeutic potential of PDO-based ASO screening, future research should focus on broadening organoid models, optimizing ASO therapeutics, and enhancing clinical translation strategies. One particularly promising direction is the extension of this methodology to other genetic disorders, such as spinal muscular atrophy, Huntington's disease, and cystic fibrosis.[8] The establishment of a diverse, patient-specific organoid biobank could facilitate high-throughput ASO screening across multiple disease paradigms, refining the identification of optimal therapeutic candidates. Further advancements in next-generation ASO design could improve therapeutic specificity and durability. Chemically modified ASOs with enhanced stability, reduced off-target effects, and superior cellular uptake will be critical for sustained therapeutic efficacy.[10] Moreover, combining ASOs with genome-editing technologies, such as CRISPR-Cas9 could offer synergistic benefits,[11] enabling permanent correction of pathogenic mutations alongside transient RNA modulation. Another crucial area of development is improving the organoid microenvironment to better recapitulate the *in vivo* physiological milieu. Present organoid models lack immune system components, which play an essential role in drug metabolism and therapeutic responses. The creation of immune-competent organoids incorporating patient-derived macrophages and T cells could enable a more nuanced evaluation of ASO-induced immune activation and toxicity. In addition, the integration of artificial intelligence (AI-driven high-throughput screening methodologies could accelerate ASO candidate optimization, enabling predictive modeling of patient-specific therapeutic outcomes and refining personalized treatment selection.

The convergence of PDO technology with ASO screening marks a paradigm shift in precision medicine, offering scalable, physiologically relevant, and patient-specific disease models for therapeutic evaluation. The study by Means *et al.*[5] provides compelling evidence for the feasibility of PDO-based ASO screening as an accelerated and effective platform for gene therapy validation. However, challenges remain, particularly in tissue representation, ASO pharmacokinetics, and clinical integration. Addressing these limitations through the expansion of organoid models, optimization of ASO therapeutics, and incorporation of advanced bioengineering and AI-driven technologies will be pivotal in establishing PDOs as the gold standard for personalized medicine. With continued advancements, organoid-based precision medicine is poised to redefine drug discovery, revolutionize gene therapy development, and transform clinical treatment paradigms for genetic disorders.

Acknowledgments

None.

Funding

None.

Conflict of interest

The authors declare no conflicts of interest.

Author contributions

Conceptualization: All authors
Writing – original draft: Xufeng Wan
Writing – review & editing: Zongke Zhou, Duan Wang

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

Not applicable.

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