Finding the Needle in the Haystack: Rapid Phenotypic-based Target Identification, Hit Stratification, and Clinical Translation with Human iPSC-derived Neurospheroids

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INTRODUCTION and METHODS

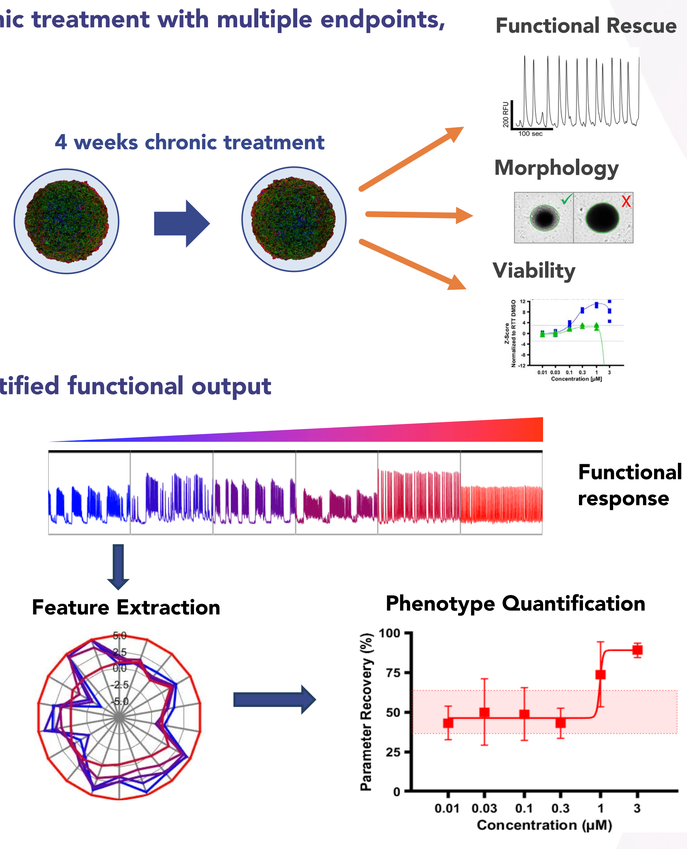
The high-risk of drug discovery and potential for late-stage failure is axiomatic and in large part due to early screens needing to sacrifice relevant biology for high-throughput approaches. Phenotypic screens offer a solution by utilizing deeper biology and have an emerging track record for successful drug development and clinical deployment.

Three dimensional human IPSC-based approaches recapitulate native behavior and lend themselves to phenotypic approaches; models generated from clinical populations often show an altered in vitro phenotype that can be 'rescued' in early screens. Crucial to this process is accurate target identification and hit stratification for further development. This is possible through using selected reference libraries and advanced combinatorial analytical approaches.

By placing human biology first in the pipeline, and deploying advanced analytical methods, three-dimensional human induced pluripotent stem cell (hiPSC)-based platforms de-risk the traditional discovery pipeline through early hit efficacy stratification and potential toxicity identification.

Here, we present a case study using an iPSC-derived neural spheroid model (microBrain3D® of Rett Disease and specifically demonstrate;
- Runctional rescue of the in vitro Rett disease phenotype.
- Target identification and hit stratification through multi-parametric analysis.
- Clinical translation of the platform through interrogation of a Phase III target.

Chronic treatment with multiple endpoints,



Results

1 microBrain® 3D: Ready-to-use HT plates

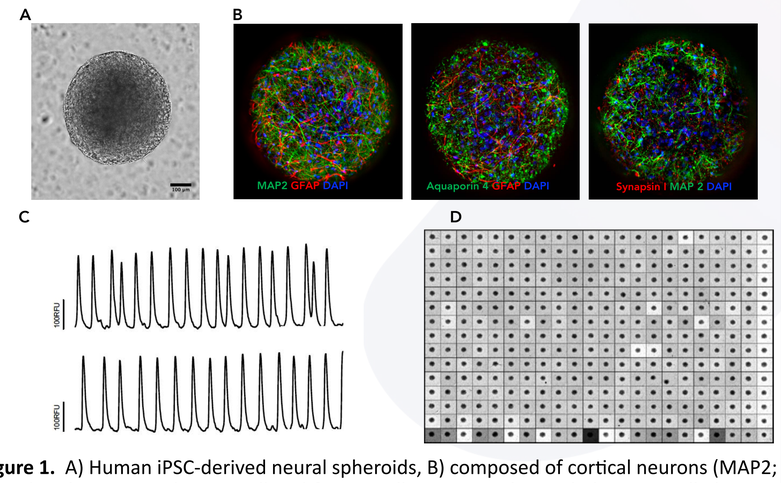


Figure 1. A) Human iPSC-derived neural spheroids, B) composed of cortical neurons (MAP2; green) and astrocytes (GFAP; red), . C) functionally active, and provided in 384-well plates

RESULTS

2 Disease modelling with microBrain 3D

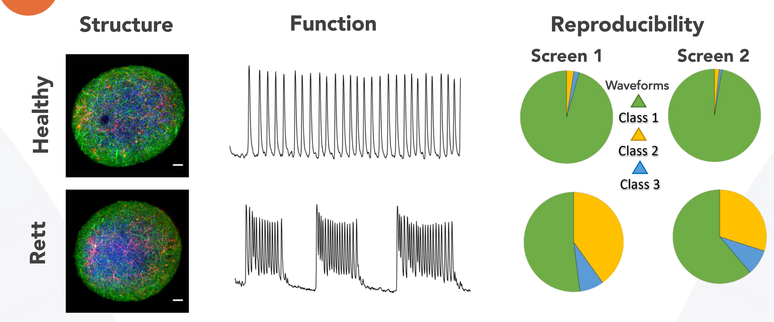


Figure 2. microBrain 3D neurospheroids were generated from a male Rett patient and unaffected parent. Neurospheroids showed similar structure, with different functional outputs, and reproducible phenotypes across screens

3 Primary screen identifies target pathways

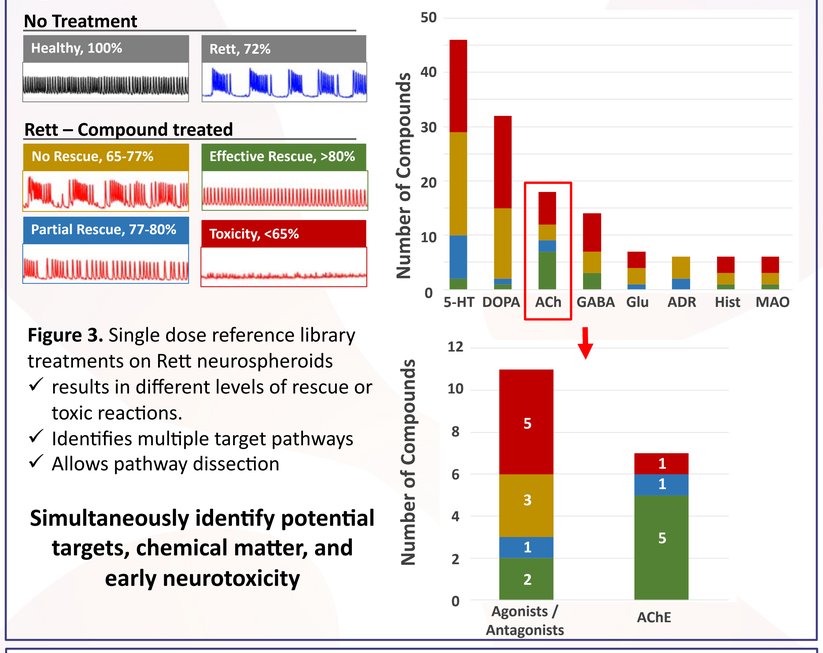


Figure 3. Single dose reference library treatments on Rett neurospheroids
- ✓ results in different levels of rescue or toxic reactions.
- ✓ Identifies multiple target pathways
- ✓ Allows pathway dissection

Simultaneously identify potential targets, chemical matter, and early neurotoxicity

4 Dose response screen verifies and stratifies responses

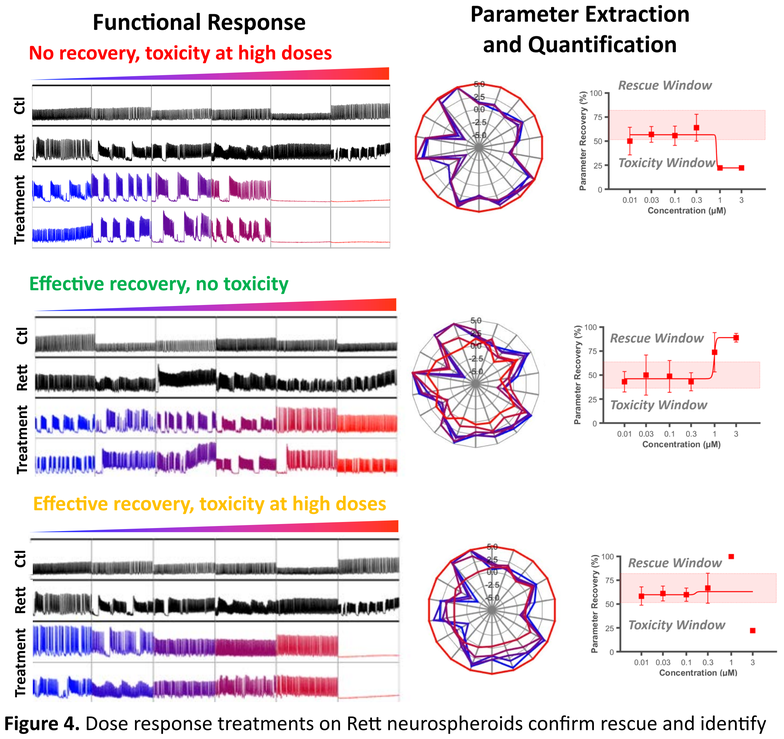


Figure 4. Dose response treatments on Rett neurospheroids confirm rescue and identify potential functional toxicity

Further stratify targets, chemical matter, and early neurotoxicity

RESULTS

5 Rank ordering compound rescue

Target	Cmpd	Rescue
A	1	100%
	2	92%
	3	86%
	4	83%
	5	64%
	6	58%
	7	67%
	8	61%
	9	56%
	10	67%
B	11	89%
	12	81%
	13	47%
	14	58%
C	15	81%
	16	69%
	17	72%
	18	58%
	19	53%
	20	50%
	21	50%
	22	47%
	23	50%

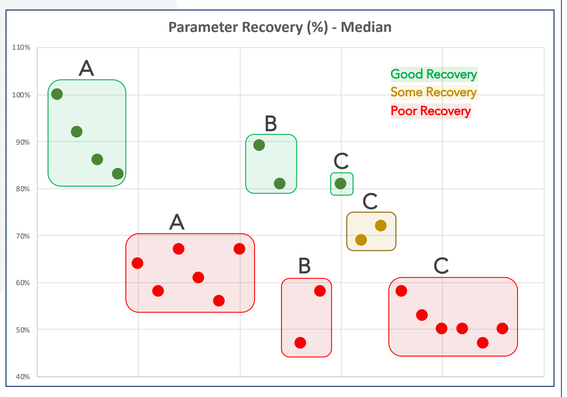


Figure 5. Compounds can be rank-ordered based on the. Percent rescue of the Rett phenotype relative to health microBrain function.

6 Holistic compound stratification

Target	Cmpd	Functional Rescue	Functional Toxicity	Structure (Diameter)	Viability (ATP)
A	1	✓	!	!	!
	2	✓			
	3	✓	!	!	!
	4	✓			
	5	X			
	6	X			
	7	X		!	
	8	X	!	!	!
	9	X			
	10	X	!	!	!
B	11	✓			
	12	✓			
	13	X	!	!	!
	14	X			
C	15	✓			
	16	X	!	!	
	17	✓			
	18	X			
	19	X		!	
	20	X	!	!	!
	21	X			
	22	X			
	23	X		!	

Figure 6. Multi-parametric assessment across functional, structural, and metabolic endpoints further stratifies compounds.

Quantitative, function-based, compound progression

7 Clinical Translation

- **Sigma-1 receptor agonist in Phase III Clinical Trial (Anavex 2-73).**
- **StemoniX screened SA4503, selective Sigma-1 receptor agonist.**

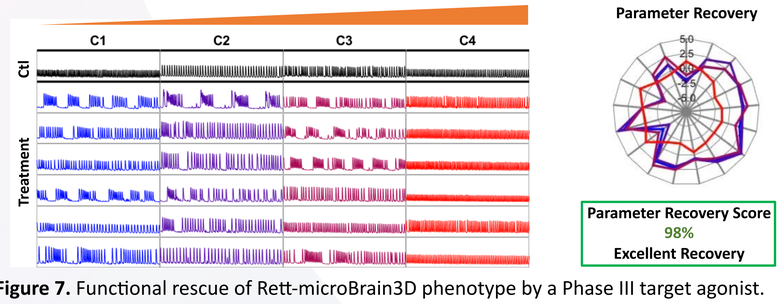


Figure 7. Functional rescue of Rett-microBrain3D phenotype by a Phase III target agonist.

microBrain3D translatability demonstrated through convergence on clinical targets

CONCLUSION and FUTURE DIRECTIONS

- **microBrain 3D de-risks Drug Discovery through early efficacy and toxicity testing with advanced AnalytiX™ relevant and translatable biology.**
- **Next steps include expanding the hit funnel across multiple targets through virtual drug discovery and empirical validation with AtomNet® and microBrain®, respectively.**

Building robust discovery pipelines by placing human biology first