PYC-002: Targeting SHANK3 with an RNA therapeutic approach for the treatment of Phelan-McDermid syndrome

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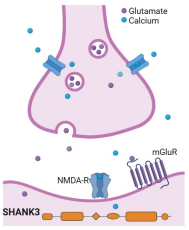
PYC Therapeutics

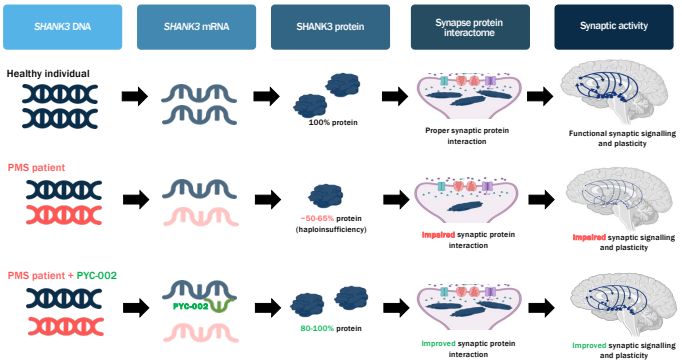
1. Phelan-McDermid syndrome is a severe neurodevelopmental disorder affecting children with no available disease-modifying treatments

- Phelan-McDermid syndrome (PMS) is associated with a variety of symptoms including intellectual disability, developmental and speech delays, autism-spectrum features, seizures, and hypotonia[1,2]
- PMS is caused by a mutation in, or deletion of, one copy of the ***SHANK3*** gene and requires a genetic diagnosis[3]
- Haploinsufficiency of SHANK3 protein, which plays a critical scaffolding role in postsynaptic neurons, results in impaired synaptic signalling and plasticity[2,3]
- PMS affects ~1 in 10,000 people with an estimated ~34,000* addressable patients[3,4]
- There is a major unmet patient need with no disease-modifying therapies currently available
- PYC-002 is a drug candidate that seeks to **precisely address the underlying cause of PMS** using antisense oligonucleotides to restore SHANK3 protein expression

* Patient estimates are based on prevalence multiplied by population in Western World (Australia, Europe, and USA) aged under 30 years.



2. PYC-002 addresses the root cause of PMS – SHANK3 haploinsufficiency



3. PYC-002 increases expression of SHANK3 protein, improves synaptic density and restores calcium-mediated synaptic signalling in PMS neurons

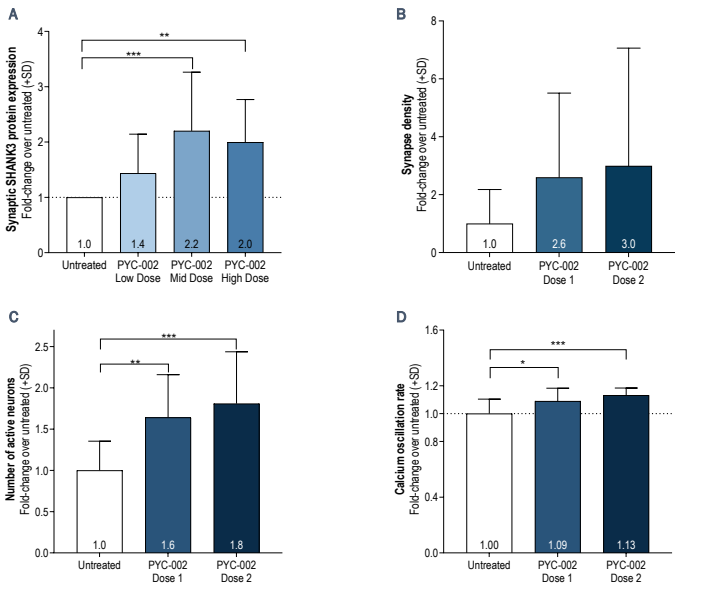


Figure 1. A) PYC-002 increases synaptic SHANK3 expression. Mean fold-change (+SD) of SHANK3 protein expression on 100 µm of neurite over untreated group after 21 days of PYC-002 gymnotic treatment of PMS patient-derived iPSC-neurons (n=2 biological replicates, each with 5 – 24 technical replicates), assessed by high content imaging. Statistical significance assessed using 2-way ANOVA. **B) PYC-002 improves synapse structure.** Mean fold-change (+SD) in synapse density in PMS patient-derived iPSC-neurons (n=2 biological replicates, each with 8 – 15 technical replicates) after 21 days of gymnotic treatment with PYC-002 compared to the untreated control. A synapse is defined as the co-localization of SHANK3 (post-synaptic marker), SYNAPSIN (pre-synaptic marker) and Tuj-1 (neurite marker) proteins. Statistical significance evaluated using 2-way ANOVA. **C) PYC-002 increases neuronal activity.** Mean fold-change (+SD) of the number of PMS patient-derived iPSC-neurons that are active by Calcium signalling pathway after 21 days of PYC-002 gymnotic treatment compared to the untreated control (n=2 biological replicates, n=6 technical replicates per group). Statistical significance evaluated using 2-way ANOVA. **D) PYC-002 increases calcium signalling.** Mean fold-change (+SD) of calcium oscillation rate in PMS patient-derived iPSC-neurons (n=3 biological replicates), after 21 days of PYC-002 gymnotic treatment compared to the untreated control. Each biological replicate contained n=13 technical replicates per PYC-002 treatment group and n=23 untreated replicates. Statistical significance evaluated using 2-way ANOVA.
*p≤0.05, **p≤0.01, ***p≤0.001, ****p≤0.0001

4. PYC-002 has desirable safety and tolerability profile in two pre-clinical species

Sprague-Dawley Rat (*Rattus norvegicus*), specific pathogen-free grade

Dose	Number of animals	Safety/Toxicology
Vehicle control	9	Well-tolerated
Low	9	Well-tolerated
Low-mid	3	Well-tolerated
Mid	11	Well-tolerated
High	6	Well-tolerated

Cynomolgus Monkey (*Macaca fascicularis*), conventional grade

Dose	Number of animals	Safety/Toxicology
Vehicle control	3	Well-tolerated
Low	3	Well-tolerated
Low-mid	3	Well-tolerated
Mid	1	Well-tolerated
Mid-high	1	Well-tolerated
High	1	Well-tolerated

5. PYC-002 shows broad distribution to disease-relevant brain regions following intrathecal administration

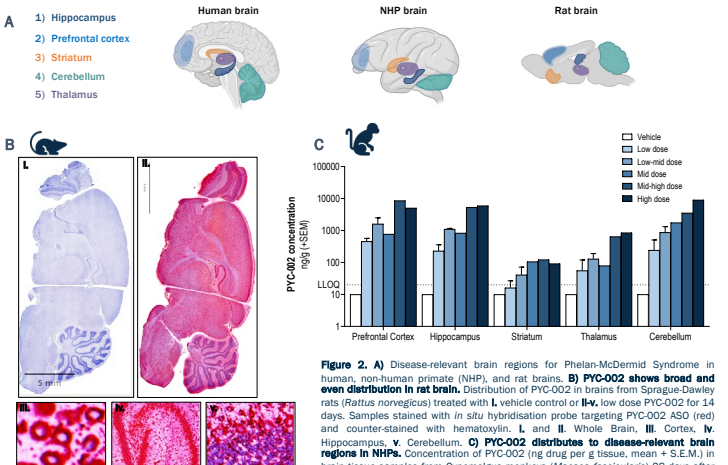


Figure 2. A) Disease-relevant brain regions for Phelan-McDermid Syndrome in human, non-human primate (NHP), and rat brains. **B) PYC-002 shows broad and even distribution in rat brain.** Distribution of PYC-002 in brains from Sprague-Dawley rats (*Rattus norvegicus*) treated with **I.** vehicle control or **II-v.** low dose PYC-002 for 14 days. Samples stained with *in situ* hybridisation probe targeting PYC-002 ASO (red) and counter-stained with hematoxylin. **I.** and **II.** Whole Brain, **III.** Cortex, **IV.** Hippocampus, **V.** Cerebellum. **C) PYC-002 distributes to disease-relevant brain regions in NHPs.** Concentration of PYC-002 (ng drug per g tissue, mean + S.E.M.) in brain tissue samples from Cynomolgus monkeys (*Macaca fascicularis*) 28 days after treatment, assessed by hy-ELISA.

6. A single dose of PYC-002 causes upregulation of multiple SHANK3 isoforms and dose-dependent upregulation in the prefrontal cortex

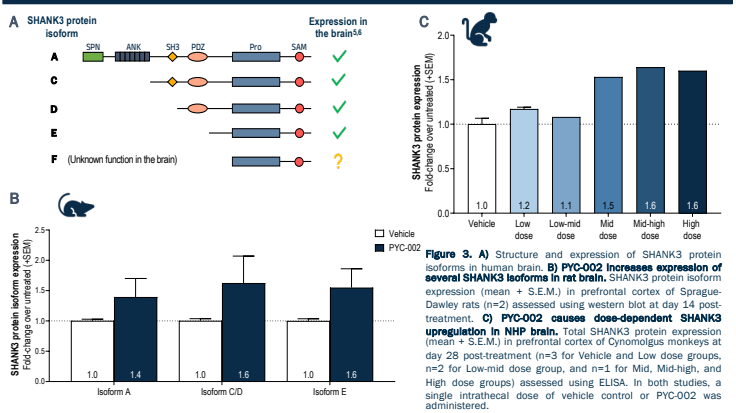


Figure 3. A) Structure and expression of SHANK3 protein isoforms in human brain. **B) PYC-002 increases expression of several SHANK3 isoforms in rat brain.** SHANK3 protein isoform expression (mean + S.E.M.) in prefrontal cortex of Sprague-Dawley rats (n=2) assessed using western blot at day 14 post-treatment. **C) PYC-002 causes dose-dependent SHANK3 upregulation in NHP brain.** Total SHANK3 protein expression (mean + S.E.M.) in prefrontal cortex of Cynomolgus monkeys at day 28 post-treatment (n=3 for Vehicle and Low dose groups, n=2 for Low-mid dose group, and n=1 for Mid, Mid-high, and High dose groups) assessed using ELISA. In both studies, a single intrathecal dose of vehicle control or PYC-002 was administered.

7. Conclusion: Preclinical findings support the development of PYC-002 as a treatment for PMS

- PYC's mutation agnostic PYC-002 drug candidate increased synaptic SHANK3 protein and improved synaptic structure and function in human iPSC-derived PMS neurons
- PYC-002 has a favourable safety profile in rats and non-human primates (NHPs) following intrathecal administration
- PYC-002 shows broad distribution throughout the brain including in disease-relevant regions in rats and NHPs
- A single, safe dose of PYC-002 upregulated multiple endogenous isoforms of SHANK3 critical for neuronal function in rats and PYC-002 caused dose-dependent upregulation in the prefrontal cortex of NHPs
- PYC is progressing through preclinical testing and is anticipated to enter human clinical trials in H2 2026.

References

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