PYC Therapeutics

Life-changing science

PYC-002: Targeting *SHANK3* with an RNA Therapeutic Approach for Phelan-McDermid Syndrome

June 2025

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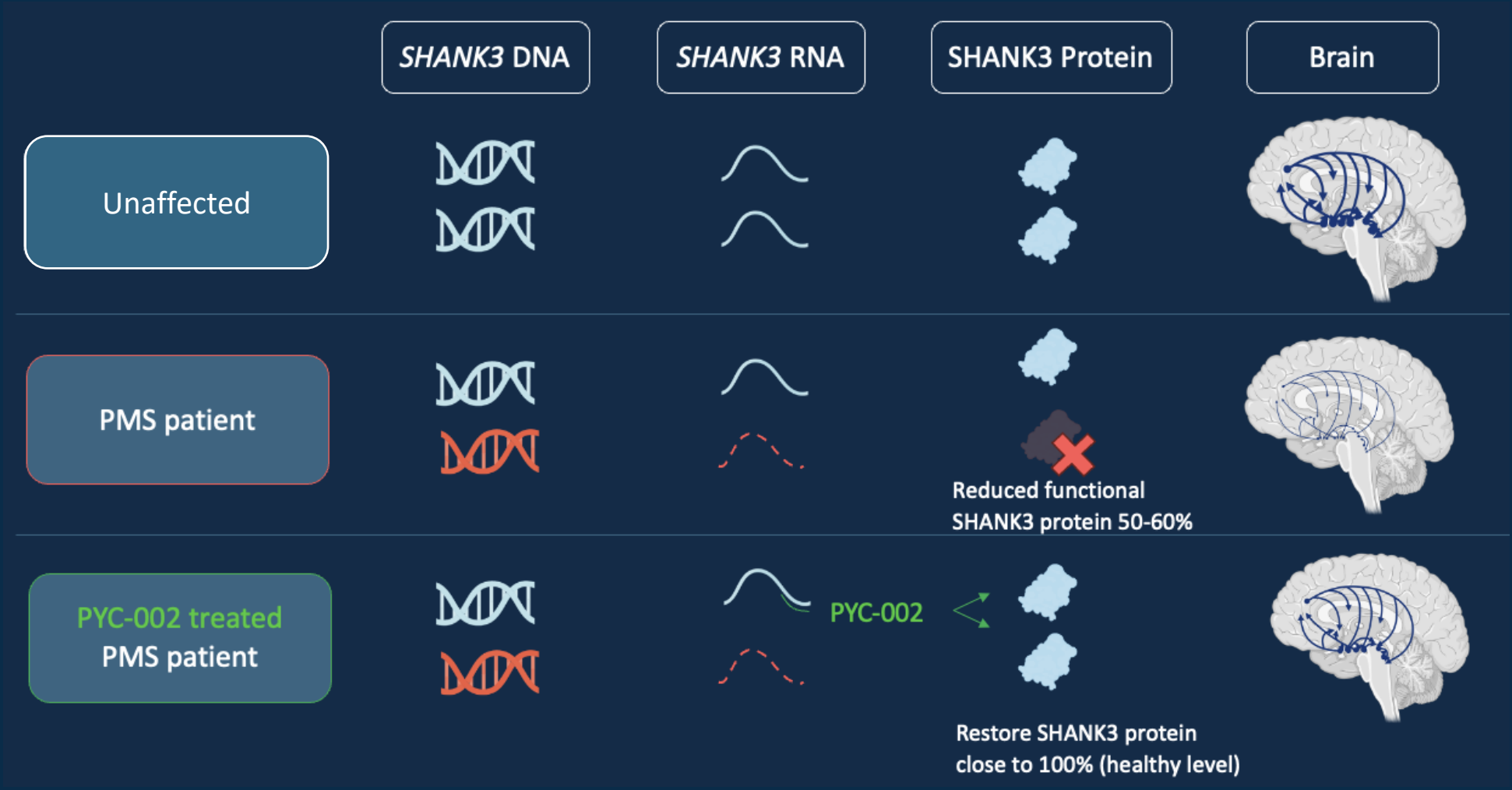
Executive Summary

- PYC-002 is a pre-clinical stage drug candidate that addresses the underlying cause of PMS (haploinsufficiency of the SHANK3 protein in the neurons of the brain)

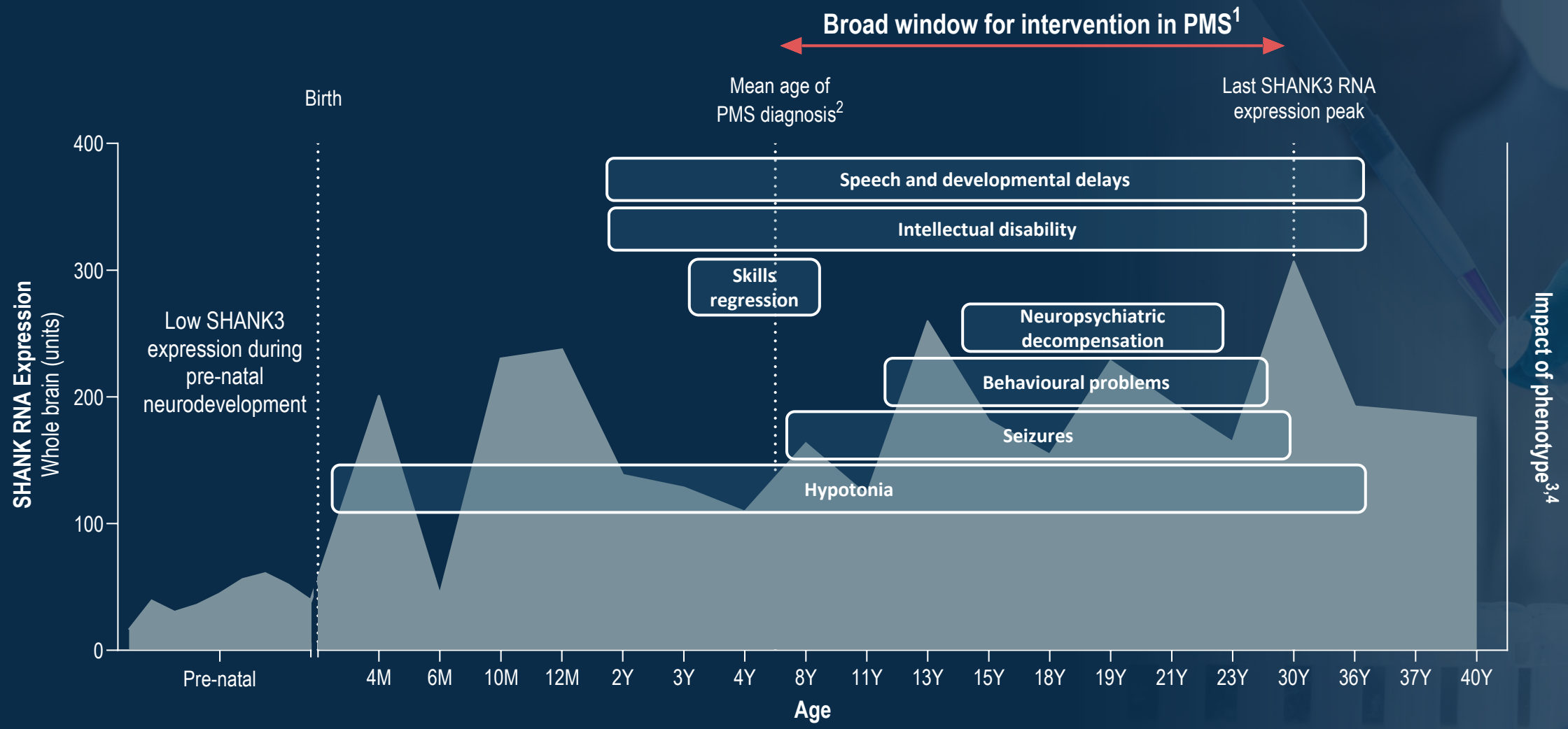
- PYC-002 combines human efficacy (*in-vitro*) with fully integrated safety/tolerability/PK/PD data *in vivo*:
  - *In vitro:* PYC-002 restores SHANK3 protein expression to wild-type/unaffected levels and leads to functional benefit in PMS patient-derived neurons
  - *In vivo:* PYC-002 reaches the target cell and modulates gene expression at safe and well-tolerated doses

- PYC-002 will progress to human trials in 2026 – further development of this program is de-risked by the ability to leverage an established clinical development pathway
  - Delivered by lumbar puncture (intrathecal) to bypass the blood-brain barrier, minimising systemic exposure
  - Treatment frequency to be confirmed - expected to be dosed every 3–6 months

PYC-002 addresses the root cause of PMS by increasing *SHANK3* expression in the target cell within the brain



The neurodevelopmental expression level of *SHANK3* enables a broad opportunity for intervention in PMS



1. Kang HJ, et al. Spatio-temporal transcriptome of the human brain. Nature. 2011 Oct 26; 478(7370):483-9.
2. Nevado J, et al. Variability in Phelan-McDermid Syndrome in a Cohort of 210 Individuals. Frontiers in Genetics. 2022;13.
3. Annemiek M. Landlust, Sylvia A. Koza, Maya Carbin, Margreet Walinga, Sandra Robert, Jennifer Cooke, Klea Vyshka, Ingrid D.C. van Balkom, Conny van Ravenswaaij-Arts, Parental perspectives on Phelan-McDermid syndrome: Results of a worldwide survey, European Journal of Medical Genetics, Volume 66, Issue 7, 2023, 104771, ISSN 1769-7212, https://doi.org/10.1016/j.ejmg.2023.104771.
4. Betancur C, Buxbaum JD. SHANK3 haploinsufficiency: a "common" but underdiagnosed highly penetrant monogenic cause of autism spectrum disorders. Mol Autism. 2013 Jun 11;4(1):17. doi: 10.1186/2040-2392-4-17. PMID: 23758743; PMCID: PMC3695795.

PMS is caused by haploinsufficiency of the SHANK3 protein

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Unaffected neurons

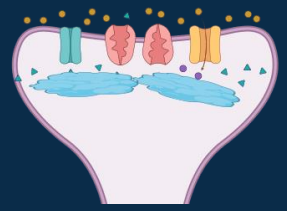
PMS neurons

Unaffected SHANK3 protein levels

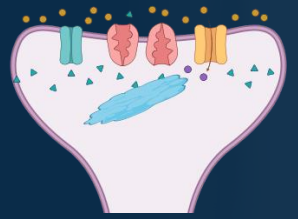
SHANK3 protein

SHANK3 haploinsufficiency (50-65% of unaffected)

Proper synaptic protein interaction



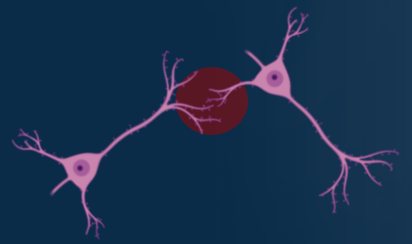
Synapse protein interactome



Impaired synaptic protein interaction

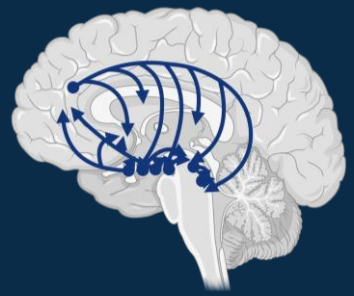
Correct synaptic function

Neuronal structure

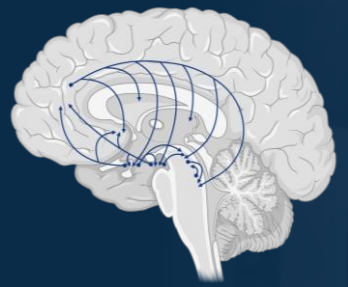


Abnormalities in synaptic structure and function

Functional neuronal communication, synaptic signalling, and plasticity



Synaptic activity



Impaired neuronal communication, synaptic signalling, and plasticity

Phelan-McDermid Syndrome

PYC-002 restores SHANK3 protein expression back to the levels seen in unaffected individuals in patient-derived models

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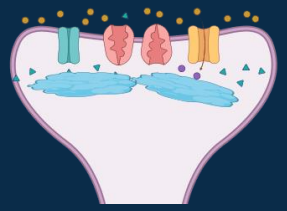
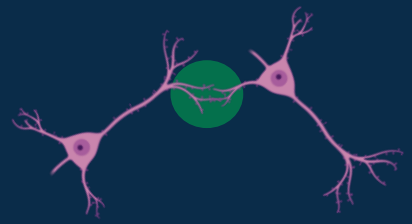
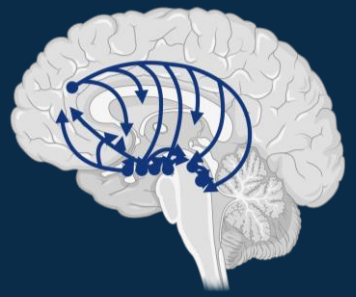
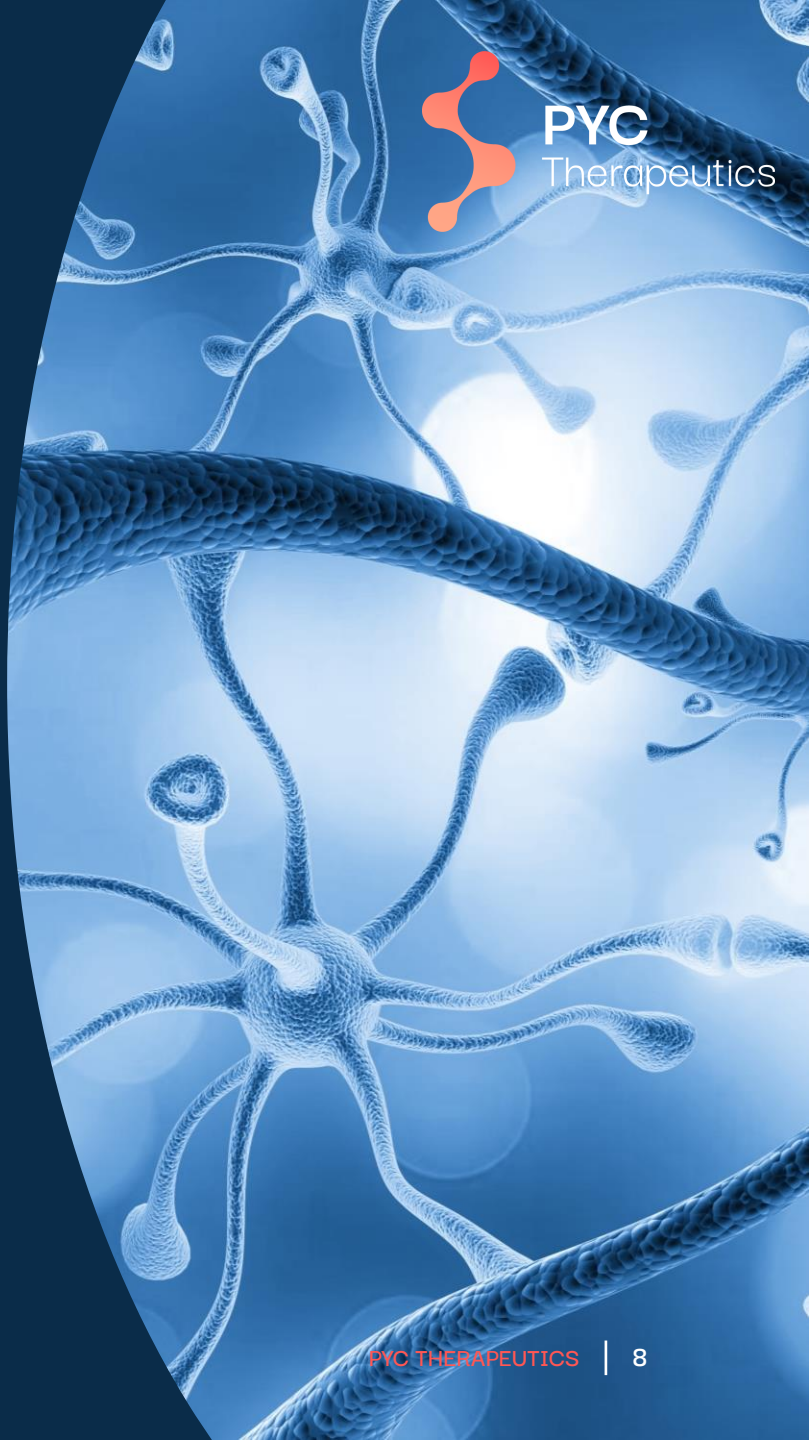
		PMS neurons + PYC-002	*Rescue in vitro assays*
SHANK3 protein		Restored SHANK3 expression in neurons	Total SHANK3 protein ✓
Synapse protein interactome		Improved SHANK3 at synapses and synaptic protein interaction	SHANK3 protein expression at synaptic site ✓
Neuronal structure		Restoration of synaptic structure and function	Neuronal structure (synapse density) ✓
neuronal activity		Improved neuronal communication, synaptic signalling, and plasticity	Neuronal signalling (active neurons count and calcium oscillation) ✓

Efficacy and Safety profile

1. PYC-002 restores *SHANK3* levels and reverses key PMS-related neuronal deficits in patient-derived neurons *in vitro*:
    a) Increases SHANK3 protein expression at the synapse
    b) Improves neuronal structure (synapse density) and restores neuronal signalling (active neuron count and calcium oscillation)

2. PYC-002 has a fully integrated safety/tolerability/PK/PD data pack *in vivo:*
    a) PYC-002 distributes to all key regions of the brain *in vivo* and can modulate gene expression at safe and well-tolerated doses



1a) PYC-002 completely rescues the deficient SHANK3 protein expression in PMS patient-derived neurons

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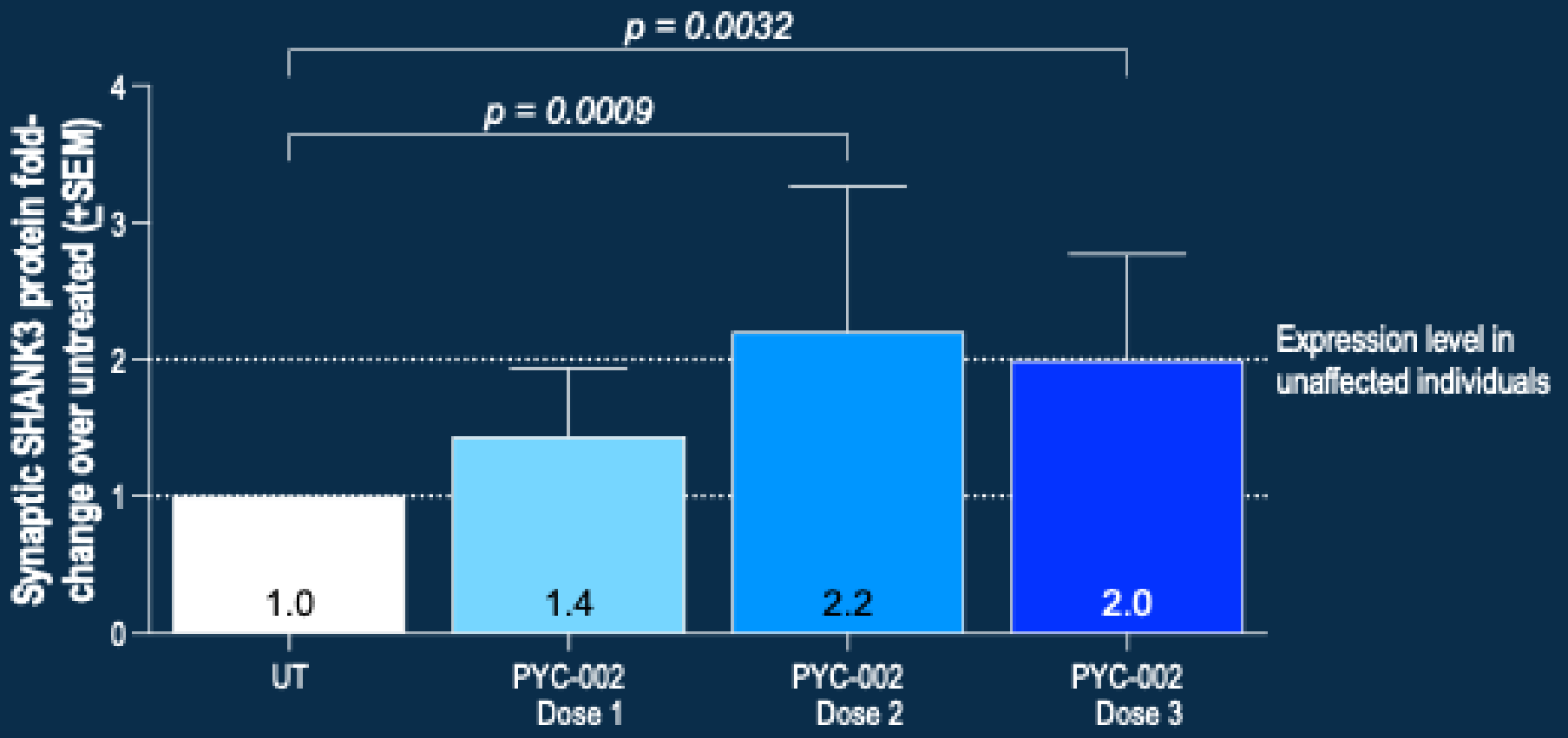
Up to 2-fold increase in synaptic SHANK3 protein expression in PMS-patient derived neurons[1]



Total SHANK3 protein

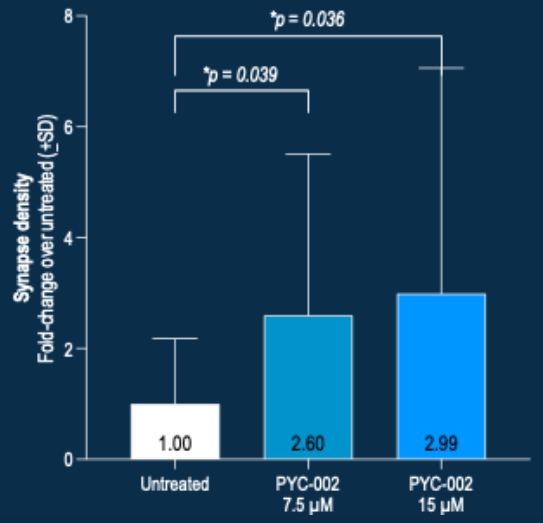
SHANK3 protein expression at synaptic site

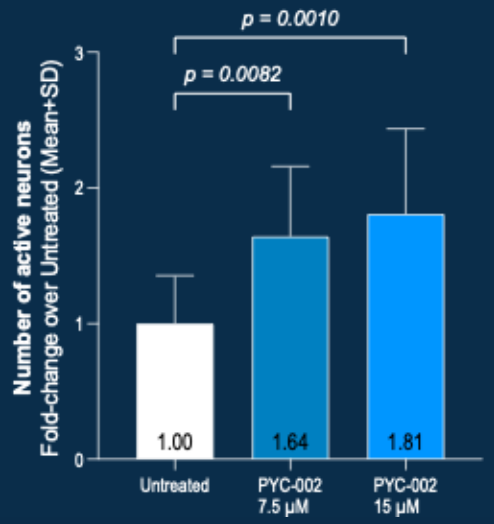
1. Mean fold-change of SHANK3 protein on 100 µm of neurite over untreated group after 21 days of PYC-002 gymnotic treatment of two PMS patient-derived iPSC-neurons, assessed by high content imaging (HCI). Each biological replicate represents the median of 5-24 technical replicates. Error bars represent standard error. **p<0.01 assessed using unpaired t-test of n=34 untreated technical replicates vs n=19 technical replicates treated with PYC-002.

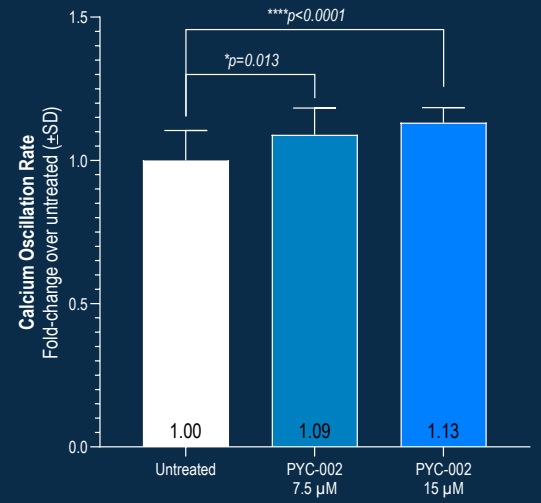
1b) Restoring SHANK3 protein expression results in improved neuronal structure and signalling

Restoration of neuronal structure and signalling in PMS-patient derived neurons

Density of synapses[1] → Number of active neurons[2] → Calcium oscillation[3]







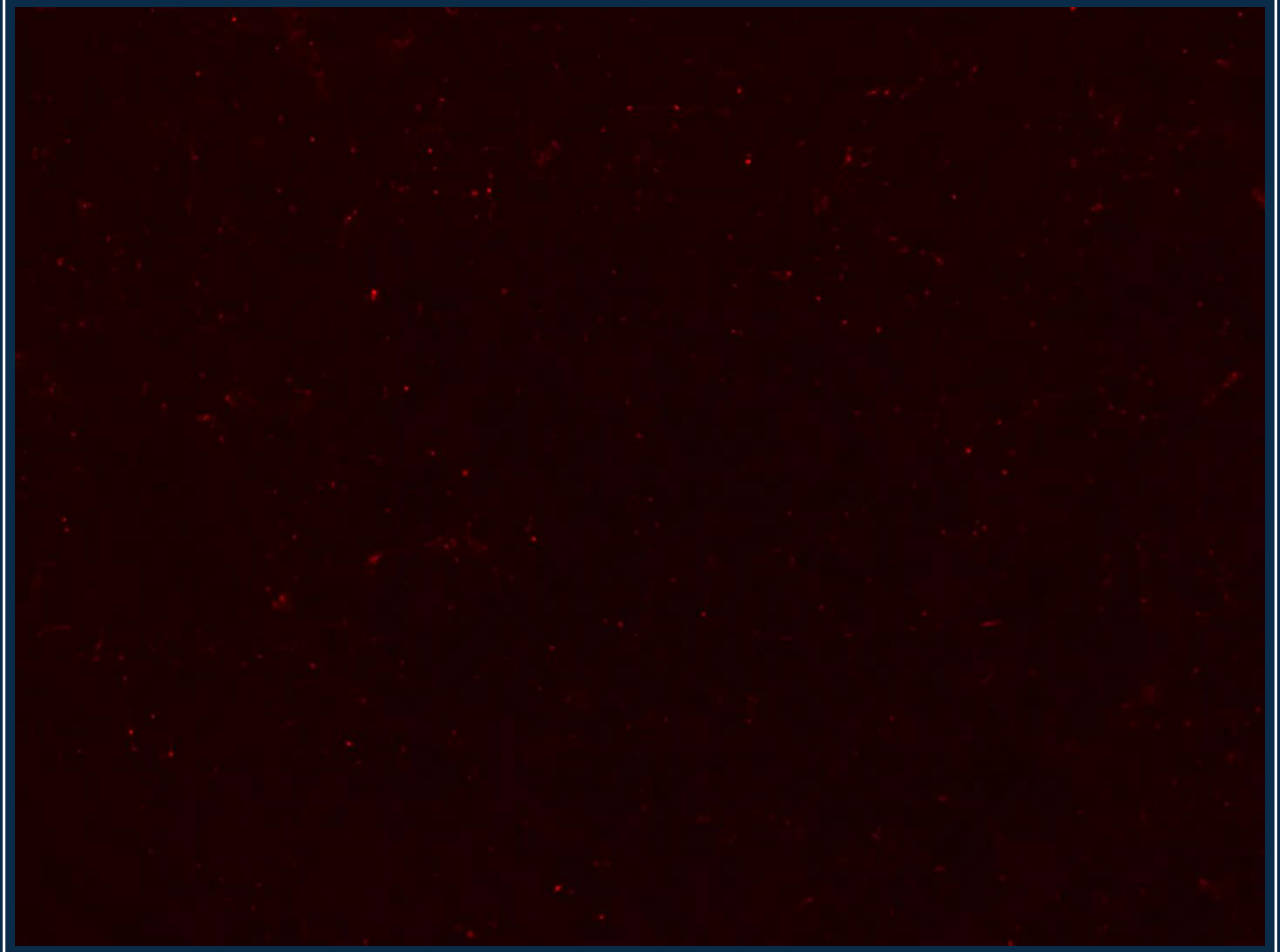
Neuronal structure (synapse count)

Neuronal signalling (active neurons count and calcium oscillation)

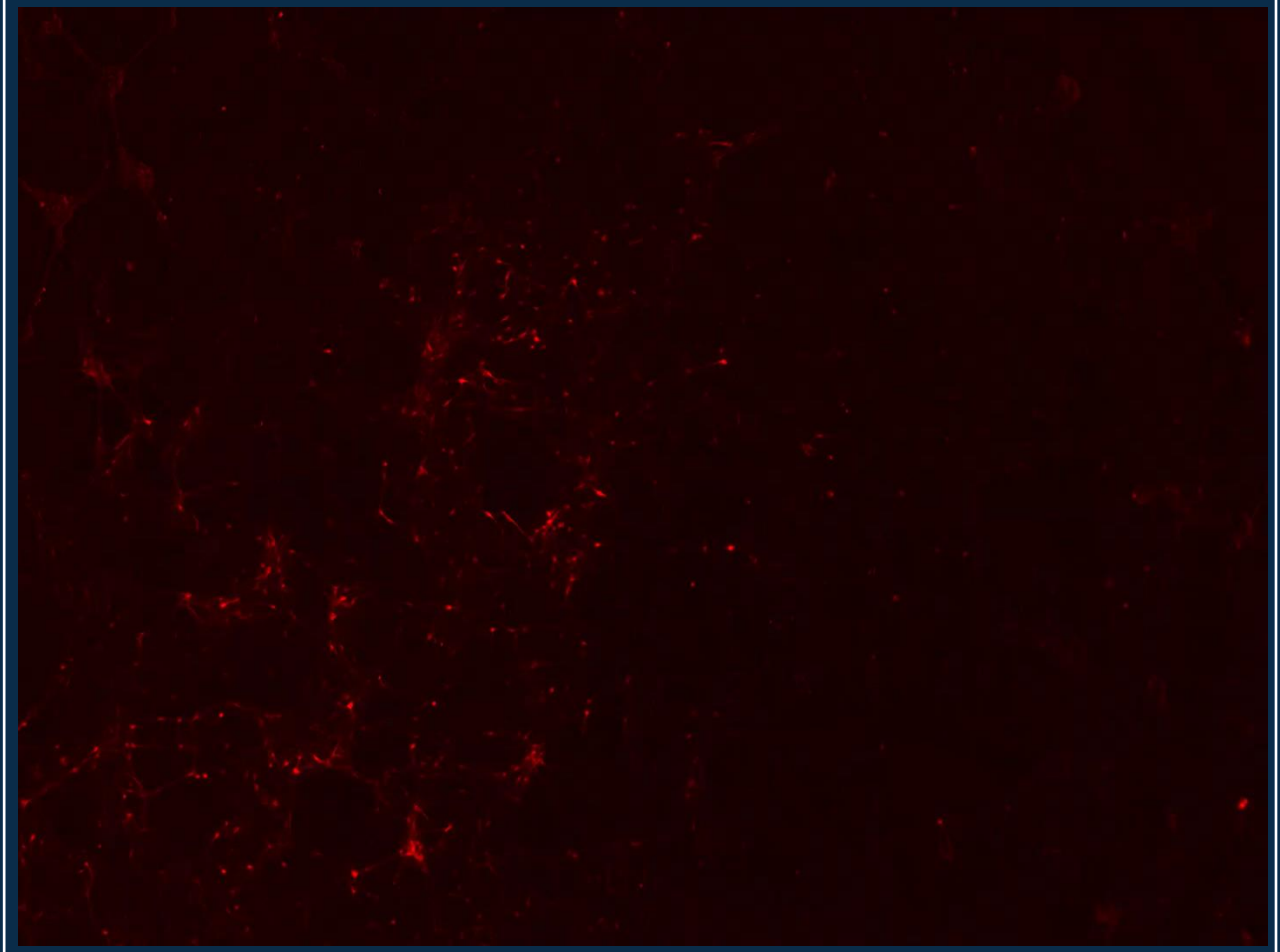
1. Bar graphs represent the mean ±SD of fold-change in synapse counts in two biological replicates of one PMS patient-derived iPSC-neurons after 21 days of PYC-002 treatment. The synapse is defined as the co-localization of SHANK3 (post-synaptic marker), SYNAPSIN (pre-synaptic marker) and Tuj-1 (neurite marker) proteins. Statistical significance evaluated using two-sided unpaired Welch's t-test comparing each treatment concentration of PYC-002 to untreated PMS-patient derived neurons (n=20 technical replicates for untreated group, n= 17 technical replicates for each PYC-002 treatment concentration)
2. Bar graphs represent mean ±SD of the fold-change of number of neurons that is active by Calcium signaling pathway over untreated. Statistical significance evaluated using two-sided unpaired Welch's t-test comparing each treatment concentration of PYC-002 to untreated PMS-patient derived neurons (n=2 biological replicates; n=6 technical replicates per biological replicate)
3. Bar graphs represent mean ± SD of the fold-change of calcium oscillation rate over untreated. Statistical significance evaluated using two-sided unpaired Welch's t-test comparing each treatment concentration of PYC-002 to untreated PMS-patient derived neurons (n=23 technical replicates for untreated group, n=17 technical replicates for each PYC-002 treatment concentration)

1b) PYC-002 rescues neuronal signalling in PMS-patient derived neurons

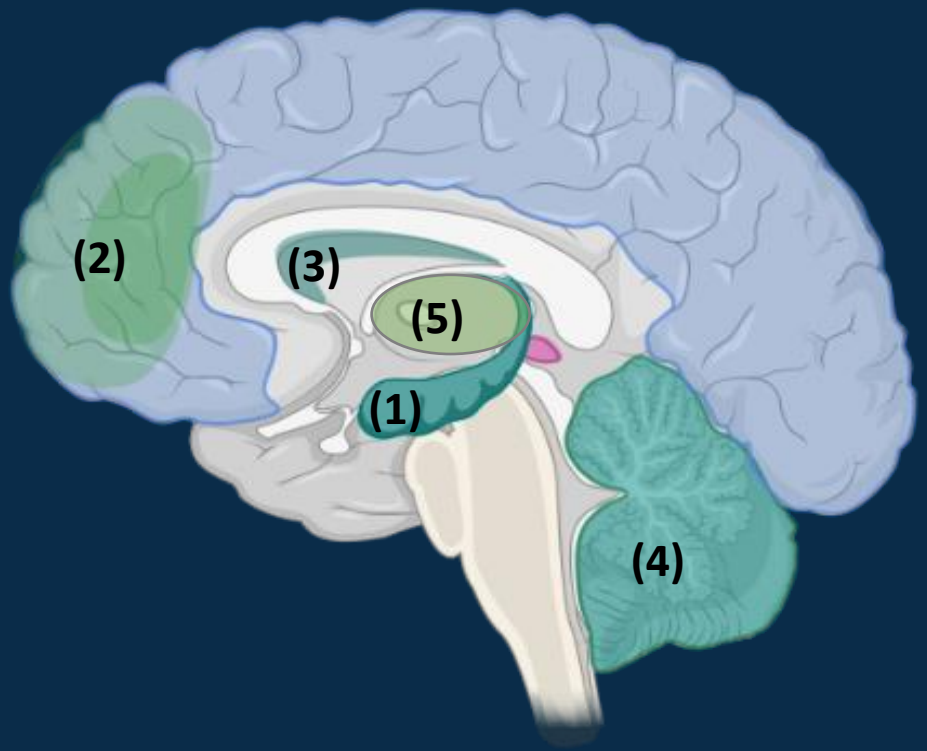
Untreated PMS-derived neurons



PYC-002 treated PMS-derived neurons (day 29)



Deficient *SHANK3* levels in multiple brain regions disrupt neuronal communication contributing to the PMS phenotype



Brain regions implicated in PMS and impact on phenotype

1. **Hippocampus:** critical for learning and memory processes
2. **Prefrontal cortex:** important for decision making, social behaviours and cognitive functions
3. **Striatum:** plays a crucial role in synaptic function and plasticity, essential for decision making, emotion and motor control
4. **Cerebellum:** functions in balance and motor/movement control
5. **Thalamus:** receiving incoming sensory and motor information

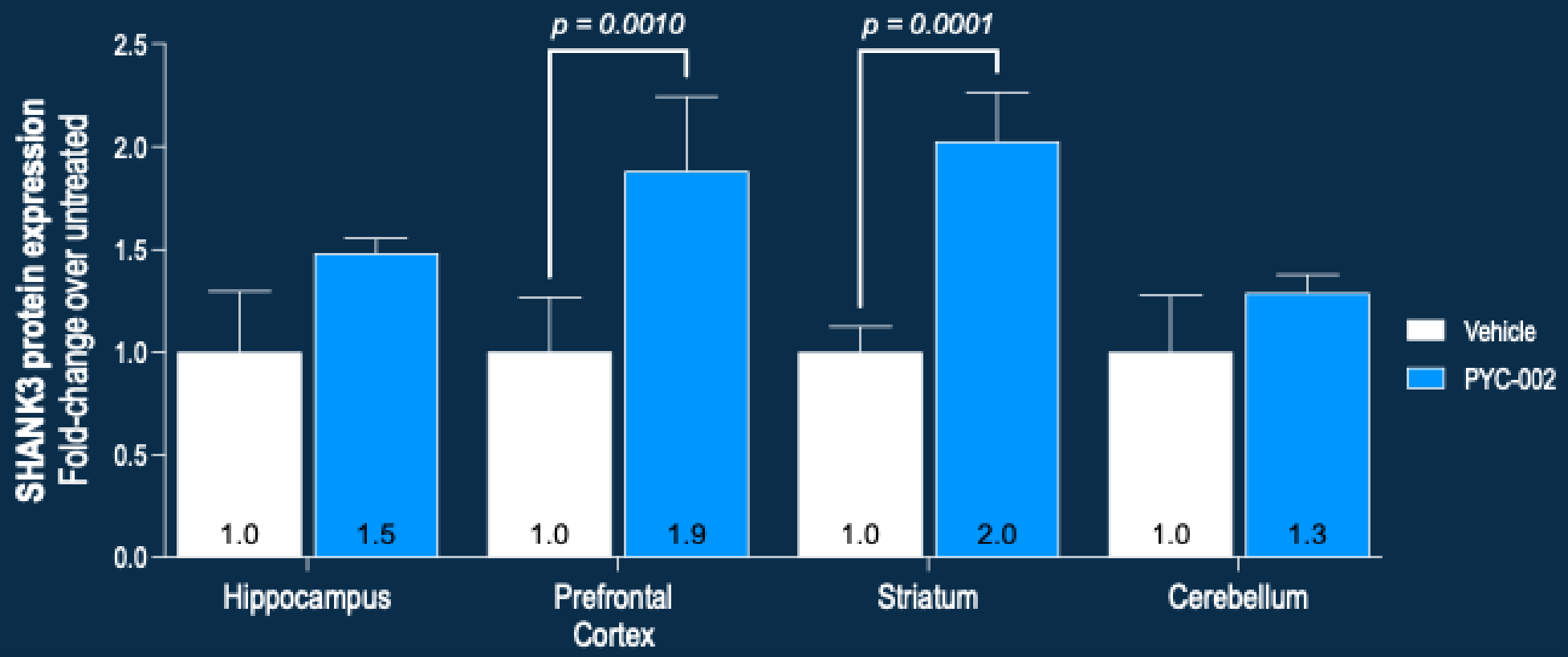
1. Kang, H. J., Y. I. et al (2011). "Spatio-temporal transcriptome of the human brain." Nature **478**(7370): 483-489.
2. Bouquier, N., et al (2022). "The Shank3(Venus/Venus) knock in mouse enables isoform-specific functional studies of Shank3a." Front Neurosci 16: 1081010.
3. Wang, X., et al (2014). "Transcriptional and functional complexity of Shank3 provides a molecular framework to understand the phenotypic heterogeneity of SHANK3 causing autism and Shank3 mutant mice." Mol Autism 5: 30.

2a) PYC-002 upregulates SHANK3 protein in key brain regions following a single safe and well-tolerated dose

Up to 2-fold increase in SHANK3 protein expression in key brain regions implicated in PMS

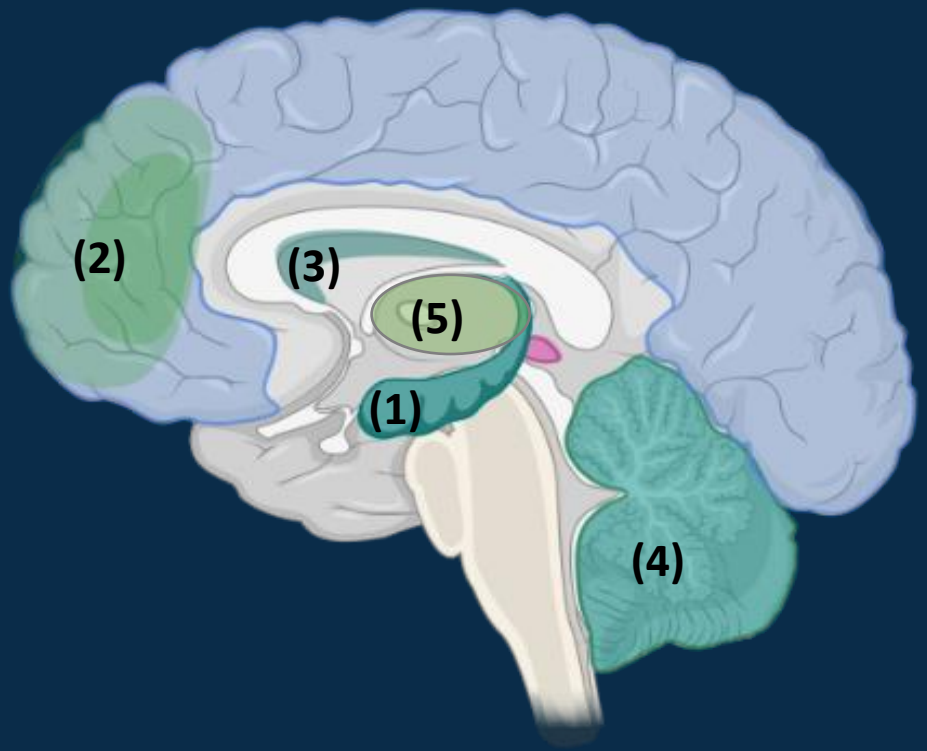
Achieved with a dose that is ~4x lower than the NOAEL

Total SHANK3 protein



1. SHANK3 expression in wild-type Sprague Dawley rats with and without treatment with PYC-002. Rats received a single intrathecal dose of vehicle control or PYC-002 (900 µg). Assessment of SHANK3 protein levels in key brain regions was completed 14 days post-treatment (n=3 treated with PYC-002 and n=6 vehicle). Samples from thalamus were not collected as part of this study. Statistical analyses using two-way ANOVA comparing PYC-002 treated to vehicle group in each brain region.

Each of the brain regions implicated in PMS has a distinct SHANK3 protein isoform expression profile

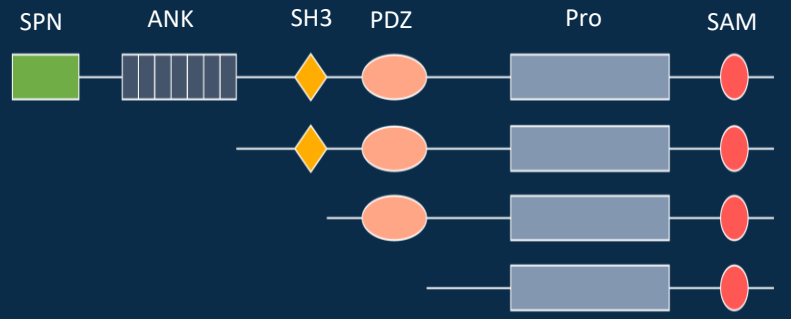


Relative expression of SHANK3 protein isoforms in the brain[1-3]

Region	Isoform A	Isoform C	Isoform D	Isoform E
1. Hippocampus	~50%	~25%	~25%	<10%
2. Prefrontal Cortex	~30%	~30%	~30%	10%
3. Striatum	~70%			30%
4. Cerebellum		~50%	~50%	
5. Thalamus	~70%		~20%	10%

Next slide

SHANK3 protein Isoform[1,2,3]



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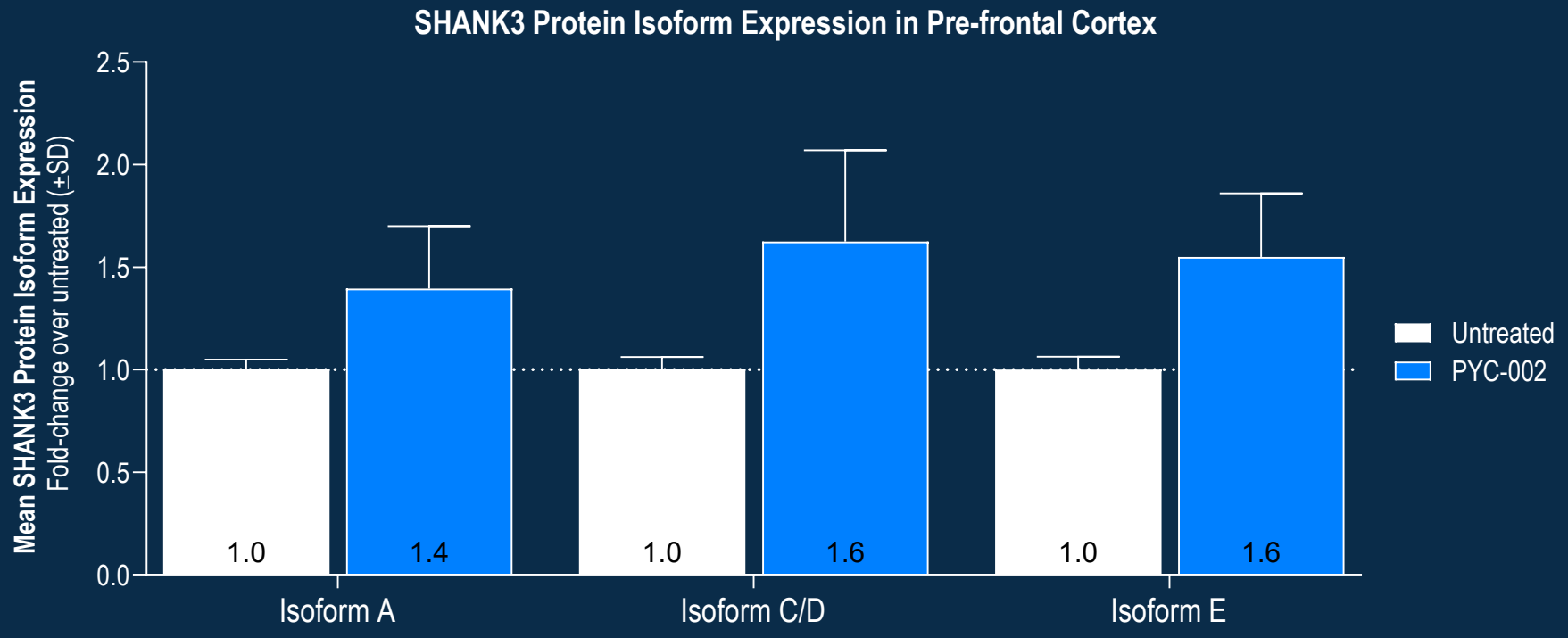
3. Wang, X., et al (2014). "Transcriptional and functional complexity of Shank3 provides a molecular framework to understand the phenotypic heterogeneity of SHANK3 causing autism and Shank3 mutant mice." Mol Autism 5: 30.

3) PYC-002 upregulates all SHANK3 protein isoforms expressed in the brain

PYC-002 increases the expression of all SHANK3 isoforms expressed in the brain[1]

An isoform agnostic mechanism of action maximizes potential for phenotypic rescue



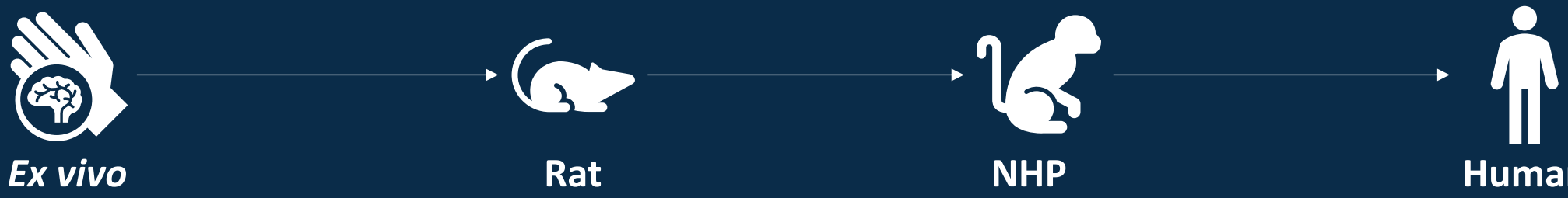
1. SHANK3 protein isoform expression in pre-frontal cortex in rats (n=2) assessed using western blot at day 14 post-treatment. Rats received a single intrathecal dose of vehicle control or PYC-002 (900 µg).

PYC-002 leverages an established clinical development path

For this combination of:

- Chemically modified ASO
- Administration: intrathecal
- Target cell: neurons

There is an **established path** through non-clinical species to **clinical validation**[1]



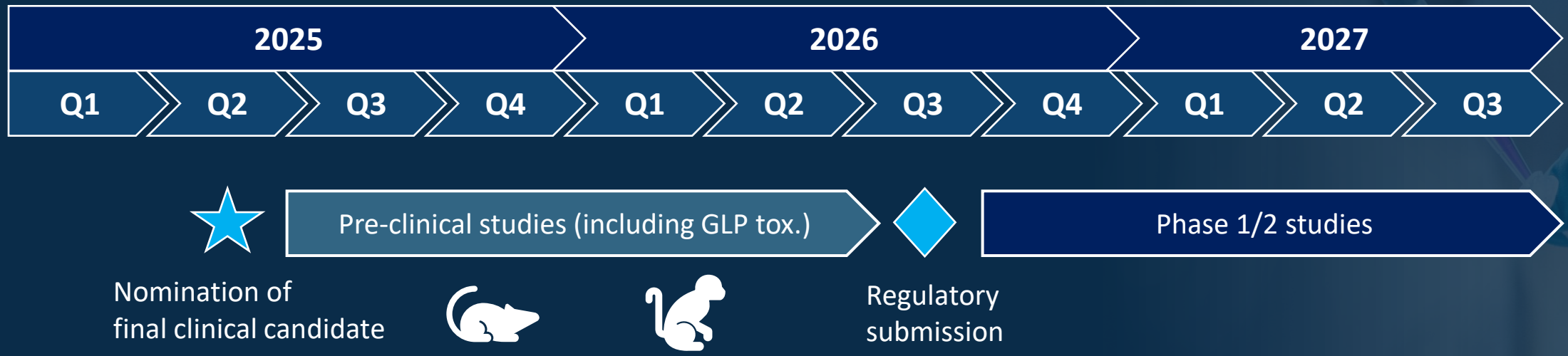
PYC-002 restores SHANK3 expression in PMS patient-derived models and has fully-integrated *in vivo* data

Established pathway

The pattern of ASO distribution and activity in the CNS of pre-clinical species translates to the human CNS[1]

1. Jafar-Nejad P, et al. The atlas of RNase H antisense oligonucleotide distribution and activity in the CNS of rodents and non-human primates following central administration. Nucleic Acids Res. 2021 Jan 25;49(2):657-673. doi: 10.1093/nar/gkaa1235.

PYC-002 will progress to human trials in 2026[1]



1. Subject to the risks and uncertainties outlined in the Company's ASX disclosures of 17 February 2025