Bespoke Gene Augmentation Therapy for Smith-Lemli-Opitz Syndrome (SLOS)

Project led by Gene2Cure Foundation

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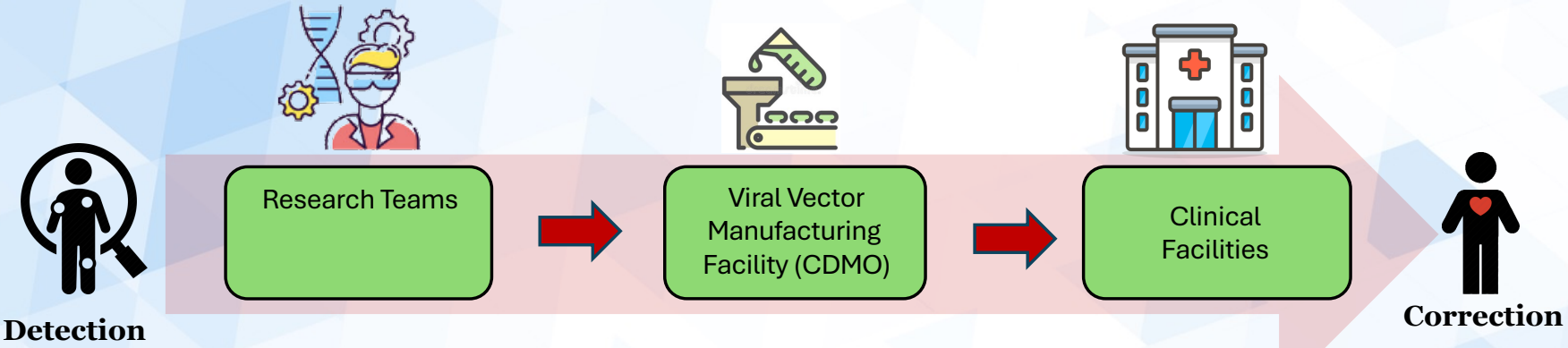
Smith–Lemli–Opitz syndrome (SLOS)

- Caused my mutations in DHCR7 gene located on chromosome 11q13
- Autosomal recessive
- Ultra-rare disease (estimated frequency of 1:20,000 to 1:70,000 newborns)
- SLOS is more common in individuals of European heritage.
- DHCR7 encodes enzyme called 7-dehydrocholesterol reductase, which is responsible for the final step in the production of cholesterol.
- SLOS is a congenital multiple-anomaly / cognitive impairment syndrome
- The SLOS phenotypic spectrum is very broad, ranging from a mild disorder with behavioral and learning problems to a lethal malformation syndrome.
- Severe SLOS is characterized by prenatal and postnatal growth restriction, microcephaly, moderate-to-severe intellectual disability, and multiple major and minor malformations including characteristic facial features, cleft palate, abnormal gingivae, cardiac defects, hypospadias, ambiguous genitalia (failure of masculinization of male genitalia), postaxial polydactyly, and 2-3 toe syndactyly.
- Individuals with milder forms may have only subtle facial characteristics, hypotonia, 2-3 toe syndactyly, and mild to no intellectual disability. Clinical variability is noted even within families, as sibs with SLOS have been reported with medical and developmental problems of different degrees.

https://www.nature.com/articles/ejhg200810

The Australian Genome Therapeutics Centre (AGTC)



The AGTC was established to help realise our dream and missions to help children affected by incurable, rare genetic conditions.

AGCT brings together the required expertise and infrastructure to enable development and translation of innovative, life-saving therapies from Detection to Correction.

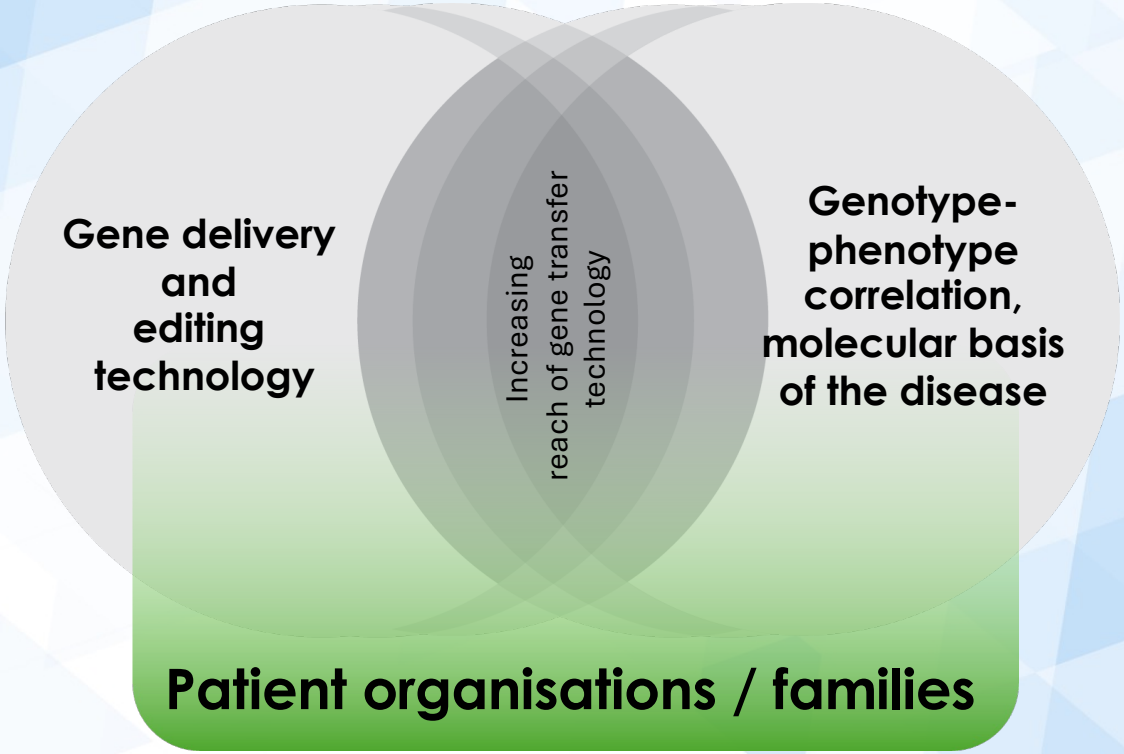
The last missing piece, the clinical-grade manufacturing facility was funded by NSW Government (>$135million) allowing us not only to develop the new therapies, but also to manufacture them at cost and bring them to the patients.

Translational Vectorology Research Unit (TVRU)

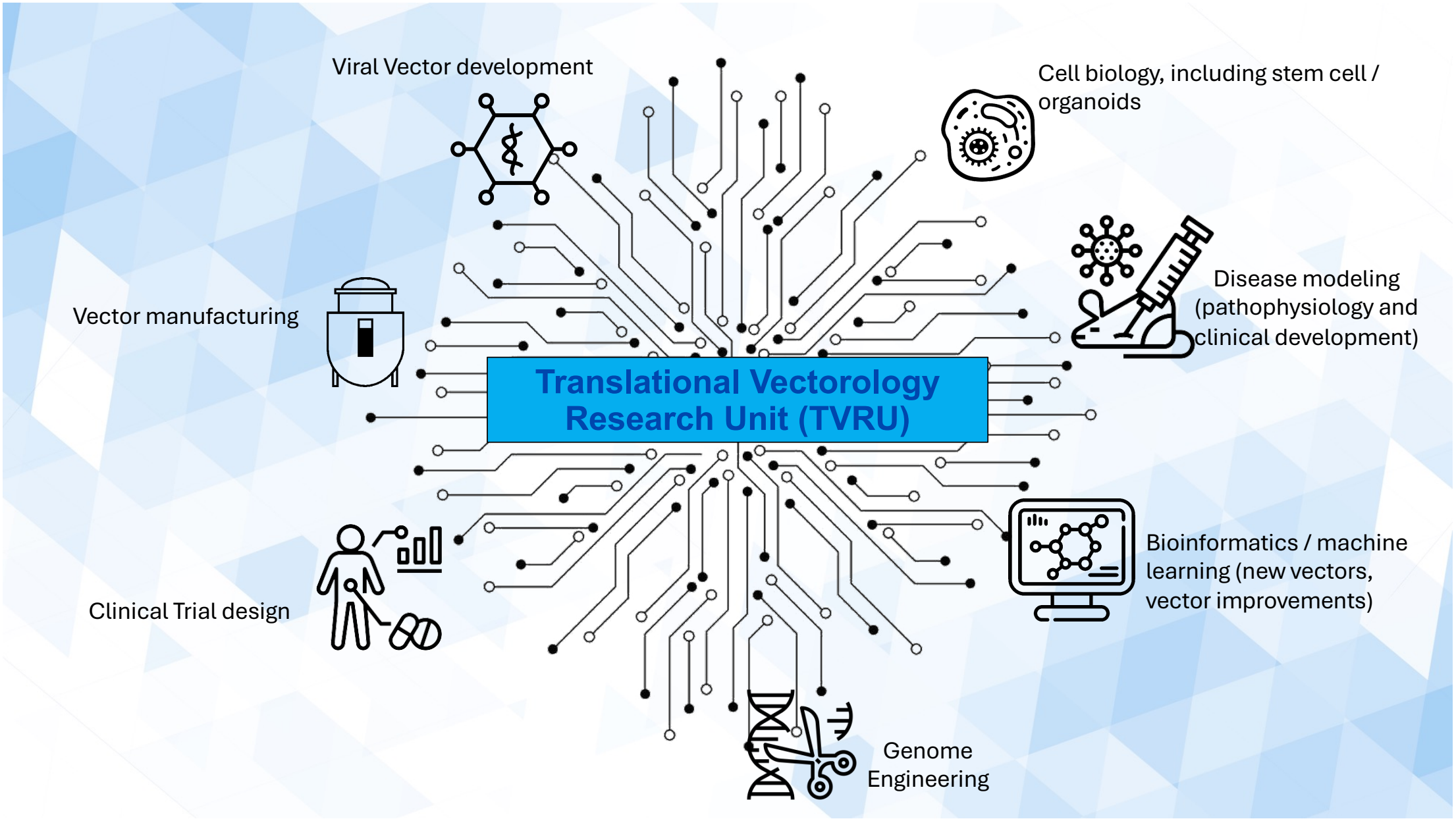
Tool makers & Tool users: the preclinical aspects of our projects are performed by highly experienced team of researchers from the Translational Vectorology Research Unit (TVRU) at Children's Medical Research Institute (CMRI) in Sydney, Australia, which brings together the highly complementary basic researchers (Tool Makers) and clinicians (Tool Users).

Venn Diagram

Tool Makers



Tool Users


Viral Vector development
Cell biology, including stem cell / organoids
Vector manufacturing
Disease modeling (pathophysiology and clinical development)
Translational Vectorology Research Unit (TVRU)
Bioinformatics / machine learning (new vectors, vector improvements)
Clinical Trial design
Genome Engineering

The Project

Our Mission:
To develop and functionally validate bespoke, world-first gene augmentation (gene addition) treatment for SLOS.

To take advantage of AGTC and the Gene2Cure Foundation to bring the therapy to the clinic.

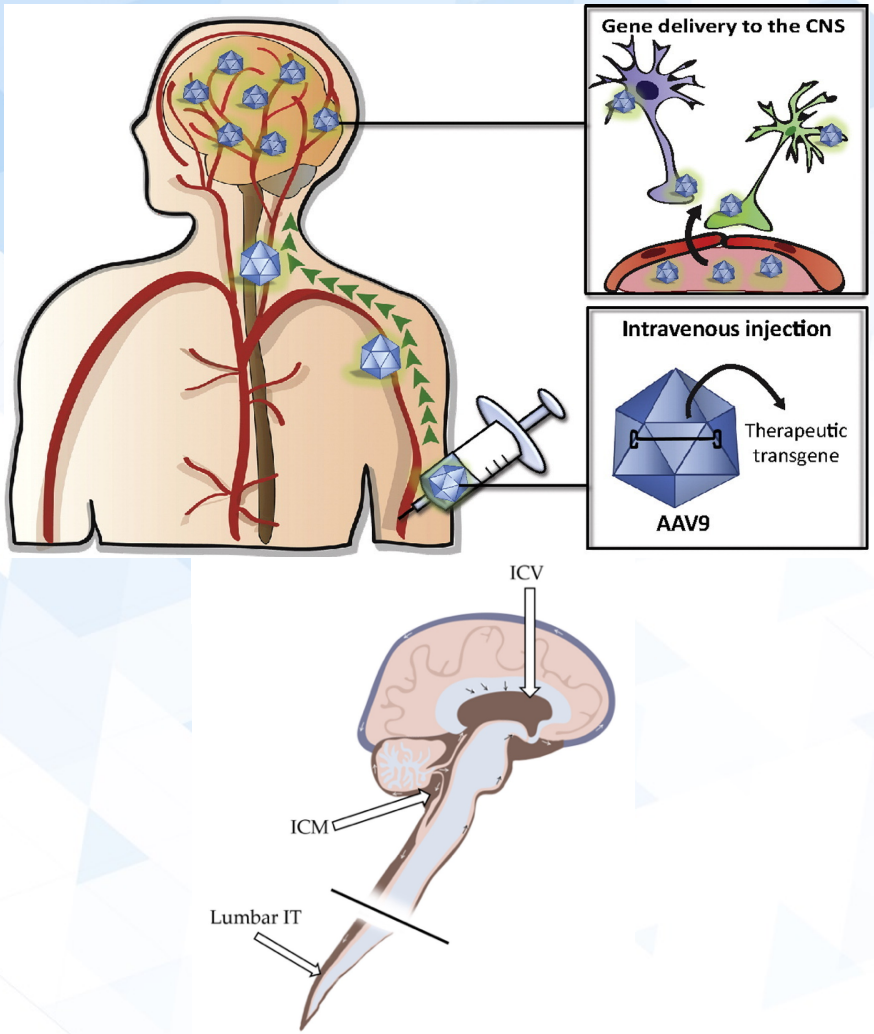
Our Strategy:
We will develop bespoke AAV-DHCR7 vector based on market-approved AAV-based gene augmentation therapy (Zolgensma™) for Spinal Muscular Atrophy (SMA). This approach will enable the most time- and cost-effective way to bring this new therapy to the clinic.

We will perform functional validation studies in patient-derived preclinical models (organoids = "brains on a dish”) using blood cells from multiple SLOS patients (no less than 5 different patients).

We will perform preliminary safety/toxicity studies in normal mice as well as functional studies in SLOS mice

Efficacy and safety data will be used to support IP filing and translational program.

Zolgensma™



Saraiva et al, 2016 and Perez et al, 2020

zolgensma® (onasemnogene abeparvovec)

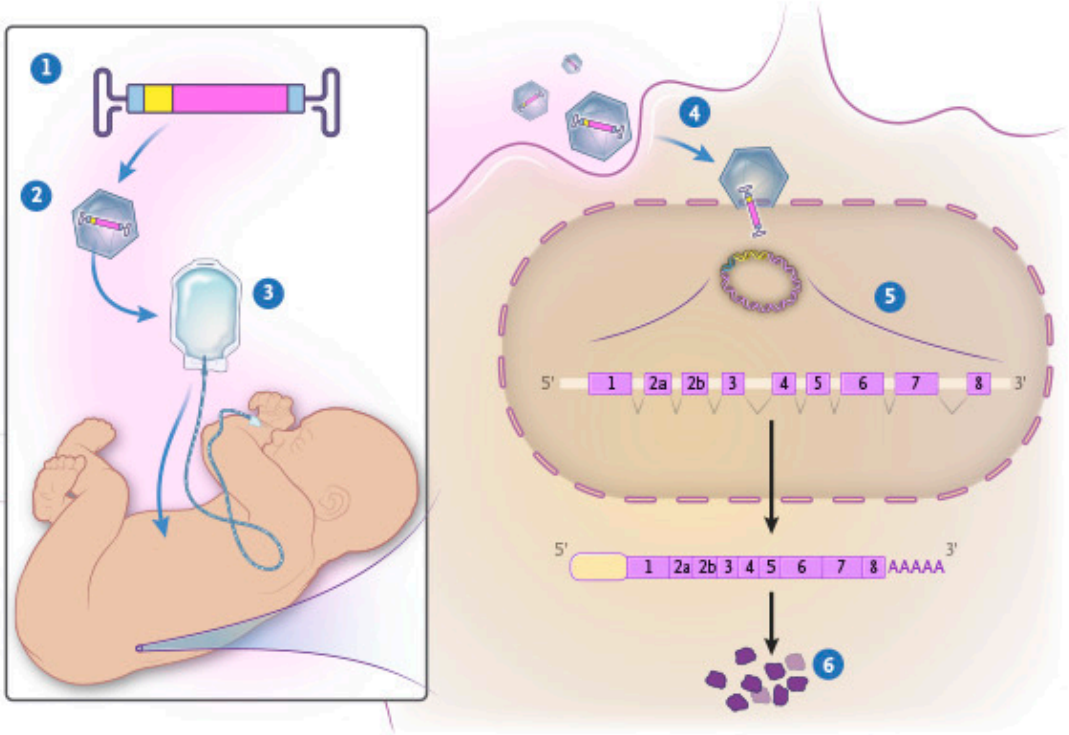
Zolgensma™ uses natural AAV variant, AAV9, which is the only AAV shown to target brain cells (neurons) following systemic administration.

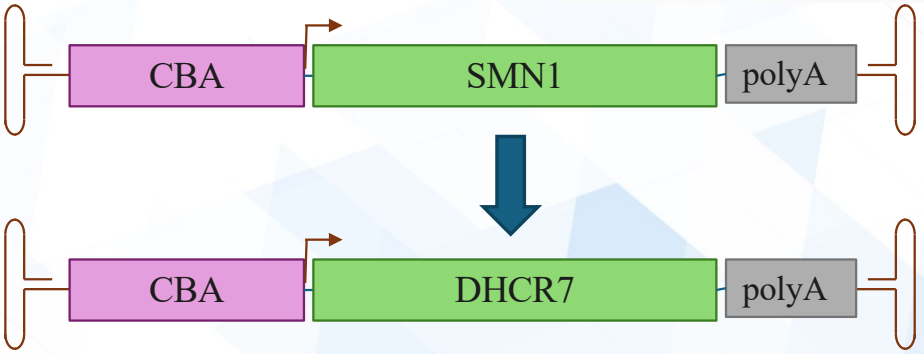
AAV9 has also been shown to have high safety and good biodistribution following more direct routes of administration: intracerebroventricular (ICV), intra-cisterna magna (ICM) and intrathecal (IT).

More direct routes increase safety while lowering the overall therapeutic dose (lower cost).

AAV9-SMN → AAV9-DHCR7

zolgensma®
(onasemnogene abeparvovec)





DHCR7 = 475aa = 1425nt

We introduce minimum changes to convert Zolgensma™ into a new drug for DHCR7 Deficiency / Smith–Lemli–Opitz syndrome (SLOS).

With the aim to improve safety and efficacy, we will also evaluate the use of endogenous promoter, which may enable restoration of physiological levels of expression in neurons and other affected cell types.

Bespoke Gene Augmentation Therapy for SLOS

Estimated Timeline (year 1-2, preclinical part only)

	Q2 2025	Q3 2025	Q4 2025	Q1 2026	Q2 2026	Q3 2026	Q4 2026	Q1 2027
Administration & Legal	Onboarding of a dedicated researcher to undertake this process (the person has already been hired)							
	Write up animal ethics and IBC applications for study							
							IP filing	
Therapeutic Constructs	in silico design of clinical AAV constructs (n=6-10 constructs). Custom synthesis.							
	cloning AAV-DHCR7 constructs into AAV backbones							
		AAV Packaging	Continue to manufacture as needed for *in vitro* and *in vivo* studies →					
		Vector manufacturing studies (titer, AAV cassette stability...)						
In vitro studies	KO DHCR7 in cell line(s)							
			Testing of AAV constructs in KO cells (western blot for transgene expression and functional assay for DHCR7 activity)					
iPSC and organoid studies	Import of patients PBMCs to CMRI (including MTAs) →							
		Reprogramming new patient cells into iPSC →						
			Differentiate patient's iPSC into 2D neurons and 3D organoids →					
				Establishing phenotype of iPSCS dervied neurons and organoids.				
				Testing AAV-DHCR7 in iPSCS dervied neurons and organoids				
in vivo studies	Identification of relevant animal models, establishing collaborations and/or bringing the model to Australia				Expression and overexpression (tox) studies in normal BL6 mice			
					Long term followup of treated BL6 mice			
					Functional evaluation in SLOS mice (at facility in Prague, CMRI and/or lab of collaborators)			

Bespoke Gene Augmentation Therapy for SLOS

Estimated Budget (year 1-2, preclinical part only)

Item	Cost (USD)
General lab reagents and consumables	$ 24,000.00
Research Officer salary for 2 years (including institutional on-cost)	$ 288,000.00
Construct synthesis (up to 10 constructs)	$ 18,000.00
Vector packaging (up to 10 constructs) for initial studies	$ 6,000.00
Vector packaging (up to 10 constructs) for in vitro/in vivo studies	$ 18,000.00
SHSY5Y KO knock out line	$ 6,000.00
In vitro study (in KO line) consumables (TC reagents and WB and functional assays)	$ 12,000.00
Reprogram PBMCs to iPSC (5 patients, 3 lines per patient)	$ 15,000.00
Differentiate iPSC to neurons and brain organoids (5 patients)	$ 18,000.00
Functional testing of AAV-DHCR7 in neurons and organoids	$ 9,600.00
In vivo tox studies (BL6 mice)	$ 18,000.00
Vector manufacturing and titre studies (compatibility with clinical manufacturing)	$ 6,000.00
AAV vectors for in vivo studies in EU and U.S.	$ 7,200.00
Shipping and other accidentals	$ 18,000.00
Travel	$ 12,000.00
TOTAL	**$ 475,800.00**

Our Exemplar Gene Therapy Program: CTNNB1 Syndrome

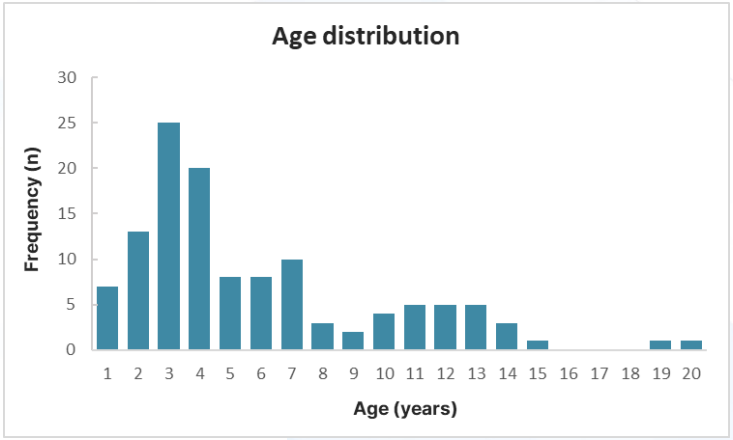
CTNNB1 Syndrome

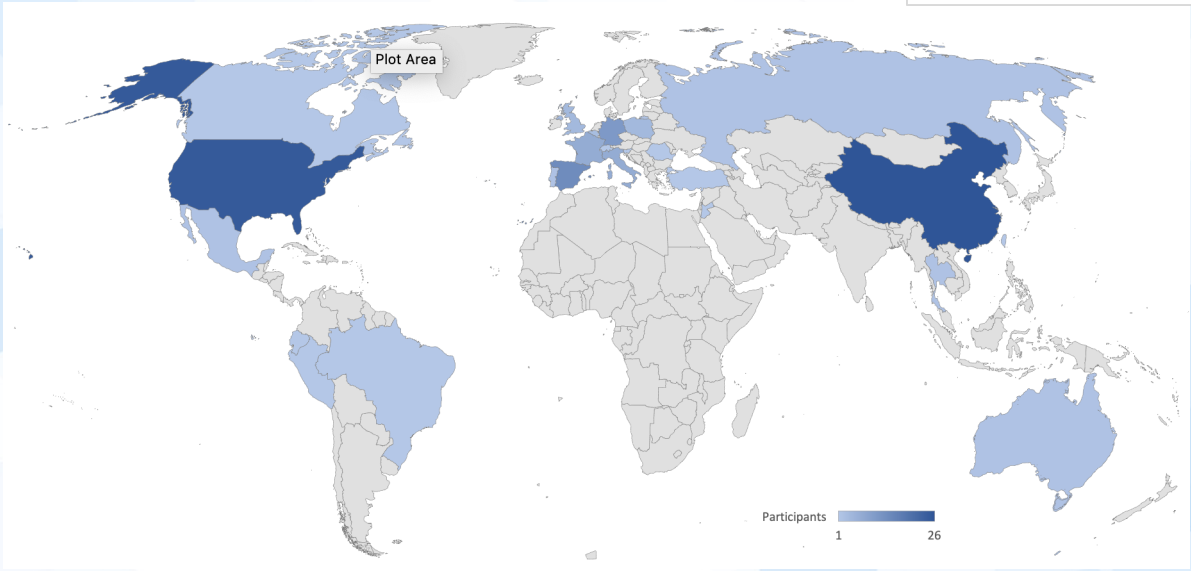
- Severe and rare neurodevelopmental disorder
- First described in 2012
- Most common cause of misdiagnosed cerebral palsy[1,2]
- Autosomal dominant, caused by mutations in CTNNB1 gene (mostly "*de novo*")
- Reduced production of β-catenin protein, with plays an important role in cell adhesion, communication and signalling (WNT pathway)
- Affects 1:50,000 individuals

Our Exemplar Gene Therapy Program: CTNNB1 Syndrome

Genotype-Phenotype Correlation Study

- ClinicalTrials.gov Identifier: NCT04812119
- 125 patients enrolled (31,3% of 400 known)
- 24 countries
- Study of genetic variants (Prof. Chan, Hong Kong)





Our Exemplar Gene Therapy Program: CTNNB1 Syndrome

Costs to date

Preclinical development	Estimated cost (in EUR)	Estimated Timeline	Notes
Vector development and initial in vitro and in vivo testing	€ 400,000.00	2 years	This is the initial part that would be performed at CMRI
Cell development (4-6 cell lines)	€ 40,000.00	4-6 months	May not be required if if sufficient number of iPSC is already available via collaborations
Mouse model development and initial phenotypization	€ 75,000.00	6-12 months	May not be required if a validated mouse model is already available and accessible
Non-GLP toxicity study	€ 100,000.00	6-12 months	
Mouse efficacy study	€ 550,000.00	6 months	
GLP Toxicology & Biodistribution			
Mouse model	€ 250,000.00	3-6 months	
NHP	€ 600,000.00	3-6 months	
Manufacturing (2l, 50l and 250l)			
CDMO: project including 2L, 50L and 250L	€ 2,500,000.00	1.5-2 years	
Plasmids required for GMP manufacturing	€ 500,000.00	6 months	
Other			
Regulatory Support	€ 50,000.00		
Natural History Study and Clinical Trial Preparation	€ 200,000.00		
Clinical Trial(s) costs	?		
Estimated total	**5,265,000.00 €**		

Note: the individual timeline items will progress in parallel and thus the times provided are to indicate the time required for each step and are not to be summed up to predict the overall timeline of the project.

Thank you