

At scale transduction of hCD34+ stem cells for Wolman disease biodistribution studies.

Presenter: Dr Stuart Ellison

WT TPA Projects for translation award

Stem cell & Neurotherapies laboratory



TranslationManchester



Wolman disease

- Wolman disease is a congenital lysosomal storage disorder characterised by impaired fat (lipid) metabolism
- Estimated incidence 1:100,000
- Mutations in the LIPA gene result in reduced or complete lack of lysosomal acid lipase (LAL)
- Symptoms include enlarged liver and spleen, vomiting and diarrhea, poor weight gain, low muscle tone, jaundice and developmental delay.
- If left untreated, patients die within the first 12 months of life.

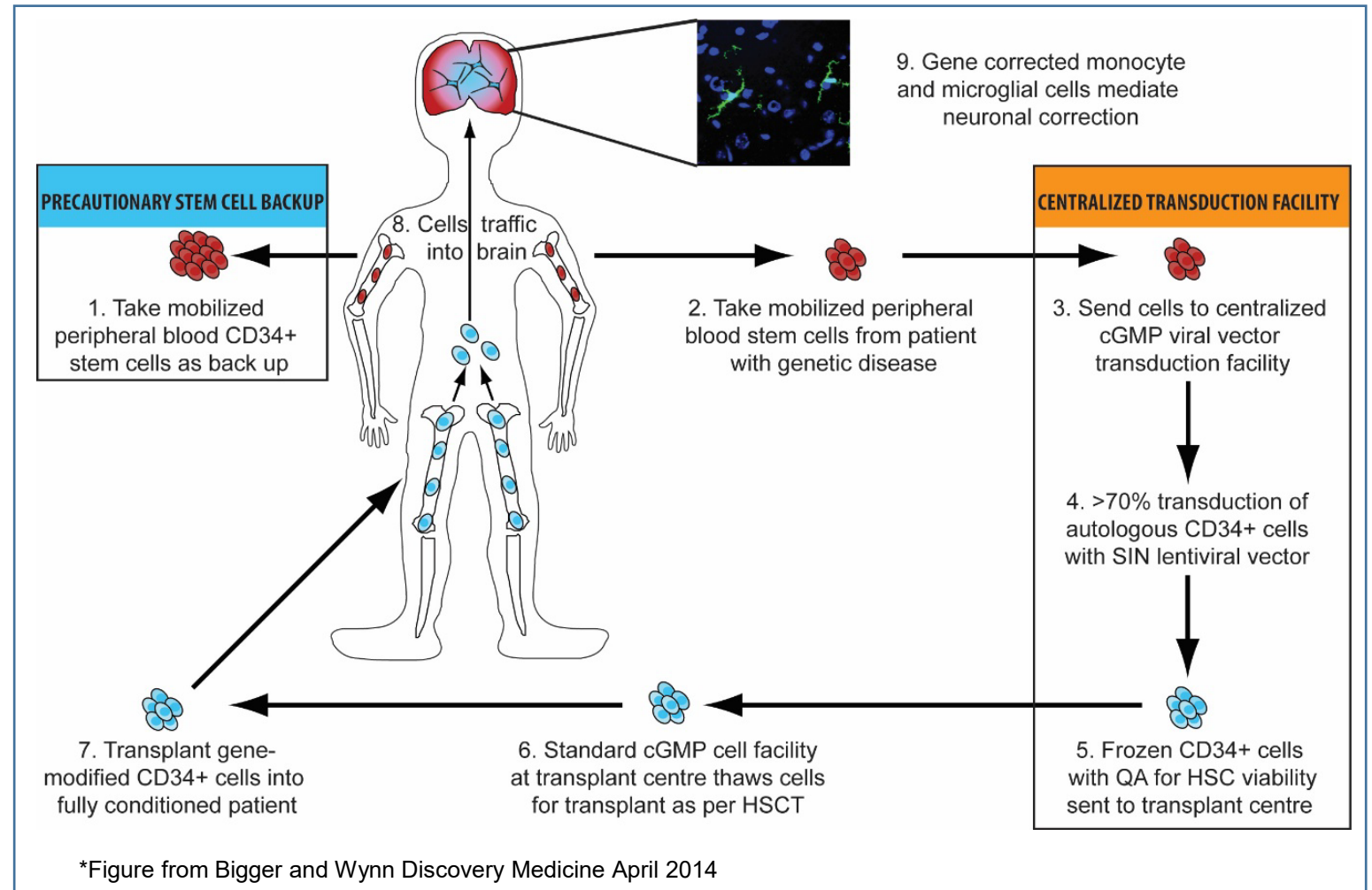


Image from Hannah et al, AJMG, 2022

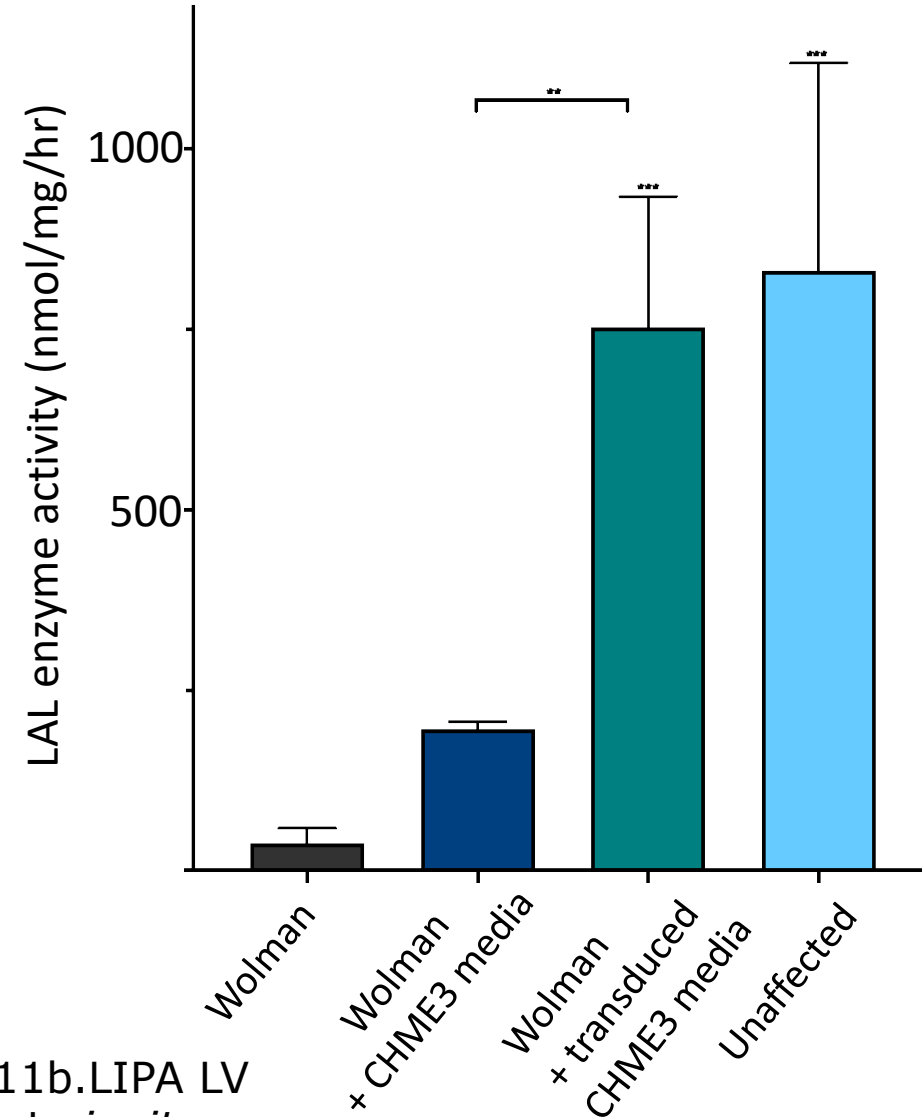
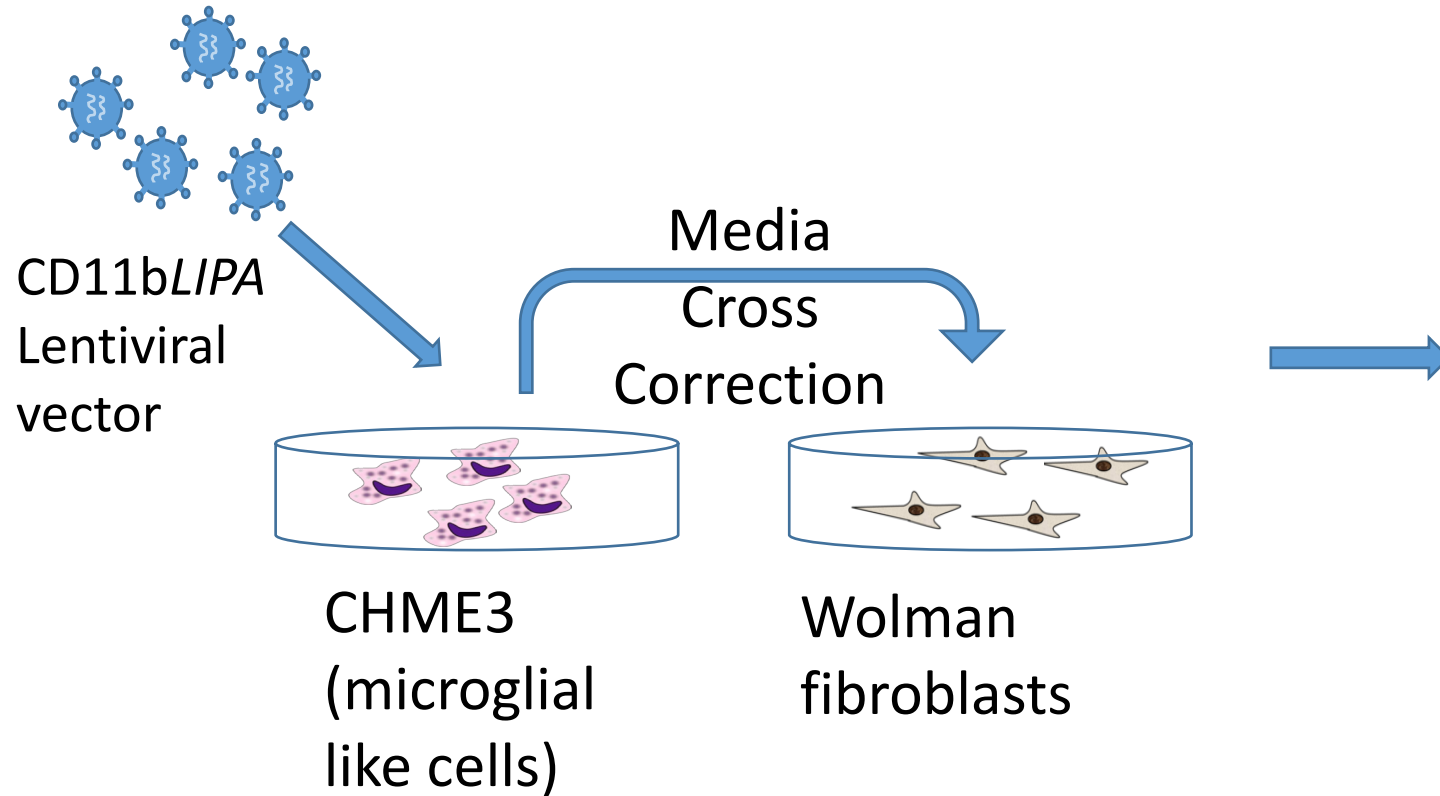
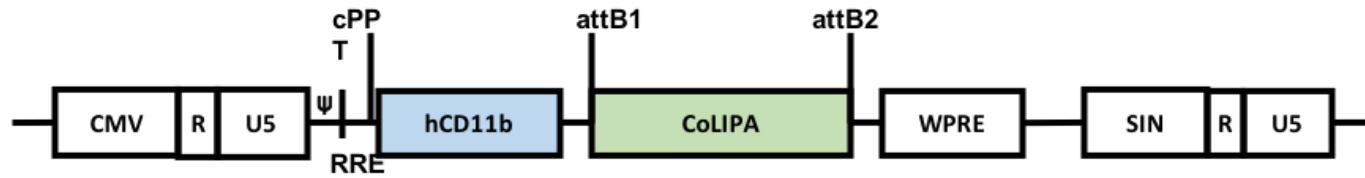
Current treatments	Pros	Cons
Bone marrow transplant (BMT)	Potential treatment effective for other diseases	high procedure-related mortality due to disease progression and disease-associated morbidities, GvH disease
Enzyme replacement therapy (ERT) -Sebelipase α	Significantly improved survival	Life long, expensive, central venous access, anti-drug antibodies

Haematopoietic stem cell gene therapy

- Autologous treatment – genetically modification of patients own stem cells
- No graft vs host disease as with standard BMT
- Can overexpress functional enzyme in the cells that traffic to the brain - ↑efficacy
- HSCGT effective for Metachromatic Leukodystrophy (MLD) and ADA-SCID

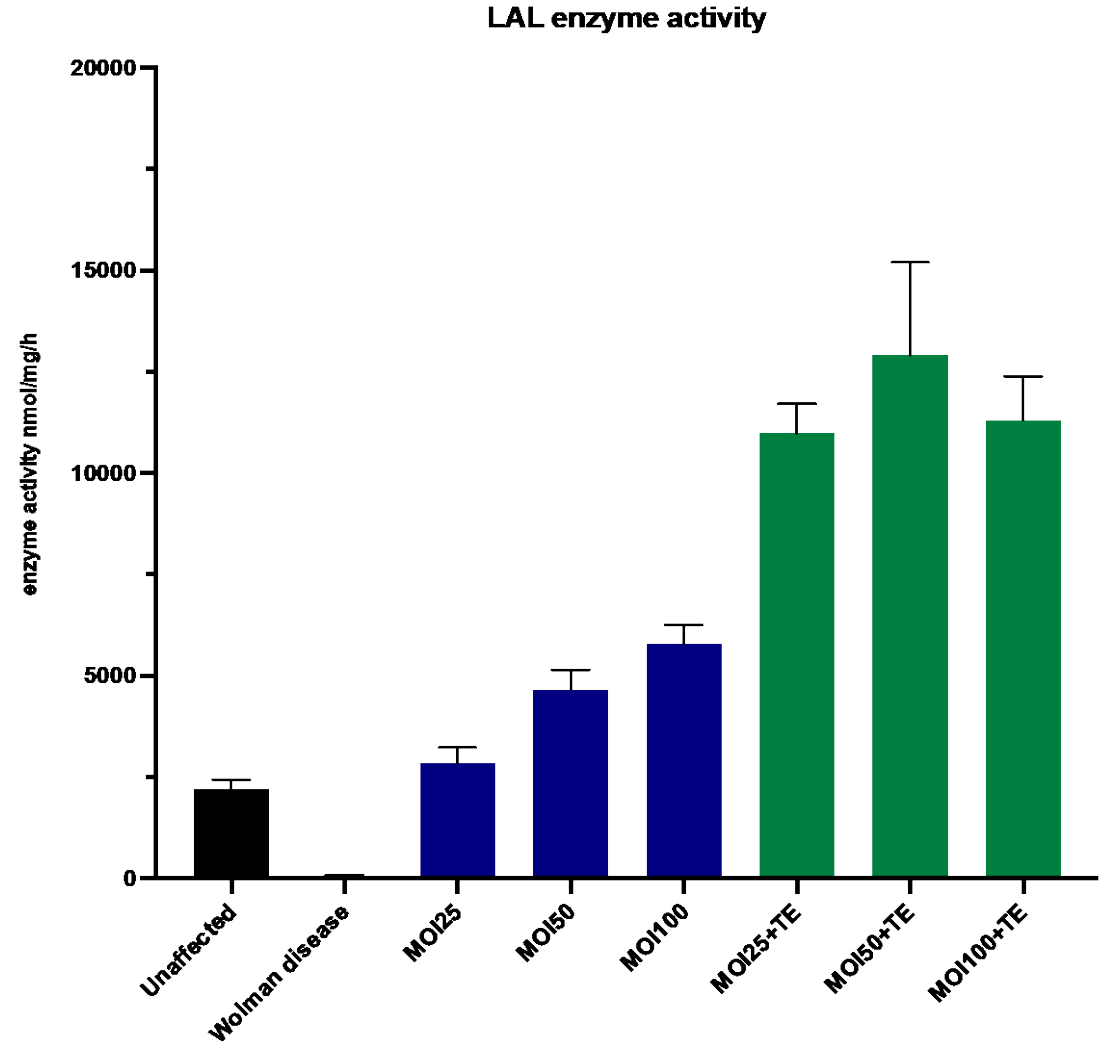
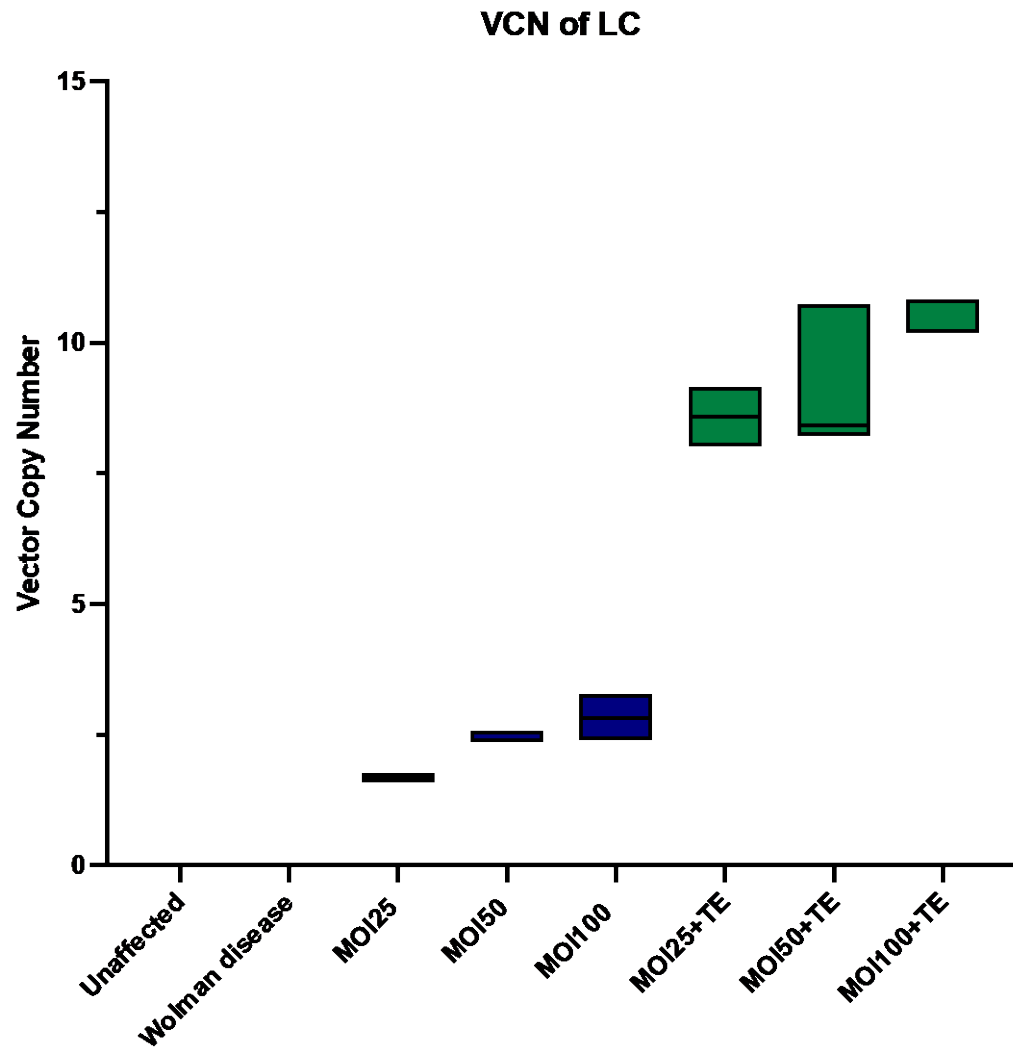


Development of HSC-GT for Wolman disease – PoC studies



microglial-like cells (CHME3 cells) can be effectively transduced with CD11b.LIPA LV and secrete function LAL enzyme that can cross-correct Wolman fibroblasts *in vitro*.

PoC studies – transduction of Wolman CD34+ stem cells



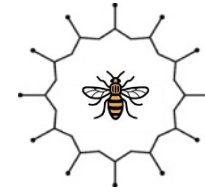
CD11b.LIPA LV transduced Wolman CD34+ cells, derived from patient BM, can over express function LAL enzyme without adverse toxicity

Project objective

Validate HSC transduction at clinical scale for Wolman disease which will also generate a cryopreserved GMP-like Investigational Medicinal Product (IMP) that can be used in future bio-distribution studies in a humanized mouse model to provide supporting *invivo* safety, efficacy and toxicology data to the regulators.

1. Manufacture large batch of R&D grade CD11b.LIPA LV with titre above 2×10^8 TU/ml
2. Optimise CD34 transduction conditions at small scale at a range of vector doses with and without transduction enhancers (MOI range 12.5, 25, 50, 100) – target 2-10 copies
3. Perform 1x 'at scale' stem cell isolation and transduction at optimal conditions followed by cryopreservation
 - Evaluate normal lineage development by colony forming unit (CFU) assay, vector copy number (VCN) and enzyme activity.
 - Perform a viability study 8-10 weeks following cryopreservation to evaluate viability, VCN and enzyme activity following product post-thawing.
 - Perform a minimal QC panel of sterility, mycoplasma and endotoxin assessment to demonstrate suitability of product for downstream *invivo* studies.

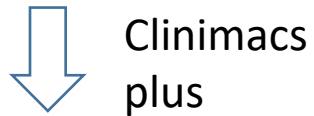
Workflow – R&D “at scale” manufacturing test runs



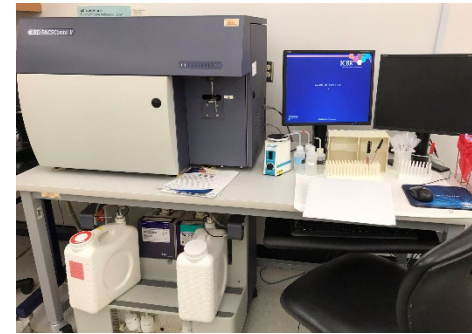
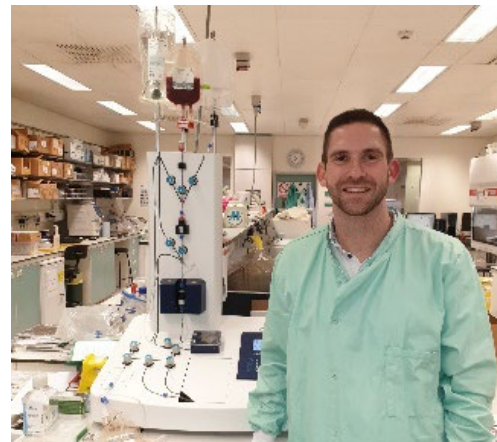
Isolate CD34+ cells from mobilised peripheral blood (leukaphoresis unit)



Anthony Nolan (UK supplier - London) – same day delivery



Count and assess purity and viability of the CD34+ cells by FACS



Evaluate purity and viability of transduced CD34+ cells by FACS



Isolate gDNA and determine VCN

↓
Prestimulate CD34+ cells O/N (1xT75 flask)

⇒ Transduce CD34+ cells with lentiviral vector For 24hrs

⇒ Wash and harvest transduced cells

⇒ 14 day culture To evaluate lineage development
⇒ Cryopreserve IMP

Cleanroom process validation and manufacturing

iMATCH (Innovate Manchester
Advanced Therapy Centre Hub)



NHSBT
Barnsley, UK

Blood and Transplant



GMP manufacturing results

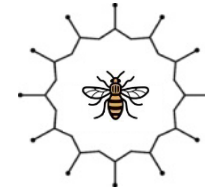
MOI of 10 + transduction enhancers

GMP Run 1	% Viability thawed CD34+	Recovered transduced CD34+ cell x 10 ⁶ /Kg *	% Overall recovery transduced CD34+ from cells seeded	VCN pooled CFU	VCN 14 day LC	Sterility	Mycoplasma Genus PCR	Endotoxin EU/mL	Meets Specification
Cells in Cryostor	97.1	123.07	88.3						Yes
Immediate Post Thaw	96.8	11.51	80.5	7.24	8.63	No growth in Final Product	Not detected	<0.1	Yes
10 Weeks	-	-	-	-	-				Yes
Thaw for NGS study (upto 52 weeks)	-	-	-	-	-	-	-	-	-
*Estimated typical patient weight 10Kg used in calculations									

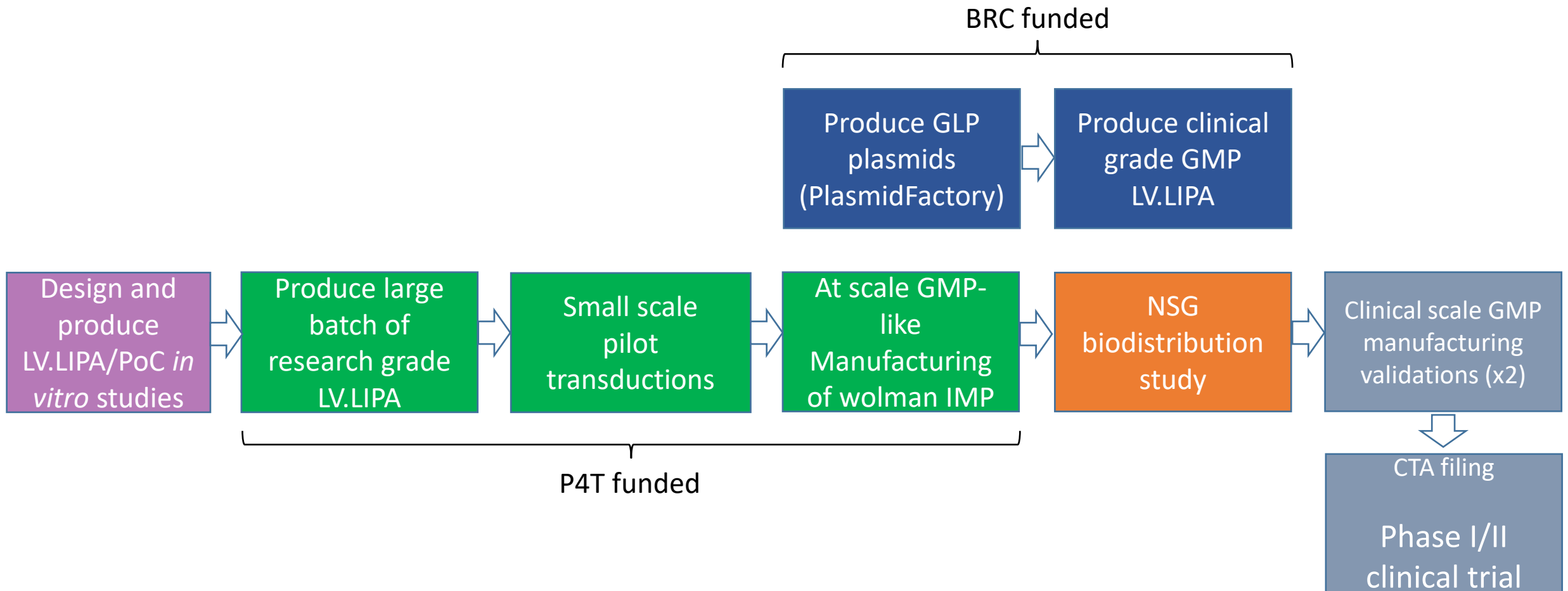
Achievements

- Manufactured sufficient quantities of LV.LIPA transduced cells for a future biodistribution study
 - 60ml at 2x10⁶ cells/ml
- GMP manufacturing validation data can contribute to the IMPD
- NHSBT can use these validation runs to obtain an MHRA licence to manufacture ATMPs in the future

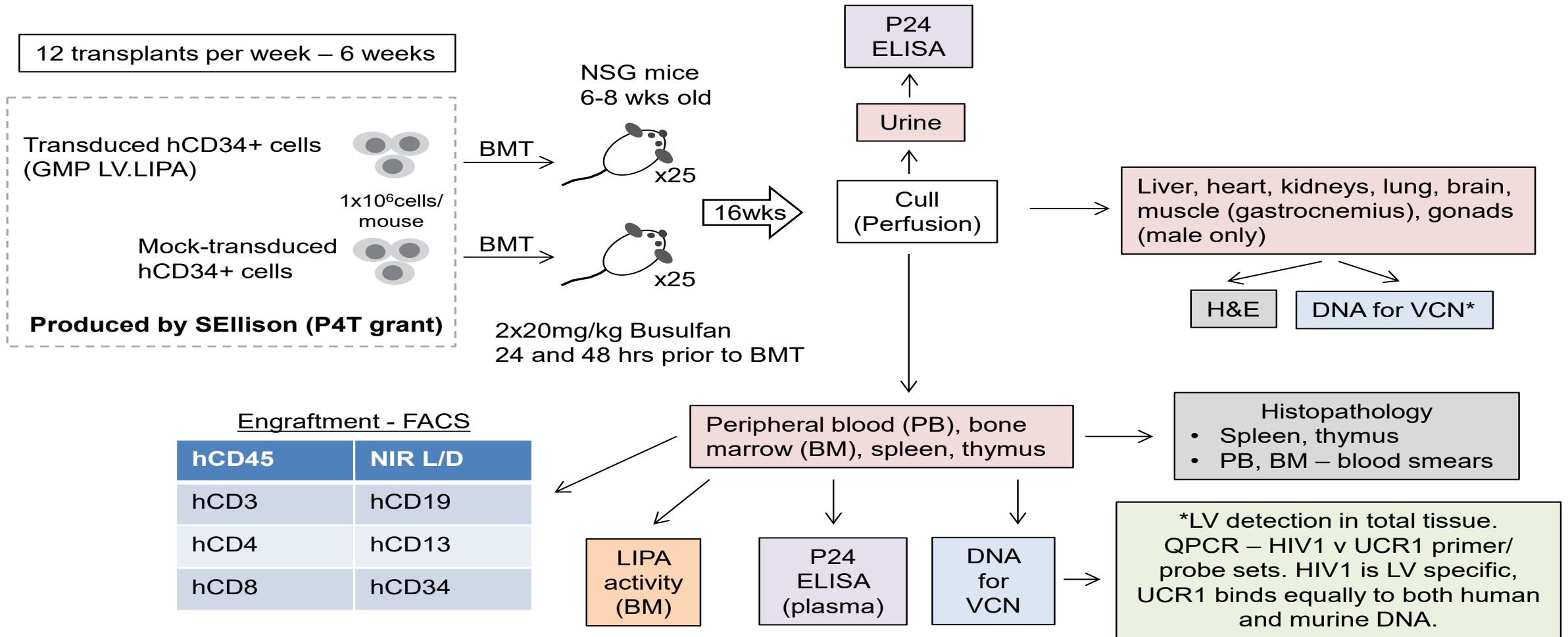
How has this work has allowed us to progress along the translational pathway?



2019 -----> 2024/25



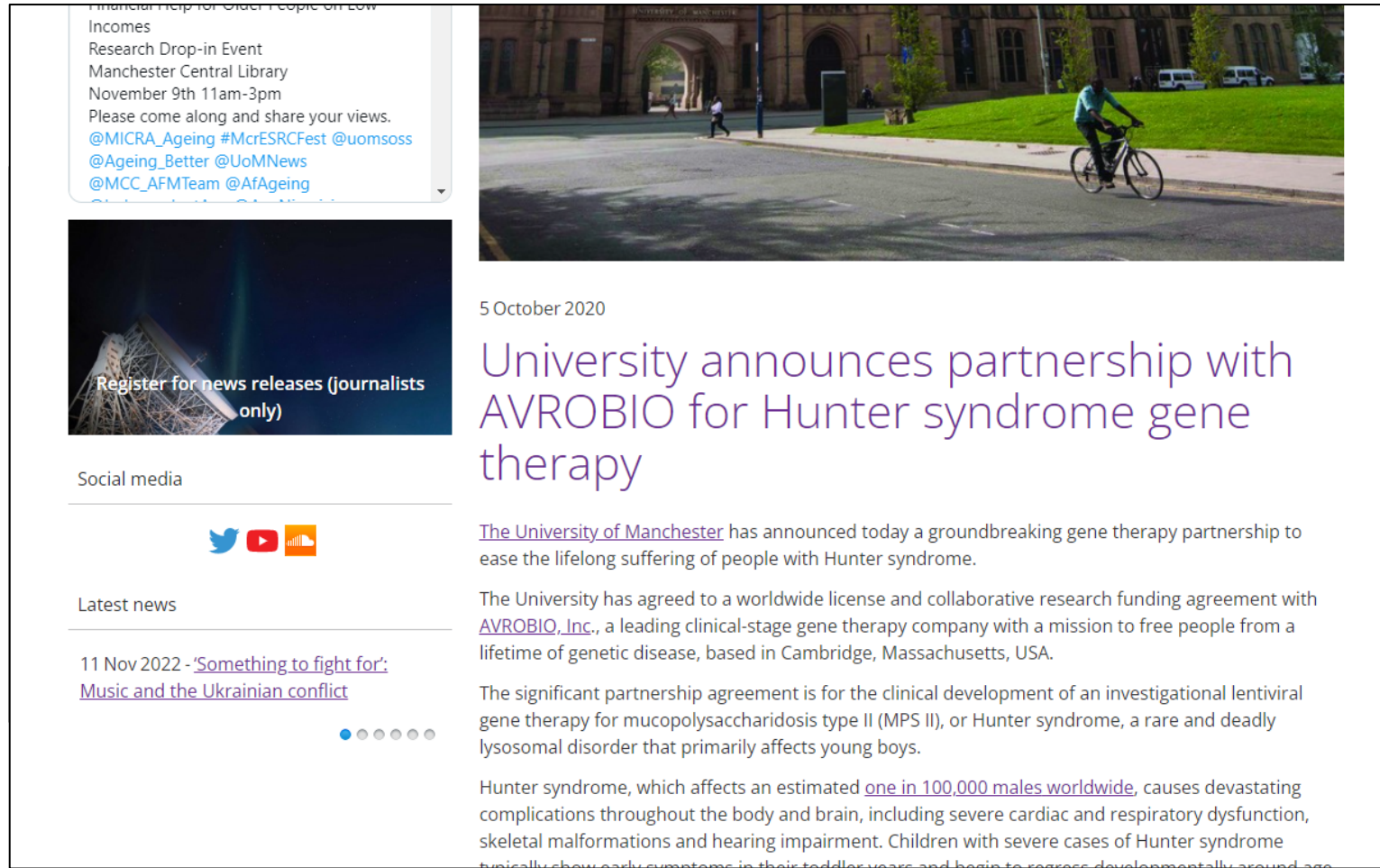
Future work – biodistribution study




Future NSG biodistribution study to evaluate efficacy and toxicity of medicinal product

Research impact

- Potential to create a new therapy for Wolman disease that surpasses current treatments
- Phase I/II clinical trial of HSC-GT for MPSIIIA for Orchard Therapeutics 2019-2024, £7.8M
- £67M licence deal with Avrobio and £9.1M clinical trial grant from Avrobio – MPSII hunter HSC-GT



Financial help for Older People on Low Incomes
Research Drop-in Event
Manchester Central Library
November 9th 11am-3pm
Please come along and share your views.
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5 October 2020

University announces partnership with AVROBIO for Hunter syndrome gene therapy

[The University of Manchester](#) has announced today a groundbreaking gene therapy partnership to ease the lifelong suffering of people with Hunter syndrome.

The University has agreed to a worldwide license and collaborative research funding agreement with [AVROBIO, Inc.](#), a leading clinical-stage gene therapy company with a mission to free people from a lifetime of genetic disease, based in Cambridge, Massachusetts, USA.

The significant partnership agreement is for the clinical development of an investigational lentiviral gene therapy for mucopolysaccharidosis type II (MPS II), or Hunter syndrome, a rare and deadly lysosomal disorder that primarily affects young boys.

Hunter syndrome, which affects an estimated [one in 100,000 males worldwide](#), causes devastating complications throughout the body and brain, including severe cardiac and respiratory dysfunction, skeletal malformations and hearing impairment. Children with severe cases of Hunter syndrome typically show early symptoms in their toddler years and begin to regress developmentally around age 10.

Acknowledgements

Stem cell and Neurotherapies group

Brian Bigger

Yuko Learmonth

Jane Potter

Laura Bonsell

Laura Booth

Oriana Mandolfo

Teresa Andreou

Shaun Wood

Willink

Heather Church

iMATCH team

Fiona Thistlethwaite

Vicky Sheard

All partners involved

MFT

Simon Jones

Rob Wynn

Anthony Nolan trust

NHSBT cleanroom Barnsley team

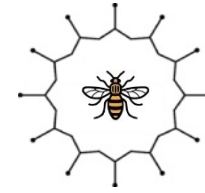
Victoria Day

Spandan Kalra

Lauren Howe



Blood and Transplant



ATTC
Advanced Therapy
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MANCHESTER
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Manchester University
NHS Foundation Trust

Funders

WT P4T award



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