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

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**WT TPA Projects for translation award**

Stem cell & Neurotherapies laboratory

MANCHESTER 1824

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# 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.

| 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 |


Image from Hannah et al, AJMG, 2022

# 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

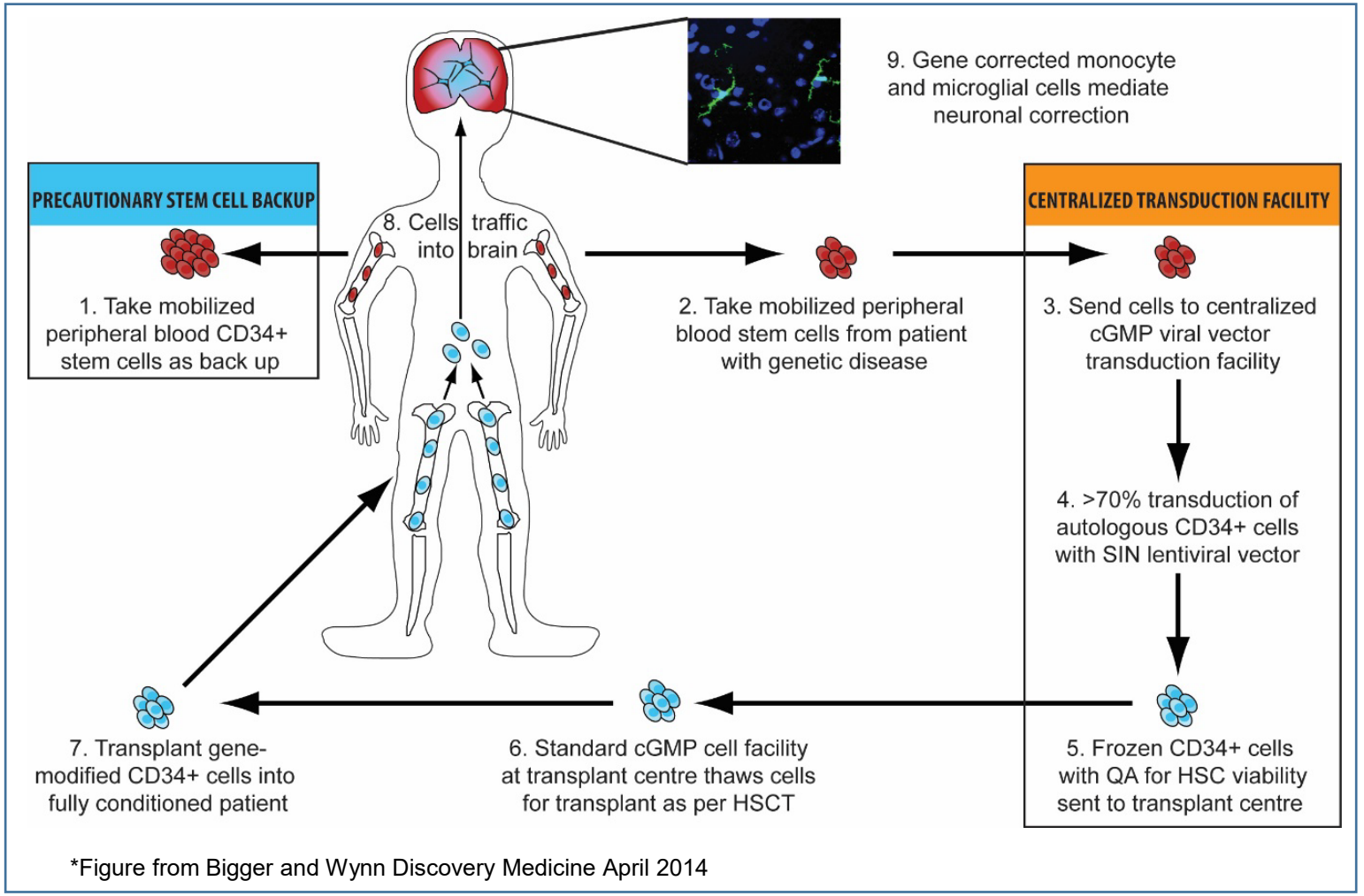
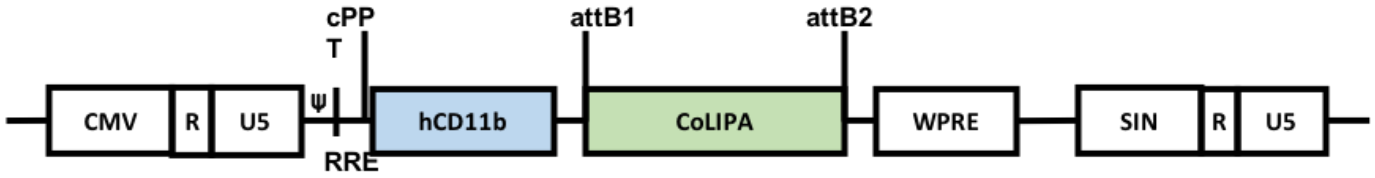
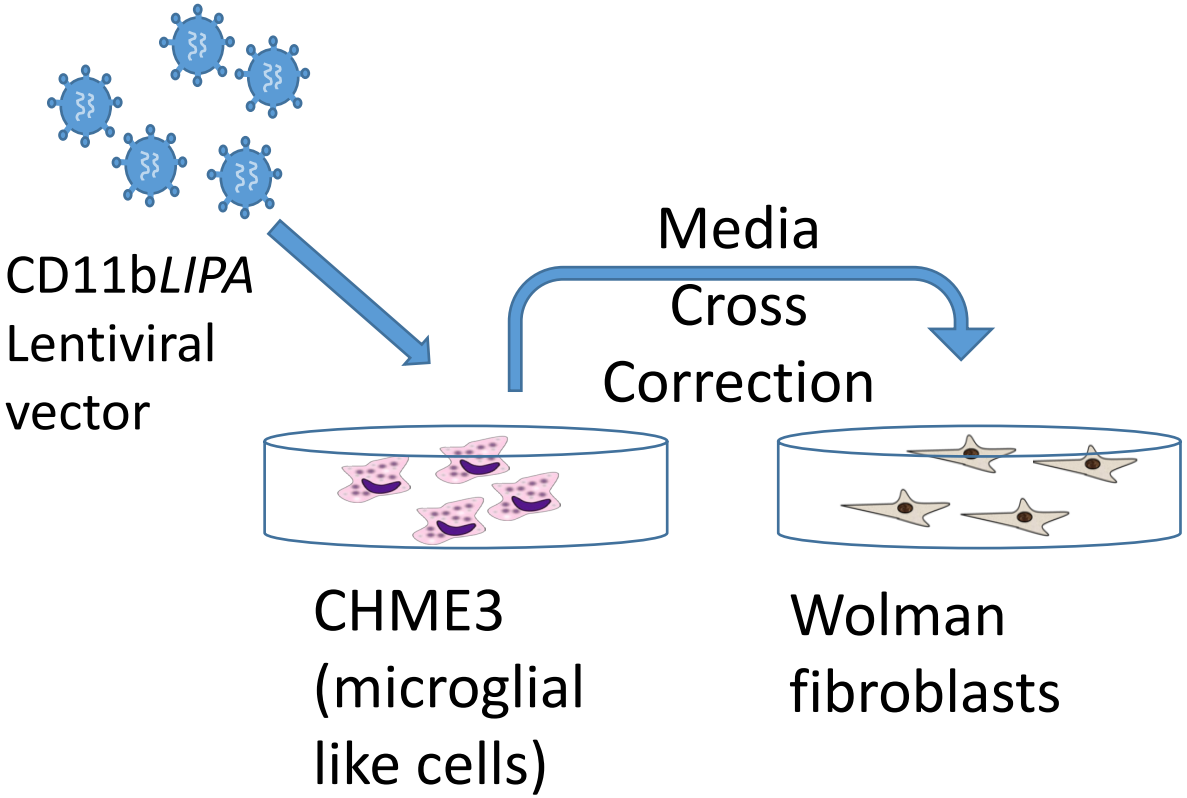
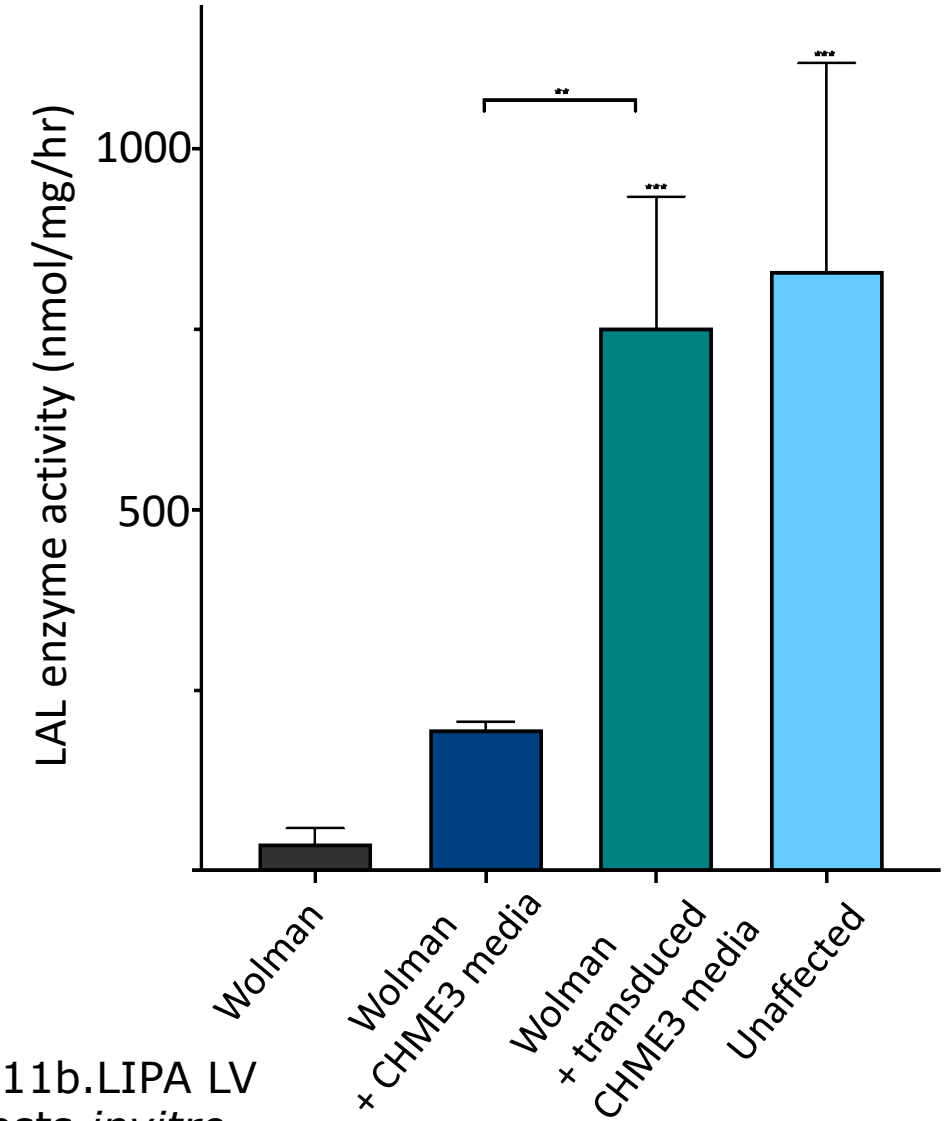



*Figure from Bigger and Wynn Discovery Medicine April 2014

# Development of HSC-GT for Wolman disease – PoC studies

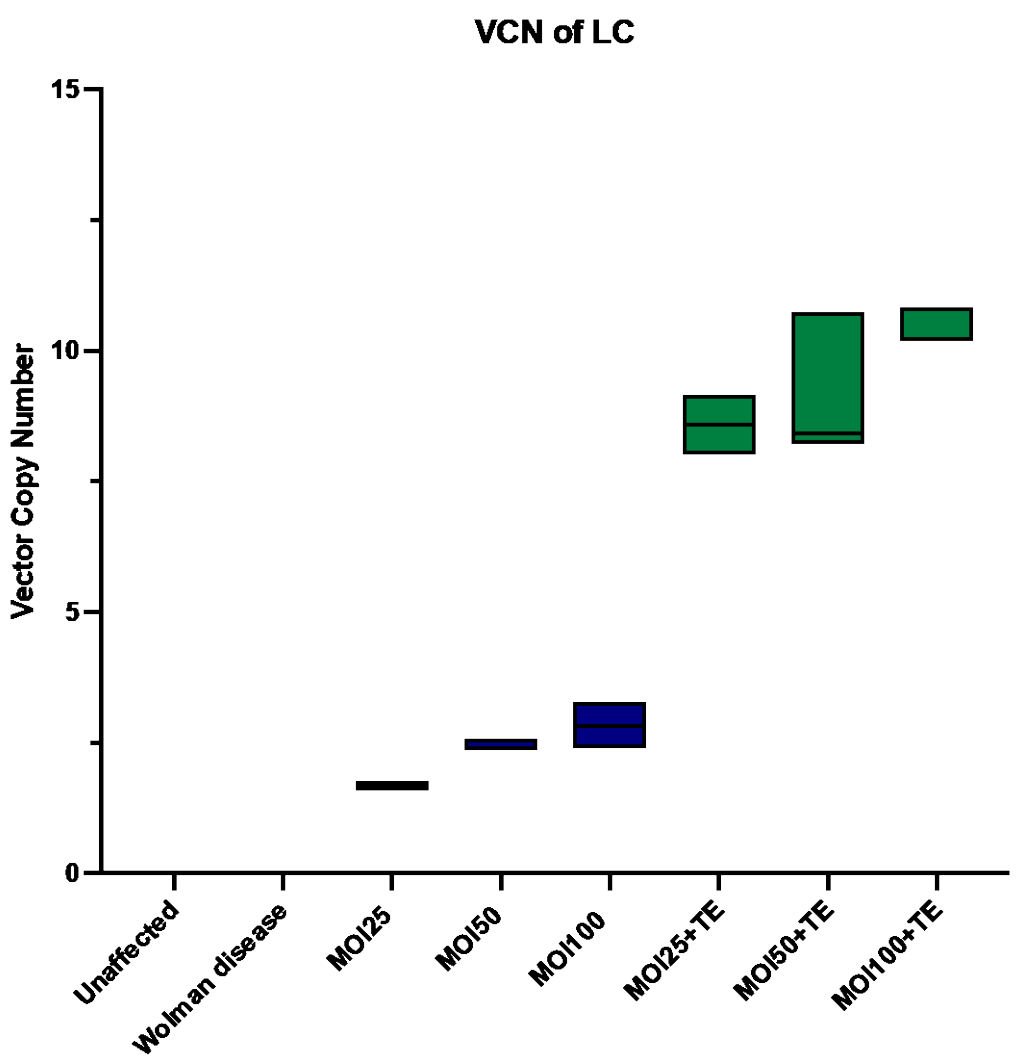


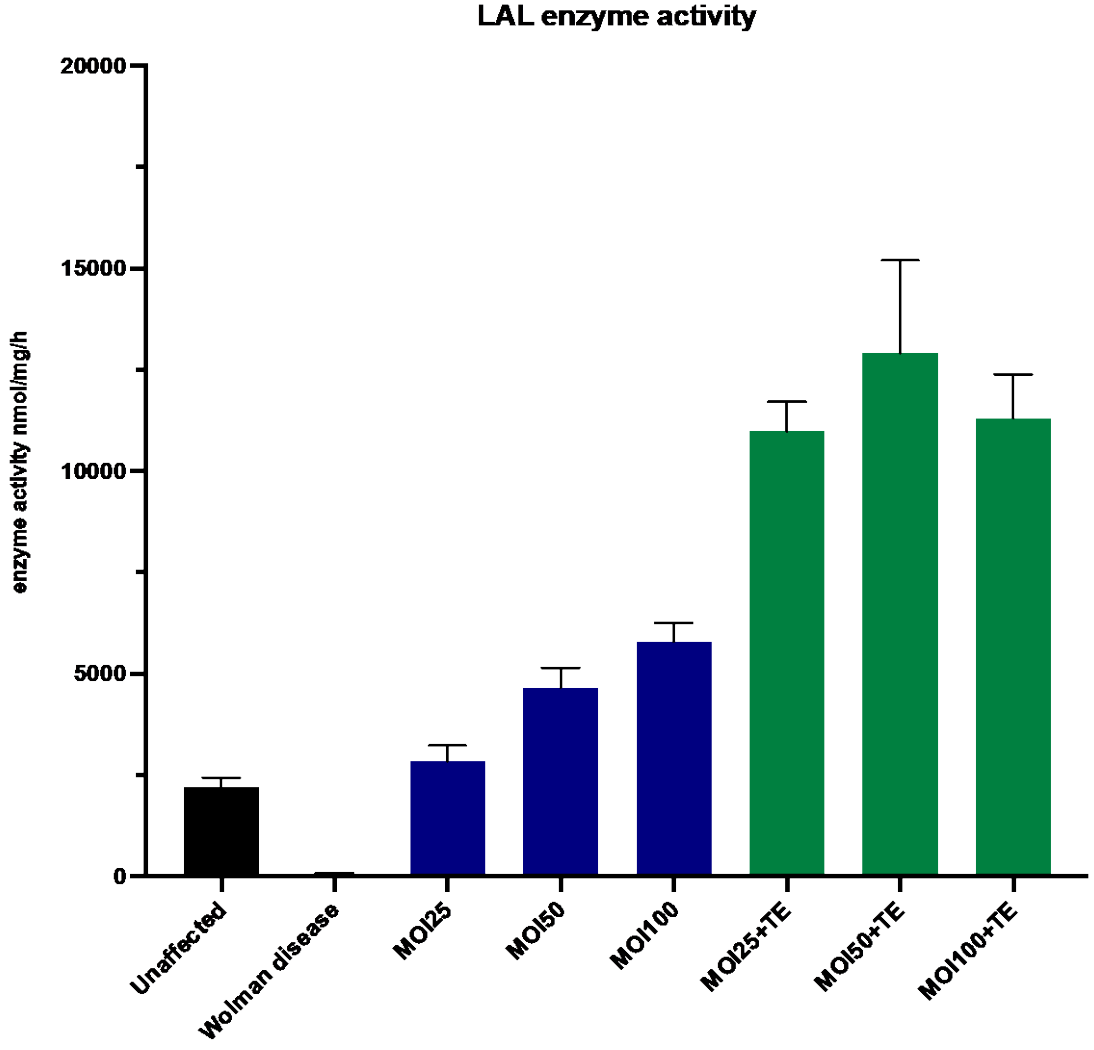




microglial-like cells (CHME3 cells) can be effectively transduced with CD11b.LIPA LV and secrete function LAL enzyme that can cross-correct Wolman fibroblasts *invitro*.

# 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 $2x10^8$ 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

Clinimacs plus

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

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

Transduce CD34+ cells with lentiviral vector For 24hrs

Wash and harvest transduced cells

Evaluate purity and viability of transduced CD34+ cells by FACS

Isolate gDNA and determine VCN

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

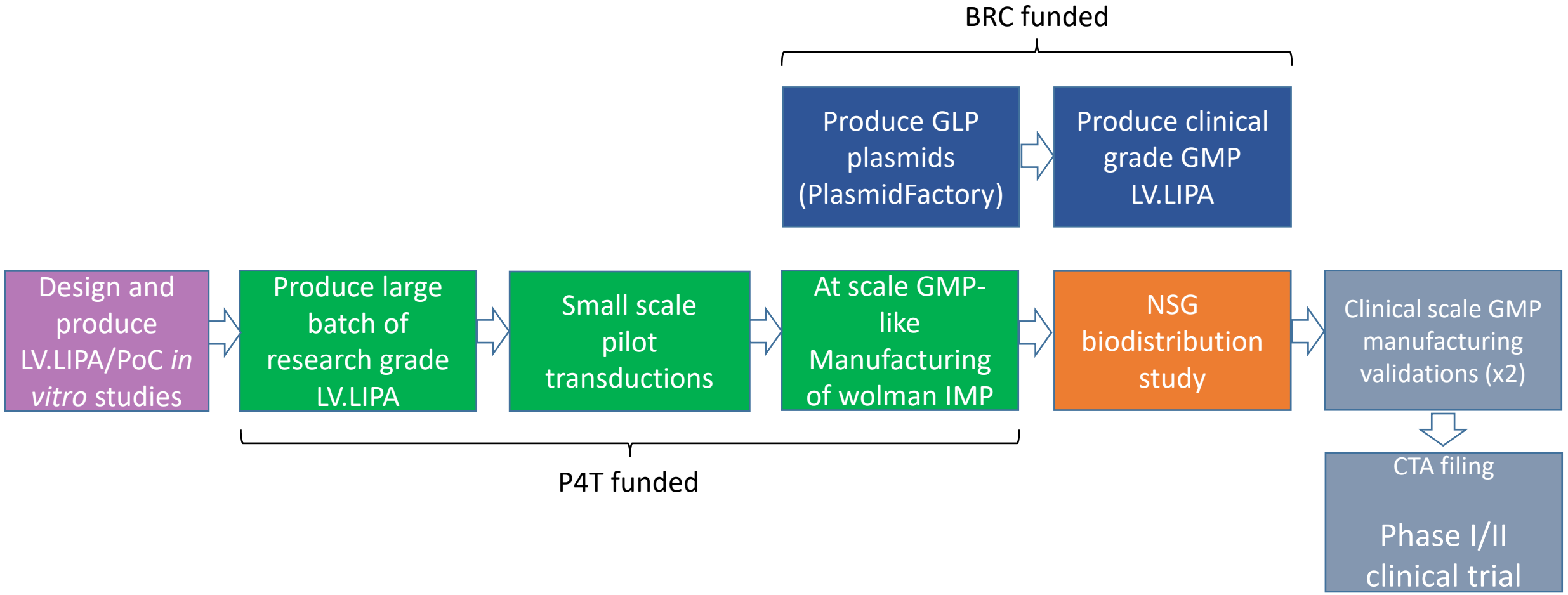
MOI of 10 + transduction enhancers

| 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 | | | | | | | | | |

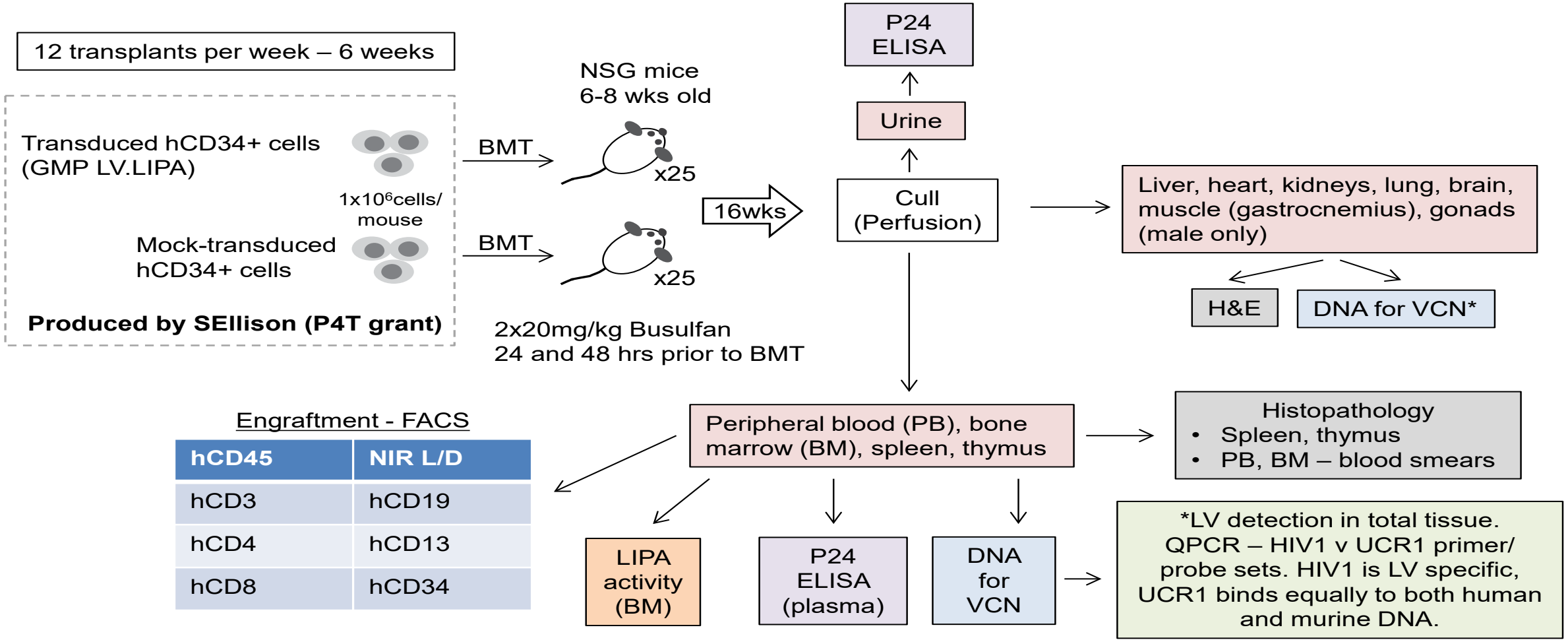
## Achievements

- Manufactured sufficient quantities of LV.LIPA transduced cells for a future biodistribution study
  - ➢60ml at $2x10^6$ 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?



# Future work – biodistribution study



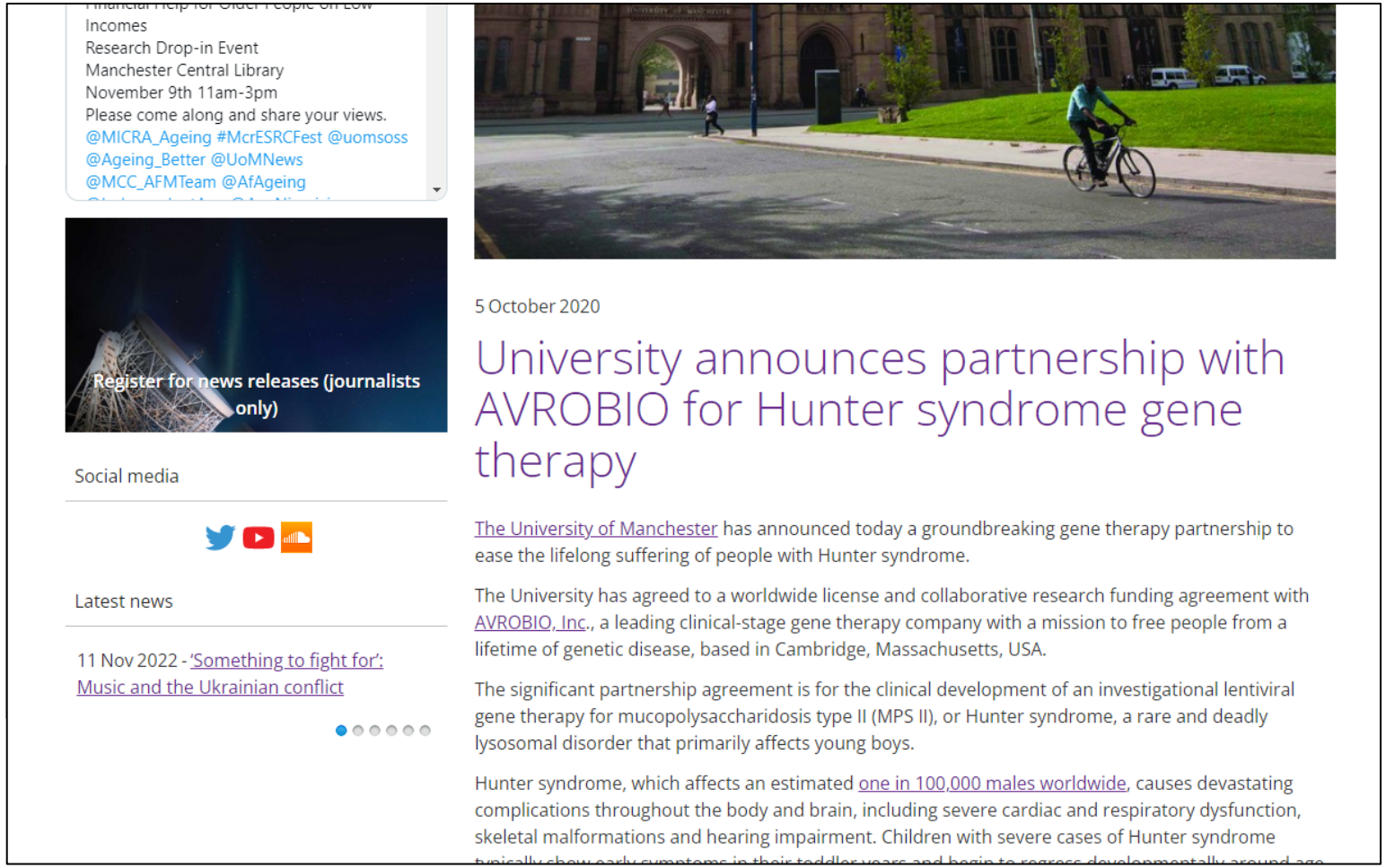

Engraftment - FACS

| hCD45 | NIR L/D |
|---|---|
| hCD3 | hCD19 |
| hCD4 | hCD13 |
| hCD8 | hCD34 |

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



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WT P4T award