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Screenable MyoScreen™ Pompe Early-Onset and Late-Onset skeletal muscle for drug discovery

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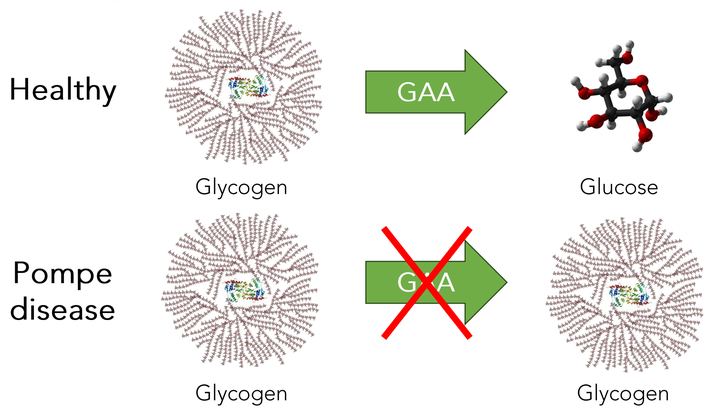
A need for new therapeutics targeting muscle defects in Pompe patients

Acid Alpha-Glucosidase: a requirement for glycogen and glucose metabolism

Glucose is stored in the form of glycogen particles within the sarcoplasm of healthy muscle cells, providing an immediate internal source of energy for exercise. Intramuscular glycogen stores targeted for glycophagy-mediated glycogenolysis traffic to the lysosome via the autophagy pathway and are broken down into glucose by the lysosomal enzyme, Acid Alpha-Glucosidase (GAA).

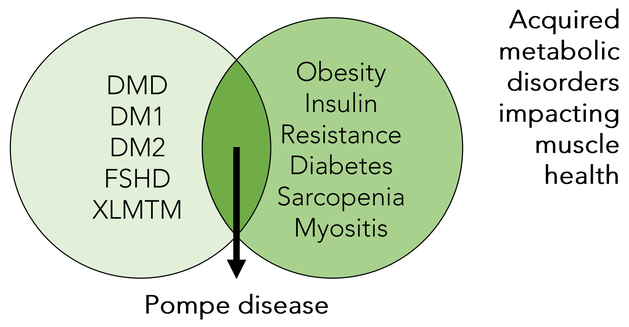
In Pompe disease (PD), GAA deficiency leads to impaired glycogen degradation, an accumulation of glycogen inside lysosomes along with a shortage of glucose and autophagic build-up. Skeletal muscles are particularly affected.



Pompe disease hallmarks and clinical unmet needs

Pompe Subtype	Early-Onset Pompe Disease (EOPD)	Late-Onset Pompe Disease (LOPD)
GAA availability	<1% enzyme activity	<30% enzyme activity
Onset	First months of age	Any age
Symptoms	• Feeding problems • Poor weight gain • Breathing issues • Muscle weakness • Enlarged heart	• Muscle weakness • Respiratory failure (progresses after several years) • No heart involvement
Incidence	1 in 40,000 people in the USA	
Available therapies	ERT is the only approved therapy for EOPD and LOPD. ERT involves infusions every 2 weeks over 4-5 hours. Gene therapy restoring GAA to the liver (AskBio) or muscle (Astellas) is in clinical trials. Yet other approaches are in preclinical stage development.	
Need for additional treatments targeting autophagy	GAA replacement fails to reverse the skeletal muscle pathology present in PD. It does not address extra lysosomal glycogen accumulation nor autophagic abnormalities that dysregulate protein synthesis pathways leading to muscle atrophy in PD patients. Furthermore, these two pathological features negatively affect muscle uptake and lysosomal delivery of recombinant GAA.	

Modeling of Pompe skeletal muscle metabolism defects *in vitro*



PD is both a genetic and metabolic myopathy since it is caused by an inherited defect in a biochemical pathway that produces ATP.

An additional characteristic of PD is lysosomal and autophagic defects however this is not always reproduced in Pompe cultured myotubes *in vitro*. Most Pompe muscle models are iPSC-derived but their unreliable maturity produces a range of phenotypes that decreases downstream assay reproducibility.

In this study, primary human myoblasts cultures from EOPD and LOPD Pompe patients were used for their human relevance and maintenance of genotype-phenotype correlation.

MyoScreen discovery platform delivers clinically relevant drug candidates

CYTOO's Myoscreen platform overcomes the assay development challenges of PHYSIOLOGICAL RELEVANCE, AUTOMATION CAPACITY and ROBUSTNESS.

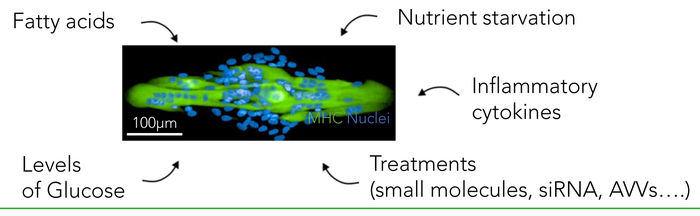
It combines the latest innovations including standardized myotubes in 96 and 384 plate format, quantitative imaging and Artificial Intelligence (AI) driven image analysis[1,2].

Four Step R&D Process Towards Screening Success

1 *In vitro* Disease Modeling

Employ new or prevalidated myoblast cell lines
- Healthy muscle (male vs. female, young vs. elderly)
- Genetic muscle disorders (DMD, DM1, DM2, FHSD, XLMTM, Pompe)

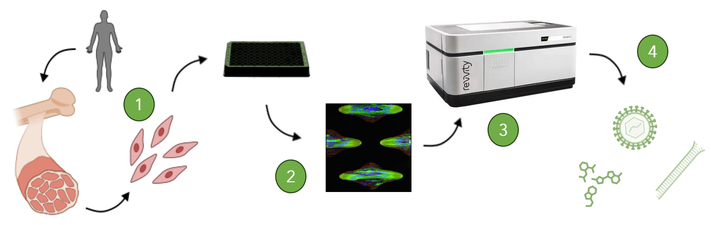
or induce pathological features through cell culture conditions



2 Differentiating Readouts Identification

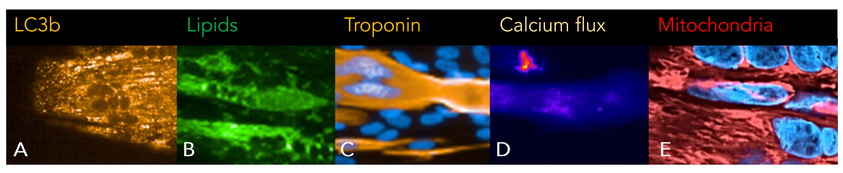
Measure altered disease-relevant proteins or functional changes
- High content phenotypic imaging
- Biochemical readouts
- qPCR and protein analysis

MyoScreen provides relevant muscle disease models for high-throughput screening



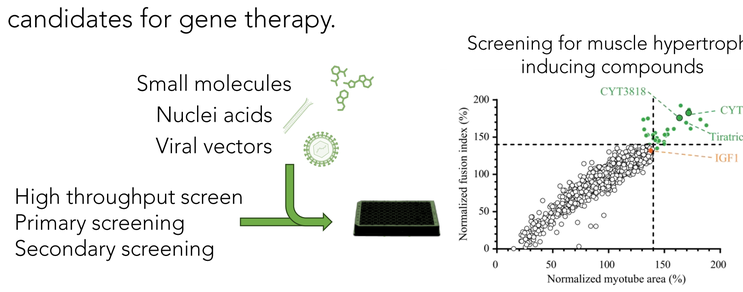
3 Assay Development

Validated assays are available monitoring different pathways such as autophagy (A), lipid accumulation (B), anabolism and catabolism impairment (C), contractility (D), mitochondria dysfunction (E). Up to three assays can be multiplexed increasing output and performance.



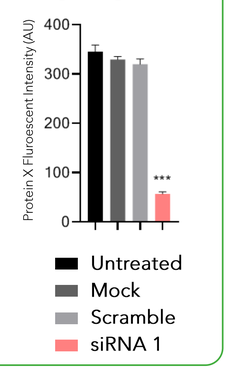
4 Screening

Experience in lead discovery, hits characterization as well as evaluation/ selection of small molecules, ASOs and AAV candidates for gene therapy.



MyoSilence™ gene silencing platform for drug target identification and validation

- High throughput and standardized: aligned and linear myotubes in 96 and 384 well format plates
- Predictive: myotubes are mature forming sarcomeric striations and acetylcholine receptor clusters
- Robust: low levels of variability and high statistical power with 100 myotubes per well (96-well plate) facilitate passing of bioassay data quality control indicators (%CV, Z'-factor, SSMD)
- Inter-experiment variability < 10%

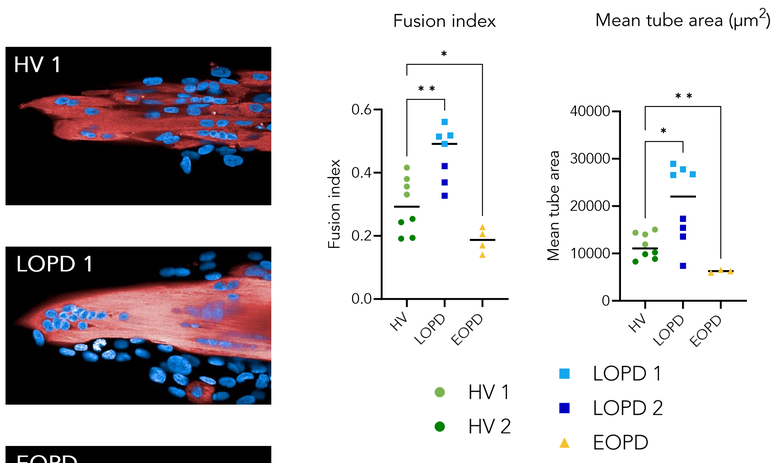


The first human muscle model distinguishing LOPD from EOPD with high content screening capacity

Lysosome accumulation affects morphology of EOPD myotubes

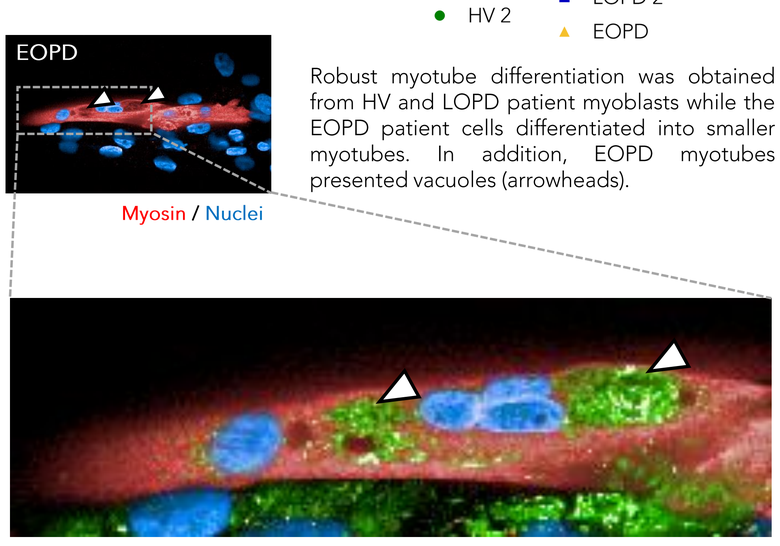
Human primary skeletal muscle myoblasts from 2 Healthy Volunteers (HV), 2 LOPD patients and 1 EOPD patient were cultured on MyoScreen micropatterned 96-well plates, differentiated for 8 days and then co-stained for myotube (Myosin), lysosomal (LAMP1) and autophagosomal (LC3B) markers.

Data are normalized to HV control wells and show mean ± SD; n = 3-4 wells unless otherwise indicated and are representative of at least N=2 experiments.



Robust myotube differentiation was obtained from HV and LOPD patient myoblasts while the EOPD patient cells differentiated into smaller myotubes. In addition, EOPD myotubes presented vacuoles (arrowheads).

Myosin / Nuclei

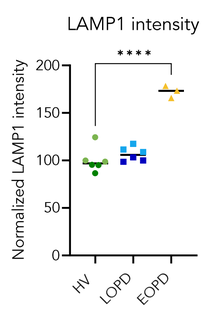


Myosin / LAMP1 / Nuclei

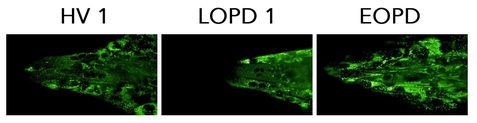
The vacuoles are filled with LAMP1-positive, LC3B-negative vesicles (arrowheads) that disturb the myosin myofibrillar architecture (red stain). Such structural abnormalities suggest massive lysosome accumulation and rupture.

These vacuoles resemble morphological alterations reported in EOPD patient biopsies[3] and are thought to be caused by the accumulation of non-degraded glycogen in lysosomes.

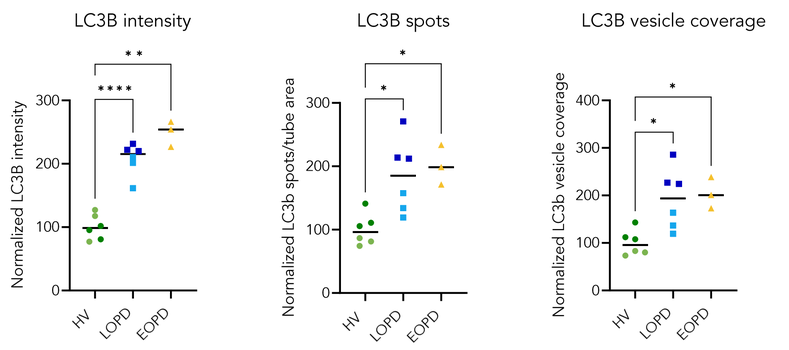
Quantification of lysosome and autophagy dysfunction



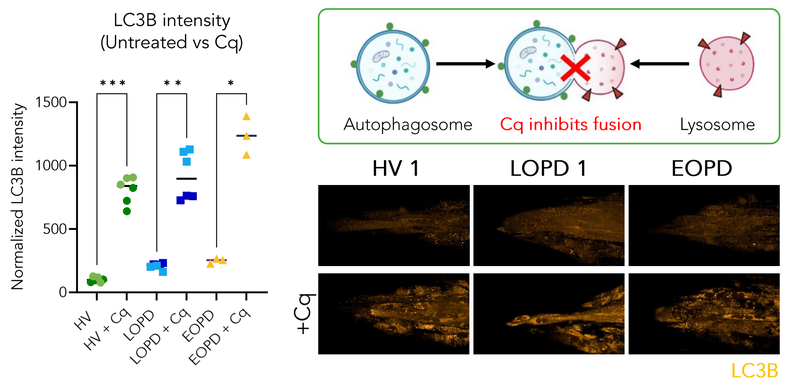
Quantitative image analysis confirmed that only EOPD myotubes present a significant increase in lysosome content (LAMP1 levels) compared to HV myotubes (1.7-fold).



Myotubes from both forms of PD showed increased levels of autophagosomes and autophagy with higher LC3B intensity (2.3-fold), LC3B spot number (1.99-fold) and LC3B vesicle coverage of myotube area (2-fold) relative to HV myotubes.



Chloroquine (Cq) treatment, which blocks autophagosome-lysosome fusion, further exacerbated autophagic build-up in all donors, including HV (4.4 to 8-fold). This strong upregulation indicates that autophagic flux is not completely impaired in PD donors in the absence of Cq.



Glycogen accumulates in EOPD myotubes

Compared to HV, EOPD cell lysates contain clearly elevated glycogen levels at baseline (4-fold).

The biochemical data correlates with severity of Pompe subtype and corroborates the quantitative high content analysis.

Due to the low throughput of sample collection, data is n=2 technical replicates from 1 sample/donor.

Supporting new muscle therapies for Pompe

– This is the first report of human primary LOPD and EOPD muscle models being established in a HT/HC screenable format

– Thanks to the development of sensitive image-based readouts that capture the complexity of the *in vitro* phenotype, we can differentiate between EOPD and LOPD pathological subtypes
- Morphological alterations (EOPD only)
- Accumulation of lysosomes (EOPD only)
- Autophagic build-up
- Abnormal glycogen levels (EOPD only)

– The MyoScreen Pompe Disease platform representative of lysosomal storage disorders provides opportunities for
- Identification and validation of new druggable targets
- Discovery of small molecules that improve the autophagic defect
- Evaluation of the positive impact of novel RNA and gene therapies

– Additional MyoScreen assays are available to investigate other components of PD pathology including oxidative stress, mitochondrial defects, metabolic shift, protein synthesis and degradation

Contact us: eventre@cytoo.com and jyoung@cytoo.com

References: [1] Young *et al.* 2018, [2] Hariharan, Lorintiu *et al.* 2023, [3] Raben *et al.* 2012

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