Pompe Disease - Biochemical Investigation and Monitoring

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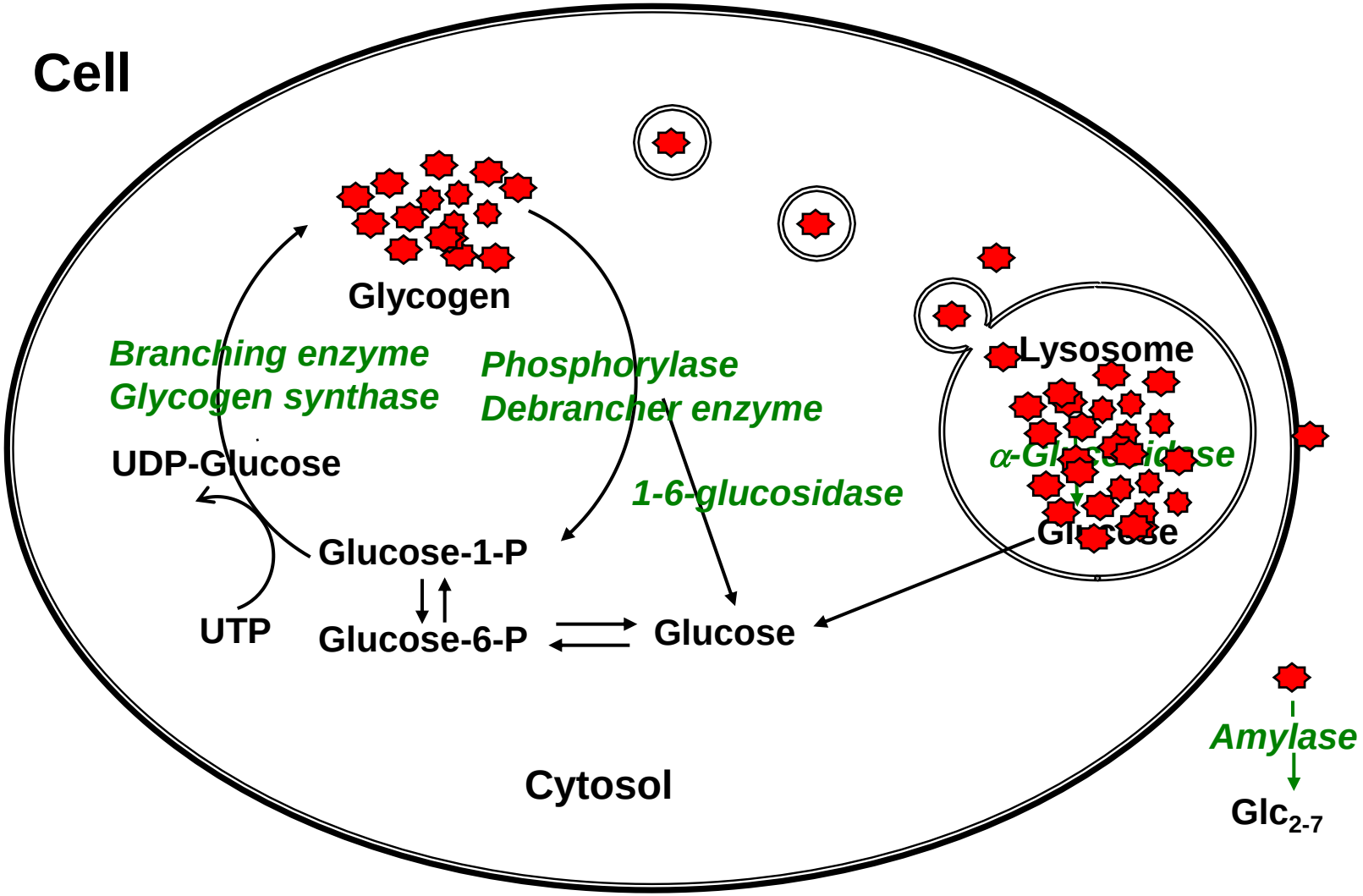
Glycogen Metabolism



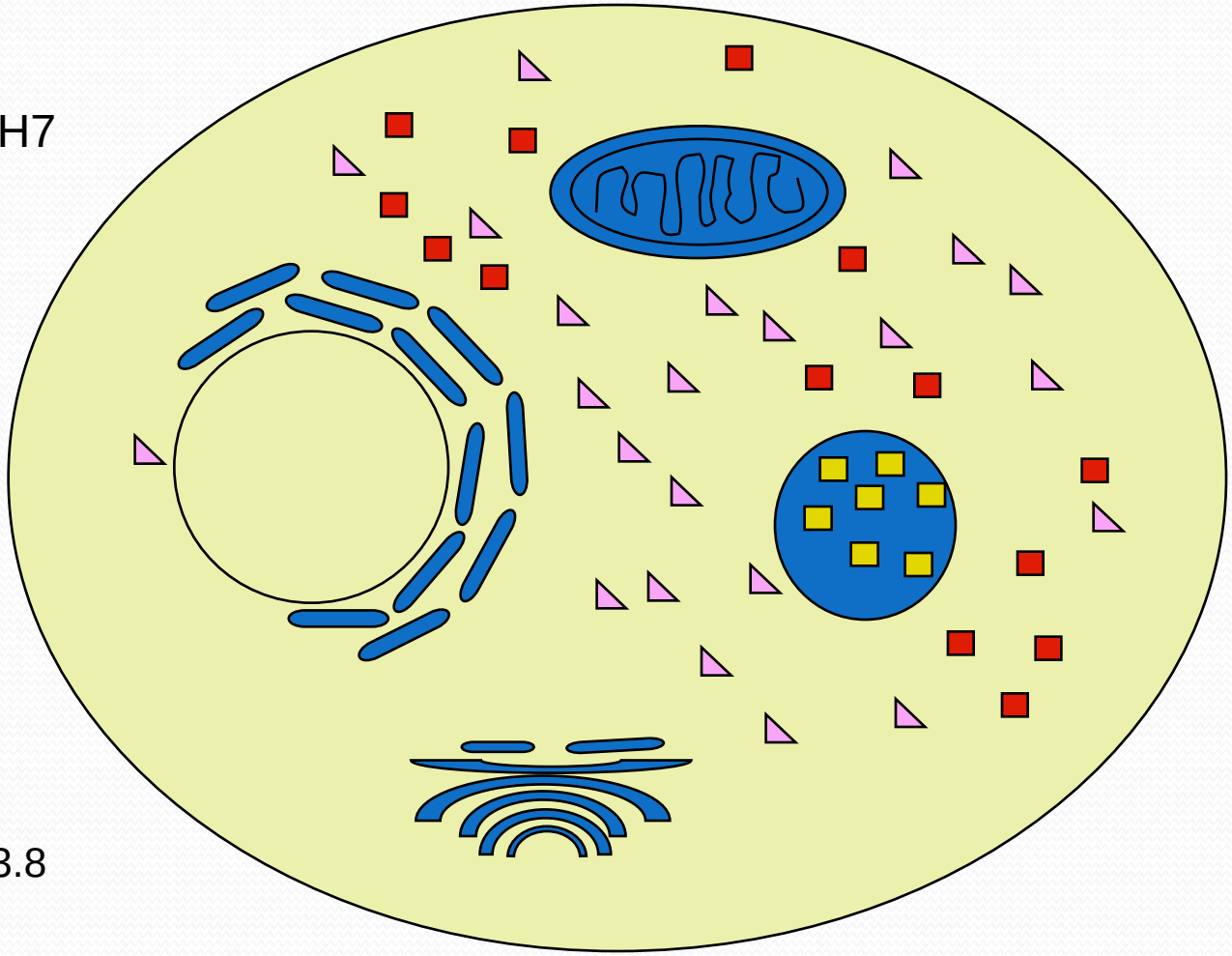
The location of glucosidase enzymes in the cell

Neutral α-glucosidase pH7

Maltase-glucoamylase

Lysosomal acid
α-glucosidase (GAA)
pH3.8 + acarbose

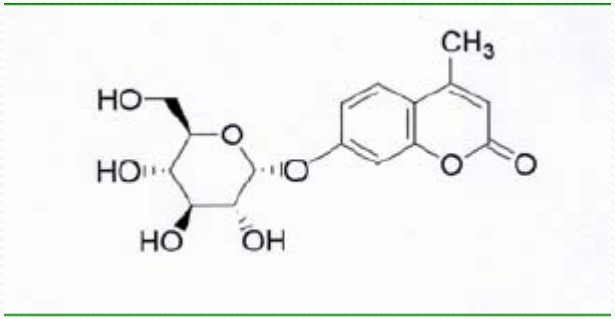
+ Enzyme activity
Measured at pH 3.8



8 week cut-off

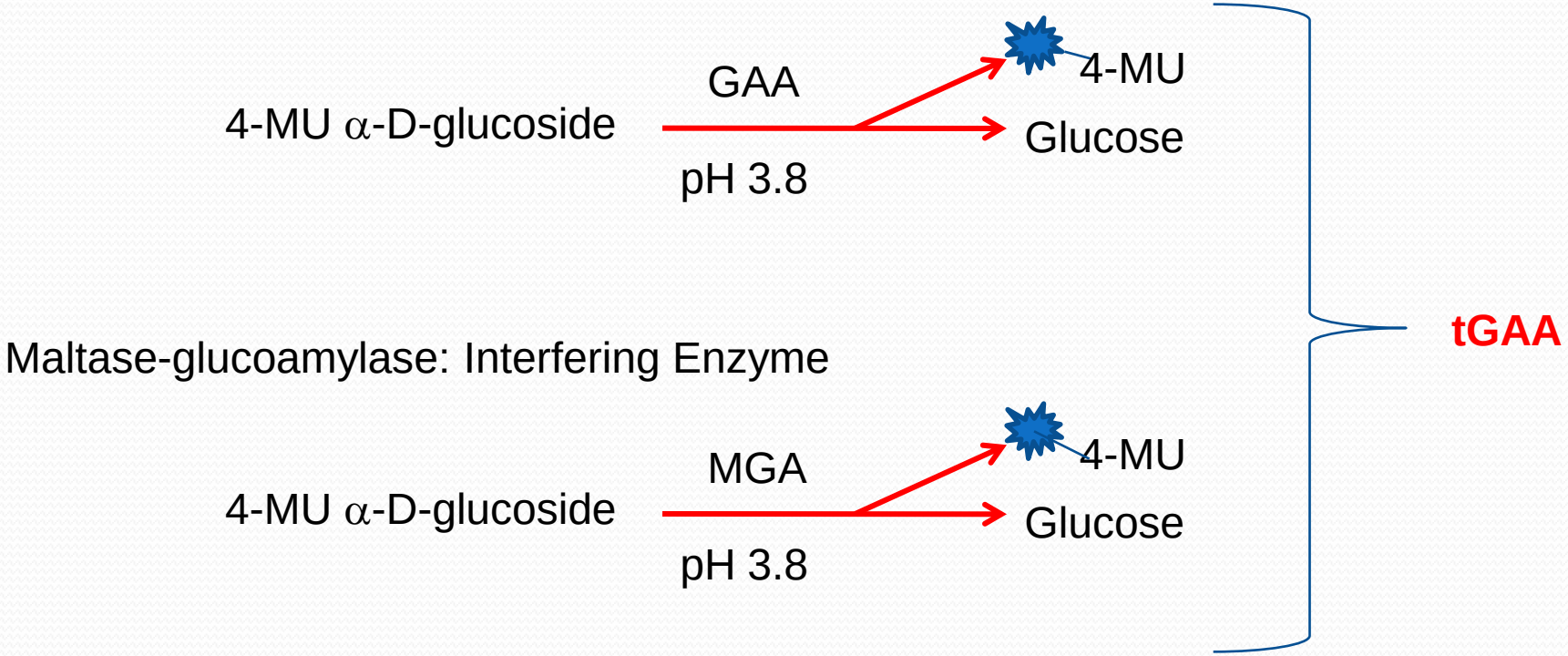
£0

Very Clear information required, including request for Pompe !
Good Quality blood spots – essential – otherwise rejection !

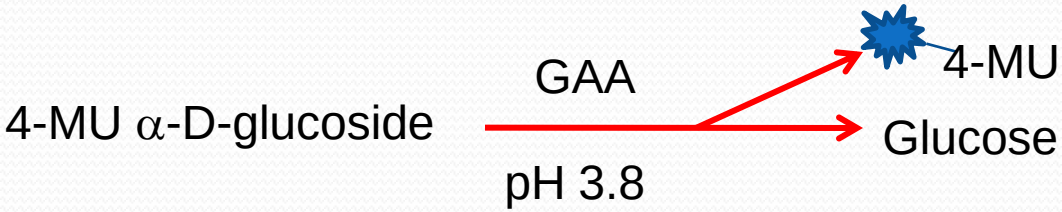


4-methylumbelliferyl α-D-glucoside

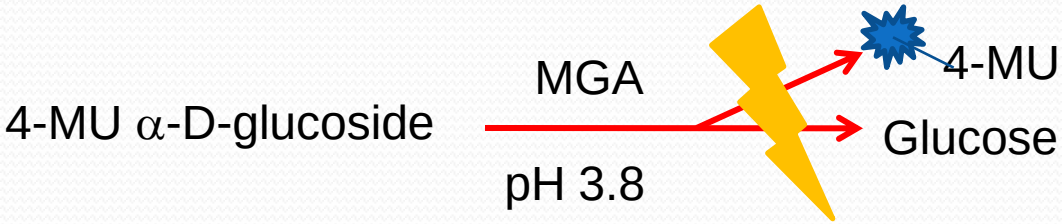
Fluorimetric GAA Assay: 4-methylumbelliferyl α-D-glucoside



Fluorimetric GAA Assay: 4-methylumbelliferyl α-D-glucoside



Maltase-glucoamylase: Interfering Enzyme

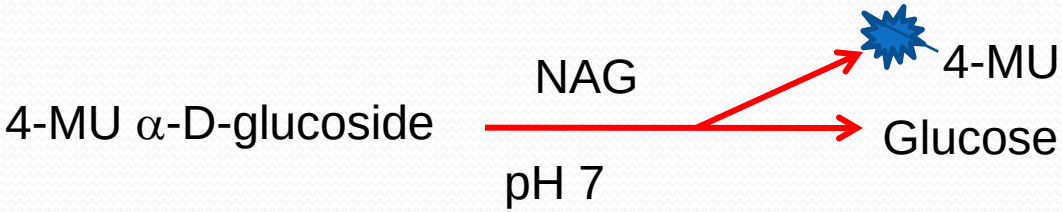


Inhibition with Acarbose

Fluorimetric GAA Assay

- Potential false positives due to specimen deterioration
- Correction for specimen deterioration:
  - Ratio of GAA/tGAA (+/- acarbose)
  - Measurement of other control enzymes

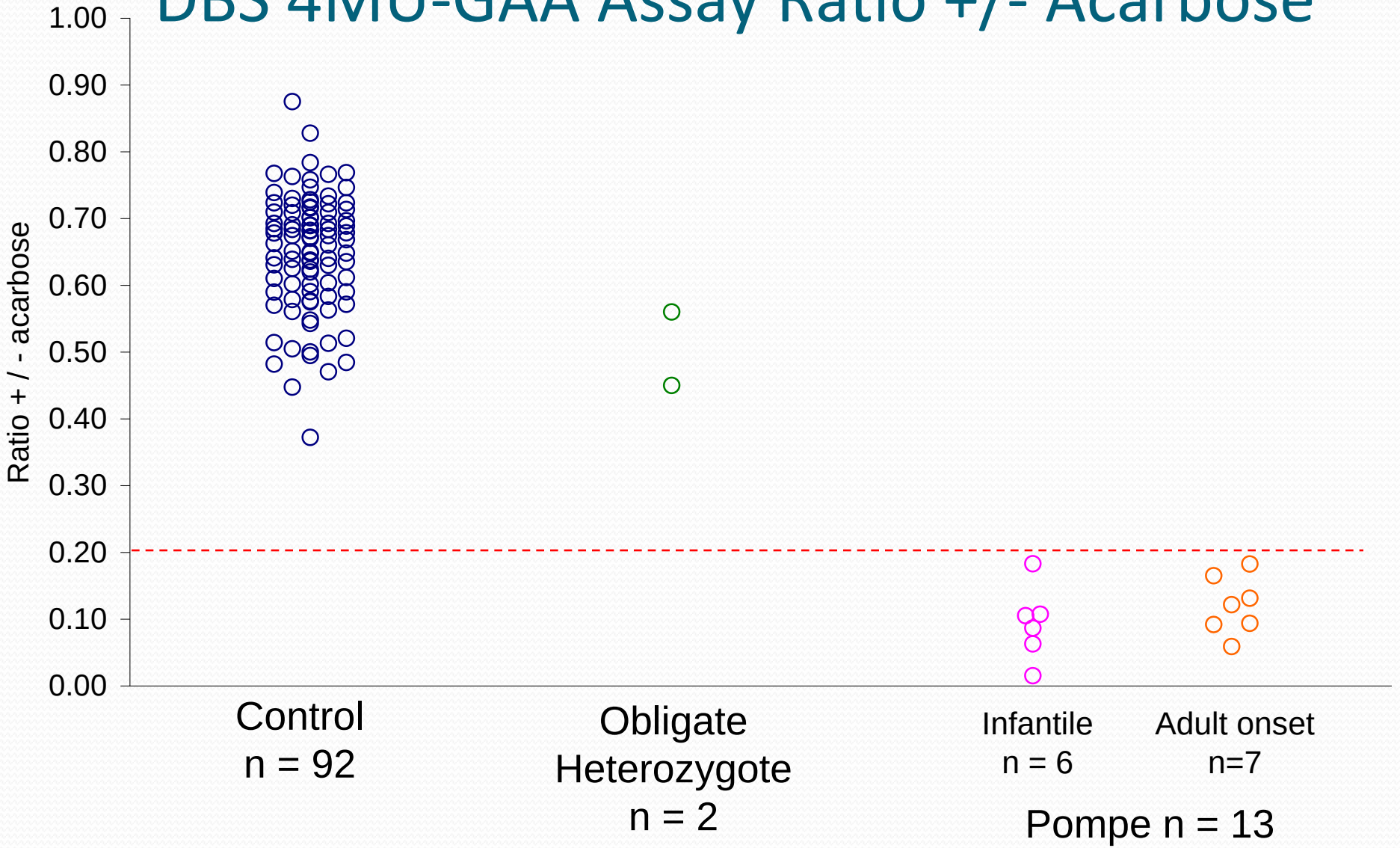
Neutral α-glucosidases: Control Enzyme



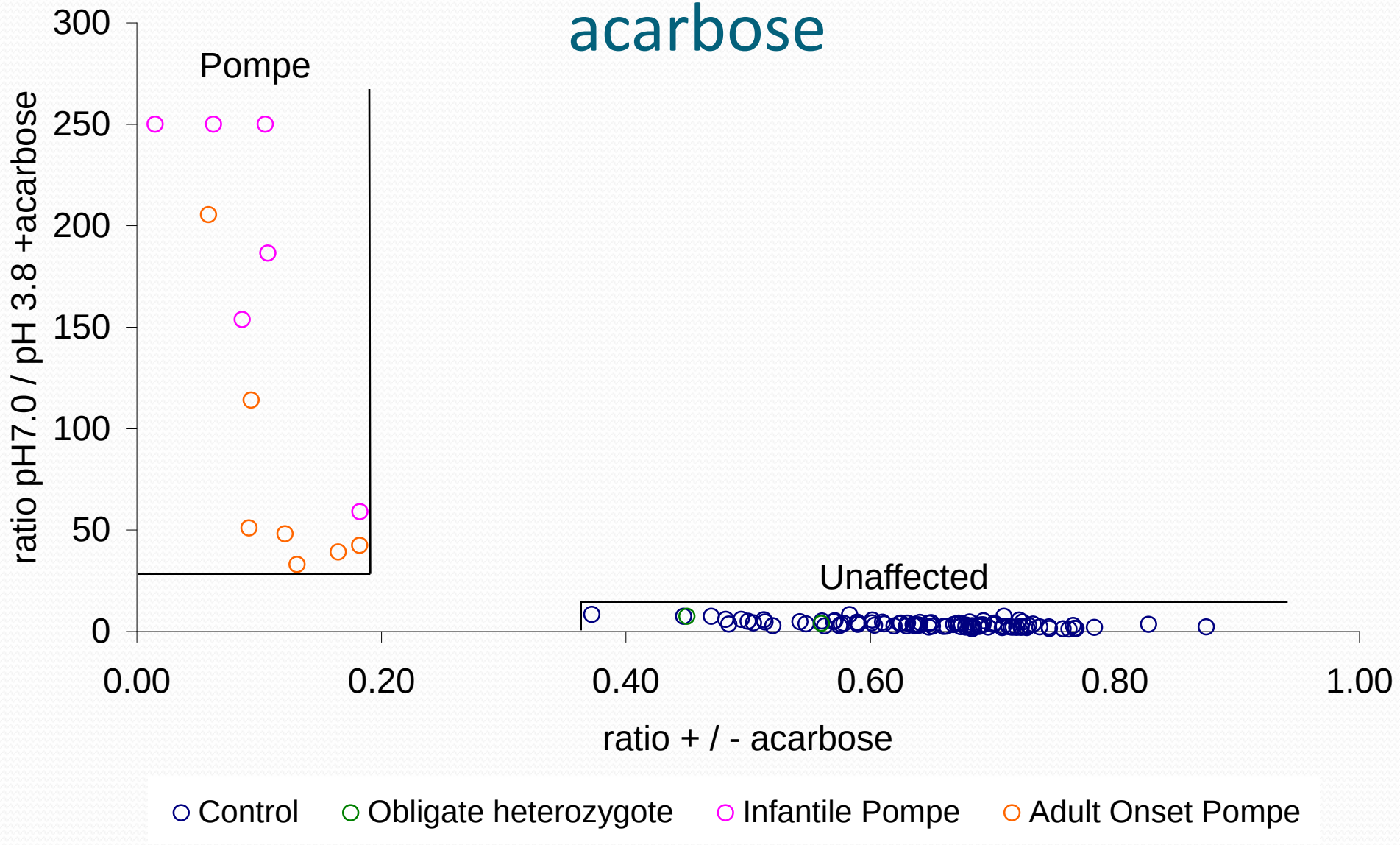
Brief protocol of the DBS Pompe assay

- Extract enzyme from blood spot with water
- Set up using the Tecan Robotic pipetting station the three assay condition in a 96 well plate
- All wells contain substrate and either:
  - pH 3.8 buffer + acarbose
  - pH 3.8 buffer
  - pH 7.0 buffer
- Add sample to test wells to start the reaction and add sample to blank wells after the reaction has terminated.
- After 20 hours incubation, stop reaction. Set up a calibration curve then read fluorescence.

DBS 4MU-GAA Assay Ratio +/- Acarbose



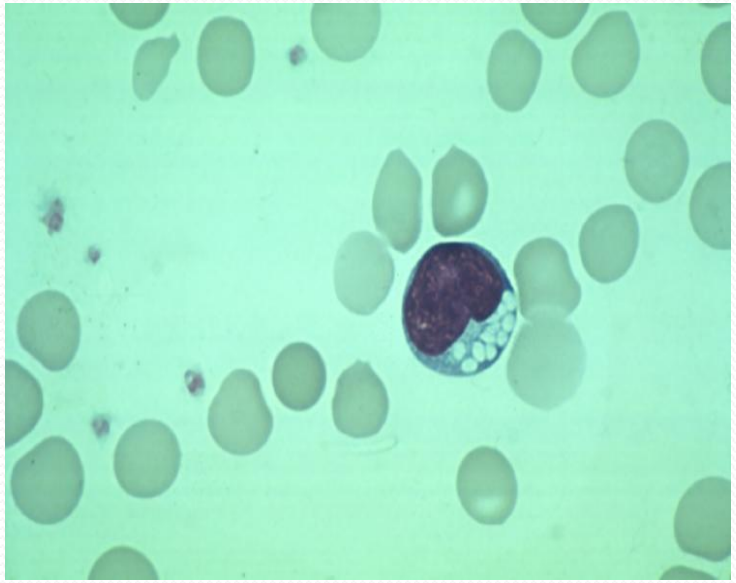
DBS 4MU-GAA Assay. Ratio pH7.0 / pH3.8 + acarbose



Pompe Disease – Post DBS Investigations

- Pseudodeficiency - Follow up testing required
  - Vacuolated lymphocytes
  - Urine tetrasacharride (Glc_4)
  - Mutation analysis

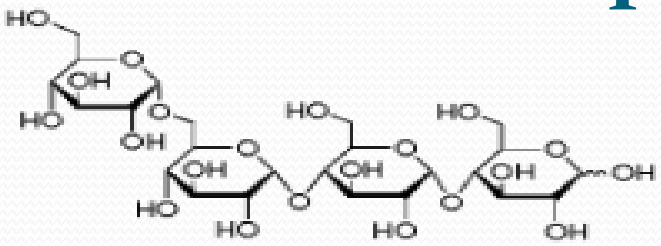
Vacuolated Lymphocytes



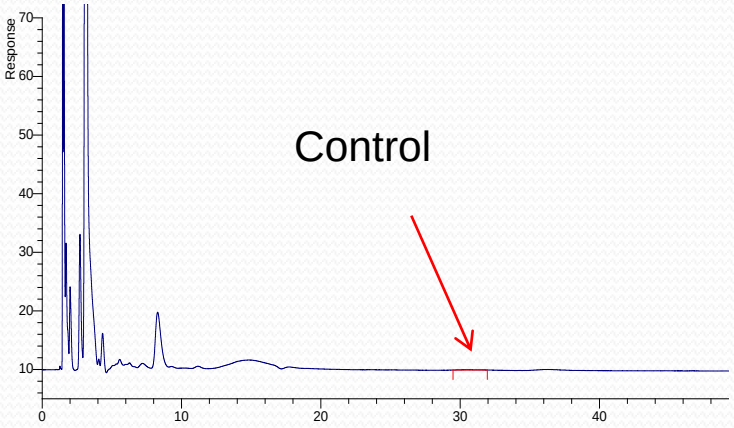
- Range of metabolic diseases lead to cytoplasmic vacuolation
- Pompe Disease
- Less frequently seen in adult form

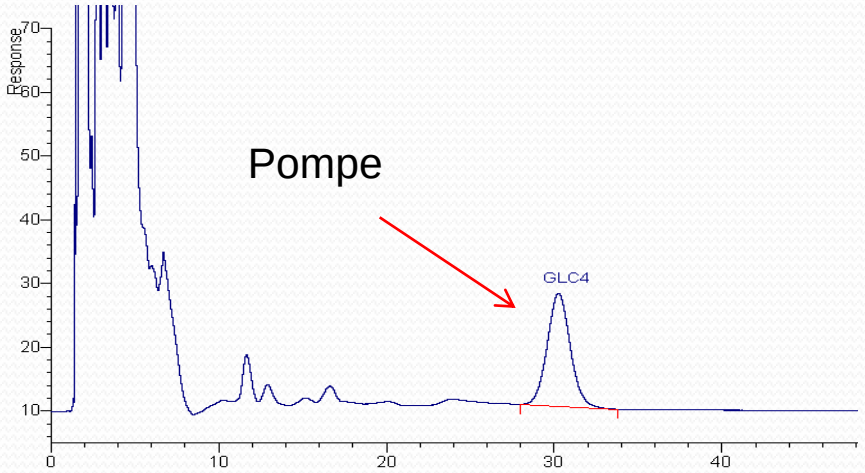
Anderson et al., 2005

Tetrasaccharide (Glc_4) as a Biomarker for Pompe Disease



Glc_4: from glycogen

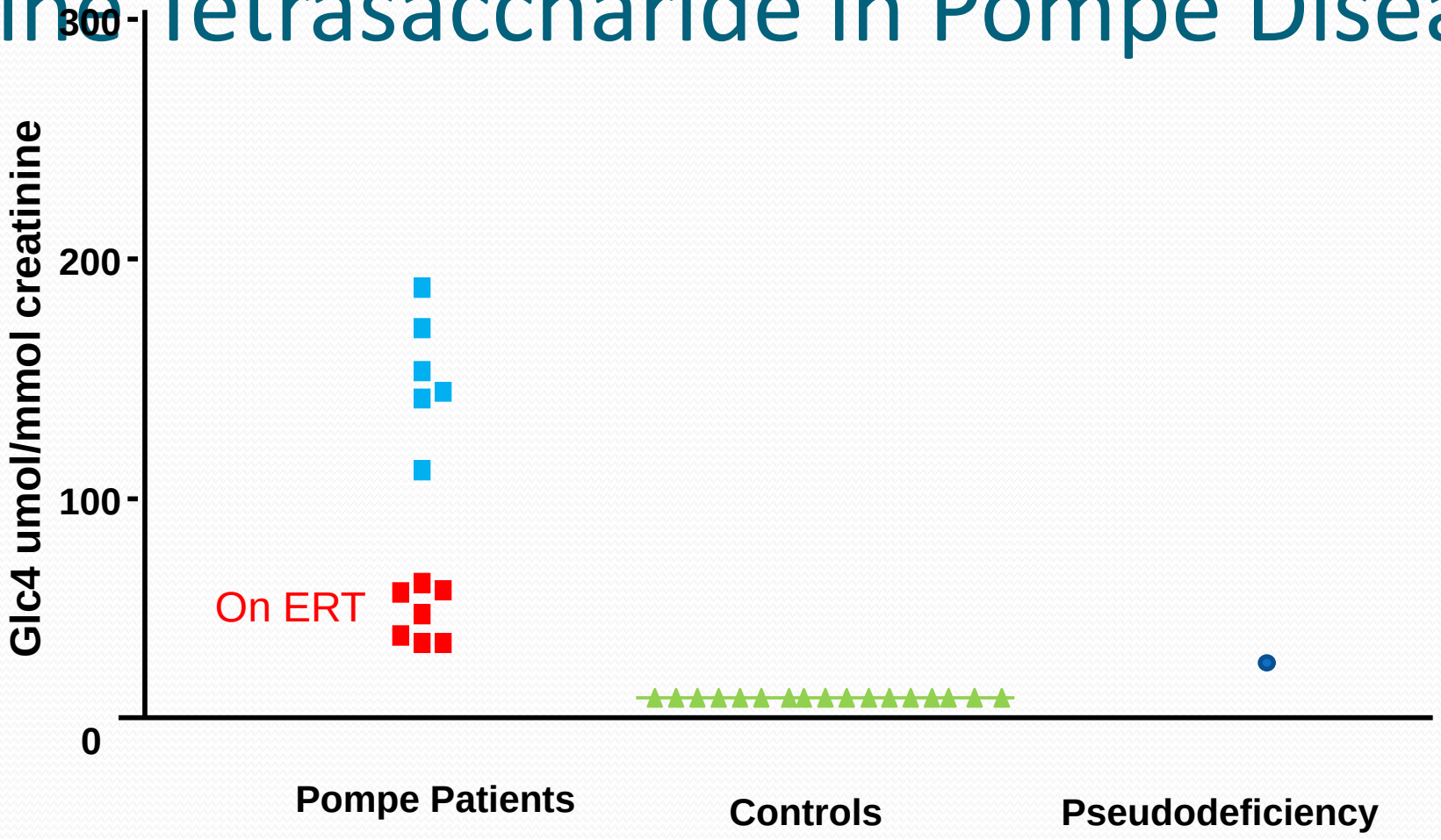




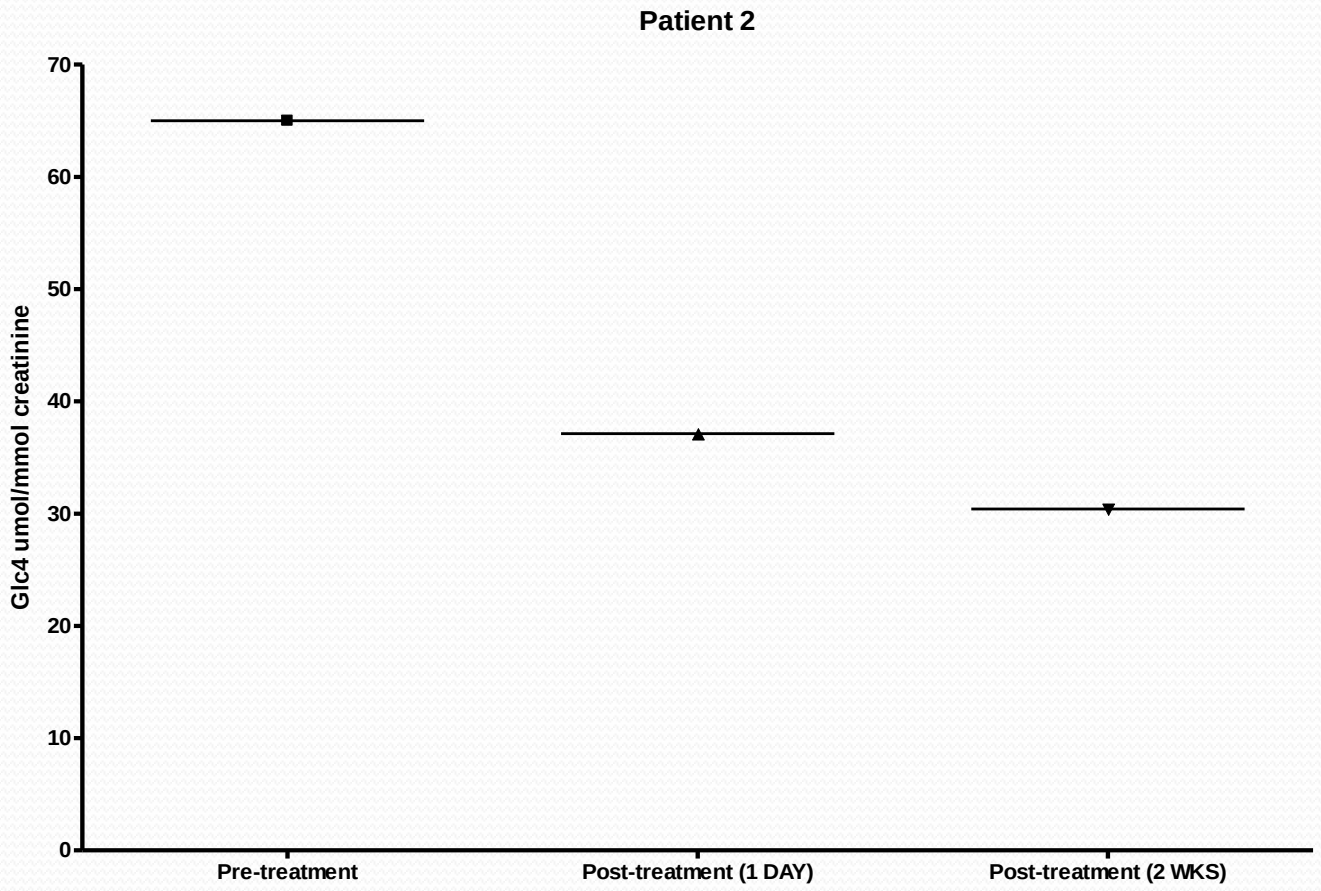
- **Urine Glc_4 reflects clinical response to treatment ?**

An et al. (2005) Molec Genet Metab 85, 247.

Urine Tetrasaccharide in Pompe Disease



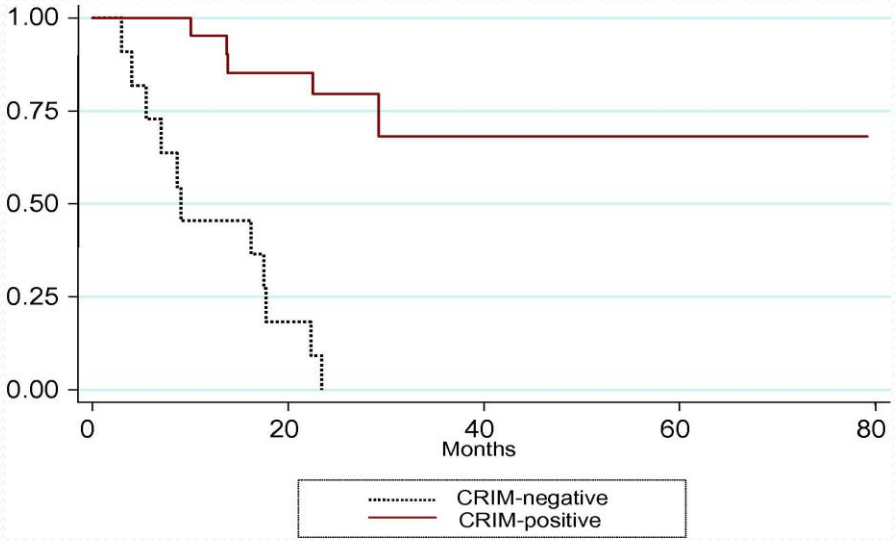
Urine Tetrasaccharide in Pompe Disease – Response to ERT



CRIM Analysis

- Cross-reacting immunologic material

- CRIM +VE patients tend to show better clinical response to ERT Klinge et al 2005, Amalfitano et al 2001, Kishani etal., 2010

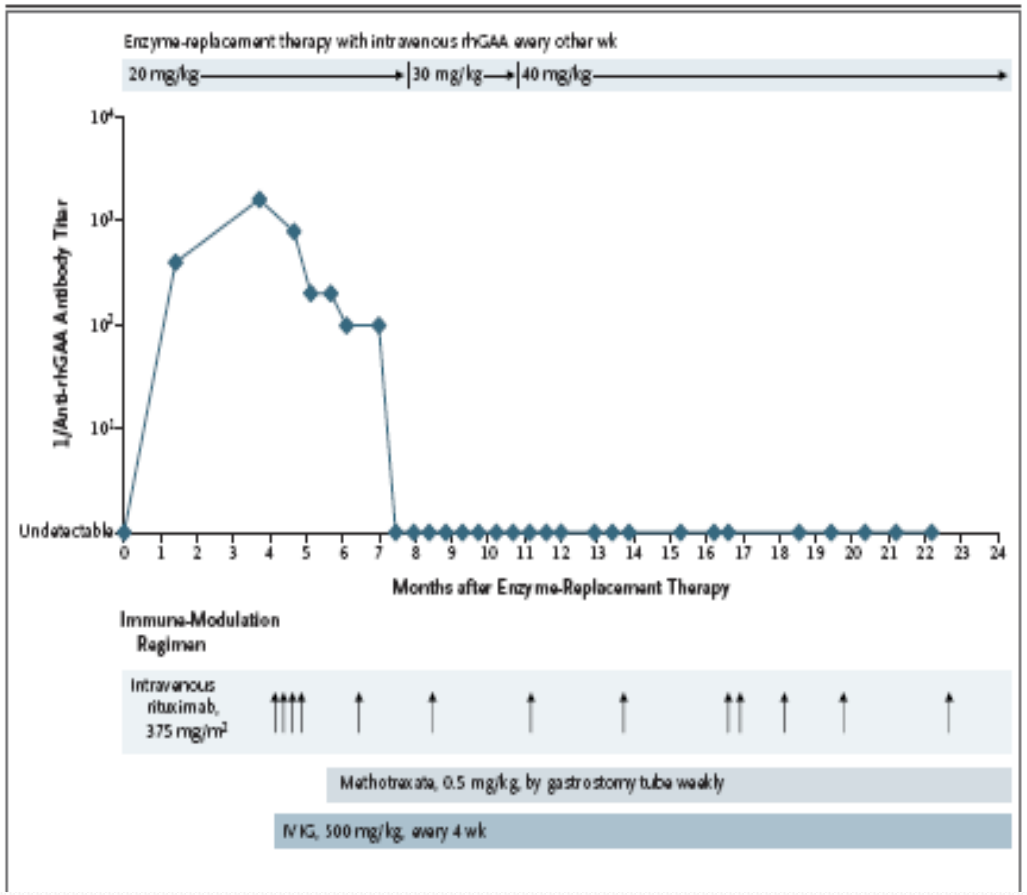


Kaplan-Meier curve of ventilator-free suvival of the CRIM-negative (n = 11) and CRIM positive (n=21) patients. (Kishnani *et al.*, 2010)

CRIM Analysis

- Currently detection of CRIM is in cultured fibroblasts by Western blotting
- Only available in a few laboratories world wide
- Long TAT: fibroblasts required (6 - 8 weeks to grow to confluence)
- In Development – CRIM analysis in white cells

Immune Modulation



Mendelsohn et al., 2009

Pompe – Diagnosis & Monitoring

Enzymology
Vacuolated Lymphocytes
Genetics
Tetrasaccharide
(CRIM)

Acknowledgements

- Katie Bainbridge
- Vicki Manwaring
- Helen Prunty
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