

# Pompe Disease - Biochemical Investigation and Monitoring

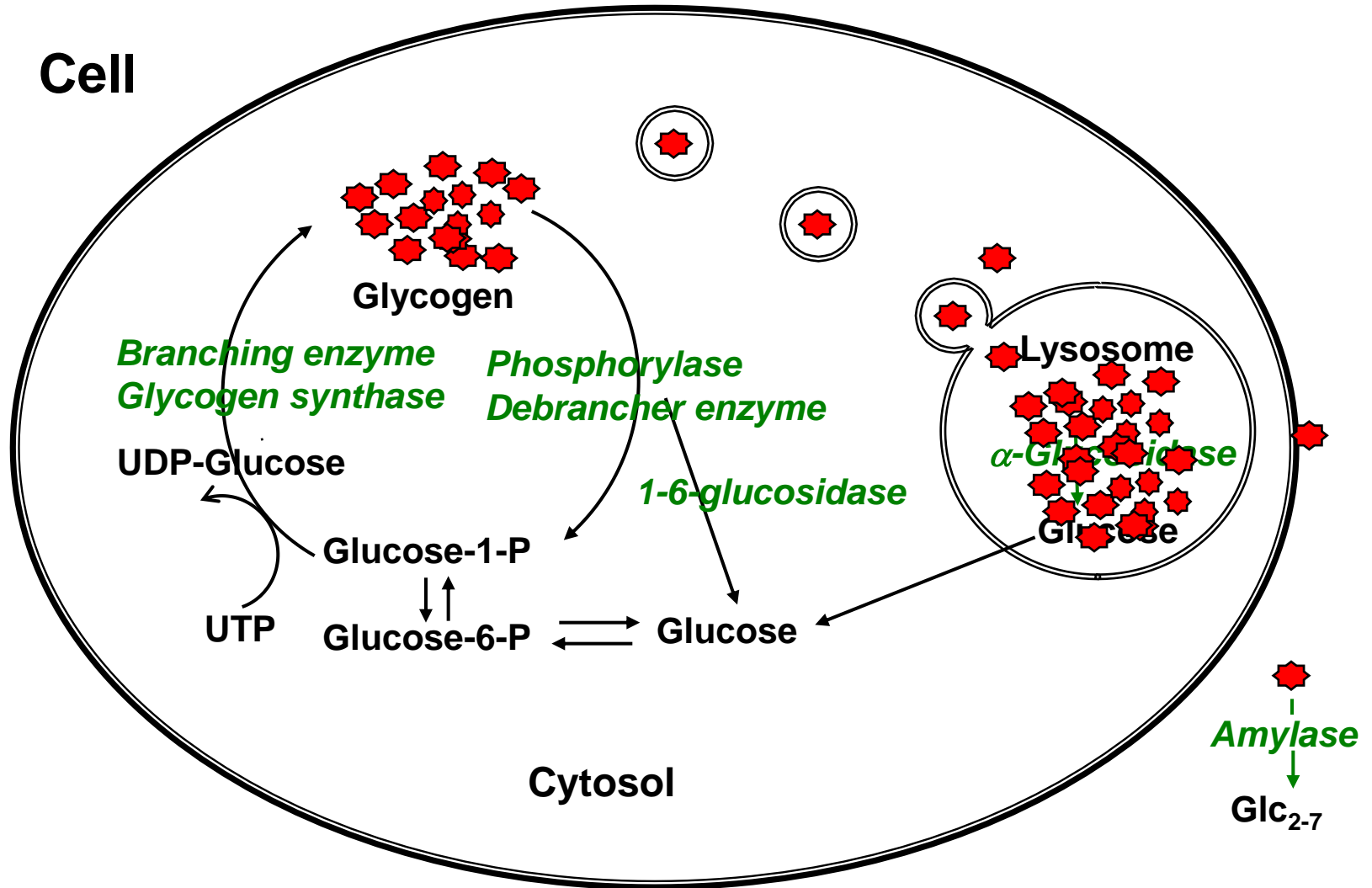
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Great Ormond Street  
Hospital for Children



NHS Trust

# Glycogen Metabolism



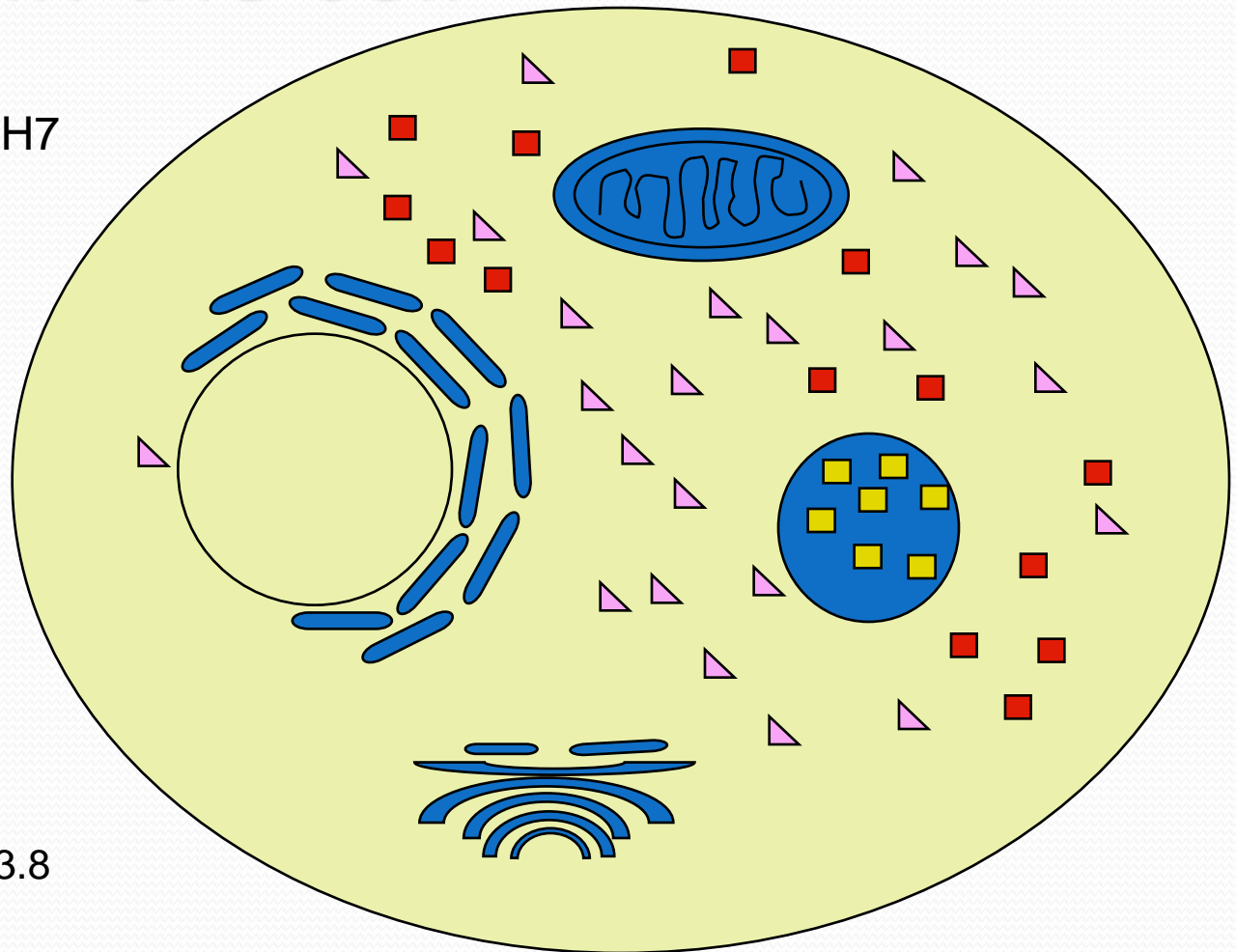
# The location of glucosidase enzymes in the cell

▲ Neutral  $\alpha$ -glucosidase pH7

■ Maltase-glucoamylase

■ Lysosomal acid  
 $\alpha$ -glucosidase (GAA)  
pH3.8 + acarbose

■ + ■ Enzyme activity  
Measured at pH 3.8



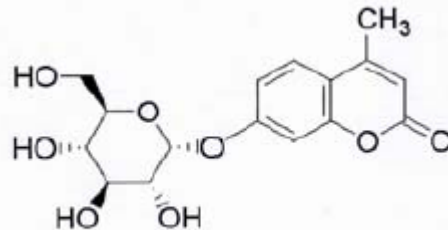
<b>NEWBORN'S</b>		<b>NHS</b>		0900394801		0900394801		Empty Dates 12-2012	
Baby's NHS No. [Barcode]		NHS No: 547 563 3118		DATE OF SPECIMEN 0 6 0 9 0 9		[Red Spot 1]		1	
SURNAME: JOHNSON Laura		01/03/2009 Female		is this a repeat (Y)?		YES NO ✓		[Red Spot 2]	
FORENAMES: [Barcode]		19 Scoopsgate Road Berwick TD15 1BW		How baby fed in last 24 hours (Y)?		YES NO ✓		[Red Spot 3]	
HOME ADDRESS: 3600 (g)		Rank: 1/1		If yes, detail last transaction		YES NO		[Red Spot 4]	
POSTCODE: [Barcode]		40+2 weeks		is the baby in hospital (Y)?		YES NO		4	
Dr. AS Smith		25/03/1967		If yes, current hospital and ward		Berwick Hospital, Ward 09		Johnson	
Dr. AS Smith		1AA222		Printed 01/03/2009		[Barcode]		Summe	
G.P. PRACTICE NAME: UKNSPC Clinic		G.P. PRACTICE ADDRESS: 130 Socklens Road Berwick		MOTHER'S FULL NAME: Christina Johnson		MOTHER'S RES NUMBER: 1 2 3 4 5 6 7 8 9 0		PARENT TELEPHONE NUMBER: 012 5099 1234	
G.P. PRACTICE CODE: 1 A   A   2   2   2		ALTERNATIVE SURNAME: N/A		MOTHER'S HOME: [Barcode]		COMMENTS: Family history eg. Mother's carrier status (National HD Code, HD-Outcome code) (temporary address)		Mother's HBE: [Barcode]	
HOSPITAL OF BIRTH: Berwick		TEL. NO. OF PERSON TAKING SAMPLE: 075 5432 2345		NAME OF PERSON TAKING SAMPLE (PRINT): Sr. Lancelot					



**8 week cut-off**

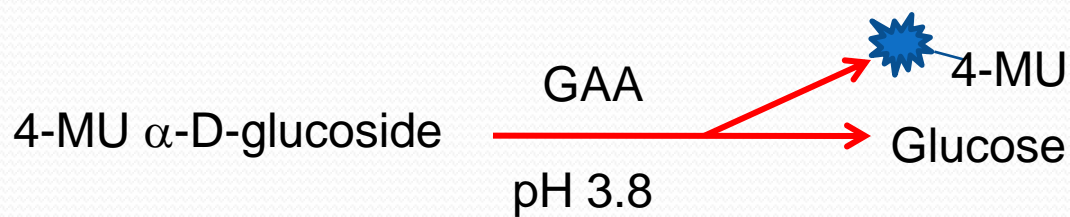
£0

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

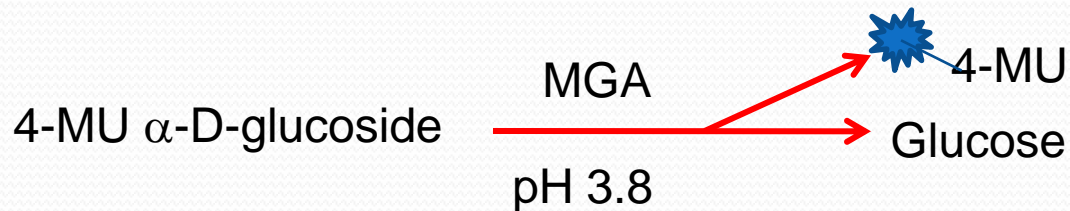


4-methylumbelliferyl  $\alpha$ -D-glucoside

# Fluorimetric GAA Assay: 4-methylumbelliferyl $\alpha$ -D-glucoside

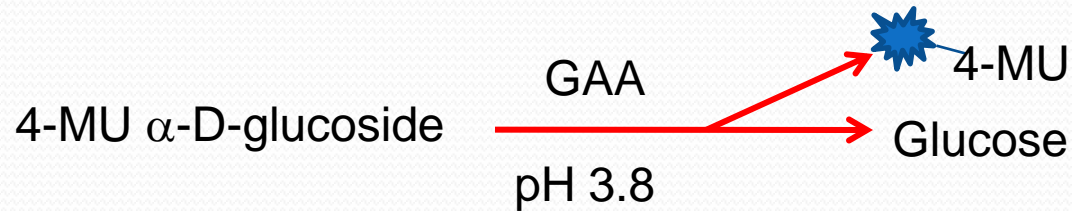


Maltase-glucoamylase: Interfering Enzyme

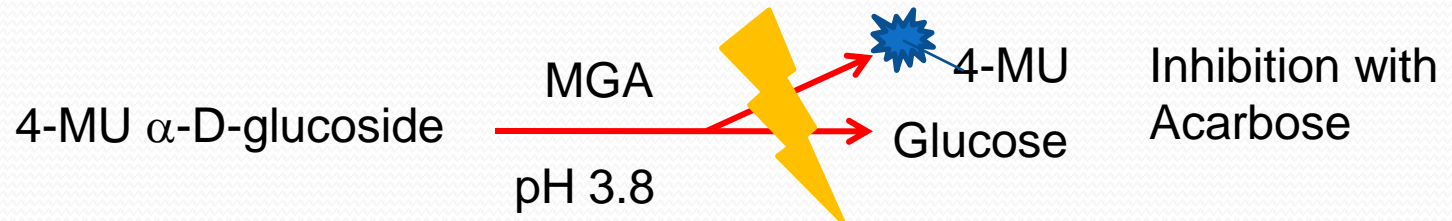


**tGAA**

# Fluorimetric GAA Assay: 4-methylumbelliferyl $\alpha$ -D-glucoside



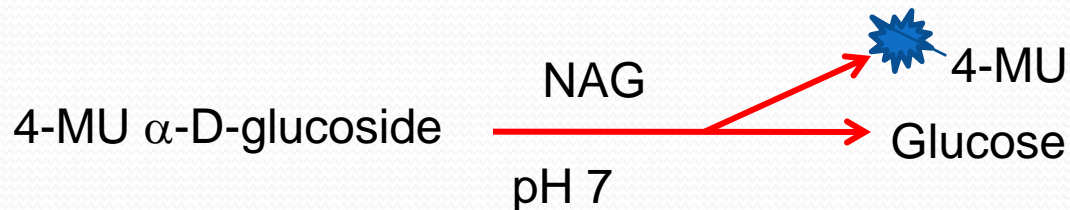
Maltase-glucoamylase: Interfering Enzyme



# 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  $\alpha$ -glucosidases: Control Enzyme

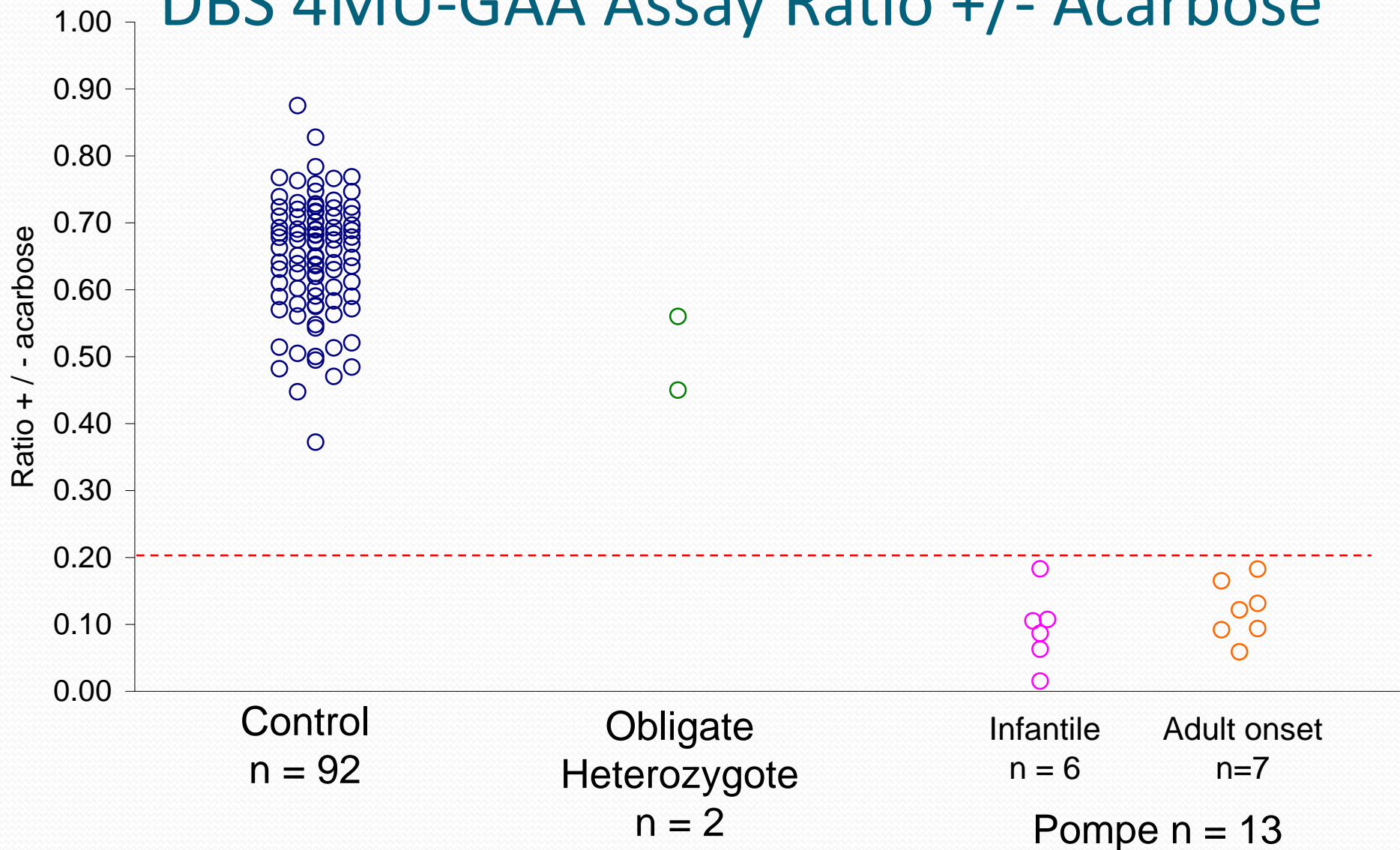


# Brief protocol of the DBS Pompe assay

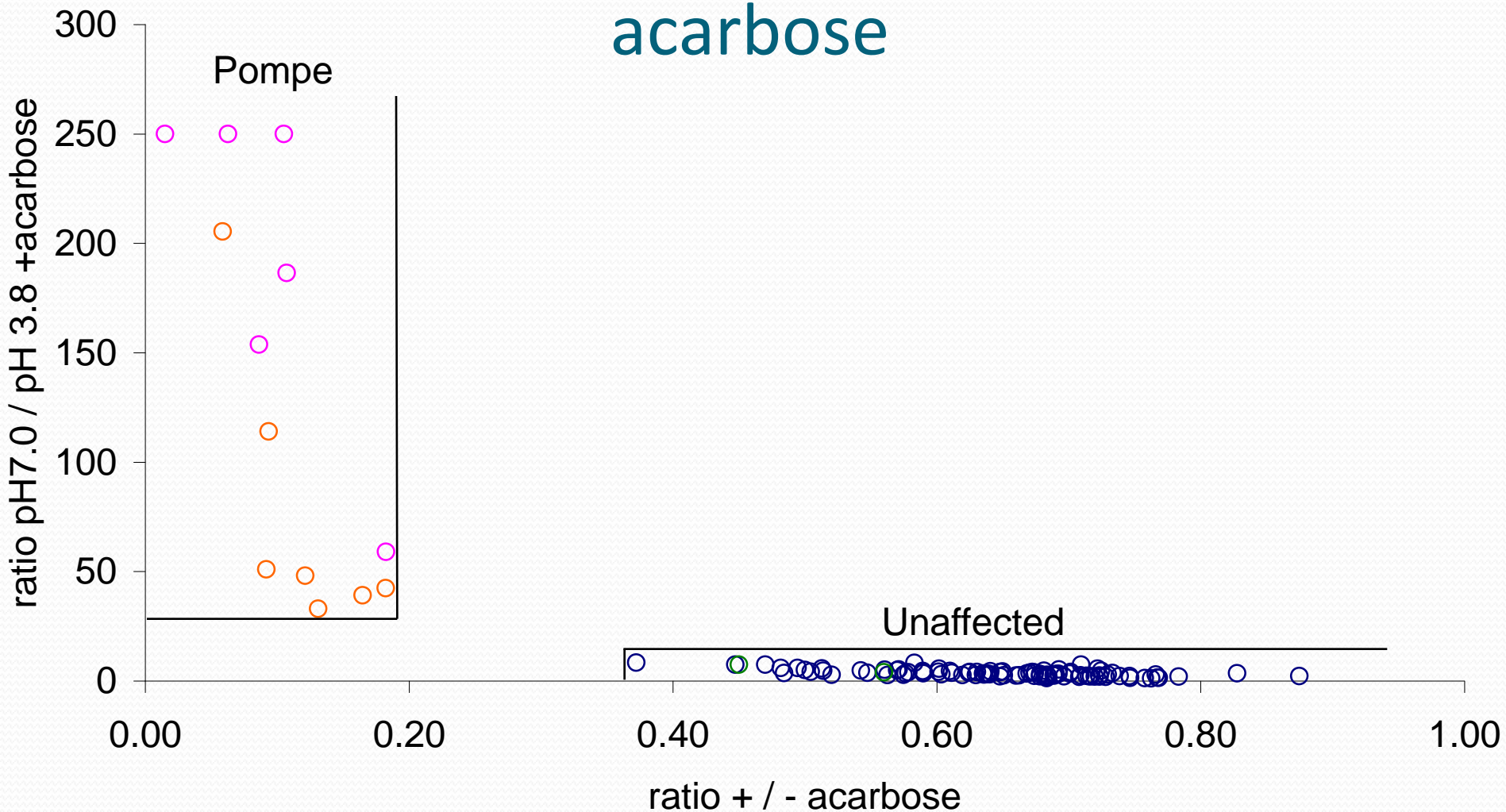
- Extract enzyme from blood spot with water
- Set up using the Tecan Robotic pipetting station the three assay condition in a 96 well plate
- All wells contain substrate and either:
  - pH 3.8 buffer + acarbose
  - pH 3.8 buffer
  - pH 7.0 buffer
- Add sample to test wells to start the reaction and add 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

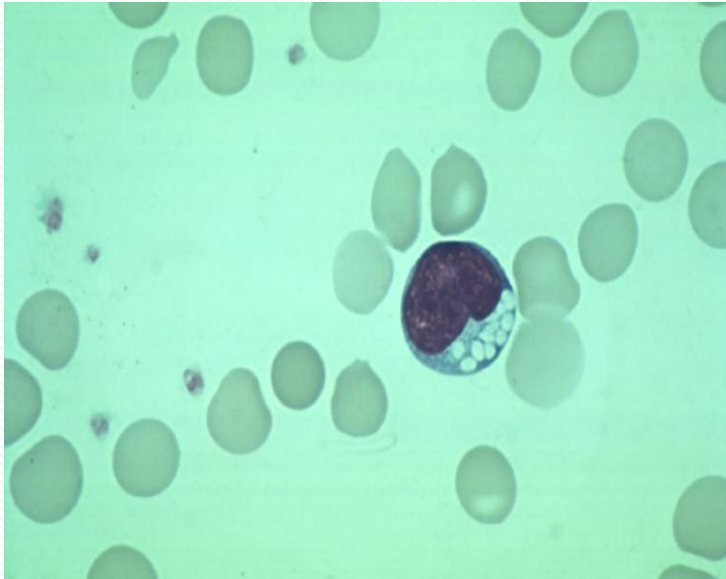


○ Control    ○ Obligate heterozygote    ○ Infantile Pompe    ○ Adult Onset Pompe

# Pompe Disease – Post DBS Investigations

- Pseudodeficiency - Follow up testing required
  - Vacuolated lymphocytes
  - Urine tetrasacharride ( $\text{Glc}_4$ )
  - Mutation analysis

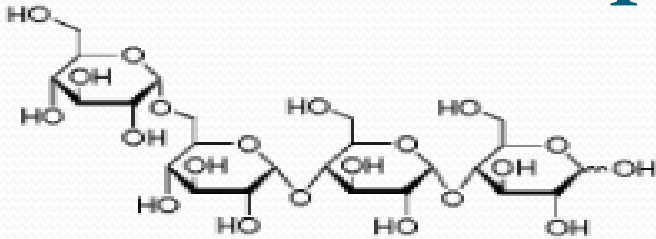
# Vacuolated Lymphocytes



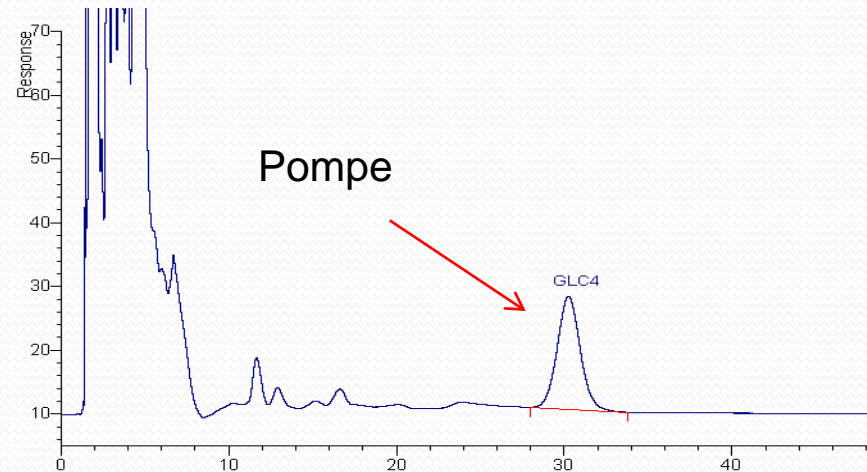
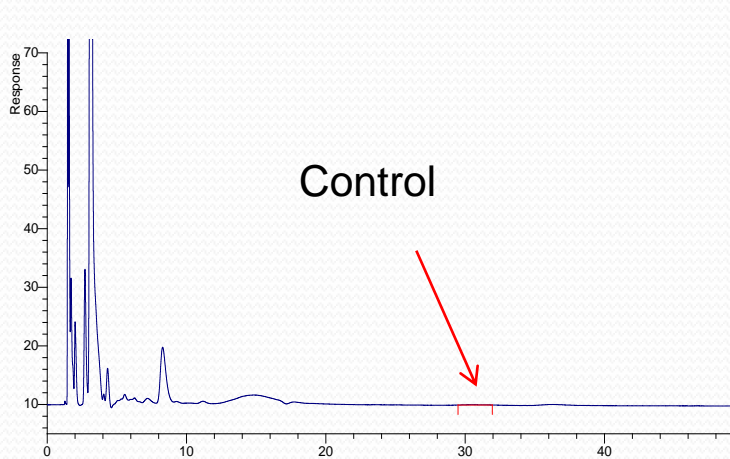
- Range of metabolic diseases lead to cytoplasmic vacuolation
- Pompe Disease
- Less frequently seen in adult form

Anderson et al., 2005

# Tetrasaccharide (Glc<sub>4</sub>) as a Biomarker for Pompe Disease



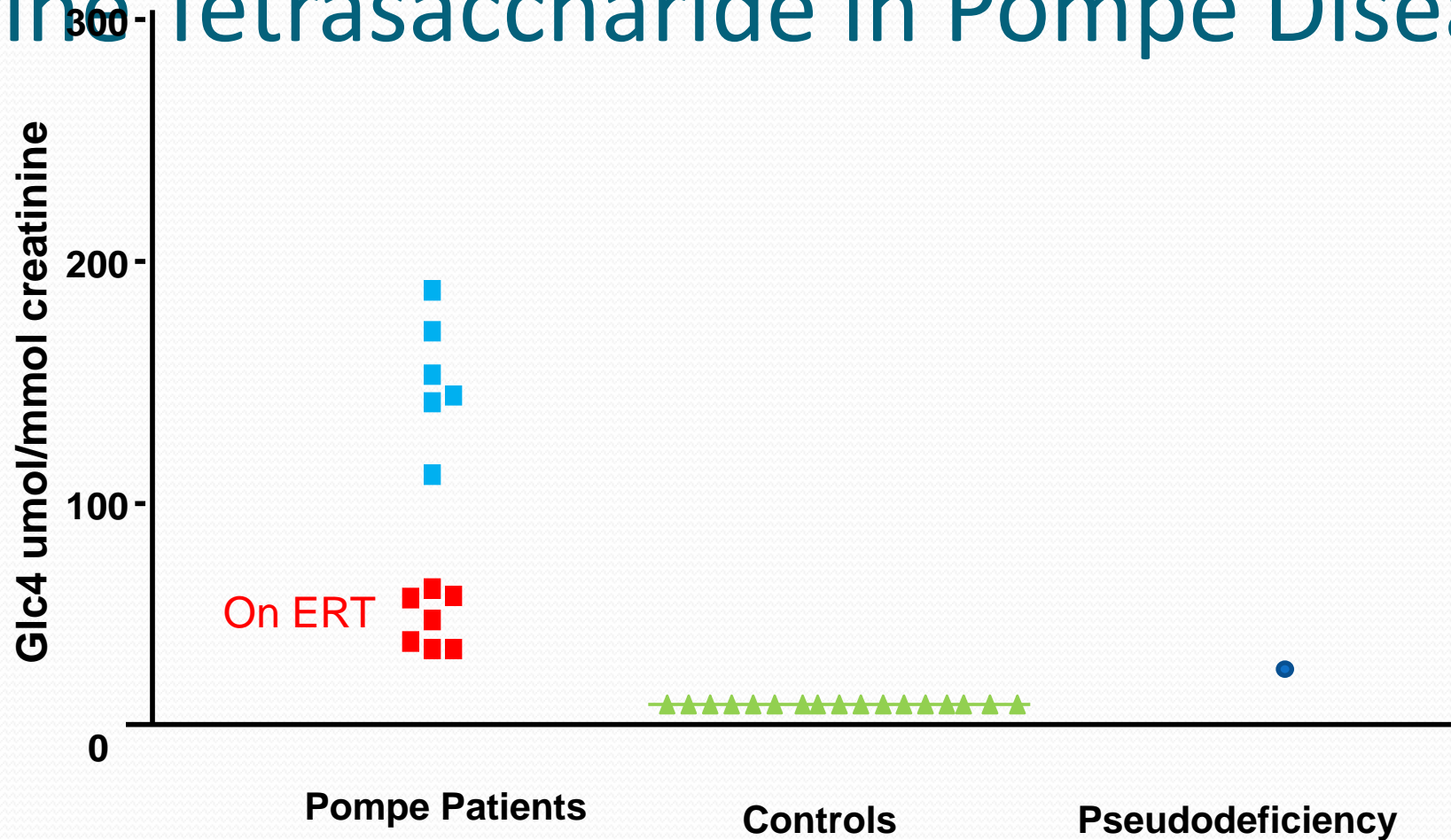
Glc<sub>4</sub>: from glycogen



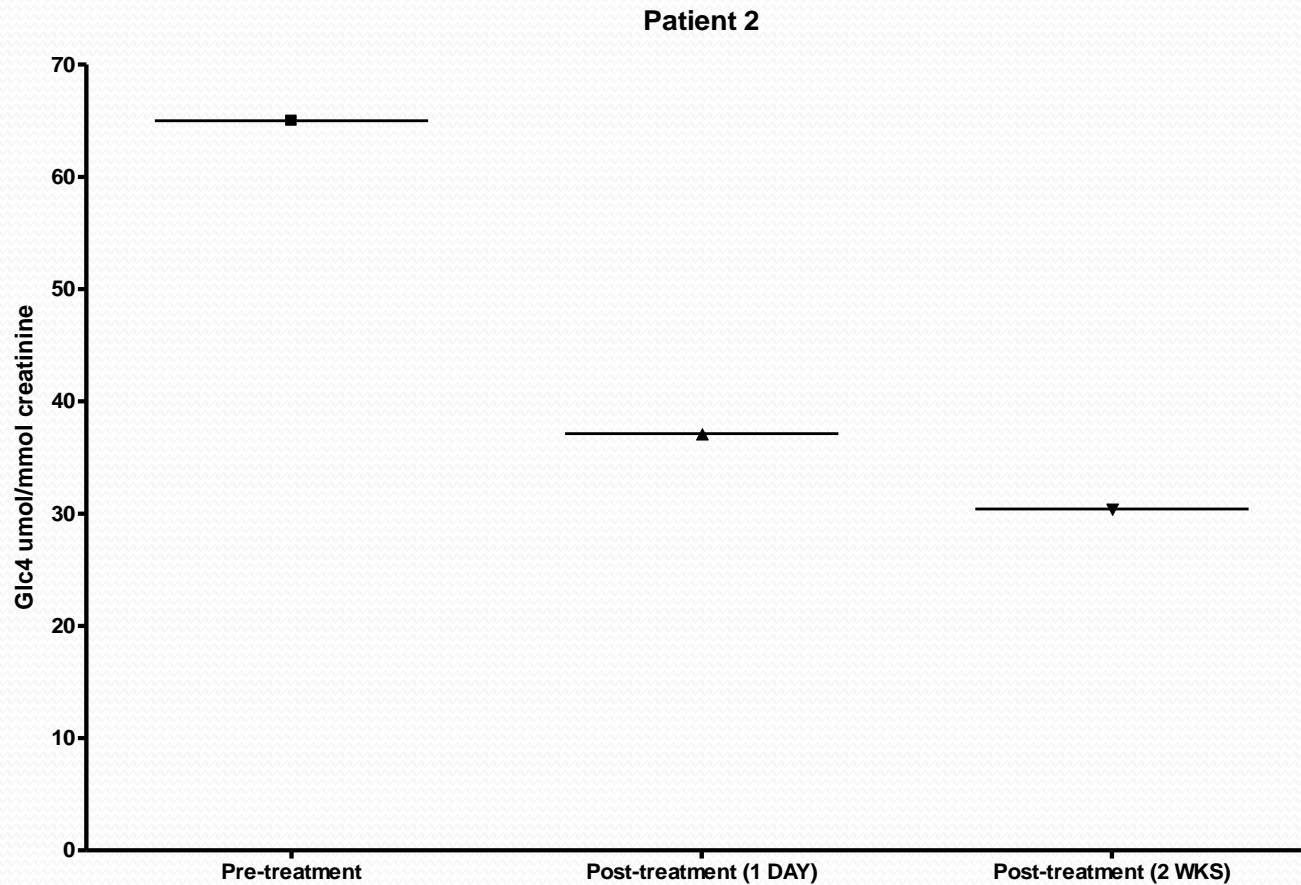
- Urine Glc<sub>4</sub> reflects clinical response to treatment ?

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

# Urine Tetrasaccharide in Pompe Disease

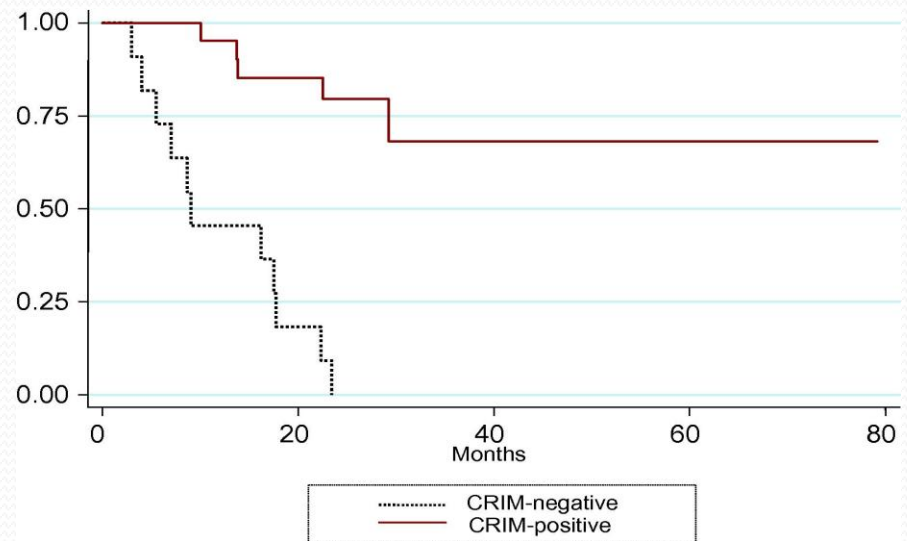


# Urine Tetrasaccharide in Pompe Disease – Response to ERT



# CRIM Analysis

- **C**ross-**r**eacting immunologic **m**aterial
- CRIM +VE patients tend to show better clinical response to ERT Klinge et al 2005, Amalfitano et al 2001, Kishani et al., 2010



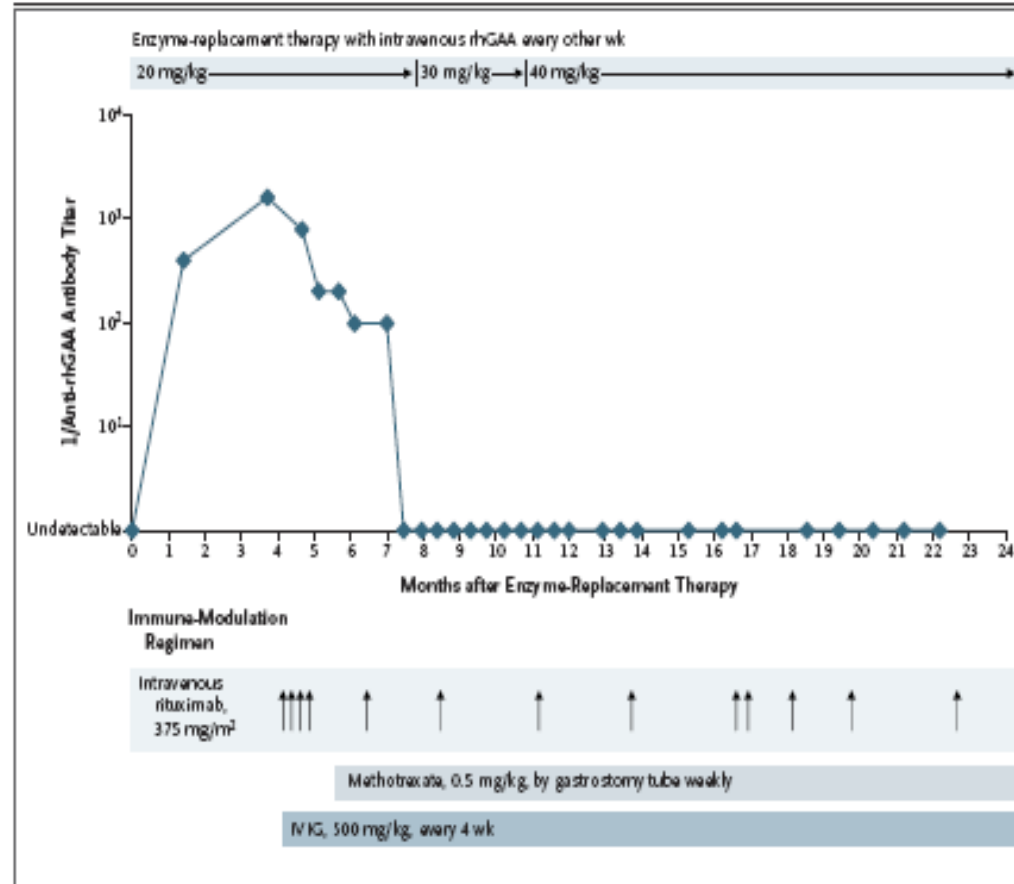
Kaplan-Meier curve of ventilator-free survival of the CRIM-negative (n = 11) and CRIM positive (n=21) patients. (Kishnani *et al.*, 2010)



# 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



# Pompe – Diagnosis & Monitoring



Enzymology  
Vacuolated Lymphocytes  
Genetics  
Tetrasaccharide  
(CRIM)

# Acknowledgements

- Katie Bainbridge
- Vicki Manwaring
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