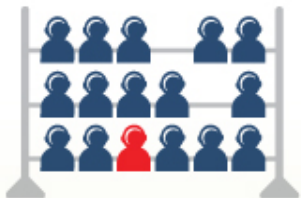




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RARE DISEASE WORKSHOP SERIES

Improving the Clinical Development Process

The use of pathology surrogate markers in Fabry Disease

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Genzyme



Fabry Disease background

- X-linked recessive disorder leading to a deficiency in the lysosomal enzyme α -galactosidase
 - Deficiency leads to the accumulation of the lipid globotriaosylceramide (GL3)
- Disease is observed in male hemizygotes and female heterozygotes (depending on the pattern of Lyonization)
- Signs and symptoms: progressive renal failure, congestive heart failure, cardiac arrhythmia, myocardial infarction, angiokeratomas, hypohydrosis, acroparesthesias, stroke.
- Average lifespan of a hemizygous male: 42 years, due to end stage renal failure
- Common etiology: ***Vascular injury***



Why Vascular Injury?

- Endothelial cells are responsible for the maintenance of a continuous anti-coagulant vascular lining
- Injury to these cells attributed to GL3 accumulation leads to vasoconstriction, intravascular inflammation, accelerated atherosclerosis and thrombosis
- Markers of endothelial cell injury and activation (ICAM-1, VCAM-1, P-selectin), leukocyte activation (CD11b) and coagulation (tPA, vWF, PAI) in Fabry patients are present at abnormal levels
- This suggests that vascular endothelial cells of Fabry patients are in a chronic pro-inflammatory and pro-thrombogenic state
- The pathognomonic lesion of Fabry disease, the angiokeratoma, is a vascular lesion



Fabry tissue biopsies: criteria for a good biomarker

- cause of disease clearly understood
 - Deficiency of lysosomal alpha-galactosidase causes an accumulation of globotriaosylceramide (GL3) due to markedly reduced metabolism
- pathophysiology reasonably well understood
 - Accumulation of GL3 in vascular endothelial cells in tissues throughout the body
- drug mechanism of action is direct and known
 - Recombinant alpha-galactosidase targets the mannose-6- phosphate receptor on cells which then delivers the enzyme to the lysosome, thus replacing the deficiency.
- biomarker has direct relationship to disease process
 - GL3 accumulation is a direct result of the enzyme deficiency
- read-out of changes in biomarker are clear
 - Presence and absence of GL3 in tissue biopsy sections are easily visualized pre- and post-treatment



Genzyme's Approach to the Clinical Trial

- Biopsies were taken from kidney, heart and skin
 - Biopsies were fixed in glutaraldehyde, processed into epoxy resin, semi-thin sections were cut and stained with Richardson's stain (methylene blue/Azure II) and examined by light microscopy
 - Ultrathin sections were processed for electron microscopy and examined as confirmation of light microscopy observations.
- Clearance of GL3 from capillary endothelial cells of renal tissue was chosen as the single, primary endpoint
- Clearance of GL3 from capillary endothelial cells of heart and skin were secondary endpoints.
- The clearance of GL3 from other cell types was also assessed



Primary Efficacy Endpoint

- **Morphologic assessment of GL-3 inclusions in renal interstitial capillary endothelium**
 - Three independent renal pathologists
 - Blinded to treatment assignment and pre/post biopsy sampling
 - Light microscopy assessment
 - 0 (none) to 3 (severe) severity scale
- **Percentage of patients with 0 scores**



Capillary Endothelial Cell Grading Criteria

Applied to the interstitial capillaries of the heart and kidney, and capillaries of the papillary dermis

0	None or Trace, Normal	Majority of vessels are clear of inclusions: minority of vessels may contain single or isolated granules; Overall impression of minimal or trace involvement.
1	Mild	Almost all clearly defined vessels contain at least a single inclusion: isolated, randomly distributed or occasional small loose clusters; rarely protruding into lumen.
2	Moderate	Multiple visible granules in most capillaries; small clusters of individually identified inclusions occasional lumen bulging.
3	Severe	Coalescence of inclusions into small masses often bulging into lumen, several sites of inclusions around vessel periphery, majority of clearly identified vessels contain numerous inclusions.

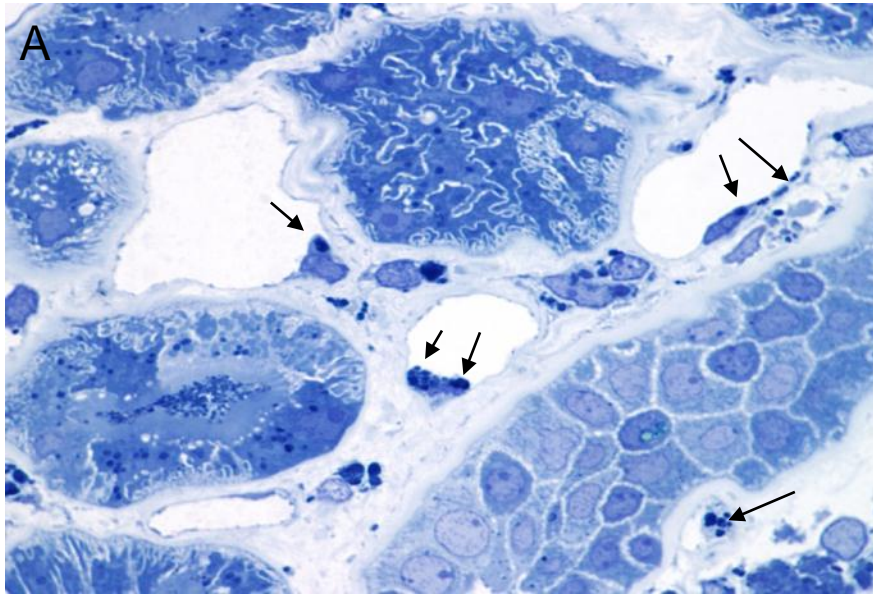


Kidney Histology Endpoints

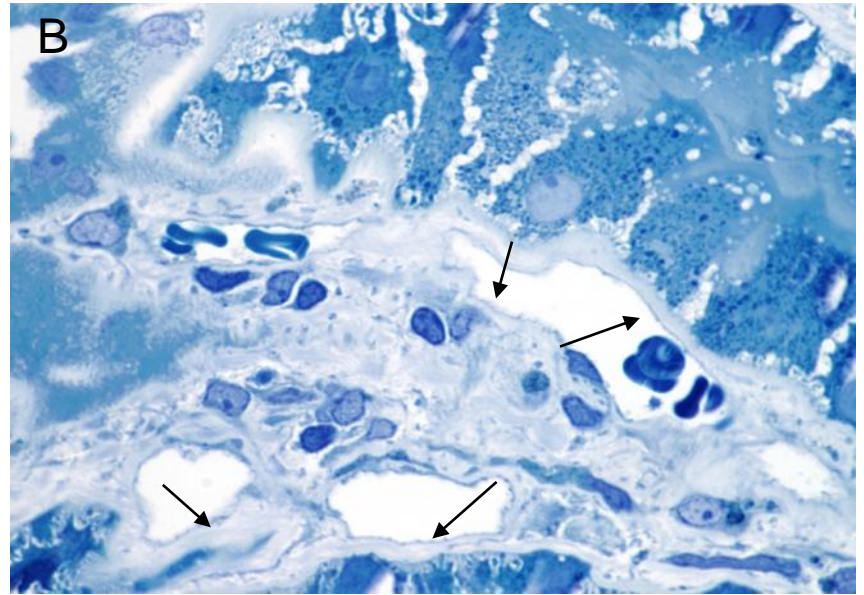
- **Kidney Interstitium**
 - **Interstitial capillary endothelium - 1° endpoint**
 - Interstitial cells (fibroblasts and phagocytic cells)
 - Arterioles: - Endothelium
 - Smooth muscle
 - Tubules (DCTs & CDs)
- **Glomeruli**
 - Capillary endothelium
 - Mesangial cells
 - Podocytes
 - Mesangium (extra-cellular matrix)



Sampling compartment predicts disease compartment: Kidney biopsy



Pre-treatment



Post-treatment

GL3 is Cleared from Interstitial Capillary Endothelium of the Renal Cortex



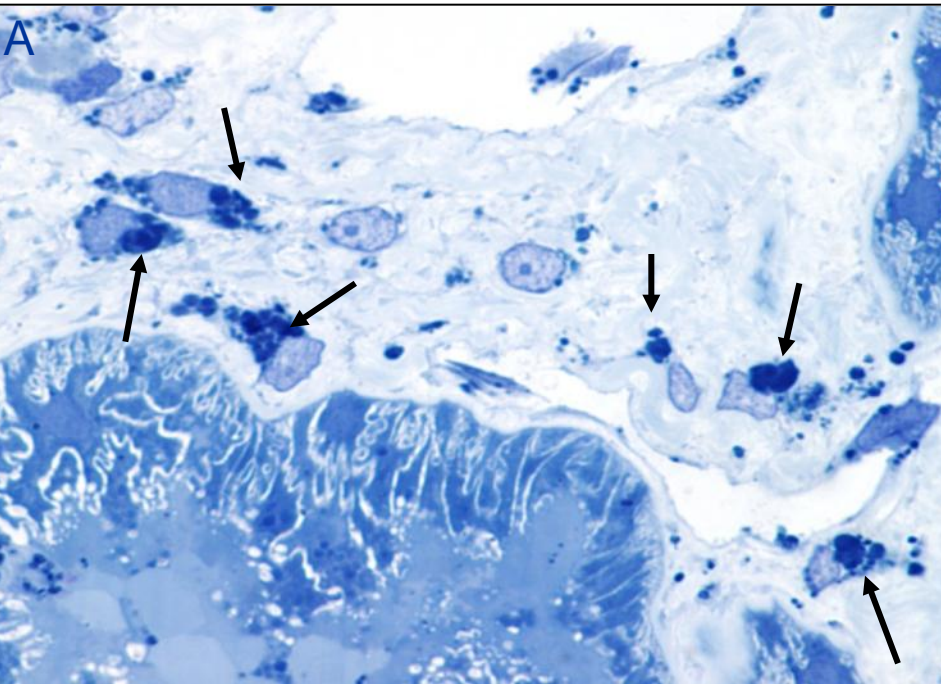
Results of primary endpoint analysis were robust

PHASE 3 EXTENSION STUDY - KIDNEY INTERSTITIAL CAPILLARY ENDOTHELIAL CELLS

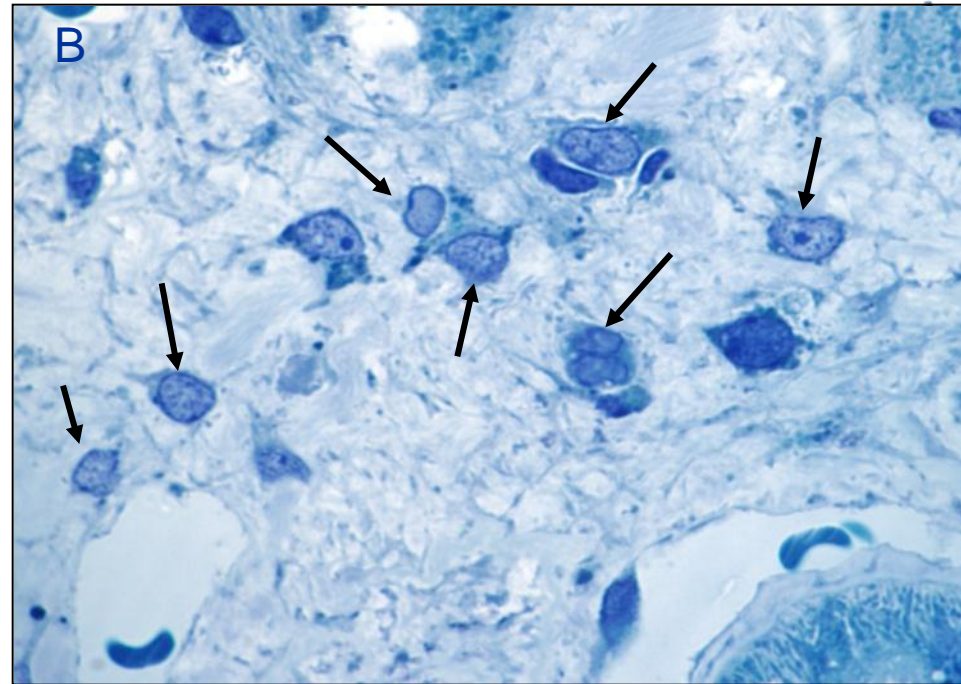
Original treatment	Patients biopsied	"0" scores	Months on α -Gal A	% "0" scores
Placebo	22	22	6	100%
α -Gal A	21	20	12	95%
Total	43	42		98%



GL-3 is Cleared from Interstitial Cells



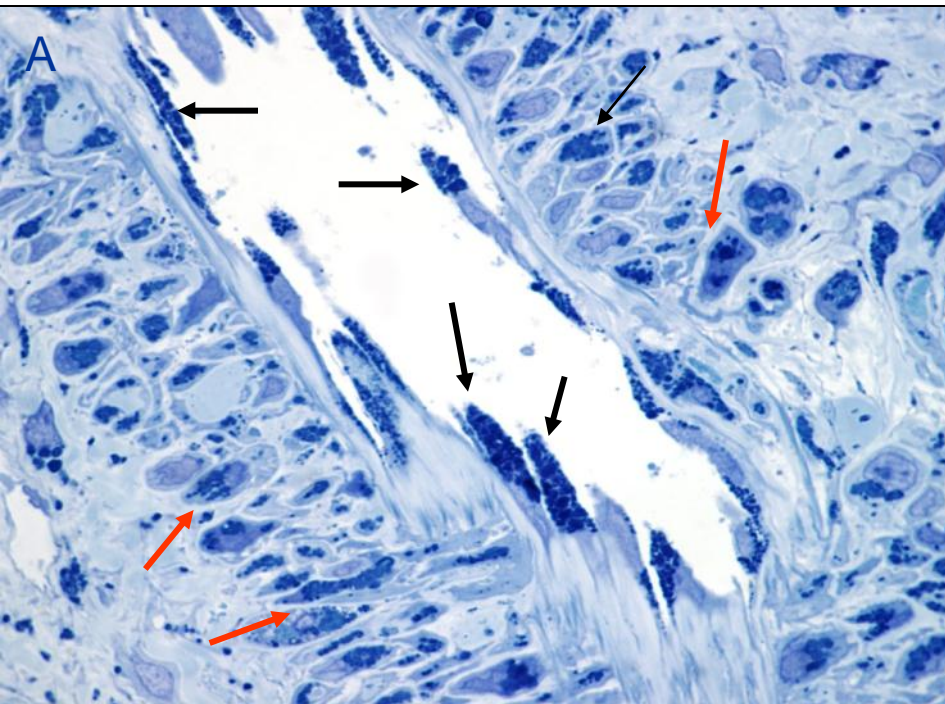
Pre-treatment



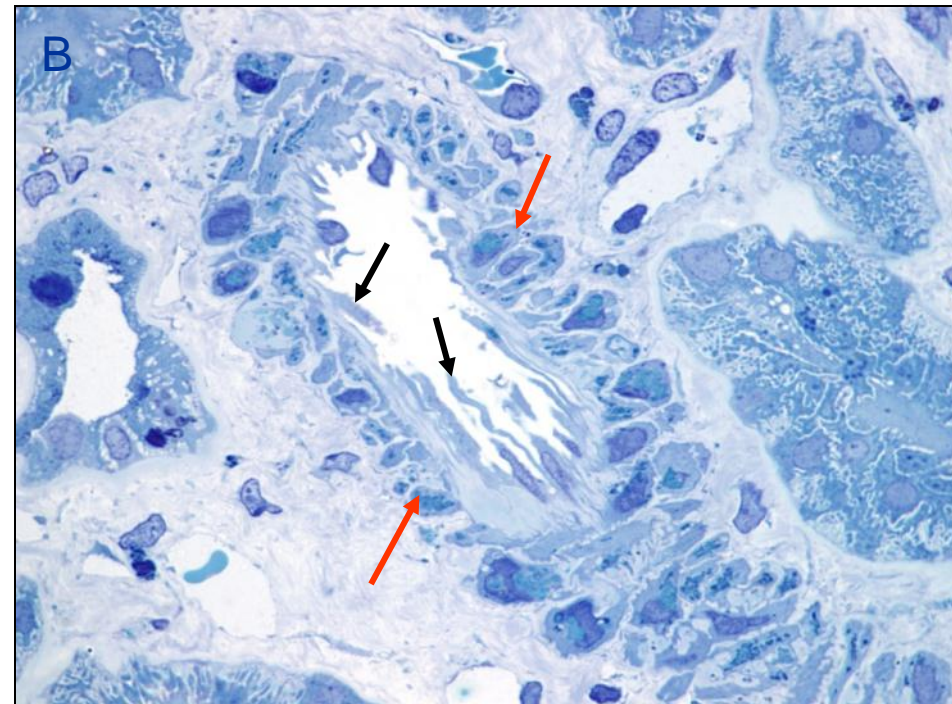
Post-treatment



GL3 is cleared from endothelial cells and vascular smooth muscle cells of renal arterioles



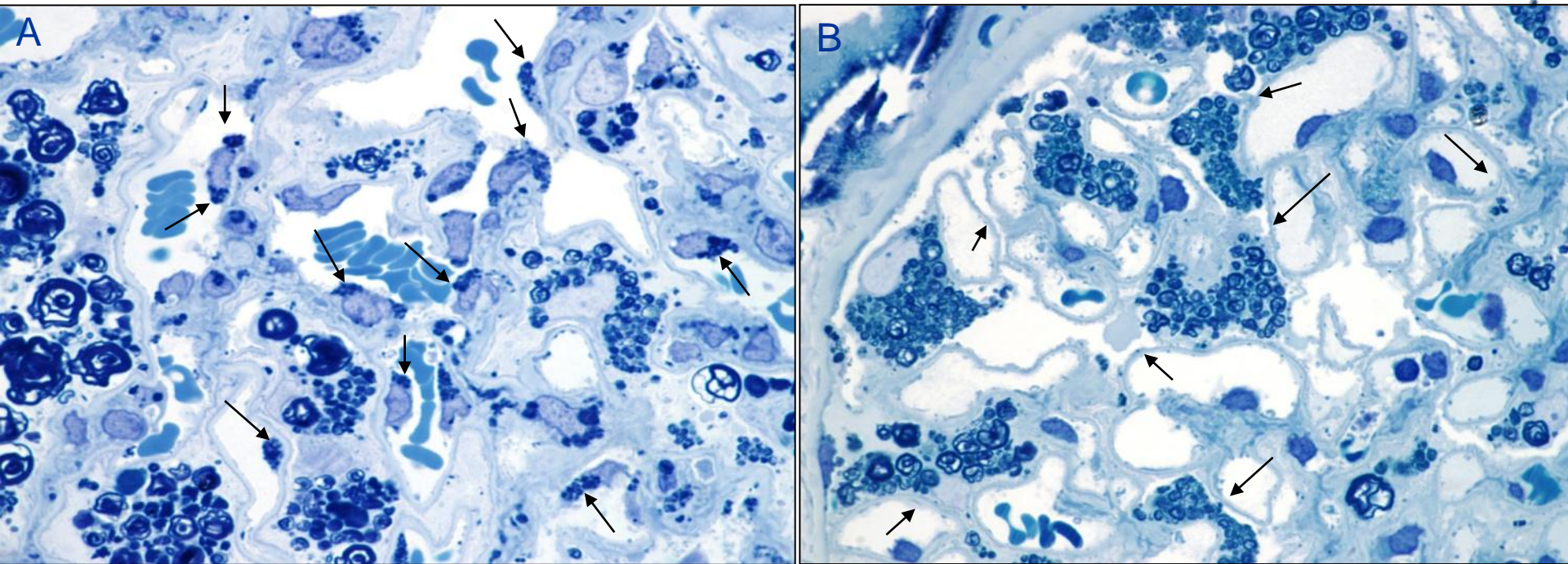
Pre-treatment



Post-treatment



GL3 is Cleared From the Glomerular Capillary Endothelium

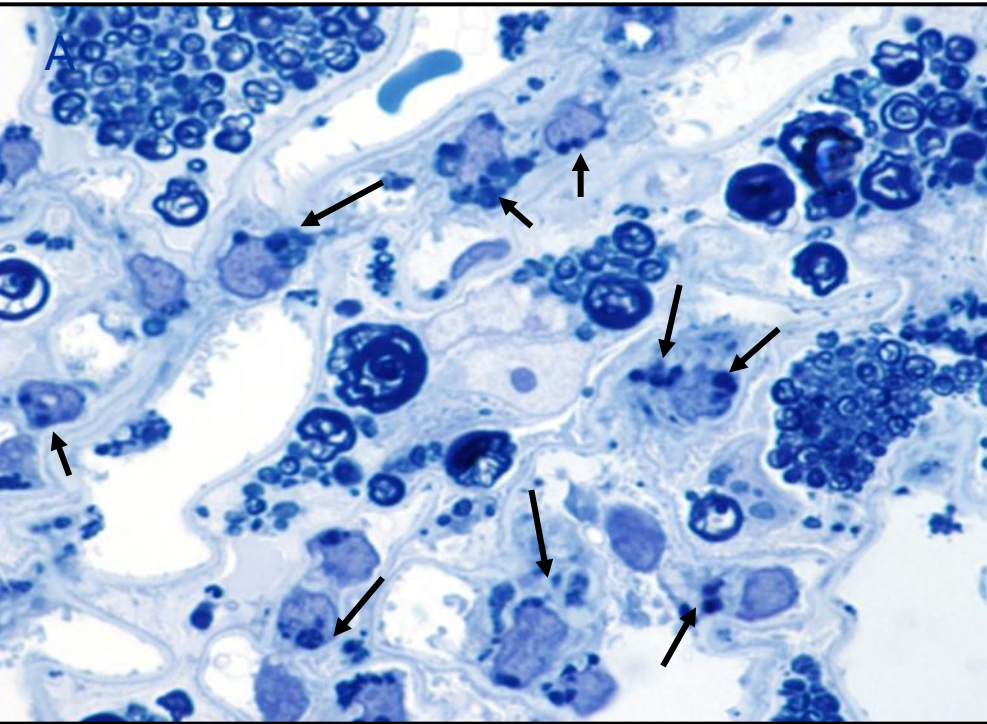


Pre-treatment

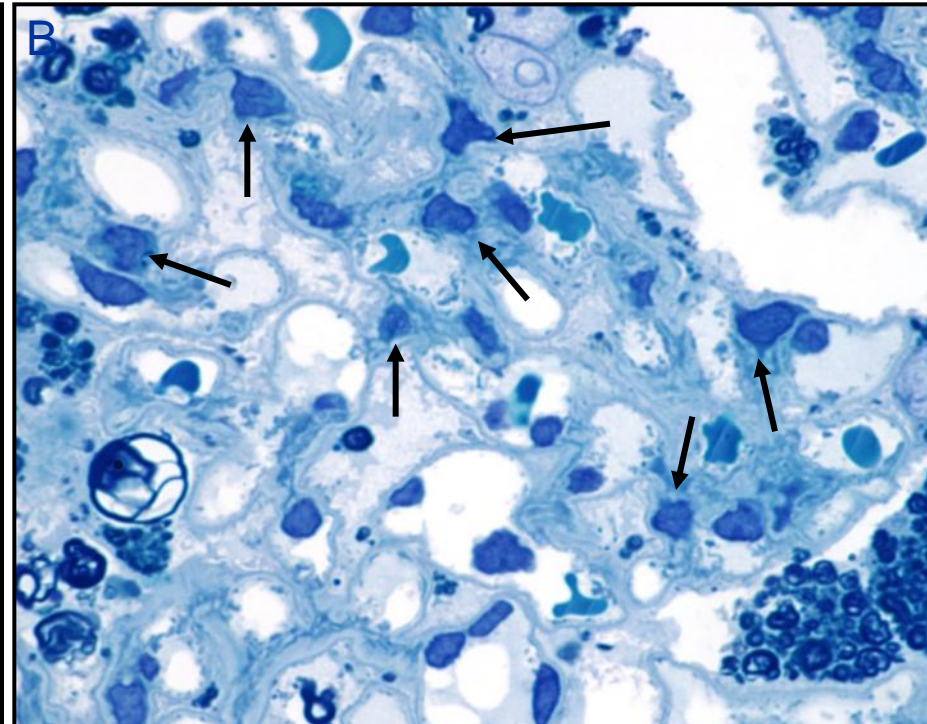
Post-treatment



GL-3 is cleared from mesangial cells



Pre-treatment

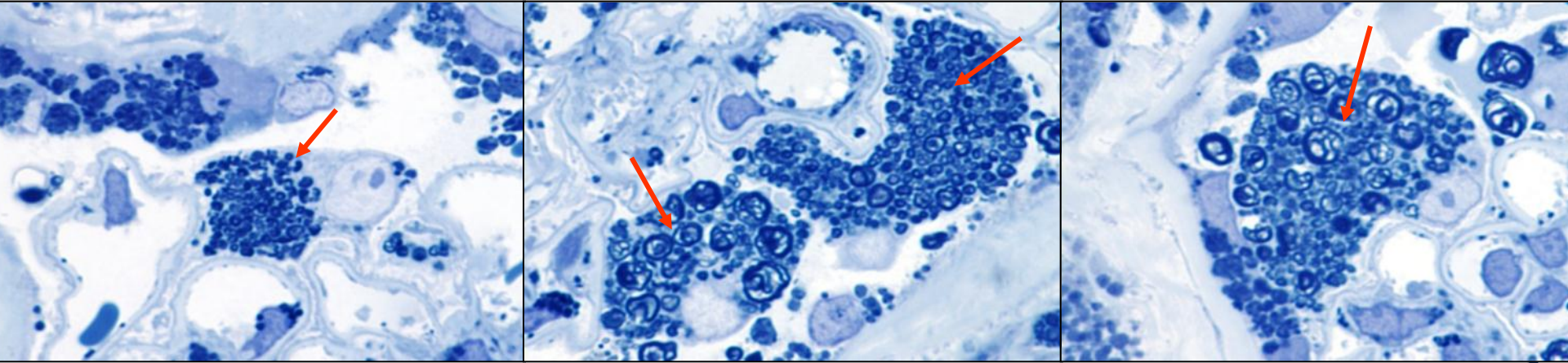


Post-treatment

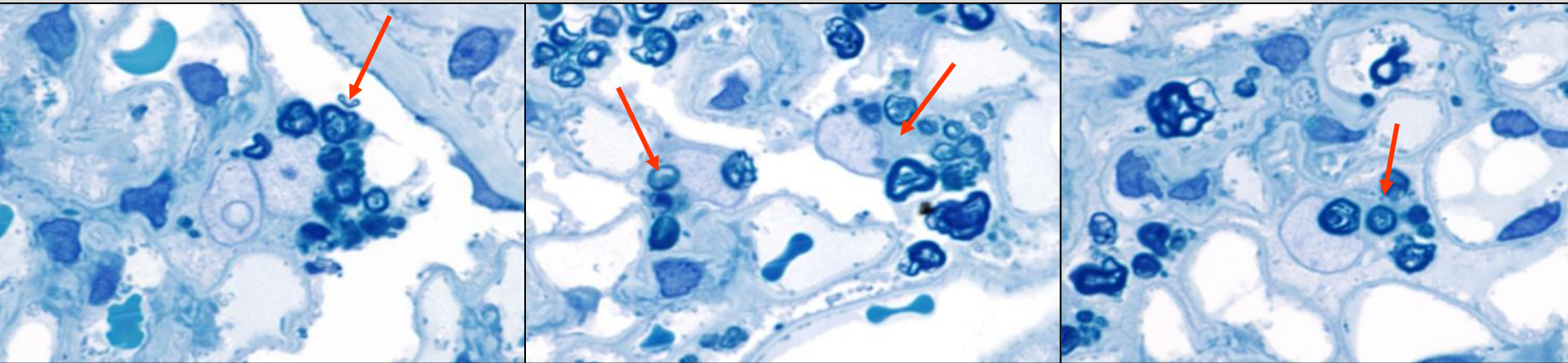


GL-3 accumulation is reduced in podocytes

Pre-treatment

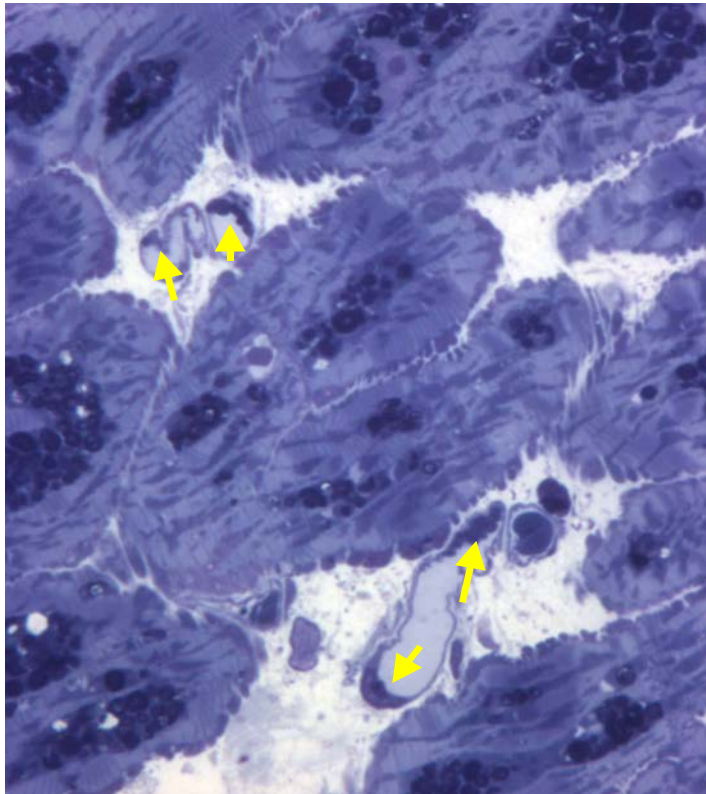


Post-treatment

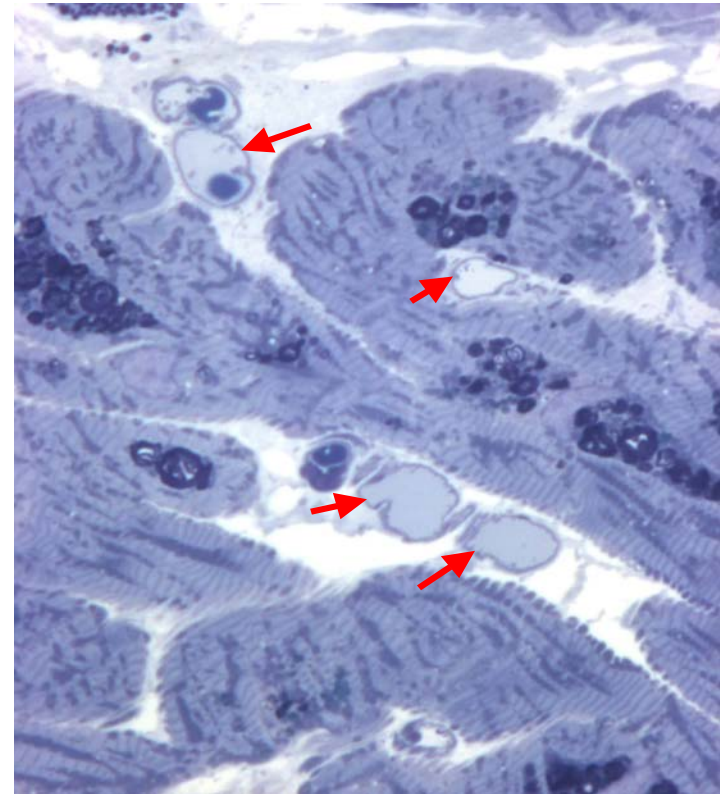




Sampling compartment predicts disease compartment: Cardiac biopsy



Pre-treatment



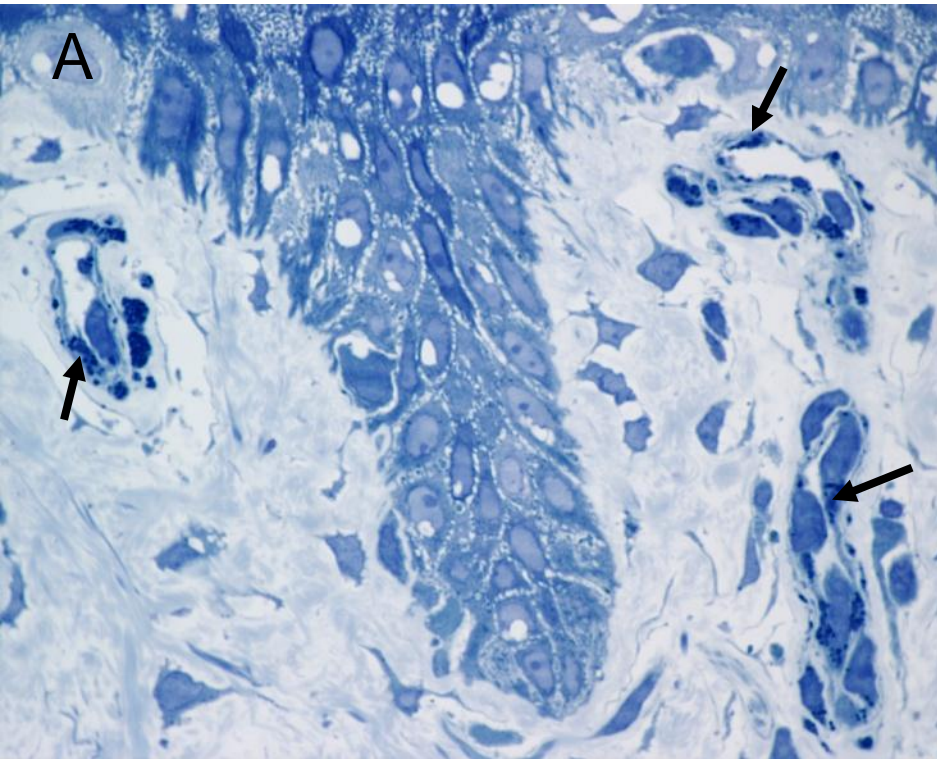
Post-treatment

GL3 is Cleared from Interstitial Capillary Endothelium of the myocardium

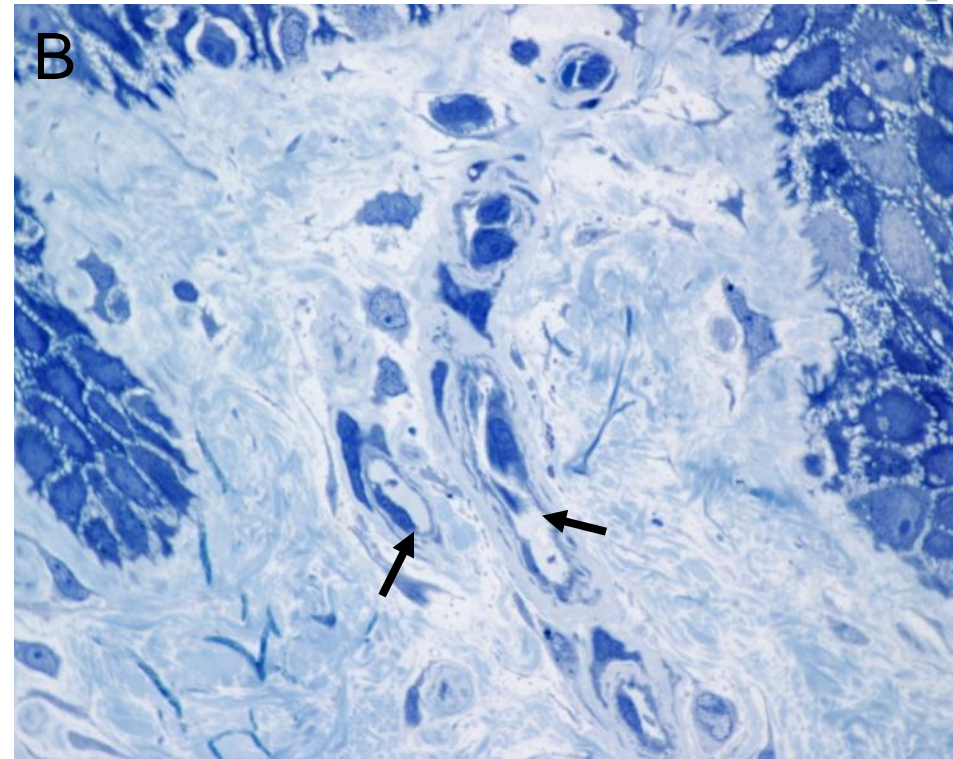
Thurberg et al Circulation 2009



Sampling compartment predicts disease compartment: skin biopsy



Pre-treatment



Post-treatment

GL3 is Cleared from superficial capillary endothelium of the skin



Summary and Conclusions

- Fabrazyme (1.0mg/kg) was highly effective in clearing GL3 from the endothelium- critical to stabilizing Fabry disease progression
- The additional pathology reading demonstrated that GL3 is cleared from multiple cells types after treatment with Fabrazyme
- Prevention in younger patients and stabilization of disease in older patients is the ultimate goal of treatment
 - Biomarker of GL3 represents primary disease which is reversible with ERT
 - Need to treat before the onset of secondary pathology (fibrosis) and end organ damage which is irreversible