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Buffy Coat Score as a Biomarker of Treatment Response in Neuronal Ceroid Lipofuscinosis Type 2

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NCL disease – currently 13 types

Classic childhood forms:

- *CLN1*: Infantile – granular osmiophilic deposits
- *CLN2*: Late infantile – curvilinear bodies
- *CLN3*: Juvenile – vacuolated lymphocytes with fingerprint profiles

Other/variant forms:

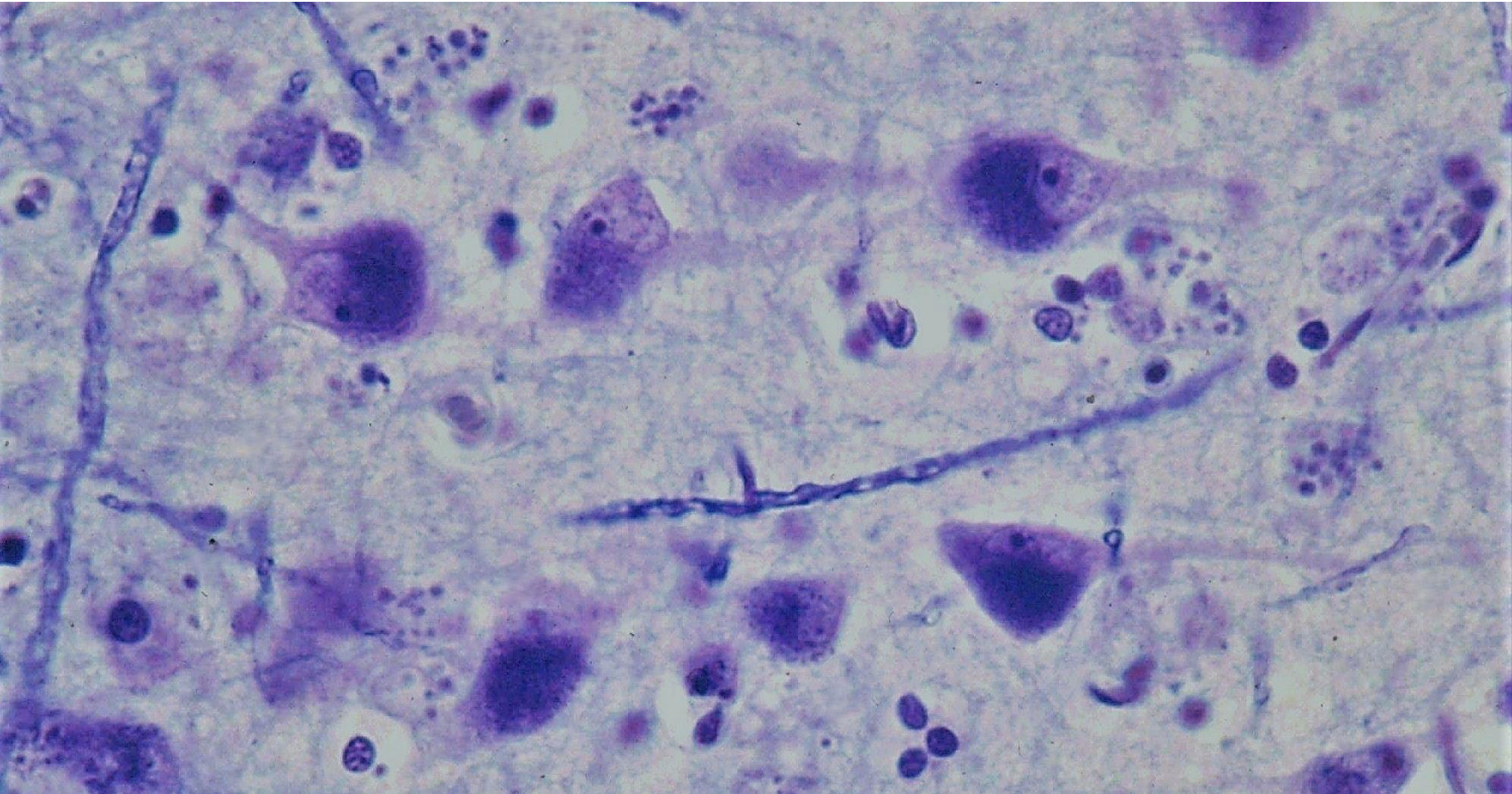
- *CLN4* – *CLN13*

Neuronal ceroid lipofuscinoses - laboratory diagnosis

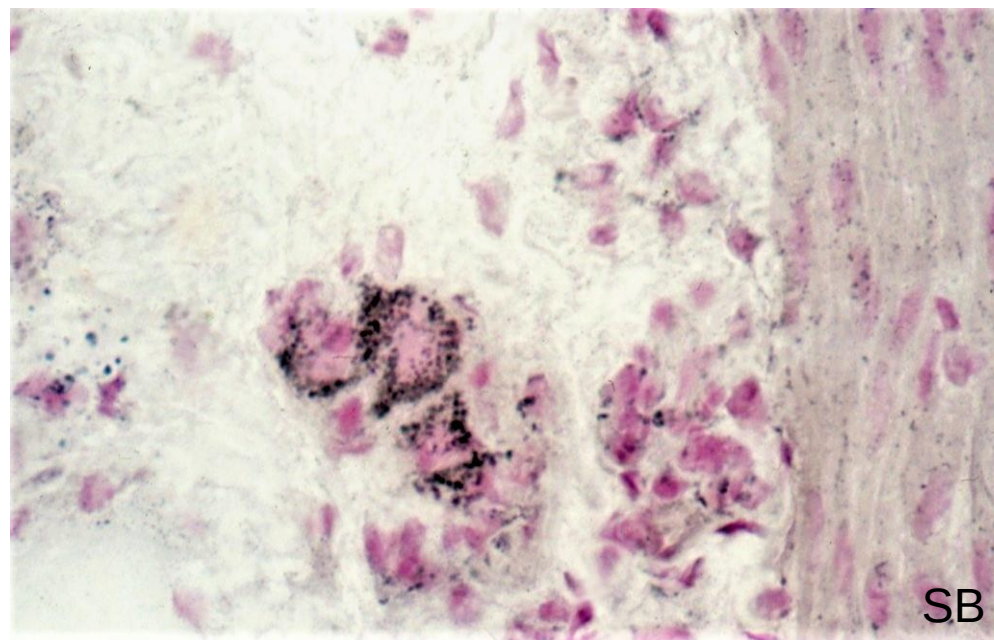
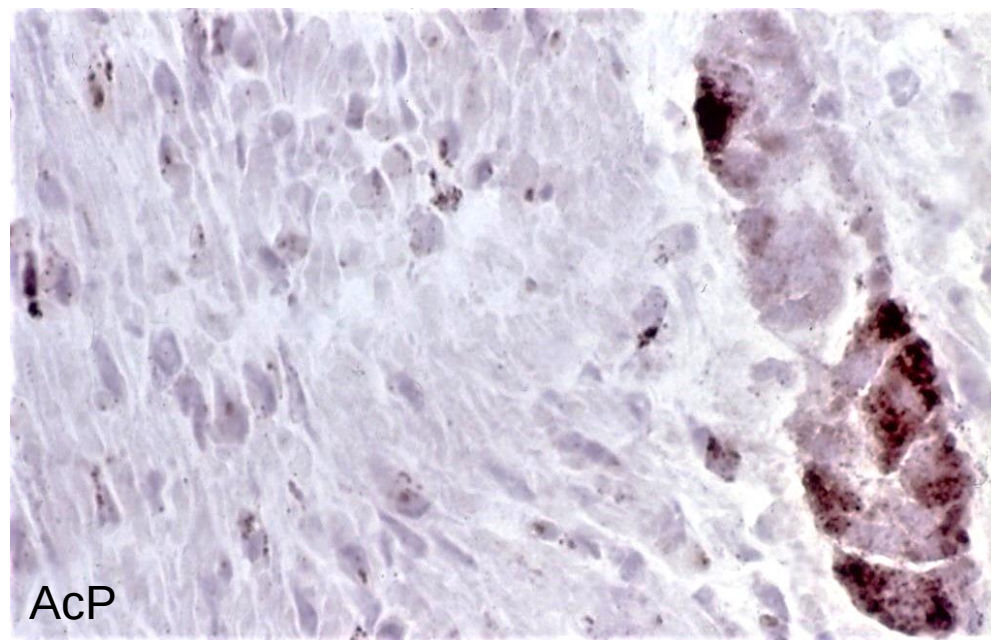
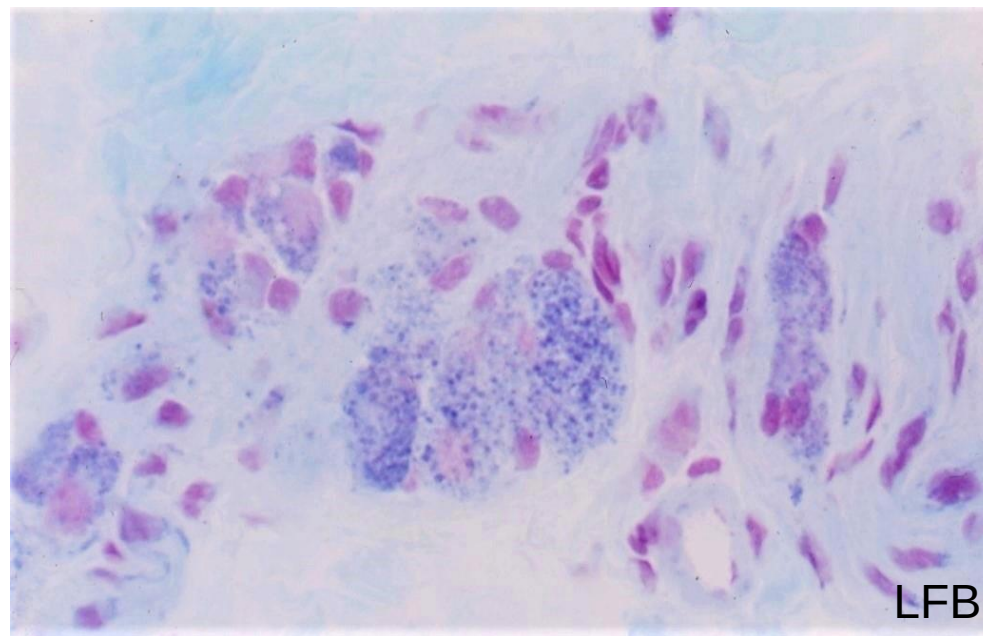
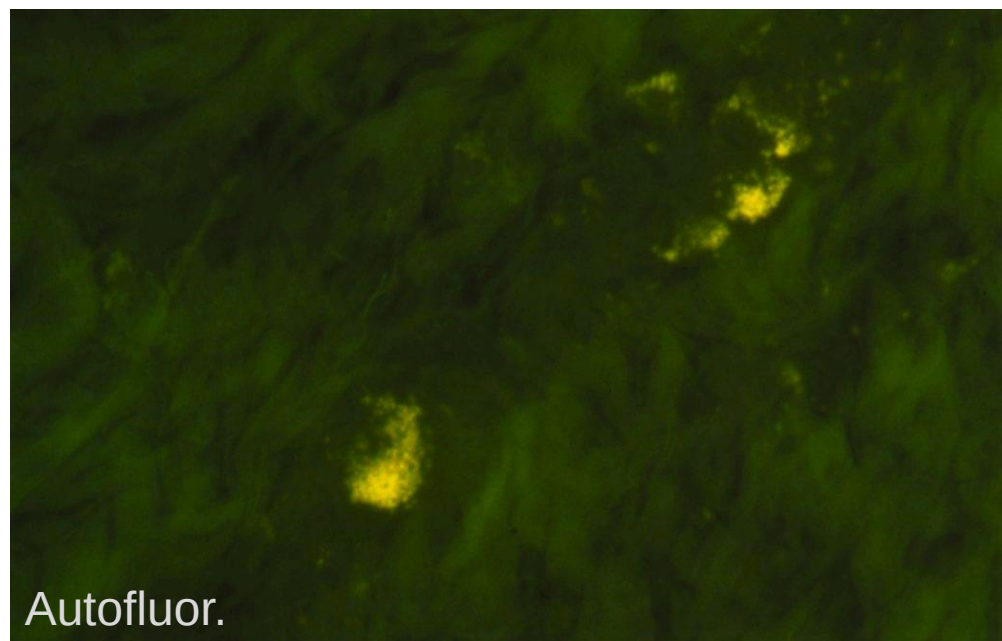
- Biochemistry - Palmitoyl protein thioesterase 1 (*PPT1*)
- Tripeptidyl peptidase 1 (*TPP1*)
- Electron microscopy – rectal biopsy, skin, blood
- Molecular genetics – confirmation of a diagnosis or establish a diagnosis where there is no straightforward result e.g. NCL's

Neuronal ceroid lipofuscinosis type 2

- Rare, rapidly progressive neurodegenerative disorder
- Autosomal recessive pattern of inheritance
- Caused by deficient activity of the enzyme tripeptidyl peptidase 1 (TPP1)
- Catastrophic neuronal degeneration throughout the CNS and retina
- Children normal until two to four years of age
- Seizures, decline in motor, language, cognitive and visual function, with death by early adolescence

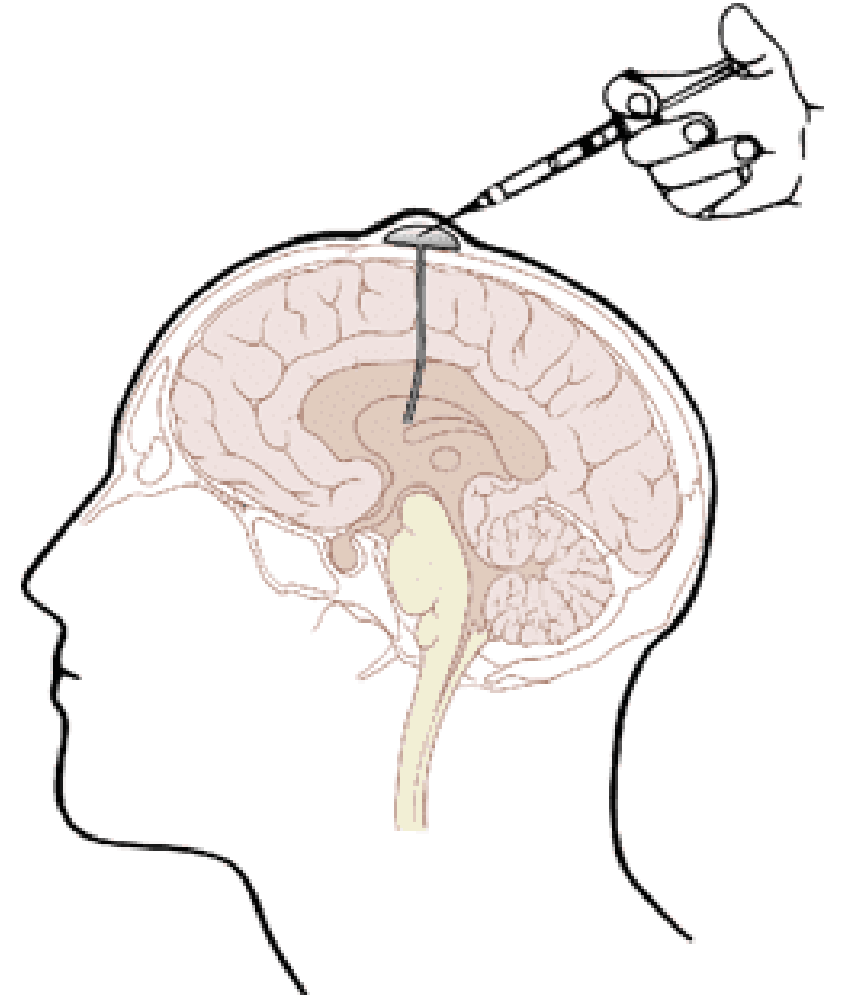


Rectal
biopsy



Intracerebroventricular ERT for treatment of Late Infantile Batten's disease

- ERT trial using cerliponase alfa – (form of human TPP1)
- Produced dramatic improvement in disease management
- Treatment was well tolerated
- Assessment of therapeutic effect – clinical observation, *subjective*
- Ultrastructural examination of buffy coat lymphocytes with storage inclusions – *objective* assessment

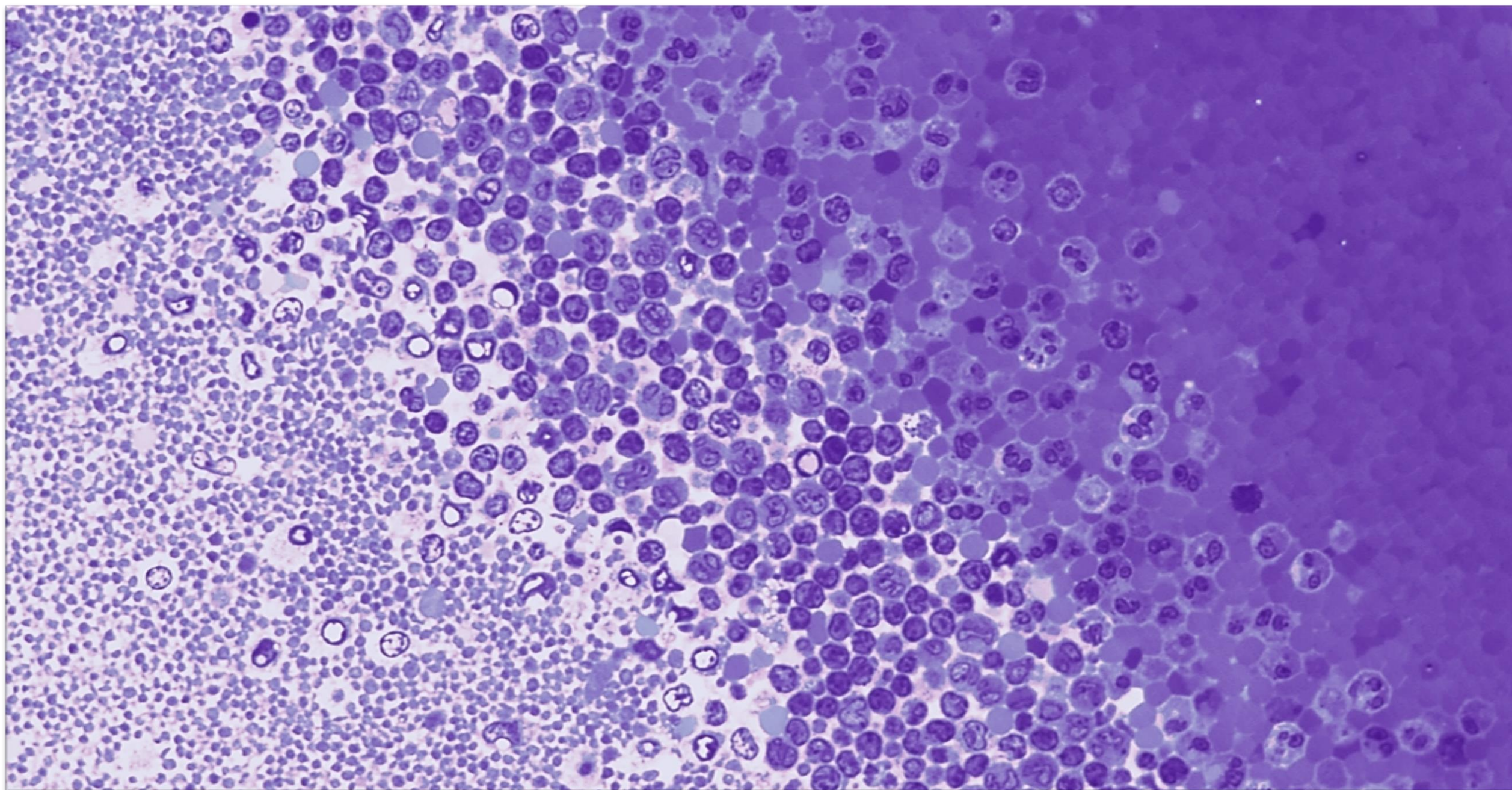


Study Patients

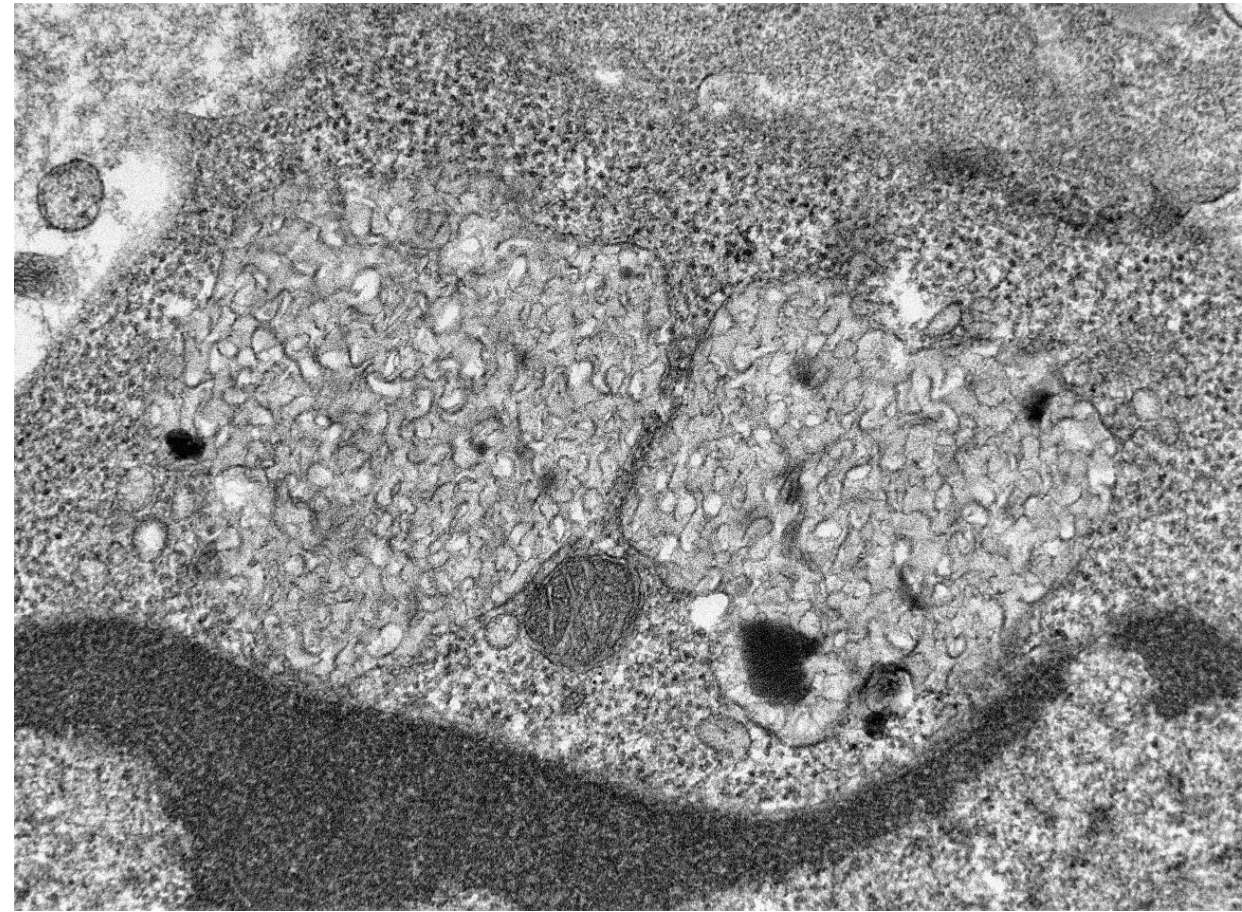
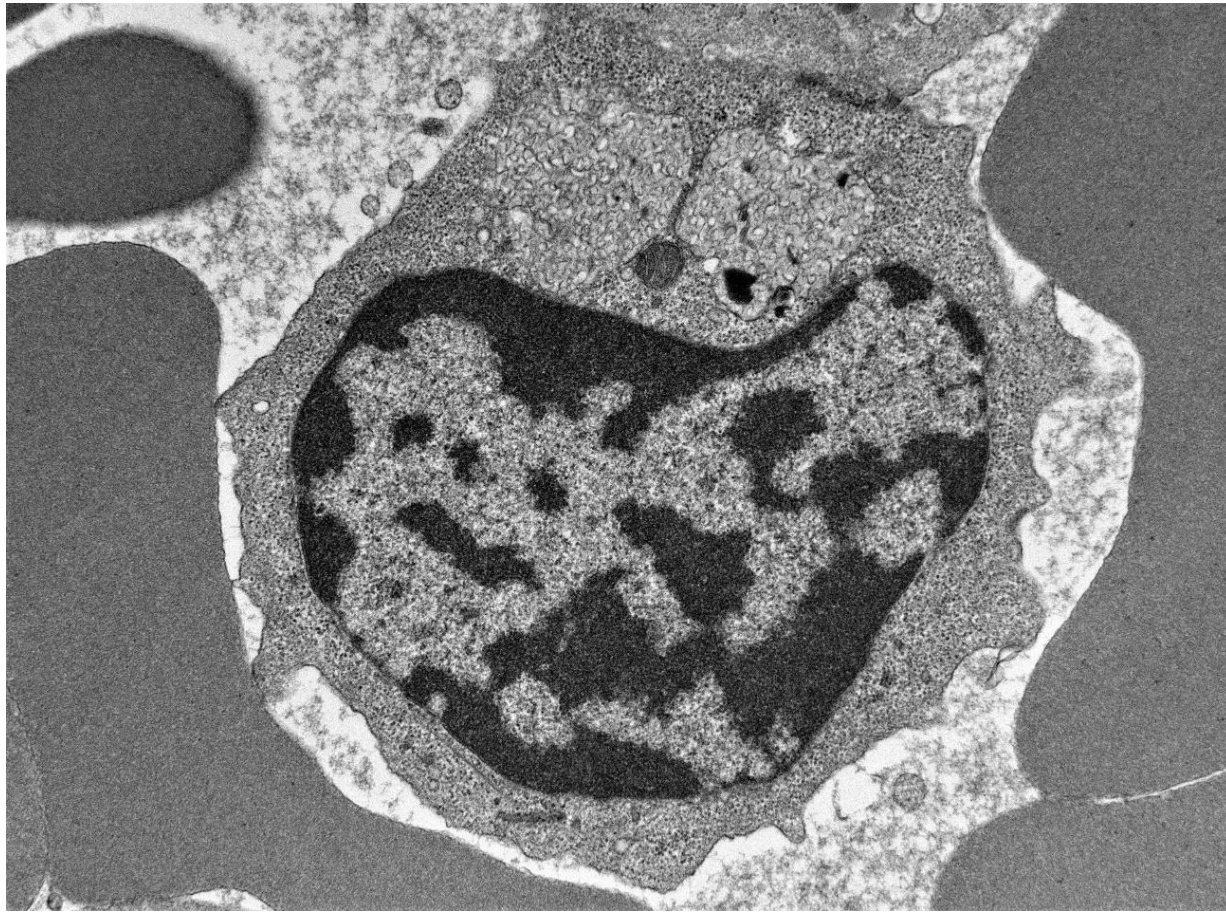
- Six patients in the initial trial
- All confirmed as CLN2 disease on genetic testing
- Cerliponase alfa administered – fortnightly
- Peripheral blood samples for buffy coat analysis taken prior to ICV infusion on an annual basis
- New patients with CLN2 are referred for ERT treatment

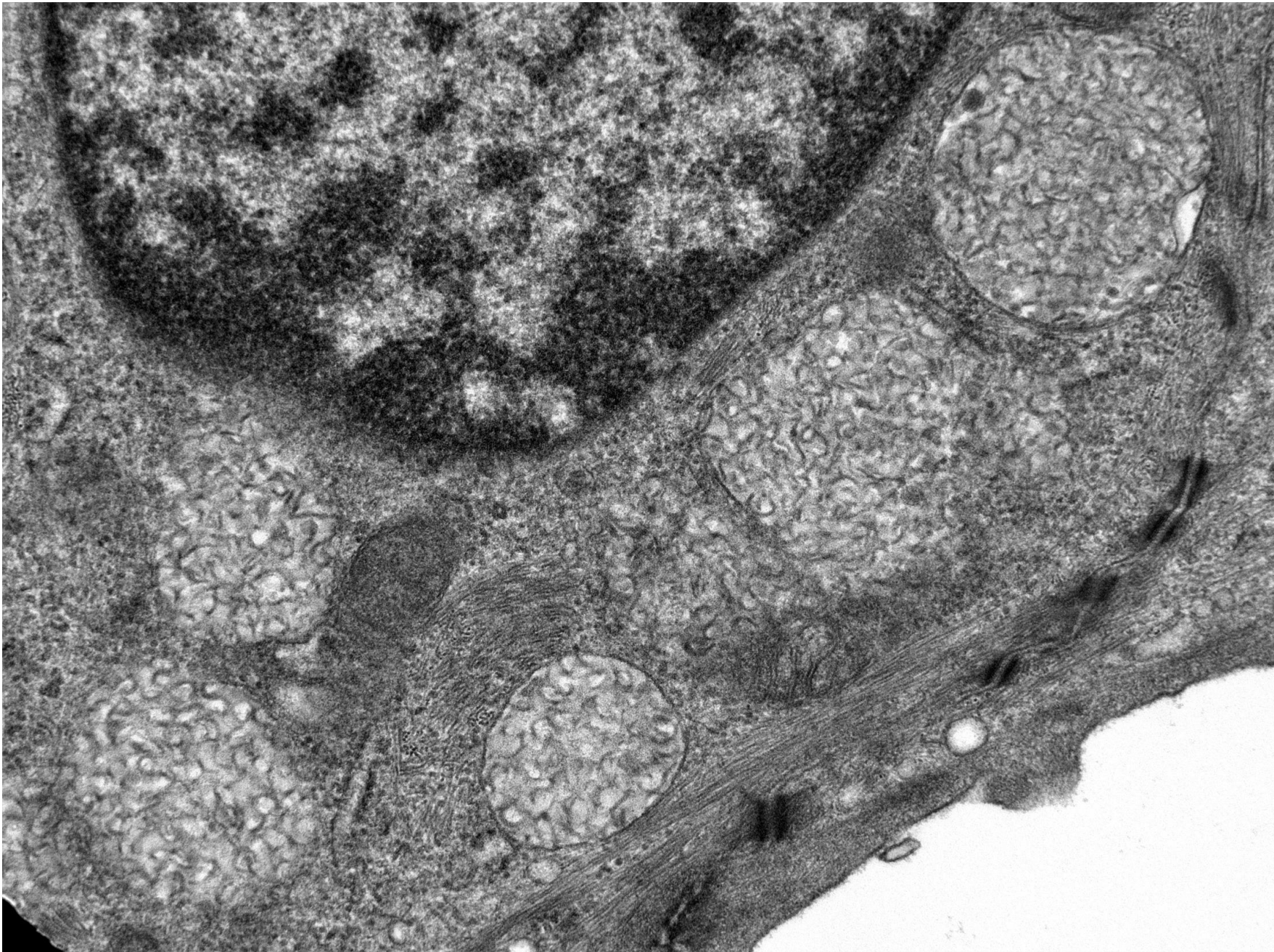
Buffy coat EM analysis

- EDTA blood centrifuged to form buffy coat
- Processed in Epon for ultrastructural examination
- One/two hundred lymphocytes examined for curvilinear storage inclusions
- Percentage of abnormal cells recorded
- Results reported and collated by Metabolic team



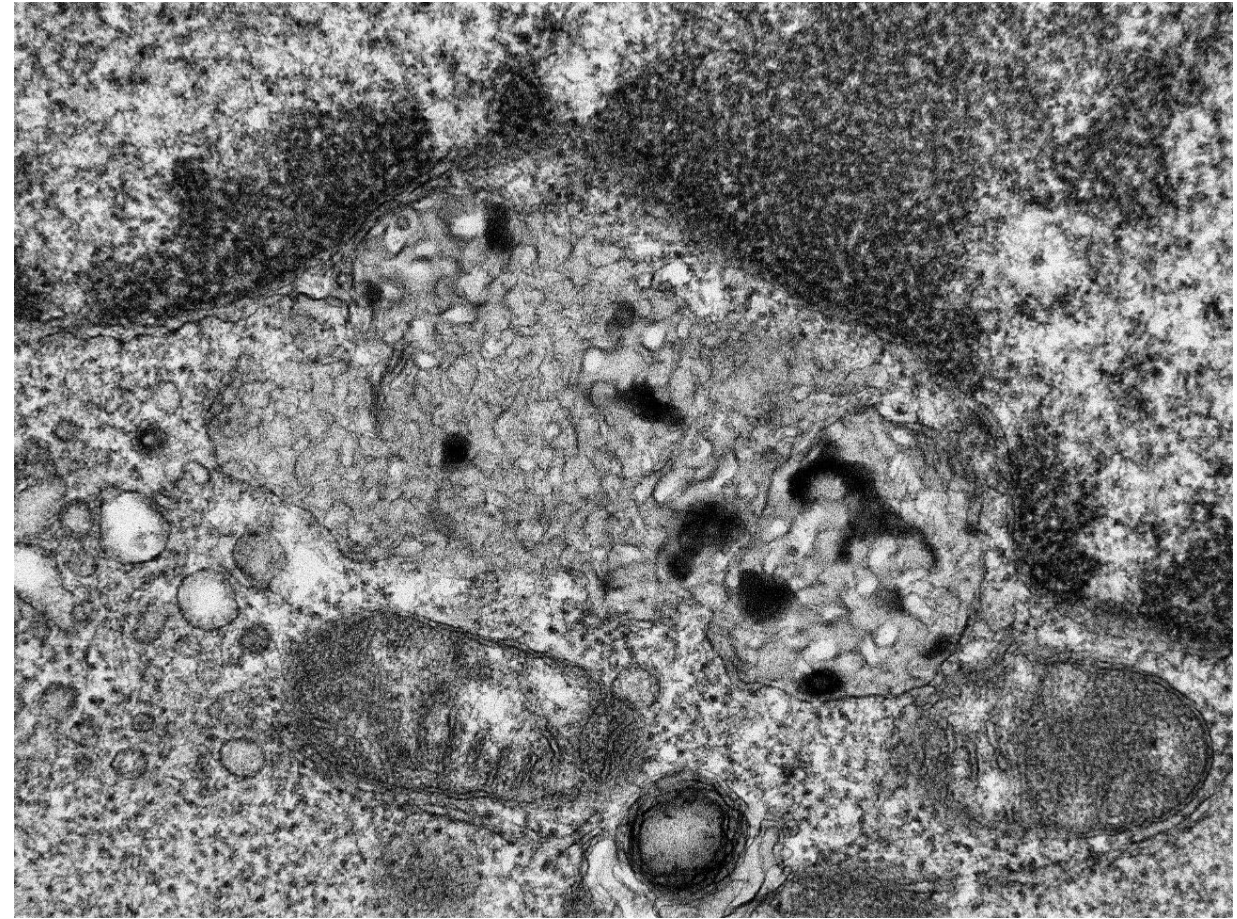
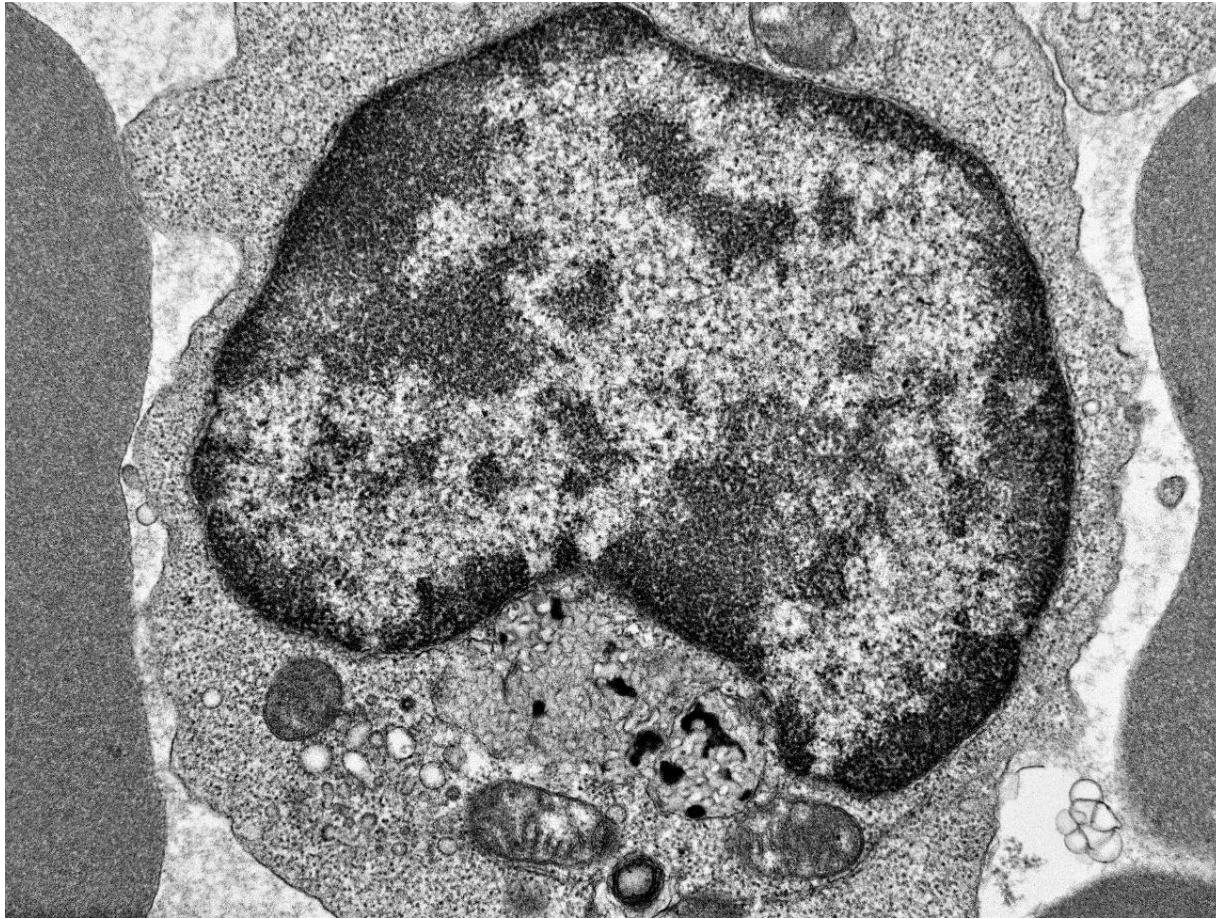
Pre ERT treatment



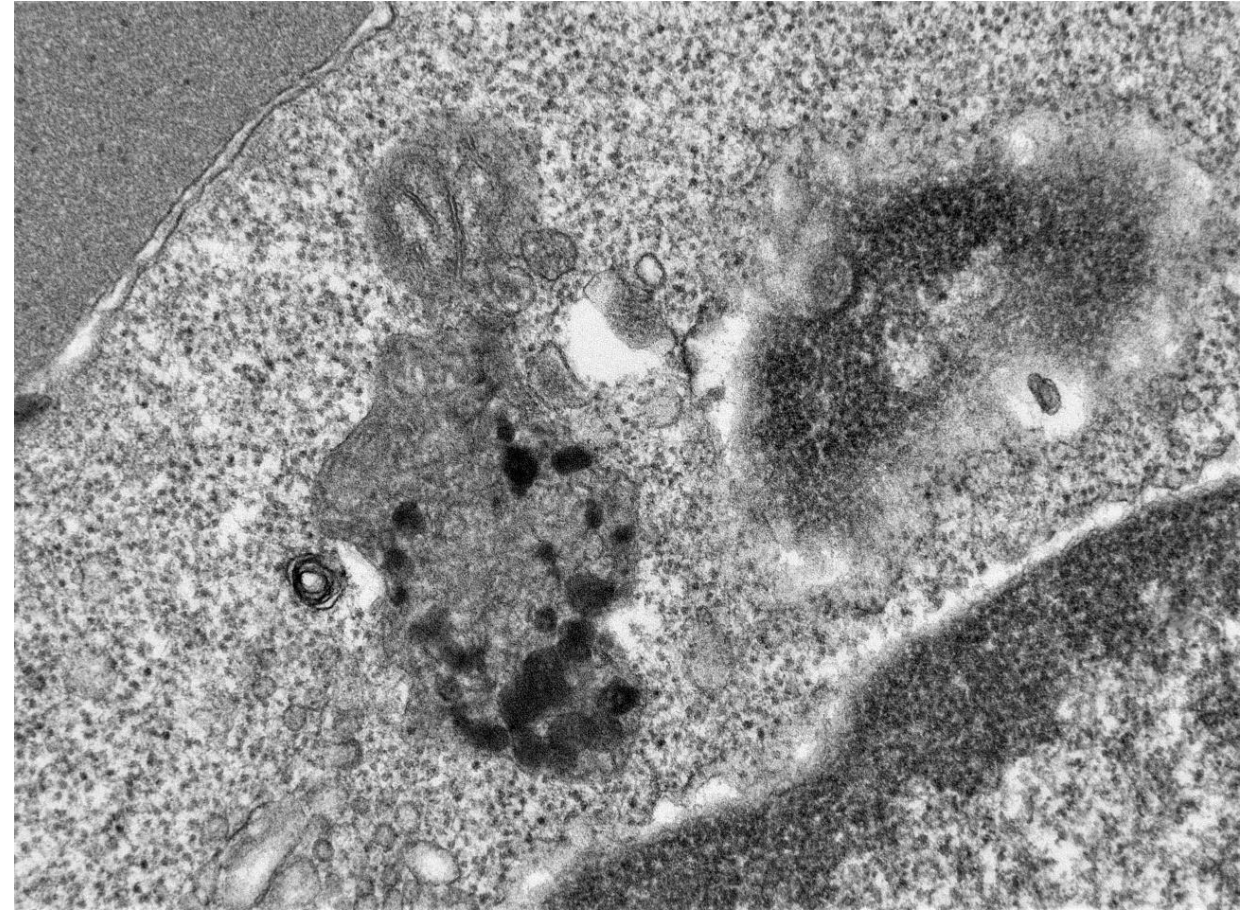
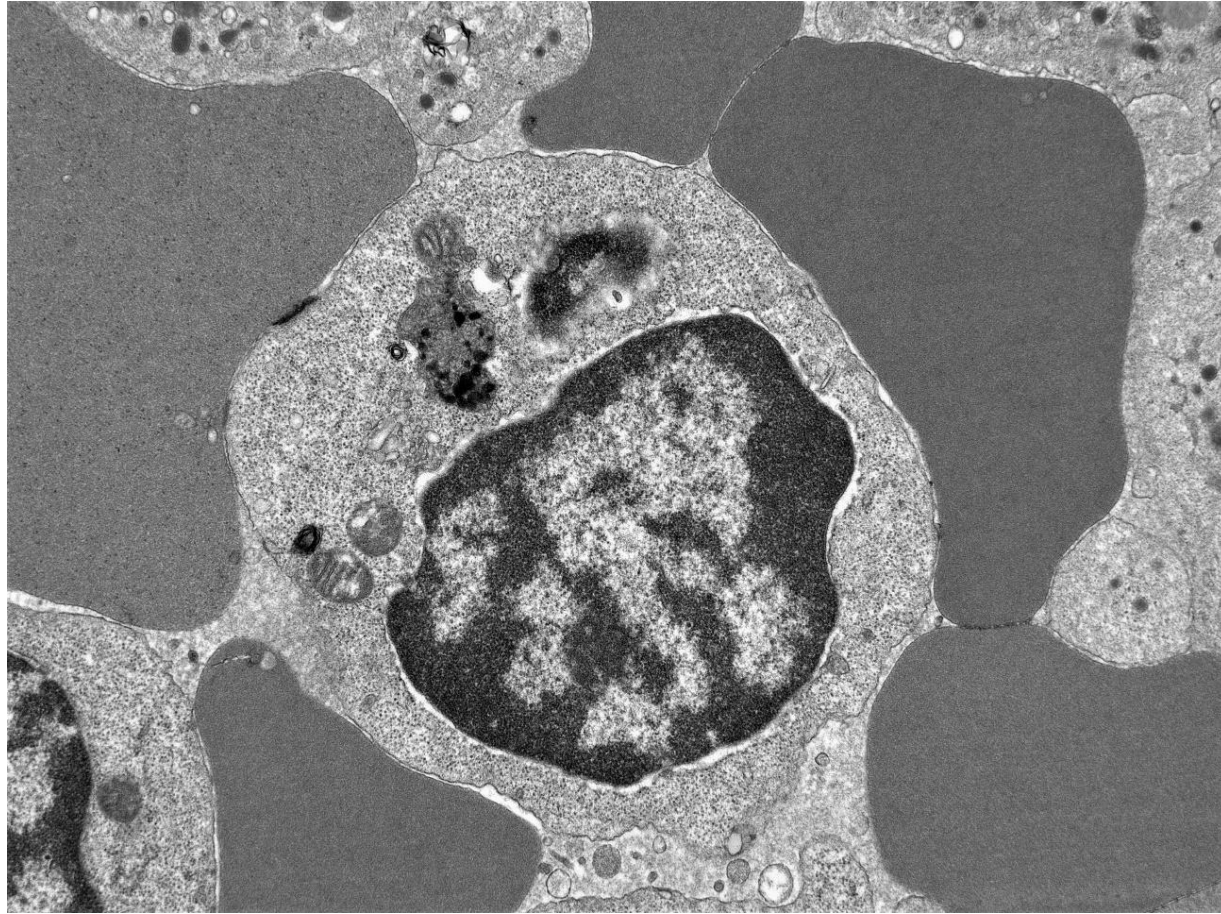


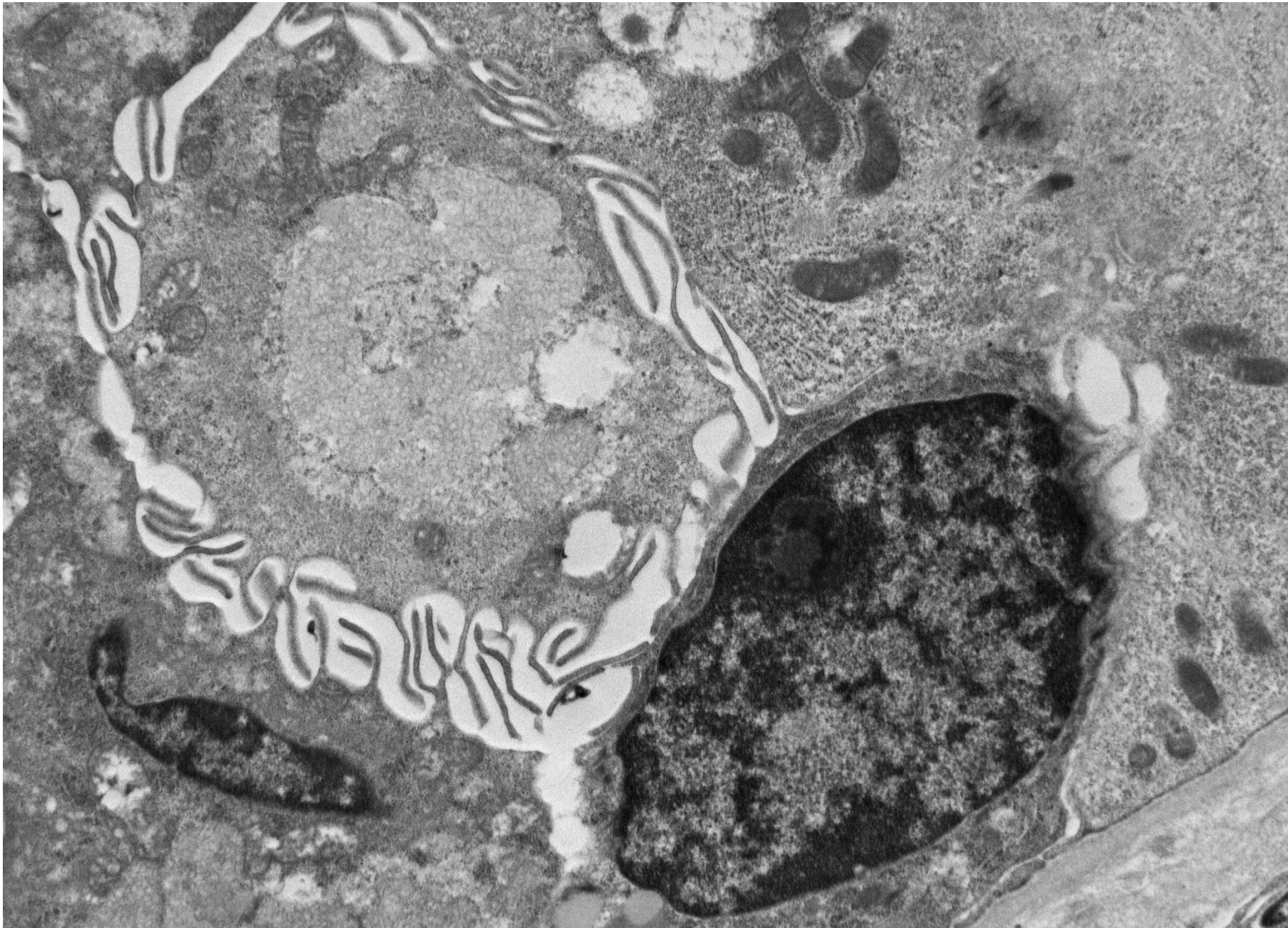
Skin – eccrine
sweat gland

One year post ERT



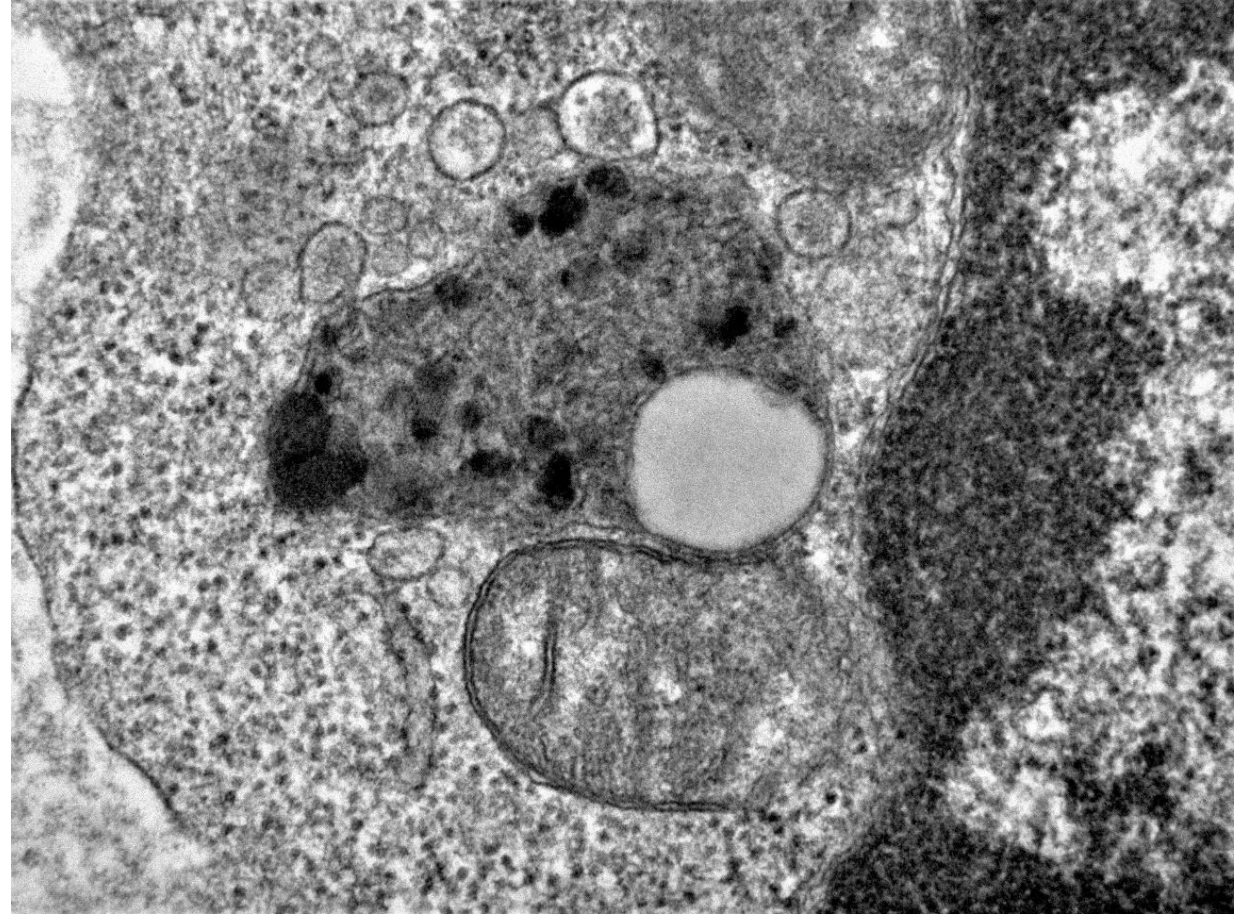
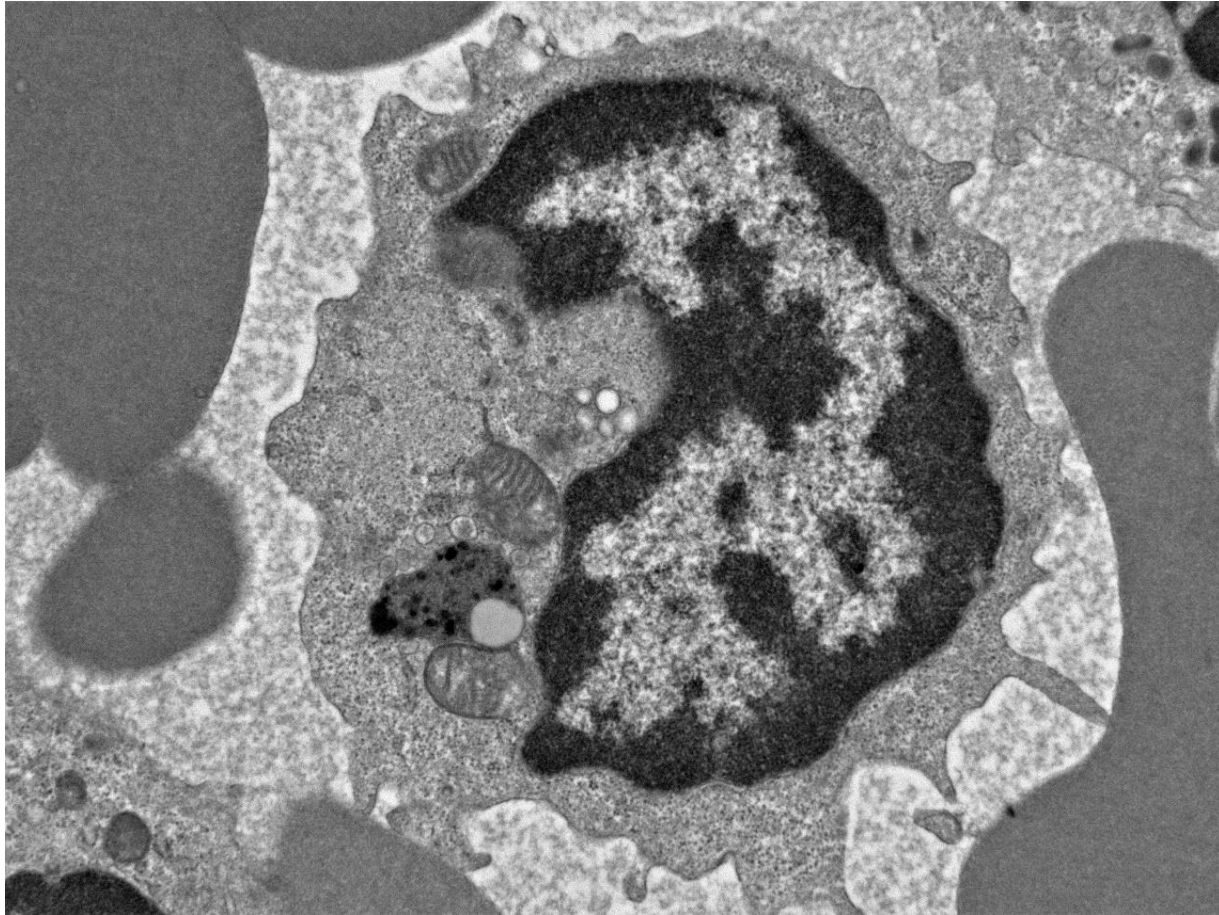
Two years post ERT





Post ERT
skin, sweat
gland

Three years post ERT



Electron microscopy results

- Clusters of membrane-bound curvilinear inclusions typical of NCL2
- Following ERT number of lymphocytes with storage inclusions declined
- Ongoing treatment, CL inclusions are poorly defined
- Prominent electron dense inclusions and associated lipid droplet
- Normal lymphocytes with no inclusions as treatment progresses

Summary

- Late infantile CLN2 caused deficient activity of enzyme tripeptidyl peptidase 1 (TPP1)
- Accumulation of ceroid lipofuscin storage in the CNS and retina
- Cerliponase alfa treatment, little to no deterioration in symptoms over a two-year period
- Ultrastructural examination of peripheral blood is reliable and minimally invasive
- EM used for diagnosis of CLN2 and other forms of NCL
- Reduction of storage inclusions in lymphocytes clearly identified
- The use of buffy coat analysis has not been previously reported and could be of value as a biomarker of therapeutic response in other LSD disorders

Article

Buffy Coat Score as a Biomarker of Treatment Response in Neuronal Ceroid Lipofuscinosis Type 2

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Abstract: The introduction of intracerebroventricular (ICV) enzyme replacement therapy (ERT) for treatment of neuronal ceroid lipofuscinosis type 2 (CLN2) disease has produced dramatic improvements in disease management. However, assessments of therapeutic effect for ICV ERT are limited to clinical observational measures, namely the CLN2 Clinical Rating Scale, a subjective measure of motor and language performance. There is a need for an objective biomarker to enable assessments of disease progression and response to treatment. To address this, we investigated whether the proportion of cells with abnormal storage inclusions on electron microscopic examination of peripheral blood buffy coats could act as a biomarker of disease activity in CLN2 disease. We conducted a prospective longitudinal analysis of six patients receiving ICV ERT. We demonstrated a substantial and continuing reduction in the proportion of abnormal cells over the course of treatment, whereas symptomatic scores revealed little or no change over time. Here, we proposed the use of the proportion of cells with abnormal storage as a biomarker of response to therapy in CLN2. In the future, as more tissue-specific biomarkers are developed, the buffy coats may form part of a panel of biomarkers in order to give a more holistic view of a complex disease.

Keywords: neuronal ceroid lipofuscinosis type 2 (CLN2) disease; lysosomal storage disorder; intracerebroventricular; enzyme replacement therapy; disease progression; neurodegeneration; biomarker; buffy coat; electron microscopy; curvilinear inclusions