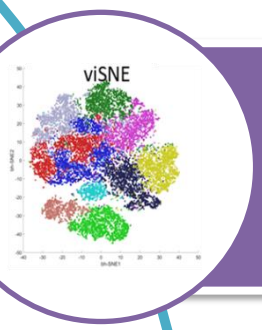
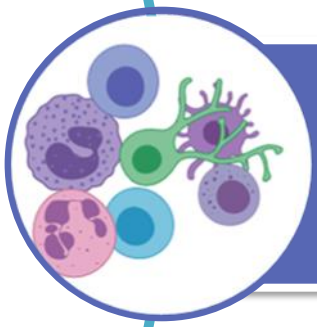
Outline



Immune response in patients with CLN2

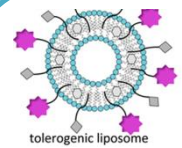
- Immune status of children affected with CLN2
- Replacement enzyme-related changes in the peripheral immune system
- Monitoring the appearance of neutralizing antibodies to ERT



Mechanisms of cell damage in CLN2 disease

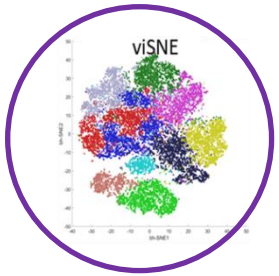
Single-cell RNAseq to assess the effects of TPP1 absence/reduction on

- Immune cells of children with CLN2
- Brain cells in the CLN2-deficient mouse



New therapeutical strategies

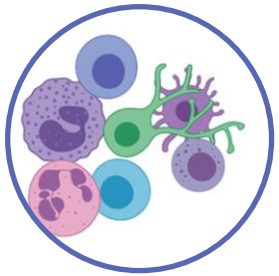
- Improve AAV-mediated delivery of TPP1 using cell-specific targeting
- Explore tolerization strategies to the replacement enzyme
- Evaluate the possibility of modifying the replacement enzyme



Immune response in patients with CLN2

- Immune status of children affected with CLN2
- Replacement enzyme-related changes in the peripheral immune system
- Monitoring the appearance of neutralizing antibodies to ERT

- Technically it is set up. Some children have already been analyzed. Collection ongoing
- Samples available (blood and CSF) ready to start analysis
- The ELISA needs to be set up. Contact lab in Texas for current protocol



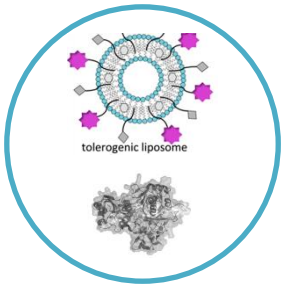
Mechanisms of cell damage in CLN2 disease

- Single-cell RNAseq
- Immune cells of children with CLN2
- Brain cells in the CLN2-deficient mouse

- Technically it is set up (Cite-Seq)
- Blood cells from untreated patients will be compared to healthy donors (n=5 each)
- CLN2 KO mouse needs to be imported. Since it will only be used as donor, no TVA required

Comments: Single cell sequencing is outsourced to a company. This part will will be started as soon as possible, to ensure that there is enough time for bioinformatic analysis, validation of the data and determination of potential therapeutic targets. Samples need to be from untreated patients.

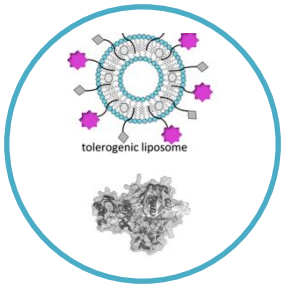
Eva Tolosa, stand December 2019



New therapeutical strategies (Phase I)

- Improve AAV-mediated delivery of TPP1 using cell-specific targeting
- Explore tolerization strategies to the replacement enzyme
- Evaluate the possibility of modifying the replacement enzyme

- Cooperation with Prof. F. Koch-Nolte (Immunology UKE). Using nanobodies to target specific cells → reduce virus titers
- Cooperation Dr. Marta Vives-Pi (IGTP Barcelona), for the encapsulation of TPP1 in lysosomes and *in vitro* testing
- Finding the immunogenic epitopes. Technically it is set up



New therapeutical strategies (Phase II)

- Improve AAV-mediated delivery of TPP1 using cell-specific targeting
- Explore tolerization strategies to the replacement enzyme
- Evaluate the possibility of modifying the replacement enzyme

- Preclinical testing: cooperation with a company to speed up (cooperation F. Koch-Nolte, UKE)
- Pre-clinical testing can be done in house using the CLN2 KO (cooperation M. Vives-Pi using liposomes)
- Directed mutagenesis for modification of the protein

To dos

- Check if ethics protocol covers all aspects of the project:
  - Monitoring the immune response to treatment
  - Single cells RNA sequencing analysis
  - Sending samples/data if necessary to our cooperators
- If necessary, write amendment

- Revise all existing samples, and acquired FACS data
- Import CLN2 animals for scRNAseq analysis of brain cells. Use Allen Brain Map for genes of interest defining brain cell types, nd for mouse/human comparison
- Angela: contact Texas lab for current ELISA protocol for neutralising antibodies
- Send TPP1 enzyme to Marta Vives to test encapsulation in liposomes