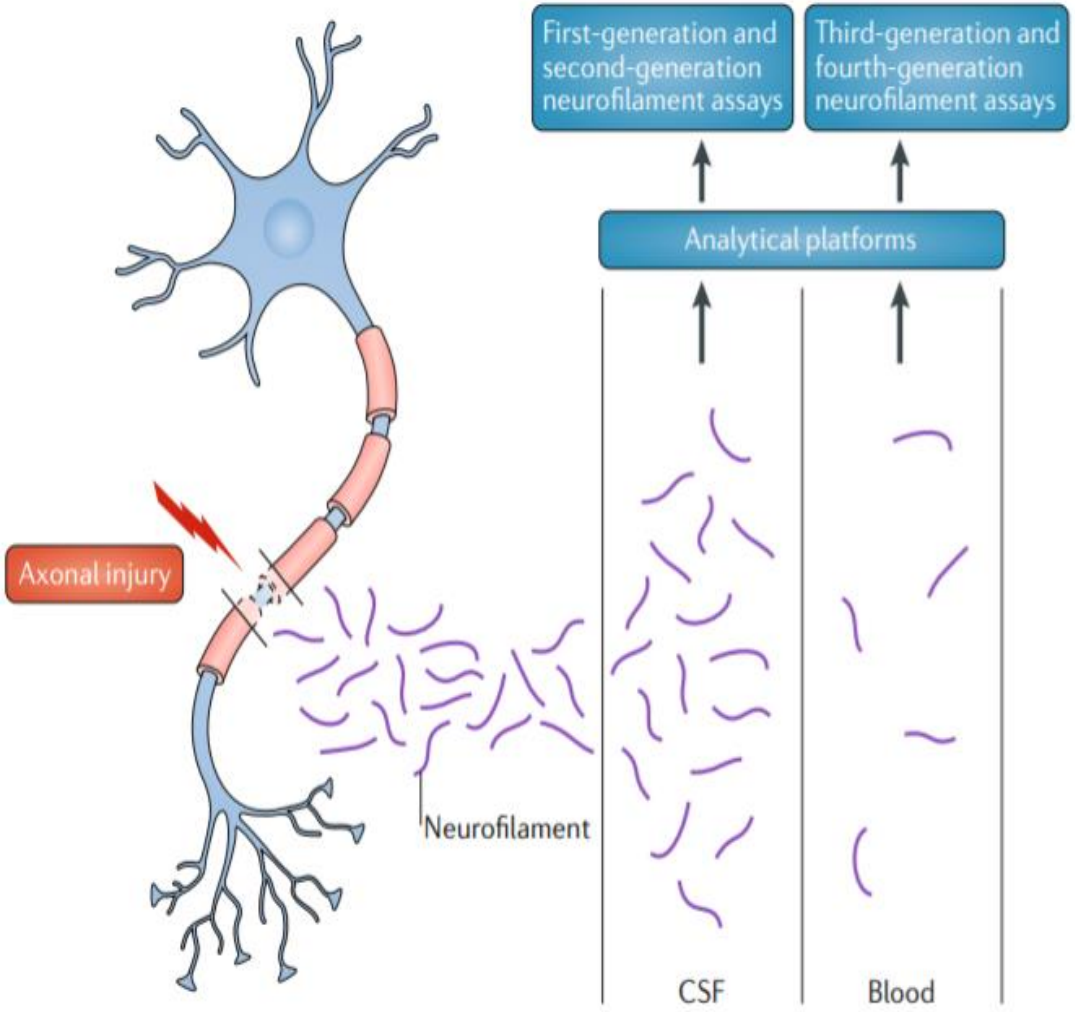
NFL et biomarqueurs

HL neurodégénérative – Neurofilament light (NFL)

Neurofilament = Protéine structurale.
Régule le diamètre et la forme des axones myélinisés.

Chaine légère des neurofilaments (NFL) :

- Marqueur de lésions neuroaxonales
- Dosage dans le plasma et le LCR
- Augmentée prouvée dans le LCR de SEP, AVC etc

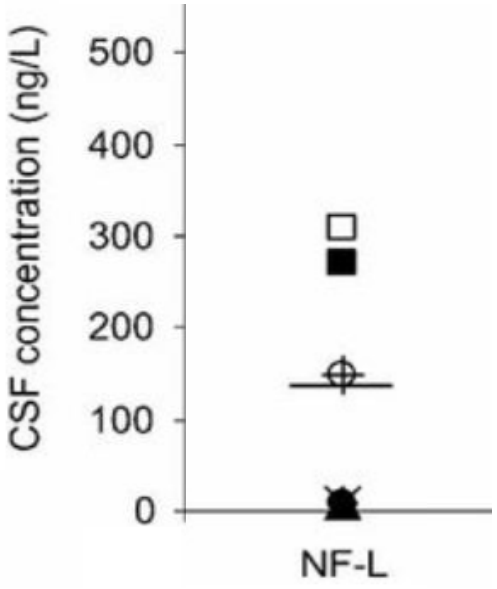


Khalil, *Nat Rev Neurol* 2018
L McClain, *Cancer* 2018

HL neurodégénérative – Neurofilament light (NFL)

Etude du NFL par ELISA chez patients HL ND (n=9)

- NFL augmenté dans le LCR : n=4
- **Inferieur au seuil de détection par ELISA : n=5**



Objectifs :

Augmenter le seuil de détection pour étude plasma (**SIMOA**)

Seuil quantif **ELISA ~80 pg/mL** ; Seuil quantif **SIMOA< 1 pg/mL**

Corrélation clinique et impact pronostic

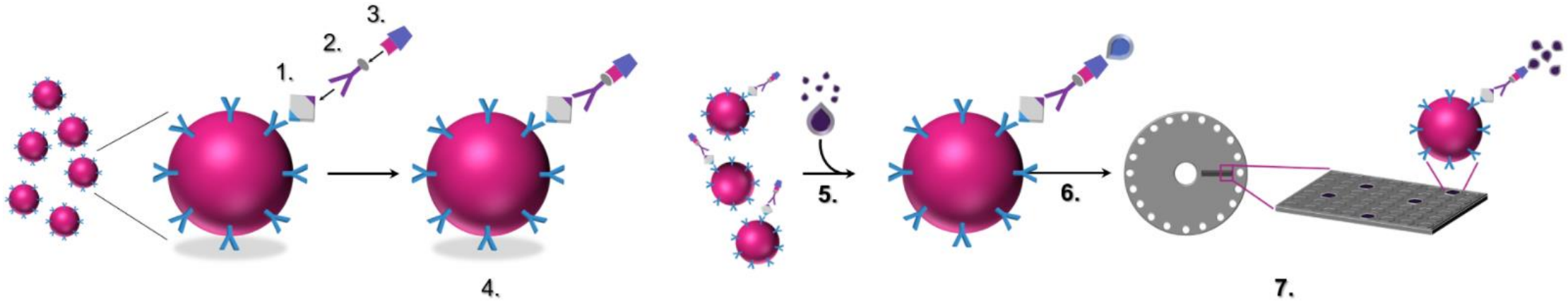
Evaluation réponse au traitement

Gavhed, PBC 2009
Kuhle, CCLM 2016

HL neurodégénérative – SIMOA

= complexes immunitaires « en sandwich » sur des billes paramagnétiques qui sont isolées dans des puits permettant une lecture «numérique» de chaque bille individuelle.

- Sensible (Fento gramme/ml)
- Permet dosage de NFL **dans le plasma/serum**



Etude Française: Temps 1 Etude pilote juin-Sept 2019

- En lien avec Equipe F Geissman / NY et une Star Up US (third rock adventure)

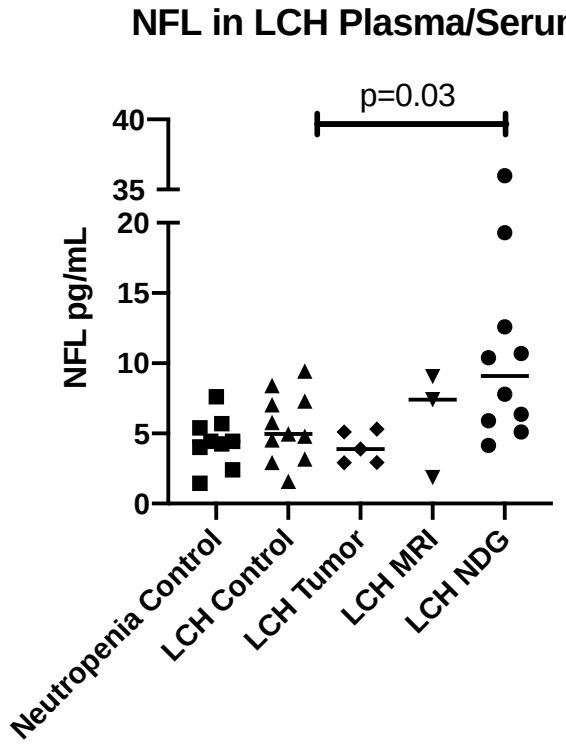
- Dosage fait à Boston / Quanterix
  - Contrôle 9 neutropenies ‘bénignes
  - 25 « LCH » diverses
    - Os sans atteinte neuro ni endocrino
    - Endocrino
    - Atteinte Neuro IRM
    - Atteinte Neuro Deg clinique

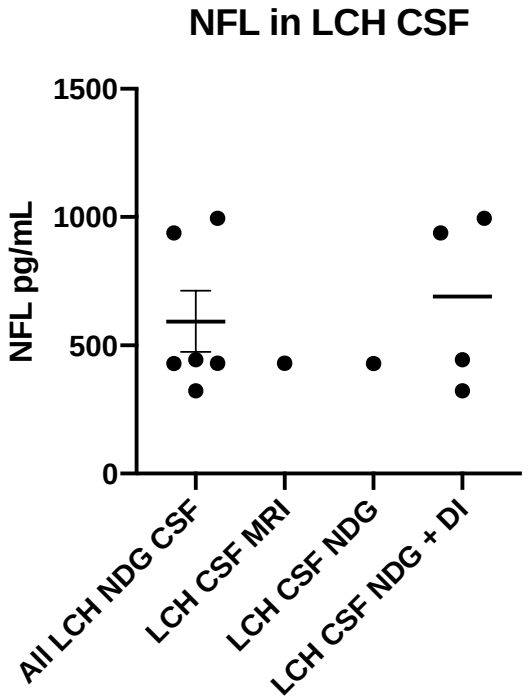
Breakdown of Pilot Study Samples

		N	M/F	Age (yr)	NFL range (pg/mL)
CSF	NDG	4	3M/1F	3.1-20.5	444-996
	NDG + DI	1	1M	27.7	323
	MRI	1	1F	7.0	431
	CSF total	**6**	**4M/2F**	**3.1-27.7**	**323-996**
Plasma	LCH Non-Neuro	2	2M	4.3-6.3	4.8-7.0
	LCH Tumor	1	1F	15.6	3.9
	LCH MRI	3	1F/2M	7.0-27.7	1.9-9.1
	LCH NDG	1	1M	3.1	10.7
	LCH NDG + DI	2	2M	18.4-18.5	5.9-7.8
	Plasma total	**9**	**7M/2F**	**4.3-27.7**	**1.9-10.7**
Serum	Neutropenia Control	9	7M/2F	1.4-32.2	1.4-7.6
	LCH Non-Neuro	9	7M/2F	1.1-10.7	1.6-9.4
	LCH DI	4	2M/2F	5.1-21.4	2.9-5.1
	LCH NDG	1	1F	15.6	6.4
	LCH NDG + DI	5	3M/2F	8.1-28.5	4.2-36
	LCH DI + Tumor	1	1M	21.4	2.9
	Serum total	**29**	**12M/7F**	**1.1-28.5**	**1.4-36**

- Sex bias in collection, 66% Male
- 4 subjects with paired CSF/plasma collections
  - 2 collected at same time
  - 1 collected day after plasma
  - 1 collected 1 weeks after plasma

NFL Levels in Plasma/Serum and CSF

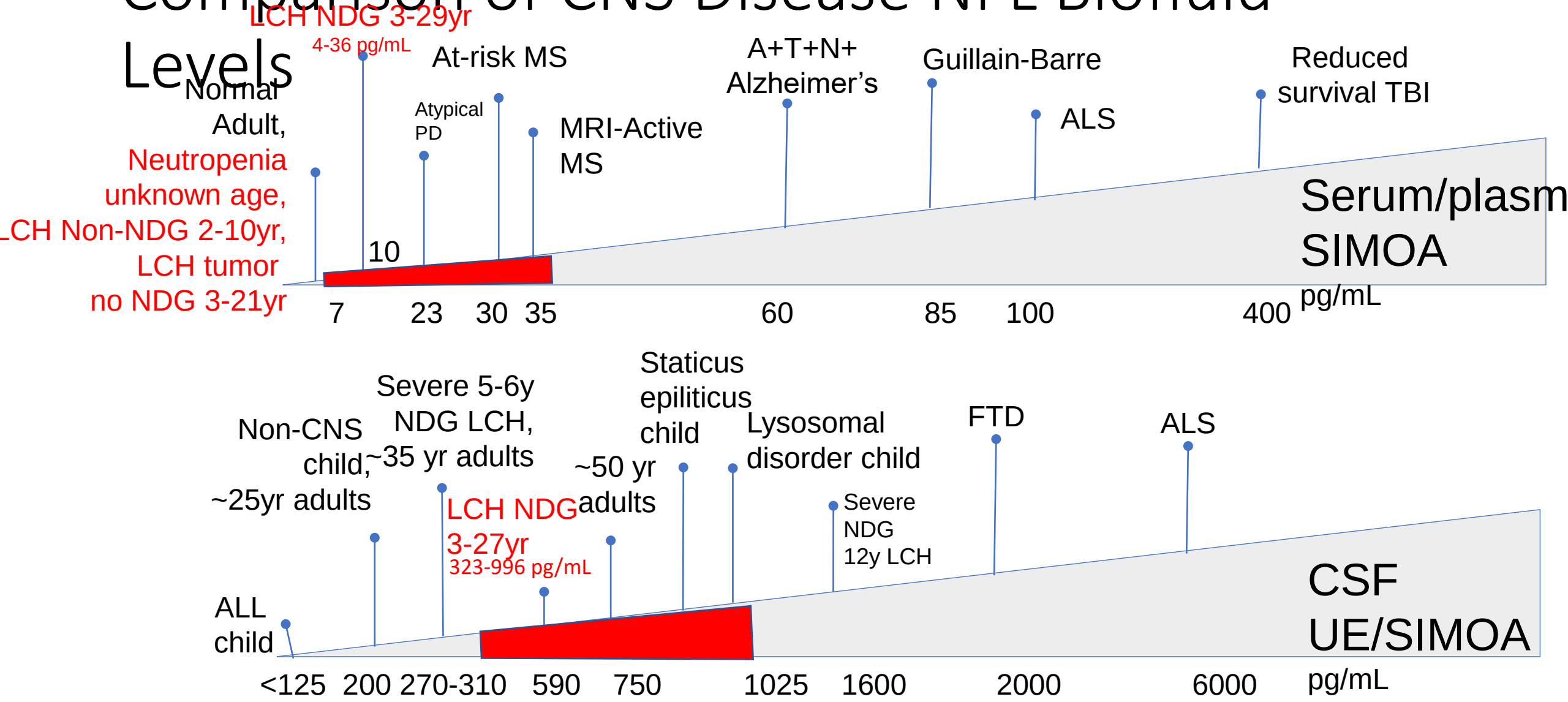




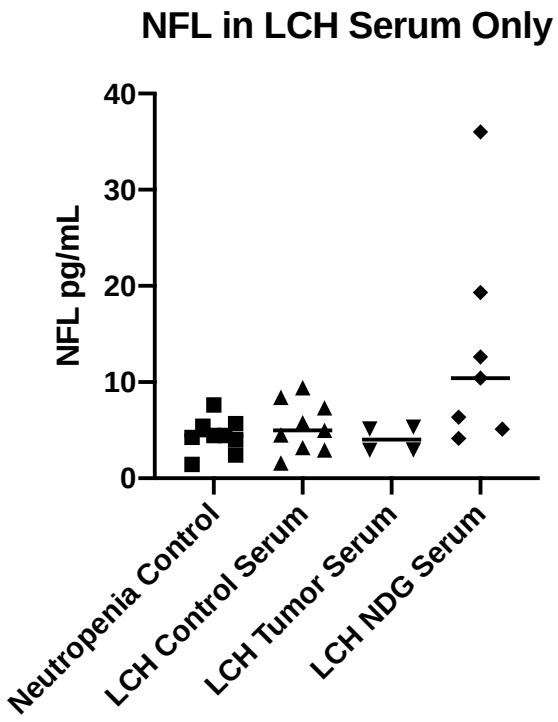
>23 pg/mL corresponds to atypical PD
>30 pg/mL corresponds to at-risk to MRI-Active MS

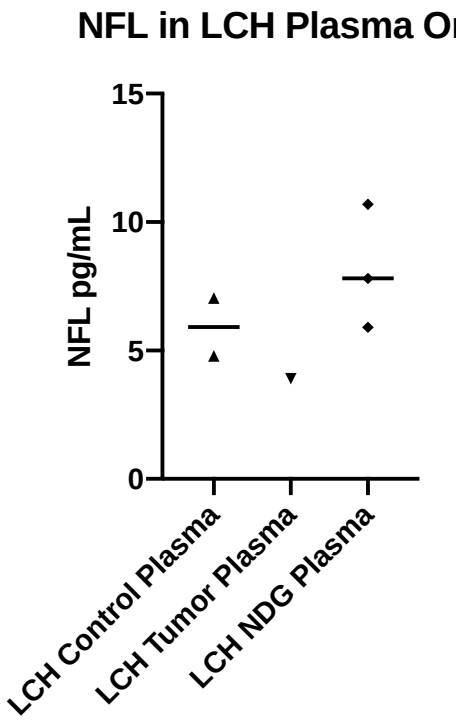
“Normal” pediatric CSF levels are <125pg/mL

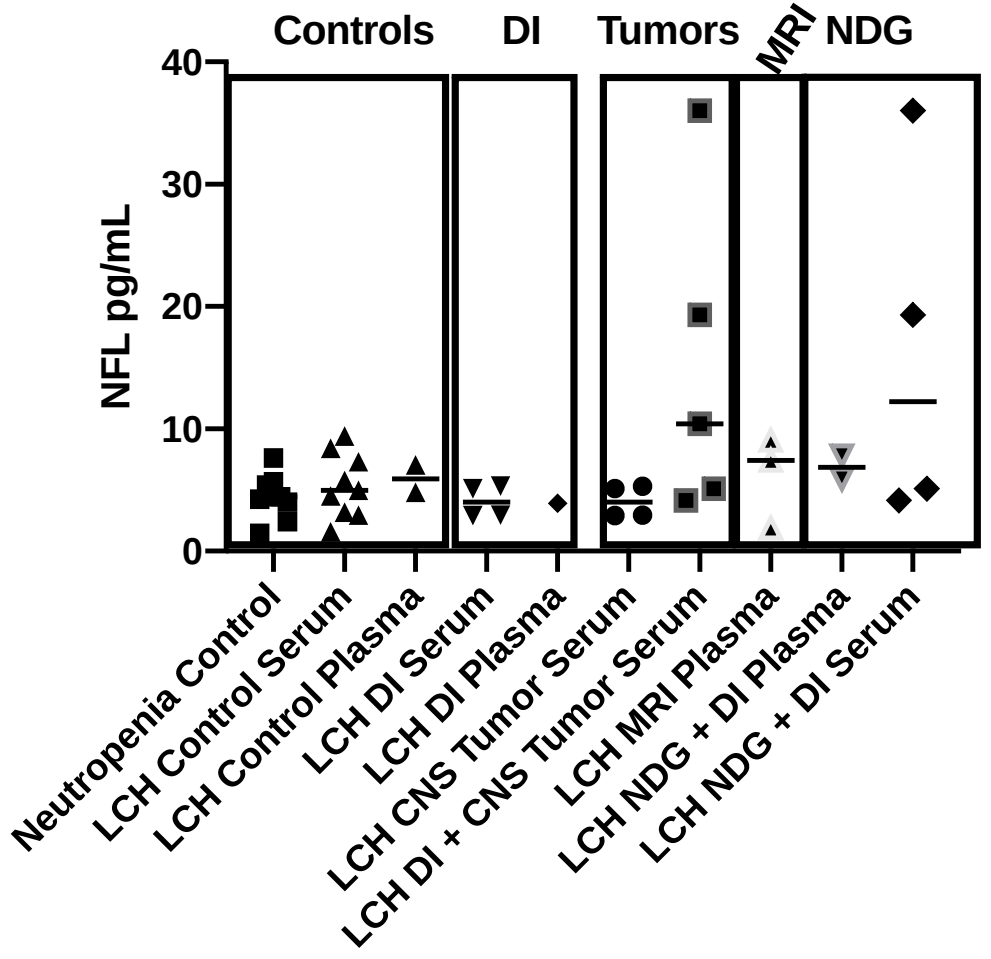
Comparison of CNS Disease NFL Biofluid Levels



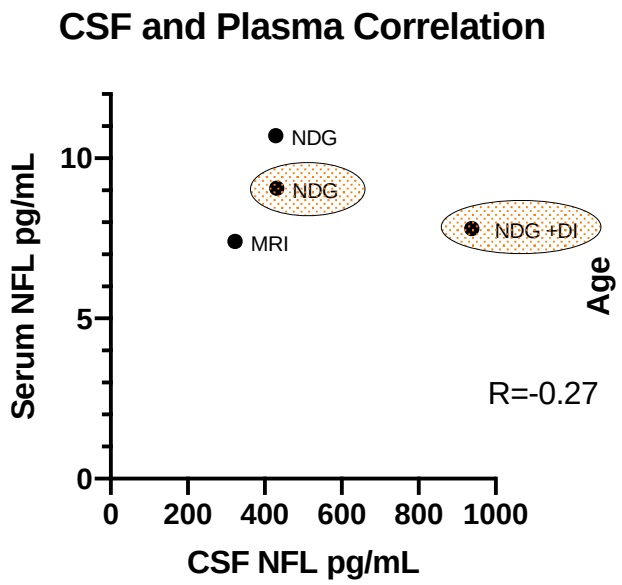
Data by Matrix, Subgroups

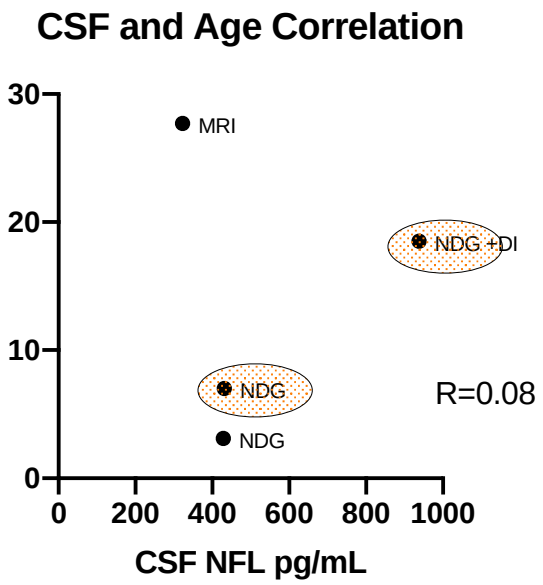


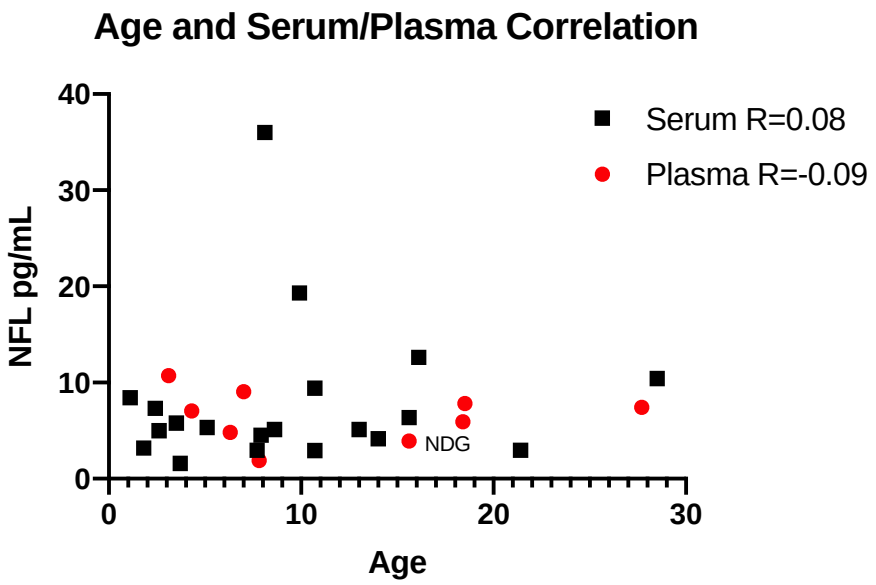




Lack of Correlation of CSF NFL and Age or to Plasma/Serum NFL







Orange shading indicates CSF, plasma samples were collected 1-7d apart

NFL LCH Pilot Results

- Topline
  - CSF NFL levels are 323-996pg/mL, corresponding to an elevation consistent children with status epilepticus or LSDs, and ~50yr old CNS normal adults
  - No obvious elevation of NFL observed in serum or plasma of LCH non-CNS controls, or LCH patients with DI or tumors but no neurodegeneration (1.6-9.4pg/mL)
  - LCH patients with neurodegeneration +/- DI or tumors have higher plasma/serum NFL levels (4.15-36.0pg/mL)
  - No trends towards correlation were observed between NFL levels and age or CSF and plasma/serum NFL, or BRAF mutation status and NFL levels

- Followup study concepts
  - Repeating the CSF NFL elevation signal, longitudinal measures
  - Expanding the specific DI or Tumor cohort to confirm lack of signal
  - Inclusion of some pediatric CSF control group
  - Study selection
    - More complete annotation/selection of cohort
    - Sole use of plasma or serum samples
  - Nice to have: more matched CSF/plasma to serum samples

NFL LCH Pilots Study Caveats & Details

- Caveats
  - Sample sizes were small in the groups, ranging from N=4 (LCH MRI) to N=9 (LCH NDG and Neutropenia Controls)
  - Groups were comprised of both plasma and serum samples, reducing the N per subgroup
  - No CSF control group
  - Lack of detailed clinical annotation: duration of disease, extent of CND tumors, CNS symptoms, Tx history, BRAF genotype for all cohort etc.
- Data details
  - Samples were run at 1:100x dilution for CSF and and 1:4 for plasma and serum
  - All test samples were reconciled
    - One extra tube that had a unclear vial designation was also sent but not included in the analysis
  - Variance was low with CV of 5% for the samplese and <5% for controls

conclusion intermédiaire:

- Il y a un signal spécifique ‘neuro dégénératif’
  >> continuer à accumuler des échantillons..

- Il faut monter une étude !

→ Histio target… des financements … des collaborations
fin 2019→ juin 2021…

→ Collaboration avec équipe Imagine / F Rieux Leucat

HL neurodégénérative – Résultat 1ère série (sept 2021)

- Réalisation au laboratoire d'immunogénétique du Dr Rieux-Laucat, **Institut Imagine**
  Ingénieur recherche : Camille Brunaud

- Etude de **la 1ère série :** n = **12 LCR**
  n = **23 Plasmas**

- Echantillons contrôles : 3 LCR (LAL)
  3 plasmas (1 LAL, 1 thalassémie, 1 neutropénie)

HL neurodégénérative – Résultat 1ère série (sept 2021)

- **Echantillons contrôles :**

LCR : valeur ≤380 pg/ml (2 patients LAL sans atteinte ND)

2759 pg/ml: LAL avec atteinte neurotoxique/ tumorale++

Plasma : valeur <7,5 pg/ml : 2 patients

valeur 20,6 pg/ml : post cure consolidation LAL (MTX HD, Ifosfamide, IT triple)

- **Echantillons patients :**

ND+ : LCR n=11 : valeur médiane **315,8** pg/mL [87-1127]

Plasma n=13 : valeur médiane **8,9** pg/mL [2,9- 35,8]

ND- : LCR n=1 : **62** pg/mL

Plasma n=10 : valeur médiane **7,2** [2,7- 133,8] pg/mL

HL neurodégénérative – Conclusion 1ère série (sept 2021)

- Etablissement de valeurs seuils

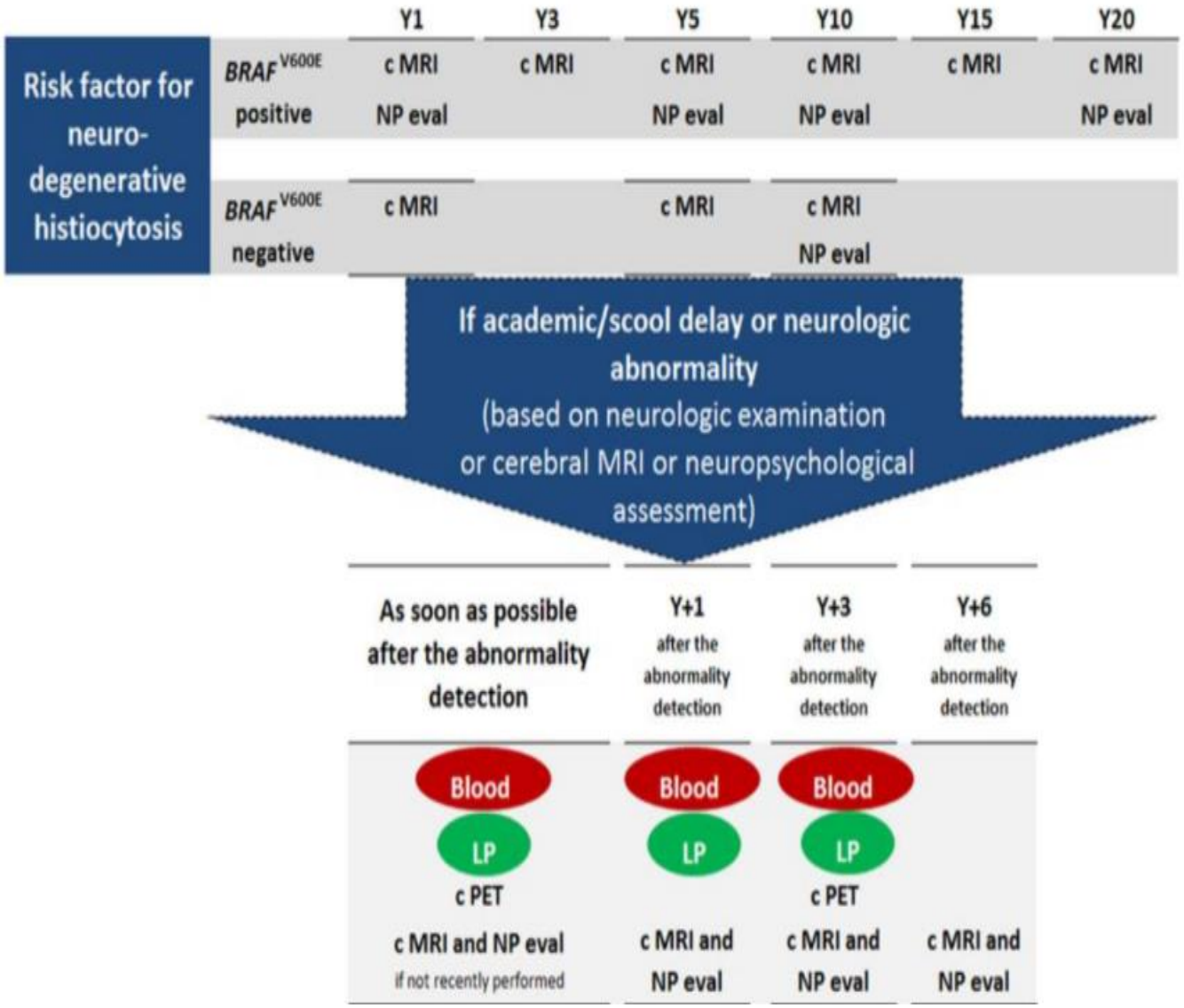
LCR (pg/ml) :
≤ 125 : strictement normal
125-380 : zone de chevauchement
≥ 380 : anormalement élevé

Plasma (pg/ml) :
≤ 4,5 : strictement normal
5-9,5 : zone de chevauchement
≥ 9,5 : anormalement élevé

- A compléter avec les échantillons témoins de séries futures

  (éviter les patients LAL traités par chimiothérapies MTX HD)
- Mise au point du dosage ostéopontine : en cours
- Suivi longitudinal +++

HL neurodégénérative – HISTIO target 2020-2030



Merci de votre attention

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