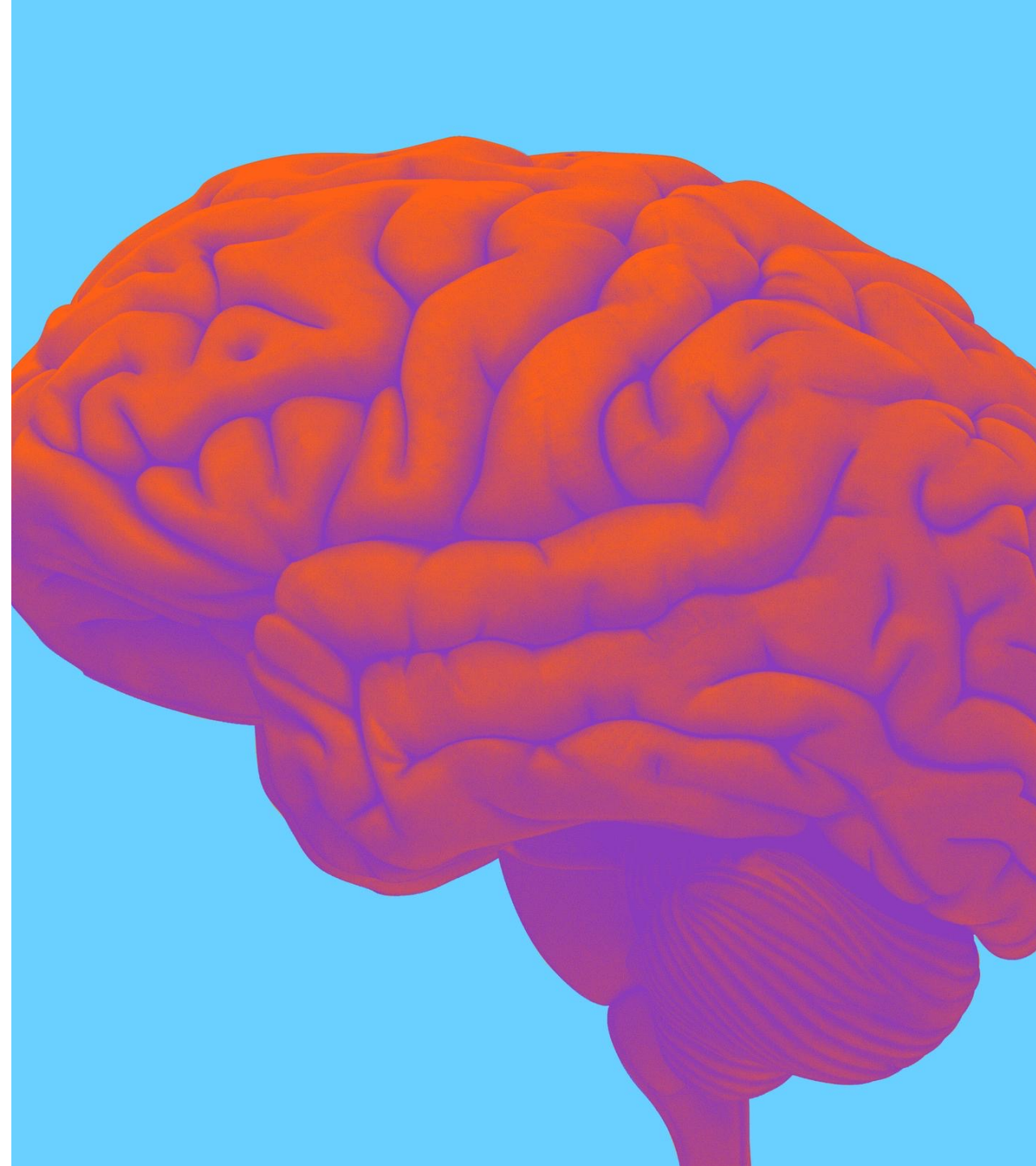


# Translational Approach of the Clinical and Nonclinical Evaluation and Qualification of Blood-based Biomarkers of Drug-induced Neurotoxicity: An IMI TransBioLine project

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May 23, 2025**

# INTRODUCTION



## Neurotoxicity

Any adverse effect on the structure or function of the central and/or peripheral nervous systems produced by a biological, chemical, or physical agent

## Neurotoxic effects

Identified and characterized at one of many levels of organization of the nervous system, including the chemical, anatomical, physiological, and neurobehavioral levels

# Neurotoxicity testing

## Preclinical Development

## Clinical trials

### Functional assessments

neurobehavioral assessment  
seizurogenic assessment

neuropsychological assessment  
patient-reported outcomes  
MRI, fMRI, CT imaging

### Morphological biomarkers

organ weights  
gross observations  
histopathology

biopsies with histopathology

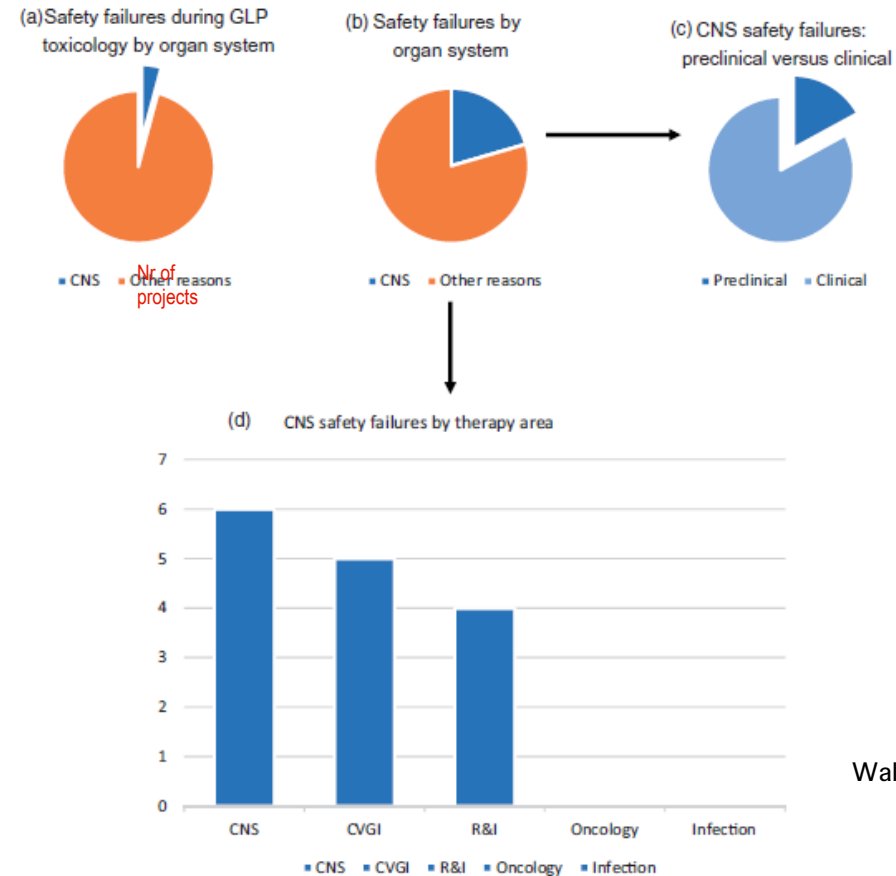
# Challenges of determining neurotoxicity of novel drug candidates

- Current gaps in the classical assessment of neurotoxicity:
  - Lack of specificity in neurobehavioral observations (physiological, pharmacological or non-nervous system actions of a compound)
  - Lack of sensitivity and quantitative nature of histology
  - Invasive nature of biopsy and lack of longitudinal measurements due to limited sampling
  - Lack of translational character from preclinical to clinical (species specificity)
- New drug candidates with novel MoA:
  - Multiple potential targets of neurotoxicity working through multiple and unknown mechanisms

# Current challenges/gaps result in high attrition rates due to neurotoxicity

Attrition rate and adverse side-effects of drugs in clinical development:

- up to 25% due to neurotoxicity
- loss of revenue
- loss of effective drugs reaching the clinic



Walker et al, 2018

**Need for easy to use, sensitive and specific fluid-based biomarkers of neurotoxicity**



 **TransBioLine**  
Translational Safety  
Biomarker Pipeline

**Translational Safety Biomarker  
Pipeline**

**TransBioLine**

<https://transbioline.com/>

<http://www.imi.europa.eu>

 TransBioLine

**Enabling development and implementation of novel safety biomarkers in clinical trials and diagnosis of disease**

# TransBioLine Beneficiaries

## University hospitals (12)



## SME (7)



## EFPIA (7)



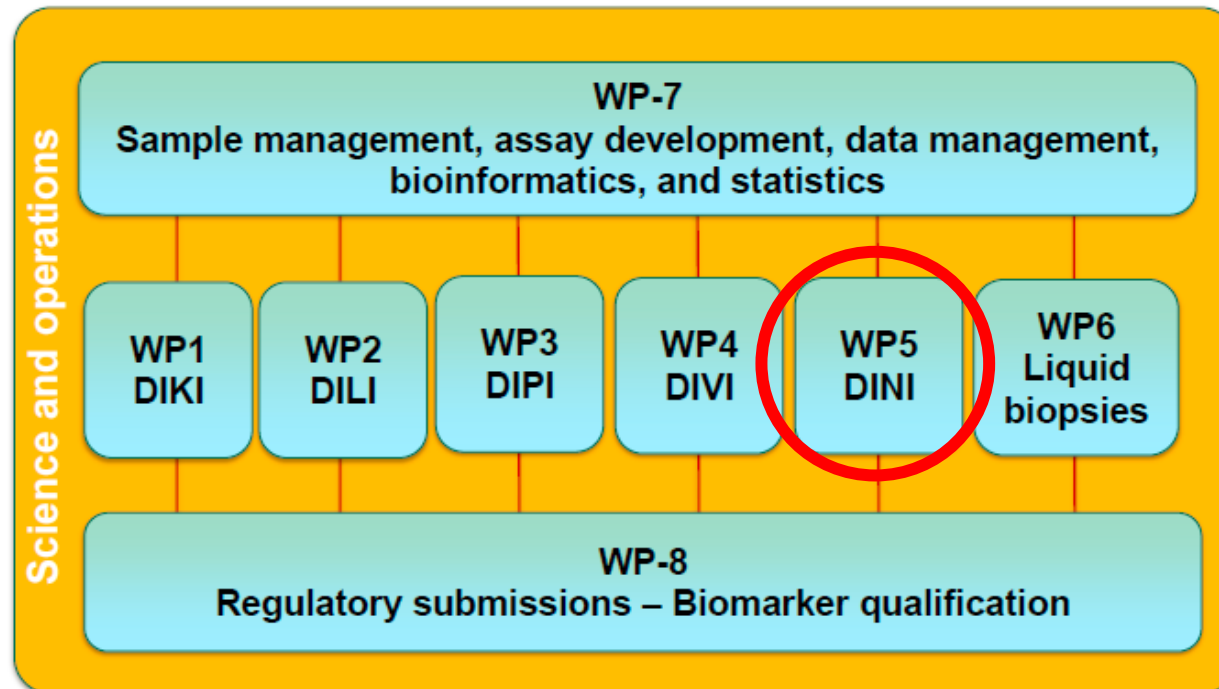
## Non-profit organisations (3)



# TransBioLine: Kick-off: Feb 28, 2019

Objective DINI [**D**rug-**I**nduced **N**eurological **I**njury]:  
General Nervous System Toxicity (CNS and PNS)

To qualify blood-based biomarkers of drug-induced neurotoxicity to enable decision-making in early clinical trials with neurotoxicity risk



# Biomarkers of Neurotoxicity

## Context of use (CoU)

- A blood-based safety biomarker or biomarker panel to aid in the detection of acute drug-induced Nervous System injury risk in Phase 1 trials in healthy volunteers when there is an a priori concern that a drug may cause nervous system injury in humans.

## Specific goals

- Characterize and validate assays for selected neurotoxicity biomarkers
- Characterize reference ranges of biomarkers in healthy volunteers, and assess influence of variables like age, gender, ethnicity, ...
- Evaluate performance/sensitivity of biomarkers in a human nervous system injury and/or disease conditions (e.g. TBI, MS, CiPN; optional: AD, PD)

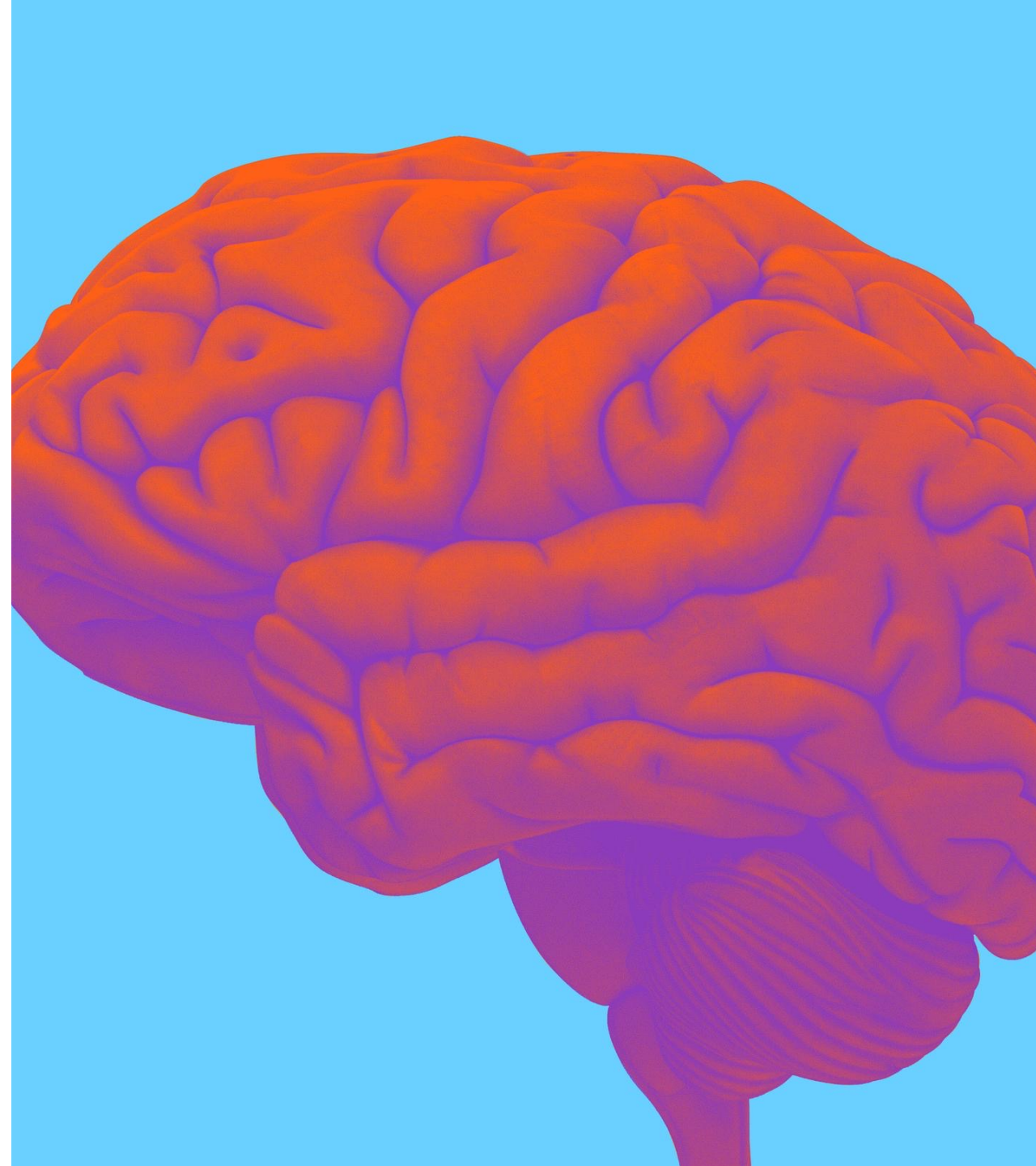
## Academic grant support

- Assay development & validation expertise, assay conduct, sample management, data analysis, ...

## In kind industry member support

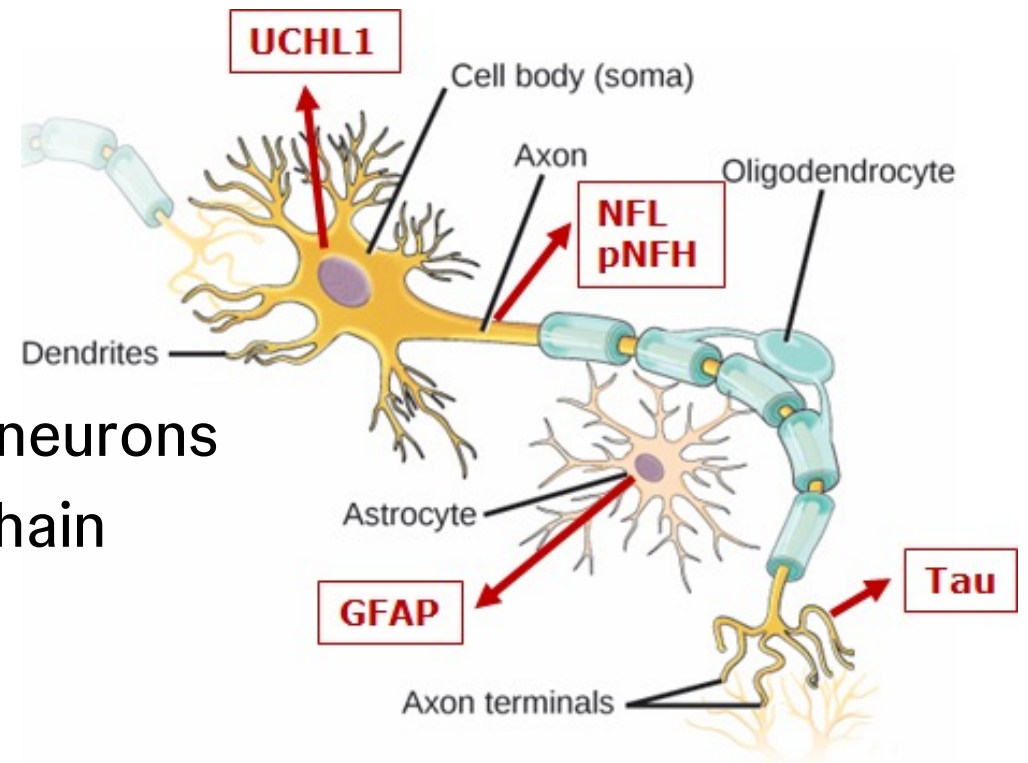
- Samples from clinical trials, preclinical studies, data analysis

# MATERIAL & METHODS



# Qualification of 5 nervous system-derived proteins as serum-based safety biomarkers of drug-induced NS neurotoxicity

<b>GFAP</b>	glial fibrillary acidic protein
<b>UCH-L1</b>	ubiquitin C-terminal hydrolase L1
<b>NfL</b>	neurofilament light chain: cytoskeletal protein expressed only in neurons
<b>pNfH</b>	phosphorylated neurofilament heavy chain
<b>Tau</b>	microtubule associated protein tau



These proteins are often associated with CNS injury or disease (literature)

[https://commons.wikimedia.org/wiki/File:Figure\\_33\\_02\\_13.jpg](https://commons.wikimedia.org/wiki/File:Figure_33_02_13.jpg)

# Background information

## **Cohorts**

Controls (healthy volunteers)

MS patients (inflammation)

Chemotherapy-induced NS toxicity (neurotoxicity)

Traumatic Brain Injury (mechanical trauma)

## **Preclinical experts**

In collaboration with clinical experts

# Planned Human Samples as per Final Research Plan (2019)

## Healthy volunteer serum samples

100 serum samples

3 time points (n=10)

For reference range of the 5 biomarkers

For variability of biomarker levels: gender, age, intra-individual variability

If low variability: 270 (x1) cross-sectional serum samples

## Patient samples

Patients with chemotherapy induced neurotoxicity:

50 serum samples from patients undergoing chemotherapy  
pre- and post-treatment

50 serum samples from control patients at baseline and after 5M

Patients with multiple sclerosis (MS):

100 CIS (Clinically Isolated Syndrome)/early MS patients at baseline and after 1 year of follow-up

Patients with traumatic brain injury (TBI):

100 serum samples collected within 12 hours of injury (observational study)

# Assays

## **Ultra-sensitive SIMOA digital ELISA assays** (Quanterix Corporation, Billerica, MA)

- Multiplexed Human Neurology 4-Plex A
  - NfL (Neurofilament Light)
  - Tau
  - UCH-L1 (Ubiquitin carboxyl-terminal hydrolase L1)
  - GFAP (Glial fibrillary acidic protein)
- Single-plex Simoa pNF-heavy assay (Stand-alone assay on SIMOA™ platform)
  - pNfH (phosphorylated neurofilament heavy chain)

SIMOA: Technology using arrays of femtoliter-sized reaction chambers (**single-molecule arrays** (SIMOA™)): Isolation and detection of single enzyme molecules  
Detection of analytes at femtogram (fg/mL) concentrations,  
1000-fold improvement in sensitivity over conventional ELISA's

Sensitivity: LLOQs 0.5 to 10 fg/ml

# Additional workstreams

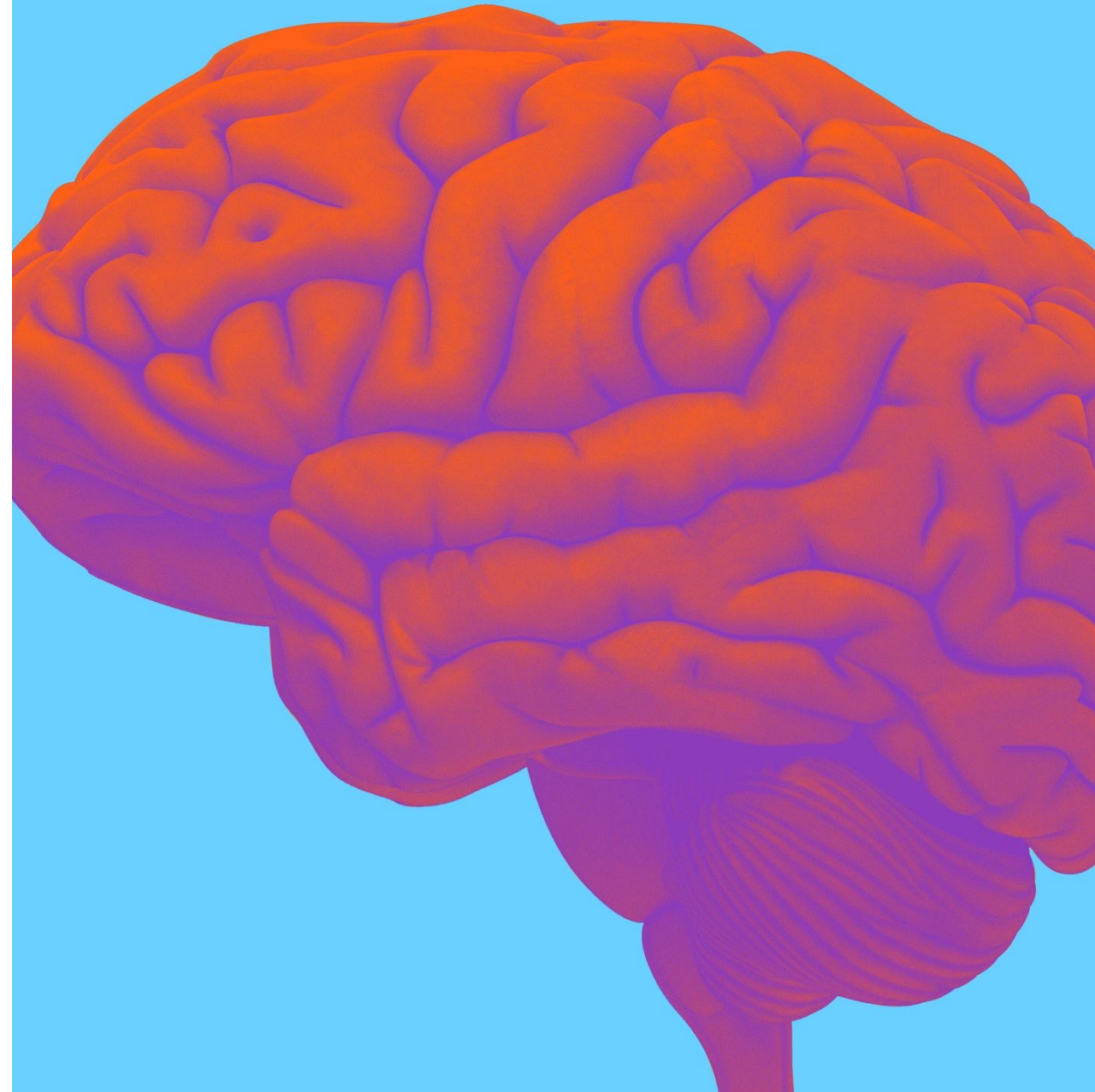
## **Supporting preclinical studies**

Rodent studies with diverse mechanisms of neurotoxicity and targets  
(linkage to certain brain cell type or PNS/CNS localization of injury)

## **Exploratory study to identify miRNAs potentially associated with neurotoxicity**

How are miRNA changes in CSF and serum correlated in patients with MS?  
[measured by Next Generation Sequencing (NGS)]

# RESULTS



# Assays

**Validation all assays in human serum completed (NMI/Signatope)  
Validation Reports finalized (NMI/Signatope)**

SIMOA 4-plex A

- NfL (Neurofilament Light)
- Tau
- UCH-L1 (Ubiquitin carboxyl-terminal hydrolase L1)
- GFAP (Glial fibrillary acidic protein)

Single-plex Simoa pNF-heavy assay

- pNfH (phosphorylated Neurofilament Heavy)

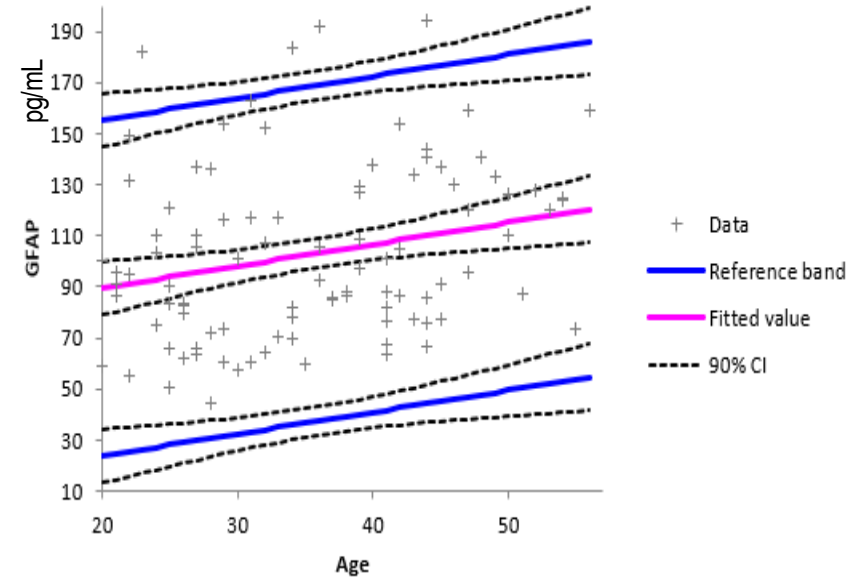
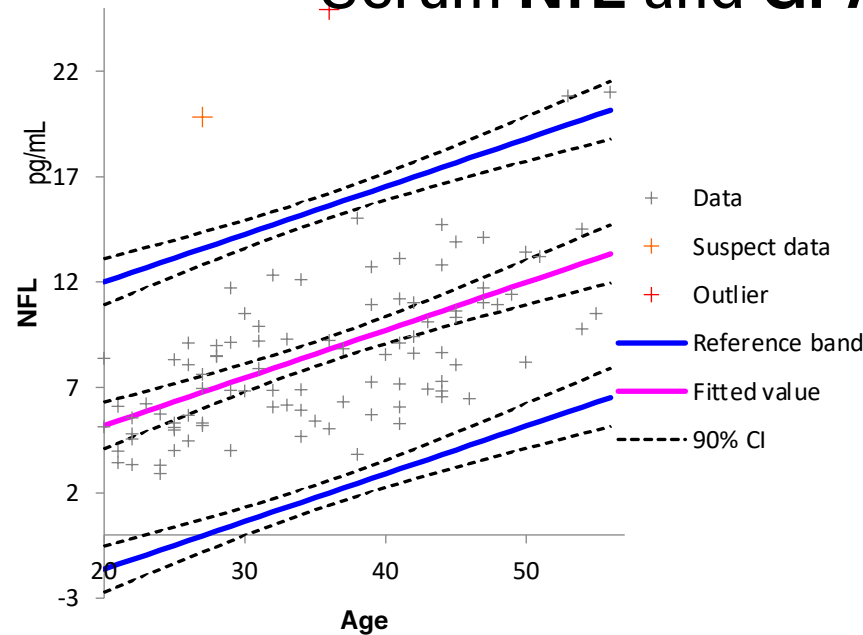
**NGS workflow developed (TAmiRNA)**

- For detection and absolute quantification of microRNAs in CSF and plasma

# Clinical Data from healthy volunteers

## Healthy Volunteers

Serum **NfL** and **GFAP** levels: dependent on age



CLSI<sup>[1]</sup>-recommended analysis, as implemented by Geffre et al<sup>[2]</sup>

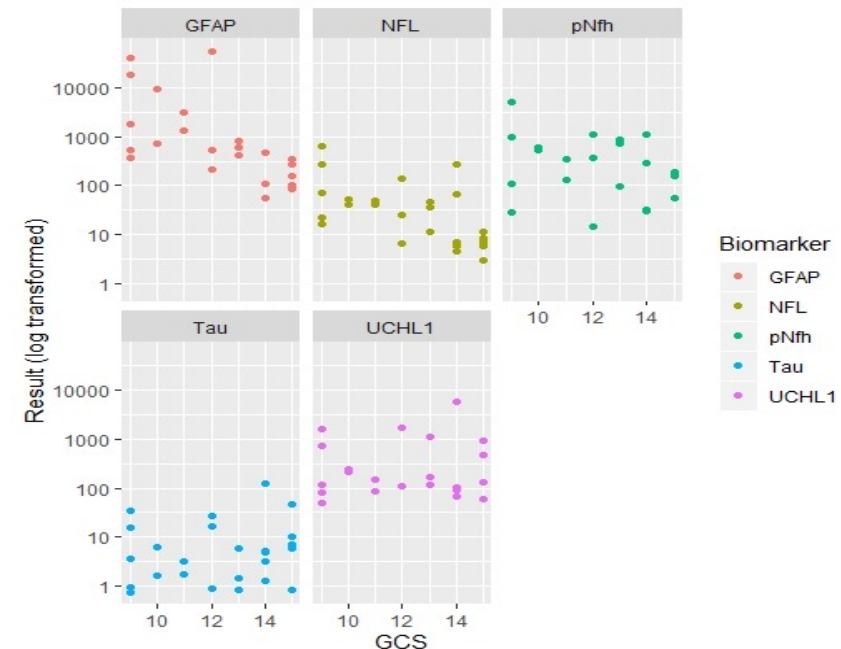
**Tau:** not affected by age (data not shown)  
**UCH-L1:** not detectable in HV (data not shown)



# Clinical Data per subset of patients

## Traumatic Brain Injury

- University of Michigan/Pfizer collaboration
- General linear model (GLM) used to assess association 5 biomarkers and GCS scores
- 4 different configurations of covariates (combinations using age, gender, hemorrhage status, intoxication, consciousness, concussion status)



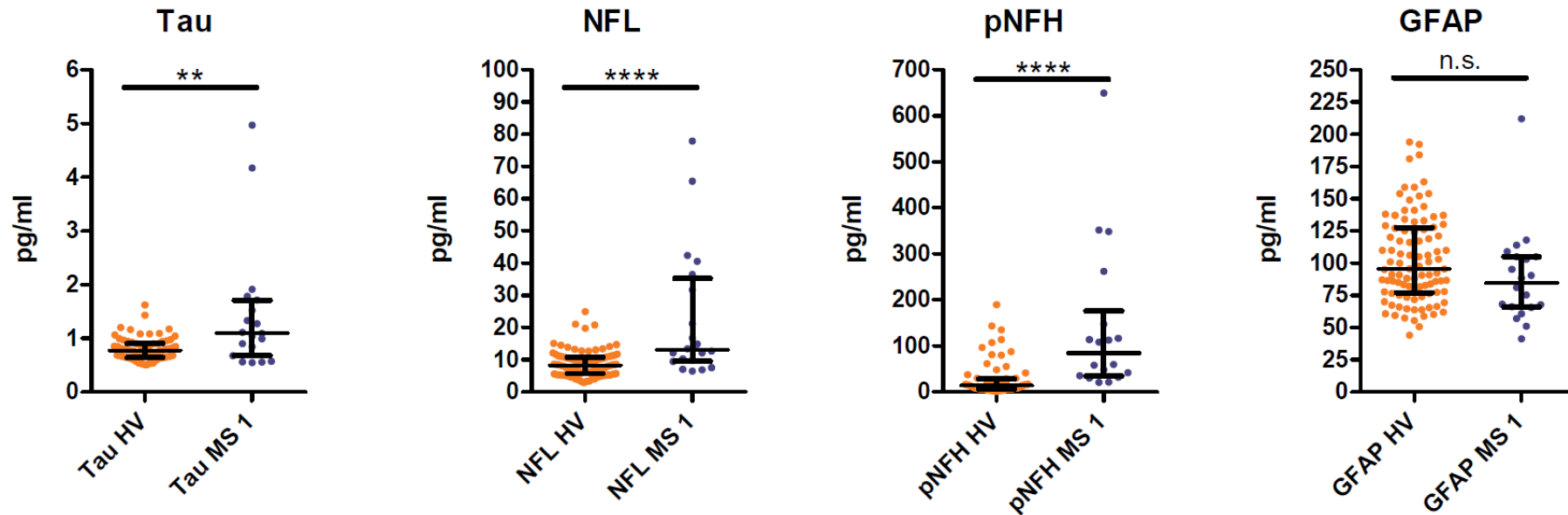
**Strong correlation (statistically significant association) for serum NfL and GFAP with GCS scores in TBI patients across models**

# Clinical Data per subset of patients

## Multiple Sclerosis

n=20 patients with MS vs. 97 healthy controls

Statistics based on age-adjusted comparisons



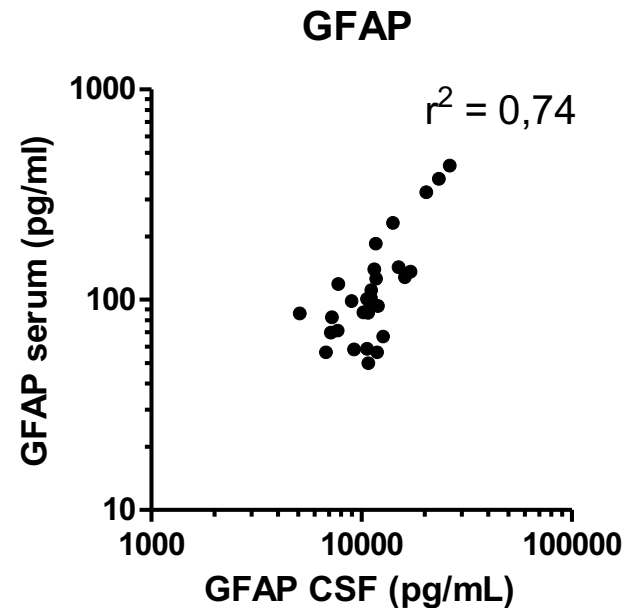
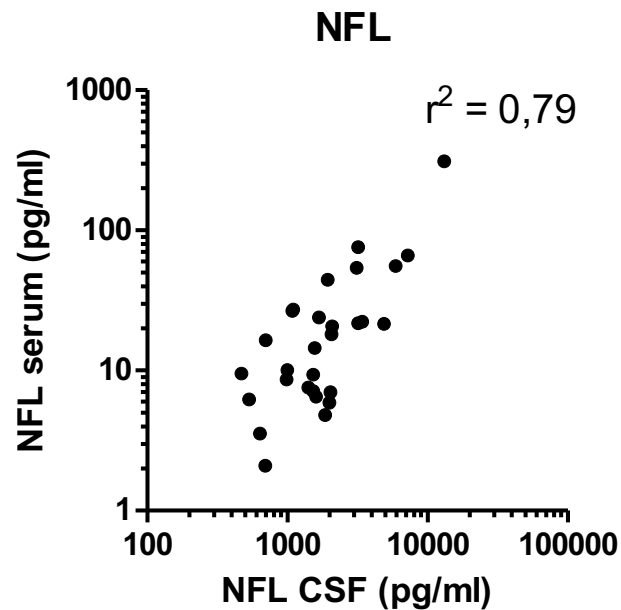
**Serum Tau, NfL and pNfH but not GFAP are significantly increased in MS cohort compared to HVs**

# Clinical Data per subset of patients

## Multiple Sclerosis/other

### Preliminary CSF/serum correlations

22 MS patients, 8 patients with other neurological diseases



**Initial data sets confirm reported CSF/serum correlations for 2 out of 5 biomarkers (NfL and GFAP) in patients with neurological diseases**

# Clinical Data per subset of patients

## Chemotherapy-induced polyneuropathy

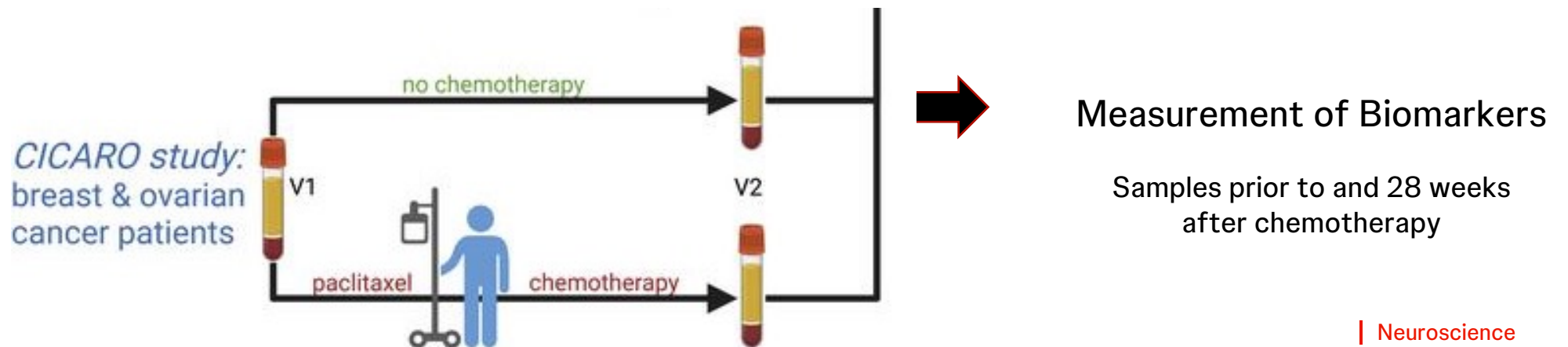
JCI insight

CLINICAL MEDICINE

Published: March 22, 2022

### Neurofilament proteins as a potential biomarker in chemotherapy-induced polyneuropathy

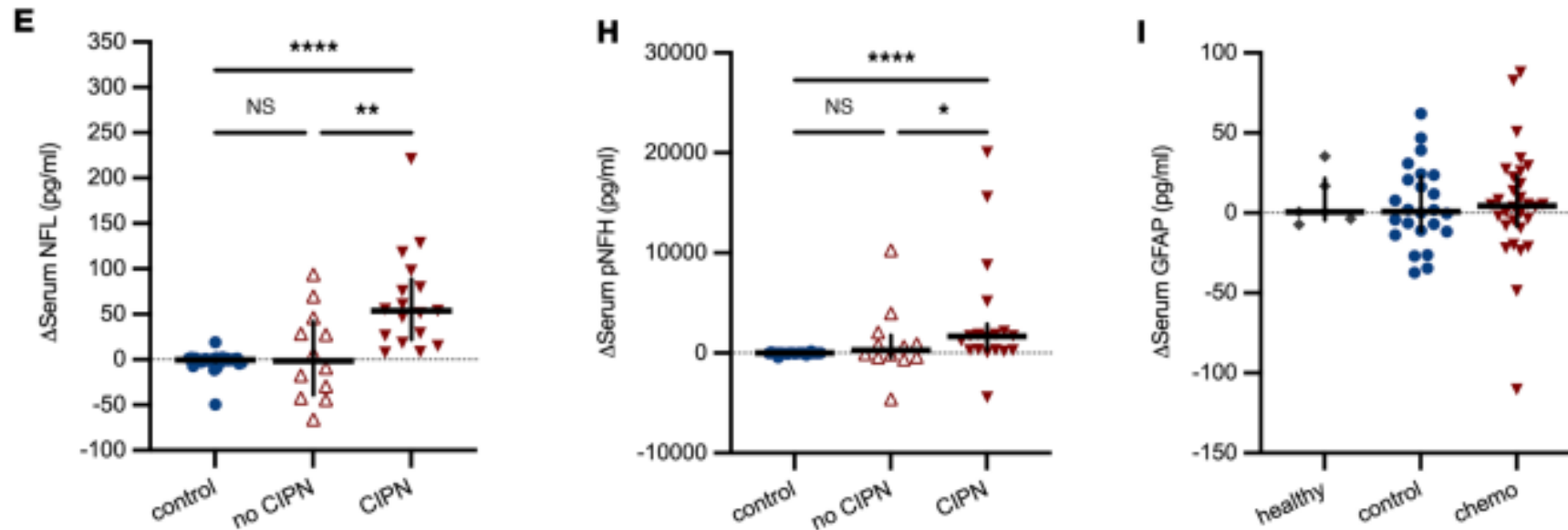
Petra Huehnchen,<sup>1,2,3</sup> Christian Schinke,<sup>1,3</sup> Nikola Bangemann,<sup>4</sup> Adam D. Dordevic,<sup>1</sup> Johannes Kern,<sup>1</sup> Smilla K. Maierhof,<sup>1</sup> Lois Hew,<sup>1,5</sup> Luca Nolte,<sup>1</sup> Peter Körtvelyessy,<sup>1,6</sup> Jens C. Göpfert,<sup>7</sup> Klemens Ruprecht,<sup>1</sup> Christopher J. Soms,<sup>8</sup> Jens-Uwe Blohmer,<sup>9</sup> Jalid Sehouli,<sup>10</sup> Matthias Endres,<sup>1,2,3,11,12,13</sup> and Wolfgang Boehmerle<sup>1,2,3</sup>



# Clinical Data per subset of patients

## Chemotherapy-induced polyneuropathy

Huehnchen et al., 2022



**Patients with paclitaxel-based chemotherapy who develop chemotherapy-induced polyneuropathy (CIPN) show increases in serum NfL and pNfH, but not GFAP (or Tau)**

# Preclinical studies

## Performance of NF-L, GFAP, NSE and TAU in in blood and cerebrospinal fluid in rat for the detection of nervous system injury

Katerina Vlasakova, Christopher J Somps, Takayuki Tsuchiya, Thomas Forest, Richard Burke, Ramesh Kovi, Cheryl Tyszkiewicz , Klemens Ruprecht, Lorenzo Di Cesare Mannelli , Warren E. Glaab

Front. Neurosci., 16 January 2024 Sec. Translational Neuroscience, Volume 17 – 2023. <https://doi.org/10.3389/fnins.2023.1285359>

### Outlines:

Evaluation NFL, GFAP, NSE (Neuron Specific Enolase) and total Tau in rats

Plasma/serum and CSF sampling

Histopathologic assessments

15 preclinical studies: CNS toxicity (a.o. trimethyltin and 2-chloropropionic acid-CPA)

PNS toxicity (a.o. doxorubicin and acrylamide)

# Preclinical studies

## Performance of biomarkers NF-L, NSE, Tau and GFAP in blood and cerebrospinal fluid in rat for the detection of nervous system injury

Katerina Vlasakova et al. 2024

### Conclusion:

**NFL:** Most sensitive biomarker with dose- and time-dependent increase after drug-induced CNS and PNS injury

Cannot inform on the original site of the injury

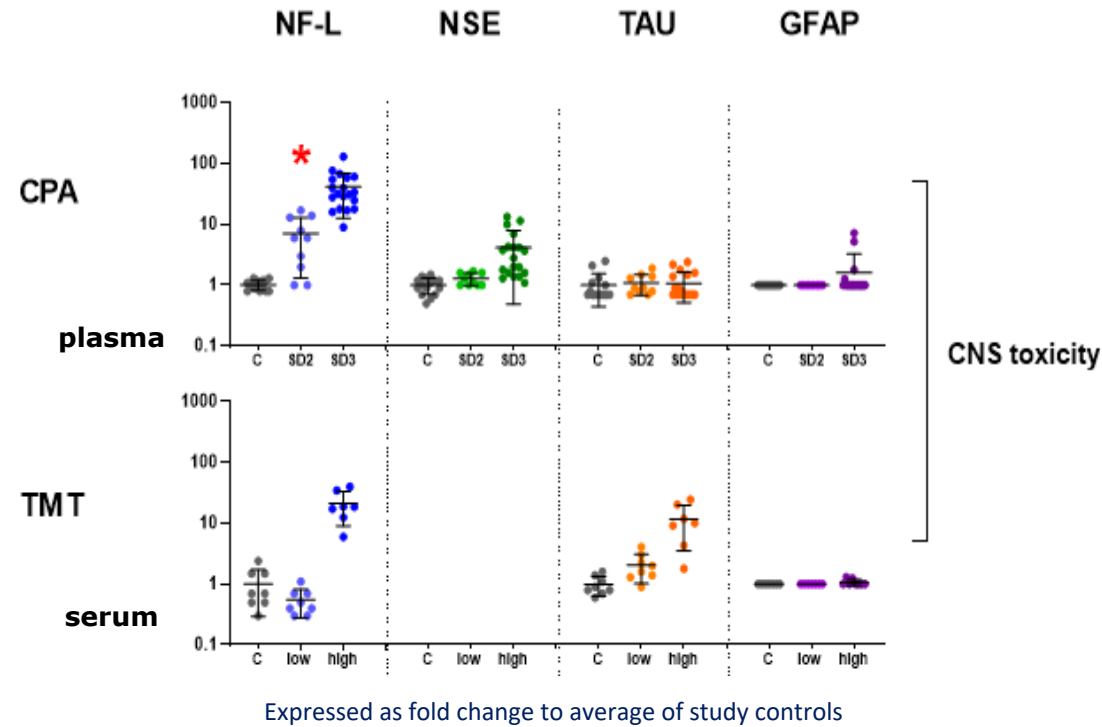
**Tau and NSE:**

Can provide additional information of the origin of NS injury (analysis in both plasma and CSF)

# Preclinical studies

Performance of biomarkers NF-L, NSE, Tau and GFAP in blood and cerebrospinal fluid in rat for the detection of nervous system injury. Katerina Vlasakova et al. 2024

Biomarker performance in plasma or serum of two studies with CNS injury



NfL, Tau and GFAP are increased with neurotoxicity in rat plasma/serum or CSF

# Exploratory study to identify miRNAs potentially associated with neurotoxicity

DINI – 32 CSF-enriched miRNAs were identified



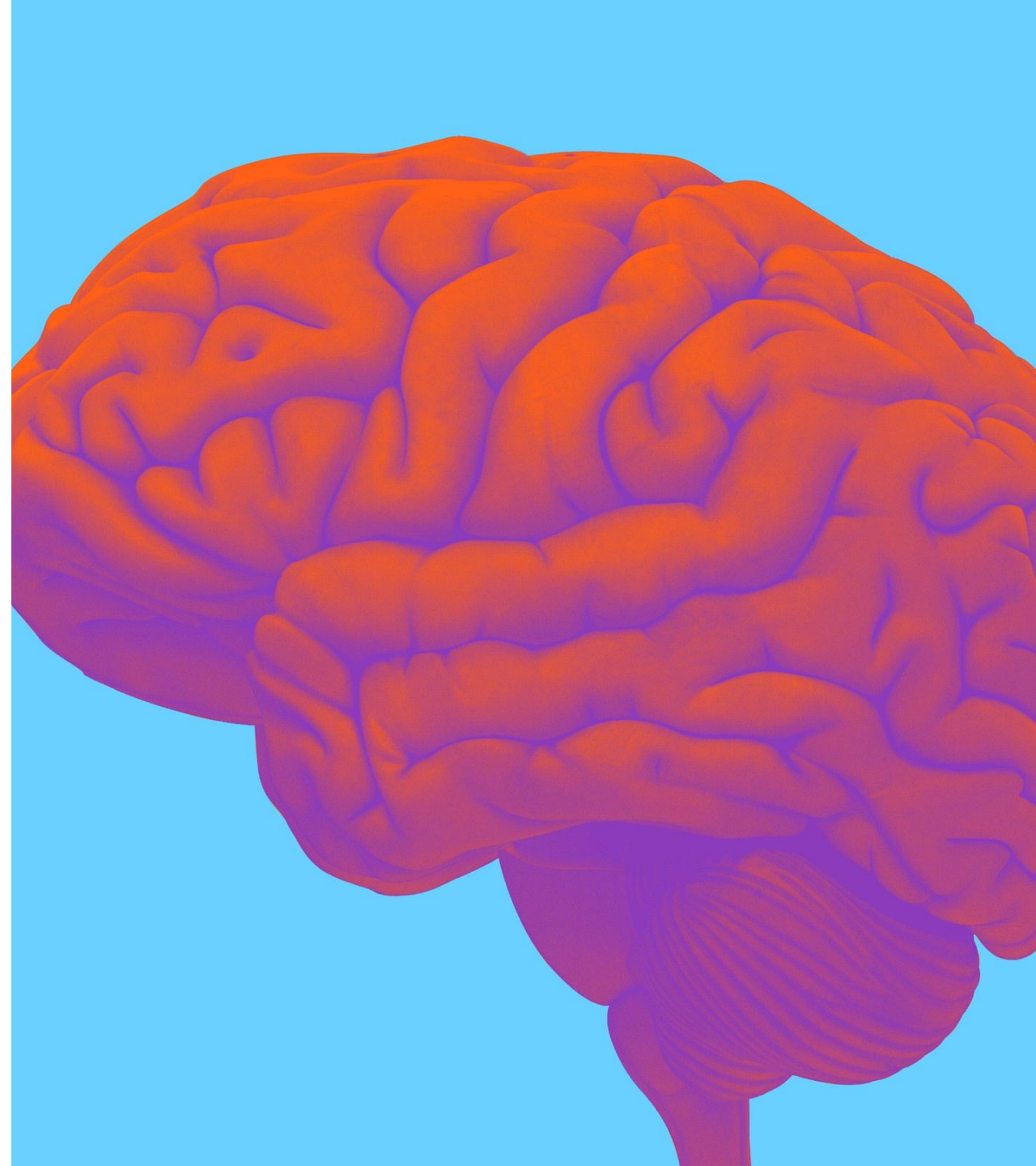
Rank	microRNA	Fold Change (CSF/Serum)	Avg concentration in CSF (molecules/ $\mu$ L)
1	hsa-miR-1911-5p	785.1	407.1
2	hsa-miR-1298-5p	270.1	380.1
3	hsa-miR-767-5p	81.6	15.1
4	hsa-miR-448	66.3	9.8
5	hsa-miR-204-5p	43.8	17170.7
6	hsa-miR-34b-3p	29.7	108.7
7	hsa-miR-1264	18.7	145.8
8	hsa-miR-4705	9.3	2.1
9	hsa-miR-124-3p	8.9	768.1
10	hsa-miR-129-2-3p	8.8	5.2
11	hsa-miR-34b-5p	7.8	1047.9
12	hsa-miR-488-3p	6.2	5.5
13	hsa-miR-219a-2-3p	6.1	11.8
14	hsa-miR-204-3p	5.7	731.2
15	hsa-miR-34c-3p	5.5	221.5
16	hsa-miR-34c-5p	5.5	1316.3
17	hsa-miR-9-3p	3.2	294.3
18	hsa-miR-449b-3p	2.9	3.4
19	hsa-miR-549a-3p	2.9	3.6
20	hsa-miR-11181-3p	2.8	1.3
...			

Top 20 candidates

$$\frac{CSF}{SERUM} > 1$$



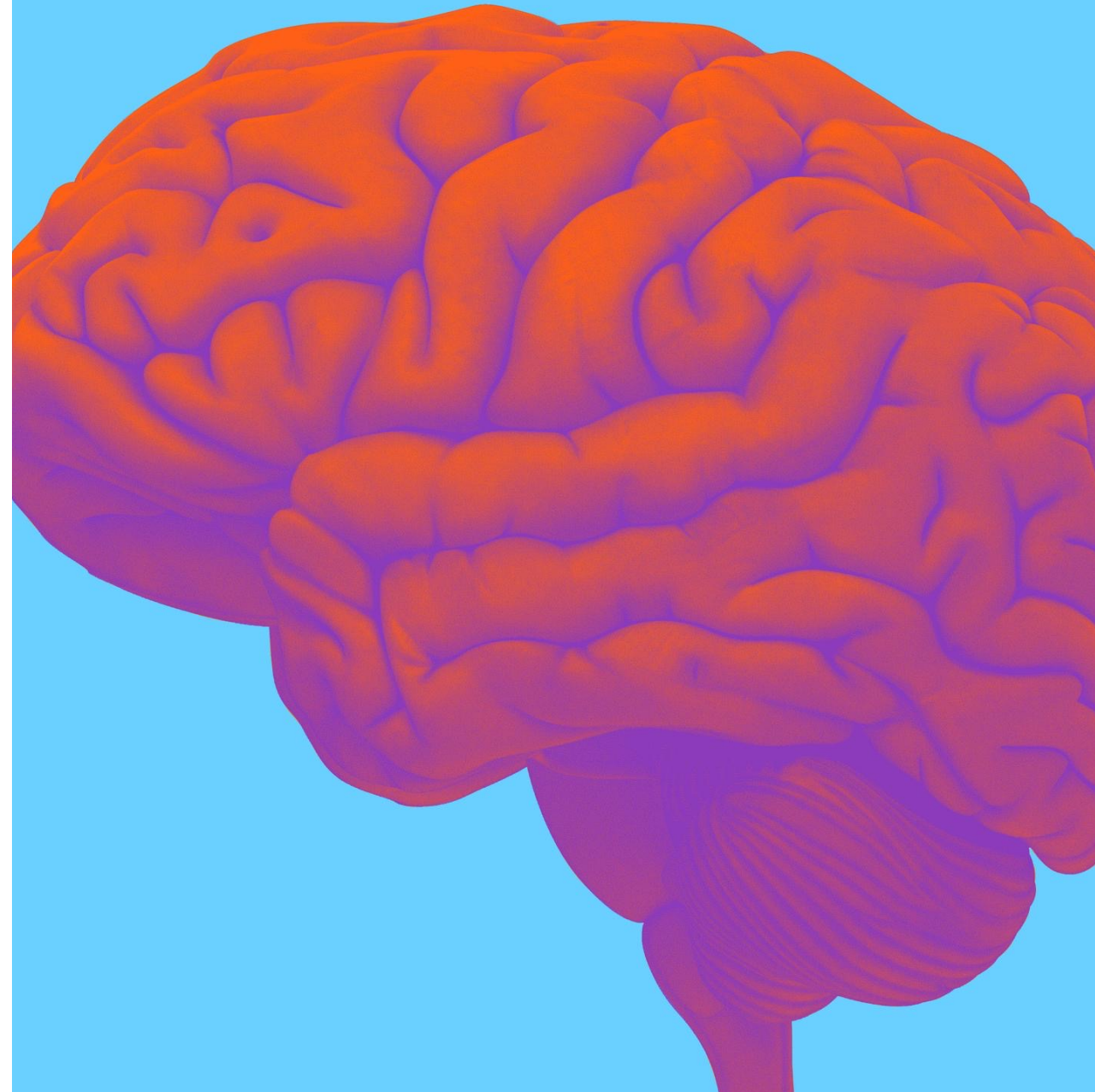
# REGULATORY INTERACTIONS



# Work Package 5: DINI : Extension granted for 6 months

- FDA: Re-submit Qualification Plan ( April 2025)
- EMA: Submit Qualification Plan (September 2025)

# CONCLUSIONS



# Serum NfL

## NeuroFilament Light chain protein

### **Currently most interesting biomarker for DINI**

Detectable in sera of healthy volunteers, stable over time

Normal values in healthy volunteers are significantly impacted by age

Sensitive marker for central and peripheral NS injury

Sensitive marker for drug-induced CNS injury

Biomarker of disease progression, prognosis, and treatment response (TBI)

Preclinically (rat): most sensitive biomarker with dose- and time-dependent increase after drug-induced CNS and PNS injury

# Decision Tree for Clinical Use of a Novel NS-injury Biomarker Panel in Drug Development

## Context of Use (CoU):

*A blood-based safety biomarker to aid in the detection of acute drug-induced nervous system injury risk in phase 1 trials in healthy volunteers when there is an a priori concern that a drug may cause nervous system injury in humans.*

Is there *a priori* concern for acute NS-injury in humans as defined in the CoU?

NO

NS-injury serum biomarkers are not appropriate for use in this context

YES

Measure serum NfL in HVs in phase 1 clinical trial

NfL exceeds cutoff thresholds

Dose is potentially unsafe

NfL does not exceed cut-off thresholds

Dose is assumed safe provided there are no other safety signals. Continue dosing and monitoring NfL in phase 1 trial

- For *a priori* CNS injury concerns, clinical decisions to stop dosing additional subjects, or stop dose escalation may be based on biomarker responses alone
- For *a priori* PNS injury concerns, physical and neurological exams, questionnaires, nerve function tests, and biomarkers used for weight-of-evidence based clinical decisions

“a priori”

means any signal indicating that HVs may be at risk of adverse nervous system injury with a new drug candidate.

Such a signal will commonly come from traditional **preclinical** toxicology studies but may also derive from an anticipated **class** safety risk based on mechanism of action (MOA).

# Acknowledgements

- **IMI2 TBL WP5 DINI team:**

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Andre Schuermann

Kseniya Khamina

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Executive TBL team

Jens Goepfert

Gesine Rieger

Lidia Mostovy

# Thank you

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