

Staging Biomarker Development

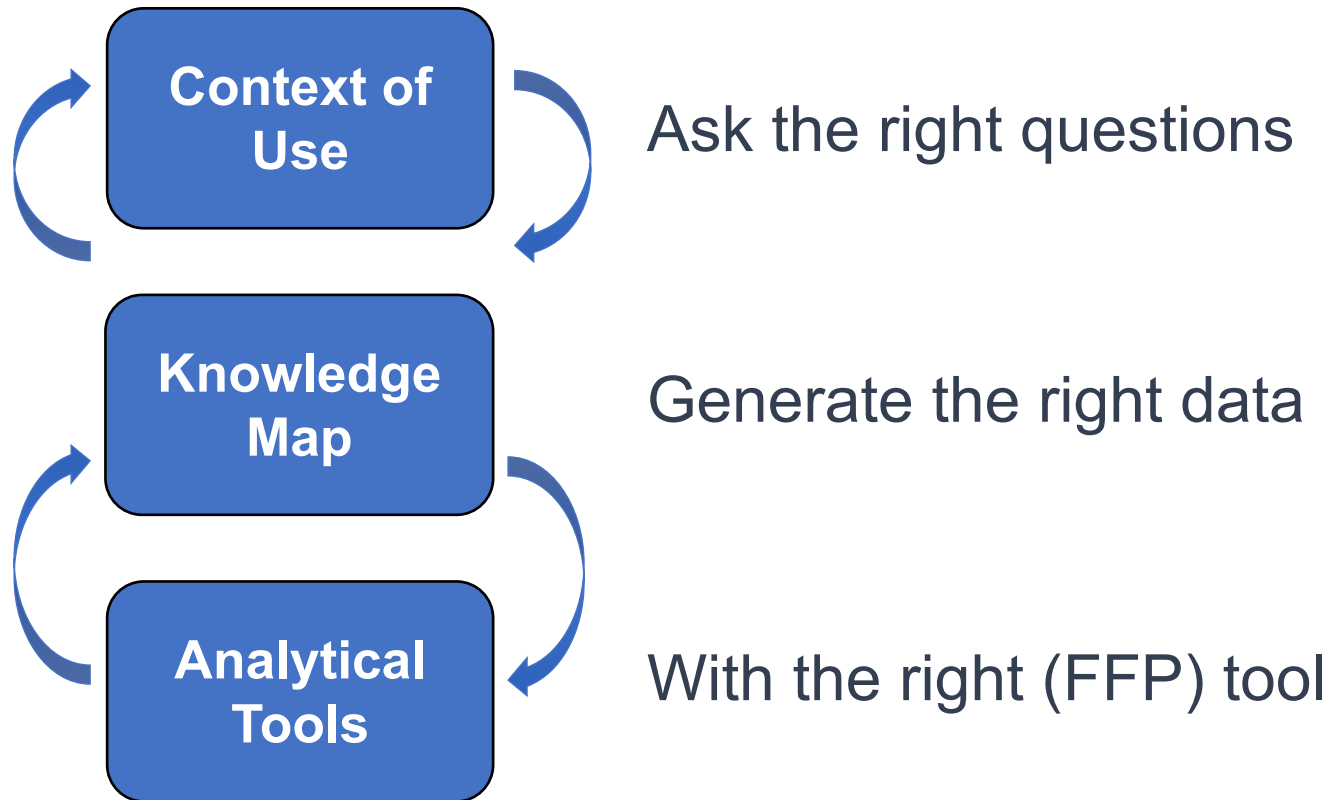
A case study on Evolving COU and Biomarker Development

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EBF - Spring Focus Workshop 2020

Overview

- Biomarker development based on “Context of Use”
- Case Study on Neurofilament: A biomarker of axonal injury
 - Aligning Fit-for-Purpose Assay Development to evolving COUs
 - Illustrated with a case study of NfL in Multiple Sclerosis

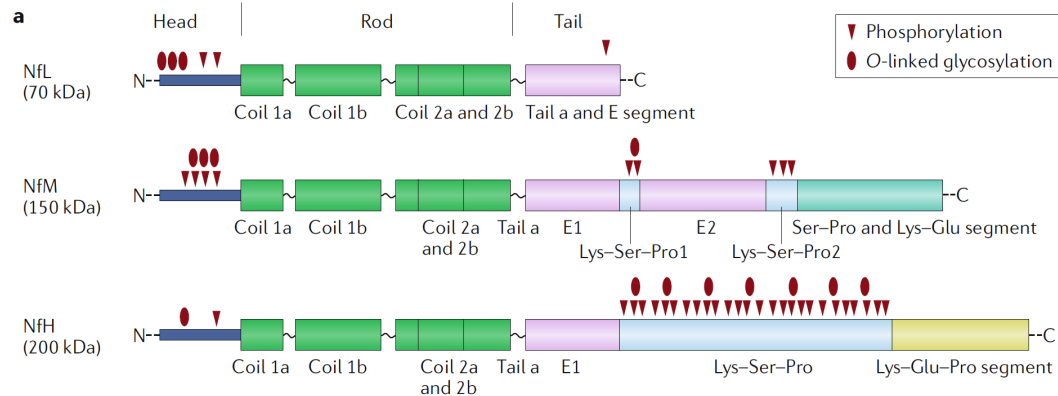
Biomarkers in 3 ~~easy~~ steps...



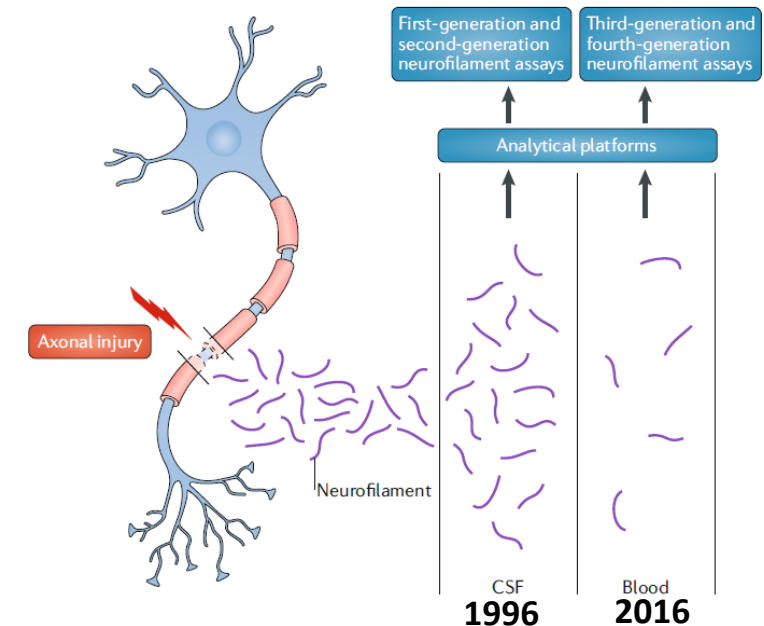
Path to biomarker utility: Iterate, iterate, iterate...

Neurofilament: Biomarker of Axonal Injury

- Intermediate filaments that serve as axonal cyto-structural units important for neuronal integrity and function (growth, maintenance, conduction, transport).
 - 3 major subunits: NfL, NfM, & NfH
 - The majority of axonal NFs are highly phosphorylated, which confers additional resistance to protein degradation

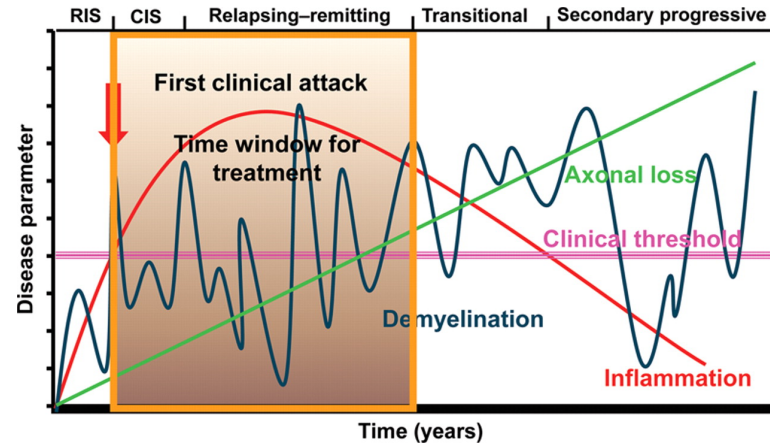


- Elevated NF levels in the CNS are associated with axonal injury, axonal loss, and neuronal death.

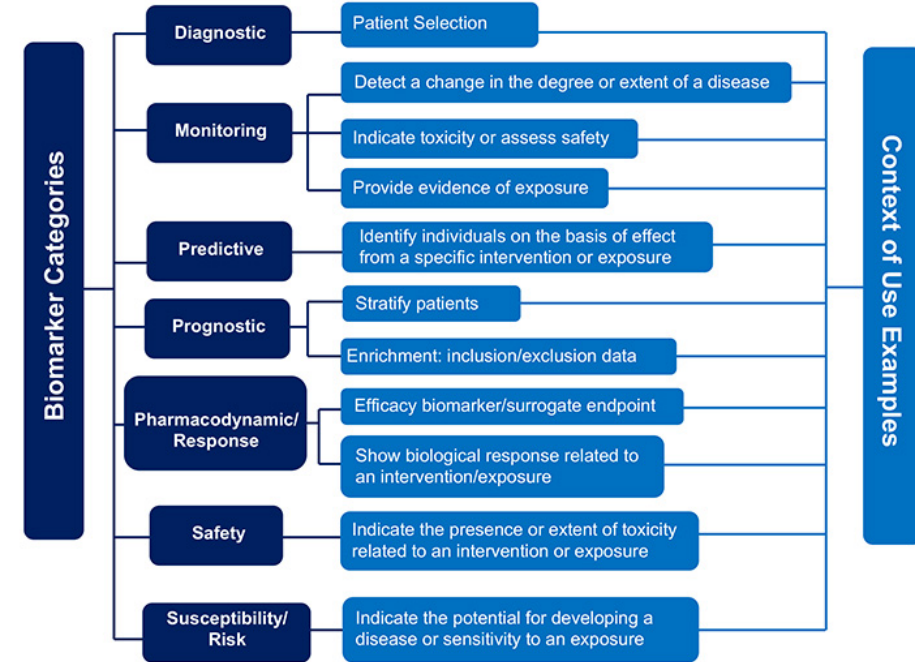


- Methodological advances have made it possible to monitor NfL and p-NfH in blood, re-invigorating its utility in multiple disease areas.

NfL: a generic marker axonal damage with utility in specific diseases



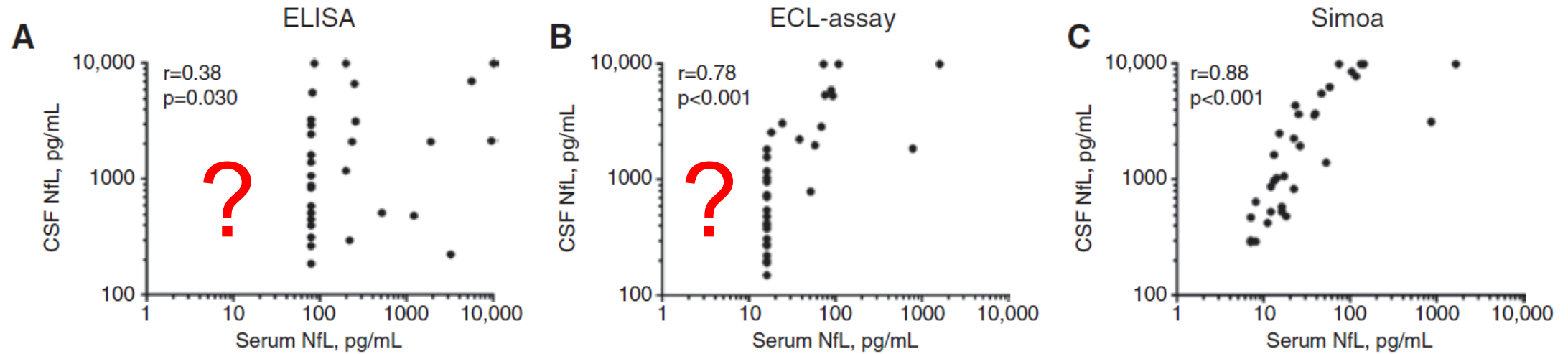
Adapted from Freedman M.; *Neur Clin Prac*, 2011



Adapted from fda.gov

Context of Use v1?

Detection of serum NfL has unlocked the potential for clinical utility in neurological diseases



Kuhle J, et al.; Clin Chem Lab Med, 2016

n=33 MS patients	ELISA	ECL-Assay	Simoa
Sensitivity (pg/mL)	78	15.6	0.62
% detected in serum	45%	39%	100%
Serum pg/mL (range)	78 - 252	15.6 - 62.5	12.5 - 45.5

Transitioning to the NfL Simoa Assay

Opportunities:

- Bead-based digital ELISA based on a count of single immunocomplexes
- Automated platform that yields high-precision between replicates (opportunity for singlicate analysis)
- Ultra-sensitivity (below the picomolar barrier)

Considerations:

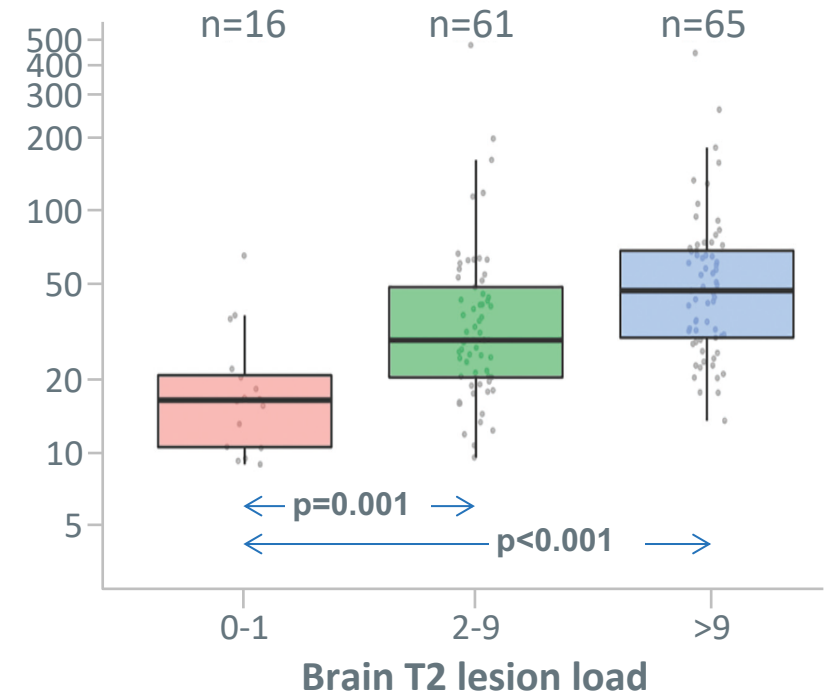
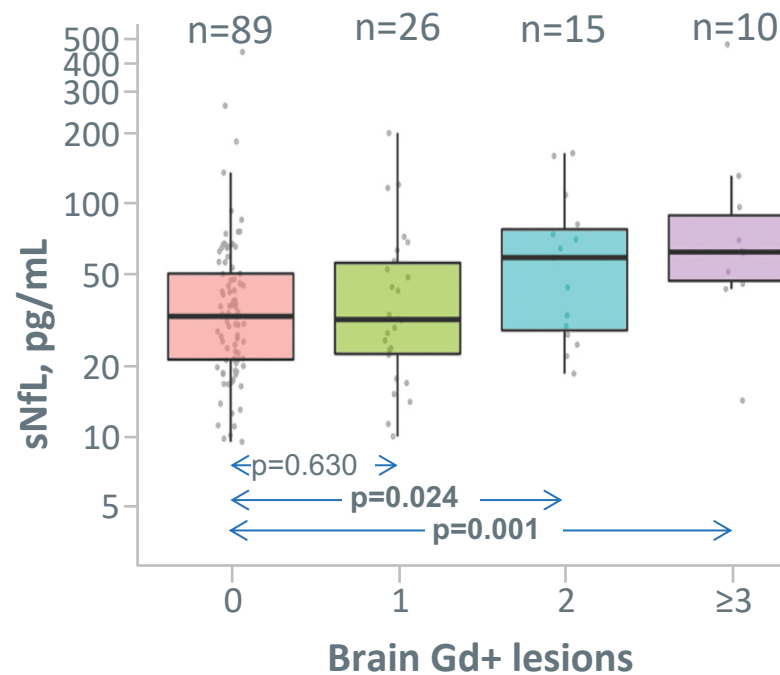
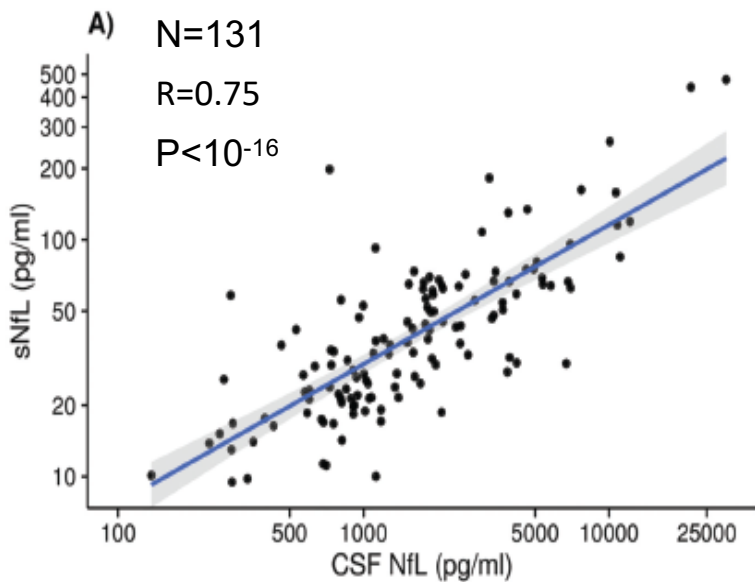
- Requires skilled operator and controlled lab conditions
- Expensive Technology
- High sample volume needed (>50 μ L per sample)
- 120 minutes per run

- Two NfL assay types: commercial kit and self-made
- Results correlate but are not equal which complicates establishment of clinically-relevant reference ranges
- Inter-lab reproducibility study of commercial kit across 17 centers demonstrated robust performance (average ~ 9% CV)

Initial analyses demonstrated association of sNfL with MS disease activity

Knowledge Map v1

Analytical Tools v1



Gd+, gadolinium-enhancing; MRI, magnetic resonance imaging; sNfL, serum neurofilament light.

Disanto G, et al. Ann Neurol. 2017

Developing a Specific COU for sNfL in Multiple Sclerosis

Context of Use v1

Descriptive:

Robust correlation and association of MRI measures and clinical outcomes with NfL values in MS patients.

Can sNfL be used to monitor MS patients more frequently to inform patient care?



Clinically Actionable:

RRMS patients with a confirmed increase in (X) pg/mL of serum NfL, as measured by **assay X**, within (X) months between assessments should have a follow-up MRI scan within (X) time to confirm inflammatory activity and inform treatment decisions (?).

Context of Use v3,4,5, etc.

Leverage learnings to inform internal drug development decisions

Context of Use v2

FFP Analytical Characterization of sNfL Biomarker Assay

Analytical Tools v2

Sharma A., et al., ECTRIMS 2018

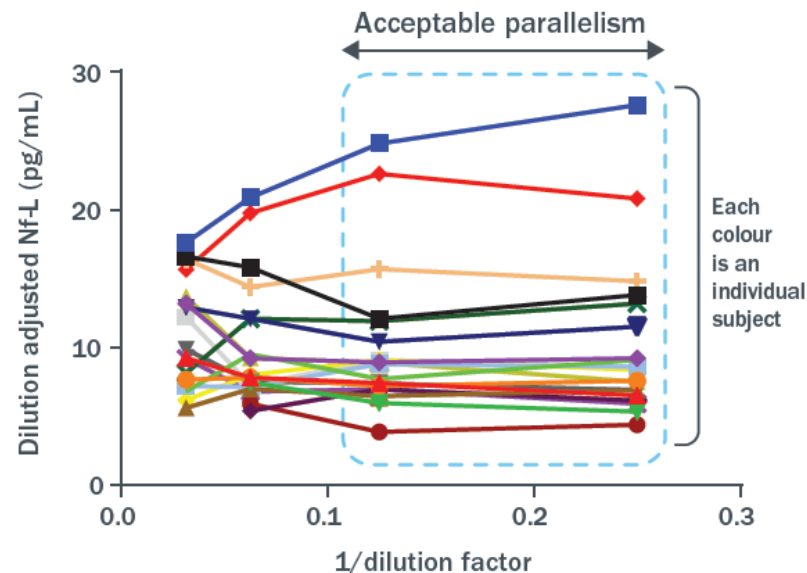
Key Parameters for Biomarker Assay Validation[^]

- Calibrators and Standard Curves
- *Parallelism*
 - *Selectivity*
 - *LLOQ*
- *Precision & Relative Accuracy*
- *Sample Stability*
(Based on the Endogenous Analyte)

[^]C-Path: 2019 Points to Consider
*Stevenson L, et al.; Bioanalysis 2014

PARALLELISM

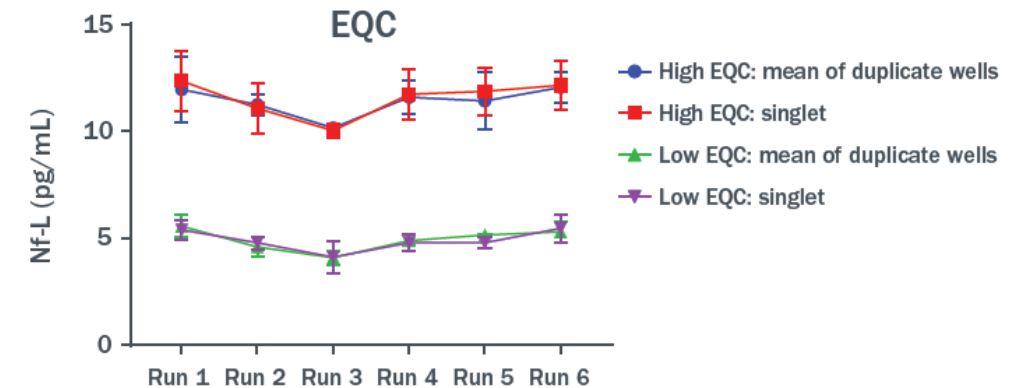
Performed on MS serum from multiple individuals



PRECISION

Assessed from 6 assay runs by 2 analysts on kits from 2 lots performed on different days

- Singlet vs. duplicate assessment performed: 3 sets of duplicate and singlet measurements were evaluated in each run and data plotted as mean with SD



- $\pm 18\%$ difference from MRD (confirmed in study parallelism)
- %CV < 10% for singlet analyses
- LLOQ = 0.7 pg/mL

FFP Analytical Characterization of sNfL Biomarker Assay

Analytical Tools v2

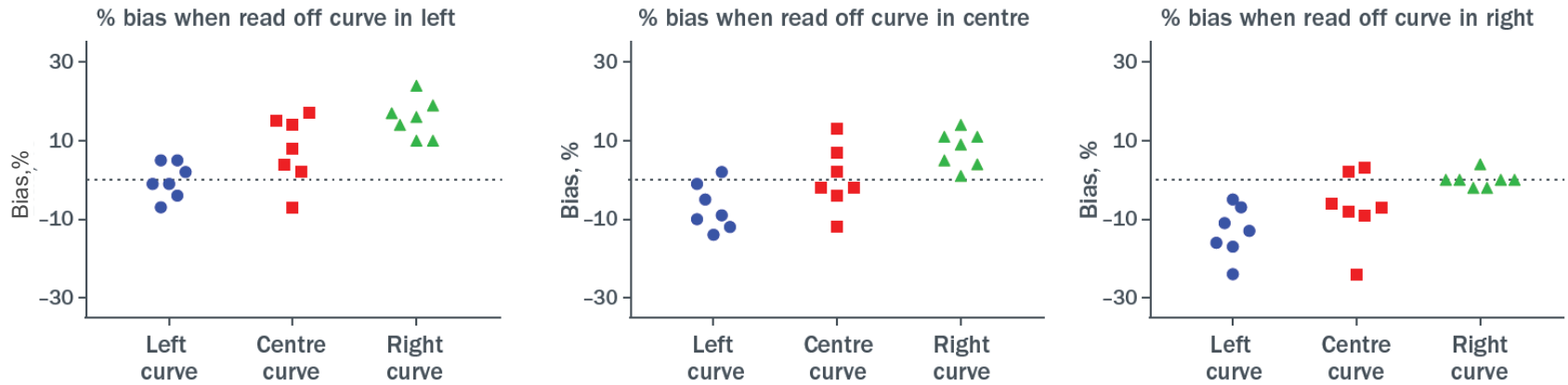
Running the curve in the centre of the plate mitigates the position effect bias

	Left curve			Centre curve			Right curve						
	1	2	3	4	5	6	7	8	9	10	11	12	
A	Blank					Blank					Blank		
B	0.686					0.686					0.686		
C	2.06					2.06					2.06		
D	6.17					6.17					6.17		
E	18.5					18.5					18.5		
F	55.6					55.6					55.6		
G	167					167					167		
H	500					500					500		

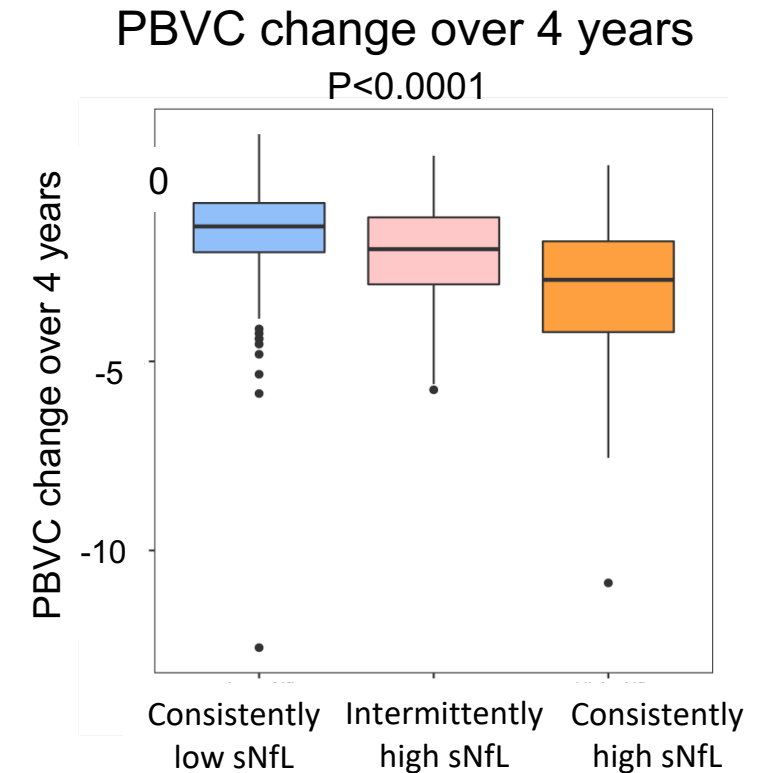
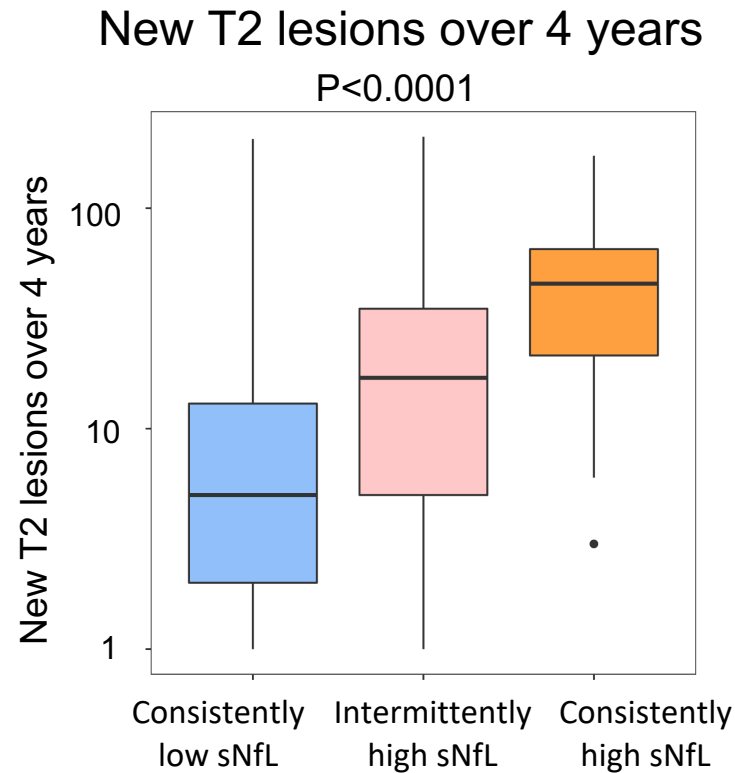
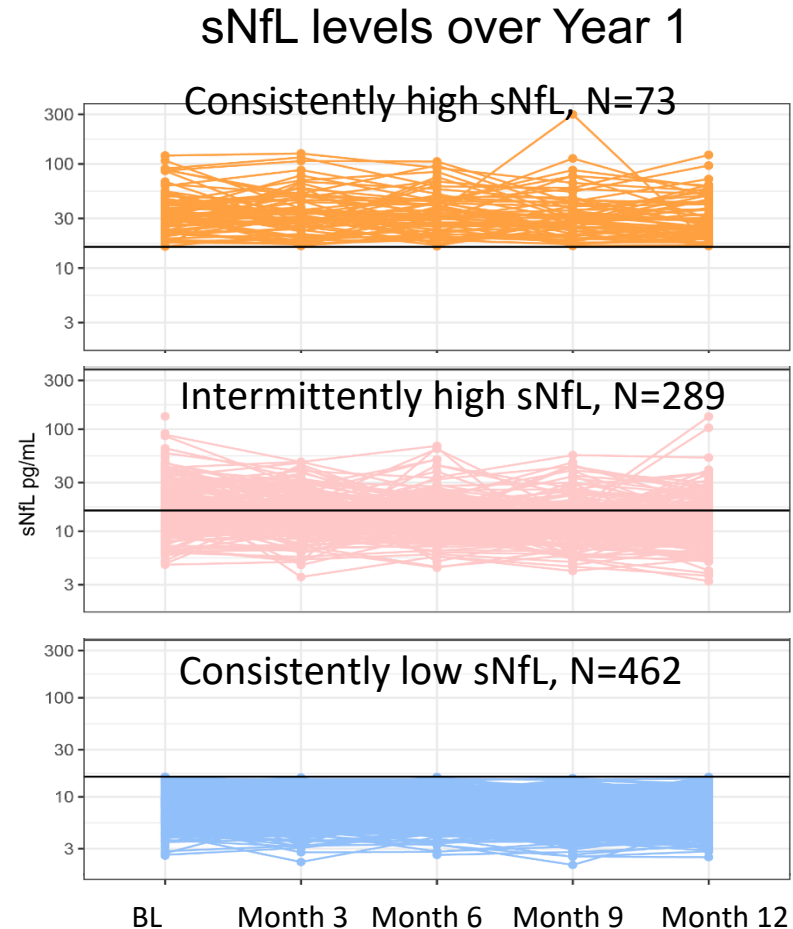
Standard curve in duplicate wells run in left, centre and right side of the plate

Impact of standard curve position on distribution of position effect bias

$$\text{Bias} = (\text{measured value} / \text{assigned value}) \times 100 - 100$$



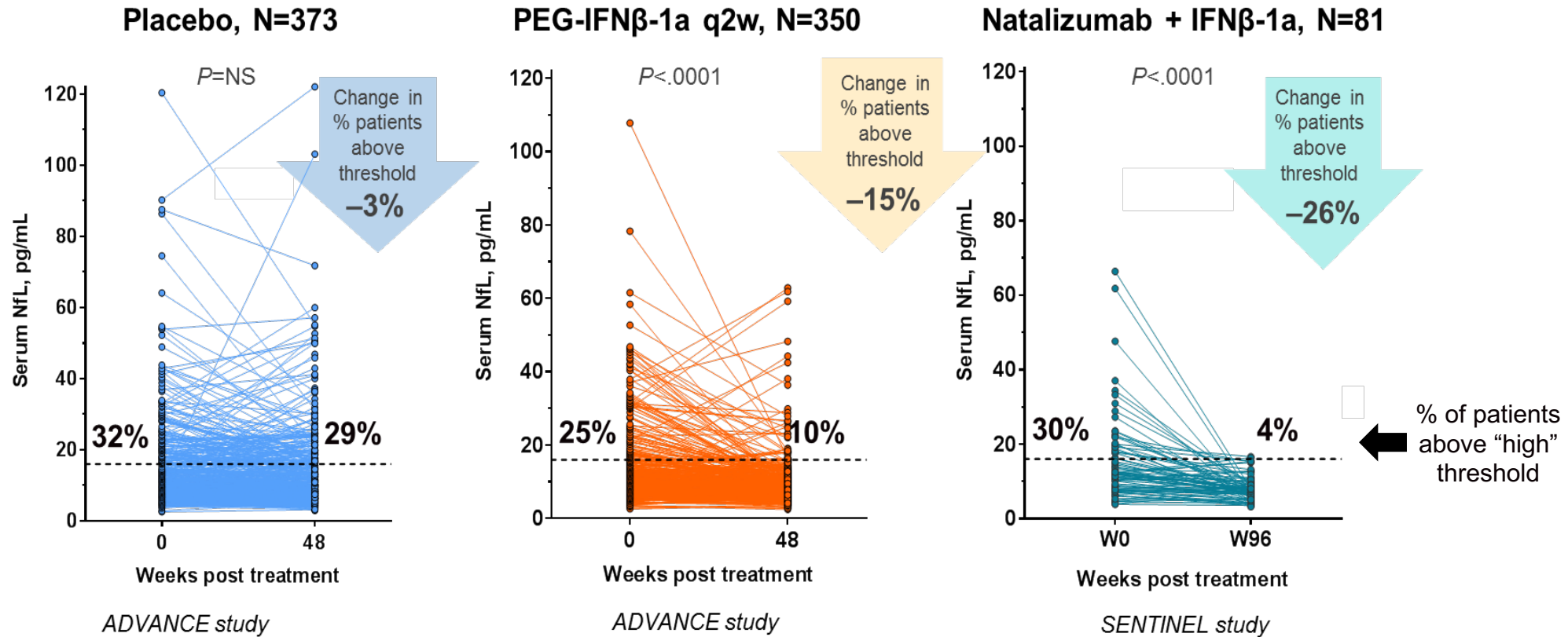
Patients With Consistently High sNfL Levels During Year 1 Had Worse MRI Outcomes Through 4 Years



Plavina T. and Calabresi P., et al., ECTRIMS 2018

BL = baseline. PBVC = percent brain volume change. ^aData from ADVANCE.

Serum NfL Levels Are Decreased on Treatment



Plavina T. and Calabresi P., et al., ECTRIMS 2018

The Next Iteration: Evaluating an NfL assay to Support Clinical Practice and Broader Use in Clinical Development

Analytical Tools v3

- Biogen-Siemens Healthineers collaboration to develop sNfL assay on the Siemens automated platform with wide access (>15,000 units worldwide)
 - Standardized, robust, and widely accessible assay to generate high quality data
 - Allows for clinical validation of sNfL in prospective and real-world cohorts to inform implementation in clinical practice.

- Repeatability >5% CV across 20 days
- LLOQ: 1.62 pg/mL
- Demonstrated parallelism
- High correlation between Quanterix Simoa and Siemens platforms ($R^2=0.838$)
- Reproducible association of Siemens NfL values with clinical and radiological disease activity (actual concentration values are shifted)

Plavina T, et. al., ECTRIMS 2019

Summary

- Biomarker assays take an iterative path as the COU, knowledge, and assays evolve.
- Assay characterization based on understanding the COU (or potential COUs) is critical to determining the validity of biomarker analyses.
- Open communication with your stakeholders is critical to success.



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