Quanterix®

Discovery Fueled by Ultra-Sensitivity

Ultrasensitive biomarker detection to transform the future of healthcare

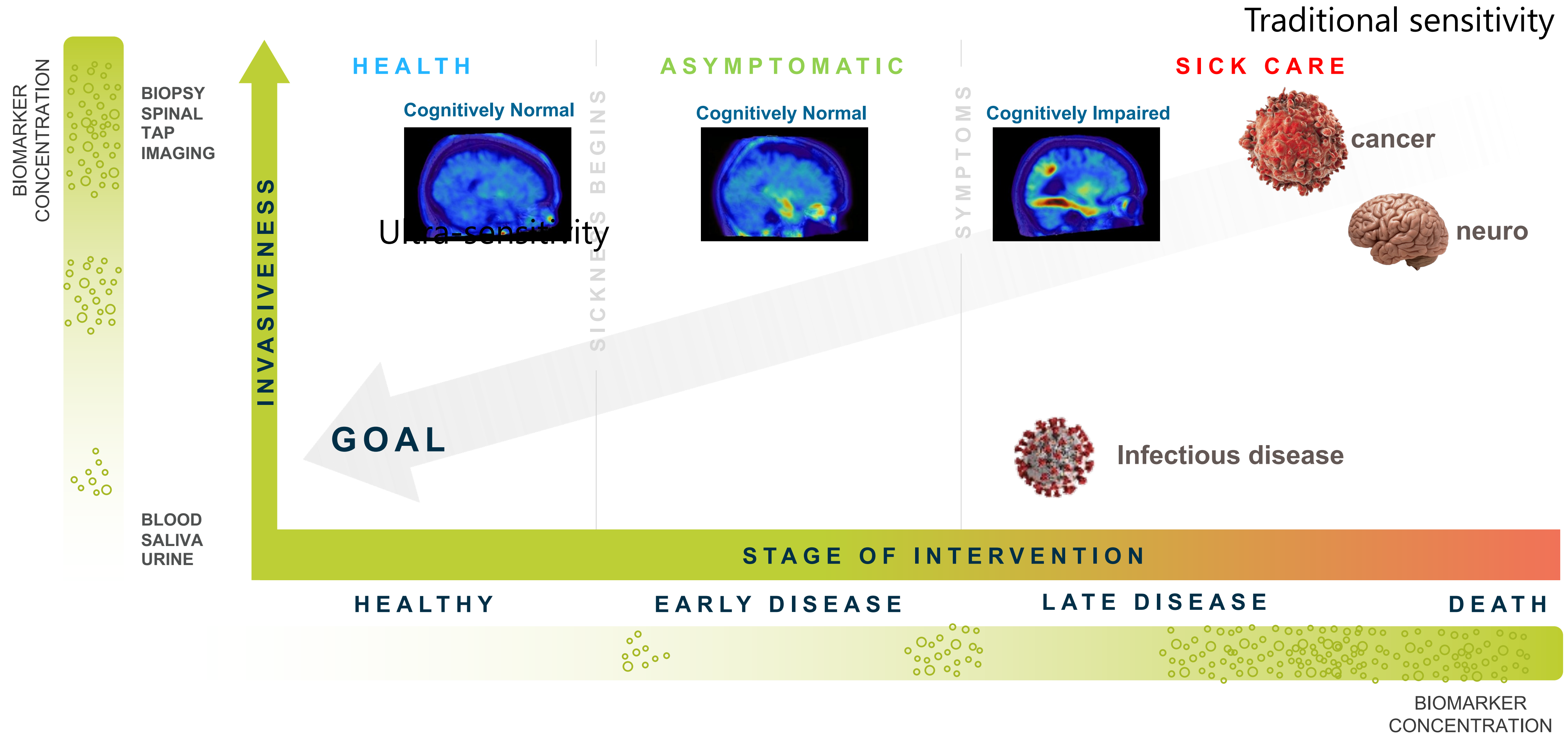
Danilo La Terra

PhD, Senior Field Application Scientist

Disclosure

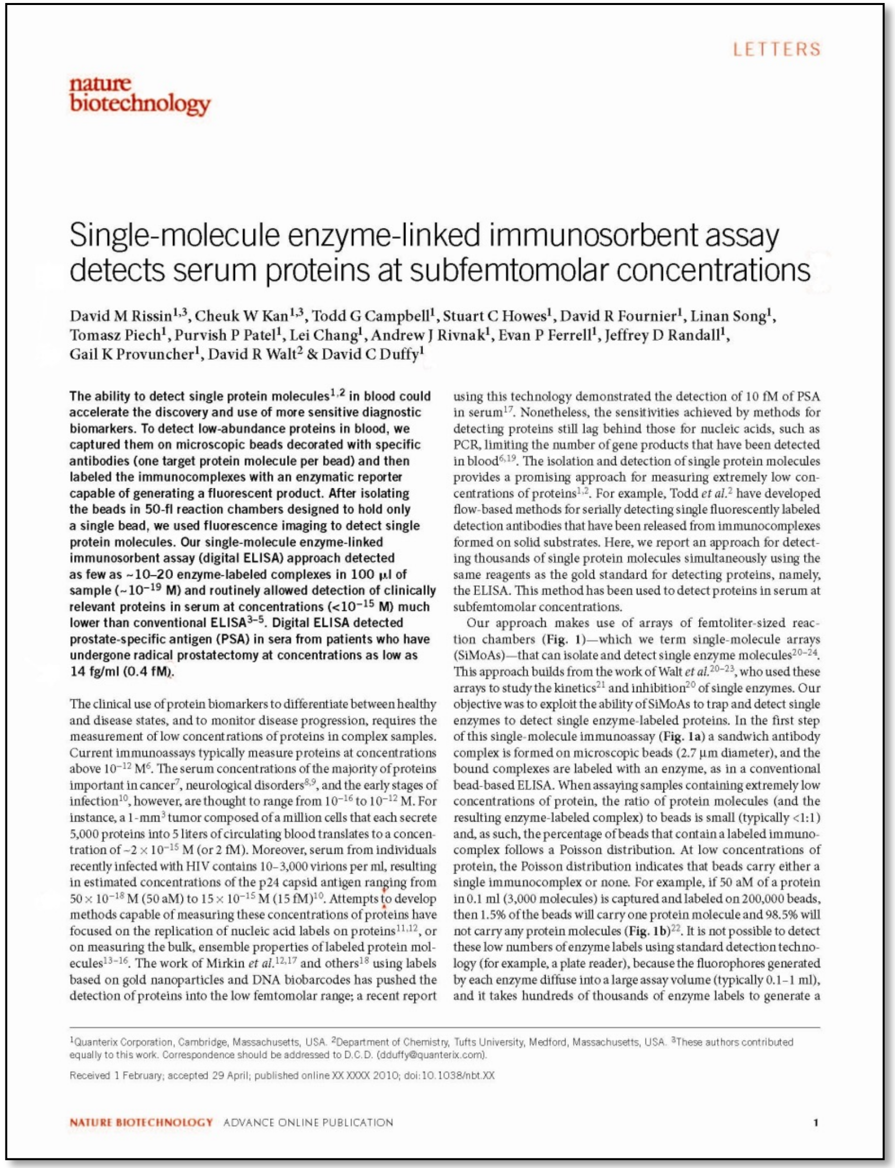
Danilo La Terra is an employee at Quanterix corporation

Measurement of fluid biomarkers with ultra-sensitivity
Why it matters?



Quanterix
Discovery Fueled by Ultra-Sensitivity

Simoa Bead Assay: Principle



nature biotechnology

Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations

David M Rissin[1,3], Cheuk W Kan[1,3], Todd G Campbell[1], Stuart C Howes[1], David R Fournier[1], Linan Song[1], Tomasz Piech[1], Purvish P Patel[1], Lei Chang[1], Andrew J Rivnak[1], Evan P Ferrell[1], Jeffrey D Randall[1], Gail K Provuncher[1], David R Walt[2] & David C Duffy[1]

The ability to detect single protein molecules[1,2] in blood could accelerate the discovery and use of more sensitive diagnostic biomarkers. To detect low-abundance proteins in blood, we captured them on microscopic beads decorated with specific antibodies (one target protein molecule per bead) and then labeled the immunocomplexes with an enzymatic reporter capable of generating a fluorescent product. After isolating the beads in 50-fl reaction chambers designed to hold only a single bead, we used fluorescence imaging to detect single protein molecules. Our single-molecule enzyme-linked immunosorbent assay (digital ELISA) approach detected as few as ~10–20 enzyme-labeled complexes in 100 μl of sample (~10^{-19} M) and routinely allowed detection of clinically relevant proteins in serum at concentrations (<10^{-15} M) much lower than conventional ELISA[3–5]. Digital ELISA detected prostate-specific antigen (PSA) in sera from patients who have undergone radical prostatectomy at concentrations as low as 14 fg/ml (0.4 fM).

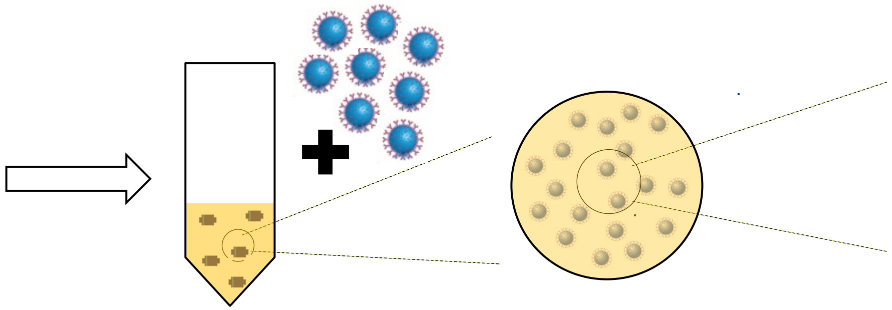
The clinical use of protein biomarkers to differentiate between healthy and disease states, and to monitor disease progression, requires the measurement of low concentrations of proteins in complex samples. Current immunoassays typically measure proteins at concentrations above 10^{-12} M[6]. The serum concentrations of the majority of proteins important in cancer[7], neurological disorders[8,9], and the early stages of infection[10], however, are thought to range from 10^{-16} to 10^{-12} M. For instance, a 1-mm^3 tumor composed of a million cells that each secrete 5,000 proteins into 5 liters of circulating blood translates to a concentration of ~2×10^{-15} M (or 2 fM). Moreover, serum from individuals recently infected with HIV contains 10–3,000 virions per ml, resulting in estimated concentrations of the p24 capsid antigen ranging from 50×10^{-18} M (50 aM) to 15×10^{-15} M (15 fM)[10]. Attempts to develop methods capable of measuring these concentrations of proteins have focused on the replication of nucleic acid labels on proteins[11,12], or on measuring the bulk, ensemble properties of labeled protein molecules[13–16]. The work of Mirkin *et al.*[12,17] and others[18] using labels based on gold nanoparticles and DNA biobarcodes has pushed the detection of proteins into the low femtomolar range; a recent report using this technology demonstrated the detection of 10 fM of PSA in serum[17]. Nonetheless, the sensitivities achieved by methods for detecting proteins still lag behind those for nucleic acids, such as PCR, limiting the number of gene products that have been detected in blood[6,19]. The isolation and detection of single protein molecules provides a promising approach for measuring extremely low concentrations of proteins[1,2]. For example, Todd *et al.*[2] have developed flow-based methods for serially detecting single fluorescently labeled detection antibodies that have been released from immunocomplexes formed on solid substrates. Here, we report an approach for detecting thousands of single protein molecules simultaneously using the same reagents as the gold standard for detecting proteins, namely, the ELISA. This method has been used to detect proteins in serum at subfemtomolar concentrations.

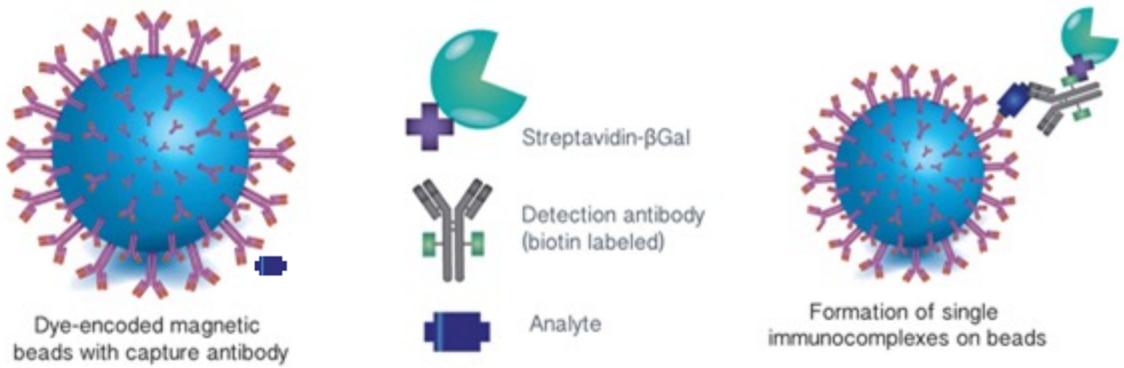
Our approach makes use of arrays of femtoliter-sized reaction chambers (**Fig. 1**)—which we term single-molecule arrays (SiMoAs)—that can isolate and detect single enzyme molecules[20–24]. This approach builds from the work of Walt *et al.*[20–23], who used these arrays to study the kinetics[21] and inhibition[20] of single enzymes. Our objective was to exploit the ability of SiMoAs to trap and detect single enzymes to detect single enzyme-labeled proteins. In the first step of this single-molecule immunoassay (**Fig. 1a**) a sandwich antibody complex is formed on microscopic beads (2.7 μm diameter), and the bound complexes are labeled with an enzyme, as in a conventional bead-based ELISA. When assaying samples containing extremely low concentrations of protein, the ratio of protein molecules (and the resulting enzyme-labeled complex) to beads is small (typically <1:1) and, as such, the percentage of beads that contain a labeled immunocomplex follows a Poisson distribution. At low concentrations of protein, the Poisson distribution indicates that beads carry either a single immunocomplex or none. For example, if 50 aM of a protein in 0.1 ml (3,000 molecules) is captured and labeled on 200,000 beads, then 1.5% of the beads will carry one protein molecule and 98.5% will not carry any protein molecules (**Fig. 1b**)[22]. It is not possible to detect these low numbers of enzyme labels using standard detection technology (for example, a plate reader), because the fluorophores generated by each enzyme diffuse into a large assay volume (typically 0.1–1 ml), and it takes hundreds of thousands of enzyme labels to generate a

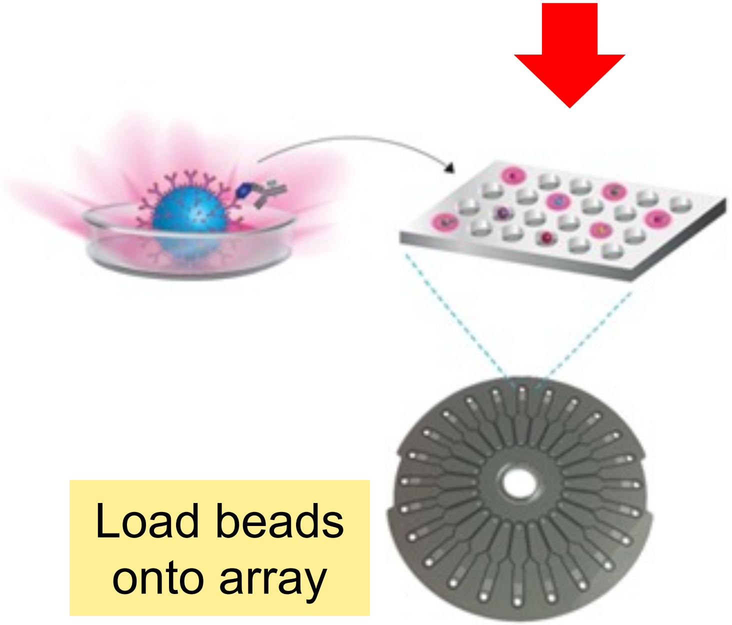
[1]Quanterix Corporation, Cambridge, Massachusetts, USA. [2]Department of Chemistry, Tufts University, Medford, Massachusetts, USA. [3]These authors contributed equally to this work. Correspondence should be addressed to D.C.D. (dduffy@quanterix.com).

Received 1 February; accepted 29 April; published online XX XXXX 2010; doi:10.1038/nbt.XX

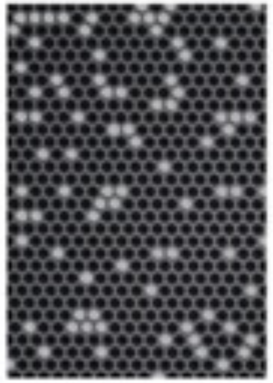
Simoa Bead Assay: Principle



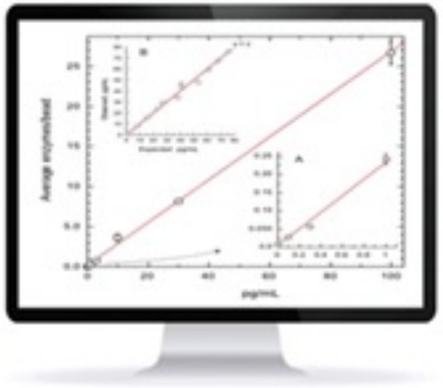




Load beads onto array



Image

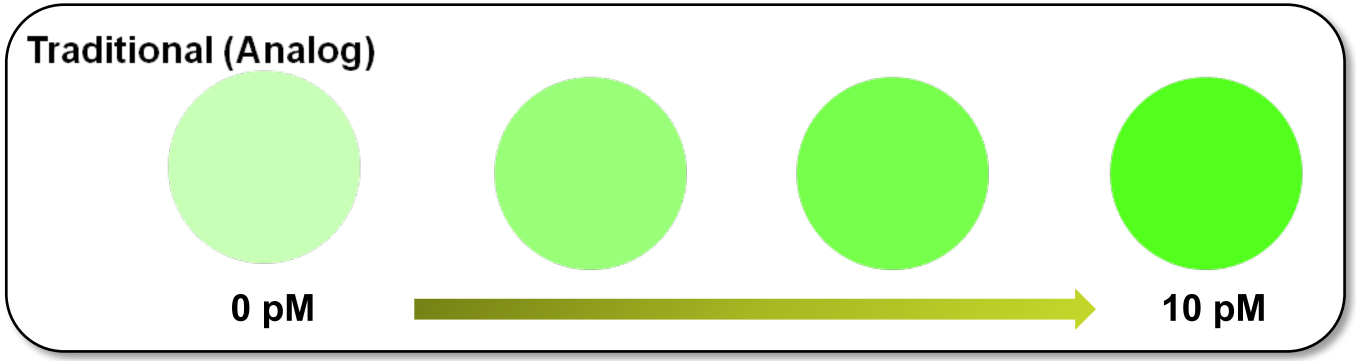


Analyze and Report

Rissin *et al.*, 2011

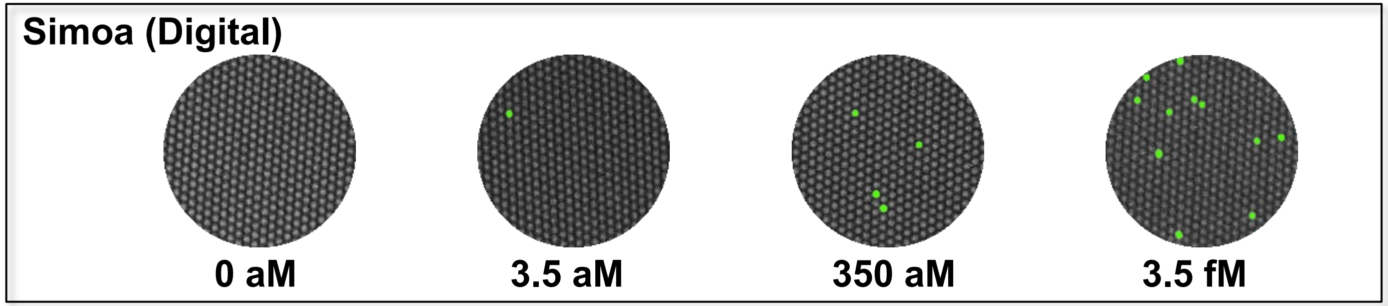
Rissin et al., 2010

Simoa Bead Assays: Digital vs. Analog Detection



- Reaction volume = 100 $\times 10^{-6}$ L
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

Microliters (µL)

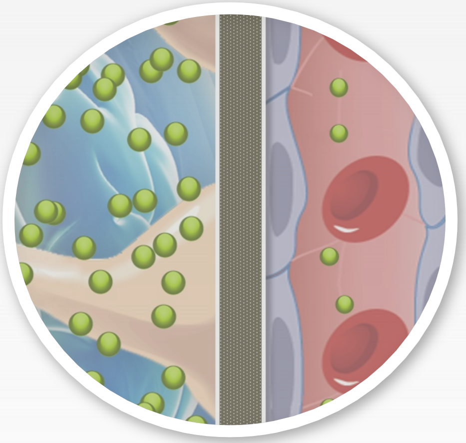


- Reaction volume = 50 $\times 10^{-15}$ L **(2 billion times smaller)**
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- **One molecule needed to reach detection limit**

Femtoliters (fL)

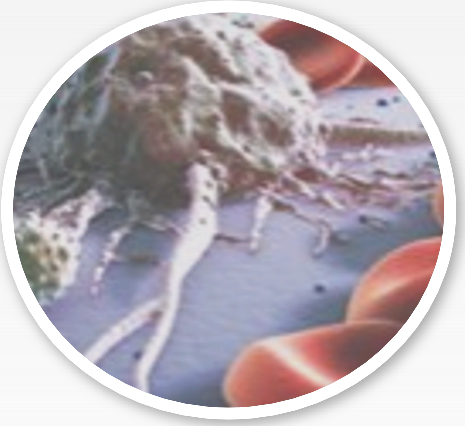
Simoa sensitivity enables research across therapeutic and disease areas

Neurology



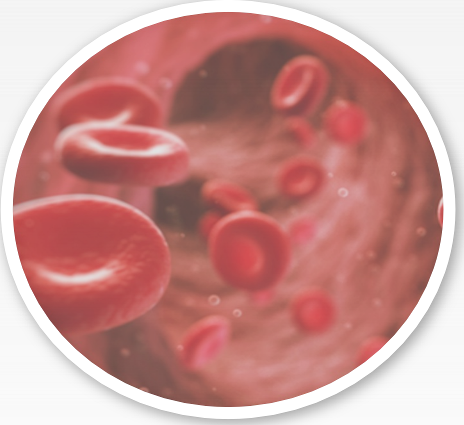
- Alzheimer's
- TBI/concussion
- MS
- Parkinson's
- ALS

Oncology



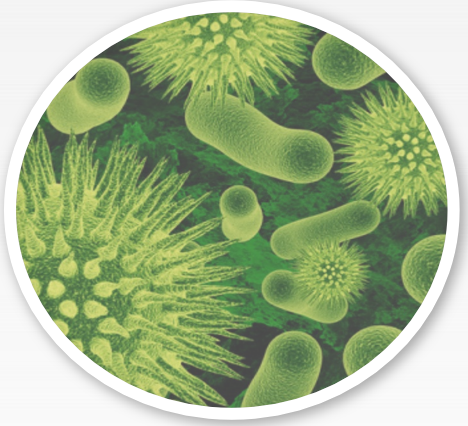
- Prostate Cancer
- Breast Cancer
- Pancreatic Cancer
- Cervical Cancer
- Lung Cancer

Inflammation



- Diabetes
- Celiac Disease
- Lupus
- Rheumatoid Arthritis
- Ulcerative Colitis

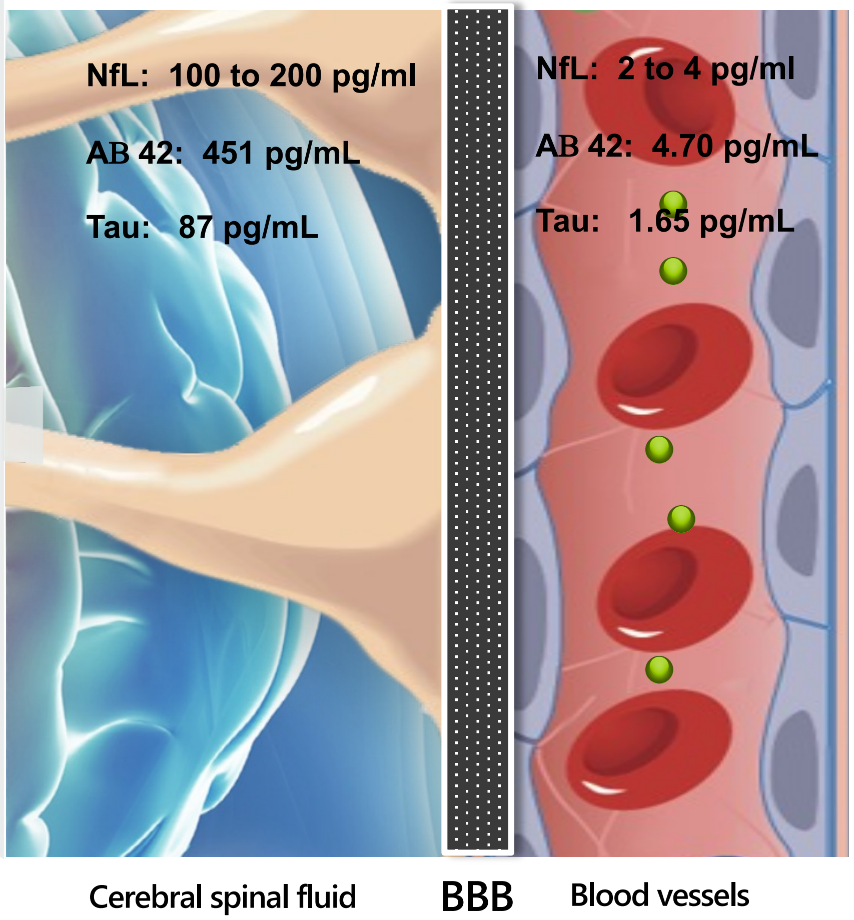
Infectious Disease



- HIV
- HCV
- *C. difficile*
- Tuberculosis
- COVID-19

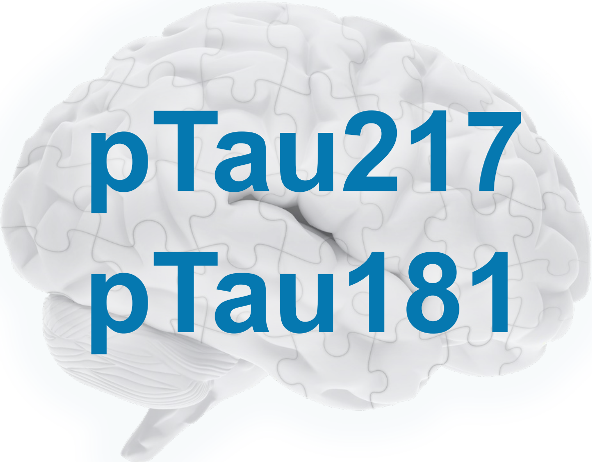
Revolution in Neuronal Biomarkers Measurement: CSF & Blood

Brain Health with a non-invasive Blood Test

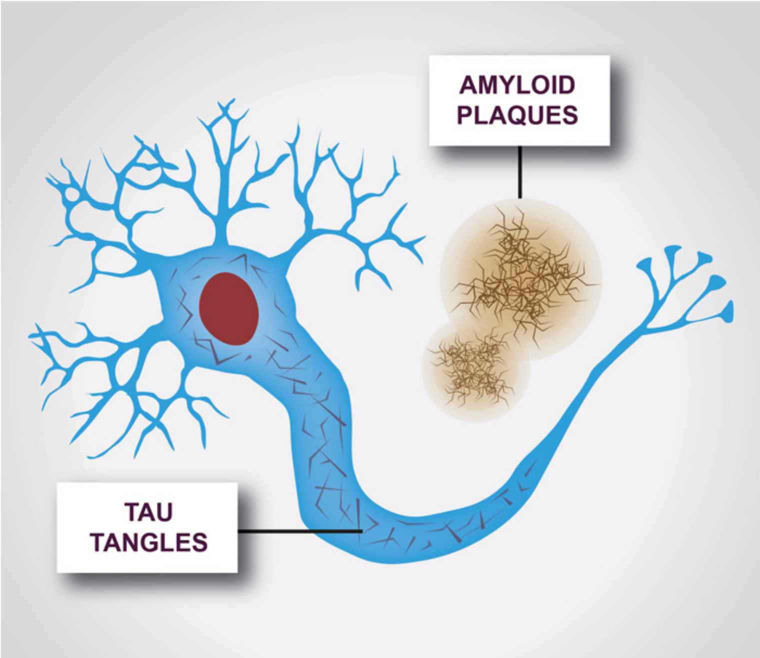


* *JAMA Neurol*. Neurochemical Aftermath of Repetitive Mild Traumatic Brain Injury September 19, 2016. doi:10.1001/jamaneurol.2016.2038

blood pTaus offer a crucial tool for the early detection and predictive assessment of Alzheimer's disease (AD)



pTau217
pTau181



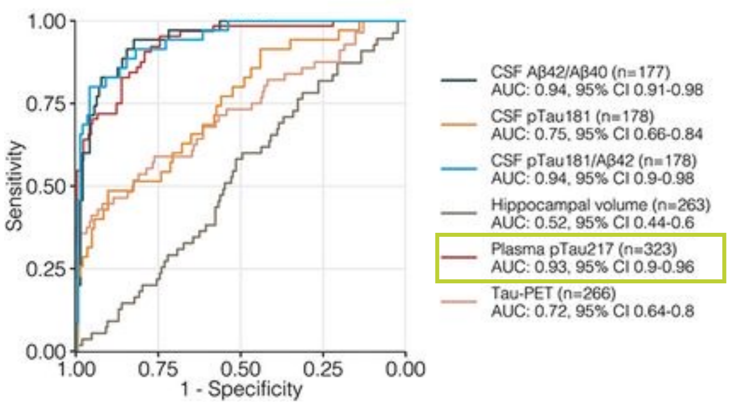
Highly accurate biomarker to assess Alzheimer's Disease

Plasma ALZpath pTau217 diagnostic accuracy in identifying Alzheimer's disease pathology

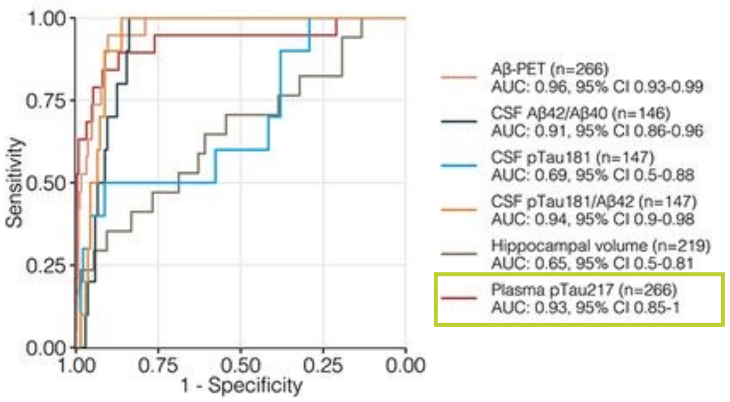
- Plasma pTau217 is comparable to CSF biomarkers in identifying AD pathology
- Plasma pTau217 accurately identifies the A T status in all cohorts, with highest levels in the A+T+ group.
- In the 8 years of longitudinal sampling in WRAP, the A+T+ group demonstrated a significantly higher annual increase in plasma pTau217 levels compared to the A-T-group.

Plasma pTau217 has similar accuracies to cerebrospinal fluid biomarkers and detects longitudinal changes.

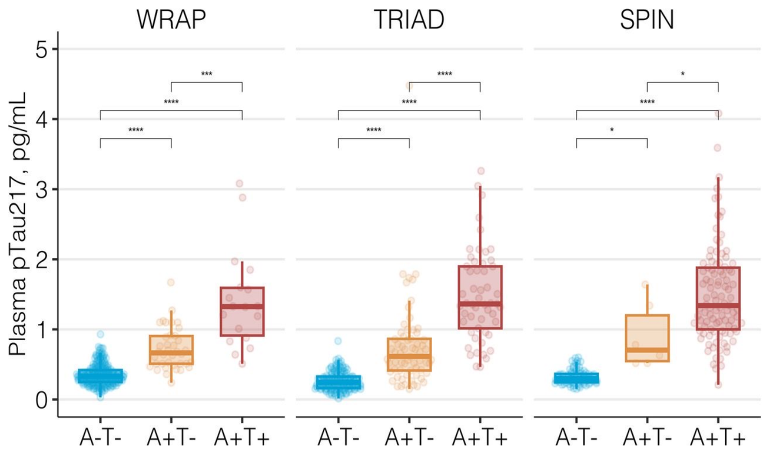
ROC curve to determine abnormal Aβ-PET (WRAP cohort)



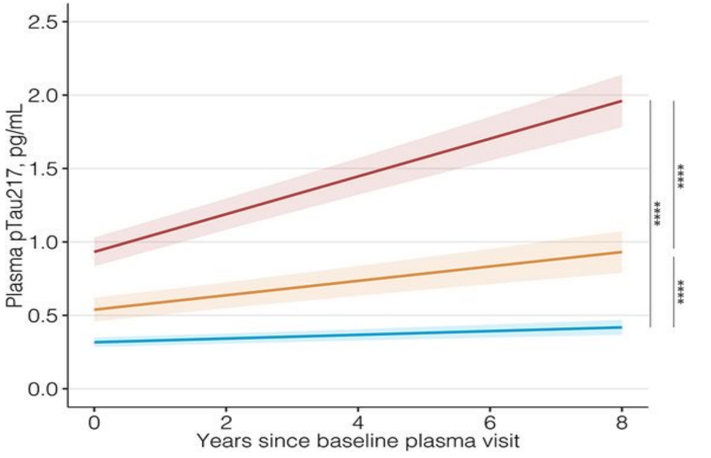
ROC curve to determine abnormal tau-PET (WRAP cohort)



Plasma p-tau217 according to amyloid and tau profiles in all the tested cohorts



Longitudinal trajectories of plasma p-tau217 according to amyloid and tau PET (WRAP cohort)



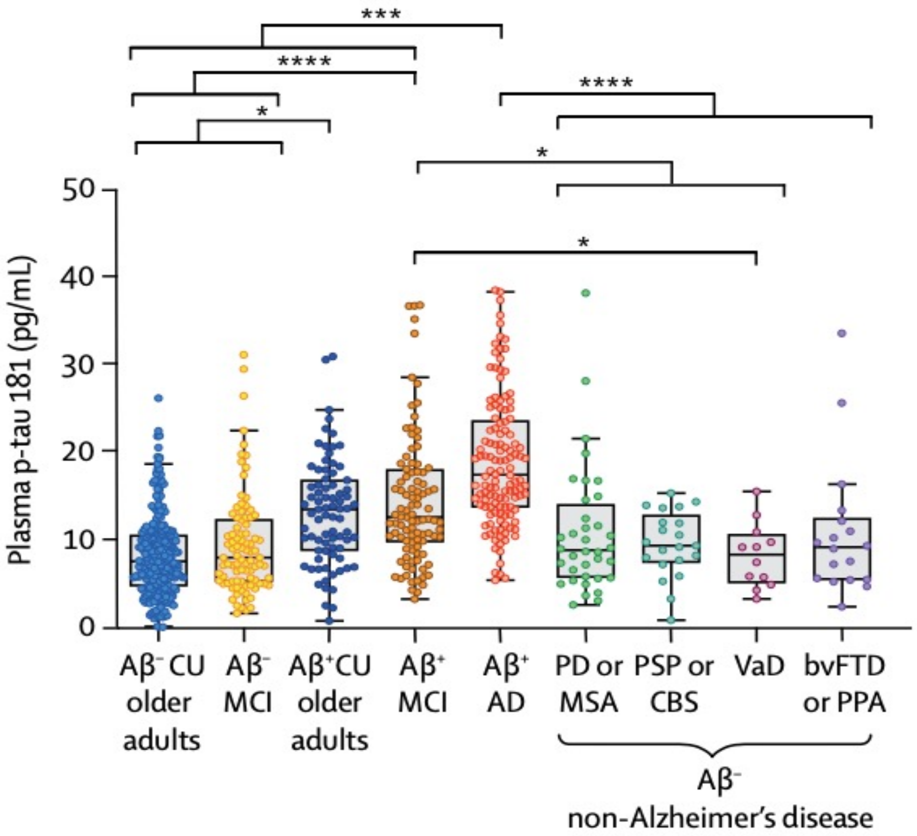
Quanterix
Discovery Fueled by Ultra-Sensitivity

Ashton. *et al.*, 2023

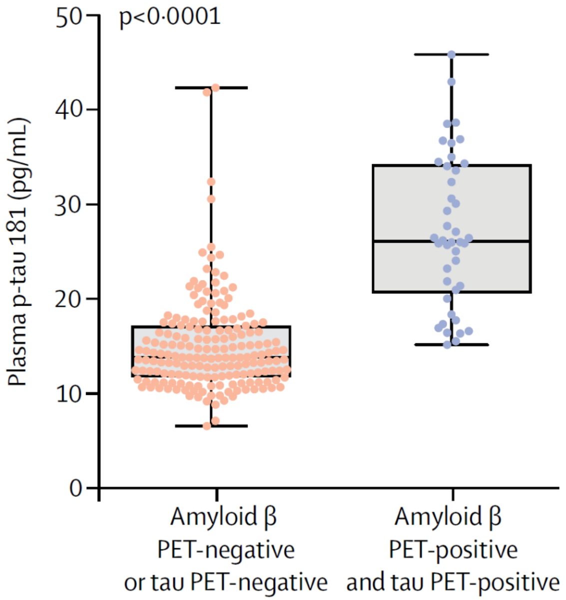
Blood phosphorylated tau 181 (p-tau181) as a specific biomarker for Alzheimer's disease

- Blood p-tau181 can predict tau and amyloid β pathologies, differentiate AD from other neurodegenerative disorders, and identify AD across the clinical continuum.

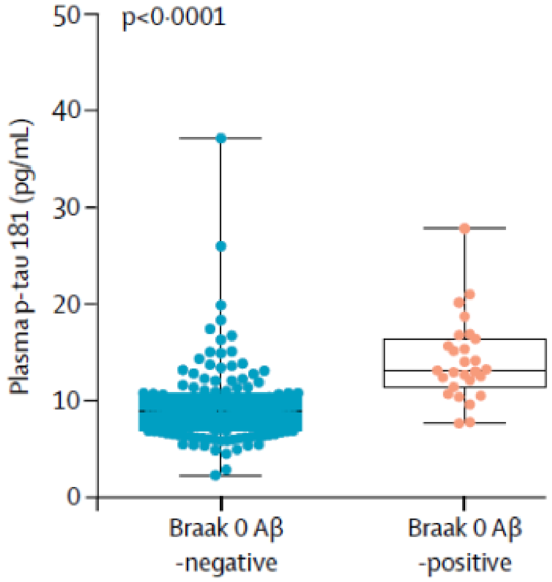
Blood p-tau181 may be used as a simple, accessible, and scalable test for screening and diagnosis of AD



Association with PET data for Amyloid and Tau pathology



Early amyloid pathology (no tau pathology)



CU: Cognitively unimpaired
MCI: Mild cognitive impairment
AD: Alzheimer disease
PD: Parkinson's disease
MSA: Multiple systems atrophy
PSP: Progressive supranuclear palsy
CBS: Corticobasal syndrome
VaD: Vascular dementia.
bvFTD: Behavioural variant frontotemporal dementia
PPA: Primary progressive aphasia.

Quanterix
Discovery Fueled by Ultra-Sensitivity

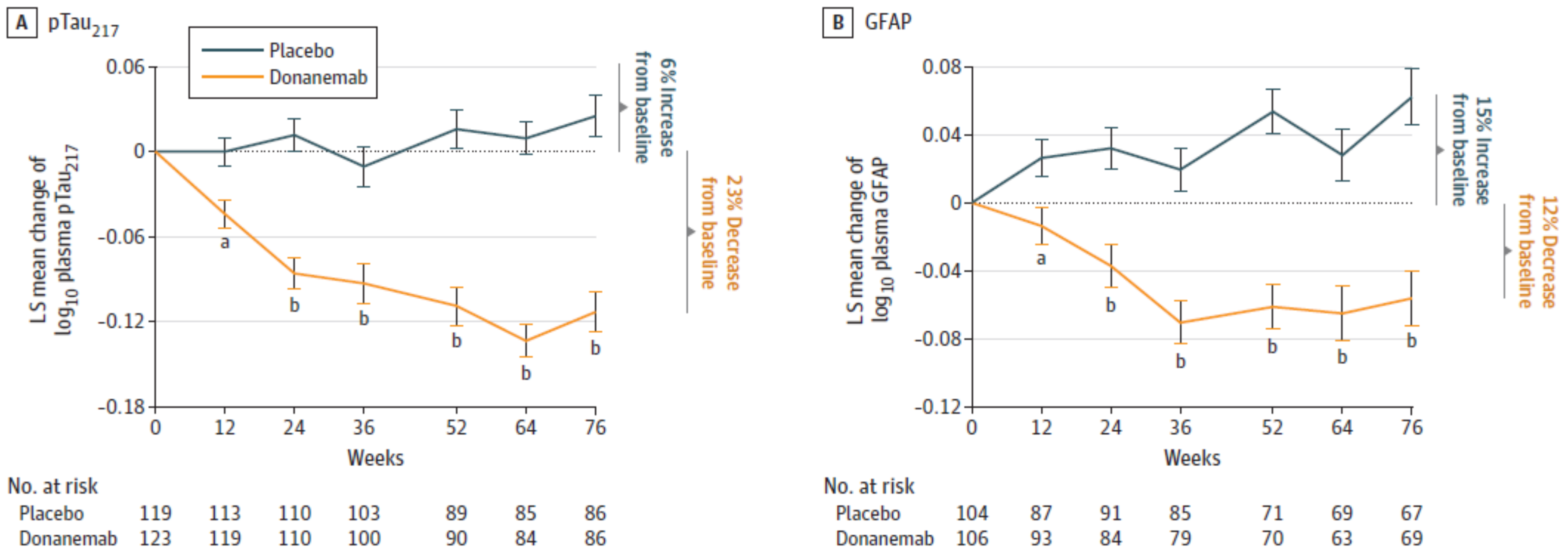
Karikari et al. Lancet Neurol, 2020

Association of Donanemab Treatment With Plasma pTau217 and GFAP in early symptomatic AD

In the TRAILBLAZER-ALZ randomized, double-blind, placebo-controlled clinical trial: 272 participants received Donanemad or placebo and changes in plasma biomarkers pTau217, GFAP, NFL, Ab42/40 were measured.

Mean plasma pTau217 and GFAP levels significantly decreased by 23% and 12% respectively after treatment.

Plasma pTau217 and GFAP reliably track treatment effectiveness and identify changes in AD pathology when using anti-amyloid therapy



Quanterix
Discovery Fueled by Ultra-Sensitivity

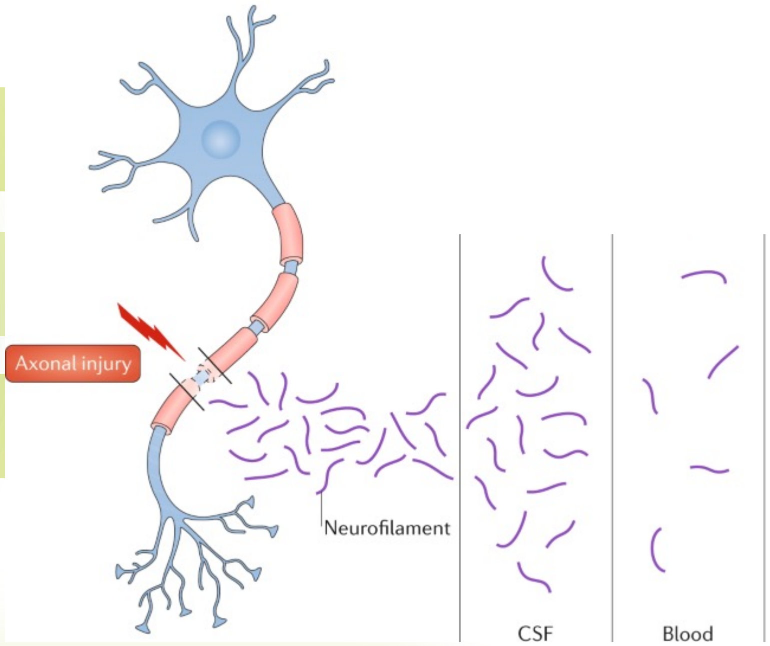
Pontecorvo et al., 2022. JAMA Neurology

NfL blood test - Key Biomarker to Assess Neural Damage

Traumatic Brain Injury

Stroke

ALS
Multiple Sclerosis



NfL is widely used in therapeutics trials with potential to become a standard for assessing brain health in clinical care

Quanterix
Discovery Fueled by Ultra-Sensitivity

NfL and Multiple Sclerosis: Disease Activity and Treatment Response

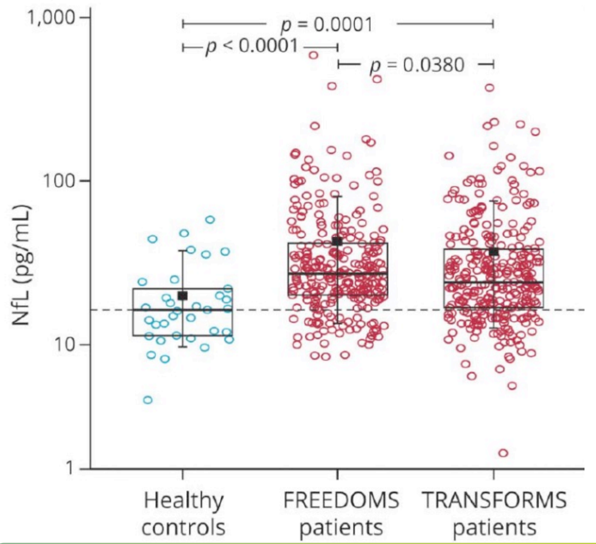
Blood NfL levels associate with clinical, MRI measures and treatment response in patients with RRMS in clinical trials

- Plasma NfL from 589 RRMS patients
- Baseline NfL higher in MS patients than in healthy controls and correlated with total lesion load, number of active lesions and treatment response

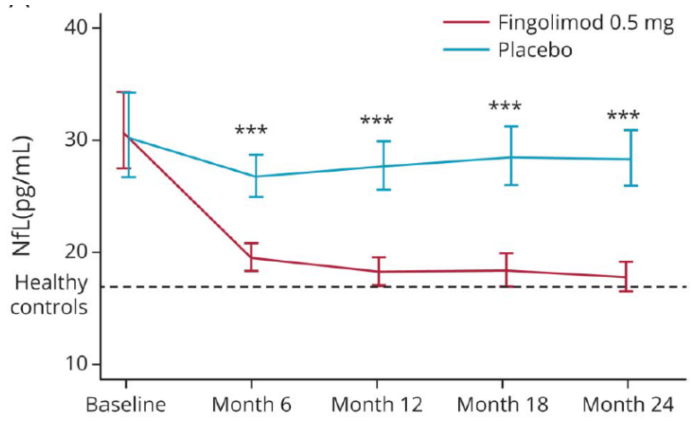
Blood NfL as an easily accessible biomarker of disease evolution and treatment response, also in a controlled clinical trial setting

RRMS: Relapsing Remitting Multiple Sclerosis
NfL: Neurofilament light

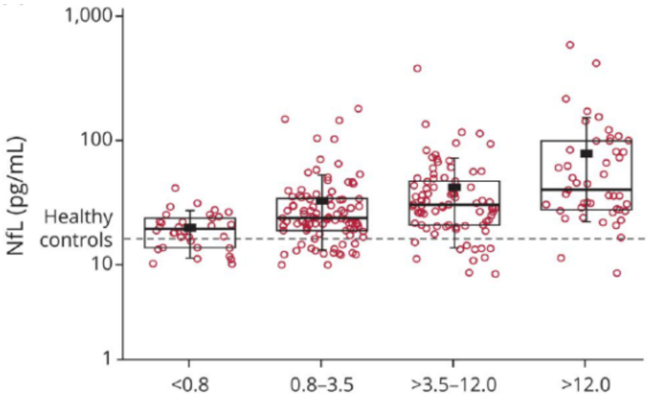
Combined FREEDOMS and TRANSFORMS



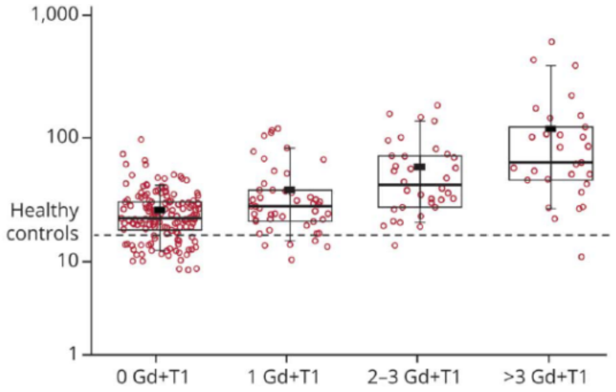
Effect of Fingolimod FREEDOMS



T2 lesion volume (total lesion load) FREEDOMS



Gd+ T1 lesion count (active lesion) FREEDOMS



Quanterix
Discovery Fueled by Ultra-Sensitivity

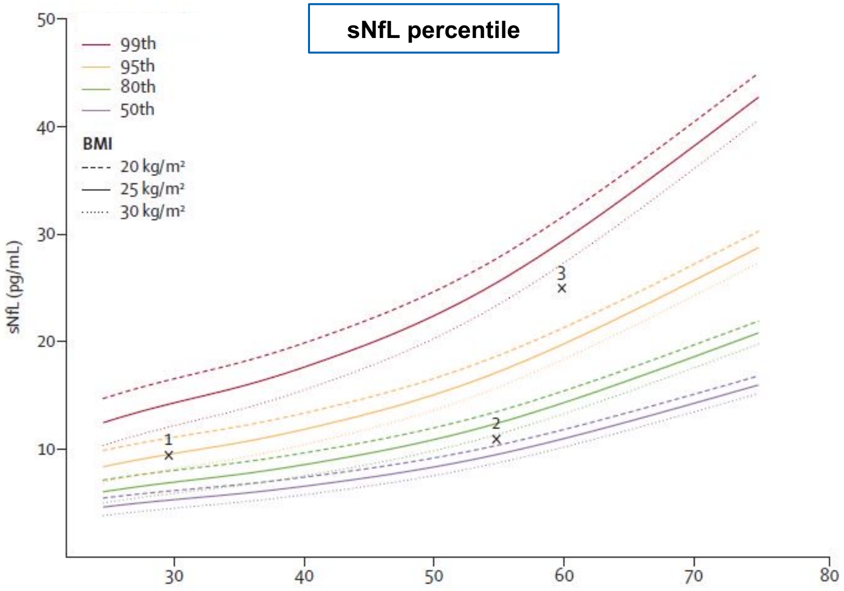
Kuhle et al. Neurology, 2019

Establishing sNfL normative ranges to aid identification of future MS risk

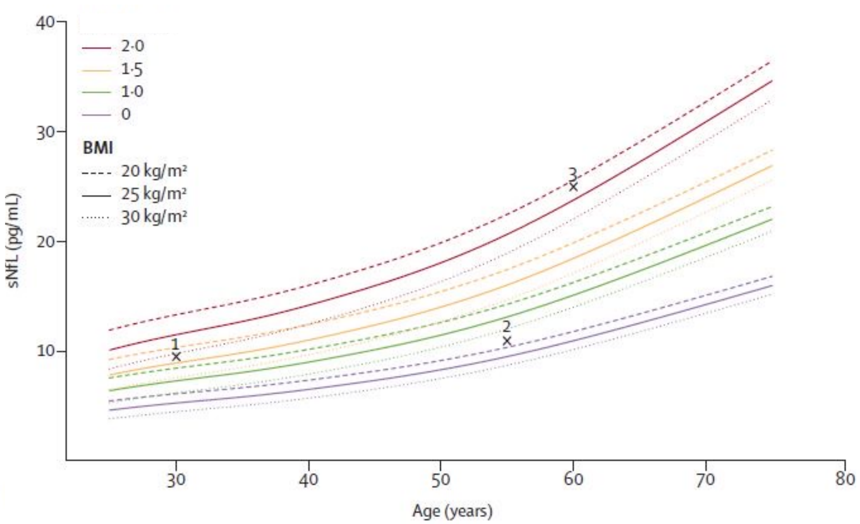
Seminal study defining sNfL reference values and development of online app

5390 healthy individuals (4 European and US cohorts)

sNfL percentile



sNfL Z score



MS: Multiple sclerosis
sNfL: serum neurofilament light
BMI: Body mass index

- Age related increase of sNfL in controls is not linear, but has a steeper increase after 50 years
- Increased Z scores outperformed absolute raw sNfL cutoff values for diagnostic accuracy

Easy identification and interpretation of elevated sNfL levels is more feasible in clinical practice

Quanterix
Discovery Fueled by Ultra-Sensitivity

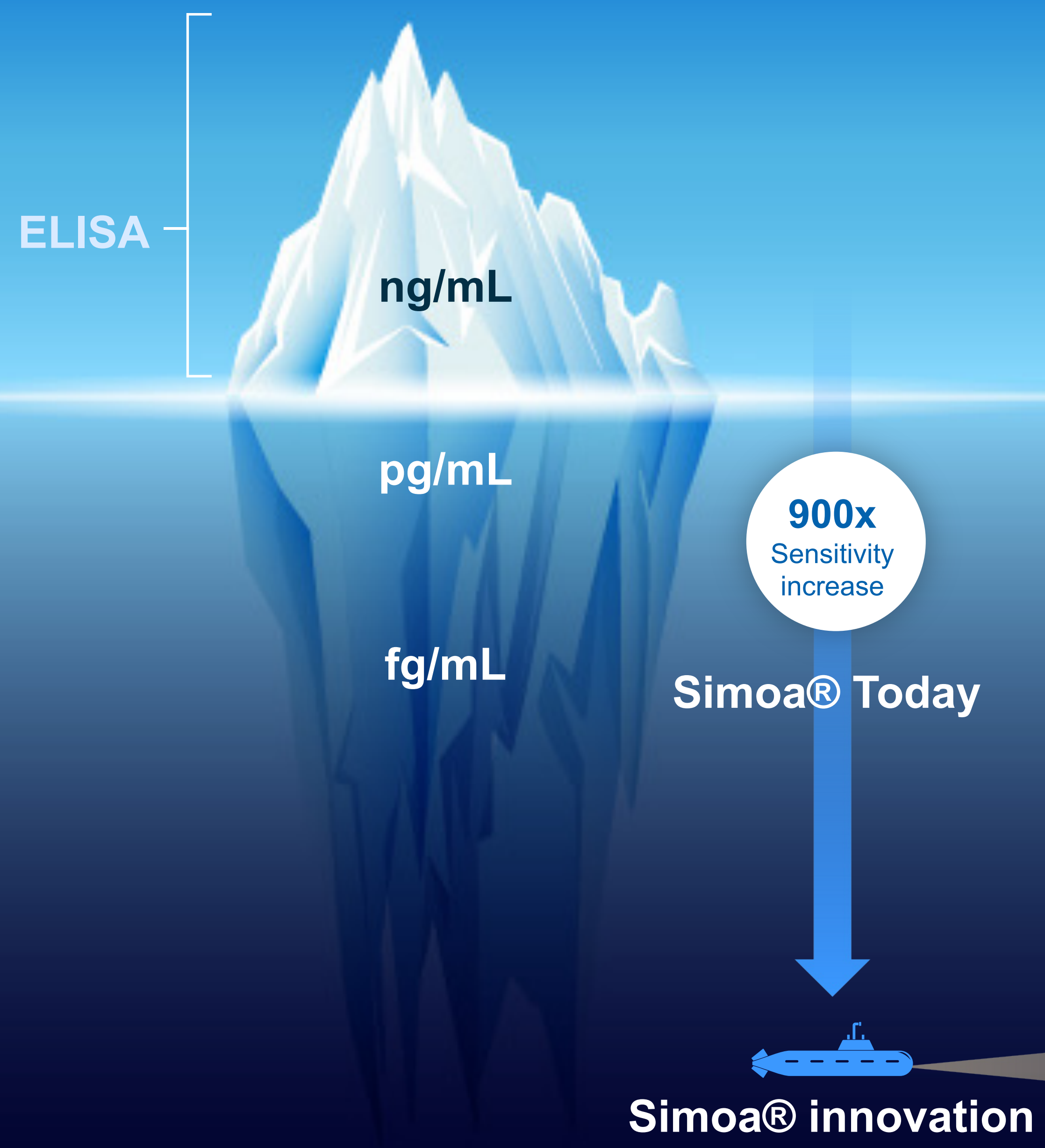
Benkert et al. Lancet Neurology, 2022

Clinical trial engagement

Non-invasive blood biomarker measurements empower pre- and post-market clinical trials

Lilly	Janssen	Eisai / Biogen	Novartis	Alnylam
Donanemab Phase 2	**JNJ-6373365 Phase 2**	**Lecanemab Phase 3**	**Ranibizumab Phase 4**	**Patisiran Phase 3**
Simoa based **pTau217**	Simoa based **pTau217+**	Simoa **pTau181**, GFAP, **NfL**	Simoa based VEGF assay	**Simoa NfL assay**
Collaboration of future multiplex biomarkers - AD	Analytical Validation - AD	Clinical trial support - AD	Clinical trial support - Macular Degeneration: Ranibizumag vs Aflibercept.	APOLLO study Polyneuropathy treatment Transthyretin-mediated (hATTR) amyloidosis

Highlights from few trials….



Thanks!

Early disease detection