celerion

Translating Science to Medicine

Tracing Biomarkers with Luminex, MSD & SIMOA

EBF 2019 Barcelona
Patrick Brennecke, PhD
Technical Director Bioanalytical Translational Science

Tracing Biomarkers: Introduction

- Introduction to Biomarkers: What are Biomarkers and why are they important?

- Human Biomarkers are endogenous molecules, which are differently regulated in healthy and diseased individuals (Diabetes, NASH, Asthma, COPD, CF and Cancer)

- Biomarkers serve to diagnose, stratify patients and monitor therapy success

- Biomarkers give key information on :

  - MOA
  - Efficacy & Safety
  - Metabolic profile

Tracing Biomarkers: Introduction

- Introduction to Biomarkers: What are Biomarkers and why are they important?

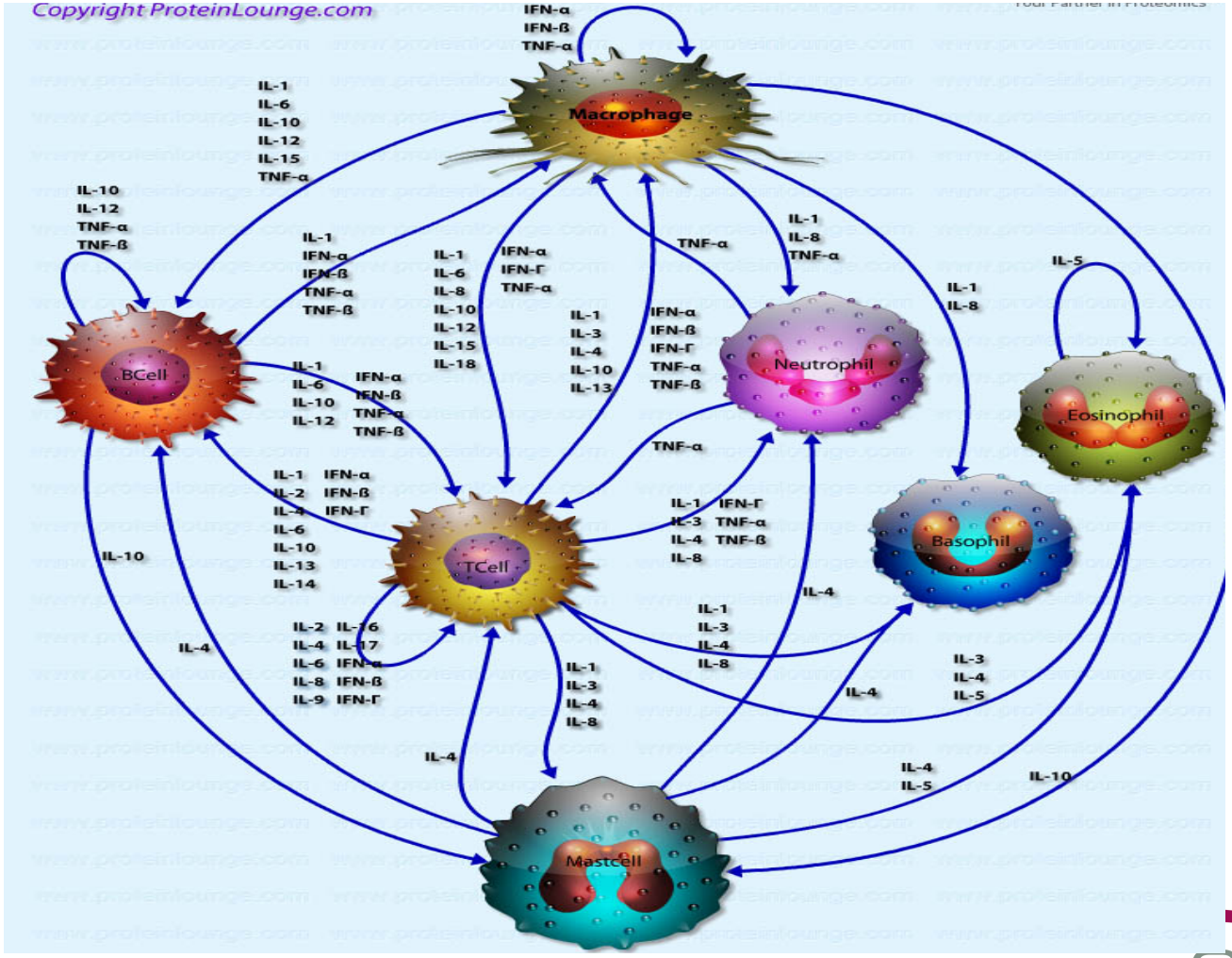
➢ Biomarkers can be used for a wide variety of purposes during drug development; therefore, a FFP approach should be used when determining the appropriate extent of method validation" U.S. FDA, CDER, CVM, Bioanalytical Method Validation, Guidance for Industry, 2018.

➢ Biomarkers for chronic diseases (Asthma, RA, Psoriasis, NASH) often fall in the category of the Inflammatory Biomarkers, which manly comprise inflammatory cytokines and chemokines

celerion

Tracing Biomarkers: Introduction

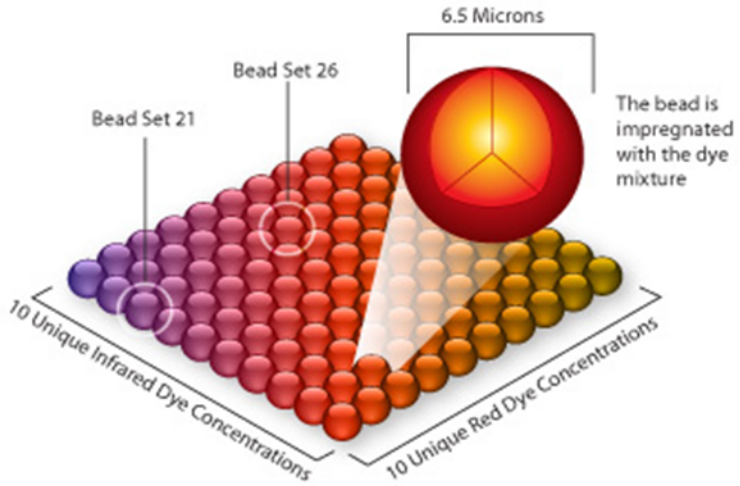
- Introduction to Biomarkers: Cytokines networks



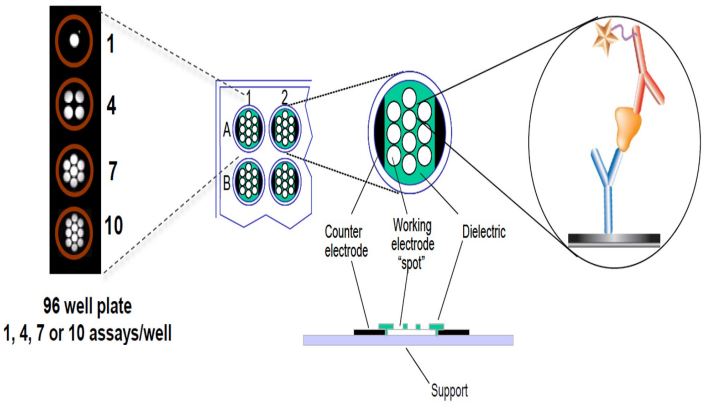
celerion

Tracing Biomarkers: Luminex & MSD

Luminex



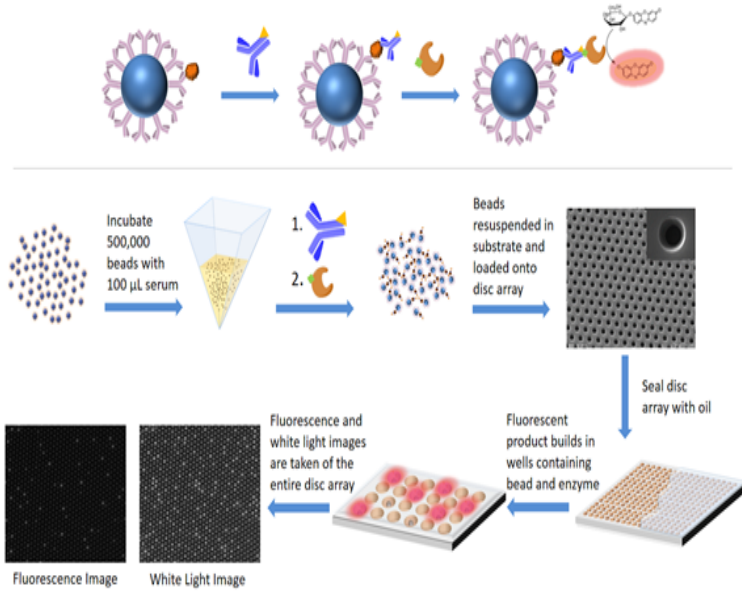
MSD



celerion

Tracing Biomarkers: SIMOA

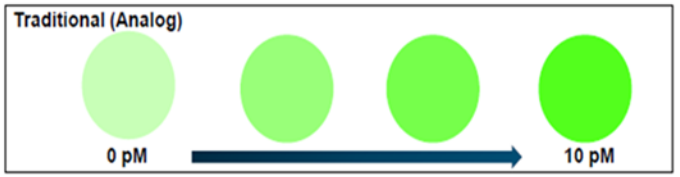
Simoa (Single Molecule Array)



celerion

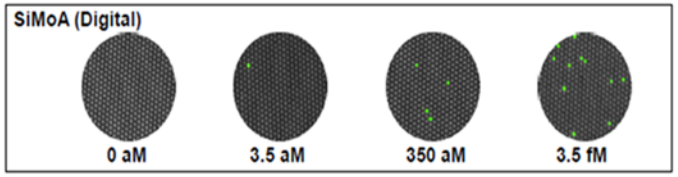
Tracing Biomarkers: SIMOA

Simoa (Single Molecule Array)



Microliters (µL)

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



Femtoliters (**f**L)

- 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

celerion

Tracing Biomarkers: SIMOA

- Why SIMOA, who needs it, and what for?

- Highly sensitive measurements in the low pg/ml or high fg/ml range

- New very potent drugs are active in this low range and SIMOA might be the only technology able to measure at such low levels

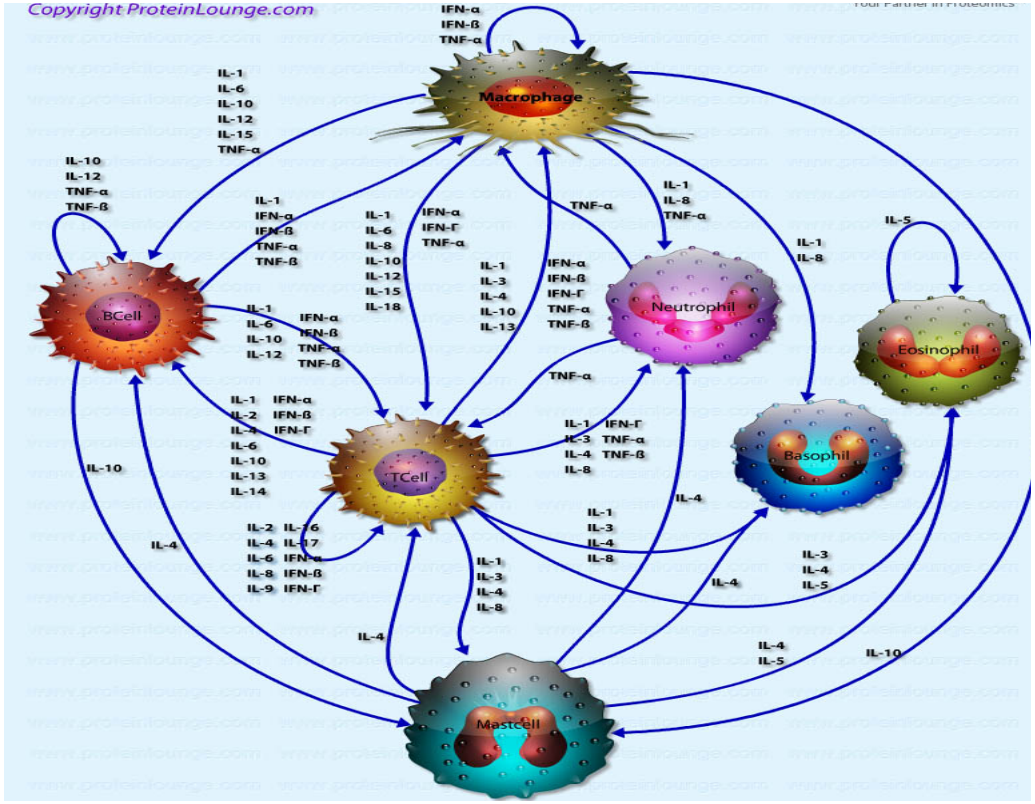
- Many Biomarkers, particularly in chronic diseases show small changes in this low analytical range (Biomarker Support)

- Alleviates patient burden- key Biomarkers can be measured in Blood instead of CSF (Cerebrospinal Fluid)

celerion

Tracing Biomarkers: Introduction

- Biomarkers of Chronic Diseases: Cytokines/Chemokines



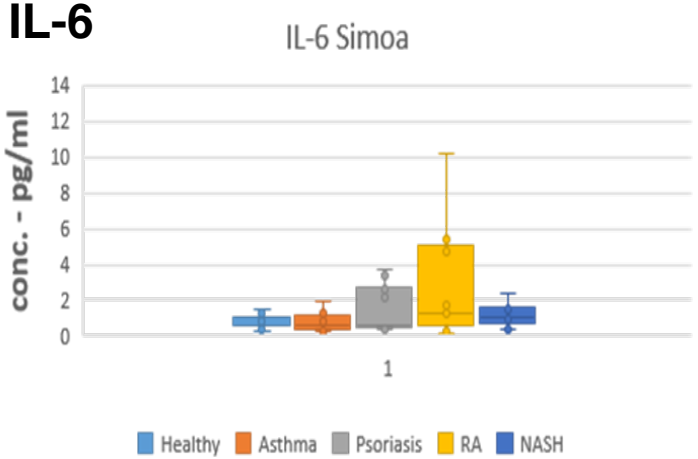
Cytokines analyzed:
IL-6, TNF-α, IL-12P70, IL-13 and IL-5

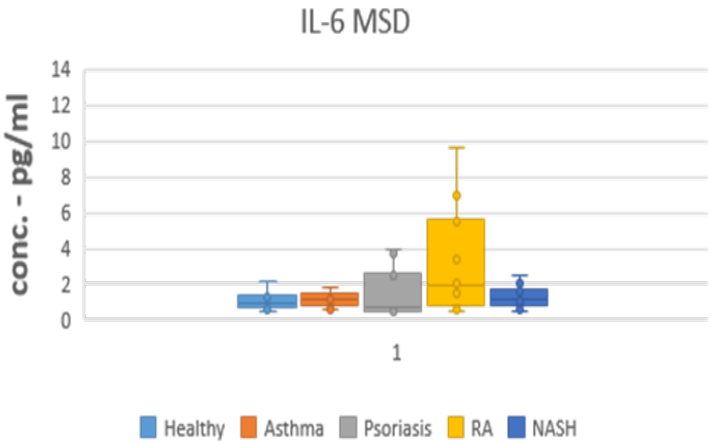
Diseases: Asthma, Psoriasis, Rheumatoid Arthritis (RA) & NASH

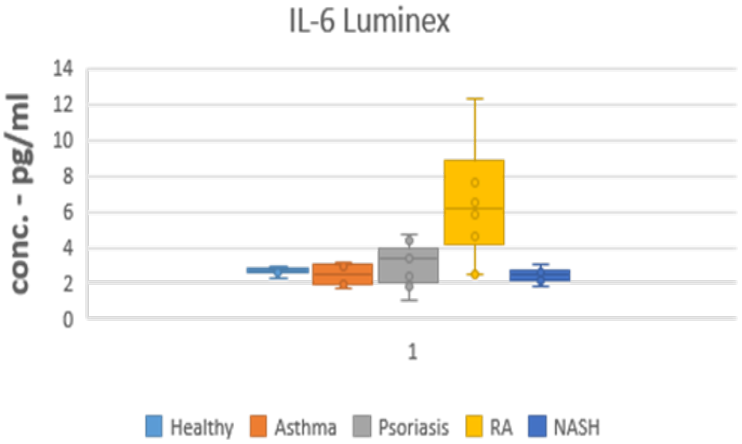
celerion

Tracing Biomarkers: Results

IL-6







	Simoa	MSD	Luminex
IL-6	0	8	43

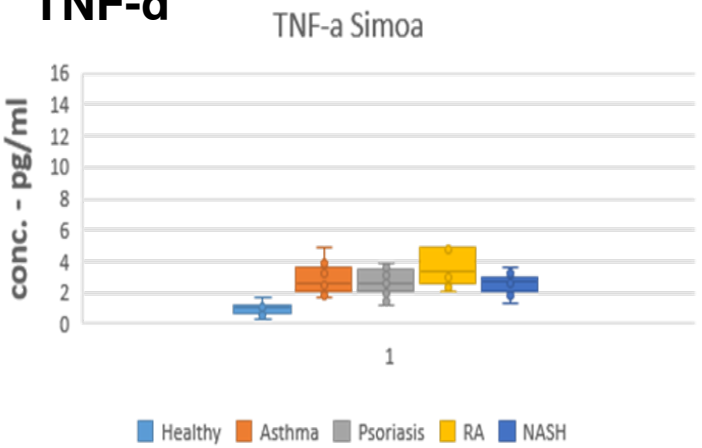
Results:

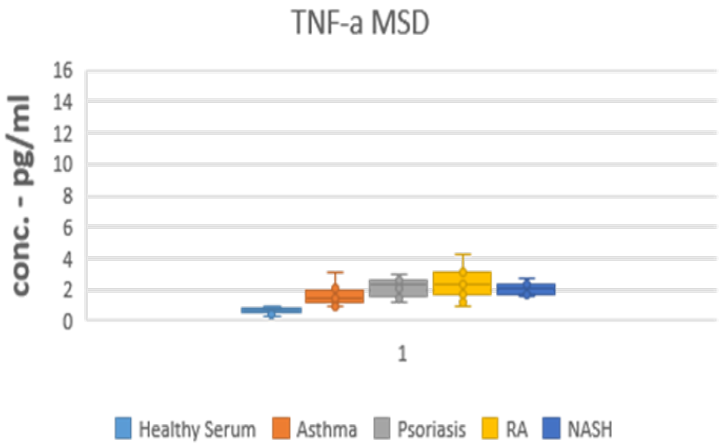
Il-6 levels were not significantly different when comparing healthy and diseased individuals; however only with SIMOA all samples could have been measured within range.

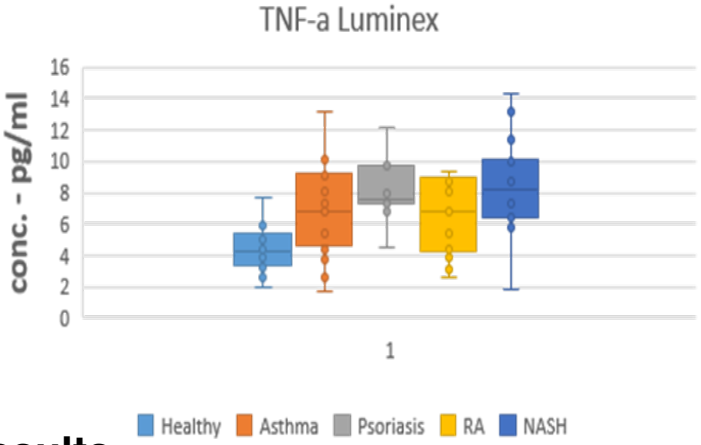
celerion

Tracing Biomarkers: Results

TNF-α







	Simoa	MSD	Luminex
TNF-α	0	2	3

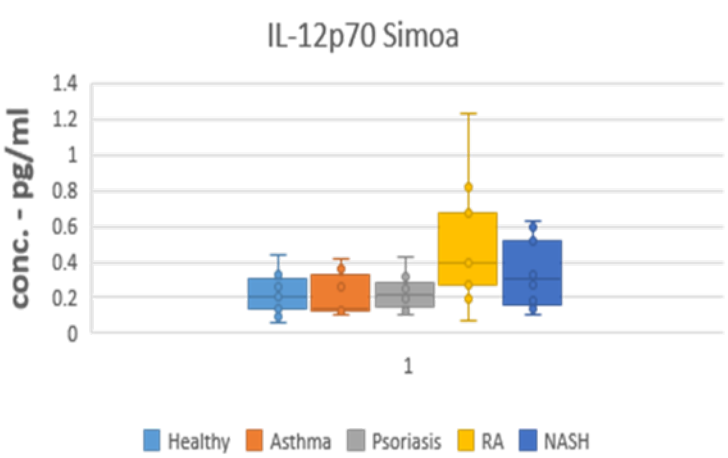
Results:

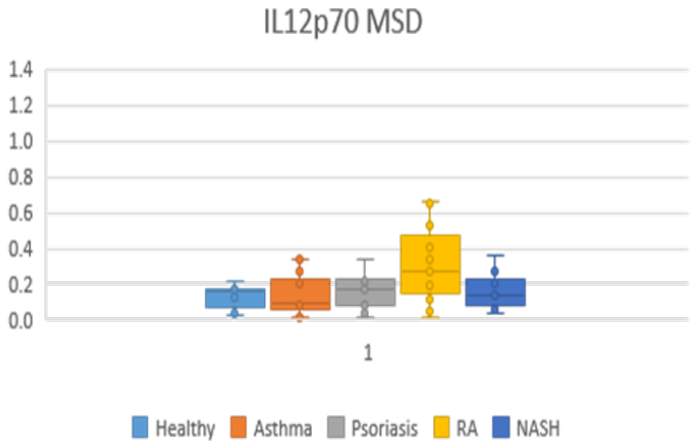
TNF alpha levels were significantly different when comparing healthy and diseased individuals; however only with SIMOA all samples could have been measured within range.

celerion

Tracing Biomarkers: Results

IL-12p70





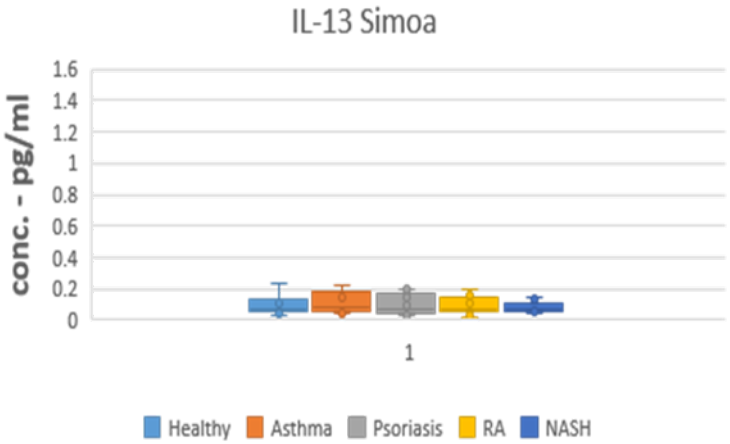
	Simoa	MSD	Luminex
IL-12p70	0	57	74

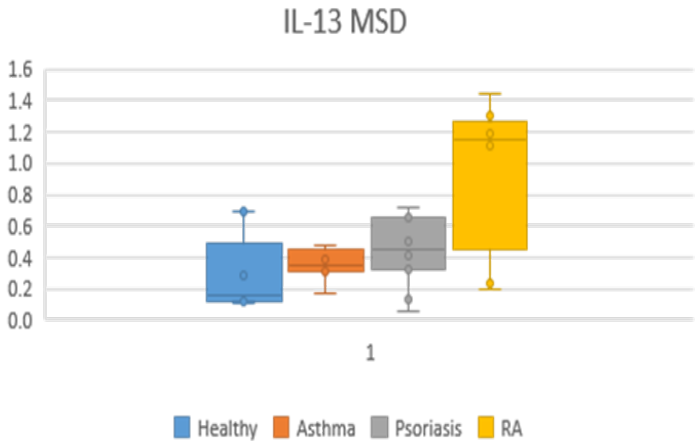
Result:
IL12p70 alpha levels were significantly different when comparing healthy and diseased individuals only for RA patients, but not for the other indications. Also here only with SIMOA all samples could have been measured within range.

celerion

Tracing Biomarkers: Results

IL-13





	Simoa	MSD	Luminex
IL-13	0	74	N/AP

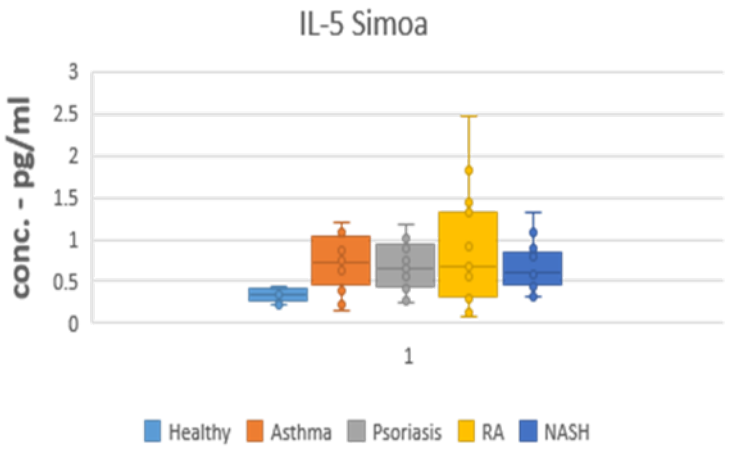
Results:

IL-13 levels were not significantly different when comparing healthy and diseased individuals. Only applying SIMOA allowed for the measurement of all samples within range; all samples measured with MSD were interpolated!

celerion

Tracing Biomarkers: Results

IL-5



	Simoa	MSD	Luminex
IL-5	0	pending	pending

Results:
IL-5 alpha levels were significantly different when comparing healthy and diseased individuals for all indications. With SIMOA all samples could have been measured within range.

celerion

Tracing Biomarkers: Results

Technology Summary

	Simoa	MSD	Luminex
IL-6	0	8	43
TNF-α	0	2	3
IL-12p70	0	57	74
IL-13	0	74	N/AP
IL-5	0	pending	pending

Results:
Comparison of SIMOA, MSD & Luminex measurements of BLQ (below quantitation) values. Applying SIMOA technology resulted in **no BLQ** values for the tested Biomarkers, whereas for MSD and Luminex various samples were not reliably measurable.

celerion

Tracing Biomarkers: Summary

- The Key Biomarkers IL-6, TNF-α, IL-12p70 IL-13 and IL-5 in were analyzed with SIMOA, MSD & Luminex in healthy and diseased individuals suffering from Asthma, Psoriasis, Rheumatoid Arthritis (RA) and Non-Alcohol-induced Steatohepatitis (NASH).

- For all Biomarkers SIMOA demonstrated to be superior compared to MSD or Luminex either because only with SIMOA all samples could be measured (no BLQ values) or because SIMOA was the only technology differentiating healthy from diseased individuals.

- Unequivocally the need for SIMOA technology was demonstrated in order to reliably detect Biomarkers for chronically ill individuals.

celerion

Tracing Biomarkers: Summary

Special thanks to:

- Petra Struwe, PhD
- LBS& CBA RnD Team
  - Marc Montjovent, PhD
  - Christopher Acuna
  - Sebastian Fleire, PhD
  - Maija Pfenniger, PhD
  - Arkadiuz Wyrzucki, PhD
  - Marita Zoma, PhD
  - Petia Doytcheva, PhD
  - Lysie Champion, PhD

celerion

Tracing Biomarkers: Summary

Thank you very much!

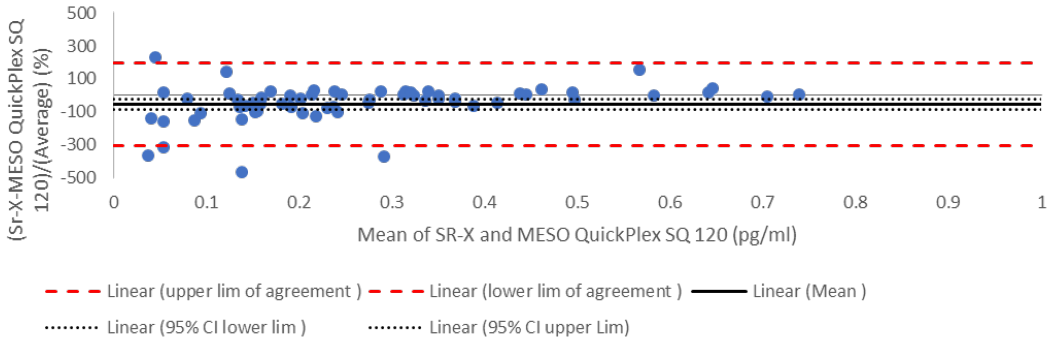
celerion

Tracing Biomarkers: Appendix

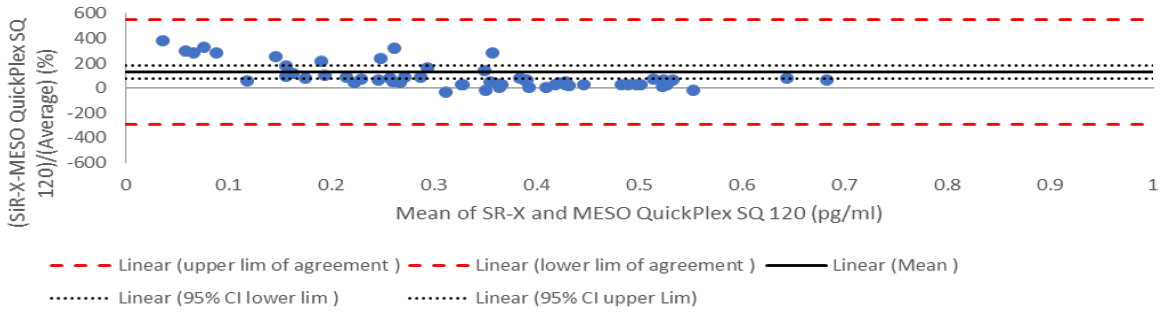
celerion

Tracing Biomarkers: Bland - Altmann

IL-6: Bland-Altman Comparison Between SIMOA & MSD



TNFα: Bland-Altman Comparison Between SIMOA & MSD



celerion