When reagents and technology matter to measure low abundant protein biomarkers

Hervé Farine - Translational Biomarkers - 19.09.2019

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Define the purpose

Assay selection
Assay development

CXCL11 **NFL**

Feasibility study

IL-5

Validation

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Measurement of IL-5 in human serum samples of asthmatics (commercial assays available)

COU

Identify the type 2 asthma patients (stratification)

Biostatistician & translational science

The assay selection based on LLOQ

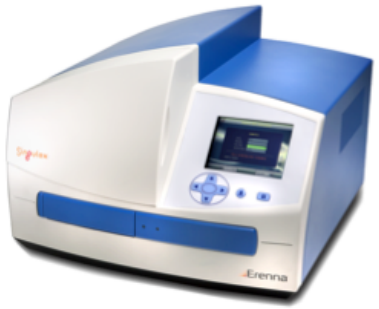
0.5 pg/ml

2014
Mesoscale x-plex

0.2 pg/ml

2015
Mesoscale V-plex

1 pg/ml

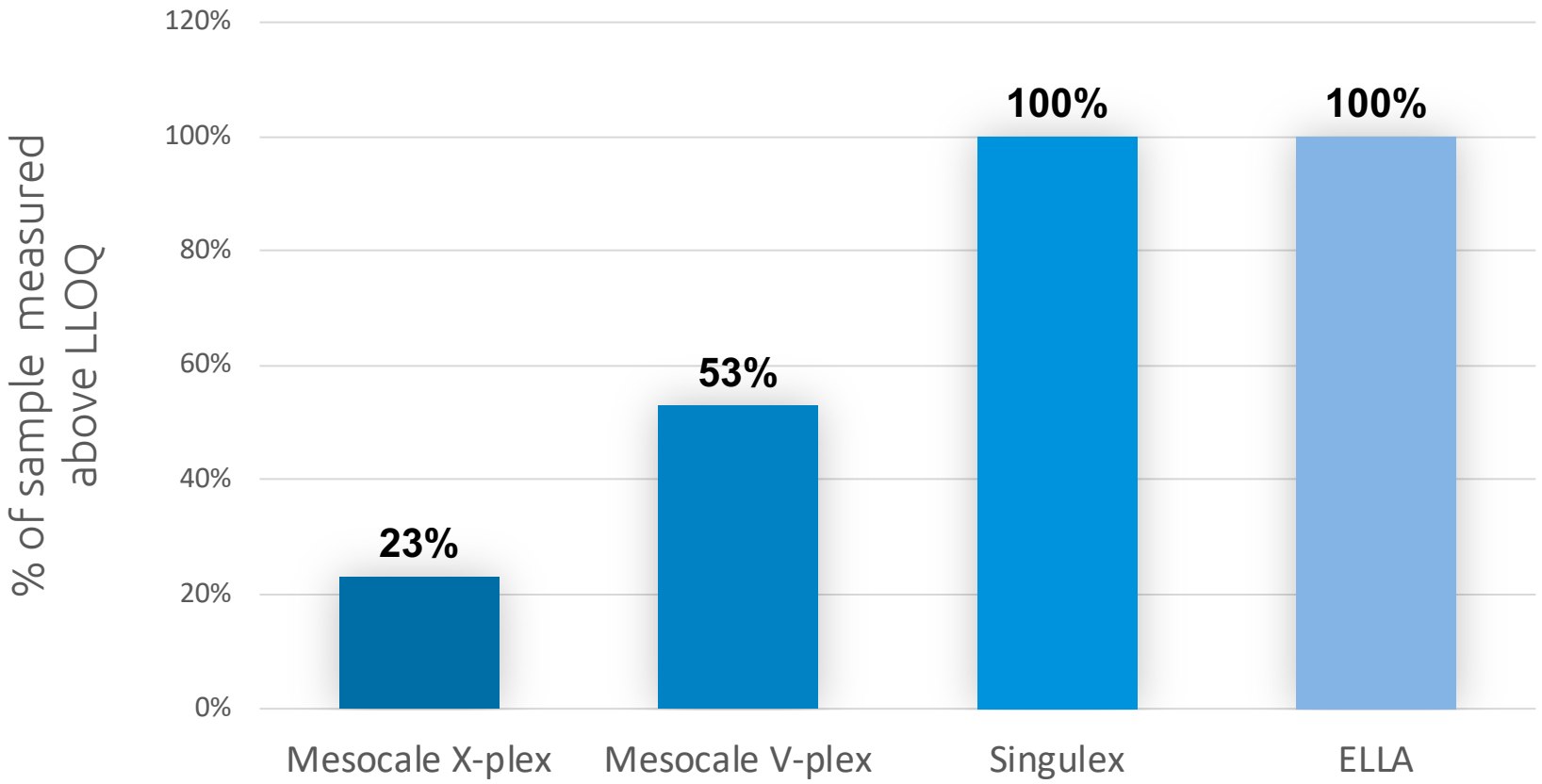


2015
Singulex/SMC

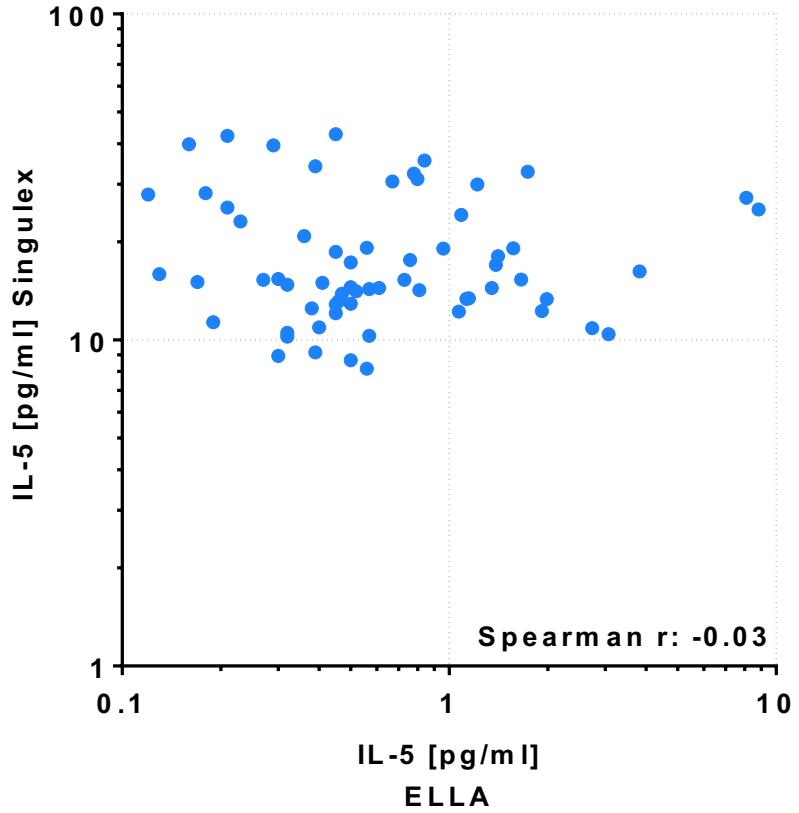
0.069 pg/ml

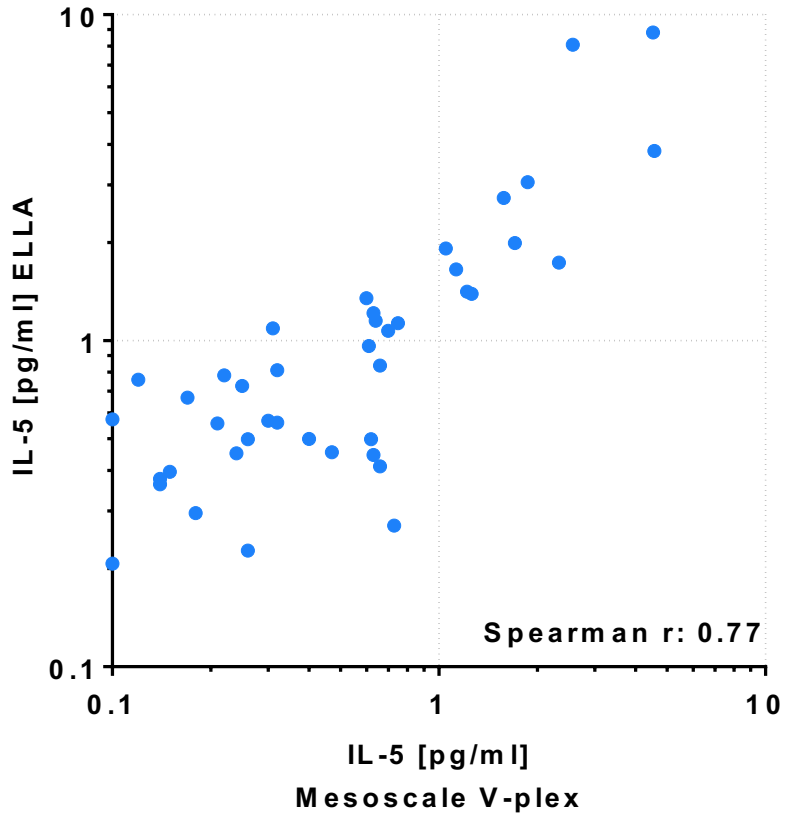
2015
ELLA

Only 2 assays allowed to measure the entire sample set.

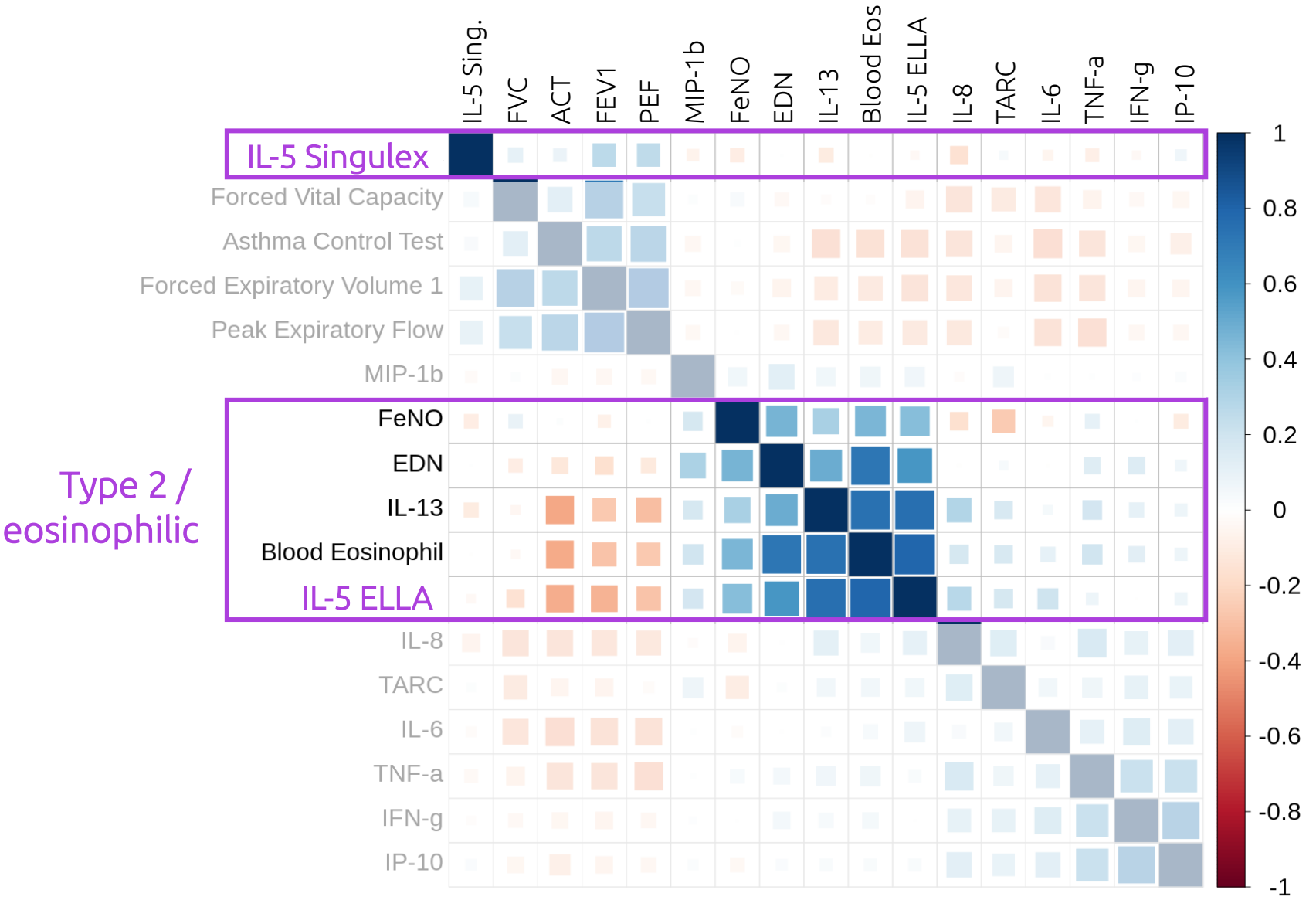


Bad correlation with Singulex and strong correlation between Mesoscale V-plex and ELLA





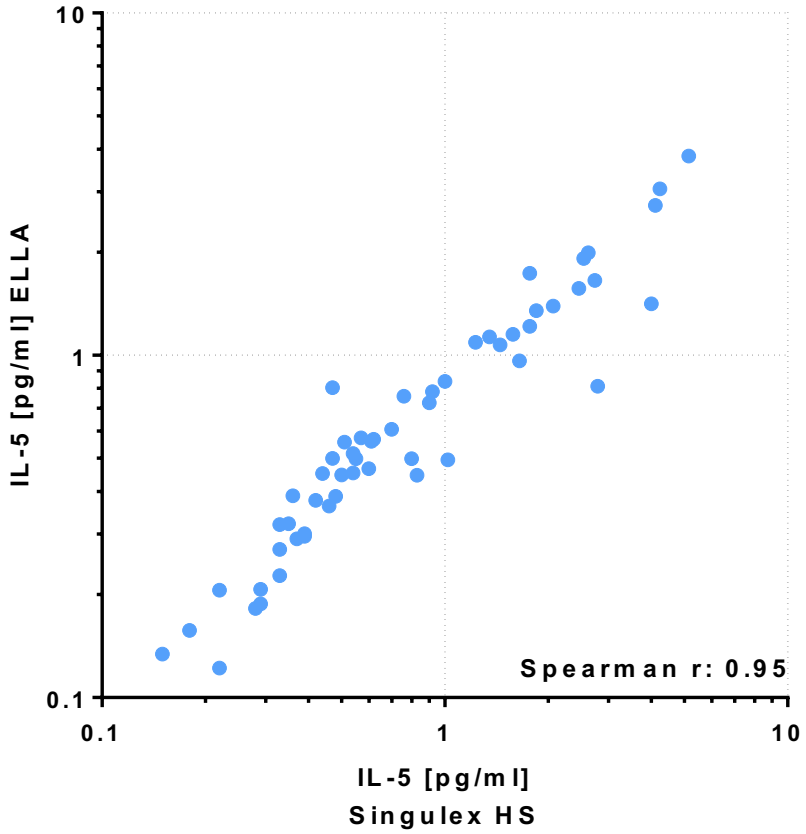
IL-5 ELLA was in the type 2 biomarkers cluster but not IL-5 Singulex

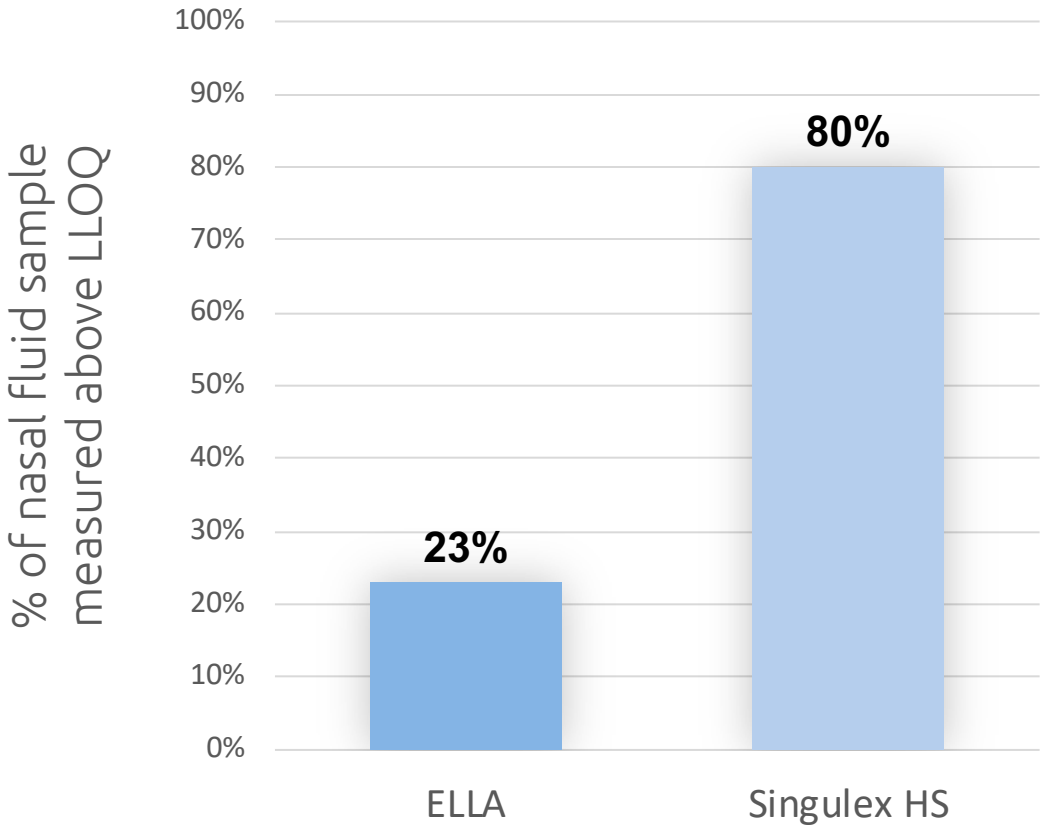


4 years later the COU changed !!

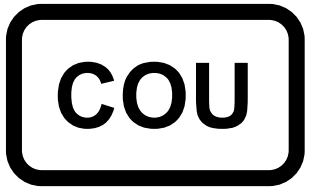
- PD Biomarker
- Sample: serum and nasal fluid
- The assays changed:
  - ELLA: LLOQ **69 fg/ml → 130 fg/ml**
  - Merck(Singulex) launched a new high sensitivity (HS) assay (with new antibodies) **LLOQ: 40 fg/ml**

Singulex/SMC HS has a strong correlation with ELLA in serum and was able to measure 80% of the nasal fluid samples.



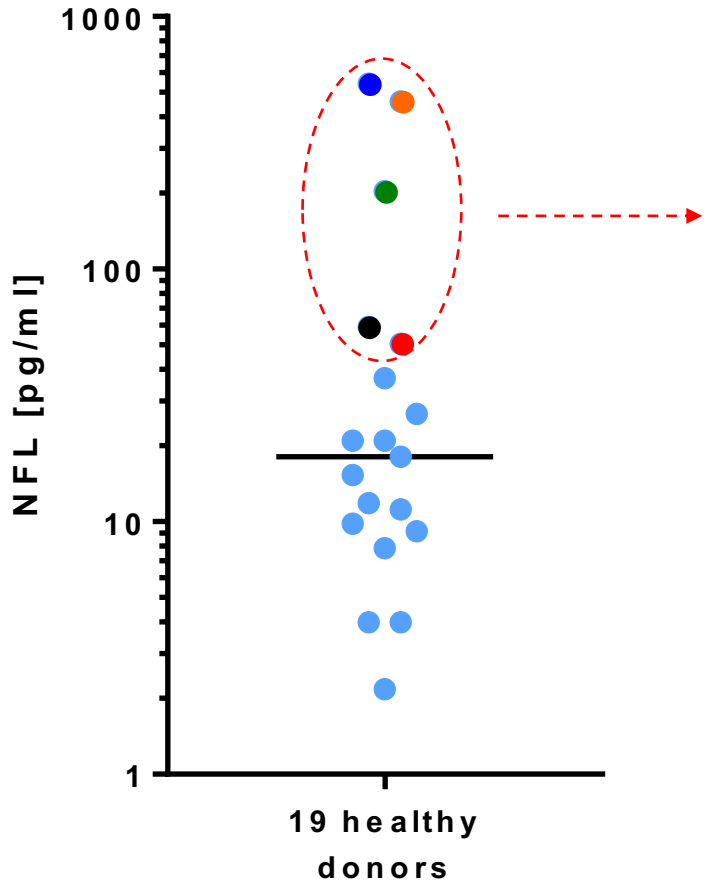


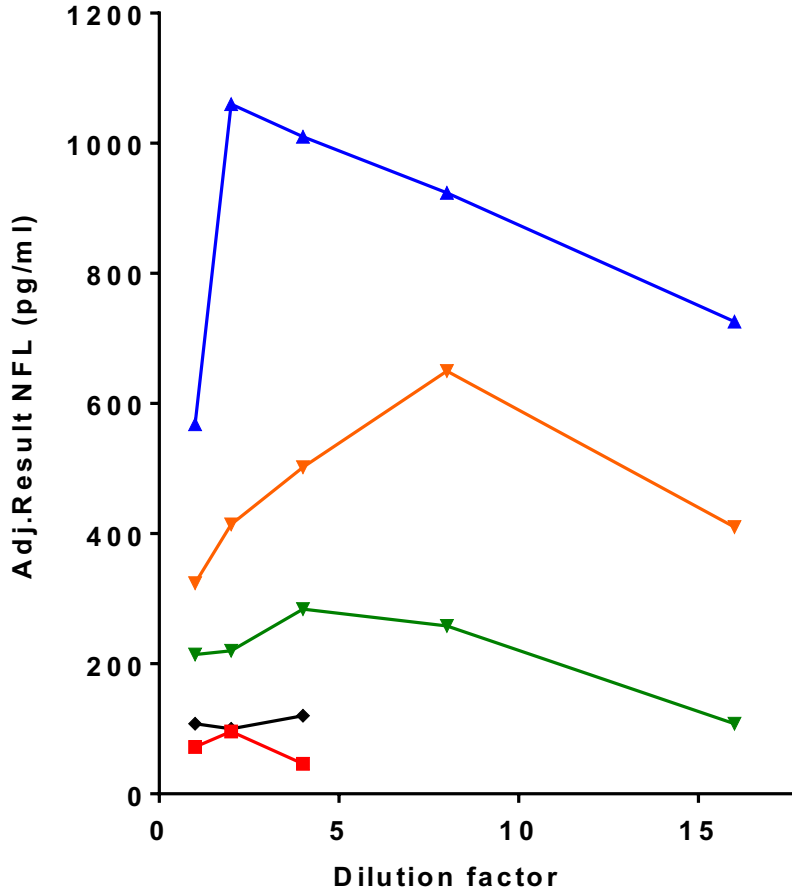
Develop an assay on MSD able to measure NFL in human and rodent in plasma and CSF



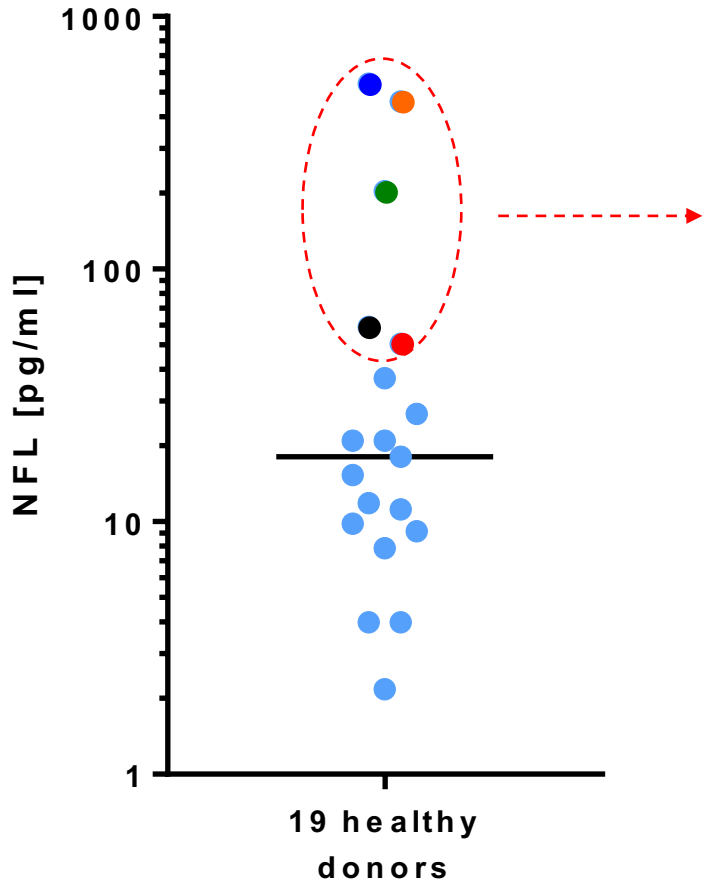
PD effect for rodent model and human disease effect

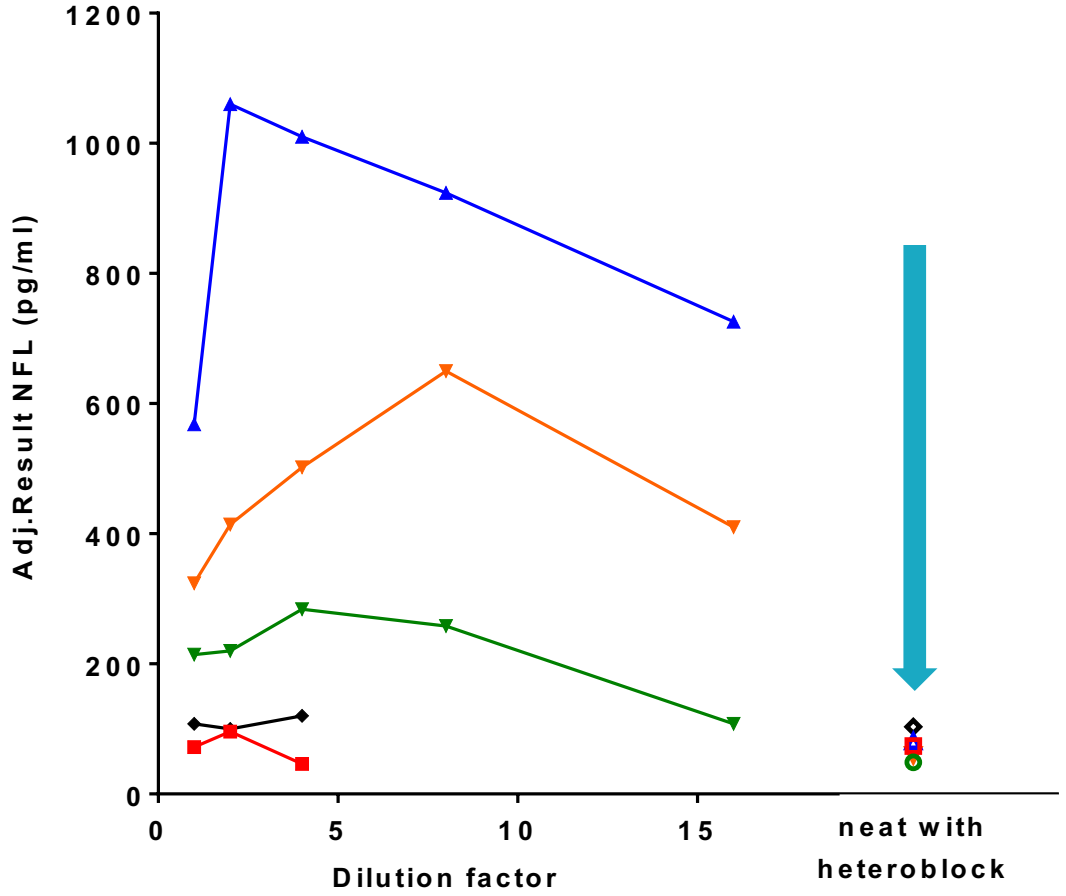
Translational science and preclinical pharmacology


1000
100
10
1
NFL [pg/ml]
19 healthy donors

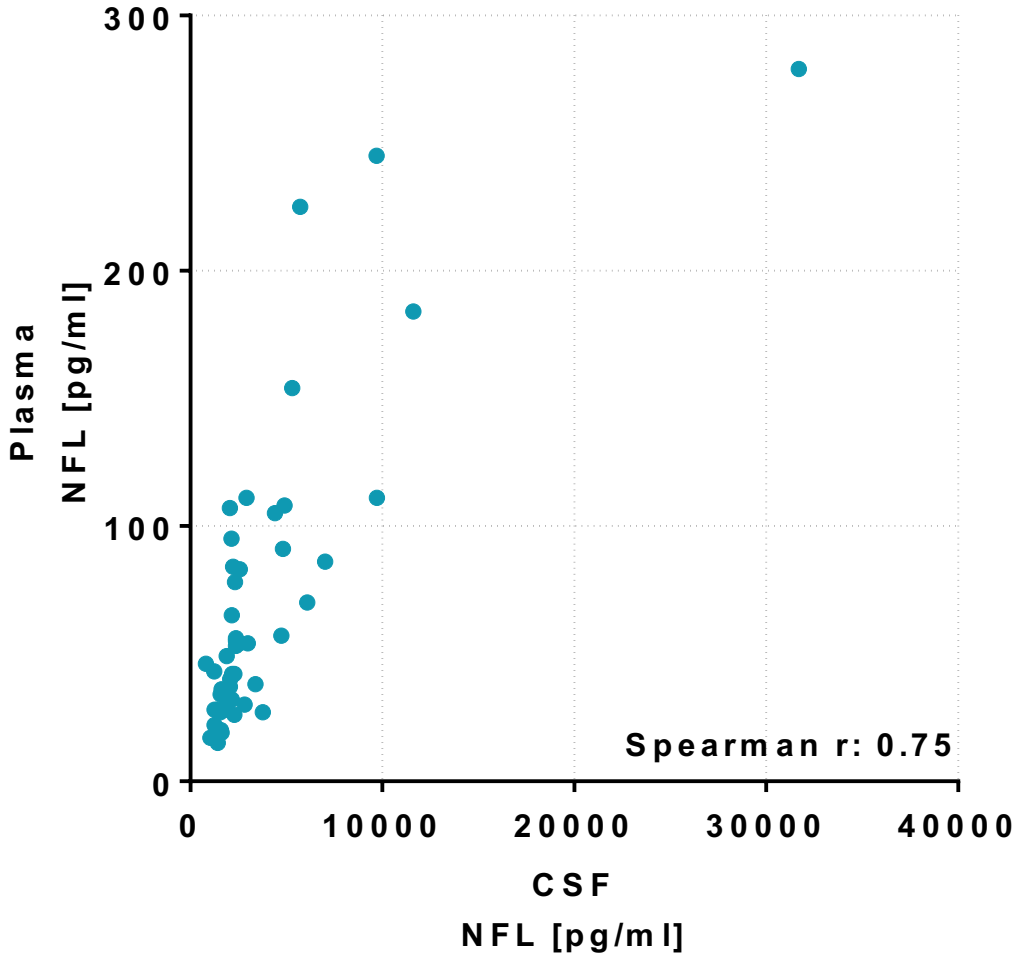

1200
1000
800
600
400
200
0
Adj.Result NFL (pg/ml)
0
5
10
15
Dilution factor

The addition of heterophilic antibodies blocker during the sample incubation reduced the level of the highest samples.

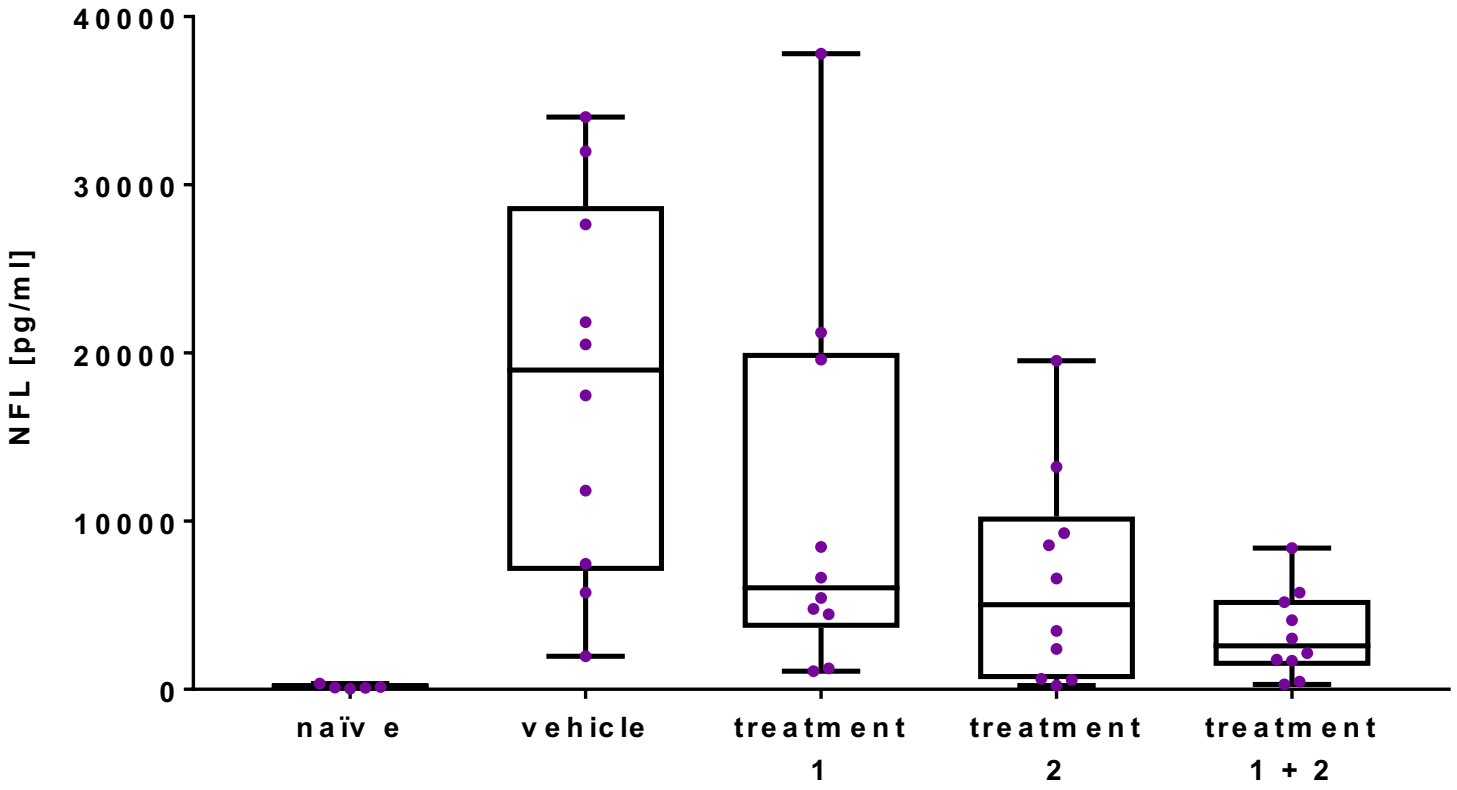


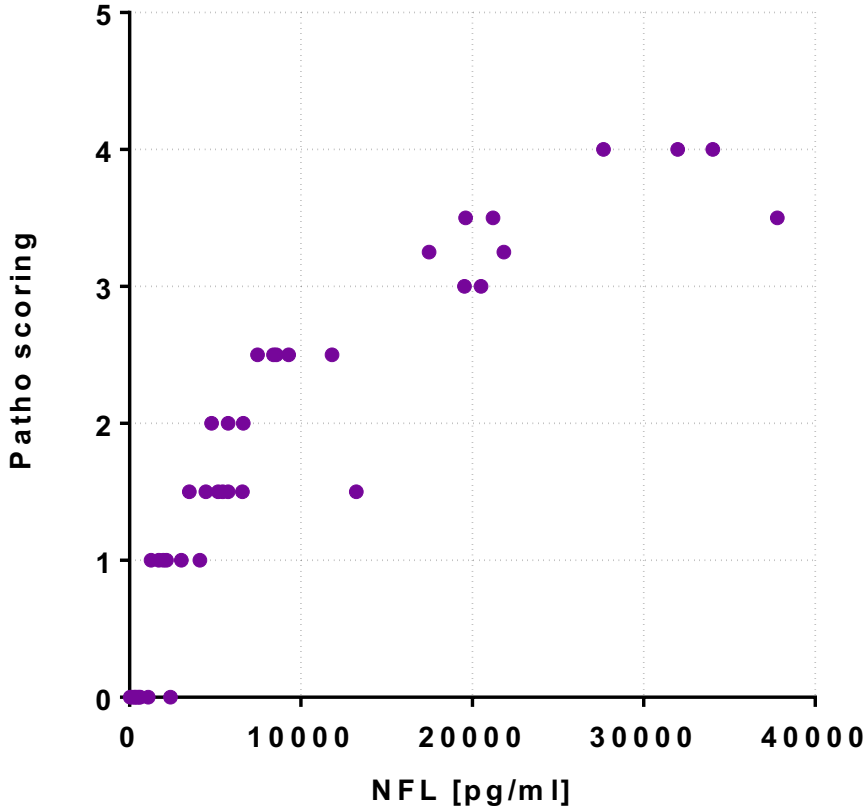


The good correlation between human CSF and plasma showed the specificity of the assay.

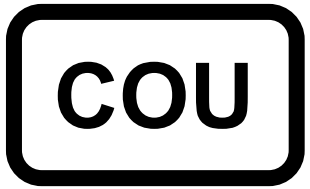


The use in mouse EAE model showed PD effect and a strong correlation with the pathology score.





Develop an assay able to measure CXCL11 in mouse plasma with 2 different high sensitivity platforms (Erenna and SR-X) with the same antibodies and the same standard.

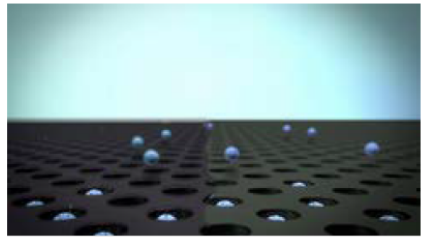


PD effect in mouse model

Preclinical pharmacology

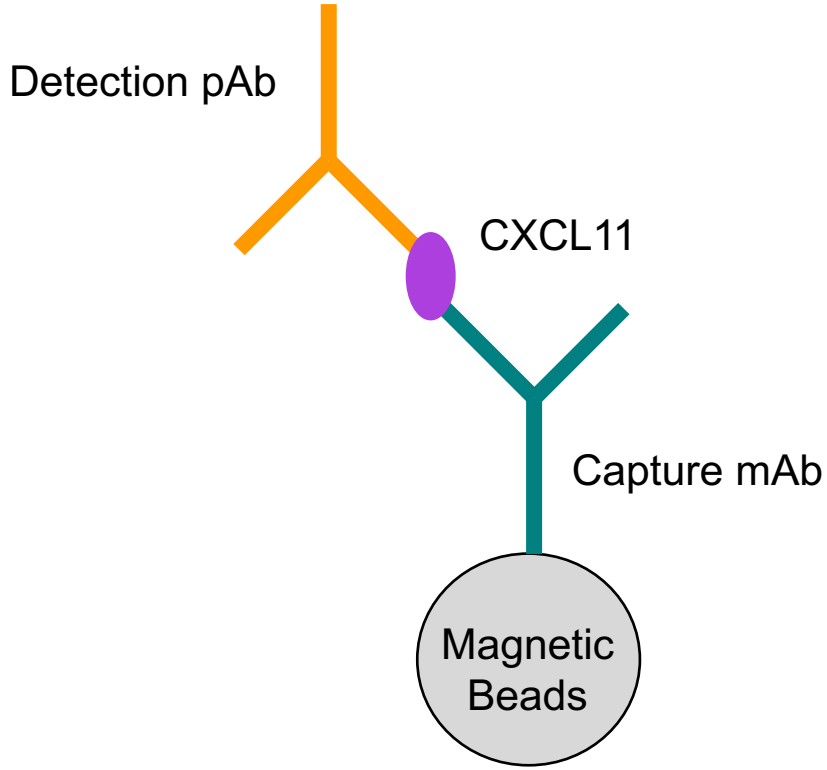
Similar assay setup but 2 different technologies

SR-X

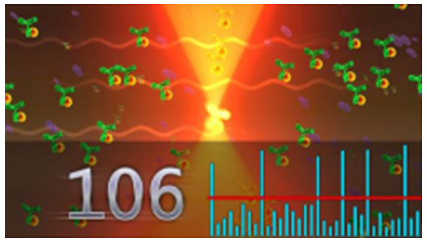


LLOQ = 0.4 pg/ml

Fluorescence signal



Erenna



LLOQ = 1 pg/ml

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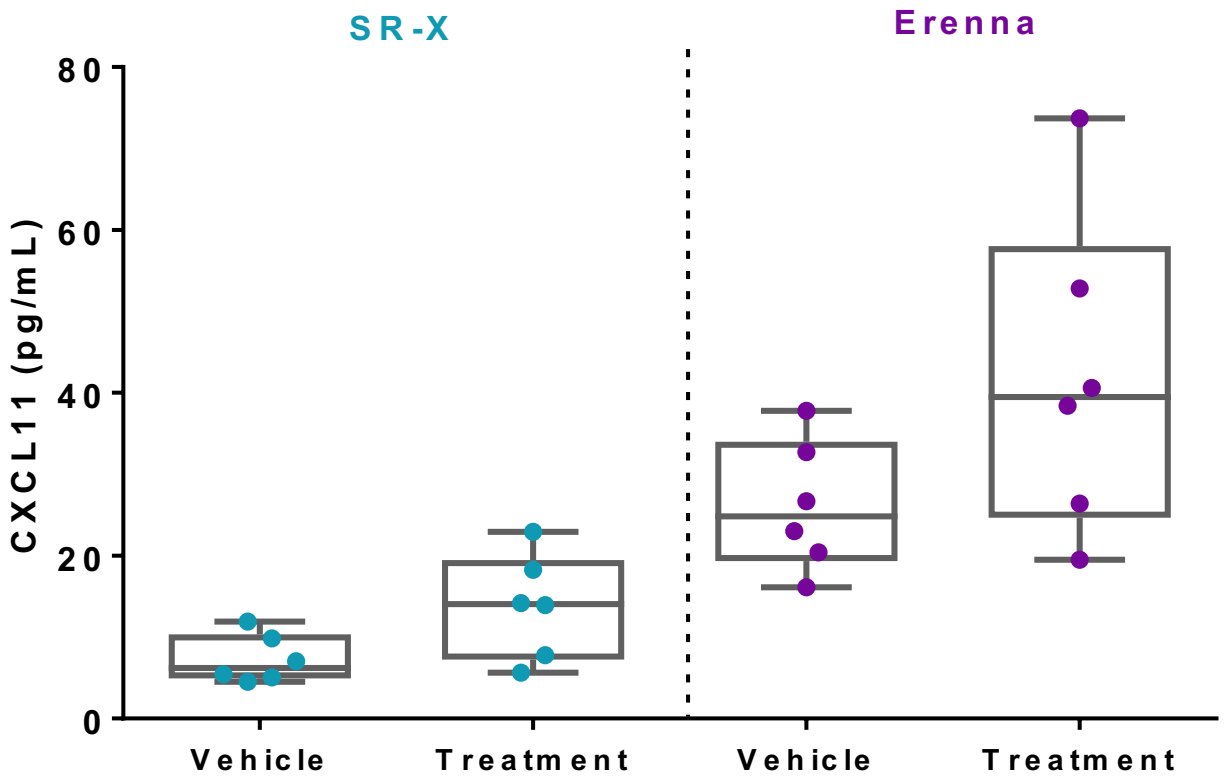
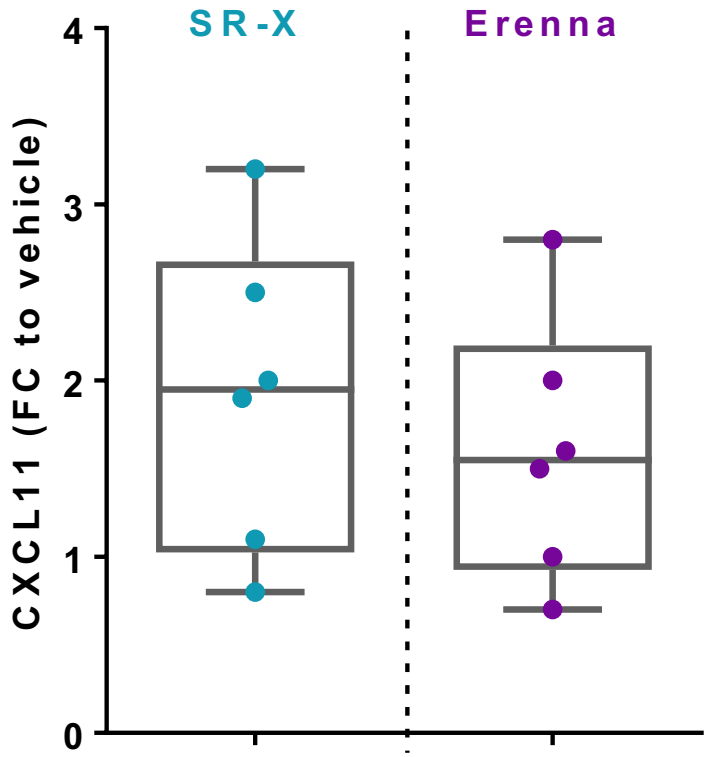
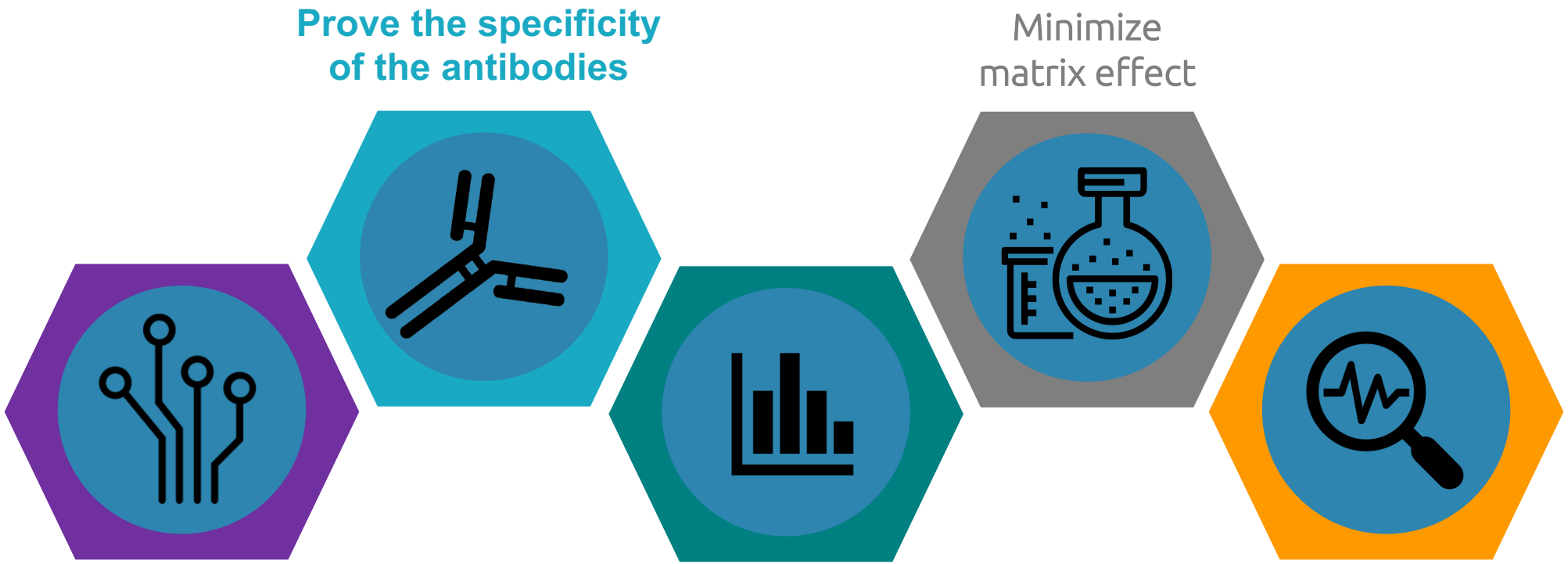
SR-X showed the same trend than Erenna but with lower absolute values



The PD effect has the same amplitude in both assays.




Prove the specificity of the antibodies
Minimize matrix effect
Technology improves the sensitivity
Multiple comparison (biomarkers and assays)
Be aware that immunoassays do not provide absolute values