



Franco-British bilateral symposium on clinical research, October 2012



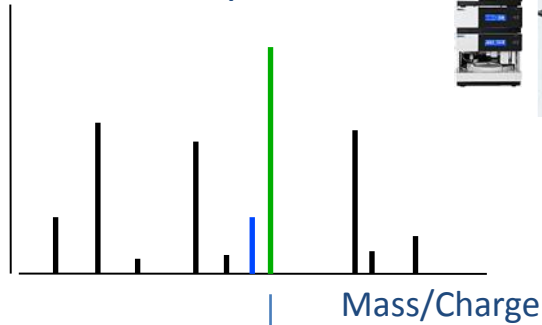
Alzheimer's disease biomarkers discovery by high resolution mass spectrometry: a challenging task of the Diatral project

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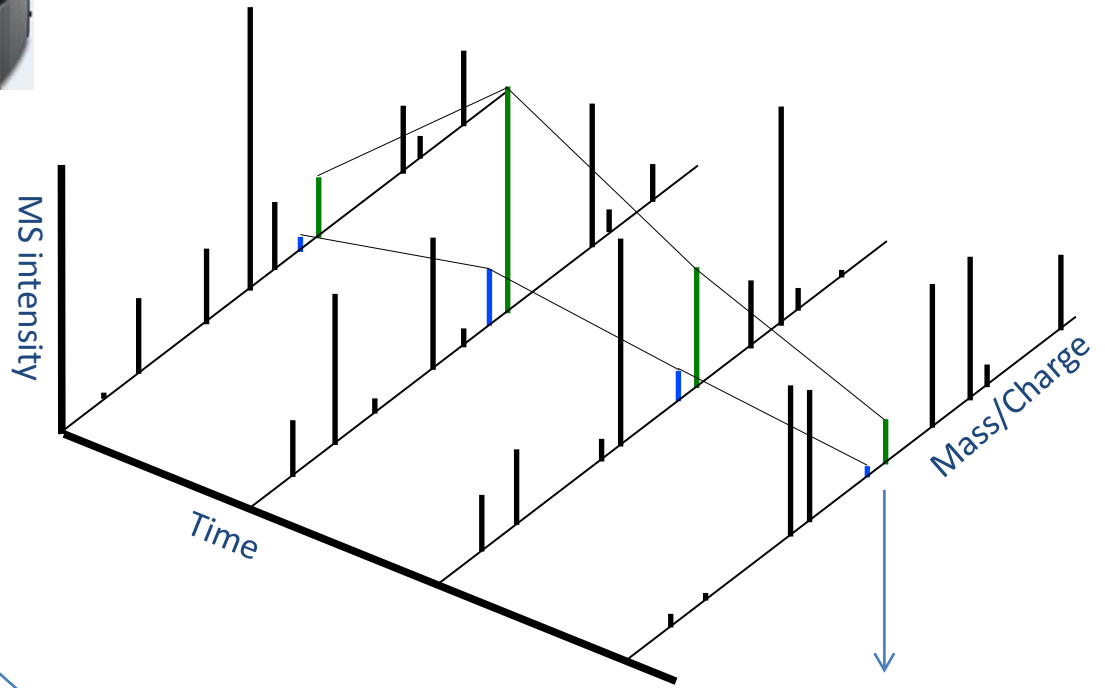


Introduction on MS peptide mapping

MS1 spectrum

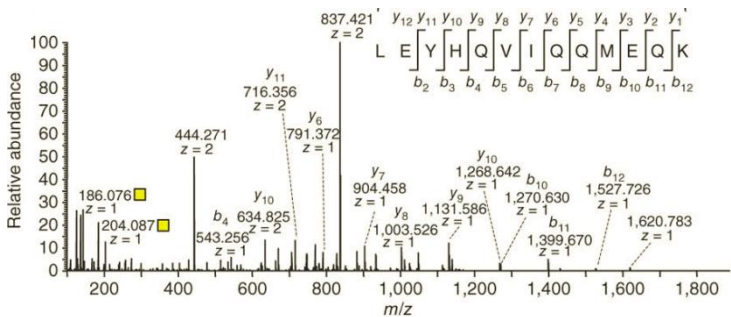


Consecutive MS1 spectra



Isolation, fragmentation

MS2 spectrum

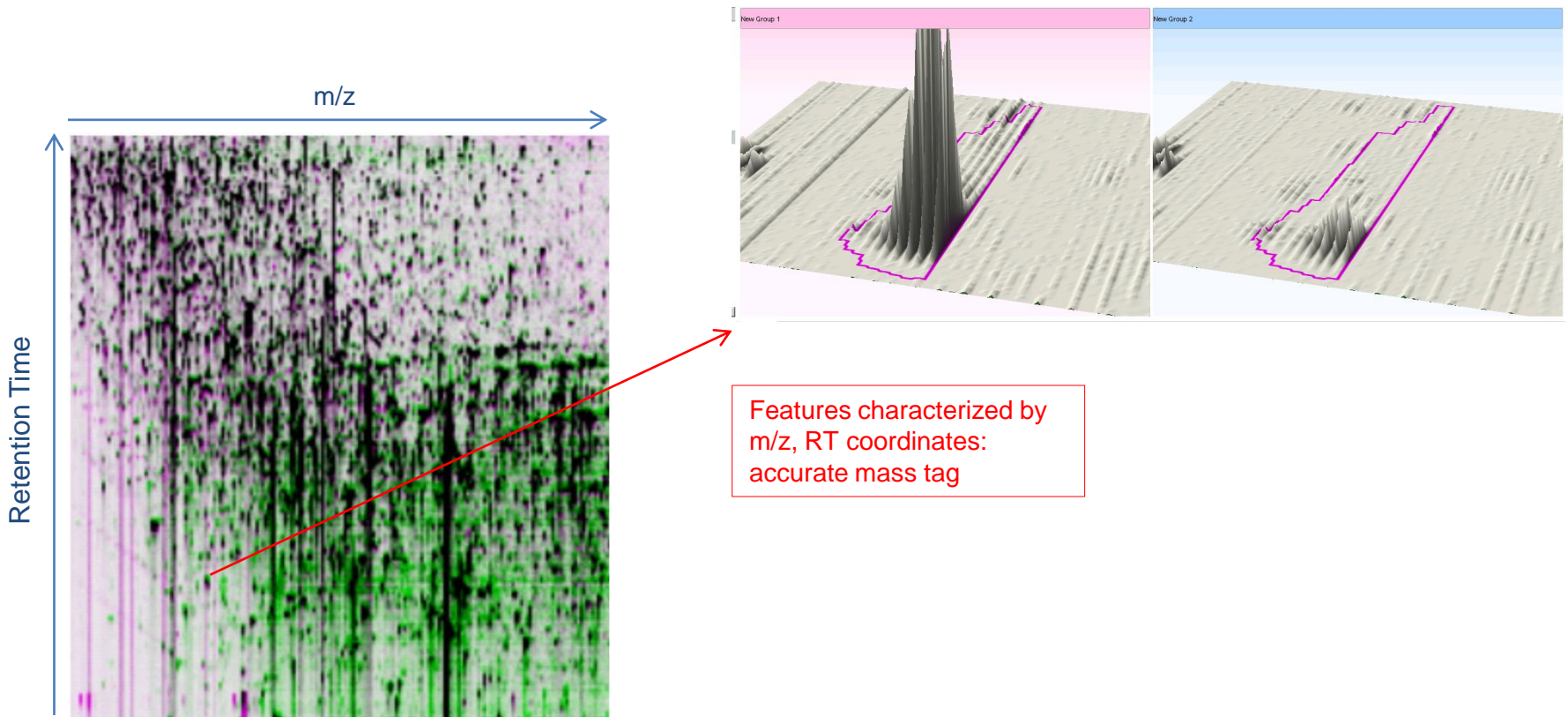


Quantification

Identification

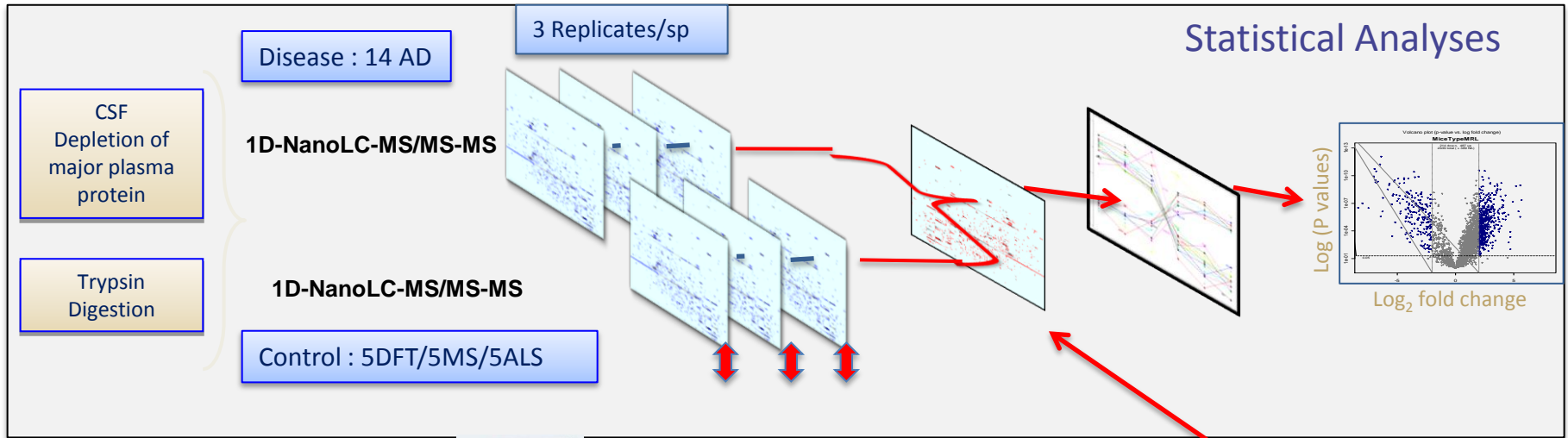
Protein sequence database

Introduction on MS peptide mapping



More than 20 000 peptide signatures in a CSF

Method for biomarker discovery in AD: high resolution peptide profiling of CSF samples by FT-MS



LC-FT MS/MS
Triplicate injections /sample



LC-FT MS/MS
Triplicate injections /sample



2D LC

Disease : 28 samples

DIFFTAL / Progenesis LCMS
Detection, Alignment/Match & sequence

Differential at the features level :
m/z, RT, Isot. Pattern, Intensity, Identification/not

SP1: CSF / Plasma samples dedicated to the project

- Collection of CSF & plasma samples (*CHRU, 24 months*)

- Biomarker identification

- HR MS peptide profiling: 15 AD (homogenous group) and 15 age-matched controls (FTD, ALS & MS): 1 ml each (split between IGF and SANOFI):

- 29 samples** (14 AD (1 with 0.5 ml) and 15 Controls

- 2D peptide map of human CSF: CSF samples with maximal heterogeneity (age, sex, pathologies, but including AD, FTD, ALS & MS): 0.5 ml each

- 28 samples**

- Biomarker validation by LC/QqQ/MRM *On going*. 24 AD at different stage of the disease (MCI, moderate & severe) and 24 age-matched controls (FTD, ALS & MS)

- Biomarker validation by Ab assays *Scheduled* 100-300 samples with clinical data

- Preclinical study / biomarker final selection *Scheduled* 100-300 samples with clinical data

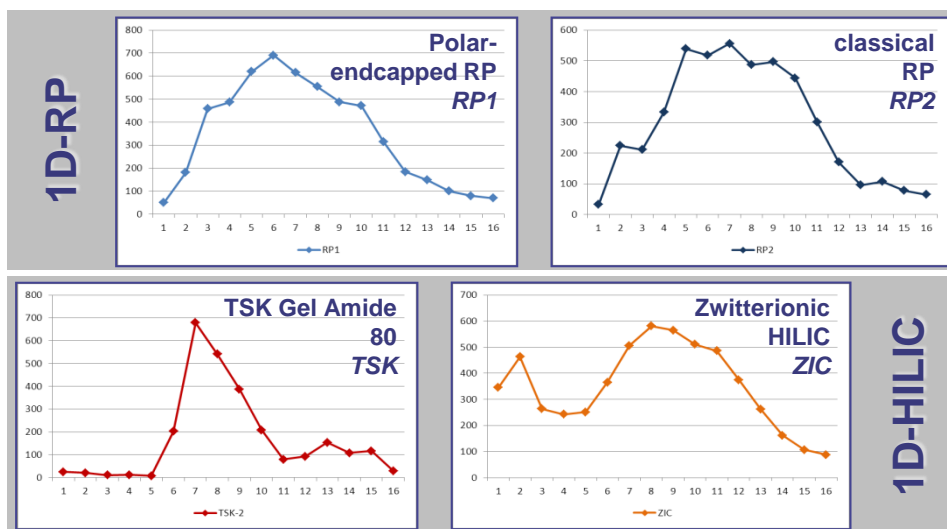
Sample preparation

- Standardized and reproducible method for CSF sample preparation
- Depletion of 20 major proteins
 - 80% of CSF proteins are common with the plasma
[97% of total protein content]
 - Immunodepletion kit ProteoPrep20 (Sigma) optimized
 - Only 3 proteins (Apo-A1; Apo-A2 and Transthyretin) are not well depleted
- Proteins quantification after depletion (for 200 μ L CSF Natif)
 - 88% depletion in protein amount
- Reproducible protein digestion on filter (FASP)
 - We now start with 200 μ L of CSF

Populating CSF MS peptide map

Bank strategy : Results 2D-LC/MS
 RP-fractionations: Global comparison

Sample used : CSF (Hydrocephalus + MS + ALS)



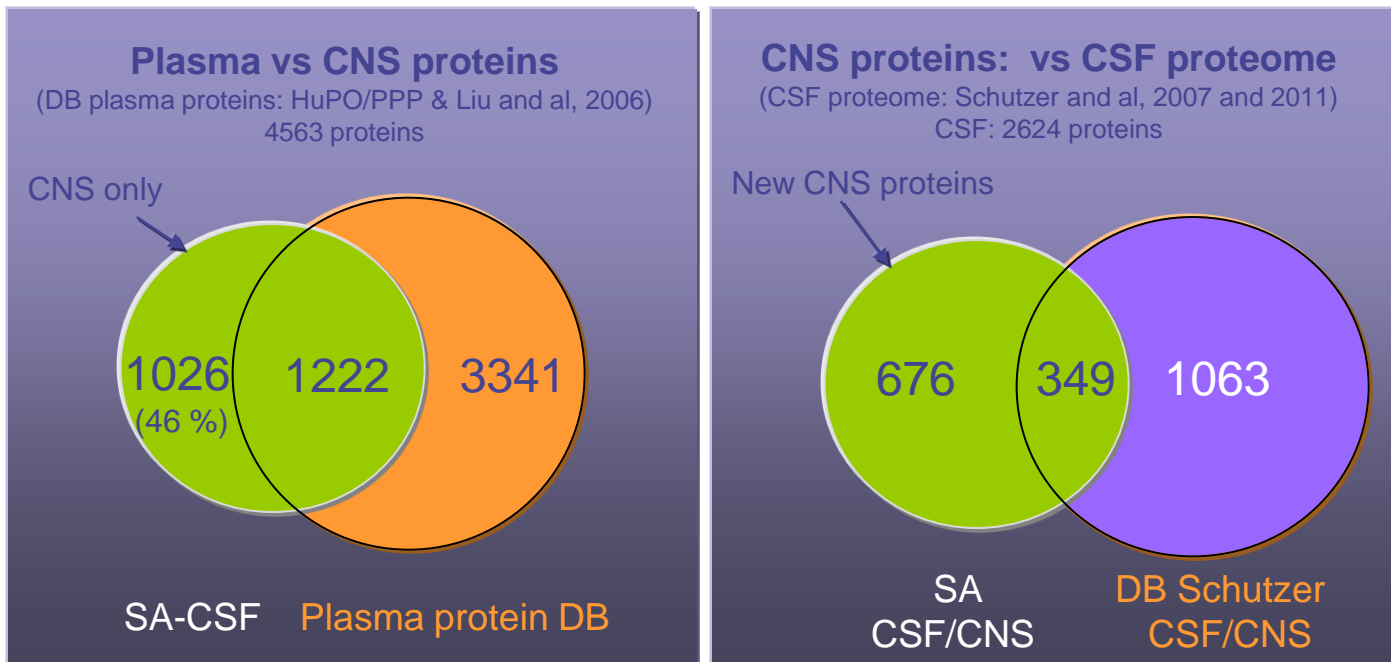
Fractionation	Cumulative Unique peptides
RP1 (PAII)	4914
RP2 (LiChrosorb)	7137
ZIC	8206
TSK	9305

Comparison of our master map with the literature

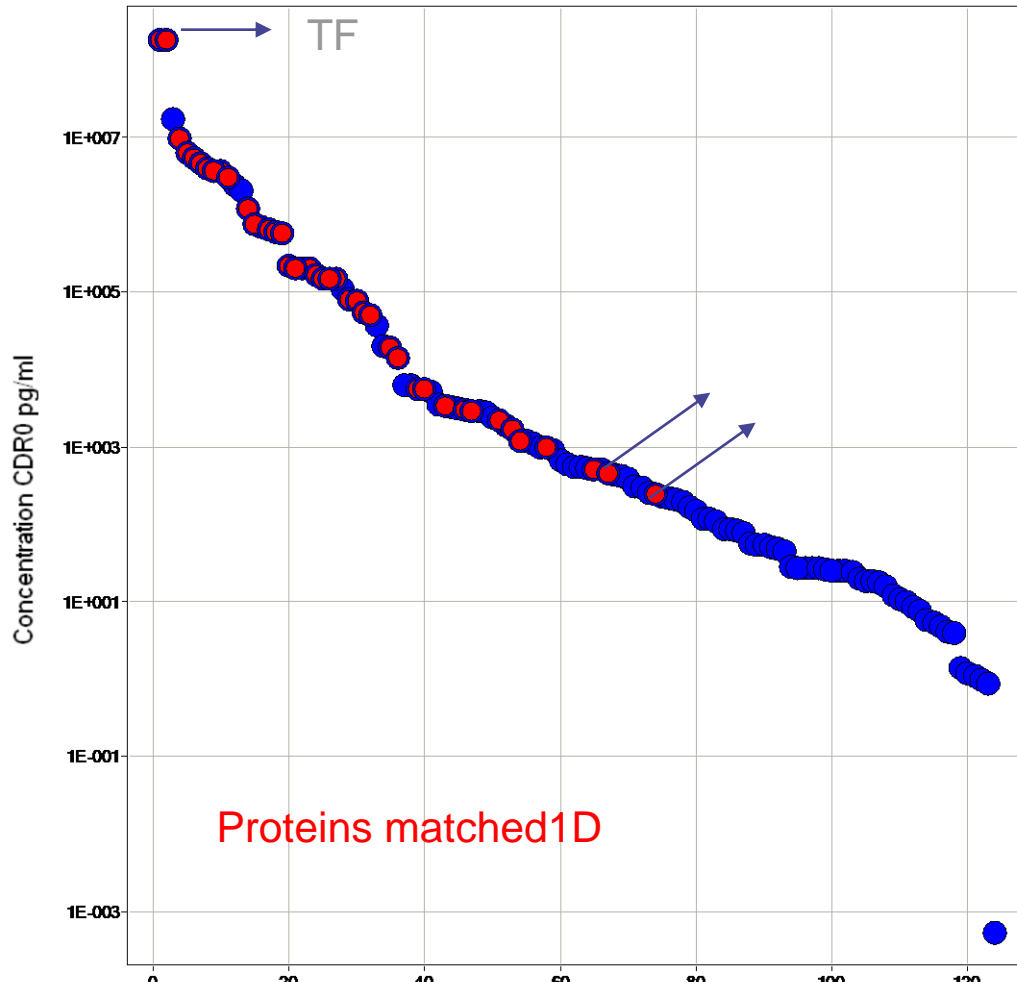
Pool of Human CSF
(hydrocephalus, ALS, MS)



2246 CSF proteins



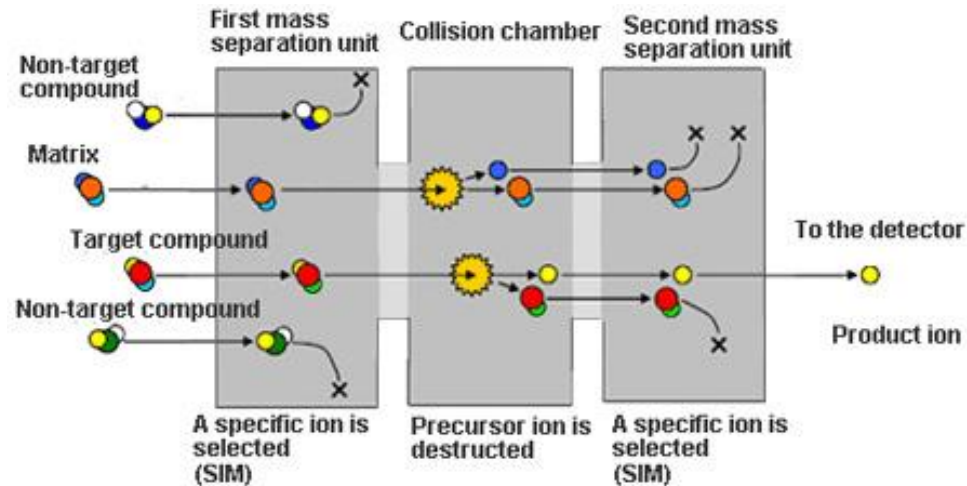
How deep do we dig in CSF proteome?



- Macrophage Migration Inhibitory Factor : 145 pg/mL
- Heparin-binding epidermal growth factor-like growth factor : 25 pg/mL

• Titration by Multiplex immunoassay of 125 proteins or peptides in 333 CSF samples (PLoS One. 2011, 12;6(1):e16032)

Multiple reaction monitoring (MRM) validation



Classically performed on a QQQ mass spectrometer

We know what we are looking for:

One precursor mass + one fragment mass = one MRM transition

Very specific

Low background noise (LOQ is very low)

Very fast (80 per second) so multiplexing is possible

Several peptides per protein and several transitions per peptides

Conclusion and perspectives

1 Collection of CSF & plasma samples (*CHRU, 24 months*)

- For biomarker identification (CSF 2D peptide map + HR profiling)
- For biomarker validation by LC/QqQ/MRM
- **For biomarker validation by Ab assays and preclinical study**

2 Method development (*SANOFI & IGF, 16 months*)

- Standardized and reproducible method for CSF sample preparation and MS analysis

3 Candidate Biomarkers identification (*SANOFI & IGF, 18 months*)

- Establishment of 2D peptide of human CSF
- HR-MS peptide profiling of CSF from AD patients and age-matched controls :

4 Candidate Biomarkers Validation by LC/QqQ/MRM (*SANOFI & IGF, 18 months*) *On going*

5 Candidate Biomarkers Validation by Ab assays (BioRad, 12 months) *Scheduled*

6 Preclinical study and final biomarker selection (BioRad, 18 months) *Scheduled*

Bonus:

2D HR MS map of LCR is now established and continuously enriched with new experiments

A new project on multiple sclerosis was started

Technology applicable to any type of protein samples

Thank you for your attention
