



Les Biologistes
Médicaux

BIO MED · j **2021**

LES JOURNÉES POUR L'AVENIR DE LA BIOLOGIE MÉDICALE

16 & 17 septembre - ASIEM, Paris

www.congres-biomedj.fr

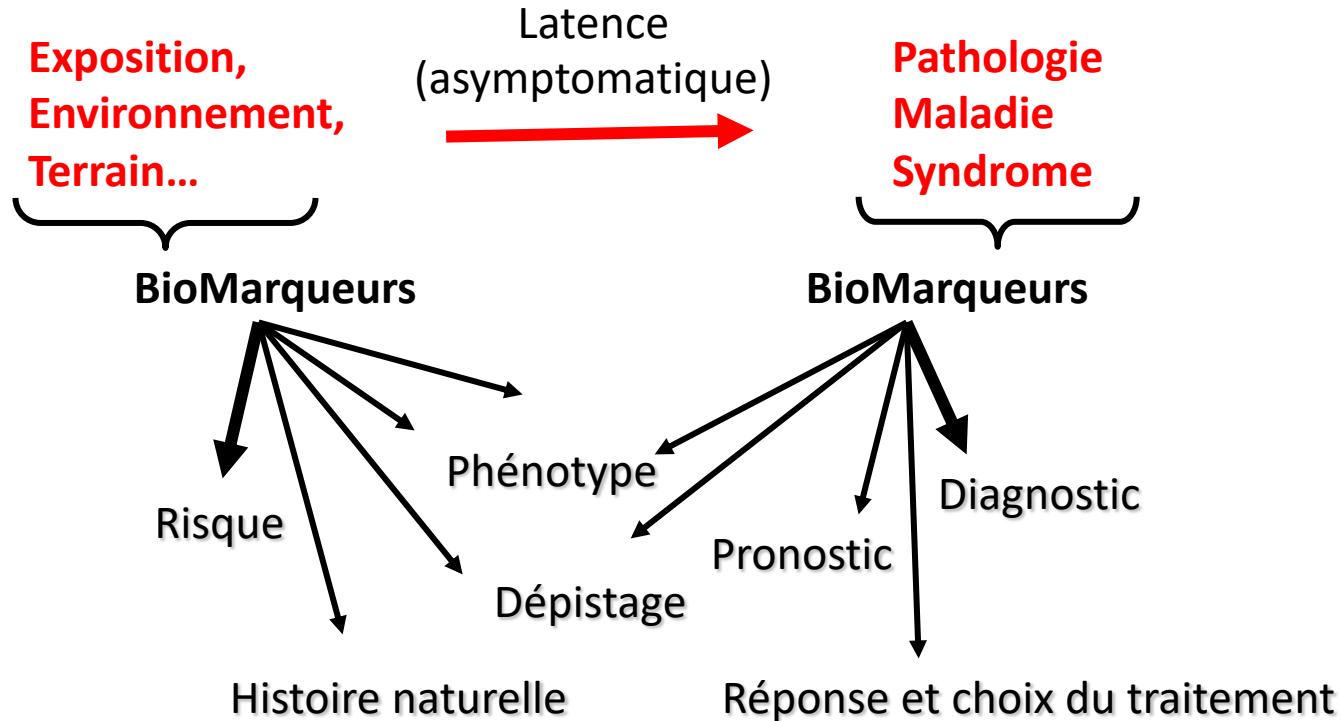


Marqueurs sanguins dans le suivi des maladies neuro-dégénératives ou neuro-inflammatoires

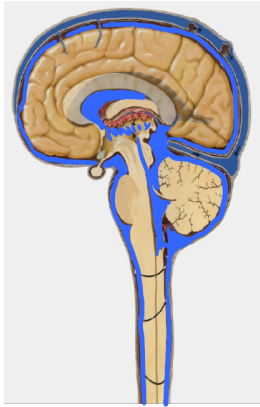
Pr Sylvain Lehmann

(Service Biochimie-protéomique clinique, Hôpital Saint-Éloi, IRMB CHU Montpellier)

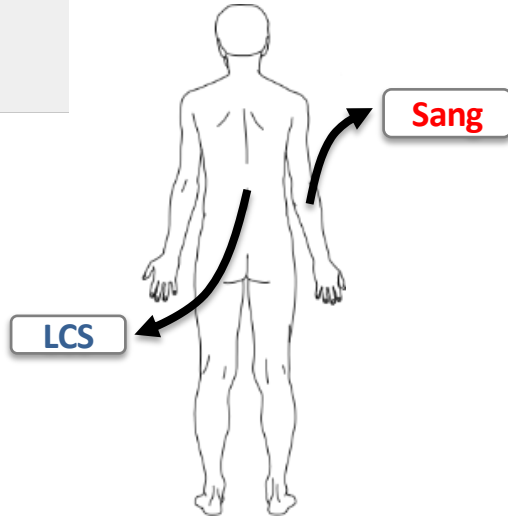
Place des biomarqueurs en médecine



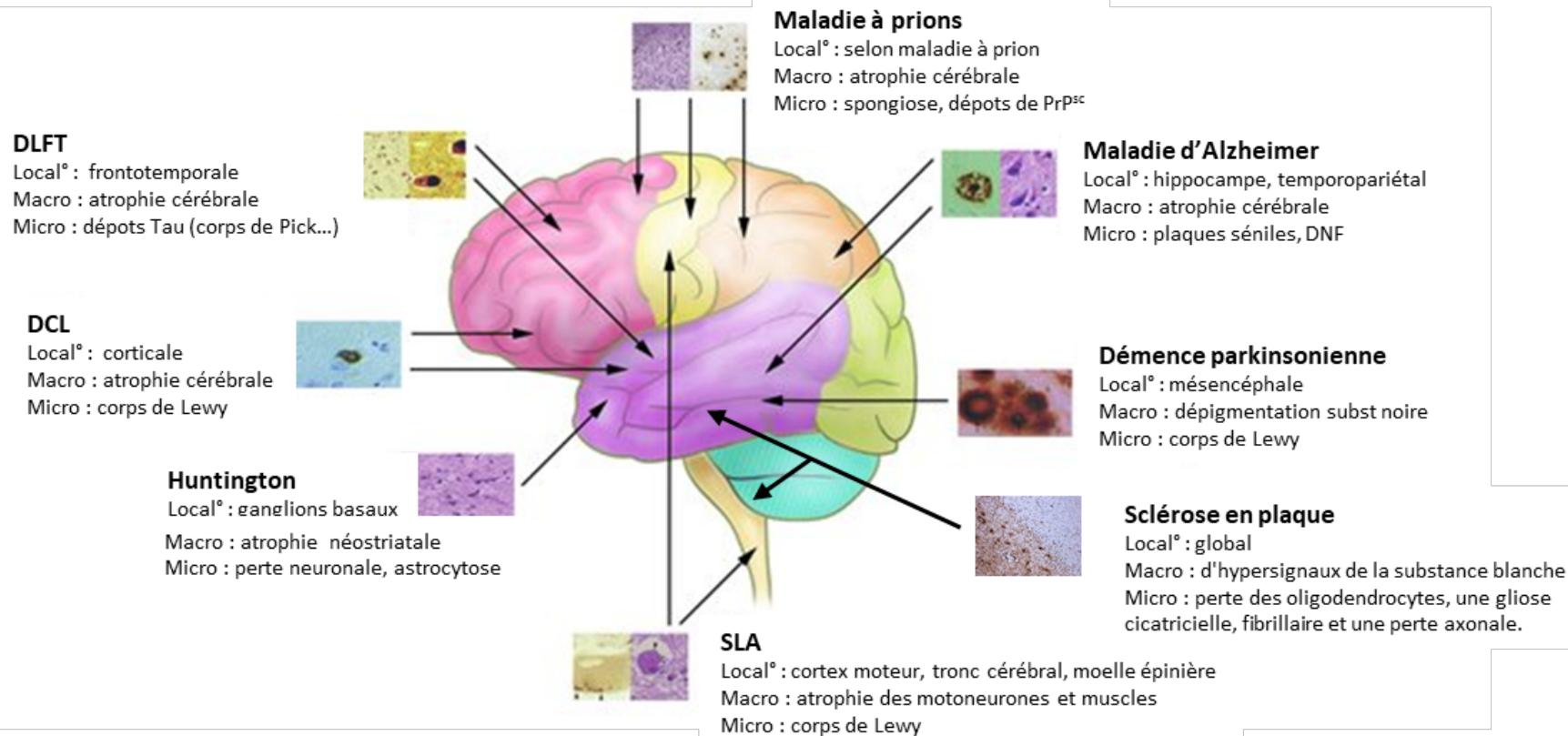
Intérêt des dosages sanguins



Liquide
Cerebro
Spinal



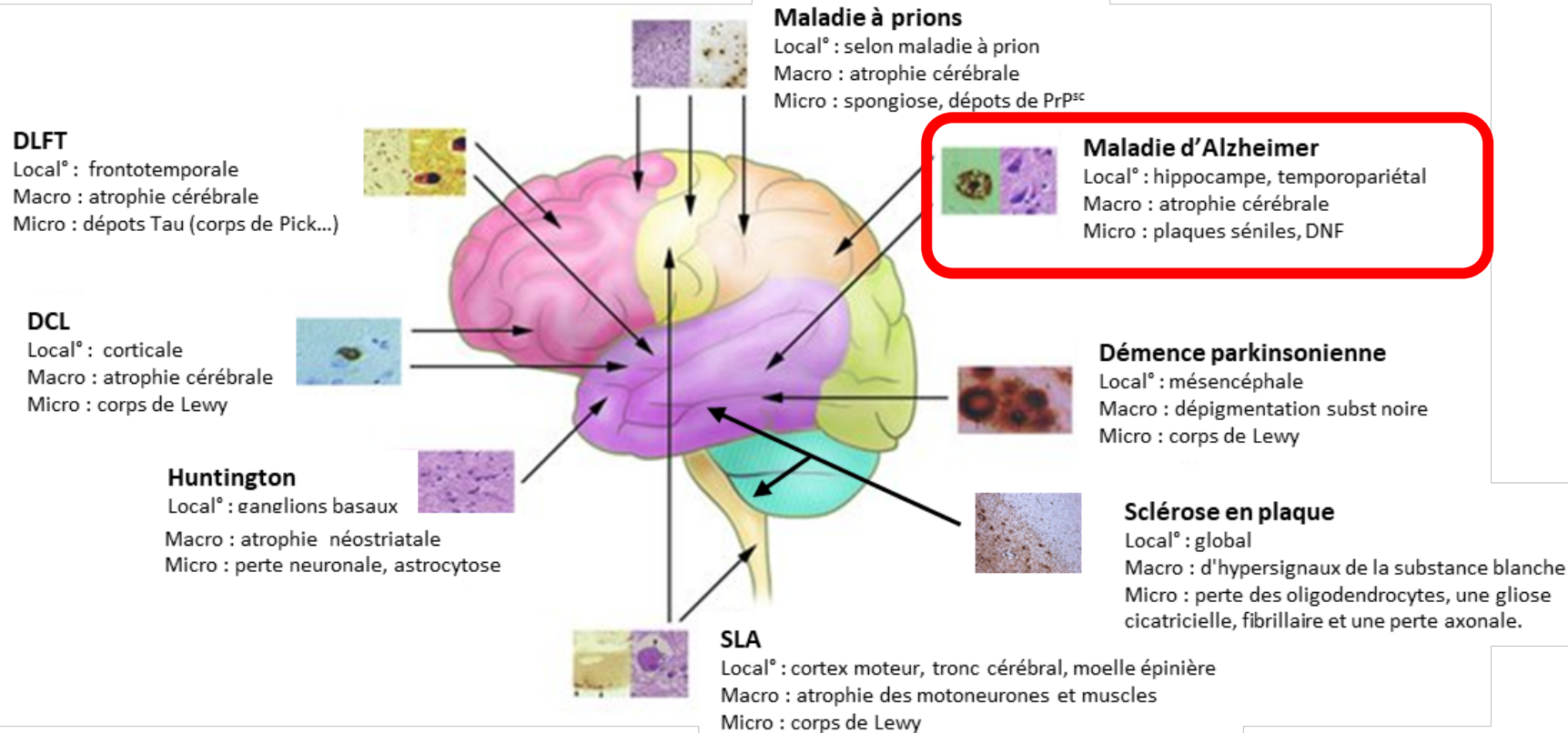
- Intérêt car **moins invasif** que la ponction lombaire → possibilité d'utilisation à plus large échelle (dépistage..) et pour le suivi
- Effet de « dilution » par rapport au LCS et d'autres origines que le cerveau (**baisse sensibilité et spécificité**)
- Nécessite souvent des méthodes innovantes et **ultrasensibles**



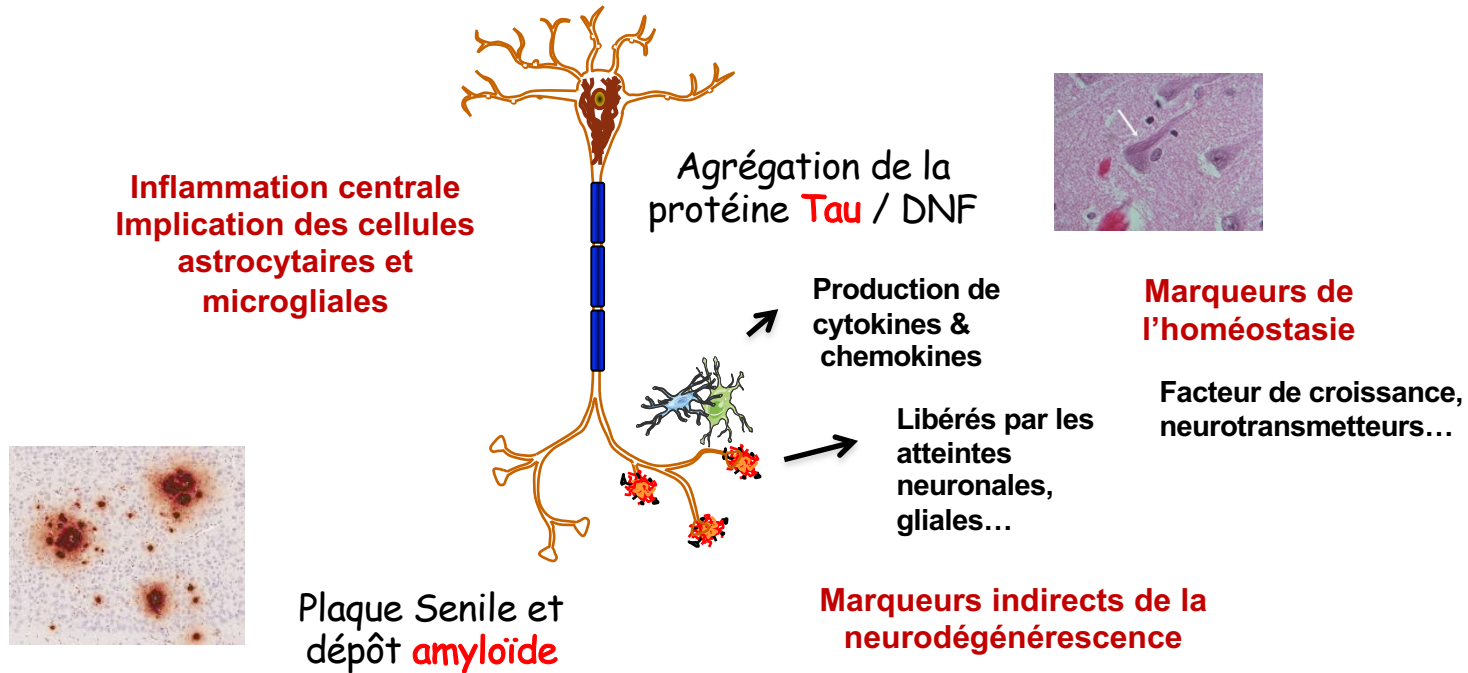


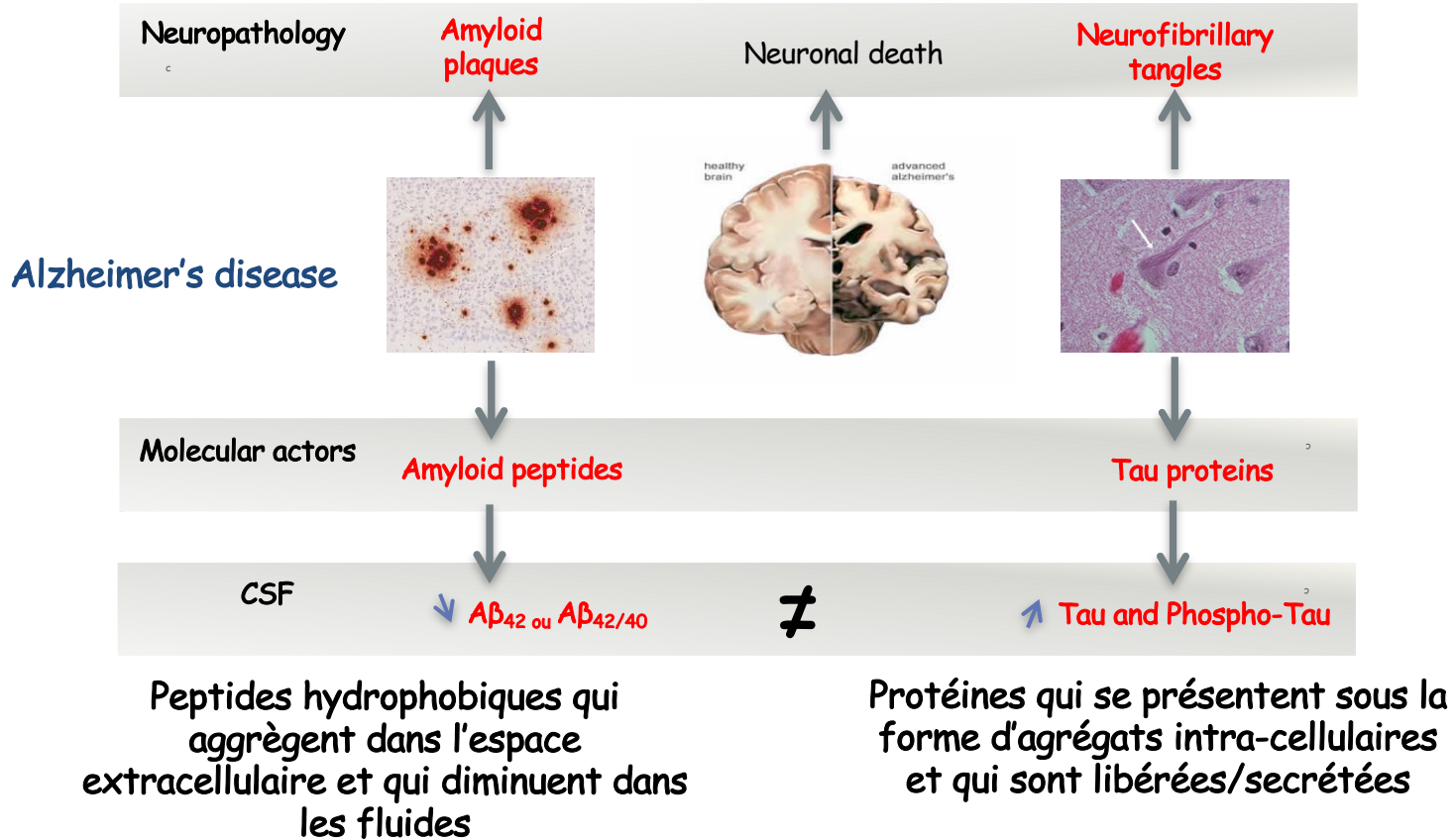
Biomarqueurs

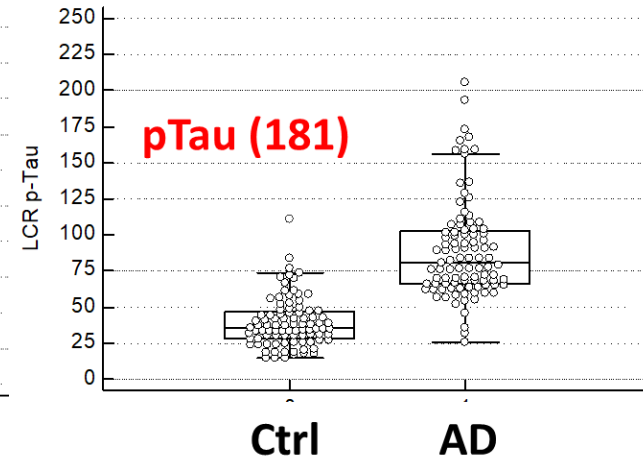
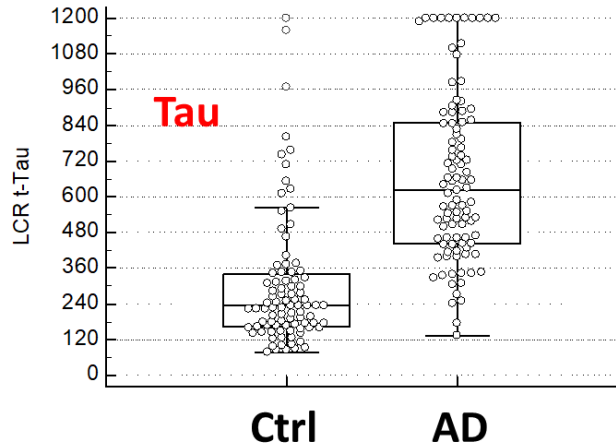
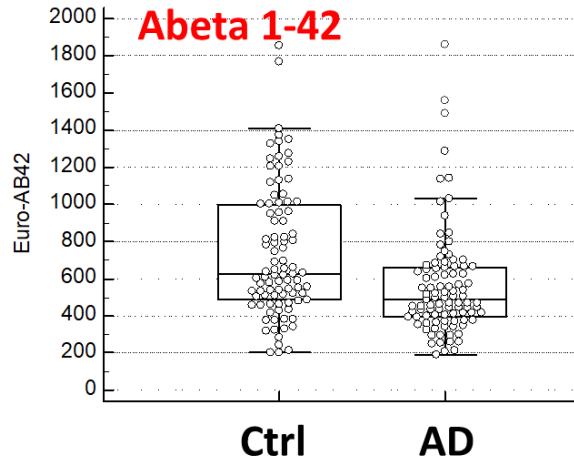
- Impliqués dans les processus pathologiques
- Témoins / réactionnels
- Lié au terrain / risque
- De retour à l'homéostasie



Physiopathologie de la Maladie d' Alzheimer (MA)





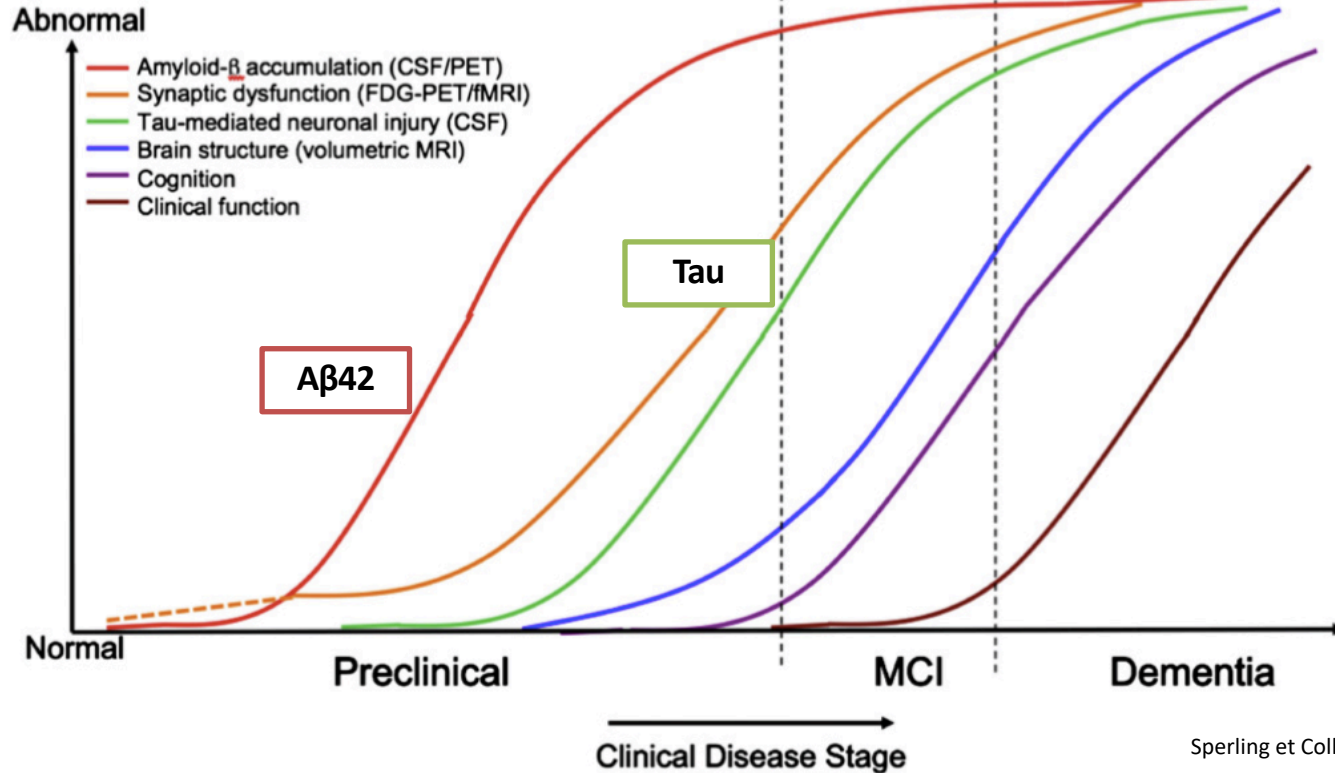


Diminution de la concentration de peptide A β 42 dans le LCS
< 500 ou 700 ng/L

Augmentation de la concentration dans le LCS :

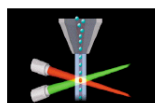
- Tau > 400 ng/L (fonction de l'âge)
- P-Tau > 60 ng/L

Modifications des biomarqueurs dans les stades précoces de la MA



Première génération de dosages sanguins des peptides amyloïdes

Luminex™ xMAP® Technology for immunoassays



ELSEVIER

Alzheimer's & Dementia (2018) 1-11

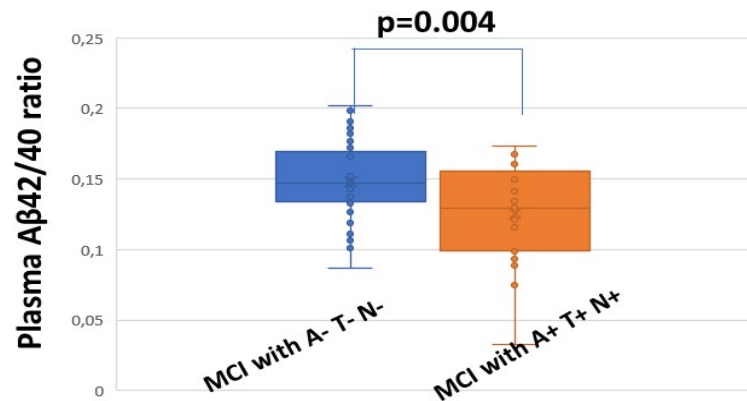
Alzheimer's
&
Dementia

Featured Article

Plasma amyloid levels within the Alzheimer's process and correlations with central biomarkers

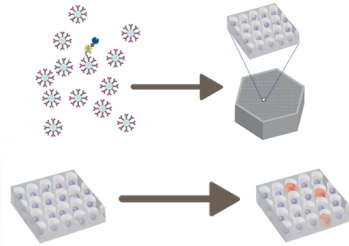
Olivier Hanon^{a,b,g}, Jean-Sébastien Vidal^{a,b}, Sylvain Lehmann^c, Stéphanie Bombois^{d,e}, Bernadette Allinquant^f, Jean-Marc Tréluyer^g, Patrick Gelé^{h,i}, Christine Delmaire^{d,e}, Frédéric Blanc^{i,k}, Jean-François Mangin^l, Luc Buée^{h,i}, Jacques Touchon^m, Jacques Hugon^{n,o}, Bruno Vellas^p, Evelyne Galbrun^q, Athanase Benetos^f, Gilles Berrut^g, Elèna Paillaud^r, David Wallon^{u,v}, Giovanni Castelnovo^w, Lisette Volpe-Gillot^x, Marc Paccalin^y, Philippe-Henri Robert^z, Olivier Godefroy^{aa}, Thierry Dantoine^{bb}, Vincent Camus^{cc,dd}, Joël Belmin^{ee,ff}, Pierre Vandel^{gg,hh}, Jean-Luc Novella^{ii,jj}, Emmanuelle Duron^{a,b}, Anne-Sophie Rigaud^{h,b}, Suzanna Schraen-Maschke^{hh,i}, Audrey Gabelle^{mm,1}, on behalf of the BALTAZAR study group

Plasma A β 42/40 ratio by groups of MCI participants classified as A-T-N- versus A+T+N+



Méthodes d'immunodéction de 2em génération

SimOa (SINgle-MOLEcule Array)
technology, Quanterix



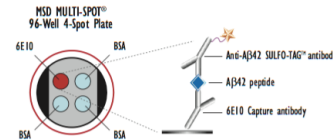
"Digital
ELISA"

Capture magnetic bead

Elecsys



Meso Scale Discovery
(MSD)



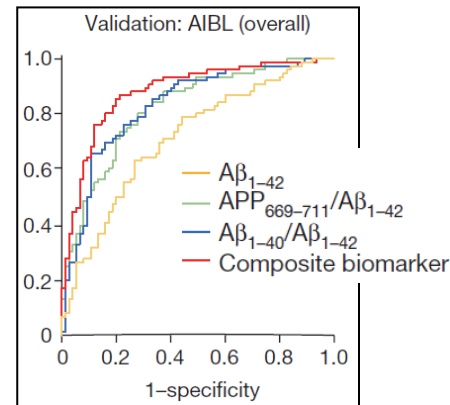
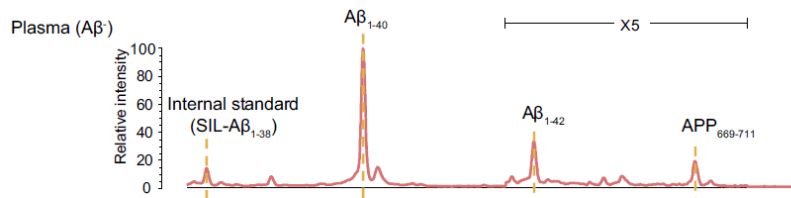
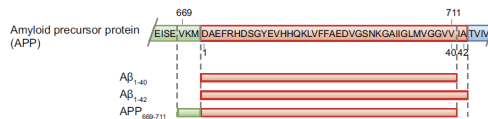
Méthodes basées sur la spectrométrie de masse

LETTER

doi:10.1038/nature25456

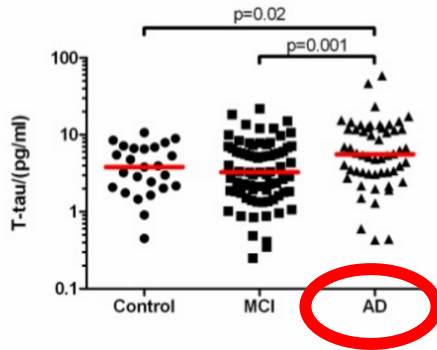
High performance plasma amyloid- β biomarkers for Alzheimer's disease

Akinori Nakamura¹, Naoki Kaneko², Victor L. Villemagne^{3,4}, Takashi Kato^{1,5}, James Doecke⁶, Vincent Doré^{3,6}, Chris Fowler⁴, Qiao-Xin Li⁴, Ralph Martins⁷, Christopher Rowe^{3,4}, Taisuke Tomita⁸, Katsumi Matsuzaki⁹, Kenji Ishii¹⁰, Kazunari Ishii¹¹, Yutaka Arahata⁵, Shinichi Iwamoto⁷, Kengo Ito^{1,5}, Koichi Tanaka⁷, Colin L. Masters⁴ & Katsuhiko Yanagisawa¹



Détection de la protéine Tau dans le sang

Plasma tau in Alzheimer's disease

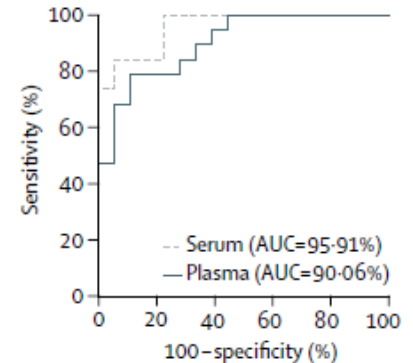
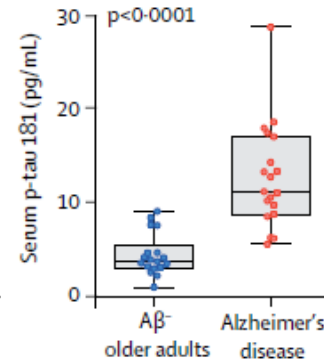
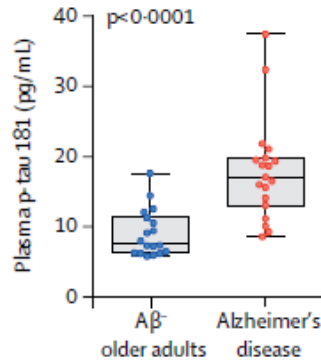


Zetterberg et al., Alz Res & Ther. 2013

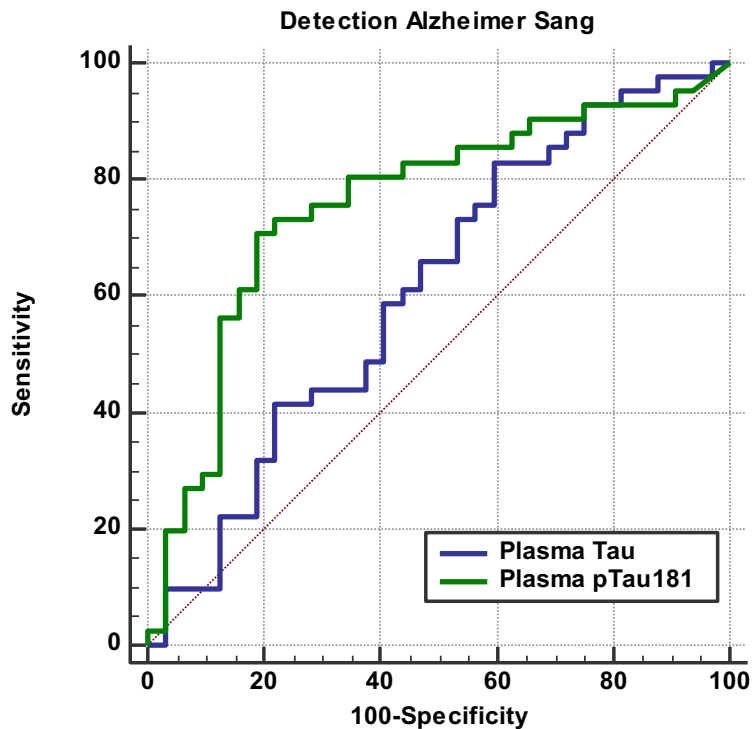
Blood phosphorylated tau 181 as a biomarker for Alzheimer's disease: a diagnostic performance and prediction modelling study using data from four prospective cohorts

Thomas K Karikari*, Tharick A Pascoal*, Nicholas J Ashton, Shorena Janelidze, Andréa Lessa Benedet, Juan Lantero Rodriguez, Mira Chamoun, Melissa Savard, Min Su Kang, Joseph Theriault, Michael Schöll, Gassan Massarweh, Jean-Paul Soucy, Kina Höglund, Gunnar Brinkmalm, Niklas Mattsson, Sebastian Palmqvist, Serge Gauthier, Erik Stomrud, Henrik Zetterberg, Oskar Hansson†, Pedro Rosa-Neto†, Kaj Blennow†

Lancet Neurol 2020; 19: 422-33



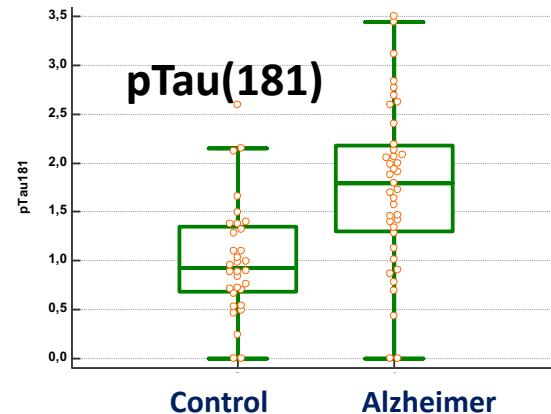
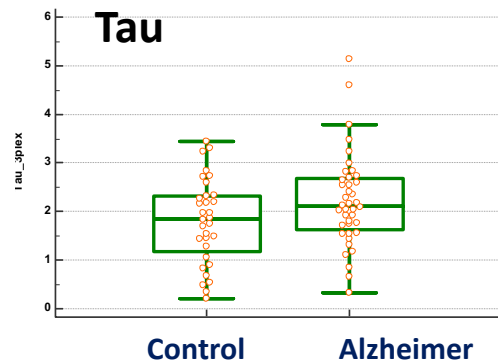
Détection de la protéine Tau dans le sang



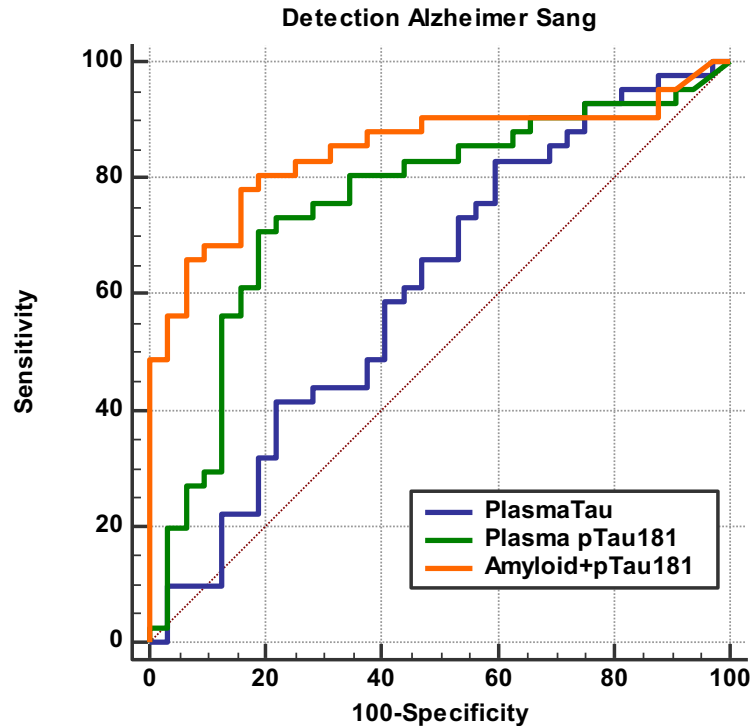
SimOa,
Quanterix



 **SHIMADZU**
Excellence in Science



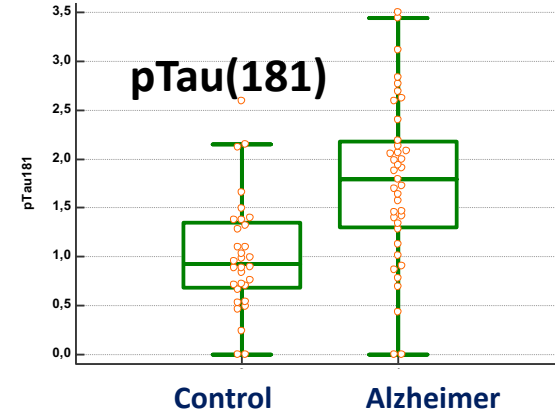
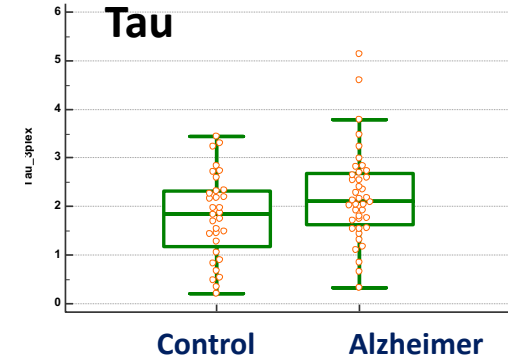
Détection de pTau+amyloïde dans le sang



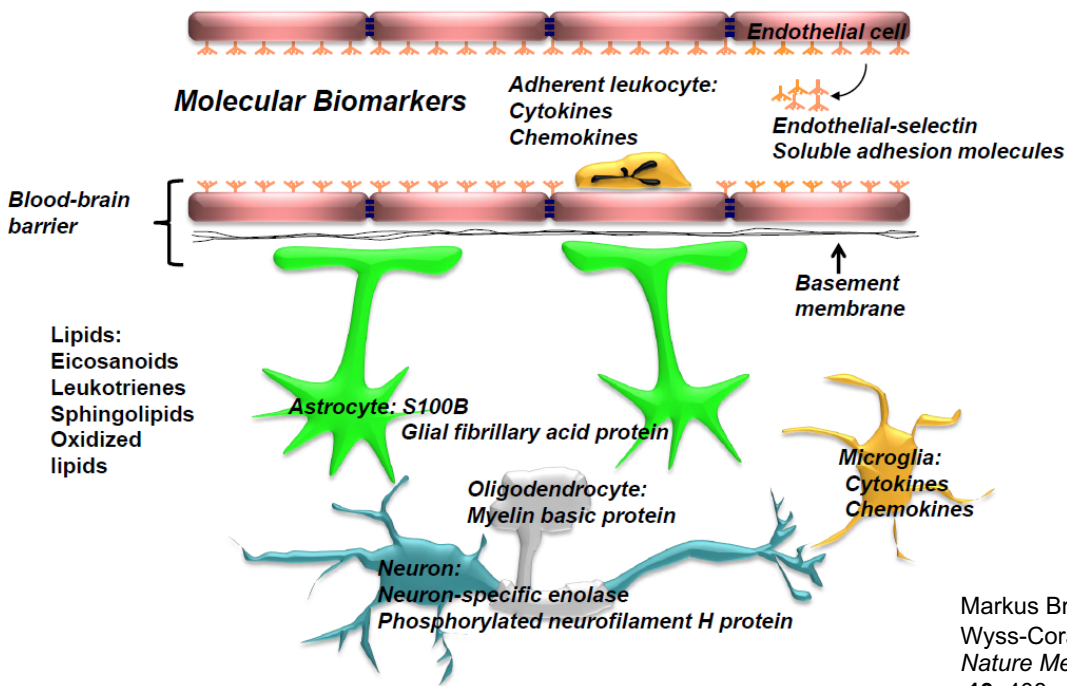
SimOa,
Quanterix



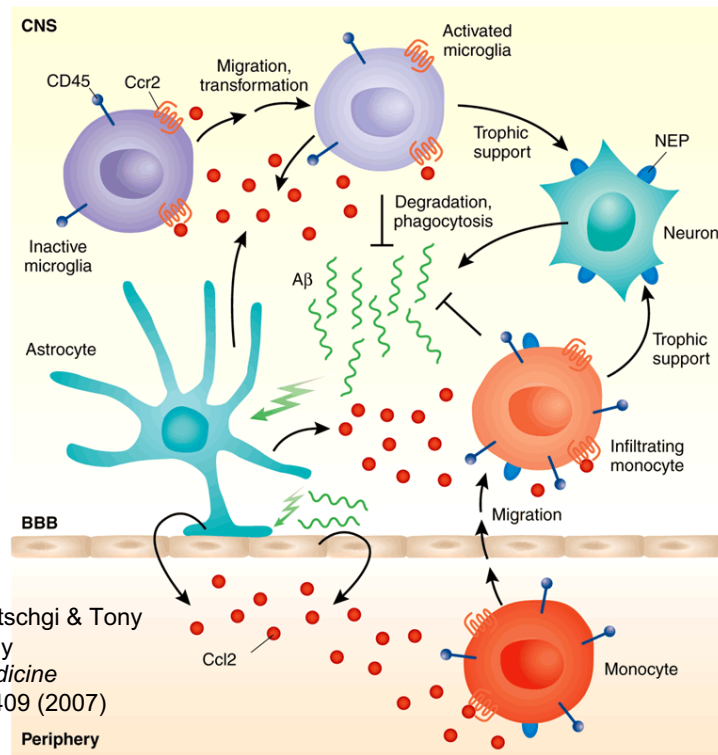
 **SHIMADZU**
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Les biomarqueurs de la neuro-inflammation



Adapted from: Jamie Hutchison, University of Toronto



Markus Britschgi & Tony
Wyss-Coray
Nature Medicine
13, 408 - 409 (2007)



Les biomarqueurs de la neuro-inflammation

Classification and prediction of clinical Alzheimer's diagnosis based on plasma signaling proteins

nature
medicine

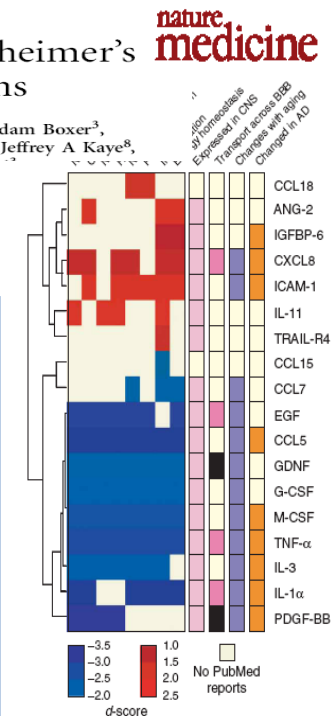
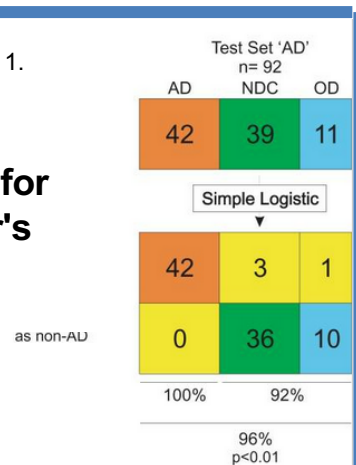
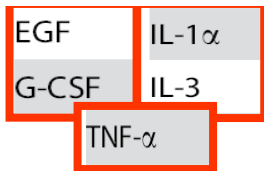
Sandip Ray^{1,16}, Markus Britschgi^{2,16}, Charles Herbert¹, Yoshiko Takeda-Uchimura², Adam Boxer³, Kaj Blennow⁴, Leah F Friedman⁵, Douglas R Galasko⁶, Marek Jutel⁷, Anna Karydas³, Jeffrey A Kaye⁸,

N= 42 AD, 47 MCI+ Screening ELISA= 120 protéines, **18 sélectionnées**
avec rôle : - dysrégulation de l'hématopoïèse, réponse immunitaire,
apoptose, neuroprotection

PLoS One. 2008 Sep 3;3(9):e3111.

Identification of a 5-protein biomarker molecular signature for predicting Alzheimer's disease.

Gómez Ravetti M, Moscato P.



Les biomarqueurs de la neuro-inflammation

ANR CytokAiz

CHU de Montpellier, S. Lehmann

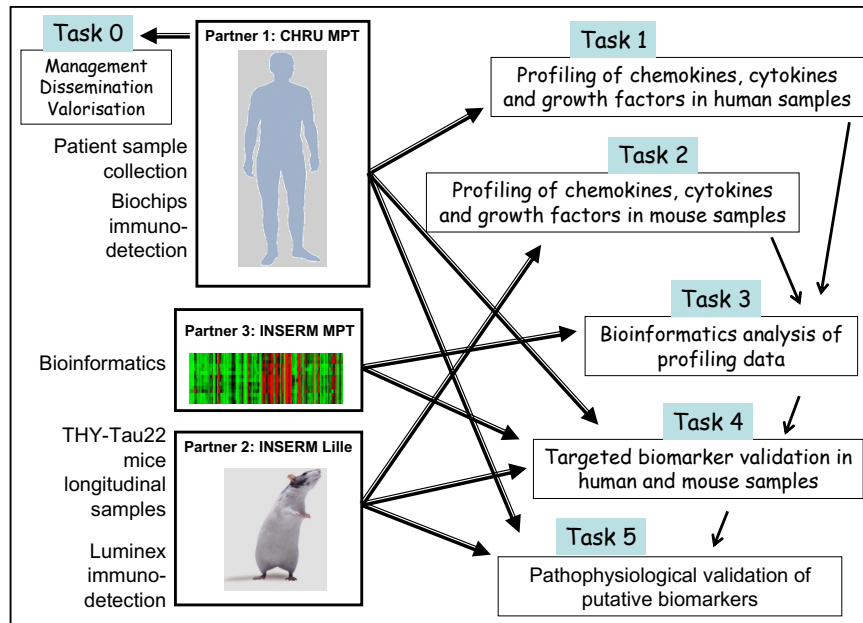
INSERM UMR837 Lille, L. Buée / S.

Schraen-Maschke / D. Blum

INSERM U1040 Montpellier, T. Reme

Central nervous system and
peripheral inflammatory processes
in Alzheimer's disease: biomarker
profiling approach

Constance Delaby^{1*}, Audrey Gabelle^{1,2}, David Blum³, Susanna Schraen-Maschke³,
Amandine Moulinier¹, Justine Boulanghien¹, Dany Séverac¹, Luc Buée², Thierry Rème²
and Sylvain Lehmann¹



Les biomarqueurs de la neuro-inflammation

OPEN ACCESS Freely available online

PLoS one

Multivariate Protein Signatures of Pre-Clinical Alzheimer's Disease in the Alzheimer's Disease Neuroimaging Initiative (ADNI) Plasma Proteome Dataset

Daniel Johnstone^{1,2}, Elizabeth A. Milward^{1,3}, Regina Berretta^{1,2}, Pablo Moscato^{1,2*}, for the Disease Neuroimaging Initiative

In conclusion, the findings of this study suggest that sets of plasma analytes can act as useful biomarkers for pre-clinical AD but can be influenced by a number of confounding variables, in particular *APOE* genotype. More research is required on larger samples which allow stratification by potential co-variables while retaining sufficient power for analyses of subgroups. It is likely that plasma biomarkers of the future will involve sets of analytes rather than individual analytes and that accurate pre-clinical diagnosis might require panels of multiple biomarkers. With technological advances in multiplexing protein assays, financial considerations relating to measuring large biomarker panels are becoming less of a barrier to implementation and more importance will instead be placed on assembling optimal panels rather than minimizing the number of proteins.

Furthermore, if costs continue to come down, it may become feasible to perform routine measurements of panels of plasma analytes in 'at risk' individuals and monitor the change over time, as is currently done in clinical biochemistry for various markers of health and disease. In addition to providing a cost-effective and

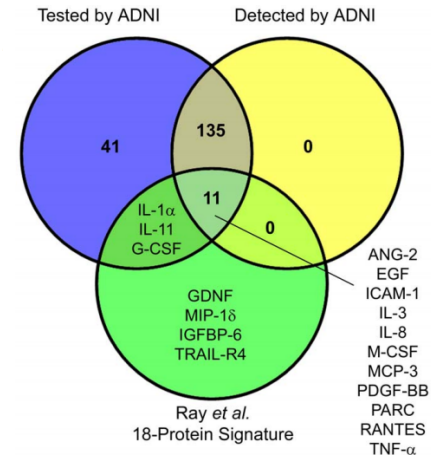
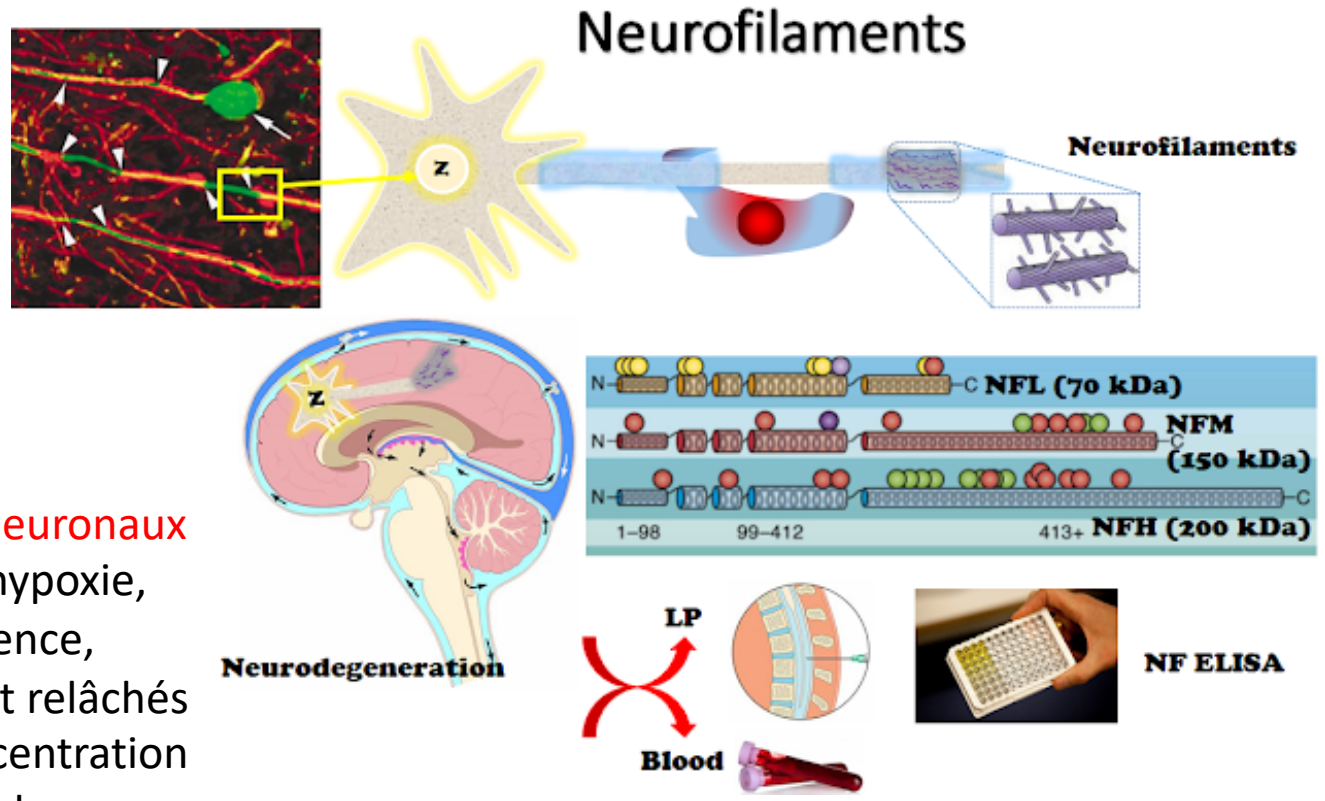


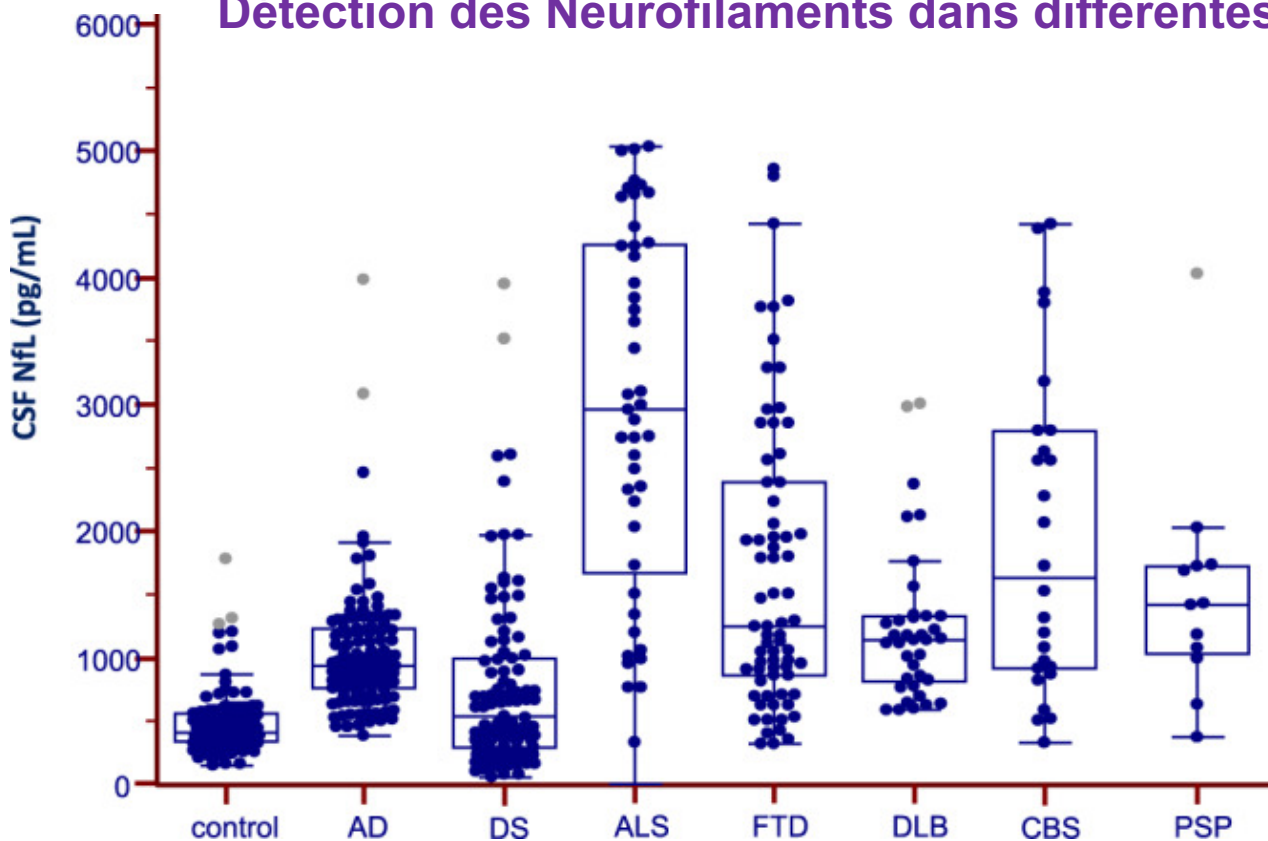
Figure 2. Detectability in the ADNI dataset of the 18 proteins highlighted by Ray et al. (2007). Of the 18 proteins in the signature highlighted by Ray and colleagues [9], three were below the detection limits of the ADNI assay, 11 were considered detectable by ADNI and four were not assessed. Protein abbreviations are defined in Table S2. doi:10.1371/journal.pone.0034341.g002

Les **neurofilaments** sont des protéines neuronales qui jouent un rôle important dans le développement axonal.

En cas de **dommages neuronaux** d'origines diverses (hypoxie, neurodégénérescence, traumatisme...), ils sont relâchés et augmentent en concentration dans le LCR... et dans le sang

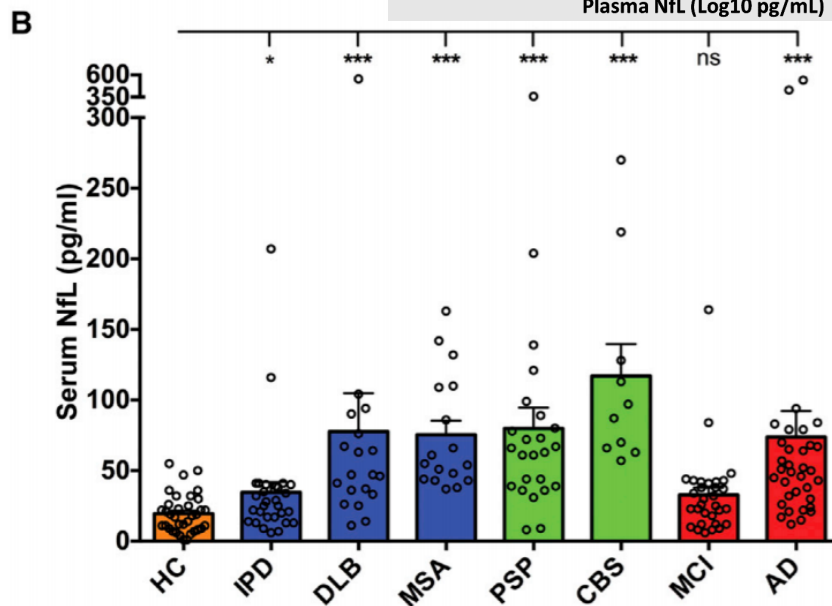
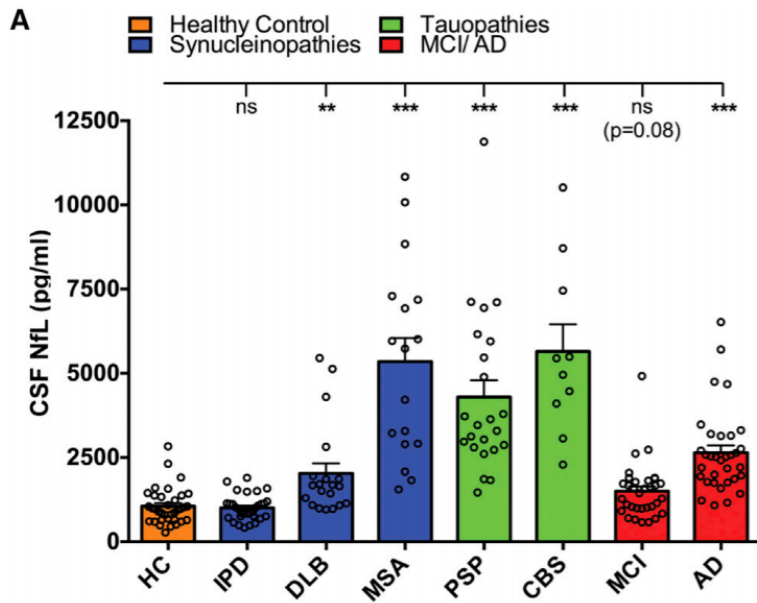
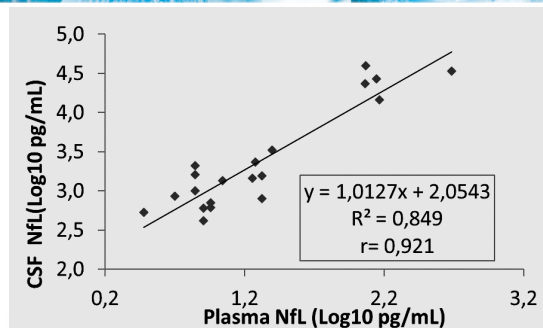


Détection des Neurofilaments dans différentes MND



Neurofilament Light Chain in Blood and CSF as Marker of Disease Progression in Mouse Models and in Neurodegenerative Diseases

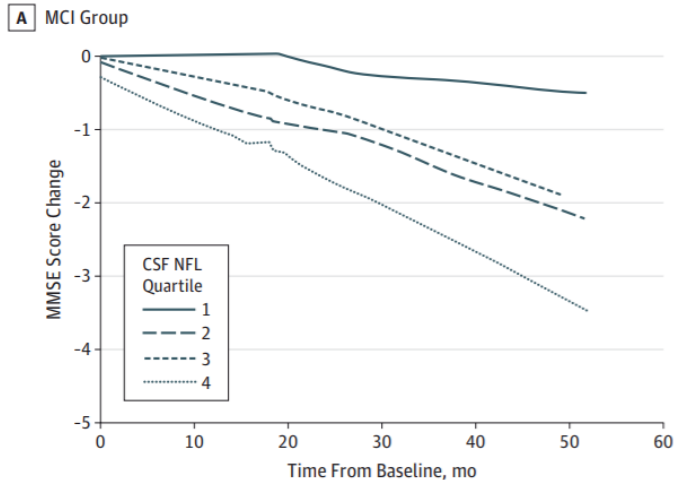
Mehtap Bacioglu,^{1,2,3,10} Luis F. Maia,^{1,2,4,10} Oliver Preische,^{1,5} Juliane Schelle,^{1,2,3} Anja Apel,^{1,6} Stephan A. Kaeser,^{1,2} Manuel Schweighauser,^{1,2,3} Timo Eninger,^{1,2,3} Marius Lambert,^{1,2} Andrea Pilotto,^{1,6} Derya R. Shimshek,⁷ Ulf Neumann,⁷ Philipp J. Kahle,^{1,6} Matthias Staufenbiel,^{1,2} Manuela Neumann,^{1,8} Walter Maetzler,^{1,6} Jens Kuhle,^{9,11,*} and Mathias Jucker^{1,2,11,*}



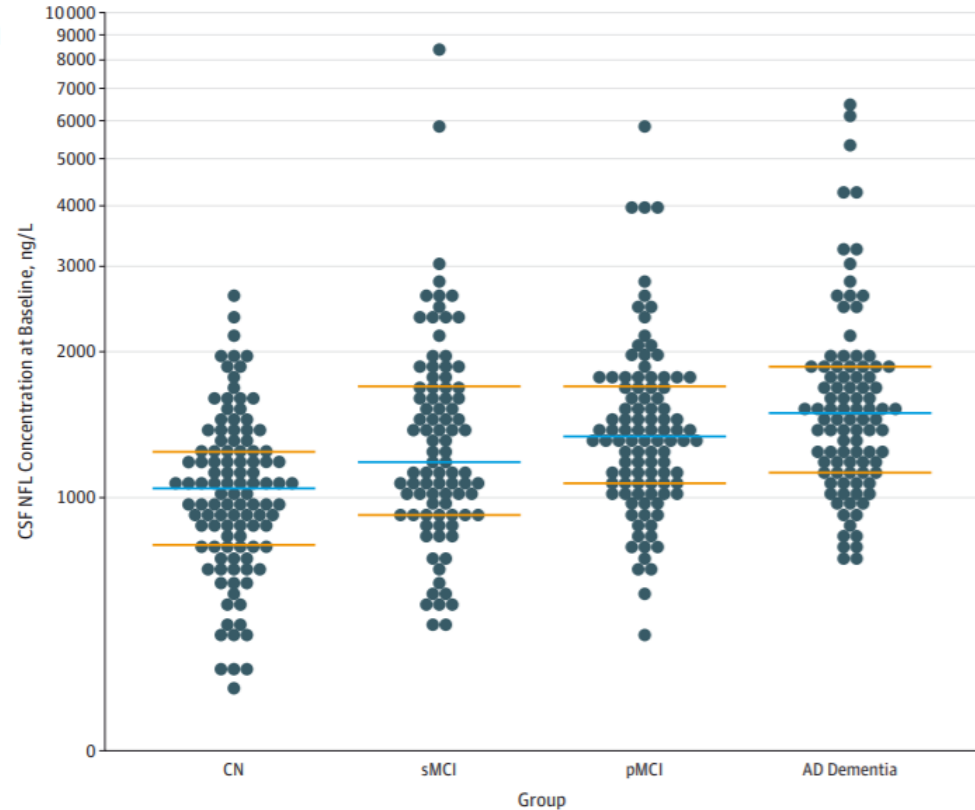
Association of Cerebrospinal Fluid Neurofilament Light Concentration With Alzheimer Disease Progression

Henrik Zetterberg, MD, PhD; Tobias Skillbäck, MD; Niklas Mattsson, MD, PhD; John Q. Trojanowski, MD, PhD; Erik Portelius, PhD; Leslie M. Shaw, PhD; Michael W. Weiner, MD, PhD; Kaj Blennow, MD, PhD; for the Alzheimer's Disease Neuroimaging Initiative

JAMA Neurol. 2016;73(1):60-67. doi:10.1001/jamaneurol.2015.3037
Published online November 2, 2015.



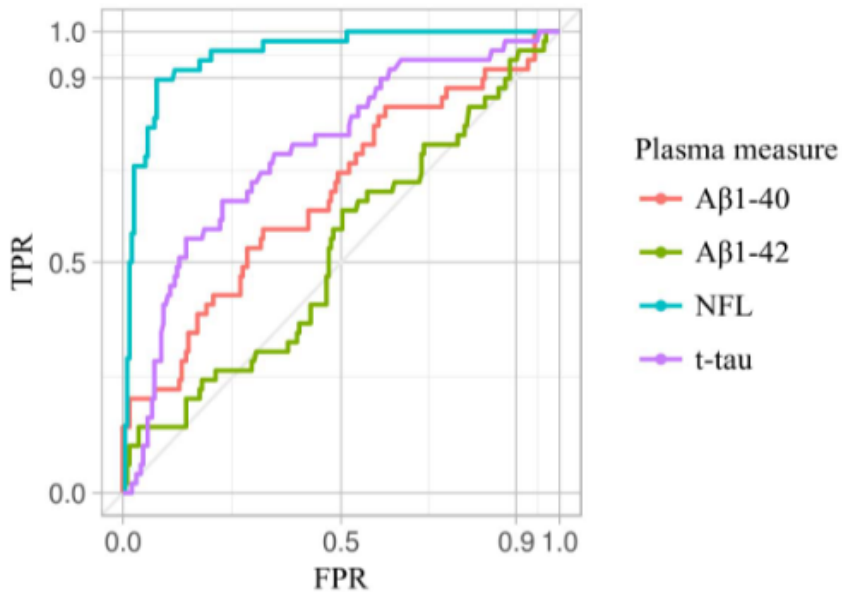
Neurofilaments



Détection des Neurofilaments dans la MA

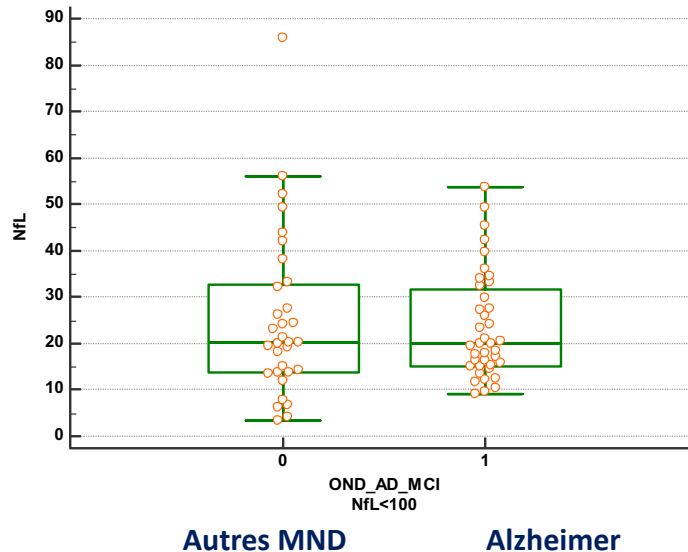
Plasma and CSF biomarkers for the diagnosis of Alzheimer's disease in adults with Down syndrome: a cross-sectional study

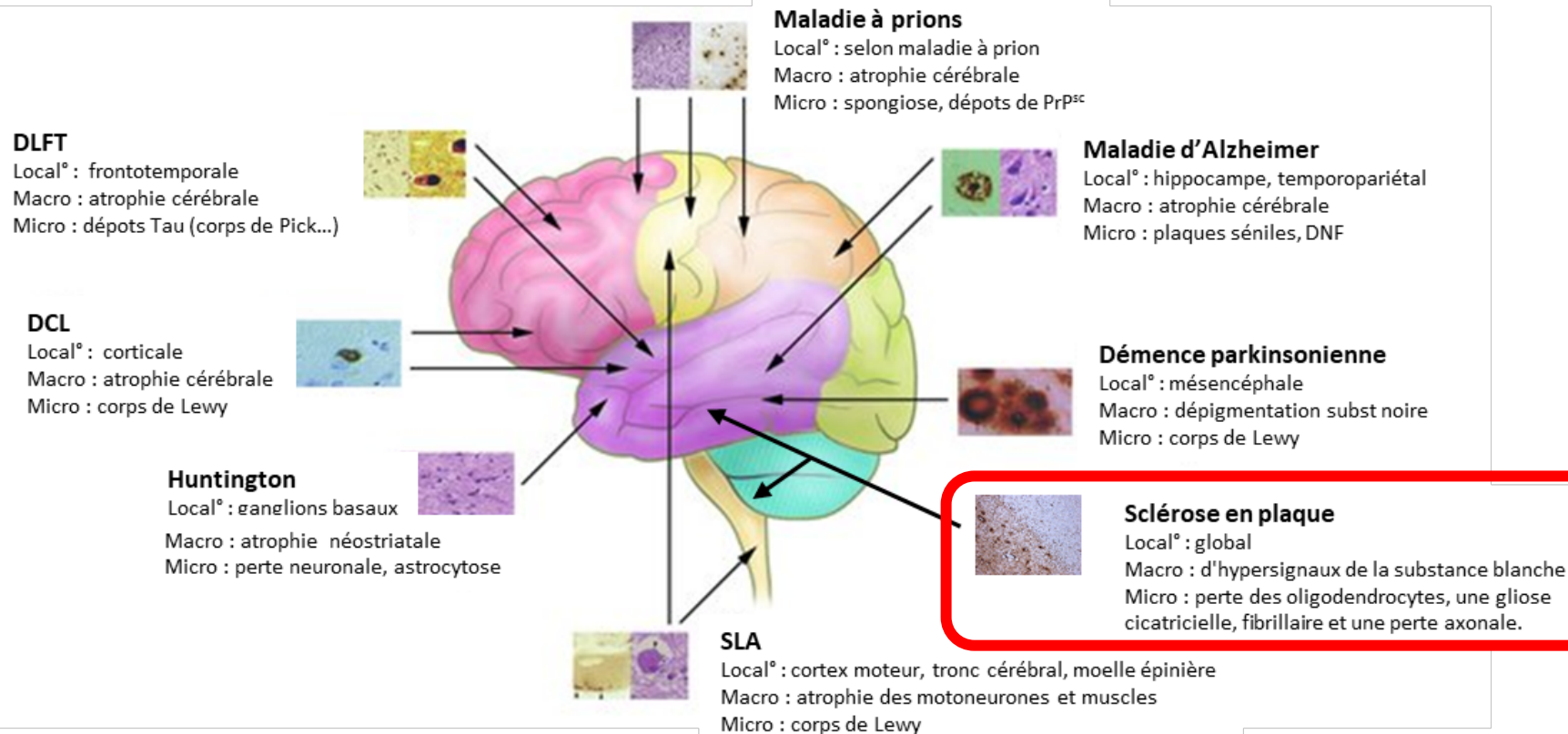
Juan Fortea, María Carmona-Iragui, Bessy Benejam, Susana Fernández, Laura Videla, Isabel Barroeta, Daniel Alcolea, Jordi Pegueroles, Laia Muñoz, Olivia Balbin, Mory J de Leon, Aleksandra Maleska Maceski, Christophe Hirtz, Jordi Clarimón, Sebastián Videla, Constance Delaby, Sylvain Lehmann, Rafael Blesa*, Alberto Lleó*



Lancet Neurol 2018

Published Online
August 29, 2018



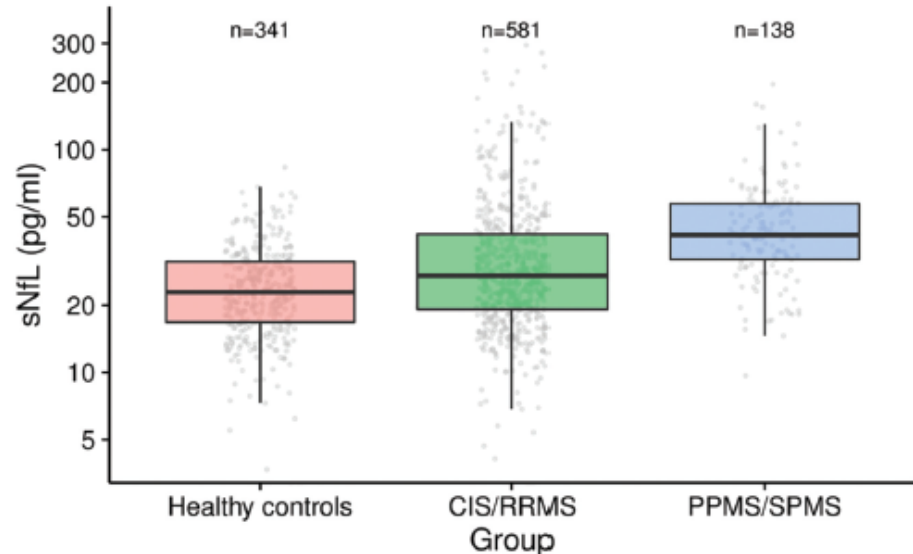


Serum Neurofilament Light: A Biomarker of Neuronal Damage in Multiple Sclerosis

Giulio Disanto, MD, PhD,¹ Christian Barro, MD,² Pascal Benkert, PhD,³
Yvonne Naegelin, MD,² Sabine Schädelin, MSc,³ Antonella Giardiello, MD,¹
Chiara Zecca, MD,¹ Kaj Blennow, PhD,⁴ Henrik Zetterberg, PhD,^{4,5}
David Leppert, MD,² Ludwig Kappos, MD,² Claudio Gobbi, MD,
Jens Kuhle, MD, PhD,² and the Swiss Multiple Sclerosis Cohort Study

ANN NEUROL 2017;81:857–870

Détection des Neurofilaments dans la SEP

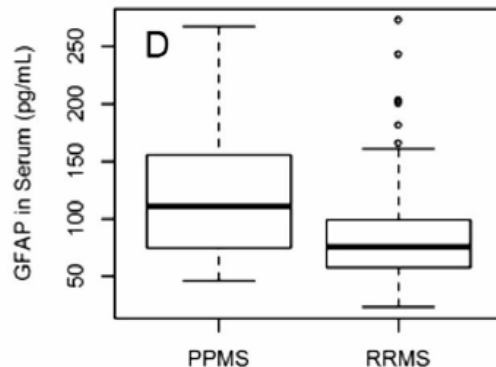


Détection des Chaines légères des Neurofilaments (NFL) et de la Glial Fibrillary Acid Protein (GFAP) dans la SEP

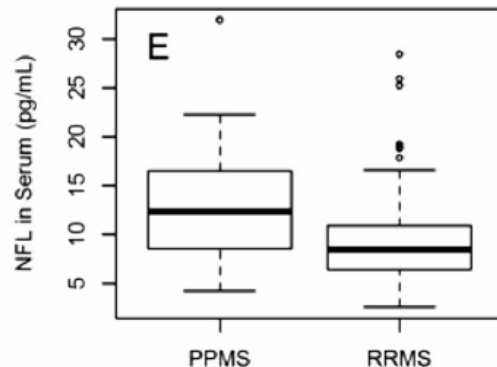
Serum GFAP in multiple sclerosis: correlation with disease type and MRI markers of disease severity

Xavier Ayrignac^{1,2}, Emmanuelle Le Bars^{2,3,4}, Claire Duflos⁵, Christophe Hirtz^{6,7},
Aleksandra Maleska Maceski^{2,7}, Clarisse Carra-Dallière¹, Mahmoud Charif⁶, Frédéric Pinna¹,
Pauline Prin¹, Nicolas Menjot de Champfleury^{2,3,4}, Jérémy Deverdun^{2,3,4}, Tobias Kober^{8,9,10},
Bénédicte Marechal^{8,9,10}, Mario Joao Fartaria^{8,9,10}, Ricardo Corredor Jerez^{8,9,10},
Pierre Labauge¹ & Sylvain Lehmann^{6,7}

GFAP



NfL

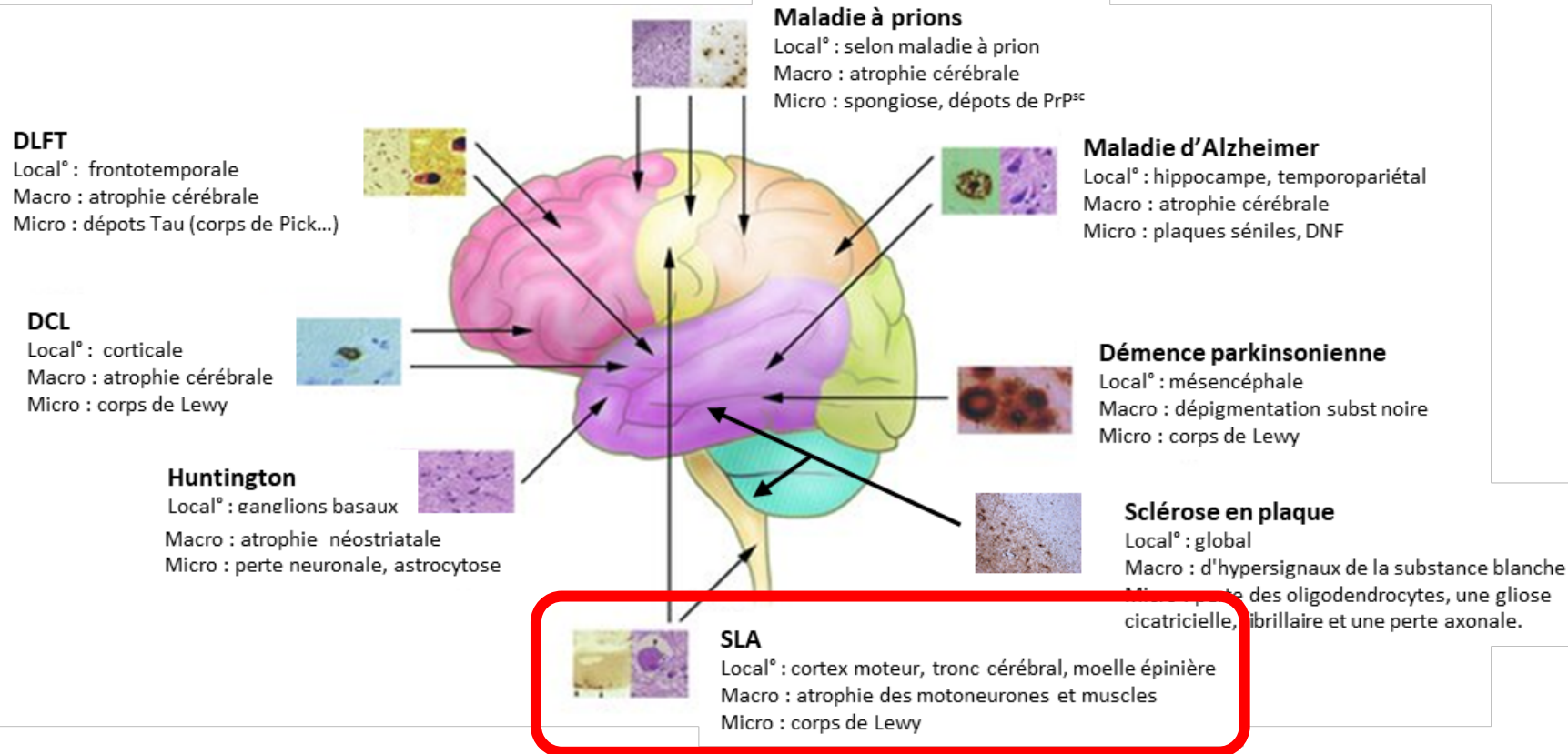


Glial fibrillary acidic protein: a blood
biomarker to differentiate
neurodegenerative from
psychiatric diseases

GFAP aussi
dans les DFT...

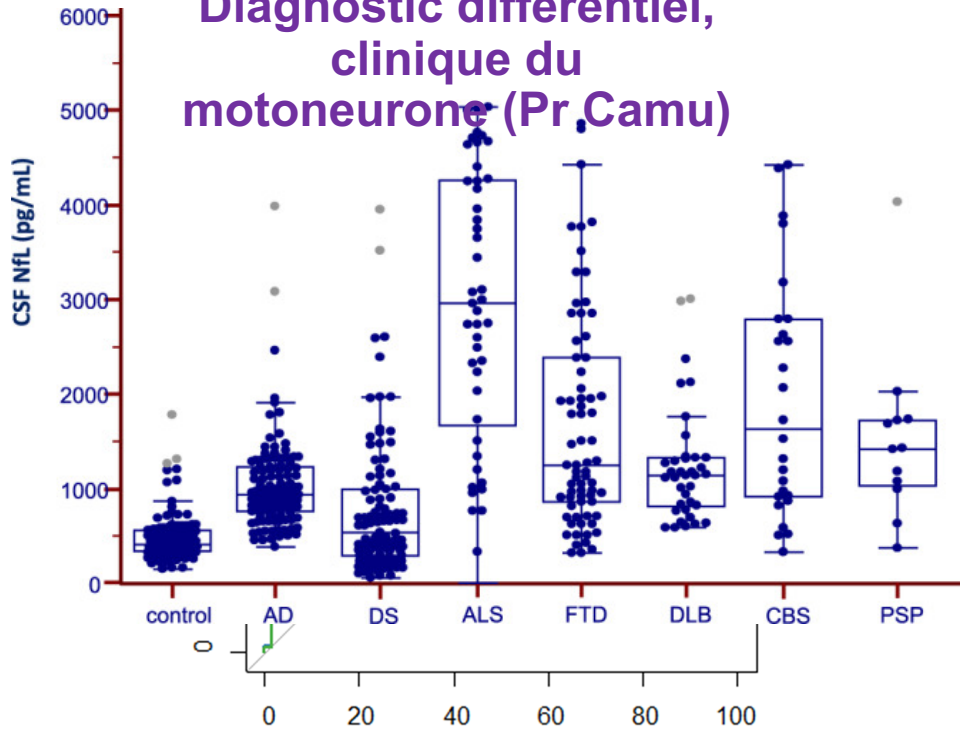
Henrik Zetterberg

Blood glial fibrillary acidic protein (GFAP) concentration is higher in frontotemporal lobar degeneration (FTLD) than in primary psychiatric disorders (PPD) and predicts disease progression in FTLD



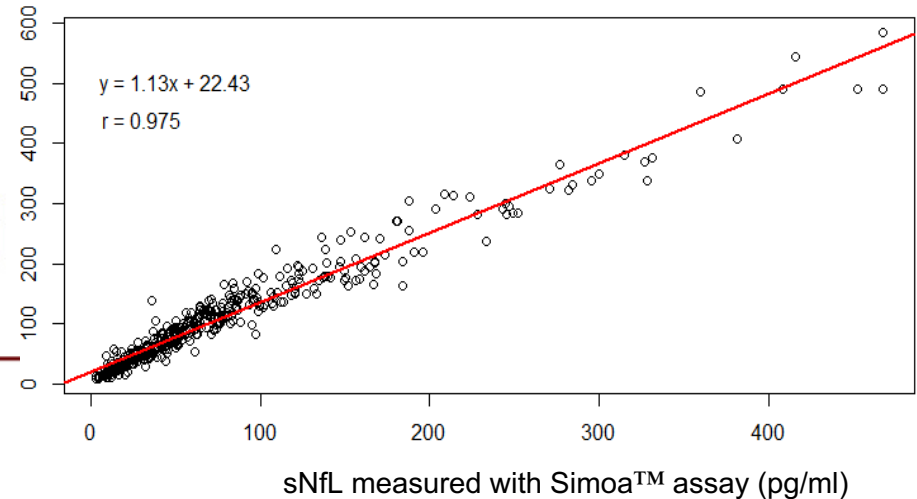
Détection des Neurofilaments dans la SLA

Diagnostic différentiel,
clinique du
motoneurone (Pr. Camu)



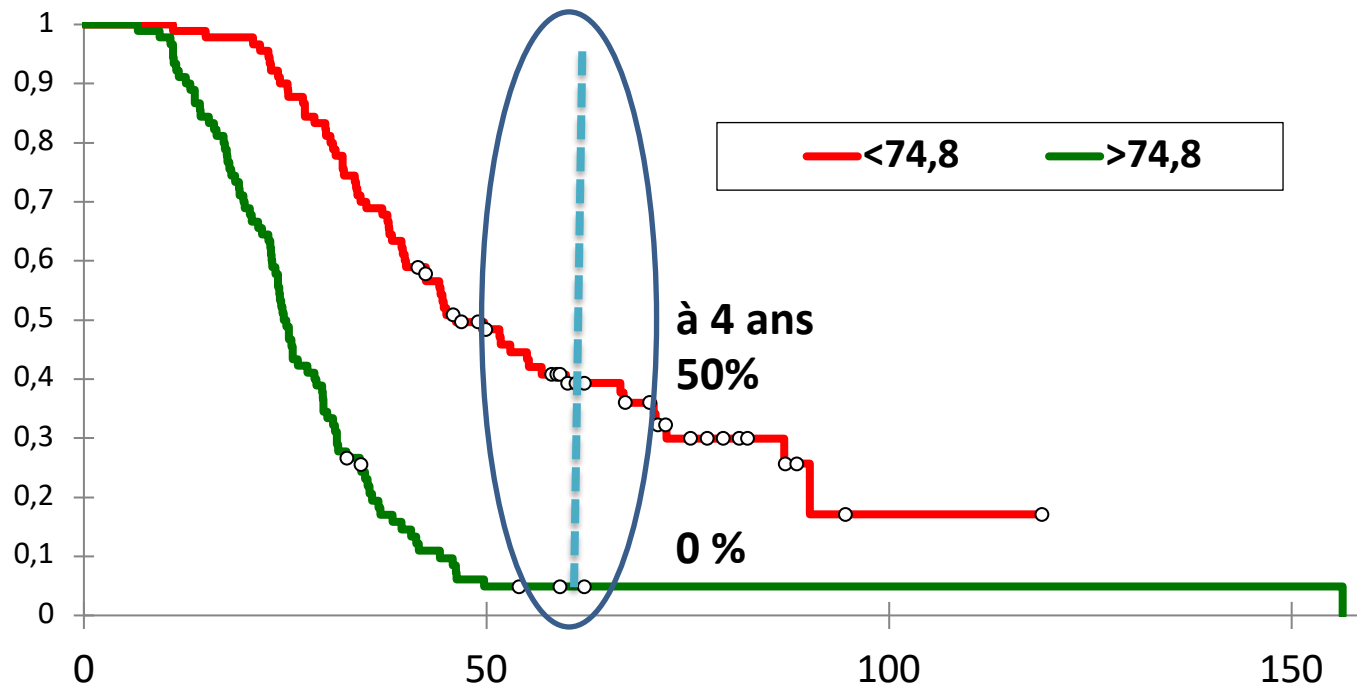
SimOa®
Quantex

ELLA®
Vs. Proteinsimple/Biotechne

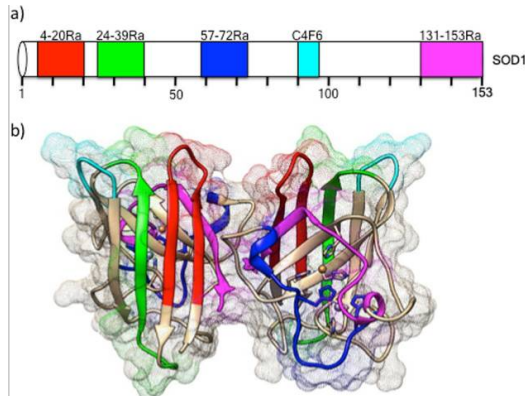
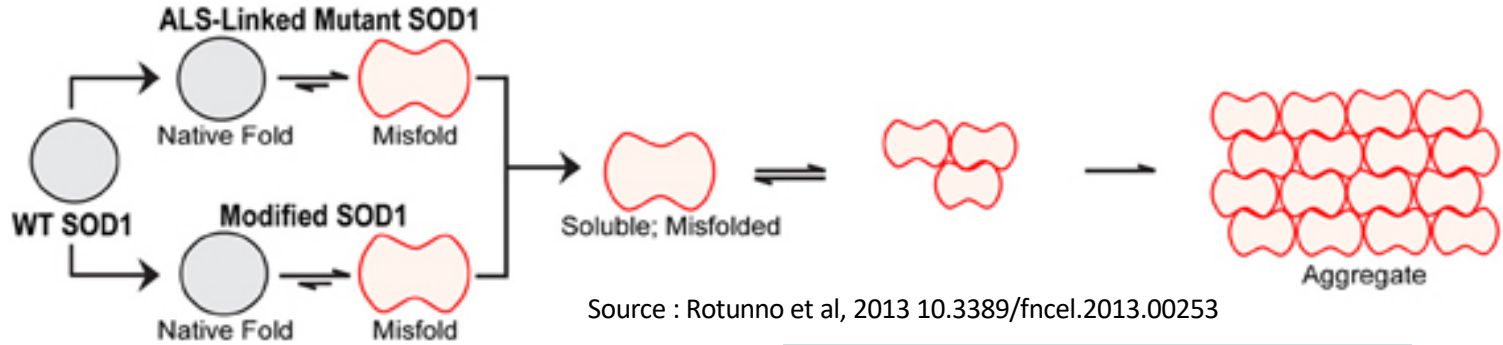


Détection des Neurofilaments dans la SLA : pronostic

Fonction de survie cumulée
(en mois) après stratification selon les valeurs des Nfl sanguines



Détection de la forme « misfolded » de la protéine SOD-1 dans la SLA



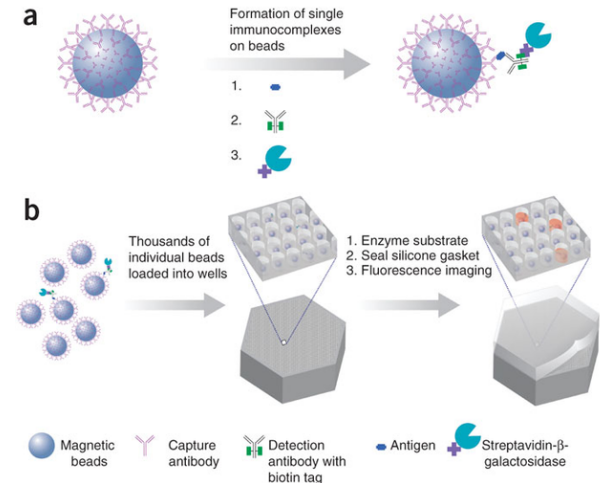
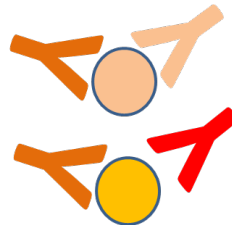
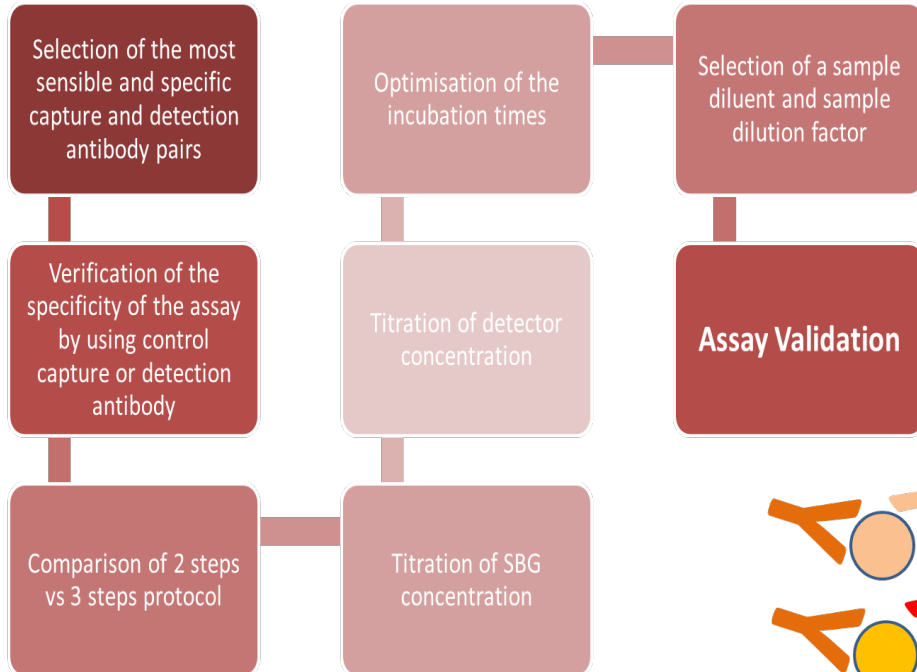
SCIENTIFIC REPORTS

Article | OPEN | Published: 21 September 2018

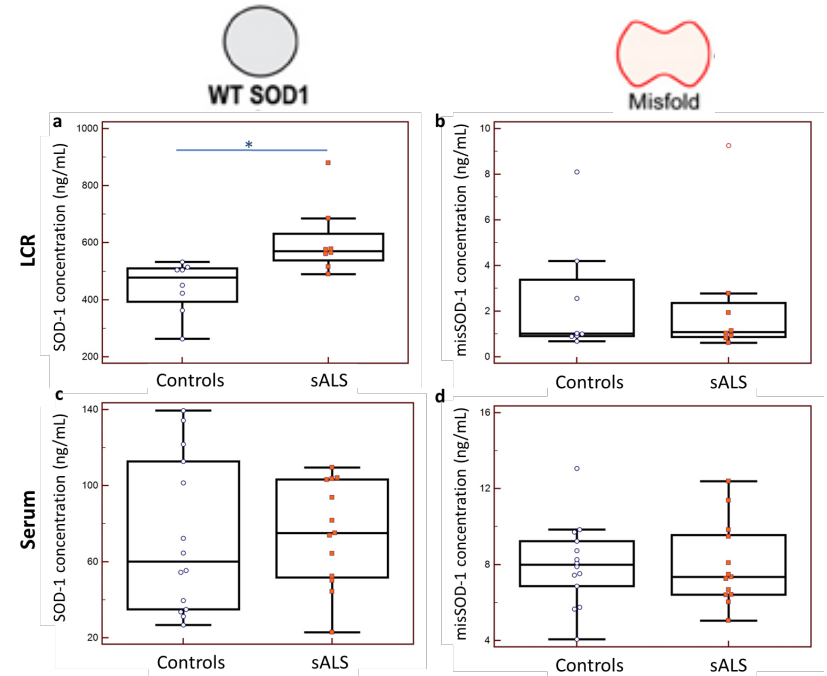
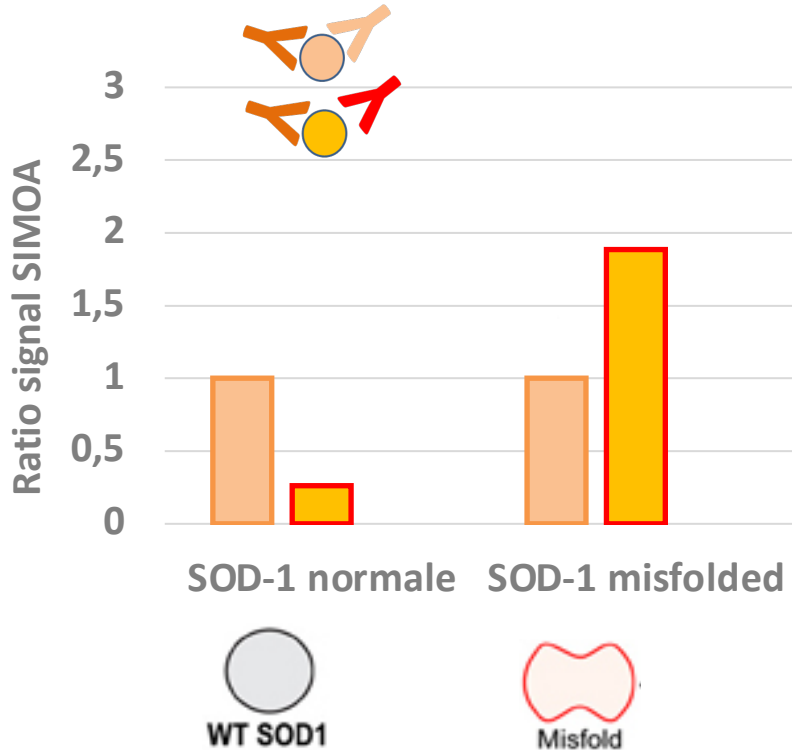
Misfolded SOD1 pathology in sporadic Amyotrophic Lateral Sclerosis

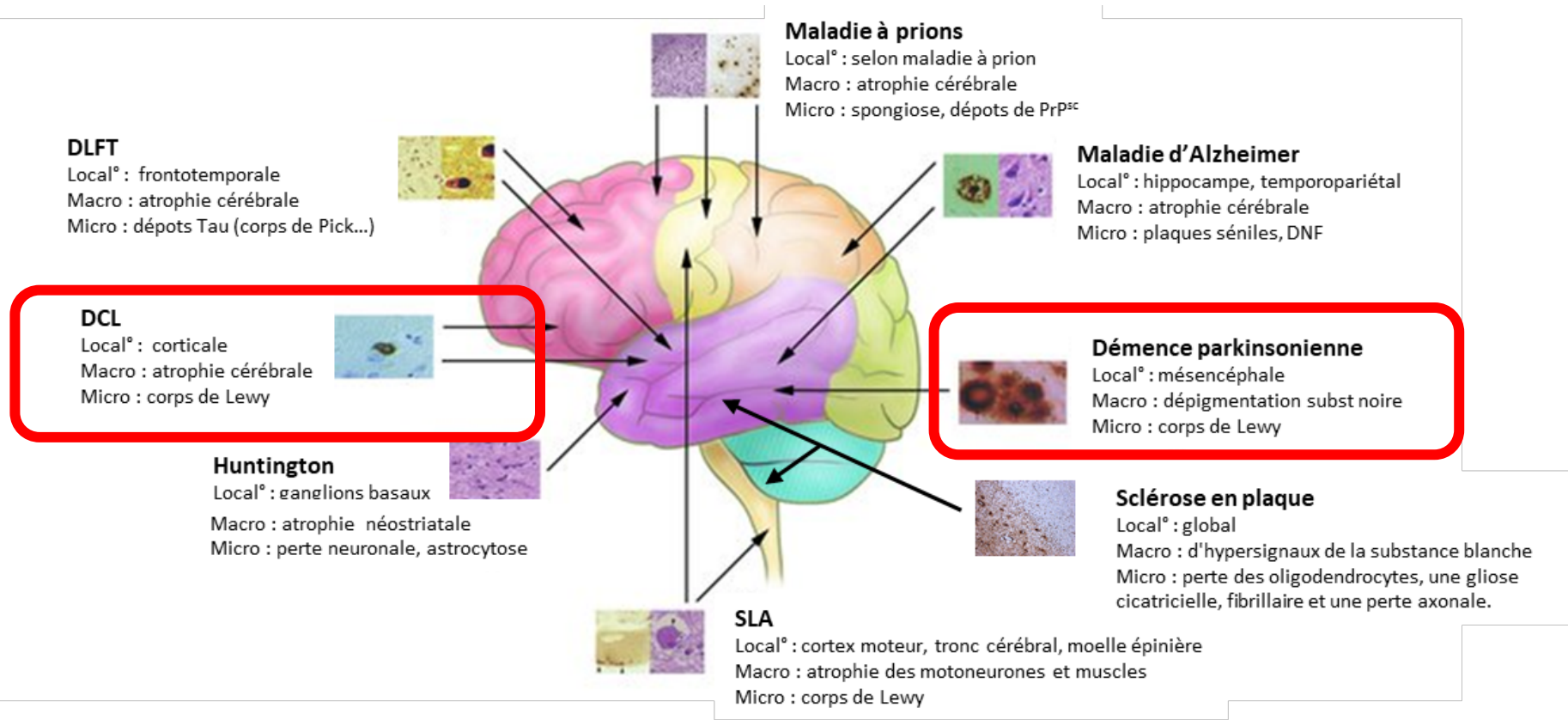
Bastien Paré, Manuela Lehmann, Marie Beaudin, Ulrika Nordström, Stephan Saikali, Jean-Pierre Julien, Jonathan D. Gilthorpe, Stefan L. Marklund, Neil R. Cashman, Peter M. Andersen, Karin Forsberg, Nicolas Dupré, Peter Gould, Thomas Brännström & François Gros-Louis

« Homebrew » ultra-sensitive assay for SOD-1

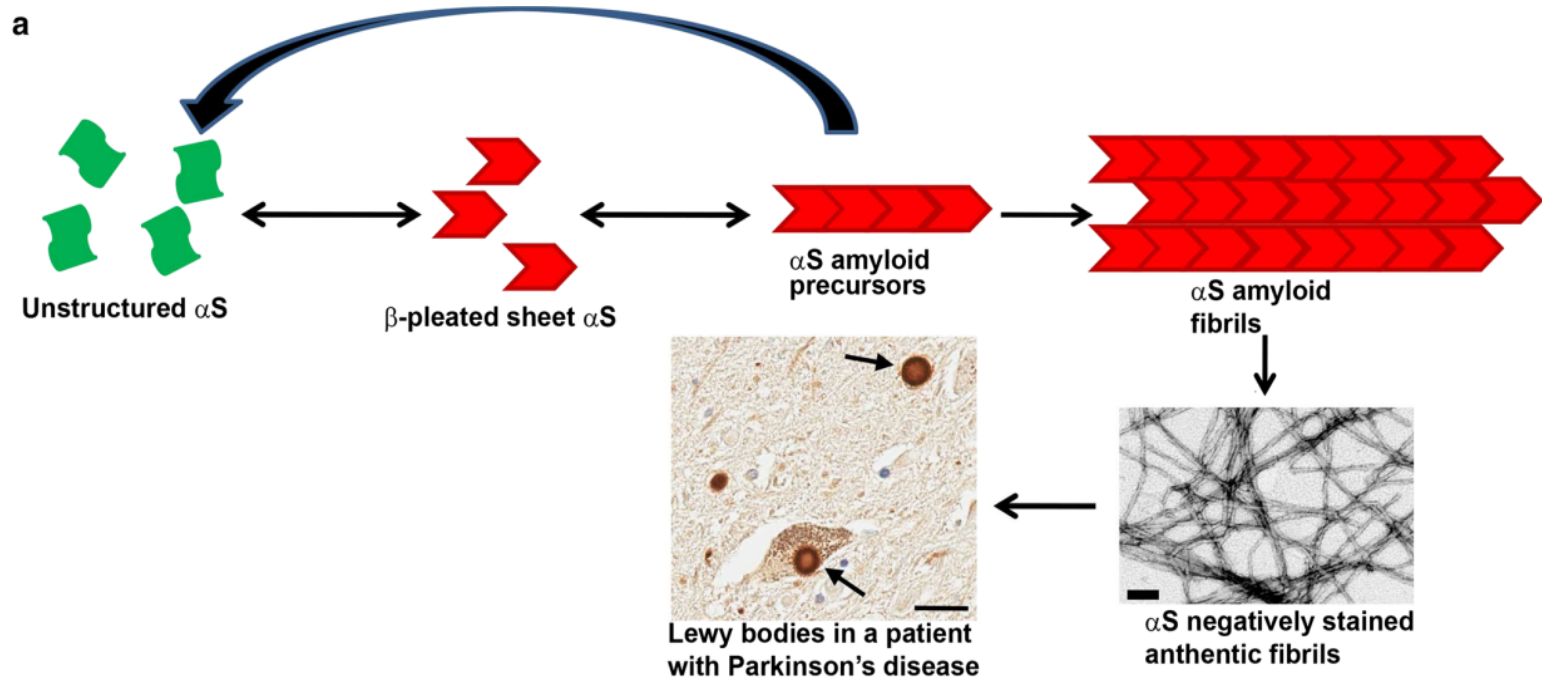


Détection de la forme « misfolded » de la protéine SOD-1 dans la SLA

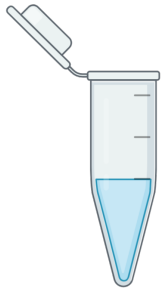




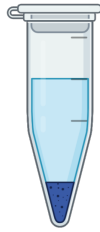
Rôle de l'alpha-synucléine dans les synucléinopathies



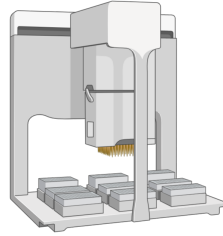
Détection de l'alpha-synucléine en spectrométrie de masse (MRM)



α , β and γ synuclein
labelled U-N15 were
added to plasma
(95 μ l) or CSF and
saliva (500 μ l each)



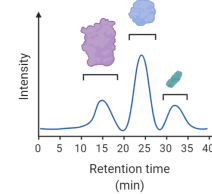
Proteins are
precipitated with
strong acid and
then **centrifuged**
at high speed



**Sample clean-up with the
AssayMap Bravo**
Agilent technologies.
Using RPW tips

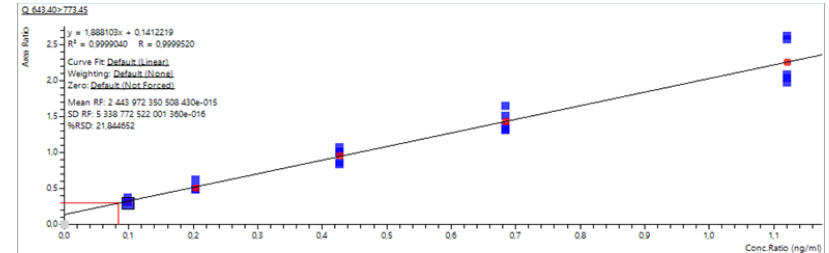
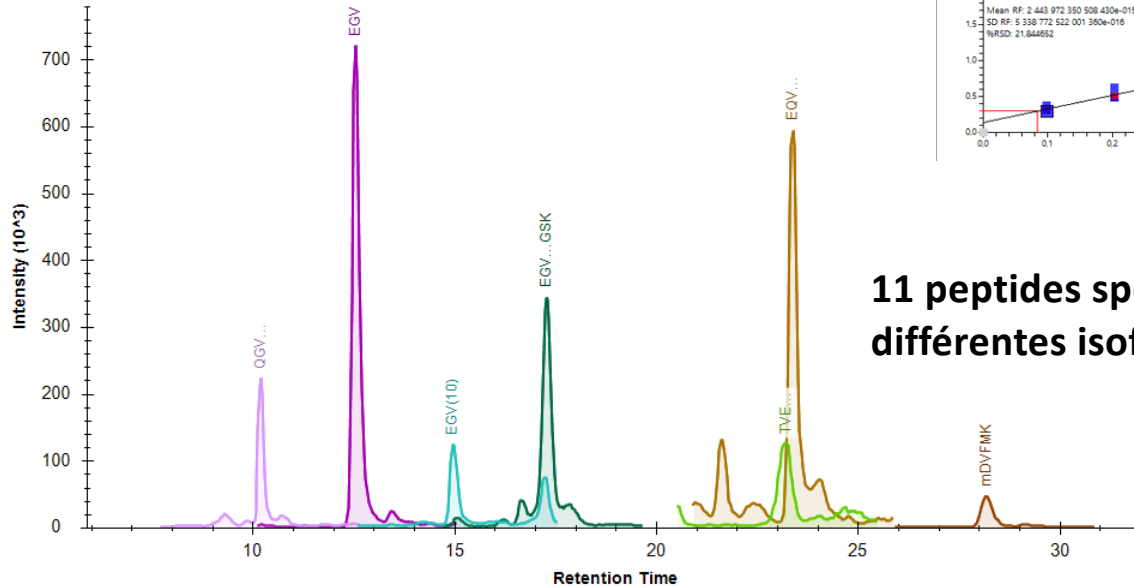


**Proteolytic digestion by
trypsin/LysC**



**Proteotypic peptides are
analyzed by multiplex
LC-MRM**
Shimadzu 8060.

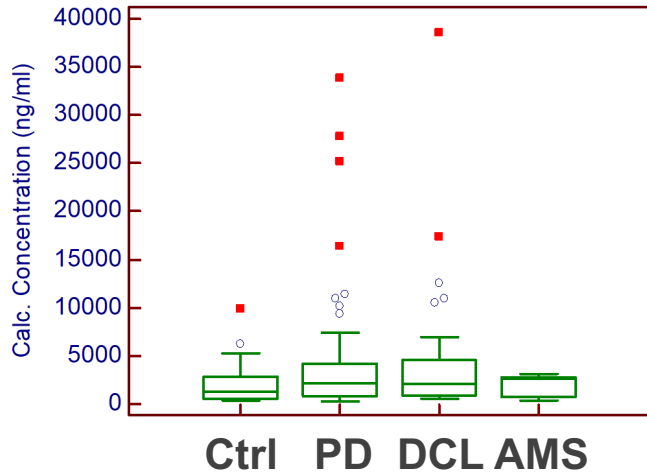
Détection de l'alpha-synucléine en spectrométrie de masse (MRM)



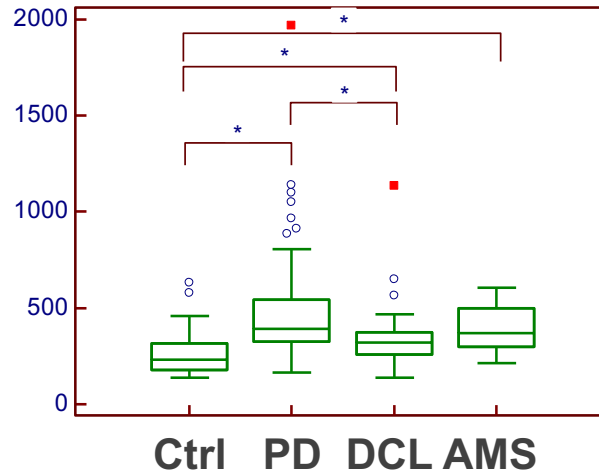
**11 peptides spécifiques des
différentes isoformes de synucléine**

Détection de l'alpha-synucléine en MRM, comparaison avec l'ELISA

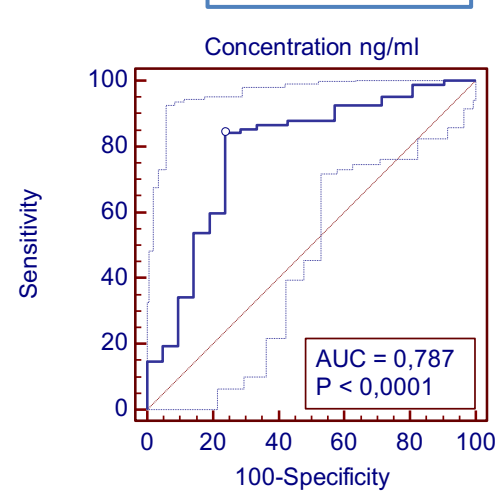
ELISA

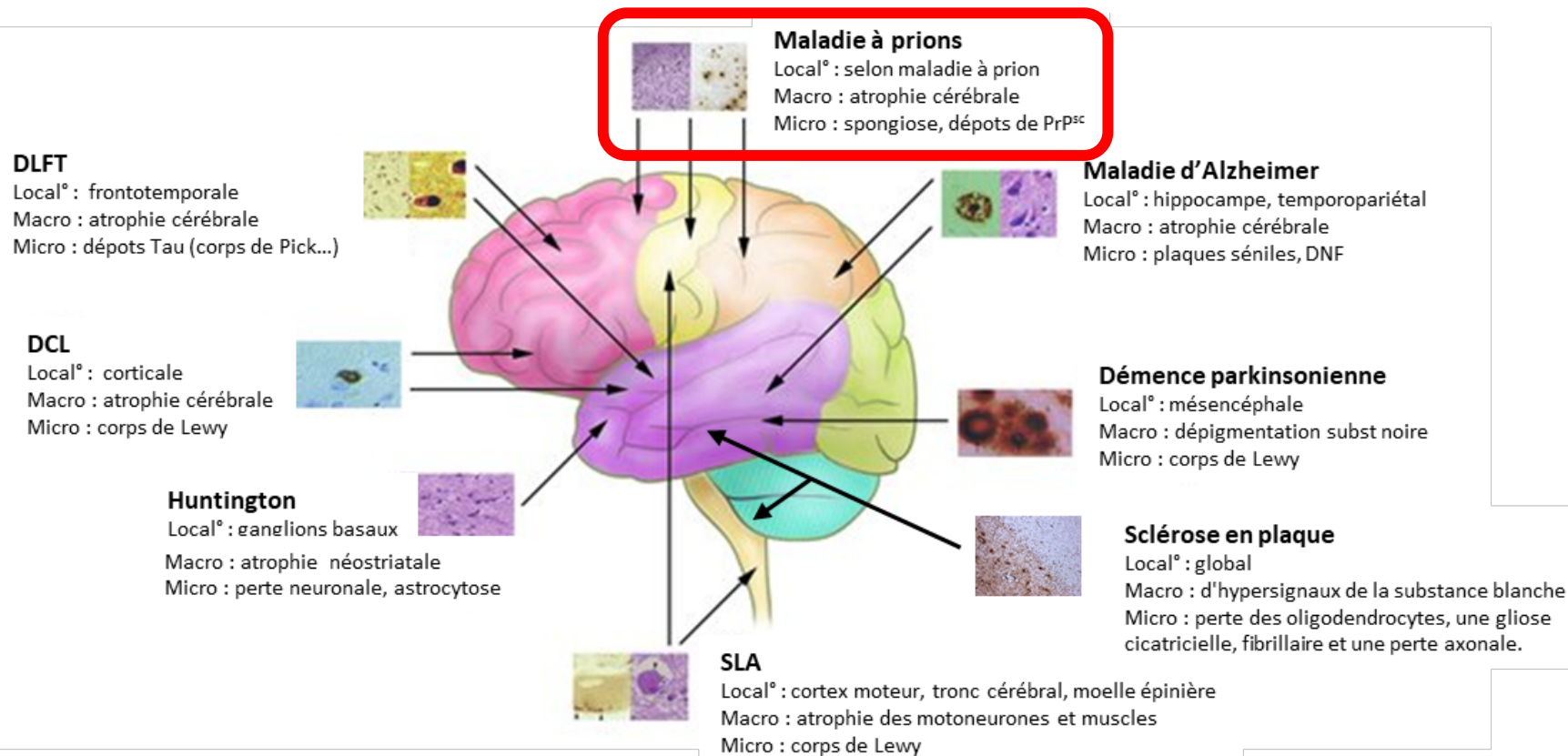
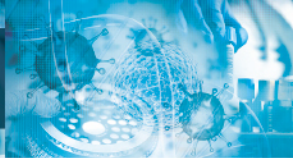


MRM

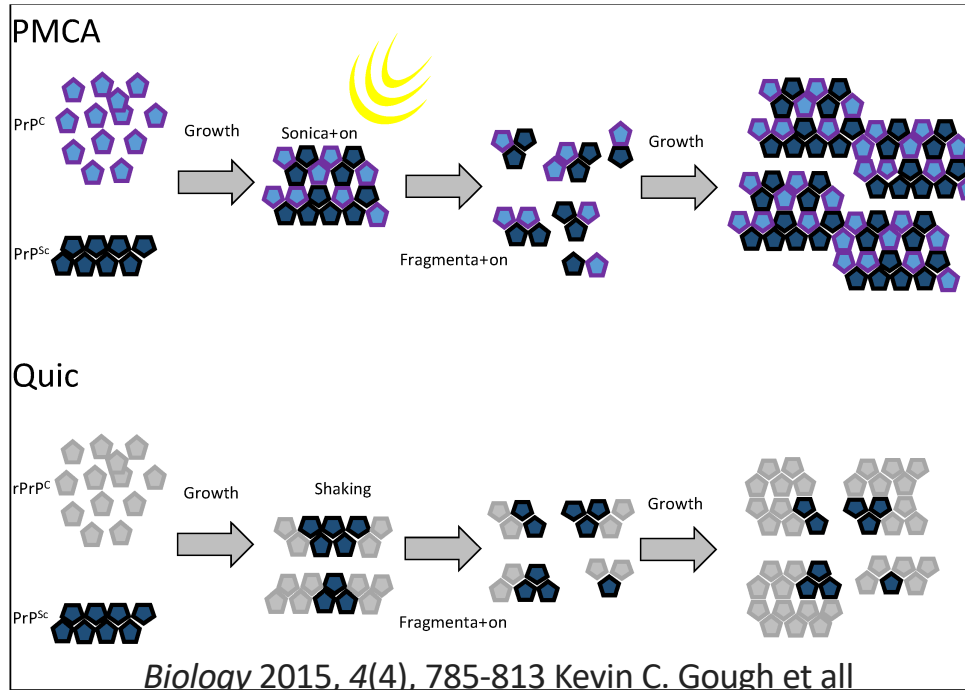


PD vs control





Amplification des agrégats de protéine prion pathologique



PMCA
Protein Misfolding
Cyclic
Amplification

QUIC
Quaking-induced
conversion

Amplification des agrégats de protéine prion pathologique

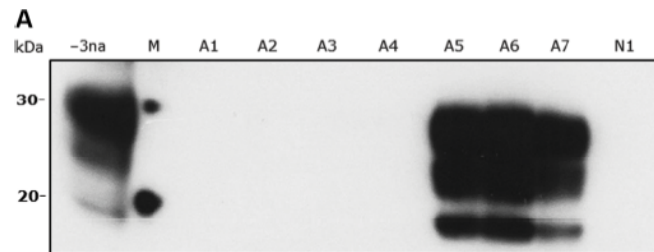
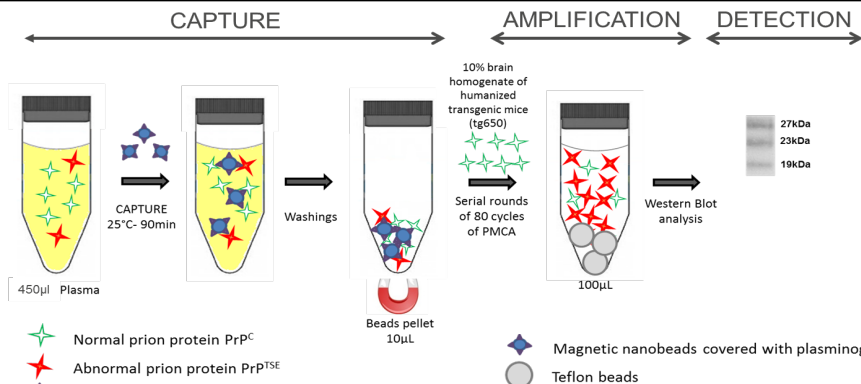
SCIENCE TRANSLATIONAL MEDICINE | RESEARCH ARTICLE

PRION DISEASES

Detection of prions in the plasma of presymptomatic and symptomatic patients with variant Creutzfeldt-Jakob disease

Daisy Bougard,^{1*} Jean-Philippe Brandel,^{2,3,4} Maxime Bélondrade,¹ Vincent Béringue,⁵ Christiane Segarra,¹ Hervé Fleury,⁶ Jean-Louis Laplanche,^{4,7} Charly Mayran,¹ Simon Nicot,¹ Alison Green,⁸ Arlette Welaratne,³ David Narbey,⁹ Chantal Fournier-Wirth,¹ Richard Knight,⁸ Robert Will,⁸ Pierre Tiberghien,^{9,10} Stéphane Haïk,^{2,3,4} Joliette Coste^{1,9*}

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- 2,6 years



Biomarqueur des maladies neurodégénératives

- Impliqués dans les processus pathologiques

Amyloïdes, protéines Tau, PrP (prion), synucléine, TDP-43, SOD1....

dans leur forme normale, oligomérique, avec des modifications post-traductionnelles (clivages, phosphorylation...)

BACE1, TACE/ADAM10, MMP9, SORL1, N-Cadherin...

- Témoins / réactionnels

Neurofilaments, GFAP, Protéines Tau (lyse), synucléine (lyse, synapse), 14-3-3 (lyse), neurogranine, YKL40/CH3L1, neuro-inflammation, cortisol, le profil immunitaire...

- Lié au terrain / risque

Apolipoprotéine E, profil lipidomique, génétique, le profil immunitaire...

- De retour à l'homéostasie (indirect)

Facteur de croissance, neurotransmetteurs...

Biomarqueur des maladies neurodégénératives

- Impliqués dans les processus pathologiques

ATTENTION.....

■ **Pré-analytique** → compatible avec pratique, variabilité (nycthémère, alimentation, médicaments..)

■ **Analytique**: variabilité, turnover, équipements...

■ **Multiparamétrique** : combinaison, multimodale (cognition.....)

■ **Vraie valeur ajoutée**: besoin clinique, intérêt médico-économique

■ **Réglementaires** : marquage CE IVD, remboursement

- De retour à l'homéostasie (indirect)

Facteur de croissance, neurotransmetteurs...

ges,

Marqueurs sanguins dans le suivi des maladies neuro-dégénératives ou neuro-inflammatoires



Dpt Neuro, Lille, Paris,
Strasbourg, Lyon, Barcelone... et
aux patients et leur famille

