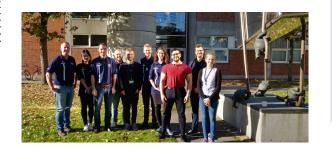


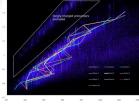
Translational Biomarker AAU















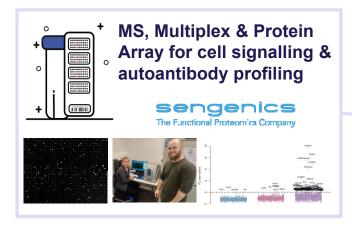
Thermo Sci Bruke Qexactive HF-X PRO Pharma-option Multi-

Bruker timsTOF F-X PRO on Multi-omics setup

Bruker UltrafleXtreme Imaging MS setup

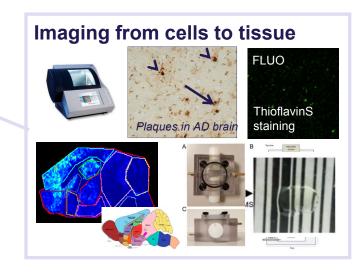
4D Setup for biomolecule characterization down to low cell number

Extensive bioinformatics platform including multiple commercial solutions for quantitative proteomics and metabolomics

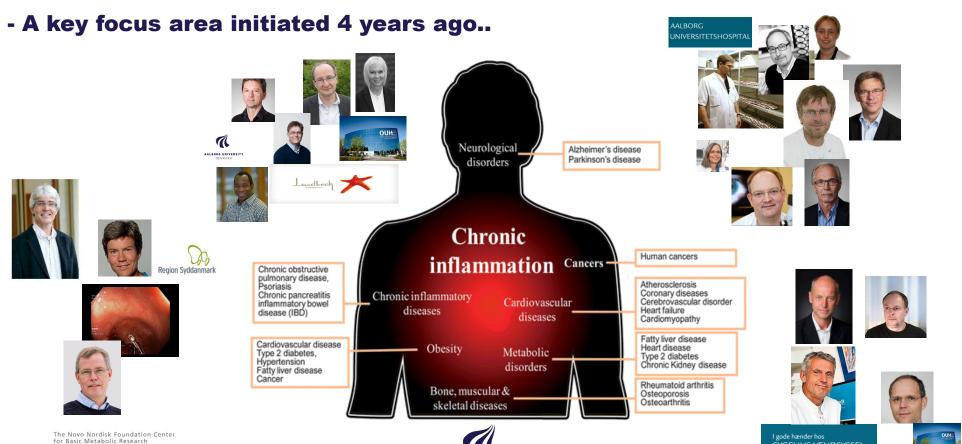








Inflammation...



AALBORG UNIVERSITY
DENMARK

SYGEHUS VENDSYSSEL

LMM PRO-MS North Jutland node

High-end for clinical Proteomics & metabolomics...

LC-MS system (PRO-MS2)

- ThermoSci QE HF-X Pharma option
- Deep proteomics and analysis of biologics
- Targeted MS by PRM

LC-MS system (PRO-MS1)

- Bruker timsTOF PRO
- Optimized for high-end proteomics and high-sensitivity metabolomics with IMS

MALDI MS

- **Bruker Ultraflex Extreme**
- **Bruker Autoflex**
- Protein & peptide profiling and MS Imaging













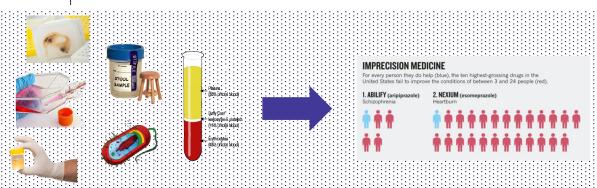
Phenotyping for enhanced diagnostics and personalized translational medicine

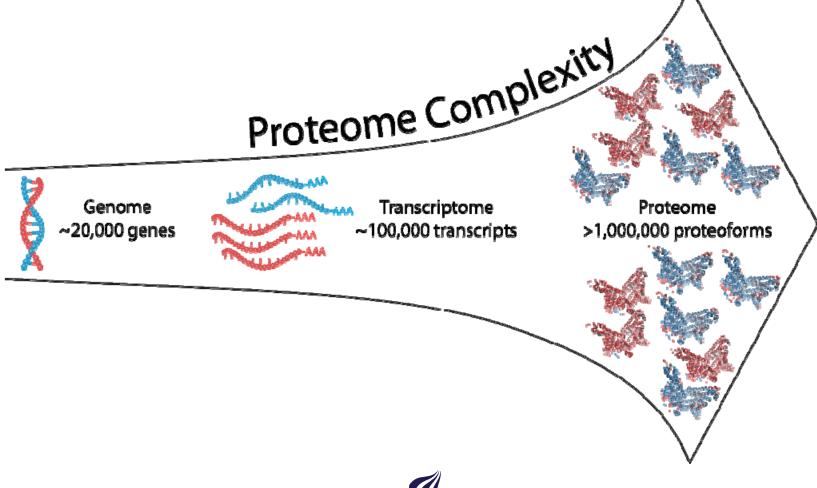
- More than 80 diverse autoimmune diseases affect 6.4% percent of women vs. 2.7 % of men
- Researchers don't know exactly what causes many autoimmune diseases.
- Genetics, diet, infections, and exposure to chemicals might be involved.
- Many subtypes remain excessively difficult to validate and no single test can diagnose most autoimmune diseases.
- Low grade inflammation & pain is central in most pathologies.

cand J Rheumatol 2019;0:1–10

Personalized Rheumatic Medicine through Dose Reduction Reduces the Cost of Biological Treatment – a retrospective intervention analysis

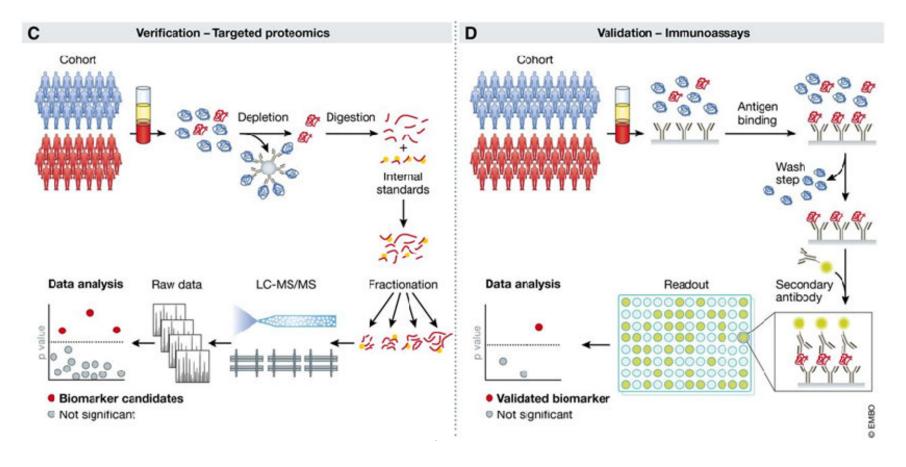
MK Meyer^{1,2,3,4,5}, M Andersen^{1,2,3}, T Ring^{3,6,7}, GN Andersen^{1,2,4}, LH Ehlers⁷, C Rasmussen^{1,2,5,7}, A Stensballe³* a) Rheumatoid Arthritis Annual bDMARD Expense/patient in EUR Annual bDMARD Expense/patient in EUR 25,000 20,000 20,000 15,000 15,000 10,000 10,000 2004 2006 2008 2010 2012 2014 2002 2006 2006 2008 2010 2012 2014 b) Spondyloarthritis Annual bDMARD Expense/patient in EUR **Accumulated saving** 25,000 over 15 years 20,000 15,000 approx. EUR 4.1mill 10,000 2008 2010 2012 2014



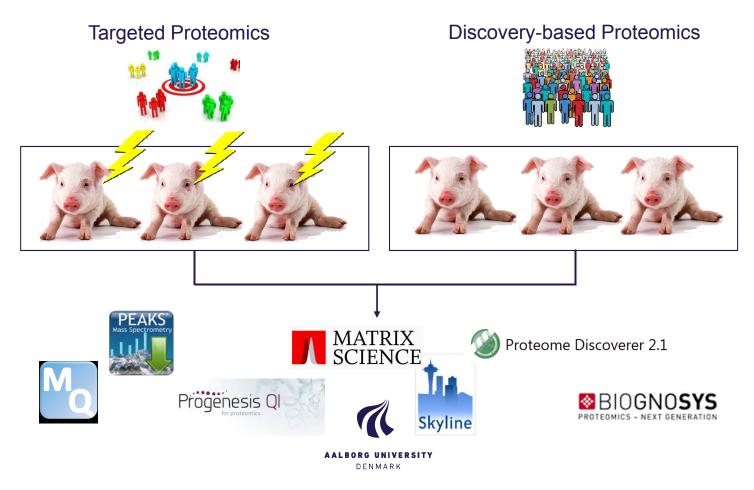




Proteomics - Key technology in Clinical research

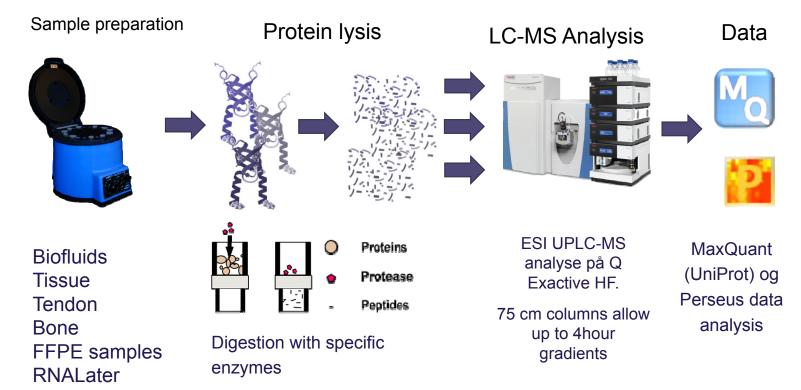


Quantitative Bioinformatics in TB



Proteome analysis pipeline

- One of seveal proteomic pipelines

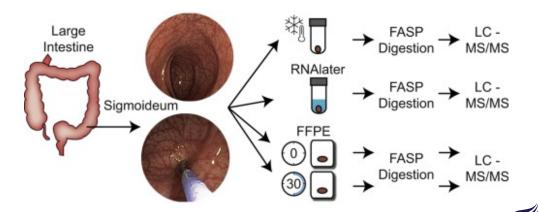


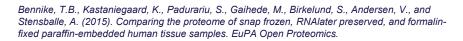


Bennike, Tue Bjerg, Thomas Gelsing Carlsen, Torkell Ellingsen, Ole Kristian Bonderup, Henning Glerup, Martin Bøgsted, Gunna Christiansen, Svend Birkelund, Allan Stensballe, and Vibeke Andersen. "Neutrophil Extracellular Traps in Ulcerative Colitis: A Proteome Analysis of Intestinal Biopsies." Inflammatory Bowel Diseases, May 19, 2015.

Improved sample preparation from Biobank samples

- Most biological samples are preferably snapfrozen and stored at -80C
- Typical samples are serum, plasma, tissue and immune cells







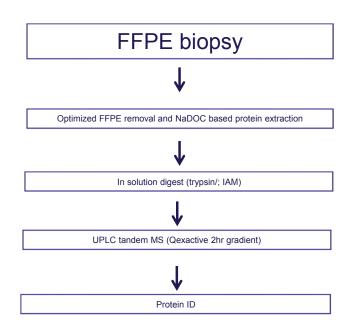
AALBORG UNIVERSITY

DENMARK

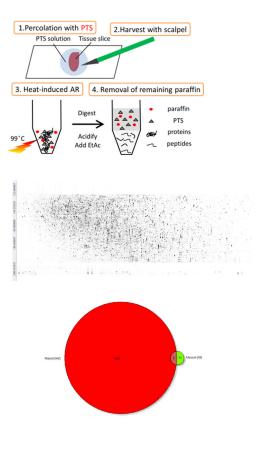




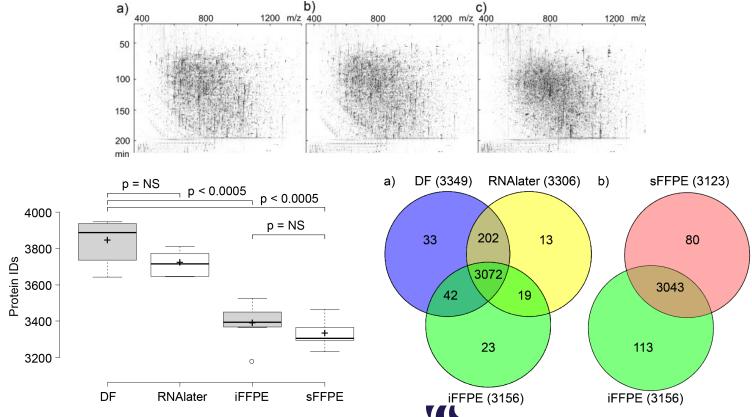
Improved sample preparation from Biobank samples







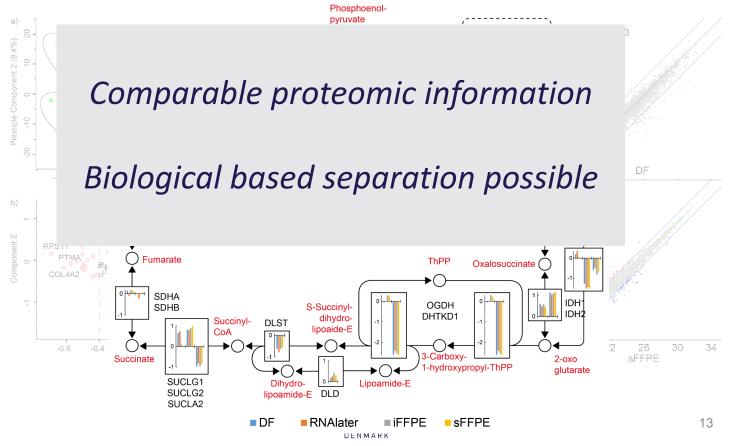
Improved sample preparation from Biobank samples



Bennike, T.B., Kastaniegaard, K., Padurariu, S., Gaihede, M., Birkelund, S., Andersel, W., and Stensballe, A. (2015). Comparing the proteome of snap frozen, RNAlater preserved, and formalin-fixed paraffin-embedded human tissue samples. EuPA Open Proteomics.

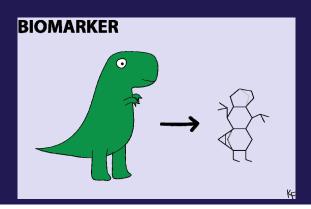
DENMARK

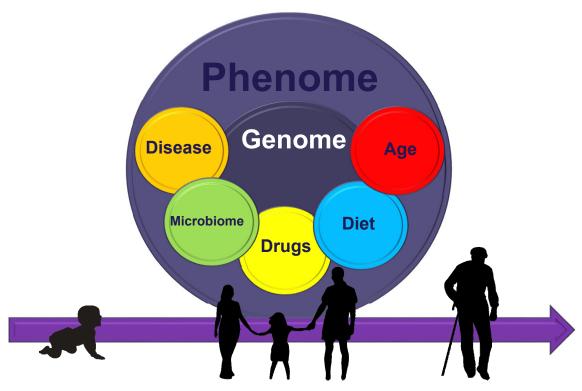
Pathway analysis of quantitative MS data



Improved and truely translational biomarkers are needed

- Enabling technologies including all Omics technologies paves the way to understanding disease etiology.
- Key to insight remains the ability to find good answers to relevant clinical questions
- Why responders and non-responders?

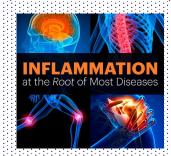




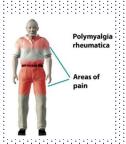
Disease risk (prognosis/Diagnosis/ prevention)



Patient stratification (Translational biomarkers)



Treatment response (Prediction/monitoring)





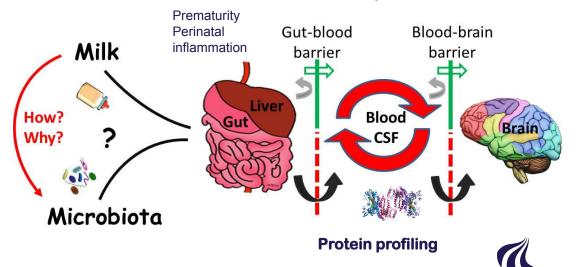


Omics technologies for premature newborns

AALBORG UNIVERSITY
DENMARK

Challenges & Opportunities

Insight into treatment and long-term outcome for healthy and perfect miracle babies if born premature

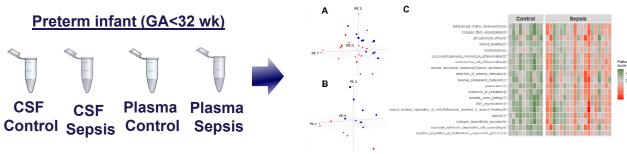


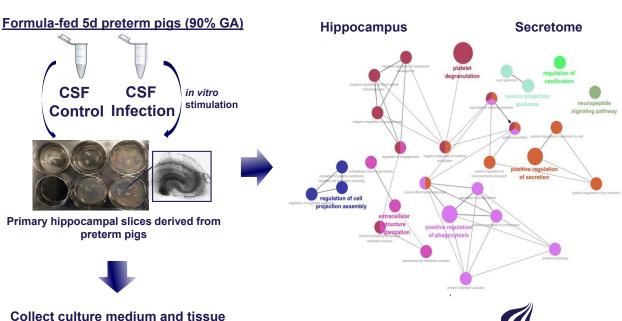
Systems Biology at Sino-Danish NEOMICS Center



22-Nov-19

15





AALBORG UNIVERSITY

DENMARK

Blood-brain

barrier

How systemic infection affects brain development and functions is poorly understood.

Normal

Blood

Lung/gut

barrier

Good biomarkers for early diagnosis and treatment for sepsis-induced brain injury are lacking.



Assistant Prof. Tue Bennike.

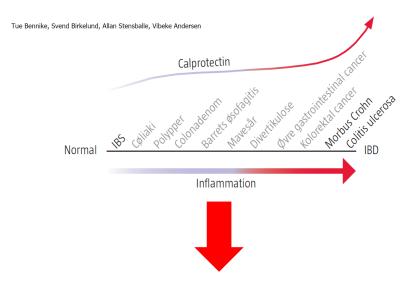
Inflammatory Bowel Diseases vs Rheumatoid Artritis

- Inflammatory Bowel Disease (IBD) comprises two major disease entities, Crohn's disease (CD) and ulcerative colitis (UC)
- IBDs are common diseases with a prevalence of 1% and of raising prevalence
- Diagnosis of IBDs depends on clinical symptoms, endoscopy, histology, MR-scanning, and the exclusion of gut microbes and may be delayed for years
- Diagnosis is difficult and 10-20% change diagnosis or are termed indeterminate colitis



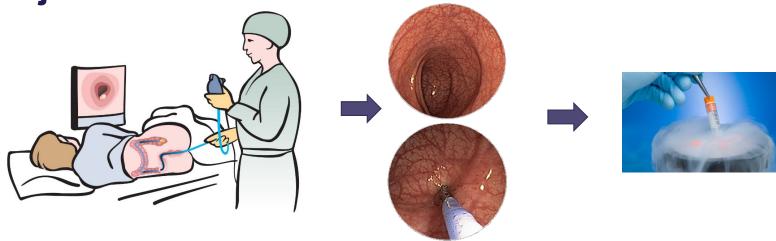
WJG 20" Anniversary Special Issues (3): Inflammatory bowel disease

Biomarkers in inflammatory bowel diseases: Current status
and proteomics identification strategies



Our Goal:
Investigate the disease related proteome changes of the large intestine associated with IBD and RA

The Silkeborg IBD vs RA Project



- 10x Ulcerative Colitis
 - Unambiguous diagnosis.
 - Medicated, no disease flairs.
- 10x Reumatoid artritis
 - Unambiguous diagnosis.
 - No gut inflammation detectable by microscopy
- 10x Controls
 - Cancer screenings with no findings

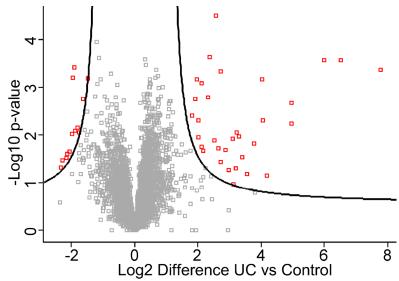


Proteomics

- Snap frozen in N_{2(L)}, stored at -140C°
- Mean weight 4.9 mg [3.6-5.9 mg; 25th-75th percentiles]

Proteome analysis reveals activation of innate immune system in "healthy-looking" UC tissue

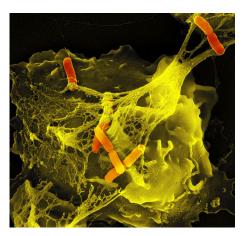
- 5,444 proteins quantifiable!
- T-tests with permutation tests to control false positives.
- 46 proteins with a statistical significant abundance change.
 - 33 more abundant in UC and 13 less.
 - 15% false positives at this cutoff.
 - Statistical significantly changed proteins biological relevant!





Discovery of NETs in gut intesitnal tissue

- Neutrophil extracellular traps (NETs) is an important line of defense against microbiota
- Eleven statistically significant proteins involved in NETs
 - all increased in ulcerative colitis!



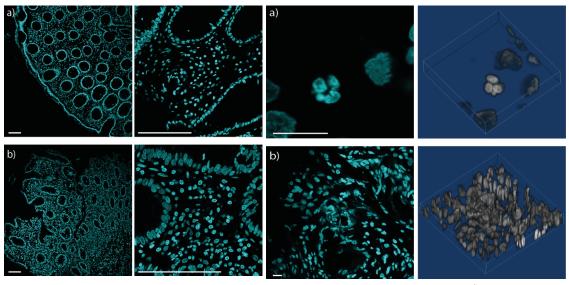
Fold Change	Protein Description	Peptides	SQ
219.2	*Lactotransferrin	51 (49)	73%
92.1	*Matrix metalloproteinase-9	30 (30)	52%
63.3	*Myeloperoxidase	48 (41)	59%
31.3	*Neutrophil elastase	11 (11)	55%
16.3	*Protein S100-A9	10 (10)	79%
11.8	*Protein S100-A12	5 (5)	36%
9.9	*Neutrophil defensin 3	5 (5)	27%
6.6	*Galectin-10	11 (11)	68%
5.9	*Eosinophil cationic protein	10 (10)	47%
4.5	*Myeloid cell nuclear diff. antigen	17 (17)	39%
3.5	*Cathepsin G	16 (16)	54%

NETs trap the invading microorganisms and facilitates interaction with other effector molecules that kill the microorganisms.

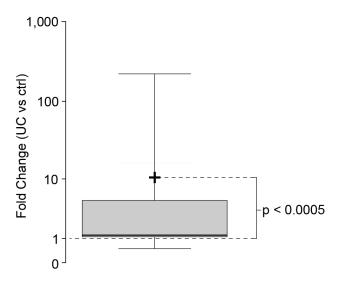


Discovery of NETs in gut intestinal tissue

NETs formation by excretion of the neutrophil DNA (TO-PRO-3 stain).







In total 49 proteins associated with NETs quantified!

Mean fold abundance: 10.9 fold increased in ulcerative colitis tissue.

Proteome analysis of RA Intestinal Mucosa reveals drug induces proteome changes and protein citrullination effects.

To gain new insight into the systemic immune manifestations of RA, we characterized the colon mucosa proteome from RA-patients and healthy controls.



THE UNIVERSITY LIBRARY
AALBORG UNIVERSITY

Subscriber access provided by Aalborg University Library

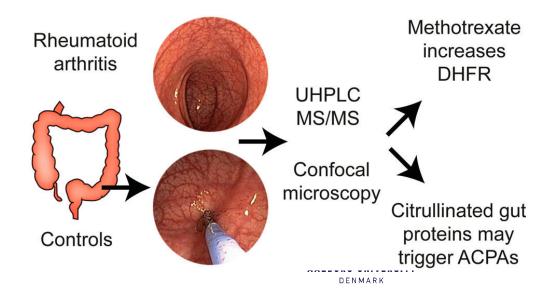
Article

A Proteome Analysis of Rheumatoid Arthritis Gut Mucosa

Tue Bjerg Bennike, Torkell Ellingsen, Henning Glerup, Ole Kristian Bonderup, Thomas Gelsing Carlsen, Michael Kruse Kruse Meyer, Martin Bogsted, Gunna Christiansen, Svend Birkelund, Vibeke Andersen, and Allan Stensballe

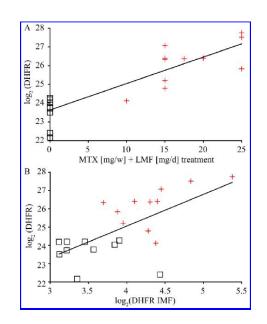
J. Proteome Res., Just Accepted Manuscript • DOI: 10.1021/acs.jproteome.6b00598 • Publication Date (Web): 14 Sep 2016

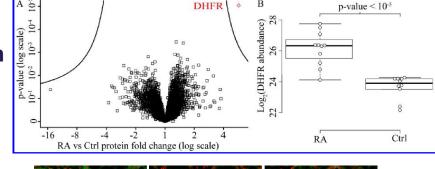
Downloaded from http://pubs.acs.org on September 27, 2016

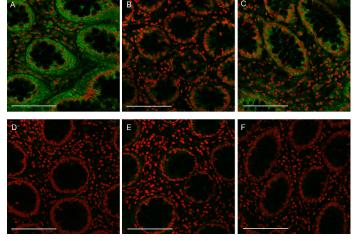


Drug treatment to affect the human immune system that affects the proteome

- Only 1 protein is highly affected by the RA diagnosis.
- The protein DHFR is linked to the treatment with Metrotraxate.



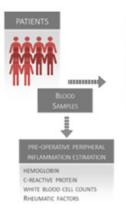


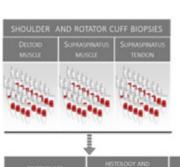




Inflammation of the supraspinatus muscle after rotator cuff lesion

- USA (200,000 procedures/year)
- Denmark (1,500 procedures /year)







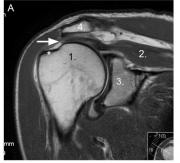


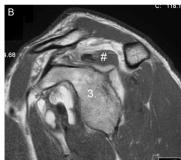


Mamil

Oversat fra engelsk - Mamil eller MAMIL er en person, der kører på en dyre racercykel til fritiden, iført udholdenheds- og præstationsfremmende body-hugging trøjer og / eller shorts. Ordet blev efter sigende myntet af det britiske markedsundersøgelsesfirm Mintel i 2010. Wikipedia (engelsk)







Results after Surgery

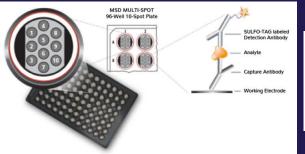
- 1/3 No healing
- 1/3 No healing but do well
- 1/3 heling but no fibrocartilago

Lars Henrik Frich, Livia Rosa Fernandes, Henrik Daa Schrøder, Eva Kildall Hejbøl, Pernille Vinther Nielsen, Puk Hvirgel Jørgensen, Allan Stensballe, Kate Lykke Lambertsen

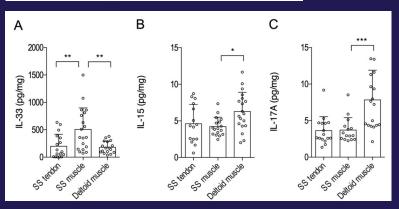
2x Manuscripts Human & murine studies (both in minor revisions)

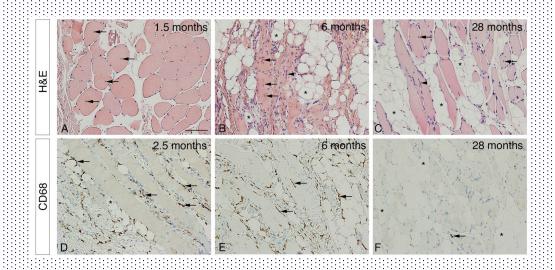
Inflammation of the supraspinatus muscle after rotator cuff lesion

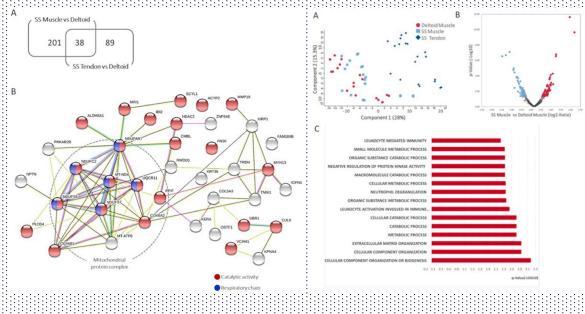
MDS MULTIPLEX (here: Inflammatory discovery)







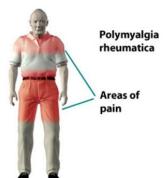




Glucocorticoid Responsive mechanisms in plasma from Polymyalgia Rheumatica patients



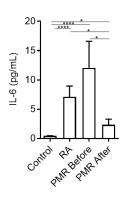
Michael Kruse Meyer

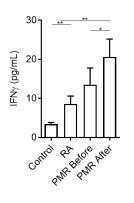


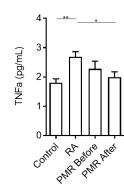
Polymyalgia rheumatica is a relatively common inflammatory rheumatic disease.

There are no validated international guidelines available for the diagnosis and treatment of PMR; however, diagnostic and classification criteria are currently being developed.

A quantitative proteome study design to compare with DMARD naïve RA patients and matched controls



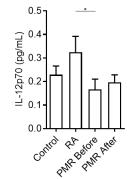


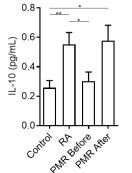


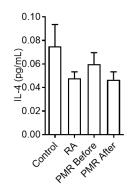






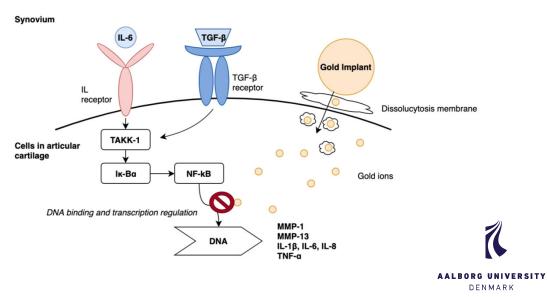




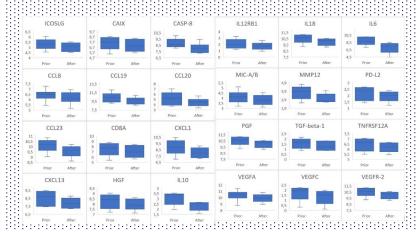


OSTEOARTHRITIS & INFLAMMATION TREATMENT BY NANO- GOLD PARTICLES

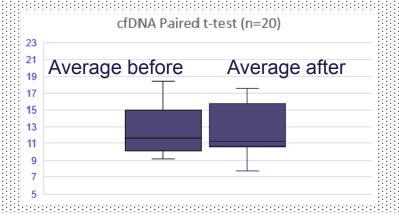
- Synovial fluid and plasma from OA patients were investigated bt low grade inflammation mapping using immuoarrys & proteomics
- Our findings confirm that that inflammation reduction in OA patients regulated through the NF-κB pathway (CCLs; IIs and MMP 12)



OLINK 92 plex immuno-oncology assay



Neutrophil-derived circulating free DNA (cf-DNA/NETs): a potential prognostic marker for posttraumatic inflammation development & sepsis.



PROCIT - Protein citrullination: A novel therapeutic target and diagnostic marker in autoimmune disease

FSS research grant 2017 together with Rigshospitalet



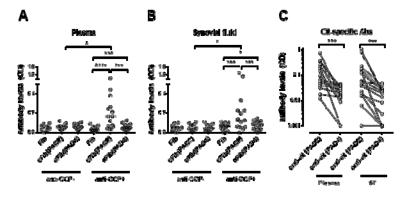
In a research collaboration with Rigshospitalet, Institute for Inflammation research we investigate the role of PAD enzymes as targets for biologics based therapy and novel diagnostics.

- That inhibitory anti-PAD antibodies, as a new class of drugs, are capable of preventing and alleviating RA, and efficacy can be proved in collagen induced arthritis (CIA), an animal model of RA.
- That PAD2 and PAD4, and specific citrullinated proteins/epitopes in blood and synovial fluid are diagnostic or prognostic markers in RA.
- That PAD2 and PAD4 catalyze formation of specific citrullination sites that are recognized by anti-citrullinated protein antibodies (ACPAs).

<u>Peptidylarginine</u> deiminase (PAD) 2 is superior to PAD4 in generating antigenic determinants recognized by anti-citrullinated protein antibodies (ACPAs) from rheumatoid arthritis patients.

Dres Damgaard^{1,2}, Allan Stensballe³, <u>Ladislav</u> Senolt⁴, Claus H. Nielsen^{1,2}

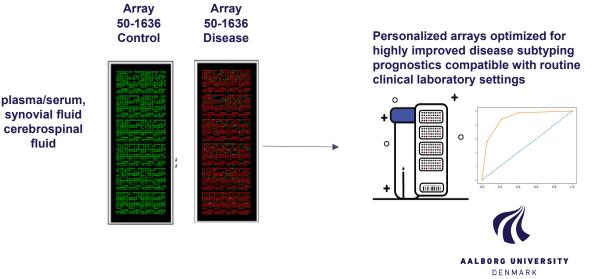


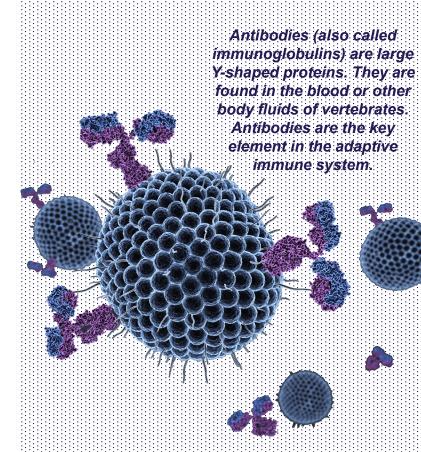


Improved phenotyping of disease subtypes by Immunome array

- Elevated levels of autoantibodies are present before the clinical diagnosis of auto immune diseases
- State-of-the-art personalized protein array technology allows phenotyping of autoantibodies.

fluid

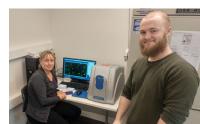




Proportion of Patients with Positive Antibody Tests Relative to the Time of Diagnosis or Appearance of the First Clinical Manifestation of Systemic Lupus Erythematosus

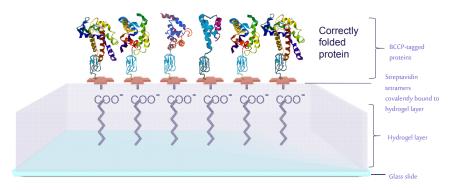
- Systemic Lupus Erythematosus Up to 28yr
 - Rheumatoid Arthritis Up to 16yr
- · Multiple sclerosis Up to 14yr





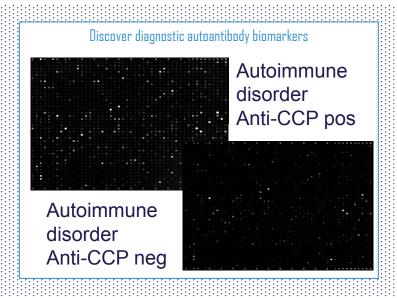
Thomas BG Poulsen Malene M. Jørgensen

- IMMUNOME Discovery Array is a protein array which utilizes the patented Sengenics KREX™ functional proteomics technology.
- ◆ The array contains human proteins from biologically significant protein families including kinases, signalling molecules, cytokines, interleukins, chemokines and cancer antigens.

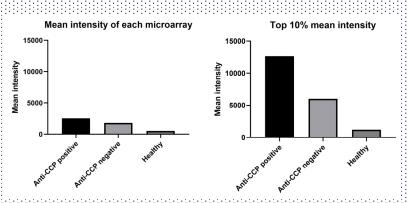


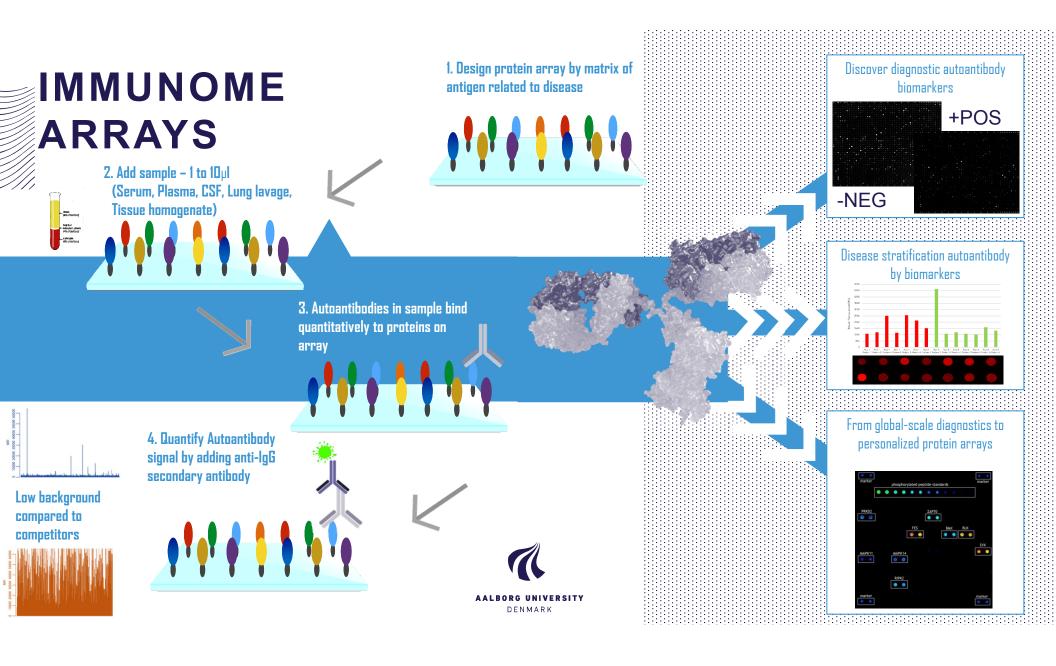
KREX technology enables autoantibody panels from single to 1600+ individual antigens.





Low noise of array technology enabling





Microproteomics in AD, PD and depression







 Laser Microdissection combined with proteomics allows investigation of minute amounts of protein

An AD mouse model system was used to investigate the plaque proteome

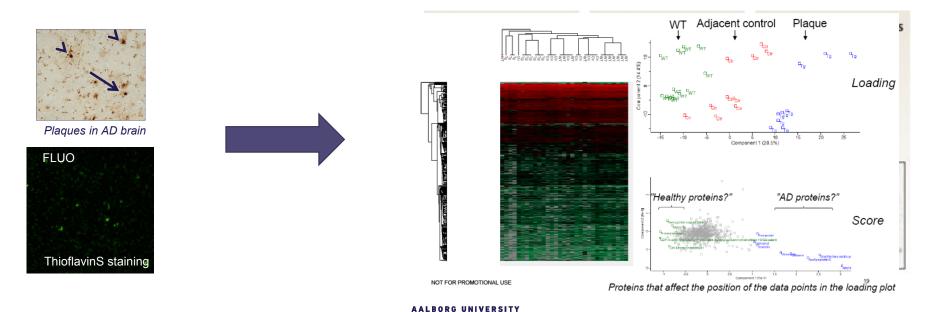
Journal of Alzheimer's Disease xx (20xx DOI 10.3233/JAD-190652 IOS Press

> Proteomic and Unbiased Post-Translational Modification Profiling of Amyloid Plaques and Surrounding Tissue in a Transgenic Mouse Model of Alzheimer's Disease

Joakim Bastrup^{x,b}, Kenneth Kastaniegaard^c, Ayodeji A. Asuni^b, Christiane Volbracht^{b,1} and Allan Stensballe^{b,c}

^aDepartment of Health Science and Technology, Aulborg University, Aulborg, Deumark

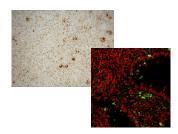
by the Company of the Co



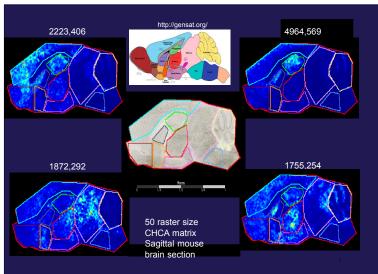
DENMARK

Avanced Imaging by combined MALDI IMS and Expansion /CLARITY microscropy



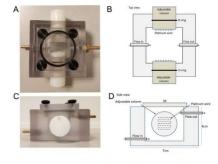




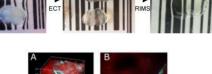






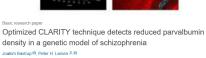










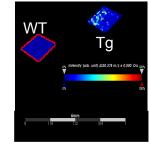












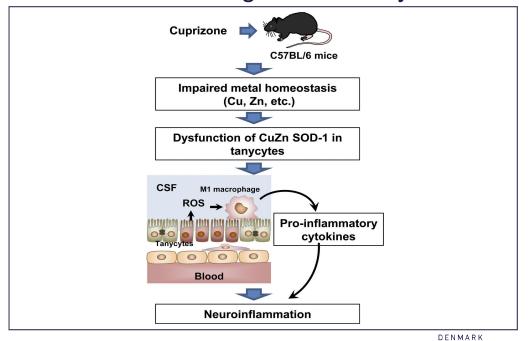
Optimized novel sample preparation enhances S/N >100x

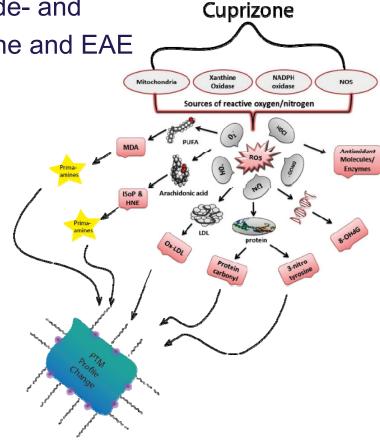
Microproteomics of MS models for investigation of disease related PTMs



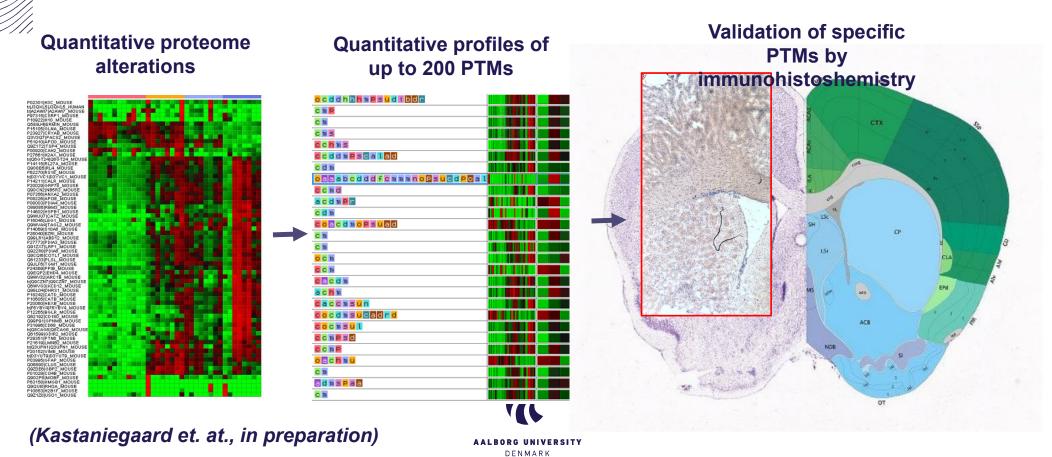
▶ In order to further understand the pathology of de- and remyelination we have investigated the Cuprizone and EAE

mouse models for regional PTM dynamics.





Microproteomics of MS models for investigation of disease related PTMs



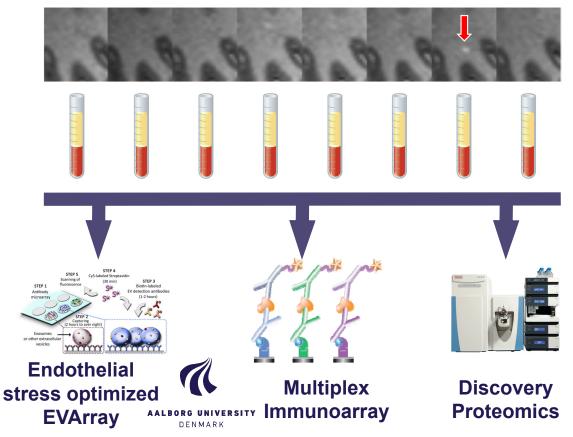
Temporal Changes Of Inflammation In Multiple Sclerosis Related To The Evolution Of Enhancing Lesions On The MRI

MULTIPLE
SCIEROSIS
JOURNAL
Original Research Paper

Multiple sclerosis lesion formation and early evolution revisited: A weekly high-resolution magnetic resonance imaging study

Charles RG Guttmann', Matthieu Rousset', Jean A Roch, Salem Hannoun, Françoise Durand-Dubief, Boubakeur Belaroussi, Michele Cavallari, Muriel Rabilloud, Dominique Sappey-Marinier, Sandra Yukusic and François Cotton

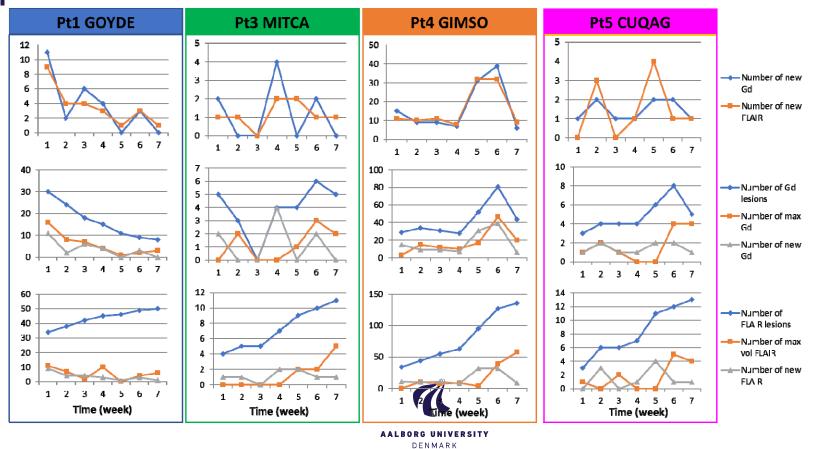
- Multiple Sclerosis
 patients were monitored
 for MS lesion activity,
 onset frequency, and
 early disease evolution.
- We investigated the inflammatory profile for correlation of disease status and biomarkers



Weekly MRI scanning (8 weeks)

Patient plasma

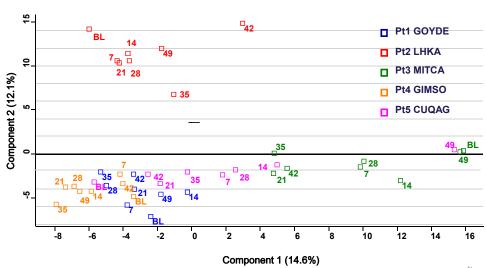
Temporal Changes Of Inflammation In Multiple Sclerosis Related To The Evolution Of Enhancing Lesions On The MRI



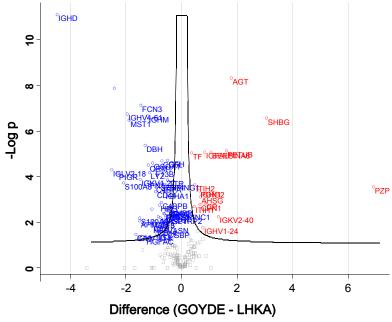
T S

Temporal Changes Of Inflammation In Multiple Sclerosis Related To The Evolution Of Enhancing Lesions On The MRI

Quantitative proteomics reveals individualized plasma profiles and FLAIR intensity related markers

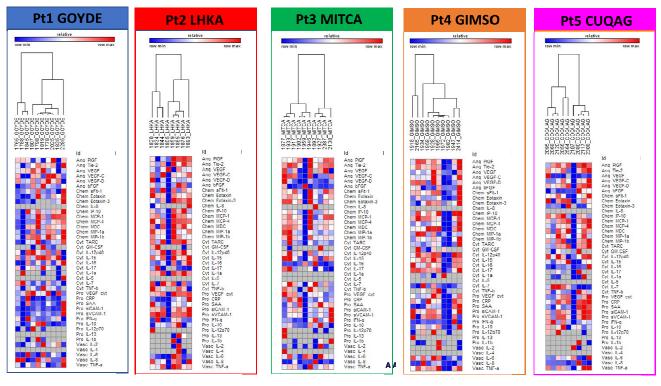






Temporal Changes Of Inflammation In Multiple Sclerosis Related To The Evolution Of Enhancing Lesions On The MRI

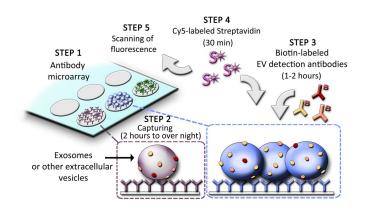
Quantitative assessment of cytokine and chemokine revealed patient to patient variation not correlating to FLAIR profiles



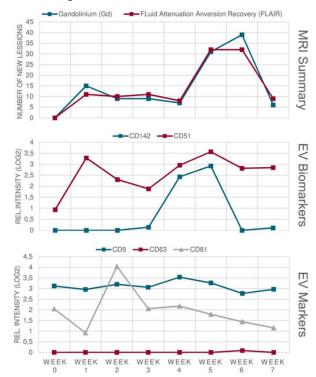
Temporal Changes Of Inflammation In Multiple Sclerosis Related To The Evolution Of Enhancing Lesions On The MRI

Quantitative assessment of EV marker profiles revealed patient to patient variation and multiple markers correlating to FLAIR profiles

EV	Immune	Endothelial	Platelet	Other
Annexin V	CD3	CD31	CD42a	LAMP2
CD9	CD8a	CD51	CD62 E	TNF RII
CD63	CD19	CD146	CD62 E/P	Tspan8
CD81	CD28	tPA	CD142	AKAP3
CD82	CD80	Thrombospondin-1		CD106
TNF RI	MIC A/B	VE-Cadherin		CD151
TSG101	CTLA4			
Alix	ICAM-1			
	HI A DR/DP/DO			







Translational Biomarker Research Unit, Aalborg University, Denmark



- Svend Birkelund
- Tue Bjerg Bennike
- Thomas B. G. Poulsen
- Azra Karamehmedovic
- Joakim Bastrup
- Ditte Kristensen
- Kenneth Kastanjegaard
- Michael Kruse Meyer
- Christopher Aboo

Sangild Lab

- Tik Muk
- Ping ping Jiang
- Ninh Duc Nguyen



- Malene Møller Jørgensen
- Rikke Bæk
- Rikke Gry Nielsen (vet)
- Jonathan Blackburn Sengenics





Danish National Hospital

- Claus H. Nielsen
- Dres Damgaard

Funding sources



















