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Therapeutic Monoclonal Antibodies: Aggregation origins & characterization - In-vitro evaluation of immunogenicity

Stéphane CORNEN
Head of Clinical Trial Manufacturing
R&D / BioPharmaceutics Development

Proteinov2016 – November 28 & 29th, 2016

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Outlook

- Sanofi R&D : BioPharmaceutics Development
- Biopharmaceutics manufacturing & aggregation
- Analytical panel to monitor aggregation
- Impact of process changes – Case studies
- ABIRISK project for “Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK”

Integrated Biotherapeutic Platform

Main Expertise:

- Oncology
- Join Research Unit(Curie)
- Biologics Research
- Preclinical Safety
- Metabolism and Pharmacokinetics
- Pharmaceutical Development
- Biopharmaceutical Development
- Translational *in vivo* models
- Translational Sciences
- Medicinal Chemistry
- Translational Medicine
- GLP / GMP Skills
- Culture Cell / Highly Active Molecules

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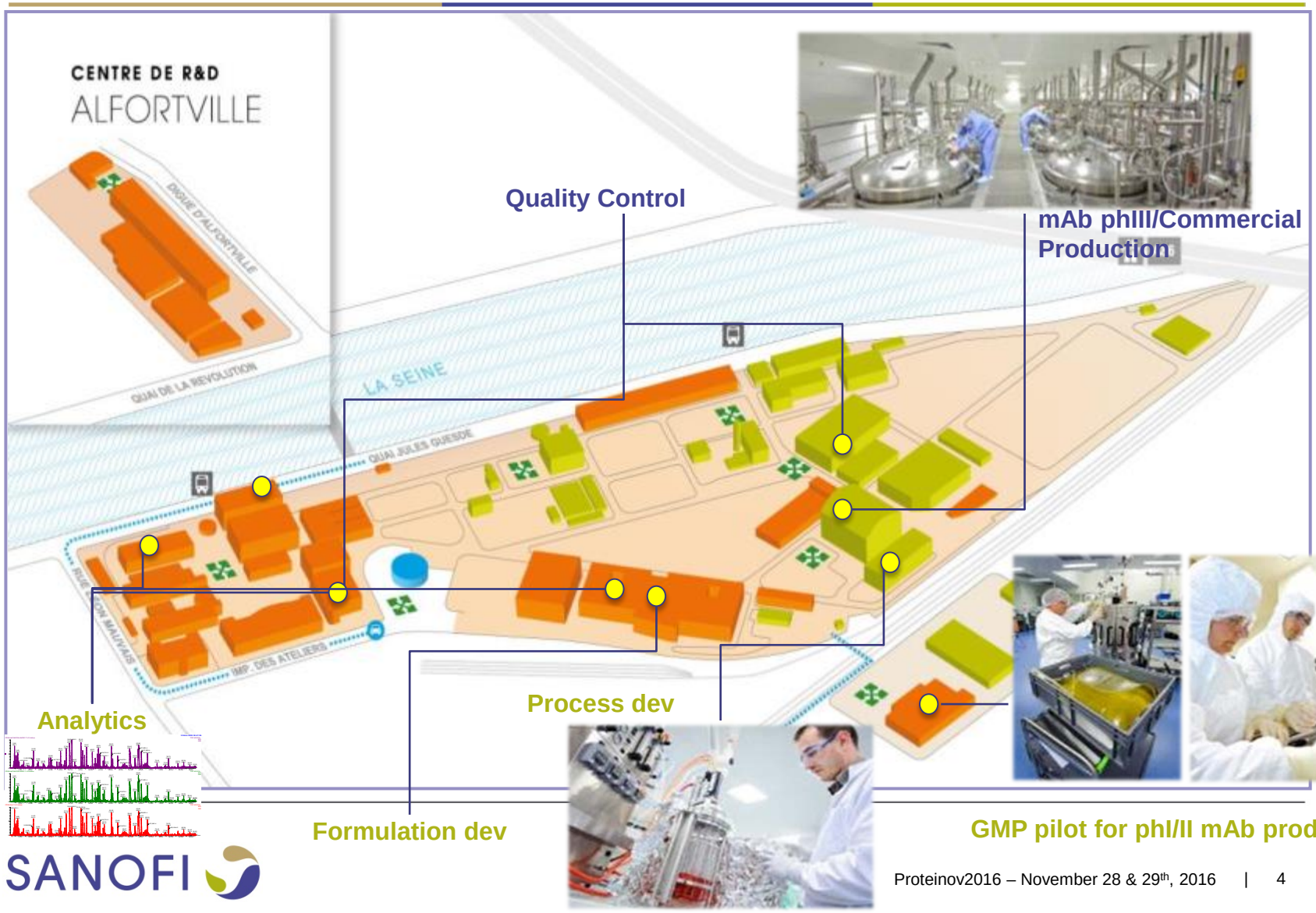
Mai 2016

Vitry BioPharmaceutics Development: An integrated platform dedicated to development and production of mAbs



Biopharmaceutical manufacturing
From cells to patients

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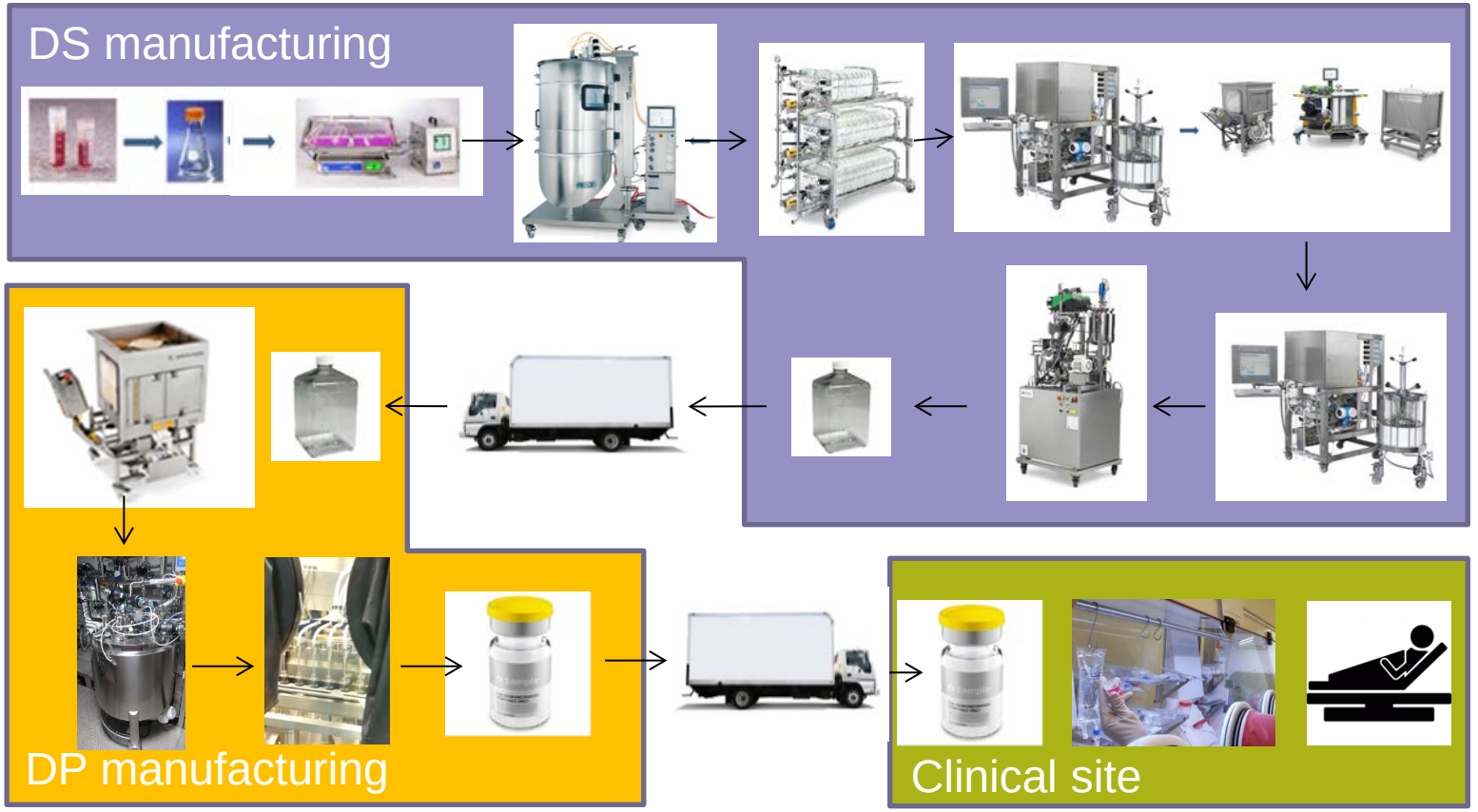

And more complex when Lyophilized form, Devices, Conjugates…

Biopharmaceutical manufacturing
Aggregation – influencing factors

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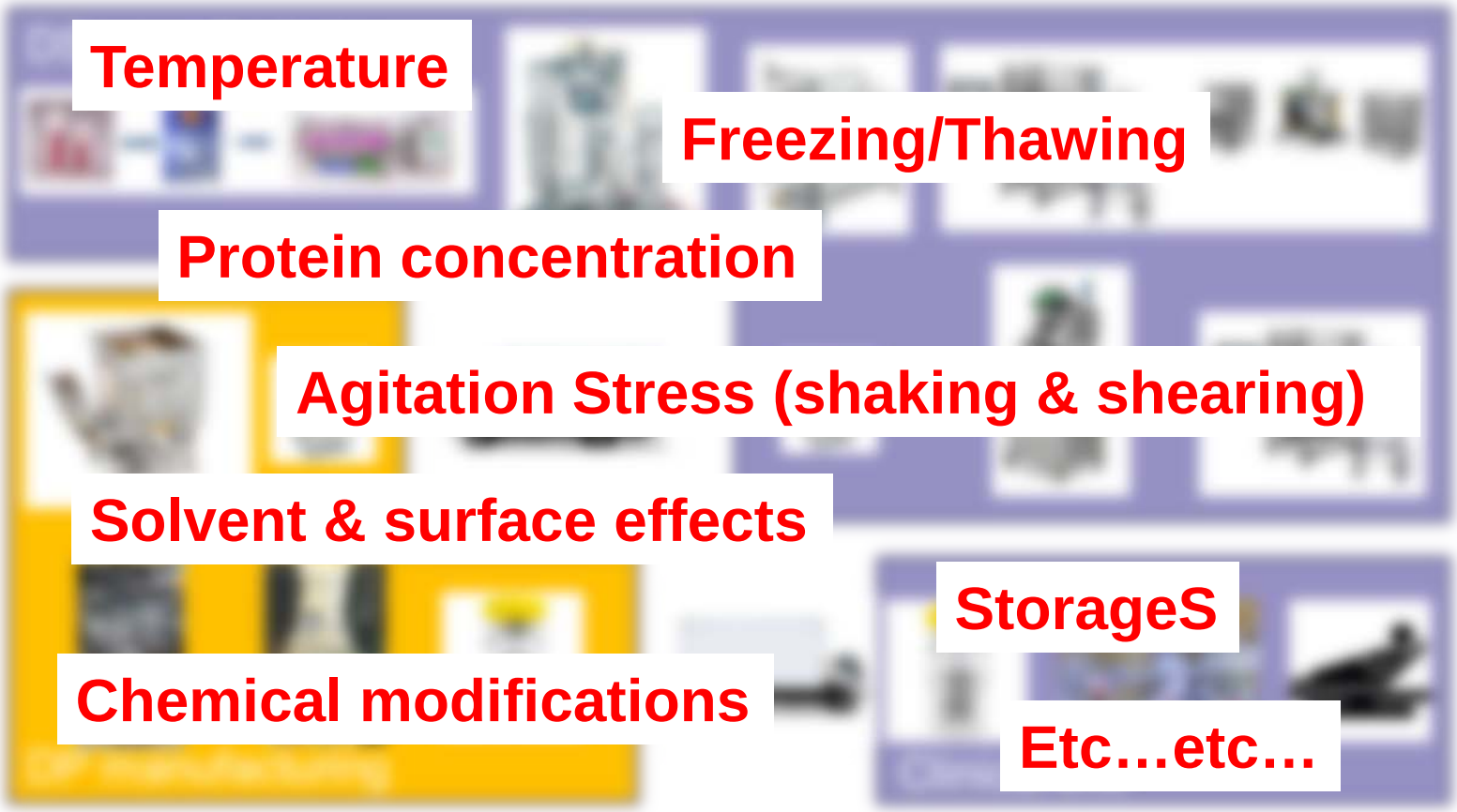

Biopharmaceutical manufacturing
Aggregation – influencing factors

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Review

Protein aggregation—Pathways and influencing factors

Wei Wang*, Sandeep Nema, Dirk Teagarden

Pfizer Inc., Global Biologics, 700 Chesterfield Parkway West, Chesterfield, MO 63017, United States

Int J Pharm, 390 (2) (2010), pp. 89–99

Protein Aggregation: Pathways, Induction Factors and Analysis

HANNS-CHRISTIAN MAHLER,[1] WOLFGANG FRIESS,[2] ULLA GRAUSCHOPF,[1] SYLVIA KIESE[1]

[1]Formulation R&D Biologics, Pharmaceutical and Analytical R&D, F. Hoffmann-La Roche Ltd., Basel, Switzerland

[2]Department of Pharmacy, Pharmaceutical Technology and Biopharmaceutics, Ludwig-Maximilians-Universitaet Muenchen, Munich, Germany

J Pharm Sci, 98 (9) (2009), pp. 2909–2934

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Aggregates & Immunogenicity

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Tungsten-Induced Denaturation and Aggregation of Epoetin Alfa During Primary Packaging as a Cause of Immunogenicity

Pharm Res (2012) 29:1454–1467

Andreas Seidl • Otmar Hainzl • Marleen Richter • Robert Fischer • Stephan Böhm • Britta Deutel • Martin Hartinger • Jörg Windisch • Nicole Casadevall • Gerard Michel London • Iain Macdougall

Table I Overview of Most Relevant Risk Factors for Immunogenicity Investigated and Evaluation of Results

Potential risk factor	Suspect batches had higher levels than?			Literature available to suggest a role in immunogenicity
	Other batches of study drug	Other batches not used as study drug	Reference product (Eprex®)	
Drug substance				
Aggregation[a,b]	No	No	N/A	Yes (10–12)
Subvisible particles[a]	No	No	N/A	Yes (13, 14)
Oxidation Met-54[c]	No	No	N/A	No
Deamidation/aspartate isomerization[d]	No	No	N/A	Yes (17)
Unfolded variants[a]	No	No	N/A	Yes (18)
Host-cell proteins	No	No	N/A	Yes (19)
Drug product				
Aggregation[a,b]	Yes	Yes	No	Yes (10–12)
Unfolded variants[a]/irreversible dimers	Yes	Yes	Yes	Yes (18)
Subvisible particles[a]	No	No	No	Yes (13, 14)
Inorganic leachates[d]	Yes (tungsten)	Yes (tungsten)	Yes (tungsten)	No[d]
Organic leachates[b]	No	No	No	Yes (8)
Oxidation Met-54[c]	No	No	No	No
Oxidation Trp-64[c]	No	No	No	No
Deamidation/aspartate isomerization[d]	No	No	No	Yes (17)
Degradation[e]	No	No	No	Yes (23, 24)
Silicone oil[f]	No	No	No	Unclear (5, 25–27)
Polysorbate 80 micelles[g]	No	No	No	No (5, 8, 28)

[a] Generally known to be a risk factor for immunogenicity of proteins

[b] Specific reports/experience available in the context of immunogenicity of epoetins

a wide range of orthogonal analytical methods, including high-performance size exclusion chromatography (HP-SEC), analytical ultracentrifugation (AUC), asymmetric-flow field-flow fractionation (AF4), micro-flow imaging (MFI) and light obscuration (LO). The selected methods make use of different separation and detection principles to cover the complete size range of soluble and insoluble aggregates as well as particles (28).

Aggregates : FDA expectations

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U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

Regulatory Expectations for Analysis of Aggregates and Particles

Susan L. Kirshner, Ph.D
Office of Biotechnology Products
Division of Therapeutic Proteins
7/12/2012

U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

Regulatory Expectations
Sub-visible particles between 2(ish) – 10 micron

- FDA is currently requesting that SVP between 2 – 10 microns be studied using a quantitative method
  - For licensed products and late stage 3/pre-BLA products this is usually a PMC
  - For products currently in early phases of development (1/2 and perhaps early phase 3) IND these studies should be incorporated into the IND
- FDA does not have a preferred method

U.S. Food and Drug Administration
Protecting and Promoting Public Health
www.fda.gov

Summary

- Specifications should be established for SVP below 0.2 micron and above 10 and 25 micron for parenteral and inhaled products
- SVP between 2(ish) and 10 micron should be evaluated using quantitative methods and an appropriate control strategy developed
- SVP between 0.2(ish) and 10 micron should be characterized and an appropriate control strategy developed

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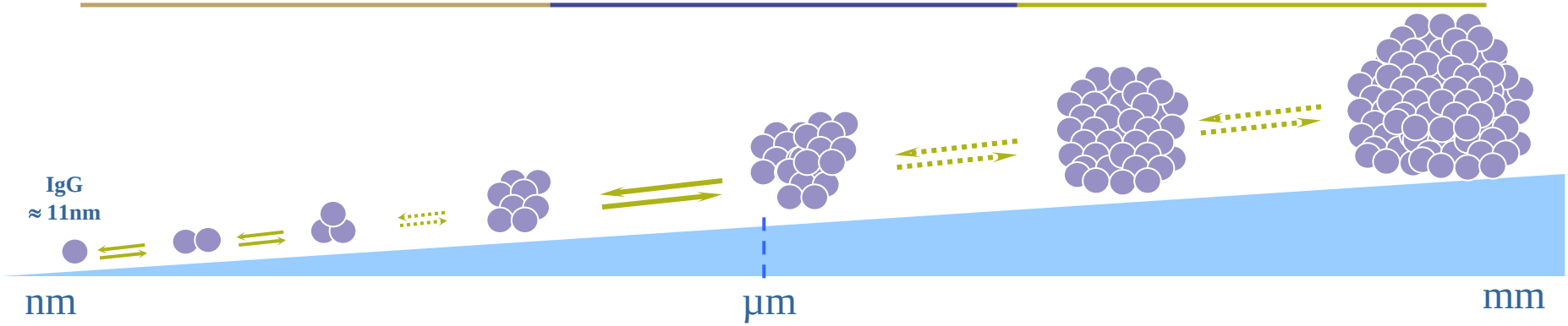
Aggregates/Particles characterization Toolbox



SEC — Release methods — Light Obscuration

A4F

svAUC

Dynamic Light Scattering

Flow-Imaging

RMM (Archimedes)

RMM: Resonant Mass Measurement
Patel A.R. & Al. Anal. Chem., **2012**, *84* (15), pp 6833–6840

Case study I
Soluble aggregates by SEC-UHPLC

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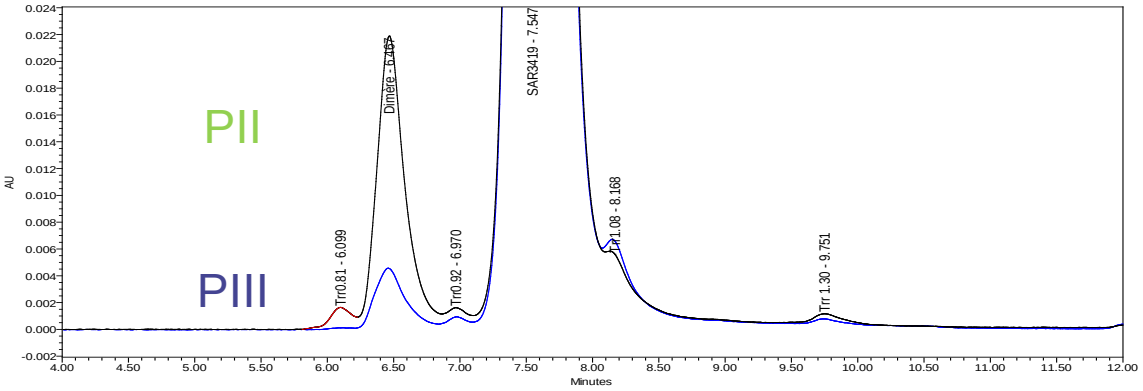
- Purity by SEC (T0)
  - **Similar SEC profile with dimer as main degradation product**



  - **Better purity for PIII material**
- Aggregation profile confirmed by orthogonal methods

SEC-MALLS			A4F-MALLS			sv-AUC		
Species	**PII**	**PIII**	**Species (MW)**	**PII**	**PIII**	**Species (S and MW)**	**PII**	**PIII**
Monomer	98.0%	99.5%	**~150kDa (monomer)**	97.9 (+/-0.2) %	98.9 (+/-0.1) %	**6S and ~150kDa (monomer)**	99.0 (+/-0.1) %	100 (+/-0.0) %
Dimer	1.7%	0.4%	**~300 kDa (Ag1=dimer)**	1.8 (+/-0.1) %	1.2 (+/-0.1) %	**9S and ~300kDa (Ag1=dimer)**	1.0 (+/-0.1) %	ND
HMW	0.2%	ND	**HMW**	0.3 (+/-0.1) %	ND	**HMW**	ND	ND

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Case study I
Soluble aggregates by SEC-UHPLC

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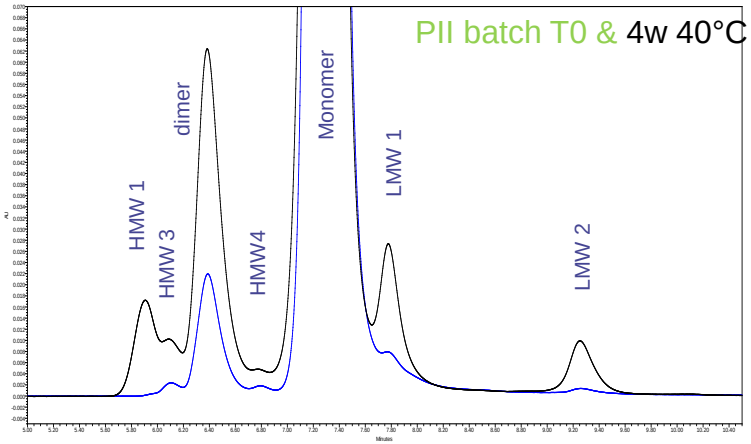
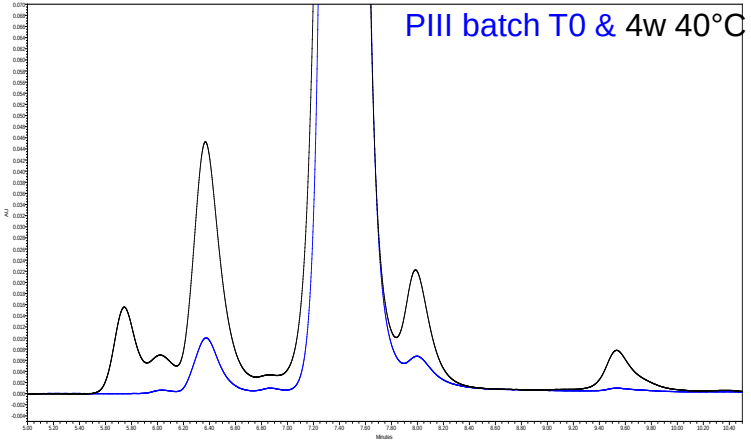
- Behavior under thermal stress





- Aggregation profile confirmed by orthogonal methods

A4F-MALS			SV-AUC		
Species (MW)	**PII**	**PIII**	**Species (S and MW)**	**PII**	**PIII**
~150kDa (monomer)	92.2 (+/-0.2)%	92.9 (+/-0.3)%	6S and ~150kDa (monomer)	94.3 (+/-1.1)%	94.9 (+/-0.3)%
~300 kDa (Ag1=dimer)	4.6 (+/-0.2)%	3.8 (+/-0.1)%	9S and ~300kDa (Ag1=dimer)	3.6 (+/-0.7)%	2.8 (+/-0.2)%
HMW	2.6 (+/-0.3)%	2.9 (+/-0.2)%	HMW	1.7 (+/-0.6)%	2.2 (+/-0.2)%
LMW	0.6 (+/-0.1)%	0.5 (+/-0.0)%	LMW	0.4 (+/-0.2)%	0.2 (+/-0.1)%

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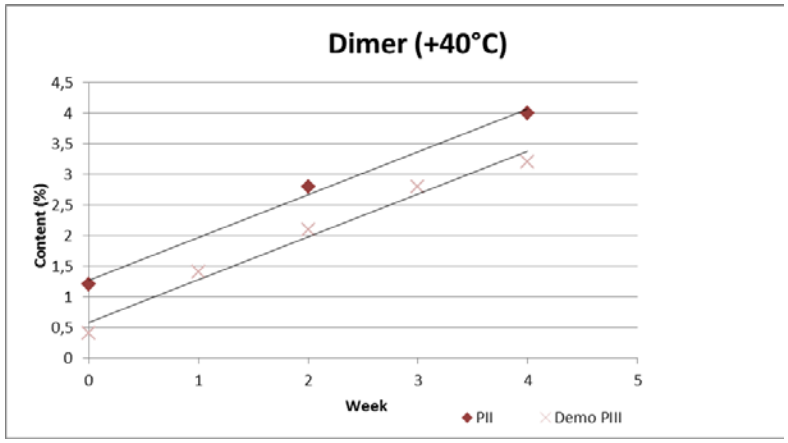
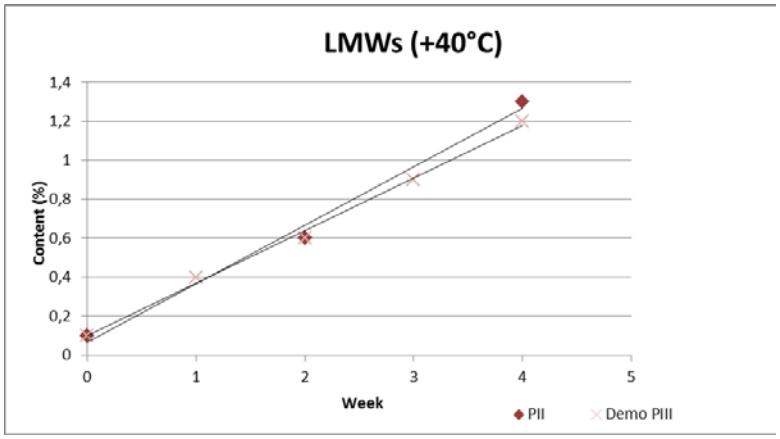
Case Study I
Soluble aggregates – stress study



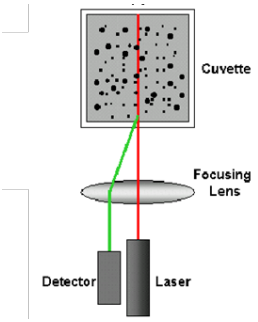


- Soluble aggregates
  - **Dimer increase**
    - Slopes ratio (PIII/PII) = **100%**
  - **Other HMWs**
    - Slopes ratio (PIII/PII) = **96%**
- Fragments
  - **Slope ratio(PIII/PII) = 90%**

Compliant with acceptance criteria proposed from HA (PI/PII outcome)
80% and 125%

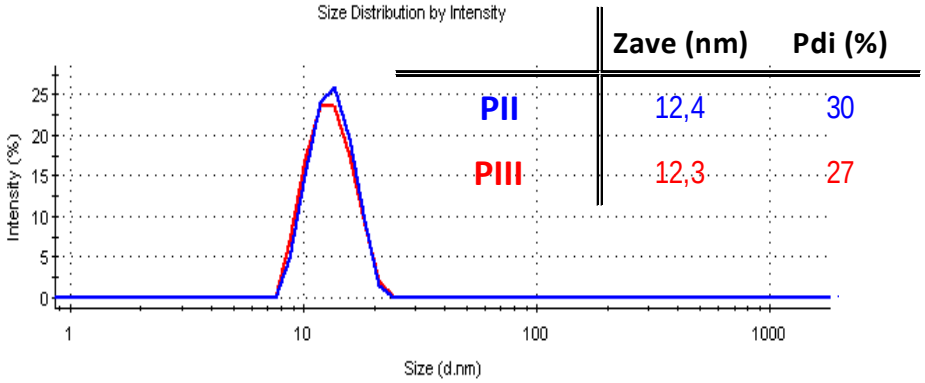
Case Study I
Soluble aggregates – stress study

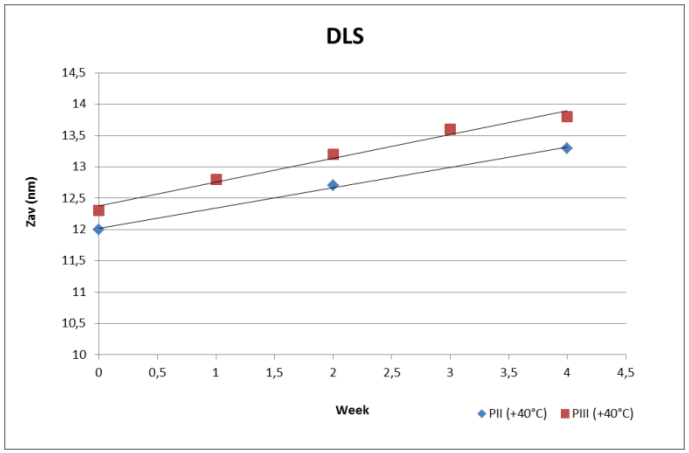
- Aggregation by DLS (sub-micron aggregation)



$$D_H = \frac{kT}{f} = \frac{kT}{3\pi\eta D}$$

D_H : Hydrodynamic diameter.
k : Boltzmann constant.
f : Particle frictional coefficient.
η : Solvent viscosity.
T : Absolute temperature.
D : Diffusion coefficient.



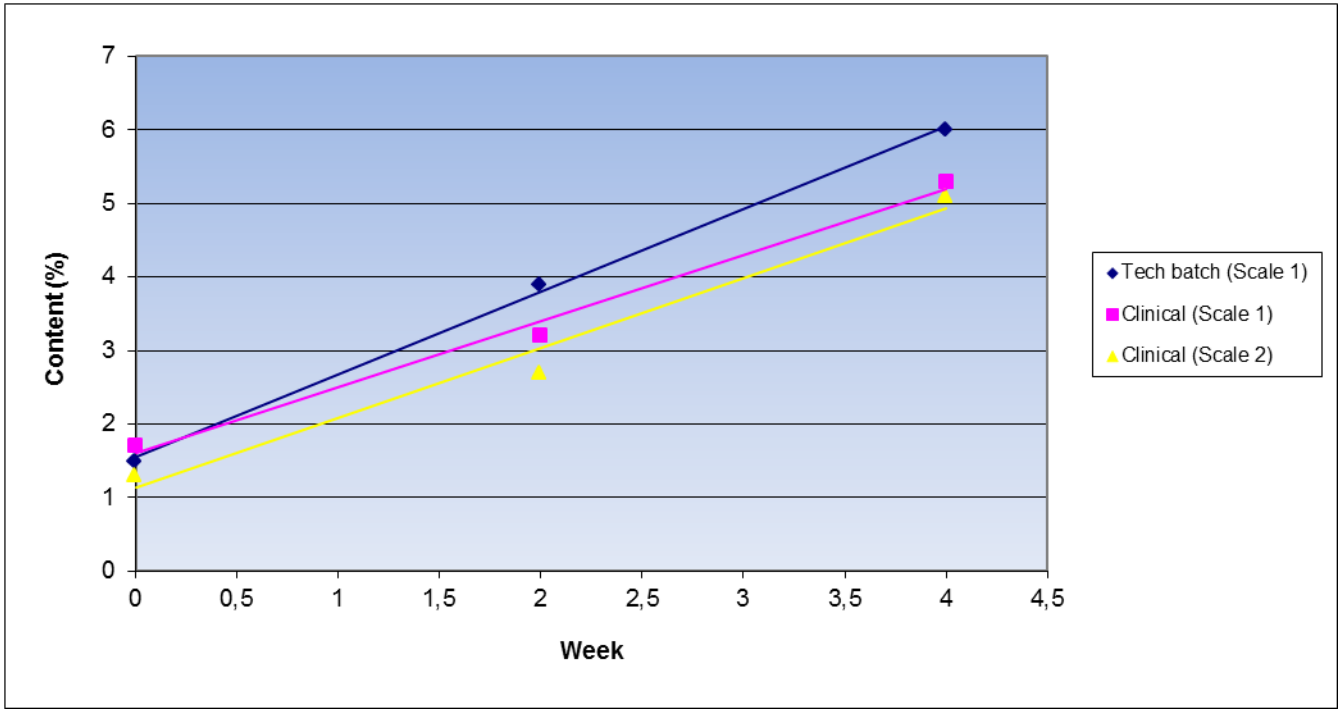


Similar Zav increase after 4W/40°C

No significant change of SVPs by Flow-Imaging

Case Study II
Soluble aggregates – stress study

- HMWs increase (SEC - 1month +40°C)



Highly similar degradation between scales: comparable

Case Study III
Submicron aggregation (DLS)

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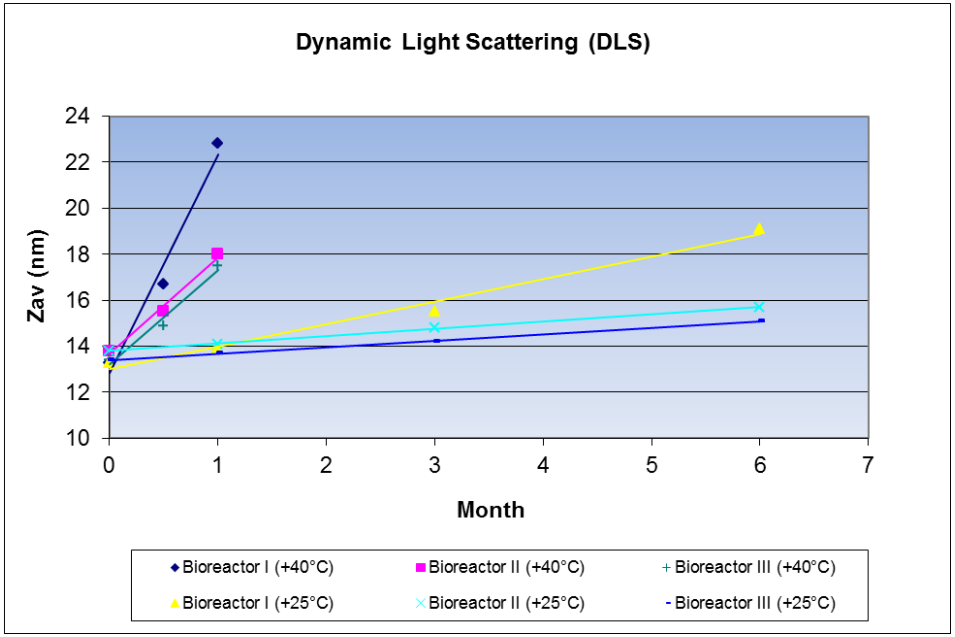
- Main degradation pathway: soluble aggregates by DLS
- Profile comparison under **stress (1m/40°C) & accelerated conditions (6m/25°C)**
  - **3 different bioreactors**



  - **Profile highly similar between 2 bioreactors**
  - **Slightly higher increase for the third one at both temperatures**

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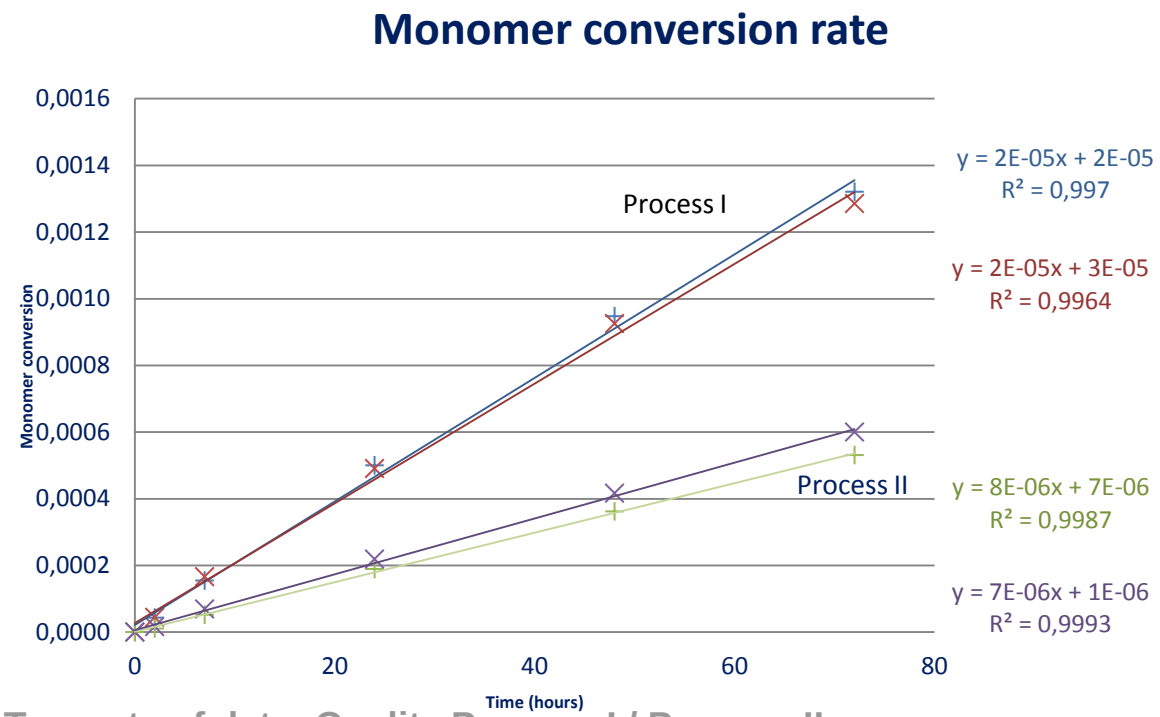
Case Study IV
Process improvement to decrease HMWs

- HMW at t=0 and Aggregation Kinetic FDS Process I vs. Process II
  - Monomer conversion plotted versus time,
  - 72h stress condition at +25°C



  - Two sets of data: Quality Process I / Process II
  - Aggregation rate lower (around 3 times) for Process II FDS batches

ABIRISK project

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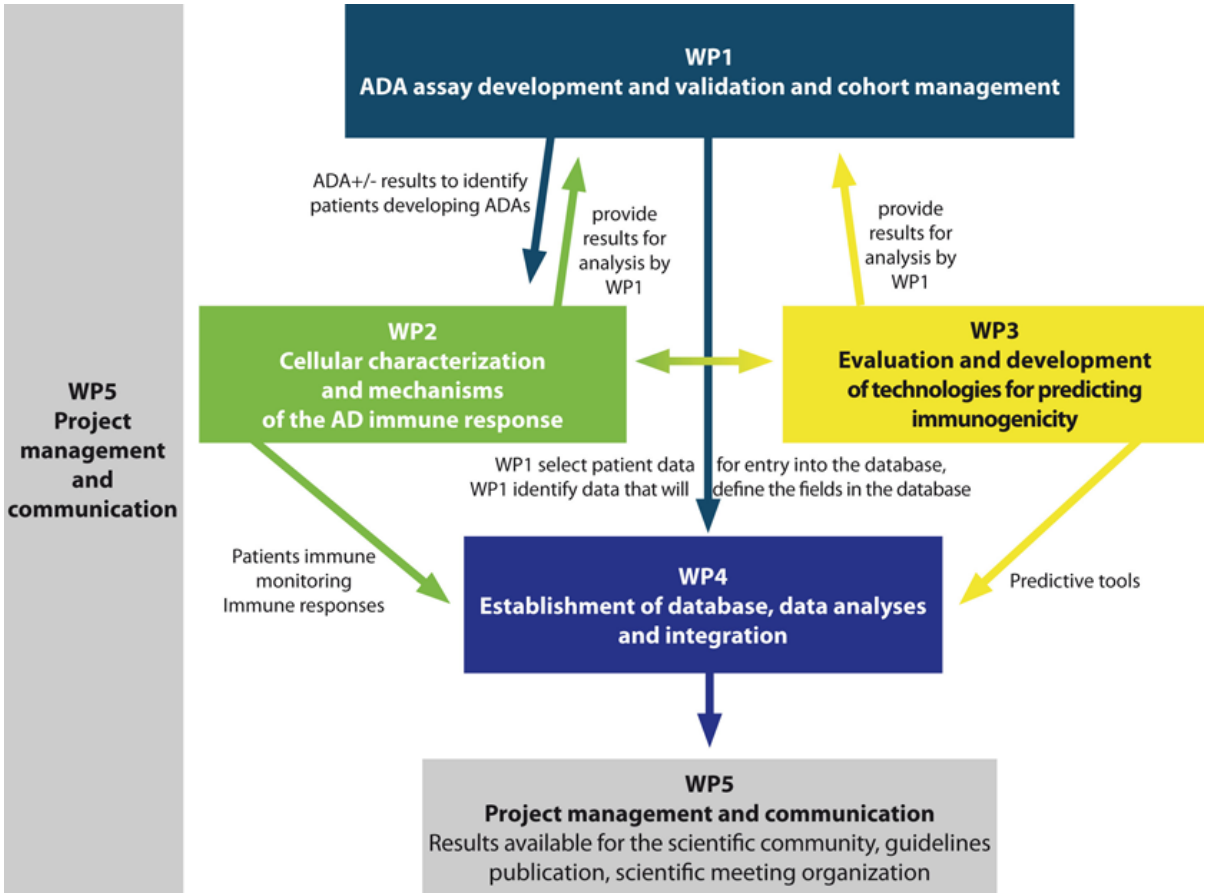
ABIRISK

Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK

imi efpia

- The ABIRISK project for “Anti-Biopharmaceutical Immunization: prediction and analysis of clinical relevance to minimize the RISK”
  - **Consortium made up of thirty-eight partners including academic institutions, EFPIA member companies and small and medium enterprises (SMEs).**
  - **Thirteen countries.**
  - **Goal: to analyze the mechanisms and consequences of immunization against biopharmaceutical products.**
  - **Organized in 5 Working Packages (WPs).**

ABIRISK project



Co-leaders

B. Maillere, CEA
C. P. Ross, Novo Nordisk
S. Spindeldreher, Novartis Pharma

Assess effects of aggregation on immunogenicity

http://www.abirisk.eu/project_organization.html

ABIRISK project
Four marketed products to be tested

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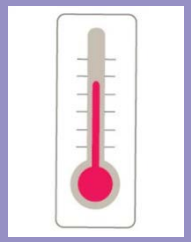
Stress conditions:

Temperature
6 & 24h @+55°C



Syringe/shear
stress: 3 &10 cycles

In-vitro methods:

- MAPPs (MHC-associated peptides proteomics)
- Dendritic Cell activation assays
- T cell assay

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ABIRISK project
Different behaviors

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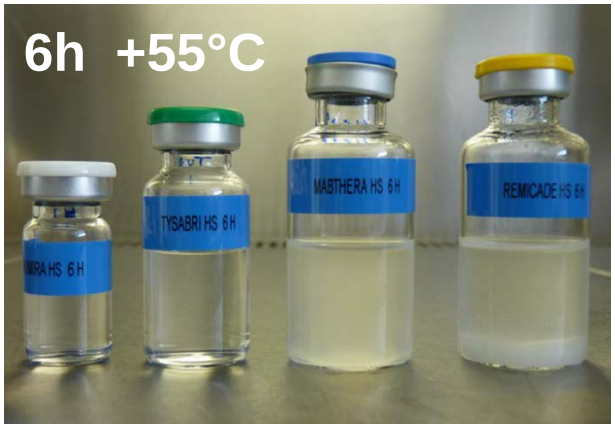
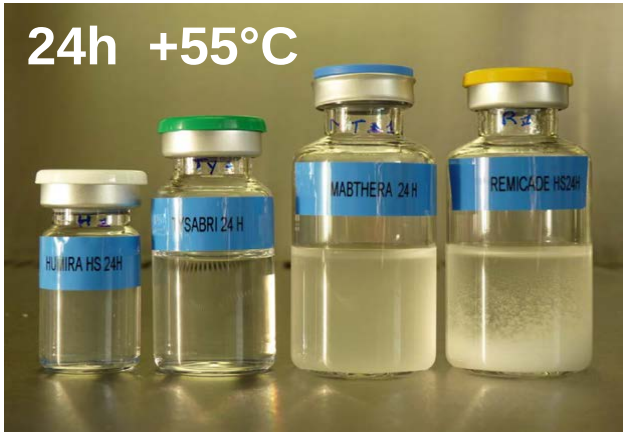
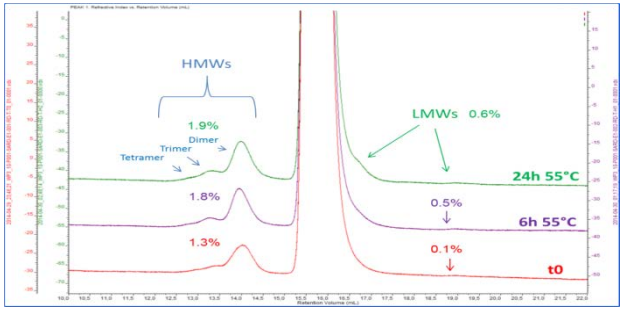
- Heat stress: significant differences



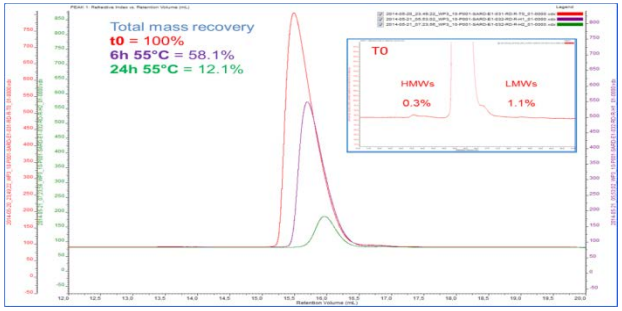


- Smooth to strong soluble aggregation observed by Size Exclusion Chromatography (SEC)

Tysabri



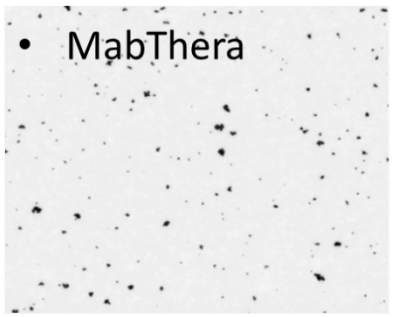
Remicade

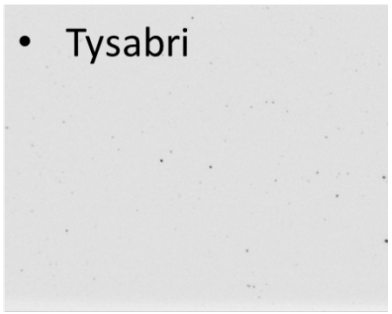


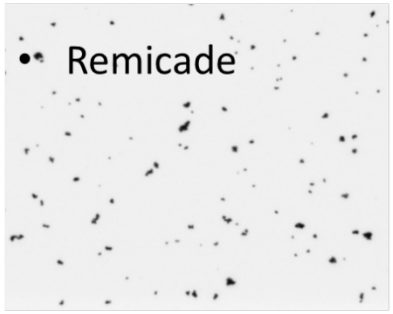
ABIRISK project
Different behaviors

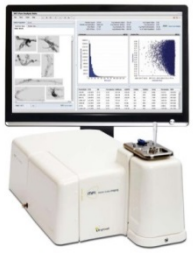
- Heat stress: significant differences observed for SVPs

Strong aggregation after 24h 55°C

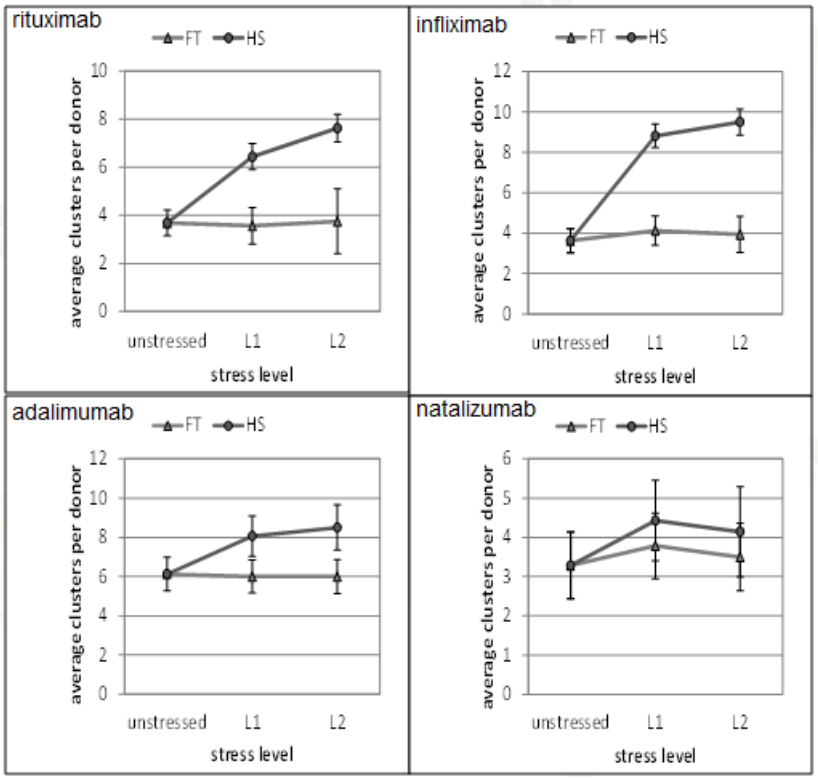








Immunogenicity evaluation
MAPPS Assay



- MAPPS assays used to identify drug-derived HLA class II peptides
- Natalizumab/Tysabri:
  - **MAPPS**: no significant change observed
  - **Stress:** minor increase in SVPs
  - **Clinics**: low immunogeneicity in the clinics
- Adalimumab/Humira:
  - **MAPPS:** Low increase (with highest baseline)
  - **Stress**: minor increase in SVPs
  - **Clinics:** low/moderate immunogeneicity in the clinics
- Rituximab (Mabthera)/Infliximab (Remicade):
  - **MAPPS:** Significant changes
  - **Stress:** high number of in SVPs
  - **Clinics:** Immunogenicity in clinics

MHC-associated Peptide Proteomics (MAPPs)

Immunogenicity evaluation
MAPPS Assay

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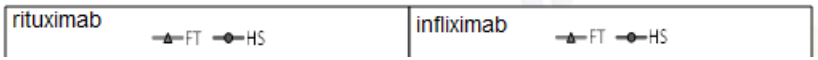
- MAPPS assays used to identify drug-derived HLA class II peptides



Regulatory Toxicology and Pharmacology 80 (2016) S1–S14

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Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

bserved

the clinics

lest

Workshop report

Non-clinical Safety Evaluation of Biotherapeutics – Challenges, Opportunities and new Insights

Guenter Blaich [a, *], Andreas Baumann [b], Sven Kronenberg [c], Lolke de Haan [d], Peter Ulrich [e], Wolfgang F. Richter [c], Jay Tibbitts [f], Simon Chivers [g], Edit Tarcsa [h], Robert Caldwell [i], Flavio Crameri [c]

Stress: high number of in SVPs

Clinics: Immunogenicity in clinics

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Summary

- **Aggregation** is one of the main degradation pathways of Biotherapeutics
- It's a complex phenomena → **multiple pathways**
- Many factors during manufacturing and lifetime impact **aggregates profile**
- Aggregates characterization required **several orthogonal analytical methods**
- **Immunogenicity prediction** is key for Biopharmaceutics development
- **Stress-induced increase** combined with in-vitro assays could be included in Immunogenicity prediction toolbox (work on-going within Abirisk)

Acknowledgment

- Sanofi R&D:
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  - Ludovic Laurent
  - Christophe Sourrouille
  - Catherine Prades
  - Thierry Ziegler
- Abirisk WP3:
  - Bernard Maillère
  - Sebastian Spindeldreher
- Novartis Pharma AG:
  - Anja Matter
  - Verena Rombach-Riegraf

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Merci

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