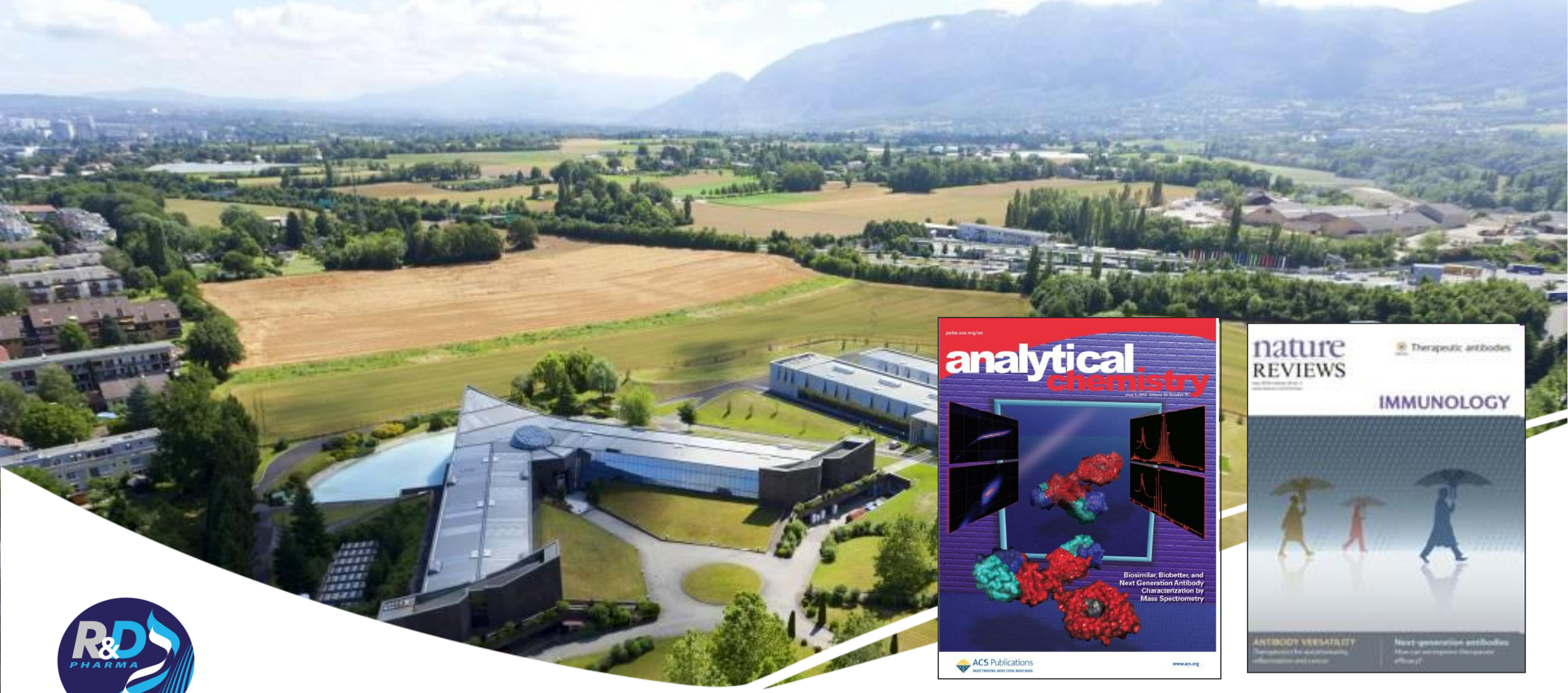
Structure, Heterogeneity and Developability Assessment of Therapeutic Antibodies

Alain BECK - CASSS MS virtual - Sep 14, 2020

Pierre Fabre

Structure, Heterogeneity and Developability Assessment of Therapeutic Antibodies

1. Cutting edge analytical methods & network
2. MS structure assessment or fingerprinting
3. CE-MS based methods
4. Multi-dimentional LC-MS methods
5. HILIC-MS
6. Take home messages



(1) Introduction:

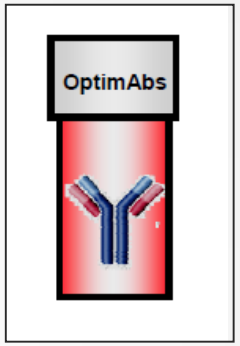
Cutting edge analytical methods & network

Pierre Fabre

OptimAbs/ADCs/bsAbs/ICs : CMC & developability

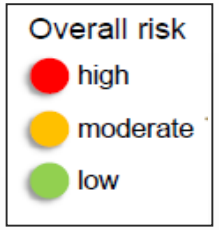
Structural assesment and sequence liabilities

- Hot spots (*in silico*): Critical Quality Attributes (CQAs, litterature)
- Chromatographic & electrophoretic profilling (size, charge, hydrophobic/philic)
- Mass spectrometry (isoforms, isotypes, macro/micro-variants, PTMs)
- Mutliple attribute methods (MAMs)
- Short stress studies (pH, heat, oxidation, glycation, light, freezing/thawing)
- Functional assays (Critical Quality Attributes ranking)
- Hits, Leads, Candidates structure optimization : iterative process



Safety/PK/PD

- Serum stability
- Half-life
- Administration schedule
- Immunogenicity
- De-conjugation
- Off-target activity



FDA APPROVED

EUROPEAN MEDICINES AGENCY

Manufacturability

- MCB, WCB
- Expression yields
- Purification yields
- Scalability
- Stability (process, long term)
- Cost of goods

➢ **Beck A, Liu H et al, mAbs 2019**

mAbs: analytical & structural methods (2013)

Analytical Chemistry — Review

MAb primary structure assessment

Charge variants
- Separation techniques
  - IEF, cIEF, icIEF
  - HPLC (IEX, RP, HIC)
  - Boronate affinity chrom.
- Mass spectrometry
  - Middle-up LC-MS
  - Peptide mapping (LC-MS/MS)
  - Top-down MS/MS

AA sequence and variants
- Intact mass (ESI-MS)
  - Glycan removal (PNGase F)
- Bottom-up peptide mapping
  - Enzyme digestion and LC-MS/MS
- Middle-up (LC-MS)
  - Red. mAb (light and heavy chains)
  - Limited proteolysis (IdeS, papain) + reduction (25 kDa fragments)
- Top/middle-down (HR-MS/MS)
  - ETD/ECD and CID
- SEC, CE-SDS

Glycovariants
- Glycan (released)
  - CE-LIF
  - HPLC (NP, HILIC, ZIC-HILIC, IEX, PGC)
  - MALDI-TOF, ESI-MS and MS/MS
  - Electronic impact-MS (with GC)
- Glyco-protein/ peptide
  - Intact/ middle-up LC-MS
  - Peptide mapping (LC-MS and MS/MS)

Cysteine-linked variants
- Ellman assay (free Cys)
- Differential peptide mapping
  - > red/ and non-red cond.
  - > CID and ETD (Cys linkage)
- IM-MS (Cys linkage)

Higher order structures, aggregates and mAb/Ag

Higher order
- XRD
- Native-MS
- IM-MS
- HDX-MS

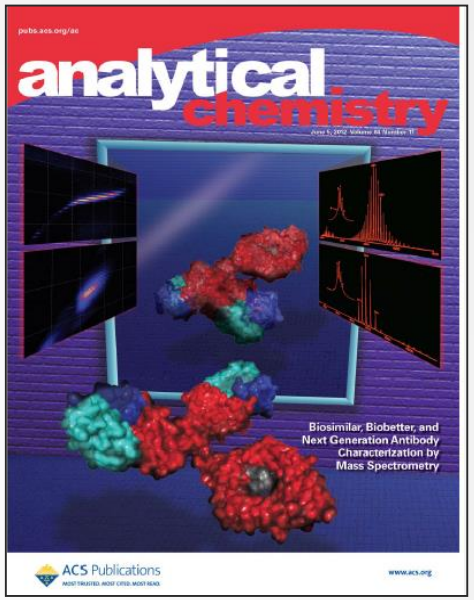
Aggregates
- SEC (UV-MALS)
- A4F (UV-MALS)
- AUC
- Native MS
- IM-MS
- HDX-MS
- Crosslinking MS

mAb/Ag complexes
- SPR
- ELISA
- FACS
- Native MS
- IM-MS
- HDX-MS
- Crosslinking MS

PK/ Quantification

- ELISA
- Radioimmuno-assay
- Immunofluorescence
- Isotope dilution – SRM
- Isotope dilution LC-MS

- **Main proteoforms**
- **HOS**

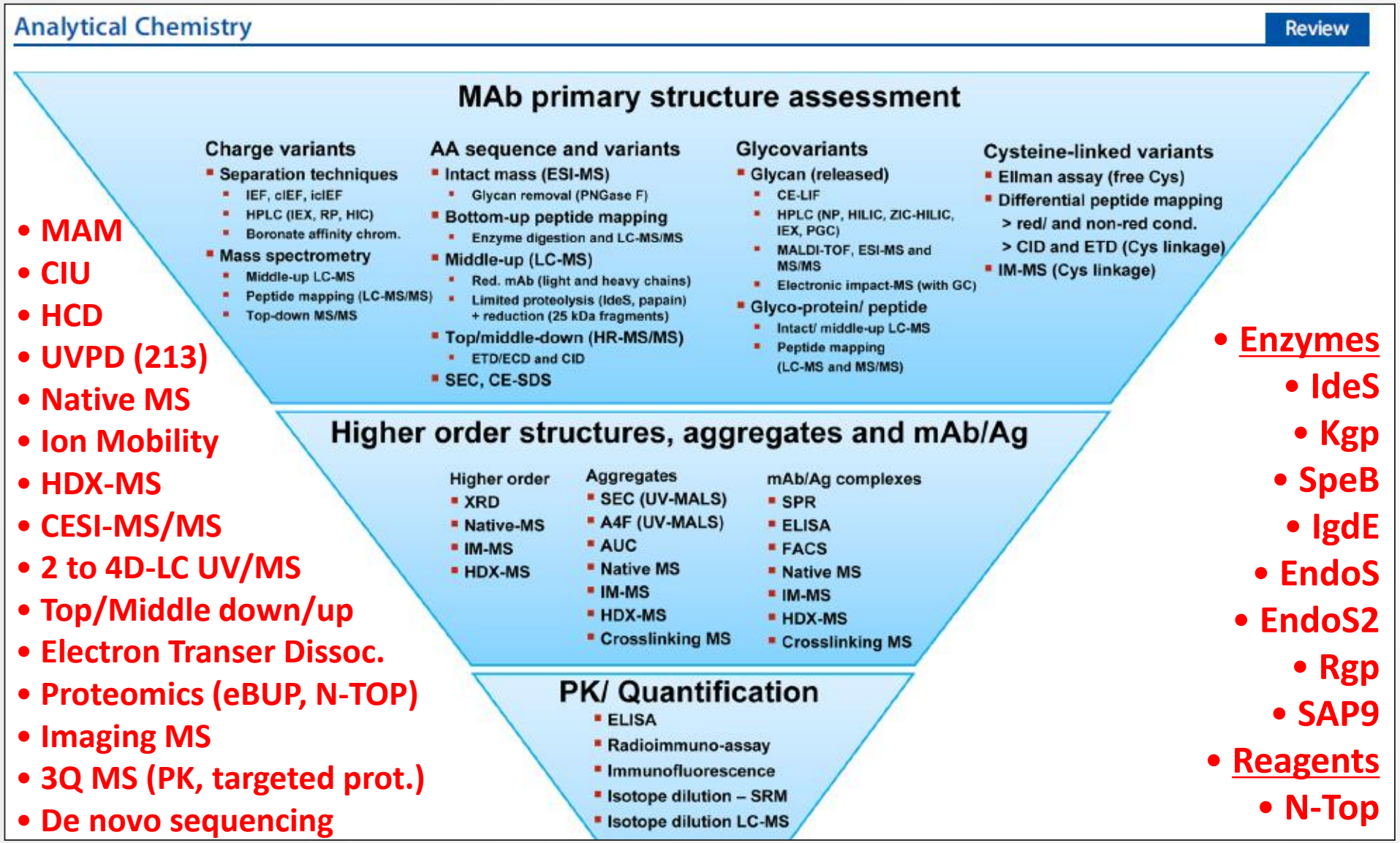


CQAs:
- **Glyco-variants**
- **Size variants**
- **Charge variants**
- **Cys-related variants**
- **Oxidized variants**

- S. Cianferani
- A. Van Dorsselaer and coll.

➢ **Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S. Anal Chem 2013**

mAbs: analytical & structural methods (2020)



- MAM
- CIU
- HCD
- UVPD (213)
- Native MS
- Ion Mobility
- HDX-MS
- CESI-MS/MS
- 2 to 4D-LC UV/MS
- Top/Middle down/up
- Electron Transer Dissoc.
- Proteomics (eBUP, N-TOP)
- Imaging MS
- 3Q MS (PK, targeted prot.)
- De novo sequencing

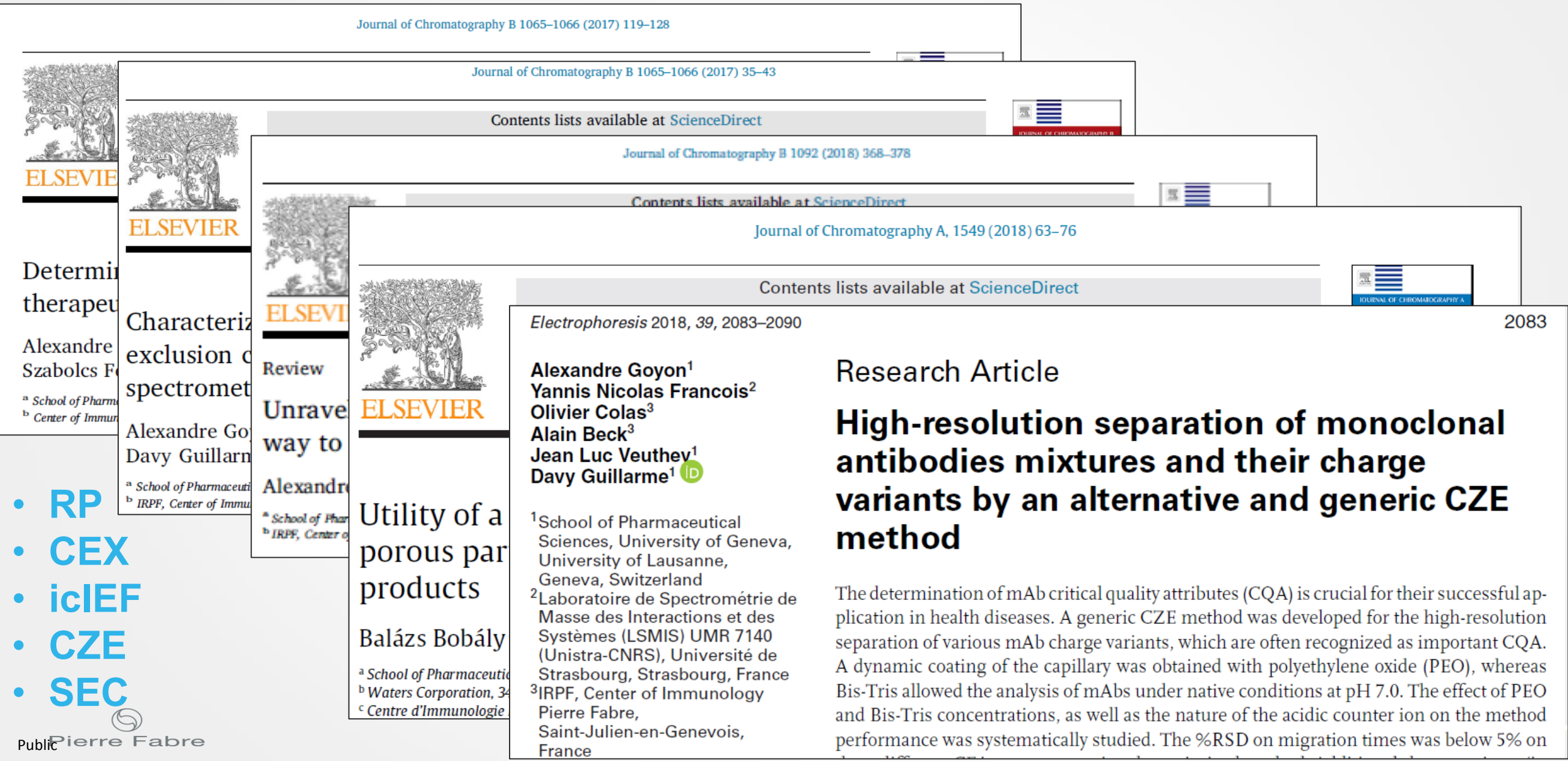
- Enzymes
  - IdeS
  - Kgp
  - SpeB
  - IgdE
  - EndoS
  - EndoS2
  - Rgp
  - SAP9
- Reagents
  - N-Top

- S. Cianferani
- D. Guillarme
- JL. Veuthey
- Y. François
- S. Heinisch
- Y. Tsybin
- D. Stoll
- A. Delobel
- J. Sjögren
- W. Chen
- D. Suckau
- H. Liu & coll.

GENOVIS

➢ Beck A, Wagner E, Ayoub D, Van Dorsselaer A, Cianferani S. Anal Chem 2013

FDA/EMA approved mAbs benchmarks: basic QC methods



- RP
- CEX
- icIEF
- CZE
- SEC

CE-SDS: 26 mAbs + 2 ADCs FDA/EMA appr (2020)

Journal of Pharmaceutical and Biomedical Analysis 184 (2020) 113166

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Determination of size variants by CE-SDS for approved therapeutic antibodies: Key implications of subclasses and light chain specificiti

Elsa Wagner[a], Olivier Colas[a], Stéphane Chenu[a], Alexandre Goyon[b], Amarande Murisie
Sarah Cianferani[c], Yannis François[d], Szabolcs Fekete[b], Davy Guillarme[b],
Valentina D'Atri[b,*], Alain Beck[a,*]

[a] Biologics CMC and Developability, IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France
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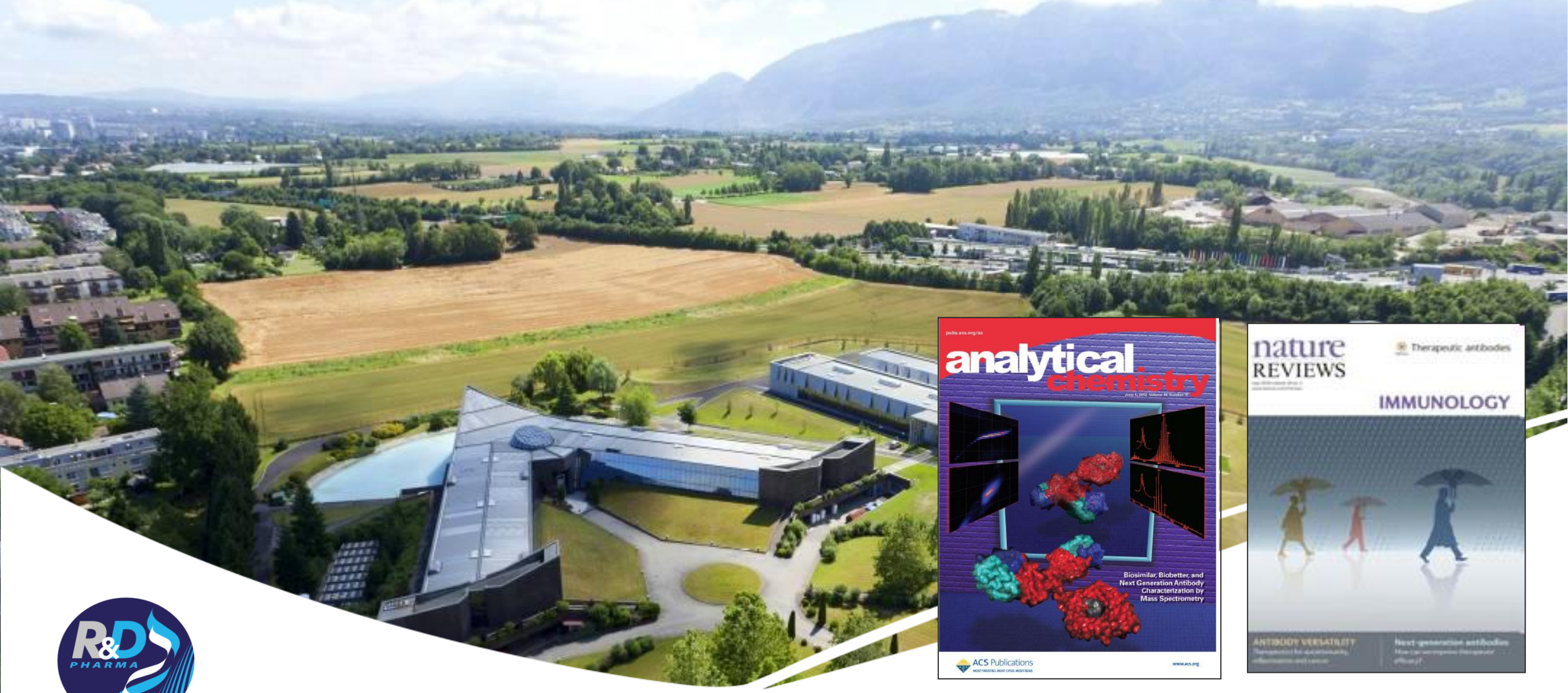
- Ch, Hz, Hu IgGs
- CHO, NS0, SP2/0
- IgG1, 2, 2/4, 4wt, 4stab
- Glyco-engineered
- A-glycosylated
- Kappa & lambda LC
- Partially reduced IgGs
- Biosimilars
- Hinge Cys & Lys ADCs
- NISTmab

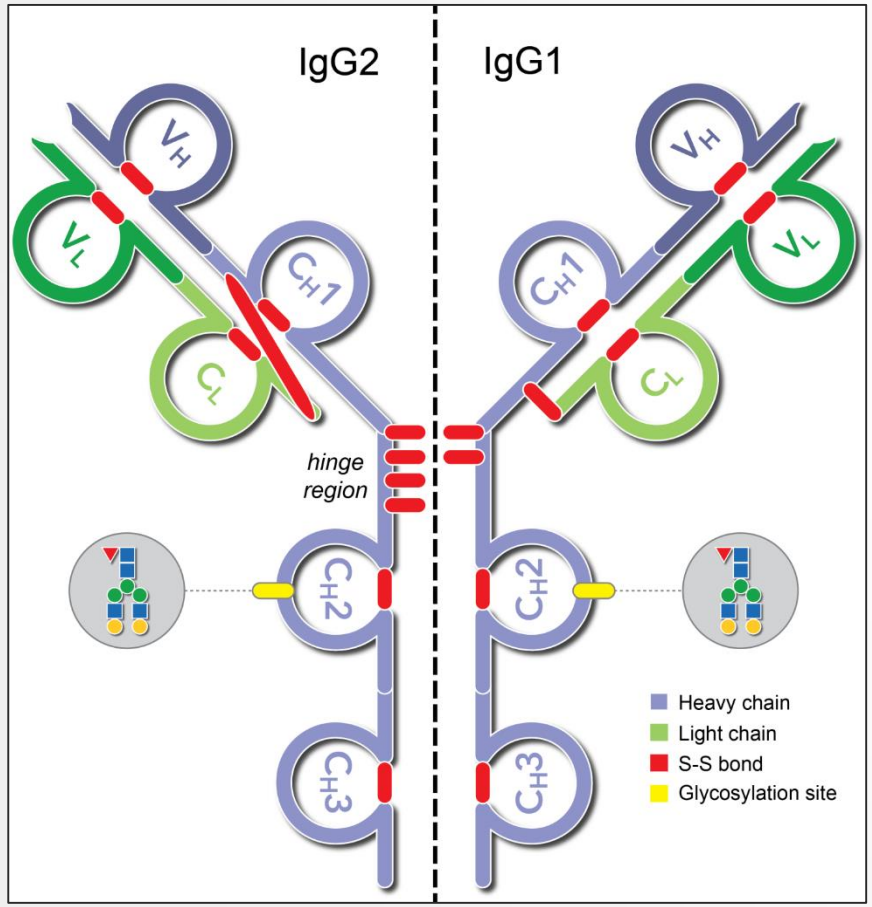


(2) Mass Spec structure assessment or fingerprinting:

TDS, HCD, ETD, UVPD, CIU

Pierre Fabre

IgG1, 2, 4 : Top & middle down MS analysis (Top Down Sequencing Consortium) (2014-20)



Dr. Y. Tsybin & coll.

- Fornelli L, Ayoub D, Aizikov, K, Beck A, Tsybin Y. Anal Chem 2014
- Srzentić K, Fornelli L, Beck A, Ayoub D, Tsybin Y. Anal Chem 2014
- Gasilova N, Beck A, Tsybin Y, Girault H et al. Anal Chem 2016
- Fornelli L, Ayoub D, Makarov A, Beck A & Tsybin YO. J Proteomics 2017
- Srzentić K, Fornelli L, Beck A, Tsybin Y et al, Anal Chem 2018
- van der Burgt Y, Tsybin Y, Beck A, Nicolardi S. et al, Anal Chem 2019
- Tsybin Y et al, JASMS 2020

Interlaboratory Study for Characterizing mAbs by Top-Down and Middle-Down Mass Spec (2020)

Journal of the American Society for Mass Spectrometry

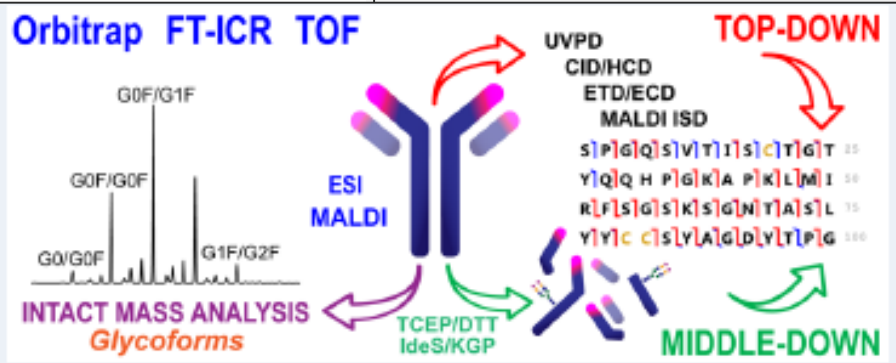
pubs.acs.org/jasms

Interlaboratory Study
Top-Down and Middle

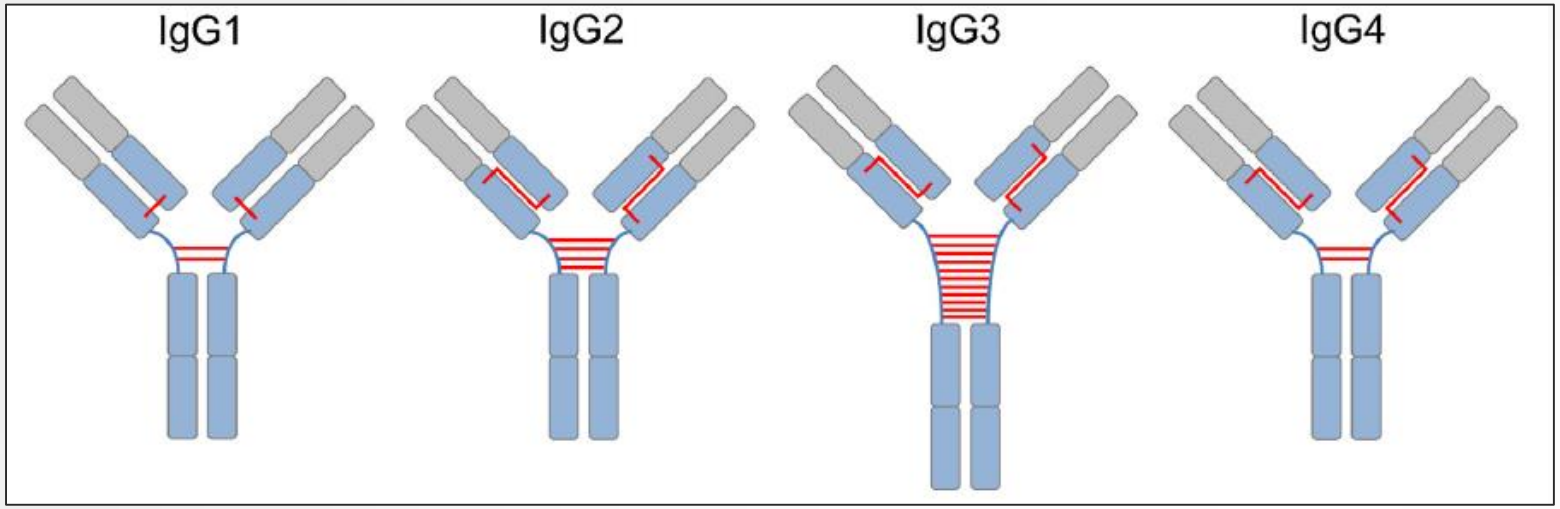
Kristina Srzentić,† Luca Fornelli,†
Lissa C. Anderson, Dina L. Bai, A
Julia Chamot-Rooke, Sneha Chatt
Robert A. D'Ippolito, Mathieu Du
Sylvester Greer, Kim F. Haselman
Matthew V. Holt, Sam Hughes, D
Christian Malosse, Alan G. Marsh
Simone Nicolardi, Ljiljana Paša-T
Wendy Sandoval, Richa Sarin, Ni
Michael R. Shortreed, Lloyd M. Smith, Frank Sobott, Detlev Suckau, Timothy Toby, Chad R. Weisbrod,
Norelle C. Wildburger, John R. Yates, III, Sung Hwan Yoon, Nicolas L. Young, and Mowei Zhou

ABSTRACT: The Consortium for Top-Down Proteomics (www.topdownproteomics.org) launched the present study to assess the current state of top-down mass spectrometry (TD MS) and middle-down mass spectrometry (MD MS) for characterizing monoclonal antibody (mAb) primary structures, including their modifications. To meet the needs of the rapidly growing therapeutic antibody market, it is important to develop analytical strategies to characterize the heterogeneity of a therapeutic product's primary structure accurately and reproducibly. The major objective of the present study is to determine whether current TD/MD MS technologies and protocols can add value to the more commonly employed bottom-up (BU) approaches with regard to confirming protein integrity, sequencing variable domains, avoiding artifacts, and revealing modifications and their locations. We also aim to gather information on the common TD/MD MS methods and practices in the field. A panel of three mAbs was selected and centrally provided to 20 laboratories worldwide for the analysis: Sigma mAb standard (SiLuLite), NIST mAb standard, and the therapeutic mAb Herceptin (trastuzumab). Various MS instrument platforms and ion dissociation techniques were employed. The present study confirms that TD/MD MS tools are available in laboratories worldwide and provide complementary information to the BU approach that can be crucial for comprehensive mAb characterization. The current limitations, as well as possible solutions to overcome them, are also outlined. A primary limitation revealed by the results of the present study is that the expert knowledge in both experiment and data analysis is indispensable to practice TD/MD MS.

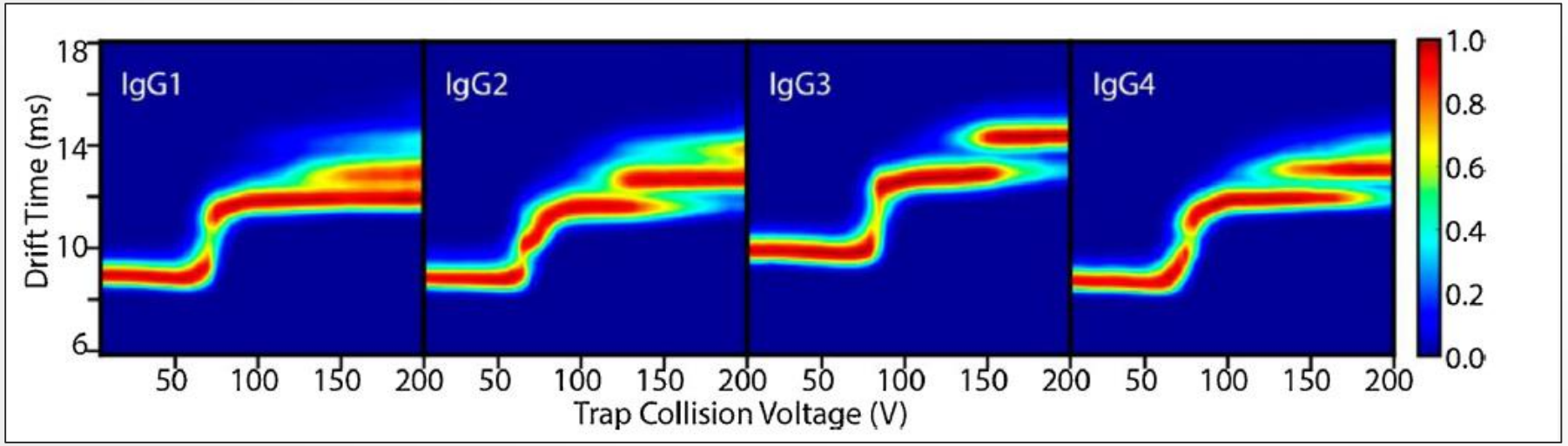
KEYWORDS: *monoclonal antibody, top-down, middle-down, intact mass measurement, mass spectrometry, glycoform*



Collision Induced Unfolding (CIU): isotype fingerprints



- Terral G, Cianferani S, Beck A, J. Chrom B 2016
- Hernandez-Alba O, Wagner E, Beck A, Cianferani S, Anal Chem 2018 (BsAb)
- Hernandez-Alba O, Wagner E, Beck A, Cianferani S et al, bioRxiv 2020



- Tian Y, Ruotolo BT et al, Anal Chem 2015

- O. Hernandez-Alba
- S. Cianférani

Middle-level-IM-MS & CIU (Anal Chem 2020)

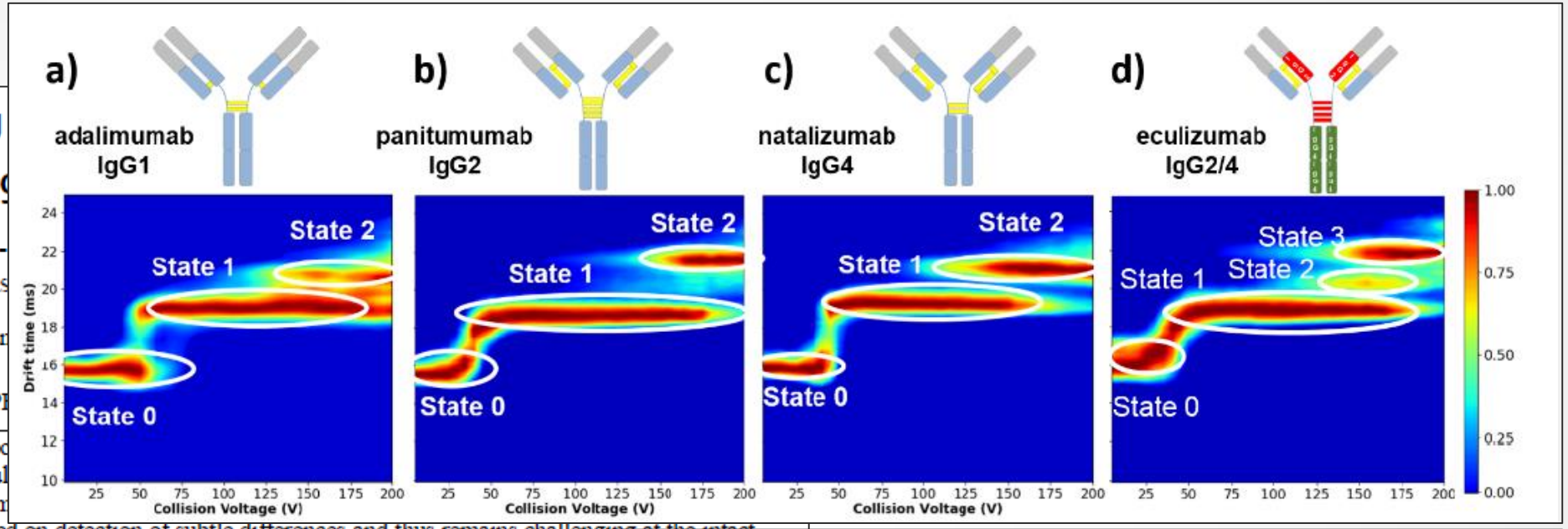
Middle-level IM-MS and CIU immunoglobulin isotype fing

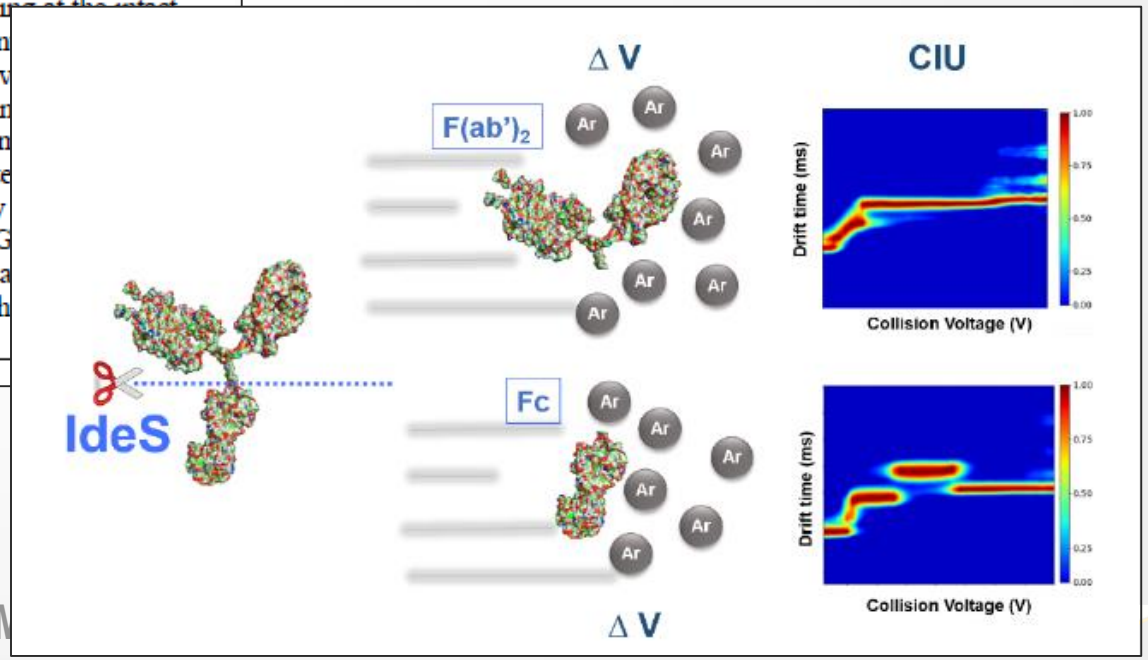
Thomas Botzanowski[1¶], Oscar Hernandez-Desligniere[1], Olivier Colas[2], Jean-François

1 Laboratoire de Spectrométrie de Masse BioOrgan bourg, France.

2 IRPF - Centre d'Immunologie Pierre-Fabre (CIPI

ABSTRACT: Currently approved therapeutic monoc types, which differ in their specific inter-chains disul for mAb isotyping, among which native ion mobility m However, mAb isotyping by these approaches is based on detection of subtle differences and thus remains challengi level. We report here on middle-level (after IdeS digestion) IM-MS and CIU approaches to afford better differen isotypes. Our method provides simultaneously CIU patterns of F(ab')2 and Fc domains within a single run. Middle-lev of F(ab')2 domains enable more reliable classification of mAb isotypes compared to intact level CIU, while CIU fin domains are overall less informative for mAb isotyping. F(ab')2 regions can thus be considered as diagnostic don specific CIU signatures for mAb isotyping. Benefits of middle-level IM-MS and CIU approaches are further illustrate IgG2/IgG4 eculizumab. While classical analytical techniques led to controversial results, middle-level CIU uniquely the challenge of eculizumab « hybridicity », highlighting that its F(ab')2 and Fc CIU patterns corresponds to an IgG respectively. Altogether, the middle-level CIU approach is more clear-cut, accurate and straightforward for canonica complex, engineered next generation mAb formats isotyping. Middle-level CIU thus constitutes a real breakthrough protein analysis, paving the way for its implementation in R&D laboratories.





- **Drs. O. Hernandez-Alba, S. Cianférani**

SEC-CIU: workflow automation (Anal Chem 2020)

Analytical Chemistry

Towards automation of Collision Induced Unfo through online Size Exclusion Chromatography Mass Spectrometry.

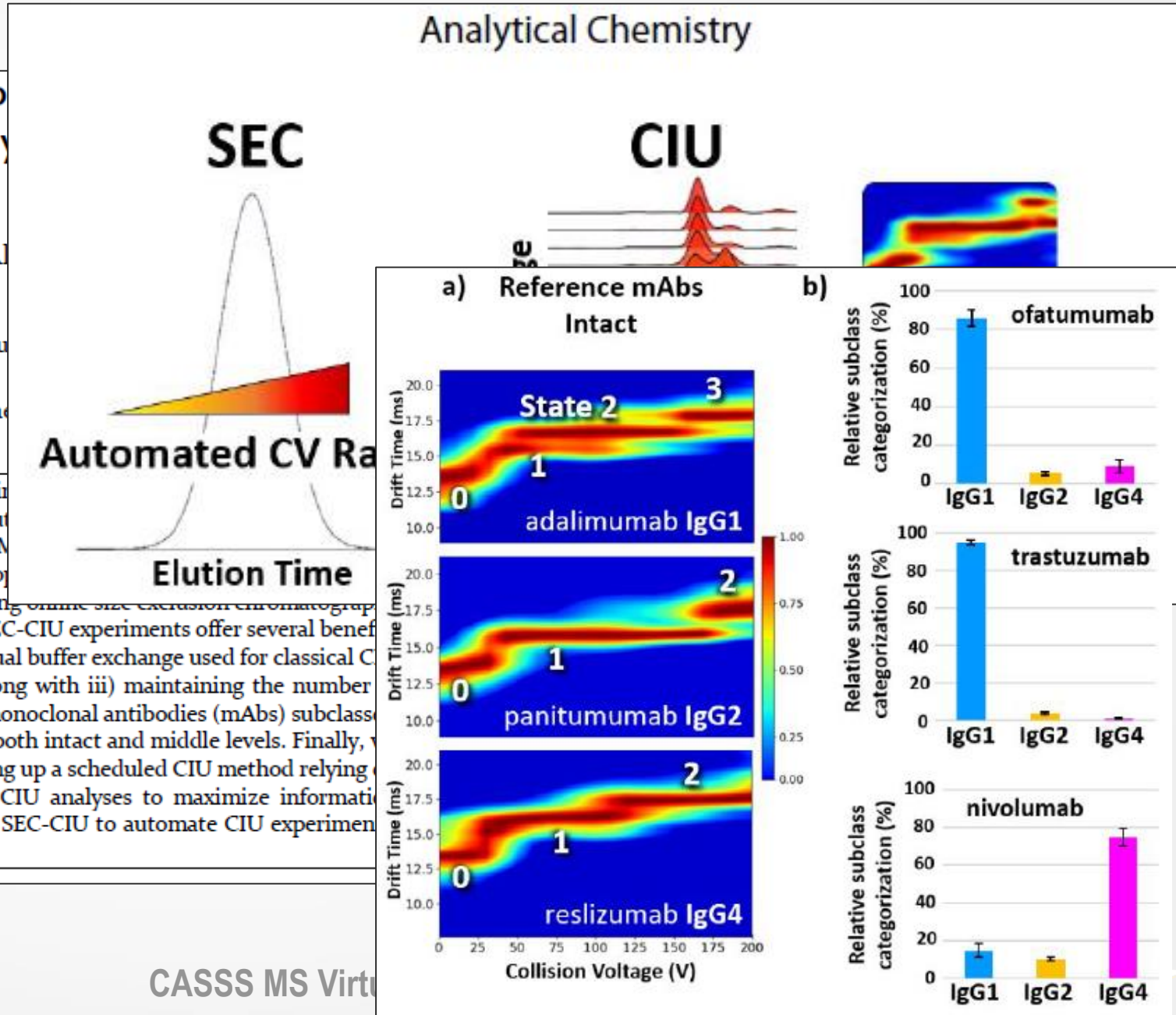
Evolène Deslignière[1], Anthony Ehkirch[1], Thomas Botzanowski[1], Al Alba[1], Sarah Cianférani[1*]

[1] Laboratoire de Spectrométrie de Masse BioOrganique, Université de Strasbou Strasbourg, France.

[2] IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 74160 Saint-Julien-en-Gen

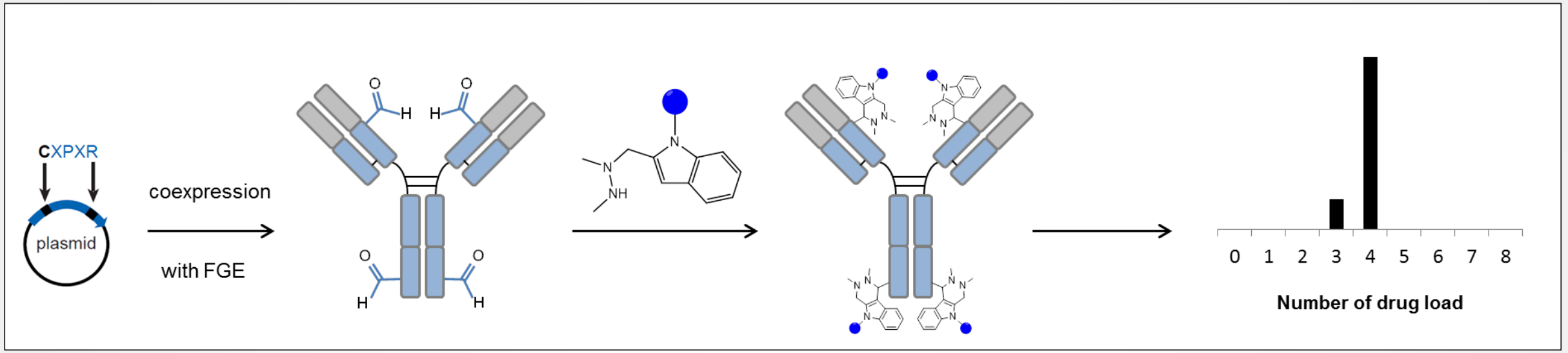
*Corresponding author: Sarah Cianférani. Email: sarah.cianferani@unistra.fr

ABSTRACT: Ion mobility-based collision induced unfolding (CIU) has gained i unfolding of proteins and their noncovalent complexes, notably for biotherapeu changes of proteins and emerges as an attractive alternative to circumvent poor IM automation for buffer exchange and data acquisition, precluding its wide ado workflow for CIU experiments, from sample preparation to data interpretation usin coupled to native ion mobility mass spectometry (SEC-CIU). Online automated SEC-CIU experiments offer several benef over nanoESI-CIU, among which i) improved and fast desalting compared to manual buffer exchange used for classical C experiments; ii) drastic reduction of the overall data collection time process along with iii) maintaining the number unfolding transitions. We then evaluate the potential of SEC-CIU to distinguish monoclonal antibodies (mAbs) subclass illustrating the efficiency of our method for rapid mAb subclass identification at both intact and middle levels. Finally, demonstrate that CIU data acquisition time can be further reduced either by setting up a scheduled CIU method relying diagnostic trap collision voltages or by implementing mAbs-multiplexed SEC-CIU analyses to maximize informati content in a single experiment. Altogether, our results confirm the suitability of SEC-CIU to automate CIU experimen particularly for the fast characterization of next generation mAb-based products.



- **E. Desligniere, S. Cianférani**

3G-ADCs: enzyme-assisted ligation
(formylglycine-generating enzyme, SMARTag®, Catalent)

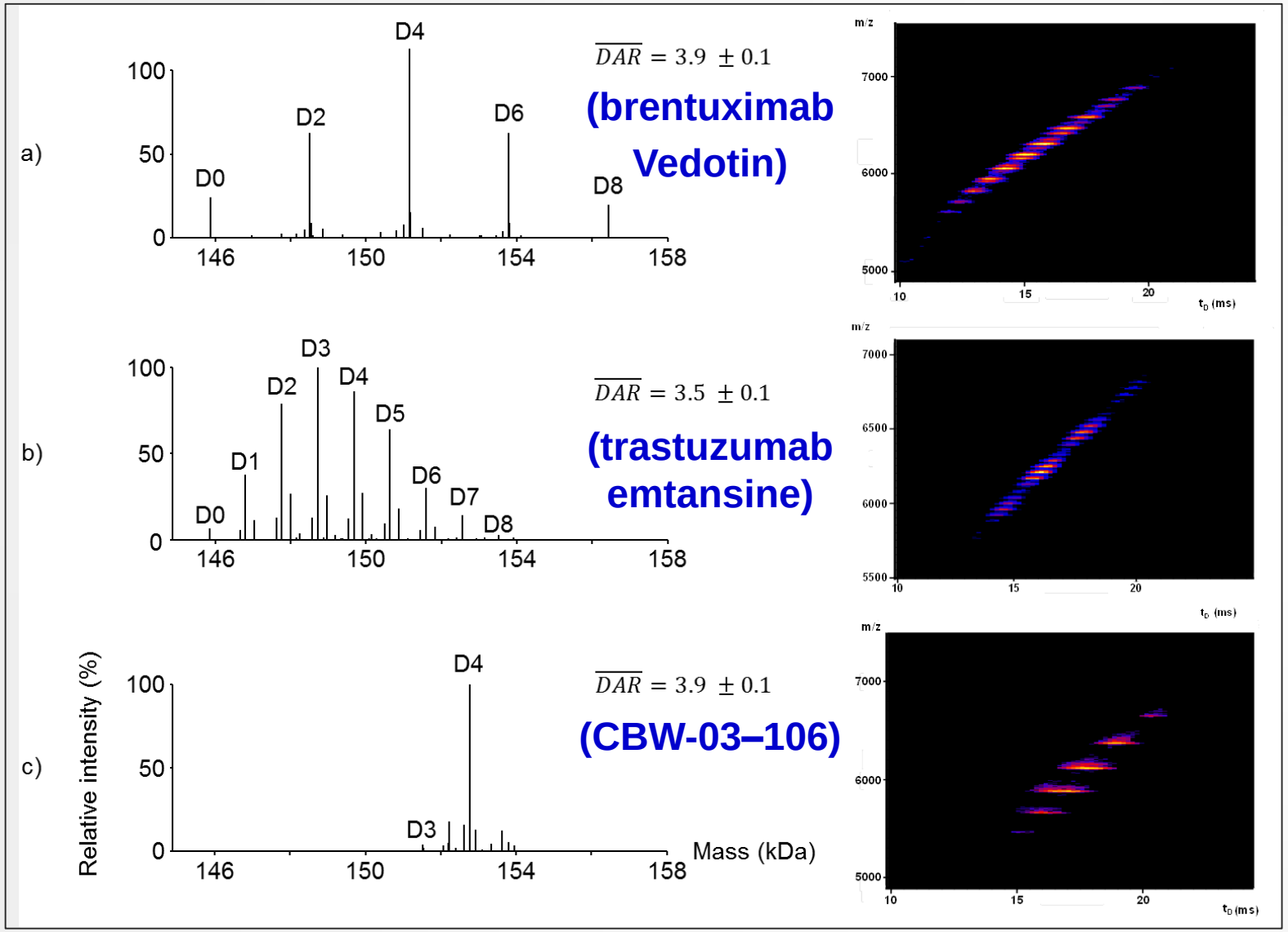


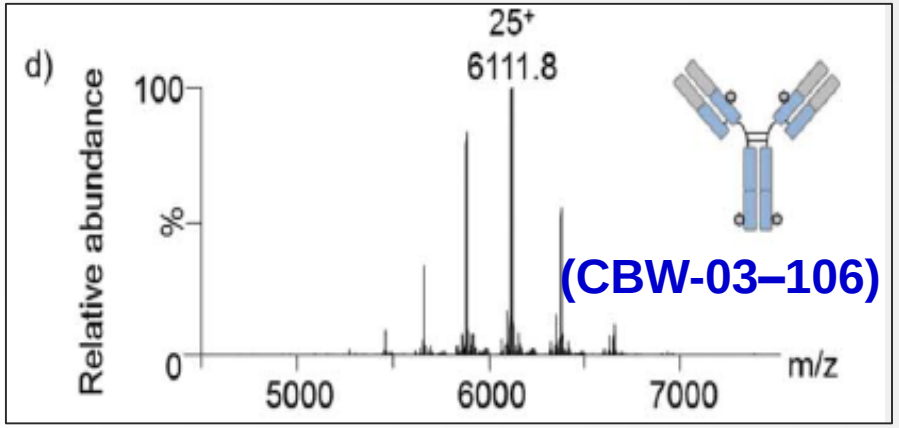
- Engineered Lys-Cys-X-Pro-X-Arg tag + formylglycine-generating enzyme (FGE)
  => Cys oxidized to formylglycine + HIPS ligation

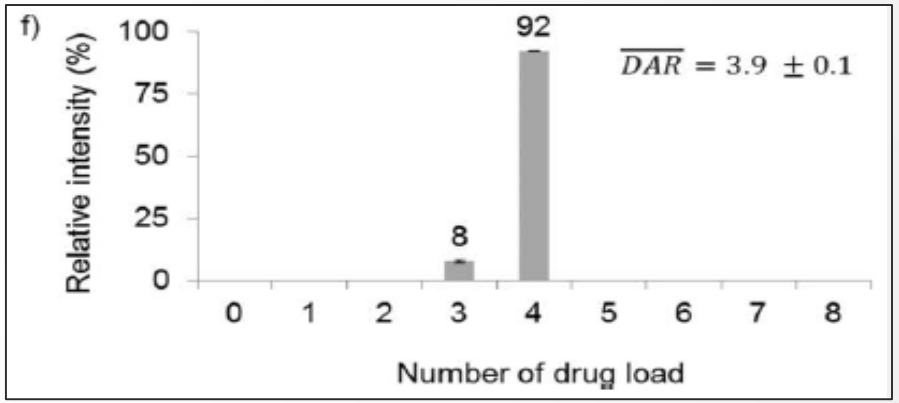
(1) native MS/ native IM-MS
(2) HCD, ETD, UVPD

- **Beck A et al, Nature Reviews Drug Discovery 2017**
- **Botzanowski T, Erb S, Rabuka D, Beck A, Drake P, Cianferani S et al, mAbs 2017**
- **Hernandez-Alba O, Houel S, Beck A, Cianferani S et al, 2019**

3G-ADCs: SMARTag® (Catalent): native MS/ IM-MS







➢ **Botzanowski T, Erb S, Rabuka D, Beck A, Drake P, Cianferani S et al, mAbs 2017**

IgG & ADC structures : HCD, ETD, UVPD (2019)

J. Am. Soc. Mass Spectrom. (2019)
DOI: 10.1007/s13361-019-02296-2

RESEARCH ARTICLE

A Case Study to Identify the Drug Conjugation Site of a Site-Specific Antibody-Drug-Conjugate Using Middle-Down Mass Spectrometry

Oscar Hernandez-Alba,[1] Stéphane Houel,[2] Steve Hessmann,[1] Stéphane E
David Rabuka,[3] Romain Huguet,[2] Jonathan Josephs,[2] Alain Beck,[4] Penelo
Sarah Cianférani[1]

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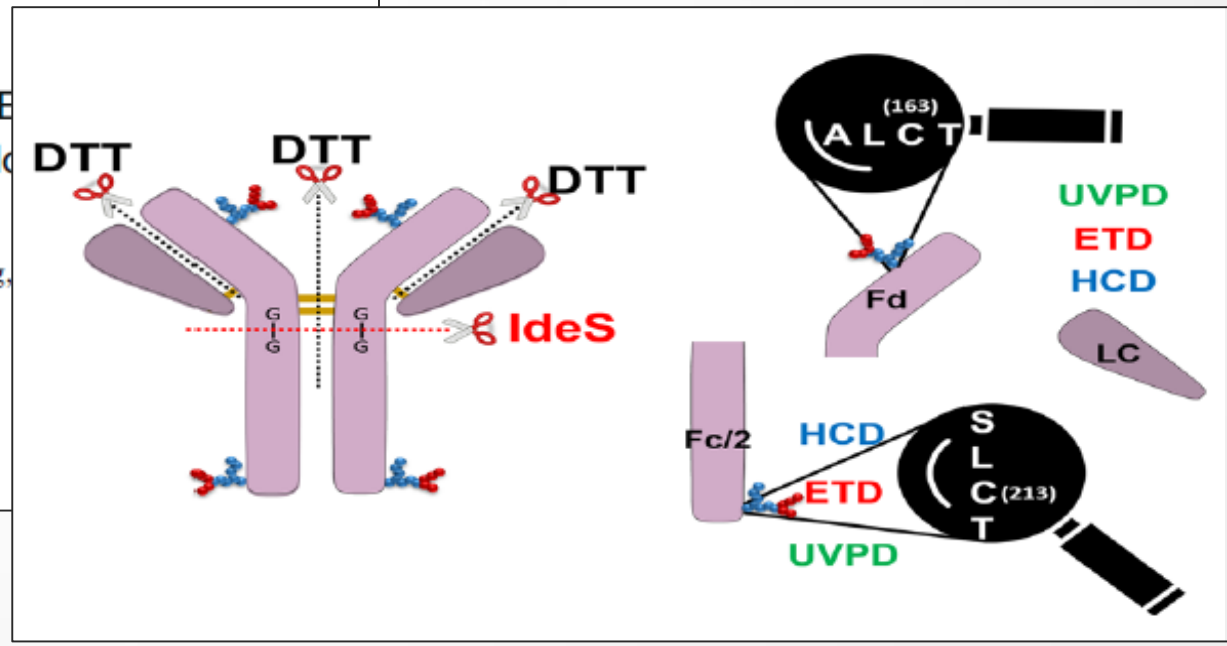
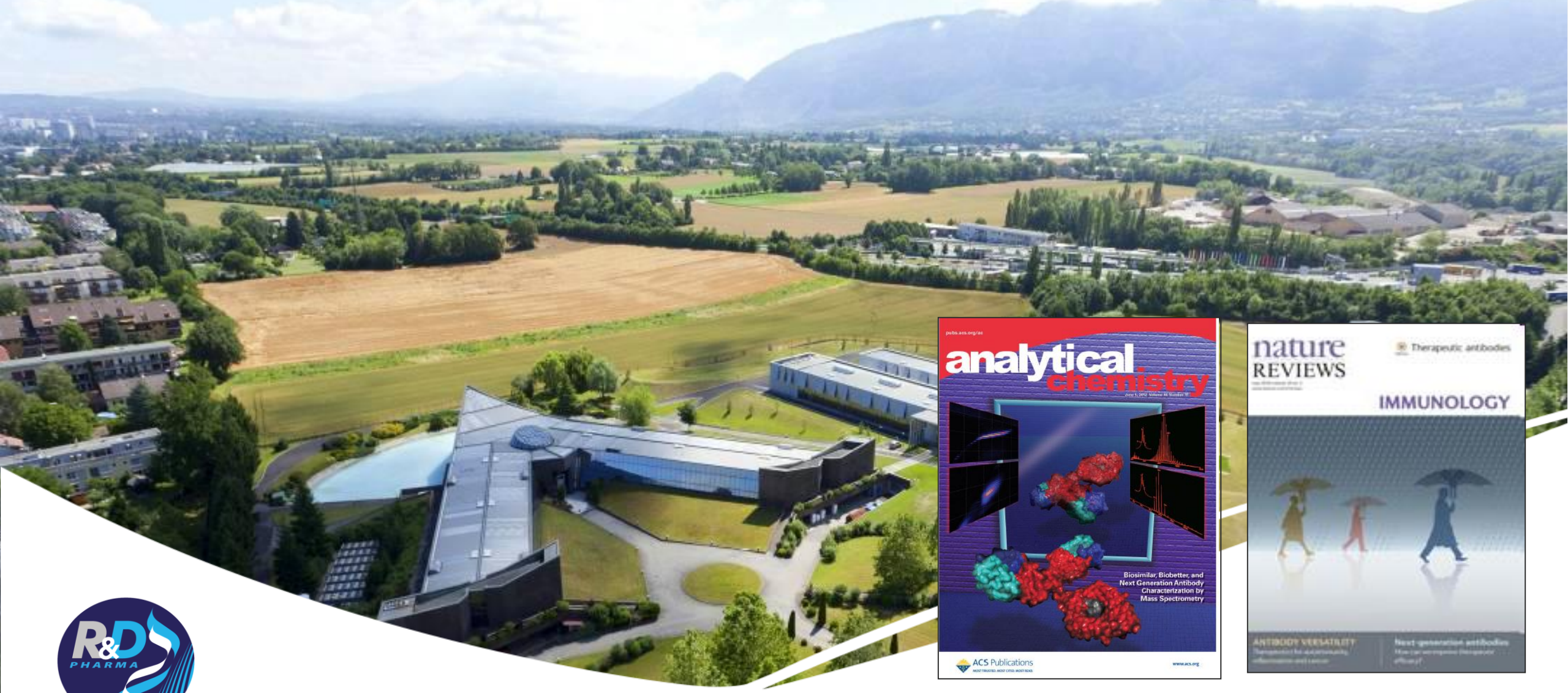
[4]IRPF, Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France

- Reduction (DTT)
- IdeS digestion
- HCD: higher-energy collisional-dissociation
- ETD: electron-transfer dissoc.
- UVPD: 213 nm UV photodissoc



- O. Hernandez-Alba, S. Cianférani



(3) Capillary Electrophoreris-MS based methods

Pierre Fabre

Capillary Electrophoreris + MS (CE-MS) (2016-20)

Journal of Chromatography B 1122–1123 (2019) 1–17

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Review

Insights from capillary electrophoresis approaches for characterization of monoclonal antibodies and antibody drug conjugates in the period 2016–2018

Antony Lechner[a], Jérémie Giorgetti[a], Rabah Gahoual[b], Alain Beck[c], Emmanuelle Leize-Wagner[a], Yannis-Nicolas François[a,*]

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[b] Unité de Technologies Biologiques et Chimiques pour la Santé (UTCBS), Paris 5-CNRS UMR8258 Inserm U1022, Faculté de Pharmacie, Université Paris Descartes, Paris, France
[c] Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France

Dr. Y. François & coll.

- **François YN, Biacchi M, Said N, Renard C, Beck A, Gahoual R, Leize-Wagner E. Anal Chim Acta 2016**
- **François YN et al, Talanta 2018**
- **François YN et al, 2019**

- **Gahoual R, Beck A, François YN, Leize-Wagner E. J Mass Spec 2016**
- **François YN, Biacchi M, Said N, Renard C, Beck A, Gahoual R, Leize-Wagner E. Anal Chim Acta 2016**
- **Gahoual R, Beck A, Leize E, Francois Y. J Chrom B 2016**
- **Said N, Gahoual R, Kuhn L, Beck A, Francois YN, Leize E. LC-GC 2017**
- **Biacchi M, Said N, Beck A, Leize-Wagner E, François YN. J Chrom A 2017**

Capillary Electrophoreris + MS (CE-MS) (2020)

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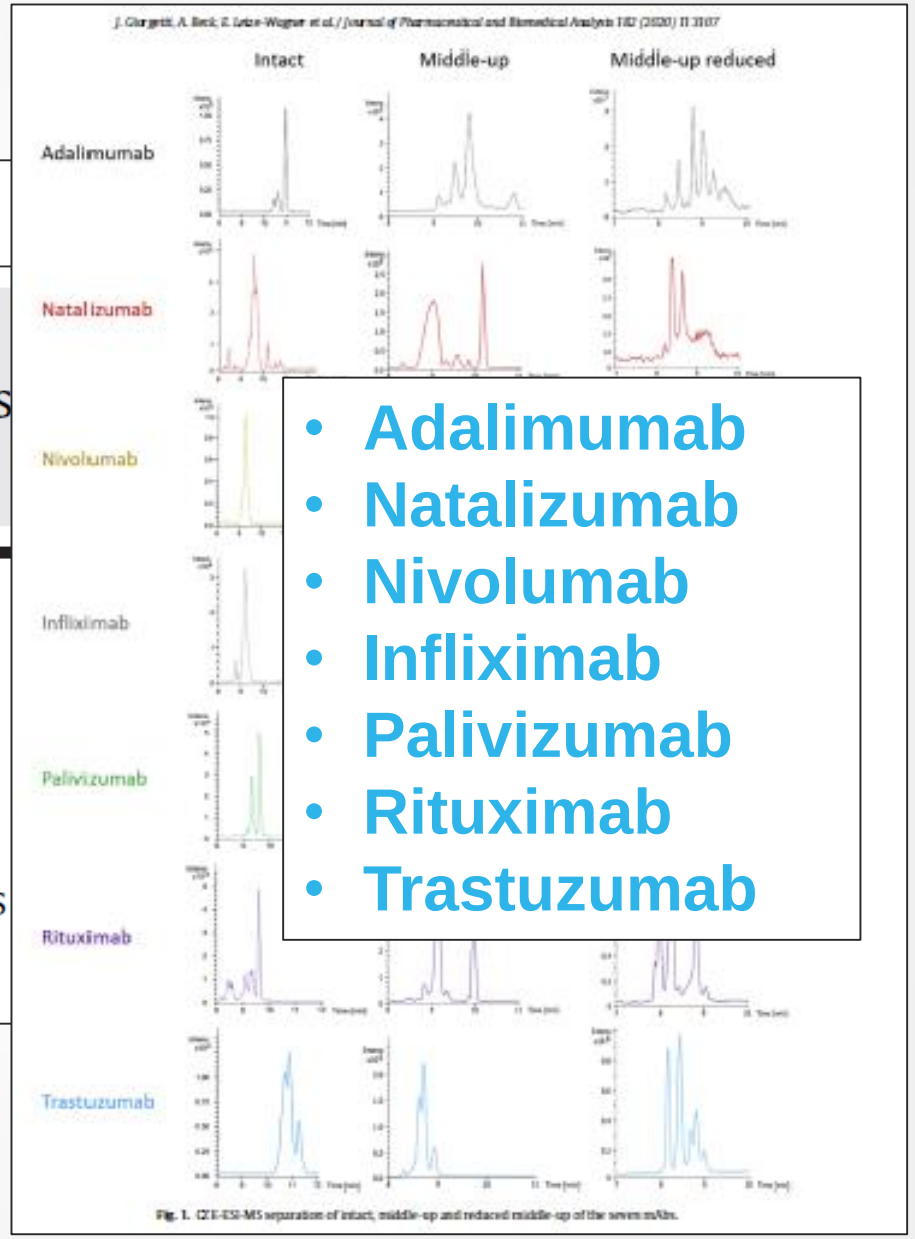
Combination of intact, middle-up and bottom-up levels to characterize 7 therapeutic monoclonal antibodies by capillary electrophoresis – Mass spectrometry

Jérémie Giorgetti[a], Alain Beck[b], Emmanuelle Leize-Wagner[a], Yannis-Nicolas François

[a] *Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS) UMR 7140 (Unistra-CNRS), Université de Strasbourg, France*
[b] *Centre d'Immunologie Pierre Fabre, Saint-Julien-en-Genevois, France*

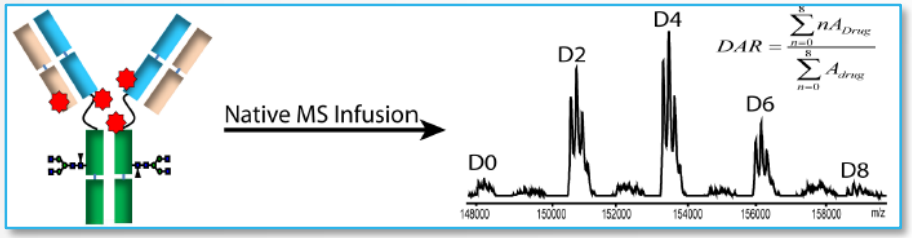


- Adalimumab
- Natalizumab
- Nivolumab
- Infliximab
- Palivizumab
- Rituximab
- Trastuzumab

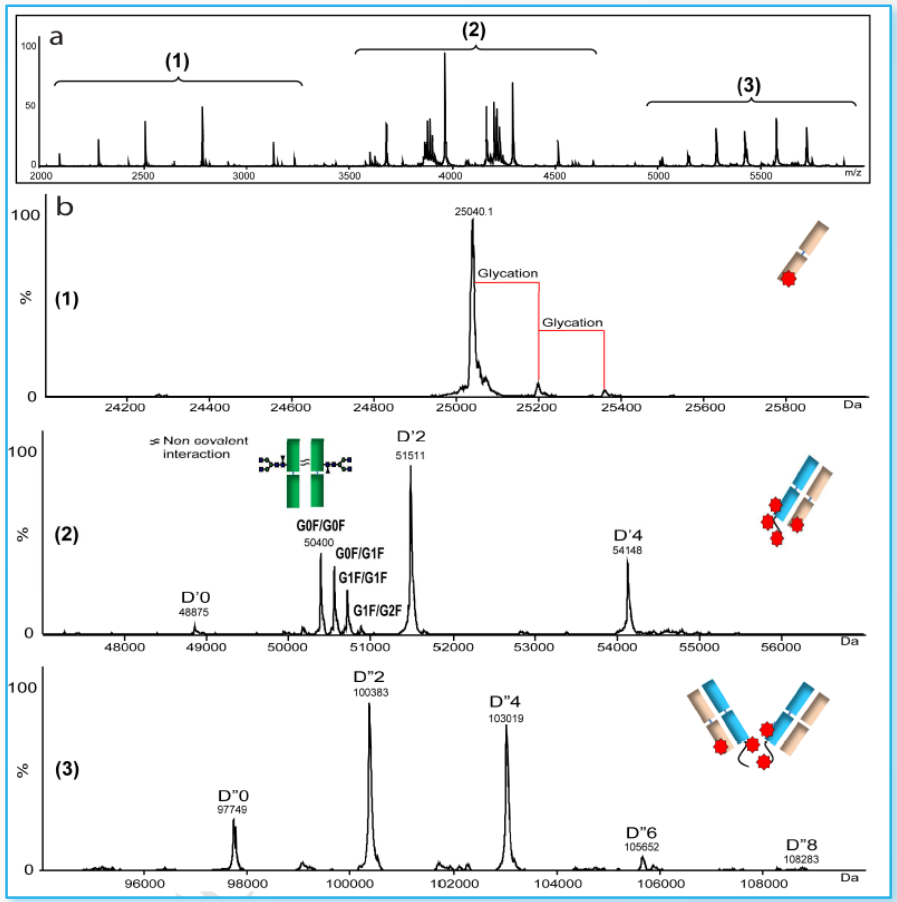
ADC characterization by sheathless CE-MS (2016-2020)

Native MS of brentuximab vedotin

1. Top level



2. Middle level



- Sheathless CE-MS used as nanoESI infusion platform
- Average DAR & distribution determination
- Structural information of the subunits $F_{c/2}$, F(ab)'2, LC, F(ab), +/- payloads
- Non-covalent dimers of ADC $F_{c/2}$ subunits

Dr Y. François & coll.

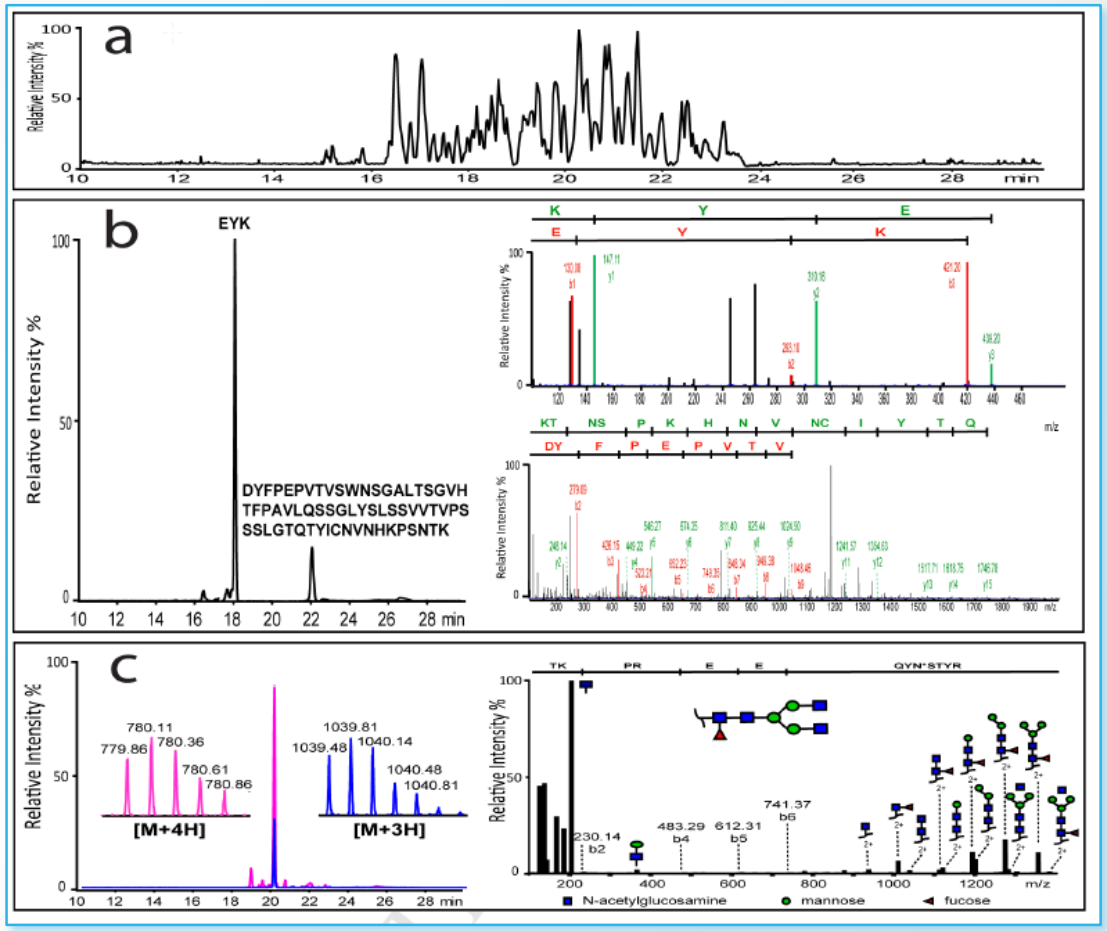
- Said N, Gahoual R, Kuhn L, Beck A, François YN, Leize-Wagner E. Anal Chim Acta 2016
- Saadé J, Gahoual R, Beck A, Leize-Wagner E, François YN, Meth Mol Biol 2020

ADC peptide mapping by sheathless CZE-ESI-MS/MS

Peptide mapping (brentuximab vedotin)

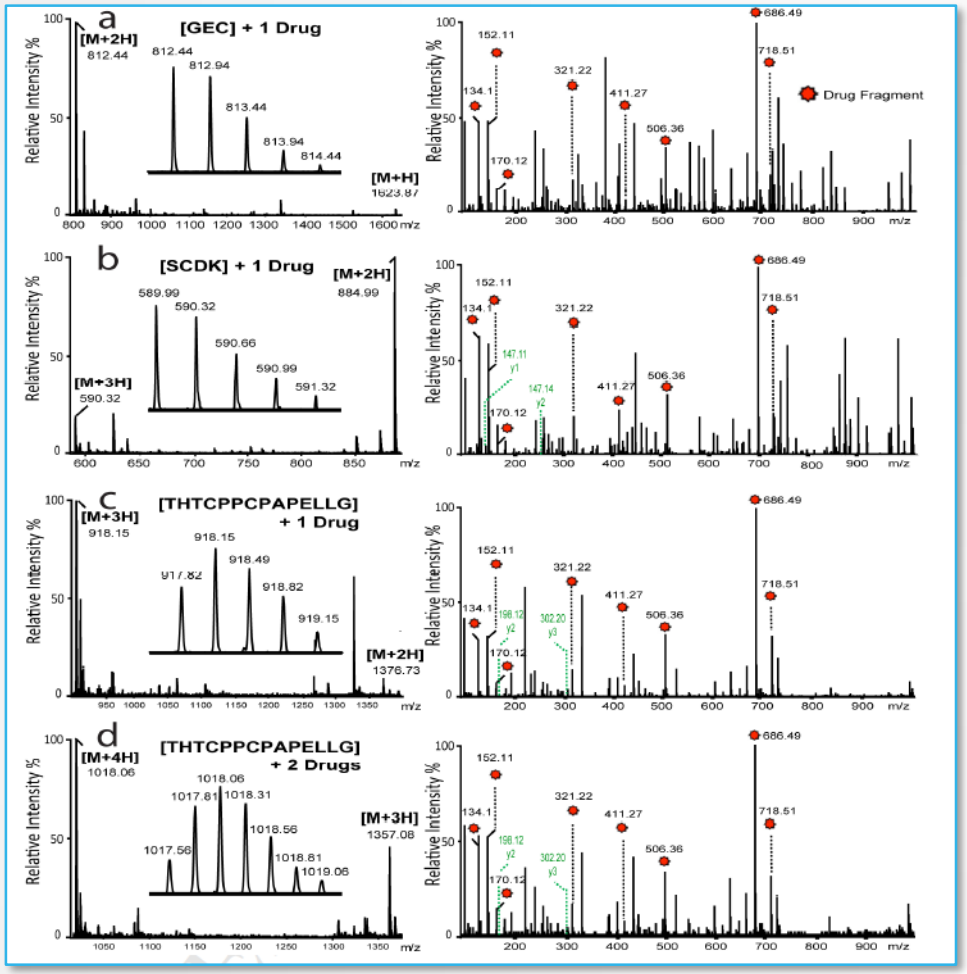
3. Bottom level

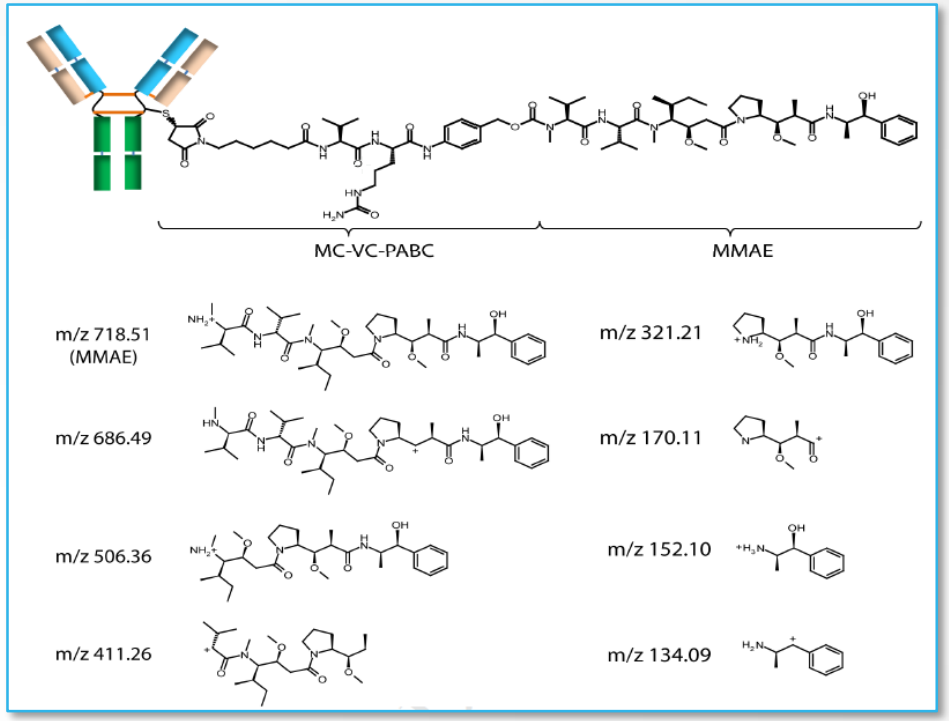
- Within a single injection of 200 fmol
- Primary structure assessment
- Identification of very small peptides (3 a.a.) alongside to a 63 a.a. peptide
- Glycosylations (11 peptides) characterized with improved sensitivity



- Said N, Gahoual R, Kuhn L, Beck A, François YN, Leize-Wagner E. Anal Chim Acta 2016
- Saadé J, Gahoual R, Beck A, Leize-Wagner E, François YN, Meth Mol Biol 2020

ADC peptide mapping CZE-ESI-MS/MS (2016-2020)

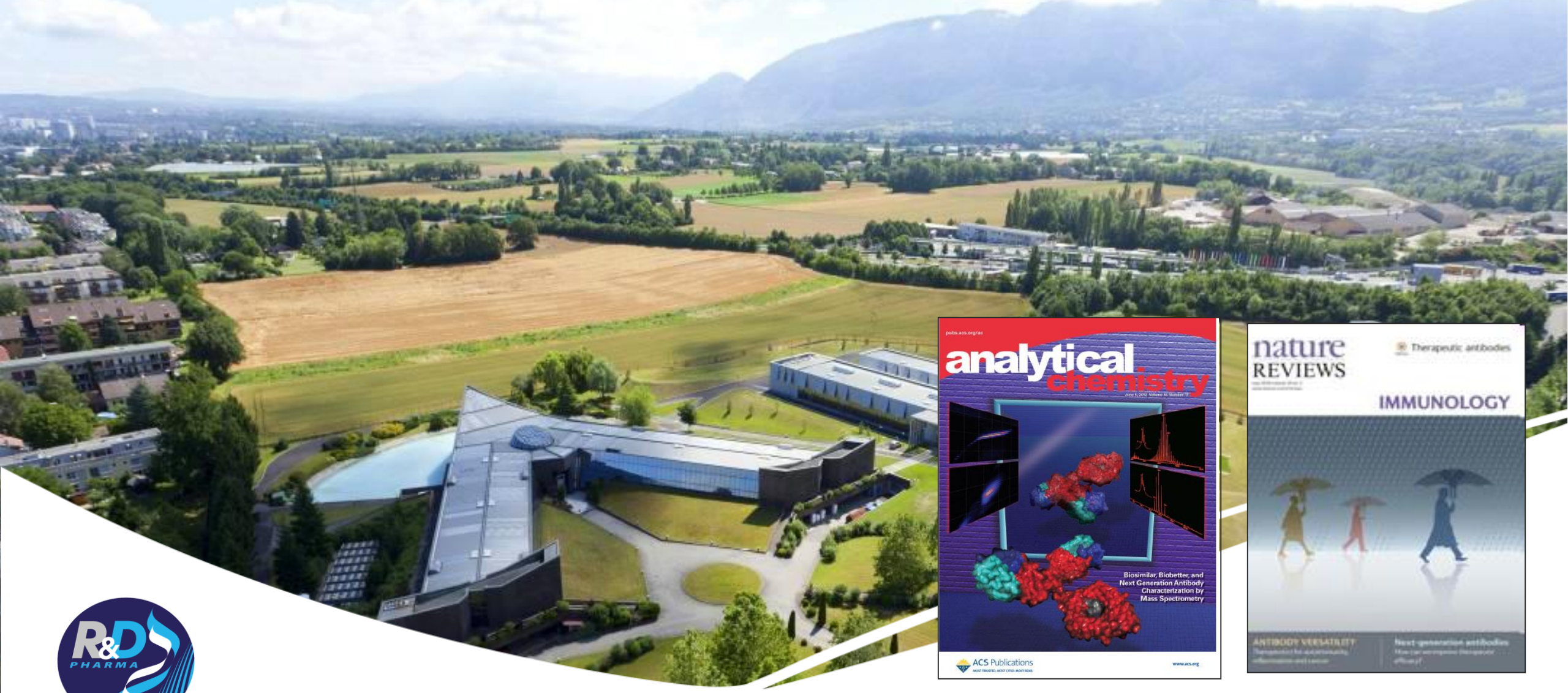




- Four drug-loaded peptides and associated MS/MS spectra
- Localization of the drugs / yield of drug incorporation
- Seven diagnostic ions identified “MMAE fragments”

➢ **Said N, Gahoual R, Kuhn L, Beck A, François YN, Leize-Wagner E. Anal Chim Acta 2016**

➢ **Saadé J, Gahoual R, Beck A, Leize-Wagner E, François YN, Meth Mol Biol 2020**

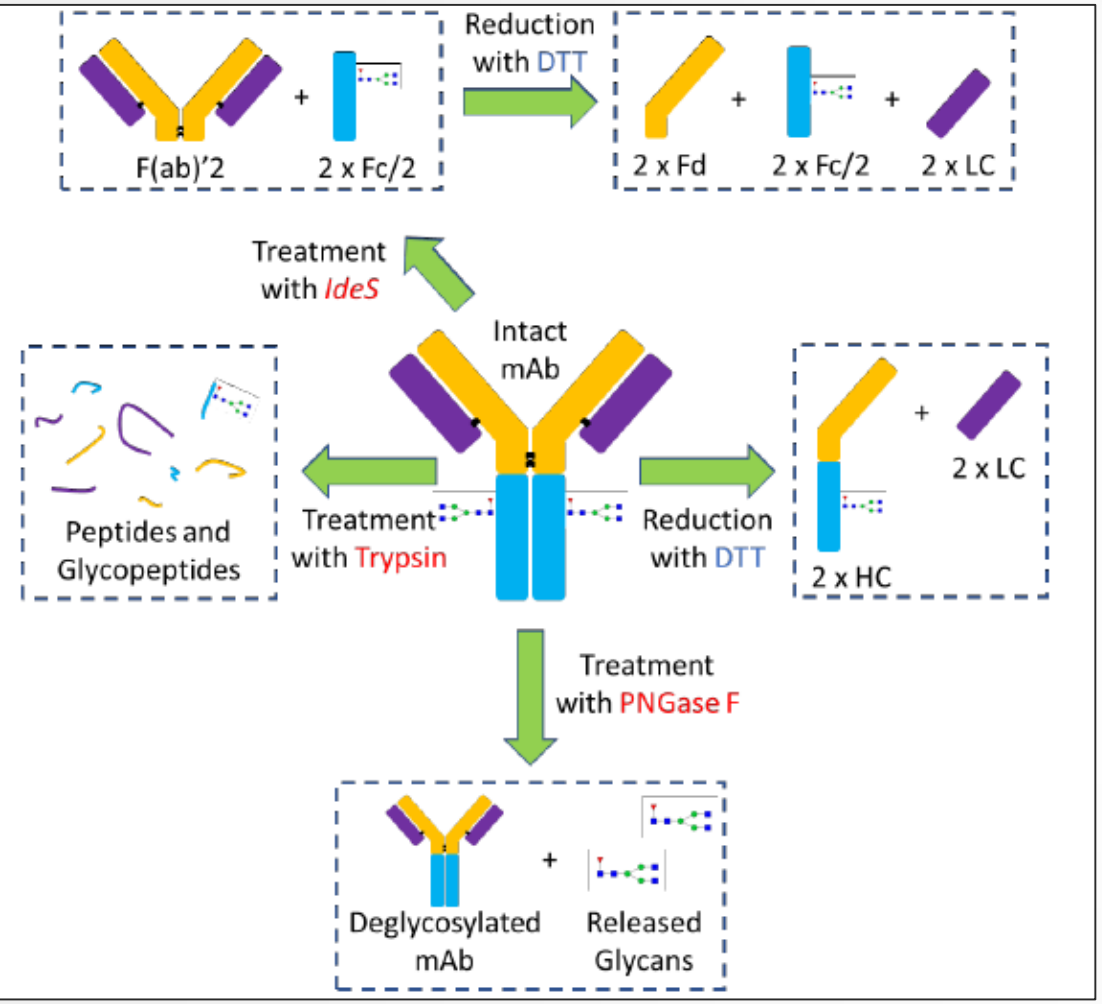


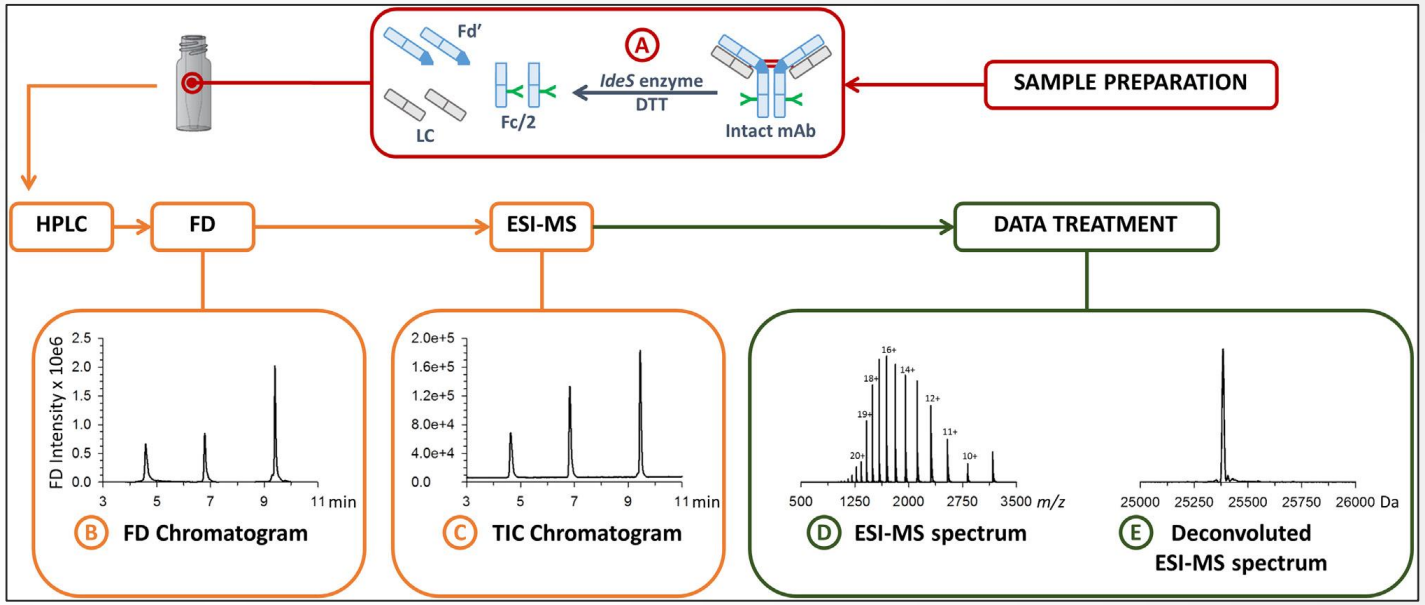
(4) Multi-dimentional LC-MS methods:

SEC-MS, SECxSEC MS, SEC-HIC-MS…

Pierre Fabre

Multilevel mAbs LC-MS characterization (2019)



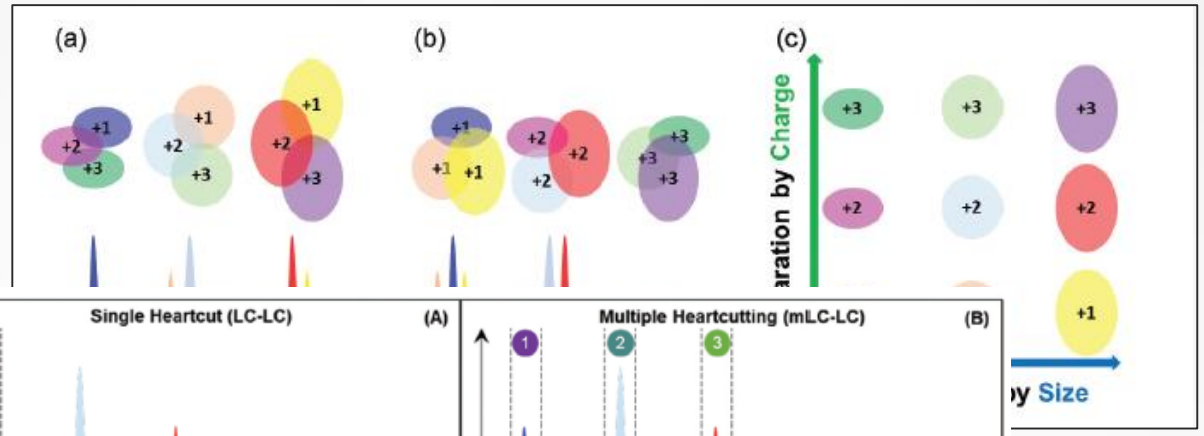


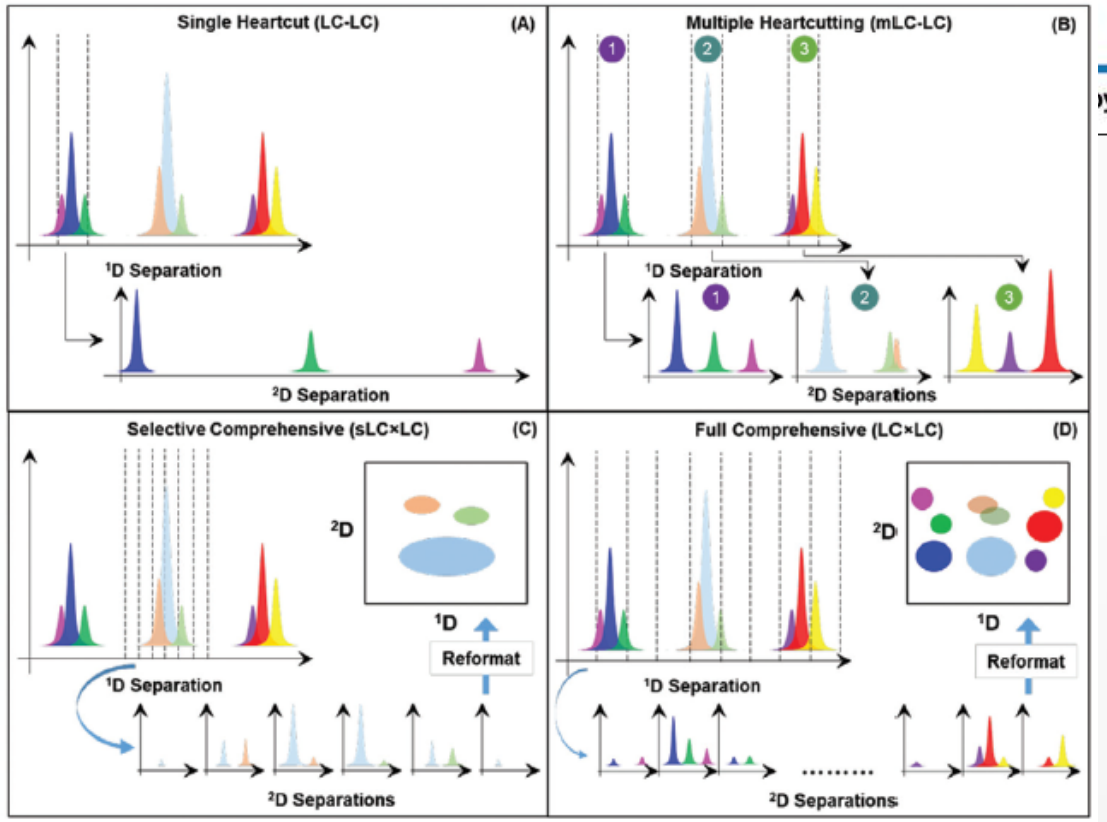
- D'Atri V, Beck A, Guillarme D et al. JCB 2018
- Bobály B, Beck A, Guillarme D, Fekete S, JCA 2018
- Bobály B, Beck A, Guillarme D, Fekete S, JCB 2018
- Ehkirch A, Goyon A et al. Anal Chem 2018
- Ehkirch A, D'Atri V et al. Anal Chem 2018
- Stoll D, Zhang K, Stapples G, Beck A. Adv Chrom 2019

2 to 4 D LC-MS: mAbs & ADCs (2019)

Recent Advances in Two-Dimensional Liquid Chromatography for the Characterization of Monoclonal Antibodies and Other Therapeutic Proteins

Dwight R. Stoll, Kelly Zhang, Gregory O. Staples, and Alain Beck





- RPxRP-MS
- CEXxRP-MS
- HILICxRP-MS
- SECxRP-MS
- SECxSEC-IMxMS
- SEC-IMxMS
- HICxSEC-IMxMS
- …

- Stoll D, Zhang K, Staples O, Beck A. Adv. in Chrom., CRC Press. 2019

Non-denaturing LC-MS: IEX, SEC, HIC-MS (2020)

Journal of Pharmaceutical and Biomedical Analysis 185 (2020) 113207

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.els

Review

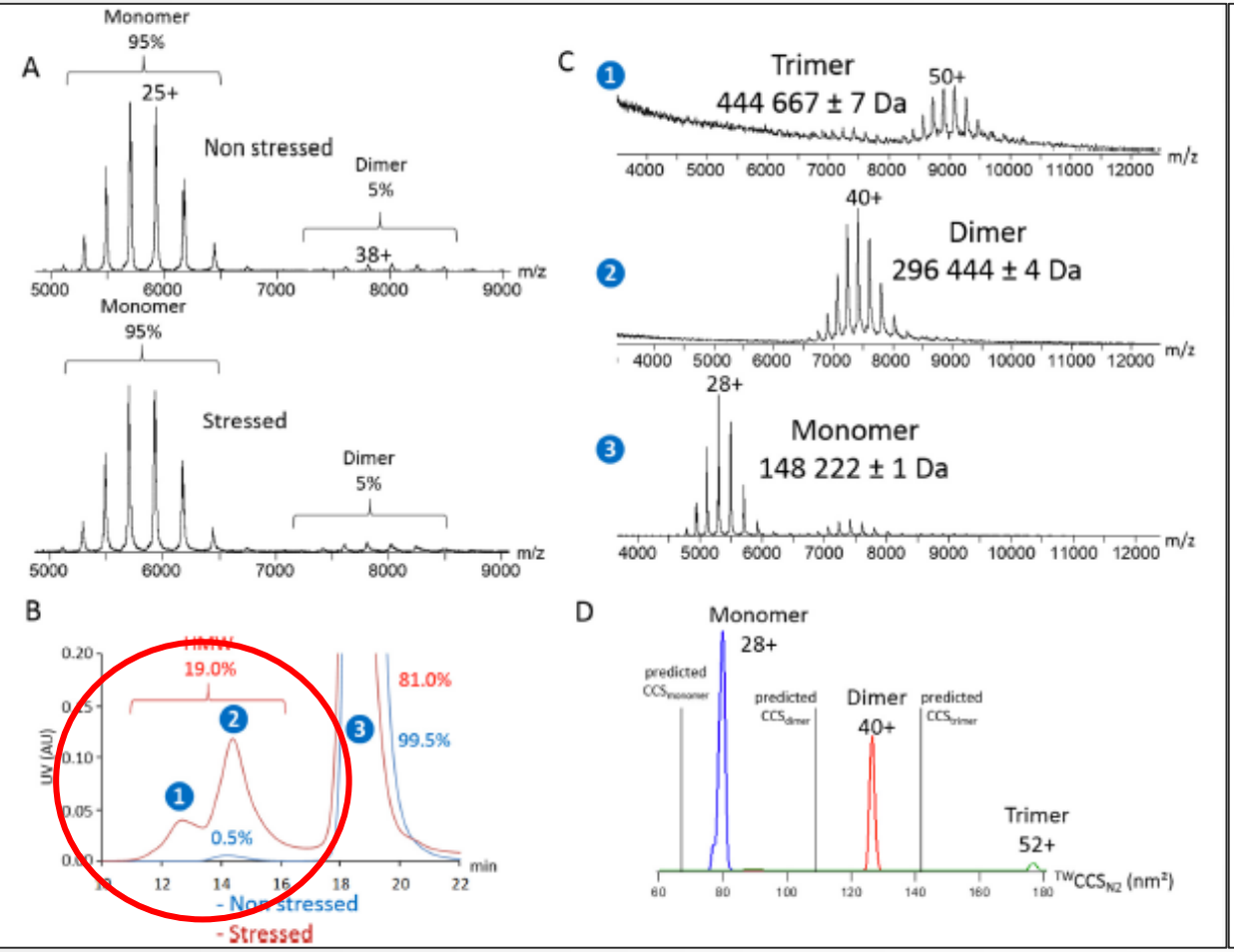
Coupling non-denaturing chromatography
the characterization of monoclonal antibo

Evelin Farsang[a], Davy Guillarme[b], Jean-Luc Veuthey[b],
Andrew Schmudlach[d], Szabolcs Fekete[b,*]

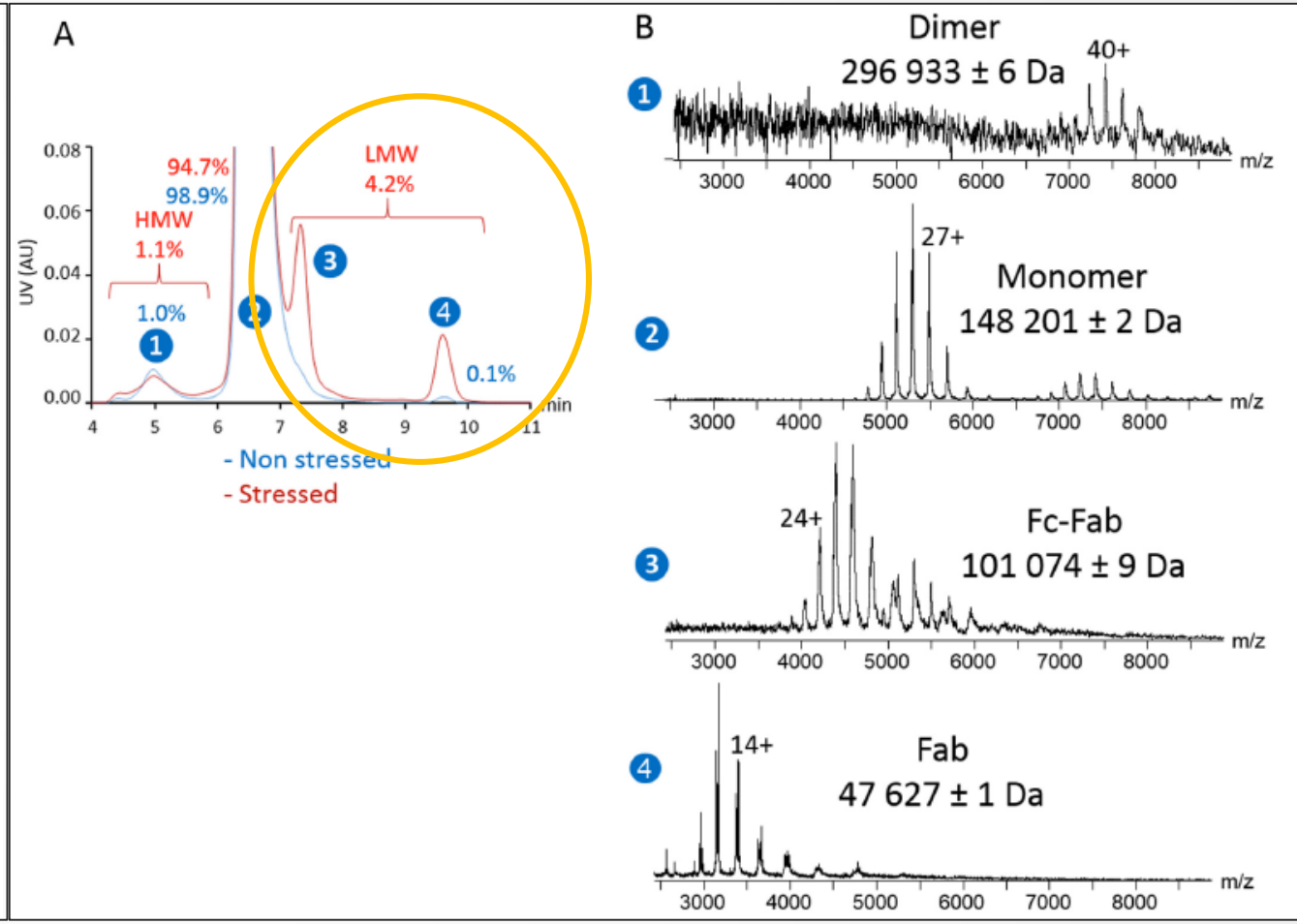
[a] Department of Analytical Chemistry, University of Pannonia, Egyetem u. 10., H-8200 Veszprém
[b] Institute of Pharmaceutical Sciences of Western Switzerland, University of Geneva, CMU-Rue
[c] Center of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, 74160, Saint-Julien-en-
[d] Waters Corporation, 34 Maple Street, Milford, MA, 01757-3696, United States

Size variants structure assessment: SEC*-native MS

(A) Monomer & HMWS (trastuzumab)**



(B) Monomer & LMWS (NISTmAb)***



- Ehkirch A, Beck A, Guillarme D, Cianferani S et al, J Chrom B 2018

*UHPLC, **pH stress,***Heat stress

On line 4D for mAbs size variants (SECxSEC-IMxMS)

Analytical Chemistry Article

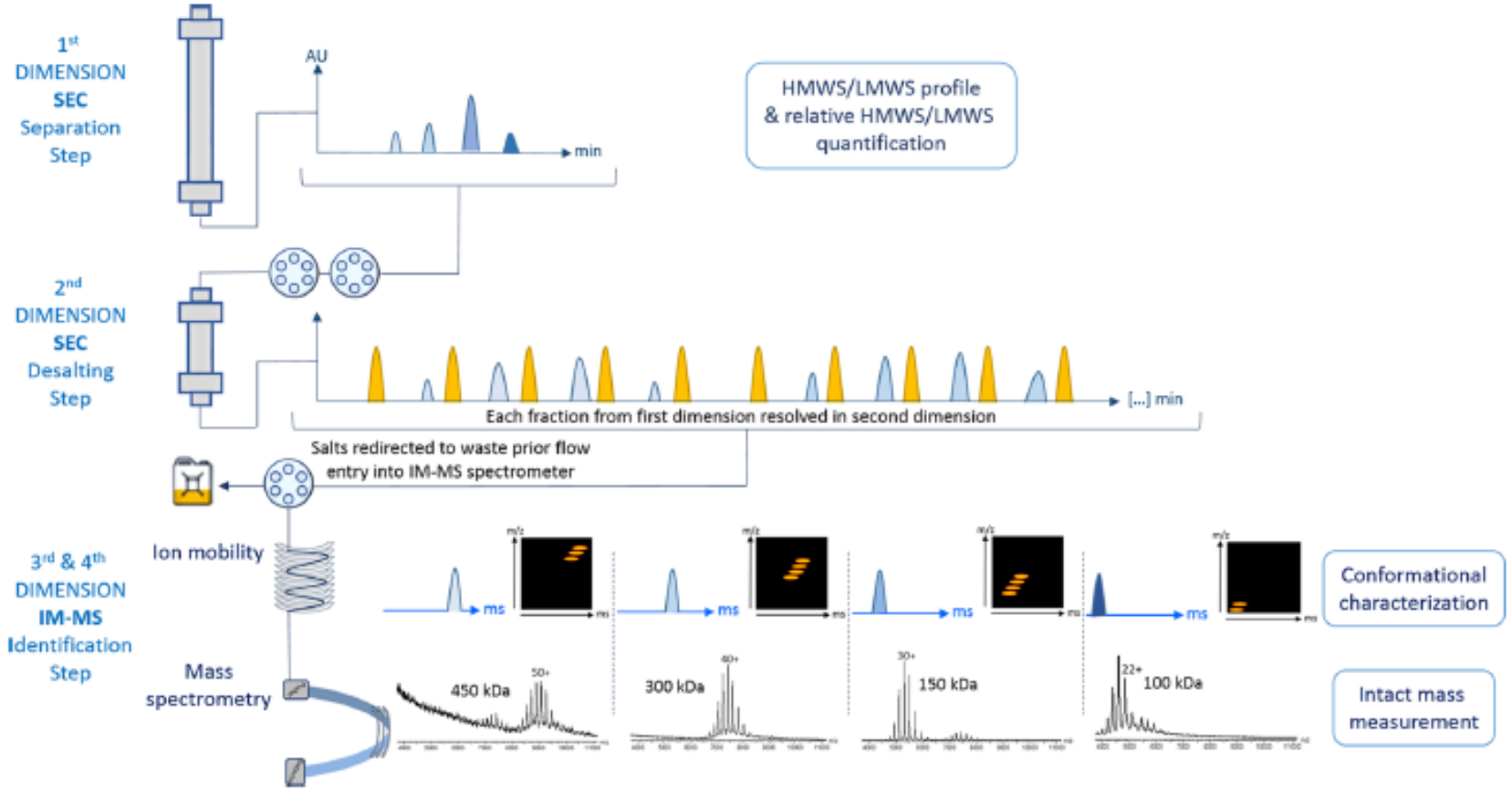
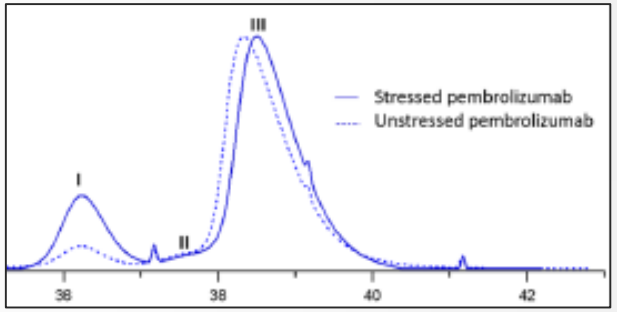


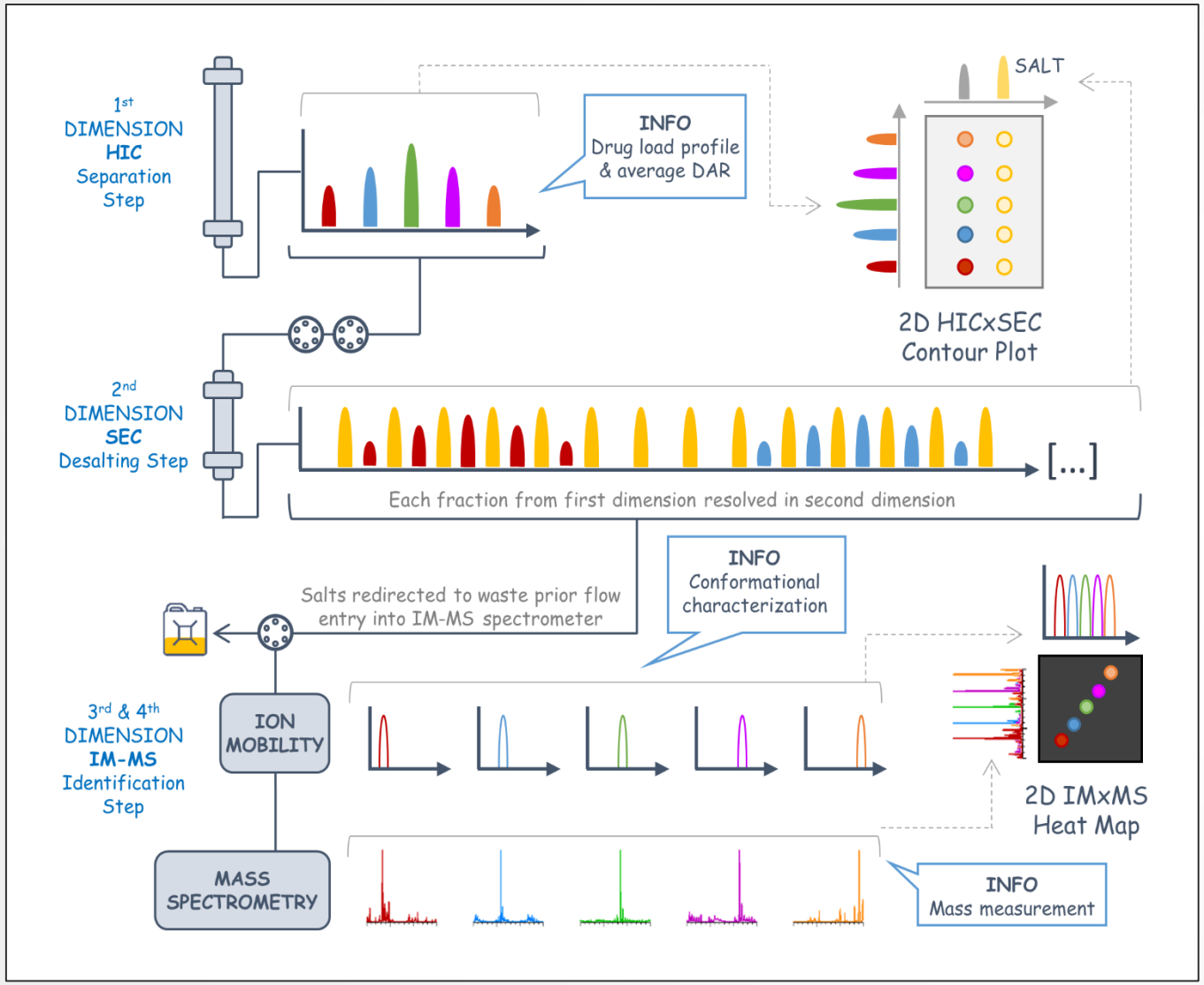
Figure 2. Flowchart of the SEC×SEC-native IM×MS for mAb analysis. The optimized SEC×SEC method was hyphenated to IM-MS. In the first dimension, SEC with nonvolatile salts allows a proper separation and quantitation of mAb HMWS/LMWS. In the second dimension, a short SEC column used with a volatile mobile phase was employed as a fast desalting step. Online native IM-MS allows conformational characterization and intact mass measurement of each individual ^{1}D-SEC peaks.

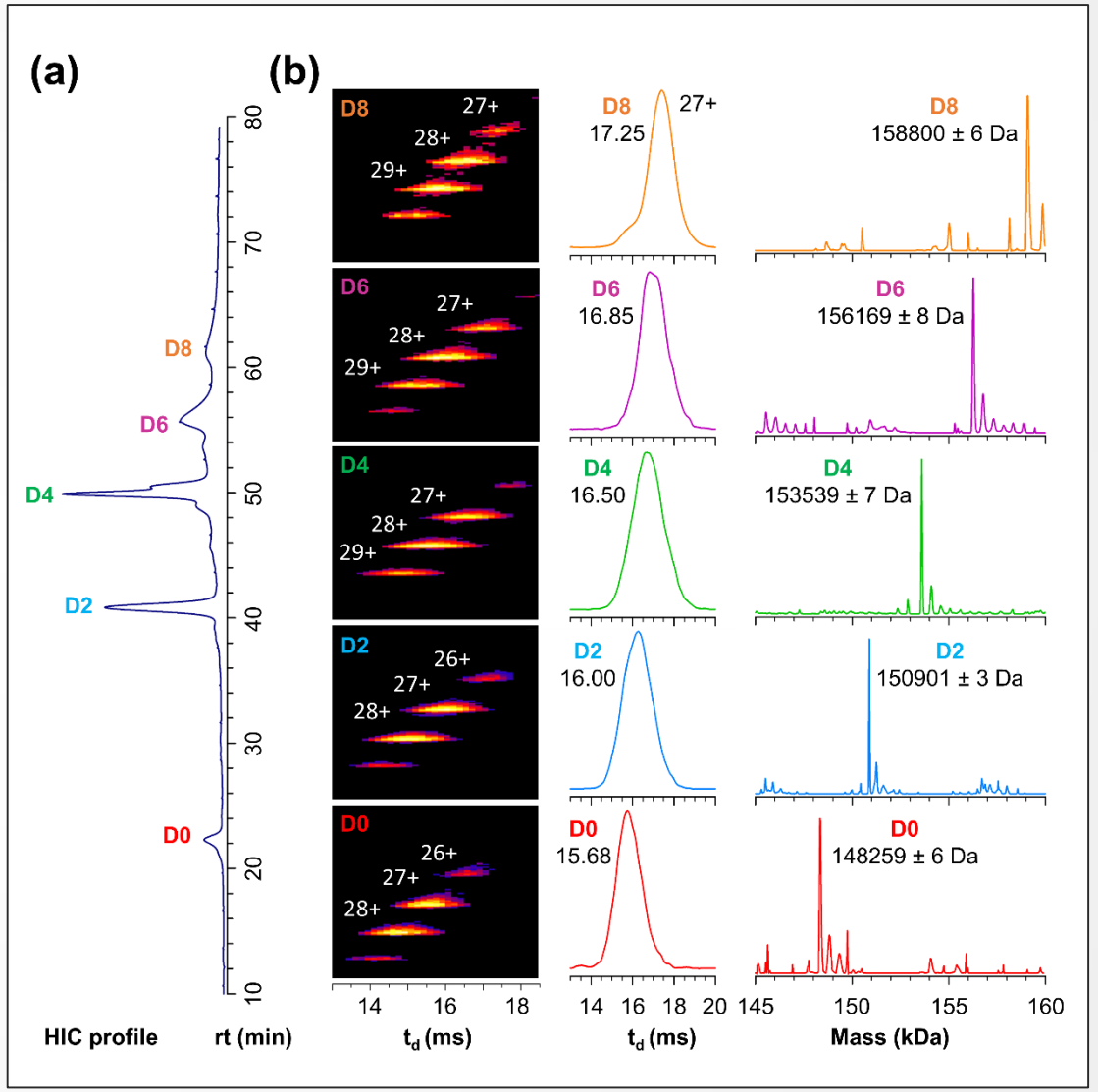


Pembrolizumab HMWS (SEC): oxidized species and not only dimers or aggregates

➢ **Ehkirch A, D'Atri V, Rouvière F, Beck A, Guillarme D, Heinisch S, Cianférani S et al. Anal Chem 2018**

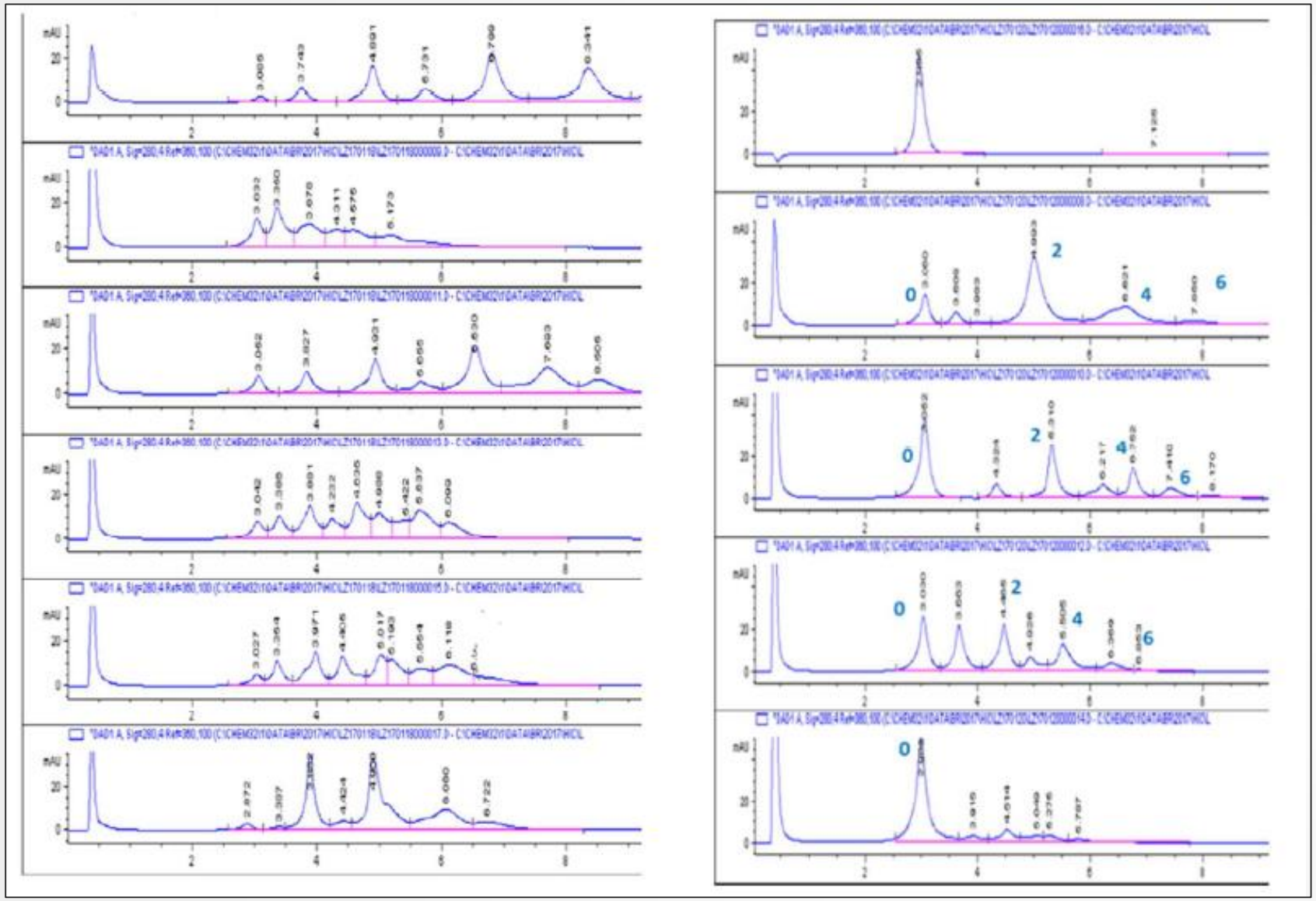
On line 4D methods for ADCs (HICxSEC-IMxMS)





➢ **Ehkirch A, D'Atri V, Rouvière F, Beck A, Guillarme D, Heinisch S, Cianférani S et al. Anal Chem 2018**

Cutting-Edge Analytical methods for ADCs: HIC

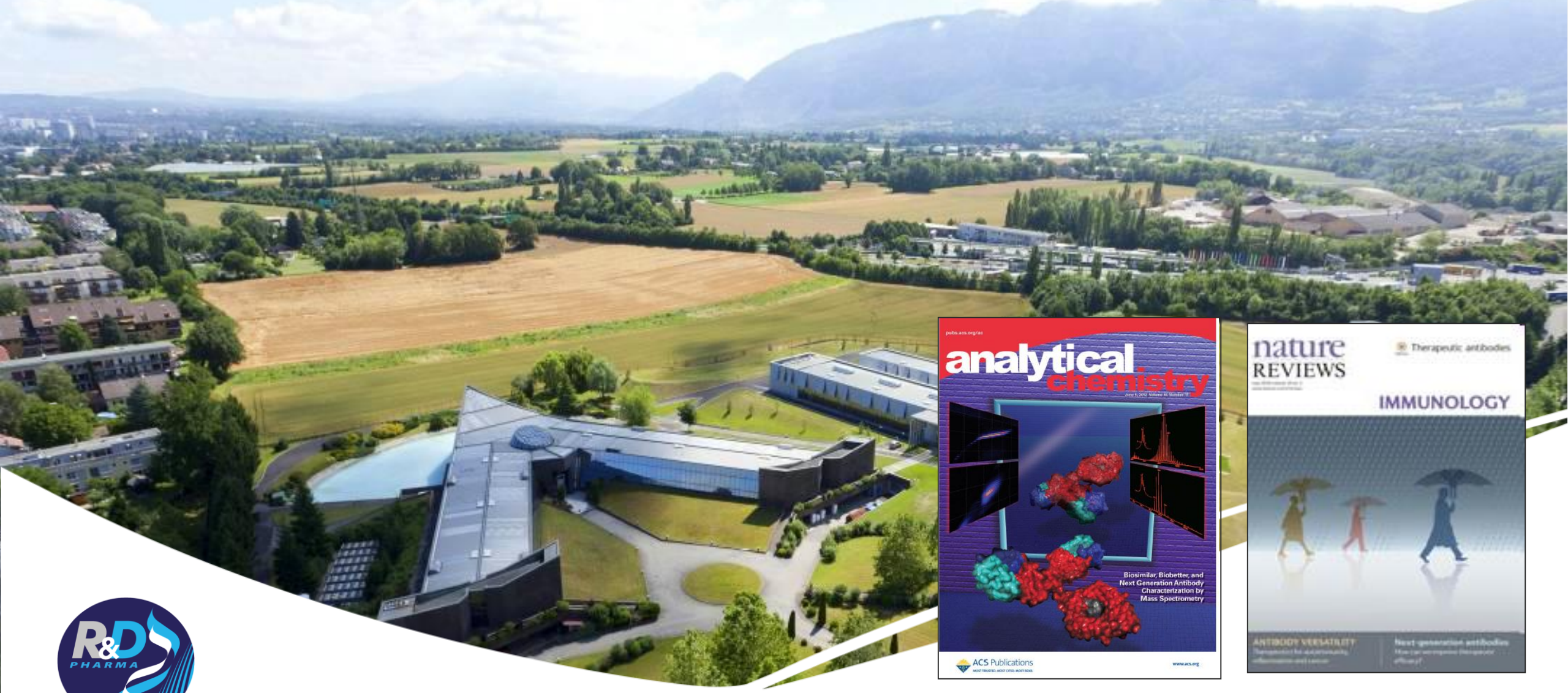


Chromatograms obtained for investigational hinge Cys-ADCs:

- **different mAbs**
- **isotypes (1, 2, 4)**
- **drug-linkers**
- **reducing agent &**
- **ratios**

➢ **Ehkirch A, D'Atri V, Rouvière F, Beck A, Guillarme D, Heinisch S, Cianférani S et al. Anal Chem 2018**

➢ **Beck A et al, Exp Rev Prot 2019**

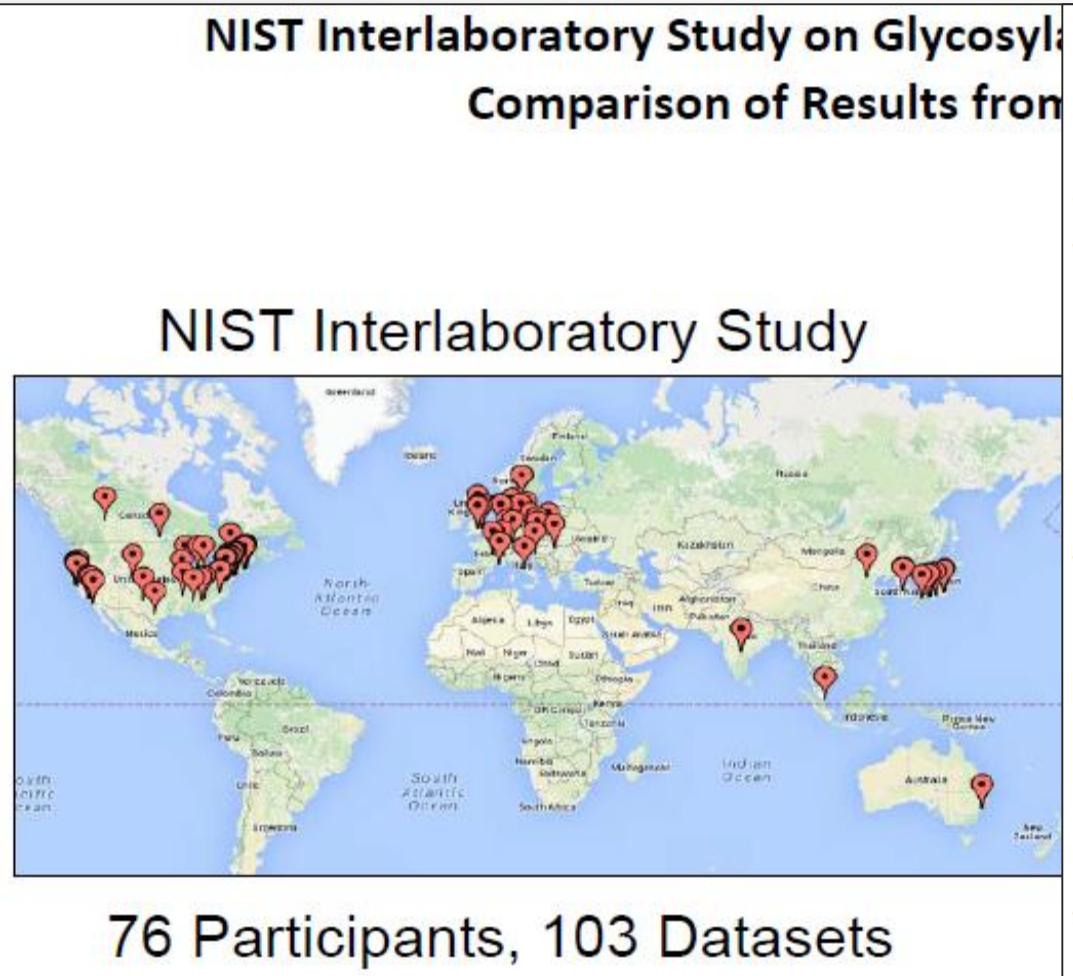


(5) HydrophILIC interaction Chromatography-MS (HILIC-MS) :

mAbs, biosimilars, Fc-fusions, ADCs…

Pierre Fabre

NISTmAb – Glyco-NIST collab. Study (2020)



NIST Interlaboratory Study on Glycosylation Analysis of Monoclonal Antibodies: Comparison of Results from Diverse Analytical Methods.

De Leoz MLA, Duewer DL, Fung A, Liu L, Yau HK, Potter O, Staples GO, Furuki K, Frenkel R, Hu Y, Sosic Z, Zhang P, Altmann F, Gruber C, Shao C, Zaia J, Evers W, Pangelley S, Suckau D, Wiechmann A, Resemann A, Jabs W, **Beck A**, Froehlich JW, Huang C, Li Y, Liu Y, Sun S, Wang Y, Seo Y, An HJ, Reichardt NC, Ruiz JE, Archer-Hartmann S, Azadi P, Bell L, Lakos Z, An Y, Cipollo JF, Pučić-Baković M, Štambuk J, Lauc G, Li X, Wang PG, Bock A, Hennig R, Rapp E, Creskey M, Cyr T, Nakano M, Sugiyama T, Leung PA, Link-Lenczowski P, Jaworek J, Yang SJ, Zhang H, Kelly T, Klapoetke S, Cao R, Kim JY, Lee HK, Lee J, Yoo JS, Kim SR, Suh SK, de Haan N, Falck D, Lageveen-Kammeijer GSM, Wuhrer M, Emery RJ, Kozak RP, Liew LP, Royle L, Urbanowicz PA, Packer N, Song X, Everest-Dass A, Lattová E, Cajic S, Alagesan K, Kolarich D, Kasali T, Lindo V, Chen Y, Goswami K, Gau B, Amunugama R, Jones R, Stroop CJM, Kato K, Yagi H, Kondo S, Yuen CT, Harazono A, Shi X, Magnelli P, Kasper BT, Mahal LK, Harvey DJ, O'Flaherty RM, Rudd P, Saldova R, Hecht ES, Muddiman DC, Kang J, Bhoskar P, Menard D, Saati A, Merle C, Mast S, Tep S, Truong J, Nishikaze T, Sekiya S, Shafer A, Funaoka S, Toyoda M, de Vreugd P, Caron C, Pradhan P, Tan NC, Mechref Y, Patil S, Rohrer JS, Chakrabarti R, Dadke D, Lahori M, Zou C, Cairo CW, Reiz B, Whittal RM, Lebrilla C, Wu LD, Guttman A, Szigeti M, Kremkow BG, Lee K, Sihlbom C, Adamczyk B, Jin C, Karlsson NG, Örnros J, Larson G, Nilsson J, Meyer B, Wiegandt A, Komatsu E, Perreault H, Bodnar ED, Said N, Francois YN, Leize-Wagner E, Maier S, Zeck A, Heck AJR, Yang Y, Haselberg R, Yu YQ, Alley W, Leone JW, Yuan H, Stein SE.

Mol Cell Proteomics. 2019 Oct 7. pii: mcp.RA119.001677. doi: 10.1074/mcp.RA119.001677. [Epub ahead of print]

PMID: 31591262 **Free Article**

- **Leoz L, Duewer D, Beck A & 100+ scientists. Mol Cell Proteomics 2020**

Glyco-analytics: originators vs biosimilars (2019)

Contents lists available at ScienceDirect

Analytica Chimica Acta

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

Glycosylation of biosimilars: Recent advances in analytical characterization and clinical implications

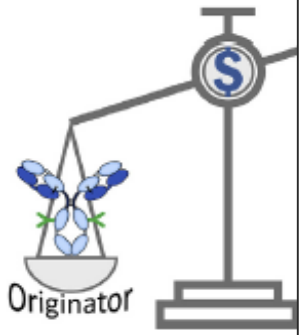
Bastiaan L. Duivelshof [a], Wim Jiskoot [b], Alain Beck [c], Jean-Luc Veuthey [a], Davy Guillarme [a], Valentina D'Atri [a, *]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU
[b] Division of BioTherapeutics, Leiden Academic Centre for Drug Research (LACDR), Leide
[c] Biologics and developability, IRPF, Center d'immunologie Pierre Fabre, St Julien-en-Gen

HIGHLIGHTS

- Multiple biosimilar products have become available for single originator biologics.
- Limitations in biological assays for the comparison of glycosylation of biosimilars.
- Novel analytical techniques for glycan analysis in biosimilar development.
- Clinical implications of glycan heterogeneity among multiple infliximab biosimilars.

GRAPHICAL A

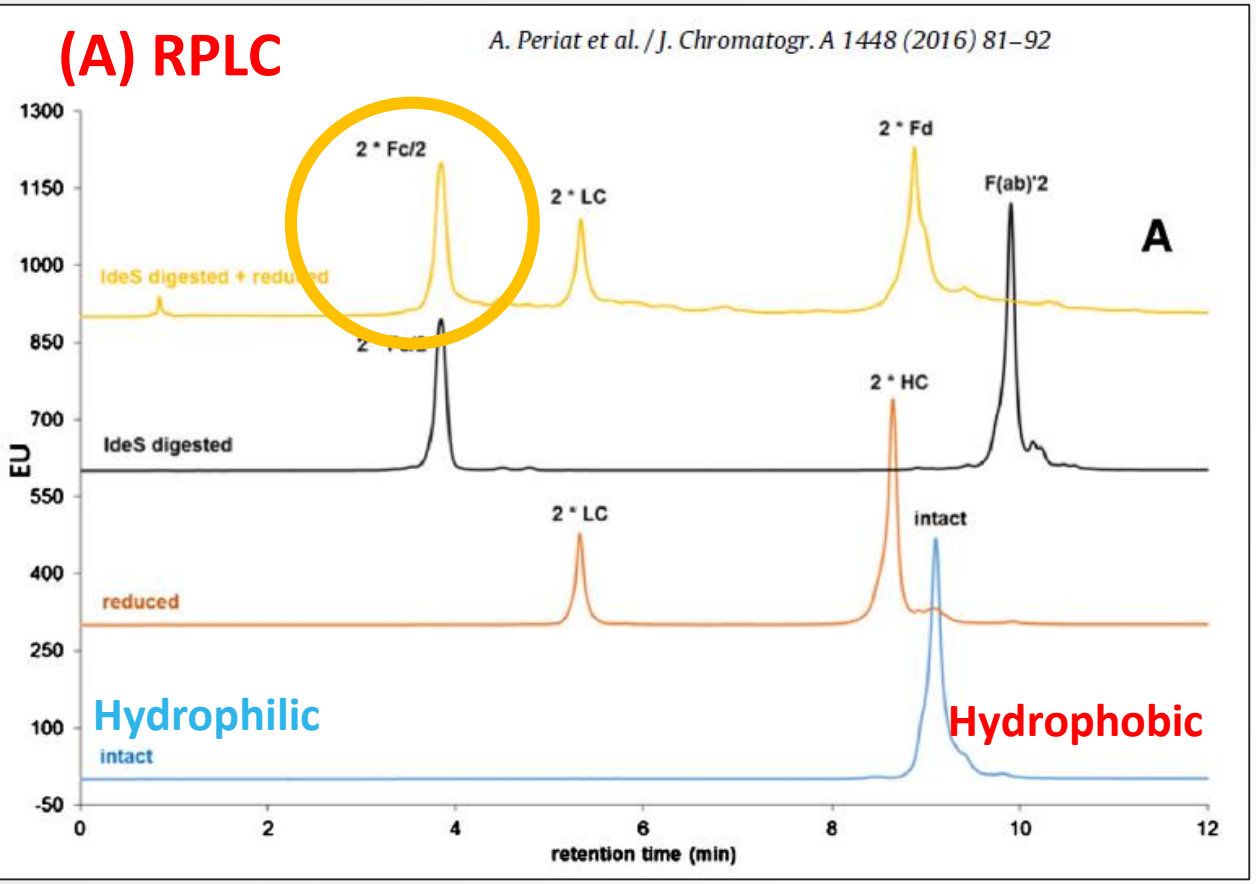


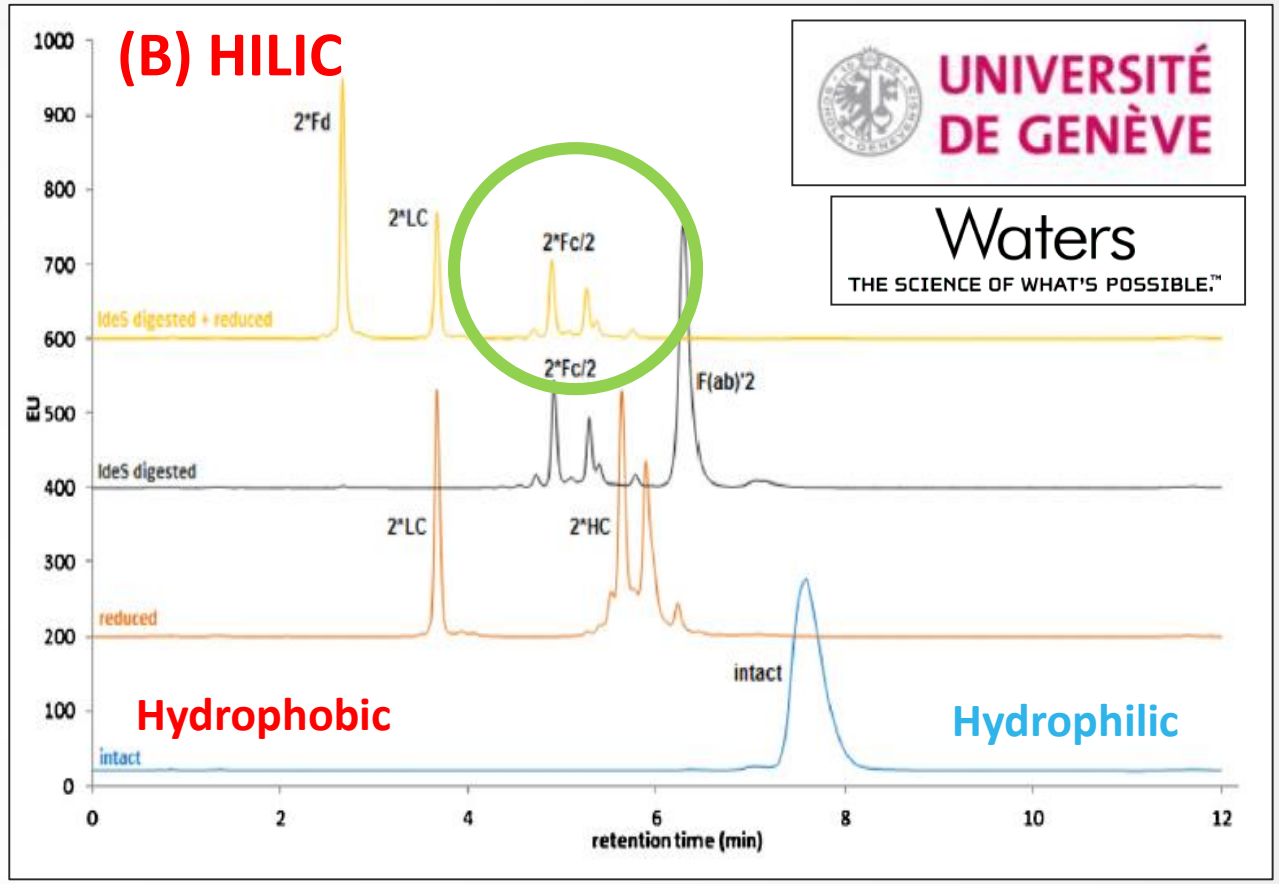
Biosimilars	EU	US
adalimumab	8	3
rituximab	6	1
trastuzumab	5	4
infliximab	4	3
etanercept	2	1
bevacizumab	2	1
Sum	27	13

Table 3
Novel analytical strategies for glycan analysis in biosimilar mAb development.

Method	Level of analysis	Obtained information	Site-specific information	Multi-attribute monitoring (MAM)	Comments	Ref.
HILIC-MS	Middle-up	Glycoform determination	No	Limited	Limited sample preparation allows the direct comparison of biosimilars.	[110,112]
2D-LC-MS	Middle-up	Glycoform determination	No	Multiple CQAs	Increased resolving power from multidimensional approach. Complex data analysis and high technical requirements.	[119,120]
IM-MS	glycopeptide	Isobaric glycopeptide and glycoform differentiation	Yes	No	Increased throughput by analysis of glycans and peptides directly after PNGase F release	[124,127]
	Intact	Glycan heterogeneity	No	No	Direct comparison of biosimilars on glycan heterogeneity and HOS differences on intact level. Limited resolving power.	[131,132]
site-specific enzymatic digestion	Peptide	Limited glycoform determination	Yes- Quantitative	No	Only differentiation between high-mannose- or complex-type glycans possible. However, site-specific glycan occupancy information is available.	[141]
	glycopeptide	Glycoform determination	Yes	No	Allows qualitative site-specific glycan determination and total glycan occupancy levels.	[75]
MAM	glycopeptide	Glycoform determination	Yes	Multiple CQAs	Fully ICH-validated platforms available for MAM-monitoring. Essential for the implementation of QbD approaches	[144,146,176]

IgGs (IdeS): RPLC vs HILIC (HydrophILIC interaction Chrom.)





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=> Orthogonal methods

Drs. D. Guillarme, V. D'Atri & coll.

- Peria A, Fekete S, Cusumano A, Veuthey JL, Beck A, Lauber M, Guillarme D. J Chrom A 2016
- Bobály B, D'Atri V, Beck A, Guillarme D, Fekete S. JPBA 2017
- D'Atri V, Fekete S, Beck A, Lauber M, Guillarme D. Anal Chem 2017
- D'Atri V, Beck A, Guillarme D, Beck A et al, J Chrom B 2018 (ADCs)
- Stoll D, D'Atri V, Guillarme D, Beck A et al, Anal Chem 2018 (2D-LC-MS)

Etanercept N & O-glycans: HILIC-MS, middle-up (2019)

analytical chemistry

Article

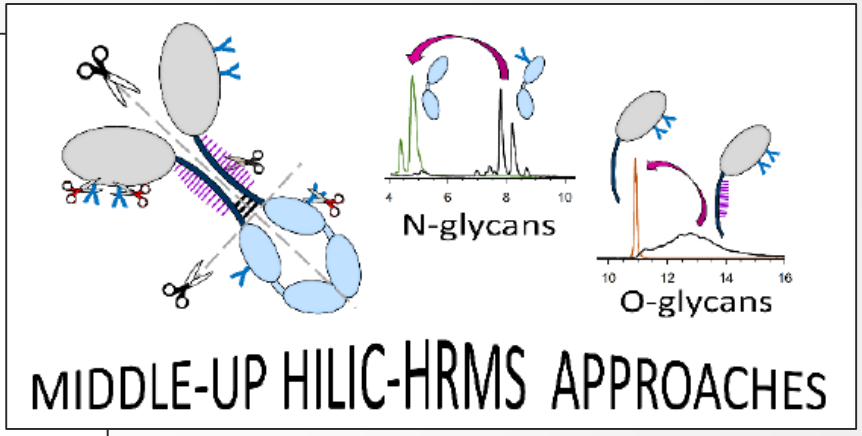
Cite This: *Anal. Chem.* 2019, 91, 873–880

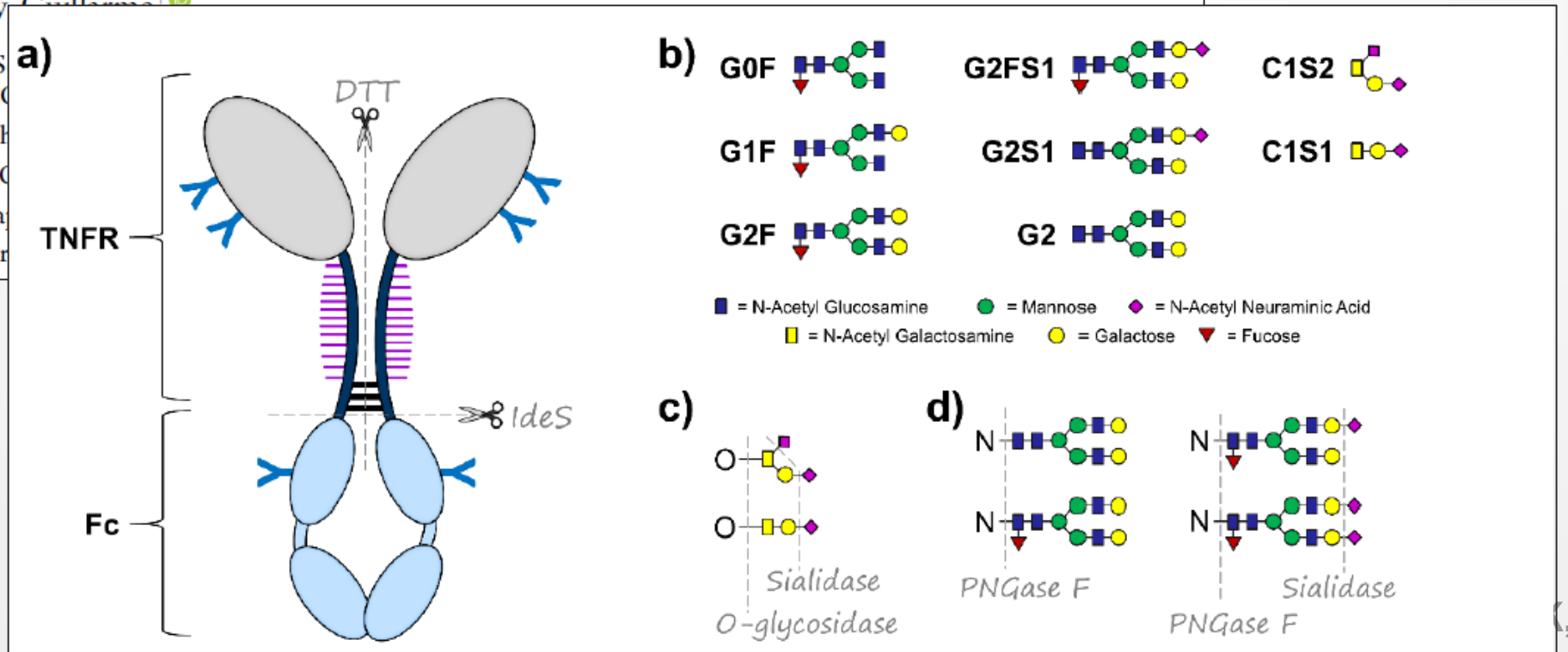
pubs.acs.org/ac

Orthogonal Middle-up Approaches for Characterization of the Glycan Heterogeneity of Etanercept by Hydrophilic Interaction Chromatography Coupled to High-Resolution Mass Spectrometry

Valentina D'Atri,[*,†,#] Lucie Nováková,[‡,#] Szabolcs Fekete,[†] Dwight Stoll,[§] Matthew Lauber,[∥] Alain Beck,[⊥] and Davy Guillarme[†]

[†]Section of Pharmaceutical S
Rue Michel Servet 1, 1211 C
[‡]Department of Analytical Ch
[§]Department of Chemistry, C
[∥]Waters Corporation, 34 Ma
[⊥]Center of Immunology Pier





ADCs (IdeS): HILIC-MS (glycans + payloads DLD)

Journal of Chromatography B 1080 (2018) 37–41

Contents lists available at ScienceDirect

Journal of Chromatography B

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

Short communication

Characterization of an antibody-drug conjugate by hydrophilic interaction chromatography coupled to mass spectrometry☆

Valentina D'Atri[a,*], Szabolcs Fekete[a], Dwight Stoll[b], Matthew Lauber[c], Alain Beck[d], Davy Guillarme[a]

[a] School of Pharmaceutical Sciences, University of Geneva, University of Lausanne,
[b] Department of Chemistry, Gustavus Adolphus College, Saint Peter, Minnesota 560
[c] Waters Corporation, 34 Maple Street, Milford, Massachusetts 01757-3696, Unite
[d] Center of Immunology Pierre Fabre, 5 Avenue Napoléon III, BP 60497, Saint-Juli

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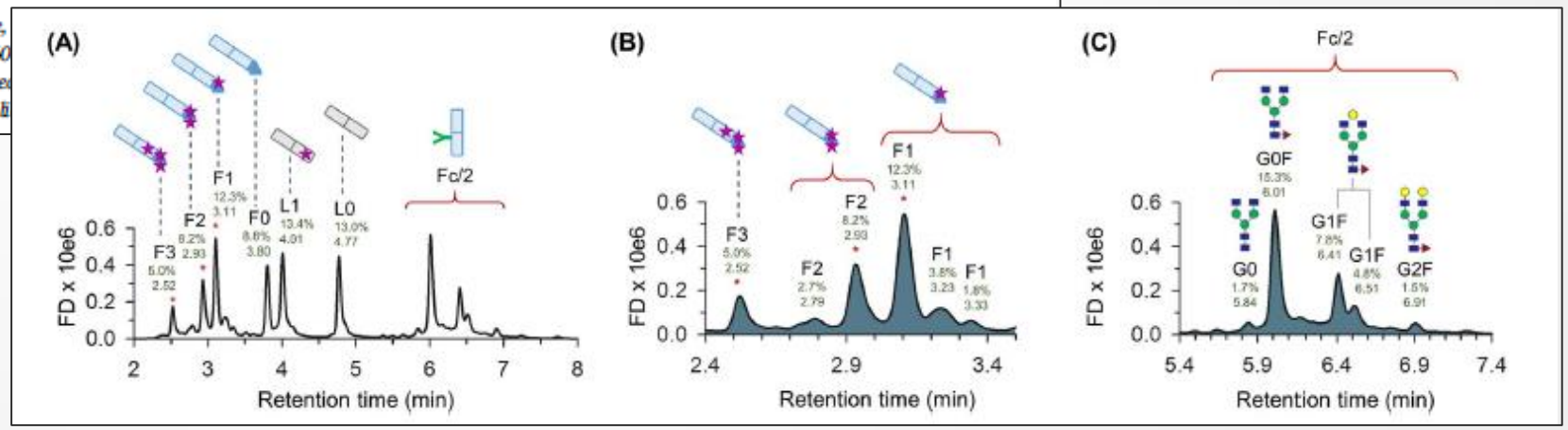
Drs V. D'Atri, D. Guillarme & coll.



⇒ **HILIC orthogonal to RP-HPLC**

Cutting-Edge Analytical methods for ADCs: HILIC-MS

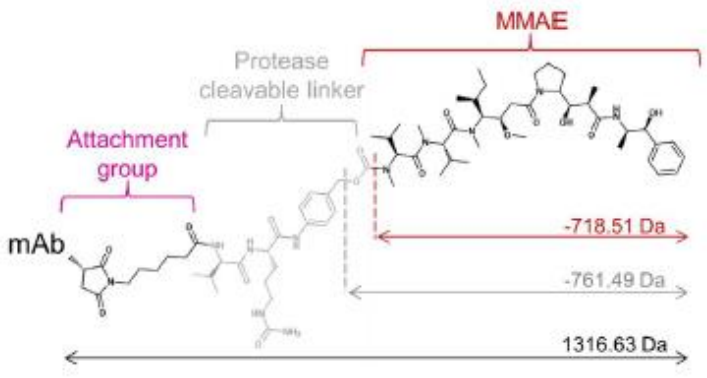
(A) Brentuximab vedotin light chain sequence

DIVLTQSPASLAVSLGQRATISCKASQSVDFDGDSY
MNWYQQKPGQPPKVLIYAASNLESGIPARFSGSGS
GTDFTLNIHPVEEEDAATYYCQQSNEDPWTFGGGT
KLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNF
YPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSF
NRGEC

(B) Brentuximab vedotin heavy chain sequence

(pE)QIQLQQSGPEVVKPGASVKISCKASGYTFTDYY
ITWVKQKPGQGLEWIGWIYPGSGNTKYNEKFKGKA
TLTVDTSSSTAFMQLSSLTSEDTAVYFCANYGNYWF
AYWGQGTQVTVSAASTKGPSVFPLAPSSKSTSGG
TAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTK
VDKKVEPKSCDKTHTCPPCPAPELLG/GPSVFLFPP
KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD
GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTL
PPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ
PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGN
VFSCSVMHEALHNHYTQKSLSLSPGK

(C) Brentuximan vedotin drug payload



HILIC-MS middle-up analysis of brentuximab vedotin. Subunit retention times and mass assignments. The following nomenclature has been used for the glycan assignments: H = hexose (mannose/galactose); N = N-acetyl glucosamine; F = fucose. Q/pE stands for pyroglutamic acid formation.

tr (min)	Assignment	Trivial assignment	Theoretical mass (Da)	Experimental mass (Da)	Δm (Da)
2.52	Fd′ (Q/pE) + 3drugs	F3	29088.22	29088.56	0.3
	Fd′ (Q/pE) + 3drugs - MMAE	F3-MMAE	28326.73	28327.05	0.3
2.79	Fd′ (Q/pE) + 2drugs	F2	27771.60	27772.16	0.6
2.93	Fd′ (Q/pE) + 2drugs	F2	27771.60	27771.48	0.1
	Fd′ (Q/pE) + 2drugs - MMAE	F2-MMAE	27010.11	27009.29	0.8
3.11	Fd′ (Q/pE) + 1 drug	F1	26454.97	26454.76	0.2
3.23	Fd′ + 1 drug	F1	26472.00	26472.00	0.0
3.33	Fd′ (Q/pE) + 1 drug + intra S-H	F1	26459.00	26459.33	0.2
3.80	Fd′ (Q/pE)	F0	25138.35	25138.06	0.3
4.01	LC + 1 drug	L1	25040.86	25040.47	0.4
	LC + 1 drug - MMAE	L1-MMAE	24279.37	24278.70	0.7
4.77	LC	L0	23724.23	23723.74	0.5
5.84	Fc/2 + H3N4	Fc/2 + G0	25054.07	25053.15	0.9
6.01	Fc/2 + H3N4F1	Fc/2 + G0F	25200.22	25199.62	0.6
6.41	Fc/2 + H4N4F1	Fc/2 + G1Fa	25362.36	25361.69	0.7
6.51	Fc/2 + H4N4F1	Fc/2 + G1Fb	25362.36	25361.75	0.6
6.91	Fc/2 + H5N4F1	Fc/2 + G2F	25524.50	25524.04	0.5

GlyGLICK ADCs (Genovis): HILIC-MS (Anal Chem 2020)

Glycan-mediated technology for obtaining homogeneous site-specific conjugated antibody-drug conjugates: synthesis and analytical characterization by using complementary middle-up LC/HRMS analysis

Bastiaan L. Duivelshof,† Evolène Deslignière,‡ Oscar Hernande
Toftevall,‡ Jonathan Sjögren,‡ Sarah Cianferani,‡ Alain Beck,§ Davy

†Institute of Pharmaceutical Sciences of Western Switzerland, University of Genev
4, Switzerland.

‡Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Un
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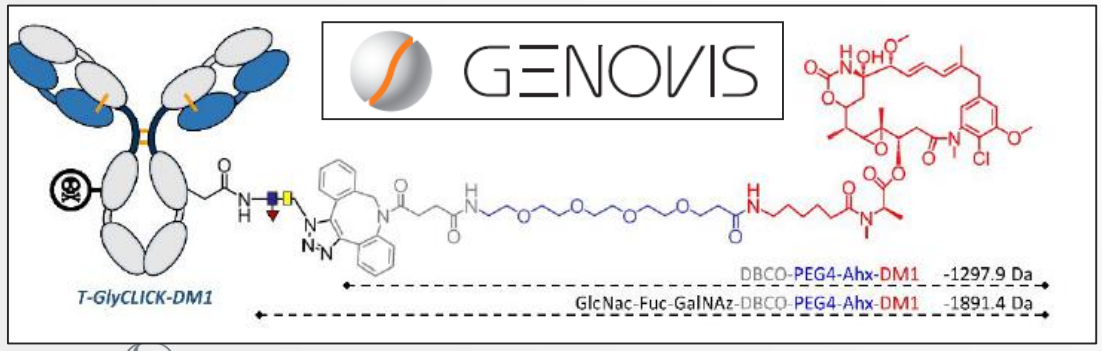
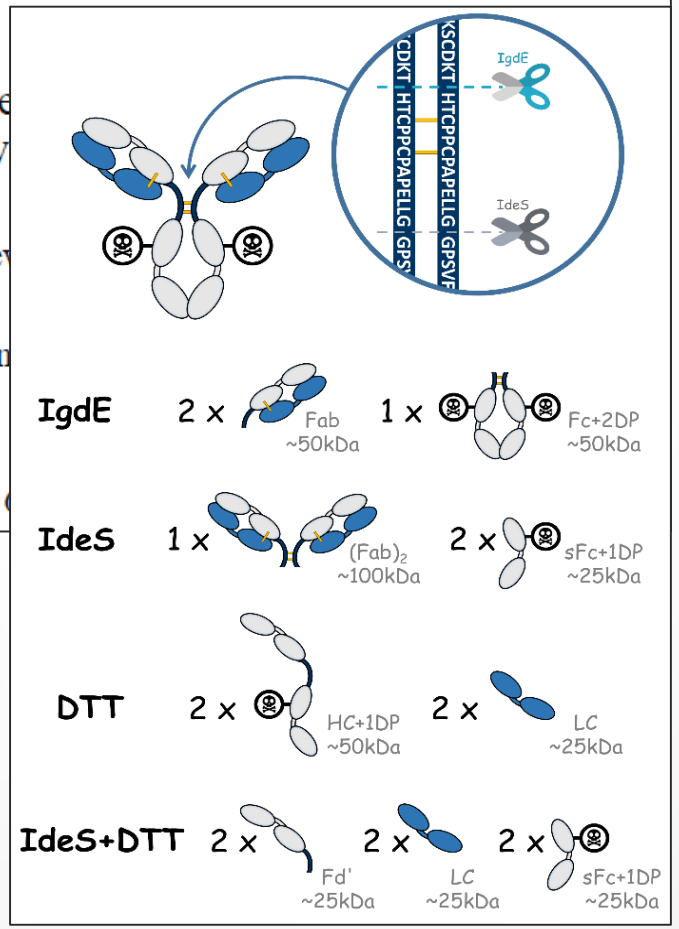
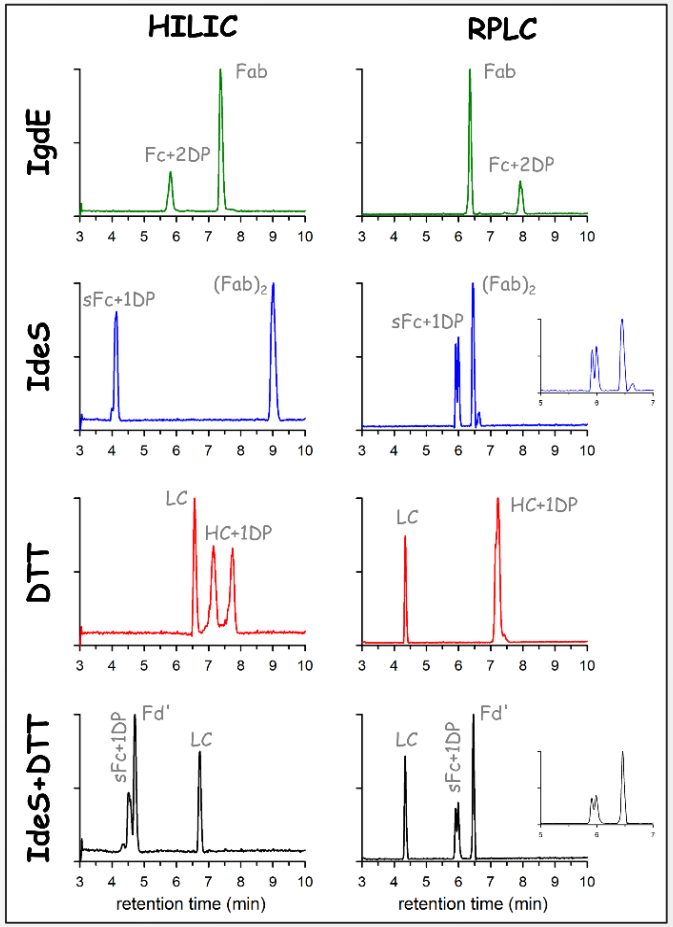
‡Genovis AB, Box 790, SE-220 07 Lund, Sweden.

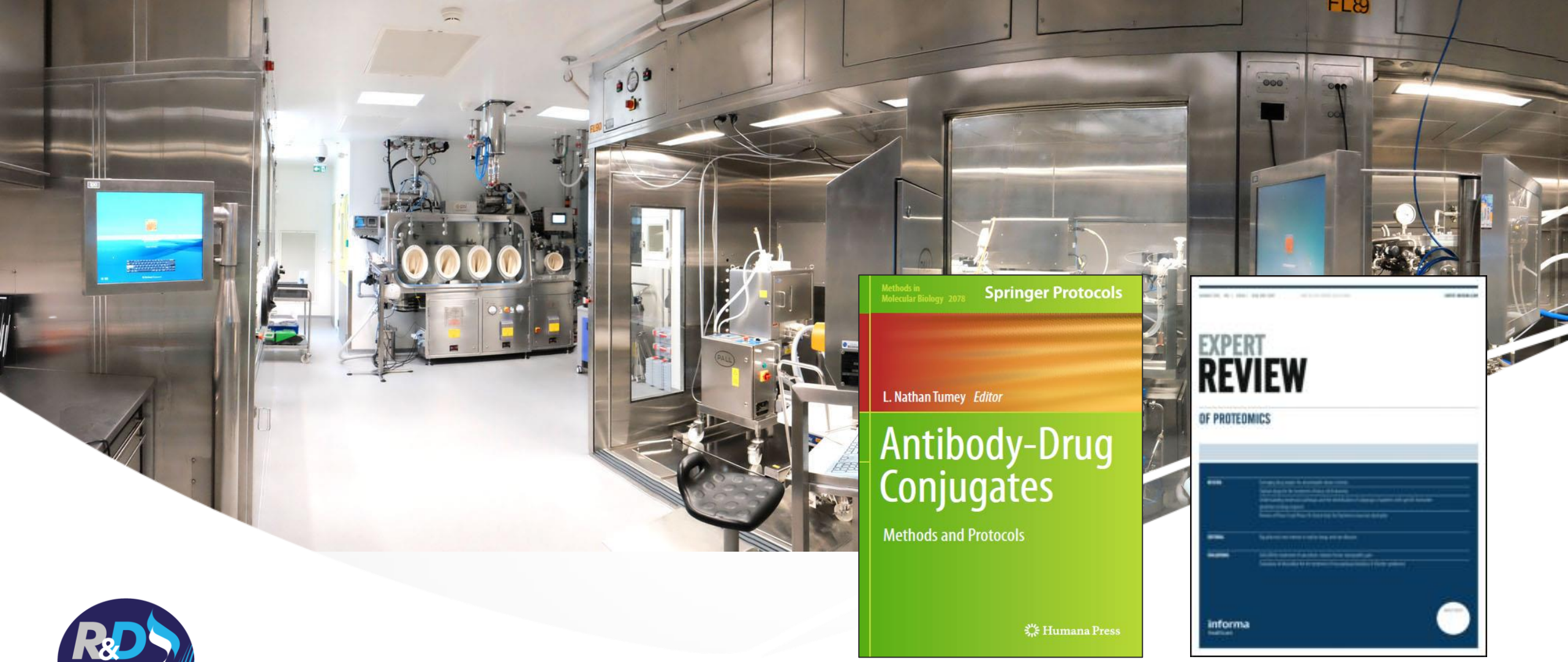
§IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), 5 Avenue Napoléon III, BP

Drs V. D'Atri, D. Guillarme & coll.









Pierre Fabre

(5) Take home messages:

networking, open research, white papers

Cutting-edge analytical & structural network: antibody-based drugs (2005-20: +200 papers*, +160 talks)**

OptimAbs

2010-13

Université de Strasbourg

Laboratoire de Spectrométrie de Masse Bio-Organique LSMBO

LSMIS

Université de Genève

EPFL École polytechnique fédérale de Lausanne

MERCK

Université de Lyon

isa Institut des Sciences Analytiques

Faculté de Pharmacie Aix-Marseille Université

Gustavus Gustavus Adolphus College

Spectroswiss

abbvie

GENOVIS

Quality Assistance Contract Research Organisation

SGS

promise Advanced Proteomics

ANAQUANT

IGENICA Biotherapeutics

FDA

Waters The science of what's possible.™

BRUKER

Thermo Scientific

eurofins

RAPID NOVOR Speed up discovery

Catalent

AB SCIEX

Agilent Technologies

Beckman Coulter

usp

PEAKS AB Antibody Characterization Service http://www.bioinfor.com/antibodyservice/

affilogic

World Health Organization

MAB Working Party

edqm European Directorate for the Quality of Medicines & HealthCare

ansm Agence nationale de sécurité du médicament et des produits de santé

NIST National Institute of Standards and Technology U.S. Department of Commerce

Chairman COSSF

MABDESIGN

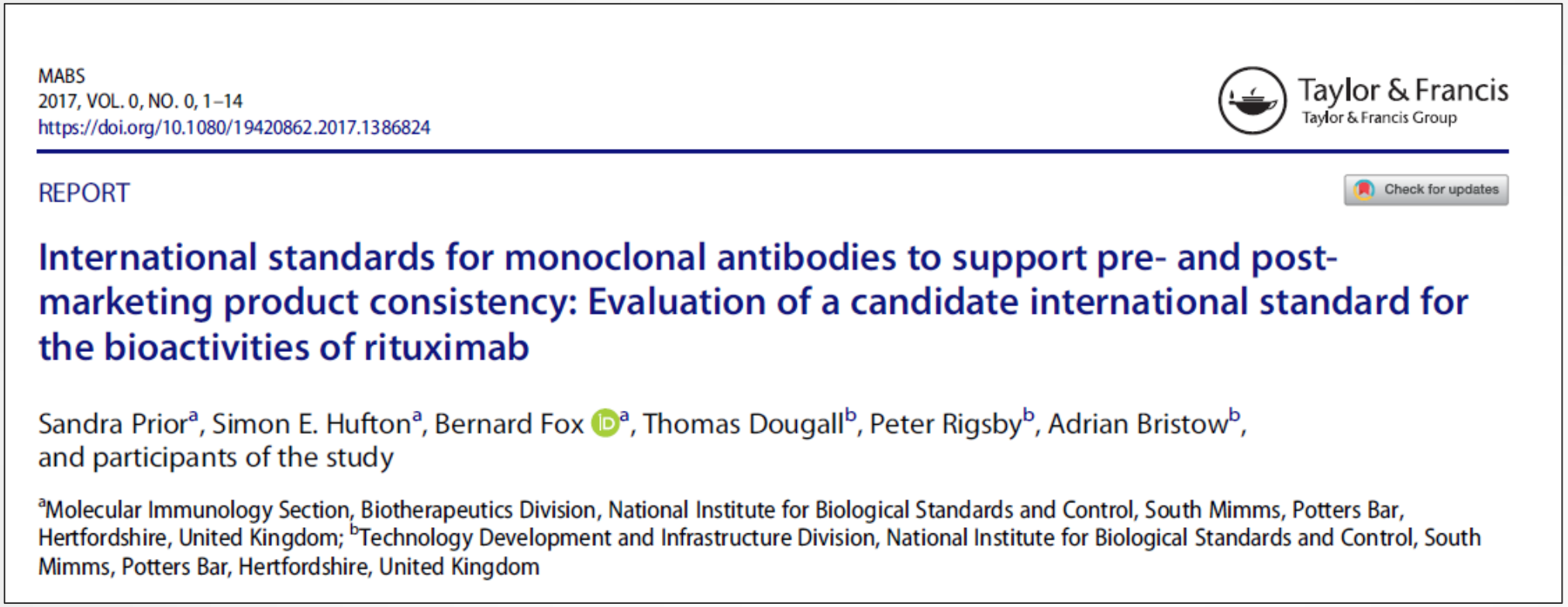
Associate Editor

mAbs

* IF50, +10,700 citations

** Open research & innovation

Member of EDQM MAB working group (2017-22)*



MABS
2017, VOL. 0, NO. 0, 1–14
https://doi.org/10.1080/19420862.2017.1386824

Taylor & Francis
Taylor & Francis Group

REPORT

International standards for monoclonal antibodies to support pre- and post-marketing product consistency: Evaluation of a candidate international standard for the bioactivities of rituximab

Sandra Prior[a], Simon E. Hufton[a], Bernard Fox[a], Thomas Dougall[b], Peter Rigsby[b], Adrian Bristow[b], and participants of the study

[a]Molecular Immunology Section, Biotherapeutics Division, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire, United Kingdom; [b]Technology Development and Infrastructure Division, National Institute for Biological Standards and Control, South Mimms, Potters Bar, Hertfordshire, United Kingdom

- αTNF
- Functional tests (Fab/Fc)
- SEC
- cIEF
- CE-SDS
- CZE

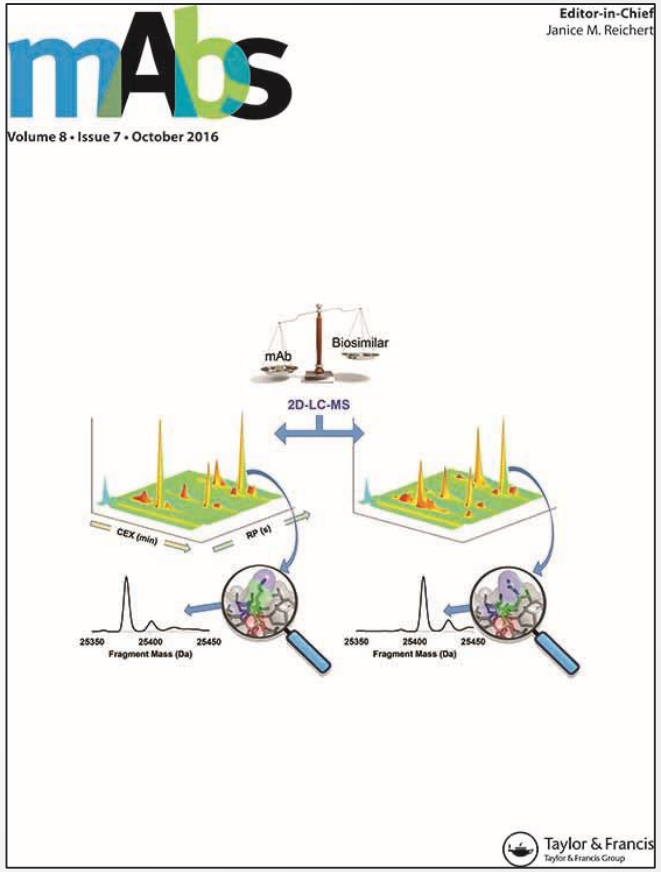
***EDQM (PhEur) +**

- **European National Competent Authorities (eg ANSM, PEI…)**
- **Australia, Canada, South Korea, Taiwan…**
- **AstraZeneca, Lilly, Lonza, Merck, Novartis, Pierre Fabre, Sanofi, UCB**

https://www.ema.europa.eu/en/partners-networks/eu-partners/eu-member-states/national-competent-authorities-human

mAbs: 2009-2020 (Open access)

2019 Impact Factor: 4.634



www.tandfonline.com/toc/kmab20/current

MABS
https://doi.org/10.1080/19420862.2018.1553476

(2019)

Taylor & Francis
Taylor & Francis Group

REVIEW

Structure, het

Yingda Xu[a], Dongc
Wei Xu[g], Smita Ra
and Hongcheng Li

[a]Protein Analytics, Adi
Pharmaceuticals, Inc., N
Development, Regener
[g]Analytical Method Dev
USA; [i]Analytical Develo
[k]Product development,
en-Genevois Cedex, Fra

ABSTRACT
Increasing attentic
evaluation of mon
development. The
development of ap
lessons learned fro
antibody quality a
methods to moni
proposed as a be
characterization a
tion with limited r

MABS
2017, VOL. 9, NO. 8, 1217–1230
https://doi.org/10.1080/19420862.2017.1368602

(2017)

Taylor & Francis
Taylor & Francis Group

REVIEW

Forced degrad

Christine Nowak[a], J
Gomathinayagam P

[a]Product Characterizatio
USA; [c]Biologics and Vacc
USA; [e]Millennium Resear
Chemistry, NBEs, Center

ABSTRACT
Forced degradatio
antibody therapeut
supporting compar
the regulatory guic
various agencies su
purposes for forced
under each conditi

MABS
2018, VOL. 0, NO. 0, 1–26
https://doi.org/10.1080/19420862.2018.1438797

(2018)

Taylor & Francis
Taylor & Francis Group

Check for updates

REVIEW

Analytical comparability study of recombinant monoclonal antibody therapeutics

Alexandre Ambrogelly[a], Stephen Gozo[b], Amit Katiyar[c], Shara Dellatore[d], Yune Kune[e], Ram Bhat[f], J
Dongdong Wang[j], Christine Nowak[j], Alyssa Neill[j], Gomathinayagam Ponniah[j], Cory King[j], Bruce M
and Hongcheng Liu[j]

[a]Analytical development, Gilead, 333 Lakeside Drive, Foster City, CA; [b]Analytical Research & Development-Biologics, Celger
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790 Memorial Drive, Cambridge, MA; [j]Product Characterization, Alexion Pharmaceuticals, 100 College Street, New Haven, C
Pharmaceuticals, 100 College Street, New Haven, CT ; [l]Analytical Chemistry, NBEs, Center d'Immunologie Pierre Fabre, St Ju
France

ABSTRACT
Process changes are inevitable in the life cycle of recombinant monoclonal antibody therapeutics. Products made using pre- and post-change processes are required to be comparable as demonstrated by comparability studies to qualify for continuous development and commercial supply. Establishment of comparability is a systematic process of gathering and evaluating data based on scientific understanding and clinical experience of the relationship between product quality attributes and their impact on safety and efficacy. This review summarizes the current understanding of various modifications of recombinant monoclonal antibodies. It further outlines the critical steps in designing and executing successful comparability studies to support process changes at different stages of a product's lifecycle.

White papers:
AbbVie
Adimab
Alexion
BioAnalytix
BMS
Gilead
Innovent
Merck
Millenium
Pierre Fabre
Regeneron

Dr. Hongcheng Liu

Pierre Fabre

antibodies

MDPI

Antibodies (2019)

Dr. Hongcheng Liu

Review

Macro- and Micro-Heterogeneity of Natural and Recombinant IgG Antibodies

Alain Beck [1,*] and **Hongcheng Liu** [2,*]

[1] Biologics CM
74160 Saint-J
[2] Anokion, 50
* Correspond

Received: 22 Dec

Abstract: Reco
be thoroughly
the structures a
are highly rele
Small structura
size, charge or
stability, pharm
as found in enc
The knowledg
of the drug and
of the current u
recombinant m

Keywords: cri
product profile

Table 1. Micro-heterogeneity natural IgGs and recombinant mAbs.

Modifications	Natural	Recombinant	Resulting Heterogeneity
N-terminal modifications			
PyroGlu	100% pyroGlu	Varied levels	Mass, charge for Gln to pyroGlu
Truncation	Not expected	Rare and low	Mass
Signal peptides	Not expected	Low	Mass and charge
Asn deamidation	Substantial level	Common, varied levels	Mass and charge
Asp isomerization	Not expected	Common, varied levels	Charge and hydrophobicity
Succinimide	Not expected	Common, varied levels	Mass, charge, and hydrophobicity
Oxidation	Low	Met, Trp, Cys, His	Mass and hydrophobicity
Cysteine related modifications			
Free cysteine	Low	Low	Mass, charge and hydrophobicity
Alternative disulfide bond linkage	Common	Common	Charge
Trisulfide bond	Extremely low	Low	Mass and charge
Thioether	Low	Low	Mass
Glycosylation	Common	Common	Mass and charge
Glycation	Common	Common	Mass and charge
C-terminal modifications			
C-terminal Lys	Complete removal	Common, varied levels	Mass, charge and hydrophobicity
C-terminal modifications	Not detected	Low varied levels	Mass and charge

ADC DS/DP: methods and monographs

EXPERT REVIEW OF PROTEOMICS, 2016
http://dx.doi.org/10.1586/14789450.2016.1132167

(2016)

Taylor & Francis
Taylor & Francis Group

REVIEW

Cutting-edge mass spectrome characterization of antibody-c

Alain Beck[a], Guillaume Terral[b,c], François De Bussat[a], Olivier Colas[a], Alain Van Dorsselaer[b]

[a]Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Ju Analytical Sciences Department, Université de Stras Strasbourg, France

EXPERT REVIEW OF PROTEOMICS
https://doi.org/10.1080/14789450.2019.1578215

(2019)

Taylor & Francis
Taylor & Francis Group

REVIEW

Check for updates

Cutting-edge multi-level analytical and structural characterization of antibody-drug conjugates: present and future

Alain Beck[a], Valentina D'Atri[b], Anthony Ehkirch[c], Szabolcs Fekete[b], Oscar Hernandez-Alba[c], Rabah Gahoual[d], Emmanuel Leize-Wagner[d], Yannis François[d], Davy Guillarme[a] and Sarah Cianférani[c]

[a]IRPF - Centre d'Immunologie Pierre-Fabre (CIPF), Saint-Julien-en-Genevois, France; [b]School of Pharmaceutical Sciences, University of Geneva, University of Lausanne, CMU, Geneva, Switzerland; [c]Laboratoire de Spectrométrie de Masse BioOrganique, IPHC UMR 7178, Université de Strasbourg, CNRS, Strasbourg, France; [d]Laboratoire de Spectrométrie de Masse des Interactions et des Systèmes (LSMIS), UMR 7140, Université de Strasbourg, CNRS, Strasbourg, France

SMD
Drug-Linker intermediate
(Intermediate, 0.3-1.5 kDa)

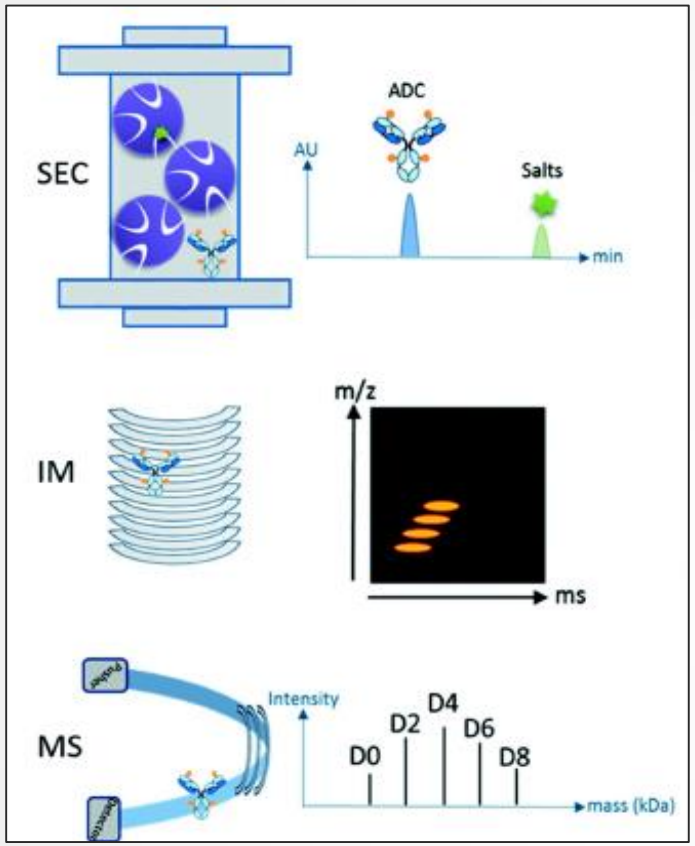
mAb
(Intermediate, 150 kDa)

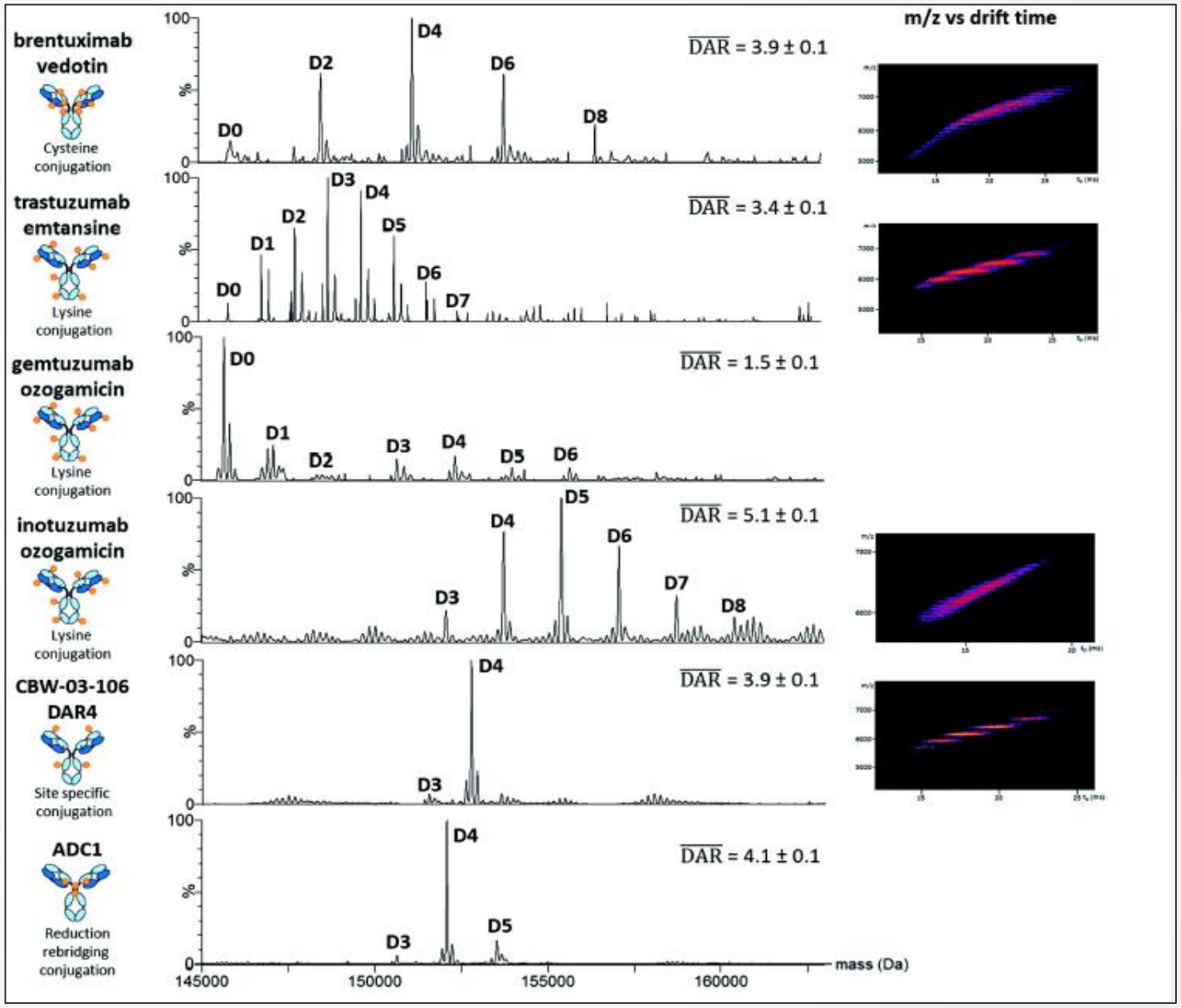
ADC-DS
(API, 156 kDa, avDAR4)

ADC-DP
(Liquid or freeze dried)

Laboratoire de Spectrométrie de LSMBO Masse Bio-Organique

1st, 2d & 3G ADCs: SEC-IM-MS (2019)





➢ **Beck A, Guillarme D, François Y,Cianferai S et al. Exp Rev Proteomics 2019**

Telisotuzumab (Hz224G4, ABT-700; cMet) - 2016

IJC
International Journal of Cancer
Int. J. Cancer: 139, 1851–1863 (2016)

A novel antagonist anti-cMet antibody with antitumor activities targeting both ligand-dependent and ligand-independent c-Met receptors

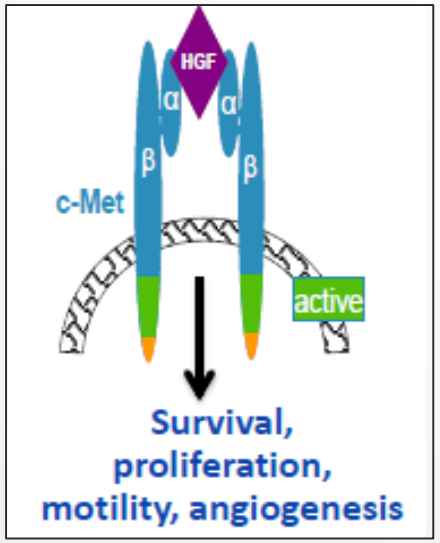
Alexandra Gonzalez, Matthieu Broussas, Charlotte Beau-Larvor, Jean-François Haeuw, Nicolas Boute, Alain Robert, Thierry Champion, Alain Beck, Christian Bailly, Nathalie Corvaïa and Liliane Goetsch

Centre D'Immunologie Pierre Fabre 5, IRPF, Av Napoléon III, F-74164, Saint-Julien-en-Genevois, France

Pierre Fabre

abbvie

NCT01472016



BMC Cancer
Wang et al. BMC Cancer (2016) 16:105
DOI 10.1186/s12885-016-2138-z

RESEARCH ARTICLE Open Access

Anti-c-Met monoclonal antibody ABT-700 breaks oncogene addiction in tumors with *MET* amplification

Jieyi Wang[1,4*], Liliane Goetsch[2], Lora Tucker[1], Qian Zhang[1], Alexandra Gonzalez[2], Kedar S. Vaidya[1], Anatol Oleksijew[1], Erwin Boghaert[1], Minghao Song[3], Irina Sokolova[3], Ekaterina Pestova[3], Mark Anderson[1], William N. Pappano[1], Peter Ansell[1], Anahita Bhathena[1], Louie Naumovski[4], Nathalie Corvaia[2] and Edward B. Reilly[1]

Telisotuzumab vedotin (cMet, PhII, NSCLC) - 2018

Cancer Therapy: Preclinical

Clinical Cancer Research

ABBV-399, a c-Met Antibody–Drug Conjugate that Targets Both *MET*–Amplified and c-Met–Overexpressing Tumors, Irr*MET* Pathway Dependence

Jieyi Wang[1], Mark G. Anderson[1], Anatol Oleks
Lora Tucker[1], Qian Zhang[1], Edward K. Han[1], J
Edward B. Reilly[1]

NCT02099058 (PhI)
NCT03539536 (PhII, NSCLC)

abbvie

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

First-in-Human Phase I, Dose-Escalation and -Expansion Study of Telisotuzumab Vedotin, an Antibody–Drug Conjugate Targeting c-Met, in Patients With Advanced Solid Tumors

John H. Strickler, Colin D. Weekes, John Nemunaitis, Ramesh K. Ramanathan, Rebecca S. Heist, Daniel Morgensztern, Eric Angevin, Todd M. Bauer, Huibin Yue, Monica Motwani, Apurvasena Parikh, Edward B. Reilly, Daniel Afar, Louie Naumovski, and Karen Kelly

Author affiliations and support information (if applicable) appear at the end of this article.

Published at jco.org on October 4, 2018.

Clinical trial information: NCT02099058.

Corresponding author: John H. Strickler,

A B S T R A C T

Purpose

This first-in-human study evaluated telisotuzumab vedotin (Teliso-V), formerly called ABBV-399, an antibody–drug conjugate of the anti–c-Met monoclonal antibody ABT-700 and monomethyl auristatin E.

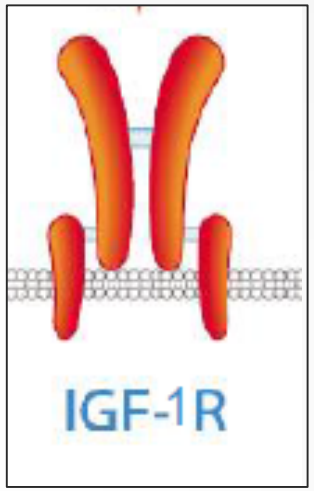
IGFR-1 ADC: W0101 (2020)

Pierre Fabre

MOLECULAR CANCER THERAPEUTICS | LARGE MOLECULE THERAPEUTICS

Efficacy of the Antibody–Drug Conjugate W0101 in Preclinical Models of IGF-1 Receptor Overexpressing Solid Tumors

Barbara Akla[1], Matthieu Broussas[1], Noureddine Loukili[1], Alain Robert[1], Charlotte Beau-Larvor[1], Martine Malissard[1], Nicolas Boute[1], Thierry Champion[1], Jean-Francois Haeuw[1], Alain Beck[1], Michel Perez[2], Cyrille Dreyfus[1], Mariya Pavlyuk[2], Eric Chetaille[2], and Nathalie Corvaia[1]



NCT03316638

ABSTRACT

The insulin-like growth factor type 1 receptor (IGF-1R) is important in tumorigenesis, and its overexpression occurs in numerous tumor tissues. To date, therapeutic approaches based on mAbs and tyrosine kinase inhibitors targeting IGF-1R have only shown clinical benefit in specific patient populations. We report a unique IGF-1R–targeted antibody–drug conjugate (ADC), W0101, designed to deliver a highly potent cytotoxic auristatin derivative selectively to IGF-1R overexpressing tumor cells. The mAb (hz208F2-4) used to prepare the ADC was selected for its specific binding properties to IGF-1R compared with the insulin receptor, and for its internalization properties. Conjugation of a novel auristatin derivative drug linker to hz208F2-4 did not alter its binding and internalization properties. W0101 induced receptor-dependent cell cytotoxicity *in vitro* when applied to various cell lines overexpressing IGF-1R, but it did not affect normal cells. Efficacy studies were conducted in several mouse models expressing different levels of IGF-1R to determine the sensitivity of the tumors to W0101. W0101 induced potent tumor regression in certain mouse models. Interestingly, the potency of W0101 correlated with the expression level of IGF-1R evaluated by IHC. In an MCF-7 breast cancer model with high-level IGF-1R expression, a single injection of W0101 3 mg/kg led to strong inhibition of tumor growth. W0101 provides a potential new therapeutic option for patients overexpressing IGF-1R. A first-in-human trial of W0101 is currently ongoing to address clinical safety.

ADC Landscape : Pharmaceuticals (2020)

pharmaceuticals

MDPI

Review

Antibody–Drug Conjugates: The Last Decade

Nicolas Joubert [1,], Alain Beck [2], Charles Dumontet [3,4,5] and Caroline Denevault-Sabourin [1]

1 GICC EA7501, Equipe IMT, Université de Tours, UFR des Sciences Pharmaceutiques, 31 Avenue Mor 37200 Tours, France; caroline.denevault@univ-tours.fr
2 Institut de Recherche Pierre Fabre, Centre d'Immunologie Pierre Fabre, 5 Avenue Napoléon III, 74160 Saint Julien en Genevois, France; alain.beck@pierre-fabre.com
3 Cancer Research Center of Lyon (CRCL), INSERM, 1052/CNRS 5286/UCBL, 69000 Lyon, France; charles.dumontet@chu-lyon.fr
4 Université de Lyon, 69000 Lyon, France
5 Hospices Civils de Lyon, 69000 Lyon, France
* Correspondence: nicolas.joubert@univ-tours.fr

Received: 17 August 2020; Accepted: 10 September August 2020; Published: in press

Abstract: An armed antibody (antibody–drug conjugate or ADC) is a vectorized chemothe which results from the grafting of a cytotoxic agent onto a monoclonal antibody via a judici constructed spacer arm. ADCs have made considerable progress in 10 years. While in 2009 gemtuzumab ozogamicin (Mylotarg®) was used clinically, in 2020, 8 Food and Drug Administ (FDA)-approved ADCs are available, and more than 80 others are in active clinical studies. This review will focus on FDA-approved and late-stage ADCs, their limitations including their toxicity and associated resistance mechanisms, as well as new emerging strategies to address these issues

Table 1. Antibody–drug conjugates (ADCs) approved by the Food and Drug Administration (FDA), in advanced clinical trials (Phase III or pivotal phase II) or recently stopped.

Company	ADC (Cytotoxic)	Isotype and Target	Indication/Approval Date (Trade Name)/Clinical Status
Pfizer	gemtuzumab ozogamicin (CAL)	IgG4 CD33	2000–2010/2017 AML (Mylotarg®)
Seattle Genetics	brentuximab vedotin (AUR)	IgG1 CD30	2011 ALCL and Hodgkin lymphoma (Adcetris®)
Roche	trastuzumab emtansine (MAY)	IgG1 HER2+	2013 metastatic HER2+++ breast cancer (Kadcyla®) **
Pfizer	inotuzumab ozogamicin (CAL)	IgG4 CD22	2017 ALL and CLL (Besponsa®)
Roche	polatuzumab vedotin (AUR)	IgG1 CD79b	2019 DLBCL (Polivy®)
Seattle Genetics	enfortumab vedotin (AUR)	IgG1 Nectin 4	2019 urothelial cancer (Padcev®) **
Daiichi Sankyo	trastuzumab deruxtecan (EXA)	IgG1 HER2+	2019 metastatic HER2+++ breast cancer (Enhertu®) **
Immunomedics	sacituzumab govitecan (IRI)	IgG1 TROP-2	2020, metastatic TNBC (Trodelvy®) **
GSK	belantamab mafodotin (AUR, MMAF)	IgG1afuc BCMA	2020, multiple myeloma (Blenrep®)

Acknowledgments

CIPF, St-Julien-en-Genevois, FR

- E. Wagner, O. Colas, MC. Janin, M. Excoffier
- N. Boute, T. Champion, M. Malissard
- B. Akla, C. Beau-Larvor, N. Loukili et al
- N. Corvaïa, JF Haeuw, P. Lowe et al

CRDPF, Toulouse, FR

- C. Lasserre, N. Regent, MF. Laliberté
- M. Nicolas, L. Liorzou
- M. Pavlyuk, P. Ferre, E. Chetaille et al
- J. Desrivot, F. Lafforgue et al

CEPC, Castres, FR

- S. Couffin, A. Grondin, MO. Roy et al

PF CDMO Biologics, St-Julien-en-Genevois, FR

- M. Culie, S. Chenu, A. Cousinet et al
- S. Demare, C. Truchy, G. Mijola et al
- S. Lauthier, J. Lhermite et al
- C. Borgne, C. Maupas

+ many more (see publications)

Pharma School, University of Geneva, CH (47 papers)

- D. Guillarme, S. Fekete, V. D'Atri, JL. Veuthey et al

LSMBO, University of Strasbourg, FR (30 papers)

- S. Cianferani, O. Hernandez, A. Ehkirch, S. Erb et al

LSMIS, University of Strasbourg, FR (18 papers)

- Y. François, R. Gahoual et al

EPFL/SpectroSwiss, CH/ Thermo, CH (7 papers)

- Y. Tsybin, K. Srzentić, L. Fornelli, D. Ayoub et al

Gustavus Adolphus College, MN/ Agilent (7 papers)

- D. Stoll et al

University of Lyon, CH (4 papers)

- S. Heinisch et al

Waters, FR, UK, US (LC-MS prototypes) (2 papers)

- W. Chen, D. Lascoux, L. Denbigh, J. Gebler et al

Bruker, GER (LC-MS prototypes) (2 papers)

- D. Suckau, A. Resemann, W. Jabs et al

Thank you for your attention





alain.beck@pierre-fabre.com

www.cipf.com