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Expectations for Bioassays
-
An Assessor´s View

CASSS - Bioassays 2017

DoubleTree by Hilton Hotel Silver Spring, Maryland, USA

May 9th 2017

Dr. Nils Jost
Quality- and Non-clinical Assessor
Section Mono- und Polyclonal Antibodies

Introduction

- Expectations for Bioassays during CTA
- Influence of Glycosylation on ADCC
- Case Study Infliximab Biosimilars
- Upcoming EDQM monographs for mAbs

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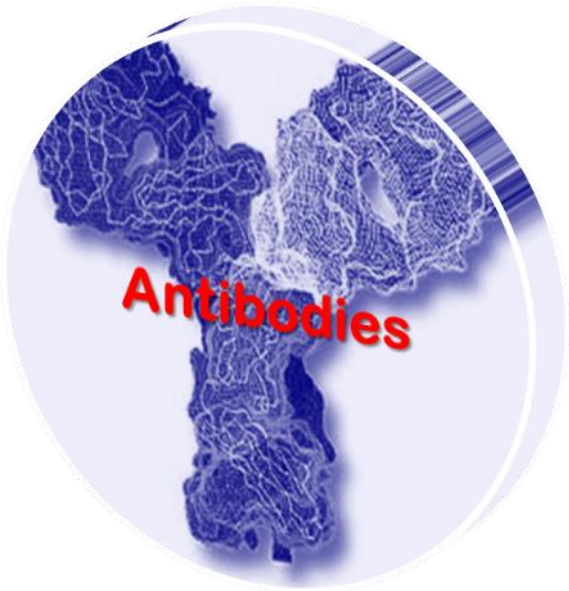
Disclaimer

The view expressed in the following is the ones of the presenter and does not necessary express the view of either the CHMP, BWP, EDQM or the Paul-Ehrlich-Institut.

Section Mono- and Polyclonal Antibodies

Mission:

Facilitate the development and authorization of safe and efficacious medicines (monoclonal and polyclonal antibodies)



Clients:

Pharmaceutical industry,

small and medium-sized enterprises

Research groups

Activities:

Marketing authorization:

National, Mutual recognition, Centralized

Clinical trial authorization

GMP Inspections

Batch release

IgG, monoclonal antibodies,

CAP Testing, Research

Scientific Advice

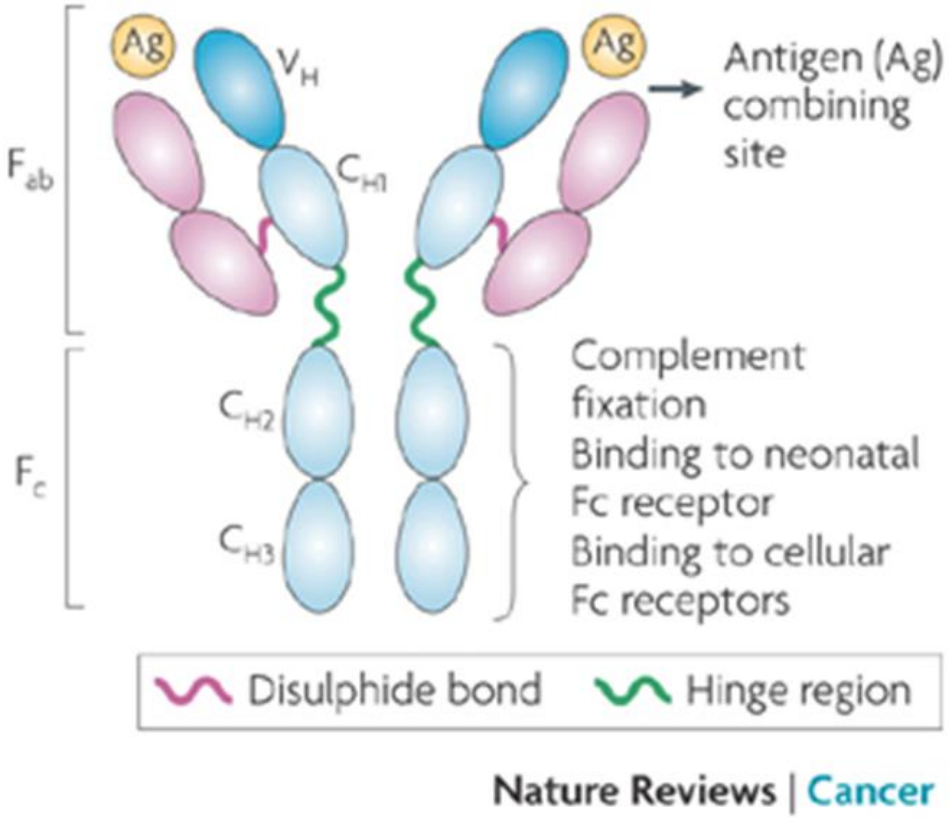
Challenge:

Biosimilars

New drug formats (ADC, multivalent antibodies, …)

Leading role addressing all aspects of product evaluation and characterization

Complexity of Monoclonal Antibodies



→ Antigen-binding (ELISA / SPR)

→ Induction of cytokines / pathways on target cell / etc. (cell based assays)

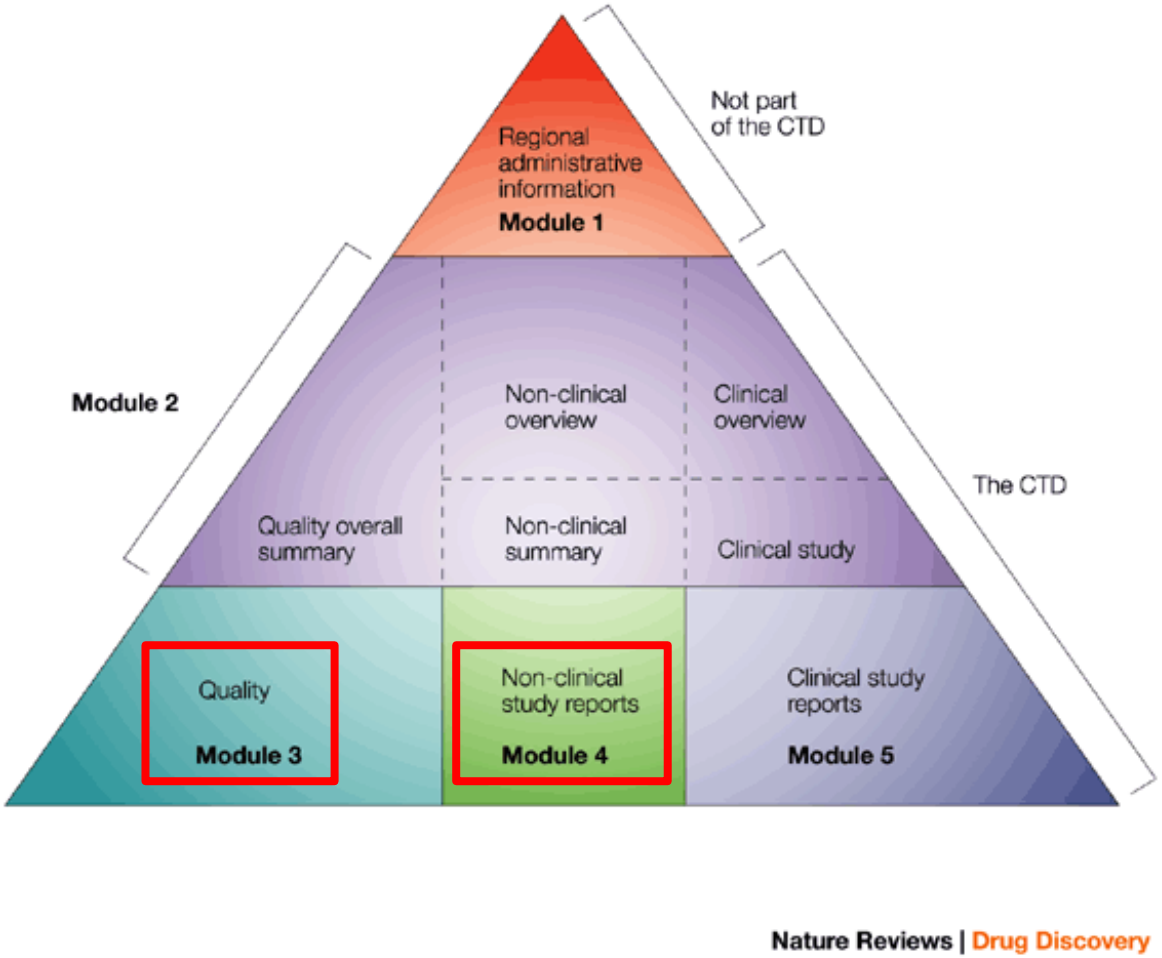
→ Fcγ-Receptor binding (ELISA / SPR)

→ ADCC / CDC cell based assays

Nature Reviews Cancer 7, 701-706 (September 2007) | doi:10.1038/nrc2209

ADCC - antibody dependent cytotoxicity
CDC - cell dependent cytotoxicity

CTD – Where to put the Data?



Description and data on Bioassays are expected in module 3 section S.3 Characterization and S.4 Specification

If data is presented in module 4 please make a reference in module 3 in order to facilitate their assessment!

Bioassays used in the comparability exercise of biosimilars should be included in the regional information section of module 3.

Nature Reviews Drug Discovery 2, 71-74 (January 2003) | doi:10.1038/nrd990

Bioassays Development during Clinical Trials

- All possible biological properties should be investigated
  - Data should be included into module 3 in section S.3 Characterization

Bioassays Development during Clinical Trials

- Data should already be provided with Phase I clinical trial application
  - “Details should be provided on the biological activity (i.e. the specific ability or capacity of a product to achieve a defined biological effect).”
  - “Usually prior to initiation of phase I studies, the biological activity should be determined using a relevant, reliable and qualified method.”
- For Phase III clinical trials and MAA also extensive validation data of bioassays is expected

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Bioassays Development during Clinical Trials

- S.4.1. Specification
  - “A test for biological activity should be included unless otherwise justified.”
    - In early development antigen-binding methods (ELISA / SPR) are acceptable for release of the medicinal product
  - “As knowledge and experience increases, the addition or removal of parameters and modification of analytical methods may be necessary. Specifications and acceptance criteria set for previous trials should be reviewed and, where appropriate, adjusted to the current stage of development.”
    - In later phases and at MAA adequate cell based bioassay needed for the release of the medicinal product

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Acceptance criteria

- The potency of bioassays for mAb are normally expressed as percentage of potency in comparison to a reference standard
- Expected acceptance criteria ranges
  - ELISA-assays → 90 – 110 %
  - Cell-based bioassays → 70 – 130 %
- Specification set according to ICH Q6B

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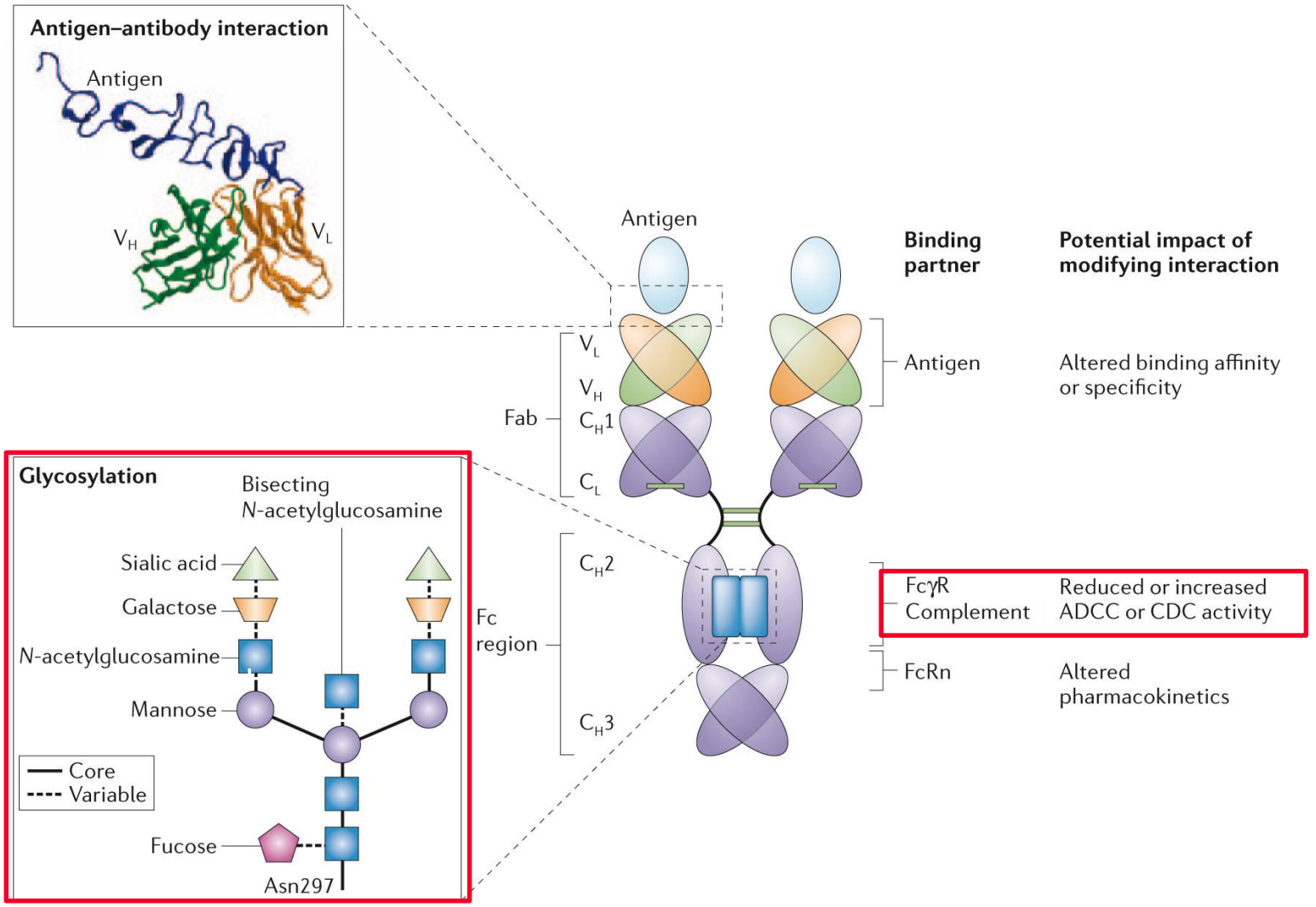
Increased Complexity by Posttranslational Modifications



Adapted from Carter PJ: Potent antibody therapeutics by design, Nature Rev Immunology 6, 343 (2006)

Effects induced by Carbohydrate to IgG Function

Without Glycan	→	No ADCC activity
Without core Fucose	→	increases ADCC activity
Bisecting GlcNAc	→	increases ADCC activity
GlcNAc/Mannose (G0)	→	Ligand for C-typ Lectin (Mannose binding Lectin) and Complement activation
α1,3Galactose	→	higher antigenicity
N-Glycoylneuramine acid	→	higher antigenicity
Galactose	→	FcRn binding, transplacental transport, increased CDC activity
NeuNAc (Sialic acid)	→	decreases ADCC activity, anti-inflammatory

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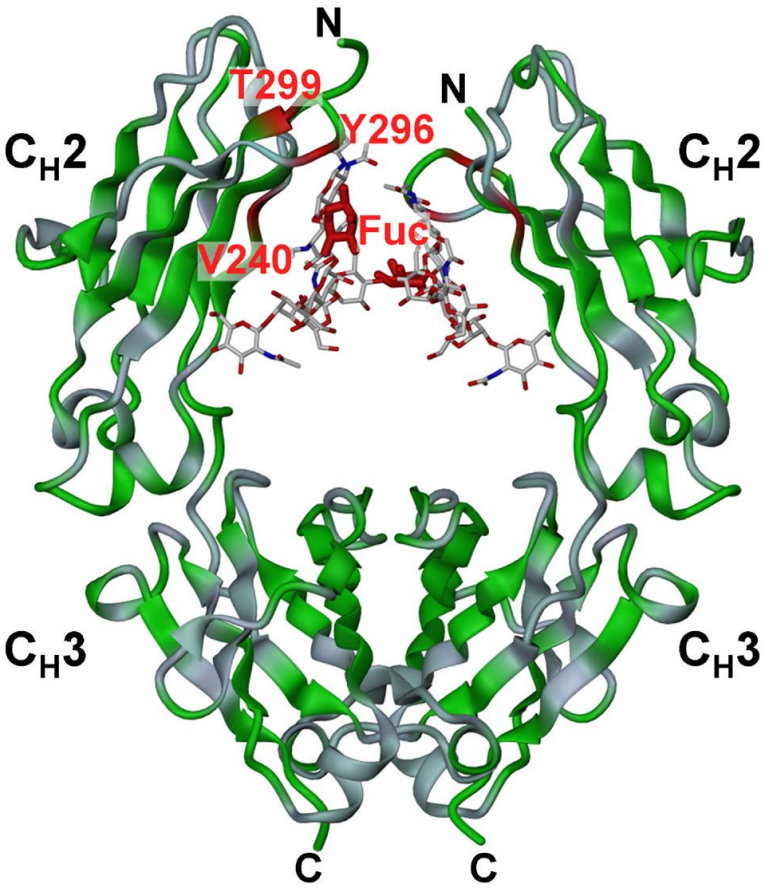
Influence of Core-Fucose

Crystal structure of IgG1-Fc with Fuc (+)Glycosylierung



Red: chemical shift difference between Fuc (+) and Fuc (-) IgG1-Fc (NMR Data)

Induced local conformational fluctuation near Tyr296 of Fuc(-) IgG-Fc

Conformation of Fuc(-) IgG-Fc more flexible and adjustable to FcgRIII A

Increased affinity to FcgRIII

Increased cellular effect: ADCC

JMolBiol-368-767

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Afucosylation as surrogate for ADCC?

THE JOURNAL OF BIOLOGICAL CHEMISTRY
© 2002 by The American Society for Biochemistry and Molecular Biology, Inc.
Vol. 277, No. 30, Issue of July 26, pp. 26733–26740, 2002
Printed in U.S.A.

Lack of Fucose on Human IgG1 *N*-Linked Oligosaccharide Improves Binding to Human FcγRIII and Antibody-dependent Cellular Toxicity*

Received for publication, March 1, 2002, and in revised form, April 19, 2002
Published, JBC Papers in Press, May 1, 2002, DOI 10.1074/jbc.M202069200

Robert L. Shields‡, Jadine Lai‡, Rodney Keck§, Lori Y. O'Connell‡, Kyu Hong¶, Y. Gloria Meng¶, Stefanie H. A. Weikert‖, and Leonard G. Presta‡**

Pharmaceuticals **2010**, *3*, 146-157; doi:10.3390/ph3010146

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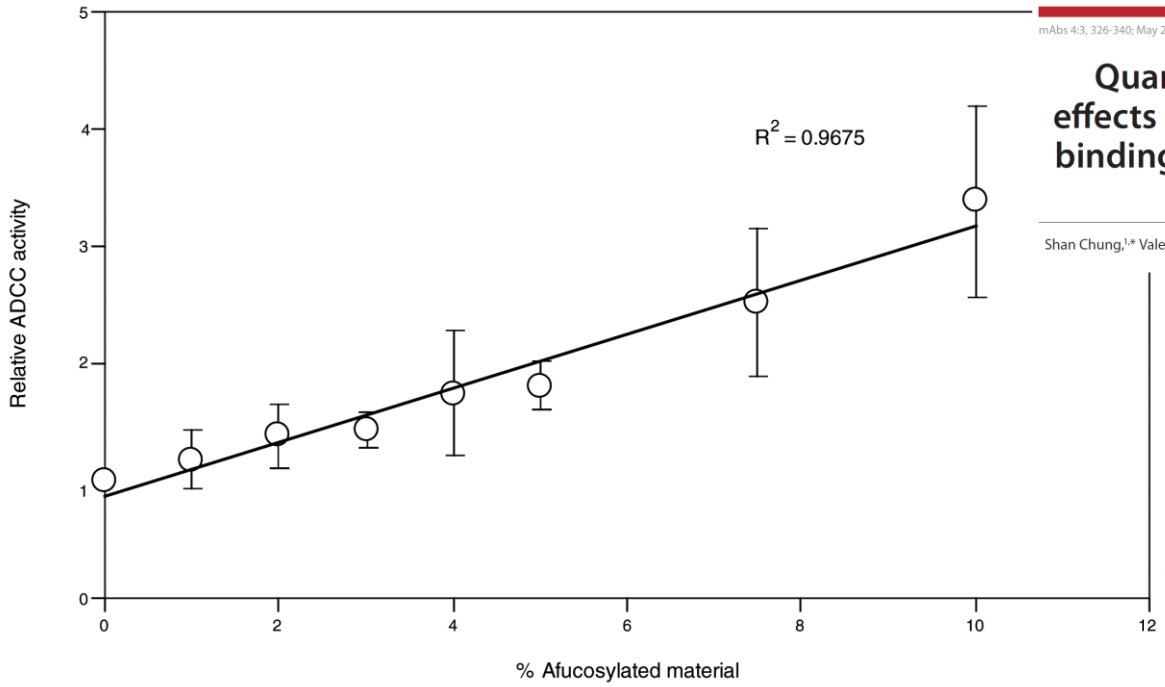
Review

Impact of Glycosylation on Effector Functions of Therapeutic IgG †

Riad Abès [1,2,3,4] and Jean-Luc Teillaud [1,2,3,*]



mAbs 4:3, 326-340; May 2012; © 2012 Landes Bioscience

Quantitative evaluation of fucose reducing effects in a humanized antibody on Fcγ receptor binding and antibody-dependent cell-mediated cytotoxicity activities

Shan Chung,[1,*] Valerie Quarmby,[1] Xiaoying Gao,[1] Yong Ying,[1] Linda Lin,[1] Chae Reed,[1] Chris Fong,[2] Wendy Lau,[3] Zhihua J. Qiu,[1] Amy Shen,[4] Martin Vanderlaan[2] and An Song[1]

mAbs 4:3, 326-340; May 2012; © 2012 Landes Bioscience

Case Study – Infliximab Biosimilars

- Infliximab
  - chimeric mAb: murine variable domaines, human IgG1 constant domaines against TNF-a
  - indications: RA, AS, PSA, Psoriasis, CD, UC
  - effector mechanism:
    - neutralization of TNFa-induced effects (soluble TNF-a in RA, Psoriasis)
      - upon binding to membrane TNF-a: ADCC, CDC and apoptosis (important for Crohn's Disease)

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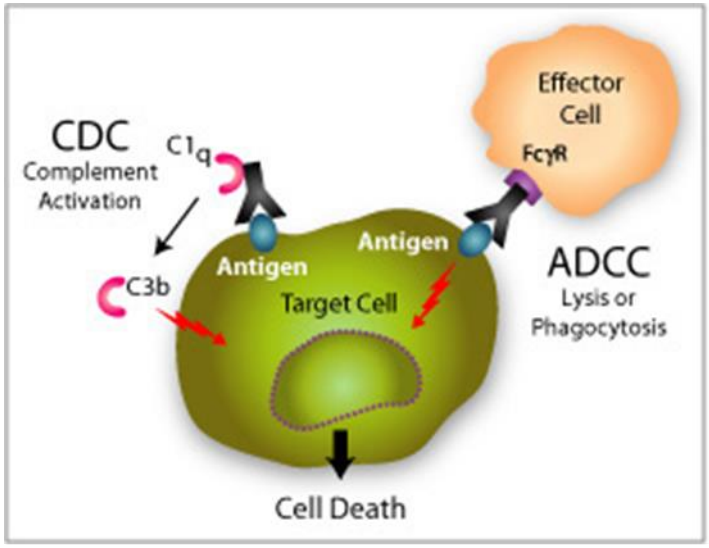
Case Study – Infliximab Biosimilars

- Infliximab biosimilars
  - Remsima (marketing authorization approved 2013)
  - Flixabi (marketing authorization approved 2016)
  - Both biosimilars showed differences in the afucosylated species
  - Differences were seen in FcγR-III binding

Questions were raised concerning ADCC function (cell based assays)



http://www.invivogen.com/images/cdc2.jpg

Case Study – Infliximab Biosimilars

http://www.celltrionph.com/eng/img/main/product01_20131213.jpg

- **Remsima**
  - 1st ADCC assay
    - Healthy donor PBMCs as effectors and Jurkat cells expressing tmTNFa as targets
      - No difference between biosimilar and reference medicinal product (RMP)
      - Was not able to dicriminate between samples with afucosylation below 12 %
  - 2nd ADCC assay
    - NK cells as effectors and Jurkat cells expressing tmTNFa as targets
      - Significant differrence between biosimilar and RMP

Source: Remsima EPAR (EMA/CHMP/589317/2013)

Case Study – Infliximab Biosimilars

- 3rd ADCC assay
  - Crohn´s disease donor PBMCs as effectors and Jurkat cells expressing tmTNFa as targets
    - No difference between biosimilar and reference medicinal product (RMP)
    - Lower response curve compared to 2nd ADCC assay (likely due to mixed cell population present in PBMC)

- 4th ADCC assay
  - LPS stimulated monocytes from healthy donors or Crohn´s disease patients as targets
    - No ADCC detected
    - Jurkat cells expressing tmTNFa have much higher tmTNFa levels
    - ADCC might be limited or absent under physiological conditions in vivo

Source: Remsima EPAR (EMA/CHMP/589317/2013)

Case Study – Infliximab Biosimilars

- **Flixabi**
  - 1st ADCC assay
    - NK cells as effectors and stable mouse cell line expressing tmTNFa as targets
    - “Artificial system”
    - Highly variable results
  - 2nd ADCC assay
    - PBMCs as effectors
    - “Physiological system”
    - Comparable results

http://www.pmlive.com/__data/assets/image/0009/1124982/Biogen-Flixabi-pack-shot-500x333.jpg

Case Study – Infliximab Biosimilars
Conclusion

- The design of ADCC assays is crucial to get reliable results
- Usage of NK-cells as effectors is considered more sensitive in comparison to PBMCs
- Usage of PBMCs reflects the physiological situation more accurately

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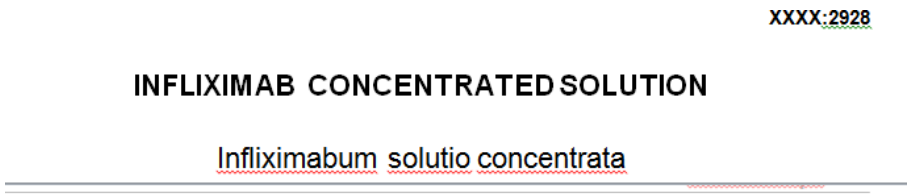
Infliximab Monograph



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INFLIXIMAB CONCENTRATED SOLUTION

Infliximabum solutio concentrata

- Public consultation ended 31st December 2016

- **Potency assay**
- In vitro cell-based assay, based on the ability of infliximab to block TNF-alpha-induced inhibition of murine fibrosarcoma WEHI-164 cell proliferation
  - Cell growth assessed through a tetrazolium-based colorimetric assay

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Etanercept Monograph

Etanercept

Étanercept

Etanerceptum

Monograph N°: 2895

- Has been adopted in November 2016
- Implementation date January 2018

- **Potency Assay**
  - Apoptosis assay in histiocytic lymphoma cell-line U937 (ATCC No. CRL-1593.2) via caspase activation.
    - “The U937 cells are incubated with varying dilutions of test and reference preparations of etanercept in the presence of TNF-α. They are then incubated with Caspase-Glo 3/7 reagent, which results in caspase cleavage of a luminogenic substrate, subsequent release of a luciferase substrate and generation of a luminescent signal.”

Im Mittelpunkt steht die Gesundheit
Our Focus is on Health

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