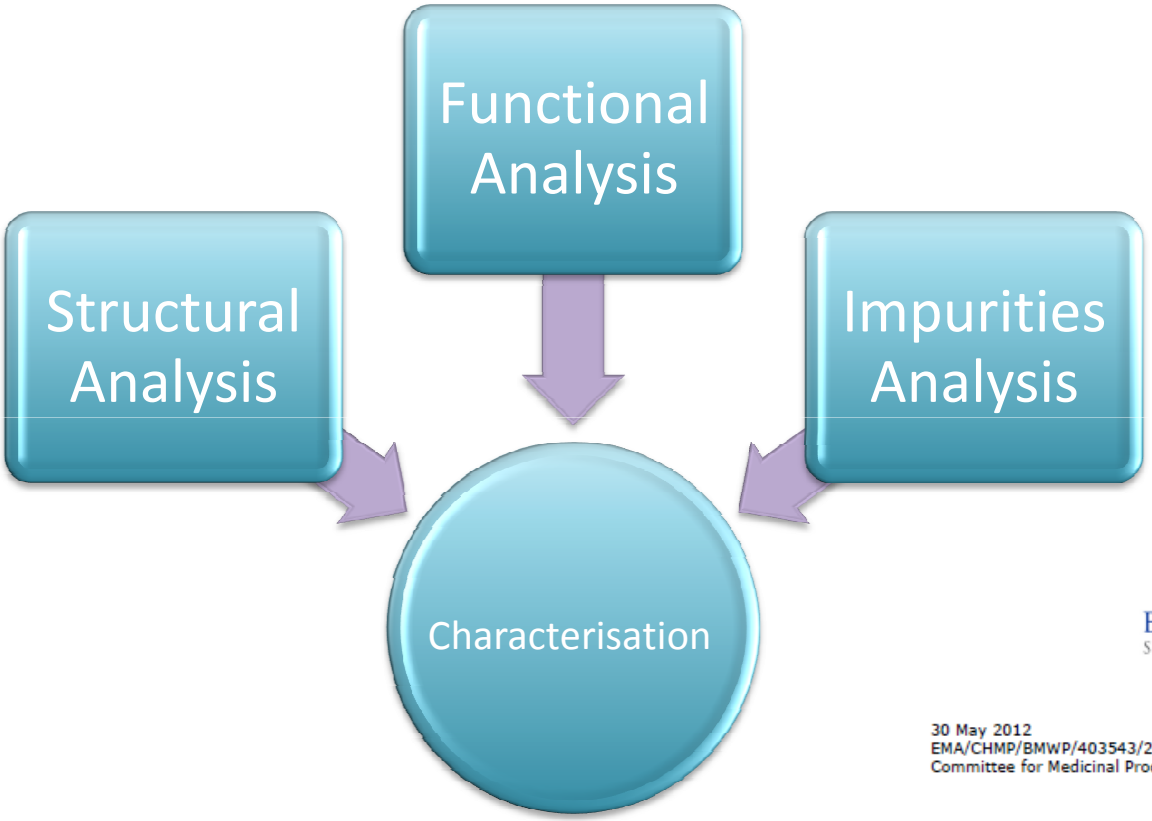
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Biological and Functional Analysis of Biosimilar TNFα Drugs

Daniel N Galbraith
Chief Scientific Officer, BioOutsource Ltd.

Drug Characterisation Activities



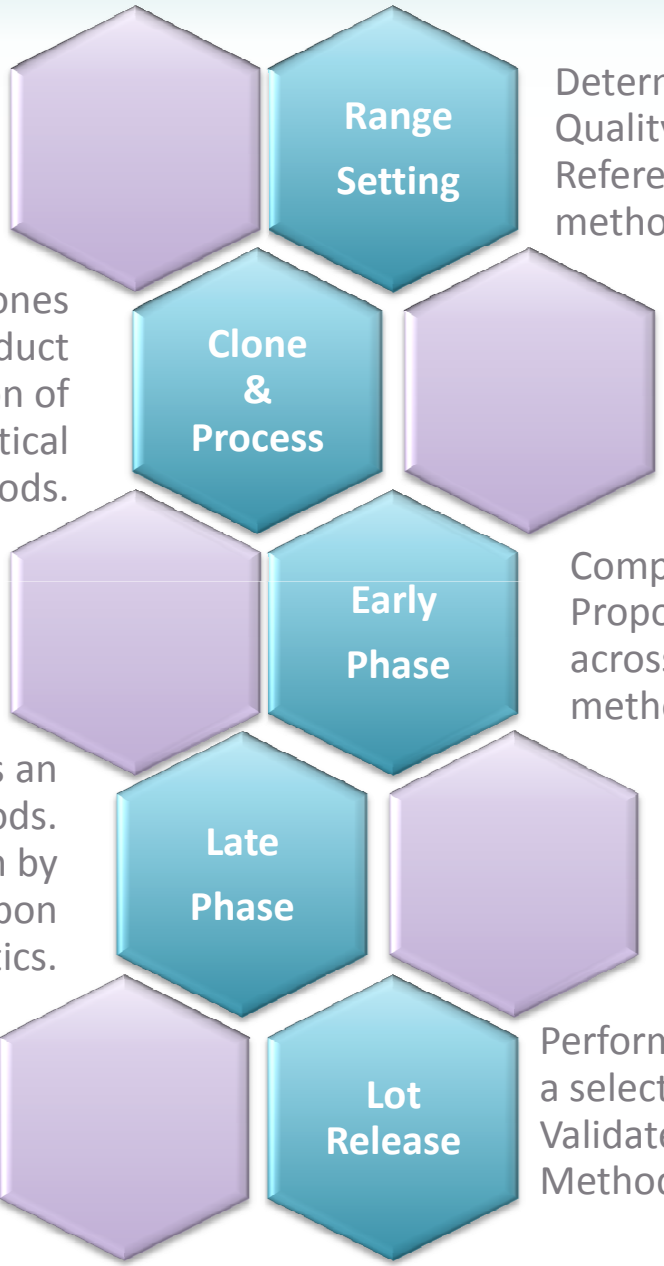
EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

30 May 2012
EMA/CHMP/BMWP/403543/2010
Committee for Medicinal Products for Human Use (CHMP)

Guideline on similar biological medicinal products containing monoclonal antibodies – non-clinical and clinical issues

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Overview of the Biosimilar Characterisation Process



Determination of Critical Quality Attributes of Reference Product using methods

Evaluation of clones against Reference Product range and evaluation of process using critical methods.

Comprehensive evaluation of Proposed Biosimilar product across a wide range of methodologies

Extended evaluation across an increased number of methods. Requirements are driven by Regulators based upon molecule characteristics.

Performance of a selection of Validated Methods

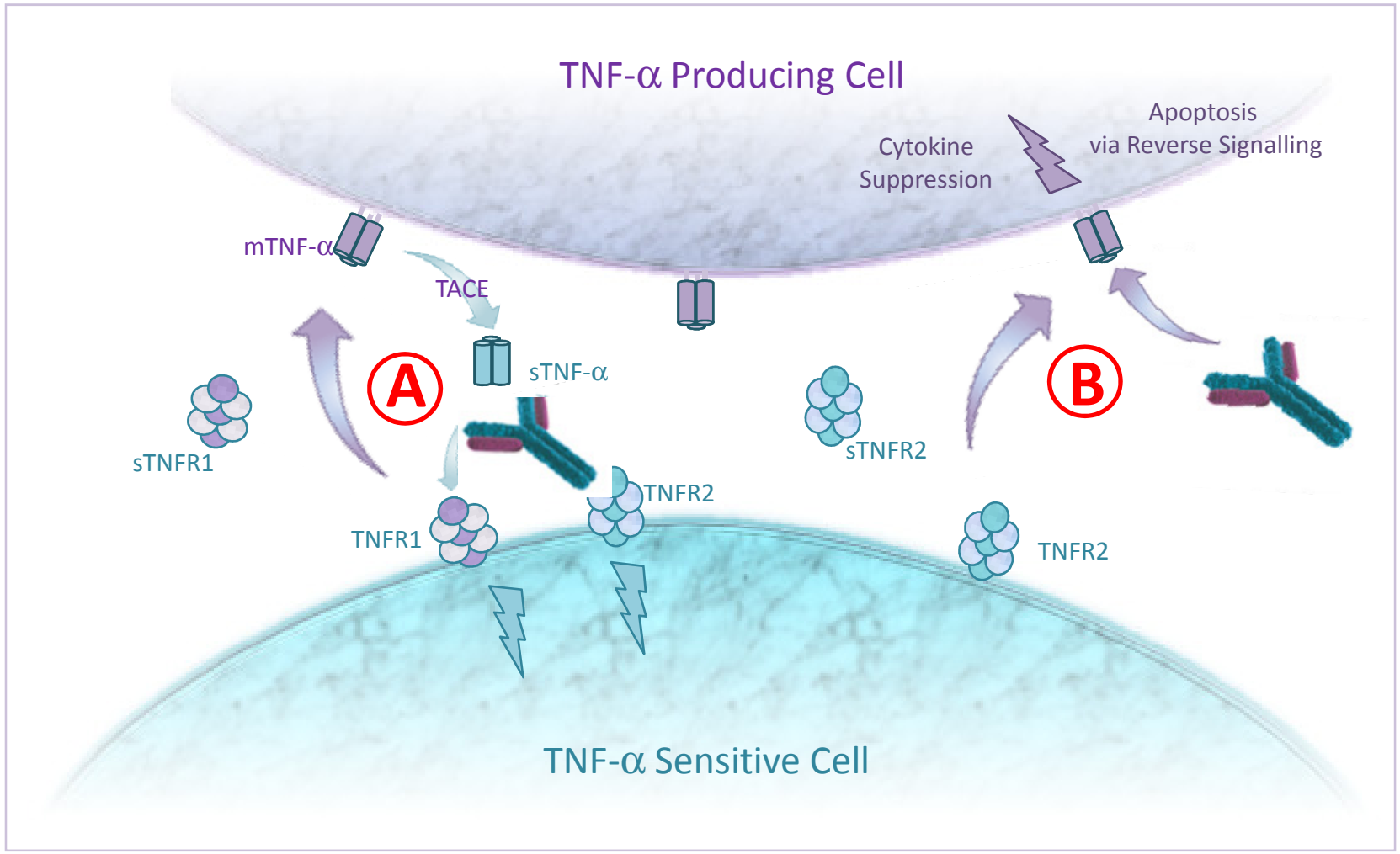
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Infliximab (Remicade)

- First approved in 1998. Now a multibillion dollar product.
- Approved for
  - Rheumatoid arthritis, psoriatic arthritis, ankylosing spondylitis, Crohn's disease, ulcerative colitis, moderate to severe chronic psoriasis and juvenile idiopathic arthritis.
- Mechanism of Action:
  - The antibody targets the TNFα protein either as a membrane bound or soluble form.
  - The primary mechanism of action is the neutralisation of circulating TNFα and reducing inflammation
  - Secondary actions include the mediation of ADCC, CDC and other methods
- Popular target as a BioSimilar after losing its patent in Europe this year and in the USA in 2018.

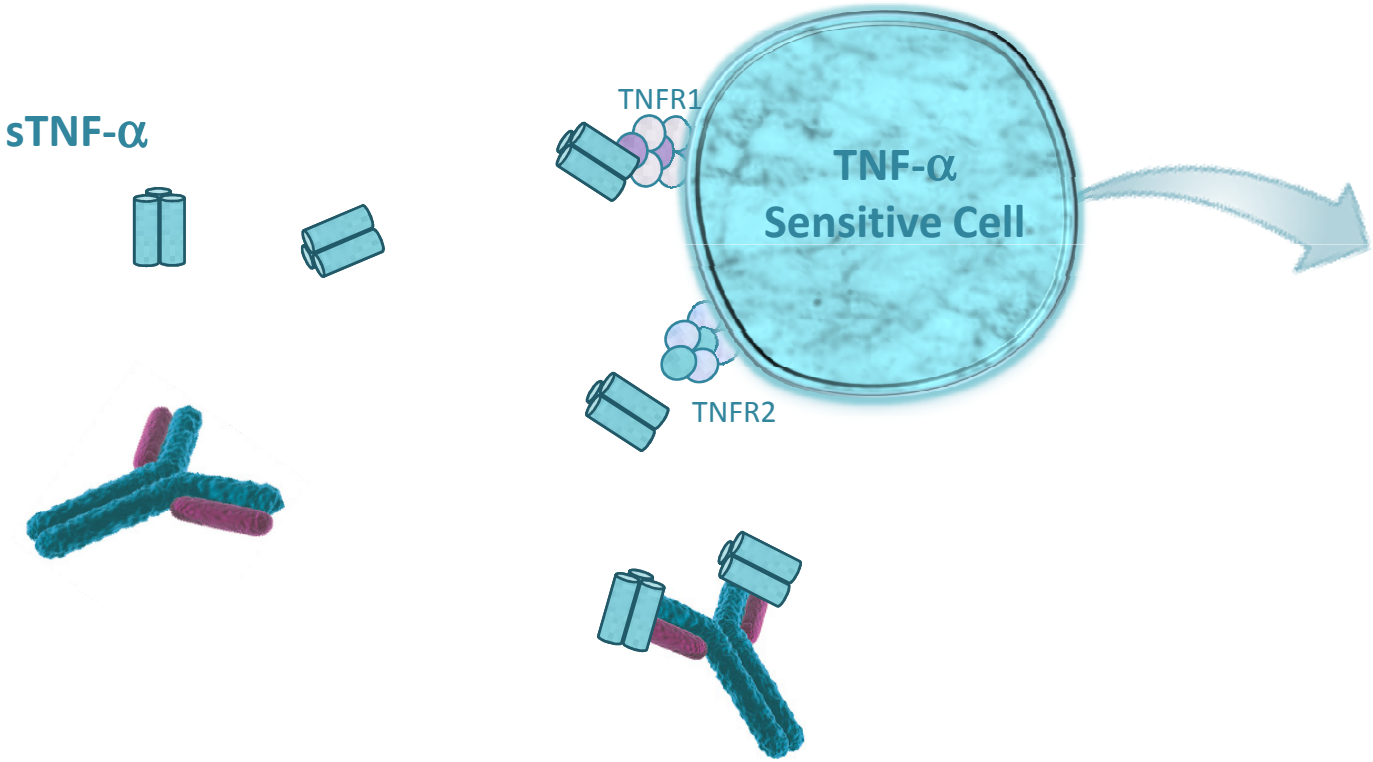
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Overview of TNF-α Biology



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Neutralisation of Soluble TNF-α



- Cytokine Production
- Apoptosis
- Adhesion Molecules
- Inflammation
- Proliferation

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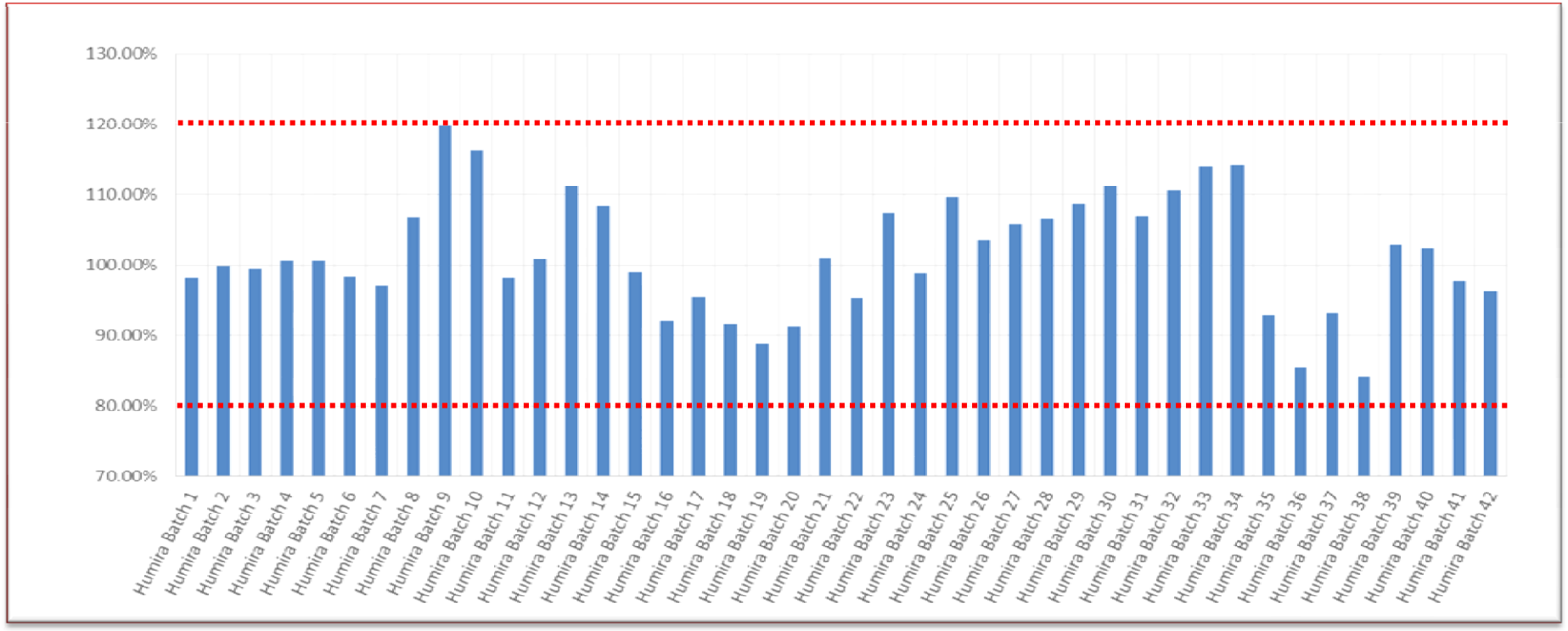
Bioassays: Key Concepts

- Measures the effects in a living system
  - The only analytical method that provides information on the biological activity
- Relative Potency
  - Comparison of test drug with a standard preparation
  - Minimises the impact of assay to assay variability ensuring consistent reporting of results
- Assay Limitations: Single Mechanism of Action
  - Multiple methods required to understand drugs with complex mechanisms of action
  - Difficulty in correlating bioassay results with therapeutic efficacy

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Bioassay Potency Measurements in Monoclonal Antibody Batches – Innovator Variability

- 42 samples of drug assessed against a common reference standard.
  - Accuracy and Precision of the assay <10%
  - Relative Potency range of 85% to 120%.
  - Primary measure of potency of these drugs is the TNF-α neutralising assay



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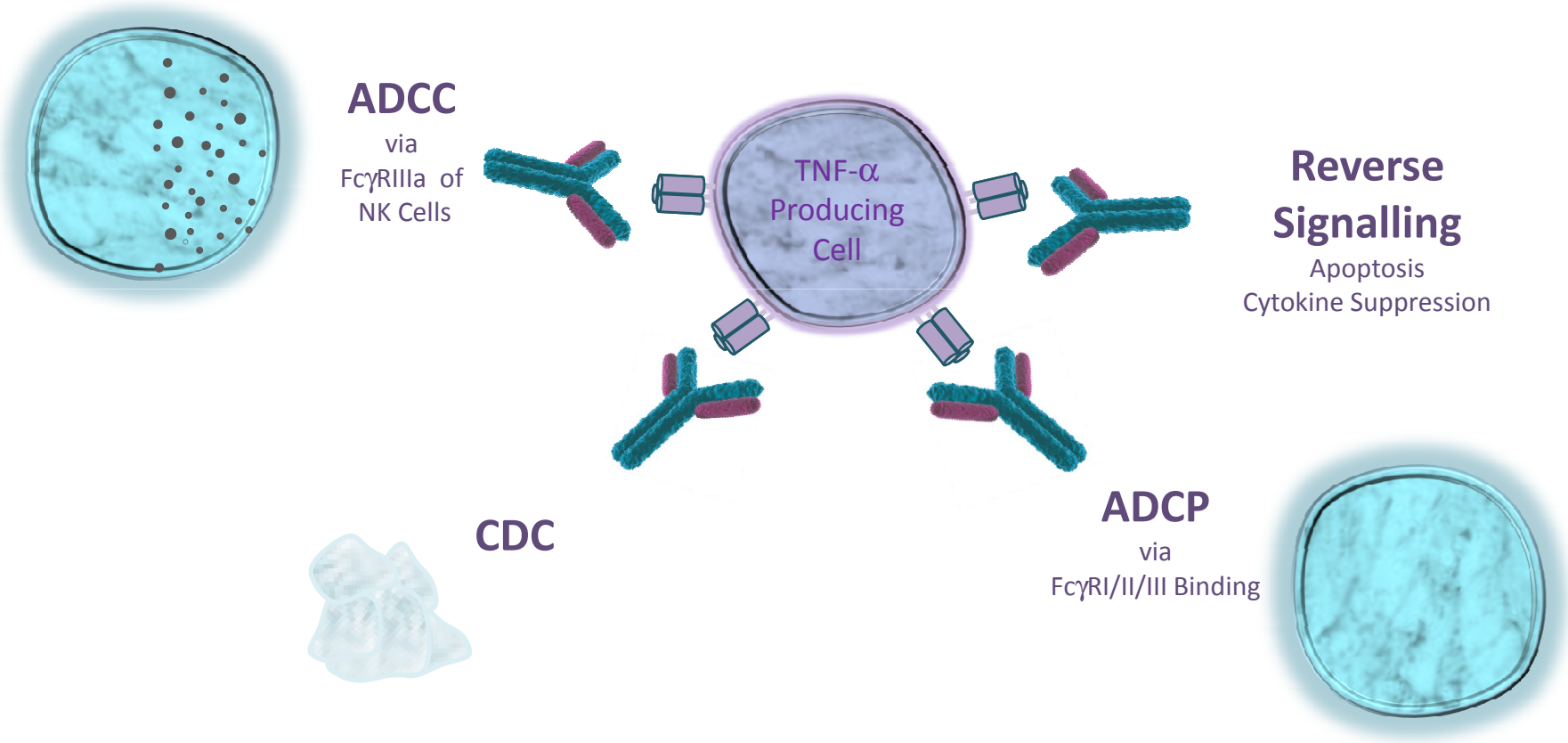
FDA Guidance

- FDA is clear that there should be comprehensive orthogonal measurement of key functional activities of the Biosimilar compared with the Innovator molecule

456 If a reference product exhibits multiple functional activities, manufacturers should perform a set
457 of relevant assays designed to evaluate the range of activities. For example, with proteins that
458 possess multiple functional domains that express enzymatic and receptor-mediated activities,
459 manufacturers should evaluate both activities. For products where a single functional activity
460 can be measured by more than one, but related, parameter (*e.g.*, enzyme kinetics or interactions
461 with blood clotting factors), comparative characterization of each parameter between products
462 should be used to provide additional valuable information.

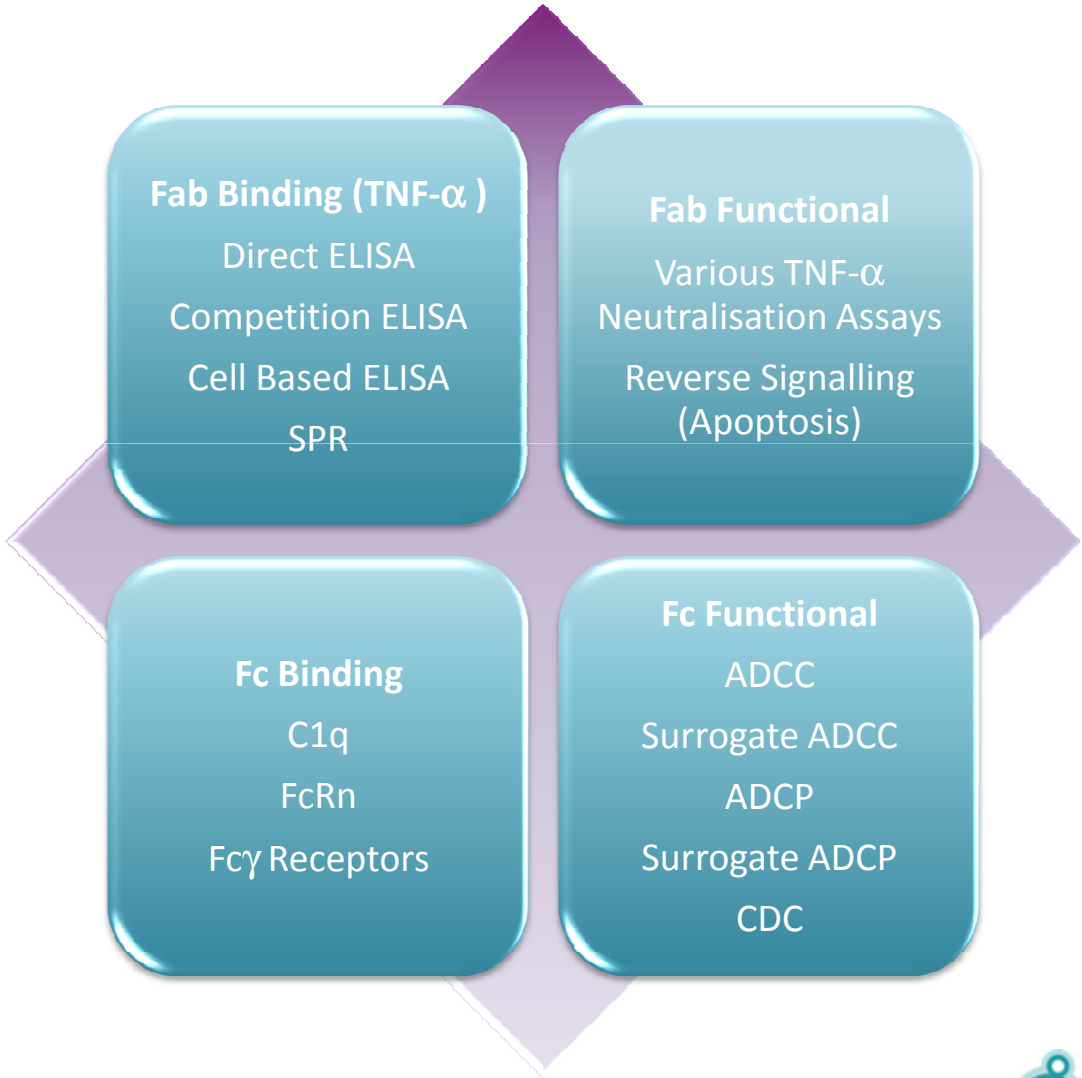
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Death of TNF-α Producing Cells



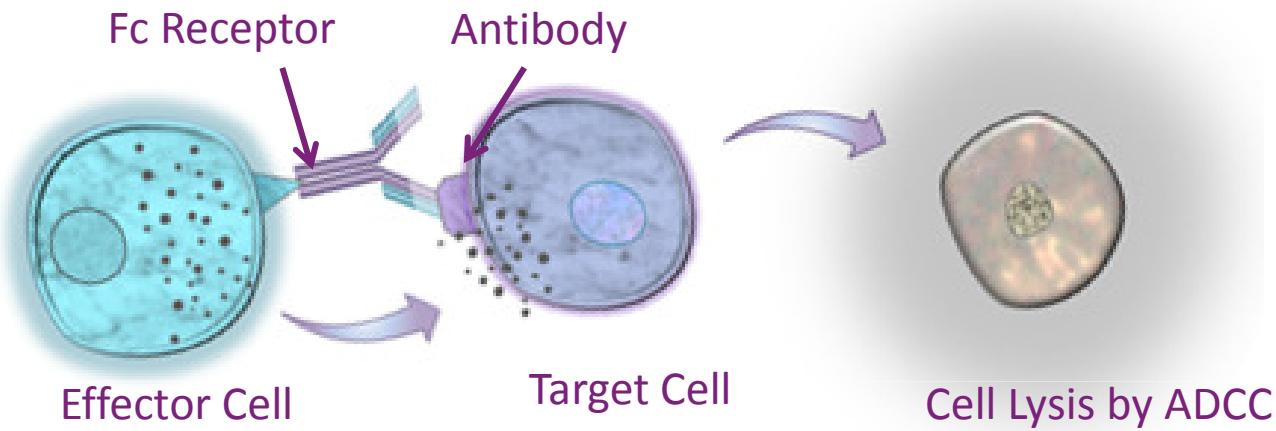
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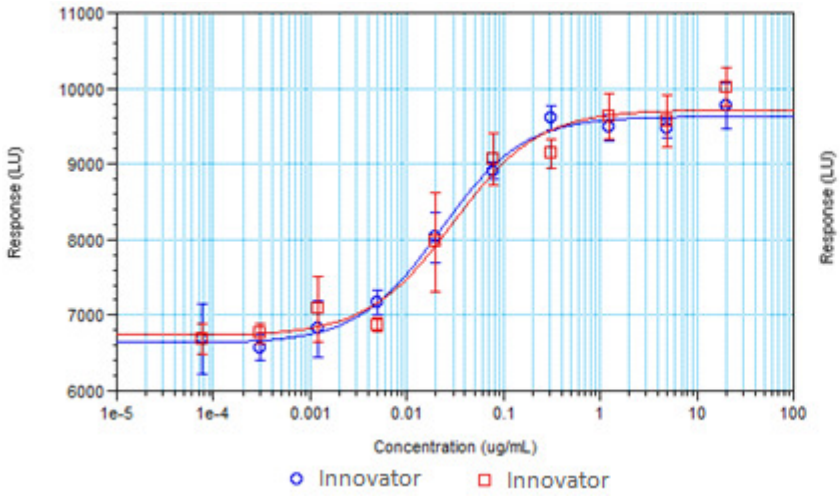
Characterisation Package

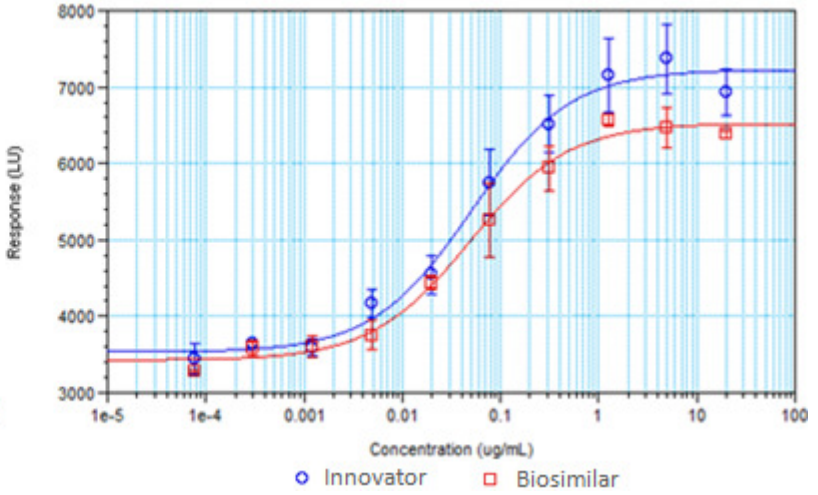


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Effector Function Assays:
Relative Potency & Relative Responses (ADCC)

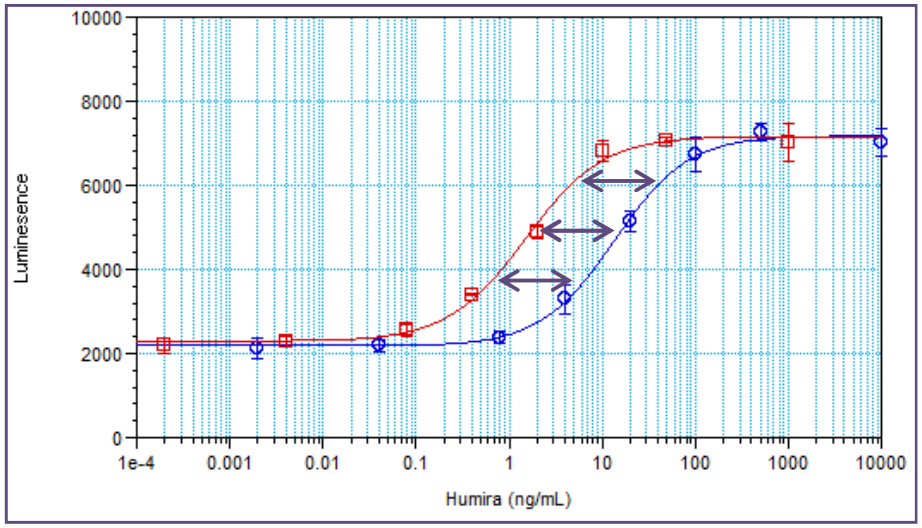






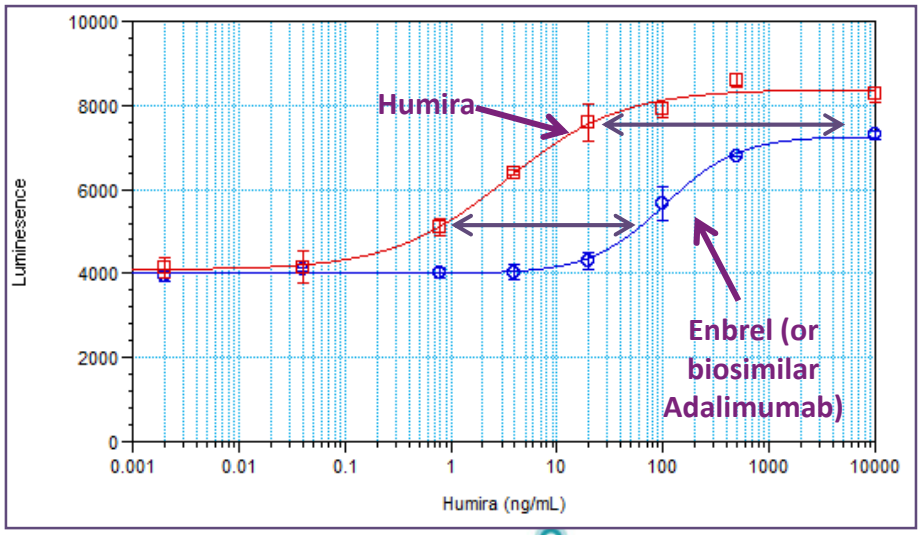
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Relative Potency and Parallelism



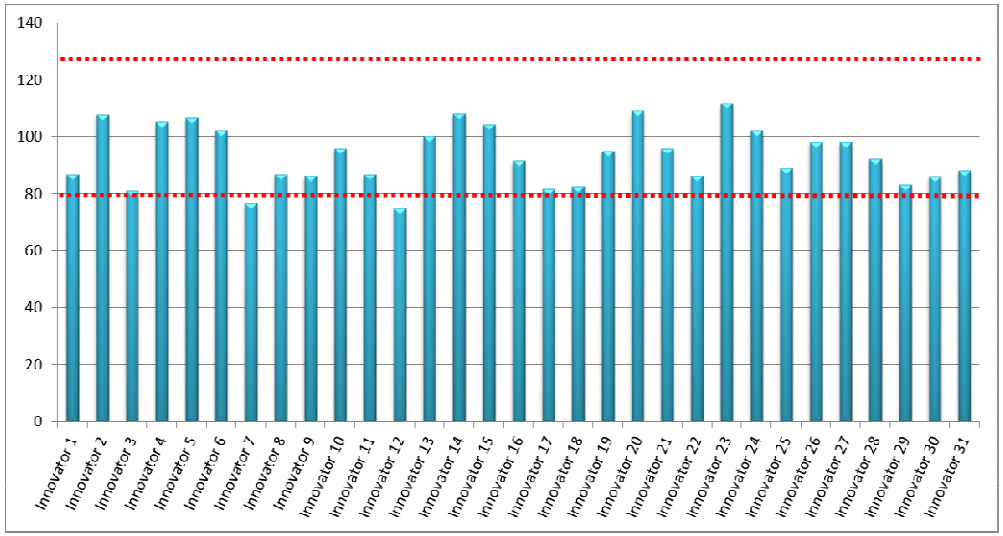
"Meaningful" Relative Potency

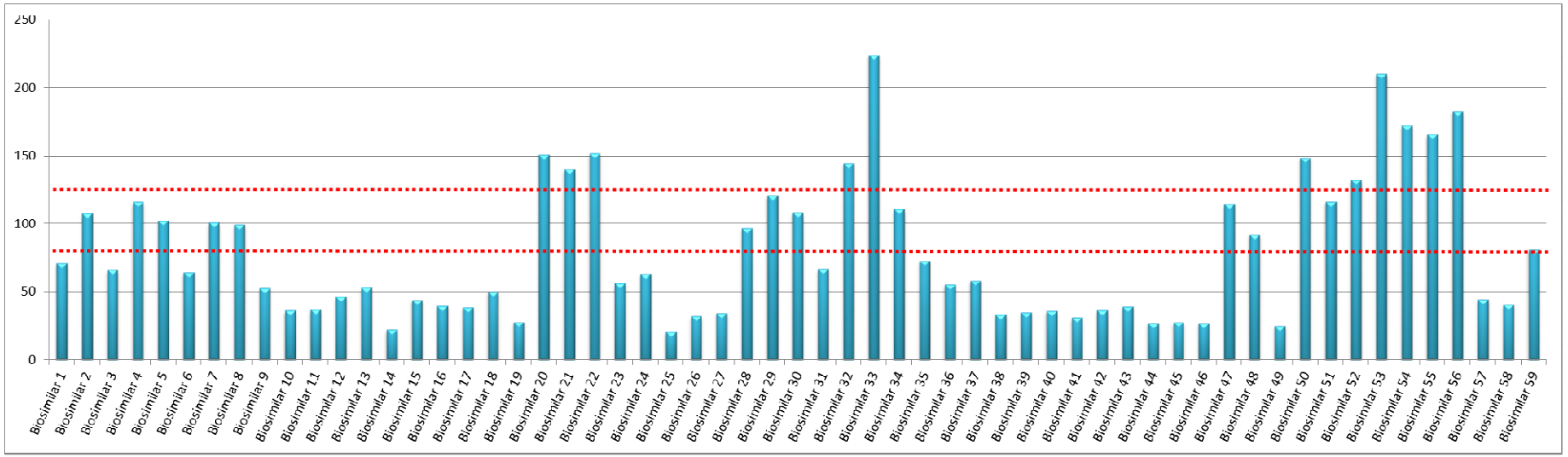
Less "Meaningful" Relative Potency



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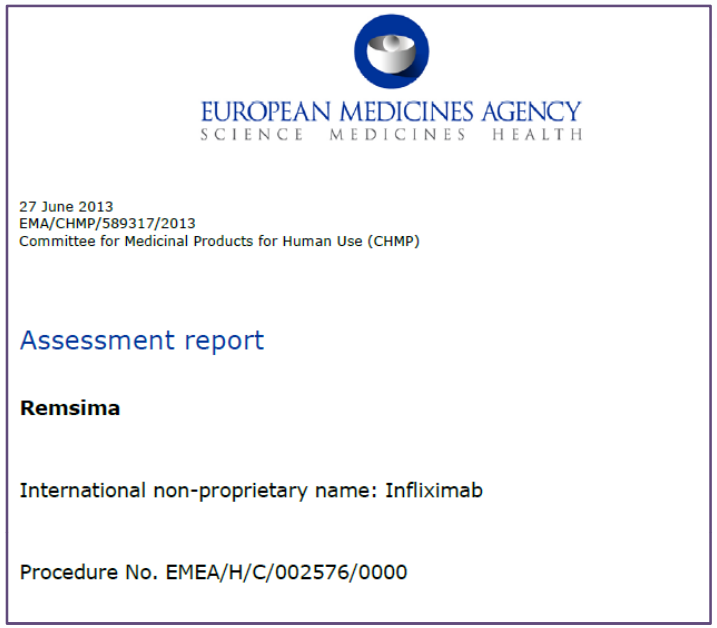
“Fc” Case Study:
Humira ADCC Comparability Data





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Remsima/Inflectra Approvals



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

27 June 2013
EMA/CHMP/589317/2013
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Remsima

International non-proprietary name: Infliximab

Procedure No. EMEA/H/C/002576/0000

Remsima™
Infliximab

- EMA first approved Monoclonal Antibody Biosimilar
- Approved June / September 2013
- Marketing Holder – Celltrion Hungary

The active substance of Remsima is infliximab, Chimeric Human Mouse monoclonal which binds sTNF-alpha and tmTNF-alpha

Approved for Psoriasis, Psoriatic arthritis, Adult and Paediatric Crohn's disease, Ulcerative colitis, Paediatric ulcerative colitis , Ankylosing spondylitis and Rheumatoid arthritis.

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Comparative ADCC Assessments

Target Cells	Effector Cells	Disease State	Genotype	Result
mTNF-α Jurkat	PBMC	Healthy Donor	Not Specified	Comparable Response
mTNF-α Jurkat	NK	Healthy Donor	V/F	Comparable Response
mTNF-α Jurkat	PBMC	Crohn's disease	V/F, F/F	Comparable Response
mTNF-α Jurkat	NK	Crohn's disease	F/F	Comparable Response
mTNF-α Jurkat	NK	Crohn's disease	V/V, V/F	**Differences Detected***
mTNF-α Jurkat	Whole Blood	Healthy	Not Specified	Comparable Response
mTNF-α Jurkat	Whole Blood	Crohn's disease	Not Specified	Comparable Response
LPS-Stimulated Monocytes	NK	Healthy	V/F	No ADCC Response
LPS-Stimulated Monocytes	PBMC	Crohn's disease	V/F	No ADCC Response

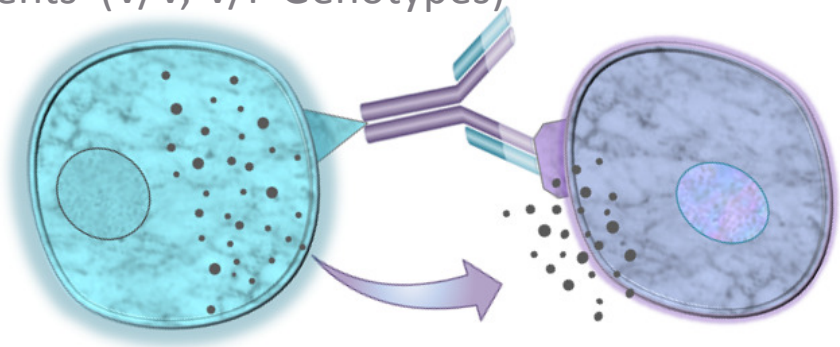
*Shown to be the most sensitive assay

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Two Dimensions to ADCC Design

Sensitivity

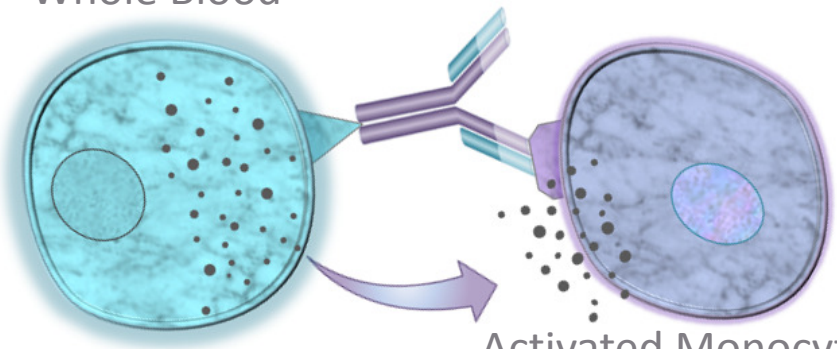
NK Cells from Crohn's disease patients (V/V, V/F Genotypes)



High Expressing tm-TNF-α Engineered cell line

Relevance

Whole Blood



Activated Monocytes / Macrophages

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FDA Guidance

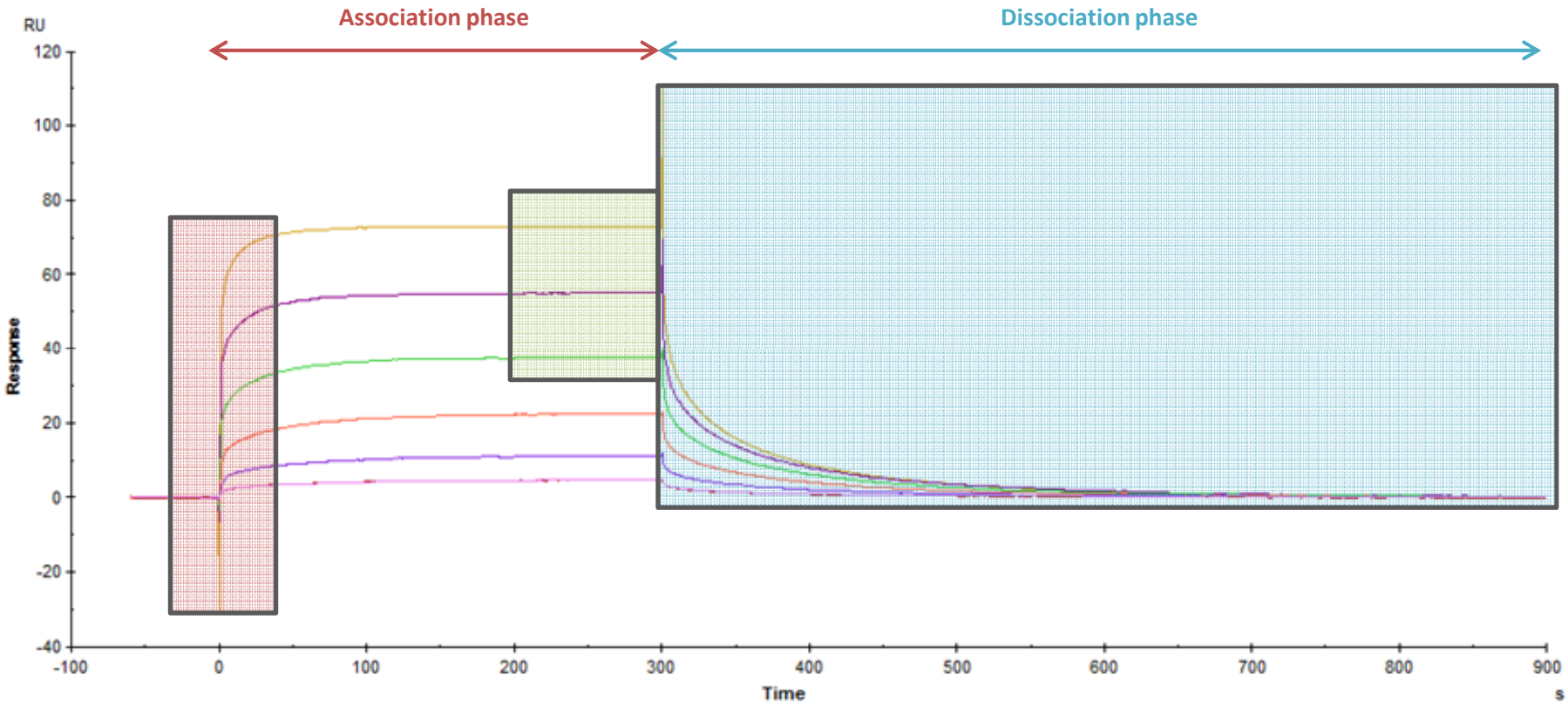
› FDA and EMA expects the binding characterisation of molecules to be understood – in relation to on and off rates and relate this to functional activity

481 When binding or immunochemical properties are part of the activity attributed to the protein
482 product, analytical tests should be performed to characterize the product in terms of these
483 specific properties (*e.g.*, if binding to a receptor is inherent in protein function, this property
484 should be measured and used in comparative studies, see ICH Q6B for additional details).
485 Various methods such as surface plasmon resonance, microcalorimetry, or classical Scatchard
486 analysis can provide information on the kinetics and thermodynamics of binding. Such
487 information can be related to the functional activity and characterization of the proposed
488 biosimilar product's higher order structure.
489

- Binding to target antigen(s)
- Binding to representative isoforms of the relevant three Fc gamma receptors (FcγRI, FcγRII and FcγRIII), FcRn and complement (C1q)
- Fab-associated functions (e.g. neutralization of a soluble ligand, receptor activation or blockade)
- Fc-associated functions (e.g. antibody-dependent cell-mediated cytotoxicity, ADCC; complement-dependent cytotoxicity, CDC; complement activation)

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Sensorgrams IV



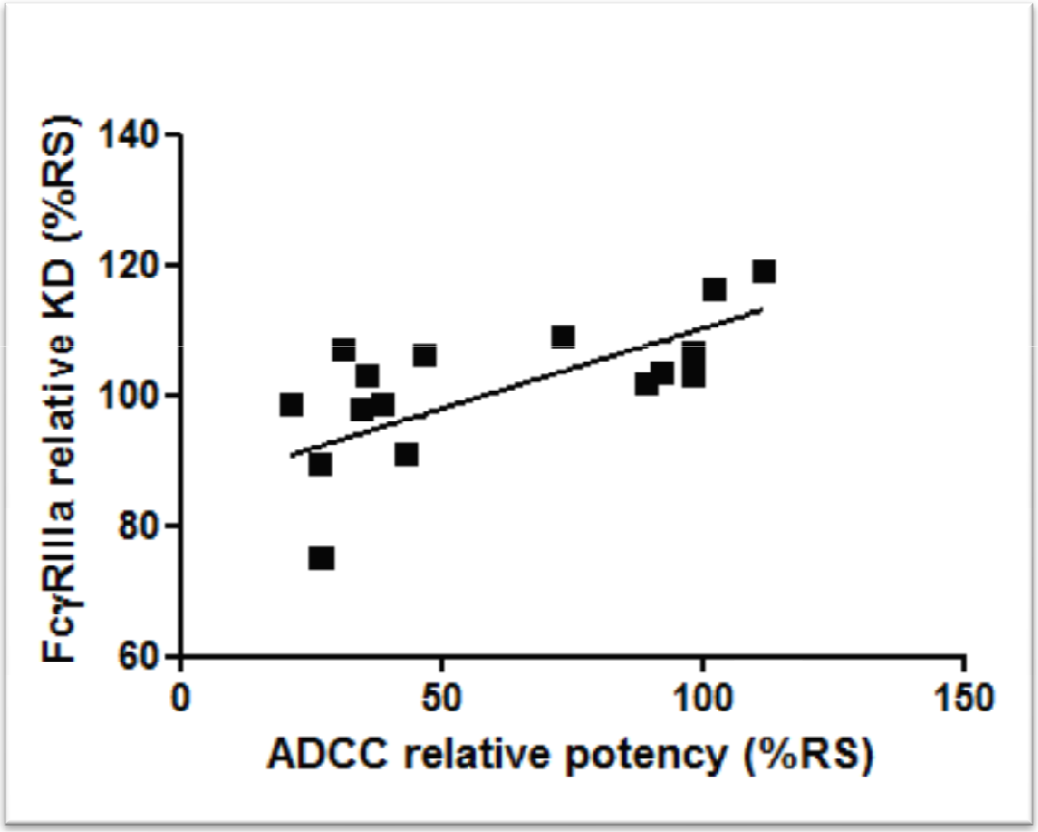
Rate of initial association is determined by Biacore evaluation software and designated k_a
K_a is measured in 1/Ms and is affected by sample concentration

Equilibrium of binding is achieved when the binding response does not change with time .
It is not always possible to reach equilibrium, particularly with high affinity interactions where the dissociation is typically very slow

Rate of dissociation is determined by Biacore evaluation software and designated k_d
K_d is measured in 1/s and is independent of sample concentration

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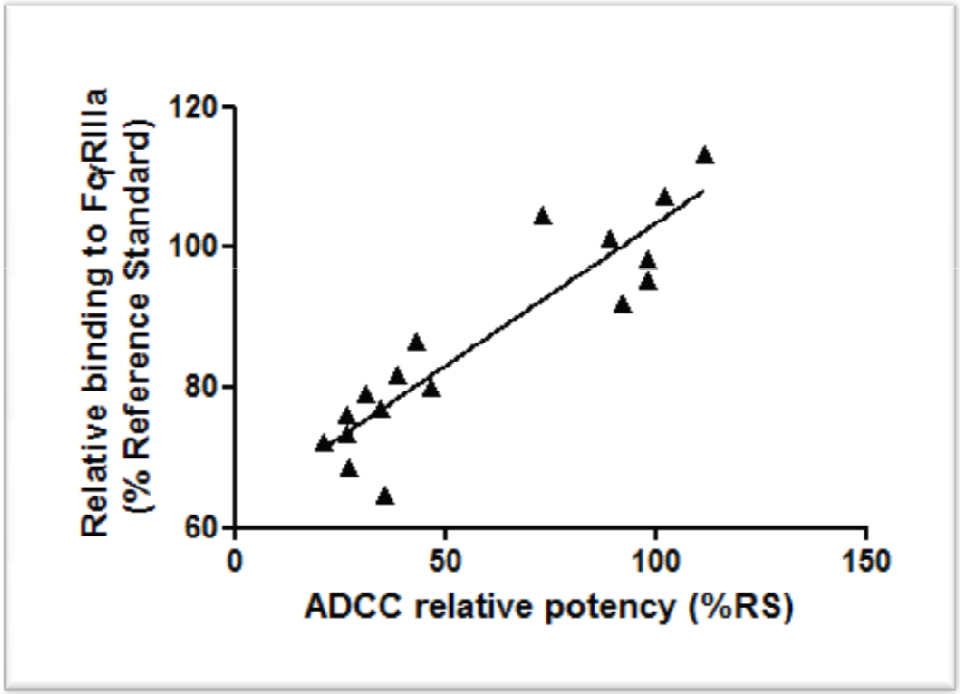
The Orthogonal Approach
Correlation between FcγIIIa and ADCC



- Relative KD – compared with a reference standard - reported as a ratio to reference standard
- Factors out assay or machine variation

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Correlation between IIIa and ADCC



- Signal at saturation point for each conc. To provide a dose response curve against the reference standard.
- Most sensitive to measure differences between samples
- Celltrion did not present this type of analysis.

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Remsima conclusions

- Remsima has the correct glycan structure from Mass Spec analysis
- SPR show differences in the binding of Remsima and Remicade to FcγIII
- ADCC data showed differences in Patient cells vs Healthy donors with a lower dose response curve. However this was not considered to be clinically relevant.
- Celltrion went to an extraordinary extent to measure these activities in very challenging assays but in the end this was useful as it showed that the difference seen in the SPR data had no clinical significance.

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Conclusions

- Understanding the Biological Activity of a Drug is the critical measure of the likely clinical efficacy.
- Biological Assays are complex and sometimes variable in quality of data.
- Biosimilars studies require these assays to be more robust and accurate than any other type of drug.
- Product variability needs to be considered carefully when assessing Biosimilars – drug data libraries become a key aspect of this
- We are still in our infancy of understanding all of the biological aspects of some drugs and this will become more important moving forward.

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Thanks to

BioSimilar Team:

- Dr Jen Lawson
- Dr Cat Thomson
- Dr Sarah Jones
- Dr Andrew Baron
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- Nathan Rendall
- Nicola McDonagh
- Hannah McLean
- Kathryn Carrick
- Sarah Marfo
- John Carruthers
- Richard McKendrick

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- Dr Debbie Allan
- Dr Terry Gray
- Dr Stefan Termen
- Dr Laura Munro

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