The Basics of the Nonclinical Comparability Exercise of Biosimilar Monoclonal Antibody for Regulatory Reviewers

IPRP Biosimilars Working Group

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I. Disclaimer

Disclaimer (1)

- This material is a compilation of publicly available information on the current approach for the nonclinical comparability exercise for biosimilars, particularly monoclonal antibodies.
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- This material is intended to help regulatory reviewers before he or she begins to review nonclinical aspects of biosimilars, who has a certain level of understanding for biotherapeutics and review experiences.

- This material could be used as an initiation step in the training of biosimilarity assessments and as a complementary tool and interactive course such as hands-on training.

- This material includes relevant guidelines currently published and cases of biosimilars approvals. These examples may not entirely reflect the current practices of the respective agencies and consultation with the agency is recommended for the most updated advice.

II. Concepts of Nonclinical Evaluation

1. Demonstration of Similarity/Biosimilarity

□ Stepwise approach

- Show similarity between the reference and biosimilar product using a stepwise approach ; quality study → nonclinical study → clinical study
- Differences between the biosimilar product and the reference product should be investigated and justified.
- Residual uncertainty needs to be addressed at all levels.

□ Totality of evidence

- The decision to license a biosimilar product should be based on a comprehensive evaluation of the whole data package, including quality, nonclinical and clinical parameters demonstrating similarity to a reference product.

(*ref: Leon van Aerts, 2016 EU and international assessor training on biosimilars*)

2. Guidelines (1)

- Demonstrating a high degree of molecular similarity between the similar biotherapeutic product (SBP) and the reference biotherapeutic product (RBP) should significantly reduce the need for nonclinical studies, since the RBP will already have a significant clinical history.

  (ref: WHO, Guidelines on evaluation of similar biotherapeutics products, 2009)

- As with all SBPs undergoing nonclinical evaluation, a stepwise approach should be applied to evaluate the similarity of biosimilar and reference mAbs. *In vitro* studies should be conducted first and a decision then made regarding the extent to which, if necessary, *in vivo* studies will be required.

  (ref: WHO, Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products, 2016)

2. Guidelines (2)

- On the basis of the totality of available quality and nonclinical *in vitro* data and the extent of residual uncertainty about the similarity of the SBP and RBP, nonclinical *in vivo* studies may not be required. If the quality-comparability exercise and nonclinical *in vitro* studies are considered satisfactory and no issues are identified that would prevent direct entrance into humans, then *in vivo* animal studies may be considered unnecessary.

  (ref: WHO Q&A: Similar biotherapeutic products, 2018)

- A stepwise approach is recommended for evaluation of the similarity of the biosimilar and the reference product**.** Analytical studies and *in vitro* studies should be conducted first and a decision then made as to the extent of what, if any, *in vivo* work in animal studies will be required.

  (ref: EMA, Guideline on similar biological medicinal products non-clinical and clinical issues, 2014)

2. Guidelines (3)

- If comparative structural and functional data using the proposed products provide strong support for analytical similarity to a reference product, then limited animal toxicity data may be sufficient to support initial clinical use of the proposed product. Such a study may be non-sacrificial and include endpoints that measure in-life parameters, pharmacodynamics (PD), and pharmacokinetics (PK) (with an assessment of immunogenicity).

(ref: FDA, Scientific considerations in demonstration biosimilarity to a reference products, 2015)

2. Guidelines : EMA

- Similar biological medicinal products (2014)
- Similar biological medicinal products containing biotechnology-derived proteins as active substance : non-clinical and clinical issues (2014)
- Similar biological medicinal products containing biotechnology-derived proteins as active substance : quality issues (2014)
- Similar biological medicinal products containing monoclonal antibodies : non-clinical and clinical issues (2012)

2. Guidelines : FDA

- Quality Considerations in demonstrating biosimilarity of a therapeutic proteins product to a reference product (2015)
- Scientific considerations in demonstrating biosimilarity to a reference product (2015)
- Clinical pharmacology data to support a demonstration of biosimilarity to a reference product (2016)
- Question and answers on biosimilar development and the BPCI Act (2018)
- New and revised draft Q&A on biosimilar development and the BPCI Act (rev.2) (2018)
- Considerations in demonstrating interchangeability with a reference product (2019)
- Development of therapeutic protein biosimilars : Comparative analytical assessment and other quality-related considerations (2019)

2. Guidelines : WHO

- Guidelines on evaluation of similar biotherapeutic product (SBPs), Annex 2, TRS No. 977 (2009)
- Guidelines on evaluation of monoclonal antibodies as similar biotherapeutic products (SBPs), Annex 2, TRS No. 1004 (2016)
- WHO Questions and Answers : Similar biotherapeutic products (2018)

2. Guidelines: HC, MFDS, PMDA, Swissmedic, TGA

☐ HC

- Guidance Document : Information and submission requirements for biosimilar biologic drug (2017)
- Biosimilar biologic drugs in Canada : Fact Sheet (2019)

☐ MFDS

- Guidelines on the evaluation of biosimilar products (2015)

☐ PMDA

- Guideline for the quality, safety, and efficacy assurance of follow-on biologics (2013)

☐ Swissmedic

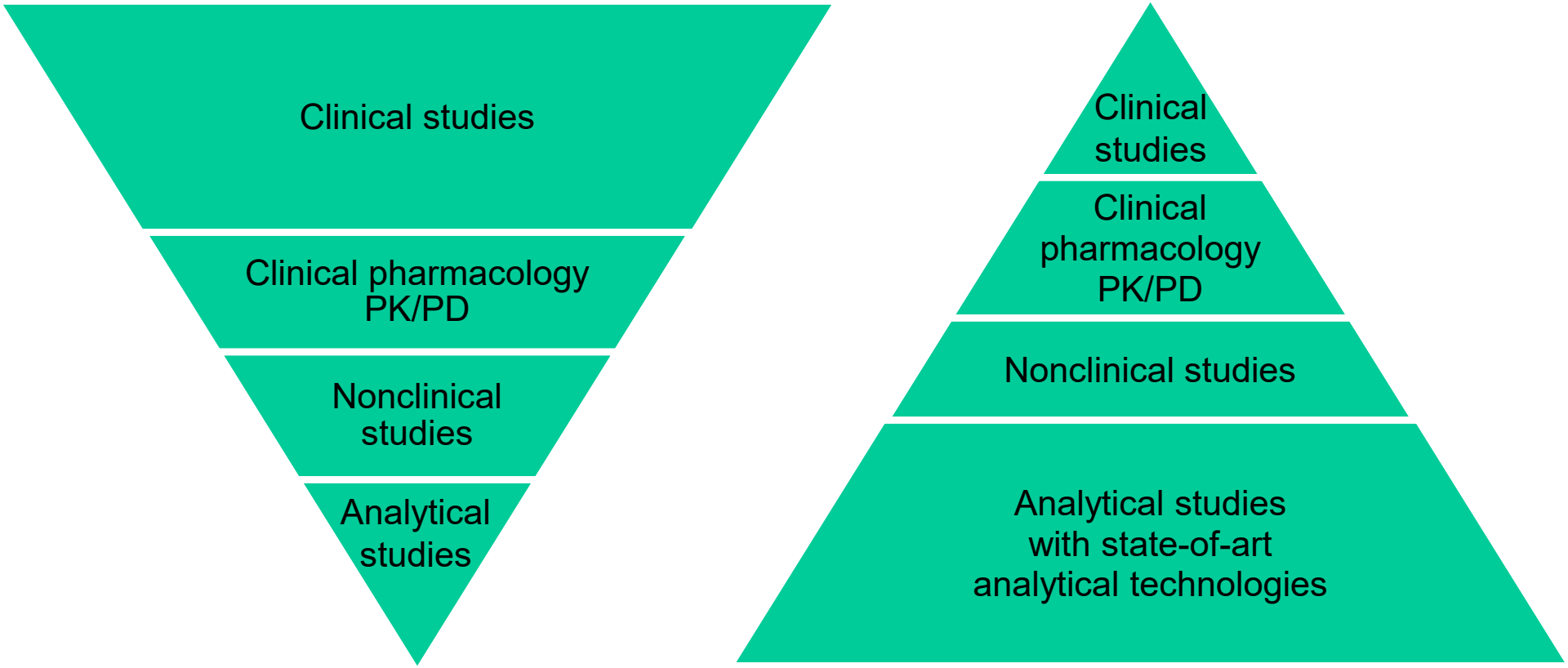
- Guidance document authorisation biosimilar (2018)
- Questions and answers concerning the authorisation of similar biological medicinal products (biosimilars) (2018)

☐ TGA

- Biosimilar medicines regulation (2018)

3. Relative Effort in Development Pathway

Originator **vs.** **Biosimilar**



(ref: Modified from Berghout A. Biologicals. 2011;39:293-6; McCamish M. Presented at EMA Workshop on Biosimilars, London, October 2013; and MacDonald J. APEC Biotherapeutics Workshop, Seoul, 2013)

4. *In vitro* Assay : Quality

- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance : quality issues (CHMP/BWP/247713 /2012. rev.1)

  - Sensitive, specific and sufficiently discriminatory

  - Methods should be appropriately qualified for the purpose of comparability.

  - Qualification for the purpose of comparability testing is the responsibility of the applicant.

- ICH Q5E

  - Characterisation studies do not necessarily entail the use of validated assays but the assays should be scientifically sound and provide results that are reliable.

- ICH Q6B

  - Normally the analytical procedures should be validated when the results are used in the specifications of the product.

5. *In vitro* Assay : Nonclinical Studies

- Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance : nonclinical and clinical issues (EMEA/CHMP/BMWP/42832/2014)

  - Comparative in nature and not just assess the response per se

  - Scientifically valid and suitable for their purpose

  - Sensitive, specific and sufficiently discriminatory

  - Compare the concentration–activity/binding relationship of the biosimilar and the reference medicinal product at the pharmacological target(s), covering a concentration range where potential differences are most sensitively detected.

  - Representative of the material intended for clinical use

  - Appropriate number of batches

  - Sufficient number of replicates to account for assay variability

6. Nonclinical Assessment : Biological Activity

- Biological activity may be evaluated using *in vitro* assays to determine which effects of the product may be related to clinical activity.

  - The use of cell lines and/or primary cell cultures can be useful to examine the direct effects on cellular phenotype and proliferation.

  - *In vitro* cell lines derived from mammalian cells can be used to predict specific aspects of *in vivo* activity and to assess quantitatively the relative sensitivity of various species (including human) to the biopharmaceutical.

    • receptor occupancy, receptor affinity, pharmacological effects

- The immunological properties of the monoclonal antibody should be described in detail.

  - Antigenic specificity, complement binding, unintentional reactivity and/or cytotoxicity towards human tissues distinct from the intended targets

(ref: ICH S6 preclinical evaluation of biotechnology-derived pharmaceuticals, 2011)

7. Nonclinical Assessment : *In vitro* Studies (1)

- WHO Questions and Answers : Similar biotherapeutic products (complementary document to the WHO guidelines on evaluation of similar biotherapeutic products)

  - Understanding of the mechanism of action of the molecule

  - Reveal difference that impacts the clinical performance (PK or efficacy)

  - Sensitive, specific and sufficiently discriminatory to provide evidence of difference

  - Justified with clinical relevance of the selected assays

  - Specific and sensitive for detecting differences between SBP and RBP unlike studies in animals

- Assays should represent all of the modes of action of the product to provide evidence useful in supporting the biosimilar for authorization on all indications.

7. Nonclinical Assessment : *In vitro* Studies (2)

- Relevant *in vitro* assay

  - Assays for evaluation of target binding

    • use of isolated ligands or receptors or cells expressing these targets

    • binding measurement

  - Assays for evaluation of biological activity

    • determination of pharmacological, functional activity (potency, efficacy)

    • measurement of signal transduction and/or functional aspect (e.g. cell death, proliferation, *etc*.)

    • one molecule may have multiple functional activities

(*ref: IPRP BWG, The basics of analytical comparability of biosimilar mAb; Leon van Aerts, 2016 EU and international assessor training on biosimilars)*

7. Nonclinical Assessment : *In vitro* Studies (3)

- *In vitro* studies specific for monoclonal antibodies

  - Fab

    - binding to target antigen or ligand : ELISA, SPR, cell-based binding assay
    - programmed cell death, neutralization assay : cell-based apoptosis assay, reporter gene assay

  - Fc effector function

    - Fc + FcγR binding : SPR, ELISA
    - Fc + C1q binding : SPR, ELISA
    - Fc effector function : Fab & Fc : ADCC, ADCP, CDC, cell-based assay

(*ref: Schiestl M, 2015 AHC Biotherapeutics Workshop*)

8. Nonclinical Assessment : *In vivo* Studies (1)

- Points to be taken into consideration when determining the need (if any) for an *in vivo* study ;

  - Presence of potentially relevant new quality attributes (e.g. new post-translational modification structures)

  - Presence of potentially relevant quantitative differences in quality attributes

  - Relevant difference in formulation

  - Inherent factors (e.g. glycosylation) that may impact PK/PD are not sufficiently characterized on a quality and *in vitro* level.

⇒ Only if a relevant animal model/species and *in vivo* study design are considered to add value/knowledge in the multidisciplinary approach.

- If the biosimilar comparability exercise for the physicochemical and biological characteristics and the non-clinical *in vitro* studies are considered satisfactory and there are no issues in this step which would block direct entrance into humans, an *in vivo* animal study is usually not considered necessary.

(*ref: WHO, Guideline on evaluation of monoclonal antibodies as similar biotherapeutic products, 2016; EMA, Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, 2014; Leon van Aerts, 2016 EU and international assessor training on biosimilars*)

8. Nonclinical Assessment : *In vivo* Studies (2)

- If *in vivo* studies are considered necessary, the following points should be taken into account ;

  - 3Rs (replace, reduce, refine) principles should be followed.
  - The design should be tailored to the type of data needed (PK, PD or safety).

- *In vivo* studies (when considered necessary) may include ;

  - Pharmacokinetics/Pharmacodynamics (PK/PD)
    - Single/repeated dose PK/PD study evaluating comparability
  - Safety studies
    - Single/repeated dose toxicity study evaluating comparability
  - Immunogenicity study (immunogenicity assessment in animals is generally not predictive for immunogenicity in humans)
    - Immunogenicity comparability

(*ref: EMA, Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues, 2014; Leon van Aerts, 2016 EU and international assessor training on biosimilars*)

III. Nonclinical Data Examples Submitted

1. Nonclinical Studies : Remicade Biosimilar (1)

	Remicade Biosimilar-C	Remicade Biosimilar-S	Remicade Biosimilar-P
Marketing authorization (approval date)	MFDS(‘12.7), EMA(‘13.9), HC(‘14.1), PMDA(‘14.7), TGA(‘15.8), Swissmedic(‘15.10), US FDA(‘16.4)	MFDS(‘15.12), EMA(‘16.5), TGA(‘16.11), US FDA(‘17.4), HC(‘17.12), ANVISA(‘18.7)	US FDA(‘17.4)
***In vitro* assay**	1) Fab related • TNF-α binding (SPR) • TNF-α binding (ELISA) • transmembrane TNF-α binding (cell-based ELISA) • TNF-α neutralizing activity • apoptosis • blockade of pro-inflammatory cytokine by reverse signaling (PBMC) • suppression of apoptosis and pro-inflammatory cytokine (IL-6, IL-8) secretion (epithelial cell line) • hTNF-β binding	1) Fab related • TNF-α binding (SPR, ELISA) • hTNF-α neutralization assay • transmembrane TNF-α binding (cell based ELISA) • apoptosis • hTNF-β binding	1) Fab related • TNF-α binding (SPR) • inhibition of apoptosis induced by TNF-α neutralizing activity • inhibition of ELAM-1 expression assay • binding to membrane-bound TNF-α by flow cytometry

1. Nonclinical Studies : Remicade Biosimilar (2)

	Remicade Biosimilar-C	Remicade Bosimilar-S	Remicade Biosimilar-P
***In vitro* assay**	2) Fc related • FcγR1 binding (SPR) • FcγR2a/FcγR2b binding (SPR) • FcγR3a binding (V and F hemizygotes, NK cell) • FcγR3b binding (SPR and neutrophil) • FcRn binding activity (SPR) 3) Fc-F(ab')2 related • C1q binding activity (ELISA) • CDC • ADCC (target cells : Jurkat cell, effector cells : PBMC, NK cells, whole blood) • suppression of T cell proliferation by induced regulatory macrophage in MLR • quantitation of the induced regulatory macrophages • induced regulatory macrophage-mediated wound healing of colorectal epithelium cells	2) Fc related • FcγR1a binding (SPR) • FcγR2a/FcγR2b (SPR) • FcRn binding (SPR) • FcγR3a binding (V and F hemizygotes, NK cell from PBMCs) • FcγR3b binding (SPR and neutrophil)) • C1q binding • CDC activity • ADCC using modified NK cell line/human PBMC • regulatory macrophage function by mixed lymphocyte reaction • cytokine (IL-8) release suppression activity in vitro IBD model • inhibitory activity of apoptosis in vitro IBD model	2) Fc related • ADCC assay using primary NK cells • CDC bioassay • FcR3a binding and membrane TNF binding by reporter gene assay • FcR3a (158V variant) binding by SPR • FcR3a (158F variant) binding by SPR • C1q binding by ELISA • FcR1 binding assay by SPR • FcR2 131H AND 131R binding assay by SPR • FcR3b binding by SPR • FcRn binding by SPR • NK cell binding assay 3) Additional *in vitro* assay • inhibition of T cell proliferation in mixed lymphocyte reaction • reverse signaling

1. Nonclinical Studies : Remicade Biosimilar (3)

	Remicade Biosimilar-C	Remicade Biosimilar-S	Remicade Biosimilar-P
***In vivo* efficacy**	• not tested	• PD : Tg197 transgenic mouse	• not tested
***In vivo* toxicology**	• 2-week, repeated dose (once weekly) toxicity : rat	• not tested	• 2-week, repeated dose (once weekly) GLP toxicology study conducted with 10 or 50 mg/kg PF-06438179 (no comparator)
***In vivo* PK**	• single dose (rat) : TK, Cmax, AUC 0 ~ 168h	• single (SD rat, Tg197 mouse) and 7 weeks, twice weekly repeated dose (Tg197 mouse) : PK, 1, 3, 10 mg/kg	• single dose (SD rat) : 10 or 50 mg/kg : TK, Cmax, AUC 0 ~ 1,344h
***In vivo* Immuno-genicity**	• not tested	• single and repeated dose (SD rat, Tg197 mouse) : immunogenicity (ADA)	• not tested

- Infliximab cross-reacts with TNF-α from humans and chimpanzees only. There are no pharmacologically relevant species in which to conduct a general toxicology assessment of Infliximab (no binding to rat TNF-α). Therefore, repeat dose toxicology studies were not conducted.

(*ref: Data retrieved from Remicade biosimilar PASIB, EPAR, FDA review report*)

2. Nonclinical Studies : Herceptin Biosimilar (1)

	Herceptin Biosimilar-M	Herceptin Biosimilar-C	Herceptin Biosimilar-S	Herceptin Biosimilar-A
MA (approval date)	US FDA('17.12), ANVISA('17.12), EMA('18.12)	MFDS('14.1), EMA('18.2), PMDA('18.3), US FDA('18.12), TGA('18.7)	EMA('17.11), MFDS('17.11), US FDA('19.1), ANVISA('19.5)	EMA('18.5) , US FDA('19.6)
***In vitro* assay**	1) Fab related • *in vitro* bioactivity (anti-proliferation assay) in SK-BR-3 cells • HER2 binding affinity in SK-BR-3 cells (FACS)	1) Fab related • *in vitro* bioactivity (anti-proliferation assay) • HER2 binding affinity (ELISA) • cell based binding affinity	1) Fab related • *in vitro* bioactivity (anti-proliferation assay) • HER2 binding affinity • combination treatment with chemotherapy, CDC assay • inhibition of AKT phosphorylation, *in vitro* angiogenesis assay	1) Fab related • ligand-independent proliferation inhibition bioassay in BT-474 cells • HER2 binding • HER2 binding kinetics • inhibition of AKT phosphorylation • inhibition of proliferation in NCI-N87 cells • inhibition of proliferation-synergy with chemotherapeutic in NCI-N87 cells • lack of proliferation inhibition in non-HER2 cells

2. Nonclinical Studies : Herceptin Biosimilar (2)

	Herceptin Biosimilar-M	Herceptin Biosimilar-C	Herceptin Biosimilar-S	Herceptin Biosimilar-A
***In vitro* assay**	2) Fc related • FcγRIa, FcγRIIa, FcγRIIab, FcγRIIIa , FcγRIIIb binding affinity (SPR) • FcRn (SPR) • ADCC (target cells : SK-BR-3 cells, effector cells : PBMC) • C1q (ELISA) • CDC • ADCP	2) Fc related • C1q binding affinity (ELISA) • FcγRI binding affinity (ELISA) • FcγRIIa binding affinity (SPR) • FcγRIIIa binding affinity (SPR) • FcRn binding affinity (SPR) • ADCC (effector cells : PBMC) 3) Additional biological assay • tissue cross reactivity	2) Fc related • C1q binding affinity (ELISA) • FcγRIa binding affinity (ELISA) • FcγRIIa binding affinity (SPR) • FcγRIIIa binding affinity (SPR) • FcγRIIIb binding affinity (SPR) • FcRn binding affinity (SPR) • ADCC 3) Additional biological assay • surface HER2 expression level, HER2 ECD shedding	2) Fc related • amplified FcRn binding • FcγRIa binding • FcγRIIa (131H) binding • FcγRIIb binding • FcγRIIIa (158V) binding • FcγRIIIa (158F) binding • FcγRIIIb binding • FcγR binding on primary macrophages • C1q binding 3) Fab- and Fc-mediated • ADCC • ADCP • lack of ADCC activity in HER2 negative cells • lack of CDC

2. Nonclinical Studies : Herceptin Biosimilar (3)

	Herceptin Biosimilar-M	Herceptin Biosimilar-C	Herceptin Biosimilar-S	Herceptin Biosimilar-A
***In vivo* efficacy**	• not tested	• inhibition of tumor xenograft growth (in nude mouse)	• inhibition of tumor xenograft growth (in mouse) : 1, 5, 15 mg/kg, 4 weeks	• inhibition of tumor xenograft growth (in mouse)
***In vivo* toxicology**	• 4-week, repeated dose (monkey) toxicity and toxicokinetic study	• 4-week, repeated dose (monkey) : 14, 42 mg/kg	• 4-week, repeated dose (monkey) (toxicity and toxicokinetic study) : 25 mg/kg	• 1 month repeated dose (monkey) : twice every week, 4 weeks, 25 mg/kg • 2-week repeated dose (rat)
***In vivo* PK**	• single and repeated dose (monkey)	• single and repeated dose (monkey)	• repeated dose (monkey)	• single and repeated dose (monkey)
***In vivo* Immuno genicity**	• not tested	• no immunogenicity was observed in toxicology test	• no difference detected in immunogenicity profile	• not tested

(*ref: Data retrieved from Herceptin biosimilar PASIB, EPAR, FDA review report*)

3. Nonclinical Studies Assessment

- The different biosimilar products of the same reference product used different pharmacology methods

  - the panels of *in vitro* tests applied were similar

  - but the *in vivo* studies performed were different between the different biosimilars

  ⇒ **Consider** that the comparability results of quality and *in vitro* assay were similar with reference, but test items of *in vitro* studies and *in vivo* studies could be different

- Recently the need for *in vivo* study has been considered with the totality of quality comparability exercise and *in vitro* data.

(*ref: Data retrieved from Remicade and Herceptin biosimilar PASIB, EPAR, FDA review report*)

Appendix : *In vitro* and *In vivo* Studies of Remicade Biosimilar

1. *In vitro* Assay

☐ Step 1 : Analyze all kinds of *in vitro* assays

- assays should discriminate the difference of biological activity and the difference of quality-related assay
- assays should cover the pharmacological/toxicological or pharmacokinetic aspect
- assays should represent and/predict the clinical situation
- assays should broadly cover all functional aspects
- assays should be tailored to the product and need to be fully justified
- assays should represent all of the modes of action of the product to provide evidence useful in supporting the biosimilar for authorization in all indications

(*ref: Leon van Aerts, 2016 EU and international assessor training on biosimilars*)

2. *In vitro* or *In vivo* Assay

☐ **Step 2 : Consider more *in vitro* assay or *in vivo* assay**

➢ Consideration of additional *in vitro* assay which is related to MoA

- presence of potentially relevant differences in the quality attributes (e.g. new post-translational modification structures)

- presence of potentially relevant quantitative differences (e.g. potency)

- relevant difference in formulation

- for the difference in glycosylation influencing ADCC activity : *in vitro* studies under more physiological considerations are more relevant than *in vivo* studies

- if the inherent factors that may impact PK/PD are not sufficiently characterized on a quality and *in vitro* level

(*ref: Leon van Aerts, 2016 EU and international assessor training on biosimilars*)

3. Mode of Action

MOA	RA	AS	PsA	PsO	CD	UC
Mechanisms involving the Fab (antigen binding) region						
Blocking TNFR1 and TNFR2 activity via binding and neutralization of s/tmTNF	Known	Known	Known	Known	Likely	Likely
Reverse (outside-to-inside) signaling via binding to tm TNF	-	-	-	-	Likely	Likely
Apoptosis of lamina propria activated T cells	-	-	-	-	Likely	Likely
Suppression of cytokine secretion	-	-	-	-	Likely	Likely
Mechanisms involving the Fc (constant) region						
Induction of CDC on tmTNF-expressing target cells (via C1q binding)	-	-	-	-	Plausible	Plausible
Induction of ADCC on tmTNF-expressing target cells (via FcγRIIIa binding expressed on effector cells)	-	-	-	-	Plausible	Plausible
Induction of regulatory macrophages in mucosal healing	-	-	-	-	Plausible	Plausible

(ref: FDA summary of existing literature on the topic of mechanisms of action of US-licensed Remicade)

4. Case Study 1 (1)

☐ *In vitro* studies of Infliximab biosimilar case 1 versus Remicade

Type of Study		Method	Result
Fab-related biological activities	TNF-α binding assay	SPR	Comparable
	TNF-α binding assay	ELISA	Comparable
	TNF-β binding assay	ELISA	No binding affinity
	TNF-α neutralization assay	WEHI bioassay	Comparable
Fc-related biological activities	FcγRI binding assay	SPR	Comparable
	FcγRIIa binding assay	SPR	Comparable
	FcγRIIIa V/F binding assay	SPR	Lower binding affinity activity
	FcRn binding affinity	SPR	Comparable
	C1q binding assay	ELISA	Comparable
	CDC	CDC	Few outlier batches exist
	ADCC	Effector cells : PMBC	Comparable

(ref: Data retrieved from infliximab biosimilar 1, EPAR, FDA review report)

4. Case Study 1 (2)

☐ Additional *in vitro* studies of Infliximab biosimilar case 1 versus Remicade

Type of Study		Method	Result
Fc-related biological activities	ADCC	Effector cells : NK cells	Comparable
	ADCC	Effector cells : LPS-stimulated monocytes as targets	Comparable
	Induction of apoptosis by reverse signaling	The binding of membrane-bound TNF-α by the antibody leads to the induction of apoptosis in Jurkat cells	Comparable
	Inhibition of pro-inflammatory cytokine release by reverse signaling (Caco-2 cells)	Evaluation of the release of inflammatory cytokine (IL-8)	Comparable
	Regulatory macrophage-mediated wound healing of colorectal epithelium cells (closure %)	Induction of closure of a gash in monolayer of HCT116 cells	Comparable
	Inhibition of T cell proliferation by induced regulatory macrophage	Mixed lymphocyte reaction (MLR)	Comparable
	Induction of regulatory macrophages	MLR : the presence CD14/CD206 cell population	Comparable

(ref : Data retrieved from infliximab biosimilar 1, EPAR, FDA review report)

4. Case Study 1 (3)

☐ Repeated dose toxicity

Species / Sex / Number	Dose (mg/kg) / Route	Duration	NOAEL (mg/kg/day)	Major findings
Rat / M&F / 5	0, 10, 40 mg/kg IV Remicade	2 doses 1 week apart	40 mg/kg	• No major toxicity • TK
Rat / M&F / 10	0, 10, 40 mg/kg IV infliximab biosimilar 1 & Remicade	2 doses 1 week apart	40 mg/kg	• No major toxicity : the two drugs are comparable • TK : Lower C_{max}, AUC_{0-168}
Rat / M&F / 10	0, 10, 50 mg/kg IV infliximab biosimilar 1 & Remicade	2 doses 1 week apart	50 mg/kg	• No major toxicity : the two drugs are comparable
Rat / 5 per dose group	10, 50 mg/kg IV infliximab biosimilar 1 & Remicade	single dose		• PK : C_{max}, AUC_t ; comparable

☐ *In vivo* pharmacology study was not performed.

- Primary PD assessed in *in vitro* studies consisted of a binding affinity comparison. No secondary PD was performed.
- No binding affinity to TNF-α from species commonly used in toxicology studies (rhesus monkey, pig, dog, rat and mouse). The hTNF- α have cross-reactivity with only chimpanzee.

5. Case Study 2 (1)

☐ ***In vitro* studies showed that biological activity of infliximab biosimilar case 2 was similar to that of Remicade.**

Type of Study		Result
Fab-related biological activities	TNF-α binding assay	TNF-α (soluble) binding activity of infliximab biosimilar was similar to that of Remicade
	TNF-β binding assay	infliximab biosimilar and Remicade showed a significant lack of TNF-β (LTα3) binding activity
	tmTNF-α binding assay	tmTNF-α binding activity of infliximab biosimilar was similar to that of Remicade
	TNF-α neutralization assay	Inhibitory activity of infliximab biosimilar on the signal pathway was similar to that of Remicade
	Apoptosis assay	Apoptosis activity of infliximab biosimilar was similar to that of Remicade
	Inhibitory activity on apoptosis in an *in vitro* IBD model	Inhibitory activity of infliximab biosimilar on apoptosis was similar to that of Remicade, which may support the extrapolation of indications to inflammatory bowel disease (IBD)
	Cytokine release activity in an *in vitro* IBD model	Cytokine IL-8 release suppression activity of infliximab biosimilar was similar to that of Remicade, which may support the extrapolation of indications to IBD
Fc-related biological activities	FcγRIa binding assay	FcγRIa binding activity of infliximab biosimilar was similar to that of Remicade
	FcγRIIa binding assay	FcγRIIa binding activity of infliximab biosimilar was similar to that of Remicade
	FcγRIIIa binding assay (V/V type)	FcγRIIIa binding activity of infliximab biosimilar was slightly higher than that of Remicade, but not biologically significant as these differences did not affect ADCC activity. ADCC activity of infliximab biosimilar was within the similarity range
	FcγRIIIa binding assay (F/F type)	FcγRIIIa (F158 allotype) binding activity of infliximab biosimilar was similar to that of Remicade

(ref: Data retrieved from infliximab biosimilar, EPAR)

5. Case Study 2 (2)

☐ ***In vitro* studies showed that biological activity of infliximab biosimilar case 2 was similar to that of Remicade.**

Type of Study		Results
Fc-related biological activities	FcγRIIIa binding assay using NK cells from PBMCs	Binding activity of infliximab biosimilar to FcγRIIIa on natural killer (NK) cells of peripheral blood mononuclear cells (PBMCs) was similar to that of Remicade
	FcγRIIb binding assay	FcγRIIb binding activity of infliximab biosimilar was slightly higher than that of Remicade, but not significant as these differences were within assay variability and did not affect ADCC activity. ADCC activity of infliximab biosimilar was within the similarity range
	FcγRIIIb binding assay	FcγRIIIb binding activity of infliximab biosimilar was similar to that of Remicade
	FcγRIIIb binding assay using neutrophils	FcγRIIIb binding activity of infliximab biosimilar using neutrophils was similar to that of Remicade
	ADCC assay using engineered NK cell line	ADCC activity (effector cell: engineered NK cell line) of infliximab biosimilar was similar to that of Remicade
	ADCC assay using healthy donor PBMCs	ADCC activity (effector cell: healthy donor PBMCs) of infliximab biosimilar was similar to that of Remicade, which may support similarity in more representative condition of *in vivo* situation
	C1q binding assay	C1q binding activity of infliximab biosimilar was similar to that of Remicade
	CDC assay	CDC activity of infliximab biosimilar was similar to that of Remicade
	FcRn binding assay	FcRn binding activity of infliximab biosimilar was slightly higher than that of Remicade, but not significant as these differences were within assay variability and were not translated into PK difference according to the PK results from the Phase I clinical study
	Evaluation of regulatory macrophage induction function	Regulatory macrophage induction function of infliximab biosimilar was similar to that of Remicade, which may support the extrapolation of indications to IBD

(ref: Data retrieved from infliximab biosimilar, EPAR)

5. Case Study 2 (3)

☐ ***In vivo* studies were conducted as supportive studies.**

Type of Study	Experimental System	Treatment	Result
***In vivo* efficacy study**	Tg197 transgenic mouse model of arthritis	• Infliximab biosimilar or Remicade • Intraperitoneal administration • Dose : 1, 3, and 10 mg/kg • 7 weeks, twice weekly	Infliximab biosimilar is similar to Remicade in its ability to inhibit, in a dose dependent manner, the arthritic pathology and histopathology of these mice
***In vivo* PK study – single dose**	Tg197 transgenic mouse model of arthritis	• Infliximab biosimilar or Remicade • Intraperitoneal administration • Dose : 1, 3, and 10 mg/kg • Single dose	Infliximab biosimilar and EU and US Remicade have similar PK behavior
***In vivo* PK study – single dose**	SD rat	• Infliximab biosimilar or Remicade • Intravenous administration • Dose : 1, 3, and 10 mg/kg • Single dose	Infliximab biosimilar and EU and US Remicade have similar PK behavior
***In vivo* PK study – repeated dose**	Tg197 transgenic mouse model of arthritis	• Infliximab biosimilar or Remicade • Intraperitoneal administration • Dose : 1, 3, and 10 mg/kg • 7 weeks, twice weekly	Infliximab biosimilar and EU and US Remicade have similar PK behavior

➢ Toxicity study was not performed since infliximab has been known to have no cross-reactivity to TNF-α from relevant species other than humans and chimpanzees.

(ref: Data retrieved from infliximab biosimilar, FDA review report)