

## Remsima Case Study

# The importance of ADCC in assessing clinically relevant differences

Authors: Dr. Daniel Galbraith, Chief Scientific Officer and Andy Upsall, Director of Technical Services – BioOutsource Ltd.

### The Regulatory Authorities Verdict

Biosimilars have caused a revolution in the development of biologic drugs. The model for biosimilars is a combination of speed to market and low cost; these are fundamental to any biosimilar development project. The biosimilars pathway to approval is not always without difficulty, biosimilars are still relatively new, and as each new applicant is reviewed we are learning more and more about these molecules. One area of interest recently has been the subject of extrapolation of data from one clinical trial within a specific indication to one or more alternative indications. This will become more and more critical as manufacturers seek to maximise the potential patient population and recoup development costs as new versions of the biosimilars enter the market. The approval of the first biosimilar monoclonal antibody, Infliximab, REMSIMA™/INFLECTRA™, in 2012 represented a significant milestone, welcomed by the biosimilar industry and patients alike. This was the world's first monoclonal antibody biosimilar approved for a developed market. There have,

however, been some issues with how different regulators have viewed this product; The European Union [1], Korean FDA [2], and Health Canada [3] have adopted different positions on the extent that extrapolation can be applied, whilst the FDA have not made their position clear (Fig. 1)[4].

A clear concern for other manufacturers has been the question; what is it about the Remsima analytical package that could cause these differing views? Based upon publicly available information, the clinical studies for the drug show good comparability in the patient populations that were assessed [5]. The physicochemical analysis revealed that all major physicochemical characteristics and biological activities of Remsima were comparable to those of Remicade. The only differences in product attributes were differing levels of afucosylation which ultimately affected the ADCC (Antibody-Dependent Cell-mediated Cytotoxicity) activity of Remsima.

Indication	European Union	Korean FDA	Canada
Rheumatoid arthritis (RA)	✓	✓	✓
Crohns Disease	✓	✓	✗
Ulcerative Colitis	✓	✓	✗
Alkolysing Spondylitis	✓	✓	✓
Plaque Psoriasis	✓	✓	✓
Psoriatic Arthritis	✓	✓	✓

Figure 1: Extrapolation of Indications of Remsima/Inflectra

For Celltrion to justify extrapolation beyond RA, the clinical significance of these differences needed to be understood and an explanation of their relevance, or lack of, was required. To achieve this, a range of modified ADCC assays (and supporting binding assays) were implemented, designed with biological relevance in mind that could determine whether the differences between the two molecules persisted in conditions closer to an *in vivo* state and to provide an indication of the clinical implications. It is likely that the degree to which these arguments were accepted by the Health Authorities influenced the extent of extrapolation, pending the result of further clinical studies.

## Antibody Dependent Cell Mediated Cytotoxicity (ADCC)

ADCC is an important part of the immune defence mechanism whereby a target cell is recognised by an antibody using specific proteins (antigens) on the cell surface. Fc receptors on the effector cells of the immune system then recognise the bound antibodies, and subsequently mediate lysis of the target cells. Different leukocytes have the ability to mediate ADCC as effector cells: natural killer (NK) cells, macrophages and neutrophilic and eosinophilic granulocytes (Figure 2).

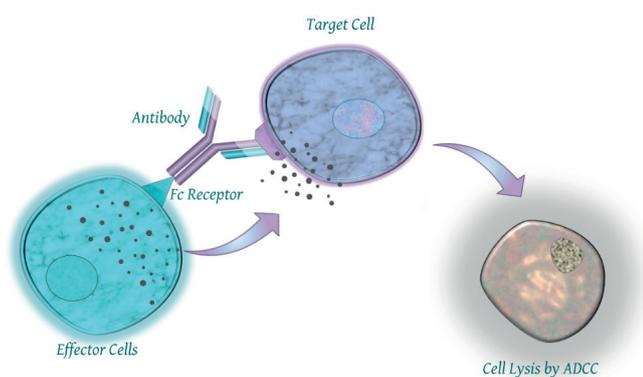


Figure 2. An Overview of ADCC

There are many formats with which to directly measure ADCC activity and these are broadly divided into classical methods in which the death of target cells is measured directly, or surrogate methods that typically rely upon a reporter cell line in which a signal cascade is activated following functional activation of the Fc receptor with the response measured via a suitable reporter. Within each of these formats, there are many variations and, when considered, the number of possible assay designs will easily reach several orders of magnitude with each of the differing methodologies imparting different information on the molecules they are used to test. Some will be more sensitive to differing glycosylation patterns, others more accurate whilst biological relevance will be the strength of other methods. However, all ADCC assays can be considered to be amongst the most challenging of bioassays, beset with practical challenges and requiring significant expertise to design and successfully perform in a routine manner.

## The relevance of ADCC activity to Remicade's clinical efficacy

TNF- $\alpha$  is an important cytokine in the pro-inflammatory pathway and a range of biologics drugs have been approved that specifically target TNF- $\alpha$ . The primary mechanism of action

(MOA) of the anti-TNF- $\alpha$  therapeutics is to neutralise soluble TNF- $\alpha$ . Although there are an increasing number of additional activities that are described, the specific involvement of ADCC as a MOA in patients is controversial. Evidence for potential ADCC mechanism *in vitro* has been published in many different forms and it is thought that for some indications these drugs have the capacity to destroy cells expressing the cytokine, via ADCC activity (amongst other mechanisms), thereby reducing the inflammatory response. However, the true *in vivo* extent of ADCC and the potential for clinical improvement in patients is unknown.

## When should ADCC potential be evaluated?

IgG1 antibodies have the potential to mediate ADCC activity provided that a cell-associated target is available. Whether or not this is specifically described for the innovator antibody, the possibility of acquired activity from a biosimilar candidate cannot be excluded. In some molecules such as Avastin there has been no recorded ADCC activity *in vitro* or *in vivo*, however the molecule does possess the Fc region capable of binding to the effector cells of the immune system. There is therefore a possibility, albeit remote, that a biosimilar version could induce ADCC and evaluation of potential ADCC activity of Avastin biosimilar molecules has been requested by the Regulators in Europe. It is expected that with this precedent set, similar requirements will be made for other molecules.

## Attributes that confer ADCC Activity

Many factors can influence ADCC, but the most likely cause will be differing glycan species on the Fc portion of the antibody and this probably represents the antibody characteristic that is hardest to control during the production process. For ADCC, the degree of fucosylation of the Fc portion is generally regarded as the most important variable, although other glycans can play a role. Many articles have discussed the variability of glycosylation profile between different batches of the same therapeutic antibody [6]. Afucosylated glycans on the Fc portion lead to higher binding affinity for CD16a (Fc $\gamma$ -Receptor IIIa) which in turn translates to the induction of higher ADCC activity. Remsima is more fucosylated than Remicade and therefore has lower affinity for CD16a which is the cause of lower ADCC potency.

## Optimal ADCC Methods: Orthogonal and Sensitive

Despite their challenging nature, ADCC assays need to be both accurate and precise. Of even more importance is the assays performance; the regulators require an understanding of the method's ability to sensitively detect specific differences in the structure of the antibodies and reveal the impact of these differences on the activity of the molecule. The assays must sit within an orthogonal process which improves our overall understanding of the molecule's activity. Such an approach is critical to fully elucidate the ADCC activity of a biosimilar candidate and the methods may include binding assays (SPR and/or ELISA) to study antibody-CD16a interactions, bridging assays that incorporate capture of the antibody by its antigen, in addition to surrogate and fully functional assays. Each of the methods will yield differing levels of sensitivity to structural differences between the innovator and biosimilar and the methods that are ultimately adopted should be based upon an understanding of the sensitivity along with throughput and the

expected level of accuracy. In figure 3, the correlation between BioOutsource methods of CD16a SPR and ADCC (classical PBMC method of mixed genotypes) data is illustrated, demonstrating higher levels of sensitivity of the classical BioOutsource ADCC methodology over the SPR.

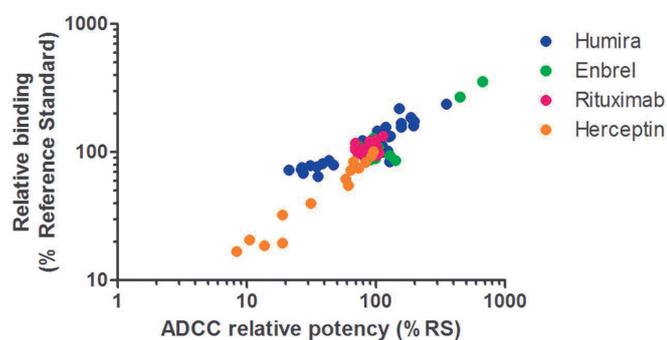


Figure 3. ADCC vs relative binding by molecules

## Biologically relevant ADCC Methods

The EMA noted that all major physicochemical characteristics and biological activities of Remsima were comparable to those of Remicade. A small difference in the amount of afucosylated infliximab was observed that translated into a lower binding affinity towards specific Fc receptors which in turn led to a lower *ex vivo* ADCC activity in the most sensitive assay [1].

Having obtained the expected results using fucose-sensitive, orthogonal methodologies to demonstrate the potential for a reduced level of ADCC activity, focus shifted to understanding the clinical relevance attributable to these differences. Celltrion employed a wide range of modified ADCC and related methodologies to provide a justification for a negligible impact on efficacy as a result of the differences.

In binding studies, the addition of serum from patients with Crohn's disease caused a reduction in the overall binding of Remsima and Remicade to the Fc receptor as well as an abrogation of the differences between the molecules when binding to primary cells, expressing different immune receptors, was assessed. Similar results were obtained using the classical ADCC format and it was suggested that these results were due to the presence of immune complexes, soluble serum factors and/or monomeric IgG in the serum with the conclusion that binding differences to CD16a, observed under stringent *in vitro* conditions, may not be extrapolated into a clinical effect in patients.

The ADCC activity under physiological conditions was further investigated with the performance of ADCC assays using whole blood in which the differences between Remsima and Remicade were abolished. The potential for ADCC was further evaluated using physiologically relevant target cells – lipopolysaccharide (LPS)-stimulated monocytes (to increase the expression of surface TNF- $\alpha$ ) – and no ADCC activity was detected. This finding suggested that ADCC activity could only be observed using cell lines, engineered to express membrane-bound TNF- $\alpha$  at a level higher than would be expected under physiological conditions and thus, that ADCC activity is likely to be limited in inflammatory settings *in vivo*. Finally, it was acknowledged, that at the time of submission, there had been no published reports describing the induction of ADCC by TNF antagonists in a patient [1].

## Conclusion

Whilst ADCC activity may not represent the primary mechanism of action of all antibodies and may be questionable, as to its clinical relevance, even if activity is shown *in vitro*. ADCC represents the biological activity that is the most difficult to characterise. This is important in the earliest stages of biosimilar development, as antibodies from different clones will almost certainly yield different glycan profiles, and simple changes to the manufacturing process have the potential to cause differences in the post translation modifications of an antibody. At early stages of development it is not cost effective to apply a full battery of tests to understand every aspect of the molecules and how Celltrion viewed the combined results of glycan analysis, CD16a binding and ADCC activity is unknown.

In choosing a clone to produce Remsima, Celltrion selected one on the basis of a good match with Remicade for the critical aspects of glycan structure, binding and biological activity. It is now known that the sensitivity of ADCC assays is key to pinpointing differences. An assay using purified NK cells harvested from patients may be most relevant clinically.

Moving forwards, the significance of potential differences between biosimilar molecules and their reference products, where ADCC is not recognised as a significant mechanism of action, remains to be resolved. Confirmation of a negative ADCC response can be conducted with relative ease; however, where ADCC potential exists the significance of differences is harder to explain. It is clear that by implementing the most sensitive methods at the earliest stages of product development, manufacturers have the ability to make informed decisions and take optimal clones and manufacturing processes forwards, eliminating or reducing the need for comprehensive evaluations of biological relevance of ADCC. From our previous experience, we now have easy access to platform technologies, with an understanding of their expected sensitivity and these can be converted to analyse new molecules with relative ease.

## References

1. European Medicines Agency - EMA. Assessment Report – Remsima. Accessed on 20 April 2015. Available from: [http://www.ema.europa.eu/docs/en\\_GB/document\\_library/EPAR\\_-\\_Public\\_assessment\\_report/human/002576/WC500151486.pdf](http://www.ema.europa.eu/docs/en_GB/document_library/EPAR_-_Public_assessment_report/human/002576/WC500151486.pdf).
2. GaBI Online – Generics and Biosimilars Initiative. Biosimilars approved in South Korea [www.gabionline.net]. Mol, Belgium: Pro Pharma Communications International. Accessed on 20 April 2015. Available from: <http://gabionline.net/Biosimilars/General/Biosimilars-approved-in-South-Korea>.
3. Health Canada. REMSIMA. Accessed on 21 April 2015. Available from: [http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd\\_smd\\_2014\\_remsima\\_160195-eng.php](http://www.hc-sc.gc.ca/dhp-mps/prodpharma/sbd-smd/drug-med/sbd_smd_2014_remsima_160195-eng.php).
4. GaBI Online – Generics and Biosimilars Initiative. FDA delays meeting to evaluate infliximab biosimilar [www.gabionline.net]. Mol, Belgium: Pro Pharma Communications International. Accessed on 22 April 2015. Available from: <http://www.gabionline.net/Biosimilars/News/FDA-delays-meeting-to-evaluate-infliximab-biosimilar>.
5. Weise M, Kurki P, Wolff-Holz E, Bielsky M, Schneider C. Biosimilars: the science of extrapolation. *Blood*. 2014; 124(22):3191-3196.
6. Schiestl M, Stangler T, Torella C, Cepeljnik T, Toll H, Grau R. Acceptable changes in quality attributes of glycosylated biopharmaceuticals. *Nature Biotechnology*. 2011; 29: 310-312.