Characterizing biosimilar molecules: Utilizing SPR as an orthogonal technique for demonstrating potency

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The biosimilar space continues to develop and grow. As of 2015, only one biosimilar monoclonal antibody (mAb) was approved in either the US or Europe. In 2016, five biosimilar mAbs were approved in these regions (Table 1). Although the launch dates remain to be seen, both the FDA and EMA have many similar applications in their pipelines with several more anticipated in 2017.

To establish biosimilarity, a sponsor is required to demonstrate that "the biological product is highly similar to the reference product notwithstanding minor differences in clinically inactive components" and that "there are no clinically meaningful differences between the biological product and the reference product in terms of safety, purity, and potency of the product" (1, 2). Regulatory agencies are taking a stepwise, "totality of evidence" approach to demonstrating biosimilarity. If the proposed and reference products show a high degree of structural and functional analytic similarity (i.e., "fingerprint-like"), then a more targeted and selective approach can be taken when designing and conducting *in vivo* animal and clinical studies. This direct path to a more targeted and selective approach creates incentive for rigorous *in vitro* structural and functional characterization in early stages of development to leverage potential cost and resource efficiencies.

While physiochemical analyses can assess the structural and physical characteristics of molecules, they do not provide information on the molecules functional capabilities: how they interact with targets and drive effector function. As such, regulatory agencies encourage sponsors to investigate the pharmacologic activity through *in vitro* and/*or in vivo* functional assays. Functional assays include, but are not limited to, ligand binding assays and cell-based assays.

Contract research organizations (CROs) continue to take on a larger role in helping their clients establish biosimilarity. Sartorius Stedim BioOutsource (3) is a leading CRO and provider of biosimilar testing services that caters to the global biopharmaceutical and biotechnology industries. They are dedicated to the biological evaluation of biotherapeutic antibodies and offer a range of off-the-shelf testing services designed to reduce the time and cost of biosimilar development programs.

In this article, Sartorius Stedim BioOutsource utilize surface plasmon resonance (SPR) as an orthogonal functional technique to investigate potency alongside cell-based assays. They investigate a variety of biosimilar mAbs that initiate antibody-dependent cellular cytotoxicity (ADCC) as an effector function. To validate SPR, they correlate potency data from both SPR and a traditional cell-based method.

Table 1. Biosimilar mAbs approved in US and Europe during 2016

Biosimilar	Benepali™	Remsima™	Flixabi™	Erelzi™	Amjevita™
Biosimilar developer	Samsung/ Biogen	Pfizer/ Celltrion	Biogen	Sandoz	Amgen
Innovator product	Enbrel™	Remicade™	Remicade	Enbrel	Humira™
Innovator developer	Amgen	J&J	J&J	Amgen	Abbvie
Region approved	Europe	US	Europe	US	US

Surface plasmon resonance

SPR is a label-free technology which monitors the formation and dissociation of biomolecular complexes in real time, allowing the measurement of binding kinetics, affinity, and binding specificity. As such, SPR is an ideal technology for functional analyses to measure the binding interactions associated with biosimilars and compare that binding to that of their reference medicinal product (RMP). SPR has several advantages over traditional end-point immunoassay approaches such as ELISA or RIA. The real-time collection of binding data enabling affinity and kinetic characterization provides a more comprehensive analysis than is possible with end-point techniques alone.

In SPR assays, target molecules (e.g., target protein or Fc receptor) are immobilized to the surface of prepared sensor chips and a sample containing a potential interacting partner (e.g., biosimilar or RMP) is injected over the surface of the sensor chip. The SPR response measured is directly proportional to the change in mass concentration close to the surface and data is displayed as a sensorgram. Mathematical models can be fitted to the sensorgram to elucidate the kinetics and affinity constants to characterize binding behaviour.

Such an approach delivers greater accuracy because it allows full flexibility of the molecule binding to immobilized ligand and minimizes the risk of differences due to artifacts of immobilization. Also, because SPR assays do not include wash steps, they are highly suitable for characterizing both low as well as high affinity interactions.

Sartorius Stedim BioOutsource

To support the development of biosimilars for clients, Dr. Sarah Stone and her team at Sartorius Stedim BioOutsource focus on biosimilar characterization. In particular, Dr. Stone develops and performs SPR-based assays analyzing Fc- and Fab-specific interactions. They use Biacore™ T200 and Biacore 4000 systems to analyze association/dissociation rates and binding constants for quantitative comparison on the binding characteristics of product candidates to the associated RMP.

Results and observations

An orthogonal approach to potency characterization

For functional testing, Dr Stone's team incorporate Biacore assays into their screening strategy to perform highly sensitive surrogate potency studies that complement traditional cell based binding assays. Adopting orthogonal modes of analysis also increases the understanding of a molecule's primary and secondary mechanisms of action, allowing it to be more accurately compared to the RMP.

Biacore FcγRIIIa screening is predictive of ADCC activity

Fc γ-receptors (FcγRs) are found on the surface of effector cells in the immune system and play an important role in mediating cellular effector functions of antibody-based therapeutics through binding to the Fc-region of IgG. Their activity stimulates phagocytic or cytotoxic cells to destroy infected cells or invading pathogens and they are an important class of cancer therapeutics that is showing positive results in early clinical trials.

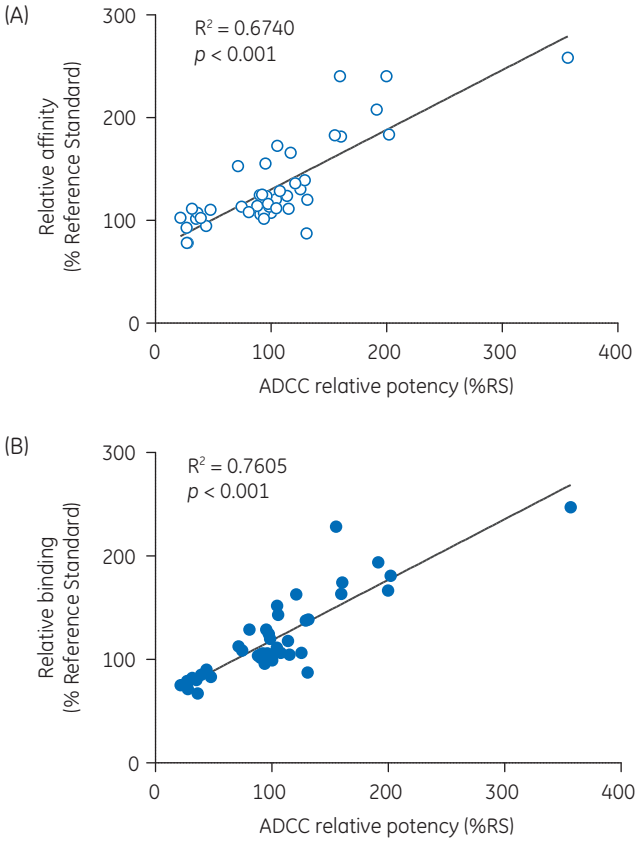

Fig 1. Correlation of FcγRIIIa and ADCC using Adalimumab.

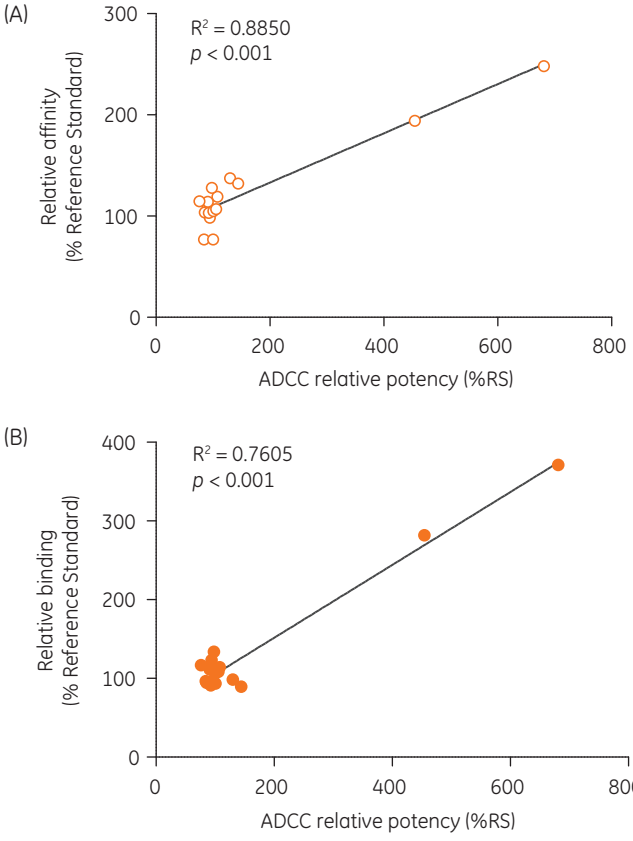

Fig 2. Correlation of FcγRIIIa and ADCC using Etanercept.

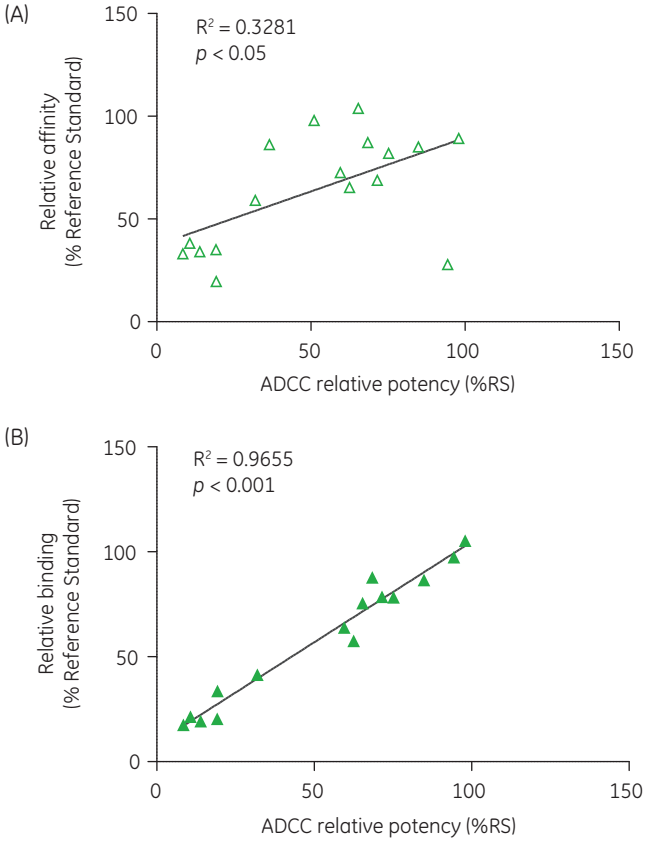

Fig 3. Correlation of FcγRIIIa and ADCC using Trastuzumab.

FcγRIIIa mediates antibody-dependent cell cytoxicity (ADCC) and to determine the value of Biacore FcγRIIIa screening in the prediction of ADCC activity, Dr Stone's team performed several correlations using reference standards such as Adalimumab, Etanercept, and Trastuzumab (Figs 1, 2, and 3).

FcγRIIIa was immobilized to a Biacore Sensor Chip CM5 surface and assays conducted to study the kinetics of the interaction between each reference standard and FcγRIIIa. Sensorgrams describing the binding behaviour were analyzed using 1:1 kinetics analysis. Figures 1A, 2A, and 3A show that when ADCC activity is high (i.e., cell death is high) there is a corresponding increase in the relative affinity. As would be expected, most molecules show a 'normal' level of cell death and this is matched by the normal distribution of the relative affinity for the test materials.

The relative binding response demonstrates an even stronger correlation than that seen with relative affinity (Figs 1B, 2B, 3B). This indicates that Biacore assays can detect molecule-specific differences over a range of responses and are suitably sensitive in both true *in vitro* conditions and in physiologically relevant ADCC conditions to be predictive of ADCC activity.

Conclusions

Sartorius Stedim BioOutsource have successfully incorporated a fast, sensitive, and reproducible Biacore FcγRIIIa assay into their functional testing regime for demonstrating biosimilarity and found it to be highly predictive of ADCC activity. Rapid turnaround times combined with the ability to check multiple analytes in an assay and determine binding kinetics not possible with other methodologies enhances the quality of data available to characterize and assess comparability of biosimilars plus aids in their development. This orthogonal approach aligns with the recommendations of the FDA and EMA and utilizes Biacore systems to perform surrogate potency assays that could be extended beyond the development process into manufacturing and QC of future antibody based therapeutics.

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