Role of cell based potency assay in functional characterization of therapeutic monoclonal antibodies (mAbs)

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INTRODUCTION

Characterization of an originator monoclonal antibody (mAb), or comparability study between originator mAb and biosimilar candidate mAb, are challenging due to the highly complex and variable structure of monoclonal antibodies. The heterogeneity that can arise in quality attributes, including structural alterations, oxidation, deamination or glycosylation, can impact the potency and the efficacy of the final product. Moreover, mAbs have multiple biological functions associated with both the specific antigen binding (Fab) region and interaction with C1q and Fc receptors via the Fc region of the antibody. For this reason, the characterization of therapeutic monoclonal antibodies should include a wide range of analytical techniques; as well as binding and cell-based potency assays in order to reflect the main mechanism of action and effector function of the mAb of interest[1,2].

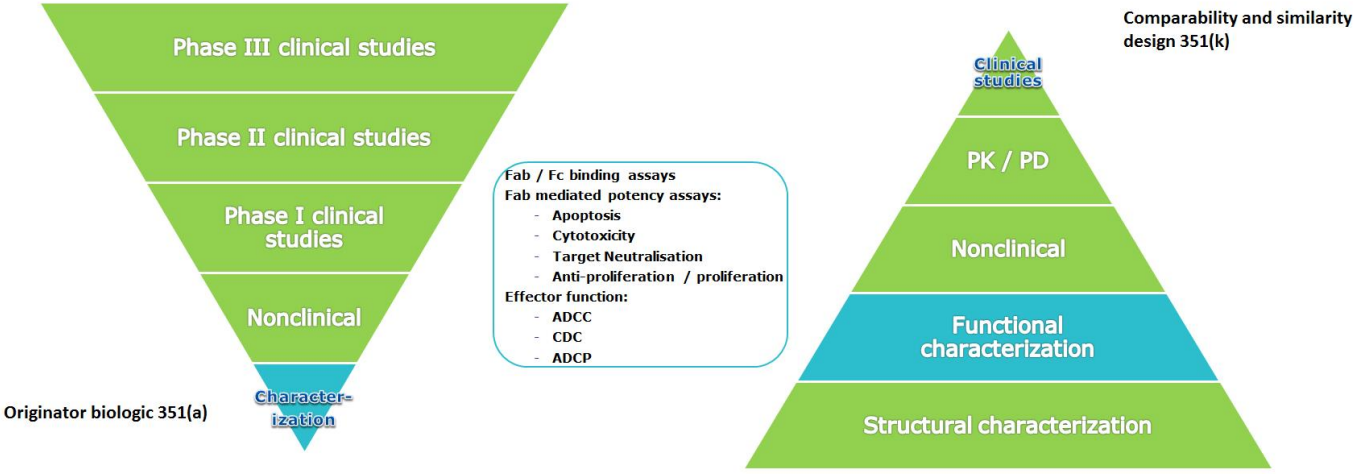
POTENCY ASSAYS

Cell based potency assays allow for quantitative determination of biological activity (relative potency), which is calculated based on a comparison of the biological response of the tested article with a reference standard (RS).

The main goal in new therapeutic mAb development is to evaluate clinical efficacy (Fig 1). The pathway to clinical evaluation is based on a foundation of thorough characterization and knowledge of the molecule, the critical quality attributes, and the possible effects of structural heterogeneity[1]. Therefore, it is imperative that appropriate cell-based potency assays are selected and developed, to support the functional characterization of mAb products, by measuring all the possible biological activities (potency). A range of cell based assays should be implemented during characterization in order to investigate all possible mechanisms of action (MoA) of the mAb, which add further information to understand the binding interactions mediated by all regions of a therapeutic mAb.

While cell-based potency assays with high sensitivity and precision are required in the early stages of development for selection of a desired clone, they are also required at later stages to fully develop the manufacturing process and evaluate the effect of any changes that are made on the desired quality attributes process.

Figure 1: Product characterization is an important activity in development of both originator biologics and biosimilars



If any changes in the manufacturing process occur (e.g., a change in manufacturing facility or process scale-up), or other changes which could affect quality attributes of the protein, it is required to assess the risk to the safety and the efficacy of the product by conducting a head to head comparability study. If process changes are implemented in late stages of development, with no additional clinical studies planned to support the BLA, the comparability exercise should be comprehensive for an approved product[3,4].

For biosimilar development, similarity between the licensed originator product and the biosimilar candidate can be proved using a step wise approach[5], which includes structural and functional characterization as well as non-clinical and clinical studies (Fig 1). For functional characterization, the bioassay should demonstrate the difference between the licensed originator product and the biosimilar candidate[6].

ASSAY DEVELOPMENT

Bioassays play an essential role for the development of an originator as well as for a biosimilar candidate.

We have years of experience in customized potency assay development, validation, and GMP testing. To expand our capabilities, two commercially available therapeutic mAbs, Adalimumab and Cetuximab, were selected as models for development of a full range of cell-based potency assays, to ensure successful analysis of both Fab-mediated and complex effector functions (Fc-mediated). Data generated from each assay is reported as relative potency (RP) values. Assessments of parallelism between product reference standards and test samples were performed using PLA software.

BioReliance®

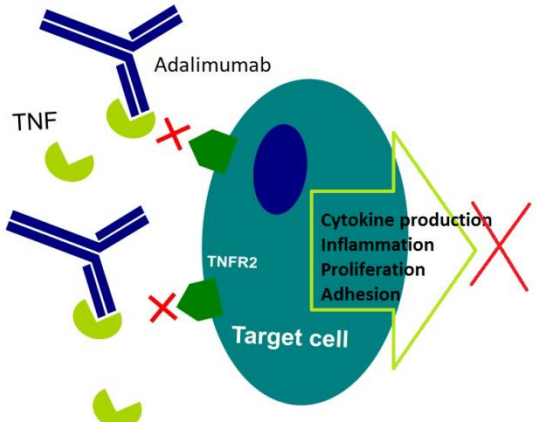
Fab-mediated Potency Assays

Target neutralization assay

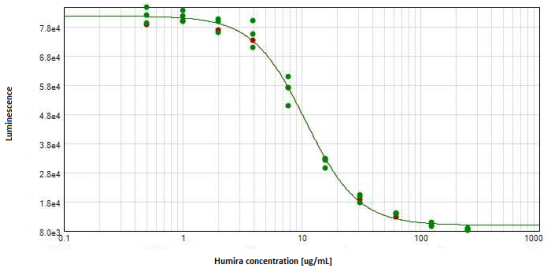
The TNFα neutralization assay was developed to measure the neutralization of TNFα mediated by Adalimumab. TNFα binds to the TNFR2 receptor, leading to apoptosis of the target cell. Adalimumab binds to sTNFα and prevents interaction of TNFα with its receptor; thereby interfering with the inflammatory signals, which initiate and maintain autoimmune inflammatory diseases. Briefly, L929 cells were treated with an Adalimumab dilution series and TNFα for 17±1h at 37ºC. Biological activity was detected using a luminescence based end-point reagent (Figure 2).

Figure 2: Adalimumab TNFα neutralization assay

Schematic diagram



Representative Adalimumab dose response-curve for TNF-neutralization

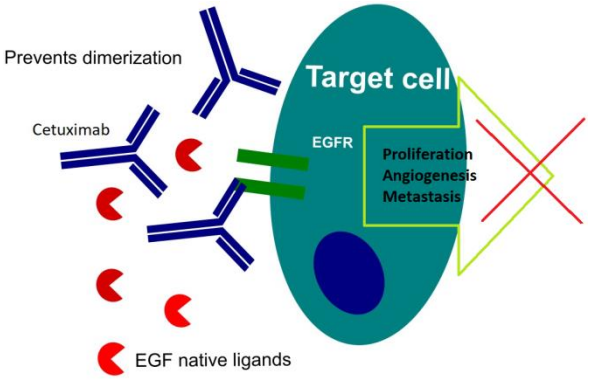


Anti-proliferation assay

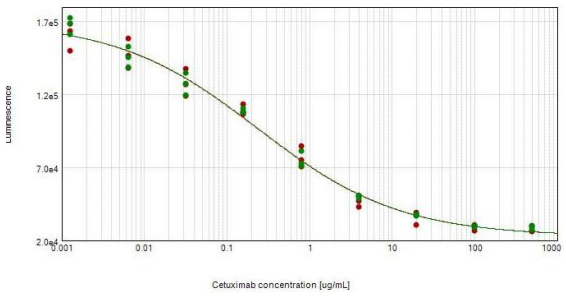
An anti-proliferation assay was developed to measure inhibition of proliferation in a cell line overexpressing epidermal growth factor (EGF) receptor mediated by Cetuximab. Cetuximab has higher affinity for the EGF receptor than the native ligands, EGF transforming growth factor-alpha (TGFα) or amphiregulin (AREG), and effectively blocks ligand binding leading to inhibition of cell proliferation.
In brief, cells overexpressing EGF were incubated with EGF native ligand and a Cetuximab dilution series for 72h at 37ºC. The anti-proliferation effect was measured using a luminescence based end-point reagent (Figure 3).

Figure 3: Cetuximab anti-proliferation assay

Schematic diagram



Representative Cetuximab dose response-curve for anti-proliferative activity



BioReliance®

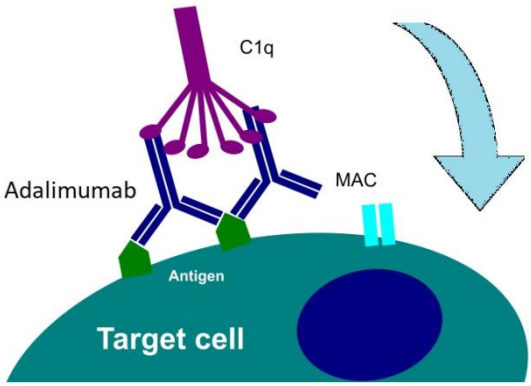
Fc-mediated Potency Assays (Immune effector functions)

CDC assay

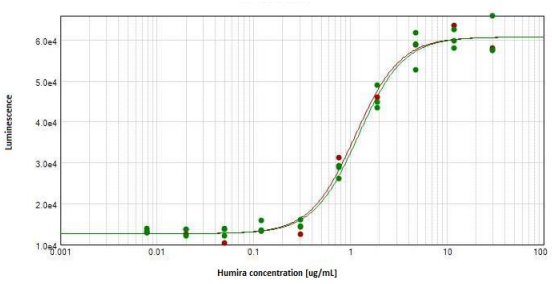
A complement dependent cytotoxicity (CDC) assay was developed to measure the activity mediated by Adalimumab, which triggers complement cascade through binding target antigen on the surface of cells, via the Fab region and C1q protein via the Fc region. The CDC assay was performed using mTNFα CHO-K1 target cell line. Briefly, cells were treated with an Adalimumab dilution series in the presence of pooled human serum complement for 2h at 37ºC. CDC activity was measured using a luminescence based end point reagent (Figure 4).

Figure 4: CDC assay

Schematic diagram of a CDC assay



Representative graph of Adalimumab dose response-curve

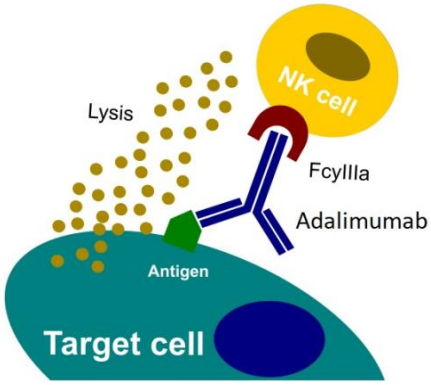


ADCC assay

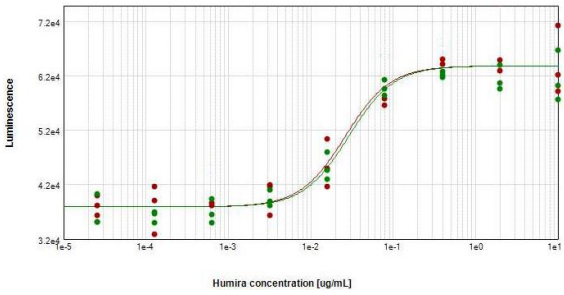
The antibody dependent cellular cytotoxicity (ADCC) assay was developed to measure cell death of target cells mediated by Adalimumab. ADCC was performed using mTNFα CHO-K1 target cell line. Fresh human peripheral blood mononuclear cells (PBMCs) were isolated from healthy blood donors by gradient centrifugation. Activation of PBMCs was followed by monocyte depletion. To detect ADCC activity of Adalimumab, the target cell line was treated with an Adalimumab dilution series followed by the addition of activated PBMC. After overnight incubation, the signal was detected using a luminescence based end-point reagent (Figure 5).

Figure 5: ADCC assay

Schematic diagram of ADCC assay



Representative graph of an Adalimumab dose response-curve



SUMMARY

The characterization of therapeutic monoclonal antibodies should include a wide range of analytical techniques, including binding and cell-based potency assays, in order to reflect the main mechanisms of action and effector functions of the mAb of interest. The assays developed for Cetuximab and Adalimumab, therapeutic mAbs with diverse mechanisms of action, enable functional characterization of both Fab- and Fc-mediated activities. The biological activities measured, using our assays, are consistent with the known binding and functional activities of each molecule as indicated in the literature. The assay methodologies used for effector function analysis can be readily adapted to accommodate alternative molecules with different target antigens.

REFERENCES

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