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BIOLOGICAL ASSAYS | KEY TO SUCCESSFUL DEVELOPMENT OF INSULIN BIOSIMILAR FOR CLINICAL APPLICATION

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BACKGROUND:

Biosimilar development is about assessment of similarity to an existing reference biologic in terms of purity, efficacy, and safety with no clinically meaningful differences. Biosimilar development is often visualized as a pyramid, based on analytical characterization (physicochemical & biological), animal testing, clinical pharmacology & clinical efficacy trials as the pinnacle (Fig. 1). The clinical efficacy endpoints for evaluation of biosimilarity are less sensitive compared to *in-vitro* biological assays which provide early signs of clinical efficacy. Immunogenicity testing by *in-vitro* bioassays is very crucial for determining the safety of a biosimilar.. Biological assays are based on sensitive techniques such as colorimetry, fluorimetry, surface plasmon resonance etc. The *in-vitro* bioassays for potency, immunogenicity & impurity studies mitigate the uncertainties & risks involved in development of biosimilar for clinical application (Fig. 2).

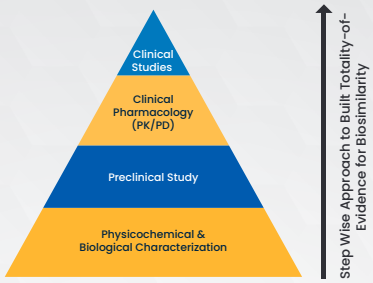


Fig.1 Pyramid based approach in development of biosimilar

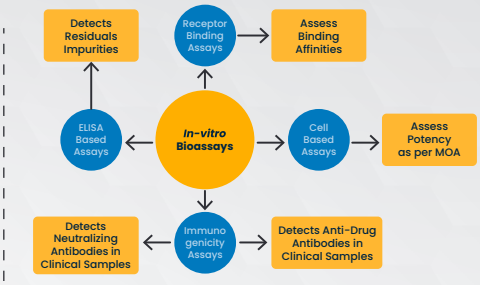


Fig.2 *In-vitro* bioassays involved in development of biosimilar

OBJECTIVE:

The objective of the study is to establish the importance of biological assays in successful development of insulin biosimilar for clinical application.

METHODS:

BioGenomics Ltd. has developed Insulin Aspart (BGL-ASP) as a biosimilar to the reference medicinal product (RMP); NovoRapid®. The *in-vitro* bioassays were done for comparative biological characterization of BGL-ASP to its innovator, NovoRapid®. The relative potencies from *in-vitro* bioassays such as kinetic binding, receptor autophosphorylation, glucose uptake, lipogenesis and mitogenicity assay were studied based on the mechanism of insulin activity (Fig 3). The residual impurities (host cell protein, trypsin & carboxypeptidase B) & immunogenicity to Insulin Aspart were determined by ELISA. The immunogenicity of BGL-ASP was investigated by multi-tiered approach for detection of Anti-Drug Antibodies (ADA) in clinical samples (Fig 4). The ADA positive samples were tested for their neutralizing potential by a cell-based glucose uptake assay; herein referred to as Neutralizing antibody assay (NAb assay).

1. Insulin binds to the insulin receptor present on target cells such as adipocytes & skeletal muscle cells.
2. Binding of insulin to its receptor leds to autophosphorylation of the receptor.
3. Insulin receptor autophosphorylation activates Phosphoinositide 3 kinase (PI3K) & Mitogen Activated protein kinase (MAPK) pathway.
4. Activation of PI3K pathway leads to glucose update, synthesis of lipids, proteins & glycogen in the target cells.
5. Activation of MAPK pathway leads to cell survival & proliferation, often mediated by off-target (IGF-1R)

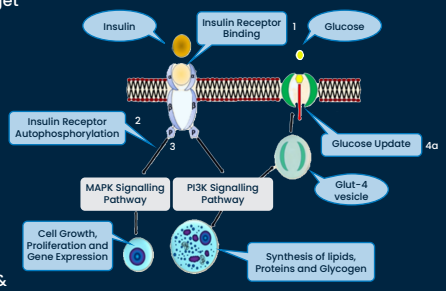


Fig. 3 Schematic representation of Insulin activity at cellular level

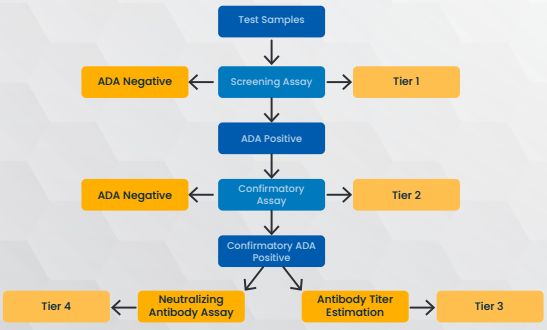


Fig. 4 Immunogenicity testing by multi-tiered approach

RESULTS:

The relative potencies observed from *in-vitro* biological assays showed apparent similarity of BGL-ASP to NovoRapid®. The sigmoidal overlay plot of BGL-ASP & NovoRapid® observed from glucose uptake assay is shown to represent biosimilarity by one of the potency assays (Fig. 5A). The apparent similarity in potency observed from glucose uptake assay correlates well with the in-vivo efficacies observed from phase-1 pharmacodynamic study (Fig 5A & 5B). The absence of residual impurities observed from ELISA tests established purity of BGL-ASP.

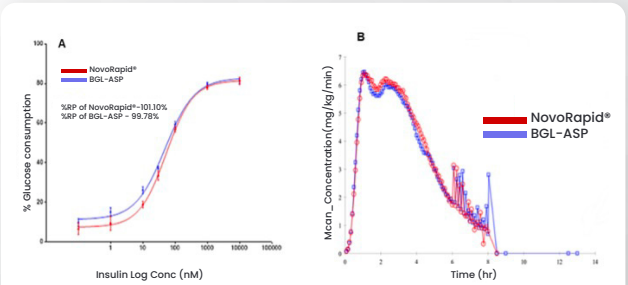


Fig. 5 A. Sigmoidal overlay plot of NovoRapid® & BGL-ASP observed from Glucose Uptake Assay (*in-vitro*), B. Comparative pharmacodynamic (PD) profile of NovoRapid® & BGL-ASP in Phase-1 study (*in-vitro*).

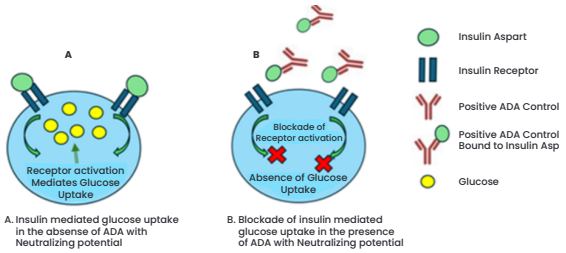


Fig. 6 Cell based glucose uptake assay showing glucose uptake activity in the absence of neutralizing antibodies (A) & blockade of glucose uptake activity in the presence of neutralizing antibodies (B).

The immunogenicity testing showed a low incidence of ADA with apparent similarity in both the treatment groups; BGL-ASP & NovoRapid®. The neutralizing potential of ADA was determined by a cell-based glucose uptake assay (NAb assay) to check for the blockade of glucose uptake activity (Fig 6). The NAb assay done with confirmed ADA positive samples showed glucose uptake activity thereby confirming the absence of neutralizing antibodies in both the treated groups. The results of biological assays clearly established purity, potency & absence of significant immunogenicity of BGL-ASP, which was confirmed by high efficacy & safety during clinical trials.

CONCLUSION:

Biological assays played a crucial role in determining purity, potency & immunogenicity of BGL-ASP in establishing it as a biosimilar & they also served as the early indicators of clinical efficacy & safety. Thus, it is clearly demonstrated that biological assays serve as the key for successful development of insulin biosimilar for clinical application.

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