THERAPEUTIC ANTIBODIES FROM CONCEPT TO CLINICAL USE

Antibody therapies have recently been established themselves as successful players in the field of biotherapeutics, showing great promises in the treatment of cancer, immune disorders, and metabolic diseases. Unlike small molecules, which penetrate cells, antibodies bind to cell membrane receptors or tumor antigens, thus activating or inhibiting a functional pathway, which in turns might lead to a therapeutic effect. The stages of therapeutic antibody development process can be divided into distinct preclinical and clinical phases, which are summarized in this poster.

Sergio Andrés Torres-Pérez[1,3], Edith Gonzalez-González[1,3], Gregorio Carballo-Uicab [1,3], Luis A. Vallejo-Castillo [1,3], Keyla M. Gómez-Castellanos [1,3], Said Vazquez-Leyva[1,3], Sonia M. Pérez-Tapia[1,3]*, and Juan C. Almagro[1,2]*

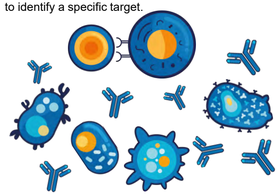
[1]UDIBI, Instituto Politécnico Nacional, Mexico City, Mexico
[2]GlobalBio, Inc. Cambridge, MA 02138
[3]Laboratorio Nacional para Servicios Especializados de Investigación, Desarrollo e Innovación (I+D+i) para Farmoquímicos y Biotecnológicos,
LANSEIDI-FarBiotec-CONACyT, Mexico City 11340, Mexico

PRECLINICAL PHASE

DISCOVERY

Target identification and validation

Molecular targets can be soluble molecules, receptors, antigens expressed on the cell surface and/or viral and bacterial antigens associated with a specific pathology. Typically, combinations of protocols including biochemical methods, genetic interactions, and computational inference are used to identify a specific target.



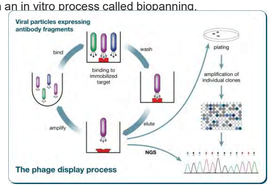
Target identification and validation include:

- Gene synthesis
- Peptides synthesis
- Customized generation of antibodies.
- Expression of personalized proteins.

Did you know that the phage display technique uses bacteriophages (viruses that infect bacteria) into which a phage coat protein gene is inserted? In fact, one of the world's best-selling drugs, Humira (adalimumab), was the first FDA-approved antibody therapy and was developed using the phage display technique.

Antibody generation and optimization

The discovery of antibodies is a fundamental part of the development of therapeutic antibodies. In this phase, validated molecular targets are used as antigens to generate antibodies with potential therapeutic use. There are technologies such as phage display that allow candidates to be selected and amplified through an in vitro process called biopanning.



THE GENERATION OF ANTIBODIES IN UDIBI INCLUDE

Generation of synthetic, natural or semi-synthetic libraries.

We build discovery platforms based on phage display technology. We have generated semi-synthetic libraries called ALTHEA Gold+ Libraries™ and others built with the repertoire of a convalescent COVID-19 patient called ALTHEA SARS-CoV-2 Libraries™.

Antibody Candidate Selection

We developed a set of methods that allow the identification of candidate antibody scFv to be developed as IgG.

- **Binding assays: target recognition by ELISA or flow cytometry**
- **Blockade: Blockage of interaction with its natural ligand or receptor.**
- **Competition: Competition tests with the natural ligand.**

DEVELOPMENT

Antibody production

In this stage, leading antibodies molecules are evaluated in multiple assays to assess the physicochemical and biological characteristics of candidate antibodies for future development.

PRODUCTION OF ANTIBODIES

High-throughput antibody expression.

Recombinant Antibody Production Service rapidly delivers high-quality, purified antibodies for all antibody screening applications.

Cell lines for antibody production

- HEK293
- Expi293T
- CHODG44

Antibody characterization and optimization

Antibody optimization is a crucial step in therapeutic antibody discovery. Here drug candidates are modified to increase their efficacy, stability and/or decrease immunogenicity. It is also in the stage where the antibodies are converted into their final therapeutic format.

ANTIBODY CHARACTERIZATION SERVICES

Physicochemical characterization platform. Determine the crucial characteristics to select the best antibodies with a complete physicochemical characterization package that includes techniques such as:

- **SDS-PAGE reductor and no reductor conditions**
- **Chromatography SEC, CEX, RP, HILIC**
- **Mass spectrometry: intact mass, intact mass glicosylated and no glicosylated reducing and non-reducing peptide mapping, disulfide bridges, carbohydrate profile.**
- **Fussion temperature (Tm), isoelectric point.**
- **Affinity constant (KD) by SPR**
- **Epitope mapping.**

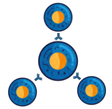
UDIBI ANTIBODY OPTIMIZATION INCLUDE

Humanization of antibodies and affinity maturation.

Humanization methods must increase the human content of therapeutic antibodies to minimize immunogenic reactions, maintain potency to minimize costs in downstream engineering processes, and secure intellectual property. Through our alliance with GlobalBio and its technology (GBM Humanization™) these objectives are met and have been applied to design more than a dozen antibodies from various academic and biotech companies.

Antibody Lead Functionality Analysis

In vitro assays involve biological testing to thoroughly evaluate potential antibody candidates for viability and functional profile.



ANTIBODIES IN VITRO FUNCTIONALITY SERVICES

Biological assays of the Fab region.

At UDIBI we have developed a series of protocols that allow an in-depth evaluation of the biological activity of the antibody:

- Apoptosis induction or blocking
- Molecular target binding
- cell proliferation
- Inhibition of cell proliferation
- Phosphorylation and activation of cell receptors
- Evaluation of mitogenic and metabolic activity
- Assays for neutralization and blockade of ligand-receptor interaction

Biological function of the Fc region.

- UDIBI offers cell-based services to determine antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC), antibody mediated phagocytosis (ADCP)

TESTING

Preclinical research

Preclinical testing and development is the phase of therapeutic antibody discovery and development in which the safety and efficacy in relevant animal models of the antibody is evaluated.

Formulation and stability

Perform formulation and accelerated stability testing to ensure the integrity of your therapeutic antibody for use in humans

Development of animal models.

Our In Vivo research protocols allow us to determine the efficacy, safety (single and repeated dose studies) and pharmacokinetics at the preclinical level.

In vivo imaging (IVIS).
Determines the kinetics of distribution and accumulation of antibodies and fluorophores in an in vivo murine model [CD1 Nude Foxn1nu mice]

We have multiple expression systems available for the production of antibodies. Generally, whole antibodies are produced in mammalian expression systems, while antibody fragments are produced using bacteriophages.

CLINICAL PHASE

CLINICAL TRIALS

Clinical manufacturing

Clinical manufacturing is the stage of therapeutic antibody development in which the candidate drug is manufactured in larger quantities under good manufacturing practices for clinical trials.

Clinical trials

There are three phases of clinical trials that must be completed before a drug can be approved by the regulatory entity:

Phase I: Evaluates the safety of the drug.

Phase II: Test the effectiveness of the drug.

Phase III: Tests the efficacy of the drug in a heterogeneous population and how it compares with current treatment options.

REGULATORY SUPPORT

UDIBI have a team of experts with extensive experience in regulating biosimilar medicines that can advice you in the registration and approval process in the COFEPRIS. We focus on understanding the regulatory needs of the Mexican pharmaceutical industry.

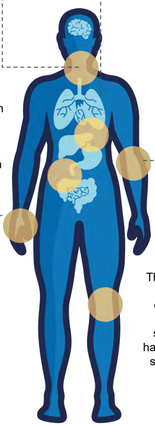
APPLICATION

THERAPEUTICS

Therapeutic antibodies are used to treat multiple diseases through a wide range of mechanisms, including immune checkpoint inhibition, immuno-oncology, cancer treatment, and neutralization of pathogens.

Covid-19
The type 2 coronavirus that causes severe acute respiratory syndrome (SARS-CoV-2) continues to threaten public health around the world, and effective therapies are being developed to combat coronavirus disease (COVID-19). Neutralizing antibodies (nAbs) have great potential for the prevention and treatment of SARS-CoV-2 infection by blocking the binding site (RBD) of the Spike protein

Rheumatoid arthritis (RA)
Being an autoimmune disease, RA is traditionally caused by excessive stimulation of a patient's immune-based inflammatory response against their own tissues. To reduce this inflammation, antibodies have been developed that attack and inhibit cytokines (tumor necrosis factor and interleukin-6).



Severe Asthma
To treat severe asthma, antibody drugs attack and inhibit immune inflammatory response proteins. to reduce the patient's risk of lung inflammation. These targets traditionally include interleukins, such as IL-5 and IL-4, which reduce eosinophilic inflammation in conjunction with traditional corticosteroids.

Acute Lymphoblastic Leukemia (ALL)
This disease is a public health problem, since it is the main cause of death by disease in children. B cells (including some leukemia cells) usually have the protein CD22 on their surface. There are antibodies that act as a search signal, carrying the chemotherapy drug to the leukemia cells, killing them.