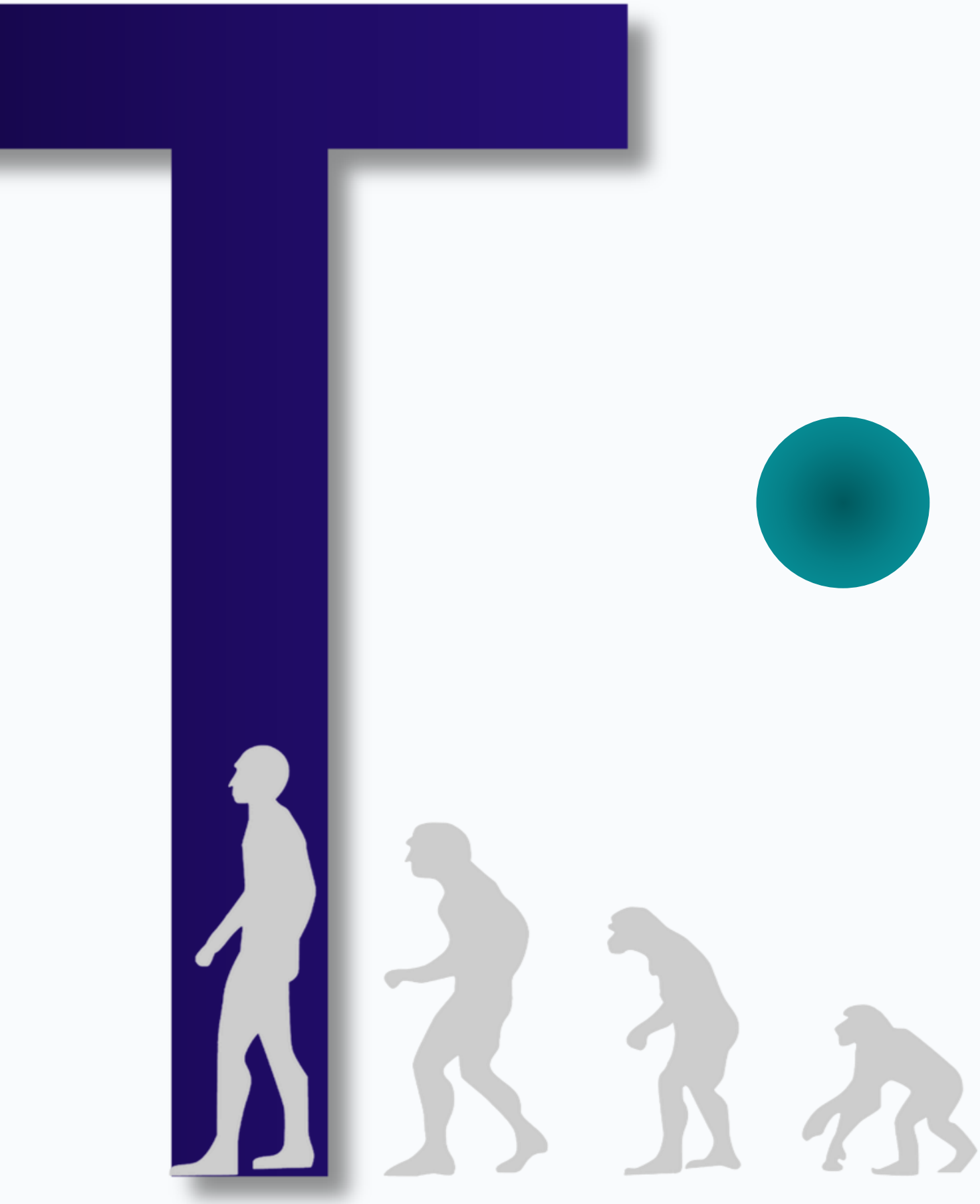
BIOTEM
Custom antibodies & services



THERAPEUTIC ANTIBODY PLATFORM

Project Strategy & Specifications

Target Typology

- Virus, bacteria, etc.
- Proteins (intracellular, membrane, post-translational modifications, etc.)
- Peptides (design, synthesis and conjugation)

Initial Materials and Project Constraints

- Available material (quantity, purity)
- Immunogenicity / Toxicity
- Cross reaction and specificity
- Timelines

BIOTEM has developed a large panel of improved strategies enabling us to complete the most challenging therapeutic antibody projects.

FULLY HUMAN *Antibodies (-mumab)*

The identification of potential donors which have been generating a specific immune response is one of the major objective of the preliminary phase. The strategy handled by BIOTEM to generate fully human monoclonal antibodies is based on the isolation of memory B cells. These cells express naturally, on their surface, specific Human antibodies. The development of monoclonal antibodies is mainly based on 2 technologies: EBV Immortalization or Phage Display (recombinant antibodies).

EBV Immortalization

Cell Selection & Enrichment

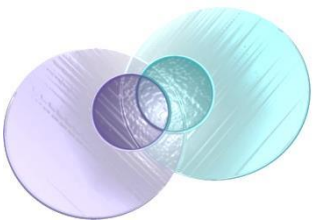
- PBMCs (Peripheral Blood Mononuclear Cells), containing the memory B cells, are extracted from whole blood using a density gradient method
- Memory B cells isolation (FACS, magnetic separation, etc.)

Activation & Immortalization

- Specific activation medium
- EBV immortalization (viral particles or virions)
- LCLs (Lymphoblastoïd Cell Line) characterization (secretion and proliferation)

Screening & Cloning

- Primary screening (polyclonal stage)
- Confirmation (polyclonal stage)
- LCLs cloning (monoclonal stage)
- Selection of sub-clones
- Validated cell bank with viability evaluation



Recombinant Antibodies

Phage Display Technology – Library Construction

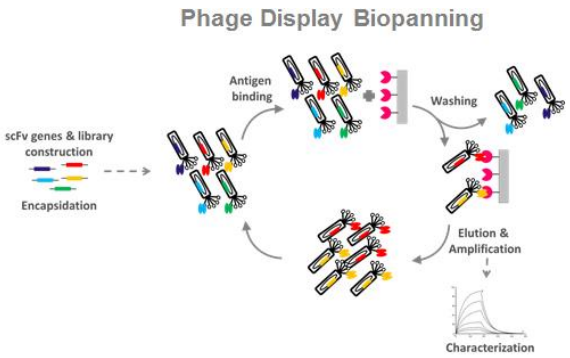
- B-cell isolation from the PBMCs
- Amplification by RT-PCR of the mRNA coding for the variable domains VLκ, VLλ and VH
- Construction of a scFv immune library focus on the antigen

Phage Display Technology – Biopanning

BIOTEM implements the best biopanning methods, adapted to the CLIENT's antigens:

- Direct
- Competitive
- Subtractive

Post-panning isolated binders are individually selected, sequenced and tested for their reactivity / specificity by different methods (ELISA, affinity analysis, …).



ULTIMATE HUMANIZATION® *Platform (-umab)*

Exclusiveness BIOTEM

By combining the best of existing *in vivo* and *in vitro* technologies, BIOTEM's Ultimate Humanization® Platform allows the generation of lead-drug antibodies with outstanding **low predicted toxicity** and **cross-reactivity** in humans. This strategy maximizes antibody success during preclinical and clinical phases.

Technology Overview

In vivo Immunization of Non-Human Primates (NHP):

- Strong and natural immune response (outbred animals)
- Antibodies naturally homologous to human antibodies
- **Low predicted toxicity and cross-reactivity in humans**

Hyper-Immune Library Construction:

- Tailored libraries highly representative of the NHP hyper-immune response (**large epitope coverage**)
- **High affinity** antibodies: 10 to 1000-fold superior affinities compared to antibodies usually obtained from naive or synthetic libraries

Phage Display & scFv Candidate Selection:

- **Customized and cutting edge high throughput screening**
- Thorough characterization of scFv candidates to select best lead

Antibody Germinalization:

- **Facilitated and extensive germinalization** of FR and CDR regions thanks to the innate high Germinality Index (see box) of NHP antibody
- Unlike germinalization of murine, rodent or lama antibodies, BIOTEM's strategy allows to generate antibodies with exceptionally high GI
- **Guaranteed GI > 92 %** while preserving parental affinity

Sequence Optimization:

To potentiate drugability and to anticipate antibody manufacturing:

- Improvement of physicochemical properties
- In vitro antibody affinity & activity maturation
- Codon optimization

Key Advantages

- ✓ Optimized & validated protocols for NHP immunization
- ✓ Large epitope coverage
- ✓ scFv and Full Ig-format
- ✓ High affinity candidates
- ✓ Reduced toxicity & cross-reactivity
- ✓ Extensive germinalization (FR and CDR)
- ✓ Sequence optimization
- ✓ Guaranteed GI >92% (up to 99%)
- ✓ Improved drugability & manufacturability

GERMINALITY INDEX

The **Germinality Index (GI)** quantifies the amino acid identity of antibody variable domains with the most homologous human germline sequences.

Typical GI for mature antibodies (IgG):

- Human: 86-96 %
- NHP: 78-90 %
- Lama: 74-82 %
- Rat: 70-80 %
- Mouse: 68-76 %

Since 1980, BIOTEM offers a large panel of immunization strategies enabling to generate immune responses against particular epitopes or challenging antigens (conserved proteins, haptens, small modifications,…). After an in depth analysis of client's specifications, BIOTEM will define the best immunization strategies.

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Immunization & Library Construction

Active immunization of outbred NHPs elicits strong and natural immune responses compared to inbred or transgenic animals in which the immune system is often impaired. BIOTEM's team benefits from a long experience in NHP immunization and isolation of its antibody-secreting cells (B-cells) which requires strong technical know-how.

For each project, BIOTEM builds a tailored and highly representative hyper-immune library which allows obtaining:

- **High affinity antibodies**: 10 to 1000-fold superior affinities compared to antibodies usually obtained from naive or synthetic libraries
- **Large epitope coverage**: with possible targeting of specific antigen epitopes
- **Antibodies with low predicted toxicity**: Specific NHP immunization strategies allow generating immune responses strongly focused on the therapeutic targets and not against NHP self-antigens (thanks to well-known tolerance mechanisms). Since NHP and human proteins are highly homologous (>95% identity on average), the risk of cross-reactivity between NHP antibodies and human antigens is very low.

The isolated scFv candidates from the phage display screening are selected and sequenced before being produced and characterized. Hence, the best candidates are identified thanks to:

- **Germinality index**
- **Antibody affinity**
- **Biological activity**
- **Biophysical characterization**
- **Expression yield**

Antibody Germinalization

Unlike germinalization of non-NHP antibodies, BIOTEM Ultimate Humanization® technology allows FR and CDR germinalization. Therapeutic antibodies generated through this technology exhibit therefore exceptionally high GI (>95%) while retaining their parental affinity and specificity. Most notably, extensive humanization is accomplished through germinalization of CDR regions which are often predicted to be highly immunogenic but essential for antigen recognition.

Sequence Optimization

- **Improvement of physiochemical properties**: Specific amino acid substitutions will be proposed in order to avoid post-translational modifications susceptible to affect antibody production, stability, solubility, aggregation, heterogeneity etc.
- **Antibody maturation**: Depending on specifications, in vitro affinity maturation and/or optimization of biological functions will be proposed.
- **Codon optimization**: Antibody coding sequence will be optimized to increase expression level in client's expression system.

Antibody Production & Purification

Depending on project specifications, BIOTEM offers different options for lead-candidate production in mammalian cells (before and/or after antibody germinalization).

HUMANIZED *Antibody (-zumab)*

Modernized CDR-grafting

Humanization by CDR-grafting consists in transferring parental (commonly rodent) complementarity determining regions (CDR) into human framework regions (FR). Parental antibody specificity and affinity are conserved thanks to the preservation of residues implicated in antigen recognition.

Modernized CDR-Grafting Including Sequence Optimization

- Identification of parental antigen-binding residues
- Selection of most adapted FR sequences
- Humanized antibody design
- Sequence optimization
- Improvement of antibody drugability & manufacturability
- Affinity & specificity optimization

Production & Purification

- scFv- or full Ig-format (several isotypes available)
- Small and large scale production / purification

Key Advantages

- ✓ CDR-grafting using human germline genes
- ✓ Sequence optimization included
- ✓ Preserved parental affinity and specificity
- ✓ 100% fee-for-service

Applications

- ✓ Humanized antibodies constitute the majority of todays FDA and/or EMA approved therapeutic antibodies

CHIMERIC *Antibody (-ximab)*

Antibody Chimerization

Chimeric antibodies are antibodies combining parental antibody variable regions with any acceptor constant region. Thanks to the domain organization of antibodies, chimerization is straightforward and does not alter antibodies affinity or specificity.

Chimeric Antibody Design

- Antibody development and/or sequencing
- Parental / Acceptor antibodies from various species and isotypes
- Codon optimization for mammalian expression system

Production & Purification

- Small and large scale production / purification

Key Advantages

- ✓ Quality validated antibodies
- ✓ Various isotypes available
- ✓ Parental antibody development and/or sequencing integrated if required

Applications

- ✓ **Therapeutics**: used as such or before humanization to secure your project.
- ✓ **Diagnostics**: replacement of serum, calibrators and positive controls.

Quality Control & Characterization

Spectrophotometry quantification, SDS-PAGE analysis, Endotoxin determination, ELISA, BLItz / Biacore, Stability studies, SEC-HPLC, DSC, etc.

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