

Developability Assessment Platform

Reduce Attrition Rates, Improve Clinical Safety and Avoid the Valley of Death



The Valley of Death

Development risk, safety and high attrition rates remain some of the key challenges to successful biopharmaceutical and vaccine pipelines. Reducing R&D costs while improving R&D productivity are essential to providing safe and effective treatments to the patients.

While designing or ranking a candidate to promote efficacy, the chemical and physical stability and safety of the product should be considered. Post translational modifications of the product can potentially lead to long term stability issues. Aggregation of the product has been shown to result in an elevated risk of immunogenicity and issues in manufacturing, which can cause an increase in production costs and delays in development timelines.

Novel biological candidates are prone to fail during preclinical and early stages of clinical development due to efficacy, stability and safety issues. The term “Valley of Death” is commonly used to refer to these early stages of development because of the extremely high attrition rates observed.

Bridging Discovery and Development

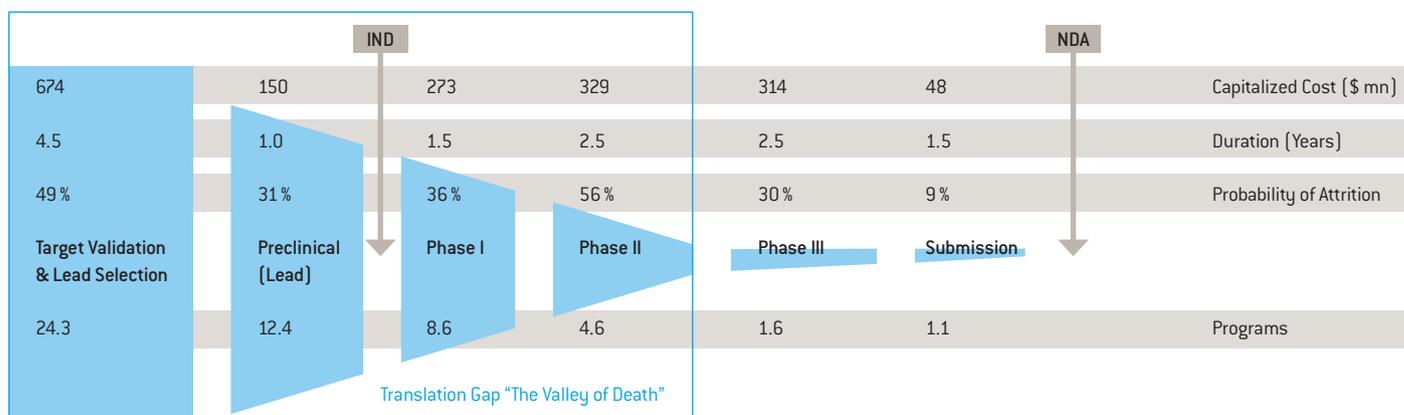
The path from discovery to commercialization is long and expensive. Driving down costs and timelines can benefit the biological manufacturers as well as the patient. Often the separation between drug discovery and development is too great to achieve success. Reducing the gap by designing or engineering candidates to optimize efficacy, safety and quality can promote reduced costs and timelines and increase success rates. An assessment of the potential manufacturing and safety risks at the early stages of the discovery and development processes can build quality into your product, by design.

Developability is an assessment of the suitability of a biological candidate to be successfully developed into an effective therapy. Can the candidate be manufactured, formulated for the appropriate route of administration and provide the desired pharmacology and pharmacodynamic characteristics? Using predictive tools to try to answer questions like these can help de-risk the later stages and bridge the gap between discovery and development.

Why Consider Developability Assessment?

- Reduce attrition rates
- Improve efficacy and safety
- Focus on most promising candidates
- Maximize the output of R&D investment
- Reduce cost and time to market
- Improve product quality, by design

The Lonza Applied Protein Services team offers a Developability Assessment Platform that is effective at improving the quality, safety and manufacturability of antibodies, therapeutic proteins, and peptides. By supporting the successful discovery, selection, optimization, and early development of promising candidates, Lonza’s Applied Protein Services can help maximize your likelihood of success.

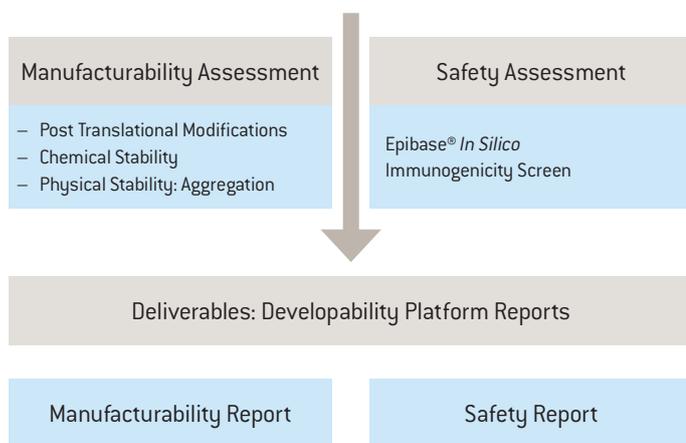


The drug development cycle is long and costly with many candidates failing early, falling into the “Valley of Death”. Adapted from Paul, et al, Nat. Rev. Drug Disc., Vol 9, pp 203-214, 2010 and the Michael J. Fox Foundation.

Lonza Developability Toolbox

Assessing the suitability of a candidate as a potential biotherapeutic or vaccine should include the analysis of the manufacturability, safety, pharmacology and pharmacodynamics of the product.

The Lonza Developability Toolbox includes *in silico* assessment tools to analyze the manufacturability and safety of potential biotherapeutic and vaccine candidates derived from either mammalian or microbial expression systems.



The manufacturability assessment includes looking at the candidate's sequence and predicting areas of risk for chemical or physical changes that could affect the ability of the candidate to be manufactured with acceptable yield, have the appropriate quality profile and be formulated for the desired route of administration.

The safety assessment uses our Epibase® *In Silico* tool to screen sequences for potential immunogenic sites. This is beneficial for both biotherapeutic and vaccine candidates. Prediction of unwanted and wanted immunogenicity is critical for the development of both types of products.

Manufacturability Assessment

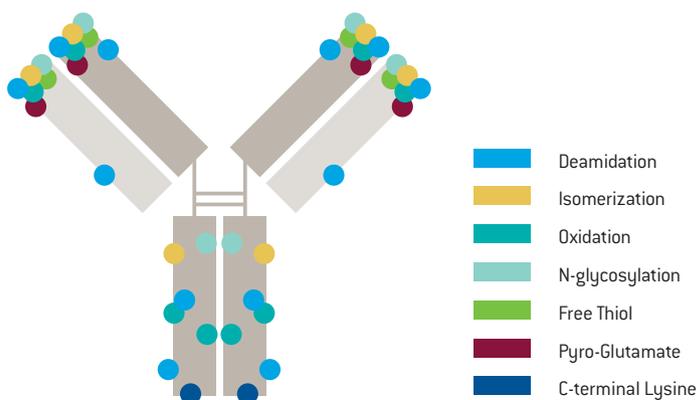
Lonza offers a set of *in silico* tools that assesses the chemical and physical stability of a biotherapeutic at a very early stage in the development pipeline. These tools can predict features critical to product quality such as post translational modifications and aggregation which can affect process yield, formulability, biological activity, and immunogenicity.

Knowing about these potential risks in advance of the start of product development can enable you to make informed decisions with regards to the choice of molecule and process development steps to help ensure a rapid and cost-effective strategy.

Post Translational Modifications

Many biotherapeutics are at risk from post translational modifications (PTMs) and lack of chemical stability. The PTMs that can occur include deamidation, N- and O-linked glycosylation and Methionine oxidation. PTMs can affect binding affinity, function and safety of the product.

PTMs in the complementarity determining regions (CDR) of antibodies, for example, can lead to loss of binding and efficacy. Some PTMs may make the biotherapeutic more susceptible to degradation, thus affecting long-term stability. PTMs usually result in multiple product variants with potentially different biological activities or safety profiles, which could negatively impact productivity, quality and regulatory compliance. *In silico* tools can be used to predict sites of potential PTMs and also to re-engineer the highlighted amino acid sequences to help mitigate identified risks.

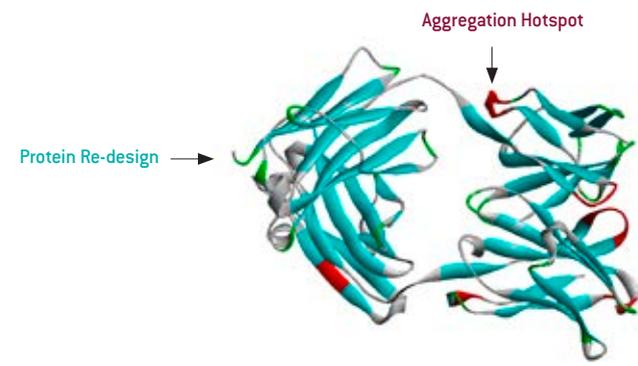
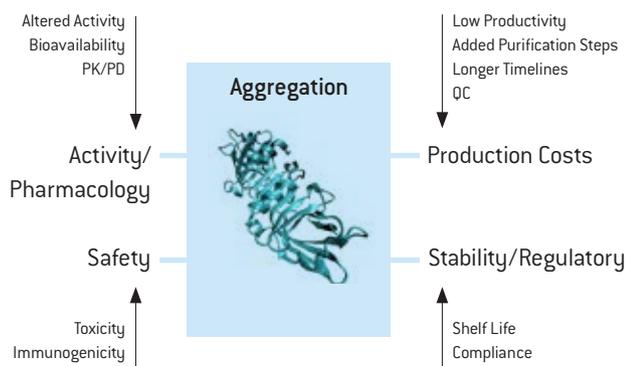


Areas at risk for PTMs that could affect chemical stability are highlighted on the heavy and light chains of an antibody.

Aggregation

Aggregation is a common and well-known problem for biotherapeutics during all stages of their production and use. The presence of aggregates has been known to increase production costs and lead to problems with product stability and regulatory compliance. More importantly, aggregation has the potential to be a significant safety risk as it can enhance immunogenicity. The *In Silico* tool is used to assess aggregation and stability risks in protein therapeutic candidates.

As important as identifying the potential problem, our *in silico* tools can also be used to guide protein re-engineering by identifying the regions prone to aggregation. With potential aggregation “hot spots” and stability “weak points” identified, amino acid substitutions can be evaluated, via modeling, for their ability to reduce the aggregation risk while maintaining the original functional and structural properties of the antibody. We also offer Sentinel APART (TM) server, a Lonza-hosted, customer-dedicated interface which enables customers to perform predictions to assess aggregation risk and to re-engineer their antibody to minimize aggregation risk.



Knowing the aggregation potential of candidates up-front may help avoid the consequences of aggregation at later stages.

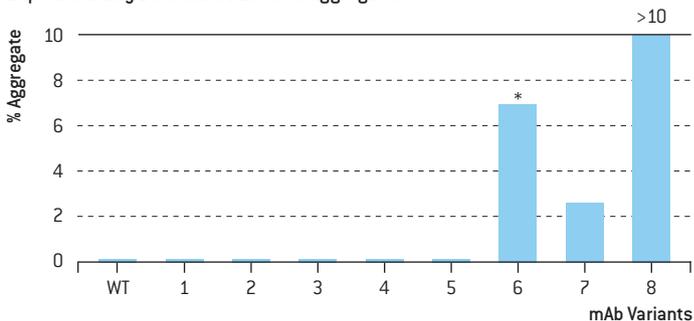
Lonza’s *In Silico* prediction tool is a suite of predictive models for the assessment of the aggregation risk of proteins such as monoclonal antibodies. The *in silico* assessment is conducted via algorithms used to identify sequence and structural motifs that have the potential to impact aggregation. For monoclonal antibodies, predictive models were developed using experimental data from more than 500 antibodies and tested on over 100 antibodies.

Predicted Risk of Aggregation

Variant	Predicted Aggregation Risk
WT	Low
1-7	Low
8	High

Aggregation risk assessment can be conducted on single or multiple sequences. For multiple sequence analysis, candidates can be ranked based on their predicted tendency or risk of aggregation. Predictive models were built on aggregation data from SE-HPLC analysis on hundreds of antibodies post protein A purification. The experimental data was used to estimate a risk threshold, with 5% aggregate as the threshold between high and low risk.

Experimentally Determined Level of Aggregation



Comparison of predicted risk and experimentally (SE-HPLC) determined levels of aggregation of wild-type (WT) and variant monoclonal antibodies (mAb). Tool correctly predicted 8 of 9 mAb. *Variant 6 showed 7% aggregates, which is close to the 5% high/low threshold.

Safety Assessment

Immunogenicity is the inherent capacity of a product to induce an immune response in a target population and has relevance in both biotherapeutic and vaccine development. For biotherapeutic development, immunogenicity is to be avoided as it has the potential to impact product safety and reduce efficacy. For vaccine development, maximum immunogenicity is desired to control disease. Using Lonza's Epibase® *In Silico* tool to perform immunogenicity assessment early in the development life cycle can help focus lead biotherapeutic candidate identification, reducing attrition rates and development costs.

Epibase® IS Epitope Screening

Epibase® *In Silico* (IS) is a patented, industry leading T cell epitope screening platform that analyzes and predicts the potential immunogenicity of proteins. Epibase® IS is a predictive tool driven by sequence information and structural bioinformatics in conjunction with experimental human leukocyte antigen (HLA) binding data. The tool uses structural characteristics of the HLA receptor along with experimentally determined binding affinities to help determine the likelihood of peptide/HLA binding, a condition leading to T cell activation. The use of structural information enhances the accuracy of the predictions when compared to approaches based on sequence alignment alone.



Determining early which candidates contain immunogenic areas (shown in red on a protein) can aid in the ranking and/or re-engineering of candidates for further development.

Broad Population Coverage

Epibase® IS addresses immunogenicity in a global population, with over 99% coverage. Eighty-five (85) different allotypes are represented encompassing the genetic variation in populations such as Caucasian, Asian, Hispanic, African American, and others. Immunogenicity can be predicted for a global population, or can be tailored to a specific population to which your biotherapeutic may be targeted.

The screenshot shows the Epibase® IS web interface. At the top, there are navigation tabs: Submission, Confirmation, History, Users, Help, and Logout. The main content is divided into three sections:

- 1. Sequences:** Includes a 'Batch name' input field, a 'Group' dropdown menu, and a text area for pasting protein sequences in FASTA or text format (up to 400 sequences, 10 to 1000 amino acid residues). There is a 'File' upload option with a 'Choose File' button and 'No file chosen' text.
- 2. Analysis parameters:** Includes an 'Allele set' dropdown menu (set to 'All DRB1 alleles'), a 'Population' section with checkboxes for Afro-American, Austronesian, Global, Indo-European, Mestizo, and Oriental, a 'Filter set' dropdown menu (set to 'Human antibody germline'), a 'Custom filter set' dropdown menu (set to 'No file chosen'), and an 'E-mail notification' dropdown menu (set to 'No').
- 3. Reports:** Includes a 'Select' section with checkboxes for 'By alleles', 'By sequences', 'Raw data', and 'Randomized'. There are 'Clear' and 'Submit' buttons at the bottom.

The Epibase® IS tool includes the Server option allowing screening to be done by your R&D team at your site.

Epibase® IS Format Options

Lonza offers two usage formats for our Epibase® IS assessment tool. Each is designed to fit your safety assessment needs.

With the Project basis format, members of Lonza's Applied Protein Services team will screen your sequence and provide the results in a customized report. Relative ranking can be incorporated at the epitope and/or whole protein level to identify the critical epitopes allowing for comparison of variants. This format is suited for analyzing a limited set of lead candidates.

Relative Epitope Ranking Criteria

- Binding strength
- Self-peptide filtering
- Promiscuity of binding to multiple HLA allotypes
- Importance of affected allotypes in a population, DRB1 primary focus

To facilitate the screening of a library or high volume of biotherapeutic candidates, we offer the Server format. The Epibase® Server is a customer specific, secure, encrypted, web-based tool that enables multiple users at the customer site to analyze an unlimited number of sequences or variants at the same time. Epitope prediction and report generation is automated and can be customized according to your specific requirements.

To enable our customers to deimmunize their molecules, the Server format also features a single substitution randomization report (deimmunization heat map). The heat map can be used to assess the impact of individual mutations on the immunogenicity of a molecule in order to help guide the redesign of the candidate.

Critical Epitope Count Difference (WT: 124)

Pos	Res	A	C	D	E	F	G	H	I	K	L	M	N	P	Q	R	S	T	V	W	Y
8	G	1	0	0	0	1	0	0	1	0	1	1	1	0	0	0	0	1	1	1	1
9	P	2	1	0	0	2	2	1	3	2	2	3	1	0	2	2	1	1	4	1	3
10	G	1	0	-1	-1	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
11	L	0	-1	-1	-1	2	0	0	1	1	0	1	1	-1	0	2	0	-1	1	1	2
12	V	-1	-3	-4	-3	0	-1	-3	0	-2	0	0	-2	-3	-2	0	-1	-1	0	-1	0
13	A	0	-1	-1	-2	0	-2	0	0	0	1	0	-1	-1	-2	1	0	-1	0	0	0
14	P	1	0	0	1	2	1	-1	2	1	2	2	1	0	1	1	1	1	2	2	2
15	S	1	-1	-1	-1	1	0	0	1	1	2	1	-1	0	-1	1	0	0	1	1	1
16	D	2	1	-1	-1	1	1	2	1	2	1	1	1	1	0	2	1	1	2	1	1
17	S	2	0	-2	-2	2	0	0	3	1	3	3	2	1	-1	1	0	0	3	1	3
18	L	-1	-1	-1	-1	-1	-2	-2	0	-1	0	0	-2	-2	-2	0	-1	-2	0	-1	-1

A deimmunization heat map provides an assessment of immunogenicity potential based on single mutations in the protein sequence and can help guide protein engineering efforts.

Fast and Cost-effective Screening

Multiple candidates and variants can be profiled rapidly at a very early stage of the discovery and development process, enabling the ranking of candidates and the early elimination of less viable molecules. Due to the potential of biotherapeutics to cause adverse clinical responses, regulators are encouraging the assessment of immunogenicity risk at a preclinical stage. Epibase® IS provides a cost-effective means to screen candidates for their potential risk of immunogenicity at an early stage supporting the design, selection, optimization, and compliance of biologicals.

Developability Assessment Can De-risk and Accelerate the Development of:

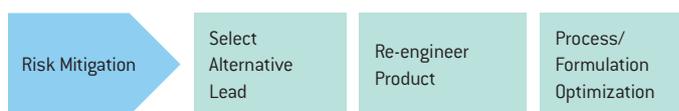
- Antibodies
- Therapeutic Proteins
- Peptides

Developability at Early and Late Stages

Benefits at Either Stage

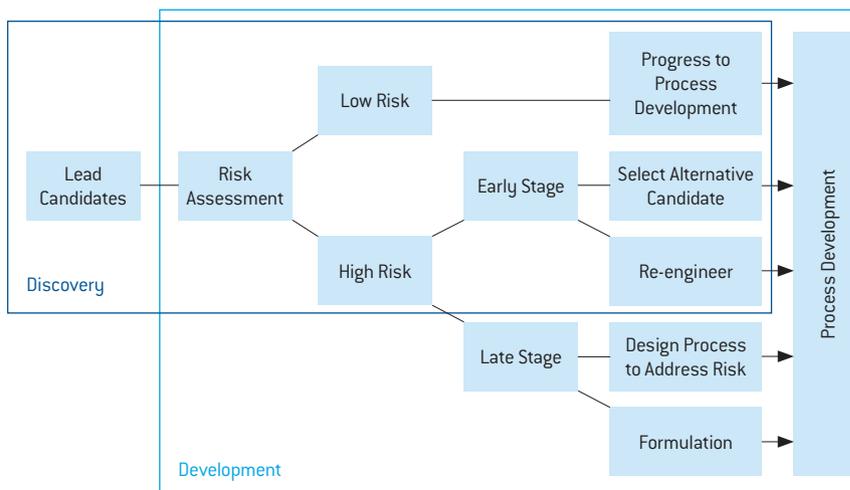
Developability assessment can be done at both the early and late stages of development. At the early stage, if you have multiple sequence candidates, they can be screened to determine whether they are at high or low risk for manufacturability or safety issues. If they are at low risk, you can proceed to process development (PD). If they are at high risk, the candidates can be re-engineered and re-validated, or alternative candidates can be chosen to move to PD.

Developability Issues	Impact					Risk
	Loss of Activity	Reduced Yield	Different Isoforms	Safety	Increased Costs of Manufacture	
Aggregation	■	■		■	■	High
Deamidation	■		■	■	■	Low to High
Isomerization	■		■	■	■	Low to High
Oxidation	■		■	■	■	Low to High
Free Thiol	■	■		■	■	High



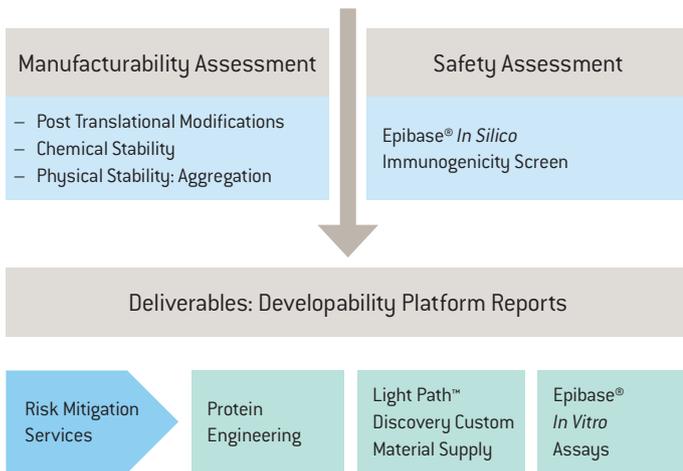
Understanding the Developability risks of your candidates allows you to implement mitigation strategies to avoid them.

If you are in the late stage of development and are committed to your molecule of choice, a developability assessment can still be beneficial. Prior to cell line construction or strain development, potential downstream issues such as aggregation can be identified and mitigated with process optimization. This will allow for streamlined scale-up processes and reduce the risk of costly troubleshooting later in product development, which ultimately will shorten the time to the clinic.



Risk Mitigation Services

Once you know the predicted risks associated with your candidates, Lonza's Applied Protein Services team can help you with the next steps in your risk mitigation strategy. Our goal at Lonza is to offer a complete service portfolio for your early discovery and development needs.

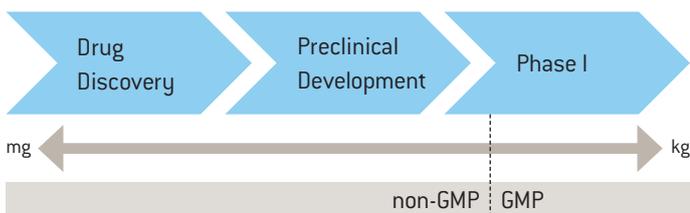


Lonza's Applied Protein Services include the Developability Assessment Toolbox and follow up Risk Mitigation Services.

Protein Engineering and Production

Lonza's Applied Protein Services team has an extensive range of protein engineering capabilities including modeling, engineering, antibody humanization, deimmunization, and deaggregation. Our experienced scientists can help you improve the structure and biological activities of proteins and antibodies, while addressing any manufacturability and safety issues. In addition, new antibodies, and potentially new intellectual property (IP), can be created through the engineering process.

Light Path™ Discovery Custom Material Supply is Lonza's streamlined manufacturing service for the production of small scale, non-GMP material for your discovery through early development stage needs.



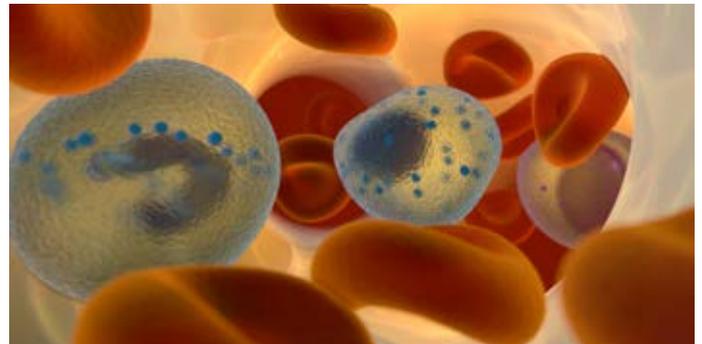
Light Path™ Discovery manufacturing services can provide non-GMP material for discovery activities and be seamlessly integrated into our Light Path™ Development services for the production of cGMP product.

Light Path™ allows you to leverage Lonza's proven GS System™ and XS Expression Technology™ platforms license free, for mammalian and microbial systems, respectively, to manufacture mg quantities of your protein candidate for *in vitro* evaluation of stability and safety. You can partner with Lonza early to benefit from regulatory approved and industry accepted expression platforms and take advantage of our industry leading experience in recombinant protein production.

Epibase® IV Immunogenicity Screening

Lonza's Epibase® *In Vitro* (IV) Screening Service can be used to evaluate the immunogenic potential of biotherapeutics by characterizing cellular responses in the target population.

As a follow up to the Epibase® IS assessment, the cellular assays can facilitate the selection of lead candidates and can support further engineering, if required. The Epibase® IV Screening Service utilizes a high throughput, multi-parameter approach to analyze T and B cell responses by Flow Cytometry, FluoroSpot and ELISA assays.



Epibase® IV assays use PBMCs to assess the human immune response to biological candidates.

Regulatory agencies are recommending the monitoring of anti-drug antibody (ADA) and other human immune responses during clinical development phases rather than relying on animal models to assess immunogenicity. The Epibase® IV assays use human peripheral blood mononuclear cells (PBMCs), which are the closest representation of the human immune system, prior to the first dose in humans. PBMCs can be isolated from healthy donors as well as from ethnic, genetic or disease groups that are representative of the target population for the biological candidate.

Combined with the Epibase® IS prediction tools, Epibase® IV assays give you insight into the immunogenicity potential of different candidates early in the development process, providing for knowledge-based selection of the "best" candidates to move forward to PD.

Avoiding the Valley of Death

Developability assessment can help reduce attrition rates and help you focus on the most promising biological candidates. The *in silico* predictive tools of Lonza's Developability Assessment Platform can provide you with information regarding both the manufacturability and safety of your target candidates. This analysis provides a predictability of product integrity and safety through the clinical development stages. Gaining a more intimate knowledge of your molecule and the optimization process will aid in regulatory filings and partnership discussions. Developability assessment can help lower overall development costs, increase speed to market and help you avoid the Valley of Death.

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