Technical Dossier : Therapeutic Monoclonal Antibody Development (mAb-X)

Cybernano

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Contents

1 Therapeutic Context and Product Objective

Rheumatoid arthritis (RA) is a chronic inflammatory joint disease characterized by persistent immune activation and elevated levels of pro-inflammatory cytokines such as IL-6, TNF-α, and IL-23. Among them, IL-23 plays a central role in the differentiation and maintenance of Th17 lymphocytes, which contribute to synovial inflammation. While IL-6 and TNF-α are well-established therapeutic targets, recent evidence indicates that the IL-23/Th17 axis contributes to disease persistence in a subset of patients, particularly those with inadequate response to existing biologics.

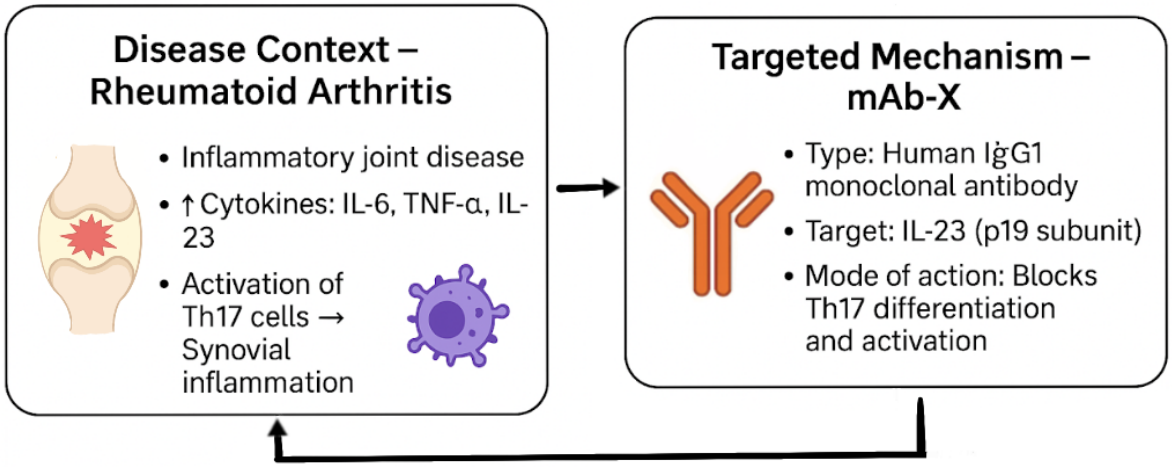


Figure 1: Context and mechanism of mAb-X: inhibition of Th17 activation via IL-23 blockade.

To counteract this inflammatory cascade, mAb-X was developed as a humanized monoclonal antibody specifically targeting the p19 subunit of IL-23, which is not shared with IL-12. By neutralizing IL-23, mAb-X inhibits the differentiation and maintenance of Th17 lymphocytes, leading to decreased production of downstream cytokines (IL-17A, IL-6, TNF-α). This mechanism is expected to reduce synovial inflammation and tissue damage, offering a therapeutic alternative for patients with Th17-driven RA.

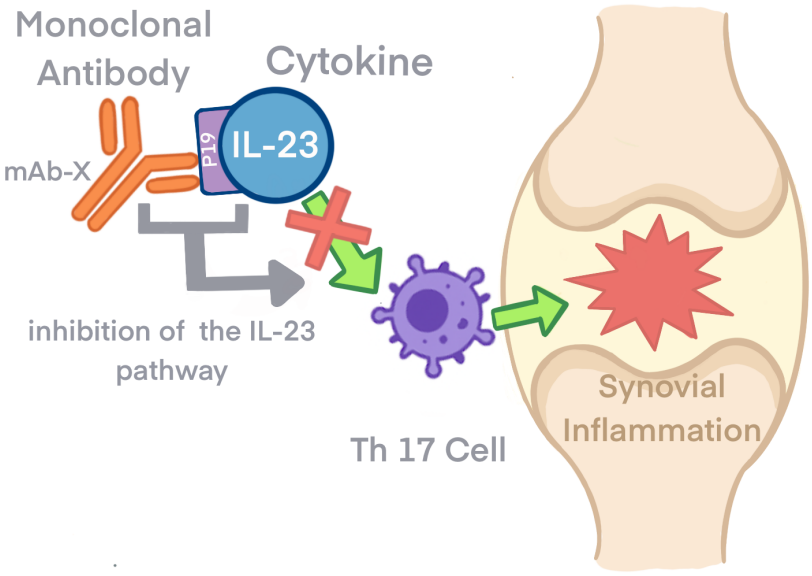


Figure 2: Mechanism of action: mAb-X binds to IL-23 (p19 subunit) to inhibit Th17-mediated inflammation.

2 Product Description

mAb-X is a recombinant human IgG1 monoclonal antibody developed for the treatment of rheumatoid arthritis. It is designed for intravenous (IV) administration at a dose of 10 mg/kg every two weeks. This regimen enables sustained systemic exposure, aligned with the kinetics required to modulate the IL-23/Th17 pathway.

Final Drug Product – mAb-X	
Concentration	10 mg/mL
Buffer	10 mM phosphate + 150 mM NaCl
Surfactant	0.01% Polysorbate 80
pH / Osmolality	6.0 ± 0.2 / ~290 mOsm/kg
Container	10 mL borosilicate vial
Shelf-life	24 months at 2–8 °C
Administration	10 mg/kg IV every 2 weeks

Figure 3: Final formulation of mAb-X.

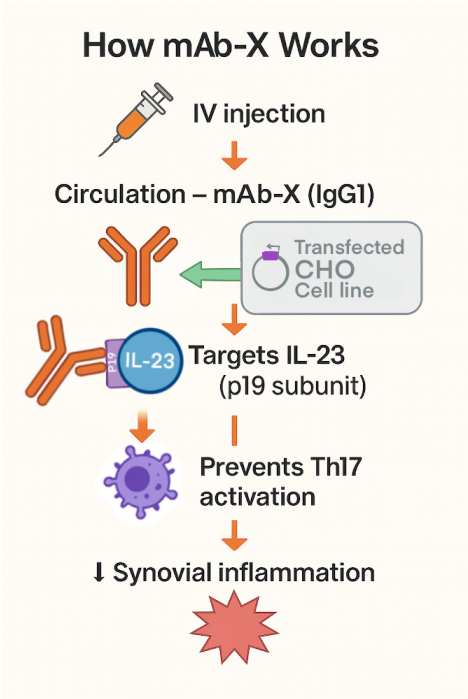


Figure 4: Mechanism of action of mAb-X.

The formulation consists of 10 mg/mL mAb-X in 10 mM phosphate buffer and 150 mM NaCl, with 0.01% polysorbate 80 as a stabilizing agent. The final pH is adjusted to 6.0 ± 0.2, and the

osmolality to approximately 290 mOsm/kg. The drug is filled in sterile 10 mL borosilicate glass vials, sealed under controlled atmosphere. Each vial contains 100 mg of antibody in the final solution.

Stability studies have confirmed a shelf-life of 24 months at 2–8°C and 30 days at room temperature.

Structurally, mAb-X consists of two heavy chains and two light chains, forming a full IgG1 immunoglobulin. Expression is driven by a bicistronic vector that incorporates both chains under the control of CMV and EF1α promoters, stably integrated into a CHO-S cell line.

As expected for an IgG1 produced in CHO cells, mAb-X is glycosylated in the Fc region, with a glycoform profile mainly composed of G0F and G1F structures, supporting controlled Fc activity and favorable pharmacokinetics.

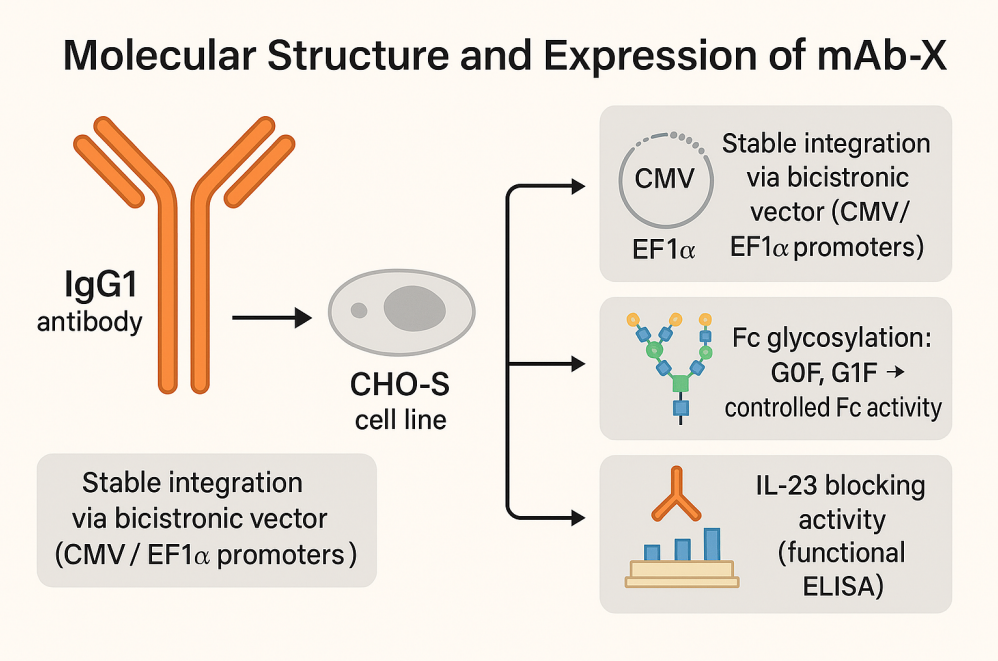


Figure 5: Molecular structure and expression of mAb-X.

Biological activity is evaluated through a functional ELISA that measures inhibition of IL-23 binding to its receptor. The potency meets the preclinical specification thresholds.

The final product meets all pharmaceutical criteria for IV administration, including requirements for stability, viral safety, and acceptable immunogenicity profile for a recombinant human antibody. https://plmlatex.math.cnrs.fr/project/68511b7059b0b606df2d7d49

3 Cell Line Origin and Bank Establishment

The CHO-S (Chinese Hamster Ovary - Suspension) cell line was selected for the stable expression of mAb-X. This cell line, widely used in the biopharmaceutical industry, offers a favorable combination of robustness, high production yield, and well-established regulatory traceability, particularly regarding viral safety and historical usage records [1, 2].

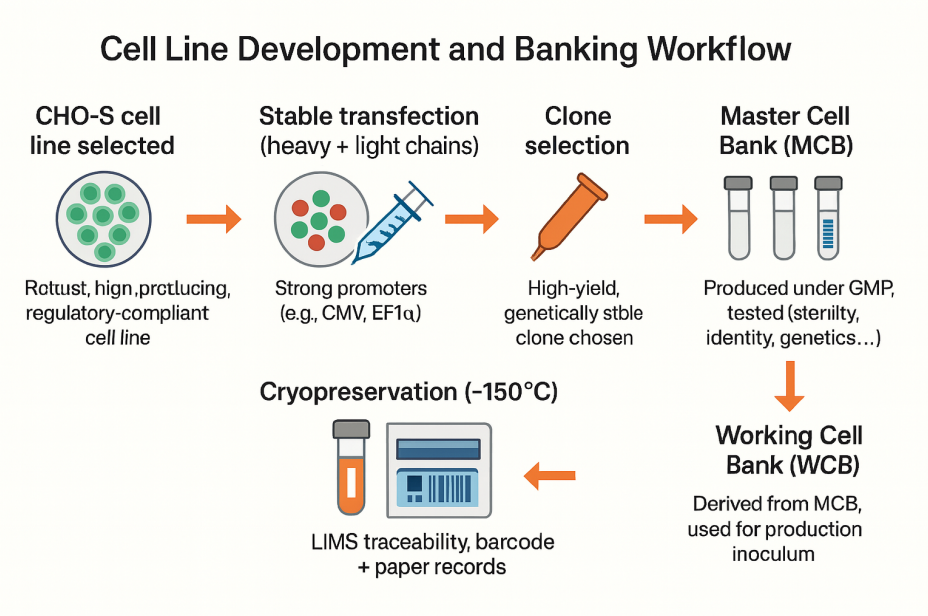


Figure 6: Development of the Cell Line and Creation of Cell Banks

Cells were stably transfected with a vector encoding the heavy and light chains of the monoclonal antibody, under the control of strong promoters. After selection, the lead clone underwent thorough characterization, including expression stability over multiple passages, growth profile, specific productivity, and absence of sequence rearrangement [**?**].

A Master Cell Bank (MCB) was established from the selected clone and produced under GMP conditions. It was then used to derive a Working Cell Bank (WCB) for inoculating production cultures. Each bank underwent a comprehensive panel of tests, including:

Table 1: Summary of tests performed on the cell banks

Tested Parameter	Method Used
Sterility	USP <71>
Mycoplasma	Cell culture + PCR
Genetic stability	Southern Blot, qPCR, targeted sequencing
Cell identity	Isoenzymes, STR (Short Tandem Repeats)
Productivity	Antibody yield in small-scale culture

Cells were cryopreserved at –150 °C in labeled cryovials, using a dual traceability system (digital barcodes + paper records). Every movement (withdrawal, testing, reissue) is logged in an electronic management system (e.g., LIMS).

Compliance with ICH Q5D [3] requirements was ensured throughout the process, and the cell bank release documents are available upon request (batch number, qualification date, responsible laboratory).

4 Upstream Process: Cell Culture and Monitoring

The upstream process is based on a 14-day fed-batch culture operated at the 2000 L scale in a stirred-tank bioreactor equipped with integrated sensors (temperature, pH, DO, pressure, conductivity). This type of equipment is standard for monoclonal antibody production, as described in both industry and academic guidelines [**?**].

For mAb-X, a CHO-S suspension cell line was used, derived from a validated WCB (Working Cell Bank) compliant with ICH Q5D [3]. The culture begins with thawing and progresses through

successive passages over 10 days with increasing volumes (2 L $\rightarrow$ 50 L) until reaching approximately 2×10^6 cells/mL and viability $> 95\%$. This strategy is well documented for high-yield GS-CHO systems [**?**].

4.1 Seed Train Strategy

The preparation of a robust high-density inoculum is a critical prerequisite for the success of the fed-batch culture. The seed train involves a sequence of amplification steps starting from a thawed vial of the WCB, progressing through shake flasks and stirred-tank reactors of increasing size. This ensures both the expansion of viable cells and their adaptation to bioreactor conditions before inoculation at the production scale.

Table 2: Typical steps in the seed train

Step	Volume (approx.)	Duration
Thawing	2–10 mL	Day 0
Pre-culture (shake flask)	50–200 mL	Day 1–3
Amplification 1	1–5 L	Day 4–6
Amplification 2	10–50 L	Day 6–9
Bioreactor inoculation	200–2000 L	Day 10

4.2 Culture Conditions and Feeding

The main bioreactor is inoculated at 0.3×10^6 cells/mL in a protein-free enriched medium, fully animal component–free. The medium contains amino acids, trace elements, vitamins, lipids, and 8 mM glutamine—typical of optimized formulations [4].

Three feeds are scheduled on days 3, 6, and 9, each adding 5–8% of the initial volume and supplying glucose, glutamine, and metabolic additives. This strategy limits lactate accumulation. At high densities, CHO cells convert glucose to lactate even under aerobic conditions. To counter this, glucose is tightly regulated, the temperature is dropped from day 5, and metabolic engineering strategies are considered to recycle lactate [**?**].

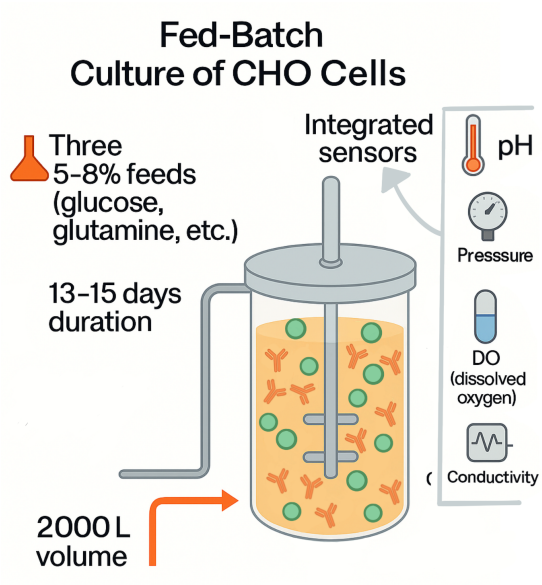


Figure 7: Fed-batch culture of CHO cells in a 2000 L stirred-tank bioreactor with integrated sensors and three 5–8% feeds.

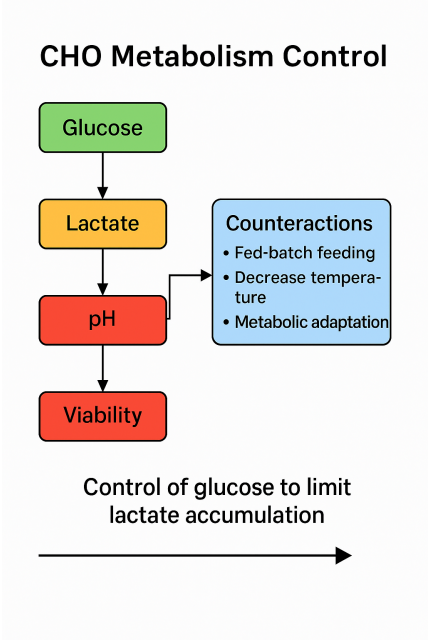


Figure 8: CHO cell metabolism control: glucose-lactate pathway, its impact on pH and viability, and associated countermeasures.

Process conditions include initial temperature at 36.5°C with a shift to 34°C on day 5 (to reduce enzymatic activity and improve glycosylation stability), pH at 7.0 ± 0.2 (controlled via CO_2/base), DO at 40%, and stirring at 100 rpm with air/O_2 mix. These settings align with those used in large-scale CHO processes [**?**].

Table 3: Recommended operating conditions for CHO cell bioreactors

Reference	Volume	CHO Agitation Speed
Kelley, 2007	2000 L	80–120 rpm
Kunert & Reinhart, 2016	1000–3000 L	90–110 rpm
Jungbauer, 2013	500–2000 L	80–100 rpm

4.3 Process Monitoring and Quality Control

Daily monitoring includes viable cell density (Vi-CELL or Neubauer), viability (Trypan Blue), metabolites (glucose, lactate, ammonium via HPLC or enzymatic assays), osmolality (freezing point osmometer), IgG titer (rapid ELISA or Protein A column), and physicochemical parameters (pH, pCO_2) using offline analyzers. These practices align with antibody manufacturing quality recommendations [**?**].

Table 4: Monitored parameters and recommended instruments

Parameter	**Recommended Method / Instrument**
Viable cell density	Vi-CELL XR, CEDEX or Neubauer chamber
Viability	Trypan Blue staining (manual or automated)
Glucose, lactate, ammonium	Enzymatic assay (YSI / Nova Flex) or HPLC
Osmolality	Freezing point osmometer (e.g., AI 3250)
IgG titer	Semi-quantitative ELISA or Protein A assay
pH and pCO_2	Offline analysis with pH/gas analyzers
Morphology	Inverted microscopy (phase contrast)

Microscopic observations are performed daily to detect potential aggregate formation or early signs of cellular stress. Culture is typically terminated between days 13 and 15, depending on viability criteria ($< 70\%$), growth stagnation, or a decline in the product titer. The supernatant is recovered by gravity through a 0.5 mm mesh before being transferred to downstream processing steps. Performance deviations have occasionally been observed from day 10 onward, particularly regarding pH stability or thermal tolerance. These deviations are currently under investigation to model their impact on process robustness.

At the end of the run, the supernatant containing the monoclonal antibody is immediately transferred to the clarification step, which marks the beginning of downstream processing. This transition is critical to preserve product integrity and minimize protein degradation or release of cellular contaminants.

5 Purification Process (Downstream)

The complete manufacturing process of monoclonal antibody mAb-X is composed of several critical stages, from early cell line development through to final formulation and packaging. Each stage is designed to ensure product quality, consistency, and compliance with regulatory requirements. Figure 9 provides a global overview of the entire process, highlighting key steps involved in the production and control of the therapeutic antibody.

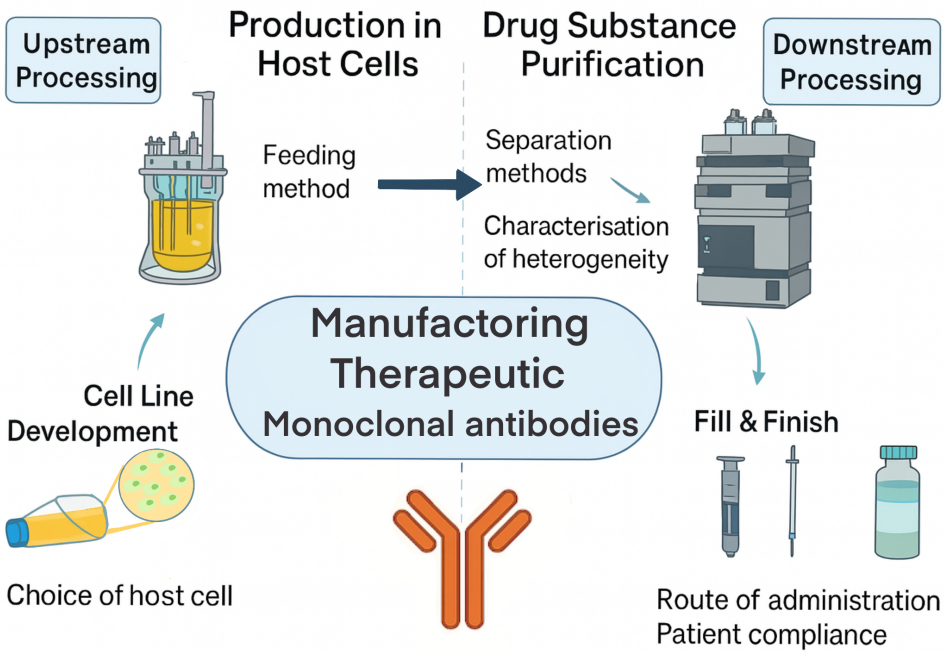


Figure 9: Overview of the monoclonal antibody manufacturing process: from cell line development to final fill & finish.

5.1 Process Steps

The purification of mAb-X follows a classic downstream process consisting of three main steps: clarification, capture, and polishing. Each step is designed to efficiently remove impurities while preserving the structural and functional integrity of the monoclonal antibody.

Clarification is performed using a dual depth filtration followed by a 0.2 µm membrane filter, efficiently removing cells, debris, and submicron particles. This step is essential to prevent clogging of downstream chromatography columns [5].

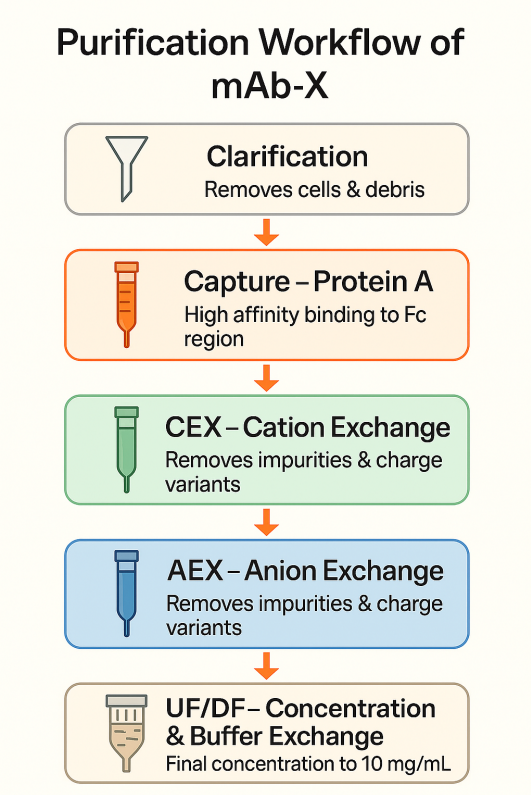


Figure 10: Clarification step using depth filtration followed by 0.2 µm membrane filtration to remove cells and debris.

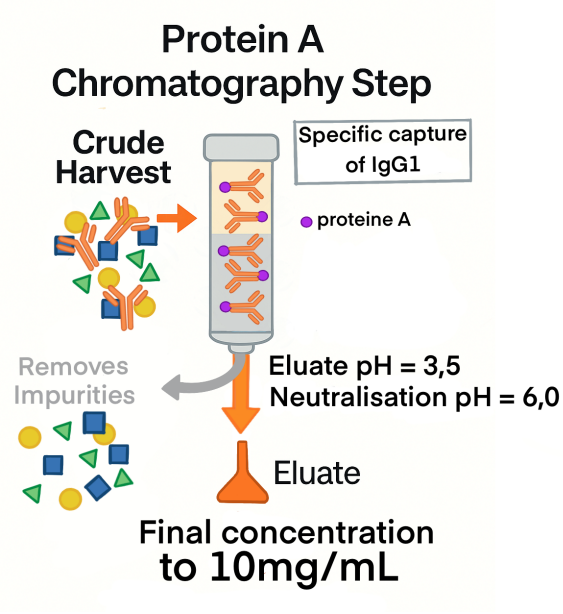


Figure 11: Protein A chromatography: specific capture of IgG1, elution at pH 3.5 and neutralization at pH 6.0.

Protein A capture chromatography is carried out using a standard industrial resin that exploits the high affinity between Protein A and the Fc region of human IgG1. Elution is performed at pH 3.5, achieving >95% recovery under mild conditions [**?**]. Immediate post-elution neutralization is applied to minimize the risk of acid-induced degradation.

Polishing involves two consecutive ion-exchange chromatography steps: first CEX (cation exchange), followed by AEX (anion exchange). These steps help reduce residual contaminants (HCPs, DNA, fragments, aggregates, leached Protein A) and separate potential charge isoforms [**?**].

5.2 Impurity Clearance

Purification performance is monitored at each stage to ensure compliance with quality specifications. The table below shows typical impurity clearance levels observed in mAb-X production batches.

Table 5: Typical impurity levels after each purification step for mAb-X.

Step	**HCP (ppm)**	**DNA (pg/dose)**	**Aggregates (%)**	**Protein A (ng/mg)**
After Protein A	100	200	3.5	300
After CEX	10	50	1.2	80
After AEX	<5	<10	<1	<10

Finally, an ultrafiltration/diafiltration (UF/DF) step is performed to concentrate the final product to 10 mg/mL and exchange the buffer with the formulation buffer. The system includes in-

line sensors (transmembrane pressure, conductivity, UV at 280 nm) to monitor process performance in real time.

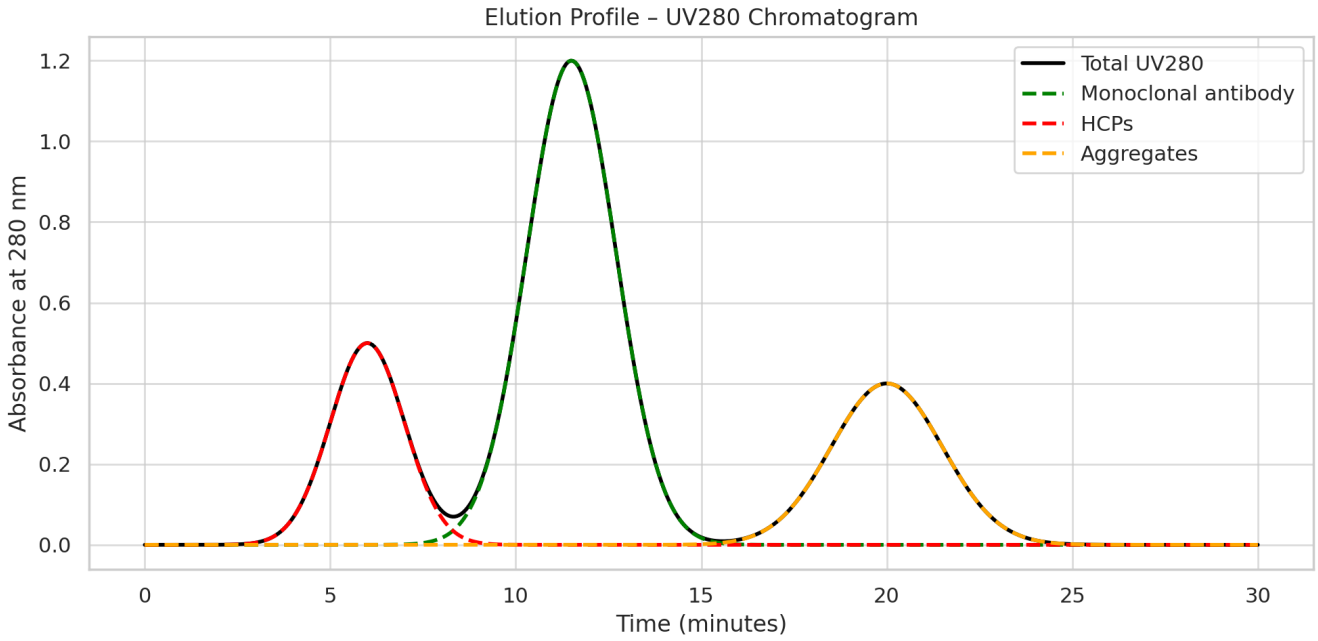


Figure 12: Elution profile during chromatography: UV280 signal showing separation of mAb, HCPs, and aggregates.

Each step of the DSP process is individually validated to ensure robustness (yield, retention capacity, elution profiles) and minimal impact on the critical quality attributes of the mAb. The overall strategy aligns with current industrial practices and complies with ICH Q6B guidelines [6].

6 Final Formulation and Packaging

The mAb-X formulation is a buffered aqueous solution optimized for stability, compatibility with intravenous administration, and container closure integrity. It contains 10 mg/mL of recombinant IgG1 antibody in 10 mM phosphate buffer and 150 mM NaCl to achieve isotonicity. Polysorbate 80 (0.01%) is included as a stabilizer to prevent aggregation and surface adsorption [7**?**]. The pH is adjusted to 6.0 ± 0.2, ensuring conformational stability of IgG1 while minimizing the risk of acid- or base-induced degradation. The formulation osmolality is maintained near physiological levels ($\sim$290 mOsm/kg).

Final Formulation of mAb-X

- IgG1 Antibody: 10 mg/mL
- Phosphate buffer: 10 mM
- NaCl: 150 mM
- Polysorbate 80: 0.01%
- pH: 6,0 ± 0.2
- Osmolality: ~ 290 mOsm/kg

Figure 13: Composition of the final formulation of mAb-X.

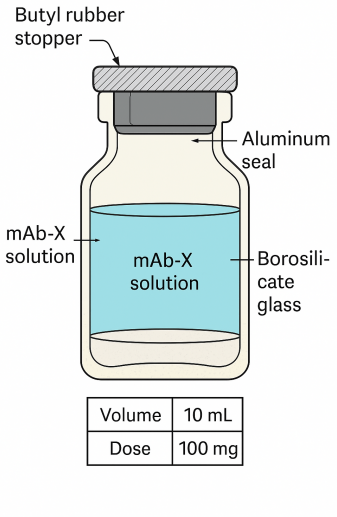


Figure 14: Primary container: 10 mL borosilicate vial sealed with butyl rubber stopper and aluminum cap.

Each vial contains 100 mg of mAb-X at a concentration of 10 mg/mL and is intended for hospital-based intravenous administration. The container system has been validated for both chemical and physical compatibility, ensuring product stability and safety during storage and handling.

The formulation was selected following a comparative screening of buffer systems (acetate, histidine, phosphate) to ensure optimal stability under thermal and mechanical stress, consistent with a Quality by Design approach.

6.1 Stability Studies

Stability studies conducted on pilot-scale batches demonstrated a 24-month shelf life at 2–8 °C. Short-term studies confirmed stability for up to 1 month at 25 °C (60% RH), supporting limited excursions outside the cold chain. Accelerated stability studies at 40 °C (75% RH) were used to model long-term degradation kinetics [8].

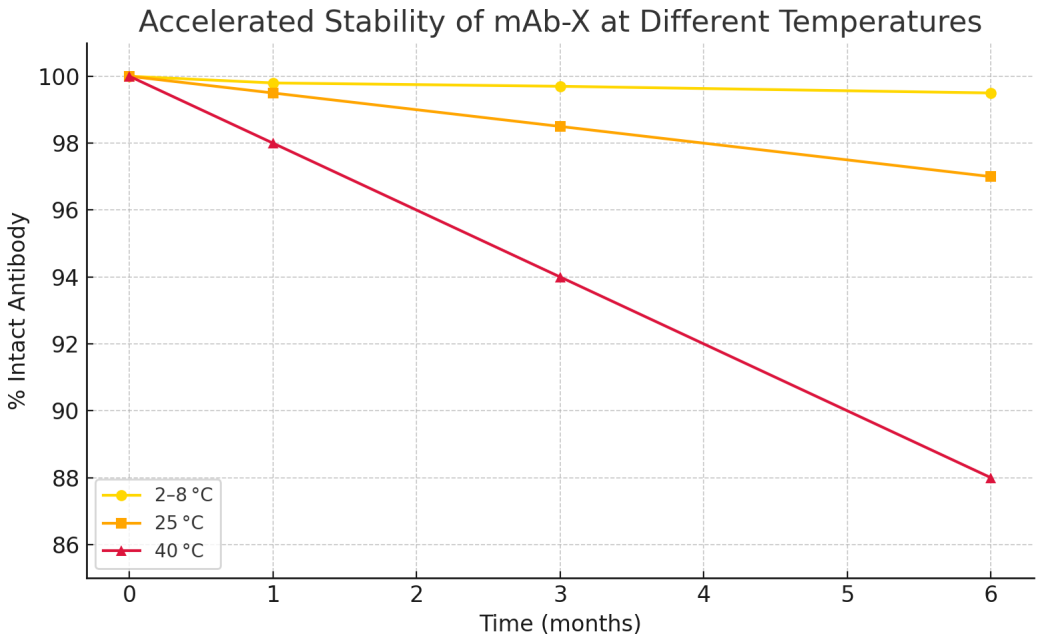


Figure 15: Accelerated stability profile of mAb-X stored at 2–8 °C, 25 °C, and 40 °C over 6 months.

6.2 Drug Product Release Testing

Final release testing of the drug product includes sterility (pharmacopoeial methods), endotoxin levels (LAL assay), pH, antibody concentration (UV_{280}), visual inspection, particle size (DLS or microflow imaging), aggregate quantification (SEC-HPLC), and biological potency (IL-23 inhibition via functional ELISA). All tests comply with ICH Q6B specifications and are supported by validated analytical methods. No visible particles or significant opalescence were detected throughout the stability period, and subvisible particle counts remained within USP ¡788¿ limits.

7 Analytical Control and Release Specifications

7.1 Overview of Analytical Strategy

Each batch of mAb-X, formulated in its final 10 mL vial presentation (100 mg/10 mL), undergoes a comprehensive analytical review before release. Quality control covers key quality attributes: physicochemical integrity, biological activity, glycosylation profile, charge heterogeneity, and process-related impurities.

All analytical methods are validated per ICH Q2(R2), and developed/maintained under an analytical lifecycle consistent with ICH Q14 [9**?**]. Validation includes specificity, precision, linearity, range, detection and quantification limits, and robustness. Approval involves cross-review between the analytical team and the Qualified Person (QP).

7.2 Specifications and Acceptance Criteria

Table 6: Release specifications and rejection criteria for mAb-X.

Parameter	**Specification**	**Rejection Limit**
Purity (%)	$\geq 98.0\%$	$< 98.0\%$
Aggregates (%, SEC)	$< 1.0\%$	$\geq 1.0\%$
IC_{50} (ng/mL)	≤ 15 ng/mL ($\geq$80% activity)	> 15 ng/mL
G2F (%)	$< 10\%$	$\geq 10\%$
HCP (ppm)	< 5 ppm	≥ 5 ppm
G0F (%)	*trend-only; target <55%*	*N/A*

Table 6 outlines the key quality attributes required for mAb-X batch release. These include purity, IC_{50} activity, aggregate level, glycosylation (G0F/G2F), and HCP content. The thresholds are based on clinical relevance and process capability, and any deviation may lead to batch rejection or investigation as part of the QbD control strategy.

7.3 Aggregates and Purity

Purity and aggregate levels are critical quality attributes due to their impact on product safety and efficacy. Aggregates, in particular, are known to increase the risk of immunogenicity and must therefore be tightly controlled. For mAb-X, both purity and aggregation are monitored using SEC-HPLC, a validated method for separating monomers from higher molecular weight species.

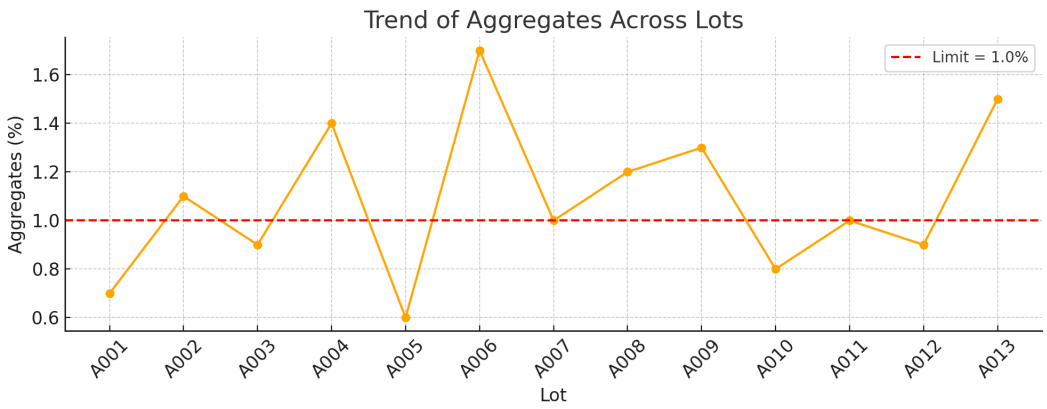


Figure 16: Aggregate levels across mAb-X clinical lots.

Figure 16 shows aggregate content across 13 clinical lots. Lots A002, A004, A006, A008, A009, and A013 exceeded the $< 1.0\%$ specification (A007 was borderline at 1.0%). Based on a multi-attribute assessment, lots A004 and A006 were rejected (concomitant failures on IC_{50} and/or HCP), whereas A002, A008, A009, and A013 were subjected to deviation-based investigations and risk assessments prior to disposition decisions.

These deviations were traced back to process excursions during the ultrafiltration/diafiltration (UF/DF) step, specifically a temperature above 25°C and a pH drop below 3.2, both known to promote protein unfolding and aggregation.

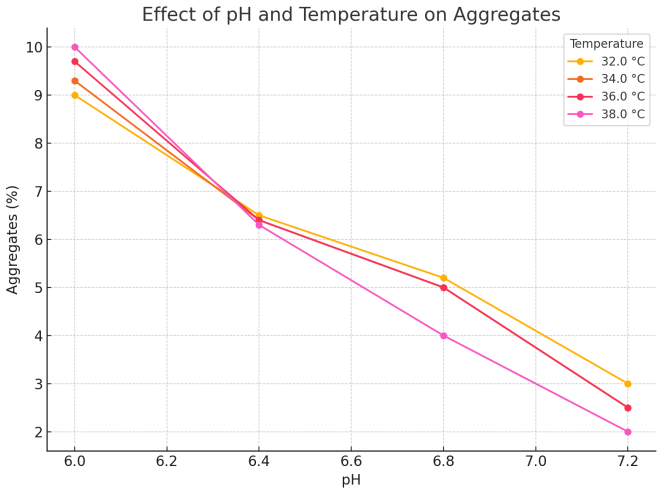


Figure 17: Impact of pH and temperature on aggregate formation.

To further understand these effects, Figure 17 illustrates how pH and temperature deviations correlate with increased aggregate levels. In a QbD context, this highlights the importance of identifying and tightly controlling critical process parameters (CPPs) such as UF/DF temperature and buffer pH. Students are encouraged to explore how design space studies could define acceptable operating ranges to prevent such deviations in future batches.

7.4 Biological Activity (IC_{50})

The biological potency of mAb-X is assessed using a neutralization bioassay, which determines the concentration required to inhibit 50% of the target activity (IC_{50}). The specification is set at a maximum of 15 ng/mL, corresponding to a minimum of 80% neutralizing activity.

As shown in Figure 18, most clinical lots are below the 15 ng/mL threshold (median 11 ng/mL). However, lots A004, A006, and A013 exceeded the limit and triggered an out-of-specification (OOS) investigation.

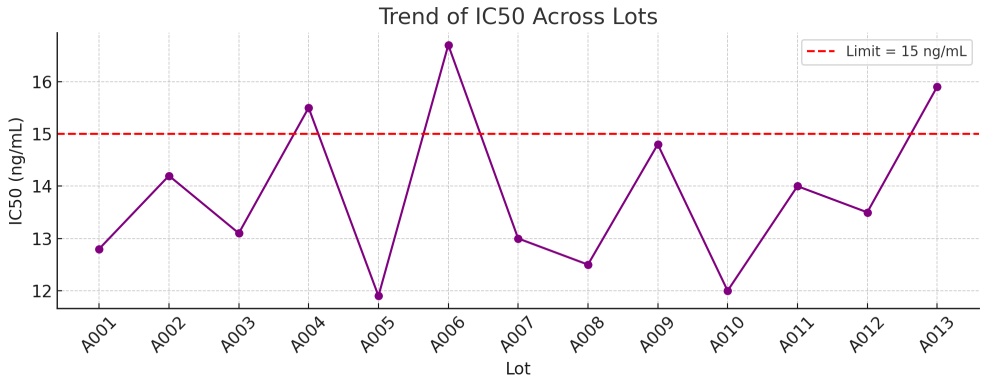


Figure 18: IC_{50} values across lots with rejection threshold.

Figure 19 provides a distribution overview via a boxplot, confirming tight clustering around the median, with a few outliers.

To investigate potential root causes of variability, correlations were analyzed between IC_{50} and two other parameters: glycosylation (G2F) and process-related impurities (HCP). As illustrated in Figure 20, both parameters show moderate to strong correlations with biological activity ($R^2 = 0.63$ for G2F, $R^2 = 0.79$ for HCP). These findings suggest that variations in glycosylation and residual impurity levels could directly impact the functional performance of the antibody.

Such correlations support the identification of Critical Quality Attributes (CQAs) and Critical Process Parameters (CPPs), and guide the implementation of control strategies to ensure consistent potency across batches.

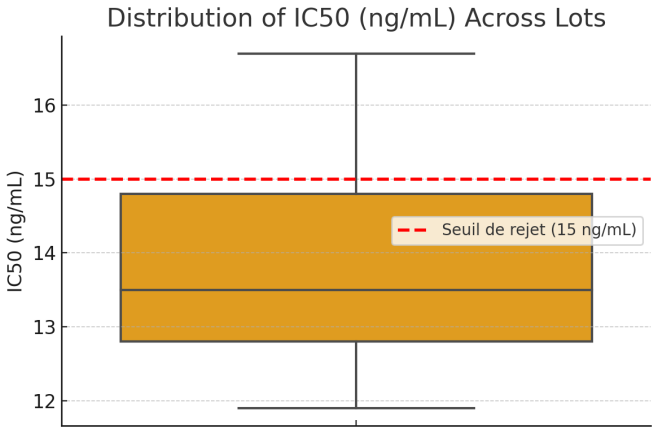
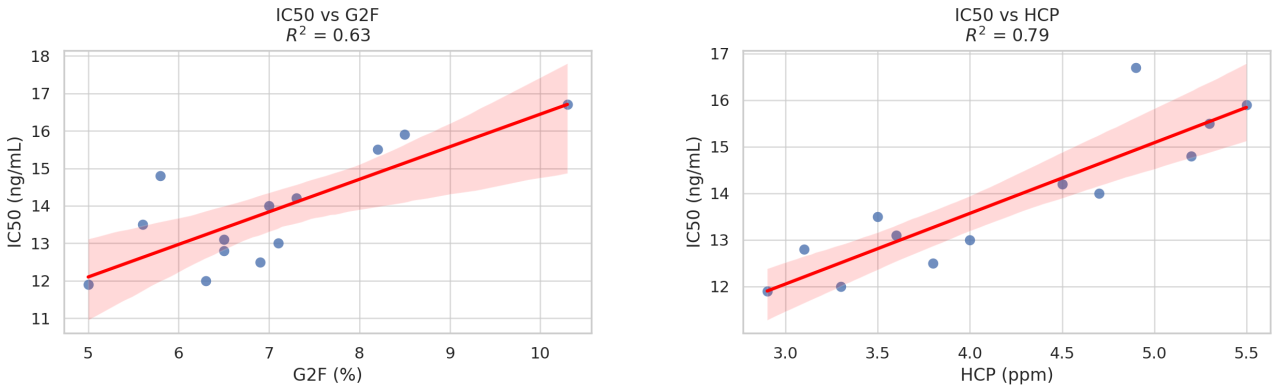


Figure 19: Distribution of IC_{50} values.

Correlations between IC_{50} and glycosylation (G2F, Figure 20a) and process-related impurities (HCP, Figure 20b) suggest possible critical quality drivers.



(a) G2F vs IC_{50} ($R^2 = 0.63$)

(b) HCP vs IC_{50} ($R^2 = 0.79$)

Figure 20: Correlations between IC_{50}, G2F and HCP.

7.5 Glycosylation Profile

The glycosylation profile of mAb-X is a critical quality attribute, especially for Fc-mediated functions such as ADCC and serum half-life. In particular, the G2F glycoform shows a strong correlation with bioactivity.

Figure 21 illustrates the impact of culture pH on both G2F levels and IC_{50} values. Lots produced at lower pH tend to show increased G2F and higher IC_{50}, suggesting a loss of potency. This trend highlights the sensitivity of glycosylation to upstream conditions and the need for tight pH control during the cell culture phase. In a QbD framework, students are invited to investigate which culture parameters most influence glycosylation, and how design space approaches can be used to maintain bioactivity within specifications.

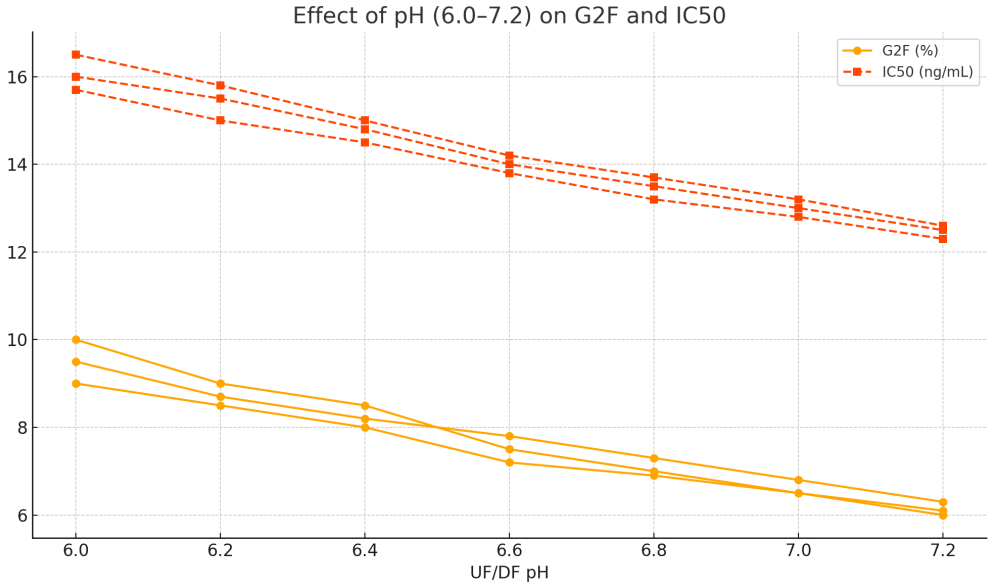


Figure 21: Effect of culture pH on G2F and IC_{50}.

7.6 Impurities and Overall Quality

Process-related impurities such as host cell proteins (HCPs) must be reduced to levels below 5 ppm to prevent potential toxicity or immune responses. Figure 22 shows an inverse relationship between product purity and HCP content: lots with lower purity generally display higher residual HCP levels. This trend reflects the efficiency of the downstream process, particularly the capture and polishing chromatography steps, in achieving impurity clearance.

In this dataset, lots A004, A009, and A013 exceeded the HCP specification, confirming the sensitivity of impurity removal to minor process deviations. Such excursions were primarily linked to suboptimal washing conditions and reduced resin performance during late campaign runs.

This observation highlights the need for robust process control and lifecycle management of chromatography media. Students are encouraged to identify which parameters—such as wash buffer composition, flow rate, and resin lifetime—most influence HCP removal, and to propose control strategies or design space studies to maintain impurity levels within specifications in future campaigns.

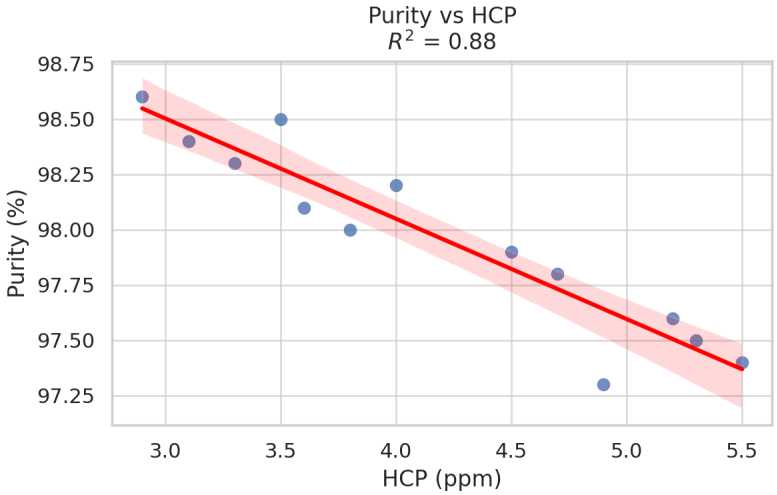


Figure 22: Purity vs. HCP levels in lots.

Finally, to provide a holistic view of product quality across the clinical campaign, Figure 23

presents a heatmap compiling all key quality attributes across the 13 lots. This visualization enables quick detection of outliers, recurring deviations, or correlations between attributes—supporting both root cause analysis and continuous improvement initiatives in a QbD mindset.

7.7 Summary Table and Lot Comparisons

Table 7 provides a comprehensive summary of key analytical parameters across the 13 clinical lots of mAb-X. The dataset includes attributes linked to product quality, efficacy, and safety, such as purity, aggregation, IC_{50}, glycosylation variants (G0F, G2F), and process-related impurities (HCP, DNA, Protein A).

These data enable cross-lot comparisons and support the identification of trends, variability, and potential quality risks. Notably, lots A004, A006, and A013 showed marginal compliance in several attributes (e.g., high G2F, low activity), suggesting upstream or downstream process deviations.

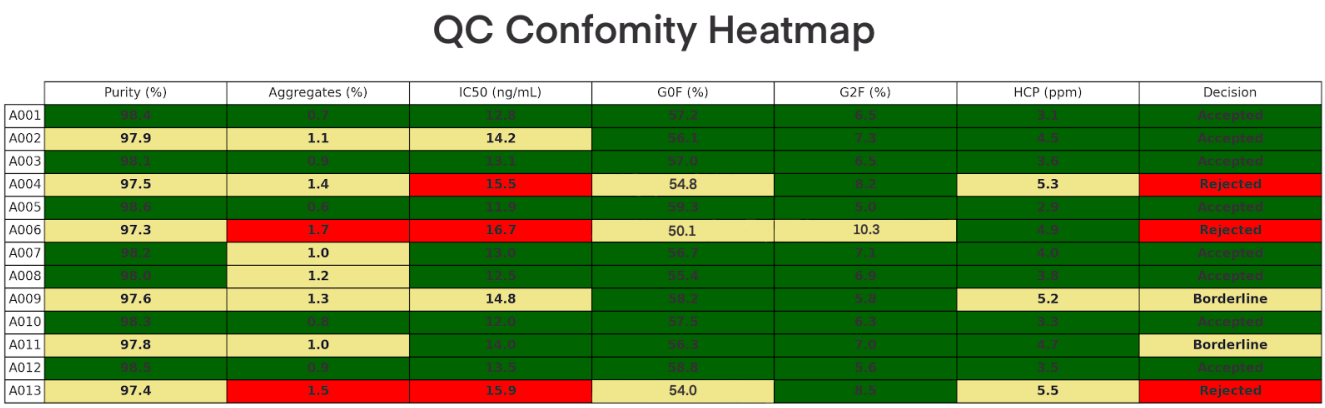
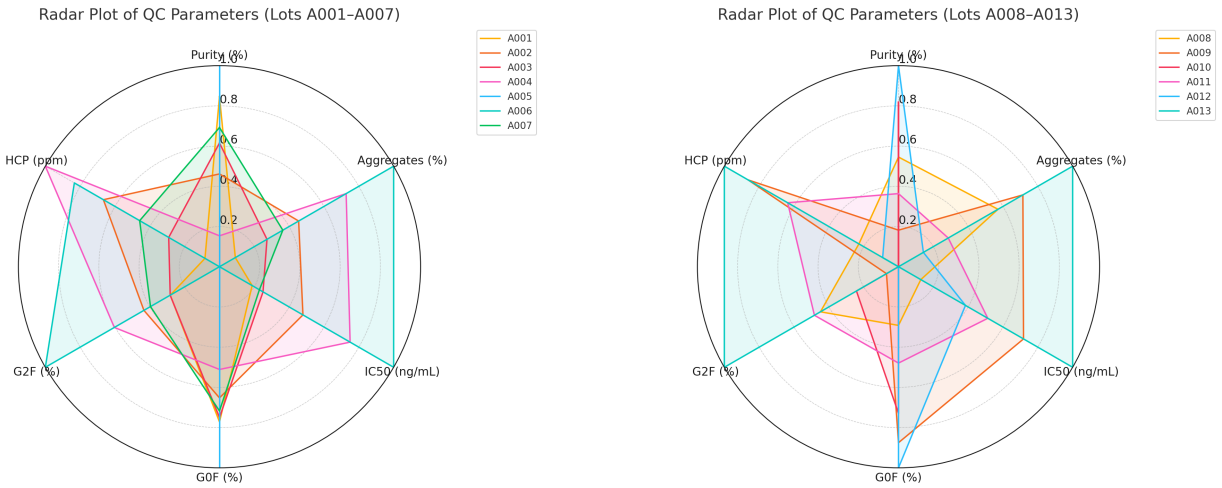


Figure 23: Quality compliance heatmap across lots.

The heatmap in Figure 23 facilitates visual identification of atypical profiles and reinforces the value of multi-attribute monitoring. This comparative analysis contributes to the refinement of control strategies and supports a QbD approach to ensure batch-to-batch consistency.

In addition to the tabular data and heatmap, radar plots offer a multidimensional visualization of the analytical profiles for each lot. They allow rapid identification of deviations, atypical attribute combinations, and overall similarity between lots, facilitating a more intuitive comparison of product consistency.



(a) Lots A001 to A007

(b) Lots A008 to A013

Figure 24: Radar plot visual comparison of lots.

Figure 25 presents a grouped bar chart comparing several critical quality parameters (excluding purity) across six representative lots. These lots include non-compliant and borderline cases (e.g., A004, A006, A013), as well as better-performing ones (e.g., A005, A011), providing a snapshot of key quality trends.

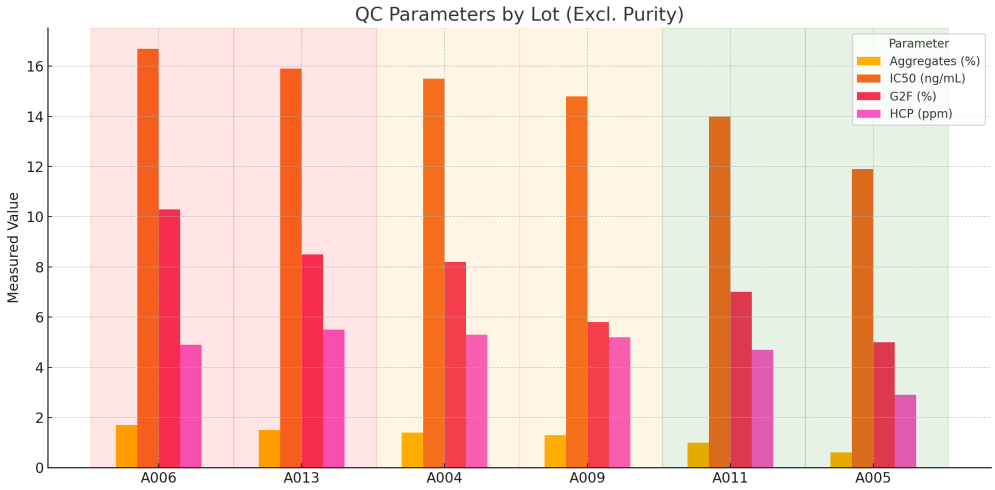


Figure 25: Comparative QC parameters (excluding purity) for selected lots.

We observe that:

- Lots A006, A013 and A004 exceed IC_{50} limits and show elevated G2F and HCP levels.
- Lots A011 and A005 maintain acceptable IC_{50} values and lower impurity levels.
- G2F and IC_{50} appear to co-vary across most lots, reinforcing earlier correlation analyses.

This visual synthesis confirms the consistency of quality drivers identified through individual analyses and supports the prioritization of G2F and HCP as potential CQAs in the QbD strategy.

This analytical dataset supports several QbD hypotheses. Elevated G2F and HCP levels are key contributors to IC50 variability. Aggregate levels respond to pH and temperature. Control of critical process parameters (CPPs) in upstream and downstream operations is vital to ensure lot consistency.

Table 7: Summary of analytical parameters measured on 13 mAb-X lots.

Lot	Purity (%)	Aggregates (%)	Activity (%)	IC50 (ng/mL)	G0F (%)	G2F (%)	HCP (ppm)	DNA (pg)	ProtA (ng/mg)
A001	98.4	0.7	89.1	12.8	57.2	6.5	3.1	8.7	3.6
A002	97.9	1.1	84.2	14.2	56.1	7.3	4.5	9.5	4.8
A003	98.1	0.9	86.5	13.1	57.0	6.5	3.6	7.8	3.9
A004	97.5	1.4	81.3	15.5	54.8	8.2	5.3	10.1	5.3
A005	98.6	0.6	90.5	11.9	59.3	5.0	2.9	7.2	3.3
A006	97.3	1.7	72.6	16.7	50.1	10.3	4.9	9.6	4.6
A007	98.2	1.0	83.0	13.0	56.7	7.1	4.0	9.0	4.2
A008	98.0	1.2	85.2	12.5	55.4	6.9	3.8	8.3	3.7
A009	97.6	1.3	82.5	14.8	58.2	5.8	5.2	9.1	4.9
A010	98.3	0.6	89.8	12.0	57.5	6.3	3.3	8.1	3.5
A011	97.8	1.0	84.7	13.8	56.8	7.0	4.7	9.3	4.4
A012	98.5	0.9	85.9	13.5	56.8	5.6	3.5	7.9	3.6
A013	97.4	1.5	81.0	15.9	54.0	8.5	5.5	10.0	5.0

8 Stability Study

The stability of mAb-X was evaluated under ICH-compliant conditions, covering long-term, intermediate, accelerated, and thermal stress studies. Both the final drug product (10 mL vial) and bulk intermediate (pre-UF/DF concentrate) were tested. At each timepoint, key CQAs were assessed, including purity, aggregates, biological activity, glycosylation profile, pH, opalescence, and impurity levels.

The tested conditions are summarized in Table 8.

Table 8: Storage conditions used for stability testing (ICH Q1A(R2)).

Condition	**Temperature**	**Relative Humidity**	**Maximum Duration**
Long-term	2–8°C	N/A	24 months
Intermediate	25°C	60%	6 months
Accelerated	40°C	75%	1 month
Thermal stress	50°C	60%	14 days

Figure 26 illustrates the evolution of key stability-indicating parameters under these storage conditions. A gradual decline in biological activity was observed over time, correlating with an increase in aggregates and a shift in the glycan profile toward higher G2F content. These changes were significantly amplified under accelerated and stress conditions, suggesting conformational sensitivity of mAb-X to thermal exposure and possible glycan remodeling under stress.

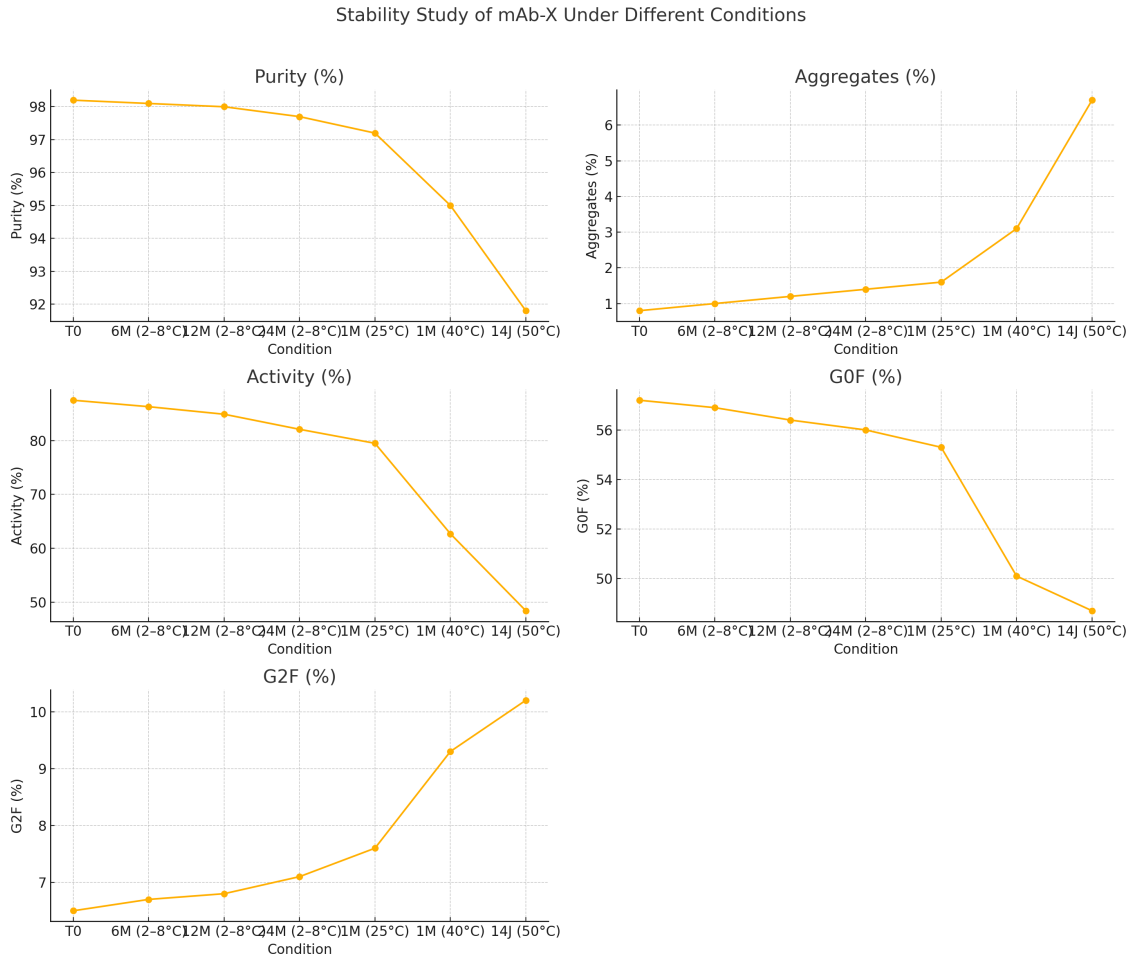


Figure 26: Stability profiles of mAb-X under long-term, intermediate, accelerated, and stress conditions.

Table 9 provides detailed analytical results. At refrigerated conditions (2–8°C), mAb-X remained within specifications up to 24 months, with only a minor increase in aggregates (<1.5%) and a slight opalescence after 12 months. In contrast, accelerated conditions (40°C, 75% RH) led to significant degradation, with purity dropping to 95% and biological activity to 63%. Thermal stress (50°C) caused marked aggregation (>6%) and visible precipitation, confirming denaturation beyond acceptable limits.

Table 9: Analytical data for the final product (10 mL vial) under various storage conditions.

Timepoint	**Purity (%)**	**Aggregates (%)**	**Activity (%)**	**G0F (%)**	**G2F (%)**	**pH**	**Observation**
T0	98.2	0.8	87.5	57.2	6.5	6.0	Compliant
6M (2–8°C)	98.1	1.0	86.3	56.9	6.7	5.9	No issue
12M (2–8°C)	98.0	1.2	84.9	56.4	6.8	5.9	Slight haze
24M (2–8°C)	97.7	1.4	82.1	56.0	7.1	5.8	Visible aggregates <1 µm
1M (25°C)	97.2	1.6	79.5	55.3	7.6	5.7	Onset of degradation
1M (40°C)	95.0	3.1	62.7	50.1	9.3	5.4	Partial denaturation
14D (50°C)	91.8	6.7	48.4	48.7	10.2	5.1	Aggregates >10 µm

Overall, mAb-X showed acceptable long-term stability under refrigerated storage (2–8°C) for at least 24 months, with predictable degradation patterns under stress. The data indicate that aggregation and changes in glycan composition (notably increased G2F) occur progressively during storage, and that both are associated with reduced biological activity.

Additional stress studies—photostability and freeze–thaw cycling—are ongoing. Preliminary results suggest that the presence of 0.01% polysorbate 80 helps to limit aggregation at the air–liquid interface, although pH fluctuations below 5.5 remain a concern during simulated cold-chain interruptions.

These experiments provided a preliminary overview of mAb-X stability prior to the implementation of the QbD framework. Several parameters were observed to vary with time and temperature, but no systematic evaluation of their criticality was performed. Students are invited to analyze these results to identify which attributes could represent potential Critical Quality Attributes (CQAs) and to discuss how process or formulation parameters might influence product stability within a future QbD strategy.

9 Potency

Before the implementation of the Quality by Design (QbD) framework, a series of experiments were conducted to characterize the biological potency of mAb-X and evaluate its stability under different process and storage conditions. The objective was to understand how environmental and process factors might influence the antibody's activity prior to formal process design.

9.1 Assay Principle

Potency was assessed using a functional ELISA inhibition assay that measures the ability of mAb-X to block the interaction between IL-23 and its recombinant receptor. The IC_{50} value, defined as the antibody concentration required to inhibit 50% of IL-23 binding, was used as the main readout. A lower IC_{50} corresponds to higher biological activity.

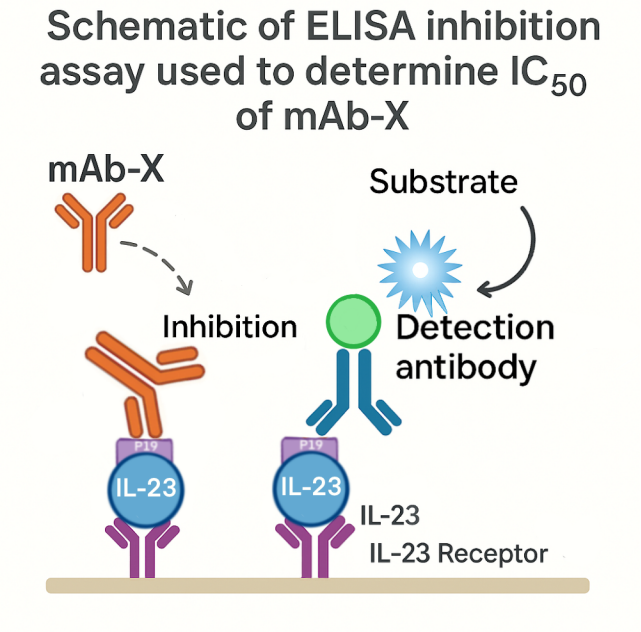


Figure 27: ELISA inhibition assay used to determine IC_{50} of mAb-X.

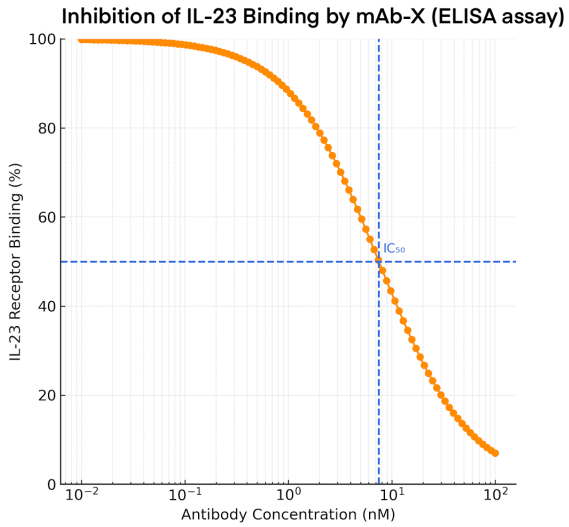


Figure 28: Inhibition curve of IL-23 receptor binding by mAb-X.

9.2 Experimental Observations

During stability and process evaluation runs, changes in potency were observed under different storage temperatures and formulation conditions. The results showed that exposure to elevated temperatures led to a progressive increase in IC_{50} values, suggesting reduced biological activity. In parallel, analytical tests (SEC-HPLC and glycan profiling) revealed changes in aggregation levels and glycoform distribution.

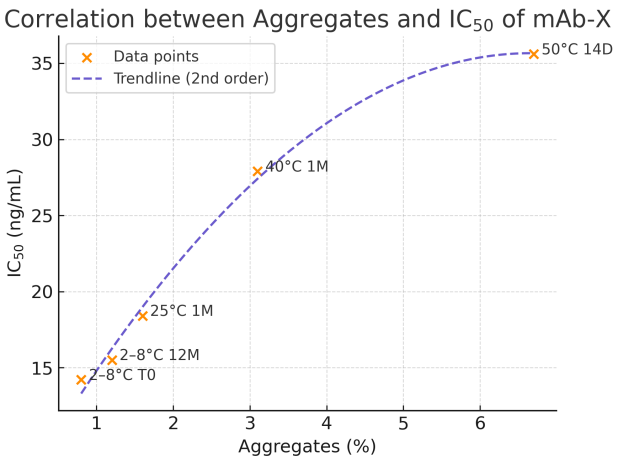


Figure 29: Evolution of IC_{50} with aggregate formation under thermal stress.

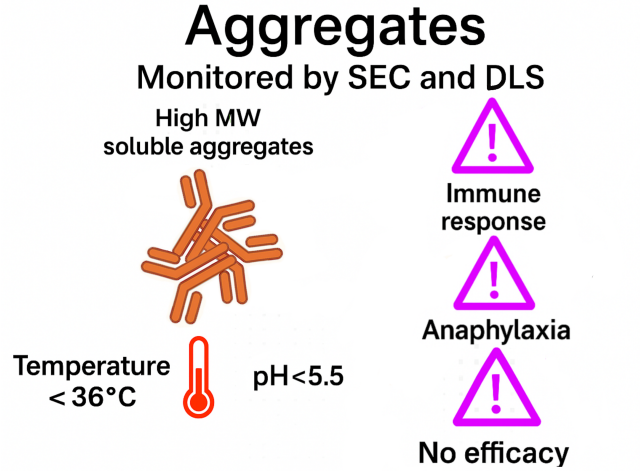


Figure 30: Observation of high molecular weight aggregates under stress conditions.

In some cases, small shifts in pH or temperature during purification and formulation steps coincided with a measurable change in potency. However, no systematic analysis was performed at this stage to establish direct causal relationships.

Table 10: Evolution of potency (IC_{50}), aggregation, and glycosylation under various conditions.

Condition	Timepoint	IC_{50} (ng/mL)	Aggregates (%)	G2F (%)
2–8°C	T0	14.2	0.8	6.5
2–8°C	12M	15.5	1.2	6.8
25°C	1M	18.4	1.6	7.6
40°C	1M	27.9	3.1	9.3
50°C	14D	35.6	6.7	10.2

9.3 Process Steps Evaluated

A preliminary review of manufacturing operations identified several process steps during which changes in potency or related attributes had been observed. These steps are listed below with the corresponding observations and the adjustments considered at the time. No formal risk assessment or design space study had yet been conducted.

Table 11: Process steps where potency variations were observed (pre-QbD phase).

Step	Observed Variation	Initial Adjustment or Note
UF/DF (diafiltration)	Slight pH drop, reduced activity	Monitoring of pH during buffer exchange
Chromatography elution	Variation in glycan pattern	Minor gradient optimization tested
Filling and storage	Increase in aggregates after hold time	Addition of surfactant under evaluation

9.4 Summary

At this stage, the data provided a first overview of the parameters potentially influencing potency, but without a clear definition of their criticality. The observed drifts in activity, aggregation, and glycosylation profiles form the experimental basis for the subsequent Quality by Design (QbD) analysis. Students are invited to use these findings to identify possible Critical Quality Attributes (CQAs), link them to the process parameters involved, and propose suitable control strategies.

10 Preclinical In Vivo Data

Two animal studies were conducted to evaluate the efficacy, pharmacodynamics, and safety of mAb-X. The key characteristics of these studies are summarized in Table 12.

10.1 Murine efficacy model: collagen-induced arthritis (CIA)

The pharmacological activity of mAb-X was evaluated in a collagen-induced arthritis (CIA) model in C57BL/6 mice, which reproduces key mechanisms of human rheumatoid arthritis. Mice ($n = 10$ per group) received intraperitoneal injections of mAb-X at 1, 3, or 10 mg/kg twice weekly for four weeks, followed by a four-week observation period without treatment.

A significant reduction in joint swelling was observed from day 10 in the 10 mg/kg group ($p < 0.01$). Serum analyses revealed marked decreases in IL-17A and IL-6 levels (about 60%

Table 12: Summary of in vivo preclinical studies

Model	**Objective**	**Route**	**Doses**	**Treatment / Follow-up**	**Key Findings**
Mouse (C57BL/6, CIA model)	Efficacy / PD	Intraperitoneal	1–10 mg/kg	2×/week – 4 w + 4 w follow-up	↓ joint swelling, ↓ IL-17A/IL-6, sustained effect
Cynomolgus monkey	Tolerance / safety / PK	Intravenous	0–50 mg/kg	1×/week – 6 w + 6 w recovery	Well tolerated, no toxicity, ADA–, $t_{1/2} \approx$ 8–10 d

reduction vs. controls), consistent with inhibition of the Th17 inflammatory pathway. Histological analysis confirmed reduced synovial hyperplasia and inflammatory cell infiltration, supporting the expected mechanism of action through IL-23 blockade and Th17 modulation.

10.2 Non-human primate safety and pharmacokinetics: repeated-dose GLP toxicity study

A 6-week repeated-dose toxicity and pharmacokinetic study was conducted under Good Laboratory Practice (GLP) in cynomolgus monkeys (3 males and 3 females per group). Animals received intravenous mAb-X at 0, 1, 10, or 50 mg/kg once weekly, followed by a 6-week recovery period.

Monitored parameters included clinical signs, hematology, biochemistry, circulating cytokines, anti-drug antibody (ADA) detection, and plasma concentrations of mAb-X. No acute or chronic toxic effects were observed, and all clinical and biological parameters remained within normal ranges. No ADA were detected.

At the highest dose (50 mg/kg), a mild periportal mononuclear infiltration was noted in the liver but was considered non-adverse. Pharmacokinetic analyses revealed a terminal half-life of approximately 8–10 days in monkeys, consistent with the expected IgG1 clearance profile.

Overall, the study confirmed good preclinical tolerance and predictable pharmacokinetics of mAb-X, supporting progression to Phase I clinical trials with an adequate safety margin.

10.3 Pharmacodynamic and exposure data

Pharmacokinetic (PK) and pharmacodynamic (PD) evaluations were performed in cynomolgus monkeys as part of the 6-week repeated-dose toxicity study, followed by a 6-week recovery phase. Animals received intravenous administrations of mAb-X once weekly at 1, 10, or 50 mg/kg. Blood samples were collected at baseline (Day 0), during the treatment phase (Days 7, 14, and 28), and during the post-treatment period (Day 56). These timepoints were selected to characterize systemic exposure, cytokine modulation, and the persistence of pharmacological activity after dosing cessation.

mAb-X administration led to a rapid and pronounced suppression of circulating IL-23, with reductions exceeding 70% within the first week of treatment. This upstream cytokine inhibition was followed by progressive decreases in downstream mediators (IL-17A, IL-6, and TNF-α), consistent with attenuation of the Th17-driven inflammatory cascade. The pharmacodynamic effects persisted for at least four weeks after the final dose, reflecting sustained target engagement and a biological duration of action coherent with the pharmacokinetic profile of an IgG1 antibody ($t_{1/2}$ 8–10 days in cynomolgus monkeys).

Plasma concentrations increased in a dose-dependent manner, with steady-state levels reached by Day 14 and a terminal elimination half-life ($t_{1/2}$) of approximately 8–10 days, consistent with

Table 13: Pharmacokinetic and pharmacodynamic parameters of mAb-X in cynomolgus monkeys (mean values, n = 6 per group).

Parameter	**Day 0 (baseline)**	**Day 7**	**Day 14**	**Day 28**	**Day 56**
IL-23 (plasma, % baseline)	100	20	12	10	11
IL-17A (pg/mL)	85	60	40	38	29
IL-6 (pg/mL)	24	19	15	10	9
TNF-α (pg/mL)	19	18	14	10	9
CRP (mg/L)	20	16	11	6	5
ESR (mm/h)	42	38	23	19	17
Th17 cells (%)	100	83	71	67	60

the expected IgG1 clearance profile. No anti-drug antibodies (ADA) were detected throughout the study.

The parallel decline in inflammatory markers (IL-23, CRP, ESR) confirmed the pharmacodynamic impact of mAb-X and its direct relationship with systemic exposure. These results demonstrate a sustained inhibition of the IL-23/Th17 axis and support the selection of the cynomolgus monkey as the relevant species for translational modeling and first-in-human dose prediction.

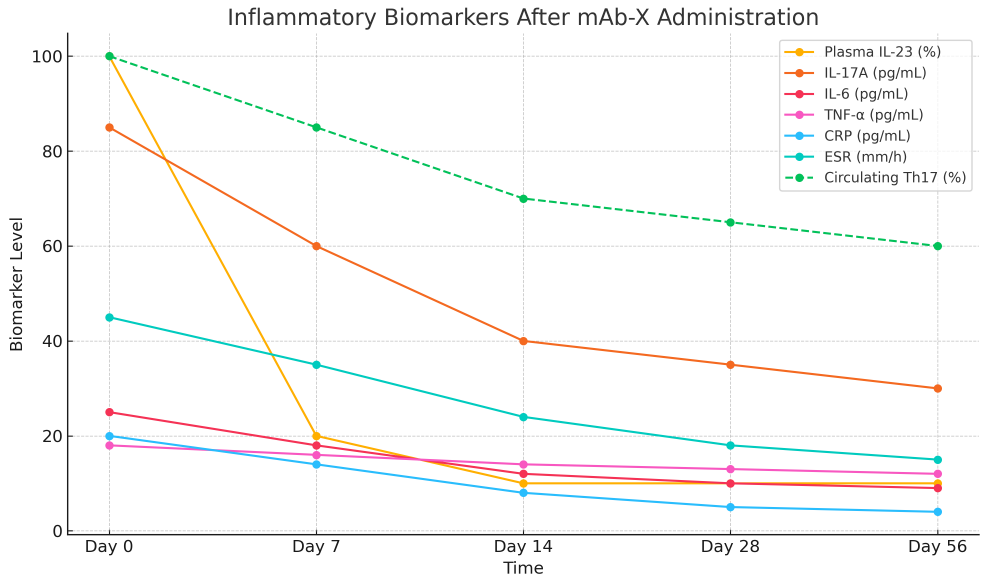


Figure 31: Evolution of inflammatory markers after mAb-X administration (IL-23, IL-17A, CRP, etc.)

Overall, the PK/PD profile in cynomolgus monkeys demonstrates that mAb-X exhibits predictable pharmacokinetics, sustained target inhibition, and no evidence of immunogenicity, supporting its selection as the species of choice for translational modeling and first-in-human dose prediction.

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