

# A Clinical Impact of Attributes (CIA) Approach for Developing Clinically Relevant Specifications for Biologics

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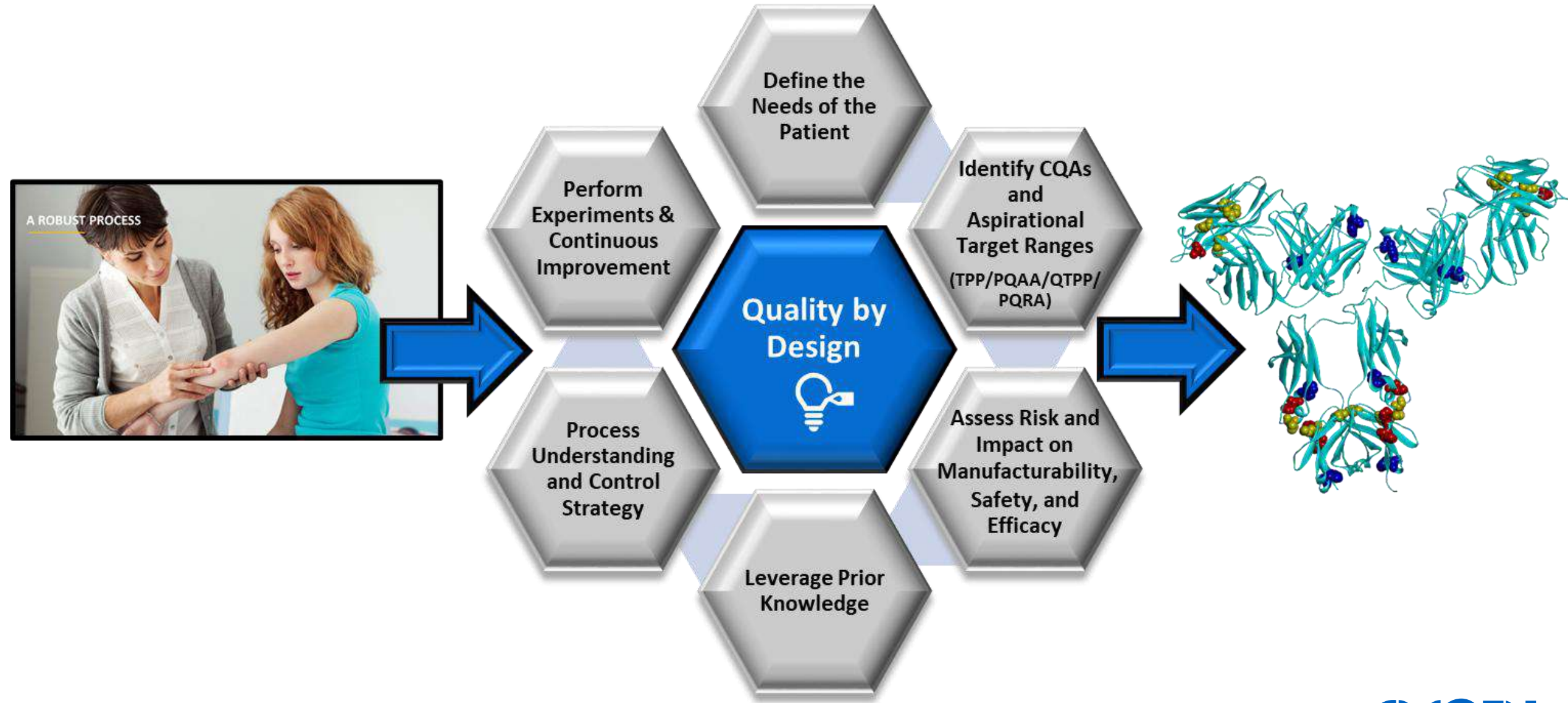
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# Outline

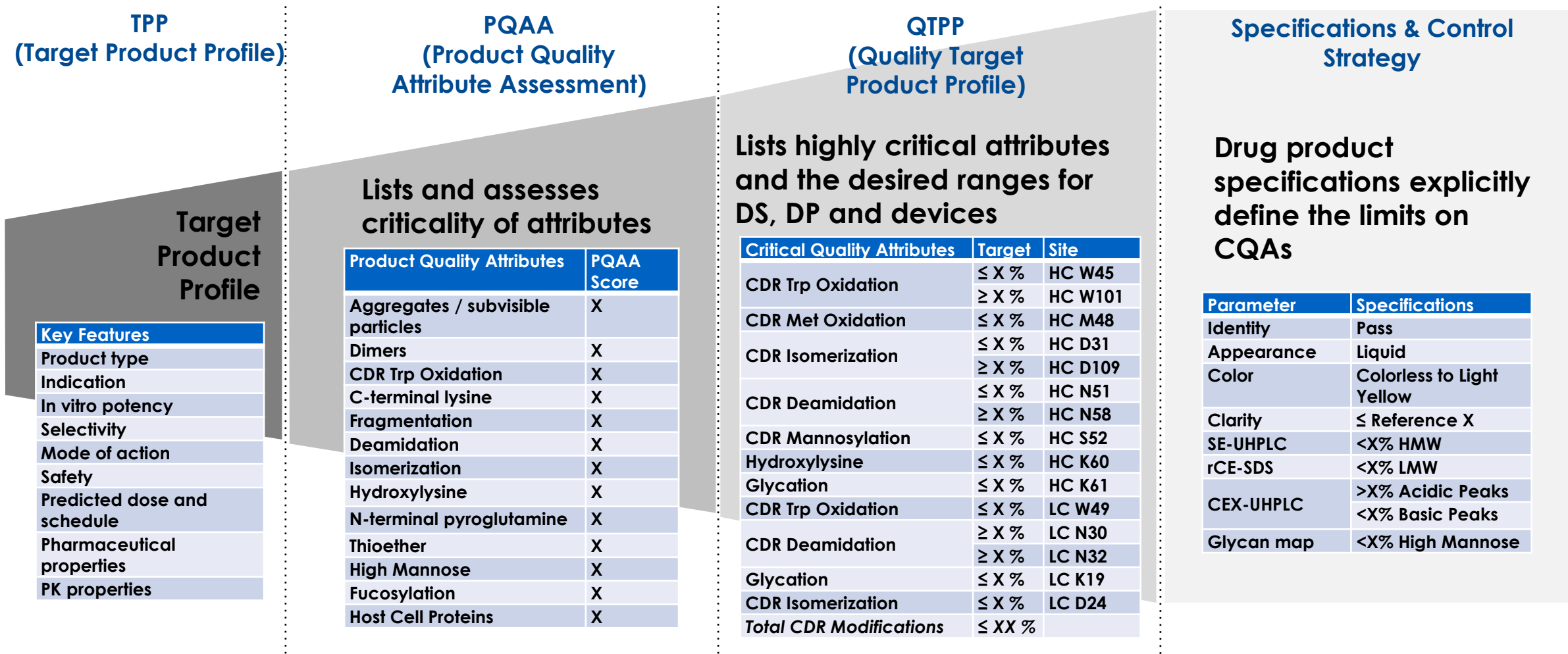
- ❑ **Science-based Risk Approach and QbD**
- ❑ **Biologically Relevant Tools**
- ❑ **Clinical Impact of Attributes (CIA) Approach**
- ❑ **Case Study #1 – Application of CIA to Justify Safe Specifications of Commercial Product 1 (CP1) to Support the Marketing Application**
- ❑ **Case Study #2 - Application of CIA to Justify Safe Specifications of Commercial Product 2 (CP2) to Support the Marketing Application**
- ❑ **Case Study #3 – Application of Biological Relevance Assays to Support a Wider Specification for Commercial Product 2 (CP3) High Molecular Weight (HMW)**

# Quality by design (QBD) principles strategically ensure quality in drug product development from the beginning



# Attribute information is built into the PQAA and QTPP which are used to inform specifications

## Patient Centric Process Development



**TPP (Target Product Profile)**

**Target Product Profile**

Key Features
Product type
Indication
In vitro potency
Selectivity
Mode of action
Safety
Predicted dose and schedule
Pharmaceutical properties
PK properties

**PQAA (Product Quality Attribute Assessment)**

**Lists and assesses criticality of attributes**

Product Quality Attributes	PQAA Score
Aggregates / subvisible particles	X
Dimers	X
CDR Trp Oxidation	X
C-terminal lysine	X
Fragmentation	X
Deamidation	X
Isomerization	X
Hydroxylysine	X
N-terminal pyroglutamine	X
Thioether	X
High Mannose	X
Fucosylation	X
Host Cell Proteins	X

**QTPP (Quality Target Product Profile)**

**Lists highly critical attributes and the desired ranges for DS, DP and devices**

Critical Quality Attributes	Target	Site
CDR Trp Oxidation	≤ X %	HC W45
	≥ X %	HC W101
CDR Met Oxidation	≤ X %	HC M48
CDR Isomerization	≤ X %	HC D31
	≥ X %	HC D109
CDR Deamidation	≤ X %	HC N51
	≥ X %	HC N58
CDR Mannosylation	≤ X %	HC S52
Hydroxylysine	≤ X %	HC K60
Glycation	≤ X %	HC K61
CDR Trp Oxidation	≤ X %	LC W49
CDR Deamidation	≥ X %	LC N30
	≥ X %	LC N32
Glycation	≤ X %	LC K19
CDR Isomerization	≤ X %	LC D24
Total CDR Modifications	≤ XX %	

**Specifications & Control Strategy**

**Drug product specifications explicitly define the limits on CQAs**

Parameter	Specifications
Identity	Pass
Appearance	Liquid
Color	Colorless to Light Yellow
Clarity	≤ Reference X
SE-UHPLC	<X% HMW
rCE-SDS	<X% LMW
CEX-UHPLC	>X% Acidic Peaks <X% Basic Peaks
Glycan map	<X% High Mannose

# Regulators are increasingly expecting that proposed specifications are clinically relevant

## Problem & Solution

Proposed specification

Clinical exposure

Clinical exposure / stability

Novel approaches (i.e. predictive modelling)

In vitro & in vivo biological assays

Prior/Cross-product knowledge & Natural occurrence

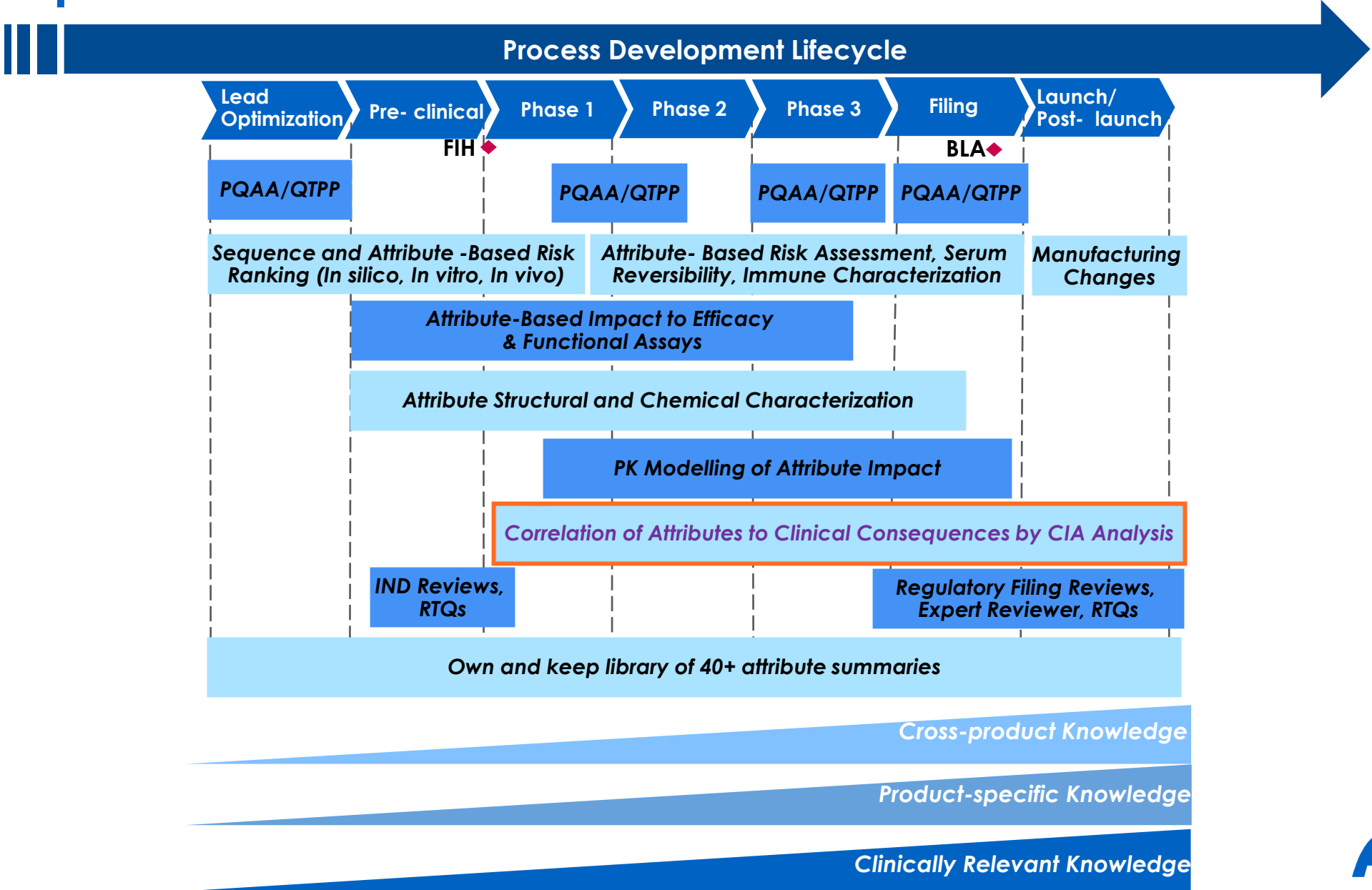
increasing attribute level

## Potential Consequences

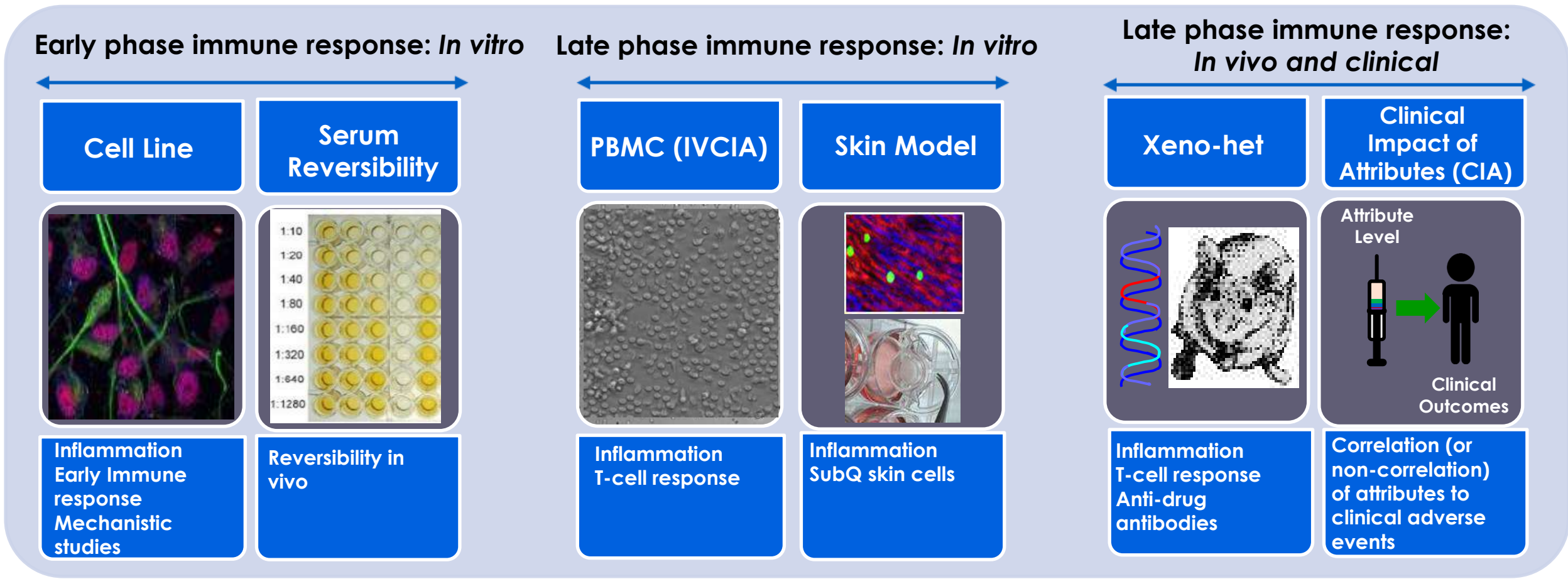
- Overly tight/costly/time consuming manufacturing boundaries
- Good batches may be rejected
- Drug shortages
- New formulations, higher concentrations, updated processes may not be approved
- Shorter expiry period
- Undesirable storage conditions
- Not understanding the impact of attributes on adverse events
- Manufacturing may not be transferable to new sites

Clinical exposure alone is often not wide enough to justify desired attribute ranges, so novel approaches (prior knowledge, predictive modeling, in vitro assays) must be developed and applied

# Strategy for attribute impact assessment to support clinically relevant specifications



# Tools for assessing biological impact



Multiple model systems have been used to study the potential impact of critical quality attributes of biotherapeutics in patients

# Clinical Impact of Attributes (CIA) Approach for developing clinically relevant specifications for biologics



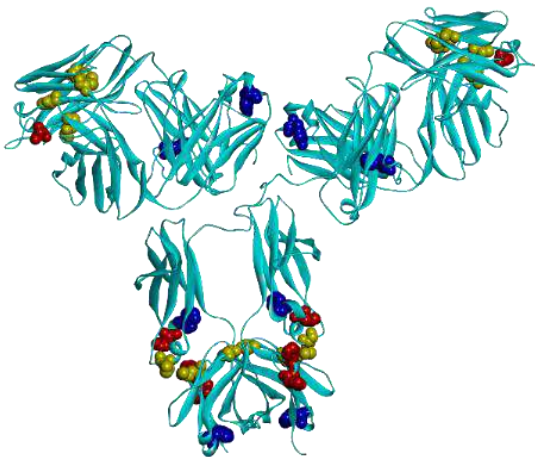
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# There is concern that attributes may have impact on adverse events or efficacy in patients

Each molecule has a unique set of attributes which vary between lots

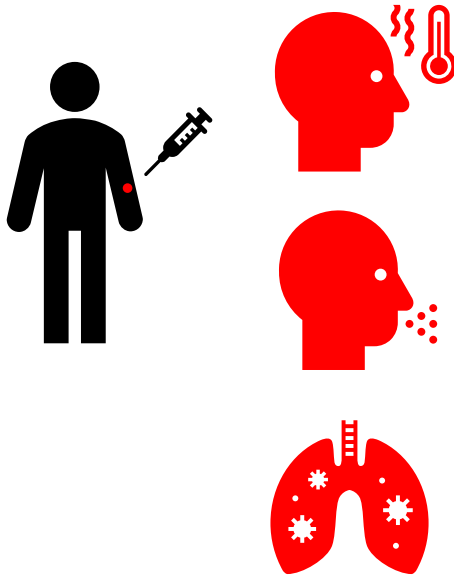
Attributes could have the potential to impact patient safety and efficacy



- CQAs**
- CDR conformers
  - Cysteine adducts
  - Deamidation
  - Disulfide isoforms
  - Fragmentation
  - Glycosylation
  - High Molecular Weight
  - Host cell proteins
  - Hydroxylysine
  - Oxidation
  - Mutations
  - Partial reduction
  - Residual protein A
  - Subvisible Particles
  - ...



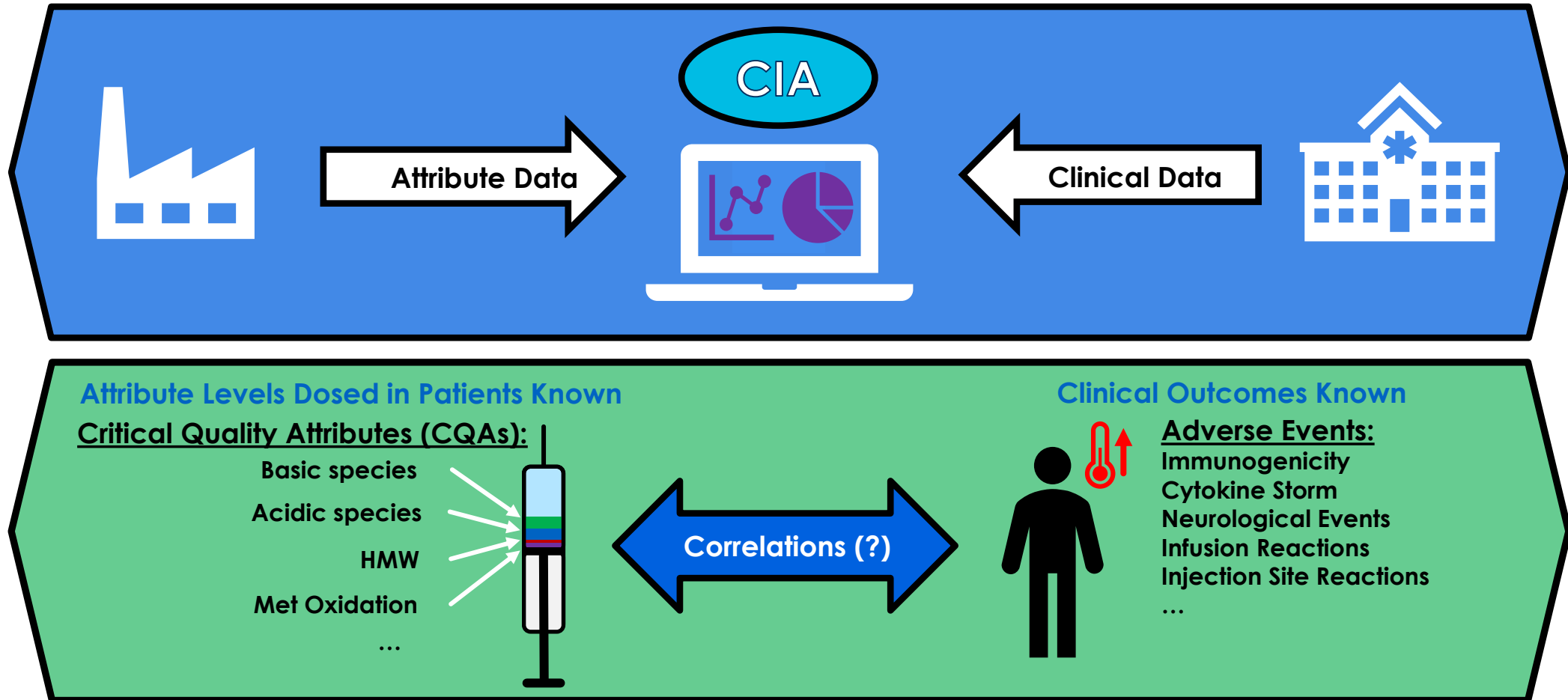
Lack of sound scientific approaches, tools, and data



- Adverse Events:**
- Anaphylactic shock
  - Cytokine storm
  - Fever
  - Immunogenicity
  - Injection site reactions
  - Lymphocytopenia
  - ....

The ability and level of attributes that cause adverse events or impact efficacy in patients are not always well understood

# CIA is a first-in-industry attempt to integrate product quality data with clinical trial data



CIA evaluates relationships between attributes administered to patients and safety

Joh NH, Joubert MK, Kleeman G, Tokuda J, Rieder N, Narhi LO, filed 2021, Methods of Manufacturing Biological Therapies..., [International Patent Application PCT/US21/63641](#).



# CIA is a data science approach that employs statistical analysis of clinical trial data

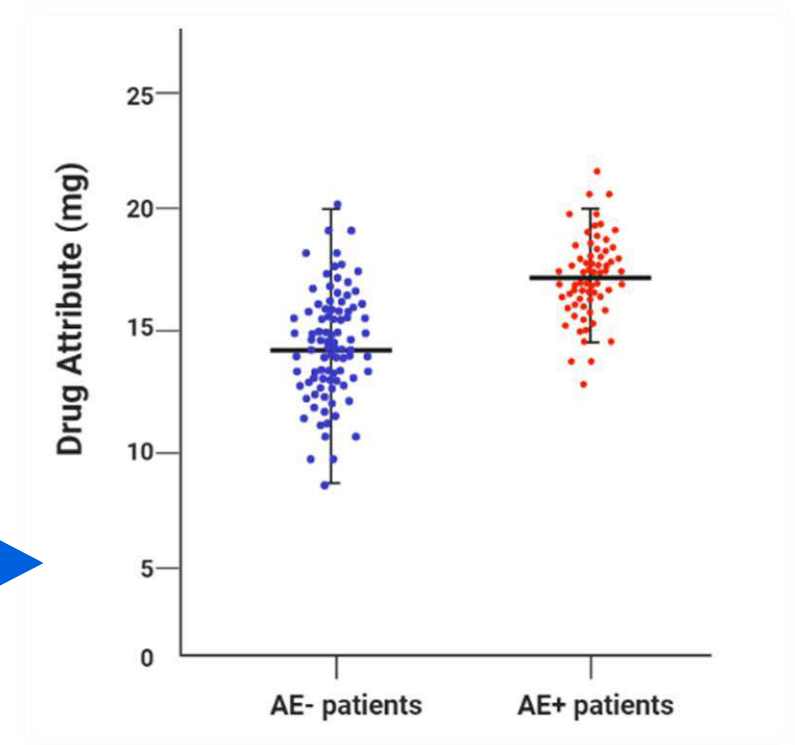
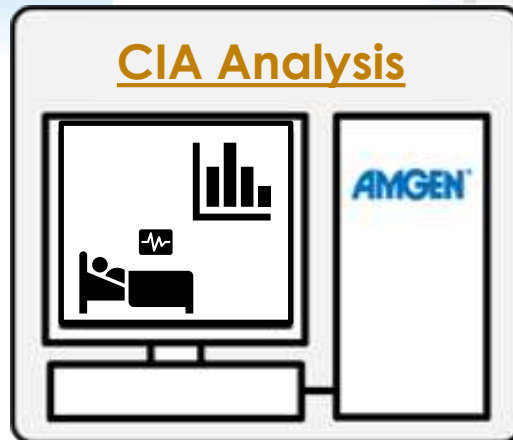
## Product Quality Attributes



## Clinical Trials Studies

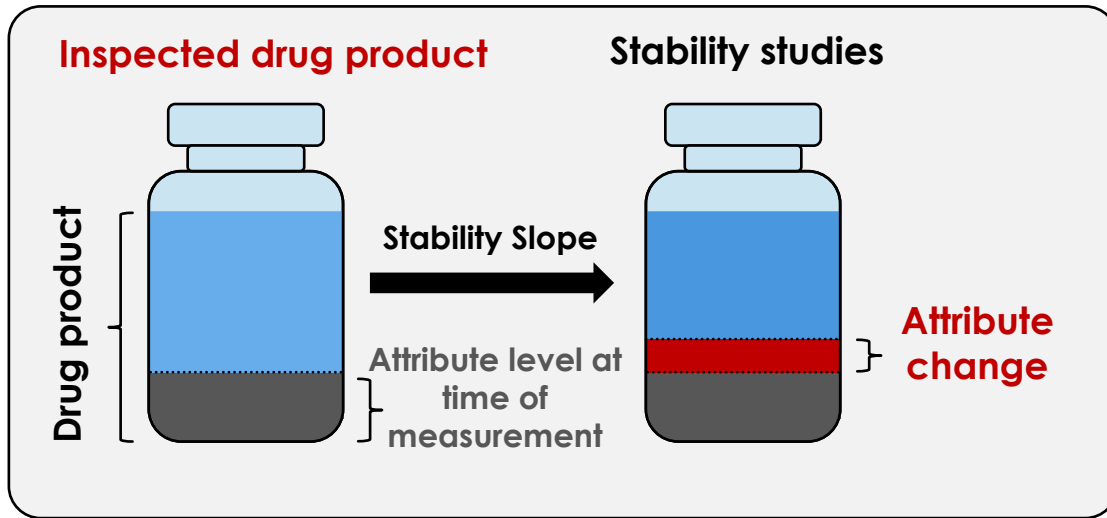


## CIA Assessment



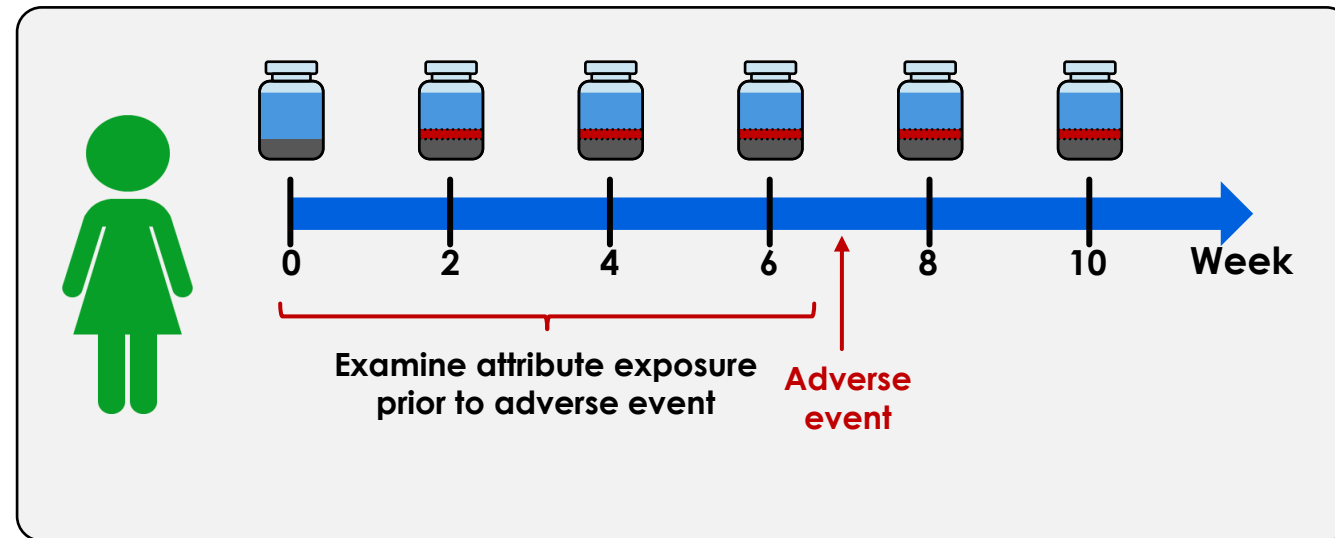
Statistical plots are used to visualize data for evaluating associations

# Attribute levels at the time of administration are estimated



**Clinical Exposure:** Attribute exposure is estimated for each dose administered to patients based on drug product lot age

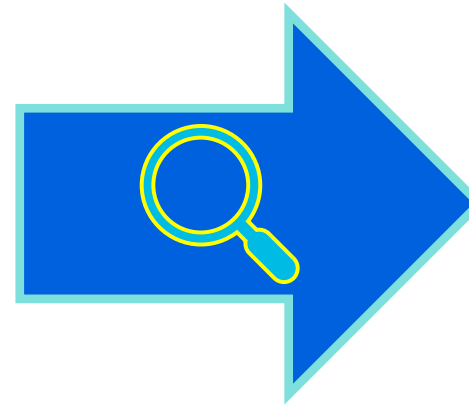
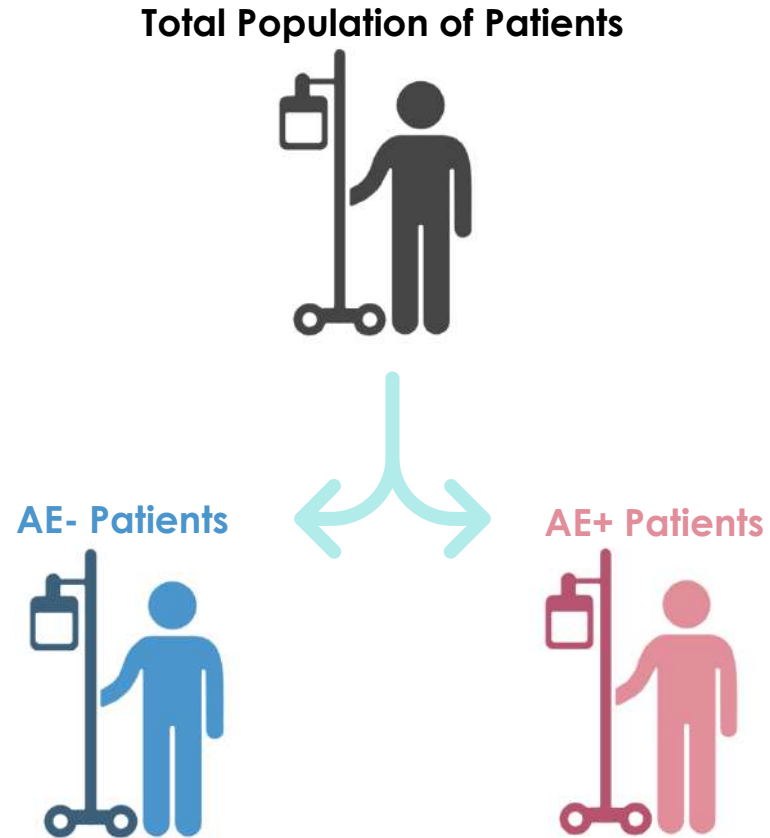
**Attribute Estimation:** Attribute levels as a function of time are calculated using various manufacturing and product quality measurements.



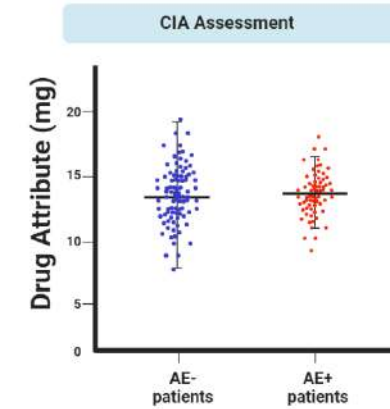
Clinical exposure to attributes may be higher than that measured at lot release

# Processed data is examined for any correlations between attribute levels exposed in patients and adverse events

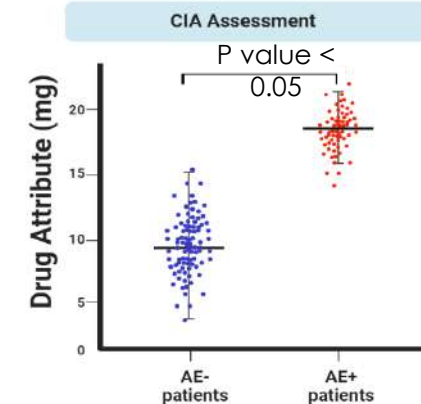
For Each Adverse Event And Each Attribute



No Correlation



Positive Correlation



CIA is a differentiating capability for generating clinically relevant evidence

# CIA Applications

CP = Commercial Product mAb = Monoclonal Antibody FP = Fusion Protein ADA = Anti-drug antibody

Program	Finding from CIA	Example Applications
CP1	No impact of 4 attributes on ADA	Included in RTQ to justify specifications. MA approved (+14-day RT storage)
CP3	No impact of 15 attributes on ADA	Included in INDa Justification of Specification section and approved for wider specification
CP4	No impact of 1 attribute on 10 AEs	Planned inclusion in Briefing Book to widen specification
CP5	1. No impact of 1 attribute on AEs (Phase 1) 2. No impact of 6 attributes on 11 AEs (Phase 3)	Support product development Intellectual property support
CP6	No attribute/potency impact on 6 AEs in observed conditions	Included in Briefing Book for new process
CP7	1. No impact of 11 attributes on ADA 2. No impact of 7 attributes on AEs	Included in RTQ to MA to justify specifications and accepted Included in post-marketing commitments to justify specifications and accepted
CP8	No impact of 20 attributes on 21 AEs	Planned inclusion in briefing book
CP9	No impact of 3 attributes on 6 AEs	Included in MA Justification of Specification section and accepted Intellectual property support
mAb1	1. No impact of 6 attributes on 12 AEs (Phase 1) 2. No impact of 6 attributes on 12 AEs (Phase 3)	Support product development Intellectual property support
mAb2	1. No impact of 6 attributes on 12 adverse events 2. Additional attributes in progress	Planned inclusion in MA and preparation for RTQs

**CIA has been applied to numerous commercial and pipeline programs at Amgen to support marketing applications, INDa, briefing books, RTQs, PMCs, and patents**

Case Study #1 – Application of CIA to Justify  
Safe Specifications of Commercial Product 1  
(CP1) to Support the Marketing Application

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# Regulators requested Clinical Qualification of Specifications in Response to the CP1 Marketing Application

## Challenge

Regulators requested additional clinical justification for 4 requested specifications for CP1 in response to the marketing application

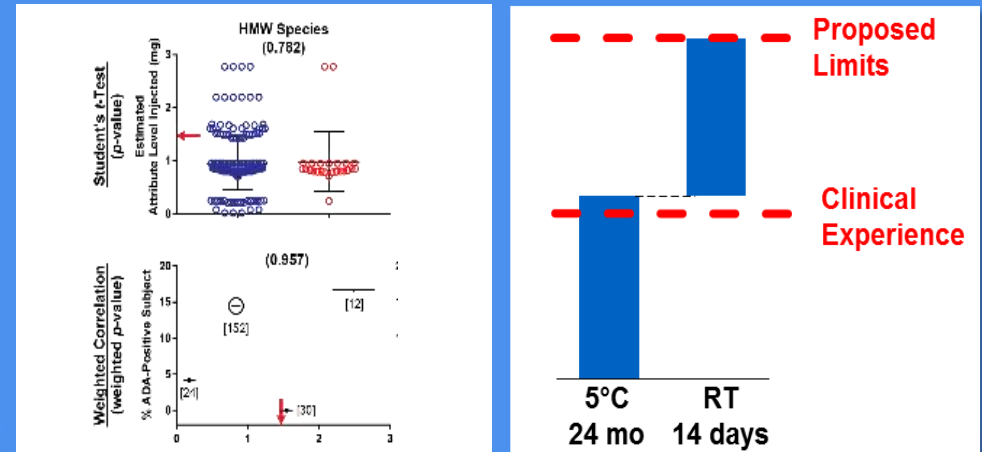
### Attributes:

- High Molecular Weight Species
- Fragments
- Acidic Species
- Asp Isomer

CIA

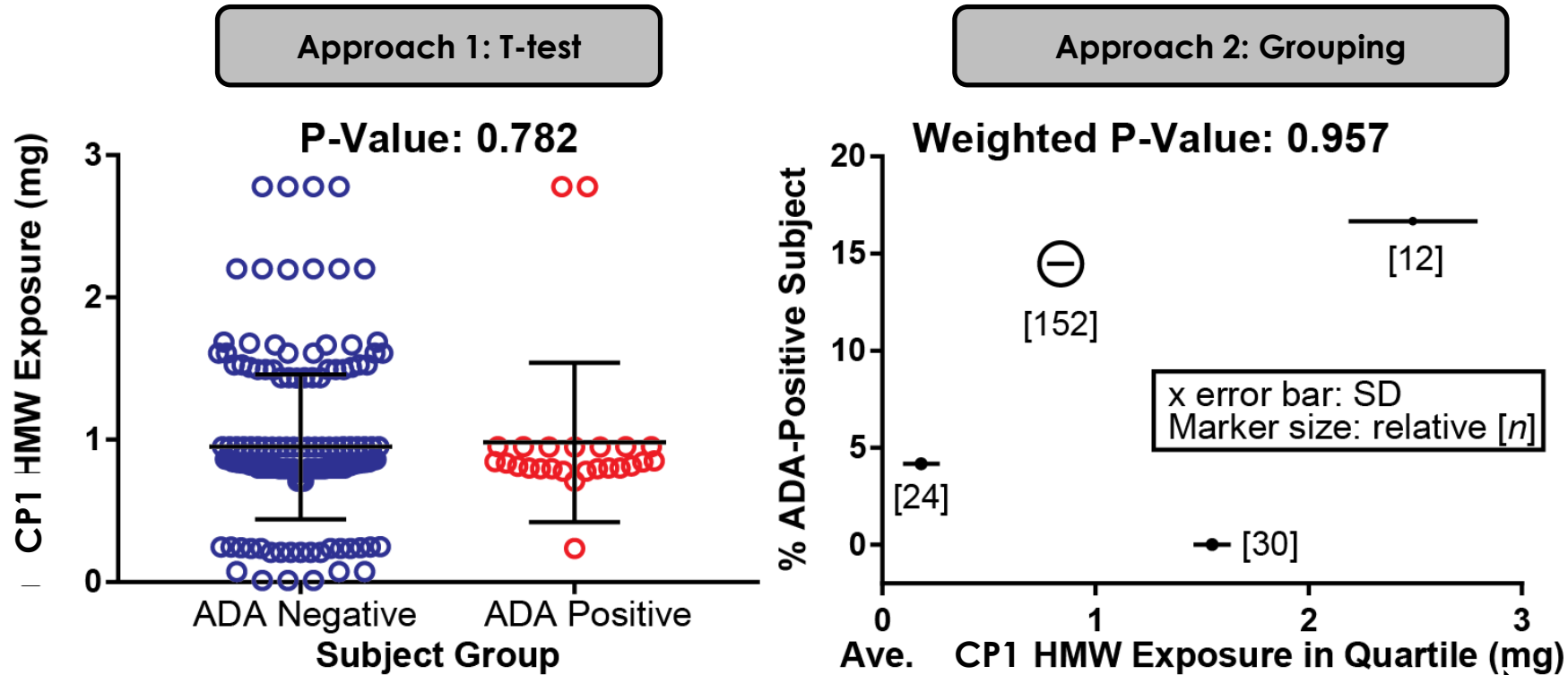
## Solution

CIA used to support approval of marketing application, with 2 weeks of RT storage



CIA Analysis, in addition to other prior knowledge arguments, was used to successfully justify specifications and gain marketing approval of CP1

# No Association was Observed between the Level of CP1 HMW Dosed in Patients and the Development of ADA



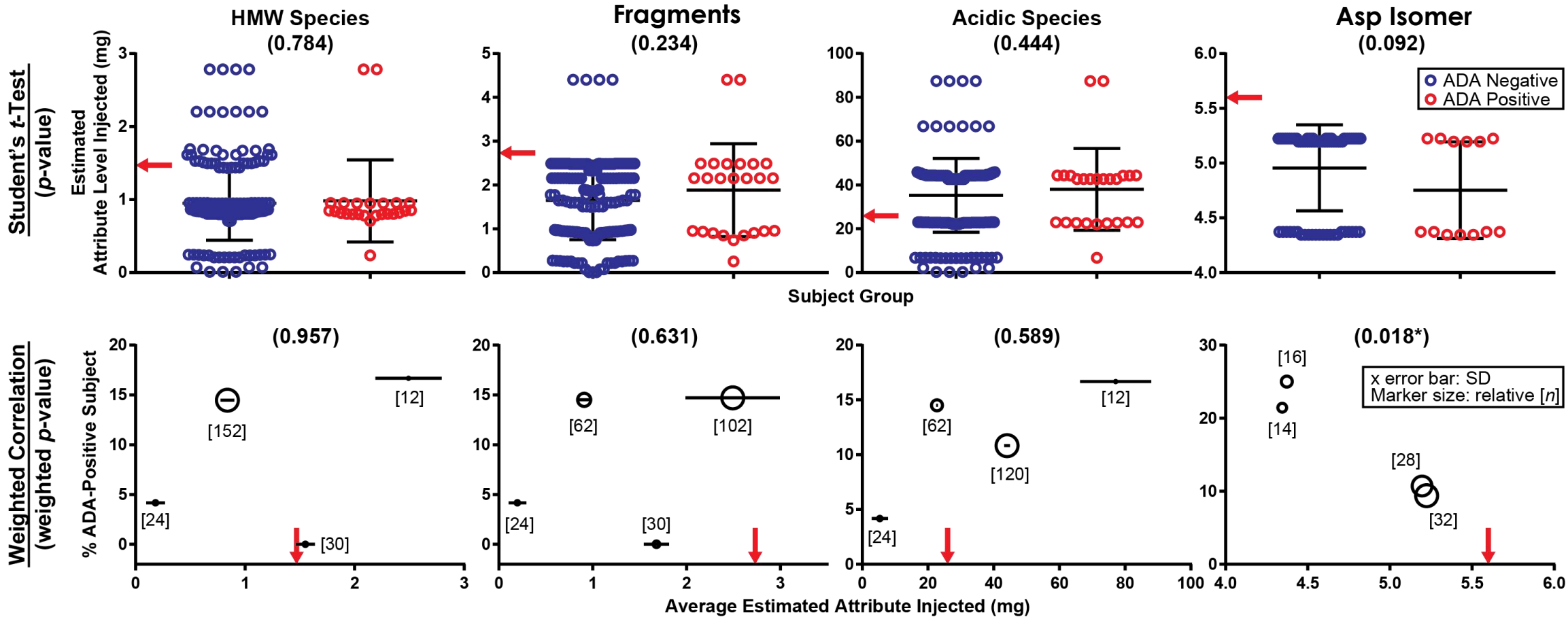
ADA = anti-drug antibodies

HMW = high molecular weight

**No association**

**This suggests that CP1 HMW has a low risk of immunogenicity**

# CIA Showed No Positive Correlation between Clinical Immunogenicity and Exposure to these Attributes



CIA was used to demonstrate that the level of all 4 attributes (HMW, fragments, acidic species, Asp isomer) that were exposed in the clinic are safe



# Conclusion from Case Study 1

**CIA was used to successfully defend the clinical relevance of the proposed specifications for this Amgen product, resulting in marketing application approval with extended room temperature storage**

Case Study #2 – Application of CIA to Justify  
Safe Specifications of Commercial Product 2  
(CP2) to Support the Marketing Application

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# Regulators Requested Clinical Qualification Of Specifications In Response To The CP2 Marketing Application

## Challenge

- Agency requested narrowing of the proposed specification for different attributes due to concerns about safety and immunogenicity.
  - HMW (High molecular weight species)
  - CEX-Acidic peaks
  - CEX-Basic peaks
  - CEX-Main peak

## Solution

- CIA analysis supported the marketing application by demonstrating the safety and immunogenicity of the proposed specifications

### Agency Assessment:

- **HMW (High molecular weight species) and high order aggregates**  
“The [clinical impact of attributes \(CIA\)](#) analysis.... provides [evidence of clinical exposure and no potential immunogenicity risk related to the higher order aggregates. Therefore, it is acceptable not to amend the HMW acceptance criteria. Issue considered resolved](#)”
- **Charge variants (CEX-Acidic, Basic, and Main peaks species)**  
“... presented [clinical impact analysis](#) supports the setting of one-sided limits as data were similar (no association between adverse effects and charge variants) for –CP2– and reference product despite of observed differences in charge variants. .... [The proposed IPC rejection limits are considered safe and justified. Issue resolved.](#)”

CIA Analysis, in addition to other biological assays, was successfully used to justify specifications and gain marketing application approval

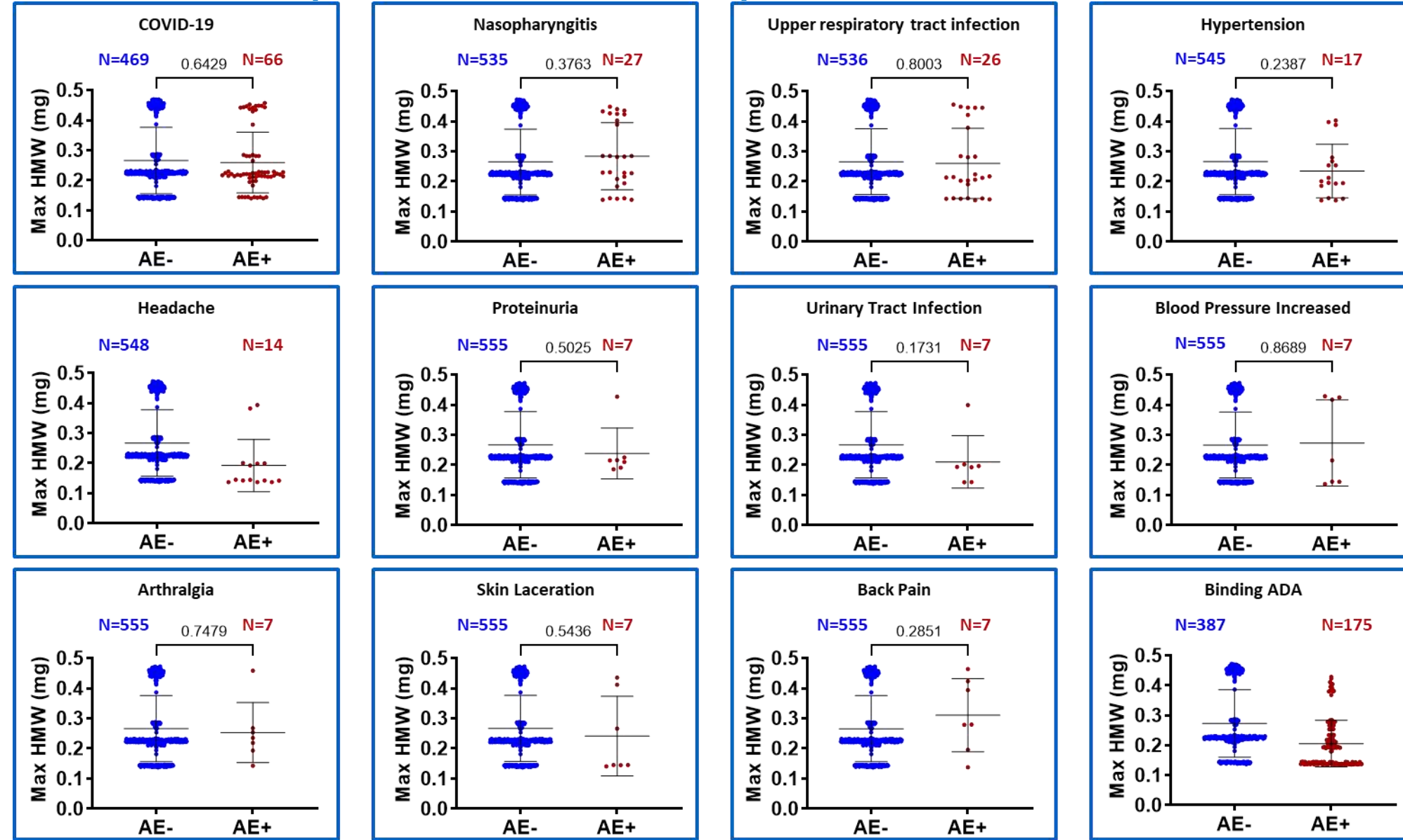
# SEC-HMW : Comparison Of Maximum Exposure For AE+/- in Phase 3

Total patients:  
562 patients

Max: maximum attribute exposure subjects received prior to AE (AE+) or any dose (AE-)

- No statistically significant\* positive correlations were observed between SEC-HMW maximum exposure and any adverse events

\*P-value >0.05 is considered not significant



HMW exposure was not associated with any adverse events

22  
Bars for each population represents average and standard deviations. N = number of subjects. P-values were calculated from Student's t-test (unpaired, equal variance)



# Conclusion From Case Study 2

CIA was used to successfully defend the clinical relevance of the proposed specifications for this Amgen product, resulting in marketing application approval

## Agency Assessment:

- **HMW (High molecular weight species) and high order aggregates**

“The [clinical impact of attributes \(CIA\)](#) analysis... provides [evidence of clinical exposure and no potential immunogenicity risk related to the higher order aggregates. Therefore, it is acceptable not to amend the HMW acceptance criteria. Issue considered resolved](#)”

- **Charge variants (CEX-Acidic, Basic, and Main peaks species)**

“... presented [clinical impact analysis](#) supports the setting of one-sided limits as data were similar (no association between adverse effects and charge variants) for –CP9– and reference product despite of observed differences in charge variants. .... [The proposed IPC rejection limits are considered safe and justified. Issue resolved.](#)”

Case Study #3 – Application of Biological  
Relevance Assays to Support a Wider  
Specification for Commercial Product 3 (CP3)  
High Molecular Weight (HMW)

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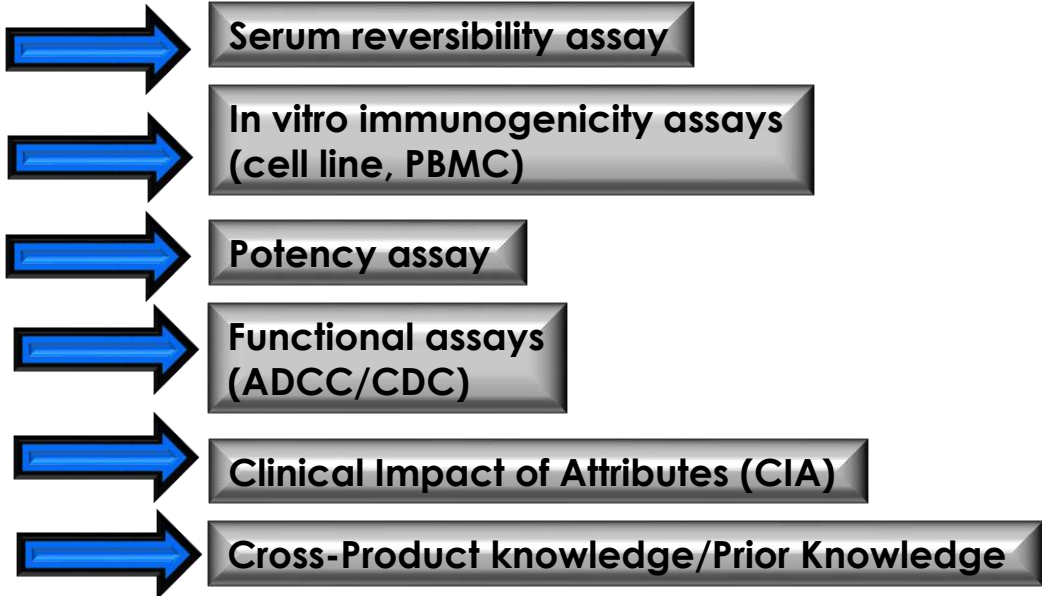
# CP3 High Concentration (HC) Formulation has Higher Levels of HMW which May Impact Safety and Efficacy

**Challenge:** A HC formulation of CP3 was developed for patient convenience and for the product to remain attractive. This led to higher levels of HMW species which might impact safety and efficacy.

## Experimental Design

CP3 HMW was enriched 75% from HC DS:

- mostly dimer (up to 90%)
- partially non-dissociable (up to 60%)
- enriched slightly in Met Ox, Met thiol loss, and NGHC, but not fragments
- similar in secondary and tertiary structure and disulfide linkages

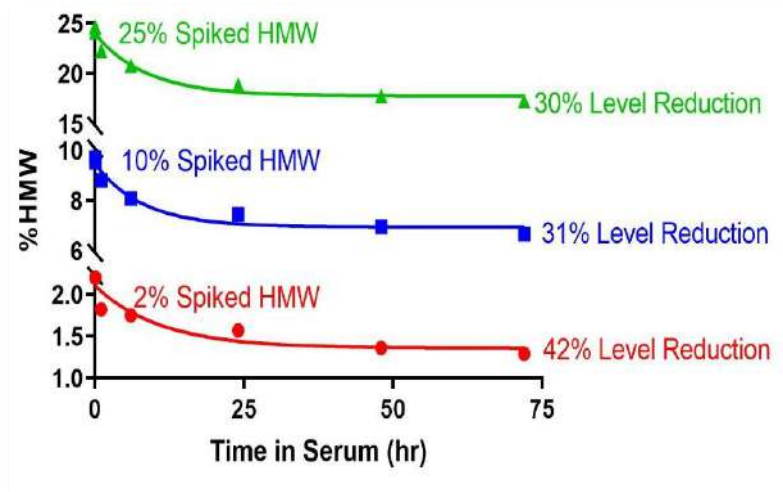


**Overall conclusion: no increased risk to safety or efficacy is posed by HMW enriched up to 25% from high-concentration (100 mg/ml) CP3 DS**

# Enriched CP3 HMW was Tested for Reversibility in Serum Depleted of Large Proteins and an In Vitro Immunogenicity Assay

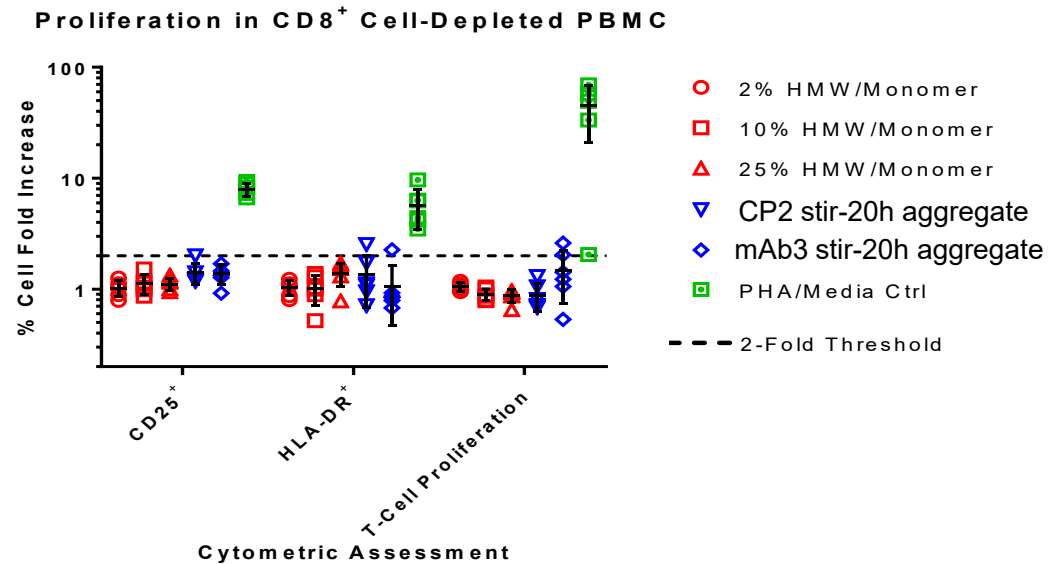
## Serum Reversibility Results

Level reduction in CP3 HMW upon incubation in serum over time



## In Vitro Immunogenicity Results

Response of a population of human PBMC (n=6) to enriched CP2 HMW



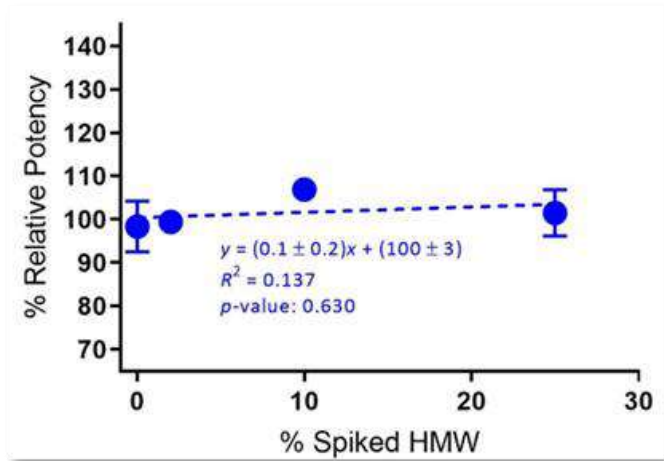
- Serum was pre-treated using a 30 kDa MWCO filter to remove large proteins that interfere with peaks for the mAb in SE-HPLC
- Levels of HMW starting at 2%, 10%, and 25% in the filtered serum at 37°C under mild agitation for up to 72 hrs was monitored by SE-HPLC

- HMW (up to 25%) did not cause an impact in the PBMC assay (shown)
- HMW (up to 25%) did not cause an impact in the cell line assay (not shown)

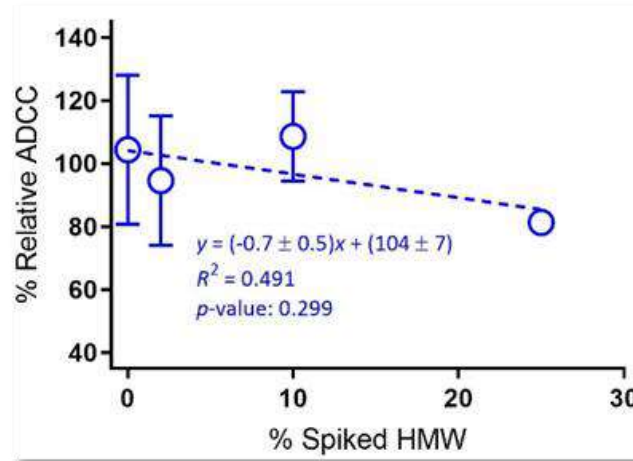
- HMW (up to 42%) was reversible in the serum environment within 72 hrs
- HMW (up to 25%) did not increase risk of immune activation in the in vitro cell-based assays

# Enriched CP3 HMW was Tested for Impact in Three Functional Assays

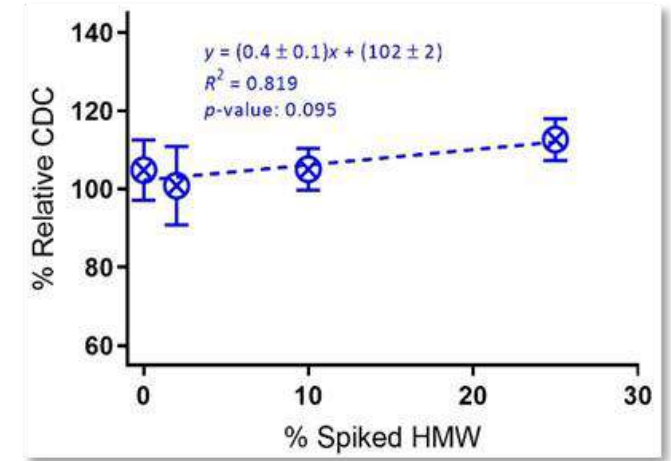
## Potency Assay



## ADCC Assay



## CDC Assay



ADCC = Antibody dependent cellular cytotoxicity  
CDC = Complement dependent cytotoxicity

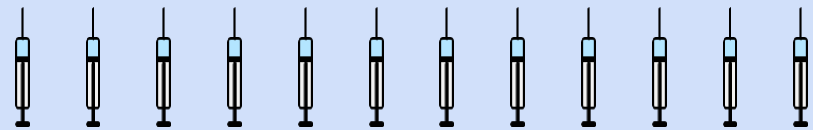
No increased response to enriched HMW (up to 25%) was detected in the potency and effector function assays (CDC/ADCC)

# CIA Analysis of CP3 HMW was Performed using CP3 Clinical Trial Data

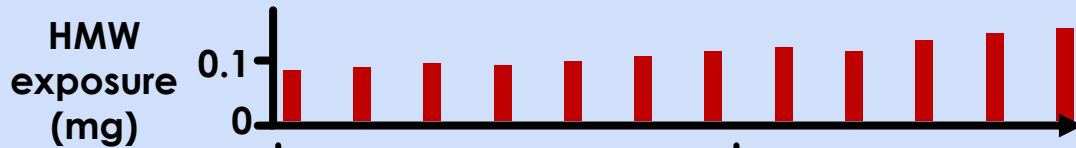
## Overview of CIA Strategy

Clinical trial (Disease Indication 1, 22-week interval studied, biweekly injection)

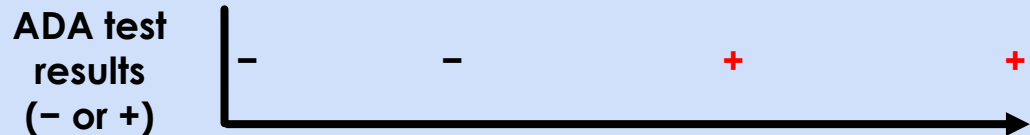
Treatment: 1 2 3 4 5 6 7 8 9 10 11 12



Patient treatment timeline



Treatments considered



Attribute Level Comparisons (ADA- vs. ADA+)

- Maximum exposure
- Average exposure

1<sup>st</sup> ADA+ test

## Additional Parameters

1) Disease Indication: Disease Indication #1

Disease Indication #2

2) Time-intervals considered

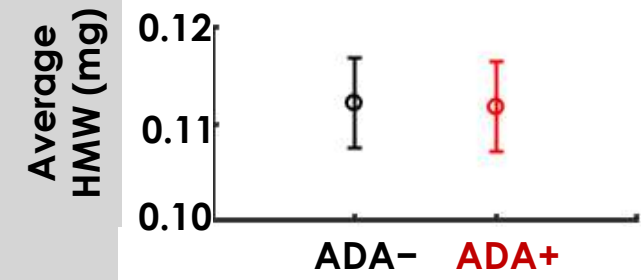
Weeks: 5, 12, 26

3) Group patients based on latest ADA test result



New ADA+ are removed from subsequent time-intervals

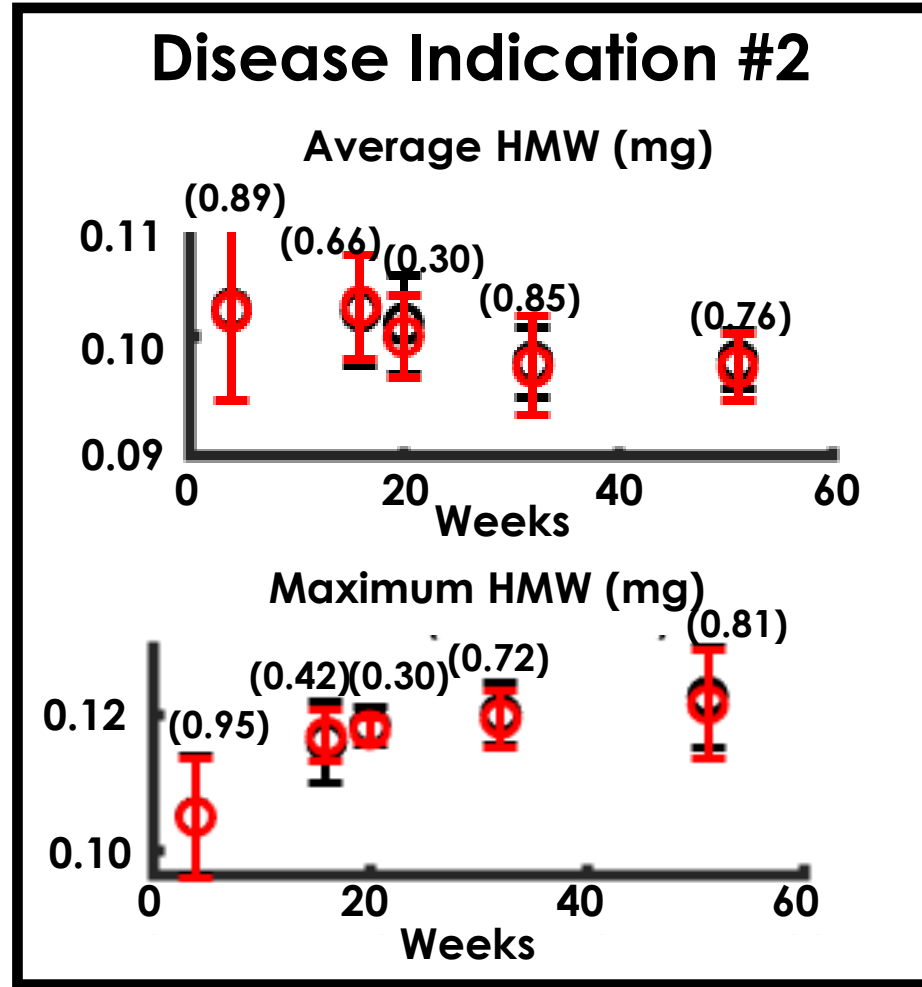
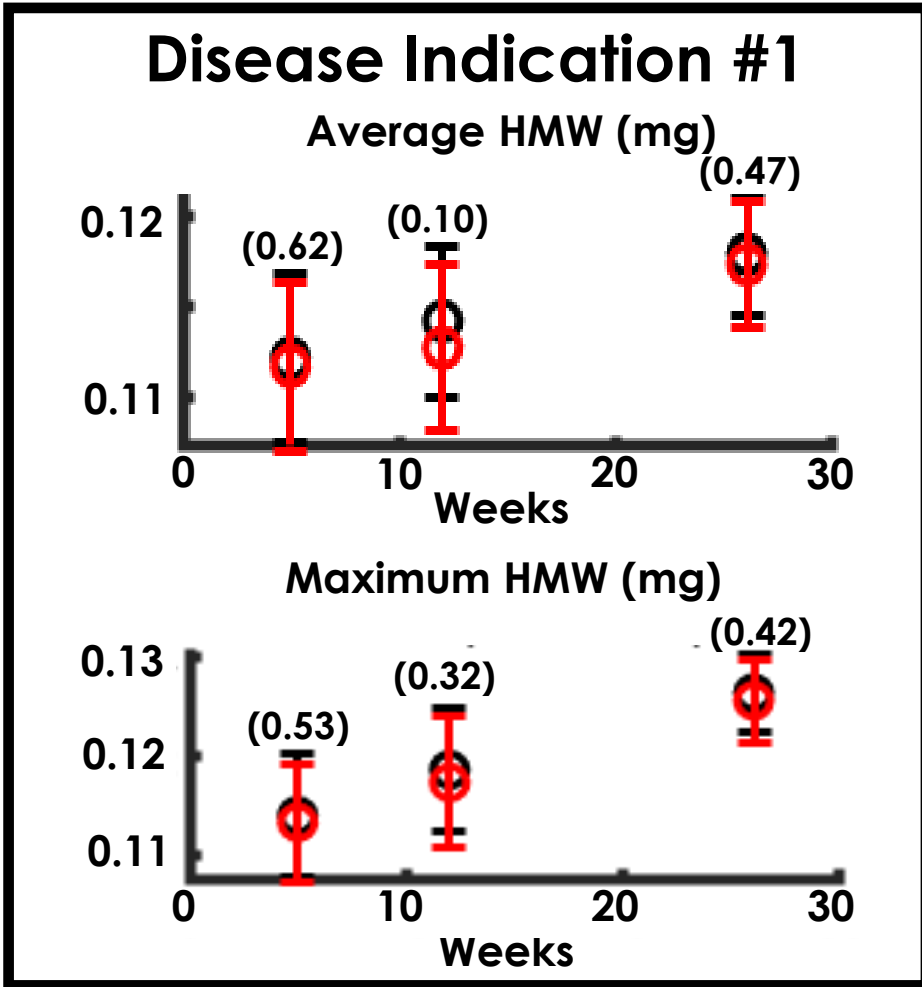
4) Calculate attribute exposure between groups



Multiple factors were taken into consideration including: dose of CP3, disease indication, level of HMW in each lot administered, stability of HMW, onset of ADA, and exposure to HMW as an average or a maximum

# CIA Analysis Results of CP3 HMW

○ ADA-  
○ ADA+ (new)  
(p-value)



No correlations were found between ADA incidence and the average or maximum levels of CP3 HMW that patients were exposed to in the clinical trials

# No Association was Observed between HMW Dosed in Patients and the Development of Adverse Events across Numerous Programs

CP = Commercial Product mAb = Monoclonal Antibody FP = Fusion Protein

Program	Treatment Route	Attribute	Maximum Exposure Range		Exposure Unit	Analyzed Clinical Outcome	Analyzed Clinical Outcome #	Correlated Clinical Outcome
			Lower Extent	Upper Extent				
CP1	SC	SEC HMW	0.010	2.78	mg	ADA	1	None
CP3	SC	SEC HMW	0.094	0.14	mg	ADA	1	None
CP4	IV	SEC HMW	0	14.79	mg	Infection, Headache, Anemia, Pyrexia, Vaccination complications, Cough, Asthenia, Fatigue, Hypertension, Back pain	10	None
CP5	IV	Agg (HMW)	0.011	0.21	µg/day	Anemia, Cytokine Release Syndrome, Fever, Infusion Related Reaction, Lymphocytopenia, Neurological event	6	None
CP6	SC	SEC HMW	1.40	2.77	mg	ADA	1	None
CP7	SC	SEC HMW	0.89	7.60	mg	Top 21 AEs (Nasopharyngitis, Headache, Backpain, Upper respiratory tract infection, Diarrhea, Arthralgia, Myalgia, Fatigue, Cough, Nausea, Muscle spasms, Pain in extremity, Urinary tract infection, Hypertension, Dizziness, Bronchitis, Oropharyngeal pain, Blood creatine phosphokinase increased, Constipation, Influenza, Injection site pain)	21	None
CP8	IV	HMW	1.57	5.73	mg	Respiratory Tract Infection, Myalgia, Headache, Oropharyngeal Pain, Dermatitis Contact, Asthma	6	None
CP8	SC	HMW	0.015	3.15	mg	Headache, Injection Site Reaction, Upper Respiratory Tract Infection, Myalgia, Back Pain, Dermatitis Contact	6	None
mAb1	SC	SEC HMW	0.132	0.332	mg	COVID-19, Headache, Nasopharyngitis, Upper respiratory tract infection, Hypertension, Diarrhoea, Proteinuria, Urinary tract infection, Blood pressure increased, Arthralgia, Skin Laceration, Back Pain, Nausea, Pruritus, Myalgia, Dizziness, Oropharyngeal Pain, and Abdominal pain.	18	None
mAb2	IVT	SEC HMW	11.7	96.36	µg	ocular hemorrhage, retinal degeneration, visual impairment, cataract, intraocular pressure increased, vitreous detachment, intraocular inflammation, thromboembolic events, hypertension, binding ADA	10	None
FP1	IV	HMW	0.0058	18.15	µg	Cytokine Release Syndrome, Neutropenia, Rash, Rash-Related Events, Pyrexia	5	None
FP2	IV	SEC HMW	0.0063	5.03	µg	Anaemia, Cytokine Release Syndrome, Lymphocytopenia, Thrombocytopenia, Efficacy	5	None



## Conclusion from Case Study 3

**HMW from CP3 posed no increased risk to safety or efficacy. Numerous additional programs showed no correlation of HMW with immunogenicity in clinical studies.**

**The data shown in this case study was successfully used to support widening of HMW release/stability specifications for clinical filing and received regulatory approval.**

**This data package was also used for supporting analytical comparability and proposing commercial specifications (FDA approval received with no questions).**

# Conclusions

- **QbD** principles are foundational to drug product development
- **The impact of attributes** on safety and efficacy is not always well understood
- An array of biological relevance tools were developed to generate scientific knowledge on attribute impact which can be used to inform clinically relevant specifications
- **The Clinical Impact of Attribute (CIA)** Approach is a powerful data science method to assess if any correlations exists between clinical attribute exposure in patients and clinical adverse events
- Leveraging this information throughout drug product development is key to developing **high quality medicine that is the most safe and efficacious for patients**

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Chetan Goudar

Margaret Ricci

Jette Wypych

Mahshid Amini

Dipa Batabyal

Nina Cauchon

Yuh-feng Chen

Hui-Ting Chou

Jill Crouse-Zeineddini

Jack Gerson

Gino Grampp

Christina Ives

Kevin Kalenian

Mark Kroenke

Scott Kuhns

Ken Lawson

Xing Ma

Trent Munro

Linda Narhi

Shane Nguyen

Rupa Padaki

Murali Pasumarthy

Barbara Rellahan

Margaret Ricci

Jason Richardson

Noel Rieder

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## Discussion/Abstract

Biological relevance tools are essential to gain knowledge about attribute impact which can be used to support clinically relevant specifications. A new data science method called the Clinical Impact of Attributes (CIA) approach will be shared that uses clinical trial information to justify clinically safe specifications. CIA analyzes clinical trial data to determine if any correlations exist between attribute levels exposed in patients and the development of adverse events. Several case studies of biologics that are commercial products will be shown.