Tailoring ADA and NAb Assays to Drug Modalities; Insights from Case Studies

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Cerba Research Canada, Montréal

Immunogenicity Summit, October 17, 2024

Agenda

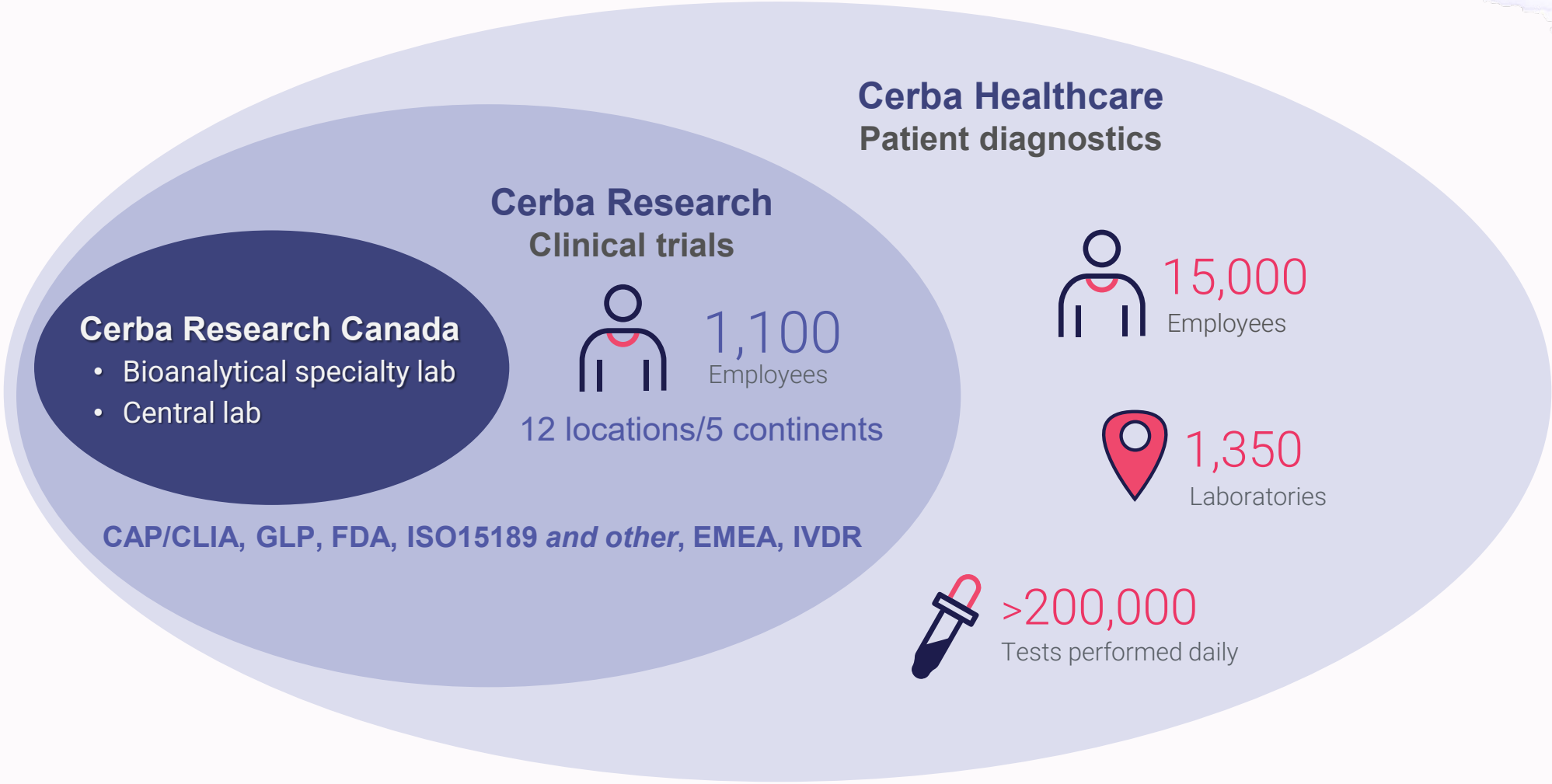
Introduction to Cerba Research

Immunogenicity assays: overview

Case study 1: ADA assay for ADC drug product

Case study 2: NAb assay for PEG-GCSF biosimilar

Cerba Research (within Cerba Healthcare organization)



CAP: College of American Pathologists; CLIA: Clinical Laboratory Improvement Amendments; GLP: Good Laboratory Practices; FDA: Food and Drug Administration; ISO: International Organization for Standardization; EMEA: European Medicines Evaluation Agency; IVDR: In Vitro Diagnostic Regulation; PK: Pharmacokinetic; IHC: Immunohistochemistry

Cerba Research is a global service company

	Bioanalytical		Biomarkers (soluble)	Molecular biology	Genomics	Virology	Flow cytometry	Histopathology/ IHC	Microbiology
	PK	Immunogenicity							
Canada (Montreal)	✓	✓	✓				✓		
USA (New York)				✓	✓	✓	✓	✓	
Ghent (Begium)			✓	✓			✓		✓
Netherlands (Schaijk, Rotterdam, Rijswijk)				✓	✓	✓			
France (Paris, Montpellier)								✓	
China (Shanghai)	✓	✓	✓	✓	✓		✓	✓	
Taiwan (Taipei)	✓		✓	✓	✓		✓	✓	
South Africa (Johannesburg)				✓	✓	✓			✓
Australia (Sydney)				✓		✓	✓		

Global program management
Central laboratories
(logistics / kit assembly / safety testing)

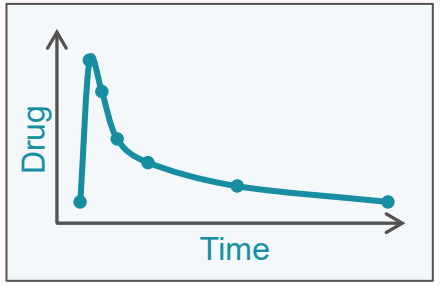
PK: Pharmacokinetic; IHC: Immunohistochemistry

Cerba Research Canada (CR-CA, legacy CIRION)

- 25+ years of recognized experience
- Focused on biologics and biosimilars
- CAP/CLIA and GLP accredited
- Method development/validation + sample analysis for 270+ trials across numerous therapeutic areas

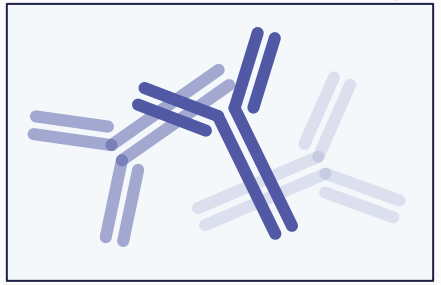
Central laboratory services

PK



- 100+ validated assays
- 70 000+ study samples

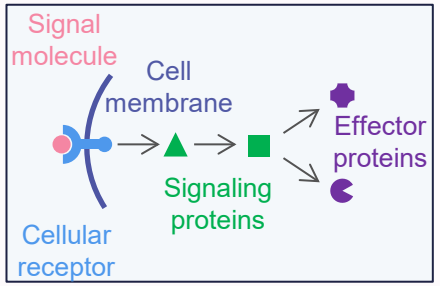
Immunogenicity



ADA/NAb

- 300+/100+ validated assays
- 77 000+/15 000+ samples

Functional assays



- Activity: 13 000+ samples
- Potency: 1500+ samples

Biomarkers (soluble)

- ELISA, MSD
- Multiple panels (single plex, multiplex)

CR-CA: Cerba Research Canada; CAP: College of American Pathologists; CLIA: Clinical Laboratory Improvement Amendments; GLP: Good Laboratory Practices; PK: Pharmacokinetic; ADA: Anti-drug antibody; NAb: Neutralizing antibody; ELISA: Enzyme-linked immunosorbent assay: MSD: Meso Scale Discovery

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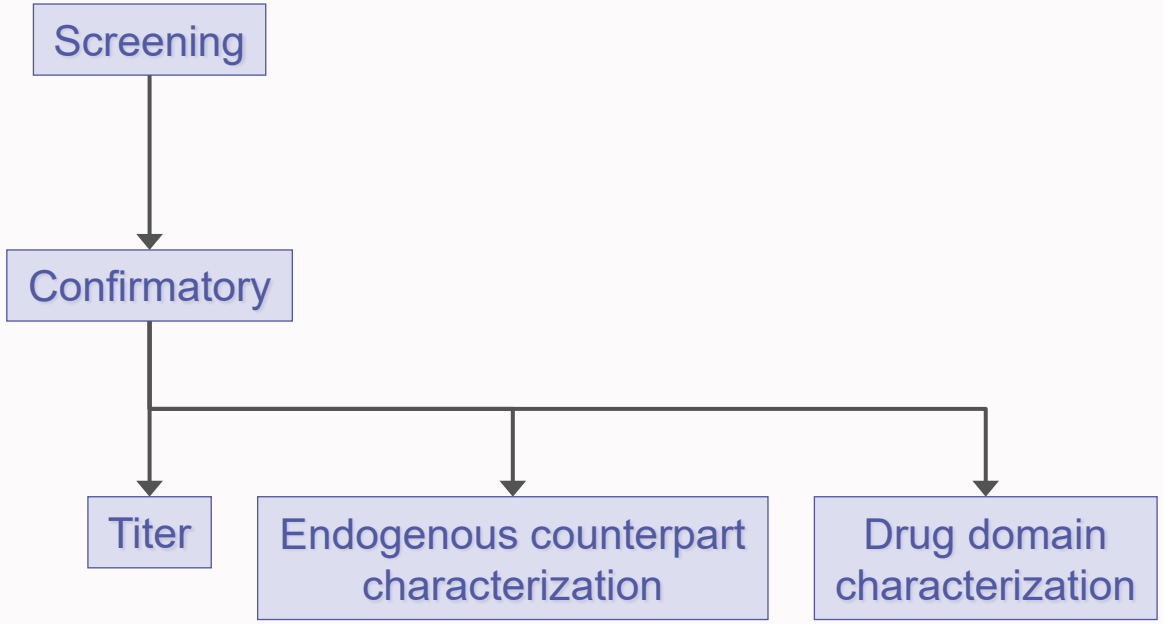
Immunogenicity overview: ADA testing cascades for clinical studies



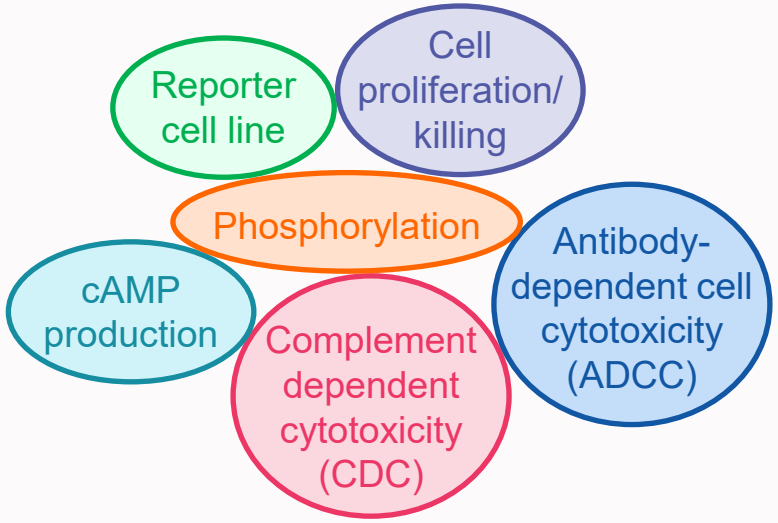
Tier adaptation based on drug product

- Antibody, peptide, CAR(T) → "traditional" cascade
- Protein (e.g.: cytokine, enzyme) → possible added endogenous counterpart characterization tier
- ADC and other conjugates (e.g.: PEG, Fc, XTEN) → possible added drug domain characterization tier
- Vectors (e.g. AAV, AdV) → many possible cascade variations

CAR(T): Chimeric antigen receptor (T-cell); ADC: Antibody-Drug Conjugate; PEG: Polyethylene glycol; Fc: Fragment crystallizable; AAV: Adeno Associated Virus; AdV: Adenovirus

Immunogenicity overview: Neutralizing antibody assays

Functional cell-based assays



Generally screening tier only

Competitive ligand binding assay

Alternate approach to cell-based assays, used when:

- Drug products bind to a soluble target
- Cell-based assay performance not suitable for intended purpose
  - Poor sensitivity (e.g. need for high dilution due to matrix effect)
  - Low drug tolerance
  - Lack of adequate reproducibility

cAMP: Cyclic adenosine monophosphate; CDC: Complement dependent cytotoxicity; ADCC: Antibody-dependent cell cytotoxicity

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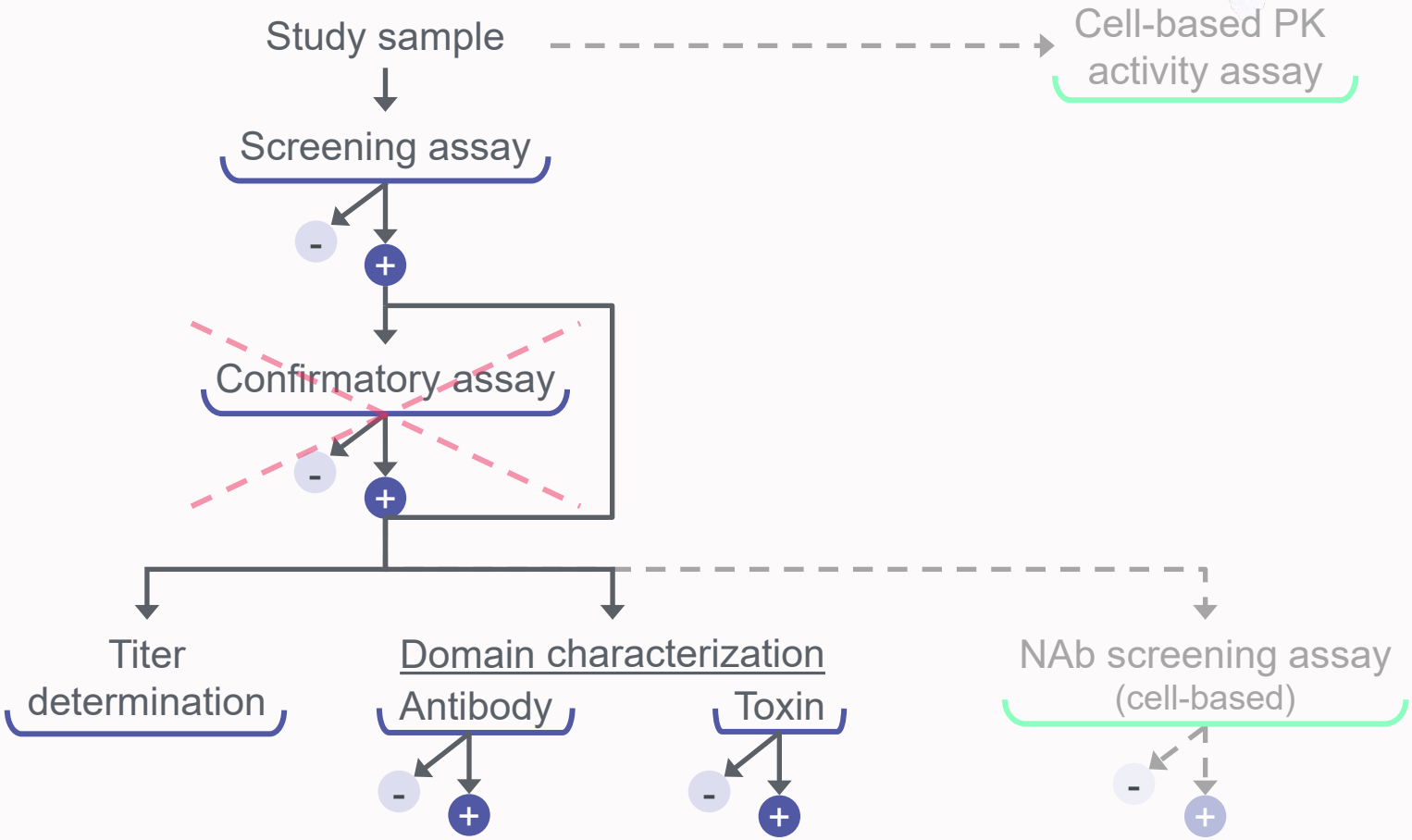
Case Study 1: Implemented cascade

Drug product

- ADC = antibody + bacterial toxin

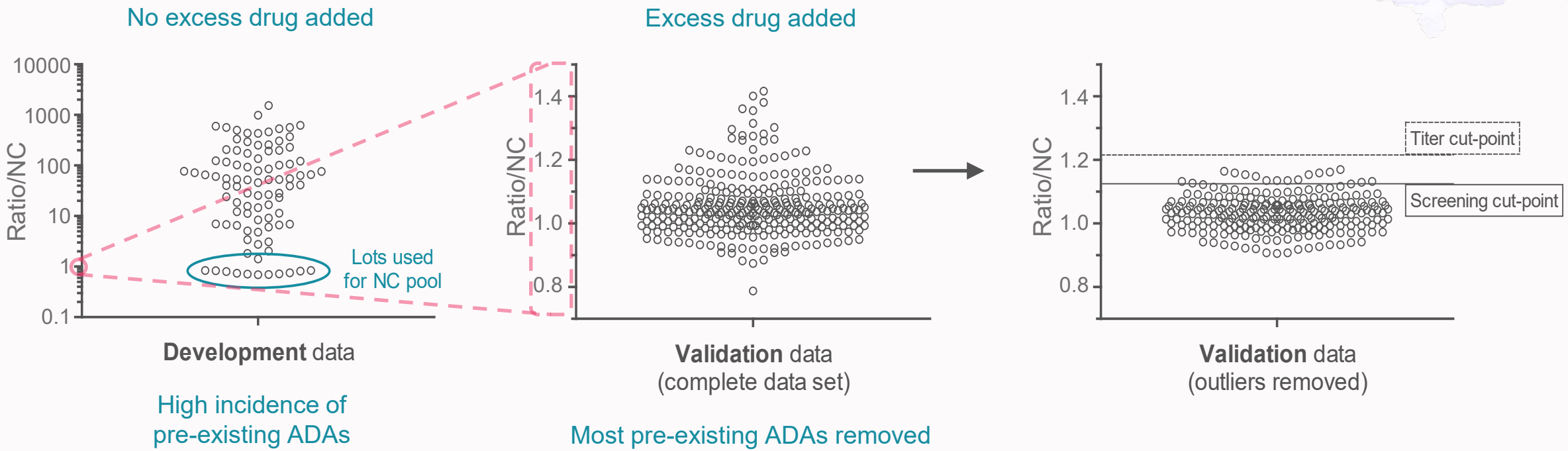
ADA assay characteristics

- Platform: MSD (ECL)
- Format: bridging + acid dissociation
- MRD: 1/40
- LOD: 23.4 ng/mL



ADC: Antibody drug conjugate; ADA: Anti-drug antibody; MSD: Meso Scale Discovery; ECL: Electrochemiluminescence; MRD: Minimum required dilution; LOD: Limit of detection; PK: Pharmacokinetic; NAb: Neutralizing antibody

Case Study 1: Cut-point determination (validation)



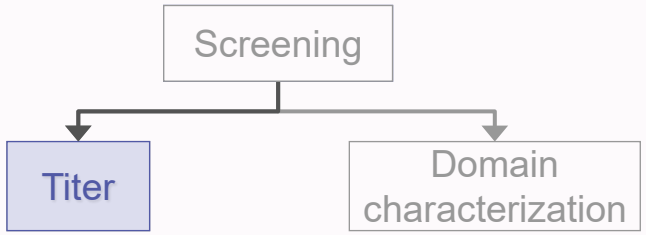
- Excess drug efficiently removes pre-existing ADAs
- Standard statistical outlier removal process was performed to calculate cut-points
- Excess drug product also added for determination of the domain characterization cut-points

NC: Negative control; ADA: Anti-drug antibody

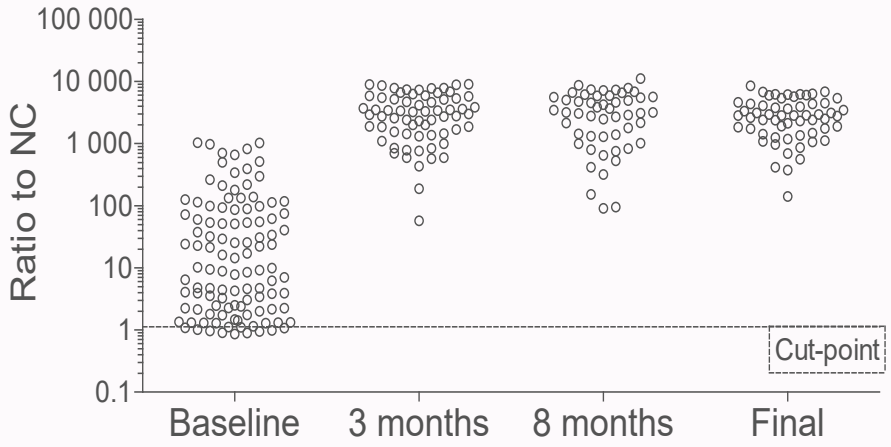
Case Study 1: Screening/titer assay results (sample analysis)

Phase 2 clinical trial

- ~300 clinical samples analyzed



Screening assay results



- Baseline: ~10% negative (similar to validation population)
- Post-dose: treatment-boosted ADAs

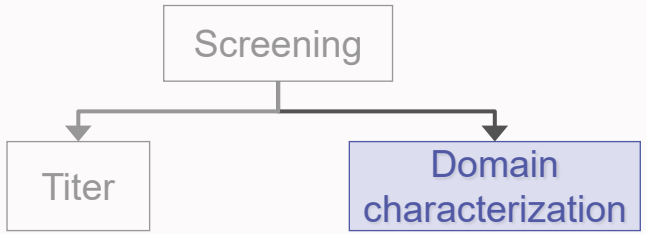
Titer assay results

- Confirmation of screening assay results

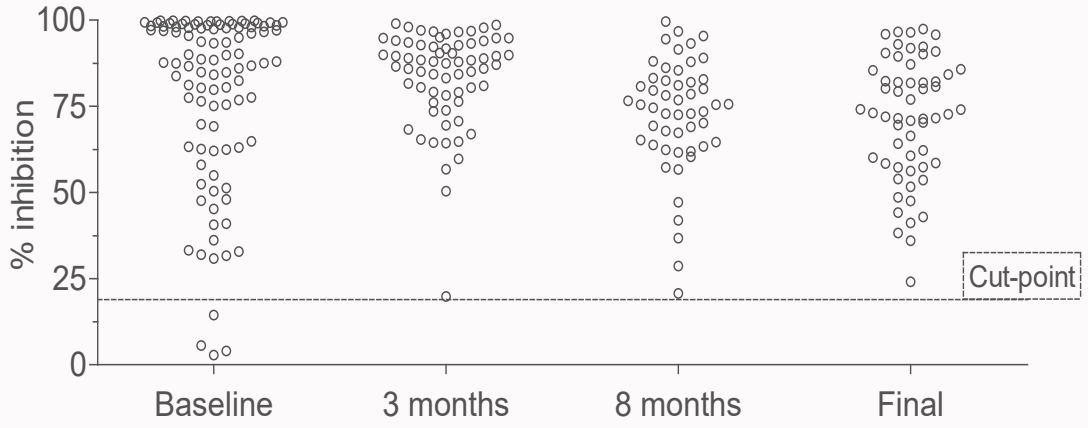
In hindsight, the screening assay tier was possibly not required for this study

NC: Negative control; ADA: Anti-drug antibody

Case Study 1: Characterization assay results (sample analysis)

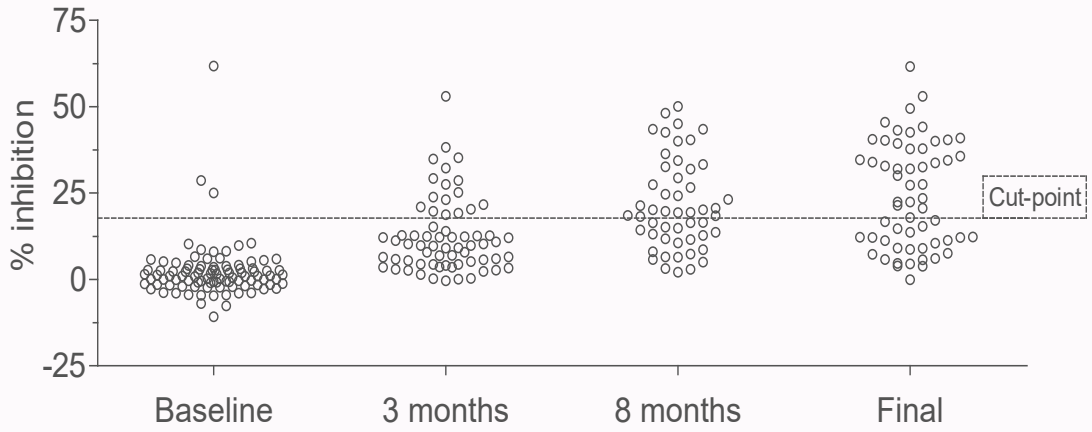


Toxin domain characterization



- Most pre-existing (baseline) antibodies are anti-toxin
- %inhibitions for toxin domain characterization are lower as time points increase → why?

Antibody domain characterization

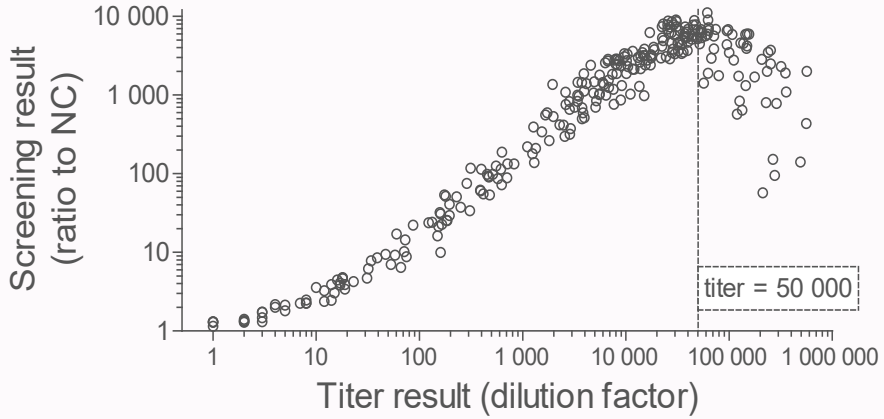


- Drug-induced antibodies directed against the antibody domain were present in many patients

All but 1 sample confirmed positive for at least one domain; confirmatory tier was not required

Case Study 1: Hook effect (sample analysis)

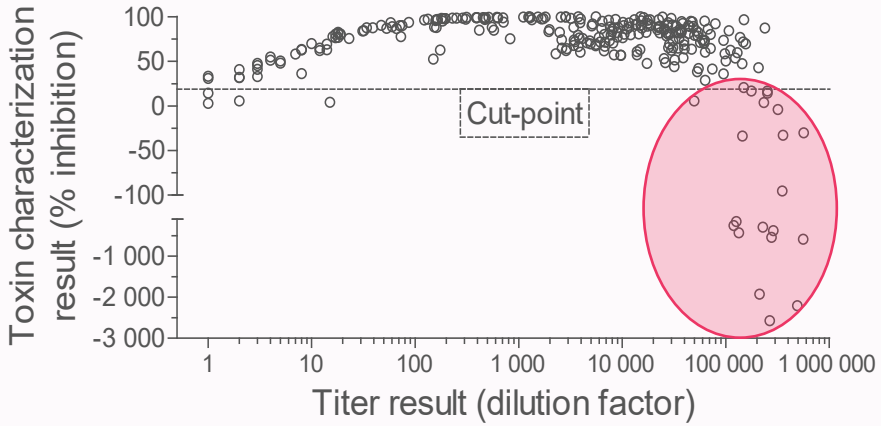
Hook effect not observed during validation, but observed during sample analysis (ECL counts up to ~200 000 vs ~1 000 000)



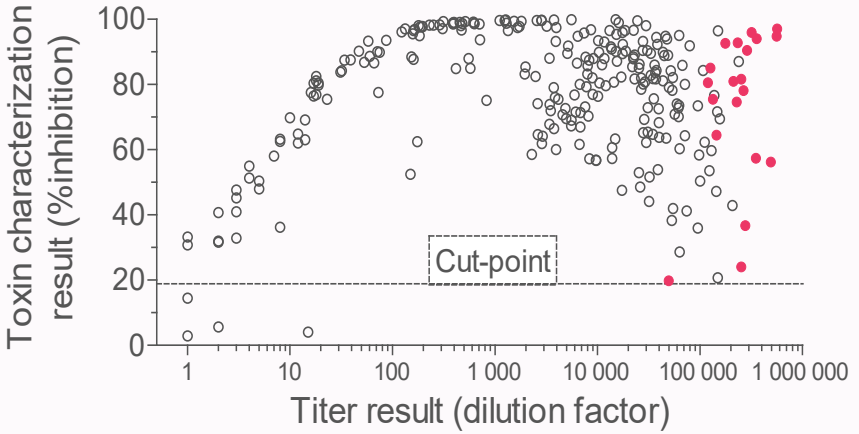
No effect on screening assay results → remained positive

Mitigation

- Samples with negative toxin characterization result and titer > 1/25 000 were retested diluted 1/1000
- 20 of 20 study samples retested in the toxin domain characterization assay went from negative to positive



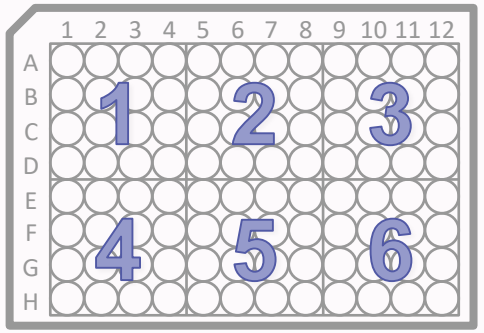
Potentially affecting characterization assay results (false-negative result?)



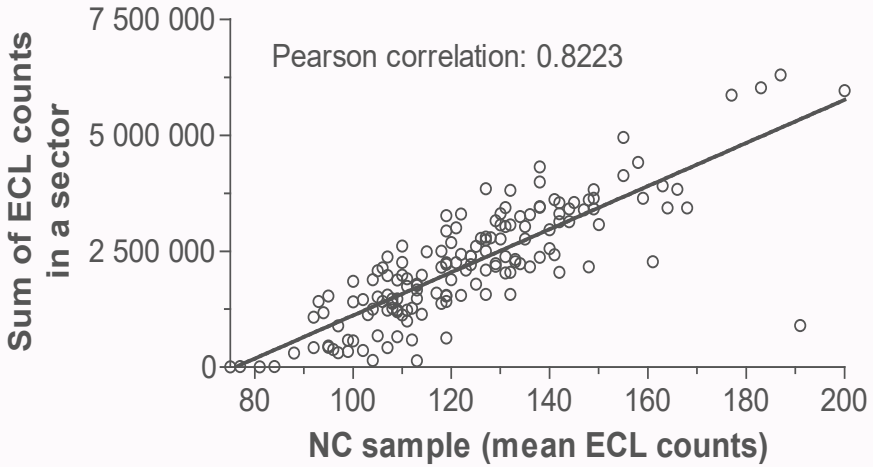
ECL: Electrochemiluminescence; NC: Negative control

Case Study 1: Signal crosstalk (sample analysis)

Plates read by sector



Crosstalk within sector observed based on NC sample signal



Effects of crosstalk

- NC samples fail threshold criteria → runs fail
- Study sample screening assay result → false-positive
- Study sample characterization assays result → false-negative
  (increased signal of sample with competitor)

Mitigation

Retested samples with low signal from sectors with high signal

Assay type	Original	Retest
Screening	21 positive	3 from positive to negative
Characterization (toxin)	20 negative	20 from negative to positive
Characterization (antibody)	80 negative	22 from negative to positive

NC: Negative control; ECL: Electrochemiluminescence

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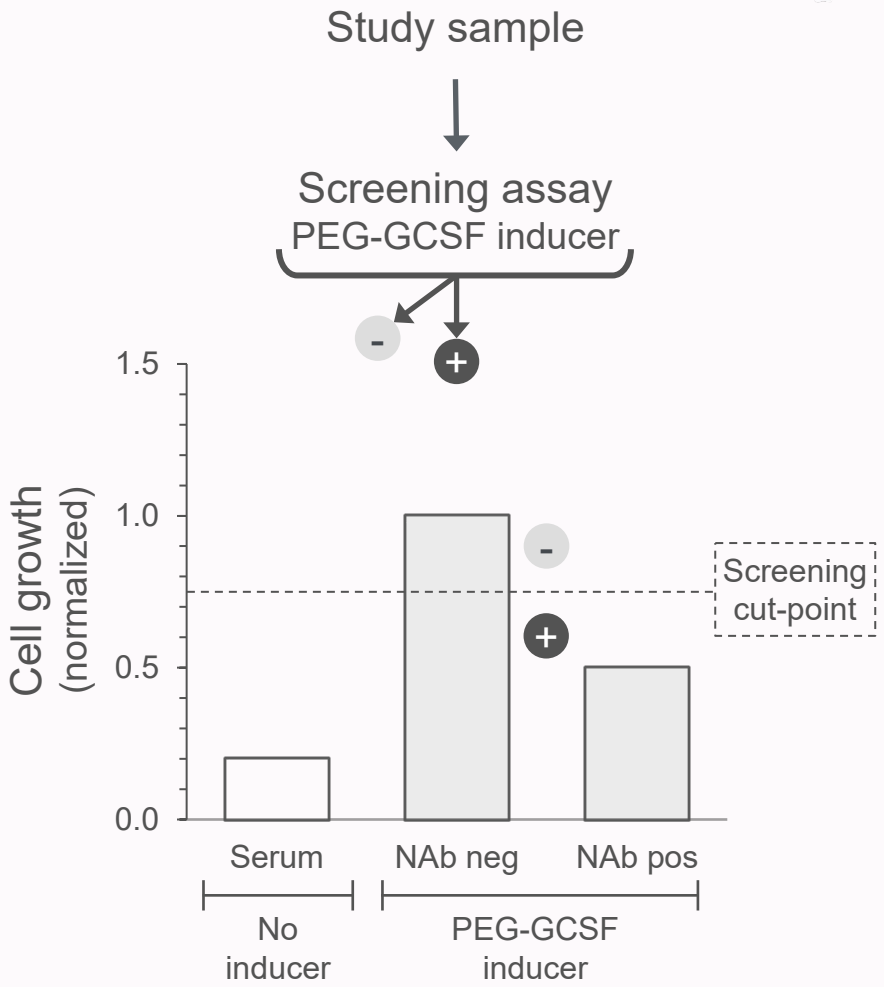
Case Study 2: NAb Assay for a PEG-GCSF Biosimilar

Standard NAb assays only include a screening tier

During development, the NFS-60 cell line was identified as an appropriate cell line for the assay based on its response to GCSF

- The cell line is also responsive to the PEG-GCSF biosimilar

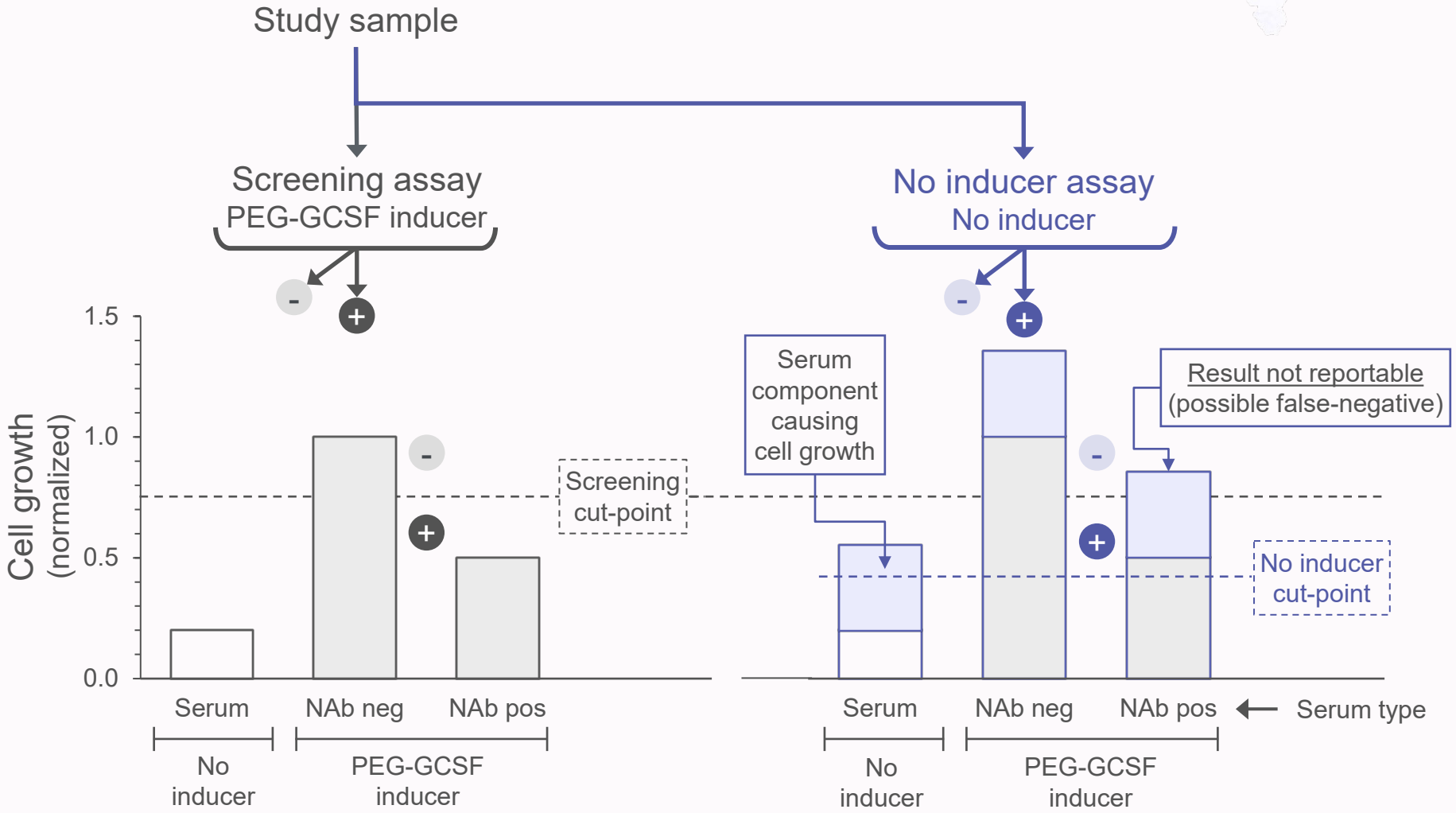


NAb: Neutralizing antibody; PEG: Polyethylene glycol; GCSF: Granulocyte colony stimulating factor

Case Study 2: “No inducer” tier

During development, some matrix components in serum (unrelated to NAbs) were identified as causing cell growth

- These components could generate false-negative results
- A “no inducer tier” was added to the testing cascade

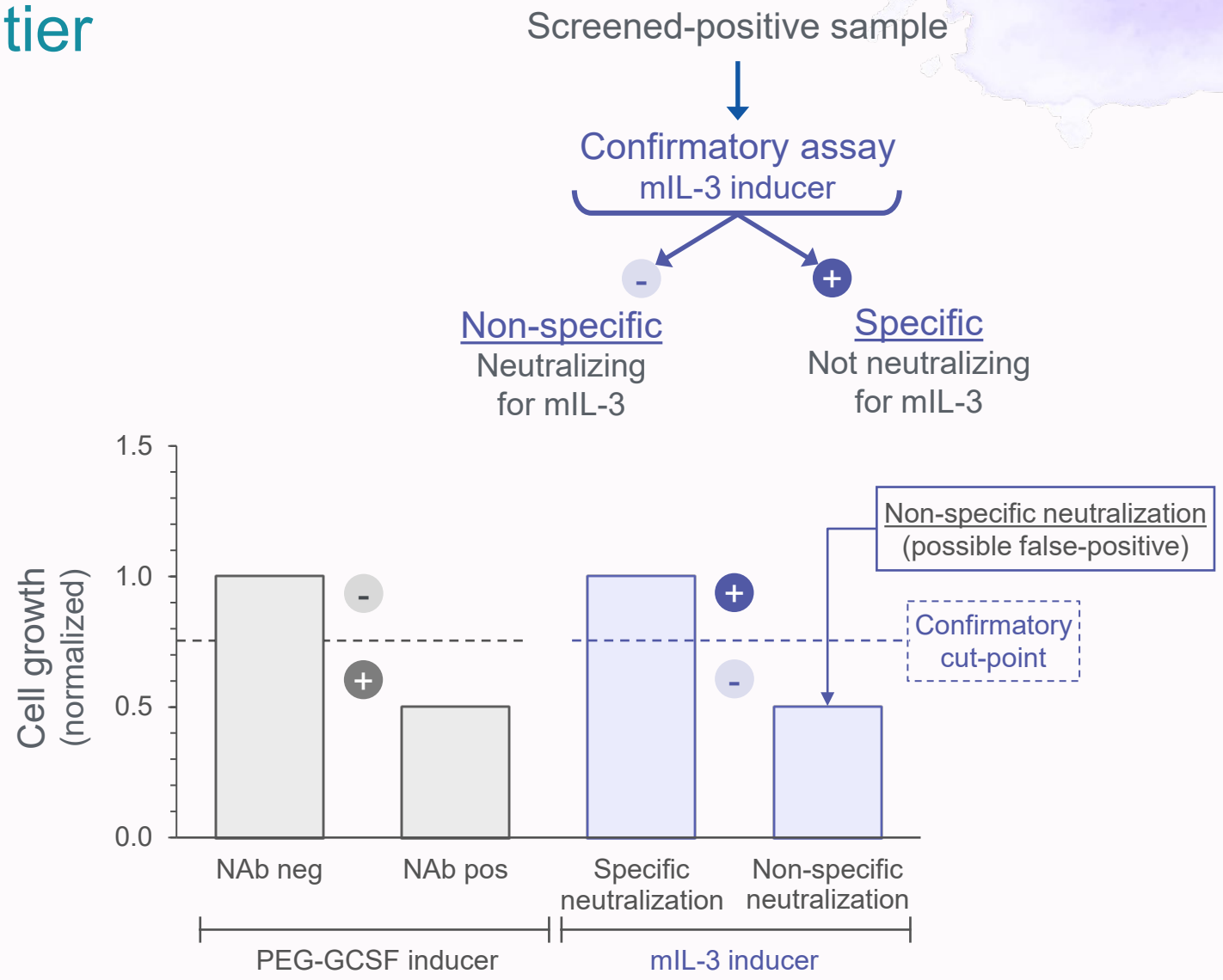


NAb: Neutralizing antibody; PEG: Polyethylene glycol; GCSF: Granulocyte colony stimulating factor; neg: Negative; pos: Positive

Case Study 2: Confirmatory tier

The NFS-60 cell line can also proliferate in the presence of mIL-3 (in addition to GCSF)

- This property allows an evaluation of the specificity of an observed positive screening result
- A non-specific neutralization can generate false-positive results
- A confirmatory tier was added to the testing cascade

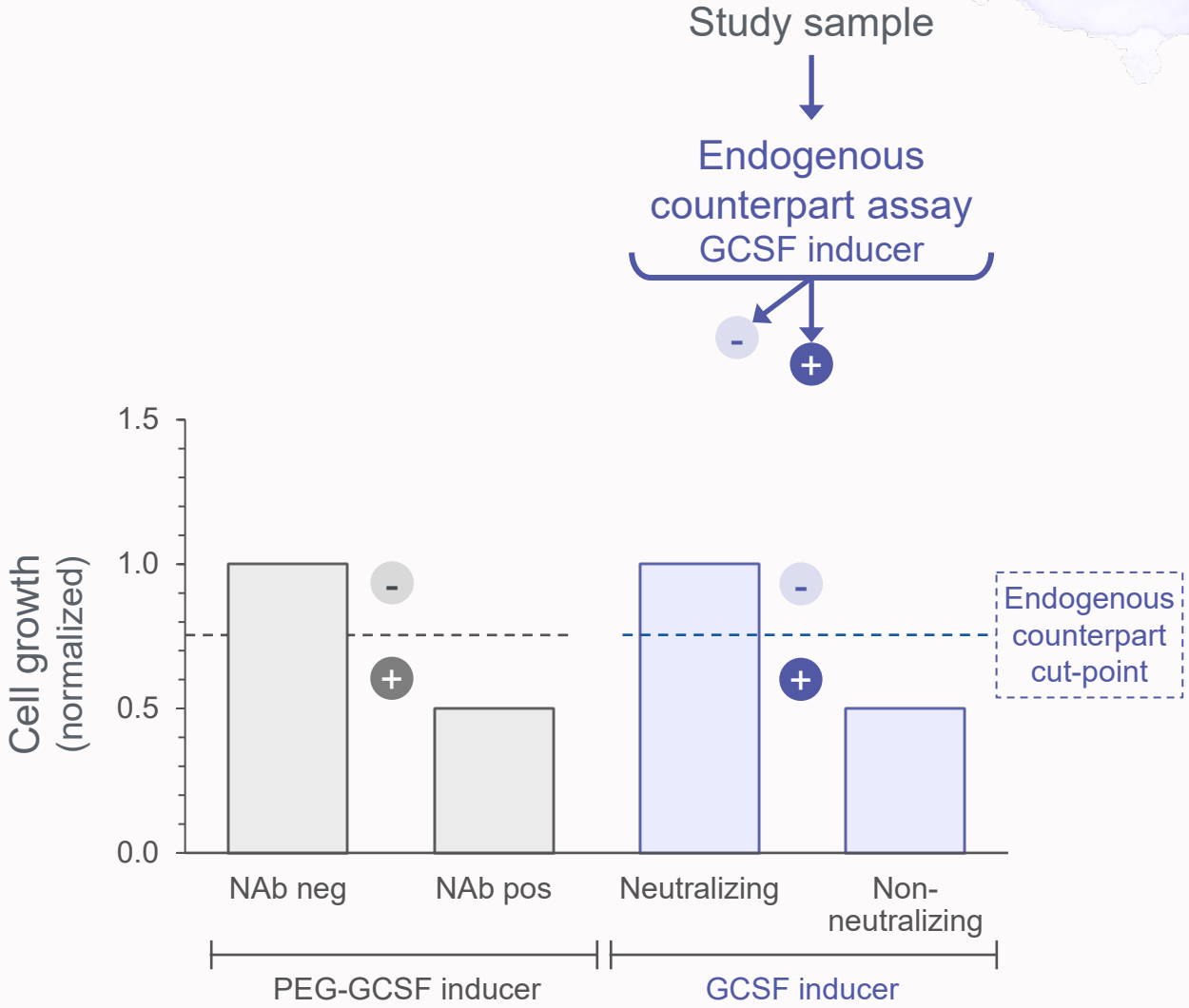


mIL-3: Mouse interleukin 3; GCSF: Granulocyte colony stimulating factor; NAb: Neutralizing antibody; neg: Negative; pos: Positive

Case Study 2: Endogenous counterpart tier

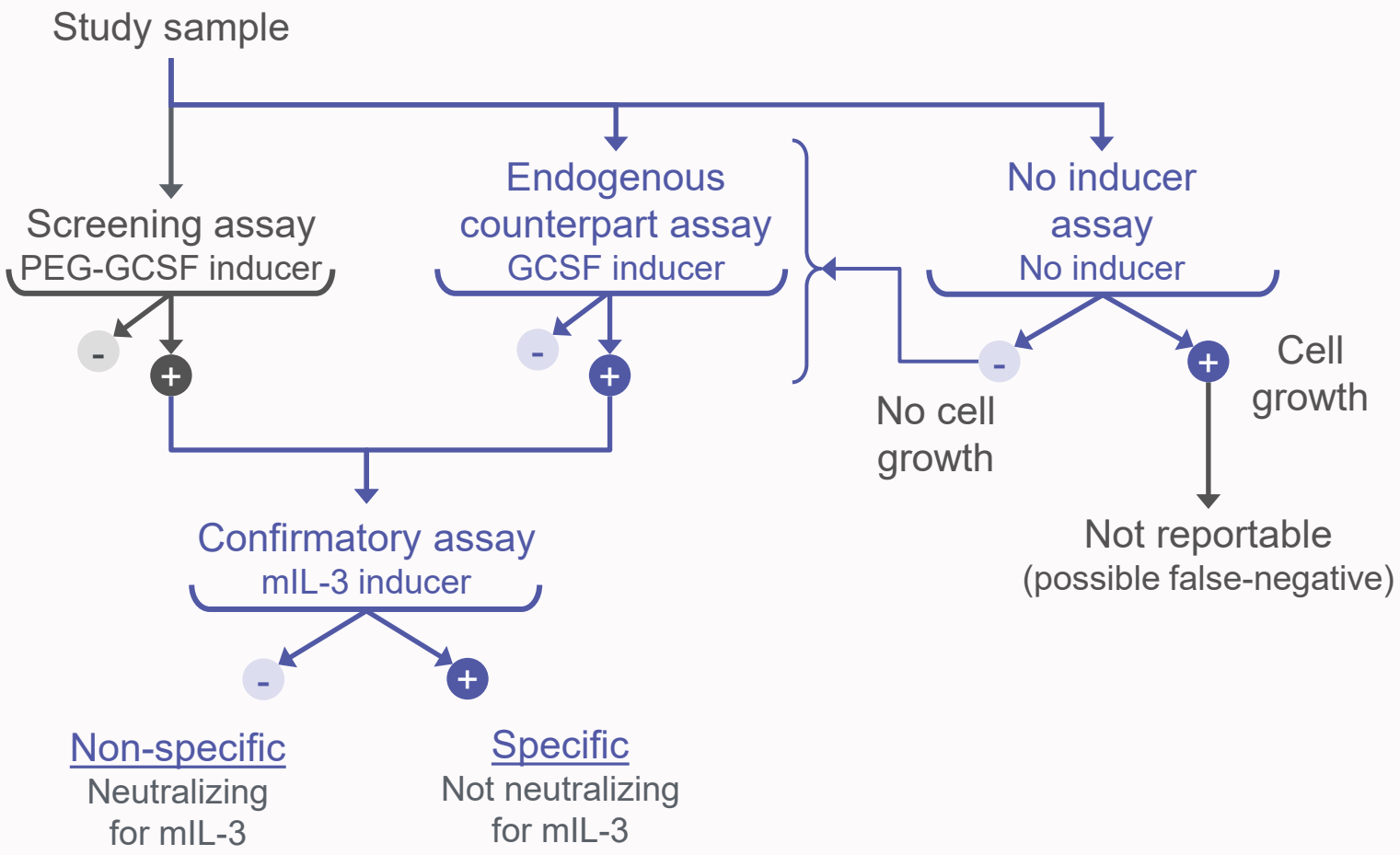
NAbs which neutralize the drug product (PEG-GCSF) might also neutralize endogenous GCSF

- Safety issue
- An endogenous counterpart tier was added to the testing cascade



NAb: Neutralizing antibody; PEG: Polyethylene glycol; GCSF: Granulocyte colony stimulating factor; neg: Negative; pos: Positive

Case Study 2: Final cascade and results summary



Summary of results for this NAb assay cascade over multiple studies

- ~400 samples tested in NAb assay
- 2% positive in No inducer assay (not reportable)
- 12% screened positive. Of which:
  - 32% neutralized mIL-3 (non-specific)
  - 13% also neutralized endogenous GCSF

PEG: Polyethylene glycol; GCSF: Granulocyte colony stimulating factor; mIL-3: Mouse interleukin 3; NAb: Neutralizing antibody

Conclusions

ADA assays

- Adapt cascades depending on drug product (e.g. domain characterization, endogenous cross-reactivity)
- New challenges can emerge during sample analysis → immediate mitigation

NAb assays

- Designed based on mechanism of action of the drug product
- Reagents and cell lines need to be characterized and well understood
- Cascades may need more than a screening tier

Cerba Research

ADA: Anti-drug antibody; NAb: Neutralizing antibody

Thank you!

Cerba Research Canada

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