The Immunogenicity Nexus:

The Predictive Relationship Between Early-Onset ADA Kinetics in NHP and Human Immune Responses

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Contents

Introduction

- It is common sense that the observed immunogenicity of therapeutic proteins in non-human primates (NHP) is not predictive for anti-drug antibody (ADA) formation in humans (“non translatable“)
- This is generally true, especially if the observed immunogenicity in NHPs is caused by the classical foreign recognition of a human protein by the monkey immune system
- However, if the immunogenicity in NHPs is rather triggered by the presence of extrinsic factors (such as impurities, aggregates, drug-target complexes), ADA formation in NHPs might translate into the human situation

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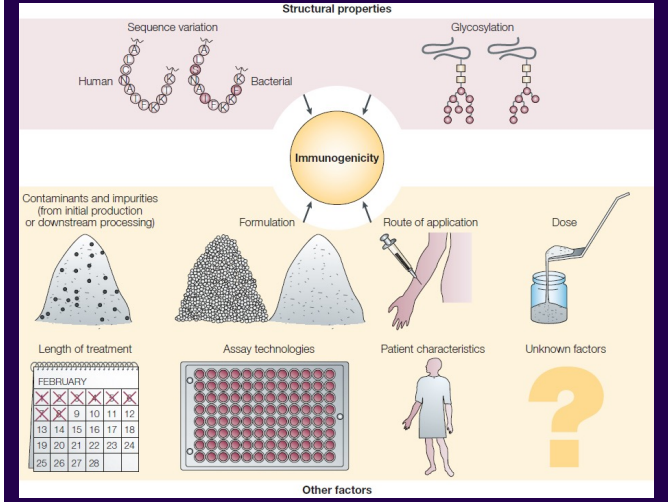
Introduction

Immunogenicity of Therapeutic Proteins



Schellekens, H. Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov 1, 457–462 (2002). https://doi.org/10.1038/nrd818

Various intrinsic and extrinsic factors can affect the propensity of a therapeutic protein to stimulate an immune response

INTRINSIC FACTORS:

- Presence of foreign T- and B-cell epitopes in therapeutic proteins

EXTRINSIC FACTORS:

- Posology related factors
  - Route of administration
  - Treatment regimen
  - Dose
- Patient related factors
  - Genetic variation of MHC-II, T-cell receptor, cytokines and cytokine receptors
  - Level of immune tolerance to therapeutic protein
- Product quality related factors
  - Protein degradation
  - Impurities
  - Aggregates
  - Drug/target complexes

Introduction

Immunogenicity of Therapeutic Proteins

INTRINSIC IMMUNOGENICITY

Primary factor: Amino Acid (AA) sequence

Mechanism:

- "Foreigness" of human(ized) compounds triggers the adaptive immune system in NHP
- T cell-dependent B cell response
- Timeline: detected after 3-4 weeks (IgG antibodies)

EXTRINSIC FACTORS

Examples: impurities, aggregates, drug-target complex formation

Mechanism:

- Trigger innate immune system ("danger signals") → early humoral immune response
- Timeline: detected earlier (early isotype switch)

WORKING HYPOTHESIS

Early onset immune responses in NHP

→ May indicate extrinsic factor-driven immunogenicity

→ potential translatability to humans

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Hypothesis testing

Retrospective analysis of biologic drug compounds

STUDY DESIGN

- Data sources: Non-clinical and clinical (Phase I/II data)
- Subject type: Healthy subjects
  - Exception: Disease population for oncology compounds
- Compound: various biological drug compounds/modalities with the exception of gene therapy products

SELECTION CRITERIA

- Timepoint requirement: early timepoint availability (**Day 15**)
- Initial approach:
  - Single administration studies only

⚠ *Challenge: For a lot of studies, no GLP-tox data*

DATASET

- Initial approach → expanded approach
- Single dose studies → Multiple dose studies included

→ Current dataset with **14 compounds**

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Hypothesis testing

Retrospective analysis of biologic drug compounds

The following information was captured, if applicable:

Healthy versus disease state

Mode of Action

Therapeutic area

Disease indication class

Route of administration

Reuse of animals

Compound

Modality

IRA classification

ADA incidence at Day 15

Target

Impact of ADA on PK, PD and safety

Note: dosing information was not included to avoid overcomplicating the exercise.

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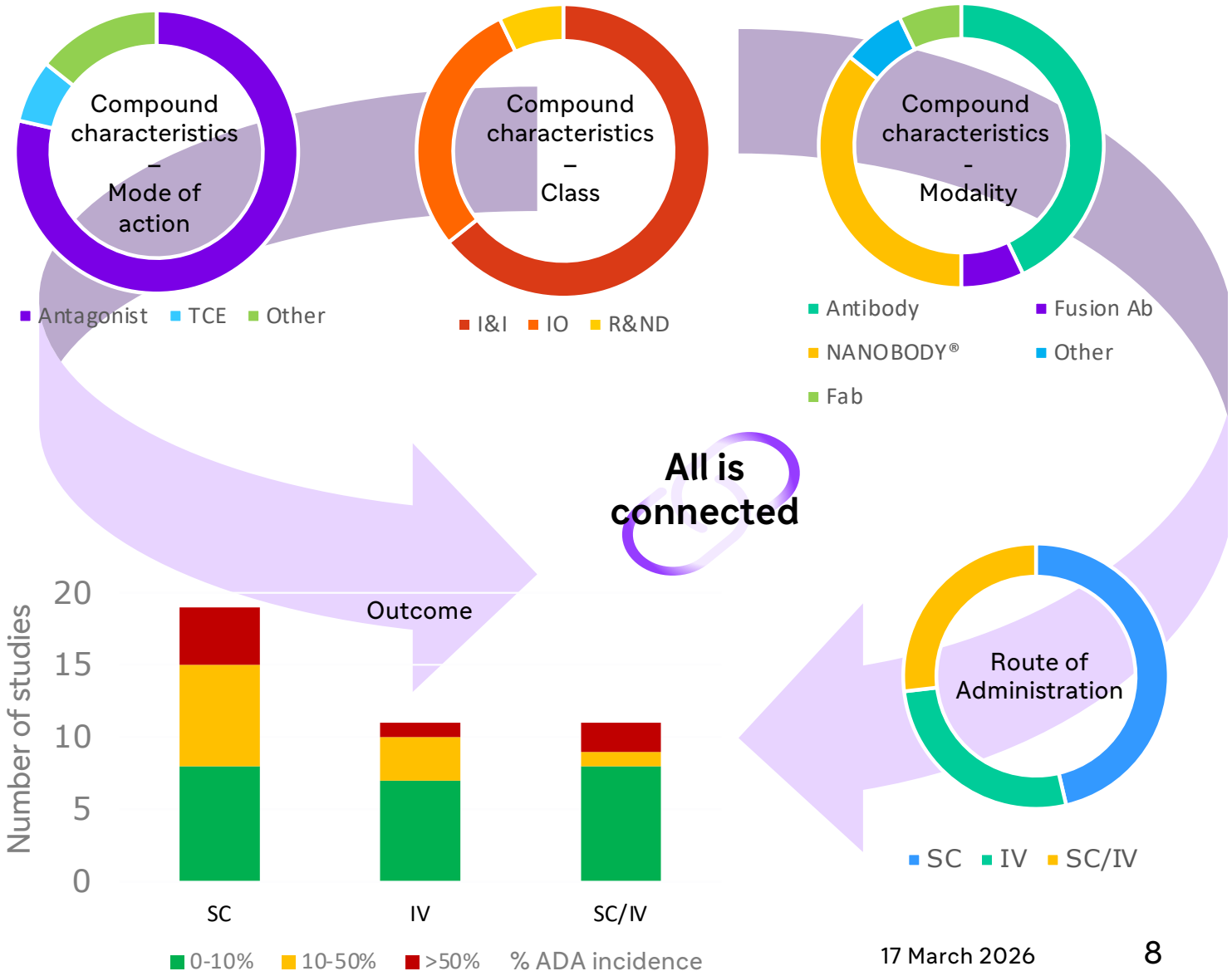
Hypothesis testing

Retrospective analysis of biologic drug compounds



Abbreviations:

TCE: T-cell engager
I&I: Inflammation & Immunology
IO: Immuno-oncology
R&ND: Rare & Neurological diseases

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Hypothesis testing

Retrospective analysis of biologic drug compounds

KEY PRINCIPLES

STUDY TYPE	METHOD	CUT-OFF	POPULATION
Non-clinical	S/N ratio	1000 ng/mL PC	Treated animals
Clinical	CSR data-ADA incidence	Treatment boosted/ induced ADA POS	Treated subjects

Abbreviations:

S/N: signal to noise
CSR: clinical study report
PC: positive control

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APPROACH FOR CALCULATING ADA INCIDENCE AT DAY 15

NON-CLINICAL STUDIES

- Sample classification method
  - ADA positive assignment: based on S/N ratio
  - Cut-off strategy: S/N at 1000 ng/mL PC used to exclude low positives (minimal required sensitivity for non-clinical studies[1])
  - Population: compound-treated animals only (excluding vehicle groups)

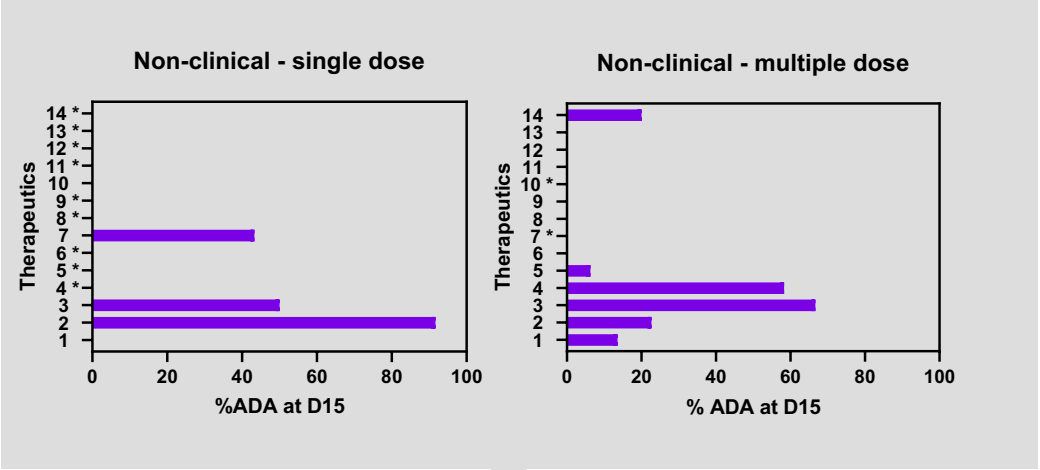
CLINICAL STUDIES

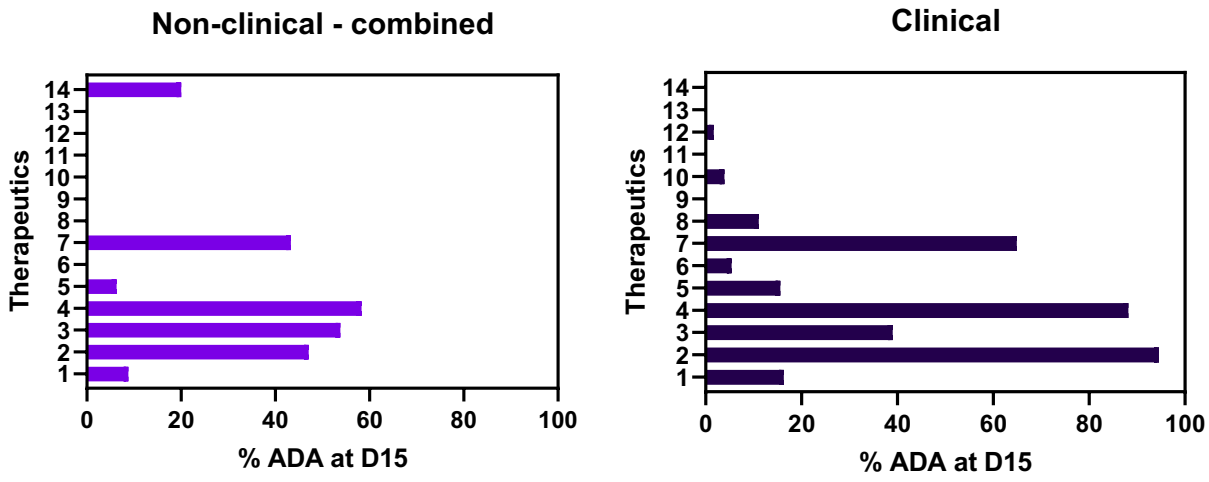
- Primary source: clinical study report
- Timepoint: Day 15 ADA incidence data
- Population: compound-treated subjects only (excluding placebo)

1: Mire-Sluis, A. R., Y. C. Barrett, et al. (2004). "Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products." J Immunol Methods 289(1-2): 1- 16

Pilot study - results

NHP (non-clinical) versus clinical ADA incidence at day 15



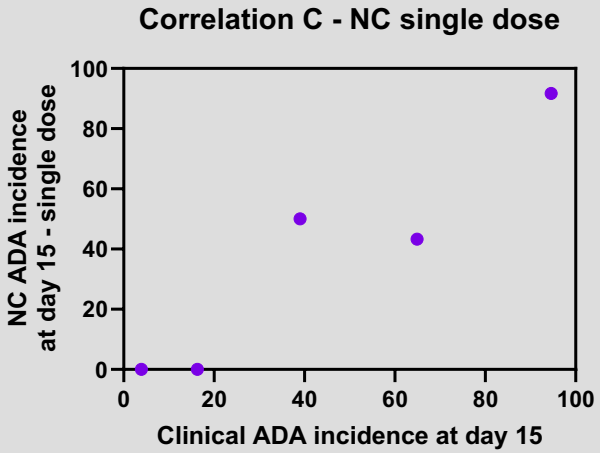


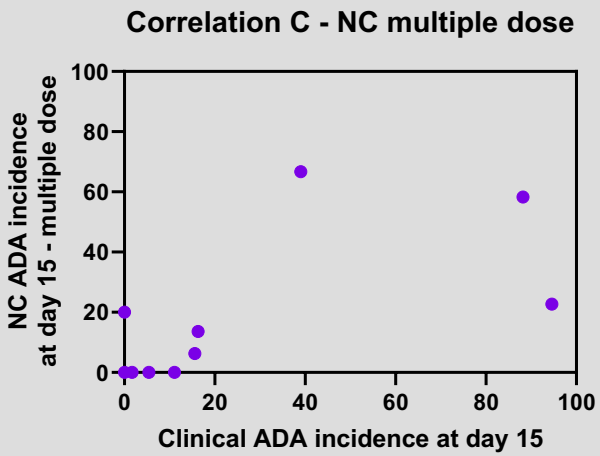
**no data available for this compound in the indicated dosing regimen*

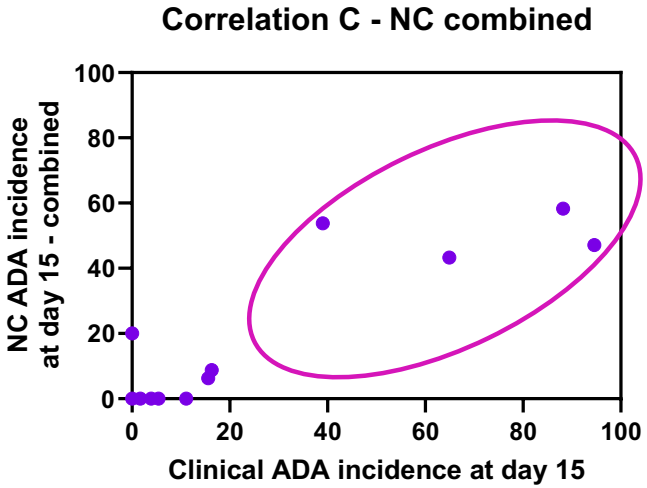
Combined: both single dose and multiple dose studies are included

Pilot study - results

Correlation clinical vs non-clinical ADA incidence at Day 15







→ The plots, although preliminary, suggest a possible correlation that points to the potential predictive value of early immunogenicity (i.e. above arbitrary LPC of 1000 ng/mL) in NHP studies towards clinical immunogenicity.

HOWEVER, exploration of the current dataset is required!!!

Abbreviations:
NC: non-clinical
C: clinical

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Example drug/target complexes

Single dose PK study

KEY PRINCIPLES

STUDY TYPE	METHOD	CUT-OFF	POPULATION
Non-clinical	S/N ratio	1000 ng/mL PC	Treated animals
Clinical	CSR data-ADA incidence	Treatment boosted/ induced ADA POS	Treated subjects

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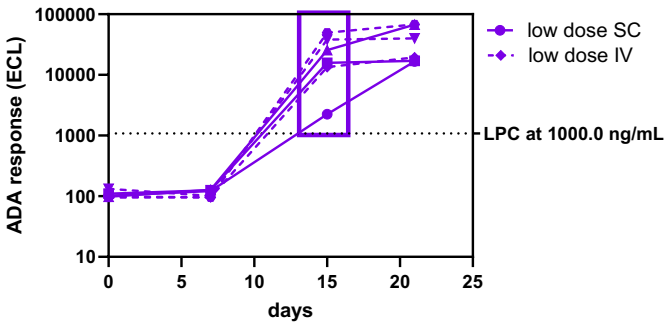
ADA

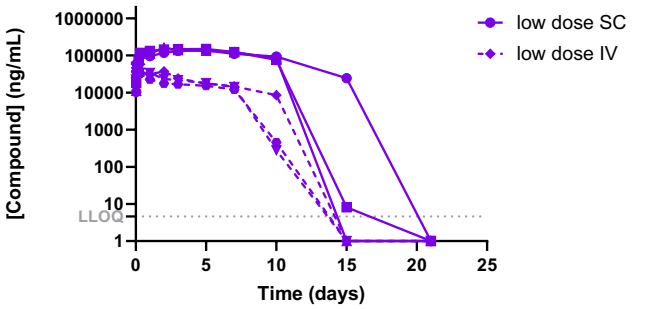


- ADA incidence at Day 15 in low dose groups: 100%
- ADA incidence at Day 15 across all dose groups: 91.7%

Safety

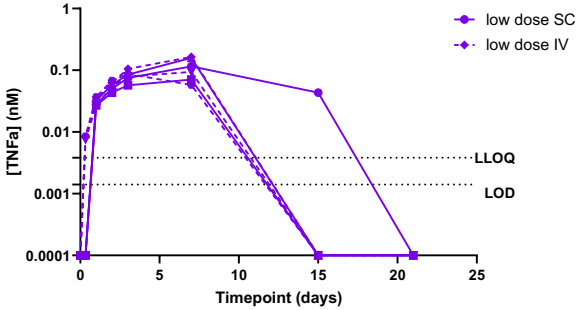
- No impact of ADA on safety

PK



- Significant impact of ADA on exposure

Total TNFα



- Significant effect of ADA on total TNFα concentration in low dose groups

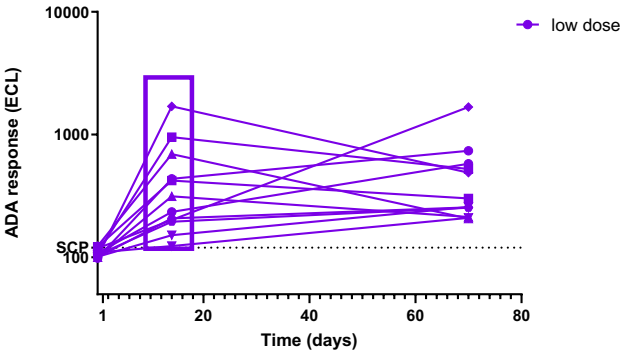
Example drug/target complexes

Phase 1 study (single dose)

KEY PRINCIPLES

STUDY TYPE	METHOD	CUT-OFF	POPULATION
Non-clinical	S/N ratio	1000 ng/mL PC	Treated animals
Clinical	CSR data-ADA incidence	Treatment boosted/ induced ADA POS	Treated subjects

ADA

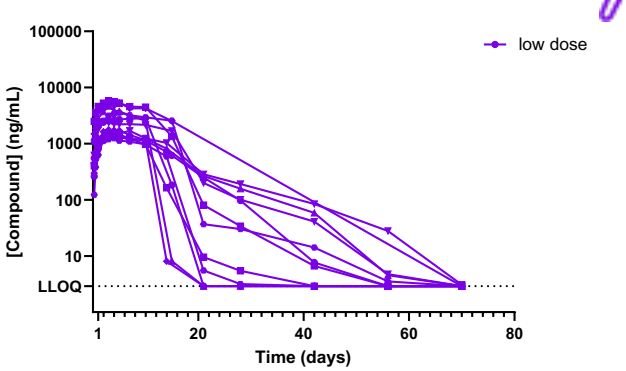


- ADA incidence at Day 15 in low dose groups: 100%
- ADA incidence at Day 15 across all dose groups: 96.6%
- Phase 1 study discontinued

Safety

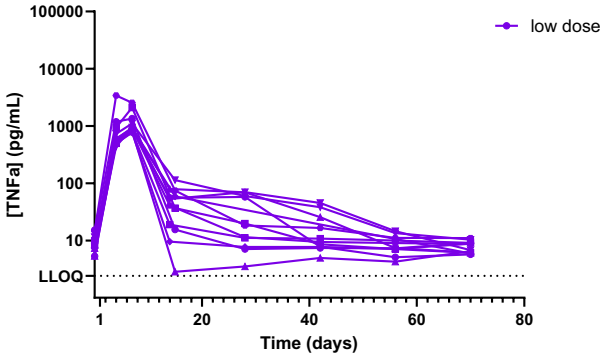
- No impact of ADA on safety

PK



- Significant impact of ADA on exposure

Total TNFα



- Significant impact of ADA on TNFα concentration

Example drug/target complexes

Compound targeting TNFα and X

- TNFα is individually a well validated target
- Dual targeting has been largely unsuccessful due to high immunogenicity impairing exposure
- Our hypothesis is that this is likely due to TNFα-driven drug/target multimerization
  - Bispecific drug & homotrimeric target (TNFα)

Examples of bispecific anti-TNF compounds suffering from immunogenicity:

- *ABT-122 (TNFα x IL17A): All subjects ADA positive in SAD from day 15 onwards*
- *AMG-966 (TNFα x TL1A): All treated subjects in SAD and all (except of one) in MAD tested ADA positive (onset on day 15 in the majority of subjects). An increase in ADA magnitude correlated with loss of AMG 966 exposure*
- *ABBV-257 (TNFα x IL17A): All subjects ADA+ from Day 15, impact on exposure in 50%*
- *COVA-322 (TNFα x IL17A): All subjects in SAD tested ADA positive (most already in week 2). Accelerated clearance starting in week 2 correlating with ADA magnitude*

Considerations and next steps

CONSIDERATIONS

- Evolving landscape: The bioanalytical field is shifting toward reduced non-clinical ADA analysis. However, more ADA analysis might be warranted for:
  - Expanding our knowledge on this hypothesis could significantly improve lifecycle management efficiency for certain biotherapeutics
  - 3R principle - animal reuse: may affect ADA outcomes – always verify prior compound exposure before study initiation

NEXT STEPS IN SUPPORT OF OUR HYPOTHESIS

- Expand the pilot study: strengthen and further explore the hypothesis
  - Interested in contributing? Please contact us!
- Explore the IDC: leverage the Immunogenicity Database Collaborative for additional insights
- Define clear guidance: establish early blood sampling timepoints for immunogenicity assessments in non-clinical NHP and clinical studies

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Take home message

Should we be ‘alerted’ by early immunogenicity with early immune responses in NHP studies?

Importance of early (D15) timepoint!

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Griet Conickx
Brendy Van Butsel
Ortwin Van de Vyver

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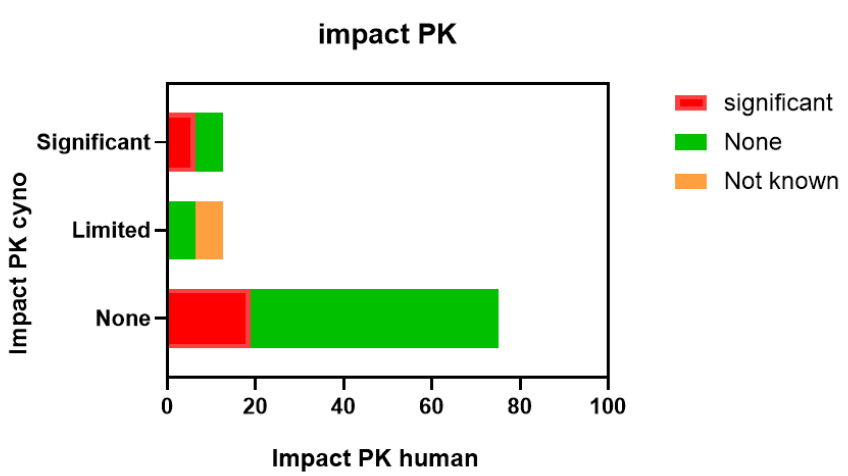
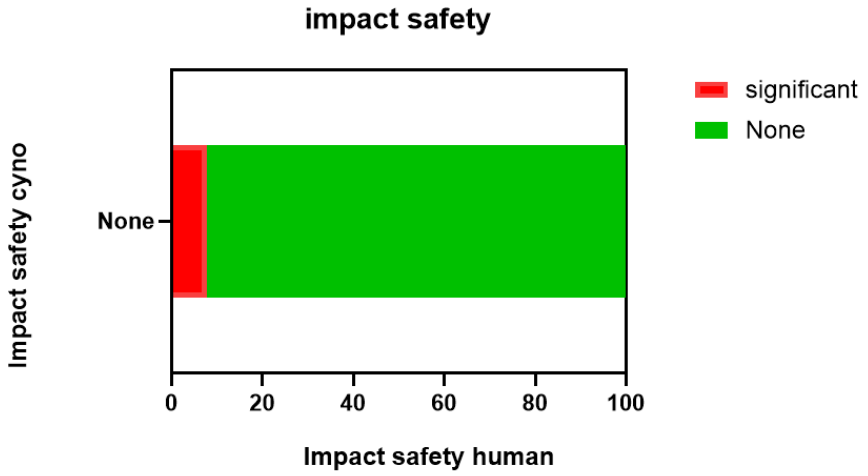
Impact of ADA on PK, PD and safety





Example drug/target complexes

Single dose PK study

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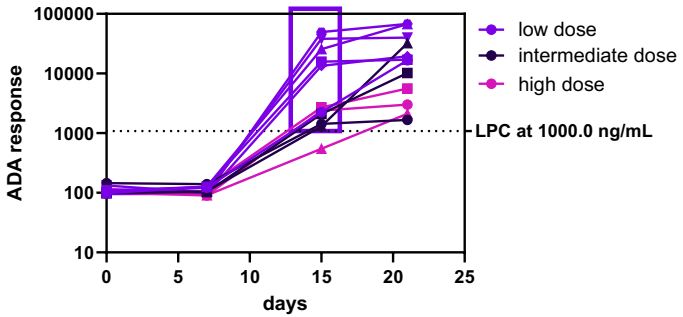
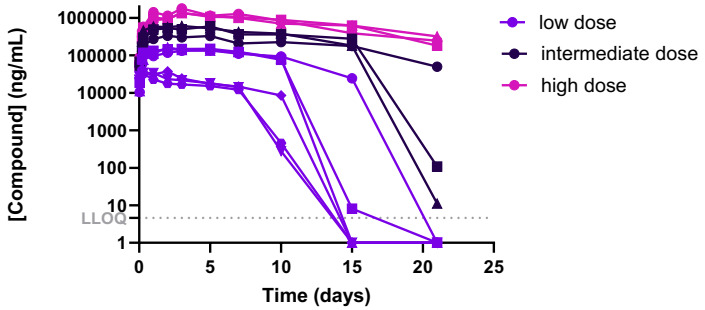
ADA



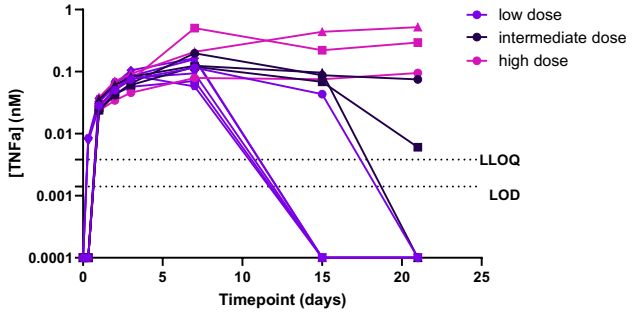
- ADA magnitude decreased with higher administered doses
- ADA incidence at Day 15
  - Using SCP: 100%
  - Using LPC at 1000.0 ng/mL: 91.7%

PK



- Significant impact of ADA on exposure in low dose groups

Total TNFα



- TNFα (target) accumulated in vivo → target engagement
- Significant effect of ADA on total TNFα concentration in low dose groups

Example drug/target complexes

Phase 1 study (single dose)

KEY PRINCIPLES

STUDY TYPE	METHOD	CUT-OFF	POPULATION
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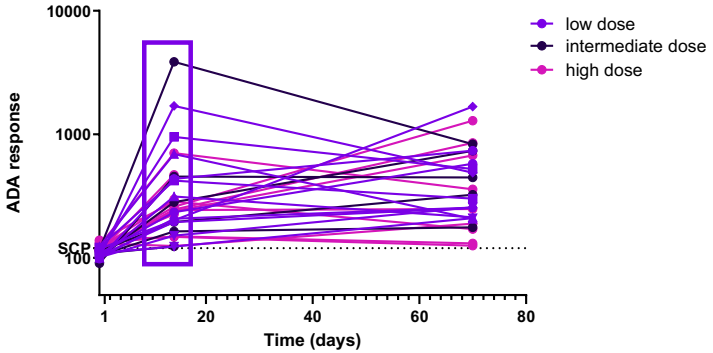
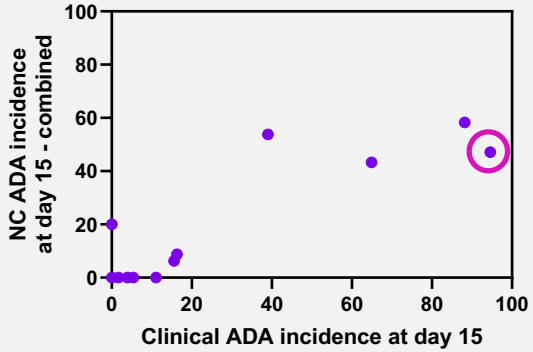
ADA



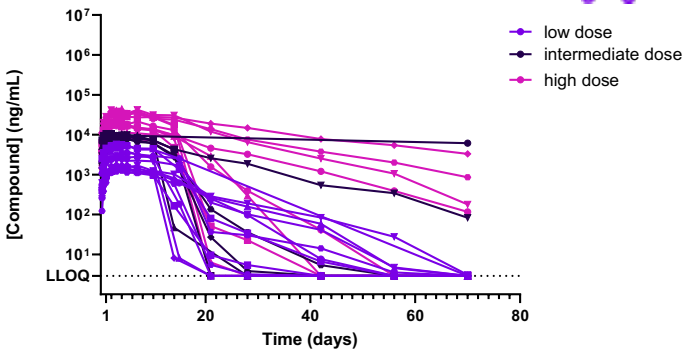
- ADA incidence at Day 15: 93.3%
- Phase 1 study discontinued

Safety

- No impact of ADA on safety

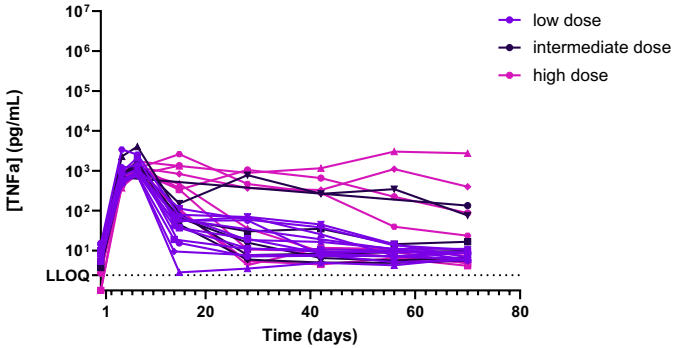


PK



- Significant impact of ADA on exposure

Total TNFα



- Significant impact of ADA on TNFα concentration