# Life Cycle Management of ADA and NAb assays during Clinical Development of a Monoclonal Antibody with Focus on Drug Tolerance Improvement – Nice to Have or Must Have?

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# Overview

- MAb – patient population A **and B**
- Population A:
  - ADA assay versions: 1 to 4
  - NAb assay version 1
- **Population B** (focus of this presentation):
  - ADA assay versions: 5 to 7
  - NAb assay version 2 and 3
- Many assay versions:
  - Improvement of drug tolerance – why and how?
  - “Nice to” or “must” have?

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# Population A, ADA assay version history

| Phase | ADA Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 1** | Version 1 | **1.0 µg/mL** | HPC at **≥5 µg/ml** drug | • Bridging assay<br>• Capture: drug coated on 96-well plate<br>• Detection: biotin-labelled drug + HRP-labelled Streptavidin + TMB |
| **Phase 2** | Version 2 (changed format) | **644 ng/mL** | 488 ng/ml PC at 1 µg/ml drug<br>1,953 ng/ml PC at **10 µg/ml** drug | • Bridging assay<br>• Capture: biotin-labelled drug captured on streptavidin coated plate<br>• Detection: Rh-(S-tag) drug<br>• 1 acid dissociation step |
| **Phase 2** | Version 3<br>(version 2 optimized) | **150 ng/mL** | 250 ng/ml PC at **2.5 µg/ml** drug | |
| **Phase 2/ Phase 3** | Version 4 (transfer to CRO and further optimization) | **76 ng/mL** | 150 ng/ml PC at **10 µg/ml** drug | |

# Nab assay, Population A

| Phase | Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 3, Population A** | Version 1 | **195 ng/mL** | 400 ng/ml PC at **0.08** µg/ml drug | • Binding assay,<br>• Capture (of labelled drug): Commercial Mab + Antigen (target)<br>• Detection: Labelled drug<br>• 1 acid dissociation step |

# Population A – assay versions

- Only ADA assay versions 3 and 4 met current regulatory requirements → optimizations of versions 1 and 2 appeared to have been a “must have”

- Nab assay version 1:
  - very poor Drug Tolerance (0.08 µg/mL)

# Population B, ADA assay version history

| Phase | Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 2** | Version 4 | **76 ng/ml** | 150 ng/ml PC at **10 µg/ml drug** | • Bridging assay<br>• Capture: biotin-labelled drug added to streptavidin coated plate<br>• Detection: s-tag drug<br>• 1 acid dissociation step |
| **Intended for Phase 3** | **Version 5 (new critical reagents + validation in new population)** | **150 ng/ml** | 150 ng/ml PC at **5 µg/ml drug** | |

# Considerations on Clinical Development

**Question from Project team:**

- *"Is it possible to improve Drug Tolerance of the ADA assay?"*
- Scope:
  - If the drug tolerance could be improved substantially, a long (16-week) safety follow up might not be needed for Phase 3
  - Direct transfer into a long-term extension trial could be allowed
  - Benefit for the patients: no off-treatment period and thus no transient reduction in treatment effect
- Answer: *"we will give it a try!"*

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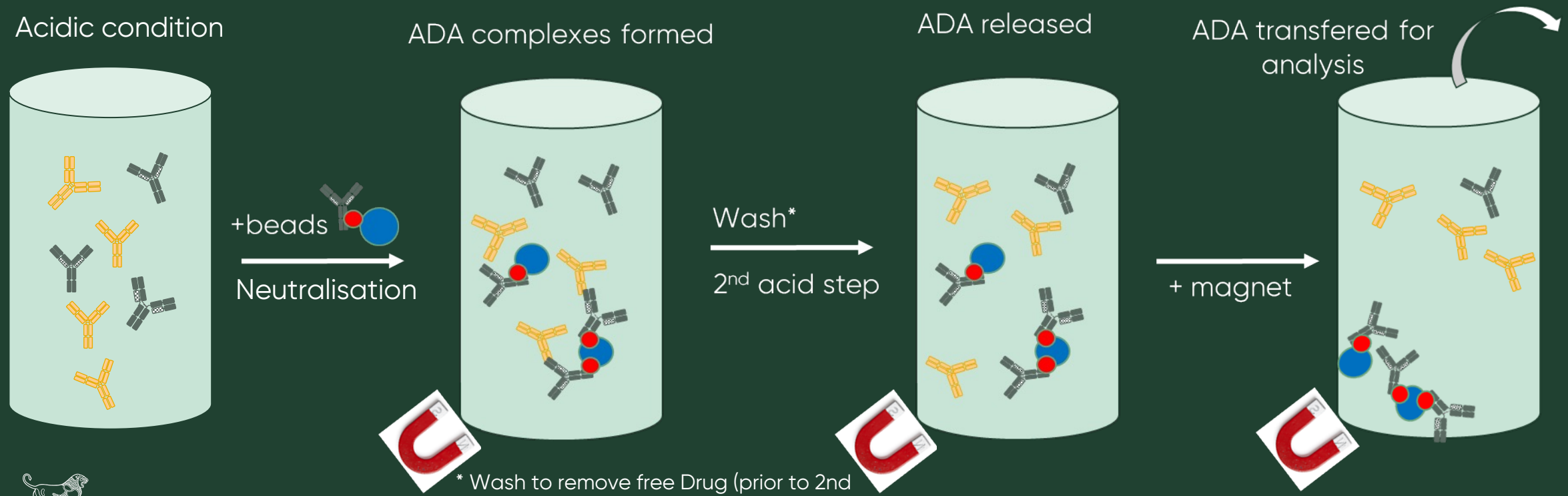
# Bead Extraction Step in ADA Assays

Drug ADA Streptavidine coated magnetic beads with biotin-Drug




* Wash to remove free Drug (prior to 2nd acid step) while ADA Complexes trapped with a magnet

# Population B, ADA assay version history

| Phase | ADA Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 2** | Version 4 | **76 ng/ml** | 150 ng/ml PC at **10 µg/ml drug** | • Bridging assay;<br>• Capture: biotin-labelled drug added to streptavidin coated plate<br>• Detection: s-tag drug<br>• 1 acid dissociation step |
| **Intended for Phase 3** | Version 5 (new critical reagents + validation in new population) | **150 ng/ml** | 150 ng/ml PC at **5 µg/ml drug** | |
| **Phase 3** | **Version 6 – introduction of bead extraction** | **50 ng/ml** | **50 ng/ml PC at 200 µg/ml drug**<br>**20 – 40 fold improvement** | • Bridging assay format;<br>• Capture: biotin-labelled drug added to streptavidin coated magnetic beads<br>• Detection: s-tag drug<br>• 2 acid dissociation steps |

# Nab assay version history

| Phase | NAb Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 3, Population A** | Version 1 | **195 ng/mL** | 400 ng/ml PC at **0.08** µg/ml drug | • Binding assay,<br>• Capture (of labelled drug): Commercial Mab + Antigen (target)<br>• Detection: Labelled drug<br>• 1 acid dissociation step |
| **Intended for Phase 3, Population B** | **Version 2 (validation in new patient population)** | **3,000 ng/ml** | 3,000 ng/ml PC at **0.9 µg/ml drug** | |

# Considerations on Clinical Development

**Add-on question  from Project team when ADA assay was optimized successfully:**

- *"Will it be possible to improve the drug tolerance for the Nab assay, too?"*
- Scope: in principle the same as for the ADA assay
- Answer: *"we will give it a try!"*

# Nab assay version history

| Phase | NAb Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Phase 3, Population A** | Version 1 | **195 ng/mL** | 400 ng/ml PC at **0.08** µg/ml drug | • Binding assay<br>• Capture (of labelled drug): Commercial Mab + Antigen (target)<br>• Detection: Labelled drug<br>• 1 acid dissociation step |
| **Intended for Phase 3, Population B** | Version 2 (validation in new patient population) | **3000 ng/ml** | 3000 ng/ml PC at **0.9 µg/ml drug** | |
| **Phase 3, Population B** | **Version 3 – change of format compared to versions 1 +2 and introduction of bead extraction** | **250 ng/ml** | **750 ng/ml PC at 25 µg/ml drug**<br>**25 – 300 fold improvement** | • Binding assay<br>• Capture (of ADA): Biotin drug on streptavidin coated magnetic beads<br>• Detection: Labelled Antigen (target)<br>• 2 acid dissociation steps |

# Population B – assay versions not used

- **ADA** assay **version 5:**
- Full validation for was deemed necessary due to:
  - change of basically all lots of critical reagents
  - need for selectivity assessment and cut point determination in matrix from population B
- Sensitivity and drug tolerance of ADA assay version 5 was poorer than for version 4 which had already been used for Phase 2B

- **Nab** assay **version 2:**
  - Development + full validation for NAb assay version 2 was necessary since NAb assay version 1 was not set up at the CRO
  - Drug tolerance of NAb assay version 2 was improved ~10 fold, but still low (0.9 µg/mL).
  - Sensitivity of was much poorer than expected also compared to NAb assay version 1

# Additional ADA assay version, population B

| Phase | ADA Assay version | Sensitivity | Drug tolerance | Assay format |
|---|---|---|---|---|
| **Intended for Phase 3** | Version 5 (new critical reagents + validation in new population) | **150 ng/ml** | 150 ng/ml PC at **5 µg/ml drug** | • Bridging assay:<br>• Capture: biotin-labelled drug added to streptavidin coated plate<br>• Detection: s-tag drug<br>• 1 acid dissociation step |
| **Phase 3** | Version 6 – introduction of bead extraction | **50 ng/ml** | **50 ng/ml PC at 200 µg/ml drug**<br>**20 – 40 fold improvement** | • Bridging assay format;<br>• Capture: biotin-labelled drug added to streptavidin coated magnetic beads<br>• Detection: s-tag drug<br>• 2 acid dissociation steps |
| **Phase 3/ Phase 4** | **Version 7**<br>**Optimization to improve robustness + full re-validation** | **50 ng/ml** | **50 ng/ml PC at 50 µg/ml drug**<br>**100 ng/ml PC at 150 µg/ml drug** | |

# Summing up

- **ADA assay version 6 and Nab assay version 3:**
  - In principle, both “Nice to” have, because you could take samples after ~5 half-lives with the older assay versions
  - However: great benefits from clinical development and patient perspectives
  - Potentially, less regulatory risk

- **ADA assay version 7:**
  - Change of critical reagents + Need for **robustness improvements**

- **Conclusion:**
  - Current ADA and Nab assay versions have acceptable Drug tolerance and sensitivity and have proven to be robust during sample analysis
  - **The many assay optimizations have been worth all the efforts!**

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