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Development of an Anti-Drug Antibody Confirmatory Assay for a PEGylated Drug using Streptavidin Magnetic Beads

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Abstract

To support the drug development for TRND00508745 (PEGylated Parathyroid Hormone 1-34), which is a PTH receptor modulator for the treatment of hypoparathyroidism, a direct ELISA anti-drug antibody (ADA) assay was designed for a non-GLP rat serum study. The aim was to have a single assay for the detection of antibodies directed against the PTH and PEG moieties, which could also be transferable to different species with minimal changes. However, in the confirmatory assay, while immunodepletion with 10 to 25 µg/mL of TRND00508745 reduced the signal of the anti-PTH positive control by at least 67%, no immunodepletion of the anti-PEG positive control signal was observed at the same drug levels.

This was unexpected since the capture of TRND00508745 on a streptavidin plate allowed the recognition of the binding epitope on the PEG moiety by the anti-PEG antibody in the screening assay. Hence, the proposed hypothesis for this lack of inhibition is a steric hindrance or conformational change of the epitope recognised by the monoclonal anti-PEG antibody when TRND00508745 is in solution. To assess this hypothesis, magnetic beads were used to immobilise TRND00508745. The presentation of the drug on the magnetic beads is a novel and creative approach to expose the anti-PEG epitopes in a direct ELISA assay when a solid support is required for antigen-antibody recognition.

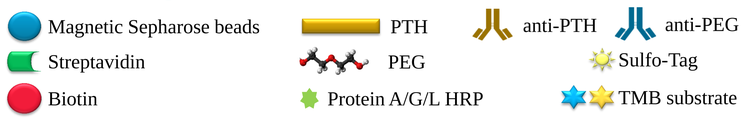
Further optimisation of the method resulted in appropriate immunodepletion of both anti-PEG and anti-PTH positive control signals, allowing the development of a qualified screening and confirmatory ADA assay for TRND00508745 using a single direct ELISA method.

Introduction

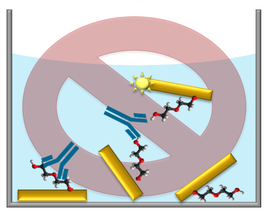
The PEGylation of PTH brings advantages such as longer half-life, owing to a better stability but at the cost of a possible immunogenic potential. Thus, developing an ADA assay for a PEGylated molecule, critical points had to be addressed during assay development:

- Detect ADAs against both the PTH and the PEG moieties of the drug since both portions of the molecule can elicit an immunogenic response.
- Develop one single assay for the detection of both the anti-PTH and anti-PEG moieties of the molecule as preclinical samples are available in limited volume.
- An easily-transferable assay to multiple species without major changes to simplify method transfer from preclinical to clinical.

Challenges

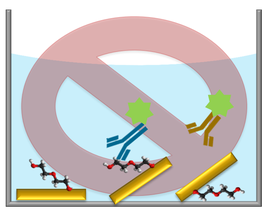


Bridge



- False negative ADA results obtained with ADAs targeting PEG moiety.
- PEG repeated units prevent ECLIA bridge format.

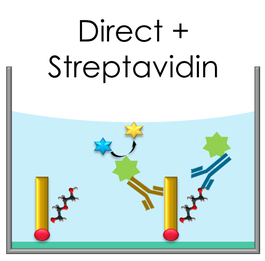
Direct



- False negative ADA results obtained with ADAs targeting PTH moiety.
- Direct absorption of TRND00508745 masks drug's PTH moiety epitopes.
- No inhibition achieved for the anti-PEG positive control when adding the drug.

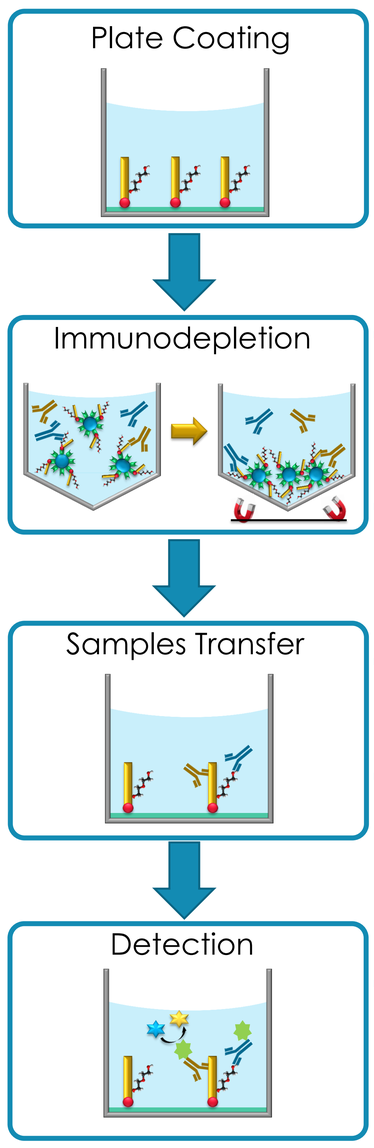
Screening Assay Method

Direct + Streptavidin



- Biotinylated TRND00508745 coated on streptavidin plate.
- Only one lysine available for biotinylation located at the C-terminal which allows epitopes access for ADAs.
- Detection is carried out using HRP coupled protein A/G/L activity on TMB substrate.

Confirmatory Assay Method



- Biotinylated TRND00508745 is added to a blocked streptavidin coated plate.
- Positive controls and samples are pre-incubated with Biotinylated TRND00508745 captured on streptavidin magnetic sepharose beads.
- Separation of ADA-TRND00508745-beads complexs using magnetic plate.
- Supernatant of immunodepleted samples are transfered to the blocked streptavidin assay plate.
- Any antibodies remaining will bind to their respective targets.
- Anti-drug antibodies are detected using HRP coupled protein A/G/L. TMB is used to evaluate HRP activity, and thus presence of ADAs.

Case Study Results

Coating of the TRND00508745

- PEG repeated units prevent ECLIA bridge format.
  - Direct coating of TRND00508745 to an assay plate was evaluated.
- Detecting ADAs against the PTH portion of the drug was difficult (Figure 1)
  - TRND00508745 is a small linear peptide (34 a.a.) and the PEG moiety can mask the epitopes.
  - Biotinylation of TRND00508745 and coating on a streptavidin plate was evaluated in order to achieve better binding, We hypothesized that this would allow coating of the molecule in an upright position, allowing better access to PTH epitopes (Figure 1).

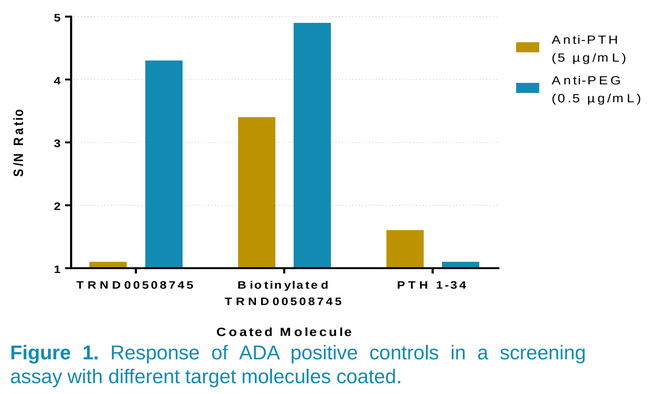


Figure 1. Response of ADA positive controls in a screening assay with different target molecules coated.

Confirmatory Assay Optimization

- Developing a confirmation assay able to confirm the response of both the anti-PEG and anti-TRND00508745 positive controls.
  - Addition of 25 µg/mL TNRD00508745 to samples and incubated at 37°C for 1 hour (Figure 2).
    - Acceptable inhibition of the anti-TRND00508745 positive control
    - No inhibition achieved for the anti-PEG positive control

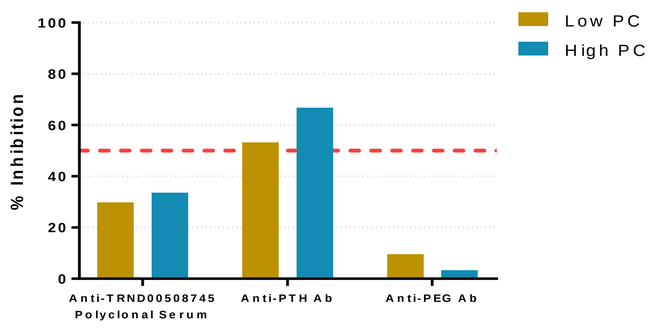


Figure 2. Percent Inhibition of the response of ADA positive controls by immunodepletion with TRND00508745.

Immunodepletion with Streptavidin Magnetic Beads

As the anti-PEG antibody had affinity for TRND00508745 in the screening assay, but neither TRND00508745 nor PEG600 could immunodeplete the anti-PEG antibodies (results not shown), we attempted to emulate the conditions used in the screening assay.

- Extract potential ADAs by the biotinylation of TRND00508745 and its addition to streptavidin magnetic beads.
  - Coat the beads with excess of biotinylated TRND00508745 which resulted in lower % inhibition than anticipated. (Figure 3).

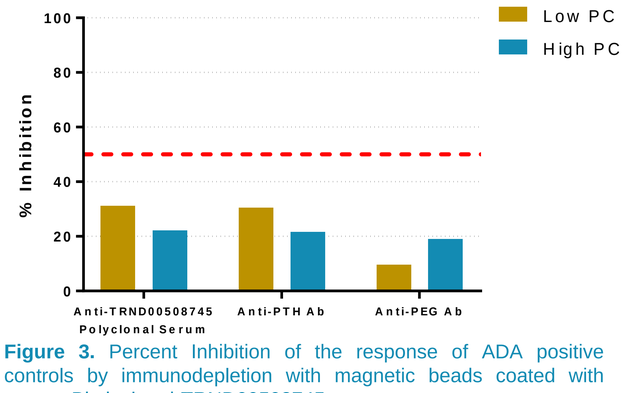


Figure 3. Percent Inhibition of the response of ADA positive controls by immunodepletion with magnetic beads coated with excess Biotinylated-TRND00508745

- Coat an excess of beads compared to the amount of biotinylated TRND00508745 and the samples were diluted 2 fold prior to the addition of the beads to mitigate any matrix effect.
  - Inhibition of signal of about 55% for the anti-PEG antibodies
  - Inhibition of signal by around 70% and 60% for the anti-PTH antibodies and rabbit serum were respectively (Figure 4).

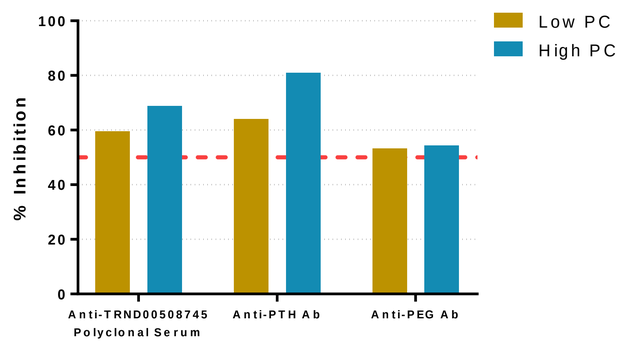


Figure 4. Percent Inhibition of the response of ADA positive controls by immunodepletion with magnetic beads (in excess) coated Biotinylated-TRND00508745

Qualification Parameters

Table 1: Qualification Parameters

Evaluation	Results
Cut-Point	▪ Floating screening cut-point based on 5% false positive rate ▪ Fixed confirmatory cut-point based on 1% false positive
Between-Run Precision for Screening Assay	▪ NC: < 17.5% ▪ Anti-PEG PC: < 9.4% ▪ Anti-TRND00508745 PC: < 9.3% ▪ Anti-PTH PC:< 10.3%
Within-Run Precision for Screening Assay	▪ NC: < 17.5% ▪ Anti-PEG PC: < 9.4% ▪ Anti-TRND00508745 PC: < 9.3% ▪ Anti-PTH PC:< 10.3%
Between-Run Precision for Confirmatory Assay	▪ Inhibited NC: < CCP ▪ Inhibited Anti-PEG PC: > CCP ▪ Inhibited Anti-TRND00508745 PC: > CCP ▪ Inhibited Anti-PTH PC:> CCP
Hook Effect	None observed for all 3 types of positives controls
Sensitivity	▪ 0.200 µg/mL for anti-PEG positive control[1] ▪ 10631237 dilution fold for the neat anti-TRND00508745 positive control
Selectivity in normal rat serum and 5% hemolysed rat serum	Meets acceptance criteria
Precision of titers	Meet acceptance criteria Anti-PEG titer: 5120 / Anti-TRND titer: 5120 / Anti-PTH titer: 640
Drug Tolerance	> 100 µg/mL
Stability	4 cycles freeze-thaw stability and 18 hrs Rmt short-term stability: Meet acceptance criteria

[1] Limit of detection

Conclusion

Biotinylating the TRND00508745 allowed the protein to coat the streptavidin plate in a way that permitted a good access to the binding epitopes of the ADAs. This allowed the detection of both the anti- PEG and PTH moieties simultaneously. As for the confirmatory assay, it appeared that the anti-PEG antibodies were only capable of binding their epitopes when the TRND00508745 molecule was bound to a solid surface.

The method for the determination, confirmation and titration of anti-TRND00508745 antibodies and anti-PEG antibodies in rat serum using a Biotek SynergyTM H4 HybridMulti-mode Microplate Reader was qualified.

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