

Approaches to Improve Sensitivity and Drug Tolerance by Reducing Target Interference During Development of an Anti-drug Antibody Assay

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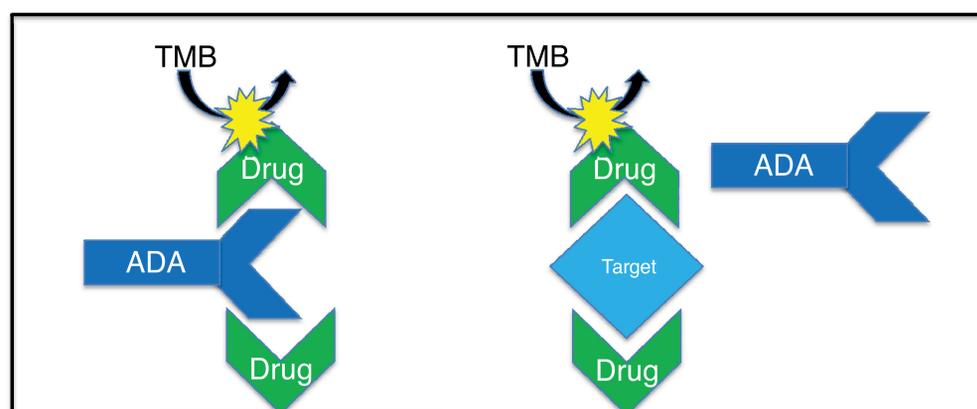
Introduction and Objective

When developing an anti-drug antibody (ADA) assay, two key challenges are overcoming high drug interference and high target interference.

- For most ADA assays with a soluble target, the endogenous target concentrations are in picograms per milliliter (pg/mL) or nanograms per milliliter (ng/mL) that has minimal interference with achieving a low sensitivity for the assay.
- An ADA assay was developed for a novel drug whose soluble target has a concentration in greater than 10 microgram concentrations ($\mu\text{g/mL}$) in normal human plasma which interferes with the assay by blocking drug binding sites.
- The objective of method development for this assay was to test various approaches remove excess soluble target to achieve the lowest 99% sensitivity possible.

Assay Format

Figure 1. Sandwich ELISA Format and Target Interference



- The ADA assay format is a typical sandwich ELISA with the drug coated to a microplate, followed step-wise by ADA positive control, biotin-conjugated drug, TMB Substrate Solution, and acid quench.
- In this assay, the high concentration of soluble target in normal human plasma interfered with the binding site on the drug.

Methods

Methods Tested to Improve Sensitivity and Drug Tolerance

- ADA purification with Melon™ Gel IgG Purification kit (Thermo Fisher) was tested, however, the kit was unable to remove the soluble target from human plasma.
- Anti-target antibodies bound to magnetic Invitrogen Dynabeads® were used to deplete the soluble target in a pretreatment step prior to sample analysis.

Table 1. Anti-Target-coated Dynabeads® pretreatment shows improved sensitivity in normal human plasma

Signal: Noise			
5000 ng/mL ADA	78.35	4.046	138.6
1000 ng/mL ADA	24.87	1.908	44.98
200 ng/mL ADA	5.616	1.163	8.847
100 ng/mL ADA	4.687	1.034	4.885
50 ng/mL ADA	3.561	0.973	3.010
25 ng/mL ADA	5.637	0.946	1.976
0 ng/mL ADA	1	1	1
	Dynabeads® pretreatment	No pretreatment	Target deficient Plasma

- Target removal with Dynabeads® does improve the sensitivity at low ADA concentrations and will be incorporated into the final assay.

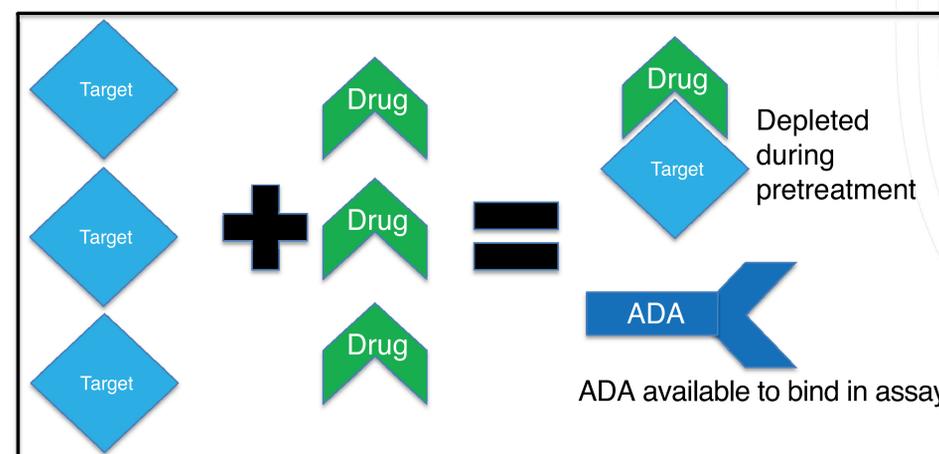
- Even after the soluble target was removed with Dynabeads®, low concentrations of drug interfered with the assay performance. Drug tolerance was first tested in target-depleted plasma, but free drug concentrations of 50 ng/mL decrease the sensitivity of the assay.

Table 2. Drug Tolerance after target-depletion pretreatment

Signal: Noise						
10,000 ng/mL ADA	37.97	35.38	40.07	36.54	35.72	14.16
2500 ng/mL ADA	29.74	23.76	22.17	7.539	1.176	1.211
500 ng/mL ADA	6.660	2.020	1.326	1.049	1.004	1.011
100 ng/mL ADA	2.167	1.123	1.127	1.055	0.9626	1.025
50 ng/mL ADA	1.576	1.011	1.122	0.9541	1.011	1.026
0 ng/mL ADA	1	1	1	1	1	1
Free Drug	0 ng/mL	50 ng/mL	100 ng/mL	200 ng/mL	500 ng/mL	1000 ng/mL

- After testing the plasma sample immediately after the target depletion step in a separate pharmacokinetic assay, it was revealed that the target depletion did not pull out excess drug. Another method would be required to improve drug tolerance.
- The Mesoscale Discovery (MSD) platform was tested for improved sensitivity and drug tolerance by coating the drug on a MSD Standard Bind plate and detecting with ruthenium-conjugated drug. Neither the sensitivity nor drug tolerance were improved using this platform, thus it was decided to proceed with the ELISA sandwich assay.
 - In a last attempt to improve drug tolerance, excess exogenous target was added and allowed to bind with the excess drug in the samples forming a complex that could be removed in the target depletion step.

Figure 2. Adding excess target to deplete free drug prior to pretreatment



Results

- For this assay, soluble target removal after acid dissociation allowed for evaluation of ADAs present in analytical samples with sufficient sensitivity to meet the clinical trial objective.
- ADA samples prepared in target-free plasma show 20-fold better sensitivity than normal human plasma verifying it is the soluble target causing the interference issues in this assay.

Conclusion & Future Work

While the assay was sufficient for the intended purpose in early stage single dose clinical trial, it is currently under evaluation. We intend to explore acid-dissociation with solid phase extraction to remove target and drug interference using various mitigation strategies such as the use of anti-target antibodies, and high affinity target-binding ligands (i.e. aptamer, lectins etc.)