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

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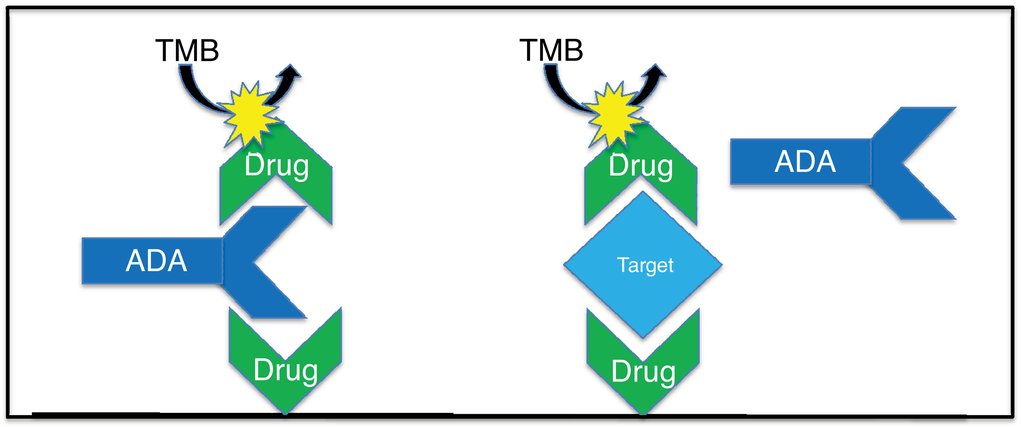
Introduction and Objective

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 (µg/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



A. 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.
B. 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

1. 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.

2. 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.

3. 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

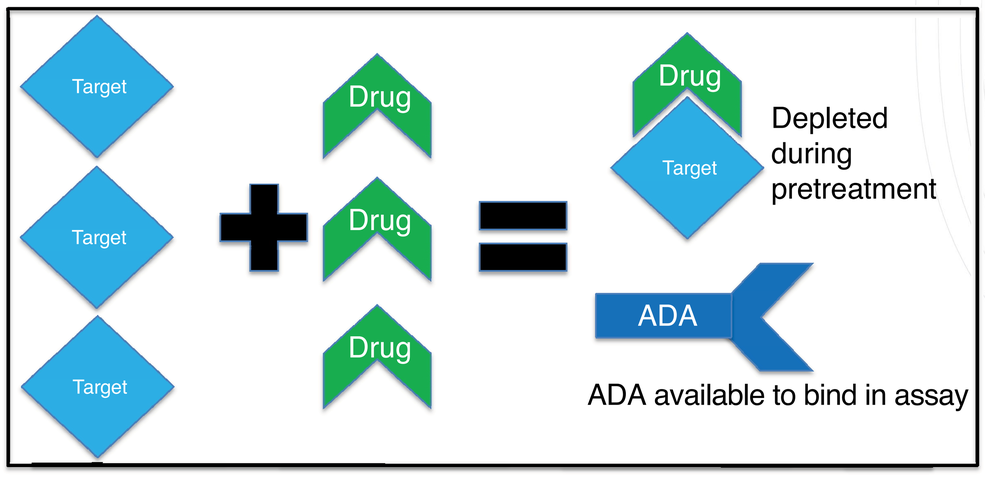
Signal: Noise						
10,000 ng/mL ADA	37.97	35.38	40.07	36.54	35.72	14.16
2500 ng/mL ADA	29.74	23.76	22.17	7.539	1.176	1.211
500ng/mL ADA	6.660	2.020	1.326	1.049	1.004	1.011
100 ng/mL ADA	2.167	1.123	1.127	1.055	0.9626	1.025
50 ng/mL ADA	1.576	1.011	1.122	0.9541	1.011	1.026
0 ng/mL ADA	1	1	1	1	1	1
Free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

4. 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.

5. 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.)