Acid Dissociation Bridging Assay for Detection of Anti-human Leptin ADA in the Presence of the Therapeutic, PEGylated Human Leptin and Endogenous Human Leptin

K. De Dios, K. Cox, R. Marsden
Ambrx, Inc.

Purpose
For proper interpretation of pharmacokinetic, pharmacodynamic, and toxicokinetic data, it is important to monitor antibody response during studies. For PEGylated human leptin, the presence of high levels of drug and endogenous leptin in matrix makes the detection of antibodies difficult. Initial method development resulted in poor assay performance, prompting exploration of an acid dissociation method.

Methods

Conventional Method: This method uses biotinylated and SULFO-tagged PEGylated human leptin at final concentration of 62.5 ng/mL incubated with sample at a final MRD of 1:20. After 2 hours, sample is added to blocked MSD Streptavidin Gold plates. MSD Read Buffer is added and plates are read.

Acid Dissociation Method: Sample is diluted to 10% with Pierce IgG Elution Buffer and incubated for 30m. Acidified sample is diluted 1:2 with labels containing 5% 1M Tris-HCL. Subsequent steps are the same as the conventional method. We used a generic cutoff of 2X background for the estimated cut-point.

Results
Using the conventional method, 250 ng/mL PC was not detectable in human serum in the presence of ≥250 ng/mL PEG-Leptin, and only tolerated up to 62.5 ng/mL recombinant human leptin. Signal to noise ratio (S/N) for a 200 ng/mL spike in normal, lean, obese, and Type II diabetic males and females (N=32) ranged from 1.23-5.71, with 22% falling below 2. Endogenous leptin levels, as measured by the MSD human leptin kit, varied per population and ranged from <0.686 – 319 ng/mL. Acid dissociation improved S/N for the 200 ng/mL spike to a range of 2.38-5.86. With acid dissociation, drug tolerance increased in rat and cyno from 500 ng/mL PEG-Leptin to 4 μg/mL, and drug tolerance improved to 2 μg/mL in human.

Conclusion
Using acid dissociation improved our bridging ADA method by increasing drug tolerance 8-fold in rat and cyno and 32-fold in human serum. Sensitivity also improved approximately 2.5-fold in human serum to 100 ng/mL.