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Development and Qualification of PK and ADA Methods for Biosimilar Trastuzumab

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Abstract

PURPOSE:
The purpose of this study was to develop and qualify immunoassay methods to quantitate trastuzumab (PK) and also detect antibodies against trastuzumab (ADA) in serum from human and preclinical species.

METHODS:
The PK method for trastuzumab was a conventional sandwich ELISA, using recombinant HER2-extracellular domain (ECD) to capture drug, and a biotinylated anti-idiotypic monoclonal antibody as secondary reagent, followed by HRP-labeled streptavidin detection. An acid pretreatment step was used to circumvent potential interference from soluble ECD in patient samples.
The ADA method used an antigen capture elution (ACE) ELISA format. After an initial acid dissociation step, anti-drug antibodies are captured on a trastuzumab-coated plate. After washing off unbound materials, the ADA are eluted using low pH and coated onto a second plate. The ADA are then detected using biotinylated drug and HRP-labeled streptavidin.

RESULTS:
The PK method was qualified for use in both human and mouse serum. The method exhibited acceptable accuracy (%RE<9.3% in both matrices) and precision (intra- and inter-assay CVs < 17% in both matrices). Selectivity was excellent, with 10 of 10 lots of human serum within 20% of nominal. No interference observed up to 4000 ng/mL sHER2.
The ADA method was qualified for use in human serum. The method had a sensitivity of 5 ng/mL using a monoclonal positive control. Precision was acceptable, with intra-assay CV of 3.4% and inter-assay CV of 19.6%. 500 ng/mL of anti-trastuzumab ADA was detectable in the presence of 62.5 µg/mL of trastuzumab

CONCLUSION:
Sensitive and precise methods were qualified to support the development of biosimilar trastuzumab. The PK ELISA was shown to detect drug in the presence of soluble target. The ADA method exhibits excellent drug tolerance.

PURPOSE:

Trastuzumab (Herceptin®) is a recombinant humanized monoclonal antibody that is directed against the human epidermal growth factor receptor 2 (HER2). Trastuzumab is indicated for the treatment of patients with metastatic breast cancer who have tumors that overexpress HER2. Treatment with trastuzumab increases overall survival and reduces the probability of recurrence after surgery. The patent for trastuzumab expired in 2014 in the EU, and is due to expire in 2019 in the US. Thus trastuzumab is an attractive target for biosimilar developers.

The evaluation of the comparability of biosimilars to the corresponding innovator molecules should follow guidelines that have been set out by both FDA and EMA. A multifactorial approach is required, including a clinical comparison of both pharmacokinetics (PK) and immunogenicity (ADA; anti-drug antibody). In this study we present the development and qualification of PK and ADA assay methods that may be useful for manufacturers developing biosimilar trastuzumab.

In developing these methods, we addressed some challenges that are specific to trastuzumab. First, it is known that the ECD of HER2 can be shed, and circulates in plasma at levels up to 1880 ng/mL in cancer patients. This soluble HER2 may interfere with detection of trastuzumab in a PK assay. Also, given the relatively high expected concentrations of therapeutic in clinical studies, with peak concentrations of approximately 150 µg/mL, drug interference in the ADA method was expected to be a challenge. The methods described here were designed to overcome both of these challenges

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Materials & Methods:

PK assay: The analytical method is a bridging ELISA with acid dissociation where serum samples containing trastuzumab are acidified to separate immune complexes. Immune complexes include trastuzumab bound to either anti-drug antibodies (ADA) and/or soluble target (sHER2). Acidified serum samples are then neutralized on an ELISA plate coated with rHER2 protein allowing trastuzumab to bind to the plate. Unbound material is then washed away. The drug is detected by a biotin labeled anti- trastuzumab monoclonal antibody and subsequent development with HRP-labeled streptavidin and enzyme substrate. Trastuzumab concentrations are interpolated from the 5parameter regressed standard curve ranging from a Lower Limit of Quantitation of 1.5 µg/mL to the Upper Limit of 80 µg/mL

ADA
The analytical method is an Affinity Capture Elution (ACE) ELISA where trastuzumab is used for capture of the anti-drug antibodies (ADA) in human sera. Unbound material is washed away and following acid dissociation and neutralization the captured ADA is transferred to a second plate and detected with biotinylated trastuzumab, followed by streptavidin-HRP, washed and visualized using TMB.

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ELISA format for total Trastuzumab PK assay

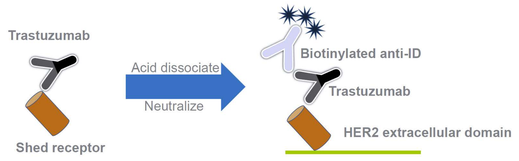

Figure 1. Bridging ELISA Format for Total Trastuzumab PK Method

Sensitivity of Total Trastuzumab PK method

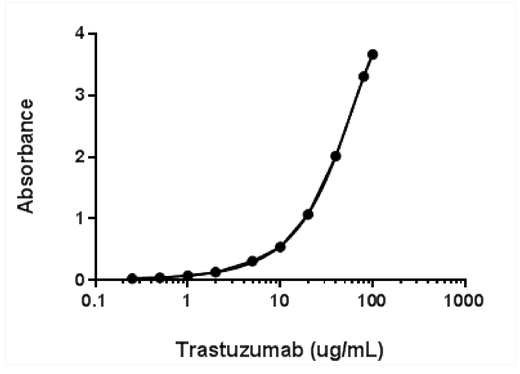

Figure 2. Representative standard curve illustrating range of detection 1.5 µg/mL to 80 µg/mL.

Effect of sHER2 on Trastuzumab recovery in PK assay

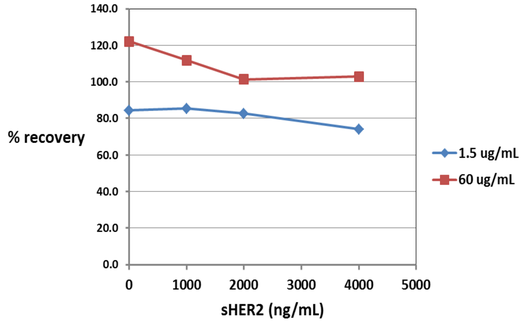

Figure 3. %Recovery of 1.5 or 60 µg/mL trastuzumab in the presence of up to 4000 ng/mL soluble HER2 (sHER2)

PK method: Performance characteristics

Performance characteristic	Results
Validated Range (LLOQ/ULOQ)	1.5 µg/mL to 80 µg/mL
Accuracy	Human Serum Range 3.3% to 9.3%
	Mouse Serum Range 3.8% to 9.1%
Precision Intra-assay Inter-assay	Human Serum Range 5.0 % to 16.1 % Range 7.3 % to 16.6 %
Intra-assay Inter-assay	Mouse Serum Range 2.6% to 9.0 % Range 6.8 % to 13.2 %
Specificity / Selectivity	10 out of 10 lots of human serum within ±20% of nominal (levels tested for each lot: unspiked, 10 and 50 µg/mL)
Dilutional Linearity	%RE Range: -8.5 % to -23.1 %; Overall %CV: 7.5 % Maximum dilution performed 1:50 (exclusive of MRD)
Soluble Target Interference (sHER2)	**No interference observed up to 4000 ng/mL sHER2**

ACE format for anti-Trastuzumab ADA assay

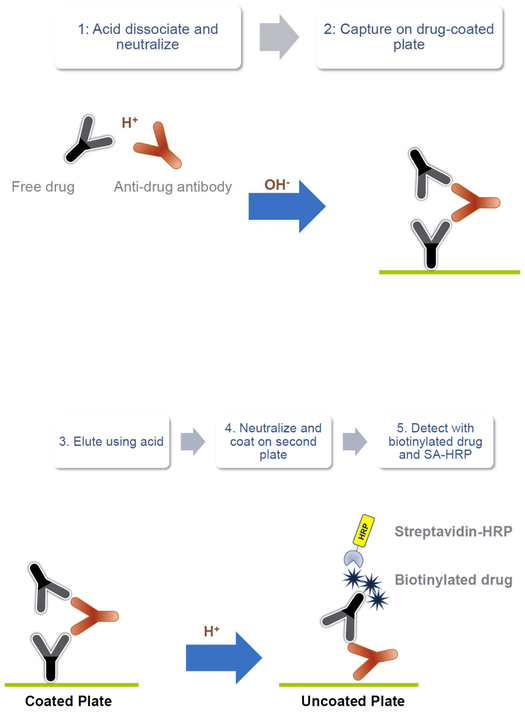

Figure 4. ACE format for anti-Trastuzumab ADA Method demonstrating use of acid dissociation followed by affinity capture elution of antibody prior to detection with biotinylated trastuzumab.

ADA method: Performance characteristics

Performance characteristic	Results
Sensitivity	5 ng/mL
Cut Point Assessment	30 individual female sera samples Floating cut point factor was established at 1.32
Selectivity (Matrix recovery)	12 out of 12 lots of human serum lots spiked with 50 ng/mL ADA were within ±25% of reference
Precision Intra-assay Inter-assay	3.4% 19.6%
Drug Tolerance	**500 ng/mL anti-trastuzumab ADA is detectable in the presence of 62.5 µg/mL of trastuzumab**

Summary

- **A PK ELISA was developed for quantification of trastuzumab in mouse or human serum.**
- **The quantitative range was 1.5 – 80 ug/mL, extended by dilution to 4 mg/mL**
- **The PK method utilized an acid dissociation step to overcome potential interference by sHER2**
- **An ACE format was used to develop an method for detection of anti-trastuzumab antibodies in human serum**
- **The ADA method exhibits drug tolerance of 62.5 ug/mL at a Positive Control level of 500 ng/mL**

Conclusion

Sensitive, robust, selective and precise methods were qualified to support the development of biosimilar trastuzumab. The PK ELISA was shown to detect drug in the presence of soluble target. The ADA method exhibits excellent drug tolerance.

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