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DATASHEET

Methods to Determine the Binding of Trastuzumab to HER2

BACKGROUND

Trastuzumab (Herceptin®) is a humanized recombinant monoclonal antibody that selectively binds to the extracellular domain of the human epidermal growth factor receptor 2, HER2, a transmembrane protein overexpressed in 25-30% of breast cancers. Trastuzumab is used for the treatment of primary breast cancers which overexpress HER2. Herceptin binds with high affinity (kDa 5nM) to tumor cells over expressing HER2 resulting in loss of malignant growth and metastasis.

Evaluation of the comparability of trastuzumab biosimilars to the innovator drug should follow the guidelines laid out by the FDA and EMA. The analysis should be multifactorial, taking into account both the physicochemical characteristics and clinical performance of the biosimilar compared to the innovator. Eurofins Bioanalytical Services offers a full range of off-the-shelf trastuzumab assays for comparability testing of biosimilars including:

- PK assay
- ADA assay
- Nab assay
- Comparability testing
  - Fc Receptor & C1q binding
  - HER2 kinetic binding assay
  - ADCC assay

A method to assess kinetics of Herceptin HER2 binding has successfully been developed and qualified. Due to the high affinity of Herceptin to HER2 and the inability of the Biacore instrument to accurately measure Kd $<1 \times 10^{-5}$ s^{-1} the approach taken was to calculate affinity (KD) by Scatchard plot.

In addition, a method to assess the potency of binding of trastuzumab to HER2 was successfully qualified at a concentration range of 0.06 µg/mL to 1000 µg/mL. The findings of the qualification are summarized below.

These methods form a suite of assays for comparability assessment of biosimilars: A reference sample and up to 3 test samples are analyzed in triplicate over a concentration range of 0.06 µg/mL to 1000 µg/mL. For the potency comparison the dose response curves compared by parallel line) for relative potency and parallelism (χ^2 (Chi-squared)). For the affinity comparison the response at equilibrium is used to plot a Scatchard plot as described in the method below.

METHOD

HER2 was immobilized to a CM5 chip and the binding of trastuzumab was measured over a 0.06 µg/mL to 1000 µg/mL concentration range at a reduced flow rate of 20µL/min. The affinity (KD) was calculated by Scatchard Plot. This was achieved by plotting Response at equilibrium (RU)/Concentration (nM) against Response at equilibrium (RU). The affinity (KD) is defined as -1/slope. A more accurate fit (larger R^2 value) was achieved by excluding the top (1000 µg/mL) and bottom (0.06 µg/mL) points from the Scatchard Plot analysis.

For the potency assay the linearity of the measured potency of the test samples (prepared at 70%, 80%, 100%, 120% and 130% of the reference samples) was used to assess the assay range. The data from were plotted and analysed by linear regression to determine the following parameters: correlation coefficient, y-intercept, slope and residual sum of squares.

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RESULTS

HER2 Binding Assay

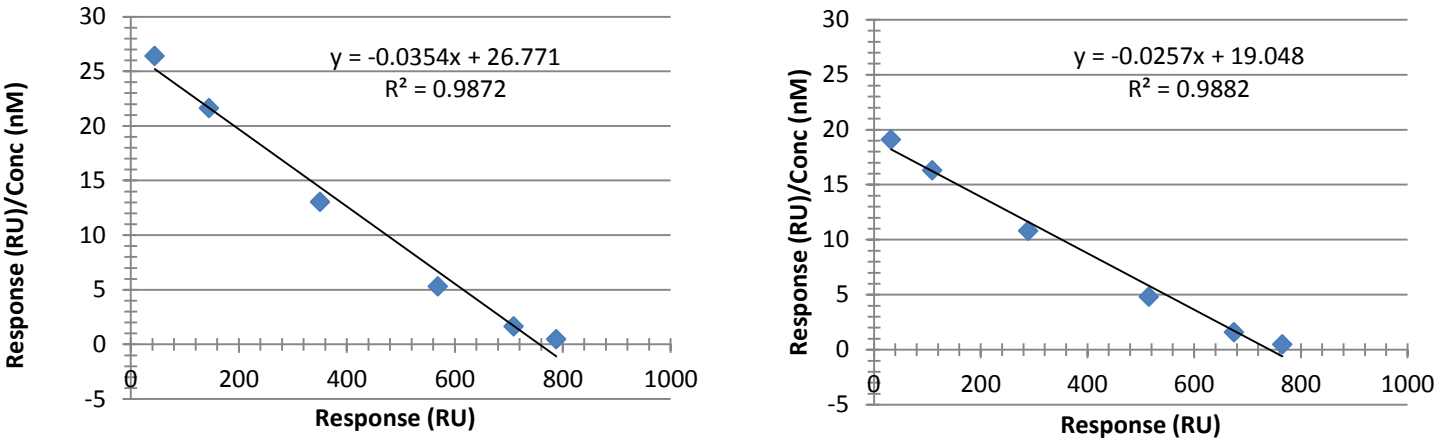

FIGURE 1. Scatchard Plot determination of KD. HER2 immobilized on a Biacore CM5 chip and the binding of trastuzumab measured over a 0.06 µg/mL to 1000 µg/mL concentration range at a reduced flow rate of 20 µL/min. The affinity (KD) was calculated by Scatchard Plot. This was achieved by plotting Response at equilibrium (RU)/Concentration (nM) against Response at equilibrium (RU). The affinity (KD) is defined as -1/slope. Samples were prepared between 70% and 130% of the reference to define the range of the assay. Representative 100% (left) and 70% (right) samples shown above.

70%		80%		100%		120%		130%	
Batch	K_D (nM)	Batch	K_D (nM)	Batch	K_D (nM)	Batch	K_D (nM)	Batch	K_D (nM)
TB07	38.9	TB08	35.6	TB07	28.2	TB08	24.5	TB07	21.0
TB08	39.2	TB09	35.5	TB09	29.2	TB09	24.8	TB09	23.5
				TB09	29.4				
				TB07	27.8				
Mean	39.1		35.6		28.7		24.7		22.3
SD	0.21		0.07		0.77		0.21		1.77
%CV	0.5		0.2		2.7		0.9		7.9
n	2		2		4		2		2
Recovery	95.4		99.3		100.0		103.2		101.0

TABLE 1. Summary of the affinity data and performance characteristics of the trastuzumab / HER2 binding Assay

Potency Assay

A further advantage of measuring equilibrium binding to determine affinity is that parallel line analysis potency data can also be generated from the same set of sensorgrams. The inter-batch precision for the potency data ranged from 0.5% to 3.9% CV and a recovery of between 99.9% and 102.9%. The assay linearity data (Figure 2 and Table 2) shows that the assay is suitable for samples between 70% and 130% of the reference at a concentration range of 0.06 µg/mL to 1000 µg/mL.

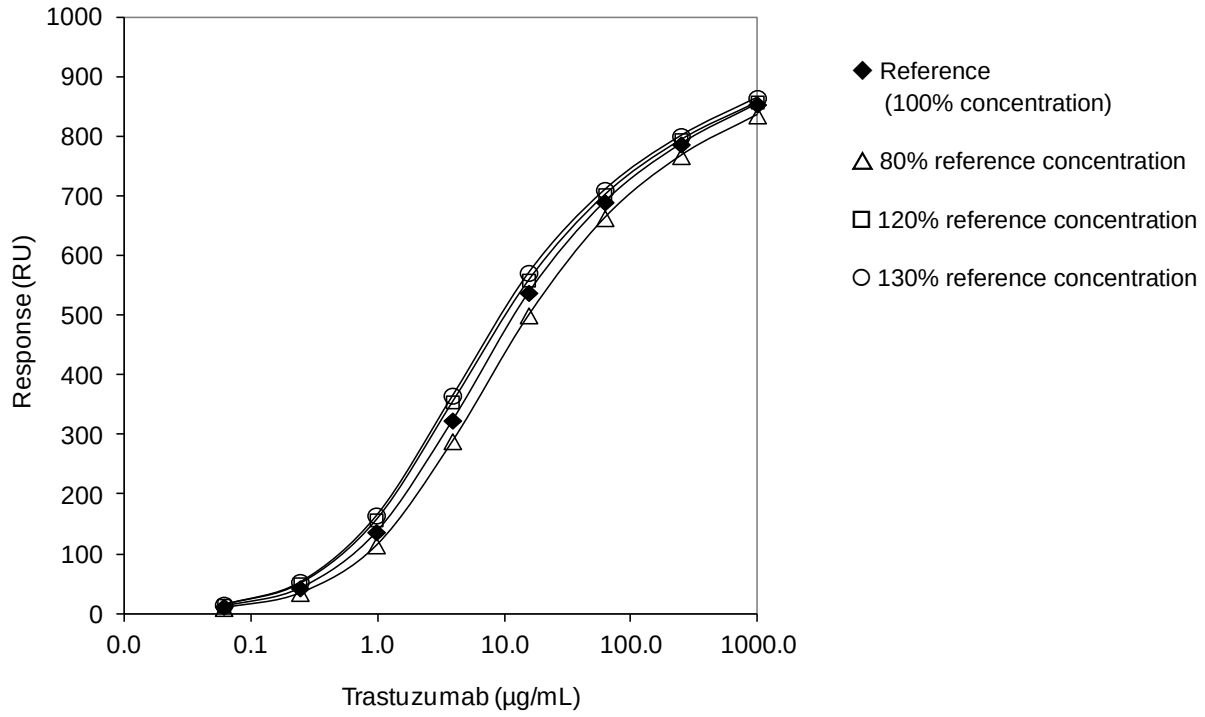

FIGURE 2. Representative analysis of trastuzumab binding to HER2 was performed on reference over eight concentrations of drug (0.06 µg/mL to 1000 µg/mL) between 70 and 130 % (80%, 120% and 130% represented) of the reference concentration. The complete dose-response curves were generated using this concentration range.

Qualification Principle	Qualification Parameters	Acceptance Criteria	Qualification Results
Assay Linearity and Range	Correlation coefficient Y-intercept Slope Residual sum of squares	> 0.95 -15% to +15% 0.8 to 1.2 report result	0.995 -3.66 1.05 31
Repeatability (Intra-assay precision)	%CV	<20%	<7%
Intermediate precision (Inter-assay precision)	%CV	<20%	<4%
Accuracy	Recovery	80% to 120%	**99.9% to 102.9%**

TABLE 2. Summary of trastuzumab potency assay performance results

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