# **Orthogonal HOS Similarity Assessment of Biosimilar Using Multi Spectroscopic Technique** and Statistical Calculation

Taiji Oyama\*, Satoko Suzuki, Ai Yamane, Yasuo Horiguchi, Ken-ichi Akao, Koushi Nagamori \*taiji.oyama@jasco.co.jp

## INTRODUCTION

The biosimilar market is expanding and regulatory authorities emphasize the importance of analytical characterization of biosimilars, and recommend orthogonal assessment of the HOS of biosimilars and their innovators using multiple techniques based on different principles<sup>1-5</sup>). Far-UV/CD and FT-IR spectroscopy are orthogonal methods for evaluating secondary structure, and near-UV/CD, Raman and fluorescence spectroscopy are orthogonal for tertiary structure. Comprehensive orthogonal assessment using these methods can provide a firm structural foundation to claims of biosimilarity.

Here we report the results of a HOS assessment of a biosimilar and its innovator using cutting edge JASCO instruments. These are the HTCD Plus for far- and near-UV/CD, the FT/IR-4X for infrared, and the NRS-4500 for Raman spectroscopy. We also use the qHOS software, which allows statistical determination of spectral similarity.



Figure 1. HOS similarity assessment for biosimilar

### EXPERIMENTAL

#### **Measurement system**



### **Materials**

Trastuzumab

mg/mL

MabThera<sup>®</sup> (Innovator)

**RIABNI<sup>™</sup> (Biosimilars)** 

Herceptin<sup>®</sup> (Innovator)

Both samples were prepared to 10 mg/mL

Additive : Sodium citrate dihydrate 7.4 mg/mL, Sodium chloride 9.0

mg/mL, Sodium hydroxide 9.0 mg/mL, Polysorbate 80 0.7 mg/mL

The powder was dissolved in  $H_2O$  to a concentration of 10 mg/mL

Additives: trehalose hydrate 4.7 mg/mL, L-histidine hydrochloride

hydrate 0.11 mg/mL, L-histidine 7.4 x 10<sup>-2</sup> mg/mL, polysorbate 2.1 x 10<sup>-2</sup>

**Rituximab** 

Methods	
Far-UV/CD	
Optical path length : 0.2 mm Band width : 1.0 nm nm/min	Auto washing : On Scanning speed : 20
Near-UV/CD	
Optical path length : 0.5 mm Band width : 1.0 nm nm/min	Auto washing : On Scanning speed : 50
ATR FT-IR	
Detector : TGS Resolution : 4cm <sup>-1</sup>	ATR crystal : Diamond Number of scans : 128
Raman	
Laser : 532 nm	Grating : 900 gr/mm
Exposure : 45 sec	Number of scans : 32
Analysis	
Distance calculation : Euclidean	Weighting : Noise

Significance testing : Welch

Significance level : 0.05





### **RESULTS**



The shapes of the near-UV/CD spectra of MabThera® and Herceptin® differ significantly (Fig. 2a). Similarly, the distribution of distances between MabThera® and Herceptin calculated from the CD spectra show a significant difference (Fig. 2b). The p-value obtained from the t-test is below the significance level of 0.05, indicating that Herceptin® has a different tertiary structure to MabThera®.



Figure 3. Orthogonal similarity assessment for the secondary structure of MabThera<sup>®</sup> and RIABNI<sup>™</sup>

The far-UV/CD and FTIR spectra of the biosimilar RIABNI<sup>™</sup> are in excellent agreement with those of the innovator MabThera® (Figs. 3a and 3b), and the distributions of the distances between MabThera® and RIABNI<sup>™</sup> are close to each other (Figs. 3c and 3d). The p-value is larger than the significance level of 0.05.

### **CONCLUSIONS**

- We performed orthogonal assessments of the secondary and tertiary structures of a biosimilar and an innovator using HTCD Plus, FT/IR-4X, NRS-4500.
- The similarity of each spectrum was statistically determined using the single qHOS platform.
- The similarity of the HOS for the biosimilar and the innovator was confirmed by orthogonal methods.
- This system can be used not only to evaluate the similarity of biosimilars but also to easily and accurately determine the changes in the HOS of antibody drugs caused by post-translational modifications and external stimuli.

Figure 4. Orthogonal similarity assessment for the tertiary structure of MabThera® and RIABNI<sup>™</sup>

Similar to the secondary structure, the tertiary structure of MabThera® and RIABNI<sup>™</sup> show excellent agreement in the near-UV/CD and Raman spectra (Figs. 4a and 4b), and the distribution of the distances between MabThera® and RIABNI<sup>™</sup> are close to each other (Figs. 4c and 4d). The p-value is larger than the significance level of 0.05.

## REFERENCES

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