Non-Parametric IND Biosimilarity Assessment for Small Analytical Sample Sizes

BioSim 1

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InsightHatch: "Non-Parametric Biosimilarity Assessment for Small Analytical Sample Sizes when dealing with IND Dossier Data"
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Contents

Revision	Date	Author(s)	Description
0.0	2023.11.15	DP	Created!
0.1	2023.11.29	DP	Update with more graphics, andded R-code blocks and calculation framework
0.2	2023.12.03	DP	With the feedback of Wang Chen expanded and clarified for YZY discussions planned on 2023-12-05
0.3	2023.12.04	DP	Clarified the role of pdf for x and y inline with linear model effect on clinical outcome
0.4	2023.12.05	DP	Finalized draft for release to client

1 BioEquivalance assessment for larger n with assumed underlying normality

We enter this discussion by following a well know reference [1: Endrenyi L, et al 2017] where the idea of using two different types of criteria for analytical similarity based on absolute difference or relative difference of a *new test material* against an *orginator reference material* is statistically formulated. In the spirit of keeping the derivation more compact we will only focus on the absolute difference approach. The more curious reader may consult the reference directly.

Basically, under the Fundamental Bio-equivalence Assumption, the "In Vitro-In Vivo Correlation" (IVIVC) is to use the dissolution test as a surrogate for human studies (i.e., when the drug absorption profiles in terms of "Area Under the Curve" (AUC) or $C_{\max}$ are similar, it is assumed that they are therapeutically equivalent). In addition, one of IVIVC's main roles is to assist in the quality control of functional and/or structural characteristics during the manufacturing process.

For simplicity and illustration purposes, we will consider the case where the relationship between CQA and clinical outcome is linear. The nonlinear case can be similarly treated, albeit perhaps more mathematically complicated.

Let x and y be the response of a "Critical Quality Attribute" CQA and the clinical outcome, respectively. In practice, if the CQA is relevant to clinical outcome, it is assumed that the clinical outcome can be predicted by the CQA accurately and reliably with some statistical assurance. One of the statistical criteria is to examine the degree of closeness (or the degree of relevance) between the observed response y and the predicted response $\hat{y}$ through an established statistical model. *It is worth noting here: We are pursuing understanding the sensitivity of a clinical outcome as related to the analytical variance of a CQA.*

To perform this examination, we will first study the association between x and y and build up a model. Then, we will validate the model based on some criteria. For simplicity, we assume that x (some analytical quality measure) and y (some measurable clinically dependent outcome) can be described by the following linear model:

$$y = \beta_0 + \beta_1 x + \varepsilon$$

where ε follows a normal distribution with a mean of 0 and a variance of σ_e^2. Suppose that n pairs of observations $(x_1, y_1), \ldots, (x_n, y_n)$ are observed in a translation process. To

define the notation, let

$$X^T = \begin{pmatrix} 1 & 1 & \dots & 1 \\ x_1 & x_2 & \dots & x_n \end{pmatrix}$$

and

$$Y^T = \begin{pmatrix} y_1 & y_2 & \cdots & y_n \end{pmatrix}.$$

Then, under model 3.1, the maximum likelihood estimates of the parameters β_0 and β_1 are:

$$\begin{pmatrix} \hat{\beta}_0 \\ \hat{\beta}_1 \end{pmatrix} = \left(X^T X\right)^{-1} X^T Y$$

with

$$\text{var}\begin{pmatrix} \hat{\beta}_0 \\ \hat{\beta}_1 \end{pmatrix} = \left(X^T X\right)^{-1} \sigma_e^2.$$

Furthermore, σ_e^2 can be estimated by the mean squared error (MSE), which is given by

$$\hat{\sigma}_e^2 = \frac{1}{n-2} \sum_{i=1}^{n} (y_i - \hat{y}_i)^2$$

Thus, we have established the following relationship:

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x.$$

For a given $x = x_0$, suppose that the corresponding observed value is given by y; however, using Equation 3.2, the corresponding fitted value is $\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x_0$. Note that $E(\hat{y}) = \beta_0 + \beta_1 x_0 = \mu_0$ and

$$\text{var}(\hat{y}) = \begin{pmatrix} 1 & x_0 \end{pmatrix} \left(X^T X\right)^{-1} \begin{pmatrix} 1 \\ x_0 \end{pmatrix} \sigma_e^2 = c\sigma_e^2,$$

where

$$c = \begin{pmatrix} 1 & x_0 \end{pmatrix} \left(X^T X\right)^{-1} \begin{pmatrix} 1 \\ x_0 \end{pmatrix}.$$

Furthermore, $\hat{y}$ is normally distributed with mean μ_0 and variance $c\sigma_e^2$, that is,

$$\hat{y} \sim N\left(\mu_0, c\sigma_e^2\right).$$

We may validate the translation model by considering how close an observed y is to its predicted value $\hat{y}$, which is fitted to the regression model 3.2. To assess the closeness, we propose the following two measures, which are based either on the absolute difference or

the relative difference between y and $\hat{y}$:

Criterion I. $p_1 = P\{|y - \hat{y}| < \delta\}$

Criterion II. $p_2 = P\left\{\left|\frac{y-\hat{y}}{y}\right| < \delta\right\}$

In this paper we only focus on Criterion I, in order to keep the overall analysis shorter.

In other words, it is desirable to have a high probability that the difference or the relative difference between y and $\hat{y}$, given by p_1 and p_2, respectively, is less than a clinically or scientifically meaningful difference δ. Then, for either $i = 1$ or 2 , it is of interest to test the following hypotheses:

$$H_0 : p_i \leq p_0 \text{ versus } H_a : p_i > p_0,$$

where p_0 is some prespecified constant. The idea is to reject H_0 in favor of H_a. In other words, we would like to reject the null hypothesis H_0 and conclude H_a, which implies that the established model is considered validated.

1.0.1 Considerations of what this is really all about - and why we are pursuing the anlysis this way

It makes sense to talk about what this above model formulation is really representing. Firstly, we are trying to build a relationship of the clinical outcome as it has some dependence on the product CQAs. And from that position talk about the allowable or acceptable variance of the CQAs with respect to this potential clinical impact. The underlying imprtance of this assumes the follwing key elemtns in the model construction:

1. The relationship of the clinical outcome and CQA to some extent can be modeled or assessed.
2. We assume this relationship for y to x, for the purposes of analysis, may be subject to MVLR (Multi-Variate Linear Regression).
3. There is enough data (i.e. n) to assess for normality of the variances of the clinical outcome, and also perhaps the CQA measure of interest.
4. The aim is to build a statistical argument that the dependence of y can be tied to a definable 't-test' on x of sorts to assess CQA differnces and the potential impact on y the clinical outcome. With the formulation of the dependence of the prediction of the clinical outcome observation $\hat{y}$we have an insightful implied coupling to the variance of the CQA x.

$$x \sim N\left(\mu_x, c^* \sigma_x^2\right) \implies \hat{y} \sim N\left(\mu_0, c\sigma_e^2\right)$$

The implication here is that the underlying variance of the CQA is closely related to the variance of the clinical outcome. Of note here is the use of c^* vs c with the former being a z-score factor for the CQAs and not the clinical outcome,

1.1 Measure of Closeness Based on the Absolute Difference

It should be noted that we have

$$(y - \hat{y}) \sim N\left(0, (1+c)\sigma_e^2\right).$$

Therefore, p_1 can be estimated by

$$\hat{p}_1 = \Phi\left(\frac{\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}\right) - \Phi\left(\frac{-\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}\right)$$

1.1.1 Understanding this formulation for p_1

- Φ represents the cumulative distribution function (CDF) of the standard normal distribution.
- δ is a clinically or scientifically meaningful difference.
- c and $\hat{\sigma}_e^2$ are parameters related to the variance in the model.
- The formula essentially calculates the probability that the absolute difference between the observed and predicted values is less than δ, under the assumption that the difference follows a normal distribution.

This formula likely comes from the properties of the normal distribution and the specific context of the study, where δ is a threshold for deciding if a difference is significant, and $\hat{\sigma}_e^2$ is an estimate of the variance of errors in the model. The use of the CDF Φ twice with δ and $-\delta$ accounts for the absolute difference being less than δ in both positive and negative directions.

To understand the derivation of the term

$$\frac{\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}$$

inside the cumulative distribution function (CDF) Φ in the equation

$$\hat{p}_1 = \Phi\left(\frac{\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}\right) - \Phi\left(\frac{-\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}\right),$$

we need to consider the context of statistical hypothesis testing and the normal distribution.

1. Context of the Problem:

- The term δ represents a predetermined margin of difference, which is considered clinically or scientifically significant.
- $\hat{\sigma}_e^2$ is an estimate of the variance of the error term in the model.
- The constant c is a scaling factor of variance in the statistical model.

2. Normalization in the Standard Normal Distribution:

- In a standard normal distribution, the variable of interest (say, y) is normalized by subtracting the mean and dividing by the standard deviation. This transforms the variable to have a mean of 0 and a standard deviation of 1 .
- In this context, δ is akin to the difference from the mean. The expression $\sqrt{(1+c)\hat{\sigma}_e^2}$ is the standard deviation of the distribution under consideration.

3. Deriving the Expression:

- The formula is normalizing the difference δ with respect to the modified standard deviation $\sqrt{(1+c)\hat{\sigma}_e^2}$. This is consistent with transforming a normal variable to a standard normal variable.
- Specifically, if the differences in the model are assumed to follow a normal distribution with a certain variance, then δ (the threshold of difference) is normalized by this standard deviation to understand its significance relative to the variability in the data.

4. Conceptual Understanding:

- This expression is essentially a Z-score, which is a common statistical measure used to describe a data point's relationship to the mean of a group of data points, in terms of standard deviations.
- In the context of hypothesis testing, this normalized value is then used with the CDF Φ to calculate the probability of observing a difference as extreme as δ, considering the variance in the data.

This transformation into a standard normal form allows the use of standard normal distribution tables (or the CDF) to determine probabilities, which is a common approach in statistical hypothesis testing.

1.2 The Variance of p_1

Using the delta method through a Taylor expansion, for a sufficiently large sample size n,

$$\text{var}\left(\hat{p}_1\right) \approx \left(\phi\left(\frac{\delta}{\sqrt{(1+c)\sigma_e^2}}\right) - \phi\left(\frac{-\delta}{\sqrt{(1+c)\sigma_e^2}}\right)\right)^2 \frac{\delta}{2(1-\delta)(n-2)\sigma_e^2},$$

where $\phi(z)$ is the probability density function of a standard normal distribution. Furthermore, $\text{var}\left(\hat{p}_1\right)$ can be estimated by V_1, where V_1 is given by

$$V_1 = \frac{2\delta^2}{(1+c)(n-2)\hat{\sigma}_e^2}\phi^2\left(\frac{\delta}{\sqrt{(1+c)\hat{\sigma}_e^2}}\right).$$

1.3 Testing the null hypothesis

By Slutsky's theorem, $\hat{p}_1 - p_0/\sqrt{V_1}$ can be approximated by a standard normal distribution. For the testing of the hypotheses $H_0 : p_1 \leq p_0$ versus $H_a : p_1 > p_0$, we would reject the null hypothesis H_0 if

$$\frac{\hat{p}_1 - p_0}{\sqrt{V_1}} > z_{1-\alpha}$$

where $z_{1-\alpha}$ is the $100(1-\alpha)$ th percentile of a standard normal distribution

1.4 Implications for CQA range limitting

This gives us a general recipe to approach these Biosimilarity assessment problems.

To limit variation in clinical outcomes, we aim to set boundaries on the range of CQAs. Based on our initial assumption of linearity:

$$\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x.$$

The differential form, where β_1 is a regression constant, becomes:

$$\partial\hat{y} = \beta_1 \partial x.$$

This supports the general notion:

$$x \sim N\left(\mu_x, c^*\sigma_x^2\right) \Longrightarrow \hat{y} \sim N\left(\mu_0, c\sigma_e^2\right).$$

From here, one might hypothesize:

$$c^* \sim \beta_1 c.$$

It is worth noting that in the absence of clinical assessment this hypothesis may challenged by health authorities. However, to-date there does not seem to be batter approach that has been put forward in the literature.

This hypothesis provides a framework for approaching Biosimilarity assessment problems:

1. We must assume that we can preserve the linear mapping $\hat{y} = \hat{\beta}_0 + \hat{\beta}_1 x$.
2. Analyze the probability of variance in x and establish control limits to ensure minimal impact of CQA changes on the clinical outcome y.
3. Leverage the relationship $c^* \sim \beta_1 c$ as a general rule
4. Tune c^* in $x \sim N\left(\mu_x, c^* \sigma_x^2\right)$ to maintain expected clinical outcomes
5. Apply a risk-based approach to adjust c^* based on the potential impact of a CQA.

In cases where the probability distribution function (pdf) is non-normal or unknown, it's reasonable to adopt a non-parametric method for variance assessment of x. This approach suggests that the linear mapping could preserve the non-parametric (nonnormal pdf) characteristics from x to y.

The leap to non-parametric methods is perhaps significant. We emphasize the conditions under which this transition is valid or more useful. For Clinical IND phase 1 studies and comparison of data - there is little room beyond this approach, unless there is access to additional data. Note that the non-parametric approach is a contingency for when normality assumptions do not hold, and when the number of samples is also low.

2 BioEquivalance assessment for smaller n with assumed underlying normality

2.1 Addressing Small Samples Sizes of n

In previous sections, we discussed scenarios involving larger sample sizes, typically $n > 12$, where the Taylor expansion is applicable for deriving estimates of the variance of p_i. This method, however, may not be suitable for smaller sample sizes.

For cases where the sample size is notably small, and the assumption of normality is questionable, an alternate estimation strategy is required for p_i.

We propose examining two distinct approaches, particularly relevant for small sample sizes:

2.2 Estimation for Small Samples Utilizing Range-Based Standard Deviation under Assumed Normal Distribution

In scenarios with small sample sizes, where it's reasonable to assume that the measure's variance is normal or nearly normal, a range-based estimate can be used to approximate the variable of interest's variance.

- A commonly used rule of thumb for estimating the sample standard deviation in small samples is:

$$\sigma \approx \frac{\text{sample range}}{4}$$

However, this method has limitations, particularly in its failure to account for the sample size dependent uncertainty of standard deviation.

A noteworthy advancement was proposed by undergraduate math researchers at Rose-Hulman in 2012 [2]. Their work, based on R simulations, suggests an improved range-based estimate:

$$\sigma \approx \frac{\text{range}}{3\sqrt{\ln n} - 1.5}$$

agin considering the general notion:

$$x \sim N\left(\mu_x, c^* \left[\frac{\text{range}}{3\sqrt{\ln n} - 1.5}\right]^2\right) \Longrightarrow \hat{y} \sim N\left(\mu_0, c\sigma_e^2\right).$$

This refined approach leverages the sample range as a surrogate for variability, a typical method in small sample size analysis. It still, however, hinges on the assumption of normal distribution for calculating probabilities using the CDF Φ. This methodology can streamline the TOST analysis z-score calculation, particularly when there is a prior basis to assert that both the reference originator material and new test articles exhibit normal variance. This situation might occur, for example, with similar antibodies under comparable testing conditions. However, in the majority of biosimilar Investigational New Drug (IND) studies, there often exists limited understanding of the original analytical method and process variance for a given Critical Quality Attribute (CQA), making this approach less commonly applicable.

3 BioEquivalance assessment for smaller n with no assumed underlying normality

When we have a small sample size that we would like to evaluate, assuming the sample pdf is not always known a priori. We consider here the approach of using a non-parametric assessment of the originator data (for some sample size n_{RP}) to our new test data (for some sample size n_{test}).

3.1 Non-Parametric Approach for Small Samples

Non-parametric methods don't assume a specific distribution for the data, making them suitable for small samples where normality cannot be assumed.

- **Boostrapping:** One approach could involve using bootstrapping or resampling techniques. By resampling the small dataset (with replacement) many times, we can create a distribution for $\hat{p}_i$ for x estimates. This distribution can then be used to determine confidence intervals or test hypotheses without relying on normal distribution assumptions.
- **Rank Based Statistics:** Another approach could be to use rank-based statistics. For example, one could calculate p_i for x based on the ranks of the observed values rather than their actual magnitudes. This could involve comparing the ranks of the observed values to the expected ranks under some null hypothesis.
- **Applied ML:** A more creative approach might involve leveraging machine learning algorithms that are robust to small sample sizes and do not assume normality. For example, decision tree-based methods or certain types of Bayesian models might be able to provide insights into the distribution and behavior of p_i under small sample conditions.

The key challenge is to adapt the method to the limitations of small sample sizes while still capturing the essence of what p_i for x represents in the context of the study. These approaches offer ways to estimate p_i for x that are less reliant on large sample theory and normality assumptions. The specific choice depends on the nature of the data and the exact requirements of the analysis.

3.2 Employing Rank Based Statstics

A rank-based approach can be particularly useful in non-parametric statistics, especially when dealing with small sample sizes or when the normality assumption is questionable.

3.2.1 Basic outline or "recipe" for using rank-based statistics to estimate p_i:

1. Understand the Context and Data:

- Identify the variable of interest (e.g., difference between observed and predicted values, response times, etc.).
- Gather your small sample data set.

2. Rank the Data:

- Assign ranks to each data point in your sample. The smallest value gets rank 1 , the next smallest rank 2, and so on.
- In case of ties (equal values), assign the average of the ranks that would have been assigned to all the tied values.

3. Calculate Rank Statistics:

- Depending on your specific hypothesis or research question, calculate relevant rank-based statistics. Examples include:
  - **Wilcoxon Signed-Rank Test:** Used for comparing two related samples or repeated measurements (aka "paired data") on a single sample to assess whether their population mean ranks differ.
  - **Mann-Whitney U Test:** Used to compare differences between two independent groups when the dependent variable is ordinal or continuous but not normally distributed. Applicable to "two groups", non-paired", with "unequal sample sizes".
  - **Kruskal-Wallis H Test:** An extension of the Mann-Whitney U Test for more than two groups. Applicable to "two or more groups", non-paired", with "unequal sample sizes".

4. Estimate p_i for x Using Rank Statistics:

- The specifics of this step depend on your hypothesis and data. For instance, if you're testing if the median of the differences is significantly different from zero, you might use the Wilcoxon Signed-Rank Test.
- The test statistic from these rank-based tests can be used to calculate a p-value, which indicates the probability of observing the obtained result, or more extreme, under the null hypothesis.

5. Interpret the Results:

- A small p-value (typically < 0.05, 95% confidence interval) suggests that the observed effect (e.g., difference between groups) is statistically significant and not likely due to chance.

- Interpret the results in the context of your research question, taking into account the limitations of rank-based methods.

6. Report Your Findings:

- Clearly report the methodology, the rank-based statistic used, the p-value, and your interpretation.
- Discuss any limitations or assumptions inherent in your analysis.

Advantages and Limitations: - **Advantages:** Rank-based methods are robust against outliers and do not assume normal distribution. They are ideal for small sample sizes. - **Limitations:** These methods might be less powerful than parametric tests when the normality assumption is met. Also, they typically focus on median differences rather than mean differences.

By following this approach, you can effectively utilize rank-based statistics for your small sample size analysis, providing a robust alternative to parametric methods that rely on normality assumptions.

4 Practical Example Ranked Based Statistics on Small n, without assuming normality

In practice, these calculations, especially for Mann-Whitney U and Kruskal-Wallis H tests, can be complex and are typically performed using statistical software like R, Python's SciPy library, or statistical calculators.

Let's proceed with a practical calculation for the Mann-Whitney U Test using R.

4.1 Application of the ranked approach for a two groups of sample size of 8 and 3 (as an example)

4.1.1 Example Dat Set:

Lets assume two sets one of 8 samples, being the reference set to create the rank based statistics. And a set of 3 additional values to compare against the reference set.

Here are the basis set of $n_{RP} = 8$ values:
{98.13, 98.39, 98.85, 98.43, 98.33, 98.98, 98.81, 99.1}
Here are $n_{test} = 3$ values to compare to the basis set (simliar set):
{98.6, 98.5, 98.2}

Lets also compare to a dis-similar set: **{97.6, 97.5, 97.2}**

To compare the two sets we will choose the Mann-Whitney U Test typically used for comparing two independent samples., which is suitable for the data.

4.1.2 Mann-Whitney U Test:

1. *Rank All Data Together:* Combine both sets and rank them from the smallest to the largest, regardless of which group they belong to.
2. *Calculate U Statistic:* Sum the ranks for each group separately. The U statistic is calculated using these rank sums and the sizes of each group.
3. *Determine Significance:* Compare the U statistic to a critical value from a Mann-Whitney U table or calculate a p-value using a statistical software.

4.1.3 Example 1 Practical Application Calculation (non-parametric unequal size, similar data sets)

R CODE TO COMPLETE THE CALCULATION:
(results of the example calculation follow below)

```
# Install necessary package if not already installed
if (!requireNamespace("stats", quietly = TRUE)) {
    install.packages("stats")
}

# Load the stats package
library(stats)

# Data Sets
n_index <- c(1,2,3,4,5,6,7,8)
basis_set <- c(98.13, 98.39, 98.85, 98.43, 98.33, 98.98, 98.81, 99.1)
comparison_set <- c(98.60, 98.50, 98.20)
max_length <- max(length(basis_set), length(comparison_set))

length(n_index) <- max_length
length(basis_set) <- max_length
length(comparison_set) <- max_length

non_param_set = cbind(n_index, basis_set, comparison_set)

np_data <- data.frame(basis_set, comparison_set)

np_data
```

```
##   basis_set comparison_set
## 1     98.13           98.6
## 2     98.39           98.5
## 3     98.85           98.2
## 4     98.43             NA
## 5     98.33             NA
## 6     98.98             NA
## 7     98.81             NA
## 8     99.10             NA
```

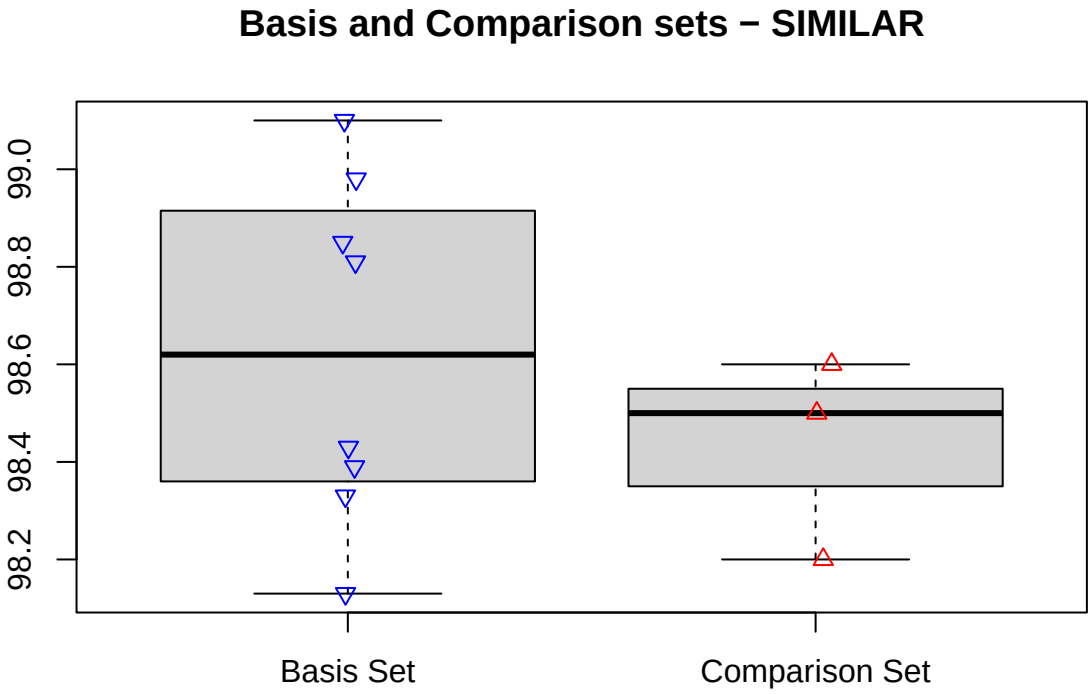
```
# table of values
knitr::kable(non_param_set, align = "ccc", caption = "Non-parametric Test
↪  Set")
```

Table 1: Non-parametric Test Set

n_index	basis_set	comparison_set
1	98.13	98.6
2	98.39	98.5
3	98.85	98.2
4	98.43	NA
5	98.33	NA
6	98.98	NA
7	98.81	NA
8	99.10	NA

```
# Boxplot
boxplot(np_data, names=c("Basis Set", "Comparison Set"))
title(main="Basis and Comparison sets - SIMILAR")

# Adding jittered points
points(jitter(rep(1, length(np_data$basis_set))), np_data$basis_set,
↪  col="blue", pch=25)
points(jitter(rep(2, length(np_data$comparison_set))),
↪  np_data$comparison_set, col="red", pch=24)
```


Basis and Comparison sets − SIMILAR
99.0
98.8
98.6
98.4
98.2
Basis Set
Comparison Set

```r
# Mann-Whitney U Test
u_test_result <- wilcox.test(basis_set, comparison_set, alternative =
↪  "two.sided")

# Output the results
cat("Mann-Whitney U Test Results:\n")
```

```
## Mann-Whitney U Test Results:
```

```r
cat("note - this is Mann-Whitney result, not Wilcoxon, \n")
```

```
## note - this is Mann-Whitney result, not Wilcoxon,
```

```r
print(u_test_result)
```

```
##
##  Wilcoxon rank sum exact test
##
## data:  basis_set and comparison_set
## W = 15, p-value = 0.6303
## alternative hypothesis: true location shift is not equal to 0
```

Interpretation: Mann-Whitney U Test: The high p-value (0.6303) suggests that there is not a statistically significant difference between the two groups. This means that the distribution of values in the basis set is not significantly different from the distribution in the comparison set.

These results suggest that, based on the data provided, there is no statistical evidence to suggest a difference between the basis set and the comparison set. It's important to note that the Mann-Whitney U Test is more appropriate here, given the two independent samples.

4.1.4 Example 2 Practical Application Calculation (non-parametric unequal size, similar data sets)

R CODE TO COMPLETE THE CALCULATION:
(results of the example calculation follow below)

```r
# Install necessary package if not already installed
if (!requireNamespace("stats", quietly = TRUE)) {
    install.packages("stats")
}

# Load the stats package
library(stats)

# Data Sets
n_index <- c(1,2,3,4,5,6,7,8)
basis_set <- c(98.13, 98.39, 98.85, 98.43, 98.33, 98.98, 98.81, 99.1)
comparison_set <- c(97.60, 97.50, 97.20)
max_length <- max(length(basis_set), length(comparison_set))

length(n_index) <- max_length
length(basis_set) <- max_length
length(comparison_set) <- max_length

non_param_set = cbind(n_index, basis_set, comparison_set)

np_data <- data.frame(basis_set, comparison_set)
```

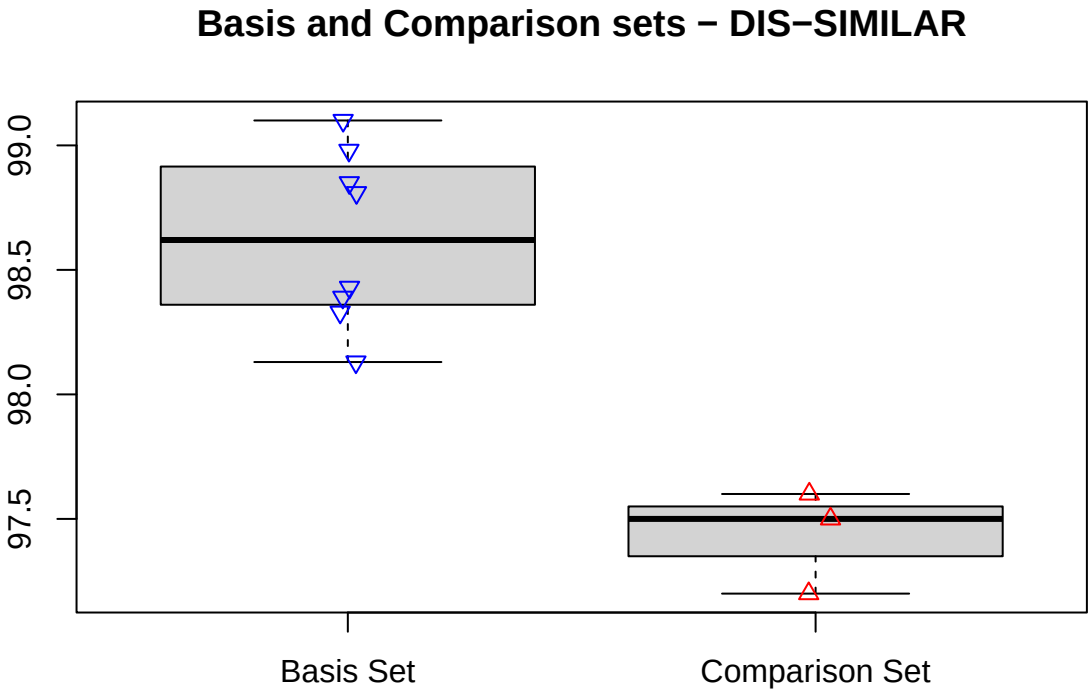
```
# table of values
knitr::kable(non_param_set, align = "ccc", caption = "Non-parametric Test
↪  Set")
```

Table 2: Non-parametric Test Set

n_index	basis_set	comparison_set
1	98.13	97.6
2	98.39	97.5
3	98.85	97.2
4	98.43	NA
5	98.33	NA
6	98.98	NA
7	98.81	NA
8	99.10	NA

```
# Boxplot
boxplot(np_data, names=c("Basis Set", "Comparison Set"))
title(main="Basis and Comparison sets - DIS-SIMILAR")

# Adding jittered points
points(jitter(rep(1, length(np_data$basis_set))), np_data$basis_set,
↪  col="blue", pch=25)
points(jitter(rep(2, length(np_data$comparison_set))),
↪  np_data$comparison_set, col="red", pch=24)
```


Basis and Comparison sets − DIS−SIMILAR
99.0
98.5
98.0
97.5
Basis Set
Comparison Set

```r
# Mann-Whitney U Test
u_test_result <- wilcox.test(basis_set, comparison_set, alternative =
↪  "two.sided")

# Output the results
cat("Mann-Whitney U Test Results:\n")
```

```
## Mann-Whitney U Test Results:
```

```r
print(u_test_result)
```

```
##
##  Wilcoxon rank sum exact test
##
## data:  basis_set and comparison_set
## W = 24, p-value = 0.01212
## alternative hypothesis: true location shift is not equal to 0
```

Interpretation: Mann-Whitney U Test: The low p-value (0.01212) suggests that there is a statistically significant difference between the two groups. This means that the distribution of values in the basis set is significantly different from the distribution in the comparison set.

These results suggest that, based on the data provided, there is statistical evidence to suggest a difference between the basis set and the comparison set. It's important to note that the Mann-Whitney U Test is more appropriate here, given the two independent samples.

5 Case Study: Henlius HLX02 (trastuzumab), BLA Tiered Biosimilarity

This case is presented to show how to assess for biosimilarity based on larger n and and the assumption of normality.

Key points to consider in this section:

1. The general notion $x \sim N(\mu_x, c^*\sigma_x^2) \Longrightarrow \hat{y} \sim N(\mu_0, c\sigma_e^2)$, is well supported here.
2. A tiered approach is employed to classify the potential level of impact of the CQAs on clinical outcome. The deatils are presented below.
3. In this case the value of c^* is set at 1.5 for Tier 1 and 3.0 for tier 2. This is consistent with NMPA and FDA guidances.

5.1 Underlying scope of the Helius published results and approach based on NMPA and USFDA Tiered Approach

For the BLA submittal of HLX02 - Henlius has openly published [3: Xie, et al, 2020] their assessment of biosimilarity. This was applied using the NMPA guidelines [4: NMPA 2021] for when n is large enough to assess normality. In this case a specific statistical analysis approach was used:

5.2 Statistical Analysis framework for Henlius HLX02 (trastuzumab)

The quality attributes of trastuzumab were ranked according to the potential impact on clinical efficacy and safety following the QbD quality study and extensive characterization of the orginator RPs. The standard deviation (σ) of the RPs is used to determine similarity acceptance criteria. Equivalence testing was applied for attributes ranked as tier 1, those with the highest potential clinical impact. An interval (-1.5σ, $+1.5\sigma$) that can support a 90% confidence interval was applied in two one-sided tests (TOST, p=0.1) to determine the similarity of HLX02 and the orginator RPs. A quality range approach was employed for attributes categorized as tier 2, those with a moderate risk ranking. Similarity to the orginator RP can be determined when 90% of HLX02 data falls into the quality range ($\mu - 3\sigma$, $\mu + 3\sigma$). Tier 3 attributes, those with the lowest risk ranking, were evaluated by visual comparison. The qualitative result of any test was evaluated by visual comparison also, regardless of its risk ranking.

The NMPA suggests a tiered approach to the assessment. [4: NMPA 2021]

The Henlius paper for HLX02 provides an extensive analysis of the biosimilar HLX02,

Table 3: Statistical Analysis Framework for Henlius HLX02 (Trastuzumab)

Tier	**Attribute Ranking**	**Statistical Rule**
Tier 1	Attributes with the highest potential clinical impact.	Equivalence testing with interval $(-1.5\sigma, +1.5\sigma)$ supporting a 90% confidence interval in two one-sided tests (TOST, $p = 0.1$) to determine similarity.
Tier 2	Attributes with a moderate risk ranking.	Quality range approach where similarity is determined if 90% of data falls into the range $(\mu-3\sigma, \mu+3\sigma)$.
Tier 3	Attributes with the lowest risk ranking.	Evaluated by visual comparison.

comparing it to both Europe-sourced and China-sourced Herceptin® (trastuzumab), a monoclonal antibody used to treat HER2-positive breast cancer. The primary objective was to assess the analytical similarity between HLX02 and Herceptin® following a quality-by-design (QbD) quality study and a tier-based quality attribute evaluation. This assessment is crucial for the biosimilar's approval for Biologics License Application (BLA) registration.

Table 4: Tiered Approach for Biosimilar Assessment

Tier	**Focus Area**	**Concepts and Logic**
Tier 1	Bio-functional Attributes	Assess the biosimilar's therapeutic action and efficacy. Examine critical functions such as binding to targets, inhibiting cell proliferation, and inducing cytotoxicity. Ensure the biosimilar's clinical effect is equivalent to the reference product.
Tier 2	Structural Properties	Investigate the molecular structure, including primary and higher-order structures. Analyze glycan profiles and their impact on the drug's efficacy and safety. Confirm structural similarity to ensure consistent biological function.
Tier 3	Stability and Consistency	Evaluate the biosimilar's stability under various conditions. Perform degradation studies to assess the consistency of the biosimilar over its shelf life. Provide additional assurance of quality and performance compared to the reference product.

5.3 Tiered approach for HLX02

Based on the paper's summary and the tiered approach to biosimilar assessment, the items were categorized as follows:

Tier I (Critical Bio-functional Attributes):
Biological and Immunological Activities: HLX02's similarity to Herceptin® in key

activities such as HER2 binding, anti-proliferation, and ADCC (antibody-dependent cell-mediated cytotoxicity).

Tier II (Structural Properties):
Analytical Similarity: Similarity of HLX02 to EU-Herceptin® and CN-Herceptin® in terms of structural, functional, and glycan profile, particularly with Herceptin® batches having high FcγRIIIa affinity.

Structural and Functional Properties: The shared amino acid sequence of HLX02 with Herceptin®, and their highly similar primary and higher-order structures. Glycan Profile Variability: Minor differences in sialylation between HLX02 and Herceptin®, with the glycoform profiles showing high similarity.

5.4 Overall Approach to the Henlius HLX02 paper findings:

Using the tiered approach, the paper concisely and effectively underscores these key points:

Analytical Similarity: HLX02 exhibits high analytical similarity to EU-Herceptin® and CN-Herceptin® in structural, functional, and glycan aspects, aligning closely with high FcγRIIIa affinity Herceptin® batches.

Structural and Functional Properties: HLX02 shares Herceptin®'s amino acid sequence. Primary and higher-order structures are highly similar. Variations in glycan moieties and charge variants are within Herceptin®'s batch variability range.

Glycan Profile: Slight difference in sialylation between HLX02 and Herceptin®, less than Herceptin®'s batch-to-batch variability. Glycoform profiles show high similarity, indicating no safety or efficacy concerns.

Biological and Immunological Activities: HLX02 matches Herceptin® in key activities: HER2 binding, anti-proliferation, and ADCC. Supports HLX02's biosimilarity in therapeutic function.

Stability and Degradation: Forced degradation studies confirm HLX02's stability and degradation patterns align with Herceptin®, ensuring product quality consistency.

FcγRIIIa Affinity: HLX02 more closely resembles high FcγRIIIa affinity Herceptin® batches. FcγRIIIa affinity chromatography enhances biosimilarity evaluation by detecting functional activity nuances.

BLA Registration: Overall, HLX02 is highly similar to Herceptin®, with no significant clinical differences. The study's thorough physicochemical, bio-functional, and degradation analyses support HLX02's BLA registration candidacy.

6 Non-parametric Approach for IND: Small Sample Size Tiered Biosimilarity without Normality

This case is presented to show how to assess for biosimilarity based on smaller n and and the without assumption of normality.

Key points to consider in this section:

1. The general notion $x \sim N(\mu_x, c^*\sigma_x^2) \Longrightarrow \hat{y} \sim N(\mu_0, c\sigma_e^2)$, is not assumed here.
2. A tiered approach is employed to classify the potential level of impact of the CQAs on clinical outcome. The deatils are presented below.
3. The underlying pdf is NOT known, and a non-parametric "two-sided" Mann-Whitney U Test approach is recommended for unequal sample sizes of un-paired data.
4. The value of $p_1 > 0.2$ (80% confidence rejection of unequal data is assumed) is set at Tier 1 and $p_1 > 0.05$ (95% confidence rejection of unequal data is assumed) for Tier 2.

Table 5: Non-paramertic Approach for IND: Small Sample Sized Tiered Biosimilarity w/o Normality

Tier	**Attribute Ranking**	**Statistical Rule**
Tier 1	Attributes with the highest potential clinical impact.	Equivalence testing with two-sided non-parametric Mann-Whitney U $p_1 > 0.2$ a 80% confidence of rejection of similarity.
Tier 2	Attributes with a moderate risk ranking.	Equivalence testing with two-sided non-parametric Mann-Whitney U $p_1 > 0.05$ a 95% confidence of rejection of similarity.
Tier 3	Attributes with the lowest risk ranking.	Evaluated by visual comparison.

7 Closing Remarks: Justification and Limitations of the Linear Model Assumption in Biosimilarity Assessment

In our biosimilarity assessment framework, we initially assume a linear relationship between Critical Quality Attributes (CQAs) and clinical outcomes. This linear model is predicated on the notion that changes in CQAs (denoted as x) linearly affect the clinical response (y), which is a simplification often used for its interpretability and ease of analysis. The model is expressed as $y = \beta_0 + \beta_1 x + \epsilon$, where β_0 and β_1 are regression coefficients and ϵ represents random error.

Justification for the Linear Model:

1. Simplicity and Interpretability: Linear models provide a straightforward way to quantify the relationship between CQAs and clinical outcomes, making it easier to interpret and communicate findings.
2. Initial Analysis Tool: In many biosimilar studies, linear models serve as an initial tool for analysis. They offer a starting point to understand trends and guide further, more complex analyses.
3. Statistical Efficiency: Linear models require fewer parameters and assumptions, making them statistically efficient for initial exploratory analysis, especially in larger datasets.

Limitations and Situations Where the Model Might Not Hold:

Nonlinearity in Biological Systems: Biological systems often exhibit nonlinear behavior, where the effect of a CQA on clinical outcomes is not proportionally constant. For instance, the relationship might be logarithmic, exponential, or follow a threshold effect.

Interaction Effects: Linear models may not adequately capture interaction effects between multiple CQAs. In reality, the combined effect of different CQAs might be synergistic or antagonistic, deviating from linearity.

Model Mis-specification: Relying solely on a linear model risks model misspecification, where the true nature of the relationship is oversimplified or inaccurately represented. This can lead to erroneous conclusions and affect the biosimilarity assessment's reliability.

Impact on Biosimilarity Assessment:

- When the linear model assumption does not hold, it can lead to incorrect estimates of the effect size, variance, and ultimately the biosimilarity conclusion.
- Nonlinearity may require more complex modeling techniques, such as polynomial regression, logistic regression, or non-parametric methods, to accurately capture the

relationship between CQAs and clinical outcomes.

- Recognizing the limitations of linear models, it is crucial to perform diagnostic checks (e.g., residual analysis) and consider alternative models when discrepancies or anomalies are observed in preliminary linear model analysis.

In summary, while the linear model assumption offers a convenient and straightforward approach to start the biosimilarity assessment, it is essential to recognize its limitations. Careful consideration and validation of this assumption, alongside exploratory data analysis and alternative modeling strategies, ensure a robust and accurate assessment of biosimilarity.

References

1. **Endrenyi L, Declerck P, Chow S-C (eds)**. Chapter 3, analytical similarity assessment. In: *Biosimilar drug product development*. CRC Press, Taylor & Francis Group; 2017. pp. 88–108.

2. **Ramirez A**, **Cox C**. Improving on the Range Rule of Thumb. *Rose-Hulman Undergraduate Mathematics Journal*;13. https://scholar.rose-hulman.edu/rhumj/vol13/iss2/1 (2012, accessed 3 December 2023).

3. **Xie L**, **Zhang E**, **Xu Y**, **Gao W**, **Wang L**, *et al.* Demonstrating Analytical Similarity of Trastuzumab Biosimilar HLX02 to Herceptin® with a Panel of Sensitive and Orthogonal Methods Including a Novel FcγRIIIa Affinity Chromatography Technology. *BioDrugs* 2020;34:363–379.

4. **NMPA**. Center for Drug Evaluation, State Drug Administration. Technical Guidelines for Biosimilars Similarity Evaluation and Indication Extrapolation. *NMPA Published Guidelines*. https://www.cde.org.cn/main/news/viewInfoCommon/d92c6507a57bee9ccfc5baa1ee87fda9 (2021, accessed 3 December 2023).