Study Notes

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Contents

1 Non-inferiority test on response rate

1.1 Study Information

- Client name: Adello biologics, LLC (Formerly known as Therapeutic Proteins, LLC)
- Celerion Study No. CA18641

NOTE FROM BIN (1): in the experiment, subjects will undergo the following steps (in order):

- ***ADA screening test***. If the screening result is *NEGATIVE*, then the result will be recoreded *NEGATIVE*, otherwise screening *POSITIVE* and continue
- ***ADA confirmatory test***. If the result is *NEGATIVE*, then the subject will have a confirmatory *NEGATIVE* result, otherwise a *POSITIVE* result with a "titer" value recorded and continue
- ***ADA neutralizing test***. If the result is *NEGATIVE*, then the subject will have a neutralizing *NEGATIVE* result, otherwise *POSITIVE* with a "titer" value recorded and there will be no more test.

NOTE FROM BIN (2): The cut-off value to determine whether a subject has *POSITIVE* or *NEGATIVE* ADA result may be different (per assay). Therefore, when (if needed) analyzing titer results, there should be a batch effect.

1.2 Background

Therapeutic proteins (sometimes also called biologics, biopharmaceuticals, biological products, or biological medicinal products) and peptides have the potential to induce immunogenicity. The consequences of product immunogenicity vary from no evidience of clinical effect to severe, life-threatening responses. Anti-drug antibodies (ADA) have been implicated in infusion reactions and anaphylaxis as well as immune complex-mediated disease. ADA have also caused secondary treatment failures (loss of efficacy) and, in rare occasions, more serious thrombocytopenia and pure red cell aplasia. Therefore, ADA are a medical concern in terms of safety and long-term efficacy of the drug and it is critical to evaluate their development in all patients during clinical studies, not just in a symptom-driven manner. With a goal of guiding medical practice, the elucidation of ADA responses and their characteristics relative to clinical consequences is vital (Shankar et al., 2014).

ADA comprises neutralizing and non-neutralizing ADA. Other terms that have been used for ADA include anti-therapeutic antibody (ATA), anti-product antibody (APA), or anti-biologic antibody (ABA).

- Neutralizing ADA (NAb): ADA that inhibits or reduces the pharmacological activity of the biologic drug molecule, as determined by an *in vitro* test or animal-based bioassay method, regardless of its *in vitro* clinical relavence (i.e., whether or not test method results relate to clinical impact in the subject).
- Non-neutralizing ADA (non-neutralizing antibody, non- NAb): ADA that binds to the biologic drug molecule but does not inhibit its pharmacological activity in an in vitro test or animal-based bioassay method, regardless of its in vivo clinical relevance (i.e., whether or not test method results relate to clinical impact in the subject).

1.3 Case Study

The goal of this study is to compare immunogenicity of Theragrastim® (new drug) and Neupogen® (reference drug) after multiple subcutaneous (SC) administrations in healthy subjects. ADA levels for Theragrastim® and Neupogen® will be estimated and compared to evaluate potential difference between the two products in the incidence of human immune responses.

This is a one center, single-blind, randomized, parallel, multiple-dose, safety and immunogenicity study. A total number of one hundred thirty four (134) healthy adult male and female subjects will be enrolled and randomized to 1 of 2 treatments (67 subjects per treatment).

The sample size is chosen based on a target of 61 subjects per arm as calculated, to which 6 subjects (~10%) were added to each arm to account for potential dropouts. With 61 subjects per arm, the trial can show, with 80% power, that the upper bound of the one-sided 95% confidence interval of the difference in ADA+ rates between the two products is below (or above) the non-inferiority margin (10%)

The power calculation for sample size is based on the following assumptions:

- The ADA+ rate of Neupogen® is 3.3%
- The ADA+ rate of Theragrastim® is 3.3%
- The mean ADA+ rate difference (δ) between the two products is zero;
- The NI margin (δ_0) is 10%.

The power calculation is based on exact method (Chan and Zhang, 1999) using δ-projected Z-statistic (i.e., the score statistic) with REML estimation procedure (Miettinen and Nurminen, 1985).

1.4 Statistical Analysis

The rate (or proportion) of subjects that have ADA+ in confimatory test and neutralizing test (if needed) will be compared between Theragrastim® and Neupogen® treatments to determine if any differences are statistically meaningful.

1.4.1 Statistical method

The rate difference between Theragrastim® and Neupogen® will be defined as:

$$\delta = \pi_1 - \pi_2 \tag{1}$$

where π_1 is the ADA+ rate of Theragrastim® and π_2 is that of Neupogen®.

In hypotheses testing, the research or alternative hypothesis represents what the study aims to show. The null hypothesis is the opposite of the research hypothesis and is what the investigator hopes to disprove (Walker and Nowacki, 2011). Therefore, the primary statistical hypothesis for the clinical trial will be tested using

$$H_0 : \pi_1 - \pi_2 \geq 0.10 \quad VS \qquad H_1 : \pi_1 - \pi_2 < 0.1 \tag{2}$$

Confidence intervals (CIs) will be calculated using the Farrington-Manning method (δ-projected Z-statistic) recommended by Chan and Zhang (1999).

Note that in SAS, the null in Expression (2) is equivalently stated as

$$H_0 : \pi_2 - \pi_1 \leq -0.10 \quad VS \qquad H_1 : \pi_2 - \pi_1 > -0.1 \tag{3}$$

Therefore, in the SAS output, the null is rejected (i.e., Theragrastim® is non-inferior to Neupogen® in terms of ADA+ rate) if the lower bound of the one-sided 95% CI of the difference is above NI margin (-0.10).

1.4.2 SAS implementation

The SAS code (Castelloe and Watts, 2015) is provided as follows:

```
proc FREQ data= test order=data;
    tables time*treat*Response / nopercent nocol nocum
    riskdiff(noninf margin=0.1 method=fmscore norisks)
    alpha = 0.05;
```

```
    exact riskdiff(method = score);
    weight Count;
    ods output CrossTabFreqs = frequency
    PdiffCLs = conf
    pdiffnoninf = pvals
    ;
run;

Programmer note: the above code produces results as desired only if
the input data set has the same structure as the data set created by
the following code. If not, please sort the test data by time treat
and response as this one.


data test;
input Time $ treat $ response $ count;
datalines;
    C2 neupogen positive 5
    C2 neupogen negative 62
    C2 theragrastim positive 3
    C2 theragrastim negative 64
    C3 neupogen positive 5
    C3 neupogen negative 62
    C3 theragrastim positive 3
    C3 theragrastim negative 64
    C4 neupogen positive 5
    C4 neupogen negative 62
    C4 theragrastim positive 3
    C4 theragrastim negative 64
    ;
Run;
```

References

Castelloe, John and Donna Watts. 2015. "Equivalence and Noninferiority Testing Using SAS/STAT Software." *SAS Institute Inc.* Paper SAS1911-2015.

Chan, Ivan SF and Zhongxin Zhang. 1999. "Test-Based Exact Confidence Intervals for the Difference of Two Binomial Proportions." *Biometrics* 55(4):1202–1209.

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Shankar, Gopi, S Arkin, L Cocea, V Devanarayan, S Kirshner, A Kromminga, V Quarmby, S Richards, CK Schneider, M Subramanyam et al. 2014. "Assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations." *The AAPS journal* 16(4):658–673.

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