RISE Primary Outcome Analysis Plan – Pediatric Medication Study
Approved by RISE Steering Committee, Sept 2017
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Often the Disposition Index (DI) – insulin sensitivity * insulin response – is used as an overall measure of beta-cell function that appropriately accounts for the reciprocal relationship of insulin sensitivity and the beta-cell's insulin response. In these analyses, the DI assumes that the product of the two variables is constant within an individual at a given time, such that changes in insulin sensitivity would be mirrored by a proportional change in the insulin response. This implies that all points along the line represent the same level of metabolic function. In many instances this relationship has been demonstrated using insulin responses to be a rectangular hyperbola (by definition, the slope of the log-log relationship equal to -1.0); however, in others this relationship has just been assumed. The power calculations for RISE were based on the DI using data provided by several investigators that used methodologies that differed from those used in RISE.

During protocol development there was thus concern that relationships underlying this constant depend on the actual measures of peptide (insulin or C-peptide) release by the beta cell. In particular, the slope of the log-log relationship between secretion and sensitivity might not be equal to -1.0 for the C-peptide measures chosen for RISE as had been observed in prior studies based on insulin measures. Therefore, the protocols specifically state that the primary outcomes would be based on two different C-peptide responses (steady state and maximal) adjusted for insulin sensitivity defined as the glucose disposal rate divided by steady state insulin (aka M/I) during the hyperglycemic clamp. However, given the likely possibility that the relationship may not be a rectangular hyperbola, the protocol did not specify details of the approach to be used for the primary outcome analysis as a decision would be based on evaluation of the baseline data.

During analysis of the baseline data, we found that the DI (i.e. sensitivity x secretion) is sometimes paradoxically lower in pediatric participants than adult participants, despite the fact that the insulin sensitivity vs. C-peptide curves describing the relationship between these two variables appears higher in children. This contradictory finding is at least in part due to the fact that the range of values for insulin sensitivity among children is narrow compared to that of adults, i.e., children are more insulin resistant than adults. Further, the log-log slopes of insulin sensitivity vs. C-peptide responses are not equal to -1 in children or adults (or overall). Although the untransformed data show a clear inverse relationship typical of a hyperbolic curve, the slopes for each of the primary outcome measures on the log scale is approximately -0.3 (not -1.0 as may have been expected); this is a hyperbola but not a square hyperbola. Thus, the approach of performing the primary outcome analysis comparing treatment groups after washout, with a test of difference in DI at Month 15 adjusted for baseline, may not be appropriate and needed to be reassessed.

Several options were considered including a simple linear regression model (on a log scale) of C-peptide (and insulin) release as a function of insulin sensitivity, with a term for treatment group and adjusting for both variables at baseline. However, this was also deemed inappropriate because that model would estimate the difference in C-peptide (and insulin) release between groups assuming that there was no difference in insulin sensitivity between groups. Rather, we want to account for movement of both variables simultaneously without forcing a specific relationship between them. This can be accomplished by performing the primary outcome analysis using two separate models: insulin sensitivity at Month 15 vs. treatment arm (adjusted for baseline) and C-peptide (and insulin) release at Month 15 vs. treatment arm (adjusted for baseline), where the two models are fit simultaneously using Seemingly Unrelated Regression techniques[1-5]. This provides an

estimate of the treatment group difference in insulin sensitivity as well as the treatment group difference in the release of the beta-cell peptides, while allowing for the correlation among the insulin sensitivity and peptide release measures. This yields an estimate of the joint covariance structure of the two models, and allows a joint statistical test of both variables using a 2-DF chi-square test of the treatment arm difference in each model. Thus, we will be able to test whether both the insulin sensitivity and C-peptide (and insulin) release variables are different across treatment groups at Month 15, adjusted for their baseline value.

This approach will provide a clear answer to the question of whether the Month 15 result differs by treatment, adjusting for baseline measures. However, given that an underlying reciprocal relationship is expected, it is possible that a significant difference could be found between groups, but that this represents a proportional shift without a specific improvement in peptide release adjusted for sensitivity. In other words, the data points could lie on a different part of a shared relationship curve such that the change represents a mutually compensated change in these terms without a separate underlying change in beta-cell function. Therefore, if the results of the two-model analysis are significant, further analysis will evaluate the patterns of change in either or both variables within each group.

Below is sample Primary Outcome R code for this primary analysis.

R code and sample output for primary outcome with Seemingly Unrelated Regression model using systemfit

```
> fit12=systemfit(list(Eq1 = log_mi ~ Treatment + log_mi_base,
                       Eq2 = log_cpeptide_steady ~ Treatment +
                                log_cpeptide_steady_base),
                method='SUR',
                data=RISEM15)

> linearHypothesis(fit12,test = "Chisq",
                   c('Eq1_TreatmentTreat2=0','Eq2_TreatmentTreat2=0'))

Hypothesis: Eq1_TreatmentTreat2 = 0    Eq2_TreatmentTreat2 = 0

Model 1: restricted model
Model 2: fit12

  Res.Df    Df    Chisq      Pr(>Chisq)
1   xxx
2   xxx-2    2     xxxx     x.xxx ← pvalue for the 2-DF joint statistical test
of both sensitivity and secretion variables of the treatment arm difference
```

References:

1. https://en.wikipedia.org/wiki/Seemingly_unrelated_regressions
2. https://www.jstatsoft.org/article/view/v023i04/v23i04.pdf
3. Zellner, Arnold. An efficient method of estimating seemingly unrelated regression equations and tests for aggregation bias". Journal of the American Statistical Association. 1962; 57: 348–368. doi:10.2307/2281644.
4. Srivastava, Virendra K.; Giles, David E.A. (1987). Seemingly unrelated regression equations models: estimation and inference. New York: Marcel Dekker.
5. Henningsen Arne, Hamann Jeff. systemfit: A Package for Estimating Systems of Simultaneous

Equations in R. Journal of Statistical Software. 2007; 23:1-40.