ITN055AI CALIBRATE

Rituximab Plus Cyclophosphamide Followed by Belimumab for the Treatment of Lupus Nephritis

Background

CALIBRATE will compare different B cell depletion strategies for the induction of tolerance in severe lupus nephritis (LN) patients. The hypothesis being tested is that inhibition of BAFF with belimumab will inhibit autoreactive B cell reconstitution in LN patients following B cell depletion with rituximab and cyclophosphamide, leading to long-term disease remission.

Both treatment arms utilize B cell depletion (with or without belimumab), therefore it is imperative to incorporate a study assay that is both precise and robust enough to detect differences in the proportion of autoreactive B cells between treatment groups at relevant tolerance checkpoints. The ideal mechanistic assay for this trial will meet the following criteria:

1. Be able to identify and enumerate autoreactive B cells in a variety of B cell compartments, including B cells that do not secrete immunoglobulin.
2. Be capable of reproducing results using the same frozen PBMC tested on different days.
3. Can demonstrate a statistically significant difference between i) patients with an autoimmune disease vs. healthy controls, or ii) patients following different treatment regimens, or iii) disease treatment status.
4. Be capable of analyzing a minimum of 80 samples and a maximum of 360 samples in a reasonable time frame.

Assay Evaluation

Please address each question below for your proposed assay and submit this form and supporting documents to Kristina Harris at kharris@immunetolerance.org by March 1st, 2014. ITN will conduct a review of proposals prior to April 1st, 2014. Promising assays will be discussed at the Network Steering Committee meeting in May of 2014; where you may be asked to present additional data in response to the review comments.

	Yes (data attached)*	**No**	**In progress (please explain)**
1. Are results from frozen PBMC consistent and reproducible?	☐	☐	☐
2. Does the assay identify & enumerate autoreactive B cells?	☐	☐	☐
3. Does the assay detect significant differences in autoreactive B cells between any of the following: i) patients with lupus (or another type of autoimmune disease) vs. healthy controls, or ii) patients following one treatment regimen vs. those following another, or iii) patients in remission vs. those with active disease or at time of flare	☐	☐	☐
4. Has the assay been performed using frozen PBMC from patients undergoing B cell depletion?	☐	☐	☐

*****For supporting data, include details such as patient status, sample size, assay controls, cell source, number of times the same end result was reproduced, and statistical considerations.

5. What technologies are required for this assay?

6. What population of B cells is evaluated, and what markers are used for identification?

7. How many cells are collected for analysis, and what proportion of B cells is evaluated?

8. What assay endpoint is used to confirm the B cells are autoreactive?

9. What is the assay throughput? Approximately, how much time will it take to test a minimum of 80 samples, and a maximum of 360 samples (40 participants x 2-9 time points)?

10. Number of supporting documents/files that have been submitted with this questionnaire to kharris@immunetolerance.org:

Investigator/Company Name: _____

Email_____ Phone_____

Signature:_____Date:_____