Plan discussed with Habil and Leslie on 07/24/19

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Study | Subject | Treatment | Sex | Type of sample | PRE-treatment | | POST-treatment | |
| # | Sample name | # | Sample name |
| 1 | 003 | placebo | M | PBMC | 9 | 003 pre pre | 1 | 003 pre post |
| 1 | 006 | Placebo | M | PBMC | 52 | 006 pre pre | 60 | 006 pre post |
| 1 | 007 | Placebo | M | PBMC | 21 | 007 pre pre | 29 | 007 pre post |
| 2 | 010 | Placebo | M | PBMC | 19 | 10v7 | 27 | 10v9 |
| 2 | 013 | placebo | M | PBMC | 12 | 13v7 | 4 | 13v9 |
| 2 | 014 | Placebo | M | PBMC | 8 | 14v7 | 16 | 14v9 |
| 2 | 018 | Placebo | M | PBMC | 33 | 18v7 | 41 | 18v9 |
| 2 | 023 | placebo | F | PBMC | 50 | 23v7 | 58 | 23v9 |
| 2 | 027 | Placebo | F | PBMC | 38 | 27v7 | 54 | 27v9 |
| 2 | 028 | Placebo | F | PBMC | 48 | 28v7 | 40 | 28v9 |
| 2 | 029 | placebo | F | PBMC | 62 | 029 v7 | 23 | 029 v9 |
| 2 | 032 | Placebo | M | PBMC | 59 | 032 v7 | 51 | 032 v9 |
| 2 | 034 | Placebo | F | PBMC | 31 | 034 v7 | 44 | 034 v9 |
|  |  |  |  |  |  |  |  |  |
| 1 | 004 | rapamycin | M | PBMC | 18 | 004 pre pre | 26 | 004 pre post |
| 1 | 005b | Rapamycin | M | PBMC | 35 | 005b pre pre | 43 | 005b pre post |
| 1 | 009 | Rapamycin | M | PBMC | 56 | 009 pre pre | 64 | 009 pre post |
| 2 | 011 | rapamycin | M | PBMC | 49 | 11v7 | 57 | 11v9 |
| 2 | 015 | rapamycin | M | PBMC | 22 | 15v7 | 30 | 15v9 |
| 2 | 016 | rapamycin | M | PBMC | 63 | 16v7 | 55 | 16v9 |
| 2 | 021 | rapamycin | M | PBMC | 2 | 21v7 | 20 | 21v9 |
| 2 | 024 | rapamycin | F | PBMC | 37 | 24v7 | 45 | 24v9 |
| 2 | 030 | rapamycin | F | PBMC | 6 | 030 v7 | 14 | 030 v9 |
| 2 | 033 | rapamycin | M | PBMC | 28 | 033 v7 | 36 | 033 v9 |
| 3 | 41 | rapamycin | M | PBMC | 17 | 41 v1 | 25 | 41 v4 |
| 3 | 42 | rapamycin | M | PBMC | 3 | 42 v1 | 11 | 42 v4 |
| 3 | 43 | rapamycin | M | PBMC | 24 | 43 v1 | 32 | 43 v4 |
| 3 | 44 | rapamycin | M | PBMC | 53 | 44 v1 | 61 | 44 v4 |
| 3 | 45 | rapamycin | M | PBMC | 42 | 45 v1 | 34 | 45 v4 |
| 3 | 46 | Rapamycin | M | PBMC | 7 | 46 v1 | 15 | 46 v4 |
|  |  |  |  |  |  |  |  |  |
| 3 | 43 | rapamycin | M | Buccal | 5 | 43D pre swab 1 | 13 | 43D 9 wk swab |
| 4 | 37yo | Rapamycin | M | Buccal | 39 | 37 yo pre swab 2 | 47 | 37 yo 10 wk swab |
|  |  |  |  |  |  |  |  |  |
| BLANKS | 10 | NA10858\_1 |  |  |  |  |  |  |
|  | 46 | NA10858\_2 |  |  |  |  |  |  |

1. Analyze only the PBMC samples (buccals can wait until we are deciding whether they would work better).

2. As there are few females and not the same number in the control groups, it may be beneficial to do two analyses: a) all subjects and b) just the male subjects.

3. There are really two questions to address:

a) Do the POST-rapamycin samples differ from the PRE-rapamycin samples (for each of the subjects)?

b) Is there an overall difference between the POST-rapamycin group and the POST-placebo group?

4. We do want to look at all of the methylation marks, but initially it may be worth interrogating genes/proteins known to be regulated either by methylation (DDAH2) or by rapamycin (foxp3, RAGE, soluble ICAM1).