RAGE Tfh Summary

Questions

1. How are frequencies of Treg populations impacted by treatment of recent onset T1D subjects with ATG or ATG/GCSF.
2. What is the ration of Treg: Teff (as well as other subsets: CM, EM, EMRA, TEMRA) in subjects receiving ATG or ATG/GCSF treatment.

Approach

1. The Tfh panel is as follows: L/D, CD3, CD4, CD8, CD45RA, CD45RO, CD127, FOXP3, **CD57**, CD38, **CCR6**, **CXCR5**, **CCR7**, **PD-1**, **CD95**, **CD27**, **CXCR3**, **ICOS**. Prioritized markers are bolded.
2. Analysis of T cells populations utilized: CD3, CD4, CD8, CD127, FOXP3 to define Treg, and non-Treg populations.
3. MFI was analyzed for CD127, CCR7, CXCR3, CD27, CD38, and CD95.

Outputs

1. 332 FCS files + associated files (single color controls)
2. 17 Workspaces + associated compensation matrices. The shared workspaces do not include adjustments to the gating for CXCR5 vs CCR6 and CXCR5 vs CXCR3 in the CD8 populations and the CD45RO vs CD45RA gating for CD4+ and CD8+ T cells.
3. 17 PDFs showing gate placement for each sample. The shared PDFs do not show adjustments to the gating for CXCR5 vs CCR6 and CXCR5 vs CXCR3 in the CD8 populations.
4. 1 Excel file including: Sheet containing outputs for each sample and acquisition notes, sheet containing gating notes, and sheet containing viability/recovery data.

Comments

1. Several T-cell subsets (CD8+, Naive, ICOS+, and CCR7) were distinct, and lacked extreme variation. MFI values for CD27, CXCR3, CD38 and CCR7 were consistent and distinguishable.
2. Differences appear to exist in frequencies of memory T-cells. Most notably the CD8+, CD45RO+ population shows this change most predominately.
3. 10 samples did not have a positive CD4 population, and the CD8- population was used for manual gating. Several (bolded) were excluded from analysis due to lack of confident downstream gating. This problem was not found to be related to technical or related to the staining process, and was sporadic in nature. (included FOXP3 populations have been italicized)
    - **TN0006430F01**, TN0007033F01, TN0007670F01, **TN0007013F01**, **TN0006097F01**, **TN0007383F01**, TN0008581F01, TN0006427F01, **TN0005954F01**, **TN0007048F01**
4. Some samples had various problems related to the physical nature of the samples under one of more of the following: The Tfh panel was the lowest priority, and "low yield and viability" samples were omitted from Tfh staining and analysis.
    - Low yield and viability after thawing for the following samples: (Data was still analyzed and reported)
        - TN0006041F01, TN0006544F01, TN0007426F01, TN0005879F01, TN0006088F01, TN0006746F01, TN0008013F01, TN0006733F01, TN0007384F01, TN0007840F01, TN0006992F01, TN0006178F01
    - High quantity red blood cell contamination of the following samples: (Data was still analyzed and reported)
        - TN0006430F01, TN0006023F01, TN0006097F01, TN0007448F01, TN0006738F01, TN0008730F01, TN0008581F01, TN0006427F01, TN0008069F01

5. MFI values for BV510 (CCR6), FITC (CD95) BV421 (PD-1) and BUV395 (CCR7) have fluorescent intensities inconsistent with data from other run dates. The reported data for this date excludes CD95 MFIs and all outputs dependent on CCR7, CCR6 and PD-1.

Contributors

1. Panel selection/prioritization – ITN/Alice Long
2. Edited x-trial panel design – ITN/Alice Long
3. Experimental design – Alice Long, Anna Kus
4. Analysis template – Alice Long, Anna Kus
5. Acquisition and analysis – Anna Kus, Bryce Fuchs, David Sierra, Jorge Pardo

Distribution and Use (for tracking of data sharing and publication to avoid overlap in analyses and include appropriate acknowledgements)

1. FCS files BRI > ITN