RAGE APC Summary

Questions

1. Does ATG therapy or ATG-GCSF combination therapy treatment lead to a more tolerogenic phenotype in antigen presenting cell subsets?

Approach

1. Based upon the following APC cell panel: **CD86**, **CD16**, CD4, CD3, **HLA-DR**, **CD11c**, CD56, **CD304**, **CD123**, **CD14**, CD19, **PDL-1**, **CD141** and Live/Dead. (Priority markers in bold.)
2. This panel has been previously used in AbATE, T1DAL, and ACCESS trials.
3. Reported values include frequency of CD3-, CD19-, CD56-, HLA-DR+ cell subsets including classical (CD16- CD14+), transitional (CD16+ CD14+) and non-classical (CD16+ CD14-) monocytes. From the CD14- CD16- subset, plasmacytoid (CD123+ CD304+) and myeloid (CD11c+ that are either CD141+ or - dendritic cell frequencies are reported. MFI of PDL-1, HLA-DR, and CD86 of all relevant cell populations are also reported.

Outputs

1. 1 Excel file including: Sheet containing outputs for each sample and acquisition notes, sheet containing viability/recovery data.

Comments

1. All monocyte and dendritic cell subsets of interest were clearly identified, with variation in their frequencies noted across subjects
2. PDL-1, HLA-DR and CD86 level of expression varied across cell types consistent with biological expectations, and also varied across subjects.
3. Panel priorities from highest to lowest for this trial were X-Trial, APC, and Tfh. In cases where cell recovery was low (TN0005879F01, Batch 2, 180427), fewer than 2 million cells were used for the APC panel, yielding fewer events. Data was not reported for % if below 25 counts, and not reported for MFI if below 50 counts. Wells with low counts are noted with ND (not determined).
4. A number of samples contained events with high SSC, likely granulocytes (n=67), and this was noted in the analysis spreadsheet in the comments section. These events were largely excluded during clean up gating; downstream analysis was performed as usual and all analytes reported.
5. Sample TN0005954F01 (Batch 16, 180622) and TN0007876F01 (Batch 9, 180523) had irregular viability over time.

Contributors

1. Panel selection/prioritization – ITN/Alice Long/TrialNet
2. APC 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, Jr.

Distribution and Use (for tracking of data sharing and publication to guide overlap in analyses and acknowledgement)

1. BRI > ITN