Investigation of CD4$^+$ CD56$^+$ T cells associated with aGvHD and CMV in naïve T cell depleted hematopoietic transplant recipients

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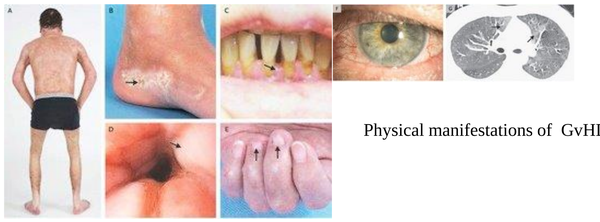
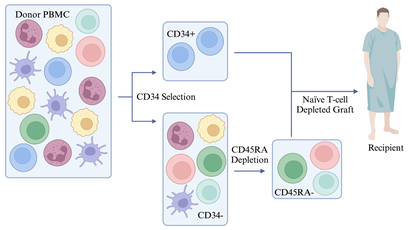
Background

Allogenic hematopoietic stem cell transplant (HCT) is the transfer of donor hematopoietic stem cells and lymphocytes to a recipient after chemotherapy and/or radiation. Although HCT is often curative for high-risk hematologic malignancies, there is still a 1/3 chance for relapse. HCT can also lead to Graft-vs-host disease (GvHD), when the donor's T cells recognize the recipient as foreign and damages healthy tissues. This leads to morbidity, mortality, and a poor quality of living which can be combatted against by various approaches, one of which being pan T cell depletion. While removing all T cells from peripheral blood stem cells (PBSC) reduces GvHD, it increases non-relapse mortality due to virus reactivation[3]. Thus, it was vital to recognize which type of T cell to deplete from this population. Naïve T cell depletion (T_ND) was chosen since T_N caused severe GvHD in murine models whereas memory T cells cause mild/no GvHD[1]. Through T_ND, patients treated for HCT had low incidences of severe aGvHD. There was prevalence of mild aGvHD, but this was associated with protection from relapse[2].



Physical manifestations of GvHD[4]



Depletion of CD45RA$^+$ cells from PBSC removes T_N (as well as T_{EMRA} & most B & NK cells)

Background data

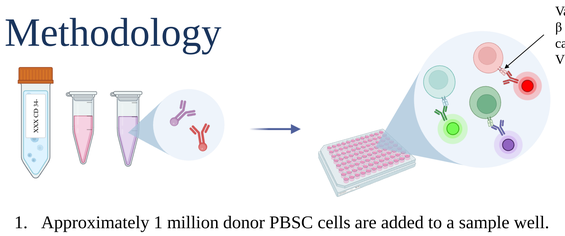
Time dependent flow cytometry studies were conducted on donor PBMC data from different time points. Results were obtained using conventional flow analysis and analyzed using Leiden-based clustering approach.

- Clustering analysis shows an interesting T cell cluster expressing CD56 ('cluster 11')
- Cluster 11 was prevalent in patients who are CMV$^+$ and have had aGvHD post transplant.
- We hypothesized that CD4+CD56+ T cells may contribute to aGVHD after T_N-depleted HCT.

Questions to answer

1. **How prevalent are the CD56$^+$ cells in donor and patient populations?**
2. **Is the CD56$^+$ population clonal or polyclonal?**
3. Are the CD56$^+$ cells responsive to human CMV?

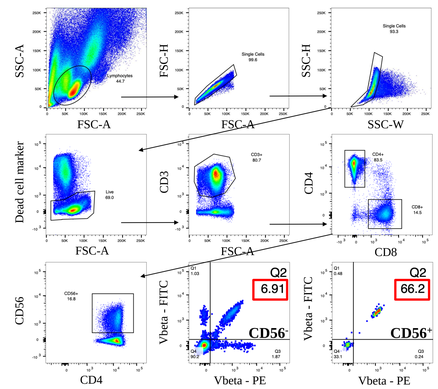
Methodology



1. Approximately 1 million donor PBSC cells are added to a sample well.
2. A surface stain master mix containing the markers to the right (excluding Vbeta antibodies) are added to a sample well.
3. Vbeta antibodies are added to corresponding sample wells. Each donor has a total of 8 sample wells for each Vbeta antibody vial.
4. Once staining is complete, samples are run through a flow cytometer.

Marker	Color	Purpose
Dead cell marker	UV440 (DAPI)	Eliminate dead cells
CD3	BUV805	Surface phenotype
CD4	BUV737	Surface phenotype
CD8	BUV496	Surface phenotype
CD27	BV605	Surface phenotype
CD56	APC-R700	Surface phenotype
CD57	APC	Surface phenotype
CD45RA	BV510	Surface phenotype
CD45RO	BV786	Surface phenotype
Vbeta	PE	Clonality
Vbeta	FITC	Clonality

Gating strategy



Results

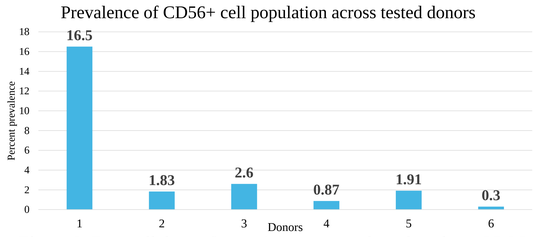


Figure 2. Extracellular surface staining was conducted on donor PBSC. Samples gated on CD56$^+$ population. Prevalence varies with the highest in donor 1. Each value captured from vial A, except donor 1, which was vial C.

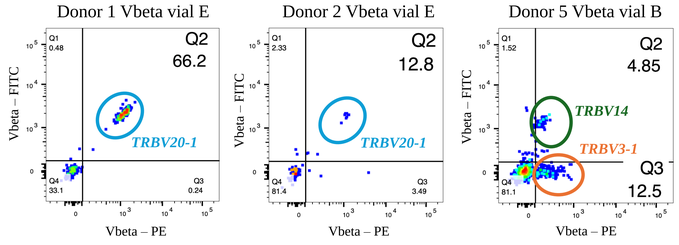


Figure 3. Samples were gated on CD56$^+$ populations as shown in the gating scheme. 48 total samples were run (6 donors, 8 Vbeta antibody vials per donor) and were measured via flow cytometry. Expanded clones were identified using this technique. Figure shows visual examples of expanded clones.

Figure 4. Flow charts were gated on CD56$^-$ and CD56$^+$ populations for each donor sample from TCR Vbeta staining. Each chart was analyzed per quadrant and graphed to compare expansion between CD56$^-$ and CD56$^+$. Vbeta usage determined by the IOTest Beta Mark Kit (see table below). Bolded Vbetas are similar between donors.

- Results indicate polyclonal CD4$^+$ CD56$^+$ population.
  - Multiple clonal expansions per donor indicates this status.
- Similar Vbeta usage between donors is seen.
  - Most expanded Vbeta: TRBV20-1.
  - Other prevalent Vbetas: TRBV3-1, TRBV6-6, and TRBV4-3.

Tube	Clone	Conjugate	Vbeta (IMGT)
A	3D11 ZOE CH92	PE FITC + PE FITC	TRBV5-5 TRBV4-1, TRBV4-2, TRBV4-3 TRBV28
B	FIN9 E17.5F3.15.13 TAMAYA1.2	PE FITC + PE FITC	TRBV3-1 TRBV19 TRBV14
C	BA62.6 IMMU157 ELL1.4	PE FITC + PE FITC	TRBV18 TRBV5-1 TRBV30
D	IMMU222 JU74.33 56C5.2	PE FITC + PE FITC	TRBV6-5, TRBV6-6, TRBV6-9 TRBV6-6 TRBV12-3, TRBV12-4
E	36213 MPB2D5 VER2.32.1.1	PE FITC + PE FITC	TRBV5-6 TRBV20-1 TRBV10-3
F	AF23 BL37.2 IG125	PE FITC + PE FITC	TRBV13 TRBV9 TRBV11-2
G	C21 IMMU546 CAS11.3	PE FITC + PE FITC	TRBV25-1 TRBV2 TRBV27
H	H132 WJF24 ZIZOU4	PE FITC + PE FITC	TRBV6-2 TRBV29-1 TRBV4-3

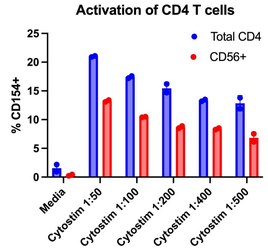
Beta Mark TCR Vbeta Repertoire Kit antibody composition and associated V-Beta according to *Wei, et al.* and IMGT nomenclature.

Conclusions

- The CD4$^+$ CD56$^+$ population is found in varying amounts across tested donor populations so far.
  - This indicates that the cell population is a relevant population.
- Multiple Vbeta usage across each donor indicates this population is a polyclonal one.
  - Most expanded Vbeta is TRBV20-1

Future Plans

- **Are these cells responsive to CMV?**
  - CD154 assay (T cell activation assay) will be used to test T cell response to CMV peptide.
  - Currently working on cytostim (positive control) dilutions.



Acknowledgements

This work is funded by P01 CA018029 and P30 CA015704. The Summer Undergraduate Research Program is supported in parts by Baylor University, the Fred Hutch Internship Program, and individual labs/research groups. Thank you to Dr. Michelle Brault for her continuous mentorship throughout the duration of this program. Thank you to Dr. Marie Bleakley and the Bleakley lab for their mentorship and support.

References

1. Anderson, Shlomchik *et al. JCI* (2003)
2. Bleakley *et al.* JCO (2022)
3. Luznik *et al. JCO* (2022)
4. Zeiser & Blazar NEJM (2017)