Analyzing T Cell Phenotypes in Chronic Graft-Versus-Host Disease After Naive T Cell-Depleted Hematopoietic Stem Cell Transplantation

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Background

Hematopoietic Stem Cell Transplantation (HCT)

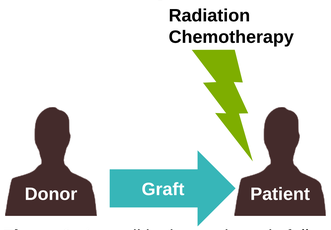


Figure 1. A conditioning regimen is followed by rescue with donor hematopoietic stem and immune cells.

HCT can be curative for high-risk leukemia. During HCT, patients are conditioned with chemotherapy and radiation followed by infusion of hematopoietic (blood-forming) stem and immune cells from a matched donor. Donor stem cells migrate to the bone marrow and repopulate the patient's blood and immune system.

Graft versus Host Disease (GVHD)

GVHD can occur when donor T cells attack healthy tissue. Chronic GVHD (cGVHD) can be life-threatening, often requiring long term immunosuppression. **If physicians can predict that a patient is at low risk of cGVHD, immunosuppression can be stopped earlier, reducing risk of infection and relapse.**

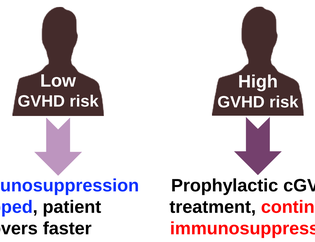


Figure 3. Identification of patients at high-risk of cGVHD would improve clinical outcomes.

Naive T (T_N) Cell Depletion for the Prevention of GVHD

Our research group conducted a clinical trial to investigate the depletion of naive T cells from donor stem cell grafts as a strategy to prevent GVHD. We are currently correlating patterns of immune reconstitution to the development of cGVHD.

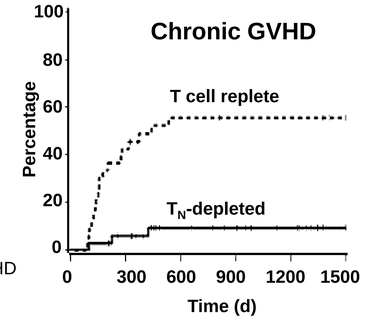


Figure 4. In a recent clinical trial from the Bleakley lab, fewer patients developed cGVHD after T_N-depleted HCT compared to T cell-replete HCT.

Gastrointestinal GVHD

Cells expressing α4 and β7 integrins home to the gut, a common site of GVHD. **Cytotoxic gut-homing T cells** ($CD8^+\alpha4^+\beta7^+$) **can cause gut GVHD.** Regulatory T cells or **T_{REGS}** ($CD4^+FoxP3^+CD127^-CD25^+$) **can suppress immune function and inhibit GVHD.**

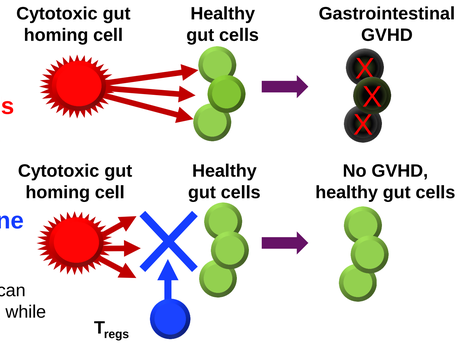


Figure 2. Cytotoxic gut homing T cells can attack healthy tissue and cause GVHD, while T_{regs} protect against GVHD.

Goals

20 color flow cytometry was used to compare **immune cell subsets at day 28 post HCT** in patients who did and did not develop cGVHD.

Figure 5. High cytotoxic gut-homing T cell: T_{REG} ratios may predict cGVHD.

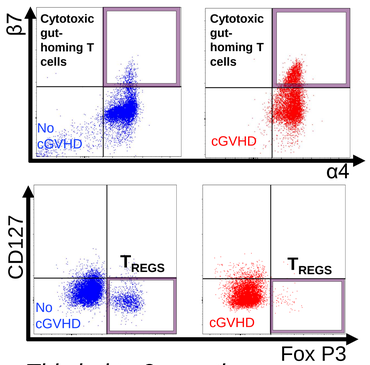
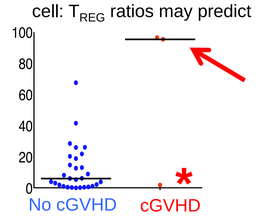


Figure 6. High cytotoxic gut-homing T cell: T_{REG} ratios may predict cGVHD.



* Why did this patient's T_{regs} not suppress cGVHD-associated gut-homing T cells? 2/3 patients who developed cGVHD had high ratios of cytotoxic gut-homing T cells to T_{REGS}.

This led to 2 questions:

- Do **cytotoxic gut-homing T cells** or **T_{REGS}** have **differing expression** of **markers** in patients who develop **cGVHD** that suggest **different cellular function**?
- Are there **phenotypic differences underlying cGVHD** development that can be used to **predict patients at high risk?**

Results

Figure 7. T cell markers differ in cGVHD patients.

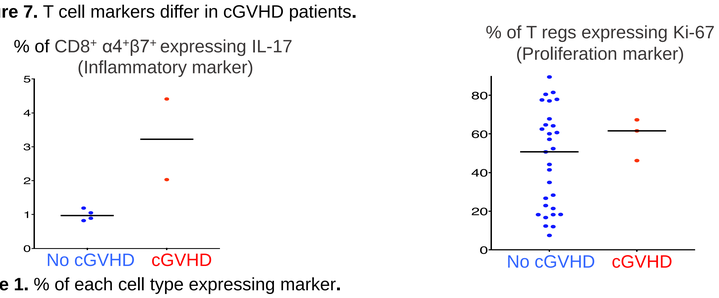


Table 1. % of each cell type expressing marker.

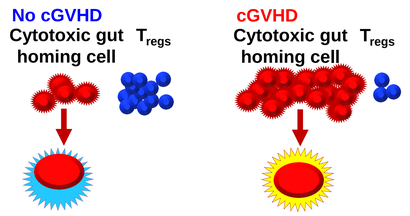
Cell Populations	$CD8^+$ T cells		$CD8^+$ $\alpha4^+\beta7^+$		$CD8^+$ $\alpha4^+\beta7^-$		$CD4^+$ T cell		$CD4^+$ T_{regs}	
Markers	No cGVHD	cGVHD	No cGVHD	cGVHD	No cGVHD	cGVHD	No cGVHD	cGVHD	No cGVHD	cGVHD
Ki-67 (Proliferation)	19	14.2	18.1	10.3	24.4	17.4	16.8	24	50.7	61.6
CCR7 (Lymph Node homing)	7.48	1.96	8.38	1.11	7.98	2.41	29.1	12.9	9.41	6.02
CD62L (Lymph Node homing)	15.7	32.1	16.1	19.7	15.4	27.7	30.9	44.6	74.8	75.8
CD28 (Activation/survival)	34.3	32.8	51.5	45.6	30.6	27.9	77.5	76.2	94.4	91
CD45RA (Naive T cell)	-	-	1.69	0.19	1.92	0.62	-	-	0.055	0
$\alpha4^+\beta7^+$ (Gut homing)	11.8	24.1	100	100	0	0	10.3	12.1	3.25	5.02
$\alpha4^+\beta7^-$ (Homing not to gut)	84.2	74.4	0	0	100	100	-	-	75	51.2
CCR9 (Thymic migrant)	9.71	8.36	8.97	6.35	9.14	8.15	7.75	3.78	45.7	41.9
I L-17 (Inflammation)	1.04	1.55	0.97	3.22	1.30	6.0	3.08	3.66	15.0	20.76
CCR5 (Chemokine receptor)	18.5	19	24.5	26.7	18.5	21.2	13.1	12.9	15.8	17.7
CD27 (Effector activation)	40.3	28.6	53.1	41.5	38.7	24.4	45.4	32.2	-	-

Conclusions

At day 28 post-HCT, **patients who later developed cGVHD** had:

- **20% more T_{regs}** expressing **Ki-67** (marker of cell proliferation)
- **3X more cytotoxic gut-homing T cells** expressing **IL-17** (marker of inflammatory activation).

Figure 8. There were phenotypic differences in patients who do and do not develop cGVHD in addition to differences in absolute number of cells.



This led to the following 2 answers:

- **Differing expression of markers** on **cytotoxic gut-homing T cells** and **T_{REGS}** in patients who later develop cGVHD suggest that these cells are **functioning differently.**
- These differences in **phenotypic expression on cytotoxic gut-homing T cells** and **T_{regs}** are from **day 28 post-HCT**, before the actual onset of the disease.

Future Directions

- **More time points**: How does the graft correlate with post-HCT cell populations?
- **More markers**: What are other phenotypes of the cells of interest?
- **Different HCT protocols**: Are these results unique to T_N –depleted HCT?

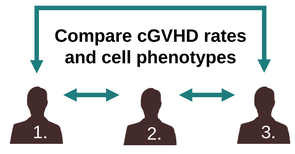


Figure 9. Randomized trial of different HCT protocols:
1. T_N-depleted HCT,
2. T cell replete HCT,
3. GVHD preventing drugs

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