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CASE STUDY

High Throughput scRNA-seq Tracks Post-AlloHSCT Immune Reconstitution and Reveals Novel Biomarkers Associated with Acute GvHD

Using scRNA-seq to profile >777,000 immune cells from AML patients, this study identified MDGA1 and ADGRG1 as complementary biomarkers associated with acute graft-versus-host disease and graft-versus-leukemia responses after allogeneic stem cell transplantation.

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Background and Objective

Allogeneic hematopoietic stem cell transplantation (alloHSCT) remains the most established curative therapy for acute myeloid leukemia (AML). While alloreactive T cells mediate graft-versus-leukemia (GvL) reactions to prevent relapse, they also mediate acute graft-versus-host disease (aGvHD), a severe and debilitating inflammatory reaction.

Despite advances in graft engineering, robust biomarkers for early diagnosis of aGvHD and risk assessment for GvL remain lacking.

Recent scRNA-seq studies identified ADGRG1 and ZNF683 as markers of alloreactive CD8+ T cells in bone marrow, but their predictive value in a more accessible tissue like peripheral blood remained unexplored.

Zheng Song, Evgeny Klyuchnikov, and colleagues led by Nicolaus Kröger and Immo Prinz used Evercode™ scRNA-seq technology to profile over 777,000 CD45+ leukocytes from ten AML patients collected pre and post transplant, enabling the largest transcriptome-wide biomarker discovery for aGvHD and GvL.

Experimental Design

Twenty-five samples were collected and cryopreserved from ten AML patients at three timepoints: peripheral blood stem cell grafts prior to graft infusion, PBMC at 30 days (D30) and at 100 days post-transplantation (D100). Live CD45+ immune cells were sorted and processed in a single experiment using one Evercode™ WT Mega to minimize batch effects (Figure 1).

> Evercode enabled us to track graft-derived T-cell persistence, expansion, and functional states at scale after allo-HSCT, providing a powerful framework to study T cell features linked to AML patient outcomes."
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> Prof. Dr. Immo Prinz
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> Director of the Institute of Systems Immunology
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> University Medical Center Hamburg-Eppendorf

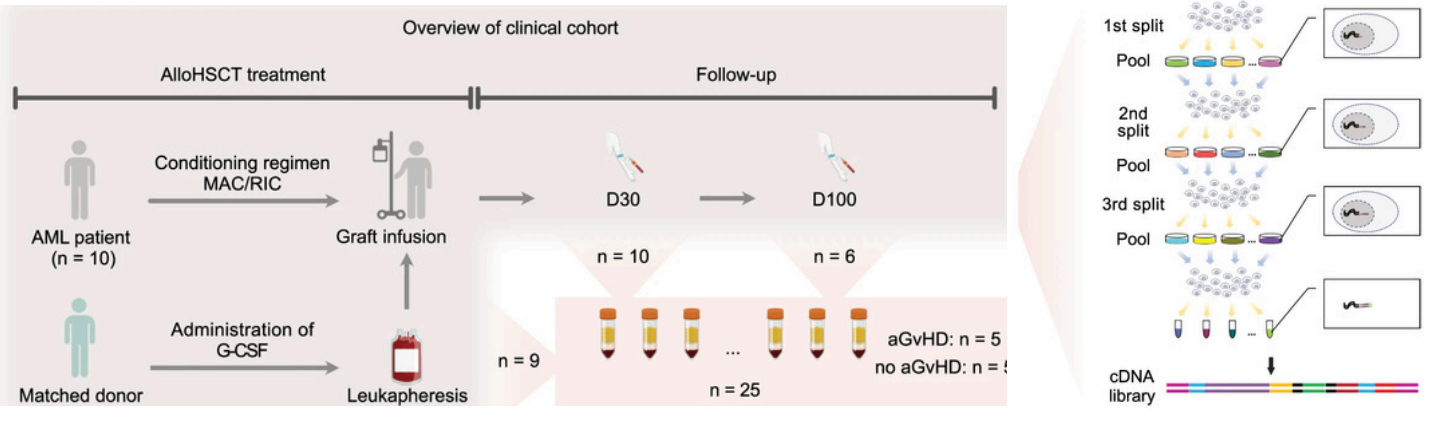

Figure 1: Schematic of experimental design. Ten patients received stem cell transplant infusion from matched donors. Graft samples were collected on the day of the transplant. Patients' PBMCs were collected and cryopreserved on day 30 and 100 post-transplant. Five patients developed aGvHD between day 19 and day 136. Two patients relapsed on day +149 and +622 respectively.

Results

Analysis of Immune Reconstitution Pattern

Over 770,000 cells from the 25 samples were classified into eight major hematopoietic cell populations and 45 subtypes including αβ T cells, γδ T cells, NK cells, B cells, monocytes and Dendritic Cells (DCs) (Figure 2).

Hematopoietic reconstitution analysis revealed a gradual increase in T cell proportions, rapid NK cell expansion at D30, near-complete B cell disappearance at D30 with slow recovery by D100, and decreasing monocytes post-transplant. The overall DCs proportion, the hematopoietic stem cell compartment, and a population characterized by expression of MKI67, remained constant at all timepoints.

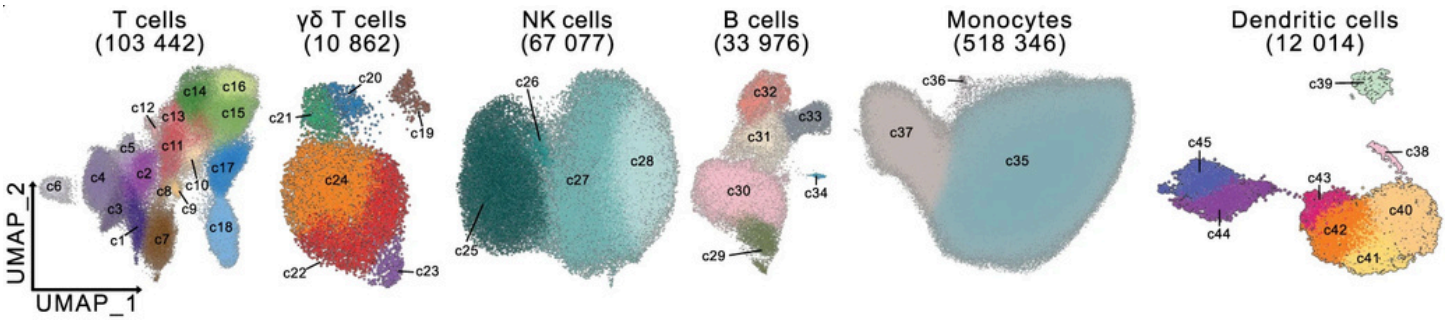

Figure 2: UMAP of T cells, γδ T cells, NK cells, B cells, monocytes, and dendritic cells (DCs) with post-QC cell number indicated. Cells are colored by subtypes.

Persistent Alloreactivity in Graft-Derived T Cells and Identification of Residual Host Cells

T and B cell receptor were used to trace the graft-derived αβ T cells, γδ T cells.

Donor-derived αβ and γδ T cell clones persisted in the host circulation after transplant confirming successful long-term survival of donor T cells.

CD8+ T cells were the most prominent persisting engrafted T cell clones, with significantly enriched cell cycle-related and metabolic pathways, indicating an activated, potentially allo-reactive status of these graft-derived T cells.

Residual host-derived T cells were identified in female patients who received male grafts using sex-chromosome-related gene expression. CD4+ but not CD8+ subsets were found in the host compartment.

MDGA1 marks aGvHD-associated T cells

Differential gene expression analysis identified several upregulated genes in the aGvHD group. Of these, only MDGA1 showed consistent, significant upregulation across all post-alloHSCT samples from aGvHD patients at both D30 and D100. MDGA1 encodes a GPI-anchored cell surface glycoprotein with an unknown immune function. It was highly expressed exclusively by lymphocytes and NK cells in aGvHD patients (Figure 3).

Reanalysis of the Parse Biosciences' 10 million PBMCs treated with 90 cytokines showed proinflammatory cytokines (IL-12, interferons, TL1A) induced MDGA1 on CD4+, CD8+, and NK cells, while anti-inflammatory cytokines downregulated it. MDGA1 was also elevated in T cells from multiple sclerosis patients, supporting its role as a broader inflammation marker.

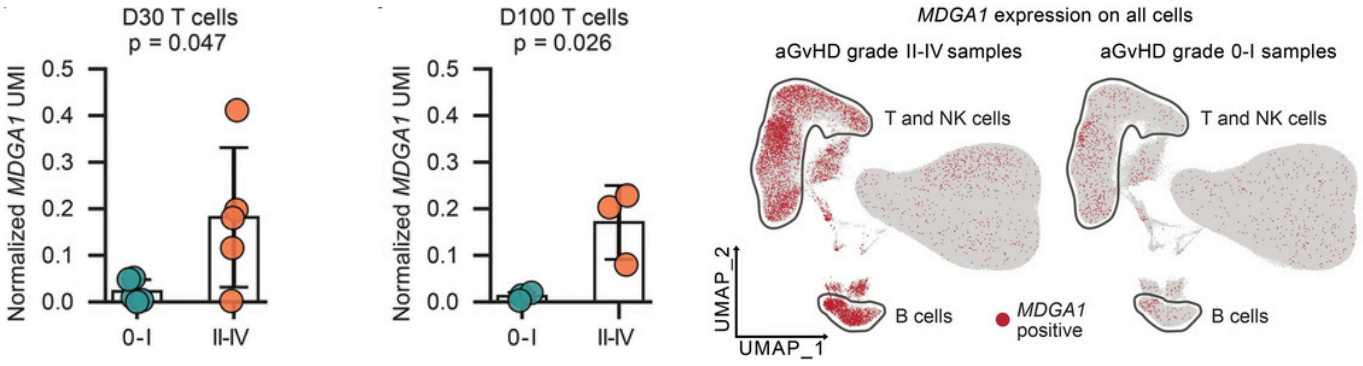

Figure 3: On the left, comparison of normalized MDGA1 UMIs of T cells between different aGvHD outcomes at D30 and D100. Each dot represents one patient. On the right, MDGA1 was distributed across T, NK and B cells of patients with aGvHD.

ADGRG1 Marks GvL-Associated Cytotoxic T Cells

Recent evidence identified ADGRG1 as a biomarker of alloreactive cytotoxic T lymphocytes mediating GvL in the bone marrow. Given the limited number of relapses in the patients' cohort in this study, a combined analysis with a public dataset suggested that ADGRG1 was significantly reduced in patients who later relapsed. This gene was not associated with aGvHD, suggesting it functions specifically as a GvL biomarker.

Conclusion

This longitudinal study demonstrates the power of scalable scRNA-seq for a comprehensive over-time immune profiling analysis after alloHSCT. The scale of the experiment enabled them to identify rare residual host-derived T cells and multiple persistent αβ T and γδ T cell clones post-alloHSCT, which displayed alloreactivity characterized by clonal expansion and increased activity of the cell cycle pathway.

Key findings include the identification of MDGA1 as a novel biomarker potentially associated with aGvHD, expressed on T cells and NK cells and induced by proinflammatory cytokines, and the validation of ADGRG1 as a marker of GvL-associated cytotoxic T cells in peripheral blood. Together, MDGA1 and ADGRG1 may function as complementary biomarkers expressed by distinct circulating T cell subsets associated with divergent outcomes in AML patients, enabling precise risk stratification of alloHSCT outcomes and presenting potential therapeutic targets.

Read the Full Study:

Song, Z., Klyuchnikov, E., Badbaran, A., et al. (2025). Mega-scale single-cell profiling reveals novel biomarkers associated with acute GvHD after allogeneic hematopoietic stem cell transplantation. Biomarker Research, 13, Article 155. https://doi.org/10.1186/s40364-025-00868-x

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