High-dimensional spectral cytometry paired with computational technology

Providing insights into the cellular features of healthy donor blood products to accelerate allogeneic cell therapy development

Ashley L. Wilson[*], Jill Thomas[2], Kurt Van Gunst[1], Arpan Neupane[1], Malisa Smith[1], Dillon Hammill[1], Fritz Lekschas[1], Mike Comeau[1], Greg Finak[1], Tim Waters[2], Evan Greene[1], Aaron Posey[2], Cherie Green[1]

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ABSTRACT

Background

Blood centers support allogeneic cell therapy development by providing access to healthy donor starting material for manufacturing. However, selecting appropriate donors remains a major challenge as these materials are not well characterized. Work using patient-derived material has shown that naive or stem-like memory phenotypes are associated with favorable product performance, while highly differentiated phenotypes are not [1–8]. Here we present data from an innovative partnership focused on identifying cellular features that predict whether a donor is a preferred candidate for allogeneic cell therapy development.

Methods

We apply a validated 48-color spectral cytometry assay paired with purpose-built machine learning to deeply characterize 3 types of starting material from healthy donors, including peripheral blood mononuclear cells, mobilized apheresis products, and T and NK cellular products (Figure 1). Single-cell data are analyzed using a novel, unbiased computational algorithm that discovers and annotates all cellular phenotypes in and across samples. For mobilized apheresis, donors are treated with G-CSF [9], and assigned a mobilization score (excellent or poor) based on absolute counts of CD34+ stem cells post mobilization.

Results

We identified over 2000 immune cell phenotypes in peripheral blood across 45 donors. CMV+ donors had higher frequencies of T cell phenotypes co-expressing KLRG1 and CD57, markers associated with chronic viral infection, terminal differentiation and reduced replicative potential (Figure 3). Of the 6 G-CSF mobilized donors, 5 donors received an excellent mobilization score, while one performed poorly. Compared to excellent mobilizers, starting material from the poor mobilizer had higher frequencies of highly differentiated T and NK cell phenotypes (Figure 5). Interestingly, these less favorable phenotypes were detected pre G-CSF, indicating they were inherent to the donor and not an effect of mobilization. The apheresis product from the poor mobilizer also had fewer stem/early progenitor phenotypes, demonstrating that blood products from different mobilized donors will have distinct immune cell compositions. Finally, we analyzed T and NK cell products purified from the starting material of 19 donors. One NK cell product was purified from a mobilized donor who received an excellent score. Their resulting product was enriched in NK cell phenotypes expressing markers associated with manufacturing potential (Figure 6).

Conclusions

We provide new insights into cellular features present across a variety of blood products generated from healthy donors. Our data suggest that starting material from CMV+ donors or suboptimal mobilizers may be less favorable for specific allogeneic cell therapy applications, given their shared immune profile consisting of highly differentiated effector cells.

PARTNERSHIP

ozette

- State-of-the-art immunology lab in Seattle, WA
- Computational platform for high-resolution discovery of cell phenotypes in single-cell cytometry data
- Rapidly surface relevant cell populations of interest in high-dimensional datasets

Accelerate cell therapy development

Bloodworks Bio

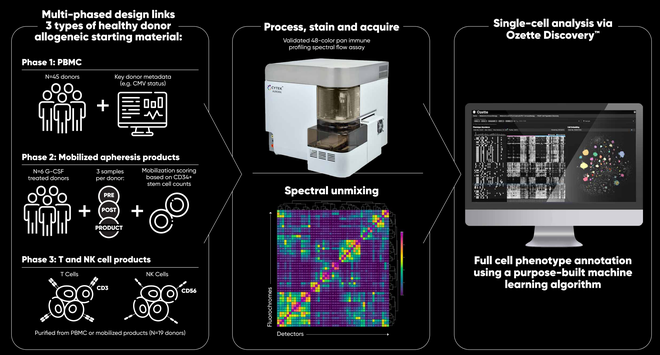
- World-class blood product provider in Seattle, WA
- Provide access to potentially life-saving starting material for allogeneic cell therapy manufacturing
- Pioneering advanced characterization of healthy donor blood products

STUDY DESIGN

FIG. 1

End-to-end sample processing and analysis of single-cell data yields interpretable insights into donor selection for allogeneic cell therapy development

Samples are stained and acquired in Ozette Lab with a 48-color pan immune profiling panel that provides comprehensive characterization of major circulating immune cells.
Data are analyzed via Ozette Discovery™. Mobilization score: Excellent = ≠71 CD34+ cells/uL, Poor = ≤25 CD34+ cells/uL blood.

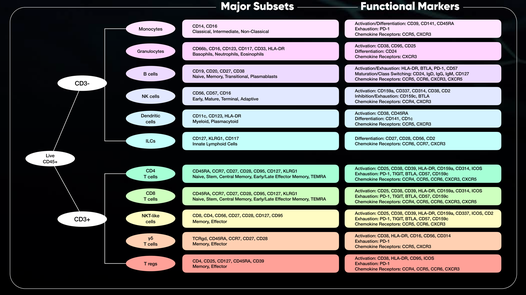


PIP-01 PANEL

FIG. 2

Pan Immune Profiling-01 (PIP-01) is a 48-color spectral flow cytometry assay that provides comprehensive insight into the state of the immune system

PIP-01 is a validated spectral flow cytometry assay designed to measure a multitude of biologically relevant markers on all major circulating immune cell subsets. It includes cell surface markers of activation, differentiation, exhaustion, cellular health and chemokine receptor expression.

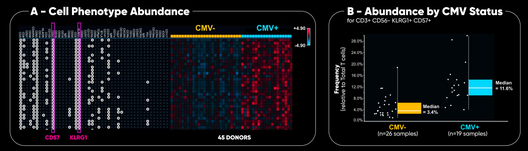


PHASE 1 CMV DATA

FIG. 3

CMV status reveals a distinct donor-specific immune profile with important considerations for downstream cell therapy manufacturing

(A) Heat map and (B) abundance of KLRG1+ CD57+ T cell phenotypes by CMV status discovered across 45 healthy donor PBMC samples. Each row is a phenotype, each column is a sample. '+' indicates positive expression. CMV negative = yellow, CMV positive = blue.

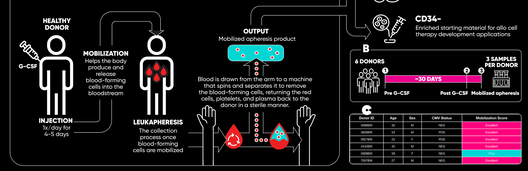


MOBILIZED APHERESIS WORKFLOW & TIMEPOINTS

FIG. 4

G-CSF mobilization yields enriched allogeneic starting material from healthy donor research participants

(A) Mobilized apheresis process. (B) Three samples were collected per donor: a peripheral blood draw pre- and post G-CSF, and a sample of the final mobilized apheresis product. The G-CSF mobilization process takes approximately 30 days to complete. (C) Table of mobilized donor demographics and key metadata. M = Male, F = Female. POS = CMV positive, NEG = CMV negative; CMV status confirmed at the time of mobilization. Each donor was assigned a "mobilization score" based on CD34+ cell counts post mobilization. Scoring: Excellent = ≠71 CD34+ cells/uL, Poor = ≤25 CD34+ cells/uL blood. Most participants mobilized well, but one did not.

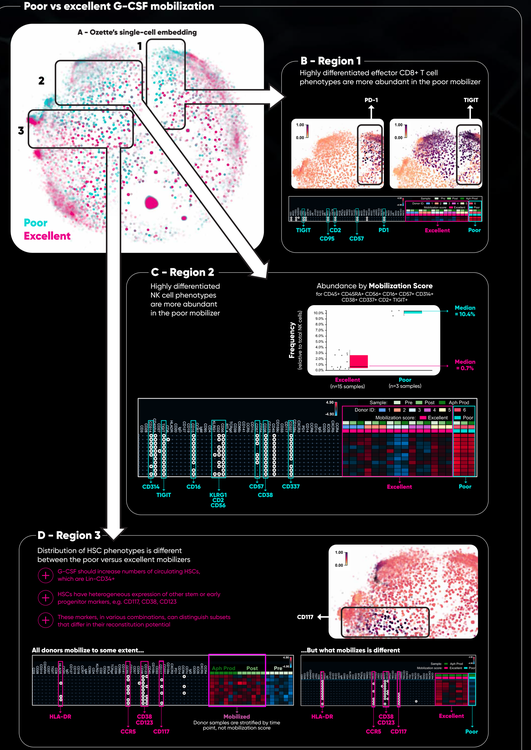


PHASE 2 DATA

FIG. 5

Immune profiles of the apheresis products are distinct between poor & excellent mobilizers

(A) Apheresis product profiles of a poor (turquoise) and excellent (pink) mobilizer are overlaid. Three regions of interest are highlighted in the upper left quadrant of the single-cell embedding visualization (numbered 1–3). (B) Region 1: Single-cell embeddings pseudo-colored by PD-1 and TIGIT expression (top) and heat map (bottom) shows highly differentiated effector CD8+ T cell phenotypes are more abundant in samples from the poor mobilizer. (C) Region 2: Heat map (bottom) and abundance by mobilization score (top) of the indicated phenotypes show that highly differentiated CD56- NK cell phenotypes are more abundant in the poor mobilizer. Interestingly, these cellular states were detectable in the poor mobilizer prior to G-CSF mobilization. (D) Region 3: [Top] Single-cell embedding pseudo-colored by CD117 expression to visualize HSC/early progenitor cell phenotypes. [Bottom left] Heat map stratified by time point shows that all G-CSF treated donors mobilize HSCs to some extent. [Bottom right] Heat map of the final mobilized apheresis products (Aph Prod) from all 6 donors, stratified by mobilization score, shows that the poor mobilizer has a lower abundance of specific HSC subsets compared to the excellent mobilizers.



Potential translational implications of G-CSF mobilization of allogeneic donors for cell therapy

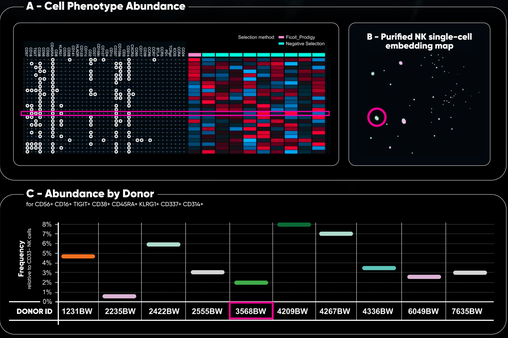
- Compared to excellent mobilizers, starting material from the poor mobilizer has:
  - Higher abundance of unfavorable T and NK cell phenotypes for allo-CAR manufacturing.
  - These phenotypes were detectable in their peripheral blood **prior to** mobilization and carried through to their apheresis product.
  - Interestingly, the poor mobilizer is confirmed **CMV negative.**
- Lower abundance (or potentially less diversity) of specific HSCs/early progenitors, which may have important implications for downstream reconstitution potential.

PHASE 3 PRODUCTS

FIG. 6

Distinct and robust NK cellular states are discovered across purified products from allogeneic donors

(A) Heat map and (B) single-cell embedding highlighting heterogeneity of NK cell phenotypes discovered across CD56+ NK cell products purified from 10 research donors. Heat map shows variability in phenotypes by donor and purification strategy. Each row is a phenotype, each column is a donor's product. '+' indicates positive expression. (C) Abundance by donor of the phenotype selected in panel A. This highly differentiated NK cell phenotype (CD56+ CD16+ TIGIT+ CD38+ CD45RA+ KLRG1+ CD337+ CD314+) constituted less than 2% of the final purified product from Donor 3568BW (boxed). This donor was an excellent mobilizer and was CMV negative.



CONCLUSION

- This first-of-its-kind collaboration begins to identify key attributes of donors that may inform whether they are preferred candidates for cell therapy development applications.
  - We provide rich insights into the immune cell phenotypes present in healthy donor starting material, and can track donors and their resulting products over time.
  - Ozette Discovery™ rapidly surfaces relevant scientific findings, particularly cellular states and phenotypes that may otherwise go undiscovered.
  - Our pairing of spectral cytometry + computational technology accelerates the analysis of these complex datasets and bolsters our ability to provide suitable starting material to researchers and drug developers.
- Future work aims to validate these preliminary findings and correlate cellular phenotypes in starting material with product manufacturing and performance outcomes.
- Together, we will continue to advance the development of safe and effective cell therapies and help deliver them to patients that need them most.

REFERENCES & ETHICS

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Ethics approval:

Under an approved IRB protocol and informed consent, Bloodworks Apheresis collects mobilized and non-mobilized leukapheresis products for both clinical and non-clinical use in compliance with cGMP/cGTPs. All donors provided informed consent before participating.

Affiliations:
[*] Presenting Author [1] Ozette Technologies, Seattle, WA, USA [2] Bloodworks Bio, Seattle, WA, USA