Immunophenotypic Characterization of AML Blast Cells: A Pilot Study for Precision Therapeutic Targeting

Shalini Gautam[1], PhD, **Greg Hayes**[2], **Chang An**[1], **Chintan Jobaliya**[1], **David Ambrose**[1], MBA, **Varun Dwivedi**[1], PhD, and **Adam Cotty**[1], PhD

[1]KCAS Bio-Philadelphia, PA, US; [2]Kurome Therapeutics, Cincinnati, OH, US;

KCAS bio
SCIENCE ACCELERATED

Introduction:

Acute Myeloid Leukemia (AML) represents one of the most challenging blood cancers, with poor patient outcomes stemming from heterogeneous blast cell populations at distinct differentiation stages that exhibit unique drug response profiles, making traditional uniform chemotherapy approaches largely ineffective. To address this complexity, we developed an advanced 15-color flow cytometry panel integrated with BD FACSymphony S6 cell sorting technology that enables simultaneous analysis and physical isolation of specific AML blast populations using systematic marker combinations including CD45dim, CD33, CD34, CD117, CD71, and HLA-DR. This ongoing pilot study focuses particularly on M4-type AML blasts, where our flow cytometry-guided sorting approach transcends single blast stage identification by employing comprehensive phenotypic signatures to distinguish various blast stages, enabling precise characterization and isolation of distinct cellular populations for downstream functional analysis. The synergy between multiparameter flow cytometry and high-precision cell sorting provides an unprecedented capability to decode AML blast heterogeneity and develop targeted therapeutic strategies tailored to specific cellular populations within individual patients. This technique was developed to serve dual purposes: first, to sort patient-specific blast populations for individual characterization, and second, to evaluate potential post-treatment "unblocking" of arrested differentiation pathways.

Table 1. Panel Composition

Antibody	Conjugate	Clone
CD11b	BUV395	D12
LD Blue		
CD13	BUV737	L138
CD117	BV421	104D2
CD45	BV510	H130
CD16	BV605	3G8
CD56	BV650	5.1H11
CD71	BV711	L01.1
HLADR	BV786	G46.6
CD33	BB515	WM52
CD15	PERCPCy5.5	H198
MPO	PE	5B8
CD7	PEVio770	CD7-6B7
CD34	APC	581
CD14	AF700	M5E2
CD64	APC Cy7	10.1

Method:

Staining was performed using a 16-color panel in AML PBMC. Acquisition was performed using BD Fortessa flow cytometers. Data analysis was performed FlowJo using the gating strategy shown below.

Gating Strategy

Figure 1. Gating Strategy

Figure 1 Legend: *AML PBMC were stained for AML panel feasibility run,. Post initial clean up gates, cells were gated for Live cells followed by CD45+ve Gate. Dim CD45 cells were considered as primarily Blasts cell containing major population. All subsequent population were gated on CD45 Dim cells as shown in Figure 1.*

Figure 2. AML feasibility run and Blast phenotyping

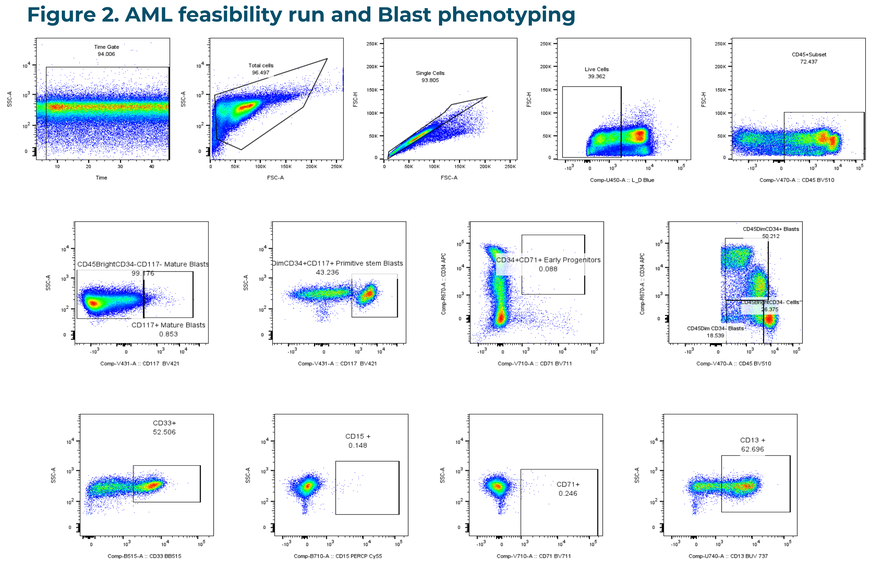

Figure 2 Legend: *To test the feasibility of Blast cell sorting, the panel was transferred and tested on BD S6 Symphony Cell Sorter. M4 (acute myelomonocytic leukemia) AML PBMC were stained with 15 color flow-panel as MPO was omitted given the fact that intracellular staining were excluded to sort viable unfixed cells. Cells were thawed and stained as per surface staining protocol and were acquired on BD S6 platform. This graph represents pre sort gating strategy, to see the AML PBMC phenotype. Analysis was done using Flow-Jo software.*

Results:

Figure 3. Post Sort: Blast Cell sorting AML PBMC (M4)

Figure 3A - AML PBMC (M4)

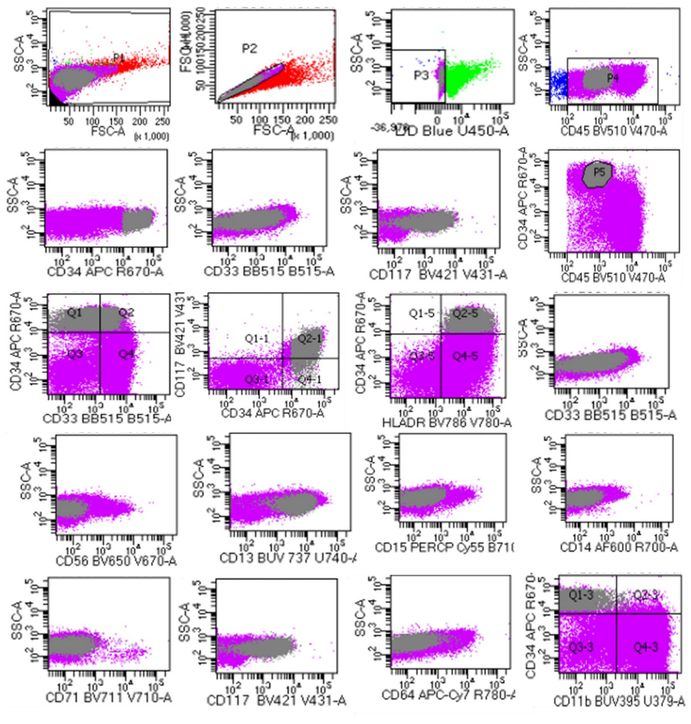

Figure 3BA - Healthy Donor PBMC

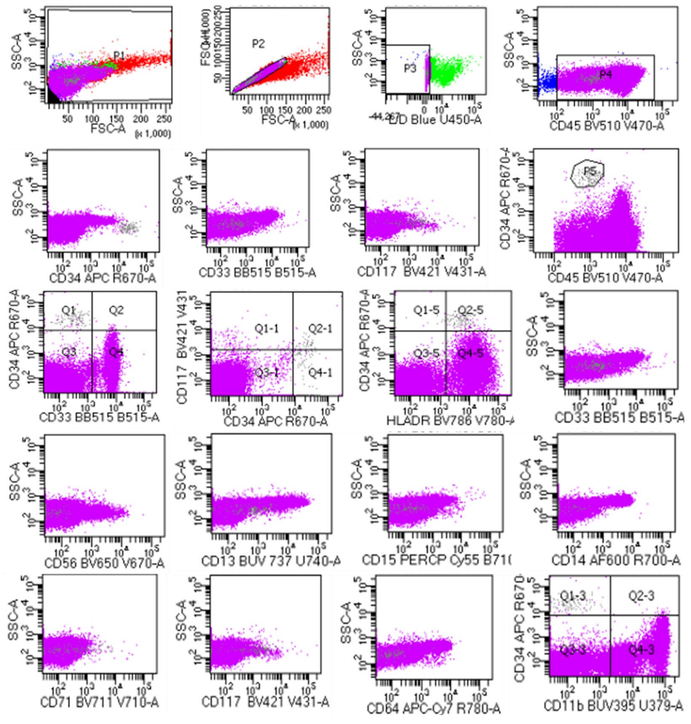

Figure 3 Legend: *AML PBMC (Figure 3A) and Healthy donor PBMC (Figure 3B) were stained with 15 color panel and samples were sorted for Blast cell population using a multi-parametric combination to phenotypically identify and sort the AML blast cells. Cells highlighted in grey, were characterized as blast cells in pre-sort analysis and were subjected to viable cell sort. Healthy donor sample were used as a negative control for blast cell identification and sorting. , CD45 dim cells show Blast cells populations, that can be further characterized with markers like CD34, CD33,CD117,CD71 primarily. Other markers for Blast cell identification are CD11b, CD64, CD13, CD56, CD15, CD14 and HLADR.*

Figure 4. Post Sort Blast Cell purity AML PBMC (M4)

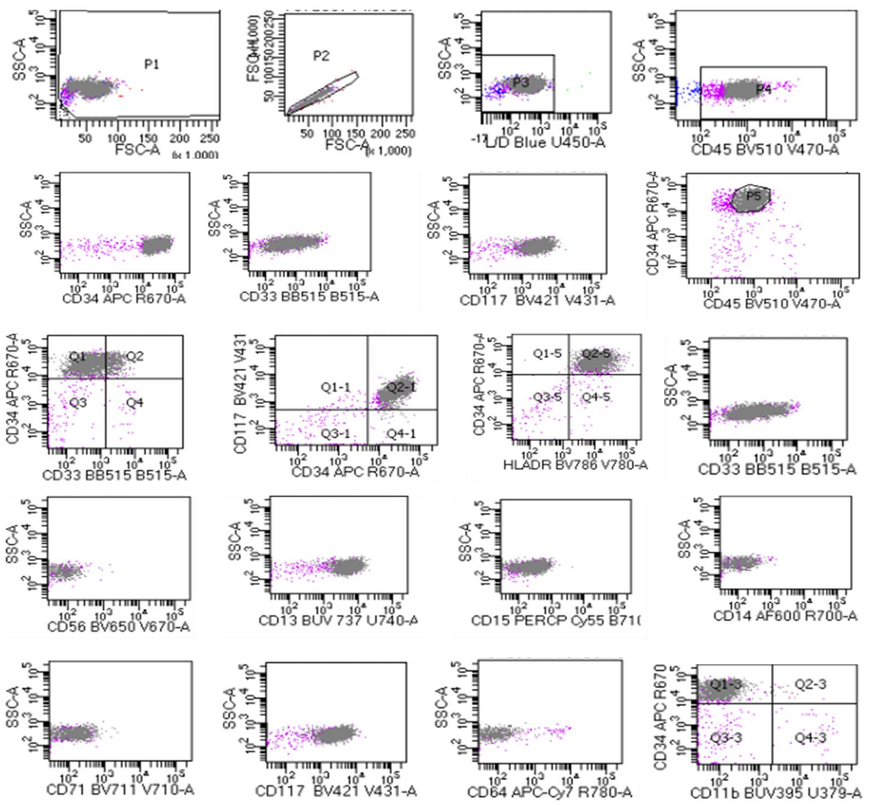

Figure 4 Legend: *Grey highlighted cells represent the pure blast cell purity post sorting. AML PBMC the purity of sorted viable AML Blast cells. Sorted Blast cells were re-acquired to show the post-sort purity of Blast cells, CD45 dim cells show Blast cells populations, that can be further characterized with markers like CD34, CD33,CD117,CD71 primarily. Other markers for Blast cell identification are CD11b, CD64, CD13, CD56, CD15, CD14 and HLADR.*

Conclusion :

The results from this pilot study indicate That using a combination of multi-parametric hierarchy, AML Blast cells can be characterized. Sorted Blast cells were re-acquired to show the post-sort purity of Blast cells, CD45 dim cells show primarily. Other markers for Blast cell identification are CD11b,CD64, CD13,CD56,CD15,CD14 and HLADR. Flow cytometric analysis using key markers CD33 (pan-myeloid), CD34 (stem /progenitor), CD117 (c-Kit receptor), and CD71 (transferrin receptor) enables precise blast population identification.

M4 (acute myelomonocytic leukemia) exemplifies therapeutic complexity, characterized by dual lineage differentiation. Our four-stage classification reveals Stage specific (CD34+CD117+CD33+CD71-) representing primitive stem-like cells; Stage II (CD34+CD117+CD33+CD71+) early committed progenitors; Stage III (CD34-CD117±CD33+CD71+) intermediate differentiating blasts; and Stage IV (CD34-CD117-CD33+CD71+) mature populations. This gating strategy is not limited to M4 Blast cell identification and can be optimized to other AML blast stages as well.

Discussion :

Flow cytometry and advanced cell sorting emerge as the cornerstone technologies transforming our understanding and treatment of Acute Myeloid Leukemia (AML), one of the most challenging hematological malignancies. While traditional morphological approaches have provided foundational insights, integrated flow cytometric analysis with precision cell sorting now reveals the true complexity hidden within AML blast populations, unlocking unprecedented opportunities for precision therapeutics. To address the limitations of conventional AML phenotyping strategies, we developed an advanced 15-color flow cytometry system - a major step forward in studying AML blast cells. This integrated technology platform provides comprehensive phenotypic fingerprints that transcend conventional FAB classification limitations, while cell sorting capabilities enable direct functional validation of identified populations. This dual approach exposes the heterogeneous nature of AML, where distinct blast cell populations coexist within individual patients, each harboring unique therapeutic vulnerabilities that remain invisible to traditional diagnostic methods. The marker panel will enable characterization and sorting of pre-treatment blast populations from individual patients. Following therapeutic intervention, this approach will be valuable for assessing the "unblocking" of AML blasts at their specific arrest points within the hematopoietic hierarchy—with particular relevance for patients who may develop differentiation syndrome, a response commonly observed with various AML treatments. While differentiation syndrome represents a serious condition managed through established clinical protocols, monitoring blast marker changes in response to treatment can enhance characterization of drug responses and provide essential data for elucidating mechanisms of action.