#P05

MULTIPLE MYELOMA FLOW CYTOMETRY PANEL VALIDATED FOR CLINICAL MONITORING OF PATIENTS

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INTRODUCTION

FLOW CYTOMETRY AND MULTIPLE MYELOMA

- Flow cytometry is a widely utilized method for measuring minimal residual disease post therapy as a prognostic marker.
- Caprion has a validated fit-for-purpose clinical assay (**MM Counting Panel**) for bone marrow aspirates (BMA) and peripheral blood (PB) that can accurately enumerate and phenotype malignant and non-malignant populations longitudinally in patients.
- This panel can support studies of approved and investigational antibody and cellular therapies targeting specific multiple myeloma (MM) antigens to direct immune cell mediated cytotoxicity.

MM COUNTING PANEL

- The MM Counting Panel detects antigens and plasma subsets that are important in MM, using fluorochrome-conjugated antibodies and flow cytometry. T cells, B cells, monocytes, and NK cells are also detected with this panel.

MM Counting Panel Markers	
Plasma and MM markers	CD138
	CD38
	BCMA (CD269)
	CD19
	CD56
Lineage markers for T cells, NK cells, and monocytes	CD4
	CD8
	CD3
	CD14
	CD16
	CD45

Plasma and MM subsets can then be identified:

POPULATION	CD45	CD3	CD19	CD14	CD56	BCMA	CD138	CD38
B cells	+	-	+	-	-	N/A	N/A	N/A
Plasma cells	+	-	+	-	-	N/A	+	+
Malignant plasma cells (CD19-)	+	-	-	-	+	N/A	+	+
Malignant plasma cells (CD19+)	+	-	+	-	+	N/A	+	+
BCMA+ plasma cells (CD19-)	+	-	-	-	+	+	+	+
BCMA+ plasma cells (CD19+)	+	-	+	-	+	+	+	+

N/A: not applicable

Figure 1. MM Counting Panel: . T cells, B cells, NK cells, and monocytes are also enumerated and phenotyped given they are prevalent in bone marrow and can be tracked longitudinally.

METHODS

ASSAY DEVELOPMENT METHODOLOGY OVERVIEW

PB and BMA samples were obtained from healthy and MM donors who consented to research testing and collected in Cyto-Chex® blood collection tubes. MM cell lines were also used to spike into donor samples to detect specific antigens. Samples were then transferred to BD TruCount™ tubes to enumerate immune populations for panel setup and characterization.

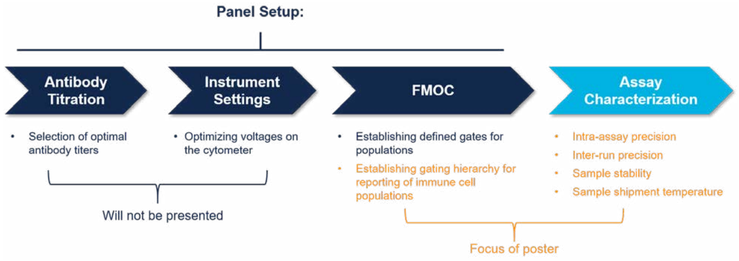


Figure 2. Assay development and characterization overview

ASSAY CHARACTERIZATION OVERVIEW

Following panel setup, the MM Counting Panel was characterized with fresh BMA and peripheral blood samples from both healthy and MM donors. The following critical parameters were assessed:

- Intra-assay precision
- Inter-run precision
- Sample stability
- Sample shipment temperature

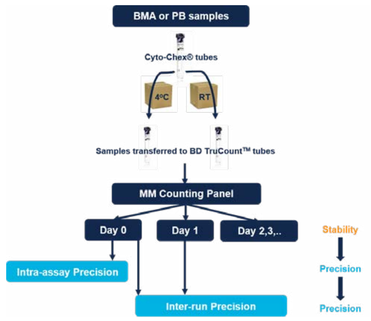


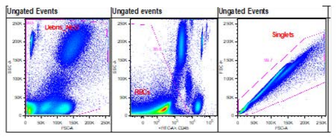
Figure 3. Assay Characterization: BMA or PB samples from MM or healthy donors received at Caprion-HistoGeneX

RESULTS

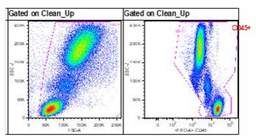
ESTABLISHING GATING HIERARCHY: IDENTIFICATION OF PLASMA AND MM CELLS

- PB samples from a healthy donor was spiked with a 1:1 mix of two MM cell lines (RPMI 8226 and NCI-H929).
  - Spiked at a level that was 5% of total leukocyte number
- Gating hierarchy was established, and a malignant plasma cell population was detected with a surface marker profile typical of MM cells: CD19-CD56+BCMA+

1 - Clean up gates



2 - Gating on CD45+ cells



3 - Identification of MM cells

4 - BCMA detection

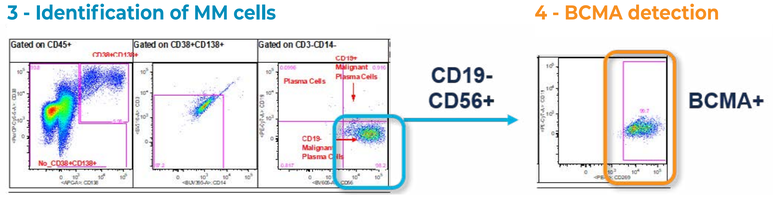


Figure 4. Gating Hierarchy in PB sample: Malignant plasma cell population defined as CD45+CD19-CD56+BCMA+

Intra-assay Precision and Limit of Quantitation

- Intra-assay precision was assessed for all readouts in PB
- Limit of quantitation was determined to be >100 absolute counts and >1% frequency

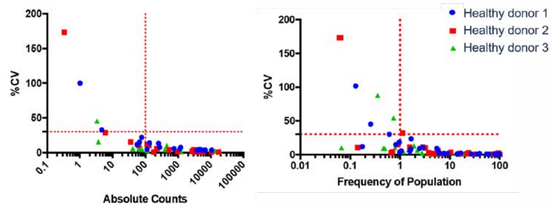


Figure 5. Intra-assay Precision

Inter-assay Precision

- Inter-assay precision was assessed for all readouts in PB
- Inter-assay precision was met for majority of readouts that were above the limit of quantitation

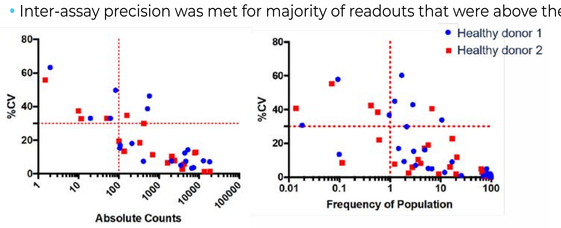


Figure 6. Inter-assay Precision

Sample Stability and Storage Conditions

- The results from a representative donor showed that the main lineage markers (along with malignant plasma cells in PB) met the acceptance criteria for both temperature conditions at all timepoints.
  - Plasma cells (both malignant and non-malignant) were not detectable above the limit of quantitation in the BMA sample.

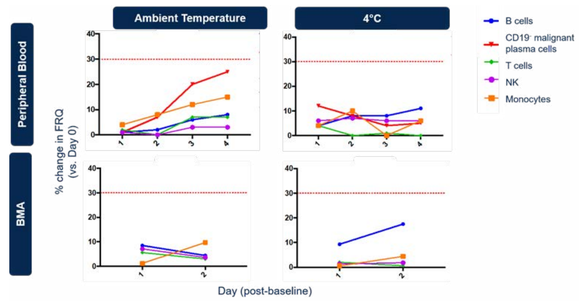


Figure 7. Sample Stability and Storage Conditions

Example of BCMA Detection in BMA Clinical Samples

- Malignant plasma cells and BCMA were detected in BMA samples from patients in the NCT03761108 clinical trial. Different BCMA expression patterns were observed amongst different subjects.

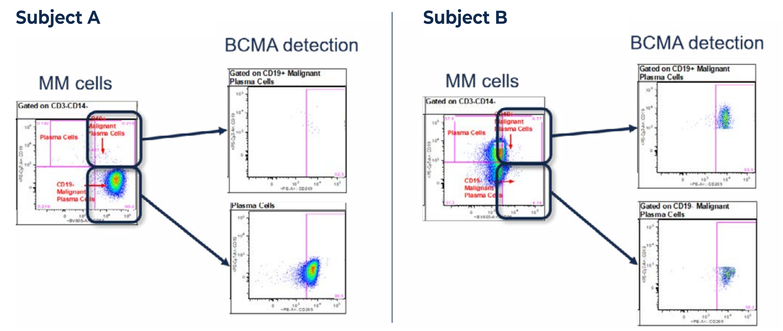


Figure 8. Two different BMA clinical samples phenotyped using the MM Counting Panel - BMA samples collected during screening (pre-treatment).

CONCLUSION

Flow Cytometry

- The MM Counting Panel was developed to enumerate and phenotype MM markers and immune cell subsets.
- MM cells were detected and assessed during both assay development and clinical immune monitoring. The assessment included the detection of the MM marker BCMA.
- This assay can be modified and deployed for other hematological malignancies.
- Similar assays detecting different immune cell subsets for other indications can easily be developed at Caprion-HistoGeneX.

Mass Spectrometry

- Serum BCMA (sBCMA) is also considered to be an important biomarker for predicting patient outcome.
- sBCMA measurement by mass spectrometry at Caprion-HistoGeneX complements our flow cytometry panel by providing an additional biomarker that may be used to predict patient outcome.
- Correlate cell surface markers with circulating markers such as sBCMA
- Stratify patients to identify the appropriate treatment based on their basal level of sBCMA
- Measure sBCMA levels pre-and post-treatment to monitor efficacy of treatment

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