Peripheral blood CMMC correlate with disease burden and can be used to characterize high-risk cytogenetics in newly diagnosed and smoldering myeloma.

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**INTRODUCTION**

There is an increasing interest in the ability to dynamically track disease burden and perform molecular subtyping of patients with plasma cell disorders without invasive bone marrow sampling.

Circulating multiple myeloma cells (CMMC) have been detected in elevated numbers in the peripheral blood of patients with plasma cell disorders using flow cytometry or circulating cell enrichment platforms.\(^1\)

We developed an automated CELLSEARCH\textsuperscript{\textregistered} assay to enrich, enumerate, and perform a triple FISH assay for t(4;14), t(14;16), and del 17p on CMMC (CD138+CD38+ CD45-CD19-) isolated from a 4 ml peripheral blood sample.\(^2\)

Here we present the enumeration and cytogenetic profiling of CMMC from separate cohorts of patients across the spectrum of plasma cell disorders.

**OBJECTIVES**

- To develop methods to enrich and enumerate CMMC from peripheral blood.
- To develop methods to characterize CMMC by transcriptional profiling and cytogenetic analysis.
- To compare CMMC to standard predictive and prognostic clinical parameters.
- To determine the ability of CMMC to predict progression from smoldering myeloma to myeloma.

**MATERIALS & METHODS**

CellSearch enrichment kits were configured to capture CMMC with CD138 and/or CD38 ferrofluid as shown. CMMC enumeration and FISH was performed by Janssen Diagnostics. For single cell isolation, pools of 10 CMMC were isolated using a micromanipulator. RNA was amplified and RNA-seq libraries were prepared using Clontech kits. FISH was performed on fixed CMMC as described.\(^3\)

**RESULTS**

**1a) CMMC isolation and detection**

Patient blood was collected in CellSave tubes from commercial vendors and University of Pennsylvania. Patients varied according to treatment status and stages of disease. Healthy donors were collected from commercial sources and the Janssen diagnostics blood draw program.

**1b) CMMC image analysis**

**1c) Linearity of CMMC recovery**

**2a) CMMC counts by stage**

**2b) Concordance between CMMC FISH and Bone Marrow**

**2c) Peripheral blood plasma cells vs CMMC method vs flow**

**3a) Frequency of CMM in high risk SMM**

**3b) CMMC FISH vs Bone marrow FISH**

**3c) ROC Analysis Comparison: CMMCs vs M Protein or % BM Plasma Cells**

**CONCLUSIONS**

- Immunomagnetic enrichment of peripheral blood CMMC represents a reproducible means to non-invasively monitor disease status in plasma cell disorders.
- CMMC FISH and transcriptional profiling indicate that CMMC share gene expression patterns and cytogenetic aberrations that are consistent with those found in tumors.
- The CMMC enrichment method is more sensitive than flow cytometry at detecting plasma cells in peripheral blood.
- In newly diagnosed myeloma, CMMC may be a useful prognostic marker at remission to delineate those patients at risk for relapse.
- Elevated numbers of CMMC are found in SMM at baseline. CMMC may be useful for predicting patients at risk of progression to MM.

**REFERENCES**