An integrated workflow for liquid biopsy of Circulating Multiple Myeloma Cells (CMMCs) with single cell resolution reveals tumor heterogeneity

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Introduction Multiple myeloma (MM) is a bone marrow derived cancer of plasma cells, which remains an incurable disease. Because of the invasive and painful nature of bone marrow biopsy, an alternate tumor monitoring strategy is needed. We have previously shown that Circulating Multiple Myeloma Cells (CMMCs) isolated by CELLSEARCH® (CS) are prognostic and indicative of disease burden through remission and relapse [Foulk et al., 2017]. Here we report, for the first time, the molecular characterization of pure single CMMCs isolated from a multiple myeloma patient, by integrating CS and DEPArray™ (DA) Nxt systems*, providing access to copy-number alteration (CNA) profiling.

Methods 4.0ml of peripheral blood from a patient with multiple myeloma was tested. CMMCs enrichment with CS was performed using antibodies anti-CD138 for cell capture and stained immunofluorescently with CD38-PE, CD19 and CD45-APC. Nuclei were stained with DAPI and detected target cells were counted (Fig.2a). The enriched sample was then analyzed using the DA Nxt system: single CMMCs (CD38+/CD19+ and CD45-/DAPI+), along with some single White Blood Cells (WBCs: CD38-/CD19+ or CD45+/DAPI+), were isolated (Fig.2b). The DNA of each single cell was amplified using the Ampli1™ WGA kit. Then, libraries were prepared using Ampli1™ Low Pass kit and sequenced on Illumina® MiSeq for obtaining a genome-wide single-cell CNA characterization.

Results CS identified 128 CMMCs. From DA Nxt we collected 20 individual cells for low-pass CNA analysis. Copy-number profiles of CMMCs confirmed their tumor origin, with several gains and few losses of chromosomal segments indicative of a high level of genome instability (Fig.3). Moreover, an unsupervised hierarchical cluster analysis highlighted a conserved pattern of alterations across CMMCs, enabling the separation between CMMCs and WBCs groups (Fig.4). The presence of subclonal alterations, shared by a subset of the CMMCs analyzed, suggests a branched evolution of different tumor subclones. WBC profiles were flat as expected.

Conclusions Cell enrichment by CS followed by individual cell sorting using DA Nxt*, enabled the isolation of single CMMCs with 100% purity. Ampli1™ single-cell analysis demonstrated CMMC molecular heterogeneity suggestive of tumor subclones presence. This platform combination provides a reliable and non-invasive method for MM characterization enabling translational research and future clinical application.

Fig.1 Complete workflow* for isolation and molecular characterization of CMMCs. Target CMMCs from peripheral blood were enriched and subsequently labeled with fluorescent markers by CELLSEARCH®. The fluorescence-labeled cells were loaded into the DEPArray™ Nxt, enabling recovery of pure single-cells exhibiting the desired fluorescence patterns and morphological characteristics. Then, single cells were firstly whole-genome amplified using the Ampli1™ WGA kit and then sequenced at shallow coverage using Ampli1™ LowPass kit and Illumina MiSeq. The genome integrity index (GII) of WGA samples was evaluated by Ampli1™ QC kit.

Fig.2 Single-cell enrichment and isolation with CELLSEARCH® and DEPArray™ Nxt*. a) Image gallery showing some of the CMMCs enumerated with the CELLSEARCH® platform b) DEPArray™ scatter plot of CS-enriched sample showing two well-defined populations, based on CD45/CD19 and CD38 intensity: CMMCs (CD38+/CD19- and CD45-) and WBC (CD45+ or CD19+/CD38-) are highlighted by a green and red circle, respectively.

Fig.3 Image gallery and low-pass copy-number profiles of 5 single-cells isolated by CS and DA Nxt*. For each cell, the figure shows the bright field and fluorescent imaging detected by DA technology on the left and low-pass copy-number profiles on the right. In copy-number tracks, significant gains and losses are highlighted in red and blue respectively. As expected, WBC shows a flat profile without copy-number changes; moreover, CMMCs clearly show aberrant profiles with some level of heterogeneity (ex. R15:chr1; R23,R10: chr13; R23:chr3, etc).

Fig.4 Cluster analysis of low-pass profiles of 20 single cells. Unsupervised clustering unveils two major clusters, underlining the clear separation between CMMCs (aberrant) and WBCs (flat). Minor CMMC subclusters depict an inter-cell variability suggesting the presence of different subclones.