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Single-Cell Multi-Omic Correlation of Single Nucleotide Variants, Copy Number Variation, and Surface Epitopes for Clonal Profiling of Myeloma

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Introduction

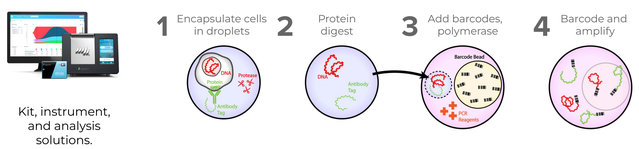
Using cryopreserved, human multiple myeloma (MM) patient samples processed on the Mission Bio Tapestri single-cell platform, subclones were simultaneously analyzed for single nucleotide variants (SNV), copy number variants (CNV), clonotypes, and surface protein expression. We show here complex clonal evolution of MM in two patient samples as copy gains and losses were sequentially acquired and correlated with expression changes of MM heme/therapeutic markers. This high-resolution, single cell assay offers a potential new modality for the diagnosis and surveillance of patients with suspected MGUS, SGUS or high-risk MM. We have demonstrated exceptional results from cryopreserved human specimens, as well as the ability to use genetic lesion profiling to positively identify subclonal MM and correlate cell surface protein expression of potential therapeutic targets with each clonal population.

Methods

Samples: Two MM patients, BMMC, CD138 enriched, diagnostic and smoldering (for patient 2) time points.

Panels: 800-plex DNA: CNV, MM hotspots, VDJ clonotyping. 50-plex antibody: heme, MM therapeutic markers.

Study: Tapestri DNA+Protein single-cell workflow (below).



Results/Conclusions

Rich branching architecture was inferred by comparing copy number and SNVs against in-sample, healthy plasma cells. Several new capabilities were demonstrated in this study:

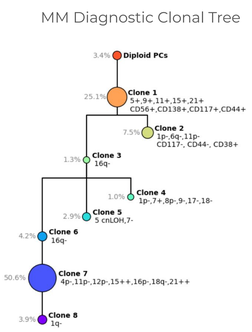
1) Therapeutic MM marker expression (e.g. FcRL5, GPRC5D, BCMA) were tracked and correlated with clonal evolution.

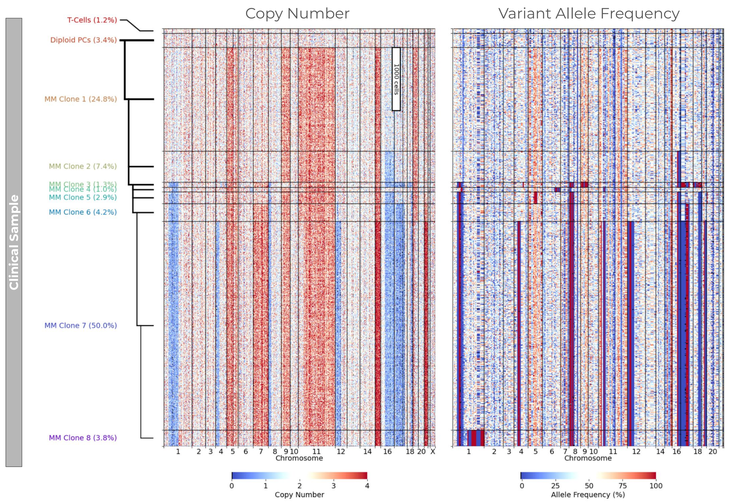
2) Clonotyping revealed a transition from diverse to clonal VDJ sequence with MM onset.

3) Comparing sample time points showed a shift in clonal population distributions with disease maturation.

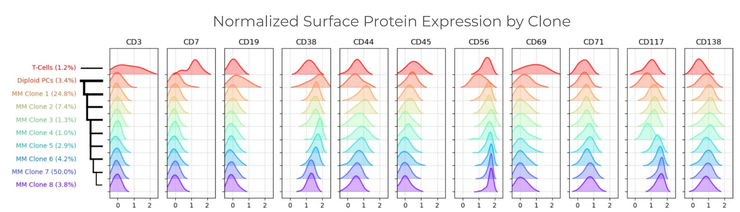
Patient 1 - Diagnostic MM Time Point

The phylogenetic tree (right) of the first patient's MM diagnostic time point shows healthy diploid plasma cells (PCs) acquired copy gain across odd-numbered chromosomes as well as increased CD56/138/117/44 expression in MM Clone 1. This was followed by further copy gain/loss events through Clones 2-8. The tree was generated from one Tapestri run analyzing copy number (below, left) and variant allele frequency (below, right) of thousands of single cells. The two plots strongly correlate.



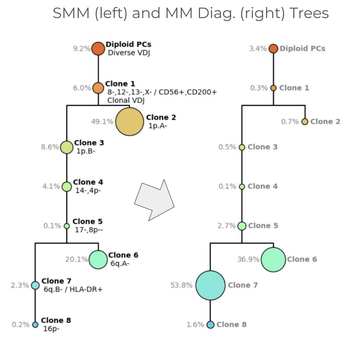


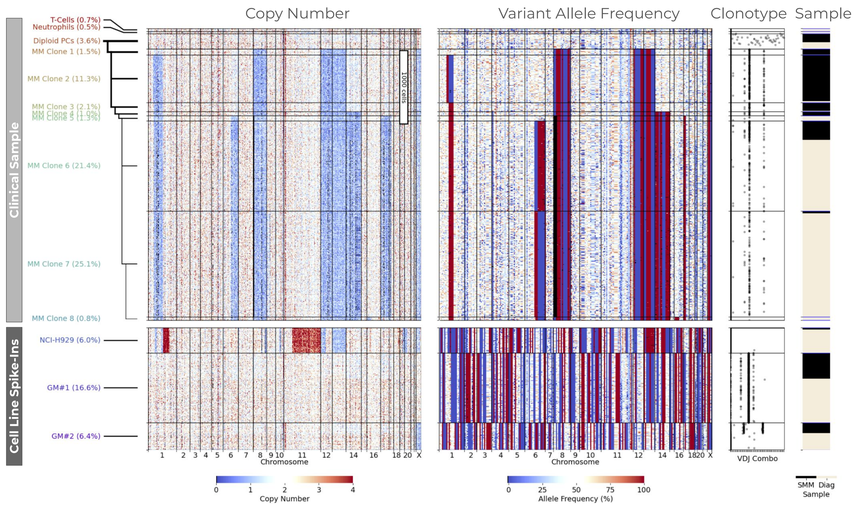
The expression of heme markers for each MM clone is shown below. As the disease progresses, CD38/44/45 declines while CD117 increases, except a noticeable dip in CD117 for Clone 5. Healthy T-cells show high CD3/7 and low CD138.



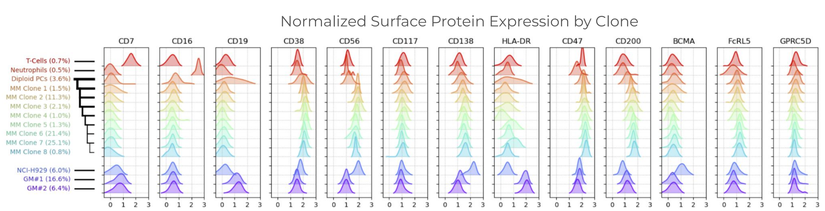
Patient 2 - Smoldering + Diagnostic MM Time Points

In patient 2, the highly mutated clones of the MM time point were more populated by fraction than in smoldering MM (SMM), as expected. The supporting CNV/SNV single-cell data are again below, and also include clonotype analysis. The latter shows a shift from VDJ-diverse healthy plasma cells to clonal MM cells. Clones 6 & 7 had two different haplotypes of Chr 6 by phasing, not apparent by CNV alone. Also included in the run were three cell lines to normalize copy number and expression, clearly distinguishable by allele frequency.





In this sample, CD56 and CD200 expression both jump with MM onset as seen below. Clones 6 & 7, differing only in Chr6 haplotype by this panel, see a large difference in HLA-DR expression. The three cell lines in purple at bottom validate most markers.



References

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