mission bio

tapestri
APPLICATION NOTE

Single-Cell DNA Analysis of Myelodysplastic Syndrome Using the Tapestri® Single-Cell DNA AML Panel

Takeaways

- Recurrent somatic mutations in MDS overlap with the Tapestri Single-Cell DNA AML Panel
- As few as 2 cells were detected at a sensitivity of 0.06%
- Single-cell genomic analysis reveals mutational co-occurrence and clonal evolution

Abstract

Myelodysplastic syndrome (MDS) is a blood disorder brought on by an accumulation of somatic mutations in hematopoietic stem cells resulting in ineffective hematopoiesis. Accumulation of genetic mutations can occur during clonal expansion of aberrant cells leading to a heterogeneous mixture of affected cells that can change a patient's prognosis. Distinguishing mutational status at the single-cell level provides precision in diagnosis and informs treatment options. Here, two MDS patient samples were analyzed with the Tapestri Platform. MDS and acute myeloid leukemia (AML) are both myeloid disorders with MDS having a chance of progressing to AML and thus can share overlapping molecular signatures[1]. Therefore, the Tapestri Single-Cell DNA AML Panel was used to comprehensively analyze the mutational landscape of MDS patient samples. Whether testing for diagnosis or disease monitoring, these results showed high sensitivity, clonal resolution, and concordance of variant allele frequency (VAF) between single-cell and bulk next generation sequencing (NGS) data. Additionally, clonal variant co-occurrence was resolved, allowing for clonal lineage reconstruction, and a more comprehensive picture of the patients' disease. These data illustrate the power of single-cell DNA analysis using the Tapestri Platform, giving researchers and clinicians more insight into MDS of individual patients for more informed treatment decisions.

Experimental Methods

Single-Cell DNA Analysis Using the Tapestri Single-Cell DNA AML Panel

Frozen PBMCs from two MDS patients were processed with the Tapestri Platform for single-cell isolation and DNA barcoding. Target amplification was performed using the Tapestri Single-Cell DNA AML Panel, which includes amplicons that assay recurrent somatic mutations in MDS[1]. Relevant splicing factors, epigenetic regulators, transcription factors, and kinases are represented within the panel (Table 1). Single-cell DNA libraries were sequenced on two Illumina MiSeq runs. Cell viabilities ranged from >75-90%. On average, 5,459 cells were analyzed per sample. Average depth of coverage was 36x, average reads mapped to cells was 88.5%, and average panel uniformity was 94.3%. Sequencing data were analyzed with Tapestri Pipeline and Tapestri Insights Software.

19-GENE AML PANEL

ASXL1	GATA2	KIT	PTPN11	TP53
DNMT3A	IDH1	KRAS	RUNX1	U2AF1
EZH2	IDH2	NPM1	SF3B1	WT1
FLT3	JAK2	NRAS	SRSF2	-

Table 1: 19-Gene Tapestri Single-Cell DNA AML Panel

Results

Disease Monitoring of MDS Research Case

Blood samples were collected at early diagnosis and post-treatment from a treatment-resistant 79-year old male diagnosed with MDS who presented with 10% bone marrow (BM) blasts at the time of diagnosis (<5% considered normal). The samples were analyzed using the Tapestri Platform for single-cell genomic analysis and NRAS G12D, KRAS G13D and JAK2 V617D mutations were detected as well as a double mutant NRAS G12D/KRAS G13D clone (Figure 1). KRAS and JAK2 variants at early diagnosis were detected at 0.3% and 0.07% VAF respectively, illustrating the platform's high level of sensitivity. Interestingly, the patient had a history of myelofibrosis. Thus the observation that the JAK2 clone is separate, and does not co-occur with the NRAS or KRAS mutations suggests this patient developed MDS separately and not from the same JAK2 clone.

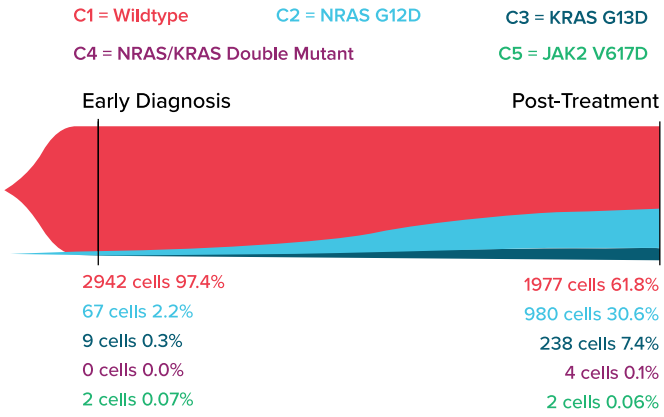

Figure 1: Fish plot illustrating progression of disease in a patient diagnosed with MDS, shown as number and percentages of variant clones over time

Clonal Variant Co-occurrence in an MDS-EB1 Research Case

A sample was taken at diagnosis from a 49-year old female with 9% BM blasts who was diagnosed with MDS-EB1, a rare type of MDS. The sample was previously analyzed with bulk NGS revealing variants DNMT3A R882H, SF3B1 H662Y, and RUNX1 R162K, however, the ratio of cells harboring each mutation, with potential co-occurrence of mutations was unattainable by bulk analysis. With single-cell genomic analysis using the Tapestri Platform, the results showed high concordance between single-cell pseudo-VAFs and bulk NGS VAFs (Figure 2). Additionally, single-cell analysis revealed clonal populations with co-occuring double and triple variants, giving the ability to reconstruct the patients' clonal lineage. With this unambiguous identification of clonal mutation co-occurrence, varied treatment options could be considered for this patient.

Gene	Variant	Bulk [%]	Single-cell [%]
DNMT3A	R882H	43.1 %	42.0 %
SF3B1	H662Y	31.0 %	35.0 %
RUNX1	R162K	63.3 %	61.2 %

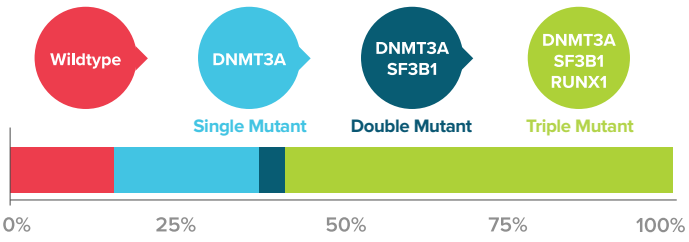

Figure 2: Correlation to bulk NGS and clonal variant co-occurrence in a patient with MDS-EB1 revealed through single-cell DNA sequencing

Conclusions

Here we show in patients with MDS that rare variants present at diagnosis at low VAFs can be detected with high sensitivity with the Tapestri Platform. In addition, single cell analysis provides a robust and explicit method for detecting co-occurrence of mutations and unraveling clonal evolution.

References

1. Walter et al., Clonal diversity of recurrently mutated genes in myelodysplastic syndromes. Leukemia, 27, 1275-82 (2013)

2. Bejar and Steensma, Recent developments in myelodysplastic syndromes. Blood, 124, 2793-803 (2014)