An Automated high-throughput custom single-cell targeted DNA sequencing platform to reveal rare clonal evolution in cancer and characterize mutation profiles in CRISPR edited cells

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Introduction

Many stages of hematopoietic differentiation provide multiple opportunities for mutations that lead to distinct tumor subtypes. However, standard bulk population sequencing is hard to identify rare alleles or determine whether multiple mutations co-occur within the same cell. Tapestri® microdroplet in microfluidics has advantages of high-throughput and high-accuracy trace detection in the field of single-cell analysis.

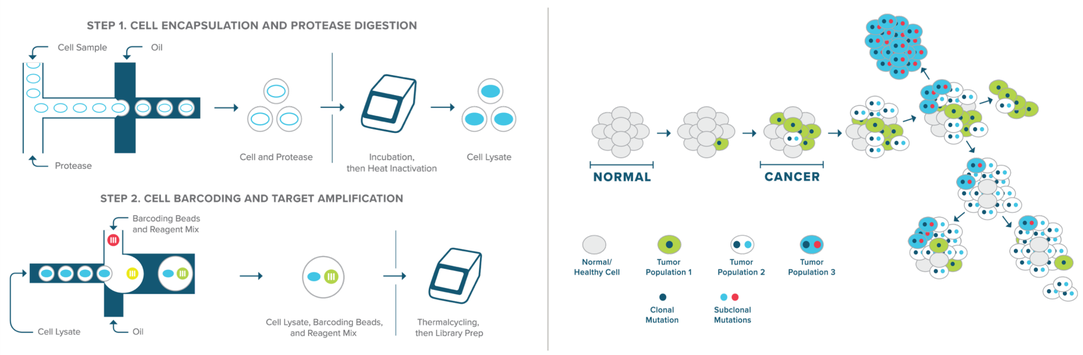


Fig. 1 Novel two-step droplet microfluidics for understanding disease at single-cell level

Objective

To identify individual cells harboring pathogenic mutations, single-cell sequencing is applied for rapid and comprehensive profiling of thousands hematological malignancy tumor cells in parallel, analyzing somatic mutations of candidate genes as markers of the neoplastic clone.

Materials & Methods

Using the Tapestri Platform, mutational burden as well as the type and frequency of genetic alterations were examined with Single-Cell DNA panels.

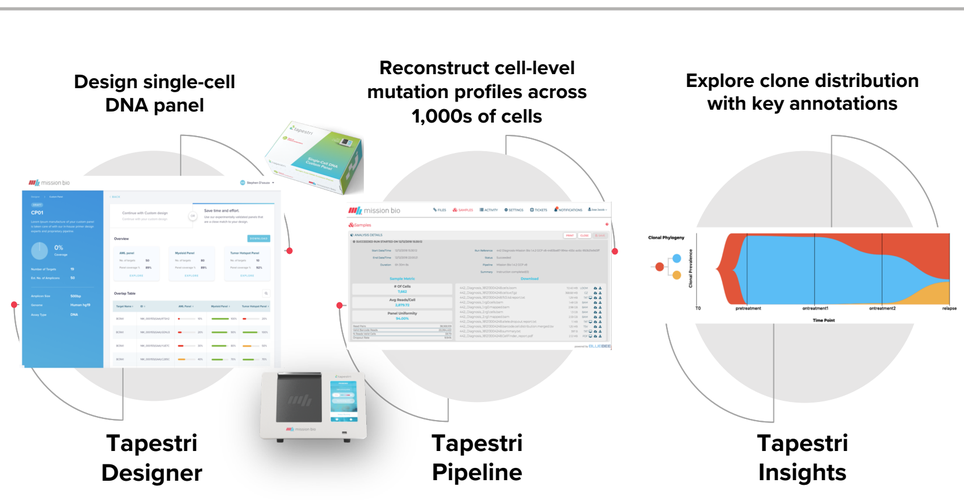


Fig. 2 Single-Cell solutions from panel design to insights

Results

Diverse patterns of clonal selection and evolution were revealed in acute myeloid leukemia (AML) patient with Gilteritinib treatment

McMahon, C.M., et al., Cancer Discovery (2019)

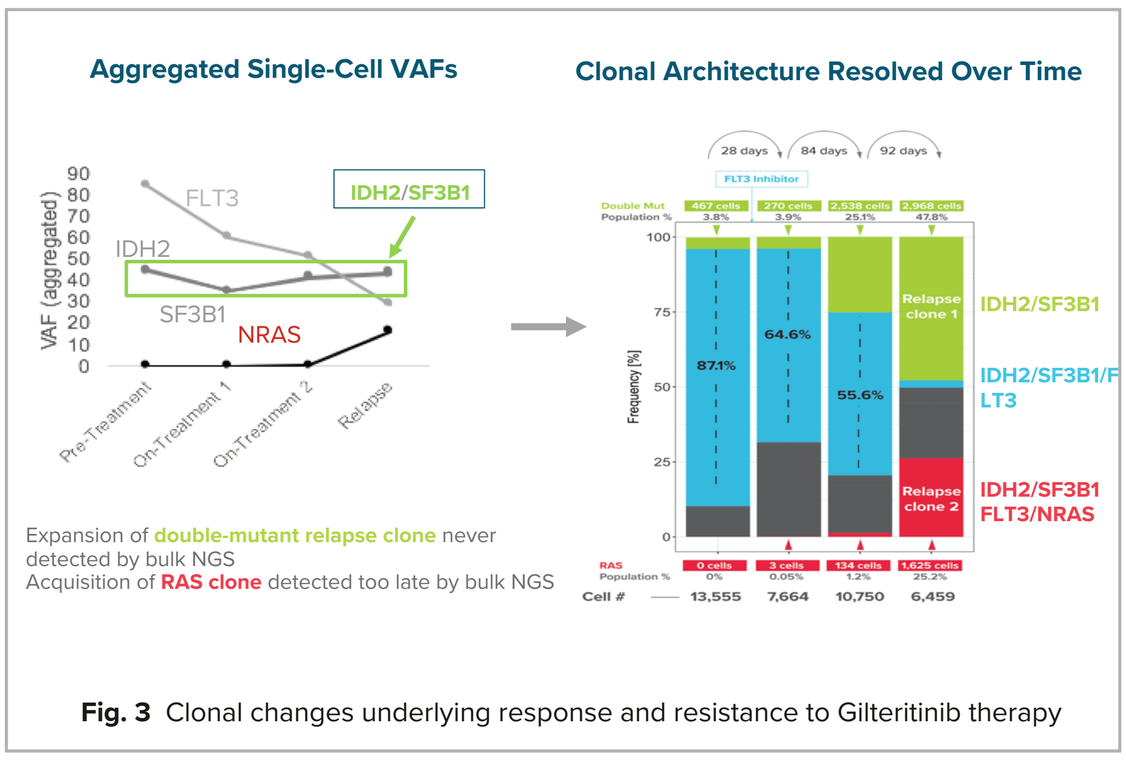


Fig. 3 Clonal changes underlying response and resistance to Gilteritinib therapy

Clonal evolution analysis on bone marrow transplantation treated AML patient's peripheral blood mononuclear cells

Xu, L. et. al., Scientific Reports (2019)

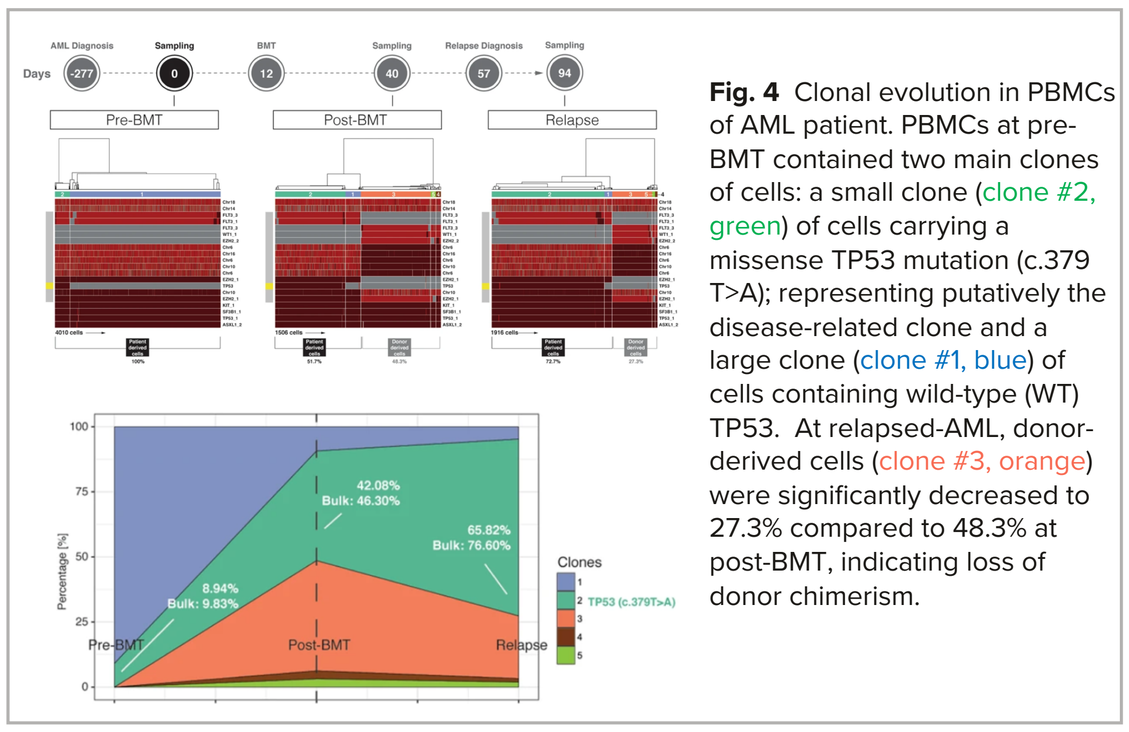


Fig. 4 Clonal evolution in PBMCs of AML patient. PBMCs at pre-BMT contained two main clones of cells: a small clone (clone #2, green) of cells carrying a missense TP53 mutation (c.379 T>A); representing putatively the disease-related clone and a large clone (clone #1, blue) of cells containing wild-type (WT) TP53. At relapsed-AML, donor-derived cells (clone #3, orange) were significantly decreased to 27.3% compared to 48.3% at post-BMT, indicating loss of donor chimerism.

Reliable CNV analysis of rare subclones

App Note, Mission Bio Inc.

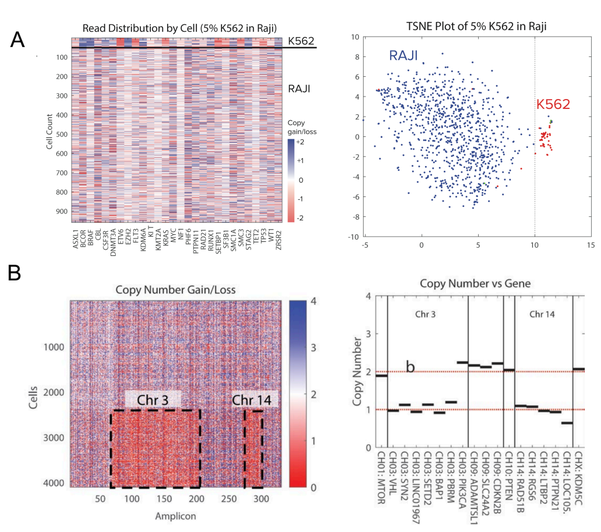
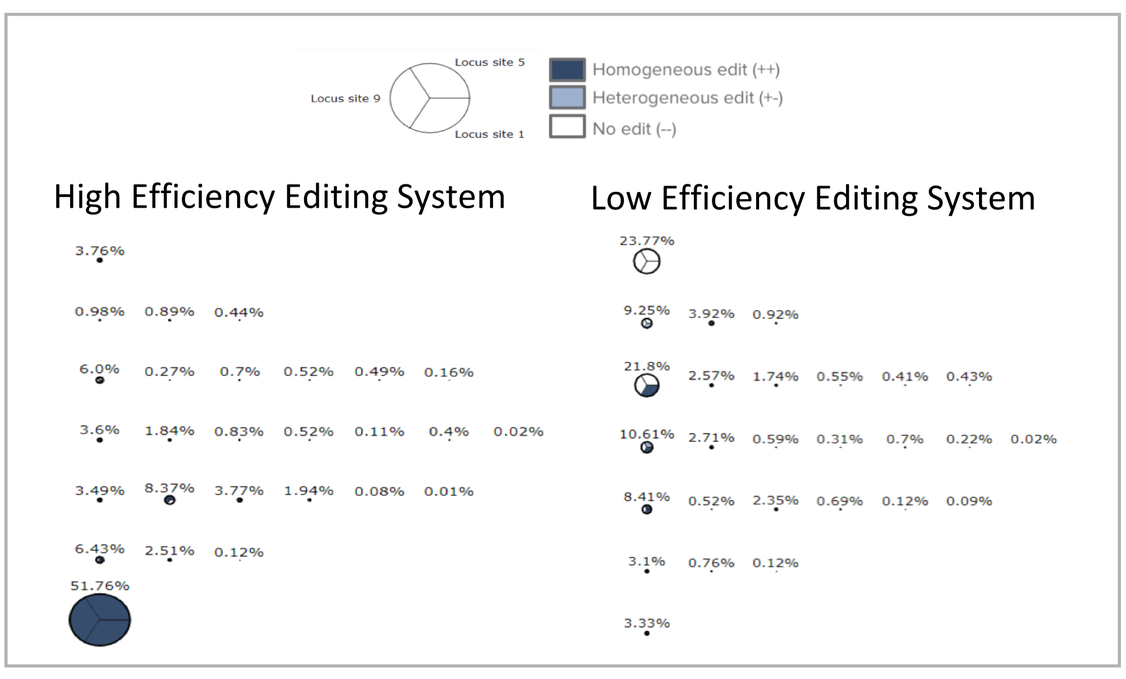


Fig. 5 To illustrate the sensitivity of CNV detection on a single-gene basis using the Tapestri platform, **A)** K562 cells were mixed with Raji cells at ratios of 5%, and run with the Myeloid panel. **B)** Clinical sample. Heat maps depicting CNV data and t-SNE plots generated from CNV data and colored by SNV data show clear clustering of the two cell types, demonstrating the ability to detect rare cell populations at 5% based on CNVs with existing panels.

Single-cell DNA analysis resolves zygosity and Co-occurrence of CRISPR genome editing events



Summary

Relevant complex clonal evolution within tumors was uncovered, and subclones impacting tumor therapeutic response and disease remission were detected, including double, triple, and quadruple mutant clones.

This novel integrated single-cell DNA sequencing system with optimized biochemistry, firmware, software, workflow, and data analysis solution, provides actionable information of clinical utilities as of diagnosis, prognosis, targeted therapy, and minimal residual disease (MRD) detection and monitoring, and facilitate clinicians to make precision medicine and improved outcomes a widespread reality.

Besides AML, CLL, Myeloid, and solid tumor panels, web-based Tapestri Designer is also available for custom genomic loci relevant to different applications for revealing genomic variation and clonal propagation in complex biological samples.

References

1. Pellegrino, M., et al., Genome Research (2018)
2. McMahon, C.M., et al., Cancer Discovery (2019)
3. Xu, L. et. al., Scientific Reports (2019)
4. https://missionbio.com/resources/#application-technical-notes