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 microfluidics has advantages of high-throughput and high-accuracy trace detection in the field of single-cell analysis.

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.

Objective

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

Results

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

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

Fig. 2 Single-CellInsights from panel design to insights

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

Fig. 4 Clonal evolution in PBMNCs of an AML patient. PBMNCs 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.

Fig. 5 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)

Fig. 6 Single-cell data reconstructed phylogenetic trees show the progression from TP1 to TP2 and highly correlated to bulk data (R² = 0.994)

Summary

AML 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.

References