Ultra-Sensitive Minimal Residual Disease (MRD) Monitoring For Cancer Patients Using SuperRCA Mutation Assays With Flow Cytometer Readout

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Background & objectives

Rare tumor-specific mutations in patient samples serve as excellent markers to monitor the course of malignant disease and responses to therapy in clinical routine, and improved assay techniques are needed for broad adoption. We describe herein - superRCA assays - which provides for rapid and highly specific detection of DNA sequence variants present at very low frequencies in DNA samples. Using a standard flow cytometer we demonstrate precise, ultra-sensitive detection of single-nucleotide mutant sequences from malignant cells against a 100,000-fold excess of DNA, to follow the course of patients treated for acute myeloid leukemia (AML) and Lung Cancer patients.

Methods

Sequence of interest are first enriched by targeted PCR amplification from a patient sample and converted to DNA circles that are subjected to rolling-circle amplification (RCA). Padlock probes specific for mutant or wild-type sequences are then used to probe the repeated sequences of the RCA products with exquisite specificity, followed by RCA of the circularized probes. The large DNA clusters that result from each starting DNA circle are referred to as superRCA products.

Results

The low detection limit and high precision of superRCA are consequences of the highly selective genotyping of the repeated target sequences in combination with the large numbers of products that may be conveniently analyzed by flow cytometry. For example, patient UPN125, NGS-analysis failed to detect the remaining IDH2 p.R172K mutation after initial treatment which was therefore paused, although later superRCA and ddPCR analyses both clearly revealed the remaining malignant clone, subsequently leading to a relapse for this patient. Even low levels of remaining leukemic markers in the post SCT-setting would prompt clinical action, mainly by reducing immunosuppressants to boost the immunological effect of

the SCT in order to eradicate remaining malignant clones that risk giving rise to leukemic relapse. For the Lung cancer patients who are under the Immune checkpoint inhibitor treatment, superRCA can precisely tracking the patients' response to the therapy by tracking recurring driver mutations presented in the patients' primary tumor. It is also demonstrated with ultra-high sensitivity, superRCA can find early sign of relapse in the blood samples than bone marrow samples analyzed with ddPCR/NGS assays.

Conclusion

The superRCA assay procedure is suitable for routine use by virtue of its high sensitivity and simplicity. The 3-hr protocol only requires a sequence of five additions to a DNA sample, separated by incubations, before reaction products are analysed using a standard flow cytometer. With ultra-high sensitivity, tt's even possible to monitoring the status of Leukaemia patient with samples with equal utility comparing to the bone marrow samples as well as in the plasma ctDNA samples from the Lung cancer patients.