Abstract: P1706

Title: DETECTION OF MEASURABLE RESIDUAL DISEASE IN AML BY NEXT-GENERATION SEQUENCING: INITIAL EXPERIENCES AND BENCHMARKING OF THE SURESEQ MYELOID MRD PANEL

Abstract Type: Poster Presentation

Topic: Novel technologies, techniques and digital analytical tools in hematology

Background:

Detection of measurable residual disease (MRD) is increasingly used to predict relapse risk and to guide therapeutic decisions in acute myeloid leukemia (AML) patients, including those undergoing allogeneic hematopoietic stem cell transplantation (HSCT). Established methods for MRD including flow cytometry and PCR-based molecular assays targeting specific gene mutations have variable sensitivity and/or are applicable to only a subset of patients. Targeted next-generation sequencing (NGS) assays covering multiple commonly mutated genes promise to allow sensitive MRD assessment in a broad range of AML patients with diverse genetic characteristics.

Aims:

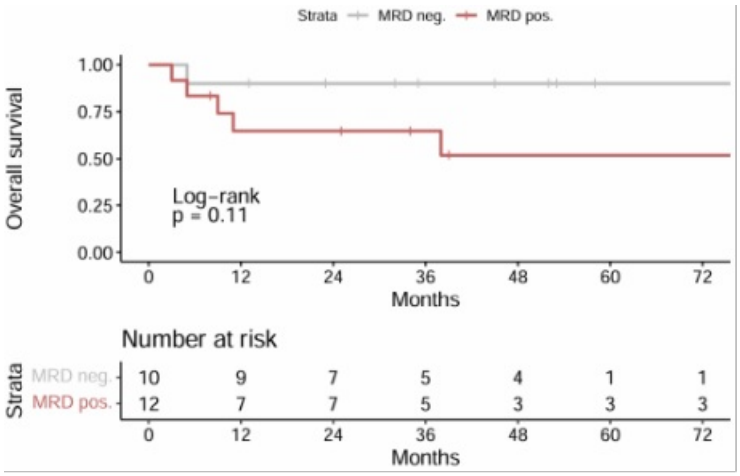
Our primary objective was to investigate the feasibility and performance characteristics of NGS-based MRD detection using the SureSeq™ Myeloid MRD panel, OGT (Oxford Gene Technology), Oxford Technology Park, Oxford, United Kingdom in comparison to a local lab-developed NGS assay, and to obtain preliminary data on the assay's prognostic value.

Methods:

We studied 22 AML patients (pts) undergoing allogeneic HSCT. Median age at HSCT was 58 (range 23-75) years, 10 pts were female; 12 had *de novo* AML, 6 had an antecedent myeloid neoplasm and 4 had AML post cytotoxic therapy. European LeukemiaNet (ELN)-2022 risk groups at diagnosis were favorable in 3, intermediate in 9, adverse in 4 and unknown in 6 pts. All pts had received intensive induction chemotherapy, 7 received a myeloablative conditioning regimen, while 15 received reduced intensity or non-myeloablative conditioning. Donors were matched related (5 pts), haploidentical (1 pt), or matched (12 pts) or mismatched (4 pts) unrelated. The median follow-up for pts alive was 35 months. Genomic DNA, derived from bone marrow obtained within 28days before HSCT, was analyzed using the SureSeq Myeloid MRD panel covering 46 regions of 13 genes (200ng input DNA), and a lab-developed assay using molecular inversion probes with unique molecular identifiers covering 92 regions in 32 genes (100ng input DNA). MRD positivity was defined as detection of ≥1 somatic variant, excluding those in *DNMT3A, TET2* or *ASXL1*.

Results:

The SureSeq Myeloid MRD panel achieved a sensitivity of 0.1% variant allele frequency (VAF) or lower across the targeted genomic regions, which was confirmed by simultaneous sequencing of diluted Myeloid DNA Reference Standard (Horizon Discovery). In our HSCT cohort, the SureSeq Myeloid MRD panel detected 16 MRD variants in 12/22 patient samples. Eleven of these variants were concordantly identified by our local lab-developed assay, while 5 additional variants (including 3 *FLT3*-ITD, and 2 single nucleotide variants in *FLT3* and *JAK2* with VAFs <0.1%) were only detected by the SureSeq Myeloid MRD panel. Only one variant was exclusively found by our lab-developed assay, in a region not covered by the SureSeq Myeloid MRD panel. Despite the limited patient number in this initial evaluation, MRD-positive pts showed a trend towards inferior overall survival compared to MRD-negative pts (Figure).



Summary/Conclusion:

This initial evaluation of the SureSeq Myeloid MRD Panel for MRD detection in AML patients undergoing allogeneic HSCT demonstrates the assay has high analytical sensitivity, including detection of *FLT3*-ITD mutations which are emerging as a clinically relevant MRD marker, and is broadly applicable to a diverse population of AML patients undergoing allogeneic HSCT. Prospective analyses of the clinical relevance of NGS-based MRD measurements are ongoing.

Keywords: Minimal residual disease (MRD), AML, Mutation analysis, Allogeneic hematopoietic stem cell transplant