P836 LONG-TERM DYNAMICS OF CLONAL HEMATOPOIESIS IN CHRONIC IDIOPATHIC NEUTROPENIA (CIN)

Topic: 12. Bone marrow failure syndromes incl. PNH - Clinical

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Background: We have previously performed next generation sequencing (NGS) analysis of genes recurrently mutated in myeloid malignancies in a cohort of CIN patients. We have estimated for the first time the frequency of clonal hematopoiesis in these patients and found that clonal CIN patients have significantly higher risk to develop MDS/AML compared to non-clonal CIN patients.

Aims: To conduct longitudinal follow-up NGS analyses of myeloid genes in CIN patients in order to assess clonal dynamics and evolution of clonal hematopoiesis in these patients.

Methods: We have performed longitudinal NGS analyses in 53 patients with CIN diagnosis (mean absolute neutrophil counts -ANCs- 1320±460/μL; median 1500, range 200-1700/μL) according to previously published criteria i.e no evidence of any underlying condition related to neutropenia after an extended clinical/laboratory investigation including bone marrow biopsy, karyotype, immunophenotype. Genomic DNA was extracted from bone marrow or peripheral blood samples at baseline and follow-up timepoints, sequencing libraries were prepared and subjected to targeted NGS on an Ion S5 Prime Sequencer (Thermo Fisher Scientific) using a panel of 38 myeloid genes.

Results: Longitudinal NGS analysis in the patients was performed over a period of 4-173 months (median 31 months). The incidence of clonal hematopoiesis was similar at baseline (16/53 i.e. 30.2%) and follow-up (16/53 i.e. 30.2%) (Figure 1A). The mutation spectrum (i.e. absence of mutations, multiple mutated genes or presence of a single mutated gene) at baseline (Figure 1B) was comparable to that at follow-up (Figure 1C). In total, 28 mutations were detected in 17 CIN patients at one or both time-points. The variant allele frequencies (VAF) did not show a significant difference at baseline (9.68%) and follow-up (13.23%) and correlation analysis showed that VAFs at both points strongly correlated (r=0.59; P<0.01), with the majority of CIN patients showing a stable clone size (Figure 1D). We performed the same analysis for the most frequently mutated genes in our cohort of patients, namely DNMT3A, TET2, SRSF2 (Figure 1E-G). Overall, DNMT3A mutations did not show any positive selection over time, with a median increase in VAF of 1.78% (P=0.67) during the follow-up period (median period of 47.5 months, range 12-164). Similarly non-significant increases in VAF were seen for TET2 (0.49%) and SRSF2 (0.04%), within a median period of 26 months (range 22-111) and 55 months (range 12-98), respectively. One patient with newly developing clonal CIN and one patient with disappearance of clonal hematopoiesis were found at follow-up after 4 and 98 months, respectively. Two patients acquired a second mutation at follow-up. At baseline they carried mutations in SRSF2 and ZRSR2, respectively and at follow-up they both developed TET2 truncating mutations. No significant changes at baseline and follow-up in ANC were observed in patients with clonal disease. Overall, four patients (7.55%) transformed to AML/MDS.

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Summary/Conclusion: In the majority of CIN patients tested for clonal evolution over time, most mutant hematopoietic clones appeared to be remarkably stable, with limited VAF expansion over the follow-up period and no acquisition of new molecular alterations. Only two patients acquired an additional mutation over time and this propensity was associated with mutations in spliceosome genes. None of the individuals bearing DNMT3A or TET2 mutations acquired additional mutations. This study contributes to the better understanding of clonal hematopoiesis in CIN.