Increased clonal hematopoiesis mutational burden and accelerated dynamic evolution of circulating clones in patients with ischemic heart failure

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Funding Acknowledgements: None.

Background: Clonal hematopoiesis (CH) - defined as the age-related acquisition of mutations in hematopoietic stem cells giving rise to mutated circulating leukocytes - is associated with increased risk of death in patients with heart failure with reduced ejection fraction (HFrEF). While the mutational landscape of CH in peripheral blood (PB) of patients with HFrEF is well characterized, the mutational burden in bone marrow (BM) samples of patients with HFrEF as well as its dynamics over time have not been investigated.

Methods/Results: We analyzed matched BM and PB cells from 68 patients with ischemic HFrEF by deep error-corrected targeted sequencing of 56 CH-driver genes with a detection threshold of VAF ≥ 0.5%. In PB, 56/68 patients carried at least one CH mutation, whereas 63/68 patients had mutations in BM. The absolute numbers of detectable mutations were significantly higher in BM compared to PB (215 mutations vs 120 mutations; p < 0.0001) (Fig. 1A). Likewise, the cumulative VAF was significantly higher in BM compared to PB (3.83 +/- 0.5% vs 3.0% +/- 0.7%; mean +/- SEM, p = 0.0025) (Fig. 1B). However, there was a strong correlation between mutation load in PB and BM (r = 0.54, p < 0.0001). Among the two most common mutations, DNMT3A mutations were either present in BM only or both compartments (PB only/BM only/both compartments 2/12/10), whereas TET2 mutations were strikingly enriched in BM cells (PB only/BM only/both compartments 0/16/2).

To study the dynamic evolution of CH clones in patients with HFrEF, we serially analyzed PB samples from 19 patients. At baseline, 16/19 patients carried at least one mutation in a CH driver gene and a total of 31 mutations. After a median follow-up of 12 months, 19/19 patients had at least one detectable mutation and a total of 43 mutations were identified (mean number of mutations per patient 1.63 +/- 0.033 vs 2.26 +/- 0.27; mean +/- SEM, p = 0.0410), indicating an increased mutation load over time (Fig. 2A). Moreover, cumulative VAF increased from 1.59 +/- 0.36 % to 2.01 +/- 0.38 %).

Conclusion: Patients with ischemic HFrEF display a high burden of CH mutations compared to values reported for healthy individuals, which is further amplified in the bone marrow compartment. Clones with new mutations arise rapidly in longitudinal samples of patients with HFrEF and clonal expansion occurs with high momentum. The overrepresentation of mutations in the BM is indicative for augmented DNA damage in the hematopoietic stem cells of patients with HFrEF, while the rapid expansion of mutated circulating clones over time suggests that heart failure itself may expedite the expansion of hematopoietic cells harboring CH-driver mutations.

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**Fig. 1:** (A) Absolute number of CHIP mutations with a VAF ≥ 0.5% and (B) cumulative VAF in BM and PB samples of patients with HFrEF. (C) Absolute numbers of CHIP mutations in BM and PB for 68 patients with HFrEF. Each dot represents the number of patients. Line = line of identity.

**Fig. 2:** (A) Longitudinal analysis of absolute number of CHIP mutations in PB samples of patients with HFrEF. Median time between analysis = 12 months (n = 19).