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**Residual hematopoietic stem cells reflect disease activity and predict outcome in acute myeloid leukemia**

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It has been demonstrated that functionally normal hematopoietic stem cells (HSCs) can be separated from leukemic cells in a subgroup of patients with acute myeloid leukemia (AML) using the $CD34^+CD38^-ALDH^+$ phenotype. Patients suitable for this separation can be identified prospectively by their low percentages of cells with high aldehyde dehydrogenase (ALDH) activity ($ALDH^+$ cells <1.9%; ALDH-rare AML). These patients also represent a cohort with favorable outcome. However, frequencies of residual HSCs vary within this subgroup and individuals with very low numbers appear to have extremely poor survival.
In this study, between 2011 and 2014 bone marrow (BM) aspirates of 73 ALDH-rare AML patients were collected after written informed consent and patients were stratified according to HSC frequencies determined by $CD34^+CD38^-ALDH^+$ cells in $HSC^-$ AML (＜0.01% of total MNCs) and $HSC^+$ AML (≥0.01% of total MNCs). HSC numbers were determined by FACS-analysis at diagnosis and various follow-up time-points. Engraftment potential was evaluated by transplantation of $2\times10^6$ bulk AML cells in immune deficient NOD/SCID-$IL2R^{\gamma null}$ (NSG) mice.
Survival analysis showed that $HSC^-$ AML represents a subgroup of patients with significantly worse overall survival (OS) (mean: 455 days) and disease-free survival (DFS) (mean: 209 days) compared to $HSC^+$ AML (mean OS: 878 days; mean DFS: 896 days). Survival analysis of patients with cytogenetic intermediate-risk also showed significantly worse OS and DFS for $HSC^-$ AML with mean OS of 439 days compared to 872 days for $HSC^+$ AML and mean DFS of 218 days compared to 848 days.
Matched sample series from diagnosis, various remission time points and relapse (if available) were analyzed, and it was found that HSCs recovered after chemotherapy in cases that achieved complete remissions. However, in cases of persistent disease HSCs remained rare. Correlation of disease status to HSC numbers during therapy revealed that residual HSCs are a reliable qualitative marker of minimal residual disease (MRD) with a negative correlation to frank or molecular relapse.
To test if HSC numbers correlate to leukemia initiation potential, 44 AML cases were transplanted in NOD/SCID-$IL2R^{\gamma null}$ (NSG) mice and only abnormal engraftment (47% AML- and 53% no engraftment) upon transplantation of $HSC^-$ cases was observed. In contrast $HSC^+$ cases mostly lead to normal, multi-lineage engraftment (79%).
Frequency of residual HSCs reliably predicts outcome in AML patients and the quality of NSG mouse engraftment. Our AML stratification strategy is especially helpful in identifying cases with poor prognosis in the intermediate-risk group with $HSC^-$ AML representing patients with poor overall and disease free survival. In addition HSC frequency can also be used as a qualitative marker of MRD status tabling it as an indicator of BM-niche occupation of persistent leukemic cells.