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ABSTRACTS & PROGRAM

1896 Characterisation of the Stem and Progenitor Cell Hierarchy in Patients with CMML

Program: 636. Myelodysplastic Syndromes – Basic and Translational Studies: Poster I
Session: 636. Myelodysplastic Syndromes – Basic and Translational Studies: Poster I
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West Building, Level 1 (Moscone Center)

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Background
Identification and characterisation of tumour-propagating cells in distinct haematological malignancies may prove decisive in the development of effective therapies. However, conventional human stem cell assays might fail to uncover the true tumour-propagating potential of different clonal cell populations. To circumvent this, we recently integrated bone marrow cellular hierarchy analysis with in vitro genetic fate mapping, to provide evidence that low and intermediate risk myelodysplastic syndromes (MDS) are only propagated by Lin−CD34+CD38−CD90+ stem cells [Woll et al Cancer Cell 2014]. We have now performed similar studies of patients with chronic myelomonocytic leukaemia (CMML).

Methods
Flow cytometry was used to identify and prospectively purify phenotypic stem cells (SCs; CD34+CD38−CD90+CD45RA−) and progenitor cells (GMPs; CD34+CD38−CD123+CD45RA+) and MEPs; CD34+CD38−CD123−CD45RA−; Pro8 cells CD34+CD19+) in vitro stem and progenitor assays, gene expression profiling and targeted DNA sequencing of the coding region of 88 genes recurrently mutated in myeloid malignancies were then performed to characterise the stem and progenitor cell hierarchy in 10 patients with CMML.

Results
CMML patients retained phenotypically distinct stem and progenitor compartments at frequencies not significantly different to that observed in normal age-matched controls. Stem cells, GMPs and MEPs were all highly clonally involved, as evidenced by high variant allele frequency for all detected mutations. Myeloid and erythroid gene expression signatures and functional capacity were restricted to the GMP and MEP compartments, respectively. A functional transcriptional stem cell signature was restricted to the stem cell compartment of CMML patients. Candidate CMML stem cells also retained a high degree of cell cycle quiescence, whereas CMML progenitors resided predominantly in G1 and S/G2/M phases of the cell cycle. Taken together, these findings establish CMML SCs, GMPs and MEPs as highly clonally involved but phenotypically, molecularly and functionally distinct cell compartments. Targeted DNA sequencing of bone marrow mononuclear cells revealed recurrent mutations in splice factors (SRG2 and ZRS2), epigenetic regulators (TET2, ASXL1 and EZH2), transcription factors (RUNX1, GATA2) and cell signaling molecules (CBL and N-RAS). The SRSF2 P95 mutation was identified in 7/10 patients. Mutations in TET2 (6/10) and ASXL1 (3/10) were the second and third most recurrently mutated genes. Notably two patients with the SRSF2 P95 mutation also had a recurrent mutation in EZH2, which some studies have reported as being mutually exclusive. Targeted mutation screening of purified stem cells and single colonies from long-term cultures established that all the mutations identified in the bulk bone marrow could be traced back to the phenotypically and functionally defined CMML stem cells in 9/10 patients.

Conclusion
Similar to our previous studies in low and intermediate-1 risk MDS cases, our investigation of CMML patients provides compelling evidence that CD34+CD38−CD90+ stem cells are the disease-propagating cells in most CMML patients.

Disclosures: No relevant conflicts of interest to declare.

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