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POSTER ABSTRACTS

631.CHRONIC MYELOID LEUKEMIA: BIOLOGY AND PATHOPHYSIOLOGY, EXCLUDING THERAPY

Multiomic Single-Cell Analysis Identifies Von Willebrand Factor and TIM3-Expressing *BCR-ABL1* $^{+}$ Chronic Myeloid Leukemia Stem Cells

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Characterization of the leukemic stem cell (LSC) population in chronic myeloid leukemia (CML) may identify therapeutic targets for sustained elimination of leukemic cells. High-throughput single-cell RNAseq (scRNAseq) has paved the way for detailed transcriptional assessment of CML LSC. Building on this technique, multiomic CITE-seq approaches carry additional benefit in that the paired protein expression information may enable identification of associated cell surface marker profiles, subsequent FACS-based isolation and functional characterization. However, detection of *BCR-ABL1* transcripts, which is arguably the only unequivocal marker to distinguish CML LSC from healthy hematopoietic stem cells (HSC), remains challenging using current high-throughput 3' end capture-based scRNAseq methods.

For the present study, we performed single-cell CITE-seq analysis of the expression of 597 genes and 51 proteins in >70,000 stem and progenitor cells from 16 chronic phase CML patients and five healthy donors. Furthermore, we developed and employed a strategy allowing parallel detection of *BCR-ABL1* transcripts at the single-cell level.

In bone marrow samples from CML patients, we observed pronounced expansion of erythroid and myeloid progenitors within the CD14 $^{-}$CD34 $^{+}$ compartment, and substantial heterogeneity within the traditionally defined CD34 $^{+}$CD38 $^{-/low}$ LSC compartment. In-depth analysis of the latter identified a group of immature *BCR-ABL1* $^{+}$cells displaying a CD45RA $^{-}$cKIT $^{-}$CD26 $^{+}$ TKI resistance phenotype sitting atop a hierarchy of myeloid progenitors. Within this group of potential LSC, expression of both previously reported (*e.g.* CD25 and CD26) and unreported gene and protein markers was found to distinguish these cells from their healthy counterparts. Unlike HSC, the immature LSC showed high surface expression of TIM3 and high transcription of the von Willebrand factor gene (*VWF*). While overexpression of *VWF* within the stem cell population may be linked to the aberrant myeloid-biased hematopoiesis characterizing the disease, the cell surface upregulation of TIM3 suggests that its targeting may have merit in CML.

In conclusion, we here performed detailed multiomic characterization of CML stem and progenitor cells, paired with *BCR-ABL1* detection at the single-cell level. Our findings revealed LSC-specific expression patterns that may have implications for the phenotypic definition of CML LSC. Additionally, the results identify TIM3 as a conceivable target for sustained elimination of immature LSC in CML.

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