Comparative gene expression analysis reveals similarities and differences of chronic myeloid leukemia phases

Text S1: Literature analysis of potential major regulators that differed in their expression between CML phases

We learned CML-specific gene regulatory networks that were associated with the observed expression differences between the three CML phases. These networks also included 24 genes with phase-specific expression behavior that had an increased connectivity to other differentially expressed genes (see Table 1 in main manuscript). We performed an in-depth literature analysis to determine known cellular functions of these genes. These functions in combination with the characteristic phase-specific gene expression patterns can contribute to a better characterization of differences between the three CML phases.

- We found that NDUFAB1, SPRR2A and AURKB could contribute to the accumulation of additional mutations. NDUFAB1 encodes a subunit of an oxidoreductase that is part of the respiratory chain, whose enhanced activity in CML can lead to increased levels of reactive oxygen species that can damage DNA [Flis et al., 2012]. SPRR2A protects cells from oxidative stress in benign and malignant liver disease [Mizuguchi et al., 2014]. AURKB is involved in chromatid segregation [Bose et al., 2019]. The observed significant upregulation of NDUFAB1 in the blast crisis, the significant downregulation of SPRR2A in the accelerated phase and the blast crisis, and the significant downregulation of AURKB in the blast crisis are in accordance with the accumulation of additional mutations in CML progression [Nowicki et al., 2004, Cramer et al., 2008, Rabian et al., 2019].

- We found that HLA-B, HLA-DMB, HLA-DRA, MUC8 and OPTN are involved in the regulation of immune responses and inflammation. Increased cytotoxic T lymphocyte responses have been reported for a negative association between the HLA-B antigen expression and the presence of the BCR-ABL fusion in leukemia patients [de Carvalho et al., 2012]. Overexpression of HLA-DMB has been reported for CML [Kremer et al., 2014]. MUC8 has been associated with anti-inflammatory processes [Cha and Song, 2018]. The observed upregulation of HLA-DMB, HLA-DRA and OPTN and the downregulation of HLA-B and MUC8 in the blast crisis could indicate a role of the immune system in CML progression.

- We found that several genes could also support the observed global deregulation of cancer-relevant signaling pathways. Reduced CDCA3 expression has led to decreased cell proliferation in acute myeloid leukemia [Bi et al., 2018]. Downregulation of EN1 has been reported to increase activity of WNT-, Notch- and Hedgehog signaling [Peluffo et al., 2019]. A targeted inhibition of RPL18A expression has led to a lack of mature red blood cells via the activation of p53 and Jak-Stat signaling in a zebrafish model [Chen et al., 2020]. ADD2 is involved in the regulation of cell proliferation and migration [Luo and Shen, 2017, Yang et al., 2018]. TMEM40 is involved in the regulation of apoptosis and cell proliferation via alterations of p53 signaling [Zhang et al., 2018]. Downregulation of TMEM40 has led to reduced cell proliferation, migration and invasion in different types of

cancer [Zhang et al., 2018, Zhang et al., 2019, Liu et al., 2020]. Overexpression of CEACAM6 has been reported to be involved in the regulation of cell adhesion, proliferation, differentiation, apoptosis, invasion and metastasis formation [Rizeq et al., 2018]. Therefore, the observed downregulation of ADD2, CDCA3, EN1 and CEACAM6 in the blast crisis, the upregulation of TMEM40 in the accelerated phase and the blast crisis, and the upregulation of RPL18A in the blast crisis could also contribute to the observed increase of differential expression of cancer-relevant signaling pathways in CML progression.

- We found that some of the genes have also been associated with survival or treatment response in leukemia and other types of cancer. The chemokine PF4 (also known as CXCL4) is involved in the regulation of hematopoietic stem and progenitor cells [Bruns et al., 2014, Sinclair et al., 2016]. A recovery of PF4 to the normal protein level has been associated with a complete remission of acute myeloid leukemia [Kim et al., 2008, Bai et al., 2013] and increased PF4 expression has been associated with better therapy response and improved survival of CML patients in blast crisis [Ryo et al., 1991]. The chemokine AZU1 has also been associated with CML therapy response and survival [Yong et al., 2006, Cha et al., 2016]. CDCA3 has been reported to influence cisplatin sensitivity in lung cancer [O'Byrne et al., 2016]. EN1 overexpression has been associated with resistance of breast cancer to chemotherapy [Beltran et al., 2014]. Thus, the observed upregulation of PF4 in the accelerated and blast phase, the downregulation of CDCA3 and EN1 in the blast phase, and the phase-wise reduction of the AZU1 expression from the chronic to the blast phase could also have an impact on treatment responses and potentially contribute to the observation that targeted treatments are less efficient in advanced CML phases [Palandri et al., 2008, Silver et al., 2009, Houshmand et al., 2019].

- We found that PRG3 and TLX3 have been identified as important driver genes in other leukemias. PRG3 encodes a proteoglykane that has been reported as major regulator associated with survival of acute myeloid leukemia patients [Tan et al., 2020]. TLX3 encodes a homeobox transcription factor that is known as a master regulator of T cell acute lymphoblastic leukemia [Gatta et al., 2012].

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