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Modelling pediatric ETO2-GLIS2-driven acute megakaryoblastic leukemia in human CD34+ cells uncovers ontogeny-related cytokine-dependency for leukemia expansion

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Background. Acute Megakaryoblastic Leukemia (AMKL) is a strongly age-related pathology. When associated with the fusion oncogene *ETO2-GLIS2* (EG), the leukemia is characterized by a low mutational burden, very young age at diagnosis and poor prognosis. Studies in mice have shown that ontogeny and cellular architecture direct phenotype and aggressiveness of EG-driven leukemia. EG-driven AMKL is thought to arise *in utero* with EG strongly affecting transcription regulation. In this project, we studied whether and how EG expression in human hematopoietic stem/progenitor cells (HSPC) from different developmental stages promotes leukemia and characterized the impact of some niche factors on leukemia propagation vs initiation.

Methods. Lentiviral vector delivery and CRISPR/Cas9-based editing were used to express EG in human fetal liver (FL) and umbilical cord blood (CB) HSPC. Cultures and xenotransplantation assays were used to test the transformation of EG cells. RNAseq (sc/bulk) analysis helped characterizing expression profiles of EG-expressing cells.

Results. EG-expressing FL and CB CD34+ cells outgrew their control counterparts and acquired an abnormal cellular and molecular phenotype *in vitro*, similar to patient cells. Strikingly, FL, but not CB, EG-expressing cells efficiently induced megakaryoblastic leukemia in NSG mice. Differences between *in vitro* and *in vivo* results were due to a differential dependency on cytokines, since injection of EG-expressing CB cells in NSGS mice that express human SCF, IL3 and GM-CSF cytokines promoted rapid leukemia onset. Cultures in presence or absence of cytokines pinpointed IL3 and SCF as major players in leukemia expansion, whereas EG alone could initiate the abnormal cell phenotype. Consistently, *in vitro* chemical inhibition of IL3/SCF downstream pathways, in combination with a BH3-mimetic, inhibited EG CD34+-derived transformed HSPC growth. *In vivo*, the combination of inhibitors delayed leukemia development in a patient-derived xenograft model, with a stronger effect in NSG vs NSGS recipients, confirming the important role of the cytokine microenvironment in leukemia expansion.

Conclusions. Through expression of the EG pediatric leukemia fusion oncogene in human fetal and neonatal HSPC, we unveiled a major ontogenic difference for human pediatric AMKL development relying on the molecular interplay between exogenous growth factors and endogenous transcription factor alterations that is essential for *bona fide* acute leukemia development *in vivo* and that can be targeted using a combination of signaling and survival inhibitors.