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ORAL ABSTRACTS

602.MYELOID ONCOGENESIS: BASIC

GATA1 Target Gene CSF2RB Is Functionally Coupled to MPL and Marks a TPO-Dependent Preleukemic TAM Cell Population Sustained By the Fetal Liver but Not Bone Marrow

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Paediatric cancers differ from adult cancers on the basis of their genetics and biology. Understanding these differences can identify factors important in oncogenesis during development. One exemplar are the genetically linked myeloid pre-leukemic and leukemic disorders in newborns and children with Trisomy 21 (T21) or Down syndrome (DS). DS children have a 400-fold increased risk of developing myeloid leukemia (ML-DS) in early life. ML-DS is preceded by a fetal/neonatal pre-leukemic phase known as transient abnormal myelopoiesis (TAM), when T21 hemopoietic cells acquire mutations in the *GATA1* gene. These mutations produce an N-terminal truncated GATA1 protein (GATA1s). Remarkably, the proliferative effect exerted by T21 and GATA1s is developmentally restricted. As hemopoiesis shifts from fetal liver to new-born bone marrow, TAM usually spontaneously regresses. The biological basis for the requirement of a fetal liver environment for TAM cells and their failure to thrive in the bone marrow is unknown. However, a clue is that TAM cells can proliferate in bone marrow if they acquire oncogenic mutations, typically in genes of the JAK-STAT signalling pathway. These mutations transform the TAM clone to an ML-DS clone.

To address the question of why TAM cells survive only in a fetal liver environment, we performed scRNA sequencing, coupled with genotyping from 3 primary TAM samples. 4 T21 and 3 disomic cord blood samples were used as controls (Fig1A). We identified a marked expansion of mutant TAM GATA1s cells, compared to controls, at the earliest GATA1-expressing progenitor stages (Fig1A). TAM GATA1s cells displayed transcriptional upregulation of the PI3K/AKT/mTOR, TNFA/NFκB and JAK/STAT pathways and enhanced STAT3 regulon activity when compared to controls (Fig1A). We hypothesized that aberrant cytokine receptor activation leading to increased STAT3 activity is essential for GATA1s cell proliferation. Differential gene expression between TAM GATA1s cells and disomic cells, pointed to CSF2RB, a cytokine receptor that directly activates the JAK-STAT signalling pathway (Fig1A). Next, we demonstrated that CSF2RB is overexpressed at the protein level in TAM and marks 80% of CD34 $^{+}$ cells compared to 5% CD34+ control cells.

Using CUT&TAG we confirmed binding of GATA1s and GATA2 at two GATA sites in the *CSF2RB* locus in CMK cells (Fig 1A). CRISPR-Cas9 disruption of either GATA2, or the -12kb GATA motif led to downregulation of CSF2RB protein (Fig1A). We are testing if GATA1 represses CSF2RB and whether GATA1s fails to repress CSF2RB, allowing sustained abnormal CSF2RB expression in TAM. CSF2RB gene knockout in GATA1s cells leads to cell death. Taken together, CSF2RB is a direct GATA target gene and abnormal sustained, CSF2RB expression in TAM cells is required for GATA1s cell survival.

Classically, CSF2RB partners with the α-chain of either IL3, IL5 or GMCSF receptors. Interestingly, none of the α-chains are expressed at the protein or RNA level in TAM CSF2RB+ cells, suggesting that CSF2RB might partner with another cytokine receptor. We nominated MPL, the thrombopoietin (TPO) receptor as a new candidate binding partner of CSF2RB for the following reasons: (i) we documented co-expression of MPL with CSF2RB at the RNA and protein level in TAM cells. (ii) we confirmed functional coupling between CSF2RB and MPL in Ba/F3 and TF-1 cells, where at equivalent MPL levels, TPO signalling via the JAK-STAT pathway is enhanced by CSF2RB. This effect is stronger at high TPO levels. (iii) A mutation in CSFR2RB (A455D) that we described in ML-DSrequires interaction with human MPL to induce autonomous growth of cytokine-dependent hematopoietic cells. Notably, direct interaction between MPL and CSF2RB A455D is detected by BRET assays.
We hypothesize that high levels of fetal liver derived TPO sustain TAM proliferation because of the functional coupling between MPL and CSF2RB (Fig1B). This proliferation signal is lost as cells migrate to the low TPO bone marrow environment. Further work will assess TPO concentration in DS fetal liver and whether TAM cells display increased proliferation in response to varying TPO doses. In summary, we propose a model whereby reduced TPO levels experienced by TAM cells as they migrate from fetal liver to bone marrow creates selective pressure for acquisition of secondary mutations that permit TAM cells to proliferate independently of the high TPO environment of the fetal liver (Fig1B).

Disclosures Constantinescu: *Novartis:* Speakers Bureau; *GSK Belgium:* Membership on an entity's Board of Directors or advisory committees; *MyeloPro Diagnostics and Research GmbH Vienna:* Other: Co-founder. **Vyas:** *Pfizer:* Honoraria; *Gilead:* Honoraria; *BMS:* Research Funding; *Auron Therapeutics:* Current holder of *stock options* in a privately-held company; *Astellas:* Honoraria; *Jazz:* Honoraria; *Abbvie:* Consultancy, Honoraria.

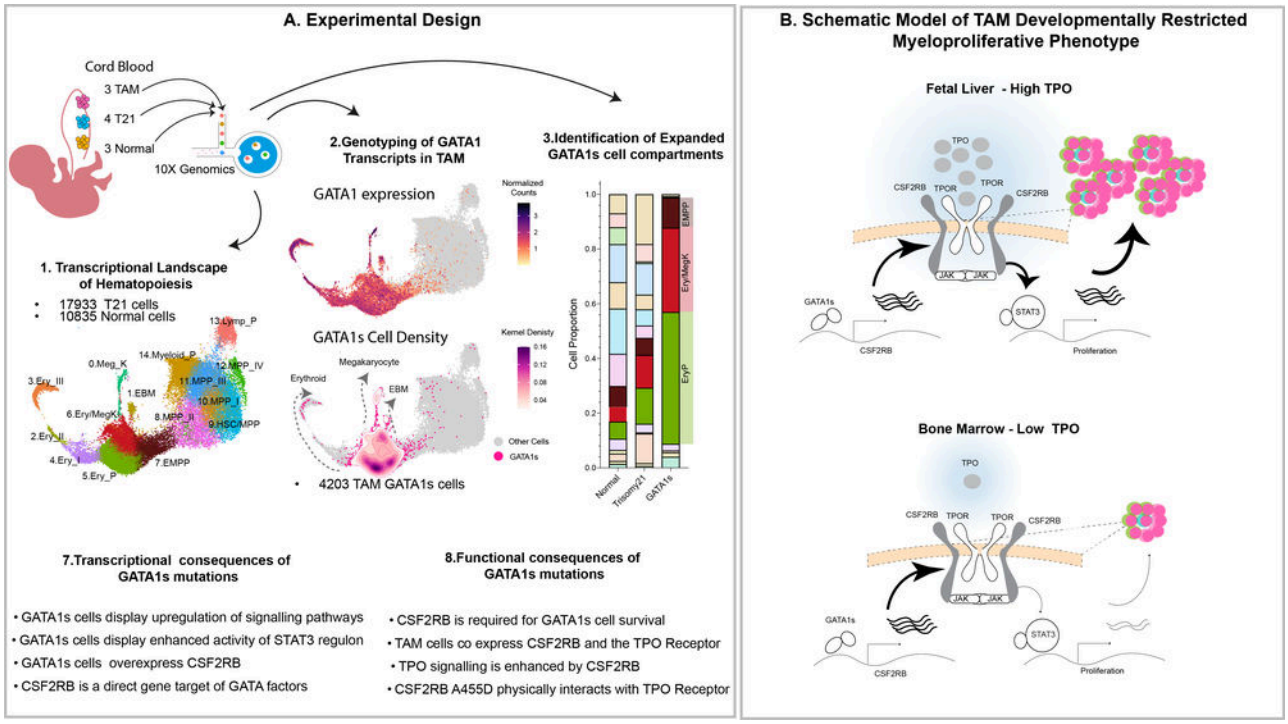

Figure 1

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