ELSEVIER

Blood 142 (2023) 995–996

The 65th ASH Annual Meeting Abstracts

ORAL ABSTRACTS

631.MYELOPROLIFERATIVE SYNDROMES AND CHRONIC MYELOID LEUKEMIA: BASIC AND TRANSLATIONAL

Aberrant Activity of the Calcium Sensor STIM1 Underlies Congenital Platelet Disorders and Myeloproliferative Neoplasms

Molly Brakhane, MSc[1], Vittorio Abbonante, PhD[2], Angelo B. A. Laranjeira[3], Christian Di Buduo, PhD[2], Brooke Sadler, PhD[4], Katrina Ashworth[5], Tim Kong, MSc[3], Fan He[3], Mary C. Fulbright[3], Dong Chen, MD PhD[6], Regina Clemens, MD[7], Elena Pegoraro[8], Michele P. Lambert, MD MTR[9], Kandace Gollomp, MD[9], Jorge Di Paola[5], Alessandra Balduini, MD[2], Stephen T. Oh, MDPhD[10]

[1] Division of Hematology, Washington University at St. Louis, Saint Louis, MO
[2] University of Pavia, Pavia, ITA
[3] Division of Hematology, Department of Medicine, Washington University School of Medicine, St Louis, MO
[4] Pediatrics, Washington University School of Medicine, Saint Louis, MO
[5] Department of Pediatrics, Washington University School of Medicine, St Louis, MO
[6] Division of Hematopathology, Mayo Clinic, Rochester, MN
[7] Departmnet of Pediatrics, Washington University at St Louis, St Louis, MO
[8] University of Padova, Padova, ITA
[9] Division of Hematology, Children's Hospital of Philadelphia, Philadelphia, PA
[10] Department of Medicine, Washington University School of Medicine, Saint Louis, MO

MB and VA equal contributors
AB and STO co-corresponding authors

This study was initiated following evaluation of a 32 year-old woman who presented with a history of thrombocytopenia identified in childhood who subsequently developed features of myelofibrosis (MF). A bone marrow biopsy demonstrated hypercellularity in conjunction with megakaryocyte hyperplasia and marked reticulin fibrosis. Molecular testing for *JAK2*, *CALR*, and *MPL* mutations was negative. Given the unusual association between congenital thrombocytopenia and MF in this patient, exome sequencing was performed, revealing a heterozygous R304W mutation in the coiled coil (CC) domain of *STIM1*. Activating mutations in the CC and EF hand domains of *STIM1* have been associated with Stormorken syndrome, a rare congenital platelet disorder associated with abnormal store operated calcium entry (SOCE). We subsequently identified a second patient with MF associated with a *STIM1* activating mutation. This individual was found to have severe thrombocytopenia at birth, and exome sequencing revealed a heterozygous S88G mutation in the EF hand of *STIM1*. A bone marrow biopsy obtained at 6 months of age revealed atypical megakaryocytes and grade 1-2 MF. A repeat biopsy at age 2 showed persistence of stable MF.

The unusual finding of MF in these two patients suggested the possibility of altered calcium signaling as a shared mechanism driving congenital platelet disorders such as Stormorken syndrome and myeloproliferative neoplasms (MPNs) including MF. In support of this notion, we identified elevated *STIM1* expression in MF vs normal megakaryocyte progenitors, as well as in platelets from patients with essential thrombocythemia (ET) vs healthy controls. Additionally, we found that *STIM1* expression was significantly elevated in CD34+ hematopoietic stem/progenitor cells (HSPCs) from both ET and MF patients vs healthy controls. Notably, *STIM1* expression was increased in both *JAK2* and *CALR*-mutant MF patients. Collectively, these findings provide evidence of aberrant *STIM1* expression in MPN patient cells.

To determine the functional role of *STIM1* in MPN disease development, colony assays and patient-derived xenograft (PDX) experiments were performed with MF patient CD34+ cells subjected to CRISPR ablation of *STIM1*. Strikingly discordant results were observed with *JAK2* vs *CALR*-mutant patient samples. Abrogation of *STIM1* in *CALR*-mutant CD34+ cells led to decreased colony formation, and NSGS mice engrafted in parallel with *STIM1*-targeted cells exhibited decreased human CD45+ cell engraftment in conjunction with prolonged survival. These findings suggest an important role for *STIM1* in *CALR*-mutant MPN disease phenotypes. In contrast, targeting of *STIM1* in *JAK2*-mutant CD34+ cells led to increased colony formation and exacerbated disease phenotypes *in vivo* as manifested by enhanced human CD45+ cell engraftment, worsened splenomegaly, and early lethality. Similar results were obtained in experiments utilizing pharmacologic inhibitors of SOCE

activity. Taken together, these findings indicate that the consequences of aberrant STIM1 activity may be context-dependent relating to specific MPN driver mutations.

To expand these observations, we identified a separate cohort of 9 family members in Italy with Stormorken syndrome and confirmed *STIM1* EF hand mutations. In *ex vivo* megakaryocytic differentiation assays, cells from affected individuals exhibited a defect in proplatelet formation. These observations were corroborated by initial analyses of a newly generated *Stim1* R304W conditional knock-in mouse which recapitulated the characteristic thrombocytopenia found in patients with Stormorken syndrome.

In summary, this study represents the first demonstration of MF development in patients with Stormorken syndrome, thereby uncovering a previously unrecognized hallmark of altered calcium signaling via aberrant *STIM1* activation underlying Stormorken syndrome and MPNs. Our findings suggest distinct mechanisms relating to the interaction between *JAK2* vs *CALR* mutation and altered STIM1 activity. Further studies of these relationships may have important ramifications for potential therapeutic approaches targeting these pathways.

Disclosures Lambert: *Octapharma:* Membership on an entity's Board of Directors or advisory committees, Research Funding; *Dova:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Principia:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding; *Rigel:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Shionogi:* Consultancy, Membership on an entity's Board of Directors or advisory committees; *Novartis:* Consultancy, Research Funding; *Argenx:* Consultancy, Research Funding; *Sobi:* Consultancy; *Sanofi:* Consultancy; *Janssen:* Consultancy; *Sysmex:* Research Funding. **Di Paola:** *CSL Behring:* Consultancy. **Oh:** *CTI BioPharma, Bristol Myers Squibb, Disc Medicine, Blueprint Medicines, PharmaEssentia, Constellation/MorphoSys, Geron, AbbVie, Sierra Oncology/GSK, Cogent, Incyte, Morphic, Protagonist:* Consultancy.

https://doi.org/10.1182/blood-2023-186413