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POSTER ABSTRACTS

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

An Atypical Somatic *Calr* Variant Acquired in *Cis* to a Germline *Calr* variant Induces Constitutive Signaling of the Thrombopoietin Receptor and Results in Essential Thrombocythemia

Laetitia Borderon[1], Jihyun Song[2], Soo Jin Kim, MS[3], Christophe Marzac[4], Christine Bellanné-Chantelot[5], Nicolas Papadopoulos[6,7], Stefan N. Constantinescu, MD PhD[8,9], William Vainchenker, MD PhD[1], Isabelle Plo, MD PhD[10], Josef T. Prchal, MD[3], Caroline Marty, PhD[1]

[1] INSERM UMR1287, Gustave Roussy, Paris-Saclay University, Villejuif, France
[2] Division of Hematology and Hematologic Malignancies, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT
[3] Division of Hematology and Hematologic Malignancies, Huntsman Cancer Institute, University of Utah, Salt Lake City, UT
[4] Laboratoire d'Immuno-Hématologie, INSERM UMR1287, Gustave Roussy, Paris-Saclay University, Villejuif, France
[5] INSERM UMR1287, Gustave Roussy, Villejuif, France
[6] Université catholique de Louvain and de Duve Institute, Brussels, Belgium
[7] Ludwig Institute for Cancer Research, Brussels, Belgium
[8] WELBIO (Wallon Excellence in Life Sciences and Biotechnology), Brussels, Belgium
[9] Oxford University, Nuffield Department of Medicine, Ludwig Institute for Cancer Research, Oxford, United Kingdom
[10] INSERM, Villejuif, France

Acquired mutations in the gene encoding the endoplasmic reticulum (ER) chaperone calreticulin (CALR) are responsible for ~ 30% of essential thrombocythemia (ET) and myelofibrosis (MF) cases. Two *CALR* mutations, a deletion of 52 base pairs (del52) and an insertion of 5 base pairs (ins5) are the most frequent in ET and MF patients. All *CALR* mutations result in a +1-frameshift leading to 1) the replacement of the negatively-charged wild-type (WT) C-terminus that binds calcium in the ER by a positively-charged mutant tail including 44 residues that are common to all CALR mutant proteins and 2) the loss of the ER retention KDEL; together leading to the deregulation of calcium homeostasis. The CALR mutants are also secreted and bind to MPL in the ER but induce its constitutive dimerization and downstream signaling pathways at the cell surface.

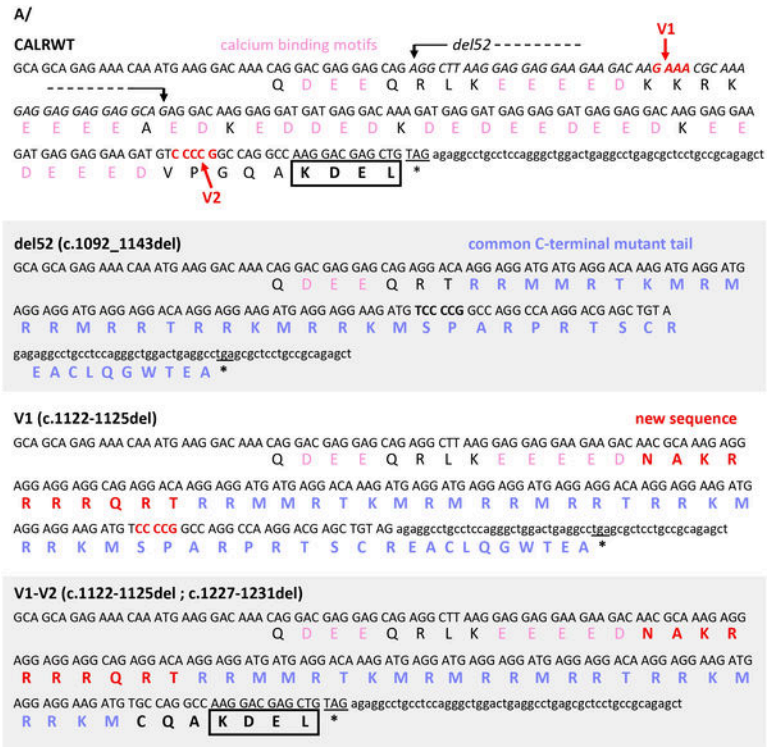
A 16-year-old girl presented with a platelet count of 797 x 10^3/mL rising to 1,228 x 10^3/mL within 2 years. After Informed Consent, we detected two atypical CALR variants in her blood cells by NGS analysis: V1 (c.1122-1125del) at 47% variant allele frequency (VAF) and V2 (c.1227-1231del) in *cis* of the V1 leading to V1-V2 (c.1122-1125del ; c.1227-1231del) at 5% VAF. The presence of V1 in blood and nails from the propositus and her healthy mother confirmed its germline origin. The V1-V2 was only found in the propositus at 5% in isolated monocytes and at very low levels in NK and B cells, confirming its somatic acquisition. The germline V1 induces a frameshift resulting in the replacement of the CALR WT C-terminus by the positively charged 44 residue-long C-terminal mutant tail present in del52 and ins5 and the loss of KDEL together with an insertion of 10 amino acids not present in CALR del52 nor ins5. In contrast, the V1-V2 generates a similar but shortened C-terminus containing the Arg-rich MPL binding site and KDEL (Fig. 1A).

To investigate the functional impact of these CALR variants, we cloned V1 and V1-V2 with a N-terminal hemagglutinin (HA) tag as well as CALR WT and del52 as controls in a retroviral plasmid. Viral particles were produced to transduce Ba/F3 cell lines expressing MPL. Structural modeling using AlphaFold was used to investigate the role of KDEL restoration in V1-V2 in producing an oncogenic protein leading to ET.

Both CALR WT and V1-V2 variants that contain the KDEL motif were detected to a similar extent in Ba/F3-MPL cell lysates. In contrast, del52 and V1, that lost the ER-retention KDEL motif, were barely detected in cell lysates. Unexpectedly, despite the presence of the KDEL motif, V1-V2 was found at a similar level than del52 at the Ba/F3-MPL cell surface. In contrast, V1 was barely detectable at the cell surface, like CALR WT. In order to investigate the role of the KDEL in V1-V2 cell surface expression, we generated a CALR WT lacking the KDEL and added the KDEL motif to del52. The presence of the KDEL motif stabilized the intracellular expression of del52 without affecting cell surface localization while the removal of the KDEL motif in CALR WT appeared to destabilize the protein and induce a slight leak to the cell surface. Structural modeling using AlphaFold predicted that the KDEL in V1-V2 structure is accessible to KDEL receptors, suggesting that the motif may be masked by the

interaction of V1-V2 with MPL while trafficking to the cell surface. Indeed, cell-surface expression of V1-V2 was decreased in Ba/F3 that do not express MPL.
We show that V1-V2, but not V1, constitutively activates the phosphorylation of STATs, ERK and AKT in Ba/F3-MPL. TPO further increases activation of STATs but not of AKT and ERK by V1-V2 and del52. Coherently, V1-V2, but not V1, sustained oncogenic MPL stimulation able to sustain cell autonomous Ba/F3-MPL cell proliferation (Fig. 1B).
Both del52 and V1-V2 up-regulated BCL-2 mRNA. Dose-response cytotoxicity assays with the BCL-2 inhibitor venetoclax showed that these two mutants are sensitive to lower doses compared to CALR WT- or V1-expressing Ba/F3-MPL cells.
This is the first report of a germline loss-of-function CALR variant (V1) expressed as an unstable protein and that might predispose to the acquisition of a somatic gain-of-function CALR variant (V1-V2) responsible for ET. The presence of KDEL in V1-V2 leads to stabilized CALR expression that does not prevent cell surface localization. Moreover, it leads to induction of MPL signaling and cytokine-independent cell growth.

Disclosures No relevant conflicts of interest to declare.



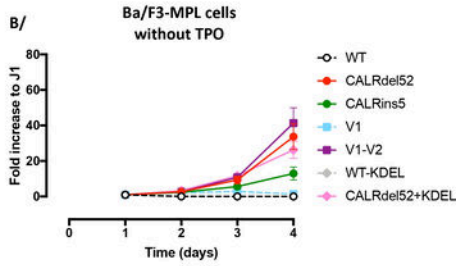


Figure 1

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