



Crispr/Cas9 Engineered 61bp Deletion in the *Calr* Gene of Mice Leads to Development of Thrombocytosis

Thomas Balligand, Younes Achouri, Ilyas Chachoua, Christian Pecquet, Jean-Philippe Defour and Stefan N. Constantinescu

Blood 2016 128:4274;

[Article](#)[Info & Metrics](#)[e-Letters](#)

Abstract

In a subset of patients suffering from myeloproliferative neoplasms (MPNs), calreticulin (CALR) exon 9 frameshift mutations are known to be responsible for the development of either essential thrombocythemia (ET) or primary myelofibrosis (PMF) (1, 2). The most prevalent mutations are a 52-bp deletion (del52, type-1 mutation) and a 5-bp TTGTC insertion (ins5, type-2 mutation). In these patients, the mutational status is almost always heterozygous. Our group and collaborators have recently shown that the pathogenic mutant CALR proteins require interaction with and activation of the thrombopoietin receptor (TpoR) for activation of the JAK-STAT pathway (3, 4). Until now, no knock-in mouse model of these diseases has been published. In this abstract, we show how we succeeded in creating such a model.

We had shown that the murine CALR mutant proteins behave just like their human counterparts (5). Specifically, the del52, ins5 and del61 (61bp deletion, type-1) *Calr* mutations were able to transform Ba/F3 cells (murine pro-B lymphocytic cells normally dependent on IL-3 for growth) expressing the thrombopoietin receptor (TpoR) and render them cytokine-independent. Importantly, we also mutated the Ba/F3 genome using the widely adopted CRISPR/Cas9 system in order to create a 61-bp deletion of the exon 9 of *Calr*. This too successfully transformed the Ba/F3 cells, showing that endogenous levels of expression of a mutant CALR protein are sufficient to induce phenotype *in vitro*.

Now, using the same approach, we injected C57BL/6J mouse zygotes with the same CRISPR/Cas9 constructs to create the same 61-bp deletion in the murine *Calr* gene. Out of 46 pups born from the procedure, one male pup was heterozygous for the 61-bp deletion. By *in vitro* fertilization, we subsequently obtained heterozygous *Calr* del61/WT pups. After inter-breeding the mice, we analyzed the blood of 12 *Calr* del61/WT males and 12 *Calr* WT/WT males (littermates) at three different timepoints (15, 18 and 22 weeks old) and found that the *Calr* del61/WT mice showed significantly higher levels of circulating platelets. Conversely, red blood and white blood cell numbers were the same between both groups at all time points. We further show that expression of a mutant CALR protein, in a heterozygous state, is sufficient to induce abnormal proliferation of megakaryocytes and develop an ET phenotype *in vivo* in mice. Follow-up in dynamics of the phenotype and bone marrow and spleen pathology (examination of myeloproliferation and fibrosis) allow comparison with the retroviral murine models of CALR-mutant MPNs and with the known features of the human disease. The only limitation of our model is the fact that the *Calr* del61 mutation is parentally acquired and widespread throughout the organism.

With this new model, we aim to test the efficiency of various drugs to prevent or cure the MPN phenotype, such as ruxolitinib, a JAK2 type-1 inhibitor that is already used in clinics in patients suffering from CALR-mutated MPNs. We also now have a means to generate a high number of *Calr* del61/WT bone marrow cells to extensively study the oncogenic properties of the *Calr* mutations at different stages of the hematopoiesis. It will also be of great interest to study, if generated, a homozygous mutational status of *Calr* del61 *in vivo*. Thus, our system will shed light on the importance of the negatively charged tail of CALR and on the effects of the novel positively charged tail on myeloproliferation.

References

1. Klampfl T, Gisslinger H, Harutyunyan AS, Nivarthi H, Rumi E, Milosevic JD, et al. N Engl J Med. 2013 Dec 10;369(25):2379-90.
2. Nangalia J, Massie CE, Baxter EJ, Nice FL, Gundem G, Wedge DC, et al. N Engl J Med. 2013 Dec 10;369(25):2391-405.
3. Chachoua I, Pecquet C, El-Khoury M, Nivarthi H, Albu RI, Marty C, et al. Blood. 2015 Dec 14;10.1182/blood-2015-11-681932.
4. Marty C, Pecquet C, Nivarthi H, Elkhoury M, Chachoua I, Tulliez M, et al. Blood. 2015 Nov 25;10.1182/blood-2015-11-679571.
5. Balligand T, Achouri Y, Pecquet C, Chachoua I, Nivarthi H, Marty C, et al. Leukemia. 2016 Feb 29;10.1038/leu.2016.47.

Disclosures Constantinescu: *Teva*: Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Novartis*: Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Shire*: Membership on an entity's Board of Directors or advisory committees, Speakers Bureau; *Personal Genetics*: Membership on an entity's Board of Directors or advisory committees, Speakers Bureau.

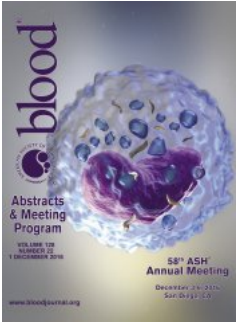
- ✖* Asterisk with author names denotes non-ASH members.

© 2016 by The American Society of Hematology

[^ Back to top](#)

Advertisement

[Previous](#)



[Table of Contents](#)

Volume: 128

Issue: 22

Pages: 4274

DOI: <https://doi.org/>

- Email

Alerts

Citation Tools

Cite This
- Request Permissions

Share

[Tweet](#) [Like](#) [G+1](#)

Jump to

- Article
- Info & Metrics
- e-Letters

Advertisement

 Related Articles

No related articles found.

Advertisement

- Current Issue
- First Edition
- Collections
- All Issues
- Abstracts

[Video Library](#)[About *Blood*](#)[Subscriptions](#)[Newsroom](#)[Copyright](#)[Public Access](#)[Permissions](#)[Submit to *Blood*](#)[Blood App](#)[Alerts](#)[RSS](#)[Contact Us](#)[Order Reprints](#)**Information for:**[Authors](#)[Subscribers](#)[Institutions/Librarians](#)[Advertising in *Blood*](#)[Terms and Conditions](#)[Blood Journals](#) ▼