Professor
Stefan N. Constantinescu

Université Catholique
de Louvain, Brussels,
Belgium

WILL THE *CALR* MUTATION BE THE NEW *JAK2*?

BIOGRAPHY

Professor Stefan N. Constantinescu is a recognized expert in signalling by cytokine receptors and the JAK-STAT pathway in hematopoiesis. He is a member of the Ludwig Institute for Cancer Research and a Professor at the University Catholic of Louvain's de Duve Institute, where he is Head of Cell Signalling Program. His background is MD, PhD followed by postdoctoral training in molecular cell biology at the Whitehead Institute for Biomedical Research at Massachusetts Institute of Technology, Cambridge, MA (with Prof. Harvey F. Lodish).

Studies performed in Prof. Constantinescu's laboratory in Brussels identified the dimeric structure, active orientation and conformational requirements for activation of cytokine receptors such as those for erythropoietin (EpoR) and thrombopoietin (TpoR, c-Mpl) in association with *JAK2*. In collaboration with Prof. William Vainchenker at Institut Gustave Roussy, Villejuif, France, he contributed to the discovery of *JAK2* V617F and to the elucidation of its oncogenic mechanism in myeloproliferative neoplasms. He identified the first constitutive active oncogenic mutants of *JAK1* (*JAK1* V658F) and *TYK2* (V678F). Work on cytokine receptor juxtamembrane domains in the laboratory of Prof. Constantinescu led to the identification of thrombopoietin receptor (TpoR) W515A/L/KR activating mutations, which were subsequently found by several teams in *JAK2* V617F-negative myelofibrosis and essential thrombocythemia patients. More recently, the group identified a pocket in *JAK2* V617F pseudokinase domain that could be targeted by small molecules for specific inhibition of mutant and not wild type *JAK2*, and described specific gene regulation induced by constitutively active STAT5 and not by physiologically activated STAT5 by cytokines. This is highly relevant for progression of myeloproliferative neoplasms.

ABSTRACT

Myeloproliferative neoplasms (MPNs) are associated at present with three phenotypic driver mutations, namely the activating *JAK2* V617F mutation, the TpoR W515/S505 and the calreticulin (*CALR*) mutations. Together they cover >92% of MPNs.

CALR mutants are the second most prevalent mutations in MPNs, being associated with a substantial fraction of Essential Thrombocythemia (ET) (16%) and Myelofibrosis (MF) (22-27%) patients. These mutations are not involved in polycythemia vera and appear to be associated with younger patients. The more than 50 known *CALR* mutants require the same frame-shift in exon 9, which is the last exon of the *CALR* gene. Via this frame-shift *CALR* mutants lose the KDEL endoplasmic reticulum localisation signal and acquire a novel unique R/K/M-rich C-terminus. Two *CALR* mutants are the most prevalent, Del 52 and Ins 5. Recent data suggests that they do not act in a completely identical manner. In ET, the allele burden of *CALR* mutants appears to be higher than that of *JAK2* V617F.

CALR mutants appear to activate the JAK-STAT pathway and transform Ba/F3 cells. Possible mechanisms by which *CALR* mutants induce ET and MF include: i) Effects on calcium metabolism in CD34++ cells, myeloid progenitors and megakaryocytes, ii) Effects on folding and activation of cytokine receptors linked to *JAK2*, iii) Defects of the macrophage "eat me" and "do not eat me" signals, which might promote platelet formation and slow their destruction.

We have observed induction by *CALR* mutants of *JAK2* dimerization and activation, and this appears to depend on certain cytokine receptors. *CALR* mutants exhibit a different cellular localization from that of wild type *CALR*, which is localised mainly in the endoplasmic reticulum. Like *JAK2* V617F and *TpoR* W515 mutants, *CALR* mutants induce continuous activation of STAT5 and pathologic induction of gene expression, especially in megakaryocytes/ platelets. We recently observed pathologic gene regulation induced jointly by STAT5 and p53. Homozygous p53 mutations/deletions appear to be the most frequent molecular event in secondary acute myeloid leukemia in MPNs.

With >92% of MPNs covered for the phenotypic driver mutations, it will be important to advance our understanding of how epigenetic mutations, like those in *TET2, EZH2* or *ASXL1* contribute to disease. Extensive sequencing efforts are being pursued to identify the remaining 6-7% of ET and MF driving mutations. The field is ready for exploring profound questions on how and when driving mutations are acquired by hematopoietic stem cells, and which avenues of prevention and therapy will be available.

CONFLICT OF INTEREST:

• SANOFI • AMGEN • SHIRE • DARFA PHARMA • PERSONAL GENETICS • TEVA

Presentation references:

• Girardot M, *et al. Oncogene*. 2014:1-10. (Epub ahead of print) • Klampfl T, *et al. N Engl J Med*. 2013;369:2379-90 • Lundberg P, *et al. Blood*. 2014;123:2220-2228 • Molecular Cell Biology Lodish, *et al*. Seventh Edition, W. H. Freeman and Co. New York • Nangalia J, *et al. N Engl J Med*. 2013;369:2391-2405 • Rotunno G, *et al. Blood*. 2014;123:1552-5. • Rumi E, *et al. Blood*. 2014 Jul 1 (Epub ahead of print) • Tefferi A, *et al. Am J Hematol*. 2014;89:E121-4 • Tefferi A, *et al. Leukemia*. 2014;28:1568-70. • Tefferi A, *et al. Leukemia*. 2014;28:1494-500 • Vainchenker W & Constantinescu SN. *Oncogene*. 2013;32:2601-13 • Vainchenker W, *et al. Blood*. 2011;118:1723-351 • Vannucchi A, *et al. Leukemia*. 2013;27:1861-9 • Wang WA, *et al. Int J Biochem Cell Biol*. 2012;44:842-846 • Walz C, *et al. Blood*. 2012; 44:842-846 • Yan D, *et al. Blood*. 2012; 120:1888-1898.