Normal and pathological erythropoiesis in adults: from gene regulation to targeted treatment concepts

Peter Valent,[1,2] Guntram Büsche,[3] Igor Theurl,[4] Iris Z. Uras,[5] Ulrich Germing,[6] Reinhard Stauder,[7] Karl Sotlar,[8] Wolfgang Füreder,[1] Peter Bettelheim,[9] Michael Pfeilstöcker,[2,10] Rainer Oberbauer,[11] Wolfgang R. Sperr,[1,2] Klaus Geissler,[12] Jürg Schwaller,[13] Richard Moriggl,[14,15] Marie C. Béné,[16] Ulrich Jäger,[1,2] Hans-Peter Horny[17] and Olivier Hermine[18]

[1]Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria; [2]Ludwig Boltzmann Cluster Oncology, Medical University of Vienna, Austria; [3]Institute of Pathology, Medizinische Hochschule Hannover, Germany; [4]Department of Internal Medicine II, Medical University Innsbruck, Austria; [5]Institute of Pharmacology and Toxicology, University of Veterinary Medicine, Vienna, Austria; [6]Department of Hematology, Oncology and Clinical Immunology, Heinrich-Heine University, Düsseldorf, Germany; [7]Department of Internal Medicine V, Medical University Innsbruck, Austria; [8]Institute of Pathology, Paracelsus Medical University Salzburg, Austria; [9]First Department of Internal Medicine, Elisabethinen Hospital, Linz, Austria; [10]3rd Medical Department, Hanusch Hospital, Vienna, Austria; [11]Department of Nephrology and Dialysis, Medical University of Vienna, Austria; [12]5th Medical Department for Hematology and Oncology, Hospital Hietzing, Vienna, Austria; [13]Department of Biomedicine, University Children's Hospital Basel, Switzerland; [14]Ludwig Boltzmann Institute for Cancer Research, Vienna, Austria; [15]Department of Biomedical Science, Institute of Animal Breeding and Genetics, University of Veterinary Medicine, Vienna, Austria; [16]Hematology Biology, University Hospital, Nantes, France; [17]Institute of Pathology, Ludwig-Maximilian University, Munich, Germany and [18]Imagine Institute, INSERM U 1163, CNRS 8654, Université Paris Descartes, Sorbonne, Paris Cité, France

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Correspondence: peter.valent@meduniwien.ac.at or ohermine@gmail.com

Supplementary Appendix to Valent et al:

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1) Faculty Discussion and the Process of Generating Consensus Statements

The Year 2017 Working Conference on Erythroid Disorders (subtitle: Normal and Neoplastic Erythropoiesis: From Gene Regulation to Targeted Treatment Concepts) was organized in Vienna in April 2017 (April 28-29, 2017). The related project with an in-depth discussion lasted from January 2017 until October 2017. The discussion phase was split into a pre-conference phase (via e-mails and smaller preparative meetings), the conference discussion and a post-conference discussion phase (April-October 2017). The consensus discussion and the consensus decision-making process were organized in accordance with published guidelines (Graham et al., 2011).

In the final discussion round, the paper-draft was discussed and adjusted based on input provided by all faculty members and available information. Open discussion points were discussed in the faculty (consensus group = co-authors) until a clear-cut result (100% of faculty members agreed) was obtained or no consensus was reached. Only those statements, criteria, and definitions that are based on a 100% consensus among all faculty members were included in the final document.

The final document and its content were approved by all faculty members (all co-authors) before submission. All actively contributing (only those) faculty members are included as co-authors on the final document.

References

Graham, R., Mancher, M., Wolman, D.M., Greenfield, S., Steinberg, E., eds, 2011. Institute of Medicine; Board on Health Care Services; Committee on Standards for Developing Trustworthy Clinical Practice Guidelines. Clinical Practice Guidelines We Can Trust. Washington, DC: National Academies Press.

Supplemental Tables

Supplemental Table S1

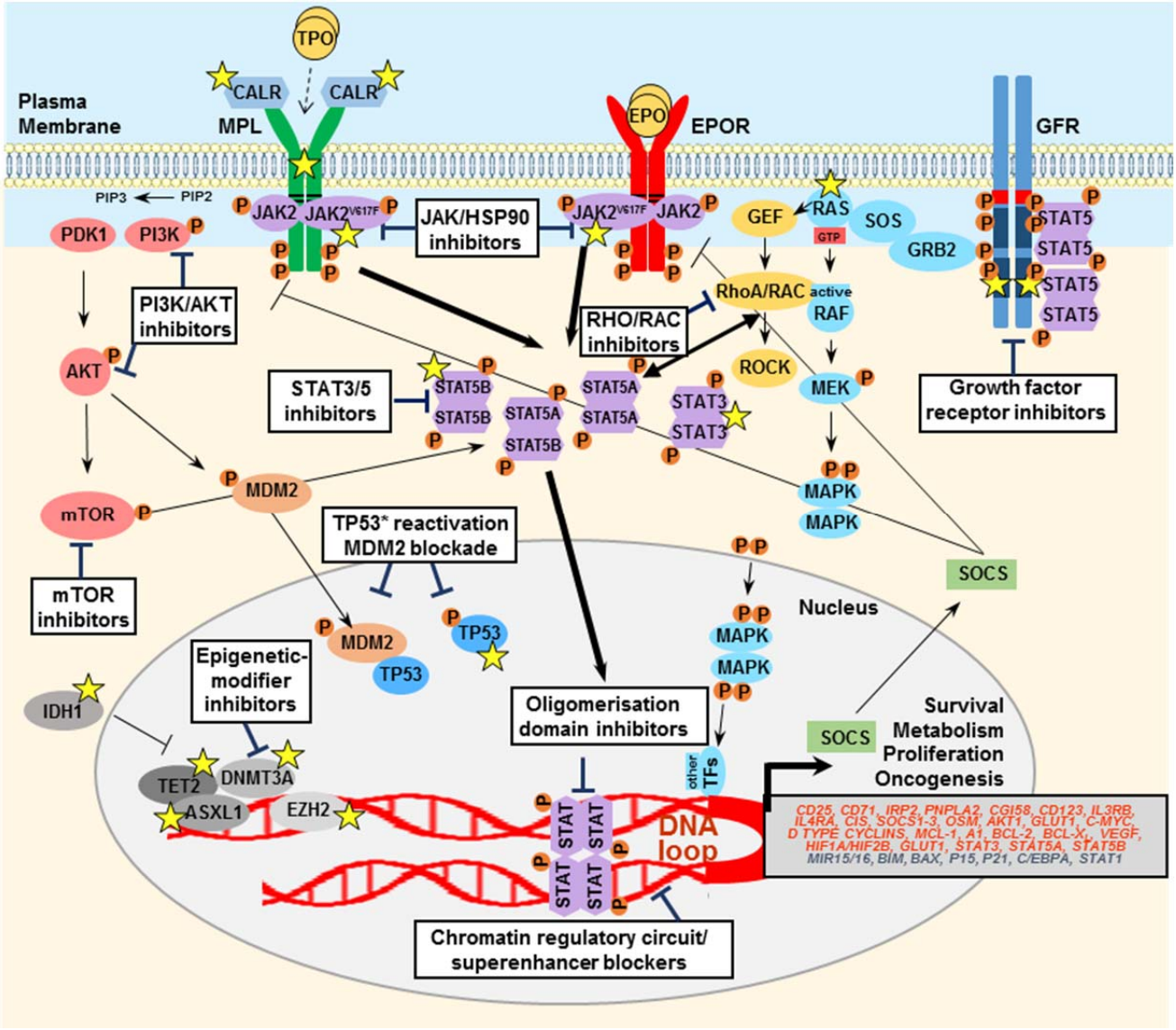
Proposed variants of erythroid leukemia*

Variant	Main clinical features
Acute erythroid leukemia	Rapid course
Chronic erythroid leukemia	Chronic/smoldering
Primary (*de novo*) erythroid leukemia	No known preceding myeloid neoplasm
Secondary erythroid leukemia	Preceding myeloid neoplasm**

*In addition to the classification proposed by the WHO, the erythroid leukemias can be sub-classified based on the clinical course of the patients and a known preceding myeloid neoplasm.
**Such preceding myeloid neoplasm usually is a myelodysplastic syndrome (MDS). However, secondary erythroid leukemia can also develop on the basis of other myeloid neoplasms such as chronic myeloid leukemia (see also Supplemental Figure S2).

Supplemental Figures

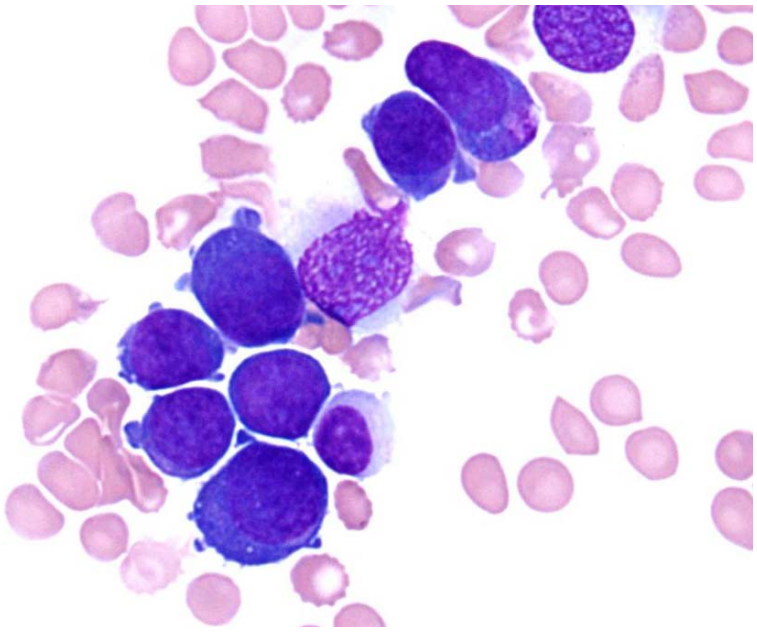
Supplemental Figure S1



Core pathways driving *JAK2*- or *CALR*-mutated polycythemia vera (PV) and other myeloproliferative neoplasm (MPN) and secondary acute myeloid leukemia (AML)
A number of somatic mutations in various signaling molecules have been described (yellow stars). In PV and other MPN, *JAK* V617F and *CALR* mutations are frequently detected. The mutated onco-proteins initiate various signal transduction cascades and thereby contribute to disease evolution and progression. JAK2 binds to the cytosolic membrane proximal region of homodimeric cytokine receptors such as MPL (TPOR that can itself be mutated in MPN) and EPOR. Mutational events promote JAK2 activation (via receptor-ligand binding or gain-of-function mutation such as JAK2V617F or Exon 8 deletion in JAK2) with hyperactive JAK2 tyrosine kinase activity that evokes subsequently high levels of STAT3 and predominantly STAT5 tyrosine phosphorylation. STAT3/5 activation stimulates the whole vicious cycle of cytokine signaling and various downstream oncogenic signaling pathways.

The two STAT5 gene products STAT5A and STAT5B form un-phosphorylated anti-parallel homo- or heterodimers. STAT5 is efficiently transported into the nucleus via tyrosine phosphorylation, where it undergoes a large conformational change to a parallel dimer. High pYSTAT5 levels can form STAT oligomers involving also STAT3/5 tetramers and as a consequence DNA loops are formed that promote high oncogene transcription (in the grey shaded box key players = STAT3/5 target genes are shown: activated genes are labeled in red, repressed genes are labeled in blue). Nuclear shuttling and efficient transformation through STAT3/5 action requires also RAS-RAF-MAPK and PI3K-AKT signaling. These pathways facilitate oncogenic gene transcription to promote MPN cell survival, proliferation, vascularization, enhanced metabolism or migration of MPN cells. The expression of negative regulators such as the SOCS proteins are also induced by the JAK-STAT pathway, however they are not sufficient to block hyperactive JAK-STAT signaling and cannot bind JAK2 V617F. MPN or AML patient cells were also described to signal via growth factor receptors (GFR). Cytokine and growth factor receptors activate efficiently the RAS-RAF-MAPK and PI3K-AKT pathway that subsequently also triggers GTPase signaling through the RhoA/RAC-ROCK pathways. RhoA and RAC provide a nuclear shuttling function for STAT5 molecules and RAS controls metabolic function of mitochondrial STAT3. Mutated CALR is frequently found in MPN patients and it interacts with the MPL receptor at the endoplasmic reticulum Golgi apparatus through a N-glycosylation motif, promoting direct dimerization oddly via binding to the extracellular domain that then causes JAK2 and STAT5 hyperactivation with other important downstream signaling. Loss-of-function mutations in the critical tumor suppressor protein TP53 are frequent in MPN patients that progress to secondary AML and the mutated TP53* boosts STAT5 transcription. Furthermore, various epigenetic-modifier proteins are found to be mutated in MPN patients, including isocitrate dehydrogenase 1 (IDH1), methylcytosine dioxygenase TET2, DNA methyltransferase 3A (DNMT3A), polycomb group protein ASXL1 and the histone methyltransferase protein of polycomb repressive complex 2 (PRC2) EZH2. Promising therapeutic agents to target these key proteins/pathways in MPN/AML have been developed and are summarized here as black boxes. CALR, calreticulin; TPO, thrombopoietin; EPO, erythropoietin; TF, transcription factor; GEF, guanine exchange factor; SOS, son of sevenless; GTP, guanine triphosphate; mTOR, mechanistic target of rapamycin.

Supplemental Figure S2



Erythroid blast phase of chronic myeloid leukemia (CML)
Wright-Giemsa-stained bone marrow smear of a 49-year old male patient with drug-resistant Ph+ CML and pancytopenia. Note the typical morphology of immature erythroid blast cells. Phenotyping confirmed the presence of CD71+ erythroblasts.