Selective deletion of Jak2 in adult mouse hematopoietic cells leads to lethal anemia and thrombocytopenia

Jak2 inhibitors are commonly used in the treatment of patients with myeloproliferative neoplasms, in particular patients with primary myelofibrosis and splenomegaly.[1] The currently available Jak2 inhibitors do not distinguish between wild-type (WT) Jak2 and mutant Jak2-V617F. Although a modest decrease in the JAK2-V617F mutant allele burden can be seen in some cases, cytopenia due to inhibiting WT Jak2 is one of the factors that limits dose increase.[1] Deleting *Jak2* by conditional knockout offers the possibility of examining the consequences of completely selective Jak2 inhibition *in vivo*, without off-target effects as seen with most Jak2 inhibitors. Since the constitutional knockout of *Jak2* was embryonically lethal due to lack of erythropoiesis,[2] it can be expected that *Jak2* is also essential in adults. Nevertheless, in some cases, the requirement for key components of hematopoiesis during embryogenesis and adult life can differ.[3,4]

To determine the role of *Jak2* in adult mouse hematopoiesis, we crossed conditional Jak2 knockout mice ($Jak2^{fl/fl}$)[5] with $SclCre^{ER}$ mice[6] that express the tamoxifen-inducible Cre-estrogen receptor (CreER) fusion protein[7] in hematopoietic stem and progenitor cells. After four weeks of a diet supplemented with tamoxifen (1 mg/g; Harlan laboratories, Venray, The Netherlands), the red cell parameters as well as the platelet counts were severely decreased in $SclCre^{ER}$;$Jak2^{fl/fl}$ mice (Figure 1A). Lymphocyte counts were not altered (*Online Supplementary Figure S1A and B*). Except for a slight reduction of neutrophils, the blood counts of wild-type (WT) and *SclCre*;$Jak2^{fl/+}$ littermates were unaffected by tamoxifen. The spleen weight was not significantly altered, although a trend towards decreased weight was noted in *SclCre*;$Jak2^{fl/fl}$ mice (Figure 1B). The survival of *Jak2*-deficient mice was drastically reduced compared to WT and *SclCre*;$Jak2^{fl/+}$ mice (Figure 1C). The efficiency of $Jak2^{fl/fl}$ excision was estimated by measuring mRNA expression of *Jak2* and target genes (Figure 1D). *Jak2* mRNA expression in bone marrow cells from tamoxifen-treated *SclCre*;$Jak2^{fl/fl}$ mice was reduced by 90% in comparison with WT mice. A similar decrease in mRNA expression was observed for the *Stat5* target genes TfR1 (CD71) and Bcl-X_L (Figure 1D). When food supplemented with tamoxifen was stopped after four weeks, some mice survived beyond six weeks (Figure 1C). These mice showed an increase in *Jak2* mRNA expression (*data not shown*). These data show that 90% reduction of *Jak2* was lethal for most mice, but in a few escapers a small number of hematopoietic stem and progenitor cells survived that were sufficient to rescue hematopoiesis. Histopathology of BM at four weeks in *SclCre*;$Jak2^{fl/fl}$ mice showed hypocellularity with only a few erythroid precursor cells (<5%) and no visible erythropoietic islands (Figure 1E). Myelopoiesis was less affected and normal histological and cellular morphology was observed for all genotypes. In the spleen, a mild decrease in hematopoiesis occurred in the red pulp, together with a relative increase in the white pulp. The morphology of the cells (especially lymphocytes) was not affected (*Online Supplementary Figure S1C*).

To exclude the possibility that the observed requirement of *Jak2* could be due to loss of Jak2 in non-hematopoietic tissues, we transplanted *SclCre*;$Jak2^{fl/fl}$ or WT bone marrow into lethally irradiated mice. To allow monitoring of autologous reconstitution, we used UBC-GFP transgenic mice[8]

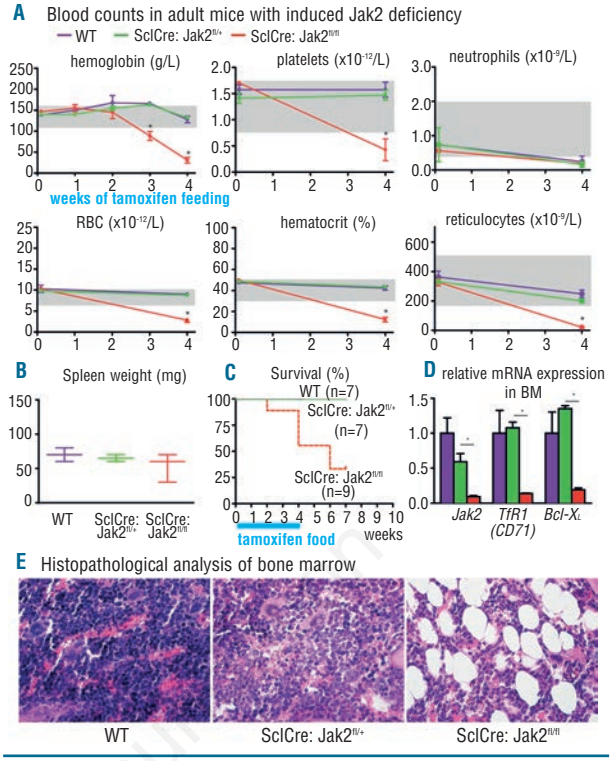

Figure 1. ***Jak2*** **excision in non-transplanted** ***SclCre;Jak2***$^{fl/fl}$ **mice. (A) Blood counts of mice exposed to four weeks tamoxifen (1 mg/g food) are shown. Hemoglobin values were determined once a week with HemoCue and complete blood counts were measured before and at the end of tamoxifen treatment with Advia hematology analyzer. Results are presented as means ± SEM. To assess statistical significance among individual cohorts, one-way ANOVA with subsequent Bonferroni post test (Graph Pad Prism, *vs.* 4.00, 2003) or Mann-Whitney rank sum test were used, and *P* values < 0.05 (*) were considered significant. (B) Spleen weights after four weeks of tamoxifen feeding. (C) Survival of mice pooled from 3 independent experiments. Tamoxifen feeding was stopped after four weeks. (D) Relative mRNA expression in bone marrow (BM) after four weeks of tamoxifen feeding determined by reverse transcription and quantitative PCR and normalized against *GusB* mRNA. (E) Histopathology of hematoxylin-eosin stained bone marrow tissue samples is shown (630x). Note lack of erythropoiesis in** ***SclCre;Jak2***$^{fl/fl}$ **mice.**

as the recipients (Figure 2A). A diet with food supplemented with tamoxifen was started three weeks after transplantation. In recipients of *SclCre*;$Jak2^{fl/fl}$ bone marrow, blood counts differentiated two subgroups of mice. Most mice (n=5) showed a decrease in red blood cell parameters and platelets in comparison with WT recipients (Figure 2B, red and blue curves, respectively). Granulocytes (Figure 2B) and lymphocytes (*data not shown*) were not affected by the excision of *Jak2*. Others, named 'rescued' (n=3), showed blood counts in the normal range (Figure 2B, orange curve). In these rescued mice, autologous reconstitution was detected, as indicated by the percentage of GFP positive cells in peripheral blood (Figure 2C). These data demonstrate that some recipient-derived hematopoietic stem and progenitor cells escaped irradiation. The spleens of *SclCre*;$Jak2^{fl/fl}$ responders showed a trend toward lower weight (Figure 2D) and survival was severely compromised (Figure 2E). Expression of *Jak2* mRNA was dramatically decreased in bone marrow cells from *SclCre*;$Jak2^{fl/fl}$ responders, but remained almost normal in rescued mice (Figure 2F). Similarly, TfR1 and Bcl-X_L mRNA expression

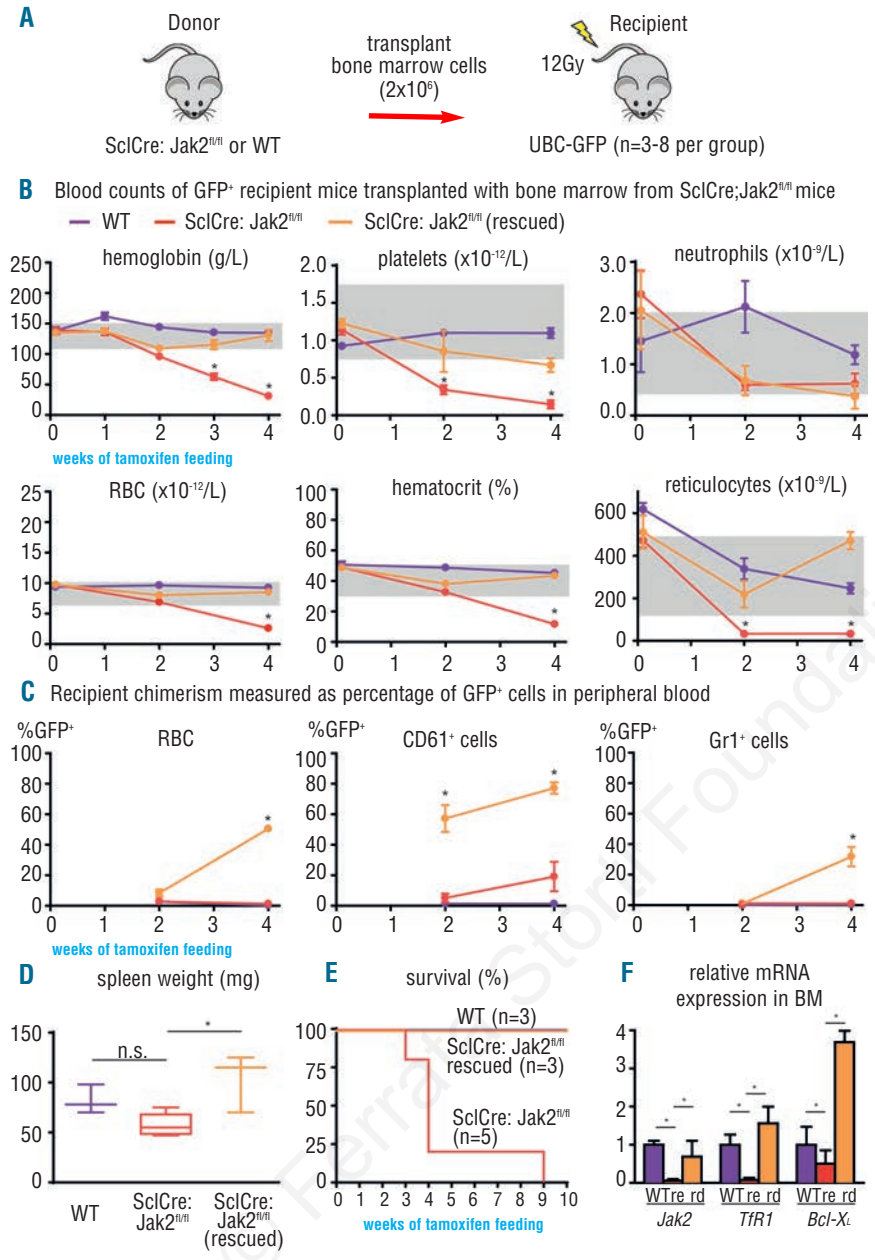

Figure 2. Deletion of *Jak2* in hematopoietic cells transplanted into UBC-GFP recipient mice. (**A**) Schematic drawing of the experimental setup. (**B**) Blood counts of transplanted mice exposed to tamoxifen (1 mg/g food supplemented with 10% sucrose) are shown. *,$P<0.05$. Hemoglobin values were determined once a week and complete blood counts were measured every two weeks. (**C**) Autologous reconstitution was determined as the percentage of GFP positive cells within peripheral blood (red blood cells, CD61+or platelets and Gr1+ or granulocytes). (**D**) Spleen weight. (**E**) Survival curve. (**F**) Relative mRNA expression in bone marrow (BM) after four weeks of tamoxifen feeding determined by reverse transcription and quantitative PCR and normalized against *GusB* mRNA. WT, wild type; re; responder *SclCre;Jak2*$^{fl/fl}$; rd: rescued *SclCre;Jak2*$^{fl/fl}$.

was decreased in responders, but showed compensatory increase in rescued mice that exceeded the levels found in wild-type controls (Figure 2F). The same results were obtained when wild-type C57BL/6N mice instead of *UBC-GFP* mice were used as recipients. The spleen weight was significantly decreased in *SclCre;Jak2*$^{fl/fl}$ responders (*Online Supplementary Figure S2*).

Our results extend the findings of a recent publication that used the ubiquitous Rosa26 promoter to express *Cre*^{ER} and delete *Jak2*.[9] The tamoxifen food regimen applied in our study, together with hematopoietic specific Cre recombinase expression, allowed a more profound deletion of *Jak2* in adult hematopoietic tissues resulting in blood counts and survival rate that were lower in our study than in the previous report. The lethal phenotype could be transferred by bone marrow transplantation, demonstrating that loss of *Jak2* solely in hematopoietic cells is sufficient to abrogate erythropoiesis and thrombopoiesis (Figure 2). Autologous reconstitution in some recipients shows the strong selection pressure that follows the loss of *Jak2*. Granulopoiesis was less affected by *Jak2* deletion, which is in part explained by the fact that the G-CSF receptor also utilizes Jak1 for signaling.[10] Tamoxifen showed an unexpected inhibitory effect on granulopoiesis in all genotypes including WT controls.

The reduction of *Jak2* mRNA expression in our *SclCre;Jak2*$^{fl/fl}$ mice correlated with reduced mRNA expression of the Stat5 target genes *Bcl-X*$_L$ and *TfR1*. Loss of *Bcl-X*$_L$ alone is sufficient to cause anemia and thrombocytopenia,[11,12] while TfR1 deficiency results in a lethal anemia.[13] Stat5 knockout mice also showed anemia due to reduced expression of *Bcl-X*$_L$ and *TfR1* mRNA.[14,15] Thus, expression of *Bcl-X*$_L$ and *TfR1* is required for adult erythropoiesis and is critically dependent on *Jak2* and *Stat5*.

These data demonstrate that strongly inhibiting Jak2 in adult hematopoiesis is incompatible with survival. To

allow more potent inhibition of Jak2-V617F, selective inhibitors that spare the WT Jak2 function will need to be developed.

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References

1. LaFave LM, Levine RL. JAK2 the future: therapeutic strategies for JAK-dependent malignancies. Trends Pharmacol Sci. 2012;33(11):574-82.
2. Neubauer H, Cumano A, Muller M, Wu H, Huffstadt U, Pfeffer K. Jak2 deficiency defines an essential developmental checkpoint in definitive hematopoiesis. Cell. 1998;93(3):397-409.
3. Tsuji-Takayama K, Tahata H, Harashima A, Nishida Y, Izumi N, Fukuda S, et al. Interferon-gamma enhances megakaryocyte colony-stimulating activity in murine bone marrow cells. J Interferon Cytokine Res. 1996;16(9):701-8.
4. Takayama M, Fujita R, Suzuki M, Okuyama R, Aiba S, Motohashi H, et al. Genetic analysis of hierarchical regulation for Gata1 and NF-E2 p45 gene expression in megakaryopoiesis. Mol Cell Biol. 2010;30(11):2668-80.
5. Krempler A, Qi Y, Triplett AA, Zhu J, Rui H, Wagner KU. Generation of a conditional knockout allele for the Janus kinase 2 (Jak2) gene in mice. Genesis. 2004;40(1):52-7.
6. Gothert JR, Gustin SE, Hall MA, Green AR, Gottgens B, Izon DJ, et al. In vivo fate-tracing studies using the Scl stem cell enhancer: embryonic hematopoietic stem cells significantly contribute to adult hematopoiesis. Blood. 20051;105(7):2724-32.
7. Feil R, Brocard J, Mascrez B, LeMeur M, Metzger D, Chambon P. Ligand-activated site-specific recombination in mice. Proc Natl Acad Sci USA. 1996;93(20):10887-90.
8. Schaefer BC, Schaefer ML, Kappler JW, Marrack P, Kedl RM. Observation of antigen-dependent CD8+ T-cell/ dendritic cell interactions in vivo. Cell Immunol. 2001;214(2):110-22.
9. Park SO, Wamsley HL, Bae K, Hu Z, Li X, Choe SW, et al. Conditional deletion of Jak2 reveals an essential role in hematopoiesis throughout mouse ontogeny: implications for Jak2 inhibition in humans. PLoS One. 2013;8(3):e59675.
10. Shimoda K, Feng J, Murakami H, Nagata S, Watling D, Rogers NC, et al. Jak1 plays an essential role for receptor phosphorylation and Stat activation in response to granulocyte colony-stimulating factor. Blood. 1997;90(2):597-604.
11. Wagner KU, Claudio E, Rucker EB 3rd, Riedlinger G, Broussard C, Schwartzberg PL, et al. Conditional deletion of the Bcl-x gene from erythroid cells results in hemolytic anemia and profound splenomegaly. Development. 2000;127(22):4949-58.
12. Rhodes MM, Kopsombut P, Bondurant MC, Price JO, Koury MJ. Bcl-x(L) prevents apoptosis of late-stage erythroblasts but does not mediate the antiapoptotic effect of erythropoietin. Blood. 2005;106(5):1857-63.
13. Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. Transferrin receptor is necessary for development of erythrocytes and the nervous system. Nat Genet. 1999;21(4):396-9.
14. Kerenyi MA, Grebien F, Gehart H, Schifrer M, Artaker M, Kovacic B, et al. Stat5 regulates cellular iron uptake of erythroid cells via IRP-2 and TfR-1. Blood. 2008;112(9):3878-88.
15. Zhu BM, McLaughlin SK, Na R, Liu J, Cui Y, Martin C, et al. Hematopoietic-specific Stat5-null mice display microcytic hypochromic anemia associated with reduced transferrin receptor gene expression. Blood. 2008;112(5):2071-80.