To the editor:

Transition to homozygosity does not appear to provide a clonal advantage to hematopoietic progenitors carrying mutations in *TET2*

TET2 mutations are frequently found in hematologic malignancies including myeloproliferative neoplasms (MPNs).[1,2] We previously reported on the clonal evolution of *TET2* mutations in 8 *JAK2*-V617F–positive MPN patients by genotyping single BFU-E colonies and found there is no strict temporal order of occurrence between the *TET2* and *JAK2* mutations.[3] The majority of patients with MPN display heterozygous *TET2* mutations, but cases with homozygous *TET2* mutations due to loss of heterozygosity (LOH) have also been described. Our clonal analysis revealed that 4/8 patients had a very small proportion (1%-3%) of colonies that were homozygous for the *TET2* mutation through a mechanism of uniparental disomy or deletion in the *TET2* locus. To determine whether cells carrying the homozygous *TET2* mutation have a competitive advantage over heterozygous cells, we examined sequential follow up samples from 3 patients with homozygous mutations (Figure 1A). The homozygous *TET2* mutation was present in the follow up sample of patient p209, but the proportion did not increase within the 11-month period between the 2 time points. In the other 2 patients (p225 and p234) we did not detect colonies homozygous for the *TET2* mutation in the follow up analyses 31 months and 33 months later, respectively. These results argue against the hypothesis that the loss of both functional copies of *TET2* provides an additional growth advantage over cells with the heterozygous mutation, as is frequently the case with tumor suppressor genes.

In our previous report, 2/8 patients showed a biclonal pattern, with mutations in *JAK2* and *TET2* representing separate clones. Our follow up analysis revealed that a third patient, p209, also acquired a second clone that carries *JAK2*-V617F without the *TET2* mutation. We excluded the possibility that this pattern was acquired through uniparental disomy in the *TET2* locus using the marker rs34402524, which remained heterozygous in this colony. Another patient (p101) with biclonal pattern of *TET2* and *JAK2*-V617F surprisingly showed a reduction and disappearance of the *JAK2*-V617F single-positive colonies accompanied by decrease in the mutant allele burden in peripheral blood granulocytes (Figure 1B). The platelet counts decreased during this period (1467×10^9/L, 864×10^9/L and 810×10^9/L), but there were no signs of leukemic transformation. A similar decrease in the *JAK2*-V617F single-positive clone was reported in a biclonal *TET2* positive MPN patient treated with pegylated interferon α.[4] However, our patient was treated with 5-hydroxyurea only and did not receive interferon. The proportion of colonies single-positive for the heterozygous *TET2* mutation increased from 16% to 40% in our patient, suggesting that the presence of the heterozygous *TET2* mutation alone may provide a growth advantage.

TET2 has recently been shown to have hydroxylase activity for 5-methyl-cytosines.[5] Our data are compatible with the hypothesis that a decrease in *TET2* hydroxylase activity through haploinsufficiency causes alterations in the DNA-methylation patterns that provide a growth advantage. Loss of the *TET2* hydroxylase activity in cells homozygous for *TET2* mutations does not appear to increase their competitive advantage, suggesting that the *TET2* hydroxylase activity is tightly regulated.

Franz X. Schaub
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Thomas Lehmann
Department of Hematology, University Hospital Basel, Basel, Switzerland

Renate Looser
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Hui Hao-Shen
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

Andre Tichelli
Division of Diagnostic Hematology, University Hospital Basel, Basel, Switzerland

Radek C. Skoda
Department of Biomedicine, Experimental Hematology, University Hospital Basel, Basel, Switzerland

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Correspondence: Radek C. Skoda, MD, Department of Biomedicine, Experimental Hematology, University Hospital Basel, Hebelstr 20, 4031 Basel, Switzerland, radek.skoda@unibas.ch.

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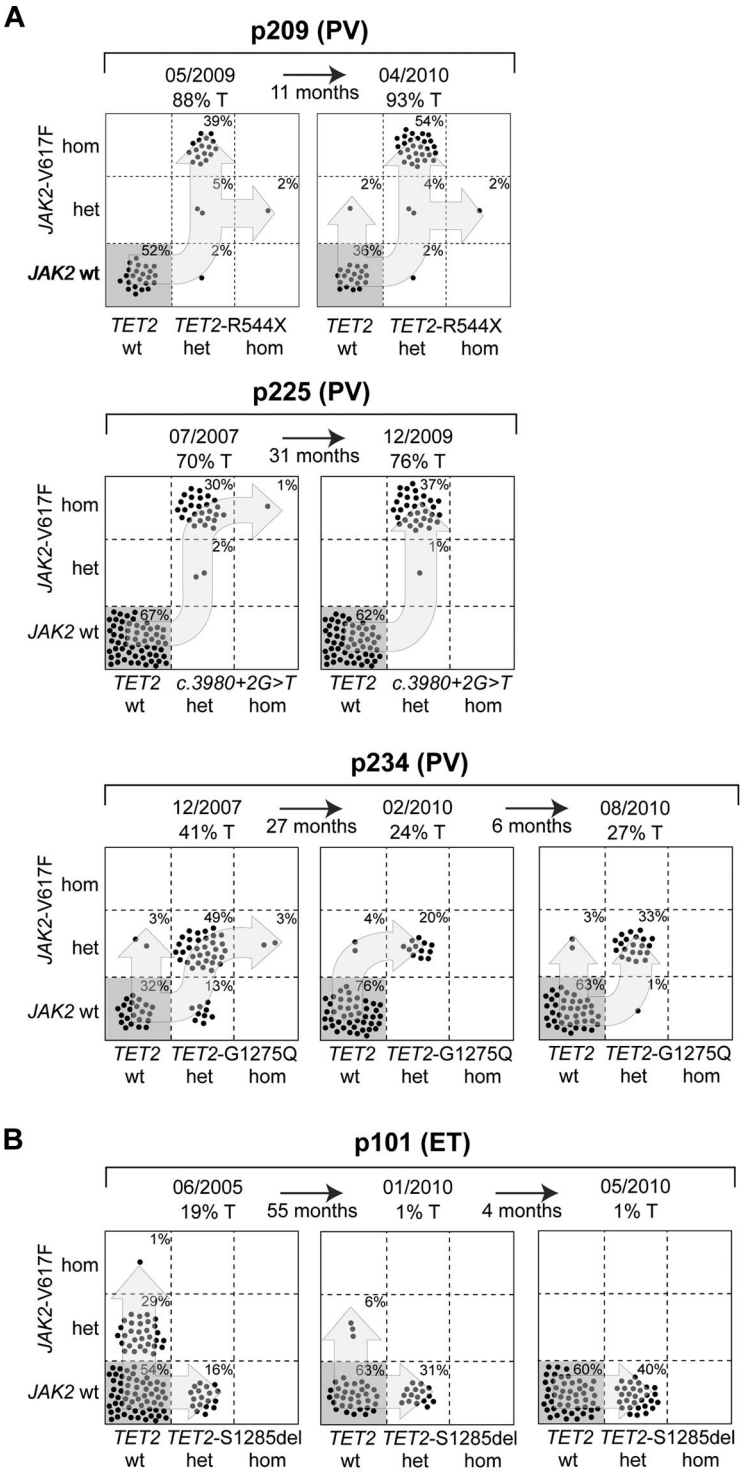

Figure 1. Analysis of single colonies for mutations in *TET2* and *JAK2*. Mononuclear cells from peripheral blood were grown in methylcellulose. Single burst forming units erythroid (BFU-E) were picked and analyzed individually for the presence of *TET2* and *JAK2*-V617F mutations by DNA sequencing and allele-specific polymerase chain reaction, respectively. Each colony is represented by a dot that is placed into one of 6 quadrangles representing the 6 possible genotypes: wild-type (wt), heterozygous (het) and homozygous (hom) for *JAK2*-V617F on the vertical axis, and for *TET2* mutations on the horizontal axis. The unique patient numbers, the diagnoses (PMF indicates primary myelofibrosis; ET, essential thrombocythemia; and PV, polycythemia vera) and the allelic ratio of *JAK2*-V617F in purified granulocytes (%T) are shown above the corresponding boxes. Light blues arrows indicate the suggested order of mutation events. (A) Patterns from patients with homozygous colonies at the first time point, which did not expand. (B) Patterns compatible with a biclonal state of the disease with a gradual decrease in *JAK2*-V617F allelic burden.