

LETTER TO THE EDITOR

Clonal evolution in UKE-1 cell line leading to an increase in *JAK2* copy number

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The *JAK2* V617F mutation is present in the vast majority of polycythemia vera patients, and in about half of patients with primary myelofibrosis and essential thrombocythemia.¹ A correlation between MPN disease phenotype and the proportion of *JAK2* V617F mutant alleles^{2,3} has led to the need for sensitive and reproducible molecular techniques to assess the so-called *JAK2* V617F allele burden in patient samples. A 2009 multicenter study conducted by Lippert *et al.*⁴ assessed the concordance of *JAK2* V617F allele burden quantitation from various diagnostic laboratories. The study demonstrated the importance of using well-defined and accurate standards to calibrate *JAK2* V617F quantitative assays. To that end, the authors suggested the use of plasmid DNA dilutions along with a known genomic DNA sample as an internal control to allow for the most precise quantification of *JAK2* V617F allele burden. As an alternative to plasmids, the diploid UKE-1 cell line, having two copies of mutated *JAK2* per cell, was suggested as a useful genomic DNA standard.⁴

There are several *JAK2* V617F-positive cell lines derived from patients with history of myeloproliferative or myelodysplastic disorders (HEL, MB-02, MUTZ-8, SET-2 and UKE-1) that differ in number of *JAK2* mutant alleles. These cell lines are good research tools for elucidating the pathobiology of MPN,⁵ and are potential standard material as well. However, HEL cells have more than two copies of *JAK2* and should not be used as a standard for quantification of *JAK2* V617F allele burden.^{3,4} The UKE-1 cell line has recently been shown to be homozygous for the *JAK2* V617F mutation^{4,5} and may therefore be a useful as a genomic DNA standard. UKE-1 was derived from an essential thrombocythemia patient transformed into acute leukemia in 1997.⁶ Phenotypic analysis of leukemic cells revealed coexpression of myeloid and endothelial antigens. Cytogenetic analysis of cells showed biclonality with the following karyotype: 48,XX,+8,+14[14]/45,XX,-7,del(11)(p14)[8]. UKE-1 cells were cultured in Iscove modified Dulbecco medium supplemented with 10% fetal calf serum, 10% horse serum and 1 μ M hydrocortisone. Morphologically, UKE-1 cells were described as heterogeneous, with coexistence of large, adherent cells with prominent vacuoles and smaller nonadherent cells.⁶

Here, we show that UKE-1 cells in our hands underwent clonal evolution during *in vitro* culture, leading to an increase of *JAK2* gene copies. Use of these cells as a standard would cause an underestimation of the *JAK2* V617F allele burden.

The UKE-1 cells used in this study had been cultured for 5 months, frozen for 16 months, then thawed. We then cultivated the UKE-1 cells and a human myeloid cell line, HL60, for 3 weeks before making serial dilutions of UKE-1 in HL60 (100% UKE-1 through 0% UKE-1). This widely used human myeloid cell line derives from leukocytes of a patient with acute promyelocytic leukemia in 1979. Initial cytogenetic analysis revealed 75% of aneuploid cells (44 chromosomes) with structural anomalies on chromosomes 7, 9, 10 and number anomalies on chromosomes 5, 8, 18, X.⁷ The cell mixtures were washed twice in

1 \times phosphate-buffered saline and pelleted. Genomic DNA was extracted with the FlexiGene DNA kit (Qiagen, Courtaboeuf, France), and *JAK2* V617F quantification was performed using the plasmid based *JAK2* Mutaquant kit (Ipsogen SA, Marseille, France) according to manufacturer recommendations.

Throughout the range of dilutions, we found that the measured *JAK2* V617F allele burden in the UKE-1/HL60 mixtures was consistently higher than expected (Figure 1). Similar findings were found in another lab. It was found that the *JAK2* V617F allele burden was consistently overestimated when Mutaquant kit was used on a range of dilution of the line UKE-1: 2.4% for 1%, 67% for 50% in mean (Cassinat, personal data). One possible explanation for this discrepancy is an abnormal *JAK2* copy number in one of the cell lines. We therefore performed conventional and molecular cytogenetic analysis of the HL60 and UKE-1 cells. Karyotypes were determined using standard cytogenetic techniques and described according to the International System for Human Cytogenetic Nomenclature (ISCN 2009).⁸ In order to assess *JAK2* copy number, fluorescence *in situ* hybridization with bacterial artificial chromosomes (FISH-BAC) was performed as previously described.⁹ A BAC-targeting *JAK2* (RP11-982A21 on 9p24.1 band) labeled in SpectrumGreen (Abbott, Rungis, France) and a reference BAC (RP11-115G2 located on 9p21.2) labeled in SpectrumOrange (Abbott) were applied to metaphase preparations and analyzed.

The karyotype of the HL60 cell line was 45,X,del(3)(p2?),add(5)(q2?3),der(8)t(4;8)(q2?;q24),del(9)(p1?2),del(10)(p1?2),add(11)(q2?3),i(13)(q10),add(17)(p11),del(18)(q1?)[22] (data not shown). FISH-BAC with *JAK2* probe found an interstitial deletion of chromosome 9 without *JAK2* deletion (data not shown) in HL60 lineage.

Cytogenetic analysis of UKE-1 revealed two clones: a pseudo-diploid clone (previously observed in 2008 in our lab) (Figure 2a) and a tetraploid clone (observed for the first time) (Figure 2b).

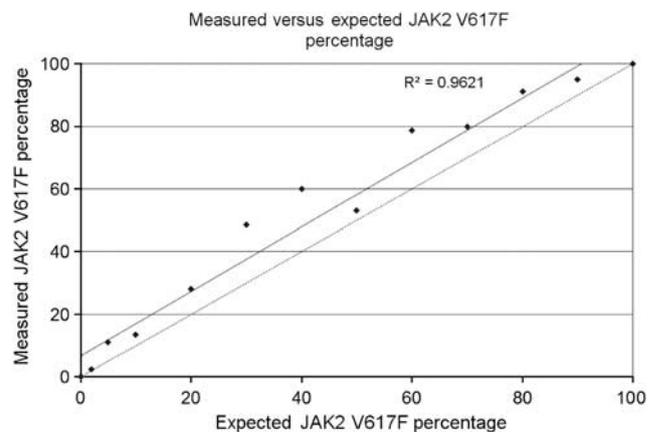


Figure 1. Measured versus expected *JAK2* V617F percentage. Serial dilutions of UKE-1 cells (supposedly homozygous for mutant *JAK2* V617F) in HL60 cells (homozygous for wild-type *JAK2*) were made and the percentage of *JAK2* V617F in each dilution was quantified using the *JAK2* Mutaquant Ipsogen kit. For each dilution, the measured *JAK2* V617F percentage was significantly greater than the theoretical ratio shown by the dotted line ($P = 0.002$).

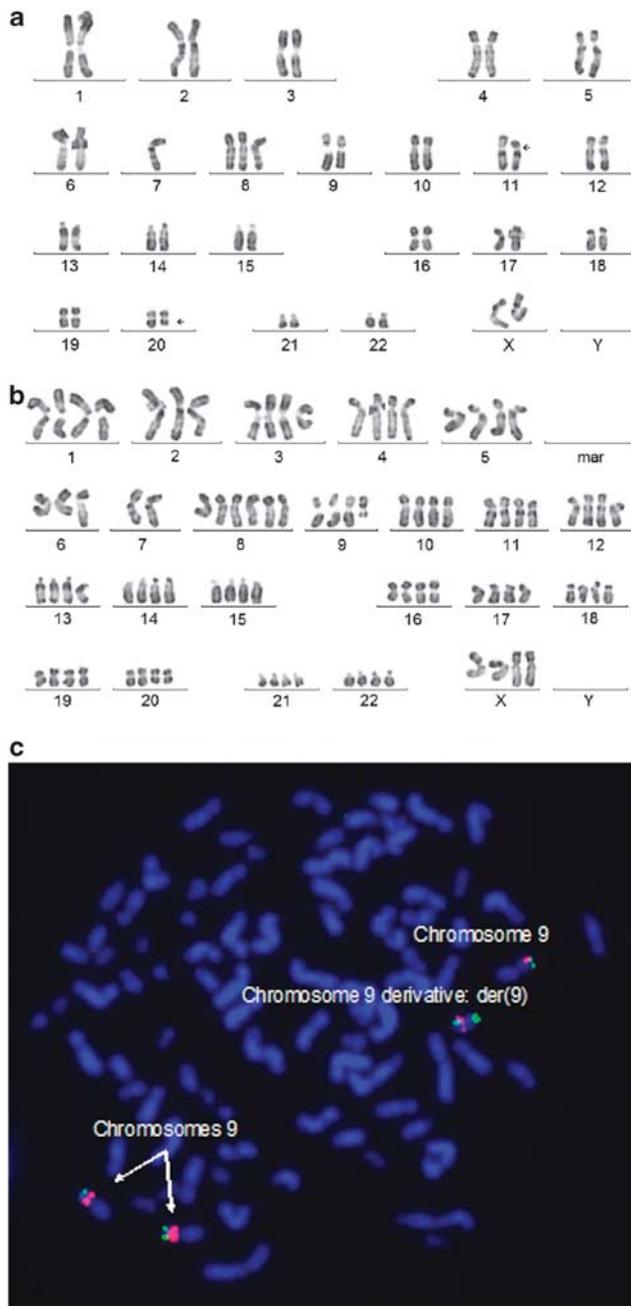


Figure 2. Cytogenetic analysis of the UKE-1 cell line used in this experiment. We observed two clones and determined the karyotype to be 46,XX,-7,+8,del(11)(p1?1p1?3),del(20q)[12]/92,idemx2,del(9)(q2?1)[8]. **(a)** RHG banding of the pseudo-diploid clone. **(b)** RHG banding of the tetraploid clone. Note the partial deletion of the long arm of chromosome 9. **(c)** FISH-BAC analysis on the UKE-1 cell line using a *JAK2* probe labeled with spectrumGreen and a reference probe on 9p21.2 labeled with spectrumOrange. The metaphase FISH shows five copies of *JAK2* in the tetraploid clone: three normal chromosomes 9, each having one copy of *JAK2* (green) and a chromosome 9 derivative having two copies of *JAK2*.

The karyotype of UKE-1 was 46,XX,-7,+8,del(11)(p1?1p1?3),del(20q)[12]/92,idemx2,del(9)(q2?1)[8]. The tetraploid clone had three normal chromosomes 9 and a derivative chromosome 9 having a partial deletion of the long arm. FISH-BAC analysis showed the expected two copies of *JAK2* in the pseudo-diploid clone (data not shown) and five copies of *JAK2* in the tetraploid clone: one copy on each of the three normal chromosomes 9 and two copies of

JAK2 on abnormal chromosome 9 (Figure 2c). As a consequence, there is an unbalanced ratio of wild-type *JAK2* and *JAK2* V617F within the quantification standard, leading to an underestimation of the *JAK2* mutational load when using this UKE-1 cell line as the standard in the analysis of patient samples.

These results highlight the possibility of clonal evolution of the UKE-1 cell line with an increase in *JAK2* gene copy number. The cell line has deviated following culture in our hands because the tetraploid clone was not present when supplying in 2008 and only two copies of *JAK2* were detectable at this time. Mechanisms of this genetic instability may be related to an excessive reactive oxygen species production that promotes DNA double-strand breaks and altered repair, as this has been reported in several myeloid diseases and cell lines.¹⁰ In MPN, it has been shown that the *JAK2* V617F mutation induces an increase in spontaneous homologous recombination leading to a hyperrecombination state.¹¹ *JAK2* V617F mutation may also affect p53 response to DNA damage.¹²

Several clinical studies show that measuring the *JAK2* V617F allele burden may be important to assess prognosis and adjust treatment in MPN patients.^{2,3,13,14} Accurate quantification of allele burden requires a well-defined genetic standard for calibration. Besides, cooperative works are underway to standardize and harmonize the q-PCR techniques. This study shows that using the UKE-1 cell line as a standard for *JAK2* V617F quantification must be avoided, as cells in culture can acquire multiple copies of the *JAK2* gene.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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