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in patients' response to TKI, since all of them, except for patient 1 with a previous PV diagnosis, achieved at least CCyR.

In summary, in this larger cohort of CML patients compared with that studied by Makishima et al,[1] we found 2.55% of cases presenting concomitant BCR/ABL rearrangement and *JAK2*V617F mutation, indicating that the simultaneous occurrence of these mutations is rare event but it is not a phoenix; however, while the pathophysiologic significance of this double mutated phenotype remains to be clarified, it seems clear that the predominant clinical phenotype is, in most cases, that of a typical BCR-ABL rearranged CML.

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Contribution: L.P., performed research, analyzed data, and wrote the manuscript; A.S., B.S., performed research; U.O., S.B., A.B, F.A., C.F., provided patient samples and clinical data; and A.M.V., designed research, analyzed data, and wrote the manuscript.

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To the editor:

Complete abolishment of coagulant activity in monomeric disulfide-deficient tissue factor

The initiator of coagulation, tissue factor (TF), resides on the cell surface in an inactive (cryptic) state that binds factor VIIa with reduced affinity and cannot activate factor X, or in an active (decrypted), conformation.[1,2] Protein disulfide isomerase-dependent regulation of the TF allosteric Cys^{186}-Cys^{209} disulfide has been proposed as a key event in TF decryption, but this remains controversial.[3-7] Although elimination of the TF disulfide by Cys^{209}-to-Ala conversion reduces affinity for VIIa and abolishes procoagulant activity, Kothari et al have questioned the validity of the disulfide switching model of TF decryption.[8] They showed that mutation of either Cys-residue to Ser led to 10-fold lower cellular TF expression compared with wild-type TF, but coagulant activity normalized per TF molecule at supraphysiologic VIIa concentrations was indistinguishable between mutants and wild-type TF.

We reasoned that excessive membrane surface in cells expressing lower levels of TF mutants led to an overestimation of procoagulant activity. To exclude this variable, we created BHK cells expressing similar amounts of surface wild-type TF (TF^{WT}) or TF containing single or double Cys-to-Ala mutations. Analysis of 40 clones per mutant showed that Cys^{186} mutation led to intracellular retention, as described before,[1] while a pair of TF^{WT} and $TF^{Cys209Ala}$ clonal cell lines with similar surface expression was identified. $TF^{Cys209Ala}$ expression was approximately 60% of TF^{WT} (Figure 1A), supported by surface-immunostaining with an antibody with similar reactivity for TF^{WT} and $TF^{Cys209Ala}$ (Figure 1B). In contrast, mAb-5G9, which has reduced affinity for disulfide-mutated TF, only recognized surface TF^{WT}. Consistent with previous results in HUVEC,[1] BHK cells expressing TF^{WT} efficiently activated X, whereas $TF^{Cys209Ala}$-expressing BHK cells did not differ from untransfected BHK cells, even at supraphysiologic VIIa concentrations (Figure 1C).

We next addressed the possibility that the differences observed in Kothari's[8] and our study were because of the

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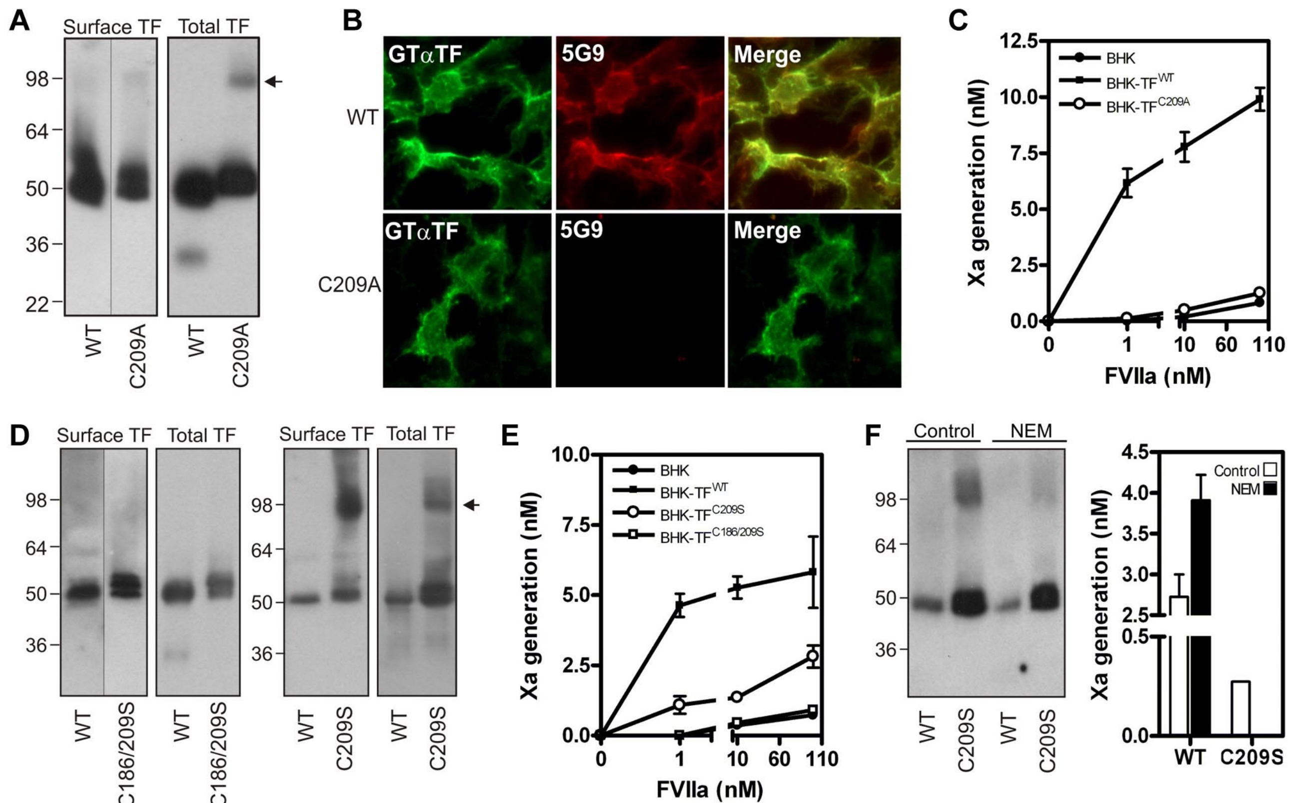

Figure 1. Absence of the TF allosteric disulfide results in abolished coagulant function. (A) BHK cells were stably transfected to express TF^{WT} or $TF^{Cys209Ala}$. TF cell surface exposure was determined by labeling with 1mM NHS-biotin in HBS, and subsequently precipitation of biotinylated TF. To obtain total TF protein, immunoprecipitation with the TF mAb 9C3 was performed with *n*-octyl β-D-glucopyranoside (OG) cell lysates. Western Blotting was performed using Goat anti-TF antibody. The arrow indicates TF dimers. (B) Reactivity of a polyclonal goat anti-TF and 5G9 (both 10 μg/mL) with BHK cell surface levels of TF^{WT} or $TF^{Cys209Ala}$. (C) TF procoagulant activity on nontransfected BHK cells, BHK-TF^{WT} and BHK-$TF^{Cys209Ala}$ was measured kinetically after addition of the indicated concentrations of VIIa and 100nM FX. (D) BHK cells expressing TF^{WT}, $TF^{Cys209Ser}$ and $TF^{Cys186/209Ser}$. Cell surface expression was determined after NHS-biotin labeling and total TF expression was determined in total lysate. Note the absence of a dimer fraction in case of the $TF^{Cys186/209Ser}$ mutant. The arrow indicates TF dimers. (E) TF procoagulant activity on nontransfected BHK cells, BHK-TF^{WT}, BHK-$TF^{Cys209Ser}$ and BHK-$TF^{Cys186/209Ser}$ was determined as described before. (F) BHK-TF^{WT} and BHK-$TF^{Cys209Ser}$ were incubated with 10mM N-ethylmaleimide for 1 hour. Presence of dimer factions was determined on Western blot and procoagulant activity was performed as described before, using 1nM VIIa.

specific replacements for these Cys-residues and generated BHK cells stably expressing TF containing Cys-to-Ser conversions. Surface biotinylation identified $TF^{Cys209Ser}$ and $TF^{Cys186/209Ser}$ clones with expression equal to the TF^{WT} clone. Interestingly, $TF^{Cys209Ser}$ showed substantial surface-exposed dimers compared with $TF^{Cys209Ala}$, while $TF^{Cys186/209Ser}$ did not form dimers, as expected from a TF mutant lacking unpaired cysteines (Figure 1D). While procoagulant activity of the $TF^{Cys186/209Ser}$ clone did not differ from untransfected BHK cells, $TF^{Cys209Ser}$ showed residual activity (Figure 1E). To prevent dimerization, $TF^{Cys209Ser}$-expressing BHK cells were incubated with N-ethyl-maleimide. Blocking free thiols impaired dimerization and also completely abolished $TF^{Cys209Ser}$ activity, while TF^{WT} activity was up-regulated (Figure 1F), consistent with previously reported effects of NEM on flippase-induced PS exposure.[9]

These data with controlled surface expression of TF support previous conclusions that disulfide-mutated TF is impaired in adopting a procoagulant conformation. Of note, Kothari et al observed dimers when expressing TF mutated at both Cys-residues.[8] Because our data demonstrate that dimerization can increase procoagulant activity of single-free-thiol mutants, their assay conditions may have favored TF dimerization by mechanisms unrelated to the mutated Cys-residues. In conjunction with increased membrane availability, residual dimer activity may have led to overestimation of TF-specific procoagulant activity. Thus, we consider general dismissal of the disulfide switching hypothesis as proposed by Kothari et al, unjustified.

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To the editor:

Haploidentical hematopoietic stem cell transplantation for graft-versus-host disease after liver transplantation

A 74-year-old woman with hepatitis C and hepatocellular carcinoma underwent deceased donor liver transplant (LT) from a male donor in August 2008. Donor and recipient were blood group A^+ and 2-loci HLA matched (DR01 and DR15). Initial immunosuppression included a corticosteroid taper and tacrolimus. On postoperative day (POD) 28, she developed a papular erythematous rash, diarrhea, and neutropenia. Skin biopsy showed perivascular lymphocytic infiltrate with necrotic keratinocytes and full thickness epidermal necrosis. FISH analysis demonstrated XY-chimerism in both peripheral blood (29.2%) and skin (18.5%, 37/200 nuclei) consistent with graft-versus-host disease (GVHD). Bone marrow biopsy showed < 10% cellularity with XY chimerism (2/10 nuclei) on karyotype analysis. Liver function tests were normal. High dose steroids and mycophenolate mofetil 500mg TID were added. The patient developed refractory neutropenia and thrombocytopenia. After failing a trial of IVIG (0.5mg/kg/d) and rabbit anti-thymocyte globulin (rATG; 1.5mg/kg/d), we sought a donor for rescue hematopoietic stem cell transplantation (HSCT) based on prior success with this strategy in GVHD following HSCT.[1]

On POD 42 she received a haploidentical $CD34^+$ selected (Baxter Isolex 300i) HSCT (2.01×10^6 cells, $CD3^+$ 0.3%) from her son (3-loci HLA match) after 7 days of rATG. A second

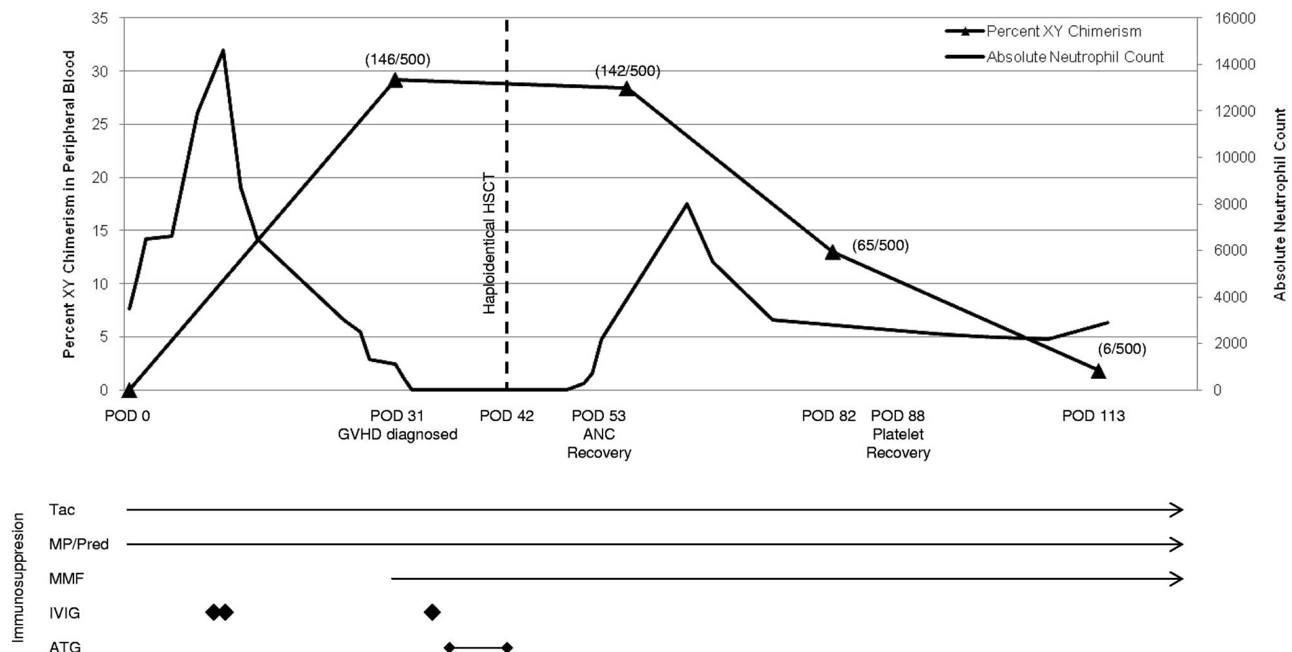

Figure 1. Trend in XY chimerism of peripheral blood relative to bone marrow function. Peripheral blood FISH analysis demonstrating XY chimerism (number of XY nuclei/total nuclei counted) with corresponding absolute neutrophil count (ANC) representing bone marrow function. Recovery of ANC occurred with XY chimerism suggesting either transient engraftment of son's cells or immunomodulatory effect of $CD34^+$ infusion facilitating resolution of GVHD with subsequent stable marrow function and near complete restoration of patient XX chimerism on POD 113. Time of ANC recovery and platelet recovery are noted as defined by CIBMTR reporting standards. Timing of immunosuppression relative to clinical course is also noted.

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