To the editor:

Episomal amplification of *NUP214-ABL1* fusion gene in B-cell acute lymphoblastic leukemia

The *NUP214-ABL1* fusion gene is found amplified as multiple (5-50) episomal copies in 6% of T-cell acute lymphoblastic leukemia (T-ALL).[1,2] Alterations of the *TLX1*, *TLX3*, *CDKN2A/B*, and *NOTCH1* genes are commonly associated with NUP214-ABL1 T-ALL. Recently, the *NUP214-ABL1* fusion gene has been reported in 2 of 15 cases of B-cell acute lymphoblastic leukemia (B-ALL)[3] identified by RNA-sequencing with no evidence of episomal amplification, suggesting an intrachromosomal rearrangement. In 1 of 2 cases, phosphoflow analysis demonstrated increased CRK-like protein phosphorylation, suggesting active ABL1 signaling, that was sensitive to tyrosine kinase inhibitors (TKIs). We now report the first case of B-ALL associated with extrachromosomal, episomal amplification of *NUP214-ABL1*. All methods can be found in supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

A 22-year-old male presented with a hemoglobin 108 g/L, white cell count 12.72×10^9/L, and platelet count 65×10^9/L. Bone marrow aspirate and trephine revealed 99% blast cells expressing CD79a, CD19, CD10, CD20, surface IgM, HLA-DR, cytTdT, and CD7 (Figure 1A-B). The latter 2 markers are more suggestive of bi-lineage blasts, but there was no cytCD3 CD2, CD4, CD5, CD7, CD8, cytMPO, CD33, CD13, CD15, and CD117 expression. The karyotype was: 47,XY,+X.

Interphase fluorescence in situ hybridization (FISH) using a *BCR-ABL1* probe demonstrated 50-80 extrachromosomal copies of *ABL1*; FISH probes targeting 3′ regions of *ABL1* and *NUP214* confirmed episomal *NUP214-ABL1* amplification in ~ 99% of cells (Figure 1C). Conversely, a probe targeting the 5′ region of *ABL1* showed a normal signal pattern. This signal configuration is the same as previously shown in T-ALL.[2] Multiplex ligation–dependent probe amplification (MLPA) confirmed amplification of both *ABL1* and *NUP214* (data not shown). Although FISH, MPLA, and SNP6.0 analysis showed no rearrangement of *TLX1* and *TLX3,* aberrant TLX1/3 expression cannot be excluded. MLPA showed focal deletions of exons 2-7 of *IKZF1* and exons 1-2 within the *CDKN2A/B* locus. SNP6.0 analysis confirmed *NUP214* and 3′ *ABL1* amplification (supplemental Figure 1). SNP data also confirmed *IKZF1* and *CDKN2A/B* loss and showed other copy number aberrations including some previously implicated in B-ALL; *FHIT*,[4] *TBL1XR1*,[5] and the histone cluster at 6p22[6] (supplemental Table 1).

The patient was treated with induction therapy (vincristine, daunorubicin, pegylated asparaginase, prednisolone, and CNS prophylaxis http://www.ctsu.ox.ac.uk/research/mega-trials/leukemia-trials/ukall-2003/). A day 29 marrow showed complete morphologic remission, but PCR-based IgVH MRD rearrangement studies revealed 1 in 10^4 cells with clonal IgVH rearrangement. The patient successfully underwent cyclophosphamide/total body irradiation myeloabalative sibling donor allogenic stem cell transplantation and is in complete remission at 4 months.

NUP214-ABL1–positive patient primary cells were cultured with low and high concentrations of imatinib (0.5 and 5μM), dasatinib (10 and 150nM), nilotinib (0.5 and 5μM), and ponatinib (10 and 100nM; Figure 1D; concentrations based on IC_{50} values and maximally achievable plasma concentrations in chronic myeloid leukemia patients). After a 72-hour culture, compared with no drug control, there was no significant reduction in viable cell numbers and no increase in apoptosis in any experimental arm. In contrast, *BCR-ABL1* lymphoblastic cell line BV-173 demonstrated anticipated TKI sensitivities. Moreover, in T-ALL the mutant fusion kinase appeared more sensitive in vitro than *BCR-ABL1* to TKIs.[7] It is noteworthy, there are only case reports documenting mixed clinical responses to TKIs in *NUP214-ABL* T-ALL.[8,9]

It is unclear to what extent subtypes of *NUP214-ABL1* seen in T-ALL, and now described in B-ALL, differ biologically. In part, these differences in drug sensitivity may arise kinase amplification in our case and differential kinase activation between *NUP214-ABL1* and *BCR-ABL1*.[7] Differences may arise not only from variable *NUP214-ABL1* copy number, but also the nature of cooperating mutations. In our case, these likely include *IKZF1* and the *CDKN2A/B* locus, important B-ALL aberrations. This variable genetic landscape may, in part, account for differential in vitro sensitivity to TKIs.

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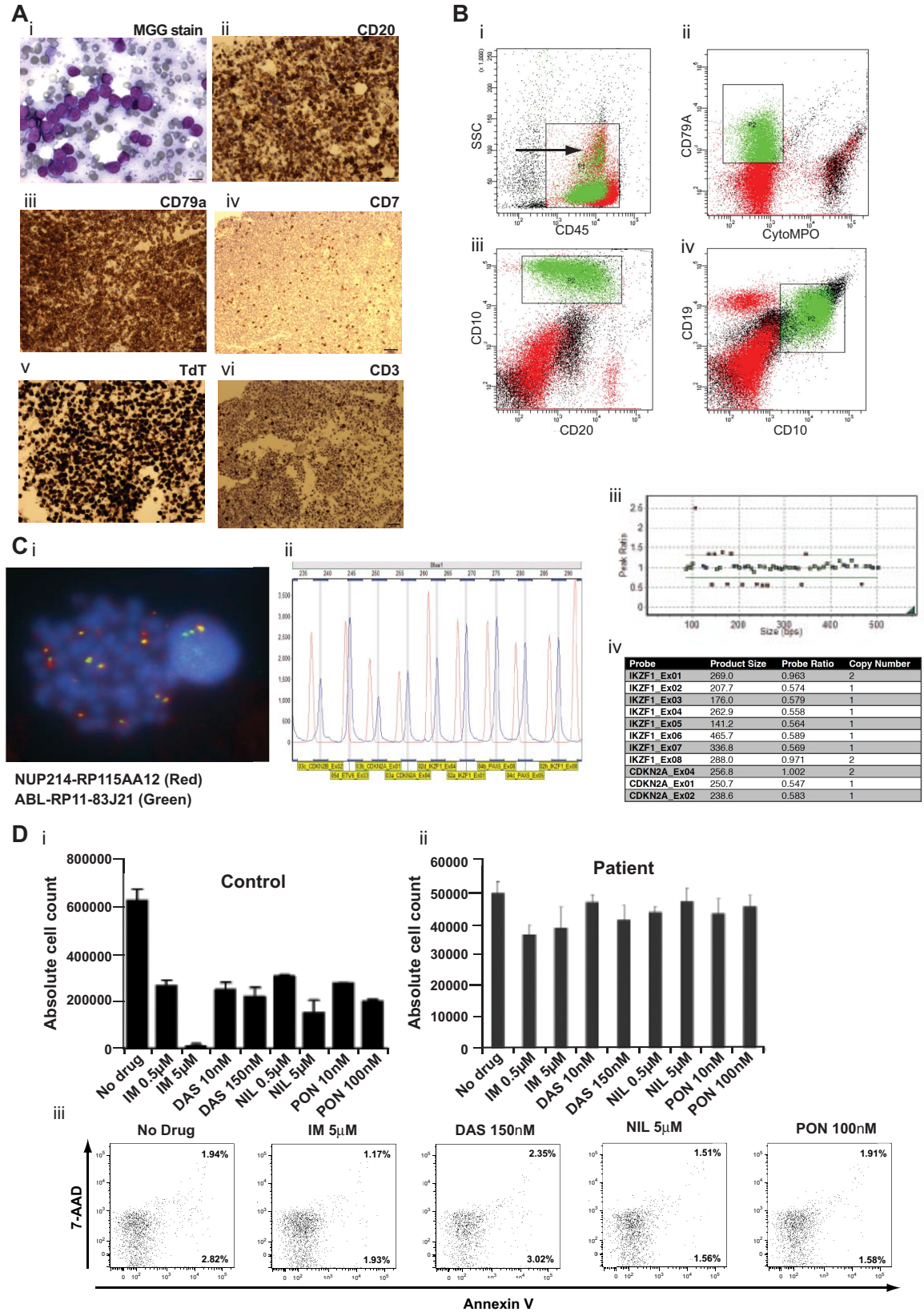

Figure 1. Characterization of episomal *NUP214-ABL1* B-ALL and in vitro sensitivity of patient blasts to TKI. (Ai) May-Grunwald-Giemsa stain of blasts from a trephine roll. (ii-vi) Immunocytochemical stains of trephine sections stained with antibodies indicated above each panel. (Bi-v) Flow cytometric analysis of marrow blasts. (i) Cell populations in the blast cell gate (CD45$^+$ and of the indicated side scatter (SSC) were studied further in subsequent panels. (ii-iv) Expression of the indicated cell surface and cytoplasmic (cyto) antigens was studied on blast cell populations. (Ci) FISH analysis of a blast cell with probes specific for *ABL1* (green) and *NUP214* (red). Two green and red signals indicate normal chromosomal *ABL1* and *NUP214*. Yellow signals indicate location of fusion gene. (ii-iv) MPLA analysis shows deletion of *IKZF1* exons 2-7 and *CDKN2A* exons 1-2. (D) Absolute cell counts of viable control BV173 (i) and patient primary blasts (ii) after 72 hours in culture with either no drug or the indicated concentration of imatinib (IM), dasatinib (DAS), nilotinib (NIL), or ponatanib (PON). (iii) FACS plots of aliquots of patient's cells after 72 hours of culture showing annexin V and 7-amino-actinomycin D (7-AAD) expression.

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To the editor:

Role of fecal calprotectin as biomarker of gastrointestinal GVHD after allogeneic stem cell transplantation

We read with interest the article of Rodriguez-Otero et al.[1] The authors studied the ability of fecal calprotectin (FC), α-1 antitrypsin, and elastase to diagnose acute gastrointestinal GVHD (GI-GVHD) after allogeneic stem cell transplantation (SCT). In their experience, FC and α-1 antitrypsin increased in patients with GI-GVHD, but there was no statistic difference compared with control groups. On the other hand, high levels of both markers at the time of diagnosis were predictive of steroid-resistant GVHD. In past years, our group also investigated the role of FC as a noninvasive biomarker of GVHD. We enrolled a cohort of 59 hematologic patients consecutively submitted to allogeneic SCT, and studied the level of FC in patients who developed GI-GVHD, non–GI-GVHD, and in patients with infective colitis. We also included a control group of 9 patients with aspecific colitis after autologous SCT. FC was detected at the onset of symptoms and before starting any therapy. Stool collection was performed by Calprest device and the protein level was measured by ELISA assay (Calprest test; Eurospital). Data were analyzed using IBM SPSS Statistics 20 Core System and Prism Version 3.0 software (GraphPad). Diagnosis and staging of acute GVHD (aGVHD) and chronic GVHD (cGVHD) was made according to current criteria.[2,3] FC was higher in patients with acute GI-GVHD (GI-aGVHD) than in non–GI-aGVHD (500 mg/Kg vs 95 mg/Kg; $P = .0003$; Figure 1A), and in stage III-IV GI-aGVHD than in the others; although, no statistic difference was observed in this case.

After treatment, in 2 of 3 responsive patients, FC value decreased to less than 15 mg/Kg. In contrast, FC was lower in patients with infective colitis compared with GI-aGVHD (106 mg/Kg vs 500 mg/Kg; $P = .0039$; Figure 1B). Comparing patients with GI-aGVHD, patients with infective enteritis and patients with both conditions, the median level of FC was 500 mg/Kg, 106 mg/Kg, and 475 mg/Kg, respectively ($P = .0096$; Figure 1C). FC was also lower in the control group of patients submitted to autologous SCT who developed mucositis and