Induction of short-term remission with single agent eltrombopag in refractory nucleophosmin-1-mutated acute myeloid leukemia

We have read with great interest the recent editorial discussing future perspectives in the treatment of patients with aplastic anemia (AA).[1] The authors emphasized the potential role of eltrombopag (EPAG), which is currently only licensed as second-line treatment for patients with immune thrombocytopenia (ITP). Notably, a recent study has shown trilineage responses in a considerable subset of patients with refractory AA to standard ATG-based therapies.[2] It is supposed that EPAG directly stimulates residual 'healthy' hematopoietic stem cells (HSCs). On the other hand, others have recently raised the potential concern[3] regarding clonal evolution to myelodysplastic syndrome (MDS) in a subset of susceptible patients.[3] However, in contrast to other thrombopoietin receptor (TPO-R, MPL) agonists, EPAG has also been shown *in vitro* to directly inhibit cell growth of leukemic cells while the underlying mechanisms are still under investigation.[5,6] There is still no formal proof of that observation in patients.

We report a 70-year old male patient with a nucleophosmin 1 mutated ($NPM1_{mut}$) acute myelogenous leukemia (AML) and a normal karyotype (NK) relapsing one year after completion of several courses of conventional chemotherapy including high-dose cytarabine-based consolidation. The patient failed subsequent treatment with azacitidine and entered a clinical trial (*clinicaltrial.gov identifier:00903422*) investigating single agent EPAG in patients with refractory AML and thrombocytopenia.[7] He received EPAG continuously starting with 100 mg/day, which was subsequently increased up to a dose of 300 mg daily. As shown in Figure 1A, the patient achieved a bone marrow response with less than 5% blasts but no recovery of peripheral counts (CRi). He remained in CRi for another three months without improvements in peripheral blood counts (Figure 1D) and ultimately progressed five months after initiation of single agent EPAG. Potential anti-proliferative effects of EPAG on leukemic cells *in vitro* have been shown to occur independently of MPL expression.[6] In line with these data, we observed a significantly lower MPL expression in our index patient as well as in a cohort of NK $NPM1_{mut}$ AML patients compared to wild-type cases (Figure 1B). Since MPL is predominantly expressed on $CD34^+$ progenitor cells, these data are also in agreement with the notion that $NPM1_{mut}$ AML often lack CD34 expression compared to wild-type cases.

In order to acquire a greater insight into the mechanisms of response and subsequent clonal evolution during single

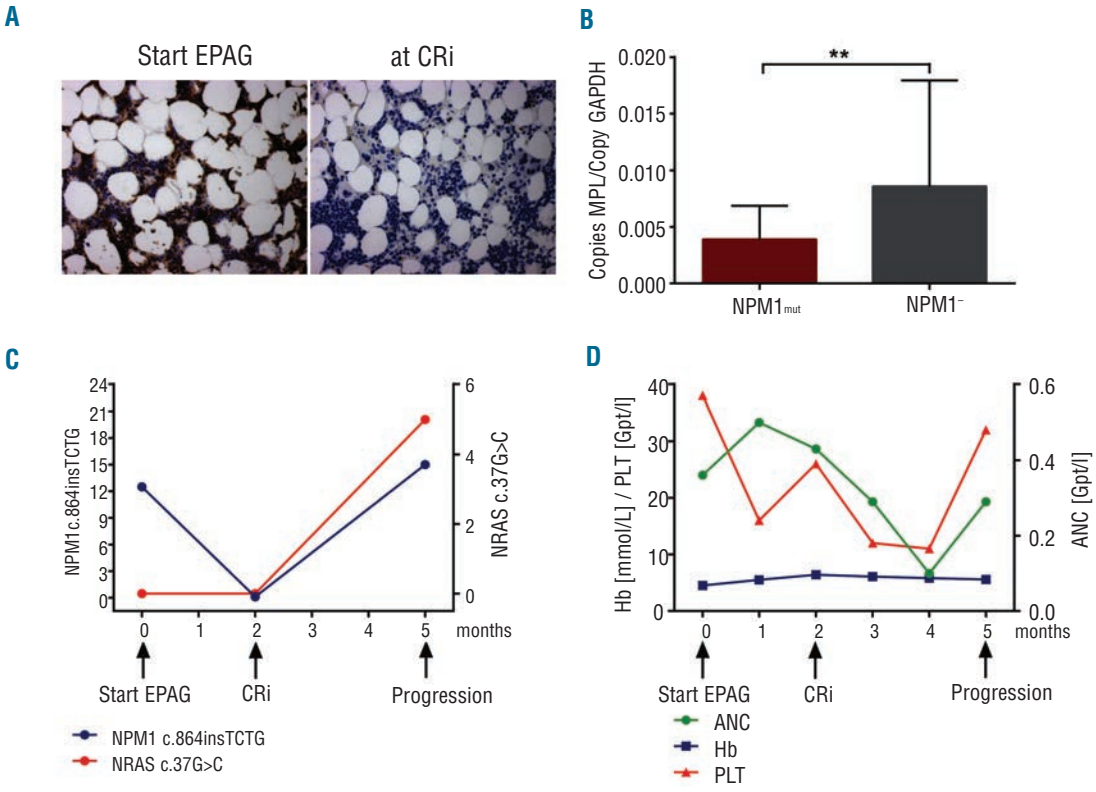

Figure 1. (A) Histological analysis of bone marrow blasts by CD163 immunostaining prior to EPAG therapy (magnification x20) and at the time of remission. Cri: complete remission with incomplete platelet recovery. Magnification x20. (B) MPL detection by Taqman-PCR in bone marrow cells from patients with *de novo* AML and normal karyotype with mutated (NPM1+, n=38) or unmutated (NPM1-, n=19) nucleophosmin-1 gene (*P*=0.0065, one-way ANOVA, Taqman PCR, Applied Biosystems, Hs_00180489_m1). (C) Next generation deep sequencing analysis of NPM1 type-A (TCTG tetranucleotide tandem duplication) and NRAS mutation load during single agent EPAG therapy of a patient with NPM1+ AML achieving a complete remission with single agent EPAG (Cri); y-axis shows mutation level. (D) Peripheral blood counts during treatment with EPAG. ANC: neutrophil count; Hb: hemoglobin; PLT: platelet count.

agent EPAG therapy, we conducted comprehensive molecular analyses of DNA derived from sequential bone marrow biopsies of this patient. Samples (prior to EPAG, at remission and on relapse) were subjected to genome-wide copy number analysis using Affymetrix SNP 6.0 arrays but no acquired copy number alterations could be detected. To screen for alterations in commonly mutated genes in AML and MDS, next generation sequencing (NGS, Roche 454 GS Junior) of the entire coding region or mutational hotspots in *ASXL1, CBL, DNMT3A, ETV6, EZH2, IDH1/2, KRAS, NPM1, NRAS, RUNX1, SF3B1, SRSF2, TET2, TP53*, *U2AF1* and *ZRSR2*, was performed. During exposure to single agent EPAG, we observed a marked decline in the NPM1 mutant clone (Mutation Type A) paralleling the morphological response [(Figure 1B; prior EPAG: 12.6%, at complete remission with incomplete platelet recovery (CRi): 1.1%)]. At the time of progression, NGS detected the emergence of an NRAS c.37G>C mutation not detected at earlier time points in addition to a sharp increase in $NPM1^{mut}$ levels (Figure 1C), consistent with either the expansion of a pre-existent EPAG resistant NRAS c37G>C mutated subclone or the *de novo* acquisition of an NRAS c37G>C mutation. These data further strengthen the potential of EPAG to stimulate early hematopoietic progenitor cells outside the common indication in ITP. In agreement with other reports,[5,6] we do not believe that EPAG promotes leukemic evolution by stimulation of the MPL receptor, but think that our case further illustrates that it may even have a direct MPL-independent (although short-lived) anti-leukemic effect *in vivo*. In the light of a recently presented clinical trial on EPAG in refractory AML,[7] it seems, however, that this effect is applicable only to a subgroup of patients, the characteristics of which still have to be defined. Our case also emphasizes that close monitoring and surveillance of these patients during treatment with a potential 'stem cell cookie'[3] are mandatory.

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