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

Running heads: Eltrombopag in refractory NPM1-mutated AML

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To the editor,

We have read with great interest the recent editorial discussing future perspectives in the treatment of patients with aplastic anemia (AA). 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. It is supposed that EPAG directly stimulates residual “healthy” hematopoietic stem cells (HSCs). On the other hand, others have recently raised their potential concern regarding clonal evolution to myelodysplastic syndrome (MDS) in a subset of susceptible patients. 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. A formal proof of that observation in patients is still missing.

We report a 70 years 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 (NCT00903422) investigating single agent EPAG in patients with refractory AML and thrombocytopenia. He received EPAG continuously starting with 100mg/day, which was subsequently increased up to a dose of 300mg daily. As shown in Figure 1A the patient achieved a bone marrow response with less than 5% blasts but in the absence of recovery of peripheral counts (CRi). He remained in CRi for another 3 months without improvements in peripheral blood counts (Figure 1D) and ultimately progressed 5 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. 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 wildtype cases (Figure 1B). Since MPL is
predominantly expressed on CD34+ progenitor cells these data are also in agreement with
the notion that NPM1mut AML often lack CD34 expression compared to wild type cases.

In order to get more insights into the mechanisms of response and subsequent clonal
evolution during single 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 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 of the NPM1 mutant clone (Mutation
Type A) paralleling the morphologic response (Figure 1B; prior EPAG: 12.6%, at 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 NPM1mut 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 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. It the lights of a recently presented clinical trial on EPAG in
refractory AML7 it seems, however, that this effect is applicable only to a subgroup of
patients, the characteristics of which remain 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.
Authorship
UP designed the research and analyzed data and wrote the paper. All others performed research, analyzed data and edited the paper.

Disclosures
UP has received honoraria for scientific presentations from GSK. All other authors declare no competing financial interests.

References
Figure 1. (A) Histologic analysis of bone marrow blasts by CD163 immunostaining prior to EPAG therapy (20x magnifications) and at the time of remission (CRi, 20x magnification). (B) MPL detection by Taqman-PCR in bone marrow cells from patients with de novo AML and normal aryotype with mutated (NPM1+, n=38) or unmutated (NPM1-, n=19) nucleophosmin-1 gene (p=0.0065, 1way 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= complete remission with incomplete recovery); y-axis displays mutation level. (D) Peripheral blood counts during treatment with EPAG (ANC= neutrophil count, Hb= hemoglobin, PLT= platelet count).