assembly GRCh37/hg19, genome.ucsc.edu) was identified in both the colorectal patients and the AML patients. The insertion, which started at codon 817 in exon 14, results in a frame shift mutation of MLL3, leading to a premature stop codon “TAA” at codon 827.

MLL3, which belongs to the human TRX/MLL family, is an important mammalian H3K4 methyltransferase. Down-regulation of MLL3 promoted cell proliferation in HCC cell lines.1 Homozygous MLL3−/− knockout mice display tumors in the innermost layer of ureter cells.5 These results suggest that MLL3 is a tumor suppressor.

Mechanistically, the MLL3 protein can selectively recognize H3K4me and affect the mechanistic readout of histone tail modifications.6 In addition, ASCOM-MLL3 has a redundant but crucial role in transactivation of p53 and participates in DNA-damage-induced expression of p53-targeted genes.5 In addition, nuclear-receptor-mediated downstream gene expression was regulated by MLL3.7

Because MLL3 is an enzyme, pharmacologic intervention may be possible. Drugs targeting this molecule may be effective in both colorectal and AML with MLL3 mutation. The restoration of balance between histone methylation and demethylation may facilitate current tumor therapy.

References


To the editor:

Low adhesion receptor levels on circulating platelets in patients with lymphoproliferative diseases before receiving Navitoclax (ABT-263)

Leukemia cells express high levels of Bcl-21 and BH3 mimetics that antagonize the prosurvival function of Bcl-2 and related proteins, thereby inducing apoptosis, are useful treatments for patients with chemotherapy-refractory leukemia.2 BH3 mimetics such as ABT-737 and ABT-263 also inhibit Bcl-xl and trigger acute thrombocytopenia in dogs,3 mice,4 and humans.5 In preclinical studies, they induced a rapid thrombocytopenia associated with shedding of GPV1 and GPV1 box ectodomains, platelet-specific adhesion receptors. This results in a loss of platelet adhesive function after ABT-263 treatment of human platelets in vitro or mice in vivo.6 The pretreatment platelet count and bleeding risk are important clinical parameters when considering BH3 mimetics as treatment options in refractory chronic lymphocytic leukemia (CLL).3

We evaluated platelet receptor levels in citrated platelet-rich plasma (PRP) samples from patients before and after receiving ABT-263 by flow cytometry using phycoerythrin-conjugated anti-GPIbα (AK2), anti-GPVI (1G5), anti-CDF9, or anti-αIIβ3 (CD41a) monoclonal antibodies. We compared data from 5 patients with lymphoproliferative diseases refractory to standard therapies who received ABT-263,5,7 with data obtained from 15 healthy donors or 7 patients with immunothrombocytopenia (ITP; chronic
Table 1. Surface levels of platelet receptors in healthy donors, ITP patients or patients with lymphoproliferative disorders before and during treatment with ABT-263

<table>
<thead>
<tr>
<th>Lymphoproliferative patients</th>
<th>Platelet count</th>
<th>GPIbα geomean</th>
<th>P</th>
<th>CD9 geomean</th>
<th>P</th>
<th>GPVI geomean</th>
<th>P</th>
<th>αIbβ3 geomean</th>
<th>P</th>
<th>sGPVI ng/ml</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presample</td>
<td>15 157 ± 43</td>
<td>271 (73) CI 165-491</td>
<td>***</td>
<td>367 (188) CI 110-376</td>
<td>ns</td>
<td>90 (24) CI 66-290</td>
<td>**</td>
<td>342 (175) CI 154-220</td>
<td>ns</td>
<td>23 (9) CI 5.9-15</td>
<td>ns</td>
</tr>
<tr>
<td>+ ABT-263 Day 1</td>
<td>5 95 ± 22</td>
<td>350 (129) CI 77-421</td>
<td>**</td>
<td>329 (205) CI 75-417</td>
<td>ns</td>
<td>105 (40) CI 50-276</td>
<td>**</td>
<td>474 (271) CI 50-380</td>
<td>ns</td>
<td>28 (17) CI 10-12</td>
<td>ns</td>
</tr>
<tr>
<td>+ ABT-263 Day 7</td>
<td>5 91 ± 30</td>
<td>296 (137) CI 199-547</td>
<td>***</td>
<td>291 (191) CI 34-452</td>
<td>ns</td>
<td>96 (38) CI 59-285</td>
<td>**</td>
<td>346 (149) CI 144-218</td>
<td>ns</td>
<td>16 (8) CI 5.4-15</td>
<td>ns</td>
</tr>
<tr>
<td>ITP† patients</td>
<td>5 35 ± 13</td>
<td>366 (187) CI 8-388</td>
<td>**</td>
<td>208 (104) CI 98-486</td>
<td>**</td>
<td>101 (53) CI 70-264</td>
<td>**</td>
<td>399 (257) CI 102-282</td>
<td>ns</td>
<td>43 (10) CI 13-32</td>
<td>***</td>
</tr>
<tr>
<td>Healthy donors</td>
<td>15 457 ± 114</td>
<td>599 (166)</td>
<td>ns</td>
<td>500 (233)</td>
<td>ns</td>
<td>268 (116)</td>
<td>ns</td>
<td>309 (172)</td>
<td>ns</td>
<td>21 (10)</td>
<td></td>
</tr>
</tbody>
</table>

Citrated PRP samples were analyzed by flow cytometry in CLL patients prior to and after receipt of 100 to 150 mg ABT-263 daily. Values reported as platelet count × 10^11/μl, mean fluorescence intensity geomeans for the entire platelet population (standard deviation), and sGPVI levels in citrated plasma ng/ml (standard deviation). P values derived from a 2-tailed unpaired t test comparing data from healthy donors with each patient group; ns indicates no significant difference. **P < .01; ***P < .001; CI = 95% confidence intervals.

*One day 7 GPIbα measurement was lost.
†Seven clinically diagnosed ITP patients (6 chronic, 1 persistent) with platelet count below 50 × 10^11/μl on the day of blood collection. Two of the 7 were receiving 50 mg or 75 mg prednisolone, the others were not receiving medication for ITP; the autoantibody target was not determined.

ITP > 6 months, 2 receiving steroid therapy) to assess the impact of reduced platelet count on our findings. All subjects provided informed consent and the study was approved by Monash University and Royal Melbourne Hospital Human ethics committees in agreement with the Declaration of Helsinki 1975 (revised 1983). Flow cytometry was performed in a FACS Calibur (BD Biosciences) using a platelet gate identified using the platelet markers GPVI, αIbβ3, and GPIbα in healthy-donor PRP. Fluorescence geomeans were ascertained using CellQuest Version 3.3 software and soluble GPVI ectodomain (sGPVI) by sandwich ELISA.

Compared with healthy donors, circulating platelets from 5 patients with lymphoproliferative disorders demonstrated stable levels of αIbβ3 and tetraspanin CD9, but significantly diminished surface levels of GPIbα and GPVI (Table 1). Loss of GPVI was not due to increased metalloproteolysis,9 because plasma sGPVI was within healthy donor ranges.10 Levels of GPIbα, GPVI, and CD9 were all diminished in PRP from ITP patients and sGPVI was significantly elevated, with a different mechanism underlying the thrombocytopenia. The progressive form of leukemias such as CLL are characterized by anemia, neutropenia, and thrombocytopenia; patients with progressive CLL may also develop secondary ITP and display a higher incidence of bleeding.11 Temporal analysis of platelet receptors in PRP from patients receiving ABT-263 (100-150 mg/d for 7 days) indicated that, despite a significant drug-associated thrombocytopenia, there was no discernible ABT-263–associated loss of platelet adhesion receptors. This apparent disparity between patient and experimental systems (using non-CLL platelets with normal base levels of adhesion receptors)9 may reflect differences in plasma drug concentrations or may mean that detectable drug-induced decreases in patients are masked by low pretreatment levels of these receptors.

This is the first report demonstrating that circulating platelets in patients with lymphoproliferative disorders display very low levels of platelet adhesion receptors. It seems probable that this is because of a platelet production defect, because receptor levels did not normalize even after the platelet count improved beyond day 7 of treatment (not shown). However, we cannot rule out peripheral mechanisms or other thrombocytopenia causing low levels of platelet receptors. How lymphoproliferative disorders at different stages affect platelet production and function is not understood, but quantitative assessment of platelet quality and receptor levels are not routinely performed clinically. Diminished GPIbα and GPVI receptor levels may contribute to the increased bleeding risk observed in these patients.11

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References


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