Response

One of the conclusions of our report is that PML/RARα itself mediates RA-dependent differentiation and that its degradation by RA is not crucial for the differentiation process to occur. Naoe and Kitamura disagree.

In general, we think that there is much direct and indirect evidence to support these conclusions: (1) PML/RARα is an RA-dependent transcriptional activator; (2) PML/RARα mediates RA-induced differentiation in both APL and non-APL cells; in particular, PML/RARα restores RSensitivity in RA-resistant cell lines carrying mutations of RARα; (3) commitment to differentiation in the presence of RA occurs within the first 12 hours, when PML/RARα is still detectable; (4) inhibition of PML/RARα degradation by caspase inhibitors (another mediator of RA-induced degradation of the fusion protein) increases RA-induced differentiation in APL cells; and (5) PML/RARα re-expression restores RA-sensitivity in RA-resistant APL cells with constitutive degradation of the fusion protein.

With respect to some specific criticisms of Naoe and Kitamura: (1) we did not sequence RARα and PML/RARα transcripts in NB4.007/6 cells. Therefore, as suggested, we cannot exclude that the PML/RARα coding sequence might affect fusion protein stability. However, we think that other mechanisms are likely more important, because exogenous PML/RARα (GFP-PML/RARα) or PML/RARα from parental NB4 cells were equally degraded in NB4.007/6 cells in infection and in vitro degradation assay, respectively. (2) Immunostaining of NB4.007/6 cells showed a micropunctuated pattern, typical of PML/RARα expression. Because we did not detect PML/RARα by Western blotting, this might reflect a better efficiency of our PG-M3 anti-PML monoclonal antibody on undenatured, versus denatured, antigens.

Finally, Naoe and Kitamura mentioned the isolation of an arsenic-resistant/RA-sensitive NB4 subline. Because the signaling pathway triggered by arsenic is yet undefined, we do not think that this model system can provide information as to the role of PML/RARα in RA-signaling.

Mirco Fanelli
Pier Giuseppe Pelicci
Department of Experimental Oncology
European Institute of Oncology, Milan, Italy

Successful Healing of Hydroxyurea-Related Leg Ulcers With Topical Granulocyte-Macrophage Colony-Stimulating Factor

To the Editor:

Hydroxyurea (HU) is a cytoreductive agent commonly used in the treatment of chronic myeloproliferative disorders. HU is usually well tolerated; however, long-term HU therapy has been associated with cutaneous side effects, such as alopecia, diffuse hyperpigmentation, erythema, skin atrophy, and nail changes. Painful skin ulcers have been also reported and their treatment modalities mainly consisted of HU discontinuation, which was generally followed by the complete or almost complete healing. We report here a successful treatment of HU-related leg ulcers with topical granulocyte-macrophage colony-stimulating factor (GM-CSF) in 4 patients affected by chronic myeloid leukemia (CML), whose clinical features are shown in Table 1. Diagnosis of CML was confirmed by both cytogenetic (Ph chromosome) and molecular (bcr-abl) findings. All of the patients, after a preliminary cytoreduction of peripheral leukemic burden with HU, were assigned to receive conventional therapy with α-interferon (α-INF), and 3 of them were also enrolled in a clinical trial of the Italian Cooperative Study Group on CML. However, because of adverse side effects caused by α-INF, we first had to reduce and then to stop it in all patients. They were then reverted to chemotherapy with HU, which was administered at a mean daily dosage of 2 g to maintain complete hematological response. After a mean time of 29.7 months (range, 19 to

Table 1. Patients’ Clinical and Laboratory Characteristics at Skin Ulcers’ Onset

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age/Sex</th>
<th>Time From Diagnosis (mo)</th>
<th>HU Therapy Duration (mo)</th>
<th>WBC (10⁹/L)</th>
<th>PLT (10⁹/L)</th>
<th>LDH (U/L)</th>
<th>Spleno-megaly</th>
<th>Hepato-megaly</th>
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<tr>
<td>1</td>
<td>68/M</td>
<td>54</td>
<td>27</td>
<td>73.5</td>
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<td>Yes</td>
<td></td>
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<td>2</td>
<td>62/M</td>
<td>75</td>
<td>36</td>
<td>29.8</td>
<td>264</td>
<td>184</td>
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<td>No</td>
</tr>
<tr>
<td>3</td>
<td>65/F</td>
<td>54</td>
<td>37</td>
<td>26.5</td>
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<td>968</td>
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<td>Yes</td>
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<tr>
<td>4</td>
<td>44/F</td>
<td>50</td>
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<td>40.0</td>
<td>312</td>
<td>1,286</td>
<td>Yes</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Abbreviations: WBC, white blood cell count; PLT, platelet count; LDH, lactate dehydrogenase.

REFERENCES

37 months), we noticed the appearance of painful perimalleolar skin ulcers. Ulcers appeared with an erythematous border, whereas the epidermidis was replaced by a fibrinous exudate and the dermis scattered by necrotic areas. Skin biopsies of the ulcerated lesions showed in all cases an histologic picture compatible with small vessel vasculitis. Circulating immune complexes were not detectable and doppler-fluximetry was always found in normal range.

Because GM-CSF has been reported to be effective in either preventing and reducing drug-induced mucositis or decreasing the healing period in cut and burn wounds, we tried a topical GM-CSF treatment after obtaining patients’ informed consent. Briefly, GM-CSF (Mielogen 150; Schering Plough, Milan, Italy) was diluted in sterile water for injection up to 5 μg/mL, and every 1 mL was dispensed in a sterile syringe and stored at 4°C until a maximum of 15 days. Skin ulcers were then douché twice a day, dried up to 20 minutes, and dressed. All patients received GM-CSF treatment for 2 weeks, with the exception of 1 patient (no. 2) who needed 2 further weeks of treatment. Topical GM-CSF was able to heal the cutaneous lesions and none of the patients required discontinuation of HU therapy.

It is noteworthy that 1 patient (no. 1) also experienced local treatment with granulocyte colony-stimulating factor (G-CSF) without any lesion improvement, whereas topical GM-CSF administration healed it. However, 2 months later, this patient entered CML blastic transformation and developed a second skin malleolar ulcer that this time showed only a partial response to an additional GM-CSF treatment.

The appearance of HU-related skin ulcers represents a serious clinical problem for CML patients in long-term continuous treatment. In the past, we have observed several CML patients with HU-related cutaneous lesions who have been unsuccessfully treated with a variety of approaches such as topical antibiotics, subcutaneous calcium-heparin injections, and hemorheologic drugs. Furthermore, the incidence of HU-related cutaneous lesions was not lowered by reducing the schedule of HU administration, suggesting a close correlation with HU cumulative dose. Long-term cytotherapeutic therapy could play a pivotal role in affecting endothelial cells’ function, therefore leading to vascular sufficiency and tissue-organ damage. In this context, we have also found abnormally high vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecule-1 (ICAM-1) serum levels in these 4 CML patients.

The mechanisms through which GM-CSF acts are not yet clarified. It might be hypothesized that its promoting activity on both recruitment and proliferation of monocytes and macrophages could modulate the in situ production of cytokines such as interferin-1, tumor necrosis factor, and macrophage colony-stimulating factor, which, in turn, can affect the healing process.

Therefore, in the search for alternative remedies for HU-related skin ulcers, our preliminary experience would suggest that topical GM-CSF therapy may be helpful in the management of these common skin lesions when discontinuation of HU treatment is not advisable.

REFERENCES


A Single-Base Deletion in the Thrombopoietin (TPO) Gene Causes Familial Essential Thrombocythemia Through a Mechanism of More Efficient Translation of TPO mRNA

To the Editor:

Deletion of a single G nucleotide (ΔG) in exon 3 of the thrombopoietin (TPO) gene was found to cosegregate with thrombocythosis and elevated TPO serum levels in a Japanese kindred with thrombocythemia. The ΔG mutation affects the 5′-untranslated region (5′-UTR) of the TPO mRNA. Cells transfected with a cDNA carrying this mutation produced more TPO protein than controls transfected with the normal TPO cDNA, strongly suggesting that the ΔG mutation is responsible for thrombocythemia in this family. However, the molecular mechanism of how this mutation causes TPO overproduction was not elucidated, and in particular, no evidence for increased RNA stability or more efficient translation of the mutant TPO mRNA was found.

We have previously shown that the 5′-UTR of TPO mRNA exerts a strong inhibitory effect on translation. This inhibition is mediated by the presence of several upstream open reading frames (uORFs) that prevent efficient translation of TPO. Loss of this inhibition due to a splice donor mutation in TPO intron 3 was shown to be the cause of hereditary thrombocythemia (HT) in a Dutch family. We noticed that the ΔG mutation described by Kondo et al results in a frameshift in the TPO 5′-UTR changing the composition of the inhibitory uORFs (Fig 1A). Only transcripts originating from the TPO promoter 2 (P2), which account for greater than 90% of total TPO mRNAs, are shown. The frameshift specifically affects uORF7, which consequently is placed into the same reading frame as the TPO protein, extending the N-terminus of the TPO signal peptide by 23 amino acids (Fig 1A).

Because uORF7 was previously shown to have a very strong inhibitory effect on translation, we reasoned that this frameshift should relieve the physiologic block on translation and thereby might cause TPO overpro-
Successful Healing of Hydroxyurea-Related Leg Ulcers With Topical Granulocyte-Macrophage Colony-Stimulating Factor

Fabio Stagno, Patrizia Guglielmo, Ugo Consoli, Paolo Fiumara, Mario Russo and Rosario Giustolisi