expressed, thus establishing Bcl-2-ASs as a critical target for AS strategies in AML.

While Drs Moreb and Zucali agree with these findings, they find the data on one of the antiapoptotic proteins, A1, "less convincing." The Bcl-2 family member A1/Bfl-1 was cloned by Choi et al.\textsuperscript{2} and by Karsan et al.\textsuperscript{3} It was found to be expressed in bone marrow cells and is induced by cytokines and differentiation inducers in leukemic cells.\textsuperscript{4}

Drs Moreb and Zucali state that they "used mainly Northern blot analysis because of the unavailability of a human A1 antibody." The A1 antibody used in our study was kindly provided by Dr John C. Reed. It generated immunoblots of better quality than those obtained with the polyclonal antibody marketed by Santa Cruz. But a paper has recently been published utilizing the Santa Cruz antibody successfully.\textsuperscript{3} We here provide our own method of A1 detection:

Cells were washed twice with phosphate buffered saline (PBS) buffer and lysed at 4 × 10^6 cells/μL in cell lysis buffer (20 mM HEPES, pH 7.4, 0.25% NP-40 containing protease inhibitor cocktail; Boehringer Mannheim, Indianapolis, IN) for 10 minutes on ice. Lysates were spun at 25 000g for 10 minutes, and an equal amount of 2 × gel-loading buffer (0.25 M Tris-HCl, 2% SDS, 4% β-mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) was added to the supernatants. Equal amounts of lysate (equivalent to 5 × 10^6 cells) were subjected to sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) onto 12% polyacrylamide gels. Afterward, proteins were transferred to Hybond-P membranes (Amersham Pharmacia Biotech, Buckinghamshire, England). The membranes were blocked overnight at 4°C with 7% milk in PBS buffer containing 0.3% Tween-20, and an equal amount of 2 × gel-loading buffer (0.25 M Tris-HCl, 2% SDS, 4% β-mercaptoethanol, 10% glycerol, 0.02% bromophenol blue) was added to the supernatants. Equal amounts of lysate (equivalent to 5 × 10^6 cells) were subjected to sodium-dodecyl-sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) onto 12% polyacrylamide gels. Afterward, proteins were transferred to Hybond-P membranes (Amersham Pharmacia Biotech, Buckinghamshire, England). The membranes were blocked overnight at 4°C with 7% milk in PBS buffer containing 0.3% Tween-20, followed by incubation with polyclonal antibody against A1 (1:1000 dilution) (kindly provided by Dr J. C. Reed) for 1 hour at room temperature. Membranes were washed 3 times with PBS buffer containing 0.3% Tween-20, probed with a horseradish peroxidase (HRP)-conjugated secondary antibody and then reacted with enhanced chemiluminescence (ECL) reagent (Amersham Pharmacia Biotech). Signals were detected by a phosphorimager (Storm 860 Version 4.0; Molecular Dynamics, Sunnyvale, CA).

Using this method, the A1 bands are clearly discernible at the correct location, and these results are supported by the detection of A1 mRNA by reverse-transcriptase polymerase chain reaction (RT-PCR) in primary AML samples (Figure 1). Oligonucleotide primers (F, forward; R, reverse) used for expression analysis by RT-PCR were as follows: human A1- F 5'-CGGCA TCA TTAACT-3' and R 5'-GA TCTTTCCTGTAACTTCTAG-3'; the expected PCR product is 250 base pairs (bp). Other pro- and antiapoptotic genes are also expressed. In a series of 135 AML analyzed for expression of A1 by semiquantitative RT-PCR, no correlation with white blood cell count or bone marrow blast count was observed.

In conclusion, we have demonstrated the presence of the antiapoptosis protein A1 in primary AML samples by RT-PCR and immunoblot analyses. We agree with Drs Moreb and Zucali that the nature of the interaction between Bcl-2 and other antiapoptotic proteins remains unknown.

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University of Texas M. D. Anderson Cancer Center
Houston, TX

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To the editor:

Ribosomal proteins S3a, S13, S16, and S24 are not mutated in patients with Diamond-Blackfan anemia

Considerable effort has been invested in identification of a gene responsible for Diamond-Blackfan anemia (DBA). The disorder is characterized by red cell hypoplasia presenting in early infancy and is accompanied in about 40% of the patients by various physical
anomalies (for review see Willig et al.). Ribosomal protein S19 (RPS19) was identified as a candidate gene for DBA and mutations in this gene have been described in 25% of DBA patients. However, the mechanism by which mutations in RPS19 can lead to DBA remains unclear.

RPS19 is a structural component of a small ribosomal subunit and is known to have two other functions. First, RPS19 homodimers are released by apoptotic cells and act as a chemotactic factor for monocytes during macrophage-dependent apoptotic cell clearance. Second, RPS19, together with ribosomal proteins S3a, S13, S16, and S24, participates in the binding of euerycaryotic initiation factor 2 (eIF-2) to ribosomes. eIF-2 plays a central role in the initiation of translation, and its function is controlled in an erythroid-specific manner by heme-regulated kinase. To investigate the possibility that DBA phenotype might result from mutations in ribosomal proteins involved in eIF-2 binding, we sequenced cDNAs for RPS3a, S13, S16, and S24 in 14 patients from the Czech National DBA Registry. Five of these patients have been previously shown to carry a mutation in the RPS19 gene.

After obtaining informed consent, total RNA was isolated from peripheral blood mononuclear cells, and reverse transcription was performed using an oligo(dT) primer. Primers specific for full-length coding regions of RPS3a, S13, S16, and S24 were used for PCR amplification. PCR products were sequenced on an automated genetic analyzer, and resulting sequences were evaluated for the presence of mutations.

In all DBA patients tested, no mutations in RPS3a, S13, S16, and S24 were found on the cDNA level. We therefore conclude that these four of the five ribosomal proteins important for eIF-2 binding to ribosomes are not involved in DBA pathogenesis.

To the editor:

Increased frequency of HLA-DRB1*1302 haplotype in patients with nonimmune chronic idiopathic neutropenia of adults

Nonimmune chronic idiopathic neutropenia of adults (NI-CINA) is a frequently seen granulocytic disorder characterized by the “unexplained” persistent decrease of the number of circulating neutrophils below the lower limit of the normal distribution in a given ethnic population. The diagnostic criteria allowing the identification of the condition among other types of chronic neutropenia are presented elsewhere. The cause of the disorder and the underlying mechanisms leading to neutropenia in the affected subjects are unknown, but recent studies in our laboratory provided strong evidence for the existence of an unrecognized low-grade chronic inflammatory process in these patients, which may be involved in the pathogenesis of NI-CINA by increasing the production of a variety of proinflammatory cytokines and chemokines and therefore affecting both neutrophil production in bone marrow and neutrophil extravasation in the periphery. Here, we describe a predisposition of HLA-DRB1*1302 haplotype—carrying individuals to develop NI-CINA.

The study was carried out on 56 NI-CINA patients and 39 healthy volunteers, all residents of the island of Crete. Venous blood was collected into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) as anticoagulant and used as a DNA source. DNA extraction was carried out by salting-out technique. For the typing of HLA alleles, polymerase chain reaction (PCR) was utilized. HLA-A, -B, and -C alleles were typed using PCR-sequence specific primers (PCR-SSP) with primer sets provided by PefFreez Clinical systems (Brown Deer, WI). HLA-DRB1 alleles were typed using the ELPHA high resolution hybridization system provided by Biostep AG (Dreieich, Germany). HLA-DQB1 and DPB1 alleles were typed using the

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<table>
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<tr>
<th>Haplotype</th>
<th>Patients (%)</th>
<th>Healthy controls (%)</th>
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</table>

†Yates continuity-corrected chi-square test. Statistically significant at P < .05. ns, nonsignificant difference. ‡Proportions of haplotype-carrying subjects are in parentheses.
Ribosomal proteins S3a, S13, S16, and S24 are not mutated in patients with Diamond-Blackfan anemia

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