it is impossible to locate in Table 7 the 4 of 7 boys who evolved to leukemia (patients 2, 3, 9, and presumably 1 or 8).

In conclusion, the proper use of the ISCN by the authors would help to avoid this confusion.

Juan Ramon Gonzalez Garcia and Juan Pablo Meza-Espinoza

The authors declare no competing financial interests.

Correspondence: Juan Ramon Gonzalez Garcia, Division de Genetica CIBO-IMSS, Sierra Mojada # 800, colonia Independencia, Guadalajara, Jalisco, Mexico; e-mail: jrgg_gene@hotmail.com.

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Response:

Use of the ISCN

Drs Garcia and Meza-Espinoza make an excellent point about the usefulness of the International System for Human Cytogenetic Nomenclature (ISCN 2005) in preventing confusion in reporting research cytogenetic results. The cytogenetic data reported in our study[1] were derived from multiple institutional clinical reports and were reported as submitted by the participating Children's Oncology Group (COG) institutions. All of the study patients were enrolled between 1994 and 1999 and thus predated the ISCN 2005 system. Obtaining cytogenetics on the leukemia cells was encouraged but was not a study requirement. Thus, there was no central reference laboratory that reviewed all of these studies. In analyzing the data, however, it appeared that the presence of cytogenetic abnormalities in addition to trisomy 21 was a potential risk factor for recurrent disease, and therefore this important finding was reported in our results.

We concur that in Table 7 for patient 1, the sex chromosomes should have been reported as "XX," and we apologize for and regret this typographic error.[1] Similarly, as exemplified by Hu et al,[2] the correct nomenclature for Table 7 patient 6 would be "+21c."

As for the total number of patients represented in Table 6, the number is indeed 42 and not 43. The one male mosaic is included in 2 columns ("Mosaic" and "Other"). All 7 karyotypes are represented in the footnote of the same table. There were 2 patients with trisomy 11 (as indicated by the 2 in parentheses), 1 mosaic, and 4 additional abnormal karyotypes. In Table 7, the 4 of 7 boys who evolved to leukemia are patients 2, 3, 6, and 9. The other 3 males had no consistent cytogenetic abnormality associated with their transient leukemia other than trisomy 21.[1]

We acknowledge that the results we reported need to be reproduced and verified by additional multi-institutional studies. For consistency and scientific accuracy, these studies should include more rigorous review of cytogenetics (through either a central review or, at the minimum, review through COG-certified cytogenetic laboratories) and reported using the ISCN 2005 nomenclature. Such a follow-up study is already ongoing in COG. Despite the nonstandardized nomenclature, we still feel that our data indicate that the presence of abnormal clonal cytogenetics in addition to trisomy 21 in neonates with transient leukemia is a risk factor for development of subsequent leukemia, and thus that these children require closer follow-up and possibly earlier intervention.

Gita Vasers Massey, Howard J. Weinstein, and Alvin Zipursky

The authors declare no competing financial interests.

Correspondence: Gita Vasers Massey, Pediatric Hematology-Oncology, PO Box 980121, Richmond, VA 23298-0121; e-mail: gvmassey@hsc.vcu.edu.

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To the editor:

PASD1 is a potential multiple myeloma–associated antigen

Immunotherapy is an important treatment option in multiple myeloma (MM), with allotransplantation demonstrating an inducible graft-versus-myeloma effect.[1] This could be potentiated by vaccination, first requiring knowledge of tumor-associated antigens.

The cancer testis antigens (CTAs) are exemplary, as normal expression is restricted to testis, an immune-privileged site. CTAs are being identified in a number of malignancies. In lymphoma, serologic analysis of recombinant cDNA expression (SEREX) analysis has revealed *PASD1,* a gene encoding the CT antigen OX-TES-1, spliced alternatively to *PASD1_v1* and *PASD1_v2*.[2] *PASD1* appears to map to chromosome band Xq28 (Unigene Hs.160594), also flanked by the cluster of *MAGE* gene families.[3] With *MAGE* antigens of importance as immunotherapeutic targets in MM, additional CTA genes at this locus may be significant. Here, we report on *PASD1* expression in MM as one such antigen.

We observed *PASD1* expression in 5 of 11 MM cell lines by reverse-transcription–polymerase chain reaction (RT-PCR), and in 8 of 11 by increasing sensitivity (Figure 1A). In primary MM, *PASD1* expression was found in 14 of 16 samples using quantitative PCR (Q-PCR), both in presentation (MM1-9) and pretreated cases (MM10-16) (Figure 1B). Protein expression was tracked immunohistochemically, using a novel monoclonal antibody

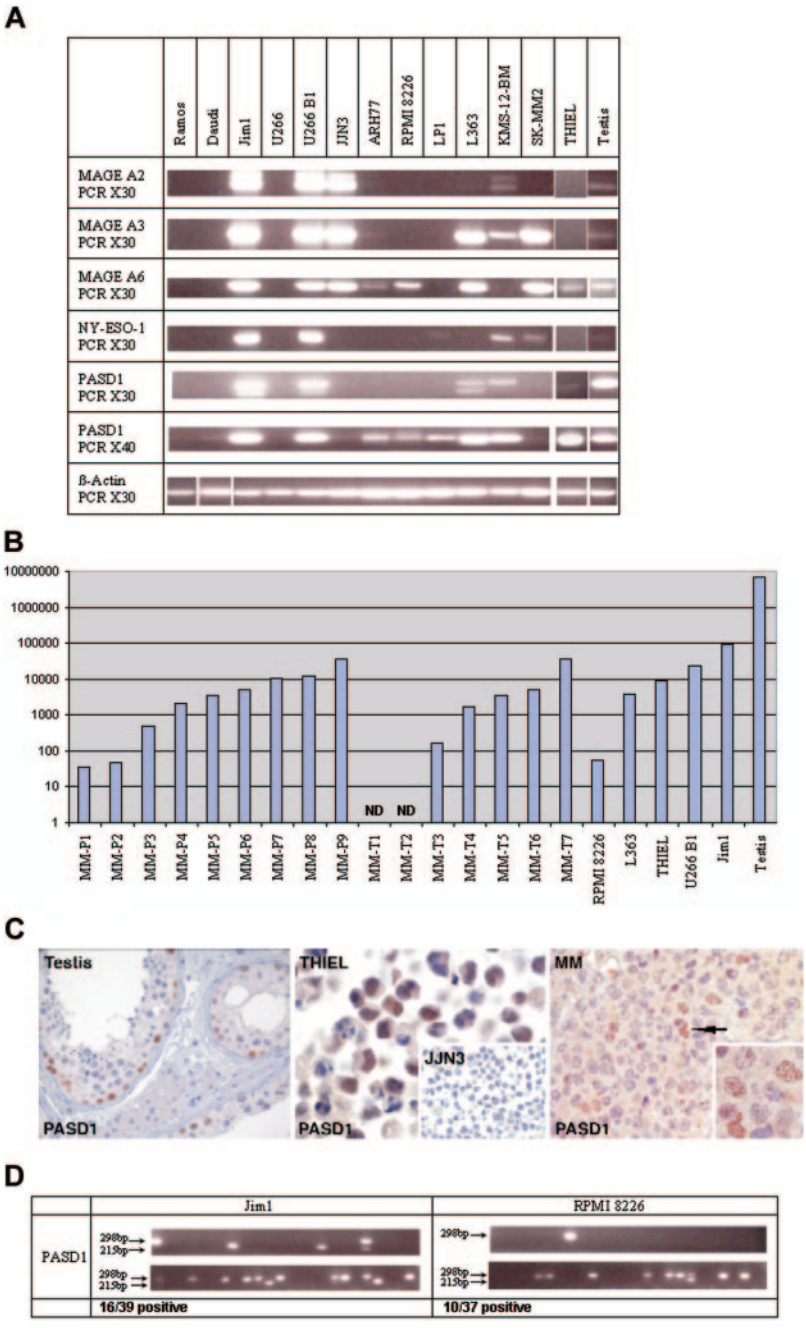

Figure 1. Analysis of CTA expression by RT-PCR, Q-PCR, and antibody staining in bulk populations and in single MM cells. Primer design for all CTAs examined spanned intronic DNA (available on request). Expression of *PASD1* was examined by RT-PCR for 30 cycles and compared with known CTAs in MM using identical amounts of cDNA input from RNA (2 μg), reverse transcribed with oligo-dT (A). *PASD1* expression was further examined after 40 cycles. Several normal lymphoid tissues were also analyzed, including specific lymphocyte populations not probed previously. In purified B cells, in vitro–generated plasmablasts, plasma cells, T cells, and spleen and bone marrow mononuclear cells (MNCs), we failed to detect *PASD1* expression either by high amplification cycle numbers or by Q-PCR (data not shown). Two Burkitt lymphoma cell lines (Ramos, Daudi) were used as negative controls (A). Q-PCR used Taqman primers (PASD1: Hs00542865_m1; β-actin: Hs99999903_m1; Applied Biosystems, Warrington, United Kingdom). Relative expression was calculated against reference sample (Ramos) as $2^{-[DCt(Sample) - DCt(Reference)]}$, where DCt indicates Ct(PASD1) − Ct(β-actin). Presentation diagnostic samples were MM-(P1-9), pretreated MM-(T10-16); ND indicates not detected (B). Immunoperoxidase labeling showing PASD1 protein expression in testis and in the nuclei of THIEL cells, with JJN3 cells negative (inset), and in the nuclei (arrowed) of tumor cells in 1 of the 2 cases of MM found to be positive (C; RPMI8226 and second MM case staining not shown). Single cells obtained by flow cytometry deposition of 2 MM cell lines expressing high (Jim1) or low (RPMI 8226) levels were analyzed by nested RT-PCR for *PASD1* expression, and for each line, upper and lower panels correspond to cell numbers 1 to 24 and 25 to 48, respectively (D). Both splice variants were detected in the 2 lines (± exon 8), yielding 298- and 215-bp amplification products. Each amplification product from bulk populations or single tumor cells was eluted and identity confirmed by DNA sequence analysis. Images were obtained using a Zeiss Axioskop microscope (Zeiss, Welwyn Garden City, United Kingdom) with nonimmersion 10×/0.25 NA (testis, JNN, and MM) and 40×/0.65 (THIEL and MM) Acroplan objectives. Image acquisition was performed using a Micropublisher 5 camera (Q-Imaging, Wokingham, United Kingdom) and Adobe Photoshop 8.0 software (Adobe Systems, San Jose, CA). All subsequent processing was performed using Adobe Photoshop 8.0.

2ALCC128, recognizing a unique C-terminal epitope in PASD1_v2.[4] The MM cell lines THIEL and RPMI8226 expressed both mRNA and protein for PASD1, whereas JJN3 lacked both (Figure 1A,C). Protein expression was also confirmed in 2 of 4 primary MM tumor samples examined (Figure 1C).[5]

To examine intraclonal *PASD1* expression, single cells were examined in 2 cell lines. *PASD1* transcripts were found in 27% to 40% of cells, a significant fraction, but not all cells (Figure 1D). Parallel findings were confirmed in 2 primary MM samples analyzed at the single-cell level (data not shown). Antibody staining in THIEL, however, indicated a more prevalent expression (Figure 1C), and together with staining in MM cases (Figure 1C) revealed variability between tumors.

PASD1 expression was compared with known MM-associated CTAs. *MAGE-A6* (8/11) was the most frequently expressed CTA in cell lines (Figure 1A). *PASD1* expression (5/11) was comparable in frequency with *MAGE-A2* (4/10) and *NY-ESO-1* (4/10) among cell lines, using 30 cycles. The levels of *PASD1* detected by RT-PCR in the 5 cell lines (Jim1, U266B1, L363, KMS-12-BM, THIEL; Figure 1A) appeared to parallel known CTA expression in MM, at this level of amplification. In 4 (25%) of 16 primary tumors (3/4 at presentation, 1/4 partial progression), *PASD1* levels by Q-PCR were comparable with the highest levels seen in THIEL, U266B1, and Jim1 (Figure 1A-B), suggesting that PASD1 levels in many primary tumors will be therapeutically relevant. However, actual levels of specific CTAs overall vary and are differentially expressed within individual tumors. This suggests a specific induction of genes that may be relevant to tumor survival. The observed intraclonal variation in *PASD1* expression paralleled known features of MAGE-A3/6 and NY-ESO-1 expression in MM.[6,7] NY-ESO-1 positivity was reported in less than 25% of tumor cells,[7] lower than the fraction observed here for *PASD1* transcripts.

These data indicate that *PASD1* is an MM-associated gene, which could be exploited as an antigen for targeted therapy.

Surinder S. Sahota, C. Madusha Goonewardena, Christopher D. O. Cooper, Amanda P. Liggins, Kamal Ait-Tahar, Niklas Zojer, Freda K. Stevenson, Alison H. Banham, and Karen Pulford

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S.S.S., K.P., and A.H.B. designed research, analyzed data, and wrote the paper; C.M.G., C.D.O.C., A.P.L., and K.A.-T. carried out research and analyzed data; N.Z. performed clinical evaluation of patients, carried out research, and analyzed data; F.K.S. analyzed data and wrote the paper. A.H.B. and K.P. contributed equally to this work.

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Correspondence: Surinder S. Sahota, Genetic Vaccine Group, Tenovus Laboratory, Cancer Sciences Division, School of Medicine, Southampton University Hospitals, Tremona Road, Southampton SO16 6YD, United Kingdom; e-mail: s.s.sahota@soton.ac.uk.

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