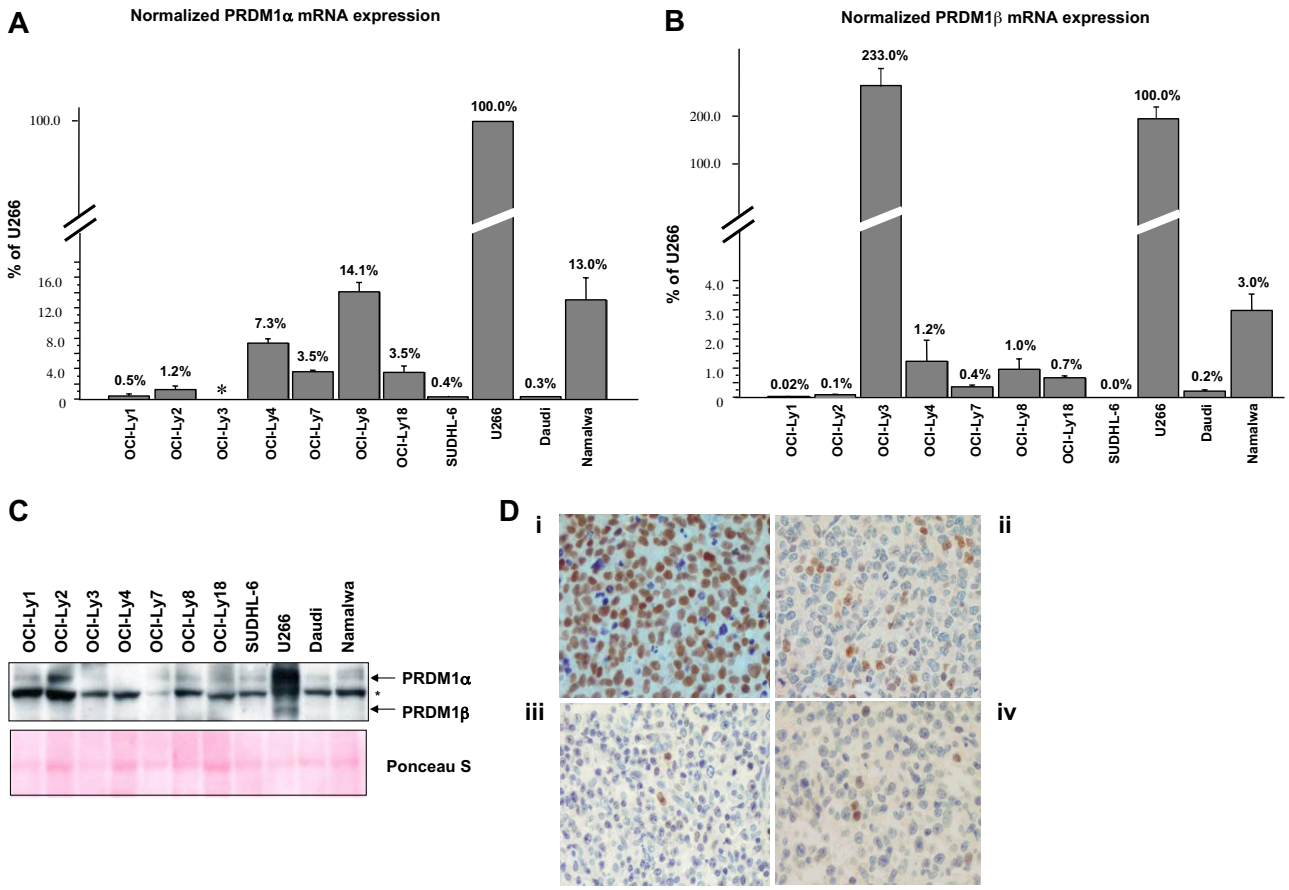


**Figure 1. Low levels of *PRDM1β* expression in B lymphoma cell lines.** (A,B) PRDM1α and PRDM1β mRNA in DLBCL cell lines (OCI-Ly1, OCI-Ly2, OCI-Ly3, OCI-Ly4, OCI-Ly7, OCI-Ly8, OCI-Ly18 and SUDHL-6) and Burkitt lymphoma cell lines (Daudi and Namalwa) were quantified by Taqman reverse-transcriptase-polymerase chain reaction, normalized with beta-glucuronidase, and expressed as a percentage relative to U266. For *PRDM1α* mRNA quantification: forward primer, TCCAGCACTGTGAGGTTTCA; reverse primer, TCAAACTCAGCCTCTGTCCA; probe, ATGGACATGGAGGATGCGGATATG. For PRDM1β mRNA quantification: forward primer, CCCGAACATGAAAAGACGAT; reverse primer, ATAGCGCATCCAGTTGCTTT; probe, TCCAGAGGGGAGCTTCACCACTTC. In OCI-Ly3, *PRDM1α* mRNA is not detectable by the primers shown above because of a chromosomal inversion breakpoint at intron 2 of the *PRDM1* gene. Error bars indicate SE. (C) Western blotting of total protein extracts using the ROS monoclonal anti-PRDM1 antibody. The positions for PRDM1α and PRDM1β are indicated. Asterisk marks the nonspecific band. Ponceau S staining of the membrane is shown for protein loading control. (D) Examples of immunoperoxidase staining on paraffin tissue sections for PRDM1 in U266 cells (i) and representative DLBCL cases (ii-iv). U266 cells show uniform strong staining, while all or most of the tumor cells in DLBCL are negative for or weakly express PRDM1. A few scattered PRDM1$^+$ cells serve as internal controls for the DLBCL cases. The DLBCL cases shown have the immunohistochemical profile of non-GCB-type DLBCL. Micrographs were acquired with a Nikon Microphot SA microscope (Nikon Instruments, Melville, NY) and a SPOT Insight Color Mosaic QE 4.2 camera and image acquisition software system (Diagnostic Instruments, Sterling Heights, MI).

marker in DLBCL needs to be interpreted with caution and requires further investigation.

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# Response

## Multiple role of PRDM1β involvement in diffuse large B-cell lymphoma

We would first like to underline that our study did not focus on the assessment of PRDM1β expression as a prognostic marker in diffuse large B-cell lymphomas (DLBCL), but on its significance on the therapeutic response. Tam et al, using quantitative real time–polymerase chain reaction (RT-PCR), found a highly increased *PRDM1* expression in U266, which is consistent with our results revealed by a semiquantitative method using the forward and reverse primer specifically located on exon 1β and exon 4 of

the *PRDM1* gene.[1] Meanwhile, we found that *PRDM1* varies according to the type of B-lymphoma cells, some of them expressing *PRDM1* at a lower level. This is the important reason why we further performed laser microdissection-based techniques to select only the lymphoma cells for the molecular studies.

At the protein level, using the monoclonal anti-PRDM1 antibody (clone ROS), Tam et al detected a negative or weak PRDM1 expression in DLBCLs. However, Garcia et al[2] had previously published their immunohistochemical results using the same antibody, showing that PRDM1 is expressed on the DLBCL lymphoma cells. Therefore, we carried out a cooperative project with Dr Garcia's group, and observed a strong expression of PRDM1 in DLBCL patient samples. A comparison of the results from Dr Garcia group with our results would imply that the same antibody and the same method should be used in the 2 series. If the discrepancy persists, a further study, sequencing the protein involved, would represent an interesting investigation.

By Western blot, as we mentioned in our article, PRDM1β protein was identified as a fragment of approximately 70 kDa in B-lymphoma cell lines and in the DLBCL patient samples we studied. The 80-kDa PRDM1β protein described by Tam et al was identified in the myeloma cell line U266. We believe that DLBCL is a malignant hematologic disease distinct from myeloma, thus protein translation disturbance and/or modification might be involved in DLBCL. We fully agree with Tam et al that "differences in identification and interpretation of the PRDM1β signal in Western blots" could happen, but we would not consider it as a "nonspecific" band, because (1) it is not detected in normal human tonsil, which is known to have no expression of PRDM1β; (2) it can be down-regulated in lymphoma cells through rituximab alone or rituximab combined with doxorubicin; and (3) it varies according to the DLBCL samples.

Moreover, Tam et al also demonstrated in their previous study that inactivating mutations of *PRDM1* occurred in 8 of 35 DLBCL patients.[3] In our series of 82 patients, despite repeated tests of sequence analysis by our experienced group,[4,5] we did not find these mutations. To interpret this discrepancy, we first have to determine if the methods of analysis were identical. If the methods were identical, we could propose an exchange of the biologic material, to repeat the sequence analyses in the 2 series. When this double technical control is achieved, if the difference persists, we should take into account the different biologic characteristics between Asian and Western populations, possibly due to genetic/environmental background. Indeed, it has recently been reported that the occurrence of the non-GCB subtype of DLBCL was significantly higher in Asian than in Western countries, as defined by immunostainings on paraffin sections using antibodies against CD10, BCL-6, and IRF4.[6,7] Therefore, instead of a single exclusive factor, we propose that inactivating mutations of *PRDM1* indicating a tumor-suppressor role and abnormal expression of functionally impaired *PRDM1β* isoform could both be involved, as well as other members of the *PRDM* gene family.

Indeed, this scientific exchange is interesting and we are open for active cooperation to investigate PRDM1 expression in DLBCL.

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*Contribution: W.-L.Z., A.J., and S.-J.C., wrote the paper.*

*Conflict-of-interest disclosure: The authors declare no competing financial interests.*

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# To the editor:

## Is early, deep free light chain response really an adverse prognostic factor?

In a cohort of 303 patients, van Rhee et al made the important observations that baseline free light chain (FLC) and early FLC reduction after 1 to 3 cycles of highly effective chemotherapy are prognostic for both overall and event-free survival.[1] First they demonstrated that patients with the highest tercile of FLC levels at baseline (in their case >75 mg/dL) had the worst overall survival, independent of high LDH and of abnormal cytogenetics. Their second observation was that patients with the deepest FLC response after 1 to 3 cycles of VDT-PACE [bortezomib, dexamethasone, thalidomide, cisplatin, doxorubicin, cyclophosphamide, and etoposide] had the worst outcomes. Immediately preceding cycle 2 of therapy, the FLC reduction terciles were less than 58%, 58% to less than 86%, and 86% to 100%, with respective 24-month estimated survival rates of 90%, 91%, and 81%. After approximately 2 to 3 cycles, the FLC reduction terciles were less than 75%, 75 to less than 96%, and 96 to 100% with respective 24-month estimated survival rates of 91%, 93%, and 79%. The negative impact of extreme drops in involved FLC on event free survival and overall survival was independent of high LDH and abnormal cytogenetics, but the authors provide no information about whether it was independent of baseline FLC. One-third of patients had baseline FLC of less than 10.7 mg/dL (lowest tercile). Given the fact that the upper limits of normal for κ and λ FLC are 1.93 and 2.64 mg/dL, respectively, a high fraction of patients in this low baseline FLC tercile group were not eligible to have a FLC reduction of 86%