

change was verified by sequencing from both the sense and antisense directions. The individual initially identified with the mutation was the mother of the proband. The husband and 8 children were then analyzed. Of the children, 4 were found to have the polymorphism (Figure 1B). One was normal at the *HFE* locus for the Cys282Tyr mutation, 2 were heterozygous, and 1 (the brother of the proband) was also homozygous for Cys282Tyr. The proband did not have the Arg455Gln mutation. Interestingly, this brother was identified in cohort A as having evidence of liver fibrosis, where his brother (the proband) did not.

In addition, analysis of exon 18 of *TFR2* showed an identically shifted band (Figure 1C) in 5 samples (1 from group A, 1 from group B, 2 from group D, and 1 in the normal controls). Direct sequencing of these samples showed a change of G>C in the 3' untranslated region (3' UTR) of exon 18. Since this change also was present in the normal control sample, we believe that it represents a previously unreported polymorphism. No correlation was found between this alteration and any of the clinical subtypes.

In summary, a group of individuals selected for unusual iron phenotypes was analyzed for evidence of mutation in the *TFR2* gene. None had mutations corresponding to those described in the Italian iron overload pedigrees. We describe a new mutation, Arg455Gln, in exon 10 of *TFR2* in a pedigree containing an individual with evidence of liver fibrosis in contrast to his *HFE* identical brother. This mutation could represent a modifier for penetrance of the hemochromatosis phenotype when present with homozygosity for Cys282Tyr. Unlike *TFR1* expression, *TFR2* expression is not down-regulated in the liver of iron-loaded mice.<sup>8</sup> Our screening for mutations in all 18 exons of *TFR2* in genomic

DNA from all of the individuals indicates that mutations of the *TFR2* gene are rare. The polymorphism in the 3' UTR that we detected was found in individuals with abnormal iron metabolism as well as in a healthy control. Further cohort studies are needed to determine if this polymorphism is associated with a subtype of hereditary hemochromatosis.

**Wolf-K. Hofmann, Xiang-Jun Tong, Richard S. Ajioka, James P. Kushner, H. Phillip Koefler**

Correspondence: Wolf-K. Hofmann, Division of Hematology/Oncology, Cedars Sinai Research Institute, UCLA School of Medicine, Los Angeles, CA 90048; e-mail: w.k.hofmann@em.uni-frankfurt.de

## References

1. Feder JN, Gnirke A, Thomas W, et al. A novel MHC class I-like gene is mutated in patients with hereditary haemochromatosis. *Nat Genet.* 1996;13:399-408.
2. Kawabata H, Yang R, Hirama T, et al. Molecular cloning of transferrin receptor 2: a new member of the transferrin receptor-like family. *J Biol Chem.* 1999;274:20826-20832.
3. Camaschella C, Roetto A, Cali A, et al. The gene *TFR2* is mutated in a new type of haemochromatosis mapping to 7q22. *Nat Genet.* 2000;25:14-15.
4. De Gobbi M, Barilaro MR, Garozzo G, Sbaiz L, Alberti F, Camaschella C. *TFR2* Y250X mutation in Italy. *Br J Haematol.* 2001;114:243-244.
5. Roetto A, Totaro A, Piperno A, et al. New mutations inactivating transferrin receptor 2 in hemochromatosis type 3. *Blood.* 2001;97:2555-2560.
6. Girelli D, Bozzini C, Roetto A, et al. A new mutation in transferrin receptor 2 gene in hemochromatosis type 3 [abstract]. *Blood.* 2001;98:4a.
7. Hofmann WK, Miller CW, Tsukasaki K, et al. Mutation analysis of the DNA-damage checkpoint gene *CHK2* in myelodysplastic syndromes and acute myeloid leukemias. *Leuk Res.* 2001;25:333-338.
8. Fleming RE, Migas MC, Holden CC, et al. Transferrin receptor 2: continued expression in mouse liver in the face of iron overload and in hereditary hemochromatosis. *Proc Natl Acad Sci U S A.* 2000;97:2214-2219.

## To the editor:

### CXCR4 expression is associated with survival in familial chronic lymphocytic leukemia, but CD38 expression is not

Recent immunogenetic studies of chronic lymphocytic leukemia (CLL) suggest a dichotomy: those developing from naive, pregerminal B lymphocytes exhibiting germline configuration of Ig V<sub>H</sub> status (poor outcome) and those stemming from more mature, postgerminal center memory B cells with mutated Ig V<sub>H</sub> genes<sup>1-3</sup> (good prognosis). Due to the cost and expertise required for this technique, a simpler, inexpensive substitute has been sought. CD38 antigen expression is a marker that strongly correlates with Ig V<sub>H</sub> gene mutational status<sup>1,4</sup> and predicts outcome in CLL,<sup>1,4-9</sup> although not all studies agree.<sup>10-12</sup> Another class of markers, chemokines, and their respective receptors, may also correlate with clinical stage and prognosis. CXCR4, a chemokine receptor found on CLL B cells, may play a role in the marrow infiltration observed in CLL.<sup>13</sup> We evaluated (1) whether CD38 or CXCR4 expression is associated with immunoglobulin gene usage and (2) whether these markers are prognostic for survival in familial CLL cases.

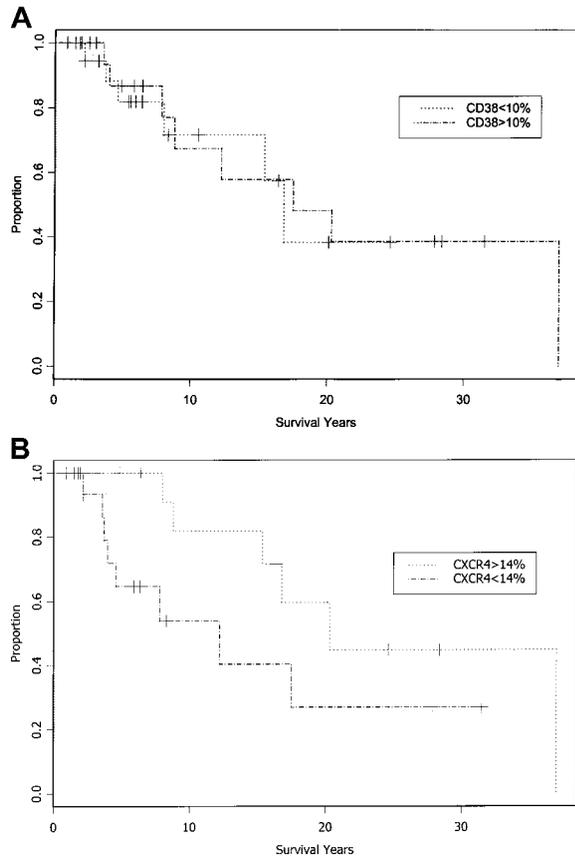
Thirty-nine individuals from the NCI Familial CLL Registry<sup>14</sup> had cells labeled, using a 4-color staining method, with monoclonal antibodies against the lymphoid antigens CD5 phycoerythrin, CD38 allophycocyanin, CD19 peridinin chlorophyll protein (BD Biosciences, San Jose, CA), and CXCR4 fluorescein (R&D Systems, Minneapolis, MN). Antibody expression was measured by FACSCaliber (Becton Dickinson, San Jose, CA) flow cytometer. CD38 and CXCR4 expressions were scored as percent positive of the B-CLL cells (CD5<sup>+</sup>/CD19<sup>+</sup>).<sup>6</sup> DNA of rearranged immuno-

globulin heavy chain genes were amplified by polymerase chain reaction and cloned and sequenced, using a previously described method.<sup>15</sup> Assays were conducted with laboratory personnel blinded to V<sub>H</sub> mutation and clinical characteristics of the patients.

Generalized linear models were used to estimate least-squared mean CD38 expression by heavy chain mutation status on 21 patients using SAS Version 8.0 (SAS, Cary, NC) and to evaluate the association with CXCR4 expression data. Survival was estimated on all 39 familial CLL patients by the Kaplan-Meier method, using SPLUS 2000 (Insightful, Seattle, WA). Median levels of CD38 and CXCR4 expression in the subset of cases with V<sub>H</sub> data were used to group the patients into lower and higher risk; differences in survival were tested by the Wilcoxon test. All tests of statistical significance were two-sided.

In agreement with earlier reports,<sup>1,3</sup> unmutated V<sub>H</sub> cases displayed a higher percentage of CD38<sup>+</sup> cells than mutated cases (23.62% versus 5.80%, *P* = .03). We did not observe an association between CD38 expression and survival in the 39 patients (Wilcoxon, *P* = .45) (Figure 1A). Rather, expression of the CXCR4 chemokine receptor was more strongly correlated with V<sub>H</sub> mutational status (9.53% versus 40.82%, *P* = .004; adjusted for age) and a better predictor of survival (Wilcoxon, *P* = .02) (Figure 1B).

CD38 expression has been proposed as a surrogate marker for V<sub>H</sub> mutation status to predict the clinical course in CLL with mixed findings.<sup>4-12</sup> Although we observed an association between CD38



**Figure 1. Survival probabilities for familial CLL patients.** (A) Survival probabilities comparing CLL patients by CD38 expression pattern. (B) Survival probabilities comparing CLL patients by CXCR4 expression pattern.

expression and  $V_H$  mutation status, CD38 expression, in contrast with  $V_H$  mutation status, did not predict survival in our familial CLL cases. The 2 assays yielded discordant results in 8 of the 21 cases, which may account for these findings. Furthermore, only 2 cases had advanced disease at the time of the specimen collection, resulting in the generally lower CD38 expression observed in this series. Variability in CD38 expression may also be lower in familial CLL than in sporadic cases, making it difficult to observe a difference with the available number of cases. Only 2 of 21 patients with  $V_H$  mutational data had CD38 expression levels greater than 30%.

The association between underexpression of the CXCR4 chemokine receptor and survival is consistent with its involvement in the trafficking and homing of B-CLL cells to bone marrow, and the strong down-modulation of CXCR4 on CLL B cells that migrate into the marrow stromal cell layer that has been reported.<sup>13</sup> Although we were unable to examine CXCR4 expression in relation to the extent of marrow involvement, our data also suggest that CXCR4 down-regulation may play a role in the marrow

infiltration observed in CLL. CXCR4 may be one factor that regulates neoplastic B cells' survival by making cells more resistant to apoptosis and allowing for unchecked proliferation of the malignant clone.

In summary, our results suggest that CXCR4 receptor expression levels could potentially distinguish CLL patients who will develop aggressive disease from those who will not. In our familial CLL cases, however, no association with prognosis with CD38 expression was observed. Future studies in a larger study population with extensive treatment and clinical information are clearly warranted.

**Naoko Ishibe, Maher Albitar, Iman B. Jilani, Lynn R. Goldin, Gerald E. Marti, and Neil E. Caporaso**

*Correspondence: Naoko Ishibe, Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, National Institutes of Health, Rockville, MD*

## References

- Damle RN, Wasil T, Fais F, et al. IgV gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94:1840-1847.
- Hamblin TJ, Davis Z, Gardiner A, et al. Unmutated Ig  $V_H$  genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94:1848.
- Maloum K, Davi F, Merle-Beral H, et al. Expression of unmutated  $V_H$  genes is a detrimental prognostic factor in chronic lymphocytic leukemia. *Blood*. 1000;96:377-379.
- Matrai Z, Lin K, Dennis M, et al. CD38 expression and Ig  $V_H$  gene mutation in B-cell chronic lymphocytic leukemia. *Blood*. 2001;97:1902-1903.
- Del Poeta G, Maurill L, Venditti A, et al. Clinical significance of CD38 expression in chronic lymphocytic leukemia. *Blood*. 2001;98:2633-2639.
- Ibrahim S, Keating M, Do KA, et al. CD38 expression as an important prognostic factor in B-cell chronic lymphocytic leukemia. *Blood*. 2001;98:181-186.
- Chevallier P, Penther D, Avet-Loiseau H, et al. CD38 expression and secondary 17p deletion are important prognostic factors in chronic lymphocytic leukemia. *Br J Haematol*. 2002;116:142-150.
- D'Arena G, Musto P, Cascavilla N, et al. CD38 expression correlates with adverse biological features and predicts poor clinical outcome in B-cell chronic lymphocytic leukemia. *Leuk Lymphoma*. 2001;42:109-114.
- Hamblin TJ, Orchard JA, Ibbotson RE, et al. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. *Blood*. 2002;99:1023-1029.
- Thunberg U, Johnson A, Roos G, et al. CD38 expression is a poor predictor for  $V_H$  gene mutational status and prognosis in chronic lymphocytic leukemia. *Blood*. 2001;97:1892-1893.
- Hamblin TJ, Orchard JA, Gardiner A, et al. Immunoglobulin V genes and CD38 expression in CLL [letter]. *Blood*. 2000;95:2455-2456.
- Morabito F, Mangiola M, Oliva B, et al. Peripheral blood CD38 expression predicts survival in B-cell chronic lymphocytic leukemia. *Leuk Res*. 2001;25:927-932.
- Burger JA, Burger M, Kipps TJ. Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood*. 1999;94:3658-3667.
- Ishibe N, Sgambati MT, Fontaine L, et al. Clinical characteristics of familial B-CLL in the National Cancer Institute Familial Registry. *Leuk Lymphoma*. 2001;42:99-108.
- Sakai A, Marti GE, Caporaso NE, et al. Analysis of expressed immunoglobulin heavy chain genes in familial B-CLL. *Blood*. 2000;95:1413-1419.

To the editor:

## Activating Ras mutations in patients with plasma-cell disorders: a reappraisal

Although several studies have been devoted to the incidence and signification of activating Ras mutations in patients with plasma-cell disorders, and especially with multiple myeloma (MM), to date it remains difficult to conclude the exact significance of such

mutations in these diseases. In this context, we have been interested in the work of Kalakonda et al<sup>1</sup> showing for the first time that codon 61 mutations of the *NRAS* gene were universal in patients with MM at presentation in a subpopulation of 12% to 100% of malignant