To the editor:

Long-term results of chemoimmunotherapy with low-dose fludarabine, cyclophosphamide and high-dose rituximab as initial treatment for patients with chronic lymphocytic leukemia

Chemoimmunotherapy with fludarabine (F), cyclophosphamide (C), and rituximab (R) is currently considered the gold standard first-line therapy for chronic lymphocytic leukemia (CLL).[1] In an attempt to reduce the neutropenia and maintain the high response rate of standard-dose FCR, we conducted a prospective phase 2 clinical trial in untreated CLL patients using lower doses of FC and higher dose of R followed by R maintenance until progression or up to 2 years (FCR-Lite). Details of the study were previously described.[2] Between June 2004 and November 2008, 65 CLL patients (median age 58 years, range 36-85 years, Rai stage I-II: 80%, Rai stage III-IV: 20%) were treated with FCR-Lite. Patients were evaluated for response using the 2008 International Workshop on CLL criteria.[3] Of the 63 evaluable patients, 46 (73%) achieved complete response (CR), 2 (3%) achieved nodular partial remission (nPR), 11 (17%) achieved partial remission (PR), and 4 (6%) did not respond. Among the 50 patients with Rai stage I-II there were 38 (76%) CRs and 10 (20%) PRs and among the 13 patients with Rai stage III-IV there were 8 (62%) CRs and 3 (23%) PRs. For patients < 60 years there were 35 (81%) CRs and 5 (12%) PRs, for patients between 60-69 years there were 6 (55%) CRs and 4 (36%) PRs, and for patients ≥ 70 years there were 5 (56%) CRs and 4 (44%) PRs. For the 40 patients with normal cytogenetics, del13q or +12 there were 31 (78%) CRs, 7 (17%) PRs, and 2 (5%) NRs. Eight of 9 (89%) patients with del11q had a CR and 1 (11%) had a PR. Three of 3 patients with del17p had PRs.

The median overall survival has not been reached and the median progression-free survival was 5.8 years and the 3- and 5-year progression-free survival probability is shown in Table 1. This compares favorably with the MD Anderson Cancer Center (MDACC) phase 2 FCR trial where the 6-year actuarial OS and failure-free survival were 77% and 51%, respectively, as well as the FCR arm of the German CLL Study Group GCLLSG) where the PFS was 4.4 years.[4-6]

Fifty-five patients (85%) completed all 6 courses of FCR-Lite with dose reduction in 2% of total cycles. Grade 3-4 neutropenia occurred in 11% of total cycles of FCR-Lite and grade 3-4 infections were seen in 6% of patients. Three patients developed myelodysplastic syndromes and 2 patients developed B-cell lymphomas after FCR-Lite therapy.

In summary, the FCR-Lite regimen represents an active front-line treatment with excellent responses and duration of response with a favorable toxicity profile. Interpretation of these data and comparisons with the MDACC and GCLLSG trials should be viewed in light of the relatively low numbers of patients, relatively early Rai stages of the patients, use of prophylactic growth factors, and maintenance rituximab in the FCR-Lite trial. Further studies with FCR-Lite will include combination therapies with promising new agents.

Table 1. One-, 3-, and 5-year survival probabilities

Year	Overall survival probability, % (95% confidence limits)	Progression-free survival probability, % (95% confidence limits)
1	96.9 (88.3%, 99.2%)	93.2 (82.9%, 97.4%)
3	87.6 (76.7%, 93.6%)	84.6 (72.6%, 91.7%)
5	85.5 (73.9%, 92.2%)	66.9 (49.7%, 79.3%)

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References

1. Foon KA, Hallek MJ. Changing paradigms in the treatment of chronic lymphocytic leukemia. *Leukemia.* 2010;24(3):500-511.
2. Foon KA, Boyiadzis M, Land SR, et al. Chemoimmunotherapy with low-dose fludarabine and cyclophosphamide and high dose rituximab in previously untreated patients with chronic lymphocytic leukemia. *J Clin Oncol.* 2009;27(4): 498-503.
3. Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: A report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood.* 2008;111(12): 5446-5456.
4. Keating MJ, O'Brien S, Albitar M, et al. Early results of a chemoimmunotherapy regimen of fludarabine, cyclophosphamide, and rituximab as initial therapy for chronic lymphocytic leukemia. *J Clin Oncol.* 2005;23(18):4079-4088.

5. Tam CS, O'Brien S, Wierda W, et al. Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood.* 2008;112(4):975-980.

6. Hallek M. Fischer K, Fingerle-Rowson G, et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomized, open-label, phase 3 trial. *Lancet.* 2010;376(9747):1164-1174.

To the editor:

Exome sequencing reveals a pallidin mutation in a Hermansky-Pudlak–like primary immunodeficiency syndrome

Partial albinism and primary immunodeficiency occur in several autosomal recessive disorders, including Hermansky-Pudlak syndrome type 2 (HPS2, Online Mendelian Inheritance in Man [MIM] #608233), Chediak-Higashi syndrome (MIM#214500), Griscelli syndrome types 1 (MIM#214450) and 2 (MIM#607624), and endosomal-adaptor protein p14 deficiency (MIM#610798).[1-6] At least 15 recessive mouse mutations have been described that also are characterized by partial albinism and immunodeficiency and/or bleeding disorders and that appear to be homologous to the human diseases.[7-10]

A 17-year-old, northern Italian female with oculocutaneous albinism, nystagmus, and normal neurologic development presented with recurrent cutaneous infections but without hemorrhagic episodes. At 6 years of age, she had a prolonged episode of fever with convulsions. At presentation, she had thrombocytopenia (111 000 platelets/μL) and leucopenia (2600 leucocytes/μL, 2300 neutrophils/μL; 300 lymphocytes/μL). Platelet aggregation tests were normal.

Nucleotides (37.7 million) of exons (the exome) were enriched 44-fold from genomic DNA from the patient and sequenced to an average, uniquely aligned coverage of 135-fold.[11] No mutations were identified in known immunodeficiency disease genes. Only one novel variant had high likelihood of pathogenicity and was unique to the patient among ~ 250 Children's Mercy Hospital exomes and the NHLBI exome collection (http://evs.gs.washington.edu/EVS/). It was a homozygous nonsense mutation, c.232C > T (p.Q78X), in exon 3 of pallidin (*PLDN*, chr15:45895305C > T, relative to human genome build 37, supplemental Figure 1A [available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article]), present in all 65 aligning sequences. This mutation was confirmed to be homozygous in the patient and heterozygous in her parents by Sanger sequencing (supplemental Figure 1B), and was associated with absent PLDN protein expression (supplemental Figure 1C).

Because intracellular trafficking and degranulation of specialized lysosomes are impaired in HPS2,[2,3,5] we sought such defects in the patient. The proportion of resting and IL-2–activated NK cells expressing the lysosomal membrane protein CD107a on the surface was increased (6% and 14% of NK cells from the patient, respectively, versus 0.6% and 2% in healthy controls, respectively, Figure 1A). However, these increases were not as marked as in HPS2 (24% in IL-2–activated NK cells [S.P., G.T., R.B., unpublished observations] but at odds with one case report[4,5]). PLDN replacement in NK cells from the patient decreased CD107a expression to normal (Figure 1B).

NK cells from the patient had intermediate cell-surface expression of CD63, another lysosomal membrane protein altered in HPS2[1,4,6] (17% in patient, 9% in controls, and 28% in an HPS2 patient, Figure 1A). Degranulation, as measured by change in surface expression of CD107a on IL-2–activated NK cells after challenge with LCL 721.221 target cells, was less than controls (Figure 1C).

Cytolytic activity of resting and IL-2–activated NK cells from the patient was reduced (Figure 1C). Low NK activity did not correlate with reduced expression of activating receptors,[12] as assessed by NKp30, NKp46, NKG2D, and CD244 (2B4) levels on NK cells cultured with IL-2 for 3 weeks.

Pallid mice have reductions in coat and eye pigmentation, lysosomal enzyme secretion, chemotactic release of neutrophil elastase, and neutrophil killing of *Leishmania,* together with prolonged bleeding because of storage pool deficiency.[7-10] Here, we

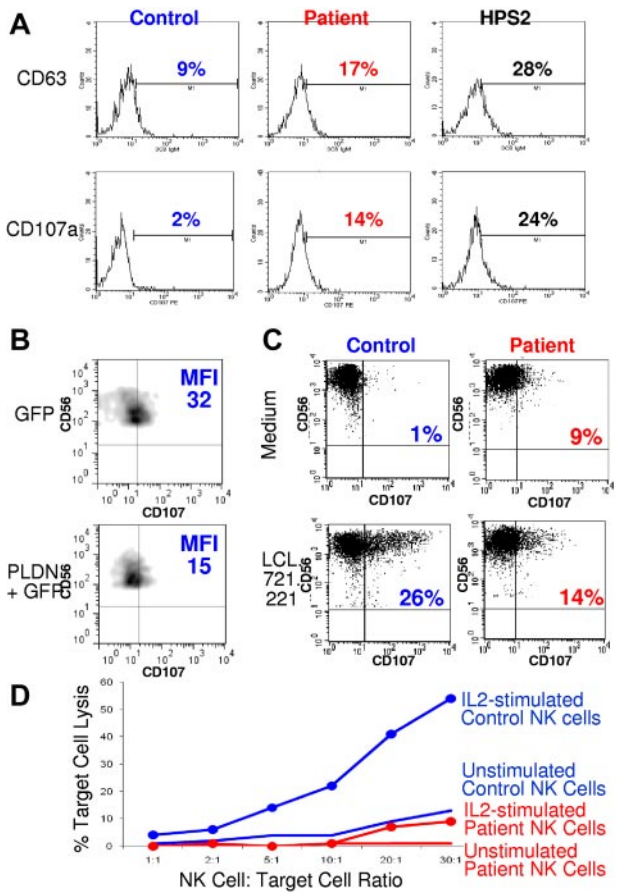

Figure 1. Effects of a *PLDN* mutation on NK-cell function. (A) Histograms of flow cytometric measurement of CD63 and CD107a on IL-2–activated NK cells from a normal control subject, the pallidin-deficient patient, and a patient with HPS2. (B) Two-color flow cytometric measurement of CD107a and CD56 on IL-2–activated NK cells from the pallidin-deficient patient, after transfection with expression vectors containing GFP or PLDN and GFP. (C) Two-color flow cytometric measurement of CD107a on IL-2 activated NK cells from a normal control subject and the pallidin-deficient patient, after culture with medium or with target cell line LCL.721.221. (D) Lysis of K562 NK target cells by freshly isolated PBMCs from the pallidin-deficient patient and a healthy control before and after overnight incubation with IL-2. Experiments were repeated 3 times in 3 independent experiments.