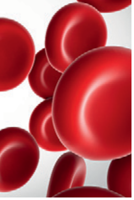

Letter to *Blood*

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**TO THE EDITOR:**

# HLA class I allele–lacking leukocytes predict rare clonal evolution to MDS/AML in patients with acquired aplastic anemia

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Acquired aplastic anemia (AA) is caused by immune-mediated destruction of hematopoietic stem and progenitor cells, resulting in bone marrow hypoplasia and pancytopenia in the peripheral blood.[1,2] HLA class I allele–lacking (HLA$^-$) leukocytes are detected in ~30% of patients with AA, and are thought to represent the involvement of a cytotoxic T lymphocyte attack against hematopoietic stem cells (HSCs) in the development of AA, based on the high response rate to immunosuppressive therapy (IST).[3-6] Similarly to glycosylphosphatidylinositol-anchored protein–deficient (GPI$^-$) leukocytes in patients with paroxysmal nocturnal hemoglobinuria, HLA$^-$ leukocytes in AA patients are often clonal or oligoclonal and expand to account for >50% of the total leukocytes.[7] Despite such overwhelming proliferation, somatic mutations in driver genes, as well as telomere shortening that portends clonal evolution, are rarely detected in HLA$^-$ granulocytes, suggesting the genetic stability of HLA$^-$ HSCs.[7]

Recent studies have reported an association between specific HLA alleles and clonal evolution to myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) in patients with AA.[8,9] However, the association between HLA loss and clonal evolution to MDS/AML has not been extensively analyzed in larger AA cohorts. We therefore addressed this issue by studying the prognosis of a large number of patients with AA with or without HLA$^-$ leukocytes who had been followed for a long period. We also studied the clonal composition of granulocytes in patients with AA with HLA$^-$ cells, wherein aberrant clones other than HLA$^-$ cells might be responsible for clonal evolution to MDS/AML.

We retrospectively analyzed the clinical characteristics of 633 patients with AA and examined peripheral blood samples for the presence of HLA$^-$ leukocytes using a high-sensitivity flow cytometry assay, droplet digital polymerase chain reaction, single-nucleotide polymorphism arrays, or next-generation sequencing between 2010 and 2020.[3-6] GPI$^-$ cells were detected using a high-sensitivity flow cytometry assay, as previously described.[10,11] Further experimental procedures are described in supplemental Methods (available on the *Blood* Web site). All patients provided their informed consent in accordance with the Declaration of Helsinki. This retrospective study was approved by the Ethics Committee of Kanazawa University School of Medical Science.

Table 1 shows the patient characteristics. The median age at diagnosis was 60 years (range, 2-91 years). The diagnoses of the patients were as follows: nonsevere AA (NSAA) (n = 385; 60.8%); severe AA (SAA) (n = 191; 30.2%), and very severe AA (n = 57; 9.0%). The median follow-up period was 55 months (range, 1-492 months). HLA$^-$ granulocytes were detected in 127 of the 633 patients (20.0%) with a median clone size of 19.7% (range, 0.04% to 100%); the aberrant granulocytes accounted for >50% of the total granulocytes in 31 of the 127 patients (24.4%) (Figure 1A; supplemental Table 1). Four hundred eighteen of the 633 patients (66.0%) possessed GPI$^-$ granulocytes with a median size of 0.20% (range, 0.003% to 100%), which was in line with previous studies (Figure 1B).[10,12] The prevalence of GPI$^-$ cells in patients with and without HLA$^-$ cells did not differ to a statistically significant extent (66.9% vs 65.8%). On the other hand, 91 of 104 patients (87.5%) with HLA$^-$ cells responded to IST, whereas 249 of 331 (75.2%) without HLA$^-$ cells responded ($P < .05$; Figure 1C; supplemental Table 2). Ten patients with HLA$^-$ cells did not receive any therapy, and only 2 patients (20%) showed spontaneous recovery without IST.

The prognosis survey revealed that clonal evolution to MDS/AML did not occur in any of the 127 patients with HLA$^-$ cells who were trackable, after a median follow-up period of 51 months. In contrast, 17 AA patients without HLA$^-$ cells eventually evolved to MDS/AML (supplemental Table 3). The median time to progression was 24 months (range, 1-309 months). The cumulative incidence of clonal evolution to MDS/AML at 10 years was 4.5% (95% confidence interval, 2.4% to 7.6%) in the overall population of AA patients (Figure 1D), 0% in patients with HLA$^-$ cells, and 5.8% (95% confidence interval, 3.1% to 9.8%) in patients without HLA$^-$ cells ($P < .05$; Figure 1E).



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**Table 1. Patient characteristics**

| Characteristics | No. of patients |
|---|---|
| Median age (range), y | 60 (2-91) |
| Sex, M/F | 295/338 |
| **Diagnosis** | |
| AA | 633 |
| **Onset, n (%)** | |
| Adult | 616 (97.3) |
| Pediatric | 17 (2.7) |
| **Severity at diagnosis, n (%)** | |
| NSAA | 385 (60.8) |
| SAA | 191 (30.2) |
| VSAA | 57 (9.0) |
| **Treatment** | |
| ATG + CsA ± TPO-RA | 230 |
| CsA ± TPO-RA | 205 |
| AS | 22 |
| PSL | 8 |
| HSCT | 3 |
| Others | 2 |
| None | 69 |
| Unknown | 94 |
| Follow-up, median (range), mo | 55 (1-492) |
| Patients with HLA$^{-}$ cells, n (%) | 127 (20.0) |
| Patients with GPI$^{-}$ cells, n (%) | 418 (66.0) |

ATG, antithymocyte globulin; AS, anabolic steroids; CsA, cyclosporine; F, female; HSCT, hematopoietic stem cell transplantation; M, male; NSAA, nonsevere aplastic anemia; PSL, prednisolone; SAA, severe aplastic anemia; TPO-RA, thrombopoietin receptor agonist; VSAA, very severe aplastic anemia.

When the age at diagnosis, sex, severity, disease duration, GPI$^{-}$ cells, and HLA$^{-}$ cells were included in the multivariate analysis, the presence of HLA$^{-}$ cells represented independent negative predictors of clonal evolution to MDS/AML (supplemental Table 4).

Interestingly, among the 127 patients with HLA$^{-}$ cells, 88 patients (69.3%) possessed aberrant clones other than HLA$^{-}$ clones, which included 0.005% to 91.6% GPI$^{-}$ cells (n = 85), del(13q) cells (n = 3), trisomy 8 cells (n = 1), t(1;10) cells (n = 1), t(9;13) cells (n = 1), and inv12 cells (n = 1) (Figure 1F). Figure 1G shows 2 representative cases in which hematopoiesis was completely replaced by HLA$^{-}$ cells and concomitant aberrant clones.

Finally, we identified 13 patients who had >90% HLA$^{-}$ cells and who had been in hematological remission for >7 years (supplemental Table 5). Even though HLA$^{-}$ cells and other concomitant aberrant cells accounted for >90% of granulocytes, these patients showed no signs of disease progression to MDS/AML. These data suggest that the escape clones were healthy enough to sustain the hematopoietic function of the patients.[7,13]

The cumulative incidence of MDS/AML increases after IST, ranging from 5% to 15% within the observation period of 5 to 11.3 years.[14-18] The relatively lower cumulative incidence of clonal evolution to MDS/AML at 10 years (4.5%) in comparison with previous studies from western countries may be attributed to the shorter follow-up period (median, 55 months) and the race of our study subjects (Japanese). Moreover, 60.8% our cohort was composed of NSAA cases, which may have also affected the incidence of clonal evolution. However, a recent study from the United States showed that the cumulative incidence of MDS/AML in moderate AA and SAA was not significantly different,[19] a finding consistent with our analysis.

Strikingly, our study showed that the presence of HLA$^{-}$ cells was an independent negative predictor of clonal evolution to MDS/AML. Our findings are consistent with the results of a previous study in that there was a lower risk of clonal evolution to MDS/AML but a higher rate of clonal hematopoiesis in patients with HLA$^{-}$ cells than in those without HLA$^{-}$ cells.[8] In line with previous reports,[20,21] 7 (5.5%) had cytogenetic abnormalities in patients with 127 HLA$^{-}$ cells, such as del(13q) and trisomy 8. The coexistence of HLA$^{-}$ cells and these cytogenetically abnormal clones supports the idea that cells with some cytogenetic abnormalities may arise as a result of immune escape.[13,22]

Retrospectives analyses using next-generation sequencing identified risk factors for post-AA MDS, including the age at the onset of AA, short leukocyte telomere length, and HLA class I risk alleles.[8,23-27] The age at diagnosis of AA was not associated with an increased risk of clonal evolution to MDS/AML in our analysis. This seems reasonable given that the cumulative incidence of MDS/AML has been reported to be similar between adults and children.[14-18] We did not have a chance to examine the telomere lengths of the patients or perform exon sequencing for all patients. However, our recent study showed no telomere attrition in HLA$^{-}$ granulocytes; it also showed that somatic mutations related to myeloid malignancies were rarely detected in HLA$^{-}$ granulocytes.[7] The present study was associated with some limitations, including the heterogeneous patient population and retrospective setting.

In summary, the presence of HLA$^{-}$ leukocytes and concomitant aberrant clones was not associated with clonal evolution to MDS/AML in Japanese patients with AA, even in those possessing a large (>90% of the total granulocyte) HLA$^{-}$ cell population. Our data suggest that HLA$^{-}$ HSC clones that escape immune attack are healthy enough to support hematopoiesis over the long-term in patients with AA.

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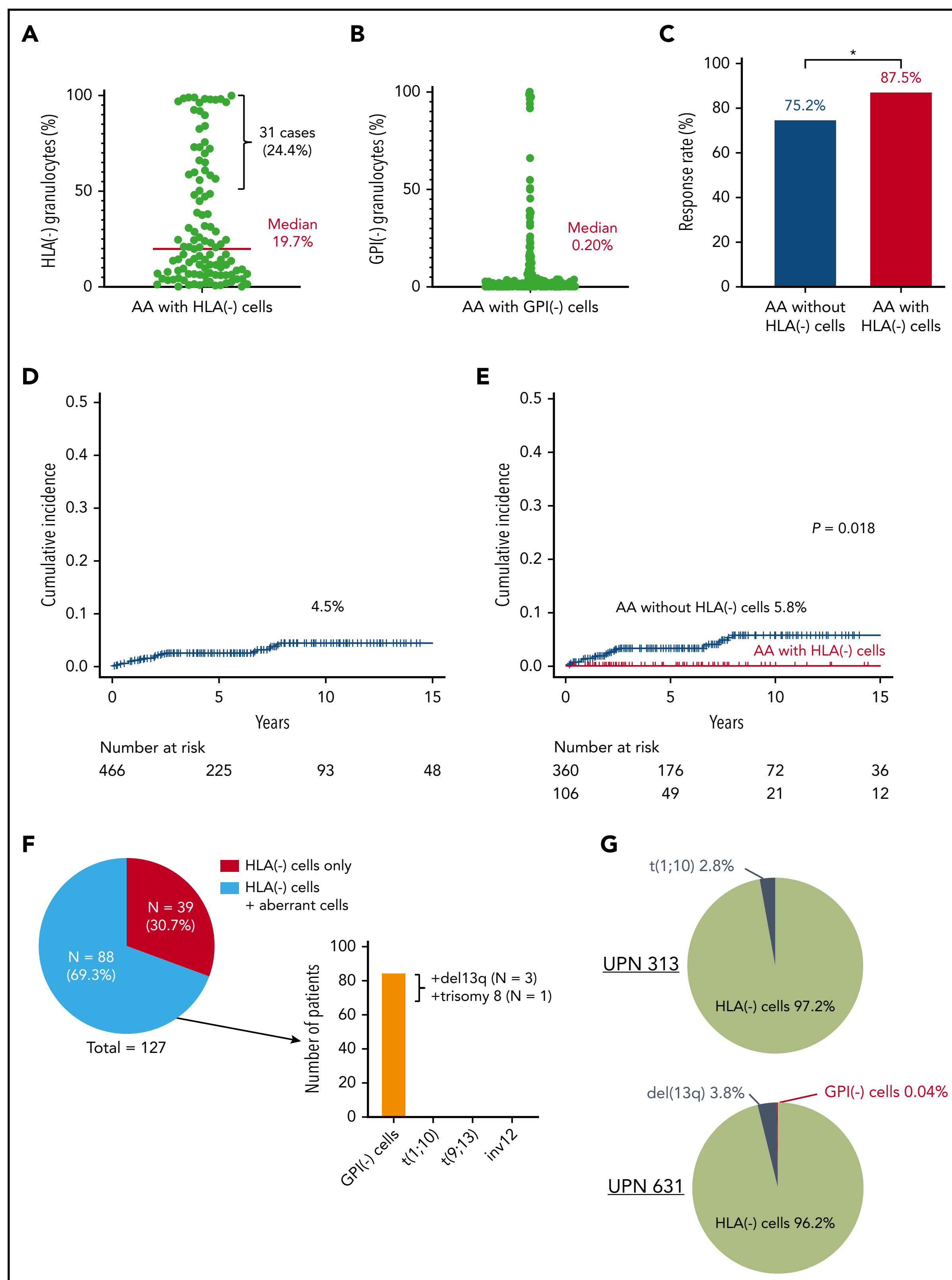


**Figure 1. Response to IST and clonal evolution to MDS/AML in patients with AA with or without HLA⁻cells.** (A) The distribution of the HLA⁻ granulocyte percentages in AA patients with HLA⁻ cells. Red bars indicate the median clone size. (B) The distribution of the GPI⁻ granulocyte percentages in AA patients with GPI⁻ cells. (C) The response to IST in AA patients with or without HLA⁻ cells. *A significant difference $P < .05$. (D) The cumulative incidence of clonal evolution to MDS/AML in the overall population of patiens with AA. (E) The cumulative incidence of clonal evolution to MDS/AML in AA patients with (red line) or without (black line) HLA⁻ cells. (F) The pie diagram indicates the proportions of concomitant aberrant clones detected in 127 patients with AA with HLA⁻ cells. The bar graphs depict the number of patients with AA with HLA⁻ cells and concomitant aberrant clones. (G) The pie diagrams indicates the proportions of HLA⁻ cells and concomitant aberrant clones in 2 representative cases (unique patient number [UPN] 313 and UPN 631).

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# Authorship

Contribution: K. Hosokawa and S.N. designed the research, analyzed the data, and wrote the manuscript; K. Hosokawa, H. Mizumaki, T.Y., H. Maruyama, T.I., N.T., R.U., M.T., Y.Z., K.I., H.Y., T.K., and S.N. recruited patients to participate in the study; H. Mizumaki, T.Y., H. Maruyama, T.I., and N.T. performed flow cytometry; H.T. generated antibodies for flow cytometry; F.A. performed HLA genotyping; S.O. conducted the single-nucleotide polymorphism array analyses; H. Mizumaki, T.I., Y.Z., K. Hosomichi, and A.T. performed deep sequencing; H. Mizumaki, Y.Z., M.A.T.N., and D.C.T. performed the droplet digital polymerase chain reaction; and all authors critically reviewed the manuscript and approved the submission of the final manuscript.

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# Footnotes


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Detailed data tables of clinical findings are available in supplemental Tables 1-5. For more information, please contact the corresponding author, Shinji Nakao, at snakao8205@staff.kanazawa-u.ac.jp.

The online version of this article contains a data supplement.

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