High Prevalence of PNH-phenotype Cells in Patients Who Received CD19-targeted CAR T-cell Therapy

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Chimeric antigen receptor (CAR) T-cell therapy targeting CD19 has emerged as a remarkably effective treatment option for relapsed/refractory B-cell acute lymphoblastic lymphoma and diffuse large B-cell lymphoma.1-3 While cytokine-releasing syndrome (CRS) and neurotoxicity are major short-term toxicities, prolonged cytopenia is one of the most important issues among late toxicities.4-5 Cytopenias manifests as a biphasic pattern.6 Early cytopenia, which occurs several days after CAR-T cell infusion, commonly resolves within 3 weeks after CAR T-cell infusion and is attributable to lymphodepletion chemotherapy (fludarabine and cyclophosphamide in most cases) as well as CRS. In contrast, late cytopenia occurs beyond 3 weeks after infusions and typically resolves in 1 to 2 months in most cases, but could persist for more than 6 months; however, the mechanisms remain unclear.7

Glycosylphosphatidylinositol (GPI)-anchored protein-deficient (GPI [−]) cells were first observed in patients with paroxysmal nocturnal hemoglobinuria (PNH). GPI (−) cells (ie, PNH-phenotype cells) were also detected at a low frequency in patients with bone marrow failure with etiologies other than PNH, including acquired aplastic anemia (AA) or low-risk myelodysplastic syndromes (MDS).8 Moreover, the presence of PNH-phenotype cells serves as a predictor of a good response to immunosuppressive therapy in AA or MDS, regardless of the frequency of those cells.9,10 Recent reports showed that high-resolution flow cytometry (observation of GPI-anchored protein-deficient Cells in Japanese Patients with Bone Marrow Failure Syndrome and in Those Suspected of Having PNH (OPTIMA method) can precisely detect clinically relevant minor PNH-type cell populations, defined as ≥0.003% CD11b-FLAER− granulocytes and ≥0.005% glycopherin A-CD55−CD59− erythrocytes, that are absent in healthy individuals.11,12 A large-scale study enrolling 1210 patients revealed that PNH-type cells were detected in 56.3% of AA, 18.5% of MDS, 100% of classical PNH, and 18.6% of undiagnosed bone marrow failure patients.13

Based on these observations, we assessed the presence of PNH-phenotype cells using OPTIMA method in a patient who developed severe and prolonged cytopenia on day 48 after CAR T-cell infusion (Table 1, patient no. 8). Surprisingly, PNH-type cells were detected at much higher levels than the threshold in the granulocyte (0.061%, Figure 1A) and erythrocyte (0.049%) populations. Bone marrow biopsy performed on day 48 (the same day as that of the assessment of PNH-type cells) showed severely hypocellular marrow with marked decrease in megakaryocytic and erythroid lineages and no evidence of lymphoma cells (Figure 1B). Chromosomal abnormalities or gene mutations suggestive of MDS were not detected. Severe cytopenia was resistant to low-dose corticosteroid, which was used for treatment of skin erythema in this patient, and repeat testing on day 92 showed persistence of the PNH-type cells (0.053% granulocytes and 0.029% erythrocytes). Finally, cyclosporine was started on day 155, which resulted in a remarkable improvement in the blood count.

Based on this experience, we assessed prevalence of PNH-phenotype cells in 17 consecutive patients (including patient no. 8 described earlier), who received CAR-T cells (Tisagenlecleucel) for relapsed/refractory acute lymphoblastic lymphoma or diffuse large B-cell lymphoma and had a partial or complete response (Table 1). The median age of the patients was 60 (range, 24-70) years. The duration from the date of CAR T-cell therapy to the assessment of PNH-type cells was a median of 84 (range, 33-252) days. Surprisingly, PNH-type cells were detected in 7 of 8 patients (87.5%) who were examined 1-2 months after infusion, when late cytopenia generally occurs. In contrast, PNH-type cells were detected in 3 of 9 patients (33.3%) who were examined beyond 2 months, by which late cytopenia generally resolves. The difference in the prevalence of PNH-type cells before and after 2 months after infusion was statistically significant (P < 0.05 with Fisher exact test). When analyzed based on the presence of neutropenia regardless of the timing of the assay, defined by an absolute neutrophil count of <1000/μL, PNH-type cells were detected in 7 of 9 patients (77.8%) with neutropenia, and 3 of 8 patients (37.5%) without neutropenia.

We demonstrated that PNH-type cells are frequently detected in peripheral blood between 1 and 2 months after CAR T-cell infusion. The association between the presence of PNH-type cells and a response to immune suppressive therapy in AA has led to a suggestion that their presence is a surrogate marker for an immunological pressure on hematopoiesis.11 PNH-type cells lack GPI-anchor proteins that can be targeted by immune effector cells, and are therefore considered to have growth advantages when immunological pressure is exerted. Consequently, immune activation of hematopoiesis 1-2 months after CAR T-cell therapy may be responsible for emergence of PNH-type...
cells in that period. However, fewer patients of those who were examined after 2 months had detectable PNH-type cells and the time-point at which cytopenia tended to resolve may correlate with decreasing levels of CAR T-cells. Thus, solely the presence of PNH-type cells around the postinfusion 1- to 2-month period may not be indicative of the level of clinical response to immuno-suppressive therapy. Of note, we observed the effectiveness of cyclosporine for severe and prolonged cytopenia in the patient no. 8, who had a persistence of PNH-type cells at high levels.

In contrast to the present study, previous studies have mainly focused on roles of cytokines, chemokines, or other aspects in hematopoiesis. Jain et al. reported that while severe CRS and immune effector cell–associated neurological syndrome were associated with the absence of blood cell recovery at 1 month, neither were associated with count recovery at 3 months. Recently, Fried et al. reported that perturbations in stromal-derived factor 1 levels were correlated with late-onset neutropenia. Interestingly, Nahas et al. reported that 2 of 8 patients presenting with persistent cytopenia had MDS. They hypothesized that an immunosuppressive environment after fludarabine-based chemotherapy following extensive prior treatment facilitated the selective evolution to MDS. They also showed

| Patient | N  | Age | Sex | Diagnosis | Lines of Treatment | Previous Transplant | Timing of Assay (d) | CBC at the Timing of Assay | PNH-type Cells in Granulocytes | PNH-type Cells in Erythrocytes |
|---------|----|-----|-----|-----------|-------------------|---------------------|---------------------|--------------------------|-------------------------------|-------------------------------|
|         |    |     |     |           |                   |                     |                     | WBC (/µL) | ANC (/µL) | HGB (g/dL) | PLT (×10³/µL) | Positivity Frequency | Positivity Frequency | Positivity Frequency |
| 1       | 61 | F   | DLBCL | 3        | No                | 252                 | 4440               | 2530       | 13.0      | 213         | 0.000           | 0.000  |
| 2       | 67 | F   | DLBCL | 3        | No                | 222                 | 3270               | 1667       | 12.5      | 225         | 0.003           | 0.005  |
| 3       | 48 | F   | DLBCL | 4        | No                | 207                 | 4220               | 3228       | 15.6      | 189         | 0.000           | 0.000  |
| 4       | 54 | M   | DLBCL | 4        | No                | 112                 | 1130               | 734        | 8.4       | 100         | 0.006           | 0.015  |
| 5       | 70 | M   | DLBCL | 3        | No                | 175                 | 2790               | 1604       | 11.3      | 88          | 0.001           | 0.000  |
| 6       | 27 | F   | DLBCL | 3        | Yes              | 91                  | 760                | 475        | 5.5       | 18*         | 0.000           | 0.000  |
| 7       | 64 | M   | DLBCL | 3        | No                | 137                 | 1710               | 735        | 11.9      | 122         | 0.002           | 0.002  |
| 8       | 54 | F   | DLBCL | 4        | No                | 48                  | 970                | 446        | 7.5       | 33*         | 0.061           | 0.019  |
| 9       | 68 | M   | DLBCL | 3        | No                | 98                  | 2660               | 1396       | 11.3      | 135         | 0.005           | 0.006  |
| 10      | 63 | F   | DLBCL | 3        | Yes              | 43                  | 7750               | 775        | 12.9      | 255         | 0.003           | 0.013  |
| 11      | 68 | F   | DLBCL | 4        | No                | 84                  | 2530               | 1290       | 12.0      | 126         | 0.002           | 0.000  |
| 12      | 52 | M   | DLBCL | 5        | Yes              | 34                  | 2060               | 185        | 7.4       | 13*         | 0.009           | 0.007  |
| 13      | 24 | F   | ALL   | 3        | No                | 40                  | 3090               | 1174       | 13.1      | 222         | 0.007           | 0.008  |
| 14      | 58 | F   | DLBCL | 3        | No                | 46                  | 3660               | 109        | 7.5       | 33          | 0.011           | 0.012  |
| 15      | 50 | M   | DLBCL | 6        | Yes              | 42                  | 1930               | 912        | 8.0       | 27*         | 0.008           | 0.005  |
| 16      | 69 | M   | DLBCL | 4        | No                | 43                  | 1230               | 424        | 6.2*      | 11*         | 0.001           | 0.000  |
| 17      | 60 | M   | DLBCL | 3        | Yes              | 33                  | 4340               | 150        | 10.6      | 18*         | 0.011           | 0.012  |

*Transfusion dependent.

ALL = acute lymphoblastic leukemia; ANC = absolute neutrophil count; CBC = complete blood count; DLBCL = diffuse large B-cell lymphoma; HGB = hemoglobin; PLT = platelets; PNH = paroxysmal nocturnal hemoglobinuria; WBC = white blood cell.

Figure 1. PNH-phenotype cells and bone marrow biopsy specimen. (A), PNH-phenotype (GPI [−]) granulocytes in patient no. 8. GPI (−) granulocytes populations were identified in the upper-left quadrant of the scattergram, on the CD11b vs fluorescein-labeled aerolysin plot. (B), Bone marrow biopsy specimen of patient no. 8. Hematoxylin-eosin image at low power view. Severely hypocellular bone marrow was observed. Bar: 500 µm. GPI (−) = glycosylphosphatidylinositol-anchored protein-deficient; PNH = paroxysmal nocturnal hemoglobinuria.
that low platelet count on the first day of lymphodepleting chemo-
therapy was an indicator of low stem cell reserve and a pre-
dictor for persistent cytopenias.

Our study is limited by its small number of patients. It is also
retrospective: the timing of the assessment of PNH-type cells
varied significantly, and longitudinal analysis for each patient
was lacking. Nonetheless, our study provides significant insight
for mechanisms underlying late cytopenia. It also suggests that
cyclosporine could be a treatment option for a severe and per-
sistent cytopenia, although it may suppress antitumor effects of
residual CAR T-cells.

In conclusion, we demonstrated that PNH-type cells are fre-
quently detected in peripheral blood between 1 and 2 months
after CAR T-cell infusion. Our results warrant an exploration
of the role of immunological pressure other than CRS in the
pathogenesis of late cytopenia after CAR T-cell therapy.

Disclosures

MO and SY have each received speaker honoraria from Novartis. All
the other authors have no conflicts of interest to disclose.

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