LETTER TO THE EDITOR

Nonengraftment donor lymphocyte infusions for refractory acute myeloid leukemia

Blood Cancer Journal (2015) 5, e371; doi:10.1038/bcj.2015.100; published online 4 December 2015

Allogeneic stem cell transplantation (allo-SCT) is an effective treatment modality for acute myeloid leukemia (AML) that exerts its therapeutic benefit via a graft versus leukemia effect. Its applicability is limited by toxicity from preconditioning chemoradiation in addition to the threat of graft versus host disease, which precludes many patients with AML from receiving this definitive consolidative therapy. 2 Previously, we have shown an ability to generate responses, some of which were complete, in patients with relapsed/refractory AML using haploidentical donor lymphocyte infusions in the absence of engraftment. 3 We present data from our FDA-approved Phase II clinical trial (BrUOG H-273, IND 015008, NCT01685606) in which haploidentical allogeneic cells are infused. Unlike our previous trial these cellular infusions occur without granulocyte colony stimulating factor (G-CSF) mobilization or preconditioning low-dose total body irradiation (TBI). The goal is to generate a robust allogeneic response that breaks host tumor tolerance without the toxicity profile seen with allo-SCT.

This trial was approved by the Rhode Island Hospital Institutional Review Board. Eligible patients had relapsed or refractory AML without curative options. Family members who were human leukocyte antigen (HLA) haploidentical donors at the A/B/DR loci were identified. Both patients and donors were consented. Donors then underwent leukapheresis without stem cell mobilization. In all, 1–2 x 10⁸ CD3+ cells/kg were infused unprocessed immediately following collection. Peripheral blood samples were collected on days 2, 7 and 14 post-cellular infusion for short tandem repeat chimerism analysis. Additional peripheral blood samples were collected pre-infusion as well as at 0–4, 8–24, 34–48, 72–96 and 168–192 h post infusion to evaluate recipient effector cells and cytokine release profiles. Following centrifugation to obtain plasma, peripheral blood mononuclear cell (PBMCN) were obtained using Ficoll–Hypaque discontinuous centrifugation. Recipient versus donor cells in the PBMCN were identified by the use of donor-specific anti-HLA antibodies that had previously been used to detect microchimerism during pregnancy. 4,5 Recipient PBMCN T-cell subpopulations were characterized for activation markers, cytokytic markers and checkpoint inhibitors. In addition, CD273 (PD-L2) and CD274 (PD-L1) expression on leukemia blasts was determined. Cytokine (IL-2, IL-6, IL-10, IFNγ) levels present in the plasma were determined using the CD8 Milliplex assay (EMD Millipore, Darmstadt, Germany). Wilcoxon rank-sum test was used to determine statistical significance using STATA 12.1 (StataCorp LP, College Station, TX, USA).

Five patients (age 52–77 years, 1–4 previous therapies) were infused with haploidentical donor cells. Four developed hyperpyrexia post infusion that lasted 24–48 h (median temperature maximum (T_max) 0–4 h post infusion 98.6 °F, median T_max 8–192 h post infusion 101.9 °F, P = 0.009). Recipient CD8 T cells demonstrated decreased perforin expression post infusion compared with pre-infusion with no changes in granzyme A/B, LAMP-1 or FasL expression. Recipient CD4 T cells showed increased LAMP-1 expression post infusion compared with pre-infusion with no changes in perforin, granzyme A/B or FasL expression. Rapid upregulation of PD-1 on host CD8 T cells was present (Table 1). The greatest difference in PD-1 expression was pre-infusion compared with 60–96 h post infusion. Non-statistically significant upregulation of surface PD-1 ligands occurred on CD33+ leukemic blasts from 0–4 (median 6.9%) to 8–24 h post infusion (median 26.9%) for PD-L1, and 0–4 (median 11%) and 72–96 h post infusion (median 28.6%) for PD-L2. No change was seen in PD-1 expression of recipient CD4 T cells. Likewise, no changes in CTLA-4, OX40, 4–1BB and CD40L expression on recipient CD8 and CD4 T cells pre- or post infusion were observed.

A statistically significant increase in IFNγ immediately post infusion and IL-2 at the onset of fever development (8–24 h post infusion) was seen (Table 2). Two patients developed grade 4 neutropenia whereas one patient developed grade 4 lymphopenia. There were no signs of graft versus host disease. No dose-limiting toxicities or durable chimerism was seen as all patients had < 2% chimerism by day 7. One of the five patients demonstrated a decrease in marrow blast counts post therapy (43% pre- to 21% 8 weeks post infusion).

Overall survival post donor lymphocyte infusion ranged from 21.5 to 46 weeks. One patient was excluded from survival analysis as the patient died before a protocol amendment to collect survival data. One patient is still alive with AML at 46 weeks post infusion. This patient had stable disease following cellular infusion. Haploidentical cellular infusions are well tolerated and demonstrate biological activity in relapsed AML. Notably, we saw less fever development as well as a decrease in clinical responses with this clinical trial compared with the prior trial. Herein, 1/5 patients had a transient response whereas previously 10/13 patients with AML had a transient response.

Laboratory correlative studies performed during the trial offer some insight into why a decrease in response was witnessed and provide a rationale by which leukemic blasts evade the host immune system. In this study, cytokine release profiles post infusion were consistent with an immune inhibitory profile with...
Table 2. Median pre versus post cytokine release profiles following haploidentical donor lymphocyte infusion

| Time from infusion | IFNγ | P-value | IL-2 | P-value | IL-6 | P-value | IL-10 | P-value |
|-------------------|------|---------|------|---------|------|---------|-------|---------|
| Pre-infusion       | 0.001| 4.5     | 12.65| 81.25   |      |         |       |         |
| 0–4 h             | 0.001| 0.37    | 2.83 | 0.10    | 4.66 | 0.81    | 73    | 0.62    |
| 8–24 h            | 7    | 0.03    | 56.3 | 0.02    | 25.8 | 0.46    | 17    | 0.8     |
| 34–48 h           | 10.25| 0.13    | 34.93| 0.08    | 22.17| 0.15    | 172.45| 0.25    |
| 72–96 h           | 6.4  | 0.13    | 5.4  | 0.46    | 12.59| 1.00    | 259.9 | 0.22    |
| 168–192 h         | 1.0  | 0.13    | 1.35 | 0.05    | 9.5  | 1.00    | 170.6 | 0.56    |

Bold numbers are statistically significant.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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