Post-transplant immunotherapy with WT1-specific CTLs for high-risk acute myelogenous leukemia: a prospective clinical phase I/II trial

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Wilms’ tumor antigen 1 (WT1) is more abundant in leukemic cells than in normal hematopoietic cells. Quantitative assessment of WT1 gene transcript abundance by real-time quantitative PCR (RQ-PCR) has been shown to be useful for predicting clinical outcome and prognosis in acute myelogenous leukemia (AML), and for detecting minimal residual disease (MRD) [1–3]. In addition, the expansion of WT1-specific CD8+ T cells was correlated with graft-versus-leukemia (GVL) effect in 10 subjects with acute lymphoblastic leukemia [4]. Autologous vaccination of AML patients with WT1 peptide or with full-length WT1 mRNA-electroporated dendritic cells (DCs) showed immunogenic and anti-leukemic activity, as evidenced by the conversion of partial remission and the induction of molecular remission [5, 6]. Adoptive transfer of WT1-specific T cells mediated antileukemic activity and persistence in relapsed or high-risk leukemia patients after hematopoietic stem cell transplantation (HSCT) [7, 8]. In the present prospective clinical phase I/II study with long-term follow-up, we demonstrated that adoptive transfer of WT1-specific cytotoxic T cells (WT1-CTLs) generated in vitro from donor-derived DCs transduced with an adenoviral vector expressing human WT1 (Adv-WT1) is a feasible therapeutic tool with acceptable safety that can induce an optimistic long-term clinical response accompanied by T-cell responses against WT1 in adult patients with relapse high-risk AML after allogeneic HSCT.

A total of 13 newly diagnosed adult patients treated for AML between 2007 and 2008 in the Catholic Blood and Marrow Transplantation Center were considered eligible for this study if they had a human leukocyte antigen (HLA)-identical sibling donor. The trial included five male and five female patients, with a median age of 40 years (range, 28–49 years), who were categorized as high-risk AML mainly based on the higher expression levels of WT1 at initial diagnosis [2, 9] and received an allogeneic sibling donor HSCT followed by anti-leukemic WT1-CTLs infusion (Table 1). For in vitro induction of WT1-CTLs from healthy donors, monocyte-derived DCs were transduced with an adenoviral vector for WT1 expression. The proportion of CD8+ and CD4+ T cells in the generated CTLs was 65.9 ± 15% and 25.9 ± 12% and the frequencies of WT1-specific IFN-γ-secreting CD8+ and CD4+ T cells were 147.3 and 305 per 106 cells, respectively. Beginning on D+35 post-transplantation, 4 × 107 WT1-CTLs were infused four consecutive times at 1-week intervals in patients without moderate to severe acute graft-versus-host-disease (GVHD). Every 1−3 months after the CTLs infusion, and for at least 1 year post transplantation, we serially monitored the clinical status, and the peripheral blood lymphocyte subpopulations using flow cytometry, the in vitro activity of interferon-gamma (IFN-γ) by enzyme-linked immunospot (ELISPOT) assay, and WT1 expression levels by RQ-PCR.

All patients were successfully engrafted; however, three of them (UPN 7, 8, and 9) died due to relapse after transplant. One of the two patients with treatment-related mortality (TRM) (UPN 2) died due to septic pneumonia and cytomegalovirus (CMV) disease in the gut combined with extensive-type GVHD at 1 year, and the other patient (UPN 6) died due to rapidly progressing gram-negative sepsis and a disseminated herpes simplex viral infection at 10 months after...
| UPN  | Diagnostic subtype | Age: D/R | Sex: D/R | Pre-HSCT status | Cytogenetics | Molecular/IP abnormality | WT1 level at Dx | AGvHD | CGvHD | TRM | Relapse | Outcome (as of May 31, 2017) |
|------|--------------------|----------|----------|----------------|-------------|------------------------|----------------|-------|-------|-----|---------|----------------------------|
| UPN1 | Hypoplastic        | 54/46    | M/F      | CR1            | 47 XX, +8   | —                     | High           | No    | Yes, extensive | No | No      | Alive, 10 y 10 m, Died, 1 y |
| UPN2 | MLD                | 37/41    | M/F      | CR1            | 46 XX, del(1q), −3, −5, +8, +11, −13, −17, 19, 3 − 3mar | CD7+       | No    | Yes, grade II | No | No      | Alive, 10 y 8 m             |
| UPN3 | M7                 | 45/43    | M/F      | CR1            | 46 XY, del(7) | FLT3-ITD +     | High           | No    | Yes, grade II | No | No      | Alive, 10 y 7 m             |
| UPN4 | M1                 | 36/39    | M/M      | CR1            | 46 XY, del(8;21) | FLT3-ITD +, CD7+ | High           | No    | Yes, grade II | No | No      | Alive, 10 y 10 m, Died, 1 y |
| UPN5 | M2                 | 26/28    | F/M      | CR1            | 46 XY, del(7) | c-kit D816V mutation+ | No            | No    | No    | No | No      | Alive, 10 y 8 m             |
| UPN6 | MLD                | 54/49    | F/M      | CR1            | 46 XY, t(11:19), der(20), (t(20:7?)) | —         | High           | No    | No    | No | No      | Alive, 10 y 4 m             |
| UPN7 | M2                 | 39/38    | M/M      | CR1            | 46 XX, t(11:15), add(18) | —         | High           | No    | Yes, extensive | No | No      | Alive, 9 y 2 m              |
| UPN8 | M0                 | 31/37    | F/F      | CR2i, CR1 after third induction | 46 XX, t(11:15), add(18) | —         | High           | No    | No    | No | No      | Alive, 9 y 2 m              |
| UPN9 | M1                 | 35/30    | M/M      | Untreated Relapse after CR2i | 46 XX, t(11:15), add(18) | —         | High           | No    | No    | No | No      | Alive, 9 y 2 m              |
| UPN10| MLD                | 41/31    |          | CR1            | 46 XX, t(6;9) | FLT3-ITD +     | High           | No    | Yes, limited | No | No      | Alive, 9 y 2 m              |

**MLD** multilinage dysplasia, **D** donor, **R** recipient, **M** male, **F** female, **WBC** white blood cell, **CRi** complete remission with incomplete recovery of CBC, **Dx** diagnosis, **HSCT** hematopoietic stem cell transplantation, **SCs** stem cell source, **IP** immunophenotype, **WT1** Wilms’ tumor gene 1, **AGVHD** acute graft-versus-host disease, **CGVHD** chronic graft-versus-host disease, **TRM** transplant-related mortality, **PBSC** peripheral blood stem cell, **BM** bone marrow, **G-BM** G-CSF-primed bone marrow, **NPM1wt** wild-type nucleophosmin gene.
transplant. However, these two patients had no evidence of relapse. The five other patients are alive, at a median follow-up of 127 months (range, 102–130 years), and the 8-year event-free survival (EFS) rate was 50%. In our previous studies, the long-term survival rates for high-risk patients who received allogeneic stem cell transplantation in CR1 without any adoptive immunotherapy is less likely around 30% [9, 10]. Among patients with complete remission 1 (CR1) pre-HSCT status, the EFS rate was 71.4%. These findings suggest that WT1-CTLs administration could induce prolonged remission only in patients without MRD, but not prevent the rapid proliferation of leukemic stem/progenitor cells even after transient hematological CR conditions established by myeloablative conditioning.

Despite the beneficial potential of WT1-CTLs therapy following allogeneic HSCT, it is necessary to consider the possibility of inducing severe chronic GVHD related to CTLs infusion. In the study, 4 out of 10 (40%) patients developed extensive type of chronic GVHD. As depicted in Table 1, UPN1 showed de novo type of chronic GVHD with skin and liver involvement and a typical manifestation of sicca 4 months after HSCT. UPN2 also showed a persistent type of chronic GVHD starting from grade I acute GVHD just early after the 3rd infusion of WT1-CTLs. UPN3 and UPN7 showed a multi-organ pattern of grade II acute GVHD involving the skin and gut after the final infusion of WT1-CTLs, and then progressed to extensive type of chronic GVHD until 6 months, 7 months after transplantation, respectively. Although, the precise causal relationship in association with the infused WT1-CTLs was not clear enough, patients having extensive type of chronic GVHD not in relapse were successfully manageable without any long-term sequelae, as shown in Table 1. Further revelation to clarify the direct effect of infused WT1-CTLs on chronic GVHD is quite anticipated in the future study.

The results from the long-term monitoring of the five living patients showed individual differences in the frequencies of WT1-specific CD8+ and CD4+ T cells and WT1 expression levels in peripheral blood mononuclear cells (Supplementary Figure 1). In UPN 1, there were predominant strong-specific T-cell responses with mostly CD4+ T cells following three peaks in WT1 expression. UPN 3 and UPN 4 showed mainly CD4+ T-cell responses around a single peak of WT1 expression. Among the patients with relapse, UPN 8 showed an increased, sustained, strong T-cell response as WT1 expression increased. Our data suggest that WT1-specific T-cell activity increases in response to an increase in the amount of WT1 antigen expressed by the leukemic cells in the patients, and that CD4+ T cells are as important as CD8+ T cells in inhibiting leukemia. Also, we measured the lymphocyte subpopulations in the patients’ blood for 140 weeks after the first WT1-CTLs infusion using multiparameter flow cytometry (MFC) (Supplement Figure 2). Various clinical features were observed in each patient and the clinical relevance of the prognosis was not observed. Supplementary Figure 1 has shown that WT1-CTLs is already present in some AML patients and dose not clearly demonstrate the persistence and efficacy of infused cells. But our previous pilot trial, we observed that frequencies of CD4+ T cells and CD8+ T cells increased progressively during serial infusion of WT1-CTLs and the pattern persisted until 9 months together with specific T-cell responses maintained against WT1 for more 2 years infusion [8]. In other previous study has shown that gene marking studies have been performed to identify cytotoxic T-cells infused after HSCT [11]. In this study, it was difficult to determine whether the engraftment and proliferation of infused cells. Therefore, further studies will be required to confirm the efficacy of infused donor cells by short tandem repeat (STR) test.

Taken together, despite the small number of patients enrolled in the study, this exploratory trial of WT1-CTLs after allogeneic HSCT for high-risk AML suggests the possibility of using this therapeutic strategy in combination with elective allogeneic or even autologous HSCT with WT1-CTL infusion. Generation of multi-antigen-specific T cells to enhance the GVL effect and prevent infection after allogeneic HSCT may be promising treatment in the near future, as well as the development of immunotherapy using T-cell receptor (TCR)-engineered T cells [12, 13].

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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