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LETTER TO THE EDITOR

Maintenance of complete remission after allogeneic stem cell transplantation in leukemia patients treated with Wilms tumor 1 peptide vaccine

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The prognosis of patients after allogeneic hematopoietic stem cell transplantation (HSCT) is still not satisfactory because, while treatment-related mortalities have decreased, relapse after HSCT remains a major concern. The effectiveness of allogeneic HSCT for hematological malignancies is the result of immunologic rejection of recipient leukemia cells by donor T cells, known as the graft-versus-leukemia (GVL) effect.[1] It is thus obviously important to be able to exploit the GVL effect while minimizing graft-versus-host disease (GVHD). A targeted anti-leukemic immunotherapy, such as use of a leukemia vaccine,[2] is a promising strategy to boost the GVL effect.

Wilms tumor 1 (WT1) protein is one of the best targets for leukemia vaccines. Overexpression of the wild-type *WT1* gene has been detected in all types of human leukemia.[3–5] We performed a phase I clinical study of immunotherapy targeting the WT1 protein in patients with leukemia, and were able to show that WT1 vaccination was safe and could induce WT1-specific cytotoxic T lymphocyte (CTL).[6] Furthermore, reduction of minimal residual disease and long-lasting complete remission (CR) was observed in some leukemia patients who were given the WT1 vaccine.[7]

This report presents the results of phase I clinical study of WT1 vaccination for HLA-A*2402-positivie post-HSCT patients who were at high risk of relapse (HSCT in non-CR and 2nd HSCT for post-transplant relapse) or had already relapsed. The HLA-A*2402-restricted modified 9-mer WT1 peptide (amino acids 235–243 CYTWNQMNL)[8] was emulsified with Montanide ISA51 adjuvant. Patients were intradermally injected with 1.0 mg (three patients: UPNs 1, 4 and 6) or 3.0 mg (other six patients) of WT1 peptide four times weekly. When no adverse effects and no obvious disease progression were observed after the fourth injection, further WT1 vaccinations at 2-week intervals were administered.

Nine patients (five with acute myeloid leukemia (AML), one each with acute lymphoblastic leukemia, chronic myelomonocytic leukemia, multiple myeloma and T-cell lymphoblastic lymphoma) were enrolled in this study (Supplementary Tables 1 and 2). Local inflammatory response was observed at the vaccine injection sites of all patients. One patient (UPN5) suffered mild hypoxia (PaO_2 65 mm Hg at room air) and restrictive pulmonary dysfunction ($FEV_{1.0}$ 40%) 65 days after the start of WT1 vaccination (day 199 after HSCT; Figure 1a). He was diagnosed with bronchioleitis obliterans (BO), which was a symptom of chronic GVHD. The patient recovered soon after administration of inhaled steroids. While early and sudden discontinuation of prednisolone and tacrolimus (day 103 after HSCT) were considered to be the reason for development of BO, the possibility of an association between BO and WT1 vaccination cannot be entirely ruled out. In other eight patients, no severe toxicities related to WT1 vaccine were observed (Table1).

Three AML patients (UPN1–3), who had undergone HSCT in non-CR, started WT1 vaccine in CR (Supplementary Tables 1 and 2). They started WT1 vaccination on post-HSCT days 141, 76 and 93 and have remained in CR for 1038, 973 and 662 days, respectively (as of 8 April 2013; Table1), suggesting the potential of WT1 vaccination as a maintenance therapy after HSCT.

Six patients started WT1 vaccination in non-CR and two of them became CR after WT1 vaccination. One B-ALL patient (UPN4) with MLL-AF4 underwent bone marrow transplantation from an HLA-matched unrelated donor during the first CR. On post-HSCT day 111, MLL-AF4 and WT1 mRNA in peripheral blood (PB) had increased to 16 000 and 15 000 copies/μg RNA, indicating that the disease had relapsed. Tacrolimus and prednisolone doses were tapered off to induce GVL effects. The expression levels of MLL-AF4 and WT1 mRNA in PB had decreased to 2700 and 190

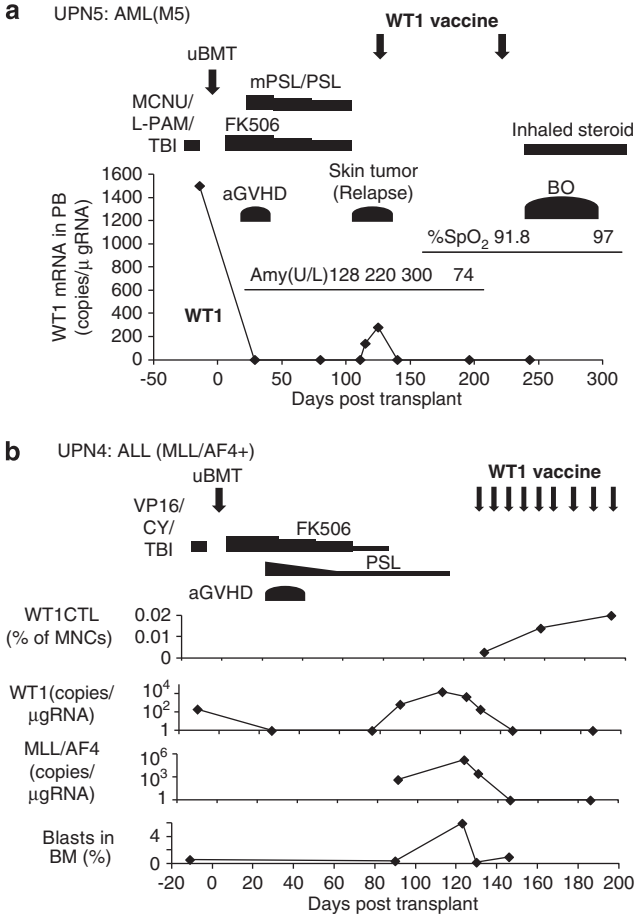

Figure 1. Clinical course of patients who attained CR after the start of WT1 peptide vaccination. (**a**) Clinical course of UPN5 who achieved CR after administration of WT1 vaccine but stopped vaccination because of the development of bronchioleitis obliterans. (**b**) Clinical course of UPN4. Residual leukemia cells that were detected by MLL/AF4 expression disappeared after the start of WT1 vaccination. In both cases, rapid tapering of immune-suppressive drugs preceded WT1 peptide vaccination.

Table 1. Patient outcomes

UPN	Disease	Status before vaccination	Adverse events	Number of vaccine doses	Outcome	Additional therapy	Survival	
							Post-HSCT	After start of vaccination
1	AML (M4)	CR	None	54	CR	−	1179+	1038+
2	AML(M4, DEK/CAN+)	CR	PLT↓	52	CR	−	1049+	973+
3	AML	CR	None	38	CR	−	759+	662+
4	B-ALL (MLL/AF4+)	Molecular relapse	None	71	CR	−	1312+	1179+
5	AML (M4)	Relapse	Amylase↑, bronchileitis obliterans (cGVHD)[a]	2	CR	−	972+	842+[b]
6	CMMoL	Relapse	None	25	PD[c]	Chemo	2265	381
7	MM	PD	None	19	PD	Chemo	1301+	804+
8	T-LBL	Relapse	None	4	PD	Second transplant	955	656
9	AML (M2)	Relapse	None	17	PD	Second transplant	1544+	749+

Abbreviations: ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CMMoL, chronic myelomonocytic leukemia; CR, complete remission; cGVHD, chronic graft-versus-host disease; HSCT, hematopoietic stem cell transplantation; MM, multiple myeloma; PD, progressive disease; T-LBL, T-cell lymphoblastic lymphoma. (8 April 2013). [a]A causal relationship between vaccination and this event was not strongly suspected, but could not be ruled out. [b]Vaccination was discontinued. (The last injection was on post-HSCT day 60). [c]Size of the subcutaneous tumor decreased, but the disease relapsed in axial lymph nodes and stomach.

copies/μg RNA by day 132, and WT1 vaccination was started on day 133. MLL-AF4 mRNA had become undetectable by day 146, and had never appeared until post-HSCT day 1312 (day 1179 after the start of WT1 vaccination as of 8 April 2013; Figure 1b).

Skin tumors appeared in UPN5 (AML-M5) on post-HSCT day 103 and was diagnosed by biopsy as leukemia relapse. Tacrolimus was discontinued on day103, and WT1 vaccination was started on day 130. Cutaneous tumors had regressed 2 weeks after the start of WT1 vaccination, but vaccination was terminated after the second injection because of the development of BO as described earlier (Figure 1a). This patient has been remained in CR until post-HSCT day 972 (day 842 after the start of WT1 vaccination at 8 April 2013). While the exact contribution of the vaccination effect to the disease remission in addition to the GVL effect was unclear, the fact that both of these two patients still have remained in CR until now is encouraging to continue this trial. In the following phase II trials, the enumeration of WT1-specific CTLs should be performed more frequently after the start of vaccination to clarify the relationship between the effect of WT1 peptide vaccination and leukemia regression.

WT1 (a natural 9-mer WT1 peptide) HLA-A*2402 tetramer assays could be performed with peripheral blood mononuclear cell in seven of the nine patients to determine whether $WT1_{235}$ peptide-specific $CD8^+$ T cells had increased after WT1 vaccination. The gates for WT1 $tetramer^+$ cells were drawn as $<0.1\%$ of $CD8^+$ T cells were included in the tetramer-positive gate in multiple healthy individuals (Supplementary Figure 1A). $WT1_{235}$ $tetramer^+$ cells increased after the start of vaccination in three (UPNs1, 2 and 4) of the four patients who have remained in CR (Figure 1b and Supplementary Figure 1B). In the cases with progressive disease, continuous increase in the frequencies of $WT1_{235}$ $tetramer^+$ cells was not observed (Supplementary Figure 1B).

Our results suggest that WT1 vaccination should be started when the leukemia burden is minimal. The timing of the start of WT1 vaccination may be also important. For the cases with good outcomes, WT1 vaccination was started 76–140 days after transplantation (UPNs1–5), and at later times (days 299–1815) for PD cases (UPNs 6–9). A lymphopenic environment a few months after transplantation may be favorable for rapid and extensive expansion of tumor antigen-specific CTLs.

In summary, this report suggests that WT1 vaccine can be safely administrated for post-HSCT patients with hematological malignancies and has potential as a maintenance therapy. Clinical benefit of WT1 vaccination for post-HSCT patients will be evaluated in the subsequent phase II trials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies this paper on Blood Cancer Journal website (http://www.nature.com/bcj)