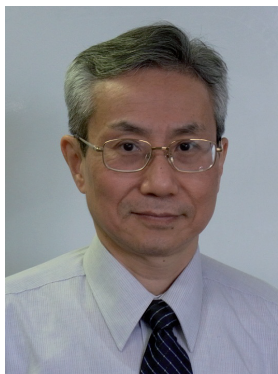


EDITORIAL

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“It is undisputed that the WT1-CTL peptide vaccine has biological activity to elicit WT1-specific immunological responses potent enough to induce clinical responses.”

A vaccine for leukemia: one step closer? Cure-oriented WT1 peptide vaccination therapy is being developed

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Leukemia antigen-targeting vaccination therapy is a naturally occurring demand & its establishment is now desired

For the treatment of hematological malignancies, including leukemia, striking advances in chemotherapy, targeted therapy such as tyrosine kinase inhibitors, and hematopoietic stem cell transplantation (HSCT), have been made. However, unfortunately, a substantial proportion of patients relapse after treatment. In this context, a novel treatment is desired and one of the more attractive candidates is a vaccine therapy.

HSCT is the most intensive treatment for leukemia. The HSCT confers a powerful T-cell-mediated graft-versus-leukemia effect to eradicate leukemic cells and, therefore, is a direct evidence for the effectiveness of T-cell-mediated immune reaction in leukemia treatment. The undisputed effect of graft-versus-leukemia provided us with an idea that autologous T lymphocytes, as well as HSCT donor-derived T cells, might eradicate leukemic cells if the autologous T cells could recognize leukemia antigens and be activated strongly enough.

How does vaccination with leukemia antigen-derived peptides show efficacy? WT1 peptides as the model

Wilms' tumor gene (*WT1*) possesses oncogenic functions, and is expressed in hematological malignancies, including leukemia, and various kinds of solid cancers. These findings led us to expect that the gene product WT1 protein should be an attractive leukemia antigen, and furthermore, a nearly universal cancer antigen. To prove that WT1 is a target antigen recognized by CD8⁺ cytotoxic T lymphocytes (CTLs); that is, WT1-specific CTLs, we had to identify epitope peptides that could induce WT1-specific CTLs among the whole WT1 amino acid sequence. Once these peptides are identified, the following scenario is expected [1]. Intradermal or subcutaneous injection of WT1 peptide vaccine, composed of WT1-CTL peptides and adjuvant, makes activated dendritic cells with 'WT1 peptide/HLA class I' complex on the cell surface, which migrate to lymph nodes, where the dendritic cells activate these complex-specific T cell receptor-bearing CTLs. These CTLs seek and recognize 'WT1 peptide/HLA class I' complexes on the surface of leukemia or solid cancer cells, and kill them [1].

In 2000, several groups, including ours, first reported identification of HLA class I-restricted WT1-CTL peptides [2–4], and this year our group also reported a mouse model for WT1-targeting cancer immunotherapy with the identification

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of a mouse major histocompatibility complex class I-restricted WT1-CTL peptide [5]. These findings demonstrated that WT1 should be a novel target antigen for cancer immunotherapy [6], and urged us to constitute clinical trials of WT1 peptide-based immunotherapy for leukemia and solid cancer.

WT1 is one of the most promising target antigens for immunotherapy against leukemia as well as solid cancers

As mentioned before, *WT1* possesses oncogenic functions, and therefore, its expression is thought to be essential for the transformed character of malignant cells, which is a theoretical advantage of WT1 as a target antigen for immunotherapy, because tumor escape from immunological attack due to down-regulation of *WT1* in tumor cells is unlikely to occur [1]. This feature of *WT1*'s oncogenic function, the gene expression in many kinds of malignancies, and WT1 protein's high immunogenicity make WT1 a superior target antigen for cancer immunotherapy [1], and a recent review article rated WT1 the most promising cancer antigen [7]. In addition, it was suggested that leukemia stem cells, which should be a key target for leukemia treatment, express WT1 [8,9], which also supports the superiority of WT1 as the target antigen for leukemia immunotherapy.

“If we appropriately choose the clinical settings for the vaccine's use, such as MRD stage, the vaccine should become a novel and powerful means to lead leukemia patients with high risk of relapse to a long-lasting remission or a cure.”

In 2003, we reported a reduction of leukemic cell burden in two cases with myelodysplastic syndromes treated by WT1 peptide with restriction of HLA-A*2402, which is the most common HLA class I type in Japanese people [10]. This is the first report of a clinical response induced by WT1 peptide vaccination. Subsequently, we demonstrated a correlation between immunological responses, that is, an increase in WT1-specific CTL frequencies in peripheral blood, and clinical responses among patients with leukemia and solid cancers treated by HLA-A*2402-restricted WT1 peptides [11]. This result, a correlation between immunological responses and clinical responses, strongly suggested that WT1 peptide vaccination induced WT1-specific immunological responses, which led to emergence of clinical responses. In other words, ‘proof-of-concept’ for WT1 peptide vaccination might be demonstrated by this study. As for patients with HLA-A*0201, which is the most common HLA class I type in Caucasians, Mailänder *et al.* first reported WT1 peptide vaccination-driven induction of a patient with acute myeloid leukemia (AML) into a durable

complete remission in 2004 [12]. Subsequently, investigations that showed the WT1 peptide vaccination-driven immunological response and/or clinical antitumor effect for patients with HLA-A*0201 or HLA-A*2402 are being accumulated [13–15]. The target diseases include not only hematological malignancies, including leukemia, myelodysplastic syndromes, and multiple myeloma, but also solid cancers [15,16]. Furthermore, tumor vessels were also shown to express WT1, indicating that tumor vessels, as well as tumor cells, might become a target for WT1 peptide vaccine.

Minimal residual disease state achieved by conventional treatment should be a clinical setting for leukemia vaccine to work best, leading to a cure

It is natural to expect that a state of minimal residual disease (MRD) is better for leukemia vaccine to work than that of high leukemia or tumor burden, because the effector/target ratio is higher in MRD state [16]. In the context of this expectation, we treated a patient with chronic myelogenous leukemia with WT1 peptide vaccine after leukemic cells were reduced to MRD state by imatinib, a molecular target-based drug [17]. After the start of vaccination without cessation of imatinib, that is, a combined therapy, the *bcr-abl* mRNA level – an essential marker of chronic myelogenous leukemia burden – was further decreased [17]. As for the usefulness of WT1 peptide vaccination performed at MRD state, our group very recently reported the following two important results [18,19].

Tsuboi *et al.* reported WT1 peptide vaccination-driven induction of three AML patients to long-term survival without relapse [18]. Before the vaccination, all three patients were in hematological remission, but had MRD, because *WT1* mRNA levels were beyond the upper limit. It is natural to think that hematological relapse was impending in these patients, and HSCT might be needed to cure them. However, continuation of WT1 peptide vaccination led the patients to more than 8-year survival without relapse, and furthermore, the normalization of *WT1* mRNA levels was achieved. It is conceivable that the three AML patients with high risk of relapse have been cured.

Hashii *et al.* reported that WT1 peptide vaccination induced a decrease in MRD levels, such as *WT1* and *AML/MTG8* mRNA levels, in three pediatric leukemia patients with high risk of relapse after HSCT, leading to durable maintenance of remission for two out of the three patients [19]. Immunological environment in patients after HSCT has distinctive features, because:

- Main effector T cells that attack leukemia cells are the donor-derived ones, which have not been exposed to leukemia cell-derived high amount of cancer antigens;

- The effector T cells are composed of peripheral blood's mature T cells, contaminated in the stem cell sources, such as bone marrow, and relatively naive T cells that have been newly generated from engrafted hematopoietic stem cells;
- A homeostatic proliferation-like state exists.

WT1 peptide vaccination after HSCT may not only be a great boon for leukemia patients but also provide us with an opportunity to further analyze cancer immunity to develop better immunotherapy.

Taken together, these two successful results strongly suggested that WT1 peptide vaccination performed in MRD stage is a very promising treatment to lead leukemia patients with high risk of relapse to long-term maintenance of remission, or possibly a cure. In the context of the usefulness of a leukemia vaccine for patients with low leukemia burden, Van Tendeloo *et al.* demonstrated that WT1-targeted dendritic cell therapy induced AML patients with partial remission to durable complete remission [20].

Conclusion & future perspective

It is undisputed that the WT1-CTL peptide vaccine has biological activity to elicit WT1-specific immunological responses potent enough to induce clinical responses [1,15,16]. So far, WT1-CTL peptides with restriction of HLA-A*0201 or A*2402 were well characterized in the

clinical as well as preclinical aspects, respectively, and a considerable proportion of the human population are considered to have either or both of the two HLA class I types. If we appropriately choose the clinical settings for the vaccine's use, such as MRD stage, the vaccine should become a novel and powerful means to lead leukemia patients with high risk of relapse to a long-lasting remission or a cure. In addition to WT1-CTL peptides, WT1 peptides that induce WT1-specific CD4⁺ helper T-cell responses, which should be able to promote induction and activation of WT1-specific CTLs, were also identified [1]. These helper T cell-inducing peptides, that is, WT1-helper peptides, are HLA class II-restricted. Since promiscuous binding of helper peptides, including the WT1₃₃₂-helper peptide we identified, to diverse HLA class II types is a generally conceivable phenomenon, the WT1-helper peptides are considered to cover a large proportion of the human population. Combined use of WT1-CTL peptides and WT1-helper peptides, which is feasible in the clinical settings, should become a further potent leukemia vaccine [1,15,16].

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