

Recent Advances of Peptide Vaccine Therapies in Hematological Malignancies

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Hematological malignancies include the acute and chronic myeloid and lymphocytic leukemia, multiple myeloma and the non-Hodgkin's lymphomas. Most are of B-cell origin (acute and chronic lymphocytic leukemia, multiple myeloma, and B-cell lymphomas), while the myeloid leukemia retain features of monocytes, macrophages, neutrophils and even platelets^[1,2,3]. While the details vary for individual cancers, treatments are generally based on cytotoxic drugs, which are variably effective and have high complication rates. New approaches to complement these existing treatments are needed to improve cure rates and decrease toxicity^[4,5,6].

One promising approach for targeting hematological malignancies involves the cellular immune system through activation of highly efficient T lymphocytes that mediate key functions such as cytotoxicity, regulation of effector cells and induction of immunologic memory^[7,8,9]. Earlier clinical studies have shown that dendritic cell vaccination for the priming of naive T cells can generate tumor-specific cytotoxic T lymphocytes (CTLs) and induce remission in pretreated patients with B-cell malignancies^[10-11]. In addition, infusion of HLA-matched allogeneic T lymphocytes has been shown to induce durable long-term remissions in relapsed lymphomas, chronic lymphocytic leukemia, or multiple myeloma after stem cell transplantation^[12]. However, only a limited percentage of patients with hematological malignancies accomplish complete remission following donor lymphocyte infusion and the patients are at risk of developing graft-versus-host disease, which can be associated with significant morbidity and mortality. Therefore, developing tumor-associated antigens (TAA)-based immunotherapies against specific TAA offer an attractive approach for boosting patients' immune system to treat hematological malignancies. Given the therapeutic potential of TAA-specific CTLs, an important issue is how best to develop clinically applicable immunotherapeutic strategies with these antigens. Recent advances in the field of molecular biology and tumor immunology have resulted in the identification of TAA-specific epitopes recognized by HLA class I or II-restricted T cells from malignant neoplasms and most translational research has focused on using those epitopes for the possibility of developing the peptide-based cancer immunotherapy.

Recent studies have identified a variety of TAA that elicit specific CD8⁺ T cell responses against hematological malignancies and the progress in the identification of

TAA has stimulated the development of vaccines to treat the specific diseases. Among them, normal self-proteins that are over-expressed on malignant cells can serve as potential target antigens for immunotherapy^[13,14,15,16]. Self-proteins have been extensively studied as target tumor-antigens, demonstrated induction of CTLs with antigen-specific immunologic activity^[17,18,19,20,21,22,23,24,25,26,27]. Surface molecules overexpressed in leukemia and lymphoma, such as CD19 and CD20, make relevant targets for developing immunotherapeutic strategies for treating hematological malignancies^[28]. CD19, a B lineage-specific transmembrane glycoprotein, functions as a central response regulator in B cells. The antigen is expressed during all stages of B-cell differentiation, is down-regulated on plasma cells, and is maintained on cells that have undergone neoplastic transformation, but importantly not in hematopoietic stem cells^[29]. It is expressed on >95% of cells in patients with B-cell lymphoma, chronic lymphocytic leukemia (CLL), and on the acute lymphocytic leukemia (ALL) progenitor cells^[30,31,32,33]. CD20 is a non-glycosylated integral membrane phosphoprotein involved in regulation of B-cell proliferation and differentiation. It is expressed slightly later in B-cell development than CD19, is not rapidly internalized, is expressed at a high surface density on the vast majority of lymphomas, and is eventually down-regulated on terminally differentiated plasma cells^[34,35,36]. Rituximab, is an antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes and is one of the first monoclonal antibodies approved for use in human cancers. It is extensively used in the treatment of B-cell malignancies, such as CLL and non-Hodgkin's lymphomas (NHLs) and induces favorable clinical responses in the patients; however, this antibody alone is not curative with most responders achieving only partial remissions with a limited mean time to disease progression following antibody treatment^[37,38,39]. Thus, combinational therapy of peptide vaccination or administration of peptide induced antigen-specific T cells in conjunction with conventional therapies could improve treatment outcome for patients with B cell malignancies. Based on this rationale, CD19- or CD20-specific immunogenic HLA-A2 peptide was identified, and the cytotoxic activity of CD19-CTLs or CD20-CTLs stimulated with respective CD19150-158 (KLMSPKLYV) or CD20188-196 (SLFLGILSV) peptide was investigated²⁸. The CTLs displayed HLA-A2-restricted cytotoxic activity against a broad range of malignant B cell lines including human CLL, multiple myeloma, and Burkitt's lymphoma cell

lines, expressing the corresponding antigen. Besides cytotoxicity, antigen-specific and HLA-A2-restricted cell proliferation and IFN- γ secretion were demonstrated in response to re-stimulation of CTLs with tumor cells. Thus, these immunogenic peptides derived from the CD19 and CD20 self-antigens identified offers promising immunotherapeutic strategies for targeting a broad range of B-cell malignancies. In developing immunotherapeutic approach by CD19 or CD20 peptides, clinical sequel of temporary B-cell lymphopenia may be an acceptable side effect, especially because prolonged ablation of normal CD20+ B cells in patients receiving Rituximab therapy does not seem to result in clinically significant complications attributable to depleted numbers of normal B cells^[40,41,42]. Future investigation needs to perform for the clinical relevance of using these peptides either for infusion of the peptide-specific CTLs or vaccination.

Alternatively, surface molecules CD33 which is highly overexpressed in AML can serve as potential immunogenic target antigen for developing immunotherapeutic strategies^[43,44,45]. CD33, a 67 kDa-size member of the sialic acid-binding receptor family and a myeloid lineage cell surface marker, offers a unique opportunity for developing an antigen-specific immunotherapy for AML due to its over-expression on greater than 90% of AML blasts and a lack of expression on pluripotent stem cells^[46,47,48]. Myelotarg, a monoclonal antibody conjugated to calicheamicin that targets the CD33 antigen, has shown efficacy in the treatment of AML patients^[49-50]. However, the treatment has serious side effects in some patients caused by the conjugated chemotherapy drug and that may be overcome using a CD33 targeted cellular based therapy. Therefore, identifying peptides that elicit the antigen-specific CTLs targeting leukemia cells could lead to a novel cellular immunotherapy with reduced toxicity for the patients. Based on the justification, immunogenic HLA-A2-specific CD33 peptide was identified which was capable of inducing CTLs targeted to AML cells^[43]. The CD33 peptide-specific CTLs displayed HLA-A2-restricted cytotoxicity against both mononuclear cells from AML patients and the AML cell line. The peptide- as well as AML cell-specificity of CD33-CTLs was demonstrated and the secretion of IFN- γ by the CTLs was detected in response to the CD33 peptide stimulation. However, a concern with CD33 antigen-based immunotherapy is the possible targeting of normal myeloid cells due to CD33 expression on mature monocytes, granulocytes, and progenitor cells during myeloid cell differentiation^[51-52]. In further study evaluated CD33-CTLs, no significant cytotoxicity of the CTLs was detected against normal CD14+ monocytes, suggesting that the level of CD33 expression on these cells might be insufficient for

recognition and lysis by the antigen-specific CTLs and the potential use of CD33 as a target antigen for cellular therapy in AML^[43]. Alteration of the native CD3365–73 peptide at the first amino acid residue from alanine (A) to tyrosine (Y) enhanced the HLA-A2 affinity/stability of the modified CD33 peptide (YIISGDSPV) and induced CTLs with increased cytotoxicity against AML cells^[43]. More effective heteroclitic analogs of the CD33 peptide were developed from the modified CD33 peptide (YIISGDSPV) through examining the effects of additional amino acid substitutions on peptide immunogenicity against AML^[44]. Further modifications of peptide were made through substitution of key amino acid residues within the CD33 peptide sequence to increase HLA-A2 affinity or interaction with the TCR. Of modified CD33 peptides tested, the YLISGDSPV epitope displayed the highest HLA-A2 affinity/stability, which correlated with the induction of highly cytotoxic CTLs response against AML cells, demonstrating that the potential of using heteroclitic CD33 peptide in developing an immunotherapy for the treatment of AML patients. Clinical benefit of the heteroclitic CD33 peptide needs to be further investigated.

BCR-ABL is a constitutively active tyrosine kinase, which has the central role in the pathogenesis of CML^[53,54,55]. The characteristic cytogenetic hallmark in CML patients is a shortened chromosome 22, which is named as Philadelphia chromosome. The disease, a clonal proliferative disorder of the primitive hematopoietic stem cell, is characterized by the [t (9;22) (q34;q11)] chromosomal translocation. This reciprocal translocation between the breakpoint cluster region gene (bcr) and the c-abl gene is translated into p210 BCR-ABL fusion protein^[56-57]. Peptides derived from the bcr-abl junction region can act as neoantigens and, thus, as targets of specific immune responses, therefore investigators have been identified peptides to bind to the specific HLA alleles and tested the immunogenicity^[58,59,60]. HLA-A2-restricted junction peptides showed to elicit CTLs responses *in vitro*, but whether or not they are naturally processed is controversial^[61,62,63]. An HLA-A3-associated tumor-specific breakpoint peptide has been eluted from primary CML cells, and specific CTLs have been detected in HLA-A3 CML patients⁶⁴. In addition, the specific MHC tetramer-positive cell population was shown to be associated with a lower tumor burden in CML patients, suggesting that BCR-ABL-specific CTLs might participate in the control of disease^[65,66,67]. Clinical trials of BCR-ABL peptides were performed in several studies. Pinilla-Ibarz J. et al evaluated the safety and immunogenicity of a multi-dose, bcr-abl breakpoint peptide vaccine in 12 adults with chronic-phase CML^[63]. Cohorts of 3 patients each received different doses of peptide mixed with QS-21. All 68 vaccinations

were well tolerated without significant adverse effects. In 3 of the 6 patients treated at the 2 highest dose levels of vaccine, peptide-specific, T-cell proliferative responses ($n = 3$) and/or DTH responses ($n = 2$) were generated, however, the antigen-specific CTLs have not been identified. Bocchia et al. has evaluated the immunogenicity of BCR-ABL peptides in a trial of 16 patients who had CML (with the b3a2 fusion point of p210) by vaccinating five b3a2 breakpoint-derived peptides^[68]. Treatment consisted of six subcutaneous vaccinations every 2 weeks with QS-21 and GM-CSF as adjuvant to increase peptide immunogenicity. In the vaccine trial outcome, all patients' cytogenetic responses were improved after six vaccinations with five reaching complete cytogenetic remission (CCR). Of ten patients on imatinib, nine started peptide vaccines having had a median of 10 months' stable cytogenetic disease, whereas one started in stable CCR. The peptide-specific delayed-type hypersensitivity (in 11 of 16 patients), CD4 T cell proliferation (13 of 14 assessed), and interferon- γ production (five of five assessed) were observed. However, this was a non-randomized trial and patients were allowed to continue imatinib or interferon therapy, it is difficult to determine the true impact of vaccination on the observed clinical efficacy.

Other studies have focused on antigens derived from normal tissue proteins that can behave as TAAs in leukemia. Primary granule proteins (PGP), a group of serine proteases or closely related molecules found in cells of the granulocyte series, is aberrantly expressed in myeloid leukemic progenitor cells, thus PGP is of great interest as a source of leukemia-restricted antigens for cancer immunotherapy. The basis of the specificity of CTLs for myeloid leukemia appears to be the aberrant expression of azurophil granule proteins in CML as well as in AML and myelodysplastic syndrome (MDS)^[14]. Among PGP, myeloid leukemia-specific antigens proteinase 3 (PR3) and neutrophil elastase (NE) are considered as the major groups in the application of immunotherapy due to their high concentrations in precursors and mature neutrophils^[69,70,71]. PR3 might be important in maintaining the leukemia phenotype because PR3 antisense oligonucleotides halt cell division and induce maturation of the HL-60 promyelocytic leukemia cell line^[72] and NE may be responsible for the clonal dominance of CML cells^[73,74]. Additionally, both PR3 and NE are associated with autoimmune diseases such as Wegener granulomatosis, and there is evidence of specific humoral and cellular immunity in patients with these diseases^[75]. PR1 (VLQELNVTV), an HLA-A2-restricted peptide derived from both PR3 and NE, was identified and demonstrated to elicit CTLs that kill myeloid leukemia cells but not normal marrow cells^[76,77,78]. PR1-specific CTLs were detected using

tetramers analysis in the peripheral blood of leukemia patients but to a lesser extent in healthy donors^[79-80]. The CTL are present at significant frequencies in CML patients in remission and the presence of PR1 responses has been found to correlate with complete remission in the patients after stem cell transplant^[81,82,79,80], suggesting that PR1-specific T-cell responses are implicated in controlling leukemia. PGP has been evaluated in clinical trials as immunotherapeutic target antigens in PR1 peptide vaccination for myeloid leukemia. In Phase I and II studies, 37 patients with AML, CML or MDS received PR1 peptide plus IFA and GM-CSF every three weeks for a total of three vaccinations^[83]. A significant increase of PR1-specific T cells after vaccination was observed in 22 out of 37 (60%) patients and clinical remission was observed in 11 (30%) patients. Durable molecular remissions were noted in three patients with refractory AML, which lasted for up to three years follow up. In addition, patients demonstrating a clinical response were shown to have PR1-specific T cells that had higher TCR avidity compared with non-responders ($p = 0.02$), supporting the previous observation that high avidity PR1-CTLs might predict long-term immunity to leukemia^[84]. The trial is still ongoing for further investigation of its efficacy in Phase III trial.

Wilms' tumor gene *WT1*, a zinc finger transcription factor expressed at low levels by immature CD34⁺ progenitor cells, is overexpressed in the leukemia cells^[85,86,87,88,89]. The *WT1* gene was originally isolated as a gene responsible for Wilms' tumor, a pediatric renal cancer. Although the *WT1* gene was first categorized as a tumor suppressor gene, Wilms' tumor 1 (WT1) may be a promising potential target antigen in immunotherapeutic trials, because of following reasons; (1) it is overexpressed in AML, ALL, CML, and MDS^[90,91,92], (2) it is apparently immunogenic as shown by spontaneous immune responses in leukemic patients^[93,94,95], (3) it induces growth inhibition of leukemic cells by treatment with WT1 antisense oligomers^[96-97], and (4) it blocks differentiation, but induces proliferation, of wild-type WT1 gene-transfected myeloid progenitor cells in response to granulocyte colony-stimulating factor^[98-99]. Immunogenic HLA-restricted T cell epitopes have been identified from WT1 and their CTLs specific for WT1 are selectively cytotoxic to myeloid leukemia. The antigen-specific CTLs were generated *in vitro* by stimulation of T lymphocytes with the peptides and the CTLs specifically lysed WT1-expressing tumor cells with HLA class I restriction^[100,101,102]. Occurrence of WT1 leukemia-reactive CD8⁺ T cells was detected in low frequencies in healthy individuals and at higher frequencies in patients (peripheral blood, tumor-draining lymph nodes) with CML or AML^[103,104,105,106]. In addition, IgM and IgG WT1 antibodies have been detected in patients with hematopoietic malignancies, indicating

that the WT1 protein was highly immunogenic, and that immunoglobulin class-switch-inducing, WT1-specific, cellular immune responses were elicited in these patients^[107]. These results provided investigators with the rationale for elicitation of CTL responses targeting the WT1 product for cancer immunotherapy. On the basis of these findings, clinical studies were performed to determine the feasibility and potential efficacy of WT1 peptide vaccination. In a small phase I clinical trial, biweekly vaccination was performed with HLA-A24 restricted WT1 peptide, 2 patients with MDS developed severe leukocytopenia in association with a reduction in leukemic blast cells and levels of WT1 mRNA after only a single vaccination of WT1 peptide. This finding indicates that the WT1-specific CTLs elicited by WT1 vaccination eradicated the antigen-expressing transformed stem or progenitor cells and that MDS patients with little normal hematopoiesis required a new strategy of WT1 vaccination to avoid severe leukocytopenia. In addition, the study showed the decrease of leukocyte and monocyte counts and increase of WT1-specific CTLs in association with a reduction in the WT1 mRNA level after the start of biweekly vaccination, which demonstrates that vaccination with WT1 peptide, can induce the antigen-specific immune responses and resultant clinical responses^[108].

Recently, the safety and immunogenicity were evaluated in a combined vaccine of 2 leukemia-associated antigenic peptides, PR1 and WT1^[109]. In the study, eight patients with myeloid malignancies received one subcutaneous dose each of PR1 and WT1 vaccines in Montanide adjuvant with GM-CSF. CTLs against PR1 or WT1 were detected in 8 of 8 patients after a single vaccination using tetramer intracellular and interferon- γ analyses. In the vaccinated patients, the emergence of PR1⁺ or WT1⁺ CTLs was associated with a decrease in WT1 mRNA expression as a marker of minimal residual disease, suggesting anti-leukemia effect by a combined PR1 and WT1 vaccine. Preliminary results of phase II trial have showed a complete remission in one patient with recurrent AML that received four biweekly and then monthly vaccinations with WT1 peptide plus KLH and GM-CSF for a total of 15 vaccinations^[110]. A Phase II study of WT1 peptide vaccination in AML and MDS patients is currently ongoing.

Human telomerase reverse transcriptase (hTERT), the catalytic subunit of telomerase, is an attractive target antigen for cancer immunotherapy due to its expression in the vast majority (>85%) of human cancers including leukemia, but not in most normal cells^[111-112]. Human telomerase is a ribonucleoprotein enzyme and plays a key role in determining telomere length and cellular

replicative life span^[113]. Since most human cancers have high levels of telomerase activity, immunotherapeutic strategies aimed at this antigen may have broad clinical applications. The major concern of utilizing hTERT as TAA would be cytolysis of the few normal cell types in which telomerase can be detected. Telomerase activity has not been detected in adult cardiac, renal, hepatic, pulmonary, neural, skeletal, and adipose tissues; however, hematopoietic stem cells and progenitors, germinal center cells, basal keratinocytes, gonadal cells, and certain proliferating epithelial cells have been reported to have measurable telomerase activity^[114,115,116]. hTERT peptides to bind to MHC class I or II molecules were identified and they were found to be able to induce primary human T-cell responses in vitro^[117,118]. It has been reported that a 9-mer peptide (ILAKFLHWL) derived from hTERT₅₄₀₋₅₄₈ is capable of eliciting HLA-A2-restricted CTLs^[119]. CTLs specific for this hTERT peptide appeared to lyse tumor cells, including leukemia cells in an HLA-A2-restricted manner. Arai et al. (2001) have identified two HLA-A24-specific peptides derived from hTERT, hTERT₃₂₄₋₃₃₂ (VYAETKHFL) and hTERT₄₆₁₋₄₆₉ (VYGFVRACL), which can elicit hTERT peptide-specific CTLs. The CD8⁺ CTL clones specific for these hTERT peptides appear to be cytotoxic against HLA-A24⁺ leukemia cells but not against HLA-A24⁻ leukemia cells or HLA-A24⁺ normal cells. The efficiency of hTERT peptide remains to be evaluated in leukemia patients although the clinical effectiveness has been examined in patients with solid tumors including breast cancer and non-small cell lung cancer.

FUTURE DIRECTIONS

Clinical achievement of donor lymphocytes infusion in patients with hematological malignancy and the high frequency of relapses in T cell-depleted bone marrow transplant provide sufficient evidence that T cells mediate an anti-tumor effect. Active cancer immunotherapy or vaccination has been proven to be an effective approach to evoke T-cell responses. In addition, this approach can overcome a number of issues with the use of passive cancer immunotherapy including the requirement for repeated dosing and its high cost, the development of resistance through loss of immunodominant epitopes and undesired immunogenicity of humanized and chimerized antibodies. A vaccine would trigger the body to produce its own antibodies and sustain immune response through long-term immunologic memory. For more than a decade, synthetic peptides representing epitopes in generation of antigen-specific CTLs have been proposed as leading candidates for vaccine development. Clinical trials with immunogenic peptides in patients with

hematological malignancies show that the vaccinations were well tolerated and induced clinical benefit in some patients. However, the effectiveness of peptide vaccine to induce anti-tumor immune response that lead into a clinical benefit depends on several factors. In comparison of utilizing single CTL epitope, the development of vaccine containing multiple epitopes derived from different target TAA could improve the clinical outcomes because targeting a single epitope may lead to antigen loss variants. CD8⁺ CTL epitopes have been mainly utilized for peptide vaccine, however it is now well established that a concomitant CD4⁺ T-helper response is also important to support robust CD8⁺ CTL responses. Besides element of antigens, it becomes critical to manipulate various components of the immune system to generate a clinically effective anti-tumor CTLs response. Generally, vaccine alone is not sufficient to evoke a potent immune response. Future challenge for successful immunotherapy is to skew the immune response towards a Th1 response and to enhance the numbers of vaccine-induced T cells that bear high-avidity T cell receptor to the specific TAA by using optimal adjuvant. Adjuvant should be important to enhance the immune response through a wide range of mechanisms including a depot action causing slow release of antigen to local inflammation causing enhanced recruitment of dendritic cells to the injection site and facilitation of cross priming and mimic a danger signal. Furthermore, administration of optimal cytokine would be supportive; for instance, IL-2 can lead to the activation of tumor-associated T-cells, enhancement of vaccine-induced T-cell expansion and potential induction of the migration of vaccine-induced circulating T-cells to the tumor site. Importantly, it would be highly potential to reverse the tolerance to tumor by blocking the CTLA-4 or by depleting regulatory T cells and this approach would be critical to selectively inhibit tumor escape pathways and in development of cancer vaccines. Additionally, type of antigen-presenting cell and its activation status in the subjects vaccinated should be considered for successful therapeutic outcomes by cancer vaccines. It has been demonstrated that excessive tumor burdens might overwhelm the immune system, even in the presence of tumor-specific lymphocytes. Clinical responses of peptide vaccines have been shown highly promising in patients with minimal residual disease to potentiate anti-tumor immunity, therefore the combination of tumor debulking treatment and treatment by vaccination has been considered as a potential strategy to lead a successful therapeutic outcome in patients. Lastly, the complexity of the immune network and of the interactions between the tumor and the immune system make the task to determine the optimal regimen including vaccine dose,

route, and schedule. It would be important to emphasize that continuing discoveries of basic immunology will lead to further new and novel strategies and clinically beneficial therapeutic outcomes.

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