Novel Immunotherapy to Eliminate Minimal Residual Disease in AML Patients

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Abstract

Even with the most sophisticated chemotherapy regimens, the majority of patients with acute myeloid leukemia will eventually experience a relapse and die from their disease. New treatments are needed to prevent relapse of disease in these patients. Immunotherapy using the host immune system to combat leukemia represents an exciting and potentially efficacious addition to standard chemotherapy for AML. Immune-based treatments may be particularly effective when administered at a time when patients are in clinical remission with normal blood counts; nevertheless, these patients often have minimal residual disease which eventually results in disease relapse. Successful vaccination-based immunotherapy targeting leukemia-specific antigens will likely require the administration of powerful immune adjuvants and removal of negative immune regulatory pathways in order to achieve maximal efficacy. This review article will focus on the rationales underlying our ongoing clinical trial to test the efficacy of WT1 peptide based immunotherapy using TLR3 agonist as adjuvant in combination of the depletion of T regulatory cells with anti-CD25 antibody in patients with hematologic malignancies.

Keywords: Acute myeloid leukemia; Immunotherapy; WT1; T regulatory cells; TLR

Introduction

Standard chemotherapy for adults with AML (Acute Myeloid leukemia) can induce remission but is not curative for the majority of patients who will eventually relapse and die of their disease. Immunotherapy represents a potentially efficacious adjunct to standard AML therapy, particularly for those in the Minimal Residual Disease (MRD) state during which immune-based therapies may be most effective. Unfortunately, the efficacy of cancer immunotherapy has been limited by a number of immune evasion pathways which suppress anti-tumor immunity and enable tumor progression. A major focus of our research group has been to characterize immune evasion pathways promoted by AML with the ultimate goal of blocking such pathways to unleash the full effectiveness of immunotherapy for leukemia.

A strong candidate mechanism for immune evasion in AML is the expansion of CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Treg) [1,2]. Treg accumulate in the blood of AML patients and their numbers correlate negatively with response to chemotherapy [2]. Depletion of Treg in murine models has been shown to improve immune-mediated tumor control [3,4]. Treg depletion has been achieved in cancer patients using an anti-CD25 monoclonal antibody [5,6], making it possible to test the impact of Treg depletion coupled with immune-based therapies in AML patients.

AML Treatment in the elderly patients

Currently, there is no universally accepted standard of care treatment for older adults with AML. Although older adults with AML often achieve a complete remission, the median disease free survival is only around 6.1 months without further consolidation chemotherapy [7], and the efficacy of standard consolidation chemotherapy for this patient cohort has not been proven to improve survival. Thus, new strategies need to be developed for post-remission management of the elderly patient with AML. Even for young AML patients who are able to complete intensive chemotherapy consolidation or allogeneic stem cell transplantation, disease relapse remains the major failure of treatment for which novel; non toxic treatments are badly needed.

Immunotherapy of cancer

Over the past decades, evidence has mounted suggesting that the immune system can play an important role in the elimination of malignant cells. Many tumors express specific antigens and allow them to be recognized by CD8⁺ T cells in particular (reviewed in [8]), and increased numbers of tumor-specific T cells can be generated in cancer patients through either vaccination with tumor-specific antigens or adoptive T cell therapy (reviewed in [9]). However, despite the fact that the immune system appears to be “aware” of a growing tumor, spontaneous clearance of established tumors is rare, suggesting the existence of downstream mechanisms that inhibit anti-tumor immune responses [10]. Several of such mechanisms have been described, including extrinsic suppression by CD4⁺CD25⁺FoxP3⁺ regulatory T cells (Treg), T cell anergy, diminished T cell activation by engagement of negative co-stimulatory T cell molecules, such as PD-1 and CTLA-4, and tryptophan catabolism by indolamine-2,3-dioxygenase (IDO) (reviewed in [10-12]). It is quite likely that these mechanisms are coordinately active in concert in established tumors. Therefore, it may be necessary to block one or more of these negative regulatory pathways in combination in order to obtain a maximally effective anti-tumor immune response in patients.

WTI is a leukemia associated antigen

The development of cancer vaccines directed against leukemia has been a research area of intense interest in the past decade [13]. Among the identified leukemia-associated antigens (LAAs), Wilms tumor 1 (WT1) is a leading candidate antigen. The WT1 protein is a zinc finger transcription factor that is normally expressed in tissues of mesodermal origin during embryogenesis. In normal adult tissues, WT1 expression is minimal, while WT1 is over-expressed in most cases of AML, CML, MDS, ALL, and in several solid tumors [11]. WT1 mRNA level in the

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Received April 15, 2013; Accepted May 10, 2013; Published May 13, 2013


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Characterization of the WT1 specific CD8+ T cell repertoire

Recent developments in deep sequencing technology make it now possible to analyze the antigen-specific T cell receptor repertoire (reviewed in [24]), which is present in hosts after peptide vaccination. Because WT1 peptide vaccination has routinely led to a robust expansion of WT1-specific CD8+ T cells [17,25], it will be interesting to analyze the clonality of WT1-specific CTLs generated in this context. We hope to gain a better understanding of the WT1-specific CTL response after WT1 peptide vaccination, and further to obtain clues as to how to enhance WT1-specific CTL responses in WT1-based immunotherapy approaches. Studies from Japanese groups clearly demonstrated biased usage of TCR-Vβ gene families in WT1 peptide vaccinated patients [26,27]; the group in Germany observed the WT1 vaccination-induced expansion of a preexisting low abundant TCR clone, which became a specific predominant clone after WT1 peptide vaccination [28]. The bias towards Vβ11 usage of the WT1-specific CTL populations was confirmed in all four patients following a single peptide vaccination [29]. In addition, the identification of a WT1-specific TCR sequence could provide the basis for adoptive transfer of ex vivo expanded WT1-specific TCR engineered CTLs [30].

The role of Treg in AML

Tregs express a high level of the FoxP3 transcription factor which delineates this subpopulation of CD4+ T cells. Tregs are a population of immune suppressive cells which are critical to prevent autoimmune diseases under physiological conditions. Tregs also expand in cancer patients and are often enriched in the tumor microenvironment. Depletion of Treg can render mice capable of rejecting tumors that normally grow progressively [31]. Several groups have shown that depletion of Treg can improve anti-tumor immunity in combination with vaccination [32]. The frequency of Treg in the peripheral blood of AML patients was found to be significantly higher than that of healthy individuals [1]. Further, Treg numbers correlate negatively with response to chemotherapy in AML patients, and patients who achieved a complete response after induction chemotherapy had lower Treg frequencies at baseline, compared with non-responders [2].

Interestingly, human AML cells also promoted the differentiation of CD4+CD25+ T cells to CD4+CD25- Treg in vitro, which might partially explain the high Treg frequencies often observed in AML patients [33]. Collectively, these data suggest that AML can not only promote the expansion of naturally-generated Treg, but also that they can mediate Treg induction. Thus, Treg appear to play important role in the pathogenesis of refractory or relapsed AML [34].

Toll-Like receptor ligands as vaccine adjuvants

Toll-Like receptors (TLR), which recognize pathogen-associated molecular patterns, have recently emerged as a critical component of the innate immune system for detecting microbial infection and activation of dendritic cell maturation programs to induce adaptive immune responses [35,36]. Stimulation of TLR signaling pathways activates dendritic cells and induces the production of pro-inflammatory cytokines, such as type-I IFN and IL-12, leading to a Th1-skewed response favoring cytotoxic T-cell differentiation. It has been reported that TLR signaling on dendritic cells by CpG or LPS renders effector cells refractory to Treg-mediated suppression [37]. Stimulation of dendritic cells with TLR ligands significantly enhances the proliferation of naive and effector T cells, making it more difficult for Treg cells to inhibit them [38,39]. These findings offer new strategies to develop more effective immunotherapy by employing TLRs agonist as vaccine adjuvants. TLR3 agonists have been used in the past, with variable efficiency, as an adjuvant to treat cancer patients, with the aim of inducing an IFN-mediated anti-cancer immune response [40,41]. Hiltonon (poly-ICLC) (Oncovir, Inc, Washington, DC), is a clinical grade stabilized TLR3 agonist containing poly-L-lysine and carboxymethyl cellulose (poly-ICLC), which has been used in several clinical trials [42-45]. An open study evaluating the safety and efficacy of long-term treatment of malignant gliomas with intramuscularly administered poly-ICLC [42] demonstrated that poly-ICLC administration was well-tolerated with little or no toxicity, and 66% patients had stable disease or disease regression by MRI. Even though poly-ICLC administration by itself was not active in advanced renal carcinoma [46] and recurrent anaplastic glioma [45], combination of poly-ICLC with radiation or with concurrent adjuvant temozolomide had improved efficacy in adults with newly diagnosed glioblastoma [43,44]. Most recently, poly ICLC was demonstrated to induce rapid immune response in ovarian cancer patient when it was used as adjuvant for tumor self-antigen [47]. These data demonstrate the safety of poly-ICLC in humans, and combined with preclinical data showing the immune potentiating effect of this TLR ligand with vaccines, support its clinical application for a vaccine adjuvant in patients.

Depletion of Treg in vivo

Because most Tregs express high levels of CD25 (IL2-receptor-alpha) on their cell surface, targeting CD25 has been exploited to deplete Tregs from humans in vivo. Ontak (denileukin diftitox) is a recombinant fusion protein between human IL-2 and a fragment of diptheria toxin [48]. Interestingly, Ontak is capable of killing normal T cells that express CD25; including the Treg subset which is contained within the CD25+ population. This was confirmed in a study from Vieweg and colleagues who observed that a single dose of Ontak did indeed decrease detectable CD4+CD25+ T cells from the circulation [3]. In addition, the magnitude of the specific immune response to the vaccine appeared to be much greater than what was seen without
Ontak [3]. However, a number of follow-up studies in which Ontak was utilized to deplete Treg did not find that Ontak led to a significant depth or duration of Treg depletion. Daclizumab (Zenapax) is a monoclonal anti-CD25 antibody which blocks the interaction of IL-2 and CD25. Rech et al. demonstrated that single dose of Daclizumab at 1 mg/kg caused marked and prolonged elimination of Treg for more than 5 weeks in patients with metastatic breast cancer when combined with a cancer vaccine [5,6]. Unfortunately, daclizumab has since been removed from the market and is no longer available. On the other hand, basiliximab (Simulect), a similar anti-CD25 antibody, is currently FDA-approved to prevent renal allograft rejection. Several reports have demonstrated that Basiliximab is capable of decreasing the number of circulating Treg in humans [49,50]. Recently, Okita et al. demonstrated that low-dose basiliximab can safely be administrated repeatedly, and can target CD4+CD25+ Treg cells while relatively preserving CD4+CD25− activated T cells, suggesting that Basiliximab could be used to deplete Treg and augment the efficacy of adoptive immunotherapy of cancer [51]. All these available data suggest that monoclonal anti-CD25 antibodies may be useful to deplete Treg and enhance the efficacy of immune response induced by peptide vaccination.

Rationale for an approach for WT1 vaccination in combination with Treg depletion in AML patients

Based on the positive clinical results with WT1 peptide immunization, along with observations regarding suppression of anti-tumor immunity by Treg, a logical strategy for improving leukemia peptide-based vaccine therapy has emerged. An open-label, randomized phase I study assessing administration of WT1 vaccine +/- TLR3 agonist (poly ICLC) alone or post-basiliximab in AML patients who are not candidates for stem cell transplant due to advanced age and co-morbidities or who refuse stem cell transplant is proposed. HLA-A0201-positive patients in complete remission or complete remission with incomplete blood count recovery after induction chemotherapy (repeat induction chemotherapy or consolidation chemotherapy is allowed), will be eligible. Each patient will undergo serial measurement of Treg cells from the peripheral blood weekly for 4 weeks prior to the stratification to Arm A (WT1 peptide vaccine in Montanide) or Arm B (WT1 peptide vaccine in TLR3 agonist, poly-ICLC).

In the initial stage of the study, 24 patients will be randomized to Arm A or Arm B (12 patients in each arm). Cellular immune responses, as measured by IFN-γ ELISPOT following stimulation with WT1 peptide, will be used to determine whether Arm A or Arm B will be superior, and basiliximab will be given prior to peptide vaccination using the superior vaccination regimen to form Arm C (12 patients will be included in Arm C). In the Basiliximab group (Arm C), a single dose of Basiliximab 20 mg will be given intravenously over 30 minutes seven days prior to the initial vaccination (Day -7, 3 weeks after confirmed CR). All patients will receive 100 ul (1000 mcg) WT1 126-134 peptide (RMFPNPAPYL), which is an HLA-A*0201-restricted peptide, in Montanide as an emulsion or in TLR3 agonist, poly ICLC, 1mg in 1ml aqueous solution administered intradermally/subcutaneously every 2 weeks x 6, starting on Day 0 of the study (4 weeks after confirmed CR). Disease re-evaluation will be performed every 6 weeks. Treg counts by flow cytometry, FoxP3 and WT1 expression by qRT-PCR, and peptide-specific immunologic responses will be monitored over time. Patients without disease progression after 6 vaccinations can be continued on additional cycles of 6 monthly vaccinations, but no further Basiliximab will be administered.

The protocol for this study was approved by IRB at University of Chicago, and the IND application for WT1 peptide was approved by the FDA. With the support by a grant from American Cancer Society, we were able to enroll five patients to the clinical trial to date. The clinical and correlative immune responses data will be presently separately in the future.

Conclusion

Relapse remains the major cause of treatment failure for patients with AML, even after allogeneic stem cell transplantation. Immunotherapy may be useful to eliminate MRD present following standard therapies, which could reduce the risk of disease relapse. The understanding of the immune evasion mechanisms and the ability to interfere with them may open the door for the delivery of effective immunotherapy. Logical combinations to suppress multiple negative regulators of tumor immune responses will likely be required in order to maximize the efficacy of immunotherapy, and thus positively affect the natural course of AML.

Acknowledgement

Dr. Hongtao Liu was supported by American Cancer Society - Institutional Research Grant; and the University of Chicago Comprehensive Cancer Center, Protocol Specific Research Award.

References


