Chapter 22
Immunotherapy for Acute Lymphocytic Leukemia

Jacalyn Rosenblatt and David Avigan

Introduction

While a majority of patients with acute lymphocytic leukemia (ALL) demonstrate response following treatment with standard chemotherapy, subsequent progression due to the emergence of resistant disease is often encountered. The failure to eradicate disease is most commonly observed in adult patients particularly with high-risk features such as adverse cytogenetics. In contrast, immunotherapy may be successful in targeting chemotherapy-resistant clones. The role of immune-based therapy in the treatment of ALL is highlighted by the observation that allogeneic hematopoietic stem cell transplantation is uniquely curative for a subset of patients in this setting [1]. The efficacy of transplant is derived from the dose intensity of the transplant conditioning regimen as well as the antitumor effect mediated by donor-derived immune effector cells. Improved outcomes as compared to standard chemotherapy have been observed in patients in first complete remission (CR) who exhibit negative prognostic factors as well as those with relapsed or refractory disease.

Allogeneic Transplantation as Cellular Immunotherapy

Standard allogeneic transplantation involves the use of high dose chemotherapy alone or in conjunction with myeloablative radiation therapy followed by the infusion of hematopoietic stem cells derived from a HLA matched sibling or alternative donor. The importance of the ablative regimen in determining outcome is supported by the lower risk of relapse observed in patients treated with total body irradiation as compared to busulfan-based regimens [2, 3]. In addition, regimens involving greater than 13 cGy or the use of etoposide as compared to cyclophosphamide have also been shown to be associated with lower relapse rate [4, 5]. However, dose intensity alone is insufficient to achieve long-term disease control in patients with high risk or recurrent ALL. A vital component of allogeneic transplantation responsible for the prevention of disease relapse is the anti-leukemia effect mediated by alloreactive lymphocytes.

Evidence of the unique efficacy of allogeneic transplant has been observed in patients undergoing transplantation in first complete remission. Patients with ALL with high-risk features such as the presence of chromosomal abnormalities including translocation of (9;22), (4;11), and (8;14) rarely
experience durable remissions following standard chemotherapy [6, 7]. In contrast, allogeneic transplantation has been associated with improved disease-free survival [3, 8]. In one study, high-risk ALL was defined by the presence of abnormal cytogenetics, elevated WBC, age older than 30, extramedullary disease, or prolonged time to achieve CR. With a median follow up of 5 years, an event-free survival of 64% was observed following allogeneic transplantation [8]. In another study, patients with ALL were assigned to receive an allogeneic as compared to autologous transplant based on the presence of an HLA-matched donor. In the subset exhibiting high-risk features, the 5-year disease free survival was 45% and 23% for the 100 patients who had a sibling donor and the 159 patients who did not, respectively [9].

The efficacy of allogeneic as compared to autologous transplant for high risk ALL was confirmed in a meta-analysis of 10 trials (1,274 patients) comparing outcomes in which patients with an available sibling donor underwent allogeneic transplantation [10]. In a retrospective analysis of 712 patients, allogeneic transplant from unrelated donor in CR1 or CR2 was associated with lower risk of relapse as compared to autologous transplant (49% vs. 14%). However, because of significant treatment related mortality, the 5 year survival was equivalent after CR1. In contrast, allogeneic transplantation was associated with improved survival (50% vs. 14%) in patients with more advanced disease (CR2) [11]. These findings highlight the importance of the donor graft in preventing disease relapse.

In a prospective joint study between the Medical Research Council (MRC) of the United Kingdom and ECOG, nearly 2,000 patients with ALL were treated with induction therapy followed by transplantation or standard chemotherapy. Those with a sibling donor were offered an allogeneic transplant while the remaining patients were randomized to receive an autologous transplant or standard chemotherapy. Risk of relapse was significantly higher for Philadelphia chromosome (Ph) negative patients not undergoing allogeneic transplant while the benefit with respect to survival was reserved for those patients younger than age 35 due to higher levels of transplant associated toxicity in older patients. Of note, autologous transplantation did not offer any benefit as compared to standard chemotherapy suggesting that improved disease control following allogeneic transplantation was not due to dose intensity.

Patients with Ph+ ALL are considered to have intrinsically chemotherapy resistant disease. In one study, 103 patients with Ph+ ALL achieved complete remission to initial therapy and were randomized to receive an autologous or allogeneic transplant based on the availability of an appropriate HLA matched donor. Those patients randomized to the allogeneic transplant arm demonstrated a better 3 year overall survival (37% vs. 12%) and decreased relapse rate (50% vs. 90%) as compared to those patients without an HLA-matched donor. On multivariate analysis, the presence of a donor and persistence of molecular evidence of BRC-ABL prior to transplant remained highly significant in predicting outcome [18]. These data suggest that while relapse remains a major concern after allogeneic transplantation, the presence of alloreactive cells following intensive therapy is potentially crucial to eliminate chemotherapy resistant disease in this setting. In several studies, disease-free survival was similar following allogeneic transplant from a sibling and unrelated donor [19, 20].

As suggested above, prevention of disease relapse is thought to be mediated, in part, by alloreactive lymphocytes that target patient-derived leukemia cells [13, 14]. The importance of the graft versus leukemia effect is supported by the association of graft versus host disease with a lower incidence of disease relapse. In one study of 192 patients with ALL who were predominantly transplanted in second complete remission, the actuarial risk of relapse was 40% for patients with grade II or higher graft versus host disease (GVHD) as compared to 80% in patients without evidence of GVHD [15]. A subsequent study of 1,132 patients with ALL also demonstrated that the presence of acute or chronic GVHD was associated with decreased risk of relapse [16]. Similarly, T cell depletion of the hematopoietic graft has been associated with decreased toxicity but a high risk of relapse [17].
Reduced Intensity Conditioning

Allogeneic transplantation following reduced intensity conditioning has been explored in an effort to minimize transplant associated toxicity while relying on the potency of the graft versus disease effect. Limited data is available regarding its efficacy in ALL. In a study of 22 patients with ALL many of which resistant disease long-term survival was only observed in patients transplanted in CR [21]. In another study of 27 patients with high risk ALL who underwent transplant with reduced intensity conditioning, the 2 year overall survival was 31% suggesting that this treatment approach was effective for a subset of patients with ALL not curable with chemotherapy [22]. One study reported on the results of 43 patients with ALL in CR2 undergoing reduced intensity conditioning with busulfan, cyclophosphamide, and fludarabine [23]. The overall survival at 3 years was 31% with a majority of deaths related to disease relapse. The authors concluded that the inferior results observed as compared to that achieved with chronic myeloid leukemia (CML) and AML using the same conditioning regimen suggested an inferior graft versus disease effect in ALL.

In contrast, a Japanese study of 33 patients with ALL demonstrated relapse-free survival even in a subset of patients treated during disease relapse (5/14) [24]. The 1 year relapse free and overall survival was 30% and 39%, respectively. The authors suggested that despite a significant risk of relapse (51% at 3 years) an important graft versus disease effect was present. In a retrospective review by the EBMT, results of 97 patients with adult ALL who underwent reduced intensity conditioning transplantation were reviewed [25]. The 2-year leukemia-free survival in this high-risk population was 21%. Not surprisingly, relapse incidence was higher in patients with more advanced disease (greater than CR1). Of note, the presence of chronic GVHD was associated with improved survival consistent with the importance of a graft versus disease effect. These data suggest that while some patients may respond to alloreactive immunity, it is often insufficient in preventing relapse particularly in the setting of advanced disease.

Donor Lymphocyte Infusion

Treatment with donor lymphocytes infusion (DLI) post-transplant has been explored in an effort to reestablish a graft versus disease effect to eliminate minimal residual or relapsed disease [26]. In patients already manifesting complete donor chimerism, DLI represents the attempt to invigorate an alloreactive anti-tumor response by the introduction of lymphocytes that have not undergone tolerization in the patient in the presence of immunosuppressive therapy such as cyclosporine. The potency of DLI is highly variable and dependent on the disease setting and has shown the greatest promise in CML. Efficacy is likely due to the sensitivity to the alloreactive response as well as the kinetics of tumor growth and the ability to wait for the establishment of a graft versus disease effect that characteristically requires 1–3 months for full expression. Results for patients with ALL have been mixed. One of the first reports of the effective use of DLI was in a child with relapsed ALL who achieved a durable remission that has persisted for greater than 15 years [27]. However, in a review of the experience of the European Group for Blood and Marrow Transplantation, DLI was ineffective in generating durable remissions for a cohort of 22 patients with ALL [28]. Similarly, in a summary report of results from US centers, 44 patients with ALL were treated with either DLI alone or following chemotherapy-induced nadir [29]. Disappointingly, only two out of 15 patients receiving DLI alone achieved a CR and only three patients achieved long-term CR for the entire cohort. In a small cohort of seven patients with ALL undergoing DLI from unrelated donors two out of four evaluable patients achieved CR and disease-free survival was 30% at 1 year for the entire group.
These data raise the question as to why DLI is not more effective in ALL despite the evidence of a significant graft versus disease effect outlined above. One issue effecting both AML and ALL is the rapid growth kinetics of the disease that may not provide the necessary time to establish and immunologically based antitumor effect. In addition, there is evidence that ALL cells directly suppress the antitumor immune response through the induction of T cell anergy [30]. ALL cells may also serve as a more resistant target for donor alloreactivity because of their lack of effective antigen presentation due to the absence of costimulatory molecules and their intrinsic resistance to killing by natural killer (NK) cells [31, 32]. These findings point to the inherent weaknesses of immune-based targeting of ALL cells through alloreactivity. Concerns remain regarding the specificity and potency of this response and overcoming the intrinsic resistance offered by ALL cells. They raise the question as to whether ALL may be more effectively targeted by immunotherapy directed against leukemia specific targets in the context of efforts to alter the immunologic milieu to favor antitumor immune response.

Augmenting Alloreactive Immunity

One approach to reduce the risk of relapse following allogeneic transplantation is to pursue methods that heighten the alloreactive response that targets leukemia cells. In a murine model for B cell leukemia, investigators have demonstrated that the post-transplant infusion of recipient antigen-presenting cells increases allosensitization of donor lymphocytes with a resultant increase in GVHD and a concomitant clearance of BCL1+ cells [33]. In another murine model, investigators have examined a strategy to amplify the potency of DLI to eradicate leukemia cells using DLI from a partially HLA mismatched donor [34]. Animals underwent reduced intensity transplantation followed by inoculation with $1 \times 10^6$ B cell leukemia cells (BCL1) and administration of mismatched DLI. Those animals who received DLI alone died of GVHD while those who received cyclophosphamide 14 days following DLI demonstrated no evidence of residual disease by PCR analysis while only experiencing self limited GVHD. These results suggest that the elimination of alloreactive cells following a period of activation may promote the graft versus disease effect. In a study using human cells, T cells derived from a haploidentical donor (parent) were cultured with the recipients ALL blasts and IL-2 [35]. The resultant cytotoxic lymphocyte (CTL) culture demonstrated a predominance of CD8+ T cells that demonstrated cytotoxicity against both ALL cells and allogeneic ConA-stimulated mononuclear cells suggesting the recognition of alloreactive targets. Infusion of these cells into a patient following haploidentical transplant resulted in clearance of circulating blasts without evidence of concurrent GVHD.

Another approach to augment the efficacy of donor lymphocytes is to amplify the alloreactive responses that more selectively target hematopoietic malignant cells. Minor histocompatibility antigens have been identified that are uniquely expressed by hematopoietic cells and are targeted by donor lymphocytes. In a murine NOD/SCID model, animals infused with T cells generated against the HLA-A2-derived HA-1 peptide demonstrated delayed growth of leukemia cells [36]. In contrast, animals receiving CTLs directed against CMV did not show disease response. Of note, tumor growth was ultimately associated with the clearance of the HA-1 recognizing T cells.

Role of NK Cells

The role of innate immunity in targeting ALL cells remains under investigation. NK cells demonstrate cytotoxicity against infectious pathogens and malignant cells mediated by cytokine expression and antigen-dependent recognition not requiring activation of adaptive immunity. NK cell response is
regulated by a series of cell surface receptors, which deliver inhibitory or stimulatory signals. The human killer Ig-like receptors (KIRs) are associated with HLA class I molecules and may deliver an inhibitory signal that prevents targeting of autologous tissue. Activation KIRs have also been identified, which may include non-HLA ligands. In addition, a series of other activation receptors are present such as NKG2D that mediate anti-tumor responses. Following haploidentical allogeneic transplantation, lack of KIR-mediated inhibitory signaling may result in the killing of leukemia cells by mismatched donor NK cells. This phenomenon has been best described in AML. In some studies, presence of KIR mismatch that facilitates NK cell activation is associated with improved outcomes in AML [37, 38]. In vitro studies demonstrate that alloreactive NK cells were capable of only lysing a minority of patient-derived ALL cells [39]. This may be due to a lack of stimulatory ligands present on ALL in contrast to AML. Despite these findings, a recent trial of suggested excellent outcomes in children with resistant ALL following haploidentical transplantation [40].

In preclinical models, investigators have examined approaches to enhance NK-cell-mediated killing of ALL cells. In a murine model, ALL cells were genetically engineered to express Murine B Defensin 2 (MBD2), a potent stimulator of innate immunity [41]. Administration of MBD2 expressing ALL cell line resulted in the production of IL-12 and IFNγ, activation of NK and CTL response, and tumor rejection. Animals were subsequently protected against challenge with the wild-type ALL cell line. Depletion of CD8 T cells or NK cells abrogated the response. These findings suggest that the intrinsic resistance of ALL cells to NK-mediated lysis may be overcome by the insertion of NK stimulatory signal.

**Generation of Leukemia Specific Immunity**

While allogeneic transplantation is effective for a subset of patients with ALL, the lack of specificity of the alloreactive response results in a significant risk of morbidity and mortality. In addition, a majority of patients with high risk and advanced disease relapse due to the lack of efficacy in eliminating the malignant clone. A major area of investigation is the identification of tumor-specific antigens that can be targeted selectively while minimizing the collateral damage on normal tissue. In addition, strategies to augment host immunity, reverse tumor-mediated immune suppression, and overcome tolerance are being pursued in an effort to develop effective immunotherapy for ALL.

**Leukemia-Associated Targets for Antibody-Mediated Immunotherapy**

Potential targets present in ALL cells include B lymphocyte-specific antigens such as CD19 and CD20, the latter of which is expressed in approximately 1/3 of B lineage precursor ALL. Correspondingly, Rituxamab has been shown to eliminate evidence of minimal residual disease in Ph+ after transplant [42]. In another study, 35 patients underwent treatment with Rituxamab in combination with cyclophosphamide and TBI in an effort to decrease risk of relapse as well as GVHD [43]. While GVHD rates may have been impacted no clear effect on relapse was documented. One approach to enhance the efficacy of antibody-mediated therapy is the alteration of the Fc receptor to amplify antibody-dependent cytotoxicity. In one study, the mutated CD19 antibody known as XmAb5574 demonstrated markedly increased binding to the Fc receptor and a 100–1,000 fold increase in ADCC against a broad array of lymphoid leukemia and lymphoma lines [44]. In a murine study, the antibody was shown to significantly inhibit lymphoma growth in a mouse xenograft model.
Another potential immunologic target is CD25, a component of the IL-2 receptor, which is expressed on activated T cells and acute T cell leukemia. Daclizumab is an antibody with specificity to the α-subunit of the IL-2 receptor blocking its interaction with IL-2 [45]. Clinical trials with the murine antibody have demonstrated modest responses in a subset of patients but the humanized form of the antibody has demonstrated greater affinity toward the target. Conjugation of the daclizumab with the radioactive isotope 90Y has resulted in clinical response in 10/18 evaluable patients. In a murine study, the combination of daclizumab and a histone deacetylase inhibitor demonstrated a markedly synergistic effect in the treatment of adult T cell leukemia [46].

The tyrosine kinase, FLT3, is expressed on leukemia cells in nearly all patients with B cell ALL and therefore may serve as a target for antibody therapy [47]. In a murine model, two anti-FLT3 antibodies (IMC-EB10 and IMC-NC7) were shown to prolong survival and reduce engraftment in animals challenged with ALL [48]. Responses were seen even when the antibody resulted in FLT3 activation and was abrogated by depletion of NK cells. Surviving cells after treatment remained sensitive to FLT3 antibody upon retransplantation in another animal.

T Cells Expressing Chimeric Antigen Receptors: Combined Humoral and Adoptive Immunotherapy

Another strategy is the development of adoptive immunotherapy that targets cell surface targets such as CD19 or CD20 while simultaneously utilizing the effector function of cellular immunity. Investigators have created chimeric antigen receptors (CARs) in which the heavy and light variable chains are joined into a single chain Fv and engrafted into the T cell receptor. In this manner, ligation of the CAR results in activation of the T cell receptor and initiation of signaling pathways that result in cytokine production and cell-mediated cytolysis. Using CARs specific for CD19, selective targeting of lymphoid malignancies has been demonstrated in animal models. To limit toxicity, T cells have been engineered to contain the suicide HY-TK gene allowing for the elimination of the infused cells using ganciclovir. In one study, primary human CD8+ cytotoxic T lymphocytes were genetically modified to express a CD19+-specific chimeric immunoreceptor. CD19+ expressing T cells were shown to proliferate, and secrete both TNF-α and interferon γ in response to stimulation by CD19+ but not CD19− tumors. In addition, genetically modified CD19+ T cells killed primary ALL tumors in a CTL assay [49].

Similarly, umbilical cord blood T cells genetically modified to express CD19 were shown to kill ALL blasts in vitro, and eliminate established tumor in a NOD/SCID mouse model [50]. In another study, human peripheral blood lymphocytes were transduced with a CD19-specific CAR called 19z1. Transduced T cells were expanded using a CD19 and CD80 expressing artificial antigen-presenting cell in conjunction with IL-15. SCID-Beige mice were inoculated with tumor and subsequently treated with 19z1+ T cells. T cell-treated mice had a significant survival advantage, demonstrating that genetically engineered human T cells can eradicate established tumors in vivo [51]. Use of T cells expressing CARs targeting CD19 eliminated cell lines that expressed the costimulatory molecule CD80 but were ineffective against an ALL cell line that did not provide co-stimulation. A series of second-generation CARs have been evaluated in vitro [52–58]. Addition of the CD28 transmembrane and signaling domain to the 19z1 CAR has been shown to enhance T cell proliferation, cytokine secretion, and survival in a murine model [52].

The sleeping beauty (SB) transposon system is an alternative, nonviral system used to genetically modify T cell populations [59]. In one study, human peripheral blood and umbilical cord blood T cells were transfected to co-express the CAR for CD19 and CD20. SB engineered T cells strongly co-expressed CD19 CAR and CD20, and effectively lysed two B ALL cell lines. Both peripheral blood and umbilical cord blood T cells engineered to express CD19 CAR and CD20 produced Th1
Immunotherapy for Acute Lymphocytic Leukemia

but not Th2 cytokines. In addition, in a NOS/SCID mouse model, adoptive transfer of SB engineered T cells resulted in reduced tumor growth and prolonged survival.

Targeting Known Tumor-Associated Antigens

Known tumor-associated antigens found in other malignancies have also been identified in ALL blasts, including MAGE-A1 [60], MAGE-A3 [61], cancer-testis (CT) antigens [62], PRAME [63–65], WT-1 [66], and Her-2/neu [67, 68] (Table 22.1). In one study, MAGE-A3 gene was shown to be expressed at the mRNA level in 20/53 ALL samples, and not in normal controls [61]. Similarly, overexpression of PRAME was found in 42% of children with ALL [63]. A trend toward higher survival rates and lower WBC was seen in patients who over-expressed PRAME, possibly due to an immune response against this tumor antigen [65, 69]. However, in contrast to melanoma, PRAME-specific CTLs generated from normal subjects did not lyse ALL cells because of lower levels of antigen expression in these cells [70]. Native humoral immune responses to WT-1 have been detected in 45.5% of ALL patients suggesting that this is a tumor antigen that is recognized by the host immune repertoire [66]. The Her-2/neu antigen is also selectively expressed by ALL blasts as evidenced by the observation that antigen-specific CTLs were shown to kill leukemic blasts from patients with ALL, but not nonmalignant B cells, dendritic cells (DCs), bone marrow, or CD34+ progenitors from healthy donors indicating that Her-2/neu is selectively expressed by ALL blasts [67].

Enhancement of Antigen Presentation Using Dendritic Cells

Despite the presence of tumor-associated antigens potentially recognizable by the immune repertoire, the generation of an effective antitumor immune response remains elusive. The primary factors thought to be responsive include the lack of co-stimulation to support effective antigen presentation, tumor-mediated suppression of host immunity through the expression of inhibitory factors, and the increased presence of suppressor cells such as regulatory T cells. A major focus of investigation is to develop strategies to enhance antigen presentation and facilitate T cell responsiveness through the reversal of tumor-associated immune suppression.

<table>
<thead>
<tr>
<th>Tumor associated antigen</th>
<th>Comment</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>MAGE</td>
<td>MAGE-A3 gene was shown to be expressed at the mRNA level in 20/53 ALL samples, and not in normal controls</td>
<td>[61]</td>
</tr>
<tr>
<td>Cancer-testis (CT) antigens</td>
<td>Testis specific antigen expression was demonstrated in 84.6% of bone marrow and peripheral blood samples obtained from patients with ALL.</td>
<td>[62]</td>
</tr>
<tr>
<td>PRAME</td>
<td>A trend toward higher survival rates and lower WBC was seen in patients who over-expressed PRAME, possibly due to an immune response against this tumor antigen</td>
<td>[65, 69]</td>
</tr>
<tr>
<td>WT1</td>
<td>Native humoral immune responses to WT-1 have been detected in 45.5% of ALL patients suggesting that this is a tumor antigen that is recognized by the host immune repertoire</td>
<td>[66]</td>
</tr>
<tr>
<td>Her2/Neu</td>
<td>Her2/Neu specific CTLs were shown to kill ALL blasts, but not nonmalignant B cells, dendritic cells (DCs), bone marrow, or CD34+ progenitors from healthy donors indicating that Her2/Neu is selectively expressed by ALL blasts</td>
<td>[67]</td>
</tr>
</tbody>
</table>
One approach has been the use of dendritic cells (DCs) to enhance antigen presentation. DCs represent a complex network of cells that play a major role in maintaining the balance between immune activation and tolerance [71]. Mature myeloid DCs are uniquely capable of initiating primary immunity through their prominent expression of co-stimulatory and adhesion molecules. Vaccination of DCs loaded with leukemia-associated antigens has been shown to induce antitumor immune responses [72]. In one model, DCs were pulsed with HB-1, a B cell lineage specific antigen that is expressed on ALL cells and recognized by the T cell repertoire [73]. DCs were generated from normal volunteers, pulsed with a HB-1-B44 peptide and used to stimulate antigen-specific CTLs. Consistent with their tumor specificity, CTLs expressed IFNγ in response to exposure to HLA matched ALL cells. RNA encoding for leukemia associated genes has also been as a source of tumor antigens for DC-based vaccination. In one study, a highly efficient transfection system was developed to load RNA onto DCs generated from CD34+ cord blood [74]. HLA-A2+ DCs were pulsed with RNA derived from the HLA-A2+ Nalm-6 ALL cell line or autologous ALL cells and shown to stimulate ALL-specific cytotoxicity.

An alternative strategy involves the use of whole leukemia cells as a source of antigens. In one study, DCs were pulsed with lysate or apoptotic bodies generated from Jurkats cell line (T cell ALL) [75]. Exposure to lysate resulted in increased DC expression of costimulatory and maturation markers. Coculture of pulsed DCs and T cells induced T cell proliferation, IFNγ expression, CTL-mediated lysis of tumor target cells. Similarly, another study demonstrated that functionally active DCs may be generated from peripheral blood mononuclear cell (PBMCs) obtained from patients with ALL in remission [76]. The DCs were pulsed with apoptotic bodies derived from ALL cells and were highly effective in stimulating T cell responses.

**Conversion of ALL Cells into Antigen Presenting Cells**

Alternatively, leukemia cells can be directly transformed into more efficient antigen presenting cells. In a murine model, transduction of the Ph+ ALL cell line BM185 with CD80 resulted in its heightened immunogenicity and the capacity of animals to withstand tumor challenge with the altered cell line [77]. The protective effect was diminished by in vivo depletion of CD4 or CD8 T cells. However, this approach was ineffective in treating established disease. While transduction with IL-2 or GM-CSF was ineffective, cotransfection with CD80 and GM-CSF induced the most prominent CTL response directed against wild type BM185 and reduction of growth of subcutaneously implanted disease. Another approach is the transformation of ALL into antigen presenting cells with phenotypic features similar to DCs. In one study, strategies for generating dendritic cells from ALL blasts containing the 9;22 translocation were examined. They demonstrated that the combination of IL-1β, IL-3, IL-7, SCF, TNF-α, and CD40L most effectively differentiated t(9;22) lymphoblasts [78]. Upregulation of costimulatory molecule expression, CCR7 and CD54 was demonstrated. Differentiated blasts induced allogeneic T cell proliferation, and killing of leukemia cells in a CTL assay. Differentiated blasts were shown to be derived from the malignant clone by FISH analysis for t(9;22). Other groups have demonstrated that ALL blasts stimulated with CD40L strongly express MHC class I, co-stimulatory and adhesion molecules [30, 79, 80]. T cells stimulated by CD40L exposed blasts have been shown to lyse autologous tumor in CTL assays [79]. A phase I clinical trial was conducted in which patients with relapsed ALL were vaccinated with autologous CD40L stimulated blasts. Vaccine was successfully generated in nine patients. Seven patients withdrew from study prior to vaccination, due to progressive disease (five patients) or to pursue other therapy (two patients). Two patients were vaccinated without evidence of toxicity, however, clinical responses were not seen [81]. This study highlights the difficulty of applying novel immunotherapeutic approaches in patients with rapidly growing disease.
Cytokine Therapy as Immune Adjuvants

Investigators have examined the role of cytokine therapy to reverse tumor mediate immune suppression and support the development of leukemia specific immunity. In a murine study, administration of rmIL-12 resulted in the eradication of established Ph+ ALL cells [82]. The schedule of treatment had a significant impact on efficacy. Depletion of CD4, CD8 or NK cells did not abrogate the response but combined removal of these cell types interfered with the activity of rmIL-12. Of note, administration of rmIL-12 did not result in immunologic memory such that animals remained susceptible to repeat challenge. However, combination of the rmIL-12 with vaccine consisting of leukemia cells modified to express CD40L/CD80/GM-CSF resulted in prolonged protection against secondary challenge with leukemia. Similarly, the role of toll-like receptor (TLR) signaling in promoting antitumor immunity has being examined [83]. In a preclinical study, B cell precursor ALL cell lines were exposed to TLR2, TLR7, and TLR9 agonists, which resulted in an increase in CD40 expression consistent with enhanced immunogenicity. Ligation of TLR2 on the ALL cell lines was most effective in altering T cell response in significantly increasing IFNγ production.

Summary

Chemotherapy-based strategies have been ineffective for many patients with ALL, particularly those with high-risk features or relapsed disease. In contrast, immunotherapeutic strategies hold potential promise as an approach to eliminate residual disease. The potency of immune based therapy in targeting ALL has been demonstrated in the allogeneic transplant setting. However, the lack of specificity of alloreactive T cells results in significant treatment associated toxicity and disease relapse remains a major concern. Investigators have examined approaches to enhance leukemia specific immunity. ALL associated antigens have been identified as targets for more selective forms of immunotherapy. Antibody therapy has been pursued and has been further augmented by the use of CARs to exploit anti-tumor cellular effector mechanisms. Efforts have also focused on stimulating anti-leukemia cellular immune responses by enhancing antigen presentation by the ALL cell or the introduction of ALL antigens onto professional antigen-presenting cells such as DCs. In addition, approaches to reverse tumor-associated immune suppression such as the use of cytokine are being examined. Defining the optimal timing of immunotherapy is of critical importance. Patients with relapsed/refractory disease demonstrate rapid disease progression, which may not allow sufficient time for an immunologic antitumor effect. As such, immunotherapeutic approaches will likely be most effective in patients with low tumor burden. Strategies that combine tumor-specific immunotherapy with chemotherapy or bone marrow transplantation warrant investigation in clinical trials.

References

allografting for acute lymphoblastic leukemia in first or second complete remission. *Biology of Blood and Marrow Transplantation, 12*, 438–453.


