Novel Therapies and Their Integration into Allogeneic Stem Cell Transplant for Chronic Lymphocytic Leukemia

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Over the past decade, numerous advances have been made in elucidating the biology of and improving treatment for chronic lymphocytic leukemia (CLL). These studies have led to identification of select CLL patient groups that generally have short survival dating from time of treatment or initial disease relapse who benefit from more aggressive therapeutic interventions. Allogeneic transplantation represents the only potentially curative option for CLL, but fully ablative regimens applied in the past have been associated with significant morbidity and mortality. Reduced-intensity preparative regimens has made application of allogeneic transplant to CLL patients much more feasible and increased the number of patients proceeding to this modality. Arising from this has been establishment of guidelines where allogeneic stem cell transplantation should be considered in CLL. Introduction of new targeted therapies with less morbidity, which can produce durable remissions has the potential to redefine where transplantation is initiated in CLL. This review briefly summarizes the field of allogeneic stem cell transplant in CLL and the interface of new therapeutics with this modality.

INTRODUCTION

Chronic lymphocytic leukemia (CLL) is the most common leukemia diagnosed in Western countries, with an incidence of about 2 to 6 cases per 100,000 people per year, increasing to a rate of 12.8 per 100,000 at age 65 [1,2]. CLL is characterized by an accumulation of leukemic cells caused, in part, because of survival signals delivered to the cells from various receptors and ligands. Improvement in initial therapies of CLL with chemoimmunotherapy in all but high-risk genomic groups has led to high response rates and longer remissions. Following relapse, remissions following second-line therapy are often shorter. CLL remains incurable outside the setting of allogeneic stem cell transplant, and therefore, identification of the appropriate time for transitioning CLL patients to transplant and having effective disease control at that time represents a major issue. For patients with high-risk disease, such as del(17p) or poor response to initial therapy, the consensus of several groups is that transition to allogeneic transplant early in the disease course is the best strategy. For those with durable first remissions the timing of transplant is more controversial. The controversy in when to proceed to a more aggressive treatment approach is in part driven by the plethora of new therapeutics available for CLL patients. Indeed, commonalities in the pathways in the development and expansion of mature B lymphocytes to clonal CLL lymphocytes have been observed allowing for the development of targeted therapies [2]. Efforts to target specific pathways in CLL have led to the development of exciting novel therapeutics, changing the way we approach patients with CLL. It is not yet known how, if at all, these therapies will change the indications for or outcomes following transplant for CLL. However, these promising results as documented herein already have started to impact on transplantation recommendations for CLL patients in a manner similar to chronic myeloid leukemia (CML) patients in the early days of imatinib treatment, where durable remissions were observed. At that time, it was common for both patients and physicians to struggle with the correct decision about continuing therapy with imatinib or proceeding to transplant. More than a decade later, with the long-term follow-up of imatinib available as well as the development of...
second generation tyrosine kinase inhibitors (TKI) including dasatinib and nilotinib, allogeneic transplant is only considered for a very small subset of high-risk CML patients. This paper provides a prospective of where CLL is relative to this therapeutic balance of targeted agents, their integration into allogeneic transplantation, and impact on choice of when to proceed with this curative but potentially more morbid treatment.

**REDUCED-INTENSITY CONDITIONING (RIC) HEMATOPOIETIC STEM CELL TRANPLANTATION (HSCT) FOR CLL**

Although myeloablative transplant in CLL can result in durable remissions, rates of transplant-related mortality (TRM) are unacceptably high, ranging from 46% to 57% [3,4]. Patients who survive, notably those who develop chronic graft-versus-host disease (cGVHD), have a good chance of developing long-term disease control, suggesting a strong graft-versus-leukemia (GVL) effect in this disease. The high mortality of myeloablative transplant in CLL even among young patients greatly reduced its application, even in the most refractory and high-risk individuals. Reduced-intensity conditioning (RIC) regimens were introduced as a way to exploit the GVL effect while reducing TRM, making transplant more available to a patient population where the median age at diagnosis is 72.

Fludarabine was evaluated by researchers at M.D. Anderson as a conditioning agent for RIC HSCT both because it is an effective treatment for CLL and because it is highly immunosuppressive as shown by others in more high risk diseases. The initial study yielded a complete response (CR) in 8 of 11 patients, and donor lymphocyte infusions were able to induce remissions following transplant, again providing good evidence of a GVL effect [5]. The Cooperative German Transplant Study Group evaluated RIC with fludarabine, busulfan, and antithymocyte globulin in 30 patients with advanced CLL, half of whom had unrelated donors. They reported 72% overall survival (OS) at 2 years, with 67% progression-free survival (PFS) and 15% TRM. Fifty-six percent of patients developed grades 2-4 acute GVHD, whereas 75% developed cGVHD. Of note, late CRs were observed up to 2 years after transplant, again providing evidence of a GVL effect [6]. The 2-year OS among 46 patients with advanced CLL who received nonmyeloablative fludarabine and busulfan conditioning at Dana-Farber Cancer Institute was 54%, with a PFS of 34%. In this analysis, the primary cause of treatment failure was relapse, and chemotherapy-refractory disease at transplant was associated with a 3.2-fold risk of death ($P = .02$). Other factors that increased the risk of relapse were low levels of donor chimerism at day 30, increased number of previous therapies, and adverse cytogenetics [7]. Five-year follow-up following nonmyeloablative transplant has been reported from a multi-institution protocol led by the Fred Hutchinson Cancer Research Center. The 5-year incidences of nonrelapse mortality, OS, and PFS were 23%, 50%, and 39%, respectively. Of the patients who survived, 76% were entirely well, while 24% continued to receive immunosuppression for cGVHD [8]. The CLL3X trial from the German CLL Study Group was a phase II study that prospectively evaluated the long-term outcome of RIC HSCT in patients with poor-risk CLL. Ninety patients received allogeneic transplant following fludarabine and cyclophosphamide-based conditioning. The 4-year nonrelapse mortality, event-free survival (EFS), and OS were 23%, 42%, and 65%, respectively. Of the patients with matched related donor monitoring available, 52% were alive and negative at 12 months following transplant, and the 4-year EFS of this group of patients was 89%. Interestingly, EFS was similar for all genetic subsets, including patients with the 17p deletion [9].

Attempts to both improve relapse-free survival following transplant and to modulate the impact of GVHD have led investigators to incorporate monoclonal antibodies into transplant regimens. Rituximab, alemtuzumab, and ofatumumab all have single agent activity in CLL, and rituximab has been shown to improve OS in previously untreated patients when added to fludarabine and cyclophosphamide compared with chemotherapy alone [10]. Immunomanipulation with rituximab for patients with persistent disease following nonmyeloablative transplant can induce responses, and in a series reported by M.D. Anderson, a survival advantage was observed among patients who received rituximab as part of their conditioning, in addition to fludarabine and cyclophosphamide, compared with those who received chemotherapy alone [11]. The current CALGB CLL transplant study incorporates rituximab into the conditioning and includes rituximab maintenance following transplant. Given alemtuzumab’s ability to deplete T cells, it has also been incorporated into conditioning, in hopes of reducing GVHD. Although Delgado et al. [12-14] report reduced incidence of cGVHD in patients who received alemtuzumab, the incidence of fungal and viral infections is increased among those patients, leading to relatively high TRM, and integration of alemtuzumab into conditioning has been associated with inferior PFS, which is consistent with our institutional observation. The CLL3X study also found T cell depletion with alemtuzumab to have an adverse impact on EFS and OS in a multivariate analysis [9].
WHEN IS THE BEST TIME TO TRANSITION CLL PATIENTS TO RIC HSCT?

 Patients with poor-risk features such as del(17p) or (11q) or immunoglobulin heavy chain variable (IGHV) unmutated disease have inferior PFS following chemotherapy. A case-control study comparing outcomes of patients who underwent RIC HSCT with patients who received conventional therapy demonstrated that the median overall survival was 113 months for HCT patients and 85 months for controls when calculated from the time of diagnosis and 103 and 67 months, respectively, when calculated from time of first therapy. Both patient groups were well balanced with respect to cytogenetics, CD38, and ZAP-70 expression, and IGHV mutational status [15]. A small retrospective analysis of outcomes among patients receiving RIC HSCT stratified by prognostic risk group suggested that RIC HSCT could overcome the adverse prognosis associated with del(11q) or del(17p), as well as unmutated IGHV [16]. A study of 44 patients with del(17p) CLL from the European Group for Blood and Marrow Transplantation database, 89% of whom received RIC, demonstrated 3-year OS and PFS rates of 44% and 37%, respectively. The cumulative incidence of progressive disease at 4 years was 34%, and no late relapses occurred in the 9 patients with follow-up longer than 4 years, suggesting a survival plateau in even this high-risk group of patients. Extensive cGVHD occurred in 53% of patients [14]. Among a series of 34 patients with unmutated IGHV, the risk of relapse at 5 years was 66% among patients who received an autologous transplant versus 17% among patients who received RIC HSCT, suggesting that allogeneic transplant may overcome the unfavorable effect of having unmutated disease [17]. Fit patients with del(17p), those who are refractory to fludarabine and alemtuzumab, and those who have a PFS of <24 months after intensive rituximab-containing therapy should all be considered for RIC HSCT if remission is achieved [18]. Further refinement of what constitutes a high-risk patient may broaden the indication for transplant in coming years. In contrast, development of targeted therapies that induce durable remission may diminish the number of CLL patients going forth for transplantation.

TARGETED THERAPY IN CML AND CONTRIBUTION TO CHANGING ROLE OF HSCT IN THIS DISEASE

Following the introduction of imatinib as a frontline strategy in CML, the rates of allogeneic HSCT quickly dropped worldwide, most notably in patients with chronic phase disease. HSCT maintained a role in the treatment of patients with high-risk disease, including patients in accelerated or blast phase or those who had failed imatinib [19]. Although patients in accelerated and blast phase frequently respond to TKIs, the response is transient, and HSCT remains an effective treatment for these patients. Results of HSCT have been reported in this patient population and have consistently shown a lack of beneficial or deleterious effect of imatinib on transplant, and a recent analysis by the Center for International Blood and Marrow Transplant showed that conventional prognostic indicators from the preimatinib era remain the strongest and that there are no new prognostic indicators for transplant outcomes in the imatinib era [20]. Another population for whom HSCT remains important is patients who harbor the T315I mutation, which confers resistance to all licensed TKIs. An analysis of 64 patients from the European Blood and Marrow Transplant registry with CML and de novo Ph+ acute lymphoblastic leukemia and harboring a T315I mutation demonstrated that survival probabilities 24 months after HSCT were 59%, 67%, 30%, and 25% for chronic phase, accelerated phase, blast phase, and Ph+ acute lymphoblastic leukemia, respectively. A myeloablative regimen was used in 60% of the patients. Multivariate analysis identified blast phase at transplant and unrelated stem cell donor as unfavorable factors [21]. As new, effective agents transition into the therapy algorithms of CLL, it remains to be seen if a similar shift toward diminishing need for allogeneic transplantation will occur.

NOVEL TARGETED THERAPIES IN CLL

Advances in the biology of CLL have brought forth a plethora of new targeted agents that ultimately have great potential to impact the treatment of CLL. Although there are more than 100 specific agents currently in clinical trials, only a select few offer both single agent activity and the potential to induce durable remissions in high-risk CLL patients making them potential candidates to delay allogeneic transplantation. These agents are summarized by class in the sections below.

MONOCLONAL ANTIBODIES

Whereas monoclonal antibodies such as rituximab have greatly improved our ability to cytorerduce and improve durable remissions in CLL, successes that will likely build to durable long-term remissions similar to that seen with imatinib are unlikely. To date, improvement in design of CD20 antibodies, including ofatumumab, which mediates improved complement-dependent cytotoxicity, and GA101, which promotes improved direct killing and natural killer (NK) cell-mediated antibody dependent cellular cytoxicity, has been the major advance made in antibody therapeutics. Ofatumumab has been provisionally
approved for use in fludarabine- and alemtuzumab-refractory CLL and is under investigation both in combination with fludarabine/cyclophosphamide versus fludarabine, cyclophosphamide, and rituximab (FCR) for younger patients and as a maintenance treatment after chemoimmunotherapy in ongoing randomized phase III trials. Ofatatumumab induces short remissions in refractory patients and should be considered only as a bridge to transition these individuals to allogeneic transplantation. GA101 is a yet unapproved, Type II glycoengineered humanized CD20 monoclonal antibody that binds CD20 in a completely different orientation than rituximab and over a larger surface area [22]. It initiates nonapoptotic cell death via an actin-dependent lysosome-mediated mechanism that is reliant on cell-to-cell contact [23]. Depletion of CLL cells in whole blood samples has been demonstrated, and it may be more potent than rituximab at similar concentrations [24,25]. The addition of GA101 to fludarabine, bendamustine, or Bcl-2 family inhibitors appears to have a synergistic effect in xenograft models [26]. A recently reported phase I study in 13 relapsed/refractory CLL patients demonstrated that GA101 is relatively well tolerated, with the most common grade 3-4 toxicity being transient neutropenia in 9 patients. One CRi, 7 partial responses (PRs), and 3 patients with stable disease were observed. No clear dose relationship was established [27]. GA101 is being evaluated in a phase II study as a single-agent in untreated and relapsed/refractory CLL and in combination with FCR as first-line therapy. A variety of other therapeutic antibodies are under clinical development at this time in CLL but at this point no emergent candidate comes forth that will induce durable remissions in CLL thereby abrogating eventual need for transplantation.

**BCL-2 FAMILY ANTAGONISTS**

BCL-2 and related family member proteins such as mcl-1 are overexpressed in CLL and thereby disrupt apoptosis in this disease. A long history of development of Genasense (G3139), a BCL-2 antisense molecule was pursued in CLL with eventual failure in a randomized phase III study but notably with some evidence of clinical benefit. Derived from this early effort was development of small molecule therapeutics that could interfere with BCL-2 protein binding to BH3 domain only proteins. The most promising of these is navitoclax (ABT-263), an orally bioavailable, BH3 mimetic that inhibits multiple antiapoptotic Bcl-2 family proteins. In phase I testing, navitoclax has a favorable pharmacokinetic profile and safety profile, with dose-limiting toxicities (DLTs) including tumor lysis syndrome (TLS) and thrombocytopenia among the CLL cohort. Although thrombocytopenia was sometimes quite profound, this was shown to be a direct consequence of inhibiting bcl-xl in platelets. Several patients were able to maintain a >50% reduction in circulating lymphocytes for over 6 months with significant reduction in lymph node enlargement [28]. The combination of navitoclax with BR, evaluated in a phase I study, has thus far been well tolerated, with no DLTs of thrombocytopenia or neutropenia, and there was evidence of antitumor activity. Data regarding the combination of navitoclax with FCR is still being collected [29]. An alternative BCL-2 antagonizing small molecule (ABT199) that lacks influence on bcl-xl, which therefore would be predicted to avoid problematic thrombocytopenia is currently beginning phase I testing in CLL. Given the durable remission identified in a subset of ABT-263 patients and enhanced ability to combine this class of drugs with other therapies used in CLL, the likelihood of future contribution of this agent to treatment of advanced CLL is high.

**CYCLIN-DEPENDENT KINASES (CDK) INHIBITORS**

One class of drugs that has promise for the treatment of CLL is those that target the cyclin-dependent kinases (CDK inhibitors). Preclinical investigation of flavopiridol by our group [30] and others [31,32] demonstrated that this agent had potent in vitro activity against CLL cells. Other studies have demonstrated that cell death promoted by flavopiridol may be, in part, because of inhibition of CDK9 and global transcriptional inhibition [33]. Based upon these studies, several clinical trials investigating a 72 hour [34], 24 hour [35], and 1 hour [34] infusion of flavopiridol were undertaken with minimal evidence of clinical benefit and with significant toxicity including diarrhea, cytokine release syndrome, and neutropenia. Likewise, poor responses were observed with flavopiridol in a multitude of solid tumor studies prompting temporary cessation of development of this compound. Our group identified differential flavopiridol protein binding between bovine and human albumin in culture media and successfully modeled a pharmacokinetic schedule of administration of flavopiridol that employed a 30-minute loading dose followed by a 4-hour infusion of drug to maintain a concentration of 2 μM for 4 to 6 hours [36]. A phase I study using this schedule of administration in 52 patients demonstrated the dose limiting side effect of hyperacute TLS. Other toxicity observed included biphasic neutropenia and diarrhea. Of the 52 patients enrolled, 21 patients (40%) achieved a PR with a median PFS of 12 months [37]. This phase I study prompted a phase II study of 64 patients, of which 34 patients (53%) responded [38]. All those with del(17p13.1) responded irrespective of bulky nodal status [38]. A multicenter phase II trial confirmed single agent flavopiridol activity albeit at a lower frequency
than observed in our single institution study. These results provided support for development of improved CDK inhibitors in CLL that have improved pharmacologic, protein binding, and off-target effects compared with flavopiridol.

Dinaciclib is one such selective inhibitor of CDKs 1, 2, 5, and 9 that was identified as having a favorable therapeutic index in an in vivo cancer screen. In CLL cells, dinaciclib promotes concentration-dependent apoptosis independent of IGHV mutational status and fludarabine-refractoriness, although CLL cells from patients with del(17p) were more resistant than cells from patients with normal cytogenetics [39]. In a phase I study, dinaciclib had an acceptable safety profile, with responses in patients with bulky disease and in 7 of 15 patients with del(17p). Two cases of tumor lysis syndrome requiring temporary dialysis were observed, and an additional cohort is currently ongoing to determine if stepped-up dosing reduces the incidence of TLS [40]. Notably different from flavopiridol is the ability of patients to receive dinaciclib for an extended period of time without undue side effects. Both flavopiridol and dinaciclib produce stable remissions after halting therapy as well, making them ideal cytoreductive therapies for patients ultimately going onto RIC HSCT.

**B CELL RECEPTOR (BCR) KINASE INHIBITORS**

Multiple studies have demonstrated the importance of BCR signaling in the survival of normal and transformed B cells. This is highlighted in particular among high risk CLL where ZAP-70 expression is abundant, which has been shown to indicate enhanced BCR signaling. Several proximal kinases including syk, lyn, PI3-kinaseδ, and Bruton’s tyrosine kinase (BTK) have been targeted by therapeutic small molecule inhibitors. Early studies with the syk inhibitor demonstrated clinical activity in CLL. Development of this agent in CLL, however, has not been pursued for uncertain reasons.

Phosphatidylinositol-3-kinases (PI3K) integrate and transmit signals from cell surface markers, including the BCR, thereby regulating key cellular functions such as growth and survival. The PI3Kδ isozyme is largely restricted to hematopoietic cells, where it plays an important role in B cell homeostasis and function [41]. This offers the opportunity to target PI3K more selectively, thereby not interfering with insulin signaling and other off target essential functions associated with alternative isoforms. CAL-101 is a highly selective PI3Kδ inhibitor that promotes apoptosis in B cell lines including CLL [42,43]. CAL-101 reduces survival signals derived from the microenvironment, and in stromal cocultures, sensitizes CLL cells toward bendamustine, fludarabine, and dexamethasone [15]. In a phase I study of patients with relapsed or refractory CLL, CAL-101 proved to be safe, with a >50% reduction in lymphadenopathy noted in 80% of patients. Medians for duration of response and PFS had not been reached as of publication of the abstract [44]. Notably, toxicity with this regimen has been quite modest to this point, with DLT in lymphoma being reversible transaminitis. Studies of CAL-101 in combination with rituximab, ofatumumab, FCR, and BR are currently ongoing. To this point, the durable remissions observed in a subset of refractory CLL patients receiving CAL-101 have mimicked the initial trials with imatinib in CML where refractory individuals gained significant benefit. The ability to transition patients off CAL-101 to RIC HSCT at this point is uncertain, particularly early on in therapy. Further studies addressing this are warranted.

BTK is a B cell-specific kinase that is located proximally in the BCR pathway and when manipulated, results in loss of BCR signaling without T or NK cell defects. PCI-32765, an orally bioavailable BTK inhibitor, antagonizes this pathway. Like CAL-101, it also abrogates protective features of the microenvironment. PCI-32765 was designed as a selective and irreversible inhibitor of the BTK protein [45]. When added directly to human whole blood, PCI-32765 inhibits signal transduction from the BCR and blocks activation of B cells. As with CAL-101, our group has demonstrated that PCI-32765 promotes apoptosis, inhibits proliferation of CLL cells and antagonizes survival signaling derived from the microenvironment [46]. Data regarding PCI-32765 in CLL has been reported from 2 clinical studies: a Phase I, first-in-human study in patients with recurrent B cell lymphoma and CLL, and a Phase Ib/II study in patients with CLL/small lymphocytic leukemia. In the phase I study, therapy was well tolerated with most adverse events being less than grade 2. T cell responses were not altered, and there was no significant depletion of peripheral blood T or NK cell counts [47]. Of the 78 subjects with CLL/small lymphocytic leukemia treated on the Phase Ib/II study as of May 2011, only 1 subject came off study because of progressive disease. Thirty-nine patients were evaluable for response when last reported, most of whom had at least 1 poor-risk molecular feature. In patients with lymphadenopathy, the rate of nodal response (>50% reduction in target lesions) was 89%. There was a 44% response rate at a median follow-up of 4 months (39% PR and 5% CR) per IWCLL/Cheson response criteria, including responses in patients with del(17p) [48]. Follow-up studies of the durability of PCI-32765 will remain quite relevant to the impact on disease control. Studies of PCI-32765 in combination with ofatumumab, FCR, and BR are currently ongoing. As with CAL-101, significant excitement in the field of CLL exists for this compound because of continued benefit documented in very refractory CLL patients. The ability to...
transition patients off PCI-32765 to RIC HSCT at this point is uncertain, particularly early on in therapy. Further studies addressing this are warranted.

**IMPACT OF NOVEL THERAPEUTICS ON RIC HSCT FOR CLL**

As the novel therapeutics discussed are in early phase clinical testing, their impact on and utility in conjunction with RIC HSCT is completely unknown at this time. Particularly because patients with high-risk disease often have inferior PFS following conventional chemotherapy, and as these agents move into phase II or III testing, it is increasing likely that patients moving on to transplant will have been exposed to one or more of these agents. It has been established that patients with diffuse large B cell lymphoma who relapse quickly following rituximab-containing therapy have inferior survival following autologous transplant [49]; it is far too early to speculate how these agents will impact the outcome following RIC HSCT. Additionally, for patients on therapies such as PCI-32765 and CAL-101 who can remain on treatment until disease progression or development of unacceptable toxicity, the timing both of transplant and when to stop the drug before transplant is not defined. It is also unknown whether any of these agents may play a role in post-transplant maintenance or as salvage for patients who relapse following transplant. Alternative target effects on NK or dendritic cells may limit applicability for some new drugs in posttransplant maintenance strategies. Indeed, the field of therapeutics in CLL is moving quite quickly and will be influenced by the longer term follow-up in trials of novel agents, including follow-up relating to safety integration with RIC HSCT. For now, our group’s approach has been to follow international guidelines for application of RIC HSCT, with the exception of patients gaining a very good early response to one of the BCR kinase inhibitor agents when alternative novel agents (ie, CDK inhibitor or BCL-2 antagonist agents) are available for future cytoreduction should progression occur. Access to multiple active agents for CLL is necessary for such an approach, emphasizing the need for continued development of therapeutics in this disease.

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**REFERENCES**


