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Educational Reviews

Back to the Future! The Evolving Role of Maintenance Therapy after Hematopoietic Stem Cell Transplantation

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A B S T R A C T

Relapse is a devastating event for patients with hematologic cancers treated with hematopoietic stem cell transplantation. In most situations, relapse treatment options are limited. Maintenance therapy offers the possibility of delaying or avoiding disease recurrence, but its role remains unclear in most conditions that we treat with transplantation. Here, Dr. Hourigan presents an overview of minimal residual disease (MRD) measurement in hematologic malignancies and the applicability of MRD-based post-transplantation interventions. Dr. McCarthy reviews current knowledge of maintenance therapy in the autologous transplantation context, with emphasis on immunologic interventions and immune modulation strategies designed to prevent relapse. Dr. de Lima discusses current lines of investigation in disease recurrence prevention after allogeneic transplantation, focusing on acute myeloid leukemia and myelodysplastic syndrome.

INTRODUCTION

The popularity of maintenance therapy has waxed and waned in the management of hematologic malignancies. Enthusiasm has often been hampered by toxicity, lack of efficacy, or difficulty proving efficacy, especially in the post-transplantation setting. The biologic revolution has brought us a plethora of less toxic new agents, creating renewed interest in intervening after auto or allogeneic hematopoietic stem cell transplantation (HCT).

In addition, new technologies are dramatically changing our ability to measure residual hematologic malignancy, allowing a more direct evaluation of potential maintenance of remission strategies. It is also important to emphasize that the problem of post-transplantation recurrence seems to be getting worse, not better, in recent years. This is possibly the reflection of increased access to HCT for older patients, who have diseases with intrinsically worse prognoses, and the use of reduced-intensity preparative regimens, which carry the trade-off of less toxicity at the expense of increased likelihood of relapse.

Therefore, it seems appropriate to highlight this rapidly evolving area of investigation in autologous HCT (auto-HCT) and allogeneic HCT (allo-HCT), where solid tumor and hematologic malignancy treatments are now joining forces with hematopoietic stem cells, T cells, NK cells, and other immunologically active types of cells.

INVESTIGATING MINIMAL RESIDUAL DISEASE

The most common cause of treatment failure after HCT is the primary malignancy for which the transplantation was performed. This includes patients after transplantation with either "refractory" disease or those previously in remission with a clinical complete response (CR) who have recurrent disease or "relapse." In patients treated for hematological cancers, the remaining total disease burden is a continuous variable, and clinical response criteria, therefore, simply represent artificial thresholds based on the technical sensitivity of standard assays to detect disease. Patients with disease classified as being in remission or reaching CR after HCT, therefore, represent a highly heterogeneous group in terms of the residual disease burden (ranging from no remaining disease to up to a billion malignant cells) and, consequently, also have heterogeneous clinical outcomes (ie, 25% to 50% will relapse). Maintenance therapy, that is, therapy given to patients in a CR after completion of standard therapy to prevent future relapse, can be effective, but it may also be associated with significant toxicity, potentially limiting its applicability. It is now clear, however, that high sensitivity measurements of remaining disease burden (minimal residual disease [MRD]) in patients with CR (ie, quantification below the traditional threshold of hematologic CR) can have significant utility in patient selection and making decisions regarding maintenance therapy after HCT.

MRD can be measured for both lymphoid and myeloid hematological malignancies (Table 1) and these measurements can be informative when taken either before or after HCT, and in both autologous and allo-HCT. MRD can be detected from a number of sources including bone marrow (BM) or peripheral blood. MRD can be measured in a variety of ways, ranging from fluorescent in situ hybridization (FISH) for cytogenetic abnormalities and (in the post–allo-HCT setting) donor cell chimerism [1] to higher sensitivity methods [2], such as flow cytometry, polymerase chain reaction (PCR)–based methods to quantify genes overexpressed in malignant clones (PCR-GE, eg, WT1) or for unique tumor-specific somatic mutations, splice variants or other

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Table 1
Methods to Detect Minimal Residual Disease (MRD) in Hematological Malignancies for Which Hematopoietic Stem Cell Transplantation is Commonly Performed

Disease	MRD Methods Available*	Notes	References
AML	NGS PCR-mut PCR-GE MFC Cytogenetics	*Standard of Care in APL;* *Multiple clinical trials ongoing using MRD. Lack of universal target in non-APL AML makes MRD assessment challenging.*	[17,18,45-48]
MDS	PCR-GE MFC FISH	*Use of donor chimerism for MDS MRD well established but generally underdeveloped area of research.*	[18,49]
ALL	PCR-mut (Ph+) NGS MFC	*25-year history of being able to detect MRD in ALL. Deeply integrated into clinical trials for a decade.*	[32,33,50]
CLL	NGS PCR MFC	*Well established, with multiple modalities available.*	[51]
CML	PCR-mut (Ph+)	*Standard of care*	[15,50]
MM	PCR-mut (BM) PCR-mut (PB) NGS MFC Imaging	*As therapy in MM has become more effective, MRD measurements have become integrated in standard response criteria definitions.*	[10]

AML indicates acute myeloid leukemia; MDS, myelodysplastic syndrome; ALL, acute lymphoblastic leukemia, CLL, chronic lymphoid leukemia; CML, chronic myeloid leukemia; MM, multiple myeloma; MFC, multiparameter flow cytometry; PCR-GE, PCR for gene overexpressed in disease compared with healthy tissue; PCR-mut, PCR for sequence, somatic mutation, or splice variant specific to tumor; Ph+, Philadelphia positive ie: Bcr:Abl translocation; NGS, next-generation sequencing; BM, bone marrow; PB, peripheral blood.

* Donor chimerism analysis post allo-HCT is an MRD testing option in all listed disease indications.

pathognomonic sequences (PCR-mut, eg, t(9;22)BCR-ABL or t(15:17) PML/RARA) and by next generation sequencing [3,4]. We review here contemporary use and capabilities of MRD testing in multiple myeloma (MM), myeloid, and lymphoid malignancies before discussing how such measurements could have utility for management of post-HCT maintenance therapy.

Over a quarter of the approximately 18,000 stem cell transplantations performed in adults in the United States in 2011 were for MM. These were predominantly auto-HCT [5]. The biology of this monoclonal plasma cell neoplasm (including nongermline rearranged receptor sequence, atypical cell surface protein phenotype and characteristic excreted proteins) allows for multiple approaches for tracking disease burden, including quantitative immunoglobulin and free light chain assays [6,7], imaging [8], multiparameter flow cytometry (MFC) [6,9,10], and immunoglobulin gene rearrangements using allele specific oligonucleotides by PCR methods [9,11,12]. As in other hematological malignancies PCR-based methods offer advantages of high sensitivity and specificity, low cost, lack of requirement for expert pathologist interpretation but with the disadvantages of need for a unique sequence target and time taken to establish and run the assays. Although appropriate questions have been raised regarding the lack of standardization of flow cytometry-based MRD assays in MM [13], and perhaps a lack of deep sensitivity compared with PCR-based methods [10,11], this modality is generally considered easy to perform with quick turnaround and sufficient sensitivity to discriminate patients into groups reflective of relapse risk. For example, a recent analysis of patients treated on the MRC Myeloma IX study demonstrated that the absence of bone marrow aspirate MFC-detected MRD at 100 days after autologous (ASCT) was strongly associated with favorable progression-free survival (PFS) and overall survival (OS) [14]. MRD status remained predictive of improved outcome when used within subgroups, such as those with adverse or favorable cytogenetics and those with or without CR defined by immunofixation. The combination of cytogenetic risk information and MRD status after ASCT was exceptionally powerful. In addition to the ability to determine efficacy of ASCT (half of MRD positive patients after induction became negative after ASCT) and predict prognosis based on day 100 post-HCT assessment, MRD could also be used to track efficacy of maintenance therapy (8 of 29 MRD positive patients after ASCT who were randomly assigned to thalidomide maintenance became MRD-negative when checked approximately 10 months after ASCT, compared with 1 of 29 patients who did not receive maintenance).

In the myeloid malignancies, MRD monitoring is already the standard of care in both chronic myeloid leukemia [15] and acute promyelocytic leukemia [16]. The literature on non–acute promyelocytic leukemia acute myeloid leukemia (AML) MRD measurement has recently been reviewed by ourselves [17] and others [2,18,19]. It is now well established that both MFC- and PCR-based MRD measurements are technically possible, predictive of relapse risk and overall survival when measured either before [20-22] or after [23,24] HCT, and can be used to guide dose escalation of chemotherapy before HCT [25] and initiation of post-HCT maintenance [26,27]. In myelodysplastic syndrome (MDS), decreasing donor chimerism post allo-HCT is well established as independently predictive of both inevitable hematologic relapse and inferior survival [1], and MFC-based [28] and PCR-based [29] MRD detection methodologies are also being developed.

MRD monitoring after HCT is also possible in the lymphoid malignancies. In acute lymphoblastic leukemia (ALL), MRD has been detectable for over 25 years [30] with the prognostic significance of MRD after treatment known [31] and the ability to use post-HCT MRD-guided therapy to reduce relapse risk available for over a decade. More recently a consensus panel defined technical standards for MRD assessment by MFC and PCR for European clinical trials [32], and the American guidelines now specifically comment on MRD assessment [33]. In chronic lymphocytic leukemia (CLL) donor chimerism [34], PCR [35], MFC [36] and next-generation sequencing [37] approaches have all been used, and MRD has been used to risk-stratify patients for

Table 2
Phase III Studies Comparing Maintenance Therapy or Consolidation versus Observation after Autologous Hematopoietic Cell Transplantation

Study	Disease	Intervention	Benefit in EFS/PFS	Benefit in OS
Furman et al. [54]	B cell NHL	B4 blocked ricin	No difference	Favors observation
Gisselbrecht et al. [55]	DLBC NHL	Rituximab	No difference	No difference
Pettengell et al. [56]	Follicular NHL	Rituximab	Favors rituximab	No difference
Thompson et al. [57]	NHL, low, int, high	IL-2	No difference	No difference
Blaise et al. [72]	AL (AML + ALL)	IL-2	No difference	No difference
Attal et al. [63]	ALL	IL-2	No difference	No difference
Bolaños-Meade et al. [58]	Poor Risk NHL	CSA IFNγ+IL-2 to generate autologous GvHD	No difference	No difference
Simonsson et al. [59]	AML	Linomide	No difference	No difference
Attal et al. [63]	MM	Thalidomide	Favors thalidomide	Favors thalidomide
Barlogie et al. [64]	MM	Thalidomide	Favors thalidomide	Trend to thalidomide
Lokhurst et al. [65]	MM	Thalidomide	Favors thalidomide	No difference
Morgan et al. [66]	MM	Thalidomide	Favors thalidomide	No difference
Morgan et al. [66]	MM	Thalidomide	Favors thalidomide	Worse in high risk cytogenetic patients
Spencer et al. [67]	MM	Thalidomide and prednisone	Favors thalidomide	Favors thalidomide
Krishnan et al. [68]	MM	Thalidomide and dexamethasone	Trend toward thalidomide	No difference
Maiolino et al. [69]	MM	Thalidomide and dexamethasone	Favors thalidomide	No difference
Stewart et al. [70]	MM	Thalidomide and prednisone	Favors thalidomide	No difference
McCarthy et al. [71]	MM	Lenalidomide	Favors lenalinomide	Favors lenalinomide
Attal et al. [72]	MM	Lenalidomide	Favors lenolinomide	No difference
Boccadoro et al. [73]	MM	Lenalidomide	Favors lenolinomide	Favors lenalinomide
Sonneveld et al. [74]	MM	Bortezomib versus thalidomide	Favors bortezomib in del17 patients and those with renal failure	Favors bortezomib in del17 patients and those with renal failure
Cavo et al. [75]	MM	Bortezomib and thalidomide versus thalidomide	Favors bortezomib and thalidomide	No difference
Mellqvist et al. [76]	MM	Bortezomib	Favors bortezomib	No difference

AL indicates aAcute leukemia; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; EFS, event-free survival; PFS, progression-free survival; NHL, non-Hodgkin lymphoma; OS, overall survival; MM, multiple myeloma; DLBC, diffuse large B cell; CsA, cyclosporine; IFN, interferon; GVHD, graft-versus-host disease.

additional therapy after HCT [34]. MRD is also quantifiable for Hodgkin's and non-Hodgkin lymphomas (NHL) [38,39], is predictive of outcome, and can be used to guide maintenance therapy [40,41].

It is important to remember that absence of detectable MRD is not necessarily equivalent to absence of disease. In all but the most sensitive of assays, MRD-negative patients are still at risk of relapse and may still benefit from maintenance therapy. Additionally, most hematological diseases are not homogenous, and both oligoclonality [42] and the presence of residual precursor cells of a different phenotype are possible [43]. MRD testing, however, with appropriate thresholds set on high sensitivity assays, may be useful in management of post-HCT maintenance therapy in at least 3 distinct ways. First, "landmark" MRD testing using high-sensitivity methods in the peri-HCT period can help stratify patients in CR to identify those at the greatest risk of relapse so that potential benefit can be weighed when the potential risks of maintenance therapy are considered. For example, the hazard ratio for post-HCT relapse for patients with MRD detectable by MFC in BM was 4.9 to 8.49, compared with those who were MRD-negative before allo-HCT for AML in CR, even after adjustment for other risk factors [20,21]. Similarly, increased relapse risk has also been noted for detection of MRD at single landmarks when measured after HCT [9,23,34,44]. Second, serial monitoring of MRD might help guide maintenance therapy in patients who have a hematological and molecular CR after HCT [45]. This group of patients has a small but significant risk of hematological relapse, and the ability to detect "molecular relapse" during surveillance with sufficient lead time to allow therapeutic intervention before the development of frank hematological relapse could be of significant benefit. Third, MRD measurements offer highly sensitive quantification of disease and can, therefore, be used as an accurate biomarker to determine the efficacy of any proposed maintenance therapy [46-51]. Adoption of this approach after HCT would potentially allow rapid drug screening of potential agents to prevent relapse and the opportunity for real-time personalization of maintenance treatment for any individual patient based on objective molecular response criteria.

PREVENTING RELAPSE AFTER AUTOLOGOUS HEMATOPOIETIC CELL TRANSPLANTATION

Auto-HCT is a primary form of therapy for high-risk malignant hematologic disorders. These include first remission high-risk or relapsed and refractory lymphomas, leukemias, and MM. Myeloablative doses of chemotherapy, with or without radiation, are given to destroy the tumor with an infusion of hematopoietic cells to rescue the patient from the marrow-toxic effects of the high-dose regimen. There is no graft-versus-tumor effect accompanying auto-HCT, unlike the case in allo-HCT. Thus auto-HCT relies on the dose-intensive effects of the treatment regimen, resulting in a higher relapse risk when compared with allo-HCT. However, allo-HCT is often complicated by graft-versus-host disease, resulting in a higher treatment-related mortality rates. Thus, strategies have been developed to increase antitumor effects after auto-HCT to decrease relapse.

An early study demonstrated that relapse can occur due to infusion of cancer cells in the graft [52]. Other studies have demonstrated that the major source of relapse after auto-HCT is endogenous tumor remaining in the patient and that positive selection for CD34+ hematopoietic cells does not result in improved survival [53]. Over the past 3 decades, different strategies have been tested in efforts to improve auto-HCT outcomes, including purging of the graft and post–auto-HCT treatments to decrease relapse incidence.

The use of post–auto-HCT treatments is attractive, since the burden of malignant cells in the recipient is frequently low after auto-HCT. In addition, the immune system has been "reset" after the dose-intensive regimen, facilitating immune manipulation against the primary cancer. Thus, post–auto-HCT could be the optimal time to use maintenance treatment.

There has been limited success using monoclonal antibodies as maintenance therapy for B cell NHL. Neither B4-blocked ricin (anti-CD19 conjugated with ricin) nor rituximab (anti-CD20) improved event-free survival or OS in this setting (Table 2) [54-56]. Rituximab improved PFS in follicular lymphoma patients but had no effect on OS.

Induction of an antitumor immune response by immunostimulatory agents has been tested. Interleukin-2 (IL-2) maintenance has been given in a variety of dosing schedules to up-regulate T cell immunity, but phase III studies showed no effect on outcome after auto-HCT for NHL, AML, or ALL [57]. Another approach was the induction of autologous graft-versus-host disease using cyclosporine A with interferon-γ and IL-2. However, a randomized trial showed no benefit with this approach in patients with poor-risk lymphoma [58]. Another randomized study compared linomide, an immune-stimulatory agent, with placebo after auto-HCT for AML and showed no difference in disease-free survival or OS [59]. Other approaches evaluated in phase I/II studies include IL-1 and granulocyte-macrophage colony stimulating factor.

Chemotherapy has been used after auto-HCT in diseases with a high probability of relapse, keeping in mind that the role of auto-HCT in ALL and AML with or without hematopoietic graft purging has been uncertain due to the persistently high-risk of post-transplantation relapse. Thus, standard chemotherapeutic strategies have been tested but are not widely used for ALL or AML. Tyrosine kinase inhibitors (TKIs) have been used as part of induction, consolidation and maintenance therapy for Ph+ ALL [60]. Generally, TKIs (imatinib and dasatinib) have been well tolerated after auto-HCT. One study showed evidence that auto-HCT followed by TKI resulted in similar outcomes as allo-HCT [61].

Preventing MM relapse after auto-HCT has been a priority because the disease recurs in most patients after transplantation. MM MRD evaluation is important for determining long-term disease control [14]. Several strategies have been studied. These have included interferon, glucocorticoids, and more recently, the immunomodulatory drugs, thalidomide, lenalidomide and the proteasome inhibitor, bortezomib [62]. Thalidomide maintenance improved PFS but had less consistent effects on OS [63-70]. Maintenance with lenalidomide improved PFS in all placebo-controlled studies [71-73] (Table 2). One study did not show an OS benefit whereas the other 2 did. Unexpectedly, the results also showed approximately a 3-fold increased risk of second primary malignancies in patients receiving lenalidomide maintenance when compared with placebo. The CALGB 100104 study showed an increased incidence of second primary malignancies in patients receiving lenalidomide, whereas there was an increased incidence of progression and death among placebo arm patients [71]. The CALGB 100104 study demonstrated an increased incidence of second myeloid malignancies, whereas the IFM05-02 study demonstrated an increased incidence of second lymphoid malignancies. The IFM05-02 study did not show an OS difference and is the only large study that discontinued lenalidomide maintenance (at approximately a median time of 32 months). Bortezomib, given as part of induction and maintenance, improved PFS and OS as compared with chemotherapy induction (vincristine/doxorubicin/dexamethasone) followed by thalidomide maintenance [74]. The major benefit was seen primarily in del17 cytogenetic disease and in patients with renal failure at diagnosis.

Chemotherapy consolidation is another strategy used to maintain disease response after auto-HCT. This approach has been studied more often in Europe than in the United States. Cavo et al. demonstrated that bortezomib/thalidomide/dexamethasone consolidation improved PFS when compared with thalidomide/dexamethasone [75]. Mellqvist et al. showed that single-agent bortezomib improved PFS when compared with no therapy [76]. The IFM05 02 study contained lenalidomide consolidation for both arms, so it was not possible to determine the effect of consolidation. So far, consolidation strategies have not shown OS benefit.

Immunomodulatory drugs have many different mechanisms of action [77] (Table 3). In addition to a direct anti-MM effect, lenalidomide and pomalidomide have potent immunomodulatory effects. Lenalidomide reverses T cell anergy at the immune synapse in CLL patients and could be utilized to enhance immune surveillance after auto-HCT [78]. Lenalidomide potentiates the action of the anti-CS1 antibody elotuzumab, resulting in clinical responses in patients with relapsed and refractory MM [79]. Phase III trials are ongoing to define the role of elotuzumab. A potential clinical trial could examine elotuzumab with lenalidomide after auto-HCT to determine whether the combination improves PFS and OS as compared with lenalidomide alone. Lenalidomide and rituximab have increased activity against relapsed or refractory mantle cell lymphoma when retrospectively compared with each agent alone [80].

Other strategies for maintaining response include the use of antibody-drug conjugates, such as brentuximab-vedotin (anti-CD30 and auristatin), inotuzumab-ozogamicin (anti-CD22 and calicheamicin), and gemtuzumab ozogamicin [81-83]. The latter is not available for clinical use in the United States, but a pediatric AML study showed that it could be used to decrease the burden of MRD [83]. Another antibody strategy employs chimeric antibodies known as bispecific T cell engagers (BiTEs) containing 2 different binding sites. One binding site is for the target of interest and the other is specific for CD3 so as to engage T cells and bring them close to the target. Blinatumomab is a novel BiTE that binds CD19, present on B cells, in NHL, CLL, and ALL [84]. The other binding target of blinatumomab is CD3, which is associated with the T cell receptor. In this way, the tumor cell functions as an antigen-presenting cell (APC) and as the T cell target. This close interaction results in T cell activation, T cell cytotoxicity, and target cell lysis. Maintenance protocols using BiTE antibodies after auto-HCT may eradicate MRD and provide prolonged disease control.

Targeted cellular therapy has been used to successfully treat B cell malignancies. Chimeric antigen receptor T cells (CAR T cells) contain a receptor with a defined specificity. The receptor is introduced into the patient's immune effector T cells in vitro. CAR T cells recognize and kill targets expressing the B cell antigen CD19, for example. The cells are expanded ex vivo and administered to the patient. The first effective CAR T cell studies demonstrated efficacy in patients with B cell malignancies [85-88]. The CAR T cells contain a lentiviral vector or a gamma-retroviral vector expressing a CD19 extracellular domain linked to T cell costimulatory receptor (CD137 or CD28) and CD3-zeta (a signal-transduction

Table 3
Summary of the Major Mechanisms of Action of Immunomodulatory Drugs

Effect	Mechanism	Relative Potency+ = Potency Factor of 10		
		Thalidomide	Lenalidomide	Pomalidomide
Immune modulation				
CD4+/CD8 + T cell co-stimulation	Increased tyrosine phosphorylation of CD28 and PI3-K signaling pathway. Increased activated protein-1 leading to increased IL-2 production.	+	++++	+++++
Treg suppression	Len and Pom inhibit Treg expansion and Foxp3 expression without affecting Treg survival and apoptosis or IL-10 and TGFβ expression.	–	+	+
Th1 cytokine production	IMiD co-stimulation effect on T and NKT cells results in increased Th1-cytokines IL-2 and IFNγ.	+	+++++	+++++
NK and NKT cell activation	IMiDs DC-induced NKT cell expansion and IFNγ secretion.	+	++++	+++++
ADCC	Enhanced NK cell ADCC with len and pom correlates with increased NK cell FasL and granzyme B but not perforin expression.	–	++++	++++
Interference with tumor microenvironment interactions				
Anti-angiogenesis (AA)	IMiDs inhibit endothelial sprout formation and vessel migration in vitro. AA occurs via modulation of chemotactic factors involved in endothelial cell migration including TNFα, VEGF and βFGF secreted by BMSC instead of inhibition of endothelial cell proliferation.	++++	+++	+++
Anti-inflammation	Thal, len and pom downregulate TNFα from LPS-stimulated monocytes, shorten the half-life of COX-2 mRNA that resulted in reduction in PGE_2. The exact signaling pathway involved is uncertain.	+	++++	+++++
Down regulation of adhesion molecules	IMiDs downregulate surface adhesion molecule expression plasma cells and PBMC, partially via the downregulation of TNFα.	+	++++	+++++
Anti-osteoclastogenic properties	IMiDs: downregulate osteoclastogenic mediator production from BMSC, including IL-6, TNFα, MIP1-α and RANKL. inhibit osteoclast maturation. inhibit Wnt/β-catenin signalling pathway, that is associated with osteoblastogenesis via the activation of DKK1, a negative regulator of Wnt signalling.	+	++++	+++++
Direct antitumor effects				
Antiproliferative activity	IMiDs induce (CDK) inhibitors: p21, p27 and p15, resulting in CDK inhibition causing cell cycle arrest in the G0/G1 phase of the cell cycle	+	+++	+++
	IMiDs induce changes in expression of Erg-1, 2 and SPARC.	+	+++	+++
	IMiDs downregulate NFκB with leading to reduction of the anti-apoptotic protein cIAP2 and FLIP expression.	+	+++	+++
	IMiDs variably inhibit caspase 3, 8 and 9.	+	+++	+++

AA indicates anti-angiogenesis; ADCC, antibody-dependent cellular cytotoxicity; βFGF, basic fibroblast growth factor; BMSC, bone marrow stromal cells; cIAP2, cellular inhibitor of apoptosis protein 2; CD, cluster of differentiation; CKD, cyclin-dependent kinase; COX-2, cyclo-oxygenase-2; DC, dendritic cell; DKK1, Dickkopf-related protein 1; Erg, early growth response genes; FasL, Fas ligand; FLIP, FLICE inhibitor protein; FOXP3, Forkhead box P3; IFN, interferon; IL, interleukin; Len, lenalidomide; MIP-1α, macrophage inflammatory protein-1α; NK, natural killer; NKT, natural killer T; NFκB, nuclear factor kappa-light-chain-enhancer of activated B cells; PBMC, peripheral blood mononuclear cells; Pom, pomalidomide; PI3-K, phosphatidylinositide 3-kinase; PGE_2, prostaglandin E_{-2}; RANKL, receptor activator of nuclear factor kappa-β ligand; SPARC, secreted protein acidic and rich in cysteine; Treg, regulatory T cells; TGFβ, transforming growth factor β; TNFα, tumor necrosis factor alpha; Wnt, wingless/integration-1; VEGF, vascular endothelial growth factors. Adapted from Quach H et al. [77].

component of the T cell antigen receptor) intracellular signaling domains. A cytokine-release syndrome has occurred with CAR T cell infusions and has been treated with glucocorticoids or tocilizumab. The former can damage the CAR T cells, whereas the latter appears to mitigate the inflammation without affecting the modified T cells. Of note, many patients develop B cell aplasia with hypogammaglobulinemia requiring gammaglobulin replacement. Another example of CAR T cell therapy utilized the Lewis-Y antigen in an AML study [89]. Other approaches include genetically modified APC with the generation of Epstein Barr virus–specific T cells to treat Epstein Barr virus–associated lymphoma [90].

Many issues require further study, including the ability to regulate or terminate CAR T cell activity. Anaphylaxis has been reported in some cases [91]. CAR T cells with an antimelanoma-associated antigen specificity for the therapy of melanoma and MM cross-reacted with the myocardial protein titin. Two patients with melanoma and MM developed fatal cardiotoxicity due to this unexpected cross-reactivity [92]. Therefore, many unanswered questions remain. What is the long-term safety of the CAR T cells? Many target antigens can be utilized for a variety of diseases, making it necessary to identify the most appropriate target for specific diseases [93]. Will auto-HCT be utilized for cytoreduction or will CAR T cell therapy decrease the need for auto-HCT? How will the cost for the generation of these treatments be managed?

Immune strategies that incorporate pharmaceuticals, antibodies directed to immunoregulatory pathways, and cellular treatments, including dendritic cell vaccines, can be used to

decrease the risk of relapse after auto-HCT. We are beginning to understand the regulation of T cell immunity. The use of immune checkpoint blockade therapy to magnify anticancer activity of T cells is summarized in Table 4 [94]. A complex and overlapping set of immunomodulatory molecules inhibits or stimulates the T cell response to antigenic stimulation. The anti–PD-1 antibody pidilzumab has already been utilized to reverse immune tolerance after auto-HCT for diffuse large B cell NHL [95]. Other strategies include blocking the killer-cell immunoglobulin-like receptor (KIR) pathway, blocking indoleamine 2,3-dioxygenase to inhibit regulatory T cell (T_{reg}) production, blocking CTLA-4 with the use of ipilimumab, or blocking the binding of Fas to Fas-ligand. Another approach under investigation includes antibody-mediated blockade of the CD200-CD200R inhibitory axis, which could magnify immune response against tumors that express CD200. Using AML cells as APC along with immune upregulation to enhance T cell activity after auto-HCT is another strategy for generating T cell immunity against AML. Strategies for preventing AML relapse are reviewed in Martner et al. [96].

A recent NCI workshop summarized strategies for preventing relapse after auto-HCT with a particular emphasis on MM [97]. The ability of lenalidomide and dendritic cell/MM fusion vaccines to enhance anti-MM immunity was among the highlighted approaches [98].

Improving our knowledge of immune tolerance and activation will likely lead to the application of new approaches that harness the immune system to treat and eradicate MRD after auto-HCT. Thus, some of the original hypotheses of immunosurveillance and growth of cancer cells may be better understood so as to prevent tumor growth in the context of immune activation without leading to anergy. Employing a spectrum of pharmacologic, immunologic cellular and antibody treatments may harness the immune system and eradicate clonogenic cells and reduce the likelihood of disease recurrence.

Table 4
Potential Immune Checkpoint Intervention Targets to Increase Antitumor Lymphocyte Activity

Antigen Presenting Cell	Ligand	Receptor	Effect on T cell Response
	PD-L1 (CD274)	PD-1 (CD279)	Negative
	PD-L2	PD-1	Negative
	B7-H3	Unknown	Negative
	B7-H4	Unknown	Negative
	GAL9, adenosine	TIM3, A2aR	Negative
	CD200	C200R	Negative
	TNFSF14 (HVEM)	BTLA	Negative
	B7-1/B7-2 (CD80/CD86)	CTLA-4 (CD152)	Negative
	MHC-Peptide	TCR (first signal)	KIR, LAG3 negative
	B7-1/B7-2 (CD80/CD86)	CD28	Positive
	B7RP1 (B7-H2 or L-ICOS)	ICOS	Positive
	CD137L	CD137	Positive
	OX40L	OX40	Positive
	CD70	CD27	Positive
Effect on APC response	Receptor	Ligand	
Negative/Positive*	CD200R	CD200 (OX-2)	

A2aR (also known as ADORA2A) indicates adenosine A2a receptor; B7RP1, B7-related protein 1; BTLA, B and T lymphocyte attenuator; CD, cluster of differentiation; CTLA4, cytotoxic T-lymphocyte antigen 4; GAL9 (also known as LGALS9), galectin 9; HVEM (also known as TNFRSF14), herpesvirus entry mediator; ICOS, inducible T cell costimulator; KIR, killer cell immunoglobulin-like receptor; LAG3, lymphocyte activation gene 3; OX-2 membrane glycoprotein, orexin receptor type 2; PD-1 (also known as PDCD1), programmed cell death protein 1; PD-L1 (also known as CD274), PD-1 ligand; PD-L2 (also known as PDCD1LG2), PD-2 ligand; TIM-3 (also known as HAVCR2), T cell membrane protein 3; TNF, tumor necrosis factor. Multiple cosignaling by costimulatory (positive) and inhibitory molecules (negative) interactions regulate T cell responses.
The major histocompatibility complex (MHC) molecule and peptide complex is recognized by the T cell receptor (TCR). This occurs via an antigen-presenting cell (APC) or a tumor cell. Several ligands and receptors have been described as inhibitory or costimulatory with multiple possibilities, including up and down regulation of TCR-mediated activation or anergy.
B7 family of co-stimulatory molecules.
* Negative: T cell coreceptors transmitting inhibitory signals after specific ligand binding expressed by APCs or cancer cells. Positive: T cell coreceptors transmitting stimulatory signals. Adapted from Ramsay AG [94].

MAINTENANCE THERAPY AFTER ALLOGENEIC TRANSPLANTATION

The hypothesis that the graft-versus-leukemia effect can be completely dissociated from graft-versus-host disease (GVHD) has driven a significant part of stem cell transplantation research over the last 20 years, with limited success. Most post–stem cell transplantation interventions have aimed at preventing GVHD while attempting to preserve antitumor activity. The principles and advances discussed in the previous sections of this review also apply here, and have increased the interest in augmenting the graft-versus-leukemia effect by pharmacologic (Table 5) or cellular approaches [99-102]. Although chronic myeloid leukemia and Philadelphia-positive ALL remain the prototypes of the diseases in which maintenance after allo-HCT has been proposed and used, the availability of new monoclonal antibodies and small molecules is leading investigators to consider post-transplantation approaches more often than in the past. In addition, refinement of conditioning regimens has led to significantly less toxicity but has not increased the cure rate for most diseases.

Azacitidine, a DNA methyl transferase inhibitor, is arguably the drug that has been most extensively studied in this scenario (and is the only one currently in phase III evaluation). In 2003, we hypothesized that low doses of this agent could decrease the risk of relapse after allo-HCT, based on a series of experiments in the 1980s and early 1990s showing increased expression of tumor antigens and HLA molecules in leukemia cells in vitro after exposure to hypomethylating agents. In addition, growing evidence at the time suggested a malignant cell differentiating effect of decitabine. Clinical and laboratory studies showed that longer exposure to lower doses were sufficient for demethylation and activation of tumor suppressor gene promoters and were more effective than higher doses that induced classic cytotoxic effects. A phase I study indicated the dose of 32 mg/m^2 daily for 5 days was well tolerated and also suggested that the risk of chronic GVHD incidence was decreased among patients receiving longer schedules of the drug (administered in 30 day cycles) [103]. Subsequently, investigators showed in murine models that decitabine or azacitidine could induce tolerance, possibly by increasing the numbers of T regulatory (Tregs) [104,105]. Craddock et al. in England then showed that AML patients receiving low-dose azacitidine had an increase in CD8 + T cell response to a variety of tumor antigens and also augmented reconstitution of T regulatory cells after T cell–depleted transplantation [106]. Longer follow-up of the British multicenter study showed that no patient developed severe acute GVHD or chronic extensive GVHD. Interestingly, azacitidine induced a CD8 + T cell response to at least 1 tumor-specific peptide in 16 of 31 patients who received

Table 5
Highlighted Studies

Trial Title	Clinicaltrials.gov Responsible Party/Sponsor
Randomized controlled study of post-transplant azacitidine for prevention of acute myelogenous leukemia and myelodysplastic syndrome relapse (VZ-AML-PI-0129)	NCT00887068 MD Anderson Cancer Center
Safety study of oral azacitidine (cc-486) as maintenance therapy after allogeneic hematopoietic stem cell transplantation in subjects with acute myeloid leukemia or myelodysplastic syndromes	NCT01835587 Celgene Corporation. Centers: University Hospitals Case Western Reserve University; MD Anderson Cancer Center; Queen Elizabeth Hospital, Birmingham, UK; Fred Hutchinson Cancer Research Center; Memorial Sloan Kettering Cancer Center
Decitabine maintenance for acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) post transplant (AML MDS)	NCT00986804 Washington University School of Medicine
Dose-finding study of post-bmt decitabine maintenance treatment in higher-risk MDS and MDS/AML (PODAC)	NCT01277484 Seoul St. Mary's Hospital
VIDAZA-DLI Pre-emptive azacitidine and donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation for high risk acute myeloid leukemia and myelodysplastic syndrome	NCT01541280 Nantes University Hospital
Phase I/II study with oral panobinostat maintenance therapy following allogeneic stem cell transplantation in patients with high risk myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) (PANOBEST)	NCT01451268 Johann Wolfgang Goethe University Hospitals
Protocol in acute myeloid leukemia with FLT3-ITD (midostaurin)	NCT01477606 University of Ulm
Standard of care +/− midostaurin to prevent relapse post stem cell transplant in patients with FLT3-ITD−mutated AML (ARMOR)	NCT01883362 Novartis Pharmaceuticals/multicenter
Sorafenib maintenance therapy for patients with AML after allogeneic stem cell transplant	NCT01398501 Massachusetts General Hospital
A study of AC220 given after transplant in subjects with acute myeloid leukemia (AML) This study is ongoing, but not recruiting participants.	NCT01468467 Sponsor: Ambit Biosciences Corporation
Dose-finding of lenalidomide as maintenance in multiple myeloma	NCT00778752 Universitätsklinikum Hamburg-Eppendorf
Safety and efficacy of lenalidomide as maintenance therapy in patients with newly diagnosed multiple myeloma following a tandem autologous-allogeneic transplant	NCT01264315 Fondazione Neoplasie Sangue Onlus
Azacitidine after allo blood and marrow transplantation (BMT) for chronic myelogenous leukemia (CML)	NCT00813124 MD Anderson Cancer Center
Allo transplant followed by lenalidomide and sirolimus maintenance in high-risk multiple myeloma (MM)	NCT01303965 Indiana University School of Medicine
Lenalidomide after donor stem cell transplant and bortezomib in treating patients with high risk multiple myeloma	NCT01954784 Case Comprehensive Cancer Center
Ofatumumab induction and maintenance in elderly patients with poor-risk CLL in the context of allogeneic transplantation	NCT01809847 Technische Universität Dresden/Multicenter
Study of dasatinib to treat Philadelphia-positive acute lymphoblastic leukemia (DASA-TRAS)	NCT01310010 Grupo Espanol de trasplantes hematopoyeticos y terapia celular
Brentuximab vedotin after donor stem cell transplantation in treating patients with hematologic malignancies	NCT01620229 Fred Hutchinson Cancer Research Center

Registered in http://clinicaltrial.gov/.
Search terms: maintenance AND allogeneic, accessed on October 30, 2013.

more than 3 treatment cycles, and this T cell response was linked to improved 1-year OS (Craddock, personal communication). These exciting findings justify further prospective evaluation of this approach. Meanwhile, a phase III study comparing 1 year of low-dose azacitidine maintenance therapy versus standard of care (no maintenance) is ongoing at MD Anderson Cancer Center (Table 5). This study aims at improving event-free survival of patients receiving allo-HCT for high-risk AML and MDS.

The complexity of analyzing the effect of post-HCT interventions is illustrated by the proposed effect of azacitidine on Tregs. Komanduri et al. at the University of Miami assessed the longitudinal recovery of Tregs in 12 patients treated with varying numbers of azacitidine cycles, after T cell−replete HCT at MD Anderson Cancer Center. Tregs were defined by the CD4+CD25+CD127 low phenotype, which was highly enriched for FOXP3 + cells, and were assessed at varying time points after 3 to 12 cycles of azacitidine (n = 23 post-treatment samples). A preliminary analysis showed no significant difference in the frequencies of Tregs within the CD4+ compartment after treatment with azacitidine, compared with the preazacitidine baseline. The Treg fractions of CD4+ cells before and after azacitidine exposure were 6.1% ± 1.37 and 4.8% ± .95, respectively (mean ± SEM; $P = .08$; NS by paired, 2-tailed t test; Komanduri, personal communication). It is possible that the T cell depletion used in the British cohort could account for the difference between the 2 studies. The data illustrate the need for controlled studies, as the Komanduri cohort did not include a comparator group not receiving azacitidine.

FLT-3 inhibitors are also under active investigation as maintenance therapy after HCT (Table 5). The rationale is similar to the use of these drugs to treat AML, ie, patients bearing the ITD mutation have a higher likelihood of relapse, and maintenance therapy with FLT-3 inhibitors could reduce recurrence rates after HCT. As with other drugs, tolerance and medication interactions after HCT are potential problems to be addressed in future studies.

Bug et al. in Germany are investigating another innovative approach, using the deacetylase inhibitor panobinostat after allo-HCT for AML or MDS. This drug has immunomodulatory effects and is moderately active against myeloid leukemias. The maximum tolerated dose is 20 mg 3 times weekly, with treatment starting at day 60 after HCT. The dose-limiting toxicity was colitis and nausea at the 30-mg dose [107].

The hypothesis that newer pharmacologic interventions could have an addictive effect with cellular treatments post-HCT is fascinating and opens a wide array of investigations, as discussed in previous sections of this review. Conceivably, antigen-specific or nonspecific cellular maintenance strategies could be magnified by concomitant administration of drugs that might enhance the effects of cellular therapy. Several groups are currently investigating this possibility.

CONCLUSION

Post-transplantation treatments are under active investigation and are an exciting field of research. Treating physicians, however, should keep in mind that the burden of the proof falls on the investigators and that significant costs are associated with maintenance therapy. Therefore, well-conducted prospective, controlled clinical trials will be necessary to demonstrate the benefits and risks of these new approaches.

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