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New approaches to manipulate minimal residual disease after allogeneic stem cell transplantation

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SUMMARY

Minimal residual disease (MRD) is a complex topic that has been studied extensively in hematologic malignancies given its clinical implications related to prognosis. However, methods to monitor and treat MRD, especially after stem cell transplantation, are not well defined and vary in different disease processes. Alternative transplant strategies, such as reduced-intensity conditioning, have altered the way we assess and address MRD after transplantation. Development of new diagnostic tools have allowed for higher sensitivity and specificity of testing. Both targeted chemotherapeutic agents and immunotherapies have been developed to treat MRD in hopes of improving patient outcomes. This article aims to address ways to define and manipulate MRD specifically after stem cell transplantation.

Minimal residual disease (MRD) is a broad term generally defined as the presence of low-level disease detectable only by advanced laboratory testing (not standard morphology and/or immunohistochemistry analysis) that remains after induction therapy or hematopoietic stem cell transplantation (HSCT). This is distinct from the issue of fluctuations in donor chimerism that may be detected for successful allogeneic transplantation. In most hematologic malignancies, MRD has prognostic significance and is a significant risk factor for overt disease relapse, especially in the post-transplant setting [1]. As new strategies such as reduced-intensity conditioning have led to a decline in nonrelapse mortality, disease relapse has become the primary cause of mortality after transplantation [2]. While early identification and treatment of MRD may prevent relapse and improve patient outcomes, there are insufficient data to form a clear consensus on appropriate strategies and evaluation of MRD after transplantation, and treatment varies [3,4]. Since there are currently no large studies that consistently show the prognostic meaning of MRD in many hematologic diseases (with the exception of acute lymphocytic leukemia [ALL] and chronic myelogenous leukemia [CML]), the role of MRD using current sensitive techniques for many other disease processes, including acute myeloid leukemia (AML) remains unclear. One should note that a very important reason for the lack of generally accepted guidelines on MRD-based treatment in this setting is the lack of standardization and the intermixture of different methodologies.

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Monitoring MRD after HSCT is achieved using two main approaches (Table 1). The malignant clone can be directly measured using tumor-specific molecular primers, flow cytometry, molecular genetics and FISH. Donor chimerism can also be used to assess MRD through characterization of the origin of hematopoiesis, although this method lacks specificity [5]. Treatment of MRD after HSCT can also be divided into two categories: adoptive immunotherapies, such as donor lymphocyte infusion (DLI) and vaccine therapy; and traditional and targeted chemotherapeutic agents. The ultimate goal of therapy is to minimize graft versus host disease (GVHD) while maintaining graft versus leukemia (GVL) effects [6,7]. This article will focus specifically on strategies to define and address MRD in acute and chronic leukemia, as well as myelodysplastic syndrome (MDS) in the post-transplant setting.

Defining & monitoring MRD after transplantation

AML & MDS

AML is a heterogeneous disease in which MRD after induction therapy has been identified as a poor prognostic marker. As in many other hematologic malignancies, we have sophisticated technologies such as reverse transcriptase PCR (RT-PCR) and multiparameter flow cytometry (MFC) to assess for MRD in the post-transplant setting, although the role and timing of testing and intervention remains unclear [8,9]. The updated International Working Group defines complete remission in AML as bone marrow blasts of <5%, platelets of $>100 \times 10^9/l$, neutrophils $>1.0 \times 10^9/l$ and disappearance of previously documented cytogenetic alteration, molecular mutation and cells with a previously documented leukemia-associated immunophenotype [10]. These same techniques used to assess for disease response can also be employed to define MRD.

MFC is a less sensitive diagnostic test but can be used for all patients with AML [11]. Another problem associated with using MFC in MRD assessment is that little consensus exists for standardized antibody panels or specimen-processing techniques, which makes reproducibility and generalizability difficult. Molecular techniques such as RT-PCR or nested PCR have the highest sensitivity, but are not universally applicable to patients without an identified mutation or translocation [12]. Gene-expression profiles can also be used to assess MRD, but this will not be discussed in this article as it is not a technique that is generally available to the practitioner today for the diseases in focus for this article. Both NPM1 and FLT3-internal tandem duplication (ITD) mutations have been studied in the post-transplant setting and studies looking at additional markers are in progress [13]. Bacher *et al.* studied NPM1 mutations in 13 patients after allogeneic HSCT (allo-HSCT) using quantitative PCR for the *NPM1* mutation. Of nine patients who relapsed, all had increases in NPM1 mutation detected, which preceded morphological relapse, suggesting that NPM1 may be an accurate marker of MRD [14]. Other molecular mutations such as MLL, WT1 and CEBPA have yet to be studied. Chimerism studies have also been used after allo-HSCT to assess the ratio of donor to recipient cells using techniques such as PCR or FISH in cases of sex-mismatched transplants. Chimerism kinetics (host vs donor) have been shown in several studies to be predictive of relapse as increasing mixed chimerism is associated with disease relapse [15,16]. Unfortunately, this technique does not provide a quantitative measure of MRD and lacks sensitivity, despite efforts for improvement such as isolation of CD34⁺ cells for chimerism analysis.

In MDSs, molecular markers and donor chimerism have also been used to detect MRD, although few studies have been done in the post-transplant setting. WT1 expression, RUNX1 (also known as AML1), NR AS and FLT3-ITD mutations have been studied although, these mutations are rare in MDSs making molecular testing of little value in many

individuals. MFC and donor chimerism may provide more valuable information although further studies are pending [4].

CML

Monitoring of MRD after transplantation is best described in CML using quantitative RT-PCR for BCR–ABL mRNA. Increasing BCR–ABL is associated with increased risk of relapse and, interestingly, studies point towards the importance of assessing MRD kinetics over time [17,18]. Frequently, patients with CML after allo-HSCT will remain with MRD detectable by RT-PCR at 3 months, although this may change to MRD negative with dynamic monitoring. Kaeda *et al.* studied 243 patients who had undergone allo-HSCT for CML and categorized patients based on levels of MRD. This group defined molecular relapse as three PCR results over a minimum period of 4 weeks with a BCR–ABL/ABL ratio in excess of 0.02%, or two results higher than 0.05% over a minimum period of 4 weeks. Patients who had a single positive PCR within the first 6 months after transplantation were not considered to have molecular relapse [19]. Identifying patients with molecular relapse before full hematologic and cytogenetic relapse provides an opportunity to intervene with tyrosine kinase inhibitors (TKIs), DLIs or both, which has proven effective in several studies [20–22].

In summary, for myeloid diseases there are a number of effective markers for MRD and identification of such post-therapy remains predictive of overt relapse, suggesting a plan for monitoring, and studies of early intervention will be valuable in assessing long-term impact.

Chronic lymphocytic leukemia

Like AML and MDS, MRD in patients with chronic lymphocytic leukemia (CLL) after HSCT has been associated with worse prognosis and increased risk of relapse. However, the clinical and prognostic significance depends on the clinical situation, such as autologous HSCT (auto-HSCT) versus T-cell depleted allo-HSCT versus T-cell replete allo-HSCT [23]. Better techniques for assessing MRD have been developed, adding complexity to interpretation, with PCR-based strategies using the hypervariable region of the clonal immunoglobulin heavy-chain gene and flow cytometry being the gold-standard techniques for detection [24,25].

In many studies, auto-HSCT leads to decreased levels of detectable disease, although durable MRD negativity is rarely achieved and detection of MRD is noted to predate overt relapse [26]. This is in contrast to allo-HSCT, which has, in part, revolutionized the way we interpret MRD and has demonstrated the importance of GVL effects [23]. Studies of MRD after transplantation for CLL suggests that MRD kinetics provide more valuable information compared with absolute MRD levels, especially in the era of reduced-intensity conditioning regimens. MRD negativity 1 year post-transplant is strongly predictive for improved overall survival (OS) and disease-free survival in this setting [27,28]. These same studies and others suggest that the delayed clearance of MRD seen after allo-HSCT with reduced-intensity conditioning is indicative of a GVL effect, which provides permanent immunotherapeutic activity in the recipient and can be manipulated with DLI or modification of immune suppression [29–31]. Farina *et al.*, for example, assessed MRD status using RT-PCR in 29 patients with relapsed CLL after allo-HSCT. A total of 16 patients achieved permanent MRD negativity or a ‘mixed pattern’ with only one patient experiencing relapsed disease. Eight of 13 patients who were MRD positive ultimately relapsed. Two-year disease-free survival was 100 versus 57% for MRD-negative versus -positive patients [32]. These and similar data have formed the basis for the use of immunomodulation for patients who remain with MRD after transplantation in CLL.

ALL

Multiple studies have shown that the presence of MRD after therapy for ALL is associated with a high risk for disease relapse [33,34]. This is especially well documented in Philadelphia chromosome-positive ALL (Ph⁺ ALL) patients (in the pre-TKI era) in whom MRD detected in bone marrow via RT-PCR after induction and consolidation therapy is associated with poor prognosis and high risk of relapse [35–37]. Post-transplant MRD evaluation, especially in ALL, may guide early intervention and impact outcomes.

A European Consensus Development Workshop on MRD Assessment in ALL summarized the current state of MRD diagnostics and constructed specific definitions of complete MRD response, persistence and reappearance to be applied specifically to clinical trials [38]. In terms of diagnostics, many techniques have been employed with sensitivities of 10⁻⁴ to 10⁻⁵. The most common assays include PCR for immunoglobulin and *TCR*-gene arrangements in precursor B-cell ALL (B-ALL) and T-cell ALL (T-ALL), PCR analysis for BCR-ABL in Ph⁺ ALL, and MFC, which applies to almost all patients with ALL [39,40].

Managing MRD after stem cell transplantation: therapeutic options

Although many immunomodulatory and chemotherapeutic treatments have been studied for treatment of MRD after transplantation, there is no consensus on standard of care. Moreover, it remains unclear as to whether early identification and treatment of MRD impacts clinical outcomes in different diseases. Thus, there is a wide array of strategies, from watchful waiting after allo-HSCT to seeing if a graft-versus-tumor effect may arise, to aggressive intervention as if the patient had relapsed. Ultimately, the decision to intervene depends on the individual patient and disease circumstances. If the decision has been made to intervene therapeutically, two general approaches exist: immunotherapy and targeted agents. Immunomodulation and adoptive immunotherapy consist of strategies that can be applied to most patients after allo-HSCT in multiple diseases and are therefore discussed together.

Immunomodulation/adoptive immunotherapy

A number of general treatment strategies can be entertained as therapies for detectable MRD, although it should be noted that most of these have been studied in relapsed disease as opposed to MRD after transplantation. Therapies such as DLI, natural killer (NK) cell infusion and withdrawal of immunosuppression are based on the premise of enhancing GVL effects. Other strategies such as chimeric antigen receptors (CARs), bispecific antibodies and vaccine therapy also harness the immune system and can be used both in the setting of allo- and auto-HSCT. While many of the interventions discussed below are under study in Phase I or II trials for relapsed disease, we believe them to be worth discussion for the potential application to MRD.

Withdrawal of immunosuppression in patients with relapsed disease after allo-HSCT is often tried. Although immunosuppression is employed to prevent GVHD, it also inhibits GVL effects. Withdrawing immunosuppression allows for increased GVL effects, and best results have been seen in myeloid malignancies [41].

DLI has been used to treat relapsed disease and tends to work best in myeloid malignancies [42]. By infusing an additional aliquot of donor lymphocytes, DLI provides a 'boost' to GVL effects. In our series of 69 patients who received DLI following matched (n = 52) or partially matched (n = 17) related donors, 38% of those with persistent disease before DLI attained remission after infusion [43]. As with withdrawal of immunosuppression, DLI has a significant risk of causing or worsening GVHD. Although controlling the dose of CD3⁺ cells, the addition of cytokine stimulation or altered immune suppression can mitigate that

risk, it may be at the expense of antitumor effect [44,45]. Other augmented DLI methods are being explored, including incorporating chemotherapy after DLI, depletion of the alloreactive T cells that may cause GVHD or modification of T cells with thymidine kinase to modify the risk of GVHD. These strategies hold great promise and may be employed prophylactically or at the detection of MRD [46,47].

NK-cell infusion may augment GVL effects without significant GVHD. In our series of 30 patients who received a total of 51 NK-cell enriched DLI following 3–6/6 HLA-matched, T-cell-depleted, nonmyeloablative allogeneic transplantation, long-term responders had significantly improved duration of response ($p = 0.0045$) and OS ($p = 0.0058$) [48]. Flow-based and functional assays showed improved NK cell numbers and activity and only two patients experienced severe acute GVHD. Miller *et al.* have shown that infusion of haploidentical NK cells in a nontransplantation setting can persist and expand *in vivo* and may result in hematologic remission without GVHD [49]. This effect is mediated by the interaction between HLA and activating and inhibitory killer immunoglobulin receptor (KIR) [50]. In a prospective study of 84 adults with high risk hematological malignancies who received reduced-intensity conditioning T-cell depleted HSCT from haploidentical related donors, we found OS higher ($p = 0.009$) and relapse-related mortality lower ($p = 0.036$) in patients with myeloid malignancies missing HLA-C group 2 ligand to donor inhibitory KIR, particularly in those with >1 missing ligand ($p = 0.018$) [51]. These results and similar reports by others suggest that the absence of appropriate HLA ligands in the recipient to donor inhibitory KIR may induce GVL effects in patients undergoing reduced-intensity conditioning T-cell depleted transplants.

Bispecific antibodies are novel constructs that simultaneously engage T cells and tumor cells, increasing cell–cell contact and T-cell mediated destruction. For example, blinatumomab has moieties that bind to CD3, expressed by T cells, and CD19, which is expressed in a majority of B-cell malignancies. A Phase II trial of blinatumomab to treat MRD in ALL showed long-lasting molecular remissions; this strategy is readily adaptable to the post-transplant setting [52].

Vaccine therapy is also a promising approach. WT1 is overexpressed in a number of malignancies including AML and MDS, and has been associated with a poor prognosis [53]. Oka *et al.* reported a WT1-vaccine strategy resulting in a decrease in the number of residual leukemic blasts or WT1 expression with repeated doses of vaccine in a number of leukemia patients. Interestingly, a correlation between increased WT1-specific cytotoxic T lymphocytes and clinical response was observed [54,55]. Although these patients were not treated with HSCT, these data suggest that WT1 vaccination may be a promising, well-tolerated immunotherapy to be considered in the post-transplant setting for eradication of MRD [56,57].

Adoptive transfer of tumor-specific T cells is another promising immunotherapy. These T cells can be obtained in a number of ways. In patients with solid tumors, autologous tumor-infiltrating lymphocytes can be obtained from biopsy specimens and cultured *ex vivo* before reinfusion [58]; these studies have shown objective cancer regression in 50% of relapsed/refractory melanoma patients [59]. Although obtaining tumor-infiltrating lymphocytes is impractical in leukemias, this principle has paved the way for other techniques such as generating CARs that are specific for molecules expressed by malignant cells [60]. Using a lentiviral vector, a CAR specific for CD19, coupled with the CD137 (also known as 4–1BB) costimulatory receptor and CD3- signal transduction component of the T-cell antigen receptor, has been used to successfully treat patients with relapsed/refractory CLL, holding promise for treatment of MRD [61]. While these CARs have been generated from autologous cells, successful murine studies suggest that these techniques can be adapted to

adoptive transfer of allogeneic T cells redirected using an antibody-based CAR [62], and Phase I/II trials are currently testing this strategy in patients with B-cell malignancies after allogeneic transplantation [101]. Other approaches involve *ex vivo* activation of cytotoxic T cells against leukemia-associated/specific antigens using peptide-pulsed dendritic cells. While these methods are still under investigation, similar techniques have been used successfully to treat cytomegalovirus- and Epstein–Barr virus-driven lymphoproliferative diseases after allo-HSCT [63].

Chemotherapeutic agents

AML & MDS—Azacitidine is a well-tolerated hypomethylating agent that has been used for relapsed disease in the post-transplant setting for both AML and MDS [64]. The RELAZA Phase II trial investigated the efficacy of azacitidine for treatment of MRD after HSCT as determined by donor chimerism analysis of CD34⁺ blood cells. A total of 80% of patients responded, with six achieving stable disease and ten achieving an increase in CD34⁺ donor chimerism to >80%, which led the authors to conclude that azacitidine is an effective therapy to delay or prevent disease relapse in patients with MRD after HSCT [65].

Many additional agents such as sorafenib, a FLT3-ITD and multikinase inhibitor, have been studied for relapsed or refractory disease both before and after HSCT [66–68]. Additional studies specifically targeting MRD are warranted.

CML—MRD in CML has been well studied in many different settings including after allo-HSCT. Fortunately, with better therapeutics and more options for first-line therapy, fewer patients are being treated with allo-HSCT for CML, especially in the chronic phase. For patients who experience disease relapse after allo-HSCT, imatinib mesylate (Gleevec®) remains as a good therapeutic option after manipulation of the immune system with cessation of immunosuppression or DLI [69]. Hess *et al.* studied 44 patients with molecular (n = 18) or cytogenetic (n = 19) relapse after allo-HSCT. Thirty nine patients were in chronic phase prior to transplantation and five patients were in accelerated or blast crisis phase. In all patients, immunosuppressives were stopped prior to enrollment on study if medically safe. A total of 70% of patients achieved a complete molecular response after initiation of imatinib mesylate, many of which were durable responses [70]. These data suggest there is a role for TKIs for treatment of MRD after allo-HSCT.

CLL—Rituximab is a monoclonal antibody against CD20, which has shown benefit and tolerability as consolidation or maintenance therapy after initial induction chemotherapy in CLL based on work done by Del Poeta *et al.* and others [71–73].

Rituximab has also been studied in nonablative allo-HSCT by Khouri *et al.*, who conclude that GVL effects may be, in part, facilitated by use of rituximab. In this study, 17 patients were treated with a nonablative regimen, ten of whom received rituximab as well. Ten patients had MRD after transplantation and underwent immunomodulation therapy with DLI or withdrawal of immunosuppression. Nine of these ten patients had a response and, interestingly, eight of nine responders had received rituximab as part of their conditioning regimen. In addition, these patients were found to have improved OS compared with patients who received chemotherapy alone. This study illustrated two pertinent concepts: immunomodulation can enhance GVL effects and lead to improved outcomes, and rituximab may facilitate this process [74,75].

Modulation of immunosuppressive agents and DLI have been shown to eliminate MRD that may remain over time. Studies have also pointed out potential augmentation of the GVL effect with use of agents such as rituximab, of atumumab and alemtuzumab, as well as other

agents, such as lenalidomide, that have previously been shown to be effective in B-cell CLL [75,76].

ALL—MRD in adult ALL has been shown to be predictive of disease relapse in several prospective studies [34,77]. Ph⁺ ALL is more aggressive and/or resistant to standard therapeutics, compared with precursor T- and B-cell ALL. Many studies have utilized the TKIs such as imatinib or dasatinib to target BCR–ABL in this patient population in both the induction phase as well as post-transplant to eradicate MRD [4]. Carpenter *et al.* prophylactically treated 22 patients with imatinib for 1 year after allo-HSCT, 15 of whom had Ph⁺ ALL and seven of whom had CML. This study confirmed the safety of this approach [78]. Wassman *et al.* subsequently followed 27 patients with MRD detected after HSCT. All patients were treated with imatinib and BCR–ABL transcripts became undetectable in 14 patients. All patients who achieved an early molecular response remained in remission for the duration of therapy. Of the 13 patients who did not achieve MRD negativity, 12 relapsed, thus leading to the conclusion that MRD positivity after starting imatinib predicts disease relapse [79]. Further studies are warranted to assess use of nilotinib, dasatinib and other potential TKIs for treatment of MRD after transplantation [80].

Conclusion & future perspective

MRD is a complex topic with different clinical implications in different situations. New technology has allowed for better diagnosis and monitoring of MRD, although in many cases it is unclear if currently available therapies can be applied at the time of MRD-only detection and impact clinically significant outcomes. Several strategies have been developed to address MRD in the post-transplant setting and test the value of these interventions. Accrual to national studies where applicable, and novel institutional studies, remains of paramount importance in order to answer these questions. Furthermore, as the clinical interventions to address the MRD issue are new, there are little data beyond Phase I and small Phase II studies. There is a requirement for large, well-carried-out trials comparing immunotherapies with targeted therapies and other approaches, along with an assessment on the safety of these approaches.

Although interventional advice is too premature at this point, it is hoped that these further studies reveal whether it would be possible to select which patients would benefit from which therapy, information from which useful recommendations could be made in clinical practice.

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Website

101. CD19 CAR T Cells for B Cell Malignancies After Allogeneic Transplant. <http://clinicaltrials.gov/ct2/show/NCT01475058?term=NCT01475058&rank=1>

Practice Points

- In acute myeloid leukemia and minimal residual disease (MRD), molecular tests, such as reverse transcriptase PCR, have the highest sensitivity, although they cannot be applied to every patient. Donor chimerism is universally applicable but lacks sensitivity.
- Reverse transcriptase PCR for BCR–ABL to detect MRD in chronic myelogenous leukemia (CML) has been well studied in the post-transplant setting. In both chronic lymphocytic leukemia (CLL) and CML, MRD kinetics provide more information as compared with an isolated MRD value.
- Both acute lymphocytic leukemia and CLL are examples of lymphoid malignancies in which MRD monitoring has evolved, although further studies of clinical implications are needed.
- Therapy for MRD after transplantation can be divided into two categories: immunotherapies versus targeted chemotherapeutic agents.
- Withdrawing immunosuppression, especially in myeloid malignancies, allows for increased graft-versus-leukemia (GVL) effects.
- Donor lymphocyte infusion and natural killer cell infusion are emerging therapies to enhance GVL effects and likely have a role in eradicating MRD when detected after transplantation.
- Bispecific antibodies and vaccine therapies are additional promising interventions that need further data.
- Azacitidine improves disease-free survival when given to patients with MRD and myeloid malignancies, such as acute myeloid leukemia and myelodysplastic syndromes after transplantation.
- Rituximab can be used to enhance GVL effects after transplantation in patients with CLL.
- There is a role for imatinib or other tyrosine kinase inhibitors for post-transplant MRD in patients with Philadelphia chromosome-positive acute lymphocytic leukemia and CML.

Table 1

Diagnostic methods to monitor residual disease and relapse after allogeneic stem cell transplantation.

Detection of MRD	Utility	Sensitivity
Karyotyping	Subset of all types of neoplasms with chromosomal abnormalities	10^{-1}
FISH	Subset of all types of neoplasms with know chromosomal abnormality	10^{-2}
Flow cytometry	ALL; most AML; CLL	10^{-3} – 10^{-4}
Antigen receptor PCR	ALL; lymphoma	10^{-4} – 10^{-5}
Translocation or other mRNA PCR	CML; subset of ALL; subset of AML; subset of lymphoma	10^{-3} – 10^{-6}
Chimerisms: XY FISH	All types of neoplasms (sex-mismatched HSCT); not specific for MRD	10^{-2}
Chimerism: qPCR/VNTR-PCR	All neoplasms (precondition differences in donor/recipient polymorphisms); not specific for MRD	10^{-3} – 10^{-6}

ALL: Acute lymphocytic leukemia; AML: Acute myeloid leukemia; CLL: Chronic lymphocytic leukemia; CML: Chronic myelogenous leukemia; MRD: Minimal residual disease; qPCR:3Real-time PCR; VNTR-PCR: Variable number tandem repeat PCR.

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