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Novel postremission strategies in adults with acute myeloid leukemia

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Abstract

Purpose of review—Given the high rates of relapse in acute myeloid leukemia (AML), there is tremendous opportunity for the development of new therapeutic strategies in the postremission state. Unfortunately, the currently available modalities for postremission therapy, namely chemotherapy, have proven largely ineffective in changing the natural history of AML. The challenges to overcome therapeutic failure in the minimal residual disease status may relate to an incomplete understanding of the mechanisms and cell populations that are directly related to disease relapse as well as suboptimal ability to identify patients at highest risk for relapse.

Recent findings—Being a heterogeneous disease, relapsed AML is unlikely to emanate from one predominant mechanism; instead, there are likely multiple biologic factors at play that allow for clinical relapse to occur. These factors likely include multidrug resistance proteins, aberrant signal transduction pathways, survival of leukemia stem cells, microenvironmental interactions, and immune tolerance. Many novel strategies are in development that target these mechanisms, ranging from chemotherapeutic modalities, to signal transduction inhibitors, to upregulation of antileukemic immune responses.

Summary—Understanding the underlying mechanisms of leukemic cell survival and resistance has spurred the development of novel therapeutic approaches to overcome these mechanisms in the hope of eradicating minimal residual disease and improving survival in AML.

Keywords

acute myeloid leukemia; leukemia stem cell; minimal residual disease; postremission therapy; targeted therapy

Introduction

Acute myeloid leukemia (AML) is a heterogeneous neo-plastic disorder of the bone marrow, associated with an abnormal proliferation of myelopoietic precursors and subsequent bone marrow failure. Although complete remission is variably achievable, prolonged remission or cures are uncommon in AML, particularly in older adults [1,2], strongly emphasizing that residual disease is a very important cause of treatment failure. Hence, effective postremission therapeutic strategies are likely of vital importance for improving cure rates of AML. The paradigm for successful postremission therapy, however, should not conflict with the incorporation of novel therapies into the upfront setting. Indeed, the very resistance

mechanisms that could be targeted in the postremission state may also be worth targeting in the 'preremission' state so as to make the currently available drugs more effective from the outset (i.e. induce a 'deeper' remission), thereby potentially attenuating the need for aggressive postremission therapy.

Components of cell survival and drug resistance

A critical yet unresolved problem in AML is the incomplete understanding of the essential components of cell survival after the completion of induction chemotherapy. Possibilities to explain these issues include altered mechanisms of resistance to chemotherapy, persistence of leukemic stem cells, and cytoprotective effects induced by leukemic cell interactions with the bone marrow microenvironment (Table 1). These individual elements of leukemic cell survival are likely not mutually exclusive, and perhaps targeting any of these mechanisms would be useful for overcoming resistance and effectively treating the patient with minimal residual disease (MRD) following chemotherapy. It should also be duly noted that targeting the cells responsible for MRD can be considered either in the postremission state or concurrently with induction therapy.

Intracellular resistance mechanisms

Although a comprehensive discussion of intracellular mechanisms of chemotherapy resistance is beyond the scope of this review, it is worthwhile to review some of the better-described and readily 'targetable' mediators of drug resistance. One well described mediator of chemotherapy resistance in AML is the family of ATP-binding membrane cassette multidrug resistance (MDR) proteins. Perhaps, the best described protein is multidrug resistance protein-1, also known as P-glyco-protein (Pgp), which serves to promote efflux of a variety of chemical substrates from the cell [3]. Pgp substrates include anthracyclines and epipodophyllotoxins, commonly used chemotherapeutic agents in AML. Pgp is frequently expressed on the blast cells of older adults with AML, particularly in patients with secondary AML, and its presence confers higher risk of induction chemotherapy failure [4,5]. Large-scale efforts have been undertaken to inhibit Pgp in the presence of chemotherapy so as to improve treatment efficacy. Some of the compounds tested include nonspecific drugs such as quinine or cyclosporine [6–8], and, more recently, highly specific and potent drugs such as PSC-833 and zosuquidar [9–11]. To date, the majority of studies have failed to demonstrate that Pgp inhibition improves response rates or survival [6,8–11]. Newer trials will study more optimal modes of Pgp inhibitor administration as well as newer, broader spectrum inhibitors. Because MDR protein inhibition requires coadministration of chemotherapeutic compounds to achieve clinical effect, the use of single-agent MDR inhibitors as a maintenance strategy is not viable, and their main role will likely be during the induction and immediate consolidation phases of treatment.

Other intracellular processes may also mediate primary chemotherapy resistance, such that inhibiting these targets, either in combination with chemotherapy or as an adjunct following remission status, may prove effective in improving patient outcomes. One specific example of an intracellular mediator of drug resistance is the phosphatidylinositol-3 (PI3K)/Akt signal transduction pathway. This ubiquitous intracellular pathway promotes cellular survival via a variety of mechanisms [12–15], and protein members of this pathway, in particular Akt, are frequently overexpressed in AML [16–18]. In primary AML cells and cell lines, it has been demonstrated that Akt activation is protective against chemotherapeutic agents, including cytarabine and etoposide [16]. When added to chemotherapeutic compounds, pharmacological inhibitors of the PI3K/Akt axis have been shown to augment and synergize with chemotherapy in eliciting apoptosis within leukemic cells, suggesting the importance of this pathway in mediating chemotherapy resistance in AML [16,19,20]. Hence, intracellular signaling pathways such as PI3K/Akt may be

upregulated in AML, such that targeting them, either alone or with chemotherapy, may be a useful adjunct to treatment of AML. To this end, many small molecule inhibitors of this pathway and others are in current clinical testing.

Leukemic stem cells

As with normal hematopoiesis, leukemic hematopoiesis follows an orderly and hierarchical process, driven by a small and defined subset of cells that are clonogenic [21,22]. In such a model, leukemic 'stem cells' are the clonal initiating cells and form only a very small population of the identifiable leukemic clone that occupies the bone marrow space. With properties of self-renewal and noncycling status [23], leukemic stem cells may have biologic similarity to normal hematopoietic stem cells. It stands to reason, therefore, that ablation of the committed cells, though enough to induce remission status, will be insufficient to permanently ablate the clone, given that the most primitive, disease-initiating cells are still present. It should be noted, however, that the precise contribution of leukemic stem cells to clinical disease relapse has not been established in experimental models to date.

Recent studies have demonstrated that leukemic progenitor cells may display unique biochemical properties that make them susceptible to specific therapeutics. One such example was demonstrated by Guzman *et al.* [24], in which it was determined that primary AML cells, including progenitor cells, overexpressed nuclear factor kappa B (NF- κ B) compared with normal progenitors and were highly sensitive to proteasome inhibitor administration. This finding has led to the development and implementation of clinical trials utilizing proteasome inhibitors in combination with cytotoxic chemotherapy, as well as trials to test the utility of single-agent proteasome inhibitors as maintenance therapy following induction. A naturally occurring compound, parthenolide, was also recently determined to be exquisitely potent against leukemic progenitor and stem cells, while sparing normal progenitors [25]. An orally bioavailable analog of parthenolide, dimethylaminoparthenolide, was also found to be highly and selectively cytotoxic to leukemic stem cells and will soon be undergoing clinical testing [26^{**}]. In light of the potential importance of leukemic stem cells in the initiation and propagation of AML, specifically targeting such a population in the postremission setting is reasonable strategy and one that is being pursued utilizing several of the above classes of agents.

Bone marrow microenvironmental interactions

Evidence suggests that leukemic cell adherence to bone marrow stroma may trigger key mechanisms of cellular survival [27–30]. As such, interruption of these physical interactions or the prosurvival signaling mechanisms triggered by these interactions may be useful strategies for killing leukemic cells that are protected via these mechanisms.

Previous studies have shown that leukemic cell adherence to stroma protects leukemic cells from drug-induced apoptosis [31–33]. This protective effect may be due, in part, to upregulation of intracellular signaling intermediates such as p-Akt or Bcl-2, such that by inhibiting these pathways, disruption of cell adhesion mediated drug resistance effect may occur [31–33]. As described above, therapeutics targeting the PI3K/Akt and Bcl-2 pathways are in development and may be a useful adjunct in abrogating the protective effect induced by cell–stromal interactions. Other studies have demonstrated that localization and adhesion of leukemic cells to the bone marrow stroma are dependent upon the interaction between chemokine stromal cell-derived factor-1 (SDF-1) and its receptor CXCR4 [34,35] and that these interactions promote survival of AML cells [35,36^{**}]. It has also been demonstrated that pharmacological inhibition of CXCR4 can not only block the homing of leukemic cells into the bone marrow, but also abrogate the prosurvival effect of stromal cells on leukemic cells [35,36^{**}], even in the presence of chemotherapy [36^{**}]. If leukemic cell survival after

chemotherapy is indeed mediated by microenvironmental adherence, then pharmacological agents that disrupt this process may restore chemosensitivity to leukemic cells or perhaps even be active as single agents. Clinical strategies utilizing chemokine receptor antagonists such as AMD3100 are clinically effective mobilizers of hematopoietic stem cells [37,38] and are also being explored as adjuncts to chemotherapy in AML. Similar to MDR modulators, the optimal role for chemokine receptor antagonists may well be in combination with chemotherapy, both in the induction and immediate postremission settings.

Assessing for minimal residual disease

Successful eradication of residual AML following remission necessarily requires not only a fundamental understanding of the critical targets in residual disease, but also an ability to adequately assess and measure MRD, in order to identify patients at the highest risk of relapse. Current techniques allow for measurement of MRD via cell surface marker assessment and highly sensitive assays for genetic alterations.

Measurement of minimal residual disease in acute myeloid leukemia

Following achievement of complete remission, it is assumed that a significant residual disease burden remains that is beyond the sensitivity limits of standard microscopic analysis. Adequate measurement of this disease burden may provide prognostic information with respect to relapse risk and provide relevant targets against which to aim and develop therapeutics. One such tool for measuring MRD is multiparametric flow cytometry. With an ability to detect one in 10 000 malignant cells, flow cytometry can be a useful tool for discovering MRD that would not be captured by morphologic marrow assessment [39]. The ability to detect MRD by flow cytometry, however, naturally depends upon the identification of a unique cell surface marker profile of leukemic blasts that differentiates this population from normal hematopoietic progenitors. Several groups have developed multiparametric flow cytometric assays that identify unique cell surface characteristics of blasts (i.e. leukemia-associated immunophenotypes), ultimately utilizing this profile to determine MRD status [40–42]. Although not absolutely predictive of relapse, the presence of MRD cell populations following induction or consolidation therapy predicts groups of patients most likely to suffer relapse and for whom novel postremission strategies should be preferentially developed [40–45].

In addition to cell surface phenotypic markers, many leukemias carry specific genetic abnormalities that can be routinely assessed via highly sensitive assays such as polymerase chain reaction (PCR). Examples of such molecular abnormalities in AML include the presence of *FLT3*, *NPM1*, *CEBPA*, and many other mutations [46]. In the case of acute promyelocytic leukemia, the presence of residual *PML-RAR* alpha transcript as detected by PCR, following completion of induction and consolidation therapy, is highly predictive for relapse [47,48] and can be used as a basis to administer salvage therapy for patients prior to full-blown morphologic relapse, in which disease burden is certainly higher and treatment may be less successful. This same paradigm for treatment of MRD based upon measurement of molecular abnormalities in other AML subtypes has not been well established to date.

Postremission clinical strategies

The remainder of this review will focus upon therapeutic strategies for AML in the MRD state. Unfortunately, the augmented knowledge about relevant disease targets has not yet translated into proven treatment strategies.

Chemotherapy trials

Many clinical studies have assessed the utility of prolonged postremission therapy in AML. Although the efficacy of multiple courses of consolidation therapy has been established in younger adults with AML, the precise number or duration of such treatment has not been well established. A pivotal study demonstrated that consolidation with high-dose cytarabine for four cycles resulted in improved disease-free survival (DFS) and overall survival (OS) compared with lower-dose consolidation regimens in younger patients [49]. However, prospective trials to study the most beneficial number of cycles to administer have not been performed. In older adults, there are minimal data to support the use of any type of prolonged chemotherapy administration in the postremission state. One recent phase 3 trial, however, published by the Acute Leukemia French Association group, demonstrated that outpatient-based daunorubicin and cytarabine, administered on a monthly basis for 6 months, resulted in superior DFS and OS for patients aged more than 60 years in first remission (CR1), compared with no further therapy [50]. These findings warrant close scrutiny and perhaps validation in another larger trial, given the predominantly negative results in other trials to date.

Demethylating agents

Demethylating agents such as 5-azacitidine and decitabine are nucleoside analogs that incorporate into DNA, exerting both a directly cytotoxic effect as well as induction of DNA hypomethylation, which may reverse silencing of genes that are critical for proliferation and differentiation. Both agents have been approved by the US Food and Drug Administration (FDA) for use in MDS, based upon results of large prospective trials [51,52]. In the arena of AML, these compounds are also active, with complete remission rates in the range of 5–20%, and with additional patients experiencing partial response or hematological improvement [53–55]. Given the acceptable toxicity profile of these agents, along with the ability to administer them in the outpatient setting, further exploration of these compounds in the postremission setting is warranted. Preliminary results from one study were presented at the 2008 American Society of Hematology Annual Meeting [56]. In this trial, patients with high-risk MDS or AML, in CR1 following induction chemotherapy, received 5-azacitidine at a dose of 50mg/m² for 5 days, every 4 weeks. The median duration of complete remission was 13 months, and 30% of patients had complete remission lasting for more than 30 months, suggesting clinical benefit to this approach and warranting larger-scale trials. Another group studied high-dose decitabine induction followed by low-dose maintenance decitabine in older adults with AML and presented preliminary results at the 2008 American Society of Hematology Annual Meeting [54]. Here, it was shown that 26% of patients survived 1 year, indicating a potential role for low-intensity demethylator maintenance.

Targeted therapies

The use of targeted therapies, particularly as single agents, in the postremission setting may be particularly appealing for a variety of reasons. One can reasonably assume that in the minimal residual disease setting, there is a higher capacity for a targeted agent to exert a clinically meaningful antileukemic effect that may not be possible in the setting of highly active and proliferative disease, in which a large heterogeneity of cells and pathogenic mechanisms likely require a more empiric approach in order to achieve disease control. In addition, targeted therapies are generally accepted as less toxic and with greater potential to be administered on a chronic basis, both appealing properties for compounds that would be administered to patients already in remission.

Given the novelty of most targeted compounds, many of which are still undergoing testing in very early phase trials, the clinical experience in treating patients in the postremission

state has been limited to date. One such trial examined the use of the farnesyltransferase inhibitor (FTI) tipifarnib in older patients in CR1, in order to establish its potential to improve DFS [57]. It was observed that patients with poor-risk AML in CR1 had a median DFS of 13.5 months and a 2-year DFS of 30%, significantly better than matched historical controls who did not receive tipifarnib. It is expected that similar trials testing the utility of targeted agents in the postremission setting will become commonplace over the next several years. As an example, a current Cancer and Leukemia group B trial of standard induction chemotherapy, combined with or without the FLT-3 inhibitor midostaurin, will employ prolonged single-agent midostaurin in the maintenance phase of therapy for patients with FLT-3-positive AML.

Immunotherapy

Identification of leukemia-specific T-cell responses in AML, along with the demonstrable effect of graft-versus-leukemia effect following allogeneic stem cell transplant in AML, has generated interest in developing approaches with both antigen-specific and nonspecific immunotherapy in AML, particularly in the postremission setting.

A recent study assessed the utility of a nonspecific immune augmentation approach with low-dose interleukin-2 (IL-2) and histamine in patients with AML in complete remission [58]. In this phase 3 trial, patients randomized to the treatment (IL-2+ histamine, administered in 3-week cycles for up to 10 cycles) arm had a 2-year median leukemia-free survival (LFS) of 41% compared with only 29% in the control arm. OS was not significantly different between the two arms, however. Reassuringly, there were no reported grade 3 or 4 hypotension or capillary-leak events. Histamine dichloride (Ceplene, Epicept Corporation, Tarrytown, New York, USA) was recently approved by the European Commission in combination with IL-2 for prevention of relapse in AML patients in CR1. A recently reported Cancer and Leukemia Group B (CALGB) study, however, failed to demonstrate significant improvement in DFS using single-agent IL-2 maintenance therapy in younger adult patients with AML in CR1 [59].

Antigen-specific immunotherapy strategies are also being developed in AML. Preclinical work has identified the peptides PR1 and WT1 to be highly expressed in AML blasts [60,61]. In addition, the immunogenicity of these antigens in eliciting T-cell responses has been established [62,63]. Clinically, a PR1 peptide vaccine has been developed and tested in AML. In one recent study, preliminary results indicated a clinical response in 36% of patients who had an immune response to the vaccine [64]. In addition, four out of 13 patients treated in remission remained in remission for a median of 30.5 months. Ongoing clinical trials are also testing the utility of WT1 peptide vaccines for patients with AML in CR1 or beyond. Clearly, these emerging strategies using antigen-specific vaccine approaches will warrant larger-scale testing in both the phase 2 and phase 3 settings.

Conclusion

Given the heterogeneity in AML, the ability to effectively develop and utilize novel postremission therapies will depend upon a more thorough understanding of the pathologic mechanisms that mediate chemotherapy resistance, and, ultimately, disease relapse. It is likely that successfully treating the MRD state will require not only novel therapies given alone during remission, but also incorporation of such therapies into the upfront setting in order to more fully eradicate the leukemic clone. Unlike the setting of active disease, measurement of the biologic effects of antileukemic therapy delivered during remission is difficult, and even the clinical effects of these treatments are measured somewhat indirectly, by assessing length of remission or OS. As such, not only will it be necessary to elucidate the mechanisms by which leukemic cell resistance develops in order to apply new

therapeutic modalities, but also it will be necessary to develop more effective tools for measuring and quantifying MRD in individual patients, so as to target new therapies to the appropriate patient and for the appropriate duration.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- • of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 150–151).

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Table 1

Potential targeted approaches to minimal residual disease based upon mechanisms of leukemic cell resistance and survival

Mediator of cell survival after chemotherapy	Examples of targeted approaches
MDR proteins	Cyclosporine PSC-833 Zosuquidar
Signal transduction pathways	FTIs (tipifamib) FLT-3 inhibitors (midostaurin) PI3K/Akt inhibitors Bcl-2 inhibitors
Leukemic stem cell	Bortezomib Parthenolide and analog
Microenvironmental interactions	Signal transduction inhibitors VLA-4 (integrin) antibodies CXCR4 inhibitors
Immune tolerance	Monoclonal antibodies IL-2/histamine Peptide vaccines

FTI, farnesyltransferase inhibitor; IL-2, interleukin-2; MDR, multidrug resistance; PI3K, phosphatidylinositol-3.