Moving Toward Targeted Therapies in Acute Myeloid Leukemia

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Abstract: Advances in genomic sequencing and insights into molecular leukemogenesis are opening the door to using targeted agents to tailor treatment for acute myeloid leukemia (AML) in individual patients. Although this shift away from traditional cytotoxic therapies represents an innovative approach to AML therapy, a number of obstacles stand in the way of widespread adoption of targeted therapy into daily practice. For example, the effects of single agents are marginal, and the degree of variability among patients is great. Some have advocated incorporation of newly identified biomarkers into clinical trials to guide patient-specific treatment, but the relevance of these biomarkers to clinical response is uncertain and requires further validation. Combining targeted agents with other targeted agents or with conventional chemotherapy to overcome the biological heterogeneity of AML may enhance treatment efficacy; however, drug toxicities also are increased and drug resistance continues to occur. Overall survival is an impractical endpoint for clinical trials of AML, which may be addressed by using the endpoint of event-free survival to evaluate novel targeted agents. Another barrier to implementation is the high cost and limited availability of targeted agents. Herein, we address the above practical questions and propose potential strategies for the future evaluation of targeted treatments.

Keywords
AML, clinical application, cost-effectiveness, leukemia, targeted therapy

Introduction

Traditional therapy for acute myeloid leukemia (AML) relies on conventional DNA-targeted chemotherapy, such as cytarabine plus either daunorubicin or idarubicin. These regimens achieve long-term survival rates approaching 25% to 50% in patients younger than 60 years, and only 5% to 15% in older patients. Traditional therapies were introduced into clinical practice several decades ago, based on evolving insight into the chemistry and biochemistry of the leukemic cells. They work by targeting AML blasts more than normal

cells to produce remission, and are highly cytotoxic. It is increasingly clear that many of these classical therapies cause genetic damage to surviving leukemia cells, which contributes to relapse via the selection of resistant clones. With the discovery of novel tumor-associated mutations and their protein antigens, and the expanding insights into the mechanisms of epigenetic gene regulation and the functions of oncogenic kinases, development of nongenotoxic, "more targeted" therapy with monoclonal antibodies or other inhibitors is being rapidly explored.[1]

One successful example is therapy with all-trans retinoic acid and arsenic trioxide for acute promyelocytic leukemia. By targeting the promyelocytic leukemia/retinoic acid receptor-alpha (PML-RARα) protein, these agents have improved survival rates in acute promyelocytic leukemia to 80% to 90%.[2] Fueled by an explosion of information about the biological underpinning of AML, new drugs that are directed at critical molecular targets have been investigated for AML treatment in recent years. For example, various kinase inhibitors have been in the process of moving from bench to bedside, following the spectacular success of the *BCR-ABL* tyrosine kinase inhibitors (TKIs) targeting Philadelphia chromosome–positive chronic myeloid and acute lymphoblastic leukemias. In addition, much effort is now being focused on reversing "leukemia-related immunosuppression" by enhancing antitumor surveillance, or blocking T-cell immune checkpoints to reverse immunologic tolerance of leukemia cells.[3-5] However, the heterogeneity of AML makes the clinical evaluation of these therapies especially challenging. Thus, it is necessary to identify suitable patients and evaluate treatment efficacy and cost appropriately.

Obstacles to Clinical Evaluation of Targeted Therapies

Prediction Biomarkers in AML-Targeted Therapies

Biomarkers are widely used to identify patients who are more likely to benefit from treatment with a specific therapeutic agent, predict outcome given the response to therapy, assess drug safety and evaluate target engagement and the immediate consequence on biological processes, and monitor disease progression or therapeutic efficacy to predict survival.[6] Proper use of biomarkers helps to ensure that patients receive the best possible therapeutic strategies, thereby avoiding unnecessary treatments and associated toxicities. Nonetheless, the best way to validate and standardize those biomarkers in AML therapy remains unknown.

Three molecular markers recently were combined with cytogenetics to further refine molecular risk stratification and treatment selection in AML. Probably the most widely used target for AML therapy in the past decade has been the FMS-like tyrosine kinase 3 (FLT3) protein. FLT3 is a transmembrane receptor tyrosine kinase that is mutated in about 30% of all AML. Two major classes of mutations have been identified in AML patients: internal tandem duplications (ITDs) of the transmembrane domain in 95% of cases, and tyrosine kinase domain (TKD) point mutations in the remainder, with other mutations rarely observed.[1,7] For cytogenetically normal AML patients, the presence of the above *FLT3* mutations is associated with a high percentage of peripheral blood and bone marrow blasts, and *FLT3-ITD* mutations correlate with an increased risk of relapse and poorer progression-free and overall survival (OS).[8-10] Given these data and the availability of several TKIs with activity against *FLT3*-mutated leukemia, clinical trials have been launched using more selective (quizartinib, crenolanib) and less selective (midostaurin, lestaurtinib) TKIs in AML.[11,12] Two studies have demonstrated that a high mutant-to-wild-type allelic ratio is closely associated with an increased risk of early relapse within the first year and significantly shorter OS, and is a strong independent prognostic factor in multivariate analysis.[13,14] Current trials have not included this ratio as an index in evaluating the clinical response and complete remission (CR) rate. Furthermore, a high CR rate and favorable OS have been observed in nucleophosmin (*NPM1*)– or CCAAT/enhancer binding protein α gene (*CEBPA*)–mutated AML patients. Those positive effects were lost in *NPM1*-mutated cases in the presence of coexisting *FLT-ITD*. Although *CEBPA* rarely coexists with *FLT-ITD*, a significant number of patients carry both *FLT3-ITD* and *NPM1* mutations. Whether the therapeutic efficacy of FLT3 TKIs could replicate the favorable outcome observed in *NPM1*-mutated AML is unclear.[9] All of these observations limit the significance of *FLT3-ITD* alone as a predictive biomarker to identify the appropriate patients to enroll in the study, and the possibility of eventually validating *FLT3* as a molecular target in AML. The prognostic relevance of *FLT3-TKD* is currently unknown, but the emergence of a gain-of-function clone with the D835 mutation in the kinase domain of FLT3 has been discovered in FLT3 TKI–resistant AML.[15] A recent publication showed efficacy of crenolanib in laboratory studies of drug-resistant *FLT-TKD*–mutated leukemia cells,[16] and a current clinical trial assessing the efficacy of crenolanib in patients without prior treatment with TKIs and those who developed resistance after TKI therapy is underway.[17]

In addition to the above 3 molecular markers, which have already entered clinical practice to guide in diagnosis and treatment, a number of other epigenetic regulators have been implicated as key components of the proliferative drive in AML in different pathways. These regulators include *NRAS*, *TP53*, ten-eleven translocation

2 (*TET2*), isocitrate dehydrogenase (*IHD*), DNA nucleotide methyltransferase 3A (*DNMT3A*), and additional sex combs–like transcriptional regulator 1 (*ASXL1*).[18] These could potentially influence biomarker validation during investigational therapy, and might therefore challenge therapeutic decisions. The heterogeneity of AML, clonal evolution during disease progression, and therapeutic effects that result in elimination of sensitive blast clone, all have been recognized in AML. Those challenges have been extensively reviewed in solid tumors, and the "fit-for-purpose" method for validating biomarkers has been proposed.[6] The validation process includes 4 parts: prevalidation, exploratory validation, in-study validation, and advanced method validation. This is a cyclical process of assay refinement, with validation criteria appropriate for the intended purpose of the biomarker.[19] Prevalidation defines the purpose of the molecular or protein biomarkers, and considers preanalytical variables and bioanalytical method feasibility. Exploratory validation assesses the basic measurement performance and characterizes the formal performance with regard to its intended use, which ensures robust use across studies according to predefined specifications and facilitates the establishment of definitive acceptance criteria for targeted therapies in the in-study and advanced validation. The process is closely entwined with the development phases of a potential targeted drug, which can be used in evaluating the targeted biomarkers in AML as well.

Relevance of the Biological Complexity of AML to Therapy

The inherent biological complexity underlying AML was illuminated by a recent study revealing that each case has an average of 13 mutations: 8 random "passenger" mutations, and 5 recurrent "driver" mutations.[20] In a large study from the Eastern Cooperative Oncology Group (ECOG) that analyzed somatic mutations in 18 genes among 398 AML patients younger than 60 years old, 97.3% of patients had at least 1 identifiable mutation, regardless of the presence of cytogenetic abnormalities.[21] All of these coexisting mutations may contribute to leukemogenesis at different levels and form a complex network of multiple interacting molecular pathways with adaptive feedback and crosstalk loops, hindering the ability of a single target agent to achieve response of the system as a whole. These data underscore the complexity of AML, which further suggests that leukemic blasts have an uncanny ability to evade highly specific targeted therapies and that less specific, multipotent "dirty" drugs may be more effective.[22]

A strategy to circumvent the low response rates and short remission duration caused by the biological complexity of AML is to combine specific targeted agents at different target levels or with standard chemotherapeutic drugs known to have activity in AML. Currently, numerous combination regimens are under investigation at either the preclinical or clinical level.[23,24] For example, phosphoinositide 3-kinase (PI3K)/AKT/mammalian target of rapamycin (mTOR) pathways are activated in most AML cells. Preclinical studies that have combined the AKT inhibitor perifosine with a MEK inhibitor, or a PI3K inhibitor with an mTOR C1/2 inhibitor, have shown therapeutic responses superior to either agent alone.[25,26] In mouse models of AML, plerixafor (Mobozil, Sanofi-Aventis), an inhibitor of CXCR4, enhances response rates to chemotherapy by removing the AML blast from its protective environment and by inhibiting survival signaling through CXCR4.[27] The combination of salvage chemotherapy and plerixafor in relapsed and refractory AML may enhance chemosensitivity; therefore, a phase 1/2 study was performed in those AML patients by combining mitoxantrone, etoposide, and cytarabine (MEC) chemotherapy with plerixafor.[28] In this study, plerixafor successfully mobilized AML blasts, resulting in encouraging rates of remission. The therapy also appeared to be safe, and did not induce hyperleukocytosis or profound aplasia. Eukaryotic translation initiation factor 4E (eIF4E) is a downstream eukaryotic translation initiation factor of the PI3K/AKT/mTOR pathway, and is highly elevated in the M4 and M5 subset of AML at both the RNA and protein levels. Increased eIF4E levels and activity may enhance translational efficiency of a specific subset of transcripts in AML that are associated with proliferation and survival signaling. Ribavirin has been used to inhibit eIF4E as a single targeted agent in refractory, relapsed, or unfit for cytotoxic chemotherapy M4 and M5 AML, and yielded promising response: three out of 11 patients achieved a partial or complete response, three had blast response, and four had stable disease.[29] When ribavirin is combined with conventional chemotherapy drugs such as cytarabine or idarubicin, it appears to further reduce the colony number in primary patient specimens. A phase 1 trial in AML with ribavirin and low-dose cytarabine was recently completed, and the combination was overall well-tolerated.[30]

NRAS is one of the most common targets of oncogenic signaling mutations in hematologic malignancies. Mutations in *NRAS* occur in 10% to 15% of AML cases, and are associated with a lower CR rate and shorter disease-free survival (DFS). Even with the challenge of directly targeting mutant *RAS* oncoproteins, mitogen-activated protein kinase (MAPK) inhibition has been shown to reduce leukocytosis and prolong survival in *Nras*-mutant AML mice models by targeting the downstream pathway of *NRAS*. The inhibition failed to cause apoptotic response depending on the variable expression of specific B-cell lymphoma/leukemia 2 (BCL-2) family

members; therefore, a combination of MAPK and BCL-2 inhibitors might be more valuable in reducing proliferation of leukemic blasts and promoting their apoptosis.[31]

Although combinations of targeted agents may not displace conventional cytotoxic regimens in AML in the foreseeable future, these combinations undoubtedly intensify the treatment efficacy and could be a therapeutic option. Several important challenges must be solved before those options finally enter clinical practice.[32] Because a large number of molecular pathways might be synergistically responsible for leukemogenesis, the contribution of each pathway may be different. It will be exhausting to test different combinations of targeted agents. Thus, further elucidation of the molecular pathogenesis of leukemia certainly will be needed to help determine which combinations will be the most effective.[33] The current molecular design of therapeutic targeted agents in AML is aimed at interacting with specific proteins identified through molecular pathways. Ongoing studies on leukemic proteomics will likely identify a large number of new biomarkers that are more directly related to the cellular protein targets of therapeutics but may reflect different epigenetics and genome alterations, warranting prompt incorporation of the increasing proteome data into targeted therapy design and evaluation in AML.[34]

Drug Toxicity and Resistance

Targeted therapies have shown activity in AML therapy, but appropriate timing, sequencing, and dosage of these agents will be crucial to the success of treatment. An obstacle to moving the most promising combinations forward is the increase in toxicity. For most phase 1 and 2 trials in the development of new targeted therapies for AML, enrolled patients are usually elderly and have relapsed AML or are unable to tolerate induction chemotherapy.[1,33] Their disease could be more resistant, with highly heterogeneous genetic changes, than that of young, newly diagnosed patients. It is still debated whether targeted agents should be used as up-front therapy in clinical trials for newly diagnosed AML. Furthermore, the pharmacokinetics of a new targeted agent need to be assessed further among patients for safety and dose determination, as preclinical studies investigating these toxicities may not fully predict the toxic range that is observed in humans.

Gemtuzumab ozogamicin is an antibody-drug conjugate consisting of a humanized monoclonal antibody directed against CD33 that is linked to a potent DNA-targeted cytotoxic agent from the class of calicheamicins. In AML, CD33 is expressed in almost 90% leukemic blast cells, and 3 open-label trials have shown that gemtuzumab ozogamicin produced a 30% overall response rate, with a favorable safety profile. Therefore, it was approved under an accelerated-approval process by the US Food and Drug Administration (FDA) in 2000 for use in patients with a first relapse of CD33-positive AML who were 60 years of age or older and who were not considered candidates for cytotoxic chemotherapy.[35] It was withdrawn from the market in 2010, however, when the Southwest Oncology Group (SWOG) S0106 study showed that the agent increased patient death and added no benefit over conventional therapy.[36] Although the postapproval SWOG study revealed a significantly higher risk of fatal adverse events with the addition of gemtuzumab ozogamicin to chemotherapy, a later study that used a different method of delivery, with lower doses but an adequate cumulative dose, observed an improved advantage in OS without an increase in the risk of death from toxicity.[37] This experience provides an example of why phase 4 trials are necessary for novel targeted agents, in order to better understand their toxicity profile, pharmacokinetics, and dose escalation scheme.

Analysis of single-agent FLT3 TKIs showed only a small number CRs and a somewhat larger number of CRs with incomplete blood count recovery, with the latter responses more associated with minimal residual disease. For several FLT3 TKIs, studies have revealed poor bioactivity due to insufficient plasma drug levels, short plasma half-lives, or hepatic metabolization, all of which may affect their treatment efficacy.[7] This ultimate lack of efficacy was explained by near uniform development of drug resistance, by 72 days on average, attributed to inherent resistance or emergence of acquired resistance after an initial response. Different solutions have been explored to deal with this issue. Because resistance to single agents occurs almost universally, multiple drugs generally are used for AML treatment. In a randomized phase 2 trial, the FLT3 TKI lestaurtinib was added to chemotherapy in AML patients experiencing a first relapse who carried *FLT3* mutations. Although FLT3 inhibition was highly correlated with remission rate, suboptimal pharmacokinetics of lestaurtinib was observed, and the upregulation of the FLT3 ligand by chemotherapy with incomplete inhibition of FLT3 autophosphorylation followed by impaired cytotoxic effects likely explains the negative results.[38] The studies on identifying multidrug resistance (MDR) have revealed 3 major mechanisms of resistance, the most common being increased efflux of a broad class of hydrophobic cytotoxic drugs mediated by ATP-binding cassette (ABC) transporters.[39] Several members of the ABC transporter family, including P-glycoprotein (Pgp, also known as MDR1), induce MDR. Development of drugs that either evade efflux or inhibit the function of efflux transporters would be a reasonable approach. In AML, although the first and second generation of antagonists targeting MDR1 have yielded conflicting results, the new

generation modulators, now rely on the structure-based design and the delineation of transcriptional regulators of survival gene cassettes, have obtained encouraging data.[40] In the clinical trials using ribavirin to treat AML, drug resistance was observed by an increase in the levels of the sonic hedgehog transcription factor glioma-associated protein 1 (Gli1) and the UDP-glucuronosyltransferase 1 family, polypeptide A complex locus (UGT1A) family of enzymes, which led to glucuronidation of ribavirin and loss of the eIF4E-ribavirin interaction.[41] These findings and others learned from mechanistic studies of cellular defense shall help us to design novel targeted therapeutics to overcome drug resistance.

Clinical Efficacy Assessment for Targeted Agents

Despite the established response criteria in AML therapy, there is no uniformly accepted clinical endpoint for the assessment of targeted therapy. OS is regarded as the gold standard primary endpoint in oncology clinical trials, and is being used to assess the efficacy of drugs submitted for FDA approval. However, several factors make OS difficult for evaluation of targeted therapies in AML. First, targeted therapies commonly take longer than cytotoxic agents to affect the blasts. Second, these agents often are used as a "bridge" for patients who will be undergoing hematopoietic stem cell transplant (HSCT). Finally, trial recruitment is more difficult as a result of patient selection criteria. As a result, the time to disease relapse or event-free survival (EFS) was used as an endpoint in the study of postremission maintenance therapy of AML, and may be considered an acceptable surrogate for OS.[42]

EFS has been used with greater frequency in pivotal randomized trials of targeted therapies for solid tumors, which can be applied in AML.[43] Compared with OS, EFS has the advantages of being reached sooner, even in smaller trials, and of not being confounded by the impact of subsequent lines of therapy. One of these subsequent lines of therapy may be HSCT, particularly in AML. However, EFS has many pitfalls compared with other measures of benefit. For example, defining what constitutes progression, determining when progression occurs, and minimizing the information bias in assessment of progression events by the treating physician, particularly in open-label studies, can influence the progression recorded and subsequently the power of the analysis.[44] To solve these issues, special attention must be paid to the design of studies that use EFS as a primary endpoint. Studies should include blinded treatment assignment whenever feasible, precise censoring, clear criteria for measuring response, and prompt interpretation of the tests. These steps are critical in reducing the variability of progression detection.

Cost-Utility Challenge

The ability to tailor treatment to individual patients through the use of predictive biomarkers provides hope that upcoming trials will support de-escalation of therapy, and in some cases, even elimination of chemotherapy. By avoiding ineffectual treatment and minimizing adverse effects, targeted agents have the potential to offer substantial clinical and economic offsets. However, the price of these novel agents is extremely high. The average cost of TKIs for AML ranges from $5000 to $10,000 per month, and the wholesale cost of sorafenib is $5600 per month.[45] Because resources are limited in most settings, publicly funded health care programs, private health plans, and policy makers need to weigh the costs and benefits of targeted treatments and make decisions about which treatments will be covered and under what circumstances. This raises important questions about the use of cost-effectiveness analysis in policy making to determine the most suitable patients for these novel agents, especially in the setting of growing concerns about health care costs and cost burden for the patient.[46]

Decision analytic modeling, including economic evaluation of targeted therapies, has been taken into clinical consideration. Many countries have authorized explicit economic evaluation guidelines to encourage appropriate conduct in decision-making. Although accurate estimation of effectiveness and cost ratio for those agents is often difficult, steps can be taken to quantify the costs and benefits of a treatment strategy by using an incremental cost-effectiveness ratio (ICER), and subsequently years of life saved, or quality-adjusted life-years (QALYs) saved. ICERs can be a useful metric for facilitating coverage decisions because they can be used to systematically compare the value of health interventions across conditions. The resulting cost-effectiveness ratios can then be compared across conditions with each other or with a threshold value, with the goal of identifying the most efficient ways of maximizing health at the population level. For example, the direct costs of decitabine vs conventional induction therapy with cytarabine plus daunorubicin in newly diagnosed AML patients with age older than 60 years were compared using ICER. The expected cost was nearly the same in both groups: $88,325 for patients receiving cytarabine plus daunorubicin, vs $91,312 with decitabine. But further interpretation of the data regarding the ICER per QALY showed the cost of decitabine was $38,839, which is superior to cytarabine plus daunorubicin.[47,48] Although there is no universally accepted cost-effectiveness benchmark in the United States, values of the conventional chemotherapy cost per QALY could be referred in the future study for new targeted therapies in AML, below which therapies may be considered cost-effective and above which

therapies be considered less cost-effective. However, these cited thresholds are theoretical rather than practical, and many agents are used in combination with others or chemotherapy. As a result, such cost-effectiveness analyses are not ideal for use as explicit criteria for coverage or regulatory decisions.[49] A systematic review that examined how economic analyses of targeted therapy were conducted, using trastuzumab (Herceptin, Genentech) as an example, supported the increased use of local data to inform model parameters to improve costing and behavioral assumptions. Regular conducting of probabilistic sensitivity analysis and the practical application of value of information methods also improve quantification and representation of decision uncertainty.[32]

Currently, the FDA considers effectiveness but not cost-effectiveness data to make decisions for drug approval. Medicare, the single largest payer of health care in the United States, is legally bound to cover treatments viewed as "reasonable and necessary," which has not been interpreted to include a consideration of the economic cost of treatment. In contrast, Canada, the United Kingdom, and other countries with single-payer health care systems have already incorporated ICERs in making decisions about what treatments they will cover. With the sophistication of cost-effectiveness analysis modeling, a standardized evaluation system could be available to better support the decision-making in this aspect.

Conclusion

Although numerous agents in development are aimed at targeting both well established and recently identified leukemogenic pathways, clinical trials face many challenges in improving survival in AML. Applicable biomarkers are integrated into clinical practice based on preclinical drug development, from target identification and validation to clinical practice, but their relevance to therapeutic efficacy will require robust validation. It is essential to recognize the importance of "fit-for-purpose" biomarker validation in AML, including for younger, newly diagnosed, and relatively fit patients. Because of the genetic complexity of leukemogenesis, a strategy that combines targeted agents and chemotherapy is likely to be a more successful intervention, with extensive attention paid to increased toxic pharmacologic profiles and drug resistance. Appropriate techniques for dosing these agents, designing a new generation of MRD-targeted agents, and identifying new drug resistance mechanisms may aid us in addressing assessment challenges brought about by the use of EFS as an endpoint in clinical trials. Some of the targeted therapies in preclinical studies have showed encouraging therapeutic outcomes. Nevertheless, they are all very expensive. Economic evaluation, preferably with ICER per QALY, is a reasonable tool that may allow policy and decision makers to address the relationship between clinical effects and costs associated with targeted treatment to identify the most cost-effective agents and their legitimate indications.

Recognizing the above challenges will encourage us to solve these problems with thoughtful and creative approaches to weighing clinical efficacy and costs against standard care. This will require collaborative effort among professional organizations, drug manufacturers, and patients who are convened around a common goal: to translate advances in leukemia biology into clinically safer and more effective products for AML therapy, and into policies that accelerate their availability.

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