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What Are the Endpoints of Therapy for Acute Leukemias? Old Definitions and New Challenges

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Abstract

Acute leukemias are complex diseases on multiple levels, and laboratory efforts over the past 3 decades have focused on better understanding of the molecular underpinnings and their stem cell biology. We now have a panoply of technologic advances that allow us to characterize individual leukemias by molecular profiles that relate directly to clinical behavior, to detect minimal residual disease, and to begin to develop “targeted” therapeutic strategies based on molecular considerations. There are a number of challenges surrounding this task: first, how to combine these agents with traditional chemotherapeutics and/or with each other to maximize leukemic cell kill and increase the cure rate; second, how to use these targeted agents in the minimal residual disease with potential curative intent; third, for patients unable to tolerate or unlikely to benefit from aggressive approaches, how to use one or more of these agents to reduce tumor bulk and either permit some restoration of normal marrow function or induce morphologic and functional differentiation of the leukemic clone to overcome the leukemia-associated bone marrow failure; and lastly, how to measure the effects of these agents on the molecular and cellular biologic levels in ways that correlate with and might even predict overall clinical outcome. These challenges are further complicated by the inherent heterogeneity in host biology; disease etiology and biology; and interactions among host, disease, and treatment that ultimately determine individual clinical outcomes. Toward this end, we will discuss selected issues surrounding new clinical trial designs and the development of clinically relevant molecular endpoints that might facilitate the development of new treatment approaches that will improve the outlook for adults with acute leukemias.

Keywords

Clinical endpoints; Drug development; Pharmacodynamic endpoints

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Introduction

Acute leukemias are complex diseases on multiple levels, and laboratory efforts over the past 3 decades have focused on better understanding of the molecular underpinnings and their stem cell biology. The diversity of these malignancies is manifest by a wide variety of morphologic subclasses, highly varied clinical presentations, and significant variation in the responses seen clinically. Unfortunately, acute leukemias have a generally poor overall clinical outcome in adults. Thirty years ago, the achievement of complete remission (CR) was determined by morphologic assessment of the individual marrow and in itself considered to be a victory. Clinically, it was realized very early on that while morphologic CR remained the first goal post of response, it provided little prognostic information and was clearly insufficient to determine a cure. Technologic advancements in marking and measuring the leukemic population have provided a clearer determination of minimal residual disease (MRD)¹⁻³ and offer a first-order assessment to discriminate between patients with drug-sensitive disease who might be cured with traditional cytotoxic chemotherapy, possibly use lower total doses, and those with inherent drug-resistant leukemia who will not be cured.

In addition, technologic advancements allow us to characterize individual leukemias by molecular profiles that not only relate to clinical behavior⁴⁻¹⁰ but also provide potential targets to direct therapeutic strategies that include blocking upstream^{11,12} and downstream¹³⁻¹⁷ signal transduction intermediaries, reversing epigenetic gene silencing,¹⁸ and evoking potential immunomodulatory approaches.^{19,20} Each of these targets provides the potential to circumvent traditional drug resistance, and each might hold promise to improve therapeutic outcomes for our patients. However, the clinical effect of such strategies might not be easily measured using traditional response criteria, and the development of criteria to better assess biologic activity and early efficacy endpoints will be critical to advancing novel agents and clinical trial designs. Moreover, our ability to define surrogate endpoints that reflect clinical response is complicated by the inherent heterogeneity in host biology; disease etiology and biology; and interactions among host, disease, and treatment that ultimately determine individual clinical outcomes. Below and as summarized in Table 1, we will discuss some critical issues surrounding our current ability to measure net clinical drug effect and accurately compare the results of these new approaches with the results being achieved with current treatment or supportive interventions.

Clinical Endpoints

The overarching endpoint by which we judge the relative success or failure of new treatment strategies is the overall duration of survival. Yet, the actual quality of the noted survival, often considered a surrogate for therapy-related toxicity, must also be evaluated and integrated into the evaluation of a treatment's success. It would seem intuitive that the effect of a treatment on duration of overall survival (OS) should directly relate to the specific measurements of treatment success such as achievement and duration of CR. Unfortunately, this is not always the case. As an example, CRs of short duration have little to no effect on OS despite achieving the time-honored endpoint of normalization of peripheral blood counts and < 5% marrow blasts by morphology. Such responses are more consistent with primary refractory disease. Moreover, the price to be paid for an intensive regimen is often significant and directly effects the patient's quality of life (QOL), particularly in the case of older adults with acute myeloid leukemia (AML). Alternatively, an exclusive endpoint of CR might not assess the full potential of an experimental agent or regimen to induce hematologic improvement that, in turn, might translate into survival advantage and/or enhancement of QOL.

To begin to define and address these burgeoning challenges to assessment and development of antileukemia agents, the US Food and Drug Administration (FDA) and the American Society

of Hematology held a joint workshop on endpoints to establish efficacy of new agents in the treatment of acute leukemia.²¹ Among the central issues are patient heterogeneity, the ability to detect and measure MRD as a surrogate of drug resistance, the clinical implications of a “less-than-CR” response or a stabilization that affords the patient a chance to move to allogeneic stem cell transplantation (a so-called bridge to transplantation) or another potential clinical intervention (ie, maintenance strategies, immunotherapy), and the individual patient's QOL assessment.

In our current configurations, it takes a long time to determine whether a new agent is a worthwhile addition to the treatment arsenal for acute leukemias and can jump the hurdles of FDA approval. Indeed, the proper selection of a “comparator” or control arm is made especially difficult by the lack of effective “standard of care,” given that the current 5-year survival rates for the “best” patients subgroups is $\leq 40\%$ and drops to $\leq 20\%$ for the remaining patients. The goal of achieving a CR in order to improve OS is further confounded by the toxicity traditionally seen with the “CR-driven” intensive chemotherapy-based induction and consolidation. In addition, our ability to understand if a new agent or approach can actually improve overall clinical outcome without increased toxicity (or possibly with less toxicity) is challenged by the heterogeneity of the biology of acute leukemias and the heterogeneity of the patients with the disease.²¹⁻²³

In this regard, we need to embrace trial designs that will hasten the drug development process and, at the same time, allow for treatment of more patients at optimal doses and fewer patients at suboptimal or toxic doses.²⁴ To allow for the treatment of more patients at or near a clinically predicted optimal dosing and fewer at suboptimal dosing, Estey and Thall have proposed the concept of adaptive randomization according to “real-time” clinical results to allow for faster and perhaps more accurate assessment of the effect of a particular therapeutic manipulation for definable subsets of patients within a heterogeneous disease population.²³ This type of Bayesian design allows more patients to be randomized to the therapeutic arm that stands the greatest chance for benefit (or the least chance for harm) and can be applied to both phase I and phase II trials, especially in the setting where multiple new agents or combinations are being compared. Moreover, the confounding effects of the inherent biologic heterogeneity of AML may be minimized by using a “biologically relevant” stratification within the randomized arms according to combinations of critical host and disease features such as patient age, underlying myelodysplasia (MDS) or treatment-related AML, and genetics.

Cure Versus Complete Response Versus Clinical Benefit

It is unfortunate that “cure” remains an ephemeral concept for most adults with acute leukemias and others with primitive “stem cell” leukemias that are characterized by genetic complexity and inherent drug resistance. These diseases not only are considered incurable outside of the allogeneic transplantation setting but are also clearly less responsive to traditional induction approaches, with less than 50% achieving CR in most series.

This is not to say that individuals with such leukemias may not reap benefits from treatments, and in fact, it is this population that may benefit from diverse therapeutic interventions (Table 2). In this regard, responses that are “less than CR” on both morphologic and molecular grounds may translate into improvements in survival duration and/or QOL but may do so in a nonlinear fashion. The ability to both recognize and define such “less-than-cure” outcomes clinically and molecularly would greatly benefit clinicians and could easily be enlisted to help determine the early efficacy for new agents under development and may help sort out the best clinical situations to use them. Ideally, such data should be available to those tasked with the arduous responsibility of determining FDA approval of such new agents. To date, quantifying “less-than-CR” responses and their effect on OS and quality of survival is a work in progress. The effect of “less-than-CR” responses has been followed most closely using gemtuzumab

ozogamicin for relapsed AML in patients over the age of 60 years where CR without full recovery of the platelet count (CRp) appears to result in similar OS compared with a traditional CR.^{25,26} A similar “equivalency” for CR and incomplete CR, of which CRp is a subclass, has been noted for relapsed/refractory AML patients treated with clofarabine.²⁷ Such is not the case, however, for newly diagnosed patients undergoing initial induction therapy, where survival is longer for those achieving true CR.^{28,29}

On the other hand, CR may not be required for clinical survival benefit when determining the effect of epigenetic modulatory therapies such as DNA methyltransferase (DNMT)-1 inhibitors (5-azacytidine and decitabine)^{30,31} or histone deacetylase (HDAC) inhibitors,^{32,33} and the immunomodulatory thalidomide derivative lenalidomide^{34,35} on disease progression and OS in myeloid malignancies, especially myelodysplastic syndromes (MDS). In these settings, the enhancement of both OS and QOL may relate to an improvement in blood counts that result in decreased transfusion requirements and an attendant decrease in complications such as iron overload and development of anti-red blood cell and/or platelet antibodies. This success might be in part because of the ability of MDS cells' ability to retain differentiation pathways that in turn can lead to multilineage hematopoiesis, which is typically lost in full-scale AML.

Molecular Activity

Many of the issues surrounding clinical endpoints apply to molecular endpoints, as well. The ultimate endpoints of cell differentiation and/or death can be described more specifically by the effect of an agent on the net expression or activity of a single molecule, or one or more integrated molecular pathways (with modulation of downstream intermediaries),^{7,11,13} or an even more global effect such as modulation of gene or protein expression profiles in response to drug exposure.¹² Ultimately, however, for molecular measurements to have clinical significance, they must reflect and/or predict clinical outcome in terms of both efficacy and toxicity. As such, specific molecular targets and the results of their modulation must be relevant to the overall cellular biology, not only for the malignant cell itself but also for the cell in the context of its microenvironment.³⁶⁻³⁸ This consideration might lead to a measurement that differs from the more conventional approach that we use for cytotoxic agents, namely, a “biologically effective dose,” which may not be the highest dose of a particular agent in terms of dose-limiting toxicities or maximal tolerated dose but rather a dose that modulates the activity of molecule(s) or pathway(s) that are critical to net cellular and clinical responses. This concept might be particularly important in epigenetic therapies, as exemplified in landmark trials of combination therapy with DNMT-1 inhibitors 5-azacytidine or decitabine and HDAC inhibitors phenylbutyrate,³⁹ valproic acid,⁴⁰⁻⁴¹ or the benzamide HDAC inhibitor entinostat (SNDX-275, formerly MS-275),⁴² and therapies aimed as inducing differentiation rather than direct apoptosis of the malignant clone.⁴³

The clinical trials of DNMT-1 inhibitors in combination with HDAC inhibitors have been accompanied by elegant correlative studies examining baseline levels and posttreatment changes in the expression of one or more selected “tumor suppressor” genes whose dysregulation might be involved in leukemogenesis and/or perpetuation of the malignant clone, including p15, p73, E-cadherin, *DAPK*, *CEBP-α*, and *SOCS1*.^{39-42,44} Though changes in the expression of these diverse genes can be documented in response to treatment, the relationship of changes (either qualitative or quantitative) to clinical response has been inconsistent. Indeed, Fandy et al measured changes in the methylation of several tumor suppressor genes in CD34⁺ bone marrow cells from patients with MDS or poor-risk AML undergoing treatment with 5-azacytidine plus entinostat and was unable to demonstrate differences in day-0, day-15, or day-29 gene expression in responders versus nonresponders.⁴⁴ The relevance of changes in specific gene expression is further complicated by seemingly contradictory findings regarding the relationship of DNA methylation to overall outcome,^{45,46} a finding that likely reflects the

inherent heterogeneity in molecular pathogenesis and pathophysiology of this complex disease plus an incomplete understanding of the full spectrum of gene expression before and after therapy.

Addressing the Challenges of Drug Development: New Paradigms in Clinical Trials Design

There is no question that all phases of clinical drug development would be well-served by the availability of molecular endpoints, or so-called biomarkers, that reflect disease activity and serve as reliable surrogate markers for drug effects (both efficacy and toxicity) on an individual patient basis.⁴⁷⁻⁴⁹ Ideally, these markers could be used in early-phase trials to guide the selection of optimal doses and schedules of investigational drugs for further studies and ultimately might even guide appropriate patient selection. Delineated in Table 3 are several requirements for using such biomarkers in an optimally informative fashion: (1) defining the full spectrum of molecules and pathways that are being targeted by a specific agent; (2) understanding how the agent in question modulates the net expression and activity of the molecular target; (3) being able to define a dose-response relationship in tumor tissue or a reliable surrogate tissue between “target modulation” and the agent under study; and (4) being able to correlate the presence and magnitude of biomarker modulation with clinical response. Identification of a surrogate tissue with biologic parameters similar to the tumor might be limited to skin or buccal mucosa for epithelial malignancies but is much less of a problem for leukemias, where the target tissue itself is easily obtained in a longitudinal fashion throughout all stages of therapy.

Phase 0 Clinical Trials: A New Concept in Drug Development

The ability to measure drug efficacy at least in part by defining the molecular consequences of drug exposure in a dose-related fashion, ie, pharmacodynamic endpoints, forms the basis for the new phase 0 trial design that integrates molecular pharmacology with traditional pharmacokinetics and offers a potentially more rapid approach to the clinical testing of novel combinations and movement of targeted agents toward FDA approval.⁴⁸⁻⁵⁰ Indeed, one of the major goals of the phase 0 “first-in-human” study is to establish the validity of one or more molecular endpoints whose behaviors are modulated by the study drug in target tumor and surrogate tissues.⁴⁸ The phase 0 approach involves limited drug exposure in terms of both dose and time with the intent of obtaining longitudinal tumor biopsies to measure drug effect. To date, this trial design has been applied to the development of veliparib (ABT-888), an inhibitor of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) which, by itself, is not expected to have significant toxicity unless it is combined with DNA-damaging agents.^{49,51,52} The PARP inhibitor is well suited to the phase 0 design, where the primary objectives are to define the interaction of the study drug with its putative molecular target in human tissue in vivo and to characterize and validate the assay of that interaction in a clinically reproducible and useful fashion.

Measuring Toxicity: Is It the Drug or Is It the Leukemia

Finally, drug development in the acute leukemias is complicated by the baseline morbidity of these diseases that relates directly to leukemia-associated bone marrow failure with an expectedly high risk for overwhelming infection and attendant multiorgan dysfunction. These complications do not accompany solid tumors without bone marrow involvement, and if such complications arise during a clinical trial, it is logical to attribute them to the study drug. Such attribution, however, is not the case for the acute leukemias. Thus, the current definitions of serious adverse events and dose-limiting toxicities (DLTs) that are customary for solid tumors may preclude full dose-escalation of new agents in the acute leukemias. To address this situation, Atallah and colleagues have proposed the establishment of a baseline toxicity rating

for patients with acute leukemias that takes into account the inherent organ dysfunctions associated with leukemia and the expected toxicities superimposed by induction therapy with cytosine arabinoside and anthracyclines.⁵³ In the phase I setting, this type of baseline might permit a clearer picture of what toxicities are truly related to the study drug and where DLTs actually occur. In the phase II and III setting, the ability to “subtract” the expected toxicities from those observed during the addition of a new agent to chemotherapy might decrease the obligatory reporting of so-called serious adverse events and therefore decrease some of the tremendous cost associated with regulatory oversight by institutional, governmental, and pharmaceutical agencies.

Future Perspectives

Our continuing challenge is to define the spectrum of clinical and molecular endpoints by which we can judge net drug efficacy and determine the optimal role of new agents in the therapeutic armamentarium for acute leukemias. The ability to recognize the heterogeneity of AML with respect to its malignant stem cell biology compared with normal hematopoietic stem cells, its cell kinetics, and its aberrant molecular pathophysiology may lead to specific curative strategies for most if not all of the AML subtypes. There are a panoply of new agents designed to target selected components of key signal transduction pathways, for instance, inhibitors of FLT-3, vascular endothelial growth factor, farnesyltransferases, components of the PI3K/Akt/mTOR pathway, or components of pathways aimed at repairing DNA damage such as CHK-1 or PARP.^{16,17,54-59} Moreover, there are agents that have been developed to target molecules, for instance, the epidermal growth factor receptor (EGFR) involved in “epithelial carcinogenesis” that might exhibit off-target effects in hematopoietic cells even though those cells lack EGFR receptors, as has been detected with erlotinib⁶⁰ and gefitinib.⁶¹ There are a number of crucial issues surrounding the optimal incorporation of these molecularly targeted agents into the therapeutic armamentarium: first, how to combine these agents with traditional chemotherapeutics and/or with each other to maximize leukemic cell kill and increase the cure rate; second, how to use these targeted agents in the MRD with potential curative intent; third, for patients unable to tolerate or unlikely to benefit from aggressive approaches, how to use one or more of these agents to reduce tumor bulk and either permit some restoration of normal marrow function or induce morphologic and functional differentiation of the leukemic clone to overcome the leukemia-associated bone marrow failure; and lastly, how to measure the effects of these agents on the molecular and cellular biologic levels in ways that correlate with and might even predict overall clinical outcome.

An ideal MRD approach would be able to discriminate normal from leukemic stem cells and thereby permit the selective destruction of the latter. This can only arise from current efforts to identify the unique characteristics that characterize each myeloid leukemia subtype stem cell on molecular and biologic levels. It is in this MRD setting that epigenetic modulatory approaches might prevent recurrence by promoting apoptosis or by reversing the silencing of genes involved in differentiation, and that immunomodulatory approaches, including vaccines directed against one or more aberrantly expressed proteins might find their greatest efficacy. Along these lines, the finding that aberrant methylation of selected genes in AML might be heightened at relapse relative to initial diagnosis provides an intriguing rationale for the use of DNMT-1 inhibitors in the MRD setting, aimed at preventing disease recurrence or progression.⁶²

Ultimately, as we come to deepen our understanding of the molecular pathogenesis of AML, particularly on the level of the leukemia-susceptible stem cell, we may be able to prevent the occurrence of AML occurring as a consequence of genomic toxicity, as in treatment-related AML. The lessons learned from treatment of patients in remission⁶³ and in preleukemic states and the molecularly targeted approaches being tested in those settings might be directly

applicable to the primary prevention setting, especially if we are able to define individuals at high risk for leukemogenesis. At all stages of disease and treatment, patients with acute leukemias should be considered for clinical trials accompanied by studies of leukemia cell biology in order to permit the fluent translation of molecular discoveries into clinical advances. It is only through such scientifically sound translation that we will be able to move the field forward in a clinically meaningful way.

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References

1. Borowitz MJ, Devidas M, Hunger SP, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia and its relationship to other prognostic factors: a Children's Oncology Group study. *Blood* 2008;111:5477–85. [PubMed: 18388178]
2. Craig FE, Foon KA. Flow cytometric immunophenotyping for hematologic neoplasms. *Blood* 2008;111:3941–67. [PubMed: 18198345]
3. Kern W, Voskova D, Schoch C, et al. Determination of relapse risk based on assessment of minimal residual disease during complete remission by multiparameter flow cytometry in unselected patients with acute myeloid leukemia. *Blood* 2004;104:3078–85. [PubMed: 15284114]
4. Bullinger L, Dohner K, Bair E, et al. Use of gene-expression profiling to identify prognostic subclasses in adult acute myeloid leukemia. *N Engl J Med* 2004;350:1605–16. [PubMed: 15084693]
5. Garzon R, Volinia S, Liu CG, et al. MicroRNA signatures associated with cytogenetics and prognosis in acute myeloid leukemia. *Blood* 2008;111:3183–9. [PubMed: 18187662]
6. Lavrencic M, Sun SM, Dijkstra MK, et al. Micro RNA expression profiling in relation to the genetic heterogeneity of acute myeloid leukemia. *Blood* 2008;111:5078–85. [PubMed: 18337557]
7. Kornblau SM, Womble M, Qiu YH, et al. Simultaneous activation of multiple signal transduction pathways confers poor prognosis in acute myelogenous leukemia. *Blood* 2006;108:2358–65. [PubMed: 16763210]
8. Loriaux MM, Levine RL, Tyner JW, et al. High-throughput sequence analysis of the tyrosine kinome in acute myeloid leukemia. *Blood* 2008;111:4788–96. [PubMed: 18252861]
9. Marcucci G, Radmacher MD, Maharry K, et al. Micro RNA expression in cytogenetically normal acute myeloid leukemia. *N Engl J Med* 2008;358:1919–28. [PubMed: 18450603]
10. Schlenk RF, Dohner K, Krauter J, et al. Mutations and treatment outcome in cytogenetically normal acute myeloid leukemia. *N Engl J Med* 2008;358:1909–18. [PubMed: 18450602]
11. Stommel JM, Kimmelman AC, Ying H, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. *Science* 2007;318:287–90. [PubMed: 17872411]
12. Tomasson MH, Xiang Z, Walgren R, et al. Somatic mutations and germline sequence variants in the expressed tyrosine kinase genes of patients with de novo acute myeloid leukemia. *Blood* 2008;111:4797–808. [PubMed: 18270328]
13. McCubrey JA, Steelman LS, Abrams SL, et al. Targeting survival cascades induced by activation of Ras/Raf/MEK/ERK, PI3K/PTEN/Akt/mTOR and Jak/STAT pathways for effective leukemia therapy. *Leukemia* 2008;22:708–22. [PubMed: 18337766]
14. Milella M, Estrov Z, Kornblau SM, et al. Synergistic induction of apoptosis by simultaneous disruption of the Bcl-2 and MEK/MAPK pathways in acute myelogenous leukemia. *Blood* 2002;99:3461–3. [PubMed: 11964319]
15. Ricciardi MR, McQueen T, Chism D, et al. Quantitative single cell determination of ERK phosphorylation and regulation in relapsed and refractory primary acute myelogenous leukemia. *Leukemia* 2005;19:1543–9. [PubMed: 16001087]
16. Wendel H-G, de Stanchina E, Fridman JS, et al. Survival signalling by Akt and eIF4E in oncogenesis and cancer therapy. *Nature* 2004;428:332–5. [PubMed: 15029198]
17. Xu Q, Thompson JE, Carroll M. mTOR regulates cell survival after etoposide treatment in primary AML cells. *Blood* 2005;106:4261–8. [PubMed: 16150937]

18. Jones PA, Baylin SB. The epigenomics of cancer. *Cell* 2007;128:683–92. [PubMed: 17320506]
19. Greiner J, Schmitt M, Li L, et al. Expression of tumor-associated antigens in acute myeloid leukemia: implications for specific immunotherapeutic approaches. *Blood* 2006;108:4109–17. [PubMed: 16931630]
20. Molldrem J. Vaccination for leukemia. *Biol Blood Marrow Transplant* 2006;12:13–8. [PubMed: 16399579]
21. Appelbaum FR, Rosenblum D, Arceci RJ, et al. End points to establish the efficacy of new agents in the treatment of acute leukemia. *Blood* 2007;109:1810–6. [PubMed: 17095617]
22. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. *Blood* 2006;107:3481–5. [PubMed: 16455952]
23. Estey EH, Thall PF. New designs for phase 2 clinical trials. *Blood* 2003;102:442–8. [PubMed: 12560224]
24. Rogatko A, Schoeneck S, Jonas W, et al. Translation of innovative designs into phase I trials. *J Clin Oncol* 2007;25:4982–6. [PubMed: 17971597]
25. Larson RA, Sievers EL, Stadtmauer EA, et al. Final report of the efficacy and safety of gemtuzumab ozogamicin (Mylotarg) in patients with CD33-positive acute myeloid leukemia in first recurrence. *Cancer* 2005;104:1442–52. [PubMed: 16116598]
26. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol* 2001;19:3244–54. [PubMed: 11432892]
27. Kantarjian HM, Gandhi V, Cortes J, et al. Phase 2 clinical and pharmacologic study of clofarabine in patients with refractory or relapsed acute leukemia. *Blood* 2003;102:2379–86. [PubMed: 12791647]
28. deGreef GE, van Putten WL, Boogaerts M, et al. Criteria for defining a complete remission in acute myeloid leukaemia revisited. An analysis of patients treated in HOVON-SAKK co-operative group studies. *Br J Haematol* 2005;128:184–91. [PubMed: 15638852]
29. Estey EH, Garcia-Manero G, Giles FJ, et al. Clinical relevance of CRp in untreated AML. *Blood* 2005;106:161a. Abstract 541.
30. Kantarjian H, Oko Y, Garcia-Manero G, et al. Results of a randomized study of 3 schedules of low-dose decitabine in higher-risk myelodysplastic syndrome and chronic myelomonocytic leukemia. *Blood* 2007;109:52–7. [PubMed: 16882708]
31. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacytidine in patient with the myelodysplastic syndrome: a study of the Cancer and Leukemia Group B. *J Clin Oncol* 2002;10:2429–40. [PubMed: 12011120]
32. Assouline S, Cortes J, et al. Phase 1 study of the oral isotype specific histone deacetylase inhibitor MGCD0103 in leukemia. *Blood* 2008;112:981–9. [PubMed: 18495956]
33. Gojo I, Jiemjit A, Trepel JB, et al. Phase 1 and pharmacologic study of MS-275, a histone deacetylase inhibitor, in adults with refractory and relapsed acute leukemias. *Blood* 2007;109:2781–90. [PubMed: 17179232]
34. List A, Kuttin S, Roe DJ, et al. Efficacy of lenalidomide in myelodysplastic syndromes. *N Engl J Med* 2005;352:549–57. [PubMed: 15703420]
35. Raza A, Reeves JA, Feldman EJ, et al. Phase 2 study of lenalidomide in transfusion-dependent, low-risk, and intermediate-I-risk myelodysplastic syndromes with karyotypes other than deletion 5q. *Blood* 2008;111:86–93. [PubMed: 17893227]
36. Garrido SM, Appelbaum FR, Willman CL, et al. Acute myeloid leukemia cells are protected from spontaneous and drug-induced apoptosis by contact with a human bone marrow stromal cell line (HS-5). *Exp Hematol* 2001;29:448–57. [PubMed: 11301185]
37. Konopleva M, Konoplev S, Hu W, et al. Stromal cells prevent apoptosis of AML cells by upregulation of anti-apoptotic proteins. *Leukemia* 2002;16:1713–24. [PubMed: 12200686]
38. Meads MB, Hazlehurst LA, Dalton WS. The bone marrow microenvironment as a tumor sanctuary and contributor to drug resistance. *Clin Cancer Res* 2008;14:2519–26. [PubMed: 18451212]
39. Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibitions in the treatment of myeloid neoplasms. *Cancer Res* 2006;66:6361–9. [PubMed: 16778214]

40. Blum W, Klisovic RB, Hackanson B, et al. Phase I study of decitabine alone or in combination with valproic acid in acute myeloid leukemia. *J Clin Oncol* 2007;25:3884–91. [PubMed: 17679729]
41. Garcia-Manero G, Kantarjian HM, Sanchez-Gonzalez B, et al. Phase 1/2 study of the combination of 5-aza-2'-deoxycytidine with valproic acid in patients with leukemia. *Blood* 2006;108:3271–9. [PubMed: 16882711]
42. Gore SD, Jiemjit A, Silverman LB, et al. Combined methyltransferase/histone deacetylase inhibition with 5-azacytidine and MS-275 in patients with MDS, CMMoL and AML: clinical response, histone acetylation and DNA damage. *Blood* 2006;108:156a. Abstract 517.
43. Matsui WH, Gladstone DE, Vala MS, et al. The role of growth factors in the activity of pharmacological differentiation agents. *Cell Growth Differ* 2002;13:275–83. [PubMed: 12114217]
44. Fandy T, Carraway HE, Licht J, et al. Reversal of methylation of candidate tumor suppressor genes is not required for clinical response in myeloid malignancy patients treated with sequential 5-azacytidine and the histone deacetylase inhibitor MS-275. *Proc Am Assoc Cancer Res* 2007;48:591.
45. Grovdal M, Khan K, Aggerholm A, et al. Negative effect of DNA hypermethylation on the outcome of intensive chemotherapy in older patients with high-risk myelodysplastic syndromes and acute myeloid leukemia following myelodysplastic syndrome. *Clin Cancer Res* 2007;13:7107–12. [PubMed: 18056190]
46. Kroeger H, Jelinek J, Kornblau SM, et al. Increased DNA methylation is associated with a good prognosis. *Blood* 2007;110 Abstract 595.
47. Goulatt BHL, Clark JW, Pien HH, et al. Trends in the use and role of biomarkers in phase 1 oncology trials. *Clin Cancer Res* 2007;13:6719–26. [PubMed: 18006773]
48. Doroshow JH, Parchment RE. Oncologic phase 0 trials incorporating clinical pharmacodynamics: from concept to patient. *Clin Cancer Res* 2008;14:3658–63. [PubMed: 18559579]
49. Murgo AJ, Kummar S, Rubinstein L, et al. Designing phase 0 cancer clinical trials. *Clin Cancer Res* 2008;14:3675–82. [PubMed: 18559582]
50. Calvert HA, Plummer R. The development of phase 1 cancer trial methodologies: the use of pharmacokinetic and pharmacodynamic end points sets the scene for phase 0 clinical trials. *Clin Cancer Res* 2008;14:3664–9. [PubMed: 18559580]
51. Ashwell S, Zabludoff S. DNA damage detection and repair pathways – recent advances with inhibitors of checkpoint kinases in cancer therapy. *Clin Cancer Res* 2008;14:4032–7. [PubMed: 18593978]
52. Ashwott A. A synthetic lethal therapeutic approach: poly(ADP) ribose polymerase inhibitors for the treatment of cancers deficient in DNA double-strand break repair. *J Clin Oncol* 2008;22:3785–90.
53. Atallah E, Cortes J, O'Brien S, et al. Establishment of baseline toxicity expectations with standard frontline chemotherapy in acute myelogenous leukemia. *Blood* 2007;110:3547–51. [PubMed: 17673605]
54. Gilliland DG, Griffin JD. The roles of FLT3 in hematopoiesis and leukemia. *Blood* 2002;100:1532–42. [PubMed: 12176867]
55. Levis M, Small D. FLT3 tyrosine kinase inhibitors. *Int J Hematol* 2005;82:100–7.
56. Karp JE, Gojo I, Pili R, et al. Vascular endothelial growth factor (VEGF) for relapsed and refractory adult acute myelogenous leukemias: therapy with sequential ara-C, mitoxantrone and bevacizumab. *Clin Cancer Res* 2004;10:3577–85. [PubMed: 15173063]
57. Karp JE, Lancer JE. Development of farnesyltransferase inhibitors for clinical cancer therapy: focus on hematologic malignancies. *Cancer Invest* 2007;25:484–94. [PubMed: 17882662]
58. Donawho CK, Luo Y, Luo Y, et al. ABT-888, an orally active poly(ADP-ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical animal models. *Clin Cancer Res* 2007;13:2728–37. [PubMed: 17473206]
59. Mesa RA, Loegering D, Powell HL, et al. Heat shock protein 90 inhibition sensitizes acute myelogenous leukemia cells to cytarabine. *Blood* 2005;106:318–27. [PubMed: 15784732]
60. Boehrer S, Ades L, Braun T, et al. Erlotinib exhibits antineoplastic off-target effects in AML and MDS: a preclinical study. *Blood* 2008;111:2170–80. [PubMed: 17925489]
61. Stegmaier K, Corsello SM, Ross KN, et al. Gefitinib induces myeloid differentiation of acute myeloid leukemia. *Blood* 2005;106:2841–8. [PubMed: 15998836]

62. Kroeger H, Jelinek J, Estecio MRH, et al. Aberrant CpG island methylation in acute myeloid leukemia is accentuated at relapse. *Blood* 2008;112:1366–73. [PubMed: 18523155]
63. Karp JE, Smith BD, Gojo I, et al. Phase II trial of the oral farnesyltransferase inhibitor tipifarnib (R115777, Zarnestra) as maintenance therapy in first complete remission in adults with acute myelogenous leukemia and poor risk features. *Clin Cancer Res* 2008;14:3077–82. [PubMed: 18483374]

Table 1**Issues Surrounding Clinical Endpoints and Drug Development**

- How do we define the target population?
 - Need to understand biology of host and biology of treatment
 - Do we need randomized trials?
 - What is the proper comparator arm in the absence of an adequate “standard of care”?
 - What clinical endpoints are meaningful?
 - Which endpoints should be used for patients with Incurable acute myeloid leukemia (eg, older adults): complete response, overall survival, quality of life?
 - How do we discriminate toxicity of the treatment regimen from toxicity of the underlying disease, especially given inherent host and disease heterogeneity?
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Table 2**Traditional Clinical Endpoints to Assess Drug Effect**

- Survival (overall, event-free, disease-free)
 - Tumor-defined responses (complete or partial remission, hematologic improvement)
 - Improved end-organ function
 - Tumor clearance (marrow, blood, extramedullary sites)
 - Increased production of differentiated lineages
 - Decreased transfusion needs
 - Improvement in clinical symptoms (eg, fever, bone pain)
 - Quality of life
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Table 3**Requirements for Biomarkers to Serve as Clinically Useful Surrogate Endpoints of Drug Effect**

- Ability to understand how the agent(s) under study modulate the net expression and activity of specific molecular targets
 - Changes in biomarker activity represent the net effects of study agent(s) on the putative targeted pathways
 - Molecular changes in biomarker activity exhibit a dose-response relationship in target tissue
 - Ability to measure the molecular effect of study agents directly in tumor or a representative tissue with similar biologic properties in “real time” during and after drug exposure
 - Ability to correlate the presence and magnitude of biomarker modulation with the observed clinical response
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