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Is the focus moving toward a combination of targeted drugs?

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Summary

The concept of combining targeted agents for the treatment of acute myeloid leukemia (AML) is a relatively new but potentially promising area of investigation. A number of targeted agents may have limited single-agent activity but could show significant promise when used in conjunction with other types of similar compounds. Combinations of targeted agents may effectively interrupt multiple pathways in either a linear or parallel fashion. There are currently numerous combination regimens under investigation at either the preclinical or clinical levels, including histone deacetylase (HDAC) and CDK inhibitors; HDAC and proteasome inhibitors; HDAC and NF- κ B (IKK β) inhibitors; CHK1 and MEK1/2 inhibitors; and BCL-2 antagonists and CDK inhibitors. Although combinations of targeted agents will not displace conventional cytotoxic regimens in AML or related disorders in the foreseeable future, these combinations clearly warrant further attention.

Keywords

targeted agents; histone deacetylase inhibitors; HDAC; NF- κ B inhibitors; CDK inhibitors; proteasome inhibitors; MEK inhibitors; BCL-2 inhibitors

INTRODUCTION

The introduction of the prototypical targeted agent imatinib mesylate (Gleevec) for the treatment of chronic myelogenous leukemia (CML) has given hope that similar approaches may be effective in the case of acute myelogenous leukemia (AML). One novel approach to the treatment of AML involves the concept of combining targeted agents, although it is only one of a number of options currently being explored. For example, the concept of combining targeted agents with more standard chemotherapy for AML is currently the focus of intense interest. Nevertheless, the introduction of targeted agents provides many new therapeutic options in this disease and has ushered in an exciting era in leukemia therapy.

The term “targeted agent” could be considered a misnomer because such agents often have multiple targets, some of which may be as or more important than the one originally intended. In fact, such agents rarely, if ever, affect only the primary target for which they were designed. There are several categories of targeted agents, and the use of each of these

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has distinct but interrelated goals, including induction of differentiation, dysregulation of the cell cycle, modulation of apoptosis, and inhibition of signal transduction pathways (Figure 1). This multiplicity of categories results in a large number of possible combinations, many of which have a rational molecular basis.

DUAL INHIBITION

The two-hit theory of leukemogenesis, as described by Gilliland,¹ provides a theoretical basis for combining targeted agents for AML therapy. According to this concept, leukemogenesis represents a cooperative process involving mutations of two types of proteins ie, Class I proteins (eg, core-binding proteins), disruption of which leads to interference with the normal differentiation process, and Class II proteins (eg, FLT3), disruption of which results in enhanced cell survival. In a limited number of cases, inhibiting a single pathway may occasionally be sufficient, as in the case of CML, in which cells that are addicted to the BCR-ABL pathway are exquisitely sensitive to imatinib mesylate, which blocks the actions of the *BCR-ABL* oncogene. However, in most cases of AML and many other cancers, redundant, complementary pathways exist that are able to circumvent the addiction phenomenon. For example, a recent study by Stommel et al² demonstrated that interrupting a single pathway was insufficient to induce cell death in a lung cancer model; instead, multiple pathways had to be inhibited to achieve this goal as a consequence of pathway redundancy and overlapping functions.

Tumor cells may not be susceptible to single inhibitors for other reasons, including pharmacodynamic or pharmacokinetic factors. In addition, the development (or pre-existence) of mutant proteins can render the inhibitor inactive due to diminished binding. In addition, constitutive activation of alternative survival pathways can render activation of the first pathway superfluous. Alternatively, inactivation of a critical survival pathway can result in the compensatory activation of a compensatory rescue pathway. A corollary of these concepts is that disruption of the second pathway, whether induced and/or constitutively activated, can render inhibition of the first pathway significantly more lethal, restoring the addiction phenomenon.

COMBINATION APPROACHES IN AML

Histone deacetylase inhibitors

From a theoretical standpoint, combination of multiple agents can potentially address the problems of oncogenic transcription factors or repressors, which induce differentiation block (Class I mutations), and constitutively active tyrosine kinases, which promote survival (Class II lesions). Moreover, certain targeted agents, such as histone deacetylase (HDAC) inhibitors, can simultaneously address both the differentiation block and enhanced survival characteristic of leukemia cells. This may reflect the ability of HDAC inhibitors to act as protein, rather than as pure histone acetylases, and thus disrupt the function of multiple proteins implicated in transformed cell survival. For example, in the case of AML, HDAC inhibitors may interact with and disrupt the function of corepressor proteins while at the same time interfering with leukemogenic tyrosine kinases by acetylating heat shock proteins (eg, Hsp90) and inducing the degradation of their client proteins.³ These actions may cooperate with HDAC inhibitor-mediated acetylation of DNA histone tails, resulting in a more open chromatin structure and the reexpression of genes encoding cell death and differentiation.⁴

HDAC inhibitors exert pleiotropic effects and may therefore kill tumor cells through multiple mechanisms. For example, as noted above, HDAC inhibitors may act through direct epigenetic mechanisms, rendering the structure of chromatin more open. This may lead to

repression of genes required for survival, or, alternatively, the induction of genes that promote cell death or differentiation. The capacity of HDAC inhibitors to disrupt the function of co-repressor proteins may also contribute to antileukemic activity. However, HDAC inhibitors may also act through indirect or nonepigenetic mechanisms.⁵ For example, HDAC inhibitors acetylate a wide variety of proteins, including HSP, DNA repair proteins (eg, Ku70), as well as multiple transcription factors (eg, NF- κ B). Modification of transcription factor activity may in fact cooperate with the more direct actions of HDAC inhibitors (eg, induction of an open chromatin structure; disruption of corepressor function) to promote the expression of genes responsible for cell death or differentiation.

Multiple determinants of HDAC-inhibitor-mediated lethality in leukemia and other transformed cells have been identified (Table 1).⁶ Given their pleiotropic mechanisms of action, HDAC inhibitors represent a prototype of a targeted agent that might rationally be combined with other agents for AML therapy.

HDAC inhibitors and hypomethylating agents—Clinical trials have combined the HDAC inhibitor valproic acid with hypomethylating agents, including 5-azacytidine and all-trans retinoic acid (ATRA),⁷ or decitabine.⁸ The concept underlying this approach is that interference with DNA methylation (ie, by hypomethylating agents) may cooperate with HDAC inhibitor-mediated disruption of co-repressors to reverse silencing of genes implicated in leukemic cell growth arrest, maturation induction, or cell death. While early results with HDAC inhibitors and hypomethylating agents appear promising, other combination strategies have been implemented that appear somewhat less intuitive, but nevertheless have a rational basis. These include combinations of HDAC inhibitors with CDK inhibitors, proteasome inhibitors, and NF- κ B inhibitors. Such newer combination approaches may represent a prototype for future approaches to combination therapy of leukemia.

HDAC inhibitors and CDK inhibitors—Flavopiridol, one of the first CDK inhibitors to enter the clinical arena, is an inhibitor of multiple cyclin-dependent kinases and a transcriptional repressor. More specifically, flavopiridol acts as a potent inhibitor of the CDK9/cyclin T complex (PTEF-b), disrupting the function of several other short-lived proteins, which are dependent upon continued transcription. Flavopiridol also inhibits phosphorylation of the C-terminal repeat domain of RNA polymerase II and disrupts initiation and elongation. Since cell cycle dysregulation is one of the cardinal features in leukemia cells, the use of CDK-inhibitors in leukemia is logical. In chronic lymphocytic leukemia (CLL), flavopiridol has shown promising results when administered according to a new infusional schedule, and attempts to combine this agent with conventional chemotherapeutic agents are underway.⁹

HDAC inhibitors acetylate and induce the promoter region of the p21 gene, which plays a critical role in HDAC inhibitor-mediated growth arrest in leukemic cells. Notably, leukemic cells that lack p21 appear to be extremely sensitive to the lethal effects of HDAC inhibitors, presumably because they lack the ability to undergo orderly cell cycle arrest and maturation in response to such agents. Instead, such cells, when exposed to HDAC inhibitors, display a marked increase in apoptosis and diminished differentiation. Interestingly, when leukemic and other malignant cells are exposed to a regimen combining flavopiridol and HDAC inhibitors, flavopiridol blocks the induction of p21 through a transcriptional mechanism (eg, by inhibition of the pTEF-b complex), thereby recapitulating the p21-null phenotype and leading to a dramatic induction of apoptosis. For example, when primary leukemic blasts were exposed to a combination of low, minimally toxic doses of flavopiridol and the HDAC inhibitor SAHA (suberoylanilide hydroxamic acid, vorinostat), there was clear apoptosis after 24 hours and necrosis after 48 hours.¹⁰ Such preclinical findings have led to the

initiation of a multi-institutional phase 1 trial (CTEP #6637)¹¹ combining vorinostat with flavopiridol in patients with refractory leukemia. A second phase of this trial, which incorporates a new hybrid infusional flavopiridol regimen shown to be effective in CLL¹² is ongoing. In this trial, patients with refractory AML/myelodysplastic syndrome (MDS) receive a loading dose and a 4-hour infusion of flavopiridol on days 1 and 8 in conjunction with vorinostat 200 mg orally three times a day for 14 days. Results of this trial are very preliminary, but further evaluation of this strategy is clearly warranted.

HDAC inhibitors and NF- κ B inhibitors—Leukemia cells, in general, and leukemic stem cells, in particular, are dependent on the NF- κ B pathway for their survival, and NF- κ B is a major determinant of HDAC inhibitor sensitivity.^{13,14} NF- κ B inhibitors, such as Bay 11-7082 or SN-50, have been shown to increase the lethality of several second generation HDAC inhibitors (eg, vorinostat, MS-275) by markedly increasing mitochondrial dysfunction.¹⁵ This phenomenon reflects the ability of HDAC inhibitors to acetylate NF- κ B (RelA), resulting in enhanced DNA binding and activation of cytoprotective NF- κ B-dependent genes, such as those encoding antioxidant (eg, *MnSOD*) and survival proteins (eg, BCL-xL). NF- κ B inhibitors block the proteasomal degradation of I κ B α , which traps NF- κ B in the cytoplasm, preventing it from translocating to the nucleus and inducing these cytoprotective responses. The net effect is a pronounced increase in HDAC inhibitor-mediated lethality.

HDAC inhibitors and proteasome inhibitors—Proteasome inhibitors, such as bortezomib, are believed to act, in part, by sparing I κ B- α from degradation, disrupting the NF- κ B pathway.¹⁶ There are a number of studies, including those involving leukemia cells, demonstrating synergism between proteasome inhibitors and HDAC inhibitors.^{17–23} HDAC inhibitors can also interact with proteasome inhibitors through several other mechanisms besides the NF- κ B pathway, including disruption of aggresome formation and induction of endoplasmic reticulum stress.²⁴ Trials combining HDAC inhibitors and proteasome inhibitors in AML are currently being planned.

HDAC inhibitors and IKK inhibitors—Recently, a mouse model has been developed that demonstrated that the MLL fusion gene was able to generate myeloid or lymphoid acute leukemias.²⁵ Interestingly, the resulting human leukemia stem cell model (MLL-ENL) recapitulated many of the features characteristically associated with leukemia stem cells. Preliminary results suggest that MLL cells are very sensitive to the combination of HDAC inhibitors and IKK β inhibitors. (Dai and Grant, unpublished results). IKK β inhibitors are currently being explored as anti-inflammatory agents, and they may represent logical candidates for combination with clinically relevant HDAC inhibitors.

Parallel inhibition

There are several different approaches to the strategy of simultaneously inhibiting signaling and cell cycle regulatory pathways. For example, a pathway can be inhibited in a linear fashion, ie, at upstream and downstream sites. An alternative approach, referred to as parallel inhibition, involves inhibition of two pathways that interact. For example, UCN-01, a multifunctional kinase inhibitor, which was originally developed as a PKC inhibitor, was subsequently found to be a CHK1 inhibitor. CHK1 is intimately involved in DNA damage responses and induces G2/M arrest. The Ras/Raf/MEK/ERK cascade is another pathway intimately involved in leukemogenesis. UCN-01 has been shown to induce the MEK/ERK pathway in human leukemia cells, a phenomenon that can be effectively blocked by coadministering a MEK inhibitor, such as PD184352.²⁶ Notably, the combination of a MEK1/2 inhibitor and UCN-01 resulted in a dramatic induction of apoptosis in human leukemia cells as well as primary AML cells. Thus, induction of the Ras/Raf/MEK/ERK

pathway may represent a compensatory response to disruption of this checkpoint. Based on that concept, it is logical to explore the use of a combination of inhibitors of CHK1 and Ras/Raf/MEK/ERK, particularly in those leukemias that are Ras-dependent.

Modulators of the apoptotic regulatory apparatus

There are currently a number of modulators of the apoptotic regulatory apparatus in development or in clinical trials. One such agent is ABT-737, a small-molecule inhibitor of BCL-2 and BCL-xL. ABT-737 has shown very impressive activity in in vitro and in vivo models²⁷ and appears to be selective for transformed cells. It has also been shown that the sensitivity of leukemia and other transformed cells to ABT-737 is inversely related to cellular levels of MCL-1.^{28–31}

CDK inhibitors, such as flavopiridol and roscovitine, inhibit the transcriptional apparatus and downregulate short-lived proteins, such as p21 and MCL-1. Interventions that downregulate MCL-1 dramatically increase the sensitivity of a variety of malignant cells, including human leukemia cells, to ABT-737.³² Such a combination may be viewed as recapitulating the normal physiologic death process. For example, certain physiologic death stimuli (eg, growth factor deprivation) act by inducing the pro-apoptotic BCL-2 family member NOXA, which degrades and interferes with the function of MCL-1. Alternatively, such stimuli may result in activation of Bad, a pro-apoptotic molecule that disrupts the function of BCL-xL, Bak, and BCL-2. Thus, ABT-737, by disrupting the function of BCL-xL, in effect, mimics the actions of Bad, whereas CDK inhibitors, by downregulating MCL-1, mimic the functions of NOXA. The concept of simultaneously disrupting BCL-2/BCL-xL function (ie, by BCL-2 antagonists such as ABT-737) in combination with MCL-1 downregulation (ie, by agents like CDK inhibitors capable of transcriptionally repressing MCL-1 expression) represents another logical approach for the treatment of leukemias, particularly those associated with high levels of anti-apoptotic proteins such as BCL-2.

Targeted agents with conventional cytotoxic therapy

It is tempting to speculate that interrupting survival pathways will lower the threshold for conventional chemotherapy-induced death, but results with this approach so far have been disappointing. This may reflect the fact that the mechanisms underlying synergism are not completely understood. However, some newer approaches appear to dramatically enhance the lethality of certain cytotoxic agents. For example, interventions that disrupt the DNA repair process may hold promise in enhancing the activity of certain DNA-damaging agents.^{33,34} In this context, it is noteworthy that HDAC inhibitors may act in part by disrupting the function of DNA repair proteins (eg, Ku70). Interfering with the NF-κB pathway represents another promising strategy to enhance the activity of established cytotoxic agents, particularly in view of its key role as a survival signaling pathway in leukemia stem cells. In addition, interference with the NF-κB pathway also perturbs the oxidative milieu, and can enhance DNA damage.^{35,36} Further studies pursuing inhibitors of pathways that might be involved mechanistically in responses to cytotoxic agents are clearly warranted.

CONCLUSIONS

Combinations of targeted agents will clearly not supplant conventional cytotoxic regimens in the near future, as conventional cytotoxic regimens currently represent the mainstays of acute leukemia treatment and have provided significant benefit for patients with this disease. However, as newer targeted agents enter the clinical arena, combinations of such agents clearly warrant further attention. An important challenge stems from the fact that, there are a virtually unlimited number of rational, mechanism-based synergistic combinations of

targeted agents to choose from. The burden now is to select criteria for moving the most promising combinations forward. For example, should the focus be placed on agents specifically directed against the pathogenic lesions in specific subtypes of AML or regimens directed against leukemia stem cells? A clearer understanding of the molecular pathogenesis of leukemia will clearly be needed to help determine which combinations will be the most effective.

In the future, it is likely regimens will involve incorporation of agents targeted against leukemia-specific mutations or fusion proteins, such as FLT3 antagonists in the case of FLT3-positive leukemias, for more individualized therapy. Further work will be needed to determine which complementary pathways confer resistance to such targeted agents and how the pathway addiction phenomenon will influence targeted strategies in leukemia. It also remains to be determined whether pathways that are not specifically implicated in leukemogenesis should be targeted. For example, it is appealing to inhibit a pathway known to be critically involved in leukemogenesis, but it is possible that the disruption of other pathways that are not clearly involved in the pathogenesis of the disease may still be effective, particularly if the initial pathogenic pathway is interrupted. Another potentially useful strategy may involve interrupting multiple interacting pathways involving a combination of three or more targeted agents. A summary of several classes of targeted agents and the potential for synergistic interactions in AML is presented in Figure 1.

Finally, as information emerges concerning the leukemic stem cell, the concept of targeting such stem cells also represents an interesting therapeutic possibility. For example, if two regimens are equally effective against the bulk population of leukemic cells, but one of the regimens is particularly effective against the leukemia stem cell population, (ie, an NF- κ B inhibitory regimen), it could be argued that the regimen that is more effective against the stem cell population may be the more logical one to pursue. Ultimately, in vivo studies, particularly those employing genetically modified animals, may prove valuable in determining the optimal approach.

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Figure 1. Categories of targeted agents used in AML

A general classification of targeted agents with possible utility in AML and their potential for combination therapy in this disease. Epigenetically acting agents include HDAC inhibitors and inhibitors of DNA methyltransferases. Cell cycle inhibitors include CDK inhibitors, such as flavopiridol, and more recently, inhibitors of CHK1 (eg, UCN-01) and aurora kinases. Small molecule inhibitors of survival signaling pathways include tyrosine kinase inhibitors, such as the FLT3 inhibitor PKC412, and serine threonine kinase inhibitors, such as the MEK1/2/ERK1/2 inhibitor AZD6244. Modulators of apoptosis include small molecule BCL-2 antagonists (ABT-737), TRAIL, and XIAP antagonists. Finally, agents which interfere with protein disposition include Hsp90 antagonists and proteasome inhibitors, such as bortezomib. In addition to combination of these agents with established chemotherapeutic drugs, numerous combinations of targeted agents are under investigation at both the preclinical and clinical levels in AML, including HDAC inhibitors and DNA methyltransferase inhibitors, HDAC inhibitors and CDK inhibitors (flavopiridol), proteasome inhibitors and HDAC inhibitors, HSP90 antagonists and tyrosine kinase inhibitors, and MEK1/2 and CHK1 inhibitors, among numerous others.

Table 1

The determinants of HDAC inhibitor-mediated lethality

Action	Effects
Generates	Reactive oxygen species (ROS); ceramide
Activates	Bid; stress-related kinase (JNK); NF- κ B
Downregulates	Antiapoptotic genes (BCL-xl, XIAP)
Upregulates	Proapoptotic genes (Bax, Bak, Bim)
Induces	Death receptors (DR4, DR5); Fas; TRAIL; p21 ^{CIP1}
Inhibits	Proteasomes
Disrupts	HSP90 function
Acetylates	Ku70 (releases Bax); NF- κ B
Disrupts	G2 and mitotic checkpoints
Inactivates	Cytoprotective pathways (Raf/Ras/MEK/ERK, Akt, BCR/ABL)