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Is Targeted Therapy Feasible in Acute Myelogenous Leukemia?

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Abstract

The prognosis for patients with acute myeloid leukemia (AML) is determined to a large degree by the biology of the leukemic cell. In recent years, the identification and characterization of genetic aberrations has vastly improved our understanding of the pathogenesis of AML. In contrast, however, there has been a lack of clinically meaningful therapeutic advances. The same chemotherapeutic strategies have been applied to AML for several decades now, and while these regimens are effective in inducing remission, most patients relapse within months after initial treatment. Hence, there is an urgent need for novel therapies. We review herein a number of lines of laboratory and clinical trial data supporting the clinical value of targeted treatment approaches that will likely result in improved outcomes for patients with AML.

Keywords

Acute myelogenous leukemia; Pathogenesis; Chemotherapeutic strategies; Relapse; Molecular markers

Introduction

Acute myeloid leukemia (AML) is a polyclonal neoplastic disease of the myeloid blood cell lineage characterized by the accumulation of somatic genetic aberrations. In past years, contemporary molecular and mutational analyses have facilitated the identification of genetic alterations relevant to the tumor and have allowed for the stratification of patient populations into different risk groups, guiding prognosis and treatment. In response to the expanded base of knowledge regarding the disease, the World Health Organization (WHO) recently revised its classification of myeloid neoplasms and acute leukemias by incorporating mutations in NPM1 and CEBPA as provisional entities in cytogenetically normal AML. The WHO did not include mutations in FLT3 as an entity but strongly recommended to assess for FLT3 mutations in all cases of cytogenetically normal AML [1]. The important role that genetic aberrations play in risk stratification and therapeutic-decision making was recently highlighted by Patel and colleagues, who proposed a revised risk stratification schema by integrating mutational profiling with cytogenetic data. This model

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was able to predict outcome independent of “traditional” markers such as age, white blood cell count, induction dose, and transplantation status [2]. Further, Grossmann et al. reported a novel prognostic classification based solely on molecular markers and which provides a more powerful model for prognostication than cytogenetics [3].

More than a decade ago, a “two-hit model” of leukemogenesis was proposed [4] which postulated that AML was associated with two general categories of mutations: Class I mutations, which primarily affect signal-transduction molecules, resulting in inhibition of apoptosis and induction of proliferation; and Class II mutations, which predominantly act as inhibitors of myeloid differentiation. This two-hit hypothesis was derived primarily from murine models of AML. In mice expressing transgenes or transplanted with marrow cells transduced with oncogenes, the so-called Class I mutations, such as a FLT3-activating mutation, induced a myeloproliferative disorder but did not result in AML unless a Class II mutation (e.g., PML-RARa) was also introduced. While this model provided a useful framework, it appears to have been overly simplistic, particularly in light of the veritable explosion of genomic information that has recently emerged. Mutations in epigenetic regulator genes (e.g., DNMT3, ASXL1) and tumor suppressor genes (e.g., TP53, WT1), for example, cannot clearly be relegated to any mutational class, and cases of AML can readily be found that lack any Class I mutation [5].

The increased knowledge of tumor biology in AML is contrasted by the lack of new anti-leukemia therapies. Two therapeutic modalities – cytarabine-based chemotherapy and allogeneic transplant – have remained central to the treatment of AML for the past 30 years. Although overall survival for patients under age 60 has risen steadily during this time, this trend is likely primarily attributable to better supportive care and risk stratification. The most commonly administered course of induction chemotherapy in all age groups has been some variation on the “7+3” regimen, which entails a seven-day course of cytarabine as continuous infusion, with the addition of an anthracycline for three of the seven days [6]. Common risks of AML induction therapy include potentially lethal infectious complications during neutropenia and anthracycline-induced cardiotoxicity. While the majority of patients achieve a CR upon intensive induction therapy, virtually all patients relapse without any further treatment, usually within a matter of a few months [7]. Therefore, the administration of a consolidation regimen to optimize therapeutic success after induction is imperative.

High-dose cytarabine is generally considered standard of care for younger patients with favorable-risk AML. While the estimated five-year overall survival rate for all AML patients is approximately 15 %, younger patients (aged 25 to 39 years) fare much better than their older counterparts (aged 60 and above), with five-year overall survival rates of 49 % versus 13 %, respectively [8]. The generally dismal prognosis of older patients is due in part to their inability to tolerate the intensive induction and consolidation regimens used in younger patients, as well as the higher incidence of unfavorable genetic alterations [9, 10]. Almost half of all patients belong to the older subgroup, thus making AML primarily a disease of the elderly, with average onset at approximately 65 years. In light of the high rates of treatment-related toxicity and multidrug-resistant phenotypes in elderly patients, optimized therapeutic concepts are urgently needed.

The enormous molecular heterogeneity and complex clonal architecture of AML has hindered the development of broadly applicable targeted therapies. The polyclonal character of AML is represented by founding clones and subclones harboring disease-initiating and cooperating mutations. In a recent study, candidates for disease initiators (as mutations in these genes were almost exclusively detected in founding clones) included *RUNX1*, *NPM1*, *U2AF1*, *DNMT3A*, *IDH1/2*, and *KIT*. Mutations in *NRAS*, *KRAS*, *TET2*, *CEBPA*, *WT1*, *PTPN11*, and *FLT3* were more commonly detected in subclones, indicating a cooperative role [5, 11].

The concept of targeting disease-initiating and cooperating mutations suggests the potential of eliminating founding clones and subclones, thereby achieving cure or, at least, clinically meaningful disease control. However, of the many known mutations, only a few are directly linked to promoting cell survival and therefore represent suitable targets. Hence, other concepts of targeted therapy have been competitively evaluated, including the identification of surface molecules expressed by AML cells, with the goal of selectively targeting leukemic cells for destruction (such as CD33 and CXCR4) and aberrant methylation.

FLT3

Mutations in the FLT3 receptor are relatively common and can be found in all age groups. Whereas point mutations in the activation loop of the kinase domain (FLT3/TKD) do not appear to have a significant prognostic impact [12], prognosis is dismal for patients harboring an internal tandem duplication (FLT3/ITD) [13, 14]. Recent data suggest up to a 35 % incidence of FLT3/ITD mutations in patients between 20 and 59 years [15].

Internal tandem duplications within the coding sequence of the juxtamembrane domain of FLT3 lead to the constitutive activation of its receptor-tyrosine kinase (RTK) function and its downstream signaling pathways, including RAS/RAF/ MEK/ERK kinases, STAT5, and PI3-kinase. Consequently, cellular proliferation is promoted, which confers a growth advantage to leukemic stem and progenitor cells. From a clinical perspective, this frequently translates into a higher percentage of blood and bone marrow blasts and a worse prognosis due to high relapse rate and adverse overall survival. Since RTK mutations have been recognized as playing an important role in the pathogenesis of AML and appear to be relatively common [16], they represent a valuable target for molecularly tailored drug therapy. In recent years, several FLT3 kinase inhibitors have been developed and tested as either single agents or in combination with conventional chemotherapy in clinical trials.

Initial phase I and II studies have revealed promising results for the second-generation TKI quizartinib (formerly known as AC220) as single agent in patients with relapsed or refractory AML, particularly those harboring a FLT3/ITD mutation. In a first-in-human study of 76 patients with either relapsed or refractory AML, 56 % (10/18) of patients with a FLT3/ITD mutation showed a clinical response, whereas patients who were found to carry a FLT3/TKD mutation (3/3) did not respond to quizartinib. The median duration of response and median OS were 13.3 and 14 weeks, respectively. Correlative studies, including the plasma inhibitory assay, demonstrated potent FLT3 inhibition at low dose levels. Quizartinib

was generally well-tolerated, and observed side effects consisted primarily of gastrointestinal symptoms and QTc prolongations [17].

More recently, data from a phase II study to assess the efficacy of single-agent quizartinib in FLT3/ITD-positive and FLT3/ITD-negative patients provided further evidence for a high degree of activity of quizartinib monotherapy in this setting. Of note, the study consisted of two cohorts. Cohort I included 134 elderly AML patients (≥ 60 years) who relapsed within less than a year or who were refractory to first-line chemotherapy; and cohort II (n=137) consisted of a younger patient population ((≥ 18 years) who relapsed or were refractory to one salvage regimen, including HSCT. While there were a few exceptions in each cohort, the majority of the patients in this study carried a FLT3/ITD mutation. The efficacy of single-agent quizartinib was encouraging in both cohorts, with a composite CR rate of 46–54 % in relapsed/ refractory FLT3/ITD-positive AML. The highest remission rates were observed in patients who had relapsed after HSCT. A number of patients who were refractory to prior treatment responded to quizartinib and were successfully bridged to potentially curative HSCT. The study confirmed the acceptable safety profile of quizartinib previously seen in other clinical trials [18, 19].

Recent data have demonstrated that potent and selective inhibition of FLT3 by quizartinib leads to rapid clearance of peripheral blasts by induction of apoptosis in most cases of FLT3/ITD-mutated AML. However, differences were observed when assessing the effects of selective FLT3 inhibition in the bone marrow compartment. Rather than inducing apoptosis of marrow blasts, with resultant aplasia, quizartinib was shown to induce terminal differentiation. For example, in 13 out of 14 patients with FLT3/ITD-mutated AML, quizartinib induced terminal differentiation of marrow blasts during the first four weeks of treatment, which was followed by a surge of leukemia-derived neutrophils in the peripheral blood during the next four weeks. While these neutrophils were morphologically normal in appearance and lacked FLT3 protein expression, they still carried the FLT3/ITD mutation, which indicated that they were derived from the malignant clone. Consistent with a differentiation process, flow cytometric analysis demonstrated a loss of expression of CD34 and CD117, along with a gain in expression of CD15 [20•]. Clinically, the surge of neutrophils into the peripheral blood was often accompanied by a steroid-responsive differentiation syndrome similar to the differentiation syndrome observed in patients with acute promyelocytic leukemia (APL) treated with all-trans-retinoic acid (ATRA). These observations were further corroborated in a report of three patients who developed characteristic skin nodules after initiation of a FLT3 inhibitor for FLT3/ITD-mutated AML. Additional evaluation of these skin lesions revealed deep dermal and subcutaneous neutrophilic infiltrates that were uniformly positive for the FLT3/ITD mutation on PCR analysis [21].

A number of other FLT3 inhibitors are under active development (Table 1). Sorafenib is a multi-targeted kinase inhibitor with activity against the FLT3/ITD receptor [22, 23•]. A retrospective analysis of 65 FLT3-ITD AML patients who received sorafenib monotherapy for relapsed or refractory disease after either chemotherapy or allogeneic HSCT reported hematological remission, bone marrow remission, complete remission (with and without normalization of peripheral blood counts), and molecular remission, with undetectable

FLT3-ITD mRNA in 37 %, 8 %, 23 %, and 15 % of patients, respectively [24]. In a phase I dose-escalation study in patients with advanced MDS or relapsed/refractory AML, sorafenib induced complete remission, with or without incomplete platelet recovery, in 10 % of patients, all of whom were positive for the FLT3/ITD mutation. Furthermore, a significant reduction in bone marrow and/or peripheral blood leukemic blasts was observed in an additional 34 % of patients [23•]. Taken together, these findings indicated that sorafenib monotherapy may have significant activity in FLT3-ITD AML and may induce durable remission. Combined with intensive chemotherapy, sorafenib induced high CR rates in both FLT3-mutant (93 %) and WT AML (66 %) patients younger than 65 years. In this combined phase I/II study, the one-year survival rate was 74 %. Potent inhibition of the FLT3 kinase activity was demonstrated by plasma inhibitory assays [25].

Midostaurin, formerly known as PKC412, is a potential inhibitor of wild-type and mutated FLT3 as well as several other molecular targets, including VEGFR2, KIT, and PDGFR [26, 27]. In a phase Ib trial of midostaurin combined with intensive chemotherapy in newly diagnosed AML patients, Stone et al. reported a CR rate of 80 % and an OS probability similar to the FLT3 wild-type (WT) population [28•]. The Cancer and Leukemia Group B (CALGB) is currently conducting a large international placebo-controlled phase III trial of midostaurin combined with induction and consolidation chemotherapy, followed by one year of midostaurin maintenance, versus chemotherapy alone in adult (aged 18 to 59 years) treatment-naïve FLT3-mutant AML patients (RATIFY trial, ClinicalTrials.gov, identifier: NCT00651261). Primary and secondary endpoints include overall survival, and EFS and CR, respectively. The RATIFY trial has completed enrollment, and final analysis is pending.

CD33

CD33 is a glycosylated transmembranous protein belonging to the family of “sialic acid-binding Ig-related lectins” (siglecs, siglec-3), and plays a role in mediating cell adhesion and interaction. The expression of CD33 appears to be restricted to myeloid lineage, with high levels on myeloid precursors in the bone marrow and circulating monocytes, and low levels on peripheral granulocytes [29]. Leukemic blasts derived from AML patients express the CD33 antigen in approximately 85–90 % of cases. Moreover, among different myeloid neoplasms, AML demonstrates the highest density of CD33 surface molecules, with a mean of 10,380 molecules per cell in the bone marrow compartment and 9,175 molecules per cell in the peripheral blood [30]. CD33 therefore represents a clinically valuable target antigen for AML therapy.

Gemtuzumab ozogamicin (GO), a conjugate of a recombinant humanized CD33 antibody and the antitumor antibiotic calicheamicin, is one of various antibody-cytotoxic agent complexes initially designed to selectively target CD33-positive leukemic cells for destruction. GO was given accelerated approval in 2001 [31], and despite considerable toxicities that eventually led to the voluntary withdrawal from the U.S. market in 2010, it demonstrated promising efficacy in single-agent and combination clinical trials [32]. Toxicities comprised primarily liver toxicity, with a subset of patients developing sinusoidal obstruction syndrome, particularly when the approved dose of 9 mg/m^2 was administered with chemotherapy [33]. In an attempt to improve therapeutic outcome, the United Kingdom

Medical Research Council conducted a clinical trial (MRC AML15) in which 1,113 patients aged less than 60 years with de novo AML (excluding APL) were randomized to receive induction chemotherapy, with or without GO, at a reduced dose of 3 mg/m^2. In remission, 948 patients were randomly assigned to receive consolidation chemotherapy alone or combined with GO. The investigators found the addition of GO to either induction or consolidation therapy to be safe and not associated with any difference in response or survival. However, predefined analysis based on cytogenetics demonstrated a significant benefit of GO in patients with favorable-risk disease (GO, 79 % OS vs. control, 51 % OS) and a non-significant trend for benefit in intermediate-risk patients [34].

A second large randomized trial (MRC AML16) randomized 1,115 elderly patients with untreated AML or high-risk myelodysplastic syndrome to induction chemotherapy with or without GO (3 mg/m^2). The authors of this trial reported that the addition of GO had no impact on remission rate, 30- or 60-day mortality, or toxicity. However, the 3-year cumulative incidence of relapse (68 % vs. 76 %; hazard ratio [HR] 0.78; 95 % CI, 0.66–0.93; p=.007) and 3-year survival (25 % vs. 20 %; HR 0.87; 95 % CI, 0.76–1.00; p=.05) were both significantly improved with GO [35]. In another trial, low-dose ara-C (LDAC) was administered with or without GO (5 mg) in 495 elderly AML patients. The authors reported that the addition of GO was associated with a significant improvement in CR rates (30 % [LDAC+GO] vs. 17 % [LDAC]) but that 12-month OS was not improved [36]. An individual patient data (IPD) meta-analysis of five randomized trials (MRC AML15/16 [34, 35], SWOG-0106 [37], ALFA-0701 [38], and GOELAMS and AML2006IR *[unpublished]*), including 3,339 AML patients aged 0–84 years showed that the survival benefit associated with administration of GO with the first course of induction chemotherapy outweighed the potential increase in early mortality. However, the survival benefit appeared to be restricted to patients with favorable- or intermediate-risk cytogenetics. The evidence, in aggregate, led the authors to conclude that the addition of GO to induction chemotherapy is beneficial in terms of improving survival, irrespective of age or FLT3/ITD mutation status, thus supporting a role for GO combined with chemotherapy for the upfront treatment of AML [39].

Another approach for targeting the CD33 antigen on leukemic blasts that has shown promising activity in preclinical studies involves bispecific T-cell engagers (BiTEs), composed of the two binding domains of two different human IgG antibodies that are interconnected by a short peptide linker, which display a unique structure within the group of bispecific antibodies. BiTEs were specifically designed to bind to a surface target antigen on cancer cells and to CD3 on T cells, thus bringing both cells to close proximity and eventually result in in T – cell activation and lysis of the attached tumor cell by cytotoxic granule fusion, cytokine release, and membrane perforation [40]. AMG330, a novel T cell-engaging BiTE antibody construct targeting CD3 and CD33, has shown promising in vitro effects in KG-1 and U937 AML cell lines, as well as in primary AML blasts. In mouse models, AMG330 was found to induce infiltration of human T cells into subcutaneous HL60 tumors and to significantly inhibit tumor growth [41]. These findings provide a strong rationale for the further development of CD33-targeted strategies and BiTE concepts in the treatment of AML.

CXCR4

C-X-C chemokine receptor type 4 (CXCR4) is a receptor protein belonging to a family of G protein-coupled cell surface receptors [42]. CXCR4 is expressed by a variety of cell types, including hematopoietic progenitors [43]. The functional importance of CXCR4 and its sole ligand, stromal cell-derived factor 1 (SDF-1), is embedded in the regulation of hematopoiesis, migration, and leukemia-stromal cell interactions [44, 45]. CXCR4 is frequently expressed in leukemic blasts [46], and retrospective analyses revealed that increased expression predicts shorter survival [47]. Analyzing the migration patterns of primary AML cells in vitro, Voermans et al. demonstrated that primary AML cells migrate towards SDF-1, irrespective of the AML subtype [48]. Disrupting the interaction between CXCR4 and SDF-1, thus "mobilizing blasts away from their protective environment," may therefore represent a novel targeted therapeutic strategy to render leukemic blasts more vulnerable to cytotoxic therapy.

Plerixafor (AMD3100) is a bicyclam derivative that reversibly blocks the binding of SDF-1 to its cognate receptor CXCR4 on $CD34^+$ progenitor cells and which was initially developed to mobilize hematopoietic stem cells into the peripheral circulation. Plerixafor exerts synergistic effects with the established mobilizing agent G-CSF [49], which indirectly induces degradation of SDF-1 by bone marrow neutrophil elastase [50]. In a single-arm phase I/II study of 52 patients with relapsed/refractory AML, plerixafor (0.24 mg/kg/d on days 0–5 of chemotherapy) combined with intensive chemotherapy (mitoxantrone, etoposide, cytarabine, MEC) yielded an overall complete remission and complete remission, with incomplete blood count recovery rate ($CR+CR_i$) of 46 % [51•], which compared favorably to previously reported data of MEC alone [52]. Furthermore, correlative studies indicated that the administration of plerixafor resulted in modestly increased mobilization (approximately 2.5-fold) of leukemic blasts into the peripheral blood [51•].

In a phase I/II trial by Roboz et al., 69 elderly patients (median age 73 years) with intermediate- or adverse-risk AML were treated with monthly cycles of decitabine (20 mg/m^2 days 1–10) combined with increasing doses of plerixafor (320–810 μg/kg days 1–5), administered every other cycle. The authors reported an overall response rate of 43 % (36 % CR, 7 % CRi). The median overall survival was significantly higher for responders than for non-responders (18 months vs. 5 months, $p<.0001$). While no difference was detected in overall response rate and overall survival associated with the administration of plerixafor, the authors noted that their study was not powered for such comparisons. Consistent with previously reported data [51•], plerixafor mobilized leukemic progenitor and stem cells which, upon staining for Ki-67, demonstrated increased proliferation. The authors concluded that the combination of plerixafor and decitabine is feasible, displays a favorable toxicity profile, and results in a high response rate in poor-prognosis elderly AML patients [53].

BMS-936564 (MDX1338), a fully humanized monoclonal antibody to CXCR4, has demonstrated potent anti-tumor effects in a variety of hematologic malignancies, including non-Hodgkin lymphoma (NHL), chronic lymphocytic leukemia (CLL), multiple myeloma (MM), and AML. Chien et al. demonstrated that BMS-936564 rapidly induces mobilization of leukemic blasts and stem cells in patients with refractory/ relapsed AML, which

frequently peaks within 2–6 hours of initiation of infusion [54]. Consistent with previously reported data in various AML cell line panels and cytarabine-resistant mouse xenograft models [55], the administration of single-agent BMS-936564 was further associated with induction of apoptosis occurring within 96 hours after drug exposure. BMS-936564 is currently being investigated in phase I trials.

Methylation

Methylation of DNA represents an important mechanism in regulating gene transcription without changing the DNA sequence. Aberrant methylation of DNA loci are commonly observed molecular lesions in cancer cells [56] and may play an important role in leukemogenesis [57]. In hematologic malignancies, several tumor-suppressor-gene promoters have been shown to be silenced by methylation [58–60]. As a result, the use of hypomethylating agents is being comprehensively evaluated for treatment of AML, particularly in older patients who are considered “medically unfit” to tolerate standard treatment regimens.

Decitabine (5-aza-2'-deoxycytidine) is a demethylating agent that acts by incorporating into DNA, thereby leading to inhibition of DNA synthesis, inhibition of proliferation, and induction of apoptotic cell death [61, 62]. There is also evidence that decitabine induces myeloid differentiation [63]. A multicenter randomized open-label phase III trial was conducted to compare decitabine with treatment choice (low-dose cytarabine or supportive care) in patients older than 65 years with newly diagnosed AML. The CR rate for patients treated with decitabine was 17.8 %, versus 7.8 % with supportive care (p=0.001). The median OS was 7.7 months in the decitabine-treated group, versus 5 months in the supportive care arm (p= 0.037) [64]. It was concluded that decitabine has activity in upfront AML therapy as well as an acceptable toxicity profile in elderly AML patients.

Azacitidine (5-azacytidine) is a cytidine analog that exerts cytotoxic effects by incorporating into both RNA and DNA, thereby interfering with nucleic acid and protein synthesis. Similar to decitabine, azacitidine has also been shown to induce myeloid differentiation [65]. Fenaux et al. reported that the administration of azacitidine significantly prolonged OS in a subgroup of high-risk MDS patients with WHO-defined AML [66]. Their phase III clinical trial compared azacitidine to conventional care regimens such as best supportive care only, low-dose cytarabine, and intensive chemotherapy. Of 113 elderly patients with a median age of 70 years, 86 % were considered unable to tolerate intensive chemotherapy. The median OS for the patients who received azacitidine was 24.5 months, as compared to 16 months for patients on conventional care regimens (p=.005). Two-year OS rates were 50 % in the azacitidine and 16 % in the conventional care arms, respectively (p=.001). Furthermore, the administration of azacitidine was associated with fewer total days in the hospital when compared to the patients treated with conventional care approaches (p<.0001).

Lines of evidence suggest that decitabine and azacitidine are generally well-tolerated, with side effects consisting primarily of myelosuppression and manageable gastrointestinal toxicities, including nausea, diarrhea, and constipation. Hypomethylating agents have become attractive compounds in the treatment of AML, either as monotherapy or combined

with other agents, based on their potential clinical benefits and favorable toxicity profile. The exact mode by which hypomethylating agents exert their antileukemic effects is not well understood, indicating that complex yet unidentified mechanisms are involved. When investigating the in vitro effects of decitabine on primary AML samples in a stromal co-culture system, Klco and colleagues found that low-dose decitabine (100 nM) induced global hypomethylation. The observed changes in the methylome, however, did not translate into a predictable pattern of gene expression changes [67]. Investigators of a phase II trial of 16 elderly AML patients treated with low-dose decitabine (20 mg/m^2/day for 10 days) reported that the methylomes of marrow samples on day 25 were significantly hypomethylated when compared to pretreatment baseline. However, there was no correlation between the global methylation level and blast percentage in the bone marrow, suggesting that methylation was related to the activity of decitabine rather than to the decrease in leukemia burden itself, indicating that the level of global methylation alone may not be an accurate predictor of treatment response [68].

In a phase II study by Ravandi et al.in 37 refractory/ relapsed, partially heavily pretreated AML patients (median age 64 years, range 24–87 years), both feasibility and efficacy were favorably reported for azacitidine and sorafenib as combination therapy. In this single-arm study, 93 % of patients harbored a FLT3/ITD mutation. Azacitidine at 75 mg/m^2 intravenously was administered daily for 7 days, and sorafenib at 400 mg orally twice daily continuously. Treatment cycles were repeated in monthly intervals. The investigators observed an overall response rate of 46 %, consisting of CRi, CR, and PR rates of 27 %, 16 %, and 3 %, respectively. CR and CRi were achieved after a median period of two treatment cycles, with median response duration of 2.3 months [69]. Further preclinical and clinical studies are currently underway to explore the synergistic antileukemic potential of combined hypomethylating and FLT-inhibitory treatment strategies [70].

The promising clinical activity of hypomethylating agents has spurred enthusiasm for the development of novel methylome-targeted therapies. To this end, several recurrently mutated genes involved in epigenetic regulation, including DNMT3A [2, 71], TET2 [2, 72, 73], IDH1/2 [2, 74–76], and ASXL1 [2, 77, 78], have been identified as prognostic markers with the potential for therapeutic modulation. While mutations in DNMT3A, TET2, and ASXL have repeatedly been associated with adverse outcomes [71, 72, 77], the reported effects of IDH mutations on outcomes have been conflicting [79]. Apart from the disparity in their prognostic impact, mutations in epigenetic modifiers have consistently been found to increase with age [80]. This is of particular interest, as older AML patients are typically unable to tolerate the intensive induction and consolidation regimens used in younger patients, and are therefore in desperate need of novel therapeutic concepts. Development of epigenetic modulators, however, has been hampered by the technical challenge of designing a drug that specifically hits one target out of a complex network of proteins and enzymes involved in regulating DNA methylation. Although therapeutic modalities specifically tailored to inhibit DNMT3A, ASXL1, or TET2 have not been developed to date, inhibitors of IDH1/2 have demonstrated promising results in preclinical studies. For example, Yen et al. reported that AGI-14100, a potent selective orally available IDH1 mutant inhibitor, induces ex vivo differentiation of primary human AML patient samples harboring an IDH1 mutation. Additionally, one month of continuous twice-daily AGI-14100 treatment of a

primary human IDH1 (R132H)/FLT3/ITD mutant xenograft model resulted in a significant reduction of leukemic blasts in the peripheral blood. These effects were further enhanced by combining AGI-14100 with a short course of low-dose ara-C, which resulted in a decrease in the bone marrow tumor burden that was maintained for several weeks beyond discontinuation of the two drugs [81]. Other novel epigenetic targeted compounds currently in early stages of clinical development (preclinical or phase I) include inhibitors of bromodomains (ClinicalTrials.gov, identifier: NCT01713582), DOT1L (NCT01684150), EZH2 [82, 83], LSD1 [84, 85], and UTX/ JMJD3 [86]. As only a limited number of epigenetic targeted drugs are currently available for the treatment of AML patients, controversy remains with regard to how deeply genomic analysis of epigenetic modifiers – other than for risk stratification – should be integrated into clinical practice to guide treatment. Little is known about the effects of methylome-targeted approaches on normal and leukemic hematopoietic stem and progenitor cells or about the potential mechanisms of resistance. Further evaluation in preclinical and clinical studies is necessary to verify and establish a significant role for these promising agents in AML therapy.

Other Targets

KIT (c-KIT) is a proto-oncogene that codes for the transmembranous tyrosine kinase receptor CD117 that is expressed on hematopoietic stem and progenitor cells. Activation of the receptor through ligand-induced dimerization results in phosphorylation of signal-transduction molecules that predominantly regulate differentiation, proliferation, and apoptosis. Mutations in c-KIT have been reported in 20–30 % of core-binding factor leukemias (CBFL) [t(8;21) and inv(16)] [87]. Several studies suggest that the presence of mutated c-KIT in CBFL is associated with adverse outcome [88, 89], highlighting a potential benefit for c-KIT-targeted approaches in distinct subgroups of AML. Dos Santos et al. found that the Src- and c-KIT inhibitor dasatinib exerts growth inhibitory effects on human AML stem and progenitor cells in vitro. Moreover, experiments conducted in mouse models suggested that in vivo administration of dasatinib further sensitized AML stem cells capable of engraftment in secondary recipients to elimination by chemotherapeutic agents. Clinical trials combining dasatinib with chemotherapy are currently underway [90].

The family of RAS proteins (H-RAS, K-RAS, and N-RAS) constitutes a class of proteins called small GTPases that are involved in intracellular signal transduction essential to cell proliferation, differentiation, and survival. Regulating a highly complex network of signaling cascades, of which the RAS-RAF-MEK-ERK pathway plays a central role, aberrant activation of RAS has been implicated in the pathogenesis of many cancers, including AML [91, 92]. Accordingly, RAS mutations have been described in approximately 25 % of AML patients, occurring most frequently in N- and K-RAS [93, 94]. In recent years, several compounds designed to disrupt RAS-RAF-MEK-ERK signaling have emerged as promising candidates for targeted antileukemic therapy [95]. To this end, the orally bioavailable selective MEK1/2 inhibitor selumetinib (AZD6244, ARRY-142886) demonstrated encouraging activity in phase I studies [96]. In a subsequent phase II study of 47 patients with relapsed/refractory AML or elderly AML patients (≥ 60 years old) with previously untreated AML, selumetinib demonstrated a favorable toxicity profile (consisting of low-grade diarrhea, fatigue, nausea, vomiting, and skin rash) and modest single-agent

antileukemic activity, suggesting that further evaluation in the context of combination studies are warranted [97•].

Conclusions

Our growing knowledge of the molecular heterogeneity of AML has formed the basis for hope of discovering better ways to effectively combat the disease. Although the advent of imatinib has led to increased optimism in cancer therapy, the exceptional success in treating chronic myelogenous leukemia (CML) is unlikely to be achieved in AML, given the diverse and complex genetic changes relevant to AML pathogenesis. However, targeted therapy in AML is viable if tailored to the individual patient based on the information obtained from systematic cytogenetic and molecular screening. A major challenge in further paving the way for targeted AML therapy will be the ability to study the efficacy of novel drugs within small subgroups and molecular entities. In light of the relatively low incidence rate of AML (3 %) as compared to prostate (28 %) or breast cancer (29 %) (http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2013/acspc-037389), it appears that large randomized trials conducted by a limited number of centers would be impracticable. The identification, recruitment, and enrollment of adequate patient numbers demand new concepts of cooperative research, including closer cooperation between clinical and research centers in order to harmonize and align research efforts, as well as novel clinical trial designs. Traditional clinical trial concepts based on large groups or cohorts have played a key role in clinical research and drug development for many years. The potential risk of obtaining inconclusive data by neglecting individual variations in molecular characteristics has been offset by the enrollment of large patient numbers. In the future, clinical trials will need to focus on small, well-defined, and carefully selected patient collectives, with the goal of changing treatment paradigms from developing “blockbuster” to “niche-buster” drugs.

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Table 1

FLT3 inhibitors under active development

TKI	Trial phase	Patient population	Single agent/ Combination	Reference
Midostaurin	3	Newly diagnosed patients with AML, FLT3-mutated, international, age 18–60 years (n=714)	Combination	NCT00651261
Midostaurin	2	Newly diagnosed patients with AML, FLT3/ITD-positive, multicenter, age 18–70 years (n=142)	Combination	NCT01477606
Midostaurin	1,2	Refractory/relapsed MDS, CMML or AML, age ≥ 18 years (n=54)	Combination	NCT01202877
Sorafenib	1	FLT3/ITD mutated patients with AML after allogeneic HSCT, age 18–75 years (n=28)	Single agent	NCT013998501
Sorafenib	1	Patients with relapsed/refractory AML with FLT3/ITD mutation, age ≥ 18 years (n=28)	Single agent	NCT00943943
Sorafenib	2	Newly diagnosed patients with FLT3-mutated AML, age ≥ 60 years (n=49)	Combination	NCT01253070
Sorafenib	1	Patients with FLT3/ITD-mutated AML, eligible to undergo BM transplant, age ≥ 18 years (n=36)	Single agent	NCT01578109
Quizartinib	2	Patients with relapsed or refractory AML, FLT3/ITD-mutated, age ≥ 18 years (n=64)	Single agent	NCT01565668
Quizartinib	1	Newly diagnosed patients with AML, FLT3/ITD and FLT3 WT are eligible, age 18–60 years (n=58)	Combination	NCT01390337
Quizartinib	1	Patients with AML after allogeneic HSCT, performed in first or second remission (n=30)	Single agent	NCT01468467
Quizartinib	1,2	Refractory/relapsed MDS, CMML or AML, age ≥ 18 years (n=64)	Combination	NCT01892371
PLX3397	1,2	Refractory/relapsed AML, FLT3/ITD-positive, age ≥ 18 years (n=45)	Single agent	NCT01349049