

Emerging Molecular and Immune Therapies in Acute Myeloid Leukemia

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Abstract

Rapid advancements in immune and targeted therapeutics coupled with improved understanding of the genomic landscape coupled with an improved understanding and clonal evolution in acute myeloid leukemia (AML) have resulted in the emergence of numerous exciting therapies for AML in the last decade. In many cases the response rates and tolerability of such targeted or immune-based approaches are superior to those achieved with standard cytotoxic therapy. The response and survival benefit may frequently be further improved by rationally combining targeted, monoclonal, or immune-activating approaches with epigenetic or cytotoxic therapies. The targeted and monoclonal-based strategies may be particularly useful in efforts to improve outcomes in traditionally poor-risk AML, including AML with poor-risk cytogenetics, *TP53* mutation, secondary AML, or AML in elderly patients (aged >60 or >65 years). While the final approval status and clinical roles of a number of these molecular and immune-targeted agents in AML will be determined by ongoing phase II/III trials, it is valuable for practicing oncologists to be aware of the scope, indications, and toxicities of these therapies, and of the significant progress in AML in the last few years. In this review, we discuss emerging molecular and immune-based therapies in AML, and how these may impact the management of AML in the near future.

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standard frontline approach in most patients with AML.² However, the last decade has seen major advances in the understanding of molecular leukemogenesis, of immune pathways, and of conjugated and bispecific antibody technology. These advances have provided crucial insights into disease pathophysiology and platforms leading to the development of novel therapies for AML.

The advent of high-throughput sequencing methods has enabled characterization of recurring, prognostically informative mutations that may serve as suitable targets for small-molecule and metabolic therapies. This is exemplified by the successful targeting by novel small-molecule inhibitors of recurrent mutations and mutation-associated pathways that play a role in leukemogenesis (eg, FMS-like tyrosine kinase-3 [*FLT3*] and isocitrate dehydrogenase [*IDH*] 1/2).³ Additional small-molecule inhibitors targeting overexpressed or aberrantly regulated pathways in AML are also showing encouraging results as single agents or in combinatorial approaches (eg, inhibition of B-cell lymphoma 2 protein [*BCL2*], of mouse double minute 2 homolog [*MDM2*], and of chromosomal maintenance 1 [*CRM1*]) (see **Figure**).

Identification and targeting of leukemia-specific antigens compose a second major area of active research in AML. Different approaches to target these antigens, with the intent of inducing preferential cytotoxicity to leukemia blasts and potentially to leukemia stem cells, are being explored. They include monoclonal antibodies; naked or antibody drug conjugates; radioimmunoconjugates; dual-affinity retargeting antibodies; and T-cell adoptive therapy, such as chimeric antigen receptor-T (CAR-T) cells.

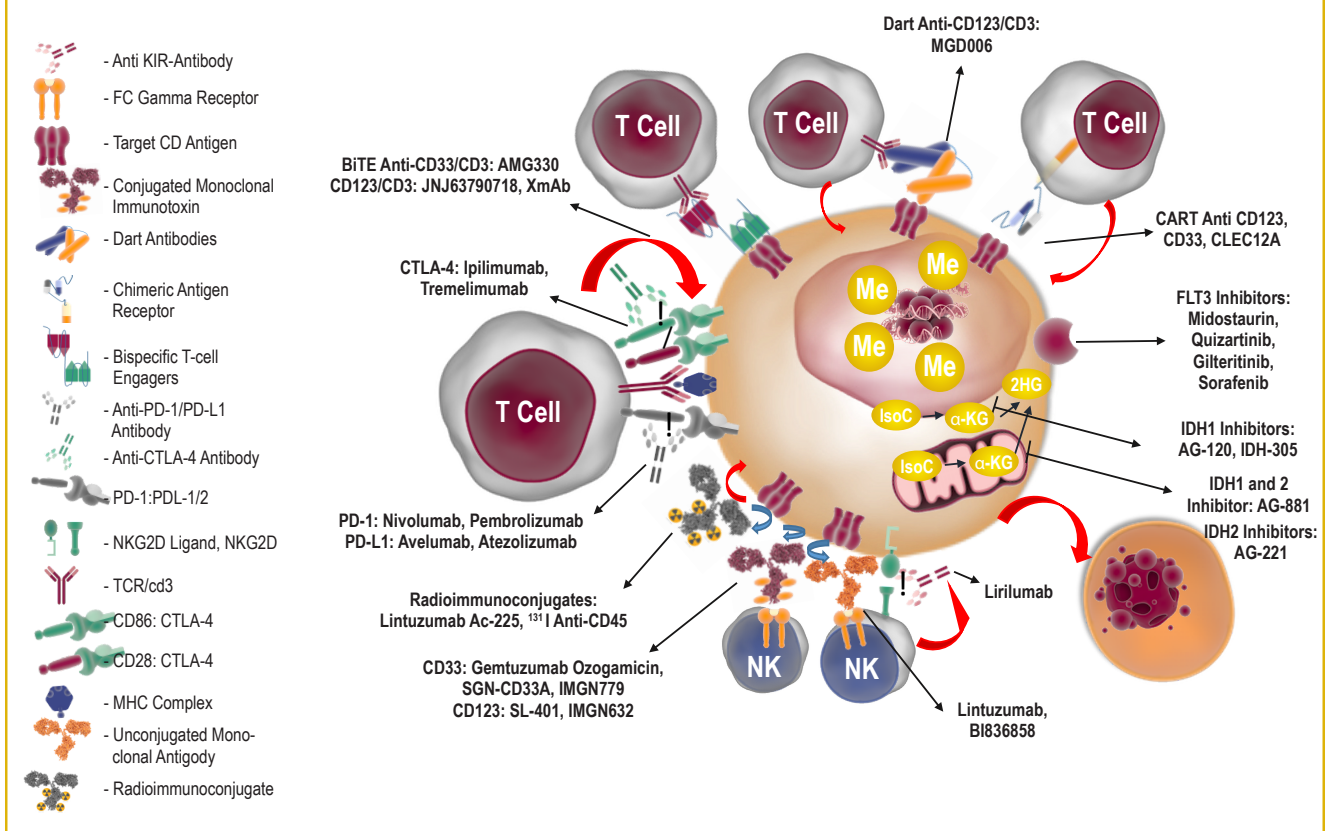
A third approach focuses on unleashing the patient's own immune system to fight against leukemic cells using immune checkpoint antibodies; bispecific T-cell engager (BiTE) antibodies; and adoptively transferred natural killer (NK) cells. Clinical trial experiences with these above-mentioned therapies (**Table**) suggest marginal therapeutic benefit when used as single agents, but suggest additive benefit, and in many instances, synergistic benefit, when implemented in rational combinations.

Molecular Targeted Therapies in Acute Myeloid Leukemia

Genome-wide sequencing on large numbers of AML patients has identified recurrent mutations in genes encoding epigenetic regulators, signaling receptors, and splicing factors, as well as

Introduction

Acute myeloid leukemia (AML) remains among the few hematological malignancies with no major drugs approved to market in the United States in the last decade. In fact, the only drug approved in the United States for AML in the past 4 decades was gemtuzumab ozogamicin (GO; Mylotarg); it was approved in 2001 and later voluntarily withdrawn from the market due to a concern for increased toxicity in a phase III study.¹ Cytarabine and anthracycline (idarubicin or daunorubicin) regimens remain the

FIGURE. Immune and Molecular Targeted Approaches in Acute Myeloid Leukemia

other genes that regulate key cellular processes.³ Notably, there are few obvious associations between a particular baseline mutational status and particular drug sensitivity, as exemplified by the sensitivity of mixed lineage leukemia (MLL)-rearranged leukemias to bromodomain inhibitors and to CDK6 inhibitors; *NPM1*-mutated leukemias to arsenic trioxide; and *IDH2*-mutated leukemias to BCL-2 inhibitors in preclinical and clinical testing.^{4,6} While drugs targeting AML molecular mutations—including *EZH*, *MLL*, *DNMT3A*, *ASXL1*, and *TET2*—are currently in preclinical or early clinical development, FLT3 and IDH inhibitors are already in an advanced phase of clinical development (Table).⁷

FLT3

Phase II studies have demonstrated that sorafenib, an FLT3 inhibitor, may improve response rates and event-free survival when used in combination with hypomethylating agents or cytotoxic chemotherapy.^{8,9} In addition, a number of other FLT3 inhibitors (quizartinib, gilteritinib, midostaurin) are currently being evaluated in phase III trials. In a large retrospective analysis, quizartinib appeared to be superior to standard-of-care regimens, both in response rates (43% vs 11%, $P = .002$) and median survival (in relapsed patients: 128 vs 53 days) when used in FLT3-internal

tandem duplication–positive (ITD+) patients with AML who relapsed after salvage chemotherapy (SCT).¹⁰ An interim analysis of 52 patients, showed the combination of quizartinib with low-dose cytarabine (LDAC) or azacitidine to be effective, with overall response rate (ORR) of 73% and a median survival of 14.8 months.¹¹ Gilteritinib (ASP2215) was evaluated in a phase I/II trial involving relapsed/refractory (R/R) AML (FLT3mut+ = 169) and was effective with an ORR of 52%, median duration of response of 20 weeks, and median survival of 7.8 months.¹² Most recently, midostaurin received breakthrough therapy designation from the FDA for newly diagnosed FLT3-mutated AML, after demonstrating significantly improved overall survival (OS) in a randomized phase III study of induction and consolidation chemotherapy combined with midostaurin or placebo.¹³ Crenolanib is another orally bioavailable pan-FLT3 inhibitor with added activity against D835H and D835Y mutants. Clinical activity has been seen even in patients who have failed prior FLT3 tyrosine kinase inhibitor (TKI) therapy. A phase II single-center study evaluating crenolanib in FLT3-mutated AML suggested modest activity with complete response (CR) rates of 23% in FLT3-TKI-naïve patients and of 5% in patients who had failed prior anti-FLT3 therapy.¹⁴ The combination of crenolanib with standard cytarabine and anthracycline

induction in newly diagnosed *FLT3*-mutant AML provided high CR rates of 88% while being well tolerated with a low incidence of AEs.¹⁵ This agent is currently undergoing evaluation in combination with other standard therapies in the treatment-naïve and salvage setting in *FLT3*-mutated AML (NCT02298166, NCT02626338, NCT02400281, and NCT02283177). Lestaurtinib was among the first *FLT3* inhibitors to be extensively evaluated as a monotherapy and in combination with SCT. Clinical activity as a single-agent therapy was shown to be modest.¹⁶ Furthermore, its addition to SCT did not improve response rates, while it was associated with a higher frequency of severe AEs and deaths (NCT00079482).¹⁷ This agent is currently not undergoing active evaluation in *FLT3*-mutated AML.

IDH 1 and 2

Two first-generation IDH inhibitors—AG-221 (IDH2 inhibitor) and AG-120 (IDH1 inhibitor)—and a second-generation pan-IDH inhibitor, AG-881, are currently undergoing active study in ongoing clinical trials. Another agent, IDH-305, targeting *IDH1* R132 mutation, is being evaluated in a phase I trial involving R/R AML (n = 24) and other malignancies. Preliminary data with the IDH1 and IDH2 inhibitors are encouraging, with an ORR in the 35% to 38% range and an acceptable safety profile.¹⁸ The drugs are oral, have been very well tolerated overall, and are now being evaluated in combination with 7 + 3 in younger patients with AML and with azacitidine in older patients with AML harboring *IDH1* or *IDH2* mutations, respectively. Differentiation is frequently seen with IDH inhibitors and often presents as increased white blood cell count, increased blasts, pulmonary infiltrates, and dyspnea. The occurrence of differentiation syndrome with IDH inhibitors does not correlate closely with the degree of leukocytosis, unlike the differentiation syndrome seen with all-trans retinoic acid (ATRA) or with arsenic trioxide in acute promyelocytic leukemia (APL). The differentiation syndrome responds rapidly to steroids, and a majority of patients are able to continue therapy with the IDH inhibitors.

IDH inhibitors are differentiation-inducers and do not frequently eliminate the malignant clone, as is the case with AG-221. In contrast, most recent data suggest that AG-120 is able to affect *IDH1* mutational clearance. In 1 study, among 63 patients with R/R AML (of 78 with hematological malignancy), 21 (33%) had objective responses: (CR = 10; complete response with incomplete hematologic recovery (CRi)/complete response with incomplete platelet recovery (CRp) = 8; marrow CR = 2; partial response (PR) = 1. Importantly, *mIDH1* clearance by next-generation sequencing was observed in 36% of CRs and 4% of non-CRs.¹⁹ Patients with an *mIDH1* clearance had improved clinical benefit from IDH inhibitors as compared with those who achieved a clinical response per International Working Group criteria but did not achieve molecular remission. Nevertheless, clonal persistence, and its therapeutic and prognostic implications for the need for continued IDH inhibitor therapy, remain unknown.

BCL2

Among the BCL-2 inhibitors, venetoclax is particularly effective and is being tested in combination with other agents, including hypomethylating agents and LDAC. Venetoclax in combination with azacitidine/decitabine produced an ORR (CR/CRi/PR) of 75% in frontline older patients with AML.²⁰ The expected response rate with azacitidine or decitabine alone is 18% to 25%, based on published data, suggesting striking synergism when the agents are combined. A phase III, randomized, registrational trial of azacitidine plus venetoclax versus azacitidine alone has begun enrollment. In a separate phase Ib study, Wei and colleagues reported on the safety and efficacy of venetoclax plus LDAC in 61 treatment-naïve patients >65 years with AML. The combination was well tolerated and produced high response rates (CR/CRi of 54%), with median survival not reached among the responders (CR/CRi/PR). Historic response rates with LDAC alone in a similar patient population have been 5% to 10%, highlighting the significant improvement when venetoclax is added to LDAC. The regimen was well tolerated. A future phase III randomized trial of LDAC with venetoclax is planned.²¹

Monoclonal Antibodies in Acute Myeloid Leukemia CD33

It has been realized that leukemic stem cells exhibit phenotypic characteristics distinct from those of normal hematopoietic stem cells.²² Of the various differentially expressed cluster differentiation (CD) antigens on leukemic blasts, CD123, CD33, and CD56 have thus far been exploited clinically as targets for monoclonal antibody-based therapies in AML.²³⁻²⁶ These monoclonal antibodies may exert their anti-AML tumor effect by varied mechanisms, including antibody-mediated neutralization, delivery of toxic payload in the case of conjugated antibodies, antibody-dependent cellular cytotoxicity, complement-mediated cytotoxicity, antibody-dependent cell-mediated phagocytosis, and enhanced T-cell antitumor efficacy by increasing T cell and tumor interaction with bispecific T-cell engaging antibodies.

GO, a humanized anti-CD33 monoclonal antibody conjugated to a DNA-damaging toxin (calicheamicin), is among the most extensively studied monoclonal antibodies in AML. It was first approved in May 2000 based on a phase II trial showing a 30% response rate in patients with AML in first relapse. However, GO was subsequently voluntarily withdrawn from the market 10 years later,²⁷⁻²⁹ based on a Southwest Oncology Group (SWOG) phase III trial that demonstrated a lack of benefit and potentially increased mortality in patients who received GO with standard chemotherapy.¹ However, multiple criticisms have since been raised against the design and dosing schema of this SWOG study. Since the SWOG study, 4 randomized trials conducted in Europe have demonstrated efficacy of GO in patients with AML with good- and intermediate-risk cytogenetics.³⁰⁻³⁴ Additionally, the higher incidence of veno-occlusive disease with the single

high dose of 9 mg/m² was significantly reduced by administering multiple split doses of 3 to 6 mg/m² in these trials. Based on these data, the use of GO is being reassessed by the European and US drug agencies and is simultaneously being further evaluated in de novo, relapsed, and pretransplant settings in clinical trials

(NCT01869803, NCT02473146, NCT02221310).

SGN-CD33A (vadastuximab), an anti-CD33 antibody conjugated to pyrrolobenzodiazepine, is a newer CD33-targeted monoclonal that demonstrated cytotoxicity in AML cell lines irrespective of multidrug-resistant status or karyotype.³⁵ This agent was evaluated

TABLE. Overview of Trials with Molecular and Immune Agents in Acute Myeloid Leukemia

Class / Agent	Study Design	Trial Regimen	Study Population, n (%)	ORR, n (%)	Treatment-Related Mortality	Median Survival	Comments
FLT3-inhibition							
Quizartinib ⁸⁵	Phase II, open label	Single arm monotherapy in FLT3-ITD positive & FLT3-ITD negative R/R AML	N = 92 (FLT3-ITD positive); N = 41 (FLT3-ITD negative)	66 (72%) (FLT3-ITD+); 17 (41%) (FLT3-ITD negative)	Not reported	25.3 weeks	High degree of activity as monotherapy, especially in FLT3-ITD positive patients in R/R setting. 13% experienced AEs requiring discontinuation
Gilteritinib ¹²	Phase III	Single arm monotherapy in R/R AML	N = 252; FLT3-ITD positive, n = 159; FLT3 D835, n = 13; FLT3-ITD & D835 positive, n = 16; other, n = 64	FLT3 ^{mut} (49%); wild-type FLT3 (12%)	7 of 252	31 weeks	Well tolerated and efficacious in FLT3 ^{mut} patients. Phase III testing in FLT3 ^{mut} R/R AML after first-line failure ongoing (NCT02421939)
AZA + sorafenib ⁹	Phase II	Single arm in R/R AML	N = 43; FLT3-ITD positive = 40	46%	8-week mortality = 16%	6.2 months	Combination effective for patients with R/R AML and FLT3-ITD. Well tolerated with very few grade 4 AEs
(7+3) + sorafenib ⁸	Phase II, multicenter, randomized, controlled	Sorafenib + (7+3) vs placebo + (7+3) in AML pts aged <60 years	Placebo, n = 133; sorafenib, n = 134	CR = 81 (60%) in the sorafenib group and CR = 78 (59%) in placebo group	30-day mortality 2% in sorafenib group vs 1% in placebo group; 60-day mortality 4% in both groups	9 months in placebo vs 21 months in sorafenib (P = .013)	AML aged ≤60 years; the addition of sorafenib to 7+3 increased efficacy but also increased toxicity; most common AEs: fever, diarrhea, bleeding, cardiac events, hand-foot-skin reactions, rash
(7+3) + sorafenib ⁸⁶	Multicenter, randomized, controlled	Sorafenib + (7+3) vs placebo + (7+3) in AML pts aged >60 years	Placebo, n = 95; sorafenib, n = 102	CR + CRi = 64 in placebo; CR + CRi = 57 in sorafenib	60-day mortality higher in the sorafenib arm vs the placebo arm (P = .035)	Median OS: 15 months for placebo vs 13 months for sorafenib	Sorafenib combined with intensive chemotherapy not effective in elderly AML pts, and also more toxic than 7+3 alone
Midostaurin + (7+3) ⁸⁷	Prospective, randomized, controlled	Newly diagnosed AML pts aged 18-60 yrs with FLT3 mutation	Midostaurin, n = 360; placebo, n = 357	CR = 59% midostaurin; CR = 54% placebo	NR	Midostaurin OS, 74.7 months vs placebo OS, 26 months (P < .01)	Very efficacious when used as a component of therapy in younger adults with mutant FLT3 AML. Also, not associated with additional toxicity
IDH Mutation							
IDH-mutation AG221 ⁸⁸	Phase I/II	mIDH2-AML/MDS	R/R-AML/MDS, n = 138 (70%); untreated AML/MDS, n = 60 (30%)	74 (41%) in overall (n = 181 evaluable); 52 (41%) in R/R AML (n = 128 evaluable)	NR	NR	Safe with few AEs, including indirect hyperbilirubinemia and nausea. Good response rates in IDH2+ AML.
AG120 ⁸⁹	Phase I	IDH1-mutant positive advanced hematologic malignancies	N = 57	ORR = 31%; CR = 15%	No drug-related deaths reported; 13 total deaths	NR	Safe with minimal toxicities. Durable response for up to 11 months
IDH305 ⁹⁰	Phase I	Advanced malignancies including R/R AML/MDS with IDH1R132 mutation	N = 81; AML/MDS = 24	ORR = 7 (33%); CR = 2 (9.5%)	NR	NR	Very favorable safety profile. Durable response in complete responders

7+3 indicates 7 days of cytarabine +3 days of daunorubicin; ABT, venetoclax; AE, adverse event; AG120, IDH1 inhibitor; AG221, IDH2 inhibitor; AML, acute myeloid leukemia; AZA, azacitidine; BCL-2, B-cell lymphoma-2 protein; CR, complete response; CRi, complete response with incomplete hematologic recovery; DAC, dacogen; FLT-3, FMS-like tyrosine kinase-3; IDH, isocitrate dehydrogenase; ITD, internal tandem duplication; LDAC, low-dose cytarabine; MDM2, mouse double minute 2 homolog; MDS, myelodysplastic syndrome; mIDH2, mutant isocitrate dehydrogenase-2; MRD, minimal residual disease; NR, no response; pts, patients; ORR, overall response rate; OS, overall survival; R/R, relapsed/refractory; and SGN 33A, vadastuximab.

TABLE. Overview of Completed Trials with Molecular and Immune-Based Agents in AML (continued)

Class / Agent	Study Design	Trial Regimen	Study Population, n (%)	ORR n (%)	Treatment-Related Mortality	Median Survival	Comments
BCL-2 inhibition							
AZA/DAC + ABT ²⁰	Phase Ib, open label	Treatment-naïve AML pts aged >65 years not eligible for intensive chemotherapy	N = 22	ORR = 75% (venetoclax 400 mg); 70% (venetoclax 800 mg)	4 deaths: 3 after treatment discontinuation, 1 from disease progression	NR	Tolerable combination. Rapid responses can be expected with this combination with high CR rates
LDAC + ABT ²¹	Phase I	Treatment-naïve AML pts aged >65 years	N = 61	CR/CRi = 54%	60-day mortality rate = 15% (9 of 61 patients)	Median not reached in responders	Highly synergistic combination. Activity in high-risk pts such as aged >75 yrs, secondary AML, adverse karyotypes
MDM2 inhibition							
RG7112 ⁹¹	Phase I	Refractory acute and chronic leukemias, including R/R AML	N = 30 evaluable AML pts	CR/CRi = 10%; PR = 13%	31 deaths in entire study, with none related to study drug (n = 116). 17 of 31 due to disease progression	NR	Clinically active in R/R leukemias. RG7388 is a more potent agent in this class and is in phase I clinical development in AML
Monoclonal antibodies							
SGN-CD33A ³⁶	Phase I	Treatment-naïve CD33+ AML	N = 27 pts	CR/CRi = 60%	60-day mortality = 15%	NR	Double the response rates expected with nonintensive therapies such as hypomethylating agents or LDAC. High MRD-negative remissions, and low early mortality rates
AZA/DAC + SGN-CD33A ³⁹	Phase I/II	Treatment-naïve CD33+ AML	N = 24 pts	CR/CRi = 65%	60-day mortality = 4%	Median OS not reached for a median of 13.5+ weeks from treatment	The combination is effective and has a favorable toxicity profile. Activity favorable over historical outcomes with single-agent hypomethylating agents
(7+3) + SGN-CD33A ⁴²	Phase Ib	Newly diagnosed acute AML	N = 42 pts	CR/CRi = 76%	30-day mortality = 2%	MRD-negative CR/CRi: not reached; MRD-positive CR/CRi = 7.46 months	Low 30-day mortality rate; good response rate; no added nonhematological toxicity with SGN-CD33A combined with (7+3)

7+3 indicates 7 days of cytarabine +3 days of daunorubicin; ABT, venetoclax; AE, adverse event; AG120, IDH1 inhibitor; AG221, IDH2 inhibitor; AML, acute myeloid leukemia; AZA, azacitidine; BCL-2, B-cell lymphoma-2 protein; CR, complete response; CRi, complete response with incomplete hematologic recovery; DAC, dacogen; FLT-3, FMS-like tyrosine kinase-3; IDH, isocitrate dehydrogenase; ITD, internal tandem duplication; LDAC, low-dose cytarabine; MDM2, mouse double minute 2 homolog; MDS, myelodysplastic syndrome; mIDH2, mutant isocitrate dehydrogenase-2; MRD, minimal residual disease; NR, no response; pts, patients; ORR, overall response rate; OS, overall survival; PR, partial response; RG7112, small-molecule MDM2 antagonist; R/R, relapsed/refractory; SGN 33A, vadastuximab.

in a phase I study of R/R CD33+ AML and demonstrated blast clearance in 48% of the 85 evaluable patients, with CR/CRi in 27% (23 of 85), of whom 73% (of 23 patients) were negative for minimal residual disease among patients treated at the recommended dose of 40 µg/kg.³⁶ Efficacy was even higher in the treatment-naïve patients (n = 27) with CR/CRi in 54% (14 of 27 evaluable patients).³⁷ The most common grade 3 adverse events (AEs), occurring in 20% of patients, were neutropenia, thrombocytopenia, and anemia. Pre-clinical and early clinical-phase studies have shown SGN-CD33A to synergize with hypomethyl-

ating agents like azacitidine, and in a recently reported phase II study, the combination produced a CR/CRi rate of >60% with an 8-week mortality of 5% in frontline older patients with AML.^{38,39} This agent is currently being studied as monotherapy in maintenance; in the pre- and postallogeic stem cell transplant (ASCT) settings; and in a phase III randomized study of SGN-CD33A with azacitidine versus azacitidine alone in untreated older patients with AML (NCT02326584, NCT02785900, NCT02706899, NCT02614560).

Another conjugated CD33 antibody that has shown marked

activity both in vitro and in vivo is IMGN779.^{40,41} Its activity appears to be more selective to leukemic stem cells while sparing normal hematopoietic stem cells (HSCs), suggesting a potential for reduced myelosuppression. It is currently in a phase Ib trial in patients with relapsed AML (NCT02674763).

CD-123

Overexpression of the interleukin-3 (IL-3) receptor α -chain (IL-3R α /CD123) on AML cells is associated with enhanced blast proliferation, disease relapse, and drug resistance in AML.^{42,43} Clinical activity was modest with the first-in-class anti-CD123 antibody (CSL360).⁴⁴ Two second-generation antibodies (CSL362, SL401) are being evaluated in phase I/II clinical trials. CSL362, a fully humanized antibody with a modified Fc-domain to enhance NK cell binding, was evaluated as postremission treatment in a phase I study involving 25 patients with CD123+ AML (in first or second CR), and it was able to prolong CRs beyond 6 months (26-52 weeks) in 10 of 20 evaluable patients.⁴⁵ SL401 (DT388IL3), a human IL-3 ligand fused to a truncated diphtheria toxin,⁴⁶ was evaluated in a phase I trial in 74 R/R or high-risk de novo AML patients, and it produced an ORR (CR + PR + disease stabilization) of 27% (20 of 74) and CR/CRi rate of 2.8% (2 of 74). The median survival of second-salvage AML patients treated in this trial (n = 33) was 3.2 months (range, 2-8.4), with 22% alive at 1 year.⁴⁷ This compound is currently being evaluated in 3 phase II trials in hematological malignancies including 1) in patients with relapsed AML and relapsed/frontline blastic plasmacytoid dendritic cell neoplasm; 2) maintenance after completion of consolidation in patients with high-risk AML patients who are not candidates for or have refused ASCT; and 3) in chronic myeloid disorders (NCT02113982, NCT02270463, and NCT02420873, respectively).

At this time, CD33- and CD123-targeted approaches are the most advanced. Monoclonal antibodies to other AML target antigens including CD25, CD37, and CD38 have recently begun clinical evaluation in phase I studies (NCT02588092, NCT02610062, and NCT01084252, respectively).

Radioimmunoconjugates

The radiosensitive nature of AML and its systemic pattern of involvement provide a rationale for radioimmunotherapy via targeted radionuclide antibody conjugates.⁴⁸ Early studies with 2 beta-emitters, ¹³¹I-M195 and ¹³¹I-HuM195, demonstrated activity in myeloid leukemia, and demonstrated safety when used at myeloablative doses in conjunction with standard chemotherapeutics as a conditioning regimen for HSC transplantation.^{49,50} Subsequent clinical studies exploring antibodies against AML targets (CD33, CD45, or CD66) with beta emitters (¹³¹iodine, ¹⁸⁸rhenium, ⁹⁰yttrium), alone or as part of a conditioning regimen pre-ASCT in patients with relapsed AML, have demonstrated tolerability and encouraging clinical efficacy.^{51,52} The ¹³¹I-labeled anti-CD45 antibody BC8 (Iomab B) is currently being tested in phase I (NCT00589316) and phase III (NCT02665065) studies to evaluate

its efficacy and safety as a part of myeloablative conditioning regimen prior to ASCT in patients with R/R AML. The properties of high-linear energy transfer and short particle length of decay has been exploited in the development of alpha emitter radioisotope conjugates. A phase I trial with the second-generation actinium-225-lintuzumab (anti-CD33) antibody demonstrated reduction of bone marrow blasts in 65% of 18 evaluable patients with R/R AML.⁵³ A phase I/II trial to determine the toxicity and efficacy of fractionated dosing of this agent in combination with LDAC in older untreated AML patients is ongoing (NCT02575963).

Immunotherapy in Acute Myeloid Leukemia

Immune Checkpoint Antibodies

The concept of targeting the immune system and not the tumor itself was initially achieved through monoclonal antibody-based inhibition of CTLA-4, a protein receptor on T cells that prevents them from unleashing an immune attack against tumor cells. Another major approach to immune checkpoint blockade involves the inhibition of PD-1/PD-L1 ligand, a co-inhibitory receptor-ligand system expressed on activated T cells and B cells.⁵⁴ Clinical trials with antibodies targeting these pathways have demonstrated marked efficacy resulting in FDA approvals in a variety of solid tumors.^{55,57} Immune checkpoint therapy has more recently been evaluated in hematologic malignancies, with robust activity and approval in Hodgkin lymphoma and modest but clear activity in certain subsets of diffuse large B-cell lymphoma and in mantle cell lymphoma. The rationale to evaluate these agents in leukemia springs from studies demonstrating reduced AML burden and improved survival in murine models on inhibition of PD-1 and CTLA-4.⁵⁸⁻⁶² Additionally, PD-1 and other clinically targetable stimulatory checkpoint receptors, such as OX40 and ICOS, are overexpressed in the bone marrow of patients with relapsed AML.⁶³

A phase I study with a PD-1/PD-L1 inhibitor, pidilizumab, demonstrated a minimal response in the form of stable disease in 1 of 8 salvage AML patients.⁶⁴ DNA methyltransferase (DNMT) inhibitors increase expression of PD-1, PD-L1, and PD-L2 in patients with AML/myelodysplastic syndrome (MDS), with higher expression in DNMT inhibitor-resistant as compared with inhibitor-sensitive patients, suggesting that PD-1 upregulation may be involved in resistance to DNMT inhibitors.⁶⁵ This has resulted in clinical trials combining PD-1/PD-L1 inhibitors with azacitidine in AML and MDS. A phase Ib/II study of nivolumab in combination with azacitidine in 53 patients with R/R AML (median prior therapies = 2, poor-risk cytogenetics = 45%) showed an ORR (CR, CRi, hematologic improvement) of 34%, and an 8-week mortality rate of 8%. The overall survival with this combination in the first salvage setting was superior to historical outcomes with other hypomethylating agent (HMA)-based salvage protocols at the same institution (9.3 vs 3.9 months, *P* = .03).⁶⁶ Furthermore, responses were durable, with 80% of relapsed patients who achieved CR/CRi alive at 1 year. PD-1 inhibitors are also being

evaluated as a maintenance therapy, post induction and consolidation, in patients with high-risk AML who are not candidates for ASCT (NCT02532231) and in combination with standard induction chemotherapy in newly diagnosed younger (aged 18–60 years) patients with AML (NCT02464657). PD-1 inhibitors are also being evaluated in phase 1 trials for patients with AML after ASCT with initial encouraging results, with CR in 5 of 12 patients with post-SCT relapsed AML (median: 3 prior therapies before ASCT), including resolution of extramedullary leukemia in 3 of the patients (NCT01822509). Activation of stimulating checkpoints such as OX40 and ICOS represents another approach to enhance anti-tumor cytotoxicity, and this may prove synergistic in combination with checkpoint inhibition. Clinical trials with such inhibitory and stimulatory combinations are being planned.

Adoptive T-Cell Therapy

The process of adoptive cell transfer involves removal of T cells from a patient and modifying them so that they express receptors specific to the patient's particular cancer. The ex vivo expanded cytotoxic T lymphocytes, now capable of targeting tumor-associated antigens, are reinfused into patients. The genetically engineered receptors consist of an extracellular domain created by the fusion between the variable region of heavy and light chains of an antigen-specific monoclonal antibody and of an intracellular T cell-activating domain, usually CD3- ζ . The tumor specificity of the monoclonal antibodies allows activation of CAR-T cells independent of major histocompatibility complex (MHC). After showing success in acute lymphoblastic leukemia, the approach was recently evaluated in the treatment of patients with AML^{67,68} with CAR-T cells targeted to Ley, a carbohydrate antigen that is overexpressed by malignant myeloid cells. The pilot study (n = 5) involved 4 evaluable patients with relapsed AML, 2 of whom had achieved stable disease and 2 who had transient responses.⁶⁹ The investigators observed that Ley was only expressed in a proportion of AML blasts, unlike epithelial tumors, in which the expression was more uniform and intense. Therefore, there has been further development of this agent in lung cancer therapy only. Preclinical studies have shown that anti-CD123 CAR-Ts are capable of targeting AML blasts and leukemia stem cells, suggesting these could be promising agents for AML CAR-T cell approaches.⁷⁰ A number of phase I clinical trials including anti-CD33, -CD7, and -CD133 CAR-Ts (NCT01864902, NCT02799680, NCT02742727, NCT02541370) and anti-CD123 and anti-NKG2D ligand CAR-T cells (NCT02159495, NCT02623582; NCT02203825) studies are ongoing in patients with R/R AML. The role and future of CAR-T cell therapy in AML remains unknown at this time.

T Cell-Engaging Antibodies

BiTE antibodies are fusion protein constructs consisting of 2 single-chain variable fragments of 2 antibodies; one binds to T cells via the CD3 receptor, and the other binds to a tumor cell

via a tumor-specific antigen. BiTE antibodies effectively recruit T cells and link them with tumor cells, thereby effectuating T cell-mediated cytotoxic activity on tumor cells independent of the presence of MHC-I or costimulatory molecules.^{71,72} After the promising clinical activity and FDA approval of blinatumomab, an anti-CD19/CD3 first-in-class BiTE antibody in precursor B-cell acute lymphoblastic leukemia, the approach has been adapted to target AML with the development of novel constructs targeting anti-CD3/CD33, such as AMG 330.^{73,74} Preclinical studies have demonstrated potent activity of this agent in CD33+ AML⁷⁴⁻⁷⁶ and a phase I trial of AMG330 is ongoing (NCT02520427). Phase I trials evaluating XmAb CD3/CD123 BiTE (NCT02730312) and JNJ-63709178 CD123/CD3 (NCT02715011) have recently begun enrollment. Early data suggest that close monitoring for cytokine release syndrome will be important with such bispecific approaches in AML.

Next-Generation Hypomethylating Agents

Guadecitabine (SGI-110), a second-generation hypomethylating agent, improves upon the pharmacokinetics of decitabine by incorporating deoxyguanosine dinucleotide into decitabine, thereby increasing in vivo exposure and potentially improving efficacy.⁷⁷ The outcomes of a phase II study involving 103 patients with R/R AML treated with SGI-110 were recently presented. CR and median survival rates on the 10-day (n = 53) and 5-day (n = 50) regimens were 30% and 16% at 7.1 and 5.7 months, respectively; the most common grade 3 AEs were anemia, thrombocytopenia, neutropenia, pneumonia, and sepsis.⁷⁸ A phase III trial comparing SGI-110 with standard of care in previously treated AML is currently ongoing (NCT02920008).

Other Therapies

DOT1L-like/histone H3K79 methyltransferase/DOT1L is a protein implicated in the development of mixed lineage leukemia (MLL) through aberrant hypermethylation-induced gene expression. A phase I trial evaluating pinometostat (EPZ-5676) in adult R/R MLL (n = 49) determined the agent to have clinical activity (composite CR [n = 2], PR [n = 1], leukemia cutis [n = 3]) with an acceptable safety profile (NCT01684150).⁷⁹ Another promising approach involves targeted inhibition of chromatin regulatory proteins such as lysine-specific demethylase-1 (LSD1), an enzyme responsible for histone H3 demethylation apart from other functions. GSK2879552 is an orally administered LSD1 inhibitor that is currently undergoing development in a phase I dose-escalation study involving R/R AML (NCT02177812), and results are eagerly awaited.

The bromodomain (BRD) family is an epigenetic class of histone-modification proteins with an ability to “read” the genome and modulate gene expression through transcriptional regulator recruitment to specific genome locations. BRDs of these reader proteins promote aberrant gene expression and sustain leukemic maintenance, thus providing a rationale for developing inhibitors

against this class.⁸⁰ Berthon and colleagues reported on outcomes with OTX105, an oral BRD inhibitor, in a phase I dose-escalation study.⁸¹ Among 36 patients with R/R AML, 3 achieved CR/CRi, and 2 had partial blast clearance. Recommended dose for further phase II studies was 80 mg on a schedule of 14 days on, 7 days off.

Histone deacetylase inhibitors provide another therapeutic approach to exploit the aberrant epigenetic alterations in AML. After showing single-agent activity in a phase I trial in AML, pracinostat was evaluated in combination with azacitidine in a phase II study involving older patients with AML deemed to not be candidates for intensive chemotherapy.⁸² The combination arm proved superior, with an estimated median OS of 19.1 months, resulting in the FDA granting a breakthrough designation for the combination's use in elderly patients with AML aged ≥ 75 years, or ineligible for intensive chemotherapy.

Pevonedistat is a novel NEDD8-activating enzyme inhibitor with single-agent clinical activity in R/R AML.⁸³ Swords and colleagues presented outcomes of a phase II study evaluating pevonedistat in combination with azacitidine in treatment-naïve older patients with AML.⁸⁴ Among 61 patients treated, ORRs were observed in 52 (18 CR, 5 CRi, 8 PR, 21 other) with a median duration of remission of 8.3 months. After a median follow-up of 16.4 months, projected 6-month survival was 52%. Single-agent and hypomethylating combination strategies with pevonedistat and MLN4924, another inhibitor in this class, are ongoing (NCT03009240, NCT02610777, and NCT01814826). NCT00911066 is completed.

Conclusion

Among available molecular targeted therapies, FLT3 inhibitors, as single agents or in combination with standard chemotherapy, in frontline or salvage settings, have already shown benefit (frontline 7+3 with midostaurin in untreated young AML patients in a phase III trial) or are being evaluated in phase III trials. Other inhibitors targeting IDH1/2 and BCL-2 among other small molecule inhibitors have shown promise as single agents and in combination with DNMT inhibitors, and are being evaluated in ongoing expanded phase II trials or soon-to-open phase III trials. Monoclonal antibody conjugates, such as SGN-CD33A and GO, are currently being evaluated in combination strategies with DNMT inhibitors or in cytotoxic induction regimens in phase III trials, while other immune- and antibody-based therapies, discussed above, are still in early phases of clinical development. The integration of informative biomarkers into clinical practice, and trials and implementation of rational combinatorial strategies of targeted, immune, monoclonal, and cytotoxic chemotherapies with each other, all while assessing for tolerability and toxicity, are important steps forward to help define and expand the scope of these novel therapies in AML.

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