

Acute Myeloid Leukemia: Advancements in Diagnosis and Treatment

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Abstract

Objective: Leukemia is the most common pediatric malignancy and a major cause of morbidity and mortality in children. Among all subtypes, a lack of consensus exists regarding the diagnosis and treatment of acute myeloid leukemia (AML). Patient survival rates have remained modest for the past three decades in AML. Recently, targeted therapy has emerged as a promising treatment.

Data Sources: We searched the PubMed database for recently published research papers on diagnostic development, target therapy, and other novel therapies of AML. Clinical trial information was obtained from ClinicalTrials.gov. For the major purpose of this review that is to outline the latest therapeutic development of AML, we only listed the ongoing clinical trials for reference. However, the published results of complete clinical trials were also mentioned.

Study Selection: This article reviewed the latest developments related to the diagnosis and treatment of AML. In the first portion, we provided some novel insights on the molecular basis of AML, as well as provided an update on the classification of AML. In the second portion, we summarized the results of research on potential molecular therapeutic agents including monoclonal antibodies, tyrosine kinase/Fms-like tyrosine kinase 3 (FLT3) inhibitors, epigenetic/demethylating agents, and cellular therapeutic agents. We will also highlight ongoing research and clinical trials in pediatric AML.

Results: We described clonal evolution and how it changes our view on leukemogenesis, treatment responses, and disease relapse. Pediatric-specific genomic mapping was discussed with a novel diagnostic method highlighted. In the later portion of this review, we summarized the researches on potential molecular therapeutic agents including monoclonal antibodies, tyrosine kinase/FLT3 inhibitors, epigenetic/demethylating agents, and cellular therapeutic agents.

Conclusion: Gene sequencing techniques should set the basis for next-generation diagnostic methods of AML, and target therapy should be the focus of future clinical research in the exploration of therapeutic possibilities.

Key words: Acute Myeloid Leukemia; Advancements; Diagnosis; Target Therapy

INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematological malignancy characterized by highly diverse genetic and epigenetic abnormalities. Traditional diagnosis of AML depends on the morphology, immunology, cytogenetics, and molecular biology (MICM) classification. As advancements in sequencing technologies are occurring in almost every natural and scientific discipline, the basis of clinical decisions is shifting progressively from the first "M" to the last "M." However, because of heterogeneity and potential gene-gene interactions, we are still exploring the

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genetic landscape and corresponding prognostic implications in AML.

Papaemmanuil *et al.*^[1] recently published a comprehensive analysis of leukemia genes in a total of 1540 patients with AML. An updated genomic classification was proposed that identified three novel subgroups: AML with mutated chromatin, RNA-splicing genes, or both; AML with tumor protein p53 (*TP53*) mutations, chromosomal aneuploidy, or both; and AML with isocitrate dehydrogenase 2 (*IDH2*^{R172}) mutations, and no other class-defining lesions.

Newly identified mutations with prognostic significance could be translated into revised risk stratification, as well as advancements in therapeutic development. Current chemotherapy agents have limited therapeutic efficacy, with an approximate 50–70% complete remission (CR) rate after induction and with only 20–30% of patients achieving long-term disease-free survival (DFS).^[2,3] Researchers have been endeavoring to improve the clinical outcome, trying out possibilities that could lead to a revolutionary change similar to what was seen with all-trans retinoid acid (ATRA) for acute promyelocytic leukemia (APL), tyrosine kinase inhibitors (TKIs) for Philadelphia chromosome-positive (Ph⁺) leukemia, and anthracyclines for AML with Down syndrome (although not directly).^[4] Thus far, target therapy is the most anticipated remedy.

For cancer, the target therapy is to “target” one or several crucial biological molecules in a chain involving the proliferation, metabolism, and apoptosis of malignant cells or treatment directed toward a certain group of patients with the same phenotypic, genetic, or epigenetic features. Drugs and agents from several categories, such as Fms-like tyrosine kinase 3 (*FLT3*) inhibitors, Aurora kinase (*AURK*) inhibitors, and immunotherapy have been or are currently being studied in prospective clinical trials. We performed a search and provided an update on the current novel molecular therapeutic agents. We also highlighted ongoing clinical trials for pediatric AML [Table 1].

The first part of this article will discuss the recent progress in genomic diagnosis. The second part will be devoted to the target therapies that have been or are currently being studied. Researches and clinical trials concerning pediatric patients will be specified.

ADVANCES IN GENOMIC DIAGNOSIS

Clonal evolution

It is important that we understand the concept of clonal evolution in the era of precision medicine and target therapies. We believed that cancer develops from somatically acquired driver mutations.^[1] However, follow-up tests throughout the disease course have shown that mutations can disappear and new ones can appear.

Kasi *et al.*^[5] reported on a 23-year-old patient who acquired a t(9;22) translocation during the administration of *FLT3* inhibitor therapy. Ismael *et al.*^[6] also reported a

similar case involving a 2-year-old boy with translocated in liposarcoma (*TLS*)/fused in sarcoma (*FUS*)-ets-related gene (*ERG*)-positive AML that relapsed as a *TLS/FUS-ERG*-negative but an runt-related transcription factor 1 (*RUNX1*)-positive genotype. Comprehensive genomic profiling of twenty pediatric AML patients revealed that only 58% of the mutations identified at diagnosis remained when patients relapsed. However, 42% of the mutations detected upon relapse were newly evolved.^[7] One case with immunophenotypic evolution after chemotherapy has also been reported.^[8]

The full spectrum and underlying mechanism of clonal evolution are still unclear. Theories include *de novo* alterations of “slippery” malignant cells and Darwinian effects (selection) involving targeting agents. Further study could augment our understanding of the disease process, relapse, and help us in choosing the right therapeutic agents.

“Pediatric-specific” genomic mapping

AML accounts for about 20% of pediatric leukemia. Childhood AML has a slightly better outcome than adult AML, with nearly 60–70% of long-term survival.^[9–11] Despite considerable variations in treatment schemes, clinical outcomes for childhood AML have not improved over the past two decades.^[12] Moreover, intensive chemotherapy is likely to render a substantial proportion of children to experience adverse effects from treatment toxicities.^[13] Therefore, new therapeutic strategies are needed for childhood leukemia.

The fact that some mutations in adult AML are rare or entirely lacking in pediatric AML suggests a different pathogenesis and thus different therapeutic strategy for children. Therefore, the understanding of “pediatric-specific” genetic alterations is critical for the development of targeted treatment.

Reports from the Japanese pediatric leukemia/lymphoma study group have confirmed that similar to adult patients with AML, enhancer binding protein α (*CEBPA*) mutations correspond to a favorable prognosis^[14] and that C-X-C motif chemokine receptor 4 (*CXCR4*) mutations are associated with less satisfactory outcomes.^[15]

However, some “unique” genetic abnormalities have also been found. A collaborative study by the Berlin-Frankfurt-Münster AML study group recently reported that pediatric AML with t(8;16)(p11;p13) is a special subgroup with a unique gene expression signature and distinct clinical features. Clinical outcomes in pediatric AML with this translocation were comparable to those of other pediatric AML.^[16] Interestingly, seven neonates with t(8;16)(p11;p13) experienced spontaneous remission, but four of them relapsed later. This self-limiting feature suggested that conservative treatment could be an option to treat neonatal AML with t(8;16)(p11;p13).

Maxson *et al.*^[17] also identified a distinct molecular subtype of pediatric AML that is defined by colony-stimulating factor 3 receptor (*CSF3R*) mutations (2.4%), which is commonly

Table 1: Molecular therapeutic agents for AML in ongoing clinical trials

Agent	Target (major)	Ongoing trials (pediatric [p])	Phase of testing
Monoclonal antibody			
Gemtuzumab ozogamicin	CD33	NCT02272478 (AML18), NCT00860639, NCT02473146, NCT02117297, NCT01869803, NCT00121303, NCT00893399, NCT00049517, NCT02221310p	I, II, III
Vadastuximab talirine (SGN-CD33A)	CD33	NCT01902329, NCT02326584, NCT02785900, NCT02706899, NCT02614560	I, II
AMG 330	CD33	NCT02520427	I
HuM195	CD33	NCT02575963	I, II
Yttrium Y 90 anti-CD45 monoclonal antibody BC8 (90Y-BC8)	CD45	NCT01300572	NA
KB004	EphA3	NCT01211691 (suspended)	I, II
Ipilimumab	CTLA-4	NCT02846376, NCT01757639	I
Brentuximab	CD30	NCT02096042	I, II
Ulocuplumab	CXCR4	NCT02305563	I
Tyrosine kinase/FLT3 inhibitors			
FLT3 inhibitors			
Lestaurtinib	FLT3	NCT00469859p	I, II
Midostaurin	FLT3	NCT00651261, NCT01883362, NCT02723435, NCT01477606, NCT01830361, NCT02624579, NCT00819546	I, II, III
Sorafenib	FLT3	NCT01398501, NCT02530476, NCT00943943, NCT02474290 (Phase IV), NCT02196857, NCT01253070, NCT02156297, NCT02728050, NCT01534260, NCT02779283, NCT01578109, NCT01620216, NCT01371981p, NCT02412475p, NCT02638428p, NCT02270788p	I, II, IV
Quizartinib	FLT3	NCT02039726, NCT02668653, NCT02834390, NCT01892371, NCT02675478, NCT02428543	I, II, III
Crenolanib	FLT3	NCT02626338, NCT01657682, NCT02298166, NCT02400255, NCT02400281, NCT02829840, NCT02283177, NCT02270788p	I, II, III
Gilteritinib	FLT3	NCT02752035	II, III
Pexidartinib (PLX3397)	FLT3	NCT01349049, NCT02390752p	I, II
AURK inhibitors			
Alisertib	AURKA	NCT02560025, NCT01779843, NCT01154816p	I, II
mTOR kinase inhibitors			
Sirolimus	mTOR	NCT01184898, NCT02583893, NCT01869114, NCT02528877, NCT01822015, NCT01885689, NCT00105001p, NCT02722668p, NCT02728700p, NCT01251575p	I, II
Temsirolimus	mTOR	NCT01611116, NCT02109744p	I, II
Everolimus	mTOR	NCT02539459, NCT01154439, NCT00819546, NCT02638428p	I, II
Epigenetic/demethylating agents			
Decitabine	Methyltransferase	NCT01786343 (Phase III), NCT01093573, NCT01846624, NCT02634827, NCT01786343, NCT02109744, NCT02252107, NCT02257138, NCT01853228p (suspended)	I, II, III
Azacitidine	Methyltransferase	NCT01305499, NCT0282984, NCT02275663	I, II
Vorinostat	Histone acetylase	NCT00948064, NCT01802333, NCT01534260, NCT01550224, NCT01617226, NCT00392353, NCT02412475p, NCT02419755p	I, II
Panobinostat	Histone acetylase	NCT01451268, NCT01463046, NCT00946647, NCT00691938, NCT02676323p	I, II
CAR-T cell therapy			
CAR-T33	CD33	NCT02799680, NCT01864902p	I, II
CAR-T123	CD123	NCT02623582	NA

The table lists ongoing or recruiting clinical trials registered on ClinicalTrials.gov only. AML: Acute myeloid leukemia; CD33: Cluster of differentiation; EphA3: Ephrin type-A receptor 3; CTLA-4: Cytotoxic T-lymphocyte-associated protein 4; CXCR4: C-X-C motif chemokine receptor 4; FLT3: Fms-like tyrosine kinase-3; AURK: Aurora kinase; mTOR: Mechanistic target of rapamycin; CAR-T: Chimeric antigen receptor T; NA: Not available.

seen in adult chronic neutrophilic leukemia, but rare in adult AML (0.5–1.0%).^[18,19] The study linked *CSF3R* mutations with a lower risk and better prognosis. The actuarial

overall survival (OS) at 5 years for those with *CSF3R* mutations versus no *CSF3R* mutations was 83% versus 65%, respectively, with an event-free survival (EFS) of 44% versus

49%, respectively, and a relapse risk (RR) of 64% versus 40%, respectively. It is worth noting that *CSF3R* mutations are sensitive to inhibition of the Janus kinase (JAK) pathway, which is downstream from the receptor.^[18] Therefore, this newly identified “pediatric-specific” mutation could also be a potential pediatric-specific therapeutic target. Clinical trials are underway to test the efficacy of JAK inhibitors.

An update in diagnostic methods naturally happens following the emergence of new genetic markers. McKerrell *et al.*^[20] recently tested a next-generation sequencing (NGS) based system called “Karyogene” in a cohort of 112 samples (62 AML, 50 myelodysplastic syndrome [MDS]). The results showed that Karyogene could successfully detect the more common fusion genes including promyelocytic leukemia (*PML*) retinoic acid receptor (*RARA*), core-binding factor beta (*CBFB*) myosin heavy chain 11 (*MYH11*), *RUNX1-RUNX1* translocation partner 1 (*RUNX1T1*), and histone-lysine n-methyltransferase 2A (*KMT2A*), with 100% sensitivity and specificity. In addition, it was able to identify the currently undetected genomic abnormalities such as a rare *KMT2A* mutation. However, the authors also admitted that it would be premature to replace standard cytogenetic testing with Karyogene. Reasons include lack of comprehensiveness (the current panel does not cover some rarer chromosomal rearrangements) and the technical limitations due to the varied level of bioinformatics expertise in medical institutions.

NEW TARGETS AND THERAPIES

Tyrosine kinase/Fms-like tyrosine kinase 3 inhibitors Fms-like tyrosine kinase 3 inhibitors

Mutations in *FLT3*, such as internal tandem duplications (*FLT3-ITD*), are common genetic alterations observed in approximately 30% of patients with AML.^[21] It has been added to the WHO risk stratification as a predictor of poor prognosis. Preclinical studies have shown that inhibiting FLT3 phosphorylation and downstream signaling could induce the apoptosis of leukemia cells.^[22] Hence, inhibiting the FLT3 pathway has been an attractive approach for target therapy.

FLT3 inhibitors are currently classified into three generations: the first generation is less selective, such as sorafenib, sunitinib, midostaurin, and lestaurtinib; the second generation includes selective inhibitors, such as quizartinib; and the third generation tackles the problem of drug resistance, such as crenolanib and gilteritinib.^[23] Among them, sorafenib and sunitinib have been approved in 2005 and 2006, respectively, to treat advanced malignancies such as renal cell carcinoma, hepatocellular carcinoma, and thyroid carcinoma.

Lestaurtinib (CEP-701) was the first FLT3 inhibitor tested in a randomized clinical trial.^[24] However, it failed to achieve a significant survival advantage over the control group, probably because of insufficient plasma levels to adequately inhibit FLT3.^[25]

Sorafenib is a multi-kinase inhibitor with activity against FLT3. In adult patients aged <60 years, an

antileukemic effect was demonstrated by adding sorafenib to standard chemotherapy in a randomized, double-blind, placebo-controlled Phase II trial.^[26] A benefit was evident with a 9-month median EFS in the placebo group versus a 21-month EFS in the sorafenib group, corresponding to a 3-year EFS of 22% in the placebo group versus 40% in the sorafenib group. Therapeutic effects were also reported in pediatric patients in a Phase I trial; six out of 11 patients with relapsed/refractory AML achieved a CR regardless of *FLT3* status after treatment with sorafenib in combination with chemotherapy.^[27] The positive results justify the incorporation of sorafenib into future pediatric AML trials.

Midostaurin is a Type III receptor TKI that inhibits FLT3 and other tyrosine kinase receptors.^[28] A single-agent clinical trial suggested that despite only a 5% partial remission (PR) rate, midostaurin was able to confer a robust anti-blast response in *FLT3*-mutated relapsed/refractory AML patients.^[29] Scientists then tried adding midostaurin to standard induction chemotherapy. At the 2015 (57th) American Society of Hematology (ASH) meeting, Stone *et al.*^[30] reported the initial results of a randomized, double-blind, Phase III study that demonstrated a survival advantage in the midostaurin group over that of the placebo group.

Quizartinib (AC220) is at least 10 folds more affinity for FLT3 than for the other receptor tyrosine kinases (RTKs).^[31] Further, it is associated with efficient and sustained FLT3 inhibition *in vivo*.^[32] In a Phase I single-agent clinical trial, 23 (30%) of 76 adult patients with relapsed/refractory AML experienced a therapeutic response, including 10 (13%) CRs and 13 (17%) PRs, after administration of quizartinib on an intermittent or continuous schedule.^[32] Recently, a first-in-child Phase I trial studying quizartinib in combination with intensive chemotherapy demonstrated that the regimen induced a CR in three of the seven evaluable *FLT3-ITD* AML patients, and an additional four patients experienced stable disease.^[33] However, only one of the seven *FLT-WT* AML patients achieved a CR, suggesting the higher selectivity of quizartinib.

Third-generation agents such as crenolanib and gilteritinib are currently in Phase I/II clinical trials, and their therapeutic value in pediatric patients is not yet clear. Additional trials with a larger number of samples are currently recruiting patients or are ongoing.

Aurora kinase inhibitors

The AURKs are serine/threonine kinases that are involved mainly in checkpoint regulation in the cell cycle.^[34] Three mammalian AURKs have been identified: AURKA, AURKB, and AURKC. The biological effect of inhibiting AURK in mitosis and its potential clinical significance were first discussed in 2003.^[35] Since then, increased consideration to this group has been garnered, and several AURK inhibitors were moved into Phase I/II clinical trials evaluating the treatment of malignancies. To date, the AURK inhibitors can be divided into two main groups: pan-Aurora inhibitors such as AMG900, SNS-314, CCT 137690,

VX-680/MK0457, VE-465, and PHA-680632, and selective inhibitors such as AZD1152, MLN8237, GSK1070916, MLN8054, PF-3814735, VX-689/MK-5108, TC-A 2317, and ZM447439.^[34]

So far, results have been modest. Alisertib (MLN8237), a selective AURKA inhibitor, induced a treatment response in six out of 35 patients (17%) with relapsed/refractory AML, while 49% achieved stable disease.^[36] Both AZD1152 (barasertib) and ZM447439, selective AURKB inhibitors, showed apoptosis-inducing effects in preclinical research.^[37] In a 2013 Phase I study, barasertib, used in combination with low-dose chemotherapy, demonstrated a therapeutic benefit in patients aged ≥ 60 years.^[38] The positive effects of barasertib were also demonstrated in advanced AML^[39] and newly diagnosed, relapsed, or refractory AML.^[40]

Epigenetic/Demethylating agents

Decitabine and azacitidine are DNA methyltransferase inhibitors used in AML, MDS, and other malignancies. Single use of these agents is often limited to patients not considered for intensive chemotherapy. A randomized Phase III study is comparing 10-day decitabine with conventional chemotherapy for patients aged >60 years (NCT01786343). However, decitabine and azacitidine were frequently tested in protocols with other chemotherapy or molecular targeting agents. Three clinical trials of decitabine or azacitidine in combination with the FLT3 inhibitor midostaurin are ongoing (NCT01093573, NCT01846624, and NCT02634827). Phase I/II trials involving decitabine used in combination with mechanistic target of rapamycin inhibitor, cytotoxic agents, or other protein kinase inhibitors such as a JAK inhibitor for high-risk or older AML patients are recruiting patients (NCT01786343, NCT02109744, NCT02252107, and NCT02257138). Phase I/II clinical trials studying azacitidine in combination with another epigenetic agent, an FLT3 inhibitor, or cytotoxic chemotherapy for older or unfit patients with AML are also ongoing (NCT01305499, NCT0282984, and NCT02275663). For pediatric patients with refractory/relapsed AML, pilot studies have demonstrated the safety and therapeutic effects of decitabine.^[41] However, a Phase I study of decitabine in combination with cytarabine for children with refractory/relapsed AML was suspended because of a lack of significant clinical benefit (NCT01853228).

Vorinostat (SAHA)^[42] and panobinostat (LBH-589)^[43] are histone deacetylase (HDAC) inhibitors that have been approved for the treatment of advanced hematologic malignancies such as lymphoma and multiple myeloma. Vorinostat did exhibit some antileukemic effects in AML patients with advanced disease.^[44] However, in a following trial testing its efficacy as a single-agent for AML, vorinostat demonstrated minimal activity.^[45] A similar modest effect was also observed with panobinostat used as monotherapy.^[46] Interestingly, a case with therapy-resistant AML who had Down syndrome experienced a transient but remarkable response to monotherapy with vorinostat, suggesting a

potential beneficial antileukemic effect in this subgroup.^[47] Clinical studies then concentrated on the usage of an HDAC inhibitor in combination with conventional chemotherapy, other targeting agents, or following allogeneic stem cell transplantation. Phase I/II studies of vorinostat and panobinostat used in combination with other chemotherapy for children with refractory/relapsed AML are both currently recruiting (NCT02419755, and NCT02676323).

Immunotherapy

Gemtuzumab ozogamicin (GO) is a cluster of difference (CD33)-specific antibody and the first immunotherapy agent that has been tested for the treatment of AML.^[48] Several clinical trials have confirmed its antileukemic effect in adult AML.^[49] With an OS of about 30%, GO was approved for the treatment of CD33-positive AML during the first relapse in patients >60 years of age who were not considered for cytotoxic chemotherapy.^[50]

Additional clinical trials focusing on the role of GO for the treatment of older AML patients unsuitable for intensive chemotherapy confirmed its therapeutic efficacy in this patient group. A Phase II study^[51] showed an improved response rate, and another study^[52] showed a beneficial single-agent therapeutic effect in patients aged >60 years. However, clinical research aimed at pediatric AML patients has proceeded to randomized trials. The Phase III Children's Oncology Group Trial AAML0531 demonstrated that pediatric AML patients with a higher CD33 expression had a significantly reduced RR and improved EFS with the addition of GO to conventional chemotherapy.^[53] Patients with a low CD33 expression experienced no benefit.

Despite the promising results of GO, its efficacy is limited by unstable drug conjugation; adverse drug reactions, especially liver toxicity; and a high incidence of multidrug resistance.^[54] To optimize the efficacy of anti-CD33 antibodies, a CD33-targeting antibody-drug conjugate, SGN-CD33A, was introduced. Preclinical testing and pilot clinical trials have verified its antileukemic activity as a single-agent or in combination with cytotoxic chemotherapy.^[55,56]

As CD33, which is expressed on malignant cells in the vast majority of AML patients, could be used as a target, other biomarkers that are recognizable by a certain antibody could be used as well. The "cancer-targeting" antibodies that are currently in Phase I/II clinical trials include CD98 antibody, killer-cell immunoglobulin-like receptor (KIR) antibody, programmed cell death protein 1 (PD1) antibody, Stage II-specific human thymocyte differentiation antigen (JL1) antibody, cytotoxic T-lymphocyte associated protein 4 (CTLA4) antibody, and CXCR4 antibody.^[54] More targets have yet to be discovered.

Cellular therapy

Cellular therapy, especially chimeric antigen receptor T-cell immunotherapy (CAR-T), is a fast-developing field in cancer therapeutics. The application of CD19 CAR-T in acute lymphoblastic leukemia (ALL) has been demonstrated to be an inspiring potential for remission induction

in refractory/relapsed patients.^[57] Likewise, with the identification of cancer-specific antibodies, cellular therapies employing not only T-cells but also natural killer cells are being tested in preclinical and clinical studies. Ehninger *et al.*^[58] studied the distribution of CD33 and CD123 in a cohort of 319 AML patients. The results showed that 87.8% of patients with AML expressed CD33, while 77.9% expressed CD123 and 9.4% expressed CD123 without CD33 expression. Hence, nearly all (potentially 97.2%) patients with AML could be treated with anti-CD33 or anti-CD123 antibodies. Therefore, the development of cellular therapy has been focusing on these two surface markers.

CD33 was among the first AML transmembrane receptors to be targeted.^[59] We have described the application of an anti-CD33 monoclonal antibody, GO, as a novel targeting agent. A similar approach using genetically engineered T-lymphocytes bearing an anti-CD33 antibody as an “identifier” and the cell as an “effector” is being tested in various studies. Preclinical studies have shown that CAR-T-cells targeting CD33 (CAR-T33) exhibited significant effector functions in the eradication of leukemia *in vitro*, but significant toxicity was a cost of the benefit. Recently, a transiently expressed “biodegradable” anti-CD33 CAR was developed using RNA modification and showed potent antileukemic activity. Whether it could be used alone or as a bridge to allogeneic transplantation in refractory/relapsed AML necessitates additional study.^[60]

CD123, another molecular target, has emerged as a more specific entity for AML blasts and AML leukemic stem cells.^[59] Human CD123-redirected T-cells (CAR-T123) showed significant blast-reducing activity in AML mice models. However, the selectivity of CAR-T123 was questioned in a recent study involving normal human fetal liver CD34⁺ cells that were also subjected to the “killing” mechanism of CAR-T123.^[61] To improve the specificity for AML blasts, a dual-affinity retargeting (DART) molecule generated from antibodies to CD3 and CD123 was designed. A recent study demonstrated that CD3 × CD123 DART induced dose-dependent killing of AML cell lines and primary AML blasts both *in vitro* and *in vivo*.^[62] Additional preclinical studies are necessary to demonstrate its safety before CAR-T123-T therapy enters clinical research.

Limitation

The role of microRNA in AML diagnosis,^[63] emerging targeting agents such as IDH inhibitors,^[64] and ongoing clinical trials is not discussed in the review due to the limitation length of this review. Readers are encouraged to use this review as a start point for further and more detailed insights into the novel developments of AML diagnosis and treatment.

CONCLUSION

A major task for medical workers is to improve the survival of AML patients while minimizing treatment-related toxicity. We are hindered by the diversity of oncogenes that evolve during the pathogenesis of malignancy, the heterogeneity of

expanding genetic subgroups, and the efficiency and costs of diagnostic tests. Gene sequencing techniques should set the basis for next-generation diagnostic methods. Further, target therapy should be the focus of future clinical research in the exploration of therapeutic possibilities.

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Conflicts of interest

There are no conflicts of interest.

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