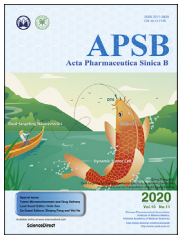
Acta Pharmaceutica Sinica B 2020;10(11):2125–2139

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com



REVIEW

Novel agents targeting leukemia cells and immune microenvironment for prevention and treatment of relapse of acute myeloid leukemia after allogeneic hematopoietic stem cell transplantation

Wei Shi[a,b,*,†], **Weiwei Jin**[c,d,†], **Linghui Xia**[a], **Yu Hu**[a]

[a]*Institute of Hematology, Union Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430022, China*
[b]*Fred Hutchinson Cancer Research Center, Seattle, WA 98109-1024, USA*
[c]*Department of Cardiovascular, Optical Valley School District, Hubei Provincial Hospital of Traditional Chinese Medicine, Wuhan 430061, China*
[d]*Hubei Province Academy of Traditional Chinese Medicine, Wuhan 430074, China*

Received 2 April 2020; received in revised form 27 May 2020; accepted 5 June 2020

KEY WORDS

AML;
Targeted therapy;
Relapse;
Immune microenvironment;
Allogeneic hematopoietic stem cell transplantation;
Oncogenic effectors;
Metabolism;
Surface markers

Abstract Relapse remains the worst life-threatening complications after allogeneic hematopoietic stem cell transplantation (allo-HSCT) in patients with acute myeloid leukemia (AML), whose prognosis has been historically dismal. Given the rapid development of genomics and immunotherapies, the interference strategies for AML recurrence have been changing these years. More and more novel targeting agents that have received the U.S. Food and Drug Administration (FDA) approval for *de novo* AML treatment have been administrated in the salvage or maintenance therapy of post-HSCT relapse. Targeted strategies that regulate the immune microenvironment of and optimize the graft *versus* leukemia (GVL) effect of immune cells are gradually improved. Such agents not only have been proven to achieve clinical benefits from a single drug, but if combined with classic therapies, can significantly improve the poor prognosis of AML patients who relapse after allo-HSCT. This review will focus on currently available and promising upcoming agents and also discuss the challenges and limitations of targeted therapies in the allogeneic hematopoietic stem cell transplantation community.

*Corresponding author.
E-mail address: shiwei076@gmail.com (Wei Shi).
†These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2020.06.012

1. Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is the backbone therapy for patients with intermediate or high-risk acute myeloid leukemia (AML) who are eligible for intensive therapy. Relapse still represents the major cause of treatment failure and up to 50% of AML patients finally relapse after allo-HSCT, about 72%−85% of relapses occur in the first year[1−3]. Their prognoses are generally poor, many of which can neither tolerate nor respond to conventional treatments. According to reports, the median overall survival (OS) after hematological relapse is only 4−6 months[2,4,5], and 1-year OS rate is about 20%[5−8]. Furthermore, even with donor cell therapy can only rescue a minority of patients in the long run. The 2-year OS rates of AML patients who relapsed after allo-HSCT and received palliative therapy, donor lymphocyte infusion (DLI), or second transplantation were 29.7%, 27.6% and 17%−22%, respectively[2,5]. The dismal success of salvage therapies means that novel strategies are needed to prevent and/or treat relapse after allo-HSCT.

Although a number of factors come into play, including resistance to traditional treatments, relapse indicates that the leukemia cells have managed to escape from the control of donor immune sytsem[9]. Leukemia cells make themselves "invisible" to donor-derived T cells by losing genomic human leukocyte antigen (HLA) or downregulating major histocompatibility complex (MHC) class II genes[10,11]. Besides loss of HLA leading to less alloantigen recognition, regulatory T cell (T_{reg}) infiltrating and leukemia-specific T cells display exhaustion markers are the other arm of immune escape. Exhausted $CD8^+$T cells accumulate in the bone marrow of relapsing patients after allo-HSCT, of whom 67.8% expressed one or more immune inhibitory receptors (IRs)[12,13]. Donor natural killer (NK) cells are the first reconstituted immune cells, both the donor and the host present all killer-cell immunoglobulin-like receptors (KIRs) in the form of donor's[14,15]. Especially, allografts from *KIR2DS1* or *KIR B* positive donor have stronger anti-leukemia effect[16−18].

Giving the rapid improving of deep sequencing techniques, the genetic driver mutations in AML are better understood and more and more novel targeting agents are synthesized. While these new developments in U.S. Food and Drug Administration (FDA) approval are welcome, more than 7 new targeted agents have received FDA approval for the treatment of AML during last three years[19]. Not only single agents but also the combination with conventional therapies has obviously improved the outcomes of high-risk AML patients after allo-HSCT. In addition, targeted immunotherapy, such as checkpoint inhibitors, engineering donor lymphocytes and chimeric antigen receptor (CAR) T cells, have been administrated to treat and/or prevent recurrence. This review will not only focus on the directly/indirectly targeted therapies to leukemia cells, but also clarify targeted strategies that interfere with the immune microenvironment and optimize the graft *versus* leukemia (GVL) effect of immune cells. Giving the rapid evolution of this field, we have selected relevant articles mainly based on the intention of current applicability.

2. Targeting leukemia cells

Recently, more and more novel agent winds have filled the sail of targeted therapy boats to leukemia cells, which don't just "direct hit" against all hematopoietic cells[20]. Targeted therapies aim to leukemia cells can be divided into three groups. Firstly, targeted agents act on oncogenic effectors of recurrent AML-associated mutations. Examples of such agents include fms-related tyrosine kinase 3 (*FLT3*), B-cell leukemia/lymphoma-2 (BCL-2), isocitrate dehydrogenase 1/2 (*IDH1/2*) and hedgehog signaling pathway inhibitors. Secondly, novel agents disrupt key metabolism of leukemia cells without directly damaging DNA. Examples include DNA hypomethylating agents and histone deacetylases inhibitors. A final group consists of leukemia epitope-targeting agents. Such immunotherapeutic strategies include antibody conjugate cytotoxic agents and antibody-based cellular therapies. Most agents will be formally introduced in the review.

2.1. Targeting oncogenic effectors of leukemia cells

2.1.1. FLT3 inhibitors

FLT3, a cytokine receptor (CD135) belonging to the receptor tyrosine kinase class III, which takes a pivotal role in myeloid and lymphoid cell proliferation and survival. It generally includes two mutations: *FLT3* internal tandem duplications (*FLT3-ITDs*) and point mutations in the tyrosine kinase activating loop of the kinase domain *FLT3-TKD*[19]. Such mutations have been reported in approximately one third of patients with AML and are associated with higher relapse rates[21]. Sorafenib, midostaurin, quizartinib, gliteritinib and crenolanib are designed to target *FLT3* and have been used to interfere with the relapse of *FLT3* positive AML after allo-HSCT.

2.1.1.1. First generation FLT3 inhibitors. Sorafenib has been used to treat relapsed *FLT3-ITD* positive AML following allo-HSCT. In a large registered study, 409 relapsed *FLT3-ITD* positive patients after allo-HSCT were analyzed. There were five arms in the study. The complete remission (CR) and 1-year OS of DLI arm were 22% and 17%, respectively, which increased to 67% and 47% when used in combination with sorafenib[22]. The studies from European Society for Bone Marrow Transplantation (EBMT) and China showed similar results that sorafenib combined with DLI obviously improved the OS and leukemia free survival (LFS) of relapsed *FLT3-ITD* positive patients following allo-HSCT[23,24]. As a preventive or maintenance medication after allo-HSCT, sorafenib decreased the 3-year incidence of relapse (CIR) of *FLT3-ITD* positive patients from more than 50%−15% in a series of retrospective studies[24−30]. For the safety of sorafenib as a prophylactic agent, a prospective study depicted that the 3-year OS

was 76% and the most common 3/4 adverse events were hepatic enzymes (23%) and thrombocytopenia (17%)[31]. In a randomized phase 3 trial, the other first generation FLT3 inhibitor, midostaurin or placebo, was used for 717 patients from induction therapy to maintenance therapy due to their capability of targeting both *TKD* and *ITD* mutations, and 57% of the patients discontinued the trial therapy because of allo-HSCT. Although patients who achieved CR1 and received allo-HSCT in both groups did not reach the median OS, the OS in the midostaurin group was significantly longer than that in the placebo group, at 69.8 and 21.8 months, respectively[32]. After that, as a prophylactic agent in a phase 2 hypothesis-generating trial, midostaurin controlled the post-HSCT 2-year CIR of *FLT3* positive patients to 13.3%[33].

2.1.1.2. The next generation FLT3 inhibitors. As the next generation *FLT3* inhibitors, quizartinib, gliteritinib and crenolanib were more targeted than the first generation, and thereby off-target-associated toxicities and side effects were decreased. Quizartinib demonstrated acceptable tolerability for *de novo* or post-HSCT AML patients in the phase 1 dose escalation studies, and the most common grade 3/4 adverse events were neutropenia, thrombocytopenia, and anemia[34,35]. For patients with relapsed/refractory (R/R) *FLT3-ITD* positive AML, the rate of quizartinib monotherapy bridging allo-HSCT reached 32%, which was significantly higher than 11% of salvage chemotherapy[36], especially in the 60 mg/day group[37]. For R/R *FLT3*-mutated patients, another inhibitor, gilteritinib, had an overall response rate (ORR) of 80% in the phase 1 clinical trial and was well tolerated[38]. In a phase 3 trial, 371 R/R *FLT3*-mutated patients were randomly assigned in a 2:1 ratio to receive either gliteritinib (at a dose of 120 mg/day) or salvage chemotherapy. Gilteritinib resulted in significantly longer LFS (2.8 *vs.* 0.7 months) and higher CR percentage (21% *vs.* 10.5%) than salvage chemotherapy[39]. As a potent typeI pan-*FLT3* inhibitor, crenolanib retains the activity against *FLT3-TKD* mutation, which is a candidate for patients resistant to the other *FLT3* inhibitors. Cumulatively, a high ORR (28%) was achieved with crenolanib monotherapy for such patients[40,41]. Since the phase 2/3 clinical trials of the next generation FLT3 inhibitors for maintenance therapy after allo-HSCT are being recruited, and none of them use a first-generation inhibitor as a control, so it is impossible to determine whether they are superior to the first-generation agents.

2.1.2. *BCL-2 inhibitors*

BCL-2 is an anti-apoptotic protein which binds to the BH3 and inhibits the apoptosis of hematologic malignancies. As a BCL-2 inhibitor, venetoclax competitively binds to the BH3 domain of BCL2, releases BH3-only proteins and induces apoptosis[42]. A phase 2 study demonstrated that venetoclax monotherapy for R/R AML or unfit for intensive chemotherapy patients achieved an ORR of 19% with an acceptable safety profile[43]. Currently, venetoclax has being investigated in a series of phase 1 studies in combination with low-dose cytarabine and decitabine or azacitidine[44]. For older *de novo* AML patients (median 75 years), venetoclax in combination with azacytidine achieved the CR/CRi rate up to 85%, compared with the 51% for conventional therapy ($P = 0.0019$)[45]. Such two-drug combination treatments reduced succinate dehydrogenase glutathionylation, impaired the tricarboxylic acid cycle, and depleted ATP in leukemia stem cells[46]. In addition, only a few patients who relapse after allo-HSCT have achieved CR after combination therapy with venetoclax and low-dose cytarabine or DNA hypomethylating agents[47,48]. Currently, there are two initiated prospective studies to evaluate the efficacy of venetoclax in combination with azacitidine to improve relapse free survival (RFS) in AML patients when given as maintenance or preemptive therapy following allo-HSCT. For patients presenting WBC above 25 G, uric acid above 7.5 mg/dL, or creatinine above 124 mmol/L, physicians need to know that the initiation of venetoclax-based therapy may elevate the risk of tumor lysis syndrome (TLS)[49].

2.1.3. *IDH1/2 inhibitors*

Somatic mutations within the conserved active site of isocitrate dehydrogenases (*IDH*) 1 and 2 occur in 6%−10% and 8%−19% of patients with AML, respectively. These mutations cause accumulation of the oncogenic metabolite *R*-2-hydroxyglutarate (2-HG), 2-HG competitively inhibits α-ketoglutarate-dependent enzymes, leading to DNA and histone hypermethylation and impairing hematopoietic differentiation[50,51]. Ivosidenib and enasidenib are oral targeted *IDH1* and *IDH2* inhibitors, respectively[52]. In a phase 1 study of *IDH1*-mutated R/R AML, administration ivosidenib at a dose of 500 mg daily achieved durable CR (21.6%, 9.3 months) and ORR (41.6%, 6.5 months) with a low frequency of grade 3 or higher adverse events[52]. For *IDH2* mutation R/R AML patients, enasidenib was well tolerated and produced an ORR of 40 % in a phase 1 trial[50,53]. Almost 20% patients attained CR, 43.1% red blood cell and 40.2% platelet transfusion-dependent patients achieved transfusion independence[51]. In addition, isocitrate dehydrogenase differentiation syndrome (IDH-DS) is a potentially lethal and recognizable complication. In a retrospective study of IDH-DS in 281 R/R AML patients, researchers recognized that approximately 12% of the patients were identified as IDH-DS, prompt diagnosis and systemic administration corticosteroids were effective for them[54].

2.1.4. *Hedgehog signaling pathway inhibitors*

The Hedgehog (Hh) signaling pathway should be silenced in adults[19], however, overexpression of Hh pathway components is observed in myeloid leukemia cells resistant to chemotherapy, especially in leukemia stem cells[55]. Aberrant Hh pathway releases transmembrane protein smoothened (SMO), which activates glioma (GLI)-associated proteins[56]. GLI is translocated into the nucleus and binds the promoter region of target DNA to express specific oncogenes, such as those encoding c-MYC, BCL2, and SNAIL[55]. Glasdegib is an oral small molecule inhibitor of SMO, which prevents SMO from translocating into the primary cilia[56]. In a phase 1b study, glasdegib was administered once daily in 28-day cycles in combination with low-dose cytarabine (arm A), decitabine (arm B) or cytarabine/daunorubicin (arm C), and the recommendation dose for glasdegib was 100 mg/day[57]. The following phase 2 study showed that 46.4% of patients achieved CR, and the median OS was 14.9 months. The most common treatment-related adverse events ($\geq$50% patients) were diarrhea and nausea[56]. In a randomized phase 2 trial of AML or high-risk myelodysplastic syndrome (MDS) unsuitable for intensive chemotherapy, patients were randomized (2:1) to glasdegib (100 mg/day) and low dose cytarabine (LDAC) or LDAC (20 mg, Bid) only arms. The CR rate and median OS of the two arms were 17% *vs.* 2.3% ($P < 0.05$) and 8.8 months *versus* 4.9 months ($P = 0.0004$), respectively[58]. In a randomized phase 3 clinical

trial, the efficacy of glasdegib maintenance *vs.* clinical observation after allo-HSCT will be evaluated until December 2026.

2.2. *Targeting key metabolism of leukemia cells*

2.2.1. *DNA hypomethylating agents (HMAs)*

HMA not only leads to hypomethylation of the promoter of silenced tumor suppressor genes, but also has the ability to enhance antigenicity and regulate immune checkpoints, and can induce $CD8^+$T cells to respond to tumor antigen after transplantation, thereby increasing GVL response without increasing the risk of graft *versus* host disease (GVHD)[59−61]. Azacytidine (AZA) and decitabine (DEC) are not really novel agents, but as the representative of HMA, clinicians have been very enthusiastic about their application in recurrence patients after allo-HSCT. In a series of retrospective studies, salvage therapies with AZA or DEC for morphological recurrence rescued very limited patients. Even with DLI booster immunotherapy, only 3%−27% of patients achieved CR, and the 2-year OS was between 11% and 29%[60−65]. In order to enhance the antitumor efficacy of HMA, 29 relapsed patients post-HSCT were treated with sequential AZA (75 mg/m^2, 7 days) followed by escalating doses of LEN from Day 10−30 in the VIOLA trial. Almost 47% of patients achieved a major clinical response and 40% achieved a CR/CRi after LEN/AZA therapy[66]. Because HMA was safe for long-term use, AZA monotherapy was given as an MRD-guided preventive treatment in a phase 2 trial to prevent morphological recurrence. A total of 198 patients were screened in the study, 60 of whom developed MRD ($CD34^+$ cell donor chimerism<80%, fusion or mutant gene>1%) during the 24-month screening period, and 53 of them were treated. Six months after initiating AZA treatment, 58% of patients were free of relapse and alive, while 75% of patients survived more than 12 months[67]. For initiating $CD8^+$ T cell response to tumor antigens, AZA maintenance therapy in high-risk AML patients after allo-HSCT was well tolerated and has the capacity to reduce the relapse risk[68,69]. In a series of preventive treatments, low dose AZA in combination with DLI reduced the CIR of high-risk patients without increasing the cumulative incidence of GVHD[70]. CC-486 is an oral AZA, which can be used more conveniently. In a prospective phase 1/2 dose-finding study, CC-486 maintenance therapy for high-risk AML patients post-HSCT was generally well tolerated with low relapse rates (21%) and low GVHD (10%)[71]. Although the double-blind, phase 3, randomized study of CC-486 has just been initiated on June 14, 2019, for patients who need long-term home medication after allo-HSCT, their compliance to it will be better than traditional AZA and more likely to benefit from it.

2.2.2. *Histone deacetylases inhibitors (HDACi)*

Myeloid oncoproteins aberrantly recruit histone deacetylases (HDAC), causing chromatin remodeling and inhibiting the expression of tumor suppressor genes such as TP53[72]. Panobinostat (PAN) is a potent oral HDACi, which can modulate the acetylation of histone proteins and protein chaperones in malignant cells[73]. In the phase 1/2 PANOBEST trial, PAN was prophylactically administrated after allo-HSCT for AML or MDS. At 2 years after the first PAN dose, the cumulative incidence of relapse, non-relapse mortality and OS across all dose levels was 20%, 5% and 81%, respectively. About 52% of patients experienced reversible PAN-related G3/4 adverse events and 29% of patients experienced GVHD[74]. PAN (30 mg)+AZA (75 mg/m^2) or AZA monotherapy was administered to high-risk MDS, CMML or AML with low burden (<30%) blasts in a phase 1b/2b trial. Although patients in PAN + AZA arm achieved better CR/CRi (27.5% *vs.* 14.3%), which resulted in more grade 3/4 adverse events (97.4% *vs.* 81.0%) and treatment-related deaths (13.2% *vs.* 4.8%)[73]. Lower dose PAN (20 mg) in combination with idarubicin and cytarabine (3 + 7) as inducing therapy for younger patients (median age, 55.5) with high-risk AML demonstrated tolerable toxicity and improved CR/CRi (60.9%)[75]. For older AML patients (median age, 69), the CR/CRi of PAN with "3 + 7" strategy was 32%, which was significantly associated with an increase in histone acetylation in peripheral blood mononuclear cells (PBMCs) after treatment[76]. In March 2020, a randomized multicenter phase 3 study was initiated to assess the efficacy of PAN 20 mg oral three times weekly every second week *vs.* standard of care following allo-HSCT. Since February 2019, another HDACi (vorinastat) combined with low-dose AZA as post-HSCT maintenance treatment has been studied in a phase 1 dose-escalation clinical trial, and the results will be announced after December 2021.

2.3. *Targeting leukemic surface markers*

2.3.1. *Targeting CD33/CD44/CD123*

2.3.1.1. Monoclonal antibodies. Some epitopes such as CD33, CD44 and CD123 are always expressed on leukemic blasts, but are not presented on normal hematopoietic stem cells. Especially, CD33 is expressed on leukemia cells in 90% of AML patients[77,78]. Clinical trials of humanized monoclonal antibodies against CD33 (SGN-33), CD44 (RG7356), and CD123 (CSL360) have generally shown good tolerability and limited antileukemia activity, but only when the burden of leukemia is small and in case of long-term infusion[77,79−81]. To improve the efficacy of antibodies against CD33 positive blasts, the researchers constructed a series of TandAbs composed of anti-CD33 and anti-CD3, which can redirect cytotoxic immune cells toward CD33 positive blasts and induce effective dose-dependent cytolysis *in vitro* and in xenograft models[82].

2.3.1.2. Antibody conjugate cytotoxic agents. Antibody conjugate cytotoxic agents provide a method for delivering cytotoxic agents to leukemia cells, thereby increasing dose intensity while reducing toxicity[83]. Gemtuzumab ozogamicin (GO) consists of a humanized anti-CD33 monoclonal antibody and the DNA intercalator calicheamicin, which received accelerated FDA approval in 2000 as a new AML monotherapy, but withdrew from the market in 2010 due to safety considerations, and gained full FDA and EMA approval for CD33 positive AML first-line and relapse treatment in 2017 and 2018, respectively[19]. A meta-analysis of 5 phase-3 trials comprising 3325 AML patients reminded that GO significantly reduced the relapse rates and improved OS in the cytogenetic favorable and intermediate risk groups without increasing toxicity[84]. A series of studies found that GO monotherapy or in combination with conventional therapies can improve the recurrence rates or OS of pediatric patients (1 month−30 years), younger (18−65 years) or older patients (62−88 years) in *de novo* or R/R AML[85−88]. There is a highly variable in the proportion of CD33-positive cells and the expression of CD33 per cell in patients with CD33 positive AML[83,89]. Patients with higher CD33 expression, *NPM1* mutation, or CD33 single nucleotide polymorphism (rs12459419 = CT/TT) may benefit more from GO, especially at a lower dose (3 mg/m^2)[90−93].

In a small sample study, cytarabine and GO (9 mg/m^2) were used as post-HSCT salvage therapy for AML patients, but only 25% of patients survived more than one year and all relapsed[94]. In a study of CD33-positive leukemia children receiving reduced-intensity conditioning (RIC) transplantation and using CD33 as maintenance therapy, patients were given two doses of GO (8 weeks apart) in a dose-escalation design (4.5, 6, 7.5, and 9 mg/m^2) 6 days after allo-HSCT. The 1- and 5-year OS probabilities were 78% and 61%, respectively, and no toxicity that might be directly or directly related to GO was observed[95]. The data of phase 1 clinical trial for the preventive use of GO by patients receiving RIC allo-HSCT will be released as early as December 2020.

Vadastuximab talirine (SGN-CD33A, 33 A) is an antibody−drug conjugate consisting of pyrrolobenzodiazepine dimers and an anti-CD33 monoclonal antibody. In a phase 1 clinical trial, 33 A monotherapy was infused to CD33-positive AML patients at a dose of 40 mg/kg, and 28% of them achieved CR/CRi, of which 14% were MRD-negative[96]. In an expansion arm of this study, 33 A subsequently in combination with HMAs were provided to *de novo* CD33-positive older AML patients (median 75 years), and 70% of them achieved CR/CRi, of whom 51% were MRD-negative. In addition, the 30- and 60-day mortality rates were 2% and 8%, respectively[97]. In the terminated phase 1/2 trial (Table 1), 33 A was used as maintenance therapy after allo-HSCT. Unpublished data showed that the most common adverse events were neutropenia and thrombocytopenia.

2.3.2. *Targeting Wilms' tumor antigen 1 (WT1)*

WT1 is a non-polymorphic intracellular protein that promotes proliferation and carcinogenesis in AML, and is overexpressed 10−1000 times in leukemia cells compared to normal CD34 positive cells[98,99]. In a phase 1 pilot study, WT1-directed peptide vaccination was injected subcutaneously to 16 heavily pretreated AML and MDS patients, which exhibited a protective immune response (IR) and was well tolerated[98]. In a phase 2 study, injection of the WT1 peptide vaccine stimulated a specific IR in patients with AML and was associated with the survival in excess of 5 years[100]. After dendritic cells (DCs) were electroporated with *WT1* mRNA and injected as a vaccine into AML patients with CR1, they induced the response of WT1-specific CD8$^+$ T, reduced the relapse rate by 25%, and were well tolerated[101]. In a small pilot study, HLA-A2 positive recipients were vaccinated with a WT1 peptide-loaded donor-derived DC vaccine every two weeks and given a DLI every four weeks to increase specific GVL effect, which was well tolerated and no grade 3 or higher adverse events directly related to the vaccine were found[102]. Because WT1-specific (*HLA-A* 24: 02*) TCR-T cells can survive *in vivo* and retain a specific immune response to WT1[103], Chapuis et al.[99]. isolated a high-affinity WT1-specific TCR (TCR_{C4}) and inserted it into EBV-specific donor CD8$^+$ T cells to make the $T_{TCR\text{-}C4}$ cells. Twelve high-risk patients after allo-HSCT received at least one preventive infusion of TCR_{C4} (total 21 infusions). Compared with 88 patients in a concurrent comparative group, the median 44-month RFS was 100% *vs.* 54% without increasing the risk of chronic GVHD. However, the data as salvage or preemptive treatment have not yet been released.

2.3.3. *Targeting chemokine (C-X-C motif) receptor 4 (CXCR4)*

CXCR4 is a key player of AML blasts, which binds with CXCL12 for retention of leukemia cells in the protective bone marrow (BM) microenvironment[104,105]. The CXCR4 antagonist BL-8040 induced the robust mobilization of AML blasts from the BM and induced the apoptosis of AML cells by the upregulation of *miR-15a/miR-16-1* resulting in downregulation of BCL-2, MCL-1 and cyclin-D1. Synergized treatment with a BCL-2 inhibitor or *FLT3* inhibitor prolonged the survival of AML-bearing mice and reduced their MRD[104]. In a preclinical study, a humanized CXCR4 immunoglobulin G1 antibody-PF-06747143 bind to CXCR4 and inhibited CXCL12-mediated signaling pathway. PF-06747143 monotherapy or synergy with standard drugs has shown strong antitumor effects in a chemo-resistant AML patient-derived xenograft model[106]. CX-01 is a low-anticoagulant heparin, which can disrupt CXCL12-mediated cell sequestration in the bone marrow. In a pilot study, 12 naive AML patients were treated by CX-01 in combination with "3 + 7" inducing chemotherapy. Eleven patients (92%) achieved morphologic CR after one cycle of induction, and the median disease-free survival was 14.8 months. No CX-01-associated serious adverse events were observed[107]. Pentixafor is an effective CXCR4 antagonist. Three patients with AML relapse after the first allo-HSCT were given targeted endoradiotherapy consisting of 68 Ga-pentixafor, and all of them achieved leukemia clearance and successfully bridged to the second allo-HSCT[105]. Leukemia stem cells always employ CXCL12/CXCR4 to home toward the protective marrow niches[106]. In a phase 1 study, one dose plerixafor was injected to 12 patients before the first dose of fludarabine and busulfan to make sure more leukemic stem cells deletion before allo-HSCT. The engraftment rate was 100%, the median OS was 67 months, and only 2 patients (17%) relapsed[108].

2.3.4. *CAR T cell therapy*

CAR T cell therapy has gained 90% of response rates in CD19$^+$ ALL and CLL, which combines a monoclonal antibody of tumor epitope to intracellular T-cell receptor signaling domain by taking advantage of a single-chain variable fragment (scFv). Because of the greater heterogeneity of AML, one of the major challenges in translating the amazing clinical success of CAR T cells in ALL into AML is to find suitable leukemia cell surface markers[109]. Over the past five years, studies have been changing from using traditional AML antigens (such as CD33, CD123, CD44, TIM-3 or CD13) to using normal hematopoietic stem cells that do not express, while leukemia cells or leukemia stem cells overexpress antigens (*e.g.*, folate receptor β, CLL-1, NKG2D, CD7 or FLT3) to reduce the off-target toxicities[109−115]. The efficacy of CAR T cells is often related to its durability *in vivo*. Zheng et al.[116] improved the persistence of CAR T cells *in vivo* by combining the PI3K inhibitor LY294002. AML is characterized by the presence of heterogeneous blast cells, and almost no antigen is present on every leukemia cell. Researchers tried to use dual targeting CAR T cells (such as anit-CD33/CD123 or anti-CD13/TIM3) for targeting leukemia cells to reduce antigen escape-associated relapse[117,118]. In addition to selecting tumor-specific targets, directly reducing tumor-associated antigens expression in normal hematopoietic stem cells can indirectly increase the specificity of tumor-associated antigens. For example, CD33 is expressed on leukemia cells in 90% of AML patients. However, almost all normal myeloid cells also express CD33[119]. The researchers generated *CD33* knockout (KO) hematopoietic stem and progenitor cells (HSPC) and demonstrated that they can be implanted and differentiated normally in immunodeficient mice and rhesus monkeys[119,120]. CD33-ablated HSPC were impervious to CD33-targeted immunotherapy (CAR T or GO), allowing for

Table 1 Summary of clinical trials for novel agents targeting leukemia cells or immune microenvironment following allo-HSCT.

Target	Agent	Intervention strategy	Therapy regimen	Identifier (phase)	Status	Complete date	With results
Oncogenic effectors							
FLT3	Sorafenib	Salvage	+/−DLI	NCT02867891 (no)	Completed	12/2016	Yes
		Maintenance	Mono	NCT01398501 (1)	Completed	8/2016	Yes
		Maintenance	Mono	NCT02474290 (2/3)	Completed	8/2019	Yes
		Maintenance	Mono	NCT01578109 (no)	Active, not recruiting	12/2025	Yes
		Maintenance	Mono	NCT03247088 (1/2)	Recruiting	7/2022	No
	Midostaurin	Preemptive	Mono	NCT03951961 (2)	Not yet Recruiting	12/2023	No
		Maintenance	Mono	NCT01477606 (2)	Active, not recruiting	6/2020	Yes
		Maintenance	Mono	NCT01883362 (2)	Completed	4/2018	No
	Quizartinib	Salvage/maintenance	Mono	NCT02039726 (3)	Active, not recruiting	12/2020	Yes
		Maintenance/ preemptive	Mono	NCT01468467 (1)	completed	3/2015	Yes
	Gliteritinib	Maintenance	Mono	NCT02997202 (3)	Recruiting	4/2025	No
	Crenolanib	Maintenance	Mono	NCT02400255 (2)	Recruiting	6/2021	No
BCL-2	Venetolax	Maintenance/ preemptive	+AZA	NCT04161885 (3)	Recruiting	8/2024	No
		Maintenance/ preemptive	+AZA	NCT04128501 (2)	Not yet Recruiting	10/2022	No
IDH1	Ivosidenib	Maintenance	Mono	NCT03564821 (1)	Recruiting	7/2024	No
IDH2	Enasidenib	Maintenance	Mono	NCT03515512 (1)	Recruiting	5/2024	No
		Maintenance	Mono	NCT03728335 (1)	Recruiting	12/2020	No
SMO	Glasdegib	Maintenance	Mono	NCT04168502 (3)	Not yet Recruiting	12/2026	No
Key metabolism							
DNA methylation	Azacitidine	Salvage	Mono	NCT01083706 (2)	Completed	12/2013	Yes
		Salvage	Mono	NCT02017457 (2)	Completed	11/2019	Yes
		Salvage	Mono	NCT00422890 (3)	Completed	12/2009	Yes
		Salvage	+DLI	NCT00795548 (2)	Completed	8/2011	Yes
		Salvage	+IFN	NCT04078399 (no)	Recruiting	5/2020	No
		Salvage	+Chemo + DLI	NCT01390311 (1)	Completed	4/2015	Yes
		Salvage	+LEN	ISCRCTN98163167 (1)	Completed	12/2018	Yes
		Salvage	+LEN + DLI	NCT02472691 (2)	Active, not recruiting	9/2020	No
		Salvage	+Chemo + DLI	NCT01369368 (1/2)	Recruiting	10/2020	No
		Preemptive	Mono	NCT01462578 (2)	Active, not recruiting	8/2020	Yes
		Preemptive	Mono	NCT03850418 (2)	Recruiting	2/2024	No
		Preemptive	+DLI	NCT01541280 (2)	Completed	7/2015	Yes
		Maintenance/ preemptive	+/-DLI	NCT02458235 (2)	Active, not recruiting	4/2020	No
		Maintenance	Mono	NCT01995578 (2)	Recruiting	11/2021	No
		Maintenance	Mono	NCT00350818 (1)	completed	8/2010	No
		Maintenance	Mono	NCT00887068 (3)	completed	8/2018	No
		Maintenance	+Nivolumab	NCT04128020 (1)	Recruiting	10/2022	No
		Maintenance	+APR-246	NCT03931291 (2)	Recruiting	9/2021	No
		Maintenance	+Valproic Acid	NCT02124174 (2)	Recruiting	1/2021	No
		Maintenance	Mono	NCT01168219 (2)	Active, not recruiting	11/2015	Yes
	Decitabine	Salvage	+DLI	NCT01758367 (1/2)	Unknown	6/2018	Yes
		Salvage	+2nd HSCT	NCT00002832 (1/2)	Completed	3/2002	No
		Salvage	+Ruxolitinib + DLI	NCT04055844 (2)	Recruiting	1/2025	No
		Preemptive	Mono	NCT03663751 (2)	Recruiting	3/2021	No
		Preemptive	+DLI	NCT03662087 (2/3)	Recruiting	12/2022	No
		Maintenance	Mono	NCT01809392 (2/3)	Unknown	12/2015	No
		Maintenance	Mono	NCT01277484 (1)	Unknown	12/2015	Yes
		Maintenance	Mono	NCT00986804 (1)	Completed	2/2016	Yes
		Maintenance	+DLI	NCT03771222 (2)	Not yet Recruiting	12/2021	No

Table 1 (*continued*)

Target	Agent	Intervention strategy	Therapy regimen	Identifier (phase)	Status	Complete date	With results
	Guadecitabine	Salvage/preemptive	+DLI	NCT02684162 (2)	Recruiting	6/2021	No
		Maintenance	Mono	NCT03454984 (2)	Not yet Recruiting	3/2022	No
	CC-486	Maintenance	Mono	NCT01835587 (1/2)	Completed	5/2017	Yes
		Maintenance	Mono	NCT04173533 (3)	Recruiting	6/2024	No
Histone deacetylases	Panobinostat	Maintenance	Mono	NCT01451268 (1/2)	Unknown	4/2018	Yes
		Maintenance	Mono	NCT04326764 (3)	Recruiting	10/2023	No
	Vorinostat	Maintenance	+AZA	NCT03843528 (1)	Recruiting	12/2021	No
Epitopes							
CD33	BI 836858	Salvage	+F16IL2	NCT03207191 (1)	Unknown	12/2019	No
	GO	Salvage	Mono	NCT00044733 (2)	Completed	9/2004	Yes
		Maintenance	Mono	NCT01020539 (1)	Active, not recruiting	12/2020	No
		Maintenance	Mono	NCT02117297 (2)	Recruiting	12/2022	No
	33 A	Maintenance	Mono	NCT02614560 (1/2)	Terminated	9/2017	No
CD38	Daratumumab	Salvage	+DLI	NCT03537599 (1/2)	Recruiting	9/2021	No
WT1	DC vaccine	Salvage/preemptive	+DLI	NCT00923910 (1/2)	Completed	11/2016	Yes
		Preemptive	+DEC	NCT01483274 (1)	Completed	6/2015	No
	WT1-sensitized T	Maintenance/ preemptive/salvage	+/−(FLU + CTX)	NCT01640301 (1/2)	Active, not recruiting	10/2030	Yes
		Salvage/preemptive	Mono	NCT00620633 (1)	Active, not recruiting	2/2021	No
	CTL	Salvage/preemptive	Mono	NCT00052520 (1/2)	Completed	6/2013	No
		Maintenance	Mono	NCT02895412 (1)	Recruiting	12/2020	No
CD123	Anti-CD123-CART	Salvage	Mono	NCT03114670 (1)	Recruiting	3/2021	No
Immune microenvironment							
CTLA-4	Ipilimumab	Salvage	+DLI	NCT00060372 (1)	Completed	4/2008	Yes
		Salvage	Or Nivolumab	NCT01822509 (1)	Active, not recruiting	12/2018	Yes
		Salvage	+LEN	NCT01919619 (2)	Recruiting	6/2021	No
		Maintenance	+/-Nivolumab	NCT02846376 (1)	Recruiting	7/2023	No
PD-1	Nivolumab	Salvage	Mono	NCT03146468 (2)	Recruiting	6/2020	No
		Salvage	+/-Ipilimumab	NCT03600155 (1)	Recruiting	6/2022	No
		Salvage	+Tocilizumab	NCT03588936 (1)	Recruiting	8/2022	No
		Maintenance	Mono	NCT02985554 (1)	Recruiting	3/2022	No
		Maintenance	Mono	NCT04361058 (1)	Recruiting	4/2025	No
	Pembrolizumab	Salvage	Mono	NCT02981914 (1)	Recruiting	2/2029	No
		Salvage	Mono	NCT03286114 (1)	Recruiting	10/2021	No
Engineering donor T	CD25hi T_{reg} depleted	Salvage	Mono	NCT00675831 (1)	Completed	1/2013	Yes
		Salvage	+Ipilimumab	NCT03912064 (1)	Recruiting	5/2024	No
	$CD8^{+}CD44hi$	Salvage	Mono	NCT01523223 (1)	Completed	10/2016	Yes
	CD45RA-delepted	Maintenance	Mono	NCT03849651 (2)	Recruiting	7/2025	No
Engineering donor NK	Purified NK	Salvage	+FLU + Ara-C or DEC	NCT04220684 (1)	Not yet Recruiting	12/2021	No
		Salvage	+CTX + FLU	NCT00526292 (2)	Completed	7/2015	Yes
		Maintenance	Mono	NCT00569283 (1)	Completed	12/2008	Yes
		Maintenance	Mono	NCT01795378 (1/2)	Completed	5/2015	Yes
		Maintenance	Mono	NCT00823524 (1/2)	Completed	2/2013	Yes
		Maintenance	Mono	NCT03300492 (1/2)	Recruiting	1/2023	No
		Maintenance	Mono	NCT04166929 (2)	Recruiting	1/2022	No
		Maintenance	Mono	NCT02727803 (2)	Recruiting	5/2021	No
		Maintenance	Mono	NCT01904136 (1/2)	Recruiting	4/2021	No
	CD4- iNKT	Maintenance	Mono	NCT03605953 (no)	Not yet Recruiting	4/2021	No
	CIML NK	Salvage	Chemo + DLI+	NCT03068819 (1)	Recruiting	11/2024	No
		Maintenance	+ALT-803	NCT02782546 (2)	Recruiting	2/2022	No
DC	DC/AML fusion cells	Maintenance	+DEC	NCT03679650 (1)	Recruiting	8/2024	No
	MiHA-loaded PD-L-silenced DC	Maintenance/ preemptive	Mono	NCT02528682 (1/2)	Recruiting	12/2020	No
CIK	IL-15 activated CIK	Preemptive	Mono	NCT02752243 (1/2)	Recruiting	3/2022	No
		Maintenance	Mono	NCT03669172 (1/2)	Recruiting	11/2021	No
IL-15	ALT-803	Salvage	Mono	NCT01885897 (1/2)	Active, not recruiting	6/2020	Yes

(*continued on next page*)

Table 1 (*continued*)

Target	Agent	Intervention strategy	Therapy regimen	Identifier (phase)	Status	Complete date	With results
	N-803	Maintenance	Mono	NCT02989844 (2)	Suspended (COVID-19)	1/2022	No

Complete date, actual or estimated study completion date; Salvage, salvage therapy for patients with hematological relapse; Maintenance, preventive therapy for high-risk patients without any sigh of underlying disease; Preemptive, preemptive therapy for patients with minimal residual disease; Mono, monotherapy; +/−, the clinical trial has two arms, monotherapy or in combination with other regimens; DLI, donor lymphocyte infusion; AZA, azacytidine; IFN, interferon; Chemo, chemotherapy; LEN, lenalidomide; APR-246, agent targeted TP53; 2nd HSCT, the second allo-HSCT; CC-486, oral azacytidine; BI 836858, a human anti-CD33 antibody; F16IL2, F16 antibody fused to human IL-2; GO, gemtuzumab ozogamicin; 33 A, vadastuximab talirine; DEC, decitabine; WT1-sensitized T, WT1-sensitized allogeneic T-lymphocytes; CTL, donor CD8+cytotoxic T lymphocyte clones specific for WT1; Tocilizumab, interleukin-6 receptor antagonist; FLU, fludarabine; Ara-C, cytarabine; CTX, Cyclophosphamide; iNKT, invariant NKT cells; CIML NK, cytokine-induced memory-like (CIML) natural killer cell; DC, dendritic cells; CIK, cytokine induced killer cells; ALT-803 or N-803, interleukin-15 (IL-15) super agonist complex; No, results unavailable.

efficient elimination of CD33 positive blasts without myelotoxicity, which provided new ideas for the application of CD33-targeted immunotherapy in combination with auto/allo HSCT.

3. Targeting immune microenvironment

With the recognition of immune-escaping driving to two-thirds of relapse post-HSCT, it seems valuable to rapidly translate it into personalized medication. For example, second transplantation from different donor for patients with genomic HLA loss[1,121,122], CPIs for patients with upregulation of IRs and inducing moderate chronic GVHD for those with downregulation of HLA class II genes[10−12]. However, in clinical practice, this translation will be subject to many restrictions, for example, alternative donor unavailable and relapse with active GVHD, etc. DLI is still an attractive treatment option for patients with high risks of recurrence or recurrence. Unfortunately, approximately 31.6%−66% of recipients obtained II−IV acute GVHD after DLI[123−126], 50% obtained chronic GVHD, and 26% of patients eventually died of GVHD related complications[127,128]. Using engineered donor T cells or NK cells to improve GVL effect and reduce GVHD is a promising strategy that is currently being extensively studied[129]. In addition, this review will describe strategies for activating endogenous IL-15 expression in the immune microenvironment to improve the GVL effect of immune cells and avoid systemic toxicity[130].

3.1. *Immune checkpoint inhibitors (CPIs)*

CTLA-4 and PD-1 are the well-known immune checkpoints for tumor immune escape[131]. CTLA-4 and PD-1 interact with ligands B7-1/B7-2 and PD-L1/PD-L2, respectively, and can inhibit the complete activation and proliferation of T cells[132,133]. Recently, monoclonal antibodies directed against immune checkpoints have benefited some patients who have relapsed after allo-HSCT. In the dose-escalation study, human anti-CTLA4 monoclonal-ipilimumab was infused in 29 patients (2 AML) who had relapsed more than 90 days after allo-HSCT or DLI. Ipilimumab did not result in clinically significant GVHD, and a response was observed in three lymphoma patients[134]. In the following phase 1/1b study, of the 22 patients receiving the 10 mg/kg dose, 23% had a complete response, including 4 patients with extramedullary AML and 1 patient with secondary AML. Because of GVHD, 4 patients had to discontinue further use of ipilimumab[133]. In a small sample study, the anti-PD1 antibody−nivolumab was given to 3 AML patients who had relapsed after allo-HSCT and failed standard therapies. One patient achieved an ongoing CR, one patient experienced disease stabilization and the side effects were tolerable[135]. In a review of 28 papers comprising 176 patients using CPIs (ipilimumab or nivolumab) after allo-HSCT, the ORR was 54% (CR 33%, PR 21%), 23% of patients developed GVHD and 28% of mortality was GVHD related[132]. However, Christopher et al.[10] reported that the expression of PD1 on T cells was undetectable in 15 AML patients who had relapsed after allo-HSCT. Therefore, it will be valuable to determine the prevalence of immune checkpoints expression to predict whether these patients will respond to CPIs.

3.2. *Engineering DLI*

DLI is one of the standard treatments for recurrence after allo-HSCT. It can play a GVL effect by identifying minor histocompatibility antigens and leukemia antigens. However, the long-term efficacy of DLI in AML patients who had relapsed after allo-HSCT was only 15%−29%, and most of them were accompanied by significant GVHD[136]. More and more studies have been using engineering DLI to improve GVL effect and reduce GVHD. In a phase 1 study, $CD25^+$ regulatory T cells were depleted from donor lymphocytes to avoid their inhibition of GVL effect. Compared to unmodified DLI, T_{reg} depletion was associated with better response rate (62.5% *vs.* 28.6%) and improved event-free survival (27% *vs.* 0%) without excessive GVHD[136]. $CD8^+$CD44hi memory T cells may have a more specific GVL effect, which were isolated and purified and given to fifteen patients in a phase 1 study. Ten patients achieved response (7 CR, 1 PR, 2 SD) for at least 3 months after infusion, and the incidence of GVHD was low[137]. CD200 is usually high expressed on leukemia cells, which binds to CD200 receptor on T-cell and negatively regulates T-cell function. Scientists used the costimulatory receptor-CD28 to replace the cytoplasmic tail of CD200R immunomodulatory fusion protein (IFP), and transduced the CD200R-CD28 IFP to CD8 T cells. It can eradicate blasts more efficiently than wild-type cells, and does not require interleukin 2 to maintain *in vivo* activity[138]. Donor NK cells treatment is the other arm to control blasts escaping. In a phase 2 study, haploidentical NK cells were successfully isolated and infused to AML patients who had relapsed following allo-HSCT. Patients with higher expression of KIR2DL2/2DL3/2DS2 at 14 days after haploidentical NK cell infusion achieved better survival[139]. Especially, allografts from donors who were positive for KIR2DS1 had more powerful anti-

leukemia effect than negative. Donor KIR3DS1 was associated with reduced mortality[16,17]. In a phase 4 study, 84 AML patients with CR1 received histamine dihydrochloride and low-dose IL-2 for 18 months or until relapse/death. Patients carrying HLA-21 M harbored better educated NKG2A + cells and displayed superior capacity to degranulate lytic granules against KIR ligand matched blasts[140]. A series of clinical trials using engineered NK cells, such as invariant NKT cells or cytokine-induced memory-like (CIML) NK cells, will provide more and more options for the preventive and salvage therapy of recurrence after allo-HSCT.

3.3. *Stimulating antitumor immunity (IL-15)*

IL-15 is a λc-chain cytokine with unique properties to stimulate antitumor immunity, including stimulation of NK cells and $CD8^+$ memory T cells[141]. In a mouse model of relapse after allo-HSCT, systemic IL-15 infusion often increases GVL activity with severe GVHD[22]. Therefore, strategies are focused on activating endogenous IL-15 expression to avoid the toxicity from a systemic administration. In a large registered study, the *FLT3* inhibitor-sorafenib was used to treat relapsed *FLT3-ITD* positive AML patients after allo-HSCT, which can indirectly activate the IL-15 transcription and induce the leukemia cells to produce IL-15. Thus, a strong GVL effect was promoted without inducing obvious GVHD[22]. Compared to the cytotoxic chemotherapy arm, sorafenib obviously improved the CR (18.5% *vs.* 32.5%) and 1-year OS (10.5% *vs.* 22%) of patients. AML blasts always lack expression of the costimulatory molecule CD80. In a preclinical study, *32Dp210* murine AML cells were engineered to express heterodimeric IL-15/IL-15Ra together with CD80 and tested as irradiated cell vaccines, which markedly increased IL-15 stability and secretion. Compared to the 100% relapse and death of control group, the OS of vaccination group was 50%[141]. In a multicenter phase 1 trial, IL-15 superagonist complex ALT-803 was administered to 33 patients who relapsed >60 days after allo-HSCT. ALT-803 was well tolerated and stimulated the activation, proliferation and expansion T cells without increasing T_{reg} cells. Responses were observed in 19% of evaluable patients, including 1 patient who achieved a CR for 7 months[142].

4. Future research questions and challenges

Once recurrence after allo-HSCT happens, patients and physicians have to face the problem of making a choice to change the dismal prognosis. Leukemia cells always acquire new oncogenic mutations, and patients may not tolerate or become resistant to conventional cytotoxicity chemotherapy[11,143]. Unavailable donor, HLA loss or active GVHD are always greatly limiting the application and the efficacy of traditional DLI and second transplantation[10,11,121,144]. Compared with traditional treatments after allo-HSCT, the novel agents have a good response, well tolerance and low toxicity, which are summarized in Fig. 1. However, scientists and clinicians need to discover new approaches to prevent and treat recurrence after allo-HSCT according to the following rules. First, we must discard the notion that donor cell booster therapy is the only definitive therapy and pay more attention to the development of targeted drugs for AML. For example, we should focus on the epigenetic therapeutic targets currently being evaluated in AML: the *MLL* rearrangements[145,146], the lysine demethylase *LSD1*[147], the protein methyltransferases EZH2,

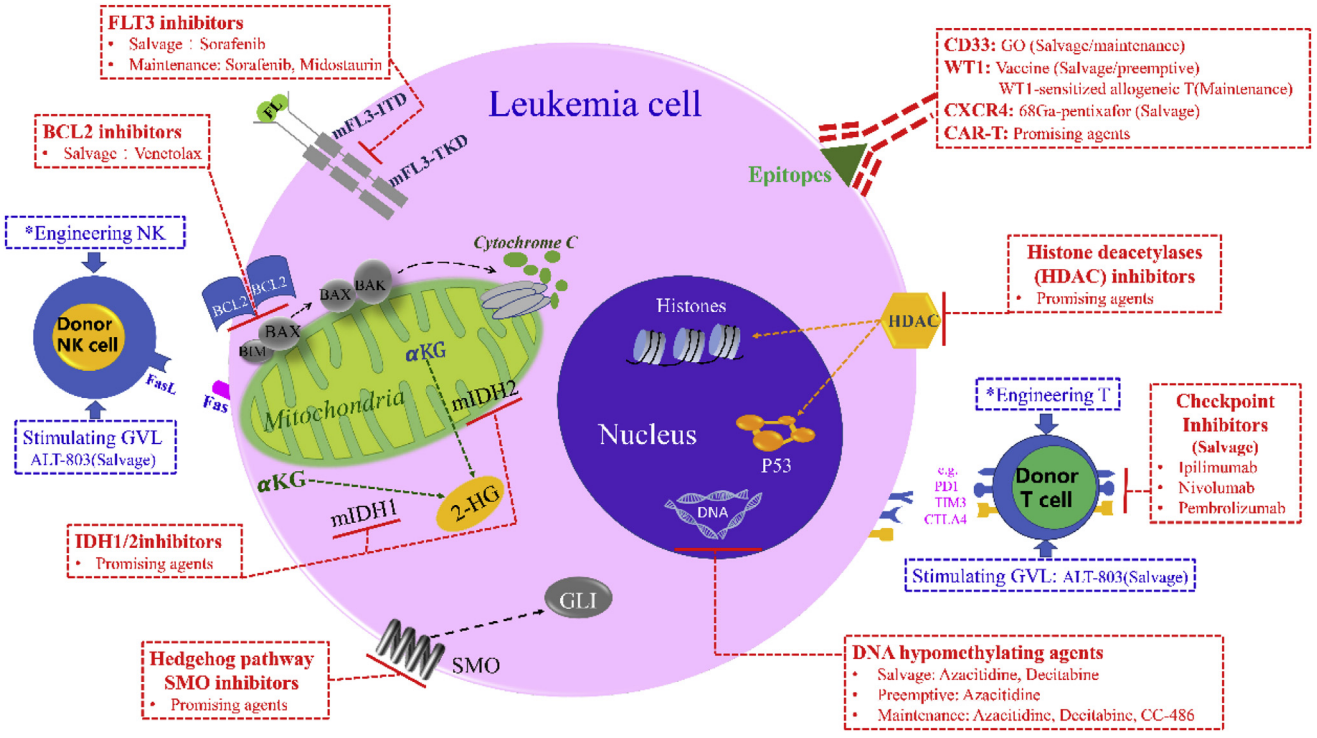


Figure 1 Summary of novel agents that have been used to prevent or treat relapse after allo-HSCT. Salvage; salvage therapy for patients with hematological relapse; Maintenance, preventive therapy for high-risk patients without any sigh of underlying disease; Preemptive, preemptive therapy for patients with minimal residual disease; Promising agents, the agents in clinical trial; *, engineering NK or T cells are introduced in Table 1.

DOT1L[148], and PRMT5149,150, and the BET bromodomain proteins[146,151−155]. Second, if safety and effectiveness are shown, it is best to use these drugs in advance in a preventive or preemptive manner, rather than just "watch and wait" for the miraculous GVL effect until the disease recurs. For example, the 3-year OS of sorafenib for post-transplant prophylaxis is 76%−84.6%, compared to 50.9% for controls[22,27,156]. Unfortunately, clinical trials using novel agents as maintenance treatment after allo-HSCT are scarce, and many uses are based on the off-protocol access. The clinical trials using novel agents are summarized in Table 1. Third, a suitable synergistic strategy can achieve better therapeutic effects without generating additional toxicity, such as coupling *FLT3* inhibitors with DLI or combining HMA with venetoclax or LEN. Finally, personalized treatment plans should be systematically formulated according to the epigenetics and immune response of AML patients, from induction therapy to maintenance treatment after allo-HSCT. However, because of the numerous and complex complications that may be encountered, clinical studies involving new drugs for *de novo* or R/R AML patients often exclude patients receiving allo-HSCT.

5. Conclusions

At present, more and more AML patients who have relapsed after allo-HSCT benefit from novel targeted agents. Clinicians must abandon the notion that anti-tumor immune effects induced by DLI or second transplantation are the only way to cure relapse after allo-HSCT, just as allo-HSCT is the definitive treatment for high-risk AML. For more individualized and systematic treatment based on the epigenetics and immunophenotype of AML patients, it should be implemented from induction chemotherapy to maintenance therapy after allo-HSCT. Scientists and clinicians must conduct more clinical trials to test novel agents earlier in allo-HSCT recipients to ensure how they can be successfully applied to improve patient outcomes.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Grant No. 81302043) and the Collaborative Innovation Center of Hematology, China. We thank Dr. Stephanie J. Lee of Fred Hutchinson Cancer Research Center for her guidance in selecting the subject of the review.

Author contributions

Weiwei Jin searched for papers and wrote the draft. Wei Shi, Linghui Xia, and Yu Hu revised and edited the manuscript. All of the authors have read and approved the final manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

References

1. Jan M, Leventhal MJ, Morgan EA, Wengrod JC, Nag A, Drinan SD, et al. Recurrent genetic HLA loss in AML relapsed after matched unrelated allogeneic hematopoietic cell transplantation. *Blood Adv* 2019;**3**:2199−204.
2. Kurosawa S, Fukuda T, Tajima K, Saito B, Fuji S, Yokoyama H, et al. Outcome of 93 patients with relapse or progression following allogeneic hematopoietic cell transplantation. *Am J Hematol* 2009;**84**:815−20.
3. Schuler E, Boughoufala S, Rautenberg C, Nachtkamp K, Dienst A, Fenk R, et al. Relapse patterns and treatment strategies in patients receiving allogeneic hematopoietic stem cell transplantation for myeloid malignancies. *Ann Hematol* 2019;**98**:1225−35.
4. Sauer T, Silling G, Groth C, Rosenow F, Krug U, Gorlich D, et al. Treatment strategies in patients with AML or high-risk myelodysplastic syndrome relapsed after Allo-SCT. *Bone Marrow Transplant* 2015;**50**:485−92.
5. Schmid C, de Wreede LC, van Biezen A, Finke J, Ehninger G, Ganser A, et al. Outcome after relapse of myelodysplastic syndrome and secondary acute myeloid leukemia following allogeneic stem cell transplantation: a retrospective registry analysis on 698 patients by the Chronic Malignancies Working Party of the European Society of Blood and Marrow Transplantation. *Haematologica* 2018;**103**:237−45.
6. Devillier R, Crocchiolo R, Etienne A, Prebet T, Charbonnier A, Furst S, et al. Outcome of relapse after allogeneic stem cell transplant in patients with acute myeloid leukemia. *Leuk Lymphoma* 2013;**54**:1228−34.
7. Bejanyan N, Weisdorf DJ, Logan BR, Wang HL, Devine SM, de Lima M, et al. Survival of patients with acute myeloid leukemia relapsing after allogeneic hematopoietic cell transplantation: a center for international blood and marrow transplant research study. *Biol Blood Marrow Transplant* 2015;**21**:454−9.
8. Piemontese S, Boumendil A, Labopin M, Schmid C, Ciceri F, Arcese W, et al. Leukemia relapse following unmanipulated haploidentical transplantation: a risk factor analysis on behalf of the ALWP of the EBMT. *J Hematol Oncol* 2019;**12**:68.
9. Zeiser R, Vago L. Mechanisms of immune escape after allogeneic hematopoietic cell transplantation. *Blood* 2019;**133**:1290−7.
10. Christopher MJ, Petti AA, Rettig MP, Miller CA, Chendamarai E, Duncavage EJ, et al. Immune escape of relapsed AML cells after allogeneic transplantation. *N Engl J Med* 2018;**379**:2330−41.
11. Toffalori C, Zito L, Gambacorta V, Riba M, Oliveira G, Bucci G, et al. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nat Med* 2019;**25**:603−11.
12. Noviello M, Manfredi F, Ruggiero E, Perini T, Oliveira G, Cortesi F, et al. Bone marrow central memory and memory stem T-cell exhaustion in AML patients relapsing after HSCT. *Nat Commun* 2019;**10**:1065.
13. Liu L, Chang YJ, Xu LP, Zhang XH, Wang Y, Liu KY, et al. Reversal of T cell exhaustion by the first donor lymphocyte infusion is associated with the persistently effective antileukemic responses in patients with relapsed AML after allo-HSCT. *Biol Blood Marrow Transplant* 2018;**24**:1350−9.
14. Shimoni A, Labopin M, Lorentino F, Van Lint MT, Koc Y, Gülbas Z, et al. Killer cell immunoglobulin-like receptor ligand mismatching and outcome after haploidentical transplantation with post-transplant cyclophosphamide. *Leukemia* 2019;**33**:230−9.
15. Zhao XY, Yu XX, Xu ZL, Cao XH, Huo MR, Zhao XS, et al. Donor and host coexpressing KIR ligands promote NK education after allogeneic hematopoietic stem cell transplantation. *Blood Adv* 2019;**3**:4312−25.
16. Venstrom JM, Pittari G, Gooley TA, Chewning JH, Spellman S, Haagenson M, et al. HLA-C-dependent prevention of leukemia relapse by donor activating KIR2DS1. *N Engl J Med* 2012;**367**:805−16.
17. Arima N, Nakamura F, Yabe T, Tanaka J, Fuji S, Ohashi K, et al. Influence of differently licensed KIR2DL1-positive natural killer cells in transplant recipients with acute leukemia: a Japanese national registry study. *Biol Blood Marrow Transplant* 2016;**22**:423−31.
18. Weisdorf D, Cooley S, Wang T, Trachtenberg E, Vierra-Green C, Spellman S, et al. KIR B donors improve the outcome for AML

patients given reduced intensity conditioning and unrelated donor transplantation. *Blood Adv* 2020;**4**:740−54.
19. Bohl SR, Bullinger L, Rucker FG. New targeted agents in acute myeloid leukemia: new hope on the rise. *Int J Mol Sci* 2019;**20**:1983.
20. Hevener K, Verstak TA, Lutat KE, Riggsbee DL, Mooney JW. Recent developments in topoisomerase-targeted cancer chemotherapy. *Acta Pharm Sin B* 2018;**8**:844−61.
21. He X, Zhu Y, Lin YC, Li M, Du J, Dong H, et al. *PRMT1*-mediated *FLT3* arginine methylation promotes maintenance of *FLT3-ITD*$^+$ acute myeloid leukemia. *Blood* 2019;**134**:548−60.
22. Mathew NR, Baumgartner F, Braun L, O'Sullivan D, Thomas S, Waterhouse M, et al. Sorafenib promotes graft-*versus*-leukemia activity in mice and humans through IL-15 production in *FLT3-ITD*-mutant leukemia cells. *Nat Med* 2018;**24**:282−91.
23. Bazarbachi A, Labopin M, Battipaglia G, Djabali A, Passweg J, Socie G, et al. Sorafenib improves survival of *FLT3*-mutated acute myeloid leukemia in relapse after allogeneic stem cell transplantation: a report of the EBMT Acute Leukemia Working Party. *Haematologica* 2019;**104**:e398−401.
24. Xuan L, Wang Y, Chen J, Jiang E, Gao L, Wu B, et al. Sorafenib therapy is associated with improved outcomes for FMS-like tyrosine kinase 3 internal tandem duplication acute myeloid leukemia relapsing after allogeneic hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2019;**25**:1674−81.
25. Pratz KW, Rudek MA, Smith BD, Karp J, Gojo I, Dezern A, et al. A prospective study of peritransplant sorafenib for patients with *FLT3-ITD* acute myeloid leukemia undergoing allogeneic transplantation. *Biol Blood Marrow Transplant* 2020;**26**:300−6.
26. Chen F, Sun J, Yin C, Cheng J, Ni J, Jiang L, et al. Impact of *FLT3-ITD* allele ratio and *ITD* length on therapeutic outcome in cytogenetically normal AML patients without *NPM1* mutation. *Bone Marrow Transplant* 2020;**55**:740−8.
27. Chappell G, Geer M, Gatza E, Braun T, Churay T, Brisson J, et al. Maintenance sorafenib in *FLT3-ITD* AML following allogeneic HCT favorably impacts relapse and overall survival. *Bone Marrow Transplant* 2019;**54**:1518−20.
28. Bazarbachi A, Labopin M, Battipaglia G, Djabali A, Forcade E, Arcese W, et al. Allogeneic stem cell transplantation for *FLT3*-mutated acute myeloid leukemia: *in vivo* T-cell depletion and posttransplant sorafenib maintenance improve survival. a retrospective acute leukemia working party-European society for blood and marrow transplant study. *Clin Hematol Int* 2019;**1**:58−74.
29. Burchert A, Bug G, Finke J, Stelljes M, Rollig C, Wäsch R, et al. Sorafenib as maintenance therapy post allogeneic stem cell transplantation for *FLT3-ITD* positive AML: results from the randomized, double-blind, placebo-controlled multicentre sormain trial. *Blood* 2018;**132**:661.
30. Battipaglia G, Ruggeri A, Massoud R, El Cheikh J, Jestin M, Antar A, et al. Efficacy and feasibility of sorafenib as a maintenance agent after allogeneic hematopoietic stem cell transplantation for Fms-like tyrosine kinase 3-mutated acute myeloid leukemia. *Cancer* 2017;**123**:2867−74.
31. Brunner AM, Li S, Fathi AT, Wadleigh M, Ho VT, Collier K, et al. Haematopoietic cell transplantation with and without sorafenib maintenance for patients with *FLT3-ITD* acute myeloid leukaemia in first complete remission. *Br J Haematol* 2016;**175**:496−504.
32. Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, et al. Midostaurin plus chemotherapy for acute myeloid leukemia with a *FLT3* mutation. *N Engl J Med* 2017;**377**:454−64.
33. Schlenk RF, Weber D, Fiedler W, Salih HR, Wulf G, Salwender H, et al. Midostaurin added to chemotherapy and continued single-agent maintenance therapy in acute myeloid leukemia with *FLT3-ITD*. *Blood* 2019;**133**:840−51.
34. Sandmaier BM, Khaled S, Oran B, Gammon G, Trone D, Frankfurt O. Results of a phase 1 study of quizartinib as maintenance therapy in subjects with acute myeloid leukemia in remission following allogeneic hematopoietic stem cell transplant. *Am J Hematol* 2018;**93**:222−31.
35. Altman JK, Foran JM, Pratz KW, Trone D, Cortes JE, Tallman MS. Phase 1 study of quizartinib in combination with induction and consolidation chemotherapy in patients with newly diagnosed acute myeloid leukemia. *Am J Hematol* 2018;**93**:213−21.
36. Cortes JE, Khaled S, Martinelli G, Perl AE, Ganguly S, Russell N, et al. Quizartinib *versus* salvage chemotherapy in relapsed or refractory *FLT3-ITD* acute myeloid leukaemia (QuANTUM-R): a multicentre, randomised, controlled, open-label, phase 3 trial. *Lancet Oncol* 2019;**20**:984−97.
37. Cortes JE, Tallman MS, Schiller GJ, Trone D, Gammon G, Goldberg SL, et al. Phase 2b study of 2 dosing regimens of quizartinib monotherapy in *FLT3-ITD*-mutated, relapsed or refractory AML. *Blood* 2018;**132**:598−607.
38. Usuki K, Sakura T, Kobayashi Y, Miyamoto T, Iida H, Morita S, et al. Clinical profile of gilteritinib in Japanese patients with relapsed/refractory acute myeloid leukemia: an open-label phase 1 study. *Canc Sci* 2018;**109**:3235−44.
39. Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, et al. Gilteritinib or chemotherapy for relapsed or refractory *FLT3*-mutated AML. *N Engl J Med* 2019;**381**:1728−40.
40. Zhang H, Savage S, Schultz AR, Bottomly D, White L, Segerdell E, et al. Clinical resistance to crenolanib in acute myeloid leukemia due to diverse molecular mechanisms. *Nat Commun* 2019;**10**:244.
41. Antar AI, Otrock ZK, Jabbour E, Mohty M, Bazarbachi A. *FLT3* inhibitors in acute myeloid leukemia: ten frequently asked questions. *Leukemia* 2020;**34**:682−96.
42. Uchida A, Isobe Y, Asano J, Uemura Y, Hoshikawa M, Takagi M, et al. Targeting BCL2 with venetoclax is a promising therapeutic strategy for "double-proteinexpression" lymphoma with *MYC* and *BCL2* rearrangements. *Haematologica* 2019;**104**:1417−21.
43. Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T, et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. *Canc Discov* 2016;**6**:1106−17.
44. Das M. Venetoclax with decitabine or azacitidine for AML. *Lancet Oncol* 2018;**19**:e672.
45. Pollyea DA, Stevens BM, Jones CL, Winters A, Pei S, Minhajuddin M, et al. Venetoclax with azacitidine disrupts energy metabolism and targets leukemia stem cells in patients with acute myeloid leukemia. *Nat Med* 2018;**24**:1859−66.
46. Nakada D. Venetolax with azacitidine drains fuel from AML stem cells. *Cell Stem Cell* 2019;**24**:7−8.
47. Gaut D, Burkenroad A, Duong T, Feammelli J, Sasine J, Schiller G. Venetoclax combination therapy in relapsed/refractory acute myeloid leukemia: a single institution experience. *Leuk Res* 2020;**90**:106314.
48. Liu B, Narurkar R, Hanmantgad M, Zafar W, Song Y, Liu D. Venetoclax and low-dose cytarabine induced complete remission in a patient with high-risk acute myeloid leukemia: a case report. *Front Med* 2018;**12**:593−9.
49. DiNardo CD, Wei AH. How I treat acute myeloid leukemia in the era of new drugs. *Blood* 2020;**135**:85−96.
50. Amatangelo MD, Quek L, Shih A, Stein EM, Roshal M, David MD, et al. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. *Blood* 2017;**130**:732−41.
51. Stein EM, DiNardo CD, Fathi AT, Pollyea DA, Stone RM, Altman JK, et al. Molecular remission and response patterns in patients with mutant-*IDH2* acute myeloid leukemia treated with enasidenib. *Blood* 2019;**133**:676−87.
52. DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, et al. Durable remissions with ivosidenib in IDH1-mutated relapsed or refractory AML. *N Engl J Med* 2018;**378**:2386−98.
53. Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, et al. Enasidenib in mutant *IDH2* relapsed or refractory acute myeloid leukemia. *Blood* 2017;**130**:722−31.
54. Fathi AT, DiNardo CD, Kline I, Kenvin L, Gupta I, Attar EC, et al. Differentiation syndrome associated with enasidenib, a selective

inhibitor of mutant isocitrate dehydrogenase 2: analysis of a phase 1/2 study. *JAMA Oncol* 2018;**4**:1106−10.
55. Terao T, Minami Y. Targeting hedgehog (Hh) pathway for the acute myeloid leukemia treatment. *Cells* 2019;**8**:312.
56. Cortes JE, Douglas Smith B, Wang ES, Merchant A, Oehler VG, Arellano M, et al. Glasdegib in combination with cytarabine and daunorubicin in patients with AML or high-risk MDS: phase 2 study results. *Am J Hematol* 2018;**93**:1301−10.
57. Savona MR, Pollyea DA, Stock W, Oehler VG, Schroeder MA, Lancet J, et al. Phase Ib study of glasdegib, a hedgehog pathway inhibitor, in combination with standard chemotherapy in patients with AML or high-risk MDS. *Clin Cancer Res* 2018;**24**: 2294−303.
58. Cortes JE, Heidel FH, Hellmann A, Fiedler W, Smith BD, Robak T, et al. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. *Leukemia* 2019;**33**:379−89.
59. Gbolahan OB, Zeidan AM, Stahl M, Abu Zaid M, Farag S, Paczesny S, et al. Immunotherapeutic concepts to target acute myeloid leukemia: focusing on the role of monoclonal antibodies, hypomethylating agents and the leukemic microenvironment. *Int J Mol Sci* 2017;**18**:1660.
60. Schroeder T, Rachlis E, Bug G, Stelljes M, Klein S, Steckel NK, et al. Treatment of acute myeloid leukemia or myelodysplastic syndrome relapse after allogeneic stem cell transplantation with azacitidine and donor lymphocyte infusions—a retrospective multicenter analysis from the German Cooperative Transplant Study Group. *Biol Blood Marrow Transplant* 2015;**21**:653−60.
61. Ueda M, El-Jurdi N, Cooper B, Caimi P, Baer L, Kolk M, et al. Low-dose azacitidine with *DNMT1* level monitoring to treat post-transplantation acute myelogenous leukemia or myelodysplastic syndrome relapse. *Biol Blood Marrow Transplant* 2019;**25**:1122−7.
62. Craddock C, Labopin M, Robin M, Finke J, Chevallier P, Yakoub-Agha I, et al. Clinical activity of azacitidine in patients who relapse after allogeneic stem cell transplantation for acute myeloid leukemia. *Haematologica* 2016;**101**:879−83.
63. Motabi IH, Ghobadi A, Liu J, Schroeder M, Abboud CN, Cashen AF, et al. Chemotherapy *versus* hypomethylating agents for the treatment of relapsed acute myeloid leukemia and myelodysplastic syndrome after allogeneic stem cell transplant. *Biol Blood Marrow Transplant* 2016;**22**:1324−9.
64. Woo J, Deeg HJ, Storer B, Yeung C, Fang M, Mielcarek M, et al. Factors determining responses to azacitidine in patients with myelodysplastic syndromes and acute myeloid leukemia with early post-transplantation relapse: a prospective trial. *Biol Blood Marrow Transplant* 2017;**23**:176−9.
65. Schroeder T, Rautenberg C, Kruger W, Platzbecker U, Bug G, Steinmann J, et al. Treatment of relapsed AML and MDS after allogeneic stem cell transplantation with decitabine and DLI—a retrospective multicenter analysis on behalf of the German Cooperative Transplant Study Group. *Ann Hematol* 2018;**97**:335−42.
66. Craddock C, Slade D, De Santo C, Wheat R, Ferguson P, Hodgkinson A, et al. Combination lenalidomide and azacitidine: a novel salvage therapy in patients who relapse after allogeneic stem-cell transplantation for acute myeloid leukemia. *J Clin Oncol* 2019; **37**:580−8.
67. Platzbecker U, Middeke JM, Sockel K, Herbst R, Wolf D, Baldus CD, et al. Measurable residual disease-guided treatment with azacitidine to prevent haematological relapse in patients with myelodysplastic syndrome and acute myeloid leukaemia (RELAZA2): an open-label, multicentre, phase 2 trial. *Lancet Oncol* 2018;**19**: 1668−79.
68. Craddock C, Jilani N, Siddique S, Yap C, Khan J, Nagra S, et al. Tolerability and clinical activity of post-transplantation azacitidine in patients allografted for acute myeloid leukemia treated on the RICAZA trial. *Biol Blood Marrow Transplant* 2016;**22**:385−90.
69. El-Cheikh J, Massoud R, Fares E, Kreidieh N, Mahfouz R, Charafeddine M, et al. Low-dose 5-azacytidine as preventive therapy for relapse of AML and MDS following allogeneic HCT. *Bone Marrow Transplant* 2017;**52**:918−21.
70. Guillaume T, Malard F, Magro L, Labopin M, Tabrizi R, Borel C, et al. Prospective phase II study of prophylactic low-dose azacitidine and donor lymphocyte infusions following allogeneic hematopoietic stem cell transplantation for high-risk acute myeloid leukemia and myelodysplastic syndrome. *Bone Marrow Transplant* 2019;**54**: 1815−26.
71. de Lima M, Oran B, Champlin RE, Papadopoulos EB, Giralt SA, Scott BL, et al. CC-486 maintenance after stem cell transplantation in patients with acute myeloid leukemia or myelodysplastic syndromes. *Biol Blood Marrow Transplant* 2018;**24**:2017−24.
72. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. *Blood* 2016;**127**: 42−52.
73. Garcia-Manero G, Sekeres MA, Egyed M, Breccia M, Graux C, Cavenagh JD, et al. A phase 1b/2b multicenter study of oral panobinostat plus azacitidine in adults with MDS, CMML or AML with 30% blasts. *Leukemia* 2017;**31**:2799−806.
74. Bug G, Burchert A, Wagner EM, Kroger N, Berg T, Guller S, et al. Phase I/II study of the deacetylase inhibitor panobinostat after allogeneic stem cell transplantation in patients with high-risk MDS or AML (PANOBEST trial). *Leukemia* 2017;**31**:2523−5.
75. DeAngelo DJ, Walker AR, Schlenk RF, Sierra J, Medeiros BC, Ocio EM, et al. Safety and efficacy of oral panobinostat plus chemotherapy in patients aged 65 years or younger with high-risk acute myeloid leukemia. *Leuk Res* 2019;**85**:106197.
76. Wieduwilt MJ, Pawlowska N, Thomas S, Olin R, Logan AC, Damon LE, et al. Histone deacetylase inhibition with panobinostat combined with intensive induction chemotherapy in older patients with acute myeloid leukemia: phase I study results. *Clin Canc Res* 2019;**25**:4917−23.
77. Perl AE. *The role of targeted therapy in the management of patients with AML. Hematology***2017**. Hematology Am Soc Hematol Educ Program; 2017. p. 54−65.
78. Pollyea DA, Jordan CT. Therapeutic targeting of acute myeloid leukemia stem cells. *Blood* 2017;**129**:1627−35.
79. He SZ, Busfield S, Ritchie DS, Hertzberg MS, Durrant S, Lewis ID, et al. A phase 1 study of the safety, pharmacokinetics and anti-leukemic activity of the anti-CD123 monoclonal antibody CSL360 in relapsed, refractory or high-risk acute myeloid leukemia. *Leuk Lymphoma* 2015;**56**:1406−15.
80. Gadhoum SZ, Madhoun NY, Abuelela AF, Merzaban JS. Anti-CD44 antibodies inhibit both mTORC1 and mTORC2: a new rationale supporting CD44-induced AML differentiation therapy. *Leukemia* 2016;**30**:2397−401.
81. Vey N, Delaunay J, Martinelli G, Fiedler W, Raffoux E, Prebet T, et al. Phase I clinical study of RG7356, an anti-CD44 humanized antibody, in patients with acute myeloid leukemia. *Oncotarget* 2016; **7**:32532−42.
82. Reusch U, Harrington KH, Gudgeon CJ, Fucek I, Ellwanger K, Weichel M, et al. Characterization of CD33/CD3 tetravalent bispecific tandem diabodies (TandAbs) for the treatment of acute myeloid leukemia. *Clin Canc Res* 2016;**22**:5829−38.
83. Godwin CD, Gale RP, Walter RB. Gemtuzumab ozogamicin in acute myeloid leukemia. *Leukemia* 2017;**31**:1855−68.
84. Hills RK, Castaigne S, Appelbaum FR, Delaunay J, Petersdorf S, Othus M, et al. Addition of gemtuzumab ozogamicin to induction chemotherapy in adult patients with acute myeloid leukaemia: a meta-analysis of individual patient data from randomised controlled trials. *Lancet Oncol* 2014;**15**:986−96.
85. Daver N, Kantarjian H, Ravandi F, Estey E, Wang X, Garcia-Manero G, et al. A phase II study of decitabine and gemtuzumab ozogamicin in newly diagnosed and relapsed acute myeloid leukemia and high-risk myelodysplastic syndrome. *Leukemia* 2016;**30**: 268−73.
86. Tarlock K, Alonzo TA, Gerbing RB, Raimondi SC, Hirsch BA, Sung L, et al. Gemtuzumab ozogamicin reduces relapse risk in

FLT3/ITD acute myeloid leukemia: a report from the Children's Oncology Group. *Clin Canc Res* 2016;**22**:1951−7.
87. Candoni A, Papayannidis C, Martinelli G, Simeone E, Gottardi M, Iacobucci I, et al. Flai (fludarabine, cytarabine, idarubicin) plus low-dose Gemtuzumab Ozogamicin as induction therapy in CD33-positive AML: final results and long term outcome of a phase II multicenter clinical trial. *Am J Hematol* 2018;**93**:655−63.
88. Amadori S, Suciu S, Selleslag D, Aversa F, Gaidano G, Musso M, et al. Gemtuzumab ozogamicin *versus* best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19 trial. *J Clin Oncol* 2016;**34**:972−9.
89. Wei AH, Tiong IS. Midostaurin, enasidenib, CPX-351, gemtuzumab ozogamicin, and venetoclax bring new hope to AML. *Blood* 2017; **130**:2469−74.
90. Burnett A, Cavenagh J, Russell N, Hills R, Kell J, Jones G, et al. Defining the dose of gemtuzumab ozogamicin in combination with induction chemotherapy in acute myeloid leukemia: a comparison of 3 mg/m^2 with 6 mg/m^2 in the NCRI AML17 Trial. *Haematologica* 2016;**101**:724−31.
91. Pollard JA, Loken M, Gerbing RB, Raimondi SC, Hirsch BA, Aplenc R, et al. CD33 expression and its association with gemtuzumab ozogamicin response: results from the randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol* 2016;**34**: 747−55.
92. Khan N, Hills RK, Virgo P, Couzens S, Clark N, Gilkes A, et al. Expression of CD33 is a predictive factor for effect of gemtuzumab ozogamicin at different doses in adult acute myeloid leukaemia. *Leukemia* 2017;**31**:1059−68.
93. Lamba JK, Chauhan L, Shin M, Loken MR, Pollard JA, Wang YC, et al. CD33 splicing polymorphism determines gemtuzumab ozogamicin response in *de novo* acute myeloid leukemia: report from randomized phase III Children's Oncology Group trial AAML0531. *J Clin Oncol* 2017;**35**:2674−82.
94. Koren-Michowitz M, Maayan H, Apel A, Shem-Tov N, Yerushalmi R, Volchek Y, et al. Salvage therapy with ARA-C and gemtuzumab ozogamicin in AML patients relapsing after stem cell transplantation. *Ann Hematol* 2015;**94**:375−8.
95. Zahler S, Bhatia M, Ricci A, Roy S, Morris E, Harrison L, et al. A phase I study of reduced-intensity conditioning and allogeneic stem cell transplantation followed by dose escalation of targeted consolidation immunotherapy with gemtuzumab ozogamicin in children and adolescents with CD33$^+$ acute myeloid leukemia. *Biol Blood Marrow Transplant* 2016;**22**:698−704.
96. Stein EM, Walter RB, Erba HP, Fathi AT, Advani AS, Lancet JE, et al. A phase 1 trial of vadastuximab talirine as monotherapy in patients with CD33-positive acute myeloid leukemia. *Blood* 2018; **131**:387−96.
97. Fathi AT, Erba HP, Lancet JE, Stein EM, Ravandi F, Faderl S, et al. A phase 1 trial of vadastuximab talirine combined with hypomethylating agents in patients with CD33-positive AML. *Blood* 2018;**132**:1125−33.
98. Brayer J, Lancet JE, Powers J, List A, Balducci L, Komrokji R, et al. WT1 vaccination in AML and MDS: a pilot trial with synthetic analog peptides. *Am J Hematol* 2015;**90**:602−7.
99. Chapuis AG, Egan DN, Bar M, Schmitt TM, McAfee MS, Paulson KG, et al. T cell receptor gene therapy targeting WT1 prevents acute myeloid leukemia relapse post-transplant. *Nat Med* 2019; **25**:1064−72.
100. Maslak PG, Dao T, Bernal Y, Chanel SM, Zhang R, Frattini M, et al. Phase 2 trial of a multivalent WT1 peptide vaccine (galinpepimut-S) in acute myeloid leukemia. *Blood Adv* 2018;**2**:224−34.
101. Anguille S, Van de Velde AL, Smits EL, Van Tendeloo VF, Juliusson G, Cools N, et al. Dendritic cell vaccination as post-remission treatment to prevent or delay relapse in acute myeloid leukemia. *Blood* 2017;**130**:1713−21.
102. Shah NN, Loeb DM, Khuu H, Stroncek D, Ariyo T, Raffeld M, et al. Induction of immune response after allogeneic Wilms' tumor 1 dendritic cell vaccination and donor lymphocyte infusion in patients with hematologic malignancies and post-transplantation relapse. *Biol Blood Marrow Transplant* 2016;**22**:2149−54.
103. Tawara I, Kageyama S, Miyahara Y, Fujiwara H, Nishida T, Akatsuka Y, et al. Safety and persistence of WT1-specific T-cell receptor gene-transduced lymphocytes in patients with AML and MDS. *Blood* 2017;**130**:1985−94.
104. Abraham M, Klein S, Bulvik B, Wald H, Weiss ID, Olam D, et al. The CXCR4 inhibitor BL-8040 induces the apoptosis of AML blasts by downregulating ERK, BCL-2, MCL-1 and cyclin-D1 *via* altered *miR-15a/16-1* expression. *Leukemia* 2017;**31**:2336−46.
105. Habringer S, Lapa C, Herhaus P, Schottelius M, Istvanffy R, Steiger K, et al. Dual targeting of acute leukemia and supporting niche by CXCR4-directed theranostics. *Theranostics* 2018;**8**: 369−83.
106. Liu SH, Gu Y, Pascual B, Yan Z, Hallin M, Zhang C, et al. A novel CXCR4 antagonist IgG1 antibody (PF-06747143) for the treatment of hematologic malignancies. *Blood Adv* 2017;**1**:1088−100.
107. Kovacsovics TJ, Mims A, Salama ME, Pantin J, Rao N, Kosak KM, et al. Combination of the low anticoagulant heparin CX-01 with chemotherapy for the treatment of acute myeloid leukemia. *Blood Adv* 2018;**2**:381−9.
108. Michelis FV, Hedley DW, Malhotra S, Chow S, Loach D, Gupta V, et al. Mobilization of leukemic cells using plerixafor as part of a myeloablative preparative regimen for patients with acute myelogenous leukemia undergoing allografting: assessment of safety and tolerability. *Biol Blood Marrow Transplant* 2019;**25**:1158−63.
109. Lynn RC, Poussin M, Kalota A, Feng Y, Low PS, Dimitrov DS, et al. Targeting of folate receptor beta on acute myeloid leukemia blasts with chimeric antigen receptor-expressing T cells. *Blood* 2015;**125**: 3466−76.
110. Lynn RC, Feng Y, Schutsky K, Poussin M, Kalota A, Dimitrov DS, et al. High-affinity FRbeta-specific CAR T cells eradicate AML and normal myeloid lineage without HSC toxicity. *Leukemia* 2016;**30**: 1355−64.
111. Wang J, Chen S, Xiao W, Li W, Wang L, Yang S, et al. CAR-T cells targeting CLL-1 as an approach to treat acute myeloid leukemia. *J Hematol Oncol* 2018;**11**:7.
112. Baumeister SH, Murad J, Werner L, Daley H, Trebeden-Negre H, Gicobi JK, et al. Phase I trial of autologous CAR T cells targeting NKG2D ligands in patients with AML/MDS and multiple myeloma. *Cancer Immunol Res* 2019;**7**:100−12.
113. Gomes-Silva D, Atilla E, Atilla PA, Mo F, Tashiro H, Srinivasan M, et al. CD7 CAR T Cells for the therapy of acute myeloid leukemia. *Mol Ther* 2019;**27**:272−80.
114. Jetani H, Garcia-Cadenas I, Nerreter T, Thomas S, Rydzek J, Meijide JB, et al. CAR T-cells targeting FLT3 have potent activity against *FLT3-ITD*$^+$ AML and act synergistically with the *FLT3*-inhibitor crenolanib. *Leukemia* 2018;**32**:1168−79.
115. Wang Y, Xu Y, Li S, Liu J, Xing Y, Xing H, et al. Targeting *FLT3* in acute myeloid leukemia using ligand-based chimeric antigen receptor-engineered T cells. *J Hematol Oncol* 2018;**11**:60.
116. Zheng W, O'Hear CE, Alli R, Basham JH, Abdelsamed HA, Palmer LE, et al. PI3K orchestration of the *in vivo* persistence of chimeric antigen receptor-modified T cells. *Leukemia* 2018;**32**: 1157−67.
117. Petrov JC, Wada M, Pinz KG, Yan LE, Chen KH, Shuai X, et al. Compound CAR T-cells as a double-pronged approach for treating acute myeloid leukemia. *Leukemia* 2018;**32**:1317−26.
118. He X, Feng Z, Ma J, Ling S, Cao Y, Gurung B, et al. Bi-specific and split CAR T cells targeting CD13 and TIM3 eradicate acute myeloid leukemia. *Blood* 2020;**135**:713−23.
119. Kim MY, Yu KR, Kenderian SS, Ruella M, Chen S, Shin TH, et al. Genetic inactivation of CD33 in hematopoietic stem cells to enable

CAR T cell immunotherapy for acute myeloid leukemia. *Cell* 2018;**173**:1439−53. e19.
120. Borot F, Wang H, Ma Y, Jafarov T, Raza A, Ali AM, et al. Gene-edited stem cells enable CD33-directed immune therapy for myeloid malignancies. *Proc Natl Acad Sci U S A* 2019;**116**:11978−87.
121. Vago L, Perna SK, Zanussi M, Mazzi B, Barlassina C, Stanghellini MT, et al. Loss of mismatched HLA in leukemia after stem-cell transplantation. *N Engl J Med* 2009;**361**:478−88.
122. Ahci M, Toffalori C, Bouwmans E, Crivello P, Brambati C, Pultrone C, et al. A new tool for rapid and reliable diagnosis of HLA loss relapses after HSCT. *Blood* 2017;**130**:1270−3.
123. He F, Warlick E, Miller JS, MacMillan M, Verneris MR, Cao Q, et al. Lymphodepleting chemotherapy with donor lymphocyte infusion post-allogeneic HCT for hematological malignancies is associated with severe, but therapy-responsive aGvHD. *Bone Marrow Transplant* 2016;**51**:1107−12.
124. Inamoto Y, Fefer A, Sandmaier BM, Gooley TA, Warren EH, Petersdorf SH, et al. A phase I/II study of chemotherapy followed by donor lymphocyte infusion plus interleukin-2 for relapsed acute leukemia after allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2011;**17**:1308−15.
125. Bar M, Flowers MED, Storer BE, Chauncey TR, Pulsipher MA, Thakar MS, et al. Reversal of low donor chimerism after hematopoietic cell transplantation using pentostatin and donor lymphocyte infusion: a prospective phase II multicenter trial. *Biol Blood Marrow Transplant* 2018;**24**:308−13.
126. Warlick ED, DeFor T, Blazar BR, Burns L, Verneris MR, Ustun C, et al. Successful remission rates and survival after lymphodepleting chemotherapy and donor lymphocyte infusion for relapsed hematologic malignancies postallogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2012;**18**:480−6.
127. Eefting M, von dem Borne PA, de Wreede LC, Halkes CJ, Kersting S, Marijt EW, et al. Intentional donor lymphocyte-induced limited acute graft-*versus*-host disease is essential for long-term survival of relapsed acute myeloid leukemia after allogeneic stem cell transplantation. *Haematologica* 2014;**99**:751−8.
128. Schroeder T, Czibere A, Platzbecker U, Bug G, Uharek L, Luft T, et al. Azacitidine and donor lymphocyte infusions as first salvage therapy for relapse of AML or MDS after allogeneic stem cell transplantation. *Leukemia* 2013;**27**:1229−35.
129. Boudreau JE, Giglio F, Gooley TA, Stevenson PA, Le Luduec JB, Shaffer BC, et al. KIR3DL1/HLA-B subtypes govern acute myelogenous leukemia relapse after hematopoietic cell transplantation. *J Clin Oncol* 2017;**35**:2268−78.
130. Moutuou MM, Page G, Zaid I, Lesage S, Guimond M. Restoring T cell homeostasis after allogeneic stem cell transplantation: principal limitations and future challenges. *Front Immunol* 2018;**9**:1237.
131. Simonetta F, Pradier A, Bosshard C, Masouridi-Levrat S, Dantin C, Koutsi A, et al. Dynamics of expression of programmed cell death protein-1 (PD-1) on T cells after allogeneic hematopoietic stem cell transplantation. *Front Immunol* 2019;**10**:1034.
132. Ijaz A, Khan AY, Malik SU, Faridi W, Fraz MA, Usman M, et al. Significant risk of graft-*versus*-host disease with exposure to checkpoint inhibitors before and after allogeneic transplantation. *Biol Blood Marrow Transplant* 2019;**25**:94−9.
133. Davids MS, Kim HT, Bachireddy P, Costello C, Liguori R, Savell A, et al. Ipilimumab for patients with relapse after allogeneic transplantation. *N Engl J Med* 2016;**375**:143−53.
134. Bashey A, Medina B, Corringham S, Pasek M, Carrier E, Vrooman L, et al. CTLA4 blockade with ipilimumab to treat relapse of malignancy after allogeneic hematopoietic cell transplantation. *Blood* 2009;**113**:1581−8.
135. Albring JC, Inselmann S, Sauer T, Schliemann C, Altvater B, Kailayangiri S, et al. PD-1 checkpoint blockade in patients with relapsed AML after allogeneic stem cell transplantation. *Bone Marrow Transplant* 2017;**52**:317−20.
136. Nikiforow S, Kim HT, Daley H, Reynolds C, Jones KT, Armand P, et al. A phase I study of CD25/regulatory T-cell-depleted donor lymphocyte infusion for relapse after allogeneic stem cell transplantation. *Haematologica* 2016;**101**:1251−9.
137. Muffly L, Sheehan K, Armstrong R, Jensen K, Tate K, Rezvani AR, et al. Infusion of donor-derived $CD8^+$ memory T cells for relapse following allogeneic hematopoietic cell transplantation. *Blood Adv* 2018;**2**:681−90.
138. Oda SK, Daman AW, Garcia NM, Wagener F, Schmitt TM, Tan X, et al. A CD200R-CD28 fusion protein appropriates an inhibitory signal to enhance T-cell function and therapy of murine leukemia. *Blood* 2017;**130**:2410−9.
139. Shaffer BC, Le Luduec JB, Forlenza C, Jakubowski AA, Perales MA, Young JW, et al. Phase II study of haploidentical natural killer cell infusion for treatment of relapsed or persistent myeloid malignancies following allogeneic hematopoietic cell transplantation. *Biol Blood Marrow Transplant* 2016;**22**:705−9.
140. Hallner A, Bernson E, Hussein BA, Ewald Sander F, Brune M, Aurelius J, et al. The HLA-B-21 dimorphism impacts on NK cell education and clinical outcome of immunotherapy in acute myeloid leukemia. *Blood* 2019;**133**:1479−88.
141. Shi Y, Dincheva-Vogel L, Ayemoba CE, Fung JP, Bergamaschi C, Pavlakis GN, et al. IL-15/IL-15Ralpha/CD80-expressing AML cell vaccines eradicate minimal residual disease in leukemic mice. *Blood Adv* 2018;**2**:3177−92.
142. Romee R, Cooley S, Berrien-Elliott MM, Westervelt P, Verneris MR, Wagner JE, et al. First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. *Blood* 2018;**131**:2515−27.
143. Ertz-Archambault N, Kosiorek H, Slack JL, Lonzo ML, Greipp PT, Khera N, et al. Cytogenetic evolution in myeloid neoplasms at relapse after allogeneic hematopoietic cell transplantation: association with previous chemotherapy and effect on survival. *Biol Blood Marrow Transplant* 2017;**23**:782−9.
144. Crucitti L, Crocchiolo R, Toffalori C, Mazzi B, Greco R, Signori A, et al. Incidence, risk factors and clinical outcome of leukemia relapses with loss of the mismatched HLA after partially incompatible hematopoietic stem cell transplantation. *Leukemia* 2015;**29**:1143−52.
145. Krivtsov AV, Evans K, Gadrey JY, Eschle BK, Hatton C, Uckelmann HJ, et al. A Menin-MLL inhibitor induces specific chromatin changes and eradicates disease in models of MLL-rearranged leukemia. *Canc Cell* 2019;**36**:660−673 e11.
146. McCalmont H, Li KL, Jones L, Toubia J, Bray SC, Casolari DA, et al. Efficacy of combined CDK9/BET inhibition in preclinical models of MLL-rearranged acute leukemia. *Blood Adv* 2020;**4**:296−300.
147. Wingelhofer B, Somervaille TCP. Emerging epigenetic therapeutic targets in acute myeloid leukemia. *Front Oncol* 2019;**9**:850.
148. Brzezinka K, Nevedomskaya E, Lesche R, Steckel M, Eheim AL, Haegebarth A, et al. Functional diversity of inhibitors tackling the differentiation blockage of MLL-rearranged leukemia. *J Hematol Oncol* 2019;**12**:66.
149. Radzisheuskaya A, Shliaha PV, Grinev V, Lorenzini E, Kovalchuk S, Shlyueva D, et al. PRMT5 methylome profiling uncovers a direct link to splicing regulation in acute myeloid leukemia. *Nat Struct Mol Biol* 2019;**26**:999−1012.
150. Fong JY, Pignata L, Goy PA, Kawabata KC, Lee SC, Koh CM, et al. Therapeutic targeting of RNA splicing catalysis through inhibition of protein arginine methylation. *Canc Cell* 2019;**36**:194−209 e9.
151. Saenz DT, Fiskus W, Manshouri T, Mill CP, Qian Y, Raina K, et al. Targeting nuclear beta-catenin as therapy for post-myeloproliferative neoplasm secondary AML. *Leukemia* 2019;**33**:1373−86.
152. Piya S, Mu H, Bhattacharya S, Lorenzi PL, Davis RE, McQueen T, et al. BETP degradation simultaneously targets acute myelogenous leukemia stem cells and the microenvironment. *J Clin Invest* 2019;**129**:1878−94.

153. Mill CP, Fiskus W, DiNardo CD, Qian Y, Raina K, Rajapakshe K, et al. *RUNX1*-targeted therapy for AML expressing somatic or germline mutation in *RUNX1*. *Blood* 2019;**134**:59−73.
154. Masse A, Roulin L, Pasanisi J, Penneroux J, Gachet S, Delord M, et al. BET inhibitors impair leukemic stem cell function only in defined oncogenic subgroups of acute myeloid leukaemias. *Leuk Res* 2019;**87**:106269.
155. Jiang Y, Hu T, Wang T, Shi X, Kitano A, Eagle K, et al. AMP-activated protein kinase links acetyl-CoA homeostasis to BRD4 recruitment in acute myeloid leukemia. *Blood* 2019;**134**:2183−94.
156. Xuan L, Wang Y, Huang F, Jiang E, Deng L, Wu B, et al. Effect of sorafenib on the outcomes of patients with *FLT3-ITD* acute myeloid leukemia undergoing allogeneic hematopoietic stem cell transplantation. *Cancer* 2018;**124**:1954−63.