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

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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.
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\(^1\). 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\(^2,3\), and 1-year OS rate is about 20%\(^4,5\). 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\(^6,8\). 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 system\(^7\). 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\(_{\text{reg}}\)) 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 (KIRs)\(^12,13\). Donor natural killer (NK) cells are the first constituted immune cells, both the donor and the host present all killer-cell immunoglobulin-like receptors (KIRs)\(^14,15\). 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\(^31\). Sorafenib, midostaurin, quizartinib, gilteritinib 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\(^32\). 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 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, respectively32. 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, gilteritinib 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 anemia34,35. 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 chemotherapy36, especially in the 60 mg/day group37. 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 tolerated38. In a phase 3 trial, 371 R/R FLT3-mutated patients were randomly assigned in a 2:1 ratio to receive either gilteritinib (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 rate (21% vs. 10.5%) than salvage chemotherapy39. As a potent type II 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 patients40,41. 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 apoptosis42. 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 profile43. Currently, venetoclax has been investigated in a series of phase 1 studies in combination with low-dose cytarabine and decitabine or azacitidine44. 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 cells46. 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 agents47,48. 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 differentiation50,51. Ivosidenib andenasidenib are oral targeted IDH1 and IDH2 inhibitors, respectively52. 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 events52. For IDH2 mutation R/R AML patients, enasidenib was well tolerated and produced an ORR of 40 % in a phase 1 trial53. Almost 20% patients attained CR, 43.1% red blood cell and 40.2% platelet transfusion-dependent patients achieved transfusion independence51. 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 them52.

2.1.4. Hedgehog signaling pathway inhibitors

The Hedgehog (Hh) signaling pathway should be silenced in leukemia stem cells53. Aberrant Hh pathway release enhances the proliferation of leukemia stem cells54. The Hedgehog (Hh) signaling pathway should be silenced in hematologic malignancies to achieve a favorable outcome. The Hedgehog (Hh) signaling pathway should be silenced in leukemia stem cells. In a randomized phase 2 trial of AML or high-risk myelodysplastic syndrome (MDS) unsuitable for intensive chemotherapy, patients were randomized (2:1) to glesdegib (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. 23% (P < 0.05) and 8.8 months versus 4.9 months (P = 0.0004), respectively55. 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). Acetylation of histone proteins and protein chaperones in malignant cells is a process known as deacetylation, which is catalyzed by HDACs. In a series of preventive treatments, low dose AZA maintenance therapy in high-risk AML patients after allo-donor chimerism expression of tumor suppressor genes such as TP53, Panobinostat (HDACi) causes chromatin remodeling and inhibiting the Myeloid oncoproteins aberrantly recruit histone deacetylases from it.

In order to enhance the antitumor efficacy of HMA, 29 relapsed patients post-HSCT were treated with sequential AZA (75 mg/m², 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. 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 chimera in <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. 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. In a series of preventive treatments, low dose AZA in combination with DLI reduced the CIR of high-risk AML patients without increasing the cumulative incidence of GVHD.

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%)

Although the double-blind, phase 3, randomized study of CC-486 following allo-HSCT. Since February 2019, another HDACi (vorinostat) 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. 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. 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.

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. 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. 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. 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. 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. 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/m²).
In a small sample study, cytarabine and GO (9 mg/m²) were used as post-HSCT salvage therapy for AML patients, but only 25% of patients survived more than one year and all relapsed. 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/m²) 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. 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. In an expansion arm of this study, 33 A subsequently in combination with HMA s 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. In the terminated 51% were MRD-negative. In addition, the 30- and 60-day mortality rates were 2% and 8%, respectively. 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 in 10–1000 times in leukemia cells compared to normal CD34 positive cells. 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. 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. 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 cells. 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. Because WT1-specific (HLA-A* 24: 02) TCR-T cells can survive in vivo and retain a specific immune response to WT1, Chapuis et al. isolated a high-affinity WT1-specific TCR (TCRCA) and inserted it into EBV-specific donor CD8⁺ T cells to make the TCR-CYA cells. Twelve high-risk patients after allo-HSCT received at least one preventive infusion of TCRCA (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. 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. In a preclinical study, a humanized CXCR4 immunoglobulin G1 antibody-PF-0674143 bind to CXCR4 and inhibited CXCL12-mediated signaling pathway. PF-0674143 monotherapy or synergy with standard drugs has shown strong antitumor effects in a chemo-resistant AML patient-derived xenograft model. 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. 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. Leukemia stem cells always employ CXCL12/CXCR4 to home toward the protective marrow niches. In a phase 1 study, one dose pentixafor 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.

2.3.4. CAR T cell therapy

CAR T cell therapy has gained 90% of response rates in CD19⁺ ALL and DLL, 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. 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, DLL-1, NK2D, CD7 or FLT3) to reduce the off-target toxicities. The efficacy of CAR T cells is often related to its durability in vivo. Zheng et al. 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 anti-CD33/CD123 or anti-CD13/ TIM3) for targeting leukemia cells to reduce antigen escape-associated relapse. 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. 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. CD33-ablated HSPC were impervious to CD33-targeted immunotherapy (CAR T or GO), allowing for...
| 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)             | Active, not recruiting | 8/2019         | Yes          |
|                |          |Maintenance             | Mono            | NCT01578109 (no)             | Recruiting        | 12/2025        | Yes          |
|                |          |Maintenance             | Mono            | NCT03247088 (1/2)            | Recruiting        | 7/2022         | No           |
|                |          |Midostaurin             | Preemptive Mono | NCT03951961 (2)              | Recruiting        | 12/2023        | No           |
|                |          |Maintenance             | Mono            | NCT01477606 (2)              | Active, not recruiting | 6/2020         | Yes          |
|                |          |Quizartinib Salvage/maintenance | Mono | NCT01883362 (2) | Active, not recruiting | 4/2018         | No           |
|                |          |Maintenance             | Mono            | NCT02039726 (3)              | Recruiting        | 12/2020        | Yes          |
|                |          |Glutetrinitib Maintenance/ preemptive | Mono | NCT01468467 (1) | Recruiting        | 3/2015         | Yes          |
|                |          |Crenolanib Maintenance | Mono            | NCT02997202 (2)              | Recruiting        | 4/2025         | No           |
|                |          |Venetolax Maintenance/ preemptive + AZA | Mono | NCT02400255 (2) | Recruiting        | 6/2021         | No           |
|                |          |Maintenance             | Mono            | NCT04161885 (3)              | Recruiting        | 8/2024         | No           |
|                |          |IDH1                    | Maintenance Mono | NCT03564821 (1) | Recruiting        | 10/2022        | No           |
|                |          |IDH2                    | Maintenance Mono | NCT03551551 (2) | Recruiting        | 5/2024         | No           |
|                |          |SMO                     | Maintenance Mono | NCT03728335 (1) | Recruiting        | 12/2020        | No           |
|                |          |Key metabolism DNA      | Azacitidine Salvage | 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    | ISRCTN981653167 (1)          | Recruiting        | 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 Mono  | NCT01541280 (2)              | Completed         | 7/2015         | Yes          |
|                |          |                       | Maintenance/ preemptive + DLI | NCT02458235 (2) | Active, not recruiting | 4/2020         | No           |
|                |          |                       | Maintenance      | NCT01995578 (2)              | Recruiting        | 11/2021        | No           |
|                |          |                       | Maintenance      | NCT00350818 (1)              | completed         | 8/2010         | No           |
|                |          |                       | Maintenance      | NCT00887068 (3)              | completed         | 8/2018         | No           |
|                |          |                       | Maintenance      | NCT04128020 (1)              | Recruiting        | 10/2022        | No           |
|                |          |                       | Maintenance      | NCT03931291 (2)              | Recruiting        | 9/2021         | No           |
|                |          |                       | Maintenance      | NCT02124174 (2)              | Recruiting        | 1/2021         | No           |
|                |          |                       | Maintenance      | NCT01168219 (2)              | Active, not recruiting | 11/2015        | Yes          |
|                |          |Decitabine Salvage + DLI | Mono            | 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 Mono  | 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 Mono | NCT03771222 (2)              | Not yet Recruiting | 12/2021        | No           |
| Target       | Agent            | Intervention strategy | Therapy regimen | Identifier (phase) | Status            | Complete date | With results |
|--------------|------------------|-----------------------|-----------------|-------------------|-------------------|---------------|--------------|
| Guadecitabine| Salvage/preemptive Maintenance | +DLI Mono | NCT02684162 (2) | Recruiting        | 6/2021 | No |
|              |                   |                       | 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      | Panobinostat     | Maintenance            | Mono            | NCT01451268 (1/2) | Unknown | 4/2018 | Yes |
| deacetylases | Vorinostat        | Maintenance + AZA      | Mono            | NCT03843528 (1) | Recruiting | 12/2021 | No |
| Epitopes     | BI 836858 GO     | Salvage + F16L2 Mono   | NCT3207191 (1) | Unknown | 12/2019 | No |
| CD33         | Maintenance Mono  | Mono                   | NCT0044733 (2) | Completed | 9/2004 | Yes |
|              | Maintenance Mono  | Mono                   | NCT01020539 (1) | Active, not recruiting | 12/2020 | No |
| CD33         | CC-486            | Maintenance Mono       | NCT01835587 (1/2) | Completed | 5/2017 | Yes |
|              | Maintenance Mono  | Mono                   | NCT04173533 (3) | Recruiting | 6/2024 | No |
| CD38         | Daratumumab      | Salvage + DLI Mono     | NCT00923910 (1) | Completed | 11/2016 | Yes |
| WT1 DC vaccine| Salvage + DLI    | Mono                   | NCT01451268 (1/2) | Recruiting | 9/2017 | No |
| WT1-sensitized T | Preemptive + DEC | Mono                   | NCT01483274 (1) | Completed | 6/2015 | No |
| CTL          | Maintenance Mono  | Mono                   | NCT00052520 (1/2) | Completed | 6/2013 | No |
| CD123        | Anti-CD123-CART  | Salvage Mono           | NCT02895412 (1) | Recruiting | 12/2020 | No |
| Immune microenvironment |                |                       | NCT03114670 (1) | Recruiting | 3/2021 | No |
| CTLA-4       | Ipilimumab        | Salvage + DLI Mono     | NCT0060372 (1) | Completed | 4/2008 | Yes |
|              | Salvage Or Nivolumab | Mono                  | NCT01822509 (1) | Active, not recruiting | 12/2018 | Yes |
| PD-1         | Salvage Mono      | +/-Nivolumab           | NCT01919619 (2) | Recruiting | 6/2021 | No |
|              | Maintenance Mono  | Mono                   | NCT02846376 (1) | Recruiting | 7/2023 | No |
|              | Salvage Mono      | +/-Ipdimumab           | NCT03146468 (2) | Recruiting | 6/2020 | No |
|              | Salvage Mono      | +/-Tocilizumab         | NCT03588936 (1) | Recruiting | 8/2022 | No |
|              | Maintenance Mono  | Mono                   | NCT02985554 (1) | Recruiting | 3/2022 | No |
|              | Maintenance Mono  | Mono                   | NCT04361058 (1) | Recruiting | 4/2025 | No |
| Pembrolizumab| Salvage Mono      | Mono                   | NCT02981914 (1) | Recruiting | 2/2029 | No |
| Engineering | CD25hi T<sub>reg</sub> depleted donor T | Salvage Mono | NCT03286114 (1) | Recruiting | 10/2021 | No |
| donor T      | CD8<sup>+</sup>CD44hi donor NK | Salvage Mono | NCT00675831 (1) | Completed | 1/2013 | Yes |
| CD45RA-depleted Purified NK | Salvage Mono | NCT03912064 (1) | Recruiting | 5/2024 | No |
| Engineering | CD4<sup>-</sup>iNKT | Salvage +/-Ipilimumab | NCT01523223 (1) | Completed | 10/2016 | Yes |
| donor NK     | Salvage +/-Ipilimumab | Mono              | NCT03849651 (2) | Recruiting | 7/2025 | No |
|              | Salvage +/-Ipilimumab | Mono              | NCT04202684 (1) | Not yet Recruiting | 12/2021 | No |
| CD4<sup>-</sup>iNKT |                |                       | NCT0060372 (1) | Completed | 4/2008 | Yes |
|              | Maintenance Mono  | Mono                   | NCT0060372 (1) | Active, not recruiting | 12/2018 | Yes |
| DC           | Salvage +/-Ipilimumab | Mono              | NCT0060372 (1) | Completed | 4/2008 | Yes |
| DC/AML fusion cells |                |                       | NCT00526292 (2) | Completed | 7/2015 | Yes |
| MICA-loaded PD-L<sub>1</sub>-silenced DC | Salvage +/-Ipilimumab | Mono              | NCT00569283 (1) | Completed | 12/2008 | Yes |
| CIK          | IL-15 activated CIK | Preemptive 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)
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 loss1,121,122. CPIs for patients with upregulation of IRs and inducing moderate chronic GVHD for those with downregulation of HLA class II genes10–12. 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 DLI123–126, 50% obtained chronic GVHD, and 26% of patients eventually died of GVHD related complications127,128. Using engineered donor T cells or NK cells to improve GVL effect and reduce GVHD is a promising strategy that is currently being extensively studied129. 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 toxicity130.

3.1. Immune checkpoint inhibitors (CPIs)

CTLA-4 and PD-1 are the well-known immune checkpoints for tumor immune escape31. 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 cells132,133. 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 patients134. 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 ipilimumab134. 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 tolerable135. 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 related136. 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 GVHD136. 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, Treg depletion was associated with better response rate (62.5% vs. 28.6%) and improved event-free survival (27% vs. 0%) without excessive GVHD136. CD8+CD44hi memory T cells may have a 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 low137. 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 activity138. 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 KIR2DL2/3/2DL3/2DS2 at 14 days after haploidentical NK cell infusion achieved better survival139. Especially, allografts from donors who were positive for KIR2DS1 had more powerful anti-
leukemia effect than negative. Donor KIR3DS1 was associated with reduced mortality. 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. 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 2c-chain cytokine with unique properties to stimulate antitumor immunity, including stimulation of NK cells and CD8+ memory T cells. In a mouse model of relapse after allo-HSCT, systemic IL-15 infusion often increases GVL activity with severe GVHD. 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 produce the leukemia cells to produce IL-15. Thus, a strong GVL effect was promoted without inducing obvious GVHD. 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%. 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 Treg cells. Responses were observed in 19% of evaluable patients, including 1 patient who achieved a CR for 7 months.

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. Unavailable donor, HLA loss or active GVHD are always greatly limiting the application and the efficacy of traditional DLI and second transplantation. 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, the lysine demethylase LSD1, the protein methyltransferases EZH2,
DOT1L, PRMT5, and the BET bromodomain proteins. 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. 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. 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.

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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.

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