Natural killer cell-based immunotherapy for acute myeloid leukemia

Jing Xu and Ting Niu*

Abstract
Despite considerable progress has been achieved in the treatment of acute myeloid leukemia over the past decades, relapse remains a major problem. Novel therapeutic options aimed at attaining minimal residual disease-negative complete remission are expected to reduce the incidence of relapse and prolong survival. Natural killer cell-based immunotherapy is put forward as an option to tackle the unmet clinical needs. There have been an increasing number of therapeutic dimensions ranging from adoptive NK cell transfer, chimeric antigen receptor-modified NK cells, antibodies, cytokines to immunomodulatory drugs. In this review, we will summarize different forms of NK cell-based immunotherapy for AML based on preclinical investigations and clinical trials.

Keywords: Acute myeloid leukemia, Natural killer cells, Immunotherapy, Adoptive NK cell transfer, Chimeric antigen receptor-modified NK cells, Antibodies, Cytokines

Background
Acute myeloid leukemia (AML) is a clinically and genetically heterogeneous disease with unsatisfactory outcomes. Over the last few years, considerable progress has been achieved in the treatment of AML with the development and implementation of new drugs [1, 2]. However, allogeneic hematopoietic cell transplantation (HCT) has been recognized as the only way to cure AML so far and relapse remains a major problem. Novel therapeutic options aimed at attaining minimal residual disease (MRD)-negative complete remission (CR) are expected to reduce the incidence of relapse and prolong survival. Thus, immunotherapy becomes an option to tackle unmet clinical needs in AML [3, 4].

Immunotherapy has been recognized as an incredibly promising therapeutic strategy for numerous cancers [5]. The adoption of this treatment modality is based on mechanisms of immune surveillance/response and cancer escape [6]. Under physiological conditions, immune cells and substances in the immune system play pivotal roles in detecting and destroying pathogen-infected or neoplastically transformed cells. But they become less potent in cancer elimination when malignant cells display the loss of antigenicity and/or immunogenicity and are surrounded by an immunosuppressive microenvironment [6]. Thus, immunotherapy with strategies of reboosting patients’ own immune system or initiating new immune response to fight cancers has been demonstrated with the capacity of producing sustainable clinical benefits against both solid and hematological malignancies [7–9].

Natural killer (NK) cell-based immunotherapy represents one of the novel immunotherapeutic strategies recently, unleashing immune suppression of NK cells to attack various cancers [10–12]. With the progressive elucidation of NK cell immunobiology and the development of manipulative techniques, the field of NK cell-based immunotherapy in hematological malignancies has been expanding and accelerating over the past years, including adoptive NK cell transfer [13–16], chimeric antigen receptor (CAR)-modified NK cells [17–22], antibodies [23–25], cytokines [26, 27] and drug treatment [28–31]. Despite remarkable progress has been made,
the application in AML is still at the initial stage. Firstly, clinical trials with results showing the efficacy and safety of these therapeutic approaches are limited, most of which are currently still in progress. Secondly, preclinical studies of NK cell-based immunotherapy are constantly emerging, in the aspect of new methodologies to utilize NK cells and strategies to enhance the response [32, 33].

Herein, in this review, we provide an overview of NK cell biology, the pathology of NK cells in AML and the recent advances in NK cell-based immunotherapy for AML based on preclinical investigations and clinical trials.

**Biology of NK cells**

NK cells belong to innate lymphoid cells that contribute to immune system’s first-line defense against infections and malignant diseases [34]. They can be categorized into two subsets on the basis of surface expression levels of CD56 and CD16, as measured by the intensity of immunofluorescence. The canonical CD56dimCD16+ NK cell subset comprises around 90% of the total population in peripheral blood and exerts strong cytolytic activity through releasing cytotoxic granules containing perforin and granzymes. The rest 10% of NK cell population, known as CD56brightCD16−, is a potent producer of immunoregulatory cytokines including interferon (IFN)-γ, tumor necrosis factor (TNF)-α/β and interleukin (IL)-10 [35].

The cytotoxic function of NK cells is finely regulated by a complex array of surface inhibitory receptors [e.g., inhibitory killer immunoglobulin-like receptors (KIRs), leukocyte immunoglobulin-like receptors (LIRs) and CD94/natural killer group 2A (NKG2A)] and activating receptors [e.g., activating KIRs, CD94/NKG2C, NKG2D and natural cytotoxicity receptors (NCRs)] that deliver suppressive and stimulatory signals, respectively (Fig. 1) [36, 37]. In line with the diversity of major histocompatibility complex (MHC) molecules in populations, KIRs are genetically determined and display a high level of polymorphism. There are two main groups of KIR haplotypes, termed as “A” and “B”, as classified by the distinct gene content. KIR A haplotypes mainly contain inhibitory KIR genes and only one activating KIR gene KIR2DS4, whereas KIR B haplotypes carry, besides inhibitory KIR genes, various numbers and combinations of activating KIR genes [38, 39]. The considerable differences of both allelic polymorphism and KIR gene content account for the high variability of KIR gene family among different individuals.

NK cell-mediated cytotoxicity is based on the notion of “missing self-recognition” and “induced self-recognition” [40]. During NK cell development, inhibitory KIR receptors encounter with MHC class I (MHC-I) ligands

![Fig. 1](image-url)

**Fig. 1** Mechanisms of immune escape from NK cell-mediated recognition in AML. Dysfunctional NK cells exhibit an imbalanced receptor expression with the overexpression of inhibitory receptors and the underexpression of activating receptors. AML cells display a defective expression of cognate ligands for NK cell activating and inhibitory receptors. The tumor microenvironment consisting of Treg cells and MDSCs can interfere with the function of NK cells through the secreting of cytokines. MDSC myeloid-derived suppressor cell, NK natural killer cell, Treg regulatory T cell
on their own hematopoietic cells, leading to the acquisition of functional competence and self-tolerance [41, 42]. Both the reduction/absence of MHC-I molecules and the upregulation/de novo expression of ligands for activating receptors on tumor cells can elicit NK cell immune response against “non-self,” through releasing cytotoxic granules, secreting cytokines and inducing death receptor-dependent apoptosis [36, 43]. Apart from the direct receptor-based recognition between NK cells and tumor cells that potentiates the anti-tumor function of NK cells, they can kill tumor cells by antibody-dependent cell-mediated cytotoxicity (ADCC) as well, which is mediated by the IgG Fc receptor CD16 [44]. 

In addition, the activation of NK cells can be induced by other immune cells such as macrophages and dendritic cells (DCs) as well, either through direct cell-to-cell contacts or the release of cytokines such as IL-12, IL-15, IL-18 and IFN-α/β, promoting NK cell cytotoxicity and IFN-γ production [45, 46].

Dysfunction of NK cell-mediated anti-leukemia responses in patients with AML
In AML, leukemia cells can escape from NK cell-mediated recognition as a consequence of NK cell abnormalities, immunosuppressive properties of AML cells or interactions between NK cells and other immune cells in favor of immune escape (Fig. 1) [47].

Since the function of NK cells is tightly regulated by their sophisticated repertoire of inhibitory and activating receptors, imbalanced receptor expressions can lead to NK cell dysfunction. Studies evaluating the expression of these molecular receptors on NK cells showed the underexpression of activating receptors such as NKG2D, NCRs and DNAX accessory molecule-1 (DNAM-1) as well as overexpression of inhibitory receptors such as KIR2DL2/L3 and NKG2A in AML patients as compared with healthy controls [48–52]. Direct contact between AML cells and NK cells, high expression of CD200 on AML cells, soluble NKG2D ligands (NKG2DLs) in the sera and suppressive tumor microenvironment are factors that lead to defective receptor expression changes [49, 53, 54].

In addition to NK cell abnormalities, leukemia cells themselves displaying a defective expression of ligands for NK cell activating/inhibitory receptors give rise to the attenuation of NK cell-mediated anti-leukemia responses as well. For instance, the low expression of NKG2DLs [MHC class I chain-related proteins (MIC) and UL16-binding proteins (ULBP)], NCR ligands and DNAM-1 ligands (CD112 and CD155) on AML cells can render them resistant to NK cell killing [55, 56]. The deficient NKG2DL expression on AML cells may be caused by aberrant epigenetic mechanisms or the release of soluble forms from the cell surface by metalloproteinases [57, 58]. Whereas, upregulation of inhibitory immune checkpoint molecules programmed cell death ligand-1 (PD-L1) and PD-L2 is observed in AML blasts [59].

The tumor microenvironment, which possesses immunosuppressive cells, such as regulatory T cells (Tregs), myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and tolerogenic DCs as well as immunosuppressive factors such as transforming growth factor (TGF)-β, IL-10 and indoleamine 2,3 dioxygenase (IDO), is another major limitation to the effectiveness of NK cells in AML [60, 61].

It is worth noting that expressions of NK receptors and their cognate ligands on leukemic cells as well as the signals deriving from tumor microenvironment are deemed to impact clinical outcomes and relapse in AML patients [47]. These NK cell function-related adverse prognostic parameters including hypomutation NK cell profile (CD56bright and KIR−/CD57−), increased NKG2A and decreased NCR on NK cells, increased CD200 and decreased ULBP1 on AML cells [49, 51, 53, 62–66]. Moreover, persistence of dysfunctional NK cells was found even in patients who achieve first CR after intensive chemotherapy [67]. Thus, the presence of dysfunctional NK cells in AML and their prognostic relevance provide the rationale for the use of NK cell-based immunotherapy to restore impaired NK cell cytotoxicity against AML.

NK cell-based immunotherapy in AML
Adoptive NK cell transfer
The strategy of adoptive NK cell transfer was put forward based on beneficial effects of NK cell alloreactivity in the setting of allogeneic HCT (allo-HCT). NK cell alloreactivity is triggered by the mismatch between KIRs on donor NK cells and human leukocyte antigen (HLA) class I molecules on recipient cells, the effectiveness of which in leukemia was initially described by Perugia group [68, 69]. Alloreactions mediated by donor NK cells can kill leukemia through graft-versus-leukemia (GvL) effect, promote engraftment through ablation of recipient T cells and protect against graft-versus-host disease (GvHD) through depleting recipient antigen-presenting cells and producing IL-10 [70, 71]. Transplantation from NK alloreactive donors is considered as a strong independent factor predicting survival in allo-HCT recipients, especially from donors with more KIR B gene-content motifs [72–75]. Besides, rapid NK cell recovery post-HCT is associated with improved outcomes, while impaired NK function may be the cause of relapse [76–79]. Taken together, given the basic notions of NK cell alloreactivity and the prognostic effects of functional NK cell counts, adoptive transfer of NK cells for the
management of AML has been explored in clinical applications (Fig. 2a).

Despite HCT has yielded a high rate of curability for AML, it is associated with transplant-related morbidity and mortality. Besides, not every patient is a candidate for HCT and relapse after HCT remains the most frequent cause of treatment failure. Therefore, adoptive NK cell transfer seems to be an ideal option as adjuvant and alternative treatment, and it has already been performed in the context of HCT as well as in the non-HCT setting.

Adoptive NK cell transfer in the context of HCT
Donor-derived NK cells are most commonly obtained from donor leukapheresis products using a magnetic cell sorting (MACS) system by CD3 depletion with or without CD56 enrichment [80–84]. They can also be generated by ex vivo differentiation from donor CD34+ hematopoietic progenitor cells [85]. NK cell transfer after HLA-haploidentical HCT is well tolerated and consolidates engraftment [80, 86]. Remarkably, a phase I study investigating the clinical effect of IL-15 plus IL-21 stimulated CD3-depleted NK cells given 2 and 3 weeks after HCT demonstrated that leukemia progression reduced compared with historical patients who have undergone HCT after the same conditioning regimen without NK cell infusion (hazard ratio 0.527, \( p = 0.042 \)) [81]. Another phase I study showed that multiple doses of NK cells (days—2, 7 and 28 post-HCT) expanded ex vivo with K562-mbIL21-41BBL feeder cells, which were genetically modified K562 leukemia cell line expressing membrane-bound IL-21 and the 41BB ligand, could be effective in controlling leukemia relapse as well [82]. However, another study showed that compared with NK cell transfer at weeks 2 and 3 post-HCT, additional early transfer (days 6 and 9 post-HCT) was associated with significant cytokine release syndrome (CRS)-related toxicity and was not associated with less leukemia progression in patients with relapsed/refractory (R/R) AML [83].
Notably, high expression of NKp30 on donor NK cells was an independent predictor of high CR and low leukemia progression [83]. In addition, NK cells are also safe and feasible to be infused prior to HCT. A phase I study infusing escalating doses of donor-derived NK cells as a component of the preparative regimen for allo-HCT (day—8 pre-HCT) demonstrated that relapse-free survival was highly associated with the number of NK cells delivered [87]. Besides, NK cell transfer can also be applied as a bridge to HCT in R/R AML, which is useful in the reduction in disease burden to make patients eligible to proceed to HCT [84].

Adoptive NK cell transfer in the non-HCT setting

Since the limitations of HCT make it not applicable to all patients, it is conceivable to propel the development of adoptive NK cell transfer outside the transplantation setting.

Miller et al. was the first to conduct NK cell transfer in adult AML patients without prior HCT, reporting that haploidentical NK cell transfer with the intense high-dose cyclophosphamide and fludarabine immune suppression regimen, CD3 depletion and IL-2 administration both ex vivo and in vivo was a safe treatment with successful NK cell proliferation and activation in R/R AML (CR 5/19) [88]. Over the years, modifications to this approach have led to remarkable progress, ranging from donor selection according to KIR-ligand mismatch to improve outcomes, NK cell purification using CD3 depletion followed by CD56 enrichment to avoid side effects caused by residual cells, to milder conditioning regimens and lower dose of IL-2 in vivo to make it a well-tolerated regimen. Adoptive NK cell transfer is a feasible strategy for AML not only to induce remission, but also to maintain CR [89–93]. The combination of consolidation therapies of NK cell transfer and chemotherapy contributed to the further remission with decreased MRD and the reduction in long-term recurrence in AML patients at CR [94]. Though a phase II trial reported that NK cell transfer as a consolidation therapy for pediatric AML in first CR did not decrease relapse and increase overall survival (OS), the result of another just concluded phase II trial (NCT02763475) with a higher number of NK cell administration is worth the wait [95, 96].

Since the higher number of donor alloreactive NK cells correlates with better outcomes, ex vivo generation and in vivo expansion of an adequate number of donor NK cells with robust anti-leukemia potential are highly warranted [92]. In terms of ex vivo manipulating methods, Miller et al. demonstrated the superiority of CD3 and CD19 depletion method compared with CD3 depletion alone and CD3 depletion followed by CD56 enrichment methods, with no cause of negative effects by co-infused monocytes [97]. NK cell expansion and functional activity can be significantly enhanced by co-culturing donor’s peripheral blood mononuclear cells (PBMC) with cytokines (mainly IL-2 and IL-15) or feeder cells bearing membrane-bound cytokines (such as K562-mbIL15-41BBL or K562-mbIL21-41BBL) [94, 98–100]. The feeder-free approach of using plasma membrane particles derived from K562-mbIL15-41BBL feeder cells resulted in great expansion of NK cells as well and avoided tumor-derived feeder cells being injected into patients [101]. Two phase I studies demonstrated NK cells primed with the lysate of CTV-1 leukemia cell line could prolong CR in high-risk AML patients [102, 103]. Despite IL-2 has the effect of stimulating NK cells, it stimulates host Treg cells in the meanwhile, which can inhibit NK cell proliferation and expansion in vivo. IL-15 was proposed as an alternative to IL-2 without such drawback [104, 105]. The first-in-human trial of using in vivo recombinant human IL-15 to potentiate haploidentical NK cell transfer in R/R AML showed better rates of NK cell expansion and remission compared with previous trials with IL-2, but CRS was observed when IL-15 was administered subcutaneously [106]. Furthermore, Miller et al. proposed a method of incorporating Treg depletion with IL-2 diphtheria toxin (IL2DT) into adoptive transfer platform. IL2DT was delivered to patients 1 or 2 days before NK cell transfer and it improved CR rate (53% versus 21%; \( P=0.02 \)) and disease-free survival (33% versus 5%; \( P<0.01 \)) for R/R AML patients [97]. It was showed that the use of IL2DT or low-dose irradiation as part of conditioning resulted in increased NK cell homing and persistence in the bone marrow, which correlated with better leukemia control [107].

Apart from quantity demands for NK cells, alternative sources for NK cells can facilitate their clinical applications as well. A phase I clinical trial evaluated the feasibility and safety of transferring activated human NK-92 cell lines to patients with R/R AML. NK-92 cells possess advantages of easy cultivation and expansion and can be repeatedly infused in the context of lymphodepletion [108]. Its derivative cell line NK-92MI without the presence of surface sialic acid-binding immunoglobulin-like lectins (siglec)-7 exhibited high and sustainable cytotoxicity against NK-92MI-resistant leukemia cells [109]. Besides, a study established the proof-of-concept of the feasibility of NK cells generated from CD34 + hematopoietic stem and progenitor cells (HSPC) isolated from cryopreserved umbilical cord blood (UCB) in a preclinical AML xenograft model [110]. The first-in-human study exploiting UCB-derived HSPC-NK cells in the treatment of elderly AML patients in morphologic CR found NK cell expansion and further maturation in vivo
as well as a reduction in MRD without the induction of NK cell-related toxicity [111]. Another study evaluating placental-derived HSPC-NK cells (PNK-007) in R/R AML demonstrated an encouraging safety profile, but larger scale studies are needed to assess clinical outcomes [112]. A clinical trial investigating the feasibility of CYNK-001, the cryopreserved successor product to PNK-007, has recently been initiated (NCT04310592). Moreover, FT516, a NK cell product derived from a clonal master engineered induced pluripotent stem cell (iPSC) line, as a monotherapy for R/R AML is in clinical investigation (NCT04023071). These “off-the-shelf” products have significant benefits over primary NK cells from adult donors in the aspect of low costs, high therapeutic dosages, immediately application, choosing appropriate KIR B haplotype alloreactive donors and doing genetic modifications.

Further clinical trials are underway to evaluate the safety and efficacy of adoptive NK cell transfer, with the exploration of optimal NK cell dosages and resources, the optimal time points in relation to HCT and potential combination therapies. A list of currently ongoing clinical trials of NK cell transfer is provided in Table 1.

**CAR-NK cell therapy**

In adoptive NK cell transfer, the ability of NK cells to mount an immune response against AML cells is largely dependent on the interactions between NK cell activating/inhibitory receptors with their cognate ligands on target cells. In order to augment the specificity and cytotoxicity, genetically modified NK cells such as CAR-modified-NK cells are designed (Fig. 2b). Since the success of CAR-T therapy in the treatment of B-lineage acute lymphoblastic leukemia and B-cell lymphoma has not yet been translated into the treatment of AML and its wide applications are limited by adverse effects such as CRS [113, 114], NK cells with short lifespan are being considered as promising alternatives to modified T cells with favorable toxicity profiles and low manufacturing costs [115]. Nowadays, the actions of CAR-NK cells are being extensively studied in a variety of tumor models, but the applications in AML are relatively limited and mainly at the preclinical stage.

The optimal choice of leukemia specific markers that can be targeted by CAR-NK cells is a major obstacle, since AML shares some phenotypic markers with normal hematopoietic stem cells (HSCs). Myeloid differentiation antigen CD33 is detected on blasts of >85% of AML patients and also on leukemia stem cells (LSCs) [116]. A preclinical investigation ascertained the targeting effect of NK cell line YT with gene transfer of a CD33-specific immunoglobulin-based humanized chimeric T cell receptor (clgTCR) to CD33+ AML cell lines [117]. The first-in-man reported phase I trial of CAR-NK cells demonstrated the safety of irradiated CD33-CD28-4-1BB-CD3ζ CAR-NK-92 cells infusion in 3 patients with R/R AML, but it did not demonstrate obvious clinical efficacy [118]. Larger-scale clinical trials are warranted to determine the effects (NCT02944162). CD4 is another antigen present on AML blasts without ubiquitous expression on HSPCs and non-hematopoietic cells. Salman et al. established the role of CD4-CD28-4-1BB-CD3ζ CAR-NK-92 cells in robustly eliminating CD4+ AML cells ex vivo and in mouse xenografts [119]. CD7 is detected in approximately 30% of AML cases and also presents as an attractive target [120, 121]. CD7-CD28-4-1BB-CD3ζ CAR-NK-92MI cells have significantly improved killing efficiency against CD7+ AML cells as compared with NK-92MI cells without genetic modifications, which provides a basis for clinical investigation (NCT02742727) [122].

As for the sources of CAR-NK cells, a preclinical study showed that CD123-CAR-NK-92 cell lines represented better CAR effector cells than primary human donor CD123-CAR-NK cells in terms of cytotoxic activities [123].

The lessons learned from CAR-T and CAR-NK cells in the treatment of other cancers are worthy to be exploited in CAR-NK cell therapy in AML in the future, including optimizing targets and structures of CAR-NK cells as well as investigating the ideal patient populations for this type of immunotherapy.

**Antibodies**

In the normal physiologic setting, the interaction of receptors-ligands and the process of ADCC are involved in the NK cell activation. Taking advantage of this functionality, monoclonal antibodies become another method of boosting patients’ NK cells against AML. On the one hand, antibodies targeting tumor-associated antigens endow NK cells with the power of activation via ADCC effects. On the other hand, antibodies targeting NK cell inhibitory receptors have the potential to weaken inhibitory signals and let activating signals dominate the process. Great progress has been made in the field of antibody therapies, and the overview of ongoing clinical trials concerning novel antibodies for AML is presented in Table 2.

**Antibodies targeting tumor-associated antigens**

Antibodies targeting tumor-associated antigens are attractive means of immunotherapy for cancers, the mechanisms of which are in great part the induction of ADCC via NK cells (Fig. 2c). The outcomes of unconjugated antibodies were generally poor when used alone [124–126]. The effects could be enhanced by engineering
antibodies’ Fc parts to increase affinity to CD16 or integrating with other therapies [127–129]. Preclinical studies investigating the efficacy of novel Fc-optimized antibodies targeting various potential antigens such as CD133, CD33, CD157 and IL-1 receptor accessory protein (IL1RAP) as well as new regimens of antibodies targeting various potential antigens such as CD133, CD33, CD157 and IL-1 receptor accessory protein (IL1RAP) as well as new regimens of antibodies...
Table 2 Overview of ongoing clinical trials of antibodies for AML

| Antibody | Target | Regimen | Indication | Phase | Identifier |
|----------|--------|---------|------------|-------|------------|
| BI 836858 | CD33 | BI 836858 + decitabine | AML | II | NCT02632721 |
| GO | CD33 | GO + CPX-351 | Relapsed AML | I | NCT03904251 |
| GO | CD33 | GO + venetoclax | R/R CD33 + AML | I | NCT04070768 |
| GO | CD33 | GO + pracinostat | R/R CD33 + AML | I | NCT03848754 |
| GO | CD33 | GO + allo-HCT | Average-risk CD33 + AML or MDS or JMML | I | NCT01020539 |
| GO, midostaurin, cytarabine and daunorubicin | CD33 | GO + talazoparib | R/R CD33 + AML | I/II | NCT04207190 |
| GO, midostaurin, cytarabine and daunorubicin | CD33 | GO, PF-04518600, venetoclax, avelumab, glasdegib and azacitidine | R/R AML | I/II | NCT03390296 |
| GO, G-CSF, cladribine, cytarabine and mitoxantrone | CD33 | GO, midostaurin, cytarabine and daunorubicin | Newly diagnosed FLT3 mutated AML | I | NCT03900949 |
| GO | CD33 | GO + allo-HCT | Newly diagnosed AML | I/II | NCT03531918 |
| GO | CD33 | GO + azacitidine | Newly diagnosed elderly AML | II | NCT00658814 |
| GO | CD33 | GO + bortezomib | R/R AML | II | NCT04173585 |
| GO | CD33 | GO + CPX-351 | R/R CD33 + AML or high-risk MDS | II | NCT03672539 |
| GO | CD33 | GO + DLI | R/R AML | II | NCT03374332 |
| GO, mitoxantrone and etoposide | CD33 | GO, mitoxantrone and etoposide | Refractory CD33 + AML | II | NCT03839446 |
| GO, cyclophosphamide, busulfan and allo-HCT | CD33 | GO, cyclophosphamide, busulfan and allo-HCT | High-risk CD33 + AML or MDS | II | NCT02221310 |
| GO, fludarabine, cytarabine, filgrastim-sndz and idarubicin | CD33 | GO, fludarabine, cytarabine, filgrastim-sndz and idarubicin | Newly diagnosed AML or high-risk MDS | II | NCT00801489 |
| GO, daunorubicin, cytarabine and glasdegib | CD33 | GO, daunorubicin, cytarabine and glasdegib | Newly diagnosed AML | II | NCT04168502 |
| GO | CD33 | GO + standard chemotherapy | Pediatric AML | II | NCT04326439 |
| GO | CD33 | GO + cytarabine | Newly diagnosed AML | II/III | NCT02473146 |
| GO | CD33 | GO + daunorubicin + cytarabine | Elderly AML | II/III | NCT02272478 |
| GO | CD33 | GO | Newly diagnosed AML | III | NCT04093505 |
| GO | CD33 | GO + standard chemotherapy | Newly diagnosed NPM1 mutated AML | III | NCT00893399 |
| GO | CD33 | GO + standard chemotherapy + HCT | AML | III | NCT00049517 |
| GO, CPX-351, gilteritinib and standard chemotherapy | CD33 | GO, CPX-351, gilteritinib and standard chemotherapy | Newly diagnosed AML | III | NCT04293562 |
| GO, liposomal daunorubicin, mitoxantrone, fludarabine, cytarabine, busulfan and cyclophosphamide | CD33 | GO, liposomal daunorubicin, mitoxantrone, fludarabine, cytarabine, busulfan and cyclophosphamide | Pediatric AML | III | NCT02724163 |
| Lintuzumab Ac-225 | CD33 | Lintuzumab Ac-225, cladribine, cytarabine, mitoxantrone and G-CSF | R/R CD33 + AML | IV | NCT03727750 |
| Lintuzumab Ac-225 | CD33 | Lintuzumab Ac-225 + venetoclax + spironolactone | R/R CD33 + AML | I | NCT03441048 |
| Lintuzumab Ac-225 | CD33 | Lintuzumab-Ac225 + venetoclax | R/R CD33 + AML | I/II | NCT03867682 |
| Lintuzumab-Ac225 | CD33 | Lintuzumab Ac-225 + venetoclax + azacitidine | R/R CD33 + AML | I/II | NCT03932318 |
| Daratumumab | CD38 | Daratumumab | R/R AML or high-risk MDS | II | NCT03067571 |
| Daratumumab + DLI | CD38 | Daratumumab + DLI | Relapsed AML after HCT | I/II | NCT03537599 |
| Isatuximab | CD38 | Isatuximab + standard chemotherapy | Pediatric R/R ALL or AML | II | NCT03808444 |
| Magrolimab | CD47 | Magrolimab + atezolizumab | R/R AML | I | NCT03922477 |
| Magrolimab + azacitidine | CD47 | Magrolimab + azacitidine | AML or MDS | I | NCT03248479 |
| Magrolimab + azacitidine + venetoclax | CD47 | Magrolimab + azacitidine + venetoclax | AML | I/II | NCT04435691 |
| Antibody     | Target | Regimen                                | Indication                                      | Phase | Identifier               |
|--------------|--------|----------------------------------------|-------------------------------------------------|-------|--------------------------|
| Cusatuzumab  | CD70   | Cusatuzumab, azacitidine and venetoclax | AML                                             | I     | NCT04150887              |
|              |        | Cusatuzumab + azacitidine              | Newly diagnosed AML or high-risk MDS            | I     | NCT04241549              |
|              |        | Cusatuzumab + azacitidine              | Newly diagnosed AML or high-risk MDS            | I/II  | NCT03090612              |
| SEA-CD70     | CD70   | SEA-CD70                               | AML or MDS                                      | I     | NCT0427847               |
| IMGN632      | CD123  | IMGN632                                | R/R CD123 + AML or other hematologic malignancies | I/II  | NCT03386513              |
| ASP1235      | FLT3   | ASP1235                                | AML                                             | I     | NCT02864290              |
| FLYSYN       | FLT3   | FLYSYN                                 | AML                                             | I/II  | NCT02789254              |
| Atezolizumab | PD-L1  | Atezolizumab + magroliamib             | R/R AML                                         | I     | NCT03922477              |
|              |        | Atezolizumab + gilteritinib            | R/R FLT3 mutated AML                            | I/II  | NCT02730012              |
| Avelumab     | PD-L1  | Avelumab, GO, PF-04518600, venetoclax, | R/R AML                                         | I/II  | NCT03390296              |
| Durvalumab   | PD-L1  | Durvalumab + azacitidine               | Newly diagnosed MDS or elderly AML              | II    | NCT02775903              |
| Antibodies targeting NK cell inhibitory receptors   |        |                                        |                                                 |       |                          |
| Pembrolizumab| PD-1   | Pembrolizumab                           | Relapsed AML or MDS after HCT                   | I     | NCT03286114              |
|              |        | Pembrolizumab + decitabine             | AML or MDS                                      | I     | NCT03969446              |
|              |        | Pembrolizumab + AMG 330                | R/R AML                                         | I     | NCT04478695              |
|              |        | Pembrolizumab                           | Non-favorable risk AML                           | I     | NCT02771197              |
|              |        | Pembrolizumab                           | Elderly AML in remission                        | I     | NCT02708641              |
|              |        | Pembrolizumab + cytarabine             | R/R AML                                         | I     | NCT02768792              |
|              |        | Pembrolizumab + azacitidine            | NPM1 mutated AML                                | I     | NCT03769532              |
|              |        | Pembrolizumab + azacitidine            | R/R AML or newly diagnosed elderly AML          | II    | NCT02845297              |
|              |        | Pembrolizumab, azacitidine and venetoclax | Elderly newly diagnosed AML                  | II    | NCT04284787              |
|              |        | Pembrolizumab, cytarabine, idarubicin, daunorubicin and HCT | Newly diagnosed AML                           | II    | NCT04214249              |
| Nivolumab    | PD-1   | Nivolumab                              | High-risk AML or MDS after HCT                  | I     | NCT04361058              |
|              |        | Nivolumab                              | Relapsed AML after HCT                          | I     | NCT01822509              |
|              |        | Nivolumab + ipilimumumab               | AML or MDS                                      | I     | NCT02846376              |
|              |        | Nivolumab + ipilimumumab               | High-risk R/R AML or MDS                        | I     | NCT03600155              |
|              |        | Nivolumab, CDX-1401, poly ICCLC and decitabine | AML or MDS                                      | I     | NCT03358719              |
|              |        | Nivolumab + azacitidine                | Pediatric R/R AML                               | I/II  | NCT03825367              |
|              |        | Nivolumab                              | AML in remission at high-risk for relapse       | I     | NCT02532231              |
|              |        | Nivolumab                              | AML in remission                                | I     | NCT02275533              |
|              |        | Nivolumab, azacitidine and ipilimumumab | AML                                             | II    | NCT02397720              |
|              |        | Nivolumab, azacitidine, midostaurin, decitabine and cytarabine | Elderly newly diagnosed AML or high-risk MDS | II/III| NCT03092674              |
| Tislelizumab | PD-1   | Tislelizumab, DNA hypomethylating agent and chemotherapy | AML                                             | II    | NCT04541277              |
| Spartalizumab| PD-1   | Spartalizumab, MBG453 and decitabine   | AML or high-risk MDS                             | I     | NCT03066648              |
| Ipilimumab   | CTLA-4 | Ipilimumab                              | Relapsed AML after HCT                          | I     | NCT01822509              |
|              |        | Ipilimumab + nivolumab                 | High-risk R/R AML or MDS                        | I     | NCT03600155              |
|              |        | Ipilimumab + nivolumab                 | AML or MDS                                      | I     | NCT02846376              |
|              |        | Ipilimumab + decitabine                | R/R AML or MDS                                  | I     | NCT02809329              |
|              |        | Ipilimumab + DLI                       | Relapsed AML, MDS or MPN after HCT              | I     | NCT03912064              |
combined with NK cell transfer exhibited promising results and these strategies can be valuable to be conducted in future clinical trials [130–136]. Antibody-drug conjugates (ADCs) and antibody-radio conjugates are promising strategies to enhance the antibody potency as well, and they yield superior clinical impacts on AML patients [137–141]. Gemtuzumab ozogamicin (GO), the combination of anti-CD33 antibody with anti-neoplastic agent calicheamicin, is currently the only ADC approved by the Food and Drug Administration (FDA) for the treatment of newly diagnosed and R/R CD33 + AML [142–144]. Latest preclinical findings of more novel ADCs targeting CD33, CD37, FLT3, C-type lectin-like molecule 1 (CLL-1; also known as C-type lectin domain family 12, member A, CLEC12A) and leukocyte immunoglobulin-like receptor subfamily B4 (LILRB4) highlight their clinical potential for the treatment of AML [145–151].

In addition, ligands of NK cell inhibitory or activating receptors on AML cells can also be the targets of antibodies. It was reported that NK-resistant feature of mixed lineage leukemia (MLL)-rearranged leukemia could be overcome by anti-CD19 antibody and anti-CD33 antibody-induced ADCC, and the effects could be further amplified with pan-MHC-I antibodies, suggesting the utilization of a triple immunotherapy approach, including KIR-mismatched NK cell transfer, antibodies against tumor-associated antigens and inhibitory KIR blockade, for the treatment of MLL-rearranged leukemia [152]. The expression level of inhibitory immune checkpoint molecule PD-L1 on AML blasts is an important negative prognostic factor [153]. Hypomethylating agents, while enhancing anti-tumor immune response, can concurrently dampen immune response by upregulating PD-1 and PD-L1 expression, providing the rationale of combination therapies of PD-L1 inhibitors and hypomethylating agents [154, 155]. Other antibodies targeting TNF family members on AML cells, such as glucocorticoid-induced TNFR-related protein ligand (GITRL) and receptor activator for NF-κB ligand (RANKL), were manifested against primary AML cells in preclinical studies through the prevention of inhibitory signals into NK cells as well as the induction of ADCC [156–158]. Despite the inevitable reduction in activating signals upon antibodies binding to ligands of activating receptors, NKG2D-Fc and NKp80-Fc fusion proteins were shown to be able to compensate for it by inducing ADCC to potentiate NK cell killing of AML cells [159, 160].

**Antibodies targeting NK cell inhibitory receptors**

Inhibitory receptors in NK cells serve as the sources of cancer immune escape, making them ideal targets for immunotherapy (Fig. 2d). Over the past decades, the number of inhibitory receptors identified in NK cells has been increasing. Apart from MHC-I-specific inhibitory receptors KIRs, LIRs and CD94/NKG2A, other immune checkpoints on NK cells have been shown to cause dysfunction such as programmed cell death-1 (PD-1), cytotoxic T lymphocyte-associated antigen-4 (CTLA-4), T-cell immunoglobulin domain and mucin domain-3 (TIM-3), T-cell immunoglobulin and immunoreceptor tyrosine-based inhibitory motif domain (TIGIT), siglec-7/9 and CD200R [161].

Just as the benefit of KIR-ligand mismatch between donors and recipients in improving the outcome of HCT, pharmacologic KIR blockade by anti-KIR antibodies can prevent the KIR-HLA-C interaction and augment NK cell function as well. IPH2101 and IPH2102 (lirilumab) are antibodies targeting KIR2D and both were reported to be safe in the treatment of elderly patients with AML in first CR, though the leukemia-free survival with lirilumab did not compare favorably to placebo in a phase II study [162–164]. The combination of lirilumab with azacitidine also did not display significant improvement in R/R AML in terms of response rate (overall response

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**Table 2 (continued)**

| Antibody | Target | Regimen | Indication | Phase | Identifier |
|----------|--------|---------|------------|-------|------------|
| MBG453   | TIM-3  | MBG453, HDM201 and venetoclax | AML or high-risk MDS | I     | NCT03940352 |
|          |        | MBG453, spartalizumab and decitabine | AML or high-risk MDS | I     | NCT03066648 |
|          |        | MBG453, azacitidine and venetoclax | Newly diagnosed AML | II    | NCT04150029 |
| BiKE or TriKE |        | CD16/IL-15/CD33 | CD33 + R/R AML or high-risk MDS | I/II  | NCT03214666 |

*ALL acute lymphoblastic leukemia, allo-HCT allogeneic hematopoietic cell transplantation, AML acute myeloid leukemia, BiKE bi-specific killer cell engager, CML chronic myeloid leukemia, CTLA-4 cytotoxic T lymphocyte-associated antigen-4, DLI donor lymphocyte infusion, FLT3 FMS-like tyrosine kinase 3, G-CSF granulocyte colony-stimulating factor, GO gemtuzumab ozogamicin, HCT hematopoietic cell transplantation, JMML juvenile myelomonocytic leukemia, MDS myelodysplastic syndrome, MPN myeloproliferative neoplasm, NPM1 nucleophosmin 1, PD-1 programmed cell death-1, PD-L1 programmed cell death ligand-1, R/R relapsed/refractory, TIM-3 T-cell immunoglobulin domain and mucin domain-3, Treg regulatory T cell, TriKE tri-specific killer cell engager*
rate, ORR 14%) or survival (median OS 4.2 months), and the relevant clinical trial (NCT02399917) was terminated early due to unsatisfactory results [165]. LIR-1 or NKG2A blockade resulted in increased NK cell cytotoxicity against AML, suggesting that the cocktail consisting of anti-KIR, anti-LIR-1 and anti-NKG2A antibodies may be a necessary option for better efficacy [166, 167]. Anti-PD-1 antibody (nivolumab and pembrolizumab) and anti-CTLA4 antibody (ipilimumab) are FDA-approved immune checkpoint inhibitors mainly for the treatment of various solid tumors, while their applications in the field of AML are still at the exploratory stage. Nivolumab in combination with idarubicin and cytarabine produced an encouraging response rate (ORR 80%) and OS (median OS 18.5 months) in patients with newly diagnosed AML [168]. The combination therapy of nivolumab and azacitidine was feasible in patients with R/R AML, and the addition of ipilimumab further upregulated the clinical efficacy (ORR 33% vs 44%; median OS 6.4 vs 10.5 months) [169, 170]. And nivolumab maintenance was safe and feasible in high-risk AML patients in CR (1-year CR duration 71%; 1-year OS 86%) [171]. The outcomes of pembrolizumab administered in combined with decitabine or following high-dose cytarabine in R/R AML (ORR 10% and 46%; median OS 7 and 8.9 months, respectively) suggested that immune checkpoint inhibitors after intensive cytotoxic chemotherapy may be a better option [172, 173]. A phase I/ib study demonstrated the safety and efficacy of ipilimumab monotherapy in AML patients with post-HCT relapse (ORR 32%; 1-year OS 49%) [174]. As for anti-TIM-3 antibody MBG453, the combination therapy with decitabine was safe and well-tolerated and exhibited encouraging preliminary response rates for AML in a phase Ib study (ORR 29% for both newly diagnosed and R/R AML) [175]. However, caution should be paid to checkpoint inhibitors, since exposure can lead to a significantly increased risk of GvHD [168, 174, 176, 177]. Furthermore, the prognostic effect of TIGIT in the bone marrow post-HCT as well as the involvement of CD137-CD137L and CD200-CD200R interactions in immune evasion raise the possibility of attacking other inhibitory receptors with antibodies as potent immunotherapeutic strategies in the near future [53, 178–180].

**BiKE and TriKE**

Bi-specific killer cell engager (BiKE) and tri-specific killer cell engager (TriKE) are the recombinant agents of bivalent and trivalent single-chain variable fragments (scFv), serving as immunologic synapses between NK cells and tumor cells. They retain the specificity of original antibodies and, at the same time, minimize the size of antibodies to increase distribution. CD16-directed BiKE and TriKE trigger NK cell activation through CD16 signaling and against tumor cells with target antigens in a highly efficient manner (Fig. 2c) [181].

Wiernik et al. designed a novel full humanized BiKE that specifically binds to both CD16 and CD33 (CD16×33 BiKE). NK cell cytotoxicity and cytokine release were specifically triggered by CD16×33 BiKE when cultured with CD33+ AML cell lines and primary AML cells, and the effector functions of NK cells were further enhanced when combined with adisintegrin and metalloprotease-17 (ADAM17) inhibitor which prevents CD16 shedding [182]. Lately, the same research group designed a TriKE by incorporating a novel modified human IL-15 crosslinker into CD16×33 BiKE, which provided a signal for NK cell self-sustaining proliferation and activation [183]. A phase I/II clinical trial of CD16×33×15 TriKE (GTB-3550) for the treatment of CD33+ R/R AML is underway (NCT03214666). TriKEs of linking anti-CD16 scFv to either two scFv against the same antigen (such as CD16×33×33 TriKE) or two scFv against two different antigens (such as CD16×33×123 TriKE) displayed greater binding affinity and superior NK cell cytotoxic potency toward AML cells compared to BiKE [184, 185]. Since CD33 is abundantly expressed on healthy myeloid cells as well, NKG2DLs, which are leukemia cell-restricted expressed, become promising targets. CD16×NKG2D BiKE displayed increased affinity to CD16 and induced superior leukemia cell killing compared to the engineered NKG2D-Fc fusion protein [186]. Besides, CD16×CLL-1×15 TriKE displayed robust NK cell activity against AML in vitro and in vivo [187]. These molecules constitute attractive candidates for personalized immunotherapy for AML based on preclinical findings.

**Cytokines**

Cytokines, including IL-2, IL-12, IL-15, IL-18 and IL-21, play an important role in NK cell proliferation, activation and effector function (Fig. 2e). Ex vivo stimulation with 10 ng/mL IL-2 or 50 ng/mL IL-15 was reported to be optimal for NK cell expansion and enable NK cells of AML patients with recovered function through upregulating activating receptors such as Nkp30, Nkp46, NKG2C and NKG2D [188–190]. IL-2 monotherapy may not be clinically efficacious in AML patients [191–194]. But, IL-2 in conjunction with histamine dihydrochloride has been proposed as a maintenance therapy in AML, resulting in improved leukemia-free survival [195, 196]. The mechanism of this therapy may partially be the induction of a striking expansion of immunocompetent CD56<sup>bright</sup> NK cell subpopulations [197]. A phase I study identified IL-15 superagonist complex ALT-803 as a safe agent in the treatment of elderly AML patients who...
relapsed after HCT and the potential efficacy is expected to be reported (NCT01885897) [198]. And the feasibility of using ALT-803 as an relapse prophylaxis for AML patients after HCT is under assessment (NCT02989844). Furthermore, genetically engineered AML cells with DNA encoding IL-12 or IL-15 have been constructed to reduce toxicities associated with systemic administration of cytokines [199, 200]. A clinical trial (NCT02483312) is ongoing to test engineered AML cells expressing IL-12 in AML patients that cannot have HCT.

Cytokines have also been widely incorporated in the NK cell transfer as a process of 'priming or arming' in order to increase NK cell proliferation and expansion. However, the effect is short-lasting and the short-term NK cell persistence within patients might limit their clinical use. Remarkably, NK cells preactivated with a cocktail of cytokines (IL-12, IL-15 and IL-18) exhibited augmented anti-leukemia responses to restimulation for weeks to months regardless of inhibitory KIR-KIR ligand interactions [201–203]. Those cytokine-induced memory-like (CIML) NK cells with adaptive immune properties represent a promising approach to enhancing adoptive NK cell transfer. The first-in-human trial of adoptive transfer of CIML NK cells in elderly patients with R/R AML showed successful induction of remission (ORR 67%) without the cause of CRS, GvHD or neurotoxicity [204, 205]. Patient outcomes were negatively associated with the frequency of CD8α + donor NK cells and the expression of NKG2A on CIML NK cells within patients [205]. Encouraging preliminary data give us confidence on more ongoing early phase clinical trials of CIML NK cells for R/R AML (NCT04354025, NCT02782546, NCT01898793, NCT03068819) [206, 207].

**Drugs with immunomodulatory function**

Many anti-tumor drugs have been illustrated with immunomodulatory properties to enhance endogenous NK cell function against AML in recent years (Fig. 2f). Since AML cells resist to NK cell-mediated killing by changing the expression of their surface ligands for various NK cell receptors and these phenotypic defects correlate with clinical outcomes, drugs that are capable of restoring ligand expressions on AML cells can render them more susceptible to NK cell killing [64].

Firstly, hypomethylating agents azacitidine and decitabine can upregulate the expression of NKG2DL on AML cells by reversing epigenetically silenced genes, resulting in enhanced NK cell-mediated immunity through the immune recognition mediated by NKG2D-NKG2DL engagement [208]. They concurrently increase the expression of tissue inhibitor of metalloproteinases-3 (TIMP3), an ADAM17 inhibitor, thus reducing the shedding of soluble NKG2DLs from AML cells [209]. Histone deacetylase inhibitors (trichostatin A and valproic acid), differentiation-promoting drugs (vitamin D3, bryostatin 1 and all-trans-retinoic acid) and hydroxyurea all somehow show the potential of upregulating the expression of NKG2DLs on AML cells, while dinaciclib-treated AML is associated with the downregulation of inhibitory NK ligand HLA-E on AML cells, consequently inducing potent NK cell anti-tumor immunity [208, 210–213]. Then, immunomodulatory drugs lenalidomide and pomalidomide exert anti-leukemia effects both directly and via NK cell-mediated immunostimulatory activities along with downregulation of HLA-class I on AML blasts [214]. The combination therapies containing the aforementioned drugs for AML are widely used in clinical practice and also in clinical trials. Besides, natural compounds or their derivatives such as safrole, α-phellandrene, casticin and ouabain can also promote NK cell activity against AML cells [215–218]. In addition, novel agents with immunomodulatory function were proposed in fundamental researches, providing therapeutic implications in AML. For instance, vascular endothelial growth factor receptor (VEGFR)-3 antagonist restored NK cell cytotoxicity with an increased IFN-γ level [219, 220], and the therapeutic efficacy of adoptive NK cell transfer could be enhanced by a TGF-β receptor kinase inhibitor galunisertib [221]. With the clarification of mechanisms of anti-tumor drugs, combining pharmacological approaches with other NK cell-based immunotherapies may strengthen the efficacy and provide a clinical benefit for AML patients.

**Conclusions and perspectives**

Results from current preclinical studies and clinical trials highlight the significant contribution of numerous NK cell-based immunotherapies in activating the reconstitution of NK cells against AML. Adoptive NK cell transfer has expanded the option of cellular immunotherapy as a feasible strategy to induce and maintain remission. Strategies of manipulating adoptively transferred NK cells, such as CAR modification and cytokine induction, may further enhance the therapeutic efficacy. Other strategies, such as immune checkpoint inhibitors, BiKE/TriKE and immunomodulatory drugs, can reverse endogenous NK cell anergy, contributing to an increasing dimensions of utilizing NK cells to fight AML.

There are several advantages in NK cell-based immunotherapy. Firstly, NK cells detect tumor cells through native receptors in a non-MHC-restricted manner and also mediate ADCC, expanding their clinical applications. Secondly, as compared with T-cell therapy, NK-cell therapy has better safety profiles with rare instances of GvHD and CRS due to limited lifespan and distinct
cytokines produced [71]. Thirdly, NK cells have the advantage of “off-the-shelf” manufacturing, making it easy to be prepared under good manufacturing practice standards and convenient to universally treat patients with increased speed of administration [222–225]. However, the field of NK cell-based immunotherapy still faces several challenges. In fact, short lifespan of NK cells narrows the therapeutic window, leading to a relatively short duration of response in most patients [88, 90, 95, 226]. Besides, tumors can escape from NK cell cytotoxicity via immunosuppressive tumor microenvironment or by shedding soluble ligands that activate NK receptors [54, 60]. Finally, transduction efficiency of CAR-NK cells is another aspect needed to be improved [227].

In the future, the efficacy of NK cell-based immunotherapy is waiting to be confirmed in large sample sizes and in great detail. The optimal dosage and schedule of adoptive NK cell transfer as well as the feasible sources and manipulation methods for NK cells have yet to be evaluated [228]. It seems logical to combine various NK cell-based immunotherapies to utilize the full potential of NK cells, such as stimulating both target-specific lysis and ADCC effects as well as simultaneously boosting endogenous NK cells and receiving exogenous NK cells [131, 135, 136, 229, 230]. Also, it is reasonable to integrate them with well-established AML treatments or novel agents which may provide synergistic effects and improve clinical response [94]. As for preclinical researches, a better knowledge of the mechanisms of NK cell dysfunction and NK cell-based immunotherapy in AML could broaden the application of NK cells and help the discovery of additional new therapeutic opportunities, including new targets and potential combination therapies. Strategies of wisely using cytokines, such as CMIL NK cells and the transduction of genes encoding cytokines into NK cells, seem to prolong the duration of NK cell persistence in some degree, but more efforts are warranted to figure out approaches to enhance tumor-immune surveillance long term [17, 183, 206, 231]. Taking advantage of multi-omics and information technology, investigation of both donor NK cell-intrinsic and host factors which may contribute to treatment response or resistance can provide an array of biomarkers in donor and patient selection. Overall, there is a bright future in NK cell-based immunotherapy for AML.

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**References**

1. Lai C, Doucette K, Norsworthy K. Recent drug approvals for acute myeloid leukemia. J Hematol Oncol. 2019;12(1):100.

2. Blum WG, Mims AS. Treating acute myeloid leukemia in the modern era: a primer. Cancer. 2020;126:4668–77.
3. Ball B, Stein EM. Which are the most promising targets for minimal residual disease-directed therapy in acute myeloid leukemia prior to allogeneic stem cell transplant? Haematologica. 2019;104(8):1521–31.
4. Lichtenegger FS, Krupka C, Haubner S, Köhnke T, Subklewe M. Recent developments in immunotherapy of acute myeloid leukemia. J Hematol Oncol. 2017;10(1):142.
5. Farkona S, Diamandis EP, Blasutig IM. Cancer immunotherapy: the beginning of the end of cancer? BMC Med. 2016;14(1):1–18.
6. Beatty GL, Gladney WL. Immune escape mechanisms as a guide for cancer immunotherapy. Clin Cancer Res. 2015;21(4):687–92.
7. Sathyanarayanan V, Neelapu SS. Cancer immunotherapy: strategies for personalization and combinatorial approaches. Mol Oncol. 2015;9(10):2043–53.
8. Vasekar M, Rizvi S, Liu X, Vrana KE, Zheng H. Novel immunotherapies for hematologic malignancies. Curr Mol Pharmacol. 2016;9(3):264–71.
9. Im A, Pavletic S. Immunotherapy in hematologic malignancies: past, present, and future. J Hematol Oncol. 2017;10(1):94.
10. Fang F, Xiao W, Tian Z. NK cell-based immunotherapy for cancer. Semin Immunol. 2017;37:53–4.
11. Cheng M, Chen Y, Xiao W, Tian Z. NK cell-based immunotherapy for malignant diseases. Cell Mol Immunol. 2013;10(3):230–52.
12. Hu W, Wang G, Huang D, Sui M, Xu Y. Cancer immunotherapy based on natural killer cells: current progress and new opportunities. Front Immunol. 2019;10:205.
13. Valipour B, Abedelali A, Naderali E, Velaei K, Movassaghpor A, Talebi M, Montazereshaieh S, Karimpour M, Darabi M, Chavoshi H. Cord blood stem cell derived CD16+ NK cells eradicated acute lymphoblastic leukemia cells using with anti-CD47 antibody. Life Sci. 2020;242:117223.
14. Tanaka J, Tanaka N, Wang Y-H, Mitsuhashi K, Ryuzaki M, Izuka Y, Watanabe A, Ichiyama M, Shinohara A, Kazama H. Phase I study of cellular therapy using ex vivo expanded natural killer cells from autologous peripheral blood mononuclear cells combined with rituximab-containing chemotherapy for relapsed CD20-positive malignant lymphoma patients. Haematologica. 2020;105(4):190.
15. Bachanova V, Sarhan D, Defor TE, Bonello GB, Wu J, Tsai P-F. Pluripotent stem cell-derived NK cells with high-affinity noncleavable CD16a mediate improved antitumor activity. Blood. 2020;135(6):399–410.
16. Liu E, Marin D, Banerjee P, Macapinlac HA, Thompson P, Barasar N, Nasrif Kerbalu B, Overman B, Thali P, Kaplan M. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382(6):545–53.
17. Liu E, Mann D, Banerjee P, Macapinlac HA, Thompson P, Barasar N, Nasrif Kerbalu B, Overman B, Thali P, Kaplan M. Use of CAR-transduced natural killer cells in CD19-positive lymphoid tumors. N Engl J Med. 2020;382(6):545–53.
18. Oelsner S, Waldmann A, Billmeier A, Röder J, Lindner A, Ullrich E, Marabelli C. Humanized anti-CD123 antibody facilitates NK cell function in chronic lymphocytic leukemia. Front Immunol. 2019;9:3152.
19. Msezouki I, Yoshimoto T, Katagiri S, Mizuguchi J, Tauchi T, Kimura Y, Inokuchi K, Ohyashiki JH, Ohyashiki K. Sustained upregulation of effector natural killer cells in chronic myeloid leukemia after discontinuation of imatinib. Cancer Sci. 2013;104(9):1146–53.
20. Cucul M, Cantafo MEG, Siciliano MA, Rillo C, Caracciolo D, Scionti F, Staropoli N, Zuccalà V, Maltese L, Di Vito A. Trabectedin triggers direct and NK-mediated cytotoxicity in multiple myeloma. J Hematol Oncol. 2019;12(1):32.
21. Sinha C, Cunningham LC. An overview of the potential strategies for NK cell-based immunotherapy for acute myeloid leukemia. Pediatr Blood Cancer. 2016;63(12):2078–85.
22. Baragano Raneros A, López-Larrea C, Suárez-Álvarez B. Acute myeloid leukemia and NK cells: two warriors confront each other. Oncoimmunology. 2019;8(2):e1539617.
23. Spits H, Arts D, Colonna M, Diefenbach A, Di Santo JP, Eberl G, Koyasu S, Locksley RM, McKenzie AN, Mebius RE. Innate lymphoid cells—a proposal for uniform nomenclature. Nat Rev Immunol. 2013;13(2):145–9.
24. Cooper MA, Fehninger TA, Caligiuri MA. The biology of human natural killer-cell subsets. Trends Immunol. 2001;22(11):633–40.
25. Waldhauer I, Steinle A. NK cells and cancer immunosurveillance. Oncogene. 2008;27(45):5932–43.
26. Campbell KS, Hasegawa K. Natural killer cell biology: an update and future directions. J Allergy Clin Immunol. 2013;132(3):536–44.
27. Xu Y, Wang Y, Jiang L, Zhu X, Chen D, Yuan L, An G, Meng H, Yang L. A novel CD7 chimeric antigen receptor-modified NK-92MI cell line targeting T-cell acute lymphoblastic leukemia. Am J Cancer Res. 2019;9(1):64–78.
28. Daher M, Basar R, Goldkemper E, Baran N, Uprey N, Nunez Cortes AK, Mendri M, Kerbalau LN, Banerjee PP, Hernandez Sanabria M. Targeting a cytokine checkpoint enhances the fitness of armed cord blood CAR-NK cells. Blood. 2020. https://doi.org/10.1182/blood.2020007748.
29. Gang M, Marin ND, Wong P, Neal CC, Marsala L, Foster M, Schappe AK, Leitner LM. Chapter 1—Antibody-dependent cellular cytotoxicity (ADCC). Antibody FC. 2014:1–27.
30. Thomas R, Yang X. NK-DC crosstalk in immunity to microbial infection. J Immunol Res. 2016;2016:6374379.
46. Zhou Z, Zhang C, Zhang J, Tian Z. Macrophages help NK cells to attack tumor cells by stimulatory NKG2D ligand but protect themselves from NK killing by inhibitory ligand Qa1. PLoS ONE. 2012;7(5):e36928.

47. Lion E, Willemen Y, Berneman Z, Van Tendeloo V, Smits E. Natural killer cell immune escape in acute myeloid leukemia. Leukemia. 2011;25(9):1920–5.

48. Costello RT, Sivori S, Marcenaro E, Lafage-Pochitaloff M, Mozziconacci M-J, Reviron D, Gastaut J-A, Pende D, Olive D, Moretta A. Defective expression of KARMA: evolution during leukemia treatment and impact of leukemia cells in NCR dull phenotype induction. Blood. 2007;109(1):323–30.

49. Sandoval-Bozeghi D, Moreno-Lafont MC, Vazquez-Sanchez EA, Gutierrez-Hoya A, Lopez-Santos R, Montiel-Cervantes LA, Ramirez-Saladana M, Vela-Ojeda J. Overexpression of CD158 and NKG2A inhibitory receptors and underexpression of NKG2D and NKP46 activating receptors on NK cells in acute myeloid leukemia. Arch Med Res. 2016;47(1):55–64.

50. Stringaris K, Sekine T, Khoder A, Alsuliman A, Razaghi B, Sargeant R, Pavlu J, Briley G, de Lalaveille H, Sarvaria A. Leukemia-induced phenotypic and functional defects in natural killer cells predict failure to achieve remission in acute myeloid leukemia. Haematologica. 2014;99(5):836–47.

51. Sanchez-Conesa B, Gayoso I, Bergua JM, Casado JS, Morgado S, Solana R, Tarazona R. Decreased expression of DNAM-1 on NK cells from acute myeloid leukemia patients. Immunol Cell Biol. 2012;90(1):109–15.

52. Coles S, Wang ECY, Man S, Hills RK, Burnett AK, Darley RL. CD200 expression suppresses natural killer cell function and directly inhibits patient anti-tumor response in acute myeloid leukemia. Leukemia. 2011;25(5):792–9.

53. Hilpert J, Grosse-Hovest L, Grunebach F, Buechele C, Nuebling T, Raum E-M, Kearney CJ, Ramsbottom KM, Voskoboinik I, Darcy PK, Oliaro J. Loss of expression and function of natural killer cell–triggering receptors in patients with acute myeloid leukemia. Leukemia. 2002;16(11):1997–1999.

54. Tajima F, Kawatani T, Endo A, Kawasaki H. Natural killer cell activity and tumor cells by stimulatory NKG2D ligand but protect themselves from NK killing by inhibitory ligand Qa1. PLoS ONE. 2012;7(5):e36928.

55. Martyr A, Rydstrom A, Riise RE, Aurelius J, Anderson H, Brune M, Foa R, Hellstrland K, Thorén FB. Re: Mission Study G. Role of natural killer cell subsets and natural cytotoxicity receptors for the outcome of immunotherapy in acute myeloid leukemia. Oncoimmunology. 2016;5(1):e1041701.

56. Martyr A, Rydstrom A, Riise RE, Aurelius J, Brune M, Foa R, Hellstrland K, Thorén FB. NK cell expression of natural cytotoxicity receptors may determine relapse risk in older AML patients undergoing immunotherapy for remission maintenance. Onco tgat. 2015;6(40):42569–74.

57. Dauquet N, Récher C, Demur C, Fournié JJ, Poupot M, Poupot R. Preemience and persistence of immature natural killer cells in acute myeloid leukemia patients in first complete remission. Am J Hematol. 2011;86(2):209–13.

58. Ruggieri L, Capanni M, Urbani E, Perotti K, Burchielli E, Martelli MF, Velardi A. Role of natural killer cell alloreactivity in HLA-mismatched hematopoietic stem cell transplantation. Blood. 1999;94(1):333–9.

59. Ruggieri L, Capanni M, Urbani E, Perotti K, Slomchik WD, Tosti A, Posati S, Roggia D, Frassoni F, Aversa F. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Sci. 2002;295(5562):2097–100.

60. Ruggieri L, Manucci A, Perotti K, Burchielli E, Martelli MF, Velardi A. Natural killer cell alloreactivity for leukemia therapy. J Immunother. 2005;28(3):175–82.

61. Chan YLT, Zuoz J, Inman C, Croft W, Begum J, Crouse J, Jinsella F, Maggs L, Nagra S, Nunnick J. NK cells produce high levels of IL-10 early after allogeneic stem cell transplantation and suppress development of acute GVHD. Eur J Immunol. 2018;48(2):316–29.

62. Ruggieri L, Manucci A, Capanni M, Urbani E, Carotti A, Aloisi T, Stem M, Pende D, Perucci K, Burchielli E. Donor natural killer cell allogeneic selection of missing self in haploidentical hematopoietic transplantation for acute myeloid leukemia: challenging its predictive value. Blood. 2007;110(1):433–40.

63. Cooley S, Weisdorf DJ, Guethlein LA, Klein JP, Wang T, Le CT, Marsh SG, Geraghty D, Spellman S, Haagenson MD, Ladhner M, Trachtenberg E, Parham P, Miller JS. Donor selection for natural killer cell receptor genes leads to superior survival after unrelated transplantation for acute myelogenous leukemia. Blood. 2010;116(4):2411–9.

64. Stringaris K, Adams S, Uribe M, Eniafe R, Wu CO, Savani BN, Barrett AJ. Donor KIR Genes 2DL5A, 2DS1 and 3DS1 are associated with a reduced rate of leukemia relapse after HLA-identical sibling stem cell transplantation for acute myeloid leukemia but not other hematologic malignancies. Biol Blood Marrow Transplant. 2010;16(9):1257–64.

65. Miller JS, Cooley S, Parham P, Farag SS, Verneris MR, McQueen KL, Guethlein LA, Trautchenberg EA, Haagenson M, Horowitz MM. Missing KIR ligands are associated with less relapse and increased graft-versus-host disease (GVHD) following unrelated donor allogeneic HCT. Blood. 2007;109(11):5038–61.

66. Savani BN, Mielek S, Adams S, Uribe M, Rezvani K, Young AS, Zeilah J, Kurlander R, Sinivasan R, Childs R, Hensel N, Barrett AJ. Rapid natural killer cell recovery determines outcome after T-cell-depleted HLA-identical stem cell transplantation in patients with myeloid leukemias but not with acute lymphoblastic leukemia. Leukemia. 2007;21(10):2145–52.

67. Pittari G, Fregni G, Rogni L, Garcia A, Vataire A, Witteble M, Amselfi C, Chouaib S, Bouchis J, Caigard A. Early evaluation of natural killer activity in post-transplant acute myeloid leukemia patients. Bone Marrow Transplant. 2010;45(5):862–71.

68. Cichocki F, Cooley S, Davis V, DeTo DE, Schlums H, Zhang B, Brunstein CG, Blazar BR, Wagner J, Diamond DJ. CD56 dim CD57+ NKGC2+ NK cell expansion is associated with reduced leukemia relapse after reduced intensity HCT. Leukemia. 2016;30(2):456–63.

69. Michelis FV, Messner HA, Loach D, Uhm J, Gupta V, Lipton JH, Selife MD, Kuruvilla J, Kim DD. Early lymphocyte recovery at 28 d post-transplant is predictive of reduced risk of relapse in patients with acute myeloid leukemia transplanted with peripheral blood stem cell grafts. Eur J Haematol. 2014;93(4):273–80.

70. Passweg JR, Tichelli A, Meyer-Monard S, Heim D, Stem M, Kühne T, Favre G, Gratwohl A. Purified donor NK-lymphocyte infusion to consolidate engraftment after haploidentical stem cell transplantation. Leukemia. 2004;18(11):1835–8.
81. Choi I, Yoon SR, Park S-Y, Kim H, Jung S-J, Jang YJ, Kang M, Yeom YI, Lee J-J, Kim D-Y. Donor-derived natural killer cells infused after human leukocyte antigen–haploidentical hematopoietic cell transplantation: a dose-escalation study. Blood Marrow Transplant. 2021;20(2):696–704.

82. Ciurea SO, Schafer JR, Bassett R, Denman CJ, Cao K, Willis D, Rondon G, Rubnitz JE, Inaba H, Kang G, Gan K, Hartford C, Triplett BM, Dallas M, Miller JS, Soignier Y, Panoskaltsis-Mortari A, McNearney SA, Yun GH, Curti A, Ruggeri L, D’Addio A, Bontadini A, Dan E, Motta MR, Trabanelli S. Haploidentical IL-15/41BBL activated and expanded natural killer cell infusion therapy after salvage chemotherapy in children with relapsed and refractory leukemia. Cancer Lett. 2018;42:107–17.

83. Shaffer BC, Le Luduc J-B, Forlenza C, Jakubowski AA, Perales M-A, Wang CJ, Huang XJ, Gong LZ, Jia JS, Liu XH, Wang Y, Yan CH, Chang II, multicentre, prospective, non-randomised clinical trial to assess the safety and efficacy of infusing allogeneic activated and expanded natural killer cells as consolidation therapy for paediatric acute myeloblastic leukaemia. BMJ Open. 2020;10(1):e029642.

97. Bachanova V, Cooley S, Defor TE, Verneis MR, Zhang B, McKenna DH, Curtis RJ, Panoskaltsis-Mortari A, Lewis D, Huppen K, McGlave P, Weidsof DJ, Blazar BR, Miller JS. Clearance of acute myeloid leukaemia by haploidentical natural killer cell is improved using IL-2 diphtheria toxin fusion protein. Blood. 2014;123(25):3855–63.

98. Vela M, Corral D, Carrasco P, Fernández L, Valentin L, González B, Escudero A, Balas A, de Paz R, Torres J, Levias A, Martinez-Lopez J, Pérez-Martínez A. Haploidentical IL-15/41BBL activated and expanded natural killer cell infusion therapy after salvage chemotherapy in children with relapsed and refractory leukemia. J Immunol. 2020;200(5):1374–85.

99. Vacca G, Tumminia V, Spinelli G, Leone M, Vescio L, Tumminia S, Menichelli L, Costanzo G. Successful adoptive immunotherapy with purified natural killer cells after haploidentical SCT: a prospective phase II study in two centers. Bone Marrow Transplant. 2013;48(3):433–8.

100. Otte D, Klempner S, Miller JS, Schaffer M. Complete remission with reducing IL-15 activity in patients with acute myeloid leukemia. J Immunother Cancer. 2019;7(1):81.

101. Boyiadzis M, Agha M, Redner RL, Sehgal A, Im A, Hou JZ, Farah R, Dorritie KA, Raptis A, Lim SH, Wang H, Lapetova N, Mei Z, Butterfield LH, Rozen CM, Whiteside TL. Phase I clinical trial of adoptive immunotherapy using ‘off-the-shelf’ activated natural killer cells in patients with refractory and relapsed acute myeloid leukemia. Cytotherapy. 2017;19(10):1225–32.

102. Cooley S, He F, Bachanova V, Vercellotti GM, DeFor TE, Curtiss RJ, Robertson P, Grzywacz B, Conlon KC, Waldmann TA. First-in-human trial of rhl-15 and haploidentical natural killer cell therapy for advanced acute myeloid leukemia. Blood Adv. 2019;3(13):1970–80.

103. Grzywacz B, Moench L, McKenna D, J Teissier KM, Bachanova V, Cooley S, Miller JS, Covell EL. Natural killer cell homing and persistence in the bone marrow after adoptive immunotherapy correlates with better leukemia control. J Immunother (Hagerstown, Md: 1997). 2019;42(2):65.

104. Boyiadzis M, Agha M, Redner RL, Seghal A, Im A, Hoz J, Farah R, Dorritie KA, Raptis A, Lim SH, Wang H, Lapetova N, Mei Z, Butterfield LH, Rozen CM, Whiteside TL. Phase I clinical trial of adoptive immunotherapy using ‘off-the-shelf’ activated natural killer cells in patients with refractory and relapsed acute myeloid leukemia. Cytotherapy. 2017;19(10):1225–32.

105. Vela M, Corral D, Carrasco P, Fernández L, Valentin L, González B, Escudero A, Balas A, de Paz R, Torres J, Levias A, Martinez-Lopez J, Pérez-Martínez A. Haploidentical IL-15/41BBL activated and expanded natural killer cell infusion therapy after salvage chemotherapy in children with relapsed and refractory leukemia. Cancer Lett. 2018;42:107–17.

106. Shook D, Gruber T, Pui CH, Leung W. Natural killer cell therapy in childhood leukemia. Zhonghua Xue Ye Xue Za Zhi. 2019;40(10):812–7.

107. Xu N, Niu J. Hematol Oncol (2020) 13:167.
112. Cooley S, Hari P, McCloskey J, Byrne M, Wang E, Hussein M, Giarritta E, Zhang X, Hariri R, Miller JS. Abstract CT079: a phase I study of PKN-107, allogeneic, off the shelf NK cell in relapsed/refractory AML (NCT02781467). In: AACR; 2019.

113. Cummins KD, Gill S. Chimeric antigen receptor T-cell therapy for acute myeloid leukemia: how close to reality? Haematologica. 2019;104(7):1302–8.

114. Yáñez L, Sánchez-Escamilla M, Perales M-A. CAR T cell toxicity: current management and future directions. HemaSphere. 2019;3(2):e186.

115. Klingemann H. Are natural killer cells superior CAR donors? Oncoimmunology. 2014;3:2589147.

116. Hauswirth AW, Florian S, Pinto D, Dotar K, Krauth MT, Fritsch G, Schernhuber GN, Wacheck V, Seiber E, Sper W. Expression of the target receptor CD33 in CD34+/CD38+ leukemia cells: safety test of CD33-CAR NK-92 cells in patients with relapsed and refractory acute myeloid leukemia. Am J Cancer Res. 2018;8(6):1083–9.

117. Salman H, Pinz KG, Wada M, Shuai X, Yoon SE, Petrov JC, Ma Y. Preclinical targeting of human acute myeloid leukemia using CD4-specific chimeric antigen receptor (CAR) T cells and NK cells. J Cancer. 2019;10(18):4408–55.

118. Chang H, Salma F, Yi Q, Patterson B, Brien B, Minden MD. Prognostic relevance of immunophenotyping in 379 patients with acute myeloid leukemia. Leuk Res. 2004;28(1):43–8.

119. Zhu MY, Zhu Y, Chen RR, Zhu LX, Zhu JH, Li YX, Zhu D, Yang XD, Zheng YL, Xie MX. CD7 expression and its prognostic significance in acute myeloid leukemia patients with wild-type or mutant CEBPA. Nature. 2020;414(2021):100–5.

120. Zhu X-Y, Liu X, Wang X-B, Wang A-Y, Wang M, Liu N-N, You F-T, Pan G-F, Zhu X-Y, Liu X, Wang A-Y, Wang M, Liu N-N, You F-T. A phase I study of the safety, pharmacokinetics and anti-leukemic activity of the anti-CD123 monoclonal antibody LS0919, an anti-BrdU/CD157 humanized antibody inducing acute myelogenous leuke

121. Koerner SP, André MC, Leibold JS, Kousis PC, Kübler A, Pal M, Haen SP, Bühring HJ, Grosse-Hovest L, Jung G, Salih HR. An Fc-optimized CD133 antibody eliminates acute myeloid leukemia stem cells in patients treated with hypomethylating agents. Nat Med. 2020;26:1–9.

122. Kayser S, Heitmeyer JS, Dorfel D, Tholf F, Heuser M, Märkl M, Müller-Tidow C, Steiner M, Grosse-Hovest L, Jung G, Schlenk RF, Salih HR. Interim results of a first in man study with the Fc-optimized FLT3 antibody Flysyn for treatment of acute myeloid leukemia with minimal residual disease. Blood. 2019;134(Supplement_1):3928.

123. Koerner SP, Andric MC, Leibold JS, Kousis PC, Kübler A, Pal M, Haen SP, Bühring HJ, Grosse-Hovest L, Jung G, Salih HR. An Fc-optimized CD133 antibody for induction of NK cell reactivity against myeloid leukemia. Leukemia. 2017;31(2):459–69.

124. Vasu S, He S, Cheney C, Gopalakrishnan B, Mani R, Lozanski G, Mo X, Groh V, Whitman SP, Konopitzky R, Kossi C,ucci B, Ducci L, Dusses J, Ul Cali
gianni MA, Blum W, Adam PJ, Borges E, Ruetter B, Heider KH, Marcucci G, Muthusamy N. Decitabine enhances anti-CD33 monoclonal antibody BI 836858-mediated natural killer ADCCD against AML blasts. Blood. 2016;127(23):2879–89.

125. Ågerstam H, Karlsson C, Hansen N, Sandén C, Askmyr M, von Paffcy S, Högberg C, Rissler M, Wunderlich M, Julisson G. Antibodies targeting human IL1RAP (IL1R3) show therapeutic effects in xenograft models of acute myeloid leukemia. Proc Nat Acad Sci. 2015;112(43):10786–91.

126. Vindetti A, Bucissano F, Maurillo L, Del Principio M, Coppola A, Palombara P, Arriga R, Bellarosa D, Bressan A, Wilson K. MEL111/12OTBT37, an anti-BrdU/CD157 humanized antibody inducing acute myelogenous leukemia (AML) blast depletion in an autologous ex vivo assay: a potential new targeted therapy for AML. Blood. 2015;126(23):788.

127. Kupka C, Lichtenegger F, Kohnke T, Bogehodz J, Buckley V, Roiss M, Aumann T, Do TU, Dusek R, Wilson K. Targeting CD157 in AML with a novel, Fc-engineered antibody construct. Oncotarget. 2017;8(22):35707.

128. Williams BA, Wang XH, Leyton JV, Maghera S, Deh B, Reilly RM, Minden MD, Keating A. CD16(+)/NK-92 and anti-CD123 monoclonal antibody prolongs survival in primary human acute myeloid leukemia xenografted mice. Haematologica. 2018;103(10):1720–9.

129. Mani R, Rapolokar G, Nunes J, Zapollini K, Wasmuth R, Mo X, Byrd JC, Lee DA, Muthusamy N, Vasu S. Fc-engineered anti-CD33 monoclonal antibody potentiates cytotoxicity of membrane-bound interleukin-21 expanded natural killer cells in acute myeloid leukemia. Cytotherapy. 2020;22(7):369–76.

130. Narayan R, Blonquist TM, Emadi A, Hasserin JP, Burke M, Lesinskas C, Neuberg DS, Brunnner AM, Hobbs G, Hack H, McFae SL, Chen YB, Attar E, Graubert TA, Bertioli C, Moran JA, Bergeron MK, Foster JE, Ramos AV, Som TT, Vartanian MK, Story JL, McGregor K, Macrae MB, Behnan T, Wey MC, Rae MC, Jaffer P, Lesho P, Duong VH, et al. A phase 1 study of the antibody-drug conjugate brentuximab vedotin with re-induction chemotherapy in patients with CD30-expressing relapsed/refractory acute myeloid leukemia. Cancer. 2020;126(6):1284–73.

131. Goldman AD, Atullare E, Rizzieri D, Panci L, Chung KY, Spira A, Stock W, Tallman MS, Cruz HG, Boni J. Camidanlumab tesirine, an antibody-drug conjugate, in relapsed/refractory CD25-positive acute myeloid leukemia or acute lymphoblastic leukemia: a phase I study. Leuk Res. 2020;95:106385.

132. Daver NG, Erba HP, Papadotmantos N, DeAngelo DJ, Wang ES, Kontolevis M, Sloss CM, Culm-Merdek K, Zweidler-Mckay PA, Kantarjian HM. A phase I, first-in-human study evaluating the safety and preliminary antileukemia activity of IMGN632, a novel CD123-targeting antibody-drug conjugate, in patients with relapsed/refractory acute myeloid leukemia and other CD123-positive hematologic malignancies. Blood. 2018;132(Supplement_1):27.

133. Agura E, Gyurkoza B, Nath R, Litzow MR, Tomlinson BK, Abyankar S, Seropon S, Siff PJ, Choi HK, Kebriaei P. Targeted conditioning of lomab-B (131I-anti-CD54) prior to allogeneic hematopoietic cell transplantation versus conventional care in relapsed or refractory acute myeloid leukemia (AML): preliminary feasibility and safety results from the prospective, randomized phase 3 Sierra trial. Blood. 2018;132(Supplement_1):1012.

134. Jurcic JG, Ravandi F, Pagel JM, Park JH, Smith BD, Douer D, Levy MY, Estey E, Kantarjian HM, Earle D, Cicic D, Scheinberg DA. Phase I trial of o-particle therapy with actinium-225 (225Ac)-lintuzumab (anti-CD33) and low-dose cytarabine (LDAC) in older patients with untreated acute myeloid leukemia (AML). J Clin Oncol. 2015;33(15_suppl):7050.

135. Castaigne S, Pautas C, Terré C, Ravandi F, Pagel JM, Park JH, Smith BD, Douer D, Levy MY, Estey E, Kantarjian HM, Earle D, Cicic D, Scheinberg DA. Phase I trial of o-particle therapy with actinium-225 (225Ac)-lintuzumab (anti-CD33) and low-dose cytarabine (LDAC) in older patients with untreated acute myeloid leukemia (AML). J Clin Oncol. 2015;33(15_suppl):7050.
myeloid leukemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379(9825):1508–16.

143. Amadori S, Sucu S, Sellecis D, Aversa F, Gaidano G, Musso M, Annino L, Venditti A, Voso MT, Mazzone C. 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(9):972–9.

144. Taksin AL, Legrand O, Raffoux E, de Revel T, Thomas X, Contentin N, Bouabdallah R, Pautas C, Turlufe P, Reman O, Gardin C, Varet B, de Botton S, Pousset F, Farhat H, Chevet S, Dombret H, Castaigne S. High efficacy and safety profile of fractionated doses of Mylotarg as induction therapy in patients with relapsed acute myeloblastic leukaemia: a prospective study of the alfa group. Leukemia. 2007;21(1):66–71.

145. Pereira DS, Guevara CI, Jin L-P, Liu BY, Zheng Q, Panuganti S, Chen R, Zhu J, Mishra M, Huang F, Sutherland MK, Yu C, Walter RB, Valliere-Douglass Kovtun Y, Noordhuis P, Whiteman KR, Watkins K, Jones GE, Harvey Brodská B, Otevřelová P, Šálek C, Fuchs O, Gašová Z, Kuželová K. High Yang H, Bueso-Ramos CE, Parmar S, Wei Y, Fang Z, Nguyen M, Fernandez Daver N, Garcia-Manero G, Yadav SS, Sharma P, Allison J, Schmiedel BJ, Baltz-Gihahremankour P, Schmiedel BJ, Steinle A, Jung G, Kübler A, Andre MC, Grossi-Hovest L, Salih HR. An FC-optimized NKG2D-immunoglobulin G fusion protein for induction of natural killer cell reactivity against leukemia. Int J Cancer. 2013;135(6):1073–84.

147. Jiang Y-P, Liu BY, Chevret S, Dombret H, Castaigne S. High resistance in MLL-rearranged leukemia expressing inhibitory KIR ligands and PD-L1 expression predicts for worse outcome of leukemia patients with acute myeloid leukemia (AML): results of the Effikir trial. Blood. 2017;130(Supplement_1):B89.

149. Schmetzer HM, Salih HR. Glucocorticoid-induced tumor necrosis factor receptor-related protein ligand subverts immunosurveillance of acute myeloid leukemia in humans. Cancer Res. 2009;69(3):1037–45.

151. Steinbacher J, Balz-Gihahremankour P, Schmiedel BJ, Steinle A, Jung G, Kübler A, Andre MC, Grossi-Hovest L, Salih HR. An FC-optimized NKG2D-immunoglobulin G fusion protein for induction of natural killer cell reactivity against leukemia. Int J Cancer. 2013;135(6):1073–84.

153. Vey N, Boushr J-H, Recher C, Etienne A, Charbonnier A, Rey J, Andre P, Calmels F, Zerbib R, Buffet R. Repeated dosing of anti-KIR (IPH2101) as maintenance therapy in elderly patients with acute myeloid leukemia. Lancet. 2013;382(9902):2169.

155. Vey N, Dumas P-Y, Recher C, Gastaud N, Boulou H, Bubulco C-E, Pautas C, Marolleau J-F, Lepretre S, Raffoux E, Thomas X, Hichens Y, Bonmati C, Quesnel B, Roussetot P, Castaigne S, Jourdan E, Malfsun JV, Guillem G, Boushr J-H, Ojeda M, Hunault M, Hraf N, Gardin C, Delanoy A, Beautier L, Paturel C, Andre P, Zerbib R, Preudhomme C, et al. Randomized phase 2 trial of lirilumab (anti-KIR monoclonal antibody, mAb) as maintenance treatment in elderly patients (pts) with acute myeloid leukemia (AML): optimized NKG2A-immunoglobulin-like receptor-negative natural killer cells after NKG2A and LIR-1 blockade. Biol Blood Marrow Transplant. 2010;16(5):612–21.

157. Daver NG, Garcia-Manero G, Cortes JE, Basu S, Ravandi F, Kadia TM, Borthakur G, Jabbour E, Dinardo CD, Pemmaraju N. Phase II/II study of lirilumab with azacitidine (AZA) in relapsed AML. Blood. 2017;130(Supplement_1):2634.

159. Goyal R, Bachanova V, Gleason M, McCullar V, Yoon GH, Cooley S, Verneris MR, McClave PB, Miller JS. Natural killer cell killing of acute myelogenous leukemia and acute lymphoblastic leukemia blasts by killer cell immunoglobulin-like receptor-negative natural killer cells after NKG2A and LIR-1 blockade. Biol Blood Marrow Transplant. 2010;16(5):612–21.

161. Khan M, Arooj S, Wang H. NK cell-based immune checkpoint inhibition. Front Immunol. 2020;11:167.

163. Vey N, Boushr J-H, Recher C, Etienne A, Charbonnier A, Rey J, Andre P, Calmels F, Zerbib R, Buffet R. Repeated dosing of anti-KIR (IPH2101) as maintenance therapy in elderly patients with acute myeloid leukemia. Lancet. 2013;382(9902):2169.

165. Daver NG, Garcia-Manero G, Cortes JE, Basu S, Ravandi F, Kadia TM, Borthakur G, Jabbour E, Dinardo CD, Pemmaraju N. Phase II/II study of lirilumab with azacitidine (AZA) in relapsed AML. Blood. 2017;130(Supplement_1):B89.

167. Vey N, Dumas P-Y, Recher C, Gastaud N, Boulou H, Bubulco C-E, Pautas C, Marolleau J-F, Lepretre S, Raffoux E, Thomas X, Hichens Y, Bonmati C, Quesnel B, Roussetot P, Castaigne S, Jourdan E, Malfsun JV, Guillem G, Boushr J-H, Ojeda M, Hunault M, Hraf N, Gardin C, Delanoy A, Beautier L, Paturel C, Andre P, Zerbib R, Preudhomme C, et al. Randomized phase 2 trial of lirilumab (anti-KIR monoclonal antibody, mAb) as maintenance treatment in elderly patients (pts) with acute myeloid leukemia (AML): optimized NKG2A-immunoglobulin-like receptor-negative natural killer cells after NKG2A and LIR-1 blockade. Biol Blood Marrow Transplant. 2010;16(5):612–21.

169. Ruggeri L, Urbani E, Andre P, Mancusi A, Tosti A, Topini F, Bléry M, Ani-mobono L, Romagné F, Wagtmann N, Velardi A. Effects of anti-NKG2A antibody administration on leukemia and normal hematopoietic cells. Haematologica. 2016;101(5):626–33.

171. Vey N, Dumas P-Y, Recher C, Gastaud N, Boulou H, Bubulco C-E, Pautas C, Marolleau J-F, Lepretre S, Raffoux E, Thomas X, Hichens Y, Bonmati C, Quesnel B, Roussetot P, Castaigne S, Jourdan E, Malfsun JV, Guillem G, Boushr J-H, Ojeda M, Hunault M, Hraf N, Gardin C, Delanoy A, Beautier L, Paturel C, Andre P, Zerbib R, Preudhomme C, et al. Randomized phase 2 trial of lirilumab (anti-KIR monoclonal antibody, mAb) as maintenance treatment in elderly patients (pts) with acute myeloid leukemia (AML): results of the Effikir trial. Blood. 2017;130(Supplement_1):B89.

173. Vey N, Dumas P-Y, Recher C, Gastaud N, Boulou H, Bubulco C-E, Pautas C, Marolleau J-F, Lepretre S, Raffoux E, Thomas X, Hichens Y, Bonmati C, Quesnel B, Roussetot P, Castaigne S, Jourdan E, Malfsun JV, Guillem G, Boushr J-H, Ojeda M, Hunault M, Hraf N, Gardin C, Delanoy A, Beautier L, Paturel C, Andre P, Zerbib R, Preudhomme C, et al. Randomized phase 2 trial of lirilumab (anti-KIR monoclonal antibody, mAb) as maintenance treatment in elderly patients (pts) with acute myeloid leukemia (AML): results of the Effikir trial. Blood. 2017;130(Supplement_1):B89.
ac}{139} of AML cells. Leukemia. 2020. https://doi.org/10.1038/s41375-020-01065-5.

Decot V, Voilllard L, Latger-Cannard V, Aissi-Rothel L, Penrier P, Stoltz JF, Bensoudas S. Natural killer cell amplification for adoptive leukemia relapse immunotherapy: comparison of three cytokines, IL-2, IL-15, or IL-7 and impact on NKGD2, KIR2DL1, and KIR2DL2 expression. Exp Hematol. 2010;38(5):351–62.

Szczepanski MJ, Sznajk M, Welsh A, Foote KA, Whiteside TL, Boyiadzis M. Interleukin-15 enhances natural killer cell cytotoxicity in patients with acute myeloid leukemia by upregulating the activating NK cell receptors. Cancer Immunol Immunother. 2010;59(1):73.

Sanchez-Correa B, Bergua JM, Pera A, Campos C, Arcos MJ, Bahas H, Duran E, Solana R, Tanzana R. In vitro culture with interleukin-15 leads to expression of activating receptors and recovery of natural killer cell function in acute myeloid leukemia patients. Front Immunol. 2017;8:931.

Baer MR, George SL, Caligiuri MA, Sanford BL, Botham SM, Morzek K, Kolitz JE, Powell BL, Moore JO, Stone RM. Low-dose interleukin-2 immunotherapy does not improve outcome of patients age 60 years and older with acute myeloid leukemia in first complete remission. Cancer and Leukemia Group B Study 9720. J Clin Oncol. 2008;26(20):4934.

Lange BJ, Smith FO, Feusner J, Barnard DR, Dinnon FD, Pegg F, Seigle HA, Arndt C, Arcucci RJ, Seibel N. Outcomes in CCG-2961, a children’s oncology group phase 3 trial for untreated pediatric acute myeloid leukemia: a report from the children’s oncology group. Blood. 2008;111(3):1044–53.

Petit A, Ducassou S, Leblanc T, Pasquet M, Rousseau A, Ragu C, Cachanado M, Nelken B, Bertrand Y, Michel G. Maintenance therapy with interleukin-2 for childhood AML: results of ELAM02 phase III randomized trial. HemaSphere. 2018;2(6):e159.

Pautas C, Merabet F, Thomas X, Raffoux E, Gardin C, Coms S, Bourhis J-H, Reiman O, Turfe F, Contentin N. Randomized study of intensified anthracycline doses for induction and combination interleukin-2 for maintenance in patients with acute myeloid leukemia age 50 to 70 years: results of the ALFA-9801 study. J Clin Oncol. 2010;28(5):808–14.

Brune M, Castaigne S, Catalano J, Gehlsen K, Ho AD, Hoffmann W-K, Hogde DE, Nilsson B, Or R, Romero AI. Improved leukemia-free survival after postconsolidation immunotherapy with histamine dihydrochloride and interleukin-2 in acute myeloid leukemia: results of a randomized phase 3 trial. Blood. 2006;108(1):88–96.

Nilsson MS, Hallner A, Brune M, Nilsson S, Thörén FB, Martner A, Hellstrand K. Immunotherapy with HDC/IL-2 may be clinically efficacious in acute myeloid leukemia of normal karyotype. Hum Vaccines Immunother. 2020;16(1):109–11.

Cuapio A, Post M, Cerny-Reiterer S, Gleineker KV, Stefand G, Basilio J, Heindlhofer S, Speer WR, Horns NH, Casanova E. Maintenance therapy with histamine plus IL-2 induces a striking expansion of two CD56bright NK cell subpopulations in patients with acute myeloid leukemia and supports their activation. Oncotarget. 2016;7(29):46466.

Rome R, Cooley S, Berenini-Elliot MM, Westervert P, Verneris MR, Wagner JE, Wendel DJ, Blazar BR, Ustun C, DeFor TE. First-in-human phase 1 clinical study of the IL-15 superagonist complex ALT-803 to treat relapse after transplantation. Blood. 2018;131(23):2515–27.

Huang J, Liu Y, Au BC, Barber DL, Arruda A, Schambach A, Rothe M, Minden MD, Paige CJ, Medin JA. Preclinical validation: IV/IL-12 transduction of patient leukemia cells for immunotherapy of AML. Mol Therapy Methods Clin Dev. 2016;3:16074.

Shi Y, Dincheva-Vogel L, Ayemoba CE, Fung JP, Bergamaschi C, Pavlakis GN, Farzaneh F, Gaensler KML. IL-15/IL-15Rα/CD80-expressing AML cell vaccines eradicate minimal residual disease in leukemic mice. Blood Adv. 2018;2(22):3177–92.

Cooper MA, Yokoyama WM. Memory-like responses of natural killer cells. Immunol Rev. 2010;235(1):297–305.

Rome R, Schneider SE, Leong JW, Chase JM, Keppel CR, Sullivan RP, Cooper MA, Fehninger TA. Cytokine activation induces human memory-like NK cells. Blood. 2012;120(4):4751–60.

Rosario M, Rome R, Schneider SE, Leong JW, Sullivan RP, Fehninger TA. Human cytokine-induced memory-like (CIML) NK cells are active against myeloid leukemia in vitro and in vivo. Blood. 2014;124(21):11117.

Rome R, Rosario M, Berenini-Elliot MM, Wagner JA, Jevela BA, Schappe T, Leong JW, Abdulla-Latif S, Schneider SE, Willey S, Neal CC, Yu L, Oh ST, Lee YS, Mulder A, Claas F, Cooper MA, Fehninger TA. Cytokine-induced memory-like natural killer cells exhibit enhanced responses against myeloid leukemia. Sci Transl Med. 2016;8(357):357ra123.

Berenini-Elliot MM, Cashen AF, Cubitt CC, Neal CC, Wong P, Wagner JA, Foster M, Schappe T, Desais S, McClain E. Multidimensional analyses of donor memory-like NK cells reveal new associations with response after adoptive immunotherapy for leukemia. Cancer Discov. 2020. https://doi.org/10.1158/2159-8290.CD-20-0312.
206. Foltz JA, Berrien-Elliott MM, Neal C, Foster M, McClain E, Schappe T, Desai S, Becker-Hapak M, Cashen AF, Desai S, Foster M, Schappe T, Bednarski JJ, Zimmerman C, Cashen AF, Desai S, Foster M, Schappe T, McClean E, Becker-Hapak M, Berrien-Elliott MM, Feher TA. Adoptively transferred donor-derived cytokine induced memory-like NK cells persist and induce remission in pediatric patient with relapsed acute myeloid leukemia after hematopoietic cell transplantation. Blood. 2019;134(Supplement_1):1594.

207. Bednarski JJ, Zimmerman C, Cashen AF, Desai S, Foster M, Schappe T, McClean E, Becker-Hapak M, Berrien-Elliott MM, Feher TA. Cytokine-induced memory-like (ML) NK cells persist for> 2 months following adoptive transfer into leukemia patients with a MHC-compatible hematopoietic cell transplant (HCT). Blood. 2019;134(Supplement_1):1954.

208. Rohner A, Lagenkamp U, Siegler J, Kalberer CP, Wodnar-Filipowicz A. Differentiation-promoting drugs up-regulate NKGD2 ligand expression and enhance the susceptibility of acute myeloid leukemia cells to natural killer cell-mediated lysis. Leuk Res. 2007;31(10):1393–402.

209. Raneros AB, Fauri AM, Rodriguez JM, Colado E, Bernal T, Angueta E, Mogorron AV, Gil AC, Vidal-Castrenie JA, Marquez-Kisinosky L. Increasing TNP3 expression by hypomethylating agents diminishes soluble MICA, MICB and ULBP2 shedding in acute myeloid leukemia, facilitating NK cell-mediated immune recognition. Oncotarget. 2017;8(19):31959.

210. Lu X, Ohata K, Kondo Y, Luis Espinoza J, Qi Z, Nakao S. Hydroxyurea treatment into leukemia patients with a MHC-compatible hematopoietic cell transplant (HCT). Blood. 2019;134(Supplement_1):1954.

211. Yun HD, Schirm DK, Felices M, Miller JS, Eckfeldt CE. Dinaciclib enhances NKG2D ligand expression on CD16A+ NK cells from acute myeloid leukemia patients. Leukemia. 2014;28(11):2026–31.

212. Shankar K, Capitini CM, Saha K. Genome engineering of induced pluripotent stem cells to manufacture natural killer cell therapies. Stem cell Res Therapy. 2020;11(1):1–14.

213. Morgan MA, Buning H, Sauer M, Schambach A. Use of cell and genome modification technologies to generate improved ‘off-the-shelf’ CAR T and CAR NK Cells. Front Immunol. 2016;5:407.

214. Suck G, Ondedahl M, Nowakowska P, Seidl C, Wels WS, Klingenho G, Tonn T, NK-92: an ‘off-the-shelf therapeutic’ for adoptive natural killer cell-based cancer immunotherapy. Cancer Immunol Immunother. 2016;65(4):485–92.

215. Spanholtz J, Preijers F, Tordoir M, Trilisbeeck C, Paardkooper J, De Witte T, Schaap N, Dolstra H. Clinical-grade generation of active NK cells from cord blood hematopoietic progenitor cells for immunotherapy using a closed-system culture process. PLoS ONE. 2011;6(6):e20740.

216. Shankar K, Capitini CM, Saha K. Genome engineering of induced pluripotent stem cells to manufacture natural killer cell therapies. Stem cell Res Therapy. 2020;11(1):1–14.

217. Zhang Y, Wallace DL, de Lara CM, Ghattas H, Asquith B, Worth A, Griffin GE, Taylor GP, Tough DF, Beverley PC, Macallan DC. In vivo kinetics of human natural killer cells: the effects of ageing and acute and chronic viral infection. Immunology. 2007;121(2):258–65.

218. Hu Y, Tian-Z, Zhang C, Chimeric antigen receptor (CAR)-transduced natural killer cells in tumor immunotherapy. Acta Pharmacol Sin. 2018;39(2):167–76.

219. Li Y, Hermansson DL, Moxiarity BS, Kaufman DS. Human iPSC-derived natural killer cells engineered with chimeric antigen receptors enhance anti-tumor activity. Cell Stem Cell. 2018;23(2):181–92.

220. Pahl JHW, Koch J, Göt t J-L, Arnold A, Reusch U, Gantke T, Rajkovic E, Tred er M, Cerverken A. CD16A activation of NK cells promotes NK cell proliferation and memory-like cytotoxicity against cancer cells. Cancer Immunol Res. 2018;6(5):517–27.

221. Cany J, Roeven MWH, Hoogstad-van Evert JS, Hobo W, Maas F, Franco Fernandez R, Blijlevens NMA, van der Velden WJ, Huls G, Jansen JH. Dectin-1 enhances targeting of AML cells by CD34+ progenitor-derived NK cells in NOD/SCID/IL2Rγnull mice. Blood. 2018;131(2):202–14.

222. Jiang W, Zhang C, Tian Z, Zhang J. Hill-15 gene-modified human natural killer cells (NK-H-15) augments the anti-human hepatocellular carcinoma effect in vivo. Immunobiology. 2014;219(7):547–53.

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