Acute myeloid leukemia (AML) is a heterogeneous disease whose therapies currently show elevated toxicity and a high rate of relapse. Recently, the burgeoning of new anti-tumor therapeutic strategies aimed at enhancing the immune response has pushed natural killer cells (NKs) into the spotlight. These cells are powerful warriors that can bring about the lysis of tumor cells through their cytotoxic ability. However, tumor cells have developed strategies to evade recognition mediated by NKs. Here, we review the mechanisms triggered by AML cells and discuss the emerging immunotherapeutic strategies that potentiate the anti-tumor functions of NKs.

1. Introduction

Acute myeloid leukemia (AML) is an aggressive malignancy of the bone marrow characterized by the uncontrolled proliferation of undifferentiated myeloid cells. It originates from a hematopoietic stem cell that acquires genetic and/or epigenetic mutations and is able to self-renew to maintain the disease. Studies based on new sequencing technologies have made it possible to determine that the majority of AML cells originate from a founder clone with a driver mutation. A study of more than 1500 AML patients found that in the majority of them, the driver mutation was located in genes involved in epigenetic mechanisms (DNMT3A, ASXL1, IDH1/2, TET2). Additionally, the founder clone can acquire subsequent mutations that generate subclones, each of which has different cellular morphologies and functionalities. These mutations are located in signaling pathways (FLT3, KIT, K-RAS), myeloid transcription factors (CEBPA, RUNX1) or in tumor suppressor genes (TP53, WT1, PHF6). The mutational pattern of each patient may be indicative of the prognosis of their disease. In this way, it has been reported that AML patients in complete remission (CR) but with persistent driver mutations have a higher incidence of relapse and poorer survival.

AML has usually been classified by the French–American–British (FAB) group into different subtypes (M0–M7) depending on the type of cell that proliferates and its state of maturation. However, this classification has no prognostic value and the World Health Organization (WHO) established a new classification in 2008 based on cytogenetic analysis that is periodically revised.

Therapeutic options for treating AML are still very limited and have changed very little in the last 30 years. The induction phase, known as “7 + 3”, based on an intensive chemotherapy regimen using standard doses of cytarabine for 7 days followed by anthracycline for 3 days. This therapy achieves CR from the disease in 60–80% of patients (age < 60 years) and in 40–60% of elderly patients (age ≥ 60 years). After CR, it is necessary to continue with an appropriate consolidation phase to eradicate the minimal residual disease and to avoid possible relapses. This treatment is based mainly on a high-dose of cytarabine followed by autologous or allogeneic hematopoietic cell transplantation (HCT). Patients unsuitable for intensive therapy, especially elderly patients, receive treatments with a low-dose of cytarabine or therapy with hypomethylating agents such as decitabine (Dacogen®, DAC). Although these therapeutic strategies have improved survival rates of younger AML patients in recent years, they are still unsatisfactory in adverse-risk and most older patients. As a consequence, current research is directed towards identifying new therapeutic strategies that improve patients’ survival and quality of life. In recent years, the greatest successes achieved in AML have been those that aim to enhance the immune response to increase the recognition and elimination of AML cells.

2. Impaired recognition of AML by natural killer cells

One of the first studies to demonstrate the role of natural killer (NK) cells in the immunosurveillance of cancer revealed that individuals who have NK cells with a low cytotoxicity capacity had a higher probability of developing some type of cancer during an 11-year follow-up. NK cells can recognize tumor cells independently of MHC, whereby inhibitory and activating receptors of NK cells bind to its ligands, which are expressed on the surface of tumor cells. Some of the first evidence that NK cells can kill AML cells was the absence of relapse of the disease after HCT transplantation containing alloreactive NKs. However, during cancer development, NK
and AML cells can develop strategies to avoid recognition and allow the tumor to propagate.

### 2.1. Repertoire of NK cell receptors and their ligands

Conventional NK cells are key lymphocytes of the innate immune system, currently considered a prototype of the innate lymphoid cell (ILC) family. They exert anti-tumor effects mediated by their cytotoxic and cytokine-secreting capacity independently of MHC-based antigen recognition. Human NK cells comprise two main phenotypes based on the CD56 and CD16 markers. The CD56$^{\text{dim}}$CD16$^{+}$ NK subset has great cytotoxic potential and is predominant in blood, whereas the CD56$^{\text{bright}}$CD16$^{-}$ subset produces abundant cytokines and is more abundant in lymph nodes. The functionality of NK cells is mediated and controlled by the balance between the expression of activating and inhibitory receptors. The main activating receptors are natural cytotoxic receptors (NCR; NKp30, NKp46, NKp44), NKG2D and DNAM-1, whereas the killer-immunoglobulin-like receptor (KIR) family and NKG2A are inhibitory receptors. During NK cell maturation, the inhibitory receptors (mainly KIRs) interact with self-molecules (self-MHC class I) to avoid the recognition of self-cells in a process known as "licensing" or "education" of NK cells. The unbalanced expression of these receptors and their ligands hinders the recognition of tumor cells and allows tumor progression.

In a high percentage of AML patients, the expression levels of some activating receptors (NKG2D, NKp30, NKp46, DNAM-1) are null or very low (Figure 1a). The inverse correlation between the DNAM-1 expression levels and one of its ligands (CD112) in AML patients suggests that the expression of activating receptors depends on their ligands being present on the surface of AML cells. Moreover, the status of AML patients also affects the repertoire of NK cell receptors. Fauriat et al. reported that the NKp30 and NKp46 expression is reduced in AML patients at diagnosis but is partially recovered after CR and decreases again in an episode of disease relapse. It is also clearly established that high levels of expression of NKp30 and NKp46 are associated with higher rates of overall, relapse-free and disease-free survival compared with patients with reduced expression of their receptors. In humans, NK cell maturation is identified by the differential expression of the CD56, CD16, NKG2A, KIRs and CD57 markers. CD56$^{\text{dim}}$CD16$^{-}$ NK cells correspond to the most immature subset (Figure 1b). In CD56$^{\text{dim}}$ NK cells, different maturation stages are characterized by the loss of NKG2A and the sequential acquisition of CD57 and KIR receptors. In AML patients, three different groups of patients were defined according to their NK maturation profile: hypomature NK cells (CD56$^{\text{bright/dim}}$ KIR$^{-/}$ CD57$^{-}$), intermediate (CD56$^{\text{dim}}$ KIR$^{+/}$ CD57$^{-/+}$) and hypermature (CD56$^{\text{dim}}$ KIR$^{+}$ CD57$^{+}$) (Figure 1b). Patients with the hypomature profile (around 9%) showed a poor 3-year overall survival and relapse-free survival, suggesting an altered maturation in some patients with relevant clinical outcomes. Defects in the NK cell maturation from leukemic mice and AML patients are associated with elevated levels of miR-29b in these cells. This microRNA inhibits the expression of the T-bet and EOMES transcription factors, which are essential for controlling the final stages of NK cell differentiation. This has a dual effect: reduced T-bet expression leads to low levels of perforin in mature NK cells, decreasing their cytolytic ability, and the damage to the development of intermediate NK cells that require expression of both transcription factors.
NK cell maturation also enables the generation of memory-like NK cells. The first time that it was observed that NKs could acquire immunologic memory was in a murine model deficient in T and B cells, in which prior contact with a hapten produced hypersensibility. 28 Subsequent studies showed that pre-activated NK cells with cytokines (IL-12, IL-15 and IL-18) induce the differentiation of memory-like NK cells, characterized by enhanced IFN-γ production and cytotoxic ability against human AML blasts. 29,30 The potent antileukemia effect was dependent on the increased expression of activating and cytokine receptors (NKG2D, NKP30, NKP44, CD25) and cytotoxic molecules, but regardless of the KIR-ligand interactions.

2.3. Expression of molecular immune checkpoint inhibitors

Immune checkpoint molecules are proteins that help maintain an adequate immune response, playing a key role in self-tolerance and the avoidance of indiscriminate lysis of self-cells. In recent years, targeting immune checkpoints with specific inhibitory antibodies has revolutionized the treatment of cancer by restoring the functionality of exhausted T cells against tumor cells. However, NK cell activity can also be regulated by immune checkpoints such as PD-1, TIM-3 or TIGIT. 31

Figure 1. Critical role of tumor microenvironment in NK-AML recognition. AML cells can create an immunosuppressive microenvironment that avoids recognition mediated by NK cells, triggering tumor immune escape. This tumor microenvironment results in decreased activity of NK cells by several mechanisms: a) Modulation of the NK receptor repertoire. NK functions are exhaustively regulated by the balance between activating and inhibitory receptors. However, during AML development the repertoire of NK cells is modified, reducing the level of expression of activating receptors (NKG2D, DNAM-1, NKP30) and increasing that of inhibitory receptors (KIRs and NKG2A). Additionally, ligands for NKG2D-activating receptor (NKG2DL) can be regulated by DNA methylation (red circles), reducing its level of expression on the cell surface of AML cells, or they can be released into the tumor environment in a soluble form that promotes the internalization and degradation of NKG2D; b) Defective NK maturation. NK cells of some AML patients feature defects in their maturation. AML patients show three different NK maturation profiles: hypomature (CD56bright/dim KIRs−/+, CD57−), intermediate (CD56dim KIR−/+, CD57−/+), and hypermature (CD56dim KIRs+/+, CD57−). Elevated levels of miR-29b in AML patients reduce the cytotoxic ability of hypermature NK cells, indicating a poor level of recognition of tumor cells, and damage NK maturation, thereby increasing the number of hypomature NK cells; and c) Lysis inhibition by immune checkpoints. PD-1, TIM3 and TIGIT expressed on the cell surface of NK cells, recognize their ligands (PD-L1, Gal-9, or CD112/CD155 respectively), which are expressed on the cell surface of AML cells. As a consequence, activating pathways involved NK cell regulation (PI3K, ERK, PKCθ) are inhibited, promoting NK cell anergy.
PD-1 is expressed in mature (CD56dim CD16+) NK cells under sustained stimulation by MHC class I-deficient tumor cells or infected cells. PD-1+ NK cells have reduced proliferative and cytolytic abilities, and the impaired cytokine production typical of an exhausted phenotype. In fact, blockade of the PD-1/PDL-1 interaction with specific antibodies restores the functions of NK cells, promoting an anti-tumor response to several cancers, such as myeloma and digestive tumors.\(^{32,33}\) Although the role of PD-1 in NK cell function in AML is poorly understood, there is clear evidence that PD-L1 is expressed in some AML patients, where it impairs immune recognition (Figure 1c).\(^{34}\) Accordingly, several clinical trials evaluating PD-1/PD-L1 blockade are under way (Table 1). Recently, high levels of DNA methylation of PD-L1 gene have been associated with reduced PD-L1 expression in some AML cells, and a consequently lower risk of relapse and prolonged overall survival in those patients.\(^{35}\) Methylation of PD-L1 could be an effective biomarker for stratifying patients who could benefit from PD-1 checkpoint inhibition.

TIM-3 is highly expressed on the surface of human NK cells and binds to its ligand Galectin-9 (Gal-9), which is expressed in AML blasts (Figure 1c). As a consequence of this interaction, TIM-3+ NK cells increase IFN-γ production, leading to increased expression of the indoleamine 2,3-dioxygenase 1 enzyme by AML cells, which, in turn, impairs NK cell degranulation activity and ensures leukemic immune escape.\(^{36}\) Moreover, high levels of soluble Gal-9 were detected in the serum of AML patients, where Gal-9 is able to bind to TIM-3 expressed on the surface of leukemic stem cells that activates the NF-κB and β-catenin pathways involved in the self-renewal of these malignant stem cells.\(^{37}\) Targeting this stimulatory loop could be an efficient target to avoid the development of human AML.

TIGIT is expressed in cytotoxic T cells and in NK cells, and binds to the same ligands as DNAM-1 (CD112 and CD155) (Figure 1c). In AML patients, a large number of dysfunctional CD8+ T cells with increased expression of TIGIT and PD-1 but low levels of DNAM-1 was reported, but TIGIT expression in the NK cells of these patients has not been characterized.\(^{38}\) As previously described, AML patients show low levels of DNAM-1 expression, while its ligands are highly expressed.\(^{39}\) This could favor the binding of TIGIT with CD112 and CD155 ligands, promoting inhibitory signaling and, thereby, tumor immune escape. Recently, it has been shown that blockade of TIGIT prevents NK cell exhaustion and promotes NK cell-mediated anti-tumor effects in several mouse models of cancer (colon, breast, melanoma and fibrosarcoma), but the therapeutic potential in AML patients needs further study.\(^{40}\)

Given these findings, the development of new immunotherapies aimed at restoring the functionality of NK cells and the recognition of AML cells is a promising strategy, and one with important clinical implications.

### 3. Targeting AML cells by NK cell-based immunotherapy

The development of immunotherapy, an approach that aims to fight cancer using the body’s own immune system, has revolutionized the treatment of cancer. Numerous lines of evidence, summarized in this review, support the use of NK cell-based immunotherapy as a strategy for better recognition of AML cells. We also review the promising strategies that are currently proposed in the context of AML.

#### 3.1. Adoptive transfer of NK cells

##### 3.1.1. Autologous NK cells

Infusing NK cells to treat AML was first proposed by the American National Cancer Institute more than thirty years ago.\(^{40}\) This therapy is based on the administration of autologous NKs (1–2 x 10^7 cells/kg) previously activated with IL-2 or IFN-γ. Subsequently, IL-2 is administered in vivo to allow NK cells to proliferate in patients (Figure 2a). The advantage of this therapy over allo-HCT is that immune suppression and HLA-matching are not necessary. Nevertheless, it also has limitations due to the high toxicity arising from the sustained in vivo administration of IL-2, and to the absence of any clear clinical benefit.\(^{41}\) The lack of improvement of immune recognition may be due to the interaction between KIR receptors expressed in autologous NKs and HLA-I molecules in AML cells.\(^{42}\) This implies that modulation of the KIR/HLA-1 axis could enhance the clinical effect of autologous NKs transplant in AML.

##### 3.1.2. Allogeneic NK cells

Further studies were performed using allogeneic NKs from healthy donors that maintain their function and can be safely administered. As previously described, during NK cell development and to guarantee ‘self-tolerance’, KIR receptors bind with their ligands to ‘license’ the NKs and avoid the recognition and lysis of self-cells.\(^{43}\) Studies were carried out in AML patients using alloreactive NKs pre-activated with IL-2 and with one or more KIR-ligands mismatched to avoid the recognition of self-HLA class I molecules (Figure 2a).\(^{44,45}\) All patients receive immunosuppressive chemotherapy before NK cell infusion, and further exogenous administration of IL-2 in order to activate and expand circulating donor NK cells. One benefit of this therapy is the low incidence of graft versus host disease (GvHD) and the production of a strong graft versus leukemia (GvL) that is associated with better survival and a lower probability of relapse. In elderly patients, whose therapeutic options are very limited, consolidation therapy with these cells is feasible and promotes a better disease-free survival rate.\(^{46}\) Moreover, allogeneic clones can persist for up to 12 months, allowing the elimination of residual blasts.\(^{47}\) Several clinical trials are currently under way using haplo-identical NKs as consolidation therapy in AML, or in combination with other therapies, to determine their anti-leukemic effect and any possible secondary effects (Table 1).

*Ex vivo* stimulation of NKs with a cytokine cocktail of IL-12, IL-15 and IL-18 is an established alternative to avoid the *in vivo* administration of IL-2 after NK cell infusion.\(^{48}\) These cells, known as CIML (cytokine-induced memory-like), proliferate and produce high levels of IFN-γ during the first week, after which its production decreases. However, the *in vitro* re-stimulation of CIML cells, with the same activation cytokine cocktail or with a target cell (cell line or primary AML cells), restores the production of IFN-γ, suggesting that these cells have memory properties.\(^{49}\) Moreover, these cells more strongly express activating receptors (NKG2D, DNAM-
| Therapy                        | NK strategy                        | Identifier       | Phase                          | AML population (n)                  | Intervention                                                                                                                                          | Estimated completion date |
|-------------------------------|-----------------------------------|------------------|--------------------------------|------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------|
| Ongoing clinical trials using NK cells to enhance the innate immune response in AML. |                                   |                  |                                |                                    |                                                                                           |                            |
| Allogeneic NK cells           | Allogeneic donor-derived NK cells | NCT02477787      | 2                              | Refractory after allo-HSCT (90)    | Biological: NK infusion, days + 13 (1–2 x 10⁷ cells/kg) and + 20 (5 x 10⁸ cells/kg). Drug: antihistamine. | JUNE 2019                   |
|                               |                                   | NCT02229266      | 2                              | High-risk (56)                     | Biological: NK infusion. Drug: immunosuppression, IL-2.                                                                                               | SEPTEMBER 2020             |
|                               |                                   | NCT02763475      | 2                              | Pediatric with cytological remission (35) | Biological: NK infusion, days 0 (5 x 10⁷ cells/kg) and 7 (5 x 10⁸ cells/kg). Drugs: immunosuppression, IL-2 (1 x 10⁷ IU/m²) 3 times/week, 2 weeks. | FEBRUARY 2022              |
|                               |                                   | NCT03300492      | 1/2                            | Adult after allo-HSCT (10)         | Biological: NK infusion (0.01–1 x 10⁷ cells/kg) on days + 10, + 15, + 20.                                                                             | DECEMBER 2021              |
|                               | CIML                              | NCT01989793      | 1/2                            | Refractory/relapsed (133)          | Biological: CIML infusion (0.5 x 10⁶ to maximum tolerable cells/kg). Drugs: immunosuppression, IL-2 every other day for 2 weeks (total of 7 doses), ALT-803 (IL-15 agonist). | DECEMBER 2020              |
|                               |                                   | NCT02782546      | 2                              | High-risk after allo-HSCT (60)     | Biological: CIML infusion, day + 7. Drugs: immunosuppression, ALT-803 (10 mcg/kg) at 4h and on days + 12, + 17, + 22 post-transplant. | FEBRUARY 2022              |
|                               |                                   | NCT03068819      | 1                              | Pediatric (24)                     | Biological: CIML infusion (0.5–10 x 10⁶ cell/kg). Next day, infusion of donor lymphocyte (1–5 x 10⁶ cell/kg). Drugs: chemotherapy (2–4 weeks) prior infusion. | NOVEMBER 2024              |
|                               | FATE-NK100                        | NCT03081780      | 1                              | Refractory/relapsed (20)           | Biological: Infusion of maximum tolerable FATE-NK100 cells. Drugs: immunosuppression, 6 IL-2 doses (6 x 10⁶ IU/kg) | JANUARY 2021                |
| Allogeneic                    | CD3/CD19+ NK cells                | NCT03152526      | 2                              | No CR after standard induction therapies (41) | Biological: CD3/CD19+ NK infusion                                                                                                                          | JUNE 2022                  |
| Immune checkpoints inhibition  | Blockage of PD-1/PD-L1 engagement | NCT03050216      | 2                              | Refractory/relapsed (24)           | Biological: CD3/CD19+ NK infusion. Drug: immunosuppression, ALT-803. New treatment cycle (6 cycles). | SEPTEMBER 2020              |
|                               |                                   | NCT02532231      | 2                              | In remission but high-risk for relapse (30) | Biological: CD3/CD19+ NK infusion. Drug: Durvalumab (anti-PD-L1) (1500 mg), day + 1 every 4 weeks, azacitidine (75 mg/m²) for 7 days every 4 weeks. | OCTOBER 2020               |
|                               |                                   | NCT02775903      | 2                              | Older (272)                        | Biological: Prembroluzumab (anti-PD-1) at day + 1 every 3 weeks (8 total doses), melphalan, cyclophosphamide.                                             | APRIL 2019                 |
|                               |                                   | NCT02771197      | 2                              | High-risk after allo-HSCT (20)     | Biological: Azacitidine (75 mg/m²), day 1–7 every 28 day cycle plus nivolumab (anti-PD-1) (3 mg/kg) and/or ipilimumab (1 mg/kg) | JUNE 2021                  |
|                               |                                   | NCT02397720      | 2                              | Refractory/relapsed and new diagnosed (182) | Biological: Single infusion of NKG2D CAR-T (0.1–3.0 x 10⁷ cells/kg) | APRIL 2020                  |
| CAR                           | NKG2D CAR-T                        | NCT0203825       | 1                              | Adult and older (12)               | Biological: Anti-CD3 CAR-NK92 infusion                                                                                                                      | COMPLETED WITHOUT RESULTS |
|                               | Anti-CD3 CAR-NK92                  | NCT02944162      | 1                              | Refractory/relapsed (10)           | Biological: Anti-CD7 CAR-pNK infusion                                                                                                                      | SEPTEMBER 2018             |
|                               | Anti-CD7 CAR-pNK                   | NCT02742727      | 1/2                            | Refractory/relapsed (10)           | Biological: Anti-CD7 CAR-pNK infusion                                                                                                                      | MARCH 2018                 |

CAR: chimeric antigen receptor; CIML: cytokine-induced memory-like cells; CR: complete remission; HCT: hematopoietic stem cell transplantation; *cyclophosphamide and fludarabine; 
₂cyclophosphamide, fludarabine, tacrolimus and mycophenolate mofetil; and ³ fludarabine, ara-C, and G-CSF (FLAG chemotherapy regimen).
1, NKp30, NKp44, NKp46) relative to unstimulated NKs, implying a greater cytotoxicity to tumor cells and, thereby, a higher frequency of CR in elderly relapsed/refractory AML patients.60 In fact, there have been several phase I and II trials based on relapsed/refractory AML patients using CIML cells, alone (0.5–10 x 10^6 cells/kg) or in combination with other treatments (Table 1).

Alternative ex vivo expansion methods for NK cells are currently being evaluated in several clinical trials for the treatment of AML patients (Table 1). One of them is the infusion of allogeneic CD3+CD19− NKs, a method that increases the proliferation and cytolytic capacity of infused NK cells in patients. Another is the use of an IL-15 antagonist (ALT-803) that increases the activation and cytotoxic capacity of NK cells with respect to IL-2. In addition, one of the most important advances is the enrichment and expansion of terminally differentiated adaptive NK cells (commercially known as FATE-NK100) and that are currently being evaluated in refractory or relapsed AML patients (Table 1). These cells are obtained from some specific NK cell subsets (CD57+ NKG2C−) that are expanded following CMV infection, creating a pool of adaptive NK cells with a lower threshold of activation.50 To generate these cells, peripheral blood mononuclear cells from CMV-seropositive donors were depleted of CD3+ T and CD19+ B cells and cultured with IL-15 (a cytokine that drives NK cell proliferation) and a small molecule inhibitor of glycogen synthase kinase 3-beta (GSK3β) for 7–9 days.51 This facilitates the generation of a large number of mature CD57+ NK cells with increased IFN-γ production. In addition to AML, two clinical trials are currently ongoing, one (NTC03319459) in subjects with advanced solid tumors (gastric, colorectal, and head and neck squamous cell carcinoma), and the other (NTC03213964) in women with advanced ovarian, fallopian tube or primary peritoneal cancer.

### 3.2. Regulation of NK cell ligands by epigenetic mechanisms

To enhance the cytotoxic ability of NK cells it is essential to recognize the engagement between activating receptors and their ligands expressed on the cell surface of target cells. However, as we have remarked above, the expression of these ligands is reduced or absent in AML cells, so their expression must be enhanced in order to facilitate immune recognition mediated by NKs.

Epigenetic modifications that modulate the expression of activating ligands are known to arise during cancer development. Several studies have reported that treatment with inhibitors of histone deacetylases (HDACi; valproic acid, trichostatin A or all-trans-retinoic acid) increases the expression of NKG2DL (MICA/B, ULBP1-3) and decreases the release of their soluble forms in cell lines and primary AML cells, thereby enhancing the lytic capacity of NKs (Figure 2b).20,52,53 Histone acetylation is also important for regulating other activating ligands in AML, such as CD48 (2B4 ligand).54

DNA methylation is another important epigenetic mechanism of gene regulation. Results from our group revealed that DNA methylation can contribute to the absence of NKG2DL expression observed during tumor development in AML patients.55 We found significantly higher DNA methylation levels in the promoter region of MICA, ULBP1 and ULBP2 genes in AML patients compared with healthy donors, and this was correlated with the absence of transcription for these genes. Moreover, 59% of AML patients had at least one methylated NKG2DL, although the combination of ligands varied between patients. DNA methylation is a reversible mechanism, so treatment with DNA methyltransferase inhibitors (DNMTi) such as azacitidine (Vidaza®, AZA) and DAC, which are commonly used to treat elderly AML patients, restores the expression of NKG2DL on the surface of AML cells (Figure 2b). Our group recently reported that treatment with these drugs also reduces the release of soluble NKG2DL, maintaining their expression on the surface of AML cells by the hypomethylation of TIMP3, an inhibitor of protease ADAM17 (Figure 2b).56 However, the role of DNA methylation in regulating other activating ligands is unknown.

All these studies suggest that the use of epigenetic treatments in combination with other conventional therapies could be an important method for enhancing the expression of activating ligands in AML blasts and thus the recognition mediated by allogeneic NKs.

### 3.3. Blockade of immune checkpoints

Given the role of immune checkpoints in inhibiting NK cell activation, the effect of blocking these molecules has been evaluated. Blockade of some immune checkpoints (LAG-3, TIM-3, PD-1) with specific antibodies increases the lytic capacity of CIML cells against AML (Figure 2c).57 In an experimental mouse model of lung cancer, the specific blockade of LAG-3 and PD-1 increases the number of mature NKS and thereby reactivates the antitumoral properties (greater production of cytotoxic granules and IFNγ) of CIML cells, allowing the control of the metastasis.58

Within immune checkpoints, PD-1 and its ligand (PD-L1) are the most thoroughly studied due to the excellent results obtained from T cells in several tumors. It has also been reported that PD-1 or PD-L1 blockade increases the degranulation and cytotoxic capacity of NKS, shrinking the tumor grown in xenograft models of digestive and myeloma cancers.33,59 In AML, several clinical trials are currently underway to evaluate the response of this blockade (Table 1). To this end, patients with relapse/refractory or high-risk AML are treated with specific antibodies against PD-1 or PD-L1 alone or in combination with other therapies (AZA, chemotherapy, dendritic cell vaccine). Unpublished preliminary data from the NCT02397720 trial show that the combined use of AZA with anti-PD-1 (nivolumab) is well tolerated and produces durable responses in relapsed AML. Therefore, the results of current clinical trials are needed to corroborate the efficiency of this therapy, as has been observed in solid tumors.

Recent significant studies have shown TIGIT and IL-1R8 to be new checkpoint molecules or negative regulators of NK cell function in several solid tumors.56,60 Blockade of these proteins reverses the exhaustion of tumor-infiltrating NK cells and reduces the threshold for NK cell activation, respectively.
The study of these mechanisms in AML, in combination or not with other therapeutic approaches, could help enhance the NK-cell mediated anti-tumor response.

3.4. Chimeric antigen receptor-based immunotherapy

Chimeric antigen receptor (CAR) therapy is an emerging immunotherapy that combines specific antigen recognition with signaling capacity to recognize specific targets in cancer cells. These CARs can be expressed in immune cells as T lymphocytes (CAR-T therapy) by viral transduction or mRNA transfection, and frequently its specificity is based on scFv regions or TCR binding domains. Several CAR-T therapies directed against some of the most important targets in AML blasts (such as CD33, FLT3 and CD123) have been assayed in recent years, revealing potent activity against tumor cells. An ideal CAR target should be expressed in a large number of AML blasts and with high-density but in as few normal tissues as possible to avoid undue toxicity. These targets are widely expressed in the majority of AML blasts but, unfortunately, also in some normal hematopoietic stem cells, myeloid cells and other tissues. CAR-T cell immunotherapy aimed at simultaneously targeting two AML antigens (CD33 and CD123) or other candidates identified by integrating proteomic and genomic datasets is a promising strategy for effectively eliminating AML cells and leukemic stem cells, while avoiding relapse and without incurring greater toxicity.

CAR technology may also be useful for enhancing the recognition and lysis mediated by NK cells (Figure 2d). Two strategies have been proposed for this: CAR-T encoding NK cell-activating receptors, and CAR-NK. In the first strategy, the use of a CAR-T with a full-length human NKG2D (NKG2D CAR-T) to target cancer cells expressing NKG2D ligands (MICA and ULBP 1–3) expression on the cell surface of AML cells. The TIMP3 gene, which is methylated in some AML patients, is also expressed, leading to the inhibition of the main protease involved in the release of NKG2DL. As a consequence, NKG2DL (MICA and ULBP 1–3) expression on the cell surface and are released in their soluble form (sMICA/B and sULBP2), maintaining the high expression levels on the AML cell surface. Specific antibodies against PD-1 (nivolumab, pembrolizumab) or its ligand PD-L1 (durvalumab) block the PD-1/PD-L1 interaction, avoiding the anergy of NK cells; and CAR technology, T cells or NK cells collected from the AML patient are transduced with CAR with specific genes (NKG2D, Nkp30) or antibodies (α-CD33, α-CD7). Further, these cells are infused in AML patients and when CAR recognizes its antigen, expressed on the surface of AML cell, CAR-T or CAR-NKs are activated.

Figure 2. Enhancing AML recognition by immunotherapy techniques. Several strategies based on the use of NK cells have been proposed to allow the recognition of AML cells, such as: a) NK infusion. Autologous NK cells or NK cells from a KIR-ligand mismatched donor, are expanded in vitro in the presence of IL-2, IFN-γ, and/or anti-CD3. AML patients are infused with these cells and treated with IL-2 to promote the expansion of NK cells. An alternative is the infusion of allogenic CIML cells that are expanded in vitro in the presence of a cytokine cocktail (IL-12, IL-15 and IL-18). An advantage of this treatment is that there is no need to treat the patient with IL-2; b) Epigenetic treatments. Treatment with HDACi and DNMTi restores NKG2DL (MICA and ULBP 1–3) expression on the cell surface of AML cells. The TIMP3 gene, which is methylated in some AML patients, is also expressed, leading to the inhibition of the main protease involved in the release of NKG2DL. As a consequence, NKG2DL (MICA and ULBP 1–3) expression on the cell surface and are released in their soluble form (sMICA/B and sULBP2), maintaining the high expression levels on the AML cell surface. c) Immune checkpoint blockade. Specific antibodies against PD-1 (nivolumab, pembrolizumab) or its ligand PD-L1 (durvalumab) block the PD-1/PD-L1 interaction, avoiding the anergy of NK cells; and d) CAR technology. T cells or NK cells collected from the AML patient are transduced with CAR with specific genes (NKG2D, Nkp30) or antibodies (α-CD33, α-CD7). Further, these cells are infused in AML patients and when CAR recognizes its antigen, expressed on the surface of AML cell, CAR-T or CAR-NKs are activated.
activated T cells.\textsuperscript{70} This could be avoided by employing the alternative strategy of using memory T cells, which are known to be less toxic after CAR-T infusion in murine models of glioblastoma and osteosarcoma.\textsuperscript{68,69} Although the antitumoral effect of NKG2D CAR-T is unknown in AML models, the Dana-Farber Cancer Institute is conducting a phase I clinical trial (NCT02203825) in which patients with AML, myelodysplastic syndrome and multiple myeloma are infused with a single dose (0.1–3 x 10\textsuperscript{7} cell/kg) of NKG2D CAR-T (Table 1). Nkp30 is another activating receptor whose use in CAR-T models has been proposed.\textsuperscript{71} This Nkp30 CAR-T increases the production of IFN-\gamma and the cytotoxic capacity towards malignant cells that express its ligand, B7H6. Nkp30 CAR-T improves survival in lymphoma murine models and prevents the development of the tumor after a subsequent challenge of malignant cells, suggesting that an immunological memory is generated. CAR-T technology can also be used to target the ligands of the activating receptors and to enhance the lysis of tumor cells. In this way, a B7H6 CAR-T therapy enhances the release of IFN-\gamma by transfected T cells against B7H6-expressing cell lines, and prolongs survival in ovarian and lymphoma murine models.\textsuperscript{72,73}

The second strategy for enhancing the recognition between NK and tumor cells is the use of CAR-modified NK cells (Figure 2d). This therapy is attracting attention because of its advantages over T lymphocytes: (1) sensitization is not necessary; (2) there is no MHC restriction; (3) antigen-specific receptors are not required; and (4) there are fewer side effects than with GvHD.\textsuperscript{74} There are currently two phase I and II clinical trials underway using CD7- or CD33-CAR NK-92 cells in AML patients with relapsed or refractory disease (Table 1). The first findings, which have recently been published, show that high doses of CD33-CAR NK cells have no significant adverse effects on the three analyzed AML patients, showing the safety of the treatment, although further studies are needed to establish the clinical efficacy.\textsuperscript{75}

4. Concluding remarks

NK cells are key effectors in cancer immunosurveillance that promote the recognition and lysis of tumor cells. However, the evolution and frequent relapses in AML patients suggest that leukemia cells can escape this recognition. As mentioned above, several mechanisms that impair NK cell functions in AML have been described in recent years that have prompted great efforts aimed at developing new therapeutic strategies that enhance the recognition and lysis of AML cells by NK cells. Currently available immunotherapeutic treatments have yielded promising results in vitro and in mouse models of AML. Nevertheless, it is important to perform in-depth studies to determine the toxicity of these immunotherapies in AML patients. The ability to inhibit AML targets specifically using immune checkpoint blockade or CAR-T/CAR-NK therapies opens up new possibilities for NK cell-based immunotherapy. A considerable number of clinical trials are currently underway using both therapies, alone or in combination with well-established AML treatments. The results of these studies will clarify the clinical value of both immunotherapies as treatments for AML.

Abbreviations

AML acute myeloid leukemia
CAR chimeric antigen receptor
CIML cytokine-induced memory-like
CR complete remission
FAB French-American-British classification
GvHD graft versus host disease
GvL graft versus leukemia
HSCT hematopoietic stem cell transplant
NK natural killer
WHO World Health Organization.

Conflicts of interest

The authors declare no conflicts of interest.

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References

1. Pandolfi A, Barreyro L, Steidl U. Concise review: preleukemic stem cells: molecular biology and clinical implications of the precursors to leukemia stem cells. Stem Cells Transl Med. 2013;2(2):143–150. doi:10.5966/sctm.2012-0109.
2. Papaemmanuel E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, et al. Genomic classification and prognosis in acute myeloid leukemia. N Engl J Med. 2016;374(23):2209–2221. doi:10.1056/NEJMoa1516192.
3. Klco JM, Spencer DH, Miller CA, Griffith M, Lamprecht TL, O’Laughlin M, Fronick C, Magrini, Demeter RT, Fulton RS, et al. Functional heterogeneity of genetically defined subclones in acute myeloid leukemia. Cancer Cell. 2014;25(3):379–392. doi:10.1016/j.ccr.2014.01.031.
4. Aziz H, Ping CY, Alias H, AbMutalib NS, Jamal R. Gene mutations as emerging biomarkers and therapeutic targets for relapsed acute myeloid leukemia. Front Pharmacol. 2017;8:897. doi:10.3389/fphar.2017.00897.
5. Rothenberg-Thurley M, Amerl S, Goerlich D, Köhnke T, Konstandin NP, Schneider S, Sauerland MC, Herold T, Hubmann M, Ksienzyk B, et al. Persistence of pre-leukemic clones during first remission and risk of relapse in acute myeloid leukemia. Leukemia. 2018;32(7):1598–1608. doi:10.1038/s41375-018-0034-z.
6. Arber DA, Orazi A, Hasserjian R, Thiele J, Borowitz MJ, Le Beau MM, Bloomfield CD, Cazzola M, Vardiman JW. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. Blood. 2016;127(20):2391–2405. doi:10.1182/blood-2016-03-643544.
7. Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood. 2017;129(4):424–447. doi:10.1182/blood-2016-08-733196.
8. Bertoli S, Tavittai S, Huynh A, Borel C, Guenounou S, Luquet I, Delahesse E, Sarry A, Laurent G, Attal M, et al. Improved outcome for AML patients over the years 2000–2014. Blood Cancer J. 2017;7(12):635. doi:10.1038/s41408-017-0011-1.
9. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. Lancet. 2000;356(9244):1795–1799. doi:10.1016/S0140-6736(00)03231-1.
10. Ruggeri L, Capanni M, Urbani E, Perruccio K, Shlomchik WD, Tosti A, Posati S, Rogaia D, Frassoni F, Aversa F, et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science. 2002;295(5562):2097–2100. doi:10.1126/science.1068440.

11. Pegram HM, Andrews DM, Smyth MJ, Darcy PK, Kershaw MH. Activating and inhibitory receptors of natural killer cells. Immunol Cell Biol. 2011;89(2):216–224. doi:10.1038/icb.2010.78.

12. Orr MT, Lanier LL. Natural killer cell education and tolerance. Cell. 2010;142(6):847–856. doi:10.1016/j.cell.2010.08.031.

13. Sandoval-Borrego D, Moreno-Lafont MC, Vazquez-Sanchez EA, Gutierrez-Hoya A, López-Santiago R, Montiel-Cervantes LA, Ramírez-Saalsa M, Vela-Ojea J. Overexpression of CD158 and NKGA2 inhibitory receptors and underexpression of NKGD2 and NKp46 activating receptors on NK cells in acute myeloid leukemia. Arch Med Res. 2016;47(1):55–64. doi:10.1016/j.arcmed.2016.02.001.

14. Sanchez-Correa B, Gayoso I, Bergua JM, Casado JG, 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):109–115. doi:10.1038/icb.2011.057.

15. Fauriat C, Just-Landi S, Mallet F, Arnoulet C, Sainty D, Olive D. Analysis of the receptor-ligand interactions in the natural killer-cell allorecognition by NK-cell lines with single KIR-HLA class I specificities. Blood. 2004;103(5):2066–2073. doi:10.1182/blood-2003-01-0019.

16. Chretien AS, Fauriat C, Orlanducci F, Rej J, Borgen GB, Gautherot E, Granjeaud S, Demerle C, Hamel JF, et al. NKp46 expression on NK cells as a prognostic and predictive biomarker for stratification of patients with intermediate-risk acute myeloid leukemia. Oncotarget. 2017;8(30):49548–49563. doi:10.18632/oncotarget.17747.

17. Chretien AS, Devillier R, Fauriat C, Orlanducci F, Harbi S, Le Roy A, Rej J, Bovier Borg G, Gautherot E, Hamel JF, et al. NKp46 expression on NK cells as a prognostic and predictive biomarker for response to allo-SCT in patients with AML. Oncoimmunology. 2017;6(12):e1307491. doi:10.1080/2162402X.2017.1307491.

18. Nowbakht P, Ionescu MC, Rohner A, Kalberer CP, Rossy E, Mori L, Cosman D, De Liberio G, Wodnar-Filipowicz A. Ligands for natural killer cell-activating receptors are upregulated upon the maturation of normal myelomonocytic cells but at low levels in acute myeloid leukemias. Blood. 2005;105(9):3615–3622. doi:10.1182/blood-2004-07-2585.

19. Pende D, Spaggiari GM, Marcenaro S, Martini S, Rivera P, Capobianco A, Falco M, Laino E, Pierni I, Zambello R, et al. Control of NK cell activation by immune checkpoint molecules. Int J Mol Sci. 2017;18(10):pii:E2129. doi:10.3390/ijms18102129.

20. Racee R, Rosario M, Berrien-Elliott MM, Wagner JA, Jewell BA, Kershaw MH. The PD-1/PD-L1 axis modulates the natural killer cell versus multiple myeloma effect: a therapeutic target for CT-011, a novel monoclonal anti-PD-1 antibody. Blood. 2010;116(13):2286–2294. doi:10.1182/blood-2010-02-271874.

21. Liu Y, Cheng Y, Xu Y, Wang Z, Du X, Li C, Peng J, Gao L, Liang X, Ma C. Increased expression of programmed cell death protein 1 on NK cells inhibits NK cell-mediated anti-tumor function and indicates poor prognosis in digestive cancers. Oncogene. 2017;36(44):6143–6153. doi:10.1038/onc.2017.209.

22. Berthon C, Driss V, Liu J, Kuranda K, Leleu X, Jouy N, Hetuin D, Quesnel B. In acute myeloid leukemia, B7-H1 (PD-L1) protection of blasts from cytotoxic T cells is induced by TLR ligands and interferon-gamma and can be reversed using MEK inhibitors. Cancer Immunol Immunother. 2010;59(12):1839–1849. doi:10.1007/s00262-010-0909-y.

23. Goltz D, Gvensleben H, Grünen S, Dietrich J, Kristiansen G, Landsberg J, Dietrich D. PD-L1 (CD274) promoter methylation of blasts from cytotoxic T cells is induced by TLR ligands and interferon-gamma and can be reversed using MEK inhibitors. Cancer Immunol Immunother. 2010;59(12):1839–1849. doi:10.1007/s00262-010-0909-y.

24. Kikushige Y, Miyamoto T, Yuja D, Jabbarzadeh-Tabrizi S, Shima H, Hajime Y, Masahiko T, Takayama Y, Hiroshi K, Tsukasa K, et al. A TIM-3/Gal-9 autocrine stimulatory loop drives self-renewal of human myeloid leukemia stem cells and leukemic progression. Cell Stem Cell. 2015;17(3):341–352. doi:10.1016/j.stem.2015.07.011.
B. Bernardini G, Magrini E, Gianni F, et al. IL-1R8 is a
2009
440. doi:
10.1182/
713.
1326. doi:
2016
2018
NKG2C
Jun 18. [Epub ahead of print].
10.1073/
2017
2017
NK cell expansion is associated with reduced
2017
2009
2005
'7071.
2012
3148.
73. doi:
1919. doi:
1647. doi:
615.
2018
2015
2018
2010
19.e5. doi:
2018
82. doi:
Cooper MA, Elliott JM, Keyel PA, Yang L, Carrero JA, Yokoyama
73. doi:
1535–
1543. doi:
10.1182/blood-2013-09-526590.
Baragáñoraneros A, Martín-Palanco V, Fernandez AF, Rodriguezm, FRama MF, Lopez-Larrea C, Suarez-Alvarez BM. Methylation
of NKG2D ligands contributes to immune system evasion in acute
malignant leukemia. Genes Immun. 2015;16(1):71–82. doi:
gene2014.58.
Raneros AB, Puras AM, Rodriguez RM, Colado E, Bernal T, AnguitaEA, Mogorron AV, Gil AC, Vidal-Castañera JF, Márquez-Kisinsonuyk L, et al. Increasing TIMP3 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–
31976. doi:
10.18632/oncotarget.16657.
Poh SL, Linn YM. Immune checkpoint inhibitors enhance cyto-
toxicity of cytokine-induced killer cells against human myeloid
leukaemic blasts. Cancer Immunol Immunother. 2016;65(5):525–
536. doi:
10.1007/s00262-016-1815-8.
Öhs I, Ducimetière L, Marinho J, Kulig P, Becher B, Tugues S. Restoration of natural killer cell antimitastatic activity by IL12 and
checkpoint blockade. Cancer Res. 2017;77(24):7059–7071.
doi:
10.1158/0008-5472.CAN-17-1032.
Guo Y, Feng X, Jiang Y, Shi X, Xing X, Liu X, Li N, Fadde B, Zheng C. PD1 blockade enhances cytotoxicity of in vitro
expanded natural killer cells towards myeloma cells. Oncotarget.
2016;7(30):48360–48374. doi:
10.18632/oncotarget.10235.
Molgora M, Bonavita E, Ponsetta A, Riva F, Barbagallo M, Jaillon
S, Popovic B, Bernardini G, Magrini E, Gianni F, et al. IL-1R8 is a
checkpoint in NK cells regulating anti-tumour and anti-viral
activity. Nature. 2017;551(7678):110–114. doi:
10.1038/nature24293.
June CH, Sadelain M. Chimeric antigen receptor therapy. N Engl J
Med. 2018;379(1):64–73. doi:
10.1056/NEJMr1706169.
Kenderian SS, Ruella M, Shestova O, Klichinsky M, Aikawa V,
Morrisette JJ, Scholler J, Song D, Porter DL, Carroll M, et al. CD33-specific chimeric antigen receptor T cells exhibit potent
preclinical activity against human acute myeloid leukemia.
Leukemia. 2015;29(8):1637–1647. doi:
10.1038/leu.2015.52.
Chen L, Mao H, Zhang J, Chu J, Devine S, Caligiuri MA, Yu J.
Targeting FLT3 by chimeric antigen receptor T cells for the
treatment of acute myeloid leukemia. Leukemia. 2017;31
(8):1830–1834. doi:
10.1038/leu.2017.147.
Mardiros A, Dos Santos C, McDonald T, Brown CE, Wang X,
Budde LE, Hoffman L, Aguilar B, Chang WC, Bretzlafl W, et al. T
cells expressing CD123-specific chimeric antigen receptors exhibit
specific cytolytic effector functions and antitumour effects against
human acute myeloid leukemia. Blood. 2013;122(18):3138–3148.
doi:
10.1182/blood-2012-12-47045.
Perna F, Berman SH, Soni RK, Mansilla-Soto J, Eyuqem J,
Hamieh M, Hendrickson RC, Brennan CW, Sadelain M. Integrating
proteomics and transcriptomics for systematic combinatorial
chimeric antigen receptor therapy of AML. Cancer Cell.
2017;32(4):506–19.e5. doi:
10.1016/j.ccell.2017.09.004.
Petrov JC, Wada M, Pinz KG, Yan LE, Chen KH, Shuai X, Liu H,
Guo X, Leung IH, Salmon H, et al. Compound CAR T-cells as a
double-pronged approach for treating acute myeloid leukemia.
Leukemia. 2018;32(6):1317–1326. doi:
10.1038/s41375-018-0075-3.
67. Sentman CL, Meehan KR. NKG2D CARs as cell therapy for cancer. Cancer J. 2014;20(2):156–159. doi:10.1097/PPO.0000000000000029.

68. Weiss T, Weller M, Guckenberger M, Sentman CL, Roth P. NKG2D-based CAR T cells and radiotherapy exert synergistic efficacy in glioblastoma. Cancer Res. 2018;78(4):1031–1043. doi:10.1158/0008-5472.CAN-17-1788.

69. Fernández L, Metais JY, Escudero A, Vela M, Valentín J, Vallcorba I, Leivas A, Torres J, Valeri A, Patiño-García A, et al. Memory T cells expressing an NKG2D-CAR efficiently target osteosarcoma cells. Clin Cancer Res. 2017;23(19):5824–5835. doi:10.1158/1078-0432.CCR-17-0075.

70. Sentman ML, Murad JM, Cook WJ, Wu MR, Reder J, Baumeister SH, Dranoff G, Fanger MW, Sentman CL. Mechanisms of acute toxicity in NKG2D chimeric antigen receptor T cell-treated mice. J Immunol. 2016;197(12):4674–4685. doi:10.4049/jimmunol.1600769.

71. Zhang T, Wu MR, Sentman CL. An NKp30-based chimeric antigen receptor promotes T cell effector functions and antitumor efficacy in vivo. Immunol. 2012;189(5):2290–2299. doi:10.4049/jimmunol.1103495.

72. Wu MR, Zhang T, DeMars LR, Sentman CL. B7H6-specific chimeric antigen receptors lead to tumor elimination and host antitumor immunity. Gene Ther. 2015;22(8):675–684. doi:10.1038/gt.2015.29.

73. Gacerez AT, Hua CK, Ackerman ME, Sentman CL. Chimeric antigen receptors with human scFvs preferentially induce T cell anti-tumor activity against tumors with high B7H6 expression. Cancer Immunol Immunother. 2018;67(5):749–759. doi:10.1007/s00262-018-2124-1.

74. Liu D, Tian S, Zhang K, Xiong W, Lubaki NM, Chen Z, Han W. Chimeric antigen receptor (CAR)-modified natural killer cell-based immunotherapy and immunological synapse formation in cancer and HIV. Protein Cell. 2017;8(12):861–877. doi:10.1007/s13238-017-0415-5.

75. Tang X, Yang L, Li Z, Nalin AP, Dai H, Xu T, Yin J, You F, Zhu M, Shen W, et al. First-in-man clinical trial of CAR NK-92 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):1083–1089.