Review

The Role of Immune Checkpoint Blockade in Acute Myeloid Leukemia

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Simple Summary: Acute myeloid leukemia is an aggressive and very lethal blood tumor. It represents a substantial percentage of leukemia patients, as well as leukemic deaths. The development and research of alternative target therapies to the detriment of conventional chemotherapy represent a new hope for these patients. In this systematic review, we aimed to explore the functioning of immune checkpoint blockers along with the data of clinical trials and their outcomes in these patients. This immunotherapy option is already used in many other solid tumors with favorable outcomes, as either monotherapy or combined therapy; however, it is not yet widely used in acute myeloid leukemias. The clinical and scientific information provided with this review allows the research community to understand how this therapy could help patients, whether it provides better outcomes when combined with other therapies, and the different side effects of checkpoint blockage.

Abstract: Immune checkpoint inhibition (ICI) has emerged as a therapeutic option for acute myeloid leukemia (AML) for patients that suffer from relapsed or high-risk disease, or patients ineligible for standard therapy. We aimed to study ICI as monotherapy and/or combined therapy (with chemotherapy (QT), for AML patients. The PRISMA statement was used. The literature used comprised clinical trials, randomized controlled trials, and systematic reviews published within the last 7 years. The blockade of CTLA-4 presented a 42% of complete remission within AML. Nivolumab in high-risk AML showed a median recurrence-free survival (RFS) of 8.48 months. The same drug on relapsed hematologic malignancies after allogenic transplantation shows a 1-year OS of 56%. The use of prophylaxis post allogenic transplantation cyclophosphamide (PTCy), following checkpoint inhibition, demonstrated different baseline disease and transplantation characteristics when compared to no-PCTy patients, being 32% and 10%, respectively. CTLA-4 blockage was a worthy therapeutic approach in relapsed hematologic malignancies, presenting long-lasting responses. The approach to AML and myelodysplastic syndrome patients with ICI before allogenic hematopoietic stem cell transplantation and the use of a graft-versus-host disease prophylaxis have shown improvement in the transplantation outcomes, and therefore AML treatment.

Keywords: acute myeloid leukemia; treatment; immune checkpoint; PD-1/PD-L1; CTLA-4

1. Introduction

Acute myeloid leukemia (AML) is a bone marrow malignancy, characterized by the expansion and differentiation of myeloid progenitor cells [1,2]. AML represents around

90 percent of leukemia cases in adults and accounts for 62 percent of leukemic deaths [3–5]. This clonal hematopoietic stem cell disorder affects people of all ages; nevertheless, its incidence increases in older adults, reporting 68 years as the median age at diagnosis [6].

AML can result from an underlying hematological disorder, prior chemotherapy, or certain chemical exposures, or, as de novo malignancy [2,7], due to genetic alterations, through well-characterized chromosomal translocations or isolated molecular changes [7,8]. The criteria to diagnose AML are either the identification of more than 20% of myeloid blasts in the bone marrow or peripheral blood or the detection of specific cytogenetic abnormalities, as observed in Figure 1 [7,9].
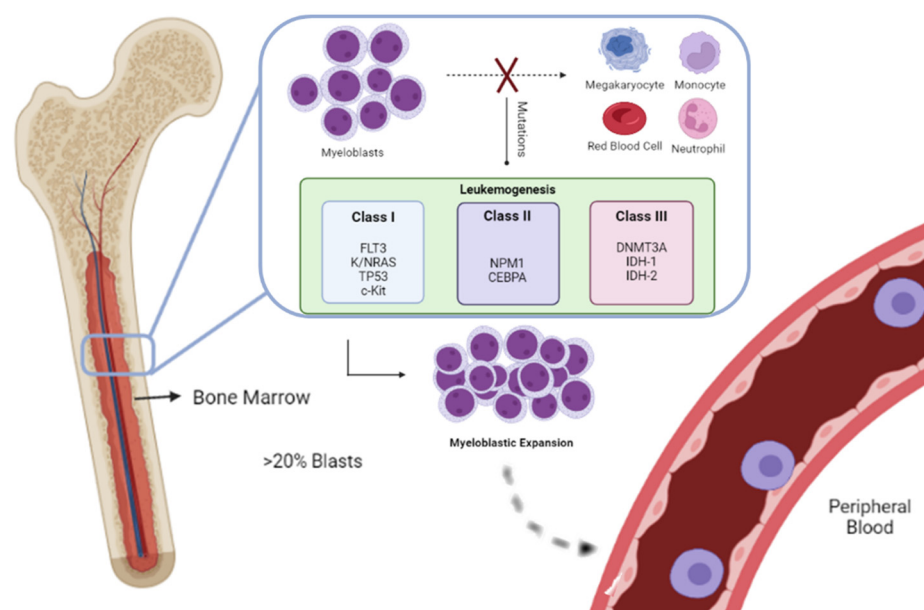
Figure 1. In Acute myeloid leukemia, due to genetic alterations in the blood cell precursors, there is an overproduction of neoplastic clonal myeloid cells, resulting in their accumulation in the bone marrow, namely, the FMS-like tyrosine kinase 3 (FLT3), Kirsten rat sarcoma viral oncogene homolog/neuroblastoma rat sarcoma viral oncogene homolog (K/NRAS), tumor protein 53 (TP53), tyrosine-protein kinase (c-KIT), nucleophosmin 1 (NPM1), CCAAT enhancer-binding protein alpha (CEBPA), DNA methyltransferase 3 alpha (DNMT3A), ten-eleven translocation-2 (TET2), isocitrate dehydrogenases 1 and 2 (IDH-1 and IDH-2). Myeloid blasts fail to differentiate into monocytes, megakaryocytes, neutrophils, and red blood cells. The expansion, over 20%, initially is in the bone marrow; however, in a later stage of the disease, blasts are detected in peripheral blood.

The subtypes of this pathology are classified by the World Health Organization. This classification system incorporates genetic information, morphology, immunophenotype, and clinical presentation. Distinguishing into six diverse groups: AML with recurrent genetic abnormalities, AML with three myelodysplasia-related changes, Therapy-related myeloid neoplasms, AML Not Otherwise Specified, Myeloid sarcoma, and Myeloid proliferations related to Down syndrome [10].

A two-hit model of leukemogenesis was developed to enable the classification of various mutations. Class I mutations, such as FMS-like tyrosine kinase 3 (FLT3), Kirsten rat sarcoma viral oncogene homolog/neuroblastoma rat sarcoma viral oncogene homolog (K/NRAS), tumor protein 53 (TP53), and c-KIT, result in the activation of pro-proliferative pathways. These must happen simultaneously to class II mutations, such as nucleophosmin 1 (NPM1) and CCAAT enhancer-binding protein alpha (CEBPA) to commit normal differentiation and develop leukemia. This model currently considers the alterations in the epigenetic regulation, the third class of mutations, in genes related to DNA methylation such as DNA methyltransferase 3 alpha (DNMT3A), ten-eleven translocation-2 (TET2), isocitrate dehydrogenases 1 and 2 (IDH-1 and IDH-2) [8,11], as observed in Table 1.

The understanding of genetic abnormalities is key for both stratifying patients and, determining appropriate treatment. The standard treatment is chemotherapy, divided into induction therapy, and consolidation therapy. Both treatment and response vary according to the patient's age, coexistence with other diseases, and genetic alterations [12–14].

Induction therapy aims at a morphologic remission, which results in the elimination of blasts of the blood and reduction in the bone marrow, to achieve the restoration of normal hematopoiesis [12]. This intensive chemotherapy approach adds in some potential complications such as prolonged marrow aplasia, profound cytopenias, transfusional support, and the risk of infections. It is often difficult to tolerate and is associated with high relapse rates [13].

Table 1. Classification of Acute myeloid leukemia mutations in Class I, Class II, and Class III.

	Mutation	Type of Mutation	Reference
Class I	FLT 3 (FLT3-ITD; FLT3-TKD)	FLT3- ITD, in-frame duplications of variable size, multiple copies of the gene in a row. FLT3- TKD, point mutations within the receptor's activation loop, single change, or gene deletions	[8,11,15]
	K-RAS and N-RAS	Activated point mutations	[8,11]
	TP53	Missense substitutions (75%); Frameshift insertions and deletions (9%); non-sense mutations (7%); Silent mutations (5%), and other rare aberrations (2%).	[8,11,16]
	c-Kit	Overexpression and point mutations	[8,11,17]
Class II	NPM1	Frameshift insertions of usually 4-bp	[8,11,18]
	CEBPA	N-terminal frame-shift insertions/deletions and/or C-terminal in-frame insertions/deletions	[8,11,19]
Class III	DNMT3A	DNA methylation	[8,11]
	TET2		
	IDH-1 and IDH-2		

Legend: FLT3—FMS-like tyrosine kinase 3; K/NRAS—Kirsten rat sarcoma viral oncogene homolog/neuroblastoma rat sarcoma viral oncogene homolog, TP53—tumor protein 53; c-KIT—tyrosine-protein kinase; NPM1—nucleophosmin 1; CEBPA—CCAAT enhancer-binding protein alpha; DNMT3A—DNA methyltransferase 3 alpha; TET2—ten-eleven translocation-2; IDH-1—isocitrate dehydrogenases 1; isocitrate dehydrogenases 2.

Consolidation chemotherapy is administrated after intensive induction therapy to remove residual leukemic cells. After chemotherapy-induced remission, post-remission therapy must avoid relapse, a stem cell transplant in younger patients, or low-dose chemotherapy in older ones [14]. Patients younger than 60 years, present a 60% to 70% rate of complete remission (CR) with chemotherapy. Nevertheless, the cure rate is around 35% to 40%, which is considerably low. Regarding older patients, and patients who suffer from adverse cytogenetic risk, these present a lower CR rate, of 35% to 50%, therefore an even lower cure rate, of 10% or less. This lower response is due to either the inability to tolerate intensive chemotherapy or the higher rates of resistance to chemotherapy [20,21].

In recent years, immunotherapy, the establishment of an anti-tumor successful response of the immune system, has played a revolutionary role in lymphoid malignancies and multiple solid tumors treatment [13,22].

Immunotherapy is not a new approach to BC treatment, as some monoclonals antibodies (mAb) have long been used as therapeutic agents to treat BC tumors, such as Trastuzumab. In recent years, due to the lack of specific target therapies, antibody–drug

conjugates (ADC) and immune checkpoint inhibitors (ICI) have increasingly been studied for BC treatment [23]. HER-2+ BC and TNBC have high levels of PD-L1 expression, and BC also can express high levels of CTLA-4, suggesting that ICI are being increasingly explored as a potential treatment strategy with promising results [24]. Atezolizumab with nab-paclitaxel was approved for patients with locally advanced or metastatic TNBC, whose tumors are positive for PD-L1 expression with progression-free survival (PFS) of 7.5 months [24,25]. Pembrolizumab has been used to treat metastatic TNBC PD-L1+ with an objective response rate (ORR) of 21.4% (Beatriz), and Pembrolizumab plus QT in early-stage TNBC demonstrated a pathological complete response of 69.5%.

In bladder cancer, immunotherapy offers an effective alternative for patients who are ineligible for cisplatin and patients with advanced disease progression after platinum-based therapy [26]. In urothelial carcinomas, monotherapy with ICIs demonstrated unprecedented results, mainly in metastatic disease [27]. In pretreated metastatic urothelial carcinoma, Atezolizumab showed a response of 40%, with a 3-year OS rate of 27% and a median overall survival of 14.6 months in patients with PD-L1 expression [28]. The effectiveness of Durvalumab was also analyzed and the subgroup with positive PD-L1 expression shows an ORR of 27.6%, with a median PFS of 2.1 months and an OS of 20 months [28], suggesting that it can be a therapeutic strategy as monotherapy and combined therapy [29].

In advanced renal cell carcinoma, immunotherapy also presents a therapeutic approach [30]. The combination of pembrolizumab plus axitinib versus sunitinib was analyzed and demonstrated that the ORR was 59.3% and the OS was 9.9% (PEM + AXI) [30,31]. A study with nivolumab in patients with refractory RCC demonstrated a median follow-up of 6.5 months, the median PFS was 4.2 months, and although the median OS was not reached, the ORR was 29%. New strategies include anti-VEGF therapies and immune-checkpoint inhibitors, either as single agents or in combination [32].

One of the promising methods of immunotherapy is the activation of the immune response through monoclonal antibodies targeting immune checkpoints on T cells [33]. Immune checkpoints are receptor-based signal cascades that result in negative regulation of T cells and, therefore, immune tolerance. This leads to immune surveillance escape and tumor evasion. Immune escape has been acknowledged as a hallmark of neoplastic proliferation. Inhibition of immune checkpoints such as cytotoxic T lymphocyte antigen 4 (CTLA-4), programmed death-1 (PD-1), and programmed death-ligand 1 (PD-L1), enhances T cell activation and tumor-associated antigens recognition enabling the lysis of tumor cells and cancer regression. Immune checkpoint molecules, such as CTLA-4 and PD-1/PD-L1, act at different stages of T-cell activation and lead to a dampened T-cell response [13,33,34].

Checkpoint inhibition's goal is to use monoclonal antibodies against checkpoint molecules to enable the pre-existing anti-tumor T cell response. To this end, the monoclonal antibodies are used to target the most prominent immune checkpoints, PD-1, PD-L1, and CTLA-4.

2. Immune System in Acute Myeloid Leukemia

Nowadays, the immune system has a significant role in tumor cell elimination. In AML, a common problem is tumor cell immune escape, being an effective survival mechanism [35,36]. AML blast cells have the capacity to block immune system communication and weaken T-cells, namely in their cytotoxic activity. The immune escape of AML blast cells in bone marrow can occur due to the burying of the malignant cells in the immune system or influencing negatively the different immune cells [37,38].

T-cells can be classified into subgroups, such as T-cell cytotoxic, T cell helper, and Regulatory T-cells (Tregs). This last one can reduce inflammatory reactions, by secreting anti-inflammatory cytokines as well as suppressing cytotoxic T-cells activity [35,39]. Therefore, Tregs' may provide protection to AML cells from the immune system. AML blasts express programmed death ligand 1 (PD-L1) as an immune checkpoint inhibitor, and produce indolamine-2,3-dioxygenase (IDO) and reactive oxygen species, which in-

duce differentiation towards Tregs, facilitating disease progression [39–42]. On the other hand, T-cell anergy can also be induced through T-cell immunoglobulin and mucin domain 3 (TIM3), which binds to and is activated by galectin-9, which is highly expressed in AML blasts [43]. Another suppressive molecule is an inducible T-cell co-stimulator ligand (ICOSL) and IDO's that contribute to Tregs expansion and an immunosuppressive environment and at the same time restrict cytotoxic activity [36,44,45]. High expression levels of PD1/PD-L1 and TIM3 are correlated with a worse prognosis in AML [36,46].

Besides decreasing T-cell cytotoxic activity, T-cell exhaustion is also described as related to significant changes in the AML microenvironment metabolically, which is described as being rich in glutamine and poor in arginine [47,48]. Another white cell subset, the B cells have not been studied profoundly; although, a recent study showed that memory B-cells have a negative impact on survival, while increased numbers of naïve B-cells seem to have a positive impact [49].

AML blasts can modify the function of the innate immune system cells, namely, macrophages and Natural Killer (NK) cells, downregulating surface molecules required for NK cells recognition through receptor natural killer group 2 member D (NKG2D), and issue altered NKG2D-ligands, therefore contributing to the reduced cytotoxic activity of NK cells; one more mechanism to evade NK-cell recognition is the interferon α (IFN-α) reduces NKG2D by AML blasts, and in this way escaping destruction by nu NK cells [50–53].

Moreover, AML blasts can promote macrophages' shift toward M2 polarization (immunosuppressive profile), associated with the promotion of tissue repair and angiogenesis [32,54].

Myeloid-derived suppressor cells (MDSC) are also influenced by AML blast cells, inducing T-cell inactivity through numerous mechanisms from PD-L1 expression to cytokines secretion such as interleukin (IL)-10 and/or tumor growth factor- β (TGF-β). Jointly with Tregs and M2-macrophages, MDSC has high numbers in AML patients [55], and, consequently, seems to be a risk factor for disease progression [56]. Ultimately, AML blasts seem to have a defective antigen-presentation, consequently downregulating the expression of human leukocyte antigens (HLA), assisting to make them unseen to the immune cells [57].

3. Acute Myeloid Leukemia Therapy

3.1. Chemotherapy

Chemotherapy is still the standard therapy used for AML. Tumor cell death is caused not only by cytostatic effects but also by the restimulation of the immune surveillance with a direct immune response towards tumor cells [58,59].

The immune response against cancer cells is increased through various processes, from enhancing antigen uptake and chemotactic response via macrophages and dendritic cells to augmenting recognition to the immune system and increasing tumor susceptibility to immune-mediated cytotoxicity [58–60].

Provoking an immune response with chemotherapy is crucial to inducing immunogenic cell death (ICD) rather than a non-immunogenic cell death (non-ICD), also known as apoptosis. To achieve this, therapy should direct to a pre-apoptotic exposure at the cell surface of calreticulin (CRT), secretion of ATP during the blebbing phase of apoptosis, and the cell death-associated release of the non-histone chromatin protein high-mobility group box 1 (HMGB1) [61]. As AML patients present a spontaneous exposure of CRT in tumor cells, this predicts an antitumor T cell response and improves patient survival [58].

However, AML is a heterogeneous and aggressive disease that may show an initial response to chemotherapy, but if not completely eradicated becomes progressively more resistant and relapses [14,62]. To overcome this adaptive resistance and reduce the risk of relapse, the combination of immune checkpoint inhibitors with traditional chemotherapy is currently being experimented [58].

3.2. Allogeneic Stem Cell Transplantation

Graft-Versus-Leukemia Effect and Graft-Versus-Host Disease

For patients with AML, the cure depends not only on the intensity of the conditioning treatment given before the transplantation but also on the immune-mediated graft-versus-leukemia (GvL) effect [63]. It is currently established that the donor immune system can mediate an effective GvL effect in many hematological malignancies [64,65].

In an allogeneic hematopoietic stem cell transplantation, the immune system of the donor aims to eliminate the residual leukemia cells that persist after prior (radio)chemotherapy. This immune-mediated response is acknowledged as graft-versus-leukemia, which represents a positive response in treating cancer. Donor T cells recognize cancer cells through the binding of T cell receptors to major histocompatibility (MHC) molecules present on the surface of cancer cells. MHC is known as human leukocyte antigens (HLA), so the donor is chosen based on the matching of HLA alleles, reducing graft rejection [63,66].

On the other hand, donor alloimmune response can target patients' healthy tissues, a response known as graft-versus-host disease (GvHD). GvHD is a multi-system disorder that commonly initially affects the skin, then the gastrointestinal tract, liver, and lungs, and in a later stage, can affect almost any organ. GvHD and GvL, regularly happen, however, do not always occur simultaneously, suggesting that is possible to promote GvL without GvHD [63,65].

This systematic review aims to study the immune checkpoint inhibitors, anti-CTLA-4 and anti-PD-1/PD-L1 as a treatment for acute myeloid patients who either suffered relapsed disease or are not considered for allo-hematopoietic stem cell transplantation (HSCT). The study of these cases, as monotherapy or as combined therapy with chemotherapy, concerning efficacy and the patient output.

3.3. Immunotherapy

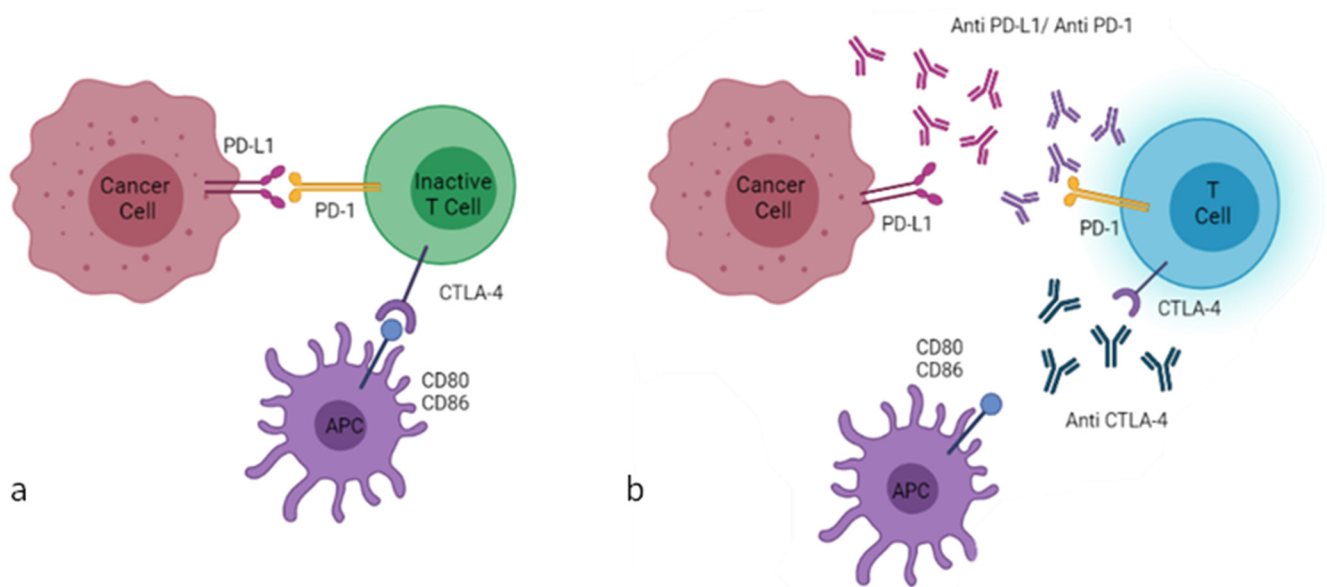3.3.1. Cytotoxic T Lymphocyte Antigen 4

CTLA-4, also known as CD152, is a protein encoded by the 4-exon CTLA4 gene, and a well-known co-inhibitory receptor expressed mostly on regulatory T cells (Treg) and activated CD4+ and CD8+ T cells. CTLA-4 is constitutively expressed in regulatory T cells, and in naïve resting T cells; it resides in the cytoplasm, until activation, when within 1 or 2 days, starts expressing on the surface. An even faster activation and expression are observed in memory T cells [60,67,68].

Although their functions are opposite, CLTA-4 and CD28, also expressed on T cells, share the same ligand, CD80 and CD86 (B7-1 and B7-2), which are expressed on antigen-presenting cells (APC). Nevertheless, both ligands link to CTLA-4, rather than CD28, with greater affinity, which causes inhibition, instead of stimulation of T cells, as shown in Figure 2. During an immune response, T cells present antigens to APC, CD80/CD86 binds to CD28 on CD4+ T cells, and these polarize to T helper 1 (Th1) cells. Th1 cells produce interferon-gamma (IFN-γ), tumor necrotic factor-alpha (TNF-α), and interleukin (IL)-2), which promote CD8+ T cells proliferation. When CTLA-4 binds APC to the IFN-γ, TNF-α and IL-2 production are reduced, and T-cell proliferation is suppressed. Tumors suppress CD4+ T cells mostly by upregulating the expression of CTLA-4. The blockade of CTLA-4 with anti-CTLA-4 monoclonal antibodies has become a great immunotherapeutic strategy for treating cancers [13,33,34,61].

3.3.2. Programmed Death-1 and Programmed Death-Ligand 1

PD-1, also known as CD279, is a surface glycoprotein encoded by the *Programmed cell death protein 1* gene. PD-1 and its ligand, PD-L1, are a vital pair of immune checkpoints. PD-1 is an inhibitory receptor expressed on effector T cells, naïve and B cells, natural killer cells, myeloid dendritic cells, and monocytes with low intensity, and plays a role in inhibiting T cell activation and effector function [13,69]. PD-1 is not present in resting T cells; however, after activation, expression can be induced within 24 h. PD-L1 is encoded

by the CD274 gene, and in non-pathologic lymphoid tissue is expressed in follicular T cells, macrophages, and a subset of dendritic cells [13,34].

Figure 2. Immune checkpoints, cytotoxic T lymphocyte antigen 4 (CTLA-4), and programmed death-1 (PD-1), interacting with the correspondent ligands in antigen-presenting cell and cancer cell, respectively: (**a**) The binding of PD-1, expressed in T cells to its ligand, programmed death-ligand 1 (PD-L1), expressed by cancerous cells, leads to the inactivation of this immune cell, representing a mechanism of evading the immune system. The binding of CTLA-4 to its ligand, CD80/CD86, in antigen-presenting cells (APC), also promotes the inactivation of the immune system, as part of the mechanism to inhibit an overactive response. (**b**) Immune checkpoint inhibitors prevent these interactions with antibodies, anti-PD-1/anti-PD-L1 and anti-CTLA-4, that link to the immune checkpoints and inhibits T cells to be inactivated.

The binding of PD-1 to PD-L1 negatively regulates both IL-2 production and T cell proliferation. This inhibitory function is a mechanism for preventing autoimmunity when APC presents self-antigens. On the other hand, tumor cells also express PD-L1, and this interaction results in the inactivation of T cells and the escape of leukemic cells, evading the immune system, as shown in Figure 2. This inhibition contributes to their long-term persistence [33,34,69,70]. Anti-PD-1/PD-L1 antibodies aim at re-establishing T cell-mediated antitumor immunity [69–71].

4. Materials and Methods

We aimed to conduct a systematic review of the use of immunotherapy to treat AML, specifically, the immune checkpoint blockade. The main goal was to compile and process information not only about this immunotherapy option provided nowadays but also about the treatment output associated with each or in combination. To achieve this goal, literature research of relevant studies was accomplished through the chosen database, PubMed. In this database, the keywords (Medical Subject Headings (MeSH) terms) used for the research were "acute myeloid leukemia," "treatment," "Immune Checkpoint", and "PD-1/PD-L1" and "CTLA-4". The strategy combination used for literature research was performed as shown in Table 2.

The setting of the inclusion and exclusion criteria helped filter the search in terms of availability, article type, and publication date. For this review, we considered free full-text and full-text articles published within the last 7 years. Only articles written in English were considered. The eligible studies included clinical trials, meta-analyses, randomized controlled trials, reviews, and systematic reviews, fulfilling the criteria above explained.

This review was performed following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [72].

Table 2. Strategy used for the combination Keywords (Medical Subject Headings terms) for the literature research of articles to include in the systematic review.

Medical Subject Headings Terms	AND	OR	NOT
Acute myeloid leukemia	Immune Checkpoint		
Acute myeloid leukemia	Treatment		
Acute myeloid leukemia	Treatment	PD-1/PD-L1	CTLA-4
Acute myeloid leukemia	Treatment	CTLA-4	PD-1/PD-L1
Acute myeloid leukemia	Treatment, PD-1/PD-L1, CTLA-4		

Legend: PD-1—programmed death-1; PD-L1—programmed death-Ligand 1; CTLA-4—cytotoxic T lymphocyte antigen 4.

The investigation strategies applied in this systematic review are detailed in Figure 3, and all the sources are accordingly referenced.
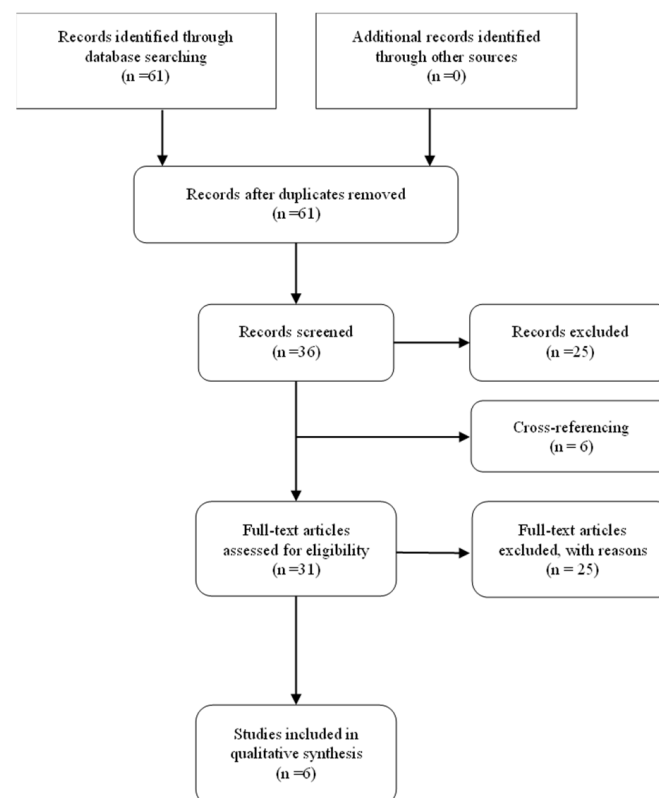
Figure 3. PRISMA Flow chart. Prism of the systematic review, where the searches are detailed in the database, the number of articles selected based on the abstract, full-text articles evaluated for eligibility, articles assessed by cross-referencing, and the total number of articles included in the review.

5. Results

The results of the clinical trials of immune checkpoint inhibitors, as monotherapy and combined therapy with chemotherapy, in acute myeloid leukemia from the last seven years, included in this study, are summarized in Table 3.

A study from 2016, by Matthew S. Davids et al., regarding the blockade of CTLA-4, with Ipilimumab, appears to be effective in relapsed AML patients after an allogeneic stem cell transplant. It was hypothesized to restore the antitumor activity through a GVL in

phase I/Ib study with ipilimumab. In this study, patients with relapsed hematologic cancer post-allo-HSCT received an intravenous dose of 3 or 10 mg per kilogram of body weight every 3 weeks for 4 cycles, followed by maintenance dosing in 12-week increments, for up to a year. The study integrated a total of 28 patients, 12 of whom had relapsed AML. Ipilimumab, at 10 mg/kg, showed promising responses, CR, in 5 (42%) of the 12 AML patients, 4 with extramedullary leukemia, and 1 with secondary AML, with 3 responses lasting over a year. Among responders, the decrease in regulatory T cells and increase in effector T cells were noted, suggesting an increase in GvL activity [73,74].

In 2021, Patrick K. Reville et al. studied the blockage of PD-1 in a pilot phase II clinical trial that used nivolumab as an immune checkpoint inhibitor that was used as maintenance therapy in high-risk AML, studying its efficacy and safety. Patients included in this study suffered from high-risk AML in remission, ineligible for allo-HSCT. Patients must have already received induction therapy and at least one cycle of consolidation therapy. Additionally, patients should have achieved a CR within 12 months. In the present study, the 15 patients received a 3 mg/kg dose of nivolumab, intravenously, every 2 weeks. In the non-appearance of disease progression, the cycles were repeated every 28 days. After cycle 6, every 4 weeks, and after cycle 12, every 3 months, until the disease relapse. With a median of six cycles of therapy and a median follow-up of 30.4 months, the estimated 6-month recurrence-free survival (RFS) was 57.1% and the median RFS was 8.48 months. The median overall survival was not yet reached. MRD was priorly assessed by multicolor flow cytometry. Nine patients were MRD positive at the time of enrolment, and seven of the nine continued to have detectable MRD with progressive disease, until disease recurrence [75].

Another study concerning Nivolumab, completed in 2020, by Matthew S. Davids et al., was performed on relapsed hematologic malignancies after allogeneic transplantation. This multicenter phase I study aimed to determine the maximum tolerated dose and safety, as well as assess the efficacy and immunologic activity. Every 2 weeks, Nivolumab was administered until either progression or unacceptable toxicity. The starting dose level was 1 mg/kg with possible de-escalation to 0.5 mg/kg or escalation to 3 mg/kg. Of the 28 patients, 19 suffered from a myeloid hematologic malignancy, 10 of which with AML. With a median follow-up of 11 months, the 1-year progression-free survival was 23% and overall survival was 56%. Regarding efficacy, 25 patients were evaluable for response and the other 3 were unevaluable due to early toxicity. From the 25, 5 were treated at 1 mg/kg and 20 were treated at 0,5 mg/kg. The overall response rate (ORR) for the efficacy on both levels was 32%. The median OS in all patients was 21.4 months, with a 1-year OS in all patients of 56% [76,77].

A 2017 three-case study, by Albright et al., reports the use of PD-1 checkpoint blockade, involving nivolumab administration, in patients with relapsed AML after allo-HSCT. Patient 1 is 61 years old, male, diagnosed with AML. Chemotherapy was performed and achieved CR for about 3 years, before relapsing. The patient received a single dose of 100 mg of nivolumab, and a few weeks after the administration developed marked pancytopenia and skin graft-versus-host-disease. Within 50 days of the administration, the marrow blast count declined to 5%. Ten months after the treatment the patient was in CR, in good performance status. Patient 2 is a 44-year-old female who was diagnosed with therapy-associated AML. The patient relapsed 8 months after the allo-HSCT and suffered a second allo-HSCT, resulting in a complete molecular remission, but a second relapse in over 2 years occurred. The patient received five low-dose nivolumab infusions (0.3–1 mg/kg) with resultant molecular disease stabilization for 6 months. Patient 3 is a 50-year-old male, diagnosed with AML, who received two nivolumab injections of 100 mg, on days 157 and 171 after the second allo-HSCT. However, these failed to show objective responses, the patient then received a haploidentical transplant and remains in incomplete molecular remission [13,21].

Table 3. Studies of immune checkpoint inhibitors cytotoxic T lymphocyte antigen 4 and programmed cell death protein 1 in monotherapy or combination with chemotherapy in acute myeloid leukemia were selected for this literature review.

Study	Antigen Target	Therapy	Patients	Results	Ref.
Davids MS et al., 2016	CTLA4	Ipilimumab	28 patients (12 AML) relapsed hematologic malignancies after allo-HSCT	GvHD in 14% of the patients; No response at 3 mg/kg; From 22 patients who received 10 mg/kg, 23% CR, and 27% decreased tumor burden; 5 of the 12 AML patients with CR, 4 having extramedullary AML.	[73]
Reville K. Patrick et al., 2021	PD-1	Nivolumab	15 patients with high-risk AML	6-month RFS is 57.1% and median RFS is 8.48 months;	[75]
Davids et al., 2020	PD-1	Nivolumab	28 patients (10 AML) with relapsed hematologic malignancies, post-allo-HSCT	3 early toxicities; 5 treated at 1 mg/kg/20 treated at 0.5 mg/kg; OS 56%. PFS 23%.	[76]
Albring et al., 2017	PD-1	Nivolumab	3 case studies in patients with relapsed AML after allo-SCT and failing standard therapy	2 of the 3 patients, previously relapsed are in CR; the other patient was in molecular stabilization for 6 months.	[21]
Ravandi et al., 2019	PD-1	Nivolumab + Idarubicin + Cytarabine	44 patients with newly diagnosed AML and high-risk MDS	Median OS was 18 months; 6 patients with immune-related adverse events; 43% achieved response and proceeded to allo-HSCT.	[78]
Oran B et al., 2020	PD-1 CTLA-4	Nivolumab Ipilimumab +Cytarabine +Idarubicin	43 AML and MDS patients treated with checkpoint blockers before allo-HSCT	34 with PD-1 blockade; 9 with CTLA-4 blockade; 24 patients CR, 6 patients CR with incomplete hematologic recovery; 5 patients showed hematologic improvement and 1 patient partial response.	[79]

Legend: CTLA-4—cytotoxic T lymphocyte antigen 4; AML—acute myeloid leukemia; allo-HSCT—allogenic hematopoietic stem cell transplantation; GvHD—graft-versus-host disease; CR—complete remission; PD-1—programmed death-1 RFS—recurrence-free survival; OS—overall survival; PFS—progression-free survival; MDS—myelodysplastic syndrome.

A single-arm, phase 2 study, combined the use of checkpoint blockers and chemotherapeutic agents. Farhad Ravandi et al. combined Nivolumab with idarubicin and cytarabine to study patients newly diagnosed with AML; 42 of the 44 enrolled patients, and the other 2 patients, had high-risk myelodysplastic syndrome. Induction therapy on the first 4 days included continuous infusions of 1.5 g/m2 cytarabine by 24 h, and 12 mg/m2 idarubicin on the first 3 days. The administration of nivolumab started on day 24, 3 mg/kg, and in responders, continued every 2 weeks for up to a year. Patients showing responses received either up to five consolidations cycles of decreased doses of idarubicin and cytarabine, or allo-HSCT if eligible. The 19 patients who received allo-HSCT had an overall survival of 25 months, whereas the median overall survival was 18.54 months. The 16 responding patients who continued idarubicin plus cytarabine and nivolumab beyond remission had a similar overall survival but tended to have worse relapse-free survival. Thirty-four patients achieved complete response, with complete or incomplete platelet recovery, and 53% of these achieved undetectable minimal residual disease [78,80,81].

Betül Oran et al. performed a clinical trial, completed in 2020, in patients with AML and myelodysplastic syndrome (MDS) who were treated with either PD-1 or CTLA-4, or

both, before an allo-HSCT, to describe transplantations outcome in what concerns GvHD and the impact of different GvHD prophylaxis approaches. Of the 43 patients, 34 received PD-1 blockade with nivolumab, and 9 received ipilimumab CTLA-4 blockade. Of the patients treated with nivolumab, 2 received it as monotherapy, 19 in combination with chemotherapy, and the rest, in combination with a hypomethylating agent. As for patients treated with ipilimumab, three were as a single agent and six in combination with the same hypomethylating agent (5-azacytidine). Among the 43 patients, 24 achieved CR, 6 achieved CR with incomplete hematologic recovery, 5 showed hematologic improvement and 1 achieved a partial response with a PD-1 inhibitor-based and CTLA-4 inhibitor-based therapies, with 41 days as the median time to best response. Of these patients, 27 suspended the checkpoint therapy and proceeded immediately to allo-HSCT at the time of their best response. The median time to undergo HSCT was within 155 days and 353 days, after the first administration of checkpoint inhibitors. GvHD prophylaxis consisted of tacrolimus and mini methotrexate with or without antithymocyte globulin in 21 patients, or post-HSCT cyclophosphamide (PTCy), or tacrolimus with or without mycophenolate mofetil, in 22 patients. The selection of prophylaxis for the patients was based on donor type. Both baseline disease and transplantation characteristics were comparable between PTCy patients and no-PTCy patients, 32% and 10%, respectively [79,82].

Other trials for AML are still recruiting or not yet completed regarding monotherapy trials with Nivolumab (NCT02275533; NCT02532231) and Pembroluzimab, an anti-PD-1 blocker (NCT02708641; NCT03286114; NCT02981914), and with the combination of checkpoint inhibitors with chemotherapy (NCT02768792) in relapsed AML [79,81].

6. Discussion

Checkpoint inhibition is a breakthrough in the treatment of AML patients [76]. The evolution in the molecular understanding of AML and in tumor immunology allows the targeting of AML with immunotherapeutic strategies such as immune checkpoint inhibitors [66]. The clinical application of checkpoint blockers has achieved modest results both as a monotherapy and when combined with chemotherapy [73,74].

Relapsed hematological diseases and post-allo-HSCT have limited treatment options. CTLA-4 blockade with ipilimumab showed clinically noteworthy remissions in patients with recurrent cancer after transplantation. No objective responses were achieved at the 3 mg/kg dosage; however, 32% who received the 10 mg/kg had a response; this accomplishment suggests that the antibody dosage may be significant after transplantation. Five patients had a complete remission and four patients who had a response maintained a remission lasting over a year. This observation suggests that CTLA-4 blockade may be effective post-allo-HSCT. Immune-related adverse events associated with ipilimumab were observed in a few patients (six/total of the patients in the study). Once the study involved patients three or more months after transplantation, it was not possible to conclude the safety of ipilimumab in the early post-transplantation period. Outlining this study, CTLA-4 blockade was an advantageous approach for the treatment of these patients, presenting durable complete remissions, even in patients with refractory myeloid cancers [73].

For high-risk AML patients not eligible for allo-HSCT, nivolumab treatment presented a recurrence-free survival similar to the mean values observed in the literature. However, a promising overall survival was observed in these patients. Furthermore, it also showed a significant effect in eradicating MRD and extending remissions, as a single agent. Nevertheless, immune-related adverse events occurred recurrently. In this setting, the provided data do not support the use of single-agent nivolumab; although, it offers background and viability for the incorporation of an immune checkpoint blockade in combination trials, as maintenance therapy for high-risk AML patients. The most adequate post-remission therapy in high-risk AML continues to be allo-HSCT [75].

The first nivolumab prospective trial for post-allo-HSCT for relapsed hematological malignancies identified the maximum tolerated dose (MTD) as a low dose of 0.5 mg/kg. PD-1 blockade involves a risk of inducing immune-related adverse events and GvHD,

as observed in 39% of the patients in this study, with two fatal patients, despite the low doses of nivolumab [76]. Not only the induced immune-related adverse events but also the demand for managing these toxicities is similar to other retrospective studies performed previously [82,83]. In this trial, patients enrolled with different periods post-transplantation, so was possible to observe that a shorter interval between transplantation and the first infusion with nivolumab was associated with an increased chance of developing GvHD. This study was heterogeneous, which enabled the observation of toxicity patterns and efficacy across the different diseases. Antitumor activity observed showed an ORR in myeloid malignancies of 21%, less activity when compared to lymphoid malignancies (ORR 44%) [76].

Multiple case studies reported three cases of AML patients. The patients who suffered from a relapse of the disease after allo-HSCT, failing standard therapy, were treated with PD-1 inhibitors through nivolumab administration at different doses. The responses achieved were a complete response, one stabilized disease and one failed to show a demonstrable response. In this study, it was observed that PD-1 inhibitors may be highly effective in the relapsed setting, after allo-HSCT, at the expense of a higher degree of GvHD [13,21,84].

The combination of nivolumab with idarubicin plus high dose cytarabine, both chemotherapeutic agents, as the frontline therapy in AML patients and high-risk myelodysplastic syndromes (MDS), led to objective responses in 80% of the patients, 64% of which CR. It was hypothesized that the induction of an antileukemic immune response through nivolumab would be efficient in increasing the durability of the response, or even that nivolumab could eliminate minimal residual disease. In this trial, it was acknowledged that the use of nivolumab did not play a role in increasing the incidence of complications after transplantation, despite the use of multiple donor sources and conditioning regimens. This trial's outcome seems promising, considering that the median overall survival and event-free survival were not reached at the time of the analysis of the data. Furthermore, the patients who continued with the therapy beyond remission had an identical overall survival and event-free survival when compared with the patients that progressed to allo-HSCT, implying that nivolumab may have the efficacy to restore the host antitumor immune surveillance. Although the study is limited by a small sample size or a short follow-up, the inclusion of nivolumab to idarubicin and cytarabine induction therapy is beneficial and safe in younger patients with AML [78].

A retrospective analysis concerning 43 patients [79] with AML and/or MDS who were previously treated with checkpoint inhibitors, before HSCT, established that the checkpoint blockade had indeed an impact on transplantation outcomes. The GvHD prophylaxis PTCy was used to deplete alloreactive T cells, and a protective effect of the PCTy on more serious GvHD was observed. Nevertheless, this may not be significant if the transplant was in HLA-matched unrelated donors, because there are no such cases observed. In this trial, it was demonstrated that the prior use of checkpoint inhibitors could be considered within the donor/hematopoietic stem cell selection and the GvHD prophylaxis for patients before HSCT. Both the overall survival and the profession-free survival were higher in patients treated with PTCy when compared with those treated with no-PTCy, with a 1-year overall survival of 81% and 34% and progression-free survival of 56% and 26%, respectively [79]. Figure 4 summarizes the main treatment options for AML, as well as the immune checkpoint inhibitors' action mechanisms.
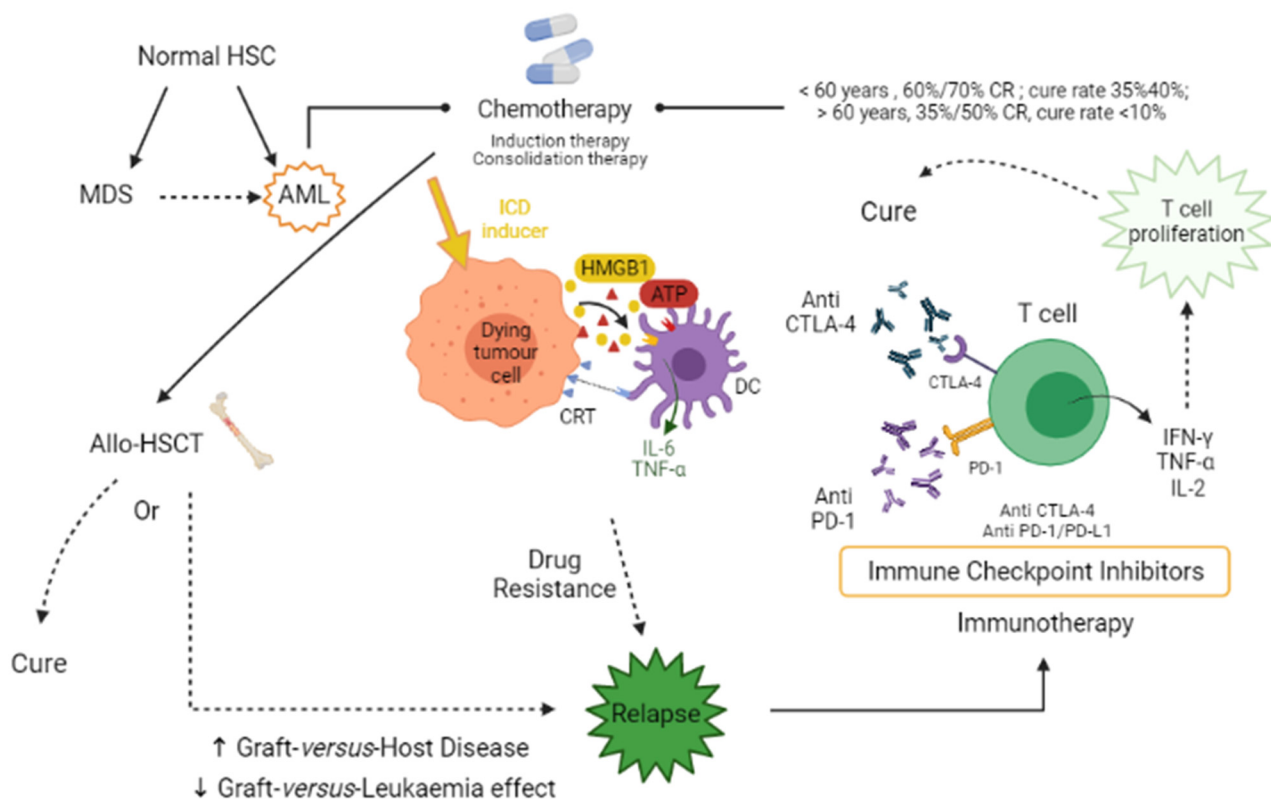
Figure 4. The treatment for acute myeloid Leukemia (AML) is chemotherapy followed by an allogenic hematopoietic stem cell transplant (allo-HSCT). Even though this is considered standard treatment, it presents low rates of complete remission, and even lower rates of cure, especially in patients over 60 years. This can be due to long-term resistance to immunogenic cell death (ICD) inducers, chemotherapeutic agents, or due to a graft rejection after allo-HSCT. ICD is characterized by an endoplasmic reticulum stress response on the dying tumor cell that results in the exposure of calreticulin (CRT) on its surface membrane and the release of chromatin protein high-mobility group box 1 (HMGB1) and ATP. These molecules interact with dendritic cells (DC) and result in DCs maturation and interleukin 6 (IL-6) and tumor necrotic factor-alpha (TNF-α) release, increasing anti-tumor response. However, due to tumor mutational burden, or, either deficiency of essential factors for ICD or expression factors that confer resistance to ICD, drug resistance may develop resulting in unresponsiveness to immunogenic actions. As for graft rejection, the installment of a prominent graft-versus-host disease rather than a graft–leukemia effect leads to relapse of the disease. To evade these events, immune checkpoint versus inhibition, using monoclonal antibodies, boosts the immune response by blocking the negative-response receptors, enabling the release of interferon-gamma (INF-γ), TNF-α, and IL-2. These stimulate T cell activation, therefore, an anti-tumor response. (Picture by Margarida Silva.).

7. Conclusions

AML treatment with monotherapy, with Ipilimumab, in relapsed hematologic malignancies, after allo-HSCT, is a worthy therapeutic approach due to the high percentage of complete remissions observed (42%). The treatment of patients in the same clinical circumstances, with nivolumab, even at a low dosage, showed significant immune-mediated toxicities in myeloid malignancies. Further investigation of this checkpoint blocker, in this setting could evaluate better strategies to decrease toxicity and increase clinical benefit. The use of nivolumab, as single-agent maintenance therapy in high-risk AML presented an interesting overall survival in these patients; however, the recurrence-free survival duration was shorter than the described results in the literature. These results, do not encourage the use of nivolumab as a single agent, in this setting; nevertheless, these data suggest that the use of checkpoint blockers in combination therapy may provide a better

outcome. In combination therapy, with chemotherapeutic agents, the use of nivolumab in newly diagnosed AML or high-risk MDS is feasible and safe in younger patients with AML and 80% of the patients presented objective responses. Meanwhile, the approach of AML and MDS patients with checkpoint blockage before allo-HSCT and the use of a GvHD prophylaxis has shown improvement in the transplantation outcomes, verifying a comparable difference between the use of PCTy and no-PCTy as prophylaxis.

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