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LEUKEMIA (A AGUAYO, SECTION EDITOR)

Immune Checkpoint Inhibitors in Acute Myeloid Leukemia: Novel Combinations and Therapeutic Targets

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Abstract

Purpose of Review Immune checkpoint therapy has dramatically changed the therapeutic landscape of solid malignancies. Here, we review the scientific rationale and current data evaluating immune checkpoint inhibitors in acute myeloid leukemia (AML).

Recent Findings Immune checkpoint inhibitor monotherapy has shown limited clinical activity in AML. Initial results from early-phase clinical trials suggest that rational combinations of immune checkpoint inhibition with hypomethylating agents (HMAs) are safe and potentially more promising. There are currently no data directly comparing immune checkpoint inhibition to standard therapies. Emerging immune targets more specific for leukemia cells including LILRB4 may improve future therapeutic efficacy.

Summary The success of immune checkpoint inhibition in AML has been modest to date. However, an improved understanding of the biology and the use of rational combinations has potential to improve rates of durable responses. Multiple clinical trials in AML are currently evaluating the use of immune checkpoints alone and in combination.

Keywords Acute myeloid leukemia · Immune checkpoints · Immunotherapy · Azacitidine · Decitabine · Pembrolizumab · Ipilimumab · Nivolumab · PD-1 · PD-L1 · CTLA-4 · LILRB4

Introduction

Acute myeloid leukemia (AML) is characterized by uncontrolled proliferation of myeloid progenitor cells, which have lost the ability to differentiate, resulting in the decreased production of normal blood cells [1]. AML is more common in older adults, with a median age of 67 and one-third of patients older than 75 years at time of diagnosis. [2] In patients who are younger than 60 years, rates of complete remission (CR) with chemotherapy are about 60% to 70%. However, cure rates remain low, around 35% to 40% [3]. Furthermore, older adults and patients with adverse cytogenetic risk have much lower CR rates of 35% to 50% and cure rates of 10% or less, with lower response rates attributed to both the inability to tolerate intensive chemotherapy and higher rates of resistance to chemotherapy [3]. In addition, patients with AML and detectable minimal residual disease (MRD) after receiving chemotherapy or allogeneic stem cell transplantation (alloSCT) have poor outcomes [4–9]. Even alloSCT cannot rescue most adults with MRD, with one recent study demonstrating that undergoing alloSCT with MRD results in equivalently poor outcomes as alloSCT with active AML [4–8]. Despite the recent approval of several novel therapies including midostaurin, gemtuzumab ozogamicin, enasidenib, and ivosidenib, the therapeutic strategy for most fit AML patients remains intensive induction and consolidation chemotherapy and has not substantially changed in the last 40 years [3, 10–12]. There is an urgent need for both more effective and more tolerable therapies for patients with AML, in particular older adults and patients with poor-risk cytogenetics and MRD after initial therapy.

Immune escape has been recognized as a hallmark mechanism of neoplastic proliferation and immunotherapeutic drugs have recently been shown to improve survival in a variety of human cancers [13, 14]. Many of these drugs target inhibitory receptors and signaling pathways such as PD-1/PD-L1 and CTLA-4 that regulate immune cell activation in response to antigen stimulation, an approach described as immune checkpoint blockade [15–18]. However, with the exception of single-agent PD-1 blockade for relapsed/refractory Hodgkin

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lymphoma, and initial data for single-agent anti-CTLA-4 ipilimumab for post-transplant relapse, the development of checkpoint inhibitors in hematologic malignancies has lagged behind solid tumors [19–21, 22••]. The lower relapse rate associated with alloSCT and the ability of donor lymphocyte infusion (DLI) to rescue some patients who have relapsed following HSCT both demonstrate the powerful role the immune system plays by inducing a graft versus leukemia effect (GVL) arguing for a potential use of more targeted immunotherapy in AML [23, 24].

In this review, we discuss both the scientific rationale and the clinical trial evidence of immune checkpoint inhibition in AML (Tables 1 and 2). We highlight the use of immune checkpoint inhibitors both as monotherapy and as combination therapy with chemotherapy, epigenetic therapy and other forms of immunotherapy (Fig. 1). We present both trials with final or preliminary results (Table 1) and trials, which are currently recruiting patients (Table 2). Finally, we outline future directions of immune checkpoint inhibition–based therapy in AML.

Immune Checkpoint Inhibition as Monotherapy

Scientific Rationale/Preclinical Studies

The importance of the PD-1/PD-L1 pathway in immune evasion of acute myeloid leukemia has been demonstrated in several murine AML models whereby PD-L1 and PD-1 were upregulated in murine leukemia cells and PD-1 knockout- or antibody-mediated blockade suppressed in vivo leukemia cell proliferation and improved survival in AML bearing mice [29, 30]. Interestingly, while at baseline low levels of PD-L1 expression were found, expression of PD-L1 was significantly upregulated in response to IFN-γ in vitro and when grown in vivo suggesting an important role of the cytokine milieu within the microenvironment in stimulating PD-L1 expression. Similarly to murine models, the expression of immune checkpoints was only found to be modestly elevated on human AML cells at baseline but increased significantly, once AML cells were exposed to IFN-γ or chemotherapy and epigenetic therapy with hypomethylating agents (HMAs) as well as at the time of relapse either after chemotherapy or after bone marrow transplant [31, 32•, 33•, 34•].

Completed Clinical Trials

A phase I clinical trial examined the use of CT-011, a humanized antibody interacting with PD-1, in 17 patients with advanced hematologic malignancies including 8 patients with AML [34]. Only one of the 8 AML patients evaluated had a response to CT-011, experiencing a reduction in peripheral blasts (50% to 5%) after the first dose and remained platelet transfusion independent for 9 months (Table 1).

Another phase I study examined the safety and efficacy of ipilimumab at a dose of 3 or 10 mg per kilogram of body weight every 3 weeks for a total of 4 doses in 28 patients with relapsed hematologic malignancies after allogeneic HSCT including 12 patients with AML (3 patients with leukemia cutis and 1 with a myeloid sarcoma) [35]. Among the 22 patients who received 10 mg of ipilimumab per kilogram in the dose expansion cohort, dose-limiting toxic effects included chronic graft versus host disease (GVHD) of the liver (in 2 patients) and grade II acute GVHD of the gut (in 1 patients), which resolved with steroid administration. While no responses were seen in patients who received a dose of 3 mg per kilogram, at a dose of 10 mg per kilogram, a complete response was achieved in 4 patients with extramedullary AML (3 patients with leukemia cutis and 1 patient with myeloid sarcoma involving lymph nodes) and 1 patient with the myelodysplastic syndrome developing into AML (Table 1). Responses appeared to be durable in the responding AML patients with continued complete remission in, which lasted 12 and 15 months for two of the three patients with leukemia cutis and 16 months for the patient with AML secondary to MDS. Serial skin biopsies in one of the AML patients with leukemia cutis responding to ipilimumab showed increased CD8 T cell infiltration associated with increased cytotoxicity as shown by perforin granules staining at the tumor site in response to treatment, which was further conformed by upregulated CD8 and perforin gene expression. Additionally, responding patients had evidence of fewer CD4+ T regulatory (T_{reg}) cells and more CD4+ conventional T cells as well as an increase in several chemokines critical for leukocyte trafficking than patients with progressive disease in the peripheral blood after ipilimumab treatment compared to patients who did not respond to ipilimumab.

Ongoing Clinical Trials

Several ongoing clinical trials are currently examining the use of immune checkpoint inhibitor monotherapy in AML patients (Table 2).

Immune checkpoint inhibition is being studied both in patients with either primary treatment refractory disease or relapse after alloSCT (NCT03291353, NCT02981914, NCT03286114). Additionally, several studies examine the role of immune checkpoint inhibition at a time when most of the leukemia bulk has been eliminated by prior therapy and patients are in remission with the goal to reduce minimal residual disease (MRD) and prevent relapse (NCT02275533, NCT02532231). Furthermore, one trial studying the combination of pembrolizumab and autologous SCT in patients who are not eligible for an alloSCT is currently enrolling patients (NCT02771197).

Table 1 Selected completed trials of immune checkpoint inhibitor–based monotherapies and combination therapies

Author/year of reference	Phase	Intervention	Patient population	*N*	Outcomes
Monotherapy					
Berger et al. Clin Cancer Res 2008 [25]	I	CT-011	Advanced hematological malignancies	17 AML:8	1/8 patients with a minimal response (reduction of peripheral blasts from 50%v to 5%)
Davids et al. NEJM 2016 [22••]	I	Ipilimumab	Hematologic malignancies with relapse after alloSCT	28 AML:12	No response with 3 mg/kg but responses observed with 10 mg/kg CR in 4 pts. with extramedullary AML and 1 pt. with AML secondary to MDS
Combination with chemotherapy					
Ravandi et al. ASH 2017 [26]	II	Nivolumab + cytarabine/-idarubicin	AML/high-risk MDS upfront therapy	32 AML:30	CR/CRi 72% CR 59% CRi 13% 28% with subsequent alloSCT Median RFS and OS not reached (at median follow-up of 8.3 months)
Zeidner et al. ASH 2017 [27]	II	High-dose cytarabine followed by pembrolizumab (including maintenance pembrolizumab in case of response)	RR-AML	13	CR/CRi 40% CR 39% 15% with subsequent alloSCT
Combination with hypomethylating agents					
Daver et al. Cancer Discovery 2018 [28••]	II	Nivolumab + azacitidine	Relapsed AML	70	ORR 33% CR/CRi 22% HI 10% Median OS 6.3 months

CR complete response, *CRi* complete response with incomplete count recovery, *RFS* relapse-free survival, *OS* overall survival, *HI* hematologic improvement, *ORR* overall response rate = CR + CRi + PR + HI

Immune Checkpoint Inhibition as Combination Therapy

Combination with Chemotherapy

Scientific Rationale/Preclinical Studies

The combination of checkpoint inhibition with chemotherapy and targeted therapy is currently being tested in multiple trials in solid tumors. One successful example is the combination of nivolumab with platinum-based doublet chemotherapy in patients with non-small cell lung cancer [36, 37]. There is a sound scientific rationale for combining chemotherapy with immune checkpoint inhibition. Chemotherapy does not cause death of tumor cells by cytostatic effects alone but also by stimulating an immune response directed towards cancer cells by reinstating immune surveillance [38, 39]. Chemotherapy has been demonstrated to augment the immune response against cancer through multiple mechanisms including improved antigen uptake and chemotactic response by macrophages and dendritic cells, improved recognition of neo-epitopes over the MHC I and T cell receptor, and increased susceptibility of tumor cells to immune-mediated cytotoxicity [40, 41]. Key in eliciting an immune response to cancer cells with chemotherapy is the induction of an immunogenic cell death (ICD) rather than a non-immunogenic cell death (non-ICD)–like apoptosis [39]. In order to induce ICD, chemotherapeutic agents need to lead to the pre-apoptotic exposure of calreticulin (CRT) at the cell surface, the 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) [39]. In AML patients, the spontaneous exposure of CRT by leukemic cells has been shown to predict antitumor T cell responses and improved patient survival [42]. Furthermore, only a small selection of chemotherapeutic agents is able to induce ICD in cancer cells as shown when cancer cells were exposed to 24 different chemotherapeutic agents with only four agents (three anthracyclines and oxaliplatin) being able of inducing ICD [43]. It fact, anthracyclines, the backbone of 7 + 3 induction chemotherapy in AML, have been demonstrated to be particularly potent inducers of ICD [44]. Induction of ICD leads to cytotoxic T lymphocyte (CTLs)

Table 2 Selected ongoing clinical trials of immune checkpoint inhibitor–based monotherapies and combination therapies

Clinical trial number status	Phase	Intervention	Patient population
Monotherapy			
NCT03291353 Recruiting	I	Pembrolizumab	Refractory AML
NCT02981914 Recruiting	I	Pembrolizumab	Hematologic malignancies (including AML) relapsed after alloSCT
NCT03286114 Recruiting	I	Pembrolizumab	AML/MDS/ALL relapsed after alloSCT
NCT02708641 Recruiting	II	Pembrolizumab	AML post remission > 60 years who are not HCT candidates
NCT02275533 Recruiting	II	Nivolumab Arm I: Nivolumab Arm II: standard of care	AML in CR
NCT02532231 Recruiting	II	Nivolumab	AML in CR at high risk of relapse
NCT02771197 Recruiting	II	Pembrolizumab + autologous SCT	AML with high risk of relapse not eligible for alloSCT
Combination with chemotherapy			
NCT03417154 Recruiting	I/II	Nivolumab + cyclophosphamide	RR-AML and high-risk MDS
NCT02768792 Recruiting	II	Pembrolizumab + high-dose cytarabine	RR-AML
NCT02464657 Recruiting	I/II	Nivolumab +idarubicin/cytarabine (7 + 3) induction chemotherapy	AML and high-risk MDS
Combination with hypomethylating agents			
NCT02890329 Recruiting	I	Ipilimumab + decitabine	RR MDS/AML
NCT02996474 Active not recruiting		Pembrolizumab + decitabine	RR-AML
NCT02845297 Recruiting	II	Pembrolizumab + azacitidine	RR MDS/AML, AML > 65 years
NCT02397720 Recruiting	II	Arm I: Nivolumab + azacitidine Arm II: Nivolumab + azacitidine + ipilimumab	RR-AML, AML > 65 years
NCT02775903 Active not recruiting	II	Durvalumab + azactidine	High-risk MDS, AML > 65 years
Combination with other immunotherapies			
NCT01822509 Recruiting	I	Nivolumab + ipilimumab	Hematologic malignancies with relapse after SCT (including AML)
NCT02846376 Recruiting	I	Arm I: Nivolumab Arm II: Ipilimumab Arm III: Nivolumab + ipilimumab	AML after alloSCT
NCT03066648 Recruiting	I	anti-TIM-3 antibody MBG453 anti-PD-1 antibody PDR001 decitabine Arm I: PDR001 + decitabine Arm II: MBG453 + decitabine Arm III: PDR001 + MBG453 + decitabine Arm IV: MBG453 Arm V: PDR001 + MBG453	RR-AML/MDS, AML not eligible for chemotherapy
NCT01096602 Active not recruiting	II	Anti-PD-1 antibody pidilizumab + dendritic cell vaccine	AML in CR

proliferation and interferon-gamma (INF-γ) release, which has been shown to result in the proliferation of T cells but also an increased expression of PD-L1 on leukemic blats [45, 46]. It is conceivable that CTLs are limited in their ability to eradicate leukemic blasts because they are inactivated by an increased expression of PD-L1 on leukemic blasts resulting in therapy-resistant leukemia cells, which will eventually lead to a relapse of the disease (Fig. 1a). One potential strategy to overcome this adaptive resistance and reduce the risk of relapse and currently is examined in clinical trials is combining immune checkpoint inhibition to chemotherapy.

Completed Clinical Trials

Preliminary results for two clinical trials examining the use of immune checkpoint inhibition in combination with chemotherapy both in the upfront and relapsed and refractory setting have been presented in abstract from [26, 27] (Table 1).

In a phase II trial, nivolumab was combined with cytarabine and idarubicin for the treatment of 32 AML and high-risk MDS patients in the frontline setting [26]. Patients were treated with nivolumab 3 mg/kg, which was started 24 ± 2 days of induction chemotherapy and was continued every 2 weeks for up to a year. A complete response (CR) or complete response with incomplete count recovery (CRi) was achieved in 72% of patients and 28% of patients went on to receive an allogeneic stem cell transplant (Table 1). Of 32 patients treated with nivolumab, 5 patients developed grade 3 or 4 immune-mediated toxicities including rash, pancreatitis, and colitis. Of 9 patients, who received a subsequent alloSCT, 4 patients developed acute GVHD, which responded to treatment in in 3 patients. The authors also showed that there was a trend towards a higher frequency of total CD3+ T cell infiltration in the bone marrow prior to start of therapy in patients who responded to therapy compared to patients who did not respond to therapy.

In the relapsed-refractory setting, a phase II trial examined the combination of high-dose cytarabine followed by pembrolizumab [27]. Thirteen patients with relapsed-refractory AML received age-adjusted high-dose cytarabine followed by pembrolizumab 200 mg IV on day 14 of induction and in the case of a response went on to receive maintenance therapy with pembrolizumab 200 mg IV Q3 weeks for up to 2 years until relapse or progression of disease. The CR/CRi rate was 40%; however, the response was short lived with a median duration of CR of 3.9 months (Table 1). There was a total of 2 patients proceeding to alloSCT; both patients developed steroid-responsive acute GVHD of skin and one patient developed a transient increase in hepatic enzymes, which was responsive to steroids, while the other patient developed moderate chronic GVHD. Prior to treatment, peripheral blood CD8+ T cells from responding patients were found to have an increased diversity of the T cell receptor compared to non-responding patients.

Ongoing Clinical Trials

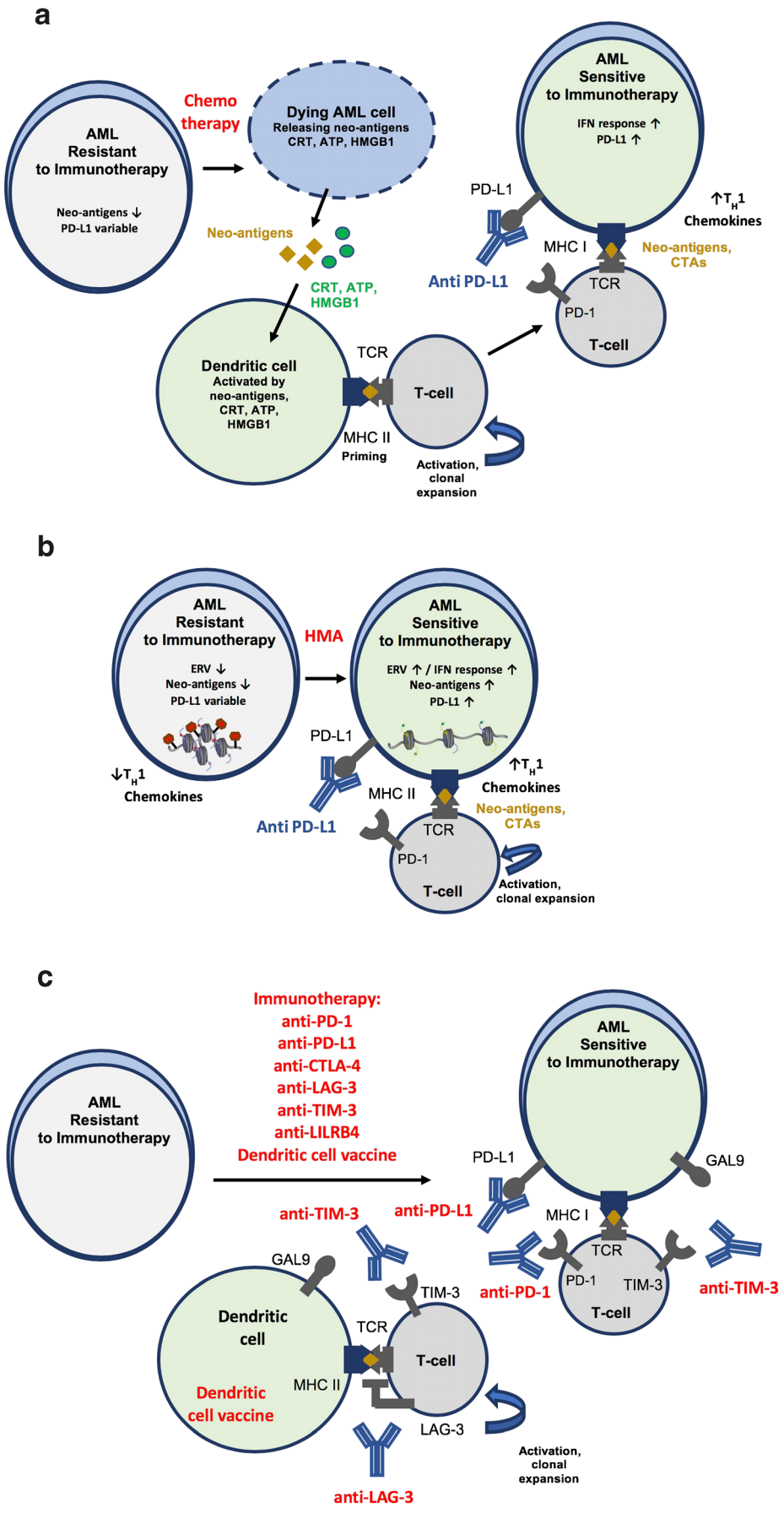
Several ongoing clinical trials are examining the combination of immune checkpoint inhibition with chemotherapy both in the relapsed and treatment refractory setting (NCT03417154, NCT02768792) as well as frontline therapy (NCT02464657).

Combination with HMAs

Scientific Rationale/Preclinical Studies

Preclinical data suggest that combining epigenetically targeted agents with immune checkpoint inhibitors in leukemia and other malignancies may enable synergistic activity and facilitate immunotherapy [47–52]. Potential rationales include upregulation of silenced leukemia neo-antigens and immune checkpoint proteins by HMAs, immunomodulatory effects of epigenetic agents on the immune system including reduction of myeloid-derived suppressor cells, and increased activity of Th1 chemokines that promote growth of cytotoxic T lymphocytes (CTLs) [48–51, 53–58]. HMAs and other epigenetic therapies upregulate a variety of genes, including previously silenced leukemia tumor-associated antigens and neo-antigens that could serve as targets for AML-specific immune responses. For example, treatment with azacitidine, the HDAC inhibitor valproic acid, or the novel HMA SGI-110 induces expression on AML cells of immunogenic cancer/testis antigens (CTAs) such as NY-ESO-1 and MAGE-A, and MAGE-specific cytotoxic T lymphocyte (CTL) activity has been associated with major clinical responses [53–55, 59]. On the other hand, inhibitory immune checkpoint proteins including PD-L1, PD-L2, PD-1, and CTLA-4 can also be upregulated by treatment with HMAs [56]. Treatment with HMAs may therefore both augment tumor-specific immune responses by upregulating neo-antigens and impair tumor-specific immune responses by upregulation of immune checkpoint proteins. However, such epigenetic drug-mediated immune checkpoint upregulation also provides a strong rationale to overcome tumor immune resistance by the use of a PD-1 or PD-L1 pathway inhibitor [56].

Animal models also provide a rationale for combination HMA and immune checkpoint inhibition. In a mouse model of metastatic breast cancer resistant to combined checkpoint blockade, co-treatment with azacitidine and the HDAC inhibitor entinostat in combination with immune checkpoint inhibitors markedly improved treatment outcomes, curing more than 80% of tumor-bearing mice [50]. Additional studies demonstrated that this combination reduced numbers of myeloid-derived suppressor cells (MDSCs), suggesting that immune-modulating effects of HMAs may potentially facilitate immunotherapy [50]. Recent preclinical data in multiple tumor types also show that HMAs upregulate endogenous retroviruses (ERVs), triggering a cellular viral defense program mediated


a
AML
Resistant
to Immunotherapy
Neo-antigens ↓
PD-L1 variable
Chemo
therapy
Dying AML cell
Releasing neo-antigens
CRT, ATP, HMGB1
Neo-antigens
CRT, ATP,
HMGB1
Dendritic cell
Activated by
neo-antigens,
CRT, ATP,
HMGB1
TCR
T-cell
MHC II
Priming
Activation,
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AML
Sensitive
to Immunotherapy
IFN response ↑
PD-L1 ↑
PD-L1
Anti PD-L1
MHC I
TCR
PD-1
T-cell
↑T_H1
Chemokines
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CTAs
b
AML
Resistant
to Immunotherapy
ERV ↓
Neo-antigens ↓
PD-L1 variable
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Chemokines
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Sensitive
to Immunotherapy
ERV ↑ / IFN response ↑
Neo-antigens ↑
PD-L1 ↑
PD-L1
Anti PD-L1
MHC II
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T-cell
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to Immunotherapy
Immunotherapy:
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anti-CTLA-4
anti-LAG-3
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TIM-3
anti-TIM-3
T-cell

◀ **Fig. 1** Facilitation of immune checkpoint inhibition by chemotherapy, epigenetic therapy, and other forms of immunotherapy-scientific rationale. **a** Immunomodulation by chemotherapy via induction of an immunogenic cell death of leukemic blasts resulting in antigen release and cross presentation of antigens by dendritic cells with a priming effect on cytotoxic T lymphocytes. Additionally, the release of calreticulin (CLR), (ATP), and HMGB-1 leads to effective activation of T lymphocytes. Interferon-gamma (INF-γ) release leads to T cell proliferation and activation but also increase in PD-L1 expression on leukemic blasts resulting in immune escape of the leukemic blasts. PD-L1 inhibition in combination with chemotherapy leads to an expansion of AML-specific T cell clones. **b** Immunomodulation by HMAs via upregulation of endogenous retroviruses (ERVs), interferon (IFN) response and viral defense pathways, neo-antigens, cancer-testis antigens (CTAs), Th1 chemokines, and PD-1/PD-L1, enabling PD-L1 inhibition, leading to expansion of AML-specific T cell clones. **c** Immunomodulation by multilevel immune checkpoint inhibition. Immune checkpoint inhibition targeted towards PD-1/PD-L1 and CTLA-4 can either be combined with each other or potentially augmented by dendritic cell vaccines and/or by antibodies blocking several other negative immune checkpoints including lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin and mucin-domain containing-3 (TIM-3). Leukocyte immunoglobulin–like receptor B4 (LILRB4) represents a novel target for immunotherapy in monocytic AML cells

by interferon-β, and strongly potentiating responses to checkpoint blockade in mouse models [48, 49, 58]. Recent data also demonstrate that epigenetic mechanisms including EZH2 H3K27 trimethylation and DNMT1-mediated DNA methylation repress tumor production of T helper 1 (TH1)-type chemokines CXCL9 and CXCL10, and treatment with epigenetic modulators including azacitidine removes this repression and increases effector T cell trafficking to the tumor microenvironment [60]. Clinical data in relapsed AML after transplant also recently showed that the combination of azacitidine and donor lymphocyte infusion could induce long-term remissions, further supporting the concept of HMA-mediated facilitation of tumor-specific immunity in AML [61].

Completed Clinical Trials

In a phase II trial, the combination of the PD-1 inhibitor nivolumab with the hypomethylating agent azacitidine was studied in 70 patients with relapsed AML [28••]. Patients were treated with azacitidine 75 mg/m^2 for 7 days in combination with nivolumab at a dose of 3 mg/kg on days 1 and 14 of each treatment cycle; treatment cycles were repeated every 4–5 weeks indefinitely. The overall response rate (ORR) was 33%; 58% in HMA naïve patients and 22% in HMA pretreated patients. Grade 3–4 immune-related side adverse effects were observed in 11% of patients.

Median overall survival was 6.3 months for all patients which per the author's institutional experience at MD Anderson Cancer Center compared favorably to historical survival with azacitidine-based salvage treatment in a similar patient population. Patients who achieved a response had a higher total CD3 T cell infiltrate in the BM pretherapy. A cut-off CD3+ T cells > 13.2% in pretherapy BM biopsies was found to have a sensitivity of 74% and a specificity of 65% for predicting response to therapy.

Ongoing Clinical Trials

Several ongoing clinical trials are dedicated to examining the combination of HMAs with immune checkpoint inhibition in both the relapsed or primary treatment refractory setting and in the upfront treatment setting for elderly patients not eligible for intensive induction chemotherapy (NCT02890329, NCT02845297, NCT02397720, NCT02996474, NCT02775903) (Table 2).

Combination with Immunotherapy

Scientific Rationale/Preclinical Studies

An improved understanding of the complexity of immune invasion in cancer has led to multiple new targets for immunotherapy in both solid and liquid malignancies [62]. Even in diseases such as melanoma, immune checkpoint inhibitors often have relatively low response rates as single agents that leave many patients without clinical benefit. One approach to improve response rates has been to combine immune checkpoint targeted towards PD-1/PD-L1 and CTLA-4, though at the cost of increased toxicity from immune-related adverse events [16]. Apart from CTLA-4, PD-1, and PD-L1, several other immune checkpoint molecules including lymphocyte activation gene-3 (LAG-3), T cell immunoglobulin, and mucin-domain containing-3 (TIM-3) have been described and inhibitory antibodies of these are currently being in clinical trial evaluation in a variety of solid tumors [63, 64]. Preclinical studies have shown that both LAG-3 and TIM-3 are promising targets in AML [65–67]. Additionally, correlative studies in a phase II clinical trial examining the effect of adding nivolumab to induction chemotherapy with cytarabine and idarubicin showed that non-responders had significantly higher percentage of CD4 T effector cells co-expressing the inhibitory markers PD1 and TIM3 and a trend towards higher percentage of CD4 T effector cells co-expressing PD1 and LAG3 compared to responders in their baseline bone marrow biopsy prior to treatment [26]. Future combination of anti-PD-1 therapy with anti TIM3 and LAG3 therapy is conceivable but will need to be balanced with a potential increase in immune-related side effects. Lastly, combining immune checkpoint inhibition with a dendritic vaccine might lead to more potent T cell stimulation and leukemia antigen recognition [68, 69].

Ongoing Clinical Trials

While there are no published results regarding combination immunotherapy available yet, multiple trials examining different combinations are currently ongoing (Table 2). Several clinical trials study the combination of inhibiting different immune checkpoints at the same time (NCT01822509, NCT02846376, NCT03066648).

One clinical study directly compares the use of nivolumab and ipilimumab monotherapy with nivolumab and ipilimumab combination therapy in three separate arms after alloSCT (NCT02846376). Another trial uses 5 separate arms to compare different combinations of the anti-TIM-3 antibody MBG453, the anti-PD-1 antibody PDR001, and the HMA decitabine (NCT03066648) in AML patients with relapsed or primary treatment refractory disease or ineligible for chemotherapy. Additionally, the anti-PD-1 antibody pidilizumab is combined with a dendritic cell vaccine as maintenance therapy of AML patients in a (NCT01096602).

Recently, the leukocyte immunoglobulin–like receptor B4 (LILRB4), which is restrictively expressed on monocytic cells and monocytic AML cells, was found to suppress T cell activity in human monocytic AML cell lines [70••]. Knockout- or antibody-mediated blockage of LILRB4 was able to reduce the for monocytic AML characteristic infiltration of internal organs (including bone marrow, liver, and brain but not skin) with leukemic cells and improve survival in monocytic AML-carrying mice. In addition, LILRB4 blockade did not significantly reduce normal hematopoiesis, opening up a potential therapeutic window, which could be tested in future clinical trials. As LILRB4 is not only expressed on T cells but also a variety of other immunosuppressive cells including MDSCs, dendritic cells, and tumor-associated macrophages, future clinical trials might combine LILRB4 blockade with other forms of immunotherapy.

Summary and Future Directions

Although immune checkpoint inhibitors have shown significant benefit in multiple solid tumors, their application in AML has shown clinical activity but also less impressive results than in patients with solid tumors like melanoma and NSCLC. Several reasons for this have been invoked including the low immunogenicity of AML due to the low mutation rate in AML compared to more immunogenic cancers like melanoma and NSCLC [71, 72], as well as an immunosuppressive tumor microenvironment in the form of a protective bone marrow niche [73]. In order to overcome these obstacles, several approaches are currently under active investigation including the use of immune checkpoint inhibitors in more selected treatment settings and the combination of immune checkpoint inhibitors with multiple other forms of therapy in order to achieve synergy.

While current immune checkpoint inhibitors might not be effective as monotherapy in highly proliferative AML, their combination with chemotherapy as upfront therapy might improve immune surveillance and reduce minimal residual disease by allowing the elimination of chemo-resistant leukemia stem cells. Similarly, their use as maintenance therapy in patients who achieved a response to chemotherapy but have evidence of minimal residual disease and are not eligible for an alloSCT might lead to a reduction in their relapse rate. Lastly, immune checkpoint inhibitors might play a role as maintenance therapy in patients after alloSCT, as relapse after alloSCT is associated with a very high mortality.

Multiple clinical trials are currently examining the addition of chemotherapy, epigenetic therapy, and other types of immunotherapy to immune checkpoint therapy in order to achieve synergy.

Early results of phase II studies demonstrate the feasibility (with encouraging response rates) and safety of adding immune checkpoint blockade to chemotherapy or hypomethylating agents both in the frontline and the relapsed-refractory setting. Lastly, a better understanding of the complex immunosuppressive microenvironment consisting of T_{regs}, myeloid-derived suppressor cells (MDSC), and metabolic dysregulation in AML offers multiple other potential targets for combination immunotherapy [74–77].

Compliance with Ethical Standards

Conflict of Interest Maximilian Stahl declares that he has no conflict of interest.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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- •• Of major importance

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