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Immune checkpoint-based therapy in myeloid malignancies: a promise yet to be fulfilled

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Abstract

Introduction: Immune system evasion is essential for tumor cell survival and is mediated by the immunosuppressive tumor microenvironment and the activation of inhibitory immune checkpoints. While immune checkpoint-based therapy yielded impressive results in several advanced solid malignancies such as melanoma and non-small cell lung cancer, its role in acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS) is still evolving.

Areas covered: Here we review the immunology in the tumor microenvironment in the bone marrow and discuss the current preclinical and clinical data for immune checkpoint-based therapy in myeloid neoplasms.

Expert opinion: Clinical trials of immune checkpoint inhibitors (ICI) in AML and MDS are still in early stages and reported results so far have been modest especially for monotherapy use in the refractory settings. However, there are preliminary data for synergistic effects for combination of multiple ICI with hypomethylating agents and conventional chemotherapy. ICI might also be effective in eradicating minimal residual disease and to prevent relapse following induction chemotherapy or hematopoietic stem cell transplant. Additional trials to provide insight into the efficacy and safety profile of immune checkpoint-based therapy, its optimal timing and potential combination with other types of therapy as well as identification of predictive biomarkers are needed.

Keywords

acute myeloid leukemia; immune checkpoint blockade; PD-1; CTLA-4; combination therapy; tumor immunology; T-cells

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1.) Introduction

Creating an immunosuppressive microenvironment is essential for malignant cells to avoid elimination by the immune system^{1–3}. Elimination of malignantly transformed cells is dependent on T-cell mediated immunity². T-cells are activated by a two-step process consisting of (I) the presentation of major histocompatibility complex (MHC)-bound tumor antigens on antigen-presenting cells (APC) to the T-cell receptor (TCR) and (II) a co-stimulatory signal triggered by binding of B7–1 (CD80) or B7–2 (CD86) on APCs to CD28 on T-cells^{2,4}. This two-step process is essential to maintain self-tolerance and protect tissues from autoimmune-mediated destruction and is further supplemented by various immune checkpoints that regulate T-cell activation⁵. These include co-stimulatory receptors that are either expressed on T-cells (CD28, 4–1BB, CD27, inducible T-cell co-stimulator (ICOS)) or APCs (CD80 and CD86), and co-inhibitory receptors (cytotoxic T-lymphocyte-associated-protein 4 (CTLA-4) and programmed cell-death protein 1 (PD-1), T-cell immunoglobulin mucin-3 (TIM-3) and lymphocyte activation gene-3 (LAG-3))⁵.

One mechanism of immune system evasion is the upregulation of immune checkpoints in the tumor microenvironment⁶. Stimulating an anti-tumor immune response by monoclonal antibodies directed mainly against CTLA-4, PD-1 and PD-1 ligand (PD-L1) has been clinically successful leading to several FDA-approvals in various advanced solid tumors^{7–9}. In hematologic malignancies, immune checkpoint inhibitors (ICI) appear promising in Hodgkin's lymphoma (HL)¹⁰ and some forms of non-Hodgkin's lymphoma (NHL)¹¹. The proof of principle for the utility of immunotherapy in acute myeloid leukemia (AML) is allogeneic hematopoietic stem cell transplant (HSCT) which remains the most effective therapy to prevent relapse in AML patients in remission following chemotherapy and relies on the graft-versus-leukemia effect to eliminate residual AML blasts¹². Additionally, the anti-CTLA4 inhibitor ipilimumab and anti-PD1 antibodies nivolumab and pembrolizumab have shown some success in treating extramedullary relapse in AML following HSCT^{13,14}. However, the results from trials using ICI as monotherapy in AML and myelodysplastic syndrome (MDS) have shown only modest results thus far^{15,16}. Besides CTLA-4 and PD-1/PD-L1-directed approaches, other T-cell immune checkpoints, such as TIM-3 and LAG-3 as well as the macrophage checkpoint CD47 are also under investigation as therapeutic targets for AML and MDS (Figure 1).

In this review, we discuss the recent clinical and pre-clinical data for ICI in AML and MDS and look forward into the future of ICB-based research in myeloid malignancies.

2.) Immune evasion in the tumor microenvironment of myeloid malignancies

The presence of T-cells in the tumor microenvironment has been shown to be a prognostic marker and to predict the response to ICI in various solid and hematologic malignancies including AML^{17–20}. While several studies showed a greater effect of immune checkpoint inhibition with a higher expression of CTLA-4 and PD-1 on tumor-infiltrating T-cells and PD-L1 on tumor cells, respectively, clinical trials in non-small-cell lung cancer and melanoma showed clinical responses even in patients with low levels of baseline PD-L1

expression^{21–23}. Furthermore, given the shared cell differentiation lineage with APCs, expression of the co-stimulatory receptors CD80 and CD86 is also seen in AML and MDS with controversial data regarding its impact on prognosis^{24–26}. Additionally, several studies have demonstrated that an increased expression of PD-L1 and PD-L2 is associated with an adverse prognosis in AML patients and a lower likelihood of response to hypomethylating agents (HMA)^{27,28}. Another study also showed an increase in PD-1⁺T-cells in the bone marrow of patients with relapsed AML²⁹. Furthermore, an increase in regulatory T-cells in the peripheral blood has been shown in patients with AML and their persistence following induction chemotherapy has been correlated with a higher risk of relapse^{30–32}.

There is a scarcity of data on the composition of the tumor microenvironment within the bone marrow in AML regarding the expression of immune checkpoint receptors, T-cell functionality, and distribution of various T-cell subsets. One requirement for the use of immune checkpoint blockade (ICB) is the presence of T-cells within the tumor microenvironment. Several studies could show that the absolute percentage of T-cells in the bone marrow from AML patients were either similar or increased compared to healthy volunteers^{4,33,34}. While peripheral T-cells appear to be functionally intact and able to respond to co-stimulatory signals, bone marrow T-cells showed an overexpression of the inhibitory immune checkpoint receptor PD-1 which could be overcome by concomitant application of a PD-1 inhibitor^{4,29,35,36}. Therefore, ideally bone marrow samples should be used to assess the expression of immune markers for clinical trials especially as there is significant heterogeneity between AML patients and T-cell function can change during the disease course and in response to treatment.

In addition to the number and distribution of T-cells in AML, their differentiation and activation status are of great importance. While CD8⁺T-cells can directly induce target cell apoptosis by secretion of granzyme B and perforin, CD4⁺T-cells can differentiate into various effector cell types including regulatory T-cells depending on the cytokines present in the tumor microenvironment¹⁸. Currently, there are conflicting results regarding the ratio of CD8⁺/CD4⁺T-cells with one study showing an increased CD8⁺/CD4⁺-ratio compared to healthy controls that normalizes after chemotherapy, while other studies could not reproduce this finding^{33,37}. One potential explanation for immune evasion in AML is T-cell exhaustion, which is characterized by increased expression of various inhibitory receptors such as TIM-3, LAG-3, PD-1, and T-cell Immunoglobulin and Immunoreceptor Tyrosine-Based Inhibitory Motif Domain (TIGIT), and leads to impaired T-cell proliferation and cytokine production¹⁸. A recent study in a mouse model and human cells which showed that tumor evasion in monocytic leukemias is mediated by LILRB4, a T-cell activity suppressing immunoreceptor tyrosine-based inhibitory motifs receptor, supports this hypothesis³⁸. This immune exhaustion seems to be related to a persistent antigen stimulation and multiple lines of prior chemotherapies and is most prevalent in bone marrow samples from patients with relapsed AML^{36,39,40}.

Lastly, immune regulation within the bone marrow is a complex interplay between T-cells, mesenchymal stromal cells, myeloid-derived suppressor cells, and various soluble factors. These interactions are highly complex and have been reviewed in detail elsewhere^{18,41,42}.

3.) Immune checkpoint inhibitors as monotherapy

3.1.) Anti-CTLA-4

CTLA-4 (CD152) is expressed on T-cells and inhibits maturation and differentiation of T-cells by competing with the costimulatory receptor CD28 for CD80 (B7.1) and CD86 (B7.2) on APCs⁴³. Additionally, CTLA-4 is constitutively expressed on and activates regulatory T-cells⁴⁴. In AML, the increased expression of CD80 and CD86 on AML blasts may contribute to immune evasion and has been associated with poor outcomes and higher rates of relapse^{24,25,45,46}. Remarkably, CTLA-4 was also found to be constitutively expressed on leukemic blasts in bone marrow samples from both untreated and chemo-resistant AML patients⁴⁷. Given the high expression of CTLA-4 on leukemia cells, blocking CTLA-4 seems to be a promising target to stimulate an anti-leukemia immune response that has been successfully tested in various preclinical models^{48–50}.

Ipilimumab is an anti-CTLA-4 monoclonal antibody that enhances T-cell-mediated cytotoxicity by abrogating the inhibitory effect of CTLA-4⁵¹. Several trials tested ipilimumab monotherapy in high-risk MDS and AML as well as in the post-HSCT relapse setting. Zeidan et al reported the results of a phase Ib study of 29 MDS patients with IPSS intermediate-1 risk and higher who had progressed while on HMAs that showed a marrow CR in 1 patient (3.4%), a prolonged stable disease >46 weeks in 24% of patients and 17% of patients were able to undergo HSCT without excessive toxicity¹⁶. Of note, patients with a response to ipilimumab had a significantly higher expression of ICOS on CD8⁺ and CD4⁺ T-cells, which has been shown to be important in enhancing T-cell activation and response, and could be potentially used as a predictive response biomarker^{13,16,52}. Of note, 20.7% of patients in this study developed immune-related adverse events (IRAE) grade 2 that required removal from the study and was more frequent at higher doses of ipilimumab. Notably, all of these resolved after discontinuation of ipilimumab and administration of corticosteroids¹⁶. Recently presented early results of ongoing trials showed more promising results using anti-CTLA4 and other ICB monotherapy in myeloid malignancies (Table 1). Should these early results hold, it would be important to understand reasons for differences in clinical activity through detailed correlative work.

3.2.) Anti-PD-1

Under physiologic conditions, interaction of PD-1 on activated T-cells and regulatory T-cells with PD-L1/2, which is expressed on various immune cells, serves to limit T-cell activation after TCR/MHC engagement and CD28 activation and can be upregulated in the presence of interferon- γ ^{53–55}. An increased expression of PD-L1 on AML blasts and PD-1 on T-cells has been found in a subset of AML and MDS patients, especially at relapse, and has been associated with a poor prognosis^{28,35}.

After mouse models showed that an increased PD-1/PD-L1 expression led to a state of T-cell exhaustion which could be overcome by ICB⁵⁶, several monoclonal antibodies targeting PD-1/PD-L1 signaling have been developed and tested in AML patients. Pidilizumab (CT-011) was one of the first monoclonal anti-PD-1 antibodies to be tested in hematologic malignancies. In a phase I study of 17 patients with advanced hematologic neoplasms (8

AML, one MDS) treated with a single dose of pidilizumab, only one AML patient achieved a reduction in bone marrow blast count but progressed subsequently⁵⁷. Other anti-PD-1 antibodies that are currently being studied as monotherapy for AML or MDS at various disease stages include nivolumab and pembrolizumab (Table 1). In the KEYNOTE-013 trial (NCT01953692), 28 MDS patients who progressed on therapy with HMAs and were treated with pembrolizumab achieved an overall response rate of only 4% (1 partial remission) with hematologic improvement in 11% of patients⁵⁸.

Given the only modest efficacy for anti-PD-1 and anti-CTLA-4 monotherapy, subsequent trials have tested combinations of either CTLA-4 and PD-1 blockade, checkpoint blockade in combination with HMAs, conventional chemotherapy or other forms of immunotherapy.

4.) Immune checkpoint inhibitors in combination with other treatment modalities

4.1) Immune checkpoint inhibitors in combination with hypomethylating agents

Gene expression is regulated by DNA methylation and DNA hydroxymethylation and mutations in genes affecting these key epigenetic pathways (e.g. *DNMT3A*, *TET2*) have been linked to malignant transformation by inactivating tumor suppressor genes and are seen in 22% and 23% of AML patients, respectively^{59–61}. HMAs, such as 5-azacytidine (5-AZA) and its analogue 5-aza-2'-deoxycytidine (decitabine) inhibit DNA methyltransferase and work by non-selectively reversing DNA methylation and restoring gene expression^{62–64}. In addition to increased expression of tumor suppressor genes, this leads to activation of the immune system by increased expression of MHC-I and other co-stimulatory receptors (CD80, CD86, ICAM1)^{65–67}. One of the potential mechanisms of resistance to 5-AZA is a dose dependent upregulation of the PD-1/PD-L1 axis, which might lead to synergistic effects of combination of HMAs with PD-1/PD-L1 inhibitors^{27,56}. Both preclinical experiments combining 5-AZA with ipilimumab as well as HMAs in combination with PD-1/PD-L1 blockade in patients with NSCLC supported this presumed synergistic effect^{68–70}.

Another potential mechanism underlying the synergy between azacitidine and ICB might be the upregulation of antiviral defense mechanisms such as interferon- γ signaling. It has previously been shown that a component of the antitumor activity of HMA is the activation of viral defense pathways by induction of HLA class I antigens and interferon- γ signaling⁷¹. Previous studies in melanoma patients treated with anti-CTLA4 antibodies showed increased expression of similar anti-viral defense genes and mouse models showed synergistic effects of anti-CTLA4 inhibitors and azacitidine, which lends additional support to combining HMA with ICB^{71,72}.

These findings provided the rationale for various clinical trials in AML and MDS of HMAs in combination with several PD-1 inhibitors (nivolumab (NCT02397720), pembrolizumab (NCT02845297)), PD-L1 inhibitors (durvalumab (NCT02775903), atezolizumab (NCT02508870)), or the CTLA-4 inhibitor ipilimumab (NCT02890329, NCT02397720) for various disease stages (Tables 2 and 3).

A recent phase II study of 70 patients with relapsed/refractory AML (RR-AML) treated with nivolumab and 5-AZA showed a sustained ORR of 33% (22% complete remission (CR) or complete remission with insufficient recovery of counts (CRi)) with a median overall survival (OS) of 6.3 months which is superior to historical controls (ORR: 20%, median OS: 4.6 months) of HMA-based therapy including a combination of 5-AZA and venetoclax⁷³. IRAEs grade 3 occurred in 11% of patients with 2 deaths due to a corticosteroid- and infliximab-refractory IRAEs (NCT02397720). Interestingly, response rates were higher in HMA-naïve patients, patients with CD3⁺, CD4⁺ T-effector cells, and CD8⁺ T-cells in the pretherapy bone marrow aspirates, and in patients with an *ASXL1* mutation, which has been previously linked to a higher immunogenicity⁷³. Of note, non-responders had an increased expression of CTLA-4 on T-cells which suggests that there might be a different efficacy of PD-1 vs. CTLA-4 inhibition. Studies investigating the combination of different ICI with or without HMAs are an interesting area of future investigation. Several of these trials are currently ongoing (nivolumab + ipilimumab + 5-AZA [NCT02397720], nivolumab + ipilimumab for AML after HSCT [NCT02846376])⁷⁴. Similar preliminary results for the combination of pembrolizumab and decitabine in RR-AML were also presented at the 2018 ASH meeting. In a phase I trial of 10 patients (NCT02996474), 1 patient achieved a minimal residual disease (MRD)-negative CR for 337 days and the median OS in the entire study population was 7 months⁷⁵. Preliminary data from a phase II study (NCT03094637) of azacitidine and pembrolizumab in 18 high-risk MDS patients presented at the 2018 ASH meeting showed 2 CRs and 3 hematologic improvements in 12 patients evaluable for response of whom 7 had progressed on HMA (1 CR and 1 HI)⁷⁶. Treatment was well-tolerated and the clinical efficacy will need to be further evaluated.

A multi-arm phase II clinical trial tested nivolumab and ipilimumab as monotherapy or in combination with 5-AZA in both the frontline setting (41 patients) or after HMA failure (35 patients) in intermediate/high risk MDS (NCT02530463). Preliminary data available in abstract form showed overall response rates of 75% (15/20; CR/CRp 50%), 71% (15/21; CR/CRp 38%), 13% (2/15; CR/CRp 0%), and 35% (7/20; CR/CRp 15%) for 5-AZA + nivolumab, 5-AZA + ipilimumab, nivolumab monotherapy, and ipilimumab monotherapy, respectively. Furthermore, the combination of 5-AZA with either nivolumab or ipilimumab was efficacious both in the frontline and in the HMA-refractory setting with a median OS of 17 months and 8 months, respectively⁷⁷.

Safety and especially IRAEs remain a major concern for checkpoint inhibitor therapy. While most IRAEs respond promptly to corticosteroids and even a re-challenge with these agents has been shown to be feasible in selected patients, fatal courses of IRAEs have been reported and a clinical trial of 5-AZA with atezolizumab (NCT02508870) had to be discontinued due to safety concerns⁷⁸. Future studies to address the safety profile of checkpoint inhibitors are therefore warranted prior to their broader clinical application especially when combining PD-1/PD-L1 and CTLA-4 blockade which has been shown to have a substantial increase in IRAEs in solid malignancies⁷.

4.2) Combination of checkpoint blockade with conventional chemotherapy

DNA damage either by cytotoxic chemotherapy or gamma-irradiation has been shown to stimulate anti-leukemia immune responses in a murine model of AML by inducing expression of the co-stimulatory receptors CD80 and CD86 and decreasing PD-L1 expression^{79,80}. An increased CD80 and CD86 expression after exposure to cytarabine could also be shown in human AML cells⁸⁰. Release of tumor antigens following cytotoxic chemotherapy might also stimulate an anti-leukemia immune response. Several trials investigating anti-PD-1 antibodies are currently active, but no results have been published yet. These include nivolumab + “7+3” induction chemotherapy (NCT02464657), nivolumab + cyclophosphamide (NCT03417154) and pembrolizumab + high-dose cytarabine (NCT02768792). Preliminary data from a phase II trial of nivolumab in combination with idarubicin and cytarabine in newly-diagnosed AML reported a 77% CR/CRi (28 CR, 6 CRi; 18/34 (53%) MRD-negative by flow-cytometry) rate and a non-significant trend towards an improved median OS (18.5 months vs. 13.2 months) with the addition of nivolumab⁸¹.

4.3) Checkpoint inhibitors for minimal residual disease eradication and post HSCT

Minimal residual disease (MRD) as measured by next-generation sequencing has been shown to have a significant prognostic effect on relapse and survival in patients in CR following induction chemotherapy⁸². Since preclinical data suggest that immune checkpoint pathways contribute to immune system evasion of dormant leukemia cells and that these cells are resistant to T-cell-mediated cytotoxicity, ICI therapy might provide a promising option to eradicate MRD in patients in CR⁴⁶. Preliminary results of a phase II study (NCT02532231) of maintenance nivolumab in AML patients in CR following induction and consolidation chemotherapy but at high risk of relapse showed promising results with a 1-year OS rate of 86% and grade 3/4 IRAEs in 5 out of 14 patients⁸³.

For patients with relapse after HSCT the prognosis is poor and treatment modalities to prevent or treat relapse are of great clinical importance^{84,85}. A potential mechanism of relapse following HSCT appears to be the downregulation of patient specific HLA haplotypes leading to immune evasion of donor T-cells^{86,87}. Inspired by the graft-versus-leukemia effect observed in HSCT and donor lymphocyte infusion and the hypothesis that this beneficial effect can be further enhanced by ICB, several clinical trials testing PD-1/PD-L1 and CTLA-4-targeted antibodies in the post-HSCT setting have been conducted. In a phase I trial of ipilimumab in relapsed hematologic malignancies following HSCT (NCT01822509), 5 out of 28 patients (4 out of 12 AML patients) had a CR with a median 1-year OS rate of 49%¹³. Additionally, ipilimumab appeared to be well-tolerated with GVHD of the liver and gastrointestinal tract in 4 patients as well as one case of IRAE being the only dose-limiting toxicities¹³. Conversely, in another trial (NCT00060372) of 29 patients who relapsed after HSCT or donor lymphocyte infusion (2 AML patients), only 3 patients with lymphoid malignancies and none of the AML patients achieved an objective response⁸⁸. Furthermore, a recent case series of 3 patients with relapsed AML post-HSCT treated with nivolumab showed a durable CR in 1 patient and disease stabilization in another patient¹⁴. Additional trials of ipilimumab, nivolumab or combination therapy to either treat (NCT01822509) or prevent relapse (NCT02846376) are currently active.

While stimulating the graft-versus-leukemia-effect by ICB might prove to be beneficial, this also poses the risk of increasing incidence and severity of GVHD. In fact, cases of fatal GVHD associated with PD-1 blockade post-HSCT have been reported repeatedly and seemed to respond poorly to corticosteroids ^{89,90}. Therefore, additional studies to evaluate both safety and efficacy of checkpoint inhibitors in the post-HSCT setting in myeloid malignancies are warranted.

5.) Future directions and other potential targets for immune checkpoint-based therapies

5.1.) TIM-3 and LAG-3

In addition to PD-1 and CTLA-4 there are several other inhibitory immune checkpoints that can be therapeutically targeted to enhance the host's immune response to malignant cells. T-cell immunoglobulin mucin-3 (TIM-3) is a glycoprotein that is expressed on the surface of regulatory T-cells, INF- γ producing T-cells, and innate immune cells and has been shown to be expressed at increased levels on AML and leukemic stem cells ⁹¹⁻⁹³. The effect of TIM-3 activation is context-dependent and includes both inhibition of T-cell-mediated immune responses and pro-inflammatory effects by activation of macrophages ^{94,95}. Galactin-9 is a major activator of TIM-3 and high levels of galectin-9-TIM-3 coexpression have been found on leukemic stem cells in AML, but not on normal hematopoietic stem cells and were linked to leukemic transformation and progression by inhibition of Th1-cell immune responses ^{96,97}. Additionally, coexpression of PD-1 and TIM-3 has been associated with immune exhaustion and relapse in AML patients after HSCT ⁴⁰. Currently, a phase I study of the anti-TIM-3 monoclonal antibody MBG453 alone or in combination with the anti-PD-1 antibody PDR001 or decitabine is being tested in patients with AML or MDS (NCT03066648). Given that TIM-3 is expressed to a higher level on leukemic stem cells than non-neoplastic hematopoietic stem cells and is often expressed in conjunction with other surface antigens such as CD33, CD123, and CLL, targeting TIM-3 might limit on-target-off-leukemia side effects both as a single agent and in combination with another target antigen ⁹².

Lymphocyte-activation gene 3 (LAG-3) is an inhibitory receptor that has been shown to be upregulated on Th-cells in bone marrow aspirates from AML and MDS patients and has been associated with immune exhaustion especially if coexpressed with PD-1 and CTLA-4 ⁹⁸. Therefore, inhibition with anti-LAG-3 antibodies alone or in combination with other checkpoint inhibitors might provide an additional therapeutic target to overcome T-cell exhaustion in AML.

LILRB4 is an immunoreceptor tyrosine-based inhibition motif-containing receptor that inhibits T-cell activity and has been shown to be associated with immune evasion of monocytic leukemia cells in both mouse models and human cells ³⁸. Importantly, antibody-mediated inhibition has been shown to inhibit AML development and makes LILRB4 another potential therapeutic target especially in monocytic AML ^{38,99}.

5.2.) OX-40

Boosting the immune response by activating co-stimulatory receptors such as 4–1BB (CD137) or OX-40 (CD134) is another promising treatment strategy. OX-40 has been shown to promote activation and proliferation of effector T-cells while suppressing the differentiation of regulatory T-cells^{100,101}. In preclinical models OX-40 agonists have been demonstrated to lead to tumor regression by stimulating cytotoxic T-cell and NK-cell activity¹⁰². OX40 is also expressed on AML cells and its expression pattern varies between patients³⁶. Application of tumor-necrosis factor in in vitro models could further increase OX40 expression¹⁰¹. Antibodies targeting OX40 have been successfully tested in various hematologic and solid malignancies^{100,102}. A multi-arm phase I trial of the OX40 agonist PF-04518600 as monotherapy or in combination with 5-AZA, PD-1 inhibitors, or 4–1BB agonists in AML patients is currently ongoing (NCT03390296). However, OX40-ligand is highly expressed on other cells within the tumor microenvironment and the effect of this OX40-OX40L interaction needs to be further elucidated¹⁰¹. Additionally, stimulating the immune response by activating OX40 may lead to increased side effects especially when combined with other checkpoint inhibitors.

5.3) Anti-CD47

While T-cells are essential in the anti-leukemia immune response, the innate immune system and especially macrophages are playing a role in immune evasion as well. CD47 is expressed by normal cells to inhibit phagocytosis by binding to SIRPα on macrophages and has been shown to be overexpressed on various tumor cells including AML^{103,104}. An increased rate of CD47 expression has also been linked to a higher rate of transformation to AML in MDS patients¹⁰⁵. There is increasing preclinical evidence that blocking the interaction between CD47 and SIRPα with anti-CD47 antibodies can promote the phagocytosis of AML cells¹⁰⁶. Of note, CD47 is expressed on normal hematopoietic stem cells and progenitor cells as well^{107,108}. In addition to acting as an antigen sink and potentially limiting the anti-CD47 antibody's ability to reach tumor cells, in vitro studies of anti-CD47 antibodies have been associated with platelet aggregation and hemolytic anemia^{104,106,109}.

Hu5F9-G4, a monoclonal anti-CD47 antibody, is currently being tested in clinical trials as monotherapy and in combination with azacitidine for RR-AML patients (NCT02678338, NCT03248479). In a phase Ib/II clinical trial (NCT02953509) in 22 patients with relapsed/refractory NHL, Hu5F9-G4 in combination with the anti-CD20 antibody rituximab showed an ORR of 50% (32% CR) with only 3 grade 3 adverse events¹¹⁰. TTI-621 (SIRPαFc), a fusion protein of the N-terminal portion of SIRPα and the IgG1 Fc region, binds to both CD47 and the Fcγ receptor on macrophages to enhance phagocytosis and has been successfully tested in NHL as well^{104,111}. However, another phase I study of CC-90002, an anti-CD47 antibody, in RR-AML and high-risk MDS has recently been terminated as “data did not offer a sufficiently encouraging profile for further development” according to a statement on www.clinicaltrials.gov (NCT02641002).

5.4) Combination of tumor vaccines and checkpoint inhibitors

As outlined above, the immunosuppressive tumor microenvironment is of great importance. Dendritic cells (DC) are potent antigen-presenting cells that function as a mediator between the innate and adaptive immune system by expressing co-stimulatory signals and presenting antigens that are required to mount an immune response ^{112,113}. Recently, dendritic cells have been shown to contribute to tumor cell immune evasion by expressing inhibitory immune checkpoints such as PD-1 leading to a reduction in proinflammatory cytokine production and proliferation of CD8⁺ T-cells ^{114,115}. Combination of dendritic cell/AML vaccination and PD-1 blockade might therefore have synergistic effects and is currently being studied in a phase II clinical trial of AML patients in remission following chemotherapy (NCT01096602).

5.5 Combination of chimeric antigen receptor (CAR) T-cell therapy and ICB

CAR T-cells, a novel therapeutic concept in which autologous T-cells are first transduced with a retro- or lentiviral vector carrying the CAR gene, are expanded in vitro and are infused back into the patient, has shown remarkable results in B-cell-derived hematologic malignancies such as B-ALL and non-Hodgkin's lymphoma ^{15,116–118}. Co-administration of ICB may increase the potency of CAR T-cells as samples from patients with B-cell malignancies treated with anti-CD19 CAR T-cells have shown that CAR T-cells can acquire an exhausted phenotype characterized by increased expression of PD-1, LAG-3, and TIM-3 ^{119,120}. Preclinical and clinical data have also shown a synergistic effect of ICB and CAR T-cells therapy ¹²¹. Notably, a case report of a patient with DLBCL who had been treated with anti-CD19 CAR T-cells but showed progressive disease on follow-up responded well to addition of pembrolizumab ¹²². Treatment of patients with CD19⁺ lymphomas who failed to respond to anti-CD19 CAR T-cells with pembrolizumab is currently studied in a phase I/II trial (NCT02650999). However, there are currently no clinical trials combining CAR T-cells and ICB in AML.

5.6) Balancing therapeutic efficacy and IRAE of ICB in AML

Unleashing the immune system can lead to a variety of adverse events ranging from colitis to pneumonitis, endocrine dysfunction, cardio- and neurotoxicity and patients treated with combinations of PD-1 and CTLA-4 inhibition tended to be more likely to develop IRAEs ^{78,123}. Fatal toxicity has been reported in 0.6% of patients with any malignancy treated with ICB ⁷⁸. While most of the safety data are from patients with melanoma and NSCLC, available data from patients with hematologic malignancies appear to be comparable and most of the IRAEs observed responded well to corticosteroids ^{16,124}. A special consideration is the concern for a higher incidence of GVHD in patients undergoing HSCT after ICB therapy. Data on the incidence and severity of GVHD are controversial and cases of fatal GVHD associated with PD-1 blockade post-HSCT have been reported which seemed to respond poorly to corticosteroids ^{13,89,90}. Therefore, additional studies are warranted to elucidate the safety profile of ICB in AML treatment especially in combination therapy and in the post-HSCT setting.

7.) Conclusion

Similar to some solid malignancies, enhancing the host's anti-tumor immune response in myeloid malignancies might be a promising therapeutic approach. While monotherapy with ICI has so far been reported to have modest effects, combination of ICI with HMAs and chemotherapy appear to offer synergistic effects in early clinical trial results. The optimal combinations and timing of ICI in the disease course need to be further elucidated by clinical trials. As treatment of AML and MDS is becoming increasingly individualized, biomarkers that predict response to immune checkpoint therapy are needed to guide patient selection. Early identification and aggressive management of immune related adverse events is necessary to minimize complications and optimize clinical results.

8.) Expert opinion

While ICI has been extremely successful even if used as monotherapy in some advanced solid malignancies, early clinical trial results in myeloid neoplasms have been underwhelming, though longer term results are still pending. Recent preclinical studies have demonstrated the presence of T-cells in an immunologically exhausted state as evidenced by the upregulation of inhibitory immune checkpoints in AML bone marrow samples which suggests that ICI might be a feasible strategy in myeloid neoplasms as well. However, results from monotherapy with ICI have been only modest thus far and predictive biomarkers are missing. Given the genetic heterogeneity of patients with AML and MDS, responses to ICI are expected to be heterogenous and additional studies to identify predictive biomarkers and to improve our understanding of the immunology in the bone marrow microenvironment are warranted to optimize patient selection.

Therefore, combination therapy of ICI and other treatment modalities such as HMAs and conventional chemotherapy seems a logical way forward. For example, upregulation of CTLA-4 and PD-1/PD-L1/2 has been found in MDS patients who failed treatment with HMAs and HMAs have been successfully combined with various ICI agents already. Similar to studies in solid malignancies that suggested tumors with a higher mutational burden had a higher likelihood to respond to ICI, the DNA damage inflicted by cytotoxic chemotherapy has been shown to stimulate anti-leukemia immune responses in a murine model of AML by inducing expression of the co-stimulatory receptors CD80 and CD86 and decreasing PD-L1 expression. Combination of ICI and conventional chemotherapy could therefore have synergistic effects as it releases the brakes from the immune system and provides additional stimulation simultaneously. Along the same lines are efforts to develop antibodies to co-stimulatory immune checkpoints like OX-40 that could be combined with ICI as well to increase the anti-leukemia immune response. However, several caveats remain especially with regard to excess toxicity associated with combination of various immune checkpoint-targeting therapies. IRAEs have been documented already in monotherapy studies and while they appear manageable with corticosteroids in most cases, fatal toxicities have been reported. Early recognition and aggressive management of IRAEs are of paramount importance in the conduction of these trials, and in myeloid malignancies impose special challenges. For example, the frequency of bacterial and fungal pneumonias in advanced myeloid malignancies can make physicians more concerned about prompt use of steroids for

suspected pneumonitis. Similarly, severe and refractory thrombocytopenia, which is common among patients with advanced MDS and AML, make obtaining lung biopsies to differentiate infectious from inflammatory pneumonitis riskier in such cases.

Finally, there has been some concern for inducing or worsening graft-versus-host-disease (GVHD) in patients treated with ICI and proceeding to, or who have relapsed after HSCT. However, early data regarding the few patients who did proceed to HSCT from different clinical trials of ICI in myeloid malignancies do not suggest an increase incidence of severe GVHD. Nonetheless, more data, longer follow-up and careful monitoring are still needed to exclude this concern.

ICI in myeloid malignancies remains in early stages and carefully conducted clinical trials are needed to assess the safety and efficacy of immune checkpoint-based therapy, its optimal timing and potential combination with other treatment modalities. Over the next 5 years we expect our understanding of the immunology of the bone marrow microenvironment to improve further and to maybe enable a correlation of various expression patterns of immune checkpoint molecules on both leukemia cells and immune cells with different disease stages. Analysis of immune checkpoint expression profiles and careful correlative studies should be performed in all such clinical trials to help identify predictive biomarkers and to inform rational combination therapies. Several large clinical trials of various combination therapies are currently ongoing and will provide additional information on the safety, efficacy and long-term outcome of ICI in AML and MDS. Additional trials to investigate the efficacy of ICI to eradicate MRD in patients post-HSCT or induction chemotherapy will also be needed. It will be interesting to see if the responses to ICI in AML are as durable as with some patients with solid malignancies. We remain optimistic that ICI especially if included in well-designed combination therapy have the potential to provide significant benefit to the treatment of patients with myeloid malignancies and various stages of the disease course.

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Article highlights

- Immune system evasion is essential for tumor cell survival in both solid malignancies as well as in myeloid neoplasms and is mediated by the activation of inhibitory immune checkpoints such as cytotoxic T-lymphocyte-associated-protein 4 (CTLA-4) and programmed cell-death protein 1 (PD-1), T-cell immunoglobulin mucin-3 (TIM-3) and lymphocyte activation gene-3 (LAG-3).
- Immune checkpoint therapy has the potential to reverse immune system evasion of myeloid malignancies. While monotherapy with immune checkpoint inhibitors has only yielded modest results, combination with hypomethylating agents, intensive chemotherapy and multiple immune checkpoint inhibitors as shown promising results.
- Further research to identify predictive biomarkers, optimize timing, combination partners and treatment settings is warranted

Figure 1:

Overview of potential targets for immune checkpoint-mediated therapy in myeloid malignancies. Antigens, including tumor antigens, are presented bound to major histocompatibility complex (MHC)-I/II molecules by antigen-presenting cells (APC) and activate T-cells by binding to the T-cell receptor (TCR). However, a co-stimulatory signal such as activation of CD28 on T-cells by CD80 or CD86 is required for T-cell activation. The activity of T-cells is further regulated by various co-inhibitory (PD-1, CTLA-4, TIM-3, LAG-3) and co-stimulatory receptors (ICOS, OX-40) which can serve as potential targets for monoclonal antibodies that mainly block the activity of co-inhibitory receptors. However, as in the case of PF-04518600, activating co-stimulatory receptor function can also be employed to increase the anti-tumor immune response. Myeloid blasts can also express various receptors on their surfaces that create an immunosuppressive microenvironment and contribute to immune system evasion. While T-cells have been the main focus of immune checkpoint-mediated therapy thus far, current research has also identified the interaction of CD47 on APCs and myeloid blasts and SIRPα on macrophages as a potentially targetable mechanism of checkpoint therapy.

Table 1:

Selected completed trials of immune checkpoint blockade monotherapy in AML/MDS

Drug	Target / mechanism	Phase [ref]	N	Patient characteristics	Outcomes
Pidilizumab	Anti-PD-1 antibody	I ⁵⁷	17 (8 AML, 1 MDS)	Advanced hematologic malignancies, age <65	1 AML patient with reduction in peripheral blasts
Nivolumab	Anti-PD-1 antibody	II ⁷⁷	15	MDS frontline and HMA failure	0% CR/CRp; 25% 1-year survival
		II ⁸³	14	HR-AML in CR ineligible for HSCT	71% 1-year CR rate
Pembrolizumab	Anti-PD-1 antibody	I ⁵⁸	28	MDS after HMA failure	ORR: 4% (1 PR); 49% 24-week survival
Ipilimumab	Anti-CTLA-4 antibody	I ¹³	28 (12 pts w/ AML)	Hematologic malignancies with relapse after HSCT	No response with 3mg/kg Response with 10mg/kg CR 23%, PR 9% 23% decrease in tumor burden CR in 1 AML patient secondary to MDS
		I ¹⁶	29	MDS patients who failed HMAs	CR 3.4%, PSD for >46 weeks 27%, >54 weeks 10% Median OS 294 days (censoring at allogeneic HSCT),
		II ⁷⁷	20	MDS frontline and HMA failure	15% CR/CRp; 45% 1-year survival

Table 2:

Selected completed clinical trials of immune checkpoint blockade as part of combination therapy in AML/MDS

Drug	Target / mechanism	Phase [ref]	N	Patient characteristics	Intervention	Outcomes
Nivolumab	Anti-PD-1 antibody	II ⁷⁷	20	MDS frontline and HMA failure	Nivolumab + 5-AZA	75% CR/CRp; 50% 1-year survival
		II ⁷³	70	RR-AML	Nivolumab + 5-AZA	ORR: 33% (22% CR/CRi); median OS 6.3 months
		II ⁸¹	41	Frontline AML or high-risk MDS; age<65	Idarubicin + cytarabine +/- nivolumab	77% CR/CRi; median OS 18.54 (nivolumab group) vs 13.2 months (I+A alone), p =0.2
Pembrolizumab	Anti-PD-1 antibody	I ⁷⁵	17	RR-AML	Pembrolizumab + decitabine	1 MRD-negative CR; median OS 7 months
Ipilimumab	Anti-CTLA-4 antibody	II ⁷⁷	21	MDS frontline and HMA failure	Ipilimumab + 5-AZA	71% CR/CRp; 68% 1-year survival

Table 3:

Selected ongoing trials of immune checkpoint therapy

Drug	Target/mechanism	Phase	NCT	Patient characteristics	Intervention
Nivolumab	Anti-PD-1 antibody	I	NCT02846376	AML/MDS after SCT at high risk for relapse	Nivolumab + ipilimumab
		II	NCT02275533	Eradication of MRD in AML in CR1	Nivolumab
		II	NCT02397720	RR-AML, ND-AML >65 years	Nivolumab + 5-AZA +/- ipilimumab
		II	NCT03417154	RR-AML, HR-MDS	Nivolumab + Cyclophosphamide
Pembrolizumab	Anti-PD-1 antibody	I	NCT02981914	Relapsed MDS, AML, B-cell lymphoma following allogeneic HSCT	Pembrolizumab
		I	NCT03286114	Relapsed MDS, AML, ALL following allogeneic HSCT	Pembrolizumab
		II	NCT02768792	RR-AML	HiDAC + pembrolizumab
		II	NCT02845297	RR MDS/AML and newly diagnosed AML patients >65	5-AZA + pembrolizumab
		II	NCT02708641	AML in CR ineligible for HSCT	Pembrolizumab
		II	NCT02771197	HR-AML ineligible for allogeneic HSCT	Pembrolizumab + Fludarabine/ melphalan conditioning + autologous HSCT
Durvalumab	Anti-PD-1 antibody	I	NCT02117219 ¹²⁵	MDS after HMA failure	Durvalumab +/- 5-AZA +/- tremelimumab
		II	NCT02775903 ¹²⁶	High risk MDS, elderly AML patients	Durvalumab + 5-AZA
Ipilimumab	Anti-CTLA-4-Antibody	I	NCT02890329	RR MDS/AML	Ipilimumab + decitabine
Hu5F9-G4	Anti-CD47 antibody	I	NCT02678338	RR-AML, HR-MDS	Hu5F9-G4
		I	NCT03248479	RR MDS/AML or unfit ND-AML/MDS	Hu5F9-G4 + 5-AZA
TTI-621 (SIRPa.Fc)	Anti-CD47 antibody	I	NCT02663518	Hematologic and solid tumors	TTI-621 +/- rituximab or nivolumab
MBG453	Anti-TIM-3 antibody	I	NCT03066648	AML, HR-MDS	MBG453 + Decitabine or PDR001 (anti-PD-1 antibody)
DC AML vaccine	Tumor cell vaccine	I	NCT01096602	ND-AML or RR-AML	PD-1 blockade + DC AML vaccine