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REVIEW

Cancer immune therapy for myeloid malignancies: present and future

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Abstract

The myelodysplastic syndromes, the chronic myeloproliferative neoplasms, and the acute myeloid leukemia are malignancies of the myeloid hematopoietic stem cells of the bone marrow. The diseases are characterized by a dysregulation of the immune system as both the cytokine milieu, immune phenotype, immune regulation, and expression of genes related to immune cell functions are deregulated. Several treatment strategies try to circumvent this deregulation, and several clinical and preclinical trials have shown promising results, albeit not in the same scale as chimeric antigen receptor T cells have had in the treatment of refractory lymphoid malignancies. The use of immune checkpoint blocking antibodies especially in combination with hypomethylating agents has had some success—a success that will likely be enhanced by therapeutic cancer vaccination with tumor-specific antigens. In the chronic myeloproliferative neoplasms, the recent identification of immune responses against the Januskinase-2 and calreticulin exon 9 driver mutations could also be used in the vaccination setting to enhance the anti-tumor immune response. This immune response could probably be enhanced by the concurrent use of immune checkpoint inhibitors or by vaccination with epitopes from immune regulatory proteins such as arginase-1 and programmed death ligand-1. Herein, we provide an overview of current cancer immune therapeutic treatment strategies as well as potential future cancer immune therapeutic treatment options for the myeloid malignancies.

Keywords Acute myeloid leukemia · Myelodysplastic syndrome · Myeloproliferative neoplasms · Immune therapy · Cancer vaccines · Antigens

Introduction

The use of cancer immune therapy for the treatment of hematological malignancies is an obvious treatment option, as the tumor cells in the blood, lymphoid tissue, and bone marrow are easily accessible to effector immune cells. However, one drawback of cancer immune therapy for hematological malignancies is that the effector immune cells may potentially be malignant themselves. One example is that T cells from patients with myelodysplastic syndrome (MDS) may originate from the malignant clone [1], and T cells from patients with chronic myeloproliferative neoplasms (MPNs) have been shown to harbor both the Janus kinase 2 (*JAK2*)- and calreticulin (*CALR*) exon 9 driver mutations [2, 3]. How this influences the effector function of the affected immune cells is yet to be clarified.

The use of allogeneic hematopoietic stem cell transplantation (alloHSCT) relies not only on the anti-neoplastic effect of total body irradiation and chemotherapy but also on the graft-versus-leukemia effect [4] and hence is essentially an immune therapeutic treatment modality, which has been used with success in several hematological cancers for decades.

Through the years, medical oncologists have often lagged behind hematologists in the search and testing of new treatment modalities. However, in the case of cancer immune therapy, the picture is the opposite, as several cancer immune therapeutic drugs, namely the immune checkpoint inhibitors, are standard first-line treatment options for several solid malignancies. But speed is catching up in the hematological departments around the globe, and several novel cancer immune therapeutic treatment options for the hematological malignancies are entering the clinic. Additionally, a plethora of novel and highly innovative treatment modalities are currently being tested in both the clinical and preclinical settings. Most

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progress has been shown in treatments for the lymphoid malignancies where, above all, chimeric antigen receptor (CAR) T cells, bispecific T cell engagers (BiTEs), and immune checkpoint inhibitors have demonstrated remarkable clinical effects, especially in acute lymphoblastic leukemia and Hodgkin lymphoma. The same rate of success has not been demonstrated in the treatment of myeloid malignancies, but several promising treatment modalities are undergoing clinical and preclinical testing and will most likely soon change the therapeutic landscape of the myeloid malignancies. In this review, we will provide an overview of current cancer immune therapeutic treatment options as well as potential future cancer immune therapeutic treatment options for the myeloid malignancies.

MDS and AML

Immune regulation in MDS and AML

MDS and acute myeloid leukemia (AML) are closely related disorders of the hematopoietic stem cells of the bone marrow. AML is characterized by dedifferentiation and hyperproliferation of myeloblasts, whereas MDS is characterized by some dedifferentiation and hyperproliferation of myeloblast in concert with dysplasia of bone marrow stem cells. AML may develop de novo or from preexisting MDS or MPN. Several studies have investigated the immune phenotype and regulation of the immune system in patients with MDS and AML. Le Dieu and coworkers identified higher amounts of $CD3^+$ T cells and $CD8^+$ T cells in peripheral blood (PB) of AML patients as well as a higher expression of activation markers such as CD25 and CD69 concurrent with a higher expression of memory markers, which made the authors conclude that in AML, the immune system is in a primed and activated state. The authors also demonstrated that effector T cells from patients with AML have reduced ability to form the immunological synapse. However, due to the disability to form the synapse, the immune system is unable to clear the malignant cells [5]. Interestingly, Vercauteren et al. showed that T cells in patients with MDS are derived from the malignant clone [1], but the functional aspects of this discovery are yet to be established. AML patients have increased expression of programmed cell death protein 1 (PD-1) on both $CD4^+$ and $CD8^+$ T cells, and at relapse, the amount of T memory cells in AML is increased compared to the levels at diagnosis [6]. A recent study showed that bone marrow T cells in AML patients have higher amounts of exhaustion markers compared to T cells from PB [7]. Studies of regulatory cells in AML and MDS have shown that immune regulation is of paramount importance in these diseases. Regulatory T cells (Tregs) are enriched in PB in patients with AML and display higher levels of the regulatory molecules cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and glucocorticoid-induced TNFR-related protein (GITR) and lower levels of HLA-DR, Fas, and CD62L [8, 9]. Moreover, Tregs from patients with AML have a higher inhibitory potential compared to Tregs from healthy donors, and most interestingly in both of the aforementioned studies, patients that reached a complete response (CR) had lower levels of Tregs in PB compared to patients who did not reach CR [8, 9]. In both AML and MDS, blasts and peripheral blood mononuclear cells (PBMCs) display increased amounts of both PD-1 and programmed cell death ligand 2 (PD-L2) [10]. Of interest, treatment with hypomethylating agents (HMA), such as 5-azacytidine (vidaza) and 5-aza-2'deoxycitidine (decitabine), which is used for treating both MDS and AML, increases the expression of PD-L1 and PD-L2 in blasts and PBMCs [10] as well as the expression of PD-1 by T cells [11]. The increased expression of PD-1 was shown to rely on demethylation of the PD-1 promoter [11], and a correlation between treatment response and low expression of PD-L1, PD-L2, and PD-1 upon treatment with HMA was demonstrated [10, 11]. In MDS, the frequency of Tregs is correlated to disease stage, since patients with high blast counts, high-risk disease, and complex karyotype show increased amount of Tregs in PB [12]. A detailed study by Chen and coworkers showed that compared to healthy donors or patients with other malignancies, patients with MDS display increased levels of myeloid derived suppressor cells (MDSCs) in the bone marrow, and the MDSCs produced more immunosuppressive substances such as interleukin (IL)-10, transforming growth factor beta (TGF-β), and arginase compared to MDSC from healthy donors [13]. Moreover, the amount of the immunosuppressive enzyme arginase-2 is increased in serum of patients with AML, and arginase-2 from the AML blasts impaired proliferation and differentiation of normal hematopoietic stem cells (HSC) [14]. Additionally, patients with AML that showed increased expression of another immunosuppressive enzyme indoleamine 2,3-dioxygenase (IDO) had significantly lower OS compared to patients with low expression of IDO [15], and AML blasts have been shown to induce formation of Tregs via an IDO-dependent mechanism [16]. As such, there is compelling evidence showing overt immune dysregulation and immune escape in AML and MDS. An overview of the some of these mechanisms is provided in Fig. 1.

Immune checkpoint inhibitors in AML and MDS

Immune checkpoint inhibitors have changed the therapeutic landscape of several solid malignancies dramatically, and these drugs are able to cure patients with advanced metastatic disease and have also shown impressive results in hematological malignancies—especially in patients with refractory Hodgkin lymphoma [17]. Accordingly, checkpoint inhibitors have also been tested in patients with AML and MDS, albeit the results have only been reported as abstracts, due to short

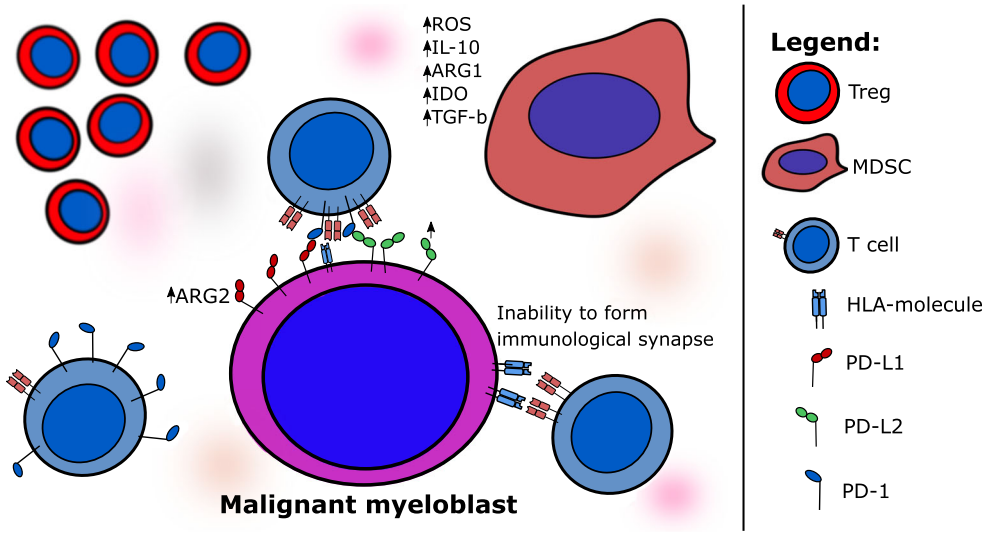

Fig. 1 An illustration of some possible immune escape mechanisms in AML and MDS. Regulatory T cells are increased in peripheral blood of patients with AML and MDS. Increased levels of MDSC in MDS produce excessive amounts of reactive oxygen species, arginase-1, indoleamine 2,3-dioxygenase, and TGF-β, which all dampen the T cell response. T cells in patients with AML are inefficient at forming the immunological synapse, and the malignant myeloblasts display increased levels of PD-L1 and PD-L2 and secrete excessive amount of arginase-2. T cells from patients with AML display increased levels of PD-1

follow-up. The anti-CTLA-4 antibody ipilimumab (IPI) was tested in 11 patients with high-risk MDS after failure with HMA. No objective responses were seen; however, three patients had disease stabilization [18]. Another trial tested the safety and toxicity and clinical effects of the PD-1 blocking antibody nivolumab (NIVO) or IPI either alone or in combination with HMA in patients with HMA refractory MDS. The combination of NIVO and HMA had impressive effects in treatment naïve patients, as an overall response rate was identified in 13/21 patients (62%) and 6 patients achieved a CR [19]. In a study from the same institution, the safety and toxicity as well as clinical effect of NIVO and HMA in combination was tested in patients with refractory AML. Of 53 evaluable patients, 11 (21%) had a CR or CR with insufficient recovery of counts. Notably, the overall survival (OS) compared favorably to historical controls, and responders had higher $CD3^+$ and $CD8^+$ infiltration in the bone marrow, as well as progressive increase of $CD4^+$ and $CD8^+$ during the study [20]. The activity of the killer-immunoglobulin-receptor (KIR) blocking antibody lirilumab is being evaluated in two clinical trials. The drug was tested as maintenance therapy in elderly non-transplant eligible patients with AML but failed to show any improvement in leukemia free survival [21]. Vidaza in combination with lirilumab is being tested as well, but no conclusions have yet been made on the efficacy [22].

Vaccination trials in AML and MDS

Given the fact that several tumor-associated antigens (TAAs) such as Wilms tumor antigen 1 (WT1), NY-ESO1, PR1 (a nonamer epitope derived from neutrophil elastase and proteinase 3), and PRAME are highly expressed in AML and MDS, several trials have tested vaccines with one or several of these epitopes. In 2004, Qazilbash et al. presented data on a clinical vaccination trial in 33 patients with either AML, chronic myelomonocytic leukemia, or MDS with the HLA-A2 restricted epitope PR1, where it was shown that patients with a vaccination-induced PR1-specific immune response had significantly longer OS compared to patients without PR1-specific immune response [23]. In 2007, Rezvani et al. presented data on the first combinatorial vaccination study in myeloid malignancies, where a combination of HLA-A2 restricted WT1 and PR1 epitopes was able to induce TAA-specific immune responses in all patients. Survival data, however, was difficult to interpret due to low amount of included patients (eight in total) and short follow-up (median follow-up 252 days) [24]. Keilholz et al. have reported on vaccination with WT1 epitopes and identified several vaccination-induced WT1-specific immune responses. One patient even had a complete cytogenetic remission, but the study did not show any correlation between immune responses and clinical response [25]. Two studies have tested a combination of different WT1 epitopes to stimulate both $CD4^+$ and $CD8^+$ T cell responses [26, 27]. The notion was that the induction of a $CD4^+$ T cell response would enhance the $CD8^+$ T cell response. The study by Uttenthal et al. failed to show any significant clinical responses, whereas the findings demonstrated by Maslak and coworkers were notable, as patients with a vaccination-induced WT1 immune response had better OS compared to patients that did not have induction of immune response [26]. Seven patients were analyzed for WT1-specific $CD4^+$ T cell responses, and of these patients, four displayed a

$CD4^+$ T cell responses. Interestingly, none of these four patients had disease progression [27]. A long-term report on a subset of WT1 vaccinated AML patients showed that the transcript levels of WT1 increased when vaccinations were stopped, and decreased when vaccinations were reinstated. Moreover, three patients achieved a complete molecular response after more than 8 years of vaccinations showing that therapeutic cancer vaccinations may cure patients with AML [28]. Another study demonstrated that vaccination with WT1 epitopes may induce immune responses in heavily pretreated patients [29], and just recently, a phase II study on a PR1 vaccine was reported in patients with AML, MDS, or chronic myeloid leukemia (CML), and PR1-specific immune responses were induced in 89% of patients. Importantly, this study showed a decline in the PR1-specific T cells through time, which could indicate that the vaccines induced exhaustion in PR1-specific T cells. Another important finding was that patients with a high disease burden (blast count) had lower frequency of immune responses compared to patients with low disease burden, thus giving impetus to the notion that therapeutic cancer vaccines have the highest potential in non-advanced/non-disseminated disease. Even more, patients who displayed a PR1-specific immune response were more likely to display a clinical response to vaccination compared to patients that did not have a PR1-specific immune response [30]. One dendritic cell (DC)-based vaccine trial employing autologous DCs porated with three WT1 mRNA constructs was tested in 30 post-remission high-risk AML patients. The trials showed better OS compared to historical controls, and data gave some indications that patients with relapse after immune therapy might still benefit through the combination of immune therapy and chemotherapy [31].

An interesting aspect in the treatment of AML and MDS is the prospect of combining HMA with peptide vaccinations, as it has been shown that HMA enhance the expression of TAA, most notably NY-ESO [32, 33], as well as increase the amount of cancer germline antigen (CGA)-specific $CD8^+$ T cells in PB [34]. As such, decitabine was combined with vaccination with a NY-ESO-1 epitope fusioned with a DEC-205 monoclonal antibody to enhance antigen uptake by DC in nine patients with MDS. Treatment with decitabine induced expression of NY-ESO-1 in all patients, and $CD4^+$ T cell responses and $CD8^+$ T cell responses were detected in 6/7 and 4/7 patients, respectively [35]. A preclinical study showed that stimulation of T cells from patients with AML with autologous blasts induced immune responses specific to the AML blasts in 5/8 patients [36]. This approach has been used somewhat differently in a clinical trial, where blasts from post remission AML patients were fusioned with autologous DCs. The fusioned cells were then irradiated and administered subcutaneously at 4-week intervals in a total of three doses, and the vaccines were able to induce immune responses against numerous TAAs. To our knowledge, this trial has generated the most impressive results in immune therapy for myeloid malignancies, as 71% of patients remain in remission after a median follow-up of 57 months. Moreover, several of the patients were older than 70 years of age, and one patient, who relapsed within 1 year and had second-line chemotherapy-induced remission and then received subsequent vaccinations, remains disease free more than 4 years in protocol [37].

Possible targets for vaccination

Vaccination with TAA may not be the only option for vaccines in AML and MDS. Considering the recent interest for neo-antigens, it might be worthwhile to test neo-antigen derived epitopes in the setting of therapeutic cancer vaccines in AML. One interesting option in AML could be the nucleophosphmine-1 (*NPM1*) mutations that are identified in 30% of patients with AML [38]. These mutations generate neo-antigens that are highly immunogenic [39], and patients with immune response to these neo-epitopes have superior survival compared to patients without an immune response [40]. Concurrently, it has been shown that certain HLA types may protect against the development of *NPM1*-mutant AML [41], giving impetus to the notion that immune-mediated rejection of *NPM1*-mutant clones may be able to prevent the emergence of overt AML. Given the fact that PD-L1 expression on AML blasts might attenuate the tumor immune response, it is interesting that leukemia stem cells from patients with *NPM1*-mutant AML show increased expression of PD-L1, hence providing a rationale for the use of PD-1- and PD-L1 blocking antibodies for the treatment of *NPM1*-mutant AML [42]. Speculations in the immunogenic potential of the neo-antigens generated by the fms-like tyrosine kinase 3 internal-tandem-duplicate mutations, which confer a dismal prognosis, have also been provided [43].

Other cancer immune therapeutic modalities for AML and MDS

Given the surge of interest in adoptive cell therapies, namely CAR T cells, this modality has also been investigated in AML and MDS. However, the results have not been nearly as promising as in acute lymphoblastic leukemia—namely due to the lack of proper extracellular targets in these diseases. CD33, which is expressed by myeloid cells and by some AML types, is a target for the antibody drug conjugate gemtuzumab ozogamicin, and second-generation anti-CD33 CAR T cells have been tested in a murine in vivo setting demonstrating convincing tumor cell killing of $CD33^+$ tumor cells [44]. CAR T cells specific for CD123 (IL-3R), which is expressed by healthy cells as well as leukemic stem cells, showed tumor cell killing both in vitro and in an in vivo murine model [45]. LeY is a difucosylated carbohydrate that binds to several LAAs, and LeY-specific CAR T cells were tested in four

relapsed AML patients. A biological effect was detected in three patients, and one patient remained disease free, but with cytogenetically detectable disease, for 23 months. Of note, CAR T cells were detectable in this patient for 23 months (longer than in any of the other patients), and the patient also displayed the highest cytokine response [46]. Other adoptive cell therapies for AML and MDS are donor lymphocyte infusions in patients with a post alloHSCT relapse, which has been shown to induce immune responses against both TAAs and TSAs as well as complete responses [47]. Just recently, a preclinical study on $CD4^-$, $CD8^-$ double-negative T cells expanded from PBMCs from patients with AML showed superior tumor cell killing compared to $CD8^+$ T cells as well as the ability to kill blasts from chemotherapy resistant patients [48]. A BiTE specific for CD3/CD33 showed remarkable tumor cell killing in in vitro testing, effectively killing $CD33^+$ tumor cells even at low effector/target ratio [49], and killing was enhanced upon combination with PD-1/PD-L1 blocking antibodies [50]. Significant NK cell activity has also been demonstrated in AML, where the administration of IL-2 combined with the the reactive oxygen species (ROS) scavenger histamine dihydrochloride may modulate the level of NK cells and NK cell receptors, which might have an impact on survival in patients [51, 52].

The chronic myeloproliferative neoplasms

Chronic myeloid leukemia

Whereas MDS is characterized by bone marrow dysplasia and accumulation of myeloblasts in the bone marrow, and AML is characterized by accumulation of myeloblasts in PB, the chronic myeloproliferative neoplasms (MPNs) are characterized by an overproduction of mature peripheral blood cells [53]. One of the MPNs, CML, is characterized by the translocation of the *ABL1* gene on chromosome 9 to the *BCR* gene on chromosome 22, hence generating the Philadelphia chromosome and the oncogenic bcr-abl fusion protein. Immune responses against both bcr-abl and abl-bcr fusion proteins have been detected [54–56], and some HLA types—HLA-A3 and HLA-A8, especially in combination—have been shown to protect against CML [57], giving impetus to the notion that patients with these HLA types may eradicate $BCR\text{-}ABL^+$ leukemia cells before the disease is able to establish in the bone marrow. Accordingly, bcr-abl epitopes have been tested in several clinical trials and has been able to induce immune responses and clinical responses [58], and it was shown that the kinase activity of the bcr-abl fusion protein enhances the expression of several TAAs [59]. In one remarkable case, a female patient achieved a major molecular response and was later cured by vaccination monotherapy with bcr-abl epitopes [60]. However, the emergence of bcr-abl-specific tyrosine kinase inhibitors such as imatinib mesylate has changed the therapeutic landscape and prognosis for patients dramatically, and research on immune therapeutic options for patients with CML has decreased dramatically.

The Philadelphia chromosome-negative chronic myeloproliferative neoplasms

The Philadelphia chromosome-negative (Ph-neg) MPNs constitute the three disorders essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF). In 2005, it was discovered that about 70% of patients with MPN share an oncogenic driver mutation in the *JAK2*-gene [61, 62], and this discovery spurred the development of a JAK2 inhibitor and gave hope that such a drug might give the same results as imatinib mesylate had in treating CML. However, ruxolitinib, the only JAK2 inhibitor approved for the treatment of Ph-neg MPN, has only showed modest results [63–65], but the drug, which is highly anti-inflammatory, is indeed able to alleviate the inflammation mediated constitutional symptoms that often torment patients with Ph-neg MPN. As such, patients with MPN display overt deregulation of the cytokine milieu, as patients with PV and ET have elevated levels of IL-6, IL-8, IL-12, tumor necrosis factor-alpha, and interferon-gamma (IFN-γ) compared to healthy controls [66]. Additionally, Tefferi and colleagues identified deregulation of 20 out of 30 investigated cytokines in patients with PMF [67], patients with PMF also display elevated levels of the inflammatory marker YKL-40 [68], and monocytes from patients with PMF display increased secretion of TGF-β [69]. Concurrently, patients with Ph-neg MPN display marked deregulation of genes related to the immune system and inflammation a s patients have lower gene expression of HLA-I, HLA-II, and beta-2-microglobulin [70]. Additionally, patients express low levels of Fas and CD40L, which could inhibit effector cell killing and attenuate the interaction between T cells and antigen presenting cells [71]. Just as patients with AML and MDS, patients with Ph-neg MPN display increased expression of several immunoregulatory proteins. Namely, it was shown that PBMCs from patients with MPN show increased expression of the immunosuppressive enzyme arginase-1 [72]. Just recently, in a very detailed and elegant study, Prestipino and colleagues showed that the *JAK2*V617F mutation, through constitutive activation of STAT3, enhances the expression of PD-L1 on *JAK2*V617F-mutant cells and that patients with *JAK2*V617F-mutant MPN display increased expression of PD-L1 in the bone marrow compared to healthy controls [73]. Moreover, patients with MPN show increased expression of MDSC [72], but not Tregs in PB [74]. One of the mainstay treatments for Ph-neg MPN is the immunostimulatory cytokine interferon-alpha (IFN-α), which is able to induce complete hematological and major molecular remissions in a substantial proportion of patients [75].

Interestingly, treatment with IFN-α induces marked alterations in the phenotype of PBMCs, as the levels of Tregs and T-effector cells have been shown to increase during treatment with IFN-α [74, 76]. Treatment with IFN-α was shown to increase the amount of $CD56^{bright}$ NK cells and decreases the amount of $CD56^{dim}$ NK cells as well as the amount of both myeloid and plasmacytoid dendritic cells [77, 78]. Additionally, IFN-α enhances the expression of HLA-genes and other genes related to antigen processing and presentation [79], and it has been speculated that one of the effects of IFN-α is that the compound is able to prime the immune system to recognize and kill the neoplastic cells in the bone marrow [80]. Hence, there is compelling evidence of a deregulation of the immune system in MPN. An overview of this immune dysregulation and of the immune escape mechanisms in MPN is provided in Fig. 2.

Immunogenic antigens in Ph-neg MPN

As in AML and MDS, tumor cell killing in MPN relies on the recognition of TAAs and TSAs which are presented by tumor cells. Xiong and coworkers identified humoral immune responses against two immunogenic antigens in patients with polycythemia vera [81]; however, these antigens were also highly expressed in neutrophils from healthy donors and thus were not suited as targets for cancer immune therapy. One important feature that distinguishes Ph-neg MPNs from the other hematological malignancies is the very heterogenic mutational landscape, as approximately 70% of patients harbor the *JAK2*V617F mutation [61, 62] and roughly 15–20% of patients harbor a mutation in exon 9 of the *CALR*-gene [82, 83]. Both of these mutations generate a mutant neo-antigen, and we just recently showed that both of these neo-antigens are recognized by effector T cells, and cells carrying the mutations are killed by these specific effector T cells [84–86]. These results have provided the preclinical rationale for targeting the driver mutations in Ph-neg MPN by cancer immune therapy such as peptide vaccinations with JAK2-mutant or CALR-mutant epitopes, and we are about to initialize a phase I clinical vaccination trial with CALR-mutant epitopes for patients with *CALR*-mutant MPN.

Other cancer immune therapeutic options for Ph-neg MPN

In the time of writing of this review, no reports have been made on trials using cancer immune therapeutic modalities in Ph-neg MPN. In total, three immune therapeutic trials in MPN are active: one trial in patients with PMF and PV, testing the clinical efficacy of the PD-1 blocking antibody pembrolizumab (NCT03065400), and another trial testing the maximal tolerated dose of IPI or NIVO in patients with relapsed hematologic malignancy following alloSCT (NCT01822509). The last trial is testing the safety profile of the PD-L1 blocking antibody durvalumab in patients with PMF (NCT02871323). None of the above has reported any results yet.

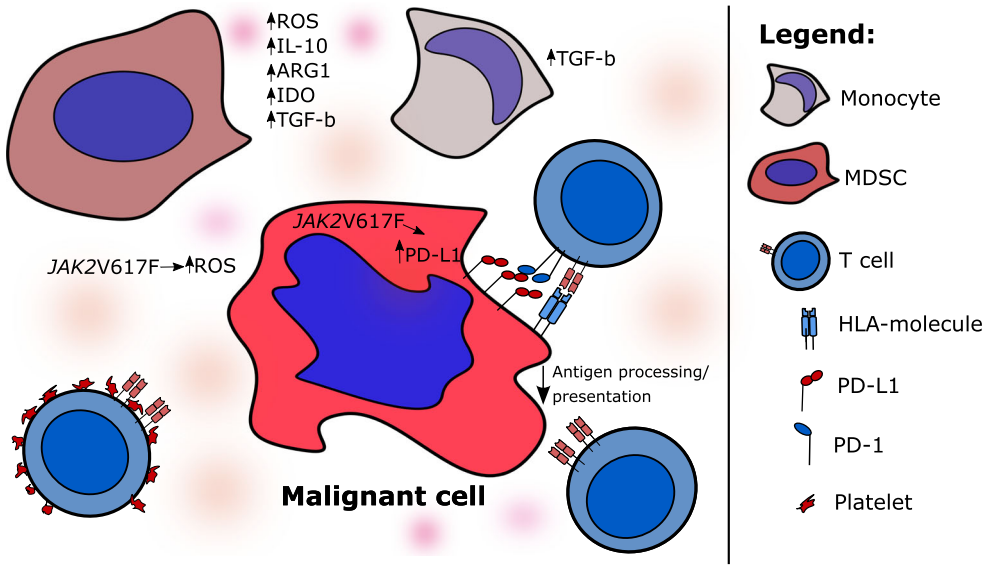

Fig. 2 An illustration of some possible immune escape mechanisms in MPN. Patients with MPN have increased levels of MDSC in peripheral blood. These MDSC secrete excessive amounts of arginase-1 and probably other immunosuppressive substances too. Monocytes from patients with primary myelofibrosis secrete elevated amounts of TGF-β. The *JAK2*V617F mutation induces PD-L1 expression through STAT3 phosphorylation. Patients with MPN show decreased expression of genes related to antigen processing and presentation. Platelets bind to T cells and probably inhibit activation. The *JAK2*V617F mutation leads to formation of reactive oxygen species, which may interfere with T cell function

Future perspectives

In light of the above, the future looks promising for the potentials of cancer immune therapy for the myeloid malignancies. Given the impressive data generated by CAR T cells for lymphoid malignancies, it is highly intriguing to identify targets for CAR T cells in myeloid malignancies. However, the lack of suitable extracellular targets that are only expressed by the malignant cells is a major issue to be solved. In the setting of MPN, it has been reported that mutant CALR is presented on the cell membrane in mutant cells [87]. Given the strong $CD4^+$ T cell responses against mutant CALR [85], it would seem most likely that patients display CALR-mutant-specific antibodies. The occurrence of such antibodies could be exploited therapeutically by both anti-CALR-mutant-specific antibodies, CAR T cells, and BiTEs. However, the presence of such antibodies has not been reported, which could either be explained by the fact that the binding of mutant CALR to the cell membrane is highly unstable, or the mutant epitope is not presented to B cells, hence preventing B cell activation.

Another form of cellular therapy would be adoptive cell therapy (ACT). Tumor infiltrating lymphocytes (TILs) expanded from malignant tissue, such as the bone marrow, may be expanded and reinfused to the patient, whom in advance to TIL infusion has received lymphodepleting chemotherapy to decrease the level of regulatory cells. The TILs, of which some are tumor-specific, will then recognize and kill neoplastic cells. Another method of adoptive cell therapy is the use of T cell receptor (TCR) transduced T cells. By this method, autologous T cells are transduced with a TCR specific for an antigen (e.g., neo-antigen) of interest. This would however require the identification and cloning of an autologous or HLA-compatible neo-antigen-specific TCR. The aspect of cross-reactivity with wild-type antigens is an issue to be carefully addressed in this setting, as cross-reactivity with wild-type epitopes would result in potentially lethal side effects. However, this form of therapy would make it possible to treat patients with advanced and disseminated disease, who are otherwise not expected to have any effect from treatment with more lenient modalities such as therapeutic cancer vaccination. In the setting of myeloid malignancies, these patients are patients with advanced PMF and MDS or relapsed/refractory AML.

Trials on therapeutic cancer vaccinations have mainly been disappointing, likely due to the fact that effects are mainly found in patients with low disease burden [88]. As such, it is obvious to start vaccination either at an early disease stage or after the patient has reached a major or even complete response to first-line therapy. In MPN, it could be speculated that the immune enhancing properties of IFN-α might enhance the anti-tumor immune response induced by therapeutic cancer vaccination. Of interest, IFN-α has shown clinical effect not only in MPN but also in AML [89], and using IFN-α as an adjuvant to therapeutic cancer vaccination could potentially have an effect in this setting.

In MPN, it is obvious to target the *JAK2* and *CALR* driver mutations by therapeutic cancer vaccination, as both the *JAK2*V617F and especially the *CALR* exon 9 mutations generate highly immunogenic neo-antigens [84–86]. In the setting of AML/MDS, there are several interesting neo-antigens that may be targeted by vaccination. Targeting of TAAs such as PR1 and WT1 has been effective in some settings, but neither are ideal targets, as both are expressed by healthy tissues leading to some degree of immune tolerance to these antigens. As such, neo-antigens generated by the *NPM1* mutations are promising targets, as these are highly immunogenic [39]. Even more, both AML and MDS are characterized by several recurrent chromosomal aberrations, which, just as the bcr-abl translocation generate neo-antigens, could be targeted by therapeutic cancer vaccination.

However, as noted above, patients with myeloid malignancies demonstrate a vast plethora of immunoregulatory mechanisms such as IDO, PD-L1, arginase, and ROS, which may thwart a vaccine-induced tumor-specific immune response. Inhibition of these mechanisms by specific inhibitors such as IDO inhibitors and blocking antibodies might induce a more effective anti-tumor immune response compared to vaccination monotherapy. Another method of targeting the aforementioned immunoregulatory mechanisms would be through vaccination, as it has been shown that both IDO, arginase-1, and PD-L1 are targets for specific T cells [90–92], and stimulation of T cell cultures with PD-L1 derived epitopes has been shown to enhance antigen-specific and leukemia-specific T cell responses in vitro [93, 94], an effect that is believed to rely on the induction of anti-regulatory T cells [95]. Most interestingly, patients with MPN display frequent and strong T cell responses against both PD-L1 [96] and arginase-1 [97], and as described above, the *JAK2*V617F mutation enhances the expression of PD-L1 on mutant cells. As such, by covaccinating with JAK2-mutant epitopes and PD-L1 derived epitopes, one may induce both a tumor-specific immune response (JAK2- and PD-L1-specific T cells) and an anti-regulatory immune response (PD-L1-specific T cells) too. Even more, Tregs, which are increased in patients with MDS and AML, display high intracellular amounts of the transcription factor FoxP3, which is also a target for specific T cells [98]. As such, vaccination against these regulatory epitopes will probably enhance the anti-regulatory immune response [95] and hence the tumor-specific immune response.

Most interestingly, treatment with HMA not only enhances the expression of cornerstone immune checkpoints such as PD-L1 and CTLA-4, but HMA also increases the expression of several TSAs—the so-called cancer germline antigens (CGA) [33] and increase the amount of circulating CGA-specific $CD8^+$ T cells [34]. This has led to the concept of combining HMAs with immune checkpoint inhibitors and

therapeutic cancer vaccination against CGAs. It is believed that vaccination with CGA-derived epitopes will induce a specific immune response, and that HMA will enhance the expression of CGA on malignant cells. However, the expression of checkpoints will be increased as well, but the negative effects of this increased expression will likely be negated through the use of immune checkpoint blocking antibodies. ROS have also been shown to attenuate the tumor-specific immune response [99], and the levels of ROS are increased in both MDS [13] and MPN. Regarding the latter, it has been shown that the *JAK2*V617F mutation per se induces formation of excessive ROS [100], which might dampen the *JAK2*V617F- and PD-L1-specific immune response. As such, the inhibition of ROS formation or simply scavenging of ROS could potentially enhance the tumor-specific immune response. One of the hallmarks of MPN is megakaryocyte hyperplasia and elevated platelets in the peripheral blood. Interestingly, elevated platelet count is an adverse prognostic factor in several cancers [101]. Recently, it has been speculated that the thrombocythemia in MPN may increase the invasiveness and metastatic potential of second cancers in MPN [102], thereby explaining the inferior survival of MPN patients with secondary cancers [103] as well as the increased frequency of secondary malignancies in patients with MPN [104]. A probable explanation beyond the detrimental effect of thrombocytosis in cancer was just recently provided in an elegant paper by Rachidi and colleagues. By a series of experiments, it was shown that platelets bind to T cells and inhibit these through release of TGF-β [105]. As such, platelet inhibition in patients with MPN could potentially enhance the anti-tumor immune response and be used in concert with other cancer immune therapeutic modalities.

One last feature that may be exploited to enhance the effects of cancer immune therapy for the myeloid malignancies is to take advantage of so-called immunogenic cell death (ICD), reviewed by Kroemer et al. [106]. In short, under normal conditions, cells that undergo apoptosis do not elicit inflammation or an immune response. However, upon exposure to certain cytotoxic agents or radiation, several kinds of apoptotic cancer cells have been described to stimulate an immune response to cancer-specific antigens—hence the term immunogenic cell death. Notably, the exposure of wild-type CALR on the plasma membrane of apoptotic cells is a sign of ICD [107], and in a study in AML, mice were transplanted with AML blasts that either expressed high or low levels of CALR. Mice transplanted with the $CALR^{high}$ expressing cells, exerted better tumor control, and mounted a stronger immune response to leukemia antigen. Of note, this enhanced immune

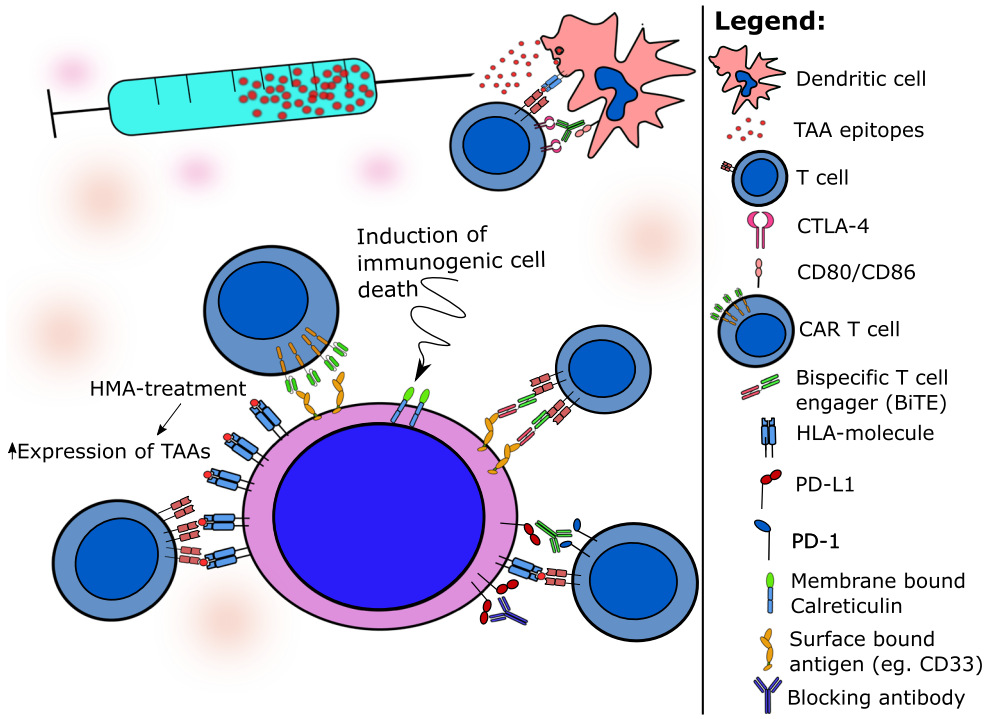

Fig. 3 An illustration of some of the described cancer immune therapeutic methods for AML and MDS. Vaccination with epitopes from tumor associated antigens will induce tumor-specific T cell responses. Blocking of CTLA-4, PD-L1, and PD-1 with specific antibodies will likely enhance the tumor-specific immune response. Bispecific T cell engagers and CAR T cells specific for surface antigens (e.g., CD33) expressed by the malignant myeloblasts could result in tumor cell killing. Treatment with hypomethylating agents increases the expression of tumor associated antigens, hence increasing the chance of T cell activation. Induction of immunogenic cell death through either radiation or chemotherapy will increase the expression of membrane-bound calreticulin, which will activate type I interferon signaling and initiate a tumor-specific immune response

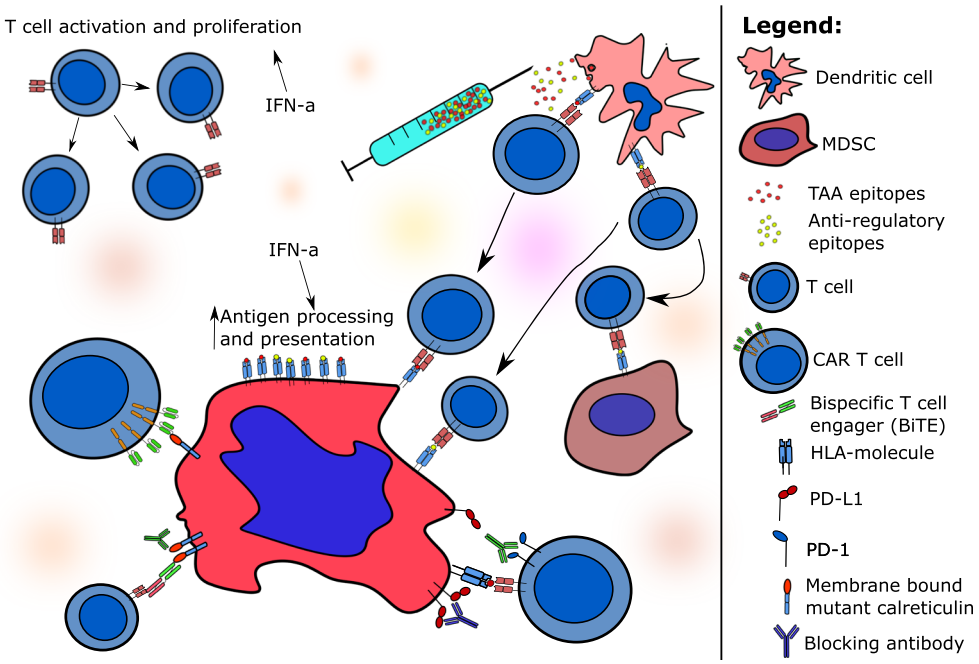

Fig. 4 An illustration of some of the described cancer immune therapeutic methods for MPN. Treatment with interferon-alpha will induce T cell activation and proliferation as well as increase antigen processing and presentation. Vaccination with JAK2- and CALR-mutant epitopes concurrent with anti-regulatory epitopes such as arginase-1 and PD-L1 derived epitopes will induce both a tumor-specific and anti-regulatory T cell response. PD-1 and PD-L1 blocking antibodies will prevent PD-L1 induced energy. If mutant calreticulin is expressed on the cell membrane, it can be used as a target for monoclonal antibodies, bispecific T cell engagers, and chimeric antigen receptor T cells

response was demonstrated to be facilitated by an enhanced type I IFN signaling conferred by CALR [108]. In patients with AML, expression of CALR had a marked effect on the immune response to TAAs, and patients with a high expression of CALR on the blasts exhibited superior survival [109]. Accordingly, the modulation and induction of immunogenic cell death in the myeloid malignancies could be another means to enhance the anti-tumor immune response. An illustration of some of the above mentioned immune therapeutic strategies for AML/MDS and MPN is provided in Figs. 3 and 4, respectively.

Conclusion

The myeloid malignancies are characterized by a dysregulation of the immune system, as both the expression of immune checkpoints, immune regulatory proteins, and genes related to immune cell function are deregulated. Several immune therapeutic modalities, namely CAR T cell, therapeutic cancer vaccination, and immune checkpoint inhibitors have been tested in AML/MDS with good results, and the combination of HMA, immune checkpoint antibodies, and therapeutic cancer vaccines looks promising. In MPN, no data on clinical trials in cancer immune therapy have yet been reported, but several trials are ongoing. The aspects of targeting the *JAK2*V617F and *CALR* exon 9 mutations by therapeutic cancer vaccination are highly intriguing. Combination with vaccines targeting regulatory mechanisms such as PD-L1 and arginase-1 will probably enhance the tumor-specific immune response. The identification of proper extracellular targets for CAR T cells and BiTEs is yet to come, as is the potential of ACT with TCR transduced T cells. The potential of ROS inhibition and/or platelet inhibition to augment the tumor-specific immune response in both AML/MDS and MPN has not yet been clarified, but could be a method to enhance the tumor-specific immune response.

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Compliance with ethical standards

Conflict of interest No authors have conflict of interest to disclose. However, it should be noted that Morten Orebo Holmström and Hans Carl Hasselbalch together with Mads Hald Andersen have filed a patent regarding the *CALR* exon 9 mutations and *JAK2*V61F mutation as a target for cancer immune therapy. The patent has been transferred to University Hospital Zealand, Zealand Region and Copenhagen University Hospital at Herlev, Capital Region according to Danish Law concerning inventions made at public research institutions.

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