Review

Immunotherapy of Acute Myeloid Leukemia: A Work in Progress

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Immunotherapy of Acute Myeloid Leukemia: A Work in Progress

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Abstract: In the last years, molecularly targeted agents and immune based treatments (ITs) have deeply changed the landscape of anti-cancer therapy. Indeed, ITs proved to be very effective in metastatic solid tumors, where outcomes were extremely poor with standard approaches. Such a scenario has been only partially reproduced in hematologic malignancies. In acute myeloid leukemia (AML), as innovative drugs are eagerly awaited in relapsed/refractory setting, different ITs have been explored, but the results are still unsatisfactory. In this work, we will discuss the most important clinical studies to date adopting ITs in AML, providing the bases to understand how this approach, although still in its infancy, may represent a promising therapeutic tool for the next future treatment of AML patients.

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1. Introduction

Anti-cancer immune-based therapeutics (ITs) rely on different approaches that have the goal to support host immune effectors to clear neoplastic clones. Although ITs have improved the prognosis of several advanced-stage solid tumors (metastatic melanoma, renal cell carcinoma, head and neck cancers, and non-small lung cancer) [1], benefits of ITs have been more limited in hematological malignancies, mostly restricted to classical Hodgkin lymphoma and primary mediastinal B cell lymphoma [2–4]. In acute myeloid leukemia (AML) several considerations need to be taken into account to correctly evaluate ITs: AML patients are severely immuno-compromised and the introduction or the addition of novel therapies may potentially alter the landscape of infectious complications with clinical practice guidelines being frequently updated by the European Conference on Infections in Leukemia (ECIL) [5,6]. Similarly, AML- and therapy-related damage can hamper immunological clearance of AML blasts by natural killer (NK) and CD8+ T cells [7]. Furthermore, AML blast can evade immune system either by reducing the expression and/or the presentation of HLA restricted leukemia associated/specific antigens (e.g., MHC class II genes loss or down-regulation after allogeneic-SCT has been extensively studied [8]). Moreover, bone marrow infiltrating T lymphocytes, although numerically similar to healthy donors, show upregulation of check point inhibitors such as PD1, TIM-3 and LAG-3 [9]. In a murine model of AML, CD8+ T cells present a typical exhaustion phenotype (TIM-3/PD-1 positive), where expression of TIM-3/PD-1 increases during AML progression and can be partially reduced by anti PD1 treatment [10]. In addition, AML blasts promote a tolerogenic immune microenviroment by releasing niche reactive oxygen species (ROS), arginase (Arg), indoleamine 2,3-dioxygenase (IDO), and extracellular vesicles. The overall effect is the inhibition of cytotoxic activity of T and NK cells, the emergence of exhausted T cells, regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs), while promoting the switch of tumor associated macrophages (TAMs) from immunogenic M1 to immune-suppressive M2 phenotype [11]. Indeed, TAMs can exert opposite functions: as part of tumor-extrinsic pathways of

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primary and adaptive resistance to ITs by expressing several immunosuppressive molecules, including checkpoint ligands (PDL-1, PDL-2, CD80, and CD86) [12]; on the other hand TAMs can promote apoptosis of neoplastic cells activated by stimulator of interferon genes (STING), CD40, and engagement of toll-like receptors [12].

Even considering these limitations, ITs represent an intensive area of clinical research. In the present work, we will provide the state-of-art of the studies on AML, highlighting the strategies adopted to overcome its natural resistance to ITs.

2. Immune Checkpoint Inhibition in Acute Myeloid Leukemia

a. PD-1/PDL-1 Blockade

PD-L1/PD-1 axis has been thoroughly studied over the past few decades and recent data indicated that CD34+ blasts from MDS and AML patients show upregulation of PDL-1, whereas PD-1 expression was raised in effector T cells and Tregs [9,13–15]. Furthermore, activated T-cells upregulate the antagonistic co-receptor PD-1. As PDL-1/PD-1 interaction dampens anti-tumor T-cell responses [16–18], the disruption of this pathway by anti-PD-1/PDL-1 monoclonal antibodies can revive worn-out T cells and prompt anti-tumor responses [19,20]. Although this strategy has provided striking data in solid malignancies, its role in AML and MDS setting needs to be further clarified.

2.2. PD-1 Inhibitors

2.2.1. Nivolumab

In a phase 2 study, 70 patients with R/R AML were treated with the combination of azacitidine 75 mg/mq days 1 to 7 with nivolumab 3 mg/Kg on days 1 and 14, every 4 to 6 weeks. The median age was 70 years old (range: 22–90); the median number of prior therapies was 2 (range: 1–7). The overall response rate (ORR) was 33%, with 7 patients reaching hematologic improvement that lasted for more than 6 months and 15 (22%) achieving full remission or complete remission with insufficient counts' recovery. Six patients (9%) showed stable disease for more than six months. When comparing hypomethylating agent (HMA)-naive patients (n = 25) and HMA-pretreated patients (n = 45), the ORR was 58% and 22%, respectively. 8 (11%) patients experienced immune-related side effects graded 3 to 4. Pretreatment bone marrow and peripheral blood CD3+ and CD8+ T cells strongly predicted response. Interestingly, after 2 and 4 doses of the drug, non-responder patients showed increased CTLA-4 expression on CD4 T cell effectors [21]. On these bases, protocol was amended allowing to introduce a second cohort, where 31 R/R AML patients with high CTLA-4 expression were treated with anti CTLA-4 moAb ipilimumab together with azacitidine and nivolumab to further improve T cell responses. With a median OS of 10.5 months, the ORR rate among efficacy evaluable patients (N = 24) was 46% (CR/CRi rate: 36%), which compared favorably to azacitidine plus nivolumab. As anticipated, 25% of patients experienced grade 3–4 immune related adverse events (ir-AEs), higher than azacitidine plus nivolumab cohort [22].

Nivolumab was also investigated by Davids et al. that focused on recurrent hematologic malignancies following allogeneic transplantation (10 AML cases out of 28). This multicenter phase I trial evaluated the effectiveness and immunologic activity as well as the maximum tolerated dose and safety of the drug. Nivolumab was given out every two weeks until either occurrence of progression or intolerable side effects. The initial dose level was 1 mg/kg, with the option to reduce to 0.5 mg/kg or increase to 3 mg/kg. The 1-year PFS rate was 23% and the OS rate was 56% with a median follow-up of 11 months. Among the 28 patients enrolled, 3 patients could not be evaluated for efficacy response whereas 25 patients could be owing to early toxicity. Twenty of the 25 received treatment at 0,5 mg/kg, while 5 received treatment at 1 mg/kg. For the efficacy on both levels, ORR was 32%. The median OS for all patients was 21.4 months, and the overall patient 1-year OS rate [23] was 56%. Two of 6 patients treated at 1 mg/kg experienced dose-limiting toxicity (DLT) from ir-AEs.

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Twenty-two patients were treated at 0.5 mg/kg, and 4 DLTs occurred, including 2 ir-AEs and 2 fatal GVHD.

A phase 2 trial combining nivolumab with idarubicin + cytarabine was carried out in patients with newly diagnosed AML (N = 42) and HR-MDS (N = 2) to evaluate the activity of nivolumab in the frontline setting. Using multiparameter flow cytometry (MFC), the composite CR rate was 78%, of which 79% showed no evidence of measurable residual disease (MRD). Nineteen patients proceeded to allo-SCT, with 13 patients (68%) experiencing GVHD. The chemo-immunotherapy regimen showed tolerable toxicity without high incidence of ir-AEs. Overall, the median OS was 18.5 months, and for those who underwent allo-SCT was 25 months. Notably, there was no difference in OS between responders who continued treatment after remission and those who were bridged to allo-SCT, suggesting that nivolumab may have the ability to restore anti-tumor immune surveillance and eliminate MRD (Ravandi et al) [24].

As observed in certain lymphoma treatment subgroups, Liu et al. [31] postulated that immunomodulation with check point inhibitors (CIs) might induce anti-leukemia immune response avoiding or postponing a potential disease relapse in intermediate/poor risk AML. They conducted a multi-center phase II trial to assess the efficacy of nivolumab as maintenance therapy for AML in first CR or CR with incomplete hematologic recovery (CRi) not eligible for HSCT (NCT02275533). Enrollment was allowed within 60 days after bone marrow biopsy confirming recovery from final chemotherapy. Eighty patients were randomized to observation or to receive nivolumab (3mg/kg every 2 weeks for forty-six doses). The 2-year PFS was 30.3% and 30% in the nivolumab arm and the observation arm, respectively (p=0.38). The median OS was 53.9 and 30.9 months in the two subgroups; however, the 2-year OS was 60.0% and 52.8% in the nivolumab and observation arm, respectively (p=0.23). AEs were more frequent in nivolumab arm with 27 (71.0%) experiencing grade 3 or higher toxicity as compared to 5 patients (12.2%) in the observation arm (p<0.001) [25]. These findings showed that nivolumab maintenance did not benefit high- and intermediate-risk AML patients after chemotherapy as it failed to improve the 2-year PFS and OS and increased the incidence of AEs.

To describe transplantation outcomes with regard to GvHD and the effects of various GvHD prophylaxis strategies, Oran et al. conducted a clinical trial in patients with AML and MDS patients treated with either PD-1 or CTLA-4 inhibitors, or both, before receiving an allo-HSCT. Of the 43 patients, 9 received ipilimumab and 34 received nivolumab. Nivolumab was administered to the patients in two cases as a monotherapy, in 19 cases in combination with chemotherapy, and in the remaining cases in combination with a hypomethylating agent. Twenty-four of the 43 patients experienced CR, 6 experienced CR with incomplete hematologic recovery, 5 showed hematologic improvement, and 1 experienced a partial response; the median time to best response was 41 days. At the time of their best response, 27 of these patients discontinued CI therapy and proceeded to allo-HSCT. Tacrolimus and mini-methotrexate with or without anti-thymocyte globulin were used as part of the GvHD prophylaxis in 21 patients, while post-HSCT cyclophosphamide (PTCy) or tacrolimus with or without mycophenolate mofetil was used in 22 individuals. The selection of prophylaxis for the patients was based on donor type. Both baseline disease and transplantation characteristics were comparable between PTCy patients and no-PTCy patients, 32% and 10%, respectively. Matched control analyses using patients with no prior exposure to CIs confirmed the increase in grade 3-4 aGVHD with those agents [26].

2.2.2. Pembrolizumab

The combination of pembrolizumab and azacitidine was evaluated in a phase 2 trial including de novo and R/R AML. Patients received azacitidine 75 mg/m^2 Days 1-7 with pembrolizumab 200 mg beginning on day 8 and every 3 weeks thereafter. Among the 37 R/R AML, 29 patients were evaluable for response showing an ORR of 55%, with 14% CR/CRi; among the 22 patients having a de novo AML who were ineligible for intensive chemotherapy, 17 resulted evaluable for response showing an ORR of 94% with 47% CR/CRi. Median OS for this cohort was 13.1 months [27]. In another trial (NCT02996474), pembrolizumab was given with 10 days of decitabine in 10 patients with R/R AML.

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The median OS was 10 months with responses observed in six patients: 2 achieved CR, 1 showed a morphologic leukemia-free state and 3 experienced a stable disease [28]. A US group conducted a phase II trial providing high-dose cytarabine followed by pembrolizumab 200 mg on day 14 to assess whether this combination could improve outcomes of R/R AML. Thirty-seven patients were enrolled with a median age of 54 years; the majority showed either refractory (43%) or relapsed disease with CR duration <1 year (43%). The median OS of the entire cohort was 11.1 months, with an ORR and a composite CR of 48% and 38%, respectively. Among patients showing refractoriness/early relapse and those who received this combination approach as first salvage therapy there were encouraging outcomes (median OS, 13.2 and 11.3 months, respectively). Rare grade ≥3 ir-AEs after pembrolizumab administration included maculopapular rash (n = 2; 5%), aminotransferase elevation (n = 2; 5%), and after the failure of HMAs, lymphocytic infiltration on liver biopsy (n = 1; 3%) [29]. In a small phase II study conducted on 20 patients affected by intermediate-risk (60%) and high-risk AML in CR, pembrolizumab was administered as consolidation after autologous-SCT for 8 doses. The study met the planned objective of a leukemia free survival of 48.4% at 2 years and the two-year OS, NRM and CIR was 68%, 5% and 46% respectively [30].

The use of pembrolizumab was also investigated in high-risk and HMA resistant MDS to define its potential efficacy in myeloid malignancies. The phase 1b KEYNOTE-013 study evaluated the use of pembrolizumab as monotherapy in 28 patients with high-risk MDS following HMA treatment failure. No patient achieved complete or partial response; 5 patients (19%) had bone marrow complete response, 12 (44%) stable disease, 10 (37%) progressive disease, 6 (22%) cytogenetic response, and 5 (19%) hematologic improvement. Median OS was 6.0 months with a 2-year OS rate of 17% [31]. Another phase 2 study explored the combination of pembrolizumab and azacitidine in patients with both de novo and HMA treatment failure intermediate to high-risk MDS. After a median follow-up of 12.8 months, the median OS was not reached and the ORR and CR rate was 76% and 18%, respectively in HMA-naïve patients (n=17). In the HMA treatment failure cohort (N = 20), the ORR was 25% with a CR rate of 5% and median OS of 5.8 months [32]. According to these data, pembrolizumab appeared to hold inefficacy in patients with high-risk MDS and in those HMA resistant both as monotherapy and in combination regimens.

2.2.3. Tislelizumab

Tislelizumab is an experimental humanized immunoglobulin G4 monoclonal antibody that has a high affinity for the PD-1 protein. It is made up to reduce binding to the Fc receptor on macrophages to prevent antibody-dependent phagocytosis, which is a potential mechanism of resistance to anti-PD-1 therapy [33]. Tislelizumab monotherapy or in combination with chemotherapy exhibited antitumor effectiveness in patients with solid tumors in early-phase clinical studies, and it also displayed a safety profile similar to other anti-PD-1 antibodies [34,35].

In a phase 2, single-arm study, R/R AML patients received azacitidine or decitabine plus CAG regimen (cytarabine, aclarubicin, G-CSF) with tislelizumab. Twenty-seven patients were enrolled; ORR was 63% (14 CR/Cri, 3 PR, 10 no response). Median OS and EFS were 9.7 and 9.2 months, respectively with grade 2–3 ir-AEs observed in 4 patients (14.8%) [36].

2.3. PD-L1 Inhibitors

The phase 2 study FUSION-AML-001 was designed to evaluate the activity and safety of durvalumab, a PDL-1 inhibitor, in combination with azacitidine for untreated patients aged ≥65 years with AML. Patients were randomized to receive azacitidine 75 mg/m^2 on days 1 through 7 with (Arm A, n = 64) or without (Arm B, n = 65) durvalumab 1500 mg on day 1, every 4 weeks. The combination regimen did not significantly improve ORR (Arm A, 31.3%; Arm B, 35.4%), duration of response (Arm A, 24.6 weeks; Arm B, 51.7 weeks) and OS (Arm A, 13.0 months; Arm B, 14.4 months). DNA methylation, mutational status, and PDL-1 expression were not associated with response to treatment [37]. Another PDL-1 antibody, avelumab, that has been FDA approved for treating Merkel cell carcinoma, renal cell carcinoma, and urothelial carcinoma, was tested in untreated and R/R AML. A phase 1 study was conducted to evaluate the combination of avelumab and decitabine as frontline

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treatment for AML patients unfit for intensive chemotherapy (N = 7). Response rate was unsatisfactory, with only one patient who reached a CR and 3 who maintained a stable disease as the best response to therapy [38]. Avelumab was also investigated in association with azacitidine in R/R setting. In a phase 1b/2 trial 19 patients received azacitidine 75 mg/m^2 on days 1 through 7 and avelumab on days 1 and 14 of 28-day cycles (dosed at 3 mg/kg for the first 7 patients and at 10 mg/kg for the subsequent 12 patients). The median age was 66 years, 100% had European LeukemiaNet 2017 adverse-risk disease, and 63% previously received a hypomethylating agent. The median OS was 4.8 months and responses with full or incomplete bone marrow recovery were 10.5%, that appeared similar to the historical CR/CRi rate of 16% observed with HMA [39,40]. A further analysis included in these studied confirmed that the overexpression of PD-L2 in myeloid blasts and monocyte-restricted increase in PDL-1 expression with therapy are the most important causes of inactivity by PDL-1 inhibitors in patients with AML [37,39].

No benefit from the employment of anti-PDL-1 inhibitors was also seen in MDS patients.

A phase 2 study including frontline azacitidine plus the ICI durvalumab (arm A) versus azacitidine monotherapy (arm B) for 84 HR-MDS was conducted. The ORR was 61.9% and 47.6% in arm A and arm B (p= .18), respectively with a median OS of 11.6 months and 16.7 months (p= .74), confirming the inefficacy of PDL-1 inhibitors also in MDS scenario [41].

3. CTLA-4 Inhibition

CTLA-4, which is expressed on the surface of T cells, prevents T-cell activation and suppresses T-cell maturation and differentiation. In T regs and naive resting T cells, CTLA-4 is expressed constitutively; it remains in the cytoplasm until activation, then it is upregulated on the surface within one or two days. In memory T cells, activation and expression occur even more quickly [42]. The potential benefit of adjuvant CTLA-4 blockade following allo-SCT in AML was suggested by preclinical studies in which CTLA-4 blockage in murine bone marrow chimeras resulted in potent anti-leukemic action while preventing GVHD. Additionally, research on AML has revealed elevated expression of co-stimulatory molecules, including CD80 and CD86, which have been linked to high relapse rates and a poor prognosis [43,44].

3.1. Ipilimumab

Ipilimumab is a human IgG1 kappa monoclonal antibody that specifically blocks CTLA-4. Davids et al. [51] postulated the hypothesis that ipilimumab can restore the antitumor activity through promoting graft-versus- leukemia (GVL) mechanisms in a phase I/Ib study. In this trial, patients with relapsed hematologic malignancies after transplantation were treated with ipilimumab at 3 or 10 mg/Kg every 3 weeks for 4 cycles, followed by maintenance dosing in 12-week increments, for up to 1 year. Twenty-eight patients were enrolled in the study (12 patients had relapsed AML). Ipilimumab given at 10 mg/kg, showed encouraging results: a CR was achieved in 5 (42%) of the 12 AML patients, 4 with extramedullary leukemia, and 1 with secondary AML, with 3 responses lasting over a year. The responders showed a significant reduction of T regs and a raise of effector T cells activities, confirming the hypothesis of an augmented GVL mechanisms [45]. The use of ipilimumab was assessed in addition to decitabine in a phase I, multicenter, investigator-initiated study (CTEP 10026). The study enrolled patients with R/R MDS/AML treated with (Arm A) and without (Arm B) prior allo-SCT. Toxicity profile was manageable, with 50% of patients experiencing a grade 1–2 ir-AEs, that were managed by steroids administration, except for the grade III acute GVHD, steroid refractory, complicated by fatal septic shock. Responses were observed in 8 of 16 evaluable patients (3 CR, 2 CRi, and 3 marrow CR), with a median OS of 18.3 months [46]. In high risk MDS setting, ipilimumab as single agent showed poor activity and did not improve outcomes. In a phase 1 study, among 29 HR-MDS patients who received Ipilimumab, 1 patient achieved a marrow CR for an ORR of 3.4%; the duration of response was 3 months [47].

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4. CD47-SIRPα Blockade

Malignant cells use the dominant macrophage immunological checkpoint CD47 to circumvent innate immunity. When CD47 binds to its receptor signal-regulatory protein alpha (SIRPα) on the surface of macrophages, it transmits a "don't-eat-me" anti-phagocytic signal. CD47-SIRP interaction promotes inhibition of myosin-IIA build-up at the phagocytic synapse through the recruitment of downstream Src homology-2 domain-containing protein tyrosine phosphatases (SHP-1 and SHP-2), thus preventing macrophage-mediated tumor phagocytosis [48]. Indeed, cellular stability requires a delicate balance between pro- and anti-phagocytic signals [49]. AML and HR-MDS have higher levels of CD47 than normal hematopoietic stem cells, contributing to poor prognosis by evasion of phagocyte-mediated immune surveillance [50,51]. The Leukemia Stem Cell (LSC) is especially vulnerable to CD47 blockage because it overexpresses calreticulin, a prominent pro-phagocytic signal [48]. Anti-CD47 monoclonal antibodies have been shown both *in vivo* and *in vitro* to revert the anti-phagocytic signal and eliminate AML LSCs, suggesting their potential role in the treatment of AML and HR-MDS [52]. Several molecules that target the CD47-SIRP pathway are now being tested in clinical trials (Table 2) [53]. These novel agents are either decoy receptors (SIRP-IgG Fc domain) or monoclonal antibodies that directly block CD47.

4.1. Magrolimab and other CD47/SIRPα inhibitors

Magrolimab is a novel humanized immunoglobulin G4 anti-CD47 antibody that improves tumor cell phagocytosis by inhibiting CD47 binding to signal-regulatory protein alpha [52]. In conjunction with rituximab, magrolimab has shown activity in R/R non-Hodgkin lymphoma [54]. Magrolimab was well tolerated and exerted modest single-agent activity in the phase 1 CAMELLIA trial, which involved 15 patients with R/R AML. Of them, 73% achieved stable disease, and 40% had lower bone marrow blast counts (mean decrease of 27%, range 5-67%) [55]. Combination regimens using drugs that boost the production of pro-phagocytic signals on leukemic cells, hence synergistically triggering leukemic phagocytosis, were devised in an effort to increase the antileukemic effect of CD47 inhibition [56]. Preclinical research showed that azacitidine significantly increased calreticulin, a cell surface pro-phagocytic marker on malignant myeloid cells [56]. Magrolimab + azacitidine also boosted phagocytosis in myeloid models both *in vitro* and *in vivo*, offering a solid mechanistic justification to explore the combination in patients [55–57].

A phase 1b trial was conducted to assess the efficacy and safety of magrolimab in combination with azacitidine in patients with previously untreated AML unfit for intensive chemotherapy (N = 52); among them, 64% had poor-risk cytogenetics, and 65% had TP53 mutations. Patients received a magrolimab priming/intrapatient dose-escalation regimen (1–30 mg/kg intravenous weekly followed by 30 mg/kg every 2 weeks in cycle 3 and beyond) in combination with azacitidine. Among the 34 patients evaluable for response, 65% showed an objective response including a CR/CRi rate of 56%, of which 37% achieved MRD negativity by multiparametric flow-cytometry. The median time to response was 2 months. The objective response rate in patients with TP53-mutant AML was 71% (15/21), including 67% (14/21) of CR/CRi. Median OS for TP53-mutant and wild-type AML patients were 12.9 and 18.9 months, respectively [58]. Based on these promising results, particularly in TP53-mutant AML, it was postulated the ongoing randomized phase 3 ENHANCE-2 trial which compares magrolimab plus azacitidine to the physician's choice of venetoclax plus azacitidine or 7 + 3 chemotherapy in untreated TP53-mutant AML (NCT04778397) [59].

Recently, Daver et al. presented data from the phase 1/2 study including the novel triplet regimen of magrolimab in combination with AZA and VEN in older/unfit or high-risk AML. The initial phase Ib enrolled only patients with R/R AML. The Phase II expansion trial included both frontline and R/R patients. The frontline cohort enrolled patients ≥75 years; patients with documented comorbidities determining ineligibility for intensive therapy or harboring adverse risk karyotype (defined by ELN 2017 criteria) and/or with TP53 mutation regardless of age/fitness. The ORR in de novo AML was comparable with prior observed responses in patients received AZA/VEN, with an ORR of 81% (35/43) including an ORR of 74% (20/27) in patients with TP53 mutations. Median OS was not reached in newly diagnosed non-secondary AML patients; the median OS was 7.6 months

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among untreated secondary AML with TP53 mutation. Responses in R/R AML were scarce, with prior VEN exposed patients faring poorly (ORR 11%), resulting in closure of this study arm. Grade 3 anemia occurred in 23% (18/79), and median hemoglobin drop was 1.2 g/dL after magrolimab infusion [60].

Evorpacept (ALX148) is a high-affinity CD47-blocking protein with a modified Fc domain that has been used in combination with magrolimab to prevent red cell agglutination. There are two ongoing trials assessing ALX148 combined with AZA in high-risk MDS (ASPEN02 trial—NCT04417517) and with AZA + venetoclax in AML (ASPEN05 trial—NCT04755244). The combination of evorpacept + AZA is tolerable, according to the preliminary findings of the phase 1 portion of the ASPEN05 trial. There were no severe treatment emergent AEs associated with evorpacept, and most of them were mild. Subjects with newly diagnosed and relapsed/refractory AML (including patients previously treated with venetoclax) showed anti-leukemic activity, with bone marrow blast reduction ranging from 20 to 100% [61]. The second phase of the trial, with a random design, will further assess the combination.

A second-generation anti-CD47 IgG4 antibody with a distinctive binding epitope and red-cell-sparing qualities was created in China and is known as lemzoparlimab (TJC4, TJ011133). A phase II clinical trial (NCT04202003) is being conducted for patients with R/R AML and MDS. One patient achieved morphologically defined leukemia-free status, and only 1 out of five enrolled patients experienced grade 3 AEs [62]. A phase III clinical trial combining lemzoparlimab and AZA has been authorized for HR-MDS (NCT05709093).

5. The TIM-3/Galectin9 Axis

A co-inhibitory receptor known as TIM-3 is expressed on CD4+ Th1 cells, CD8+ cytotoxic T-cells (CTLs), and other innate immune cells like dendritic cells, monocytes, macrophages, mast cells, and NK cells as well as on other cancerous cells [63,64]. The TIM-3 gene is located among the IL4 and IL-5 genes on chromosome 5q33.2. TIM-3 is a single transmembrane (TM) protein that has an N-terminal IgV domain and a mucin domain with glycosylation sites in its extracellular tail. The TM domain and the cytoplasmic tail in the C-terminus follow this one. Although TIM-3 lacks the traditional tyrosine-based inhibitory motifs, it does have a conserved region with five tyrosine residues that are phosphorylated following TIM-3 interaction with its ligands [65]. The earliest and most researched of the four TIM-3 ligands is galectin-9 (gal-9), which causes Th1 cells to undergo apoptosis [66] and is essential for tumor cell immune evasion. T-cell dysfunction is caused by TIM-3 overexpression in human and mouse tumor models [67]. TIM-3 and PD-1 are frequently co-expressed, and inhibiting TIM-3 by itself or in combination with other co-inhibitory molecules can restore T-cell exhaustion. High amounts of TIM-3 have been observed in immune cells, especially T-cells and NK cells, which promote immunological exhaustion, as well as on LSCs, where it serves as a unique marker, in AML [68]. The ability of AML cells to proliferate was decreased when TIM-3/gal-9 binding was inhibited *in vitro* [69], while in murine models, an anti-human TIM-3 MoAb eradicated LSCs without impairing regular hematopoiesis [70]. Several MoAbs, including MBG453 (sabatolimab), TSR-022, BMS-986258, LY3321367, SYM023, BGB-A425, and SHR 1702 [71] are currently being evaluated in studies for solid malignancies, but only MBG453 has so far demonstrated preliminary efficacy and safety in AML and MDS. Eight phase I/II trials of MBG453 are now being conducted, either as a monotherapy or in combination with HMAs, PD-1 inhibitors, the MDM2 inhibitor HDM201, and venetoclax.

The preliminary data from the NCT03066648 trial (MBG453 + HMAs) showed an ORR of 58% in MDS and 38% in newly diagnosed AML patients for the MBG453 + DAC arm and 70% in MDS and 27% in AML patients for the MBG453 + AZA arm, respectively. The most common 3/4 AEs were thrombocytopenia, anemia and neutropenia; in the MBG453 + DAC group, four ir-AEs were reported (ALT increase, arthritis, hepatitis and hypothyroidism), compared to none in the MBG453 + AZA cohort [72,73].

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6. The LAG-3/MHC pathway

LAG-3 (CD223) gene is located on the short arm of chromosome 12 (12p13.32), coding for a 70 kDa type I membrane CD4-like protein with higher affinity for binding MHC class II than CD4, whose engagement prevents T-cell activation [1]. Given that the CD4 co-receptor locus is nearby and has a similar intron/exon structure, it is likely that the CD4 and LAG-3 genes diverged from a single common ancestor gene by gene duplication. The mature protein is composed of two metalloproteases (ADAM 10 and ADAM 17) that are activated by TCR signaling, a TM region with four external immunoglobulin-like domains, and a cytoplasmic tail that enables the intracellular transduction of inhibitory signals [74,75].

While information on AML is currently limited, LAG-3 and PD-1 co-expression in solid tumors has been linked to poor susceptibility to PD-1 blockage and has been recommended as a biomarker for predicting the success of immunotherapy [76,77]. It should be kept in mind that immune suppression and antigen presentation mechanisms can both be impacted by MHC class II expression in AML blasts. PD-1 inhibitors are currently being tested in conjunction with antibodies targeting LAG-3 in solid tumors, lymphomas, and multiple myeloma, with encouraging results, particularly in metastatic melanoma, where the combination of nivolumab + relatlimab significantly improved PFS [68].

The AARON study (NCT04913922), which will test the safety and tolerability of a triplet of AZA, nivolumab (anti-PD-1) and relatlimab (anti-LAG-3), in patients with relapsed/refractory AML and newly diagnosed AML aged > 65 years, is the only clinical trial that has been activated in AML patients to date. Recruitment began in November 2022, and no results are available at the time of writing.

7. The CD27/CD70 Axis

CD27 belongs to the tumor necrosis factor superfamily, it is a strong costimulatory molecule for T-cell activation [78]. CD27 signal is regulated by its unique ligand CD70 that is alternately upregulated on immune cells upon activation, but it is not expressed in normal tissues and hematopoietic system, indicating that the early hematopoietic phases show independency from the CD27/CD70 axis [79]. Abnormal CD70 expression alone (solid tumors) or together with CD27 co-expression (hematologic malignancies) favors the development of a suppressive microenvironment [80]. Indeed, CD27 expression allows immune escape as high levels of sCD27 were linked to a poor prognosis for AML, and abnormal CD27 expression was seen in LSCs in both AML and chronic myeloid leukemia (CML) [81]. In LSCs, CD27/CD70 pathway induces the abnormal activation of the Wnt pathway, leading to disease progression, drug resistance, and LSC proliferation [89]. Additionally, CD27/70 mediated activation of MEK pathway, transcription factor AP-1 and beta-catenin activation (by Wnt pathway) can promote blast survival. The anti-CD79 antibody cusatuzumab (ARGX-110) *in vitro* cleared LSCs by causing differentiation and apoptosis [82].

A phase I/II trial (NCT0030612) evaluating the safety and effectiveness of cusatuzumab as monotherapy and in combination with AZA in untreated AML patients but ineligible for intensive therapy, has been designed based on these preclinical evidences. A durable response was seen in six patients, with a median PFS that was not reached, and the best hematological response was CR/CRi (2/8 patients), with a median time to response of 3.9 months. Another study investigating cusatuzumab plus AZA in HR-MDSs or newly diagnosed AML not eligible for intensive therapy has been completed but the data are not yet available (NCT042415499). Furthermore, there are two ongoing but not recruiting clinical studies examining the combination of cusatuzumab + AZA (CULMINATE trial—NCT04023526). In this trial a total of 38 patients were enrolled: 12 in phase I and 26 in phase II. An objective response (≥partial remission) was achieved by 19/38 patients (50%); 14/38 (36%) achieved complete remission [83]. Cusatuzumab + AZA + venetoclax (ELEVATE trial—NCT04150887) for newly diagnosed AML ineligible for intense chemotherapy is ongoing.

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8. Bispecific antibodies (T cell Engager): BiTE.

Bispecific T cell-redirecting antibodies are typically designed to bind to the CD3 chain, the signaling invariant component of the TCR complex and to a selected Tumor Associated Antigen (TAA) [84], that in case of AML are mainly directed against: CD123 [85], CD33 [86], and CLL1 [87]. The most relevant BiTE tested so far is flotetuzumab, an anti CD123xCD3, that in a phase 1 study in 88 patients with r/r AML after at least 2 lines of therapy showed a 30% ORR. Most AE reported were cytokine release syndromes (CRS) and infusion-related reactions, mostly of grade 1-2 [88]. Moreover, JNJ-63709178, a CD123xCD3 targeting antibody was tested in a phase 1 study on 62 AML patients, with disappointing results characterized by minimal clinical activity (non-sustainable reduction in blasts) and substantial toxicity in terms of CRS, heavy to manage also with step-up dosing [89]. In a phase I study of vibecotamab (XmAb14045), another CD123xCD3 BiTE, preliminary data showed an ORR of 14% in AML patients [90]. Therefore, a phase 2 study (NCT05285813) for MRD-positive AML and MDS after HMA treatment failure is recruiting patients at MD Anderson [91].

CD33 appears an alternative feasible target for BiTE treatment strategies. In a phase 1 study, AMG 330, an anti CD33xCD3 with short half-life (it requires continuous infusion like blinatumomab), achieved evaluable responses in 8 of 42 (19%) patients: 3 CR, 4 CRi, and 1 morphologic leukemia free state. The most frequent AEs included CRS (67%; ≥ grade 3 in 13%) and nausea (20%) [92]. AMG 673 is similar to AMG 330, but with extended half-life, thanks to a linked Fc molecule. In a phase I study of AMG 673, 12/27 (44%) evaluable patients had reduction in blasts compared with baseline but only one patient achieved CRi with 85% reduction in bone marrow blasts [93]. AMV564 is a novel bivalent, bispecific (2:2) CD33xCD3 T cell engager (TCE), that was tested in a phase 1 study: among 35 efficacy evaluable patients, bone marrow blast reductions have been observed in 17 (49%), but only 1 CR and 1 CRi were attained [94]. These data discouraged further development in hematologic malignancies, thus AMV564 is being explored (in combination with anti PD-1) in solid cancers [95].

In 2020, it was presented the last Update from the ongoing phase I multinational study of MCLA-117, a bispecific CLL1xCD3 TCE, in AML patients (pts). Out of 26 evaluable pts with post-baseline BM assessment, 4 (15%) showed ≥ 50% blast reduction including 1 with morphological leukemia free state [96]. These poor results have been related to CLL1 bimodality expression, occuring in about 25% of AML patients and hence limiting the effectiveness of CLL-1-targeted therapies [97].

Moreover, bispecific Abs targeting FLT3xCD3 are under investigation in early phase I studies: CLN-049 (NCT05143996) for use in the treatment of patients with R/R AML, and AMG 427 (NCT03541369).

9. Conclusions

ITs have opened a new era in solid cancer therapy, attaining unprecedented long-lasting responses even in metastatic diseases [1]. In hematologic malignancies, similar results have been observed in lymphomas [2–4]. In AML, although analogue studies have been conducted, most of them have failed to advance to phase 3 studies and currently no drug is approved for AML, yet.

Several explanations can be provided to unveil the reasons for the poor results attained in AML. Indeed, cancer immune therapy, especially immune CIs (ICIs), have shown strong activity in high mutational burden carrying tumors, such as melanoma, lymphoma, and myeloma, where the increased production of private neoantigens represents the ideal targets of immune effectors. In contrast, AML, although presenting with molecular heterogeneity, falls within low mutational burden tumors [105,106]. Moreover, AML bone marrow is a "tolerogenic" microenvironment, that can impair immune effectors activity even when boosted by ITs. [98,99]. These observations may explain the failure to "re-ignite" exhausted T cells by ICIs in AML, although this T cell subset is abundant in the bone marrow of AML patients [109]. On the other hand, the presence of exhausted T cell populations represent a peculiar problem for TCE treatments and alternative solutions, beside combination with ICIs and pauses in the administration of a TCE [100], are eagerly awaited.

Nevertheless, encouraging data of anti CD47 blocking agents indicated that AML can be targeted with alternative ITs as compared to other tumors. Indeed, magrolimab has shown activity on TP53

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mutated AMLs, a very poor prognosis AML category. Anti CD47 blocking agents are particularly effective when combined with innovative drugs introduced in AML therapy such HMA and BCL2 inhibitors, which render them appealing for next future development in clinical studies. Finally, post-transplant setting needs to be further evaluated by ad hoc designed trials as this specifical condition should hypothetically magnify the power of ITs by promoting a "reset" of tolerogenic bone marrow microenvironment and favoring boosted immune effectors, although at the putative cost of increased GVHD.

In conclusion, our knowledge on the efficacy of ITs in AML requires further pre-clinical/clinical research to better underline the patterns of immune escape/resistance of AML blasts, which will help for the design of new clinical studies and maximize current data to successfully apply these innovative drugs also on AML patients.

Figure 1. 1) MHC presents Leukemia associated antigens to T lymphocytes, giving the first signal to the immunological synapse. 2) PD1-L and PD2-L can induce T cell anergy by linking PD1 present on T lymphocytes. Several anti PD1 (nivolumab, pembrolizumab, tislelizumab) or anti PD1L (durvalumab , avelumab) inhibit this crucial axis. 3) The CTLA4/CD86 axis can be inhibited by ipilimumab. 4) The TIM3/Galectin9 axis can be inhibited by sabatolimab, MBG453 5)The SIRPα/CD47 axis represent the «don't-eat-me» signal and can halt phagocytosis of macrophages. Monoclonal Ab. Like magrolimab can silence this signal and restore phagocytosis. 6) BITE AMG 330 is an anti CD33xCD3 7) JNJ-63709178, a CD123/CD3 Targeting Antibody.

Table 1. Trials including PD-1 inhibitors in patients with AML.

Reference	Therapeutic approach	Type of AML	Number of patients	Response	Survival
Daver et al	Azacitidine + nivolumab	R/R AML	70	ORR: 33% CR/CRi:22%	Median OS 9.2 months
Daver et al	Azacitidine + nivolumab + ipilimumab	R/R AML	24	ORR: 46% CR/CRi:36%	/
Davids et al.	Nivolumab	AML and myeloid malignancies after transplant	10 AML 19 myeloid malignancies	/	Median OS 21.4 months 1-year OS: 56%
Ravandi et al.	Nivolumab+ idarubicin + cytarabine	Newly diagnosed AML and HR-MDS	42 AML 2 HR-MDS	composite CR: 78%,	Median OS 18.5 months
Liu et al.	Nivolumab	Maintenance on AML in first CR or CR or CRi	26	/	Median OS 53.9 months; 2-year OS 60.0%
Gojo et al.	Pembrolizumab + azacitidine	Newly diagnosed AML and R/R AML	37 R/R AML 22 de novo AML	ORR: 55%, with 14% CR/CRi in R/R AML ORR: 94% with 47% CR/CRi in de novo AML	Median OS for de novo AML 13.1 months
Goswami et al.	Pembrolizumab + decitabine	R/R AML	10	/	Median OS 10 months
Zeidner et al.	Pembrolizumab + high-dose cytarabine	R/R AML	37	ORR: 48% with a composite CR 38%,	median OS 13.2 months
Gao et al.	tislelizumab + azacitidine or decitabine + CAG regimen (cytarabine, aclarubicin, G-CSF)	R/R AML	27	ORR: 63%	Median OS: 9.7 months

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