Chapter 4
Harnessing the Immune System Against Leukemia: Monoclonal Antibodies and Checkpoint Strategies for AML

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Abstract Acute myeloid leukemia (AML) is the most common leukemia among adults and is associated with a poor prognosis, especially in patients with adverse prognostic factors, older age, or relapsed disease. The last decade has seen a surge in successful immune-based therapies in various solid tumors; however, the role of immune therapies in AML remains poorly defined. This chapter describes the rationale, clinical data, and toxicity profiles of immune-based therapeutic modalities in AML including naked and conjugated monoclonal antibodies, bispecific T-cell engager antibodies, chimeric antigen receptor (CAR)-T cells, and checkpoint blockade via blockade of PD1/PDL1 or CTLA4. Monoclonal antibodies commonly used in AML therapy target highly expressed "leukemia" surface antigens and include (1) naked antibodies against common myeloid markers such as anti-CD33 (e.g., lintuzumab), (2) antibody-drug conjugates linked to either, (a) a highly potent toxin such as calicheamicin, pyrrolobenzodiazepine, maytansine, or others in various anti-CD33 (gemtuzumab ozogamicin, SGN 33A), anti-123 (SL-401), and anti-CD56 (lorvotuzumab mertansine) formulations, or (b) radioactive particles, such as ^{131}I, ^{213}Bi, or ^{225}Ac-labeled anti-CD33 or CD45 antibodies. Novel monoclonal antibodies that recruit and promote proximity-induced cytotoxicity of tumor cells by T cells (bispecific T-cell engager [BiTE] such as anti CD33/CD3, e.g., AMG 330) or block immune checkpoint pathways such as CTLA4 (e.g., ipilimumab) or PD1/PD-L1 (e.g., nivolumab) unleashing the patients T cells to fight leukemic cells are being

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evaluated in clinical trials in patients with AML. The numerous ongoing clinical trials with immunotherapies in AML will improve our understanding of the biology of AML and allow us to determine the best approaches to immunotherapy in AML.

Keywords Acute myeloid leukemia • Immunotherapy • Monoclonal antibody • Immune checkpoint blockade

4.1 Introduction

Acute myeloid leukemia (AML) is the most prevalent acute leukemia among adults with an annual incidence of 19,000 new cases in the United States. AML comprises a heterogeneous group of diseases with differential behavior and overall survival impacted by numerous clinical, cytogenetic, and molecular factors. Despite extensive research efforts, the therapy of AML has improved modestly over the last four decades. Standard frontline treatment still represents a combination of cytarabine and daunorubicin ("3 + 7") introduced in the 1970s [1]. The 7 + 3 regimen produces complete remissions (CR) in approximately 70% of patients and long-term overall survival (OS) in 40% of young adults with AML. The results are worse in older patients or those with adverse karyotypes, where CR and OS rates are 50% and 15%, respectively [2, 3]. Furthermore, despite intensified consolidation after remission, most patients experience subsequent relapse, likely from persistence of chemorefractory leukemic "stem" cells. Improved induction regimens producing long-term remissions and/or the addition of maintenance therapy to high-risk patients in remission are warranted.

A significant number of patients with AML (especially those with adverse cytogenetic features, adverse molecular mutations, or antecedent hematological disorder) will be refractory or relapse after initial response to induction therapy. Patients with relapsed AML have further dismal outcomes, with response rates ranging from 2 to 30% and OS of 1.5 to 3.8 months with salvage therapy [4, 5]. The only therapy offering long-term survival and a potential for cure in relapsed AML is allogeneic stem cell transplantation (ASCT), but age, performance status, and organ function requirements coupled with considerable morbidity and mortality of this procedure limits routine applicability of this approach. Therefore, improved therapeutic approaches in salvage AML are urgently needed.

Targeted immune therapies such as antibodies, CAR-T cells, and checkpoint inhibitors aim to increase antitumor activity without the burden of systemic toxicities encountered with cytotoxic chemotherapies. Redirecting the patients' own immune system to target cancer cells is a highly attractive treatment option and has become a standard and approved anticancer modality in solid tumors including melanoma, lung cancer, bladder cancer, and renal cancer. Although the role of

antibodies that target CTLA-4 and PD-1, oncolytic viruses and adoptive T-cell therapy is well established in solid tumor malignancies, the experience in incorporating similar immune therapies for the treatment of leukemias remains limited. This is surprising for many reasons. Firstly, leukemias were the first tumor type to demonstrate the success of allogeneic stem cell transplant, an immunotherapeutic approach that depends on graft versus leukemia effect to eradicate leukemia cells [6]. Secondly, having an immune cell lineage, leukemias often express immune checkpoint molecules that are absent in solid tumor cells thereby offering direct targets for immune checkpoint inhibition. In the recent years, a number of immunotherapy approaches are under investigation in numerous clinical trials in patients with hematologic malignancies including AML, myelodysplastic syndrome (MDS), and acute lymphoblastic leukemia (ALL). These include monoclonal antibodies, naked or antibody-drug conjugates (ADC) targeting leukemia-specific antigens on AML cells (e.g., anti-CD33, anti-CD38, anti-CD123, anti-56) or immune checkpoint blocking molecules (e.g., anti-PD-1, anti-PD-L1, or anti-CTLA-4), bispecific antibodies (e.g., bispecific T-cell engagers, BiTEs, e.g., CD3/CD33), T-cell adoptive therapy including chimeric antigen receptor (CAR) T cells and adoptively transferred natural killer (NK) cells.

This chapter focuses on the rationale, clinical data, and toxicity profiles of these immunotherapies for patients with AML.

4.2 Monoclonal Antibodies

Antibodies as cancer-targeting therapies have been investigated since the early 1980s and a number of antibodies have successfully been used in the therapy of solid and hematologic malignancies [7]. Monoclonal antibodies work by a number of different mechanisms to target tumor cells, of which one of the most important is antibody-dependent cellular cytotoxicity (ADCC) mediated by activation of NK cells, neutrophils, and macrophages. Following ADCC, fragments of tumor cells are released and taken up by antigen-presenting cells (APCs), where they are presented on the surface by the major histocompatibility complex class II and I (MHC) to cytotoxic T-lymphocytes with subsequent killing of cells containing tumor antigens [8]. An ideal targetable cluster of differentiation (CD) surface antigen has to be highly expressed on leukemic blasts with minimal to no expression on other cells, especially hematopoietic stem cells (HPSC) to allow for recovery of normal hematopoiesis. CD33, CD123, CD32, CD38, CD47, CD44, CD96, and CLL-1 [9] have differential expression on AML and leukemia stem cells (LSC) when compared with normal HPSC and represent potential targets.

Most of the clinical efforts thus far have focused on exploiting CD33, CD123, and CD56 as targets, as they have been shown to be frequently expressed on AML cells including AML stem cells making them ideal markers for eradicating malignant stem cell while sparing normal HPSC [10–12].

4.2.1 Anti-CD33 Antibodies

CD33 is a member of the sialic acid-binding immunoglobulin-like lectins (Siglecs) and is a myeloid differentiation antigen [10] primarily expressed at very early stages on myeloid progenitors. CD33 is highly (>90%) expressed on AML blasts [13]. Unconjugated antibody lintuzumab (SGN-33) and several antibody-drug conjugates (ADCs) such as gemtuzumab ozogamicin (GO), AVE9633, and SGN-33A that target CD33 have been evaluated in the treatment of patients with AML. Conjugated antibodies were engineered with an intention to improve the antitumor efficacy of CD33 antibodies by leveraging the endocytolytic property of CD33.

4.2.1.1 Unconjugated Anti-CD33 Antibodies

Lintuzumab (SGN-33, HuM195), an unconjugated anti-CD33 antibody exerts its anti-leukemic activity through ADCC, complement-dependent cytotoxicity, and inhibition of inflammatory cytokines. Early clinic studies [14] with lintuzumab demonstrated promising activity with good tolerability. Subsequently, a phase 2B trial comparing low-dose cytarabine with or without lintuzumab in the frontline setting and a phase 3 trial comparing mitoxantrone, etoposide, and cytarabine with or without lintuzumab in the salvage setting were conducted and both demonstrated no significant survival benefit. This resulted in cessation of further development of this agent in AML [15, 16].

Despite the initial disappointing results with unconjugated anti-CD33 antibodies in AML, recent research showed promising preclinical anti-leukemic efficacy with a new unconjugated Fc-engineered (enhanced binding affinity to Fcγ receptor IIIa on NK cells), CD33 antibody, *BI836858.* This fully humanized anti-CD33 antibody promoted more robust NK-cell-mediated anti-AML activity in patients treated with 10 day decitabine [17]. The observed higher lysis of AML cells at day 28 post-decitabine was due to up-regulation of NK-activating receptor NKG2D ligands (NKG2DL) by the DNA-methyltransferase inhibitor decitabine resulting in enhanced NK-cell-mediated cytotoxicity against AML blasts. This agent will be entering clinical trials in the United States in late 2016 (Clinicaltrials.gov: NCT02632721).

4.2.1.2 Conjugated Anti-CD33 Antibodies

AVE9633 (ImmunoGen, USA) was the first anti-CD33 antibody conjugated to a cytotoxic toxin to be evaluated in clinical trials for patients with AML. The conjugated toxin was maytansine, a highly potent tubulin inhibitor. *AVE9633* showed limited clinical activity in three phase 1 trials performed on 54 patients with refractory/relapsed AML, with only one CRp (CR with incomplete platelet recovery) and one PR (partial remission) observed [18].

Gemtuzumab ozogamicin (GO; Mylotarg) (Pfizer, USA) is the best-known monoclonal antibody in AML therapy. Thus far, the largest clinical experience with a monoclonal antibody in AML has been with gemtuzumab ozogamicin (GO), a humanized anti-CD33 monoclonal antibody covalently linked to a semisynthetic derivative of a potent DNA-damaging toxin calicheamicin. In 2000, GO was granted accelerated approval by the United States FDA [19] on the basis of a 30% overall response rate (CR + CRi) in phase II clinical trials [20] in 142 and 277 patients with de novo AML in first relapse, respectively [21]. Response duration was difficult to determine due to the high prevalence of post-remission therapies; however, responses were relatively short. However, no difference in OS was observed in the phase III SWOG S0106 trial designed to meet FDA post-approval requirements [22]. The lack of clear clinical benefit, concerns about increased side effects, and slightly increased early death rate with GO in this SWOG trial [22], led to voluntary withdrawal of the drug from US markets in 2008. Particular concerns were related to life-threatening sinusoidal obstruction syndrome or veno-occlusive disease, which was more likely to occur when the drug was used in higher concentration, in combination with hepatotoxic agents, or within 3 months of allogeneic SCT (incidence rate 9–14%) [23]. The mechanisms included either dissociation of calicheamicin from the anti-CD33 antibody causing direct toxic effect to hepatocytes or uptake of GO by CD33(+) cells residing in the hepatic sinusoids [24]. The potential benefits of GO in this trial might have been masked due to a suboptimal dosing schema as well as failure to perform patient subgroup analysis. Subsequently, large randomized trials conducted in the United States and Europe investigated GO in addition to standard induction chemotherapy in adults with newly diagnosed AML. These studies [25–27] showed statistically improved OS when GO was added to standard induction, particularly in younger patients with intermediate and/or favorable risk cytogenetics. In older patients, the addition of GO to cytotoxic induction regimens improved the relapse risk, event-free survival, and overall survival without improving the response rate or early mortality rate [26–27]. In a meta-analysis of these randomized clinical trials, the addition of GO significantly reduced the risk of relapse (HR 0.8; 95%CI 0.72–0.89, $p < 0.001$), improved relapse free (HR 0.8; 95%CI 0.76–0.94, $p = 0.001$) and overall survival (HR 0.89; 95%CI 0.82–0.97, $p = 0.01$), particularly in patients without adverse cytogenetics [28]. These data suggest that the use of GO in AML in the United States and Europe should be reassessed as suggested by experts in the field [29, 30]. Currently, clinical trials are ongoing to evaluate the efficacy and toxicity of GO either as a monotherapy or in combination with chemotherapy in frontline (France) and relapsed (United States) patients with AML, including its addition to standard conditioning prior to ASCT [ClinicalTrials.gov: NCT01869803, NCT02473146, NCT02221310].

SGN33A (vadastuximab talirine, Seattle Genetics, USA) is a promising new anti-CD33 antibody conjugated to a highly potent, synthetic pyrrolobenzodiazepine, producing DNA damage and cell cycle arrest with subsequent leukemic cell apoptosis. In preclinical studies, SGN33A demonstrated greater cytotoxic potency against AML cell lines and primary AML cells than GO, regardless of multi-drug-resistant status or cytogenetic risk group [31]. Furthermore, 5-azacitidine was

shown to significantly enhance the tumor killing ability of SGN33A through enhanced ADCC and phagocytosis [32]. This compound is currently being tested in phase I dose escalation studies as a single agent and in combination with chemotherapy, including DNA-methyltransferase inhibitors (DNMTi) (decitabine or 5-azacitidine) in the pre- and post-ASCT setting, or as a monotherapy in maintenance [Clinicaltrials.gov: NCT02326584, NCT02785900, NCT02706899, NCT02614560]. Initial results from an ongoing phase II study combining SGN-CD33A with a DNMTi in elderly, treatment-naïve patients with AML are promising, with a CR plus CRi rate of 71% (including CR rate of 41%), ≥50% reduction in blasts in 85% of treated patients, and a low early mortality (8-week mortality of 4%) [33].

IMGN 779 (ImmunoGen, USA) is another humanized anti-CD33 antibody conjugated to a novel DNA-alkylating IGN payloads, DGN462 that acts as an alkylating cytotoxic agent without DNA crosslinking [34]. In preclinical studies, the compound showed highly potent activity against AML cell lines in vitro and in primary AML patient samples isolated from peripheral blood or bone marrow. In long-term cultures, it has also demonstrated a dose-dependent decrease in leukemic stem cell colony formation without affecting normal HPSC thereby avoiding prolonged myelosuppression [35, 36]. IMGN 779 is currently being tested in a phase 1 clinical study for patients with relapsed or refractory AML [clinicaltrial.org: NCT02674763].

4.2.2 Anti-CD123

The CD123 antigen is another ideal target for monoclonal antibody-based therapy in patients with AML. Binding of CD123 to interleukin-3 (IL-3Rα) results in increased cell survival and proliferation [37]. Overexpression of the interleukin (IL)-3 receptor α-chain (IL-3 Rα/CD123) on AML cells was found to be associated with enhanced blast proliferation, poor prognosis [38], and a major cause of leukemia relapse and chemotherapy resistance.

The first anti-CD123 antibody *CSL360* was a recombinant, chimeric immunoglobulin G1 against CD123 that prevented IL-3Rα from binding to its receptor. CSL360 had underwhelming clinical efficacy when tested in relapsed, refractory or high-risk AML with only one CR observed among 26 treated patients [39]. These results resulted in cessation of further development of this compound in AML.

A second-generation anti-CD123 antibody, *CSL362* is a fully humanized, genetically engineered antibody containing a modified Fc-domain to enhance binding to NK cells through Fcγ receptors (FcγR) of CD16 to enhance antibody-dependent cellular toxicity (ADCC). This agent showed potent activity in patients with CD123+ AML with a tolerable safety profile in a phase I study of 25 patients with AML in first or second CR/CRp with adverse risk factors conferring a high risk of early relapse. Among 20 patients evaluable for a response, 10 had maintained their CR, with a median duration of CR of 34+ weeks (range, 26–52 weeks) and ongoing at the last follow up. Furthermore, three out of six patients, who were MRD positive, converted to MRD negative. Related adverse events observed in ≥10% of patients

included infusion reaction/hypotension, hypertension, and increased C-reactive protein, three of these were classified as dose-limiting toxicities. Pharmacodynamic correlative studies showed rapid, complete, and durable in vivo depletion of cells highly expressing CD123 by induced ADCC [40].

Another anti-CD123 antibody currently in phase 2 clinical trials is *SL-401 (DT388IL3) (Stemline Therapeutics, Inc. USA)*—a recombinant fusion protein composed of the truncated diphtheria toxin and a human IL-3 ligand [41], which after binding to CD123 get internalized, and leads to inactivation of protein synthesis, and cell death. Encouraging results were shown in a phase I trial of SL401 in 74 AML/MDS patients (56 with relapsed and refractory AML, 11 with de novo poor risk elderly AML, and 7 high-risk MDS), where ORR was observed in 6 patients (2 CRs and 4 PRs) and a minor response with blasts reduction was observed in 14 patients, including $a > 50\%$ reduction in bone marrow blasts in four patients. Moreover, disease stabilization was observed between 43 and 55% of patients. The median survival and overall survival at 12 months in patients with relapsed AML (≥2nd salvage) was 3.2 months and 22%, respectively, both favorable when compared to historical results. Toxicities did not differ from those observed in patients with BPDCN. Severe grade 3/4 adverse events were only transient and included elevation in transaminases (20%) and capillary leak syndrome (4%) [42, 43]. Recently reported data from the early expansion stages of an ongoing pivotal phase 2 clinical trial confirmed an overall response rate (ORR) of 87% in all patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN) [44]. In the frontline setting, the response rate was 100% with majority of responses CR or CRc. The responses have been durable in all cases. The most common toxicities included fever, chills, hypotension, edema, transaminase elevation, and hypoalbuminemia. The notable toxicity was capillary leak syndrome in 2/18 treated patients; this was reversible in one case and fatal in one case. The study continues to accrue and may be a breakthrough in the management of BPDCN. The phase II study evaluating SL401 in AML shows disease stabilization in heavily pretreated patients with relapsed refractory AML and is ongoing [45]. The results in AML have thus far been less impressive than those see in BPDCN. This agent is also being evaluated in a phase 2 trial designed as a consolidation therapy for patients with high-risk AML in first complete remission to determine whether targeting CD123 improves the duration of response and survival in patients who would traditionally be at a high risk of relapse [clinicaltrials.gov: NCT02270463].

4.2.3 Anti-CD56

CD56, also known as NCAM1, is a member of the neural cell adhesion molecule family [46] that plays an important functional role during nervous system differentiation, and immune surveillance. Although primarily expressed in neuroendocrine, NK, and T cell lineages [47], aberrant CD56 expression is seen in a variety of hematological malignancies [11] as well as solid tumors [48].

IMGN901 (lorvotuzumab mertansine) (ImmunoGen, Inc., USA) is a humanized anti-CD56 antibody conjugated to tubulin inhibitor maytansinoid DM1 via a stable disulfide linker. On binding to the CD56 antigen, IMGN901 is internalized with intracellular release of toxin DM1 with subsequent microtubule disruption, cell cycle arrest, and ultimately cell apoptosis [49]. In preclinical models, IMGN901 demonstrated high-affinity, antigen-specific binding, and antitumor activity in CD56-positive tumors [35]. And, open label phase 1/2 clinical trial was conducted in patients ($n = 97$) with relapsed CD56+ solid tumors in combination with chemotherapy. The drug had an acceptable tolerability profile with CR/PR observed in four patients and disease stabilization in 25% of evaluable patients [50]. This compound is currently being evaluated in a phase 2 clinical trial in CD56+ hematologic malignancies, including AML, myelofibrosis, and BPDCN [clinicaltrials.gov: NCT02420873].

4.2.4 Radioimmunotherapy via Targeted Antibodies Conjugated to Radioactive Particles

The radiosensitive nature of AML best seen in the setting of a stem cell transplantation [51], the diffuse and widespread pattern of involvement, and the high expression of specific antigens on AML blasts, suggests that radioimmunotherapy via targeted antibodies conjugated to radionuclides may be an attractive alternative to antibodies conjugated to toxins. This approach has been explored in patients with AML for over two decades. Since the first phase 1 clinical trial published in 1991 demonstrated the feasibility of using radiolabeled ^{131}I anti-CD33 in patients with relapsed AML [52], several clinical studies have explored antibodies carrying beta (^{131}Iodine, ^{188}Rhenium, ^{90}Yttrium) or alfa (^{213}Bismuth, ^{225}Actinium) emitters, alone or as part of a conditioning regimen for ASCT in patients with relapsed AML against different AML targets (CD33, CD45, or CD66). Early clinical studies with easily accessible radionuclide ^{131}I targeted against CD45 (^{131}I-labeled anti-CD45 antibody) or CD33 (^{131}I-labeled anti-CD33 antibody—murine M195 and humanized Hu195) showed feasibility, efficacy, and acceptable toxicity when used in combination with standard conditioning regimen prior to ASCT in patients with refractory/high-risk AML [53–55]. ^{131}I-labeled anti-CD45 antibody BC8 *(Iomab-B, Actinium Pharmaceuticals, USA)* is currently being tested in a phase 2 [clinicaltrials.gov: NCT00589316] and phase 3 registration trial [clinicaltrials.gov: NCT02665065] to evaluate the efficacy and safety of this agent in patients of all ages with relapsed or refractory AML as a part of myeloablative conditioning regimen prior to ASCT (phase 2) or in older patients with relapsed or refractory AML prior to ASCT in comparison to standard conventional care (phase 3).

In order to reduce the toxicity and improve the efficacy, especially in the settings of minimal residual disease (MRD), several studies have evaluated radionuclides emitting high energy or short range alfa particles, such as ^{213}Bi and ^{225}Ac. Preclinical studies followed by early clinical phase 1 studies showed the safety, feasibility, and

anti-leukemic activity of ^{213}Bi anti-CD33 (^{213}Bi-labeled HuM195). However, a very short half-life of only 48 min limited its widespread clinical testing [56]. To circumvent this problem, second-generation immunoconjugates, such as ^{225}Ac (half-life of 10 days), were developed. A phase 1 clinical trial with ^{225}Ac-labeled anti-CD33 antibody lintuzumab demonstrated clinical activity with reduction of the peripheral blood/bone marrow blasts in 63–67% of 18 evaluable patients with relapsed refractory AML. Dose-limiting toxicities included prolonged myelosuppression and death due to sepsis in three patients [57]. Based on these findings, a multicenter, phase I/II trial is now underway to determine the toxicity and efficacy of fractionated-dose ^{225}Ac-lintuzumab (A*ctimab-A*) (*Actinium Pharmaceuticals, USA*) in combination with low-dose cytarabine in untreated older (>60) patients with AML [clinitaltrials.gov: NCT02575963].

4.3 T-Cell-Engaging Antibodies

A novel class of antibody-based immunotherapy in AML includes monoclonal antibodies designed to promote antitumor activity by engaging and enhancing T- cell activation. These agents are called bispecific T-cell engagers (BiTEs). BiTE antibodies are able to effectively recruit antigen-experienced T cells, without the requirement of pre- or co-stimulation, and lead to direct killing of tumor-associated antigen cell (TAA) [58]. BiTEs are composed of a single polypeptide chain consisting of two light and heavy chains of targeted antibodies. The first-in-class BiTE antibody, anti-CD19/CD3 Blinatumomab, demonstrated significant clinical activity against CD19-positive malignancies [59]. Single agent blinatumomab tested in phase 2 clinical study in 189 relapsed refractory ALL patients showed 43% CR/CRi rate (95% CI 36–50), with median OS and RFS of 6.1 and 5.9 months, respectively, and served as an excellent bridge to potentially curable allo-SCT in 40% of patients who achieved CR/CRi. These data resulted in the FDA approval of blinatumomab for the treatment of relapsed/refractory B-ALL. Based on these promising results, a similar construct targeting CD3/CD33 has been developed to target AML, *AMG 330 (Amgen, USA)* [60]. In preclinical studies, AMG 330 demonstrated potent CD33-dependent cytolytic activity in vitro [61]. The drug is currently being evaluated in phase 1 clinical trial in patients with relapsed/refractory AML [clinicaltrials.gov: NCT02520427]. Another CD123/CD3 BiTE, *JNJ-63709178 (Janssen, USA)* is soon to enter phase 1 clinical trials in patients with relapsed refractory AML [clinicaltrial.gov: NCT02715011].

In an effort to improve the efficacy, stability, and valency of BiTEs, a novel class of Bivalent Dual Affinity Re-Targeting Bispecific Antibodies (DARTs) has been developed. DARTs are composed of heavy and light chain variable domains of two antigen-binding specificities connected to two independent polypeptide chains via a disulfide linker [62]. Recently, a CD123/CD3 DART has been developed for AML (MGD006) and demonstrated promising anti-leukemic activity in preclinical studies [63]. This compound is currently being evaluated in a first-in-human phase I dose escalation study in patients with relapsed AML or International Prognostic Scoring system (IPSS) intermediate-2/high-risk MDS [clinicaltrials.gov: NCT02152956].

4.4 Adoptive T-Cell Therapy

Adoptive cell therapy (ACT) is a highly personalized therapy that involves transfer of ex vivo expanded cytotoxic T-lymphocytes (CTLs) capable of targeting TAA into tumor-bearing patients. It was first recognized >20 years ago that some T cells from patients with cancer could immunologically recognize and kill the patients cancer cells [64]. Researchers found that patient lymphocytes stimulated in vitro with interleukin 2 and tumor cells were able to lyse autologous tumor cell lines through major histocompatibility complex II (MHC II). These tumor-reactive T cells have been extensively investigated over the past years and may revolutionize out current approach to cancer therapy in hematologic and possibly in solid malignancies. The biggest advantage of ACT is that a large number (up to 10^{11}) of lymphocytes can be grown in vitro and genetically engineered to express the binding site of specific antibodies. These T cells with engineered chimeric antigen receptor, also called CAR-T cell, are then able to directly bind to a specific TAA producing highly targeted and robust tumor killing [65].

CARs consist of an extracellular domain created by the fusion between the variable region of heavy and light chains of an antigen-specific monoclonal antibody (ScFv) separated by a short peptide linker and an intracellular T cell-activating domain, usually CD3-ζ of the TCR receptor, and a co-stimulator molecule. This allows CAR-T cells to manifest the tumor specificity of monoclonal antibodies while simultaneously activating effector T-cells independent of MHC [66]. Various CAR-T constructs have different co-stimulatory molecules to increase their efficacy and longevity (CD28, OX40, or 4-1BB in the second and third-generation constructs; additional cytokines such as IL-2, IL-15, IL-12, and IL-21 in the fourth-generation constructs) [67]. Anti-CD19 CAR-Ts have already shown remarkable success in the treatment of B-cell malignancies [68], and it remains to be established whether similar activity can be reproduced in AML.

Only one clinical study testing anti-LeY CAR-T cells (Australia) in patients with AML has been completed and reported to date. This study reported the feasibility, safety, and persistence of CAR-T cells for up to 10 months post infusion as tested in five patients with relapsed AML (first salvage). Two patients achieved stable disease (duration of 23 months in one patient), and an additional two had transient response (blasts reduction/cytogenetic remission). Overall, infusion were well tolerated with no severe (grade 3 or 4) adverse events or tumor lysis syndrome observed [69]. A number of phase I clinical trials with CAR-T cells in relapsed, refractory AML patients are ongoing including anti-CD33, CD7, and CD133 CAR-T cell studies in China [clinicaltrials.gov: NCT01864902, NCT02799680, NCT02742727, NCT02541370] and anti-CD123 and anti-NKG2D ligand CAR-T cell studies in the United States [clinicaltrials.gov: NCT02159495, NCT02623582, NCT02203825].

Alternative T-cell-engaging antibody constructs, cytokine-induced killers (CIK), involving CD56 + NK like cells with a potent killing activity, showed activity in reducing refractory AML blasts and cell lines in preclinical studies when combined with anti-CD33 and/or anti-CD123 CAR-T [70].

4.5 Checkpoint Inhibitors

Maintenance of immune homeostasis, self-tolerance, and prevention of autoimmunity requires strict regulation of immune response, especially its quality and amplitude, provided by T cells and multiple interactions between co-stimulatory and co-inhibitory signals [71]. T-cell-mediated immunity includes many steps involving initial presentation of antigen peptide on MHC through the T-cell receptor (TCR) with sequential activation of T cells. All the steps in this pathway are regulated by careful counterbalancing of the co-stimulatory and co-inhibitory signals and receptors, resulting in appropriate T-cell effector function. The most important receptors promoting final activation of T cells are co-stimulatory signals CD28, 4-1BB (CD137), and CD27 (expressed on T cells), and CD80, CD86 (expressed on APC). These stimulatory signals are antagonized by inhibitory receptors (the so-called checkpoint inhibitors)—cytotoxic T-lymphocyte-associated protein 4 (CTLA4) and programmed cell-death protein (PD-1).

A major impediment to cancer immunotherapy with the previously discussed antibody-based approaches in this chapter is tumor-induced immune suppression and evasion of anti-tumor immune responses, rendering the host tolerant to tumor-associated antigens [72, 73]. The true potential of cancer immunotherapy came to the fore with James Allison's breakthrough discovery of cytotoxic T-lymphocyte antigen 4 (CTLA-4), a receptor on the surface of T cells that blocks the immune response by inhibiting T-cell activation and the subsequent development of an anti-CTLA-4 antibody, ipilimumab, that blocks this "immune checkpoint" protein, thereby freeing the immune system to attack tumors [74].

Under normal physiological conditions, immune checkpoints regulate self-tolerance and protect tissues from damage by restraining the immune systems response to pathogenic infection. Deregulation of immune checkpoint proteins including up-regulation of negative co-stimulatory receptors and downregulation of positive co-stimulatory receptors plays a central role in tumor-mediated evasion of T-cell immune response [71]. Targeting CTLA4 and other immune checkpoint molecules represented a major breakthrough for immunotherapy in solid tumors and more recently in hematologic malignancies. These agents target inhibitory pathways on T cells thereby unleashing antitumor immune responses.

The two major approaches to immune checkpoint blockade that have been clinically investigated in large numbers of patients, primarily in solid tumors and more recently in hematologic malignancies, focus on targeting the co-inhibitory receptors, CTLA4 and PD-1, or its ligands PD-L1/PD-L2. These two inhibitory molecules work on different levels and by different mechanisms. CTLA4 is expressed predominantly on the T cells in lymph nodes where it primarily regulates early T-cell activation. CTLA4 is sequestered in intracellular vesicles in T cells and is transported to the surface only after antigen recognition. The level of CTLA4 induction depends on the amplitude of the initial T-cell receptor (TCR)-mediated signaling, further amplified by co-stimulatory receptor CD28. The stronger the stimulation through the TCR, the greater the amount of CTLA4 deposited on the T-cell surface.

CTLA4 then binds to the same ligands as the co-stimulatory receptor CD28, namely, CD80 and CD86 and counteracts the stimulatory activity of CD28 by competitive inhibition. CTLA4 has higher affinity to CD80 and CD86 ligands and serves as a signal dampener to maintain a consistent level of T-cell activation, primarily by downregulation of T-helper cells and up-regulation of T-regulatory cells [74, 75]. In contrast, the major role of the PD-1 pathway (PD-1 receptor and its ligands) is to regulate inflammatory responses in the peripheral tissues by inhibiting effector T cells [76]. Inflammatory signals activate T cells and up-regulate expression of PD-1 and PD-1L in the tissue. PD-1 expression inhibits the T-cell effector activity by decreasing the duration of interaction between the T cell <– > APC or T cell <– > target cell and enhancing Treg proliferation [77]. Moreover, chronic inflammation leads to excessive production of inhibitory co-signals in tumor cells or their microenvironmental components, resulting in an exhausted or anergic state among co-signaling antigen-specific Tcells leading to immune escape of the tumor [78]. This may possibly be reversed by PD-1 or PD-L1 pathway blockade [79].

Clinical trials with anti-CTLA-4 antibodies, the first immune checkpoint targeted antibody [74], have shown encouraging responses in melanoma, advanced mesothelioma, gastric cancer, non-small cell lung cancer, bladder cancer, and prostate cancer [80–82]. CTLA4 inhibitor, ipilimumab, demonstrated overall survival benefit in patients with metastatic melanoma, and more importantly, revealed an important concept of immune-based therapies which seem to re-educate the immune system to keep tumors under control even in patients with multiple prior therapeutic intervention as was noticed by increased proportion of long-term survivors [80]. The responses with anti-CTLA4 occurred slowly after treatment initiation, in many patients were delayed up to 6 months, and were often maintained for many years after completion of a relatively short course of treatment. Toxicity mostly involved immune-mediated pneumonitis, colitis, hepatitis, or thyroiditis, and seemed to be manageable with steroids. Identification and targeting of additional positive co-stimulatory receptors (4-1BB, CD27, ICOS, OX40, GITR) and negative co-stimulatory receptors (PD-1, CTLA4, TIGIT, BTLA, LAG3, TIM3) regulating T-cell activation and dual blockade of concurrently expressed receptors produced synergistic antitumor responses in mouse models [78].

Since the basic immunologic principles behind immune checkpoint therapy can be applied to other tumor types, it is plausible that immune checkpoint therapy can also be beneficial for patients with leukemias and other hematologic malignancies, specifically AML. Firstly, leukemias are one of the first tumor types to be successfully treated with immunotherapy approaches as proven by the success of allogeneic stem cell transplantation. Secondly, leukemias have an immune cell lineage and may express immune checkpoint molecules thereby offering direct targets for immune checkpoint therapy. For example, there is frequent expression of PD-L1 and PD-L2 ligands on various hematopoietic cells—activated and non-activated T cells, B cells, and NK cells [83]. Similarly, markers typically associated with antigen-presenting cells, such as CD80 and CD86, are commonly overexpressed in hematologic malignancies owing to a common lineage shared by leukemia cells and APC [84–88]. Thirdly, a number of studies have demonstrated encouraging results

with immune checkpoint inhibition in other hematologic malignancies including Hodgkin's lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, and multiple myeloma. Specifically in leukemia, PD-1 and CTLA4 have been shown to play a role in leukemia, and graft versus host disease (GvHD), and their overexpression was clearly associated with a more aggressive leukemia [89, 90]. Researchers have demonstrated that PD1 plays a role in immune evasion and exhaustion of tumor-infiltrating lymphocytes (TILs) and that blocking CTLA4 and PD-1/PD-L1 pathways enhances the anti-leukemia responses with decreased tumor burden and increased survival in murine models [91, 92, 93, 94]. Additionally, PD-1 positive T cells were shown to be significantly increased in the bone marrow aspirates of patients with relapsed AML as compared to healthy adult donors [95].

The initial clinical results of a phase I study of PD-1/PDL-1 inhibitor *pidilizumab (MDV9300)* in patients with various solid and hematologic malignancies included a small number of patients with AML. Among eight patients with AML and one patient with MDS, minimal response was seen in one patient with AML in the form of a decrease in the blast percentage from 50 to 5% [96]. In order to improve the response rate and the durability of response in patients with AML treated with checkpoint inhibitors, combinations of these agents with standard anti-leukemic therapy may be needed. 5-azacitidine, an epigenetic drug approved by FDA for the treatment of MDS, up-regulated PD-1, PD-L1, and PD-L2 ($\geq$ 2-fold) in >50% of 61 evaluable patients with AML/MDS during their first course of therapy. There was a trend toward increased expression of all three genes in azacytidine-resistant patients compared with sensitive patients, suggesting up-regulation of immune makers as a potential mechanism of resistance to 5-azacytidine and that concomitant inhibition of the PD-1/PD-L1 axis may be a potential mechanism to prevent or overcome resistance to 5-azacytidine [97]. These data have resulted in currently ongoing clinical trials combining epigenetic therapy with PD-1/PD-L1 inhibitor *nivolumab* (*Opdivo, BMS-936558*) (*Bristol-Myers Squibb, USA*) in relapsed and frontline elderly AML, relapsed and frontline MDS and epigenetic therapy in combination with CTLA4 inhibitor *ipilimumab (Yervoy, BMS-734016)* in relapsed and frontline MDS patients [ClinicalTrials.gov: NCT02397720, NCT02530463]. Phase 1/2 trials are evaluating the combination of PD-1 inhibitor nivolumab with standard induction chemotherapy in newly diagnosed AML patients [CTI: NCT02464657] or single agent nivolumab as a maintenance in high-risk AML patients to reduce the incidence of relapse (clinicaltrials.gov: NCT02532231). CTLA4 inhibitor is being evaluated as a monotherapy in high-risk MDS failing HMA therapy and AML with minimal residual disease [CTI: NCT01757639]. Both PD1 and CTLA4 are also being tested in phase 1 trials for patients with AML after ASCT [CTI: NCT01822509]. Results from ongoing phase 1/2 trial of PD1 inhibitor nivolumab with 5-azacitidine [NCT02397720] are encouraging. Preliminary data on the 22 evaluable patients were recently presented by Daver et al. [98] and showed significantly improved overall response rate, 8-week mortality, and median progression-free survival as compared to historical outcomes with 5-azacitidine-based therapies from the same institution. A phase 1 trial with CTLA4 inhibitor ipilimumab [CTI: NCT00060372] in patients with solid and hematologic malignancies, including patients with relapsed AML after allo-SCT

has been completed. Results on 28 patients with hematologic malignancies after stem cell transplant, including 12 patients with AML and 2 with MDS, were recently presented, and showed very encouraging activity with a CR/CRi rate of 33% and an overall disease reduction in 48%. Five of 12 (42%) patients with AML achieved CR, including 4 patients with chemorefractory leukemia cutis and/or myeloid sarcoma with the longest duration of response of 8 months and still ongoing. Typical immune-related grade 2–4 toxicities were observed in four patients, three of them were able to resume the therapy after management with steroids. One patient died due to sepsis presumably related to severe adverse events (pneumonitis and colitis), four others had to be withdrawn from the study due to treatment-related adverse events (acute and chronic GVHD of gastrointestinal tract) [99].

Evaluation of the clinical efficacy of targeting immune checkpoint pathways beyond PD-1/PD-L1 and CTLA4, such as 4-1BB, OX40, and ICOS, is currently ongoing in patients with advanced or metastatic carcinomas [CTI: NCT02315066]. Agonistic antibodies to these co-stimulatory signals, such as 4-1BB, or OX40 may result in increased immune effector cytotoxicity. OX40 (CD134), 4-1BB (CD137) receptors, and the inducible co-stimulator receptor (ICOS) belong to tumor necrosis factor (TNF) receptors family members, and are potent co-stimulators in T-cell activation and promote expansion and proliferation of CD8+ and CD4+ T cells. They are transiently up-regulated on APC, B cells, macrophages, and T cells following their activation, and play a significant role in the functional maturation of T cells [100–102]. They were also found to be overexpressed on leukemic cells [103], and in the bone marrows of patients with AML [95] as compared to healthy donors. These data suggest that evaluation of these immune system accelerators in hematologic malignancies, especially leukemias is warranted and may improve the responses when rationally administered in combination with checkpoint inhibitors. A number of ongoing phase 1/2 of clinical trials are evaluating these molecules (anti-4-1BB antibody PF-05082566; anti-OX40 antibody MEDI-6469, and anti-ICOS antibody MEDI-570) in patients with advanced solid malignancies or lymphomas as single agents or in combinations [ClinicalTrials.gov: NCT02554812, NCT02559024, NCT02315066, NCT02520791]. Hopefully, these will soon be evaluable in hematologic malignancies.

Currently, ongoing clinical trials testing checkpoint inhibitors and monoclonal antibodies in patients with AML are summarized in Table 4.1 and Figs. 4.1 and 4.2.

4.6 Discussion

Immunotherapy is undoubtedly a breakthrough in cancer therapy, and emerging data suggests that immunotherapeutic approaches hold the potential to become one of the cornerstones of treatment strategies in AML. In spite of the rapid development of monoclonal antibodies and other immunotherapeutic agents for AML in clinical trials, none of these agents are approved for standard use and there remains limited experience in incorporating these therapies in routine clinical practice. Historic

Table 4.1 Ongoing trials of monoclonal antibodies and immune checkpoint blockade in AML

Type	Therapy	Primary endpoint	Inclusion[a]	Clinicaltrials.gov Identifier
Phase 2	GO	Efficacy—ORR, Toxicity	R/R AML	NCT01869803
Phase 2,3	GO + Cytarabine vs "7 + 3"	Efficacy—ORR, OS, Toxicity	Frontline AML, >65 years	NCT02473146
Phase 2	GO + Busulfan + CFA → ASCT	Efficacy—ORR, OS	Salvage High-Risk AML, MDS—1st CR or R/R	NCT02221310
Phase 1b	SGN-CD33A + "7 + 3", 1SGN-CD33A + HDAC; SGN-CD33A	Toxicity/DLT	Frontline AML Maintenance	NCT02326584
Phase 3	SGN-CD33A + DAC/AZA[b]	Efficacy—OS	Frontline AML	NCT02785900
Phase 1/2	SGN-CD33A + AZA[b]	Toxicity, Efficacy—ORR	Frontline Int-2/High-Risk MDS	NCT02706899
Phase 1/2	SGN-CD33A + Fludarabine + Melphalan → ASCT; SGN-CD33A	Toxicity, Efficacy—OS	R/R AML pre-ASCT Post-ASCT maintenance	NCT02614560
Phase 1/2	SL 401	Toxicity, Efficacy—OS	Consolidation—High-Risk AML, or MRD+ AML in 1st CR	NCT02270463
Phase 2	IMGN 901	Efficacy—ORR	R/R CD56+ AML	NCT02420873
Phase 1	AMG 330	Toxicity	R/R AML	NCT02520427
Phase 1	JNJ-63709178	Toxicity	R/R AML	NCT02715011
Phase 1	IMGN 779	Toxicity	R/R AML	NCT0267463
Phase 1	MGD 006	Toxicity	R/R AML, Int-2 and HR MDS	NCT02152956
Phase 1/2	CAR-T CD33	Toxicity	R/R AML	NCT01864902
Phase 1/2	CAR-T CD7 (NK cells)	Toxicity	R/R AML	NCT02742727
Phase 1	CAR-T CD123	Toxicity	R/R AML	NCT02159495
Phase 0, pilot	CAR-T CD123	Toxicity	R/R AML	NCT02623582
Phase 1	CAR-T NKG2DL	Toxicity	R/R AML, MDS RAEB	NCT02203825
Phase 1	CAR-T CD33	Toxicity	R/R AML	NCT02799680
Phase 1	CAR-T CD133	Toxicity	R/R AML	NCT02541370

(continued)

Table 4.1 (continued)

Type	Therapy	Primary endpoint	Inclusion[a]	Clinicaltrials.gov Identifier
Phase 2	Iomab-B, ^{131}I anti-CD45 + conditioning prior SCT	Toxicity	R/R AML pre allo-SCT	NCT00589316
Phase 3	Iomab-B, ^{131}I anti-CD45 vs. standard care	Efficacy—ORR, OS	Active, R/R AML >55 years Pre allo-SCT	NCT02665065
Phase 1/ 2	Actimab-B, ^{225}Ac anti-CD33 + LDAC	Toxicity	Frontline AML, >60 years	NCT02575963
Phase 1	Ipilimumab	Toxicity	R/R AML, High-Risk MDS	NCT01757639
Phase 1/1b	Ipilimumab or Nivolumab	Toxicity, MTD	R/R AML after ASCT	NCT01822509
Phase 2	Pidilizumab + DC vaccine	Toxicity	AML in CR prior to cell collection for DC generation	NCT01096602
Phase 2	Nivolumab + AZA	Efficacy—ORR	R/R AML Frontline AML, >65 years	NCT02397720
Phase 2	Nivolumab + "7 + 3"	EFS	Frontline AML, <60 years	NCT02464657
Phase 2	Nivolumab	EFS	AML in CR	NCT02532231

Abbreviations: *R/R* relapsed/refractory, *AML* acute myeloid leukemia, *ORR* overall response rate, *OS* overall survival, *EFS* event-free survival, *GVHD* graft versus host disease, *ASCT* allogeneic stem cell transplantation, *MDS* myelodysplastic syndrome, *DC* dendritic cells, *DAC* decitabine, *AZA* azacitidine, *HDAC* high-dose Cytarabine, *GO* gemtuzumab ozogamicin, Data was compiled from ClinicalTrials.gov (https://clinicaltrials.gov) assessed on 6/14/2016

[a]Only AML indications listed

[b]Placebo controlled trials

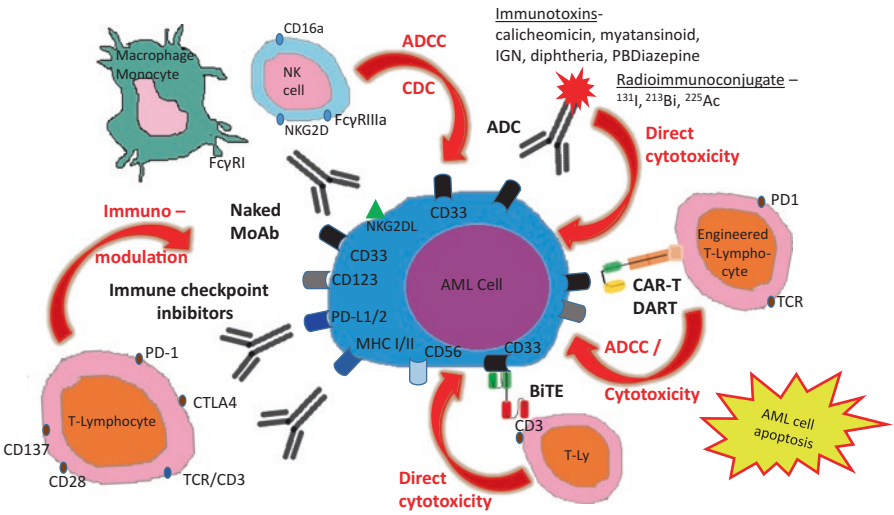

Fig. 4.1 Mechanism of action of immunotherapy in AML. Abbreviations: *ADCC* antibody-dependent cellular cytotoxicity, *CDC* complement-dependent cytotoxicity, *NK cell* natural killer, *MoAb* monoclonal antibody, *BiTE* bispecific T-cell engager

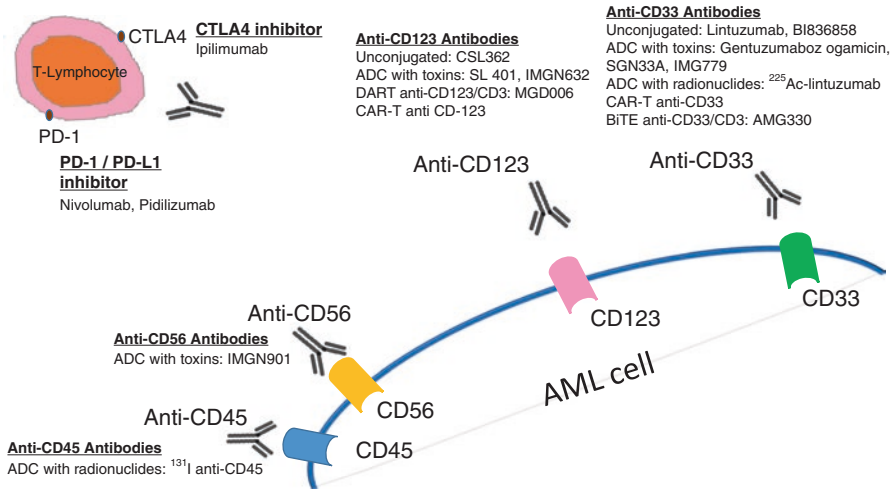

Fig. 4.2 Current immunotherapeutic in development for AML

monoclonal antibodies showed encouraging efficacy albeit with a potential for significant toxicity. The new generation of monoclonal antibodies with more effective payloads and better-selected targets are showing further enhanced activity with abrogated toxicity profiles. BiTEs, CAR-T cells, and other T-cell engaging agents along with immune checkpoint inhibitors are designed to harness the patient's own immune system to target and kill leukemic cells, and the preclinical and early

clinical data are very promising. With rationally designed biomarker driven clinical trials these agents may well find a place in frontline treatment of high-risk AML as well as in salvage or maintenance setting.

Immunotherapy research in solid tumors has significantly enhanced our understanding of solid tumor cancer biology, and we hope that the ongoing research in leukemia will similarly help us better understand the underlying mechanisms of AML. However, several critical issues need to be addressed before immunotherapy is widely used in clinical practice for AML, including (1) defining the best targets in order to eradicate the disease while sparing the normal tissue, (2) accurately timing systemic therapy which may be lymphodepleting and may limit the efficacy of T cells required for immunotherapy effect, (3) timing of the immunotherapy in the context of high tumor burden with rapid proliferation often seen in hematologic malignancies and leukemias, (4) identification of ideal T-cell antigens to enable the development of targeted adoptive T-cell strategies with maximum potency and limited collateral organ damage, (5) improving the technology of targeted therapies to ensure better stability, delivery, and efficacy, (6) defining the ideal approach for combining immunotherapy with standard chemotherapy or other anti-leukemic therapies, (7) recognizing and developing standardized management of immune-mediated toxicities in leukemias, (8) and identification of resistance mechanisms with development of strategies to overcome such mechanisms. The gamut of ongoing and future clinical trials with extensive biomarker assays will likely help answer a number of these questions and allow immunotherapy to find its true niche in AML therapy.

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