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REVIEW ARTICLE

Novel Therapies for Acute Myeloid Leukemia: Are We Finally Breaking the Deadlock?

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Abstract Acute myeloid leukemia (AML) is one of the best studied malignancies, and significant progress has been made in understanding the clinical implications of its disease biology. Unfortunately, drug development has not kept pace, as the '7+3' induction regimen remains the standard of care for patients fit for intensive therapy 40 years after its first use. Temporal improvements in overall survival were mostly confined to younger patients and driven by improvements in supportive care and use of hematopoietic stem cell transplantation. Multiple forms of novel therapy are currently in clinical trials and are attempting to bring bench discoveries to the bedside to benefit patients. These novel therapies include improved chemotherapeutic agents, targeted molecular inhibitors, cell cycle regulators, pro-apoptotic agents, epigenetic modifiers, and metabolic therapies. Immunotherapies in the form of vaccines; naked, conjugated and bispecific monoclonal antibodies; cell-based therapy; and immune checkpoint inhibitors are also being evaluated in an effort to replicate the success seen in other malignancies. Herein, we review the scientific basis of these novel therapeutic approaches, summarize the currently available evidence, and look into the future of AML therapy by highlighting key clinical studies and the challenges the field continues to face.

Key Points

An improved understanding of the key genetic, epigenetic, metabolic, and immunological dysregulation driving AML has catalyzed the development of novel therapies.

The multikinase inhibitor midostaurin is the first targeted therapy to improve OS in AML. Multiple FLT-3 inhibitors are currently tested in clinical trials.

IDH 1/2 inhibitors have shown early promise but their benefit needs to be examined in randomized trials.

Cell- and antibody-based immunotherapy in AML is still in its infancy but has the potential to radically change the therapeutic landscape for AML.

1 Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy characterized by clonal proliferation of myeloid precursors, which have a reduced capacity to differentiate into more mature cellular elements, and impaired normal hematopoiesis [1]. AML is a disease of elderly adults with a median age at diagnosis of 67 years and one-third of AML patients are older than 75 years [2]. As the general population ages, AML is becoming a more common problem; it is expected that there will be 21,380 patients diagnosed with AML and more than 10,000 deaths in the US in 2015 [2, 3]. Standard treatment in AML is intense induction chemotherapy upfront, with the goal of achieving complete remission (CR) before proceeding to either consolidation chemotherapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT) based on the patient's individual risk for relapse [1]. Elderly patients who are unfit

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for intense chemotherapy (IC) with curative intent often receive less intense therapy with palliative intent (e.g., hypomethylating agents) or best supportive care (BSC) [2].

Unfortunately, prognosis remains relatively poor. In patients younger than 60 years, the CR rates are 60–70%, but the overall cure rates are only 35–40% [4]. Elderly patients and those with adverse karyotypes have CR rates of 35–50% and cure rates of 10% or less [4].

If patients relapse, the goal is to achieve a second remission (CR2) with re-induction chemotherapy followed by allo-HSCT, which is thought to have a cure rate of approximately 30–40% [5]. However, among patients who relapse, only a minority are able to achieve CR2 to become eligible for allo-HSCT, and therefore the 5-year survival rate after first relapse is only 11% [6]. In a model identifying three risk groups based on the patient age at relapse, relapse-free interval from first CR, cytogenetic risk, and previous allo-HSCT, patients in the good risk group (only 9% of patients) had a CR rate of 85% and overall survival (OS) of 46% at 5 years, 5 years, while patients in the intermediate (25% of patients) and poor risk groups (67% of patients) had CR rates of only 60% and 34% and OS of 18% and 4% at 5 years, respectively [6].

Over the last decade, improved DNA sequencing techniques have led to a better understanding of the key genetic drivers of AML. It has become evident that AML is not one homogenous disease but rather a heterogeneous disease consisting of many genetically unique subtypes that correlate with clinical outcomes [7, 8]. Furthermore, we now have a better understanding of the sequence of genetic mutations leading to leukemogenesis, and the clonal evolution during treatment resulting in resistance to therapy and disease relapse [8–11]. Improved genetic testing has already revolutionized risk stratification and is of vital importance for identifying which patients would best benefit from allo-HSCT [1]. However, despite our rapidly increasing understanding of the biology underlying AML, the general therapeutic strategy has not substantially changed in the last 40 years. Aside from all-*trans*-retinoic acid and arsenic trioxide, which were approved in 1995 and 2001, respectively, for the treatment of acute promyelocytic leukemia, the last new US Food and Drug Administration (FDA) drug approval for AML was idarubicin in 1990 [4, 12, 13]. While gemtuzumab ozogamicin (GO) was approved in 2000, it was subsequently withdrawn from the market in 2010 [14].

In this review, we shed light on advances in different areas of drug development in AML, including chemotherapy, targeted therapy, epigenetic therapy, metabolic therapy, immune therapy, and therapy targeting leukemia stem cells (Fig. 1, Tables 1, 2, 3, 4, 5, 6, 7, and 8). In each section, we first discuss the progress that has been made and highlight results from completed clinical trials before overviewing the ongoing clinical trials. Lastly, we emphasize strategies that combine different modalities in order to achieve therapeutic synergism. We are not able to discuss all of the drugs currently in preclinical or clinical development in AML but rather focus on compounds that are most advanced in clinical evaluation and more likely to pave the way towards success in improving patient outcomes.

2 Advances in Chemotherapy

Our improved understanding of the underlying biology of AML has opened the possibility for developing targeted and more effective treatments. However, it is important to keep in mind that the efficacy of traditional cytostatic chemotherapy is less dependent on the mutational complexity of each patient than precision medicine drugs [15]. Dose intensification of cytarabine or daunorubicin, changing the anthracycline administered, and adding other agents such as cladribine to induction chemotherapy have slightly improved response rates and led to modest improvement in long-term outcomes, but the details of these studies are not discussed in this review [16–24]. Instead, we discuss novel chemotherapeutic drugs currently in different stages of clinical development, which aim to improve the potency and tolerability of traditional cytotoxic regimens.

2.1 CPX-351

As a liposomal formulation of cytarabine and daunorubicin in a 5:1 fixed molar ratio, CPX-351 aims to increase the exposure of leukemic cells to both agents at concentrations optimal for synergy and to prevent antagonism, optimize delivery to the bone marrow, and reduce normal organ toxicity [25]. After CPX-351 showed promising response rates in refractory/refractory or refractory AML patients in a phase I study [25], two phase II studies compared CPX-351 to physician's choice of IC in refractory AML patients as salvage therapy [26] and to '7+3' induction chemotherapy in elderly AML patients as frontline therapy [27] (Table 1). Outcomes have been mixed, as in the first study CPX-351 resulted in increased relative risk (RR) [CRc and CR with incomplete neutrophil count recovery (CRi): 49.3% vs. 40.9%] but no statistically significant increase in OS [26], while the second study showed no statistically significant difference in either RR or OS between CPX-351 and 7+3 [27]. In a subsequent subgroup analysis, CPX-351 did result in a statistically significant benefit in OS in poor-risk patients as defined by the European Prognostic Index when compared to physician's choice IC (OS 6.6 vs. 4.2 months) [26] and in patients with secondary AML when compared to 7+3 (12.1 vs. 6.1 months) [27] (Table 1).

A phase III clinical trial compared CPX-351 versus 7+3 induction chemotherapy in patients with newly diagnosed, secondary AML who were between 60 and 75 years old [28, 29]. Secondary AML included patients with prior cytotoxic

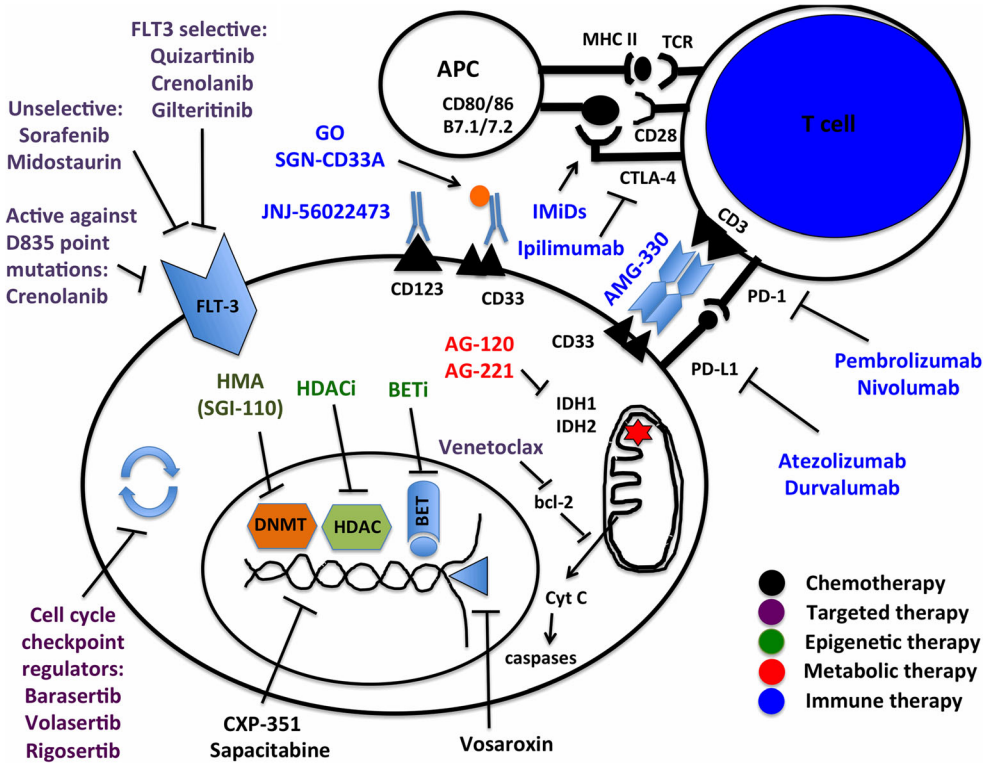

Fig. 1 Novel approaches to treatment for AML. A remarkable spectrum of different therapeutic approaches for AML is currently being tested in clinical trials including novel chemotherapeutic drugs, targeted therapy, epigenetic therapy, metabolic therapy, and immunotherapy. Novel chemotherapeutic drugs with significant potential to improve the current therapeutic landscape are the liposomal formulation of cytarabine and daunorubicin (CPX-351) and the nucleoside analog sapacitabine, as well as the topoisomerase II inhibitor vosaroxin (Sect. 2, Table 1). Targeted therapy includes several different generations of FLT3 inhibitors, the BCL-2 inhibitor venetoclax, as well as the cell cycle inhibitors barasertib, rigosertib, and volasertib (Sect. 3, Tables 2, 3, and 4). Epigenetic therapy consists of HMAs and HDACi as well as newer oral and extended-release HMAs (guadecitabine) and BETi (Sect. 4, Table 5). The most successful candidates interfering with leukemia cell metabolism are the IDH1 inhibitor AG-120 as well as the IDH2 inhibitor AG-221 (Sect. 5, Table 6). Immunotherapy in AML is based on multiple approaches to enhance the immune system's ability to recognize and kill AML cells (Fig. 2, Sect. 6, Tables 7 and 8). Peptide and dendritic cell vaccination enhance leukemia antigen recognition and T cell priming (not shown here). Immune surveillance is suppressed in AML by increased expression of CTLA-4 leading to ineffective T cell priming through APCs by interfering with the interaction of B7.1 and B7.2 with CD28. At the level of the tumor cells, overexpression of PD-1 on leukemia cells and PD-L1 on T cells leads to T cell anergy and exhaustion. Immune checkpoint inhibitors directed towards CTLA-4 (ipilimumab), PD-1 (nivolumab, pembrolizumab), and PD-L1 (durvalumab, atezolizumab) are currently tested for their ability to reconstitute immune surveillance. Furthermore, leukemia antigens (e.g., CD33 and CD123) can be specifically targeted by monoclonal antibodies, which either use antibody-dependent, cell-mediated cytotoxicity (JNJ-56022473) or are conjugated with cytostatic drugs (GO and SGN-CD33A). Alternatively, BiTE antibodies such as AMG-330 directly connect cytotoxic T cells with leukemia cells by binding to both CD3 on T cells as well as antigens on the surface of leukemia cells (such as CD33). Lastly, adoptive cell therapy with CAR T cells, NK cells, and CIK cells are potent new immunotherapy strategies using effector cells targeting AML blasts (not shown here). Particularly promising are approaches that combine chemotherapy, targeted therapy, epigenetic therapy, and metabolic therapy with immunotherapy in order to achieve synergism between these different approaches (Fig. 2, Sect. 6). *AML* acute myeloid leukemia, *APC* antigen-presenting cell, *BCL-2* B-cell lymphoma 2, *BET* bromodomain and extra-terminal, *BETi* bromodomain and extra-terminal inhibitors, *BiTE* bispecific T cell engager, *CAR* chimeric antigen receptor, *CIK* cytokine-induced killer, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *Cyt C*, cytochrome C, *DNMT* DNA methyltransferase, *FLT3* FMS-related tyrosine kinase 3, *HDAC* histone deacetylase, *HDACi* histone deacetylase inhibitors, *HMAs* hypomethylating agents, *IDH* isocitrate dehydrogenase, *IMiDs* immunomodulatory drugs, *MHC* major histocompatibility complex, *NK* natural killer, *PD-1* programmed cell death protein 1, *PD-L1* programmed death-ligand 1,*TCR* T cell receptor

treatment, antecedent myelodysplastic syndrome (MDS) [± prior treatment with hypomethylating agents (HMAs)], or AML with World Health Organization (WHO)-defined MDS-related cytogenetic abnormalities. In this cohort of elderly, secondary AML, CPX-351 treatment resulted in significantly improved RR as well as prolonged OS when compared to 7+3 induction chemotherapy without an increase in 60-day mortality or adverse effects (AEs) frequency or severity [28] (Table 1). Furthermore, a subgroup analysis of patients who subsequently received allo-HSCT after CPX-351 and 7+3 induction, although not randomized, showed that the CPX-351 group patients were older (≥70 years: 31% vs. 15%) and had 53% fewer deaths within 100 days of transplant compared with the 7+3 group [29] (Table 1). Based on the favorable results of the phase II trial, the FDA gave the drug a breakthrough designation in May 2016, and it is currently being

Table 1 Novel chemotherapeutic agents

Drug	Molecular target	Completed and recruiting clinical trials				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
CPX-351	Liposomal formulation of cytarabine and daunorubicin Optimal synergy of both drugs in a 5:1 combination	I [25]	48	RR-AML, HR-MDS	CPX-351	CR 21%, CRp 2%
		II [26]	125	Relapsed AML, first salvage	CPX-351 vs. physician choice IC (2:1)	CR/CRi 49.3% vs. 40.9% OS 8.5 vs. 6.3 months (NS) Poor risk pt: OS 6.6 vs. 4.2 months
		II [27]	127	Frontline AML, >60 years	CPX-351 vs. 7+3 (2:1)	CR/CRi 66.7% vs. 51.2% (NS) OS 14.7 vs. 12.9 months (NS) 2nd AML pt: OS 12.1 vs. 6.1 months
		III [28, 29]	309	2nd AML and 60–75 years old	CPX-351 vs. 7+3 (1:1)	CR/CRi 47.7% vs. 33.3% OS 9.56 vs. 5.95 months Pts who received an HSCT: mortality at 100 days 9.6% vs. 20.5%
Vosaroxin	DNA topo-isomerase II inhibitor No free radical formation without cardiotoxicity	I/II [32]	108	RR-AML	Vosaroxin + cytarabine	CR/CRi 28%
		II [33]	116	>60 years and adverse prognosis	Vosaroxin	CR/CRp 35% OS 7.7 months
		III [34]	711	RR-AML	Vosaroxin + cytarabine vs. cytarabine	CR 30.1% vs. 16.3% OS 7.5 vs. 6.1 months (NS) Pt >60 years: 7.1 vs. 5 months
		II		Upfront setting	Vosaroxin + cytarabine	Enrolling pts (NCT02658487)
Sapacitabine	Nucleoside analog	I [35]	47	RR-AML and MDS	Sapacitabine	Overall response rate 28% CR 9%
		II [36]	86	>70 years, frontline and in first relapse	Sapacitabine	1-year OS 35% 200 mg twice daily, 7 days
		II [37]	143	Upfront setting	Sapacitabine vs. LDAC	CR/CRi 27% vs. 16% (NS) OS at 2 years 12% vs. 11% (NS)
		III [38]	485	>70 years, unfit for IC	Sapacitabine + decitabine vs. decitabine alone	Press release: no statistically significant difference in OS

2nd AML secondary AML, *AML* acute myeloid leukemia, *CR* complete remission, *CRi* complete remission with incomplete neutrophil count recovery, *CRp* complete remission in the absence of total platelet recovery, *HR-MDS* high-risk myelodysplastic syndrome, *HSCT* hematopoietic stem cell transplantation, *IC* intensive chemotherapy, *LDAC* low-dose Ara-C (cytarabine), *MDS* myelodysplastic syndrome, *NS* not statistically significant, *OS* overall survival, *pt(s)* patient(s), *RR-AML* relapsed and refractory AML,

considered by the FDA for a frontline therapy indication in elderly patients with secondary AML. It remains to be investigated whether younger patients with high-risk features such as poor karyotype or adverse mutational profiles would also benefit from CPX-351.

2.2 Vosaroxin

Vosaroxin is a first-in-class quinolone derivative that, like anthracyclines, intercalates into DNA and inhibits topoisomerase II inhibitor, thereby inducing double-stranded DNA breaks [30]. However, unlike anthracyclines, vosaroxin is not associated with free radical production and therefore does not lead to cardiotoxicity, which is a feared complication associated with cumulative anthracycline usage [31]. Furthermore, vosaroxin exerts an effect independent of the p53 pathway. Vosaroxin has shown encouraging efficacy in two early-phase clinical trials as both monotherapy and in combination with cytarabine (Table 1): in refractory and relapsed AML (RR-AML) patients, vosaroxin plus cytarabine led to an RR of 28%, with stomatitis being the dose-limiting toxicity (DLT) [32]. Vosaroxin alone resulted in an RR of 35% when studied in patients who were older than 60 years and who had one of the following adverse prognostic factors: age ≥70 years, antecedent hematological disorders, ECOG (Eastern Cooperative Oncology Group) performance status (PS) of 2, or intermediate or unfavorable karyotype [REVEAL-1 (Response Evaluation of Vosaroxin in Elderly AML)] [33].

Based on these promising results, a phase III clinical trial comparing cytarabine with or without vosaroxin in patients with first RR-AML was conducted [VALOR (Vosaroxin and

Table 2 FMS-related tyrosine kinase 3 (FLT3) inhibitors

Drug	Generation	Clinical trials with results				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
Sorafenib (BAY 43–9006)	1st	I [288]	15	AML, ALL	Sorafenib	Stable disease 11/15
		I [51]	65	FLT3-ITD AML	Sorafenib	CR/CRi 23% Resistance in previous no-SCT 47% vs. allo-SCT 38% Days to resistance 136 vs. 197 days ($p = 0.03$)
		I/II [289]	51	ND-AML <65 years	Induction (Ara-C + Ida) + sorafenib	CR 75% CR/CRp FLT3-ITD 100% (CR 93%) CR FLT3-wt 66% 1-year OS 74%
		II SORAML [57]	276	ND-AML	Sorafenib vs. placebo (1:1) + induction (7+3)/consolidation (HiDAC)	EFS 21 vs. 9 months ($p = 0.013$) No correlation with *FLT3* mutation status 1-year EFS 64% vs. 50%
		II [59]	37	RR FLT3-ITD AML	Sorafenib + AZA	ORR 46%, CR 16%, CRi 27%, PR 3% Adequate (>85%) FLT3 inhibition 64%
		II [58]	201	ND-AML >60 years	Sorafenib + induction (7+3)/consolidation	CR 48% vs. 60% Early death 17% vs. 7% Worse outcomes due to higher treatment-related mortality
		II [290]	23	ND, FLT3+ AML >60 years	Sorafenib + AZA	CR 32%, CRi/CRp 41% Remission experimental 16 vs. historical 3.8 months ($p = 0.08$) Statistically similar to historic data on HMA
Sunitinib (SU11248)	1st	I/II [53]	17	ND, FLT3 AML >60 years	Sunitinib + induction (7+3)	FLT3-ITD CR/CRi 57% (8/14), FLT3-TKD 62.5% (5/8)
Lestaurtinib (CEP-701)	1st	I/II	14	RR, FLT3 AML	Lestaurtinib	Clinical activity in 5/14
		II	29	ND-AML >60y	Lestaurtinib	Clinical activity in FLT3+ 3/5, FLT3-wt 5/22
		III	224	RR-FLT3	Lestaurtinib + re-induction	No difference in CR, OS Only 58% had sustained FLT3 inhibition in vivo
		III	500	ND, FLT3 AML	Induction/consolidation ×4 cycles ± lestaurtinib	No difference in 5-year OS, 5-year RFS Reduced relapse if sustains >85% FLT3 inhibition
Midostaurin (PKC412)	1st	Ib CONSORT [291]	29	ND-AML	Midostaurin + induction (7+3)	CR FLT3+ 92% vs. FLT3-wt 74%
		I [292]	17	ND-AML or RR-AML	Midostaurin + AZA	CR 3/14, count improvement 2/14
		I [293]	10	RR-AML	Midostaurin + ATRA + CLAG	CR 2/10, CRi 1/10
		I [294]	16	ND-AML >60 years or RR-AML	Midostaurin + DEC	Stable disease 9/16, hematologic response 4/16
		I/II [295]	54	AML, HR-MDS	Midostaurin + AZA	ORR 26% Mdn RD 20 weeks

Table 2 (continued)

Drug	Generation	Clinical trials with results				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
						Longer RD if FLT3 not previously treated with FLT3i ($p = 0.05$) Longer RD if not previously transplanted ($p = 0.01$)
		II [52]	20	RR FLT3+ AML or high-grade MDS	Midostaurin	CR 70%
		IIb [296]	95	AML or MDS	Midostaurin	ORR FLT3-mt 71% vs. wt 56% BR FLT3-mt 71% vs. wt 42% HI FLT3-mt 46% vs. 35%
		III RATIFY [60]	717	ND-AML <60 years	Midostaurin vs. placebo + conventional chemotherapy	5-year OS 51.4% vs. 44.2% No difference in 60-day CR increased survival in all pts
Quizartinib (AC220; ASP2689)	2nd	I [61]	76	RR, FLT3-ITD+ AML	Quizartinib	CR/CRi/CRp 13% FLT3-ITD+ 9/17 responders FLT3– 5/37 responders Mdn response duration 13.3 week Mdn OS 14 weeks
		I/II [297]	52	AML, MDS, CMML	Quizartinib + AZA vs. LDAC	Actively recruiting ORR 67% (AZA arm 77%, LDAC arm 23%) ORR FLT3-ITD+ 73%
		II [62]	154	RR-AML >60 years	Quizartinib	CRc FLT3+ 54% vs. FLT3– 32% Mdn response 12.7 weeks vs. FLT3– 22.1 weeks Mdn OS 25.3 vs. 19 weeks
		II [298]	137	RR-AML	Quizartinib	CR/CRp/CRi FLT3+ 44% vs. FLT3– 34% Mdn response 25.6 vs. 11.3 weeks CRc RR 47% vs. 31%
		III [299]	52	ND-AML >60 years	Quizartinib + ADE	CR 60% Mdn OS 15 months
Crenolanib (CP-868596)	2nd	I/II [300]	8	RR-AML	Crenolanib + HAM	Actively enrolling (expanded to MEC, FLAG-Ida) CR + CRi 4/8
		I/II [70]	13	RR FLT3+ AML	Crenolanib + salvage (HiDAC + Ida)	ORR 36% (1 CR, 3 CRi) CR/CRi prior FLT3i 67% No CR in pts who received ≥3 past treatment
		II [67, 68]	55	RR FLT3+ AML	Crenolanib	Actively enrolling Mdn OS overall 19 weeks FLT3-naïve 55 weeks vs. prior 13 weeks Mdn EFS overall 8 weeks FLT3-naïve 13 weeks vs. prior 7 weeks
		II [69]	25	ND FLT3+ AML	Crenolanib + induction (7+3)	Actively enrolling CR/CRi 96% (CR 88%)

Table 2 (continued)

Drug	Generation	Clinical trials with results			Intervention	Outcomes and subpopulation
		Phase [reference]	*N*	Patient characteristics		
Gilteritinib (ASP2215)	2nd	I/II Chrysalis [71]	252	RR AML	ASP2215	DR 6/25 ORR FLT3+ 49% vs. FLT3-wt 12% Mdn OS FLT3+ 31 weeks Mdn duration response 20 weeks Best if sustained concentrations >100 ng/mL

ALL acute lymphoblastic leukemia, *allo-SCT* allogeneic hematopoietic stem cell transplantation, *AML* acute myeloid leukemia, *Ara-C* cytarabine, *AZA* azacitidine, *BR* blast reduction, *CLAG* cladribine, cytarabine, granulocyte colony stimulating factor, *CMML* chronic myelomonocytic leukemia, *CR* complete remission, *CRc* composite complete remission = CR + CRp + CRi, *CRi* complete remission with incomplete neutrophil count recovery, *CRp* complete remission in the absence of total platelet recovery, *DEC* decitabine, *EFS* event-free survival, *HAM* high-dose Ara-C (cytarabine)/mitoxantrone, *HI* hematologic improvement, *HiDAC* high-dose cytarabine, *HMA* hypomethylating agent, *HR-MDS* high-risk myelodysplastic syndrome, *Ida* idarubicin, *ITD* internal tandem repeats, *LDAC* low-dose Ara-C (cytarabine), *Mdn* median, *ND* new diagnosis, *ORR* overall response rate, *OS* overall survival, *PR* partial response, *pt(s)* patient(s), *RD* remission duration, *RFS* relapse-free survival, *RR* relapsed/refractory, *SCT* stem cell transplant, *wt* wild-type

Ara-C combination evaLuating Overall survival in refractory/refractory AML)] [34]. While the addition of vosaroxin to cytarabine resulted in a higher CR (30.1% vs. 16.3%), OS was not statistically significantly different between both treatment arms (Table 1). In a preplanned intention-to-treat analysis, vosaroxin was associated with a statistically significant improvement in OS for patients ≥60 years (7.1 vs. 5 months). A phase II single-arm clinical trial [VITAL (Vosaroxin and Infusional cytarabine in Treating patients with untreated Acute myeloid Leukemia)] examining vosaroxin in combination with cytarabine in patients with previously untreated AML is currently enrolling patients (Table 1).

2.3 Sapacitabine

Sapacitabine is an oral deoxycytidine nucleoside analog that showed promising activity in elderly AML patients in phase I and II clinical trials [35, 36] (Table 1). However, when sapacitibine was compared to low-dose cytarabine (LDAC) in a randomized controlled trial (RCT) of 143 untreated AML and MDS patients, there was no difference between sapacitabine and LDAC in terms of remission rate or OS [37] (Table 1). Based on a recent press release, the phase III (SEAMLESS) study comparing sapacitabine in combination with decitabine (administered in alternating cycles) versus decitabine alone also failed to meet its primary endpoint of significant improvement in OS [38] (Table 1).

3 Advances in Targeted Therapy

Since the establishment of tyrosine kinase inhibitors as the gold standard for treating chronic myelogenous leukemia, the field has sought to replicate similar therapeutic successes in other malignancies. Given the complex mutational landscape found in AML, such robust and sustained responses have proven difficult to be realized. Nonetheless, several novel agents are currently under development and represent a promising approach to improving outcomes.

3.1 FMS-related tyrosine kinase 3 (FLT3) Inhibitors

FMS-related tyrosine kinase 3 (FLT3) represents an attractive therapeutic target in AML. As a class III family receptor tyrosine kinase, FLT3 is strongly expressed in hematopoietic progenitors, including leukemic myeloblasts, and acts as a cytokine receptor for the FLT3 ligand. FLT3 activation promotes cell proliferation and pro-survival properties via downstream cascades, including the RAS/MEK, PI3K/AKT/mTOR (mechanistic target of rapamycin), and STAT5 (signal transducer and activator of transcription 5) pathways [39, 40].

Clinically, *FLT3* mutations are one of the most frequently observed mutations in AML and are found in upwards of 30% of patients [41]. The most prevalent mutation, seen in

Table 3 Active FMS-related tyrosine kinase 3 (FLT3) inhibitor clinical trials

Drug	Generation	Recruiting clinical trials			
		Phase [reference]	*N*	Patient characteristics	Intervention
Sorafenib (BAY 43–9006)	1st	II	54	ND-FLT3+ AML >60 years	Sorafenib + chemotherapy (NCT01253070)
		II	52	ND, FLT3-ITD AML or MDS	Sorafenib + AZA (NCT02196857)
Midostaurin (PKC412)	1st	I	36	RR/poor-risk AML or MDS	Midostaurin + RAD001 (NCT00819546)
		I	34	RR-AML	Midostaurin + MEC + bortezomib Closed (NCT01174888)
		II	18	ND, c-KIT or FLT3-ITD t(8;21) AML	Midostaurin + induction (NCT01830361)
		II	26	ND-AML ≥60 years	Midostaurin + DEC (NCT02634827)
		II	36	ND-AML ≥60 years	Midostaurin + DEC (NCT01846624)
Quizartinib (AC220; ASP2689)	2nd	I	536	FLT3-ITD, international	Quizartinib + chemotherapy (NCT02668653)
		I	19	ND-AML	Quizartinib + induction (7+3)/consolidation Closed (NCT01390337)
		Ib	N/S	ND-AML	Quizartinib + induction/consolidation (NCT02834390)
		II	76	RR, FLT3-ITD+ AML	Quizartinib + induction/consolidation Closed (NCT01565668)
		II	41	RR, FLT3-ITD+ AML	Quizartinib (NCT02984995)
		III	363	RR FLT3-ITD+ AML QUANTUM-R	Quizartinib vs. salvage chemotherapy (NCT02039726)
		III	536	ND FLT3-ITD+ AML QUANTUM-First	Quizartinib vs. placebo + induction/cnsolidation (NCT02668653)
Crenolanib (CP-868596)	2nd	I	10	Pedi, AYA RR FLT3+ AML	Crenolanib + sorafenib (NCT02270788)
		I/II	72	RR-AML	Crenolanib + salvage (HAM, MEC, FLAG-Ida) (NCT02626338)
		I/II	88	RR FLT3 AML or HR-MDS	Crenolanib + standard chemotherapy (MEC, FLAG-Ida) vs. AZA (NCT02400281)
		II	48	ND FLT3+ AML	Induction (7+3) + crenolanib (NCT02283177)
		II	70	RR FLT3+ AML	Crenolanib (NCT01657682)
		II	20	RR FLT3+ AML	Crenolanib (NCT01522469)
		II	48	FLT3+ AML	Crenolanib maintenance (NCT02400255)
		III	276	RR FLT3+ AML	Crenolanib vs. placebo + salvage (HAM) (NCT02298166)
Gilteritinib (ASP2215)	2nd	I	21	ND-AML	ASP2215 + Induction (7+3) (NCT02236013)
		II/III	540	ND FLT3+ AML	ASP2215 ± AZA (NCT02752035)
		III	369	RR FLT3+ AML	ASP2215 vs. salvage (LDAC, MEC, FLAG-Ida) (NCT02421939)
		III	354	FLT3+ AML in CR1	ASP2215 vs. placebo maintenance

Table 3 (continued)

Drug	Generation	Recruiting clinical trials			
		Phase [reference]	*N*	Patient characteristics	Intervention
					(NCT02927262)
		III	346	FLT3+ AML s/p allo-SCT	ASP2215 vs. placebo maintenance (NCT02997202)
Ponatinib (AP24534)	N/S	I/II	40	ND FLT3+ AML	Ponatinib + Ara-C consolidation (NCT02428543)
		I/II	132	FLT3+ AML (both ND and RR)	Ponatinib + AZA (NCT02829840)
		II	24	RR FLT3+ AML	Ponatinib (NCT01620216)
		Ib	24	ND-AML	Ponatinib + induction (7+3) (NCT02779283)
PLX3397	N/S	I/II	90	RR FLT3-ITD+ AML	PLX3397 (NCT01349049)
AMG925, FLX925	3rd TKI	I/Ib	123	RR FLT3+ AML	FLX925 (NCT02335814)

allo-SCT allogeneic hematopoietic stem cell transplantation, *AML* acute myeloid leukemia, *Ara-C* cytarabine, *AYA* adolescents and young adults, *AZA* azacitidine, *CR1* first complete remission, *DEC* decitabine, *FLAG* fludarabine + cytarabine + G-CSF *HAM*, high-dose Ara-C (cytarabine)/mitoxantrone, *HR* high-risk, *Ida* idarubicin, *ITD* internal tandem repeats, *LDAC* low-dose cytarabine, *MDS* myelodysplastic syndrome, *ND* new diagnosis, *N/S* not specified, *Pedi* pediatrics, *RR* relapsed/refractory, *TKI* tyrosine kinase inhibitor

approximately 25% of AML patients, involves duplicated coding sequences within the juxtamembrane domain, termed internal tandem repeats (ITD), that result in disruption of the auto-inhibitory function [42, 43]. The *FLT3*-ITD mutation is a poor prognostic marker, with higher relapse rates and reduced OS [44]. The length of the ITD and the allelic ratio of mutant:wild-type (wt) correlate with worse survival [45]. Point mutations within the tyrosine kinase domain (TKD), most commonly at the residue aspartate 835 (D835), are observed in ~7% of patients [46]. The prognostic implication of FLT3-TKD in AML is less certain.

Agents targeting FLT3 are generally categorized by target specificity. The first-generation agents (sunitinib, sorafenib, midostaurin, lestaurtinib) mainly comprise multi-kinase inhibitors, many of which were originally studied for use in other malignancies. For example, while sunitinib (SU11248) is a potent FLT3 inhibitor [47], it also has activity against platelet-derived growth factor receptor (PDGFR), vascular endothelial growth factor receptor (VEGFR), and KIT [48], and was originally approved for use in metastatic renal cell carcinoma. Initial studies (Table 2) with sunitinib and other first-generation agents as monotherapy in AML demonstrated activity [49–52]; however, such responses were short-lived and associated with significant toxicities and acquisition of secondary mutations [53].

Early studies also demonstrated the importance of sustained FLT3 inhibition on clinical outcomes (Table 3). Two large phase III studies involving the first-generation FLT3 inhibitor lesaurtinib (CEP-701) in the upfront [54] and salvage [55] settings showed no overall difference in CR, 5-year relapse-free survival (RFS), or 5-year OS. However, analyses found improved OS and reduced relapse rates in patients where lesaurtinib was able to sustain over 85% FLT3 inhibition [56]. Further characterization of patients who would best benefit from lesaurtinib is therefore warranted.

Combination therapy with first-generation agents and conventional chemotherapy regimens demonstrated more promising results both as upfront and salvage therapies. The SORAML trial was a German randomized, double-blind, placebo-controlled phase II study that enrolled 276 newly diagnosed younger (18–60 years) patients to either induction chemotherapy with daunorubicin and cytarabine (7+3) with or without sorafenib (BAY 43–9006) [57]. Event-free survival (EFS) was significantly prolonged at 1 year (64% vs. 50%). Interestingly, no correlation between *FLT3* mutation status and outcome was observed. The most common significant (grade ≥3) adverse events were fever, infection, pneumonia, and pain. However, the survival benefits seen in the SORAML trial may not apply to all populations, as demonstrated by Serve et al., who reported worse outcomes in elderly patients treated with sorafenib in combination with standard induction (7+3) [58]. Treatment-related toxicities resulted in higher treatment-related mortality, lower CR rates, and less consolidation chemotherapy due to higher induction toxicity in the sorafenib arm. Sorafenib in combination with less toxic regimens were better tolerated. Ravandi et al. described the addition of sorafenib to azacitidine in 43 patients with refractory/refractory disease [59]. The overall response was

Table 4 Targeted therapies other than FMS-related tyrosine kinase 3 (FLT-3) inhibitors

Drug	Molecular target	Completed and recruiting clinical trials				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
Venetoclax (ABT-199; GTC-0199)	BCL-2	Ib [301]	260	ND-AML ≥60 years	ABT-199 + DEC or AZA	Actively recruiting Interim results (n = 22) CR/CRi/PR DEC/VEN 9/12 (CR2), AZA/VEN 7/10 (CR3) (NCT0220377)
		II [302]	32	AML (RR 93.8%)	ABT-199	Closed CR/CRi 5/28 evaluable pts 3/5 CR/CRi pts had IDH-mt
		I/II	91	ND-AML ≥65 years	ABT-199 + LDAC	Actively enrolling (NCT02287233)
		III	400	ND-AML ≥60 years	ABT-199 vs. AZA	Actively enrolling (NCT02993523)
Barasertib (AZD-1152)	Aurora B kinase inhibitor	I [83]	22	ND-AML	LDAC + AZD-1152	ORR 45%
		II [84]	74	ND-AML ≥60 years	LDAC vs. AZD-1152	CR/CRi 35.4% vs. 11.5% ($p < 0.05$) Mdn OS 8.2 vs. 4.5 months (NS)
Volasertib (BI-6727)	PLK1 inhibitor	I/IIa [89]	180	RR-AML	LDAC ± BI-6727	Closed Interim results (n = 87) ORR LDAC + V 31% vs. LDAC 13.3% (p = 0.052) independent of cytogenetics OS Mdn 8.0 vs. 5.2 months (p = 0.047) EFS Mdn 5.6 vs. 2.3 months (p = 0.021)
		I	19	AML (ND or RR)	BI-6727	Closed (NCT01662505)
		I	127	ND-AML ≥65 years	BI-6727 + DEC	Terminated (NCT02003573)
		I	30	ND-AML	Induction (7+3) + BI-6727	Not yet enrolling (NCT02527174)
		I	28	ND-AML	Induction (7+3) + BI-6727	Not yet enrolling (NCT02905994)
		III	660	ND-AML ≥65y	LDAC ± BI-6727	Closed (NCT01721876)
Rigosertib (ON-01910)	PLK1 inhibitor	I [95]	14	RR-AML and HR-MDS	ON-01910 IV	Closed ORR 4/14 pts (NCT00533416)
		I/II [97]	40	RR-AML 1 prior salvage, MDS, CMML	ON-01910 PO + AZA	Actively enrolling Interim results (n = 18) CR/CRi 5/18 pts (NCT01926587)
		I/II	28	RR-AML, ALL, MDS, CML, CLL	ON-01910 IV	Closed (NCT00854945)
		I/II	34	RR-AML/ALL, MPD, CML	ON-01910 IV/PO	Closed (NCT01167166)

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *AZA* azacitidine, *BCL-2* B-cell lymphoma 2, *CLL* chronic lymphocytic leukemia, *CML* chronic myelogenous leukemia, *CMML* chronic myelomonocytic leukemia, *CR* complete remission, *CR2* second complete remission, *CR3* third complete remission, *CRi* complete remission with incomplete neutrophil count recovery, *DEC* decitabine, *EFS* event-free survival, *IDH-mt* IDH-mutant, *IV* intravenous, *LDAC* low-dose cytarabine, *Mdn* median, *MDS* myelodysplastic syndrome, *MPD* myeloproliferative disease,*ND* new diagnosis, *NS* notstatistically significant,*ORR* overall response rate, *PLK1* polo-like kinase I, *PO* oral, *PR* partial response, *pts* patients, *RR* relapsed/refractory, *VEN* venetoclax

Table 5 Epigenetic therapies

Drug	Molecular target	Completed and recruiting clinical trials				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes
Guadecitabine (SGI-110)	HMA resistant to deamination	I [120]	93	RR-AML (80%) and MDS (20%)	SGI-110	SGI-110 60 mg/m^2 well-tolerated dose
		II [121]	51	Elderly AML pt not eligible for IC frontline therapy	SGI-110	CRc 57% CR 37% OS 10.5 months
		II [122]	103	RR-AML	SGI-110	CRc 23% OS 6.6 months
		III		AML pts not eligible for IC frontline therapy	SGI-110 vs. conventional therapy (AZA, DAC, LDAC)	ASTRAL-1 trial Active (NCT02348489)
		III		RR-AML	SGI-110 vs. conventional therapy (HiDAC, MEC, Flag-Ida, AZA, DAC, LDAC)	ASTRAL-2 trial Not yet recruiting (NCT02920008)
Pinometostat (EPZ-5676)	DOT1L inhibitor	I [130]	49	RR-AML, MDS, ALL, and CMML with MLL1 rearrangement	EPZ-5676	Morphologic CR 1 pt Cytogenetic CR 1 pt Partial response 1 pt Resolution of leukemia cutis 3 pts
OTX-015	BET inhibitor	I [134]	41	RR leukemia (88% with AML)	OTX-015	CR/CRi 7.3% PB clearance 4.8%
GSK525762	BET inhibitor	I		RR-AML and other myeloid malignancies	GSK525762	Recruiting (NCT01943851)
CPI-0610	BET inhibitor	I		AML, MDS, Myelofibrosis	CPI-0610	Recruiting (NCT02158858)
TEN-010	BET inhibitor	I		AML, MDS	TEN-010	Recruiting (NCT02308761)

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *AZA* azacitidine, *BET* bromodomain and extraterminal, *CMML* chronic myelomonocytic leukemia, *CR* complete remission, *CRc* composite complete remission (CR + CRi + CRp), *CRi* complete recovery with incompleteneutrophil count recovery, *CRp* complete recovery with incomplete plateletrecovery and normal neutrophil count, *DAC* intermediate-dose cytarabine,*DOT1L* disruptor of telomericsilencing 1-like, *HiDAC* high-dose cytarabine, *HMA* hypomethylating agent, *IC* intensive chemotherapy, *Flag-Ida* fludarabine, AraC, idarubicin, *LDAC* low-dose cytarabine, *MDS* myelodysplastic syndrome, *MEC* mitoxantrone, etoposide, cytarabine, *MLL1* mixed lineakge leukemia protein-1, *OS* overall survival, *PB* peripheral blasts, *pt(s)* patient(s), *RR* relapsed/refractory

46%, including CR in 16% of patients and CRi in 27% of patients. Based on the responses seen in the SORAML and other clinical trials, sorafenib is now frequently used for selected patients with RR-AML.

Similarly, the RATIFY trial (Randomized AML Trial in FLT3 the Young patients) was a phase III, randomized, double-blind study that enrolled newly diagnosed, younger (<60 years) FLT3-positive (FLT3+) (both *ITD* and *TKD* mutations) AML patients to either conventional chemotherapy [induction with 7+3, consolidation with high-dose cytarabine (HiDAC) with or without midostaurin (PKC412)] [60]. Though no difference in CR was observed, both EFS (24.2% vs. 21.8%) and OS (51.4% vs. 44%) at 5 years was significantly higher in the midostaurin group, with no difference in toxicity. Based on these results, on 28 April 2017, the FDA approved midostaurin for the treatment of adult patients with newly diagnosed AML who are *FLT3* mutation-positive, in combination with standard cytarabine and daunorubicin induction and cytarabine consolidation.

Ongoing trials are now investigating the role of first generation FLT3 inhibitors, primarily sorafenib (NCT02196857) and midostaurin (NCT02634827, NCT01846624), in combination with HMAs in treating patients who are poor candidates for intensive induction regimens (Table 2).

The newer second-generation agents (quizartinib, crenolanib, and gilteritinib) are more specific, more potent, and generally better tolerated (Table 2). Quizartinib (AC220, ASP2689), a selective small-molecule inhibitor, has a relatively longer half-life and sustained inhibition in vivo compared

Table 6 Metabolic therapies

Drug	Molecular target	Completed and recruiting clinical trials				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
Enasidenib (AG-221)	IDH2 inhibitor	I/II [303]	198	IDH2+ myeloid malignancies	AG-221	Closed (NCT01915498) Interim: RR-AML 138 pts RR-AML: CR 18%, CRc 41% Mdn response 6.9 months
		III [302]	280	IDH2+, RR-AML ≥60y	AG-221 vs. conventional therapy (AZA, LDAC, IDAC, or BSC only)	IDHENTIFY trial Recruiting (NCT02577406)
AG-120	IDH1 inhibitor	I [158]	236	$IDH1^{R132}$ myeloid malignancies	AG-120	Recruiting (NCT02074839) Interim: 66 pts CR 18%, ORR 36% Mdn response 5.6 months
Multiple	IDH1 or IDH2 inhibitor	I	90	IDH1/IDH2+ ND-AML	AG-120 or AG-221 + induction (7+3)/consolidation (Ara-C, ME)	Recruiting (NCT02632708)
		Ib/II	123	IDH1/IDH2+ ND-AML	AG-120 or AG-221 + AZA	Recruiting (NCT02677922)
FT-2102	IDH1 inhibitor	I/Ib	78	$IDH1^{R132}$ AML or MDS	FT-2102 vs. FT-2102 + AZA	Recruiting (NCT02719574)
AG-881	IDH1/2 inhibitor	I	46	IDH1 or IDH2 myeloid malignancies	AG-881	Closed (NCT0492737)
CB-839	Glutaminase inhibitor	I	43	RR-AML/ALL, ND-AML/ALL ≥60 years	CB-839, CV-839 + AZA	Complete (NCT02071927)
Erwinaze®	Glutamine depletion	I	5	RR-AML	Erwinase	Closed (NCT2283190)
		II	20	RR-hematologic malignancy	Fludarabine + Ara-C + Erwinase	Not yet open (NCT02718755)
Indoximod	IDO inhibitor	Ib/IIa	138	ND-AML	Ida + Ara-C (7+3) ± indoximod	Recruiting (NCT02835729)

ALL acute lymphoblastic leukemia, *AML* acute myeloid leukemia, *Ara-C* cytarabine, *AZA* azacitidine, *BSC* best supportive care, *CR* complete remission, *CRc* composite complete remission, *HiDAC* high-dose cytarabine, *Ida* idarubicin, *IDAC* intermediate-dose cytarabine, *IDH* isocitrate dehydrogenase, *IDO* indoleamine 2,3-dioxygenase, *LDAC* low-dose cytarabine, *MDS* myelodysplastic syndrome, *ME* mitoxantron/etoposide, *Mdn* median, *ND* new diagnosis, *ORR* overall response rate, *pts* patients, *RR* relapsed/refractory

with first-generation agents [61]. When used as monotherapy in the refractory/refractory setting, the composite CR (CRc)—defined as CR + CRi + CR with incomplete platelet recovery and normal neutrophil count (CRp)—was observed in 54% of FLT3-ITD patients compared with 32% in FLT3-wt patients, though notably 58/63 patients who obtained CRc had incomplete peripheral blood counts [62]. Quizartinib was generally well-tolerated, with corrected QT interval (QTc) prolongation, cytopenias, fatigue, and hypoalbuminemia being the only significant (grade ≥3) toxicities. The ongoing phase III, QUANTUM trials are now investigating the efficacy of quizartinib in the upfront (NCT02668653) and salvage (NCT02039726) settings (Table 2). Additional studies are also investigating the use of quizartinib as maintenance therapy (NCT01390337, NCT012468467).

Unfortunately, resistance to quizartinib has rapidly emerged via acquired *TKD* mutations, most commonly at the *D835* and *F691* sites [63, 64]. The *D835* mutation has also been associated with treatment-resistance against sorafenib, underscoring the need for additional agents to be developed. PLX3397, a potent and selective inhibitor of FMS, KIT, and FLT3-ITD, initially demonstrated disease activity even in the presence of *F691 L* mutations [65], providing the basis for a phase I/II trial (NCT01349049) studying the role of PLX3397 in the refractory/refractory setting. However, an interim mutagenesis screen of patients who relapsed after initial response demonstrated resistance via acquired mutations in the FLT3 activation loop and TK1 domains [66]. Final results from the phase I/II trial will help determine which patients may benefit from PLX3397 therapy.

Table 7 Immunotherapy trials with results

Drug	Molecular target	Completed clinical trials				
		Phase [reference]	*N*	Patient characteristics	Intervention	Outcomes and subpopulation
Vaccine therapy						
WT-1 peptide vaccine	Peptide vaccination	I/II [206]	8	Poor-risk AML in remission	WT-1 peptide vaccine	WT-1-specific CTL responses were detected in 6 pts, but re-stimulation failed to elicit secondary expansion
		II [205]	14	AML in CR but with detectable WT-1 levels	WT-1 peptide vaccine	Immune response detected in 9 pts, pts with no response had worse OS and LFS
WT-1 DC vaccine	DC vaccination	I/II [207]	10	AML in remission (8 pts in CR, 2 pts in PR)	WT-1 DC vaccine	2 pts with PR → CR 6 pts in CR → molecular remission (normalization of WT-1 mRNA levels)
Antibody drug conjugates (ADC)						
SGN-C-D33A	Anti-CD33 antibody conjugated to PBD	I [227]	27	Frontline AML, unfit for chemotherapy	SGN-CD33A	CR/CRi 54% -MRD for pts with CR/CRi: 46% **On full clinical hold by FDA**
		I [228]	42	Frontline AML	SGN-CD33A + 7+3 chemotherapy	CR/CRi 78% -MRD for pts with CR/CRi: 74% **On partial clinical hold by FDA**
		I [229]	53	Frontline AML, unfit for chemotherapy	SGN-CD33A + azacitidine/-decitabine	CR/CRi 73% -MRD for pts with CR/CRi: 47% **On partial clinical hold by FDA**
Antibody-dependent cell-mediated cytotoxicity (ADCC)						
CSL360	Chimeric anti CD123 antibody	I [233]	40	RR-AML, frontline AML unfit for chemotherapy	CSL360	93% with persistent disease 2 pts achieved CR with 1 of them achieving durable CR (maintained after 12 doses)
CSL362	Completely humanized anti-CD123 antibody	I [234]	25	AML pts in CR with a high risk of relapse	CSL362	50% of pts remained in CR at 6 months' follow-up 50% of evaluated pts with +MRDS converted to -MRD
Adoptive cell transfer therapy						
Anti-LeY CAR T cells	CAR T cells targeting LeY	I [253]	4	Relapsed AML	CAR T cells	Stable disease: 2 pts Reduction blasts: 1 pt Cytogenetic remission: 1 pt
CIK cells	CIK cell therapy	II [264]	74	Advanced hematological malignancies (55% AML)	CIK after DLI	CR 28% Early death in 32% of pts (5.4% during DLI)
Immune checkpoint inhibition						
Nivoluma-b	Anti-PD-1 antibody	I [270]	51	Refractory AML	Nivolumab + azacitidine	CR/CRi 18% HI 15% OS 9.3 months
Ipilimum-ab	Anti-CTLA-4 antibody	I [271]	28	Hematological malignancies with relapse after HSCT (12 pts with AML)	Ipilimumab	No response with 3 mg/kg Response with 10 mg/kg: CR 23% PR 9% Decrease in tumor burden 23% CR in all pts with leukemia cutis (3 pts), 1 pt with myeloid sarcoma, and 1 pt with AML secondary to MDS

AML acute myeloid leukemia, *CAR* chimeric antigen receptor, *CIK* cytokine-induced killer cells, *CR* complete remission, *CRi* complete remission with incomplete neutrophil count recovery, *CTL* cytotoxic T lymphocyte, *CTLA* cytotoxic T lymphocyte-associated antigen, *DC* dendritic cell, *DLI* donor lymphocyte infusion, *FDA* US Food and Drug Administration, *HI* hematologic improvement, *HSCT* hematopoieticstem cell transplantation, *LeY* Lewis Y, *LFS* leukemia-free survival, *MDS* myelodysplastic syndrome, *+MRD* presence of minimal residual disease, *-MRD* absence of minimal residual disease, *OS* overall survival, *PBD* pyrrolobenzodiazepine dimer, *PR* partial response, *pt(s)* patient(s), *RR-AML* relapsed/refractory acute myeloid leukemia, *WT* Wilms' tumor protein 1

Table 8 Selection of immunotherapy trials either recruiting or ongoing

Drug	Recruiting clinical trials				
	Drug class	Phase	Clinical trial number	Patient characteristics	Intervention
Vaccine therapy					
WT-1 vaccination	DC vaccine	II	NCT01686334	AML in remission	Autologous DC vaccine presenting WT-1
WT-1/PRAME vaccination	DC vaccine	I/II	NCT01734304 NCT02405338	AML in remission	Autologous DC vaccine presenting 2 leukemia-associated antigens (WT-1 and PRAME)
Antibody drug conjugates (ADC)					
SGN-CD33A	Anti-CD33 antibody conjugated to PBD	III	NCT02785900	Newly diagnosed AML	SGN-CD33A + azacitidine/decitabine **On hold by the FDA**
Antibody-dependent cell-mediated cytotoxicity (ADCC)					
JNJ-56022473	Anti-CD123 antibody	II	NCT02992860	MDS/AML after HMA failure	JNJ-56022473
		II	NCT02472145	AML ineligible for intense chemotherapy	Decitabine + JNJ-56022473 vs. decitabine alone
Bispecific T cell engager (BiTE) antibody and dual affinity retargeting (DART) molecules					
AMG-330	CD33/CD3 BiTE	I	NCT02520427	RR-AML	AMG-330
MGD006	CD123/CD3 DART	I	NCT02152956	RR-AML	MGD006
Adoptive cell transfer therapy					
Anti-CD33 CAR T cells	CAR T cell therapy	I/II	NCT01864902 NCT02799680	RR-AML	CAR T cells targeting CD33
Anti-CD123 CAR T cells	CAR T cell therapy	I	NCT02159495	RR-AML	CAR T cells targeting CD123
Anti-NKG2D CAR T cells	CAR T cell therapy	I	NCT02203825	MDS, AML, MM	CAR T cells targeting NKG2D
Anti-CD33 CAR-NK cells	CAR NK cell therapy	I/II	NCT02944162	RR-AML CD33+	CAR-NK cells targeting CD33
Anti-CD7 CAR-NK cells	CAR NK cell therapy	I/II	NCT02742727	CD7-positive leukemias and lymphomas	CAR-NK cells targeting CD7
CIK	CIK cell therapy	I	NCT01898793	RR MDS/AML	CIK cells stimulated with interleukin-2
Immune checkpoint inhibition					
Ipilimumab	Anti-CTLA-4 antibody	I	NCT02890329	RR MDS/AML	Ipilimumab
		I	NCT01757639	RR-AML	Ipilimumab
Nivolumab	Anti-PD-1 antibody	II	NCT02532231	AML with high risk of relapse	Nivolumab
		II	NCT02275533	AML in remission with MRD	Nivolumab
		I	NCT01822509	Hematological malignancies with relapse after SCT	Nivolumab/ipilimumab
		I/II	NCT02464657	AML/MDS	Nivolumab + induction (7+3)
		II	NCT02397720	RR-AML, AML >65 years old	Nivolumab + azacitidine
		I	NCT02846376	AML after SCT	Nivolumab ± ipilimumab
Pembrolizumab	Anti-PD-1 antibody	II	NCT02708641	AML post-remission >60 years old	Pembrolizumab
		I	NCT02981914	AML with relapse after SCT	Pembrolizumab
		II	NCT02768792	RR-AML	Pembrolizumab + high-dose cytarabine

Table 8 (continued)

Drug	Recruiting clinical trials				
	Drug class	Phase	Clinical trial number	Patient characteristics	Intervention
		II	NCT02845297	RR-AML and AML >65 years old	Pembrolizumab + azacitidine
		I/II	NCT02996474	RR-AML	Pembrolizumab + decitabine
Durvalumab	Anti-PD-L1 antibody	II	NCT02775903	High-risk MDS, elderly AML patients	Durvalumab + azacitidine
Atezolizumab	Anti-PD-L1 antibody	II	NCT02892318	RR-AML, elderly AML patients unfit for chemotherapy	Atezolizumab + guadecitabine

AML acute myeloid leukemia, *CAR* chimeric antigen receptor, *CIK* cytokine-induced killer cells, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *DC* dendritic cell, *HMA* hypomethylating agent, *MDS* myelodysplastic syndrome, *MM* multiple myeloma, *MRD* minimal residual disease, *NK* natural killer, *PBD* pyrrolobenzodiazepine dimer, *PD-1* programmed cell death protein 1, *PD-L1* programmed death-ligand 1, *PRAME* preferentially expressed antigen in melanoma, *RR-AML* refractory/relapsed acute myeloid leukemia, *SCT* stem cell transplant, *WT* Wilms' tumor protein 1

Newer second-generation FLT3 inhibitors, including crenolanib (CP-868596) and gilteritinib (ASP2215), appear to be effective in overcoming acquired mutations [67]. Each also provides activity against both *FLT3-ITD* and *FLT3-TKD* mutations, are not associated with QTc prolongation, and are less myelosuppressive.

Initial results from a phase II study of 55 relapsed/refractory FLT3+ patients treated with crenolanib monotherapy demonstrated median OS of 19 weeks and EFS of 8 weeks [68]. Another ongoing phase II trial pairing crenolanib with induction 7+3 chemotherapy demonstrated even more encouraging preliminary results, showing CR/CRi in 24/25 patients, with 22/25 achieving CR [69]. A phase I/II study evaluating the combination of salvage therapies (HiDAC + idarubucin) and crenolanib in patients with FLT3+ RR-AML found an overall response rate (ORR) of 36% with no DLTs [70]. Thus far, periorbital edema, delayed count recovery, transaminitis, nausea, and rash have been reported in association with crenolanib use.

Gilteritinib has also emerged as a promising, potent, and selective FLT3 inhibitor. A phase I/II dose-escalation study investigated the effects of ASP2215 monotherapy in 252 RR-AML patients, of whom 77% carried *FLT3* mutations. CRc in FLT3+ patients was higher (49%) than in FLT3-wt patients (ORR 12%) [71]. Diarrhea and fatigue were the most commonly reported adverse reactions. Importantly, QTc prolongation was reported in <5% of patients. Several additional phase II and III studies are actively enrolling patients to investigate the role of both these agents as monotherapy (NCT02927262, NCT0242193, NCT02997202) and in combination with other agents (NCT02752035).

Additional agents currently under investigation to overcome secondary mutations in FLT3 disease include the third-generation BCR-ABL multi-kinase inhibitor ponatinib (APS24534), and the small-molecule inhibitor dual FLT3/CDK4/6 inhibitor AMG925 (FLX925). Ponatinib (Iclusig®) was originally FDA approved for use in treating CML in 2012, though it was temporarily withdrawn from the market due to concerns of an increased risk of vascular occlusive events [72]. Initial in vitro studies in FLT3+ AML demonstrated activity against several secondary point mutations, including the *F691 L* mutation [73, 74], providing the basis for several ongoing phase I and II trials investigating its efficacy both as monotherapy (NCT01620216) and in combination with various agents (NCT02428543, NCT02779283, NCT02829840). AMG925 has shown in vitro and ex vivo effects against AML, particularly those carrying *ITD*, *D835Y*, and *ITD/D835Y* mutations. The exact mechanism of the favorable resistance profile of AMG925 compared to other agents, including quizartinib and gilteritinib, is still under active investigation [75]. It is hypothesized that the combination of CDK4-mediated retinoblastoma phosphorylation and inhibition of FLT3 pathways enhance the potency of AMG925 [76]. A phase I/Ib first-in-human trial is now actively investigating the efficacy of AMG925 in FLT3+ AML in the relapsed/refractory setting.

The mechanism appears to differ from traditional hypocellularity resulting from chemotherapy-associated myelosuppression. Instead, patients treated with these agents undergo a type of differentiation syndrome that results in a neutrophil surge, hypercellular marrow comprised of terminally differentiated myeloid cells, and inflammatory tissue infiltrates [77]. Regardless, the impressive clinical remission rates induced by some of the newer FLT3 inhibitors provide

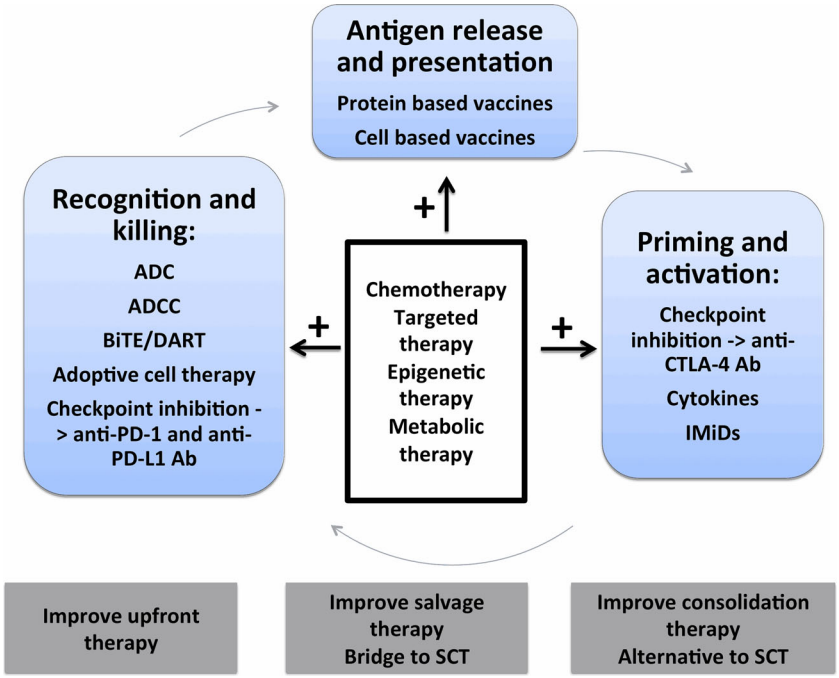

Fig. 2 Simplified cancer immunity cycle and immunotherapeutic as well as combination treatment strategies for AML. In order to effectively stimulate the immune system to kill leukemic cells each step of the cancer immunity cycle can be targeted. Protein and cell based vaccines result in improved antigen release and presentation, while cytokines, the CTLA-4 inhibitor ipilimumab and IMiDs such as lenalidomide enhance T cell priming and activation. Lastly, multiple approaches exist to improve recognition of tumor cells and their destruction. Antibody-based approaches include ADC, ADCC, BiTE, and DART. Adoptive cell therapies include CAR T cells and CIK cells. Recognition of leukemia cells by T cells can be promoted by inhibiting immune checkpoints with anti-PD-1 and anti-PD-L1 antibodies. Immunotherapy is envisioned to be used at different phases of treatment including induction therapy, salvage therapy, and as either a bridge or instead of HSCT. Currently available treatment options including chemotherapy, targeted therapy, epigenetic therapy, and metabolic therapy have been shown to independently promote antigen release and presentation as well as T cell priming and leukemic cell recognition and destruction. Their ability to work in synergism with immunotherapy in promoting the immune system to find and kill leukemia cells is currently being tested in several clinical trials. *Ab* antibodies, *ADC* antibodydrug conjugates, *ADCC* antibody-dependent cellular cytotoxicity, *AML* acute myeloid leukemia, *BiTE* bispecific T cell engager, *CAR* chimeric antigen receptor, *CIK* cytokine-induced killer, *CTLA-4* cytotoxic T lymphocyte-associated antigen 4, *DART* dual affinityretargeting molecules, *HSCT* hematopoieticstem cell transplantation, *IMiDs* immunomodulatory drugs, *PD-1* programmed cell death protein 1, *PD-L1* programmed death-ligand 1, *SCT* stem cell transplantation

cautious optimism for improved outcomes in FLT3+ disease. Furthermore, the duration of responses is limited with a median of a few months as resistance eventually emerges. Therefore, in the relapsed/refractory setting, these agents should ideally be used as a bridge to allo-HSCT for patients who are candidates for the procedure.

3.2 Cell Cycle Checkpoint Regulators

3.2.1 Barasertib (ACD1152)

The aurora kinases are important mitotic regulators, playing critical roles in chromosome alignment, segregation, and cytokinesis [78]. Aurora A and B kinases are frequently overexpressed in AML and correlate with poor outcomes [79, 80]. Preclinical experiments showed that barasertib, a selective aurora B kinase inhibitor, not only possessed anti-proliferative effects on AML cells but also potentiated the effects of conventional chemotherapies, such as vincristine and daunorubicin [81, 82]. A phase I dose-escalation study in 22 newly diagnosed elderly (≥60 years) AML patients who were not candidates for intensive chemotherapy showed an ORR of 22% [83]. DLTs were observed in two patients (grade 3 stomatitis/mucositis). The SPARK-AML 1 trial (Table 4), a phase II study comparing barasertib to LDAC in newly diagnosed elderly AML patients, showed higher CR/CRi (35.4% vs. 11.5%) and median OS (8.2 vs. 4.5 months) [84]. The major AEs were stomatitis and febrile neutropenia in both trials.

3.2.2 Volasertib (BI6727)

Polo-like kinase I (PLK1) is a highly conserved, master mitotic regulator that also promotes DNA repair during stress conditions [85]. Given the observed overexpression in numerous cancer types and association with poor prognosis [86], PLK1 has been hypothesized to be a key player in carcinogenesis and an attractive therapeutic target [87]. In this setting, volasertib was developed as a potent small-molecule PLK1 inhibitor that induces Polo arrest and apoptosis. Despite

promising preclinical data, early clinical studies investigating volasertib as monotherapy in solid tumors showed only modest responses, which were initially attributed to a poor pharmacokinetic profile [88]. Its application in AML, however, was of particular interest given the high proliferation rate of malignant cells, which thereby provides an optimal target substrate. A phase I/IIa study in RR-AML (Table 4) patients comparing LDAC with or without volasertib showed enhanced response rates (31% vs. 13.3%), prolonged EFS (5.6 vs. 2.3 months) and prolonged OS (8 vs. 5.2 months) in those treated with volasertib [89]. Importantly, these benefits were seen regardless of cytogenetics. No difference in death was seen at 60 or 90 days. The major AEs reported were neutropenic fever and gastrointestinal symptoms (e.g., diarrhea). A phase III study investigating the effects of volasertib in newly diagnosed elderly (≥65 years) AML patients ineligible for intensive chemotherapy (POLO-AML-2, NCT01721876) and two other trials combining volasertib with conventional induction chemotherapy (7+3) in newly diagnosed patients (NCT02905994, NCT02527174) were in development. However, the clinical development of this drug has been recently discontinued by the pharmaceutical company due to manufacturing problems.

3.2.3 Rigosertib (ON 01910.Na)

Another PLK1 inhibitor currently under development is rigosertib, which has inhibitory effects in the phosphatidylinositol 3-kinase (PI3K) pathway [90] in addition to the RAS/MEK/ERK pathway, as recently described by Athuluri-Divakar et al. [91]. Rigosertib was therefore shown to induce tumor cell G2/M arrest and apoptosis while sparing non-malignant cells [92, 93]. Given its minimal myelosuppressive effects and favorable safety profile, both oral and intravenous forms of rigosertib are being investigated as a therapeutic option in patients who are not candidates for induction chemotherapy, particularly patients with MDS. In a safety trial, 557 patients with MDS or AML received intravenous ($n = 335$) or oral ($n = 222$) rigosertib either as monotherapy or in combination with azacitidine (oral rigosertib only) [94]. The most common AEs in those receiving intravenous rigosertib were gastrointestinal symptoms (nausea, diarrhea, constipation). Significant effects reported in more than 10% of patients included cytopenias, febrile neutropenia, and pneumonia. The most common AEs in those receiving oral rigosertib as either monotherapy or combination therapy were urinary symptoms, including urinary frequency, urinary urgency, hematuria, dysuria, and urinary tract infections. Only anemia was reported as a significant AE in over ≥10% of patients receiving oral monotherapy, whereas significant cytopenias and pneumonia were mainly seen in combinatory therapy.

An initial phase I trial (NCT0053341) investigated the effects of intravenous rigosertib in 14 elderly (median age 73 years) patients (12 high-risk MDS, two RR-AML with trisomy 8) [95]. Responses (bone marrow or hematologic blast reduction) were observed in four patients. Significant AEs reported included febrile neutropenia, radiation recall, and metabolic derangements (decreased calcium, elevated lactate dehydrogenase, elevated bilirubin). No grade 4 toxicities were reported. Results of a phase III trial comparing intravenous rigosertib to supportive care in high-risk MDS following HMA failure demonstrated no statistically significant improvement in OS (8.2 vs. 5.9 months, $p = 0.33$), though improvements were noted in several subgroups [96]. An ongoing phase I/II trial (NCT01926587) including RR-AML with ≤1 prior salvage therapy is investigating the combinatory effect of oral rigosertib and azacitidine. Preliminary results in 53 evaluable patients showed responses in 55% of patients (CR 24%, concurrent marrow CR + hematologic improvement 27%, marrow CR alone 21%, hematologic improvement alone 3%). Notably, responses were seen in 70% of patients who were HMA-naïve. The most frequent adverse events reported include gastrointestinal effects (nausea, diarrhea, constipation, anorexia), fatigue, and urinary symptoms (hematuria, dysuria) [97].

3.3 Pro-Apoptotic Agents

3.3.1 Venetoclax (ABT-199)

The anti-apoptotic protein BCL-2 plays an essential role in the maintenance and survival of AML cells [12]. Overexpression of BCL-2 has been implicated in the chemoresistance observed in AML [98]. Small molecules targeting the BH3 domain of BCL-2 proteins, also known as BH3-mimetics, have therefore been developed to stimulate this essential mitochondrial apoptotic pathway. Early agents, such as ABT-737 and ABT-263 (navitoclax), initially showed disease activity but subsequently developed resistance through up-regulation of other anti-apoptotic proteins [e.g., myeloid cell leukemia 1 (MCL-1)] or were associated with DLTs [99]. Venetoclax (ABT-199), a modified derivative of ABT-263, was therefore developed as a selective BH3 mimetic with a more favorable disease profile. Pan et al. demonstrated ex vivo drug activity in multiple AML models [100], providing the basis for a phase II trial (NCT0199483) in 32 mostly RR-AML patients treated with venetoclax monotherapy [101]. IDH mutations were present in 12/32 (38%) patients, of whom four (33%) achieved CR/CRi. Febrile neutropenia (28%) and pneumonia (16%) were the most common grade 3/4 toxicities. *IDH2*-mutant susceptibility to venetoclax was mechanistically confirmed by Chan et al., who described ABT-199-induced cyclo-oxygenase (COX) suppression [102]. *IDH*

mutation status may therefore provide a predictor for response. Combination trials are now underway to investigate the effects of venetoclax with LDAC (NCT02287233) and HMAs (NCT0220377; NCT02993523). The latter trials are of particular interest, as data in high-risk MDS/secondary AML suggest a synergistic effect even after HMA failure [103]. Early results from the phase Ib trial (NCT0220377) are encouraging, as CR/CRi was achieved in 16/22 (CR in 5 patients), with nausea, constipation, and cough as the most common treatment-emergent AEs. If validated, venetoclax would provide a valuable alternative for patients who are poor candidates for intensive regimens and potentially even after failing HMA therapy.

4 Advances in Epigenetic Therapy

Epigenetic dysregulation is a hallmark of cancer and is particularly prevalent in myeloid malignancies [104, 105]. Epigenetic writers [e.g., DNA methyltransferases (DNMTs), histone acetyltransferases (HATs)] place epigenetic marks (e.g. methylation, acetylation) on DNA and histones, whereas epigenetic erasers [e.g., histone deacetylases (HDACs)] remove these marks [104]. Finally, epigenetic reader proteins [e.g., bromodomain and extraterminal (BET) family of proteins] survey the genetic landscape, bind to epigenetic marks and recruit other epigenetic regulators, which either induce or inhibit gene transcription [106]. This tightly regulated process of adding and removing epigenetic marks is disrupted in AML, subsequently leading to decreased transcription of genes involved in differentiation of myeloid cells and promotion of leukemogenesis [104]. Mutations in epigenetic regulators, including *DNMT3A*, *TET2*, *EZH2*, and *ASXL1*, are common in AML patients, enriched in patients with normal cytogenetics, and frequently associated with an adverse prognosis [105, 107–110].

Epigenetic therapy has shown benefits in patients with MDS and AML: the hypomethylating DNMT inhibitors azacitidine and decitabine are FDA approved for the treatment of MDS and have also shown significant activity as frontline therapy in elderly AML patients, who are considered not fit for intensive induction chemotherapy [2, 111]. The use of azacitidine and decitabine in patients with RR-AML leads to a response in only a minority of patients, although responses are associated with significantly prolonged OS [112]. HDAC inhibitors (HDACi) have shown only modest activity in patients with MDS and AML, and the combination of HDACi with HMA has not led to significant synergism [113, 114]. However, the mechanism of action of HMA and HDACi is incompletely understood: HMA and HDACi not only result in transcription of prior epigenetically silenced genes but also have pleiotropic effects on cell differentiation, senescence, apoptosis, angiogenesis, and, most intriguingly, the immune system [104, 113]. Combining existing epigenetic drugs with other treatment strategies, particularly immunotherapy, is an attractive platform for pharmacological synergism and is currently being tested in multiple clinical trials (see Sect. 6). Additionally, there are multiple novel epigenetic drugs in preclinical and different stages of clinical development [104, 115]. For the sake of this review, we focus on the next-generation HMA guadecitabine as well as disruptor of telomeric silencing 1-like (DOT1L) and BET inhibitors (Fig. 1 and Table 5).

4.1 SGI-110 (Guadecitabine)

The therapeutic effects of HMAs are dependent on their incorporation into DNA (and RNA in the case of azacitidine) during the S-phase of the cell cycle and therefore a sufficient overlap between intracellular drug half-lives and S-phase entries of malignant cells is required for HMA to work properly [116, 117]. A limitation of HMA is their short half-life; therefore, it is not surprising that an increase of HMA drug exposure during S-phase through a more frequent administration schedule of HMA results in improved response rates to in MDS [118, 119]. Guadecitabine, a hypomethylating dinucleotide of decitabine linked to guanosine, is resistant to degradation by cytidine deaminase and thereby increases exposure of the drug during S-phase [120]. In a phase II study of guadecitabine as frontline therapy in elderly AML patients who were not eligible for intensive chemotherapy, guadecitabine led to a CR in 37% of the patients with late responses being common (28% of responses occurred after six cycles) [121] (Table 5). Another phase II study of guadecitabine in 103 RR-AML patients resulted in CRc of 23% [122] (Table 5). Given these promising results, two phase III RCTs will be dedicated to examining the effect of guadecitabine in patients with treatment naïve AML (ASTRAL-1) and RR-AML (ASTRAL-2) (Table 5). ASTRAL-1 compares SGI-110 with azacitidine, decitabine, or low-dose cytarabine as frontline therapy in AML patients not eligible for IC (Table 5). ASTRAL-2, comparing the efficacy of guadecitabine with conventional modes of therapy in patients with RR-AML, will be recruiting patients shortly (Table 5).

4.2 Disruptor of Telomeric Silencing 1-Like (DOT1L) and Bromodomain and Extraterminal (BET) Inhibitors

Rearrangements of the mixed lineage leukemia (*MLL*) gene are found mainly in infant leukemia but also in about 10% of adult AML, where they are associated with secondary AML and confer a poor prognosis [123]. MLL fusion proteins have been shown to associate with the H3K79 histone methyltransferase DOT1L complex, which leads to the up-regulation of several genes directly involved in leukemogenesis (e.g.,

HoxA9 and *MEIS1*) [124–126]. Inactivation or inhibition of DOT1L and the associated loss of H3K79 methylation have been demonstrated to inhibit leukemia development in animal models of *MLL*-rearranged AML [127–129]. The DOT1L inhibitor pinometostat (EPZ-5676) was recently tested in a phase I study in 49 patients with RR-AML, MDS, acute lymphoblastic leukemia (ALL), or chronic myelomonocytic leukemia with *MLL* rearrangements or *MLL*-partial tandem duplication [130]. Pinometostat showed reductions in the methylation of target genes of the MLL fusion protein following drug exposure and modest clinical activity, with marrow responses in three patients and resolution of leukemia cutis in three other patients (Table 5).

MLL fusion proteins not only bind to the histone methyltransferase DOT1L but also to the superelongation complex (SEC), which phosphorylates the RNA polymerase II facilitating its recruitment to the promoters of crucial oncogenes such as *MYC*, *BCL-2*, and *CDK6* [106]. The BET epigenetic reader proteins, which include BRD2, BRD3, and BRD4, are part of the SEC complex and allow it to bind to acetylated histones on chromatin, thereby interacting with RNA polymerase II [131]. BET inhibitors have shown significant activity in preclinical models of AML with MLL translocations by preventing the BET-associated SEC complex to bind to chromatin [131–133].

In a phase I clinical trial using the BET inhibitor OTX-015 in 41 patients with leukemia (36 with AML), who had received two prior lines of therapy, only 7.3% of patients achieved either CR or CRi [134] (Table 5). While dosing at 120 mg did not show any DLT, gastrointestinal and cutaneous AEs as well as fatigue reduced patient compliance. It remains to be seen whether dosing at 80 mg will be more efficacious in a phase II study. Several other BET inhibitors, including GSK525762, CPI-0610, and TEN-010, are currently being evaluated in patients with AML and other myeloid malignancies in phase I clinical trials (Table 5). Additionally, BET inhibitors have shown particular activity in preclinical models of AML with *NPM1* mutations, *FLT3-ITD*, as well as *MLL* translocations [132, 135, 136].

5 Advances in Metabolic Therapy

Ever since Otto Warburg first observed the unexpectedly high rates of anaerobic metabolism in cancer cells even in non-hypoxic environments—now known as the Warburg effect—understanding the mechanisms behind cancer metabolism and how alterations in the microenvironment may be beneficial for therapeutic purposes has been of great interest [137–139]. While the exact molecular mechanisms behind this phenomenon are still being elucidated, it is now understood that the Warburg effect is a consequence of mutations in tumor suppressors and oncogenes, all of which have important roles in metabolic pathways [140]. Furthermore, the finding that mutations in metabolic enzymes alone are sufficient to induce cancer growth has challenged the view of metabolic genes as simply housekeeping genes. Mutations in key metabolic enzymes, such as isocitrate dehydrogenase 1/2 (IDH1/2) and indoleamine 2,3-dioxygenase (IDO), provide modifiable targets in cancer therapy. We describe several agents that are currently under clinical investigation, though several additional agents are anticipated to emerge in the coming years [141, 142].

5.1 Isocitrate Dehydrogenase (IDH) Inhibitors

The IDH family consists of nicotinamide adenine dinucleotide phosphate (NADP)-dependent enzymes that play an essential role in cellular aerobic respiration. IDH catalyzes the conversion of isocitrate to α-ketoglutarate (α-KG) and in turn NADPH through oxidative decarboxylation during the tricarboxylic acid cycle. Mutations in the IDH family were first identified in gliomas and glioblastomas [143–145]. Mutations in IDH1 and IDH2 isoforms were subsequently identified in 15–20% of adult AML through a large mutation analysis [146], and have become of particular interest as a therapeutic target. *IDH1* and *IDH2* mutations invariably alter the catalytic site. *IDH1* mutations occur at the R132 codon (R132H, R132C, R132G, R132S) while *IDH2* mutations occur at the R140 (R140Q, R140W) or R172 (R172K, R172G) codons [147, 148]. As a result, mutant *IDH* fails to catalyze isocitrate to α-KG. Instead, mutant *IDH* converts α-KG, which is primarily derived from glutamine, to (D)-2-hydroxyglutarate (2-HG) with concurrent consumption of NADPH [149]. As 2-HG is a competitive inhibitor of several α-KG-dependent histone demethylases, the accumulation of 2-HG disrupts normal DNA methylation [150–152]. 2-HG has also been shown to inhibit histone demethylation and arrest lineage-specific progenitor cell differentiation, providing the basis for leukemogenesis [153].

Several pre-clinical studies targeting IDH demonstrated promising anti-leukemic effects. From these studies emerged AG-221 (enasidenib), which is a first-in-class selective small-molecule IDH2 inhibitor. AG-221 was shown to suppress 2-HG production and induce cellular differentiation in in vitro and ex vivo models [154]. In vivo data suggest AG-221 reverses DNA hypermethylation in leukemic stem cells (LSCs) [155]. A dose-dependent survival benefit was also seen in a xenograft mouse model [156], prompting the initiation of a phase I/II multicenter, open-label trial (NCT01915498) investigating the role of AG-221 in AML with *IDH2* mutations. Interim analyses showed that of 181 evaluable patients—including RR-AML, newly diagnosed AML, and MDS—who received AG-221, the CRc of 41%, and CR of 17% [157]. For the RR-AML subset, the CRc was 41% and CRc was 18%. The duration of response was 6.9 months [15]. Overall, AG-221 was fairly well-tolerated, with the most common AEs

being hyperbilirubinemia and nausea. The most common severe treatment-related AE was leukocytosis, which was seen in seven patients. Additional clinical trials are currently investigating the role of AG-221 as monotherapy (IDHENTIFY trial, NCT02577406) or in conjunction with conventional chemotherapies and HMA (NCT02632708, NCT02677922).

For patients with *IDH1* mutations, an IDH1-selective small-molecular inhibitor, AG-120, is currently under investigation (Table 6). Interim results from a phase I trial in elderly $IDH1^{R132}$ patients with myeloid malignancies (NCT02074839) showed objective responses in 36% of patients, with CR of 18% [158]. The majority of AEs were grade 1 or 2 (diarrhea 23%, fatigue 22%, pyrexia 22%), with febrile neutropenia (11%) the most common serious AE. Differentiation syndrome (DS) is a potentially fatal treatment effect that has been described in association with AG-120 monotherapy [159]. As with DS classically described in the treatment of acute promyelocytic leukemia with all-*trans*-retinoic acid, DS associated with AG-120 may present with non-specific constitutional symptoms and was mitigated with steroid and hydroxyurea therapy.

Additional trials with AG-120 in combination with conventional therapies and HMA are currently underway (NCT02677922, NCT02577406). Other agents with ongoing clinical trials include FT-2101 (NCT NCT02719574), an IDH1-specific inhibitor, and AG-881 (NCT0492737), a CNS-penetrant IDH1/2 inhibitor.

Additional agents targeting the glutamine metabolic pathway include CB-839 and Erwinaze®. CB-839 is an orally available glutaminase inhibitor that was shown to inhibit AML cell growth by reducing intracellular glutamate levels in vitro [160]. Results from a phase I trial investigating CB-839 in relapsed/refractory-hematologic malignancies showed that CB-839 was relatively well-tolerated, with asymptomatic, reversible transaminitis being the most common significant AE [161]. Responses were observed, though final analyses are pending. CB-839 is now also being studied in conjunction with azacitidine. Erwinaze®, which is asparaginase *Erwinia chrysanthemi*, provides an alternative approach by depleting intracellular glutamine, which also inhibits mTORC1 activity and protein synthesis [162]. These promising preclinical results provided the basis for phase I (NCT02283190) and phase II (NCT02718755) trials.

5.2 Indoleamine 2,3-Dioxygenase (IDO) Inhibition in Acute Myeloid Leukemia (AML)

Another emerging metabolic target is IDO. IDO is an intracellular, heme-containing enzyme that catalyzes tryptophan degradation. As a result, IDO plays an essential role in immune homeostasis, as it takes part in both immune counter regulation and regulating T cell tolerance. When activated, IDO depletes tryptophan, which can activate a stress response involving general control nonderepressible 2 (GCN2). GNC2 has inhibitory effects on T cell proliferation and can induce the differentiation of conventional T cells (Tcon) into regulatory T cells (Treg) [163, 164]. IDO also results in the production of soluble factors, such as kynurenine, which can bind aryl hydrocarbon receptor (AhR) and induce antigen-presenting cells (APCs) into an immunosuppressive phenotype [165]. Therefore, a shift in IDO expression, as can occur during inflammation or in the presence of IDO-expressing cancer cells, can create a microenvironment amenable to tumorigenesis.

IDO overexpression has been associated with poor prognoses in AML [166]. Furthermore, IDO overexpression in AML blasts is correlated with lower rates of CR, higher rates of relapse, and overall worse survival [167–169]. IDO inhibition is therefore an attractive approach towards metabolic therapies. Indoximod (1-methyl-D-tryptophan) is an IDO inhibitor that has shown promising pre-clinical results [170, 171]. While IDO inhibition did not show direct anti-tumor cytotoxic effects or spontaneous immune stimulation, when combined with conventional chemotherapies, tumor regression was observed [172]. A phase Ib/IIa trial (NCT02835729) investigating the effects of induction chemotherapy (fludarabine, cytarabine) with or without indoximod in newly diagnosed AML is underway. Numerous additional trials investigating IDO inhibitors in combination with chemotherapies and/or immunotherapies are currently underway in solid tumors [173].

6 Advances in Immunotherapy

The idea of directing the immune system towards AML blasts is not new as Powles et al. successfully combined chemotherapy with immunotherapy consisting of irradiated allogeneic AML cells in 1973 [174, 175]. The success of allogeneic stem cell transplantation (allo-SCT) suggests the importance of anti-tumor immune responses in curing AML [176, 177]. However, relapse after allo-HSCT is common and allo-HSCT causes inevitable AEs such as graft-versus-host disease (GvHD) by alloimmune response to normal tissues [176, 178]. Novel immunotherapeutic approaches in AML attempt to enhance different aspects of the so-called cancer immunity cycle: antigen release and presentation; T cell priming and activation; and leukemia cell recognition and killing (Fig. 2) [175, 179].

Antigen release and presentation is augmented through improved protein- and cell-based vaccines (Sect. 6.1, Fig. 2, Tables 7 and 8) [180, 181]. Stimulatory cytokines as well as immune checkpoint inhibitors targeting cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) enhance T cell priming and activation (Section 6.2 and 6.5, Fig. 2, Tables 7 and 8). Finally, antibody- and cell-based therapy as well as

immune checkpoint inhibition targeting programmed cell death protein 1 (PD-1) or programmed death-ligand 1 (PD-L1) has been shown to improve recognition and killing of leukemic cells (Sects. 6.3, 6.4, and 6.5, Fig. 2, Tables 7 and 8) [182, 183].

Immunotherapy is envisioned for use in a variety of different settings, including as upfront therapy to improve CR rates as salvage therapy, a bridge to allo-HSCT (i.e., antibody-based, cell-based, and checkpoint inhibitor therapy) or to prevent relapse as consolidation therapy as an alternative to allo-HSCT (i.e., vaccine therapy and checkpoint inhibitor therapy) (Fig. 2) [175].

Furthermore, traditional chemotherapy, targeted therapy, and epigenetic therapy have all been shown to lead to a stimulation of the immune system, either at the level of antigen release and presentation or T cell priming and effector function, and therefore may work synergistically when combined with immunotherapy (Sect. 6, Fig. 2, Tables 7 and 8) [184–187].

6.1 Targeting Leukemia Antigens

A multitude of antigens have been recognized on AML cells, which can either be targeted by active immunotherapy through tumor-antigen based vaccination or by passive immunotherapy through either monoclonal antibodies or adoptive cell transfer [188]. AML-related antigens are generally grouped into four categories based on their expression patterns of leukemia and normal cells: ubiquitous antigens, leukemia-specific antigens (LSAs), leukemia-associated antigens (LAAs), and cancer-testis antigens (CTAs) [188]. While ubiquitous antigens, which are both expressed in leukemic cells and in multiple normal human tissues, are not suitable targets for immunotherapy, LSAs, LAAs, and CTAs are all potential immunotherapy targets [188].

LSA are restricted to leukemia cells and consist of either fusion proteins (e.g., AML1-ETO or PML-RARα) or gene mutations resulting in mutant leukemia-specific proteins (e.g., *FLT3*-ITD and *NPM-1* mutations) [189–191]. Given that LSAs are restricted to leukemic cells, they are generally considered preferred targets for antigen directed immunotherapy [192]. However, the restricted expression of LSAs in specific subgroups of AML patients limits the use of many LSAs for broad application in vaccine therapy [188].

While LAAs are expressed by leukemic cells, they are also expressed by normal cells, raising concerns that immune therapy targeted towards these antigens could either cause autoimmune AEs or have limited efficacy given natural immunological tolerance towards self-antigens [188]. However, since LAAs are aberrantly overexpressed on leukemic blasts, the immune system is generally able to accurately discriminate leukemic cells from their normal counterparts arguing for tumor-associated antigens (TAAs) as a useful target in immunotherapy despite their roles as self-antigens [188]. Wilms' tumor protein (WT) 1 is an LAA highly overexpressed in the majority of AML cells (including LSCs) showing low levels of expression in various normal tissues including the kidney, the gonads, and the hematopoietic system [193–195] CTAs are expressed on germ cells (i.e., testes, ovaries, and placental trophoblasts) but are also aberrantly expressed on leukemia cells [196]. As germ cell tissues are protected from immune therapy-induced autoimmune AEs by the blood–testes barrier, CTAs are an ideal target for antigen-directed immunotherapy. While the majority of CTAs are absent on AML blasts, the preferentially expressed antigen in melanoma (PRAME) is present on AML blasts and is currently targeted by vaccine therapy in AML [197].

6.2 Vaccine Therapy

Key to deciding the optimal antigen for vaccination is the immunogenicity of the antigen, including its ability to elicit a potent humoral and even more importantly a cellular immune response. Several factors determine the immunogenicity of a potential antigen used as a vaccine in AML therapy: longer antigens such as proteinase 3, WT-1, and PRAME possess multiple epitopes and are therefore are more immunogenic [198–200]. Furthermore, epitopes with the capacity to bind to several different major histocompatibility complex (MHC) molecules (promiscuity in MHC binding) and to elicit a critical CD4 T helper cell response by binding to MHC II molecules are preferred [188, 201, 202]. In that sense, $WT_{331–352}$ is one of the most immunogenic epitopes in AML, as it is able induce specific CD4+ T cell immune responses in most AML patients and contains another peptide fragment ($WT\text{-}1_{332–347}$) which is recognized by a broad range of human leukocyte antigen (HLA)-DR and HLA-DP molecules [188, 201].

Early results from investigational DNA and peptide vaccine approaches using antigens such as WT-1 and proteinase 1 supported the potential therapeutic benefit of recruiting the endogenous immune system by vaccination [203, 204].

In a phase II trial of monthly WT-1 peptide vaccination in 14 AML patients in CR but with reverse transcription–polymerase chain reaction (RT-PCR)-detectable WT transcript levels, nine patients had an immunological response, which was associated with better OS than in patients who did not have an immunological response [205]. However, in another phase I/II trial using the WT-1 peptide, vaccination in eight patients with AML induced only short-lived WT-1-specific immune responses as re-stimulation failed to elicit secondary expansion of T cells and failed to induce clinical responses [206] (Table 7).

There is hope that using dendritic cell (DC) vaccination instead of peptide vaccines will lead to improved outcomes [175]. DCs can either be differentiated in vitro from

monocytes or CD34+ hematopoietic progenitor cells or they can be directly obtained from leukapheresis products [181]. Subsequently, they are loaded with tumor lysate, tumor antigen-derived DNA, RNA peptides, or whole protein and then transfused back into the patient after being activated in vitro [181]. In a phase I/II trial using a WT-1-targeted DC vaccine in ten AML patients in remission, the vaccine induced a CR in two patients who were previously in partial remission and molecular remission in six other patients who were previously in CR, as demonstrated by the normalization of WT-1 mRNA levels [207] (Table 7).

Currently, several clinical trials testing DC vaccines in AML are enrolling patients: while a phase II trial examines the effect of a DC against WT-1 (NCT01686334), two phase I/II trials examine the efficacy of a combined DC vaccine against the LAA WT-1 and the CTA PRAME in AML patients in remission (NCT01734304, NCT02405338) (Table 8).

Additional improvements in antigen loading on DC, DC maturation and activation, as well as the route of administration of the DC vaccines are potential ways to further increase the potency of DC vaccines [181, 208, 209]. DC vaccination also seems to be most efficacious in the context of low disease burden and should therefore preferentially be used as a consolidation/post-remission therapy when minimal residual disease (MRD) is present [210].

6.3 Cytokine Therapy

Treatment of AML patients with interleukin-2 (IL-2) has shown no significant success, which is likely due to the limited ability of IL-2 to mount a significant anti-leukemia immune response in a highly immunosuppressive microenvironment [211]. To maximize the chances for success, the use of IL-2 has been examined in patients with MRD as maintenance therapy after achieving CR or after transplant [211]. A Cochrane review including nine phase III randomized clinical trials (with a total of 1665 participants) comparing IL-2 with no treatment showed no difference in disease-free survival or OS of patients with AML in first CR [212].

Combining IL-2 with histamine dihydrochloride (Ceplene®) as post-consolidation therapy was shown to improve leukemia-free survival but not OS when compared with no treatment in a randomized phase III trial [213]. This has led to the approval of IL-2 plus histamine dihydrochloride in Europe; however, the FDA denied approval unless an improvement in OS could be shown in another phase III trial comparing IL-2 plus histamine dihydrochloride with IL-2 alone [214].

6.4 Antibody-Based Immunotherapy

Monoclonal antibodies directed towards antigens expressed on leukemic blast cells as well as LSC's and myeloid progenitors, particularly CD33 and CD123, are in different stages of clinical development [215, 216]. As unconjugated monoclonal antibodies have shown limited potency in AML, newer approaches focus on monoclonal antibodies, which are either conjugated with chemotherapeutic drugs [antibody drug conjugates (ADC)] or are manipulated in the Fc portion to enhance antibody-dependent cellular cytotoxicity (ADCC) [182].

Furthermore, bispecific T cell engager (BiTE) antibodies and dual affinity retargeting (DART) molecules are bispecific monoclonal antibody constructs that direct cytotoxic T cells (by binding to CD3) into the proximity of leukemia cells (by binding to a specific leukemia antigen), which subsequently leads to the destruction of the leukemia cell [217] (Fig. 1).

6.5 Antibody Drug Conjugates (ADC)

6.5.1 Gemtuzumab Ozogamicin (GO)

The development of GO (Mylotarg®), a humanized anti-CD33 monoclonal antibody conjugated with the DNA-damaging toxin calicheamicin, is a story filled with success, disappointment, and confusion [14]. The FDA first approved GO in 2000 for AML patients older than 60 years who were in first relapse and not candidates for aggressive chemotherapy [218–220]. GO was withdrawn from the market in 2010 after a phase III clinical trial comparing the combination of GO with standard induction chemotherapy consisting of daunorubicin and cytarabine with standard induction chemotherapy alone in patients younger than 60 years showed no additional clinical benefit, and instead increased mortality [221]. However, the dose of daunorubicin in the study group was only 45 mg/m^2 compared with 60 mg/m^2 in the standard group (with higher anthracycline doses being known to be associated with a survival advantage) and the induction mortality in the study group was consistent with other studies while mortality in the control group was uncharacteristically low (5% vs. 1%) [14]. Furthermore, four other randomized studies have shown improved OS rates with the addition of GO in patients with favorable and intermediate-risk cytogenetics while demonstrating no increased rates in induction mortality [222–225]. These results were confirmed in a recent meta-analysis, which included five RCTs with a total of 3325 patients, showing that the addition of GO to induction chemotherapy is safe and results in a benefit in OS in patients with favorable and intermediate-risk cytogenetic characteristics while not benefiting patients with adverse cytogenetic characteristics [223]. Given the multitude of clinical trial data supporting the use of GO, both the FDA and European Medicines Agency (EMA) are currently re-evaluating the role of GO in appropriate subpopulations of AML patients with a favorable or intermediate cytogenetic risk profile.

6.5.2 SGN-CD33A (Vadastuximab Talirine)

Since the development of GO, significant efforts have been made to improve ADC targeting CD33 by eliminating linker instability problems and toxin-related off-target toxicities [175]. Optimizing the linker technology of a monoclonal antibody directed towards CD33 conjugated with a potent, synthetic DNA-crosslinking pyrrolobenzodiazepine dimer (PBD) by using engineered cysteine moieties at linker attachment sites in order to allow homogenous and precise drug loading resulted in the development of SGN-CD33A [226]. SGN-CD33A proved to be about three times more potent than GO in preclinical models and, unlike GO, was able to eliminate AML cells with the multidrug-resistant phenotype and independent of cytogenetic risk group in xenotransplantation models [226]. This has resulted in several phase I clinical trials examining the effect of SGN-CD33A monotherapy as frontline therapy in AML patients unfit for IC [227] as well as frontline therapy in combination with 7+3 induction chemotherapy [228], azacitidine, or decitabine [229] (Table 7). These trials have shown promising results with response rates of 54%, 78%, and 73% with SGN-CD33A monotherapy, in combination with chemotherapy and in combination with HMA, respectively. More importantly, of patients with a clinical response, 46%, 74%, and 47% achieved negative MRD with SGN-CD33A monotherapy, chemotherapy, and HMA combination therapy, respectively (Table 7). This resulted in the opening of a phase III clinical trial examining the effect of azacitidine and decitabine in combination with SGN-CD33A versus in combination with placebo for elderly patients with newly diagnosed AML (CASCADE trial, NCT02785900) (Table 8). Unfortunately, six patients who had received allo-HSCT either before or after treatment with SGN-CD33A developed hepatotoxicity secondary to veno-occlusive disease, including four fatal events related to hepatotoxicity [230]. Subsequently, the FDA placed a full clinical hold on the phase I/II trial of SGN-CD33A monotherapy in pre- and post-allogeneic transplant AML and placed a partial clinical hold on the two phase I trials investigating SGN-CD33A in combination with chemotherapy and HMA in newly diagnosed patients (meaning no new enrollment; existing patients may continue treatment with re-consent) [230]. The phase III CASCADE trial of SGN-CD33A in combination with HMA in older AML patients was allowed to proceed with patient enrollment [230].

6.6 Depleting Antibodies

6.6.1 CSL360

CD123 is an ideal target for therapeutic antibody therapy in AML as CD123 is highly expressed on LSC but shows only low levels of expression on normal hematopoietic stem and progenitor cells [216, 231]. Indeed, preclinical studies of anti-CD123 directed monoclonal antibody therapy have been promising in eliminating LSC [232]. Unfortunately, a phase I clinical study of CSL360, a recombinant chimeric IgG1 monoclonal antibody directed towards CD123, in 40 patients with poor prognosis AML showed only two achieving a response [233] (Table 7).

6.6.2 CSL362

After disappointing results with CSL360, CSL362 was developed as an updated version with complete humanization to reduce immunogenicity and additional optimization in the Fc portion to improve ADCC with a high affinity for NK cell CD16 [234]. CSL362 was tested in AML patients in CR with a high risk for relapse and was shown to be able to keep half of the patients in CR after 6 months of follow-up [234] (Table 7). Furthermore, six patients with MRD converted to undetectable MRD [234].

6.6.3 JNJ-56022473

JNJ-56022473, a CSL362 variant, was generated from a new cell line and showed similar activity to CSL362 in preclinical assays [235]. The efficacy of JNJ-56022473 either as monotherapy in AML patients after HMA treatment failure or in combination with decitabine in AML patients ineligible for IC is currently tested in two phase II clinical trials (NCT02992860 and NCT02472145) (Table 8).

6.7 Bispecific T Cell Engager (BiTE) Antibodies and Dual Affinity Retargeting (DART) Molecules

6.7.1 AMG-330

The advantage of BiTE antibodies is that they are able to recruit cytotoxic T cells directly to a specific target antigen independent of their antigen specificity, which leads to T cell-mediated killing of the leukemia cell without pre- or co-stimulation [175, 217]. Blinatumomab, which is a BiTE antibody connecting the CD19 surface antigen on B cells with the CD3 component of the T cell receptor (TCR) complex, resulted in impressive response rates in patients with B cell ALL and B cell lymphomas [236, 237]. AMG-330, a monoclonal bispecific antibody directed at CD33 and CD3, demonstrated potent antibody-mediated cytotoxicity in preclinical experiments involving AML cell lines and xenotransplantation experiments [238–240]. Importantly, AMG-330 does not lead to CD33 internalization after binding to it, and remains active even in the presence of circulating CD33 or presence of single-nucleotide polymorphisms in the *CD33* gene and on cells expressing only low levels of CD33 without significantly reducing normal hematopoietic progenitor cells [238, 240,

241]. AMG-330 is currently being evaluated in AML patients, who either relapsed after initially achieving a remission or were refractory to prior therapy (NCT02520427) (Table 8).

6.7.2 MGD006

In comparison to BiTE antibodies, which consist of four variable domains of heavy and light chains linked to each other like pearls on a string of polypeptide linkers, bispecific antibodies based on the DART technology place cognate heavy and light chain variable domains on two separate polypeptides, which are stabilized by a C-terminal disulfide bridge, giving them a theoretic advantage over BiTE antibodies [242]. In in vitro cytotoxicity assays with human B cell lines comparing CD19/CD3 DART constructs with CD19/CD3 BiTE constructs, the DART construct outperformed the BiTE construct; however, in vivo side-by-side comparisons are not available yet [242, 243]. MGD006 is a DART molecule with affinity for CD123 on leukemia blasts cells and LSC as well as CD3 and has been shown to successfully inhibit expansion of AML cells in a xenograft mouse model [244]. MGD006 is tested in RR-AML patients in a phase I clinical trial (NCT02152956) (Table 8).

6.8 Adoptive Cell Therapy

6.8.1 Chimeric Antigen Receptor (CAR) T Cells

Chimeric antigen receptor (CAR) T cell therapy combines gene therapeutic, immunotherapeutic, and cell therapy approaches: a patient's own T cells are transduced with a retroviral vector carrying the CAR (as well as other non-viral based approaches) and after in vitro expansion (and often lymphodepletion of the patient with high-dose chemotherapy) are infused back into the patient [245, 246]. The CAR in its most basic formulation is made of an antigen-binding element consisting of the extracellular single chain immunoglobulin variable fragments (scFvs), a trans-membrane spacer element, and an intracellular signaling domain, which usually exists of the CD3 zeta (CD3ζ) chain of the TCR complex [247]. However, unlike TCRs, CARs target only surface proteins (not processed antigens) and their antigen recognition is HLA-independent, resulting in universal application [247]. Major advantages to monoclonal antibodies are better tumor penetration and the generation of a long-lasting immunological memory mediated by ongoing stable persistence of the engineered cells after initial engraftment [245]. Since the development of the first-generation CARs, significant progress has been made in second- and third-generation CARs improving T cell activation by adding additional co-stimulatory intracellular domains or alternatively additional receptors: second-generation CARS have a single co-stimulatory domain derived from either CD28 or 4-1BB (CD137) while third-generation CARs have two co-stimulatory domains (CD28, 4-1BB, ICOS, OX40, and others) [247]. Most of the clinical data come from trials with CAR T cells targeting CD19 or CD20 on B cell malignancies including ALL, chronic lymphocytic leukemia, and aggressive non-Hodgkin's lymphoma, which have shown impressively high remission rates and durable responses in patients with highly advanced and refractory disease [248–252].

While targeting CD19 in B cell malignancies results in prolonged B cell aplasia (the associated risk for infection can be medically managed), using CAR T cells in myeloid malignancies including AML is more challenging, as many of the LAAs are also expressed on normal myeloid cells which would result in strong on-target–off-leukemia effects including prolonged neutropenia [175].

A phase I clinical trial using CAR T cells targeting the Lewis-Y (Le-Y) antigen in four patients with relapsed AML showed that CAR T cells persisted for up to 10 months and led to a clinical response [253]. While one patient died because of sepsis after receiving induction chemotherapy, three patients had evidence of cytogenetic MRD at the time of CAR T cell infusion and showed a biological response after receiving CAR T cells. One patient achieved a cytogenetic remission for 5 months, a second showed an extended remission for 23 months, and a third patient with evidence of active leukemia despite re-induction chemotherapy had a reduction in peripheral blood blasts (Table 7). Several more phase I clinical trials testing CAR T cells in AML are currently enrolling patients including CAR T cells directed towards CD33 (NCT01864902 and NCT02799680), CD123 (NCT02159495), and natural killer (NK) group 2D (NKG2D) antigen (NCT02203825) (Table 8).

Apart from the on-target–off-leukemia effects, there are several other safety concerns of CAR T cells including tumor lysis syndrome (TLS); neurologic toxicities, including fatal brain edema; and cytokine release syndrome (CRS), which is caused by cytokine release from T cells or macrophages resulting in fever, tachycardia, and hypotension and can lead to distributive shock with multi-organ failure [254]. CRS is associated with increased levels of soluble IL-2R, IL-6, ferritin, C-reactive protein as well as higher levels of blood CAR T cells. CRS also seems to be related to higher disease burden, although better predictors based on cytokine profiles are currently being developed [255]. In the summer of 2016, it became known that two patients included in the ROCKET trial, which examined the efficacy of JCAR015 (CAR T cells directed against CD19) in ALL, had died of brain edema [256]. Subsequently, the trial was placed on hold by the FDA but then reopened after the preconditioning regimen was changed from fludarabine, which was thought to be the culprit, to cyclophosphamide [257]. However, in November 2016, two more patients died of brain edema and the clinical trial was stopped [256]. Results from the phase II ZUMA-1 trial using

anti-CD19 CAR T cells (KTE-C19) in patients with diffuse large B cell lymphoma (DLBCL) and follicular lymphoma showed that 25% of patients had neurologic events and 18% of patients had grade three or higher CRS, with two out of 62 patients dying of the consequences [252]. The prevention and treatment of CAR T cell-mediated toxicities is out of the scope of this paper and has been described in detail elsewhere [254].

6.8.2 CAR Natural Killer Cells (NK) Cells and Cytokine-Induced Killer (CIK) Cells

As innate immune cells, NK cells are able to attack malignant cells without prior antigen presentation and without need for human leukocyte antigen matching. NK cells are therefore not restricted to autologous use but can also be acquired from allogeneic donors [258]. This makes them an attractive cell population to use in adoptive cell therapy in AML, particularly in combination with CARs redirecting NK cells towards specific antigens on AML blasts [258, 259]. Currently, several clinical trials are testing the application of CAR NK cells targeting CD33 (NCT02944162) and CD7 (NCT02742727) on leukemic blasts in patients with RR-AML (Table 8). Recently, FATE Therapeutics presented a method for large-scale ex vivo expansion of terminally differentiated adaptive NK cells (FATE-NK100), which are characterized by the expression of CD57 and the activating NK cell receptor NKG2C [260]. They used peripheral blood mononuclear cells from cytomegalovirus seropositive donors, removed CD3+ T cells and CD19+ B cells and then cultured the cells in the presence of IL-15 and a small-molecule inhibitor of glycogen synthase kinase 3-beta (GSK3β) leading to enhanced NK cell maturation and expansion. Using this method, NK cell expansion was enhanced 6.4-fold with the final product FATE-NK100 containing 142.2×10^8 CD57+ NK cells and 15.8×10^8 CD57+ NKG2C+ adaptive NK cells. FATE-NK100 has been cleared by the FDA to be tested in a phase I clinical trial in patients with advanced AML [261].

Cytokine-induced killer (CIK) cells are a heterogeneous NK cell-like cell population (co-expressing CD3 and CD56), which can be expanded from peripheral mononuclear cells in the presence of inverted formin (INF) and IL-2 and has been shown to have less risk of inducing GvHD than donor lymphocyte infusion (DLI) [262, 263]. In a phase II clinical trial using CIK cells in adult and pediatric patients with advanced hematologic malignancies, including 41 patients with AML, despite a CR of 28% after the initial DLI, early death occurred in 24 patients and acute GVHD was observed in 11 patients [264] (Table 7). Another study of CIK in combination with IL-2 is currently recruiting patients with refractory and high-risk AML as well as MDS (NCT01898793) (Table 8). Using CARs to target CIK might lead to more effective targeting of leukemia cells [265, 266].

6.9 Immune Checkpoint Inhibitors

Immune checkpoints, including CTLA-4 and PD-1, have been recognized as important mechanisms for tumor cells to escape immune surveillance. Blocking these checkpoints resulted in impressive clinical effects in solid cancers, particularly melanoma, non-small cell lung cancer, and Hodgkin lymphoma [267, 268].

Programmed cell death ligand-1 (PD-L1) as well as programmed cell death 1 (PD-1) are overexpressed on murine AML blasts and on bone marrow stromal cells, respectively, leading to a suboptimal antitumor T cell response with AML blasts subsequently evading immune surveillance [269]. Additionally, PD-L1, programmed death-ligand 2 (PD-L2), PD-1, and CTLA-4 expression is upregulated on AML blasts after treatment with HMA [187].

Early results of a phase I clinical trial combining azacitidine with the anti-PD1 antibody nivolumab in 51 AML patients refractory to prior therapy were recently presented [270]. Patients received azacitidine on days 1–7 and nivolumab on day 1 and 14 with courses repeated as long as patients did not have significant AEs. A median OS of 9.3 months compared favorably to historical survival data achieved with salvage azacitidine monotherapy in a comparable patient population. An immune cell infiltrate in the baseline bone marrow consisting of a larger proportion of CD8+ effector T cells and a lower proportion of Treg cells was associated with a higher response rate to therapy. A phase I trial examined the safety and efficacy of ipilimumab in 28 patients with a variety of hematological malignancies (including 12 patients with AML) after relapse from HSCT [271]. While there were no responses observed at a dose of 3 mg/kg, five patients achieved a CR at a dose of 10 mg/kg. All five CRs were achieved by patients with AML including all three patients with AML and leukemia cutis, one patient with AML and myeloid sarcoma, and one other patient with smoldering MDS developing in AML with marrow involvement. In three of the five patients the response was sustained, with patients remaining in CR at 15 months. Patients who responded to ipilimumab were found to have an increased CD8 T cell infiltration at the site of leukemic involvement as well as systemic activation of T cell immunity with an increase CD4+ Tcon populations and a decrease in CD4+ Treg populations.

Several other clinical trials examining the application of the anti-PD-1 antibodies nivolumab and pembrolizumab, the anti-PD-L1 antibodies durvalumab and atezolizumab, and anti-CTLA-4 antibody ipilimumab either as monotherapy or in combination with induction chemotherapy, epigenetic therapy (azacitidine, decitabine, guadecitabine), or other checkpoint inhibitors are currently recruiting patients (Table 8). These clinical trials will test immune checkpoint inhibitors in a variety of different settings, including in elderly patients unfit for IC, patients refractory to other therapies or who relapsed after allo-HSCT, and patients

who are in remission but at high risk of relapse because of MRD (Table 8).

7 Combination Therapy

While all these novel approaches to AML therapy are promising, monotherapy alone is unlikely to result in a cure for AML as the genetic landscape of AML is complex. Often more than one driver mutation is present and multiple other mutations are acquired during disease evolution [272–275]. While discussing all the combination approaches currently tested is out of the scope of this paper, we want to illustrate the potential of combination approaches by using the example of combining immunotherapy with chemotherapy and epigenetic therapy (Fig. 2).

Chemotherapy does not cause death of tumor cells by cytostatic effects alone but also by stimulating an immune response directed towards cancer cells by reinstating immune surveillance [184, 276]. Chemotherapy has been demonstrated to augment the immune response against cancer through multiple mechanisms including improved antigen uptake and chemotactic response by macrophages and DCs, improved recognition of neo-epitopes over the MHC I and TCR, and increased susceptibility of tumor cells to immune-mediated cytotoxicity [185, 277]. Key in eliciting an immune response to cancer cells with chemotherapy is the induction of an immunogenic cell death (ICD)- rather than a non-ICD-like apoptosis [276]. In order to induce ICD, chemotherapeutic agents need to lead to the pre-apoptotic exposure of calreticulin (CRT) at the cell surface, the secretion of adenosine triphosphate (ATP) during the blebbing phase of apoptosis, and the cell death-associated release of the non-histone chromatin protein high-mobility group box 1 (HMGB1) [276]. In AML patients, the spontaneous exposure of CRT by leukemic cells has been shown to predict antitumor T-cell responses and improved patient survival [278]. Interestingly, only a small selection of chemotherapeutic agents is able to induce ICD in cancer cells: when cancer cells were exposed to 24 different chemotherapeutic agents, only four agents (three anthracyclines and oxaliplatin) were able to induce ICD, while all agents were able to induce apoptosis [279]. It seems that anthracyclines, the backbone of 7+3 induction chemotherapy in AML, are particularly potent in inducing ICD [280].

The combination of checkpoint inhibition with chemotherapy and targeted therapy is currently being tested in multiple trials in solid tumors. One successful example is the combination of nivolumab with platinum-based doublet chemotherapy in patients with non-small cell lung cancer [281, 282]. In AML, an example of combining chemotherapy and immunotherapy is the combination of SGN-CD33A with 7+3 chemotherapy as frontline therapy, which resulted in a response rate of 78%, with 74% of patients with CR/CRi also achieving MRD [228] (Table 7). Two clinical trials are dedicated to examining the combination of chemotherapy with immune checkpoint inhibition: the first trial examines the combination of 7+3 chemotherapy and nivolumab as frontline therapy (NCT02464657) while the second study looks at HiDAC in combination with pembrolizumab in patients with RR-AML (NCT02768792) (Table 8). Another trial will examine the efficacy of giving the immunomodulatory derivative pomalidomide at the time of early lymphocyte recovery after induction chemotherapy (NCT1510016699) (Table 8).

The effect of HMAs on the immune system is complex as HMAs have both immune stimulatory properties and immunosuppressive effects [186]. HMAs enhance multiple aspects of the immune response against malignant cells by augmenting antigenicity (tumor antigen expression, processing, and presentation) as well as T cell priming and effector function [186]. HMAs induce the expression of multiple prior suppressed CTAs such as melanoma-associated antigen (MAGE)-A, NY-ESO-1, and synovial sarcoma X (SSX)-2 through promoter region hypomethylation, which leads to their recognition by CTA-specific CD8+ cytotoxic T cells [197, 283–286]. Furthermore, HMAs enhance antigen presentation by up-regulating the expression of the MHC class I molecule and the co-stimulatory molecules CD80 and CD86 [285–287].

On the other hand, HMAs result in an up-regulation of the PD-1/PD-L1 and CTLA-4/CD80/86 axis as well as expansion of Treg in MDS and AML, which leads to immune escape of malignant cells [186, 187]. Relatively higher expression levels of immune checkpoint genes were associated with resistance to HMA, which argues for combining HMAs with immune checkpoint inhibitors [187]. Several clinical trials are dedicated to examining the combination of HMAs with antibody based immunotherapy (NCT02785900, NCT02472145) and immune checkpoint inhibition (NCT02397720, NCT02845297, NCT02996474, NCT02775903, NCT02892318) (Table 8).

8 Conclusion

An improved understanding of the biology underlying AML has revealed a complicated, heterogeneous disease landscape. As a result, multiple rationally designed agents have shown promise and are in advanced clinical trial testing. Furthermore, the pipeline is rich with many other targeted agents.

Among the most advanced agents in development is CPX-351, which has shown excellent activity in older patients with secondary AML. Although vosaroxin was not superior to anthracyclines for most patients in a phase III study, the absence of cardiotoxicity will make this drug attractive for

patients with cardiomyopathies unable to receive anthracycline therapy.

The FLT3 inhibitor midostaurin has been recently approved by the FDA in combination with 7+3 chemotherapy in the frontline setting for *FLT3*-mutated AML based on the RATIFY trial results. Several other FLT3 inhibitors are in phase III trials. The approval of midostaurin will probably lead to a change in the standard of care of these patients with the mutation to receive 7+3 + midostaurin and therefore will likely affect the design of the control arm in the randomized studies of other FLT3 inhibitors in the upfront setting. IDH1/2 inhibitors and BCL-2 inhibitors are also showing meaningful clinical activity. Based on the results of a large phase I trial in the refractory/relapsed setting, an application of accelerated approval of the IDH2 inhibitor AG-221 has been submitted to the regulatory authorities and a decision is expected in 2017.

Several new epigenetic drugs including the next-generation HMAs as well as DOT1L inhibitors and BET inhibitors are in clinical trials.

Immunotherapies have changed the therapeutic landscape of many solid tumors and will likely have important impact on AML management as well. The rapidly increasing arsenal of immunotherapies tested in AML encompass every step of the cancer immunity cycle. Vaccine-based therapy, antibody-based therapy, CAR T cells, and immune checkpoint inhibitors have all shown early promising results but more data are needed to understand the best setting and combinations of these agents.

Overall, the future of drug development in AML is bright with multiple new avenues of therapies and possible combination approaches.

Compliance with Ethical Standards

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