REVIEW

# Translating recent advances in the pathogenesis of acute myeloid leukemia to the clinic

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**Despite FDA approval of nine new drugs for patients with acute myeloid leukemia (AML) in the United States over the last 4 years, AML remains a major area of unmet medical need among hematologic malignancies. In this review, we discuss the development of promising new molecular targeted approaches for AML, including menin inhibition, novel IDH1/2 inhibitors, and preclinical means to target *TET2*, *ASXL1*, and RNA splicing factor mutations. In addition, we review progress in immune targeting of AML through anti-CD47, anti-SIRPα, and anti-TIM-3 antibodies; bispecific and trispecific antibodies; and new cellular therapies in development for AML.**

Acute myeloid leukemia (AML) is a genetically diverse myeloid neoplasm and the most common form of acute leukemia in adults, with >20,000 new cases in the United States in 2021 (Shallis et al. 2019; National Cancer Institute-Surveillance 2021, https://seer.cancer.gov/statfacts/html/amyl.html). With a 5-yr relative survival of only 29.5% between 2011 and 2017, there continues to be a high clinical need for novel and more effective therapies in both the frontline and relapsed/refractory (R/R) setting (National Cancer Institute-Surveillance 2021, https://seer.cancer.gov/statfacts/html/amyl.html).

Thanks to a better understanding of the molecular pathophysiology of AML, AML treatment is becoming increasingly individualized based on molecular features enabling improved risk stratification and more targeted therapies (Papaemmanuil et al. 2016; Döhner et al. 2017). In contrast to the genetically agnostic treatment with conventional cytotoxic chemotherapy, seven out of the nine novel therapies that have been approved for the treatment of newly diagnosed or R/R AML since 2017 act via a molecularly defined target (Fig. 1; Castaigne et al. 2012; Stein et al. 2017; Stone et al. 2017; DiNardo et al. 2018, 2020a; Cortes et al. 2019a; Perl et al. 2019; Wei et al. 2020). However, despite the introduction and success of these novel agents for AML therapy, areas of unmet need persist. These include the fact that there are still limited treatment options for many patients with R/R AML who do not harbor currently targetable mutations. Additionally, none of the recently approved therapies are curative except for the small minority of patients who proceed to allogeneic hematopoietic cell transplant (allo-HCT) (Bewersdorf et al. 2021a).

Several novel therapeutic approaches—including menin inhibitors in *MLL1* (or *KMT2A*)-rearranged and *NPM1* mutant AML, harnessing synthetic lethality in patients with loss-of-function mutations (e.g., *TET2*, *RUNX1*, and other *DNMT3A* mutations), and splicing modulators—are in various stages of clinical development (Issa et al. 2021). Additionally, the immune landscape within the bone marrow microenvironment in AML is becoming increasingly characterized, and immune escape mechanisms have been identified as a potential cause of disease relapse, leading to the development of various immunotherapeutic approaches such as immune checkpoint inhibitors, bispecific antibodies, and cellular therapies (Bewersdorf et al. 2020; Sallman et al. 2020b; Vadakekolathu et al. 2020b; Uy et al. 2021). This review focuses on how recent insights into the pathogenesis of AML are informing molecularly targeted therapeutics based on genomic alterations in AML as well as cellular and immunotherapeutic approaches.

## Advances in the molecular pathogenesis enable individualized treatment of AML patients

Thanks to advances in diagnostic technologies, molecular testing based on next-generation sequencing is becoming an increasingly routine part of management of AML patients and has implications for prognostication and treatment selection (Papaemmanuil et al. 2016; Döhner et al.

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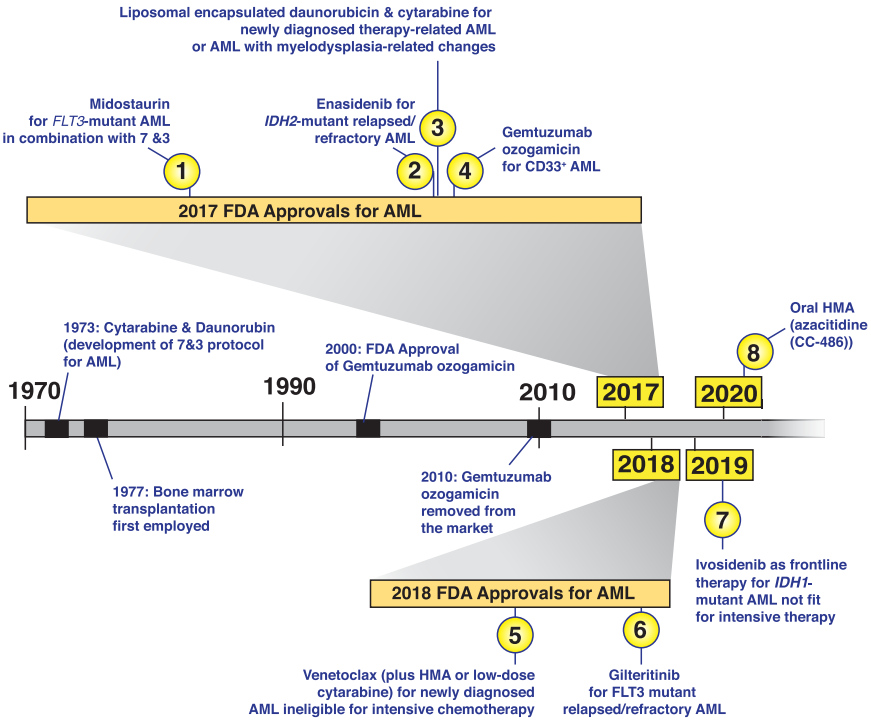


**Figure 1.** Time line of FDA-approved therapies for the treatment of acute myeloid leukemia (AML). (HMA) Hypomethylating agent.

2017). While the standard of care for newly diagnosed AML patients eligible for intensive chemotherapy has remained induction and consolidation chemotherapy with a cytarabine/anthracycline combination, the addition of the FLT3 inhibitor midostaurin in patients with *FLT3* mutations or of gemtuzumab ozogamicin in patients with CD33-positive, favorable risk AML has improved outcomes (Castaigne et al. 2012; Stone et al. 2017; Tallman et al. 2019). Additionally, combining the BCL2 inhibitor venetoclax with either hypomethylating agents (azacitidine and decitabine) or low-dose cytarabine has significantly improved the outcomes of older patients and those ineligible for intensive chemotherapy in randomized placebo-controlled clinical trials compared with either azacitidine or low-dose cytarabine alone (DiNardo et al. 2020a; Wei et al. 2020). Generally well-tolerated oral inhibitors of mutant FLT3 and IDH1/2 have also been approved for patients with R/R AML (Fig. 1; Stein et al. 2017; DiNardo et al. 2018; Perl et al. 2019). Several ongoing clinical trials seek to further increase the response rate and extend the durability of responses by the addition of targeted therapies to either intensive chemotherapy or venetoclax-based combinations. Preclinical studies have suggested synergy between FLT3 inhibitor-mediated down-regulation of the antiapoptotic mediator MCL1 and enhanced sensitivity to BCL2 inhibition by venetoclax, lending support to combining FLT3 inhibitors with hypomethylating agents and venetoclax in AML patients with *FLT3* mutations (Ma et al. 2019; Singh Mali et al. 2021). Similarly, conventional cytotoxic chemotherapy such as idarubicin and cytarabine has been shown to suppress MCL1 and to synergize with venetoclax in murine AML models (Teh et al. 2018). While early results from single-arm clinical trials appear promising, larger, randomized clinical trials with longer follow-up are necessary, and adverse events (especially myelosuppression) remain a concern (Daver et al. 2020; Lin et al. 2020; Reville et al. 2020; DiNardo et al. 2021a; Maiti et al. 2021).

Besides identifying a potential therapeutic target, molecular testing may also have the potential to guide therapy selection in AML patients. For example, *TP53* mutations occur in 10%–15% of patients with AML and are enriched in patients with therapy-related AML or other adverse prognostic features such as complex or monosomal karyotypes (Rücker et al. 2012; Papaemmanuil et al. 2016). Additionally, *TP53* mutations have been shown to confer a higher rate of resistance to conventional cytotoxic chemotherapy but to potentially be more susceptible to treatment with hypomethylating agents (Kadia et al. 2016; Welch et al. 2016). Whether *TP53* mutations could serve as a marker of adverse "genomic fitness" in AML patients that would provide the rationale for using venetoclax-based combinations in otherwise chemotherapy-eligible patients requires additional studies. It is important to note that *TP53* mutations are also associated with lower response rates to venetoclax/azacitidine and constituted one of the few patient subgroups that do not experience a statistically significant survival benefit with venetoclax/azacitidine compared with azacitidine alone (hazard ratio [HR]: 0.76; 95% CI: 0.40–1.45). However, this was a likely underpowered subgroup analysis, which should be interpreted cautiously. *TP53* mutations also retained their adverse prognostic impact in AML patients treated with decitabine/venetoclax in a recent phase II trial (median overall survival [OS]: 5.2 mo vs. 19.4 mo; HR: 4.67; 95% CI: 2.44–8.93; $P < 0.0001$) (DiNardo et al. 2020a,b,d; Kim et al. 2021).

Given that the majority of AML patients does not harbor a mutation in *FLT3, IDH1*, or *IDH2*, novel therapeutic

strategies for patients harboring the large number of other AML-associated genetic alterations are needed and are discussed in the sections below.

## Novel molecularly targeted therapies in AML

### *Menin inhibitors*

Although leukemogenic translocations of the mixed-lineage leukemia (*MLL*, also known as lysine methyltransferase 2A [*KMT2A*]) gene on chromosome 11q23 are found more frequently in infants, 10% of adult AML patients also harbor this genetic alteration (Krivtsov and Armstrong 2007). *KMT2A* rearrangements are also common in patients with mixed-lineage (biphenotypic) leukemias and have been associated with a variable, context-dependent prognosis (Caligiuri et al. 1998; Krivtsov and Armstrong 2007; Issa et al. 2021). *KMT2A* encodes an essential regulator of HOX genes via methylation of histone H3 lysine residue 4 (H3K4) (Chen et al. 2006; Krivtsov and Armstrong 2007). Although menin was initially described as a tumor suppressor in the context of multiple endocrine neoplasia type I, subsequent studies have established menin's requirement in the maintenance of *KMT2A*-rearranged AML (Chandrasekharappa et al. 1997; Yokoyama et al. 2005; Chen et al. 2006).

Oncogenic fusions of *KMT2A* occur with >90 different partners, several of which influence leukemia phenotype and prognosis (Meyer et al. 2018). Such rearrangements have also been associated with emergence of therapy-related AML following treatment with topoisomerase II inhibitors such as etoposide (Super et al. 1993). Compared with other AML subtypes, AML with *KMT2A* rearrangements is characterized by fewer co-occurring genetic alterations, highlighting the role of the *KMT2A* rearrangement as a driver of leukemogenesis and underscoring this oncogenic fusion as a potentially promising target (Andersson et al. 2015; Bill et al. 2020).

While *KMT2A* rearrangements only affect a minority of AML patients, *NPM1* mutations constitute one of the most common genetic abnormalities in adult AML, affecting up to 30% of patients (The Cancer Genome Atlas Research Network 2013; Papaemmanuil et al. 2016). Mutations in *NPM1* have also been associated with the up-regulation of *HOXA* genes, similar to gene expression patterns observed in patients with *KMT2A* rearrangements (Mullighan et al. 2007; Andreeff et al. 2008). These findings have led to the hypothesis that AML patients with *NPM1* mutations might also benefit from menin inhibition. Figure 2 illustrates the pathogenesis of *KMT2A*-rearranged and *NPM1* mutant AML and the role of menin inhibition in this context.

Small molecules inhibiting the interaction of menin with KMT2A by binding to the KMT2A binding pocket have been developed and successfully tested in preclinical models (Shi et al. 2012; Borkin et al. 2015). Similarly, menin inhibitors showed antileukemic activity in mouse and patient-derived xenograft (PDX) models of *NPM1* mutant AML (Klossowski et al. 2020; Uckelmann et al. 2020). Based on those encouraging preclinical findings, several early phase trials have been initiated. KO-539 is an oral inhibitor of the menin–KMT2A protein–protein interaction that is currently being tested in a phase I/II trial (NCT04067336). While data on the efficacy and safety

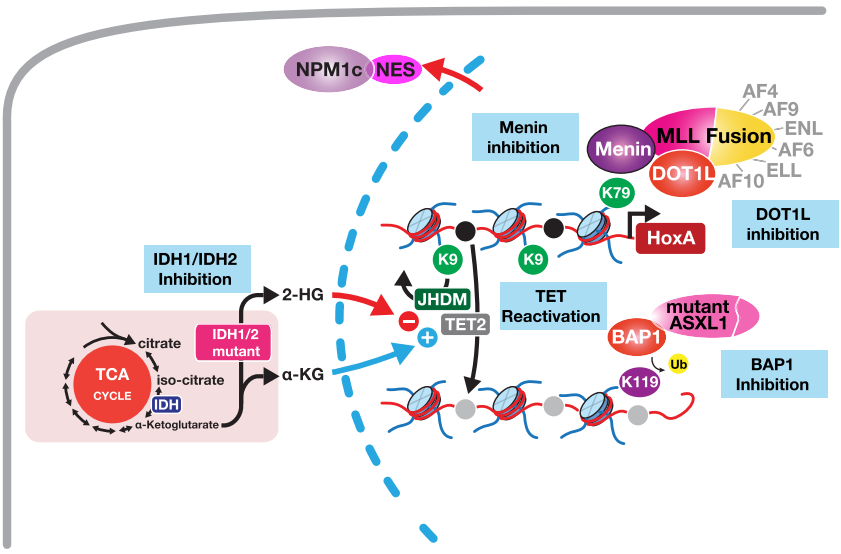


**Figure 2.** Known and novel epigenetic targets for the treatment of acute myeloid leukemia (AML). AML-associated genetic alterations in IDH1, IDH2, TET2, and ASXL1, as well as chromosomal rearrangements in MLL1 (KMT2A), alter histone post-translational modifications and/or DNA cytosine modifications and represent exciting therapeutic targets. AML-associated mutations in NPM1 enforce cytoplasmic localization of the mutant NPM1 (NPM1c) and result in up-regulated *HOXA* gene cluster expression via a mechanism that is not entirely clear. Menin inhibitors have demonstrated promising efficacy and safety in ongoing phase I/II trials for MLL-rearranged and NPM1 mutant AML. In addition, it is known that KMT2A translocations alter the enzymatic activity of KMT2A from a histone H3 lysine 4 (H3K4) methyltransferase to gain the ability to methylate H3K79 via association with the H3K79 methyltransferase DOT1L. As such, DOT1L inhibitors continue to be evaluated for KMT2A-rearranged AML. IDH1/2 mutations alter the enzymatic activity of IDH1/2 to generate the oncometabolite 2-hydroxyglutarate (2-HG) from isocitrate and reduce levels of α-ketoglutarate (α-KG). This alteration in α-KG levels impacts numerous α-KG-dependent enzymes, including TET2 and the Jumonji family of histone lysine demethylases (JHDM). Currently, IDH1 and IDH2 inhibitors are FDA-approved. A number of methods are being tested to boost TET2 enzymatic activity in AML patients with haploinsufficient TET2 mutations (including increasing levels of the TET2 cofactor vitamin C). Finally, recent data identify that certain ASXL1 mutations promote the activity of the H2AK119 deubiquitinase BAP1. As such, the first class of BAP1 catalytic inhibitors has been developed very recently.

are limited to date, KO-539 showed biologic activity among six patients with R/R AML (including one patient who achieved a measurable residual disease [MRD]-negative complete remission [CR]) (Wang et al. 2020). Of note, patients enrolled in this trial were not required to have *KMT2A* rearrangements or *NPM1* mutations. SNDX-5613 is another orally available menin–KMT2A binding inhibitor that has demonstrated single-agent activity in PDX models of *NPM1* mutant AML (Krivtsov et al. 2019). This agent is currently being tested in a phase I/II trial in patients with R/R AML with *KMT2A* rearrangement or *NPM1* mutations. Preliminary data from 45 patients with *KMT2A*-rearranged or *NPM1* mutant AML presented in abstract form showed an overall response rate (ORR) of 44% with 22% CR or CR with partial hematologic recovery (CRh) (Stein et al. 2021). However, safety and efficacy data are still immature, and longer follow-up is needed. Table 1 provides an overview of selected ongoing clinical trials of menin inhibitors in AML.

Potential options to enhance the therapeutic efficacy of menin inhibitors include the combination with FLT3 or DOT1L inhibitors; both of which have shown efficacy in preclinical models (Kuhn et al. 2015; Dafflon et al. 2017; Dzama et al. 2020). However, such combinations have not been tested clinically to date. Additionally, biomarkers predicting response to menin inhibition as well as the impact of co-occurring mutations are areas of ongoing research needs.

### *Novel IDH1/2 inhibitors*

Mutations in *IDH1* or *IDH2* occur in 8%–12% of AML patients and have been shown to promote leukemogenesis by the production of the oncometabolite 2-hydroxyglutarate (Fig. 2; The Cancer Genome Atlas Research Network 2013; Chan et al. 2015). The IDH1 inhibitor ivosidenib and the IDH2 inhibitor enasidenib have been approved for the treatment of R/R AML patients with *IDH1* or *IDH2* mutations, respectively, based on single-arm phase I/II studies. Both agents demonstrated overall response rates (ORR) of 40% and median duration of response of ~6 mo (Stein et al. 2017; DiNardo et al. 2018). While these results are promising in a pretreated patient population, efforts to both improve the response rates and duration by combination with synergistic agents and use them in the first-line setting have been launched (Dinardo et al. 2020c; Lin et al. 2020; Roboz et al. 2020; Stein et al. 2020a). Additionally, substantial progress has been made in the identification of resistance mechanisms to IDH inhibitors. Studies have identified co-occurring RAS pathway mutations as being associated with decreased IDH1/2 inhibitor response as well as the emergence of second site mutations within IDH1/2 and isoform switching between IDH1/2 mutations (Amatangelo et al. 2017; Intlekofer et al. 2018; Choe et al. 2020).

Several second-generation IDH inhibitors have been developed and demonstrated mixed results. BAY1436032 has shown in vitro and in vivo activity against various *IDH1* mutations and also demonstrated changes in DNA methylation patterns (Chaturvedi et al. 2017). However, in a phase I clinical trial, BAY1436032 yielded an ORR of 15% and median duration of response of 3.0 mo among 27 AML patients with *IDH1* mutations (Heuser et al. 2020). This compound is not going to be developed further for the treatment of AML given this limited efficacy.

FT-2102 (olutasidenib) is another IDH1 inhibitor in clinical development for IDH1 mutant AML (Caravella et al. 2020). As a single agent, FT-2102 showed an ORR of 41% (18% CR) among R/R AML patients with *IDH1* mutations enrolled in a phase I study (NCT02719574) (Watts et al. 2019). Vorasidenib (AG-881) is a combined IDH1 and IDH2 inhibitor that is currently being tested in low-grade IDH mutant glioma and could eventually be studied in AML as well (Mellinghoff et al. 2021). Finally, LY3410738 is a novel covalent IDH1-R132 inhibitor that has increased potency against *IDH1*-R132 mutations and has activity against common second site IDH mutations thanks to its binding outside of the dimer interface, which is a known resistance mechanism to first-generation IDH inhibitors. LY3410738 is currently being studied in a phase I clinical trial but no results are available to date (Stein et al. 2020b). Figure 2 illustrates the pathogenesis of *IDH1/2* mutant AML and mechanism of action of approved and novel IDH1/2 inhibitors.

Based on changes in DNA methylation observed following treatment with IDH1/2 inhibitors, combination with the hypomethylating agent azacitidine could have combinatorial efficacy. Preclinical experiments supported this potential synergy, showing substantial antitumor activity of azacitidine plus BAY1436032 against leukemic stem cells (Chaturvedi et al. 2021). This synergy has also been explored in early phase clinical trials. In a phase I trial of 23 newly diagnosed *IDH1* mutant AML patients ineligible for intensive induction chemotherapy (NCT02677922), the combination of ivosidenib and azacitidine led to an ORR of 78.3% (95% CI: 56.3%–92.5%) and CR rate of 60.9% (95% CI: 38.5%–80.3%) (DiNardo et al. 2021b).

**Table 1.** *Ongoing trials of menin inhibition in AML*

| Agent | NCT | Phase | Patient population |
|---|---|---|---|
| KO-539 | NCT04067336 | I/II | R/R AML; independent of cytogenetic and molecular characteristics |
| JNJ-75276617 | NCT04811560 | I | AML and ALL patients with *KMT2A* or *NPM1* mutations refractory to or ineligible for all standard therapies |
| DSP-5336 | NCT04988555 | I/II | R/R AML or ALL with *MLL* rearrangement or *NPM1* mutations (phase I enrollment independent of cytogenetic or molecular characteristics) |
| SNDX-5613 | NCT04065399 | I/II | R/R AML or ALL with *MLL* rearrangement or *NPM1* mutations |

Responses appeared durable and deep in a subset of patients, with 10 out of 14 patients achieving *IDH1* mutation clearance by PCR and a 12-mo OS estimate of 82.0% (95% CI: 58.8%–92.8%) (DiNardo et al. 2021b). The combination of azacitidine and ivosidenib has also been evaluated in a randomized, placebo-controlled phase III trial (AGILE; NCT02677922), which randomized 146 newly diagnosed AML patients with IDH1 mutations who were not eligible for intensive chemotherapy. Median OS (24 mo vs. 7.9 mo; HR = 0.44 [95% CI: 0.27–0.73]; $P = 0.0005$) and CR rate (47.2% [95% CI: 35.3%–59.3%] vs. 14.9% [95% CI: 7.7%–25.0%]; $P < 0.0001$) were both superior with ivosidenib + azacitidine compared with placebo + azacitidine (Montesinos et al. 2021). Similar preliminary results have been presented from a phase I/II trial (NCT02719574) of the combination of FT-2102 with azacitidine in patients with myelodysplastic syndromes (MDS) (Cortes et al. 2019b; Watts et al. 2019). Additionally, results from a phase I/II trial of ivosidenib plus venetoclax with or without azacitidine in newly diagnosed and R/R AML patients with *IDH1* mutations have demonstrated an ORR of 78% (Lachowiez et al. 2020). Table 2 provides an overview of ongoing clinical trials of IDH inhibitors in AML.

### *RNA splicing as a novel therapeutic target in AML*

Mutations in genes encoding RNA splicing factors such as *SF3B1*, *SRSF2*, *U2AF1*, and *ZRSR2* are encountered frequently in patients with AML, especially in those with an antecedent MDS (Inoue et al. 2016; Chen et al. 2021). RNA splicing is a tightly regulated process during which nucleotide segments are enzymatically removed from precursor messenger RNA (pre-mRNA) and the remaining nucleotides are ligated to form the mature mRNA (Chen et al. 2021). This process is essential for regulation of gene expression, and alternative splicing is a major contributor to proteome diversity.

*SF3B1* mutations are the most common splicing mutations in hematologic malignancies and define a distinct subtype of MDS (MDS with ringed sideroblasts) (Papaemmanuil et al. 2011; Malcovati et al. 2020). While *SF3B1* mutations in MDS have been associated with a favorable prognosis, *U2AF1* and *SRSF2* mutations are enriched in high-risk MDS and AML and confer an adverse prognosis (Thol et al. 2012; Bejar et al. 2015; Ohgami et al. 2015). Mutations in genes encoding RNA splicing factors affect a wide variety of transcripts and signaling pathways via differential splicing such as DNA damage response, epigenetic regulation, and immune signaling (Chen et al. 2021). It therefore remains to be fully elucidated what specific aberrant splicing events lead to the development of myeloid neoplasms, and *SRSF2*, *SF3B1*, and *U2AF1* mutations are not felt to be leukemogenic in isolation based on various murine knock-in models (Chen et al. 2021).

Based on the finding that splicing factor mutations are often mutually exclusive, it has been proposed that cells with splicing factor mutations are dependent on the normal function of the residual wild-type splicing factors (Lee et al. 2016, 2018). Thus, using pharmacologic interference with splicing could lead to synthetic lethality and constitute a potent, novel therapeutic approach to

**Table 2.** *Ongoing trials of IDH1 or IDH2 inhibition in AML*

| Agent | Specific regimen | NCT | Phase | Patient population |
|---|---|---|---|---|
| Enasidenib | Enasidenib monotherapy | NCT04203316 | II | Pediatric R/R *IDH2* mutant AML patients |
| | Enasidenib monotherapy following allo-HCT | NCT03728335 | I | *IDH2* mutant AML in remission following allo-HCT |
| | Enasidenib monotherapy following allo-HCT | NCT04522895 | II | *IDH2* mutant AML, MDS, or CMML in remission or relapse following allo-HCT |
| | Enasidenib + CPX-351 | NCT03825796 | II | R/R *IDH2* mutant AML patients |
| | Enasidenib + venetoclax | NCT04092179 | I/II | *IDH2* mutant AML patients |
| | Enasidenib or ivosidenib + decitabine/cedazuridine + venetoclax | NCT04774393 | I/II | R/R AML or MDS with *IDH1* or *IDH2* mutation |
| | Enasidenib or ivosidenib vs. placebo + induction and consolidation chemotherapy | NCT03839771 | III | Newly diagnosed AML or MDS with *IDH1* or *IDH2* mutation |
| Ivosidenib | Ivosidenib + combination chemotherapy | NCT04250051 | I | R/R *IDH1* mutant AML patients |
| | Ivosidenib + CPX-351 | NCT04493164 | II | *IDH1-R132* mutant AML patients or MDS |
| | Ivosidenib | NCT02074839 | I | *IDH1* mutant advanced hematologic malignancies |
| | Ivosidenib + venetoclax ± azacitidine | NCT03471260 | I/II | *IDH1* or *IDH2* mutant myeloid neoplasms |
| FT-2102 | FT-2102 ± azacitidine | NCT02719574 | II | R/R *IDH1-R132* mutant AML patients |
| SH1573 | SH1573 | NCT04806659 | I | R/R *IDH2* mutant AML patients |
| HMPL-306 | HMPL-306 | NCT04272957 | I | *IDH1* or *IDH2* mutant R/R myeloid neoplasms |
| | HMPL-306 | NCT04764474 | I | *IDH1* or *IDH2* mutant R/R myeloid neoplasms |
| LY3410738 | LY3410738 monotherapy | NCT04603001 | I | *IDH1* or *IDH2* mutant R/R myeloid neoplasms |
| SLE24/MEN1703 | SLE24/MEN1703 monotherapy | NCT03008187 | I/II | *IDH1* or *IDH2* mutant R/R AML |

splicing factor mutant AML and MDS. Several compounds binding to the SF3b complex have been developed and reviewed in detail recently (El Marabti and Abdel-Wahab 2021).

Figure 3 provides an overview of potential therapeutic approaches for splicing factor mutant AML. Among these compounds, H3B-8800 is the agent furthest along in clinical development. H3B-8800 is an orally bioavailable modulator of the SF3b complex that has been shown to preferentially kill spliceosome mutant cells in vitro (Seiler et al. 2018). However, data from a subsequent phase I clinical trial (NCT02841540) that enrolled 84 patients with AML, MDS, or CMML independent of the presence of splicing mutations were disappointing, with no complete or partial responses per 2006 IWG response criteria reported, although 15% of patients achieved red blood cell transfusion independence (RBC-TI) (Steensma et al. 2021). Treatment with H3B-8800 was generally well tolerated, with diarrhea, nausea, fatigue, and vomiting being the most common treatment-related, treatment-emergent adverse events (Steensma et al. 2021). While these results are disappointing at first glance, it is important to note that this trial enrolled a genetically and clinically diverse patient population and that patients with *SF3B1* mutations achieved higher rates of RBC-TI than *SF3B1* wild-type patients (Steensma et al. 2021). Further studies are needed to identify predictive biomarkers and to optimize treatment schedules.

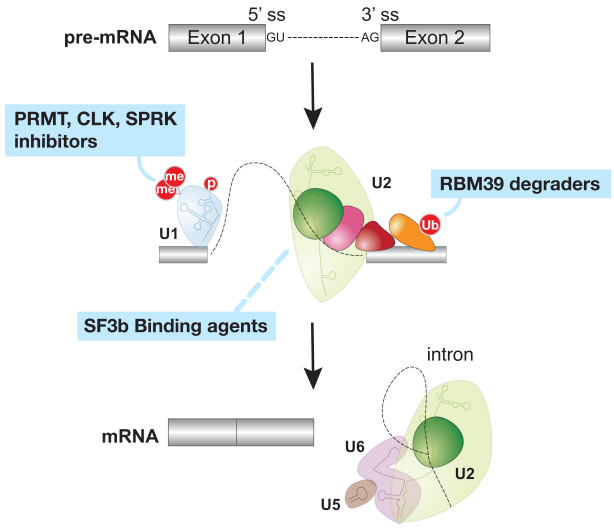


**Figure 3.** Therapeutic modalities for targeting aberrant RNA splicing in acute myeloid leukemia. Based on data identifying that leukemia cells with change of function mutations in RNA splicing factor genes are preferentially sensitive to chemical modulators of splicing, a number of means to perturb splicing have been developed. These include SF3b binding agents, RBM39-degrading compounds, and inhibitors of a series of enzymes that place critical post-translational modifications on splicing factors, such as inhibitors of protein arginine methyltransferases (PRMTs), CDC2-like (CLK) protein kinases, and SR protein kinases (SRPKs).

Besides SF3b binding agents, RBM39 degraders represent another class of drug candidates that target splicing. RBM39 is an accessory RNA splicing factor that can be targeted by aryl sulfonamide molecules (e.g., indisulam) that mark RBM39 for proteasomal degradation leading to splicing defects (Han et al. 2017; Chen et al. 2021). As RBM39 is required for the survival of various types of cancer cells, including splicing factor mutant leukemias, degradation of RBM39 has been shown to have antileukemic effects in preclinical models (Wang et al. 2019). Indisulam was studied in a phase II trial in combination with idarubicin and cytarabine in patients with R/R AML or MDS and demonstrated an ORR of 35%. However, this trial was not restricted to patients with splicing factor mutations and did not use biomarkers such as DCAF15 for patient selection (Han et al. 2017; Assi et al. 2018). Other RBM39-degrading compounds such as E7820 are currently in clinical trials and are specifically enrolling patients with splicing factor mutant AML or MDS (NCT05024994).

Finally, targeting RNA splicing via inhibiting enzymes that place post-translation modifications on splicing factors has emerged as another potentially clinically viable means to target splicing factor mutant leukemias. The furthest approach of this category is protein arginine methyltransferases (PRMT) inhibitors. PRMT inhibitors interfere with the arginine methylation of splicing factors required for various steps of the splicing process, leading to the preferential killing of splicing factor mutant leukemia cells (Fong et al. 2019). Several PRMT5 inhibitors are being tested in clinical trials (e.g., NCT03886831 and NCT03614728) but no results have been published to date. Attempts to increase the response rate to single-agent splicing modulators are ongoing and include combination with azacitidine (NCT03614728). Additionally, the combination with venetoclax or immune checkpoint inhibitors could have synergistic effects based on encouraging in vitro results (Lu et al. 2021; Stahl et al. 2021).

### *Targeting other AML-associated mutations*

While there has been considerable debate on the mechanistic effects of leukemia-associated *ASXL1* mutations, several recent studies (Balasubramani et al. 2015; Asada et al. 2018; Wang et al. 2021) have identified that the most common *ASXL1* mutations generate a stable truncated protein that promotes the activity of the histone H2A lysine 119 deubiquitinase BAP1 (Fig. 2). These observations motivated Wang et al. (2021) to screen for BAP1 catalytic inhibitors. A first-in-class BAP1 inhibitor abrogated truncated ASXL1 gene expression and tumor growth in vivo. Future studies elucidating the dependency of ASXL1 mutant cells on BAP1 or BAP1 inhibitors will be an important next step in evaluating this new therapeutic target.

Similar to *ASXL1* mutations, *RUNX1* mutations have also been associated with an adverse prognosis in AML patients (Döhner et al. 2017). *RUNX1* mutations have been shown to lead to impaired biogenesis of ribosomes and to be more susceptible to treatment with venetoclax or the protein translation inhibitor homoharringtonine (Mill

et al. 2021). In vitro experiments demonstrated reduced levels of c-Myc, c-Myb, MCL1, and BCL-XL in *RUNX1* mutant AML cells treated with homoharringtonine. Additionally, cotreatment with venetoclax or BET inhibitors with homoharringtonine had synergistic effects, suggesting homoharringtonine-based combination therapies as a potential therapeutic option in AML patients with *RUNX1* mutations (Mill et al. 2019, 2021).

Loss-of-function mutations in *TET2* are found in ~10% of AML patients but are more common in patients with MDS or CMML (Delhommeau et al. 2009). *TET2* loss has been associated with DNA hypermethylation and changes in gene expression in hematopoietic stem and progenitor cells leading to increased self-renewal and myeloid differentiation (Moran-Crusio et al. 2011). It is also notable that *TET2* mutations are one of the most common mutations in otherwise healthy people with clonal hematopoiesis and confer a preleukemic lesion that can promote development of myeloid neoplasm (Busque et al. 2012; Jaiswal et al. 2014; Xie et al. 2014). Thus, restoration of TET2 activity not only could be beneficial in patients with AML and MDS but could potentially even prevent the development of an overt myeloid neoplasm in patients with clonal hematopoiesis. In inducible and reversible Tet2 knockdown mouse models, treatment with vitamin C was able to restore Tet2 expression and to reverse the aberrant self-renewal of hematopoietic stem and progenitor cells (Cimmino et al. 2017). Additionally, vitamin C treatment increased the susceptibility of leukemia cells to treatment with poly-ADP-ribose polymerase (PARP) inhibitors (Cimmino et al. 2017). As *TET2* mutations have also been associated with increased response rates to hypomethylating agents (Bejar et al. 2014), using vitamin C as an adjunct to treatment with hypomethylating agents or PARP inhibitors could lead to therapeutic synergy. The combination of azacitidine and vitamin C has been studied in a phase II clinical trial (NCT03397173) but no results have been published yet.

It has also been shown that the oncometabolite 2-hydroxyglutarate is lethal to *TET2*-deficient cells, which also explains the mutual exclusivity of *TET2* and *IDH1/2* mutations. Therefore, TET-selective dioxygenase inhibitors have been developed and shown to have antileukemic effects in xenografts from *TET2* mutated human leukemia, but additional testing in clinical trials is necessary (Guan et al. 2021).

## Immune therapies in AML

Beyond cell-intrinsic genetic alterations driving AML development, avoiding elimination by the immune system via the up-regulation of inhibitory immune checkpoints such as programmed death (PD)-1 and cytotoxic T lymphocyte-associated protein (CTLA)-4 has been associated with disease persistence in AML (Williams et al. 2019). Several additional inhibitory immune checkpoints, including TIM-3 and LAG-3, have also been implicated in immune evasion in AML, and the double expression of PD-1 and TIM-3 or LAG-3 is a characteristic feature of T-cell exhaustion at the time of disease relapse (Kong et al. 2015; Schnorfeil et al. 2015; Williams et al. 2019; Bewersdorf et al. 2020).

In an era of increasingly individualized, genetically driven treatment approaches to AML patients, recent efforts have focused on the characterization of the immune landscape in genetically defined patient subsets. For example, emerging data suggest that *TP53* mutations confer an immunosuppressive phenotype in patients with secondary AML or MDS that is characterized by the up-regulation of PD-L1 and other markers of $CD8^+$ T-cell exhaustion, an expanded population of immunosuppressive regulatory T cells, and increased interferon-γ signaling (Sallman et al. 2020b; Vadakekolathu et al. 2020a,b). Thus, clinical trials to increase antitumor immune responses using immune checkpoint inhibitors, bispecific antibodies, and cellular therapies have been conducted with mixed results (Table 3; Boddu et al. 2018; Liu et al. 2019). Figure 4 illustrates various immune targets in development for AML.

While treatment with anti-PD-1/PD-L1 or anti-CTLA-4 antibodies has reshaped the treatment for various solid malignancies, results in myeloid malignancies have largely been disappointing, with limited efficacy if used as monotherapy (Berger et al. 2008; Zeidan et al. 2018). One of the few trials showing clinical efficacy of anti-CTLA-4 monotherapy with ipilimumab enrolled 28 patients (12 with AML and two with MDS) who relapsed after allo-HCT (Davids et al. 2016). Five patients (23%) achieved a CR in this trial, with four of these patients presenting with extramedullary disease, suggesting that ipilimumab is able to reinvigorate the "graft versus leukemia" effect in some patients. However, it is unclear what specific characteristics of the immune microenvironment in patients with extramedullary disease render these tumors more susceptible to immune checkpoint blockade. Of note, exacerbation of "graft versus host disease" constituted a dose-limiting toxicity in this trial, and fatal "graft versus host disease" with the use of immune checkpoint inhibitors in the post-transplant setting have been reported (Davids et al. 2016; Gros et al. 2017).

Preclinical studies showing up-regulation of PD-1, PD-L1, CTLA-4, and PD-L2 in $CD34^+$ cells from patients treated with hypomethylating agents have suggested potential synergy of hypomethylating agents with immune checkpoint inhibitors (Yang et al. 2014; Ørskov et al. 2015). While single-arm studies supported synergistic effects of azacitidine with anti-PD-1 and/or anti-CTLA4 antibodies, the only randomized trial that compared the anti-PD-L1 antibody durvalumab plus azacitidine with azacitidine alone did not show a survival benefit in newly diagnosed older AML or MDS patients (Zeidan et al. 2019a; Chien et al. 2020; Morita et al. 2020; Saxena et al. 2021). A potential barrier to the success of these trials is the absence of a validated biomarker predicting response to immune checkpoint inhibitors. While PD-1 expression on T cells does not appear to predict response, the absolute $CD3^+$ and $CD8^+$ T-cell count prior to therapy, a higher number of ICOS (inducible T-cell costimulator)-expressing T cells, and a low bone marrow blast percentage have been suggested as potential response predictors (Zeidan et al.

**Table 3.** *Results of selected trials on immune therapies in AML and MDS*

| Reference | Therapy | Phase | Study population | Outcomes |
|---|---|---|---|---|
| **Immune checkpoint inhibitors** | | | | |
| **Anti-PD1** | | | | |
| Berger et al. 2008 | Pidilizumab | I | R/R AML ($n = 8$) | 1 patient with reduction in peripheral blasts from 50% to 5% |
| Daver et al. 2019 | Nivolumab + AZA | Ib/II | R/R AML ($n = 70$) | 33% ORR (22% CR/CRi) |
| Ravandi et al. 2019 | Nivolumab + cytarabine/ idarubicin induction and consolidation | II | Frontline AML and MDS ($n = 44$) | CR/CRi 78%; median OS: 18.5 mo |
| Tschernia et al. 2021 | Pembrolizumab as bridge to allo-HCT | II | 9 AML patients receiving high-dose cytarabine followed by pembrolizumab prior to allo-HCT | 1-yr OS: 67% |
| Chien et al. 2021 | Pembrolizumab + AZA | II | Frontline ($n = 17$) and R/R ($n = 20$) MDS | Frontline: 76% ORR, median OS not reached<br>R/R: 25% ORR, median OS 5.8 mo |
| **Anti-PD-L1** | | | | |
| Zeidan et al. 2019a | Durvalumab + AZA vs. AZA monotherapy | II | Frontline MDS ($n = 84$) and AML ($n = 129$) | ORR (MDS: 61.9% for AZA + durvalumab vs. 47.6% with AZA alone; AML: 31.3% vs. 35.4%)<br>Median OS (MDS: 11.6 mo vs. 16.7 mo; AML: 13.0 mo vs. 14.4 mo) |
| **Anti-CTLA4** | | | | |
| Zeidan et al. 2018 | Ipilimumab | Ib | R/R MDS ($n = 29$) | 1 patient with mCR, 24% with prolonged stable disease |
| **Anti-CD47** | | | | |
| Sallman et al. 2020a | Magrolimab + AZA | II | Frontline MDS ($n = 39$) and AML ($n = 29$) | MDS: 91% ORR (42% CR): AML: 64% ORR (40% CR) |
| Zeidan et al. 2022 | CC-90002 | I | R/R AML ($n = 24$) and MDS ($n = 4$) | No objective responses |
| **Anti-TIM3** | | | | |
| Brunner et al. 2021 | Sabatolimab + HMA | Ib | Frontline AML ($n = 48$), MDS ($n = 51$) | AML: 40.0% ORR; MDS: 56.9% ORR |
| **Bispecific antibodies** | | | | |
| Uy et al. 2021 | Flotetuzumab (anti-CD3 × CD123 DART) | I/II | R/R AML ($n = 88$) | ORR: 30% among AML patients with primary induction failure or early relapse; 24% in unselected cohort |
| Ravandi et al. 2020a | Vibecotamab (anti-CD3 × CD123 BiTE) | I | R/R AML ($n = 104$) | ORR: 14% (4% CR) |
| Ravandi et al. 2020b | AMG 330 (anti-CD3 × CD33 BiTE) | I | R/R AML ($n = 55$) | ORR: 19% (7% CR) |
| Subklewe et al. 2019 | AMG 673 (anti-CD3 × CD33 BiTE) | I | R/R AML ($n = 30$) | 1 patient with CRi; 6 with ≥50% bone marrow blast reduction |
| **CAR T cells** | | | | |
| Liu et al. 2018 | Anti-CLL1-CD33 compound CAR T cells | I | R/R AML | 1 patient with MRD-negative CR |
| Sallman et al. 2018 | NKG2D CAR T cells | I | R/R AML ($n = 8$), multiple myeloma ($n = 3$), MDS ($n = 1$) | AML: ORR 42% (1 patient with CRh) |

(Allo-HCT) Allogeneic hematopoietic cell transplantation, (AML) acute myeloid leukemia, (AZA) azacitidine, (BiTE) bispecific T-cell engager, (CMML) chronic myelomonocytic leukemia, (CR) complete remission, (CRi) complete remission with incomplete count recovery, (CTLA-4) cytotoxic T lymphocyte-associated protein-4, (DART) dual-affinity retargeting, (HMA) hypomethylating agent, (mCR) marrow CR, (MDS) myelodysplastic syndrome, (MRD) minimal residual disease, (ORR) overall response rate, (OS) overall survival, (PD1) programmed cell death protein 1, (R/R) relapsed/refractory, (TIM3) T-cell immunoglobulin and mucin domain-containing.

2018; Daver et al. 2019). On a genetic level, the presence of *ASXL1* mutations has been associated with higher response rates to combination treatment with azacitidine plus the anti-PD1 antibody nivolumab (Daver et al. 2019). However, additional validation of immunophenotypic and molecular biomarkers is necessary.

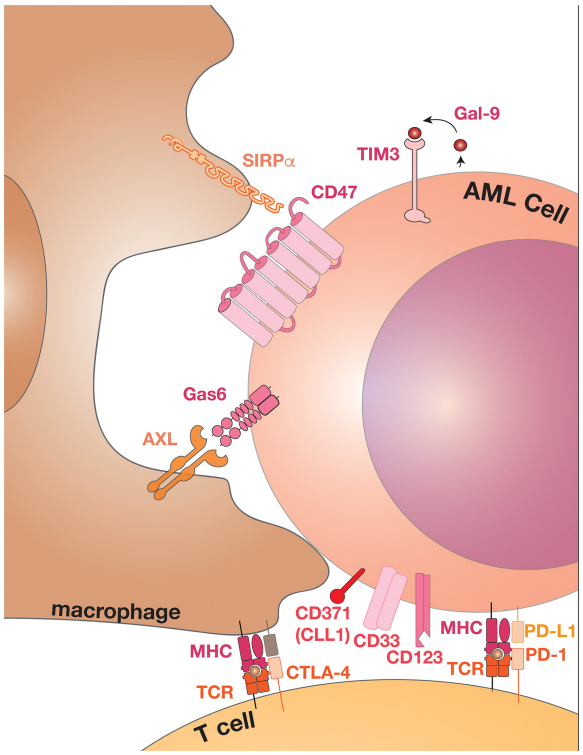


**Figure 4.** Innate and adaptive immune targets in clinical evaluation for the treatment of acute myeloid leukemia. Currently, clinical trials combining anti-CD47 antibodies (which block CD47 interaction on AML cells with the receptor SIRPa on macrophages) with DNA hypomethylating agents and the triplet of HMAs and venetoclax are ongoing. In addition, blocking SIRPα is being pursued clinically, and inhibiting signaling downstream from the AXL receptor tyrosine kinase on macrophages has been shown in preclinical settings to promote innate immune killing of AML. While treatment with anti-PD-1/PD-L1 or anti-CTLA-4 antibodies has had limited efficacy if used as monotherapy or combined with hypomethylating agents in MDS and AML to date, targeting adaptive immune signaling via blocking the TIM-3 immune checkpoint is currently being evaluated in AML. Preclinical data suggest a tumor cell-autonomous effect of TIM-3 signaling in AML where the TIM-3 ligand Gal-9 is secreted by AML cells and supports AML survival via an autocrine loop. Finally, a number of AML-associated surface antigens are being targeted via T-cell-engaging approaches, including CD33, CD123, and CD371 (CLL1). Currently, the safety and utility of these latter approaches are unclear, as many of these antigens are expressed on normal myeloid cells and/or hematopoietic precursors.

### *Novel immune checkpoint inhibitors and combination approaches*

Following the lackluster results with anti-PD-1/PD-L1- and anti-CTLA-4-directed therapies, novel therapeutic targets such as TIM-3, LAG-3, and CD47 have been explored. As the presence of exhausted immune effector cells that are characterized by PD-1 and TIM-3, TIGIT, or LAG-3 expression have been linked to AML relapse, targeting both either sequentially or simultaneously could have the potential to reverse this immune-exhausted state (Kong et al. 2016; Lichtenegger et al. 2018; Armand et al. 2019; Toffalori et al. 2019; Williams et al. 2019). The expression of TIM-3 on leukemic stem cells in particular makes TIM-3 a promising target in AML (Kikushige et al. 2010; Haubner et al. 2019).

MBG453 (sabatolimab) is an anti-TIM-3 monoclonal antibody currently being explored in clinical trials in combination with hypomethylating agents and/or venetoclax in AML, MDS, and CMML (NCT04150029 and NCT03946670) (Zeidan et al. 2020a). Data from a phase I trial that combined MBG453 with decitabine or azacitidine in patients with MDS, CMML, and AML showed response rates ranging from 40.0% among newly diagnosed AML patients to 56.9% for MDS patients (Brunner et al. 2020; Brunner et al. 2021). Grade 3 or higher treatment-emergent adverse events were primarily hematologic with thrombocytopenia (43.4%–45.8%), neutropenia (47.2%–50.0%), and febrile neutropenia (29.2%–35.8%) in both cohorts. Bispecific antibodies and chimeric antigen receptor (CAR) T cells targeting both TIM-3 and other leukemic stem cell markers such as CD13, CLL1, and CD33 have also been tested preclinically and could enable the eradication of leukemic stem cells while minimizing on-target, off-leukemia side-effects (Haubner et al. 2019; He et al. 2020). However, these agents have only been tested in vitro to date.

The role of the innate immune system in the antitumor immune response has received increasing attention recently, with studies showing the role of CD47 in the immune evasion of leukemic cells. CD47 is overexpressed on AML blasts and leukemic stem cells and has been associated with adverse outcomes in AML and MDS patients (Majeti et al. 2009; Chao et al. 2020). Via interaction with SIRPα on macrophages, CD47 functions as a "do not eat me" signal that protects leukemic cells from phagocytosis (Russ et al. 2018). Several antibodies targeting anti-CD47 (e.g., CC-90002, Hu5F9-G4 [magrolimab], and TTI-621) have been developed and showed only limited efficacy when used as single agents (Petrova et al. 2017; Zeidan et al. 2019b, 2022). However, the addition of azacitidine to magrolimab has been shown to significantly enhance the therapeutic efficacy with ORR of 91% (42% CR) and 64% (40% CR) in newly diagnosed MDS and AML patients, respectively (Sallman et al. 2020a). While not specifically directed against *TP53* mutations, combining magrolimab with azacitidine also showed significant efficacy in *TP53* mutant AML patients with a 75% ORR (Sallman et al. 2020a). Treatment was generally well tolerated, with anemia (38%; an expected on-target adverse event due to expression of CD47 on erythrocytes), fatigue (21%), neutropenia (19%), and thrombocytopenia (18%) being the most common adverse events (Sallman et al. 2020a). However, these promising results need to be validated in the ongoing randomized placebo-controlled phase III trial (NCT04313881). Besides targeting CD47, ablation of the AXL receptor in macrophages has been shown to enable NK cell- and T-cell-mediated antitumor immune responses and to restore sensitivity to PD1 inhibition in preclinical leukemia models (Tirado-Gonzalez et al. 2021).

*Bispecific antibodies*

Bispecific antibodies constitute a heterogenous class of antibody constructs that simultaneously bind CD3 on T cells and another specific tumor-associated antigen on the surface of tumor cells, leading to an HLA-independent, endogenous immune response against the target cell (Huehls et al. 2015). This has the advantage of minimizing off-target toxicity but also inducing an antitumor response independent of MHC-I expression on tumor cells or costimulatory signals (Dreier et al. 2002; Brischwein et al. 2007; Huehls et al. 2015).

The first approval for this novel class of therapeutics was granted to the anti-CD3/CD19 bispecific T-cell engager (BiTE) blinatumomab in acute lymphoblastic leukemia (ALL) (Kantarjian et al. 2017; Gökbuget et al. 2018). In contrast to ALL, target antigen selection in AML is more difficult given the expression of potential targets on both leukemic cells and normal hematopoietic stem and progenitor cells. Several potential therapeutic targets for bispecific antibodies, including CD33, CD123, and CLEC12A, have been explored in AML, but early constructs had limited efficacy in clinical trials (Clark and Stein 2020; Slade and Uy 2020). As a comprehensive review of the various different constructs would be beyond the scope of this review, we refer the reader to a recent review on T-cell-based therapies in AML (Daver et al. 2021).

AMG 330 is an anti-CD3/CD33 BiTE that has been studied in a phase I trial (NCT02520427) that enrolled 55 patients with R/R AML and demonstrated objective responses in eight out of 42 evaluable patients (19.1%; four patients with CR), with 67% of patients experiencing cytokine release syndrome (grade 3 or higher in 13%) (Ravandi et al. 2020b). Several other anti-CD3/CD33 BiTE constructs (e.g., AMG 673 and AMV 564) tested in early stage clinical trials in patients with R/R AML showed acceptable safety and some antileukemic efficacy (Subklewe et al. 2019; Westervelt et al. 2019). These latter agents offer the advantage of an extended half-life compared with AMG 330, which requires a continuous infusion. Preclinical studies showed that AMG 330 led to up-regulation of PD-L1 on primary AML cells, which could represent an escape mechanism by tumor cells, underlying the limited response rates seen in clinical trials thus far. As addition of PD-1/PD-L1 blockade was able to restore T-cell proliferation and AMG 330-mediated leukemic cell lysis preclinically, combination treatment of anti-PD-1/PD-L1 antibodies and BiTEs could be a potent therapeutic option that is currently being explored in a clinical trial (NCT04478695) (Krupka et al. 2016).

CD123 is expressed on both normal hematopoietic stem and progenitor cells but also on AML blasts and leukemic stem cells (Ehninger et al. 2014). Among the various anti-CD123 targeted bispecific antibodies, flotetuzumab, an anti-CD3/CD123 dual-affinity retargeting antibody, is the furthest advanced in clinical development (Uy et al. 2021). Flotetuzumab has been studied in 88 patients with R/R AML in a phase I/II study and showed an ORR of 30.0% among patients with primary induction failure or relapse within 6 mo as well as 24% in the total, unselected patient cohort (Uy et al. 2021). Given the otherwise very limited prognosis in patients with induction failure or early relapse, a 6- and 12-mo OS estimate of 75% and 50%, respectively, among responding patients appears encouraging (Uy et al. 2021). Correlative studies from this trial also revealed up-regulation of inhibitory immune checkpoints following treatment with flotetuzumab, which highlights the immunosuppressive microenvironment in the bone marrow and provides the rationale for combination of flotetuzumab with PD-1/PD-L1-directed therapies (Vadakekolathu et al. 2020b). The investigators also identified specific interferon-γ-related gene signatures as a potential biomarker for response to flotetuzumab that could help with patient selection based on response likelihood (Vadakekolathu et al. 2020b). Vibecotamab is another anti-CD3/CD123 BiTE that has demonstrated dose-dependent antileukemic activity in a phase I study of 104 R/R AML patients, with 14% of patients responding at the higher dose level with frequent but manageable cytokine release syndrome (85% grades 1–2, 15% grade 3 or higher) (Ravandi et al. 2020a).

While early results with bispecific antibodies appear promising, several challenges pertaining to antigen target selection, dose/schedule optimization, and management of toxicities (e.g., cytokine release and neurotoxicity) remain. Additionally, resistance mechanisms such as the up-regulation of inhibitory immune checkpoint or antigen loss warrant further exploration.

*Chimeric antigen receptor (CAR) T-cell therapy*

CAR T cells have been approved for various advanced B-cell malignancies (Park et al. 2018; Schuster et al. 2019). In contrast to bispecific antibodies, CAR T cells are genetically modified autologous T cells that expand and persist after infusion to induce both short- and long-term antileukemic immune response. However, the development of CAR T cells in AML has been hindered by the lack of a tumor-specific antigen and the clonal heterogeneity of AML (Daver et al. 2021). Several early phase clinical trials targeting CD33, CD123, or NKG2D are ongoing and objective responses have been reported, but the data available currently are too immature and limited to draw any definitive conclusions (Liu et al. 2018; Sallman et al. 2018). Multiple efforts to improve the efficacy of CAR T cells in AML are ongoing and include the identification of alternative target antigens, the development of compound or bispecific and split CAR T cells that target two distinct antigens, combination with azacitidine, and gene-editing strategies using CRISPR–Cas9 to reduce on-target, off-leukemia toxicity (Kim et al. 2018; Liu et al. 2018; El Khawanky et al. 2019; Leick et al. 2019; He et al. 2020). However, these efforts remain in the preclinical space at this point.

*Neoantigens and tumor vaccines*

Neoantigens are derived from cancer-associated aberrant proteins that are presented bound to human leukocyte

antigen (HLA) molecules on the cell surface and elicit an antitumor T-cell response (Biernacki and Bleakley 2020). Identification of AML-associated neoantigens could therefore provide a new target for immunotherapies. Compared with various solid malignancies, AML has a low tumor mutational burden, which may be associated with a relatively lower number of candidate neoantigens (Chalmers et al. 2017). However, subsets of AML patients are characterized by recurrent fusions and mutations in RNA processing machinery, which may serve as neoantigens (although their immunogenicity remains to be systematically assessed) (Biernacki and Bleakley 2020). The neoantigen burden in genetically defined AML subsets is being increasingly elucidated, with the most data available for patients with *NPM1* mutations and fusions such as *MLL-KMT2A*, *CBFB-MYH11*, or *PML-RARA* (Meyer et al. 2018; van der Lee et al. 2019; Biernacki et al. 2020). For example, mutant NPM1, when presented on HLA-A*02:01 on leukemic blasts, has been shown to elicit a $CD8^+$ T-cell response in vitro. Similar data exist for *CBFB-MYH11,* which results in an immunogenic fusion protein in *HLA-B*40:01*$^+$ donors. However, whether this could offer a targeted immunotherapy option in genetically defined subsets of AML patients requires additional studies, and whether these advances could pave the way for vaccine therapy in AML is an area of ongoing research (Berlin et al. 2015; Rosenblatt et al. 2016; Pearlman et al. 2021).

Pharmacologic modulation of RNA splicing has also been shown to generate immunogenic neoantigen peptides that are presented on MHC-I and elicit a $CD8^+$ T-cell response (Lu et al. 2021). Furthermore, combination of the splicing modulator indisulam and an anti-PD1 antibody was able to induce an antitumor T-cell response in murine solid tumor syngeneic models (Lu et al. 2021). As mutations in genes involved in splicing regulation such as *SF3B1* are frequently encountered in MDS and AML, the possibility of RNA splicing factor mutation-induced neoantigens is intriguing. While data from MDS and AML patients are limited, *SF3B1* mutations have been associated with the generation of putative neoantigens in myeloproliferative neoplasm as well as uveal melanoma, which could serve as potential targets for immunotherapy (Schischlik et al. 2019; Bigot et al. 2021). Evaluation of these fusion and RNA-derived neoantigens for therapeutic targeting of MDS and AML will be an important area of research in the near future.

## Conclusion and future directions

Thanks to technological advances and wider availability, individualized treatment selection based on genetic and molecular features is increasingly becoming routine clinical practice. While only *FLT3*, *IDH1*, and *IDH2* mutations are currently targetable by specific small molecule inhibitors, several other compounds targeting dependencies caused by mutations in RNA splicing factors, *TET2*, *RUNX1*, and *ASXL1* might become available in the future and expand the proportion of AML and MDS patients eligible for targeted therapies. Additionally, a multitude of antibody and cellular therapies are in various stages of clinical development, including randomized phase III trials that could lead to the approval of a new class of therapeutic agents in myeloid malignancies.

Although the rate of early mortality with AML induction chemotherapy has been declining over the last decades, a substantial subset of older patients with AML remains untreated due to concerns for treatment-related toxicity. Indeed, early mortality with cytarabine/anthracycline-based induction chemotherapy has been reported to be >10% in population-based studies (Zeidan et al. 2019c, 2020b). With the introduction of venetoclax-based therapies, an effective, lower-intensity option has become available for older AML patients, but responses are time-limited, and myelosuppression remains a concern that may preclude use of this regimen in frail, very elderly patients (DiNardo et al. 2020a). Therefore, the development of well-tolerated and effective novel therapies in both the frontline and R/R setting is still needed. Such targeted and safe therapies are especially important when given as maintenance therapy following induction chemotherapy or allo-HCT or pre-emptively in patients with clonal hematopoiesis of indeterminate potential (CHIP). Recent studies with the multikinase inhibitor sorafenib have demonstrated an OS benefit of sorafenib maintenance therapy after allo-HCT, but treatment discontinuation due to adverse events remains a concern (Burchert et al. 2020; Xuan et al. 2020). Several other agents, including FLT3 inhibitors and venetoclax, are currently being evaluated in randomized clinical trials in this setting as well (Bewersdorf et al. 2021b).

An additional important area of future research will be potential preventive approaches against AML, with the increasing recognition of CHIP and its risk of progression to MDS and AML (Genovese et al. 2014; Jaiswal et al. 2017). CHIP refers to the presence of somatic mutations in genes associated with myeloid neoplasms in otherwise healthy individuals (Steensma et al. 2015). While certain mutations (e.g., *TP53*) and the presence of cytopenias are associated with a higher risk of evolution into an overt myeloid neoplasm, genes involved in epigenetic processes (e.g., *TET2*, *DNMT3A*, and *ASXL1*) are most frequently encountered in CHIP (Malcovati et al. 2017; Sperling et al. 2017; Desai et al. 2018). As peripheral blood-based next-generation sequencing is becoming increasingly performed for other indications (e.g., patients with solid tumors), CHIP is likely going to become a more prevalent clinical scenario in the future, and it will be interesting to determine whether preemptive therapy can prevent the development of overt myeloid neoplasms. For example, patients with *IDH2* mutations have a very high risk of eventually developing AML but could benefit from suppression of clonal evolution by treatment with enasidenib (Desai et al. 2018). This exact concept is currently being evaluated in a multicenter, phase I clinical trial (NCT05102370) in patients with IDH2 mutant clonal cytopenia of undetermined significance (CCUS). Other open questions that could be worth exploring are whether treatment of chronic inflammation or use of vitamin C can delay or even abrogate development of myeloid neoplasms (Cimmino et al. 2017; Jaiswal et al. 2017).

In addition to careful selection of high-risk patients who are most likely to benefit from preventive treatment, such agents would need to have limited side-effects given their prolonged use in (currently) healthy individuals.

## Competing interest statement

O.A.-W. has served as a consultant for H3B Biomedicine; Foundation Medicine, Inc.; Merck; Prelude Therapeutics; and Janssen, and is on the Scientific Advisory Board of Envisagenics, Inc.; AIChemy; Harmonic Discovery, Inc.; and Pfizer Boulder. O.A.-W. has received prior research funding from H3B Biomedicine and LOXO Oncology unrelated to the current manuscript. J.P.B. declares no competing interests.

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## References

Amatangelo MD, Quek L, Shih A, Stein EM, Roshal M, David MD, Marteyn B, Farnoud NR, de Botton S, Bernard OA, et al. 2017. Enasidenib induces acute myeloid leukemia cell differentiation to promote clinical response. *Blood* **130:** 732–741. doi:10.1182/blood-2017-04-779447

Andersson AK, Ma J, Wang J, Chen X, Gedman AL, Dang J, Nakitandwe J, Holmfeldt L, Parker M, Easton J, et al. 2015. The landscape of somatic mutations in infant MLL-rearranged acute lymphoblastic leukemias. *Nat Genet* **47:** 330–337. doi:10.1038/ng.3230

Andreeff M, Ruvolo V, Gadgil S, Zeng C, Coombes K, Chen W, Kornblau S, Barón AE, Drabkin HA. 2008. HOX expression patterns identify a common signature for favorable AML. *Leukemia* **22:** 2041–2047. doi:10.1038/leu.2008.198

Armand P, Zinzani PLL, Palcza J, Healy JA, Nahar A, Marinello P, Gregory GP. 2019. Phase 1–2 study of pembrolizumab combined with the anti-LAG-3 antibody MK-4280 for the treatment of hematologic malignancies. *Blood* **134:** 1548. doi:10.1182/blood-2019-127474

Asada S, Goyama S, Inoue D, Shikata S, Takeda R, Fukushima T, Yonezawa T, Fujino T, Hayashi Y, Kawabata KC, et al. 2018. Mutant ASXL1 cooperates with BAP1 to promote myeloid leukaemogenesis. *Nat Commun* **9:** 2733. doi:10.1038/s41467-018-05085-9

Assi R, Kantarjian HM, Kadia TM, Pemmaraju N, Jabbour E, Jain N, Daver N, Estrov Z, Uehara T, Owa T, et al. 2018. Final results of a phase 2, open-label study of indisulam, idarubicin, and cytarabine in patients with relapsed or refractory acute myeloid leukemia and high-risk myelodysplastic syndrome. *Cancer* **124:** 2758–2765. doi:10.1002/cncr.31398

Balasubramani A, Larjo A, Bassein JA, Chang X, Hastie RB, Togher SM, Lähdesmäki H, Rao A. 2015. Cancer-associated ASXL1 mutations may act as gain-of-function mutations of the ASXL1–BAP1 complex. *Nat Commun* **6:** 7307. doi:10.1038/ncomms8307

Bejar R, Lord A, Stevenson K, Bar-Natan M, Pérez-Ladaga A, Zaneveld J, Wang H, Caughey B, Stojanov P, Getz G, et al. 2014. TET2 mutations predict response to hypomethylating agents in myelodysplastic syndrome patients. *Blood* **124:** 2705–2712. doi:10.1182/blood-2014-06-582809

Bejar R, Papaemmanuil E, Haferlach T, Garcia-Manero G, Maciejewski JP, Sekeres MA, Walter MJ, Graubert TA, Cazzola M, Malcovati L, et al. 2015. Somatic mutations in MDS patients are associated with clinical features and predict prognosis independent of the IPSS-R: analysis of combined datasets from the international working group for prognosis in MDS-molecular committee. *Blood* **126:** 907. doi:10.1182/blood.V126.23.907.907

Berger R, Rotem-Yehudar R, Slama G, Landes S, Kneller A, Leiba M, Koren-Michowitz M, Shimoni A, Nagler A. 2008. Phase I safety and pharmacokinetic study of CT-011, a humanized antibody interacting with PD-1, in patients with advanced hematologic malignancies. *Clin Cancer Res* **14:** 3044–3051. doi:10.1158/1078-0432.CCR-07-4079

Berlin C, Kowalewski DJ, Schuster H, Mirza N, Walz S, Handel M, Schmid-Horch B, Salih HR, Kanz L, Rammensee HG, et al. 2015. Mapping the HLA ligandome landscape of acute myeloid leukemia: a targeted approach toward peptide-based immunotherapy. *Leukemia* **29:** 647–659. doi:10.1038/leu.2014.233

Bewersdorf JP, Shallis RM, Zeidan AM. 2020. Immune checkpoint inhibition in myeloid malignancies: moving beyond the PD-1/PD-L1 and CTLA-4 pathways. *Blood Rev* **45:** 100709. doi:10.1016/j.blre.2020.100709

Bewersdorf JP, Derkach A, Gowda L, Menghrajani K, DeWolf S, Ruiz JD, Ponce DM, Shaffer BC, Tamari R, Young JW, et al. 2021a. Venetoclax-based combinations in AML and high-risk MDS prior to and following allogeneic hematopoietic cell transplant. *Leuk Lymphoma* **62:** 3394–3401. doi: 10.1080/10428194.2021.1966788

Bewersdorf JP, Tallman MS, Stahl M. 2021b. Maintenance therapies in acute myeloid leukemia: the renaissance of an old therapeutic concept. *Curr Opin Oncol* **33:** 658–669. doi:10.1097/CCO.0000000000000778

Biernacki MA, Bleakley M. 2020. Neoantigens in hematologic malignancies. *Front Immunol* **11:** 121. doi:10.3389/fimmu.2020.00121

Biernacki MA, Foster KA, Woodward KB, Coon ME, Cummings C, Cunningham TM, Dossa RG, Brault M, Stokke J, Olsen TM, et al. 2020. CBFB-MYH11 fusion neoantigen enables T cell recognition and killing of acute myeloid leukemia. *J Clin Invest* **130:** 5127–5141. doi:10.1172/JCI137723

Bigot J, Lalanne AI, Lucibello F, Gueguen P, Houy A, Dayot S, Ganier O, Gilet J, Tosello J, Nemati F, et al. 2021. Splicing patterns in *SF3B1*-mutated uveal melanoma generate shared immunogenic tumor-specific neoepitopes. *Cancer Discov* **11:** 1938–1951. doi:10.1158/2159-8290.CD-20-0555

Bill M, Mrózek K, Kohlschmidt J, Eisfeld A-K, Walker CJ, Nicolet D, Papaioannou D, Blachly JS, Orwick S, Carroll AJ, et al. 2020. Mutational landscape and clinical outcome of patients with de novo acute myeloid leukemia and rearrangements involving 11q23/*KMT2A*. *Proc Natl Acad Sci* **117:** 26340–26346. doi:10.1073/pnas.2014732117

Boddu P, Kantarjian H, Garcia-Manero G, Allison J, Sharma P, Daver N. 2018. The emerging role of immune checkpoint based approaches in AML and MDS. *Leuk Lymphoma* **59:** 790–802. doi:10.1080/10428194.2017.1344905

Borkin D, He S, Miao H, Kempinska K, Pollock J, Chase J, Purohit T, Malik B, Zhao T, Wang J, et al. 2015. Pharmacologic inhibition of the menin–MLL interaction blocks progression of MLL leukemia in vivo. *Cancer Cell* **27:** 589–602. doi:10.1016/j.ccell.2015.02.016

Brischwein K, Parr L, Pflanz S, Volkland J, Lumsden J, Klinger M, Locher M, Hammond SA, Kiener P, Kufer P, et al. 2007. Strictly target cell-dependent activation of T cells by bispecific single-chain antibody constructs of the BiTE class. *J Immunother* **30:** 798–807. doi:10.1097/CJI.0b013e318156750c

Brunner AM, Esteve J, Porkka K, Knapper S, Vey N, Scholl S, Garcia-Manero G, Wermke M, Janssen J, Traer E, et al. 2020. Efficacy and safety of sabatolimab (MBG453) in combination with hypomethylating agents (HMAs) in patients with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (HR-MDS): updated results from a phase 1b study. *Blood* **136:** 1–2. doi:10.1182/blood-2020-136855

Brunner A, Esteve J, Porkka K, Knapper S, Traer E, Scholl S, Garcia-Manero G, Vey N, Wermke M, Janssen J, et al. 2021. Efficacy and safety of sabatolimab (MBG453) in combination with hypomethylating agents (HMAs) in patients (Pts) with very high/High-Risk myelodysplastic syndrome (vHR/HR-MDS) and acute myeloid leukemia (AML): final analysis from a phase Ib study (Abstract 244). *Blood* **138:** 244. doi:10.1182/blood-2021-146039

Burchert A, Bug G, Fritz LV, Finke J, Stelljes M, Röllig C, Wollmer E, Wäsch R, Bornhäuser M, Berg T, et al. 2020. Sorafenib maintenance after allogeneic hematopoietic stem cell transplantation for acute myeloid leukemia with *FLT3*-internal tandem duplication mutation (SORMAIN). *J Clin Oncol* **38:** 2993–3002. doi:10.1200/JCO.19.03345

Busque L, Patel JP, Figueroa ME, Vasanthakumar A, Provost S, Hamilou Z, Mollica L, Li J, Viale A, Heguy A, et al. 2012. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet* **44:** 1179–1181. doi:10.1038/ng.2413

Caligiuri MA, Strout MP, Lawrence D, Arthur DC, Baer MR, Yu F, Knuutila S, Mrozek K, Oberkircher AR, Marcucci G, et al. 1998. Rearrangement of ALL1 (MLL) in acute myeloid leukemia with normal cytogenetics. *Cancer Res* **58:** 55–59.

The Cancer Genome Atlas Research Network. 2013. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med* **368:** 2059–2074. doi:10.1056/NEJMoa1301689

Caravella JA, Lin J, Diebold RB, Campbell AM, Ericsson A, Gustafson G, Wang Z, Castro J, Clarke A, Gotur D, et al. 2020. Structure-based design and identification of FT-2102 (olutasidenib), a potent mutant-selective IDH1 inhibitor. *J Med Chem* **63:** 1612–1623. doi:10.1021/acs.jmedchem.9b01423

Castaigne S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie JN, Legrand O, Thomas X, Turlure P, Reman O, et al. 2012. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. *Lancet* **379:** 1508–1516. doi:10.1016/S0140-6736(12)60485-1

Chalmers ZR, Connelly CF, Fabrizio D, Gay L, Ali SM, Ennis R, Schrock A, Campbell B, Shlien A, Chmielecki J, et al. 2017. Analysis of 100,000 human cancer genomes reveals the landscape of tumor mutational burden. *Genome Med* **9:** 34. doi:10.1186/s13073-017-0424-2

Chan SM, Thomas D, Corces-Zimmerman MR, Xavy S, Rastogi S, Hong WJ, Zhao F, Medeiros BC, Tyvoll DA, Majeti R. 2015. Isocitrate dehydrogenase 1 and 2 mutations induce BCL-2 dependence in acute myeloid leukemia. *Nat Med* **21:** 178–184. doi:10.1038/nm.3788

Chandrasekharappa SC, Guru SC, Manickam P, Olufemi SE, Collins FS, Emmert-Buck MR, Debelenko LV, Zhuang Z, Lubensky IA, Liotta LA, et al. 1997. Positional cloning of the gene for multiple endocrine neoplasia-type 1. *Science* **276:** 404–407. doi:10.1126/science.276.5311.404

Chao MP, Takimoto CH, Feng DD, McKenna K, Gip P, Liu J, Volkmer J-P, Weissman IL, Majeti R. 2020. Therapeutic targeting of the macrophage immune checkpoint CD47 in myeloid malignancies. *Front Oncol* **9:** 1380. doi:10.3389/fonc.2019.01380

Chaturvedi A, Herbst L, Pusch S, Klett L, Goparaju R, Stichel D, Kaulfuss S, Panknin O, Zimmermann K, Toschi L, et al. 2017. Pan-mutant-IDH1 inhibitor BAY1436032 is highly effective against human IDH1 mutant acute myeloid leukemia in vivo. *Leukemia* **31:** 2020–2028. doi:10.1038/leu.2017.46

Chaturvedi A, Gupta C, Gabdoulline R, Borchert NM, Goparaju R, Kaulfuss S, Görlich K, Schottmann R, Othman B, Welzenbach J, et al. 2021. Synergistic activity of IDH1 inhibitor BAY1436032 with azacitidine in IDH1 mutant acute myeloid leukemia. *Haematologica* **106:** 565–573. doi:10.3324/haematol.2019.236992

Chen YX, Yan J, Keeshan K, Tubbs AT, Wang H, Silva A, Brown EJ, Hess JL, Pear WS, Hua X. 2006. The tumor suppressor menin regulates hematopoiesis and myeloid transformation by influencing Hox gene expression. *Proc Natl Acad Sci* **103:** 1018–1023. doi:10.1073/pnas.0510347103

Chen S, Benbarche S, Abdel-Wahab O. 2021. Splicing factor mutations in hematologic malignancies. *Blood* **138:** 599–612. doi:10.1182/blood.2019004260

Chien KS, Borthakur G, Naqvi K, Daver N, Montalban Bravo G, Cortes JE, DiNardo CD, Jabbour E, Alvarado Y, Andreeff M, et al. 2020. Final results from a phase II study combining azacitidine and pembrolizumab in patients with higher-risk pyelodysplastic syndrome after failure of hypomethylating agent therapy. *Blood* **136:** 23–24. doi:10.1182/blood-2020-141100

Chien KS, Kim K, Nogueras-Gonzalez GM, Borthakur G, Naqvi K, Daver NG, Montalban-Bravo G, Cortes JE, DiNardo CD, Jabbour E, et al. 2021. Phase II study of azacitidine with pembrolizumab in patients with intermediate-1 or higher-risk myelodysplastic syndrome. *Br J Haematol* **195:** 378–387. doi:10.1111/bjh.17689

Choe S, Wang H, DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, Watts JM, Pollyea DA, et al. 2020. Molecular mechanisms mediating relapse following ivosidenib monotherapy in IDH1-mutant relapsed or refractory AML. *Blood Adv* **4:** 1894–1905. doi:10.1182/bloodadvances.2020001503

Cimmino L, Dolgalev I, Wang Y, Yoshimi A, Martin GH, Wang J, Ng V, Xia B, Witkowski MT, Mitchell-Flack M, et al. 2017. Restoration of TET2 function blocks aberrant self-renewal and leukemia progression. *Cell* **170:** 1079–1095.e20. doi:10.1016/j.cell.2017.07.032

Clark MC, Stein A. 2020. CD33 directed bispecific antibodies in acute myeloid leukemia. *Best Pract Res Clin Haematol* **33:** 101224. doi:10.1016/j.beha.2020.101224

Cortes JE, Heidel FH, Hellmann A, Fiedler W, Smith BD, Robak T, Montesinos P, Pollyea DA, DesJardins P, Ottmann O, et al. 2019a. Randomized comparison of low dose cytarabine with or without glasdegib in patients with newly diagnosed acute myeloid leukemia or high-risk myelodysplastic syndrome. *Leukemia* **33:** 379–389. doi:10.1038/s41375-018-0312-9

Cortes JE, Wang ES, Watts JM, Lee S, Baer MR, Dao K-H, Dinner S, Yang J, Donnellan WB, Schwarer AP, et al. 2019b. Olutasidenib (FT-2102) induces rapid remissions in patients with IDH1-mutant myelodysplastic syndrome: results of phase 1/2 single agent treatment and combination with azacitidine. *Blood* **134:** 674. doi:10.1182/blood-2019-124360

Dafflon C, Craig VJ, Méreau H, Gräsel J, Schacher Engstler B, Hoffman G, Nigsch F, Gaulis S, Barys L, Ito M, et al. 2017. Complementary activities of DOT1L and menin inhibitors in MLL-rearranged leukemia. *Leukemia* **31:** 1269–1277. doi:10.1038/leu.2016.327

Daver N, Garcia-Manero G, Basu S, Boddu PC, Alfayez M, Cortes JE, Konopleva M, Ravandi-Kashani F, Jabbour E, Kadia T, et al. 2019. Efficacy, safety, and biomarkers of response to azacitidine and nivolumab in relapsed/refractory acute myeloid leukemia: a nonrandomized, open-label, phase II study. *Cancer Discov* **9:** 370–383. doi:10.1158/2159-8290.CD-18-0774

Daver N, Altman JK, Maly J, Levis M, Ritchie E, Litzow M, McCloskey JK, Smith CC, Schiller GJ, Bradley T, et al. 2020. Efficacy and safety of venetoclax in combination with gilteritinib for relapsed/refractory *FLT3*-mutated acute myeloid leukemia in the expansion cohort of a phase 1b study. *Blood* **136:** 20–22. doi:10.1182/blood-2020-139705

Daver N, Alotaibi AS, Bücklein V, Subklewe M. 2021. T-cell-based immunotherapy of acute myeloid leukemia: current concepts and future developments. *Leukemia* **35:** 1843–1863. doi:10.1038/s41375-021-01253-x

Davids MS, Kim HT, Bachireddy P, Costello C, Liguori R, Savell A, Lukez AP, Avigan D, Chen YB, McSweeney P, et al. 2016. Ipilimumab for patients with relapse after allogeneic transplantation. *N Engl J Med* **375:** 143–153. doi:10.1056/NEJMoa1601202

Delhommeau F, Dupont S, Valle VD, James C, Trannoy S, Massé A, Kosmider O, Le Couedic J-P, Robert F, Alberdi A, et al. 2009. Mutation in *TET2* in myeloid cancers. *N Engl J Med* **360:** 2289–2301. doi:10.1056/NEJMoa0810069

Desai P, Mencia-Trinchant N, Savenkov O, Simon MS, Cheang G, Lee S, Samuel M, Ritchie EK, Guzman ML, Ballman KV, et al. 2018. Somatic mutations precede acute myeloid leukemia years before diagnosis. *Nat Med* **24:** 1015–1023. doi:10.1038/s41591-018-0081-z

DiNardo CD, Stein EM, de Botton S, Roboz GJ, Altman JK, Mims AS, Swords R, Collins RH, Mannis GN, Pollyea DA, et al. 2018. Durable remissions with Ivosidenib in *IDH1*-mutated relapsed or refractory AML. *N Engl J Med* **378:** 2386–2398. doi:10.1056/NEJMoa1716984

DiNardo CD, Jonas BA, Pullarkat V, Thirman MJ, Garcia JS, Wei AH, Konopleva M, Döhner H, Letai A, Fenaux P, et al. 2020a. Azacitidine and venetoclax in previously untreated acute myeloid leukemia. *N Engl J Med* **383:** 617–629. doi:10.1056/NEJMoa2012971

DiNardo CD, Maiti A, Rausch CR, Pemmaraju N, Naqvi K, Daver NG, Kadia TM, Borthakur G, Ohanian M, Alvarado Y, et al. 2020b. 10-day decitabine with venetoclax for newly diagnosed intensive chemotherapy ineligible, and relapsed or refractory acute myeloid leukaemia: a single-centre, phase 2 trial. *Lancet Haematol* **7:** e724–e736. doi:10.1016/S2352-3026(20)30210-6

Dinardo CD, Schuh AC, Stein EM, Montesinos P, Wei A, Botton SD, Zeidan AM, Fathi AT, Quek L, Kantarjian HM, et al. 2020c. Effect of enasidenib (ENA) plus azacitidine (AZA) on complete remission and overall response versus AZA monotherapy in mutant-*IDH2* (*mIDH2*) newly diagnosed acute myeloid leukemia (ND-AML). *J Clin Oncol* **38:** 7501. doi:10.1200/JCO.2020.38.15_suppl.7501

DiNardo CD, Tiong IS, Quaglieri A, MacRaild S, Loghavi S, Brown FC, Thijssen R, Pomilio G, Ivey A, Salmon JM, et al. 2020d. Molecular patterns of response and treatment failure after frontline venetoclax combinations in older patients with AML. *Blood* **135:** 791–803. doi:10.1182/blood.2019003988

DiNardo CD, Lachowiez CA, Takahashi K, Loghavi S, Xiao L, Kadia T, Daver N, Adeoti M, Short NJ, Sasaki K, et al. 2021a. Venetoclax combined with Flag-IDA induction and consolidation in newly diagnosed and relapsed or refractory acute myeloid leukemia. *J Clin Oncol* **39:** 2768–2778. doi:10.1200/JCO.20.03736

DiNardo CD, Stein AS, Stein EM, Fathi AT, Frankfurt O, Schuh AC, Döhner H, Martinelli G, Patel PA, Raffoux E, et al. 2021b. Mutant isocitrate dehydrogenase 1 inhibitor ivosidenib in combination with azacitidine for newly diagnosed acute myeloid leukemia. *J Clin Oncol* **39:** 57–65. doi:10.1200/JCO.20.01632

Döhner H, Estey E, Grimwade D, Amadori S, Appelbaum FR, Büchner T, Dombret H, Ebert BL, Fenaux P, Larson RA, et al. 2017. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood* **129:** 424–447. doi:10.1182/blood-2016-08-733196

Dreier T, Lorenczewski G, Brandl C, Hoffmann P, Syring U, Hanakam F, Kufer P, Riethmuller G, Bargou R, Baeuerle PA. 2002. Extremely potent, rapid and costimulation-independent cytotoxic T-cell response against lymphoma cells catalyzed by a single-chain bispecific antibody. *Int J Cancer* **100:** 690–697. doi:10.1002/ijc.10557

Dzama MM, Steiner M, Rausch J, Sasca D, Schönfeld J, Kunz K, Taubert MC, McGeehan GM, Chen CW, Mupo A, et al. 2020. Synergistic targeting of *FLT3* mutations in AML via combined menin–MLL and FLT3 inhibition. *Blood* **136:** 2442–2456. doi:10.1182/blood.2020005037

Ehninger A, Kramer M, Röllig C, Thiede C, Bornhäuser M, von Bonin M, Wermke M, Feldmann A, Bachmann M, Ehninger G, et al. 2014. Distribution and levels of cell surface expression of CD33 and CD123 in acute myeloid leukemia. *Blood Cancer J* **4:** e218. doi:10.1038/bcj.2014.39

El Khawanky N, Hughes A, Yu W, Taromi S, Clarson J, Lopez AF, Brown MP, Duyster J, Hughes TP, White DL, et al. 2019. Azacytidine sensitizes AML cells for effective elimination by CD123 CAR T-cells. *Blood* **134:** 3904. doi:10.1182/blood-2019-124684

El Marabti E, Abdel-Wahab O. 2021. Therapeutic modulation of RNA splicing in malignant and non-malignant disease. *Trends Mol Med* **27:** 643–659. doi:10.1016/j.molmed.2021.04.005

Fong JY, Pignata L, Goy PA, Kawabata KC, Lee SC, Koh CM, Musiani D, Massignani E, Kotini AG, Penson A, et al. 2019. Therapeutic targeting of RNA splicing catalysis through inhibition of protein arginine methylation. *Cancer Cell* **36:** 194–209.e9. doi:10.1016/j.ccell.2019.07.003

Genovese G, Kähler AK, Handsaker RE, Lindberg J, Rose SA, Bakhoum SF, Chambert K, Mick E, Neale BM, Fromer M, et al. 2014. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med* **371:** 2477–2487. doi:10.1056/NEJMoa1409405

Gökbuget N, Dombret H, Bonifacio M, Reichle A, Graux C, Faul C, Diedrich H, Topp MS, Brüggemann M, Horst H-A, et al. 2018. Blinatumomab for minimal residual disease in adults with B-cell precursor acute lymphoblastic leukemia. *Blood* **131:** 1522–1531. doi:10.1182/blood-2017-08-798322

Gros FX, Cazaubiel T, Forcade E, Lechevalier N, Leguay T, Servant V, Tabrizi R, Clement L, Dumas PY, Bidet A, et al. 2017. Severe acute GvHD following administration of ipilimumab for early relapse of AML after haploidentical stem cell transplantation. *Bone Marrow Transplant* **52:** 1047–1048. doi:10.1038/bmt.2017.78

Guan Y, Tiwari AD, Phillips JG, Hasipek M, Grabowski DR, Pagliuca S, Gopal P, Kerr CM, Adema V, Radivoyevitch T, et al. 2021. A therapeutic strategy for preferential targeting of *TET2* mutant and TET-dioxygenase deficient cells in myeloid neoplasms. *Blood Cancer Discov* **2:** 146–161. doi:10.1158/2643-3230.BCD-20-0173

Han T, Goralski M, Gaskill N, Capota E, Kim J, Ting TC, Xie Y, Williams NS, Nijhawan D. 2017. Anticancer sulfonamides target splicing by inducing RBM39 degradation via recruitment to DCAF15. *Science* **356:** eaal3755. doi:10.1126/science.aal3755

Haubner S, Perna F, Köhnke T, Schmidt C, Berman S, Augsberger C, Schnorfeil FM, Krupka C, Lichtenegger FS, Liu X, et al. 2019. Coexpression profile of leukemic stem cell markers for combinatorial targeted therapy in AML. *Leukemia* **33:** 64–74. doi:10.1038/s41375-018-0180-3

He X, Feng Z, Ma J, Ling S, Cao Y, Gurung B, Wu Y, Katona BW, O'Dwyer KP, Siegel DL, et al. 2020. Bi-specific and split CAR T cells targeting CD13 and TIM3 eradicate acute myeloid leukemia. *Blood* **135:** 713–723. doi:10.1182/blood.2019002779

Heuser M, Palmisiano N, Mantzaris I, Mims A, DiNardo C, Silverman LR, Wang ES, Fiedler W, Baldus C, Schwind S, et al. 2020. Safety and efficacy of BAY1436032 in IDH1-mutant AML: phase I study results. *Leukemia* **34:** 2903–2913. doi:10.1038/s41375-020-0996-5

Huehls AM, Coupet TA, Sentman CL. 2015. Bispecific T-cell engagers for cancer immunotherapy. *Immunol Cell Biol* **93:** 290–296. doi:10.1038/icb.2014.93

Inoue D, Bradley RK, Abdel-Wahab O. 2016. Spliceosomal gene mutations in myelodysplasia: molecular links to clonal abnormalities of hematopoiesis. *Genes Dev* **30:** 989–1001. doi:10.1101/gad.278424.116

Intlekofer AM, Shih AH, Wang B, Nazir A, Rustenburg AS, Albanese SK, Patel M, Famulare C, Correa FM, Takemoto N, et al. 2018. Acquired resistance to IDH inhibition through *trans* or *cis* dimer-interface mutations. *Nature* **559:** 125–129. doi:10.1038/s41586-018-0251-7

Issa GC, Ravandi F, DiNardo CD, Jabbour E, Kantarjian HM, Andreeff M. 2021. Therapeutic implications of menin inhibition in acute leukemias. *Leukemia* **35:** 2482–2495. doi:10.1038/s41375-021-01309-y

Jaiswal S, Fontanillas P, Flannick J, Manning A, Grauman PV, Mar BG, Lindsley RC, Mermel CH, Burtt N, Chavez A, et al. 2014. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med* **371:** 2488–2498. doi:10.1056/NEJMoa1408617

Jaiswal S, Natarajan P, Silver AJ, Gibson CJ, Bick AG, Shvartz E, McConkey M, Gupta N, Gabriel S, Ardissino D, et al. 2017. Clonal hematopoiesis and risk of atherosclerotic cardiovascular disease. *N Engl J Med* **377:** 111–121. doi:10.1056/NEJMoa1701719

Kadia TM, Jain P, Ravandi F, Garcia-Manero G, Andreef M, Takahashi K, Borthakur G, Jabbour E, Konopleva M, Daver NG, et al. 2016. *TP53* mutations in newly diagnosed acute myeloid leukemia: clinicomolecular characteristics, response to therapy, and outcomes. *Cancer* **122:** 3484–3491. doi:10.1002/cncr.30203

Kantarjian H, Stein A, Gökbuget N, Fielding AK, Schuh AC, Ribera JM, Wei A, Dombret H, Foà R, Bassan R, et al. 2017. Blinatumomab versus chemotherapy for advanced acute lymphoblastic Leukemia. *N Engl J Med* **376:** 836–847. doi:10.1056/NEJMoa1609783

Kikushige Y, Shima T, Takayanagi S-i, Urata S, Miyamoto T, Iwasaki H, Takenaka K, Teshima T, Tanaka T, Inagaki Y, et al. 2010. TIM-3 is a promising target to selectively kill acute myeloid leukemia stem cells. *Cell Stem Cell* **7:** 708–717. doi:10.1016/j.stem.2010.11.014

Kim MY, Yu KR, Kenderian SS, Ruella M, Chen S, Shin TH, Aljanahi AA, Schreeder D, Klichinsky M, Shestova O, et al. 2018. Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. *Cell* **173:** 1439–1453.e19. doi:10.1016/j.cell.2018.05.013

Kim K, Maiti A, Loghavi S, Pourebrahim R, Kadia TM, Rausch CR, Furudate K, Daver NG, Alvarado Y, Ohanian M, et al. 2021. Outcomes of *TP53*-mutant acute myeloid leukemia with decitabine and venetoclax. *Cancer* **127:** 3772–3781. doi:10.1002/cncr.33689

Klossowski S, Miao H, Kempinska K, Wu T, Purohit T, Kim E, Linhares BM, Chen D, Jih G, Perkey E, et al. 2020. Menin inhibitor MI-3454 induces remission in MLL1-rearranged and NPM1-mutated models of leukemia. *J Clin Invest* **130:** 981–997. doi:10.1172/JCI129126

Kong Y, Zhang J, Claxton DF, Ehmann WC, Rybka WB, Zhu L, Zeng H, Schell TD, Zheng H. 2015. PD-$1^{hi}$)TIM-$3^{+}$ T cells associate with and predict leukemia relapse in AML patients post allogeneic stem cell transplantation. *Blood Cancer J* **5:** e330. doi:10.1038/bcj.2015.58

Kong Y, Zhu L, Schell TD, Zhang J, Claxton DF, Ehmann WC, Rybka WB, George MR, Zeng H, Zheng H. 2016. T-cell immunoglobulin and ITIM domain (TIGIT) associates with $CD8^{+}$ T-cell exhaustion and poor clinical outcome in AML patients. *Clin Cancer Res* **22:** 3057–3066. doi:10.1158/1078-0432.CCR-15-2626

Krivtsov AV, Armstrong SA. 2007. MLL translocations, histone modifications and leukaemia stem-cell development. *Nature Reviews Cancer* **7:** 823–833. doi:10.1038/nrc2253

Krivtsov AV, Evans K, Gadrey JY, Eschle BK, Hatton C, Uckelmann HJ, Ross KN, Perner F, Olsen SN, Pritchard T, et al. 2019. A Menin-MLL inhibitor induces specific chromatin changes and eradicates disease in models of MLL-rearranged leukemia. *Cancer Cell* **36:** 660–673.e11. doi:10.1016/j.ccell.2019.11.001

Krupka C, Kufer P, Kischel R, Zugmaier G, Lichtenegger FS, Köhnke T, Vick B, Jeremias I, Metzeler KH, Altmann T, et al. 2016. Blockade of the PD-1/PD-L1 axis augments lysis of AML cells by the CD33/CD3 BiTE antibody construct AMG 330: reversing a T-cell-induced immune escape mechanism. *Leukemia* **30:** 484–491. doi:10.1038/leu.2015.214

Kuhn MW, Hadler MJ, Daigle SR, Koche RP, Krivtsov AV, Olhava EJ, Caligiuri MA, Huang G, Bradner JE, Pollock RM, et al. 2015. MLL partial tandem duplication leukemia cells are sensitive to small molecule DOT1L inhibition. *Haematologica* **100:** e190–e193. doi:10.3324/haematol.2014.115337

Lachowiez CA, Borthakur G, Loghavi S, Zeng Z, Kadia TM, Masarova L, Takahashi K, Tippett GD, Naqvi K, Bose P, et al. 2020. Phase Ib/II study of the IDH1-mutant inhibitor ivosidenib with the BCL2 inhibitor venetoclax ± azacitidine in IDH1-mutated hematologic malignancies. *J Clin Oncol* **38:** 7500. doi:10.1200/JCO.2020.38.15_suppl.7500

Lee SC, Dvinge H, Kim E, Cho H, Micol JB, Chung YR, Durham BH, Yoshimi A, Kim YJ, Thomas M, et al. 2016. Modulation of splicing catalysis for therapeutic targeting of leukemia with mutations in genes encoding spliceosomal proteins. *Nat Med* **22:** 672–678. doi:10.1038/nm.4097

Lee SC, North K, Kim E, Jang E, Obeng E, Lu SX, Liu B, Inoue D, Yoshimi A, Ki M, et al. 2018. Synthetic lethal and convergent biological effects of cancer-associated spliceosomal gene mutations. *Cancer Cell* **34:** 225–241.e8. doi:10.1016/j.ccell.2018.07.003

Leick M, Scarfò I, Choi BD, Larson R, Bouffard AA, Castano A, Cabral ML, Schmidts A, Frigault MJ, Maus MV. 2019. Use of CD70 targeted chimeric antigen receptor (CAR) T cells for the treatment of acute myeloid leukemia (AML). *Blood* **134:** 4443. doi:10.1182/blood-2019-127154

Lichtenegger FS, Rothe M, Schnorfeil FM, Deiser K, Krupka C, Augsberger C, Schlüter M, Neitz J, Subklewe M. 2018. Targeting LAG-3 and PD-1 to enhance T cell activation by antigen-presenting cells. *Front Immunol* **9:** 385. doi:10.3389/fimmu.2018.00385

Lin T, Mannis GN, Erba HP, Levis M, Zou H, Faderl S, Cheung RS, Pullarkat VA. 2020. V-FAST: a phase 1b master trial to investigate CPX-351 combined with various targeted agents in patients with previously untreated acute myeloid leukemia. *Blood* **136:** 26–28.

Liu F, Cao Y, Pinz K, Ma Y, Wada M, Chen K, Ma G, Shen J, Tse CO, Su Y, et al. 2018. First-in-human CLL1–CD33 compound CAR T cell therapy induces complete remission in patients with refractory acute myeloid leukemia: update on phase 1 clinical trial. *Blood* **132:** 901. doi:10.1182/blood-2018-99-110579

Liu Y, Bewersdorf JP, Stahl M, Zeidan AM. 2019. Immunotherapy in acute myeloid leukemia and myelodysplastic syndromes: the dawn of a new era? *Blood Rev* **34:** 67–83. doi:10.1016/j.blre.2018.12.001

Lu SX, De Neef E, Thomas JD, Sabio E, Rousseau B, Gigoux M, Knorr DA, Greenbaum B, Elhanati Y, Hogg SJ, et al. 2021. Pharmacologic modulation of RNA splicing enhances anti-tumor immunity. *Cell* **184:** 4032–4047.e31. doi:10.1016/j.cell.2021.05.038

Ma J, Zhao S, Qiao X, Knight T, Edwards H, Polin L, Kushner J, Dzinic SH, White K, Wang G, et al. 2019. Inhibition of Bcl-2 synergistically enhances the antileukemic activity of midostaurin and gilteritinib in preclinical models of FLT3-mutated acute myeloid leukemia. *Clin Cancer Res* **25:** 6815–6826. doi:10.1158/1078-0432.CCR-19-0832

Maiti A, DiNardo CD, Daver NG, Rausch CR, Ravandi F, Kadia TM, Pemmaraju N, Borthakur G, Bose P, Issa GC, et al. 2021. Triplet therapy with venetoclax, FLT3 inhibitor and decitabine for FLT3-mutated acute myeloid leukemia. *Blood Cancer J* **11:** 25. doi:10.1038/s41408-021-00410-w

Majeti R, Chao MP, Alizadeh AA, Pang WW, Jaiswal S, Gibbs KD Jr., van Rooijen N, Weissman IL. 2009. CD47 is an adverse prognostic factor and therapeutic antibody target on human acute myeloid leukemia stem cells. *Cell* **138:** 286–299. doi:10.1016/j.cell.2009.05.045

Malcovati L, Gallì A, Travaglino E, Ambaglio I, Rizzo E, Molteni E, Elena C, Ferretti VV, Catricalà S, Bono E, et al. 2017. Clinical significance of somatic mutation in unexplained blood cytopenia. *Blood* **129:** 3371–3378. doi:10.1182/blood-2017-01-763425

Malcovati L, Stevenson K, Papaemmanuil E, Neuberg D, Bejar R, Boultwood J, Bowen DT, Campbell PJ, Ebert BL, Fenaux P, et al. 2020. *SF3B1*-mutant MDS as a distinct disease subtype: a proposal from the international working group for the prognosis of MDS. *Blood* **136:** 157–170. doi:10.1182/blood.2020004850

Mellinghoff IK, Penas-Prado M, Peters KB, Burris HA, Maher EA, Janku F, Cote GM, de la Fuente MI, Clarke JL, Ellingson BM, et al. 2021. Vorasidenib, a dual inhibitor of mutant IDH1/2, in recurrent or progressive glioma; results of a first-in-human phase I trial. *Clin Cancer Res* **27:** 4491–4499. doi:10.1158/1078-0432.CCR-21-0611

Meyer C, Burmeister T, Gröger D, Tsaur G, Fechina L, Renneville A, Sutton R, Venn NC, Emerenciano M, Pombo-de-Oliveira MS, et al. 2018. The MLL recombinome of acute leukemias in 2017. *Leukemia* **32:** 273–284. doi:10.1038/leu.2017.213

Mill CP, Fiskus W, DiNardo CD, Qian Y, Raina K, Rajapakshe K, Perera D, Coarfa C, Kadia TM, Khoury JD, et al. 2019. RUNX1-targeted therapy for AML expressing somatic or germline mutation in RUNX1. *Blood* **134:** 59–73. doi:10.1182/blood.2018893982

Mill CP, Fiskus W, DiNardo CD, Birdwell C, Davis JA, Kadia TM, Takahashi K, Short NJ, Daver NG, Ohanian M, et al. 2021. Effective therapy for AML with RUNX1 mutation by co-treatment with inhibitors of protein translation and BCL2. *Blood* **139:** 907–921. doi:10.1182/blood.2021013156

Montesinos P, Recher C, Vives S, Zarzycka E, Wang J, Bertani G, Heuser M, Calado RT, Schuh AC, Yeh S-P, et al. 2021. AGILE: a global, randomized, double-blind, phase 3 study of ivosidenib + azacitidine versus placebo + azacitidine in patients with newly diagnosed acute myeloid leukemia with an *IDH1* mutation. *Blood* **138:** 697. doi:10.1182/blood-2021-147805

Moran-Crusio K, Reavie L, Shih A, Abdel-Wahab O, Ndiaye-Lobry D, Lobry C, Figueroa ME, Vasanthakumar A, Patel J, Zhao X, et al. 2011. Tet2 loss leads to increased hematopoietic stem cell self-renewal and myeloid transformation. *Cancer Cell* **20:** 11–24. doi:10.1016/j.ccr.2011.06.001

Morita K, Kantarjian HM, Montalban Bravo G, Sasaki K, Daver N, Jabbour E, Alvarado Y, Chien KS, DiNardo CD, Ravandi F, et al. 2020. A phase II study of double immune checkpoint inhibitor blockade with nivolumab and ipilimumab with or without azacitidine in patients with myelodysplastic syndrome (MDS). *Blood* **136:** 7–9. doi:10.1182/blood-2020-142003

Mullighan CG, Kennedy A, Zhou X, Radtke I, Phillips LA, Shurtleff SA, Downing JR. 2007. Pediatric acute myeloid leukemia with NPM1 mutations is characterized by a gene expression profile with dysregulated HOX gene expression distinct from MLL-rearranged leukemias. *Leukemia* **21:** 2000–2009. doi:10.1038/sj.leu.2404808

Ohgami RS, Ma L, Merker JD, Gotlib JR, Schrijver I, Zehnder JL, Arber DA. 2015. Next-generation sequencing of acute myeloid leukemia identifies the significance of TP53, U2AF1, ASXL1, and TET2 mutations. *Mod Pathol* **28:** 706–714. doi:10.1038/modpathol.2014.160

Ørskov AD, Treppendahl MB, Skovbo A, Holm MS, Friis LS, Hokland M, Grønbæk K. 2015. Hypomethylation and up-regulation of *PD-1* in T cells by azacytidine in MDS/AML patients: a rationale for combined targeting of PD-1 and DNA methylation. *Oncotarget* **6:** 9612–9626. doi:10.18632/oncotarget.3324

Papaemmanuil E, Cazzola M, Boultwood J, Malcovati L, Vyas P, Bowen D, Pellagatti A, Wainscoat JS, Hellstrom-Lindberg E, Gambacorti-Passerini C, et al. 2011. Somatic *SF3B1* mutation in myelodysplasia with ring sideroblasts. *N Engl J Med* **365:** 1384–1395. doi:10.1056/NEJMoa1103283

Papaemmanuil E, Gerstung M, Bullinger L, Gaidzik VI, Paschka P, Roberts ND, Potter NE, Heuser M, Thol F, Bolli N, et al. 2016. Genomic classification and prognosis in acute myeloid leukemia. *N Engl J Med* **374:** 2209–2221. doi:10.1056/NEJMoa1516192

Park JH, Rivière I, Gonen M, Wang X, Sénéchal B, Curran KJ, Sauter C, Wang Y, Santomasso B, Mead E, et al. 2018. Long-term follow-up of CD19 CAR therapy in acute lymphoblastic leukemia. *N Engl J Med* **378:** 449–459. doi:10.1056/NEJMoa1709919

Pearlman AH, Hwang MS, Konig MF, Hsiue EH-C, Douglass J, DiNapoli SR, Mog BJ, Bettegowda C, Pardoll DM, Gabelli SB, et al. 2021. Targeting public neoantigens for cancer

immunotherapy. *Nature Cancer* **2:** 487–497. doi:10.1038/s43018-021-00210-y

Perl AE, Martinelli G, Cortes JE, Neubauer A, Berman E, Paolini S, Montesinos P, Baer MR, Larson RA, Ustun C, et al. 2019. Gilteritinib or chemotherapy for relapsed or refractory *FLT3*-mutated AML. *N Engl J Med* **381:** 1728–1740. doi:10.1056/NEJMoa1902688

Petrova PS, Viller NN, Wong M, Pang X, Lin GH, Dodge K, Chai V, Chen H, Lee V, House V, et al. 2017. TTI-621 (SIRPαFc): a CD47-blocking innate immune checkpoint inhibitor with broad antitumor activity and minimal erythrocyte binding. *Clin Cancer Res* **23:** 1068–1079. doi:10.1158/1078-0432.CCR-16-1700

Ravandi F, Assi R, Daver N, Benton CB, Kadia T, Thompson PA, Borthakur G, Alvarado Y, Jabbour EJ, Konopleva M, et al. 2019. Idarubicin, cytarabine, and nivolumab in patients with newly diagnosed acute myeloid leukaemia or high-risk myelodysplastic syndrome: a single-arm, phase 2 study. *Lancet Haematol* **6:** e480–e488. doi:10.1016/S2352-3026(19)30114-0

Ravandi F, Bashey A, Stock W, Foran JM, Mawad R, Egan D, Blum W, Yang A, Pastore A, Johnson C, et al. 2020a. Complete responses in relapsed/refractory acute myeloid leukemia (AML) patients on a weekly dosing schedule of vibecotamab (XmAb14045), a CD123 × CD3 T cell-engaging bispecific antibody; initial results of a phase 1 study. *Blood* **136:** 4–5. doi:10.1182/blood-2020-134746

Ravandi F, Walter RB, Subklewe M, Buecklein V, Jongen-Lavrencic M, Paschka P, Ossenkoppele GJ, Kantarjian HM, Hindoyan A, Agarwal SK, et al. 2020b. Updated results from phase I dose-escalation study of AMG 330, a bispecific T-cell engager molecule, in patients with relapsed/refractory acute myeloid leukemia (R/R AML). *J Clin Oncol* **38:** 7508. doi:10.1200/JCO.2020.38.15_suppl.7508

Reville PK, Kantarjian HM, Borthakur G, Yilmaz M, Montalban-Bravo G, DiNardo CD, Andreeff M, Daver N, Jain N, Kornblau SM, et al. 2020. Cladribine, idarubicin, cytarabine (ara-C), and venetoclax in treating patients with acute myeloid leukemia and high-risk myelodysplastic syndrome. *Blood* **136:** 7–9. doi:10.1182/blood-2020-141075

Roboz GJ, DiNardo CD, Stein EM, de Botton S, Mims AS, Prince GT, Altman JK, Arellano ML, Donnellan W, Erba HP, et al. 2020. Ivosidenib induces deep durable remissions in patients with newly diagnosed IDH1-mutant acute myeloid leukemia. *Blood* **135:** 463–471. doi:10.1182/blood.2019002140

Rosenblatt J, Stone RM, Uhl L, Neuberg D, Joyce R, Levine JD, Arnason J, McMasters M, Luptakova K, Jain S, et al. 2016. Individualized vaccination of AML patients in remission is associated with induction of antileukemia immunity and prolonged remissions. *Sci Transl Med* **8:** 368ra171.

Rücker FG, Schlenk RF, Bullinger L, Kayser S, Teleanu V, Kett H, Habdank M, Kugler CM, Holzmann K, Gaidzik VI, et al. 2012. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. *Blood* **119:** 2114–2121. doi:10.1182/blood-2011-08-375758

Russ A, Hua AB, Montfort WR, Rahman B, Riaz IB, Khalid MU, Carew JS, Nawrocki ST, Persky D, Anwer F. 2018. Blocking 'don't eat me' signal of CD47–SIRPα in hematological malignancies, an in-depth review. *Blood Rev* **32:** 480–489. doi:10.1016/j.blre.2018.04.005

Sallman DA, Kerre T, Poire X, Havelange V, Lewalle P, Davila ML, Wang ES, Dekker D, Snykers S, Sotiropoulou PA, et al. 2018. Remissions in relapse/refractory acute myeloid leukemia patients following treatment with NKG2D CAR-T therapy without a prior preconditioning chemotherapy. *Blood* **132:** 902. doi:10.1182/blood-2018-99-111326

Sallman DA, Al Malki M, Asch AS, Lee DJ, Kambhampati S, Donnellan WB, Bradley TJ, Vyas P, Jeyakumar D, Marcucci G, et al. 2020a. Tolerability and efficacy of the first-in-class anti-CD47 antibody magrolimab combined with azacitidine in MDS and AML patients: phase Ib results. *J Clin Oncol* **38:** 7507. doi:10.1200/JCO.2020.38.15_suppl.7507

Sallman DA, McLemore AF, Aldrich AL, Komrokji RS, McGraw KL, Dhawan A, Geyer S, Hou HA, Eksioglu EA, Sullivan A, et al. 2020b. TP53 mutations in myelodysplastic syndromes and secondary AML confer an immunosuppressive phenotype. *Blood* **136:** 2812–2823. doi:10.1182/blood.2020006158

Saxena K, Herbrich SM, Pemmaraju N, Kadia TM, DiNardo CD, Borthakur G, Pierce SA, Jabbour E, Wang SA, Bueso-Ramos C, et al. 2021. A phase 1b/2 study of azacitidine with PD-L1 antibody avelumab in relapsed/refractory acute myeloid leukemia. *Cancer* **127:** 3761–3771. doi:10.1002/cncr.33690

Schischlik F, Jäger R, Rosebrock F, Hug E, Schuster M, Holly R, Fuchs E, Milosevic Feenstra JD, Bogner E, Gisslinger B, et al. 2019. Mutational landscape of the transcriptome offers putative targets for immunotherapy of myeloproliferative neoplasms. *Blood* **134:** 199–210. doi:10.1182/blood.2019000519

Schnorfeil FM, Lichtenegger FS, Emmerig K, Schlueter M, Neitz JS, Draenert R, Hiddemann W, Subklewe M. 2015. T cells are functionally not impaired in AML: increased PD-1 expression is only seen at time of relapse and correlates with a shift towards the memory T cell compartment. *J Hematol Oncol* **8:** 93. doi:10.1186/s13045-015-0189-2

Schuster SJ, Bishop MR, Tam CS, Waller EK, Borchmann P, McGuirk JP, Jäger U, Jaglowski S, Andreadis C, Westin JR, et al. 2019. Tisagenlecleucel in adult relapsed or refractory diffuse large B-cell lymphoma. *N Engl J Med* **380:** 45–56. doi:10.1056/NEJMoa1804980

Seiler M, Yoshimi A, Darman R, Chan B, Keaney G, Thomas M, Agrawal AA, Caleb B, Csibi A, Sean E, et al. 2018. H3B-8800, an orally available small-molecule splicing modulator, induces lethality in spliceosome-mutant cancers. *Nat Med* **24:** 497–504. doi:10.1038/nm.4493

Shallis RM, Wang R, Davidoff A, Ma X, Zeidan AM. 2019. Epidemiology of acute myeloid leukemia: recent progress and enduring challenges. *Blood Rev* **36:** 70–87. doi:10.1016/j.blre.2019.04.005

Shi A, Murai MJ, He S, Lund G, Hartley T, Purohit T, Reddy G, Chruszcz M, Grembecka J, Cierpicki T. 2012. Structural insights into inhibition of the bivalent menin–MLL interaction by small molecules in leukemia. *Blood* **120:** 4461–4469. doi:10.1182/blood-2012-05-429274

Singh Mali R, Zhang Q, DeFilippis RA, Cavazos A, Kuruvilla VM, Raman J, Mody V, Choo EF, Dail M, Shah NP, et al. 2021. Venetoclax combines synergistically with FLT3 inhibition to effectively target leukemic cells in $FLT3^{-}ITD^{+}$ acute myeloid leukemia models. *Haematologica* **106:** 1034–1046. doi:10.3324/haematol.2019.244020

Slade MJ, Uy GL. 2020. CD123 bi-specific antibodies in development in AML: what do we know so far? *Best Pract Res Clin Haematol* **33:** 101219. doi:10.1016/j.beha.2020.101219

Sperling AS, Gibson CJ, Ebert BL. 2017. The genetics of myelodysplastic syndrome: from clonal haematopoiesis to secondary leukaemia. *Nat Rev Cancer* **17:** 5–19. doi:10.1038/nrc.2016.112

Stahl M, Menghrajani K, Derkach A, Chan A, Xiao W, Glass J, King AC, Daniyan AF, Famulare C, Cuello BM, et al. 2021. Clinical and molecular predictors of response and survival

following venetoclax therapy in relapsed/refractory AML. *Blood Adv* **5:** 1552–1564. doi:10.1182/bloodadvances.2020003734

Steensma DP, Bejar R, Jaiswal S, Lindsley RC, Sekeres MA, Hasserjian RP, Ebert BL. 2015. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood* **126:** 9–16. doi:10.1182/blood-2015-03-631747

Steensma DP, Wermke M, Klimek VM, Greenberg PL, Font P, Komrokji RS, Yang J, Brunner AM, Carraway HE, Ades L, et al. 2021. Phase I first-in-human dose escalation study of the oral SF3B1 modulator H3B-8800 in myeloid neoplasms. *Leukemia* **35:** 3542–3550. doi:10.1038/s41375-021-01328-9

Stein EM, DiNardo CD, Pollyea DA, Fathi AT, Roboz GJ, Altman JK, Stone RM, DeAngelo DJ, Levine RL, Flinn IW, et al. 2017. Enasidenib in mutant IDH2 relapsed or refractory acute myeloid leukemia. *Blood* **130:** 722–731. doi:10.1182/blood-2017-04-779405

Stein EM, DiNardo CD, Fathi AT, Mims AS, Pratz KW, Savona MR, Stein AS, Stone RM, Winer ES, Seet CS, et al. 2020a. Ivosidenib or enasidenib combined with intensive chemotherapy in patients with newly diagnosed AML: a phase 1 study. *Blood* **137:** 1792–1803. doi:10.1182/blood.2020007233

Stein EM, Konopleva M, Gilmour R, Szpurka AM, Hill E, Ward R, Kantarjian HM, Dinardo CD. 2020b. A phase 1 study of LY3410738, a first-in-class covalent inhibitor of mutant IDH in advanced myeloid malignancies (trial in progress). *Blood* **136:** 26. doi:10.1182/blood-2020-134307

Stein E, Aldoss I, DiPersio JF, Stone RM, Arellano ML, Rosen G, Meyers ML, Huang Y, Smith S, Bagley RG, et al. 2021. Safety and efficacy of menin inhibition in patients (Pts) with MLL-rearranged and NPM1 mutant acute leukemia: a phase (Ph) 1, first-in-human study of SNDX-5613 (AUGMENT 101). *Blood* **138:** 699. doi:10.1182/blood-2021-146944

Stone RM, Mandrekar SJ, Sanford BL, Laumann K, Geyer S, Bloomfield CD, Thiede C, Prior TW, Döhner K, Marcucci G, et al. 2017. Midostaurin plus chemotherapy for acute myeloid leukemia with a *FLT3* mutation. *N Engl J Med* **377:** 454–464. doi:10.1056/NEJMoa1614359

Subklewe M, Stein A, Walter RB, Bhatia R, Wei AH, Ritchie D, Bücklein V, Vachhani P, Dai T, Hindoyan A, et al. 2019. Preliminary results from a phase 1 first-in-human study of AMG 673, a novel half-life extended (HLE) anti-CD33/CD3 BiTE® (bispecific T-cell engager) in patients with relapsed/refractory (R/R) acute myeloid leukemia (AML). *Blood* **134:** 833. doi:10.1182/blood-2019-127977

Super HJ, McCabe NR, Thirman MJ, Larson RA, Le Beau MM, Pedersen-Bjergaard J, Philip P, Diaz MO, Rowley JD. 1993. Rearrangements of the MLL gene in therapy-related acute myeloid leukemia in patients previously treated with agents targeting DNA-topoisomerase II. *Blood* **82:** 3705–3711. doi:10.1182/blood.V82.12.3705.3705

Tallman MS, Wang ES, Altman JK, Appelbaum FR, Bhatt VR, Bixby D, Coutre SE, De Lima M, Fathi AT, Fiorella M, et al. 2019. Acute myeloid leukemia, version 3.2019, NCCN clinical practice guidelines in oncology. *J Natl Compr Canc Netw* **17:** 721–749. doi:10.6004/jnccn.2019.0028

Teh TC, Nguyen NY, Moujalled DM, Segal D, Pomilio G, Rijal S, Jabbour A, Cummins K, Lackovic K, Blombery P, et al. 2018. Enhancing venetoclax activity in acute myeloid leukemia by co-targeting MCL1. *Leukemia* **32:** 303–312. doi:10.1038/leu.2017.243

Thol F, Kade S, Schlarmann C, Löffeld P, Morgan M, Krauter J, Wlodarski MW, Kölking B, Wichmann M, Görlich K, et al. 2012. Frequency and prognostic impact of mutations in *SRSF2*, *U2AF1*, and *ZRSR2* in patients with myelodysplastic syndromes. *Blood* **119:** 3578–3584. doi:10.1182/blood-2011-12-399337

Tirado-Gonzalez I, Descot A, Soetopo D, Nevmerzhitskaya A, Schaffer A, Kur IM, Czlonka E, Wachtel C, Tsoukala I, Muller L, et al. 2021. AXL inhibition in macrophages stimulates host-versus-leukemia immunity and eradicates naive and treatment resistant leukemia. *Cancer Discov* **11:** 2924–2943. doi:10.1158/2159-8290.CD-20-1378

Toffalori C, Zito L, Gambacorta V, Riba M, Oliveira G, Bucci G, Barcella M, Spinelli O, Greco R, Crucitti L, et al. 2019. Immune signature drives leukemia escape and relapse after hematopoietic cell transplantation. *Nat Med* **25:** 603–611. doi:10.1038/s41591-019-0400-z

Tschernia NP, Kumar V, Moore DT, Vincent BG, Coombs CC, Van Deventer H, Foster MC, DeZern AE, Luznik L, Riches ML, et al. 2021. Safety and efficacy of pembrolizumab prior to allogeneic stem cell transplantation for acute myelogenous leukemia. *Transplant Cell Ther* **27:** 1021.e1–1021.e5. doi:10.1016/j.jtct.2021.08.022

Uckelmann HJ, Kim SM, Wong EM, Hatton C, Giovinazzo H, Gadrey JY, Krivtsov AV, Rücker FG, Döhner K, McGeehan GM, et al. 2020. Therapeutic targeting of preleukemia cells in a mouse model of *NPM1* mutant acute myeloid leukemia. *Science* **367:** 586–590. doi:10.1126/science.aax5863

Uy GL, Aldoss I, Foster MC, Sayre PH, Wieduwilt MJ, Advani AS, Godwin JE, Arellano ML, Sweet KL, Emadi A, et al. 2021. Flotetuzumab as salvage immunotherapy for refractory acute myeloid leukemia. *Blood* **137:** 751–762. doi:10.1182/blood.2020007732

Vadakekolathu J, Lai C, Reeder S, Church SE, Hood T, Lourdusamy A, Rettig MP, Aldoss I, Advani AS, Godwin J, et al. 2020a. TP53 abnormalities correlate with immune infiltration and associate with response to flotetuzumab immunotherapy in AML. *Blood advances* **4:** 5011–5024. doi:10.1182/bloodadvances.2020002512

Vadakekolathu J, Minden MD, Hood T, Church SE, Reeder S, Altmann H, Sullivan AH, Viboch EJ, Patel T, Ibrahimova N, et al. 2020b. Immune landscapes predict chemotherapy resistance and immunotherapy response in acute myeloid leukemia. *Sci Transl Med* **12:** eaaz0463. doi:10.1126/scitranslmed.aaz0463

van der Lee DI, Reijmers RM, Honders MW, Hagedoorn RS, de Jong RC, Kester MG, van der Steen DM, de Ru AH, Kweekel C, Bijen HM, et al. 2019. Mutated nucleophosmin 1 as immunotherapy target in acute myeloid leukemia. *J Clin Invest* **129:** 774–785. doi:10.1172/JCI97482

Wang E, Lu SX, Pastore A, Chen X, Imig J, Chun-Wei Lee S, Hockemeyer K, Ghebrechristos YE, Yoshimi A, Inoue D, et al. 2019. Targeting an RNA-binding protein network in acute myeloid leukemia. *Cancer Cell* **35:** 369–384.e7. doi:10.1016/j.ccell.2019.01.010

Wang ES, Altman JK, Pettit K, De Botton S, Walter RP, Fenaux P, Burrows F, Tomkinson BE, Martell B, Fathi AT. 2020. Preliminary data on a phase 1/2A first in human study of the menin-KMT2A (MLL) inhibitor KO-539 in patients with relapsed or refractory acute myeloid leukemia. *Blood* **136:** 7–8.

Wang L, Birch NW, Zhao Z, Nestler CM, Kazmer A, Shilati A, Blake A, Ozark PA, Rendleman EJ, Zha D, et al. 2021. Epigenetic targeted therapy of stabilized BAP1 in ASXL1 gain-of-function mutated leukemia. *Nature Cancer* **2:** 515–526. doi:10.1038/s43018-021-00199-4

Watts JM, Baer MR, Yang J, Prebet T, Lee S, Schiller GJ, Dinner S, Pigneux A, Montesinos P, Wang ES, et al. 2019. Olutasidenib (FT-2102), an IDH1m inhibitor as a single agent or in

combination with azacitidine, induces deep clinical responses with mutation clearance in patients with acute myeloid leukemia treated in a phase 1 dose escalation and expansion study. *Blood* **134:** 231. doi:10.1182/blood-2019-123920

Wei AH, Montesinos P, Ivanov V, DiNardo CD, Novak J, Laribi K, Kim I, Stevens D, Fiedler W, Pagoni M, et al. 2020. Venetoclax plus LDAC for patients with untreated AML ineligible for intensive chemotherapy: phase 3 randomized placebo-controlled trial. *Blood* **135:** 2137–2145. doi:10.1182/blood.2020004856

Welch JS, Petti AA, Miller CA, Fronick CC, O'Laughlin M, Fulton RS, Wilson RK, Baty JD, Duncavage EJ, Tandon B, et al. 2016. *TP53* and decitabine in acute myeloid leukemia and myelodysplastic syndromes. *N Engl J Med* **375:** 2023–2036. doi:10.1056/NEJMoa1605949

Westervelt P, Cortes JE, Altman JK, Long M, Oehler VG, Gojo I, Guenot J, Chun P, Roboz GJ. 2019. Phase 1 first-in-human trial of AMV564, a bivalent bispecific (2:2) CD33/CD3 T-Cell engager, in patients with relapsed/refractory acute myeloid leukemia (AML). *Blood* **134:** 834. doi:10.1182/blood-2019-129042

Williams P, Basu S, Garcia-Manero G, Hourigan CS, Oetjen KA, Cortes JE, Ravandi F, Jabbour EJ, Al-Hamal Z, Konopleva M, et al. 2019. The distribution of T-cell subsets and the expression of immune checkpoint receptors and ligands in patients with newly diagnosed and relapsed acute myeloid leukemia. *Cancer* **125:** 1470–1481. doi:10.1002/cncr.31896

Xie M, Lu C, Wang J, McLellan MD, Johnson KJ, Wendl MC, McMichael JF, Schmidt HK, Yellapantula V, Miller CA, et al. 2014. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med* **20:** 1472–1478. doi:10.1038/nm.3733

Xuan L, Wang Y, Huang F, Fan Z, Xu Y, Sun J, Xu N, Deng L, Li X, Liang X, et al. 2020. Sorafenib maintenance in patients with FLT3-ITD acute myeloid leukaemia undergoing allogeneic haematopoietic stem-cell transplantation: an open-label, multicentre, randomised phase 3 trial. *Lancet Oncol* **21:** 1201–1212. doi:10.1016/S1470-2045(20)30455-1

Yang H, Bueso-Ramos C, DiNardo C, Estecio MR, Davanlou M, Geng QR, Fang Z, Nguyen M, Pierce S, Wei Y, et al. 2014. Expression of PD-L1, PD-L2, PD-1 and CTLA4 in myelodysplastic syndromes is enhanced by treatment with hypomethylating agents. *Leukemia* **28:** 1280–1288. doi:10.1038/leu.2013.355

Yokoyama A, Somervaille TC, Smith KS, Rozenblatt-Rosen O, Meyerson M, Cleary ML. 2005. The menin tumor suppressor protein is an essential oncogenic cofactor for MLL-associated leukemogenesis. *Cell* **123:** 207–218. doi:10.1016/j.cell.2005.09.025

Zeidan AM, Knaus HA, Robinson TM, Towlerton AMH, Warren EH, Zeidner JF, Blackford AL, Duffield AS, Rizzieri D, Frattini MG, et al. 2018. A multi-center phase I trial of ipilimumab in patients with myelodysplastic syndromes following hypomethylating agent failure. *Clin Cancer Res* **24:** 3519–3527. doi:10.1158/1078-0432.CCR-17-3763

Zeidan AM, Cavenagh J, Voso MT, Taussig D, Tormo M, Boss I, Copeland WB, Gray VE, Previtali A, O'Connor T, et al. 2019a. Efficacy and safety of azacitidine (AZA) in combination with the anti-PD-L1 durvalumab (durva) for the Front-Line treatment of older patients (pts) with acute myeloid leukemia (AML) who are unfit for intensive chemotherapy (IC) and Pts with higher-risk myelodysplastic syndromes (HR-MDS): results from a large, international, randomized phase 2 study. *Blood* **134:** 829. doi:10.1182/blood-2019-122896

Zeidan AM, DeAngelo DJ, Palmer JM, Seet CS, Tallman MS, Wei X, Li YF, Hock N, Burgess MR, Hege K, et al. 2019b. A phase I study of CC-90002, a monoclonal antibody targeting CD47, in patients with relapsed and/or refractory (R/R) acute myeloid leukemia (AML) and high-risk myelodysplastic syndromes (MDS): final results. *Blood* **134:** 1320. doi:10.1182/blood-2019-125363

Zeidan AM, Podoltsev NA, Wang X, Bewersdorf JP, Shallis RM, Huntington SF, Gore SD, Davidoff AJ, Ma X, Wang R. 2019c. Temporal patterns and predictors of receiving no active treatment among older patients with acute myeloid leukemia in the United States: a population-level analysis. *Cancer* **125:** 4241–4251. doi:10.1002/cncr.32439

Zeidan AM, Esteve J, Giagounidis A, Kim H-J, Miyazaki Y, Platzbecker U, Schuh AC, Sekeres MA, Westermann J, Xiao Z, et al. 2020a. The STIMULUS program: clinical trials evaluating sabatolimab (MBG453) combination therapy in patients (Pts) with higher-risk myelodysplastic syndromes (HR-MDS) or acute myeloid leukemia (AML). *Blood* **136:** 45–46. doi:10.1182/blood-2020-134718

Zeidan AM, Podoltsev NA, Wang X, Zhang C, Bewersdorf JP, Shallis RM, Huntington SF, Neparidze N, Giri S, Gore SD, et al. 2020b. Patterns of care and clinical outcomes with cytarabine-anthracycline induction chemotherapy for AML patients in the United States. *Blood Adv* **4:** 1615–1623. doi:10.1182/bloodadvances.2020001728

Zeidan AM, DeAngelo DJ, Palmer J, Seet CS, Tallman MS, Wei X, Raymon H, Sriraman P, Kopytek S, Bewersdorf JP, et al. 2022. Phase 1 study of anti-CD47 monoclonal antibody CC-90002 in patients with relapsed/refractory acute myeloid leukemia and high-risk myelodysplastic syndromes. *Ann Hematol* **101:** 557–569. doi:10.1007/s00277-021-04734-2