Which are the most promising targets for minimal residual disease-directed therapy in acute myeloid leukemia prior to allogeneic stem cell transplant?

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ABSTRACT

Minimal residual disease has emerged as an important prognostic factor for relapse and survival in acute myeloid leukemia. Eradication of minimal residual disease may increase the number of patients with long-term survival; however, to date, strategies that specifically target minimal residual disease are limited. Consensus guidelines on minimal residual disease detection by immunophenotypic and molecular methods are an essential initial step for clinical trials evaluating minimal residual disease. Here, we review promising targets of minimal residual disease prior to allogeneic stem cell transplantation. Specifically, the focus of this review is on the rationale and clinical development of therapies targeting: oncogenic driver mutations, apoptosis, methylation, and leukemic immune targets. We review the progress made in the clinical development of therapies against each target and the challenges that lie ahead.

Introduction

For over 45 years, standard therapy for fit patients with newly diagnosed acute myeloid leukemia (AML) has been induction chemotherapy with cytarabine and an anthracycline.[1] Despite most patients achieving morphological remission with intensive chemotherapy, the prognosis for long-term survival in AML remains poor. Advances in multiparameter flow cytometry and molecular testing, including real-time quantitative polymerase chain reaction, digital polymerase chain reaction and next-generation sequencing, have enabled detection of minimal or measurable residual disease (MRD) far below a threshold of 5% blasts required for morphological remission.[2] Among patients receiving induction chemotherapy, complete remission (CR) with persistent MRD occurs in a substantial 40% of patients.[3] Mounting evidence has shown that the presence of MRD detectable prior to myeloablative allogeneic stem cell transplantation (SCT) is associated with shorter survival and increased risk of relapse that is similar to the risk in patients with active disease.[4-7]

Eradication of MRD prior to allogeneic SCT has the potential to increase long-term survival in AML. However, few studies have reported on the outcomes of patients converting from MRD-positive to MRD-negative disease after treatment with consolidation therapies. In the HOVON/SAKK AML 42A study, post-remission treatment with either chemotherapy, autologous or allogeneic SCT led to a change from MRD-positive to MRD-negative status in 7/21 (33%) patients.[8] In the GIMEMA study, late MRD clearance (induction positive, consolidation negative MRD status) was observed in 15/134 (11%) patients and was associated with similar rates of 5-year overall survival and relapse-free survival as those of patients with early MRD clearance (induction negative, consolidation negative MRD status). MRD status after consolidation was the only factor independently associated with both a shorter duration of relapse-free survival and overall survival in multivariate analaysis, suggesting a more favorable outcome from MRD conversion after post-remission chemotherapy.[9] Given the modest rates of MRD conversion with consolidation chemotherapy, more effective therapies capable of eradicating MRD prior to transplantation are urgently needed.

As a reservoir for relapse, MRD would ideally be targeted by therapies that

reduce the potential for recurrence by eliminating leukemia regenerating cells. AML is a heterogeneous disease that includes populations of bulk leukemic blasts and leukemic stem cells that are thought to be more refractory to treatment than others.[10] Leukemic stem cells were initially defined phenotypically by specific cell surface markers $CD34^+$ $CD38^-$ and functionally by an ability to initiate leukemia in animal transplant models.[11] Cellular tracking of leukemic cell populations demonstrated the persistence of either leukemic stem cell subclones or more committed leukemia cells that retained stemness transcriptional programs from disease initiation to relapse.[12] Therefore, central to the development of MRD targeting is the ability of the novel therapies to eradicate leukemic stem cells.

In this review, we discuss MRD targets of therapeutic potential. We focus on the therapies that have been developed for each target and, if available, evidence of efficacy in reducing MRD prior to allogeneic SCT.

Targeting oncogenic driver mutations

Fms-like tyrosine kinase 3 (*FLT3*)

Fms-like tyrosine kinase 3 (*FLT3*) is the most commonly mutated gene in AML with *FLT3* internal tandem duplications (ITD) and FLT3 tyrosine kinase domain (TKD) mutations occurring in 22-32% and 8% of newly diagnosed cases, respectively.[13,14] In a large population-based study the incidence of *FLT3*-ITD mutations was lower at 18.9% and decreased with age.[15] *FLT3*-ITD mutations are associated with worse prognosis and increased risk of relapse with allogeneic transplantation.[13,14,16-18] As monotherapy, FLT3 inhibitors are capable of inducing molecular remissions and gilteritinib (Xospata) is approved for relapsed or refractory *FLT3*-mutated AML.[19,20] Quizartinib has also demonstrated efficacy as monotherapy in patients with relapsed or refractory *FLT3*-ITD-mutated AML.[21] The combination of FLT3 inhibitors with chemotherapy has the potential to induce deeper remissions than induction chemotherapy alone. Midostaurin (Rydapt) is a first-generation FLT3 inhibitor that was originally developed as a protein kinase C inhibitor and found to have inhibitory activity against multiple tyrosine kinases including FLT3.[22] The phase III RATIFY trial randomized younger patients with newly diagnosed *FLT3*-TKD or *FLT3*-ITD mutated AML to midostaurin in combination with induction and consolidation chemotherapy or placebo with standard chemotherapy. Patients in the midostaurin arm had a significantly longer median overall survival (74.7 *vs.* 25.6 months, *P*=0.009) leading to approval of the regimen. In this study, MRD was not assessed; however, among patients undergoing allogeneic SCT, midostaurin in combination with chemotherapy led to a near significant increase in overall survival (*P*=0.07) and a significant decrease in cumulative incidence of relapse [hazard ratio (HR) 0.47, *P*=0.02].[23,24]

Next-generation FLT3 inhibitors have greater specificity and higher potency. Type I inhibitors such as gilteritinib and crenolanib are active against *FLT3*-TKD or *FLT3*-ITD mutations. In contrast, *FLT3*-TKD mutations in the activation loop and gatekeeper domain confer resistance to type 2 inhibitors such as quizartinib.[25] Active clinical trials evaluating next-generation FLT3 inhibitors in combination with induction and consolidation include NCT02283177 for crenolanib, NCT02236013 for gilteritinib, and

NCT02668653 for quizartinib. In a single-arm, phase II study (NCT02283177) of crenolanib in combination with standard induction and consolidation chemotherapy followed by crenolanib maintenance for 1 year, 24 out of 29 (83%) patients achieved CR and 20 out of 25 evaluable patients (80%) achieved MRD-negative disease, as determined by multiparameter flow cytometry.[26,27] Similarly, in a phase I study in patients with newly diagnosed *FLT3*-mutated AML, gilteritinib in combination with induction and consolidation led to a high CR rate of 77% (n=23/30).[28] A phase I study of quizartinib in combination with induction and consolidation in newly diagnosed AML led to CR in six of nine (67%) patients and a morphological leukemia-free state in two of nine (22%) patients with *FLT3*-ITD mutations.[29] The high response rates of next-generation FLT3 inhibitors in combination with chemotherapy in early phase studies led to the development of randomized studies comparing gilteritinib (NCT03836209) and crenolanib (NCT02283177) to midostaurin in combination with induction and consolidation chemotherapy.

Isocitrate dehydrogenases (*IDH1* and *IDH2*)

Mutations involving the isocitrate dehydrogenase-1 (*IDH1*) and -2 (*IDH2*) genes occur in about 6-10% and 9-13% of newly diagnosed cases of AML, respectively.[30-35] Mutant IDH has neomorphic enzyme activity leading to aberrant production of the oncometabolite 2-hydroxyglutarate.[33,36] Accumulation of 2-hydroxyglutarate competitively inhibits α-ketoglutarate-dependent enzymes including TET2, a DNA hydroxymethylase resulting in global hypermethylation, a block in cellular differentiation, an increase in self-renewal and enhancement of leukemic transformation.[36-38] Ivosidenib (Tibsovo) and enasidenib (Idhifa) are oral inhibitors of mutant IDH1 and IDH2, respectively and are approved for relapsed or refractory *IDH1*- and *IDH2*-mutant AML.[39,40] In relapsed or refractory AML, ivosidenib led to clearance of *IDH1* mutations in seven out of 25 (28%) patients who achieved either CR or CR with incomplete count recovery (CRi).[39] Similarly, treatment with enasidenib in relapsed or refractory AML led to *IDH2* mutation clearance in nine out of 29 (31%) patients achieving a CR.[41] Preliminary results from a phase I study of ivosidenib or enasidenib in combination with standard induction and consolidation chemotherapy in patients with newly diagnosed *IDH*-mutated AML demonstrated that the combination was well tolerated. Among patients treated with ivosidinib, responses [CR, CRi or CR with imcomplete platelet recovery (CRp)] occurred in 26 out of 28 (93%) and 33 out of 45 (73%) patients with *de novo* and secondary *IDH1*-mutated AML, respectively. In the enasidenib group responses occurred in 33 out of 45 (73%) and 20 out of 32 (63%) patients with *de novo* and secondary *IDH2*-mutated AML, respectively. Furthermore, *IDH*-mutation clearance was observed in nine out of 22 (41%) of the patients with *IDH1* mutations and in 11 out of 31 (30%) of those with *IDH2* mutations. MRD negativity by multiparameter flow cytometry was observed in eight out of nine (89%) patients with *IDH1* mutations and seven out of 12 (58%) of those with *IDH2* mutations.[42] Although IDH inhibitors and chemotherapy may increase MRD-negative rates, further studies are needed to determine the impact of the combination on survival after allogeneic SCT. A phase III, randomized study of ivosidenib or enasidenib in combination with

induction and consolidation chemotherapy followed by maintenance therapy in newly diagnosed AML or myelodysplastic syndrome (MDS) with excess blasts-2 with an *IDH1* or *IDH2* mutation (NCT03839771) will soon begin enrollment.

The observation that cancer stem cells are resistant to therapies targeting BCR-ABL in chronic myeloid leukemia and JAK2 V617F in myeloproliferative neoplasms raises concern regarding the ability of targeted therapies to eradicate leukemic stem cells.[21,22] If indeed FLT3 and IDH1/2 inhibitors are unable to eradicate leukemic stem cells, then targeted therapy may reduce or maintain low levels of bulk disease but will likely not be curative unless combined with allogeneic SCT or other therapies targeting leukemic stem cell. A leukemic stem cell population that is refractory to targeted therapy may also contribute to clonal evolution and the acquisition of secondary resistance mutations. Clinical studies evaluating FLT3 and IDH inhibitors as maintenance therapy after induction and consolidation and allogeneic SCT are also essential to determine the optimal duration of treatment. In the phase II AMLSG 16-10 trial, treatment with midostaurin in combination with induction and consolidation chemotherapy followed by maintenance midostaurin for 1 year after allogeneic SCT was associated with improved 1-year event-free survival when compared to that of historical controls with *FLT3*-ITD-mutated AML [HR 0.58; 95% confidence interval (95% CI): 0.48-0.7; *P*<0.001].[43]

Targets of apoptosis evasion

B-cell lymphoma 2 (BCL2)

Evasion of apoptosis is a hallmark of malignant tumor progression, allowing for tumor survival and resistance to cancer treatments.[37] The anti-apoptotic protein B-cell lymphoma 2 (BCL2) is overexpressed in AML and associated with resistance to chemotherapy and poor outcomes.[44] The prosurvival BCL2 family of proteins such as BCL2 and MCL1 sequester the apoptosis initiator protein BIM to prevent initiation of apoptosis.[45] Aberrant BCL2 expression is also essential for maintaining oxidative phosphorylation in quiescent leukemic stem cells. BCL2 inhibition reduces oxidative phosphorylation and preferentially induces cell death in leukemic stem cells.[46,47]

Venetoclax is an oral, BH3 mimetic that selectively binds BCL2, displacing pro-apoptotic proteins leading to apoptosis.[48] Monotherapy with venetoclax demonstrated clinical activity in early phase studies but was associated with modest response rates and a short duration of response.[49] Combinations of venetoclax with both low-dose cytarabine and hypomethylating agents in previously untreated, newly diagnosed elderly patients not eligible for chemotherapy resulted in high response rates and durable remissions leading, to Food and Drug Administration (FDA) approval of these regimens.[50,51] Venetoclax and hypomethylating agents led to a CR or CRi with MRD-negative disease by multiparameter flow cytometry in 45% of patients.[52] Similarly, treatment with venetoclax and low-dose cytarabine led to MRD-negative disease in 32% of patients in CR or CRi.[53] This spurred the development of trials evaluating venetoclax in combination with 7+3 (NCT03709758), CPX-351 (NCT03629171), or FLAG-IDA (NCT03214562) based induction regimens in newly diagnosed patients eligible for chemotherapy. In a phase I study of venetoclax in combination with FLAG-IDA in relapsed or refractory AML, treatment was well tolerated and eight of 11 patients achieved a CR or CRi.[54] The high MRD-negative rates associated with venetoclax combinations are encouraging; however, additional phase III studies are needed to determine if there is a survival benefit, in particular among patients who undergo allogeneic SCT.

Tumor protein 53 (*TP53*)

p53 is a transcription factor that is activated by cellular stress and promotes cell cycle arrest, senescence and apoptosis.[55] Loss of p53 induces oncogenic self-renewal in mouse hematopoietic progenitor cells.[56] In AML, inactivating mutations in the *TP53* gene occur in 7-18% of patients with newly diagnosed AML and are enriched in patients with other poor prognostic features including complex karyotype and therapy-related disease.[57,58] The co-occurrence of *TP53* mutations and a complex karyotype is associated with an especially dismal prognosis and a high rate of relapse after allogeneic SCT.[59] In AML, p53 inactivation more commonly results from overexpression of negative regulators.[60,61] MDMX and MDM2 inhibit p53 transactivation and induce its ubiquitination with subsequent degradation.[62] Idasanutlin is an oral selective MDM2 inhibitor capable of activating apoptosis in a p53-dependent manner.[63] Current trials evaluating the combination of this MDM2 inhibitor with chemotherapy include a phase I/II study (NCT03850535) of idasanutlin in combination with standard induction chemotherapy in newly diagnosed AML and a phase III study (NCT02545283) of idasanutlin with or without cytarabine in relapsed or refractory AML.

Despite many patients achieving deep and durable remissions with apoptosis inhibitors, primary and secondary resistance is known to occur. In particular, *RAS* pathway mutations and *TP53* mutations are associated with decreased responses to venetoclax.[47,51,64,65] MCL-1 also serves as a redundant pro-survival pathway that mediates resistance to venetoclax.[48,49] In cell lines resistant to BCL2 inhibition, idasanutlin led to induction of apoptosis through p53 activation and MCL1 degradation.[52] MCL1 mimetics currently in active trials as monotherapy and in combination with venetoclax include S64315 (Servier) (NCT02979366, NCT03672695), AMG 176 (Amgen) (NCT02675452, NCT03797261), and AMG 397 (Amgen) (NCT03465540). Additionally, *TP53*-mutant AML are resistant to MDM2 inhibitors and prolonged exposure to idasanutlin in cancer cell lines has been associated with the development of *TP53* mutations. APR-246 is a prodrug that is converted to the Michael acceptor methylene quinuclidinone, which covalently binds mutated p53 cysteine residues 124 and 277, leading to refolding and restoration of p53 function.[66,67] In a phase Ib study of APR-246 in combination with azacitidine in patients with *TP53*-mutant MDS and AML, all 11 evaluable patients responded with nine patients achieving CR (82%) and eight having clearance of p53 mutations (73%).[68]

Methylation

The hypomethylating agents 2'deoxy-5-azacitidine (decitabine) and 5-azacitidine (azacitidine) are approved for the treatment of MDS and newly diagnosed AML patients unfit for chemotherapy.[69-71] Azacitidine and

decitabine are nucleoside analogs that irreversibly bind the methylase DNMT1 leading to global hypomethylation, resulting in altered expression and cell death.[72,73] Low doses of hypomethylating agents disrupt immune evasion by inducing expression of tumor-associated antigens such as cancer/testis antigens in AML cell lines and antigen presentation molecules such as human leukocyte antigen class I antigens.[74-77] Hypomethylating agents also upregulate expression of endogenous retroviruses that activate viral recognition and interferon response pathways.[78,79] In contrast, treatment with hypomethylating agents induced expression of programmed cell death protein 1 (PD1), programmed death-ligand 1 and 2 (PD-L1 and PD-L2) and cytotoxic T-cell ligand antigen 4 (CTLA-4) in patients with MDS, AML and chronic myelomonocytic leukemia and was associated with resistance to treatment with hypomethylating agents.[80]

In the RELAZA2 trial, patients with advanced MDS or AML who achieved a CR after conventional chemotherapy or allogeneic SCT but had MRD, detected by either quantitative polymerase chain reaction for mutant *NPM1* or other leukemia-specific fusion genes or by flow cytometry, were treated with azacitidine.[81] The study met its primary endpoint with 31 out of 53 (58%) patients being relapse-free at 6 months. Reassessment of MRD status revealed that 19 out of 53 patients achieved MRD negativity and 12 out of 19 MRD-negative patients maintained MRD negativity without hematologic relapse during the median follow-up time of 23 months. *Post-hoc* analysis demonstrated a difference in relapse-free survival (HR 0.2, $P<0.0001$), but not overall survival (HR 0.4, $P=0.112$), between responders and non-responders to azacitidine.[81]

Immunotherapy targets

Immunotherapy is an approach that uses the potency of the immune system as a therapeutic modality against cancer.[82,83] The rationale for immunotherapy in AML lies in the curative potential of allogeneic SCT as post-remission therapy mediated by a graft-*versus*-leukemia effect. Similarly, immunotherapy leverages the adaptive immune system, specifically antibodies from B cells and the T-cell receptor on T cells to recognize antigens expressed on the cancer cells. In AML, immunotherapy has the potential to target unique leukemic stem cell surface antigens, thereby selectively eradicating these cells.

Immune checkpoints: PD1, PD-L1, CTLA-4

Immune modulating antibodies against negative regulators of T-lymphocyte activation, including anti-CTLA-4 and anti-PD1/PD-L1 have produced unprecedented rates of durable responses in a variety of malignancies.[83] In AML, responses to checkpoint inhibitors as monotherapy have been modest. A phase I study of patients treated with the anti-PD1 antibody pidilizumab revealed a response in only one out of eight patients with AML with a reduction in blast percentages from 50% to 5%.[84] A phase I/Ib study of 28 patients with relapsed hematologic malignancies after allogeneic transplantation, including 12 patients with AML, evaluated the anti-CTLA-4 antibody ipilimumab, given at doses of 3 mg/kg and 10 mg/kg. Responses were only observed with the ipilimumab 10 mg/kg dose in seven out of 22 (32%) patients and included CR in four patients with extramedullary AML and one

patient with MDS that progressed to AML. Dose-limiting chronic graft-*versus*-host disease of the liver or gut occurred in four patients but resolved when the treatment was withheld and steroids were administered.[85] Active phase II studies evaluating anti-PD1 therapy as post-remission treatment include NCT02532231 with nivolumab and NCT02708641 with pembrolizumab.

In order to enhance responses to checkpoint inhibition in AML, combinations with chemotherapy, hypomethylating agents, and other checkpoint inhibitors are under investigation. In a phase II study (NCT02464657) patients with newly diagnosed AML received induction chemotherapy with idarubicin and cytarabine followed by nivolumab 3 mg/kg starting on day 24 and continued every 2 weeks for up to 1 year; 34 out of 44 patients (77%) achieved a CR or CRi and 18 out of 43 (53%) had undetectable MRD by multiparameter flow cytometry. Responses were durable and the median overall survival was 18.5 months, which compared favorably to that of a contemporary cohort of patients treated with idarubicin and cytarabine induction alone. Among 18 patients who underwent allogeneic SCT, 13 (72%) developed graft-*versus*-host disease and eight responded to treatment.[86] Increased expression of PD1, PD-L1, and CTLA-4 is associated with resistance to treatment with hypomethylating agents but has the potential to sensitize leukemia cells to checkpoint-blocking monoclonal antibodies.[74,80] In a phase II study of azacytidine and nivolumab 3 mg/kg on days 1 and 14 in relapsed/refractory AML, responses occurred in 23 patients (overall response rate, 33%) including 15 patients (22%) with CR or CRi. The median overall survival for all patients enrolled was 6.3 months, while that of the patients who achieved any type of response (CR, CRi, partial response or hematologic improvement) or had stable disease was 16.2 months. When compared to controls from historical hypomethylating agent-based clinical trials, patients receiving nivolumab and hypomethylating agents had an increased response rate (33% *vs.* 20%) and significantly longer median overall survival (6.3 *vs.* 4.6 months).[87] The phase II PEMAZA study is evaluating azacitidine in combination with pembrolizumab in patients achieving CR after induction chemotherapy but with detectable MRD (NCT03769532).

Dendritic cells

Dendritic cells are the most potent antigen-presenting cells capable of priming new responses or enhancing existing antigen-specific immune responses.[88,89] Mature dendritic cells facilitate cytotoxic T-lymphocyte activation through antigen presentation on major histocompatibility complex class 1 molecules, termed cross-presentation and by upregulating co-stimulatory molecules, such as CD80 and CD86.[89,90] Dendritic cell vaccination approaches differ in the source of dendritic precursors, maturation methods, target antigen, antigen loading, and in the administration of the vaccine.[89] A phase II study of patients with AML in first CR after induction chemotherapy at high risk for relapse and without a matched sibling donor for allogeneic hematopoietic SCT revealed that treatment with *WT1* mRNA-electroporated dendritic cell vaccine led to a clinical response in 13 out of 30 patients (30%) with nine patients achieving molecular remission by *WT1* transcript levels.[91] The 5-year overall survival rate was 40% among vaccine recipients and compared favorably to a 5-year overall survival rate of 24.7% observed in historical con-

trols.[91] Additionally, the dendritic cell vaccine elicited *WT1*-specific CD8^{+} T-cell responses resulting in expression that correlated with long-term survival.[91] Another prospective study of a vaccine composed of patient-derived AML cells fused with autologous dendritic cells in patients in CR after induction chemotherapy not eligible for allogeneic SCT led to sustained remission in 12 of 17 patients receiving at least one dose of vaccine and a 4-year progression-free survival rate of 71%: the median progression-free and overall survival had not been reached.[92] The vaccine was well tolerated with the most common adverse events being erythema, pruritis and/or induration at the vaccine site.[92] The dendritic cell/AML fusion also induced CD8^{+} T-cell specific responses and an increased circulating leukemia-reactive T-cell population that persisted for more than 6 months.[92]

Antibody drug conjugates and bispecific T-cell engaging therapy

Cluster of differentiation 33 (CD33)

The development of an antibody-based therapy targeting antigens expressed on leukemic blasts to eradicate MRD is supported by the efficacy of the CD19/CD3 bispecific antibody, blinatumomab in B-cell acute lymphoblastic leukemia.[73] CD33 is a transmembrane sialic acid-binding immunoglobulin-like lectin (SIGLEC) family protein that is expressed by cells of the myeloid lineage but not hematopoietic stem cells.[93-95] CD33 is expressed on leukemic blasts as well as CD34^{+}/CD38^{-} leukemic stem cells.[96] CD33 levels are highest in acute promyelocytic leukemia and AML with *NPM1*, *FLT3*-ITD and *KMT2A* mutations and lower in those with core-binding factor translocations or complex cytogenetics.[97] Gemtuzumab ozogamicin (GO) is a human antibody conjugated to a DNA-damaging calicheamicin derivative by an acid-labile linker.[98] Based on promising results from three single-arm phase II studies at a dose of 9 mg/m^2 given every 2 weeks, GO was initially granted FDA approval for patients >60 years of age with CD33^{+} AML who were not candidates for aggressive chemotherapy.[99] However, GO was later withdrawn from the commercial market in October 2010 after the confirmatory phase III SWOG S0106 study showed no survival benefit and increased treatment-related mortality in patients treated with GO compared to those given standard induction.[100] Subsequent studies have evaluated reduced and fractionated dosing of GO to decrease treatment-related toxicity.[100-103] A large meta-analysis from five randomized controlled trials of patients with newly diagnosed AML receiving GO with induction chemotherapy revealed that the addition of GO was associated with a reduced risk of relapse (odds ratio 0.81, $P=0.0001$) and improved overall survival at 5 years (odds ratio 0.9, $P=0.01$), especially in patients with favorable and intermediate-risk cytogenetics.[104] Additionally, the NCRI AML17 trial demonstrated a lower rate of veno-occlusive disease and early mortality but no difference in relapse or survival at 4 years between patients given GO at a dose of 3 mg/m^2 or a dose of 6 mg/m^2.[105] As a result GO received FDA approval for adults with newly diagnosed AML, whose tumor expresses the CD33 antigen. Retrospective analysis of adult patients with *NPM1*-mutated AML enrolled in the ALFA-0701 trial revealed that GO in combination with induction chemotherapy increased the proportion of patients with MRD-negative disease at the end of treatment, as determined by *NPM1* gene transcript levels, when compared to those treated with chemotherapy alone (91% *vs.* 61%, $P=0.028$).[106] This has led to a phase II trial of fractionated GO on days 1, 4, and 7 in patients with MRD after at least one cycle of induction chemotherapy. (NCT03737955)

Clinical trials evaluating MRD-directed therapies

Intensive chemotherapy combinations

Target	Therapy (Clinical trial ID)
FLT3 ITD/TKD	Gilteritinib (NCT02236013, NCT03836209)
FLT3 ITD/TKD	Crenolanib (NCT02236013, NCT03258931)
FLT3 ITD	Quizartinib (NCT02668653)
IDH1/IDH2	Ivosidenib or enasidenib (NCT03839771)
IDH2	Enasidenib (NCT03825796)
BCL2	Venetoclax (NCT03709758, NCT03214562, NCT03629171)
MDM2	Idasanutlin (NCT03850535, NCT02545283)
PD1	Nivolumab (NCT02464657)
PD1	Pembrolizumab (NCT02768792)
CD33	Gemtuzumab (NCT03531918, NCT00801489, NCT03839446, NCT03900949, NCT03904251)

Non-chemotherapy post-remission therapies

Target	Therapy (Clinical trial ID)
PD1	Pembrolizumab + azacitidine (NCT03769532)
PD1	Pembrolizumab (NCT02708641)
PD1	Nivolumab (NCT02532231)
WT1	WT1 mRNA DC vaccine (NCT01686334)
AML antigens	DCP-001 DC vaccine (NCT03697707)
AML antigens	DC/ AML cell fusion vaccine (NCT03059485)
CD33	Gemtuzumab (NCT03737955)
CD123	SL-401 (NCT02270463)

Figure 1. Active clinical trials evaluating minimal residual disease-directed therapies arranged by trial design. Trials with induction and consolidation-based combinations are shown on the left, non-chemotherapy post-remission therapies are shown on the right. Studies evaluating post-allogeneic transplant minimal residual disease therapies are not included.

Table 1. Outcomes of clinical trials targeting minimal residual disease with induction chemotherapy or as post-remission therapy.

MRD target	Drug name	Trial	Combination	Population	Clinical phase	Efficacy	MRD negative rate	Ref.
FLT3 TKD *FLT3* ITD	Gilteritinib (ASP 2215)	NCT02236013	Gilteritinib with induction and consolidation chemotherapy	Newly diagnosed AML	I	Among *FLT3* mutated patients: CR 23/30 (77%) CRc (CR/CRp/ CRi) 27/30 (90%) CRc 100% at 120 mg dose	Not reported	(28)
FLT3 TKD *FLT3* ITD	Crenolanib	NCT02283177	Crenolanib with induction and consolidation chemotherapy	Newly diagnosed *FLT3*-mutated AML	II	CR 24/ 29 (83%) 2 patients relapsed with a median follow-up of 14 months	20/25 (80%) by MPFC	(26, 27)
FLT3 ITD	Quizartinib	NCT01892371	Quizartinib with induction and consolidation chemotherapy	Newly diagnosed AML	I	Among *FLT3*-ITD mutated patients CR 6/9 (67%) MLFS 2/9 (22%)	Not reported	(29)
IDH1 *IDH2*	Ivosidenib Enasidenib	NCT02632708	Ivosidenib or enasidenib in combination with induction and consolidation chemotherapy	Newly diagnosed AML with an *IDH1* and/or *IDH2* mutation	I	Ivosidenib *De novo* AML CRc (CR, CRi, CRp) 26/28 (93%) Secondary AML CRc 6/13 (46%) Enasidenib *De novo* AML CRc 33/45 (73%) Secondary AML CRc 20/32 (63%)	*IDH1* MC: 9/22 (41%) of responding patients by NGS *IDH2* MC: 11/37 (30%) of responding patients by NGS	(42)
BCL-2	Venetoclax	NCT03214562	Venetoclax in combination with FLAG-IDA	Relapsed or refractory AML	I	CR+CRi 8/11 (73%)	Not reported	(54)
DNMT1	Azacitidine	NCT01462578	None	Advanced MDS or AML in CR, MRD⁺ after induction or allo-SCT	I/II	Primary endpoint: relapse-free at 6 months post- treatment 31/53 (58%)	19/53 (36%) by *NPM1* or fusion gene transcript levels	(81)
PD1	Nivolumab	NCT02464657	Nivolumab in combination with standard induction and consolidation chemotherapy	High-MDS or AML, chemotherapy naïve	II	CR+CRi 34/44 (77%)	18/34 (53%) by MPFC after induction	(86)

AMG 330 is a bispecific T-cell engager (BiTE) antibody construct that binds CD33 on leukemic blasts and CD3 on T cells.[107] Preliminary results from a phase I study (NCT02520427) of AMG330, revealed serious adverse events in 23 out of 35 patients (66%) including cytokine release syndrome in 11 patients. The cytokine release syndrome was mitigated with step-up dosing, corticosteroid pretreatment, intravenous fluids, tocilizumab, and drug interruption. Two patients had a CR and two had a CRi during dose escalation.[108]

Cluster of differentiation 123 (CD123)

CD123 is the alpha chain of the interleukin-3 receptor heterodimer and is expressed at higher levels in leukemic stem cells than on normal hematopoietic bone marrow stem cells.[109,110] CD123⁺CD34⁺CD38⁻ leukemic cells are capable of initiating and maintaining leukemia in NOD/SCID mice.[110] IMGN632 is a CD123⁻targeting antibody-drug conjugate consisting of a CD123 antibody linked to a DNA alkylating indolino-benzodiazepine dimer (IGN) via a protease cleavable linker.[111] In a phase I trial of IMGN632 (NCT03386513) in patients with relapsed or refractory CD123⁺ hematologic malignancies, four out of 12 (33%) patients with AML achieved a CR or CRi.[112] Elzonris (tagraxofusp or SL-401) is a recombinant fusion protein consisting of human interleukin-3 fused via a Met-His linker to a truncated diptheria toxin that is currently FDA-approved for the treatment of blastic plasmacytoid dendritic-cell neoplasm.[113,114] The interleukin-3 domain binds to the interleukin-3 receptor leading to translocation of the diphtheria A fragment and thus to inactivation of protein synthesis and cell death. A phase I/II study of SL-401 as consolidation therapy for patients in first or second CR is ongoing. (NCT02270463)

C-type lectin-like molecule-1 (CLL1 or CLEC12A)

C-type lectin-like molecule-1 (CLL1 or CLEC12A) is a transmembrane glycoprotein that functions as an inhibitory receptor. CLL-1 is expressed on leukemic blasts in the majority of cases and selectively expressed in leukemic CD34⁺CD38⁻ cells but not normal hematopoietic stem cells. Moreover, CLL1⁺ CD34⁺ cells are serially trans-

MRD target	Drug name	Trial	Combination	Population	Clinical phase	Efficacy	MRD negative rate	Ref.
Dendritic cells	WT1- mRNA dendritic cells	NCT00965224	None	AML or MDS RAEB1/2 in PR or CR or smoldering course with high risk of relapse	II	Clinical response rate 13/30 (43%) 5-year OS 40% *vs*. 24.7% historical control	9/30 (30%) by *WT1* transcript levels	(91)
Dendritic cells	hTERT-dendritic cells	NCT00510133	None	AML in first or second CR after induction or consolidation	II	Recurrence free at a median 52 months follow up 11/19 (74%)	Not reported	(125)
Dendritic cells	AML/ dendritic cell fusion	NCT01096602	None	Newly diagnosed or first relapsed AML in CR ineligible for allo-SCT	II	4-year progression-free survival 71%	Not reported	(92)
Dendritic cells	DCPrime (DCP-001)	NCT00965224	None	AML in CR after ≥ 1 course of chemotherapy and age >60 years or < 60 years without allo-SCT donor	II	Clinical response rate 12/20 (43%) 5-years OS 40%	9/13 by normalization of *WT1* transcript levels	(126)
CD33	Gemtuzumab ozoganicin	NCT00927498	GO in combination with standard induction and consolidation	Untreated *de novo* AML	III	GO *vs.* control Median as: 27.5 *vs*. 21.8 months (*P*=0.16) Median event-free survival: 17.3 *vs*. 9.5 months (*P*<0.01)	Post- induction GO *vs.* control 39% *vs*. 7%, *P*<0.01 Post-treatment GO *vs.* control 91% *vs.* 61%,*P*=0.03 by *NPM1* mutation transcript levels	(106, 127)

MRD: minimal residual disease; Ref.: references; *FLT3* TKD: fms-like tyrosine kinase 3 (*FLT3*) gene tyrosine kinase domain mutations; *FLT3* ITD: *FLT3* internal tandem duplications; AML: acute myeloid leukemia; CR: complete remission; CRi: complete remission with incomplete count recovery; CRp: complete remission with incomplete platelet recovery; CRc: complete remission - composite; MPFC: multiparameter flow cytometry; MLFS: morphological leukemia-free state; *IDH1/ IDH2*: isocitrate dehydrogenase-1 and -2; MC: mutation clearance; NGS: next-generation sequencing; BCL-2: B-cell lymphoma-2; FLAG-IDA: fludarabine, cytarabine, idarubicin, and granulocyte colony-stimulating factor; *DNMT1*: DNA methyltransferase 1; allo-SCT: allogeneic stem cell transplant; *NPM1*: nucleophosmin 1; PD1: programmed death protein 1; MDS: myelodysplastic syndrome; *WT1*: Wilms tumor 1; RAEB 1/2: refractory anemia with excess blasts; PR: partial response; OS: overall survival; hTERT: human telomerase reverse transcriptase; GO: gemtuzumab ozogamicin

plantable in NOD/SCID mice suggesting a self-renewal ability.[115] MCLA-117 is a potent bispecific T-cell engager that directs CD3+ T cells to leukemia cells expressing CLL1.[116] A phase I clinical trial of MCLA-117 in patients with relapsed or refractory AML or in elderly patients not eligible for chemotherapy is currently recruiting patients (NCT03038230).

Chimeric antigen receptor therapy

Chimeric antigen receptors (CAR) are engineered extracellular receptors joined to intracellular signaling domains that reprogram immune cells for therapeutic purposes.[117] The development of second-generation CAR with an additional CD28 or 41BB co-stimulatory domain has allowed for effective responses.[117] CAR-T cells kill tumor cells and promote immune surveillance directly by persisting and indirectly by cross-priming tumor-infiltrating lymphocytes through antigen release.[10-12] CAR therapy targeting CD19 is extremely effective in B-cell malignancies, resulting in the approval of tisagenlecleucel (Kymriah) for the treatment of pediatric B-cell acute lymphoblastic leukemia that is refractory or in second relapse and axicabtagene ciloleucel (Yescarta) in large B-cell lymphomas after two or more lines of systemic therapy.

A phase I study of autologous CAR-T cells with specificity for a difucosylated carbohydrate antigen Lewis (Le)-Y coupled to the cytoplasmic domains of CD28 and TCR-ζ chain produced a transient cytogenetic remission in one out of three patients with MRD at the time of infusion. Another patient with MRD prior to the infusion of CAR-T cells had persistent cytogenetic MRD but sustained MRD negativity by multiparameter flow cytometry for 23 months. Although LeY CAR-T cells persisted up to 10 months after infusion, most patients relapsed within the first 5 months suggesting possible antigen escape. None of the patients developed grade 3 or 4 toxicity.[118]

In AML, the ideal CAR target that is highly expressed in myeloid blasts and spares normal myeloid progenitor cells and vital tissues has not yet been identified. In preclinical studies anti-CD33 CAR-T cells resulted in a reduction of normal myeloid progenitors.[119,120] Similarly, anti-CD123 CAR-T cells have demonstrated myeloablation in a xenograft mouse model.[121] CLL1 CAR-T cells are cytotoxic to normal mature myeloid cells but not to normal myeloid progenitor cells or hematopoietic stem cells.[122] An extensive proteomic and transcriptomic analysis revealed four potential CAR targets, ADGRE2, CCR1, CD70, and LILRB2, with high expression in AML, AML leukemic stem cells, and low expression in normal tissues, normal hematopoietic stem and progenitor cells and resting/acti-

vated T cells. However, none of the targets showed a profile comparable with that of CD19 in B-cell malignancies.[123] This suggests that combinatorial strategies may be necessary for targeting AML with CAR-T cells. An approach for combination includes bispecific T cells that co-express two CAR or a dual-specific CAR (CAR/CAR T cells) allowing T-cell recognition of target cells that express any of two given antigens.[123] Alternatively, the combination of a CAR that alone is insufficient to activate a T cell and a chimeric co-stimulatory receptor (CAR/CCR T cells) restricts T-cell recognition to dual antigen-expressing target cells. The latter approach requires pan-expression of CAR targets on AML cells, which was not seen by Perna and colleagues.[117] Persistent CAR-T-cell mediated myelotoxicity may necessitate incorporation of CAR-T cells with conditioning regimens prior to allogeneic SCT. An alternative approach currently in development is the use of genetically modified donor allografts that lack expression of CAR-T-cell targets, such as CD33, followed by administration anti-CD33 CAR-T cells after transplantation.[124]

Conclusion

Advancements in flow cytometry, quantitative polymerase chain reaction analysis and more recently next-generation sequencing continue to push the limits of detection of residual disease and open the door to therapies aimed at eradicating it. As MRD is a significant negative prognostic factor for relapse and survival in AML following allogeneic SCT, therapies capable of eliminating MRD are urgently needed to increase the number of patients cured of their disease. Here, we have reviewed the most promising MRD targets with therapeutic potential based on efficacy in reducing MRD and potential for targeting leukemia repopulating cells mediating relapse. The targets discussed are by no means an exhaustive list and will continue to be refined as single-cell sequencing and xenograft studies better characterize leukemia populations in MRD that mediate relapse. Ultimately, incorporation of MRD into clinical practice will require pivotal trials that demonstrate an improvement in survival with MRD-directed approaches. Moving forward with MRD-targeted therapies will require a standardized method for detecting MRD and rigorous assessment of the safety and efficacy of these therapies.

The European LeukemiaNet MRD working group has recently provided recommendations for assessment of MRD by multiparameter flow cytometry and molecular testing.[2] These consensus recommendations aid the standardization of MRD testing should be incorporated into all AML clinical trials. Additional issues that will need to be addressed include the optimal timing of MRD assessments. MRD after induction, second induction and consolidation may have varying prognostic impact. Differences in time to initial response and the duration of response among MRD therapies may also affect the interval of MRD assessments. In particular, IDH inhibitors typically take a longer time to produce an initial response and may warrant later MRD assessments at later timepoints than MRD therapies with a faster onset of effect.

The use of MRD as a surrogate endpoint for survival for clinical trials in AML has the potential to accelerate drug development. Although MRD has a significant impact on prognosis, the mortality associated with treating MRD also needs to be considered. The experience with CD33-targeted therapies demonstrates that toxicities associated with treatment may outweigh the potential benefit associated with eradicating MRD. In addition, MRD as a surrogate endpoint would not capture the impact of MRD therapies on transplant outcomes. For example, vadastuximab and GO were associated with an increased risk of veno-occlusive disease after transplantation. T-cell-activating therapies such as checkpoint inhibitors, dendritic cell vaccines and CAR-T cells have the potential to increase the risk of graft-*versus*-host disease after transplantation. Therefore, initial studies evaluating the safety of MRD-directed therapies should include post-transplant outcomes to identify late toxicities. The development of MRD-directed therapies may be facilitated in other ways. Similar to clinical trials in acute lymphoblastic leukemia and pediatric AML, current and future clinical trials in patients with AML who are fit for allogeneic SCT should include an intensification arm with MRD-directed therapies. This has the potential to increase the number of trials evaluating MRD therapies.

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