The new perspectives of targeted therapy in acute myeloid leukemia

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Abstract

Acute myeloid leukemia (AML) is a heterogeneous disease and the results of previous treatment with cytotoxic drugs have not been satisfactory. This situation has prompted investigations into novel approaches. The breakthrough in therapy brought by all-trans retinoic acid (ATRA) in acute promyelocytic leukemia (APL) and tyrosine kinase inhibitors in neoplasms with the Philadelphia chromosome has encouraged the search for other effective targeted therapies. Among the tested substances are higher molecular mass drugs such as antibodies and various small molecules: kinase inhibitors, cell pathway inhibitors and epigenetic modulators. So far, the U.S. Food and Drug Administration (FDA) has approved the antibody-drug conjugate gemtuzumab ozogamycin (GO), the tyrosine kinase inhibitor midostaurin and the IDH2 inhibitor enasidenib. These studies have led to a better understanding of the mechanisms of leukemogenesis and may soon allow for differentiating treatments depending on baseline mutational complements. Some innovative drugs described in this article have strong therapeutic potential, but there is still a long way to go before actual success in targeted treatment.

Key words: immunotherapy, target therapy, acute myeloid leukemia
Acute myeloid leukemia (AML) occurs in 3–4 people out of 100,000 with a median age of 67 years. The 5-year survival rate is 20%. The course of the disease depends on many factors, including cytogenetics, molecular genetics, comorbidity score, and the patient’s age. Long-term survival rates for patients <65 years of age and ≥65 years of age are 40% and 5%, respectively. Complete remission (CR) is achieved in 66% of elderly patients; in this group, the disease reoccurs in 16% of cases. Allogenic stem cell transplantation provides a chance for recovery from AML and longer overall survival (OS). The unsatisfactory results of previous AML treatment have encouraged the study of intracellular mechanisms that prolong the survival of leukemic cells and their resistance to apoptotic stimuli.

These genetic changes have inspired the search for an effective targeted therapy. The first drug of this kind was all-trans retinoid acid (ATRA) in acute promyelocytic leukemia (APL), directed against the fusion of the genes RARA and PML, which is present on the surface of the myeloblasts in over 90% of AML cases and is toxic to DNA calicheamicin. Gemtuzumab ozogamicin (GO, Mylotarg) is an immunono conjugate compound created by the CD33 antibody, which is present on the surface of the myeloblasts in over 90% of AML cases and is toxic to DNA calicheamicin. This epitope to CD33 antigen, occurs in many expression and functional variants, and only some of these epitopes are sensitive to the cytotoxicity caused by GO. Despite GO withdrawal caused by toxicity in early clinical trials, subsequent trials have renewed the interest in this drug. In a meta-analysis of prospective phase III trials, it was proven that the use of GO in inductive therapy in variable age groups prolongs relapse-free survival (RFS) with tolerable adverse effects. Overall survival elongation thanks to GO was proven in most clinical trials in age-differentiated groups, but the benefit for patients with adverse cytogenetics is controversial. Promising effects were observed among fit patients >50 years old and ≥60 years old not qualified for allogenic stem cell transplantation (alloSCT). The results of these trials suggest the advantage of using GO in bridge therapy before alloSCT with other cytostatics. Promising results were achieved with GO applied in AML relapse after stem cell transplantation (SCT) therapy. On September 1, 2017, the U.S. Food and Drug Agency (FDA) approved GO for treatment in adults with newly diagnosed CD33+ AML.

BI 836858, lintuzumab (SGN-33; HuM195) and vadas tuximab talirine are new anti-CD33 antibodies. Lintuzumab used with standard chemotherapy resulted in OS prolongation in a group of previously untreated patients who were unfit for intensive chemotherapy, aged 60–87 years, with an intermediate or adverse prognosis. The use of vadsatuximab talirine is undergoing a phase III trial in a group of elderly patients.

AMG 330 (bispecific T-cell engager antibody [BiTE]) is a new antibody directed against both CD33 and CD3, which are present on the surface of T lymphocytes. Bi-specific T-cell engager antibody was created to engage the cytotoxic response of T cells against leukemic cells.

### Table 1. Targeted drugs in AML treatment

<table>
<thead>
<tr>
<th>Target</th>
<th>Drug</th>
<th>Group</th>
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<tbody>
<tr>
<td>CD33</td>
<td>gemtuzumab ozogamicin, lintuzumab, vadas tuximab talirine</td>
<td>high molecular mass drugs</td>
</tr>
<tr>
<td>CD33, CD3</td>
<td>AMG 330</td>
<td></td>
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<tr>
<td>FLT3</td>
<td>1st-generation: sorafenib, midostaurin, lestaurtinib, sunitinib, tivantinib, pacritinib; 2nd-generation: quazartinib, crenolamid, ponatinib, PLX3397, gletirtinib, JH-Ix-179</td>
<td>tyrosine kinase inhibitors</td>
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<tr>
<td>PLK1</td>
<td>volasertib</td>
<td>low molecular mass drugs</td>
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<tr>
<td>CDK</td>
<td>flavopiridol</td>
<td>cell pathway inhibitors</td>
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<tr>
<td>AURK</td>
<td>alisertib, barasertib</td>
<td></td>
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<tr>
<td>PIM</td>
<td>AZD1208, SGI-176</td>
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<tr>
<td>IDH</td>
<td>cenasidenib</td>
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<tr>
<td>GLI</td>
<td>GANT61</td>
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<tr>
<td>BCL-2</td>
<td>navitoclax, veno tixax</td>
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<tr>
<td>NAE</td>
<td>penovidistat</td>
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<tr>
<td>topoisomerase</td>
<td>vosaroxin</td>
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<tr>
<td>BET</td>
<td>OTX015, ARV-825</td>
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<tr>
<td>LSD1</td>
<td>ORY-1001, GSK2879552</td>
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<td>HDAC</td>
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<tr>
<td>DOTL1</td>
<td>pnometostat</td>
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<tr>
<td>PD1/PDL1</td>
<td>nivolubum</td>
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<tr>
<td>MDM2</td>
<td>RG7112, idasanutin</td>
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</tbody>
</table>

FLT3 – FMS-like tyrosine kinase-3; PLK1 – polo-like kinase 1; CDK – cyclin-dependent kinases; AURK – aurora kinase; PIM – proviral insertion in +murine; IDH – isocitrate dehydrogenase; GLI – glioma; BCL-2 – B-cell lymphoma 2; NAE – NEDD8 activating enzyme; BET – bromodomain and extraterrormal; LSD1 – lysine-specific demethylase; HDAC – histone deacetylase; PD1/PDL1 – programmed death-1/programmed death-1 ligand.
in order to avert their immunological escape.\textsuperscript{13} It showed the best results among previously untreated AML patients with standard prognosis. In addition, tetravalent bispecific anti-CD3/CD3, bispecific anti-CD3 and C-type lectin-like molecule-1 (CCL-1), which can be found on most leukemic cells, are being investigated in animals.\textsuperscript{14}

Ulocuplumab (BMS-936564/MDX-1338) is a monoclonal antibody which inhibits the binding of the CXC chemokine receptor 4 (CXCR4) to stimulate migration from the bone marrow to peripheral blood stromal cell-derived chemokine CXC motif ligand 12 (CXCL12). CXCR4 is overexpressed on AML blasts, among others. CXCR4 inhibition restricts AML cell growth and induces their apoptosis. In the first clinical trial on patients with relapsed/refractory AML, ulocuplumab in combination with mitoxantrone, etoposide and cytarabine led to CR with incomplete marrow recovery (CRi) in 51% of the group of 73 patients.\textsuperscript{15} In December 2015, the FDA decided to use ulocuplumab as an orphan drug.

Low molecular mass drugs

The FMS-like tyrosine kinase-3 (FLT3) gene mutations FLT3-ITD and FLT3-TDK occur in 30% and 7% of AML cases, respectively. The FLT3 kinase inhibitors may be divided into 1\textsuperscript{st}- and 2\textsuperscript{nd}-generation drugs. The first group (1\textsuperscript{st}-generation) are polykinase inhibitors, while the newer drugs are more selective molecules, which makes them safer and more effective.\textsuperscript{16} The mutated FLT3 gene has variable sensitivity to different drugs.\textsuperscript{17}

Sorafenib is a multikinase inhibitor. It inhibits C-RAF, FLT3, VEGFR2, VEGFR3, and PDGFR family kinases. The action of sorafenib is amplified by the activation of p-AMPK by metformin, which potentiates the proapoptotic and antiproliferative effect. Glycolysis inhibition also plays a synergistic role, which has been proven in an animal model.\textsuperscript{18} According to the National Comprehensive Cancer Network (NCCN) guidelines, sorafenib is used in the treatment of refractory/recurrent AML, in monotherapy or with other drugs. Despite a higher response rate in FLT3+ patients than in FLT3- patients, sorafenib does not influence OS. In addition, it is not effective in elderly patients. When added to standard chemotherapy in patients younger than 60 years, sorafenib prolongs DFS.\textsuperscript{19} Moreover, sorafenib is effective before and after SCT it prolongs DFS and OS, maintains remission in sustained therapy after SCT in 100% of patients, and has a hematological response >90% in AML recurrence after SCT.\textsuperscript{20–22} Promising effects have been reported from combining sorafenib with hypomethylating agents, ATRA or homoharringtonin, especially in refractory AML.\textsuperscript{23–25} The greatest antiproliferative and proapoptotic accuracy in preclinical trials on human leukemia cell lines was demonstrated with a composite of 3 kinase inhibitors: FLT3 (sunitinib), PI3K (PF-04691502) and GLI1/2 (GANT61).\textsuperscript{26} The use of sunitinib with standard inductive and life-sustaining therapy showed no benefits because of toxicity. The combination of FLT3 and AKT inhibitors is associated with the induction of resistance due to the protective effect of stroma on leukemia cells.

Lestaurltinib is a multikinase inhibitor whose targets include JAK-2. Added to a standard first-line FLT3+ AML therapy, it does not provide any benefits.\textsuperscript{27}

Midostaurin used in monotherapy or in combination with different cytostatics in intensive chemotherapy has prolonged OS with tolerable toxicity in FLT3+ AML patients: studies include the addition of midostaurin in inductive or consolidative therapy, in sustaining therapy, in AML relapse, and in bridge therapy before SCT.\textsuperscript{28–31} The greatest benefits were observed in a group of patients who did not qualify for SCT previously untreated with FLT3 inhibitors. A promising effect was achieved in preclinical trials by a combination of ATRA and midostaurin due to the synergic effect against leukemic cells. Midostaurin combined with other drugs was registered by the FDA in the treatment of refractory/relapsed AML with the FLT3 mutation.\textsuperscript{32}

Crenolamid represents a new, selective FLT3 kinase inhibitor group. It is currently in phase II trials. There is a possible synergy in using it with sorafenib against leukemic cells.

Quizartinib (AC220) has a high affinity for wild-type and mutated FLT3 kinase and has successfully completed phase I trials on a pediatric FLT3+ AML population.\textsuperscript{33} Its activity was demonstrated in refractory/reapse AML. AKN 028, a dose-dependent FLT3 kinase inhibitor that stops the cell cycle, is still being investigated. Glitertitinib is a selective FLT3/ASXL1 inhibitor. Used in a group of 80 patients with refractory/relapse FLT3+ AML, it resulted in a 55% response rate and it doubled OS. Glitertitinib is currently under investigation in supportive care and rescue therapy. Kinase inhibitors may generate secondary mutations.\textsuperscript{34} In trials on human leukemic cells, FLT3 mutations resistant to AC220 and sorafenib succumbed to a new kinase inhibitor, TT-3002. New molecules are being investigated, for example, AMG 925.

Volasertib (BI6727) is a polo-like kinase (PLK) inhibitor. Polo-like kinases play a key role in mitosis. There is higher PLK expression in AML, Hodgkin lymphoma (HL), non-small-cell lung cancer (NSCLC), and breast cancer, and its concentration correlates with mortality.\textsuperscript{35} In preclinical trials on leukemic cells acquired from patients, volasertib proved effective in monotherapy and with antimetabolites, hypomethylating agents and quizartinib. Associated with small doses of cytarabine, it increased CR and DFS in a previously untreated group of patients who, in the investigators’ opinion, were unfit for intensive chemotherapy. In 2016, volasertib was called a breakthrough drug in the treatment of AML by the FDA.\textsuperscript{36}

Flavopiridol (alvocidib) is a cyclin-dependent kinase (CDK), which induces cell cycle arrest and apoptosis.
in leukemic cells. The latest studies show no benefits over standard chemotherapy in previously untreated AML patients with an intermediate or adverse prognosis.

Alisertib (MLN8237) is an orally taken aurora kinase A (AurKA) inhibitor. Its synergy with cytarabine has been proven in preclinical tests. In phase II studies on a group of refractory/relapsed AML patients who did not qualify for standard chemotherapy, the disease was stabilized in nearly half of the patients.

Barasertib is an aurora kinase B (AurKB) inhibitor. It is more effective in prolonging OS in patients >60 years of age, but is more toxic in comparison with cytarabine. AZD1208 is an inhibitor of all proviral insertion in +murine (PIM) kinases, which in correlation with PIM1 expression inhibits the growth of 5 of the 14 AML cell lines, including FLT3-ITD+. The PIM kinase inhibitors show synergy with mTOR and AKT inhibitors in suppressing leukemic cells and in sensitizing AML cells to topoisomerase II inhibitors. SGI-1776 acts similarly; it has also been tested in NCL2 inhibition.

**Cell pathway inhibitors**

Isocitrate dehydrogenase (IDH) takes part in lipid metabolism and the Krebs cycle, and it catalyzes the transformation of isocitrate to α-ketoglutarate. The IDH1 and IDH2 gene mutations occur in 11% and 12% of AML cases, respectively. Enasidenib (AG-221/CC-9007) is the first selective IDH2 inhibitor to induce the differentiation of leukemic cells. Enasidenib is taken orally and is active in monotherapy. It has been well-tolerated in phase II studies on patients with refractory/relapsed AML and has achieved an overall response rate (ORR) of 40% with a median response duration of 6 months. On August 1, 2017, enasidenib was approved by the FDA for the treatment of adult patients with relapsed/refractory AML with the IDH2 mutation.

The expression of the glioma (GLI) family transcription factors, which are the last part of the Hedgehog proliferative signal pathway, is a negative prognostic factor in AML. This finding has inspired the search for GLI inhibitors. The small-molecule inhibitor GANT61 is currently being studied.

Navitoclax (ABT-263) is a BCL-2, BCL-XL and BCL-W protein family inhibitor. Its antitumor activity is restricted by adverse effects. Venetoclax (ABT-199) is a small-molecule antiapoptotic BCL-2 protein inhibitor which is registered by the FDA for treating chronic lymphocytic leukemia (CLL) and AML. In a high-risk recurrent/refractory AML patient group, a 38% response rate was achieved, half of these being complete responses according to the International Working Group (IWG) criteria.

There have been studies on molecules influencing the suppressor protein p53 pathway, for instance, the HDML2 inhibitor CGM097, which neutralizes the p53-inhibiting effect of HDML2 on AML cells.

Tosedostat is an aminopeptidase inhibitor that blocks the destruction and rebuilding of intercellular proteins. It is undergoing phase II trials. It was demonstrated on a group of patients >60 years of age with relapsed/refractory AML that tosedostat is active in monotherapy, dose-independently. In the same group with a negative prognosis, tosedostat combined with cytarabine and azacitidine achieved a 30% ORR. Used on elderly patients in inductive therapy with cytarabine or decitabine, it resulted in a CR or CRi of more than 50%.

Pevonedistat (MLN4924) is an NEDD8-activating enzyme (NAE) inhibitor that controls the destruction of many proteins taking part in the cell cycle, signal transduction, the destruction of DNA, or the stress response, for example, p53, p27, cyclin E, c-MYC, phospho-IκBα, CDT-1, NRF-2, and HIF-1α. In preclinical tests, pevonedistat was effective in monotherapy in amplifying cytarabine action, but there was only a 20% response rate.

Vosaroxin is a topoisomerase II inhibitor which is essential for cell survival. Vosaroxin induces DNA destruction and is most effective among elderly patients diagnosed with AML or myelodysplastic syndrome (MDS). Phase III trials showed that vosaroxin prolongs survival by about 6 weeks.

OTX015 is a BRD2/3/4 inhibitor indispensable for leukemic clone survival of c-MYC. OTX015 used in conjunction with paminostat and azacitidine showed synergic activity towards KASUMI AML cell lines. ARV-825 was more effective against AML post-myeloproliferative cell lines than OTX015. Both drugs are in phase I trials.

**Epigenetic modulators**

Lysine-specific demethylase 1 (LSD1) is a histone demethylase. Its expression has been demonstrated in many neoplasms and it plays a role in the self-renewal of AML stem cells. LSD1 inhibition leads to the inhibition of tumor growth and metastasis. ORY-1001 and GSK2879552 are tranylcypromine-derivative LSD1 inhibitors, both in phase I trials.

Panobinostat (LBH589) induces AML cell apoptosis in vitro by inhibiting the expression of repair proteins (e.g., BRCA1, CHK1 and RAD51), increasing the efficiency of cytarabine and daunorubicin, and it is promising in t(8;21) AML due to the pathological AML1/ETO protein that recruits histone deacetylases.

Vorinostat (suberoylanilidehydroxamic acid [SAHA]) promotes cell cycle inhibition and arrested growth, and induces differentiation and AML cell apoptosis. In phase II trials with cytarabine on AML/MDS patients with severe concomitant diseases, there was a median OS >7 months with acceptable toxicity.

Histone deacetylase inhibitors, such as pracinostat and entinostat, are under investigation in AML patients. Rearranged mixed lineage leukemia (rMLL) is associated with an aggressive disease course and a poor response
to multidrug chemotherapy, which is caused by a higher expression of HOXA9 and MEIS1. Pinometostat (EPZ-5676) is a histone methyltransferase DOT1L enzyme inhibitor. DOT1L is the rMLL target gene. Pinometostat is undergoing phase I trials.

The programmed death-1 (PD-1) receptor occurs on activated T cells and after binding with its programmed death 1 or 2 ligand (PD-L1, PD-L2), it suppresses T cell cytotoxic activity. This immunological escape has been presented in many cell lines, including AML. High PD-L1 expression correlates with an unfavorable course. Nivolumab is a PD-L1 inhibitor which has been approved by the FDA for treating non-small-cell lung cancer, melanoma and renal cancer. Nivolumab with azacitidine is now in phase II studies in relapsed AML patients. A remission rate of 18% was achieved in elderly patients with tolerable side effects.

The suppressor gene p53 is called a genome warden due to its prevention of the replication of defective genome material and it leads to apoptosis. The destruction of the MDM2 protein is called a genome warden due to its prevention of the replication of defective genome material and it leads to apoptosis. The destruction of the p53 protein is proceeded by ubiquitination after connecting with the MDM2 protein. A high MDM2 concentration in many cell lines, including AML. High PDL-1 expression correlates with an unfavorable course. Nivolumab is a PD-L1 inhibitor which has been approved by the FDA for treating non-small-cell lung cancer, melanoma and renal cancer. Nivolumab with azacitidine is now in phase II studies in relapsed AML patients. A remission rate of 18% was achieved in elderly patients with tolerable side effects.

Idasanutlin (R7388) is a selective, next-generation MDM2 inhibitor. There were promising effects of phase I trials in refractory/relapsed AML: the higher the MDM2 expression was, the better the response to the drug was. Idasanutlin is better tolerated than RG 7112.

Despite the growing interest awakened by targeted therapy in AML treatment, the current results are unsatisfactory. Undoubtedly, this is due to the complexity of leukemogenic mechanisms. There is potential for further investigations and clinical studies to improve AML therapy. All in all, each study brings us closer to achieving success in AML therapy.

References

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