The Development of Personalized Medicine: Acute Myeloid Leukemia as a Model

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ABSTRACT

The term personalized medicine has been employed in widely different contexts and has acquired status as one of the most often used keywords recently. In this review we take it to understand the application of modern diagnostic medicine and therapeutics to patient with the purpose of eradicating disease or alleviating symptoms in a manner, where all actions are based on detailed knowledge of the condition of the individual patient. Applying these concepts should lead to optimization of clinical decision-making and, in its utmost consequence, a substantial decrease in costs incurred for hospitalization and follow-up. The latter is based on the evidence that for many disorders "less but more targeted" will mean improved outcome. Acute myeloid leukemia (AML) is the most common acute leukemia in adults and is a major challenge in terms of diagnosis, care, follow-up and therapy. Thus, in population-based analyses, overall survival is only just exceeding 40% with major reasons for treatment failure. For these reasons, AML has been intensely studied during the recent decades. With the development of multiparametric flow cytometry, it allows us to get an accurate diagnosis and immunophenotypic profiles of AML. In addition, there is now an abundance of knowledge regarding its cytogenetic and molecular background. These enable us to follow the amount of disease down to the minutest quantity with a high resolution of molecular details. Finally, based on knowledge of these variables in the single patient cytoreduction is now being refined to therapies targeted to the molecular changes in the patient.

Keywords: Acute myeloid leukemia; developing country; precision medicine; targeted therapy; Thailand (Siriraj Med J 2019; 71: 414-425)

INTRODUCTION

Acute myeloid leukemia (AML) is a highly malignant blood cancer characterized by rapid accumulation of abnormally differentiated immature myeloid cells in the bone marrow (BM) and peripheral blood (PB), resulting in disruption of the production of normally functioning blood cells.[1] Thus, patients present with severe symptoms of BM failure, *i.e.* anemia, bleeding and often life-threatening infections. AML can arise *de novo*, secondary to antecedent myelodysplastic syndrome (MDS) or myeloproliferative neoplasm (MPN), known as secondary acute myeloid leukemia (sAML), or following cytotoxic therapy (tAML). The incidence of AML is generally reported to be 3-5 per 100,000 per year, but varies according to age and study. Despite the fact that initial therapy induces complete remission (CR) in up to 80% of patients, the relapse-rates are high in AML other than acute promyelocytic leukemia (APL) subtype. The

five-year survival rate of younger patients (age < 60 years) is approximately 40% and less than 20% in the elderly.[2] Unfortunately, data from less developed countries, such as Thailand, showed an even worse outcome. The 2-year overall survival (OS) rates of younger and elderly groups in Thai AML patients were merely 28.6% and 10.3%, respectively.[3] Surprisingly, the Thai cohort showed a lower proportion of patients with good-risk cytogenetics in comparison to other reports.[3]

The diagnostic tool box in AML

There are several diagnostic tools that should be included in the investigation of newly-diagnosed AML patients (Fig 1). Firstly, blast counts from PB and BM smear are simple assays and instantly provide the provisional diagnosis of AML. Standard guidelines recommend counting from 200 total white blood cells from PB or 500 total nucleated cells from BM smear.[4,5] 20% myeloblasts or more in PB or BM is compatible with AML; however, physicians should keep in mind that AML can be diagnosed in the patient who has t(15;17), t(8;21) or inv(16) – even if blast count is less than 20%.[4,5] In addition, for AML-M4, a total blast counts must include monoblasts and promonocytes.

Secondly, immunophenotyping from multiparametric flow cytometry (MFC) is an essential investigation for AML

subtype categorization. However, it is required that the MFC machine should be well-equipped with ≥2 lasers and ≥6-8 photomultiplier tubes and operated with technical expertise. Immunophenotypic markers can separate precursor-, granulocytic-, monocytic-, megakaryocytic- and erythroid lineages. MFC is an important part of the diagnostic work up in daily clinical practice, as it provides a rapid blast count together with immunophenotyping capable of confirming the myeloid origin of blasts. In some cases, the immunophenotype can be indicative of underlying cytogenetic aberrations, e.g. co-expression of the lymphoid marker CD19 on myeloid blasts in t(8;21) AML or lack of CD34 and HLA-DR antigens in APL.[6] Moreover, a baseline immunophenotypic result before treatment is important for treatment response evaluation and measurable residual disease (MRD) monitoring.[7-9]

Cytogenetic and molecular analyses are also required for all newly-diagnosed AML to predict patient prognosis and guide treatment decision.[4] Besides, these genetic data are also used to further classified patients into different subtype according to WHO 2016 classification criteria.[10]

The molecular panel that is mandatory to be screened by polymerase chain reaction (PCR) based method includes *PML-RARA, CBFβ-MYH11, RUNX1-RUNX1T1* and *BCR-ABL1* genes. In addition, other gene mutations

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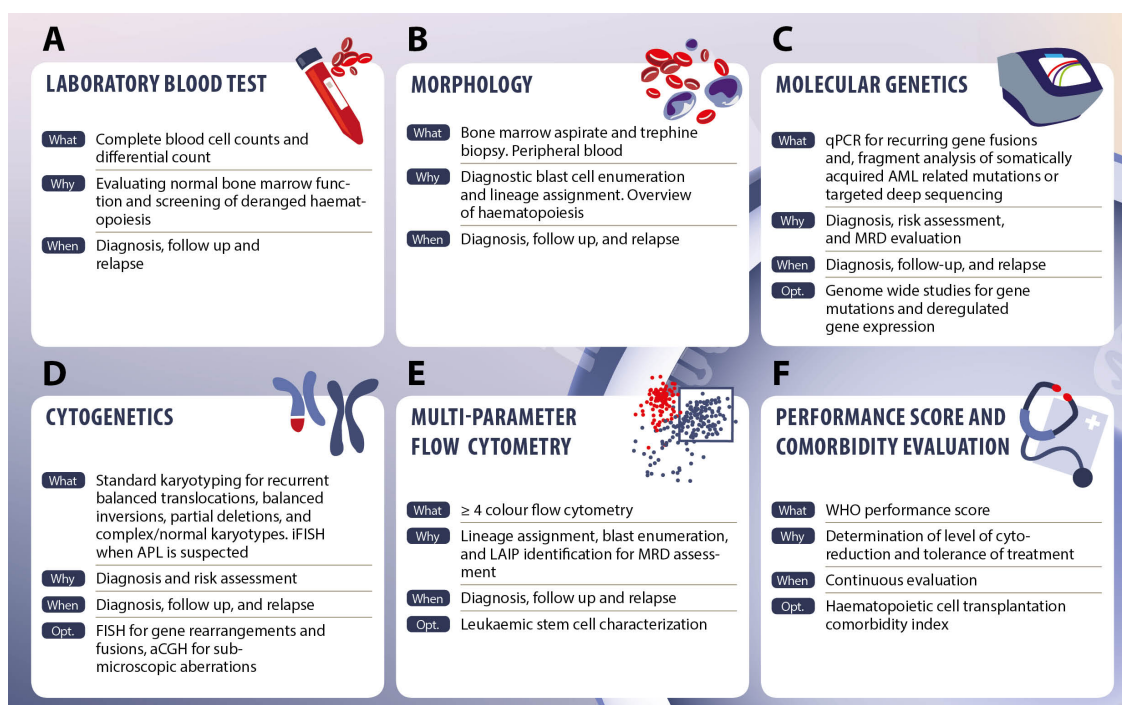
LABORATORY BLOOD TEST

What — Complete blood cell counts and differential count

Why — Evaluating normal bone marrow function and screening of deranged haematopoiesis

When — Diagnosis, follow up and relapse

B

MORPHOLOGY

What — Bone marrow aspirate and trephine biopsy. Peripheral blood

Why — Diagnostic blast cell enumeration and lineage assignment. Overview of haematopoiesis

When — Diagnosis, follow up, and relapse

C

MOLECULAR GENETICS

What — qPCR for recurring gene fusions and, fragment analysis of somatically acquired AML related mutations or targeted deep sequencing

Why — Diagnosis, risk assessment, and MRD evaluation

When — Diagnosis, follow-up, and relapse

Opt. — Genome wide studies for gene mutations and deregulated gene expression

D

CYTOGENETICS

What — Standard karyotyping for recurrent balanced translocations, balanced inversions, partial deletions, and complex/normal karyotypes. iFISH when APL is suspected

Why — Diagnosis and risk assessment

When — Diagnosis, follow up, and relapse

Opt. — FISH for gene rearrangements and fusions, aCGH for sub-microscopic aberrations

E

MULTI-PARAMETER FLOW CYTOMETRY

What — ≥ 4 colour flow cytometry

Why — Lineage assignment, blast enumeration, and LAIP identification for MRD assessment

When — Diagnosis, follow up and relapse

Opt. — Leukaemic stem cell characterization

F

PERFORMANCE SCORE AND COMORBIDITY EVALUATION

What — WHO performance score

Why — Determination of level of cyto-reduction and tolerance of treatment

When — Continuous evaluation

Opt. — Haematopoietic cell transplantation comorbidity index

Fig 1. The diagnostic toolbox in acute myeloid leukemia. Blood counts **(A)** and morphology of peripheral blood and bone marrow **(B)** are still frontline assays, when diagnosing acute myeloid leukemia, whereas as molecular genetics/genomics **(C)**, cytogenetics **(D)**, flow cytometry **(E)** are of highly important for subtype classification and prognosis. This information, together with performance score **(F)**, forms the basis of clinical decision-making and treatment strategy for the individual patient. Cytogenetics are also used in diagnosis as specific chromosomal aberrations may override the lower threshold of blasts in the peripheral blood or bone marrow.

including *NPM1, FLT3*-ITD, *CEBPA, KIT, RUNX1, TP53, ASXL1, IDH1* and *IDH2* genes are also highly recommended from the latest standard guidelines as an initial investigation.[4,5] The turn-around time of these workups should not exceed more than 3 days for fusion gene rearrangements, *NPM1* mutation and *FLT3*-ITD mutation and not more than one week for cytogenetic analyses.[4] More advanced technologies, such as next-generation sequencing (NGS), is also now integrated for molecular characterization of AML patients.[11,12] With this method, we can obtain multiple or complete mutational data in a single assay. In the future, we plan to expand the molecular work-up for Thai patients to see if there are any population-based differences from other ethnicity cohorts. This information may arguably be useful in the personalized therapy era. In addition, cost-effectiveness of generalized NGS for routine implementation is to be validated. Table 1 illustrates diagnostic tools for newly-diagnosed AML patients.

While previous prognostic classification systems have primarily been based on cytogenetic findings[13], the revised risk stratification from the European Leukemia Net (ELN) incorporates the most significant prognostic mutations[7] (Table 2).

Genetic risks along with patient related factors, *e.g.* age, comorbidity or performance status, and post-treatment factors, such as the presence or absence of MRD[7], guide the choice and intensity of therapy, which will be discussed further.

Conventional treatment and follow up in AML

While targeted therapy is evolving rapidly for hematological malignancies, the general treatment of AML has not changed substantially the past 30 years.[2] For patients with AML eligible for intensive chemotherapy, standard regimens consist of 1-2 courses of chemotherapy to induce complete remission (CR). CR is defined by a presence of less than 5% blasts in BM accompanied by a complete hematologic recovery. The most commonly used regimen for AML other than APL subtype, referred to as the *7+3 regimen*, is comprised of 7 days continuous intravenous cytarabine infusions and 3-day anthracycline infusions.[11,15] The response is evaluated two weeks after the onset of chemotherapy. Younger and physically fit patients, belonging to intermediate or adverse risk groups, will be referred for allogeneic hematopoietic stem cell transplantation (allo-HSCT) in first CR. Patients with favorable risk or without donor eligibility may continue to receive further 2-4 cycles of consolidation chemotherapy. Older patients, not eligible for intensive treatment, will receive best supportive care, *e.g.* low-dose cytarabine (LDAC) or alternatively therapy with a hypomethylating agent (HMA) such as 5-azacitidine[4]. With the continuously poor outcome for AML-patients,[13] it is clear that new and better treatment options are warranted in order to improve survival. In that regard, targeted treatment strategies are attractive in order to obtain efficient kill of the leukemic cells without extensive toxicity on normal cells.

TABLE 1. Diagnostic tool for newly-diagnosed AML patient.

Strongly recommended	Turnaround time
Complete blood count with blood smear	1 hour
Bone marrow aspiration for assessment of blast number	1 day
Multiparametric flow cytometry	3 days
Screening for gene rearrangements including *PML-RARA, CBFβ-MYH11, RUNX1-RUNX1T1* and *BCR-ABL1* genes by RT-PCR	3 days
Cytogenetics	7 days
Mutations of NPM1 and FLT3-ITD	3 days
Recommended or optional	**Turnaround time**
Specific gene mutations including *CEBPA, KIT, RUNX1, TP53, ASXL1, IDH1* and *IDH2* genes	7-14 days
Next-generation sequencing for myeloid panel	7-14 days

TABLE 2. Risk stratification according to the 2017 ELN guidelines.

Risk category	Genetic abnormality
Favorable	t(8;21)(q22;q22.1); *RUNX1-RUNX1T1*
	inv(16)(p13.1q22) or t(16;16)(p13.1;q22); *CBFB-MYH11*
	Mutated *NPM1* without *FLT3*-ITD or with FLT3-ITD^low
	Bi-allelic mutated *CEPBA*
Intermediate	Mutated *NPM1* and *FLT3*-ITD^high
	Wild-type *NPM1* without *FLT3*-ITD or with *FLT3*-ITD^low (without adverse-risk genetic lesions)
	t(9;11)(p21.3;q23.3); *MLLT3-KMT2A*
	Cytogenetic abnormalities not classified as favorable or adverse
Adverse	t(6;9)(p23;q34.1); *DEK-NUP214*
	t(v;11q23.3); *KMT2A* rearranged
	t(9;22)(q34.1;q11.2); *BCR-ABL1*
	Inv(3)(q21.3q26.2) or t(3;3)(q21.3;q26.2); *GATA2, MECOM(EVI1)*
	-5 or del(5q); -7; -17/abn(17p)
	Complex karyotype, monosomal karyotype
	Wild-type *NPM1* and FLT3-ITD^high
	Mutated *RUNX1*
	Mutated *ASXL1*
	Mutated *TP53*

Modified from[4], where further details are available

Taking aim at personalized therapy in AML: clinical considerations cost-benefit

In contrast to the stagnant situation in conventional induction cytoreduction in AML, treatment therapies are now becoming more personalized. In recent years, much focus has been put on mapping genetic and epigenetic heterogeneity of the malignancies. Consequently, the information of specific antigens expressed on leukemic cells together with somatic mutations have been included in drug investigations to improve outcomes in AML.[16] The treatment approaches are summarized in Fig 2 and 3.
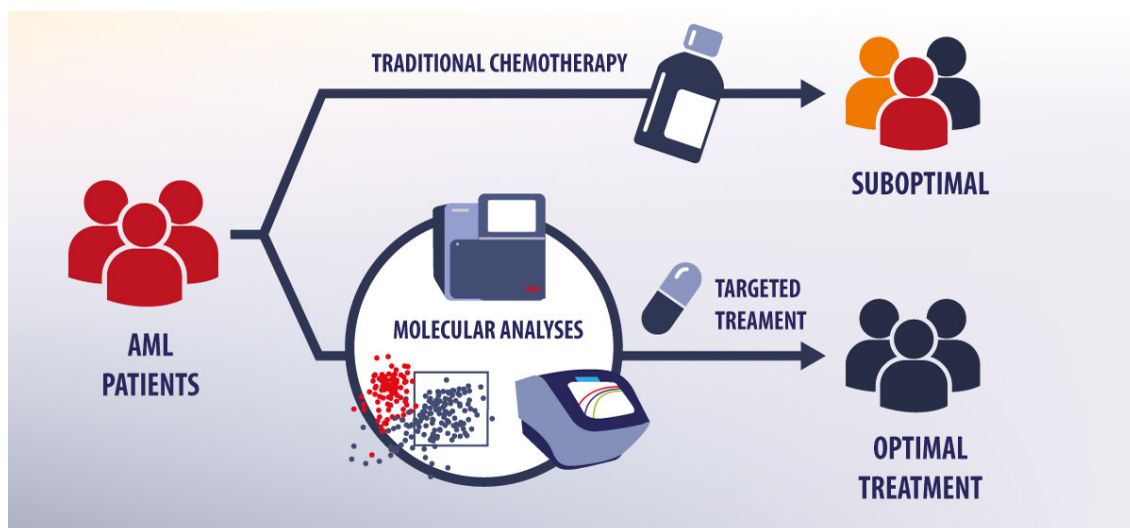

Fig 2. Chemotherapy versus targeted treatment. Targeted therapy is based on the most optimal individual treatment for specific types or combinations of molecular aberrations. The current molecular diagnostics toolbox to guide precision medicine includes both next generation sequencing, digital droplet PCR and flow cytometry, each with different sensitivities, strengths and weaknesses.
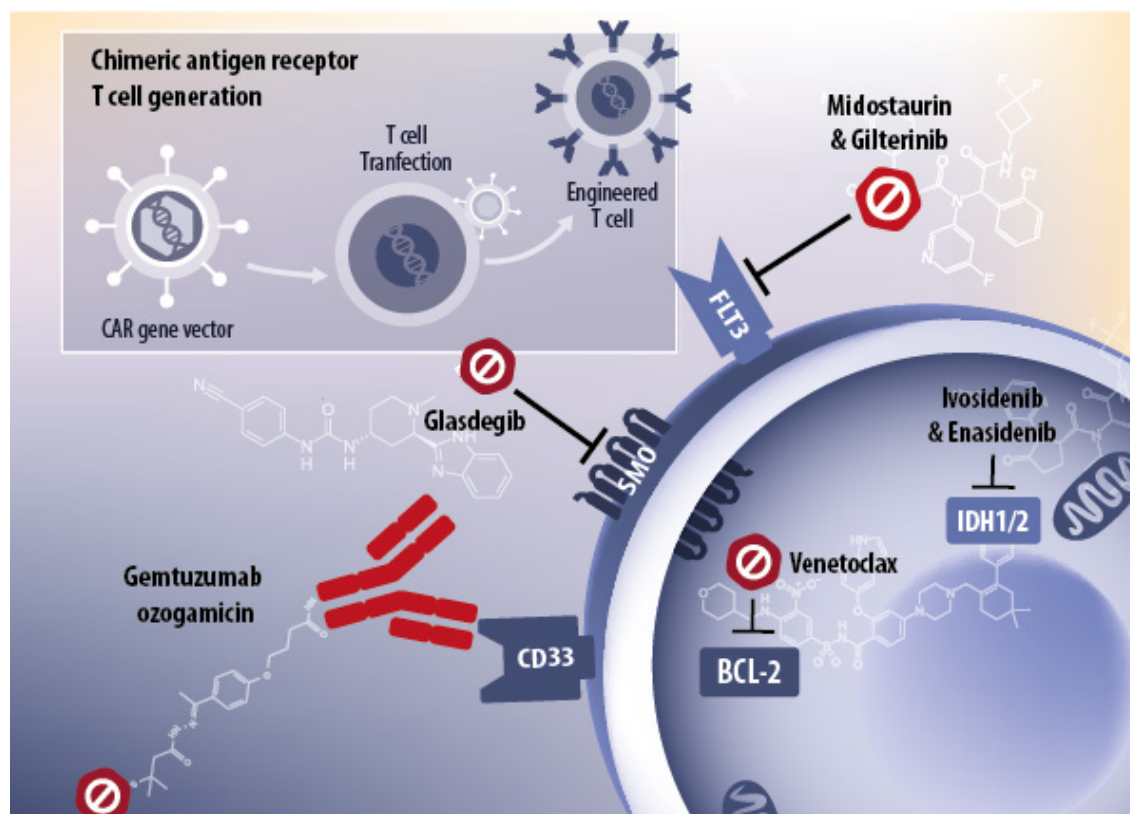
Fig 3. Examples of targeted therapy in acute myeloid leukemia. Targeted therapy or precision medicine has the purpose of eradicating leukemic cells with specific molecular aberrations without hitting nonspecific targets, such as normal hematopoietic cells. These drugs include monoclonal antibodies, such as antibody-drug conjugate gemtuzumab ozogamicin (Mylotarg), which targets CD33, or selective small molecule inhibitors, such as venetoclax for older patients that respond poorly to standard therapy. Other promising strategies of precision medicine include the use of chimeric antigen receptor T cells, where patient T cell are engineered to target the malignant cells (insert).

The studies on novel therapeutic targets, including surface antigen, driver oncogenes and cellular pathways have been an active field of AML research in recent years. Thus, there are many pathway specific agents and chemotherapy with new drug delivery system being approved by the US Food and Drug Administration (FDA). These include midostaurin, CPX-351, enasidenib and gemtuzumab ozogamicin in 2017 and glasdegib, venetoclax, ivosidenib, and gilteritinib in 2018. Here, we review the data of these promising agents along with the benefit to the patients.

Approved drugs
FMS-like tyrosine kinase 3 (FLT3) Inhibitors

FLT3 mutations are found in 30% of AML patients, leading to ligand-independent activation of the receptor, thereby promoting proliferation, survival, and resistance to apoptosis. It is known that patients with a *FLT3* mutations have significantly lower OS.[17] Several drugs that can inhibit FLT3 kinases have been tested in AML. Midostaurin works through inhibition of multiple receptor tyrosine

kinases including the activity of wild-type FLT3 and kinases with either internal tandem duplications (ITD) or a mutated tyrosine kinase domain (TKD). In phase III study, where the drug has been given in addition to standard chemotherapy and as a maintenance therapy, OS and event-free survival (EFS) were significantly higher in comparison to placebo (74.7 vs. 25.6 months and 8.2 vs. 3 months, respectively).[18] Due to its survival benefit, US FDA has approved its use in combination with standard induction and consolidation therapy in adults with newly diagnosed AML, who are *FLT3*-mutation positive. Another approved agent is gilteritinib. Gilteritinib is a highly potent inhibitor which has been approved in fast track according to preliminary phase I/II data.[19] Nonetheless, the drug as a monotherapy ultimately showed improved survival in relapsed or refractory AML patients with *FLT3*-mutation in comparison to standard chemotherapy (9.3 VS 5.6 months) in complete Phase III analysis. However, long term survival was still uncommon on either treatment arm. The longest survival in the gilteritinib arm was seen among patients

who underwent HSCT followed by maintenance therapy. Therefore, ongoing study has been done to see whether using the drug as a first-line treatment would give more survival benefit to the patients. Other drugs in this category including sorafenib, quizartinib and crenolanib, have shown benefit in preclinical and early clinical studies and awaits for further validation in various settings.[20]

Isocitrate dehydrogenase (IDH) Inhibitors

IDH1 and IDH2 are enzymes that catalyze the conversion of isocitrate to α-ketoglutarate. *IDH1* and *IDH2* mutations occur in 5-10% and 10-15% of adult AML patients, respectively, with higher frequencies in older patients. These result in an abnormal enzyme activity, lead to the competitive inhibition of α-ketoglutarate dependent enzymes, target genes hypermethylation and impaired hematopoietic differentiation.[21] So far, two agents targeting these mutant enzymes have been approved. The first drug, enasidenib, which is a selective IDH2 inhibitor, provided overall response rate (ORR) of 40.3% and impressive median time to first response of 1.9 months in relapsed and refractory AML patients.[22] Data also confirmed that enasidenib can salvage a number of patients (50% ORR) and provided survival benefits.[23] There is one particular study of enasidenib in newly diagnosed elderly patients, which showed that there were relatively low toxicities (cytopenic rate 21%), good response rate (30.8%) and acceptable OS (11.3 months). Ivosidenib, on the other hand, inhibits *IDH1* mutant enzyme, demonstrated ORR of 42% and CR of 22% in relapsed and refractory AML patients.[24] Therefore, both were approved for treatment of relapsed or refractory AML with a corresponding *IDH* mutation. The durations of responses varied from an average of 6 months to more than 2 years. Additional studies will give more information on the benefit of these drugs in terms of a long-term survival as a frontline treatment. Studies has now been expanded to test these drugs in combination with chemotherapy in newly diagnosed patients and gave a promising results.[25]

B-cell leukemia/lymphoma-2 (BCL2) inhibitor

Venetoclax is a selective, oral small-molecule *BCL-2* inhibitor, leading to cell apoptosis[21] (Fig 3). *BCL2* is overexpressed in hematologic malignancies and implicated in AML cell survival, chemoresistance, and is linked to poor OS in AML patients.[26] As a monotherapy, it demonstrated activity in relapsed refractory or unfit AML (19% overall response rate) with a tolerable safety profile.[27] *IDH* mutational status was correlated with good responses, however, all patients eventually relapse despite the initial response. Lately, the studies showed that rapid resistance may occur from *MCL1* and *BAX* upregulation. Many agents can reduce *MCL1* expression in vitro including chemotherapy and HMA.[28] Venetoclax in combination with HMA in newly diagnosed elderly AML patients demonstrated 67% CR and median OS of 17.5 months.[29] The responses occurred in patients with poor risk mutations; *TP53* and *FLT3* mutations, similar to those with *IDH* and *NPM1* mutations. These impressive results led to recent approval of drug combination with HMA or LDAC for frontline treatment of elderly unfit patients. New combination therapy such as MCL1 inhibitor and IDH inhibitors are lining up to be tested.[28]

Glasdegib

This drug works through inhibition of sonic hedgehog pathway in leukemic stem cells (Fig 3). In phase II, randomized, open-label, multicenter study, 132 patients with AML or high-risk MDS unsuitable for intensive chemotherapy were evaluated for the efficacy of glasdegib plus LDAC in comparison to LDAC alone.[30] It showed OS benefit (8.8 vs. 4.9 months) and clinical efficacy across patients with diverse genetic profiles.[30] This led to its approval by US FDA in 2018 as a treatment in unfit AML/MDS patients.

CPX-351

CPX-351 is a dual-drug liposomal encapsulation of cytarabine and daunorubicin packaged at a synergistic dose of 5:1 molar ratio.[31] A nanoliposomal carrier of the drug leads to prolonged exposure and intracellular delivery. In comparison to a conventional 7+3 induction of cytarabine and daunorubicin treatment in high risk elderly AML patients (60–75 years of age), CPX-351 arm demonstrated higher CR and CR with incomplete recovery (CRi) (57% vs 40% and 73% vs 52%) and led to higher number of patients undergoing HSCT. CPX-351 also improved OS compared with 7+3 (median OS 9.56 vs 5.95 months), regardless of age, therapy-related or MDS-related subgroup.[32] Consequently, it has been approved as a frontline treatment in patients of all ages with therapy-related AML or AML with MDS-related changes.

Gemtuzumab ozogamicin

CD33 antigen is a transmembrane receptor and myeloid differentiation marker variably expressed on AML cells in almost all patients.[33] Gemtuzumab ozogamicin is a humanized CD33 antibody-toxin conjugate toxic to CD33-expressing leukemic cells[32] (Fig 3B). After binding to the antigen on the surface of leukemic blasts

the antibody is internalized and binds to DNA leading to double-strand break and cell death. It has been previously withdrawn from American market in 2010 due to its toxicity and limited benefit.[34] However, with recently more efficacy data and desperate needs to improve treatment of elderly AML patients, the drug has been resubmitted to US FDA regulatory review again.[35] In newly diagnosis adult AML patients age 50 to 70 years, a randomized phase III trial of daunorubicin and cytarabine with or without gemtuzumab ozugamycin in induction and consolidation chemotherapies (ALFA-0701) has shown the benefit of increase median EFS of 15.6 months in the gemtuzumab ozogamicin arm versus 9.7 months in control arm except in patients with adverse cytogenetic risk.[36] In addition, in de novo or sAML patients age >75 years or age 61-75 years with WHO performance status >2 who are unwilling to receive intensive chemotherapy, gemtozumab ozogamycin monotherapy resulted in a significant improvement in median OS 1.3 months comparing to best supportive care arm. The toxicities did not differ between treatment arms.[37] This agent was approved in newly diagnosed CD33-positive adult AML and relapsed or refractory CD33-positive AML in adult and pediatric AML aged 2 years and older.[35] New CD33 antibody and drugs targeting CD33 are currently tested in various studies.[38]

Investigational therapy
Cellular therapy targeting surface targets

Chimeric antigen receptor T cell (CAR-T) therapy targeting surface marker of AML, e.g. CD33, CD123 and FLT3, have been developed in preclinical and early clinical phase with some concerns of serious toxicities.[39] Many leukemia associated antigens are differentially expressed by MHC on tumor cells and have been investigated in small clinical studies to be used as a vaccine or dendritic cell-based therapy. These targets include Wilm's tumor 1 (WT1), New York esophageal squamous cell carcinoma 1 (NY-ESO1), PRAME and survivin (BIRC5).[40] However, the main challenge of vaccine-based therapy is MHC restriction. Immune checkpoint inhibitors are also of great interests and have currently been studied in different combination at various phases of treatment.[41]

Ongoing investigation therapies

Many more inhibitors that targeting different pathways such as DNA repair and epigenetic modification, including polyadenosine diphosphate ribose polymerase (PARP), lysine-specific demethylase 1 (LSD1), bromodomain and extra terminal (BET) and mutant TP53 pathway have now been tested in clinical studies.[41,42]

Innovative approach for precision medicine

As a result of novel targeted therapy discovery, we may select the proper treatment to the patients according to their molecular abnormalities. Not only specific mutation can be targeted directly but rather several features can also predict drug response. For instance, co-occurring IDH2 and DNMT3A mutations result in distinct DNA methylation pattern, leading to upregulation of RAS signaling and sensitivity to MEK inhibition.[43] This proved to be feasible in Beat AML study which included 273 elderly patients. Ninety-five percent of the study group could be assigned to treatment by mutation stratified algorithm within 7 days.[44] Apart from matching genetic data to targeted therapies, drug sensitivity testing (DST) approaches such as ex vivo drug sensitivity screening can guide the individualized treatment more precisely. By using a sample from each patient tumor as an avatar to evaluate patient-specific drug sensitivity profiles. This method is attractive due to easy accessibility of malignant cells in the PB or BM and will be very useful – especially for relapse/refractory patient. Preliminary data from a pilot study showed that it can be done within short turnaround time. Even though initial results have showed a promising outcome, further studies should be performed.[45] However, due to molecular heterogeneity of AML, possible target mutations may be one of several potential targets. Many challenging questions still need to be answered including how to identify the key pathway, whether a combination of targeted drug would be beneficial and how to manage the toxicities.

Costs of novel treatment in AML and clinical benefits

Advanced therapies have changed the way of treatment especially to patients with high risk features who have low chance of survival. Most of them are currently approved to be used as a last resort in patients who fail or deem unsuitable to conventional treatment. Even though they provide more opportunities to increase OS the treatment, however, rarely lead to cure as monotherapy. These effective drugs also come with high costs leading to an increase of the economic burden in AML treatment. The prices of these drugs are summarized in Table 3. In countries with limited resources treatment decision should always be made by evaluating the cost-effectiveness to patient survival and quality of life. Choosing a drug, which provides the best response to the right group of patients at the right time and administered in a brief duration as a bridging therapy to curative HSCT, would be the most optimal way to limit costs and provide the best outcome. In contrast, we can save cost by not giving

TABLE 3. Novel drug costs in AML: dose recommendations and average prices.

Drug name	Dose recommendations	Average price*
Midostaurin (FLT3 inhibitor)	50 mg twice daily, days 8–21 of induction and consolidation	5,448 baht per 25-mg tablet
Gilteritinib (FLT3 inhibitor)	120 mg once daily	9,600 baht per 40 mg
Enasidenib (IDH2 inhibitor)	100 mg once daily	32,953 baht per 100 mg
Ivosidenib (IDH1 inhibitor)	500 mg once daily	16,713 baht per 250 mg
Venetoclax (BCL2 inhibitor)	Day 1, 100 mg once daily; Day 2, 200 mg once daily; Day 3, 400 mg once daily; Day 4 and beyond, 400 mg once daily	357 baht per 10 mg; 1,784 baht per 50 mg; 3,568 baht per 100 mg
Glasdegib	100 mg once daily	10,832 baht per 25 mg
CPX-351	Induction, 44 mg/m²–100 mg/m² on days 1, 3, 5; Reinduction (if patient not in remission), 44 mg/m²–100 mg/m² days 1, 3; Consolidation, 29 mg/m²–65 mg/m² on days 1 and 3 for 2 cycles	306,528 baht per 44–100-mg vial
Gemtuzumab ozogamicin (humanized CD33 antibody-toxin conjugated)	Induction (in combination with "7+3" regimen, 3 mg/m² (max 4.5mg/dose) on days 1, 4, 7; Consolidation, 3 mg/m²* day 1	314,880 baht per 4.5-mg vial

*Costs are reported as average wholesale price and calculated from US dollar price (1 US dollar equals 32 Thai Baht). They are not meant to represent true costs.
Modified from[46]

drugs to patient with predictive low response profile. Nowadays, technologies for precision medicine, and especially next generation sequencing, have become much more accessible than a decade ago. With more information and tools, they will guide the clinicians to the treatment that provides maximum benefits – not only as a salvage but as a frontline therapy. If we can use these regimens upfront, we may prevent more patients from relapsing and avoid the side effects and costs from chemotherapy and HSCT. Nevertheless, novel therapies give us treatment options, which were previous unavailable, and possibly with new side-effects. Therefore, information with regards to risks and benefits should be given to the patient and should be able to reach a final decision that match their interests and expectations under solid counselling from the clinician.

Follow-up in the age of personalized medicine

Relapse in AML patients may be caused by a persistence or an evolution of therapy-resistant leukemic clones in the bone marrow. To capture low level of cancer cells, referred to as MRD, high sensitivity laboratory investigations are required. As discussed above, relapse is still common even in patients who have reached CR states. The goal of MRD monitoring is early detection of patients at risk and guide treatment decisions to minimize the risk of clinical relapse by intensifying the therapy. Alternatively, in the era of targeted therapy, we may be able to target the specific pathway in resistant leukemic clones. Conventional methods using morphology and cytogenetic assay have low sensitivities and only allow the detection of leukemic cells greater than one cell in 20 leukocytes. Therefore, even in a CR state, up to 1011

residual leukemic cells may still exist.[47] Currently, two widely accepted methods to detect MRD are; MFC and real time quantitative PCR (RT-qPCR) which can detect MRD leukemic cells in white blood cells component with a sensitivity down to $10^{-3} – 10^{-6}$.[48] However, MRD detection is not a straightforward process, because of the immunophenotypic and molecular heterogeneity between patients and clonal heterogeneity that may emerge as novel resistant clones under selective pressure – especially in response to single targeted therapy.[49,50] Identifying the appropriate markers that differentiate leukemic cells from normal hematopoietic progenitor, to evaluate disease burden or MRD and to predict relapse, is still challenging. Follow-up approaches are summarized in Fig 4.

Multiparametric flow cytometry

By labelling cells with multiple fluorescent markers and determining the light emission from the cells as they flow against laser beam, MFC can characterize phenotypes and immunophenotypes of malignant cells down to a single level. Apart from being used as a diagnostic tool, MFC can also be used as a disease monitoring method. MFC has the sensitivity to detect MRD of leukemic cells down to 10^{-3}-10^{-5}.[48] It can be applied for almost all patients. However, due to the complexity of leukemic immunophenotype described previously, the markers need to be tailored. A large panel of antibodies of more than 8 colors are recommended for characterizing the leukemic cells by MFC. The marker should include early hematopoietic markers (e.g. CD34, CD45, CD117), myeloid-lineage associated markers (e.g. CD4, CD11b, CD11c, CD33, CD64), aberrant differentiation markers (e.g. CD2, CD7, CD19, CD56).[51] MRD can be defined by either tracking leukemia associated immunophenotypes (LAIP) from the diagnosis or by identifying new emerging clone that express aberrant differentiation profiles called different-from-normal (DfN) approach. ELN MRD working party recommends a combination of both approaches to best define MRD burden.[51] Other markers that could be used to define leukemic stem cell population, e.g. CD38, CD123, CD133, are also of interests. There are also some technical considerations including the source of specimen, sample collection and number of collected cells. For example, MRD level in PB may be one-tenth the frequency in BM. Therefore, a detection of MRD by MFC in PB is not currently recommended and bone marrow specimen should be collected from the first pull to avoid hemodilution. Because of the complicated process and interpretation, a validation of the method is essential. It
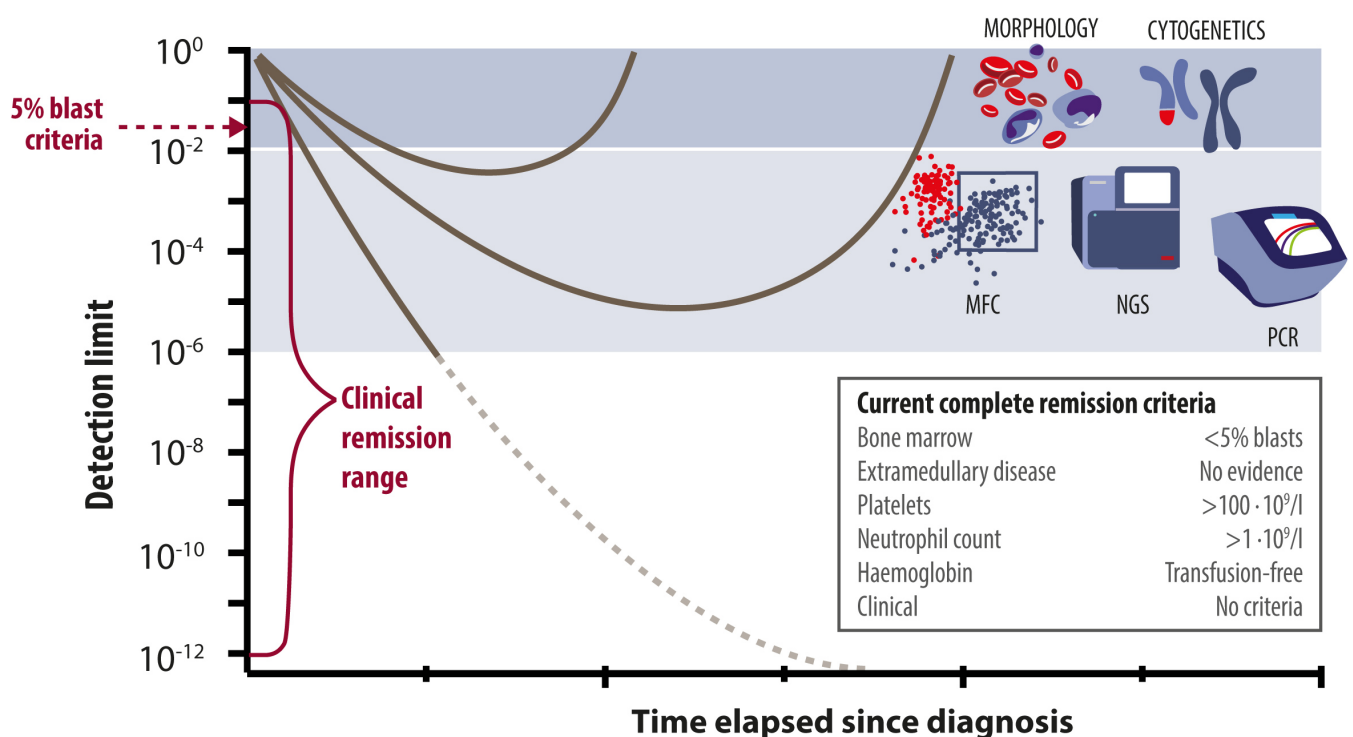

Fig 4. Detection of minimal/measurable residual disease (MRD). Currently, PCR is still the most sensitive method to detect MRD. Next generation sequencing (NGS) is being investigated as a potentially highly sensitive method for detection of residual disease, while increasing the informational content for clonal assessment.

is recommended that there should be a central institute for an evaluation and a final interpretation and should not be done in a single-centered fashion. Various cut-off values have been applied but MRD level below 0.1% is the most widely used in the clinical studies, with more than 500,000 CD45+ cells acquired.[51]

Molecular MRD

Molecular abnormalities of the leukemic cells can be used as a marker to monitor the level of malignant cells with the help of PCR-based methods. qRT-PCR is a laboratory technique based on the PCR to measure the amplification of a targeted DNA molecule during the PCR in real-time. It can be used quantitatively or semi-quantitatively. This approach has a high sensitivity for detecting one malignant cell in 10^4 to 10^6 leukocytes. It can detect target genes representing malignant cells and is considered as the gold standard for MRD detection. However, in contrast to MCF, this approach is applicable for only around 40% of patients who have suitable abnormalities. Target genes could be fusion, recurrent mutated or overexpressed genes. However, only some verified markers associated strongly with relapse including *NPM1* mutation, *RUNX1-RUNX1T1*, *CBFB-MYH11* and *PML-RARA* fusion.[51] Some other mutations may persist after treatments but do not relate to real disease burden. For example, mutations that occur in the preleukemic clone which can be found in aging population e.g. *DNMT3A, ASXL1, TET2*[52,53] or in germline e.g. *RUNX1, GATA2, CEBPA, DDX41, ANKRD26*[54]. Apart from those genetic markers, *FLT3*-ITD, *FLT3*-TKD, *NRAS, KRAS, IDH1, IDH2, MLL*-PTD and the expression of *EVI1* are not recommended to use as a single marker but rather in combination.[51] Despite being shown to relate with patient outcome in many studies, using WT1 expression as a MRD marker is still controversial due to its low sensivity.[51,55-57] More recent technologies such as Next generation sequencing (NGS) and droplet digital PCR (ddPCR) are ideal to be used in all patients. NGS gives the whole genetic profile of the patient while ddPCR allows simultaneous multiple gene expression quantification. The main shortcoming of NGS is that it has low specificity in low allelic burden setting.[48] While ddPCR require the establishment of specific set of primers according to sequencing data. Even though promising, more studies is needed to verify and standardize the methods.[58]

CONCLUSIONS AND PERSPECTIVES

AML is often regarded as a signature disease in hematology. Thus, it is the most common leukemia in adulthood and though advances have been made, both in terms of diagnosis, follow-up and therapy, the long-term survival of patients is still unsatisfactory, with treatment failure being the reason. The concepts of personalized and precision medicine have been coined after the availability of genome sequencing and the increased realization that each patient has different in genetic mutational background. A targeted treatment points to the specific mutation which causes better treatment outcome compared to conventional therapy.

The standard AML treatment in last several decades has changed, expecting to improve patient outcomes and quality of life. However, having more available choices might lead to additional concerns, such as drug costs versus clinical significance. Comprehensive or complex decision-making in personalized medicine are highly relevant matters in these situations. However, it is important to note that not all these techniques and the clinical decisions following their results need to increase the costs of treating AML patients. In fact, the presence of some molecular phenotypes, such as the CBF subset, will mean that allo-HSCT is not needed for certain patient groups. In addition, it should be remembered that current therapy of AML, like the time-honored *7+3 regimen*, while not being expensive in term of drugs, is accompanied by very severe side effects, mostly related to the deep immunosuppression and highly correlated with serious bacterial and fungal infection. In this setting, more expensive personalized therapies developed though knowledge about the phenotype and molecular profile of the given patient's malignant cells, might be more efficient and not accompanied by similar side-effect, resulting in fewer days in hospital and minimize antibiotic or antifungal usage. This applies not only to agents targeting molecular drivers, but also to the recently developed therapeutic options relating to redirecting cytotoxic NK- and T-cells (CAR cells) or unmasking immunogenic molecules on the AML cells in the exciting field of checkpoint immunotherapy. In all these situations it is the job of health care providers to enlighten administrators and funding authorities about such potentially cost-saving actions.

It is noteworthy that these issues pertain not only to affluent countries, but equally to those with strained economies. Pharmacoeconomics is to be investigated with regards to appropriation of novel therapy for AML patients in each country.

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