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Review

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Genomics in leukaemia in clinical practice: past, present and the future

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Abstract

Acute myeloid leukaemia (AML) is a heterogeneous group of diseases with diverse genetic drivers. The conventional one-size-fits-all approach with chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) has reached an impasse, and only about 40% of patients can achieve long-term survival. Disease heterogeneities have also hampered the development of effective therapy applicable to the multitude of AML subtypes. Recent advances in cancer genetics and genomics have shed light on the genetic underpinnings of AML and both inter-individual and intra-tumoral heterogeneities. These new pieces of knowledge have begun to impact the management and prognostication of AML. They also provide the foundation for personalized treatment for this group of diseases.

Keywords: Acute myeloid leukaemia, next-generation sequencing, measurable residual disease, personalized medicine

INTRODUCTION

Advances in genome sequencing technologies in the past two decades have resulted in an unprecedented increase in knowledge of cancer genetics and genomics. The information arising has shed important light on the genetic underpinnings of oncogenesis and the complexity of inter-individual and intra-tumoral

heterogeneity that tends to evolve during the course of cancer treatment[1]. This information has also formed the theoretical basis of personalized cancer treatment based on the unique mutation compositions of cancers at diagnosis and relapse.

Leukaemia has long been the foundational paradigm for new concepts in cancer biology and innovation in therapeutic targeting. Phenomenal observations of chromosomal translocations in chronic myeloid leukaemia (CML)[2] and acute promyelocytic leukaemia (APL)[3], and the subsequent identification of pathogenic fusion genes in these diseases, have led to the development of tyrosine kinase inhibitors and differentiation agents. Both of these therapies have transformed the outcomes of patients, who can now be cured based on chemotherapy-free regimens.

Acute myeloid leukaemia (AML) is a devastating disease worldwide, for which treatment outcomes are unsatisfactory overall. The considerable inter- and intra-tumoral heterogeneities in AML have hampered the development of effective treatments applicable to the multitude of AML subtypes. However, advances in cancer genetics and genomics in recent years have empowered clinicians and scientists with the ability to analyze leukaemia heterogeneities and clonal evolution at different treatment stages. This information has begun to influence the clinical management of AML, improving outcomes for some of these patients[4].

We review the history of genomic research in AML in the past and how this knowledge has led to improvements in the treatment outcomes of patients. We also discuss the future development of a personalized approach to AML management.

ACUTE MYELOID LEUKAEMIA IN THE CLINICS – CURRENT STATE OF AFFAIRS

AML is a group of heterogeneous diseases with diverse morphologies, immunophenotypes, cytogenetics, and genetics, sharing in common an abnormal increase in blasts in blood and bone marrow (BM). It occurs in 3-5 patients per 100,000 individuals every year, and its incidence has increased in the past few decades. It is a highly lethal disease and is the fifth deadliest cancer of all kinds, particularly in elderly patients who are unfit to receive standard treatment. About 50% of AML cases carry normal cytogenetics, and they show on average 2-4 recurrent mutations in different combinations, some of which are considered drivers and others passengers in leukemogenesis[5]. Some of these mutations are of prognostic significance. In particular, *NPM1* mutation and bZIP in-frame mutation of *CEBPa* are associated with a favorable response to conventional chemotherapy, whereas *FLT3*-ITD is associated with a less favorable response. About 10%-15% of AML cases carry translocation t(8;21) (*RUNX1::RUNX1T1*) or inversion of chromosome 16 (CBFβ::MYH11), both of which involve components of the core-binding factor (CBF), which is a heterodimeric transcription factor comprising the non-DNA-binding CBFβ chain and the DNA-binding CBFα chain RUNX1. Half of these patients carry KIT mutations, which confer an inferior prognosis in this AML subtype, which hitherto had a favorable response to conventional chemotherapy[6]. Another 10% of AML cases carry complex (≥ 3 karyotypic abnormalities) or monosomy karyotype (≥ 2 monosomies or 1 monosomy and one structural abnormality), and this subtype portends an extremely poor prognosis, particularly those carrying *TP53* mutations, which happen in half of the patients with this subtype[7]. The rest of AML cases are made up of diverse diseases with different karyotypic and genetic abnormalities [Figure 1].

Despite the heterogeneity, AML has been managed by a one-size-fits-all approach in the past 4 decades. In young and fit patients, intensive chemotherapy and allogeneic hematopoietic stem cell transplantation (HSCT) are the mainstays of treatment. However, this approach has reached an impasse, and only 40% of patients can survive long-term. A number of agents, when added to the chemotherapy regimen, were shown

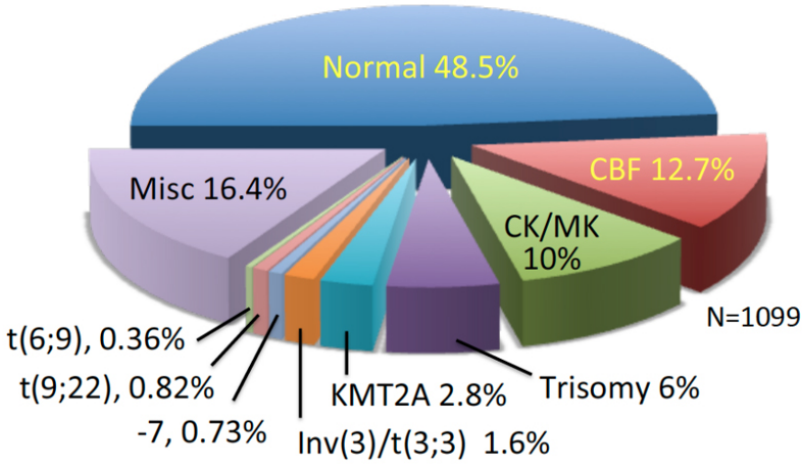

Figure 1. Cytogenetic landscape of acute myeloid leukaemia (AML) patients younger than 60 years old in Hong Kong. Adapted from Leung *et al.*[7]

to improve overall survival based on randomized control trials or meta-analyses, including *FLT3* inhibitor midostaurin[8] and monoclonal antibody against CD33[9], and were approved by the US FDA for such indications. Old and frail patients who are ineligible for standard treatment are primarily treated by low-intensity treatment of palliative intent, including hypomethylating agents or low-dose cytarabine. More recently, the addition of BCL2 inhibitor venetoclax to these agents was shown to improve overall survival, with 30% of these patients able to live beyond 30[10].

AML GENOMICS - A HISTORICAL PERSPECTIVE

Modern genomics in leukaemia research owes much to the phenomenal observation of recurrent chromosomal translocations in patients with AML[11], APL[3], and CML[2] by Dr. Janet Rowley and others about 50 years ago, leading to the identification of oncogenic fusion genes, i.e., *RUNX1-RUNX1T1*, *PML-RAR*, and *BCR-ABL1*, respectively. These discoveries have been instrumental in our understanding of the molecular mechanisms of leukaemogenesis and formed the foundation for the subsequent development of targeted therapies, including all-trans retinoic acid and tyrosine kinase inhibitors for APL and CML, both of which have transformed the outcome of these patients. Since then, more chromosomal translocations in AML have been described, many of which were predictive of clinical outcomes upon conventional treatment. In addition, specific mutations have been identified, including those of *CEBP* and *NPM1*, which were associated with a favorable prognosis, and those of *RUNX1* and *FLT3-ITD*, which were associated with an unfavorable prognosis[12].

The advent of next-generation sequencing (NGS) has made it possible to rapidly discover genetic mutations in the cancer genome. Since the first report of whole-genome sequencing in AML in 2008[13], the list of novel gene mutations has expanded at an unprecedented rate [Figure 2]. In particular, mutations of genes encoding for isocitrate dehydrogenase 1 and 2 were identified by NGS in patients with cytogenetically normal AML[14,15], and specific inhibitors are now available in clinics for AML carrying these mutations, attesting to the power of genomic information in the development of target-specific therapy[16,17]. The technologies have been generalized to solid cancers, forming the basis of The Cancer Genome Atlas (TCGA). TCGA comprises more than 20,000 primary and matched normal samples spanning more than 30 cancer types and has become an important reference for cancer genome research(https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga).

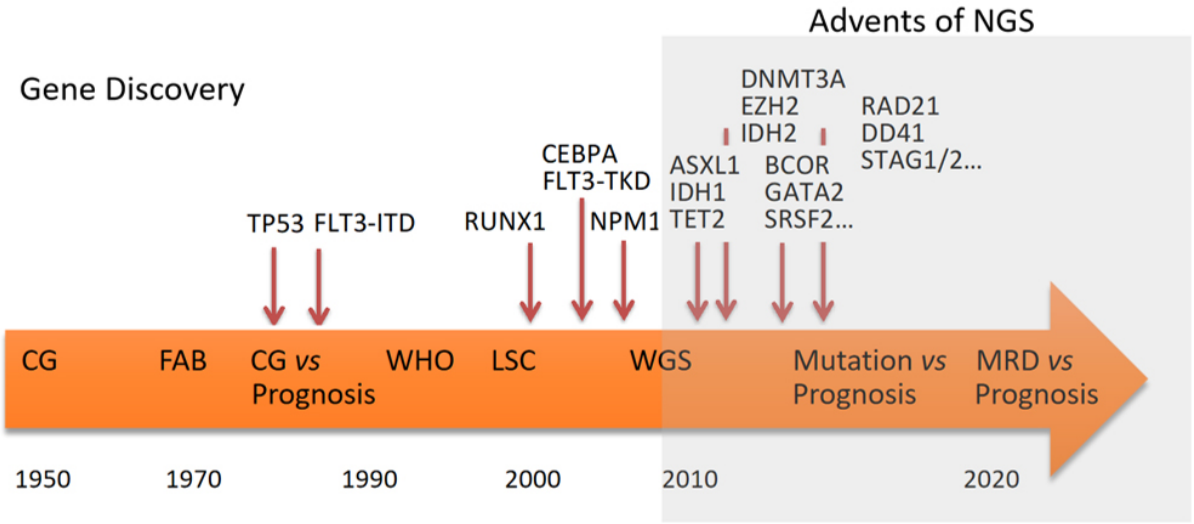

Figure 2. Timeline of gene discovery and evolution of concepts and management principles in acute myeloid leukaemia. NGS: Next Generation Sequencing, CG: cytogenetics; FAB: French, American and British classification; WHO: World Health Organization classification; LSC: leukaemia stem cells; WGS: whole genome sequencing; MRD: measurable residual disease.

CURRENT APPLICATION OF GENETICS AND GENOMICS IN AML

NGS has now become the standard of care for AML in tertiary institutes, where facilities and expertise are available. In most clinical practices, targeted DNA sequencing is used to identify mutations at diagnosis. This method focuses on a panel of 50-70 recurrent mutations in myeloid malignancies. Briefly, the first step involves library preparation, which entails the fragmentation of the genome into DNA fragments of about 150 base pairs and the addition of specialized adaptors to both ends of the fragments. The second step involves DNA sequencing, in which the library is loaded onto a flow cell and DNA fragments are amplified, resulting in millions of copies of single-stranded DNA. The third step involves data analysis, in which the nucleotides in the amplified products are confirmed through base-calling, and the presence of genetic variants is identified [Figure 3]. Gene mutations at diagnosis often predict patient outcomes upon treatment with conventional therapy, and hence inform clinical decisions on allogeneic HSCT at first complete remission (CR1). Patients who are at high risk of relapse should receive HSCT, which is of curative intent, and those with predictably favorable outcomes could be observed, to avoid the morbidity and occasional mortality associated with HSCT. Using deep learning algorithms, predictive modeling has been developed in cytogenetically normal AML based on genetics and clinicopathologic characteristics, which could be helpful in guiding the HSCT decision[18]. Furthermore, patients whose diseases show a high likelihood of relapse, even after HSCT, for instance, *TP53* mutated AML, should be given the option of clinical trials. The importance of genetic mutations and the growing number of mutations identified in AML and myelodysplastic syndrome (MDS), often a harbinger of AML, were underscored by the increased recognition of mutation-defined AML subtypes in the new WHO 2022 classification. Many of these subtypes were shown to define prognosis upon conventional treatment [Table 1][12,19].

In addition to gene mutations at diagnosis, the detection of measurable residual disease (MRD) has become the standard of care in the management of AML, providing real-time information about the depth of remission during the course of treatment[20,21]. In newly diagnosed AML, patients typically carry 10^{12} leukaemia cells in the body. Traditionally, morphologic assessment of BM and peripheral blood post-chemotherapy has been used to define disease remission based on a cutoff of blasts < 5%, amounting to approximately 10^{9} leukaemia cells in the body, which is substantial. MRD detection based on flow cytometry

Table 1. 2022 European LeukemiaNet (ELN) risk classification by genetics at initial diagnosis[21]

Risk category	Genetic abnormality
Favorable	RUNX1::RUNX1T1 [t(8;21)]
	CBFB::MYH11 [Inv(16)]
	Mutated NPM1 without FLT3-ITD
	bZIP in-frame mutated CEBPA
Intermediate	FLT3-ITD
	MLLT3::KMT2A [t(9;11)]
	Cytogenetic or mutations not classified as favourable or adverse
Adverse	DEK::NUP214 [t(6;9)]
	KMT2A rearranged (other than MLLT3::KMT2A)
	BCR::ABL1 [t(9;22)]
	KAT6A::CREBBP [t(8;16)]
	MECOM(EVI1) rearranged [incl. Inv(3)]
	ASXL1, BCOR, EZH2, RUNX1, SF3B1, SRSF2, STAG2, U2AF1, ZRSR2, TP53
	Complex and/or monosomy karyotype
	-5/del(5q); -7; -17/abn(17p)

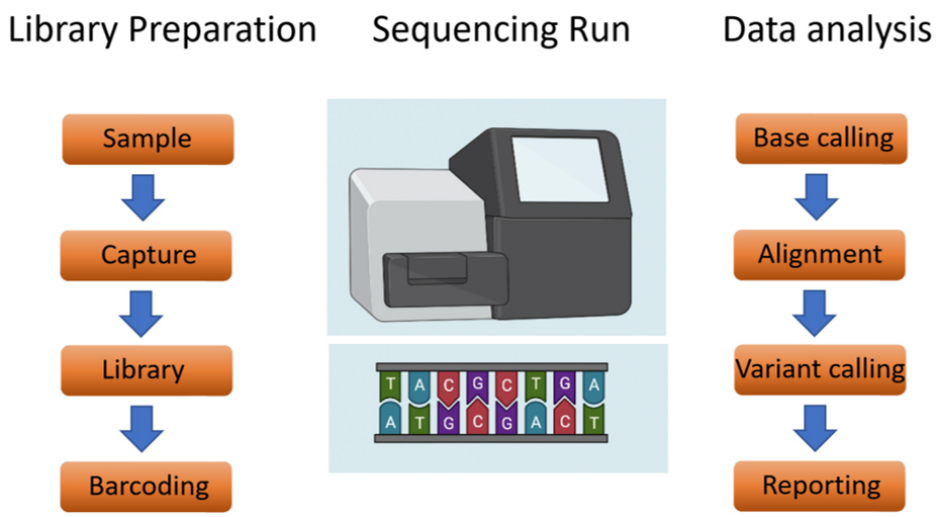

Figure 3. Workflow of myeloid focused next-generation sequencing.

or molecular means can achieve a sensitivity of at least 10^{-3}, and a persistently negative MRD during the course of treatment can predict disease eradication and long-term survival. In most tertiary centers, MRD is evaluated by either one of two methods. Flow cytometry offers a more rapid evaluation of patient samples but requires a higher level of technical expertise and standardization. Molecular methods are widely used and encompass quantitative RT-PCR, droplet digital PCR, and NGS platforms, choices of which often depend on institutional experience and expertise and the genes of interest.

PERSONALIZED APPROACH OF AML TREATMENT – FUTURE PERSPECTIVE

An important question in AML management is whether personalized treatment based on genomic information of individual patients, if available, would confer benefits to patients over the one-size-fits-all approach based on the "standard of care" (SOC). This is being addressed by the Beat AML trial, in which untreated patients older than 60 years received either the SOC or clinical studies founded on the mutation

profiles of these patients. Reportedly, the latter resulted in less frequent 30-day mortality and overall survival compared with those patients receiving SOC[22]. While the impact of this study remains to be seen, it highlighted the potential application of genomic information in guiding the upfront treatment of AML.

Another approach to personalized treatment of AML involves *in vitro* testing of drug sensitivity, with the goal of using the results to guide the clinical treatment of patients in real-time. This approach is akin to culture and sensitivity testing that guides antibiotic treatment for infectious diseases in clinics. Interestingly, leukaemia blasts grow very slowly *ex vivo* once they are taken out of their hosts, and optimization of culture conditions becomes critical for their maintenance and evaluation of drug sensitivity. When coupled with genomic information of the samples, *in vitro* drug sensitivity provides a powerful platform for identifying novel biomarkers predictive of drug response and personalized treatment through drug repurposing. Using these platforms, homoharringtonine was shown to be effective in FLT3-ITD AML through protein translation inhibition[23]. Importantly, clinical response to FLT3 inhibitors and homoharringtonine in patients receiving such treatments were correlated with the *in vitro* drug sensitivity of their samples, attesting to the potential application of the latter in predicting clinical responses. More recently, results from *in vitro* drug sensitivity testing of blood and BM samples from AML patients with relapsed and refractory disease against 515 anticancer drugs were used to guide the treatment of 37 patients using a customized combination of 2-3 drugs based on patient-specific sensitivity to single drugs and molecular data. Reportedly, nearly 60% of evaluable patients showed clinically meaningful responses, of whom the majority achieved complete remission (CR) or CR with incomplete hematologic recovery (CRi)[24]. A similar approach was also reported in pediatric AML patients[25].

Recent advances in transcriptomic analysis at the single-cell level have enabled the evaluation of cellular heterogeneity and hierarchy in AML, as well as the simultaneous examination of non-leukemic immune cell populations. It is now technically possible to enhance its analytic power by simultaneously measuring immunophenotype using barcoded antibodies and epigenetic state based on single-cell ATAC-sequencing. Bioinformatic analyses can enable the clustering of distinct cell populations based on their transcriptome profile[26]. Serial monitoring of the BM transcriptome upon leukaemia treatment at the single-cell level may shed light on the therapeutic responses in the leukaemia and microenvironment compartments.

CONCLUSION

Recent advances in genome sequencing technologies have empowered scientists and clinicians with the ability to examine inter-individual and intra-tumoral heterogeneities in acute myeloid leukaemia in great detail. The information has begun to influence the clinical management and prognostication of the disease. Transcriptome analyses at a single cell level are ideally suited to examine cellular heterogeneity in leukaemia samples and will shed important light on the dynamic changes of the microenvironment in the course of leukaemia therapy.

DECLARATIONS

Authors' contributions

Performed the literature search and wrote the manuscript: Leung HC
Outlined the scope of the review and wrote the manuscript: Leung AYH

Availability of data and materials

Not applicable.

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Conflicts of interest

Both authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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