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Epigenomic insights and computational advances in hematologic malignancies

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Abstract

Hematologic malignancies (HMs) encompass a diverse spectrum of cancers originating from the blood, bone marrow, and lymphatic systems, with myeloid malignancies representing a significant and complex subset. This review provides a focused analysis of their classification, prevalence, and incidence, highlighting the persistent challenges posed by their intricate genetic and epigenetic landscapes in clinical diagnostics and therapeutics. The genetic basis of myeloid malignancies, including chromosomal translocations, somatic mutations, and copy number variations, is examined in detail, alongside epigenetic modifications with a specific emphasis on DNA methylation. We explore the dynamic interplay between genetic and epigenetic factors, demonstrating how these mechanisms collectively shape disease progression, therapeutic resistance, and clinical outcomes. Advances in diagnostic modalities, particularly those integrating epigenomic insights, are revolutionizing the precision diagnosis of HMs. Key approaches such as nano-based contrast agents, optical imaging, flow cytometry, circulating tumor DNA analysis, and somatic mutation testing are discussed, with particular attention to the transformative role of machine learning in epigenetic data analysis. DNA methylation episignatures have emerged as a pivotal tool, enabling the development of highly sensitive and specific diagnostic and prognostic assays that are now being adopted in clinical practice. We also review the impact of computational advancements and data integration in refining diagnostic and therapeutic strategies. By combining genomic and epigenomic profiling techniques, these innovations are accelerating biomarker discovery and clinical translation, with applications in precision oncology becoming increasingly evident. Comprehensive genomic datasets, coupled with artificial intelligence, are driving actionable insights into the biology of myeloid malignancies and facilitating the optimization of patient management strategies. Finally, this review emphasizes the translational potential of these advancements, focusing on their tangible benefits for patient care and outcomes. By synthesizing current knowledge and recent innovations, we underscore the critical role of precision medicine and epigenomic research in transforming the diagnosis and treatment of myeloid malignancies, setting the stage for ongoing advancements and broader clinical implementation.

Keywords Epigenetics, Hematologic malignancies, DNA methylation, Machine learning

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Introduction

Hematologic malignancies (HMs) present unique diagnostic and treatment challenges due to molecular and cellular heterogeneity of these diseases. These malignancies can occur over many stages throughout hematopoiesis and are caused by a diverse group of chromosomal, genetic, and epigenetic aberrations resulting in aberrant functional consequences. Much of the complexity in caring for HMs arises from the multifaceted nature of hematopoiesis (blood cell generation). During hematopoiesis, multipotent hematopoietic stem cells (HSCs) in the bone marrow give rise to erythrocytes, platelets, granulocytes, monocytes, and lymphocytes; mutations prior to and during this process can result in malignancy [1]. While there have been substantial advancements in the field of cancer diagnostics and treatment, the ability to generate a broadening range of molecular information is outpacing our ability to understand the impact of this information in diagnosis and management of patients with HMs. Personalized medicine promises to merge the cumulative impact of diverse genomic, epigenomic and environmental etiologies with consequent and equally complex clinical phenotypes.

Definition and classification of hematologic malignancies

With any malignancy, the primary essential tool in clinical management and treatment is a specific diagnosis obtained in a timely manner. Matching a patient with personalized treatment and support options relies on accurately diagnosing the specific subtype of cancer the patient has.

As of 2022 there are two main classification systems for HMs: The World Health Organization 5th Edition (WHO5) and the International Consensus Classification (ICC). These two systems categorize HMs based on morphology, clinical attributes, immunophenotype, cytogenetic features, molecular mechanisms, and genetic profiles [2, 3]. It is recommended that diagnostic specialists and treating physicians use consensus classifications of HMs from WHO5 and ICC whenever possible [2].

Both the ICC and WHO5 separate HMs into two large categories based on cell line– lymphoid neoplasms and myeloid neoplasms. Of the myeloid neoplasms, which this review will focus on, there are four classes which each possess their own subclasses. These four classes are: myeloproliferative neoplasms (MPN), acute myeloid leukemias (AML), myelodysplastic neoplasms/syndromes (MDS), and myelodysplastic/myeloproliferative neoplasms (MDS/MPN) [2, 5]. Figure 1 illustrates the natural progression of hematopoiesis, as well as the possible malignant transformations and associated myeloid neoplasms.

The relatively recent increase in use of genomic analysis ranging from single gene to whole-genome sequencing has contributed to the predominantly genetic variant-based categorization of HMs in the most recent edition of the World Health Organization Classification of Tumours, the WHO5. Comparatively, the previous

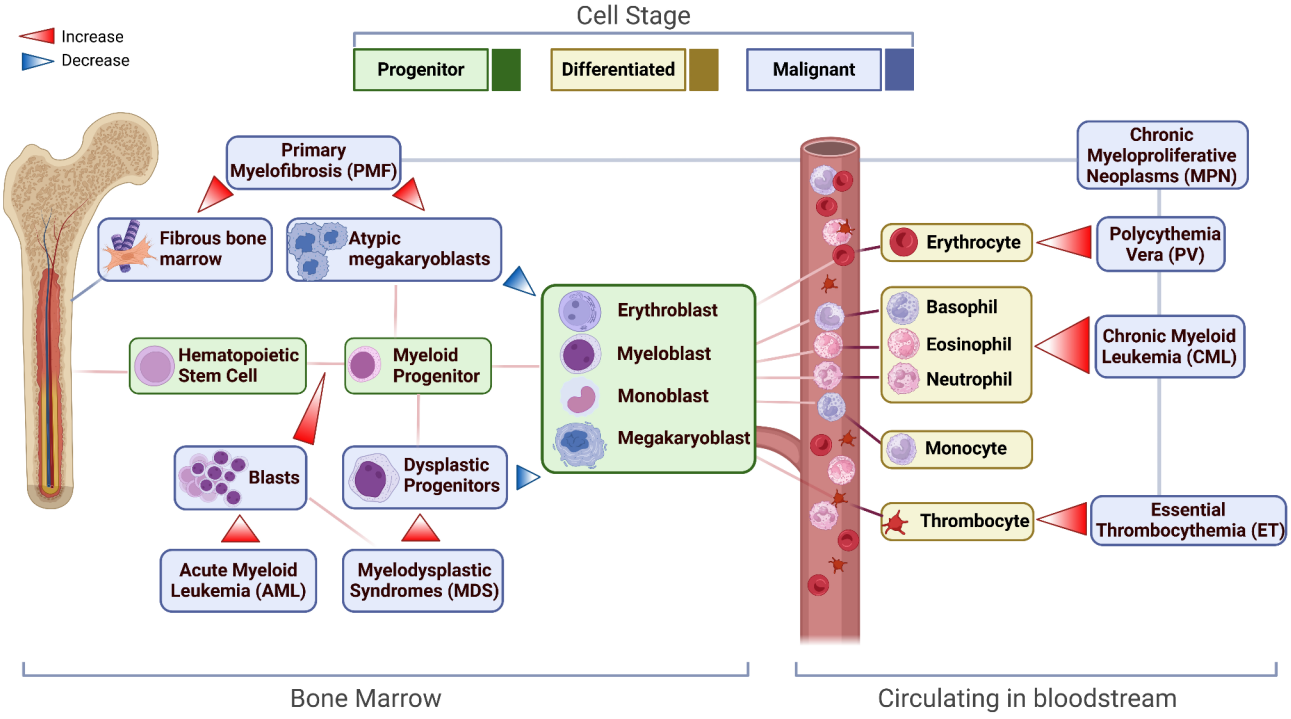

Fig. 1 Physiological stages of hematopoiesis and associated malignant transformations

versions of classification were based mostly on morphology and cytogenetics [3]. While no longer the primary classification determinant, morphological and cytogenetic aspects of HMs remain key confirmatory factors following identification of suspicious genetic findings.

The increase in availability and use of molecular profiling in oncology has led to great strides in cancer classification and treatment; however, the identification and classification of different myeloid malignancies remains a point of difficulty in the research and clinical community due to the overlap in mutations and clinical presentation, and molecular heterogenity [3]. When analyzing genetic information diagnostic specialists including molecular geneticists and pathologists are frequently presented with genomic variant data that they are unable to interpret. Variants of unknown clinical significance (VUS) present the greatest challenge with available testing as their interpretation often depends on extensive bioinformatic, clinical, functional and reference database information, by multidisciplinary teams [6]. Unfortunately, despite the extensive effort over the past few decades, most genomic changes and their consequences in tumors remain difficult to conclusively interpret. One review focusing on Next-Generation Sequencing (NGS) in breast and ovarian cancers found that VUSs accounted for over 40% of total variants identified through screening [7]. Another assessment performed at LHSC looking at the clinical utility of implementing a frontline NGS-Based DNA and RNA fusion panel test in patients with suspected myeloid malignancies also found greater than 40% yield of VUS [6]. Nearly half of variants screened resulting in mutations which are not clinically reported leaves an immense gap in people who could be receiving access to treatment and care associated with a definitive diagnosis.

In addition to diagnostic biomarkers, it is equally important to be able to identify molecular biomarkers related to prognosis and clinical management. Molecular classification associated with treatment outcomes has led to individualized therapeutic regimens and drug development, increasing patient survival rate and decreasing the rate of non-response [8, 9]. Relapse still occurs frequently in HMs; to illustrate - up to 30–50% of Acute Lymphoblastic Leukemia (ALL) patients are found to relapse. This pervasive issue indicates a need to establish further diagnostic and prognostic classification based on risk, treatment response, and consideration of prognosis [6, 8–11].

Blood cell generation in humans begins in the red bone marrow with multipotent stem cells called hematopoietic stem cells (HSCs), from which all cells of the lymphoid and myeloid cell lines are produced. HSCs destined to become myeloid cells differentiate to become myeloid progenitors, which further differentiate to terminal progenitor cells including erythroblasts, myeloblasts, monoblasts, and megakaryoblasts. The mature myeloid blood cells then enter the bloodstream as erythrocytes, granulocytes, monocytes, and thrombocytes to perform a wide variety of immunological and physiological tasks throughout the body. MPN occur when genes are affected which control expression and cellular proliferation, such as *JAK2*, resulting in overproduction (cytosis) of mature blood cells. MDS occur when genes involved in the regulation of hematopoiesis and transcription are non-functional, such as *SF3B1*, resulting in morphologic dysplasia of progenitors and subsequent mature cell cytopenia's. AML is largely characterized by the presence of non-functional, undifferentiated cells called blasts, which proliferate in the bone marrow and rapidly reduce the capability of HSCs to produce functional mature blood cells. These blasts are also present in MDS to a lesser extent than found in AML, with increased volumes indicative of poor prognosis and possible transformation to more severe disease. AML also presents similar genetically to MDS, with mutations commonly found in chromatin and spliceosome genes important to transcription such as *NPM1* and *FLT3*, along with chromosomal rearrangements involving transcriptional factors not seen as commonly in MDS.

Prevalence and incidence

Hematologic cancers are of great concern due to their prevalence in aging populations. Globally the average life expectancy has increased by an average of almost 6 years between 2000 and 2019 [12, 13]. This increase in life expectancy correlated with an increase in age-standardized incidence rate (ASIR) of some HMs, though variances based on country, sociodemographic indices (SDI), and healthcare systems cannot be overlooked.

As of 2019, malignancies under the class of leukemia possessed both the highest incidence and mortality rates of all HMs. There is a notable relationship between sex, ASIR and age standardized death rate (ASDR), wherein males have both a higher incidence and mortality rate. Countries with high SDI, which correlates to higher average life-expectancies and easier access to healthcare, have the highest ASIR of leukemia with Western Europe, North America, and Australasia having the top 3 incidence rates respectively. Interestingly, though considered a high SDI region, North America also holds the highest ASDR of leukemia, followed by North Africa, and the Middle East [14].

Of the leukemia subtypes, AML is the most common, holding both the highest incidence and death rates among the leukemia's, exponentially so in middle to high SDI regions [14, 15]. AML's increasing ASIR and ASDR is due to aging populations, previous exposure to carcinogens, and cytotoxic therapies [12, 16]. Aging populations, aside from the general increased risk of malignancy

experienced as humans age, were also more likely to have experienced workplace exposure to carcinogens and to have smoked– each of these are noted as leading risk factors for AML development. Due to the nature of AML and its oncogenic transformation of hematopoietic progenitor cells, treatment of primary tumours with cytotoxic chemotherapy often results in transformation of the primary cancer type to AML over time [12].

Genetic and epigenetic basis of malignancies

Genetic alterations and disease pathogenesis

Over decades of investigation and technological advancements a multitude of common genetic alterations associated with various HMs have been identified, many of which form the basis of WHO and ICC classifications [17, 18].

Hematopoiesis occurs from HSC's which exist in human bone marrow and can self-renew and differentiate, giving rise to lymphoid and myeloid progenitor cells that continue differentiation through the blood cell lines. The myeloid line, which develops from myeloid progenitor cells, generates three different cell lines: megakaryocytes, the precursors of platelets; erythrocytes, known as red blood cells; and myeloblasts, which become granulocytes and monocytes. To achieve functional hematopoiesis free of neoplastic changes a tightly maintained order of events without mutations and other disruptive effects must occur, along with a well-balanced ratio of stem cell differentiation vs. self-renewal [1, 19]. When disruptions occur causing the ratio to become unbalanced it results in HMs of a dysplastic and/or proliferative nature. MPN result in the excessive proliferation of myeloid progenitors leading to an increase in peripheral blood components, the classification of which depends on the affected genes. MDS, and by extension AML, result in dysplasia, cytopenia, and increased blasts in the bone marrow. Identification of gene specific variances has increased the variety of AML types and the staging at which it can be detected, which is of vital importance as acute disease has a far worse prognosis [2, 20, 21].

It is well established that cancer is a genetic disease, however, which genes are affected has a heavy influence on which type/subtype of cancer will occur; the rate at which the disease will progress; and the likelihood of response to treatment or relapse [19, 22, 23]. HMs present many diagnostic and management challenges differentiating them from solid tumour neoplasms, as hematopoiesis occurs in multiple sites across most large bone marrow deposits in the human body [1, 24]. The identification of genetic drivers of disease pathogenesis in HMs can help decrease this diagnostic burden by providing answers with much less extensive testing and permitting the formation of simpler guidelines for diagnosis and treatment [22, 25–27]. Key discoveries that have improved diagnosis and management of HMs began in the 1960's with the identification of the *BCR/ABL1* fusion that remains a diagnostic factor in chronic myeloid leukemia (CML) to date [28]. Looking forward to the early 2000s when sequencing technologies became more available, *NPM1* is identified as a driver mutation in AML [29] and *JAK2* is discovered as a driver in most MPN subtypes [30, 31]. In the following years many genetic discoveries of clinical significance in relation to prognosis and treatment were achieved, such as the identification of *SF3B1* as a noteworthy mutation in MDS which corresponded to the presence of ring sideroblasts and favorable prognosis [32], and *FLT3* mutations in AML and MDS which are associated with poor clinical outcomes [33, 34]. Investigation into the genetic and epigenetic basis of HMs as technology advances provides valuable functional insights that continue to positively transform HM diagnostics and treatment.

Epigenetic modulations and their association with genetic factors

Broadly stated, epigenetics is the study of heritable changes in gene expression that occur without an underlying change in DNA sequence [35]. DNA methylation (DNAm) and histone modifications are two main mechanisms for establishing epigenetic patterns in human genomes. DNAm is a covalent modification of cytosine bases in DNA which plays a pivotal role in the pathogenesis of cancer, with general trends of hypomethylation and localized hypermethylation near promoter regions seen across the genome of affected individuals [36–39]. Epigenetic DNAm changes are central to the regulation of DNA transcription and translation through the presence or absence of chemical modification of the genome, initiating or repressing transcription, respectively [36]. Trends of hypermethylation at key regulatory elements lead to cellular oncogenesis through silencing of protection mechanisms, as seen in the case of DNA hypermethylation at tumour suppressor genes. The opposite is also true, with hypomethylation at commonly methylated promoters, leading to the activation of oncogenes, excessive cellular proliferation, and chromosomal instability [35, 40, 41].

It is known that the functionality of genetic disease pathogenesis is influenced by epigenetic regulation [42–45]; HMs are no exception to this phenomenon, with mutations in key epigenetic modification genes such as *TET2*, *DNMT3A*, *ASXL1*, and *EZH2* noted in every class of HM [18, 25]. Epigenetic mutations of *DNMT3A*, *TET2*, and *ASXL1* have even been identified in preleukaemic haemopoietic stem cells decades before the development of AML, suggesting that these are early founder events that precede leukemogenic transformation [15]. Changes in epigenetic machinery genes are not the only genetic

alterations that inevitably result in epigenetic changes, as DNAm ultimately effects chromatin conformation and thereby gene expression. Thus, changes in DNAm at any number of regulatory elements and genetic regions can result in malignant cellular changes [40]. In myeloid malignancies the typical patterns of hypo- and hypermethylation are observed in combination with a multitude of malignancy specific epigenetic changes. When these changes are viewed from a genome-wide perspective, patterns in DNAm across CpG islands can be found and correlated to clinical diagnoses or underlying genetic aberrations [26, 40].

An additional layer to the complex web of HMs is the presence of germline mutations and constitutional disorders which predispose individuals to HMs. Disorders such as Fanconi anemia, Shwachmann-Diamond syndrome, telomere disorders, severe congenital neutropenia, Diamond-Blackfan anemia, Bloom syndrome, Noonan syndrome, and Down syndrome, as well as germline mutations in *CEBPA*, *DDX41*, *TP53*, *RUNX1*, *ANKRD26*, *ETV6*, *GATA2*, *SAMD9*, *SAMD9L*, *CSF3R*, *CHEK2*, and *MBD4* have all been identified as predisposing to HMs [46]. These germline predisposing mutations operate through the impairment of general cellular processes and by altering epigenetic mechanisms regulating those processes. For example, mutations in *MBD4* affect mismatch-specific DNA repair and methylated DNA binding/protein interactions. Mutations in *RUNX1* alter a key transcriptional mechanism in hematopoiesis and effect DNAm due to the genes function in recruiting site-specific DNA demethylating machinery. *CEBPA*, *TP53*, *ETV6*, and *GATA2* are part of cellular transcriptional machinery; *SAMD9*, *SAMD9L*, and *CSF3R* function in cellular proliferation; and *DDX41* and *ANKRD26* are not well understood in their mechanisms of predisposition but believed to play a role in tumour suppression [47]. This further substantiates the interconnectedness of genetics, epigenetics, and functional outcomes.

The interplay between genetic and epigenetic mechanisms is crucial in understanding the pathogenesis of myeloid malignancies. While genetic mutations serve as fundamental drivers of disease, epigenetic modifications, including DNA methylation and histone modifications, play a regulatory role in disease progression, treatment resistance, and clinical heterogeneity. Importantly, genetic testing alone often yields inconclusive results, particularly when identifying VUS, which require additional layers of molecular data for proper classification. This gap highlights the need for an integrated diagnostic approach that incorporates both genomic and epigenomic profiling to enhance disease stratification, refine prognostic models, and inform personalized treatment strategies. As research advances, the integration of these molecular insights is becoming essential for optimizing precision medicine applications in HMs.

Precision diagnosis and computational innovations

Key methods in diagnosis techniques

Nano-based contrast agents for medical imaging

Advancements in molecular techniques, particularly nano-based contrast agents, have significantly influenced preclinical and clinical diagnostics [48–51]. These agents, known for their multimodal capabilities, are revolutionizing bioimaging by enhancing precision across various imaging modalities, including X-ray, magnetic, nuclear, optical, and photoacoustic imaging. Nanoparticles, particularly those sized between 10 and 60 nm, demonstrate enhanced cellular uptake, making them highly valuable for diagnostic applications [48].

Iron oxide nanoparticles, especially magnetite (Fe3O4), are used in the diagnosis and treatment of different cancers, including HM, due to their distinct magnetic properties [52]. They play a crucial role in magnetic resonance imaging, targeted drug delivery, and cancer cell detection [52]. The chemical co-precipitation process is the most used synthesis method, ensuring efficiency in nanoparticle production [52, 53]. However, challenges such as aggregation and instability persist, necessitating surface modifications to improve stability, biocompatibility, and targeting capabilities [52].

Emerging nanotechnology-based biosensors have also shown promise in HM diagnostics. A novel electrochemical nanobiosensor incorporating reduced graphene oxide and gold nanoparticles has demonstrated high sensitivity in detecting microRNA-128, a key biomarker distinguishing ALL from AML. This sensor has been successfully validated with real serum samples from leukemia patients, highlighting its potential as a reliable diagnostic tool [54].

Additionally, noble metal nanoparticles have exhibited considerable potential in HM diagnostics. Techniques such as localized surface plasmon resonance and surface-enhanced Raman scattering utilize these nanoparticles for highly sensitive biomarker detection and imaging. Hollow gold-silver nanoparticles, when functionalized with antibodies, enable precise imaging of lymphoma cells, while fluorescent nanoclusters—including gold, silver, and platinum nanomaterials—offer stable photoluminescence for leukemia cell bio-labeling [55]. These nanotechnology-driven approaches contribute to early, non-invasive, and accurate HM identification, improving disease monitoring and treatment planning.

Targeting strategies further enhance the efficacy of nanoparticles in imaging and therapy. Active targeting involves ligands such as antibodies, aptamers, and peptides to improve specificity, while physical targeting strategies exploit pH and temperature variations to direct

nanoparticles to tumor sites. Surface modifications, such as polyethylene glycol encapsulation, extend circulation time, thereby enhancing contrast signals [50].

Nanoparticles hold significant promise for early disease detection in HM, functioning as contrast agents across various imaging platforms and facilitating precise biomarker identification [51]. Their application has the potential to revolutionize non-invasive diagnostic approaches, and continued advancements in nanotechnology may lead to the development of tumor-targeting contrast agents with enhanced sensitivity and specificity for metastasis detection in clinical practice [48–51].

Optical imaging

In the landscape of HM diagnosis, Optical Genome Mapping (OGM) emerges as a promising advancement, bringing notable improvements to conventional methods. OGM offers enhanced resolution for detecting structural variations (SVs) compared to standard techniques like karyotyping and fluorescence in situ hybridization (FISH). Particularly, OGM eliminates the need for cell culture and DNA amplification, streamlining the diagnostic process [56, 57].

The clinical validation of OGM as a laboratory-developed test marks significant progress in HM diagnostics. Compared to conventional techniques, OGM provides superior sensitivity and resolution for identifying SVs, achieving high concordance with standard methods while also uncovering additional submicroscopic alterations [56, 58]. Its ability to detect clinically relevant variants in AML, MDS, and CMML, such as *KMT2A* partial tandem duplication, supports more precise prognostic assessments and treatment decisions [59].

Moreover, OGM has demonstrated strong analytical performance across multiple SV types, including duplications, inversions, and isochromosomes, while identifying novel fusions missed by traditional approaches [58]. While these findings underscore OGM's potential as diagnostic tool, it remains an evolving technology with certain limitations, particularly in detecting alterations in centromeric and telomeric regions [58]. Nonetheless, its ability to provide a more comprehensive view of genomic abnormalities reinforces its growing relevance in HM diagnostics.

Flow cytometry

Flow cytometry (FCM) is an essential tool in hematologic diagnostics, playing a critical role in various conditions such as MDS/MPN and myeloid/lymphoid neoplasms with eosinophilia and tyrosine kinase gene fusions [60–62]. In CMML, FCM detects abnormal partitioning of peripheral blood monocyte subsets, aiding in diagnosis and subtype differentiation. It also shows promise in predicting outcomes, as seen in clonal monocytosis of undetermined significance [60]. Furthermore, FCM enables the identification of neoplastic mast cells with distinct immunophenotypes, reinforcing diagnostic criteria for systemic mastocytosis [60].

In AML, FCM remains crucial for rapid diagnosis, precise lineage assignment, and minimal residual disease (MRD) assessment. By identifying hematopoietic blast cells, it facilitates tailored clinical follow-up for therapy management and prognostication [61]. Flow cytometric immunophenotyping plays a pivotal role in distinguishing myeloid lineage blasts from B- or T-cell blasts in ALL, using markers such as CD34, CD117, HLA-DR, CD13, CD33, and MPO [63].

In MDS, FCM enhances diagnostic precision by detecting clonal hematopoiesis and immunophenotypic abnormalities. The Ogata score and integrated FCM, which incorporate erythroid markers such as CD45, CD36, CD71, CD105, CD117, and CD235a, improve diagnostic accuracy [64–66]. In CMML, FCM further differentiates monocyte subsets, notably classical monocytes ($CD14^{++}$, $CD16^{-}$) which are elevated in CMML cases [60, 67]. The addition of markers like CD56 and CD64, along with myeloid antigen abnormalities and an increase in immature monocytes (≥ 20%), enhances diagnostic precision [60].

FCM also plays a role in neutrophil subset analysis. In AML and MDS, alterations in the distribution of low-density granulocytes and normal-density neutrophils indicate a shift toward immature forms, supporting a pro-inflammatory environment and emergency hematopoiesis [68]. This highlights the growing diagnostic potential of neutrophil immunophenotyping in these diseases.

In addition, FCM guides targeted therapies, including chimeric antigen receptor T-cell treatments, by identifying cell surface antigens on leukemic blasts [60]. The integration of artificial intelligence (AI) and machine learning (ML) into FCM data analysis is further enhancing its capabilities, promising standardized and harmonized diagnostic applications [60–62]. As a versatile technique, FCM continues to provide invaluable insights into HMs, improving diagnostic accuracy and supporting personalized treatment strategies.

Circulating tumour DNA (ctDNA)

The examination of peripheral blood for circulating tumour DNA (ctDNA) offers a non-invasive approach to diagnose, profile, and monitor cancer in individual patients, showing promise as a prognostic biomarker for immunotherapy across various cancer types [69–71]. Dynamic changes in ctDNA concentrations serve as a potential surrogate endpoint for assessing clinical efficacy during adjuvant immunotherapy. Standardizing

ctDNA assessments in interventional clinical trials is crucial to substantiate its clinical utility [69].

In hematopoietic tumours, ctDNA analysis is emerging as a potential application in both myeloid and lymphoid malignancies. Recent research underscores ctDNA's efficacy in identifying genetic mutations, evaluating MRD, and tracking treatment responses, as substantiated through clinical trials [70, 71]. Sequencing techniques like high-throughput deep sequencing and cancer personalized profiling by deep sequencing enable comprehensive analysis of ctDNA, increasing genotyping sensitivity [70, 72, 73].

Multiple myeloma, known for clonal plasma cell proliferation, presents challenges in molecular profiling due to its multifocal nature. Comprehensive analysis of cell-free DNA (cfDNA) is essential to capture the entire tumour landscape, especially in MRD monitoring. Studies have explored cfDNA's potential in detecting immunoglobulin rearrangements and focusing on SNV/Indel analysis targeting genes like *KRAS*, *NRAS*, and *BRAF* [70, 74, 75].

Somatic mutation testing

The era of advanced molecular techniques, particularly NGS, has brought about a transformative understanding of the genetic landscape in HMs. In comparison to medical imaging techniques, the potential advances and advantages of somatic mutation testing are significant. Classically, mutation testing relied on cytogenetic techniques, specifically karyotype analysis and FISH, which enabled detection of large chromosomal, targeted chromosomal copy number, and structural variations involving genomic regions relevant to HMs. NGS has revolutionized our understanding of HMs by providing detailed insights into much higher resolution of the genetic landscape [76]. Through somatic mutation testing, clinicians can identify specific genetic alterations that play crucial roles in prognosis, treatment selection, and risk stratification for patients with various HMs [77–79].

In MDS about 80% of patients harbor mutations, with over 40 recurrent somatic mutations identified, playing a pivotal role in prognosis, guiding therapeutic choices, and offering targeted treatment avenues [80]. Notably, mutations in *DNMT3A* are associated with accelerated progression to acute myelogenous leukemia and reduced median survival [17, 18]. Multivariate analyses identify several mutations, including *ASXL1*, *RUNX1*, *TP53*, *EZH2*, and *ETV6*, as independent predictors of poor outcomes. Proposals for prognostic tools integrating mutational status, age, and gender underscore the potential of molecular data in refining risk stratification for MDS patients [80]. In AML, NGS aids in diagnosis by supplementing traditional assessments, and specific mutations identified through NGS contribute to risk stratification and provide valuable prognostic information [81]. Similarly, in CML, somatic mutation testing reveals unexpected mutation dynamics post-treatment and predicts treatment responses, highlighting the importance of incorporating molecular data into treatment decision-making processes [82].

Somatic mutation testing offers a personalized approach to cancer care, allowing for tailored treatment strategies based on individual genetic profiles. This can lead to more effective therapies, improved patient outcomes, and better management of HMs. Table 1 lists some of the key genetic mutations and phenotypic attributes of the various myeloid malignancies according to current literature and ICC/WHO5 classification guidelines.

Although recent advancements in diagnostic techniques—such as FCM, somatic mutation testing, and ctDNA analysis—have significantly improved the identification and classification of myeloid malignancies, these methods often lack the ability to contextualize results across multiple molecular dimensions. For example, genetic profiling can identify mutations but may not predict their impact on gene expression or disease phenotype. Epigenetic data, when analyzed alongside genetic variants, provides crucial insights into how these mutations affect gene regulation, ultimately influencing disease progression and response to therapy. ML and AI are now being leveraged to integrate these diverse datasets, enabling the discovery of hidden patterns that refine disease classification, predict patient outcomes, and guide targeted treatment strategies. By applying ML algorithms to multi-omics data, clinicians and researchers can gain a more holistic understanding of myeloid malignancies, driving precision diagnostics forward.

Machine learning and epigenetics for precision decision-making

ML is transforming precision decision-making in oncology by harnessing robust algorithms capable of processing vast datasets. ML empowers clinicians with tools for early disease detection, accurate diagnosis, and personalized treatment strategies. Deep learning (DL), a subset of ML, has shown remarkable efficacy in tasks like brain tumour detection and classifying gene expression patterns in cancerous tissues. Additionally, AI methods such as support vector machines and neural networks contribute to precise risk stratification, diagnosis, and treatment outcome prediction, offering invaluable insights into HMs [88–90].

Despite advancements, integrating ML into cancer diagnosis and treatment poses challenges due to the complexity and scale of genomic and epigenomic data. Accurate data representation and feature extraction,

Table 1 Current clinical classifications of myeloid malignancies and their associated driver mutations, secondary mutations, and physiology [3, 5, 16, 18, 21, 41, 83–87]

Condition	Subtype	Driver mutations	Other mutations	Physiology
MPN	ET[5,83–85]	*JAK2V617F*, *CALR*, *MPL*	*ASXL1*, *SRSF2*, *IDH2/EZH2*, *SETBP1*, *TET2*	Thrombocytosis; proliferation of mature megakaryocytes in BM
	PV [5,83–85]	*JAK2*, *JAK2V617F*	*ASXL1*, *SRSF2*, *IDH2/EZH2*, *SETBP1*, *TET2*	Elevated hemoglobin, hematocrit, or red blood cell mass; panmyelosis; no megakaryocytic atypia
	PMF [5,83–85]	*JAK2*, *CALR*, *MPL*	*ASXL1*, *SRSF2*, *IDH2/EZH2*, *SETBP1*, *TET2*	Megakaryocytic proliferation and atypia in BM; fibrosis in BM; anemia, leukocytosis, high LDH
	CNL [5,83–85]	*CSF3R*	*SETBP1*, *ASXL1*, *SRSF2*	Persistent neutrophilia; granulocytes at segmented stage in PB
	CML[5,21,41,83–85]	*BCR/ABL1*	**Rare - ABL1*	Proliferation of mature myeloid cells; Philadelphia chromosome t(9;22) translocation.
MDS	SF3B1, del(7q)[3,5,16,83,85]	*SF3B1*, *del(7q)*	*TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *EZH2*	Morphologic dysplasia; <5% BM blasts; <2% PB blasts; ≥1 cytopenia; no cytoses
	del(5q) [3,5,16,83,85]	*del(5q)*	*TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *EZH2*	Morphologic dysplasia; <5% BM blasts; <2% PB blasts; ≥1 cytopenia; possible thrombocytosis
	EB[5,21,83,86]	*SF3B1*, *del(5q)*, *del(7q)*	*TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *EZH2*	Morphologic dysplasia; 5–9% BM blasts; 2–9% PB blasts; ≥1 cytopenia; no cytoses
	TP53[3,5,83,85,86]	*Multi-hit TP53*	*Del(17p)*	Morphologic dysplasia; 0–9% BM and PB blasts; ≥1 cytopenia
MDS/AML[5,21,86]		*SF3B1*, *del(5q)*, *del(7q)*	*TET2*, *SRSF2*, *ASXL1*, *DNMT3A*, *RUNX1*, *U2AF1*, *EZH2*	10–19% BM or PB blasts
AML	Gene or Chromosome[3,5,18,41,83,85–87]	*NPM1*, *DNMT3A*, *FLT3*	*NRAS*, *KRAS*, *KIT*, *SF3B1*, *ZRSR2*, *U2AF1*, *SRSF2*, *IDH1*, *IDH2*, *TET2*, *ASXL1*, *RUNX1*, *GATA2*, *CEBPA*, *BCOR*, *EZH2*, *WT1*	Clonal expansion of myeloid precursors with ≥ 20% blasts in BM or PB; inhibition of normal hematopoiesis, causing cytopenias; variable differentiation in myeloid lineages
	APL[5,18,83,85–87]	*PML/RARA*	*FLT3-ITD*, *FLT3-D835*, *NRAS*, *KRAS*, *DNMT3A*, *IDH1/2*, *TET2*, *ASXL1*	Severe bleeding; coagulopathy due to DIC; promyelocytes dominate BM
	TP53[3,5,18,85–87]	*Multi-hit TP53*	-	Complex karyotypes; associated with poor prognosis and resistance to standard chemotherapy
	Myelodysplasia-related[3,5,18,21,83,86,87]	*TP53*, *RUNX1*, *ASXL1*, *EZH2*, *STAG2*	*SF3B1*, *SRSF2*, *U2AF1*, *ZRSR2*	Arises from MDS; characterized by dysplasia in ≥ 50% of cells in at least 2 lineages
	t-AML[5,16,18,83]	*NPM1*, *DNMT3A*, *FLT3*	-	Previously treated with leukemogenic therapies
	Secondary AML[5,16,18,87]	*ASXL1*, *SRSF2*, *SF3B1*, *U2AF1*, *ZRSR2*,	*EZH2*, *BCOR*, *STAG2*	Arises from antecedent hematologic disorder
MDS/MPN	CMML[5,83,86]	*SRSF2*, *TET2*, *ASXL1*	*SETBP1*, *NRAS/KRAS*, *RUNX1*, *CBL*, *EZH2*, *NPM1*	Monocytosis; cytopenia; presence of clonality; <20% blasts in PB and BM
	RS [5,83]	*SF3B1*	*TET2*, *SRSF2*, *U2AF1*	Ring sideroblasts present in BM; dyserythropoiesis; anemia
	aCML[5,21,83]	**BCR/ABL1*$^{-}$	*SETBP1*, *ASXL1*, *EZH2*, *NRAS*, *KRAS*	Persistent leukocytosis; <20% blasts in PB; dysgranulopoiesis; no *BCR/ABL1* fusion

particularly concerning interrelated epigenetic events, are essential for ML efficacy [91, 92].

Epigenetic modifications are pivotal in ML for precise diagnosis [93], and have become an increasingly important target in the discovery of biomarkers for HMs. ML and DL analyses uncover unique disease-specific DNAm patterns, aiding diagnosis, staging, and prognosis [94–96].

Overall, ML algorithms distinguish between cancer stages, classification, and/or risk stratification through DNAm analysis, showcasing high discriminative capacity among malignancy types [94–97]. However, integrating ML into cancer diagnosis and treatment poses significant

challenges. The complexity and scale of genomic and epigenomic data necessitate sophisticated analytical tools to derive meaningful insights and predictions. An integrated approach that combines multi-layered omics data acquisition, feature selection, and ML techniques can facilitate a deeper understanding of cancer biology and drive advancements in precision oncology.

DNA methylation episignatures

DNAm is an ideal candidate for biomarker development to combat the diagnostic and prognostic complexity of HMs for a multitude of reasons. Firstly, DNAm changes are widespread and pathologically significant in malignancies of all kinds, with effects seen across all stages of carcinogenesis from neoplastic transformation to metastasis [36]. Secondly, the DNAm changes detected in blood are biologically stable and cancer specific, even in early stages [98]. Thirdly, genomic DNAm profiling using microarray technology is relatively affordable, technically robust, reproducible, and scalable, and enables assessment of genome wide changes [39, 93]. DNAm profiling targets areas such as promoters of oncogenes and tumour suppressor genes, differentially methylated regions (DMRs) associated with prognostic outcomes, and large-scale changes in hyper- or hypo- methylation in identified diagnostically relevant CpG's [39].

DNAm profiling including assessment of episignatures, which are recurrent, sensitive, and specific DNAm biomarkers detectable in patient's tissues associated with a common genetic or environmental etiology [99, 100], have been investigated for biomarker utility in many major cancer types [38, 39, 41–44, 98, 101, 102]. These include assessment of targeted genomic regions for DMRs and differentially methylated genes in ovarian cancers [103–106], and gene-specific methylation changes in head and neck cancers [107], colorectal [108], lung [109], breast [7], prostate [110, 111], and hematologic cancers [17, 40, 45, 112, 113]. Genome-wide DNAm episignatures have been studied for their utility as biomarkers in sarcoma's [114], brain and central nervous system (CNS) tumours [115–117], and AML [118]. One genome-wide episignature classifier that has seen significant adoption in solid tumours is a CNS cancer DNAm classifier, which uses methylation microarray in combination with Randon Forest ML techniques to classify brain tumours [116]. Large scale studies on the use of DNAm biomarkers in HM have not been performed to date, however, there is evidence of the utility of testing peripheral blood and bone marrow DNAm to develop biomarkers for specific subtypes of HMs, including CML and AML [26, 118, 119]. These studies support the hypotheses that DNAm profiling is an effective method for cancer biomarker development, though there remains limited implementation of DNAm biomarker discovery in cancer, possibly due to the lack of clinical guideline incorporation of current technology [120].

While research in classification of HMs using DNAm classifiers is just emerging, the use of DNAm episignatures in peripheral blood has been extensively studied in hereditary genetic and environmentally induced disorders. More than 100 rare disorders related to over 140 genes have distinct diagnostic episignatures [99, 100, 121–129]. In addition to enabling patient screening, episignatures can be used for reclassification and interpretation of VUS in genetically unsolved patients [99, 125, 126, 128, 130]. The ability to detect DNAm episignatures in easily accessible peripheral blood samples has facilitated implementation of episignature testing in a standardized fashion across clinical laboratories [93, 127, 129]. This highlights the critical role of epigenomic research in transforming diagnosis and treatment of genetic disease, the principles and technology of which can be extrapolated to apply to HMs and other cancers.

The increasing complexity of HMs necessitates a shift from conventional diagnostic approaches toward integrative computational frameworks that can analyze multi-omics data. Traditional methods, while effective at detecting genetic and cytogenetic abnormalities, often fail to capture the nuanced regulatory effects of epigenetic modifications. The integration of genomic, transcriptomic, epigenomic, and proteomic datasets allows for the construction of more comprehensive disease models, improving diagnostic precision and therapeutic decision-making. Advances in bioinformatics and computational oncology are facilitating this shift, enabling the identification of molecular signatures that correlate with disease subtypes, treatment responses, and patient outcomes. As a result, integrating multi-omics data through computational techniques is emerging as a cornerstone of modern HM diagnostics, bridging the gap between molecular research and clinical application.

Data integration and computational advances

Impact of integrated genomic and epigenomic profiling techniques in HMs

Integrated genomic and epigenomic profiling represents a crucial advancement in understanding and managing HMs, significantly enhancing disease characterization and treatment strategies [131–133]. Through integration of comprehensive genetic and epigenetic alteration analyses, we obtain invaluable insights into disease subtypes and prognosis, transforming personalized medicine in hematology [133]. This approach facilitates the identification of actionable alterations, guiding targeted therapies and advancing precision medicine tailored to individual patients [131–133]. Integrated profiling improves patient outcomes and addresses challenges inherent to traditional diagnostic and treatment modalities in HMs,

establishing itself as a cornerstone in advancing clinical care [131–133].

Over decades of study and technological advancements our ability to identify causative variations in cellular processes leading to malignancy has improved exponentially. The relationship between large scale genetics to functional consequences in cancer began in 1960 through cytogenetic analysis, when the first chromosomal abnormality associated with cancer was discovered; this abnormality is known as the Philadelphia chromosome, and to this day it is a diagnostic component for CML [134]. Following this initial discovery researchers determined that the chromosomal rearrangement which occurs resulting in the Philadelphia chromosome impacts gene function and forms the gene fusion *BCR/ABL1*. Subsequent analysis showed that this gene codes for an aberrant kinase protein, with the functional consequence of unrestricted cellular growth [135]. The knowledge of kinase activity led to treatment for CML patients with kinase inhibitors, which were highly effective until treatment resistance began to emerge. Studies investigated and found that the DNAm of the s*FRP1* gene, causing epigenetic silencing, was responsible for the resistance to kinase inhibition and de-methylating agents could be used to sensitize previously resistant CML cases [119]. The story of CML illustrates the profound relationship between cytogenetics, genetics, epigenetics, and functional outcomes in cancer, and notably in myeloid malignancies.

Epigenetic variations contribute to the missing understanding of heritability in complex diseases and reveal the regulatory roles of non-coding genomic regions, providing insights into dynamic gene expression control through chromatin packaging [136]. Key epigenetic mechanisms such as DNAm, histone modifications, and non-coding RNA are vital components of gene regulation, influencing gene silencing, chromatin remodeling, and post-transcriptional control [136, 137]. Integrated genetic and epigenetic profiling techniques, from array-based assays to sequencing-based and single-cell analyses, enable a detailed understanding of these regulatory processes at different levels of resolution [138]. Statistical and data integration methods identify functional epigenetic alterations and their associations with gene expression. Pathway analysis and network-based approaches highlight the biological implications of these changes, uncovering therapeutic targets and advancing personalized treatment strategies for HMs [137].

This integration aids in identifying epigenetic biomarkers and drug targets, while also providing insights into gene-environmental interactions. With ongoing advancements in computational tools and experimental methods, collaborative efforts in integrated profiling promise to revolutionize clinical practice, enhancing disease prevention, diagnosis, and treatment strategies. Studies have found significant improvement in identification of actionable target discovery in HMs using integrated profiling techniques, as evidenced in high-risk pediatric cancers [79], ALL [139], blast-crisis CML [140], and *TP53* AML and MDS [141], with the possibility of further applications well-supported by conceptual review [88].

Precision oncology has revolutionized cancer treatment by tailoring therapies to molecular alterations, enhancing patient-specific approaches. Tumor characterization techniques like immunohistochemistry and DNA/RNA sequencing rely on molecular pathology biomarkers for initiating treatment. Aligning pharmacological interventions with molecular findings improves progression-free survival, as evidenced by randomized trials [142–144].

Next-generation sequencing has transformed cancer research by enabling simultaneous examination of gene sequences. While these advancements have expanded diagnostic capabilities, rigorous validation remains essential for the clinical application of large gene panels and whole-genome analyses. In HMs, molecular testing technologies such as cytogenetics, FISH, and PCR play pivotal roles in influencing prognosis and treatment decisions, improving diagnostic precision and advancing therapeutics [142, 144]. Although cytogenetics offers comprehensive insights, FISH and PCR provide quicker, more specific alternatives. FISH excels at analyzing non-dividing cells, while PCR remains indispensable for DNA analysis. NGS identifies driver mutations, expediting therapeutic decisions in HMs [142].

Molecular testing extends to evaluating germline and somatic mutations, essential for assessing cancer risk and guiding treatment. Understanding concepts like "drivers," "passengers," and "actionable" mutations is key to optimizing therapy. In MRD monitoring, molecular methods such as flow cytometry, PCR, and NGS detect low cell levels post-therapy, serving as critical indicators for prolonged survival. MRD status, influenced by molecular sensitivity, predicts progression-free and overall survival in diseases like chronic lymphocytic leukemia, ALL, and multiple myeloma [142, 144]. These molecular techniques underscore their indispensable role in refining diagnostic precision and guiding therapeutic decisions in HMs. Additionally, advanced molecular methods like gene-level copy-number alteration (CNA) analysis enable a comprehensive understanding of the genomic landscape in HMs. By identifying CNAs and allele-specific states, these approaches enable large-scale sequencing efforts in HMs, offering refined diagnostic, prognostic, and therapeutic strategies [145].

The translational potential of integrating genomic and epigenomic data into clinical practice is already being realized through the development of epigenetic biomarkers and AI-driven diagnostic tools. DNA

methylation-based classifiers, for example, demonstrate high sensitivity and specificity in differentiating HM subtypes, aiding in early detection and risk stratification. Furthermore, computational models that incorporate both genetic mutations and epigenetic alterations are improving the accuracy of prognostic predictions, allowing clinicians to tailor treatment regimens based on an individual's molecular profile. These advancements are ushering in a new era of precision oncology, where treatment decisions are no longer solely based on histopathology or genetic mutations but are informed by a comprehensive molecular landscape. By continuously refining these integrative approaches, the field is moving closer to a paradigm in which every patient benefits from a truly personalized treatment strategy.

Conclusion

The molecular and cellular heterogeneity of HMs causes difficulty in diagnostic subclassification. Great strides have been made in recent years with molecular profiling for diagnostic subclassification of HMs, however, challenges persist with the multitude of malignancies with overlapping molecular and clinical features, and VUS. Information related to prognosis, treatment response, and disease progression is also limited based on currently available molecular and cytogenetic profiling technologies.

The ability of DNAm to influence leukemogenic processes implies that a deeper understanding of epigenetic regulation in HMs can provide insights into malignant transformation, disease progression, genetic etiology, VUS classification, biomarker development and therapeutic strategies to ultimately overcome the challenges posed by heterogeneity in HMs.

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