

Application of precision medicine in clinical routine in haematology—Challenges and opportunities

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Precision medicine is revolutionising patient care in cancer. As more knowledge is gained about the impact of specific genetic lesions on diagnosis, prognosis and treatment response, diagnostic precision and the possibility for optimal individual treatment choice have improved. Identification of hallmark genetic aberrations such as the *BCR::ABL1* gene fusion in chronic myeloid leukaemia (CML) led to the rapid development of efficient targeted therapy and molecular follow-up, vastly improving survival for patients with CML during recent decades. The assessment of translocations, copy number changes and point mutations are crucial for the diagnosis and risk stratification of acute myeloid leukaemia and myelodysplastic syndromes. Still, the often hetero-

geneous and complex genetic landscape of haematological malignancies presents several challenges for the implementation of precision medicine to guide diagnosis, prognosis and treatment choice. This review provides an introduction and overview of the important molecular characteristics and methods currently applied in clinical practice to guide clinical decision making in haematological malignancies of myeloid and lymphoid origin. Further, experimental ways to guide the choice of targeted therapy for refractory patients are reviewed, such as functional precision medicine using drug profiling. An example of the use of pipeline studies where the treatment is chosen according to the molecular characteristics in rare solid malignancies is also provided. Finally, the future opportunities and remaining challenges of precision medicine in the real world are discussed.

Keywords: drug screening, haematology, MRD, precision medicine

Introduction

The term precision medicine refers to the strategy of predicting which patients are most likely to respond to specific cancer therapies based on high-resolution molecular diagnostics and, increasingly, functional and mechanistic understanding of individual tumours [1]. The field of haematology has

been pivotal in the development and implementation of precision medicine. This is partly because tumour material is readily available in the blood and bone marrow. Further, haematologic malignancies have been suitable candidates for pioneering precision medicine due to the presence of some disorders with a homogeneous genetic landscape and characteristic driver mutations. For example,

one of the first largely successful targeted cancer therapies was tyrosine-kinase inhibitors (TKIs) for chronic myeloid leukaemia (CML) [2]. While genetic analyses such as karyotyping have been essential diagnostic procedures for a long time in haematology [2, 3], gene panel sequencing has been introduced more recently in clinical routine, to identify genetic alterations with diagnostic, prognostic and predictive impact [4–7]. In addition, more sensitive techniques to assess minimal residual disease (MRD) are being developed. However, since the first whole-genome sequencing (WGS) of leukaemia in 2008 [8], the mutational landscape of haematological malignancies has proven to be both much more complex and heterogeneous with a shortage of targets as uniformly present and druggable as in CML. Also, intratumour heterogeneity and clonal evolution are increasingly recognised and likely to play a role in tumour progression as well as therapy resistance [9–12].

Despite rapid advances, a multitude of challenges remains in implementing precision medicine in clinical routine. How do we best identify genetic alterations of importance for an individual patient? How do we ascertain who will benefit from novel and often costly targeted therapies? How do we best evaluate the treatment effect and progression to stay ‘on top’ of the tumour and modify treatment schemes if needed?

This review provides an overview and examples of the key methods of molecular characterisation currently applied to guide diagnosis, prognosis and individual treatment choice in myeloid and lymphoid malignancies in real-world clinical practice. Whereas genetic characterisation is a cornerstone of the clinical work-up of myeloid malignancies, we have yet to understand the full benefit in several lymphoid tumour subtypes. Further, we will also provide examples of more experimental ways to choose optimal treatment using functional precision medicine (FPM; drug profiling) and examples of how precision medicine has been used to improve survival in rare solid malignancies. Finally, we will discuss the remaining challenges and future opportunities with precision medicine in haematological malignancies.

Current precision diagnostics in myeloid haematological malignancies

For the diagnosis, classification, prognostication and therapy selection of myeloid malignancies, a

comprehensive genetic workup is required in current clinical practice. Currently, a combination of different techniques such as chromosome banding analysis, fluorescence in situ hybridisation (FISH) and panel sequencing is performed to comprehensively assess translocations, copy number changes and point mutations. The extent of the genetic analyses, especially of the set of genes to be analysed, is dependent on the disease entity as the diagnostic relevance of translocations, copy number changes and point mutations vary between entities. The WHO classification and several entity-specific guidelines provide recommendations [13].

CML is an example of a disease in which both diagnostics and therapeutics have been revolutionised by advances in genetic characterisation [2]. This is largely thanks to the fact that CML is quite a simple disease from a genetic point of view, driven by the *BCR::ABL1* fusion. Following the availability of TKIs, young patients with CML responding optimally to TKI therapy have a near-normal life expectancy [14–16]. Thus, at diagnosis, a combination of chromosome banding analysis and molecular techniques to confirm the presence of the *BCR::ABL1* fusion is sufficient for diagnosis and treatment initiation [17]. For follow-up, measurement of the *BCR::ABL1* real-time quantitative reverse transcriptase polymerase chain reaction (PCR) is the current gold standard [17]. In the case of TKI resistance, a more comprehensive genetic workup including sequencing of *BCR::ABL1* and the detection of additional structural variants, copy number alterations (CNAs) and single nucleotide variants (SNVs) are helpful for further treatment decisions. However, the main focus in personalizing treatment in CML is currently to select TKIs based on patients’ comorbidities and to monitor treatment response [18].

The group of *BCR::ABL1* negative myeloproliferative neoplasms (e.g., polycythaemia vera, essential thrombocythaemia, primary myelofibrosis) is much more heterogeneous from a clinical point of view but also in terms of genetics. Much progress has been made in the genetic characterisation of these diseases and the presence of point mutations in genes such as *JAK2*, *CALR* and *MPL* is now crucial for diagnosis and prognosis, but translation into targeted treatment approaches based on genetic markers has yet to occur [19].

Myelodysplastic syndrome (MDS) and acute myeloid leukaemia (AML) are very complex disease

groups including subtypes with rather few genetic abnormalities up to more complex changes including a variety of structural variants, CNAs and SNVs. The genetic landscape of MDS and AML has been evaluated in detail by several groups [4, 20, 21]. Internationally accepted prognostication scoring systems have been established for both diseases that guide treatment decisions—in MDS including karyotype and AML based on karyotype and molecular mutations [22–24]. More recent prognostication models in MDS also include gene mutations [25].

Examples of specific genetic abnormalities that currently guide treatment choice in MDS include deletion 5q as the sole abnormality and *SF3B1* mutations in MDS with less than 5% blasts, influencing selection to treatment with lenalidomide and luspatercept, respectively [26, 27]. In AML, treatment assignment based on genetic markers such as *FLT3*-ITD and mutations in *IDH1* and *IDH2* is both implemented in clinical routine and studied in ongoing trials [28, 29].

Due to the genetic complexity of MDS and AML, a combination of chromosome banding analysis and panel sequencing is required to capture the relevant genetic alterations. WGS could be a great step forward in the genetic characterisation of myeloid malignancies as it captures all different types of genetic aberrations in one assay. Recently, a cohort of AML and MDS patients was prospectively analysed by WGS and results were compared to the gold standard genetic work-up demonstrating the feasibility and clinical utility of WGS [30].

While current diagnostics to aid precision medicine are focused mainly on identifying variants at the DNA level, the ability to identify gene fusions and to measure gene expression by RNA sequencing, also referred to as whole-transcriptome sequencing (WTS), holds great promises, as recently reviewed [31]. For example, many gene fusions in AML constitute class-defining and treatment predictive entities, for example, *PML::RARA*, *BCR::ABL1*, *CBFB::MYH11* and *RUNX1::RUNX1T1*, that are readily detectable by WTS [32]. In addition, data are forthcoming that classification based on transcriptional profiles can be used to subdivide class-defining subtypes (e.g., *NPM1*-mutated AML) into separate entities of prognostic and possibly therapeutic relevance and as a proxy to predict response to a large variety of drugs [33, 34].

Collectively, depending on the entity of myeloid malignancies, the complexity and spectrum of genetic aberration vary as well as the progress in adapting treatment decisions based on genomics. Depending on these parameters, the value of comprehensive diagnostics up to WGS varies and needs to be re-evaluated and adjusted periodically based on the evolution of knowledge and availability of new drugs.

Current precision diagnostics in lymphoid haematological malignancies

For lymphoid haematological malignancies, genetic categorisation and treatment choice have come the furthest in chronic lymphocytic leukaemia (CLL). In CLL, the prognostic role of genetic markers has been established for a long time and the presence of *TP53* alterations as well as the IGHV mutation status have been included in the international prognostic index for patients with CLL [35]. Current clinical practice guidelines state that *TP53* mutations (analysed by Sanger sequencing or next generation sequencing (NGS)) and *del(17p)* (evaluated by FISH) are of the strongest prognostic and predictive relevance. Strong prognostic evidence and predictive evidence for chemoimmunotherapy (CIT) have been confirmed for the IGHV mutation status. A complex karyotype determined by chromosome banding analysis was categorised as possible prognostic and predictive relevance still requiring prospective clinical trials for confirmation [36].

Additional genetic markers have been analysed in CLL for their prognostic impact by different techniques such as FISH [37, 38], genomic arrays [39, 40] or whole exome sequencing (WES)/WGS [41–43]. The next step is to evaluate these genetic markers in relation to new therapeutic concepts. For gene mutations such as *NOTCH1*, *SF3B1*, *BIRC3* or *RPS15*, as well as complex karyotype, it was demonstrated that they predict an unfavourable prognosis also in the absence of *TP53* deletion/mutation, but it still has to be determined in clinical trials which drug/drug combinations can improve outcome in a certain genomic setting [36].

Another lymphoid disease in which genetic aberrations guide diagnosis and prognosis is acute lymphoblastic leukaemia (ALL) [1, 44]. According to the current WHO classification, nine genetic subgroups of B-cell precursor ALL are defined as requiring a comprehensive genetic work-up currently based mainly on the karyotype and the

detection of gene fusions by FISH or molecular techniques [45]. However, alterations below the resolution of chromosome banding analysis such as submicroscopic gene deletions or even intragenic deletions such as deletion within *IKZF1* were also shown to be relevant for the pathogenesis and prognosis [1, 46, 47]. Also, SNVs were shown to be relevant [1]. However, in contrast to AML, screening for mutations in a panel of genes is not yet considered standard in ALL. While risk assessment and risk-adapted treatment based on genomics have been implemented for many years in paediatric ALL, this approach is lagging in adult ALL [44, 48, 49]. The availability of new drugs and the need to identify which patients will benefit the most from these will likely require increasingly detailed genomic diagnostics. As ALL harbours a broad spectrum of structural variants, CNAs and SNVs, technologies that can assess all of these, such as WGS, might turn out to be the method of choice, especially as chromosome banding analysis is hampered by low in vitro proliferation of certain ALL subtypes as well as the cytogenetically cryptic nature of specific abnormalities such as *ETV6::RUNX1*, *IGH::CRLF2*, *EP300::ZNF384*, *DUX4*-rearranged and others [31, 50]. In this context, more widespread WTS in a clinical setting is likely to improve precision diagnostics further given that ALL is a disease entity characterised by very distinct gene expression signatures and a large number (greater than 150) of recurrent gene fusions [31, 51–53]. Of particular importance for improved precision medicine in the management of ALL is the detection of gene expression signatures (e.g., Philadelphia-like or *BCR::ABL1*-like ALL) or gene fusions involving *CRLF2(IGH::CRLF2, P2RY8::CRLF2)*, *ABL*-class genes (*ABL1*, *ABL2*, *CSF1R*, *LYN*, *PDGFRA*, *PDGFRB*) or alterations resulting in the activation of JAK-STAT and RAS signalling [54–56]. Clinical trials are currently ongoing in which kinase inhibitors against *ABL*-class and *JAK1/2* family members are tested as treatment additions depending on the underlying genetic alteration (ClinicalTrials.gov: NCT03117751, NCT02883049 and NCT02723994).

In lymphomas, detection of chromosomal translocations through FISH has been an important part of the diagnostic workup for several decades, including, t(14;18) in follicular lymphoma, t(11;14) in mantle cell lymphoma and t(8;14) in Burkitt lymphoma. Since then, the broader WES and WGS techniques and their detection of both transloca-

tions, SNV and CNAs, have paved the way for further understanding of the molecular biology of the diseases and refinements of the lymphoma subtype classification. About 10 years ago, the *BRAF*^{V600E} point mutation was identified as a molecular hallmark of hairy cell leukaemia and its detection is now used in routine diagnostics by real-time PCR or targeted NGS [57]. Additionally, this finding has created possibilities for potential treatment of relapsed/refractory (R/R) cases with *BRAF* inhibitors [58]. In Waldenström's macroglobulinemia, the *MYD88*^{L265P} point mutation has been found to be highly recurrent, although it is not exclusive to this lymphoma subtype, and may have implications for response to ibrutinib [59, 60]. The *CXCR4* mutation has also been identified specifically in Waldenström albeit in a lower proportion of the cases (30%) [61].

In aggressive lymphomas, the distinction of high-grade B-cell lymphomas with translocations involving *MYC* and *BCL2* and/or *BCL6* (often referred to as double- or triple-hit lymphomas) is of high clinical relevance as these patients are now recommended to receive more intensified treatment up front [62]. Also, gene-expression profiling and exome sequencing analysis has shed additional light on the aggressive grey-zone lymphomas [63]. This uncommon entity can further be dichotomised into a more primary mediastinal B-cell lymphoma-like and a more diffuse large B-cell lymphoma (DLBCL)-like molecular subtype strongly correlated with the presence or absence of anterior mediastinal involvement, and with implications for the choice of treatment. The former displays a mutational profile resembling classical Hodgkin lymphoma with *SOCS1* and *B2M* mutations, whereas the latter nonmediastinal subtype commonly displays mutations in *TP53* and *BCL2* [63]. In another subtype of DLBCL, the T-cell/histiocyte-rich large B-cell lymphoma, *PD-L1/PD-L2* alterations are common and provide a genetic basis for immune evasion, and treatment with immune checkpoint inhibitors [64]. These alterations resemble those of Hodgkin Reed-Sternberg cells where gains of chromosome band 9p24.1 encompassing *PD-L1/PD-L2/JAK2* contribute to an inefficient antitumour microenvironment and an 'exhausted' T-cell phenotype. It is possible that a broad gene panel or WGS incorporated in the early diagnostics of lymphoid malignancies will prove to be efficient in both recognising rare subtypes early on as well as guiding primary and/or secondary treatment lines [65].

Precision medicine in the clinical routine in AML—Progress and challenges

The intensive regimen of a combination of cytarabine and anthracycline, termed '7 + 3' chemotherapy, has long been considered a standard of care for 'fit' younger patients, yet the long-term cure rates remain at only 30%, with consistently poor outcomes in patients aged >65 years. The examples of data-driven therapy for molecular subsets were, for a long time, limited to the use of all-trans retinoic acid/arsenic trioxide in acute promyelocytic leukemia [66] and subsequent recognition of the benefits of anti-CD33 antibody-drug conjugate gemtuzumab ozogamicin (GO) in core-binding factor leukaemias [67], followed by FDA re-approval of GO in 2017. Recent advances in understanding the pathophysiology and the molecular basis of leukemogenesis led to accelerated discovery and introduction in clinical practice of novel agents for AML therapy.

The affordable and broad use of NGS has enabled the definition of refined molecular subsets of AML. This has not only improved risk assignment but also fine-tuned and increased knowledge regarding prognostic markers to further understand the role of stem cell transplantation (SCT). Ultimately, it has also led to FDA approvals of several molecularly defined targeted agents clinically useful for patients of all ages. The era of new drug approvals in the setting of molecularly defined personalised medicine for AML was pioneered by the RATIFY trial, which showed a survival benefit of adding the first generation FLT3 inhibitor midostaurin to 7 + 3 chemotherapy for newly diagnosed patients with *FLT3* mutations, followed by multiple trials with second and third generation FLT3 inhibitors [68]. From these, single-agent gilteritinib was FDA approved as salvage therapy for *FLT3*-mutated AML based on results of the phase 3 ADMIRAL trial, which demonstrated higher response rates and longer survival with gilteritinib therapy compared with salvage chemotherapy (complete remission [CR] rates of 54% vs. 22%, and median survival 9.3 vs. 5.6 months; hazard ratio 0.637; $p = 0.0007$) [69]. Several ongoing randomised studies are studying the efficacy of gilteritinib and other FLT3 inhibitors, such as quizartinib and crenolanib, when added to the 7 + 3 backbone.

Recurrent mutations in two IDH isoforms, *IDH1* (6%–10%) and *IDH2* (9%–13%), have been iden-

tified in AML and are more often seen in elderly patients. Specific inhibitors for AML with an *IDH1* mutation (ivosidenib) and *IDH2* mutation (enasidenib) are both FDA approved now for use in patients with R/R AML harbouring these respective mutations, based on acceptable safety and impressive activity with overall response rates of approximately 40% and median overall survival (OS) of 9 months [70, 71]. Furthermore, ivosidenib is also approved for frontline therapy based on efficacy in patients with newly diagnosed *IDH1*-mutated AML [72]. These agents induce leukaemia cell differentiation, which may manifest as on-target 'differentiation syndrome' toxicity.

Given that the median age at diagnosis of AML is high—approximately 68 years—a majority of AML patients are considered 'older' [73]. In 'fit' older patients, a phase III randomised trial has compared the intensive regimen CPX-351 (liposomal daunorubicin and AraC) to the 7 + 3 regimen in patients aged 60–75 with secondary AML. With CPX-351, both CR rates and OS were improved compared to 7 + 3 (2-year OS 31.1% with CPX-351 vs. 12.3% with 7 + 3), and 34% of patients treated with CPX-351 underwent SCT [74]. This led to FDA approval of CPX-351 for adult newly diagnosed therapy-related AML and AML with MDS changes, with no age restriction. For older AML patients ineligible for intensive chemotherapy, the combination of hypomethylating agents (HMA) or low-dose cytarabine (LDAC) and the BCL2 inhibitor venetoclax (VEN) has become a standard-of-care nonintensive option. AML cells are highly dependent on the anti-apoptotic BCL2 family of proteins for survival, and venetoclax is a specific and potent inhibitor of the anti-apoptotic protein BCL2 [75]. The addition of the BCL2 inhibitor venetoclax to HMA was highly effective in the pivotal randomised Phase III VIALE-A study, extending OS from 9.6 months in the AZA group to 14.7 months in the AZA/Ven group and increasing the CR/CRi (CR with incomplete recovery) rate from 28.3% to 66.4%, with an overall acceptable safety profile notable for higher rates of myelosuppression [76]. LDAC with venetoclax is also approved for AML based on VIALE-C results [77]. Glasdegib is an oral inhibitor of the Hedgehog signalling pathway, which has been shown to play a role in maintaining the leukaemia stem cell compartment. Among AML patients, the addition of glasdegib to LDAC improved the median OS from 4.3 months to 8.3 months and improved the overall response rate (ORR) from 5.3% to 26.9% [78].

Apart from FDA-approved novel agents, multiple exciting immunotherapy approaches are being exploited in AML clinical trials. T-cell engaging molecules that co-engage the CD3 receptor on T cells and a myeloid surface marker include the CD3-CD123 DART flotetuzumab and the CD3-CD33 BiTE AMG 330. In a phase I/II study of flotetuzumab in patients with R/R AML, the ORR in 30 patients with early relapse or primary induction failure was 30% [79]. Early phase clinical trials are currently assessing the role of CAR-T cells against CD33 or CD123 in patients with R/R AML. The potential roles of anti-CD47 antibody magrolimab in the treatment of *TP53*-mutated AML, and of menin inhibitors in the treatment of *KMT2A*- and *NPM1*-rearranged AML, are under evaluation [80]. The clear benefit of oral azacitidine (CC-486) maintenance therapy in AML in the first CR in a QUAZAR AML-001 study with a median survival of 24.7 months with CC-486 versus 14.8 months with placebo (hazard ratio 0.69, $p = 0.0009$) heralded renewed interest of maintenance strategies as a valid therapeutic approach in AML armamentarium [81].

Despite these encouraging advances, several roadblocks await a resolution to improve the efficacy of novel and established therapies. Recognition of the complex molecular patterns of clonal architecture and mutation order in AML supports the notion of using rotating schedules of combination therapies, ideally focusing on driver molecular events [82, 83]. One example of such a strategy could be the rational use of 'triplets' with HMA/VEN backbone and targeted agents (FLT3, IDH inhibitors, immune therapies), with judicious attention to overlapping toxicities. Shared antigens between AML and normal haematopoietic stem cells yield risks of on-target off-tumour toxicity (cytokine-release syndrome and profound myelosuppression) and antigen escape (leading to early relapse). For any type of therapy, utilisation of sensitive and specific assays of minimal residual disease (MRD) may improve the early detection of tumour escape and offer opportunities for MRD-directed therapies. Identification of novel targets for both AML and immune microenvironment using single-cell approaches will broaden our horizons of personalised therapy in AML.

Precision medicine in clinical routine in lymphoid malignancies—Progress and challenges

Although genetic aberrations have improved and been implemented in the diagnostic work-up for

several lymphoid disorders, few lymphoid malignancies are as yet managed with a precision medicine approach in the first-line setting. One exception is CLL. As reviewed above, *TP53* alterations are associated with the poorest prognosis if patients are treated with CIT. Prognosis has significantly improved under treatment with B-cell receptor inhibitors, such as ibrutinib, and the *BCL2* inhibitor venetoclax. Thus, the presence of *TP53* deletion or mutation currently mandates treatment with BTK-inhibitor ibrutinib in the first line [84, 85]. Moreover, VDJ mutation status impacts treatment choice with the combination of *BCL2*-inhibitor venetoclax and the CD20 antibody obinutuzumab constituting a treatment option for IGVH unmutated patients [86].

Several studies now suggest that *TP53* aberrations are also associated with worse prognosis in other lymphoma subtypes, most notably mantle cell lymphoma, but also, for example, Waldenström's macroglobulinemia [61]. Recently, four distinct clusters of mantle cell lymphoma were defined based on WES and RNA sequencing and the group with the worst outcome was characterised by *TP53*/del(17)p mutations, del(13q) and del(9p) and active *MYC* and hyperproliferation signatures [87]. However, we have yet to learn how these findings should be implemented in clinical practice. In follicular lymphoma, a prognostic model has been suggested, called the m7-FLIPI, that integrates established clinical prognostic determinants such as advanced disease stage and number of involved lymph node regions with the mutation status of seven genes (*EZH2*, *ARID1A*, *MEF2B*, *EP300*, *FOXO1*, *CREBBP* and *CARD11*) [88]. However, the m7-FLIPI was defined among patients treated with first-line immunochemotherapy and was not validated in a cohort of patients treated with single rituximab [89].

The most commonly occurring lymphoma is DLBCL. That DLBCL constitutes an extremely genetic and phenotypic heterogeneous entity has made precision medicine a challenge in DLBCL [65]. Genetic studies have elucidated that DLBCL is much more heterogeneous than the previous stratification into germinal centre B-cell (GCB) and non-germinal centre (GC) subtypes [90]. The findings have led to the proposal of two major classifications according to genetic signature [91, 92]. These new classifications have led to the development of the 'LymphGen' classifier, in which cases of DLBCL are clustered according to probability algorithms

belonging to a certain genetic subtype based on present genetic aberrations [65]. Approximately 5.7% of patients are classified as strongly belonging to more than one genetic subtype and classified as genetically composite, while approximately 35% remain unclassifiable due to a lack of mutations, atypical mutations or very few genetic aberrations identified [65]. Still, stratification according to these subgroups has already led to the identification of subgroups that may benefit from specific therapies. For example, in a subgroup analysis of the phase III PHOENIX trial, OS was 100% for patients classified in the genetic subgroup MCD and aged ≤ 60 years, when the BTK-inhibitor ibrutinib was added to standard treatment with R-CHOP [93]. As BTK activity was one of the essential survival mechanisms found for DLBCL in the MCD subtype, the augmented efficacy of ibrutinib in this setting appears plausible [65]. Thus, treatment stratifications according to genetic subgroups now represent a feasible future possibility. To enable this development, the LymphGen algorithm is publicly available for use to support treatment stratification according to genetic variants in future clinical trials [65]. Further supporting this claim are the interim results of a prospective study evaluating R-CHOP + X, where X is chosen according to stratification with the LymphGen algorithm. With 128 patients included thus far, CR rates were 85% in the R-CHOP + X group compared to 65% in the comparator arm [94].

Apart from the genetic heterogeneity, several challenges further remain in the application of precision medicine in lymphoid malignancies. To be clinically feasible, genetic analyses need to be succinct with a short turnaround, especially in aggressive lymphoma subtypes, to facilitate prompt treatment initiation. Further, some lymphomas are not easily accessible for biopsy. Here, analyses of circulating tumour DNA (ctDNA) in the blood may offer a potential solution [95, 96]. Lastly, the complex genetic landscape of lymphomas and the as-yet-unknown significance of specific aberrations highlight the need for extensive collaboration between clinicians, pathologists and geneticists through molecular tumour boards and easily accessible and updated support tools [97].

How to best assess treatment response and follow longitudinal tumour evolution?

Apart from guiding diagnosis and prognosis, genotypic and phenotypic characterisation of malignant

clones at diagnosis provides prerequisites to monitor haematological disease during treatment. Capturing remaining disease at low levels offers an opportunity to follow treatment response and progression and to enable swift treatment initiation and adjustment, if needed. An increasingly utilised method to do this is by assessment of MRD. MRD refers to the presence of a detectable small population of malignant cells upon treatment and has revolutionised the possibility to assess disease progression and treatment response. MRD positivity indicates that there are still remaining malignant cells in the body while MRD negativity means that no cancer cells are detectable even using very sensitive methods. MRD negativity is associated with longer remissions and potentially longer rates of survival for certain haematological malignancies [23, 98–100]. In general terms, MRD testing can show how well patients respond to treatment and it can be used to monitor remission, predict relapse and identify patients in need of alternative therapies. Depending on the specific malignancy, some or all of these potential goals can be met when measuring MRD, but technological considerations and improvements, as well as careful clinical studies in specific disease entities, are warranted for successful clinical implementation [101–105].

By definition, MRD methods are highly sensitive and can detect very low amounts of malignant cells in a large background of normal cells, at levels under morphologic detection. The most widely used methods are based on cells using flow cytometry (FC), genome detection with NGS, quantitative real-time PCR (qPCR) and droplet digital PCR (ddPCR), among others. The choice of MRD markers and choice of tissue to examine often varies by type of malignancy and specific clinical needs. FC is widely used in most haematological malignancies [106] and measures protein markers at the surface of individual malignant cells and often a fresh bone marrow sample is required. Immunophenotypic evaluation by multiparameter FC can detect one malignant cell in 10^3 – 10^5 normal cells [107], as it uses a combination of leukaemic specific markers. The advantages of FC methods include its fast turnaround time, within 1 day, but it requires skilled pathologists and standardised procedures.

PCR methods search for low amounts of specific aberrant genomic sequences characteristic of the leukaemic clone, which include SNVs to translocation breakpoints in DNA and fusion transcripts

in RNA. Quantitative PCR methods can measure levels of the mutant genotype compared to the wildtype gene or a control gene and are widely applicable to many different types of genetic changes identified at diagnosis by NGS. qPCR methods are sensitive (10^3 – 10^5), well standardised and with established routines for quality assurance [99, 108]. Among the limitations are their low multiplexing potential and the need for standard curves for each specific assay. ddPCR can overcome the need for standard curves, as absolute quantification of targets can be achieved, but it also offers simpler and faster laboratory workflows [109–112].

Considering the large number of genetic aberrations that the NGS method can identify at diagnosis, a sensitive follow-up using NGS methods with high multiplexing capacity should be optimal. However, due to intrinsic errors in PCR and sequencing, variants with allele fractions under 1% are difficult to detect and measure using standard NGS [113]. Several tag-based error correction approaches exist to distinguish errors from true mutations [114, 115] and can detect variant allele frequencies (VAF) as low as 0.1% VAF but these require ultra-deep sequencing at a high cost or pooling of many samples in larger sequencing instruments, compromising fast turnaround times, often needed for follow-up samples. Notwithstanding, novel NGS approaches that circumvent the need for high sequencing depths, able to detect variants in multiple genes at low VAF and affordable sequencing depths, are being developed [116]. The technological advances of NGS will certainly lead to easily standardised, sensitive and robust MRD methods. However, method development should be undertaken in the context of specific treatment protocols to fully evaluate their clinical utility and limitations. Furthermore, the value of MRD as a guide for therapy should be further evaluated in prospective studies.

How to choose the right treatment for the right patient?

Certain genetic aberrations have a direct impact on treatment choice, but so far the majority do not. Further, that some tumours lack mutations detectable with current methods is well known [65, 91, 92]. Despite treatment advances, there are still patients for whom all standard treatment options have been exhausted without obtaining disease control. Here, taking advantage of genetic characteristics to guide experimental treatments, ideally

in a clinical trial setting, is appealing. In the following sections, novel methods to guide the choice of the right treatment for the right patient are discussed.

FPM as a complement to comprehensive molecular profiling

FPM means the direct measurements of drug effects on primary neoplastic cells to profile the potential efficacy of a specific treatment for a patient [117–120]. This may be performed in situations where available treatment options have been exhausted to find other potential drugs with possible activity in an individual patient. Measuring drug effect on cells has been around since the 1950s; however, FPM brings forward important advances such as disease relevance by using primary cells *ex vivo* and by including controls to distinguish general toxicity [119, 120]. Often, the *ex vivo* drug tests are done in multiwell plates to test tens to hundreds of drugs in dose-measuring cell viability or death as a readout [117, 119]. These bulk assays are useful to profile cell population-level responses but are limited in providing information on the specific cell types that respond to the drug. To exclude general toxicity and to provide for disease selectivity, calculation of the difference to a control sample, such as healthy bone marrow, is often included in the hit scoring [117, 121]. Viability-based FPM has been used to profile drug responses and to create large cohorts of leukaemia cases often including molecular omic profiling [34, 122, 123]. These systems biology data sets can readily be generated from both prospective fresh samples and viably frozen biobanked samples with good technological success rates. In one of the recent studies on AML, actionable drugs were found for 97% of the 252 included patient samples [123]. The same study also suggested individually tailored therapies for 37 relapsed or refractory AML patients and reported that 59% of the patients had objective responses, which are praising for the added value of the FPM approach.

In addition to measuring the cell viability by ATP-content, the apoptotic priming of a cell can be measured using dynamic BH3-profiling (DBP) (Fig. 1) [124, 125]. The DBP assay is a pathway-specific approach and interrogates the BCL2 family of proteins that regulates commitment to the mitochondrial pathway of apoptosis. This mode of program of cell death is commonly used by cancer cells in response to most chemotherapeutic agents [118]. The apoptotic priming is measured by the

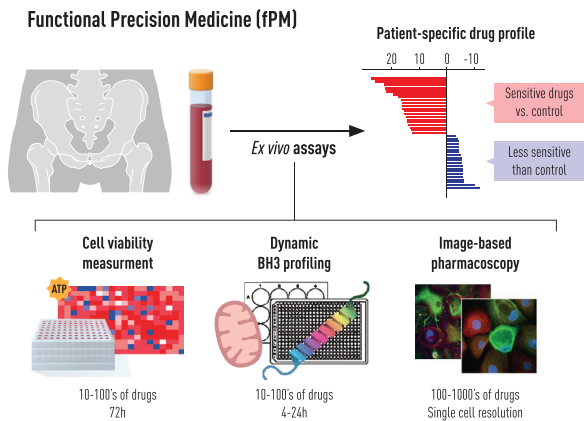


Fig. 1 Graphic overview of the workflow in functional precision medicine.

level of mitochondrial outer membrane permeabilisation induced by standardised concentrations of synthetic peptides derived from the BH3 domains of the pro-apoptotic BCL2 family proteins. The DBP assays are generally shorter (4–24 h) in comparison to the ATP-dependent viability assays (24–72 h) but have had limited throughput to tens of drugs tested. The throughput is important if one wishes to move beyond hypothesis-driven drug testing and perform unbiased screening of compound libraries to discover new therapies or new indications for existing therapies. Recently, a novel high-throughput DPB was developed using microscopy, thereby also offering single cell resolution [126].

Single-cell resolution provides the ability to measure cell type-specific responses and use non-malignant cells from the same specimens as general toxicity controls. This approach was pioneered by the pharmacology imaging-based assay on a retrospective cohort of 20 newly diagnosed and previously untreated patients with AML [127]. This microscopy-based approach predicted the clinical response of AML patients to their initial therapy with 88% accuracy. Recently, the clinical feasibility of pharmacology was shown in the first prospective single-cell FPM (scFPM) trial called EXALT [128]. Here, 56 patients received scFPM guided treatment and 30 of them (54%) demonstrated clinical benefit of more than 1.3-fold enhanced progression-free survival (PFS) compared to their previous therapy. Twelve patients experienced exceptional responses lasting three times longer than expected for their respective

diseases. This trial is a milestone for FPM by demonstrating the feasibility and clinical relevance in guiding treatment and thereby providing proof for a novel single-cell resolution tool for precision haematology/oncology. In summary, today's FPM approaches have advanced away from the early days of drug testing when cancer cell lines were used to assess in vivo therapy [129]. All the described FPM assays have demonstrated an important contribution to the field of precision medicine and are particularly feasible for blood-based cancer with easily attainable disease material. They are not costlier than genome-based assays and have quick turnover times (hours to days). Implementation into a clinical routine can be done at diagnosis and/or more advanced disease stages whenever enough sample (blood, bone marrow) is available. FPM provides an important complement to genomic profiling by direct efficacy measurement of drugs on the malignant cells and could be used to design and assess treatment response throughout the disease.

Comprehensive molecular profiling as a tool for improving diagnostic precision and selection of treatment—Lessons learned from rare cancers

Although haematologic malignancies, for reasons outlined in the previous section, are disease entities that have pioneered precision diagnostics and medicine, several lessons can also be learned from other rare cancer types. The translational and clinical potential of comprehensive molecular profiling to improve the stratification of patients to treatments is well illustrated by the example of rare cancers. However, determining the clinical consequences of a tumour's molecular profile is not always straightforward. For example, the question often arises whether the 'druggability' of a genetic variant can be transferred from one tissue context to another. This problem is well illustrated by the example of monotherapy with the mutation-specific BRAF inhibitor vemurafenib in more common malignancies with *BRAF*^{V600E} mutations, such as melanoma, where these alterations were first described [130], which leads to an objective response in approximately half of the entities studied [131]. In contrast, in colorectal carcinoma, rational combination therapies that take into account the physiologic expression profile of the tissue of origin are required to suppress oncogenic RAF-MEK-ERK signalling [132]. Another barrier to implementing precision oncology approaches in rare cancers is that negative evidence of drug

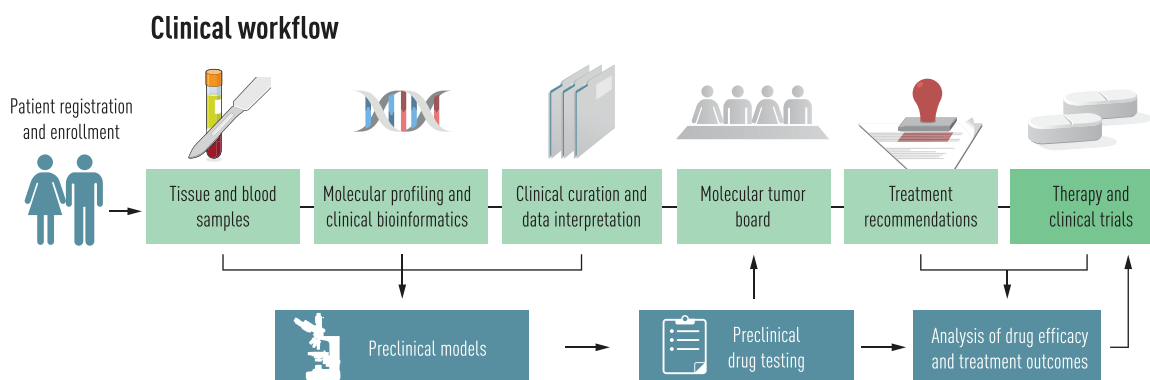


Fig. 2 Workflow for guiding a therapeutic decision based on comprehensive genomic and transcriptomic profiling in patients with rare cancers, with courtesy of NCT Heidelberg [135].

efficacy in unstratified clinical trials that followed a ‘one-size-fits-all’ strategy likely underestimated the value of many therapies, leaving the efficacy of most approved targeted drugs in rare cancers unexplored [133].

The problem of context dependence complicates the study of targeted therapies in rare cancers, defined as entities with an incidence of less than six per 100,000 persons per year, and prevents their use despite known molecular alterations in these diseases that are, in principle, addressable. This caveat is also frequently present in haematological malignancies, as many are also very rare. To overcome these limitations and advance the discovery of effective biomarker–drug pairs, including their tissue dependence, new adaptive trial designs have been introduced [134]. To further address this unmet clinical need, the National Center for Tumor Diseases, the German Cancer Research Center and the German Cancer Consortium have established a multicentre, prospective observational study that uses a standardised workflow to inform clinical management of these patients and to identify starting points for molecularly stratified clinical trials (Fig. 2). This workflow ranges from patient selection, tissue processing and WGS/WES and RNA sequencing under accredited conditions to clinical decision making in semiweekly molecular tumour boards. The molecular profiles and clinical outcomes of more than 1300 patients first enrolled in this study, approximately three quarters of whom represented rare cancers and were enrolled by more than 100 partners from all cancer care settings in Germany, were recently published [135]. The results demonstrate that comprehensive molecular analysis has tangible diagnostic

and therapeutic implications in this prognostically unfavourable patient population. First, the molecular profile was inconsistent with the clinical diagnosis in approximately 5% of the tumours studied, particularly in specific disease groups such as soft-tissue sarcomas. This usually triggered a re-evaluation of the particular case, sometimes leading to refinement or even revision of the diagnosis with immediate therapeutic consequences. Second, systematic analysis of autosomal dominant or recessive cancer predispositions in the germline revealed pathogenic variants in approximately 10% of patients. Most of these alterations were not previously known, had immediate consequences for affected families and were sometimes suitable therapeutic targets. Third, comprehensive molecular profiling had relevant therapeutic implications. Based on the categorisation of patients into distinct molecular intervention baskets using several hundred individual genes and six composite biomarkers, the molecular tumour board made recommendations for further clinical management in nearly 90% of the cases. These could be implemented in about one third of cases, indicating that access to medications and clinical trials is an unresolved issue for patients with rare cancers. Among patients who could be treated according to molecular tumour board recommendations, the disease control rate was 55% (response, 24%; stabilisation, 31%) and a PFS ratio >1.3 was achieved in 36% [135]. While these results are encouraging overall, given the prognostically unfavourable patient population with a median survival of less than 1 year and generally exhausted standard therapy, they also identify patient subsets, such as bone sarcomas, in which current precision oncology strategies have not yet been successful.

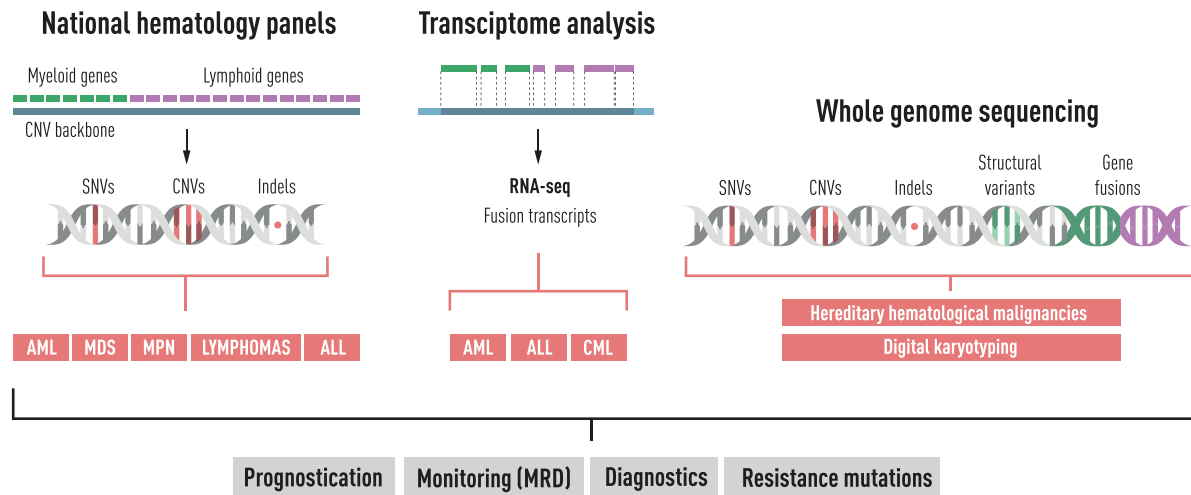


Fig. 3 An overview of current workflows and available methods for genetic analyses in haematological diseases.

Overall, these recent developments illustrate the feasibility and added value of team research in multicentre, sometimes even nationwide, precision oncology networks and demonstrate that WGS/WES and RNA sequencing can enable molecularly informed treatments that lead to clinical benefit in a substantial proportion of patients with rare cancers. In addition, they demonstrate how cross-entity approaches can help understand which molecular alterations and pathways are valid pan-cancer targets and which have strong tissue dependency. Hopefully, even stronger interactions between precision oncology in haematological and solid-organ neoplasms will develop in this regard in the future.

Discussion and concluding remarks

It is clear that genetic characterisation is already pivotal for both diagnosis and prognosis and sometimes treatment choice for many haematological malignancies and implemented in clinical practice, as summarised in Table 1. A summary of techniques used for various haematological malignancies is summarised in Fig. 3. Still, a lot remains to be learned with regard to how best to choose treatment according to an individual patient's genetic tumour landscape. Drug screening and a structured categorisation pipeline, as described above, may offer two novel alternative options. Also, the application of novel therapies based on the presence of certain aberrations will require organised follow-up and registration to ascertain which patients benefit from specific interventions

and when, and to enable robust evaluations across centres.

In addition to the tumour heterogeneity between patients, intratumour heterogeneity is increasingly recognised and a lot remains to be uncovered with regard to longitudinal changes in genetic aberrations. That subclones of a tumour harbour different genetic aberrations and that clonal evolution often drives disease progression and relapse have been demonstrated for several malignancies [10–12]. Thus, monitoring and precise characterisation of clonal evolution and diversity will be important to understand the development of relapse and resistance mechanisms and to choose subsequent second-line treatment and enable a deeper understanding of the biology and evolution of haematological diseases. This can be achieved through close monitoring of MRD, for which there are several different techniques available, as covered in this review. Further refinement of methods to measure MRD may provide more accurate and sensitive treatment response information and increase its prognostic value [136]. Moreover, methods that allow measurement of genetic, epigenetic, transcriptomic and proteomic alterations as well as drug efficacies at the single-cell level are already dramatically improving our understanding of the cellular ecosystem of haematologic malignancies, but several challenges remain before the implementation in a clinical setting [12, 83, 137, 138]. Increasing possibilities to measure disease burden from other sites than the tumour, through, for example, measurement of ctDNA, is also likely

Table 1. Overview of advances in implementation of molecular characterisation and its impact on diagnosis, prognosis, follow-up and treatment choice for major myeloid and lymphoid haematological malignancies

Disease entity	For diagnosis		For prognosis		For longitudinal follow-up		Targeted therapies in clinical practice		Impact on survival
	In use	Impact	In use	Impact	In use	Impact	In use	Examples	
Acute myeloid leukaemia	Yes	Required according to WHO classification	Yes	Improved risk stratification; guides treatment choice	Yes	MRD used to assess treatment response and identify treatment failure and relapse	Yes	FLT3-inhibitors IDH1/2-inhibitors Anti-CD33 antibody-drug conjugate BCL2-inhibitor	Yes
Chronic myeloid leukaemia	Yes	Required according to WHO classification	Yes	Provides information regarding TKI resistance	Yes	MRD used to assess treatment response and identify treatment failure and relapse	Yes	TK-inhibitors	Yes
Myelodysplastic syndrome	Yes	Required according to WHO classification	Yes	Improved risk stratification; guides treatment choice	Yes	MRD used to assess treatment response and identify treatment failure and relapse	Yes	BCL2-inhibitor Luspatercept Lenalidomide	Yes
Myeloproliferative neoplasms	Yes	Required according to WHO classification	Yes	Improved risk stratification	No	Not yet in clinical practice	Yes	JAK2-inhibitor	Yes
Chronic lymphocytic leukaemia	No	Not yet used in clinical practice for diagnosis but necessary for risk stratification	Yes	Improved risk stratification; guides treatment choice	Yes	Longitudinal addition of genetic alterations guides subsequent treatment choice	Yes	BTK-inhibitors, BCL2-inhibitor,	Yes
Acute lymphoblastic leukaemia	Yes	Improved diagnosis and of help to differentiate between ALL, AML and mixed phenotype leukaemia	Yes	Improved risk stratification	Yes	MRD used to assess treatment response and identify treatment failure and relapse	Yes	CD19/CD3 BiTcs Anti-CD22 antibody-drug conjugate TK-inhibitors	Yes

(Continued)

Table 1. (Continued)

Disease entity	For diagnosis		For prognosis		For longitudinal follow-up		Targeted therapies in clinical practice		Impact on survival
	In use	Impact	In use	Impact	In use	Impact	In use	Examples	
Aggressive lymphomas	Yes	Identification of double-hit lymphomas. Can help guide diagnosis in unclear cases	Yes	Improved risk stratification; granular Diffuse large B-cell lymphoma classifications underway using genetic alterations	No	Not yet used in clinical practice	Yes	CD79-antibody BTK-inhibitor	Yes
Mantle cell lymphoma	Yes	Presence of genetic aberrations function as diagnostic criteria (t[11;14], SOX11)	Yes	Improved risk stratification (TP53-mutation)	No	MRD to guide follow-up is being evaluated in clinical trials	Yes	BTK-inhibitors BCL2-inhibitor	Yes
Hairy cell leukaemia	Yes	Presence of genetic aberrations function as diagnostic criteria (BRAF ^{V600E})	No	Not yet in clinical practice	No	Not yet in clinical practice	Yes	BRAP-inhibitor	Yes
Waldenström macroglobulinemia	Yes	Presence of genetic aberrations function as diagnostic criteria (MYD88)	Yes	Can help guide response to BTK-inhibitor (MYD88, CXCR4)	No	Not yet in clinical practice	Yes	BTK-inhibitor	Yes
Follicular lymphoma	Yes	Presence of genetic aberrations function as diagnostic criteria (t[14;18])	No	Not yet in clinical practice	No	Not yet in clinical practice	Yes	P13K-inhibitor	Yes

Abbreviations: ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; MRD, minimal residual disease; TKI, tyrosine-kinase inhibitor; WHO, World Health Organisation.

to become a valuable tool to capture genetic aberrations more comprehensively [96, 139]. This may especially be useful for diseases such as lymphomas, where recurrent analyses of tumour material may not readily be available [140, 141]. In addition, in studies using ctDNA, specific genetic lesions have been identified in samples from the blood of patients with aggressive lymphoma that were not found in the original tumour, indicating both intratumour heterogeneity and clonal evolution [96]. Still, most current approaches to personalised medicine do not account for intratumour heterogeneity. In studies using single-cell sequencing to describe this phenomenon, a drastically different treatment response to *ex vivo* anticancer treatment was seen in malignant subpopulations from the same patient [10]. Further, the impact of the tumour environment and its diverse composition of cell types and the cell states of these (also possible to depict using single-cell sequencing and transcriptome deconvolution methods) have shown prognostic impact and may also offer additional therapeutic opportunities [142].

A challenge in the implementation of precision medicine is its cost. TKI for the treatment of CML has proven to be cost effective [143]. For other disorders, it is less clear, although systematic reviews indicate that the application of precision medicine overall is cost neutral in comparison to standard care [144]. Evaluation of the cost versus both survival and the potential quality of life benefit of precision medicine in haematology, and continued efforts to assess which patients will benefit from which intervention, will be of paramount importance. This is ongoing in clinical studies, but real-world evaluation will be needed to ultimately assess its role. Here, register-based evaluation may be a feasible option and such efforts are ongoing, for example, in the Lymphoma Epidemiology of Outcomes (LEO) study in the United States [145] and the REAL-LYSA study currently ongoing at lymphoma centres in France [146].

Overall, the easy access to blood to monitor leukaemic diseases provides a unique opportunity for longitudinal follow-up of disease burden and genetic aberrations in many haematological malignancies and may offer a means to overcome the heterogeneity of tumours. Thus, we are likely to see several expansions to the field of precision medicine in the near future. As haematological malignancies are caused by an interplay of genetic, behavioural and environmental risk factors, mod-

els for prediction and diagnosis that incorporate genetic and nongenetic data will likely remain necessary for a long time.

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Conflicts of interest

L. B. has held an advisory role and/or received honoraria from AbbVie, Amgen, Astellas, BristolMyers Squibb, Celgene, Daiichi Sankyo, Gilead, Hexal, Janssen, Jazz Pharmaceuticals, Menarini, Novartis, Pfizer, Roche, Sanofi and Seattle Genetics, and has received research support from Bayer and Jazz Pharmaceuticals.

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Author contributions

K. E. S., T. F. and T. W. planned and prepared the manuscript structure. All authors contributed to the writing of the manuscript and critically reviewed and revised the manuscript. All authors approved the final manuscript.

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