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# High-throughput sequencing for noninvasive disease detection in hematologic malignancies

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**Noninvasive monitoring of minimal residual disease (MRD) has led to significant advances in personalized management of patients with hematologic malignancies. Improved therapeutic options and prolonged survival have further increased the need for sensitive tumor assessment that can inform treatment decisions and patient outcomes. At diagnosis or relapse of most hematologic neoplasms, malignant cells are often easily accessible in the blood as circulating tumor cells (CTCs), making them ideal targets to noninvasively profile the molecular features of each patient. In other cancer types, CTCs are generally rare and noninvasive molecular detection relies on circulating tumor DNA (ctDNA) shed from tumor deposits into circulation. The ability to precisely detect and quantify CTCs and ctDNA could minimize invasive procedures and improve prediction of clinical outcomes. Technical advances in MRD detection methods in recent years have led to reduced costs and increased sensitivity, specificity, and applicability. Among currently available tests, high-throughput sequencing (HTS)–based approaches are increasingly attractive for noninvasive molecular testing. HTS-based methods can simultaneously identify multiple genetic markers with high sensitivity and specificity without individual optimization. In this review, we present an overview of techniques used for noninvasive molecular disease detection in selected myeloid and lymphoid neoplasms, with a focus on the current and future role of HTS-based assays. (*Blood*. 2017;130(4):440-452)**

## Introduction

For any given malignancy, the success of disease detection from peripheral blood (PB; ie, noninvasive disease detection) generally requires several considerations: knowledge of tumor biology and its molecular characteristics, accessibility of tumor material in circulation, and technical factors. Most hematologic neoplasms are circulating cancers; therefore, circulating tumor cells (CTCs) are often easily accessible in the blood at times of overt disease. Other malignancies, including lymphomas, often manifest in noncirculating tissue compartments and organs, thus making CTCs rare. Here, circulating tumor DNA (ctDNA) in the bloodstream can be used for noninvasive tumor detection (Tables 1 and 2; Figure 1).[1,2] Unfortunately, the fraction of CTCs and ctDNA in the pool of normal PB mononuclear cells (PBMCs) or cell-free DNA is often very small. Thus, the analytical sensitivity and specificity of a noninvasive test must be sufficiently high to detect minimal tumor loads that almost invariably lead to future disease progression. Furthermore, to minimize costs and allow clinical implementation, a test should apply to a wide range of patients without patient-specific optimization.

Another important consideration is the type of genetic aberration being detected. Some malignancies are characterized by a single stereotypic genetic variant, which can be assessed in nearly every patient by highly sensitive approaches.[3,4] Molecular aberrations in other hematological neoplasms are heterogeneous, and detailed knowledge of the underlying genetic landscape is required. Finally, sensitivity strongly relies on input material quantity and quality. For example, a single gene test can only achieve a sensitivity of 1 in 20 000 if the input material matches or exceeds this threshold (Figure 2).[5]

In hematologic neoplasms, several technologies have been established for noninvasive minimal residual disease (MRD) detection and response assessment: flow cytometry (eg, multicolor flow cytometry [MFC]), polymerase chain reaction (PCR)-based methods that do not rely on sequencing (quantitative real-time PCR [qPCR], digital PCR [dPCR], reverse transcription PCR [RT-PCR]), and high-throughput sequencing (HTS, next-generation sequencing [NGS]) methods, which allow massive parallel sequencing of DNA molecules in a single flow cell and produce millions or billions of sequences concurrently (Figure 2; Table 3). For balancing brevity with clarity in this review, we focus on increasingly common HTS approaches and encourage readers to consult dedicated sources for MRD methods that do not rely on HTS.[6-11]

HTS methods are powerful tools for noninvasive disease detection with several dedicated advantages over other approaches. They can detect the entire spectrum of genetic alterations, including single nucleotide variants, insertions/deletions, chromosomal rearrangements, and copy number changes, and thus can overcome the limitations of assays covering single somatic variants (Table 3).[12-14] Moreover, HTS assays are usually applicable to a broader population of patients and patient-specific optimization or knowledge of tumor genotypes is generally not required. To achieve sequencing depths that allow sensitive noninvasive disease detection similar to modern qPCR or dPCR assays (0.001% to 0.0001% allele frequency [AF]), targeted approaches are generally preferred over whole genome or exome sequencing, especially when considering current costs and sequencing

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**Table 1. Clinical impact of noninvasive disease detection at distinct disease milestones in lymphoid malignancies**

| Diagnosis | Precancerous condition | Diagnosis/pretreatment | During therapy | Posttreatment/surveillance | Relapse/progression |
|---|---|---|---|---|---|
| ALL | | CTC or malignant BM cell: levels predict outcome before allo-SCT[38,41,43,55] | | CTC or malignant BM cell: positivity predicts clinical outcome[29-35,38-43,54,55]; positivity predicts relapse[39,56]; positivity might guide treatment decisions[33,35] | Malignant BM cell: positivity identifies relapse[39] |
| HL | | | | ctDNA: positivity tends to predict clinical outcome[66,68] | |
| MM | | CTC or malignant BM cell: genotyping defines clinical risk (eg, t(4:14))[71]<br>Autograft: positivity predicts clinical outcome[82] | | Malignant BM cell: positivity predicts clinical outcome[72,73,77-79]; positivity pre-lenalidomide maintenance predicts clinical outcome[78] | |
| MCL | | CTC or malignant BM cell: positivity before auto-SCT predicts survival[87] | | CTC or malignant BM cell: positivity predicts clinical outcome[85,86,88]; detection might guide treatment decisions[84] | |
| CLL | | CTC: genotyping defines clinical risk (eg, del(17p13), *TP53*)[92,93]; assessment might identify therapeutic targets (eg, del(17p13))[92,93] | | CTC or malignant BM cell: levels predict clinical outcome[68,94-99,103,104]; dynamics predict relapse[97] | |
| FL | Healthy PBMC: *BCL2-IGH* rearrangement levels >0.01% are associated with a 23-fold higher risk for malignant transformation[112] | ctDNA or malignant BM cells: levels correlate with clinical outcome[122,132] | | CTC or malignant BM cells: positivity predicts clinical outcome[116-120,125,127,129,131] | CTC: positivity identifies relapse[116] |
| DLBCL | | ctDNA: levels correlate with tumor burden and PFS[22,134,135]; genotyping identifies COO subtypes[22]; profiling identifies DH and TH lymphomas[22] | ctDNA: profiling identifies resistant clones[22,137]; negativity after 2 cycles predicts PFS[134] | CTC or ctDNA: predicts relapse with 3-6 month lead time[22,134,135]; positivity predicts clinical outcome[22,134] | CTC or ctDNA: positivity identifies relapse[22,134,135,137]; profiling detects histological transformation[22] |

Role of PBMC, CTC, ctDNA, and BM cell profiling for detection of premalignant states in healthy individuals, identification of clinically relevant biomarkers, and prediction of outcome in lymphoid malignancies at distinct disease milestones.

COO, cell of origin; DH, double hit; DLBCL, diffuse large B-cell lymphoma; TH, triple hit.

error rates. These targeting methods include amplicon-based or targeted hybrid-capture techniques to select and enrich the genomic regions of interest.[15-18] The ability to simultaneously detect and monitor multiple aberrations and recent methodological advances in reducing errors have improved analytical sensitivity of targeted HTS assays down to ~0.0001% (Figure 2).[16,19,20] Furthermore, HTS-based assays facilitate assessment of intra- and intertumor heterogeneity, allowing individualized detection of clonal evolution.[21,22] Although HTS methods are becoming more common, they are not widely used in clinical laboratories yet and therefore are not prominently featured in disease management guidelines. Methodological challenges, both in molecular biology and bioinformatics analyses, must be overcome as these methods become routinely used.

## Noninvasive disease detection in lymphoid malignancies

### Acute lymphoblastic leukemia

Lymphoid malignancies are characterized by clonal immunoglobulin (Ig) or T-cell receptor (TCR) gene rearrangements, which take place during early B- and T-cell development. This genetic feature is highly specific to each individual tumor and thus represents an ideal molecular marker for sensitive disease detection by PCR- and HTS-based techniques. Consequently, MRD detection using Ig or TCR rearrangements has become widely adopted in lymphoid cancers.

Acute lymphoblastic leukemia (ALL) is exemplary for how MRD assessment can be incorporated into treatment protocols and clinical guidelines. Nearly 80% to 95% of adult ALL patients and more than 95% of pediatric patients achieve complete remissions (CRs) after induction chemotherapy.[23,24] However, a substantial proportion of patients will relapse and ultimately succumb to their disease. Clinical evidence for the utility of MRD in ALL has led to its broad adoption for patient risk stratification and early therapeutic response monitoring. Current standard approaches include flow cytometry (sensitivity, ~0.01%), allele-specific oligonucleotide (ASO) qPCR of Ig/TCR gene rearrangements (~0.001%), and qRT-PCR of fusion transcripts (eg, *BCR-ABL1*, *MLL-AF4*; 0.01% to 0.001%).[8,25-28] Although flow cytometry and ASO-qPCR are applicable to virtually all patients, the use of qRT-PCR is limited to patients with detectable fusions.[25] However, MRD status assessed by those techniques is one of the most powerful predictors of survival and guides treatment decisions (Table 1).[29-43] For detailed reviews, see van Dongen et al,[25] Brüggemann et al,[44] and Campana et al.[45]

All of these methods, despite demonstrating utility in clinical settings, have limitations. For example, they typically rely on invasive bone marrow (BM) sampling and cannot capture clonal heterogeneity.[46,47]

**Table 2. Clinical impact of noninvasive disease detection at distinct disease milestones in myeloid malignancies**

| Diagnosis | Precancerous condition | Diagnosis/pretreatment | During therapy | Posttreatment/surveillance | Relapse/progression |
|---|---|---|---|---|---|
| AML | Healthy PBMC: harbor age-dependent aberrations associated with overt AML/MDS[138-142] | CTC or malignant BM cell: genotyping defines molecular prognostic factors[150,151]; genotyping might identify therapeutic targets (eg, *FLT3*)[151,152] | CTC or malignant BM cell: positivity and kinetics during therapy predict risk of relapse[155,157] | CTC or malignant BM cell: positivity postinduction and postconsolidation predicts clinical outcome[156,157,161,164] | CTC or malignant BM cell: profiling identifies emergent clones at relapse[163]; profiling identifies relapse[161] |
| CML | | | CTC or malignant BM cell: *BCR-ABL1* levels predict clinical outcome[168-170]; profiling identifies resistance mutations[176,177,180,181] | | CTC or malignant BM cell: rising *BCR-ABL1* levels indicate progression[178] |
| MPN | | CTC or malignant BM cell: genotyping is part of diagnostic criteria[189] | | CTC or malignant BM cell: levels predict clinical outcome[190,191] | |

Role of PBMC, CTC, ctDNA, and BM cell profiling for detection of premalignant states in healthy individuals, identification of clinically relevant biomarkers, and prediction of outcome in myeloid malignancies at distinct disease milestones.

Modern approaches combining universal PCR primers for rearranged Ig/TCR regions with HTS have the potential to overcome those shortcomings. Proof-of-principle analyses applying this technique to PB and BM samples demonstrated high sensitivity (0.001% to 0.0001%) and high concordance with ASO-qPCR and flow cytometry.[48-53] Moreover, it allows identification of oligoclonality at diagnosis and noninvasive disease monitoring over time.[49,50] Logan et al,[54] Pulsipher et al,[55] and Mannis et al[56] demonstrated that MRD assessment by this HTS approach accurately predicts relapse and patient survival before and after allogeneic stem cell transplantation (allo-SCT) or autologous SCT (auto-SCT), using either noninvasively acquired CTCs or invasive BM biopsies. Additionally, it can provide information on the physiological repertoire of B and T cells, which appears to be prognostically relevant.[57,58]

However, HTS analysis of BM still appears superior to PB in ALL, possibly from a higher concentration of tumor cells.[51,54] Nevertheless, the reported sensitivities of MRD monitoring using less invasive blood samples seems to be promising and is moving forward into future clinical trials.[48-51,54] International standardization and validation of noninvasive HTS-based methods in multicenter studies is needed to demonstrate robust clinical utility.

### Hodgkin lymphoma

Despite high initial response rates to combination chemotherapy, 20% to 30% of patients with Hodgkin lymphoma (HL) will experience either primary refractoriness to chemotherapy or disease relapse.[59] Identifying genetic features for disease monitoring and response assessment has been challenging in this disease, as malignant cells are usually very rare in bulk tissue (<5%). However, by enriching tumor cells through microdissection, some recurrent aberrations could be identified that can be followed for noninvasive MRD monitoring, including mutations in specific genes (eg, *TNFAIP3, XPO1*) and pathways (NF-κB, JAK/STAT), as well as chromosomal aberrations, translocations, and rearrangements involving Ig.[60-66]

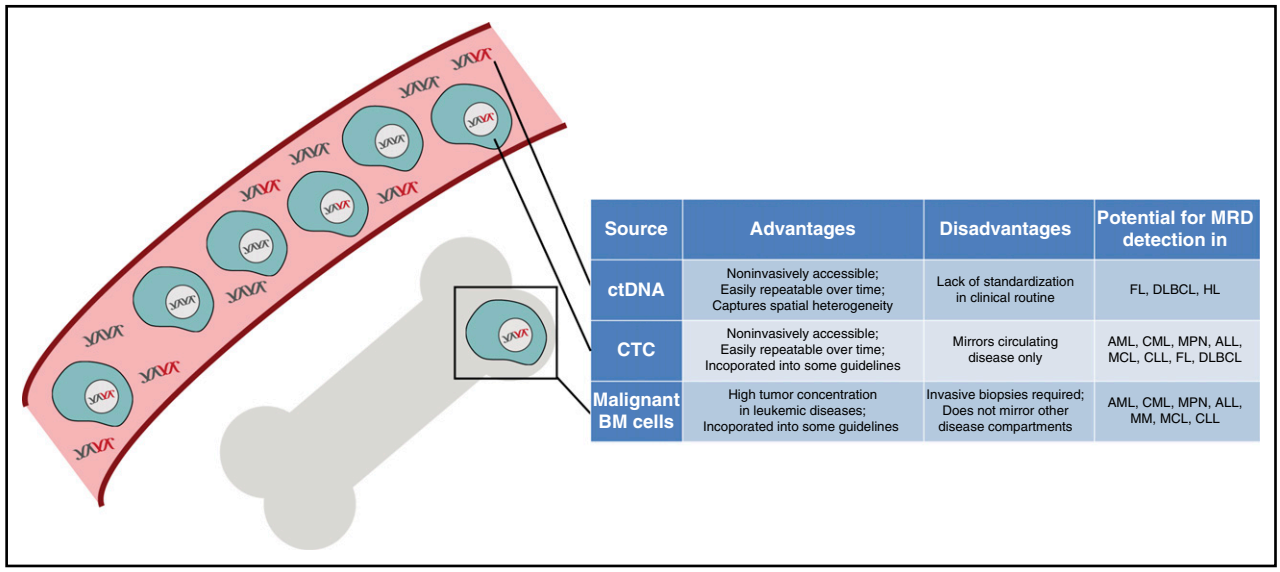
Camus et al demonstrated that patients with *XPO1* mutations detected in plasma by dPCR (0.1%) at the end of standard chemotherapy had a shorter progression-free survival (PFS) than *XPO1*-negative cases.[67] In a large study conducted by Herrera et al, ctDNA positivity assessed by HTS of Ig rearrangements (IgHTS) after allo-SCT predicted clinical outcomes.[68] However, Oki et al showed that IgHTS fails to identify Ig clonotypes at diagnosis in more than one-quarter of patients, limiting its use as a ubiquitous biomarker.[69] Vandenberghe et al identified chromosomal aberrations by pretreatment ctDNA profiling using whole genome sequencing and observed that normalization of ctDNA after initiation of therapy mirrors treatment response.[66]

These studies demonstrate feasibility of noninvasive MRD monitoring in HL. However, improvements are needed to increase sensitivity and broaden the applicability of PCR- and HTS-based methods.

### Multiple myeloma

Major therapeutic advances in the past decade have significantly improved outcomes of patients with multiple myeloma (MM).[70] Consequently, both the role of diagnostic genotyping to define prognostic markers (eg, del(17p13), t(4;14)) and MRD assessment to monitor treatment efficacy has become increasingly important.[71] Current standard approaches for MRD detection in clinical trials and research studies include MFC and ASO-qPCR of rearranged Ig regions (0.01% to 0.001%). Both have demonstrated clinical utility: MRD negativity by MFC at day 100 after auto-SCT was associated with improved survival.[10,72] Similarly, Ferrero et al showed that patients achieving major MRD response (<0.01%) by PCR-based methods had a favorable prognosis and excellent disease control.[73] However, caveats still remain: despite high sensitivity and reasonable turnaround time, both methods are more applicable to invasive BM specimens than PB samples (for detailed reviews, see Mailankody et al[74] and Paiva et al[75]).

Approaches using IgHTS in MM can assess MRD in BM aspirates, PBMCs, and cell-free DNA with sensitivities down to 0.0001%.[48,49,76] Several studies applying IgHTS to invasive BM biopsies showed improved sensitivity over conventional methods, demonstrated prognostic relevance of MRD detection, and identified substantial oligoclonality at diagnosis with emergence of subclones over time.[77-80] On the other hand, studies investigating noninvasive disease detection in PB are relatively rare. In a proof-of-principle analysis, Gimondi et al were able to identify *IGH* clonotypes in the plasma of 6 MM patients at diagnosis, demonstrating 100% concordance with tumor V(D)J sequences.[81] Wee et al analyzed autografts by 3 different methods (IgHTS, qPCR, and dPCR) in patients undergoing auto-SCT. Here, IgHTS demonstrated the highest sensitivity and prognostic value, suggesting a potential future role for noninvasive MRD detection.[82]



| Source | Advantages | Disadvantages | Potential for MRD detection in |
|---|---|---|---|
| ctDNA | Noninvasively accessible; Easily repeatable over time; Captures spatial heterogeneity | Lack of standardization in clinical routine | FL, DLBCL, HL |
| CTC | Noninvasively accessible; Easily repeatable over time; Incoporated into some guidelines | Mirrors circulating disease only | AML, CML, MPN, ALL, MCL, CLL, FL, DLBCL |
| Malignant BM cells | High tumor concentration in leukemic diseases; Incoporated into some guidelines | Invasive biopsies required; Does not mirror other disease compartments | AML, CML, MPN, ALL, MM, MCL, CLL |

**Figure 1. DNA sources for MRD detection in hematologic malignancies.** Graphic overview of distinct DNA sources for molecular disease profiling in hematologic neoplasms, which include circulating tumor DNA as part of the circulating cell-free DNA pool and circulating tumor cells in the bloodstream as well as malignant cells in the BM compartment. Their main characteristics and role in hematologic cancers are displayed in the table next to the graphic.

### Mantle cell lymphoma

Patients with mantle cell lymphoma (MCL) are genetically characterized by clonal Ig rearrangements and translocation t(11;14) (*CCND1/IGH*), resulting in overexpression of CCND1 that enables malignant transformation by cell-cycle deregulation.[83] Consequently, the gold standard for MRD detection in current clinical trials is qPCR of *CCND1/IGH* and/or V(D)J rearrangements with sensitivities down to 0.001%. Numerous studies have demonstrated that MRD assessment by qPCR applied to PB or BM samples in MCL after induction therapy is a strong prognostic factor, even guiding therapeutic decisions in response to molecular relapse after transplant.[84-87] However, these assays are applicable to only 40% and 90% of all patients.[88,89]

Similar to ALL and MM, IgHTS has improved noninvasive disease detection compared with qPCR, offering an alternative for MRD detection.[48,52] However, the clinical utility of this greater sensitivity needs to be evaluated prospectively before IgHTS potentially replaces standardized qPCR assays as the gold standard in clinical trials. For further reviews covering this topic, see Hoster et al[90] and Pott et al.[91]

### Chronic lymphocytic leukemia

Chronic lymphocytic leukemia (CLL) is an incurable disease with a heterogeneous but often indolent course.[92] An increased understanding of the genetic landscape has led to significant advances in assessing clinical outcomes and defining therapeutic procedures (eg, del(17p13), *TP53*).[92,93] Furthermore, MRD detection in PB or BM above a threshold of 0.01% by standardized MFC or ASO-qPCR of Ig rearrangements seems to be an independent predictor of survival.[94-96] For example, high-risk CLL patients undergoing allo-SCT have a favorable prognosis if they achieved MRD negativity 12 months after transplant.[97-100] However, the use of MRD to tailor treatment in CLL in clinical routine is currently discouraged (for detailed reviews, see Wierda et al,[100] Thompson et al,[101] and Böttcher et al[102]).

Thus far, there have been only a few studies applying IgHTS to PB in CLL. Rawstron et al[103] and Logan et al[104] reported concordance between IgHTS and MFC or ASO-qPCR, with increased sensitivity. They also demonstrated that MRD detection >0.0001% at 9, 12, and 24 months after allo-SCT in high-risk patients was associated with subsequent relapse. Moreover, doubling of MRD levels within 12 months after transplant was prognostic for relapse.[103] This suggests 2 conclusions: first, an increased sampling frequency might help to predict disease progression; and second, the increased sensitivity of IgHTS over conventional methods might be clinically relevant. Larger studies are needed to validate both of these hypotheses.

Yeh et al applied a targeted HTS approach to serial plasma samples of 32 CLL patients, capturing 7 recurrently mutated genes.[105] They revealed that noninvasive ctDNA analysis allows detection of different disease compartments and discovery of emerging genomic changes over time (eg, toward Richter's transformation), highlighting the advantages and potential of methods covering multiple genes over single gene assays (Table 3).

### Follicular lymphoma

Chromosomal translocations involving *BCL2* and *IGH* (t(14;18)) are present in >90% of follicular lymphoma (FL) patients. This genetic hallmark occurs during B-cell development and gives rise to constitutive expression of the antiapoptotic protein BCL2.[106,107] *BCL2/IGH* translocation is considered the founding event of FL lymphomagenesis, but is not sufficient for malignant transformation.[108] Consequently, t(14;18) can be detected at low frequencies in healthy subjects who will never develop FL.[109-111] Roulland et al screened prediagnostic blood from a large cohort of healthy individuals for the presence of t(14;18). By applying qPCR, they found that people with t(14;18) >0.01% AF had a 23-fold higher risk for developing FL, suggesting a potential role as a predictive biomarker.[112] However, specificity at this threshold was relatively low (96.5%), which limits the clinical utility of *BCL2/IGH* detection in healthy individuals; therefore, the use of this test outside research studies is not recommended. Additional recurrent driver mutations have recently been described and are thought to represent early initiating events required for malignant transformation.[113-115] Yet, these events have not been identified before overt malignancy; therefore, their role in detecting premalignant states is unclear.

Clinically, FL is the most common indolent NHL; however, recurrent relapses and histological transformation into aggressive

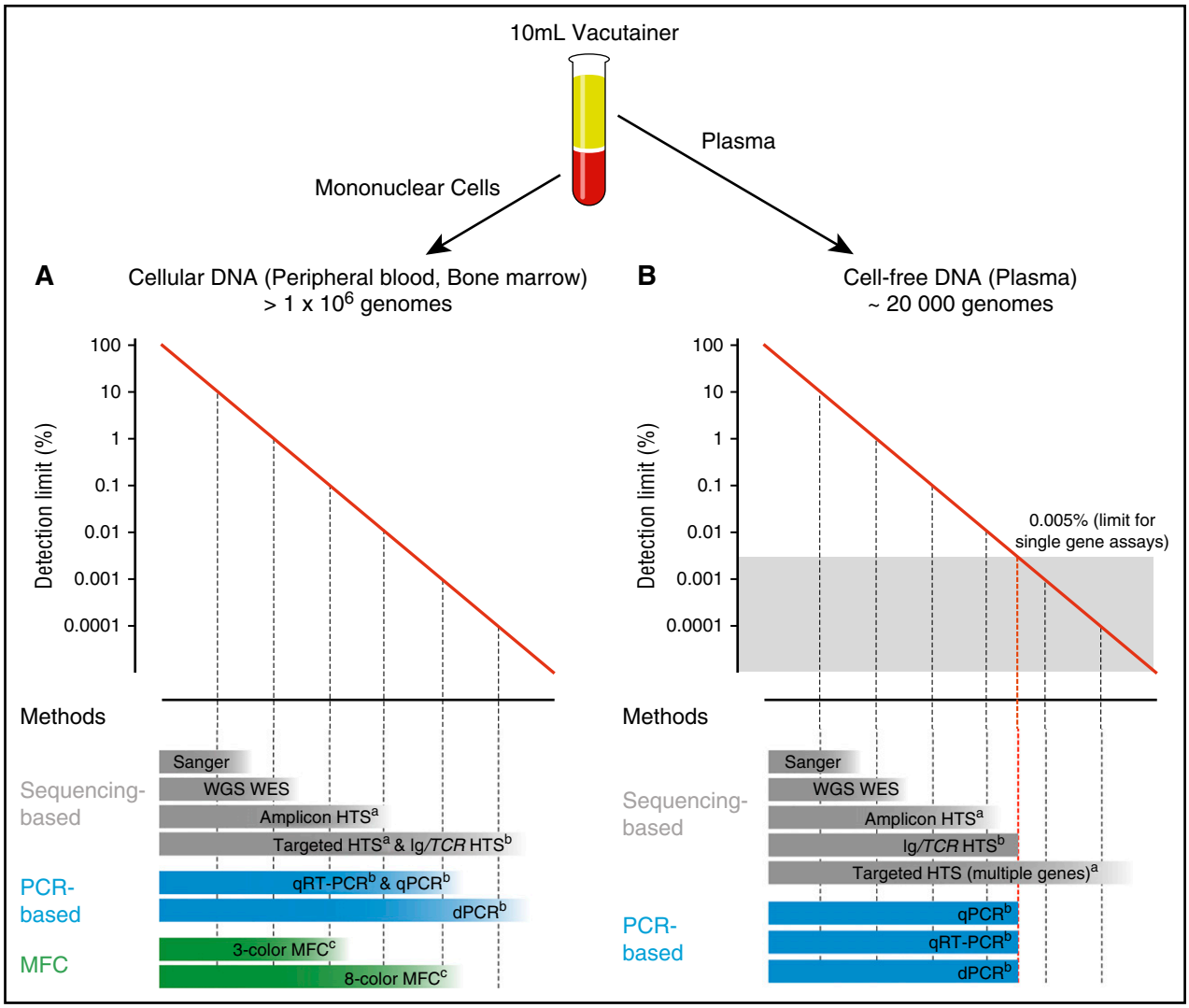



**Figure 2. Detection limits of methods used for DNA identification in PB and BM.** Diagrams depicting the range of detection of methods used for identification of cellular DNA in PB and BM (A) and cell-free DNA in plasma (B). PB input material for each assay was considered from a normal 10 mL EDTA vacutainer. [a]Analytical sensitivity highly depends on panel width, sequencing depth, and technical conditions (eg, barcoding, duplex sequencing). [b]Analytical sensitivity substantially varies depending on the target/targets interrogated and number of input genomes. [c]Analytical sensitivity depends on the method used and number of markers tested. WES, whole exome sequencing; WGS, whole genome sequencing.

disease can lead to decreased survival. Conflicting results have been reported as to whether PCR-based methods for detection of *BCL2/IGH* or Ig rearrangements in BM or PB can be used for prediction of treatment response and outcome at diagnosis or after therapy.[12,116-129] The reported methods have several shortcomings and probably contribute to these controversies: most PCR assays for *BCL2/IGH* identification are restricted to the major breakpoint region and minor cluster region of *BCL2*; therefore, only a subset of patients (50% to 65%) can be assessed. Detection of Ig rearrangements can also be ineffective in FL because of high rates of somatic hypermutation (SHM), a characteristic of germinal center lymphomas. Alcaide et al developed a digital droplet PCR method targeting recurrently mutated genes in FL and DLBCL and demonstrated in a proof-of-principle study high sensitivity and specificity for noninvasive ctDNA detection.[130]

Modern HTS-based approaches also use clonal V(D)J rearrangements for noninvasive detection of FL. Pott et al demonstrated good concordance between t(14;18) qPCR and IgHTS for MRD monitoring in PB samples; however, applicability of IgHTS was not optimal because clonotype sequences could not be identified in 25% of patients.[131] Sarkozy et al applied IgHTS to tumor biopsies and plasma samples at diagnosis. They identified tumor clonotypes in 83% of diagnostic ctDNA and showed that patients with high levels of ctDNA have a shorter PFS than patients with low levels.[132]

Both studies again illustrate that ongoing SHM significantly limits the use of V(D)J sequencing in this disease. Better strategies are therefore needed to overcome these problems in FL.

## DLBCL

DLBCL is the most common NHL subtype. A hallmark of this disease is its molecular and clinical heterogeneity. Assessment of ctDNA in the plasma or serum of DLBCL patients by HTS- and PCR-based approaches to evaluate therapeutic response and clinical outcome has been extensively studied in recent years. In a proof-of-concept study, Camus et al used both dPCR and amplicon HTS to detect ctDNA in diagnostic and follow-up plasma samples. They showed that both

**Table 3. Key characteristics of methods used for noninvasive disease detection in hematologic malignancies**

| | Method | Advantages | Disadvantages |
|---|---|---|---|
| Flow cytometry | MFC (3-8 colors) | High sensitivity, no individual optimization required, broad availability across institutions | No standardized workflows and data interpretation across laboratories, requires fresh samples, limited number of tested markers |
| PCR-based methods | qPCR | Ease of use, high sensitivity, standardized for certain applications (eg, Ig detection in ALL) | Requires individual optimization, time-consuming, limited number of tested aberrations, limited standardization for most applications |
| | qRT-PCR | Ease of use, high sensitivity, standardized for certain applications (eg, fusion detection in AML or ALL) | Requires individual optimization, time-consuming, limited number of tested aberrations |
| | dPCR | High sensitivity, quantitative | Requires individual optimization, time-consuming, limited number of tested aberrations, limited standardization |
| Sequencing-based methods | Sanger sequencing | Ease of use, standardized for certain applications (eg, detection of resistance mutations in CML) | Low sensitivity, requires individual optimization, can test only limited number of aberrations |
| | Whole genome sequencing | Broadly applicable, full characterization of genome landscape, detection of clonal heterogeneity | Low sensitivity, expensive, no standardized workflows and data interpretation |
| | Whole exome sequencing | Broadly applicable, full characterization of exome landscape, detection of clonal heterogeneity | Low sensitivity, expensive, no standardized workflows and data interpretation |
| | Amplicon HTS | High sensitivity, can test multiple mutations simultaneously, less expensive than WGS/WES | Inability to detect CNVs and fusions without optimization, no standardized workflows and data interpretation, less comprehensive than WGS/WES |
| | Ig/TCR HTS | High sensitivity, no individual optimization required | Captures only one genetic marker, no standardized workflows and data interpretation |
| | Targeted HTS | High sensitivity, captures all types of genetic aberrations without individual optimization, broadly applicable, less expensive than WGS/WES | No standardized workflows and data interpretation, less comprehensive than WGS/WES |

Colors in column 1 refer to the colors in Figure 2.
CNVs, copy number variations; WES, whole exome sequencing; WGS, whole genome sequencing.

methods were highly concordant and identified ctDNA in 93% of diagnostic plasma samples, with a sensitivity of 0.05%.[133]

Roschewski et al and our group set out to evaluate the value of ctDNA as a prognostic biomarker in larger cohorts of patients by applying IgHTS to a series of tumor biopsies, diagnostic and follow-up plasma/serum samples as well as CTCs. Both studies showed a detection rate of ~85% in tumor biopsies and 82% to 92% in pretreatment ctDNA.[134,135] Levels of ctDNA correlated with other measures of tumor burden, including lactate dehydrogenase, metabolic tumor volume, and International Prognostic Index. Furthermore, they suggested a prognostic role for monitoring ctDNA during and after therapy. In patients achieving an initial CR, ctDNA was detectable 3 to 3.5 months before clinical relapse, outperforming positron emission tomography/computed tomography.[134,135] In addition, patients with undetectable ctDNA on the first day of cycle 3 of therapy had a superior PFS (but not overall survival) compared with patients with positive ctDNA.[134] Importantly, disease detection from plasma significantly outperformed detection from CTCs.[135] Despite its value as a prognostic tool, IgHTS has several shortcomings. This includes limited sensitivity in low tumor burden settings and reduced applicability because of SHM, leading to difficulty identifying clonotypic sequences. Furthermore, IgHTS monitors only a single genetic marker and cannot capture complex genetic landscapes.

To overcome some of these challenges, our group recently applied Cancer Personalized Profiling by Deep Sequencing, a targeted capture HTS method, to DLBCL. In this effort, we targeted 268 genes to assess the whole variety of different genetic aberrations simultaneously (point mutations, translocations, insertions/deletions, Ig rearrangements) and to improve sensitivity and applicability to ctDNA.[22] In a cohort of 92 patients, we identified somatic alterations in 100% of pretreatment ctDNA samples with a specificity of 99.8% when tumor mutation profiles were known (87% when tumor genotypes were unknown). Pretreatment ctDNA levels correlated with clinical measures of tumor burden and were prognostic of patient outcomes. Relapses could be noninvasively detected in 100% of cases with a mean lead time exceeding 6 months, including detection of disease burden as low as 0.003% AF.[22,136] Importantly, clinically relevant tumor heterogeneity was observed in plasma. This included emergence of subclones harboring resistance mutations to targeted therapies, identification of mutation patterns that allowed noninvasive classification of DLBCL cell-of-origin subtypes, and discovery of clonal evolution, distinguishing transformation of FL from indolent FL progression.[22] Using a related approach focused on genotyping but not optimized for monitoring, Rossi et al targeted 59 recurrently mutated genes for ctDNA analysis.[137] In their study, 85% to 95% of patients had detectable ctDNA at diagnosis when tumor genotypes were unknown, with 83% of all tumor mutations identified in pretreatment plasma. Their longitudinal ctDNA analyses also showed emerging mutations at relapse, suggesting evolution of resistant subclones.

These studies demonstrate the great potential of ctDNA assessment at various disease milestones by HTS. We anticipate a growing role of such technologies to complement positron emission tomography/computed tomography imaging and tumor biopsies for precision patient management within the next few years, initially in trials and ultimately as part of clinical routine.

## Noninvasive disease detection in myeloid malignancies

### Acute myeloid leukemia and myelodysplastic syndrome

Over the past 5 years, systematic genetic profiling of PB cells in healthy individuals by HTS-based methods has led to a growing understanding of potential precancerous conditions in hematologic cancers.[138-142] Several research groups have identified an increase of somatic mutations in the blood of individuals without overt signs of a hematologic malignancy and normal blood counts, including *DNMT3A, TET2,* and *ASXL1* mutations.[138-142] These variants seem to occur in hematopoietic stem and progenitor cells and confer survival advantage to clones as they expand. Consequently, they can be detected in mature circulating cells with increasing prevalence in older healthy people (up to 20% of people ≥90 years) in a condition called clonal hematopoiesis of indeterminate potential (CHIP; for detailed reviews, see Link et al and Steensma et al).[139,143-145]

Recurrent mutations related to age-dependent CHIP have also been described in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML).[146,147] This suggests that they represent potential early genetic events in the clonal selection process leading to hematologic cancers. Indeed, by studying whole exome sequencing DNA from nearly 30 000 PB samples, Genovese et al[138] and Jaiswal et al[139] demonstrated that elderly individuals harboring CHIP-associated mutations have a significantly higher risk for developing hematologic neoplasms (hazard ratio, 11.1; 95% confidence interval, 3.9-32.6) and death (hazard ratio, 1.4; 95% confidence interval, 1.1-1.8). These studies suggest a potential future role of population-based screening assays to identify healthy individuals at risk for malignant transformation.

At the same time, there is evidence that only a minority of persons carrying CHIP-associated mutations develops myeloid malignancies, calling into question the potential clinical value of such wide-scale screening approaches. In fact, individuals older than age 80 years seem to have a normal life expectancy despite harboring aforementioned aberrations.[148] Furthermore, Young et al demonstrated by using a more sensitive HTS approach that clinically silent clonal hematopoiesis can be detected in nearly all individuals between ages 50 and 60 (95%) and that malignant transformation in these cases is exceptionally rare.[149] Those conflicting results highlight the need for future prospective longitudinal studies incorporating genetic tests to accurately define the risk of precancerous genomic conditions, to understand their clinical relevance, and to distinguish between benign and malignant clonal hematopoiesis. Consensus and guidelines for how to use detection of mutations in healthy individuals are required to inform clinical decision-making.

At AML diagnosis, profiling of certain gene rearrangements (eg, *PML-RARA, CBFB-MYH11, RUNX1-RUNX1T1, BRC-ABL1*) and mutations (eg, *NPM1, FLT3*-ITD, *RUNX1, CEBPA*) in BM or PB has become increasingly relevant for patient risk assessment and therapeutic decision-making.[150-155] In addition, achieving deep CR without MRD is of key importance for assessment of clinical outcomes.[155-157] MFC- and PCR-based technologies allow a highly sensitive (0.1%-0.001%), objective, and standardized assessment of treatment response over time (Figure 2A; Table 3). They provide independent prognostic information and are therefore recommended in trials and clinical practice for MRD detection.[155-161] However, detection limits of those assays differ substantially between molecular markers, mainly because of large variations in expression levels of fusion genes and mutant alleles analyzed.[155,162] Furthermore, it remains unclear what the best time to test for MRD might be; kinetics of MRD response differ considerably depending on treatment, methods, and markers tested (for a detailed review, see Döhner et al[155]).

MRD detection by HTS in AML/MDS is a relatively new field. Thol et al used amplicon HTS to follow *FLT3*-ITD and *NPM1* mutations in BM and PB samples of a small cohort of AML patients. They demonstrated high concordance with qRT-PCR and robust detection of MRD, including the identification of emergent mutations during relapse.[163] Kohlmann et al performed amplicon sequencing of *RUNX1* in a cohort of 103 AML patients (both BM and PB samples), demonstrating that reduction of *RUNX1* mutant alleles below a threshold predicts clinical outcome (Table 2).[164] Most recently, Yeh et al showed that ctDNA analysis by targeted HTS accurately mirrors diagnostic BM genotypes, reflects clonal evolution over time, and predicts treatment failure in patients with MDS, indicating a potential future role for noninvasive disease monitoring.[165]

However, most of these studies either restricted their analyses to a subset of AML/MDS patients known to harbor one specific aberration, or applied HTS approaches with a relatively high lower limit of detection, allowing robust mutation detection only at AFs of 1% to 2%. Technical improvements will be needed to demonstrate a significant prognostic effect of HTS-based methods in large and genetically diverse patient cohorts.

In a screening study of 59 individuals from 17 families with familial AML/MDS, Churpek et al were able to identify recurrent pathogenic germ line alleles in 30% of cases by targeted HTS, including variants in *GATA2* and *RUNX1*. Furthermore, the authors found that the spectrum of emerging somatic alterations in individuals with overt AML/MDS seems to be different from de novo AML.[166] This suggests that high-risk families could potentially benefit from HTS for both recognition of pathogenic germ line alleles in asymptomatic carriers and early detection of emerging somatic alterations in individuals developing AML/MDS.

### Chronic myeloid leukemia

Chronic myeloid leukemia (CML) is generally defined by the reciprocal chromosomal translocation between chromosomes 22 and 9, which results in an oncogenic *BCR-ABL1* gene fusion. *BCR-ABL1* is the target of highly effective tyrosine kinase inhibitors (TKIs; eg, imatinib) with excellent response rates.[167] Monitoring of *BCR-ABL1* transcripts under TKI treatment at certain disease milestones ("major molecular response" [MMR]) is greatly relevant for prediction of clinical outcomes.[168-170]

Established clinical tests to detect *BCR-ABL1* and assess MMR include conventional cytogenetics, fluorescence in situ hybridization, and qRT-PCR.[171] While cytogenetics is relatively insensitive and limited to cultured dividing BM cells, fluorescence in situ hybridization and qRT-PCR can be performed using noninvasively acquired PB cells. Moreover, qRT-PCR with a limit of detection of ~0.001% also allows for the identification of "deep molecular remissions," which may define a subgroup of patients who stay in unmaintained remission after treatment discontinuation.[168,171-174] HTS-based approaches capturing *BCR-ABL1* currently play only a minor role for response assessment in CML. Once fully established, they could facilitate MRD detection with even higher sensitivities. For example, Alikian et al used a coupled HTS and dPCR assay for detection of *BCR-ABL1* breakpoints and demonstrated that this method outperforms qRT-PCR and DNA-based qPCR for MRD monitoring at deep molecular remissions.[175]

Resistance mutations scattered across the *BCR-ABL1* kinase domain under TKI treatment occur frequently and are associated with

disease progression, particularly in patients with rising *BCR-ABL1* levels.[176-178] Sanger sequencing is presently the primary method for identifying TKI resistance, but remains limited because of poor sensitivity (~10%).[179,180] HTS methods covering all known resistance mutations might overcome these limitations and facilitate prediction of TKI resistance and clinical progression. Soverini et al demonstrated that amplicon HTS allows detection of *BCR-ABL1* kinase domain mutations down to 1% AF.[181] In a study by Machova Polakova et al, amplicon-based HTS detected emergent TKI-resistant mutations in CTCs earlier than conventional sequencing, even at the time of MMR.[180] Increasingly refined methods including duplex sequencing are likely to further improve detection and monitoring of such resistance mechanisms.[182]

#### Myeloproliferative neoplasms

*BCR-ABL1* negative myeloproliferative neoplasms (MPNs) are characterized by BM proliferation of 1 or more myeloid lineages with no alterations in cellular maturation. Primary disease entities are polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[183] At molecular level, mutations in *JAK2* V617F are frequently observed, with ~95% of PV and 50% to 60% of ET/PMF patients harboring this aberration. However, variants in other genetic regions are also common, including *CALR* exon 9 (~25% ET, ~35% PMF), *MPL* exon 10 (~4% PMF, ~1% ET), and *JAK2* exon 12 (~4% PV).[184-188]

All those aberrations are part of diagnostic criteria in MPN and represent attractive targets for MRD monitoring.[189] For tracking of *JAK2* V617F, qPCR currently represents the most reliable and sensitive method (~0.01%) and is widely used in clinical trials and routine laboratories to assess patient outcomes.[190,191] For example, patients with *JAK2* V617F levels >1% in PB or BM 1 month after allo-SCT appear to have a significantly higher risk of relapse and death than patients with deeper responses.[191]

Aberrations in *JAK2* exon 12, *MPL*, and *CALR* are less established as markers for disease monitoring. However, a variety of different methods have been developed to track mutations in those genes, including qPCR and digital droplet PCR, achieving sensitivities of 0.01% to 2.5%.[192-194] Some assays have already been used in clinical settings, demonstrating potential future utility for noninvasive disease monitoring.[183,195-198]

HTS-based approaches are not broadly established yet for routine MPN molecular diagnostics, but their capacity to simultaneously cover multiple genetic variants make them a promising tool for monitoring the genetic complexity of MPN.[184,185,197] Abdelhamid et al demonstrated in a proof-of-principle study robust detection of *JAK2* V617F in PB by HTS with high concordance to qPCR, even at low AFs.[199] Lundberg et al applied a targeted HTS panel (104 genes) to serial blood samples and showed that the number of emergent mutations over time was low, characterizing these diseases as genetically stable. This study also illustrated the capability of HTS to detect the expansion of preexisting clones harboring *TP53* and *TET2* mutations toward transformation to AML.[185] Similarly, Ferrer-Marín et al found the emergence of an *ASXL1* mutation in a patient with *JAK2* V617F–positive PMF, leading to leukemic transformation.[200] A study performed by Fu et al suggested that identification of mutations in PB after allo-SCT by amplicon-based HTS results in a higher rate of MPN relapses.[201]

Because of the genetic complexity of MPN, we anticipate a growing role of HTS platforms in the future, both for mutation identification at diagnosis and disease monitoring.

## Conclusions

Modern HTS approaches for noninvasive disease detection are not part of standardized procedures in hematologic malignancies yet. However, they have several decisive advantages over conventional approaches such as MFC and PCR-based assays, at least for selected applications (Figure 2; Table 3). First, targeted capture or amplicon HTS approaches are not restricted to one or only a few genetic markers and can capture hundreds or even thousands of genomic regions without sacrificing sensitivity. Second, the ability to detect and monitor multiple aberrations at the same time allows assessment of intra- and interpatient tumor heterogeneity, which facilitates the identification of clinically relevant subclone evolution. Finally, HTS approaches do not require individual and mutation-specific optimization or a priori knowledge of the patient's tumor genotype.

Given the momentum of these advances, we envision that noninvasive HTS approaches will soon be shown to have clinical utility for precision and personalized therapeutic strategies in several hematologic cancers. For example, we anticipate a growing role in ALL, in which qPCR assays of clonal Ig/TCR gene rearrangements are currently considered the gold standard for assessment of MRD and are already included in clinical guidelines, despite several limitations. It has been demonstrated in numerous studies that HTS assays might overcome those limitations and thus have the potential to replace conventional methods as gold standard for MRD detection.[57] Furthermore, we expect ctDNA analysis will soon be tested in trials for its clinical use in DLBCL and FL. Both neoplasms primarily manifest in lymph node tissue, hampering repeated sampling of tumor DNA. Applying IgHTS and targeted HTS to plasma samples at different disease milestones has demonstrated great potential for noninvasive risk assessment in several studies. Last, the majority of myeloid malignancies are characterized by a complex genetic landscape (eg, AML, MPN). Conventional PCR-based methods are highly limited by the proportion of patients they are able to cover. HTS platforms capturing the whole variety of genetic aberrations have particular advantages in these diseases and are expected to become part of patient management in the near future.

However, before HTS technologies can be implemented into routine clinical testing, certain challenges and limitations have to be addressed. For example, assays must undergo careful validation in multiple prospective clinical trials and prove that patients significantly benefit from their use. Moreover, disease-specific standardized workflows need to be defined by consortia bringing together specialists with clinical and technical experience and expertise. This must include a concerted effort to standardize the way samples are collected and quantified, methods for quality control of sequencing libraries, bioinformatics platforms, and data interpretation.

## Authorship

Contribution: F.S. and D.M.K. wrote the manuscript and designed tables and figures; M.D. and A.A.A. revised the manuscript; and all authors reviewed and edited the final draft of the manuscript and made substantial contribution to discussion of the content.

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## References

1. Mandel P, Metais P. Les acides nucléiques du plasma sanguin chez l'homme [in French]. *C R Seances Soc Biol Fil*. 1948;142(3-4):241-243.
2. Schwarzenbach H, Hoon DS, Pantel K. Cell-free nucleic acids as biomarkers in cancer patients. *Nat Rev Cancer*. 2011;11(6):426-437.
3. Radich J, Gehly G, Lee A, et al. Detection of bcr-abl transcripts in Philadelphia chromosome-positive acute lymphoblastic leukemia after marrow transplantation. *Blood*. 1997;89(7):2602-2609.
4. Cross NC, White HE, Müller MC, Saglio G, Hochhaus A. Standardized definitions of molecular response in chronic myeloid leukemia. *Leukemia*. 2012;26(10):2172-2175.
5. Parpart-Li S, Bartlett B, Popoli M, et al. The effect of preservative and temperature on the analysis of circulating tumor DNA. *Clin Cancer Res*. 2017;23(10):2471-2477.
6. van der Velden VH, Hochhaus A, Cazzaniga G, Szczepanski T, Gabert J, van Dongen JJ. Detection of minimal residual disease in hematologic malignancies by real-time quantitative PCR: principles, approaches, and laboratory aspects. *Leukemia*. 2003;17(6):1013-1034.
7. Gabert J, Beillard E, van der Velden VH, et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia - a Europe Against Cancer program. *Leukemia*. 2003;17(12):2318-2357.
8. van der Velden VH, Cazzaniga G, Schrauder A, et al; European Study Group on MRD detection in ALL (ESG-MRD-ALL). Analysis of minimal residual disease by Ig/TCR gene rearrangements: guidelines for interpretation of real-time quantitative PCR data. *Leukemia*. 2007;21(4):604-611.
9. Dworzak MN, Fröschl G, Printz D, et al; Austrian Berlin-Frankfurt-Münster Study Group. Prognostic significance and modalities of flow cytometric minimal residual disease detection in childhood acute lymphoblastic leukemia. *Blood*. 2002;99(6):1952-1958.
10. Paiva B, Vidriales MB, Cerveró J, et al; GEM (Grupo Español de MM)/PETHEMA (Programa para el Estudio de la Terapéutica en Hemopatías Malignas) Cooperative Study Groups. Multiparameter flow cytometric remission is the most relevant prognostic factor for multiple myeloma patients who undergo autologous stem cell transplantation. *Blood*. 2008;112(10):4017-4023.
11. Paiva B, Martinez-Lopez J, Vidriales MB, et al. Comparison of immunofixation, serum free light chain, and immunophenotyping for response evaluation and prognostication in multiple myeloma. *J Clin Oncol*. 2011;29(12):1627-1633.
12. van Oers MH, Tönnissen E, Van Glabbeke M, et al. BCL-2/IgH polymerase chain reaction status at the end of induction treatment is not predictive for progression-free survival in relapsed/resistant follicular lymphoma: results of a prospective randomized EORTC 20981 phase III intergroup study. *J Clin Oncol*. 2010;28(13):2246-2252.
13. Lanza C, Gottardi E, Gaidano G, et al. Persistence of E2A/PBX1 transcripts in t(1;19) childhood acute lymphoblastic leukemia: correlation with chemotherapy intensity and clinical outcome. *Leuk Res*. 1996;20(5):441-443.
14. Pløen GG, Nederby L, Guldberg P, et al. Persistence of DNMT3A mutations at long-term remission in adult patients with AML. *Br J Haematol*. 2014;167(4):478-486.
15. Newman AM, Bratman SV, To J, et al. An ultrasensitive method for quantitating circulating tumor DNA with broad patient coverage. *Nat Med*. 2014;20(5):548-554.
16. Newman AM, Lovejoy AF, Klass DM, et al. Integrated digital error suppression for improved detection of circulating tumor DNA. *Nat Biotechnol*. 2016;34(5):547-555.
17. Narayan A, Carriero NJ, Gettinger SN, et al. Ultrasensitive measurement of hotspot mutations in tumor DNA in blood using error-suppressed multiplexed deep sequencing. *Cancer Res*. 2012;72(14):3492-3498.
18. Kinde I, Wu J, Papadopoulos N, Kinzler KW, Vogelstein B. Detection and quantification of rare mutations with massively parallel sequencing. *Proc Natl Acad Sci USA*. 2011;108(23):9530-9535.
19. Kennedy SR, Schmitt MW, Fox EJ, et al. Detecting ultralow-frequency mutations by Duplex Sequencing. *Nat Protoc*. 2014;9(11):2586-2606.
20. Schmitt MW, Kennedy SR, Salk JJ, Fox EJ, Hiatt JB, Loeb LA. Detection of ultra-rare mutations by next-generation sequencing. *Proc Natl Acad Sci USA*. 2012;109(36):14508-14513.
21. Chabon JJ, Simmons AD, Lovejoy AF, et al. Circulating tumour DNA profiling reveals heterogeneity of EGFR inhibitor resistance mechanisms in lung cancer patients. *Nat Commun*. 2016;7:11815.
22. Scherer F, Kurtz DM, Newman AM, et al. Distinct biological subtypes and patterns of genome evolution in lymphoma revealed by circulating tumor DNA. *Sci Transl Med*. 2016;8(364):364ra155.
23. Rowe JM, Buck G, Burnett AK, et al; ECOG; MRC/NCRI Adult Leukemia Working Party. Induction therapy for adults with acute lymphoblastic leukemia: results of more than 1500 patients from the international ALL trial: MRC UKALL XII/ECOG E2993. *Blood*. 2005;106(12):3760-3767.
24. Pui CH, Campana D, Pei D, et al. Treating childhood acute lymphoblastic leukemia without cranial irradiation. *N Engl J Med*. 2009;360(26):2730-2741.
25. van Dongen JJ, van der Velden VH, Brüggemann M, Orfao A. Minimal residual disease diagnostics in acute lymphoblastic leukemia: need for sensitive, fast, and standardized technologies. *Blood*. 2015;125(26):3996-4009.
26. van Dongen JJ, Macintyre EA, Gabert JA, et al. Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. *Leukemia*. 1999;13(12):1901-1928.
27. Grimwade D, Jovanovic JV, Hills RK, et al. Prospective minimal residual disease monitoring to predict relapse of acute promyelocytic leukemia and to direct pre-emptive arsenic trioxide therapy. *J Clin Oncol*. 2009;27(22):3650-3658.
28. Theunissen P, Mejstrikova E, Sedek L, et al; EuroFlow Consortium. Standardized flow cytometry for highly sensitive MRD measurements in B-cell acute lymphoblastic leukemia. *Blood*. 2017;129(3):347-357.
29. Raff T, Gökbuget N, Lüschen S, et al; GMALL Study Group. Molecular relapse in adult standard-risk ALL patients detected by prospective MRD monitoring during and after maintenance treatment: data from the GMALL 06/99 and 07/03 trials. *Blood*. 2007;109(3):910-915.
30. van Dongen JJ, Seriu T, Panzer-Grümayer ER, et al. Prognostic value of minimal residual disease in acute lymphoblastic leukaemia in childhood. *Lancet*. 1998;352(9142):1731-1738.
31. Cavé H, van der Werff ten Bosch J, Suciu S, et al. Clinical significance of minimal residual disease in childhood acute lymphoblastic leukemia. European Organization for Research and Treatment of Cancer–Childhood Leukemia Cooperative Group. *N Engl J Med*. 1998;339(9):591-598.
32. Conter V, Bartram CR, Valsecchi MG, et al. Molecular response to treatment redefines all prognostic factors in children and adolescents with B-cell precursor acute lymphoblastic leukemia: results in 3184 patients of the AIEOP-BFM ALL 2000 study. *Blood*. 2010;115(16):3206-3214.
33. Vora A, Goulden N, Wade R, et al. Treatment reduction for children and young adults with low-risk acute lymphoblastic leukaemia defined by minimal residual disease (UKALL 2003): a randomised controlled trial. *Lancet Oncol*. 2013;14(3):199-209.
34. Vora A, Goulden N, Mitchell C, et al. Augmented post-remission therapy for a minimal residual disease-defined high-risk subgroup of children and young people with clinical standard-risk and intermediate-risk acute lymphoblastic leukaemia (UKALL 2003): a randomised controlled trial. *Lancet Oncol*. 2014;15(8):809-818.
35. Roberts KG, Pei D, Campana D, et al. Outcomes of children with BCR-ABL1–like acute lymphoblastic leukemia treated with risk-directed therapy based on the levels of minimal residual disease. *J Clin Oncol*. 2014;32(27):3012-3020.
36. Attarbaschi A, Mann G, Panzer-Grümayer R, et al. Minimal residual disease values discriminate between low and high relapse risk in children with B-cell precursor acute lymphoblastic leukemia and an intrachromosomal amplification of chromosome 21: the Austrian and German acute lymphoblastic leukemia Berlin-Frankfurt-Munster (ALL-BFM) trials. *J Clin Oncol*. 2008;26(18):3046-3050.
37. Eckert C, Biondi A, Seeger K, et al. Prognostic value of minimal residual disease in relapsed childhood acute lymphoblastic leukaemia. *Lancet*. 2001;358(9289):1239-1241.
38. Bader P, Kreyenberg H, Henze GH, et al; ALL-REZ BFM Study Group. Prognostic value of minimal residual disease quantification before

allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia: the ALL-REZ BFM Study Group. *J Clin Oncol*. 2009; 27(3):377-384.

39. Bader P, Kreyenberg H, von Stackelberg A, et al. Monitoring of minimal residual disease after allogeneic stem-cell transplantation in relapsed childhood acute lymphoblastic leukemia allows for the identification of impending relapse: results of the ALL-BFM-SCT 2003 trial. *J Clin Oncol*. 2015;33(11):1275-1284.
40. Eckert C, Hagedorn N, Sramkova L, et al. Monitoring minimal residual disease in children with high-risk relapses of acute lymphoblastic leukemia: prognostic relevance of early and late assessment. *Leukemia*. 2015;29(8):1648-1655.
41. Eckert C, Henze G, Seeger K, et al. Use of allogeneic hematopoietic stem-cell transplantation based on minimal residual disease response improves outcomes for children with relapsed acute lymphoblastic leukemia in the intermediate-risk group. *J Clin Oncol*. 2013;31(21):2736-2742.
42. Ravandi F, Jorgensen JL, O'Brien SM, et al. Minimal residual disease assessed by multi-parameter flow cytometry is highly prognostic in adult patients with acute lymphoblastic leukaemia. *Br J Haematol*. 2016;172(3):392-400.
43. Pieters R, de Groot-Kruseman H, Van der Velden V, et al. Successful Therapy Reduction and Intensification for Childhood Acute Lymphoblastic Leukemia Based on Minimal Residual Disease Monitoring: Study ALL10 from the Dutch Childhood Oncology Group. *J Clin Oncol*. 2016;34(22):2591-2601.
44. Brüggemann M, Raff T, Kneba M. Has MRD monitoring superseded other prognostic factors in adult ALL? *Blood*. 2012;120(23):4470-4481.
45. Campana D. Minimal residual disease in acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program*. 2010;2010:7-12.
46. van der Velden VH, Jacobs DC, Wijkhuijs AJ, et al. Minimal residual disease levels in bone marrow and peripheral blood are comparable in children with T cell acute lymphoblastic leukemia (ALL), but not in precursor-B-ALL. *Leukemia*. 2002;16(8):1432-1436.
47. Gawad C, Pepin F, Carlton VE, et al. Massive evolution of the immunoglobulin heavy chain locus in children with B precursor acute lymphoblastic leukemia. *Blood*. 2012;120(22): 4407-4417.
48. Ladetto M, Brüggemann M, Monitillo L, et al. Next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in B-cell disorders. *Leukemia*. 2014; 28(6):1299-1307.
49. Faham M, Zheng J, Moorhead M, et al. Deep-sequencing approach for minimal residual disease detection in acute lymphoblastic leukemia. *Blood*. 2012;120(26):5173-5180.
50. Wu D, Sherwood A, Fromm JR, et al. High-throughput sequencing detects minimal residual disease in acute T lymphoblastic leukemia. *Sci Transl Med*. 2012;4(134):134ra63.
51. Torra OS, Othus M, Williamson DW, et al. Next-generation sequencing in adult B cell acute lymphoblastic leukemia patients. *Biol Blood Marrow Transplant*. 2017;23(4):691-699.
52. Malnassy G, Geyer S, Fulton N, et al. Comparison of deep sequencing and allele-specific oligonucleotide PCR methods for MRD quantitation in acute lymphoblastic leukemia and mantle cell lymphoma: CALGB 10403 and CALGB 59909 (Alliance). *Blood*. 2013;122:2547.
53. Wu D, Emerson RO, Sherwood A, et al. Detection of minimal residual disease in B lymphoblastic leukemia by high-throughput sequencing of IGH. *Clin Cancer Res*. 2014; 20(17):4540-4548.
54. Logan AC, Vashi N, Faham M, et al. Immunoglobulin and T cell receptor gene high-throughput sequencing quantifies minimal residual disease in acute lymphoblastic leukemia and predicts post-transplantation relapse and survival. *Biol Blood Marrow Transplant*. 2014; 20(9):1307-1313.
55. Pulsipher MA, Carlson C, Langholz B, et al. IgH-V(D)J NGS-MRD measurement pre- and early post-allotransplant defines very low- and very high-risk ALL patients. *Blood*. 2015;125(22): 3501-3508.
56. Mannis GN, Martin TG III, Damon LE, et al. Quantification of acute lymphoblastic leukemia clonotypes in leukapheresed peripheral blood progenitor cells predicts relapse risk after autologous hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant*. 2016;22(6):1030-1036.
57. Kotrova M, Trka J, Kneba M, Brüggemann M. Is next-generation sequencing the way to go for residual disease monitoring in acute lymphoblastic leukemia? [published online ahead of print 27 April 2017]. *Mol Diagn Ther*. doi:10.1007/s40291-017-0277-9.
58. Kotrova M, Muzikova K, Mejstrikova E, et al. The predictive strength of next-generation sequencing MRD detection for relapse compared with current methods in childhood ALL. *Blood*. 2015;126(8):1045-1047.
59. Canellos GP, Rosenberg SA, Friedberg JW, Lister TA, Devita VT. Treatment of Hodgkin lymphoma: a 50-year perspective. *J Clin Oncol*. 2014;32(3):163-168.
60. Slovak ML, Bedell V, Hsu YH, et al. Molecular karyotypes of Hodgkin and Reed-Sternberg cells at disease onset reveal distinct copy number alterations in chemosensitive versus refractory Hodgkin lymphoma. *Clin Cancer Res*. 2011; 17(10):3443-3454.
61. Reichel J, Chadburn A, Rubinstein PG, et al. Flow sorting and exome sequencing reveal the oncogenome of primary Hodgkin and Reed-Sternberg cells. *Blood*. 2015;125(7):1061-1072.
62. Martín-Subero JI, Klapper W, Sotnikova A, et al; Deutsche Krebshilfe Network Project Molecular Mechanisms in Malignant Lymphomas. Chromosomal breakpoints affecting immunoglobulin loci are recurrent in Hodgkin and Reed-Sternberg cells of classical Hodgkin lymphoma. *Cancer Res*. 2006;66(21): 10332-10338.
63. Kato M, Sanada M, Kato I, et al. Frequent inactivation of A20 in B-cell lymphomas. *Nature*. 2009;459(7247):712-716.
64. Tiacci E, Penson A, Schiavoni G, et al. New recurrently mutated genes in classical hodgkin lymphoma revealed by whole-exome sequencing of microdissected tumor cells [abstract]. *Blood*. 2016;128(22). Abstract 1088.
65. Marafioti T, Hummel M, Foss HD, et al. Hodgkin and Reed-Sternberg cells represent an expansion of a single clone originating from a germinal center B-cell with functional immunoglobulin gene rearrangements but defective immunoglobulin transcription. *Blood*. 2000;95(4):1443-1450.
66. Vandenberghe P, Wlodarska I, Tousseyn T, et al. Non-invasive detection of genomic imbalances in Hodgkin/Reed-Sternberg cells in early and advanced stage Hodgkin's lymphoma by sequencing of circulating cell-free DNA: a technical proof-of-principle study. *Lancet Haematol*. 2015;2(2):e55-e65.
67. Camus V, Stamatoullas A, Mareschal S, et al. Detection and prognostic value of recurrent exportin 1 mutations in tumor and cell-free circulating DNA of patients with classical Hodgkin lymphoma. *Haematologica*. 2016; 101(9):1094-1101.
68. Herrera AF, Kim HT, Kong KA, et al. Next-generation sequencing-based detection of circulating tumour DNA After allogeneic stem cell transplantation for lymphoma. *Br J Haematol*. 2016;175(5):841-850.
69. Oki Y, Neelapu SS, Fanale M, et al. Detection of classical Hodgkin lymphoma specific sequence in peripheral blood using a next-generation sequencing approach. *Br J Haematol*. 2015; 169(5):689-693.
70. Kristinsson SY, Anderson WF, Landgren O. Improved long-term survival in multiple myeloma up to the age of 80 years. *Leukemia*. 2014;28(6): 1346-1348.
71. Fonseca R, Bergsagel PL, Drach J, et al; International Myeloma Working Group. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. *Leukemia*. 2009;23(12):2210-2221.
72. Rawstron AC, Child JA, de Tute RM, et al. Minimal residual disease assessed by multiparameter flow cytometry in multiple myeloma: impact on outcome in the Medical Research Council Myeloma IX Study. *J Clin Oncol*. 2013;31(20):2540-2547.
73. Ferrero S, Ladetto M, Drandi D, et al. Long-term results of the GIMEMA VEL-03-096 trial in MM patients receiving VTD consolidation after ASCT: MRD kinetics' impact on survival. *Leukemia*. 2015;29(3):689-695.
74. Mailankody S, Korde N, Lesokhin AM, et al. Minimal residual disease in multiple myeloma: bringing the bench to the bedside. *Nat Rev Clin Oncol*. 2015;12(5):286-295.
75. Paiva B, van Dongen JJ, Orfao A. New criteria for response assessment: role of minimal residual disease in multiple myeloma. *Blood*. 2015;125(20):3059-3068.
76. Logan AC, Gao H, Wang C, et al. High-throughput VDJ sequencing for quantification of minimal residual disease in chronic lymphocytic leukemia and immune reconstitution assessment. *Proc Natl Acad Sci USA*. 2011;108(52):21194-21199.
77. Martinez-Lopez J, Lahuerta JJ, Pepin F, et al. Prognostic value of deep sequencing method for minimal residual disease detection in multiple myeloma. *Blood*. 2014;123(20): 3073-3079.
78. Avet-Loiseau H, Corre J, Lauwers-Cances V, et al. Evaluation of minimal residual disease (MRD) by next generation sequencing (NGS) is highly predictive of progression free survival in the IFM/DFCI 2009 Trial [abstract]. *Blood*. 2015;126(23). Abstract 191.
79. Takamatsu H, Murata R, Zheng J, et al. Prognostic value of sequencing-based minimal residual disease detection in patients with multiple myeloma who underwent autologous stem cell transplantation [abstract]. *Blood*. 2015; 126(23). Abstract 1788.
80. Munshi N, Minvielle S, Tai Y-T, et al. Deep Igh sequencing identifies an ongoing somatic hypermutation process with complex and evolving clonal architecture in myeloma [abstract]. *Blood*. 2015;126(23). Abstract 21.
81. Gimondi S, Cavanè A, Vendramin A, et al. Identification of clonal Igh gene rearrangements by high-throughput sequencing of cell free DNA in multiple myeloma patients [abstract]. *Blood*. 2015;126(23). Abstract 2987.
82. Wee R, Takamatsu H, Murata R, et al. A comparison of minimal residual disease detection among ASO-PCR, Dd-PCR and deep-sequencing in patients with multiple myeloma who underwent autologous stem cell

transplantation [abstract]. *Blood*. 2015; 126(23). Abstract 1782.

83. Campo E, Rule S. Mantle cell lymphoma: evolving management strategies. *Blood*. 2015; 125(1):48-55.
84. Andersen NS, Pedersen LB, Laurell A, et al. Pre-emptive treatment with rituximab of molecular relapse after autologous stem cell transplantation in mantle cell lymphoma. *J Clin Oncol*. 2009;27(26):4365-4370.
85. Pott C, Hoster E, Delfau-Larue MH, et al. Molecular remission is an independent predictor of clinical outcome in patients with mantle cell lymphoma after combined immunochemotherapy: a European MCL intergroup study. *Blood*. 2010;115(16): 3215-3223.
86. Pott C, Macintyre E, Delfau-Larue M-H, et al. MRD eradication should be the therapeutic goal in mantle cell lymphoma and may enable tailored treatment approaches: results of the Intergroup Trials of the European MCL Network [abstract]. *Blood*. 2014;124(21). Abstract 147.
87. Callahan M, Delfau M-H, Macintyre E, et al. Monitoring in peripheral blood and bone marrow in patients with mantle cell lymphoma following autologous stem cell transplantation with or without rituximab maintenance; interim results from the LyMa-MRD Project, conducted on behalf of the Lysa Group [abstract]. *Blood*. 2015; 126(23). Abstract 338.
88. Pott C, Schrader C, Gesk S, et al. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission in mantle cell lymphoma. *Blood*. 2006;107(6): 2271-2278.
89. Cheminant M, Derrieux C, Touzart A, et al. Minimal residual disease monitoring by 8-color flow cytometry in mantle cell lymphoma: an EU-MCL and LYSA study. *Haematologica*. 2016; 101(3):336-345.
90. Hoster E, Pott C. Minimal residual disease in mantle cell lymphoma: insights into biology and impact on treatment. *Hematology Am Soc Hematol Educ Program*. 2016;2016:437-445.
91. Pott C. Minimal residual disease detection in mantle cell lymphoma: technical aspects and clinical relevance. *Semin Hematol*. 2011;48(3): 172-184.
92. Jain N, O'Brien S. Initial treatment of CLL: integrating biology and functional status. *Blood*. 2015;126(4):463-470.
93. Burger JA, Tedeschi A, Barr PM, et al; RESONATE-2 Investigators. Ibrutinib as initial therapy for patients with chronic lymphocytic leukemia. *N Engl J Med*. 2015;373(25): 2425-2437.
94. Böttcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. *J Clin Oncol*. 2012;30(9):980-988.
95. Strati P, Keating MJ, O'Brien SM, et al. Eradication of bone marrow minimal residual disease may prompt early treatment discontinuation in CLL. *Blood*. 2014; 123(24):3727-3732.
96. Kwok M, Rawstron AC, Varghese A, et al. Minimal residual disease is an independent predictor for 10-year survival in CLL. *Blood*. 2016;128(24):2770-2773.
97. Dreger P, Döhner H, Ritgen M, et al; German CLL Study Group. Allogeneic stem cell transplantation provides durable disease control in poor-risk chronic lymphocytic leukemia: long-term clinical and MRD results of the German CLL Study Group CLL3X trial. *Blood*. 2010;116(14): 2438-2447.
98. Moreno C, Villamor N, Colomer D, et al. Clinical significance of minimal residual disease, as assessed by different techniques, after stem cell transplantation for chronic lymphocytic leukemia. *Blood*. 2006;107(11):4563-4569.
99. Kovacs G, Robrecht S, Fink AM, et al. Minimal residual disease assessment improves prediction of outcome in patients with chronic lymphocytic leukemia (CLL) who achieve partial response: comprehensive analysis of two phase III studies of the German CLL Study Group. *J Clin Oncol*. 2016;34(31):3758-3765.
100. Thompson PA, Wierda WG. Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL. *Blood*. 2016;127(3):279-286.
101. Böttcher S, Hallek M, Ritgen M, Kneba M. The role of minimal residual disease measurements in the therapy for CLL: is it ready for prime time? *Hematol Oncol Clin North Am*. 2013;27(2): 267-288.
102. Wierda WG. Minimal residual disease provides treatment focus for next chronic lymphocytic leukemia advances. *J Clin Oncol*. 2016;34(31): 3722-3723.
103. Logan AC, Zhang B, Narasimhan B, et al. Minimal residual disease quantification using consensus primers and high-throughput IGH sequencing predicts post-transplant relapse in chronic lymphocytic leukemia. *Leukemia*. 2013; 27(8):1659-1665.
104. Rawstron AC, Fazi C, Agathangelidis A, et al. A complementary role of multiparameter flow cytometry and high-throughput sequencing for minimal residual disease detection in chronic lymphocytic leukemia: an European Research Initiative on CLL study. *Leukemia*. 2016;30(4): 929-936.
105. Yeh P, Hunter T, Sinha D, et al. Circulating tumour DNA reflects treatment response and clonal evolution in chronic lymphocytic leukaemia. *Nat Commun*. 2017;8:14756.
106. Shaffer AL III, Young RM, Staudt LM. Pathogenesis of human B cell lymphomas. *Annu Rev Immunol*. 2012;30:565-610.
107. Kridel R, Sehn LH, Gascoyne RD. Pathogenesis of follicular lymphoma. *J Clin Invest*. 2012; 122(10):3424-3431.
108. Roulland S, Faroudi M, Mamessier E, Sungalee S, Salles G, Nadel B. Early steps of follicular lymphoma pathogenesis. *Adv Immunol*. 2011; 111:1-46.
109. Limpens J, Stad R, Vos C, et al. Lymphoma-associated translocation t(14;18) in blood B cells of normal individuals. *Blood*. 1995;85(9): 2528-2536.
110. Dölken G, Illerhaus G, Hirt C, Mertelsmann R. BCL-2/JH rearrangements in circulating B cells of healthy blood donors and patients with nonmalignant diseases. *J Clin Oncol*. 1996; 14(4):1333-1344.
111. Roulland S, Navarro JM, Grenot P, et al. Follicular lymphoma-like B cells in healthy individuals: a novel intermediate step in early lymphomagenesis [published correction appears in *J Exp Med*. 2006;203(11)L2563]. *J Exp Med*. 2006;203(11):2425-2431.
112. Roulland S, Kelly RS, Morgado E, et al. t(14;18) translocation: a predictive blood biomarker for follicular lymphoma. *J Clin Oncol*. 2014;32(13): 1347-1355.
113. Green MR, Kihira S, Liu CL, et al. Mutations in early follicular lymphoma progenitors are associated with suppressed antigen presentation. *Proc Natl Acad Sci USA*. 2015; 112(10):E1116-E1125.
114. Weigert O, Weinstock DM. The evolving contribution of hematopoietic progenitor cells to lymphomagenesis. *Blood*. 2012;120(13): 2553-2561.
115. Okosun J, Bödör C, Wang J, et al. Integrated genomic analysis identifies recurrent mutations and evolution patterns driving the initiation and progression of follicular lymphoma. *Nat Genet*. 2014;46(2):176-181.
116. Ladetto M, De Marco F, Benedetti F, et al; Gruppo Italiano Trapianto di Midollo Osseo (GITMO); Intergruppo Italiano Linfomi (IIL). Prospective, multicenter randomized GITMO/IIL trial comparing intensive (R-HDS) versus conventional (CHOP-R) chemoimmunotherapy in high-risk follicular lymphoma at diagnosis: the superior disease control of R-HDS does not translate into an overall survival advantage. *Blood*. 2008;111(8):4004-4013.
117. López-Guillermo A, Cabanillas F, McLaughlin P, et al. The clinical significance of molecular response in indolent follicular lymphomas. *Blood*. 1998;91(8):2955-2960.
118. Corradini P, Ladetto M, Zallio F, et al. Long-term follow-up of indolent lymphoma patients treated with high-dose sequential chemotherapy and autografting: evidence that durable molecular and clinical remission frequently can be attained only in follicular subtypes. *J Clin Oncol*. 2004; 22(8):1460-1468.
119. Apostolidis J, Gupta RK, Grenzelias D, et al. High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: long-term clinical and molecular follow-up. *J Clin Oncol*. 2000;18(3):527-536.
120. Pott C, Hoster E, Kehden B, et al. Minimal residual disease in patients with follicular lymphoma treated with obinutuzumab or rituximab as first-line induction immunochemotherapy and maintenance in the phase 3 GALLIUM study [abstract]. *Blood*. 2016; 128(22). Abstract 613.
121. Mandigers CM, Meijerink JP, Mensink EJ, et al; Interzol (South-East Netherlands Comprehensive Cancer Centers Cooperative Group). Lack of correlation between numbers of circulating t(14;18)-positive cells and response to first-line treatment in follicular lymphoma. *Blood*. 2001;98(4):940-944.
122. Rambaldi A, Carlotti E, Oldani E, et al. Quantitative PCR of bone marrow BCL2/IgH+ cells at diagnosis predicts treatment response and long-term outcome in follicular non-Hodgkin lymphoma. *Blood*. 2005;105(9):3428-3433.
123. Gribben JG, Neuberg D, Barber M, et al. Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. *Blood*. 1994;83(12): 3800-3807.
124. Price CG, Meerabux J, Murtagh S, et al. The significance of circulating cells carrying t(14;18) in long remission from follicular lymphoma. *J Clin Oncol*. 1991;9(9):1527-1532.
125. Lambrechts AC, Hupkes PE, Dorssers LC, van't Veer MB. Clinical significance of t(14; 18)-positive cells in the circulation of patients with stage III or IV follicular non-Hodgkin's lymphoma during first remission. *J Clin Oncol*. 1994;12(8): 1541-1546.
126. Ladetto M, Corradini P, Vallet S, et al. High rate of clinical and molecular remissions in follicular lymphoma patients receiving high-dose sequential chemotherapy and autografting at diagnosis: a multicenter, prospective study by the Gruppo Italiano Trapianto Midollo Osseo (GITMO). *Blood*. 2002;100(5):1559-1565.
127. Moos M, Schulz R, Martin S, Benner A, Haas R. The remission status before and the PCR status after high-dose therapy with peripheral blood

stem cell support are prognostic factors for relapse-free survival in patients with follicular non-Hodgkin's lymphoma. *Leukemia*. 1998; 12(12):1971-1976.

128. Gribben JG, Neuberg D, Freedman AS, et al. Detection by polymerase chain reaction of residual cells with the bcl-2 translocation is associated with increased risk of relapse after autologous bone marrow transplantation for B-cell lymphoma. *Blood*. 1993;81(12):3449-3457.
129. Hardingham JE, Kotasek D, Sage RE, et al. Significance of molecular marker-positive cells after autologous peripheral-blood stem-cell transplantation for non-Hodgkin's lymphoma. *J Clin Oncol*. 1995;13(5):1073-1079.
130. Alcaide M, Yu S, Bushell K, et al. Multiplex droplet digital PCR quantification of recurrent somatic mutations in diffuse large B-cell and follicular lymphoma. *Clin Chem*. 2016;62(9): 1238-1247.
131. Pott C, Monitillo L, Genuardi E, et al. A comparative analysis of next-generation sequencing and real-time quantitative PCR for minimal residual disease detection in follicular lymphomas. *Blood*. 2013;122:4293.
132. Sarkozy C, Huet S, Carlton V, et al. Quantitative assessment of circulating clonal IG-VDJ sequences in plasma of follicular lymphoma at diagnosis is highly predictive of progression free survival (PFS). *Blood*. 2015;126:2675.
133. Camus V, Sarafan-Vasseur N, Bohers E, et al. Digital PCR for quantification of recurrent and potentially actionable somatic mutations in circulating free DNA from patients with diffuse large B-cell lymphoma. *Leuk Lymphoma*. 2016; 57(9):2171-2179.
134. Roschewski M, Dunleavy K, Pittaluga S, et al. Circulating tumour DNA and CT monitoring in patients with untreated diffuse large B-cell lymphoma: a correlative biomarker study. *Lancet Oncol*. 2015;16(5):541-549.
135. Kurtz DM, Green MR, Bratman SV, et al. Noninvasive monitoring of diffuse large B-cell lymphoma by immunoglobulin high-throughput sequencing. *Blood*. 2015;125(24):3679-3687.
136. Scherer F, Kurtz DM, Newman AM, et al. Noninvasive genotyping and assessment of treatment response in diffuse large B cell lymphoma [abstract]. *Blood*. 2015;126(23). Abstract 114.
137. Rossi D, Diop F, Spaccarotella E, et al. Diffuse large B-cell lymphoma genotyping on the liquid biopsy. *Blood*. 2017;129(14):1947-1957.
138. Genovese G, Kähler AK, Handsaker RE, et al. Clonal hematopoiesis and blood-cancer risk inferred from blood DNA sequence. *N Engl J Med*. 2014;371(26):2477-2487.
139. Jaiswal S, Fontanillas P, Flannick J, et al. Age-related clonal hematopoiesis associated with adverse outcomes. *N Engl J Med*. 2014;371(26): 2488-2498.
140. McKerrell T, Park N, Moreno T, et al; Understanding Society Scientific Group. Leukemia-associated somatic mutations drive distinct patterns of age-related clonal hemopoiesis. *Cell Reports*. 2015;10(8):1239-1245.
141. Busque L, Patel JP, Figueroa ME, et al. Recurrent somatic TET2 mutations in normal elderly individuals with clonal hematopoiesis. *Nat Genet*. 2012;44(11):1179-1181.
142. Xie M, Lu C, Wang J, et al. Age-related mutations associated with clonal hematopoietic expansion and malignancies. *Nat Med*. 2014; 20(12):1472-1478.
143. Welch JS, Ley TJ, Link DC, et al. The origin and evolution of mutations in acute myeloid leukemia. *Cell*. 2012;150(2):264-278.
144. Steensma DP, Bejar R, Jaiswal S, et al. Clonal hematopoiesis of indeterminate potential and its distinction from myelodysplastic syndromes. *Blood*. 2015;126(1):9-16.
145. Link DC, Walter MJ. 'CHIP'ping away at clonal hematopoiesis. *Leukemia*. 2016;30(8):1633-1635.
146. Haferlach T, Nagata Y, Grossmann V, et al. Landscape of genetic lesions in 944 patients with myelodysplastic syndromes. *Leukemia*. 2014; 28(2):241-247.
147. Cancer Genome Atlas Research Network, Ley TJ, Miller C, Ding L, et al. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013; 368(22):2059-2074.
148. van den Akker EB, Pitts SJ, Deelen J, et al; Genome of The Netherlands Consortium. Uncompromised 10-year survival of oldest old carrying somatic mutations in DNMT3A and TET2. *Blood*. 2016;127(11):1512-1515.
149. Young AL, Challen GA, Birmann BM, Druley TE. Clonal haematopoiesis harbouring AML-associated mutations is ubiquitous in healthy adults. *Nat Commun*. 2016;7:12484.
150. Döhner H, Weisdorf DJ, Bloomfield CD. Acute myeloid leukemia. *N Engl J Med*. 2015;373(12): 1136-1152.
151. Patel JP, Gönen M, Figueroa ME, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. *N Engl J Med*. 2012;366(12):1079-1089.
152. Grossmann V, Schnittger S, Kohlmann A, et al. A novel hierarchical prognostic model of AML solely based on molecular mutations. *Blood*. 2012;120(15):2963-2972.
153. Coombs CC, Tallman MS, Levine RL. Molecular therapy for acute myeloid leukaemia. *Nat Rev Clin Oncol*. 2016;13(5):305-318.
154. Grimwade D, Ivey A, Huntly BJ. Molecular landscape of acute myeloid leukemia in younger adults and its clinical relevance. *Blood*. 2016; 127(1):29-41.
155. Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. *Blood*. 2017;129(4):424-447.
156. Ommen HB. Monitoring minimal residual disease in acute myeloid leukaemia: a review of the current evolving strategies. *Ther Adv Hematol*. 2016;7(1):3-16.
157. Grimwade D, Freeman SD. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? *Hematology Am Soc Hematol Educ Program*. 2014;2014:222-233.
158. Buccisano F, Maurillo L, Del Principe MI, et al. Prognostic and therapeutic implications of minimal residual disease detection in acute myeloid leukemia. *Blood*. 2012;119(2):332-341.
159. Buckley SA, Appelbaum FR, Walter RB. Prognostic and therapeutic implications of minimal residual disease at the time of transplantation in acute leukemia. *Bone Marrow Transplant*. 2013;48(5):630-641.
160. Campana D, Leung W. Clinical significance of minimal residual disease in patients with acute leukaemia undergoing haematopoietic stem cell transplantation. *Br J Haematol*. 2013;162(2): 147-161.
161. Ivey A, Hills RK, Simpson MA, et al; UK National Cancer Research Institute AML Working Group. Assessment of minimal residual disease in standard-risk AML. *N Engl J Med*. 2016;374(5): 422-433.
162. Grimwade D, Freeman SD. Defining minimal residual disease in acute myeloid leukemia: which platforms are ready for "prime time"? *Blood*. 2014;124(23):3345-3355.
163. Thol F, Scherr M, Kirchner A, et al. Clinical and functional implications of microRNA mutations in a cohort of 935 patients with myelodysplastic syndromes and acute myeloid leukemia. *Haematologica*. 2015;100(4):e122-e124.
164. Kohlmann A, Nadarajah N, Alpermann T, et al. Monitoring of residual disease by next-generation deep-sequencing of RUNX1 mutations can identify acute myeloid leukemia patients with resistant disease. *Leukemia*. 2014; 28(1):129-137.
165. Yeh P, Dickinson M, Ftouni S, et al. Molecular disease monitoring using circulating tumor DNA in myelodysplastic syndromes. *Blood*. 2017; 129(12):1685-1690.
166. Churpek JE, Pyrtel K, Kanchi KL, et al. Genomic analysis of germ line and somatic variants in familial myelodysplasia/acute myeloid leukemia. *Blood*. 2015;126(22):2484-2490.
167. Druker BJ, Guilhot F, O'Brien SG, et al; IRIS Investigators. Five-year follow-up of patients receiving imatinib for chronic myeloid leukemia. *N Engl J Med*. 2006;355(23):2408-2417.
168. Baccarani M, Deininger MW, Rosti G, et al. European LeukemiaNet recommendations for the management of chronic myeloid leukemia: 2013. *Blood*. 2013;122(6):872-884.
169. Branford S, Kim DW, Soverini S, et al. Initial molecular response at 3 months may predict both response and event-free survival at 24 months in imatinib-resistant or -intolerant patients with Philadelphia chromosome-positive chronic myeloid leukemia in chronic phase treated with nilotinib. *J Clin Oncol*. 2012;30(35):4323-4329.
170. Neelakantan P, Gerrard G, Lucas C, et al. Combining BCR-ABL1 transcript levels at 3 and 6 months in chronic myeloid leukemia: implications for early intervention strategies. *Blood*. 2013;121(14):2739-2742.
171. Cross NC, White HE, Colomer D, et al. Laboratory recommendations for scoring deep molecular responses following treatment for chronic myeloid leukemia. *Leukemia*. 2015; 29(5):999-1003.
172. Hehlmann R, Müller MC, Lauseker M, et al. Deep molecular response is reached by the majority of patients treated with imatinib, predicts survival, and is achieved more quickly by optimized high-dose imatinib: results from the randomized CML-study IV. *J Clin Oncol*. 2014; 32(5):415-423.
173. Branford S, Yeung DT, Parker WT, et al. Prognosis for patients with CML and >10% BCR-ABL1 after 3 months of imatinib depends on the rate of BCR-ABL1 decline. *Blood*. 2014; 124(4):511-518.
174. Mahon FX, Réa D, Guilhot J, et al; Intergroupe Français des Leucémies Myéloïdes Chroniques. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. *Lancet Oncol*. 2010;11(11):1029-1035.
175. Alikian M, Ellery P, Forbes M, et al. Next-generation sequencing-assisted DNA-based digital PCR for a personalized approach to the detection and quantification of residual disease in chronic myeloid leukemia patients. *J Mol Diagn*. 2016;18(2):176-189.
176. Gorre ME, Mohammed M, Ellwood K, et al. Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. *Science*. 2001;293(5531):876-880.
177. Parker WT, Lawrence RM, Ho M, et al. Sensitive detection of BCR-ABL1 mutations in patients with chronic myeloid leukemia after imatinib resistance is predictive of outcome during subsequent therapy. *J Clin Oncol*. 2011;29(32): 4250-4259.

178. Press RD, Galderisi C, Yang R, et al. A half-log increase in BCR-ABL RNA predicts a higher risk of relapse in patients with chronic myeloid leukemia with an imatinib-induced complete cytogenetic response. *Clin Cancer Res*. 2007; 13(20):6136-6143.

179. Poláková KM, Polívková V, Rulcová J, et al. Constant BCR-ABL transcript level >or=0.1% (IS) in patients with CML responding to imatinib with complete cytogenetic remission may indicate mutation analysis. *Exp Hematol*. 2010; 38(1):20-26.

180. Machova Polakova K, Kulvait V, Benesova A, et al. Next-generation deep sequencing improves detection of BCR-ABL1 kinase domain mutations emerging under tyrosine kinase inhibitor treatment of chronic myeloid leukemia patients in chronic phase. *J Cancer Res Clin Oncol*. 2015;141(5):887-899.

181. Soverini S, De Benedittis C, Machova Polakova K, et al. Unraveling the complexity of tyrosine kinase inhibitor-resistant populations by ultra-deep sequencing of the BCR-ABL kinase domain. *Blood*. 2013;122(9):1634-1648.

182. Schmitt MW, Fox EJ, Prindle MJ, et al. Sequencing small genomic targets with high efficiency and extreme accuracy. *Nat Methods*. 2015;12(5):423-425.

183. Haslam K, Langabeer SE. Monitoring minimal residual disease in the myeloproliferative neoplasms: current applications and emerging approaches. *Biomed Res Int*. 2016;2016:7241591.

184. Skoda RC, Duek A, Grisouard J. Pathogenesis of myeloproliferative neoplasms. *Exp Hematol*. 2015;43(8):599-608.

185. Lundberg P, Karow A, Nienhold R, et al. Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. *Blood*. 2014;123(14):2220-2228.

186. Klampfl T, Gisslinger H, Harutyunyan AS, et al. Somatic mutations of calreticulin in myeloproliferative neoplasms. *N Engl J Med*. 2013;369(25):2379-2390.

187. Passamonti F, Rumi E, Pietra D, et al. A prospective study of 338 patients with polycythemia vera: the impact of JAK2 (V617F) allele burden and leukocytosis on fibrotic or leukemic disease transformation and vascular complications. *Leukemia*. 2010;24(9): 1574-1579.

188. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. *Blood*. 2006;108(10):3472-3476.

189. Arber DA, Orazi A, Hasserjian R, et al. The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood*. 2016;127(20):2391-2405.

190. Jovanovic JV, Ivey A, Vannucchi AM, et al. Establishing optimal quantitative-polymerase chain reaction assays for routine diagnosis and tracking of minimal residual disease in JAK2-V617F-associated myeloproliferative neoplasms: a joint European LeukemiaNet/ MPN&MPNr-EuroNet (COST action BM0902) study. *Leukemia*. 2013;27(10):2032-2039.

191. Lange T, Edelmann A, Siebolts U, et al. JAK2 p.V617F allele burden in myeloproliferative neoplasms one month after allogeneic stem cell transplantation significantly predicts outcome and risk of relapse. *Haematologica*. 2013;98(5): 722-728.

192. Kjær L, Westman M, Hasselbalch Riley C, Høgdall E, Weis Bjerrum O, Hasselbalch H. A highly sensitive quantitative real-time PCR assay for determination of mutant JAK2 exon 12 allele burden. *PLoS One*. 2012;7(3):e33100.

193. Furtado LV, Weigelin HC, Elenitoba-Johnson KS, Betz BL. Detection of MPL mutations by a novel allele-specific PCR-based strategy. *J Mol Diagn*. 2013;15(6):810-818.

194. Verger E, Cassinat B, Chauveau A, et al. Clinical and molecular response to interferon-$\alpha$ therapy in essential thrombocythemia patients with CALR mutations. *Blood*. 2015;126(24):2585-2591.

195. Alchalby H, Badbaran A, Bock O, et al. Screening and monitoring of MPL W515L mutation with real-time PCR in patients with myelofibrosis undergoing allogeneic-SCT. *Bone Marrow Transplant*. 2010;45(9):1404-1407.

196. Baerlocher GM, Oppliger Leibundgut E, Ottmann OG, et al. Telomerase inhibitor imetelstat in patients with essential thrombocythemia. *N Engl J Med*. 2015;373(10):920-928.

197. Langabeer SE, Andrikovics H, Asp J, et al; MPN&MPNr-EuroNet. Molecular diagnostics of myeloproliferative neoplasms. *Eur J Haematol*. 2015;95(4):270-279.

198. Mansier O, Migeon M, Saint-Lézer A, et al. Quantification of the mutant CALR allelic burden by digital PCR: application to minimal residual disease evaluation after bone marrow transplantation. *J Mol Diagn*. 2016;18(1):68-74.

199. Abdelhamid E, Figeac M, Renneville A, et al. Quantification of JAK2V617F mutation by next-generation sequencing technology. *Am J Hematol*. 2013;88(6):536-537.

200. Ferrer-Marín F, Bellosillo B, Martínez-Avilés L, et al. Leukemic transformation driven by an ASXL1 mutation after a JAK2V617F-positive primary myelofibrosis: clonal evolution and hierarchy revealed by next-generation sequencing. *J Hematol Oncol*. 2013;6:68.

201. Fu Y, Schroeder T, Zabelina T, et al. Postallogeneic monitoring with molecular markers detected by pretransplant next-generation or Sanger sequencing predicts clinical relapse in patients with myelodysplastic/ myeloproliferative neoplasms. *Eur J Haematol*. 2014;92(3):189-194.