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REVIEW ARTICLE

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Realizing precision medicine in chronic lymphocytic leukemia: Remaining challenges and potential opportunities

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Abstract

Patients with chronic lymphocytic leukemia (CLL) exhibit diverse clinical outcomes. An expanding array of genetic tests is now employed to facilitate the identification of patients with high-risk disease and inform treatment decisions. These tests encompass molecular cytogenetic analysis, focusing on recurrent chromosomal alterations, particularly del(17p). Additionally, sequencing is utilized to identify *TP53* mutations and to determine the somatic hypermutation status of the immunoglobulin heavy variable gene. Concurrently, a swift advancement of targeted treatment has led to the implementation of novel strategies for patients with CLL, including kinase and BCL2 inhibitors. This review explores both current and emerging diagnostic tests aimed at identifying high-risk patients who should benefit from targeted therapies. We outline existing treatment paradigms, emphasizing the importance of matching the right treatment to the right patient beyond genetic stratification, considering the crucial balance between safety and efficacy. We also take into consideration the practical and logistical issues when choosing a management strategy for each individual patient. Furthermore, we delve into the mechanisms underlying therapy resistance and stress the relevance of monitoring measurable residual disease to guide treatment decisions. Finally, we underscore the necessity of aggregating real-world data, adopting a global perspective, and ensuring patient engagement. Taken together, we argue that precision medicine is not the mere application of precision diagnostics and accessibility of precision therapies in CLL but encompasses various aspects of the patient journey (e.g., lifestyle exposures and comorbidities) and their preferences toward achieving true personalized medicine for patients with CLL.

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INTRODUCTION

In recent years, chronic lymphocytic leukemia (CLL) has emerged as a paradigmatic disease where both patients and doctors entered the era of precision medicine (PM).[1] In this review, we first emphasize the essential role of precision diagnostics and prognostic/predictive tests and highlight how treatment can be tailored and monitored based on biomarkers (Figure 1). More importantly, we extend our concept of PM into broader terms, arguing that to achieve a personalized approach we need to include a thorough medical assessment to balance safety with efficacy and consider all practical and logistical issues, including patient's preferences and adherence. Finally, we conclude by highlighting the importance of generating real-world data (RWD) to advance the concept of PM and offer perspectives about the global dimensions of PM in CLL.

PRECISION DIAGNOSTICS AND PROGNOSTICATION

Diagnostic considerations

The diagnosis of CLL is usually straightforward, using immunophenotyping by flow cytometry to demonstrate the expression of CD5 and CD23 on a $CD19^+$ B-cell population with low levels of CD20 and monoclonal kappa or lambda light-chain expression. Expression of CD79B and surface immunoglobulin is characteristically weak.[2] All efforts should be made to rule out any other differential diagnosis when facing an atypical phenotype on a $CD5^+$ B-cell population, including additional phenotyping (CD43, CD200, and ROR1) or molecular work-up (*CCND1* or *BCL2* rearrangement), as a precise diagnosis is a prerequisite for the implementation of a PM approach.

Biomarker assessment before treatment initiation

After diagnosis, most patients will experience a treatment-free watch-and-wait period, ranging from a few months to decades.[3] If and when a patient meets the criteria for progressive disease according to the iwCLL guidelines, specific genetic biomarkers need to be assessed before starting treatment in order to inform treatment choice (Figure 1).[3] Among others, *TP53* aberrations and immunoglobulin heavy variable (IGHV) gene mutation status are the cornerstones and considered mandatory in both general practice and clinical trials.

TP53 aberrations

Evidence indicating shorter overall survival (OS) and poor therapy response in patients with *TP53* mutations and/or del(17p) has accumulated since the early 1990s, finally leading to the incorporation of their detection before each line of therapy in international guidelines.[3–5] Earlier studies employing Sanger sequencing and fluorescent in situ hybridization (FISH) identified *TP53* aberrations in 5%–10% of patients with CLL at diagnosis and 10%–15% at front-line treatment. While del(17p) typically co-occurs with *TP53* mutation on the other allele, they may also be present independently, with a sole *TP53* mutation being more common than a sole del(17p).[6]

Next-generation sequencing (NGS) uncovered *TP53* mutations below the detection limit of Sanger sequencing, corresponding to a variant allele frequency (VAF) of ~10%.[7,8] Such *TP53* micro-clones often expand during the disease course, positively selected by chemoimmunotherapy (CIT).[9] Responding to the need for method validation, the European Research Initiative on CLL (ERIC) has invested in

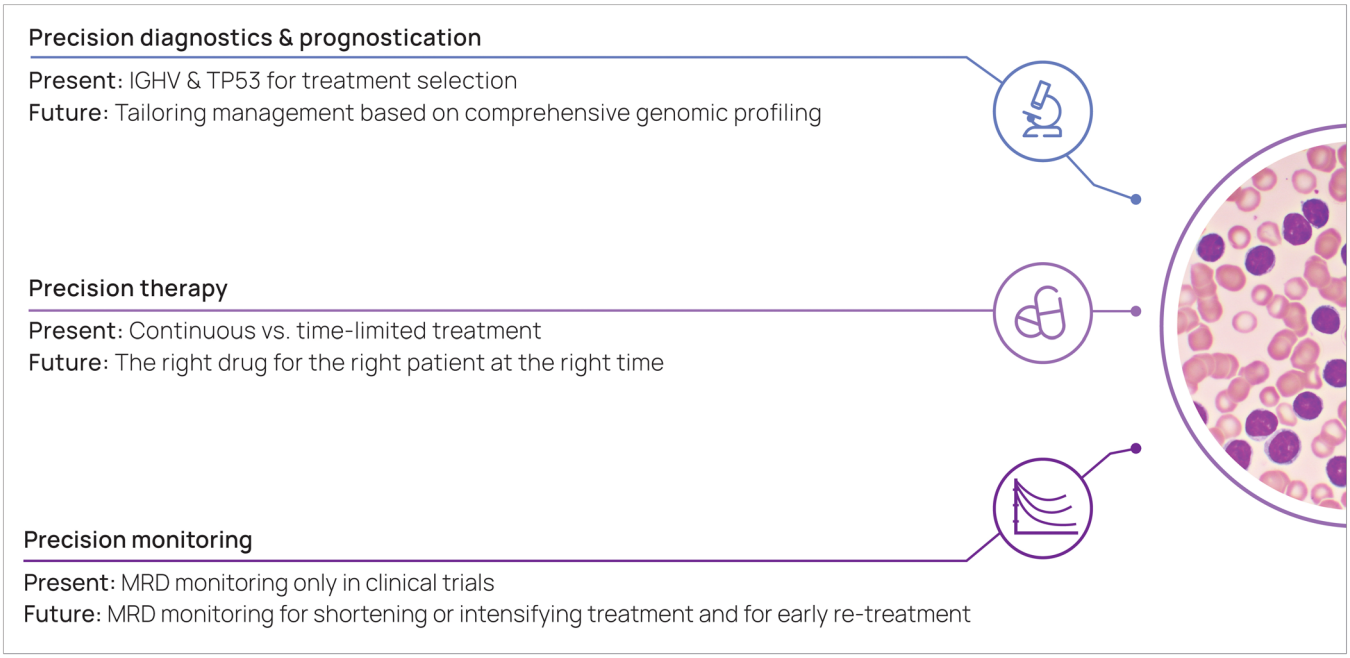

FIGURE 1 Core concepts of precision medicine in patients with chronic lymphocytic leukemia (CLL) involve precision diagnostics and prognostication as a basis for selecting targeted agents (precision therapy) and following treatment response at the individual patient level (precision monitoring).

offering guidance on *TP53* mutation screening, including by NGS,[10] while also running an external quality assessment scheme for laboratories performing the test and holding dedicated educational workshops.

The negative impact of *TP53* aberrations manifests mainly in limited sensitivity and consequent clonal expansion upon CIT, thus worsening patient prognosis. In contrast, both Bruton's tyrosine kinase inhibitors (BTKi) and BCL2 inhibitors (BCL2i) are effective in patients with *TP53* aberrations.[11] This fact challenged *TP53* alterations as independent prognostic and predictive markers, at least for patients receiving frontline treatment with kinase inhibitors. Nevertheless, they retain prognostic relevance in relapsed and refractory (R/R) patients even when treated with novel agents as well as when using fixed-duration regimens as frontline treatment.[12–14]

In terms of PM, not all *TP53* aberrations are functionally equal[15] and targeted therapies do not appear to favor clonal expansion as compared to CIT.[16] Hence, the challenge ahead involves defining which types and combinations of *TP53* alterations (e.g., mono-allelic versus bi-allelic versus multiple subclones) are predictive for tailoring targeted treatment and what is the relevance of *TP53* micro-clones. Evidently, this will require large-scale collaborative studies and harmonization of relevant methodologies, already pioneered by ERIC, combined with functional assays to better characterize the prevised functional impact of *TP53* aberrations, especially concerning the (rare) variants that are difficult to interpret.

IGHV gene analysis

In CLL, the somatic hypermutation (SHM) status of the IGHV gene can directly predict patient survival.[17,18] In more detail, patients with CLL cells carrying mutated IGHV genes (M-CLL, <98% identity to germline) generally follow a more indolent course than those with unmutated IGHV genes (U-CLL, ≥98% identity), who tend to show advanced disease, adverse cytogenetic features, and less favorable outcome, although the latter has changed dramatically, thanks to the advent of targeted therapies. Of note, IGHV gene SHM status remains stable during the clinical course, thus contrasting genomic aberrations which may change over time.[19] It is, therefore, unsurprising that current recommendations mandate that IG gene analysis is performed in all patients with CLL prior to frontline treatment, as the results from this test may have a profound impact on clinical decision-making.[3,20] In fact, in recent prospective clinical studies, chemo-free approaches proved to benefit U-CLL patients significantly more compared to CIT.[20–28]

The IG gene repertoire of CLL is nonrandom, culminating in the existence of subsets of cases with (quasi)identical or stereotyped B-cell receptor IG (BcR IG).[29–31] Accumulating evidence supports that major stereotyped subsets likely represent distinct molecular and clinical variants of CLL.[32,33] A prime example is stereotyped subset #2, expressing restricted IGHV3-21/IGLV3-21 BcR IG.[34] Subset #2 represents almost 8% of M-CLL requiring treatment and, in contrast to the remaining M-CLL cases, does not benefit from CIT,[35,36] that is still considered as a valid treatment option for M-CLL.[37]

IGHV gene analysis has traditionally been performed by combining PCR amplification and Sanger sequencing, but more recently started to be replaced by NGS-based assays.[38] ERIC has put effort into harmonizing IGHV gene analysis, ensuring that methodological recommendations by experts are regularly updated,[20,39,40] interested laboratories have access to an external quality assessment scheme, scientists involved in CLL diagnostics receive hands-on training in dedicated workshops, and an expert panel is available for online troubleshooting (www.ericll.org/ignetwork/).

In terms of PM, the forthcoming challenge involves defining whether other stereotypes might be predictive of differential responses to current or future treatments and, as highlighted by the case of subset #2, which other features may account for the heterogeneous outcomes of M-CLL patients.[37] As a concrete measure to address these issues, ERIC has recently launched two relevant studies, of which the first focuses on stereotyped subsets that are closely similar (satellites) to major subsets #1, #2, and #8, while the second aims to address the heterogeneity of M-CLL by detailed genomic and immunogenetic characterization.

Complex karyotype (CK)

FISH-detected recurrent chromosomal abnormalities, that is, deletion of 13q, 11q, 17p, and trisomy 12, are associated with different clinical outcomes, contributing to risk stratification and treatment decision-making.[4,41] More recently, accumulating evidence supports that karyotypic complexity, as measured either by chromosome banding analysis (CBA) or microarray analysis, is associated with worse outcomes in CLL.[42,43] A large study by ERIC found that a highly CK, defined as ≥5 abnormalities, was predictive of poor prognosis in patients with CLL treated with CIT.[42] A caveat is that CK-related studies were mostly retrospective and heterogeneous in methodologies. Moreover, it remains uncertain whether CK has an independent prognostic value as CK and *TP53* aberrations most often coexist.[44] That said, high CK has been associated with inferior outcomes both using BTKi and BCL2i treatment.[45,46] For instance, data obtained from the randomized GAIA/CLL13 study (NCT02950051), which excluded patients with *TP53* aberrations, confirmed an adverse impact of CK defined as ≥3 abnormalities with CIT, while only high CK (≥5 abnormalities) appeared to be a negative prognostic marker in case of time-limited BCL2i treatment.[22]

Key outstanding questions are whether CBA or array analysis holds similar prognostic power and the exact relevance of major structural chromosome abnormalities, such as translocations, but also certain numerical aberrations (e.g., multiple trisomies), in particular in relation to targeted drugs. To address these challenges, ERIC has recently launched a study to evaluate the predictive impact of CKs in patients undergoing targeted therapies. We therefore recommend only assessing CK in clinical trials but, for now, refrain from guiding treatment decisions based on CK in the real-world setting.[44]

Clinical impact of other genomic aberrations

Genome sequencing in CLL has unveiled numerous recurrent genetic aberrations, currently impacting >2000 genes.[47,48] There are a few more frequently mutated genes (*ATM*, *NOTCH1*, *SF3B1*, and *TP53*) followed by a long list of less commonly mutated genes, often occurring in <1%–5% of patients.[49,50] Recently, a large-scale sequencing study identified 202 drivers, including 109 new ones, encompassing point mutations, indels, and copy-number variants (CNVs).[51] Another recent investigation employing whole-genome sequencing (WGS) revealed 56 recurrent driver alterations, of which 33 were affected by CNVs and noncoding mutations in regulatory elements.[52]

Today, more than 50 genetic aberrations have been linked to disease outcomes in CLL.[19,53] The great majority have been associated with shorter time-to-first-treatment (TTFT) and OS. Interestingly, the genomic landscapes seem to differ in M-CLL and U-CLL.[51] While *SF3B1* and *XPO1* mutations appear to be strong independent prognostic factors in both U-CLL and M-CLL, alterations of *TP53*,

BIRC3, and *EGR2* only impact U-CLL patients, and *NOTCH1* and *NFKBIE* mutations predominantly affect M-CLL patients.[53] These findings emphasize the need for a compartmentalized approach, considering genetic aberrations in the context of IGHV mutation status to identify high-risk patients.

Today, besides *TP53*, no other gene mutation is recommended for routine diagnostics. More research needs to be performed, and, particularly, patients with the more common mutations should start being stratified in the context of prospective clinical trials to answer this important aspect of PM in CLL.

Beyond genomics

In recent years, genomic analysis has emerged as key to understanding CLL pathogenesis and stratifying patients. That said, genomics represents only one layer of the multilayer biology of CLL cells. Evidently, therefore, much remains to be revealed from the deeper investigation of other layers, for example, the epigenome and the proteome. Particularly regarding the former, ample evidence supports that key epigenetic features such as the DNA methylation profile can discriminate patients with CLL with markedly different prognoses and outcomes.[54–58] As for the latter, pioneering proteogenomic studies have offered relevant proof-of-principle, for example, by identifying a novel subtype of patients with poor prognosis associated with aberrant BcR signaling.[59]

Despite the undisputed biological and prognostic significance of the novel information acquired through the aforementioned studies, translation to the clinic is yet to be achieved. Hence, the challenge ahead lies in developing and validating laboratory protocols ready for routine clinical application as well as dedicated software that would allow integrative multiomics analysis toward refining patient stratification and advancing the prospects of PM in CLL.

PRECISION THERAPY AND CLINICAL DECISION-MAKING IN CLL

Tailoring treatment based on biomarkers

In CLL, the need exists for biomarkers that can help discriminate between patients who will experience a stable disease with no treatment requirement during their lifetime from those who will eventually progress and need to be treated (Figure 1). This would allow tailoring the management of patients at the time of diagnosis, sparing unnecessary visits and tests let alone the psychological burden for them and their carers. However, the possibility of applying biomarker-based information to the individual patient is hampered by the fact that the association, for example, between IGHV gene SHM status and clinical outcome, only reaches 80% concordance.

In contrast, one of the main successes of PM in CLL is the possibility to utilize distinct disease features (predictive biomarkers) that indicate how likely a patient is to benefit from a specific treatment, thus providing valuable information for patient stratification (Figure 1), as exemplified by *TP53* aberrations and the IGHV gene SHM status, both of which should be assessed before the start of treatment[3,60,61] Finally, on- and posttreatment biomarkers, in particular measurable residual disease (MRD) status, allows a refined prediction of the outcome but only after treatment and beyond known pretreatment characteristics.[62,63]

Nowadays, the treatment of CLL can be tailored in two ways: (i) choice of treatment, for example, selecting whether to use CIT versus targeted therapy (i.e., BTKi and venetoclax containing regimens; Table 1) or continuous versus time-limited targeted therapy: this should be based on combining tumor- and host-related features, such as comorbidities and polypharmacy, availability of treatment options, as well as patients' preferences; and (ii) the duration (fixed versus response-adapted) of a chosen treatment regimen, for example, based on depth of MRD levels, to further improve treatment outcomes. While the former is already part of routine CLL management, the latter is still under exploration in clinical trials and has yet to enter routine healthcare.

Tailoring treatment decisions by balancing safety and efficacy

Indications for treatment in patients with CLL continue to rely exclusively on the occurrence of active and/or symptomatic disease.[3] In the past, no clear benefit for early initiation in asymptomatic patients was demonstrated with CIT.[64–66] More recent attempts to improve the outcome of patients deemed at higher risk of CLL progression by starting treatment with targeted agents earlier have not produced a tangible OS benefit either.[67]

Once the need for treatment is established, surveys have shown that patients with CLL pay more attention to the occurrence of adverse events rather than to survival advantages when discussing therapy initiation.[68] Efficacy and safety are strongly dependent upon patients' situation at the time of treatment decision, and their balance should be a priority for personalized approaches, especially since patients with CLL are elderly with a life expectancy that might be dependent on other concomitant diseases rather than CLL itself.

When balancing efficacy and safety between BTKi and BCL2i, both the clinical presentation and the biological background must be considered. Long-term BTKis are often preferred in the presence of *TP53* alterations (often associated with CK),[69] but the possibility of cardiac toxicity (arrhythmias, hypertension, and heart failure) suggests caution in patients with a history of cardiovascular disease or ill-controlled hypertension.[70] Second-generation BTKis are better tolerated,[71] but cardiac toxicity (particularly hypertension) is not erased, and rare cases of unexplained sudden deaths still occur. Bleeding risk precludes the use of BTKis with concomitant double antiplatelet therapy, and careful follow-up is mandatory in case of concomitant administration of one antiplatelet or anticoagulant.[72] There are other milder adverse events, such as cutaneous, musculoskeletal, or digestive, that might lead to discontinuation if not taken care of.[73] Hence, adherence becomes crucial, particularly considering oral targeted therapies where patients are more independent in taking the drug. Even time-limited treatments span over a year or even longer, thus personalization of therapy entails providing accurate information to each patient according to their expectations and their way of living.

The combination of the BCL2i venetoclax and the anti-CD20 antibody obinutuzumab is also highly effective and well tolerated, even in unfit patients with comorbidities, but should be used with caution in case of compromised renal function considering the risk of

TABLE 1 Approved targeted agent-based first-line treatments in chronic lymphocytic leukemia (CLL).

Continuous	Fixed-duration
Ibrutinib ± anti-CD20 monoclonal antibody	Venetoclax + obinutuzumab
Acalabrutinib ± obinutuzumab	Ibrutinib + venetoclax
Zanubrutinib	

tumor lysis syndrome for both agents.[74] The risk of tumor lysis syndrome is limited if the well-established rules of administration, dose adaptation, and biological surveillance are followed. Its efficacy is optimal in M-CLL cases.[21] It is also preferred in rapidly progressive, nonbulky CLL, and its tolerance and time-limited administration are favorable for patients with comorbidities.[75] Neutropenia is frequent in the first months of treatment but does not lead to an elevated risk of severe infections.[76]

The combination of venetoclax and BTKi combines the toxicity of each agent which appears to be counterbalanced by an enhanced efficacy and a time limited dosing.[77] However, it is still too early for the evaluation of long-term toxicity. "Triplet" therapy (BCL2i, BTKi, anti-CD20) is effective but leads to excess toxicity in unfit patients.[22,78]

PRECISION MONITORING IN CLL

Development of therapy resistance

In a significant fraction of patients treated with pathway inhibitors, therapy resistance may be acquired by the emergence of mutations in genes that are directly targeted by the drugs or belong to the targeted pathway. Resistance to covalent BTKi is associated with point mutations targeting the *BTK* gene in the cysteine residue (C481) of the kinase domain, thus preventing the drug from binding covalently to BTK.[79,80] Importantly, noncovalent BTK inhibitors can overcome the resistance conferred by C481 mutations.[81] While mutations at other sites of the *BTK* gene have also been reported,[82,83] the predictive value of low-VAF *BTK* mutations in patients clinically responding to BTKi is still a matter of research.[84] In addition, resistance can be caused by gain-of-function *PLCG2* mutations that lead to constitutive activation of BcR signaling downstream to BTK.[80,85] Nonetheless, *BTK* and *PLCG2* gene mutations do not explain resistance to BTKi in all cases.[16,86] In fact, ~30% of patients with CLL relapsing on ibrutinib do not carry such mutations even when investigated with droplet digital PCR (ddPCR).[16] Alternative mechanisms, including *EGR2*, *BIRC3* and *NFKBIE* mutations as well as del(8p), might cooperate in promoting BTKi resistance.[16]

The molecular mechanisms of venetoclax resistance include mutations of the *BCL2* gene.[87–89] The most common mutation is G101V, which falls in the BH3-binding groove and causes a marked reduction in the BCL2 affinity for venetoclax, preventing the drug from displacing proapoptotic BH-3 only proteins (e.g., BIM). The novel BCL2i sonrotoclax appears to have the potential to overcome venetoclax resistance in preclinical models and is currently being tested in clinical trials.[90] Because *BCL2* mutations are restricted to a subset of venetoclax-refractory patients, the involvement of other molecular mechanisms of resistance is a matter of active research.[91] Importantly, *BCL2* mutations (as well as *BTK* and *PLCG2*) are absent in CLL relapsing after first-line treatment with fixed-duration ibrutinib plus venetoclax, suggesting that treatment duration has an impact on the acquisition of these mutations, although limited numbers of patients have been evaluated to date.[92]

Current guidelines do not recommend regular monitoring of resistance mutations during treatment with pathway inhibitors or the use of mutation testing for making decisions in clinical practice outside of clinical trials. Considering the expanding therapeutic landscape of CLL, however, resistance mutations may eventually become an important set of biomarkers for PM management of the disease. Toward this aim, a more precise definition of resistance along with standardization of testing technologies (e.g., gene panel deep-sequencing or ddPCR analysis) as well as harmonization of interpretation of the results across laboratories will be a prerequisite. In parallel, the inclusion of resistance mutation testing in clinical trials with pathway inhibitors is highly desirable.

MRD detection

MRD is an essential tool for determining treatment response in clinical trials and is often a primary endpoint in hypothesis-generating trials and a secondary endpoint in registration trials (Figure 1). MRD assessment is not yet applied in routine practice but may be used to provide supportive information for monitoring patients in remission after treatment. The iwCLL guidelines have established $0.01\%/10^{-4}$, that is, 1 CLL cell in 10.000 leukocytes, as an appropriate threshold for the assessment of MRD in CLL.[3] However, other thresholds may hold significance in specific contexts. For instance, a threshold of $>1\%/10^{-2}$ is important for identifying individuals at risk of early relapse or progression, while thresholds of $<0.001\%/10^{-5}$ or $<0.0001\%/10^{-6}$ are relevant for evaluating disease eradication.[13,62,93]

Flow cytometry can be used to identify CLL cells using a core set of 6 markers, as proposed by ERIC,[94] in a single tube, to enable a detection limit of $0.001\%/10^{-5}$. Flow cytometry MRD is relatively quick, simple, cost-effective, and reproducible but has the disadvantage of requiring a high number of cells (~7-fold more than molecular approaches for equivalent detection limit) that need to be analyzed fresh (<48 h from collection).[94–96]

IG real-time quantitative PCR (RQ-PCR) or ddPCR are also sensitive and well-validated approaches suitable for identifying residual disease to a limit of detection (LOD) of $0.001\%/10^{-5}$. The assay requires design of primers specific to the clonotypic IGH gene rearrangement in each patient, thus pretreatment disease material is required for assay design, and the detection limit can vary between patients. NGS approaches for MRD detection in CLL instead use approaches targeting rearranged IGH (± immunoglobulin kappa [IGK]/immunoglobulin lambda [IGL]) sequences enabling to detect MRD at the $0.0001\%/10^{-6}$ level.[97–99] To date, only one commercial approach incorporating such calibration has been authorized by the Food and Drug Administration (FDA),[100] while more recently, an academic assay has been published using primers targeting the IGHV-leader sequence, which allows complete characterization of the IGHV sequence.[97]

MRD-guided therapy

MRD is affected both by the type (CIT vs. targeted therapy) and duration (continuous vs. time-limited) of treatment as well as by biological features such as the presence of high-risk genomic alterations. MRD status has been found to correlate with PFS or even OS in most time-limited combination regimens, including CIT and targeted therapy.[21,101,102] Based on the strong prognostic impact of the MRD status, it can be a key enabler of precision therapy in CLL (Figure 1). Several phase 2 studies have demonstrated that treatment duration and intensity can be modulated based on peripheral blood (PB) and/or bone marrow (BM) assessments and most commonly with thresholds of 10^{-4} and with flow cytometry.[103–105] Additionally, some studies implement integrated assessments of clinical response and MRD, for example, by requiring reaching a complete remission as per iwCLL in addition to BM and PB MRD $<10^{-4}$.[106,107] However, this plethora of phase 2 studies can only serve as a proof-of-principle and is insufficient to change current practice. Several key uncertainties need to be overcome to establish the clinical benefit of MRD guidance. First, the method of measuring MRD requires standardization. While the international efforts by ERIC have led to standardized analysis of MRD by flow cytometry, regulatory bodies commonly also require standardization of each assay component, which so far has not been achieved. Second, a consensus on the most

appropriate MRD cutoff is required to establish safe MRD guidance; currently, most studies have identified a threshold of 10^{-4} as the most feasible and still highly prognostic cutoff. However, a fraction of patients show MRD $<10^{-4}$ and still detectable disease in BM, which can potentially be overcome by an MRD cutoff of 10^{-5} in PB.[108] Importantly, while highly sensitive NGS-based assays can call levels down to 10^{-6}, the added prognostic information of these very deep responses is not yet clear.[62,109] Finally, to definitively establish the clinical benefit of MRD-guided precision treatment of CLL, prospective randomized comparisons are warranted. So far, only the FLAIR study has compared an MRD-guided treatment to a fixed-duration treatment, but since MRD-guided targeted treatment with ibrutinib plus venetoclax was compared to fixed-duration FCR, the contribution of the MRD-guidance is difficult to isolate.[24] Hence, the comparison to the continuous ibrutinib arm in the FLAIR protocol (NCT00614315) as well as ongoing or planned studies like CLL18 (venetoclax-obinutuzumab vs. pirtobrutinib-venetoclax vs. MRD-guided pirtorutinib-venetoclax) or RESOLVE (MRD-guided shortening vs. standard venetoclax-obinutuzumab or venetoclax-ibrutinib) will likely pave the way toward understanding the clinical benefit of MRD-guided treatment of CLL.

RWD AGGREGATION

While randomized controlled trials (RCT) remain the cornerstone of generating evidence in medicine, real-world evidence (RWE) can complement RCTs and provide profound insights into improving the quality and delivery of services in medical care, becoming crucial to evaluate personalized approaches in diseases like CLL (Figure 2). Although lacking key attributes of RCTs, real-world studies can include large cohorts of patients with long follow-ups and better portray the reality of everyday clinical practice. To this point, real-world studies have revealed the clinical importance of several biomarkers in CLL (e.g., CK and BcR stereotypy), helped us understand the adherence to guidelines in terms of biomarker testing, and gave insights into long-term complications.[42,110–112] In the era of targeted agents, RWE gave us insights into the tolerability and effectiveness of these drugs while also revealing the unmet need for effective treatments in patients relapsing after receiving both BTKi and venetoclax-based treatments.[113,114]

Another advantage of RWE is the faster preparatory phase. Swiftly conducted real-world studies by ERIC and others during the COVID-19 pandemic exemplify this potential, providing useful information on the outcomes of patients with CLL infected with SARS-COV-2 and their responses to COVID-19 vaccines.[115–121] Last but not least, RWD has also been used in more elaborate approaches involving artificial intelligence (AI). Indicatively, Agius et al. have reported a machine-learning approach for identifying patients with a high risk of infection, underlining the myriad possibilities of utilizing RWD toward the realization of PM.[122] AI and machine learning approaches are the way forward in incorporating a large amount of complex RWD data to make more refined predictions for our patients. However, data quality and methodology of real-world studies remain the cornerstone even in the presence of these powerful tools.

ERIC has recognized the importance of RWD in CLL and designed the ERIC CLL database, which represents an ongoing effort to answer relevant clinical questions through the collection of RWD on a project basis.[123] ERIC has also provided solutions for RWD collection, management, and analysis, ranging from offering technical support to working toward a common data model in CLL and exploring analyses using federated learning approaches.

WHAT DOES PM MEAN TO PATIENTS?

Although the goal of PM can be easily explained in layman's terms: "Provide the right treatment in the right dose to the right patient at the right time," the complexity of what patients and carers need to be able

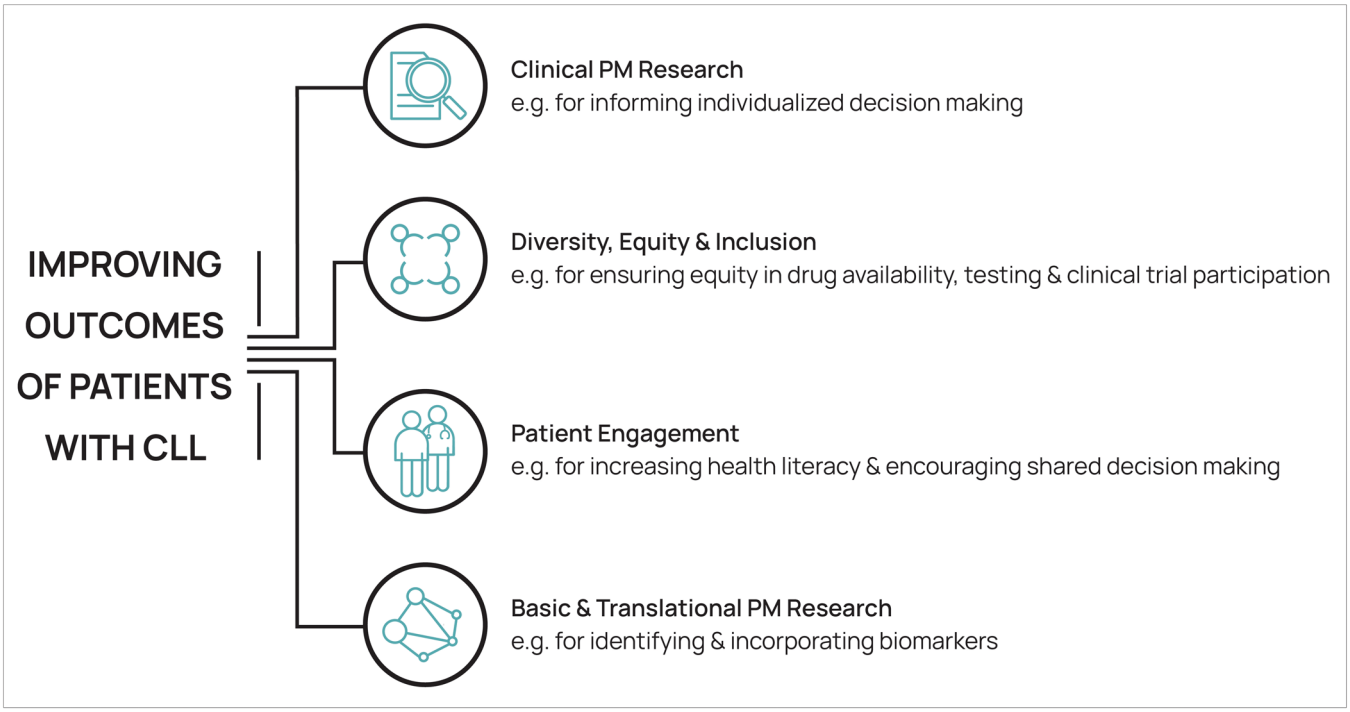

FIGURE 2 Key areas to improve patient outcomes and ensure equal and resource-efficient implementation of PM in CLL. CLL, chronic lymphocytic leukemia; PM, precision medicine.

to know and understand can be confusing and often overwhelming. PM introduces highly specialized concepts, such as genes, genomic testing, and targeted therapy, that are unknown to the general public. With this in mind, ERIC has produced printed/online material to allow easy access to this information to the large public, including affected individuals (https://ericll.org/for-patients/people-with-cll/).

Even after understanding the basic concepts of PM, patients and their carers will have to deal in practice with the various tests, procedures, and decisions that it entails. They need to be able to navigate through this experience by being empowered to ask questions and make informed decisions within the context of a specialized care team. Managing expectations and the various emotions that may arise during this time is also important and can affect decision-making and overall outcomes. Research implemented by ERIC confirms that coping with feelings of sadness, grief, anxiety, but also hope, determination and resolve, needs to be supported through discussions with the healthcare team, support groups and patient advocacy groups.[124,125] Moreover, ERIC has developed multimedia content on various complex medical topics for various audiences (cllempowerment.com).

Patients and their families may have concerns regarding PM in terms of privacy, security, availability, equity, and ethics. Since targeted therapies are generally more costly than traditional treatment options, issues over insurance coverage and socioeconomic inequalities need to be addressed. Patients may also worry about genetic discrimination based on the genomic testing results, spanning from long-term care provision to insurance and employment discriminatory measures from companies and employers in the future. The perceived lack of privacy can be alleviated by communicating the strict regulatory framework, robust privacy preserving, and security measures encompassing genetic testing.

The CLL scientific community needs to concentrate efforts on the following: (i) the education of patients and carers about PM and their empowerment with regards to rights and benefits as well as their familiarization of the regulatory framework around PM (Figure 2), (ii) support to patients and carers in coping and resilience before, during and after their involvement in PM experiences, and (iii) education of healthcare professionals (HCP) on the introduction of targeted treatment approaches and how to convey the information to patients. An important aspect of this could be the development and consensus of a clear and consistent vocabulary for PM to allow oncology specialists, HCPs, patients, and carers to speak a common language.

FINAL CONSIDERATIONS

Some of the promise of PM for CLL has already been realized. Indeed, PM concepts are routinely applied to establish a correct diagnosis (precision diagnostics), decide about the treatment among different therapeutic options (precision therapy), and assess response (precision monitoring) (Figure 1). At the same time, basic and translational research is gradually identifying new potential biomarkers and druggable targets to further improve and differentiate PM approaches. In fact, the emerging concept of functional PM, where genomics data is combined with ex vivo drug sensitivity testing to identify treatment targets and guide treatment decisions, has been applied in a few clinical trials in acute leukemia with promising results[126,127] and more recently in a drug repurposing study including CLL (NCT04817956). Against this progress, however, major challenges remain to be addressed.

First, considering the immense clinicobiological heterogeneity of CLL, global efforts are warranted toward amassing huge and longitudinal cohorts. ERIC contributes to this endeavor in tangible ways: a prime example is the ERIC ImMunoGeneTics (IMGT)/CLL-DB database,[128] a joint initiative between ERIC and IMGT currently including immunogenetic information from ~70,000 patients with CLL attended in 51 different institutions from 27 different countries in four different continents. In a similar vein, related ERIC initiatives are underway (e.g., the ERIC *TP53* database).

An even bigger challenge concerns the lack of diversity in populations involved in CLL research. Indicatively, ~90% of cases in the ERIC IMGT/CLL-DB originate from Europe and the United States, meaning that Caucasians represent the overwhelming majority. This may reflect the lower incidence and prevalence of CLL in certain parts of the world (most notably the Far East) but also less access to testing both between high versus middle/low-income countries and within high-income countries.[129] This is just one of the many factors exacerbating health inequity for patients with CLL throughout the world (Figure 2). Even more important is the limited or no access to innovative medicines in many geographic areas, preventing the application of PM and still forcing the use of CIT for many patients.[130] This fact should prompt an intense discussion into the causes of inequity in the care of CLL. Unless this happens, PM would regrettably come to represent an option for a small proportion of patients with CLL living in a few wealthy nations while remaining a dream for the great majority of those with fewer resources.

In conclusion, as for any disease, PM for CLL should rely not only on biological information but also on clinical phenotypes, exposures, and lifestyle data. Currently, this is largely overlooked in CLL, where (multi)omics are commonly misunderstood as the key to realizing PM whereas it is simply one piece of the puzzle of PM for CLL. Integration of various data sources, however, represents another major challenge as electronic health records are still largely unavailable, databases and registries are not always interoperable, and the use of wearables varies considerably. Hence, there is an urgent need to reappraise how we conceptualize and practice PM in CLL, including how we strive for inclusivity in research and equity in access to testing and medications.

AUTHOR CONTRIBUTIONS

Kostas Stamatopoulos: Conceptualization (equal); writing—original draft preparation (equal); writing—review and editing (equal). **Sarka Pavlova**: Writing—original draft preparation (equal); writing—review and editing (equal). **Othman Al-Sawaf**: Writing—original draft preparation (equal); writing—review and editing (equal). **Thomas Chatzikonstantinou**: Writing—original draft preparation (equal); writing—review and editing (equal). **Christina Karamanidou**: Writing—original draft preparation (equal); writing—review and editing (equal). **Gianluca Gaidano**: Writing—original draft preparation (equal); writing—review and editing (equal). **Florence Cymbalista**: Writing—original draft preparation (equal); writing—review and editing (equal). **Arnon P. Kater**: Writing—original draft preparation (equal); writing—review and editing (equal). **Andy Rawstron**: Writing—original draft preparation (equal); writing—review and editing (equal). **Lydia Scarfò**: Writing—original draft preparation (equal); writing—review and editing (equal). **Paolo Ghia**: Conceptualization (equal); writing—original draft preparation (equal); writing—review and editing (equal). **Richard Rosenquist**: Conceptualization (equal); writing—original draft preparation (equal); writing—review and editing (equal).

CONFLICT OF INTEREST STATEMENT

Kostas Stamatopoulos has received honoraria from AbbVie, AstraZeneca, Janssen, Lilly and BMS and research support from AbbVie, AstraZeneca, Janssen. Othman Al-Sawaf has received honoraria from AbbVie, Adaptive, AstraZeneca, Ascentage, BeiGene, Gilead, Eli Lilly, Janssen, Roche, and research funding from AbbVie, BeiGene, Janssen and Roche. Gianluca Gaidano has received honoraria from Abbvie, AstraZeneca, BeiGene, Hikma, Incyte, Janssen, and Lilly. Florence Cymbalista has performed advisory board activities for AstraZeneca, Lilly, AbbVie, and

BeiGene. Arnon P. Kater reports research grants from AbbVie, Astra Zeneca, BMS, Janssen, and Roche Genentech and has performed advisory board activities for AbbVie, Astra Zeneca, BMS, Janssen, LAVA, and Roche Genentech. Lydia Scarfò has received honoraria from AbbVie, AstraZeneca, BeiGene, Janssen, and Lilly was part of the speakers bureau of Octapharma. Paolo Ghia has received honoraria from AbbVie, AstraZeneca, BeiGene, BMS, Galapagos, Janssen, LoxoOncology@Lilly, MSD, Roche; research support from AbbVie, AstraZeneca, BMS, Janssen, and is an Editor of HemaSphere. Thomas Chatzikonstantinou has received honoraria from AbbVie. Richard Rosenquist has received honoraria from AbbVie, AstraZeneca, Janssen, Illumina, and Roche. The remaining authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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REFERENCES

1. Mollstedt J, Mansouri L, Rosenquist R. Precision diagnostics in chronic lymphocytic leukemia: past, present and future. *Front Oncol*. 2023;13:1146486. doi:10.3389/fonc.2023.1146486
2. Alaggio R, Amador C, Anagnostopoulos I, et al. The 5th edition of the World Health Organization classification of haematolymphoid tumours: lymphoid neoplasms. *Leukemia*. 2022;36(7):1720-1748. doi:10.1038/s41375-022-01620-2
3. Hallek M, Cheson BD, Catovsky D, et al. iwCLL guidelines for diagnosis, indications for treatment, response assessment, and supportive management of CLL. *Blood*. 2018;131(25):2745-2760. doi:10.1182/blood-2017-09-806398
4. Döhner H, Stilgenbauer S, Benner A, et al. Genomic aberrations and survival in chronic lymphocytic leukemia. *N Engl J Med*. 2000;343(26):1910-1916. doi:10.1056/NEJM200012283432602
5. Stilgenbauer S, Schnaiter A, Paschka P, et al. Gene mutations and treatment outcome in chronic lymphocytic leukemia: results from the CLL8 trial. *Blood*. 2014;123(21):3247-3254. doi:10.1182/blood-2014-01-546150
6. Malcikova J, Tausch E, Rossi D, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. *Leukemia*. 2018;32(5):1070-1080. doi:10.1038/s41375-017-0007-7
7. Malcikova J, Pavlova S, Kunt Vonkova B, et al. Low-burden TP53 mutations in CLL: clinical impact and clonal evolution within the context of different treatment options. *Blood*. 2021;138(25):2670-2685. doi:10.1182/blood.2020009530
8. Rossi D, Khiabanian H, Spina V, et al. Clinical impact of small TP53 mutated subclones in chronic lymphocytic leukemia. *Blood*. 2014;123(14):2139-2147. doi:10.1182/blood-2013-11-539726
9. Lazarian G, Cymbalista F, Baran-Marszak F. Impact of low-burden TP53 mutations in the management of CLL. *Front Oncol*. 2022;12:841630. doi:10.3389/fonc.2022.841630
10. Malcikova J, Pavlova S, Baliakas P, et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-2024 update. *Leukemia*. 2024. Published online May 16, 2024. doi:10.1038/s41375-024-02267-x
11. Woyach JA, Perez Burbano G, Ruppert AS, et al. Follow-up from the A041202 study shows continued efficacy of ibrutinib regimens for older adults with CLL. *Blood*. 2024;143:1616-1627. doi:10.1182/blood.2023021959
12. Byrd JC, Furman RR, Coutre SE, et al. Ibrutinib treatment for first-line and relapsed/refractory chronic lymphocytic leukemia: final analysis of the pivotal phase Ib/II PCYC-1102 study. *Clin Cancer Res*. 2020;26(15):3918-3927. doi:10.1158/1078-0432.CCR-19-2856
13. Kater AP, Wu JQ, Kipps T, et al. Venetoclax plus rituximab in relapsed chronic lymphocytic leukemia: 4-year results and evaluation of impact of genomic complexity and gene mutations from the MURANO phase III Study. *J Clin Oncol*. 2020;38(34):4042-4054. doi:10.1200/JCO.20.00948
14. Tausch E, Schneider C, Robrecht S, et al. Prognostic and predictive impact of genetic markers in patients with CLL treated with obinutuzumab and venetoclax. *Blood*. 2020;135(26):2402-2412. doi:10.1182/blood.2019004492
15. Kennedy MC, Lowe SW. Mutant p53: it's not all one and the same. *Cell Death Differ*. 2022;29(5):983-987. doi:10.1038/s41418-022-00989-y
16. Bonfiglio S, Sutton LA, Ljungström V, et al. BTK and PLCG2 remain unmutated in one-third of patients with CLL relapsing on ibrutinib. *Blood Adv*. 2023;7(12):2794-2806. doi:10.1182/bloodadvances.2022008821
17. Damle RN, Wasil T, Fais F, et al. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. *Blood*. 1999;94(6):1840-1847. doi:10.1182/blood.V94.6.1840
18. Hamblin TJ, Davis Z, Gardiner A, Oscier DG, Stevenson FK. Unmutated Ig V(H) genes are associated with a more aggressive form of chronic lymphocytic leukemia. *Blood*. 1999;94(6):1848-1854. doi:10.1182/blood.V94.6.1848
19. Baliakas P, Hadzidimitriou A, Sutton LA, et al. Recurrent mutations refine prognosis in chronic lymphocytic leukemia. *Leukemia*. 2015;29(2):329-336. doi:10.1038/leu.2014.196
20. Agathangelidis A, Chatzidimitriou A, Chatzikonstantinou T, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: the 2022 update of the recommendations by ERIC, the European Research Initiative on CLL. *Leukemia*. 2022;36(8):1961-1968. doi:10.1038/s41375-022-01604-2
21. Al-Sawaf O, Zhang C, Jin HY, et al. Transcriptomic profiles and 5-year results from the randomized CLL14 study of venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab in chronic lymphocytic leukemia. *Nat Commun*. 2023;14(1):2147. doi:10.1038/s41467-023-37648-w
22. Eichhorst B, Niemann CU, Kater AP, et al. First-line venetoclax combinations in chronic lymphocytic leukemia. *N Engl J Med*. 2023;388(19):1739-1754. doi:10.1056/NEJMoa2213093

23. Kater AP, Owen C, Moreno C, et al. Fixed-duration ibrutinib-venetoclax in patients with chronic lymphocytic leukemia and co-morbidities. *NEJM Evid*. 2022;1(7):EVIDoa2200006. doi:10.1056/EVIDoa2200006
24. Munir T, Cairns DA, Bloor A, et al. Chronic lymphocytic leukemia therapy guided by measurable residual disease. *N Engl J Med*. 2024;390(4):326-337. doi:10.1056/NEJMoa2310063
25. Shanafelt TD, Wang XV, Kay NE, et al. Ibrutinib-rituximab or chemoimmunotherapy for chronic lymphocytic leukemia. *N Engl J Med*. 2019;381(5):432-443. doi:10.1056/NEJMoa1817073
26. Sharman JP, Egyed M, Jurczak W, et al. Efficacy and safety in a 4-year follow-up of the ELEVATE-TN study comparing acalabrutinib with or without obinutuzumab versus obinutuzumab plus chlorambucil in treatment-naïve chronic lymphocytic leukemia. *Leukemia*. 2022;36(4):1171-1175. doi:10.1038/s41375-021-01485-x
27. Tam CS, Brown JR, Kahl BS, et al. Zanubrutinib versus bendamustine and rituximab in untreated chronic lymphocytic leukaemia and small lymphocytic lymphoma (SEQUOIA): a randomised, controlled, phase 3 trial. *Lancet Oncol*. 2022;23(8):1031-1043. doi:10.1016/S1470-2045(22)00293-5
28. Burger JA, Barr PM, Robak T, et al. Long-term efficacy and safety of first-line ibrutinib treatment for patients with CLL/SLL: 5 years of follow-up from the phase 3 RESONATE-2 study. *Leukemia*. 2020;34(3):787-798. doi:10.1038/s41375-019-0602-x
29. Agathangelidis A, Chatzidimitriou A, Gemenetzi K, et al. Higher-order connections between stereotyped subsets: implications for improved patient classification in CLL. *Blood*. 2021;137(10):1365-1376. doi:10.1182/blood.2020007039
30. Agathangelidis A, Darzentas N, Hadzidimitriou A, et al. Stereotyped B-cell receptors in one-third of chronic lymphocytic leukemia: a molecular classification with implications for targeted therapies. *Blood*. 2012;119(19):4467-4475. doi:10.1182/blood-2011-11-393694
31. Messmer BT, Albesiano E, Efremov DG, et al. Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. *J Exp Med*. 2004;200(4):519-525. doi:10.1084/jem.20040544
32. Agathangelidis A, Chatzikonstantinou T, Stamatopoulos K. B cell receptor immunoglobulin stereotypy in chronic lymphocytic leukemia: key to understanding disease biology and stratifying patients. *Sem Hematol*. 2024;61:91-99. doi:10.1053/j.seminhematol.2023.12.005
33. Stamatopoulos K, Agathangelidis A, Rosenquist R, Ghia P. Antigen receptor stereotypy in chronic lymphocytic leukemia. *Leukemia*. 2017;31(2):282-291. doi:10.1038/leu.2016.322
34. Tobin G, Thunberg U, Johnson A, et al. Chronic lymphocytic leukemias utilizing the VH3-21 gene display highly restricted Vλ2-14 gene use and homologous CDR3s: implicating recognition of a common antigen epitope. *Blood*. 2003;101(12):4952-4957. doi:10.1182/blood-2002-11-3485
35. Baliakas P, Agathangelidis A, Hadzidimitriou A, et al. Not all IGHV3-21 chronic lymphocytic leukemias are equal: prognostic considerations. *Blood*. 2015;125(5):856-859. doi:10.1182/blood-2014-09-600874
36. Jaramillo S, Agathangelidis A, Schneider C, et al. Prognostic impact of prevalent chronic lymphocytic leukemia stereotyped subsets: analysis within prospective clinical trials of the German CLL Study Group (GCLLSG). *Haematologica*. 2020;105(11):2598-2607. doi:10.3324/haematol.2019.231027
37. Thompson PA, Bazinet A, Wierda WG, et al. Sustained remissions in CLL after frontline FCR treatment with very-long-term follow-up. *Blood*. 2023;142(21):1784-1788. doi:10.1182/blood.2023020158
38. Davi F, Langerak AW, de Septenville AL, et al. Immunoglobulin gene analysis in chronic lymphocytic leukemia in the era of next generation sequencing. *Leukemia*. 2020;34(10):2545-2551. doi:10.1038/s41375-020-0923-9
39. Ghia P, Stamatopoulos K, Belessi C, et al. ERIC recommendations on IGHV gene mutational status analysis in chronic lymphocytic leukemia. *Leukemia*. 2007;21(1):1-3. doi:10.1038/sj.leu.2404457
40. Rosenquist R, Ghia P, Hadzidimitriou A, et al. Immunoglobulin gene sequence analysis in chronic lymphocytic leukemia: updated ERIC recommendations. *Leukemia*. 2017;31(7):1477-1481. doi:10.1038/leu.2017.125
41. Juliusson G, Oscier DG, Fitchett M, et al. Prognostic subgroups in B-cell chronic lymphocytic leukemia defined by specific chromosomal abnormalities. *N Engl J Med*. 1990;323(11):720-724. doi:10.1056/NEJM199009133231105
42. Baliakas P, Jeromin S, Iskas M, et al. Cytogenetic complexity in chronic lymphocytic leukemia: definitions, associations, and clinical impact. *Blood*. 2019;133(11):1205-1216. doi:10.1182/blood-2018-09-873083
43. Leeksma AC, Baliakas P, Moysiadis T, et al. Genomic arrays identify high-risk chronic lymphocytic leukemia with genomic complexity: a multi-center study. *Haematologica*. 2020;106(1):87-97. doi:10.3324/haematol.2019.239947
44. Baliakas P, Espinet B, Mellink C, et al. Cytogenetics in chronic lymphocytic leukemia: ERIC perspectives and recommendations. *HemaSphere*. 2022;6(4):e707. doi:10.1097/HS9.0000000000000707
45. Fürstenau M, Thus YJ, Robrecht S, et al. High karyotypic complexity is an independent prognostic factor in patients with CLL treated with venetoclax combinations. *Blood*. 2023;142(5):446-459. doi:10.1182/blood.2023019634
46. Kittai AS, Miller C, Goldstein D, et al. The impact of increasing karyotypic complexity and evolution on survival in patients with CLL treated with ibrutinib. *Blood*. 2021;138(23):2372-2382. doi:10.1182/blood.2020010536
47. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. *Nature*. 2015;526(7574):525-530. doi:10.1038/nature15395
48. Puente XS, Beà S, Valdés-Mas R, et al. Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2015;526(7574):519-524. doi:10.1038/nature14666
49. Puente XS, Pinyol M, Quesada V, et al. Whole-genome sequencing identifies recurrent mutations in chronic lymphocytic leukaemia. *Nature*. 2011;475(7354):101-105. doi:10.1038/nature10113
50. Wang L, Lawrence MS, Wan Y, et al. SF3B1 and other novel cancer genes in chronic lymphocytic leukemia. *N Engl J Med*. 2011;365(26):2497-2506. doi:10.1056/NEJMoa1109016
51. Knisbacher BA, Lin Z, Hahn CK, et al. Molecular map of chronic lymphocytic leukemia and its impact on outcome. *Nat Genet*. 2022;54(11):1664-1674. doi:10.1038/s41588-022-01140-w
52. Robbe P, Ridout KE, Vavoulis DV, et al. Whole-genome sequencing of chronic lymphocytic leukemia identifies subgroups with distinct biological and clinical features. *Nat Genet*. 2022;54(11):1675-1689. doi:10.1038/s41588-022-01211-y
53. Mansouri L, Thorvaldsdottir B, Sutton LA, et al. Different prognostic impact of recurrent gene mutations in chronic lymphocytic leukemia depending on IGHV gene somatic hypermutation status: a study by ERIC in HARMONY. *Leukemia*. 2023;37(2):339-347. doi:10.1038/s41375-022-01802-y
54. Beekman R, Chapaprieta V, Russiñol N, et al. The reference epigenome and regulatory chromatin landscape of chronic lymphocytic leukemia. *Nat Med*. 2018;24(6):868-880. doi:10.1038/s41591-018-0028-4
55. Giacopelli B, Zhao Q, Ruppert AS, et al. Developmental subtypes assessed by DNA methylation-iPLEX forecast the natural history of chronic lymphocytic leukemia. *Blood*. 2019;134(8):688-698. doi:10.1182/blood.2019000490

56. Queirós AC, Villamor N, Clot G, et al. A B-cell epigenetic signature defines three biologic subgroups of chronic lymphocytic leukemia with clinical impact. *Leukemia*. 2015;29(3):598-605. doi:10.1038/leu.2014.252
57. Tsagiopoulou M, Papakonstantinou N, Moysiadis T, et al. DNA methylation profiles in chronic lymphocytic leukemia patients treated with chemoimmunotherapy. *Clin Epigenetics*. 2019;11(1):177. doi:10.1186/s13148-019-0783-1
58. Wojdacz TK, Amarasinghe HE, Kadalayil L, et al. Clinical significance of DNA methylation in chronic lymphocytic leukemia patients: results from 3 UK clinical trials. *Blood Adv*. 2019;3(16):2474-2481. doi:10.1182/bloodadvances.2019000237
59. Herbst SA, Vesterlund M, Helmboldt AJ, et al. Proteogenomics refines the molecular classification of chronic lymphocytic leukemia. *Nat Commun*. 2022;13(1):6226. doi:10.1038/s41467-022-33385-8
60. Banerji V, Aw A, Robinson S, Doucette S, Christofides A, Sehn LH. Bruton tyrosine kinase inhibitors for the frontline treatment of chronic lymphocytic leukemia. *Curr Oncol*. 2020;27(6):645-655. doi:10.3747/co.27.6795
61. Rossi D, Gerber B, Stüssi G. Predictive and prognostic biomarkers in the era of new targeted therapies for chronic lymphocytic leukemia. *Leuk Lymphoma*. 2017;58(7):1548-1560. doi:10.1080/10428194.2016.1250264
62. Al-Sawaf O, Zhang C, Lu T, et al. Minimal residual disease dynamics after venetoclax-obinutuzumab treatment: extended off-treatment follow-up from the randomized CLL14 study. *J Clin Oncol*. 2021;39(36):4049-4060. doi:10.1200/JCO.21.01181
63. Kurtz DM, Esfahani MS, Scherer F, et al. Dynamic risk profiling using serial tumor biomarkers for personalized outcome prediction. *Cell*. 2019;178(3):699-713.e19. doi:10.1016/j.cell.2019.06.011
64. Dighiero G, Maloum K, Desablens B, et al. Chlorambucil in indolent chronic lymphocytic leukemia. *N Engl J Med*. 1998;338(21):1506-1514. doi:10.1056/NEJM199805213382104
65. Herling CD, Cymbalista F, Groß-Ophoff-Müller C, et al. Early treatment with FCR versus watch and wait in patients with stage Binet A high-risk chronic lymphocytic leukemia (CLL): a randomized phase 3 trial. *Leukemia*. 2020;34(8):2038-2050. doi:10.1038/s41375-020-0747-7
66. Hoechstetter MA, Busch R, Eichhorst B, et al. Early, risk-adapted treatment with fludarabine in Binet stage A chronic lymphocytic leukemia patients: results of the CLL1 trial of the German CLL study group. *Leukemia*. 2017;31(12):2833-2837. doi:10.1038/leu.2017.246
67. Langerbeins P, Zhang C, Robrecht S, et al. The CLL12 trial: ibrutinib vs placebo in treatment-naïve, early-stage chronic lymphocytic leukemia. *Blood*. 2022;139(2):177-187. doi:10.1182/blood.2021010845
68. Laurenti L, Gaidano G, Mauro FR, et al. What are the attributes prioritized in the choice of therapy in chronic lymphocytic leukemia? A patient-physician cross-matching analysis of a discrete choice experiment. *HemaSphere*. 2022;6(9):e771. doi:10.1097/HS9.0000000000000771
69. Lévy V, Delmer A, Cymbalista F. Frontline treatment in CLL: the case for time-limited treatment. *Hematology*. 2021;2021(1):59-67. doi:10.1182/hematology.2021000233
70. Salem JE, Manouchehri A, Bretagne M, et al. Cardiovascular toxicities associated with ibrutinib. *J Am Coll Cardiol*. 2019;74(13):1667-1678. doi:10.1016/j.jacc.2019.07.056
71. Byrd JC, Hillmen P, Ghia P, et al. Acalabrutinib versus ibrutinib in previously treated chronic lymphocytic leukemia: results of the first randomized phase III trial. *J Clin Oncol*. 2021;39(31):3441-3452. doi:10.1200/JCO.21.01210
72. Brown JR, Moslehi J, Ewer MS, et al. Incidence of and risk factors for major haemorrhage in patients treated with ibrutinib: an integrated analysis. *Br J Haematol*. 2019;184(4):558-569. doi:10.1111/bjh.15690
73. Lipsky A, Lamanna N. Managing toxicities of Bruton tyrosine kinase inhibitors. *Hematology*. 2020;2020(1):336-345. doi:10.1182/hematology.2020000118
74. Gribben JG. Practical management of tumour lysis syndrome in venetoclax-treated patients with chronic lymphocytic leukaemia. *Br J Haematol*. 2020;188(6):844-851. doi:10.1111/bjh.16345
75. Al-Sawaf O, Zhang C, Tandon M, et al. Venetoclax plus obinutuzumab versus chlorambucil plus obinutuzumab for previously untreated chronic lymphocytic leukaemia (CLL14): follow-up results from a multicentre, open-label, randomised, phase 3 trial. *Lancet Oncol*. 2020;21(9):1188-1200. doi:10.1016/S1470-2045(20)30443-5
76. Prosty C, Katergi K, Nguyen A, et al. Risk of infectious adverse events of venetoclax therapy for hematologic malignancies: a systematic review and meta-analysis of RCTs. *Blood Adv*. 2024;8(4):857-866. doi:10.1182/bloodadvances.2023011964
77. Tam CS, Allan JN, Siddiqi T, et al. Fixed-duration ibrutinib plus venetoclax for first-line treatment of CLL: primary analysis of the CAPTIVATE FD cohort. *Blood*. 2022;139(22):3278-3289. doi:10.1182/blood.2021014488
78. Huber H, Tausch E, Schneider C, et al. Final analysis of the CLL2-GIVe trial: obinutuzumab, ibrutinib, and venetoclax for untreated CLL with del(17p)/TP53mut. *Blood*. 2023;142(11):961-972. doi:10.1182/blood.2023020013
79. Furman RR, Cheng S, Lu P, et al. Ibrutinib resistance in chronic lymphocytic leukemia. *N Engl J Med*. 2014;370(24):2352-2354. doi:10.1056/NEJMc1402716
80. Woyach JA, Furman RR, Liu TM, et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. *N Engl J Med*. 2014;370(24):2286-2294. doi:10.1056/NEJMoa1400029
81. Mato AR, Woyach JA, Brown JR, et al. Pirtobrutinib after a covalent BTK inhibitor in chronic lymphocytic leukemia. *N Engl J Med*. 2023;389(1):33-44. doi:10.1056/NEJMoa2300696
82. Estupiñán HY, Wang Q, Berglöf A, et al. BTK gatekeeper residue variation combined with cysteine 481 substitution causes superresistance to irreversible inhibitors acalabrutinib, ibrutinib and zanubrutinib. *Leukemia*. 2021;35(5):1317-1329. doi:10.1038/s41375-021-01123-6
83. Maddocks KJ, Ruppert AS, Lozanski G, et al. Etiology of ibrutinib therapy discontinuation and outcomes in patients with chronic lymphocytic leukemia. *JAMA Oncol*. 2015;1(1):80-87. doi:10.1001/jamaoncol.2014.218
84. Quinquenel A, Fornecker LM, Letestu R, et al. Prevalence of BTK and PLCG2 mutations in a real-life CLL cohort still on ibrutinib after 3 years: a FILO group study. *Blood*. 2019;134(7):641-644. doi:10.1182/blood.2019000854
85. Liu TM, Woyach JA, Zhong Y, et al. Hypermorphic mutation of phospholipase C, γ2 acquired in ibrutinib-resistant CLL confers BTK independency upon B-cell receptor activation. *Blood*. 2015;126(1):61-68. doi:10.1182/blood-2015-02-626846
86. Woyach JA, Ghia P, Byrd JC, et al. B-cell receptor pathway mutations are infrequent in patients with chronic lymphocytic leukemia on continuous ibrutinib therapy. *Clin Cancer Res*. 2023;29(16):3065-3073. doi:10.1158/1078-0432.CCR-22-3887
87. Blombery P, Anderson MA, Gong J, et al. Acquisition of the recurrent Gly101Val mutation in BCL2 confers resistance to venetoclax in patients with progressive chronic lymphocytic leukemia. *Cancer Discovery*. 2019;9(3):342-353. doi:10.1158/2159-8290.CD-18-1119
88. Blombery P, Thompson ER, Nguyen T, et al. Multiple BCL2 mutations cooccurring with Gly101Val emerge in chronic lymphocytic leukemia progression on venetoclax. *Blood*. 2020;135(10):773-777. doi:10.1182/blood.2019004205

89. Tausch E, Close W, Dolnik A, et al. Venetoclax resistance and acquired BCL2 mutations in chronic lymphocytic leukemia. *Haematologica*. 2019;104(9):e434-e437. doi:10.3324/haematol.2019.222588
90. Liu J, Li S, Wang Q, et al. Sonrotoclax overcomes BCL2 G101V mutation-induced venetoclax resistance in preclinical models of hematologic malignancy. *Blood*. 2024;143:1825-1836. doi:10.1182/blood.2023019706
91. Khalsa JK, Cha J, Utro F, et al. Genetic events associated with venetoclax resistance in CLL identified by whole-exome sequencing of patient samples. *Blood*. 2023;142(5):421-433. doi:10.1182/blood.2022016600
92. Jain N, Croner LJ, Allan JN, et al. Absence of BTK, BCL2, and PLCG2 mutations in chronic lymphocytic leukemia relapsing after first-line treatment with fixed-duration ibrutinib plus venetoclax. *Clin Cancer Res*. 2023;30(3):OF1-OF8. doi:10.1158/1078-0432.CCR-22-3934
93. Kater AP, Seymour JF, Hillmen P, et al. Fixed duration of venetoclax-rituximab in relapsed/refractory chronic lymphocytic leukemia eradicates minimal residual disease and prolongs survival: post-treatment follow-up of the MURANO phase III study. *J Clin Oncol*. 2019;37(4):269-277. doi:10.1200/JCO.18.01580
94. Rawstron AC, Böttcher S, Letestu R, et al. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. *Leukemia*. 2013;27(1):142-149. doi:10.1038/leu.2012.216
95. 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. doi:10.1038/leu.2015.313
96. Rawstron AC, Villamor N, Ritgen M, et al. International standardized approach for flow cytometric residual disease monitoring in chronic lymphocytic leukaemia. *Leukemia*. 2007;21(5):956-964. doi:10.1038/sj.leu.2404584
97. Hengeveld PJ, van der Klift MY, Kolijn PM, et al. Detecting measurable residual disease beyond 10-4 by an IGHV leader-based NGS approach improves prognostic stratification in CLL. *Blood*. 2023;141(5):519-528. doi:10.1182/blood.2022017411
98. Schilhabel A, Szczepanowski M, van Gastel-Mol EJ, et al. Patient specific real-time PCR in precision medicine—validation of IG/TR based MRD assessment in lymphoid leukemia. *Front Oncol*. 2023;12:1111209. doi:10.3389/fonc.2022.1111209
99. van der Velden VHJ, Cazzaniga G, Schrauder A, et al. 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. doi:10.1038/sj.leu.2404586
100. FDA. Accessed February 20, 2024. https://www.accessdata.fda.gov/cdrh_docs/reviews/K200009.pdf
101. 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. doi:10.1200/JCO.2016.67.1305
102. 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. doi:10.1182/blood-2016-05-714162
103. Cramer P, von Tresckow J, Bahlo J, et al. Bendamustine followed by obinutuzumab and venetoclax in chronic lymphocytic leukaemia (CLL2-BAG): primary endpoint analysis of a multicentre, open-label, phase 2 trial. *Lancet Oncol*. 2018;19(9):1215-1228. doi:10.1016/S1470-2045(18)30414-5
104. Hillmen P, Rawstron AC, Brock K, et al. Ibrutinib plus venetoclax in relapsed/refractory chronic lymphocytic leukemia: the CLARITY study. *J Clin Oncol*. 2019;37(30):2722-2729. doi:10.1200/JCO.19.00894
105. Wierda WG, Allan JN, Siddiqi T, et al. Ibrutinib plus venetoclax for first-line treatment of chronic lymphocytic leukemia: primary analysis results from the minimal residual disease cohort of the randomized phase ii CAPTIVATE study. *J Clin Oncol*. 2021;39(34):3853-3865. doi:10.1200/JCO.21.00807
106. Davids MS, Lampson BL, Tyekucheva S, et al. Acalabrutinib, venetoclax, and obinutuzumab as frontline treatment for chronic lymphocytic leukaemia: a single-arm, open-label, phase 2 study. *Lancet Oncol*. 2021;22(10):1391-1402. doi:10.1016/S1470-2045(21)00455-1
107. Kater AP, Levin MD, Dubois J, et al. Minimal residual disease-guided stop and start of venetoclax plus ibrutinib for patients with relapsed or refractory chronic lymphocytic leukaemia (HOVON141/VISION): primary analysis of an open-label, randomised, phase 2 trial. *Lancet Oncol*. 2022;23(6):818-828. doi:10.1016/S1470-2045(22)00220-0
108. Rawstron A, Webster N, Dalal S, et al. Using peripheral blood (PB) measurable residual disease (MRD) levels to predict <0.01% bone marrow disease (BM uMRD4): identification of effective PB targets for CLL treatment cessation in the ibrutinib+venetoclax arm of the FLAIR trial. *Blood*. 2023;142(Suppl 1):632. doi:10.1182/blood-2023-187943
109. Fürstenau M, Ritgen M, Robrecht S, et al. First-line venetoclax combinations in fit patients with CLL: 4-year follow-up and NGS-based MRD analysis from the phase 3 GAIA/CLL13 trial. *Blood*. 2023;142(Suppl 1):635. doi:10.1182/blood-2023-173709
110. Baliakas P, Hadzidimitriou A, Sutton LA, et al. Clinical effect of stereotyped B-cell receptor immunoglobulins in chronic lymphocytic leukaemia: a retrospective multicentre study. *Lancet Haematol*. 2014;1(2):e74-e84. doi:10.1016/S2352-3026(14)00005-2
111. Chatzikonstantinou T, Scarfò L, Karakatsoulis G, et al. Other malignancies in the history of CLL: an international multicenter study conducted by ERIC, the European Research Initiative on CLL, in HARMONY. *EClinicalMedicine*. 2023;65:102307. doi:10.1016/j.eclinm.2023.102307
112. Mato AR, Barrientos JC, Ghosh N, et al. Prognostic testing and treatment patterns in chronic lymphocytic leukemia in the era of novel targeted therapies: results from the informCLL registry. *Clin Lymphoma Myeloma Leukemia*. 2020;20(3):174-183.e3. doi:10.1016/j.clml.2019.10.009
113. Mato AR, Hess LM, Chen Y, et al. Outcomes for patients with chronic lymphocytic leukemia (CLL) previously treated with both a covalent BTK and BCL2 inhibitor in the United States: a real-world database study. *Clin Lymphoma Myeloma Leukemia*. 2023;23(1):57-67. doi:10.1016/j.clml.2022.09.007
114. Roeker LE, Fox CP, Eyre TA, et al. Tumor lysis, adverse events, and dose adjustments in 297 venetoclax-treated CLL patients in routine clinical practice. *Clin Cancer Res*. 2019;25(14):4264-4270. doi:10.1158/1078-0432.CCR-19-0361
115. Antic D, Milic N, Chatzikonstantinou T, et al. Thrombotic and bleeding complications in patients with chronic lymphocytic leukemia and severe COVID-19: a study of ERIC, the European Research Initiative on CLL. *J Hematol Oncol*. 2022;15(1):116. doi:10.1186/s13045-022-01333-0
116. Campanella A, Capasso A, Heltai S, et al. Additional booster doses in patients with chronic lymphocytic leukemia induce humoral and cellular immune responses to SARS-CoV-2 similar to natural infection regardless ongoing treatments: a study by ERIC, the European Research Initiative on CLL. *Am J Hematol*. 2024;99:745-750. doi:10.1002/ajh.27218
117. Chatzikonstantinou T, Kapetanakis A, Scarfò L, et al. COVID-19 severity and mortality in patients with CLL: an update of the

international ERIC and Campus CLL study. *Leukemia*. 2021;35(12):3444-3454. doi:10.1038/s41375-021-01450-8

118. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood*. 2021;137(23):3165-3173. doi:10.1182/blood.2021011568
119. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020;136(10):1134-1143. doi:10.1182/blood.2020006965
120. Scarfò L, Chatzikonstantinou T, Rigolin GM, et al. COVID-19 severity and mortality in patients with chronic lymphocytic leukemia: a joint study by ERIC, the European Research Initiative on CLL, and CLL Campus. *Leukemia*. 2020;34(9):2354-2363. doi:10.1038/s41375-020-0959-x
121. Visentin A, Chatzikonstantinou T, Scarfò L, et al. The evolving landscape of COVID-19 and post-COVID condition in patients with chronic lymphocytic leukemia: a study by ERIC, the European research initiative on CLL. *Am J Hematol*. 2023;98(12):1856-1868. doi:10.1002/ajh.27093
122. Agius R, Brieghel C, Andersen MA, et al. Machine learning can identify newly diagnosed patients with CLL at high risk of infection. *Nat Commun*. 2020;11(1):363. doi:10.1038/s41467-019-14225-8
123. Chatzidimitriou A, Minga E, Chatzikonstantinou T, Moreno C, Stamatopoulos K, Ghia P. Challenges and solutions for collecting and analyzing real world data: the eric CLL database as an illustrative example. *HemaSphere*. 2020;4(5):e425. doi:10.1097/HS9.0000000000000425
124. Karamanidou C, Xochelli A, Ghia P, Stamatopoulos K. How do hematologists communicate with patients suffering from chronic lymphocytic leukemia? Insights from the ERIC pilot study in greece. *Eur J Health Commun*. 2021;2(3):110-135. doi:10.47368/ejhc.2021.306
125. Kyrou D, Stavrogianni K, Koulierakis G, Vrontaras N, Stamatopoulos K, Karamanidou C. The looming cancer: a qualitative study on the experience of living with chronic lymphocytic leukemia (CLL) before the initiation of treatment. *Eur J Cancer*. 2024. Published online February 28, 2024. doi:10.1155/2024/4034801
126. Kuusanmäki H, Kytölä S, Vänttinen I, et al. Ex vivo venetoclax sensitivity testing predicts treatment response in acute myeloid leukemia. *Haematologica*. 2023;108(7):1768-1781. doi:10.3324/haematol.2022.281692
127. Malani D, Kumar A, Brück O, et al. Implementing a functional precision medicine tumor board for acute myeloid leukemia. *Cancer Discovery*. 2022;12(2):388-401. doi:10.1158/2159-8290.CD-21-0410
128. CLLDB. Accessed February 20, 2024. https://www.imgt.org/CLLDBInterface/query
129. Yang S, Varghese AM, Sood N, et al. Ethnic and geographic diversity of chronic lymphocytic leukaemia. *Leukemia*. 2021;35(2):433-439. doi:10.1038/s41375-020-01057-5
130. Chiattone CS, Gabus R, Pavlovsky MA, Akinola NO, Varghese AM, Arrais-Rodrigues C. Management of chronic lymphocytic leukemia in less-resourced countries. *Cancer J*. 2021;27(4):314-319. doi:10.1097/PPO.0000000000000533