Present and future of personalized medicine in CLL

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Abstract

Medicine has been ‘personalized’ (i.e. centred in persons) since its foundation. Recently, however, the term ‘personalized medicine’ (or, better, ‘precision medicine’) has been introduced to define ‘a form of medicine that uses information about a person’s genes, proteins, and environment to prevent, diagnose, and treat disease’. This concept has gained momentum thanks to next-generation-sequencing (NGS) techniques that allow identification of molecular characteristics unique to the patient and to the tumour. It is hoped that NGS will not only contribute to a better understanding of chronic lymphocytic leukaemia (CLL), but will identify disease subsets that could benefit from specific treatment interventions. Recent advances in diagnosis (e.g. high-resolution immunophenotyping, markers of genetic abnormalities), prognosis (e.g. biomarkers), response predictors [e.g. del(17p)/TP53 mutations even at subclonal level], treatment (e.g. BCR signalling inhibitors, BCL2 antagonists, CAR-T cells) and methods to evaluate minimal residual disease constitute good examples of tools facilitating ‘personalized’ management of patients with CLL.

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Introduction

Chronic lymphocytic leukaemia (CLL) is a common disorder characterized by the accumulation of neoplastic CD5⁺ B lymphocytes with a characteristic immunophenotype (CD19⁺, CD20<sup>weak</sup>, CD23⁺) in blood, bone marrow and lymphoid tissues. The median age of patients at diagnosis is approximately 70 years. The incidence of CLL is approximately 4/100,000 people per year, and this increases markedly with age up to more than 20/100,000 per year in people aged >70 years. Despite significant progress in its management, CLL remains an incurable disease [1,2]. Continued improvement in understanding of the clinical and biological heterogeneity of CLL, and the development of non-cytotoxic targeted therapies are driving the management of this form of leukaemia based on an individualized, personalized approach [3,4].

What is personalized medicine?

Personalized medicine does not have a unique definition. The National Cancer Institute defines personalized medicine as ‘a form of medicine that uses information about a person’s genes, proteins, and environment to prevent, diagnose, and treat disease’ [5]. Other terms such as ‘individualized medicine’, ‘precision medicine’, ‘sequential medicine’, ‘stratified medicine’ and ‘genomic medicine’ have been coined to describe what, in practical terms, is comparable, if not the same, to personalized medicine [6]. It can be argued that medicine has been ‘personalized’ (i.e. centred in people) since its foundation, as classically underlined by the maxim ‘there are no diseases but people suffering from a given disease’. Currently, there is increasing confidence that modern techniques to study the immunophenotype, genetics, epigenetics, pharmacogenetics and all corresponding present and future ‘omics’, along with new and non-cytotoxic treatments will allow identification of the ‘right treatment for the right patient’; one of the objectives of personalized medicine. Indeed, personalized medicine should be considered as part of, if not equivalent to, comprehensive patient management (Fig. 1). Readers interested in the history, development and conceptual aspects of personalized medicine are

Fig. 1. Comprehensive, personalized and precision medicine.
referred to some recent reviews [6–9]. The following sections discuss various examples of how personalized medicine is shaping and could further influence the management of patients with CLL, together with some of its drawbacks and challenges.

**Personalized medicine in CLL**

**Quest for more precise diagnostic markers**

The diagnosis of CLL is supported by the following criteria: (1) presence of >5 × 10^9/l monoclonal B lymphocytes in peripheral blood, persisting for at least 3 months; (2) demonstration of the clonality of the population (k/λ analysis); and (3) characteristic morphology and immunophenotype: SmIg-weak, CD5^+, CD19^+, CD20^weak and CD23^+ [10]. In 1994, Matutes et al. devised an immunophenotypic score based on a few markers (CD5^+, CD23^+, FMC7^-, SmIgweak and CD22weak), each of them receiving a score of 1 if present or 0 if absent. A total score of 4 or 5 is classic of CLL, whereas cases scoring 0 or 1 correspond to other B-cell lymphoproliferative disorders, mainly lymphomas [11]. This score has undoubtedly been useful and is still employed in some centres. However, markers used for the diagnosis of CLL should be reviewed due to the discovery of new antigens that are specific for CLL B cells, such as CD200 and, to a lesser extent, ROR1 [12,13]. In a recent project, the European Research Initiative on CLL (ERIC) and the European Society for Clinical Cell Analysis identified the optimal markers for CLL diagnosis. A minimum requirement for diagnosis was defined as follows: CD19^+, CD5^+, CD23^+, CD20^weak and IgG/κ light chain weak and restricted. In addition, recommended markers include: CD43^+, CD79bweak, CD81weak, CD22weak, CD200^+, D10- and ROR1^+ [14]. An important finding was that approximately 5% of the samples did not meet CLL diagnostic criteria when reviewed centrally before enrolling patients in clinical trials. This is of concern because a precise diagnosis is fundamental for patient management and the interpretation of data from a clinical trial. As the methods to diagnose chronic B-cell disorders improve, the term ‘atypical CLL’ should be avoided; ‘chronic B-cell leukaemia — unclassifiable’ might be a preferable name when a precise diagnosis is elusive. In addition to flow cytometry markers, genetic aberrations identified by either conventional techniques [e.g. fluorescence in-situ hybridization (FISH), conventional karyotype] or next-generation sequencing (NGS) may contribute to the diagnosis of B-cell disorders (Table 1). Once the diagnosis is established, patient samples should be preserved whenever possible as this may be useful for research purposes and also for studying new biomarkers useful for patient management.

**CLL: how many clinic-biological forms?**

**Disease entities and the case of monoclonal B-cell lymphocytosis**

CLL and small lymphocytic lymphoma (SLL) are considered to be the same disease, only differing in terms of the degree to which they involve blood (CLL) or lymphoid tissues (SLL) [10]. As part of the same spectrum, monoclonal B-cell lymphocytosis (MBL) describes the presence of <5 × 10^9/l monoclonal B cells in the peripheral blood of an asymptomatic individual with no lymphadenopathy nor organomegalies [15]. Of note, CLL and MBL are separated on the basis of a cell count above or below

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CLL: chronic lymphocytic leukaemia; B-PLL: B-cell prolymphocytic leukaemia; HCL: hairy-cell leukaemia; LPL: lymphoplasmacytic lymphoma; SMZL: splenic marginal zone lymphoma; MCL: mantle-cell lymphoma; FL: follicular lymphoma.
5 × 10^9/l monoclonal B-cells, respectively; an arbitrary threshold established because of the need for strict diagnostic criteria in CLL trials [10]. MBL can be differentiated into two different forms: (1) low-count MBL (<0.5 × 10^9/l clonal CD5^+ B lymphocytes) and (2) high-count MBL (>0.5 × 10^9/l clonal CD5^+ B lymphocytes). In most cases, but not all, the immunophenotype is the same of CLL. The prevalence of MBL ranges from 1% to 18% depending on the sensitivity of the flow cytometry technique, patient’s age, and whether or not there is a first-degree relative with CLL. Immunophenotypically, MBL cases can be: (1) CLL-like, (2) CD5^- or (3) atypical.

While some experts support that MBL is an early form of CLL, others consider MBL to be a condition with peculiar features of its own. This issue is far from being settled. NGS has shown that MBL shares the same biological features as CLL, with low-count MBL being enriched for favourable biomarkers [e.g. mutated IGHV, del(13q)] and high-count MBL occasionally presenting with high-risk markers [e.g. del(17p)/TP53 mutations, NOTCH1 mutations], most frequently at subclonal levels. In most cases, low-count MBL is detected in healthy individuals as part of population-based or research studies, and rarely progresses to CLL. Moreover, some cases are biclonal or express markers other than those of CLL; spontaneous disappearance of the clonal population may occur. In contrast, high-count MBL is mainly discovered as a result of a routine blood analysis showing some degree of lymphocytosis, presents biological features similar to those of CLL, and evolves into CLL at an annual rate of 1–2%. NGS studies have shown that as the clonal population increases, genetic lesions and clonal heterogeneity expand [16]. People with high-count MBL require therapy less frequently than patients in early stage (Rai O); this is not surprising because clonal blood lymphocyte counts behave as a continuous prognostic variable — the higher the count, the greater the probability of progression. In this regard, in studies comparing high-count MBL with Rai O CLL, the best B-cell cut-off to predict progression has been found to be 10−11 × 10^9/l rather than 5 × 10^9/l [17,18]. Based on the current information, it has been suggested that low-count MBL and high-count MBL, although related, constitute two different clinic-biological situations, but this requires further investigation. The management of patients with MBL should be the same as for Rai 0 CLL patients independent of blood lymphocyte counts and molecular features.

**IGHV mutational status differentiates two forms of CLL**

One of the most important, and still unparalleled, breakthroughs in the understanding of CLL was the discovery that IGHV genes can be either mutated (approximately 60% of cases) or unmutated (approximately 40% of cases) in CLL [19,20]. While patients with mutated IGHV genes have indolent disease, no high-risk genetic lesions, rarely require therapy and have a good prognosis, those with unmutated IGHV genes tend to have aggressive disease, high expression of CD38, ZAP-70 and CD49d, short telomers, poor-risk genetics and short survival (Fig. 2) [3,19-21]. Interestingly, the subset of IGHV-mutated cases using V_{H}3-21, particularly those expressing the BCR-antigen receptor subset 2, show a similar clinical course as unmutated IGHV cases [22]. In contrast with other markers, IGHV mutational status remains unchanged over the course of the disease. In approximately one-third of patients, non-clonally-related different mutations have been observed; this observation needs further investigation [23]. Micro-array studies have demonstrated that mutated and unmutated IGHV CLL share the same genetic signature (although ZAP-70 expression is higher in unmutated cases); accordingly, CLL is considered to be a single disease with two different forms [24,25]. It is worth emphasizing that IGHV mutational status is strongly correlated with the pace of the disease, genome complexity and patient outcome. Therefore, IGHV mutational status is a cornerstone not only of the biology but also of the evolution of the disease. Finally, studying IGHV-mutated and unmutated CLL cases separately could translate into further progress in CLL, including the development of specific treatment strategies.

**Genetics and clinic-biologic forms of CLL**

Genetic lesions, particularly gene mutations, are useful for the identification of different forms of tumours with unique features, sensitivity to specific targeted therapies, and predictable outcome. Although far away from what happens in other haematological malignancies (e.g. acute myeloblastic leukaemia, myelodysplastic syndromes), different forms of CLL can be outlined [26] (Table 2). The ultimate goal of delineating different forms of CLL is to apply specific interventions to each subgroup.
NGS techniques, however, are unveiling the complexity and evolving nature of genetic lesions in CLL. As such, potentially ‘actionable’ (i.e. susceptible of a direct intervention) gene mutations largely conform a ‘moving target’. The interaction between different clones and subclones, and the development of resistant clones (either spontaneously or secondary to therapy) is a key area in CLL research. A better understanding of clonal and subclonal evolution is a necessary first step to prevent disease progression and resistance to therapy [27–31].

**BCR-antigen receptor stereotypes**

BCR-antigen signalling regulates the development of normal and leukaemic B cells [32]. B cells play an essential role in innate immunity by producing antibodies against foreign and autologous antigens. For that, B cells need to generate a diverse repertoire of antigen receptors and thereafter select B cells for specific antigens. The probability that two independent B-cell clones carry exactly the same BCR is extremely low. However, in approximately 30% of cases, CLL cells from different patients express similar, if not identical, BCRs with common (‘stereotypic’) features and/or structural similarities. Many studies have correlated BCR stereotypes with clinical features in CLL [33,34]. In a recent analysis of 8593 patients, the clinical implications of BCR immunoglobulin stereotyping were assessed [34]. BCR-stereotyped subsets showed significant differences regarding age, sex, disease burden, CD38 expression and cytogenetic aberrations of prognostic significance. Patients within a specific subset had similar clinical behaviours, whereas those in different stereotyped subsets showed different times from diagnosis to first treatment. By integrating BCR stereotypes (subsets #1, #2 and #4) into the well-established cytogenetic Döhner’s prognostic model [26], different clinic-biological forms of CLL could be separated, ranging from very indolent (subset #4) to aggressive disease (subsets #1 and #2). Also, the molecular classification of CLL based on BCR stereotypes improved cytogenetics as prognostic

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**Fig. 2.** Scheme of the two main forms of chronic lymphocytic leukaemia: mutated IGHV vs non-mutated IGHV. Markers enriched in each one of these two forms are shown. Consistent combinations of cytogenetic and molecular features are presented in boxes.
markers regardless of IGHV mutational status (33,34). Further research and harmonization studies are needed in this area.

**Prognosis**

The median survival of patients with CLL has improved over the last years and is now close to 10 years. Individual prognosis, however, remains extremely variable and ranges from a few months to a normal life expectancy. Prognostication is an essential component in the management of patients with CLL. Although overlapping, it is useful to distinguish those parameters that foresee disease progression, and hence need for therapy (prognostic factors), and those factors that predict which therapy a given patient will respond to (predictive factors) [35,36]; a core element of personalized medicine.

Prognosis is related to many factors, and thus a single parameter rarely predicts outcome. As such, prognosis is based on the combination of different variables in the form of stages, prognostic groups or scores. The best and oldest examples are the Binet and Rai staging systems [37,38]. A plethora of parameters has been proposed to refine the prognostic strength of clinical stages or to replace them; many of these ‘new prognostic factors’, however, do not fulfill the requirements to be considered as such [36,39,40]. Despite this caveat, patients with CLL can be stratified into different prognostic groups based on several parameters (i.e. clinical stages, IGHV mutational status, ZAP-70, CD38, FISH cytogenetics, serum beta-2 microglobulin, white blood cell count and lymphocyte doubling time). Importantly, new prognostic parameters should be more robust than previously identified indicators for the same outcome [36,39,40]. A detailed discussion of prognostic factors in CLL is presented in Chapter 8: Chronic Lymphocytic Leukemia (CLL).

**Treatment**

One of the goals of personalized medicine is the identification of the ‘right treatment for the right patient’. Nevertheless, there are very few predictive factors for CLL that help to reach this goal. In fact, del(17p)/TP53 mutations represent the only (negative) response predictor currently used in clinical practice. Patients showing del(17p)/TP53 mutations immediately before therapy (genetic abnormalities may change over time) respond poorly to immunochemotherapy ([41–45]). The same is true for complex karyotype, although this bears del(17p)/TP53 mutations in most cases [43,46]. Patients harbouring these mutations should be treated with the new compounds (e.g. ibrutinib, idelalisib or...
venetoclax), keeping allogeneic stem cell transplantation or other forms of T-cell therapy (i.e. CAR-T cells) as treatment alternatives for refractory cases [47–49]. Treatment of CLL is discussed extensively in Chapter 8: Chronic Lymphocytic Leukemia (CLL).

Next-generation sequencing and new mutations

NGS studies have widened the landscape of genetic lesions in CLL, including those involving MYD88, NOTCH1, SF3B1, BIRC3, NFKBIE and POT1 genes which are present in 5–20% of cases depending on the phase of disease. Correlation of these mutations with patient outcome has mainly been investigated in retrospective studies with disparate results. However, there is convincing information correlating NOTCH1, SF3B1 and BIRC3 mutations with advanced disease (including Richter’s syndrome), refractoriness to fludarabine-based therapy and poor survival. In a large study, newly discovered del(20p), gains in chromosomes 2p16 and 5q34, and aberrations of BRAF, ZMYM3, IRF4 and NFKB2 genes have been found to predict shorter time to treatment, while those mutations in ASXL1, POT1 and del(14q24) correlated with overall survival (OS) [50–53]. Another study has shown that patients whose disease expresses TP53, NOTCH1 and SF3B1 mutations simultaneously (‘multiple hit’ CLL) have inferior outcome compared with patients carrying a single mutation [54]; this concept is reminiscent of what happens with some lymphomas. Additionally, it has been reported that patients with NOTCH1 mutations do not benefit from rituximab-combining therapies, which could be related to low expression of CD20 in NOTCH1-mutated cases [55]. In the UK LRF CLL4 trial, patients with mutations in NOTCH1 or SF3B1 showed survival comparable to patients with del(11q), but better survival than patients with del(17p) [56]. On the other hand, results from the CLL8 trial confirmed SF3B1 mutations as an independent factor for disease progression, while NOTCH1 mutations had no significant prognostic role [55]. The discordant results regarding the prognostic significance of NOTCH1 mutations in these two trials underlines that the prognostic significance of biomarkers may differ depending on the study population and type of therapy. Therefore, predictive markers should be studied separately for each new treatment agent. Moreover, a prerequisite for a marker to be incorporated into clinical practice is to be actionable (subject to a specific clinical intervention), and this is not a possibility at present for NGS-identified gene mutations and other genomic aberrations. To complicate matters further, somatic mutations may evolve over time, not all mutations within the same gene have equivalent clinical consequences, mutations and cytogenetic lesions are often found in combination (Fig. 2), and the micro-environment may modulate the function and prognostic impact of mutated genes [3,31]. As such, there is an urgent need to dissect critical molecular pathways, identify synergisms, establish a hierarchical model for biomarkers, and retain only those that have direct consequences in clinical management. Furthermore, well-designed, prospective clinical studies are needed to better determine the relationship between newly described mutations and clinical outcomes.

Minimal-residual disease as response surrogate

In CLL, the level of minimal residual disease (MRD) after therapy is an independent predictor of progression-free survival (PFS) and OS, and consequently a desirable treatment objective [57]. Although not yet recommended in general clinical practice, MRD-guided therapy could facilitate personalized, more precise treatment (e.g. disease eradication, maintenance therapy). Different studies have demonstrated that achieving a complete response with no detectable. In the CLL8 trial, comparing fludarabine, cyclophosphamide and rituximab (FCR) with FC, MRD levels were quantified prospectively in 1775 blood and bone marrow samples from 493 patients. They were categorized based on MRD levels into low- (<10⁴), intermediate- (10⁴ to 10²) and high-level (<10²) groups. Low MRD levels were significantly associated with longer PFS and OS. Notably, PFS and OS did not differ between treatment arms within each MRD category. However, FCR induced low MRD levels more frequently than FC. On multivariate analysis, MRD status and del(17p) were the most important factors for PFS and OS [58].

In another study in which 237 patients with CLL received FCR as a first-line treatment, MRD was assessed prospectively using four-colour flow cytometry in bone marrow after the third cycle of
therapy and at the final response assessment. After the third cycle and at the response assessment, 17% and 43% of patients achieved MRD negativity in the bone marrow, respectively. Importantly, MRD-negative patients had comparable PFS and OS, independent of the number of cycles received. This study suggests that MRD eradication with fewer cycles of treatment may be a plausible endpoint, prompting consideration of early discontinuation of treatment, thus avoiding unnecessary toxicity [59]. This concept warrants study in randomized clinical trials.

One of the difficulties for progress in CLL treatment is the high number of new agents with the potential for therapeutic usefulness, and the enormous costs associated with designing and conducting clinical trials. There is an urgent need to expedite the study of new treatments for CLL using new models for clinical trials and MRD status as response surrogate [60]. To that end, the method to determine MRD should be reliable, easy to perform, and simple to interpret so that it can be applied routinely. In a number of studies, ERIC has harmonized flow cytometry methods to detect residual disease in CLL [61]. NGS techniques may have an important role for the measurement of residual disease. In a recent study, ERIC identified and validated a flow cytometry approach to reliably quantify CLL cells to the level of 0.001% (10^-5); a lower detection level than that achieved by current methods (0.01%/10^-4). It is conceivable that achieving an MRD level below 0.001%/10^-5 will translate into better clinical outcome, but this requires prospective studies. The same study also found that high-throughput sequencing using the ClonoSEQ assay showed good concordance with flow cytometry results. Therefore, it is likely that high-throughput sequencing, either by itself or in combination with flow cytometry, may prove to be a valuable resource to improve MRD detection [62].

Conclusions

Medical practice is shifting to a model of management known as ‘personalized’ medicine that incorporates unique biological and clinical features of the patient and the disease into clinical management. CLL is no exception to this approach. The extent to which, thanks to newer technologies, knowledge of molecular lesions in CLL has arrived is impressive and would have been difficult to imagine just a few years ago. This progress has already resulted in better diagnostic tools and effective, relatively well tolerated, targeted treatments. Notably, these therapies are useful in frail patients and in those with refractory/progressing disease, including cases with del(17p)/TP53 mutations. In addition, a number of genetic mutations, particularly NOTCH1, SF3B1 and BIRC3 mutations, could be incorporated into clinical practice in the near future as outcome predictors. Findings based on NGS techniques accumulate exponentially, and identification of those that are meaningful from a clinical standpoint is a complex task. A multidisciplinary approach from different professional scopes (epidemiologists, molecular biologists, pharmacologists and clinicians) using strict methodology based on the ‘discovery, analysis, harmonization, validation and clinical utility’ road map is necessary for the development of strong personalized medicine.

Practice points

- Management of patients with CLL should be based on the characteristics of the patient, tumour and environmental factors
- The ultimate goals of therapy are to improve quality of life and to prolong survival
- Personalized medicine refers to patient management based on unique molecular features of the disease and the patient, thus being part of comprehensive clinical management
- Personalized medicine complements, but does not replace, clinical expertise
- Whenever possible, patients should be managed within large, well-designed clinical trials
- Storing samples from patients with CLL at diagnosis can be useful, not only for investigational purposes but also to determine new biomarkers as they are identified
Research agenda

- prospective validation of molecular abnormalities for which clinical correlations have been established
- identify key molecular pathways that are biologically relevant and potentially actionable
- collect data for a large number of patients and biological features through international collaborations and bio-bank data platforms
- investigate mechanisms of resistance to treatment, including the clinical significance of cell subclones in evolution of the disease
- identify environmental factors correlated with patient outcome and susceptible of modification
- establish hierarchical models for molecular abnormalities and to integrate genomic, epigenetic, transcriptomic and proteomic data, and also to develop multiparametric outcome predictors
- design research programmes focused on elderly patients with CLL

Conflict of interest statement

None declared.

References


