On the architecture of translational research designed to control chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) has been 1 of the most dynamic fields of clinical research over the last 2 decades. Important advances in understanding the biology of CLL have led to the development of new prognostic and diagnostic tools. Concurrently, several recently approved new agents hold the potential to fundamentally change the management of this leukemia and have started to improve clinical outcomes for patients. This conceptual review summarizes the major recent insights regarding the biology of CLL, the technological advances that have allowed refinement of the prognostication of the clinical course, and the new therapeutic strategies that are currently under investigation to further ameliorate the outcome for patients with CLL.

Learning Objectives

- Understand the principle mechanisms of the pathogenesis of CLL
- Understand the recent progress and the current, risk and age adapted treatment concepts for CLL patients
- Understand the strategies for the trial design using the currently available agents
- Increase the awareness of the disparity of CLL therapy around the world

Introduction

Chronic lymphocytic leukemia (CLL) represents 1 of the most active fields of clinical research at the present time. Reviews thoroughly summarizing this impressive progress have been published recently.[1-3] Therefore, rather than generating an additional potentially redundant review of the state of the art in biology and management of CLL, I intend to describe the major forces and achievements that have inspired my own work and have driven the dynamic progress in this disease. Moreover, I wish to sketch the "architecture" of the translational and clinical research that is needed to achieve full control over this formerly incurable form of leukemia.

Biology of CLL: discoveries driven by technological progress

At least 3 recent technological advances and improvements have critically contributed to progress in the discovery and preclinical research in this area over the last decades: the progress of molecular genetics and molecular biology, the availability of reliable animal models, and the tremendous increase in our understanding of the central pathways regulating the development of B cells in their microenvironment.

Molecular genetics and genomics

The genetic and genomic technologies that have become available since the second half of the last century have allowed fundamental discoveries that improved our understanding of the biology of CLL. Studies using fluorescent in-situ hybridization and chromosome banding have described recurrent and frequent aberrations in CLL, some of which, such as del(17p), have demonstrated profound prognostic impact.[4] Almost simultaneously it has been shown that the mutational composition of *immunoglobulin heavy chain variable region* (*IGHV*) genes separates 2 apparently related, but biologically and clinically different, forms of CLL.[5,6] These findings suggested a central role for B-cell receptor (BCR) signaling for this leukemia (see Functional understanding of BCR signaling and B-cell development section). In a search for the relevant genes disrupted by the frequent del(13q), occurring in almost 50% of CLL cases, it was discovered that this deletion causes the loss of microRNAs (miR-15a and miR-16-1), which initiates leukemogenesis.[7,8] It was suggested that these microRNAs induce the upregulation of Bcl2 protein that is usually highly overexpressed in CLL.[9] More recently, whole-exome sequencing of datasets from large annotated clinical CLL databases has enabled a description of the genomic landscape of CLL.[10,11] From these studies, we have learned that inflammatory pathways, BCR signaling and differentiation, Notch signaling, Wnt signaling, DNA damage control, chromatin modification, and RNA and ribosomal processing are frequently altered in CLL.[11]

The importance of animal models

CLL cells are difficult to culture in vitro, because they do not survive for more than a few hours without considerable support and seem "addicted to the host."[12] Accordingly, only very few CLL cell lines are available, and their relevance is highly disputed because they often represent CLL variants with multiple genetic variations. To conduct meaningful in vitro experiments, coculture methods with feeder cells or stimulation with CpG oligonucleotides have facilitated the culture of freshly isolated CLL cells over several days to weeks and resulted in important insights.[13-15] This situation was significantly improved with the advent of reliable animal models, such as the Eμ-TCL1 mouse,[16] which allowed the complexity of the dialogue between CLL cells and their microenvironment to be addressed in a more comprehensive manner. From these studies, we

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have learned that the interaction of CLL cells with their microenvironment is essential for leukemogenesis. Leukemia-associated macrophages were shown to be important components of the microenvironment and support the growth of CLL cells.[17,18] Even the efficacy of conventional therapeutics, such as chemotherapy with alkylators and monoclonal antibodies, seem to mediate their effects through compartment-restricted interactions with specific cells (eg, macrophages).[19] More recently, we could show that agents intended to target BCR-associated kinases, such as LYN tyrosine kinase or Bruton tyrosine kinase (BTK), seem to exert essential effects through the modulation of the leukemic niche, because targeted deletions of these kinases reduce the capacity of macrophages and of other cell types to "feed" CLL growth.[20]

Functional understanding of BCR signaling and B-cell development

Using some of the above technologies has allowed a deeper understanding of the B-cell biology and the essential signaling pathways that modulate these processes. BCR signaling seems to play an important role in the survival of CLL cells.[21] This is underscored by the observation that the mutational status of *IGHV* genes defines different forms of CLL and has prognostic impact. Moreover, continuous or repetitive BCR signaling supports CLL cell survival (reviewed in Stevenson et al[21]). The BCR signaling in CLL cells is transmitted by different tyrosine kinases, such as BTK, spleen tyrosine kinase (SYK), Src family kinases (in particular LYN tyrosine kinase), as well as phosphoinositide 3-kinases.[12] These BCR-associated kinases are crucial signaling transducers that play a role in B cell maturation, as well as in the initiation and progression of B-cell lymphoma.[22] BCR-associated kinases are activated by the binding of antigen to the BCR. Upon antigen ligation, the BCR becomes rearranged and translocated into lipid rafts, initiating the binding of the BCR to LYN that is densely located within these rafts.[23] LYN phosphorylates the immunoglobulin heterodimers of the BCR, leading to the recruitment and phosphorylation of SYK.[24] Thereafter, activated LYN and SYK induce the formation of the BCR signalosome, leading to the phosphorylation and activation of SYK, BLNK, BTK, PLCγ, and phosphoinositide 3-kinases.[25,26] Together, these early events trigger a signaling cascade, which, in turn, activates a series of kinases and pathways, including MAPK/ERK, AKT/mTOR, PKCβ, and BCL-10/CARD11/MALT1, and the mobilization of calcium ions (reviewed in Young and Staudt[27] and Shaffer et al[28]). As a result, BCR signaling may initiate transcription processes via transcriptional factors, such as NF-κB, MYC, and NFAT. Depending on the nature of the BCR stimulus (tonic or antigen dependent), as well as on the duration and strength of BCR activation and subsequent calcium release, signaling through the BCR may result in the activation of a specific transcriptional factor, thus leading to distinct outcomes, such as B-cell proliferation, differentiation, or apoptosis.[22,27]

These insights into the signaling pathways regulating B-cell function and development have undoubtedly contributed to the creation of novel therapeutics for CLL and other lymphoid malignancies.

Combined assessment of clinical, biological, and genetic information to predict outcome

For a long time, Rai or Binet stages have helped to stratify patients according to a disease-specific risk. With the new therapies, the prognostic value of these staging systems has decreased, no longer differentiating intermediate from advanced stages.[29] Over the last decades, a large number of biomarkers have been identified that provide additional prognostic information.[30-32] The most relevant prognostic parameters extracted from the clinical trials with long follow-up are *IGHV* mutational status, serum β_2-microglobulin, and the presence of del(17p) and/or *TP53* mutations. Usually, high-risk CLL is defined, at least in part, by genetic aberrations of the *TP53* gene [ie, del(17p) or *TP53* mutations]. The plethora of genetic markers obtained by next-generation sequencing has not yet provided additional prognostic or predictive markers that are sufficiently validated, and these need to be further tested in clinical trials.

Using some of these markers, a number of prognostic scores and stratification systems have been proposed based on multivariate analyses to extract the most significant independent prognostic information from the plethora of known prognostic markers.[29,33,34] These models are very useful for identifying high-risk patient populations for experimental protocols, as well as those patients with a very good prognosis, even at advanced stages. One of these prognostic scores, the CLL international prognostic index, consists of a weighted score that includes the clinical stage, age, *IGHV* mutational status, serum β_2-microglobulin, and presence of del(17p) and/or *TP53* mutations.[34] It was originally developed using datasets of >4500 patients treated within or outside of clinical trials, separates 4 prognostic subgroups, and has been validated extensively.[35-41]

Design of clinical trials: the art of being creative and systematic at the same time

The recent above-described progress in our understanding of the biology of CLL has allowed us to develop new more-targeted therapeutic options for CLL. Within <20 years, these improvements have profoundly changed the management and the outcome of patients with this disease. The following paragraphs summarize these improvements and describe principal insights that we have gained by several generations of clinical trials since the 1990s (Figure 1).

From chlorambucil monotherapy to chemoimmunotherapy (German CLL Study Group [GCLLSG] trials CLL1-11)

Intensification of chemotherapy in elderly or unfit CLL patients does not improve their outcome. Chlorambucil was the standard treatment of CLL for several decades, and attempts to improve the outcome by combinations of cytotoxic agents, even when using polychemotherapy regimens that were highly active in other lymphoid malignancies, such as CHOP (cyclophosphamide, doxorubicin, vincristine, and prednisone) or CVP (cyclophosphamide, vincristine, and prednisone), remained frustrating.[42-44] Although the use of purine analogs (fludarabine), alone or in combination with cyclophosphamide, improved the quality and duration of responses in younger CLL patients,[45-47] it failed to produce a meaningful benefit in elderly or unfit patients with CLL.[44,48,49] From these trials, particularly from the CLL5 and CLL11 protocols, we have learned that elderly patients with CLL do not benefit from intensified chemotherapy (including purine analogs) and that the biology of CLL may be different in elderly patients compared with younger patients. This was also supported by sequencing data in elderly patients treated within the CLL11 protocol, which yielded a somewhat different spectrum of genomic aberrations compared with younger patients.[50]

Collectively, this generation of clinical trials demonstrated that younger patients could be safely treated with chemotherapy

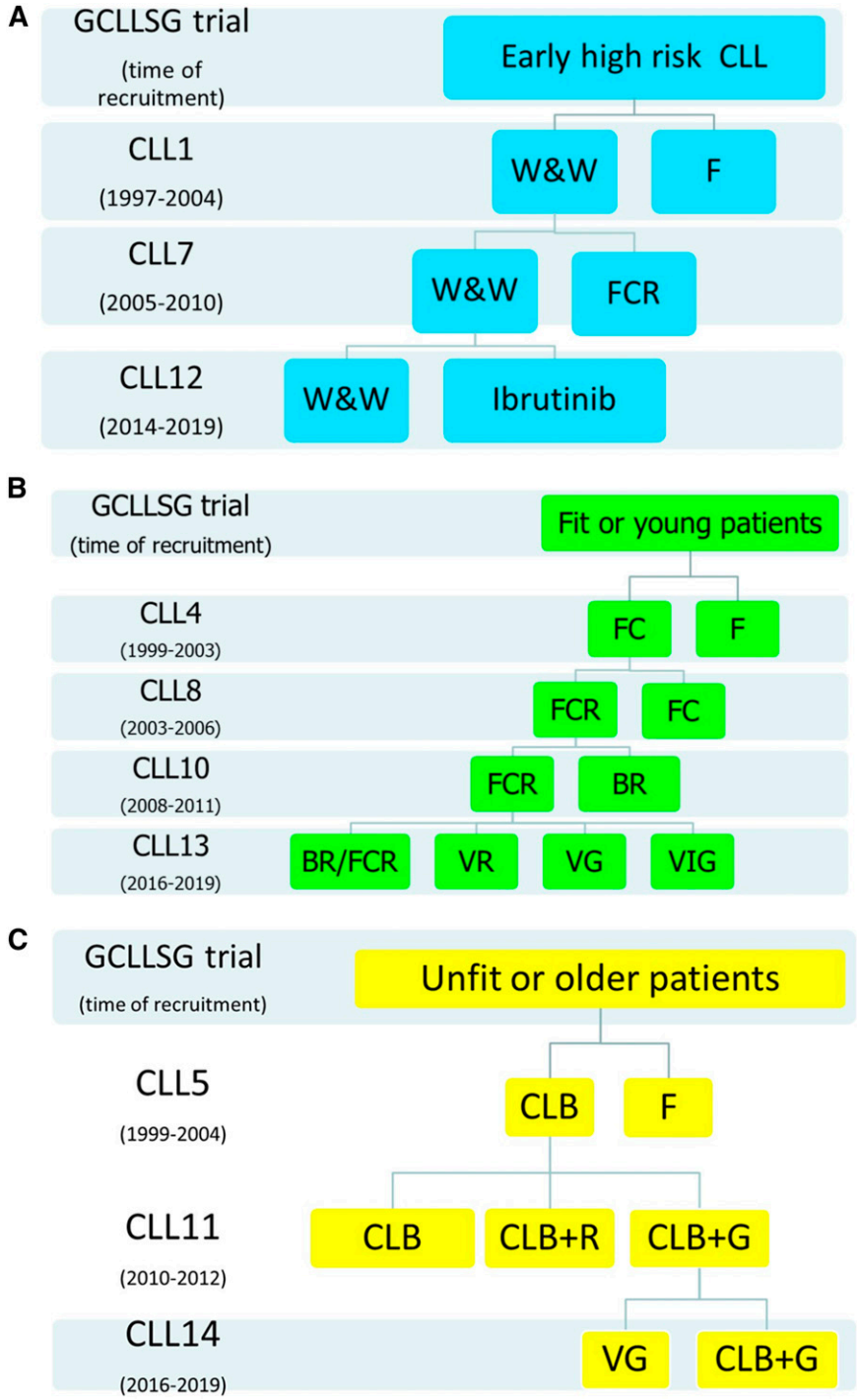

Figure 1. Pedigree of the different clinical studies of the GCLLSG from 1997 until today. Note that the treatment arm yielding a superior outcome in a trial systematically defines the standard or control arm of the next trial. (A) Studies of early asymptomatic, but high-risk, CLL. (B) Studies of symptomatic CLL in fit or young patients. (C) Studies of symptomatic CLL in unfit or elderly patients. Numbers in brackets indicate the start and end of recruitment of patients into the trials. B, bendamustine; C, cyclophosphamide; CLB, chlorambucil; F, fludarabine; G, obinutuzumab (formerly called GA101); I, ibrutinib; R, rituximab; V, venetoclax; W&W, watch and wait.

combinations, such as fludarabine and cyclophosphamide (FC; without yielding very relevant differences in overall survival), whereas the gold standard for elderly unfit patients remained monotherapy with chlorambucil. Finally, it became apparent that different trials and treatment concepts were needed for young fit CLL patients vs elderly unfit CLL patients.

Addition of anti-CD20 antibodies to therapy prolongs overall survival. Building on the success of combinations using purine analogs and cyclophosphamide, initial attempts to add the anti-CD20 antibody rituximab to the FC chemotherapy backbone (FCR regimen) proved surprisingly successful,[51] in contrast to the somewhat disappointing results obtained with rituximab monotherapy.[52-54] Based on

these results, the GCLLSG initiated the CLL8 protocol, a phase 3 trial evaluating the addition of rituximab to FC (FCR vs FC) (Figure 1B). This randomized protocol was the first to show that the choice of first-line therapy could improve overall survival of CLL patients.[55]

Consequently, the addition of anti-CD20 antibodies was also evaluated in a trial for elderly unfit CLL patients with comorbidities using chlorambucil as a standard comparator arm (CLL11 protocol) (Figure 1C). Again, the combination of chlorambucil with both anti-CD20 antibodies, rituximab and obinutuzumab, produced a survival benefit compared with chlorambucil monotherapy, even in these elderly CLL patients.[56,57] Interestingly, the introduction of the more potent type II antibody, obinutuzumab, yielded a survival benefit compared with rituximab (V. Goede, K. Fischer, M. J. Dyer, L. Müller, L. Smolej, M. C. Di Bernardo, A. Knapp, T. Nielsen, and M.H., manuscript submitted). Based on these results, chemoimmunotherapy using anti-CD20 monoclonal antibodies has become a standard first-line treatment of CLL patients, independent of their age or fitness.

The long-term follow-up of the CLL8 trial and the MD Anderson patient cohort treated with FCR has been reported recently. A very good outcome was demonstrated for specific subgroups of patients, in particular those with a mutated IGHV, del(13q), trisomy 12, or del(11q), or for those patients achieving a remission without detectable minimal residual disease (MRD; commonly called MRD-negative remission).[58-60] The CLL8 protocol demonstrated that FCR treatment of CLL patients that showed a combined occurrence of mutated *IGHV* genes plus del(13q), del(11q) or trisomy 12 yielded an overall survival rate $>$ 90% at 5 years. The CLL10 protocol showed that the combination of bendamustine with rituximab was much less efficient in achieving lasting remissions.[61] Together, the results suggest that relevant genetically defined subgroups of fit CLL patients (~25%-30%) may be cured or experience long-term remissions when treated using FCR chemoimmunotherapy.[62]

Concerns have been raised regarding an increased frequency of secondary malignancies, in particular acute myeloid leukemia (AML) and myelodysplastic syndrome (MDS), following fludarabine-based therapies. In a trial of 278 CLL patients, 13 cases (4.7%) of therapy-related myeloid neoplasms were reported at a median of 5 years from initial therapy: 9 after FC and 4 after fludarabine alone.[63] Therefore, a careful assessment of all secondary neoplasias was conducted in 800 patients receiving FC or FCR at a median observation time of 5.9 years.[59] A total of 136 cases of secondary malignancies was reported in 122 (15.3%) patients, including 40.4% solid tumors (including melanoma, 55 cases), 27.9% Richter transformation (38 cases), 17.6% hematologic neoplasias (24 cases), and 14% other skin cancers, such as squamous cell basalioma (19 cases). Only 14 cases of MDS or AML were observed in 13 patients (6 [1.5%] for FCR and 7 [1.8%] for FC) with a median time to onset of 39 and 40 months after last dose of study treatment with FC and FCR, respectively. There was no significant difference in the time to development of MDS/AML between the treatment arms. Secondary malignancies occurred in 53 (13.1%) patients after FCR and 69 (17.4%) patients after FC therapy, with a median time to onset of 2 years after the start of treatment. At 5 years after the start of treatment, 89.1% vs 83.2% of FCR- or FC-treated patients were free of secondary malignancies. Richter transformations were observed twice as often in the FC arm (13 [3.2%] for FCR and 25 [6.3%] for FC). In summary, the risk for secondary AML or MDS following FCR (or FC) therapy may have been somewhat overestimated in the past, whereas the potential benefit of preventing Richter transformation by FCR may have been underestimated.

The principal lessons learned from this generation of clinical trials were that the choice of first-line therapy in CLL is relevant and changes the natural history of the disease; therefore, we need to use our best treatment first. Also, anti-CD20 antibodies are very relevant components of CLL therapy, and long-term control (or cure?) of CLL is possible by choosing the optimal first-line therapy.

Early therapy does not change the outcome of CLL. It was shown in the 1990s that the early use of chlorambucil did not generate a meaningful improvement for CLL patients.[64] The GCLLSG has generated 2 trials, CLL1 and CLL7 (together with the French CLL study group), which have systematically tested the use of fludarabine or FCR in high-risk early-stage patients (Figure 1A). So far, these trials have failed to substantially improve the outcome of CLL patients.[65,66] Therefore, a watch-and-wait policy remains the standard of care for early-stage asymptomatic CLL patients. The role of early intervention with novel inhibitors like ibrutinib remains to be evaluated; trials regarding this question are currently underway (CLL12 protocol) (Figure 1A).

MRD is a highly relevant end point. Like in other malignancies, the complete eradication of the leukemia is an obvious and desired end point.[67] At least 3 different methods, sensitive multicolor flow cytometry, polymerase chain reaction, and next-generation sequencing, are able to detect MRD in CLL patients who otherwise achieve a complete response. Efforts to refine and harmonize these technologies have established that a typical flow cytometry–based assay comprises a core panel of 6 markers (CD19, CD20, CD5, CD43, CD79b, and CD81).[68] Patients are defined as having undetectable MRD (MRD-negative) remission if they have blood or marrow with $<$1 CLL cell per 10 000 leukocytes.

There is ample evidence from prospective controlled clinical trials with long-term follow-up that therapies that are able to achieve MRD-negative remissions consistently result in a significant improvement in clinical outcome, including a longer overall survival.[69-74] From studies of MRD in patients treated with chemo(immuno)therapy within the CLL10 and CLL8 protocols, we have learned that the assessment of MRD seems more relevant than the clinical response assessment of CLL to predict the outcome.[70]

Combinations of novel targeted agents (CLL12-CLL14)

More recently, the advent of targeted agents, such as ibrutinib,[75] idelalisib,[76] and venetoclax,[77,78] has improved our therapeutic armamentarium in a very impressive way. Based on our experience from previous generations of clinical trials, we sought to systematically combine different mechanisms of action rather than testing monotherapy for CLL patients. Moreover, it rapidly became clear that single agents would not achieve long-lasting complete remissions.

Therefore, the fourth generation of clinical trials of the GCLLSG is currently comparing various combinations of venetoclax with obinutuzumab or rituximab and ibrutinib for the first-line therapy for CLL against the previous standard therapies for fit and unfit CLL patients (CLL13 and CLL14 protocol) (Figure 1). Preliminary results obtained from the safety run-in phase of the CLL14 protocol that included 12 patients have shown that the combination of venetoclax and obinutuzumab produces a very high number of complete remissions, as well as MRD-negative responses ($>$90%).[79] It is anticipated that this represents a new treatment paradigm for CLL. Encouraging preliminary data have also been reported recently regarding the combination of venetoclax plus ibrutinib, with or without obinutuzumab.[80-83] Therefore, we have entered a new era in which

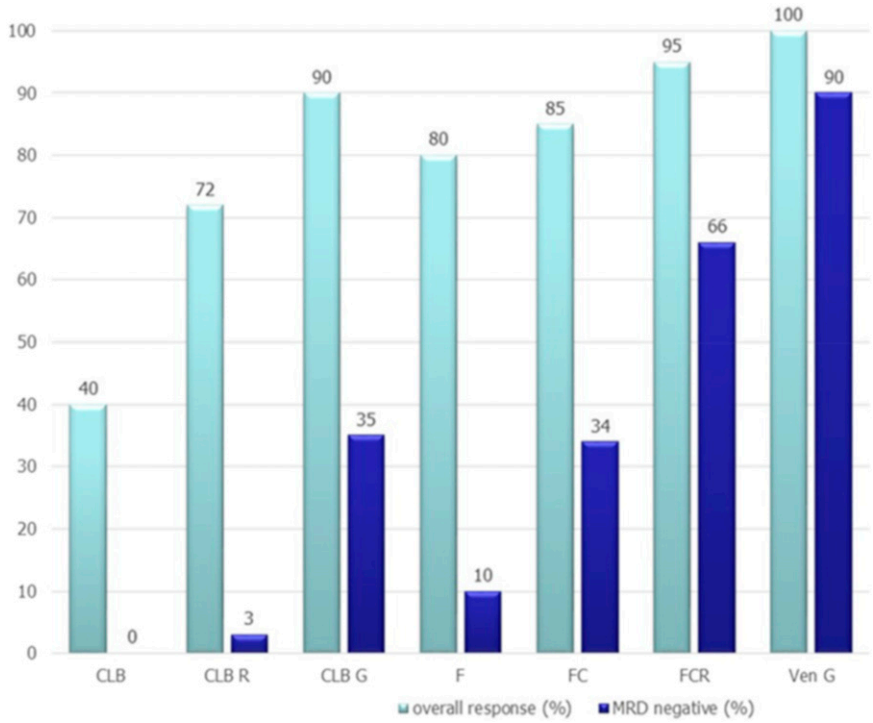

Figure 2. Overall responses and MRD-negative responses obtained with various CLL therapies in the randomized controlled trials of the GCLLSG, from CLL1 to CLL14. Results are shown as reported by the protocols CLL4, CLL8, CLL10, CLL11, and CLL14 run-in study (Figure 1). C, cyclophosphamide; CLB, chlorambucil; F, fludarabine; G, obinutuzumab (formerly called GA101); R, rituximab; Ven, venetoclax.

combinations of targeted noncytotoxic agents achieve long-lasting remissions for the majority of CLL patients. Figure 2 illustrates the impressive progress in overall responses and MRD-negative responses that we have achieved over the last 2 decades in this disease.

Given this impressive progress, one may discuss whether a precision medicine approach will finally prevail for CLL. Because the efficacy of the current therapeutic combinations seems so high and valid across all major subgroups, we may not need to further personalize future therapies in CLL to a high degree. Only carefully conducted trials with long-term follow-up and a precise description of the side effects will clarify this question, given the impressive short-term efficacy of these novel therapies.

Future trials and concepts

It has been demonstrated recently that the development of CLL follows various patterns of clonal evolution.[84] This has opened up the possibility to monitor the clonal composition of even single leukemia cells under selective pressure by novel therapeutic combinations. Therefore, the GCLLSG has started to design clinical trials that carry different elements to prevent clonal evolution. These concepts attack the clonal evolution of this leukemia at different time points. The first strategy consists of "cutting the stem" of the evolutionary tree, and the second consists of "cutting the branches" that are recognized during monitoring of the clonal development (Figure 3).

A therapeutic concept to prevent clonal evolution has been proposed that uses sequential targeted therapies to eradicate MRD and a maintenance phase for total (MRD-negative) eradication of CLL.[85] The treatment intensity is tailored by assessing MRD.[85] Importantly, in these trials we have included a tumor-debulking treatment with bendamustine, which may sound like an old-fashioned concept. However, it is possible that this debulking step reduces the heterogeneity of the malignant clones at the beginning of the therapy. Moreover, the debulking has proven beneficial in terms of reducing the initial treatment side effects (tumor lysis syndrome and infusion-related side effects) of potent agents, such as venetoclax and obinutuzumab. Initial results obtained with this type of therapy have been very promising. In particular, the BAG protocol (1-2 courses of bendamustine, followed by ABT-199 [venetoclax] and GA-101 [obinutuzumab]) was able to achieve excellent overall response and MRD-negative response rates of ~90% in treatment-naive and pre-treated patients.[86] In future trials, we will monitor leukemic evolution by targeted sequencing and eventually treat upcoming resistance-defining mutations with novel agents. This concept is inspired by the findings that some of the resistance-defining mutations (such as BTK^{C481S} for ibrutinib or *TP53* mutations for chemotherapy) may exist prior to the start of therapy or can be recognized very early and might be prevented by the (pre-emptive) use of agents targeting these mutations.[87-89] It is highly likely that we will be able to predict the onset of resistance-defining mutations at the beginning of therapy (in high-risk CLL [ie, with *TP53* gene aberrations]) and design combination therapies to prevent the development of unfavorable conditions, such as transformation into diffuse large cell lymphoma (Richter syndrome),[90] with the eventual help of probabilistic modeling of the clonal evolution.

Render therapies available to everyone in need

During my lecturing activities on CLL in many regions and countries of the world, I have come to realize that the therapeutic concepts used for CLL patients strongly depend on the availability of novel drugs. Like in other cancers, the increasing disparity is creating profound differences in the management and outcome worldwide.[91] I am convinced that it is our task as physicians to ensure that every CLL patient can obtain the optimal treatment (ie, according to the

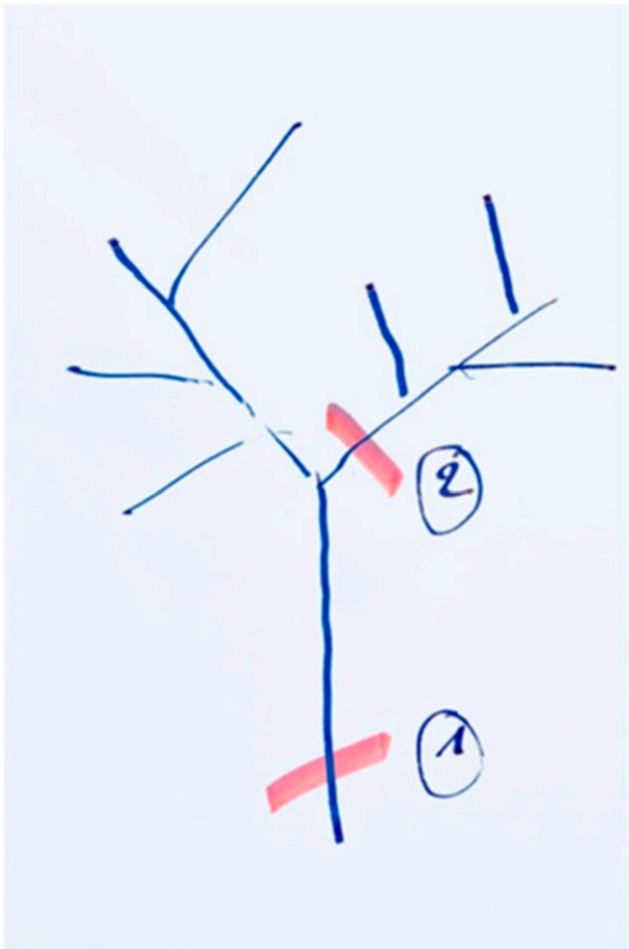

Figure 3. Treatment strategies to prevent clonal evolution of CLL. Therapies may aim to cut the stem (1) or cut the branches (2) of the phylogenetic tree. The reader is referred to the section on Future trials and concepts for a detailed explanation of the 2 concepts.

current evidence). Therefore, I advocate to create national or international study groups or pressure groups of our profession, together with our patients, to influence our health care systems, politicians, and the companies acting in these markets to realize their responsibility for providing novel effective therapies to all patients with CLL.

Conclusion and prospects for future improvement

The past 30 years have created an impressive progress in CLL therapy and management. We understand the biology of the disease much better than 30 years ago. By using a systematic approach that incorporates, in a comprehensive way, improved technologies and novel insights from basic discoveries, we have constructed a series of clinical trials that led to an improved outcome for CLL.

Creating clinical trials in an era of dynamic innovation has similarities to the comprehensive concept of designing architecture, where construction needs to incorporate novel technologies, materials, changing needs of future users and inhabitants, and trends in fashion and style into 1 "Gesamtkunstwerk" (ideal work of art).[92] The same type of effort is needed to incorporate very different aspects, such as setting up and managing a study group, negotiations with health care providers and pharmaceutical companies, and the incorporation of novel insights from basic and translational research, to create meaningful practice-changing trials. In contrast with laboratory experiments, these clinical studies cannot be repeated quickly, if ever; therefore, they need to be planned meticulously to ensure the greatest value.

With the most recent advances in CLL therapy, in particular the new targeted agents and antibodies, there is justified hope that our united effort will ultimately lead to the control or cure of CLL in most of our patients.

Acknowledgments

The author is grateful to all of his collaborators, the patients, and his family for their support of this important project over many years. He looks forward to the next steps that will hopefully achieve the long-lasting control or cure of this leukemia over the next few years to come. The author thanks Kirsten Fischer and Nisha de Silva for very helpful suggestions and comments on this manuscript.

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