Keynote Lectures

Henry Kaplan Memorial Lecture

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001 | Towards Control of Chronic Lymphocytic Leukemia with Targeted Agents

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Chronic lymphocytic leukemia (CLL) represents one of the most active fields of clinical research at the present time.12 This short review summarizes some of the basic, translational and clinical research that has led to substantial improvements of the management of patients with CLL over the past two decades.

Biology: The use of genetic and genomic technologies has led to an improved understanding of the biology of CLL. Studies using fluorescent in-situ hybridization (FISH) and chromosome banding have described recurrent and frequent aberrations in CLL, some of which, such as del(17p) have demonstrated profound prognostic impact.3 Moreover, the mutational composition of immunoglobulin heavy chain variable region (IGHV) genes separates two apparently related, but biologically and clinically different forms of CLL.4,5 These findings suggested a central role of B-cell receptor (BCR) signaling in this leukemia. In a search for the relevant genes disrupted by del(13q), found in almost 50% of CLL cases, it was discovered that this deletion causes the loss of miRNAs (miR-15a and 16-1), which initiate leukemogenesis.6,7 It was suggested that these miRNAs induce the up-regulation of Bcl2 protein that is usually highly overexpressed in CLL.8 More recently, whole exome sequencing has enabled the description of the genomic landscape of CLL.9,10 From these studies we have learned that genes regulating inflammatory pathways, BCR signaling and differentiation, Notch signaling, Wnt signaling, DNA damage control, chromatin modification, RNA and ribosomal processing are frequently altered in CLL.10 Signaling through the BCR seems to play an important role for the survival of CLL cells.11 BCR activation in CLL cells induces the activation of several tyrosine kinases, such as Src family kinases (in particular LYN), Bruton tyrosine kinase (BTK), Spleen tyrosine kinase (SYK), as well as phosphoinositide 3-kinases (PI3K).12 In addition to BCR signaling, CLL receive essential growth support through various cell-membrane-bound and soluble factors produced in their cellular microenvironment.12 Leukemia associated macrophages are particularly important components of the microenvironment and support the growth of CLL cells.13,14 Even the efficacy of conventional therapeutics such as chemotherapy with alkylators and monoclonal antibodies seem to mediate their effects through compartment restricted interactions with macrophages.15 More recently, we could show that targeted disruption of BCR associated kinases such as LYN or BTK reduces the capacity of leukemia-associated macrophages or fibroblasts to “feed” CLL growth.16

Prognosis: The clinical staging systems of Rai or Binet stratify patients according to their disease-specific risk. With the new therapies, the prognostic value of these staging systems has decreased, no longer differentiating intermediate from advanced stages.17 A large number of biomarkers have been identified that provide additional prognostic information.18-20 The most relevant prognostic parameters extracted from the clinical trials with long follow up are IGHV mutational status, serum ß2-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 (i.e. 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.17,21,22 These models are useful to identify high-risk patient populations for experimental protocols, but also those patients with a very good prognosis even at advanced stages. The CLL international prognostic index (CLL-IPI) consists of a weighed score that includes the clinical stage, age, IGHV mutational status, serum ß2-microglobulin, and the presence of del(17p) and/or TP53 mutations.22 It separates four different prognostic subgroups, and has been validated extensively. A system for predicting the time to first treatment (TTFT) in patients with CLL with early, asymptomatic disease was recently proposed (International Prognostic Score for Early-stage CLL [IPS-E]).23 Three covariates, unmutated IGHV gene, absolute lymphocyte count higher than 15 x 10⁹/L, and presence of palpable lymph nodes were combined and predict a 5-year cumulative risk for treatment start of 8.4%, 28.4%, and 61.2% for low-risk, intermediate-
risk, and high-risk patients, respectively. The IPS-E will be helpful to counsel patients with early stage CLL.

**Minimal residual disease:** Like in other malignancies, the complete eradication of the leukemia is an obvious and desired endpoint. At least three different methods, sensitive multicolor flow cytometry, PCR, or next-generation sequencing are able to detect minimal residual disease (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 six markers (i.e. CD19, CD20, CD5, CD43, CD79b and CD81). Patients are defined as having undetectable MRD (MRD-neg) remission if they have blood or marrow with less than one CLL cell per 10,000 leukocytes.

Today, there exists ample evidence from prospective, controlled clinical trials with long-term follow-up, that therapies that are able to achieve MRD-neg remissions consistently result in a significant improvement of clinical outcome, including a longer overall survival.

From studies of MRD in patients treated with chemo(immuno)therapy protocols, we have learned that the assessment of MRD seems more relevant than the clinical response assessment of CLL to predict the outcome. The Combined Use of Initial prognostic information, as provided by the CLL-IPI and the dynamic assessment of the treatment response (by MRD assessment) may provide methods to dynamically determine outcome probabilities for individual patients utilizing risk predictors acquired over time. The Continuous Individualized Risk Index (CIRI) shows that in CLL a dynamic risk assessment allows to accurately predict the outcome following chemoimmunotherapy.

**Therapy: Chemoimmunotherapy:** Progress in CLL therapy has been initiated by using combinations of purine analogs and cyclophosphamide, in particular fludarabine and cyclophosphamide (FC). Thereafter, the anti-CD20 antibody rituximab was added to this FC chemotherapy backbone (FCR), yielding impressive response rates. Based on these results, the GCCLSG initiated the CLL8 protocol, comparing FCR to FC. This randomized protocol was the first to show that the choice of first line therapy, FCR, could improve overall survival of CLL patients. The benefit of anti-CD20 antibodies was also shown for CLL patients with comorbidities using chlorambucil as a standard comparator arm (CLL11 protocol). Interestingly, obinutuzumab, a more potent type II antibody, yielded a survival benefit when compared with rituximab. The long-term follow up of patients treated with FCR demonstrated that for specific subgroups with a mutated IGHV, del (13q), trisomy 12 or del(11q), or for those patients achieving a remission without detectable minimal residual disease (commonly called MRD-negative remission), FCR treatment of CLL patients with the combined occurrence of mutated IGHV genes plus del(13q), del(11q) or trisomy 12 yielded an overall survival rate above 90% at 5 years. Together, the lessons learned from these clinical trials were: 1) The choice of first line therapy in CLL is relevant and changes the natural history of the disease. 2) We should give our best treatment first. 3) Anti-CD20 antibodies are relevant components of CLL therapy. 4) Long-term control (or cure) of CLL seems possible.

**Targeted agents:** More recently, the advent of targeted agents such as ibrutinib, idelalisib, or venetoclax has further improved the armamentarium of CLL therapies. As it became rapidly apparent that single agents would not achieve long-lasting complete remissions, we sought to systematically combine different mechanisms of action rather than testing monotherapy for CLL patients. In the CLL2-BAG trial patients received sequential treatment of debulking with two cycles of bendamustine followed by induction and maintenance with obinutuzumab and venetoclax, yielding very high response rates of 95%. Therefore, CLL14 protocol investigated a fixed-duration treatment with venetoclax and obinutuzumab (VO) compared with chlorambucil-obinutuzumab and showed that the progression-free survival at 24 months was significantly higher in the VO group than in the comparator arm (88.2% vs. 64.1%). This benefit was also observed in all major subgroups. Very encouraging results have also been reported recently regarding the combination of venetoclax plus ibrutinib.

**Future prospects:** We have entered a new era where combinations of targeted agents achieve long-lasting remissions for the majority of CLL patients. In addition, therapy with ibrutinib as a single agent delivers long-term disease control, even in high-risk CLL. Therefore, one of the most important questions regarding CLL therapy is the comparison of two different treatment concepts, fixed duration therapy aimed at achieving maximal response (undetectable MRD) versus long-term disease control with single agent BTK inhibitors. The CLL17 protocol that has just opened recruitment will address this important question. Moreover, we need to intensify our effort to understand and neutralize the cellular mechanism of resistance to the new inhibitors.
Finally, and most importantly, we need to make sure that these novel therapies will become available to all patients with CLL world-wide.

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GIANNI BONADONNA MEMORIAL LECTURE
(in collaboration with American Association for Cancer Research, AACR)
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002 | THE ARCHITECTURE OF LIQUID BIOPSY RESEARCH FOR LYMPHOMA MONITORING

Quantification and qualification of circulating tumor material is an emerging biomarker of lymphoma that may be used for tumor load estimation, clonal evolution monitoring, treatment response assessment and early identification of clinically occult relapse. Circulating