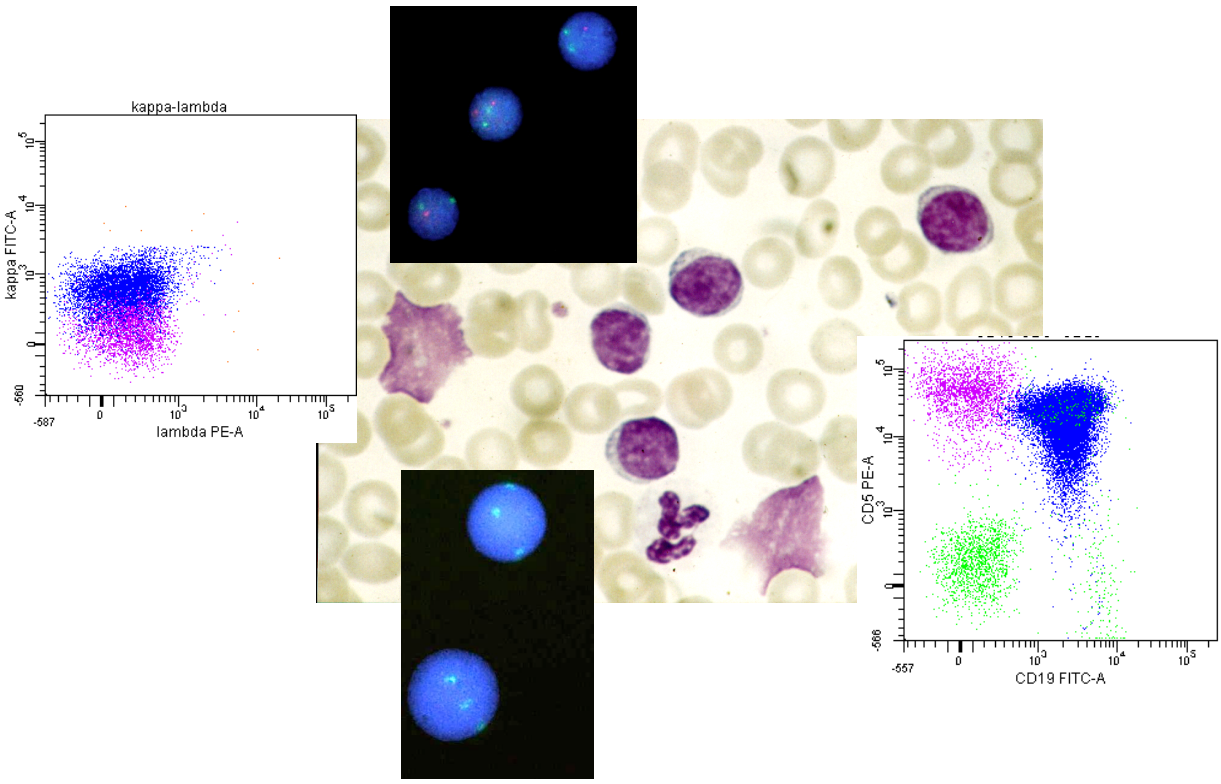
Suppl. n. 1 April 2018 of LETTERE GIC

ITALIAN CYTOMETRY SOCIETY
SCHOOL

FOCUS ON
CHRONIC LYMPHOCYTIC LEUKEMIA



Guest Editor
Giovanni D'Arena, MD, PhD

FOCUS ON
CHRONIC LYMPHOCYTIC LEUKEMIA
Copyright © GIC Società Italiana di Citometria

I diritti di traduzione, di memorizzazione elettronica,
di riproduzione, e di adattamento totale o parziale con qualsiasi mezzo
(compresi i microfilm, le copie fotostatiche e copie informatiche)
sono riservati per tutti i paesi.

Supplemento n. 1 a "Lettere GIC"
Periodico della Società Italiana di Citometria
Autorizz. del Trib. di Roma n. 512/92 del 17/09/1992
ISSN 2280-8663
Vol. 27, n. 1 aprile 2018
Direttore Responsabile
Raffaele De Vita

Segreteria GIC:
Società Italiana di Citometria
c/o Divisione Tecnologie e Metodologie per la Salvaguardia della Salute
ENEA Centro Ricerche Casaccia, s.p. 016
Via Anguillarese, 301 - 00123 ROMA
☎ 06/30484671
e-mail: gic@enea.it http://www.citometriagic.it

PROCEEDINGS
OF THE MEETING
OF THE ITALIAN CYTOMETRY SOCIETY

FOCUS
ON
CHRONIC LYMPHOCYTIC LEUKEMIA

April 28th, 2018
Palinuro
Italy

Giovanni Pizzolo, Luca Laurenti, Nicholas Chiorazzi, Dimitar Efremov,
Giovanni D'Arena, Mario Luppi, Gianmatteo Rigolin

Giuliano Mazzini

Degree in Biology (110/110 cum laude)
The main research lines included fluorescence methodologies for quantitative analyses, mainly devoted to DNA and technical developments of instrumentation for fluorescence microscopy and cytometry. In particular he had been a national pioneer in the "Flow cytometry" research in both methods and instruments development and upgrade.
1985-89 Italian member of the CEE Concerted Action "Automated and analytical Cytology".
1979-87 Co-resp. and resp.of Research Groups of CNR Project "Control of Neoplastic Growth" (sub-projects "Chemotherapy" and "Cell biology") and later involved in the Project "Biotechnology and bioinstrumentation".
1986 Active founding member of the Scientific Society "Gruppo Italiano di Citometria GIC" (today turned to "Società Italiana di Citometria GIC), President of the Society and still Council member.
1986 Scientific responsible of the "Laboratorio di Citofluorimetria" of the " Centro Grandi Strumenti" of the Pavia University.
1997 Co-resp. of "Corso di Perfezionamento in Citometria a flusso applicata all'Oncologia Medica" of the Pavia University Medical Faculty.
1998 Head of the "National School of Cytometry" hold by "Italian Society of Cytometry".
Author and co-author of more than 200 scientific articles published on qualified international journals.

FOREWARD

This workshop in Palinuro is the third in a series that can be defined a "satellite event" of the GIC Italian Society of Cytometry. The GIC, which boasts a history of almost half a century, has had since its beginnings a mission of didactic-formative nature as well as promoting the development of cytometric applications at national level. Despite being a multidisciplinary scientific society that gathers members in all the application areas of Flow Cytometry (from Biomedical Sciences to Biotechnology and Environmental Science including Plant Sciences and Marine Biology) has obviously a "particular focus" on the clinico-medical area where the impact of FC is decisive in the diagnostic process of many pathologies. In the field of clinical applications, the hematologic area and even more the onco-hematologic one has become more and more important year by year. Since the evolution of technology and methodology on the one hand and the knowledge of the most fine mechanisms of development of the disease and the improvement of the therapy, have seen FC more and more unbeteable analytical approach. The GIC President elect Giovanni D'Arena internationally recognized as a qualified hematologist and great supporter (since the beginning of his professional adventure in S. Giovanni Rotondo) of the role of cytometry in the field of hematology, wanted to dedicate this event to the theme of the Chronic Lymphocytic Leukemia. The analytical impact of FC in the diagnosis of these hematological deseases is universally recognized. It is therefore important (for the GIC mission) that a continuous and constant improvements of the knowledge and of the methodological approaches will spread and up-grade national wide. Great is in fact the number of young operators who apply in this area and, to them, the GIC addresses a special formative attention.
As head of the National School of Cytometry GIC I would like to thank the President Giovanni Arena for the effort and commitment he has also made this time in the organization of this event. We are also all in debt with our friend Raffaele De Vita (GIC Secretary/Treasurer) for the great efforts he always spend supporting all the Meeting and GIC events.
Giovanni has managed to bring to the beautiful site of Palinuro a large number of young haematologists eager to learn the "state of the art" on the subject, but also to consolidate their basic knowledge on the best diagnostic strategies allowed by multiparametric FC. Giovanni has also managed to coordinate the best of the faculty available on this subject inviting the most qualified experts in the field not only from all over Italy but also from overseas! The venue of the Conference (beautiful scenery of nature and sea) is also an ideal place of interchange between teachers and "students" who have had many moments (in addition to front-teaching in the classroom) to confront and exchange professional experiences.

Giovanni D'Arena

After obtaining his MD at "La Sapienza" University in Rome, he completed his post-graduate training in Hematology at the "Catholic University of Sacred Heart" in Rome and PhD in Medical and Surgical Advanced Therapy at the "Federico II" University of Naples.
He worked at the Hematology Department of IRCCS "Casa Sollievo della Sofferenza" Hospital, San Giovanni Rotondo and at the Hematology Department of National Cancer Institute Fondazione "Pascale", Naples.
Since 2011 he works at the Hematology Department of IRCCS "Centro di Riferimento Oncologico della Basilicata", Rionero in Vulture, Italy.
He is particularly interested in diagnosis and treatment of acute and chronic leukemias, lymphomas, multiple myeloma, and in autologous stem cell transplantation. Special field of interest is flow cytometry and chronic lymphocytic leukemia, the management of its autoimmune complications, and new treatment approaches.
He is author of 146 peer reviewed scientific articles.
In April 2017 he was elected President of the Italian Cytometry Society.

THE RELEVANCE OF FLOW CYTOMETRY TO DIAGNOSE CHRONIC LYMPHOCYTIC LEUKEMIA

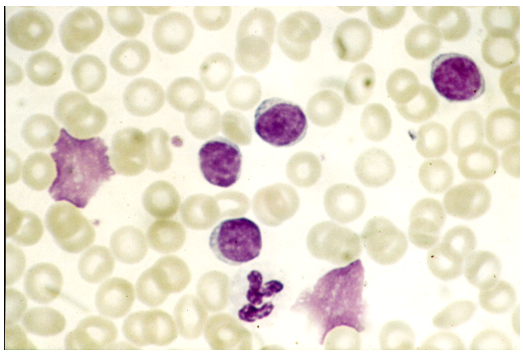
Chronic lymphocytic leukemia (CLL) is a clonal disorder characterized by the expression of small, mature-appearing, immunologically competent, long-lived B cells, that accumulate in the blood, bone marrow, and lymphoid tissues. CLL is recognized as a neoplasm of mature B cells in the World Health Organization (WHO) classification[1,2].

CLL is the most common type of leukemia in developed countries with an incidence of 4.2:100000/year. The incidence increases with age and is higher in men that women. The median age at diagnosis is 72 years, while about 11% of patients are diagnosed under the age of 55 years.

According to the International Workshop on Chronic Lymphocytic Leukemia/National Cancer Institute-Working Group guidelines[3], to diagnose CLL the following criteria must be meet.

presence of at least 5 x10^9 monoclonal B lymphocytes/L.

leukemic cells found in the blood smear are characteristically small, mature-appearing lymphocytes with a narrow border of cytoplasm and a dense nucleus lacking discernible nucleoli, and having partially aggregated chromatin (Figure 1, see below).



Larger, atypical lymphocytes or prolymphocyes may be seen but must not exceed 55% of the blood lymphocytes (in the latter case the diagnosis of prolymphocytic leukemia is made). Gumprecht nuclear shadows, or smudge cells, are other characteristic morphologic features of CLL.

On physical examination, lymphadenopaty (single or diffuse node involvement) can be observed. Enlargement of spleen and liver may be also seen. Bone marrow infiltration by CLL cells can cause anemia and/or thrombocytopenia, as well.

These clinical and laboratory features are usually used to stage CLL. Patients can be assigned to each stage according to clinical (node, spleen, and liver enlargement) and laboratory (hemoglobin level and platelet count) features. Each stage has a prognostic relevance as clearly showed by the different overall survival.

The differential diagnosis includes primarily small lymphocytic lymphoma (SLL) and monoclonal B-cell lymphocytosis (MBL). In the former, the number of circulating neoplastic B cells must be less than 5 x10^9/L, while the presence of lymph nodes, liver or spleen enrlargement is a typical feature of SLL that is considered the lymphomatous counterpart of CLL. However, MBL is an asymptomatic condition characterized by the presence of a circulating small clonal B-lymphocyte population in persons who do not have CLL, other B-cell lymphoproliferative disorder, or underlying conditions such as infectious or autoimmune disorders[4]. As showed in Tab. 1, the distinction between MBL and Rai stage 0 CLL resides only on the number of absolute neoplastic B-cells. Furthermore, Landgren et al demonstrated that virtually all patients with CLL have a MBL phase several years before the CLL diagnosis [5].

Tab. 1

Differential diagnosis among CLL, MBL and SLL

	Neoplastic B-cells <5 x10^9/L	**Lymph nodes and/or spleen and/or liver enlargement**
MBL	+	-
CLL	-	+/-
SLL	+	+

CLL cells typically coexpress the T cell antigen CD5 and B cell surface antigens, such as CD19, CD20, and CD23. Surface immunoglobulins, CD20, CD22, and CD79b levels are characteristically lower than found on normal B cells[6] (Fig. 2 and 3).

Fig. 2

CD19 and CD5 co-expression in B-CLL cells (blue) and normal T-cells (purple)

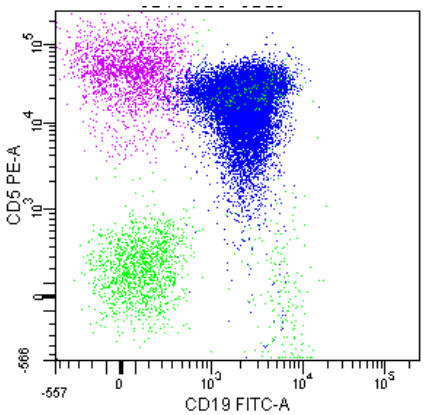
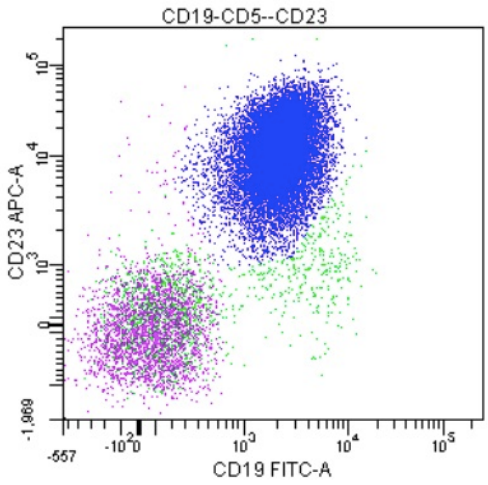
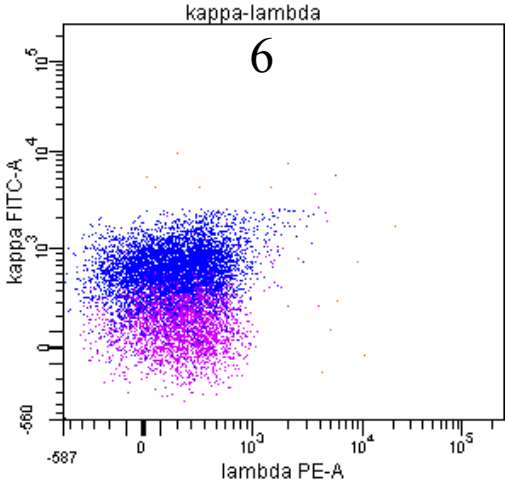


Fig. 3



The clonality of the circulating B lymphocytes needs to be confirmed by flow cytometry. As showed in Fig. 4, clonality is detected by the restricted expression of either kappa or lambda immunoglobulin light chain. A clonal B cell population is arbitrarely defined as having greater than 3:1 or less than 1:2 κ/λ surface immunoglobulin light chain ratio.

Fig. 4



Despite the morphology and the immunophenotypic profile of CLL cells is now well defined and significant progresses in cytogenetics and molecular biology, morphological cell identification and immunological marker analysis remain cornerstones in distinguishing between the different disease entities belongig to the category of mature B-cell neoplasms in leukemic phase, and difficulties in defining some cases still exist. Immunophenotyping techniques result in a better characterization of a number of disease variants, some of which may benefit from different therapeutic approaches. However, because there is no single marker characteristic of a single disease entity, a composite immunophenotypic panel including different B-cell markers is used to distinguish CLL from other mature B-cell neoplasms (Tab. 2).

Tab. 2
Immunological markers for the differential diagnosis of chronic B-cell leukemias

	CLL	**PL**	**HCL**	**HCL-v**	**MZL**	**FCL**	**MCL**
CD19	+	+	+	+	+	+	+
CD20	+ (LD)	+	+	+	+	+	+ (HD)
CD5	+(HD)	-	-	-	-	-	+ (ID or LD)
CD10	-	-	-	-	-	+/-	-/+
CD22	-/+ (LD)	+	+	+	+	+	+ (HD)
CD23	+	-	-	-	-/+	-/+	-
CD25	+/-	-	+ (HD)	-	+/-	-	-/+
CD11c	+/-	-	+ (HD)	+/-	-	-	-
CD103	-	-	+	+/-	-	-	-
CD43	+		+	+			+
CD200	+		+	+			+
SmIg	+ (LD)	+ (HD)	+ (HD)	+ (HD)	+ (HD)	+ (HD)	+ (HD)

Abbreviations: SmIg: surface membrane immunoglbulins; CLL: chronic lymphocytic leukemia; PL: prolymphocytic leukemia; HCL: hairy cell leukemia; HCL-v: variant form of HCL; MZL: marginal zone lymphoma; FCL: follicular cell lymphoma; MCL: mantle cell lymphoma; SmIg: surface membrane immunoglobulin; LD: low density; ID: intermediate density; HD: high density

In the early 90s, the British group from the Royal Marsden Hospital in London proposed a scoring system based on flowcytometric evaluation of 5 membrane markers, namely, CD5, CD22, CD23, FMC7 (that recognizes an epitope of CD20), and surface membrane immunoglobulin (SmIg) (7). A score of 0 or 1 was attributed to each marker based on their characteristic expression on chronic lymphocytic leukaemia (CLL), based on its most classical immunological profile (CD5 positive, CD22 weak or negative, CD23 positive, FMC7 negative, and SmIg weak). The total score—defined as Matutes score—is usually 4 or 5 for typical CLL cases, while other mature B-cell lymphoid neoplasms score 3 or less. In 1997, the same British group improved the score replacing CD22 with CD79b, with an increase in the diagnostic accuracy from 91.8% to 96.8% [8] (Tab. 3).

Table 3

Scoring system for the differential diagnosis of CLL

Points	**1**	**0**
Marker		
CD5	+	-
CD23	+	-
FMC7	-	+
SmIg	Low	Medium/High
CD22/CD79b	Low/-	Medium/High

However, despite Matutes score is a very useful tool in distinguishing typical CLL from its atypical form and non-CLL B-cell neoplasms, some difficulties persist in discriminating atypical CLL from some cases of mantle cell lymphoma (MCL) in leukemic phase.

More recently, CD200, a transmembrane type Ia glycoprotein belonging to the immunoglobulin superfamily and expressed by various cell types (ie, B cells, a subset of T cells including activated T cells, thymocytes, endothelial cells, and neurons), has been shown to have differential expression in B-cell neoplasms. In particular, CD200 is useful in distinguishing CLL from MCL and in discriminating hairy cell leukemia (HCL) from its variant form (v-HCL). Very recently, Kohnke et al revisited the British scores including CD200 and proposed the so-called CLLflow score that retained high sensitivity but showed markedly increased specificity with respect to Matutes score[9]. This German group found that if CLLflow score (= $\%CD200^{+} + \%CD5^{+}/CD23^{+} - \%CD79b^{+} - \%FMC7^{+}$) is higher than zero, a diagnosis of CLL is likely, while a score ≤ 0 suggest a non-CLL entity. We also recently performed a large Italian multicenter retrospective cohort of patients with chronic B-cell leukemias to test the diagnostic relevance of CD200[10]. Moreover, our group recently identified a simplified score for the differential diagnosis of chronic B-cell leukemias which only requires 4 markers (CD5, CD23; CD200, and SmIg).

Finally, several other markers have been tested in CLL and some of these have been shown their prognostic relevance, such as CD38, CD49d, CD69, and ZAP-70.

References

1) Chiorazzi N, Ferrarini M. Cellular origini(s) of chronic ymphocytic leukemia: cautionary notes and additional considerations and possibilities. Blood 2011; 117: 1781-1791.

2) Swerdlow SH, Campo E, Pileri SA, et al. The 2016 revision of the World Health Organization classification of lymphoid neplasms. Blood 2016; 127: 2375-2390.

3) Hallek M, Cheson BD, Catovsky D, et al. Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 2008; 111: 5446-5456.

4) D'Arena G, Musto P. Monoclonal B-cell lymphocytosis. Transl Med UniSa 2014; 8: 75-79.

5) Landgren O, Albitar M, Ma W, et al. B-cell clone sas early markersfor chronic lymphocytic leukemia. N Engl J Med 2009; 360: 659-667.

6) D'Arena G, Keating MJ, Carotenuto M. Chronic lymphorpoliferative disorders: an integrated point of view for the differential diagnosis. Leuk Lymphoma 2000; 36: 225-237.

7) Matutes E, Owusu-Ankomah K, Morilla R, et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994; 8: 1640-1645.

8) Moreau E, Matutes E, A'Hern RP, et al. Improvement of the chronic lymphocytic leukemia scoring system with the monoclonal antibody SN8 (CD79b). Am J Clin Pathol 1997; 108: 378-382.

9) Kohnke T, Wittmann VK, Veit L, et al. Diagnosis of CLL revisited: increased specificity by a modified five-marker scoring system including CD200. Br J Haematol 2017; 179:480-487.

10) D'Arena G, Vitale C, Rossi G, et al. CD200 included in a 4-marker modified Matutes score provides optimal sensitivity and specificity for the diagnosis of chronic lymphocytic leukemia. Hematol Oncol (in press).

THE EVOLUTION OF A STEREOTYPED ANTIGEN-BINDING DOMAIN IN CHRONIC LYMPHOCYTIC LEUKEMIA:DUELLING SELECTIONS FOR FOREIGN AND AUTO-ANTIGENS

Nicholas Chiorazzi

The Feinstein Institute for Medical Research
New York, USA

Dr. Chiorazzi and his group demonstrated that CLL cells respond to signals from the microenvironment, in particular those from the B-cell antigen receptor (BCR), leading to leukemic cell proliferation and maturation or death; that BCR-induced signals are likely delivered by (self) antigens, and, for ~33% of patients, are delivered by BCRs of remarkably similar amino acid structure; that patients with CLL fall into two subgroups based on BCR structure and these differ dramatically in clinical outcome. These findings led to the view that (auto) antigen drive promotes CLL and laid the groundwork for BCR inhibition therapy.

Introduction. The unique features of the BCRs expressed by CLL B cells have led to the hypothesis that BCR -mediated signaling promotes the transformation of a normal B lymphocyte to a CLL B cell. This is best illustrated by the 30% of CLL patients grouped into stereotyped subsets based on HCDR3 similarity and the use of similar IGHV-D-J rearrangements, leading to the assumption that B cells from each subset encountered distinct sets of shared antigenic epitopes that led the way to leukemia. In light of this, a number of autoantigens and exoantigens have been found to bind to CLL BCRs and hence could be involved in the survival and proliferation of CLL B-cell clones. However, this view needs to be reconciled with the finding that CLL BCRs appear to universally undergo homotypic interactions and consequently lead to autonomous signaling in the absence of additional non-IG (self)antigens.

We will discuss the relationship of these two types of specificities (non-self-IG and self-IG) and delineate their effects on the development of the structural elements of the BCRs from a specific stereotyped subset by focusing on stereotyped subset #4. Clones in this subset bear *IGHV4-34/IGHD5-18/IGHJ6* and *IGKV2-30/IGKJ2* rearrangements and are always class-switched to IgG and somatically mutated; they also often exhibit amino acid replacement mutations at defined positions within the antigen-binding site. Hence, this subset provides an opportunity to study the influences of a specific *IGHV* genes and recurrent, restricted somatic mutations on selection for structure and specificity.

Antigen-binding properties of subset #4 monoclonal antibodies (mAbs). We have evaluated the (auto)antigenic specificities of subset #4, using 3 recombinant mAbs belonging to this stereotyped subset. We have demonstrated that these subset #4 mAbs bind to two antigenic epitopes: [1] those on the surface of viable memory human B cells as well as on naïve B cells (the latter already known for other *IGHV4-34*-expressing IGs from patients with systemic autoimmunity, and [2] those on hemagglutinins of influenza A viruses. Moreover, we have documented that the same subset #4 mAbs do not bind to two expected, natural targets of IGs using the *IGHV4-34* gene: [1] i/I-type blood group glycans and [2] ss- and ds-DNA.

Although we have not yet definitively identified the nature of the surface antigen on viable memory B cells and on B-cell line (Ramos) cells, our findings indicate that this is distinct from that on the surface of naïve B cells bound by non-subset #4 Abs that use the IGHV4-34 gene.

Regarding the reactivity with influenza hemagglutinins, we have found that this is directed to two sets of hemagglutinins belong to phylogenetically distinct sets, indicating that subset #4 IGs bind to an epitope shared by multiple influenza viruses and hence are "broadly-reactive". Moreover, subset #4 IGs were able to protect target cells from influenza virus infection. Notably, influenza viruses expressing these two types of hemagglutinins have been responsible for several pandemics over the past 70 years. Thus, since CLL is a disease of aging, it is not unreasonable to postulate that repetitive, temporally-spaced encounters with influenza viruses expressing these hemagglutinins were involved in the evolution of the selection of those normal B cells that eventually transformed to CLL.

Finally, since our preliminary findings suggest that the viable memory B-cell surface epitope is a carbohydrate and since the immunogenetic epitopes on viral hemagglutinins are usually carbohydrate in nature, it is also not unreasonable to postulate that the same glycan epitope is shared by these two targets. This is provocative in light of the fact that other non-IGHV4-34^{+} Abs can react with blood group glycans. Also intriguing is the finding that subset #4 IGs lack reactivity with I-/i- carbohydrate antigens, despite the binding site in FR1 of *IGHV4-34* gene classically involved in such binding being intact.

The impact of CSR on the structure, antigen-binding properties, and function of BCRs. It is well accepted that IG heavy (H) chain class switching provides a mechanism to change effector function. However, a few studies have suggested that CSR can also affect the degree of antigen reactivity and even specificity. This is of particular interest since subset #4 IGs are always class switched to IgG. To understand the impact of class-switching on the specificity of subset #4 BCRs, all 3 CLL subset #4 variable domains were recombinantly expressed with a Cμ region and their capacity for self-association and binding to the aforementioned antigens were evaluated.

In contrast to the induction of cell autonomous signaling induced by IgG molecules, the surface membrane IgM molecules were not capable of inducing such signaling, regardless the presence/absence of the somatic mutations. This suggests that the original subset #4 B cells underwent CSR and thereby developed autonomous signaling. Moreover, in contrast to subset #4 IgGs, Cμ-linked subset #4 molecules lost reactivity to viable B cells and to influenza A virus, and concomitantly lost the capacity of cell protection from infection. These findings indicate that the IgG isotype is required for binding to these antigens. However, a non-self-associable mutant of subset #4 linked with IgG, did not bind to viable B cells or influenza A virus, suggesting that the recognition of these antigens by subset #4 IgGs requires an IG - IG complex formed by self-association.

In this setting, the stability of the self-associated "receptor/antigen" complex is important. Therefore we addressed this by testing the stability of the surface expressed homodimer by probing with soluble subset #4 IgGs. When the surface expressed IgGs were exposed to the soluble IgGs, no interaction occurred. Thus, the homodimer forms stable, relatively long-lived complexes that could provide a stable binding site for the antigens on viable B cell surfaces and influenza viruses. Once we have identified the nature of the antigens on these two target, we will perform co-crystallization studies to address this possibility.

In contrast to wild type subset #4 mAbs that exhibited minimal binding to DNAs and showed no binding to I-/i- carbohydrate sequences, switching the Cγ region back to Cμ region dramatically increased the binding to both types of antigens.

Collectively these results demonstrate that IG isotype determines antigen specificity and reactivity for subset #4 IGs. CSR impacted specificity in 3 ways. First, it created a binding site for IG self-association that led to the ability to signal in the absence of foreign antigen. Second, it permitted the creation a scaffold for a new type binding site not involving the original variable domains. Third, it helped reduce and escape from autoreactivity. Thus, for subset #4 IGs, isotype switching provided the ability of an autoreactive naïve B cell clone to survive and proliferate, by developing structural elements that are beneficial and tolerated.

The impact of somatic mutations on the structure and antigen-binding properties of subset #4 BCRs. The formation of a self-associating subset #4 IG did not involve those amino acids typically generated by SHM. Hence, these recurrent somatic mutations suggest selection by antigen(s) other than self-IG.

By reverting somatic mutations back to their germline amino acids, we found that the development of binding to viable memory B-cell surfaces is independent of somatic mutations in the H chain variable domain. This was always the case regardless of which individual mutation was reverted, including the stereotyped "E28G". Instead, binding was created by a single, CLL subset #4-biased somatic mutation "N66D" immediately adjacent to VL CDR2 position of *IGKV2-30*. Reverting "D66" back to its germline amino acid "N" disrupted binding to memory B cells, without impacting binding to naïve B cells. This supports our hypothesis that the antigens/epitopes on naïve and memory B cells recognized by subset #4 IGs are distinct.

However, somatic mutations had a different impact on binding to influenza virus. The L chain somatic mutation made only a moderate contribution to this binding, whereas the introduction of somatic mutations to the sequences outside of *IGHV4-34* gene (revertant rIGHV4-34) more strikingly increased reactivity to flu virus. However these also enhanced autoreactivity to DNA. Although wild-type subset #4 mAbs and the germline configuration rH (IgG) did not bind DNA, introduction of the somatic mutations outside of *IGHV4-34* dramatically increased such binding. This, however, was reduced by the introduction of the stereotype mutation "G28E" in the *IGHV4-34* gene, and completely eliminated when other non-stereotyped mutations were introduced. Eliminating this strong autoantigen binding, however, significantly compromised (20-fold less affinity) the collective increased anti-HA activity that somatic mutations made in *IGHV4-34* genes.

Together these data suggest that BCR engagement with influenza virus led to somatic mutations and their selection. However this selection also led to unacceptable autoreactivity, which when eliminated by additional mutations, compromised reactivity to influenza.

Hypothetical model for the evolution of subset #4 BCRs. In summary, these findings indicate that the original subset #4 BCR contained germline-like *IGHV4-34/IGHD5-18/IGHJ6* and *IGKV2-30/IGKJ2* rearrangements linked to a Cμ region. These BCRs were autoreactive, binding to DNAs and i-type glycans. These autoreactivities were subsequently significantly reduced (in case of i-type antigens) or completely eliminated (in case of DNAs) by CSR to IgG. Class-switching to IgG also resulted in IgG self-association, which consequently induced autonomous signaling.

Meanwhile/concomitantly, the "N66D" somatic mutation in the L chain rearrangement enabled binding to viable B-cell surfaces and mutations made outside *IGHV4-34* on the H chain rearrangement increased reactivity to influenza. However, the same mutations re-enabled strong binding to DNAs, albeit due to a different set of residues than in the original rearrangement, and this was reduced by the stereotyped mutation "G28E" on *IGHV4-34* and was diminished further by other mutations made throughout this gene. These collective mutations impaired considerably the affinity for influenza virus but made any residual autoreactivity tolerable.

Although we cannot be sure of the relative timing of the effects that CSR and SHM had on BCR structure and function, overall these findings illustrate the importance of positive and negative selection for the structural changes in CLL subset #4 IGs that ultimately determined the unique antigen-reactivities of these CLL B-cell clones.

References.

1. N. Chiorazzi, M. Ferrarini, B cell chronic lymphocytic leukemia: lessons learned from studies of the B cell antigen receptor. *Annu Rev Immunol* **21**, 841-894 (2003).
2. N. Chiorazzi, K. R. Rai, M. Ferrarini, Chronic lymphocytic leukemia. *N Engl J Med* **352**, (2005).
3. F. K. Stevenson, F. Caligaris-Cappio, Chronic lymphocytic leukemia: revelations from the B-cell receptor. *Blood* **103**, 4389-4395 (2004).
4. G. Tobin *et al.*, Subsets with restricted immunoglobulin gene rearrangement features indicate a role for antigen selection in the development of chronic lymphocytic leukemia. *Blood* **104**, 2879-2885 (2004).
5. F. Ghiotto *et al.*, Remarkably similar antigen receptors among a subset of patients with chronic lymphocytic leukemia. *J Clin Invest* **113**, 1008-1016 (2004).
6. K. Stamatopoulos *et al.*, Over 20% of patients with chronic lymphocytic leukemia carry stereotyped receptors: Pathogenetic implications and clinical correlations. *Blood* **109**, 259-270 (2007).
7. R. Catera *et al.*, Chronic lymphocytic leukemia cells recognize conserved epitopes associated with apoptosis and oxidation. *Mol Med* **14**, 665-674 (2008).
8. C. C. Chu *et al.*, Chronic lymphocytic leukemia antibodies with a common stereotypic rearrangement recognize nonmuscle myosin heavy chain IIA. *Blood* **112**, 5122-5129 (2008).
9. C. C. Chu *et al.*, Many chronic lymphocytic leukemia antibodies recognize apoptotic cells with exposed nonmuscle myosin heavy chain IIA: implications for patient outcome and cell of origin. *Blood* **115**, 3907-3915 (2010).
10. A. Lanemo Myhrinder *et al.*, A new perspective: molecular motifs on oxidized LDL, apoptotic cells, and bacteria are targets for chronic lymphocytic leukemia antibodies. *Blood* **111**, 3838-3848 (2008).

11. M. Duhren-von Minden *et al.*, Chronic lymphocytic leukaemia is driven by antigen -independent cell-autonomous signalling. *Nature* **489**, 309-312 (2012).

12. C. Minici *et al.*, Distinct homotypic B-cell receptor interactions shape the outcome of chronic lymphocytic leukaemia. *Nature communications* **8**, 15746 (2017).

13. B. T. Messmer *et al.*, Multiple distinct sets of stereotyped antigen receptors indicate a role for antigen in promoting chronic lymphocytic leukemia. *J Exp Med* **200**, 519-525 (2004).

14. L. A. Sutton *et al.*, Extensive intraclonal diversification in a subgroup of chronic lymphocytic leukemia patients with stereotyped IGHV4-34 receptors: implications for ongoing interactions with antigen. *Blood* **114**, 4460-4468 (2009).

15. R. Catera *et al.*, Binding of CLL subset 4 B-cell receptor immunoglobulins to viable human memory B lymphocytes requires a distinctive IGKV somatic mutation. *Mol Med* **23**, (2017).

16. G. Smith, M. Spellerberg, F. Boulton, D. Roelcke, F. Stevenson, The immunoglobulin VH gene, VH4-21, specifically encodes autoanti-red cell antibodies against the I or i antigens. *Vox sanguinis* **68**, 231-235 (1995).

17. F. K. Stevenson *et al.*, Utilization of the VH4-21 gene segment by anti-DNA antibodies from patients with systemic lupus erythematosus. *J Autoimmun* **6**, 809-825. (1993).

18. A. J. Cappione, A. E. Pugh-Bernard, J. H. Anolik, I. Sanz, Lupus IgG VH4.34 Antibodies Bind to a 220-kDa Glycoform of CD45/B220 on the Surface of Human B Lymphocytes. *The Journal of Immunology* **172**, 4298-4307 (2004).

19. M. Torres, R. May, M. D. Scharff, A. Casadevall, Variable-region-identical antibodies differing in isotype demonstrate differences in fine specificity and idiotype. *J Immunol* **174**, 2132-2142 (2005).

20. M. Torres, N. Fernandez-Fuentes, A. Fiser, A. Casadevall, Exchanging murine and human immunoglobulin constant chains affects the kinetics and thermodynamics of antigen binding and chimeric antibody autoreactivity. *PLoS ONE* **2**, e1310 (2007).

Dimitar Efremov

Staff Scientist and Head of the Molecular Hematology Group of the International Centre for Genetic Engineering and Biotechnology (ICGEB) in Trieste, Italy. Dr. Efremov obtained his MD degree at the Faculty of Medicine in Skopje, Republic of Macedonia, where he also completed his specialization in internal medicine and training in hematology. He started his research career in 1987 at the Medical College of Georgia, Augusta, GA, USA, and obtained his PhD degree from the Faculty of Medicine, Maastricht University, Maastricht, The Netherlands, in 1994. The same year he became Staff Scientist at the International Centre for Genetic Engineering and Biotechnology in Trieste, Italy, where he remained until 1998. He subsequently returned to the Department of Hematology, Faculty of Medicine, Skopje, Republic of Macedonia, where he was appointed Associate Professor of Internal Medicine. In 2003 he moved again to Italy and established the ICGEB Molecular Hematology Group and the ICGEB Outstation in Rome, which he headed until his move to the ICGEB Headquarters in Trieste in November 2015. Dr. Efremov's primary research interest is chronic lymphocytic leukemia, particularly the molecular mechanisms governing the development and progression of the disease and identification of novel targeted therapies. He has authored more than 100 articles in peer-reviewed medical journals.

NOVEL TARGETED THERAPIES IN CLL: MECHANISMS OF ACTION AND RESISTANCE

Various pathways involved in normal B cell growth and survival are aberrantly or excessively activated in chronic lymphocytic leukemia (CLL) B cells as a consequence of genetic lesions or chronic stimulation by signals from the microenvironment. Such pathways most notably include the B cell receptor (BCR), NOTCH, NF-kB and Toll-like receptor (TLR) pathways, as well as aberrant expression of apoptosis regulatory proteins, among which most notable is overexpression of BCL-2. Drugs that can target these aberrantly activated pathways or molecular defects have been developed and have shown considerable activity in preclinical models and clinical trials. Moreover, several of these drugs have recently been approved for clinical use in CLL or other B cell malignancies, including the BTK inhibitors ibrutinib and acalabrutinib, the PI3K inhibitors idelalisib and copanlisib, and the BCL2 inhibitor venetoclax. However, complete responses with these drugs as single agents are infrequent and many patients progress during treatment, highlighting the need to develop more effective rational drug combinations and to better understand the mechanisms of resistance (1,2).

Acquired resistance to ibrutinib is primarily caused by missense mutations in BTK at the ibrutinib binding site (Cys481) or in BTK's downstream target PLCG2 (3,4). The mutations in BTK prevent covalent binding of ibrutinib, whereas mutations in PLCG2 yield a constitutively active enzyme, which no longer needs to be phosphorylated and activated by BTK. Recently, it was demonstrated that multiple subclones with BTK and PLCG2 mutations are often present in resistant patients and that these subclones can be present prior to initiating ibrutinib treatment (5,6). The subclonal heterogeneity of CLL progressing on ibrutinib has important clinical implications. For example, although reversible BTK inhibitors might be effective against BTK-mutant clones, they would not be effective against PLCG2 mutants. In addition, BTK and PLCG2 mutations are often present in minor subclones at progression, suggesting that there are additional mechanisms of ibrutinib resistance (6).

Resistance to venetoclax has been primarily associated with increased expression of the anti-apoptotic proteins BCL-xL and MCL-1, which are both BCL-2 homologues that do not bind to venetoclax (7-10). Increased expression of these proteins in some cases may be driven by genetic alterations in cancer associated genes, such as B-RAF (11), but appears to be mainly caused by pro-survival signals from the microenvironment. Examples of such signals include BCR, CD40 and TLR9 stimulation, which have all been shown to confer significant protection from venetoclax-induced apoptosis (8-10). Drugs that can inhibit these pathways show synergistic activity with venetoclax in experimental models *in vitro*. Moreover, ongoing clinical trials with venetoclax and ibrutinib are reporting complete responses in approximately 60% of patients, suggesting that combinations of venetoclax with drugs that target microenvironmental signals could represent an effective strategy to overcome resistance *in vivo* (12-14). Interestingly, ongoing preclinical studies in our lab suggest that such combinations may also be effective in a subset of cases with diffuse large B cell lymphoma characterized by CD79A/B mutations or deficiency of the phosphatase SHP1.

Another potential therapeutic target in CLL is the TLR pathway. This pathway is activated in a small percentage of patients (<3%) by mutations in the MYD88 adaptor protein, but appears to be activated in a much greater proportion of patients by signals received in the lymph node microenvironment. Ongoing studies in our lab suggest that such signals can be effectively blocked by an inhibitor of the kinase IRAK4, resulting in significant inhibition of leukemic cell proliferation *in vitro* and significantly prolonged survival of mice with adoptively transferred leukemia. These data suggest that inhibitors of IRAK4, currently in development for treatment of inflammatory diseases, could represent a novel class of therapeutic agents that warrant further study in CLL.

References

1. Burger JA, Wiestner A. Targeting B cell receptor signalling in cancer: preclinical and clinical advances. Nat Rev Cancer. 2018;18(3):148-167.

2. Davids MS. Targeting BCL-2 in B-cell lymphomas. Blood. 2017;130(9):1081-1088.

3. Woyach JA, Furman RR, Liu TM, Ozer HG, Zapatka M, Ruppert AS et al. Resistance mechanisms for the Bruton's tyrosine kinase inhibitor ibrutinib. N Engl J Med 2014; 370: 2286–2294.

4. Furman RR, Cheng S, Lu P, Setty M, Perez AR, Guo A et al. Ibrutinib resistance in chronic lymphocytic leukemia. N Engl J Med 2014; 370: 2352–2354.

5. Burger JA, Landau DA, Taylor-Weiner A, Bozic I, Zhang H, Sarosiek K et al. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun 2016; 7: 11589.

6. Ahn IE, Underbayev C, Albitar A, Herman SE, Tian X, Maric I, et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood. 2017;129(11) :1469-1479.

7. Choudhary GS, Al-Harbi S, Mazumder S, Hill BT, Smith MR, Bodo J, Hsi ED, Almasan A. MCL-1 and BCL-xL-dependent resistance to the BCL-2 inhibitor ABT-199 can be overcome by preventing PI3K/AKT/mTOR activation in lymphoid malignancies. Cell Death Dis. 2015 Jan 15;6:e1593.

8. Thijssen R, Slinger E, Weller K, Geest CR, Beaumont T, van Oers MH, Kater AP, Eldering E. Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors. Haematologica. 2015;100(8):e302-6.

9. Bojarczuk K, Sasi BK, Gobessi S, Innocenti I, Pozzato G, Laurenti L, Efremov DG. BCR signaling inhibitors differ in their ability to overcome Mcl-1-mediated resistance of CLL B cells to ABT-199. Blood. 2016;127(25):3192-201

10. Jayappa KD, Portell CA, Gordon VL, Capaldo BJ, Bekiranov S, Axelrod MJ, et al. Microenvironmental agonists generate de novo phenotypic resistance to combined ibrutinib plus venetoclax in CLL and MCL. Blood Adv. 2017;1(14):933-946.

11. Herling CD, Abedpour N, Weiss J, Schmitt A, Jachimowicz RD, Merkel O, et al. Clonal dynamics towards the development of venetoclax resistance in chronic lymphocytic leukemia. Nat Commun. 2018;9(1):727.

12. Hillmen P, Munir, T, Rawstron, A, et al. Initial Results of Ibrutinib Plus Venetoclax in Relapsed, Refractory CLL (Bloodwise TAP CLARITY Study): High Rates of Overall Response, Complete Remission and MRD Eradication after 6 Months of Combination Therapy. ASH; 2017; Atlanta, abstract 428.

13. Jain N, Thompson, PA, Ferrajoli, A, et al. Combined Venetoclax and Ibrutinib for Patients with Previously Untreated High-Risk CLL, and Relapsed/Refractory CLL: A Phase II Trial. ASH; 2017; Atlanta, abstract 429.

14. Rogers K, Huang, Y, Stark, A, et al. Initial Results of the Phase 2 Treatment Naive Cohort in a Phase 1b/2 Study of Obinutuzumab, Ibrutinib, and Venetoclax in Chronic Lymphocytic Leukemia. ASH; 2017; Atlanta, abstract 431.

THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: GUIDELINES

Luca Laurenti, M.D.

Physician and resercher at the Hematology Department of the Universita' Cattolica del Sacro Cuore in Rome since 2002.
He got habilitation to Associate Professor in 2013. He is involved in assistential activity in Hematology Department and Bone Marrow Unit and he is interested in biological and clinical aspects of chronic lymphocytic leukemia and in autologous/allogeneic transplantation.
He teaches at Medical school of the Universita' Cattolica Sacro Cuore in Rome.
He is author of about 200 papers published by international journals.

Chronic Lymphocytic Leukemia (CLL) is the most common leukaemia in adults with an incidence of 4.2/100.000/year. As life expectancy is increasing, most of the patients with CLL are older, with a median age at diagnosis of 72[1].

Once established the presence of ≥ 5.000 monoclonal lymphocytes/μL confirmed by flow cytometry with an antigenic pattern which overlaps normal B-cells (CD19+, CD23+ and CD20+ and light chain restriction) diagnosis of CLL is made.

At this point, CLL patients must undergo a clinical and biological baseline assessment at diagnosis and before starting treatment since leukaemic clones may acquire further mutations.

Clinical recommended examinations included blood count, serum chemistry and physical examination, in order to detect any enlargement of lymph nodes, spleen or liver, allowing patients to be staged and history of infections and comorbidities which could restrict and contraindicate the use of certain drugs in CLL therapy.

So far, biological assessment draws upon detection of IGHV mutation status, p53 mutation and FISH for detection of chromosomal aberrations such as trisomy 12, del11q, del13q or del17p.

Nowadays, more recent genetic mutations such as BIRC3, SF3B1 and NOTCH1[2] further contributed to the stratification of prognosis and outcome in CLL patients along with the parameters above reported.

In the therapeutic scenario in patients with active and progressive CLL, two great arms have to be defined according to the presence or absence of del17p/TP53 mutation.

The confirmation of one of these alterations defines a cohort of patients with shorter progression free survival/overall survival (PFS /OS) and a worse prognosis. Since the low response rate to traditional chemoimmunotherapy, first line treatment recommended which proved to have impact on the outcome of CLL patients are BCR inhibitors (BCRi) such as Ibrutinib[3] and Idelalisib alone or in combination with Rituximab. Therapeutic options, once excluded del17p/TP53 mutation, may differs on grounds of comorbidities according to CIRS scale. Fit (CIRS<6) and young patients get benefit

from the association of Fludarabine, Cyclophosphamide and Rituximab (FCR)[4]. A valid alternative regimen in fit older patients (>65) or when fludarabine is contraindicated (e.g. infections) is the association of other alkylating agent (e.g. Bendamustine, Chlorambucil) with antiCD20 (Rituximab, Ofatumumab, Obinutuzumab)[5].

At this point, patients may keep a response for a variable span of time. Relapses require a reassessment of the biologic profile of the disease and necessity to retreat patients.

Patients relapsed after 24-36 months, may get benefit with first line treatment or switching to other regimens or BCRi.

Early relapses, within 24-36 month from chemoimmunotherapy, switch to a target therapy with BCRi and, if previously used, to BCL2 antagonists with the chance of complete remission and MRD negativity, even in relapsed/refractory CLL.[6]

References:

[1] Eichorst B et al. Chronic lymphocytic leukaemia: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. *Annals of oncology 26: v78-v84, 2015*

[2] Sabina Chiaretti et al. NOTCH1, SF3B1, BIRC3 and TP53 mutations in patients with chronic lymphocytic leukemia undergoing first-line treatment: correlation with biological parameters and response to treatment. *Leukemia & Lymphoma Volume 55, 2014: 2785-2792.*

[3] Hun Ju Lee et al. Developing Therapeutic Strategies for CLL with Mutant p53. *Blood 2015 126:2476;*

[4] Chatree Chai-Adisaksopha and Jennifer R. Brown. FCR Achieves Long-Term Durable Remissions in Patients with *IGHV*-Mutated CLL. *Blood 2017 :blood-2017-07-731588;*

[5] Idanna Innocenti et al. Treatment approach for elderly and unfit patients with chronic lymphocytic leukemia. *Expert review of Hematology: >Volume 10, 2017- Issue 12.*

[6] Stephan Stilgenbauer et al. Venetoclax (ABT-199/GDC-0199) Monotherapy Induces Deep Remissions, Including Complete Remission and Undetectable MRD, in Ultra-High Risk Relapsed/Refractory Chronic Lymphocytic Leukemia with 17p Deletion: Results of the Pivotal International Phase 2 Study. *Blood 2015* 126:LBA-6

THE RELEVANCE OF MINIMAL RESIDUAL DISEASE

Gianmatteo Rigolin

Hematology Section, Department of Medical Sciences,

Azienda Ospedaliero-Universitaria, Arcispedale S. Anna, University of Ferrara, Italy

1998-1999: Honorary Research Fellow – King's College School of Medicine - London

2013 - present
Member of the Ethics Committee

2016 - present
Associate professor in Hematology – University of Ferrara

Research in the field of molecular cytogenetic lesions in hemopoietic neoplasms

Bibliometric index (Scopus may 2018)
h-index 30;
citations 2988

In chronic lymphocytic leukemia (CLL) the assessment of residual disease has been correlated with improved outcomes. Randomized clinical trials have shown that achieving a complete remission (CR) was associated with a better overall (OS) and progression free survival (PFS). However, in a significant proportion of patients achieving clinical CR, residual disease may still be detected when sensitive flow cytometry (FLC) and molecular methods are used in the study of peripheral blood (PB) and bone marrow (BM) samples. The demonstration of residual CLL leukemic cells, the so-called minimal residual disease (MRD), has been demonstrated to be responsible for disease relapse and therefore to correlate with a worse clinical outcome (Thompson, 2016).

Methods for MRD study

In CLL, MRD can be assessed both by sensitive FLC and PCR methods. The techniques for assessing MRD have undergone a critical evaluation and have become well standardized (Rawstron, 2016). MRD negativity has been commonly defined as PB or BM with less than 1 CLL cell per 10000 leukocytes (<10−4). Multicolor FLC methods have the advantage of being more commonly available and more rapid than PCR methods that, by contrast, have a higher sensitivity (10-6). Disparities were observed in MRD evaluation between BM and PB samples with MRD negativity that is less likely to be present in BM than in PB. The site of MRD sampling may therefore affect the predictive ability of the test as the detection of MRD in PB was associated with shorter response duration while MRD negativity had the highest negative predictive value for predicting subsequent relapse risk (Thompson 2018). Disparities have also been observed between clinical staging and MRD evaluation. Overall, there is evidence that achieving post treatment MRD-negative remission may be more important than achieving clinical CR as no difference was observed in PFS between patients in MRD negative CR vs MRD-negative PR while patients in MRD-negative PR had superior PFS than those in MRD-positive CR (Kovacs, 2014)

MRD, chemoimmunotherapy (CIT) and novel agents.

Prospective clinical trials have provided substantial evidence that CITs are able to eradicate MRD usually resulting in an improved clinical outcome (Thompson, 2018). Several potential benefits may derive from the study of MRD in patients undergoing CIT including; (i) the possibility to avoid the cumulative toxicity deriving from unnecessary adjunctive treatments or maintenance therapies; (ii) the reduced risk of developing resistance mutations; (iii) the reduction in the financial burden due to delay in the use of expensive targeted therapies; (iv) the possibility to reverse the immune dysfunction that characterize CLL patients with an increased risk of infections, autoimmune disorders and second cancers. However, these possible beneficial benefits must be balanced with the toxicities deriving from CIT regimens particularly those fludarabine based that include immune depression with neutropenia and lymphopenia, long-term risk of secondary AML/MDS (Thompson, 2016).

As far as novel agents are concerned, MRD negativity rates with ibrutinib monotherapy are low both in the front-line and in the relapsed/refractory settings (Ahn, 2018). Higher rates of MRD negativity may be obtained when ibrutinib is used in combination with chemotherapy and monoclonal antibodies. Similarly, high rates of MRD negativity can be achieved with venetoclax in combination. In the recently published Murano trial MRD negativity at 9 moths was observed in 62.4% of the patients in the venetoclax -Rituximab group as compared to 13.3% in the bendamustine groups (Seymour, 2018).

MRD negativity as a surrogate for PFS in CLL

The European Medicines Agency (EMA) has accepted MRD negativity as a surrogate marker for PFS in clinical trial. The importance of using MRD negativity as a primary end-point in clinical trial design is that differences in the rate of MRD negativity between treatment arms can be assessed after only 9 to 15 months, thus potentially allowing more rapid regulatory approval of novel therapies. With this aim, recently Dimier et al (Dimier, 2018), for the first time, quantitate the relationship between MRD negativity and PFS in patients receiving CIT as first-line treatment, establishing its validity as a surrogate marker for PFS. In their model, they demonstrated a statistically significant relationship between treatment effect on PB-MRD and treatment effect on PFS. As the difference between treatment arms in PB-MRD response rates increased, a reduction in the risk of progression or death was observed. This surrogacy model supports use of PB-MRD as a primary end-point in randomized clinical trials of CIT in CLL although additional CLL trial data are required to establish a more precise quantitative relationship between MRD and PFS, and to support general applicability of MRD surrogacy for PFS across diverse patient characteristics, treatment regimens, and different treatment mechanisms of action.

MRD and 2018 NCI-sponsored guidelines from the International Workshop on CLL

According to the recently published guidelines (Hallek, 2018), MRD assessment is not generally indicated in general practice while in clinical trials aimed at maximizing the depth of remission, the presence of MRD after therapy should be assessed because the lack of leukemia persistence using sensitive tests has a strong, positive prognostic impact. The sensitivity of the method used to evaluate for MRD should be reported, as should be the tissue studied (blood or marrow). The blood generally can be used for making this assessment, as the marrow will have detectable CLL when it is also found in the PB. However, it may be important to confirm that the marrow aspirate is also MRD-neg when the blood is found to be MRD-neg. Patients are defined as having undetectable MRD (MRDneg) remission if they have PB or BM with less than one CLL cell per 10.000 leukocytes. The proportion of patients achieving undetectable MRD should be reported with the total number of patients treated with the specific therapy as the denominator (not as a proportion of responders or those in complete remission). Of note, for patients in clinical trials to demonstrate that a CR may have been achieved, the cytological or pathological evaluation of the BM smear or biopsy is not based on a FLC based MRD assessment. Therefore, in clinical trials aimed at maximizing the response rate, the quality of the response should also be assessed in the BM for MRD by highly sensitive molecular based assays or immunophenotyping.

Selected References

Ahn IE, Farooqui MZH, Tian X, et al. Depth and durability of response to ibrutinib in CLL: 5-year follow-up of a phase II study. Blood. 2018 Feb 26. pii: blood-2017-12-820910. doi: 10.1182/blood-2017-12-820910. [Epub ahead of print]

Dimier N, Delmar P, Ward C, et al. A model for predicting effect of treatment on progression-free survival using MRD as a surrogate end point in CLL. Blood. 2018;131(9) :955-962.

Hallek M, Cheson BD, Catovsky D, et al. Guidelines for diagnosis, indications for treatment, response assessment and supportive management of chronic lymphocytic leukemia. Blood. 2018 Mar 14. pii: blood-2017-09-806398. doi: 10.1182/blood-2017-09-806398. [Epub ahead of print]

Kovacs G, Boettcher S, Bahlo J, et al. Value of minimal residual disease (MRD) negative status at response evaluation in chronic lymphocytic leukemia (CLL): combined analysis of two phase III studies of the German CLL Study Group (GCLLSG). Blood. 2014;124(21):23.

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;3 0(4):929-936.

Seymour JF, Kipps TJ, Eichhorst B, et al. Venetoclax-Rituximab in Relapsed or Refractory Chronic Lymphocytic Leukemia. NEJM. 2018 Mar 22;378(12):1107-1120.

Thompson M, Brander D, Nabhan C, Mato A. Minimal Residual Disease in Chronic Lymphocytic Leukemia in the Era of Novel Agents: A Review. JAMA Oncol. 2018;4(3) :394-400.

Thompson PA, WierdaWG. Eliminating minimal residual disease as a therapeutic end point: working toward cure for patients with CLL. Blood. 2016;127 (3):279-286.

EXPANDING THE USE OF BCR AND BCL-2 INIBITHORS

Maurizio Cavallari, Antonio Cuneo
Section of Hematology
Department of Medical Sciences
University of Ferrara

Prof. Antonio Cuneo
MD, PhD

Hematology Section, Department of Medical Sciences, Azienda Ospedaliero-Universitaria, Arcispedale S. Anna, University of Ferrara, Italy

2005
Full Professor of Hematology at the University of Ferrara

2010
Coordinator of the PhD Program in molecular medicine and pharmacology at the University of Ferrara

2012-2017
Director Dept. Specialistic Medicine
University Hospital S.ANNA – Ferrara

Research funds
FIRB – PRIN – Ricerca Finalizzata MOH

Research in the field of molecular cytogenetic lesions in hemopoietic neoplasms

Bibliometric index
(Scopus march 2018)
h-index 43; citations 5943

Target therapy combinations in CLL.

Long-term treatment with any of the novel agents was shown to lead to the emergence of resistant clones in high risk CLL (Woyach, 2017a; Ahn, 2017). While single agent therapy will remain an important option for elderly and unfit patients, it may represent a suboptimal option for younger and fit patients. Furthermore, limited response durability in patients with TP53 disruption and/or complex karyotype, limited compliance to long-term therapy and unsustainable costs related to prolonged treatment should be considered. Fixed-duration regimens may allow patients to take advantage of the benefits associated with a target therapy without the commitment to chronic therapy until progression.

Novel agents plus chemoimmunotherapy combinations in CLL

Considering that only FCR has shown a curative potential for fit and younger CLL patients with mutated *IGHV* thus far, combination regimens with chemoimmonutherapy (CIT) plus novel agents have been considered. One study offered CIT plus Ibrutinib in relapsed/refractory (R/R) CLL (Brown, 2015). 30 patients were treated with Ibrutinib plus BR for up to 6 cycles, with ibrutinib subsequently continued until progression or toxicity. The ORR was 93%, and the PFS at 1 year was 90%. 3 patients who received ibrutinib plus FCR were also included in this study. All 3 patients tolerated this combination well and achieved CR, including 2 with bone marrow MRD negativity. This led to the development of a phase 2 study of ibrutinib plus FCR (iFCR) for previously untreated younger, fit patients with CLL (Davids, 2016). The patients in this protocol are treated with up to 6 cycles of Ibrutinib given concurrently with FCR, followed by at least 2 years of Ibrutinib maintenance. Patients who are MRD-negative in the bone marrow (BM) after 2 years of maintenance discontinue Ibrutinib. This approach has been well-tolerated and achieved thus far a rate of CT-confirmed CR with bone marrow MRD negativity of 39%, which compares favorably to the 20% rate observed historically with FCR alone in the CLL8 trial (Hallek, 2010). Moreover, 89% of patients on iFCR achieved MRD negativity in the marrow, including 76% of patients who achieved PR, most of whom had small residual lymph nodes.

The iFCR study will also provide data on whether *IGHV* unmutated CLL patients can also achieve durable response with a time-limited regimen.
Another phase 2 study was designed with the intent to limit chemotherapy to 3 courses, potentially reducing toxicity while maintaining efficacy. In this regimen Ibrutinib plus Fludarabine, Cyclophosphamide and Obinutuzumab (iFCG) was administered to 32 previously untreated patients with M-IGHV CLL without TP53 disruption. The primary endpoint was CR/ CRi with undetectable BM MRD (4-color FCM, sensitivity 10^{-4}) after 3 courses of iFCG. Patients who obtained MRD-negativity received Ibrutinib with Obinutuzumab (iG) for 3 cycles, then Ibrutinib for further 6 cycles. Patients not achieving the primary endpoint received iG for 9 cycles. After a median follow up of 10.9 months, the ORR was 100% and the median PFS was not reached. 86% of patients achieved MRD-negative remission in the BM at 3 months and 46% achieved CR/CRi with undetectable MRD at 3 months. Responses continued to improve over time (6 months: CR/CRi rate 74%, MRD neg rate 91%; 12 months: CR/CRi rate 75%, MRD neg rate 100%). This regimen has been globally well-tolerated with grade 3-4 AEs mostly related to hematological toxicity (G3-4 neutropenia 68%; G3-4 thrombocytopenia 48%). (Jain, 2017)

Adding novel agents to anti-CD20 monoclonal antibodies in CLL

"Chemo sparing" regimens were developed during last years, through associations of anti-CD20 monoclonal antibodies and target therapies. Venetoclax was firstly evaluated in a highly effective combination with Rituximab (VR), which may produce enhanced ADCC (Souers, 2013). In a phase 1b study of VR on 49 R/R CLL patients (Seymour, 2017), the combination showed similar toxicities to those seen with Venetoclax single agent, apart for a slightly higher incidence of neutropenia. This combination regimen attained a 86% ORR, including 51% CR/CRi, leading to a 2-year PFS of 82% and a 57% BM MRD-neg rate by FCM. Among 13 responders elected to discontinue Venetoclax, all 8 who were MRD-neg remain in ongoing remission after a median of 9.7 months off Venetoclax. Overall, these data suggest that time-limited therapy is able to produce deep responses without increasing toxicity significantly.
A randomized phase-3 trial the VR regimen for 2 years vs BR X 6 cycles in R/R CLL is ongoing (MURANO, NCT02005471). It is noteworthy that a highly significant PFS advantage in the VR arm compared to BR has been recently reported in the planned interim analysis (Seymour, LBA-2, ASH 2017). However, in this trial an OS benefit has not yet been shown according to the predefined statistical model. Similarly, the CLL14 study, a randomized, phase 3 trial comparing 1 year of Obinutuzumab/Venetoclax (GV) to Obinutuzumab/Chlorambucil (G-Clb) in older patients with untreated CLL is ongoing (NCT02242942). An early report on 13 patients was recently published (Fischer, 2017). The GV regimen was well-tolerated, and 7/12 patients had CR/CRi, with 11/12 MRD-neg in the blood. Ibrutinib, Idelalisib have also been studied in combination with rituximab (Furman, 2014; Jain, 2017) and acalabrutinib has been studied in combination with Obinutuzumab, all studies showing promising results (Woyach, 2017b).

Target therapies combinations

Studies on combinations of novel agents with different activities and non-overlapping toxicities are ongoing. One promising combination is Ibrutinib plus Venetoclax. It has been shown that Venetoclax increases the propensity for CLL cells to undergo apoptosis, whilst Ibrutinib causes CLL cells to selectively become more dependent on BCL-2 for their survival.

These complementary effects likely underlie the potent CLL cell-killing that is observed with the combination of these two drugs ex vivo (Cervantes-Gomez, 2015; Deng, 2017). Clinical trials are already underway evaluating this combination, including a phase 2 study in previously untreated CLL showing a 100% ORR at a in preliminary analysis (Jain, 2017; NCT02756897) and 2 separate phase-2 studies looking at the 3-drug combination of venetoclax, Ibrutinib, and Obinutuzumab in previously untreated patients with del(17p) CLL (NCT02758665) or in CLL patients in all cytogenetic risk groups (Rogers, 2017; NCT02427451) with promising results.

BCR and BCL-2 inhibitors in hematological malignancies other than CLL

Considering BCR-pathway inhibitors in hematological malignancies other than CLL, Ibrutinib have obtained FDA approval for patients with mantle cell lymphoma (MCL), Waldenstrom Macroglobulinemia (WM) and Marginal Zone Lymhoma (MZL), while Idelalisib is currently indicated in CLL and Follicular Lymphoma (FL). Both agents have a satisfactory efficacy in the aforementioned diseases (Byrd, 2013; Furmann, 2014; Wang, 2013; Kahl, 2014; Gopal,2014; Noy, 2017; Treon, 2015), while in other B-cell neoplasms responses are limited to a few cases (Wilsom, 2015) with wide variations in ORRs and response duration. In R/R MCL, ibrutinib achieve responses in a substantial fraction of cases with a median PFS of 13,9 months with In Diffuse Large B Cell Lymphoma (DLBCL), BCR inhibitors' activity is limited to the ABC subtype (Davis, 2010; Yang, 2012) with limited ORRs and short PFS (Wilsom, 2015). The response variability in B cell malignancies depend on the strength of BCR signaling dependence (Burger, 2018). In indolent lymphomas BCR inhibitors have also been added to chemotherapeutic regimens trying to improve responses (e.g. NCT02629809, NCT02251548 and NCT01974440). Similarly, in ABC-DLBCL the addition of Ibrutinib to R-CHOP or to G-CHOP is being investigated (NCT01855750 and NCT02670317).

Finally, the *BCL-2* inhibitor Venetoclax have been studied in Non-Hodgkin Lymphomas (NHL), multiple myeloma (MM) and acute myeloid leukemia (AML). In a phase 1 study in R/R NHL, Venetoclax single agent (200–1200 mg) showed a 44% ORR among all subtypes (75% in MCL, 38% in FL, 18% in DLBCL) with a median PFS of 17 months (Gerecitano, 2015). In R/R MM a phase 1 study on 48 patients, Venetoclax (300-1200 mg QD) demonstrated a 24% ORR in patient bearing t(11;14) (Kumar, 2015) probably in relation with a higher BCL2/MCL1(Punnoose, 2016). Any data on PFS (No dati di sopravvivenza: fase 1) In 32 R/R or unfit AML patients, Venetoclax single agent (800 mg/die) was studied in a phase II study (Konopleva, 2016) with a 19% ORR, 6% CR rate and 13% of CRi (higher response rate in patients carrying IDH1/2 mutations). The median OS was 47 months with a median time to progression of 25 months. Synergy has been shown between hypomethylating agents and BCL-2 inhibitors in AML cell lines in preclinical models (Bogenberger, 2014) providing the basis for combination studies. Venetoclax in combination with low-dose cytarabine (LDAC) (NCT02287233) and decitabine or azacitidine (NCT02203773) is currently being investigated in previously untreated and elderly patients with promising results (DiNardo, 2017; Wei, 2017).

References

Ahn IE, Underbayev C, Albitar A, Herman SE, Tian X, Maric I et al. Clonal evolution leading to ibrutinib resistance in chronic lymphocytic leukemia. Blood. 2017 Mar 16;129(11):1469-1479

Bogenberger JM, Kornblau SM, Pierceall WE, Lena R, Chow D, Shi CX et al. BCL-2 family proteins as 5-Azacytidine-sensitizing targets and determinants of response in myeloid malignancies. Leukemia. 2014 Aug;28(8):1657-65.

Brown JR, Barrientos JC, Barr PM, Flinn IW, Burger JA, Tran A et al. The Bruton tyrosine kinase inhibitor ibrutinib with chemoimmunotherapy in patients with chronic lymphocytic leukemia.Blood. 2015 May 7;125(19):2915-2922.

Burger JA, Wiestner A. Targeting B cell receptor signaling in cancer: preclinical and clinical advances. Nat Rev Cancer. 2018 Mar;18(3):148-167.

Byrd JC, Furman RR, Coutre SE, Flinn IW, Burger JA, Blum KA et al. Targeting BTK with ibrutinib in relapsed chronic lymphocytic leukemia. N Engl J Med. 2013 Jul 4;369(1):32-42.

Cervantes-Gomez F, Lamothe B, Woyach JA, Wierda WG, Keating MJ, Balakrishnan K, Gandhi V. Pharmacological and Protein Profiling Suggests Venetoclax (ABT-199) as Optimal Partner with Ibrutinib in Chronic Lymphocytic Leukemia. Clin Cancer Res. 2015 Aug 15;21(16):3705-15.

Davids MS, Kim HT, Brander DM, Bsat J, Savell A, Francoeur K et al. Initial Results of a Multicenter, Phase II Study of Ibrutinib Plus FCR (iFCR) As Frontline Therapy for Younger CLL Patients. Blood 2016 128:3243.

Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB et al. Chronic active B-cell -receptor signalling in diffuse large B-cell lymphoma. Nature. 2010 Jan 7;463(7277):88-92.

Deng J, Isik E, Fernandes SM, Brown JR, Letai A, Davids MS. Bruton's tyrosine kinase inhibition increases BCL-2 dependence and enhances sensitivity to venetoclax in chronic lymphocytic leukemia. Leukemia. 2017 Oct;31(10):2075-2084.

DiNardo CD, Pratz KW, Letai A, Jonas BA, Wei AH, Thirman M et al. Safety and preliminary efficacy of venetoclax with decitabine or azacitidine in elderly patients with previously untreated acute myeloid leukaemia: a non-randomised, open-label, phase 1b study. Lancet Oncol. 2018 Feb;19(2):216-228.

Fischer K, Al-Sawaf O, Fink AM, Dixon M, Bahlo J, Warburton S et al. Venetoclax and obinutuzumab in chronic lymphocytic leukemia. Blood. 2017 May 11;129(19):2702-2705.

Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, et al. Idelalisib and rituximab in relapsed chronic lymphocytic leukemia. N Engl J Med. 2014 Mar 13;370(11) :997-1007.

Gerecitano JF, Roberts AW, Seymour JF, Wierda WG, Kahl BS, Pagel JM Davis RE, Ngo VN, Lenz G, Tolar P, Young RM, Romesser PB et al. al. A phase 1 study of venetoclax (ABT-199/GDC-0199) monotherapy in patients with relapsed/refractory non-Hodgkin lymphoma. Blood. 2015; 126:254.

Gopal AK, Kahl BS, de Vos S, Wagner-Johnston ND, Schuster SJ, Jurczak WJ et al. PI3Kδ inhibition by idelalisib in patients with relapsed indolent lymphoma. N Engl J Med. 2014 Mar 13;370(11):1008-1018.

Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J et al. Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. Lancet. 2010 Oct 2;376(9747) :1164-1174.

Jain N, Thompson PA, Ferrajoli A, Burger JA, Borthakur G, Takahashi K et al. Combined Venetoclax and Ibrutinib for Patients with Previously Untreated High-Risk CLL, and Relapsed/Refractory CLL: A Phase II Trial. Oral Abstract #429: ASH 59th Annual Meeting and Exposition, December 2017, Atlanta, GA

Jain P, Keating MJ, Wierda WG, Sivina M, Thompson PA, Ferrajoli A et al. Long-term Follow-up of Treatment with Ibrutinib and Rituximab in Patients with High-Risk Chronic Lymphocytic Leukemia. Clin Cancer Res. 2017 May 1;23(9):2154-2158.

Kahl BS, Spurgeon SE, Furman RR, Flinn IW, Coutre SE, Brown JR et al. A phase 1 study of the PI3Kδ inhibitor idelalisib in patients with relapsed/refractory mantle cell lymphoma (MCL). Blood. 2014 May 29;123(22):3398-4405.

Konopleva M, Pollyea DA, Potluri J, Chyla B, Hogdal L, Busman T et al. Efficacy and biological correlates of response in a phase II study of venetoclax monotherapy in patients with acute myelogenous leukemia. Can Disc, 2016; 6:1106–1117.

Kumar SVR., Kaufman JL, Mikhael J, Facon T, Moreau P, Amiot M et al. Venetoclax (Abt-199/gdc-0199) monotherapy for relapsed/refractory multiple myeloma: phase I safety and efficacy. Haematologica, 2015; 100:256–257a.

Kumar SVR., Kaufman JL, Mikhael J, Facon T, Moreau P, Amiot M et al. Phase I interim safety and efficacy of venetoclax (ABT-199/GDC-0199) monotherapy for relapsed/refractory (R/R) multiple myeloma (MM). Journal of Clinical Oncology, 2015; 33:8576.b

Noy A, de Vos S, Thieblemont C, Martin P, Flowers CR, Morschhauser F, et al. Targeting Bruton tyrosine kinase with ibrutinib in relapsed/refractory marginal zone lymphoma. Blood. 2017 Apr 20;129(16):2224-2232.

Punnoose EA, Leverson JD, Peale F, Boghaert ER, Belmont LD, Tan N et al. Expression profile of BCL-2, BCL-XL, and MCL-1 predicts pharmacological response to the BCL-2 selective antagonist venetoclax in multiple myeloma models. Mol Can Ther. 2016; 15:1132 –1144.

Rogers KA, Huang Y, Stark A, Awan FT, Maddocks KJ, Woyach JA et al. Initial Results of the Phase 2 Treatment Naive Cohort in a Phase 1b/2 Study of Obinutuzumab, Ibrutinib, and Venetoclax in Chronic Lymphocytic Leukemia. Abstract #431: ASH 59th Annual Meeting and Exposition, December 2017, Atlanta, GA

Seymour JF, Ma S, Brander DM, Choi MY, Barrientos J, Davids MS et al. Venetoclax plus rituximab in relapsed or refractory chronic lymphocytic leukaemia: a phase 1b study. Lancet Oncol. 2017 Feb;18(2):230-240.

Seymour JF, Kipps TJ, Eichhorst BF, Hillmen P, D'Rozario JM, Assouline S et al. Venetoclax Plus Rituximab Is Superior to Bendamustine Plus Rituximab in Patients with Relapsed/ Refractory Chronic Lymphocytic Leukemia - Results from Pre-Planned Interim Analysis of the Randomized Phase 3 Murano Study. LBA-2: ASH 59th Annual Meeting and Exposition, December 2017, Atlanta, GA

Souers AJ, Leverson JD, Boghaert ER, Ackler SL, Catron ND, Chen J et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013 Feb;19(2):202-208.

Treon SP, Tripsas CK, Meid K, Warren D, Varma G, Green R et al. Ibrutinib in previously treated Waldenström's macroglobulinemia. N Engl J Med. 2015 Apr 9;372(15):1430-1440.

Wang ML, Rule S, Martin P, Goy A, Auer R, Kahl BS et al. Targeting BTK with ibrutinib in relapsed or refractory mantle-cell lymphoma. N Engl J Med. 2013 Aug 8;369(6):507-516.

Wei A, Strickland SA, Roboz GJ, Hou JZ, Fiedler W, Lin TL et al. Phase 1/2 Study of Venetoclax with Low-Dose Cytarabine in Treatment-Naive, Elderly Patients with Acute Myeloid Leukemia Unfit for Intensive Chemotherapy: 1-Year Outcomes. Abstract #890: ASH 59th Annual Meeting and Exposition, December 2017, Atlanta, GA.

Wilson WH, Young RM, Schmitz R, Yang Y, Pittaluga S, Wright G et al. Targeting B cell receptor signaling with ibrutinib in diffuse large B cell lymphoma. Nat Med. 2015 Aug;21(8) :922-926.

Woyach JA, Ruppert AS, Guinn D, Lehman A, Blachly JS, Lozanski A et al. BTKC481S -Mediated Resistance to Ibrutinib in Chronic Lymphocytic Leukemia. J Clin Oncol. 2017 May 1;35(13):1437-1443.a

Woyach JA, Awan FT, Jianfar M, Rogers KA, Jones J, Covey T, et al. Acalabrutinib with Obinutuzumab in Relapsed/Refractory and Treatment-Naive Patients with Chronic Lymphocytic Leukemia: The Phase 1b/2 ACE-CL-003 Study . Abstract #432: ASH 59th Annual Meeting and Exposition, December 2017, Atlanta, GA.b.

Prof. Mario Luppi
MD, PhD

Hematology Section, Department of Medical and Surgical Sciences, University of Modena and Reggio Emilia, and Azienda Ospedaliero-Universitaria di Modena, Italy

2016, President of the Italian Society of Experimental Hematology (SIES)

2012-2015, Director of Department of Medical and Surgical Sciences, and Elected Member of Academic Senatus, University of Modena and Reggio Emilia

2010, Full Professor in Hematology at the University of Modena and Reggio Emilia and Chief of Section of Haematology, AO-U Modena.

Grants from E-R Region, PRIN, Italian Ministry of Defence, the European Community and Private Associations (A.I.R.C.).

Research in the field of: genetic and immunologic control of acute and chronic leukemias; pathogenesis and immunologic diagnosis of viral and fungal diseases; cancer pain therapy; innovative clinical models of early-supportive-palliative care in oncology and hematology.

Bibliometric index
(Scopus, may 2018)

h-index 45;
citations 7466

CONCLUDING REMARKS

Most recent evidences from biological and clinical studies have been summarised and discussed during «**The Focus on Chronic Lymphocyitic Leukemia (CLL)**» by leading investigators in the field. The pathogenesis of CLL recognizes major mechanisms involving chromosomal alterations, mutations, alterations in the expression of miRNAs and epigenetic modifications. Moreover, CLL cells depend on survival signals that they receive in lymphoid tissues from neighboring non-neoplastic cells within the so-called cancer microenvironment. (*Kipps et al., Nat Rev Dis Primers 2017 Jan 19;3:16096. doi: 10.1038/nrdp.2016.96*).

CLL is also a B-cell malignancy with a strong familial component and an ~8.5-fold increased relative risk in first-degree relatives. Genome-wide association study have identified multiple risk loci for CLL and the proximity of several of these loci to genes involved in apoptosis has also suggested a plausible underlying biological mechanism (*Berndt et al., Nat Genet 2013; 45: 868-78; Goldin et al., Blood 2016; 128: 2261-3*).

CLL is known to be preceded by a condition termed monoclonal B-cell lymphocytosis (MBL) that is characterized by the presence of circulating monoclonal B cells with a CLL phenotype, however, at a lower concentration than required for a clinical diagnosis of CLL ($\geq 5x10^9$/L). Low count MBL, high-count MBL and highly stable CLL (follow-up more than 10 years) share a similar mutational signatures, which include the presence of mutations in known drivers associated with poor outcome, such as *NOTCH1* and *SF3B1,* indicating that these mutations are not sufficient to drive the aggressiveness of the disease by themselves. A number of somatic mutations have been detected in both the patients' MBL/CLL cells and polymorphonuclear cells suggesting that the MBL/CLL clone may derive from a common hematopoietic precursor, capable of both lymphoid and myeloid differentiation, supporting the notion that age-related clonal hematopoiesis may associate not only with the expansion of myeloid clones and precede myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML), but also with expansion of B-cell clones with CLL phenotype. Whether the occurrence of age-related clonal hematopoiesis in CLL patients receiving immuno-chemotherapy is a risk factor for therapy-related MDS/AML, remains to be investigated (*Condolucci & Rossi, Haematologica 2018; 103:751-2*).

The immunogenetic and molecular heterogeneity of the disease is also reflected by the different incidence in populations with diverse ethnic and geographic distribution, being the age-adjusted Incidence CLL rate in Asians about 10 times lower than in US people and unchanged among Asians residing in the US and in their descendants. The *IgHV* gene repertoire in Taiwanese patients results biased and distinct from that observed in the West patients, with the most common *IgHV* genes being *IgHV3-23, IgHV3-7*, and *IgHV3-48,* suggesting different pathogenetic factors implicated in the development of chronic lymphocytic leukemia, and a potential different antigenic exposure between Eastern and Western CLL, while the higher frequency of *TP53* mutations in Taiwanese patients could in part explain the worse outcome of Asian CLL patients (*Marinelli et al., Oncotarget 2016; 7: 20520-531, Wu et al., Haematologica 2017; 102: 1085-90*).

The availability of novel agents for CLL therapy has several implications. First, in the routine diagnostic-therapeutic algorithm, both *del(17p)* and/or *TP53*-mutation and *IGHV* mutation testing are now mandatory and test standardization needs to be implemented on a large scale (*Malcikova et al., Leukemia https://doi.org/10.1038/s41375-017-0007-7*). Second, minimal residual disease assessment has become an indispensable tool for clinical research studies in CLL patients (*Ghia & Rawstrom Leukemia 2018 Mar 26. doi: 10.1038/s41375-018-0109*). Third, new clinical competences are required to recognize and manage potential known extra-hematologic toxicities associated with idelalisib, ibrutinib and venetoclax (*Ahn & Davids, Hematology Am Soc Hematol Educ Program. 2017 Dec 8; 2017:354-357. doi: 10.1182/asheducation-2017.1.354*). More recent and recurrent reports of invasive fungal infections related to ibrutinib, often in combination with steoids, need confirmation in prospective studies (*Chamilos, et al., Clin Infect Dis,* 2018; 66**:** 140-89), but have important practical consequences, being ibrutinib a potential cause of drug-related pneumonitis responsive to prednisone (*Mato et al., Blood 2016; 127: 1064-7).*

Additionally, several next generation novel therapeutic agents are showing promise, such as selective BTK inhibitors like acalabrutinib, PI3K inhibitors like umbralisib and duvelisib, and CAR-T–based therapies (*Davids, Hematology Am Soc Hematol Educ Program. 2017 Dec 8;201 7(1):346-353. doi: 10.1182/asheducation-2017.1.346*). Contrary to relapsed or refractory acute lymphoblastic leukemia, in which anti-CD19 CAR T cells induce complete remission in over 90% of cases, clinical trials of CD19-targeted T cell (CTL019) therapy have shown durable antitumor responses in CLL, but in only 26% of patients. Recent data by transcriptomic profiling have revealed that CAR T cells from complete-responding patients with CLL are enriched in memory-related genes, including IL-6/STAT3 signatures, whereas T cells from non-responders upregulate programs involved in effector differentiation, glycolysis, exhaustion and apoptosis, being a relevant population of CD27+PD-1–CD8+ CAR T cells, expressing high levels of the IL-6 receptor, able to predict therapeutic response and to control the tumor cells (*Fraietta et al., Nat Med 2018 Apr 30. doi: 10.1038/s41591-018-0010-1*).

Novel therapies are altering the paradigm of cancer care, including CLL care. What has not changed is patients' need to have accurate information about their prognosis and to engage discussions about their care options. The integration of palliative/supportive care, early in the course of illness, enables patients to understand their prognosis more accurately, improves clinician-patient communication about future care programs, including end-of-life care preferences, and improves both patients' quality of life and survival (*Bandieri et al., Ann Oncol 2012; 23: 2016-20; Bakitas et al., J Clin Oncol 2015; 33: 1438-45; Temel et al., J Clin Oncol 2016; 34: 3605-7*). Early palliative/supportive care models should be implemented not only in oncology but also in hematology wards, as widely recommended by International Societies. (*Thienprayoon & LeBlanc Hematology 2015 2015:479-483; doi:10.1182/asheducation-2015.1.479*).

INDEX

FOREWARD page 5
GIULIANO MAZZINI

THE RELEVANCE OF FLOWCYTOMETRY TO DIAGNOSE CHRONIC LYMPHOCYTIC LEUKEMIA 6
GIOVANNI D'ARENA

THE EVOLUTION OFA STEREOTYPED ANTIGEN-BINDING DOMAIN IN CHRONIC LYMPHOCYTIC LEUKEMIA:DUELLING SELECTIONS FOR FOREIGN AND AUTO-ANTIGENS 12
NICHOLAS CHIORAZZI

NOVEL TARGETED THERAPIES IN CLL: MECHANISMS OFACTION AND RE 17
DIMITAR EFREMOV

THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: GUIDELINES 20
LUCA LAURENTI

THERAPY IN CHRONIC LYMPHOCYTIC LEUKEMIA: GUIDELINE LUCA LAURENTI THE RELEVANCE OFMINIMAL RESIDUAL DISEASE 22
GIANMATTEO RIGOLIN

EXPANDING THE USE OF BCR AND BCL-2 INIBITHORS 25
MAURIZIO CAVALLARI, ANTONIO CUNEO

CONCLUDING REMARKS 31
MARIO LUPPI