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Con riferimento alla Sua richiesta, a nome del Presidente e
della Commissione Attività Formative dell’Associazione,
sono lieta di comunicarLe che
la SIE, Società Italiana di Ematologia
ha concesso il Patrocinio al seguente evento
da Lei organizzato:
Chronic lymphocytic leukemia: advances in
pathogenesis and treatment
che si svolgerà
a Venezia dall’8 al 10 marzo 2018
Distinti saluti
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Loss of miR-15/16 is the most common genetic lesion in chronic lymphocytic leukemia (CLL), promoting overexpression of BCL2, which factors in leukemia pathogenesis. Indeed, an inhibitor of BCL2, venetoclax, is highly active in the treatment of patients with CLL. However, single-agent venetoclax fails to eradicate minimal residual disease (MRD) in most patients. Accordingly, we were interested in other genes that may be regulated by miR-15/16, which may target other drivers in CLL. We found that miR-15/16 targets ROR1, which encodes an oncoembryonic surface protein expressed on the CLL cells of over 90% of patients, but not on virtually all normal post-partem tissues. CLL with high-level expression of ROR1 also have high-level expression of BCL2, but low-to-negligible miR-15/16. Moreover, CLL cases with high-level ROR1 have deletion(s) at the chromosomal location of the genes encoding miR-15/16 (13q14) more frequently than cases with low-to-negligible ROR1, implying that deletion of miR-15/16 may promote overexpression of ROR1, in addition to BCL2. ROR1 is a receptor for Wnt5a, which can promote leukemia-cell proliferation and survival, and can be targeted by cirmtuzumab, a humanized anti-ROR1 mAb. We find that this mAb can enhance the in vitro cytotoxic activity of venetoclax for CLL cells with high-level expression of ROR1, indicating that combining these agents, which target ROR1 and BCL2, may have additive, if not synergistic, activity in patients with this disease.
Basal phosphorylation of SYK, upstream of BTK in the BCR pathway, is also reduced by ibrutinib, but inducibility by anti-IgM increases with IgM recovery.

In this presentation:
I) the role of surface IgM in affecting CLL behavior and
II) the dynamic adaptability of tumor IgM to precision monotherapy will be discussed from direct investigations of CLL patients.

SIGNIFICANCE OF B-CELL RECEPTOR VARIATIONS IN CLL PATIENTS

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The B-cell receptor (BCR) influences the behavior of CLL, and BCR-associated kinases are now targets of effective therapeutic drugs including BTK-inhibitor ibrutinib for patients.

By analysis of the tumor BCR rearrangements, CLL can be divided into two major biological subsets arising at different stages of differentiation, one with unmutated IGHV derived from naive CD5+ B-cells (U-CLL) and poorer prognosis, and another with mutated IGHV derived from post-follicular CD5+ B-cells (M-CLL) and good prognosis.

The two subsets are characterized by a variable degree of anergy, defined by low sIgM levels and reduced signaling capacity. They also differ in the incidence of high-risk genetic aberrations and in the DNA methylation profile, preserved from the cell of origin, that associate with IgM levels and signaling capacity.

However, sIgM levels and function recover in vitro, which strongly claims a variable and reversible influence by antigen in vivo. The sIgM levels and signaling capacity are higher and less variable in U-CLL than in M-CLL and correlate with natural disease progression between and within U-CLL and M-CLL.

The persistent CLL cell redistribution in the blood caused by ibrutinib therapy now offers a 'tool' to investigate if there is BCR engagement by putative antigen at tissue sites directly in patients. We find that during ibrutinib therapy, IgM recovers surface expression on the circulating tumor cells and remains higher than pre-therapy in the initial months of therapy, despite gradual cell size reduction and ongoing autophagy and apoptotic activity.

Conversely, IgD and other receptors do not increase and subsequently reduce expression. Recovered surface IgM is fully N-glycosylated, another feature of escape from antigen, and does not increase further during culture in vitro.
omalous signaling mediated by auto-recognition. More recently, through combined crystallographic and functional analysis, we showed that different CLL-derived BcR IG bind homotypically via their combining sites to specific, distinct epitopes to initiate intracellular signaling. Interestingly, the avidity of BcR self-recognition was found to be associated with clinical outcome, in that tight, persistent binding was identified in cases with indolent disease whereas weaker interactions characterized the aggressive progressive cases. Hence, these findings reinforce the importance of BcR signaling in the natural history of CLL, while also linking the BcR IG structure with particular modes of signaling and eventual clonal behavior.

REFERENCES


BCR SIGNALING: THE LESSON OF MURINE MODELS

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CLL B cells display a number of features that point to an important role of the BCR in the development and behavior of the disease. Such features include the frequent expression of stereotyped BCRs, the aberrant expression of regulatory molecules involved in antigen-receptor signaling and the peculiar pattern of reactivity with external and cell-autonomous antigens. To understand how these features influence disease activity, a number of mouse models have been developed in recent years. Studies with transgenic BCRs in the TCL1 model have demonstrated that BCR signals are absolutely required for CLL development and have pinpointed cell-autonomous BCR-BCR interactions and interactions with low affinity external autoantigens as primary drivers of the disease (1,2). In addition, these studies have shown an inverse correlation between the capacity of the leukemic cells to respond to external antigen and time to leukemia development, recapitulating findings in human CLL. TCL1 transgenic mice with targeted deletion or overexpression of signaling molecules that are aberrantly expressed in CLL B cells have shown that multiple mechanisms determine BCR signaling capacity of CLL B cells and have provided further evidence that the quality of the BCR signal can influence the aggressiveness of the disease. Ongoing studies are exploiting these models to identify mechanisms of resistance to drugs that target the BCR pathway and test potential strategies to overcome this resistance.

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Chronic lymphocytic leukemia (CLL) is defined as monoclonal expansion of morphologically mature B cells expressing characteristic set of surface markers and remarkably reduced amounts of the B cell antigen receptor (BCR). Several mechanisms that mediate the malignant phenotype of CLL B cells have been characterized suggesting that both cell intrinsic factors as well as signals from the microenvironment contribute to the pathogenesis and prognosis of CLL. The crucial role of the BCR in CLL is demonstrated by signs of chronic BCR activation and by the fact that inhibitors of BCR signaling belong to the most efficient drugs in the therapy of CLL patients. Since BCR activation is usually achieved by binding to cognate antigen, it was assumed that external antigens induce BCR signaling and determine the survival of CLL B cells. We have recently shown that CLL-derived BCRs possess the unique capacity of autonomous signaling, which is mediated by mutual interaction of adjacent BCRs on the same cell in an antigen-independent manner1-2. Importantly, crystallographic analysis of CLL-derived BCRs confirmed the proposed mutual BCR-BCR interaction and identified the crucial residues involved in this interaction3. These data suggest that the structural constraints of a BCR molecule define the limits for a mutual BCR-BCR interaction leading to unusual similarity in the primary sequences of CLL-derived BCRs from patients that can therefore be subdivided in distinct subsets. In addition, these results also show that the constant region of the BCR, which determines the isotype, is involved in the mutual recognition and might be important for CLL development4.

THE ROLE OF NURSELIKE CELLS

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B cell receptor (BCR) signaling is primarily activated in secondary lymphoid organs, presumably by interactions between CLL cells and the microenvironment, resulting in activation of key survival pathways for CLL cells, including c-MYC and NF-κB proteins. Importantly, when CLL cells are co-cultured in the presence of monocyte-derived nurse-like cells (NLC), an in vitro model system for the lymphoid tissue microenvironment, gene signatures associated with BCR signaling are recapitulated, along with activation of BCR signaling kinases and IgM internalization, suggesting direct antigenic engagement of the BCR.

Functionally, nurse-like cells can activate a number of pathways by secreting chemokines (CXCL12 and CXCL13) and expressing tumor necrosis factor (TNF) family members (B cell activating factor [BAFF; also known as TNFSF13B] and a proliferation-inducing ligand [APRIL; also known as TNFSF13]) and ligands for the BCR. Gene expression studies indicated that BCR signaling is the most prominent pathway activated in CLL cells upon contact with nurse-like cells. The mechanisms that trigger BCR activation in CLL have not been fully elucidated, although BCR activation in the lymphoid tissues by autoantigens and microbial antigens is the most plausible mechanism. Relevant antigens for CLL have been characterized, including self-antigens, such as non-muscle myosin heavy chain IIA, vimentin, dsDNA, oxidized lipoproteins, calreticulin, and fungal antigens. In support of a crucial role for nurse-like cells in CLL progression, deletion of LYN kinase disables the ‘nursing’ function of macrophages and thereby delayed disease progression in a mouse CLL model. Accordingly, targeting of macrophages, either by colony-stimulating factor 1 receptor (CSF1R) signaling blockade, by using clodrolip (the bisphosphonate known as clodronate encapsulated in liposomes), or by deletion of migration inhibitory factor (MIF) also delayed leukemia development in other CLL mouse models. These data collectively demonstrate that CLL-NLC interactions play a central role in BCR pathway activation and CLL pathogenesis.
THE ROLE OF THE VLA-4 INTEGRIN

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CD49d, the alpha chain of the CD49d/CD29 integrin heterodimer very late antigen-4 (VLA-4), emerged as one of the main biological predictors of overall survival and progression free survival in CLL. About 40% of CLL cases are classified as CD49d+ according to the well established cutoff of 30% positive cells to discriminate between CD49d expressing and non-expressing cases [1].

VLA-4 mediates both cell-cell and cell-matrix interactions by binding respectively to vascular cell adhesion molecule-1 and fibronectin. Through the binding with its ligands, the VLA-4 integrin is responsible for the localization of CLL cells in protective niches in the bone marrow or in the lymph nodes, and exerts a prominent role in the recirculation of CLL cells with a high-risk phenotype [2].

The VLA-4-dependent CLL cell interactions also involve other microenvironmental receptors, such as CD38, NOTCH1, and B-cell receptor (BCR), that are able to modulate both the expression and the function of VLA-4, eventually influencing CLL cell aggressiveness. CD38, usually co-expressed with CD49d, has been described to increase the VLA-4 adhesive properties through physical interactions in the context of cell membrane molecular complexes, and to functionally cooperate with VLA-4 in the bone marrow milieu where the concomitant presence of CD38 and CD49d favor the delivery of pro-survival signals for CLL cells.

CD49d is expressed at the highest levels in CLL with trisomy 12, a CLL subset characterized by a high incidence of cases with NOTCH1 mutations. Of note, the NOTCH1 pathway, which is particularly active in NOTCH1 mutated CLL, is involved in CD49d expression regulation through NF-kB dependent mechanisms. The overexpression of CD49d in trisomy 12 CLL may help explain the specific tropism toward lymph nodes of trisomy 12 CLL cells and the peculiar clinical features of this CLL subset, in which massive lymph node enlargement is often observed and the final transformation in Richter’s syndrome is more frequent than in other cytogenetic categories [3].

VLA-4 is usually present on the cell surface of resting leukocytes in an inactive conformation, but can be activated in response to different stimuli, becoming competent for high-affinity and high-avidity interactions [4]. In CLL, the VLA-4 integrin can be inside-out activated upon BCR triggering, resulting in enhanced adhesive capacities of cells to VLA-4 ligands. Such a BCR-VLA-4 interplay is particularly relevant in CLL given the central role played by the BCR pathway in this disease, as witnessed by the emerging remarkable clinical activities of several inhibitors that interfere with the action of key BCR-related intracellular enzymes [5]. In this setting, in-vitro and in-vivo ibrutinib treatment, although reducing the constitutive VLA-4 activation and cell adhesion, can be overcome by exogenous BCR triggering in a BTK-independent manner involving PI3K. The clinical implications of the VLA-4 activity also during ibrutinib treatment are that CLL cases expressing CD49d usually fail to display the canonical ibrutinib-induced lymphocytosis, and experience a lower nodal response; consistently, CD49d expression is associated with shorter PFS in the context of ibrutinib-treated CLL.

Altogether, CD49d is a molecule whose exploitation can be useful in the clinical management of CLL patients in the context of both conventional and novel therapies.

RECURRENT GENE MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Genomic studies have provided a comprehensive view of somatic mutations and epigenetic changes in chronic lymphocytic leukemia revealing a remarkable molecular heterogeneity with only few genes mutated in up to 10-15% of the patients and a relatively large number of genes recurrently mutated at low frequency. Approximately 60 candidate driver genes have been identified that tend to cluster in different pathways including NOTCH1 signaling, RNA metabolism, BCR signaling, DNA damage response, cell cycle regulation, chromatin modification and NFkB signaling among others. Recurrent chromosomal translocations are uncommon with the exception of BCL2 rearrangements and the 13q14 region associated with deletions of the miR-15a/miR-16 cluster. These genetic alterations are differentially distributed in clinical and biological subsets of the disease indicating that they may drive at least in part their heterogeneous evolution. Genome wide methylation analysis has identified three epigenetic subgroups with a distinct distribution of genetic changes, IGHV gene repertoire and stereotyped B-cell receptors. The genomic landscape of CLL is highly dynamic in the evolution of the disease. The distribution of mutations in monoclonal B-cell lymphocytosis is similar to those of population-based CLL. However, mutations in TP53, SF3B1, POT1, ATM and RPS15 increase their frequency in progressed disease. Chemotherapy and new target treatments also modulate the landscape of mutations of the disease with the selection of resistant clones. Most CLL have a complex intratumor heterogeneity that influences the evolution of the disease. Some mutated genes and structural alterations provided relevant prognostic information. The accumulative number of driver alterations and the subclonal heterogeneity of the disease discriminate patients with differences in clinical behavior. These global genomic and epigenomic studies of CLL provide new perspectives for clinical interventions that may improve the management of these patients.

MECHANISMS OF IMMUNOSUPPRESSION IN THE CHRONIC LYMPHOCYTIC LEUKEMIA NICHE

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Chronic lymphocytic leukemia (CLL) is the most common leukemia in the western world and is characterized by accumulation of mature B cells in the peripheral blood and in the lymphoid organs. Disease outcome is influenced by both a complex pattern of genetic lesions and by a network of stimuli coming from non tumoral neighboring cells in the microenvironment. Tumor-host interactions are particularly important in CLL as leukemic cells strongly depend on external factors for proliferation and survival. Indeed, whilst the fraction of CD5+ leukemic cells circulating in the PB are arrested in the G0 phase of the cell cycle, those in the BM or residing in the lymphoid tissues actively proliferate at a rate of 0,1-1% of the total leukemic clone per day. This is the result of tumor-favorable local conditions that CLL cells themselves contribute to create.

Over the past years, increasing number of studies highlighted a role for hypoxia-mediated signals in re-shaping tumor microenvironment towards immune suppression and tumor support. Accordingly, CLL niche has been found to be a highly hypoxic environment. We observed that hypoxia orchestrates local immune tolerance by suppressing T-cell functions and by altering the correct differentiation and homeostasis of T cell populations and macrophages. These effects are, at least in part, mediated by the adenosinergic system. Functionally, under low O2 tension, CLL cells undergo a metabolic adaptation that increases the levels of extracellular ATP and ultimately enhances adenosine production and signaling through adenosine receptors, expressed by leukemic B cells, T lymphocytes and macrophages. Therefore, autocrine and paracrine signalings cooperate to confer pro-survival stimuli to CLL cells and to create a tumor-supportive environment. In line with this, targeting the adenosinergic axis, by acting either on adenosine production or signaling, reverts the effects on cell differentiation and opens the way to specific inhibitors as a new therapeutic strategy in CLL.
NOTCH1 AND SF3B1 MUTATIONS: PHYSIOPATHOLOGY AND CLINICAL IMPLICATIONS

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The NOTCH receptor genes encode a family of heterodimeric transmembrane proteins (NOTCH1 to NOTCH4) that function as ligand-activated transcription factors. When the NOTCH receptors interact with their ligands through the extracellular subunit, two consecutive proteolytic cleavages of the NOTCH proteins are initiated and lead to pathway activation. Upon activation, the cleaved intracellular portion of the NOTCH receptors (ICN) translocates into the nucleus where it recruits a transcriptional complex that modifies the expression of a number of target genes, including MYC and NF-κB signaling components. The most prominent mechanism of NOTCH signal suppression is operated through its PEST domain of the ICN, which is recognized by the FBXW7 ubiquitin protein ligase and directed towards proteasomal degradation.

NOTCH1 mutations characterize ~15% of unselected CLL and are represented by frameshift or non-sense events clustering within exon 34, including the highly recurrent c.7544_7545delCT deletion (~80% of all mutations), as well as by non-coding mutations affecting the 3' UTR region of NOTCH1, which cause aberrant splicing events resulting in a deletion of the last 158 coding bases of exon 34. NOTCH1 mutations in CLL are selected to disrupt the PEST domain of the protein, resulting in NOTCH1 impaired degradation, stabilization of the active ICN, and deregulated NOTCH signaling. NOTCH1 is preferentially targeted in specific biological groups of CLL. In fact, NOTCH1 mutations are significantly more common in CLL with unmutated IGHV genes, and are enriched in CLL harboring +12.

The active intracellular portion of NOTCH1 (ICN) is also detectable in ~50% of peripheral blood CLL cases lacking gene mutations. NOTCH1 deregulated genes in CLL include key mediators of B-cell proliferation, survival, and signal transduction. In particular, NOTCH1 transactivates MYC via binding to B-cell-specific regulatory elements, thus implicating this oncogene in CLL development.

The clinical implication of NOTCH1 mutations affecting exon 34 has been clarified to a certain extent. Conversely, though mutations in the 3' UTR of the gene seem to behave similarly to exon 34 mutations, their role as prognostic biomarker needs further validation. At the time of CLL presentation, the presence of NOTCH1 mutations in exon 34 identifies a group of patients with intermediate-risk disease (~40% of cases are alive at 10 years, accounting for a ~50% reduction of the expected survival compared to the general population) and those in whom CLL is more likely to transform into RS (cumulative incidence of transformation at 10 years of ~50%). Among CLL requiring treatment, cases harboring NOTCH1 mutations in exon 34 seem not to benefit from the addition of an anti-CD20 monoclonal antibody to chemotherapy. Indeed, among CLL harboring NOTCH1 mutations, treatment with FCR does not result into the expected increase in minimal residual disease (MRD) response nor into an improvement in PFS or OS compared to treatment with the sole FC.

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This oncogene in CLL development.
SF3B1 mutation was also found to dysregulate the splicing of multiple genes involved in cellular functions including DNA damage response, telomere maintenance, and Notch signaling. Among CLL requiring treatment, SF3B1 mutations can potentially help refining prognostication of treatment relapse, though they do not represent a predictive biomarker for treatment tailoring. Indeed, the SF3B1 status does not impact on the chance of achieving responses to chemo+/-immunotherapy, though patients harboring SF3B1 mutations show a shorter PFS than SF3B1 wild type cases.

**NOVEL AND MORE RARE MUTATIONS IN CLL: PHYSIOPATHOLOGY AND CLINICAL IMPLICATIONS**

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With the introduction of next-generation sequencing technologies almost ten years ago, the genomic landscape of CLL was rapidly deciphered. Already in the pivotal studies, it became apparent that only a handful of ‘driver’ genes were frequently mutated (i.e. TP53, ATM, SF3B1 and NOTCH1), while a seemingly endless ‘tail’ of low-frequent, recurrently mutated genes was identified (e.g. BIRC3, MYD88, NFKBIE, POT1, SETD2 and XPO1). Today, more than 1000 CLL patients have been whole-exome sequenced and more than 3000 mutated genes have thus far been reported, most of which were detected in less than 5% of patients. One example concerns EGR2 mutations, which were initially reported in a study on early hematopoietic progenitor cells and linked to clinically aggressive disease. In a recent, large-scale study (including more than 2400 patients), EGR2 mutations were detected in about 4% of cases. These mutations were associated with younger age, advanced stage, and independently predicted inferior time-to-first-treatment and overall survival, similar to patients with TP53-aberrations. Hence, EGR2 mutations appear to define a new high-risk group of CLL. Another example refers to recurrent mutations in RPS15, a gene encoding a component of the 40S ribosomal subunit, that was identified in overtime studies of patients relapsing after chemoimmunotherapy (i.e. FCR). In extended cohorts, RPS15 mutations were detected in 4-6% of cases and linked to poor outcome; one-third of patients also carried concurrent TP53 aberrations. In pilot functional studies, RPS15 mutations appear to impact ribosome fidelity, but may also be involved in p53 dysregulation. Taken together, the increasing number of mutated genes occurring only in a minor proportion of cases makes it particularly demanding to study the clinical impact of more rare mutations without analyzing large cohorts of patients, but also poses a challenge to select which gene mutations to be further studied regarding their functional role. In order to gain insight into the former, the European Research Initiative on CLL (ERIC) is currently performing a large-scale study including 10 genes and more than 4000 CLL patients. This effort will hopefully inform us as to which genes to include in the future diagnostic set-up in CLL.
The entity monoclonal B cell lymphocytosis (MBL) was defined just over a decade ago but has become one of the most extensively studied and recognized pre-malignant entities in hematologic diseases. Curiously the entity of MBL was first detected because of a Center for Disease Control environmental study that found a percentage of individuals with a circulating clonal B cell population. The phenotype resembled a clonal chronic lymphocytic leukemia (CLL) B cell population and subsequent studies found that MBL is the precursor to CLL and that the prevalence of MBL is approximately 200 fold that of CLL. The incidence of MBL is 5% in individuals over the age of 40 and appears to rise to very high levels in aging otherwise healthy individuals (75% > age 90). The diagnosis of MBL is now well characterized and features a total B-cell count of <5x10^9/L with no other features of a lymphoproliferative disorder. There are two subtypes of MBL; low count and high count (clinical) MBL where the latter is often detected on routine blood work. Of note in patients with familial CLL (at least one blood relative with CLL) the incidence of MBL is significantly increased for first degree blood relatives (approximately 17 fold increase risk). Currently genome wide association studies (GWAS) have found 40 genetic susceptibility loci in CLL and that MBL share 9 of these loci. The progression of clinical MBL to overt CLL is 1% per year requiring therapy and has been the main primary health concern when MBL has been identified in otherwise healthy individuals. However more recently an additional clinical phenotype has been identified in clinical MBL and includes; a) significantly increased risk for serious bacterial infections requiring hospitalization and b) increased risks for second malignancies. These latter analyses indicate that the risk for infections and second malignancies is greater than the progression to CLL. Recent work has also shown that low count MBL have increased risk for bacterial infections. Investigations into clinical MBL finds that while some aspects of immune system are within normal levels others resemble CLL such as increased levels of exhausted T cells and decreased levels of immune synapse capacity as in overt CLL. Genetic analyses of high count MBL find a similar usage of IGHV genes and similar known CLL specific mutation sites as in overt CLL. There is also an association of mutations in known CLL driver genes for high count MBL who have subsequent disease progression. Risk of progression to CLL requiring therapy in clinical MBL is still not precisely defined but current biomarkers for that risk include the level of the blood clonal B cell count and some of the known prognostic factors in CLL. Despite the significant advances in both low count and high count MBL entities major questions remain including the etiology for both low and high count MBL, the exact relationship if any of low count to high count MBL and what are the important clinical implications of having MBL?

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4) Immunogenetics shows that not all MBL are equal: the larger the clone, the more similar to CLL. Vardi A, Dagklis A, Scarfò L,
The typical genome of unselected chronic lymphocytic leukemia (CLL) carries ~2000 molecular lesions. Few mutations recur across patients at a frequency >5%, while a large number of biologically and clinically uncharacterized genes are mutated at lower frequency. In total, more than 40 recurrently mutated driver genes have been identified in CLL. Recurrent mutations are not homogeneously spread across the CLL genome, but, rather, affect genes that can be integrated into a small set of pathways. These include microenvironment-dependent signaling through NOTCH (NOTCH1, FBXW7), inflammatory receptors (MYD88), MAPK-ERK (BRAF, KRAS, NRAS, MAP2K1) and NF-κB pathways (BIRC3, TRAF3, NFKBIE), as well as intracellular programs such as DNA damage and cell cycle control (ATM, TP53, SAMHD1, POT1), chromatin modification (HIST1H1E, CHD2, ZMYM3), transcription (EGR2, IRF4, BCOR, MED12), and ribosomal processing (XPO1, SF3B1, RPS15). Approximately 80% of CLL carry at least one of four common chromosomal alterations, namely deletion 13q14, deletion 11q22-23, deletion 17p12 and trisomy 12. Deletion 13q14 is the most frequent genetic lesion of CLL occurring in 50-60% of cases. In ~25% CLL, deletion of 13q14 occurs in the absence of any concomitant driver genetic lesion. Patients harboring solely 13q14 deletion have an excellent clinical outcome with a progression rate of less than 1% per year, longer PFS after therapy, and low risk of transformation, which overall translate into an expected survival only slightly lower than that of the general population. Deletion of 11q22-
23 always includes ATM and occurs in less than 10% newly diagnosed CLL, while its prevalence rises to ~20% at the time of first treatment and 30% at disease relapse. Mutations of the SF3B1 and NOTCH1 genes are observed in 10-15% newly diagnosed CLL, while their prevalence rises to ~20% in progressive or relapsing CLL. Deletion of 11q22-23, SF3B1 mutations and NOTCH1 mutations identify patients with intermediate-risk disease (~40% of cases are alive at 10 years) whose survival is ~50% less than that expected for the general population. Patients with deletion of 11q22-23, SF3B1 mutations or NOTCH1 mutations have short time to progression if managed with “watch and wait”, and faster relapse if treated with chemoimmunotherapy, but not if treated with ibrutinib. The risk of transformation is specifically affected by NOTCH1 mutations, but not by 11q22-23 deletion or by SF3B1 mutations. The TP53 gene is disrupted in 4-8% unselected CLL by deletions, mutations or a combination of both. The incidence rises to 10% at the time of first line treatment, 30-40% in relapsed/refractory CLL, and 50-60% in Richter syndrome, because TP53 abnormalities can be acquired/selected during the course of the disease. TP53 abnormalities mark the worst outcome in CLL, with an estimated median OS of 3-5 years that is ~70% less than that expected for the general population. Patients having TP53 abnormalities have short time to progression if managed with “watch and wait”, and do not respond or progress quite fast if treated with chemoimmunotherapy. The risk of transformation is also increased among patients with TP53 abnormalities. The negative impact of TP53 abnormalities is only smoothed, but not overcome, by ibrutinib, idelalisib or venetoclax. Integration of the most recurrent mutations onto the backbone of the FISH hierarchical model has allowed an improvement in outcome discrimination. Consistently, CLL patients with 17p13 deletion or TP53 mutation have a very poor response to chemoimmunotherapy regimens, including fludarabine, cyclophosphamide and rituximab (FCR), bendamustine and rituximab (BR), obinutuzumab and chlorambucil, ofatumumab and chlorambucil or rituximab and chlorambucil. Ibrutinib, idelalisib and venetoclax do not exert their anti-leukemic activity through genotoxic mechanisms, and are therefore active irrespective of TP53 dysfunction. Consistently, the response rate in TP53 disrupted patients is superimposable to that observed in patients with a wild type TP53 gene, and better than that observed in every previous historical control treated with chemoimmunotherapy.

Four groups of patients are hierarchically classified by the integrated mutational-cytogenetic model: i) high-risk patients, harboring TP53 and/or BIRC3 abnormalities independent of co-occurring genetic lesions, that account for ~15-20% newly diagnosed CLL and show a 10-year survival of 29%; ii) intermediate-risk patients, harboring NOTCH1 and/or SF3B1 mutations and/or del11q22-23 in the absence of BIRC3 and TP53 abnormalities, that account for ~15-20% newly diagnosed CLL and show a 10-year survival of 37%; iii) low-risk patients, harboring +12 or a normal genetics, that account for ~40% of newly diagnosed CLL and show a 10-year survival of 57%; and iv) very low-risk patients, harboring del11q1314 only in the absence of any additional abnormality, that account for ~25% newly diagnosed CLL and have a nearly normal life expectancy with a 10-year survival of 69%. The German CLL Study Group (GCLLSG) developed a comprehensive prognostic index by taking advantage of a large population of prospectively collected patients within its clinical trials. The IGHV genes of CLL can accumulate variations as a consequence of the SHM process. Patients with mutated IGHV genes, when compared to patients with unmutated IGHV genes, experience longer time to first treatment when managed with “watch and wait”, longer progression free survival (PFS) when treated with chemoimmunotherapy, lower risk of transformation and thus, in the end, better overall survival (OS). Among patients receiving chemoimmunotherapy, the IGHV mutation status affects the kinetics of relapse and thus PFS, which is longer in IGHV mutated versus IGHV unmutated patients. Accordingly, approximately 50-60% of patients with mutated IGHV genes who receive potent chemoimmunotherapy regimens maintain disease remission on the long term, which translates into a plateau on the PFS curve, no relapses beyond 10 years, and an OS similar to the one expected in normal healthy subjects. Conversely, on the long term, almost all IGHV unmutated CLL patients are projected to progress after chemoimmunotherapy.
strategies (3-5). The results demonstrate that TAMs critically support the survival and proliferation of CLL cells in vivo and suggest therapeutic strategies based upon manipulating TAM/CLL-cell interactions. GEP shows that BM monocytes/macrophages exposed to leukemic cells in vivo are enriched for specific genes involved in a number of monocyte/macrophage functions and that also the transcriptome of leukemic cell is modified underlying the cross talk bi-directionality (4). The scenario emerging from mouse findings emphasizes that the microenvironment provides critical niches where the engraftment and progression of leukemic clones occur with the help of monocytes/macrophages and that at the same time the leukemic infiltration modifies the function of normal myeloid cells during leukemia development and progression. A critical molecule on the surface of monocyte/macrophages is colony stimulating factor-1 receptor (CSF1R). In mouse models the anti-CSF1R monoclonal antibody (moAb) emactuzumab has been found to impair CLL cell engraftment and to associate with a striking anti-leukemic effect significantly improving mouse survival (4). Mechanistically macrophage targeting sensitizes leukemic cells to apoptosis via induction of TNF signaling pathway and triggers their death through a TNF-dependent mechanism. A critical intracellular signalling molecule has been shown to be Lyn (5), which had been found to be overexpressed and constitutively activated in human CLL. Evidence has been provided (5) that, while Lyn deficiency in CLL B cells does not influence the leukemia evolution, it supports CLL pathogenesis by operating in the leukemia microenvironment, as the loss of Lyn in the macrophages fails to support CLL growth.

Taken together these findings shed some light onto which are the key cells in the CLL cell neighbourhood and how they communicate. The improved understanding of the microenvironment complexity is paving the way toward novel treatment approaches.

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**CHEMOIMMUNOTHERAPEUTIC APPROACHES TODAY**

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Chemotherapeutics have been the backbone for treatment of CLL during the past decades. Combination therapies consisting of CD20 antibody (rituximab, ofatumumab or obinutuzumab) plus chemotherapy have been introduced into frontline therapy 15 years ago and are still the standard therapy in most Western countries, if del(17p) and/or TP53 mutation have been excluded by genetic analysis before treatment initiation. In relapsed CLL chemoimmunotherapy has been demonstrated to be inferior to novel agents and is now used only in patients with very long remission after frontline therapy or not suitable for novel agents. For physically fit patients the combination of fludarabine, cyclophosphamide and rituximab (FCR) is the frontline treatment of choice. Particularly patients with mutated IGHV Status benefit from this regimen with long progression-free survival (PFS) rates (54% after 12.8 years in a phase II trial, 67% 5-year PFS rate in a phase III trial).

Clinically significant toxicities associated with FCR therapy are up to 39% severe infections and 4% secondary MDS/AML, particularly affecting patients above the age of 65 years. Though bendamustine plus rituximab (BR) has been shown to be inferior to FCR with regard to PFS this regimen can be considered as an alternative in patients >65 years, because of better tolerability of BR over FCR (in patients > 65 years 21% versus 48% severe infections). Additionally overall survival (OS) is not different between BR and FCR. Patients with relevant coexisting conditions may alternatively receive combination therapy based on chlorambucil. All three abovementioned anti-CD20 antibodies have been demonstrated to be superior to chlorambucil alone with regard to PFS and for obinutuzumab also for OS, when combined with chlorambucil in elderly and less fit patients. In a head to head comparison obinutuzumab demonstrated also superiority to rituximab in PFS (29.2 versus 15.4 months; p< 0.001).
B-CELL RECEPTOR SIGNALING / BRUTON’S TYROSINE KINASE INHIBITORS IN CLL

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Chronic lymphocytic leukemia (CLL) is characterized by the clonal expansion of auto-reactive B cells whose proliferation and survival are dependent on the tissue microenvironment and B-cell receptor (BCR) signaling. Spleen tyrosine kinase (SYK), Bruton’s tyrosine kinase (BTK), and the phosphatidylinositol 3-kinase (PI3K) δ isoform are essential for BCR signal transduction but also mediate the effect of pathways engaged in CLL cells in the tissue-microenvironment. Orally bioavailable inhibitors of SYK, BTK, or PI3Kδ, induce high rates of durable responses.1 The BTK inhibitor, ibrutinib is the only kinase inhibitor currently approved as a single agent for CLL, regardless of prior treatment status or cytogenetic findings. The Pi3Kδ inhibitor idelalisib in combination with rituximab is approved for treatment of relapsed CLL. Several other BTK and Pi3K inhibitors, as well as SYK inhibitors are in clinical development.

Immediate effects of the kinase inhibitors include mobilization of CLL cells from lymphoid tissues resulting in a transient increase in lymphocytosis that has not been associated with any untoward effects in CLL. Adverse events of kinase inhibitors have mostly been grade 1 and 2; among grade 3 adverse events, infections are most common. For ibrutinib there is an increased rate of atrial fibrillation that infrequently leads to treatment discontinuation. Idelalisib has been associated with autoimmune complications, including colitis and hepatitis that lead to interruption or discontinuation of therapy.2 ibrutinib covalently attaches to a Cys481 in BTK leading to sustained target inhibition and abrogation of critical signaling pathways in the CLL cells, in particular BCR signaling. Immediate effects in addition to the transient increase in lymphocytosis, include inhibition of tumor proliferation, and an increase in apoptosis.3 In randomized clinical trials, ibrutinib induced higher objective response rates (ORR) and extended survival compared to the comparator treatment for both relapsed/refractory and treatment-achieved 22.4 months median PFS.4 Ongoing randomized studies will show if therapies based on novel agents will replace chemoimmunotherapy also in frontline in the near future.

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naïve CLL patients. Notably, the rate of infections decreases with time on ibrutinib and IgG levels appear to be mostly stable, while IgA levels tend to rise, suggesting an improvement in immune function. Remarkably, despite the covalent inhibition of BTK, humoral immune responses to vaccines are possible. While initial response rates are high, most responses are partial, and disease progression on continuous therapy with ibrutinib has been described. Early progression, within the first couple of years, often presents as Richter transformation, while late progression is most often associated with the emergence of CLL clones that carry mutations in BTK affecting the cysteine (Cys481) that serves as the binding site of ibrutinib and/or PLCG2. Unanswered questions about BTK and PLCG2 mutations include the prevalence of mutations over time, the impact of the mutations on CLL biology, and the identification of at risk populations. Clinically, there is a need to develop effective salvage therapies for patients with disease progression on ibrutinib. Combinations of ibrutinib with monoclonal antibodies, chemoimmunotherapy, and the BCL-2 inhibitor venetoclax are actively being investigated and early results show high rates of response. Longer follow-up is required to fully appreciate the safety and efficacy of combinations compared to single-agent therapy.

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rituximab versus bendamustine with rituximab (BR) for relapsed patients with CLL.7 Patients were randomized to venetoclax 400mg daily for up to 2 years with 6 monthly doses of rituximab (VenR) versus 6 cycles of standard BR. Independent Review Committee-assessed PFS was markedly improved for patients who received VenR versus BR (HR=.19, p<.0001). All subgroups benefited with VenR, including del(17p) and unmutated IGHV CLL. In fact, there was no significant difference in PFS between those who received VenR with versus without del(17p) CLL. Myelosuppression with neutropenia is the most common adverse event with VenR, and was manageable.

Venetoclax monotherapy was also evaluated in patients who were refractory or intolerant to ibrutinib 8. The ORR was 70%, with 67% PR and 2% CR, and estimated 12-month PFS was 80%. My preference is to give venetoclax with rituximab, including in this setting, based on this MURANO trial data. Venetoclax-based treatment provides an excellent standard of care treatment option for ibrutinib-refractory CLL.

Early results of novel combinations of venetoclax plus BTK-inhibitor, with or without CD20 mAb were recently reported at ASH. These studies report highly effective and well-tolerated therapy with deep remissions occurring rapidly, particularly in treatment naïve patients. Confirmation of these early results will come as these studies mature.

Venetoclax is administered once daily and can be associated with mild toxicities, including gastrointestinal intolerance and neutropenia.3 It is a highly potent inducer of CLL cell apoptosis, accounting for the most notable potential toxicity of tumor lysis syndrome (TLS) that occurs if patients are started at too high a dose, or if they dose-escalate too rapidly. Therefore, patients are started at a low daily dose of 20 mg, and escalated weekly over 5 weeks to the target 400 mg daily, to mitigate significant TLS risk. Even with this strategy, patients at high-risk for TLS by virtue of having bulky lymph nodes and high leukemia count, must be aggressively hydrated prior to and during initiation and escalation and closely monitored.

The era of small molecule inhibitors has ushered in remarkable advances in treatments and improved survival for patients with CLL, particularly in high-risk disease. The Bcl-2 small molecule inhibitor venetoclax is
playing a major role in this progress, providing a highly active agent that induces deep remissions and which is well-tolerated and can effectively be combined with potentially synergistic agents for time-limited therapy.

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COMORBIDITIES AND OTHER CANCERS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL), the most common leukemia in the western world, is primarily a disease of older individuals. In the United States in 2017 there will be 20,110 new cases of CLL, the median age of the patients at diagnosis is 70 years and the estimated survival at 5 years is 83%.

Older patients tend to have a large number of co-morbidities and it has been reported that 89% of the patients with CLL will have one or more co-morbidities at the time of diagnosis. The most frequently reported co-existing medical diseases are rheumatological diseases, cardiovascular complications and other cancers. Co-morbidities are often severe and approximately half of the patient will have at least one co-morbidity defined as major (1, 2,).

Patients with CLL have an extended survival and are vulnerable to the occurrence of other cancers (OC). OC have an increased incidence in patients with CLL compared to the general population (3). The demographics of CLL patients per se (typically elderly, white males) portend a higher risk for cancer. In addition, disease-related immune perturbations cause defective immune surveillance, further heightening the risk of OC. Other potential contributing factors to the development of OC include genetic predisposition, shared risk factors, and the use of chemotherapy.

Not only patients with CLL are at increased risk of OC, but the overall and cancer-specific survival from several common cancers is shorter in patients with pre-existing CLL at the time of cancer diagnosis compared to matched patients without CLL, according to an analysis of the SEER database (4).
MOLECULAR PATHOGENESIS OF CHRONIC LYMPHOCYTIC LEUKEMIA

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Recent investigations have provided a comprehensive picture of the CLL genome (1), revealing its relatively low burden of genetic lesions, which include chromosomal gains (trisomy 12) and deletions (17p, 11q, and 13q14), as well as recurrent somatic non-synonymous mutations of a few genes, including the NOTCH1 oncogene, the splicing regulator SF3B1, the tumor-suppressors TP53 and ATM, and several BCR/NF-κB regulators such as MYD88, BIRC3 and NFKBIE (1). CLL can transform (Richter syndrome, RS) into an aggressive lymphoma, most commonly of the diffuse large B cell lymphoma (DLBCL) type, which typically arises from the predominant CLL clone by acquiring an average of ∼20 genetic lesions/case, which include those involved in CLL progression and chemo-refractoriness (TP53 disruption and NOTCH1 activation) as well as some not previously implicated in CLL or RS pathogenesis. Notably, the genomic landscape of RS is different from that of de novo DLBCL, from DLBCL deriving from Follicular Lymphoma transformation, as well as from that of CLL undergoing clinical progression (1).

Deletions of 13q14 are the most common aberration in CLL (55%) and are found at a similar percentage in MBL and less frequently in diffuse large B-cell lymphoma, multiple myeloma, and several types of non-B cell tumors. The extensive characterization of 13q14 deletions has demonstrated that the break points are heterogeneous and that the minimal deleted region (MDR) comprises the deleted in leukemia 2 (DLEU2) gene encoding a sterile RNA transcript and the microRNA 15a/16-1 cluster that is located in an intron of DLEU2. The causative role of miR-15a/16-1 in CLL pathogenesis has been demonstrated in a knockout mouse model in which these microRNAs were specifically deleted in B cells (1). However, a larger deletion (MDR) including DLEU2 shows a more aggressive phenotype, suggesting that additional genetic elements in addition to miR-15a/16-1 loss, including DLEU2, may contribute to CLL progression.

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pathogenesis. Consistent with this notion, we will describe initial results indicating that the long non-coding DLEU2 RNA has biological activity. NOTCH1 has emerged as the most commonly mutated gene in CLL at diagnosis, accounting for ~4-13% of patients (1). In contrast with T-ALL, where the majority of mutations are represented by constitutively activated ligand-independent alleles affecting the heterodimerization (HD) domain of the protein, most NOTCH1 mutational events in CLL are represented by PEST-truncating events removing the phosphodegron sequence required for FBXW7-mediated ICN1 proteasomal degradation. However, CLL cells in the lymph node frequently express ICN1 independent of NOTCH1 PEST-truncation, especially within the proliferation centers. We have analyzed the functional status of ICN1 in normal mature B-cells and in a panel of peripheral blood CLL cells including both NOTCH1 mutated and wild-type cases, and found broader NOTCH1 activation significantly extending beyond the mutated cases (2). The implications of these findings for CLL pathogenesis and NOTCH1 therapeutic targeting will be discussed.

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