
# RESEARCH IN LATIN AMERICA

## abstracts

## Selected abstracts for oral presentation



## Accepted abstracts

## CHRONIC LYMPHOCYTIC LEUKEMIA: PROTEIN KINASE AKT AND MICRORNAS GENE EXPRESSION EVALUATION AND THEIR IMPORTANCE IN DISEASE PATHOGENESIS

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Chronic lymphocytic leukemia (CLL) is recognized by heterogeneous clinical course and cannot be accurately predicted by clinical staging systems. This leads to investigation of prognostic markers that can add predictive value to staging systems. The present study aimed to evaluate markers with potential for diagnosis and prognosis focusing on AKT protein kinase and microRNAS expression in individuals with CLL compared to controls. We evaluated 60 individuals diagnosed with CLL at the Hematology Service. The same analyzes were performed in 44 individuals, apparently healthy and without previous history of leukemia (control group). The consent term was obtained from all participants according to the privacy law. AKT and microRNAs gene expression was assessed by qPCR. There was a significant increase in AKT gene expression in patients when compared to controls (p= 0,017). Considering Binet staging groups, a significant difference was observed between the groups, being higher in the B+C group (p= 0,013). The miR-27a, miR-let-7b, miR-21 and miR-26a microRNAs were also evaluated in CLL patients and in the control group. No significant differences were observed in miR-27a and miR-21 gene expression when compared to controls. On the other hand, there was a significant increase in miR-let-7b (p= <0,001) and miR-26a (p= <0,001) gene expression in controls when compared to CLL patients. These miRNAs appear to be related to tumor suppression. Increase of AKT protein kinase and miR-let-7b and miR-26a reduction in CLL patients may explain, at least in part, the increase in lymphocytes survival in these patients.

## DEVELOPMENT OF ARTIFICIAL BINDING PROTEINS FOR SPECIFIC ROR1 BINDING AND TUMOR TARGETING

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Chronic Lymphocytic Leukemia (CLL) follows a variable course, with survival ranging from months to years and despite significant progress in treatment, it remains incurable. Absence of a powerful prognostic marker and the complete remission by gold standard treatment leads to investigate new strategies for diagnosis and therapy. In the last years, the surface protein receptor tyrosine kinase orphan receptor 1 (ROR1) has appeared as one of the most specific marker for CLL malignant cells. Taking this into account, we aimed to develop a new prognostic method and a possible novel therapeutic strategy based on the targeting of ROR1 in CLL using artificial binding proteins (ABPs). ABPs are non-immunoglobulin protein scaffolds that belong to the new generation of combinatorial protein engineering technologies and can combine the high affinity and specificity of antibodies with a small size, high yield production and high thermal and chemical stability. In this regard we expressed the extracellular domain of ROR1 and performed Ribosome Display selections for obtaining ABPs derived from the affitin scaffold against ROR1. Binders presenting strong interaction with ROR1 and not to unrelated proteins as seen by ELISA, were selected and expressed in a larger scale for further characterization. In parallel, we set up an *in vitro* ligation method to covalently ligate the anti-ROR1 selected binders with ULBP2-Fc molecules in order to redirect the citotoxic effects of NK cells to the malignant B cell. Presently, anti-ROR1 ABPs are being tested on primary CLL cells using flow cytometry and microscopy approaches.

## AUTOLOGOUS T CELL ACTIVATION FOSTERS VENETOCLAX RESISTANCE IN CLL: RATIONALE FOR A COMBINED THERAPY WITH THE BCR KINASE INHIBITOR GS-9973 AND ANTI-CD20 MABS

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Leukemic B cells from CLL patients survive and proliferate within lymphoid tissues in contact with activated T cells, myeloid cells and receiving signals through the BCR. Venetoclax (ABT-199) is a potent selective inhibitor of BCL-2, which rapidly induces apoptosis in unstimulated CLL cells *in vitro*. We here investigated whether resistance to venetoclax can be conferred by autologous T cell activation. Cell survival was evaluated by flow-cytometric alterations of light-scattering properties and confirmed by Annexin-V-FITC assay. The expression of cell activation markers was determined by flow cytometry, BCL-XL and MCL-1 by western blot and phagocytosis of CFSE-labeled CLL cells by flow cytometry and confocal microscopy. We confirmed that unstimulated leukemic cells are highly sensitive to venetoclax, compared to T cells, NK cells, and monocytes ($n = 30$, $p < 0.001$). Leukemic cells cultured in the presence of activated T lymphocytes were clearly less sensitive to the drug and this resistance was overcame by GS-9973 ($n = 18$, $p < 0.01$). Autologous activated T cells enhanced the activation of CLL cells ($n = 19$, $p < 0.0001$) and the upregulation of MCL-1 and BCL-XL which are not targeted by venetoclax ($n = 8$). Finally, we found that venetoclax increased the phagocytosis of rituximab-coated CLL cells ($n = 20$, $p < 0.01$) even in presence of GS-9973 ($n = 13$, $p < 0.05$). In conclusion, our results suggest that leukemic cells from the supportive microenvironment might not be properly targeted by venetoclax monotherapy and encourage the combination of the drug with BCR-kinase inhibitors, such as GS-9973 and anti-CD20 mAbs.

## METABOLOMICS APPROACH IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Chronic lymphocytic leukemia (CLL) is one of the most common lymphoid malignancies that result in quantitative and qualitative changes in blood cells (B lymphocytes). Metabolomics is a recent analytical approach that aims to identify and quantify metabolites present in biological samples. In this context, an application of these analyzes in patients with leukemias is still incipient. Plasma samples from 22 patients with CLL and of 19 healthy individuals who constituted the control group were analyzed (the consent term was signed according to privace law). Metabolites were analyzed using a quantitative and controlled metabolomic target based on Absolute/DQ® p180 Kit (Biocrates Life Sciences AG, Innsbruck, Austria) instructions. The Ultra Performance Liquid Chromatography (UPLC) technique associated with the tandem quadrupole mass analyzer was used. The validated assay allowed a comprehensive identification and quantification of 186 endogenous metabolites, including 21 amino acids, 19 biogenic amines, 40 acylcarnitines, 90 glycerophospholipids, 15 sphingolipids and 1 sum of hexose. The data were based on multivariate statistical methods (SIMCAP + (14.0.1, MKS) and univariate (MATLAB). The PCA, PLS-DA and OPLS-DA statistical models were used to create a metabolic ranking. Metabolic routes were analyzed through HMDB, KEGG and MBROLE databases. Some metabolites were selected according to increased (citruline, glutamate, threonine, asparagine) or decrease levels (glycerophospholipids) in CLL patients when compared to control group. Metabolic profile preliminary analysis of this experiment allowed identification of metabolites related to cancer metabolism processes, which may be considered possible targets for future research of diagnostic and prognostic markers as well as therapeutic targets.

## MARCADORES MOLECULARES CON VALOR PRONOSTICO EN PACIENTES CON LEUCEMIA LINFOCITICA CRONICA (LLC) EN UNA COHORTE DE PACIENTES ARGENTINOS.

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OBJETIVO: evaluar la frecuencia de marcadores moleculares en pacientes con LLC en nuestra población. METODOLOGIA: se realizó análisis retrospectivo de 79 pacientes diagnosticados con LLC por morfología y citometría de flujo multiparamétrica. En ADN se realizó el análisis del rearreglo y status mutacional de los genes IGHV según protocolo BIOMED-2, y se evaluó por hibridación fluorescente in situ (FISH): deleción de 17p13, deleción de 11q22, deleción de 13q14 y trisomía del cromosoma 12. Se empleó estadística descriptiva para el análisis de los datos. RESULTADOS: la mediana de edad fue de 66 años (rango: 35-84), relación varón/mujer 1.9:1. Distribución según score RAI (disponible en 53 pacientes): 0, 21 (40%) pacientes; I/II, 21 (40%) y III/IV 11 (20%). Cuarenta y nueve pacientes (62%) fueron mutados (LLC-M) y 30 (38%) no mutados (LLC-NM). El análisis del score clínico mostró 76% (16/21) de pacientes LLC-M con RAI 0. Las familias de IGHV más frecuentes fueron IGHV3>IGHV1>IGHV4>IGHV2, y los reordenamientos génicos más usados: IGHV4-34 (14.3%), IGHV 1-69 (11.7%), IGHV3-30 (10.4%) e IGHV1-02 (9.09%). La frecuencia de deleciones halladas por FISH fue: 5% P53 (3/66), 17% ATM (6/30), 35% 13q14 (6/23). Se detectaron 11% de trisomías del cromosoma 12 (2/19). CONCLUSIONES: La distribución de familias IGHV en esta cohorte de pacientes presentó características comparables a las de los países occidentales tal como se ha descripto en otras series de nuestro país. Se destaca la importancia de considerar el status clínico del paciente al diagnóstico para orientar el estudio de los factores pronósticos necesarios en cada momento.

## RESULTS OF MUTATIONAL STATUS OF IMMUNOGLOBULIN HEAVY-CHAIN VARIABLE GENE ANALYSIS IN A COHORT OF PATIENTS WITH B-CLL. A SINGLE CENTRE EXPERIENCE.

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BACKGROUND: The mutational status of the variable region of the Immunoglobulin heavy-chain (IGHV) gene in Chronic Lymphocytic Leukemia (CLL), allows the identification of 2 groups with different clinical behavior. European Research Initiative on CLL (ERIC) has defined guidelines for Immunoglobulin gene sequence analysis. MATERIALS AND METHODS: Patients with CLL were studied for productive IGHV rearrangements and its mutational status. Genomic DNA was used, and ERIC guidelines were followed. DNA was isolated and amplified using forward Leader primers. If Leader primers were unsuccessful at providing a product that could be sequenced, VH FR1 primers were used. Direct bidirectional sequencing was performed and Stereotype B cell receptors were analyzed. RESULTS: 102 samples were analyzed between 6/2016 and 4/2018, 94 productive rearrangements were identified, 8 patients had insufficient leukemic cells. Of 94 patients 35 (37%) were UM-CLL and 59 (63%) were M-CLL. Within this last group, 8 were considered "borderline" M-CLL. The most frequent IGHV family in this series was IGHV3, followed by IGHV4 and IGHV1. Double rearrangements were detected in 10 of the cases. Stereotype B cell receptors were found in 10 patients. CONCLUSION: These results show a higher incidence on M-CLL than the published data. We believe this is due to a high proportion of asymptomatic patients with no need of treatment, in this cohort. The most frequent used IGHV family was IGHV-3. Differing from a cohort of Argentinean patients and of the western countries, in this series IGHV-4 was the second most frequent IGHV family.

## ASSOCIATION BETWEEN MICRONUCLEUS FORMATION AND GENOMIC COMPLEXITY IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.

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Genomic instability is a hallmark of human cancers, promoting oncogenesis and clonal evolution. Basal chromosome instability measured by micronucleus (MN) frequency was evaluated in 74 patients with chronic lymphocytic leukemia (CLL) and 15 normal controls (NC). Cytogenetic, FISH and *IGHV* mutational status analysis were performed. The study was approved by the local Ethics Committee. All patients provided their written informed consent. An increased MN frequency in patients (3.1 ± 1.7%) compared to NC (0.7 ± 0.3%) (p = 0.0001) was observed. No difference between cases with normal (2.94 ± 1.83%) and abnormal karyotypes (3.08 ± 1.5%) was found. Considering FISH risk groups, the highest MN frequency was detected in patients with ≥ 2 FISH alterations (3.71 ± 1.87%) followed by cases with del13q14 (2.78 ± 1.83%), trisomy 12 (2.79 ± 1.7%), and no alterations (NA) (2.39±1.14%), with differences between ≥ 2 FISH alterations and NA groups (p=0.018). When the cohort was divided using the cut-off obtained by ROC analysis (2.2%), a higher MN frequency in patients with ≥ 2 FISH alterations compared to the other FISH groups (p = 0.04) was found. Interestingly, cases with high MN frequency had shorter time to first treatment than those with low frequency (p = 0.011). No differences between cases with mutated and unmutated *IGHV* status were observed. Our results support the strong association between MN formation and genomic complexity as well as their influence on poor outcome in this pathology.

## ESTUDIO DE SOBREEXPRESIÓN DE AID Y LPL VINCULADO AL ÍNDICE CLL-IPI Y SU IMPACTO SOBRE EL TIEMPO DE INICIO DE TRATAMIENTO.

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La búsqueda de marcadores pronósticos que permitan anticipar la progresión en la Leucemia Linfoide Crónica ha sido uno de los principales objetivos científicos recientes. Con dicho fin se propuso un Índice Pronóstico Internacional (CLL-IPI) *(The International CLL workinggroup, Lancet, 2016)* que define riesgo evolutivo. Se evaluó el beneficio de agregar al CLL-IPI dos marcadores pronósticos: la expresión del RNA mensajero de la proteinalipoprotein lipasa (LPL) y de la citidinedeaminase inducida por la activación (AID), descritos como marcadores de progresión tumoral (Oppezzo and Vasconcelos et al., Blood 2005) (Heintel et al., Leukemia 2004). El objetivo fue correlacionar el CLL-IPI con AID y LPL en la población de estudio y evaluar si agrega mejoras en el valor predictivo. La cohorte analizada está compuesta de 123 pacientes con una media de edad de 67 años (58% IgVH mutado). Los resultados muestran que el 68% de los pacientes de riesgo CLL-IPI bajo son AIDneg/LPLneg mientras que el 56% de los pacientes de riesgo CLL-IPI muy alto son AIDpos/LPLpos. Se identifica dentro de los de riesgo CLL-IPI intermedio y alto dos subgrupos, AIDpos/LPLpos y AIDneg/LPLneg con diferencias significativas respecto al tiempo de inicio de tratamiento. La incorporación de AID y LPL al CLL-IPI podría generar un método pronóstico adicional que le permita al médico extremar los controles clínicos ante la previsión de pacientes que requerirán tratamiento precozmente.

## AID-RELATED SOMATIC SIGNATURES AND THEIR ROLE IN TUMOR PROGRESSION OF CLASS SWITCHED VS NON-SWITCHED B-CELL NEOPLASMS

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The two most common indolent B-cell neoplasms are Chronic Lymphocytic Leukemia (CLL) and Follicular lymphoma (FL). CLL and FL are characterized by distinctive pathogenic processes. Nevertheless, current evidence indicates that activation-induced cytosine deaminase (AID) activity may constitute a shared pathogenic mechanism. AID initiates somatic hypermutation and class-switch-recombination of immunoglobulin. However, off-target AID activity may induce mutations that affect tumor suppressor and proto-oncogenes associated with malignant transformation. FL is characterized by constitutive AID expression; we and others have demonstrated that continuous AID activity results in clonal evolution and mutation of proto-oncogenes. We also described that, in IgM expressing FL, the mutation load of immunoglobulin genes can be described as a function of the AID expression. In contrast, in cases that underwent class-switch-recombination AID expression and somatic hypermutation are dissociated. In CLL high expression of AID and active class-switch-recombination might account for a more aggressive disease. A subset of CLL B-cells with AID expression display dissociation between class-switch-recombination and somatic hypermutation, resembling FL observations. We therefore hypothesize that class switched malignant B-cells display a distinctive mutational landscape as compared to non-switched malignant B-cells, which can be related to AID activity.

We will present our work in progress as to:

1. The development of a bioinformatics pipeline to identify AID-related mutation signatures.
2. The *in vivo* validation of AID-related mutation signatures detection.
3. The differential contribution of AID-related mutations in FL and CLL cases.
4. The relation between AID-related genomic changes with the clinical outcome of FL and CLL patients.

## A PAUCITY OF VH3-21 AND A DISTINCT PATTERN OF STEREOTYPED B-CELL RECEPTORS IN CHRONIC LYMPHOCYTIC LEUKEMIA FROM BRAZIL

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The mutational status of the immunoglobulin heavy chain (IGHV) genes is one of the most important prognostic/predictive biomarkers in CLL. Stereotyped B-cell receptors (sBCR) are described in ~30% of cases, associated with distinct clinical-biological characteristics. Brazil has continental dimensions and marked ethnic contrasts, and its IGVH diversity has not been described. We describe IGVH mutation status and sBCR in 306 CLL patients (Rio de Janeiro N=122, São Paulo N=73, Minas Gerais N=34, and Pernambuco N=77). Sequence data were analyzed using the IMGT database, the ARResT/Teiresias clustering tools and a databank of 2508 stereotyped sequences. In diagnostic samples, UM cases were slightly more frequent (54%) than M cases (46%). A scarcity of VH3-21 rearrangements (1.8%) along with a lack of stereotyped subset#2 (the most frequent in international series) was observed. With our comprehensive approach, 69 BCRs were defined as stereotyped (20.8%; 8.46 major and 9.37% minor subsets) The frequency of major subsets was inferior to the international series (~12%) for all localities except São Paulo (24.7% total; 12.3% major subsets). The most frequent subsets were #1 (1.5%, represented in all 4 localities) and #5 (1.5%), followed by #3, #4, #8. Minor subsets, including new stereotyped sequences characteristic of Brazilian CLL were described. We describe for the first time a diverse Brazilian series of CLL patients, which exhibited particular immunogenetic characteristics, likely reflecting particular pathogenic stimuli or ethnic composition in the geographical regions.

## CHRONIC LYMPHOCYTIC LEUKEMIA CELLS FROM PROGRESSIVE PATIENTS INHIBIT INFLAMMASOME AND AVOID PYROPTOSIS CELL DEATH

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Chronic Lymphocytic Leukemia (CLL) is characterized by a reinforcement of malignant cell/microenvironment interactions. Relationships between leukemic cells with non-tumor cells and/or soluble factors in lymphoid organs drive disease progression. The hypothesis of this work is that an inflammatory scenario sustained in time in chronic neoplasm, could be pathologically relevant during disease progression. In CLL, our results show that in progressive cases S100A9 protein, a damage-associated molecular pattern (DAMP) is associated with NF-kB activation and disease progression. Since DAMP proteins are implicated in regulating immune response and inflammation, this work try to deep insight on the role of inflammation during CLL progression.TMEM176A is an intracellular channel molecule that regulates inflammasome, a principal actor in inflammation. Inflammasome respond to cellular stress by activating caspase-1 and triggering pyroptosis. The role of this inflammatory pathway has not been yet studied in CLL. In this work we demonstrate that progressive patients have less leukemic cells with active caspase-1 than from indolent cases and that TMEM176A is highly expressed in progressive CLL cases. Our results show that this overexpression regulates caspase-1 in CLL cells and that pharmacological inhibition of TMEM176A induces caspase-1 dependent-death. Herein we propose that progressive CLL downregulates caspase-1 trough TMEM176A overexpression and that this can be beneficial for disease progression. Our results underline the importance of pyroptotic death and inflammation in CLL in-vivo, opening new questions about current and innovative therapies for this leukemia.

## CHARACTERIZATION AND EXPRESSION OF THE TCR/CD3 CHAINS OF THE EXTRACELLULAR VESICLES FROM SUPERNATANT EX VIVO CELL CULTURE, BEFORE AND AFTER EXPOSURE TO FLUDARABINE, OF PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Immune disorders in chronic lymphocytic leukemia (CLL) is a clinical problem. In order to elucidate the feature of extracellular vesicles (EVs) in this CLL dusruption, culture of peripheral mononuclear cells from 28 CLL patients and 24 controls were performed to evaluate EVs: size distribution by dynamic light scattering and nanoparticle tracking; interference on gene expression of CD3/TCR complex (γ, δ, ε, and ζ) by qPCR; immunophenotyping by flow cytometry; and protein profile on SDS-PAGE. All participants of this study signed an informed consent form accordingly to the privacy law. The EVs sizes among patients, their clinical classifications and controls showed no significant difference. Significant lower expression levels of CD3 ε, and ζ chain genes were found in patients compared to the controls ($p = 0.014$ and $p = 0.008$ respectively), and there was a greater carry of these genes by the patients EVs than controls subjects. There was a subtle difference of protein profile in EVs from moderate risk patients (Binet B). Immunophenotyping confirmed predominance of EVs from lymphocytes. The estimated concentration of EVs of controls, the non-exposed and exposed EVs to fludarabine in culture was 9.66e + 008 (+/- 0.35e + 007); 1.19e + 009 (+/- 0.73e + 007); and 7.61e + 008 (+/- 0.48e + 007) particles per ml respectively. In conclusion, the data suggest the potential role of EVs in the CLL on maintenance of pathogenic microenvironment due to their increase in number, interference in the expression of CD3 chain genes and variation in their protein profile.

## INNATE IMMUNE RECEPTORS STIMULATION MODULATES CELL DEATH INDUCED BY CHEMOTHERAPEUTICS DRUGS IN CHRONIC LYMPHOCYTIC LEUKEMIA CELLS.

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INTRODUCTION: The treatment of patients with Chronic Lymphocytic Leukemia (CLL) can include chemotherapy, chemoimmunotherapy, or use of agents targeting specific B-lymphocyte pathways (BCR signaling, BCL-2, etc.). However, CLL remains incurable, drug resistance is a major cause of treatment failure and other unknown biologic factors may contribute to treatment failure. Different groups of CLL patients show heterogeneous response to Toll-like receptor (TLR) 9 stimulation in terms of proliferation and apoptosis, suggesting that additional molecular characterization of functional roles of different TLRs is needed. We previously reported that stimulation of TLR2 promote cell death by inducing autophagy in phagocytes. In cancer, autophagy plays dual roles, acting as a tumor suppressor mechanism and a pro-survival response, promoting tumor growth. In this context, our objective was to study if the stimulation innate immune receptors stimulation, such as TLRs and Nucleotide-binding oligomerization domain-containing protein 2 (NOD2) modulates fludarabine or BCl-2 inhibitor (ABT-199) induced CLL cell death. MATERIALS AND METHODS: Purified blood mononuclear cells (PBMC) isolated from CLL patients were incubated with Fludarabine or ABT-199 in the presence of a TLR2 ligand (Pam3CSK4) or a NOD2 ligand (MDP). Then, cell death was evaluated by flow cytometry and LC3BII expression by western blot. RESULTS: Pam3CSK4 modulated Fludarabine-induced CLL cell death and increased LC3BII expression. Interestingly, this effect was potentiated by co-stimulation with Pams3CSK4 plus Fludarabine. On the other hand, MDP induced similar effects on LC3BII expression and modulated CLL cell death by ABT-199. CONCLUSIONS: These preliminary results suggest that innate receptors may affect autophagy and leukemia cell survival.

## IMPACT OF DELETION 17P CLONE SIZE ON OUTCOME IN CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS IN A POPULATION-BASED ANALYSIS IN RIO DE JANEIRO, BRAZIL

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OBJECTIVES: Chronic lymphocytic leukemia (CLL) presents a heterogeneous clinical course. Treatment mainly aims to control clinical manifestations and improving survival. Some biological markers have significant prognostic value. Cytogenetic studies in CLL have central role to refine the follow-up of disease evolution, to guide treatment options and monitoring response. Due to low mitotic index in conventional cytogenetic studies, fluorescence *in situ* hybridization (FISH) became an indispensable tool. The main objective of this study was analyze the presence of del(17p13) using FISH and the percentage of positive cells that have impact on survival, along with clinical characteristics from CLL patients. METHODS: Seventy-one CLL patients were studied from 2010 to 2017. Median age was 57 years old and male patients were 56,4%. FISH analysis was performed using peripheral blood samples from patients and TP53 spectrum srange/CEP 17 spectrum green probe (Vysis). Statistical analysis was performed with SPSS20.0 software. RESULTS: Twenty-eight of 71 patients had positive FISH for del(17p13) and the evidence of more than 20% del(17p13) cells was related to a worse overall survival ($p<0,05$). No difference in survival was observed between negative FISH patients and those with less than 20% del(17p13) cells. CONCLUSIONS: Patients presenting del(17p13) in more than 20% of cells had worse prognosis with reduced survival. Our results suggest the prognostic relevance of deletion17p clone size detected by FISH in CLL patients.

## EFFECT OF THE BRUTON TYROSINE KINASE (BTK)-INHIBITORS SPEBRUTINIB (CC-292) AND ACALABRUTINIB (ACP-196) ON MACROPHAGE ´ S PHENOTYPE AND FUNCTIONS.

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Ibrutinib is a first-in-class Btk-inhibitor used in the treatment of CLL. Besides its effects on leukemic B-cells, ibrutinib also affects functions on T cells, NK cells and macrophages, in some cases due to inhibition of off-target kinases. Second generation more selective Btk-inhibitors have been developed and are being evaluated in clinical trials. Here we aimed to evaluate the effect of the second-generation Btk-inhibitors, spebrutinib (CC-292/AVL-292) and acalabrutinib (ACP-196), on macrophages´ phenotype and functions. We found that spebrutinib and acalabrutinib did not reduce the phagocytosis of rituximab-coated CLL cells by macrophages, as it was reported for ibrutinib (n=7). Interestingly we found that acalabrutinib impaired macrophage polarization into the M1 profile, associated with an effective anti-microbial immune response, by up-regulating M2-markers (CD206-CD14-CD16 and CD163), by down-regulating M1-markers (CD86-HLA-DR) and by affecting glucose metabolism, with a decrease in glucose consumption and lactate production (n=6, $p<0.05$). In contrast, we found that spebrutinib did not modify M1 markers while it affects glucose metabolism (n=6, $p<0.05$). Then, we analyzed the effect of the Btk-inhibitors on macrophage´s response to microbial stimulation and found that ibrutinib and acalabrutinib, but not spebrutinib, significantly decreased TNF-α secretion in response to Pam3CSK4, LPS and irradiated-*Mycobacterium tuberculosis* (n=10, $p<0.05$). Altogether, our findings show that spebrutinib, acalabrutinib and ibrutinib have different effects on macrophages, probably due to their differences in kinase-selectivity, with potential consequences on combination strategies with anti-CD20 antibodies as well as on the innate-immune system of treated patients.

## LPL PROTEIN IN CHRONIC LYMPHOCYTIC LEUKEMIA HAVE DIFFERENT ORIGINS IN MUTATED AND UNMUTATED PATIENTS. ADVANCES FOR A NEW PROGNOSTIC MARKER IN CLL.

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Among different prognostic factor in Chronic Lymphocytic Leukemia (CLL), lipoprotein lipase (LPL) has been associated to an unmutated immunoglobulin profile and poor clinical outcome. To date, the anomalous expression at mRNA level of *LPL* and the functional role of this protein in CLL are still open and controversial issues. In this work we evaluated the subcellular localization of LPL protein in CLL cells expressing LPL mRNA and compared these levels with negative CLL cells for LPL mRNA. Our results show that LPL protein inside CLL cells could have different localizations depending on the *IgVH* mutational status. LPL protein in unmutated cases is principally found associated with markers of endoplasmic reticulum, while in mutated cases, which are negative for LPL at the mRNA, this protein is incorporated from the extracellular medium and remains associated with endosome/lysosome vesicles. This different subcellular localization of endogenous LPL in CLL cells reveals that it is possible to set up a specific method aiming to quantify endogenous LPL protein levels to be used as a prognostic marker in this leukemia. For this, we present an easy and accessible flow cytometry protocol based on the single immunolabeling of LPL protein on fresh PBMCs of CLL patients which is related to *LPL* mRNA expression levels and to the mutational *IgVH* status. At present, we are starting a validation phase comparing specificity, sensibility, and predictive power of LPL, Zap-70 and CD49d prognosis markers in a regional study in Latin America.

## S100A9 AS A NOVEL TARGET IN CLL. LINKING INFLAMMATION, MICROENVIRONMENT AND CLINICAL EVOLUTION.

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Chronic Lymphocytic Leukemia (CLL) develops through accumulation of malignant B cells that circulate in the peripheral blood and are continuously supported by microenvironmental signals. In this context, antigen stimulation, immune cells signals, and inflammation are key events involved in the initiation and progression of chronic lymphoid malignancies of B-cell type. We recently described that the S100A9 protein in exosomes from progressive CLL patients promotes NF-kB activity. S100A9 is a damage-associated molecular protein (DAMP) associated with chronic inflammation, tumor progression, recruitment of myeloid-derived suppressor cells (MDSC), and activation of NF-kB, and JNK kinase pathways. To further investigate of role of S100A9 we used a double transgenic (DT) CLL mouse model recently developed in our laboratory (DT-Eμ-TCL1/Actin-AID), which develops a more aggressive leukemia similar to what happens in progressive CLL patients. Consistently, preliminary results show a higher percentage of leukemic cells expressing S100A9 in DT-Eμ-TCL1/Actin-AID when compared to aging Eμ-TCL1. These results encourage us to develop a new DT model overexpressing S100A9 in the leukemic scenario of the TCL1 mice. In this project, we hypothesize that: ***i)*** in an inflammatory context, progressive CLL overexpresses S100A9 promoting leukemic proliferation and recruitment of MDSC, ***ii)*** inhibition of S100A9 could become a therapeutic opportunity to treat progressive/refractory CLL patients. Presently, we are evaluating the first generation of DT-Eμ-TCL1/S100A9 mice and we start new experiments in primary CLL cell cultures and leukemic mice cells designed to test these hypothesis.

## IMPACT OF IBRUTINIB IN QUALITY OF LIFE (QOL) IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA (CLL): PRELIMINARY DATA FROM THE REAL WORLD

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CONTEXT: Data from controlled trials report QoL improvement with ibrutinib monotherapy. Real-life evidence is necessary to confirm this hypothesis. DESIGN: Prospective, longitudinal, single-arm study enrolling consecutive CLL patients under ibrutinib as first or further-line treatment. INTERVENTIONS: QoL was explored with FACIT-Fatigue and EQ5D visual-analogue-scale (VAS) questionnaires (copyright permission) at baseline and after 3 months of treatment. PRIMARY-OBJECTIVE: evaluate impact of ibrutinib treatment in QoL. We defined a clinically meaningful improvement ≥3 points in FACIT score. Secondary-Endpoints: Detect 10% improvement by EQ5D-VAS. Correlate baseline and follow-up hemoglobin levels. Statistical analysis: analyzed with Sign Test (Binomial Test). Study approved by Institutional Committee. Patients signed informed consent. RESULTS: Since 2016; 21 patients were included. Median age: 75 years (range 57-84); follow-up: 7 months (range 1-28). Ibrutinib was first-line in 7 patients (33%), second-line in 7 patients (33%) and ≥3 previous lines in 7 patients (33%). After 3 months, median change in FACIT score ≥3 points was reached in 13 patients (62%) as compared to baseline (p=0.024); by EQ5D-VAS we detect improvement (1-5%) in 15 patients (71%) (p=<0.001), but 10% improvement was not reached (p=0.593). Regarding hemoglobin, from 13 patients evaluable at 6 months, 10 (77%) improved their levels in ≥ 0.5g/dl compared to baseline (p=0.046). CONCLUSION: We detected an early fatigue improvement within the first months of treatment and a tendency of QoL improvement coincident with hemoglobin recovery. Longer follow-up and larger enrollment are necessary to determine further improvements of QoL with ibrutinib.

## IBRUTINIB THERAPY DOWNREGULATES ACTIVATION-INDUCED CYTIDINE DEAMINASE AND PROLIFERATIVE FRACTIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Activation-induced cytidine deaminase (AID) initiates somatic hypermutation and class switch recombination of the immunoglobulin genes. As a trade-off for its physiological function, AID can contribute to tumor development and progression through its mutagenic activity. In Chronic Lymphocytic Leukemia (CLL), AID is over-expressed in the peripheral blood of patients with poor prognosis, primarily in the proliferative fractions (PFs) of the tumor clone. Recent preclinical data suggested that kinase inhibitors targeting B cell receptor signaling increase AID expression and genomic instability. To determine whether these findings translate into the clinical situation, we analyzed AID expression and PFs in serial CLL samples from patients before and during treatment with the BTK inhibitor ibrutinib. We found that Ibrutinib administration reduced AID expression in treated-CLL cases after 1 and 4 weeks of continuous therapy. In addition, flow cytometry analyses of the four previously described PFs showed that the proportion of CD38+; CD86+; IgM+/IgG+ and CXCR4lowCD5high leukemic cells was also significantly decreased at 4 weeks after Ibrutinib treatment. Possible reasons for the discrepancy between preclinical and clinical findings, and their impact for treatment safety are discussed.

## CYTOTOXIC AND REGULATORY EFFECTS OF LURBINECTEDIN ON B-CELL FROM CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS

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Lurbinectedin (Lur) is a synthetic alkaloid that induces DNA damage even in quiescent cells and is currently in phase II/III clinical trials for solid tumors. We aimed to evaluate the cytotoxicity of Lur on circulating mononuclear cells (PBMC) from CLL patients and healthy donors and to determine whether it could alter the cross-talk of CLL cells and tumor microenvironment. By flow cytometry analysis, we found that Lur induced a dose- and time-dependent death of all cell types, being B cells and monocytes the most susceptible populations at clinical relevant doses (Lur ≤ 10 nM). Interestingly, nurse like cells differentiated in vitro from CLL-monocytes showed a greater resistance compared to monocytes ($p < 0.05$, $n = 5$). To mimic microenvironment conditions, we incubated CLL cells with activated T cells or with the stromal cell line HS5 before exposure to Lur. Under these conditions, CLL cells became less sensitive to Lur ($n = 10$ $p < 0.05$). At subapoptotic doses, Lur decreased the expression of CCR7 on CLL cells ($n = 14$ $p < 0.001$) and impaired their migration towards CCL19 or CCL21 ($n = 7$ $p < 0.05$). Interestingly, low concentrations of Lur stimulated the synthesis of pro-IL1b in macrophages. Although Lur did not activate the inflammasome, the addition of ATP induced the release of IL-1b. These results suggest that Lur could impair the migration of CLL cells to the protective lymphoid microenvironment, favoring their death in the peripheral blood compartment. Our findings encourage further investigation of Lur as a potential therapy for CLL.

## REVISITING THE ROLE OF INTERLEUKIN-8 IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Proliferation and survival of malignant B cells in chronic lymphocytic leukemia (CLL) depends on signals from the microenvironment in lymphoid tissues. Among a plethora of soluble factors, IL-8 has been considered one of the most relevant to support CLL-B cell progression in an autocrine fashion, even though the expression of IL-8 receptors, CXCR1 and CXCR2, on leukemic B cells has not been reported. Our aim was to re-examine the role of IL-8 in CLL evaluating the expression of IL-8 receptors in leukemic cells and their capacity to produce it. Our results show that CLL-B cells, resting or activated, do not express CXCR1 or CXCR2 (n=56). Moreover, CLL cells did not show increased cell survival in response to exogenous IL-8 when cultured *in vitro* alone or in the presence of monocytes/nurse like cells (n=9). We next determined if CLL-B cells were able to produce IL-8. PBMC were activated with anti-IgM plus CD40 ligand for 24-72 hs and IL-8 production was assessed by flow cytometry (n=17). We found that CLL-B cells do not produce IL-8 spontaneously or upon activation, while monocytes did. Therefore, we compared by ELISA, the capacity of PBMC and highly purified CLL cells to release IL-8. We found that a minor proportion of monocytes was responsible for IL-8 levels in supernatants ($n=13$ $p<0.001$). Altogether our results indicate that CLL-B cells are not able to secrete or respond directly to IL-8 and highlight the importance of methodological details in *in vitro* experiments.

## DISTINCT CLINICAL CHARACTERISTICS AND MOLECULAR GENETICS OF CHRONIC LYMPHOCYTIC LEUKEMIA IN A TERTIARY CANCER CENTER IN BRAZIL

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The characteristics of chronic lymphocytic leukemia patients may vary according the clinical and epidemiological settings of diagnosis. We characterized a cohort of 113 patients from a single tertiary center in Brazil in respect of: (i) IGVH gene usage and mutational status, (ii) CDR3 stereotypes, and (iii) TP53 (exons 2-11), NOTCH1 (exon 34), SF3B1 (exons 14-16), BIRC3 (exons 6-9), and MYD88 (exon 5) mutations by Sanger sequencing. TP53 was analyzed in 30 cases by NGS sequencing (IonTorrentPGM®). 70 patients were assessed before and 44 after treatment, 15% of cases had a previous cancer while ~20% developed a second cancer during the disease course; 11% had chronic infectious diseases. Regarding *IGVH* mutational status, 62% of patients carried unmutated (UM) rearrangements, 18% were assigned to stereotyped subsets (7.9% to major subsets and 10% to minor ones). Six patients had two or more IGVH expressed; all of them underwent disease progression. Frequencies of *NOTCH1*, *SF3B1, BIRC3* and *MYD88* mutations were 16.2%, 6.1%, 4%, and 1%, respectively; while frequency of *TP53* mutations was significantly higher (19%). *TP53* was found concurrently mutated with *SF3B1*, *BIRC3*, and *NOTCH1,* associated to a dismal disease course. NOTCH1 mutations were detected mostly pre-treatment (80%), compared with TP53 mutations (35%). TP53 and NOTCH1 mutations, and UM-IGVH had a worse outcome compared to the other patients. Our data point to the need to better known the characteristics of patients at diagnosis and previously to the treatment in Latin America, in order to develop more efficient diagnostic and therapeutic approaches.

## CLINICAL AND PATHOLOGIC FINDINGS IN A PATIENT BEARING SUBSET#4 STEREOTYPED BCR AND A CODING NOTCH1 MUTATION (P.I2550V)

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Male patient diagnosed in 2003, at the age of 35 years. No anemia or thrombocytopenia; WBC 10,100/mm3; 52% lymphocytes; B2 microglobulin 1.6 mg/l, Binet A. IGVH of the clonotypic immunoglobulin was IGHV4-34- IGHD5-18- IGHJ6, mutated (95%), whose CDR3 was characterized by being stereotyped, subset#4. Immunophenotyping of the leukemic clone showed a profile characteristic of CLL, but negative for IgM and positive for IgG/kappa. At diagnosis and in 2 different time points, a sole mutation of NOTCH1 (p.I2550V) in heterozygosis was detected. The clinical decision was "watch and wait". In 2009, the patient was re-staged to Rai 3. In 2016, WBC was 44,740/mm3; 85% lymphocytes, B2 microglobulin 4.38 mg/l. Clinical decision making is underway. Subset #4 patients are characterized by IGHV mutated, and absence of unfavorable cytogenetic lesions, and NOTCH1 or SF3B1 mutations, and clinically are characterized by an indolent disease course. The herein described case has been free from treatment for the last 15 years, however, the progression of hematological parameters raises a note of caution, especially considering that coding NOTCH1 mutations have been associated with shorter time to first treatment, shorter overall survival, and resistance to anti-CD20 immunotherapy in the FluCy plus rituximab combination. However, coding mutations other than the more frequent NOTCH1 c.7544-7545delCT appear not to have the same negative functional and clinical impact. All this considerations need to be integrated in the clinical management of this case.

## SEGUNDOS TUMORES EN LEUCEMIA LINFÁTICA CRÓNICA; A PROPÓSITO DE UN CASO.

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La Leucemia Linfatica Cronica (LLC) es el tipo de leucemia mas frecuente en los paises de occidente con un curso clinico heterogeneo. Segun distintos reportes los pacientes con LLC tienen mayor incidencia de segundas neoplasias (SN), principalmente hematologicas, pero tambien tumores solidos (TS) en pulmon, piel, higado, prostata entre otros. Presentamos el caso de una paciente de 64 anos con diagnostico de LLC en 2012 que realiza esquema de 1º linea FCR logrando remision completa (RC) con Enfermedad Minima Residual (EMR) negativa por citometria de flujo (CMF). En 2015 tras hallazgo de imagen nodular pulmonar por TAC se realiza lobectomia con diagnostico de Carcinoma Epidermoide, estadio IA N0 MO. Continua en remision por 4 anos y en marzo de 2017 se diagnostica Linfoma Burkitt (LB) e inicia tratamiento con esquema R-DA-EPOCH x 6 ciclos con buena respuesta. Sin lograr recuperacion hematologica completa luego de 4 meses de finalizado el tratamiento se realiza biopsia de medula osea (BMO) compatible con Sindrome Mielodisplasico asociado a tratamiento (SMD-t) e inicia tratamiento con Azacitidina. Es sabido que los pacientes con LLC tienen mayor incidencia de SN. Esta descripto que el riesgo global de desarrollar SN luego del esquema FCR como primera linea aumentaria el riesgo 2.38 veces con respecto a la poblacion general, especialmente LMA-SMD t aunque tambien se ha observado mayor incidencia de TS (entre ellos, cancer de pulmon). Es importante tener en cuenta el efecto pro tumoral de esquemas como el FCR para la deteccion temprana de SN durante el seguimiento.