Prognostic roles of both CD49d and trisomy12 in chronic lymphocytic leukemia (CLL)

Review article

Eman M. Zaki [1], Sahar A. El-Gammal [2], Noha G. Sayed [3], Mayada F. Sedek [4] and Asmaa A. Mohamed [5].

1- Professor of Clinical Pathology department, South Egypt Cancer Institute, Assiut University. E-mail: eman_mosaad@hotmail.com
2- Assistant professor of Clinical Pathology department, faculty of medicine, Assiut University. E-mail: saharelgammal@hotmail.co.uk
3- Lecturer of Clinical Pathology department, South Egypt Cancer Institute, Assiut University. E-mail: noha.gaber@yahoo.com
4- Lecturer of Medical oncology department, South Egypt Cancer Institute, Assiut University. E-mail: mayadaelzohri@gmail.com
5- Demonstrator of Clinical Pathology department, South Egypt Cancer Institute, Assiut University. E-mail: asmaa.ah1190@gmail.com (corresponding author)

A b s t r a c t

Introduction: Chronic lymphocytic leukemia (CLL) is considered the most common type of leukemia in the western world and in Egypt CLL is the most common subtype of leukemia's. The National Cancer Registry reported over 80% of lymphoid leukemia's are CLL. Chronic lymphocytic leukemia (CLL) is characterized by heterogeneous clinical course. Therefore, the identification of prognostic and predictive factors for CLL is critical and this is a field of active investigation. Over the past several years, a group of so-called new prognostic factors has been developed. This several prognostic factors associated with the immunophenotypic profile, cytogenetic features and mutational status of the immunoglobulin heavy chain (IGVH).

Aim of the work: The aim of this review is to discuss the prognostic role of both CD49d and trisomy12 in chronic lymphocytic leukemia (CLL).

Conclusion: Combination of CD49d and trisomy12 may have very strong prognostic roles in the prognosis of CLL and add a prognostic value to CLL patients.

Keywords: Chronic lymphocytic leukemia, CD49d, Trisomy12.

Conflict of interest: The authors declare no conflict of interest

Introduction:

Role of immunophenotyping in CLL

The most reliable methodology for the diagnosis of chronic lymphocytic leukemia (CLL) is immunophenotyping by flow cytometry. Flow cytometry is usually performed in all patients with lymphocytosis, in order to confirm the Diagnosis of CLL suspected by morphology and clinical data.

Application of immunological markers primary determines B cells by showing surface immunoglobulin (sIg) light chain restriction **(1).** CLL cells express B-cell markers, like CD19 along with low levels of CD20, positive for CD5 and CD23 and Weak to negative expression of CD10, CD11c, CD79b, CD25, and FMC7 **(2).**

In addition to the diagnostic value, by flow cytometry a large number of cellular biomarkers have been found to correlate with the prognosis in CLL patients. Some of These biomarkers, the expression of CD38 on the surface of CLL cells, the intracellular expression of zeta-associated protein 70 **(3)**, and a surface markers like CD49d that not only correlate

with prognosis, but also with genetic aberrations like trisomy 12 and are possibly involved in mobilization and homing of CLL cells **(4).**

1- CD38:

Is a surface protein with a wide pattern of expression in cells of hematological and non-hematological origin **(5)**. A number of hematological malignancies expresses CD38, including CLL. CD38 is a useful and reliable factor because it typically remains stable over time, even in the face of chemotherapy. It behaves simultaneously as a cell surface enzyme and as a receptor. As an ectoenzyme, CD38 presents multiple enzymatic activities that involved in signal transduction through the regulation of cytoplasmic Ca^{+2} levels. As a receptor, CD38 binds CD31 that is expressed by a variety of immune cells including B and T cells subsets, and by vascular endothelial cells **(6).**

CD38 expression identifies two subgroups of CLL patients with different clinical outcomes; this distinction is based on the percentage of CD38 positive leukemic cells within a CLL clone. In the majority of studies, the threshold is considered as > 30% CD38 positive clonal members **(7).** CD38 positive patients (the threshold >30% of CD38 positive CLL cells) were reported to have significantly worse prognosis regarding progression free survival (PFS) and overall survival (OS) than those who were CD38 negative. CD38 expression is an important prognostic indicator when combined with ZAP70 and IgVH mutation analysis and CD49d **(8).**

2- ZAP70:

Is a member of the syk family Protein Tyrosine Kinases (PTKs) which plays a critical role in T-cell antigen receptor (TCR) signaling and T-cell development. It was found also to be associated with the B-cell receptor (BCR) in CLL **(9).**

Intracellular expression of the ZAP-70 protein above a certain threshold of cells by flow cytometry (≥ 20%) has proven to be an important indicator of time-to treatment and survival in CLL. The cutoff to classify patients as ZAP-70 positive (negative prognostic factor, correlating with unmutated IGHV status) or ZAP-70 negative. Positive patients have poor prognosis with shorter progression free survival, decreased overall survival and aggressive disease course. Whereas patients with ZAP-negativity have shown good prognosis, prolonged treatment free survival **(10).**

Some studies suggest that ZAP-70 retards internalization of surface membrane immunoglobulin M (smIgM) and CD79b from the cell membrane, leading to prolonged BCR pathway signaling. In addition, ZAP-70 positive CLL cells are more likely to express adhesion molecules such as CD49d and chemokine receptors, in particular CCR7, which promote migration toward a series of chemokines and inhibit apoptosis **(11).**

3- CD49d:

A molecule belonging to the integrin superfamily, highly expressed in normal peripheral blood and bone marrow B lymphocytes. It is a surface molecule, which binds to the β-integrin CD29 to form very late antigen-4 (VLA-4), the expression of which promotes microenvironment, mediated proliferation of CLL leukemic cells and identifies subgroup of about 40% of CLL patients characterized by progressive course and short survival **(12).**

CD49d is known to operate as one of the master molecules mediating both cell-cell and cell-matrix interactions by binding respectively to vascular cell adhesion molecule-1 (VCAM-1), non-RGD sequences (Arg– Gly–Asp) of fibronectin (FN), and C1q-like domain of elastin microfibril interfacer-1 (EMILIN-1). These features are reflected in the independent prognostic impact of CD49d expression in CLL **(13).** Recent multicenter study identified CD49d as the strongest factor within flow cytometry-detected markers and also predictive of OS and treatment-free survival in patients with CLL **(14).**

CD49d can also serve as a signaling receptor that influences B-cell survival via up regulation of Bcl-2 family members which was suggested to be related to the chemo resistant phenotype of CLL. In CLL, ligation of CD49d by FN was demonstrated to prevent apoptosis due to an increase in the BCL-2/BCL-2-associated X protein (BAX) ratio and to protect CLL cells from fludarabine-induced apoptosis, this effect correlated with an increased expression of BCLXL **(15).**

The relevance of CD49d as prognosticator for progressive disease evaluation was compared with CD38, ZAP-70, IGHV mutational status, β2M, Soluble CD23, modified-Rai and the presence of specific genomic aberrations **(16).** It should be noted that the expression of CD49d correlates with some other prognostic factors. Specifically, higher expression of CD49d is associated with unmutated IGHV, CD38 and ZAP70 **(17).**

It was reported that, positive CD49d CLL when associated with high serum levels for β2M or sCD23 or with a modified Rai high /intermediate-risk group, identified the subsets of patients with the shortest survival. Conversely, a CD49d negative CLL, if paralleled with low serum levels for β2M or sCD23, or in the context of a modified Rai low-risk group, always identified the subgroups with the longest survival **(16).**

Cytogenetic abnormalities in CLL

Acquired genetic aberrations, as in other types of cancer, have an important role in CLL pathogenesis. Since the late 1970s, numerous genetic studies using a wide range of laboratory techniques (conventional G-banding cytogenetics, fluorescence in situ hybridization, microsatellite analysis to detect loss of heterozygosis, Sanger sequencing, genomic arrays and more recently next generation sequencing methodologies, among others) have identified a broad spectrum of genomic aberrations **(18).** Recurrent cytogenetic abnormalities in CLL cells are the most reliable such markers currently available. Fluorescent in situ hybridization (FISH) is the standard method used to detect genomic aberrations in CLL **(19).**

Cytogenetic abnormalities can be detected in 80% of cases. Some patients have more than one cytogenetic defect, displaying the heterogenic nature of the disease. Five major prognostic categories have been defined; trisomy 12 was reported in 10–20% of cases, deletion of 13q was reported in approximately 55% of cases, deletion of 11q was reported in 10–25% of cases, and deletion of 17p was reported in 5–10% of cases, also Del (6q) in 6% **(20).**

These cytogenetic abnormalities are conventionally used to sub-classify patients into three prognostic ally relevant subgroups:

1-The favorable risk group, includes those with deletion 13q14 as the sole abnormality,

2-The intermediate group, includes a normal karyotype, trisomy 12, or a 6q deletion.

3- The unfavorable group includes an 11q22/ATM deletion, deletion of17p13/ TP53, or complex karyotypes.

1- 13q14 Deletion

Deletion of 13q14 region is the most frequent genetic change in CLL, as it was reported in 55% of the disease. As the sole aberration, it is associated with good prognosis **(21).** In the last few years, it has been reported that patients with CLL and 13q deletion may differ in their outcomes depending on the percentage of cells displaying this aberration **(22)**, being monoallelic in 70% of cases and biallelic in 30% **(23).** Several studies have found that biallelic 13q deletion is associated with a shorter OS than mono-allelic deletion **(24),** while recent work has suggested no significant difference between patients with biallelic or monoallelic 13q deletion with respect to OS **(19).**

Because microRNAs miR-15a and miR-16-1 are located within the commonly deleted region on chromosome13, their deletions contribute to the pathogenesis of this subtype of CLL. Both of these microRNAs have been shown to accelerate the proliferation of human B cells by modulating the expression of genes controlling cell-cycle progression; their deletion might partially explain the good prognosis associated with 13q deletion **(23).**

2- 11q23 Deletion

Deletions of the long arm of chromosome 11 (del11q) can be found in about 25% of chemotherapy-naïve patients with advanced disease stages and 10% of patients with early stage disease **(25).** The prognostic significance of this deletion results mainly from mutations in the ATM (ataxia telangiectasia mutated) gene, located on the long arm of chromosome 11. The deletion may be distinguished as the more common 'classical or large deletion' or an 'atypical or small deletion', which is uncommon and more frequently associated with ATM mutations **(26).**

ATM gene mutations have been largely studied in CLL patients with Del (11q); however, they have been found in only 8–30% of 11q- patients **(27)**, indicating that other genes could play a role in the pathobiology of 11q deletions in CLL. One of these genes is baculoviral IAP repeat-containing 3(BIRC3), a negative regulator of the non-canonical nuclear factor κ-light-chain-enhancer of activated B cells (NF-κB) protein, which is located near to ATM gene at 11q22. However, a recent study by Rose-Zerilli et al. has shown that ATM mutations rather than BIRC3 deletion and/or mutation had impact on overall and progression-free survival in 11q-deleted CLL patients treated with first- line therapy **(28).**

From a clinical point of view, CLL patients with Del (11q) are identified by large and multiple lymphadenopathies and have been associated with poor prognostic factors, such as unmutated IGHV genes. The presence of Del (11q) implies clinically progressive disease in almost all cases. In addition, cases with Del (11q) have been associated with shorter remission durations, and shorter OS following standard chemotherapy compared to non-deleted 11q cases **(29).**

3- Trisomy12

Trisomy 12 is the most common abnormality identified by chromosome banding analysis (CBA) **(30).** It is the third most common abnormality identified by fluorescence in situ hybridization (FISH) occurring in up to 10–20% It may also be associated with trisomy 18, deletion 14q, t (14; 19) (q32; q13), and/or trisomy 19 **(31).**

The prevalence of this cytogenetic abnormality is significantly higher in small lymphocytic lymphoma (SLL) where it is present in 28% of cases, also may increase the risk of Richter syndrome (RS) and second cancers **(32).**

In some studies proposed by Döhner et al. found that, trisomy 12 CLL carry an intermediate prognostic risk, with median overall survival **(33)**. These results found to be contra directory to results reported by González-Gascón y Marín et al. who found that, overall survival was shorter in patients with high trisomy12 expression in comparison to those with low trisomy12 expression **(31).**

CLL cells with trisomy 12 tend to have atypical morphology, defined as more than 15% of cells with cleaved nuclei and/or lymphoplasmacytoid features. This cytogenetic abnormality results in the "atypical" CLL; patients with the "atypical" CLL frequently have an atypical phenotype, with loss of CD5 or expression of FMC7, a marker usually absent in CLL **(34).**

Regarding the pathogenesis of trisomy 12, it has been difficult to establish a set of candidate genes since the affected region is the whole chromosome instead of a smaller critical region. However, the effects of trisomy 12 may be due to gene dosage effect, with increased expression of a number of genes, including: huntingtin interacting protein-1 related (HIP1R), myogenic factor 6 (MYF6), Cyclin-dependent kinase 4 (CDK4), and murine double minute 2 (MDM2) protein located on chromosome 12 **(35).**

CLL patients with trisomy12 rarely show TP53 mutations and rarely acquire these over time, finding that may explain the benign course after treatment **(36).** However, recently the presence of NOTCH-1 mutations can be identified in 30–40% of patients carrying trisomy12 particularly in cases with somatically unmutated immunoglobulin heavy chain variable region (IGHV) genes. NOTCH1 mutation may be associated with a shorter OS **(37).** However, the impact of NOTCH1 mutation on prognosis may be influenced by other chromosomal aberrations. For example, NOTCH1 mutation occurs more frequently in patient's carrying trisomy12 as the sole abnormality, but a worse outcome is observed among patients with trisomy12 associated with additional chromosomal abnormalities, irrespective of NOTCH1 mutation status **(38).**

4- 17p13 deletion

Deletion of 17p is found in approximately 5–10% of CLL patients at diagnosis (de novo deletions), but can also be acquired during the evolution of the disease, particularly in patients who have received chemotherapy. Indeed, the incidence of 17p deletion in patients with relapsed or refractory CLL can be up to 30% (**39).**

Patients with 17p deletion have always been included into the highest risk prognostic category, showing the shortest OS. This finding can be explained not only because of the cell-cycle deregulation caused by the loss of TP53 but also the usual requirement of chemotherapy, both independent predictors of a reduced OS **(40).**

Recent studies have shown clinical heterogeneity in 17p deletion patients according to the appearance of this abnormality during follow-up: as an early event (de novo) or, as a secondary alteration, which is more frequent. Patients with de novo 17p deletion have a longer median OS (4-5 years) whereas those who acquired 17p deletion during clonal evolution have a decreased survival (1–1.5 years) **(41).**

It has been shown that approximately 90% of patients with Del (17p) carry a TP53 mutation; conversely, only 60–70% of patients with TP53 mutation also harbor Del (17p), as detected by FISH. However, TP53 mutations also occur in the absence of Del (17p) in about 5% of untreated patients and are associated With a poor outcome, **(42).** Patients harboring biallelic inactivation of TP53 have a significantly poorer outcome, with shorter OS than those with TP53 mutation or deletion of a single 17p allele **(43).**

Patients with del17p have unusual immunophenotype with strong CD20, CD79b, FMC7, surface Ig, expression, in addition, an increased expression of CD38, ZAP-70, and unmutated IGHV was reported in 17p deletion cases, which agrees with the poor prognosis of this group of patients **(44).**

5- 6q deletion

6q deletion seems to occur with relatively low frequency (3–6%) and it is detected by chromosome banding analysis (CBA) as well as FISH **(45).** The abnormalities on chromosome 6 typically involve Deletions at 6q23, but can also involve deletions at 6q25–27 and/or 6q21 **(46).**

Patients with abnormalities between 6q21 and 6q24 generally have higher proportions of blood prolymphocytes, higher than average expression of CD38, and more aggressive disease **(47).** Lately, it was observed that deletion in 6q16 appears to be the most frequent region in CLL and could be associated with a more widespread disease when present as the sole abnormality **(45).**

Association of trisomy 12 with CD49d

Although expression of the integrin CD49d was decreased on circulating CLL cells in general, uniquely among the main cytogenetic categories, its expression was relatively preserved on trisomy 12 CLL cells (**48**), 90% of trisomy12 cases were found to express CD49d. Moreover, trisomy12 CLL cases were characterized by the higher mean fluorescence intensity levels of CD49d compared with cases belonging to the other cytogenetic categories **(49).**

These differences in surface integrin expression were associated with up regulation of molecules involved in intracellular integrin signaling. These changes were of

functional significance, as trisomy 12 CLL cells exhibited increased Intercellular Adhesion Molecule 1(ICAM-1) and VCAM-1 binding on integrin activation, and showed enhancedVLA-4-mediated adhesion and motility. The effect of trisomy 12 was dominant, with up regulation of integrin signaling also present in trisomy 12 with other cytogenetic abnormalities including del-11q or del-17p **(48).** CD49d overexpression found in trisomy 12 CLL could explained by DNA hypo methylation, a well-known epigenetic mechanism regulating gene transcription in tumors including CLL. It was found that CD49d+/trisomy 12 CLL completely lacked CPG methylation, while a significant CPG methylation was detected in the negative cases. Among this hypo ethylating agents, deoxyazacytidine (DAC) that operates by inhibiting DNA methyltransferase activity, thus preventing methylation of newly replicated DNA, leading to DNA demethylation and subsequent gene activation **(49).**

An interesting observation is the strong correlation between CD49d expression and trisomy 12 since it might anticipate a putative general feature of CLL cells **(13).**

Conclusion:

Combination of CD49d and trisomy12 may have very strong prognostic roles in prognosis of CLL and add a prognostic value to CLL patients.

References:

1-DRAGOVIC-IVANCEVI C, T., KRAGULJAC-KURTOVIC, N., KNEZEVIC, V., BOGDANOVIC, A., MIHALJEVIC, B., BOZIC, B. & GOTIC, M. 2014. The role of immunophenotyping in differential diagnosis of chronic lymphocytic leukemia. Srpski arhiv za celokupno lekarstvo, 142, 197-203.

2-RODRIGUES, C. A., GONCALVES, M. V., IKOMA, M. R., LORAND-METZE, I., PEREIRA, A. D., FARIAS, D. L., CHAUFFAILLE, M. L., SCHAFFEL, R., RIBEIRO, E. F., ROCHA, T. S., BUCCHERI, V., VASCONCELOS, Y., FIGUEIREDO, V. L., CHIATTONE, C. S., YAMAMOTO, M. & BRAZILIAN GROUP OF CHRONIC LYMPHOCYTIC, L. 2016. Diagnosis and treatment of chronic lymphocytic leukemia: recommendations from the Brazilian Group of Chronic Lymphocytic Leukemia. Rev Bras Hematol Hemoter, 38, 346-357.

3-GOMES, L. C., EVANGELISTA, F. C. G., SOUSA, L. P., ARAUJO, S., CARVALHO, M. D. G. & SABINO, A. P. 2017. Prognosis biomarkers evaluation in chronic lymphocytic leukemia. Hematol Oncol Stem Cell Ther, 10, 57-62.

4-JEON, Y. W. & CHO, S. G. 2016. Chronic lymphocytic leukemia: a clinical review including Korean cohorts. Korean J Intern Med, 31, 433-43.

5-VAISITTI, T., AUDRITO, V., SERRA, S., BUONINCONTRI, R., SOCIALI, G., MANNINO, E., PAGNANI, A., ZUCCHETTO, A., TISSINO, E., VITALE, C., COSCIA, M., USAI, C., PEPPER, C., GATTEI, V., BRUZZONE, S. & DEAGLIO, S. 2015. The enzymatic activities of CD38 enhance CLL growth and trafficking: implications for therapeutic targeting. Leukemia, 29, 356-68.

6-DEAGLIO, S. & VAISITTI, T. 2012. CD38 (CD38 molecule). Atlas of Genetics and Cytogenetics in Oncology and Haematology.

7-MALAVASI, F., DEAGLIO, S., DAMLE, R., CUTRONA, G., FERRARINI, M. & CHIORAZZI, N. 2011. CD38 and chronic lymphocytic leukemia: a decade later. Blood, 118, 3470-8.

8-CRAMER, P. & HALLEK, M. **2011.** Prognostic factors in chronic lymphocytic leukemia-what do we need to know? Nat Rev Clin Oncol, 8, 38-47.

9-ZUCCHETTO, A., VAISITTI, T., BENEDETTI, D., TISSINO, E., BERTAGNOLO, V., ROSSI, D., BOMBEN, R., DAL BO, M., DEL PRINCIPE, M. I., GORGONE, A., POZZATO, G., GAIDANO, G., DEL POETA, G., MALAVASI, F., DEAGLIO, S. & GATTEI, V. 2012. The CD49d/CD29 complex is physically and functionally associated with CD38 in B-cell chronic lymphocytic leukemia cells. Leukemia, 26, 1301-12.

10- ZEESHAN, R., IRFAN, S. M., SULTAN, S. & BHIMANI, S. 2015. ZAP-70 protein expression in B-cell chronic lymphoid leukemia: a single center experience from Pakistan. Asian Pac J Cancer Prev, 16, 1587-90.

11- BOMBEN, R., GOBESSI, S., DAL BO, M., VOLINIA, S., MARCONI, D., TISSINO, E., BENEDETTI, D., ZUCCHETTO, A., ROSSI, D., GAIDANO, G., DEL POETA, G., LAURENTI, L., EFREMOV, D. G. & GATTEI, V. 2012. The miR-17 approximately 92 family regulates the response to Toll-like receptor 9 triggering of CLL cells with unmutated IGHV genes. Leukemia, 26, 1584-93.

12- ***BULIAN, P., SHANAFELT, T. D., FEGAN, C., ZUCCHETTO, A., CRO, L., NUCKEL, H., BALDINI, L., KURTOVA, A. V., FERRAJOLI, A., BURGER, J. A., GAIDANO, G., DEL POETA, G., PEPPER, C., ROSSI, D. & GATTEI, V.*** 2014. CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. J Clin Oncol, 32, 897-904.

13- ***DAL BO, M., TISSINO, E., BENEDETTI, D., CALDANA, C., BOMBEN, R., DEL POETA, G., GAIDANO, G., ROSSI, F. M., ZUCCHETTO, A. & GATTEI, V.*** 2014. Micro environmental interactions

in chronic lymphocytic leukemia: the master role of CD49d. Semin Hematol, 51, 168-76.

14- BAUMANN, T., DELGADO, J., SANTACRUZ, R., MARTINEZ-TRILLOS, A., ROZMAN, M., AYMERICH, M., LOPEZ, C., COSTA, D., CARRIO, A., VILLAMOR, N. & MONTSERRAT, E. 2016. CD49d (ITGA4) expression is a predictor of time to first treatment in patients with chronic lymphocytic leukaemia and mutated IGHV status. Br J Haematol, 172, 48-55.

15- ***HENDY, O., EL SHAFIE, M., ALLAM, M., MOTALIB, T., KHALAF, F. & GOHAR, S.*** 2016. The diagnostic and prognostic value of CD38 and CD49d expressions in chronic lymphocytic leukemia. The Egyptian Journal of Haematology, 41, 70.

16-Gattei, V., Bulian, P., Del Principe, M. I., Zucchetto, A., Maurillo, L., Buccisano, F., Bomben, R., Dal-Bo, M., Luciano, F., Rossi, F. M., Degan, M., Amadori, S. & Del Poeta, G. 2008. Relevance of CD49d protein expression as overall survival and progressive disease prognosticator in chronic lymphocytic leukemia. Blood, 111, 865-873.

17-Strati, P., Parikh, S. A., Chaffee, K. G., Achenbach, S. J., Slager, S. L., Call, T. G., Ding, W., Jelinek, D. F., Hanson, C. A., Kay, N. E. & Shanafelt, T. D. 2017. CD49d associates with nodal presentation and subsequent development of lymphadenopathy in patients with chronic lymphocytic leukaemia. British Journal of Haematology, 178, 99-105.

18-Puiggros, A., Blanco, G. & Espinet, B. 2014. Genetic abnormalities in chronic lymphocytic leukemia: where we are and where we go. Biomed Res Int, 2014, 435983.

19-Davids, M. S., Vartanov, A., Werner, L., Neuberg, D., Dal Cin, P. & Brown, J. R. 2015. Controversial fluorescence in situ hybridization cytogenetic abnormalities in chronic lymphocytic leukaemia: new insights from a large cohort. Br J Haematol, 170, 694-703.

20- ***Hallek, M.*** 2015. Chronic lymphocytic leukemia: 2015 Update on diagnosis, risk stratification, and treatment. American Journal of Hematology, 90, 446-460.

21-GRYGALEWICZ, B., WORONIECKA, R., RYGIER, J., BORKOWSKA, K., RZEPECKA, I., LUKASIK, M., BUDZILOWSKA, A., RYMKIEWICZ, G., BLACHNIO, K., NOWAKOWSKA, B., BARTNIK, M., GOS, M. & PIENKOWSKA-GRELA, B. 2016. Monoallelic and biallelic deletions of 13q14 in a group of CLL/SLL patients investigated by CGH Hematological Cancer and SNP array (8x60K). Mol Cytogenet, 9, 1.

22- DAL BO, M., ROSSI, F. M., ROSSI, D., DEAMBROGI, C., BERTONI, F., DEL GIUDICE, I., PALUMBO, G., NANNI, M., RINALDI, A., KWEE, I., TISSINO, E., CORRADINI, G., GOZZETTI, A., CENCINI, E., LADETTO, M., COLETTA, A. M., LUCIANO, F., BULIAN, P., POZZATO, G., LAURENTI, L., FORCONI, F., DI RAIMONDO, F., MARASCA, R., DEL POETA, G., GAIDANO, G., FOA, R., GUARINI, A. & GATTEI, V. 2011. 13q14 deletion size and number of deleted cells both influence prognosis in chronic lymphocytic leukemia. Genes Chromosomes Cancer, 50, 633-43.

23-Nabhan, C., Raca, G. & Wang, Y. L. 2015. Predicting Prognosis in Chronic Lymphocytic Leukemia in the Contemporary Era. JAMA Oncol, 1, 965-74.

24-ORLANDI, E. M., BERNASCONI, P., PASCUTTO, C., GIARDINI, I., CAVIGLIANO, P. M., BONI, M., ZIBELLINI, S. & CAZZOLA, M. 2013. Chronic lymphocytic leukemia with del13q14 as the sole abnormality: dynamic prognostic estimate by interphase-FISH. Hematological Oncology, 31, 136-142.

25- ***QUESADA, V., CONDE, L., VILLAMOR, N., ORDONEZ, G. R., JARES, P., BASSAGANYAS, L., RAMSAY, A. J., BEA, S., PINYOL, M., MARTINEZ-TRILLOS, A., LOPEZ-GUERRA, M., COLOMER, D., NAVARRO, A., BAUMANN, T., AYMERICH, M., ROZMAN, M., DELGADO, J., GINE, E., HERNANDEZ, J. M., GONZALEZ-DIAZ, M., PUENTE, D. A., VELASCO, G., FREIJE, J. M., TUBIO, J. M., ROYO, R., GELPI, J. L., OROZCO, M., PISANO, D. G., ZAMORA, J., VAZQUEZ, M., VALENCIA, A., HIMMELBAUER, H., BAYES, M., HEATH, S., GUT, M., GUT, I., ESTIVILL, X., LOPEZ-GUILLERMO, A., PUENTE, X. S., CAMPO, E. & LOPEZ-OTIN, C.*** 2011. Exome sequencing identifies recurrent mutations of the splicing factor SF3B1 gene in chronic lymphocytic leukemia. Nat Genet, 44, 47-52.

26-ALHOURANI, E., OTHMAN, M. A., MELO, J. B., CARREIRA, I. M., GRYGALEWICZ, B., VUJIC, D., ZECEVIC, Z., JOKSIC, G., GLASER, A., POHLE, B., SCHLIE, C., HAUKE, S. & LIEHR, T. 2016. BIRC3 alterations in chronic and
B-cell acute lymphocytic leukemia patients. Oncol Lett, 11, 3240-3246.

27-OUILLETTE, P., LI, J., SHAKNOVICH, R., LI, Y., MELNICK, A., SHEDDEN, K. & MALEK, S. N. 2012. Incidence and clinical implications of ATM aberrations in chronic lymphocytic leukemia. Genes Chromosomes Cancer, 51, 1125-32.

28-ROSE-ZERILLI, M. J., FORSTER, J., PARKER, H., PARKER, A., RODRIGUEZ, A. E., CHAPLIN, T., GARDINER, A., STEELE, A. J., COLLINS, A., YOUNG, B. D., SKOWRONSKA, A., CATOVSKY, D., STANKOVIC, T., OSCIER, D. G. & STREFFORD, J. C. 2014. ATM mutation rather than BIRC3 deletion and/or mutation predicts reduced survival in 11q-deleted chronic lymphocytic leukemia: data from the UK LRF CLL4 trial. Hematological, 99, 736-42.

29-WIERDA, W. G., O'BRIEN, S., WANG, X., FADERL, S., FERRAJOLI, A., DO, K. A., GARCIA-MANERO, G., CORTES, J., THOMAS, D., KOLLER, C. A., BURGER, J. A., LERNER, S., SCHLETTE, E., ABRUZZO, L., KANTARJIAN, H. M. & KEATING, M. J. 2011. Multivariable model for time to first treatment in patients with chronic lymphocytic leukemia. J Clin Oncol, 29, 4088-95.

30-HAFERLACH, C., DICKER, F., SCHNITTGER, S., KERN, W. & HAFERLACH, T. 2007. Comprehensive genetic characterization of CLL: a study on 506 cases analyzed with chromosome banding analysis, interphase FISH, IgV (H) status and immunophenotyping. Leukemia, 21, 2442-51.

31-GONZALEZ-GASCON, Y. M. I., HERNANDEZ-SANCHEZ, M., RODRIGUEZ-VICENTE, A. E., SANZO, C., AVENTIN, A., PUIGGROS, A., COLLADO, R., HERAS, C., MUNOZ, C., DELGADO, J., ORTEGA, M., GONZALEZ, M. T., MARUGAN, I., DE LA FUENTE, I., RECIO, I., BOSCH, F., ESPINET, B., GONZALEZ, M., HERNANDEZ-RIVAS, J. M., HERNANDEZ, J. A., GRUPO ESPANOL DE LEUCEMIA LINFATICA, C. & GRUPO COOPERATIVO ESPANOL DE CITOGENETICA, H. 2016. A high proportion of cells carrying trisomy 12 is associated with a worse outcome in patients with chronic lymphocytic leukemia. Hematol Oncol, 34, 84-92.

32-STRATI, P., ABRUZZO, L. V., WIERDA, W. G., O'BRIEN, S., FERRAJOLI, A. & KEATING, M. J. 2015. Second cancers and Richter transformation are the leading causes of death in patients with trisomy 12 chronic lymphocytic leukemia. Clin Lymphoma Myeloma Leuk, 15, 420-7.

33-Dohner, H., Stilgenbauer, S., Benner, A., Leupolt, E., Krober, A., Bullinger, L., Dohner, K., Bentz, M. & Lichter, P. 2000. Genomic aberrations and survival in chronic lymphocytic leukemia. The New England Journal of Medicine, 343, 1910–1916.

34-CRO, L., FERRARIO, A., LIONETTI, M., BERTONI, F., ZUCAL N, N., NOBILI, L., FABRIS, S., TODOERTI, K., CORTELEZZI, A., GUFFANTI, A., GOLDANIGA, M., MARCHESELLI, L., NERI, A., LAMBERTENGHI-DELILIERS, G. & BALDINI, L. 2010. The clinical and biological features of a series of immunophenotypic variant of B-CLL. European Journal of Haematology, no-no.

35-Porpaczy, E., Bilban, M., Heinze, G., Gruber, M., Vanura, K., Schwarzinger, I., Stilgenbauer, S., Streubel, B., Fonatsch, C. & Jaeger, U. 2009. Gene expression signature of chronic lymphocytic leukaemia with Trisomy 12. Eur J Clin Invest, 39, 568-75.

36-RODRIGUEZ-VICENTE, A. E., DIAZ, M. G. & HERNANDEZ-RIVAS, J. M. 2013. Chronic lymphocytic leukemia: a clinical and molecular heterogeneous disease. Cancer Genet, 206, 49-62.

37-BALATTI, V., LERNER, S., RIZZOTTO, L., RASSENTI, L. Z., BOTTONI, A., PALAMARCHUK, A., CASCIONE, L., ALDER, H., KEATING, M. J., KIPPS, T. J., PEKARSKY, Y. & CROCE, C. M. 2013. Trisomy 12 CLLs progress through NOTCH1 mutations. Leukemia, 27, 740-3.

38-LOPEZ, C., DELGADO, J., COSTA, D., CONDE, L., GHITA, G., VILLAMOR, N., NAVARRO, A., CAZORLA, M., GOMEZ, C., ARIAS, A., MUNOZ, C., BAUMANN, T., ROZMAN, M., AYMERICH, M., COLOMER, D., COBO, F., CAMPO, E., LOPEZ-GUILLERMO, A., MONTSERRAT, E. & CARRIO, A. 2012. Different distribution of NOTCH1 mutations in chronic lymphocytic leukemia with isolated trisomy 12 or associated with other chromosomal alterations. Genes Chromosomes Cancer, 51, 881-9.

39-DELGADO, J., ESPINET, B., OLIVEIRA, A. C., ABRISQUETA, P., DE LA SERNA, J., COLLADO, R., LOSCERTALES, J., LOPEZ, M., HERNANDEZ-RIVAS, J. A., FERRA, C., RAMIREZ, A., RONCERO, J. M., LOPEZ, C., AVENTIN, A., PUIGGROS, A., ABELLA, E., CARBONELL, F., COSTA, D., CARRIO, A., GONZALEZ, M., GRUPO ESPANOL DE LEUCEMIA LINFATICA, C. & GRUPO ESPANOL DE CITOGENETICA, H. 2012. Chronic lymphocytic leukaemia with 17p deletion: a retrospective analysis of prognostic factors and therapy results. Br J Haematol, 157, 67-74.

40-MARÍN, I. G. G. Y., HERNÁNDEZ-SÁNCHEZ, M., VICENTE, A.-E. R. & HERNÁNDEZ-RIVAS, J.-Á. 2015. New Insights in Prognosis and Therapy of Chronic Lymphocytic Leukaemia.

41-LANDAU, D. A., CARTER, S. L., STOJANOV, P., MCKENNA, A., STEVENSON, K., LAWRENCE, M. S., SOUGNEZ, C., STEWART, C., SIVACHENKO, A., WANG, L., WAN, Y., ZHANG, W., SHUKLA, S. A., VARTANOV, A., FERNANDES, S. M., SAKSENA, G., CIBULSKIS, K., TESAR, B., GABRIEL, S., HACOHEN, N., MEYERSON, M., LANDER, E. S., NEUBERG, D., BROWN, J. R., GETZ, G. & WU, C. J. 2013. Evolution and impact of sub clonal mutations in chronic lymphocytic leukemia. Cell, 152, 714-26.

42-MALCIKOVA, J., TAUSCH, E., ROSSI, D., SUTTON, L. A., SOUSSI, T., ZENZ, T., KATER, A. P., NIEMANN, C. U., GONZALEZ, D., DAVI, F., GONZALEZ DIAZ, M., MORENO, C., GAIDANO, G., STAMATOPOULOS, K., ROSENQUIST, R., STILGENBAUER, S., GHIA, P., POSPISILOVA, S. & EUROPEAN RESEARCH INITIATIVE ON CHRONIC LYMPHOCYTIC LEUKEMIA, T. P. N. 2018. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. Leukemia, 32, 1070-1080**.**

43-MALCIKOVA, J., PAVLOVA, S., KOZUBIK, K. S. & POSPISILOVA, S. 2014. TP53 mutation analysis in clinical practice: lessons from chronic lymphocytic leukemia. Hum Mutat, 35, 663-71.

44-QUIJANO, S., LOPEZ, A., RASILLO, A., SAYAGUES, J. M., BARRENA, S., SANCHEZ, M. L., TEODOSIO, C., GIRALDO, P., GIRALT, M., PEREZ, M. C., ROMERO, M., PERDIGUER, L. & ORFAO, A. 2008. Impact of trisomy 12, Del (13q), Del (17p), and Del (11q) on the immunophenotype, DNA ploidy status, and proliferative rate of leukemic B-cells in chronic lymphocytic leukemia. Cytometry B Clin Cytom, 74, 139-49.

45-DALSASS, A., MESTICHELLI, F., RUGGIERI, M., GASPARI, P., PEZZONI, V., VAGNONI, D., ANGELINI, M., ANGELINI, S., BIGAZZI, C., FALCIONI, S., TROIANI, E., ALESIANI, F., CATARINI, M., ATTOLICO, I., SCORTECHINI, I., DISCEPOLI, G. & GALIENI, P. 2013. 6q deletion detected by fluorescence in situ hybridization using bacterial artificial chromosome in chronic lymphocytic leukemia. Eur J Haematol, 91, 10-9**.**

46-STILGENBAUER, S., BULLINGER, L., BENNER, A., WILDENBERGER, K., BENTZ, M., DÖHNER, K., HO, A. D., LICHTER, P. & DÖHNER, H. 1999. Incidence and clinical significance of 6q deletions in B cell chronic lymphocytic leukemia. Leukemia, 13, 1331-1334.

47-CUNEO, A., RIGOLIN, G. M., BIGONI, R., ANGELI, C. D., VERONESE, A., CAVAZZINI, F., BARDI, A., ROBERTI, M. G., TAMMISO, E., AGOSTINI, P., CICCONE, M., PORTA, M. D., TIEGHI, A., CAVAZZINI, L., NEGRINI, M. & CASTOLDI, G. 2003. Chronic lymphocytic leukemia with 6q− shows distinct hematological features and intermediate prognosis. Leukemia, 18, 476-483.

48-RICHES, J. C., O'DONOVAN, C. J., KINGDON, S. J., MCCLANAHAN, F., CLEAR, A. J., NEUBERG, D. S., WERNER, L., CROCE, C. M., RAMSAY, A. G., RASSENTI, L. Z., KIPPS, T. J. & GRIBBEN, J. G. 2014. Trisomy 12 chronic lymphocytic leukemia cells exhibit up regulation of integrin signaling that is modulated by NOTCH1 mutations. Blood, 123, 4101-10

49-RICHES, J. C., O'DONOVAN, C. J., KINGDON, S. J., MCCLANAHAN, F., CLEAR, A. J., NEUBERG, D. S., WERNER, L., CROCE, C. M., RAMSAY, A. G., RASSENTI, L. Z., KIPPS, T. J. & GRIBBEN, J. G. 2014. Trisomy 12 chronic lymphocytic leukemia cells exhibit up regulation of integrin signaling that is modulated by NOTCH1 mutations. Blood, 123, 4101-10.