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# ABSTRACT BOOK

## EHA2021 Virtual Congress

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## EHA2021 Virtual Congress

## 26th Congress of the European Hematology Association

# ABSTRACT BOOK

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**Methods:**
Two patient groups were recruited from three institutions (St. James's Hospital, University Hospital Limerick and the Midlands Regional Hospital) from October 2017 - September 2018. Group 1 included consecutive patients with newly diagnosed CLL and group 2 included previously-diagnosed patients who required treatment during the study time period. Ethics approval was granted and written informed consent was sought for biobanking, immunophenotypic and molecular analysis (extended flow panel, *TP35* and *NOTCH1* mutational status). If consent was not provided, the patients were included for epidemiological purposes only.
**Results:** 148 patients were included: 111 newly-diagnosed (1) and 38 known cases of known CLL pre-treatment (2). An incidence of 5 per 100,000 (females) and 11 per 100,000 (males) was established. The median age was 67.5 years with 58 patients (39%) aged ≥ 70 years. The male to female ratio was 2.2:1. Immunophenotyping and mutational analysis of groups 1 and 2 revealed: CD62L (1-63.5%; 2- 64%), CD49d (1- 27.1%; 2- 34.6%), ROR1 (1- 85.9%; 2- 88.5%), mutated *TP53* (1- 9.2%; 2- 11.8%) and mutated *NOTCH1* (1- 9.4%; 2 16.7%). The association between CD49d expression and lymphadenopathy was demonstrated. In cases where expression levels fell between 10-30%; CD62L had two (11.7%) and CD49d had nine (90%) cases of bimodal distribution patterns.

**Table: Baseline characteristics.**

| Demographic, clinical and molecular Characteristics | All patients N=148 | | Group 1 N=111 | | Group 2 N=37 | |
|---|---|---|---|---|---|---|
| Men | 102 | 68.9% | 76 | 68.5% | 26 | 70.3% |
| Women | 46 | 31.1% | 35 | 31.5% | 11 | 29.7% |
| Median age at diagnosis (Years) | 67.5 (29-91) | - | 69 (41-91) | - | 60 (29-79) | |
| Binet stage A | 97/148 | 65.6% | 94/111 | 84.7% | 3/37 | 8.1% |
| Binet stage B | 28/148 | 18.9% | 14/111 | 12.6% | 14/37 | 37.8% |
| Binet stage C | 23/148 | 15.5% | 3/111 | 2.7% | 20/37 | 54.1% |
| *TP53* mutated | 12/121 | 9.9% | 8/87 | 9.2% | 4/34 | 11.8% |
| *NOTCH 1* mutated | 13/115 | 11.3% | 8/85 | 9.4% | 5/30 | 16.7% |
| ROR1 ≥ 30% | 96/111 | 86.5% | 73/85 | 85.9% | 23/26 | 88.5% |
| ROR1<30% | 15/111 | 13.5% | 12/85 | 14.1% | 3/26 | 11.5% |
| CD62L ≥ 30% | 70/110 | 63.6% | 54/85 | 63.5% | 16/25 | 64.0% |
| CD62L<30% | 40/110 | 36.3% | 31/85 | 36.4% | 9/25 | 36.0% |
| CD49d ≥ 30% | 32/111 | 28.8% | 23/85 | 27.1% | 9/26 | 34.6% |
| CD49d <30% | 79/111 | 71.2% | 62/85 | 72.9% | 17/26 | 65.3% |

**Summary/Conclusion:** We have established in-depth epidemiological and molecular analysis of an Irish patient cohort in an effort to redress the paucity of published Irish data. We have established that our CLL cohort and biobank benchmarks from a clinical and laboratory perspective to other international cohorts. A longitudinal study of the patient cohort will establish whether CD49d expression ≥10% is prognostically useful and whether cases of CD62L with bimodal patterns of expression have clinical relevance.

## EP629 INFLUENCE OF INFLAMMATORY CYTOKINES ON S100A PROTEINS EXPRESSION IN CLL PATIENTS

O. Mitrovic [1,*], M. Diklic [1], D. Antic [2,3], B. Mihaljevic [2,3], V. Vukovic [2], V. Otasevic [2], K. Tomic [2], V. Cokic [1]

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**Background:** S100A proteins possess a broad range of intra- and extracellular functions. The involvement of these proteins in inflammation-mediated responses is of particular interests, considering that inflammation represents one of the landmarks of malignancy. Processes such as inflammation and cellular stress trigger the release of S100A proteins to extracellular space, interacting with their receptors and activating numerous intracellular signaling pathways, for instance NF-κB and AP1. Through them, S100A proteins take part into regulation of some of the most essential cellular processes: cell differentiation, apoptosis, inflammation, proliferation, etc.
**Aims:** The aim of the study is to assess the role of inflammation in activation of S100A proteins via proliferation and inflammation related signaling pathways.
**Methods:** We observed 60 CLL patients' samples to isolate mononuclear cells (MNC) and CD19⁺ cells. MNC of CLL patients were treated with pro-inflammatory IL-6 and anti-inflammatory IL-10 cytokines, and inhibitors of JAK1/2, NF-κB and PI3K signaling pathways, to evaluate S100A4, S100A8, S100A9, S100A12 and NF-κB protein expression by immunoblotting. Also, we used immunocytochemistry to analyse the number of the S100As immunopositive MNC of CLL patients.
**Results:** S100A8 showed higher level of protein expression in MNC and CD19⁺ cells in comparison to healthy control. The number of immunopositive S100A4 ($p<0.05$) and S100A9 cells ($p<0.001$) was significantly decreased in CD19⁺ cells and MNC, respectively of CLL patients in comparison to healthy control. In addition, S100A4 and S100A9 proteins expression had statistically significant lower level of expression in MNC. Also, IL-6 stimulated expression of S100A8 and S100A4 in MNC of CLL, while the expression of latter one was prevented by NF-κB and JAK1/2 inhibitors. IL-10 reduced expression of S100A8 and S100A12 in MNC of CLL.
**Summary/Conclusion:** Pro-inflammatory IL-6 and anti-inflammatory IL-10 cytokines have opposite effect on inflammatory S100A8 protein in CLL, with a potential to be a prognostic marker.

## EP630 RELEVANCE OF NRF2/KEAP1 AXIS IN CHRONIC LYMPHOCYTIC LEUKEMIA DEVELOPMENT

E. Gomes [1], J. Jorge [1,2,3,4], J. P. Carda [1,2,3,4,5], M. I. Costa [1], N. Magalhães [1,6], D. Roque [1,6], R. Alves [1,2,3,4], B. Lapa [1,2], S. Marini [5], S. Duarte [5], A. Pereira [2,3,7], A. C. Gonçalves [1,2,3,4], A. B. Sarmento-Ribeiro [1,2,3,4,5,*]

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**Background:** Chronic lymphocytic leukemia (CLL) is characterized by the accumulation of long-lived resting clonal B lymphocytes in blood, bone marrow, and secondary lymphoid organs. Oxidative stress is known to be one of the mechanisms important in CLL development and progression. Specifically, NRF2/KEAP1 axis plays a crucial role in redox balance, inducing the expression of target genes, namely those related to antioxidant defenses.
**Aims:** The goal of this study was to clarify the relevance of NRF2/KEAP1 axis in CLL development, particularly through the evaluation of its target genes expression levels and their correlation with clinical and laboratory data, in order to identify potential new CLL diagnostic biomarkers.
**Methods:** We evaluated the expression levels of *NFE2L2*, *KEAP1*, *NFKB1*, *NQO1*, *HMOX1*, *GPX1*, *SQSTM1*, *TXNRD1*, *BCL2*, *GSTM1* and *HPRT* (endogenous control) genes in peripheral blood of 25 CLL patients and 30 healthy controls (CTL) using qPCR. Informed consent was obtained in accordance with the Helsinki Declarations. Mann-Whitney U and Kruskal-Wallis tests were used to assess the statistical significance between groups. Receiver operating characteristic (ROC) curves analysis was performed to analyze variables accuracy as diagnostic biomarkers. Patients were dichotomized according to the cut-off points obtained from the ROC curves constructed to predict death. All statistical analyses were two-sided and a $p<0.05$ was considered statistically significant.
**Results:** The 25 CLL patients studied were 13 males and 12 females with a median age of 73y, ranging between 48 and 83 years, and the 30 healthy controls, were 15 males and 15 females with a median age of 75 years, ranging between 54 and 99 years. At diagnosis, all CLL patients were Binet A (low risk). 24% of CLL patients presented low-risk cytogenetic abnormalities, 19% presented intermediate-risk, and 24% high-risk and 33% did not present any cytogenetic abnormalities. Our results showed, in CLL patients, an increase on gene expression levels of *NQO1* [CLL: m=0.161; interquartile range (IR)=0.283; CTL: m=0.023; IR=0.098; p=0.005] and *BCL2* [CLL: m=3,400; IR=6,353; CTL: m=0,434; IR=1,039; p<0,001) and a decrease of *NFKB1* (CLL: m=0,900; IR=0,690; p=0,014; CTL: m=1,752; IR=5,220; p=0,014) and *SQSTM1* (CLL: m=0,144; IR=0,395; CTL: m=0,880; IR=3,153; p<0,001), when compared to controls. None of the other evaluated genes showed significant differences between CLL patients and controls.

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ROC analysis for these genes suggested that their expression levels might be possible diagnostic biomarkers for CLL *NQO1* (AUC:0,733; IC95%:0,593-0,873; cut-off>0,159; sensitivity=57%; specificity=85%; p=0,005); *BCL2* (AUC:0,882; IC95%:0,786-0,978; cut-off>0,532; sensitivity=91%; specificity=74%; p<0,001); *NFKB1* (AUC:0,695; IC95%:0,553-0,837; cut-off<1,030; sensitivity=64%; specificity=77%; p=0,014); *SQSTM1* (AUC:0,799; IC95%:0,676-0,921; cut-off<0,443; sensitivity=68%; specificity=87%; p<0,001)]. No correlation was found between gene expression levels and CLL patients' survival, as well as with other clinical and laboratory data.

**Summary/Conclusion:** The decrease of *NFKB1* and *SQSTM1* gene expression levels observed in our study confirms the importance of oxidative stress in CLL, and the increase of *BCL2* and *NQO1* reflect the ability of tumor cells to resist cell death. Furthermore, as these genes are target of the NRF2/KEAP1 axis, our study suggests the relevance of the NRF2/KEAP1 axis for CLL development, being the levels of *NFKB1*, *SQSTM1*, *BCL2* and *NQO1* genes potential new diagnostic biomarkers.

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## Chronic lymphocytic leukemia and related disorders - Clinical

### EP631 OTHER MALIGNANCIES IN THE HISTORY OF CHRONIC LYMPHOCYTIC LEUKEMIA – A RETROSPECTIVE, MULTICENTER COHORT STUDY BY ERIC, THE EUROPEAN RESEARCH INITIATIVE ON CLL, IN HARMONY

T. Chatzikonstantinou [1,2,*], L. Scarfò [1,3], J. Kotašková [4,5], G. Iacoboni [6], E. Minga [2], D. Chammou [2], G. Karakatsoulis [2], M. Alcoceba [7], S. El-Ashwah [8], F. Bacchiarri [9], M. Ali Khan [10], R. Cassin [11], S. Chatzileontiadou [12], A. Christian [13], R. Collado [14], M. D. De Deus Santos [15], Z. Davis [13], C. Demosthenous [16], M. Dimou [17], D. Donaldson [18], G. Dos Santos [19], B. Dreta [20], M. Efstathopoulou [21], A. Enrico [22], A. Fresa [23], S. Galimberti [24], R. García-Serra [14], I. González-Gascón-y-Marín [25], A. Gozzetti [9], V. Guarente [26], S. Harrop [27], E. Hatzimichael [28], Y. Herishanu [29], M. Iskas [16], O. Jaksic [30], S. R. Janssen [31], E. Kalicińska [32], V. Karakus [33], A. P. Kater [31], B. Kho [34],?. Konstantinou [35], M. Koren-Michowitz [36,37], I. Kotsianidis [38], R. J. Kreitman [39], U. K. Nath [40], J. Labrador [41], D. P. Lad [42], M.-D. Levin [43], I. Levy [44], A. Lopez-Garcia [45], J. Marquet [46], S. Mašlejová [4], C. Mayor-Bastida [47], B. Mihaljevic [48,49], I. Milosevic [50], F. Miras [51], R. Moia [52], M. Morawska [53,54], A. Navarro-Bailón [7], D. Oscier [13], A. C. Oliveira [55], J. Olivieri [56], T. Papajík [57], M. Papaioannou [12], C. Pierie [31], A. Puiggros [58], G. Reda [11], G. M. Rigolin [59], R. Ruchlemer [60], G. Sevindik [61], S. Al-Shemari [62], Y. Shen [63], M. Šimkovič [64], S. Smirnova [65], D. S. A. Soliman [66], M. Špaček [67], T. Tadmor [44], L. Tourjeman [60], E. Tse [68], A. Visentin [69], K. Tomic [48], M. van Gelder [70], T. Vassilakopoulos [35], C. Vitale [71,72], J. von Tresckow [73], G. Vrachiolias [38], V. Vukovic [48], Z. Xu [74], L. Yañez [75], M. Yagci [76], M. Yassin [77], J. Zuchnická [78], M. Angelopoulou [35], D. Antic [48,49], B. Biderman [65], M. Catherwood [18], M. Coscia [71,72], A. Cuneo [59], F. Dermikan [79], B. Espinet [58], G. Gaidano [52], L. Laurenti [23], S. Mulligan [63], E. Nikitin [80], P. Panagiotidis [17], G. Pangalis [21], V. M. Popov [81], Š. Pospíšilová [4,5], L. Smolej [64], P. Sportoletti [26], N. Stavrogianni [16], C. Tam [27], L. Trentin [69], M. Trněný [67], A. Chatzidimitriou [2], P. Ghia [1,3], F. Bosch [6], M. Doubek [4,5], K. Stamatopoulos [1,2]

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**Background:** Other malignancies occurring in patients with CLL are a well-known, but insufficiently investigated area, highlighting the need for further research.

**Aims:** To leverage the understanding of other malignancies in CLL, particularly regarding potential links to chemoimmunotherapy (CIT) and/or novel agents, as well as associations with relevant clinicobiological factors.

**Methods:** Data from consecutive sets of patients diagnosed with CLL between 2000-2016 were collected by affiliated members through ERIC and subjected to detailed statistical analysis. Collected variables included: demographics, stage at diagnosis, treatment, treatment response, death, cause of death, other malignancy (type, date of diagnosis, outcome), biomarker profiles.

**Results:** Overall, 13,808 patients from 64 centers were evaluated: 8,517 males, 5,291 females; median age: 66 (range, 27-92). At diagnosis, 74/16/10% of cases were classified as Binet stages A/B/C. Biomarker profiling (performed before frontline treatment): 1,055/6,310 cases (14.1%) with del(11q); 843/6,928 cases (10.8%) with *TP53* aberrations