ratios within VHCDR2, VHFR2 and VHCDR3 in stereotyped, while VHFR3 was the most involved in non-stereotyped rearrangements. Trisomy 12 was more frequent in non-stereotyped cases (57%) compared to stereotyped ones (20%) while abnormal karyotypes were only found in non-stereotyped patients (33%), suggesting a better outcome for IGHV4-34 stereotyped patients. Both subsets expressed similar mutational pattern and aminoacid changes at codons P45-S, E55Q and S64-I while codon 40 S40T mutation was not found in our series. Our data showed substantial differences in the mutational profile of stereotyped and non-stereotyped IGHV4-34 CLL patients, supporting that cells react in a specific way in face to the antigen selection.

527. (135) **NOTCH1 AND TP53 MUTATIONS IN CHRONIC LYMPHOCYTIC LEUKEMIA. THEIR RELATIONSHIP WITH CYTOGENETIC, FISH AND IGHV STATUS OF PATIENTS.**

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Molecular studies have revealed a number of recurrently mutated genes in chronic lymphocytic leukemia (CLL). Among them, the study of NOTCH1 and TP53 gene mutations showed significant importance in CLL prognosis. The aim of this study was to evaluate NOTCH1 and TP53 mutations in our CLL patients, in order to analyze the type and frequency of these alterations. Results were correlated with cytogenetics, FISH and IGHV mutational status studies. A total of 60 patients were evaluated. Mutational status was analyzed by PCR followed by bidirectional sequencing, and compared with public databases. The study was approved by the Institutional Ethics Committee. All individuals provided their informed consent. Three (5%) cases showed the NOTCH1 c.7541_7542delCT mutation. These patients had unmutated (UM) IGHV (100% germinal identity) and two of them showed very complex karyotypes. For TP53 mutation analysis, a selected group of 24 patients with TP53 deletion by FISH analysis was evaluated. Nine (37.5%) cases showed mutated TP53 (TP53-M), all of them with more than 20% of cells with TP53 deletion; exons 4-8 were involved. Two insertions and 2 deletion with change of reading frame, and 5 replacement point mutations (4 transitions and 1 transversion), were observed. The polymorphism analysis of codon 72 (rs1042522) that encodes arginine (Arg) or proline (Prol) showed association of the Pro/Pro genotype with TP53-M (p=0.047). The presence of TP53-M was not significantly related to IGVH mutational status. Cytogenetic analysis showed that total cases with TP53-M had abnormal karyotypes, compared to 25% of patients with TP53-UM. One case had both NOTCH1 and TP53 mutations. Our data constitute the first evaluation of NOTCH1 and TP53 mutations in patients with CLL in our country and provide information about the molecular heterogeneity of this pathology.

528. (438) **ANALYSIS OF BIRC3 ALTERATIONS IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA.**

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BIRC3 (Baculoviral IAP repeat containing 3) gene (11q22.2), located ~6Mb centromeric to the ATM (Ataxia telangiectasia mutated) locus (11q22.3), is a negative regulator of non-canonical NF-kB signaling pathway. We evaluated BIRC3 mutations and deletions in chronic lymphocytic leukemia (CLL) patients in order to have a better biologic characterization of the disease. Results were correlated with cytogenetics, FISH and IGVH (immunoglobulin heavy chain variable region) mutational status. A total of 80 patients were evaluated. Mutational status of exons 7, 8 and 10 was analyzed by PCR followed by bidirectional sequencing and compared with public databases. BIRC3/MALT1 Dual Color Dual Fusion Probe (Zytovision, Bioars) was used. The study was approved by the Institutional Ethics Committee. All individuals provided their informed consent. No BIRC3 mutations were observed. Three patients showed variants: rs17881197, rs7124969 and rs1055088, within exons 7, 8 and 10, respectively, all of them without clinical significance. The latter variant was associated to abnormal karyotype, TP53 deletion and unmutated (UM) IGHV. BIRC3 deletion was evaluated by FISH analysis in a selected group of 17 patients with ATM deletion. BIRC3 deletion was observed in 16/17 cases with the following distribution: 58% showed similar values in both genes, 25% had higher percentage of ATM deletion and 17% of cases showed increased frequency of BIRC3 deletion, suggesting clonal evolution. In addition, 77.8% of patients expressed UM-IGHV and 76.5% had abnormal karyotypes, 53.8% of them complex karyotypes. Interestingly, 64.3% of cases also showed TP53 deletion, associated to bad prognosis. To our knowledge, this is the first evaluation of BIRC3 alterations in CLL patients in our country. These results suggest very low frequency of BIRC3 mutations and highly variable size of deletions at 11q22 chromosomal region in our series. We identified a high-risk molecular group whose heterogeneity warrants further studied in the search for clinical correlations.

529. (459) **DELETIONS OF THE LONG ARM OF CHROMOSOME 6 IN MULTIPLE MYELOMA PATIENTS. ASSOCIATION WITH PROGNOSTIC FACTORS.**

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Multiple myeloma (MM) is a malignancy of mature plasma B cells. In this pathology, classical parameters are not accurate enough to predict the outcome of patients at early-stage disease. Nowadays, the genetic profile of tumor cells is one of the most relevant prognostic factors that could contribute to the identification of different risk groups. Chromosome unbalances, are common events, being the most frequent gains on 1q, 19p and 9q and losses on 1p, X, 13q, 14q, and 6q. We investigated the impact of deletionsof the long arm of chromosome 6 (del6q) in MM patients. Results were correlated with clinical parameters and overall survival (OS). A total of 150 bone marrow samples of newly diagnosed patients were studied: 89 (59.3%) cases with structural abnormalities(SA) and 61(40.6%) with normal karyotype (NK). Among the SA group, 35 samples showed del6q (39.3%) (17 with complex karyotype; CK), and 54 (60.7%) with other alterations than del6q (19 with CK). Clinical parameter comparisons showed that all patients with del6q (with simple or CK) showed increased levels of creatinine (p=0.0378) and beta2 microglobulin (B2M) (p=0.0039) with respect to NK group. A higher percent of cases with kappa light chain, bone marrow infiltration and lytic bone lesions was detected in cases with del6q with respect to those with NK (p=0.0022, p=0.0004 and p=0.0364, respectively). No significant differences in OS were found between cases with del6q as the only abnormality (87.7 months) respect to those with NK (123.7 months).However, a significant short OS (28.4 months) for patients with CK and del6q, was found (p=0.0021).Our data showed the association of del6q with adverse prognostic factors, but without clinical impact by itself, supporting the importance of genomic complexity in MM clinical evolution.

530. (715) **F8 GENOTYPE CHARACTERISATION OF THE FIRST ARGENTINE SERIES OF PATIENTS WITH MILD HAEMOPHILIA A: NOTABLE PREVALENCE OF RECURRENT MUTATIONS.**

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Haemophilia A (HA) is the commonest X-linked coagulopathy caused by deleterious mutations in *F8*. Mild-HA associates with minor reduction in the clotting activity of factor VIII (FVIII:C) to 5-40 UI/dL. Perhaps due to their mild phenotype expression, mild-HA pa-

tients are rarely genotyped although they represent 35-40% of HA cases worldwide. The scarce published data indicate that mild-HA shows notable differences with severe-HA (FVIII:C<1 IU/dL) in its population genetics and mutational pool turnover.
Objective: to characterise the *F8*-genotype in a large series of Argentine patients with mild-HA and to discuss its mutational dynamics.
Population: 64 apparently unrelated families affected with mild-HA countrywide, 97 individuals including index-cases and relatives. Our *F8* analysis algorithm includes: -genomic DNA extraction from peripheral blood leukocytes, -a mutational screening by PCR-amplification of all coding and regulatory regions of *F8* over all 26 exons (38 amplimers) and conformation sensitive gel electrophoresis (CSGE), -mutational characterisation by Sanger sequencing of CSGE anomalous amplimers. Duplication of exon 13 (Dup13) was detected by tail-to-head PCR-analysis.
The mild-HA-causative mutation (established by genotype/phenotype assignment criteria) was identified in 61 families (detection efficiency 95%). Thirty-four families (56%) showed 14 recurrent mutations (repeated 2-5 times), whereas the remnant 27 families, non-recurrent *F8*-defects. Among the recurrent mutations, we found 11 missense in 26 families highlighting p.Arg612Cys* (n=5) and p.Arg550Cys (n=3) among others (n=2), a splicing defect on c.601+5G>A (n=3), the Dup13* (n=3) and a synonym change (n=2) (*reported with Italian origin). Non-recurrent mutations included 24 missense, an *ins-del* and two splicing defects.
Our findings demonstrate that our practical approach is adequate to characterise the mild-HA-causative *F8*-genotype in patients and relatives, highlight the prevalence of missense defects in mild-HA (50/61, 82%) and indicate that the higher frequency assessed for recurrent mutations in mild-HA respect to severe-HA may reflect the higher mutational turnover of the severe-HA pool.

531. (542) **ALTERNATIVE END-JOINING REPAIR PATHWAY INDUCES CHROMOSOMAL REARRANGEMENTS IN HUMAN CELLS TREATED WITH ETOPOSIDE**

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Chromosomal rearrangements (CR) involving the MLL (mixed-lineage leukemia) gene cause secondary malignancies associated with the treatment of human tumors with etoposide (ETO). The incorrect repair of DNA double-strand breaks (DSB) by alternative end-joining (alt-EJ) pathway generates CR, genomic instability and tumorigenesis. The role of alt-EJ in the generation of CR induced by ETO in human cells deficient in the cohesion subunit Rad21 (homologous recombination defective) and in DNA-PKcs (one of the main factors of classical nonhomologous end-joining) was evaluated. HeLa Rad21kd cells and their non-silencing NS control cells were treated with ETO 2μg/ml for 1-2h in the presence or absence of the chemical inhibitor of DNA-PKcs, NU7026 10μM. After 2h of treatment, an increase in the percentage of G2 cells with DSB (88.2%-93.4%), analyzed by flow cytometry, was observed. At 10h post-treatment (PT), the immunofluorescence analysis of nuclei with more than 20 γH2AX foci (DSB biomarker) in G1 post-mitotic binucleated cells showed a significant increment in HeLa Rad21kd and HeLa NS exposed to NU7026-ETO (82.8±2.1% vs. 21.7±3.2%, p=0.0001) compared to cells treated with ETO alone (Rad21kd =36.4±5.9% vs. NS=7.7±1.8%). Abnormal repair of ETO-induced DSB led to inter-chromosomal exchanges and mainly, dicentric chromosomes at second metaphases (28h PT), being this frequency 3.1-times higher in NU7026-ETO-treated HeLa Rad21kd cells than in NS (2.42±0.33 vs. 0.78±0.16, p=0.0001). Moreover, MLL gene rearrangements at band 11q23 using fluorescence in situ hybridization procedure were found in 7.2% and 4.9% interphase nuclei of Rad21kd and NS cells exposure to the combination of NU7026-ETO, respectively. Meanwhile, the percentage of MLL gene rearrangements after ETO treatment was similar in both cell lines (NS= 6.3% and Rad21kd= 5.9%). These results indicate that ETO-induced DSB go through the successive cell division and that alt-EJ plays an important role in the CR formation involving MLL gene.

532. (236) **MITOCHONDRIAL HAPLOGROUPS IN ONCOHEMATOLOGICAL, COLORECTAL AND BREAST CANCER SAMPLES FROM CABA AND BUENOS AIRES PROVINCE (ARGENTINA)**

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Mitochondrial DNA (mtDNA) variants -a unique SNPs combination- define specific haplotypes, which are gathered in haplogroups showing ethnic differences and a continental-specific distribution. mtDNA haplogroups are suggested to be associated with certain pathologies, including cancer. Aims: 1-Identify mtDNA haplogroups in patients with oncohematological (OncoHemCa), colorectal (CRC) or breast cancer (BrCa) and in control samples, 2-Compare frequencies with those published for the Argentine population, and 3-Analyze the possible role of mtDNA haplogroups as risk factors. Sample set (blood): 96 cases with OncoHemCa and 272 controls, from a public hospital in La Plata (DsAs, Argentina), plus 68 CRC and 54 BrCa cases, recruited at private hospitals from CABA (Argentina) and La Plata, respectively. A sequencing approach and multiplex PCR-APLP assays were used to determine mitochondrial haplogroups. Assignments were performed using bioinformatic tools, phylogeographical criteria and local databases. Regarding OncoHemCa cases, 57.3% were assigned to Amerindian, 36.5% to European/Middle Eastern and 6.2% to African lineages. Controls showed 63.5% of Amerindian, 33.9% of European/Middle Eastern and 2.6% of African ancestry. About CRC cases, 25% were assigned to Amerindian, 69.1% to European/Middle Eastern and 5.9% to African lineages. mtDNA ancestry of BrCa cases was 29.6% Amerindian, 64.8% European/Middle Eastern and 5.6% African. Maternal lineage distribution showed significant differences among cases from the public versus private healthcare system (p<0.001). Adjusted multivariate logistic regression models estimated an OR=2.96 (CI95%,0.9-9.65, p=0.07) for individuals carrying African mtDNA when analyzing OncoHemCa. No differences in haplogroups distribution were seeing among CRC and BrCa samples. Observed frequencies in cases and controls from public and private institutions are in line with those reported for this region. More samples will be added in future studies, particularly controls from private hospitals, which will allow us to better analyze the role of mtDNA haplogroups as risk factors, a research area that was not yet deeply studied.

533. (239) **EVALUATION OF GERMLINE AND SOMATIC VARIANTS OF 141 CANCER PREDISPOSITION GENES IN PATIENTS OF HBOC**

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Hereditary Breast and ovarian cancer syndrome (HBOC) is a genetic condition. The majority of HBOC risk is due to germline variants in BRCA1 and BRCA2 genes, however, around 60% of high-risk