

369 SIGNAL TRANSDUCTION IN CHRONIC LYMPHOCYTIC LEUKEMIA (CLL) CELLS BY MULTIPARAMETER FLOW-CYTOMETRY

M.L. Palomba¹, J.P. Patel¹, U. Rao¹, N. Rastalsky¹, S.X. Lu¹, A.N. Houghton¹, A.D. Zelenetz¹, M.R. van den Brink¹

¹Medicine, Mem. Sloan-Kettering Cancer Center, New York, United States

Introduction: CLL is the clonal expansion of mature CD5⁺/CD23⁺/sIg^{low} lymphocytes. Most B-CLL cells express on their surface surface IgM or IgD, both of which function as B-cell antigen receptor (BCR). The majority of CLLs originate from antigen-experienced, memory B cells with mutated variable region (V) genes (M-CLL) and have a more indolent course. CLL cells with unmutated V_H genes (U-CLL), more aggressive, might derive from B-cells in which antigenic stimulation did not result in somatic mutations. The prognostic difference between M-CLL and U-CLL might originate from differences in responsiveness of the mutated or unmutated BCR to stimulation. We interrogated signaling pathways in primary CLL cells using a novel multi-parameter flow cytometric technique.

Materials and methods: The peripheral blood mononuclear fraction of healthy volunteers (PBMCs) and CLL patients was stimulated with anti-IgM antibodies. After fixation and permeabilization, the samples were stained with fluorochrome-conjugated anti-CD-20, -CD5 and -CD38 surface antibodies and intracellular antibodies against BLNK(pY84), Syk(pY352)/Zap-70(pY319), PLCY2(pY759), JNK(pT183/pY185), p38-MAPK(pT180/pY182) and ERK1/2(pT202/pY204). In some experiments, H₂O₂ was added to maximize kinase activation via inhibition of cellular phosphatases.

Results: The CD20⁺/CD5⁺ population of the CLL samples was compared to the CD20⁺ population in PBMCs. We found greater variability among CLL patients in baseline kinase activity and response to activation than in PBMCs. The overall level of constitutive phosphorylation was similar in the two groups. Stimulation of the BCR resulted in greater activation of pSyk/Zap70 and pPLCY2 in the PBMCs samples than in the CLL samples. Both pERK1/2 and pP38-MAPK however were more responsive to IgM stimulation in 3/6 CLL patients compared with PBMCs. Treatment with H₂O₂ resulted in significantly higher activation of pBLNK, pPLCY2, Syk/Zap70 and p38 in CLL cells than in PBMCs.

Conclusions: Phospho-specific flow cytometry identifies altered signaling nodes in CLL. Correlation with clinical parameters and V gene status of individual patients and analysis of non-BCR signaling pathways is under way.

370 CD31 DENSITY IS A NEW RISK FACTOR FOR PATIENTS WITH B-CELL CHRONIC LYMPHOCYTIC LEUKAEMIA

T. Mainou-Fowler¹, A. Porteous¹, A. Nicolle¹, S. J. Proctor¹, J. Anderson¹, G. Summerfield¹

¹Academic Haematology, Newcastle University, Newcastle upon Tyne, United Kingdom

Background: CD31 is the physiological ligand for CD38. CD38 expression in a high percentage of malignant cells is a risk factor for patients with B-cell chronic lymphocytic leukaemia (B-CLL). A previous investigation demonstrated that quantification of CD38 improves upon the prognostic value of the percentage expression (Br J Haematol 2002, 118, p755). A recent study states that the percentage of CD31 expression is not predictive in B-CLL (Cancer 2003, 97, p1914).

Materials and Methods: We re-assessed the predictive power of CD31 in a cohort of 120 patients with B-CLL. Peripheral blood cells were stained with PCP-labelled anti (a) CD19, FITC-aCD5 and PE-aCD31. CD31 expression was quantified using beads of specific antibody binding capacity and the density was correlated with clinical outcome (Br J Haematol 2002, 118, p755). End points were disease-specific survival and time to treatment (TTT). The inclusion criteria were availability of samples and clinical data.

Results: We report that CD31 density was significantly lower in the group of patients with Binet stage B and C; P=0.0003. There was an inverse, significant correlation between CD31 and CD38 densities; R=-0.281, P=0.002. All CLL-related deaths occurred in patients with low CD31 density. While, with respect to CD38 expression, CLL-related deaths were equally distributed between the groups with high and that with low CD38 density. Low CD31 predicted for poor disease outcome (all patients: survival: P=0.0087; TTT: P=0.0064) and identified Binet stage A patients (survival: P=0.0350; TTT: P=0.0718) and those with low CD38 (survival: all patients: P<0.0001; stage A: P=0.003) who followed a more aggressive clinical course. Disease-specific survival of patients with low CD31 and high CD38 densities was significantly shorter than all other groups. In addition, low CD31 density was a poor risk factor irrespective of patient age (survival: all patients: P=0.045; stage A: P=0.021) and identified patients with Binet stage B/C as the highest risk group (survival: P<0.0001).

Conclusions: Low CD31 density is an adverse prognostic indicator in B-CLL. The interaction between CD31 and CD38 and its clinical significance in B-CLL merits further investigation.

371 HEMATOPOIETIC LINEAGE CELL SPECIFIC PROTEIN 1 (HS1) IS A NEW PROGNOSTIC MARKER AND IS INVOLVED IN CELL GROWTH OF B-CELL CHRONIC LYMPHOCYTIC LEUKEMIA

F. Frezzato¹, C. Gattazzo¹, A. Brunati², M. Frasson², V. Martini¹, E. Scquizzato¹, S. Carraro¹, A. Teramo¹, M. Miorin³, R. Zambello¹, G. Semenzato¹, L. Trentin¹

¹Department of Clinical and Experimental Medicine, Hematology and Clinical Immunology Branch, Venetian Institute for Molecular Medicine (VIMM), Centro di Eccellenza per la Ricerca Biomedica, University of Padova, Padova, Italy,

²Department of Biological Chemistry and Institute of the Neuroscience of the Italian National Research Council (CNR), University of Padova, Padova, Italy,

³Department of Clinical and Experimental Medicine, Hematology and Clinical Immunology Branch, University of Padova, Padova, Italy

Introduction: B-cell chronic lymphocytic leukemia (B-CLL), the most common form of leukemia in adults and it is characterized by the accumulation of clonal CD5⁺ B lymphocytes due both to uncontrolled growth and resistance to apoptosis. Several protein kinase pathways have been claimed to be involved in this regulation of cell survival. We previously demonstrated that the Src kinase Lyn is overexpressed, constitutively active and anomalously distributed in malignant B cells as compared to normal B lymphocytes. Our attention was subsequently focused on the 75 kDa HS1 protein which is one of the major substrate of Lyn kinase upon BCR cross-linking.

Methods: In the present study HS1 protein level was measured by Western blotting and RT-PCR analysis in 43 untreated B-CLL patients and in 26 normal controls. In some patients HS1 levels were measured before and following therapy. The distribution of HS1 in leukemic cells was investigated by confocal microscopy and subcellular cell fractionation.

Results: We found a significant difference in HS1 protein level between normal and CLL B cells, being mainly expressed in the leukemic patients with respect to normal controls (p<0.01). Furthermore, we observed that subjects with unfavourable prognostic factors had an higher expression of HS1 protein as compared to those with a better prognosis. We also analyzed HS1 in 10 CLL patients following treatment with fludarabine and cyclophosphamide and in 7 responder patients we found a reduction of HS1 protein and its mRNA levels, while in refractory patients we didn't find any change. Using confocal microscopy and subcellular cell fractionation, we also observed an abnormal distribution of HS1 in leukemic cells with respect to normal B cells. In particular, the pattern of expression of HS1 appeared with a spotting distribution and a 4-7% aliquot of HS1 was present in the nucleus of leukemic B cells but not in normal B lymphocytes. This nuclear localization disappeared following BCR engagement. By pre-incubating B-CLL cells with PP2, which is a Src kinase inhibitor, we were able to prevent the IgM-mediated redistribution of HS1. Finally, we investigated whether HS1 could interact with cytoskeletal components because of its structure and its well-known interaction with actin and Arp2/3 complex. We found that either in normal and leukemic B cells cytosolic HS1 colocalizes with beta-actin.

Conclusions: All these findings suggest a pivotal role for HS1 in the regulation of cell survival of leukemic B cells and hint that this protein might represent a target for the development of new therapeutic clinical strategies.

372 EXPRESSION OF AID PROTEIN IN SMALL B-LYMPHOCYTIC LYMPHOMA IS ASSOCIATED WITH POOR PROGNOSIS AND COMPLEX GENETIC ALTERATIONS

M. Tinguely¹, M. Leuenberger¹, S. Frigerio¹, P.J. Wild¹, F. Noetzi¹, D. Korol², D.R. Zimmermann¹, N.M. Probst-Hensch²

¹Department of Pathology, Institute of Surgical Pathology, Zurich, Switzerland,

²Department of Pathology, Institute of Social and Preventive Medicine, Division of Molecular Epidemiology/Cancer Registry, Zurich, Switzerland

Introduction: The clinical course of B-CLL/B-SLL is heterogenous and difficult to predict. Two prognostic subtypes have been identified, based on the mutation status of the IgV_H gene, being more favourable for the mutated ones. AID is indispensable for somatic hypermutation and class switch recombination. So far, investigations on mRNA level were contradictory in predicting the mutation status of B-CLLs. The aim of the study was to analyze AID protein in SLL to 1) determine the immuno-architecture and assess the frequency of AID expression 2) clarify its prognostic role 3) determine the relationship with chromosomal aberrations and 4) to investigate whether AID is a surrogate marker for the IgV_H mutation status.

Methods: B-CLL/B-SLL of 71 patients were investigated on a TMA for IHC and FISH. IHC markers included: AID (clone EK2-5G9; E, Kremmer, GSF, München and clone ZA001, Zymed), ZAP70 (2F3.1), CD38, CD20, Mib1. FISH probes for 17p13.1 (p53), 11q22.3 (ATM), 13q14.3 (D3S319), 13q34 and 12p11.1-q1 (all Vysis) were applied. For mutation analysis, V_{H1}-V_{H6} FRI and JH consensus primers were used. PCR products were directly sequenced.

Results: AID was found in 17/71 (23%) of patients. Interestingly, its expression was preferentially seen in proliferation centres. The median survival in the AID positive cohort was significantly shorter ($p=0.015$) as compared to median survival of the whole study population (24 vs 44 months). Moreover, AID expression was associated with more complex genetic aberrations, particularly deletion of p53 and ATM. However, it was not predictive of the IgH mutation status.

Conclusion: We demonstrate the prognostic role of AID expression on protein level in B-CLL/B-SLL. Particularly, AID expression is associated with a complex genetic background. Moreover, we were able to disclose proliferation centres as interesting microenvironment possibly promoting AID expression.

373 NEW INSIGHTS INTO GENETICS OF CLL BASED ON CHROMOSOME BANDING ANALYSIS

C. Haferlach¹, F. Dicker¹, S. Schnittger¹, W. Kern¹, T. Haferlach¹
¹Munich Leukemia Laboratory, MLL, Munich, Germany

Introduction/Background: In CLL data from chromosome banding analysis have been scarce due to the low proliferative activity in vitro. Therefore, fluorescence in situ hybridization using a limited number of probes has become the standard for cytogenetic characterization of CLL. However, chromosome banding analyses provides more detailed information with respect to the spectrum of abnormalities, clonal evolution and the coexistence of independent clones.

Material and Methods: We improved the cultivation technique using an immunostimulatory CpG-oligonucleotide DSP30 and IL-2 leading to a sufficient number of analyzable metaphases in 98.8% of cases and the detection of an aberrant clone in 83.0%.

Results: Based on a series of 506 CLL cases it was demonstrated that chromosome banding analysis detected 1.7 fold more abnormalities than the standard FISH panel (832 vs. 502). Furthermore, cases showing a 13q deletion in interphase FISH could be divided into 3 subgroups based on chromosome banding analysis data: 1. del(13q) sole, 2. del(13q) with additional abnormalities and 3. del(13q) due to a reciprocal translocation. This genetic heterogeneity might account for differences in clinical outcome. 16.4% of cases showed a complex aberrant karyotype which was associated with an unmutated IgV_H status and CD38 expression ($p=0.034$ and $p=0.02$, respectively). Gain of 2p was identified as a new recurrent abnormality occurring in 2.7% of cases and was also strongly associated with an unmutated IgV_H status and CD38 expression. Gene expression analysis revealed a significantly higher expression of 10 genes located on 2p (LOC56902, PPP3R1, MSH6, RTN4, COX7A2L, HADHA, TTC32, ACP1, CPSF3, PDIA6) in cases with 2p gain. These genes are involved in mismatch repair and in negative regulation of anti-apoptosis. Interestingly, ATM showed a significantly lower expression in the group with 2p gain. In addition, a correlation between TP53 deletions and a higher number of chromosome abnormalities was observed. Cases with TP53 deletion showed in mean 5.0 aberrations as compared to 1.5 in cases without TP53 deletion ($p<0.0001$).

Conclusions: Chromosome banding analysis reveals that CLL is more heterogenous on the genetic level as assumed based on FISH data. A more detailed subdivision of CLL based on cytogenetic data might allow a more precise prognostication and treatment selection.

374 P53 MUTATIONS IN A LARGE COHORT OF CLL PATIENTS WITH 17P DELETION: DETAILED ANALYSIS OF MUTATION PROFILE, ALTERNATIVE MECHANISMS OF INACTIVATION, CLONE SIZE AND CLONAL EVOLUTION

T. Zenz¹, T. Denzel¹, S. Häbe¹, J. Mohr¹, D. Winkler¹, D. Kienle¹, A. Bühler¹, H. Döhner¹, S. Stilgenbauer¹
¹Internal Medicine III, University of Ulm, Ulm, Germany

Background: The prognosis of CLL with 17p deletion (17p-) is very poor. While it is generally accepted that inactivation of p53 (by mutation) underlies refractoriness of CLL with 17p-, no study has analysed a large cohort of CLL patients with 17p- with respect to TP53 mutations.

Methods: In order to assess the incidence of TP53 mutations in CLL with 17p- we studied TP53 mutations in a large cohort (n=94 patients) of these patients. We used DHPLC to screen for mutations (Exons 2-11). A sub-group of cases were also studied with an array based p53 mutation platform to confirm the absence of mutations. Detailed genetic studies (IgVH, ZAP70, FISH) were available for the patients.

Results: We found mutations in the protein coding region of p53 in 71 of 94 CLL patients (76%) with 17p deletion. In the majority of the cases the clone size (mutated) correlated very closely with the FISH results. Only few cases had more than one mutation (2/73). The majority of mutations were located in the DNA binding domain of p53 (88% of the mutations in exon 5 to 8). We also observed mutations in cases with less than 20% of cells carrying the 17p-. The analysis of follow-up samples in cases with low grade 17p- showed definite evidence for the selection of the p53 deficient clone (mutation and deletion). In spite of this clear evidence for a classical tumor suppressor mechanism underlying the resistance to chemotherapy in cases with 17p-, there remain cases where no mutation in the exons of TP53 can be detected by DHPLC, sequencing or array based mutation analysis, suggesting that in these cases alternative

mechanisms lead to inactivation of p53. These mechanisms (e.g. alternative splicing) are currently under investigation.

Conclusion: The current study supports the role of p53 inactivation (by mutation) underlying the chemoresistance of CLL with 17p deletion. The extend of mutations of the remaining allele and the demonstration of coexisting mutations even in cases with deletions in only the minority of cells suggests that p53 is the main biological target of 17p- and its clinical consequence.

375 MOLECULAR AND CLINICAL PREDICTION OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) ARISING IN THE CONTEXT OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

D. Rossi¹, D. Capello¹, M. Cerri¹, C. Deambrogi¹, S. Cresta¹, S. Rasi¹, V. Spina¹, A. Conconi¹, V. Gattei², G. Gaidano¹
¹Hematology, Amedeo Avogadro University, Novara, Italy, ²Onco-Hematology Unit, CRO, Aviano, Italy

Background. DLBCL occurs in 5-15% CLL, depending on follow-up length and re-biopsy policy. The value of biological and clinical risk factors at CLL diagnosis in predicting subsequent DLBCL transformation is unknown and represented the aim of this study.

Methods. The analysis was based on a consecutive series of 185 CLL. DLBCL was diagnosed in 17 cases, all EBV negative. Cumulative incidence of DLBCL at 10 years was 16.2%.

Results. By univariate analysis, biological variables at CLL diagnosis predicting DLBCL were: IGHV4-39 usage (HR=6.00; $p=.002$), IGHV homology $\geq 98\%$ (HR=4.33; $p=.003$), CD38 (HR=6.01; $p=.002$) and ZAP70 expression (HR=11.24; $p=.002$), and absence of del13q14 (HR=5.27; $p=.009$). Clinical variables predicting DLBCL included: lymph node ≥ 3 cm (HR=9.99; $p<.001$), involvement of 3 nodal areas (HR=5.51; $p=.001$), LDH >1 x ULN (HR 5.89; $p=.001$), and advanced Binet stage (HR=4.18; $p=.004$). Multivariate analysis including biological variables at CLL diagnosis selected CD38 expression (HR=4.26; $p=.018$) and IGHV4-39 usage (HR=4.29; $p=.018$) as independent risk factors of DLBCL. Accordingly, CLL characterized by CD38 expression and IGHV4-39 usage displayed the highest risk of DLBCL (5-year risk: 72.2%). Multivariate analysis including both biological and clinical variables at CLL diagnosis selected lymph node size ≥ 3 cm (HR=6.51; $p=.001$) and absence of del13q14 (HR=4.08; $p=.031$) as independent risk factors of DLBCL. Accordingly, CLL characterized by lymph node size ≥ 3 cm and absence of del13q14 displayed the highest risk of DLBCL (5-year risk: 78.9%). Based on landmark analysis, the type of first progression influenced the risk of DLBCL. Risk of subsequent transformation was restricted to patients who underwent nodal progression ($p<.001$), while patients who developed cytopenia had a negligible risk of subsequent transformation ($p=.964$). By landmark analysis, risk of DLBCL did not differ after exposition to fludarabine or alkylating agents ($p=.070$).

Conclusions. Close monitoring and a careful biopsy policy may help prompt recognition of DLBCL in CLL carrying lymph node >3 cm in the absence of del13q14 CLL patients and in CLL expressing CD38 and utilizing IGHV4-39.

376 THE ACTIVITY OF ALEMTUZUMAB IS INDEPENDENT OF P53 MUTATIONAL STATUS IN FLUDARABINE REFRACTORY CLL: FINAL ANALYSIS FROM THE CLL2H STUDY OF THE GCLLSG

T. Zenz¹, S. Häbe¹, T. Denzel¹, D. Winkler¹, M. Hallek², M. Hensel³, E. Lengfelder⁴, P. Dreger³, U. Jäger⁵, H. Döhner¹, S. Stilgenbauer¹
¹Internal Medicine III, University of Ulm, Ulm, Germany, ²Department of Internal Medicine, University of Cologne, Cologne, Germany, ³Internal Medicine, University of Heidelberg, Heidelberg, Germany, ⁴Internal Medicine, University of Mannheim, Mannheim, Germany, ⁵Internal Medicine, University of Vienna, Vienna, Austria

Background: The prognosis of fludarabine-refractory CLL is poor with a median survival time of 12 months. Campath-1H (Alemtuzumab) is approved for fludarabine refractory CLL and there is growing evidence that Alemtuzumab is active irrespective of high-risk genetic subgroups.

Methods: To assess the incidence of TP53 mutations in fludarabine refractory CLL we studied TP53 mutations in the CLL2H trial of the GCLLSG. The CLL2H trial of the GCLLSG was initiated to evaluate the subcutaneous application of 3 x 30 mg alemtuzumab weekly in fludarabine refractory CLL. We used DHPLC to screen for TP53 mutations in 103/108 patients (Exons 2-11). Aberrant DHPLC profiles lead to sequencing of the respective exons. In addition, detailed clinical history and genetic studies were available for all patients.

Results: At an interim analysis we found TP53 mutations in 37% of patients with completed mutational analysis. There was a very high concordance rate between 17p deletions and mutations. In addition, TP53 mutations were found in patients with deletion 13q (single), with normal karyotype, 1 of 7 with trisomy 12, but interestingly none of 18 patients with deletion 11q (without 17p-). The mutations were mainly missense mutations including some of the known hot spots (e.g. codon 173, 220, 248, 273) but also small deletions (n=10). The analysis of the impact of the presence of a TP53 mutation showed no difference in overall survival for the subgroup with

currently confirmed TP53 mutation ($p=0.36$), suggesting that alemtuzumab is active irrespective of TP53 mutation status. The overall response (CR and PR) rate for patients with TP53 mutation was not different.

Conclusion: Campath-1H is effective in genetic high risk subgroups including those patients with TP53 mutations. The functional consequences of 17p deletions with and without TP53 mutation are under investigation.

377 INTERIM RESULTS OF THE COMBINATION RITUXIMAB, FLUDARABINE, CYCLOPHOSPHAMIDE AND MITOXANTRONE (R-FCM) FOLLOWED BY RITUXIMAB MAINTENANCE IN PREVIOUSLY UNTREATED CHRONIC LYMPHOBLASTIC LEUKEMIA (CLL)

F. Bosch¹, A. Muntanola¹, N. Villamor¹, M. Terol¹, E. Gonzalez-Barca¹, J. Ribera¹, M. Gonzalez¹, E. Abella¹, J. Delgado¹, F. Carbonell¹, J. Garcia Marco¹, L. Escoda¹, S. Ferrer¹, E. Monzo¹, Y. Gonzalez¹, J. de la Serna¹, P. Abrisqueta¹, E. Gine¹, O. Salamero¹, E. Montserrat¹

¹Hematology, Hospital Clinic, University of Barcelona, Barcelona, Spain, On Behalf of Grupo Español de leucemia linfática crónica (GELLC)

Immunochemotherapy is emerging as the new gold standard for CLL treatment. However, the best combination chemotherapy to be given along with rituximab has not yet been determined. In untreated CLL, FCM results in a response rate of 90%, including a high proportion of MRD-negative CRs. Against this background, we have conducted a prospective clinical trial in which treatment-naïve CLL receive R-FCM followed by rituximab maintenance. We included pts younger than 70 yrs with active disease (NCI-WG criteria) and adequate performance status. R-FCM consists of rituximab 500 mg/m² on day 1, fludarabine 25 mg/m² on days 1 to 3, cyclophosphamide 200 mg/m² on days 1 to 3, and mitoxantrone 6 mg/m² on day 1, given at a 4-week intervals up to six courses. All pts receive G-CSF and cotrimoxazole. Pts achieving response (CR or PR) are subsequently treated with rituximab 375 mg/m² every 3 months up to two years. Response is assessed 3 months after R-FCM treatment including MRD evaluation by flow cytometry. Out of 85 pts included in the study, 47 (65% male, median age 58 years) are evaluable for response to the first part of the treatment (R-FCM induction therapy). At study entry, 85% of the pts were in advanced (B and C) Binet's clinical stage and 56% had increased (>20%) ZAP-70 expression. Ninety-one per cent of the pts have received the entire planned treatment. Overall response rate is 94%. MRD-negative CR is 37%, MRD-positive CR 37% (CR rate, 74%), nPR 7%, and PR 13%. Two out of 4 PR cases are MRD-negative. Toxicity has been manageable, with grade III-IV neutropenia being observed in 12% of the cases. In conclusion, R-FCM is a well tolerated regimen that induces a high CR rate, including a high proportion of MRD-negative CRs. Whether these already extremely promising results are improved by maintenance therapy with rituximab remains to be seen.

378 TREATMENT OF PATIENTS WITH RELAPSED/REFRACTORY CLL USING A COMBINATION OF FLUDARABINE, CYCLOPHOSPHAMIDE AND ALEMTUZUMAB: FIRST SAFETY ANALYSIS OF THE CLL2L TRIAL OF THE GERMAN CLL STUDY GROUP

T. Elter¹, R. James¹, C. M. Wendtner¹, S. Stilgenbauer², D. Winkler², M. Ritgen³, M. Hallek¹, A. Engert¹

¹Department of Hematology and Oncology, University of Cologne, Cologne, Germany, On Behalf of German CLL Study Group, ²Department of Hematology and Oncology, University of Ulm, Ulm, Germany, ³Department of Hematology and Oncology, University of Kiel, Kiel, Germany

Background: Purine nucleoside combination therapy has become the standard treatment approach in B-CLL. In order to enhance efficacy, we developed a multicenter phase II trial combining fludarabine, cyclophosphamide and alemtuzumab in a 4-weekly schedule.

Methods: Fludarabine 25mg/m² iv, cyclophosphamide 200mg/m² iv and alemtuzumab 30 mg sc were given on days 1 – 3 and repeated on day 29 for up to six cycles. A 2-day escalation of alemtuzumab was administered prior to the first cycle. Minimal residual disease (MRD) was measured by 4-color flow cytometry. Antiinfective prophylaxis and CMV-monitoring was done according to established standards.

Results: A total of 39 patients of a planned sample size of 61 patients were included in this phase-II study so far, of which 20 patients are evaluable for response and safety analysis. Median age is 64 (range 48-78) years with a median number of 1 prior regimen; 14/20 patients completed at least four cycles with a median number of 4.5 cycles. Pretreatment consisted of fludarabine (8/20), fludarabine and cyclophosphamide (12/20) or rituximab combination (1/20). Thrombocytopenia and neutropenia were the most serious side effects and detailed data will be presented. 2 CMV-reactivations, 1 Herpes-zoster-reactivation and 12 fever of unknown origin have been reported. The overall response rate was 70% with 5 CR (25%), 9 PR (45%), 2 SD and 2 PD. All complete responders became MRD negative in peripheral blood or bone marrow; response was independent of FISH status. A correlation of response with prior treatment was observed, with 88% ORR for fludarabine pre-treatment versus only 65% for those patients pretreated with fludarabine and cyclophosphamide.

Conclusions: The concomitant application of fludarabine, cyclophosphamide and alemtuzumab appears as a safe and effective approach for patients with relapsed CLL.

379 BENDAMUSTINE VS. FLUDARABINE AS SECOND-LINE TREATMENT FOR PATIENTS WITH CHRONIC LYMPHOBLASTIC LEUKEMIA – FIRST INTERIM RESULTS OF A RANDOMIZED STUDY

N. Niederle¹, L. Balleisen², W. Heit³, W. Knaut⁴, J. Weiß⁵, W. Freier⁶, S. Ibach⁷, H. Eimermacher⁸

¹Medizinische Klinik III, Klinikum Leverkusen, Leverkusen, Germany, ²Med. Klinik, Ev Krankenhaus, Hamm, Germany, ³Innere Med., Kliniken Essen-Süd, Essen, Germany, ⁴Praxis, Frankfurt, Germany, ⁵Praxis, Weiden, Germany, ⁶Praxis, Hildesheim, Germany, ⁷WiSP, Langenfeld, Germany, ⁸Hämatologie/ Onkol., Marien-Hosp., Hagen, Germany

Background: Bendamustine (B) has both a bifunctional mustard and a purine analogue moiety showing a unique mechanism of action distinct from classical alkylating agents with no cross-resistance. B demonstrated clinical activity in a wide range of hematological and non-hematological malignancies.

Materials and methods: Patients (pts) with relapsed CLL requiring treatment after one previous systemic regimen (not including fludarabine (F) or B) were randomized centrally to receive either B 100 mg/m² as 30-minute-infusion on days 1 + 2 of a 4week (w) cycle, or as standard treatment F 25 mg/m² on days 1 to 5 q 4w. Treatment was repeated until diagnosis of best response or up to a maximum of 8 cycles. The primary objective was to achieve comparable progression-free survival (PFS) to F.

Results: Out of a total of 96 pts randomized, 89 were eligible for the interim analysis, 46 allocated to B and 43 to F. B/F median age: 67.5/69 years, male pts: 65/60%, Binet C stage: 54/49%, B symptoms: 42/38%, respectively. Bulky disease (12/14%) was equally distributed, while a favourable performance status was more frequent in the B group (47/29%). First-line treatment consisted of chlorambucil or the Knosp regimen in 95% of pts. About half of the pts received six or more cycles in either treatment arm. Overall response rates (ORR) were 78% (B) and 65% (F), clinical CR rates 29/10%. After a median follow-up of two years and 54 events, median PFS was 83/64 w (hazard ratio 0.93; 90% confidence interval: 0.59 – 1.47). Hematotoxicity was marginally more frequent in the B arm, while non-hematological toxicities were generally rare, with grade 3/4 infections of 15% in both arms.

Conclusions: These data suggest at least comparable efficacy of B to F with respect to ORR and PFS, pointing to an alternative treatment option to F in relapsing CLL pts after first line chemotherapy containing chlorambucil.

380 LENALIDOMIDE LEADS TO A PRO-INFLAMMATORY CYTOKINE RELEASE SYNDROME IN CLL: IMPLICATIONS FOR PATIENT MANAGEMENT AND A POSSIBLE ROLE OF INNATE IMMUNITY

A. Wiestner¹, B. Vire¹, N. Njuguna¹, S. Soto¹, C. Boss¹, S. Pittaluga², G. Aue¹
¹Hematology Branch, NHLBI, National Institutes of Health, Bethesda, United States, ²Laboratory of Pathology, NCI, NIH, Bethesda, United States

Introduction: Lenalidomide induces clinical responses in 1/3 of patients with relapsed CLL. The mechanism of action is ill defined but could involve immune responses directed at tumor cells, suppression of cytokines, notably TNF α , and inhibition of signaling pathways. To better define the immune modulatory effect of lenalidomide in CLL we systematically analyzed cytokine levels and immune cell subsets *in vivo* during lenalidomide treatment.

Patients and Methods: 10 patients with CLL treated on an IRB approved study of single agent lenalidomide 20mg daily cycled for 3 weeks on, 3 weeks off for up to 8 cycles. Serum levels of 16 cytokines were measured in the first 2 cycles, changes in lymphoid cell subsets and activation markers were assessed by standard flow cytometry.

Results: Most patients had rapid reduction in absolute lymphocyte count. Tumor flare reactions were observed in cycle 1, 2, or 3 in 8, 4, and 2 patients, respectively. Pre-renal azotemia likely due to vasodilatation was observed in 3 patients. Clinical findings and elevated CRP were consistent with inflammation. Pro-inflammatory cytokines and chemokines, including IL1 α , IL6, IL10, TNF α , CCL2, CCL3, CCL4, and CXCL8 were rapidly upregulated, remained elevated while on drug and increased again in the second cycle. We found no differences in cell surface expression of activation markers (CD25, CD69, CD86 or FAS-ligand) on circulating T, B or NK cells and their cell count did not increase during the first 2 cycles, on the contrary, they often decreased in parallel with the leukemic cells. Analysis of matched lymph node biopsies taken pre and on treatment is ongoing.

Conclusions: Lenalidomide released pro-inflammatory cytokines that can cause constitutional symptoms, and lead to pre-renal azotemia, which may be clinically important because lenalidomide is renally cleared. Despite increased cytokine levels we were unable to detect any evidence of expansion or functional activation of lymphoid cells *in vivo*. Most cytokines induced by lenalidomide play prominent roles in macrophage biology indicating a possible role of the innate immune system.

381 THE HISTONE DEACETYLASE INHIBITOR VALPROIC ACID INCREASES CHEMOSENSITIVITY TO SEVERAL DRUGS, INDUCES APOPTOSIS, AND CAN INHIBIT PROLIFERATION OF CHRONIC LYMPHOCYTIC LEUKEMIA CELLS

B. Stamatopoulos¹, N. Meuleman¹, P. Martiat¹, D. Bron¹, L. Lagneaux¹
¹Experimental Hematology, Bordet Institute, Brussels, Belgium

Background: Valproic acid (VPA), a well-known antiepileptic drug, has been identified recently as a histone deacetylase inhibitor that can induce apoptosis, modulate the cell cycle, and sensitize cancer cells to other chemotherapeutics. Therefore combination of VPA with others drugs is being discussed as a promising treatment in Chronic Lymphocytic Leukemia (CLL), disease remaining incurable despite introducing new therapies.

Methods: CLL cells were treated in vitro by a combination of VPA at a physiological dose (1mM) and other classical therapeutic drugs. We also induced CLL cell proliferation using IL2 and CpG oligonucleotides and analyzed VPA effect using WST1 assay. We investigated gene expression profiles of 14 CLL-patient samples treated in vitro during 4 hours with VPA at 1mM and compared them with their untreated counterpart using Affymetrix technology. Several genes were validated by real time PCR.

Results: VPA treatment induced apoptosis with similar mean rates between good and poor prognosis patients. Moreover, VPA significantly increased in vitro cytotoxic effects of fludarabine, bortezomib, and flavopiridol allowing the reduction of effective concentration 50% (EC50). VPA can also inhibit proliferation in stimulated CLL cells. Finally, in order to understand the early mechanism of VPA action, we performed gene expression profile comparison that revealed (i) no difference in gene modulation between poor and good prognosis patients after VPA treatment, (ii) an upregulation of pro-apoptotic mRNA (APAF1, PTEN, CASP6), (iii) a downregulation of anti-apoptotic mRNA (FLIP, AVEN, XIAP) and (iii) a modulation of cell cycle linked mRNA (CNND1, CNNE1, CDK4, CDK2, p18).

Conclusions: Valproic acid induced CLL cell apoptosis at clinically relevant concentration by targeting several pro- and anti-apoptotic genes. Furthermore, VPA enhanced apoptosis when associated with other chemotherapeutic drugs and can also inhibit CLL cell proliferation by cell cycle regulatory protein perturbation. Therefore, the combination of VPA with other drugs might be considered as a potential strategy for CLL treatment.

382 EPSTEIN-BARR VIRUS REACTIVATION IS A POTENTIALLY FATAL COMPLICATION IN CLL PATIENTS WITH POOR PROGNOSTIC BIOLOGICAL MARKERS AND FLUDARABINE REFRACTORY DISEASE

J.C. Rath¹, C. Geisler¹, C.B. Christiansen², N. Hastrup³, H. Madsen⁴, J. Jurlander¹

¹Department of Hematology, The Leukemia Laboratory, Rigshospitalet, Copenhagen Ø, Denmark, ²Department of Microbiology, Rigshospitalet, Copenhagen, Denmark, ³Department of Pathology, Rigshospitalet, Copenhagen, Denmark, ⁴Department of Clinical Immunology, Rigshospitalet, Copenhagen, Denmark

Introduction: Severe immunodeficiency is a major problem in advanced CLL, resulting in a high incidence of severe opportunistic infections.

Method: We identified 11 CLL cases with measurable plasma EBV DNA and EBV-reactivation retrospectively over a period of two years (2005-2007).

Results: All patients had biological high-risk disease, i.e. unmutated IgV_H rearrangement and/or high-risk cytogenetic aberrations by FISH. Nine of the 11 patients had Fludarabine refractory disease and six had received alemtuzumab based therapy prior to EBV-reactivation. We note that EBV-reactivation occurred before exposure to alemtuzumab in four patients, and in one patient prior to any CLL treatment. The clinical presentation of the patients ranged from fever of unknown origin, to hemophagocytic syndrome and B-cell lymphoproliferative disease (BLPD). Four patients were either asymptomatic, or presented with FUO, or signs of pneumonia. These patients had EBV-levels below 2500 copies/mL plasma, and were considered having low-grade EBV-reactivation. Three patients were symptomatic at presentation with fever, fatigue, night sweats and/or enlarged lymph nodes. These patients had EBV-levels from 1700 to 6600 copies/mL plasma and were in retrospect all considered having possible BLPDs. Four patients had proven EBV-associated disease with very high EBV-levels from 45.000 to 3.700.000 copies/mL plasma. Of these patients one presented with EBV-associated hemophagocytic syndrome and three developed BLPD. In two of the BLPDs we found CLL independent clones, in one of these cases detectable in samples obtained prior to overt BLPD. Five of the 11 patients died, despite aggressive treatment with Rituximab-based chemo-immunotherapy. The median overall survival was 274 days, with a follow-up time of 730 days.

Conclusion: We conclude that the possibility of EBV-reactivation must be considered in the management of high-risk CLL patients presenting with fever.

383 AUTOLOGOUS STEM CELL TRANSPLANTATION (ASCT) IN 72 PATIENTS WITH SMALL LYMPHOCYTIC LYMPHOMA (SLL): THE SPANISH EXPERIENCE

A. Oliveira¹, Á. León², R. Varela³, E. Conde⁴, J. Lahuerta⁵, M. Gandarillas⁶, E. González-Barca⁷, D. Caballero⁸

¹Hematology, Institut Català d'Oncologia, Barcelona, Spain, On Behalf of GEL/TAMO, ²Hematology, HG Jerez, Jerez de la Frontera, Spain, ³Hematology, HU Juan Cantalejo, Coruña, Spain, ⁴Hematology, HU Marqués Valdecilla, Santander, Spain, ⁵Hematology, HU 12 de Octubre, Madrid, Spain, ⁶Hematology, HU Marqués Valdecilla, Santander, Spain, ⁷Hematology, IC Oncología, Barcelona, Spain, ⁸Hematology, HU Salamanca, Salamanca, Spain

Introduction: High-dose therapy followed by ASCT has become the accepted therapy for patients with relapsed large B cell lymphoma. SLL however, does not have standard treatment and ASCT has not a well-defined role in this pathology. We have reviewed the results of ASCT for patients with SLL in Spain.

Patients and Methods: Seventy-two patients classified according to the Working Formulation as SLL underwent ASCT in 21 Spanish centres from June 1989 to July 2006. Patients were registered in GEL/TAMO database. Data were analysed using SPSS v.11 program. Survival was analysed using Kaplan-Meier method and comparisons were made using Log-rank test.

Results: Median age was 52 (27-68) years and 46 (64%) were male. The characteristics of disease at diagnosis were: ECOG > or = 2: 10 (7.2%), stage III-IV: 63 (45.3%), bone marrow infiltration: 53 (38.1%), B symptoms: 17 (12.2%), abnormal value of LDH level: 22 (15.8%) and IPI 3-4: 15 (10.8%). Status of the disease at transplantation was: 26 (36%) patients in 1st CR, 11 (15%) in 2nd CR and 35 (49%) presented chemo sensitive disease (13 in 1st RP and 22 in 2nd RP or beyond). Stem cells for engraftment were obtained from bone marrow in 15 cases and from peripheral blood in the remaining 57. All patients engrafted. The main conditioning regimen used was BEAM (51%). One patient died of toxicity during the procedure. Response rates after transplantation were: CR in 61 (84%) patients and PR in 10 (14%). The median follow-up was 45 months. Twenty-one patients have relapsed. Estimated 10-year OS and DFS for all 72 patients was 49% (CI95: 33-75) and 32% (CI95: 18-46), respectively. Actuarial probability of relapse for 61 valuable patients was 38% (CI95: 22-54), at 10 years. The major prognostic parameter associated with OS was status of disease at transplantation (p<0,0002). Twenty-four (33%) patients died, 17 from lymphoma progression, 4 due to toxicity and 3 from secondary neoplasias.

Conclusions: ASCT is a safe procedure and could be a feasible treatment option in selected patients with SLL. Patients in CR at transplantation have a better clinical outcome.

384 ALLOGENEIC STEM-CELL TRANSPLANTATION AS A CURATIVE APPROACH FOR CHRONIC LYMPHOCYTIC LEUKEMIA (CLL). THE RELEVANCE OF LATE RELAPSES. THE CLINIC OF BARCELONA EXPERIENCE AND A SUCCINCT REVIEW OF THE LITERATURE

C. Moreno¹, J. Esteve¹, P. Abrisqueta¹, C. Martínez¹, N. Villamor¹, M. Aymerich¹, M. Rovira¹, F. Bosch¹, E. Giné¹, E. Carreras¹, E. Montserrat¹

¹Hematology, Hospital Clinic. IDIBAPS. University of Barcelona, Barcelona, Spain

Background: Allogeneic stem cell transplantation (allo-SCT) is considered to be potentially curative in CLL. Although late relapses (> 4 years after transplant) are considered rare, their real incidence, characteristics, and outcome are not well known. The aim of this work is to report patients with CLL having undergone allo-SCT in our center focusing on late relapses. In addition, we succinctly review late relapses in other series.

Material and Methods: Thirty-two patients transplanted between 1991 and 2006. Median age: 50 years (range, 29-63); 62% males. Twenty-five patients received allo-SCT from an HLA-identical sibling donor and 7 from an unrelated donor. Eleven received RIC and 12 (37%) a T cell-depleted graft. Median follow-up is 6 years. Besides, we reviewed 15 series accounting for 976 patients.

Results: In our series, NRM was 31% at 1 year (CI, 19,52). DFS and OS at 5 years were 86% (77,95) and 56% (47,65). After a median follow-up of 6 years, four patients relapsed, one within 4 years (30 m.) and three more than 4 years after transplantation (62, 68, and 120 m). The relapse risk (RR) was 23% at 10 years. Three of 12 patients receiving a T-cell depleted graft relapsed vs. one of 20 patients receiving an unmanipulated graft. In other reported series, NRM ranged from 15% to 40%. RR was 20% - 68% at 2 to 10 years. In only two series median follow-up was 6 years or longer. Altogether, seven late relapses could be identified. With the limitations of a literature review, T-cell depletion appears to be the strongest factor associated to clinical relapse independently of when it occurs. Also the detection of MRD and particularly its increase over time herald such an event.

Conclusions: In patients with CLL submitted to allograft, late relapses (> 4 years after transplant) could be more frequent than what is usually recognized. This calls for a careful and continued follow-up of these patients.

385 OTHER MALIGNANCIES IN CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA (CLL/SLL): ANALYSIS OF 2028 PATIENTS

A.M. Tsimberidou¹, S. Wen², S. O'Brien¹, P. McLaughlin³, W.G. Wierda¹, H. Kantarjian¹, S. Lerner¹, S. Strom⁴, E.J. Freireich¹, L. Medeiros⁵, M.J. Keating¹
¹Leukemia, MDACC, Houston, United States, ²Biostatistics, MDACC, Houston, United States, ³Lymphoma, MDACC, Houston, United States, ⁴Epidemiology, MDACC, Houston, United States, ⁵Pathology, MDACC, Houston, United States

Introduction: Other malignancies reportedly occur with increased frequency in patients (pts.) with CLL.

Patients and Methods: We reviewed the records of pts. Diagnosed with CLL at MD Anderson from 1985 to 2005 to determine the frequency, clinical outcomes and factors predicting development of other cancers. The number of observed cancers was compared with the expected number based on the Surveillance Epidemiology and End Results data.

Results: Among 2,028 consecutive pts, 324 (16%) had a history of other cancers before CLL diagnosis and 227 (11.2%) developed second cancers after CLL diagnosis during the follow-up period (median 2.9 yrs, range 0-17). Overall, 625 cancers were observed

in the 551 pts, including skin (30%), prostate (13%), and breast cancers (9%), melanoma (8%), lymphoma (8%), gastrointestinal (9%), lung (6%) and others (17%). Overall, 88.4% pts had 1 other malignancy, 10.9% 2, .5% 3, and .02% had 4 other cancers. The risk of having a second cancer for pts with CLL was 2.2 times higher than the expected risk. The overall response rates in pts with and without history of other cancers were 86% (109/127) and 92% (736/804), respectively (p=.04); and the 5-yr survival rates were 70% and 82%, respectively (p<.001). In Cox analysis, independent factors predicting development of other cancers were older age [relative risk (RR)=2.1, p<.001], male sex (RR=1.6, p=.002), higher (> 3 mg/L) β_2 -microglobulin (RR=1.5, p=.01), LDH > 618 IU/L (RR=1.5, p=.02), and creatinine > 1.6 mg/dL (RR=1.9, p=.07). In treated pts, the number of new cancers was proportionate to the duration of follow-up, but treatment regimen did not affect the risk of developing other cancers (p=.49). In pts with Rai II, III, or IV CLL, there was no difference in development of second cancers between those who required CLL treatment and those who did not require treatment (p=.47, p=.21, and p=.15, respectively). Among pts with Rai 0-I disease, those who subsequently required CLL treatment had a higher risk of developing another cancer (from 1st visit at MD Anderson) compared with those who did not require treatment (p=.004).

Conclusion: Other malignancies were noted in 27.2% of pts. with CLL. Independent factors predicting second cancers were identified.