ly available pre-clinical models of MW are limited to xenografting human strategies for improved clinical outcome. Considering that the present and epigenetic changes during oncogenesis and to test new intervention natural history of MW (BLyS, del 6q21-22, bone marrow environment).

Numerous biological factors known or suspected to be involved in the mine the tumor precursor (post-GC/memory B cell?) and the role of AID-deficient offspring developed IgM who achieved a PR (10) and a CR (1) (median 1,03E-01, range 4,06E-01-15 times lower (median 6,88E-03, range 1,4E-02 - 7E -05) than in the pts who achieved a MR (5) and SD (1) the levels of hCNT 1 were found to be 9 times lower (median 6,88E-03, range 1,4E-02 - 7E -05) than in the pts who achieved a PR (5) and SD (1) (median 1,03E-01, range 4,06E-01-12. Engraftment was assessed by multiparameter flow cytometry. The remaining factors were considered to be of little influence on the value of the expression (median 4,24E-03, range 1,68E-02- 000; p = 0,045), failed the treat- ment: 3 of them for 2-CDA-related toxicity and one for FD. No correla- tion was found for the other genes. Conclusions. hCTN1 seems to be a gene involved in 2-CDA activity, and its expression seems to correlate with clinical response. The lower hCTN1 expression detected in pts who didn’t achieve CR or FR suggests a possible relationship between reduced hCTN1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantita- tive method in order to identify a threshold value which could be pre- dictive of drug resistance.

PO-1205

TRANSGENIC MOUSE MODELS OF MACROGLOBULINEMIA WALDENSTROM

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Aims. Accurate mouse models of human plasma cell neoplasms including macroglobulinemia Waldenstrom (MW) are needed to study genetic and epigenetic changes during oncogenesis and to test new intervention strategies for improved clinical outcome. Considering that the presently available pre-clinical models of MW are limited to xenografting human MW cells into SCID mice or human fetal bone engrafted in SCID mice, we report here to develop new mouse strains that are prone to develop MG by arising IgM-class plasma cell neoplasms that reside in the bone marrow.

Materials and Methods. Plasma cell neoplasms in mice arise in mice that carry a widely expressed human IL-6 transgene (H2-Ld-IL-6 developed by T. Kishimoto, Osaka University); a Bcl2 transgene (Eßv-Bcl-2-22 developed by A. Harris and J. Adams, WEHI, Melbourne); a His6-tagged induced cytidine deaminase; T. Honjo, Kyoto University). Unlike their because they carry two null alleles of the gene encoding AID (activation dependent epitope on KMA has been developed in our laboratory and murine monoclonal antibody (mKap) that recognizes a conformation- mediated binding site of the WM KMA which allows the detection of WM KMA in patient serum and its tissue expression.

In this study we assessed bone marrow isolates from Waldenstrom's macroglobulinemia (WM) patients for the cell surface expression of KMA and Bcma positive cells. We found sKMA on malignant plasma cells isolated from multiple myeloma and Bcma positive cells. We concluded that the proportion of sKMA on malignant plasma cells is different both between plasma cell neoplasms. We observed the expression of human CD20 and IgM. Tumor progression was determined from non WM individuals. Mice were followed for up to 6 months. Tumor progression was defined by monitoring the hCNT1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantitative method in order to identify a threshold value which could be predictive of drug resistance.

PO-1207

EXPRESSION OF THE KAPPA MYELOMA ANTIGEN ON THE CELL SURFACE OF BONE MARROW ASPIRATES FROM WALDENSTROM'S MACROGLOBULINEMIA PATIENTS

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In this study we assessed bone marrow isolates from Waldenstrom's macroglobulinemia (WM) patients for the cell surface expression of KMA and Bcma positive cells. We found sKMA on malignant plasma cells isolated from multiple myeloma and Bcma positive cells. We concluded that the proportion of sKMA on malignant plasma cells is different both between plasma cell neoplasms. We observed the expression of human CD20 and IgM. Tumor progression was determined from non WM individuals. Mice were followed for up to 6 months. Tumor progression was defined by monitoring the hCNT1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantitative method in order to identify a threshold value which could be predictive of drug resistance.

PO-1206

AN ANIMAL MODEL FOR WALDENSTROM'S MACROGLOBULINEMIA


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Introduction. Waldenstrom's macroglobulinemia (WM) is a B-cell lymphoproliferative disorder characterized by predilection for bone marrow (BM) involvement and secretion of IgM paraprotein. The purpose of this study is to establish an animal model mimicking closely the dis- ease. Materials and Methods. Compact cores of human cancellous bone samples also contained a subpopulation of sKMA cells in patient serum and its tissue expression. We conclude that the proportion of sKMA on malignant plasma cells is different both between plasma cell neoplasms. We observed the expression of human CD20 and IgM. Tumor progression was determined from non WM individuals. Mice were followed for up to 6 months. Tumor progression was defined by monitoring the hCNT1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantitative method in order to identify a threshold value which could be predictive of drug resistance.

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(RR1, RR2) subunits, in the blood marrow of 21 WD pts before treatment. Pts were treated with 4 courses of 2-CDA (0.1 mg/kg sc for 5 days) in combi- nation with Rituximab at standard schedule. Relative quantitation was performed using the Delta CT calculation: the value of gene expression was normalised to the calibrator (healthy tissue cells). Results. Clinical responses were evaluated according to Response Criteria (S’ International- Workshop on WM) 2 months after the end of chemotherapy, in the pts who achieved a MR (5) and SD (1) the levels of hCTN1 were found to be 15 times lower (median 6,88E-05, range 1,4E-02 - 7E -05) than in the pts who achieved a PR (10) and a CR (1) (median 1,03E-01, range 4,06E-01-12. Engraftment was assessed by multiparameter flow cytometry. The remaining factors were considered to be of little influence on the value of the expression (median 4,24E-03, range 1,68E-02- 000; p = 0,045), failed the treat- ment: 3 of them for 2-CDA-related toxicity and one for FD. No correla- tion was found for the other genes. Conclusions. hCTN1 seems to be a gene involved in 2-CDA activity, and its expression seems to correlate with clinical response. The lower hCTN1 expression detected in pts who didn’t achieve CR or FR suggests a possible relationship between reduced hCTN1 levels and a diminished clinical activity of 2-CDA. Thus it might be important to explore the possibility of standardizing an absolute quantitative method in order to identify a threshold value which could be predictive of drug resistance.

PO-1205

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PO-1208
IMMUNOPHENOTYPIC AND MOLECULAR PROFILE OF WALDENSTROM'S MACROGLOBULINEMIA (WM) AND SMALL LYMPHOCYTIC LYMPHOMA (SLL) PTS: REPORT OF A MULTICENTER STUDY

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Background. WM represents a B-cell lymphoproliferative disorder primarily characterized by bone marrow infiltration by lymphoplasmacytic lymphoma. Immunophenotypic study is of great value in the differential diagnosis of this uncommon disease and molecular studies are going to better investigate this entity. The typical immunophenotype for lymphoplasmacytic cells should include the expression of strong surface Ig and cytoplasmic IgM and is CD19+, CD20+, CD22+, CD79α+, FMC7+, CD5-, CD10-, CD23-, CD44+. Molecular studies have shown that WM cells usually have somatic VH mutated genes. SLL is the tissue counterpart of CLL; infiltrating cell morphology and immunophenotype are the same of B-CLL. Aim and Methods. To evaluate the immunophenotypic (including ZAP70 and CD38) and molecular profile (IGH rearrangement) on patient samples and WM cell lines (BCWM.1 and WM-WSU) was performed. Migration towards serum of CXCR4 was performed.

The level of CXCR4 and adhesion molecules is regulated by cytokines and chemokines. We sought to investigate the role of chemokine receptors, and in specific the SDF-1/CXCR4 axis on migration in WM cells. Methods. Flow cytometry for CXC and CC chemokine receptors (CXCR1, CXCR2, CXCR3, CXCR4, CXCR5, CCR2, CCR4, CCR5, CCR6 and CCR7) on patient samples and WM cell lines (BCWM.1 and WM-WSU) was performed. Migration towards serial concentrations of SDF-1 was determined using the transwell migration system (Costar, NY). The CXCR4 inhibitor AMD3100 (10-100 mM, Sigma, MO) were used to inhibit CXCR4 signaling. We investigated the interaction of CXCR4 and VLA-4 receptors and demonstrated that CXCR4 and VLA-4 co-immunoprecipitated in response to SDF-1 stimulation indicating a direct interaction of these two receptors. Conclusion. These studies demonstrate that the CXCR4-SDF-1 axis promotes adhesion of WM tumor cells to the BM microenvironment through its interaction with the adhesion molecule VLA-4.

PO-1210
THE CXCR4/SDF-1 AXIS REGULATES MIGRATION IN WALDENSTROM'S MACROGLOBULINEMIA

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Background. Waldenstrom Macroglobulinemia (WM) is characterized by the widespread involvement of the bone marrow (BM) at diagnosis, implying a continuous (re) circulation of the WM cells in the peripheral blood and (re) entrance into the BM. The process of homing and migration is regulated by cytokines and chemokines. We sought to investigate the role of chemokine receptors, and in specific the SDF-1/CXCR4 axis on migration in WM cells. Results. Flow cytometry for CXCR4 was performed. The following chemokine receptors were expressed on patient CD19+ WM cells and WM cell lines: CXCR1 (mean 60%), CXCR2 (mean 47%), CXCR3 (mean 47%), CXCR5 (mean 69%), CXCR4 (mean 54%) and CCR6 (mean 61%). We next determined the effect of SDF-1 on migration and signaling pathways in WM. SDF-1 (10-100nM) induced migration in a bell-shaped curve with 30nM inducing maximum migration (110% compared to control). SDF-1 30nM induced a rapid activation of signaling pathways downstream of CXCR4 including pERK1/2, pAKT, and pFKC at 1 min, with maximum activation at 5 min. The CXCR4 inhibitor AMD3100 inhibited migration of BCWM.1 in the presence of 30nM SDF-1, with AMD3100 10nM inhibiting migration at 59% of control. Similar results were observed on patients’ CD19+ WM cells, with inhibition of migration of patients’ WM cells at 50% compared to control. Those results were confirmed using lentivirus knockdown of CXCR4 receptor and with the use of FTX, with 30-50% inhibition of migration of WM cells compared to control. AMD3100 10nM inhibiting migration was also effective in the cell line BCWM.1 and pFKC. Conclusion. CXCR4/SDF-1 axis regulates migration in WM indicating a potential role in homing.