(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 combination 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 (3rd International Workshops on WM) 2 months after the end of chemotherapy; in the pts who achieved a MR (5) and SD (1) the levels of hCNT1 were found to be 15 times lower (median 6,88E-03, 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-01-2,46E-02 p=0.014).The remaining four, showing very low values of expression (median 4,24E-03, range 1,68E-02- 00E0 p=0.045), failed the treatment: 3 of them for 2-CDA-related toxicity and one for PD. No correlation was found for the other genes. *Conclusions*. hCNT1 seems to be a gene involved in 2-CDA activity and its expression seems to correlate with clinical response. The lower hCNT1 expression detected in pts who didn't achieve CR or PR suggests a possible relationship between reduced 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-1205

TRANSGENIC MOUSE MODELS OF MACROGLOBULINEMIA WALDENSTROM

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Aims. Accurate mouse models of human plasma cell neoplasms including macroglobulinemia Waldenstom (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 sought to design new mouse strains that are prone to spontaneously arising IgM$^+$ plasma cell neoplasms that reside in the bone marrow. *Materials and Methods*. Plasma cell neoplasms 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 (EiSV-Bcl-2-22 developed by A. Harris and J. Adams, WEHI, Melbourne); a His6-tagged mouse Myc gene inserted in three different locations of the mouse Ig heavy-chain locus (iMyc transgenes); or one of the iMyc genes plus a second, cooperating transgene: IL-6, Bcl-2, or the Bcl-XL transgenes developed by B. Van Ness and T. Behrens, University of Minnesota, respectively. Plasma cell tumors of this sort undergo immunoglobulin isotype switching and express, therefore, IgG or IgA in the great majority of cases. We hypothesized the tumorigenesis might be arrested at the MW-typical IgM$^+$ stage if the tumors arose on a genetic background in which isotype switching has been abrogated. *Results*. We crossed IL-6, iMyc, and Bcl-XL transgenic mice with mice deficient in isotype switching because they carry two null alleles of the gene encoding AID (activation induced cytidine deaminase; T. Honjo, Kyoto University). Unlike their AID-proficient counterparts that developed IgG$^+$ or IgA$^+$ plasma cell tumors, the AID-deficient offspring developed IgM$^+$ tumors that typically exhibited lymphoplasmacytoid features with a variable potential to undergo terminal plasmacytic differentiation. Bone marrow infiltration by IgM$^+$ tumor cells, splenomegaly and, in advanced cases, leukemic dissemination of tumor cells were routinely observed. *Conclusions*. Our findings define the first step toward a new mouse model of human MW. Additional studies of the present mouse strains are warranted to determine the tumor precursor (post-GC/memory B cell?) and the role of numerous biological factors known or suspected to be involved in the natural history of MW (BLyS, del 6q21-22, bone marrow environment).

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 disease. *Materials and Methods.* Compact cores of human cancellous bone obtained from adults undergoing total hip arthroplasty were implanted in the hindlimb muscles of NOD-SCID mice and were allowed to mature for eight to twelve weeks. Freshly obtained WM cells were transplanted employing three methods: a) i.m. injection of Ficoll-separated BM aspirate from WM patients (3×10^6 cells) close to the bone implant; b) i.v. injection of similarly obtained cells into the tail vein (1×10^6cells) and c) implantation of freshly harvested un-manipulated BM core biopsy from WM patients to the contralateral hindlimb of animals carrying bone fragment from non WM individuals. Mice were followed for up to 6 months. Tumor progression was determined by monitoring human immunoglobulin M (IgM) levels in murine plasma and by histopathologic evaluation, including immunohistology for expression of human CD20 and IgM. *Results*. All animals regardless of their treatment (a: i.m. injections, n=10; b: i.v. injections, n=6; c: bone biopsy, n=10) showed elevated levels of human IgM one month after the introduction of WM cells. However, only a small minority (10%) of mice injected i.m. (group a) maintained elevated IgM beyond 5 months. In all i.v. injected mice IgM was gradually diminishing over time. On the other hand, over a half (60%) of mice implanted with the bone marrow core biopsies showed a steadily increasing level of IgM, indicative of the development of the disease. Positive cells for both CD20 and IgM were found in the BM core biopsies from the WM patients and the human bone graft opposite to the implanted site suggesting of metastasis. Murine tissue histopathologic evaluation is ongoing. *Conclusions*. The implantation of whole BM core biopsies from WM patients enables the creation of a successful WM model, as it preserves the essential interaction of WM cells with their microenvironment. The colonization by WM cells of the uninvolved human bone graft may allow the study of factors related to its aggressiveness and adhesion to stroma.

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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 kappa myeloma antigen (KMA), which has previously been described in multiple myeloma. KMA is a membrane associated kappa light chain present on malignant plasma cells isolated from kappa type multiple myeloma patients and multiple myeloma (MMkappa) cell lines. A murine monoclonal antibody (mKap) that recognizes a conformation-dependent epitope on KMA has been developed in our laboratory and a chimeric version of the antibody was shown to mediate significant ADCC of MMkappa cells using human PBMCs as effectors.[1] A chimeric version of this antibody is due to enter a Phase I/II clinical trial for MMkappa patients in Australia in late 2007. Although the immunnophenotypic profile of the malignant clone in WM and MM is different both are lymphoproliferative disorders in which the neoplastic cells express monoclonal immunoglobulin light chain.[2] Demonstration of KMA on the surface of malignant cells from WM patients would provide a rationale for treatment with the chimeric antibody. Archived bone marrow aspirates from eleven confirmed WM patients were studied by flow cytometry for the presence of surface (s)KMA positive cells. Seven of the patient samples were WMkappa, and three of these contained a subpopulation of sIgM$^+$ sKMA$^+$ cells. The proportion of sIgM$^+$ cells that were sKMA$^+$ ranged from 10 to 15 percent. Interestingly, 2 out of the 3 patient samples also contained a subpopulation of sKMA$^+$ cells that were sIgM negative. None of the four WMlambda patient samples analysed contained sKMA$^+$ cells. These preliminary results demonstrate that KMA is expressed in some WMkappa patients. We therefore propose that the chimeric version of mKap should be considered for assessment as a potential therapeutic in WMkappa. Further characterisation of the phenotype of the WM KMA$^+$ subpopulation is ongoing in our laboratory. *Acknowledgements.* Biospecimens were provided by the Peter MacCallum Cancer Centre Tissue Bank, a member of the ABN-Oncology group,

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PO-1208

IMMUNOPENOTYPIC 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 cytoplasmatic IgM and is $CD19^+$, $CD20^+$, $CD22^+$, $CD79a^+$, $FMC7^+$, $CD5^-$, $CD10^-$, $CD23^-$, CD43±. 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 by PCR) on marrow and peripheral blood of 43 newly diagnosed/pretreated pts affected by WM (28) or SLL (15) requiring a treatment enrolled in a multicenter trial evaluating a combination therapy with Rituximab and 2CdA. *Results*. Considering WM pts, we found that only 50% of them presented a typical immunophenotype profile. On the contrary, in 82% of the pts with a diagnosis of SLL the immunophenotype was according the histopatologic diagnosis. Simultaneous expression of $CD5^+$, $CD23^+$, $CD43^+$ was found only in the 7% of WM pts versus 87% of SLL pts. Considering ZAP70, CD38 and IgH rearrangement, 61% of WM pts resulted ZAP70 positive with a concordance between $ZAP70^+$ and $CD38^+$ of 62% and 46% of pts showed a monoclonal IgH rearrangement suggestive for pre germinal centre status origin. On the contrary, 86% of evaluable SLL pts were positive for ZAP70 expression, with a concordance between $ZAP70^+$ and $CD38^+$ of 55% and 73% of them presented a IgH rearrangement. When both marrow and peripheral blood were evaluated for immunophenotypic study, the concordance between bone marrow and peripheral blood immunophenotype was 55% in WM versus 100% in SLL pts. *Conclusions*. In our experience, the typical immunophenotypic panel confirmed only half of histopatologic diagnosis of WM compared to 82% of SLL diagnosis. Molecular study of IgH rearrangement does not seem to confirm the exclusive post germinal status origin for WM cells.

PO-1209

CXCR4 AND VLA-4 INTERACTION PROMOTES ADHESION OF WALDENSTROM'S MACROGLOBULINEMIA CELLS

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Background. Waldenstrom Macroglobulinemia (WM) is characterized by widespread involvement of the bone marrow (BM) and lymphadenopathy in 20% of the patients. Adhesion of malignant cells to the bone marrow microenvironment induces proliferation and resistance to therapy. Chemokines and adhesion molecules regulate the interaction of WM cells with their microenvironment. We hypothesized that the SDF-1/CXCR4 axis plays an important role on the regulation of adhesion in WM. *Methods*. The level of CXCR4 and adhesion molecules (VLA-4 and LFA-1) was determined using flow cytometry and RT-PCR in patient samples and WM cell lines (BCWM.1 and WM-WSU). Adhesion was determined using an adhesion assay coated with the VLA-4 ligand fibronectin (EMD Biosciences, San Diego, CA) in the presence or absence of SDF-1 a 10-100 nM, R&D, MN). Co-immunoprecipitation was performed with CXCR4 and VLA-4 antibodies (BD pharmingen, San Diego, CA). The CXCR4 inhibitor AMD3100 (10-100 mM, Sigma, MO), the Gi protein inhibitor pertussis toxin PTX (10-200 ng/mL, Sigma, MO), and anti-VLA4 antibody (Calbiochem, CA) were used to inhibit adhesion in WM cells and downstream signaling pathways. These studies were confirmed using CXCR4 knockdown with lentivirus infection (RNA Consortium, MA). Immunoblotting for proteins downstream of CXCR4 was performed. *Results*. WM tumor cells from patients and cell line expressed high surface expression of CXCR4 (mean 70%) and VLA-4 (mean 95%). Adhesion of WM cells to fibronectin was significantly increased compared to BSA control, and SDF-1 induced a significant increase in adhesion of WM cells to fibronectin, up to 85% increase compared to control. AMD3100 20 mM inhibited adhesion by 50% compared to SDF-1 stimulated control. Similar results were obtained with PTX 200 ng/mL and anti-VLA-4 antibody (10 ng/mL). These results were confirmed using CXCR4 knockdown in WM cells. To identify the mechanism of regulation of adhesion by CXCR4, 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. *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 assay (Costar, NY). The CXCR4 inhibitor AMD3100 (10-100 mM, Sigma, MO), Gi protein inhibitor pertussis toxin PTX (10-200 ng/mL, Sigma, MO) were used to inhibit CXCR4 signaling. These studies were confirmed using CXCR4 knockdown with lentivirus infection (RNA Consortium, MA). Immunoblotting for proteins downstream of CXCR4 was performed. *Results*. The following chemokine receptors were expressed on patient $CD19^+$WM cells and WM cell lines: CXCR1 (mean 60%), CXCR2 (mean 47%), CXCR4 (mean 47%), CXCR5 (mean 69%), CCR4 (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 pPKC 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 10mM inhibiting migration at 59% of control. Similar results were observed on patients' $CD19^+$WM cells, with inhibition of migration of patients' cells at 50% compared to control. Those results were confirmed using lentivirus knockdown of CXCR4 receptor and with the use of PTX, with 30-50% inhibition of migration of WM cells compared to control. AMD3100-inhibition of migration was through inhibition of pERK1/2, pAKT and pPKC. *Conclusion*. CXCR4/SDF-1 regulates migration in WM indicating a potential role in homing.