lymphoma biology

273 EXONIC MUTATIONS ARE COMMONLY FOUND IN PATIENTS WITH WALDENSTRÖM’S MACROGLOBULINEMIA AND FAMILY MEMBERS OF WM PATIENTS WITH HYPER-IGM SYNDROME

S. Adamz1, L. Xu1, L. Ioakimidis1, Z. Hunter1, R. Manning1, B. Ciccarelli2, E. Hatjiharissi3, C. Patterson4, S. Treon5
1Bing Center for Waldenstrom’s macroglobulina, Dana Farber Cancer Institute, Boston, MA, United States, 3Malmo¨ University Hospital, Department of Medicine, Malmo¨, Sweden

Introduction: Waldenstrom’s macroglobulinemia (WM) is an indolent B-cell disorder characterized by accumulation of IgM in serum of patients. Familial disease is common with 29% of WM patients demonstrating a first degree relative with WM or a closely related B-cell disorder. Moreover, IgA and IgM hypogammaglobulinemia are commonly encountered, and persist despite therapy suggesting that common variable immunodeficiency disorder (CVID) may be a predisposition to WM. Consistent related B-cell disorder. Moreover, IgA and IgG hypogammaglobulinemia are well known as one of the characteristic features of the CVID as seen in our series. Given these findings, we sought to determine if factors with WM harbored CD40 mutations akin to those reported in patients with Hyper-IGM Syndrome.

Methods: We performed cloning and sequencing of CD40 gene from bone marrow (BM) CD19 cells from WM patients, family members of WM patients with polyclonal hyper-IgM, IgA and IgG hypogammaglobulinemia as well as healthy donors.

Results: In WM patients we detected at least one novel recurring mutation which predicted for amino acid changes. These mutations—3 missense (on exon 3), and one frame-shift deletion leading to protein truncation (on exon 5)—occurring in WM cells taken from 5/14 (36%) WM patients. Overall mutated allele frequencies for the 3 missense mutations were 14%(Phe>Ser) at position 3997 and 21%(Phe>Ser) at position 4036 of exon 5, and 7%(Try>Arg) at position 4946 of exon 5, while no mutations were detected in any of the healthy donors (HD). Importantly, we detected the C>T missense mutation at position 4036 of exon 3 in individuals with polyclonal hyper-IgM who belonged to a family with familial WM.

Conclusions: Mutations in Exon 3 and 5 of CD40 are commonly found in patients with WM, as well as family members of WM patients with polyclonal hyper-IgM, suggesting that Hyper-IGM Syndrome may be a predisposition for WM.

274 FAMILIAL CHARACTERISTICS OF WALDENSTRÖM’S MACROGLOBULINEMIA (WM) AND LYMPHOPLASMACYTIC LYMPHOMA (LPL): A POPULATION-BASED STUDY IN SWEDEN

S. Y. Kristensson1, M. Björlin2, L. P. Goldin3, L. M. McMaster4, L. Turesson3
1Karolinska University Hospital, Hematology Centre, Stockholm, Sweden, 2NH, National Cancer Institute, Bethesda, United States, 3Malmo¨ University Hospital, Department of Medicine, Malmo¨, Sweden

Background: The causes of WM/LPL are largely unknown. A role for genetic factors in the etiology of WM and LPL has been suggested based on prior single center observations indicating evidence of familial aggregation. The aims of this large population-based familial case-control study were to quantify risks of WM, LPL, monoclonal gammopathy of undetermined significance (MGUS) and related lymphoproliferative malignancies (LP) among first-degree relatives of WM/LPL patients and to define characteristics of familial aggregation.

Methods: We identified 2,671 WM/LPL patients diagnosed in Swedish hospitals 1983-2005 (Cancer and local hospital-based registries), with linkable relatives; 8,279 frequency-matched controls (Population registry); and first-degree relatives of cases (n=6,172) and controls (n=24,612) (Multigenerational registry). Relatives of WM/LPL patients and controls were linked with the Cancer and local hospital-based registries to define occurrence of MGUS, WM/LPL and related LP tumors. Using a proportional survival model, we calculated relative risks (RR) and 95% confidence intervals (CI) as measures of familial aggregation.

Results: First-degree relatives of WM/LPL patients had a significantly increased risk for developing WM/LPL (RR=20.0; 95% CI, 4.1-98.4), chronic lymphocytic leukemia (34; [1.7-66.0]), non-Hodgkin lymphoma (3.0; [2.0-4.4]), and MGUS (5.0; [1.5-18.9]). We observed no increased risk for developing multiple myeloma or Hodgkin lymphoma. The risk estimates were virtually the same when we conducted analyses by gender of proband, by type of first-degree relative (parent, sibling, offspring), and by age at WM/LPL diagnosis (below/above 70 yrs) for probands.

Conclusions: In this first large population-based study we found over 3-fold increased risks of developing WM/LPL and associated LP disorders (CML, NHL, and MGUS) among relatives of WM/LPL patients. These novel results support the hypothesis that there are common, strong, shared susceptibility genes that predispose to WM/LPL and related LP malignancies.

275 DETECTION OF EPSTEIN-BARR VIRUS INTEGRATION LOCI IN BURKITT’S LYMPHOMA CELL LINES

E. Murga Perus1, J. Hirkki1, G. Schilling1, P. Behrmann1, M. Kückow1, N. Albert1, C. Birner1, J. Diefler1
1Center of Oncology, Department of Medicine II, University Hospital Hamburg-Eppendorf, Hamburg, Germany

Introduction: Epstein-Barr virus (EBV) is an oncogenic virus found in about 95% of the endemic Burkitt’s lymphomas (BL). In latently infected cells, the EBV DNA can be maintained in episomal form, but integrated EBV could also be present. We herein report the identification of the EBV integration loci in the BL cell lines Seraphina and Nakala using long distance-PCR (LD-PCR) and fluorescence in situ hybridization (FISH).

Material and Methods: For the synthesis of an EBV-DNA probe for FISH experiments, DNA of the EBV-positive BL cell line Raji was isolated and LD-PCR with specific primers for EBV was performed. The 3,000 bp fragment was further labelled with Biotin-16-DUTG by nick translation, and FISH was performed according to standard methods. FISH on metaphase spreads of the BL cell line Namalwa was performed to validate the quality of the EBV-FISH probe. Integration of EBV was defined by the presence of symmetrical doublet hybridization signals at the same chromosomal loci in both sister chromatids.

Results: The cell line Namalwa showed symmetrical doublet hybridization signals of our EBV-FISH probe on chromosome 1p35, as it has been shown in previous reports. In the cell line Seraphina, 11 out of 16 analyzed metaphases revealed a unique integration locus of EBV on chromosome 2p23. FISH experiments on 8 metaphase spreads of the cell line Nakala revealed two integration loci of EBV. In the latter cell line, double hybridizations signals of the EBV-DNA probe were detected on chromosomes 3q11 and 17q25.

Conclusions: Our results identify for the first time chromosome 2p23, 3q11, and 17q25 as the integration locus of EBV in the BL cell lines Nakala and Seraphina. Whether the integration of EBV in these loci affects the expression of genes important for the pathogenesis of Burkitt’s lymphomas remains to be determined.

This work was supported by the Wilhelm Sander-Stiftung.

276 HUMAN HERPESVIRUS 8 K1-DERIVED PEPTIDES DISRUPT THE INHIBITORY FAS-K1 COMPLEX AND RESTORE FAS RECEPTOR-MEDIATED APOPTOSIS

H. Maeng1, Z. Berkova1, S. Wang1, S. Wang1, P. Samaria1
1Lymphoma Myeloma, UT MD Anderson Cancer Center, Houston, United States

Background: Human herpesvirus 8 (HHV-8) infection is associated with the development of primary effusion lymphoma, Kaposi’s sarcoma, and multicentric Castleman’s disease. The K1 gene of HHV-8 is expressed in tumor cells as a transmembrane protein with an immunoglobulin-like domain in its ectodomain and an immunoreceptor tyrosine-based activation motif (ITAM). We demonstrated that K1 protein activates nuclear factor-kappa B (NF-kB), and K1 expression in transgenic mice showed accumulation of lymphatic cells and development of lymphoma. How K1 blocks apoptosis and induces hyperplasia and lymphomas is not known. We hypothesized that K1 contributes to lymphoma development partly by suppressing apoptosis, and that this suppression combined with its NF-kB activation produces lymphoma.

Results: We found that K1 binds to Fas and in turn, inhibits Fas-mediated apoptosis. We mapped the region that K1 uses to bind to Fas as an immunoglobulin (Ig) chain-like domain by expressing deletion mutants of K1. Overexpression of an Ig domain-containing protein CD79b competed with K1-Fas binding in a dose-dependent manner. Two 20-amino acid peptides (N251, N253) representing the K1 Ig-like domain mediated this effect. The N251 peptide (K1dIg) did not form complexes with Fas, suggesting that the Ig domain is not required for the inhibitory effect of K1. Whether the integration of EBV in these loci affects the expression of genes important for the pathogenesis of Burkitt’s lymphomas remains to be determined.

Conclusions: Collectively, these results indicate that K1 potently blocks apoptosis, and that this effect is mediated through the Ig-like domain of K1. Because viral proteins mimic cellular proteins, these results predict the presence of functional cellular homologs of K1 that have key roles in death receptor regulation.

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11q23.1 amplification was defined at genomic level with Affymetrix GeneChip Human Mapping 10K arrays, including over 10 possible known transcripts. FISH analysis revealed different patterns of 11q23.1 structural rearrangement. Tiling hybridization is a powerful initial approach to identify DNA copy number changes and to detect candidate genes. We have previously analysed a series of MM and DLBCL clinical cases and cell lines with the Affymetrix GeneChip Human Tiling 2.0R arrays, using an RNA pool derived to a hierarchy: Recirculating ISC recover rapidly to normal steady state levels, while B cells numbers, while B cells were still reduced by 100-fold. Only 4 to 6 months after therapy human naive and memory B cells were detected in peripheral blood again. Conclusions: Regeneration of human peripheral blood B lymphocytes thus may follow a hierarchy: Recirculating ISC recover rapidly to normal steady state levels, while B cells with naïve or memory phenotype require longer time periods. This hierarchy may represent two elements within the B cells system: a primary system sustaining constant antibody levels and a secondary system providing a diverse repertoire for adaptive immune responses.

278 MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF 11023.1 AMPLIFICATION IN MULTIPLE MYELOMA (MM) AND DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) F. Dartois, L. Kiewel, M. Titiello, B. Bernasconi, A. Rinaldi, E. Zucca, A. Neri, F. Bertoni 1ICSI, Bellinzona, Switzerland, 2IDSSA, Milano, Switzerland, 3Ospedale di Circolo, Università dell’Insubria, Varese, Italy, 4Fondazione Policlinico, Milano, Italy

Background: Genomic profiling by microarray-based comparative genomic hybridization is a powerful initial approach to identify DNA copy number changes and to detect candidate genes. We have previously analysed a series of MM and DLBCL clinical cases and cell lines with the Affymetrix GeneChip Human Mapping 10K arrays (Rinaldi et al, 2006; Lombardi et al, 2007). Here, we present genetic and functional analysis of a region at 11q23.1 identified as recurrent site of DNA amplification. The involved region overlaps with the breakpoint of a t(11;15)(q23;q12) chromosomal translocation that we previously cloned in a chronic lymphocytic leukemia patient (Auer et al, 2005).

Material and Methods: 11q23.1 amplification was identified at genomic level with Affymetrix GeneChip Human Mapping 250K arrays, by quantitative genomic PCR and by FISH using three BAC clones overlapping the amplicon on two DLBCL (Karpas 422 and U-2932) and one MM cell line (F2). Transcriptome mapping was performed with the Affymetrix GeneChip Human Tiling 20K arrays, using an RNA pool derived from normal lymph node, thymus and spleen, as reference sample. Validation of candidate genes expression was done by real-time PCR on lymphoma and MM cell lines and clinical samples.

Results: The minimally overlapping amplicon was 480 kb large, comprising the region from POU2AF1 to PPP2R1B, including over 10 known transcribed FISH analysis revealed different patterns of 11q23.1 structural rearrangement. Tiling expression array and real-time PCR identified three target genes: POU2AF1, PPP2R1B and RUNX2. Experiments aimed to silence the genes in DLBCL and MM cell lines are being performed using custom and validated Ambion siRNA molecules to evaluate the role of the genes in cell growth and proliferation.

Conclusions: Genomic profiling allowed the identification of a recurrent site of DNA amplification at 11q23.1. Detailed results will be presented on the molecular and functional characterization of the affected region.


1Hematology, Centre Henri Becquerel, INSERM U918, Rouen, France, 2Institut de recherche sur le cancer, INSERM U537, Université de Lille-IFR 114, Lille, France

Materials and Methods: A genomic multiplex PCR (Multiplex PCR of Short Fluorescent Fragments, QMPSF) was designed to analyse the 9p21 locus. This assay contains 10 primer pairs that cover a 2.8 Mb region and 5 relevant genes (miR-31/CDKN2A/CDKN2B/DMRTA1). 93 DLBCL patients were analysed. QMPSF was validated by comparison with CGH-array (n=60). Allelic status was correlated to the GCB/non-GCB phenotype and p16+/+ expression. The prognostic value was assessed in 35 patients uniformly treated by CHOP plus rituximab (R-CHOP).

Conclusion: A concordance between QMPSF and CGH was observed in all cases. Total or partial deletions of 9p21 locus were observed in 34 cases (36%). CDKN2A or CDKN2N homozygous deletions were detected in 16 cases and CDKN2A/CDKN2B were mostly contexted in combination with CDKN2N loss. Telomeric breakpoints are mostly located between miR-31 and CDKN2A exon 3 (47%). Centromeric breakpoints are mainly located between CDKN2A and DMRTA1 (47%). A hot spot was identified between CDKN2A exon 1a and alternative exonb. CDKN2A/CDKN2B deletions were predominantly observed in the non-GCB subtype (81%, p=0,008) and correlated to a lower p16+/+ expression. CDKN2A/CDKN2B deletions strongly correlated to a shorter EFS and OS in patients treated by R-CHOP (p <0.05, median follow-up 28 months).

Conclusion: Our 9p21 QMPSF assay provided new information regarding molecular anatomy of the deletions involving this locus in DLBCL. CDKN2A/CDKN2B losses detected in one third of patients and mainly in the non-GCB subtype, may contribute to the R-CHOP resistance.
Results: In hyperplastic lymph nodes, both BTLA and HVEM are expressed in T lymphocytes. Usually, T lymphocytes are found in the inner areas and in mantle zone and marginal zone B-lymphocytes. Within germinal centers (GC), B-lymphocytes are negative for both BTLA and HVEM, whereas T lymphocytes are BTLA positive and follicular dendritic cells positive for both markers (0/20). Neither BTLA nor HVEM was expressed in Chronic Lymphoid Leukemia/Small Lymphocytic Lymphoma (CLL/SLL, 15/15), and LEUKEMIA PATIENTS CARRYING BOTH BCL2 AND MYC 282 CLINICOPATHOLOGICAL FEATURES OF LYMPHOMA/LEUKEMIA DO NOT CARRY BOTH MARKERS (0/20).

Conclusion: The extinction of BTLA and HVEM expression in normal GC B cells suggests that downregulation of BTLA/HVEM pathway may be involved in the process of antigen-driven B-cell activation. The preferential expression of BTLA and HVEM in follicular lymphoma is correlated with the GC and the involvement of neoplastic lymphoid tissues.

Material and Methods: We developed monoclonal antibodies against BTLA and HVEM and studied their expression in frozen tissues from benign lymph node (n=13) and lymphomas (n=86) using immunohistochemistry.

Results: Nearly all peripheral T Cell Lymphomas were BTLA positive (7/7), whereas Marginal Zone Lymphomas (MZL, 8/8) were positive for BTLA. Neither BTLA nor HVEM was expressed in Chronic Lymphoid Leukemia/Small Lymphocytic Lymphoma (CLL/SLL, 15/15), and LEUKEMIA PATIENTS CARRYING BOTH BCL2 AND MYC 282 CLINICOPATHOLOGICAL FEATURES OF LYMPHOMA/LEUKEMIA (n=15). In Hodgkin disease, tumour cells were negative for both markers (0/20).

Conclusion: The extinction of BTLA and HVEM expression in normal GC B cells suggests that downregulation of BTLA/HVEM pathway may be involved in the process of antigen-driven B-cell activation. The preferential expression of BTLA and HVEM in follicular lymphoma is correlated with the GC and the involvement of neoplastic lymphoid tissues.
Diffuse large B-cell lymphoma (DLBCL) is the most common type of non-Hodgkin’s lymphoma. The highly variable outcome reflects a heterogeneous group of tumours, with different genetic abnormalities and response to therapy. A series of 74 primary nodal DLBCL from four different Swiss and Italian Institutions was investigated using a FISH approach to detect BCL2, BCL6, MYC, BCL10 and MALT1 rearrangements with the aim to validate a new panel of signal FISH probes. A gene specific abnormality was identified in 62% of our series and the most involved gene was BCL6 (44%). Twenty percent showed rearrangements of more than one gene. The results obtained demonstrated that FISH analysis on paraffin embedded sections using the DAKO set probes are useful for a routine definition of a gene rearrangements in DLBCL even if the sensitivity of FISH on histological section may be reduced due to a peculiar feature of the histological sample. Practical strategies for using FISH analysis to detect cytogenetic rearrangements in lymphoma histological sections were pointed out. This study demonstrated chromosome translocations affecting BCL2, BCL6, BCL10 and MYC in a multi-centric trials of paraffin embedded samples of DLBCL. The pattern of the observed translocations highlighted that not only DLBCL represent a heterogenous entity, but that even individual cases may contain different subgroups bearing different chromosomial translocations. When DLBCL were subdivided into GC and ABC groups, an inverse pattern of BCL2 and BCL6 rearrangements was observed. Of interest the presence of chromosome rearrangements was associated with a worse prognosis.

286 BLOCKADE OF PD-1/PD-LIGAND PATHWAY RESTORES FUNCTION OF INTRATUMORAL EFFECTOR T CELLS IN FOLLICULAR LYMPHOMA

S.S. Neeleman1, D. Nattamia1, M. Foglietta1, L.W. Kwak1

Lymphoma and Myeloma, The University of Texas M. D. Anderson Cancer Center, Houston, United States

Background: The inhibitory receptor programmed death 1 (PD1), a negative regulator of activated T cells was recently shown to be upregulated on the surface of HIV-specific CD4+ and CD8+ T cells in humans and was associated with reversible impairment of T-cell function. Here, we examined the role of PD-1 on the function of intratumoral T-cells in patients with follicular lymphoma.

Materials and Methods: PD-1 expression was determined on peripheral blood and intratumoral T-cells in patients with follicular lymphoma at the time of initial diagnosis by flow cytometry. To test the function, intratumoral T-cells were isolated and cultured in the absence or presence of PMA/ionomycin or autologous tumor cells with or without anti-human PD-1 blocking antibody or isotype control antibody (R&D Systems). Supernatants were harvested after 48 hours and Th1/Th2 cytokine (IL-2, IL-4, IL-5, IL-10, IL-13, GM-CSF, IFNg and TNFa) production was measured by multiplex cytokine assay.

Results: PD-1 expression was significantly upregulated on peripheral blood and intratumoral CD4+ and CD8+ T cells in patients with follicular lymphoma as compared with normal donor PBMC (mean 5% for CD4+ T cells and 7% for CD8+ T cells). Furthermore, PD-1 expression was significantly higher on intratumoral CD4+ T cells (mean 67% for CD4+ T cells and 47% for CD8+ T cells) compared with peripheral blood (mean 33% for CD4+ T cells and 22% for CD8+ T cells) T cells in follicular lymphoma. PD-1 expression was associated with impaired cytokine production and blocking of the PD-1/PD-ligand pathway with antibodies against PD-1 significantly enhanced intratumoral T-cell Th1 but not Th2 cytokine production in response to polyclonal or autologous tumor cell stimulation.

Conclusions: PD-1 is markedly upregulated on intratumoral and peripheral blood CD4+ and CD8+ T-cells of patients with follicular lymphoma, associated with impaired T-cell function, and blocking PD-1 restored the function of these T-cells. These data suggest that blockade of PD-1/PD-ligand pathway may be used in combination with other immunomodulatory strategies as vaccines and adoptive T-cell therapies to enhance their efficacy.

287 CD74 ASSOCIATES WITH FAS AND INHIBITS FAS-MEDIATED APOTOTIC SIGNALING

Z. Berkova1, H. Maeng1, S. Wang1, S. Wang1, J. Wise1, D. Hawke1, M. Campbell1, L. Fayad1, L. Kwak1, F. Samaniego1

Lymphoma/Myeloma, UT MD Anderson Cancer Center, Houston, United States

Introduction: Resistance to Fas-mediated apoptosis in hematopoietic cancers interferes with the efficacy of currently available chemotherapy. Our prior research on human herpesvirus 8 oncprotein K1 showed that K1 binds to Fas and interferes with activation of Fas-mediated apoptotic signaling (Wang, W., et al, Blood 2007; 109:5455-62). Herpesvirus proteins often mimic host proteins or their functions, we thus searched for endogenous host proteins associated with inactive Fas in order to identify potential regulators of Fas signaling. Potential inhibitors of Fas were identified by immunodepletion of cell extracts from B-cell lymphoma-derived BJAB cells with activating anti-Fas antibody and by subtractive coimmunoprecipitation of proteins associated with nonactivated Fas. CD74, the invariant light chain of MHC-II, was identified in complex with inactive Fas by liquid chromatography tandem mass spectroscopy. Interestingly, overexpression of CD74 was previously reported in hematopoietic cancers in 43 of 66 cases of pediatric non-Hodgkin’s lymphomas (Miles, R.R., et al, Jr. of Haematology 2007; 138:64-71), in 8 out of 14 multiple myeloma-derived cell lines and B-cell lymphoma cell lines Raji and Ramos (Burton, J.D., et al, Clin Cancer Res. 2004; 10: 6660-6611), in 11 of 16 cases of leukemia (Kaddu, S., et al, J Am Acad Dermatol. 1998; 40: 986-978), as well as in 9 out of 11 cases of non-small-cell lung carcinoma (Ioschim, H.L., Am J Surg Pathol. 1996; 20: 64-71).

CD74 expression in cancers was thus suspicious.

Methods/Results: Through overexpression of CD74 in Fas-positive HEK 293 cells and suppression of CD74 expression in BJAB cell using siRNA technology we have determined that cells overexpressing CD74 are more resistant to agonistic antibody CH-11-induced Fas-mediated apoptosis then their relative controls. We have also mapped the domain of CD74 required for association with Fas to a membrane-proximal region of CD74 by expressing deletion mutants. Transfection of mice with plasmid encoding full length CD74 protected mice from lethal challenge with agonistic anti-Fas antibody Jo2. All 5 of vector transfected mice died within 6 hours from challenge, while 4 out of 5 CD74 transfected mice survived the challenge (P<0.05).

Conclusions: Our results support the idea of an endogenous regulatory system of Fas-mediated apoptosis that utilizes transmembrane proteins interacting with Fas. We anticipate that the specific ligand(s) for CD74 in human hematopoietic cancers remains to be elucidated. This knowledge may have implications for both the use of Fas agonists and CD74 antagonists as therapeutic targets in multiple hematopoietic malignancies.

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289 CHANGES IN SEROLOGICAL BIOMARKERS (BM) OF CELL DEATH DURING CHEMOTHERAPY (CT) IN PATIENTS WITH LYMPHOMA

A. Greytak1, K. Linton2, T. Rider3, R. Coward4, J. Cummings5, T. Ward6, C. Hamer3, M. Ranson7, A. Hughes2, J. Radford2, C. Dave5

CSEP, University of Manchester, Manchester, United Kingdom

Introduction: Early identification of chemoresistant disease and a focus on toxicity is important in patients (pts) with lymphoma where there is the potential for long term survival. The Cell Death Detection ELISA kitTM (Roche) detects nucleosomal DNA (nDNA) shed from dying nucleated cells whilst the M65TM assay detects cytokeratin 18 (CK18) products specific to dying epithelial. The utility of these assays as biomarkers of early therapeutic response and toxicity in lymphoma is unknown.

Methods: Serum samples from 22 patients with lymphoma (7 Hodgkin (HL) treated with ABVD, 7 follicular (FL) treated with R-CHOP and 8 diffuse large B cell (DLBCL) treated with R-CHOP) were analysed for nDNA and CK18. Samples were taken throughout therapy (days 1, 3, 8, 15 cycle 1, days 1, 3, subsequent cycles). Assays were performed in accordance with manufacturers’ instructions. Control samples were taken from healthy volunteers. Toxicity was graded (CTCAE version 3.0). Statistical analysis was non-parametric with significance at P<0.05. The area under the curve for CK18 during cycle 1 was estimated using the trapezoid method.

Results: nDNA was significantly higher in patients than in healthy controls (median 1.34 vs 0.30 (p<0.0001). Baseline CK18 was not elevated (285 vs 258 U/L (NS)) in contrast to elevations reported in patients with epithelial tumours. There was no significant difference between nDNA in HL, FL or DLBCL, however nDNA was higher in patients with stage II and III disease than stage I (1.38 vs 0.85 P<0.005). nDNA levels fell after therapy (median day 8, 0.55 P<0.05) and remained low (median day 22, 0.42 P<0.05). Administration of chemotherapy provoked significant rises in CK18 by day 3 (median +3 U/L, p<0.05) which returned to baseline by day 8 (median -0.1 U/L NS). Persistent elevations in CK18 throughout cycle 1 were seen in patients experiencing the worst epithelial toxicity (Cumulative CTCAE score ≥3 median increase 1% vs ≥3 median increase 38% P<0.05).

Conclusion: This pilot study reveals the potential of these serological assays of cell death as response and toxicity biomarkers in lymphoma. The high baseline levels of nDNA pre-therapy which fall post therapy suggest nDNA as a biomarker of early tumour response. CK18 release peaking day 1 post therapy reflects epithelial damage. Persistent CK18 elevation in patients reporting more side-effects supports serum CK18 as a useful toxicity biomarker. Integration of these biomarkers into future therapeutic trials in lymphoma is warranted.

290 ARE SERUM ANGIOGENIC (VEGF AND BFGF) AND ANTIANGIOGENIC (ENDOSTATIN) FACTORS PREDICTIVE OF CLINICAL OUTCOME IN PATIENTS WITH LYMPHOMAS?

T. Grinyty1, J. Abou Yared2, S. Kusieryt1, S. Cesapa1, F. Griscelli4, C. Ferme2, A. Ghanouni1, P. Carder1, F. Pilzinger3

1Radiation Oncology, Institut Gustave Roussy, Villejuif, France, 2Department of Hematology, Institut Gustave Roussy, Villejuif, France, 3Biostatistics, Institut Gustave Roussy, Villejuif, France, 4Biology, Institut Gustave Roussy, Villejuif, France

Purpose: To evaluate the predictive value of angiogenic and antiangiogenic factors in the clinical outcome.

Volume 19 | Supplement 4 | June 2008
doi:10.1093/annonc/mdn242 | iv169
Patients and Methods: Frozen samples were obtained from patients treated between March 1987 and September 1988. Thereafter patients entered the study prospectively. Accrual ended at the end of August 2001. Serum bFGF, VEGF and endostatin concentrations were measured using ELISA assays before treatment.

Results: Thirty patients had Hodgkin lymphoma. Fifty four patients and eighty two patients had indolent and aggressive lymphoma respectively. Treatments of lymphoma patients mostly consisted of ACVBP or CHOP-like treatments. Treatments of Hodgkin lymphoma patients were EBVP and MOPP/ABV. The median follow-up is 7.4 years (range 0.1-15.9 years). The 5-year overall survival for patients with Hodgkin, aggressive non Hodgkin and indolent lymphoma were 100%, 60% and 72% respectively. In multivariate analyses, VEGF, endostatin, the ratio of VEGF/endostatin and performance status were highly significant in terms of progression - free survival and overall survival.

Conclusions: Serum angiogenic and antiangiogenic factors were found to be independent prognostic factors on overall survival and progression-free survival.

Background: The retrovirus, SL3-3 Murine Leukemia Virus (MLV), induces T-cell lymphomas when injected into newborn mice of the NMRI inbred strain making it at present, the most reliable model of MLV-induced T-cell lymphomagenesis. The oncogenic effects of MLV are caused by insertional mutagenesis of the host genome and often result in alteration of the product of genes involved in lymphoma development. Integration sites in the NMRI mouse genome thus provide an efficient screening method for identifying genes involved in the progress of murine and potentially also human lymphomas. The proto-oncogene, Gfi1, is a frequent target of retroviral integration and its disruption is associated with both murine and human cancers.

Materials and Methods: By high-throughput sequence identification of retroviral integration sites in MLV infected NMRI inbred mice, we have mapped 182 out of 1565 insertions to the genomic locus of Gfi-1, making it a hotspot for MLV integration. Forty five tumors harboring integrations in the gfi1 locus were screened for alternative transcripts by RT-PCR and sequencing. The impact of MLV integration on Gfi1 gene and protein expression was analyzed by qPCR, Northern and Western Blot.

Results: We have identified four tight SL3-3 integration clusters in the gfi1 locus. A new SL3-3-specific cluster of 30 integrations was located within a 1kb window in the 3'UTR of the Gfi1 gene. Three new Gfi1 transcripts were identified and gene expression was demonstrated to be increased up to 80-fold in tumors possessing SL3-3 integrations in the gfi1 locus. We have observed major variations in the Gfi1 protein expression and a potential new protein isoform.

Conclusion: We have identified a new proviral insertional cluster in the Gfi1 gene using the NMRI inbred mouse model and demonstrated that proviral insertions in the Gfi1 gene result in deregulation of both its gene and protein expression, establishing its oncogenic properties.