

# hematology meeting reports

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**3<sup>rd</sup> International Symposium on  
Childhood, Adolescent and Young  
Adult Non-Hodgkin's Lymphoma**

**Frankfurt, Germany, June 11-13, 2009**

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# HEMATOLOGY MEETING REPORTS

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2. Eliassen AH, Missmer SA, Tworoger SS, Spiegelman D, Barbieri RL, Dowsett M, et al. Endogenous steroid hormone concentrations and risk of breast cancer among premenopausal women. *J Natl Cancer Inst* 2006;98:1406-15.
3. Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. *J Natl Cancer Inst* 2006;98:1382-96.
4. Reed E. ERCC1 measurements in clinical oncology. *N Engl J Med* 2006; 355:1054-5.
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6. Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, eds. *Clinical oncology*. 2<sup>nd</sup> ed. Churchill Livingstone. 2000.
7. Pizzo PA, Poplack DG. *Principles and Practice of Pediatric Oncology*. 4th ed. Philadelphia, Lippincott Williams & Wilkins, 2001.
8. Coleman RE, Rubens RD. Bone metastases. In: Abeloff MD, Armitage JO, Lichter AS, Niederhuber JE, eds. *Clinical Oncology*. 2nd ed. Churchill Livingstone. 2000. pp 836-871.
9. Lung LKW, Hui AMY, Leung WK, Sung JY, Ng EKW. Gene expressions of human peritoneal mesothelial cells in

gastric cancer. Proceedings of the 97th AACR Annual Meeting, April 1-5, 2006, Washington, DC, USA, *Proc Amer Assoc Cancer Res* 2006;47: [Abstract #122].

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14. Morse SS. Factors in the emergence of infectious diseases. *Emerg Infect Dis* [serial online] 1995 Jan-Mar [cited 1996 Jun 5];1(1):[24 screens]. Available from URL: <http://www.cdc.gov/ncidod/EID/eid.htm>

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**3<sup>rd</sup> International Symposium on Childhood,  
Adolescent and Young Adult  
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## ORAL PRESENTATIONS

### SCIENTIFIC SESSION 1: EPIDEMIOLOGY AND ETIOLOGY

#### 001

##### HERITABLE T CELL MALIGNANCY MODELS FROM A ZEBRAFISH MUTAGENESIS SCREEN

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**Background.** T cell lymphoblastic lymphoma (T-LBL) and leukemia (T-ALL) are common childhood cancers. One key transformation mechanism in these diseases is known, as NOTCH1 activation occurs in about 50% of T-ALL cases. However, many other genetic lesions are unknown. To address this deficiency, we pioneered a T cell neoplasia screen using the vertebrate zebrafish (*Danio rerio*) as a model. Transgenic oncogenes can induce T-LBL/-ALL in *D. rerio*, but are limited in scope to the known pathways used to create them. Because *D. rerio* are ideal for 'forward genetic' approaches, they are potentially useful to discover new oncogenic mutations. Yet to date, they have not been used to search for novel genetic lesions in T cell neoplasia. We set out to use zebrafish to create completely new genetic models of T-LBL/-ALL. **Objectives:** To use random mutagenesis to create *D. rerio* lines with heritable T cell cancer predisposition, to characterize their cancers, and to employ these models to define the molecular pathogenesis of T cell transformation, disease progression, and relapse. **Design.** To visualize *D. rerio* T cells, we used a transgenic line where the *p56<sup>lck</sup>* promoter directs T cell-specific expression of green fluorescent protein (*lck::EGFP*). We then performed germline mutagenesis, screened progeny for GFP+ tumors, and isolated mutant lines with heritable T cell cancer predisposition. **Results.** In each line, disease incidence, infiltration patterns, histology, and morphology resembled human T-LBL/-ALL. Expression studies confirmed that neoplasms were T lineage, but suggested that *D. rerio* Notch1 did not underlie oncogenesis. Analyses of T cell receptor rearrangements from tumor cells verified their clonality. Neoplastic cells were transplantable, radiation-sensitive, relapse-prone, and contained leukemia-initiating cells, like their human correlates. Ongoing investigations are focused on cloning the underlying mutated genes and examining the genomic, epigenetic, and expression profiles of these neoplasms in comparisons between pre-malignant and cancerous T cells, pre- and post-transplant samples, and relapsed isolates. **Conclusion.** We have identified zebrafish mutants that recapitulate human T cell malignancies and show heritable transmission. These represent entirely new animal models of T-LBL/-ALL, and provide exciting experimental platforms for the study of this important class of human cancers.

#### 002

##### SERO-EPIDEMIOLOGICAL STUDIES FOR HERPES VIRUSES IN CHILDREN AND ADOLESCENTS WITH NON-HODGKIN'S LYMPHOMA IN CENTRAL EUROPE

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**Background.** The majority of endemic Burkitt's lymphoma (BL) is infected with the Epstein-Barr virus (EBV), whereas only a subset of sporadic BLs is EBV-positive. The exact role of EBV in the pathogenesis of endemic and sporadic BLs remains uncertain. **Objective.** The aim of our study was to analyze the seroprevalence for herpes viruses in pediatric patients with Non-Hodgkin's lymphoma (NHL) enrolled in NHL-BFM (Berlin-Frankfurt-Muenster) studies. **Study design.** We determined the prevalence of antibodies to EBV, human cytomegalovirus (CMV), varicella-zoster virus (VZV) as well as herpes simplex virus (HSV) at the time of diagnosis in pediatric NHL patients in a retrospective study. **Sero-epidemiological data** of 1131 out of 2612 (43.3%) patients were available from centres of the NHL-BFM study group between 1/1990 and 12/2007. Patients between 6 months and 18 years of age with BL, anaplastic large cell lymphoma (ALCL), diffuse large B-cell lymphoma (DLBCL), lymphoblastic precursor B-cell lymphoma (PreB) and T-cell lymphoblastic lymphoma (T-LBL) were included. **Results.** We found a significant difference in the EBV-seropositivity of patients, with 315 (68.5%) EBV-seropositives out of 460 patients with BL as compared to 98 (58.3%) EBV-seropositives out of 168 patients with T-LBL ( $p=0.018$ ;  $\chi^2=5.63$ ). Interestingly, analysis of different age groups (6 month to 5.9 years, 6 to 9.9 years, and 10 to 17.9 years) demonstrated, that antibodies against EBV were significantly more prevalent in patients with BL younger than 6 years of age (62/105 EBV-seropositives [59.1%]) as compared to patients with T-LBL (11/34 EBV-seropositives [32.4%]) ( $p=0.007$ ;  $\chi^2=7.34$ ) or with DLBCL (7/20 EBV-seropositives [35.0%]) ( $p=0.048$ ;  $\chi^2=3.93$ ). These differences were not seen in the older age groups (6 to 9.9 years or 10 to 17.9 years). The seroprevalence of antibodies against CMV and HSV revealed no significant differences for the entire study population or with regard to different age groups. The VZV antibody prevalence showed only differences for the entire population when patients with PreB were compared to ALCL, DLBCL or T-LBL. **Conclusion.** EBV might be more involved in the pathogenesis of sporadic BL than originally assumed.

003

### GLOBAL PROTEOMIC PROFILING OF ENDEMIC VERSUS SPORADIC EPSTEIN-BARR VIRUS (EBV) POSITIVE BURKITT'S LYMPHOMA

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**Background.** Overexpression of c-myc is the sine qua non of Burkitt's Lymphoma (BL). Endemic BL (eBL) is characteristically positive (100%) for EBV, compared to sporadic BL (sBL) (30%). eBL vs. sBL have significantly different breakpoint regions within c-myc (Shiramizu/Magrath *et al*, Blood 1991). Apoptotic pathway disruption is propelled by EBV and is critically important in c-myc deregulation and subsequent lymphomagenesis that occurs in EBV+ eBL vs. sBL (Ruf *et al*, J Vir, 2000). Global analysis of proteins expressed in EBV+ eBL vs. sBL may provide novel insights into biologic, molecular differences between the two subtypes of lymphoma. **Objectives.** To examine and compare the proteomic expression profile of EBV+ eBL vs. sBL. **Methods.** Global proteome profiling was compared between the EBV+ eBL cell line Raji and the EBV+ sBL cell line NC37 using the iTRAQ™ method of liquid chromatography tandem mass spectrometry (MS/MS) as we have previously described (Elenitoba-Johnson/Lim *et al*, XIV Euro Assoc of Hematopath, 2008). The MS/MS data was analyzed using SEQUEST to search the UniProt database, and analyzed by ProteinProphet and INTERACT. Proteins were analyzed using pathway analysis tools. **Results.** Over 400 proteins were identified as being differentially expressed by a ≥1.25 fold difference between the two cell lines. They included proteins that are implicated in the p53 apoptosis pathway (PCNA, MSH6, C1QBP, MAP4, and BAX), proteins linked to the caspase network of apoptosis (HCLS1, ACIN1, and AIFM1), proteins that regulate the tumor suppressor protein RB network (MCM7, PA2G4, and API5), proteins associated with general apoptotic pathways (HSP90 and PDCD4), proteins involved in B-cell differentiation and proliferation (TPD52 and IKBKG), and proteins involved in ubiquitination (UBE2J1, UBE2C, and UBE2S). Ten of these proteins are c-myc target genes. **Conclusion.** Although on a global level we observed fundamentally similar proteome expression profiles between EBV+ eBL vs. sBL, this study illustrates a distinct differential expression of specific proteins that are critical to various apoptotic pathways. This suggests that there are potentially different pathogenetic mechanisms driving c-myc deregulation and apoptotic resistance in eBL vs. sBL, and that EBV infection may be involved in these processes that drive lymphomagenesis.

004

### THE DLEU1 NETWORK PROTEIN TUBULIN $\beta$ 2C IS EXPRESSED IN A SUBSET OF PEDIATRIC BURKITT LYMPHOMAS

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**Background.** In an international study of pediatric Burkitt lymphoma (PBL), we identified a cytogenetic abnormality, del(13q), which was significantly associated with a 4.8 fold risk of relapse and almost a 20% decrease in event free survival (Poirel/Cairo *et al*. Leukemia, 2009). Cytogenetic analysis of patients treated on the FAB/LMB 96 international pediatric mature B-NHL trial (COG-5961) showed approximately 14% of PBL patients had a del(13q), while FISH analysis utilizing 13q14.3 and 13q34 loci specific FISH probes demonstrated loss of 13q14.3 in 14%. Deleted in leukemia 1 (DLEU1), located on 13q14.3, is one of the most commonly over-expressed c-MYC target genes that distinguishes BL from diffuse large B-cell lymphoma (DLBCL) (Staudt *et al*. NEJM, 2006). The association between loss of 13q14.3 and inferior overall survival suggests a tumor suppressor role for DLEU1. The DLEU1 network gene tubulin  $\beta$  2C (TUBB2C) was differentially expressed in BL vs. DLBCL cell lines by quantitative real time-polymerase chain reaction, and expression of TUBB2C showed a significant reduction following DLEU1 siRNA treatment (Day/Cairo *et al*. SIOP, 2008). **Objectives.** The objectives of this study were to develop new diagnostic methods for identifying the expression of the DLEU1 network protein TUBB2C in PBL. **Design/Methods.** We optimized immunohistochemistry (IHC) for TUBB2C on paraffin embedded tissues and then characterized the expression of TUBB2C on archived diagnostic biopsies of 30 PBL patients treated on COG-5961. In addition, we performed FISH for del(13q) on a subset of these cases to examine the correlation between del(13q) and TUBB2C expression. **Results.** In reactive tonsillar tissue, TUBB2C showed strong, cytoplasmic staining in germinal center B-cells and interfollicular immunoblasts. TUBB2C was positive by IHC in 28/30 cases (93%) of PBL. 11 of these cases were evaluated by FISH for del(13q); 6 were positive and 5 were negative. All 11 cases were positive for TUBB2C. **Conclusion.** TUBB2C is positive in the majority of PBL cases, including those with del(13q). This suggests that, although regulated by DLEU1, TUBB2C expression can be seen in PBL with deletion of the DLEU1 locus.

005

**SOCIOECONOMIC DISPARITIES IN SURVIVAL BY RACE/ETHNICITY FOR ADOLESCENTS AND YOUNG ADULTS WITH NON-HODGKIN LYMPHOMA**

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**Background & Objectives.** Shorter survival is associated with low socioeconomic status (SES) among elderly patients with non-Hodgkin lymphoma (NHL); however, it is unknown if the same relationship holds for younger patients. Further, it is difficult to determine whether variability in survival is primarily the result of SES or due to other variables such as tumor staging, insurance status, treatment differences. We explored the California Cancer Registry (CCR), a large, population-based database, to investigate this relationship in adolescent and young adult (AYA) NHL patients. **Methods.** A case-only survival analysis of first primary NHLs diagnosed in 15-39 year olds from 1996-2005 was conducted to examine demographic and clinical variables hypothesized to be related to survival. The area SES variable used was derived in a previous study from census block group level data. Univariate analyses of overall survival (OS) and lymphoma-specific survival (LSS) were conducted using the Kaplan-Meier method. Multivariate survival analyses using Cox proportional hazards methods were also performed to calculate adjusted hazard ratios. **Results.** The analysis included 3,489 incident cases of first primary NHL in those 15-39 at diagnosis (1,930 non-Hispanic Whites (NHW), 281 African Americans (AA), 1,131 Hispanics (H), 380 Asian Americans/Pacific Islanders (API)). In the multivariate analysis, OS was reduced in those with later stage diagnosis ( $p < 0.05$ ) those with extranodal disease ( $p < 0.01$ ), those who did not receive chemotherapy as a first course of treatment ( $p < 0.05$ ), and in each older year of age at diagnosis ( $p < 0.001$ ). There was also a significant gradient in survival, with higher all-cause mortality at each decreasing quintile of SES ( $p < 0.001$ ), but no significant difference in survival time by insurance status ( $p = 0.47$ ). In stratified analyses, only NHWs had a significant SES-OS trend ( $p < 0.001$ ). Results were similar for LSS, except that patients not receiving chemotherapy as a first course of treatment experienced significantly longer LSS ( $p < 0.001$ ). **Conclusions.** Reduced overall and lymphoma-specific survival was associated with lower SES in AYAs with NHL, after controlling for stage and treatment factors, although a significant trend was observed only for NHWs. These findings warrant further investigation and intervention for possible vulnerable patient groups.

006

**CLINICAL CHARACTERISTICS OF CHILDREN WITH SYSTEMIC ANAPLASTIC LARGE CELL LYMPHOMA ENROLLED IN THE EUROPEAN INTERGROUP FOR CHILDHOOD NON-HODGKIN LYMPHOMA TRIAL ALCL99**

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**Background.** Despite recent progress in the field of anaplastic large cell lymphoma (ALCL), our clinical knowledge is still based on limited patient series. The international ALCL99 study included a randomized trial aiming to assess the impact of the addition of vinblastine maintenance to NHL-BFM90 therapy in high risk patients (pts). Pts were classified as high-risk or standard-risk if they had or not involvement of mediastinum, lung, liver, spleen and/or biopsy proven skin lesions. **Objective.** To define the clinical features of systemic ALCL of childhood in a large pts population. **Methods.** Analysis included all pts registered in the ALCL99 trial from 11/1999 to 06/2006 with systemic ALCL (N=463). Pts with isolated skin lesions (N=30), diagnosis rejected after histological review (N=33) and ALK negative disease without histological review (N=3) were excluded. Data were collected prospectively and centrally checked for consistency. A centralized pathology review was done in 442/463 pts, including 371 cases reviewed by an international panel. **Results.** Lymph-node involvement was documented in 399 pts (86%), mediastinal mass in 212 (46%). 324 pts had extra-nodal disease (70%) involving lung in 96, liver in 72, spleen in 82, biopsy proven skin lesions in 87, soft tissue in 72, bone in 77/393, bone marrow in 51 and CNS in 12. Distribution according to St. Jude staging system was: 36 st-1, 78 st-2, 290 st-3 and 58 st-4. 152 pts (44%) had impaired performance status

(ECOG  $\geq 2$ ) at diagnosis, 231 had fever and 104 weight loss. ALCL was a second malignancy in only 1 pt. Distribution according to WHO classification (available in 428 cases) was: 272 cases of common pattern (64%), 135 small-cell or lymphohistiocytic component, 21 others. 442/463 cases were ALK positive. None had B-cell phenotype. Complete remission was achieved in 414 patients (91%). With a median follow-up of 55 months (13-103) the 5-year overall survival was 89% (SE=1.5) and disease-free survival 71% (SE=2.2). *Conclusion.* This is the largest series of pediatric ALCL enrolled in a single clinical trial. We confirmed the peculiarity of ALCL within childhood NHL, including a high prevalence of extra-nodal localizations and of B-symptoms at diagnosis, the very low frequency of CNS disease and the favorable outcome with short pulse chemotherapy.

007

**COMPOUND HETEROZYGOUS GERMLINE SEQUENCE VARIANTS IN MLH1 IN A GIRL WITH EARLY ONSET AND SEQUENTIAL OCCURRENCE OF TWO DISTINCT LYMPHOMAS AND CLINICAL SIGNS OF KLIPPEL-TRENAUNAY SYNDROME**

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*Background.* Heterozygous germline mutations in the mismatch repair (MMR) genes MLH1, MSH2, MSH6 and PMS2 cause the autosomal dominant hereditary non-polyposis colorectal cancer syndrome (HNPCC) in adults. Patients with biallelic mutations in one of the MMR genes suffer from a rare distinctive syndrome, the childhood cancer syndrome (CCS), which is characterized by the development of miscellaneous childhood cancers, mainly hematological malignancies and brain tumors, and early onset of intestinal tumours. Additionally, most patients show signs of neurofibromatosis type 1. We describe a 13-year old child with the diagnosis of a mediastinal lymphoblastic T-Non-Hodgkin Lymphoma (NHL) at the age of 14 months and a centroblastic B-NHL of the nasopharynx at the age of 12 years. A large cutaneous hemangioma, varicosis and hemihypertrophy of the right lower leg, but no café au lait spots were noticeable by physical examination. The family history was positive for HNPCC with several affected members in the maternal branch. The mutation A21E in MLH1 was identified in the maternal aunt. *Objectives.* The sequential occurrence of two different NHLs in a 13 year old girl together with the family history led to the hypothesis of biallelic mutation in MLH1. *Methods.* Sequencing and *Multiplex Ligation-dependent Probe Amplification* was performed on genomic DNA in the patient and her parents. *Results.* Compound heterozygous MLH1 variants were found in the in our patient. The girl carried the maternal pathogenic mutation A21E in exon 1 as well as the unclassified variant V716M in exon 19 inherited from her father. MLPA gave no evidence of genomic rearrangements in *MLH1* and *MSH2*, sequencing of *MSH2* revealed no mutations in our patient. *Conclusion.* For the first time, we describe a girl with sequential occurrence of two distinct lymphomas and clinical signs of Klippel-Trenaunay syndrome. We hypothesize that the V716M is a hypomorphic variant which has a reduced penetrance and therefore only a weak effect on its own, but in this case aggravates the phenotype of A21E causing CCS. Due to the defective MMR system additional somatic changes in the hypothetical gene causing symptoms of Klippel-Trenaunay syndrome might be responsible for the hitherto unknown phenotype.

**SCIENTIFIC SESSION 2:  
DIAGNOSIS AND CLASSIFICATION OF NHL**

**DIAGNOSIS AND CLASSIFICATION OF NHL-STATE OF THE ART**

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The process of diagnosing Non-Hodgkin-Lymphoma (NHL) has changed over the last decades substantially. On the one hand side, the former exclusive morphological procedure of diagnosing NHL has been developed to an interdisciplinary process in which clinical, genetical, immunphenotypical and morphological information is integrated to come to a final diagnosis. On the other hand side, the number of diagnostic entities has increased and existing entities have been subdivided, making the diagnosis of NHL to a more and more challenging task. Moreover, development of new diagnostic technologies will change the work of scientists and physicians involved in diagnosis of NHL substantially in the future. Herein, I summarise the current status of NHL diagnosis with a special focus on paediatric NHL. The new WHO-classification will be presented, which – for the first time in the history of lymphoma classifications – emphasises the distinctiveness of pediatric lymphomas. A special focus will be put on the European approaches among paediatric lymphoma pathologists to standardise diagnostic criteria for paediatric NHL within the European Intergroup for Childhood Non-Hodgkin Lymphoma (EICNHL) international clinical trials. The impact of new technologies on the diagnostic process will be discussed in the light of the question: What is necessary for a state of the art diagnosis of paediatric NHL in daily practise?

**TYPE SPECIFIC GENETIC ALTERATIONS IN PEDIATRIC AND YOUNG ADULT NHL**

R. Siebert

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The WHO classification highlights the importance of recurrent genetic changes in the diagnosis of malignant lymphomas. In adult lymphomas, recurrent chromosomal aberrations are closely associated with defined subtypes of malignant lymphomas like the t(11;14)(q13;q32) present in almost all mantle cell lymphomas or the t(14;18)(q32;q21) present in approximately 85% of follicular lymphomas and 30% of diffuse large B-cell lymphomas (DLBCL), predominately of germinal center B-cell type. In contrast to adult lymphomas, the pattern of chromosomal aberrations in pediatric and young adult lymphomas is less well defined with the notable exception of Burkitt lymphoma (BL). The latter is characterised by the chromosomal translocation t(8;14)(q24;q32) and its variants, juxtaposing the MYC oncogene next to one of the immunoglobulin loci, i.e. IGH and 14q32, IGK in

2p12 and IGL in 22q11. In BL, these so called “Burkitt translocations” mostly form part of a rather simple karyotype in the absence of other oncogenic translocations (e.g. BCL2, BCL6). The usually few secondary aberrations show a quite typical pattern being gain in 1q most prominent. BL with a complex karyotype might be associated with clinically more aggressive course. The translocation t(14;18)(q32;q21) juxtaposing IGH and BCL2 is virtually absent below the age of 18 years, and, thus, pediatric FL and DLBCL lack this aberration in contrast to their adult counterparts. Whereas a rare subset of pediatric DLBCL expressing ALK is characterised by translocations affecting the ALK gene, primary mediastinal B-cell lymphomas (PMCBCL) in children seem to show a pattern of aberrations with gains in 2p and 9p resembling adult PMBCL. Though recurrent imbalances have been detected in DLBCL in children and young adults, the type specificity e.g. with regard to gene expression groups awaits further analyses. With regard to T-cell lymphomas, anaplastic large cell lymphoma (ALCL) in childhood and young adulthood is mostly ALK-positive due to translocations of the ALK gene to various partners. T-lymphoblastic lymphomas show recurrent translocations affecting the T-cell receptor (TCR) genes, aberrations of the NOTCH gene as well as deletions in the long arm of chromosome 6. In summary, with the exception of BL the patterns of genetic alterations in pediatric and young adult lymphomas are less well characterised than in adult lymphomas. To obtain a full genome-wide overview on chromosomal aberrations in pediatric lymphomas systematic karyotyping of banded chromosomes should be performed within clinical trials. Moreover, new genome wide methods in part applicable to archived material might widen the knowledge on the pathogenetic and clinical importance of genetic aberrations in pediatric lymphomas in the near future.

**008**

**DIFFERENT PATTERN OF CHROMOSOMAL ALTERATIONS ACCORDING TO THE MORPHOLOGY AND THE AGE: REPORT OF THE FRENCH LMB2001/03 STUDY**

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**Background.** We recently showed the prognostic impact of chromosomal alterations in childhood mature B-cell lymphomas enrolled in the international FAB/LMB96 trial [Poirel HA *et al*, Leukemia, 2009]. **Objectives.** The aim of our study is to confirm these results on a new series of patients treated with the next French LMB2001/03 trial. **Results.** A karyotype has been performed in 216/564 (38%) patients registered in the ongoing LMB2001/03 trial for children and adolescent mature B-cell lymphomas. It failed to detect any relevant clonal alteration in 51 cases. Karyotype was informative in 118 cases (21%), while in 47 cases, only the information on 8q24 status was available (cytogenetics or FISH). 84% were locally classified as Burkitt lymphoma (BL), 7% as DLBCL, the morphological diagnosis was not specified or unavailable for 9%. MYC/8q24 locus was translocated in 132/139 (95%) BL while in 2/10 DLBCL. It was associated with 1 or more alteration in 67% cases in BL: 1q gain (32%), der(13q) (16%), 7q+ (10%), del(17p) & del(6q) (7%). The more frequent alterations detected in complex karyotype with 8q24 rearrangement were: del(6q), der(7q), der(13q), der(11q), +12q & del(17p). Der(11q) & der(13q) were more frequent in diploid karyotypes while der(7q) & +12q in hyperdiploid karyotypes. The karyotype was more frequently aneuploid in DLBCL than in BL (86% vs. 28%). In the DLBCL without MYC rearrangement, 3 other chromosomes were altered in at least 3 cases: 1p, 4q & 15q. Chromosome alterations tended to vary according to the age. Lymphomas without MYC alteration were more frequently detected in older patients (20% >10 yr-old vs. 7% ≤10 yr-old). Complexity of the karyotype in BL increased with age: average number of chromosome alterations was 2.5 for children ≤10 yr-old vs. 3.5 for older patients. **Conclusion.** This descriptive analysis of childhood mature B-cell lymphoma confirms the different patterns of chromosomal alterations according to the morphology and suggests variations with age which may explain the worse prognosis of adolescents. Multivariate analysis is ongoing to evaluate the prognostic significance of these genetic biomarkers. Although its limitation (low resolution and need for cell division), cytogenetics remains useful for screening tumoral genomic alterations in complement to CGH-array which allows molecular characterization.

009

**A STUDY OF L-PLASTIN (LYMPHOCYTE CYTOSOLIC PROTEIN 1) EXPRESSION IN LYMPHOMA CELL LINES AND PRIMARY TUMORS IDENTIFIES ITS OVEREXPRESSION IN PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA (PMBL) AND SELECTIVE LOSS IN THE TUMOR CELLS OF CLASSICAL HODGKIN LYMPHOMA (CHL)**

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**Background.** PMBL is a rare subtype of extranodal diffuse large B-cell lymphoma (DLBCL) that can be difficult to distinguish from other types of DLBCL and cHL. We have compared the proteomes of PMBL (Karpas 1106P), cHL (L428) and DLBCL (SUDHL-9) cell lines using a differential isotopic strategy, iTRAQ and tandem mass spectrometry to identify the overexpression of L-plastin in PMBL vs. cHL and DLBCL. The expression of L-plastin, a 65kDa actin-bundling protein from the fimbrin family is normally restricted to the hematopoietic lineage, but it becomes overexpressed in a high proportion of epithelial and mesenchymal malignancies. The expression of L-plastin in lymphomas is largely uncharacterized. **Objectives.** In this study, we sought to evaluate L-plastin expression in several lymphoma cell lines (B and T) by Western blot (WB) and primary lymphoma tissue by immunohistochemistry (IHC) on tissue microarrays (TMAs). **Design/Methods.** L-plastin expression was validated by immunoblotting (clone LPL4A.1, NeoMarkers, Fremont, CA) on PMBL, cHL, DLBCL, and T-cell lymphoma (TCL) cell lines and IHC of tissue microarrays (TMAs) of PMBL, cHL and NLPHL. In addition, a TCL TMA and several follicular lymphoma (FL) tissue cores were evaluated. **Results.** By WB, high L-plastin expression was observed in the PMBL cell lines K1106P and MedB1, relative to cHL cell lines. All other cell lines (DLBCL, n=15), ALCL (n=4), T-ALL (n=4) and NK (n=1) showed uniform intermediate expression. By IHC using a three-tier scheme, 32/37 (86%) of PMBLs were strongly immunoreactive, 73% of cHLs were negative or weakly + ( $p < 0.0001$ ). 12/16 (75%) of NLPHL cases were also strongly positive. Of the TCLs, 6/9 (66%) of PTCLs and 7/7 (100%) of T-ALLs showed weak to no expression, while 2/2 AITLs were strongly +. FLs showed uniform intermediate staining. **Conclusion.** We identified L-plastin, a 65 kDa leukocyte-specific actin bundling protein to be highly expressed in PMBL and selectively lost in cHL and may be useful in the distinction of these two neoplasms. IHC studies on a larger array of B and T-cell lymphoma cases are underway to better define the pattern and significance of the selective regulation of this protein in lymphoma.

010

**EPIDEMIOLOGY OF IMMUNOPHENOTYPING AND SPECIFIC GENETIC ALTERATIONS IN YOUNG BRAZILIANS WITH T-CELL MALIGNANCIES**

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**Background.** SIL-TAL1 fusion gene, ectopic expression of HOX11L2 and NOTCH1 mutations are somatic abnormalities found in T-cell acute lymphoblastic

leukemia/lymphoma (T-ALL/LBL). Immunophenotyping and molecular subgroups profiling of T-ALL/LBL correlate with cell arrest at specific stages of thymocyte development and they are of interest for prognosis prediction. *Objectives.* To identify molecular abnormal pattern among immunophenotypic subgroups of T-ALL/LBL in Brazilian patients, and its correlation with clinical features and disease free survival. *Methods.* We analysed malignant cells, collected at diagnosis from 320 children, consecutively diagnosed and treated from January 2000 to November 2008. The immunophenotypes were defined according to standard criteria for leukemia and/or lymphoma clinical onset; RT-PCR technique was used to identify the presence of SIL-TAL1 and HOX11L2 in tumor specimens. Genomic DNA approaches were used for screening NOTCH1 mutations. HTLV-1/2 serological tests were performed by ELISA. Statistical analysis to test the association among immunophenotypes and molecular markers was calculated by mean of odds ratios followed by 95% confidence interval. Two-sided p values with a significance limit of 0.05 were used throughout the study. EFS and CCR rates were estimated by Kaplan and Meier's method. *Results.* There were 101 females and 219 males, age range 0-21 years old; during the period of the study, T-ALL/LBL accounted for 16.6% of incident malignant cases. There were 40 T-LBL and 240 T-ALL cases. Immunophenotyping analysis revealed that 30.7% of T-ALL cases were CD10+. The most frequent maturation stage was T-IV (40.1%). SIL-TAL1+ and HOX11L2+ accounted for 26.7% and 10.3% of T-ALL, respectively. NOTCH1 mutations were found in 67.2%, being the mutations found in HD only (16.2%), PEST only (8.8%) and HD+PEST sequences in 57.4% of studied cases. The combination of molecular aberrations were found with SIL-TAL1+ and NOTCH1 mutations in 28.7% of cases. The overall survival (OS) found was 74% in 80-month follow-up. Mean OS in patients younger than 9 years-old was 47 months, while in the older ones it was 52 months. The survival analysis reported here demonstrated that CD1a had no significant association with outcome. HOX11L2+ was not predictive factor for T-ALL outcome. Considering T-ALL patients <9 years-old, those with SIL-TAL1+ presented a poorer outcome than the SIL-TAL1- ones ( $p=0.02$ ). EFS (36 months) were 18.5 % in NOTCH1 wild-type whereas 62.8 % for NOTCH1 mutation group; better survival when these association were found in children in young adults aged >9 years of age (61.7%). *Conclusion.* The result of this study suggests that in the Brazilian population only the presence of SIL-TAL1 and NOTCH1 might predict outcome in a restricted group of young patients.

## 011

### PEDIATRIC NON-HODGKIN'S LYMPHOMA IN JAPAN: WHO CLASSIFICATION AND DIAGNOSTIC PROBLEMS

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*Objectives.* To investigate the morphologic and phenotypic characteristics of pediatric non-Hodgkin's Lymphoma (NHL) in Japan and to assess the diagnostic problems in WHO classification. *Methods.* total of 391 cases clinically-suspected of having NHL between 11/2004 and 02/2009 were reviewed by the central pathology panel. These cases were classified according to the 2001 WHO classification. Immunophenotyping included antibodies against CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD15, CD20, CD30, CD34, CD56, CD68, CD79a, ALK1, BCL2, BCL6, EMA, granzyme B, MPO, TdT, and TIA1. EBV-DNA and c-myc translocation were detected by EBER-ISH and FISH, respectively. *Results.* The diagnosis of NHL was confirmed in 333 cases (85%), 185 cases (56%) had a B-cell phenotype and 148 (44%) had a T-cell phenotype. These cases were classified as follows: 38 cases were precursor B lymphoblastic lymphoma (B-LBL), 55 were diffuse large B-cell lymphoma (DLB), 71 were Burkitt lymphoma (BL), 15 were mature B-cell lymphoma, high grade (DLB or BL), 6 were other B-cell lymphomas, 74 were T-LBL, 60 were anaplastic large cell lymphoma (ALK+ALCL), 8 were primary cutaneous CD30-positive T-cell lymphoproliferative disorders (CD30+ T-LPD), 4 were peripheral T-cell lymphoma, unspecified, 2 were other T-cell lymphomas and 16 were other hematopoietic disorders including 8 EBV-LPD and 5 immature non-T non-B lymphomas. C-myc translocation was detected in 44/55 (80%) of BL and 3/24 (12.5%) of DLB. Mature B-cell lymphoma, high grade (DLB or BL) were positive for CD10 and BCL6, negative for BCL-2 and had a high proportion of Ki-67-positive cells. The EBV-LPD cases showed a morphological spectrum of the reactive process phase leading to T-cell lymphoma. Immature non-T non-B lymphomas with a blastic morphology showed CD34 and CD7 immunoreactivity were not classifiable by WHO classification standard. *Conclusion.* In present study, there were less BL cases and more DLB and ALCL cases than were previously reported from Western countries. The EBV-LPD was also more frequently present in Japan. Since pediatric DLB is a predominantly germinal center immunophenotype, the same as BL, it is sometimes difficult to differentiate them by only morphology and immunophenotyping. C-myc translocation was less frequent in both BL and DLB than in previous reports. Immature non-T non-B lymphomas seemed to be unique in children.

## 012 EXPRESSION OF GROWTH FACTOR RECEPTOR-BOUND PROTEIN 2 (GRB2) IN T CELL LYMPHOMAS

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**Background.** Grb2 is a key adaptor molecule that mediates signaling between activated cell surface receptors such as the B-cell receptor or receptor tyrosine kinases to downstream targets. Grb2 signaling is critical for cell cycle progression and actin-based cell motility. In a previous study using mass spectrometry-driven proteomics we identified the overexpression of Grb2 in NPM/ALK transfected cells as compared to vector. Grb2 also functions in the ALK interactome as evidenced by its presence in the ALK immunocomplex. Evaluation of reactive lymph nodes tissue by immunohistochemistry (IHC) had revealed that Grb2 expression was restricted to B-cells and histiocytes and was not observed in T-cells. **Objectives.** In this study, we sought to determine the prevalence of Grb2 expression in T-cell lymphomas and to determine its correlation with ALK expression. **Design/Methods.** We performed Grb2 IHC on tissue microarrays and whole tissue sections from 180 diagnostic biopsies of cutaneous, extranodal and nodal T and NK/T-cell malignancies (WHO classification). Cases were considered positive when greater than 25% of the tumor cells were immunoreactive. Western blot analysis for Grb2 expression was performed on ten T-lymphoma cell lines. **Results.** Prevalence of Grb2 expression in T cell lymphoma subtypes:

**Table.**

	ALK+ ALCL	ALK- ALCL	cut.CD30+ LPD <sup>a</sup>	PTCL NOS <sup>b</sup>	AITL <sup>c</sup>	T-ALL	NKT	EATL <sup>d</sup>
Grb2+	12/23 (52%)	10/32 (31%)	0/22	2/23 (9%)	0/16	9/13 (69%)	3/51 (6%)	3/4 (75%)

<sup>a</sup>cutaneous CD30+ lymphoproliferative disorder; <sup>b</sup>peripheral T-cell lymphoma, not otherwise specified; <sup>c</sup>angioimmunoblastic T cell lymphoma; <sup>d</sup>enteropathy-associated T-cell lymphoma

in addition, hepatosplenic T-cell lymphoma and adult T-cell leukemia/lymphoma (one case each) strongly expressed Grb2. Western blotting showed uniform expression of Grb2 in eight cell lines derived from T-cell malignancies (ALK+ALCL, T-ALL, cutaneous TCL) while its expression is lower in MOLT-4 T-ALL and NK92 NK cell lines. **Conclusion.** Expression of Grb2 in T-cell neoplasms is largely restricted to T-ALL and nodal ALCL irrespective of ALK status. Its expression in other T cell malignancies is rare. The discrepancy between Grb2 expression observed in

tissues and cell lines in culture may reflect *in vitro* mechanisms of Grb2 upregulation. Our results warrant further investigation to evaluate the biologic significance of Grb2 in the pathophysiology of nodal ALCL and T-ALL.

## 013 STRONG BCL2 PROTEIN EXPRESSION IN PEDIATRIC BURKITT LYMPHOMA: IMPLICATIONS FOR WHO 2008 CLASSIFICATION

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**Background.** Burkitt lymphoma (BL) is an aggressive B-cell lymphoma that can be difficult to distinguish from diffuse large B-cell lymphoma (DLBCL). BL typically lacks BCL2 expression, but we have previously described rare cases of pediatric BL with weak (3/142, 2%) as well as strong (1/142, 1%) BCL2 protein expression. The 2008 WHO Classification introduced a new category for overlapping cases: B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and BL (Intermediate BL/DLBCL). Although weak BCL2 protein expression is allowed in BL, tumors with morphology and immunophenotype of BL but strong BCL2 expression should be considered intermediate BL/DLBCL. A subset of these cases have rearrangements of *C-MYC* and *BCL2* and are called "double hit" lymphomas, which are aggressive and respond poorly to treatment. **Objectives.** The objective of this study was to further characterize an unusual case of pediatric BL with strong BCL2 expression in light of the revised 2008 WHO classification. **Design/methods.** Formalin-fixed, paraffin-embedded archival tissue was available from the diagnostic biopsy of a patient with BL entered into the FAB-LMB96 trial of the treatment of pediatric B-cell non-Hodgkin lymphoma (Patte/Cairo *et al.* Blood, 2007). Immunohistochemistry (IHC) for BCL2, CD10, and CD20, and fluorescence *in situ* hybridization (FISH) for *MYC* rearrangement and *BCL2/IGH* (immunoglobulin heavy chain) translocation were performed on paraffin tissue sections by standard methods. **Results.** The patient was a 4-year-old female who presented with bilateral cystic and solid renal masses. The morphology was consistent with BL by international consensus review, and the tumor was positive for CD10, CD20, and strong BCL2 by IHC. FISH was positive for *MYC* rearrangement and negative for the *BCL2/IGH* translocation. The patient has no evidence of disease with four years of follow-up. **Conclusions.** This case is best classified under the WHO 2008 system as intermediate BL/DLBCL, but it did not demonstrate a *BCL2/IGH* translocation. These findings demonstrate a role for BCL2 IHC in the precise sub-classification aggressive B-cell lymphomas in pediatric patients. Further studies of intermediate BL/DLBCL will be required to determine the incidence and clinical significance of this subtype as well as of "double hit" lymphomas in pediatric patients.

014

**FLOW CYTOMETRIC IMMUNOPHENOTYPING FOR RAPID DIAGNOSIS OF PEDIATRIC NON-HODGKIN'S LYMPHOMAS**

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**Background.** Childhood Non-Hodgkin's Lymphomas (NHL) are mainly undifferentiated, clinically aggressive, high-grade malignancies. Therefore, rapid establishing of NHL diagnosis is of utmost importance. In Poland, average time to obtain histopathology and immunohistochemistry report ranges from a few hours to several days. **Objectives.** We aimed to assess the usefulness of multiparameter flow cytometry to speed up initial diagnostics of childhood NHL. **Patients and Methods.** The study group comprised 84 patients aged 3 to 20 years (mean 10.4 years), 60 boys and 24 girls, with the suspicion of malignant lymphoma, which was indication for lymph node biopsy or lymphoid tissue sampling. The children were treated in two pediatric hemato-oncology centers in Upper Silesia in Poland. All biopsy specimens or effusion fluids were analyzed with multicolor flow cytometry. **Results.** Flow cytometric immunophenotyping showed the presence of clonal lymphoid populations in 26 of 29 patients (90%) with final diagnosis of NHL. In three patients, flow cytometry did not reveal any clonal lymphoid population, while the histopathologic report described low frequencies of malignant cells with abundant infiltrate of normal lymphocytes and histiocytes. Flow cytometric evaluation in 21 patients with Hodgkin Lymphoma did not show any clonal lymphoid population. However, the significant percentage of T-cell infiltrate was comprised by CD4<sup>+</sup> T cells, so the mean CD4/CD8 ratio was significantly higher in Hodgkin Lymphoma when compared to reactive lymph nodes. In 31 patients, the final histopathologic diagnosis was reactive lymphadenopathy. There was no evidence of clonal lymphoid population in any of the patients. In three patients, flow cytometric analysis did not show any expression of tested surface leukocyte markers. Histopathologic investigation confirmed non-leukocytic origin of these malignancies. **Conclusion.** The introduction of flow cytometry has significantly speeded up establishing the diagnosis of childhood NHL and initiation of appropriate treatment. Importantly, multiparameter flow cytometric immunophenotyping is highly specific (no false-positive results). Nevertheless, histopathologic evaluation clearly remains the gold standard and ultimate criterion for diagnosis of NHL, because clonality testing with flow cytometry might cause false-negative results in a small subset of patients.

015

**FLOW CYTOMETRY AS A DIAGNOSTIC PROCEDURE IN NON-HODGKIN LYMPHOMA, CASE REPORT**

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**Background.** For diagnosing of non-Hodgkin lymphomas (NHL), except standar diagnostic procedures we can performe immunophenotyping of pathological cells from body fluids. **Case report.** We are presenting a four years old boy, who was admited in our hospital because of cough, dyspnea, oedema of upper eye lids, lost of appetite and fatigue. Phisical finding established that he was conscious, afebrile, with highly elevated respiratory rate and retraction of inetrcostal spaces. Auscolatory finding showed silent respiratory sound on the chest left side. Imiging procedures ( chest x ray, ECHO sound of the thorax and CT scan of the thorax ) confirmed pleural effusion on the left side. Than thoracocentesis was performed and control chert x ray showed large mediastinal tumor mass. He was transfered to the thoracal surgery department for biopsy of mediastinal tumor. Few days latter, the general condition and respiratory function were compromised due to upper vena cava syndrome and enlargement of mediastinal tumor mass (seen on the chest x ray). Due to increased risk of surgical intervention , we performed immunophenotyping of pathological cells from pleural effsion by flow-cytometry. Finally, we confirmed that pathological cells are intermediate-cortical T lymphoblasts, and established the diagnosis of non-Hodgkin T cell lymphoma. After that, we started therapy according AIEOP LNH 97- non B protocol for induction phase. Four days latter, we registrated remarkably improvement with normal chest x ray and regular respiratory function. **Conclusion.** Although of the patohistological diagnoses being essential for diagnosing NHL, in urgent situations when the surgical procedure is risky, due do tumor compression of the trachea, immunophenotyping of pathological cells from body fluids may help for establishing the diagnosis .

016

**VALUE AND LIMITATIONS OF FINE-NEEDLE ASPIRATION CYTOLOGY IN DIAGNOSIS AND CLASSIFICATION OF LYMPHOMAS**

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**Background.** The fine needle aspiration (FNA) cytologic diagnosis of non-Hodgkin's lymphoma (NHL) depends upon finding a relatively monotonous population of lymphoid cells in smears. Lymphomas have successfully been classified by FNA cytology following the prevalent histologic classifications. **Methods.** The success rate of FNA cytology ranges from 80%-90% in diagnosis of NHL and from 67.5%-86% in its sub typing. Yet, the role of cytology in primary diagnosis, sub classification and management of patients with lymphoma remains contro-

versial. The differential diagnostic problems for NHL include a group of small round cell tumors, nonlymphoid acute leukemia and HD. Reservations have been expressed regarding the efficacy of cytology in separating florid reactive hyperplasia from low-grade malignant lymphoma. *Results.* The reported cytodiagnostic accuracy for follicular lymphomas and nodular sclerosis type of HD is less compared to other subtypes of NHL and HD respectively since nodular pattern and sclerosis are strict histologic criteria which can not be appreciated in cytologic preparations. Entities like atypical lymphoproliferative disorders, peripheral T-cell lymphomas and Ki-1 positive anaplastic large cell lymphomas pose diagnostic challenges to cytologists. *Conclusion.* Despite these limitations, FNA cytology remains the first line of investigations used in cases of lymphadenopathy. Various special ancillary techniques are now being performed on lymph node aspirates to diagnose lymphoma versus other malignancies, and to decide the functional character of lymphomas and their clonal nature.

### SCIENTIFIC SESSION 3: STAGING AND RESPONSE EVALUATION

#### STAGING OF CHILDREN AND ADOLESCENTS WITH NON-HODGKIN LYMPHOMA REVISITED

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A staging classification is primarily a means to describe the anatomic extent of cancer and, although with significant differences, is adopted for all types of cancers, including non-Hodgkin lymphomas (NHL). The main objectives of a staging system are to help clinicians and researchers to plan treatment, assess prognosis, stratify patients for therapeutic decisions and evaluate treatment results. In this context staging should be regarded as a tool facilitating communication among physicians and investigators involved in the diagnosis and treatment of cancer patients. In addition to describing extent of disease, staging also provides a means to evaluate non-anatomic prognostic factors in specific disease stages. As medical and biological knowledge has made significant progress recently, cancer staging is increasingly integrated with additional information to identify subgroups of patients with homogeneous anatomic disease extent, but differing prognosis, that may warrant distinct treatments. In the field of pediatric NHL, the St. Jude or Murphy staging system has represented since 1980 an invaluable tool for pediatric oncologists, but since then significant changes in diagnosis, management and therapy of NHL have occurred. New biological information led to the identification of novel entities with peculiar histological/immunological profiles and clinical behavior. Modern imaging and recent biological assays can discriminate the presence of otherwise unidentifiable tumor localizations, including sub-microscopic disease. Consequently, the St. Jude staging classification has become less effective in orienting therapeutic decisions and in some cases "risk groups" were devised that combine stage and biological and/or clinical features to aid treatment decision. Based on this evidence, some efforts were made by a number of physicians involved in diagnosis and treatment of childhood NHL to critically revisit each of the St. Jude staging system definitions, to review its interpretation and implementation in the actual clinical activity and to propose adjustments that reflect the state-of-the-art medical and biological knowledge in the field, maintaining a continuity with the St. Jude staging system. As a staging system should hold applicability and comparability with time and through different socio-economic conditions and geopolitical borders, thus making it possible to compare large populations, we used caution in considering

changes that may prevent the functional use of a revised staging system world-wide. As a preliminary result of our work, it was recognized that the St Jude staging system is still a valid tool to describe localization and disease extent in pediatric NHL. Nevertheless, it is felt that specific sites of disease with recognized impact on prognosis, including central nervous system, skin, bone, and recently developed technology to assess and quantify minimal disseminated disease, should be considered in a modern staging system. The principles and preliminary conclusions for a revised pediatric NHL staging system will be presented and discussed.

#### **RESPONSE EVALUATION: PEDIATRIC NON-HODGKIN LYMPHOMA**

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An accurate and standardized response evaluation of tumor response in a child receiving therapy for non-Hodgkin lymphoma has important clinical implications. Specifically, the response evaluation has used in the following ways: 1. to determine effectiveness of therapy; 2. to modify treatment based on early and late response; and 3. making comparisons between differing treatment approaches. Historically, very general designations have been made, which include complete response (CR), partial response (PR) and no response (NR). Although it is generally understood what these designations indicate (CR, disappearance of all disease; PR, reduction in tumor size by 50%; NR, no change in tumor size), there have often been varying criteria used to make these designations. In some cases, degree of response has been determined by change in transverse diameter and in others by the sum of the products of the largest diameters (perpendicular). The results from nuclear imaging studies has also been incorporated by some investigators (gallium and bone scans have been used historically, and more recently replaced by PET imaging with an FDG tracer at some centers). A number of protocols have included additional response designations such as CRu (complete response unconfirmed) and minor response (MR). Obviously, there needs to be a clear, reproducible and mutually agreed upon system for response evaluation. Toward this end, the International Harmonization Project convened to address these issues and subsequently published their recommendations (Cheson, *et al.*, *J Clin Oncol* 25:579-86). These evaluation guidelines incorporated PET findings, IHC and flow cytometric data. These guidelines were not meant to be age specific; they can be applied to both children and adults. However, the differing spectrum of histologic subtypes occurring in children versus adults, and the recently developed technology to quantify minimal residual disease for the NHL subtypes frequently encountered in children (egs., lymphoblastic lymphoma, Burkitt lymphoma and anaplastic large cell lymphoma) must now be considered in contemporary response evaluation of pediatric non-Hodgkin lymphoma. These issues as well as other challenges in refining the response evaluation of children being treated for NHL will be discussed.

#### **017**

#### **MINIMAL DISSEMINATED DISEASE IN CHILDHOOD T-CELL LYMPHOBLASTIC LYMPHOMA: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP**

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**Background.** Disease dissemination to the bone marrow is detected at diagnosis in approximately 15% of children with T-cell lymphoblastic lymphoma (T-LL). It is unclear whether the remaining patients have submicroscopic systemic disease and, if so, what is the clinical significance of this finding. The characteristic immunophenotype of T-LL cells with coexpression of T-cell markers and TdT is not found among normal bone marrow and peripheral blood cells, and has been used to monitor minimal residual disease in patients with T-cell acute lymphoblastic leukemia. Therefore, it should be possible to identify circulating lymphoma cells by this phenotype. **Objectives.** To determine the extent of bone marrow involvement at diagnosis in patients with T-LL, and assess the feasibility of monitoring treatment response using peripheral blood. **Design/Methods.** Using a flow cytometric method that can detect 1 T-LL cell among 10,000 normal cells, we examined bone marrow and peripheral blood samples collected from 99 children with T-LL at diagnosis, as well as blood samples collected from 42 patients during treatment. **Results.** In 71 of the 99 (71.7%) marrow samples obtained at diagnosis, T-LL cells represented 0.01% to 31.6% (median, 0.22%) of mononuclear cells; 57 of the 71 T-LL-positive samples were from patients with stage II/III disease. Results of studies in bilateral marrow aspirates were highly concordant. Two-year event-free survival (EFS) was 68.1%±11.1% for patients with ≥1% T-LL cells in bone marrow versus 90.7%±4.4% for those with lower levels of marrow involvement ( $p=0.031$ ); EFS for patients with ≥5% lymphoblasts was 51.9%±18.0% ( $p=0.009$ ). T-LL cells were as prevalent in blood as in marrow; monitoring residual T-LL cells in blood during remission induction therapy identified patients with slower disease clearance. **Conclusions.** More than two-thirds of children with T-LL have disseminated disease at diagnosis, a proportion much higher than previously demonstrated. Measurements of disease dissemination at diagnosis might provide useful prognostic information, which can be further refined by monitoring response to therapy through blood testing.

**018  
PROGNOSTIC SIGNIFICANCE OF CIRCULATING  
TUMOR CELLS IN BONE MARROW OR  
PERIPHERAL BLOOD DETECTED BY QUALITATIVE  
PCR IN NPM-ALK-POSITIVE ANAPLASTIC  
LARGE CELL LYMPHOMA (ALCL):  
RESULTS OF THE ALCL99 STUDY**

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**Background.** The association of minimal bone marrow involvement detected by qualitative polymerase chain reaction (PCR) for NPM-ALK with a higher risk of failure has been shown in only two national series of patients (pts). **Objectives.** To study the incidence and the prognostic value of the presence of circulating tumor cells (CTC) in bone marrow (BM) or peripheral blood (PB) detected by qualitative PCR in systemic NPM-ALK-positive ALCL (sALK+ALCL). **Methods.** Among the 442 pts included in the ALCL99 study between 11/1999 and 06/2006 for a sALK+ALCL, 144 had BM samples and 91 had PB samples assessed by PCR at diagnosis, including 86 with BM and PB. Qualitative PCR for NPM-ALK was applied as previously described. Cox models were used to study the impact of PCR-BM+ on the risk of failure (progression or relapse) controlling for clinical characteristics and histological pattern. **Results.** CTC were detected by PCR in 74/144 BM samples (51% BM+) and 54/91 PB samples (59% PB+). Both results strongly correlated (Odds-Ratio=20,  $p<.0001$ ) but were not in complete agreement: identical in 70/86 cases (29 BM-PB- and 41 BM+PB+), different in 16 cases: 6 BM+PB- and 10 BM-PB+ ( $\kappa=0.62$ ). BM+ was associated with mediastinal ( $p=.008$ ) and visceral involvement ( $p=.007$ ). Among the 132/144 pts with histological pattern available, BM+ was found in 38/55 cases containing small cell or lymphohistiocytic components (SC-LH) vs. 33/77 other cases (69% vs. 43%,  $p=.003$ ). Median follow-up was 55 months. 3y DFS for BM/PCR+ pts was 57% compared to 88% for BM/PCR- pts whereas OS were very similar in both groups (92% vs. 93% at 3y). In multivariate analysis, SC-LH pattern and BM/PCR+ but not clinical characteristics were significantly associated with a high risk of failure (HR=2.5,  $p=.01$  and HR=2.5,  $p=.007$  respectively). Pts with SC-LH pattern and/or BM/PCR+ (88/132 pts) had a 3.4 fold increased risk of relapse compared to pts with no risk factor, resulting in 62% vs. 89% 3y DFS ( $p=.003$ ). Results did not differ significantly between the three participating laboratories. Results were quite similar considering PCR-PB results instead of PCR-BM results. **Conclusion.** BM/PCR+ was detected in 51% of the assessed pts and was strongly associated with the risk of failure.

**019  
MINIMAL DISSEMINATED DISEASE IDENTIFIES A  
POOR PROGNOSIS SUBGROUP AMONG HIGH  
RISK BURKITT'S LYMPHOMA PATIENTS**

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**Background.** The chromosomal translocation t(8;14) (q24;q32) represents a specific tumor marker in Burkitt's lymphoma (BL). This chromosomal aberration involves the MYC oncogene on chromosome 8 and the immunoglobulin heavy-chain (IgH) locus on chromosome 14. We have previously demonstrated that this genetic abnormality can be used as a marker of Minimal Disseminated Disease (MDD) in BL. **Objectives.** The aim of the study was to assess of the prevalence of MDD in BL at diagnosis in children enrolled in the AIEOP LNH-97 clinical protocol and the evaluation of its impact on prognosis. **Methods.** We established a simplified long-distance PCR (LD-PCR) assay which can amplify up to 15-20 Kb DNA sequence making it possible to detect the t(8;14) at the genomic level with the sensitivity of 10<sup>-4</sup>. The assay was based on 4 separate PCR reactions in which one primer complementary to the first exon of the MYC gene is used with one of four primers for the IgH locus (1 for the JH region and 1 for each of the 3 constant regions). **Results.** LD-PCR was applied to prospectively study 124 BL biopsies and detected a specific PCR product in 88 of them (71%). Of the 88 positive BL patients we studied both the tumor and the bone marrow (BM) at diagnosis in 76: BM was positive by LD-PCR in 25 patients (33%), whereas only 10 (13%) were positive at the standard morphological and/or immunophenotypical analyses. Most of the MDD positive patients (88%) belonged to the R4 Risk Group according to BFM definition (stage III or stage IV according to St. Jude staging classification and LDH>1000 U/L). The 3-year progression-free survival (PFS) was 68% (SE 10%) in MDD positive R4 patients compared with 96% (SE 4%) in MDD negative R4 patients ( $p=0.02$ ), whereas there was no difference in PFS between children with morphological involvement of BM at diagnosis versus those who had negative BM (PFS=62.5% (SE 17%) vs. PFS= 87% (SE 6%), respectively,  $p=0.09$ ). By multivariate analysis (including MDD, gender, LDH, CNS involvement) MDD was predictive of higher risk of failure (Hazard Ratio: 8.4,  $p=0.04$ ). **Conclusions.** We demonstrated that MDD has a negative prognostic impact in R4 BL patients and suggest that a more effective risk-adapted therapy, possibly including anti-CD20 monoclonal antibody, should be considered in this selected cohort of patients.

**019A**

**IgGENE REARRANGEMENTS AS PCR TARGETS FOR MRD ANALYSIS IN PEDIATRIC B-ALL AND BURKITT'S LYMPHOMA AND COMPARISON WITH T(8;14) DETECTION BY LD-PCR**

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**Background.** We recently reported that minimal residual disease (MRD), assessed by Long-Distance PCR (LD-PCR) for t(8;14), is a negative prognostic factor in B-cell acute lymphoblastic leukemia (B-ALL). However, t(8;14) is detectable in about 70% of B-ALL and Burkitt's lymphoma (BL) patients, thus preventing MRD studies by this method in the remaining cases. **Objectives.** The aim of our study was to evaluate the feasibility of the Ig-based RQ-PCR assay for MRD analysis in B-ALL and BL and to compare the sensitivity of this method with LD-PCR for t(8;14). **Methods.** We studied a total of 48 B-ALL and 45 BL patients, mostly positive for t(8;14) at different time-points during treatment according to AIEOP LNH97 protocol: at diagnosis, before the second cycle of chemotherapy and at stop therapy. Diagnostic samples were amplified using multiple primer sets for IGH and IGK rearrangements. Clonal rearrangements were identified by heteroduplex analysis. Based on the sequence of the junctional region, patient-specific oligonucleotides were developed. The RQ-PCR assay was performed following the guidelines of the European Study Group on MRD in ALL. High molecular weight DNA was used to investigate the presence of t(8;14) by LD-PCR in all samples. **Results.** In 90% and 73% of the patients at least one sensitive RQ-PCR target (detection limit  $\leq 10^{-4}$ ) and two sensitive targets were found, respectively. The sensitivity level was  $10^{-5}$  in 45% of the targets and  $10^{-4}$  in 45%. Forty-six percent of the selected targets were IGHV-D-J rearrangements, followed by IGHD-J (25%) and IGKV-J (17%). The sensitivity of the two assays was similar, although in 7/35 cases of t(8;14) positive BL only the RQ-PCR approach detected BM involvement at diagnosis, while in one case of B-ALL MRD positivity was detected only by the LD-PCR assay. **Conclusion.** Our results suggest that RQ-PCR for Ig rearrangements and LD-PCR for t(8;14) can be used for MRD analysis. To study MRD we propose to screen all B-ALL and BL patients at diagnosis by LD-PCR: in t(8;14) positive patients, MRD could be analyzed by LD-PCR, whereas in negative patients the Ig-based assay could be performed. Considering the mature phenotype of B-ALL and BL cells, we hypothesize that MRD could be successfully studied with a single sensitive clonotypic target in these malignancies.

**020**

**QUANTIFICATION OF CIRCULATING TUMOR CELLS BY QUANTITATIVE PCR FOR NPM-ALK IN ANAPLASTIC LARGE CELL LYMPHOMA DEFINES A VERY HIGH RISK GROUP OF PATIENTS – UPDATE OF THE BFM-EXPERIENCE**

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**Background.** Circulating tumor cells (CTC) can be detected in bone marrow (BM) or blood (PB) by qualitative RT-PCR for NPM-ALK in about 45-60% of patients with NPM-ALK positive ALCL. Detection of CTC confers a relapse risk of 50%. Quantification of CTC by quantitative Real-time-PCR in BM applying a cut-off of 10 copies NPM-ALK/104 copies ABL (NCN) identified 20% of patients with a relapse risk above 60% among 74 patients in our previously reported series (Damm-Welk *et al.*, 2007). **Objective.** We extended our analysis to a cohort of 116 patients with NPM-ALK positive ALCL to substantiate the prognostic value of the quantification of CTC. **Design and methods.** Qualitative and quantitative PCR for NPM-ALK were performed as previously described. CTC could be analyzed by qualitative PCR and quantified in BM-samples from 116 and 105 pts, respectively, and PB-samples from 79 and 76 of them. The pts were treated between 1996 and 6/2008 according to the protocols NHL-BFM95 and ALCL99. **Results.** NPM-ALK transcripts could be detected in initial BM of 45% (52/116) and in initial PB of 48% (38/79) of patients. The cumulative incidence of relapses (CI-R) of patients CTC positive in BM and PB was: BM positive 53±7%, PB positive 46±8% compared to BM negative 21±6%, PB negative 12±7%. More than 10NCN were measured in 20% of BM-samples (21/105) and 24% of PB-samples (18/76). The cumulative incidence of relapses (CI-R) and overall survival (OS) of patients with  $> \text{or} \leq 10\text{NCN}$  in BM or PB were:

BM CI-R:  $>10\text{NCN}$  71±11%,  $\leq 10\text{NCN}$  24±5% ( $p < 0.001$ )  
BM OS:  $>10\text{NCN}$  54±11%,  $\leq 10\text{NCN}$  90±4% ( $p < 0.001$ )  
PB CI-R:  $>10\text{NCN}$  69±12%,  $\leq 10\text{NCN}$  15±6% ( $p < 0.001$ )  
PB OS:  $>10\text{NCN}$  68±12%,  $\leq 10\text{NCN}$  91±5% ( $p = 0.01$ ).

**Conclusion.** These extended data corroborate that quantification of CTC by RQ-PCR in initial BM or blood allows identifying 20% of patients experiencing 50% of all relapses with a relapse risk of almost 70%.

**021  
EARLY ASSESSMENT OF MINIMAL RESIDUAL DISEASE (MRD) BY QUALITATIVE PCR FOR NPM-ALK IDENTIFIES PATIENTS AT VERY HIGH RISK OF RELAPSE IN ANAPLASTIC LARGE CELL LYMPHOMA - A BFM GROUP REPORT**

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**Background.** 25-35% of children and adolescents with anaplastic large cell lymphoma (ALCL) suffer a relapse. Circulating tumor cells (CTC) can be detected in bone marrow (BM) or blood (PB) by qualitative RT-PCR for NPM-ALK in about 50-60% of patients. Detection of CTC confers a relapse risk of 50%. **Objective.** We asked whether detection of minimal residual disease (MRD) by qualitative RT-PCR for NPM-ALK in BM or PB early during treatment allows identification of patients at highest risk of relapse. **Design and methods.** The qualitative RT-PCR for NPM-ALK with a sensitivity of 10-5 was applied as previously described (Damm-Welk *et al.*, 2007). Patients were treated according to the BFM-type protocols NHL-BFM95 and ALCL99 with the same cytoreductive prephase between 8/1998 and 10/2007. Peripheral blood (PB) and/or bone marrow (BM) of 118 patients with NPM-ALK-positive ALCL were screened for the presence of NPM-ALK transcripts at diagnosis. BM or PB was requested from those patients with detectable CTC in BM or PB for the measurement of MRD at the end of the cytoreductive prephase (day 6) and before the second course of chemotherapy (course 2), respectively. **Results.** CTC were detected in BM or PB or both media in 64 of the 118 patients with NPM-ALK positive ALCL. The cumulative incidence of relapse (CIR) of CTC-positive pts was 48±7% (29/64) compared to 20±7% (7/54) of patients who were CTC-negative in both BM and PB. MRD at day 6 was measured in either BM or PB of 21 patients positive for CTC: 12 of the 15 pts with detectable MRD relapsed compared to 1 of the 6 pts negative for MRD (CIR 80±11% vs. 25±25%, p=0.04). 26 pts were evaluated for MRD before course 2: 14 of the 16 pts with still detectable MRD relapsed compared to 3 of 7 pts without detectable MRD (CIR 92±10% vs. 33±17%, p=0.005). **Conclusion.** Our data suggest that early evaluation of MRD by a sensitive RT-PCR in NPM-ALK positive ALCL with detectable CTC at diagnosis identifies patients with a very high risk of relapse.

**22  
MINIMAL RESIDUAL DISEASE (MRD) ASSESSMENT IN CHILDREN AND ADOLESCENTS WITH MATURE B-CELL NON-HODGKIN LYMPHOMA (B-NHL): A CHILDREN'S ONCOLOGY GROUP REPORT**

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**Background.** We recently reported the feasibility of using immunoglobulin heavy chain primer pools to assess MRD in children with B-NHL (Agsalda/Shiramizu *et al.*, Adv in Hem, 2009). The PCR-based method uses immunoglobulin heavy chain (IgVH) primer pools from IgVH1-IgVH7 regions. The major advantage of this method lies in eliminating the need for original tumor DNA to design patient-specific primers (Sabesan/Shiramizu *et al.*, JPHO, 2003). Previous studies suggested that MRD studies using qRT-PCR of cMYC rearrangements may be prognostic in childhood Burkitt lymphoma (BL) (Mussolin *et al.*, JCO, 2007). **Methods.** Children with B-NHL underwent therapy consisting of FAB Group B4 and Rituximab as previously described (Patte/Cairo *et al.*, Blood, 109:2773, 2007; Cairo *et al.*, ASH, 2008). Entry specimen DNA was first screened by PCR for VH family usage using the following primer pools: VH1/VH2; VH3/VH4; VH5, VH6/VH7. If MRD-positive results were found, then individual VH primers were used to identify the variable region involved and in sequential specimens. **Results.** Twelve of 20 evaluable patients had tumor and staging specimens (PBMC, BM, and/or CSF) with detectable tumor DNA; the other 8 cases had only staging specimens, but were positive for clonal DNA. Eighteen cases had follow-up specimens. The sensitivity of the assay was previously shown to detect 1 malignant cell in 105 PBMC. Eighteen subjects were in clinical remission at the end of therapy (EOT); 17/18 had MRD-negative specimens prior to EOT. Two subjects experienced relapse while on therapy and 1 case had MRD-positive PBMC prior to relapse. The other relapse case had no follow-up specimens (Table). **Conclusions.** Use of IgVH primer pools appears feasible to assess MRD in childhood mature B-NHL. Subjects who remained in remission, had MRD-negative results on specimens prior to EOT. Limitations of the study include small number of cases and specimens; and B-NHL involving IgH rearrangements. The data however support future studies to assess the validity and significance of MRD-positive and -negative specimens in childhood mature B-NHL. (Supported by NIH CA121955)

**Table. MRD Assessment on Clinical Specimens and Disease Status**

MRD Status On Last Specimen	Disease Status	
	Remission	Relapse
Positive	0	2 (1 subject with only entry specimens)
Negative	17	0
Indeterminate	1	0

**023**

**PROPOSAL FOR A REVISION OF THE ST. JUDE STAGING SYSTEM CLASSIFICATION FOR PEDIATRIC NON-HODGKIN LYMPHOMAS**

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**Background.** Since 1980 the St. Jude or Murphy staging system has represented an invaluable tool for clinicians dealing with pediatric non-Hodgkin's lymphomas (NHL). Since then significant changes in diagnosis, management and therapy of NHL have occurred. New biological information led to the identification of novel entities with peculiar histological/immunological profiles and clinical behavior. Consequently the St Jude staging classification has become less effective in orienting therapeutic decisions and in some cases, "risk groups" were devised that combine stage and biological and/or clinical features to aid treatment decisions. **Objective.** To elaborate a modified staging classification for pediatric NHL that reflects the state-of-the-art medical and biological knowledge in the field, maintaining a continuity with the St Jude staging system. **Methods.** A series of meetings among professionals involved in diagnosis and treatment of childhood NHL were held to analyze each of the St Jude staging system definitions and to critically review its interpretation and implementation in the actual clinical activity. Whenever, based on the current knowledge and clinical practice, definition was felt to be incomplete, ambiguous or in case a clinically relevant disease site or a combination of sites were not clearly identifiable, a change or addition was proposed. Great attention was given to maintain a continuity with the original staging system and to assure the widest applicability of the modified staging approach to different NHL subgroups and to different conditions where children with NHL are taken care of. **Results.** It was recognized that the St Jude staging system is still a valid tool to describe localization and disease extent in pediatric NHL. However, selected extranodal sites, including bone and skin should be annotated for localized disease as they could identify different clinical entities. Except for localized-resectable tumors, abdominal parenchymatous localizations should be regarded as stage III disease, independent of their number. Stage IV disease should be further distinct between BM and CNS and type of BM and CNS involvement should be specified as supplemental information. Caution should be used in adopting new imaging technologies to define stage. **Conclusion.** On the backbone of St Jude staging system, some adaptations should be introduced that reflect the recent medical, biological and technological progress. Much caution should be used to guarantee reproducibility and comparability with time.

**024**

**LONG-TERM OUTCOME OF CHILDREN WITH B-NON-HODGKIN'S LYMPHOMA: RESULTS FROM BRAZILIAN NATIONAL CANCER INSTITUTE**

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**Background.** Brazil is a very large country with a remarkable socio-economic heterogeneity. The survival rates of children and adolescents with B-Non-Hodgkin's lymphoma (B-NHL) in Brazil remain unknown. There is a compelling requirement for finding treatment strategies applicable to centers or regions with different health care resources. In this scenario, the challenge in Brazil is to plan treatment intensity so that morbidity is manageable while increasing the survival rate. **Objectives.** To evaluate the efficacy and toxicity of the modified B-NHL Berlin-Frankfurt-Münster (BFM) protocol in Brazilian children with B-NHL. **Methods.** From September 1997 to December 2007, 85 untreated patients (age less than 16 yr) were enrolled and treated at the National Cancer Institute, Rio de Janeiro, Brazil. B-NHL subtypes were classified according to the WHO classification. Clinical stage was based on the St. Jude staging system. The patients were stratified by risk factors (stage and LDH level) and treated with B-NHL-BFM 86-90-based protocol with reduction of metotrexate dose from 5 g/m<sup>2</sup> to 2 g/m<sup>2</sup> for patients with advanced disease, and LDH level more than double the normal level. **Results.** The median age of the patients was 6 years (range 1-16 yr); 61 patients were male and 24 patients were female. Of these patients, 69 (81%) had Burkitt's lymphoma, 9 (11%) had diffuse large B-cell lymphoma (DLBL), 3 (3%) Burkitt-like lymphoma, and 4 (5%) were not further classified. According to the St Jude staging system, 18% of patients had stage I/II, and 82% stage III/IV disease. At a median follow up of 43 months, the event free survival (pEFS) for all patients was 80±4%, with 93% for stages I/II, and most notably, 78±5% for stage III/IV. There was statistically significant difference ( $p=0.009$ ) in pEFS between patients with LDH level less (69±7%), and more than double the normal level (94±3%). The major toxicity complications were myelosuppression and mucositis, but these conditions were manageable. Events were as follows: progression during therapy, n=8; relapse after therapy, n=4; second malignancy, n=1. There was only one death from sepsis related to treatment. **Conclusions.** This modified B-NHL-BFM protocol was very effective for Brazilian children with B-NHL, and represented an increase in the cure rates in childhood B-NHL in Brazil.

**025**

**TREATMENT RESULTS OF 1110 PATIENTS WITH NON-HODGKIN'S LYMPHOMA**

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**Objective.** To evaluate the clinical characteristics, treatment regimens, and outcome of the patients with non-Hodgkin's lymphoma. **Patients and methods.** 1459 patients diagnosed with non-Hodgkin's lymphoma between 1971 and 2008 were retrospectively evaluated. Staging was done according to Murphy classification system. All patients were treated with chemotherapy with or without radiotherapy. Chemotherapeutic regimens included COP or derivatives, COMP, LMB/LMT, modified COP+Adriamycine, BFM, and others. **Results.** 1110 newly diagnosed patients out of 1459 patients were eligible for analysis. There were 816 males and 294 females with a median age of 6 years (0.3 to 19). Stage distributions were 20, 121, 746, and 223 patients in stage I, II, III, and IV, respectively. Primary tumor localisations were abdomen, mediastinum, head and neck, peripheral lymph nodes, generalized disease, and extranodal disease, and others in 591, 133, 207, 50, 96, 17, and 16 patients, respectively. COP+Adriamycine was used in 424 patients, LMB in 216, LMT in 102, COP and derivatives in 231, COMP in 29, BFM in 26, others in 82. Overall (OS), and event-free survival (EFS) rates in whole group were 50 and 46% at 10 years, respectively. Median survival time was 13 years. OS rates were 67%, 61%, 48%, 48% in regimens of LMB/LMT, COMP, COP+Adriamycine, and BFM ( $p=0.0001$ ), respectively. The survival improved in last decade to 70% with the LMB/LMT regimens. The other significant prognostic factors on survival were stage ( $p=0.0001$ ), histopathology ( $p=0.003$ ), and tumor localizations ( $p=0.0008$ ). **Conclusion.** This is one of the largest series in a single center. LMB/LMT regimens have achieved the best survival rate in last decade. High dose methotrexate regimens should be used in treatment of NHL.

**026**

**PRIMARY BONE LYMPHOMA: DIFFICULTIES IN DIAGNOSIS AND FOLLOW UP**

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**Background.** Primary bone lymphoma (PBL) is a rare condition accounting for 3-7% of extra-nodal non-Hodgkin lymphoma (NHL). It can occur at any age. Common sites of involvement are the femurs, the tibia and the vertebrae, with no association to visceral or lymph node involvement. The more frequent histotype is diffuse large-cell lymphoma. The chief complaint is usually pain, sometimes a palpable mass or a pathological fracture. Systemic symptoms are generally absent. **Objectives.** To report on 2 children with PLB and descri-

be our difficulties in follow-up strategy. **Case reports.** Patient 1: a 12 year old boy complained of pain at his right knee, ankle and foot for 6 months. Radiograph performed 2 month before revealed fracture of the first metacarpus, but despite appropriate treatment, walking became painful. MRI revealed multiple osteolytic lesions with osteoclerotic border (left shoulder, humer and tibia bilaterally, right foot). Patient 2: a 11 year old boy had a long history of isolated left leg pain. Radiographs were negative; after 2 months he started walking with a limp. MRI revealed osteosclerotic lesions of left tibia. Needle biopsy revealed lymphoblastic NHL of B-cell lineage in both children. Physical examination was normal and staging (total body CT, bone marrow examination, lumbar puncture) excluded other sites of involvement. They were treated according to EURO-LB 02 protocol. Symptoms disappeared in a couple of weeks; MRI pattern improved after 3 months and resolved after 10 months from starting chemotherapy; anyway clinical interpretation of the serial follow-up MRI was not always immediate. Presently, patients are in complete remission with a follow up of 13 and 4 months after stop-therapy, respectively. **Conclusion.** Non-specific pain was the only complaint, that caused a long delay in reaching the correct diagnosis, which was not even suspected until biopsy: radiographs underestimated the extent of disease in both cases; not even MRI findings suggested the lymphomatous nature of the disease. Follow-up was particularly troublesome since biopsy could not be used repeatedly in the routine clinical work-up; MRI abnormalities, the only specifically related to the disease, may persist long after completion of treatment, due to a remodelling/reossification process of lytic lesions; differentiating residual disease from healing process of the bone is not easy and only time gives the answers.

**027**

**THE ADDITIONAL USE OF RITUXIMAB TO CONVENTIONAL CYTOTOXIC CHEMOTHERAPY IN CHILDHOOD NON-HODGKIN LYMPHOMAS: A SINGLE CENTER EXPERIENCE FROM TURKEY**

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**Background.** The development of rituximab, an anti-CD20 monoclonal antibody, represents a revolutionary advance in the therapy of hematological malignancies. Although, rituximab has been shown to significantly add to the response rate and survival of adult patients with CD20 positive B-cell lymphomas; minimal pediatric data have been published. **Objectives.** We summarize our experience about the response rate and therapy related toxicities with 9 patients who used conventional chemotherapy combined with rituximab during the last 4 years. **Design/Methods.** 9 Patients (4 girl, 5 boys) ages varied from 3.5 to 12 years (median age 7 years) with Non-Hodgkin Lymphomas (NHL) (4 Burkitt, 5 diffuse large B-cell lymphomas) received 4 to 8 doses of rituximab and additional chemotherapy. Rituximab 375

mg/m<sup>2</sup> was administered. The concomitant chemotherapy protocols were generally EPOCH-Rituximab (etoposide, prednisone, vincristine, cyclophosphamide, and doxorubicin plus rituximab) for the diffuse large B-cell lymphoma; however the addition of Rituximab was preserved for refractory or recurrence Burkitt Lymphomas, so the frontline chemotherapy for these patients were NHL-BFM 90. Use of growth factors was permitted. *Results.* The all of the diffuse large B-cell lymphoma (In five of the out of nine patients), rituximab therapy led to complete response. The median follow-up period was 33 months (2 to 48 months). In contrast, none of the four children treated with rituximab for Burkitt Lymphoma responded, unfortunately the entire patient died because of progressive disease. Noted toxicities included febrile neutropenia and prolonged hypogammaglobulinemia. *Conclusion.* The combination of rituximab and cytotoxic chemotherapy was associated with an encouraging response rate and an acceptable toxicity profile. Furthermore, risk-adapted and rationally targeted subtype-specific therapy for patients with NHL will serve more comfortable outcomes in the future.

#### **NEW INSIGHTS INTO THE PATHOGENESIS OF BURKITT LYMPHOMA**

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Burkitt Lymphoma (BL) originates from the malignant transformation of mature B cells. In particular, BL derives from centroblasts within germinal-centers (GC), the site where B cells are selected for high-affinity antibody production and undergo somatic hypermutation (SHM) and class switch recombination (CSR) of Immunoglobulin (Ig) genes (Klein and Dalla-Favera, 2008). The key genetic lesion associated with BL development is the chromosomal translocation juxtaposing Ig genes to the c-MYC oncogene, which are present in virtually all cases of BL (Dalla-Favera *et al.*, 1982, 1983). These translocations lead to the constitutive expression of the c-MYC transcription factor under the control of Ig transcriptional control elements. *In vitro* experiments as well as studies in transgenic mice have provided evidence that c-MYC deregulation contributes to tumorigenesis, although it is not sufficient to BL development. However, the mechanism(s) leading to chromosomal translocation as well as the role of c-MYC deregulation are still poorly understood. Recent results will be presented that shed some lights into some of these issues. In particular: 1) chromosomal translocations derive from mistakes of the SHM and CSR mechanisms (Pasqualucci *et al.*, 2008); 2) translocation induce ectopic expression of c-MYC in GC B cells, where its expression is actively repressed by BCL6; 3) once expressed in BL cells, c-MYC forms a pathologic protein complex with BCL6, which regulates the transcription of novel target genes and may represent a tumor-specific therapeutic target; 4) c-MYC has a novel non-transcriptional role in promoting DNA replication, and its deregulation may be responsible for abnormal replication and genomic instability in BL pathogenesis (Dominguez-Sola *et al.*, 2007).

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**SCIENTIFIC SESSION 4: MATURE B-CELL NHL**

**CURRENT MANAGEMENT OF B-NHL IN CHILDREN AND ADOLESCENTS**

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B-NHL are the most frequent lymphoma in children, with a majority of Burkitt lymphoma (BL). The latest generally arise in abdomen and head and neck region and present as advanced stage disease. Bone marrow and CNS are involved in about 20-25% of the cases. Its outcome greatly improved due to several prospective (multinational studies, especially the LMB and the BFM studies.

Treatment of BL must be done by intensive pulse polychemotherapy courses. The French LMB studies showed that :a) HDMTX is a very effective drug for prevention of CNS disease b) B-ALL and CNS positive patients benefited from the increase of the dose of HD MTX and the introduction of HD ARA-C (CYVE courses), c) treatment intensity can be adapted to stage and resection, but also to response to chemotherapy (at D7 and after 3 courses), d) early dose intensity is essential. The German BFM studies showed that a) treatment intensity can be adapted to stage and resection, but also to LDH level, b) HDMTX is a very effective systemic drug, and exposition to the drug must be all the more long as the disease is more advanced. They also confirmed that HD Ara-C is important in the advanced diseases. These and other studies showed that treatment can be of short duration, relapses occurring early within the first year, and confirmed that cyclophosphamide, HDMTX and Ara-C are the major drugs in BL, in addition to vincristine, doxorubicin, VP16, corticosteroids. CNS prophylaxis must be done by HDMTX±HD Ara-C and IT injections of MTX±Ara-C, but not by cranial irradiation. Adequate supportive care is necessary. With all these current strategies in Western countries, EFS of BL increased to 80-90%. Large B-cell lymphoma represent 10 to 20% of B-cell lymphoma in children and occur more frequently in adolescents. Although relapses can occur later, their outcome is similar to that of Burkitt lymphoma with the LMB and BFM protocols, except for primary mediastinal large B-cell lymphoma (PMLBCL) which have a worse outcome and need a different therapeutic strategy. The current questions are the following: 1/ In countries with less resources, how to reach these cure rates? Cooperation are needed between "privileged" and "less privileged" countries. 2/ Relapses are very difficult to cure, new approaches need to be developed. 3/ Will biology help to recognize prognostic factors to adapt therapy, and to find new therapeutics approach? 4/ One major question concerns rituximab, an anti-CD20 antibody, largely used in adult B-cell lymphoma, but for which there are no data in Burkitt lymphoma. Interestingly, the analysis of the pooled data of the 2 recent BFM and LMB studies showed similar results. A group of "worse" prognosis can be identified: stages III with high LDH level, stages IV and L3ALL whose EFS is around 85%. For these patients, the European and North American groups are discussing a phase III trial on the

efficacy of rituximab added to the current chemotherapy regimens, on the model of the MinT trial done in adult DLBCL. In conclusion, although EFS of B-cell lymphoma in children and adolescent increased to 80-90%, while weight of treatment decreased for the majority of the patients, questions remain for the next coming years, especially the necessity to demonstrate the benefit of rituximab in children and the necessity to improve outcome in countries with less resources where Burkitt lymphoma is frequent.

**THE ROLE OF RITUXIMAB IN SENSITIZATION TO CHEMOTHERAPY**

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Rituximab, chimeric anti-CD20 mAb, alone or in combination with chemotherapy, is successfully used in the treatment of patients with B-NHL and autoimmune diseases. Treatment with rituximab alone results in clinical response of 50% of patients whereas >90% of patients respond when used in combination with chemotherapy. The mechanism of rituximab-mediated chemosensitization was examined *in vitro* using drug-resistant B-NHL cell lines. We first observed that treatment with rituximab inhibited selectively the anti-apoptotic Bcl-2/Bclxl gene products and these were responsible for chemoresistance. Hence, we examined upstream the various signaling pathways that regulate the transcription, expression and activities of these gene products. We have found that several survival/anti-apoptotic pathways were inhibited by rituximab and all of which regulated downstream Bcl-2/Bclxl expressions and activities. These included the Raf-1/MEK/ERK1/2, NF-κB and Akt pathways. The direct role of each pathway that was inhibited by rituximab was confirmed by the use of specific chemical inhibitors and which mimicked rituximab for chemosensitization when used in combination with drugs. Upstream, we also show that rituximab inhibits Src kinases following lipid raft mobilization and which were responsible, in part, downstream in the inhibition of survival pathways. In addition, we show that rituximab induces the expression of Raf-1 kinase inhibitor protein (RKIP) and demonstrate that RKIP was involved in the inhibition of both the Raf-1 and NF-κB pathways. To examine the mechanism of rituximab resistance, we generated rituximab-resistant clones that still expressed CD20 on the cell surface. These clones no longer responded to rituximab nor can they be chemosensitized by rituximab. There was no detectable cell triggering nor intracellular gene modification compared to wild type cells. However, drug resistance could be circumvented by the use of chemical inhibitors of intracellular survival pathways in combination with chemotherapeutic drugs. Overall, our findings demonstrate that rituximab signals the tumor cells and induces several intracellular modifications of survival/anti-apoptotic pathways leading to chemosensitization to drug-induced apoptosis. Further, we demonstrate that rituximab resistance can be circumvented by the

use of chemical inhibitors in combination with drugs. The clinical relevance of these findings will be discussed.

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## IMMUNE EFFECTOR MECHANISMS OF CD20 MONOCLONAL ANTIBODIES

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CD20 is a B cell-specific molecule that is first expressed on the cell surface during the pre-B to immature B cell transition, but is lost upon plasma cell differentiation. CD20 is an ideal target for monoclonal antibodies (mAb) as it is expressed at reasonably high levels on most B non-Hodgkin's lymphomas (NHL), but is not internalized after mAb binding and does not circulate in the plasma as free protein. CD20 can serve as a membrane-embedded target for lymphoma destruction *in vitro* through activation of the innate immune system by initiating complement- and mAb-dependent cytotoxicity. Furthermore, CD20 mAb treatment alters transmembrane Ca<sup>2+</sup> transport and B cell progression through cell cycle and can induce B cell apoptosis alone or following further cross-linking. Rituximab and other CD20 mAbs induce classical pathway complement activation and complement-dependent cytotoxicity (CDC) of fresh B lymphoma cells and cell lines. Rituximab also activates complement in patients and CDC is involved in the cytokine-released syndrome and its toxicity. Although CD20 mAb depletes human lymphoma cells *in vitro* through CDC, tumor susceptibility to CDC and expression of complement inhibitor proteins does not always predict the outcome of CD20 therapy. Moreover, recent studies in mice have shown that complement does not contribute to Burkitt's like BL3750 lymphomacidal activity *in vivo* even though CD20 mAb efficiently binds C1q and activates complement *in vitro*. Other Ab-dependent effects also appear important since a chimeric CD20 mAb of an isotype different than that used clinically does not deplete normal B cells in non-human primates and the anti-tumor effect of CD20 mAb depends in part on immune activation through Fc receptors for immunoglobulin (Ig) (FcγR). Although it is clear that FcγR play a critical role in human mAb therapy, due to the recruitment of effectors for antibody dependent cellular cytotoxicity (ADCC), we knew much less about which cells are responsible for mediating such effects *in vivo*. Recent studies using mouse lymphoma models demonstrated that FcγR-bearing macrophages are necessary and sufficient to mediate this process. Moreover, human studies in follicular lymphomas confirmed that tumor-associated macrophages play a critical role in rituximab efficacy. Finally, rituximab was found to rapidly activate complement *in vivo* and induce chemokines that activates the innate immune network to eradicate human lymphoblastoid cell lines in nude mice. Thus,

there is evidence for both ADCC and complement mediated lymphoma depletion following CD20 mAb treatment *in vivo*. At least, after a conventional 4-dose course of rituximab therapy, B lymphocyte counts recover in 9 to 12 months, while Ig serum concentrations remain normal. B cells play multiple roles during immune responses in addition to antibody production. Regulatory B cells have been recently described that significantly inhibit autoimmune disease and inflammation. Depleting normal B cells during anti-CD20 immunotherapy has been associated with a significant increase in infectious risk and may also have consequences on tumor immunity. These recent findings allow for mechanism-based predictions of the biological outcome of CD20 mAb therapy and treatment optimization.

## 028

### REDUCED EXPRESSION OF DLEU1 IN PEDIATRIC BURKITT LYMPHOMA (PBL) IS ASSOCIATED WITH SIGNIFICANT DECREASE IN CYCLOPHOSPHAMIDE (CY) INDUCED APOPTOSIS: POTENTIAL ROLE OF A TUMOR SUPPRESSOR GENE

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**Background.** Deletions of 13q have been associated with a significantly poorer outcome in children with BL treated on the FAB/LMB 96 study (Poirel/Cairo *et al* Leukemia 2008). DLEU1, a gene within the Burkitt classifier genes as reported by Dave/Staudt *et al*. NEJM, 2006, is located on 13q. DLEU1 interacts with proteins including c-Myc, histone acetylase (HTATIP), tumor antigen p53, histone-lysine N-methyltransferase (SETDB1), Tubulin β-2C (TUBB2C), RASSF1A, and E3 ubiquitin-protein ligase (UBR1). We previously report that RASSF1, ERG, UBR1, and TUBB2C were expressed significantly higher in BL than in DLBCL (Day/Cairo *et al*, AACR 2008) and that levels of UBR1 and TUBB2C are significantly reduced in DLEU1 siRNA transfection BL study (Day/Cairo *et al* SIOP 2008). **Objective.** We examined the effect of DLEU1 siRNA transfection on CY-induced BL apoptosis and determined the DLEU1-interactome network in BL. **Methods.** Ramos BL cell lines were transiently transfected (24 hrs) with DLEU1 and control siRNA previously described (Day/Cairo SIOP 2008). The siRNA transfected cells (4 samples per group) were then treated with CY (0, 89.5, 895, 8950 nM) for additional 4 hrs. Cells were evaluated for apoptosis using Annexin V-FITC (Q2 and Q4) and Propidium Iodide (Q1 and Q2) followed by FACS using BD LSRII.

Statistics was conducted by one-way ANOVA followed by Dunnett multiple comparisons test. **Results.** DLEU1 siRNA S3 transfected cells vs. control or siRNA-negative controls showed significantly reduced both early (Q4) and late (Q2) apoptosis (16.90±0.37%,  $p<0.001$  and 14.70±0.27%,  $p<0.0001$ ). The early apoptosis (Q4) in CY treated BL presented profound inhibition in the DLEU1 siRNA-s3 knockdown group (89.5 nM CY: 10.26±0.23% reduction,  $p<0.05$  to negative control; 895 nM CY, 10.86±0.67% reduction,  $p<0.01$ ; 8950 nM, 9.85±0.32% reduction,  $p<0.05$ ). Pathway analysis of the DLEU1 network genes identified 34 genes involved in hypoxia signaling, protein ubiquitination, and NOTCH signaling. **Conclusion.** These data suggest that DLEU1 may in part regulate programmed cell death in BL. Deletion of 13q14.3, which contains DLEU1, in pediatric Burkitt lymphoma may confer a phenotype of drug resistance and predispose pediatric patients with BL to a significantly decreased EFS following standard chemotherapy.

## 029

### RISK FACTORS ASSOCIATED WITH POOR OUTCOME IN CHILDREN AND ADOLESCENTS (C & A) WITH MATURE B-NHL: REPORT OF THE FAB/LMB 96 STUDY

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**Background.** We recently reported the results in C & A with low risk (Group A), intermediate risk (Group B) and high risk (Group C) mature B-NHL treated on FAB/LMB 96 (Gerrard *et al*, BJH, 2008; Patte *et al*, Blood, 2007; Cairo *et al*, Blood, 2007, respectively). Adolescent age (15-21 yrs) has historically been considered to be an independent risk factor for poor outcome in subsets of mature B-NHL (Hochberg/Cairo *et al*, BJH, 2009; Burkhardt *et al*, BJH 2005; Cairo *et al*, BJH, 2003). Other known risk factors have included tumor burden (LDH), stage and primary site. **Objective.** To determine if age, LDH, stage, primary site and histology were independent risk factors in C & A with newly diagnosed mature B-NHL treated on FAB/LMB 96. **Methods.** We analyzed the EFS of all pts treated on FAB/LMB 96 and the following risk factors were investigated in a univariate and Cox multivariate analysis: age (<15 vs. ≥15 yrs), primary sites (BM/CNS, PMBL, other), LDH <2 vs. ≥2 NL, stage I/II vs. III/IV, and histology (DLBCL vs. BL/BLL). **Results.** 1111 pts (15%, 15-21 yrs) were treated with Group A (N=132), Group B (N=744), and Group C (N=235) therapy. 5yr EFS (CI95) for all, A, B, C pts was 86% (84%, 88%), 98% (93%, 100%), 87% (84%, 89%), and 79%

(73%,84%), respectively. Age (≥15 yrs), LDH ≥2NL, stage III/IV, and BM+/CNS+ and histology were significant univariate risk factors for decreased EFS ( $p<0.045$ , <0.0001, <0.0001, <0.0001, and <0.0001 respectively). Multivariate analysis demonstrated age ≥15 yrs and DLBCL histology were no longer independent significant risk factors ( $p=.82$  and 0.08, respectively), but LDH (RR 2.0,  $p=.001$ ), stage III/IV (RR 3.8,  $p<0.001$ ), and primary sites including PMBL (RR 4.0,  $p<.001$ ) and BM+/CNS+ (RR 2.8,  $p<0.001$ ) were independent significant risk factors for poorer EFS. **Conclusion.** With the use of FAB-LMB 96 therapy, adolescent age and histology are no longer poor risk factors in C & A with mature B-NHL. The independent risk factors identified in this study (stage, LDH, BM+/CNS+ and PMBL) for decreased EFS in C & A mature B-NHL will form the basis of the next risk adapted international pediatric mature B-NHL trial.

## 030

### PEDIATRIC B-CELL NON-HODGKIN LYMPHOMAS: PRELIMINARY RESULTS OF THE ITALIAN AIEOP LNH97 PROTOCOL

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**Background.** B-cell non-Hodgkin lymphomas (B-NHL) comprise highly aggressive NHL subtypes that need high dose-intensive CT regimens. The early Italian AIEOP LNH92 protocol, based on BFM90 strategy, showed good results compared with concomitant international trials, but rather high toxicity. **Objective.** With the AIEOP LNH97 protocol for B-NHL we aimed to improve the EFS of the previous Italian protocol, intensifying CT in high stage disease, and to reduce early life-threatening toxicity. **Patients and methods.** Patients aged up to 18 y with newly diagnosed B-NHL were included. Therapy consisted of a 5-days standard-dose chemotherapy (pre-phase) followed by 2-6 cycles of high dose-intensity CT. Treatment was stratified on 4 different risk-groups, based on stage, tumor resectability and LDH value. CNS prophylaxis was accomplished by lumbar intrathecal CT. Patients with more than 25% of blast in the BM were excluded from the study. **Results.** From 1997 to 2008, 379

(297 M, 82 F) out of 402 children with newly diagnosed B-NHL were eligible for the protocol; median age at diagnosis was 8.8 y (0.56-17.8); LDH median value 634 UI/L. Distribution according to St. Jude staging system was: 22 st-1, 110 st-2, 199 st-3 and 48 st-4 (17 with CNS involvement). With regard to risk groups, 17 were R1, 143 R2, 84 R3 and 135 R4. Histo-pathology diagnosis showed 284 cases (75%) of Burkitt or Burkitt-like NHL, 27 B-large cell NHL (including 10 patients with PMLB-CL), and 68 other sub-types. CR was obtained in 91% of cases. Overall, there were 26 remission failures, 17 relapses (median time 5 mo from diagnosis), and 1 second malignancy. With a median follow-up of 4 y, 348 patients are alive and 31 died: 26/31 for disease progression and 5 for septicemia (1 before and 4 after CR). OS was 91% (SE 2%) and EFS 86% (SE 2%). Multivariate analysis for different parameters (including age, gender, LDH, histology, stage, risk group, CNS) demonstrated a statistically significant impact on EFS of age and LDH above the median value. WHO grade III-IV hematologic and gastro-intestinal toxicities were the most frequent. *Conclusion.* The preliminary results of AIEOP LNH-97 protocol for B-cell NHL demonstrated an improved EFS compared with the previous AIEOP LNH92 protocol (EFS 81.8%). Early toxicity appeared to be acceptable.

### 031

#### RESULTS OF A PHASE II WINDOW STUDY ON RITUXIMAB IN NEWLY DIAGNOSED PEDIATRIC MATURE B-CELL NON-HODGKIN LYMPHOMA (B-NHL) / BURKITT'S LEUKEMIA (B-ALL)

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*Background.* Pediatric mature B-NHL differ from aggressive B-NHL of adults in terms of biology and treatment outcome. In contrast to adults, Rituximab (Ri) is not established in the treatment of pediatric B-NHL yet. Even the activity is not determined. *Objectives.* We conducted a phase II window study to examine the activity and tolera-

bility of Ri in children and adolescents with newly diagnosed mature B-NHL/AL. *Methods.* Eligibility: age <19 years, CD20+ B-NHL/AL, >1 measurable lesion, informed consent. Exclusion: Lansky performance scale 5, impaired renal-, heart-, liver-function, known allergy against foreign proteins, hepatitis B, pre-treatment, pre-existing disease, pregnancy. Treatment: Ri 375 mg/m<sup>2</sup> IV on day -4 prior to chemotherapy; concomitant therapy: Rasburicase, steroids only for anaphylaxis, intrathecal (IT) triple drug at days 1, 3 for central nervous system (CNS)+ patients (pts) only. Response evaluation: product of 2 largest perpendicular diameters of 1-3 index lesions/% blasts in bone marrow/peripheral blood within 24 hours prior to Ri and at day 5. Responder (RP): at least one lesion with at least objective effect (decrease of >25%) and no progress (increase of >25%) at other sites. Study plan: Simon 2-stage phase II with  $\alpha$  and  $\beta$  5%. Response rate (RR) for poor activity was set to 45%, for good activity to 65%. 33 pts entered the first stage, final evaluation was scheduled after at least 79 evaluable pts. *Results.* 136 pts were enrolled from 04/04-08/08. CTC <sup>3/4</sup> toxicities reported for the 136 pts: general condition 16%, fatigue 13%, anaphylaxis (chills/fever/bronchospasm) 6 (1/2/4)%, infection 3%, S-GOT/GPT 10%, acute tumor lysis (ATL) 7%, capillary leakage (0), toxic death (0). 49 pts were not evaluable for response due to withdrawal (causes: anaphylaxis (8), ATL (2), suspected progression, not verified (4), other (2)), IT therapy in CNS-pts (8), corticosteroids (3), technical inadequacy (21), no index lesion (1). Of the 87 evaluable pts 37 were RP (42.5%, 95%-CI 32% - 54%). RP by histology: Burkitt/B-AL 29/68, diffuse large B-cell lymphoma (DLBCL) 6/14, juvenile follicular lymphoma 1/2, primary mediastinal B-cell lymphoma (PMBCL) 1/1, B-NHL not further specified 0/2. 50 pts were non-RP. *Conclusion.* Ri 375 mg/m<sup>2</sup> is active as single agent in more than 40% of pediatric B-NHL.

### 032

#### INVESTIGATING MATURE B-CELL LYMPHOMAS BY MICRORNA PROFILING

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In order to understand the role of microRNA (miRNAs) in mature B cell function and lymphomagenesis, we analyzed the miRNA expression profile of B cells at different stages of differentiation by cloning of short RNA and by array-based miRNA expression studies. We generated libraries representative of miRNAs expressed in normal germinal center (GC), naïve and memory B cells isolated from human tonsils and in a Burkitt lymphoma cell line (Ramos). Short RNA libraries were sequenced and computationally analyzed toward the identification of candidate miRNAs expressed in B cells. The results show that 178 (103 previously known and 75 newly discove-

red) mature miRNAs were cloned from normal B cells and Ramos cell line. miRNA expression profiling was also performed using a commercial array platform designed to interrogate known miRNA. The cloning data show a good degree of concordance with the array-based analysis leading to the identification of a distinct miRNA expression profile for each normal B cell population. Using the same miRNA array platform we performed a comprehensive analysis of miRNA expression profiles of GC-derived lymphomas (Burkitt lymphoma, diffuse large B cell lymphoma, follicular lymphoma). Each tumor type shows a distinct miRNA profile that separates them from their normal counterpart. Interestingly, initial results indicate that a set of miRNAs is expressed in normal GC cells, but not in lymphoma samples and cell lines, suggesting that structural and/or functional alterations of miRNAs occur during lymphomagenesis.

### 034

#### **RARE CASES OF B-ALL WITH MYC REARRANGEMENT AND PRECURSOR B-CELL IMMUNOPHENOTYPE**

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**Background.** Burkitt's leukemia (B-ALL) is characterised by rearrangement of the MYC oncogene and a mature immunophenotype with expression of surface immunoglobulins (sIg). **Objectives.** To demonstrate rare cases of B-ALL with precursor B-cell immunophenotype and MYC rearrangement. **Case reports.** 1) 13-year-old boy presented with cytopenia, hepatosplenomegaly, markedly elevated LDH and bone marrow infiltration. Immunophenotype was consistent with precursor B-ALL (expression of TdT, CD10, CD19, lack of sIg, light chains  $\kappa/\lambda$  and CD20). However, the blasts had typical L3 morphology and chromosome rearrangement t(2;8)(p12;q24) involving the MYC locus. The cytogenetic features characteristic of mature B-ALL led to change of treatment. Although the patient received full courses of B-type therapy, he relapsed 14 months after initial dia-

gnosis. 2) An 8-year-old girl presented with anemia, splenomegaly, LDH of 14.070U/l, paravertebral and CNS-lymphoma. Bone marrow revealed a blast population with L3 morphology but also atypical myeloid blasts. Immunophenotyping showed a precursor B-cell phenotype and a population of myeloid cells with atypical expression of CD56 and lack of CD64. Cytogenetics revealed a t(8;14)(q24;q32) with additional MYC-IGH fusions. The girl initially received 1 block of AML-induction and then 5 blocks of NHL-therapy. She has been off therapy and in complete remission for 3 months. 3) A 4-year-old boy presented with an upper respiratory tract infection, cervical lymphadenopathy hepatomegaly, pancytopenia and markedly increased LDH and uric acid. Bone marrow showed extensive infiltration by L3 lymphoblasts. Immunophenotype was consistent with precursor B-ALL (expression of CD19, CD20, cyIgM, lack of sIgM,  $\kappa/\lambda$  light chains and TdT. Because of typical L3 morphology and acute tumor lysis syndrome the patient was treated according to the B-NHL BFM protocol; he had a complete remission on day 10 after rituximab. FISH result, received after start of therapy, showed a fusion of MYC and IGH. The patient has been in complete remission for three years. **Conclusion.** These unusual cases of B-ALL demonstrate the importance of careful evaluation of leukemia by morphology, immunophenotyping and cytogenetics. Future identification of patients with similar profile can lead to a better understanding of the biology, prognostic significance and optimal treatment for this rare subtype of leukemia.

### 035

#### **SAFETY OF LIPOSOMAL CYTARABINE (DEPOCYT®) PROPHYLAXIS IN PEDIATRIC ALLOGENEIC STEM CELL TRANSPLANT (ALLOSCT) RECIPIENTS WITH ACUTE LEUKEMIA (AL) AND NON HODGKIN LYMPHOMA (NHL) AT HIGH RISK OF CNS RELAPSE**

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**Background.** DepoCyt® is a liposomal derivative of cytarabine designed for intrathecal or intraocular treatment of leukemia/lymphomatous meningitis (LLM). This formulation allows for sustained release with prolonged therapeutic concentration and prolonged drug exposure of 8 to 14 days in children and adults, respectively. Adult trials have shown Depocyt® 50mg to be both safe and effective in the treatment of LLM (Glantz *et al*, JCO 1999). Bomgaars *et al*. investigated the use of Depocyt® in children in a phase I dose escalation trial and demonstrated the maximal tolerated dose to be 35mg and that it was safe, well tolerated and effective (Bomgaars *et al.*, JCO 2004). However, there remains a paucity of data of Depocyt® prophylaxis to prevent LLM in pediatric patients. **Objective.** To determine the safety and tolerability of Depocyt® as prophylaxis prior to and following AlloSCT in pediatric recipients with AL and NHL. **Methods.** Pediatric AlloSCT recipients with a history of

AL or NHL and high risk of CNS disease received Depocyt® 35mg (<21yr) or 50mg (>21yr) via lumbar puncture or omya reservoir pre and approximately every 3mo x 1-2yrs post AlloSCT. Patients received Decadron (0.15mg/kg/dose BID) concomitantly x 5days. *Results.* 11 patients (7ALL, 2AML, 1DLBCL and 1T-LL) received a total of 36 doses (median=2, range 1-12). Median age:15yr (range 6-22). Mean follow up: 323 days (median=173 days). Seven patients had a history of CNS AL/NHL involvement. No patients had evidence of LLM at the time of AlloSCT. Five and 6 patients had matched related and unrelated donors, respectively. Two patients experienced Grade II-III headache and vomiting. Three patients developed relapsed systemic disease, but none relapsed in the CNS. Six patients are deceased, however, none of these had CNS AL/NHL at time of death. *Conclusion.* These preliminary results suggest that prophylactic Depocyt® is tolerable and effective in pediatric AlloSCT recipients at risk for CNS relapse. Larger randomized studies are needed in the future to compare this to other CNS prophylaxis regimens post AlloSCT. In addition, trials are in development to investigate the combination of intraomaya Rituximab followed by liposomal Cytarabine in children with recurrent/refractory CNS CD20<sup>+</sup> AL/NHL.

**036**  
**SERUM IMMUNOGLOBULIN LEVELS IN CHILDREN AND ADOLESCENTS AFTER B-NHL BFM CHEMOTHERAPY WITH AND WITHOUT ONE DOSE OF RITUXIMAB**

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*Background.* In contrast to adults there are no data regarding the impact of Rituximab (Ri) on the immunoglobulin (Ig) levels in children and adolescents treated for B-cell non-Hodgkin lymphoma (B-NHL). In children treated with Ri for non-malignant disorders no hypogammaglobulinemia was observed (Bennett *et al.*, Blood 2006). However, in pediatric NHL patients (pts) IgM was reported to be significantly decreased until 2 years after chemotherapy (CT) alone (Abrahamsson *et al.*, Acta Paediatr 1995). *Objectives.* To study Ig levels, frequency of infections and Ig substitution in pediatric mature B-NHL/Burkitt's leukemia (B-AL) after CT alone or combined with Ri. *Methods.* We analyzed 344 pts, treated with CT alone (n=233) or combined with Ri (Ri+CT; n=111). Pts were diagnosed between 2004 and 2007.

Median age was 10.6 (range 1.5-18.6) years. CT consisted of 4-7 five-day-courses over 3-5 months (mth). Ri was given as window therapy in a dose of 375 mg/m<sup>2</sup> on day -4 prior to CT. *Results.* Ig before therapy: Ig levels were available in 184 pts. At least 1 Ig subclass (IgG and/or IgM and/or IgA) was below the lower normal limit (LNL) in 62 pts (34%). Ig later than 12 mth after starting Ri/CT: Ig levels were available in 145 pts (71 CT pts, 74 Ri+CT pts). 73 pts (50%) had >1 Ig below LNL, 49% of CT pts, 51% of Ri+CT pts. All Ig subclasses were below LNL in 25% of pts, IgM alone in 22% of pts. Ig levels below LNL were observed in 37% of the pts treated with 4 courses CT + Ri and in 59% of the pts treated with 5-6 courses CT + Ri, respectively. 21 of the 73 pts with Ig below LNL after therapy had already Ig below LNL at diagnosis. 75% of pts with >1 Ig below LNL were clinically asymptomatic, 19% suffered from frequent, mostly mild infections (no data in 6%). 75% of the 73 pts never received Ig infusions, 25% were temporarily substituted. 24-32 mth after starting therapy Ig were still below LNL in 26% of CT pts and in 18% of Ri+CT pts. *Conclusion.* 1/3 of B-NHL/AL pts already revealed Ig levels below LNL at diagnosis. 50% had Ig below LNL >1 year after beginning therapy. The frequency of Ig below LNL increased with the intensity of CT. One dose of Ri did not augment the effects of B-NHL CT on Ig levels.

**037**  
**MODIFIED MCP-842: A NOVEL HIGHLY EFFICACIOUS, SAFE AND COST-EFFECTIVE PROTOCOL FOR B-NHL TREATMENT IN RESOURCE POOR COUNTRIES**

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*Background.* To improve the outcome of childhood NHL in India, the MCP 842 Protocol, a short duration intensive chemotherapy protocol was initiated in 1987. We present the results of this protocol in patients with B-NHL. *Objective.* To characterize the clinical features and treatment response of children & young adults (≤24 years) with B-NHL prospectively treated with TMH-MCP-842 protocol. *Methods.* 160 previously untreated patients ≤24 years of age with B-NHL (Burkitt's lymphoma [BL]: 107 and Diffuse large B-cell NHL [DLBL]: 53) were enrolled between 1987 and 2006. Treatment consisted of eight alternating cycles of two regimens, A (Cyclophosphamide, Adriamycin, Vincristine and Cytosine-arabioside) and B (Etoposide, Vincristine, Methotrexate, and Ifosfamide). Intrathecal methotrexate and cytosine arabinoside were administered in the first 4 cycles. No radiotherapy or high dose methotrexate was given. The protocol was modified in 2003 with addition of COP prophase, low-dose rasburicase in patients with clinical tumor lysis syndrome and optimization of dose intensity with granulocyte colony stimulating factors. *Results.* The median age was 9.3 years (range 2-24 years), M: F ratio of 4:1, Sites: abdomen-66%, head and neck region-13%, peripheral lymph nodes-11%, mediastinum-5% and others-5%; stage I-20 (12%), II-40 (25%), III-89(56%) and IV-

11(7%). The response rate was 94% (151/160) with complete responses in 83% (133/160) patients. There were 22 relapses (13%) at a median time of 6 months from the diagnosis. Majority (75%) of the relapses occurred on treatment and most of these (88%) occurred in the primary sites of disease. 10 year EFS analyzed stage wise was 76% and 73% for localized stages (I & II) and advanced stages (III & IV) respectively with an overall EFS of 74% and OS of 82.7%. The EFS improved from 68% to 86% and relapse rate decreased from 17% to 3% after modification of protocol in 2003 ( $p < 0.05$ ). The toxic death rate in this study was less than 5%. The average cost of protocol was \$400 for the entire therapy. **Conclusions.** These results prove that Modified MCP-842 protocol is highly efficacious, safe, and cost-effective in patients with B-NHL. Minimal supportive care requirements, outpatient therapy, absence of high dose methotrexate and low-cost make it ideal for resource poor countries.

### 038

#### **ANALYSIS UPDATE OF THE PROGNOSTIC ROLE OF MINIMAL RESIDUAL DISEASE IN B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA OF CHILDHOOD TREATED IN THE AIEOP PROTOCOLS**

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**Background.** We recently reported the prevalence of t(8;14) at diagnosis and the response kinetics to treatment of Minimal Residual Disease (MRD) in B-cell acute lymphoblastic leukemia (B-ALL) patients treated according to the protocol of the Italian Association of Pediatric Hematology and Oncology (AIEOP) LNH-97. **Objectives.** The aim of this study was to update the prospective analysis of MRD in a larger series of B-ALL patients and to evaluate its impact on prognosis. **Patients and Methods.** A total of 81 children affected by *de novo* B-ALL enrolled in the AIEOP LNH-97 clinical protocol were studied. Bone marrow aspirate from all of the patients was analyzed for the presence of IgH-MYC rearrangements derived from the t(8;14)(q24;q32) by LD-PCR at diagnosis, after the first chemotherapy cycle and after subsequent cycles until negative for MRD. Morphological and immunophenotypical analyses were centrally reviewed. **Results.** A total of 55/81 cases (68%) were positive for IgH-MYC rearrangements. MRD response kinetics was determined in 45/55 patients. All of them reached clinical CR and 37/45 became MRD negative after the first chemotherapy cycle. The 3-year relapse free survival (RFS) was 38% (SE=17%) in patients MRD positive after the first chemotherapy cycle compared to 86% (SE=6%) in MRD negative patients ( $p < 0.0001$ ).

There was no difference in RFS for children who reached a clinical CR after the first chemotherapy cycle vs. those who became negative later: 77% (SE=8%) vs. 80% (SE=10%) ( $p = 0.9$ ), respectively. In multivariate analysis (taking into account CNS involvement, age, gender, white blood cell counts, LDH, organ involvement, tumor mass, MRD status after first cycle) only MRD was predictive of higher risk of failure: Hazard Risk 8.3 (2.4-29.3)  $p = 0.0009$ . **Conclusions.** This study confirmed on a larger series of patients that MRD has a negative prognostic impact in B-ALL and suggests that a better risk-adapted therapy, possibly including the use of anti-CD20 monoclonal antibody, should be considered in this selected group of patients.

### 039

#### **UCL1: A DE-UBIQUITINATING ENZYME THAT CONTRIBUTES TO THE DEVELOPMENT OF BURKITT LYMPHOMA**

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**Background.** We previously observed a dramatic overactivity of the de-ubiquitinating enzyme UCHL1 in various forms of B-cell malignancy. Whether UCHL1 expression in these cells contributes to, or is a consequence of, malignant transformation is unclear. **Objectives.** Our primary objective was to determine the role of UCHL1 in the malignant transformation of B-lymphocytes *in vivo*. Our secondary objective was to identify the molecular mechanisms behind any observed tumorigenic effects. **Methods.** We generated a transgenic mouse expressing UCHL1 in all tissues (UCHL1HATa). Two clones containing single transgene integrations were analyzed. To assess the impact of excess UCHL1 on the development of Burkitt lymphoma we mated UCHL1HATa mice to the well-described model E?-MYC that harbors the immunoglobulin heavy chain enhancer upstream of the Myc oncogene. To determine if UCHL1 can contribute to the development of other cancers we performed a tumor bioassay in which we treat pups with the carcinogen DMBA and observe for tumors at 5 months of age. Mechanistic studies were performed using several malignant B-cell lines derived from patients with multiple myeloma. RNA interference involved lentivirus-encoded miR30-based constructs targeting UCHL1 compared to non-silencing controls. **Results.** UCHL1HATa:E?-MYC mice developed lymphoma with 100% penetrance and at an accelerated rate compared with E?-MYC littermates (median lifespan 12 vs. 21 wks respectively). 35% of UCHL1HATa:E?-MYC mice develop abdominal masses compared with less than 5% of E?-MYC mice. Tumors from UCHL1HATa:E?-MYC mice have a starry sky histology and are comprised of B220+, CD3- lymphocytes. In the DMBA tumor bioassay we observed a striking increase in the number of skin tumors in UCHL1HATa mice compared with wild-type littermates. In mechanistic studies, depletion of UCHL1 in malignant B-cell lines led to a dramatic loss of cell viability with cell death occurring coincidentally with UCHL1

depletion. UCHL1 knockdown was accompanied by a dramatic loss of IL-6 or IGF-1 induced phosphorylation of AktSer473. **Conclusion.** UCHL1 is an oncogene that contributes to the development of Burkitt lymphoma and perhaps other cancers. UCHL1 provides a potent survival signal to malignant B-cells at least in part due to enhanced Akt signaling. These data may support the development of UCHL1 inhibitors as anti-neoplastic agents.

#### 040

##### **IS CYCLOPHOSPHAMIDE ALONE SUFFICIENT FOR THE PATIENTS WITH ADVANCED STAGE BURKITT LYMPHOMA IN AFRICA? A STUDY OF THE FRENCH-AFRICAN PEDIATRIC ONCOLOGY GROUP (G.F.A.O.P.)**

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**Background.** a first prospective multicentric study was performed by GFAOP in 2001-2004, aiming to assess the feasibility of adapted LMB 89 protocol in Africa. Two schemes of different intensity were tested. Overall survival (OS) was 61%. (Harif, BPC, 2008) It was concluded that adapted multidrug chemotherapy could be used successfully in Africa. However toxicity and cost were a matter of concern. In parallel, P.Hesseling (SIOP meeting, 2005) reported the possibility of curing about 50% of African patients with cyclophosphamide (CPM) alone: 1.2 g/m<sup>2</sup> D1, 8, 15, +3 additional courses in st 3+4. **Objectives.** to reproduce and to improve results of P Hesseling *et al* with CPM alone, by adding a "rescue" in cases of poor response to CPM. Rescue is made of 2 COPDM followed by 2 CYM courses. Six sub-Sahara units participated to the study. **Results.** From 04/05 to 03/09, 227 patients (pts) are evaluable: 22 st 1, 34 st 2, 165 st 3, 6 st 4 CNS neg (CNS+ and ALL were not included). 907 CPM were given, with toxicity in 18% of courses. 15 pts died of toxicity (7%). The response to CPM was CR in 91 pts (43%), but 25 relapsed. In total, 104 pts (46%) received the "rescue" either for PR or for relapse: 36 pts of them attained CR and 22 died of toxicity. The OS after CPM alone (rescue being considered as failure) is 30% for all pts, 41% for st 1+2, and 25% for st 3. The OS including rescue is 53% for all pts, 74% for st 1+2, 43% for st 3. To notice that during the last year, rescue was started earlier after 3 courses and outcome seems to be better. **Conclusion.** Inclusion rate was satisfactory.

Limited toxicity was observed with CPM alone, but the strategy was not satisfactory for st 3. Starting earlier the rescue for pts not in CR after 3 courses might be a direction to improve outcome.

#### 041

##### **POOLED DATA OF THE SFCE LMB 96, LMB 2001, BFM 95 AND BFM 04 STUDIES: SIMILAR RESULTS WERE OBSERVED**

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**Background.** In order to prepare a common study on rituximab and to study the possibility of doing a study on the model of the MinT study, each country continuing to use its protocol, data of the 2 more recent French LMB and German-Austrian-Switzerland NHL-BFM studies were pooled to analyse and compare their results. In the LMB regimen, patients (pts) are stratified in 3 risk groups (A, B,C) depending on resection, stage (st) and CNS involvement, and receive 2, 4/5 or 8 courses of chemotherapy (CT). In the BFM regimen, pts are stratified in 4 groups (R1, R2, R3, R4) adding LDH level in the stratification and receive 2, 4, 5 or 6 courses. Although courses are differently designed and drug dosages are different in the 2 regimens, drugs are the same: HDMTX, corticosteroid, vincristine, cyclophosphamide ( $\pm$ fosfamide), ara-C (HD in advanced st), doxorubicine, and  $\pm$ VP16. **Methods.** Data of the BFM 95 (Woessmann, Blood 2005), the ongoing 04 studies, the SFCE part of the FAB LMB 96 (Cairo *et al*, Patte *et al*, Blood 2007, Gerrard *et al*, BJH 2008) and the ongoing LMB 2001/03 studies were merged. **Results.** there were 691 pts in the LMB (07/96-12/07) and 935 pts in the BFM (04/96-12/07) studies. For the following analysis, PMLBCL were excluded (21 and 21), and results given for LMB and BFM in this order. 4y EFS was 90% and 89% (SE=0.01) respectively (NS). The 4y EFS for patients diagnosed between 1995 and 1999 was 89% for LMB and BFM, for 2000-2002 it was 89% and 87% and for 2003-2005 90.5 and 89.5% respectively. By stage (st), 4y EFS was: 98% and 97% for st1, 96% and 98% for st2, 92% and 88% (NS) for st3, 85% and 76% (NS) for st4, 81% and 81% (NS) for L3ALL, 79% and 72% for the CNS+(NS). If considering the higher risk (HR) pts: st3 with high LDH (defined either as more than twice the upper normal value, or >500), st 4 and ALL (=R3 and R4 in BFM), 4y EFS is 85% (n=366 pts) and 84% (n=393 pts). While overall results were stable, variation was observed in subgroups over time within the same regimen: in BFM 95, with MTX 24h, 4y EFS of R3 and R4 was 92% during and 83% after the end of randomisation. In LMB, 3y EFS of gr C CNS neg was 89% in LMB 96 and 77% in LMB 2001. **Conclusion.** Results are similar in the LMB and BFM studies allowing doing a common study, e.g. on the question of rituximab for HR pts.

**042**  
**CIRCULATING EPSTEIN-BARR VIRUS DNA AS A MARKER IN PEDIATRIC B-NON-HODGKIN'S LYMPHOMA PATIENTS UNDERGOING CHEMOTHERAPY**

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**Background.** The presence of tumour-derived DNA in plasma and serum of cancer patients opens up new possibilities for detecting and monitoring cancer. Several studies have detected the presence of Epstein-Barr virus DNA (EBV-DNA) in the plasma of patients with EBV-associated lymphomas, but only few studies have presented quantitative data regarding the concentration of circulating EBV-DNA and the quantitative-temporal relationship during the therapy. **Objectives.** To determine the value of circulating EBV-DNA as a marker in patients with B Non-Hodgkin's lymphoma (B-NHL). **Methods.** A total of 32 patients with B-NHL were prospectively recruited for the study. Blood samples were collected at diagnosis, between chemotherapy cycles and at the end of treatment. The negative controls comprised 10 blood samples collected from healthy individuals, and as positive control, plasma samples were selected from 2 patients with EBV-positive NHL identified previously by *in situ* hybridisation (ISH). A Real-time quantitative method was developed for EBV-DNA detection toward the EBNA-1 region. The presence of EBV was confirmed by ISH in tumour cells. **Results.** In all cases, paired ISH assay and plasma EBV viral load quantification were blindly tested. EBV was detected by ISH in 7/32 paediatric patients with B-NHL. Quantitative estimates of EBV-DNA in plasma were obtained from 7 EBV+ patients while plasma EBV-DNA was not detected in 25 EBV-negative patients and 10 healthy controls. The median EBV-DNA level at diagnosis in EBV+ patients equalled 1280,0 copies/mL (range: 23-3436,0). EBV-DNA concentration in all controls and EBV-patients equalled 0 (zero) copies/mL. There was a strong correlation between plasma EBV viral load and EBV detection by ISH methodology (7/7 EBV+ and 25/25 EBV- patients,  $p=0.000$ ). EBV+ patients who attained a complete response to therapy became EBV negative in plasma after initiating therapy. EBV-DNA remained undetectable through all time points. Despite complete clinical and radiologic remission, one patient relapsed 3 months after the end of treatment when an increase of EBV viral load was detected (6355,0 copies/mL). **Conclusion.** This study indicated that both detection and monitoring of the viral load in plasma from B-NHL patients may be a useful non-invasive tool as well it may provide information related to diagnosis and treatment response.

**043**  
**RESULTS OF A MODIFIED BFM STRATEGY FOR THE TREATMENT OF B-CELL MALIGNANCIES IN ARGENTINA**

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**Background.** The use of BFM-based protocols for the treatment of B-cell malignancies in developing countries is successful. However, with increasing intensity, death on induction (DOI) became a challenge. In our previous study, the pEFS was 0.79 and the DOI was 9%. With the aim of reducing the DOI and improve pEFS, a modified strategy was developed at our center. **Methods.** This was a prospective study including 44 consecutive patients (2002- 2007). Risk groups included: R1 (completely resected), R2 (extra-abdominal incompletely resected or abdominal and LDH <500 UI/L), R3 (abdominal >500 UI/L, stage IV and B-ALL). R1 received a prephase followed by blocks A and B. R2 received a prephase followed by AA, BB, AA, BB blocks (MTX 2g/m<sup>2</sup>, 24 hour infusion) and R3 received a prephase and CC, AA, BB, CC, AA, BB. CNS+ patients received intensified intrathecal therapy. **Results.** Median age was 69 months. 5 patients had diffuse large B cell lymphoma and the remaining ones had Burkitt lymphoma. With a median follow up of 34 months, the overall 3-year pEFS was 0.83. R1 and R2 (n=17) pEFS=1. R3 (n=27 CNS=2) pEFS for patients with LDH<1000 UI was 0.89 while for those with LDH>1000 UI/L was 0.6. Events included: Relapse-no response in 4, DOI=2, death in CR in 1 and early death in 1. Patients with LDH >1000 UI had a 10% DOI rate. **Conclusions.** pEFS and DOI improved comparing our previous results. However, DOI remains high in patients with higher tumor burden. A new strategy will be pursued for reducing it.

**044**  
**B-CELL NON-HODGKIN'S LYMPHOMA IN 2 INDIAN CHILDREN WITH ATAXIA TELANGIECTASIA**

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**Background.** Ataxia telangiectasia (AT) is a rare inherited disorder that affects 1 in 40,000 to 100,000 people worldwide. It is caused by mutations in the ATM gene which provide instructions for making proteins that plays an important role in the normal development and activity of several body systems, including the nervous system and immune system. Hence, children with this disorder has multiple neurological problems such as progressive ataxia beginning in early childhood, chorea, myoclonus, neuropathy and oculomotor apraxia. Small clusters of enlarged blood vessels called telangiectases, which occur

in the eyes and on the surface of the skin, are also characteristic of this condition. Due to their weakened immune system, many develop chronic lung infections and are at an increased risk of developing cancer, particularly leukemia and lymphoma. We report 2 cases of B-cell non-hodgkin's lymphoma (B-cell NHL) in 2 children with AT in the last 3 years, both from the Indian ethnic group treated at our centre. *CASE 1.* A 9 and a half-year-old Indian boy presented with a 2 month history of weight loss and increasing abdominal pain followed by billous vomiting. Six months earlier, he started to have unsteady gait and progressive weakness. About the same time he started to have abdominal pain he was already unable to walk unaided. His parents are second cousins. On examination he was weak and cachexic. He has bilateral scleral telangiectasia and some cerebellar signs such as past pointing and truncal ataxia but no nystagmus. He had generalized weakness but no significant sensory deficit. A large abdominal mass was felt in the right iliac fossa. An ultrasound abdomen was done followed by a computed axial tomographic (CT) scan of the abdomen which revealed a large enhancing mass measuring 8x12x9 cm arising from the caecum/appendix region with mesenteric lymph node enlargement just superior to the mass measuring 2.9x4.9 cm. A biopsy of the mass confirmed it to be B-cell NHL. A raised  $\alpha$ -feto protein level supports the diagnosis of AT. He was treated with the COMP chemotherapy regime consisting of vincristine, cyclophosphamide, methotrexate and prednisolone. Unfortunately after the first course of chemotherapy, he had a loss of vision and seizure. Magnetic Resonance Imaging scans suggests him to have reversible posterior leucoencephalopathy (RPLE). At this point his abdominal mass was not palpable anymore. His parents decided to seek alternative treatment in India. He was still alive 1 year 4 months after the chemotherapy. *CASE 2.* A 5-year-old Indian girl presented to the ear, nose and throat (ENT) surgeon for snoring for 3 months associated with symptoms of allergic rhinitis and followed later by dysphagia. She was also on follow-up by the paediatric neurology and genetic team for AT and is being treated for bronchial asthma as well. Her parents are non-consanguineous. On examination she had obvious truncal ataxia and was breathing from the mouth. There were bilateral conjunctival telangiectasia. She had unilateral enlarged tonsil, the right one crossing the midline. There were no masses and lymph nodes elsewhere. Bilateral tonsillectomy was done and it showed that the right tonsil was infiltrated by malignant lymphoid cells, histochemistry staining for positive for CD 30 and CD 71-???Radiological assessment showed that she has only some small lymph nodes in the posterior cervical region. The girl was and treated as Stage I disease and chemotherapy was given according to the COMP protocol for 6 months. She was well on follow-up. *Discussion.* Although individuals with ataxia-telangiectasia usually live into adulthood, their life expectancy is reduced. The major cause of death is sinopulmonary infection and cancer. B-cell lymphoma are known to occur more in children and T-cell lymphoma occurs more in adults with AT. Taylor *et al.* however suggests that there is a five-fold increase in frequency in T-cell tumours in AT patients compared to B-cell tumours and it can occur at any age.

This is because they are preferentially more deficient in T-cell compared to B-cell function. Reports from the west suggests that the risk of malignancy is higher in blacks compared to whites. We have no such data from the Asian region but both our patients are from the Indian ethnic group, the third largest in Malaysia after the Malays and the Chinese.

**045  
PRIMARY MEDIASTINAL LARGE B-CELL  
LYMPHOMA (PMBCL) AFTER NODULAR  
SCLEROSIS (NS) HODGKIN'S LYMPHOMA (HL)  
IN CHILDHOOD: REPORT OF 3 CASES**

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Since 1983 to December 2008 the AIEOP registered 1609 pts in 4 consecutive protocols for therapy of HL in childhood. Although the good prognosis of HL, 190 of these patients relapsed; PMBCL was identified in 3 boys (1.6%). The incidence of secondary (s) NHL following HL in adults varies between 0.5% and 5.9%, with a higher risk rate in lymphocytic predominance subtype. In childhood and adolescence sNHL is rare and informations on outcome are scarce. Primary site of HL in our 3 pts, aged 13, 16 and 17 yrs respectively, was cervical/supraclavicular lymph nodes, associated with mediastinal mass (MM) (stage IIA), in two cases with M/T > 0.45 and jugular thrombosis (stage IIB because of fever) or multiple chest nodules (stage IVA). Diagnostic biopsy showed a NS subtype, CD20<sup>+</sup> in all cases and CD15<sup>+</sup> in 2/3 pts. First line therapy was successful for 2 pts: the stage IIA pt achieved CR after 3 ABVD; the stage IVA pt received 6 COPP-ABV and subsequent local 14.4 Gy RT. PMBCL was diagnosed 6 and 8 mos after stop therapy respectively. Both pts presented a MM, which was biopsied. Further therapy of the stage IVA pt consisted in AA & BB cycles, according to the AIEOP-LNH'97 protocol (BFM oriented), followed by 2 ICE+ 4 Rituximab; MM surgical removal and autologous SCT completed treatment and the pt is in IICR after 30 mos of FUP. The stage IIA 18-yr-old pt underwent mediastinal resection and received a 6-month CT in a hematologic unit for adults; he is off-therapy in IICR after 8 mos. The last boy showed a PD during the 4<sup>o</sup> cycle of COPP-ABV. Initially suspected as relapse of HL, the MM was biopsied because resistant to IEP and DHAP CT and sPMBCL was identified. AA-CC-CC+ 6 Rituximab were given but PD with renal and cerebral involvement was observed and the pt died after 12 mos from HL diagnosis. Specimens revision by an expert pathologist panel confirmed the primary diagnosis of NS-HL and sPMBCL in all three cases. Characteristics of our experience are the occurrence in NS-HL and a very short interval between HL and sNHL, that could support the hypothesis that sNHL is caused by a clonal expansion of a common B-precursor cell. However, it should be confirmed by histopathological analyses. From a clinical point of view, when a relapse or a resistant PD is suspected, biopsy is mandatory.

**046**  
**BURKITT-LIKE LYMPHOMA AND SUBSEQUENT**  
**APLASTIC ANEMIA IN AN EBV NEGATIVE**  
**16-YEAR OLD BOY WITH X-LINKED**  
**LYMPHOPROLIFERATIVE DISEASE**

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*Background.* X-linked lymphoproliferative disease (XLP) is a rare primary immunodeficiency most commonly manifesting after Epstein-Barr virus (EBV) infection. The typical phenotype includes fulminant infectious mononucleosis, dysgammaglobulinemia and NHL. Aplastic anemia is among its other rare symptoms. *Case.* We present an adolescent patient with Burkitt-like lymphoma (BLL). In pre-school age he suffered from recurrent middle ear infections, eufunctional goitre and mild growth retardation were noted 2 years before BLL diagnosis. He presented with generalized lymphadenopathy, pleural effusions and poor nutritional status at the time of lymphoma diagnosis. His immunoglobulin levels and basic lymphocytic subpopulations were normal; he was EBV and CMV seronegative. Treatment according to BFM B-NHL 2004 protocol was administered. Due to neutropenic enterocolitis after the 1st cycle, 2 doses of rituximab (375 mg/m<sup>2</sup>) were given for chemotherapy delay. The treatment was finished in January 2008. Three months later, he presented with pancytopenia (WBC 1.6x10<sup>9</sup>/L, Hb 46 g/L, platelets 55x10<sup>9</sup>/L). Based on the findings in bone marrow (BM) aspirate and biopsy, diagnosis of severe aplastic anemia (SAA) was made, while excluding BLL relapse. Excess of activated TCR  $\gamma/\delta$  positive T cells (CD8<sup>+</sup>) with no clearly documented clonality tested by PCR was found. Serology due to IVIg administration was not informative, EBV, CMV and HHV6 PCR was negative in the periphery and BM, copies of parvovirus B19 were found in low numbers in BM. The coincidence of BLL and SAA initiated an analysis of SH2D1A gene, a deletion of exon 1 was found. The patient was transplanted with peripheral blood stem cells from his healthy 9/10 HLA-matched brother. Currently, he is 8 months after transplantation, clinically well, not requiring any immunosuppression neither substitution. The same deletion in SH2D1A was found in 24-year old patient's maternal cousin who suffers from mutilating epidermolysis-like skin anomaly and mild psychomotor retardation. He is EBV negative and has never suffered from symptoms ascribed to XLP. *Conclusions.* A molecular defect in SH2D1A can be underlying cause in EBV-negative BLL. The genetic testing may reveal family members at risk of disease and

they should be followed closely.

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**047**  
**TREATMENT OUTCOME IN CHILDREN WITH**  
**B-NON HODGKIN'S LYMPHOMA WITHOUT AND**  
**WITH MONOCLONAL ANTIBODIES IN CROATIA**

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*Background.* B-non-Hodgkin's lymphomas (B-NHL) are a group of highly aggressive malignant lymphoproliferative diseases that require rapid diagnosis and prompt therapy initiation. *Objectives.* The aim of the study was to confirm excellent results recorded in the BFM-NHL trial in Croatia, and to identify appropriate measures to prevent the life-threatening events frequently accompanying the early phase of treatment. *Patients and methods.* During the 1990-2008 period, 29 children with B-NHL (15 male and 14 female) aged 2-16 (mean age 9.3) years, were treated according to the NHL-BFM protocol. Therapy consisted of 5-day pretreatment (standard chemotherapy dosage) combined with 2-6 cycles of high-dose chemotherapy; in addition, five patients received monoclonal antibodies (Mabtera) in 2006. Patients were divided into three risk groups. *Results.* Complete remission was achieved in all 29 (100%) patients; disease relapse and lethal outcome were recorded in four (13.7%) patients, i.e. three patients on chemotherapy alone and one patient also administered monoclonal antibodies (meningeal relapse 2 months of treatment completion); in 25 (86.2%) patients, the first complete remission has been persisting to the present. Grade III and IV toxicity was mostly observed after the first and second cycle of chemotherapy. The level of toxicity associated with the first cycle of chemotherapy was considerably lower in patients administered monoclonal antibodies. In one patient, secondary tumor disease (AML) developed 4 years of treatment discontinuation. *Conclusion.* Although referring to a relatively small number of patients, therapeutic results were very good and consistent with those reported from other European centers. However, many questions remain to be answered. For instance, should monoclonal antibodies be administered in all patients with B-NHL, or in the high risk group only, or during the first chemotherapy cycle only, or in patients with disease relapse only, or as maintenance therapy in patients with B-ALL/NHL?

**048**  
**RITUXIMAB IN THE FIRST LINE THERAPY OF**  
**ADVANCED B-MATURE LYMPHOMA AND**  
**LEUKEMIA IN CHILDREN AND ADOLESCENTS:**  
**PRELIMINARY RESULTS**

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*Background.* To improve survival rate while decrease toxicity of induction chemotherapy was the aim of inclu-

ding of rituximab in to the first line therapy of high risk childhood B-NHL/ALL. *Methods and patients.* Since October 2004 28 patients (pts) diagnosed with advanced B-cell neoplasms were treated with modified B-NHL-M-2004 (Russia) protocol. Including criteria were stage III-IV/B-ALL if LDG>2N, and primary mediastinal B large-cell lymphoma (PMBLCL). M/F ratio was 3/1, median age -14.3. Histologically Burkitt lymphoma (BL) was in 12 cases (43%), immunoblastic B large-cell in 1(4%), PMBLCL in 7(25%) and B-ALL in 8 (29%). Median LDG level was 2600MU/l. Patients received 6 courses of chemotherapy similar to NHL-BFM(R4) regimen added with rituximab 375mg/m<sup>2</sup> on day 0 in blocks 1-4. The dose of MTX was reduced to 1g/m<sup>2</sup> in the first and the second courses. *Results.* We observed moderate infusion reactions on rituximab in 4 pts,  $\gamma$ -globulin level less then 2.5 g/L in 4 pts, late neutropenia less then 1000/ $\mu$ L in 2 pts. Time to restore CD20<sup>+</sup> cells level ranged from 1.5 to 16 months. With a median FU of 22 months the probability of EFS for 28 pts was 0.79, FFS-0.92. Complete remission was achieved in 25(89%) pts, 11 of them had early and 14 - late response. Three (11%) pts died because of toxicity. Tumor progression/relapse occurred in 3(11%) pts. Probability of EFS was 0.75 for 8 children with B-ALL (no cases of death because of toxicity) and 0.86 for 7 pts with PMBLCL (no cases of disease failure). *Conclusions.* Rituximab is well tolerable in children. High efficiency of combined therapy is obvious, especially for pts with PMBLCL. Dose rescue in the first course of induction is an advantage, but not in the second one for pts with BL and B-ALL. The optimal number of injections of rituximab remains unclear.

**049**  
**RETROSPECTIVE STUDY OF B-CELL NON HODGKIN'S LYMPHOMAS ON CHILDHOOD: 20 YEARS EXPERIENCE**

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*Background.* Favourable results had been reported from previous studies on childhood and adolescent B-cell Non Hodgkin lymphoma, with overall survival rates of 90%. *Objectives.* The purpose of our study was to analyze epidemiological data, clinical presentation, event free and overall survival after 20 years of experience in the treatment of Burkitt and Diffuse large B-cell lymphomas. *Methods.* A retrospective revision was made to identify all B-cell Non Hodgkin's lymphoma cases diagnosed in children  $\leq$ 18 years old in the Niño Jesus Hospital from 1.987 to 2.007. Statistical analysis was performed with SPSS 15.0 program. Event free survival (EFS) and overall survival (OS) were calculated using Kaplan-Meier analysis and Logrank tests. *Results.* Between 1987 and 2007, sixty five patients were diagnosed of B-cell Non Hodgkin's lymphoma. Mean age was 6.6 years (range 2-16) and median age 7 years. Overall, a majority of patients were male (77%). The distribution of stages was 7, 8, 38 and 12 patients with stages I, II, III, and IV, respectively. Fifty one patients (78%) achieved complete remission with current therapy. Eleven of them relapsed.

The median time to relapse from the start of treatment was 11.9 months (range 3-44). In spite of rescue therapy, seven of these patients died from their disease. With a median follow-up of 4.4 years from diagnosis, fifty seven patients are alive in complete remission. Eight have died, six from their disease, one from infectious and therapy complications, and the last one from a second tumor. The probabilities of overall and event free survival at 5 years were 85 $\pm$ 5% and 80 $\pm$ 5%, respectively. *Conclusion.* Prognosis of childhood B-cell Non Hodgkin's lymphomas has improved considerably on the last twenty years, with similar overall survival rates than those achieved for acute lymphoblastic leukemia. Our sample shows a comparable event free and overall survival with other previous studies.

**050**  
**IMPROVED OUTCOME OF RELAPSED/REFRACTORY CHILDHOOD B-CELL NON-HODGKIN'S LYMPHOMA WITH RITUXIMAB PLUS INTENSIVE CHEMOTHERAPY FOLLOWED BY AUTOLOGOUS TRANSPLANT**

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*Background.* Children with B-cell Non-Hodgkins Lymphoma have an event free survival of over 90%, but prognosis following relapse or primary refractory disease is poor. Rituximab is a chimeric anti CD20 monoclonal antibody which has shown single agent efficacy in adult studies and synergy with chemotherapy. Since standard chemotherapy has such good results, no data exists in children. However given the results from adult studies it is being used more and more in children with relapsed/refractory disease who have traditionally had a survival of less than 10%. We present 5 cases treated with Rituximab plus intensive chemotherapy followed by autologous transplant showing improved outcome. *Patients and Methods.* Five children with relapsed/refractory disease, were treated at the Royal Marsden Hospital between February 2004 and January 2009. Three patients were male and 2 female. Age range was 2.6-17 years (mean 12.8 years). Four patients had diffuse large B cell lymphoma (DLBC) and one Burkitt's Lymphoma (BL). One out of four patients with DLBCL, was initially treated as Anaplastic large cell Lymphoma (ALCL). Four patients were treated according to with FABLM96 group B and one on the group C protocol. At end of the primary treatment, three patients achieved complete remission (CR), and two had progressive disease. Out of three patients who achieved CR, the duration of the CR was between 3 and 7 months (mean 4.6 months). At relapse one patient was treated with 2 courses of CYVE, and two patients were treated with 2 courses of CYVE followed by HD Methotrexate and Rituximab. All achieved complete remission. Two patients were treated with Rituximab plus Ifosphamide, Carboplatin and Etoposide (R-ICE) for 2 cycles. One patient achieved partial remission and the other is undergoing treatment. Four patients have gone on to have (CCNU, Etoposide, Cytarabine and Melphalan (BEAM) conditioned autologous peripheral

stem cell transplant and are currently in CR after a follow-up of 17-40 months (mean 25 months). The fifth patient will have an autologous about 2 months time. *Conclusion.* Most relapses in patients with DLBCL/BL, still occur within the first 12 months of coming off treatment. Patients respond to second line chemotherapy and response rate has improved with the addition of Rituximab. This allows patients to have consolidation therapy with autologous peripheral stem cell transplant, and achieve long term disease control. Further larger studies are required, but our data shows that in contrast to published data, even patients with relapsed/refractory disease can be salvaged.

**51**  
**PRIMARY MEDIASTINAL LARGE B-CELL LYMPHOMA: THERAPEUTIC OPTIONS AND EVALUATION OF REFRACTORY DISEASE**

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*Background.* The primary mediastinal large B-cell lymphoma usually presents as mediastinal mass. Other therapeutic options can be associated to chemotherapy: radiotherapy, the monoclonal antibody anti-CD20 (Rituximab) or stem cells transplantation. *Objectives.* Case report of a primary mediastinal large B cell lymphoma treated with rituximab. We present a 14-year-old with a mediastinal mass. Computer tomography (CT) shows a mediastinal mass of 7x5x4 cm. Biopsy of the mass was done by thoracostomy, being the diagnosis a diffuse large cell lymphoma. Immuno-histochemical analysis shows positivity for LCA and antigen of the B cell line (CD20 and CD79) being negative CD15, CD30, EMA, ALK-1 and prot S-100. *Results.* With the diagnosis of primary mediastinal large B cell lymphoma chemotherapeutic treatment begun with COP and two cycles with Rituximab at 375 mg/m<sup>2</sup>. At re-evaluation by PET hypermetabolic lesions were suggestive of active tumoral mass in mediastinum. Consolidation therapy was continued with CYVE 1 and 2 and with Rituximab. After maintenance treatment (x4:) persisted a paramediastinal mass of 1.2x1.1 cm in CT and positive capitation in PET. Due to the absence of complete remission treatment with local radiotherapy was started (40 Gy in 2 Gy/day) and transplant of hematopoietic stem with high-dose chemotherapy with BEAM (Carmustine, Cytarabine, Etoposide and Melphalan). At re-evaluation a CT was done with residual mediastinal mass seen with similar characteristics, and PET, in which no enhanced glucose capitation, so that the patient was considered in non-confirmed complete remission. He is now 18 months off therapy. *Conclusion.* In B cell lymphomas with positive immunohistochemistry for CD20 the use of monoclonal antibody anti-CD20 (Rituximab) should be considered in combination with standard chemotherapy. In our case Rituximab was well tolerated although complete remission was not obtained. In those cases, in which no complete remission after chemotherapy was obtained the use of local radiotherapy on the residual mass and consolidation therapy with hematopoietic stem cell transplant have to be weighed up like in

our patient. -Considering the poor relationship between the presence in CT of the residual mass after chemotherapy and the activity of the disease, PET can be very useful in evaluating treatment response and the need of additional treatment.

**052**  
**INVASIVE FUNGAL INFECTION (IFI) AND FECAL PERITONITIS IN PATIENT WITH B CELL LYMPHOMA**

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*Background.* Invasive fungal infection (IFI) are frequent and life threatening complication in patient with haematological malignancies. Patient at high risk for IFI are those with acute myeloid leukaemia (AML) and patients undergoing bone marrow transplantation. Patient receiving treatment for B-Cell Non-Hodgkins Lymphoma (B NHL) are susceptible to IFI due to use of steroid and neutropenia. Fungal sepsis associated with ileal perforation complicating therapy for B-Cell Non-Hodgkins Lymphoma (B NHL) is very rare. We report these complications and their management together with a review of current literature. *Objectives.* To highlight clinical features, management and outcome of a rare complication (IFI with ileal perforation) in a case of B NHL treated with UKCCSG B NHL 2003 Guidelines Group B. *Design/Methods.* Interventional case report of a 14 year old boy diagnosed with abdominal B NHL who received COP (Cyclophosphamide/Vincristine/Prednisolone) induction chemotherapy followed by first cycle of COPADM (Cyclophosphamide/Vincristine/Prednisolone/High dose Methotrexate) one week later. He had greater than twenty percent (20%) response at day 8 of treatment and was stable at discharge. He presented five days later with diarrhoea, vomiting and fever associated with profound hypotension. Patient required cardio respiratory support. Investigations revealed *Candida albicans* septicaemia along with ileal perforation resulting in fecal peritonitis. Appropriate antifungal therapy along with surgical intervention were instituted. *Results.* Patient died despite treatment after 43 days in Paediatric Intensive Care Unit. *Conclusion.* Approximately 90% of paediatric patients with B NHL treated with current intensive chemotherapy regimen have long term disease free survival. It is crucial that therapy is instituted promptly and without any interruption for such results. Patients with B NHL are at high risk of getting IFI and this can complicate ongoing treatment. New strategies for IFI prevention such as use of protected environment and use of broad spectrum antifungal prophylaxis should be complimented with due vigilance in patients receiving treatment for B NHL according to UKCCSG B NHL 2003 Guidelines.

**053**

**TREATMENT OF PEDIATRIC B-NHL LYMPHOMA WITH PROTOCOL NHL-BFM-95 AND RITUXIMAB: A SINGLE CENTRE EXPERIENCE**

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**Background.** Rituximab mediates complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity of CD20-positive human B cells and sensitizes B-NHL cells to cytotoxic chemotherapy. In adults rituximab is effective in patients with aggressive B-NHL. In pediatric population, data of rituximab clinical effect are not adequately documented. **Objectives.** The aim of this study was to evaluate the therapeutic efficacy and toxicity of the modified Berlin-Frankfurt-Munster (BFM)-95-based protocol + rituximab in children with Burkitt's lymphoma (BL) and diffuse large B cell lymphoma (DLBCL). **Design/Methods.** From Jan 2006 to Dec 2007, 6 patients with B-NHL (age 4-16 yr) were enrolled in the study: 3 with BL (2 patients with BL and 1 with L3-ALL, respectively), and 3 patients with DLBCL (2 patients with disease gr II and one patient with disseminated disease gr IV, respectively). In all patients the lymphoma cells expressed CD20 cells. The patients have not been previously treated, and at the beginning of therapy there were no blasts in cerebrospinal fluid. The patients received infusion of rituximab at a dose of 375 mg/m<sup>2</sup> 5 days before of each block of chemoth. Four patients with grade IV of disease received 6 doses of rituximab and 6 blocks of chemoth. Two patients with grade II of disease received 4 doses of rituximab +4 blocks of chemoth. **Results.** Complete remission of NHL/ALL was achieved in all six patients. Four of them achieved remission after 2 doses of rituximab +2 blocks of chemotherapy, and two patients after 4 doses of rituximab +4 blocks of chemotherapy (one patient with grade IV BL and one patient with grade IV of DLBCL, respectively). Five patients are still in complete remission (16, 17, 19, 20 and 21 months after the completion of therapy). One patient (gr IV BL) experienced meningeal relapse 2 months after completion of therapy, followed by hematological dissemination of disease and death 8 months after first relapse. Major adverse side effect of rituximab therapy was prolonged B-cell depletion. **Conclusion.** Rituximab plus B-NHL-BFM-95 protocol was well tolerated and proved to be effective in children and adolescents with B-NHL. However, a larger group of patients is needed to draw more precise conclusions of rituximab efficacy in the treatment of B-NHL.

**054**

**B CELL NHL IN A CHILD WITH HYPER IGE SYNDROME: TREATMENT RELATED INFECTIVE AND NEUROLOGICAL COMPLICATIONS**

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**Background.** Hyper IgE syndrome (HIES) is a primary

immunodeficiency syndrome characterized by high serum IgE level, eczema and infections. An association between lymphoma and HIES has been recognized. Significant complications of therapy are anticipated in these patients but not well reported in the literature. Autosomal recessive type of HIES (AR-HIES) is associated with severe neurological complications of unknown origin but such complications in relation to anti lymphoma treatment are not reported. **Objectives.** To report a case of B-NHL in a child with HIES which was successfully treated and to highlight infective and neurological complications during treatment. **Design/Methods.** A case study of a 14 year old girl with HIES who was diagnosed and treated for Diffuse large B cell Lymphoma (DLBCL) based on CCLG B-NHL guidelines, Group B disease. **Results.** A 14 year old girl was diagnosed to have HIES based on NIH scoring system. She is negative for STAT-3 gene mutation and has no family history of HIES. She developed DLBCL; bone marrow and CNS negative disease. She was treated with intensive systemic and intrathecal chemotherapy. During her treatment she had multiple bacterial (Staphylococcus aureus, Klebsiella), viral (HSV, Planar wart) and presumed fungal chest infections which were appropriately treated. She also received regular intravenous immunoglobulin. After the last dose of chemotherapy she developed an encephalopathic process with general neurological deterioration. Various investigations did not yield any evidence of infective, metabolic or autoimmune causes. As a diagnosis of exclusion it was considered to be secondary to treatment; possibly Methotrexate related toxicity. She is 12 months from the end of treatment and has remained in remission. Her neurological condition has improved slightly, but she remains incapable of independent living and significantly neurologically impaired. **Conclusion.** Children with HIES have higher chance of developing lymphoid malignancies. They also have a higher risk of infective complications during treatment. Patients with AR-HIES are known to develop severe neurological complications of unknown origin and this report further highlights that during anti cancer treatment clinicians should be alert to such complication and families must be made aware of such risks.

**055**

**T-CELL CD38 EXPRESSION IN B-CHRONIC LYMPHOCYTIC LEUKEMIA**

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**Background.** B-cell chronic lymphocytic leukemia (B-CLL) is a heterogeneous disease with some patients having an indolent course never needs treatment, while others having rapidly progressive one requires intensive treatment. In recent decades, numerous prognostic markers, such as IgVH mutational status, ZAP-70 and the expression of CD38 on leukemic cells were introduced to screen for patients likely to have progressive course of B-

CLL bearing the potential to facilitate risk-adapted treatment strategies. In B-CLL, T cell function is shown to be dysregulated. CD38 has been demonstrated to be an important transmembrane signaling molecule of T cell with a direct effect on its function. *Objective.* The present study was conducted to analyze CD38 expression on T cells in B-CLL patients. *Design and methods.* By using flow cytometry, CD38 expression on T cells were analyzed to evaluate its impact on the clinical course of 88 unselected B-CLL patients and correlate it with other risk factors. *Results.* CD38 expression level on T cells was shown to predict the clinical course of B-CLL in male patients but not in female patients. Male patients showed CD38 expression on T cells in a stage-dependent manner, in contrast to female patients who showed higher expression irrespective to clinical staging. CD38 expression on T cells negatively interacted with treatment-free survival in male patients. Multivariate analysis revealed that CD38 expression level on T cells is an independent prognostic factor in B-CLL male patients. *Conclusion.* A simultaneous evaluation of CD38 expression on both B-CLL cells and T cells allowed predicting male patient groups with the most favorable prognosis as well as those with the worst.

## SCIENTIFIC SESSION 5: LYMPHOBLASTIC LYMPHOMA

### BIOLOGY OF T CELL LYMPHOBLASTIC LYMPHOMA

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T Lymphoblastic Lymphomas (T-LBL) represent rare expansions of immature T lymphoid cells which occur preferentially in older children and younger adults and which frequently present with a mediastinal mass. They are closely related to T-Acute Lymphoblastic Leukemia (T-ALL) but the fact that they differ by the absence of significant blood and bone marrow (<25%) involvement complicates understanding of oncogenic mechanisms, in conjunction with their rarity and anatomic localisation. We adapted a TCR-based classification of T-ALL to T-LBL and demonstrated in a retrospective, predominantly adult, series that T-LBL with an intermediate, thymic cortical stage of maturation arrest demonstrated TCRG and TCRD rearrangements and HOXA or TLX1/3 transcripts and were of relatively good prognosis compared to T-LBL with an earlier or later stage of maturation arrest (Baleyrier *et al.* Clin. Can. Res. 2008). The predominant oncogenes which determine the stage of maturation arrest in T-ALL were also found in T-LBL, suggesting that other factors determine whether these immature T cell disseminate from the thymus or alternative sites of origin. We have therefore compared the mutational status of NOTCH1 and JAK1 and the neuropilin/semaphorin interface in T-LBL with T-ALL in both adults and children. Appropriate evaluation of the implication and exploitation of abnormalities of these pathways can only be undertaken within the context of prospective, multicenter clinical trials.

### CURRENT MANAGEMENT OF LYMPHOBLASTIC LYMPHOMA

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Lymphoblastic Lymphoma (LBL) account for approximately 25-30% of Non-Hodgkin's Lymphoma of childhood and adolescence. In the majority of cases the lymphoma cells derive from precursor T-lymphocytes of thymic or pre-thymic origin, while in approximately 25% of patients the lymphoma cells display a precursor-B-cell phenotype. Although LBL is widely considered biologically similar or even identical to acute lymphoblastic leukemia (ALL) recent molecular genetic studies suggest biological heterogeneity especially between T-LBL and T-ALL. The majority of patients present with advanced stage III or IV disease. Initial CNS-involvement can be diagnosed in 3-4% of patients. In large multicenter studies therapeutic programs based on treatment strategies for acute lymphoblastic leukemia (ALL) resulted in event-free survival probabilities of over 80 % even for

patients with advanced stage disease. Most of these combination chemotherapy programs contain corticosteroids, vincristine, anthracyclines, L-asparaginase, cyclophosphamide and anti-metabolites administered over several weeks and ordered in phases of induction, consolidation, re-intensification, and maintenance therapy. The impact of individual drugs to the patients cure remains largely unknown, however, due to the lack of randomized trials asking such questions. Intrathecally applied methotrexate is a standard procedure for prevention of CNS relapse while high-dose methotrexate is used in some strategies but not in others. Local treatment modalities such as local radiotherapy are rarely applied although their role in the treatment program was never prospectively evaluated in randomized trials on the basis of current highly efficient chemotherapy protocols. The same is true for pre-symptomatic cranial radiotherapy for prevention of CNS relapse while cranial radiotherapy is performed in most patients presenting with overt CNS disease at diagnosis. In the remaining patients who suffer from tumor failure relapse occurs very early, especially in T-LBL patients. In those patients mediastinal relapse alone or combined with other sites are the most frequent site of failure. The chance to survive after relapse to current intensive front-line therapy is poor, especially for T-LBL patients. Only patients with sufficient response to salvage chemotherapy followed by allogeneic haematopoietic stem cell transplantation have a significant chance to survive. There is a scarcity of prognostic factors applicable for the early identification of patients at highest risk for tumor failure allowing risk adaptation of therapy intensity. Especially genetic markers associated with treatment outcome are almost completely lacking mainly due to the lack of suitable tumor material for such analysis. Recent retrospective studies revealed that loss of heterozygosity at 6q14-24 confer a significantly increased risk of relapse of T-LBL patients to current treatment. This might be a first indication of the promise of more comprehensive genetic and biological studies for the future development of the treatment of these patients. Similar to ALL the kinetics of early response to treatment might have highly predictive value for treatment outcome also for LBL patients. However, in contrast to ALL, attempts to determine and evaluate the prognostic impact of early response to treatment by means of e.g. minimal residual disease monitoring or functional imaging using FDG PET are still in an initial stage of investigation. Thus, important challenges remain to be solved. Among them the identification of highly predictive prognostic parameters to early identify patients at increased risk for tumor failure, the evaluation of the impact of individual drugs to patients cure, optimal duration of treatment, the possible role of local therapy modalities, and the evaluation of novel treatment options for patients with highly refractory disease.

## 056

### **HIGH-DOSE METHOTREXATE AND EARLY INTENSIFICATION OF THERAPY DO NOT IMPROVE 3 YEAR EFS IN CHILDREN AND ADOLESCENTS WITH DISSEMINATED LYMPHOBLASTIC LYMPHOMA. RESULTS OF THE RANDOMIZED ARMS OF COG A5971**

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**Background.** The treatment of pediatric lymphoblastic lymphoma (LL) has developed in parallel with treatment strategies for childhood acute lymphoblastic leukemia using a BFM backbone. **Objective.** The excellent results of the NHL/BFM 90 trial prompted us to design this randomized factorial study to determine whether a regimen without high dose methotrexate (HDMTX) the CCG BFM will result in the same outcome as NHL/BFM-90 and whether intensification with anthracycline and cyclophosphamide would further improve disease free survival. **Design/Method.** From June 2000 to October 2005, 257 patients with Murphy's Stage III and IV (excluding CNS disease) LL were randomized to one of the four regimens. All regimens used the BFM/NHL95 backbone. The CCG BFM regimen had intrathecal (IT) methotrexate throughout interim maintenance and maintenance without IV methotrexate. The NHL BFM utilized I.V. Methotrexate 5 Gms/m<sup>2</sup> and intrathecal MTX every 2 weeks for four doses during interim maintenance without further intrathecal MTX during maintenance. One of each backbone regimens was further intensified with anthracycline and cyclophosphamide early in induction and delayed intensification. **Results.** The median age was 10.3 years, 195 (76%) were males; 43 (17%) had >5% bone marrow involvement. Twelve patients with CNS disease were not randomized and received intensification and HD HDMTX with delayed CNS radiation (data not reported here). Major toxicities have been related to bone marrow suppression with 4 toxic deaths, 3 due to sepsis and 1 from cerebral hemorrhage. The frequency of grade III/IV neutropenia (alone, with fever or with infection), anemia, and thrombocytopenia were higher in the intensified arms during induction. Three of the four toxic deaths occurred on the intensified arms. The three year EFS of the HDMTX vs. none is 84.5±3.5% vs. 82.7±3.8% (=0.93) and the intensification vs. none is 83.4±3.7% vs 83.0±3.6% (=0.66). Therefore, there was no significant difference between treatment arms.

**Conclusion:** These results suggest that neither HDMTX nor early intensification in the setting of BFM type ALL therapy improves EFS in LL. Future direction should

focus on identifying biological factors early in therapy so alternative therapies may be investigated.

**057  
FREQUENCY AND CLINICAL RELEVANCE OF DNA  
MICROSATELLITE ALTERATIONS OF THE  
CDKN2A/B, ATM AND P53 GENE LOCI:  
A COMPARISON BETWEEN PEDIATRIC  
PRECURSOR T-CELL LYMPHOBLASTIC  
LYMPHOMA AND T-CELL LYMPHOBLASTIC  
LEUKEMIA**

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**Background.** Deletions at cell cycle regulatory gene loci have long been reported in various malignancies. However, little is known regarding the relevance of these deletions in pediatric T-cell lymphoblastic lymphoma (T-LBL) and T-cell lymphoblastic leukemia (T-ALL). **Objectives.** The study focused on microsatellite alterations at three key loci of cell cycle regulatory genes. The frequency of alterations and their association with clinical and prognostic parameters were analyzed in T-LBL and T-ALL patients treated uniformly according to ALL-BFM-type strategies. **Design/Methods.** Eleven DNA microsatellites were analyzed in each patient, with markers localized at chromosome 9p flanking the CDKN2A/B locus encoding for p16INK2A, p15INK2B and p14ARF, at chromosome 11q flanking the ATM gene locus, and at chromosome 17p at the p53 gene locus. **Results.** 113 T-LBL patients and in 125 T-ALL patients were successfully analyzed. Loss of heterozygosity (LOH) at chromosome 9p was detected in 47% of T-LBL and 51% of T-ALL patients. LOH at 9p was more frequent in male than in female pts, both for T-LBL and T-ALL. In T-ALL, LOH at 9p was associated with favorable initial treatment response at day 8 and day 15. A tendency for favorable event-free-survival was observed in LOH 9p positive T-LBL patients but did not reach statistical significance. Frequency of LOH at chromosomes 11q and 17p was 5% or less for both diseases. **Conclusions.** In patients with pediatric precursor T-cell lymphoblastic lymphoma and leukemia, chromosome 9p with the cell cycle regulatory gene locus CDKN2A/B was affected by loss of heterozygosity in 47% and 51% of patients respectively. LOH at the ATM and p53 gene loci were rare events in both diseases. These findings indicate that the two diseases share common patterns of LOH of critical cell cycle regulatory gene loci. Interestingly, LOH at chromosome 9p was associated with favorable initial treatment response in T-cell lymphoblastic leukemia.

**058  
NOTCH1/FBXW7 MUTATIONAL STATUS DIFFERS  
QUALITATIVELY AND QUANTITATIVELY IN  
T-LINEAGE LYMPHOBLASTIC LYMPHOMA (T-LL)  
AND LEUKEMIA (T-ALL)**

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**Background.** The Notch pathway is a major determinant in hematopoietic and particularly T lymphoid lineage commitment and development. Constitutive activation of the Notch1 pathway as a result of activating mutations of NOTCH1 in the heterodimerisation (HD) and/or PEST domains and/or mutation of the ubiquitin-ligase FBXW7, is found in the majority of T ALL, where it is associated with a favorable prognosis. The clinical rationale for evaluating Notch1 status is also based on the availability of therapeutic  $\gamma$ -secretase inhibitors of this pathway. Notch1 pathway activation has not been extensively studied in T-LL, which is considered to be closely related to T-ALL. **Objectives.** To assess the incidence and the mechanisms of Notch pathway activation in T-LL compared to T-ALL. **Method.** 336 T-ALL (116 children/220 adults) and 96 T-LL (34 children/62 adults) were screened for Notch1 HD and PEST mutations and for FBXW7 mutations. Data were analyzed according to the stage of maturation arrest and to oncogenesis. **Results.** Notch1 pathway activation by Notch1 and/or FBXW7 mutation (N/Fmut) was significantly less frequent in T-LL (51%) than in T-ALL (69%);  $p=0.002$ . This was true for both Notch1 (48% vs. 58%;  $p=0.037$ ) and FBXW7 mutations (11% vs. 29%;  $p=0.035$ ) individually. PEST domain truncation alone or combined to HD mutation were more frequent in T-LL than in T-ALL ( $p=0.03$ ); HD mutations predominated in T-ALL and were more often combined with FBXW7 than PEST mutation. This is likely to reflect differences in ligand interaction. Incidence of double mutations (DB), known as the most potent activators of the Notch1 pathway, was similar in T-LL and T-ALL. N/Fmut were found at all stages of maturation arrest, but tended to be less frequent in mature cases, as defined by sTCR expression in T-ALL or TCR G+/D- PCR gene rearrangement profile in T-LL. With respect to cooperating oncogenes, T-ALL and T-LL N/Fmut cases tend to cosegregate with those demonstrating TLX1/TLX3 deregulation. In T-LL, all HD+PEST DB are associated with additional oncogenic abnormalities. **Conclusion.** Notch1/FBXW7 mutational status differs between T-LL and T-ALL both qualitatively and quantitatively. This suggests different oncogenic mechanisms, despite overlap for classical, maturation arrest defining, oncogenes. The prognostic impact of status in T-LLs is being addressed.

**059**

**OUTCOME OF NEWLY DIAGNOSED CHILDREN AND ADOLESCENTS WITH LOCALIZED LYMPHOBLASTIC LYMPHOMA TREATED ON COG A5971: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP**

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**Background.** Therapy for localized lymphoblastic lymphoma historically is similar to acute lymphoblastic leukemia. Localized lymphoblastic lymphoma is relatively rare and patients are typically included in small numbers on trials with patients diagnosed with disseminated disease. Shorter duration of therapy produced five-year event-free survival (EFS) of around 60%. **Objective.** The objective of this study was to determine the event-free survival for patients with localized lymphoblastic lymphoma treated on uniform acute lymphoblastic leukemia based therapy. **Design/Methods.** From June 2000 to July 2005, newly diagnosed patients with Murphy Stage I or II lymphoblastic lymphoma over the age of 1 year were enrolled. Pathology was centrally reviewed. Treatment was Children's Cancer Group BFM without the 4 additional day 28 intrathecal methotrexates during maintenance.<sup>1</sup> Duration of therapy was 24 months. **Results.** Fifty-seven eligible patients enrolled with localized disease (31 Stage I, 20 Stage II, and 6 unknown). Thirty-four (59%) were males and twenty-three (41%) were female. The median age was 7.3 years (range 1.4-24.5 years). Forty-five (79%) had pre-B immunophenotype, eight (14%) pre-T cell immunophenotype, and 4 (7%) were neither or unknown. Eleven (19%) had bone primaries. Median follow up on surviving patients was 4.5 years (range 1.4-8.1). Five-year EFS for localized disease was 85.2±7.5%. Five-year overall survival was 94.0±4.0%. No deaths occurred on therapy. **Conclusions.** This is the largest study of localized lymphoblastic lymphoma treated with uniform acute lymphoblastic leukemia based therapy. Most of the patients with localized disease had pre-B immunophenotype. With 24 months of CCG BFM therapy, 5 year EFS for localized lymphoblastic lymphoma was 85%. The outcome is similar to that of patients with advanced disease supporting the need for intensive treatment in this population and the importance of studying biological predictors of treatment failure.

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children with high-risk acute lymphoblastic leukemia treated with and without cranial irradiation: a report from the Children's Cancer Group. *Journal of Clinical Oncology* 1998;16(3):920

**060**

**T-LBL AND T-ALL: DIFFERENCES AND SIMILARITIES DETECTED BY GENOMIC AND TRANSCRIPTOMIC ANALYSES**

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T-cell acute lymphoblastic leukemia (T-ALL) and lymphoma (T-LBL) share common morphologic and immunophenotypic features and are treated with similar therapeutic approaches. Nonetheless, they show distinct clinical presentations suggesting that they may represent two different biological entities. In order to investigate T-ALL and T-LBL genetic characteristics we used genomic and transcriptional profiling approaches. Genome-wide gene expression profiling was performed on diagnostic specimens from 20 patients affected by T-LBL and 10 patients affected by T-ALL. In order to control for normal cells contamination present in the nodal biopsies (T-LBL) and bone marrow aspirates (T-ALL), we used gene expression profiles of B-cell lymphoblastic lymphoma and common (CD10<sup>+</sup>) lymphoblastic leukemia, respectively. Upon elimination of genes differentially expressed in nodal versus bone marrow-derived samples, we were able to identify a subset of genes which appeared to be differentially expressed in T-LBL versus T-ALL. This gene signature includes genes involved in chemotactic responses and angiogenesis which may play a role in the different tumor cell localization. Copy number analysis was performed using single nucleotide polymorphism (SNP) arrays (Human Mapping 100K arrays, Affymetrix) on a subset of the samples (9 T-ALL and 9 T-LBL) analyzed by gene expression profiling. This analysis detected approximately 200 genetic loci recurrently affected by copy number alterations in T-ALL and/or T-LBL. The most common aberration was the 9p21.3 deletion which includes CDKN2A/B. Consistent with previous reports amplifications involving MYB were identified in two cases of T-ALL. Although most aberrations were commonly found in both entities several were recurrently detected in T-LBL but not in T-ALL and vice versa. Taken together these results suggest that T-LBL and T-ALL share a large fraction of their biological features; nevertheless each malignancy displays also a unique pattern of genetic lesions affecting their transcriptome.

**061**  
**PRECURSOR T-LYMPHOBLASTIC LYMPHOMA IN CHILDREN AND ADOLESCENTS: AN IMMUNOPHENOTYPIC ANALYSIS OF A LARGE COHORT (N=186); A CHILDREN'S ONCOLOGY GROUP REPORT**

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**Background.** Precursor T-acute lymphoblastic leukemia (T-ALL) and precursor T-lymphoblastic lymphoma (T-LBL) are neoplasms derived from immature lymphoid cells of T-cell lineage. These neoplasms are biologically similar, however, significant differences may exist between the two given the differences in their clinical presentation and genomic phenotype. Although ample data regarding the immunophenotypic characterization T-ALL are available, there is a paucity of such data in children and adolescents who present with T-LBL. **Objectives.** Our study aims to further characterize the immunoprofile of childhood and adolescent T-LBL. **Design/Methods.** We present a review of flow cytometric data from 186 patients with a diagnosis of T-LBL derived from the Children's Oncology Group (study A5971) which included lymphoblastic lymphomas and required less than 25% bone marrow lymphoblasts as a study criterion. Multiple T-cell, B-cell, myeloid, and other markers were evaluated. **Results.**

**Table.**  
**Immunoprofile of T-LBL (n=186)**

Marker	Number of Cases (n)	Positive (%)
CD1a	67	72.8
CD2	127	91.4
cCD3	109	84.5
sCD3	64	46.0
CD4	110	71.9
CD5	145	95.4
CD7	143	96.0
CD8	117	78.0
CD4+/CD8+	129	79.1
CD4-/CD8-	129	18.6
TdT	174	89.7
CD10	131	56.5
CD34	109	20.2
Any B-cell marker	138	8.7
Any myeloid marker	73	15.1
Multiple myeloid markers	73	4.1

cCD3: Cytoplasmic CD3 expression, sCD3: Surface CD3 expression

**Immunoprofile of TdT-Negative T-LBL (n=17)**

Marker	Number of Cases (n)	Positive (%)
CD1a	11	72.7
CD2	13	76.9
cCD3	13	100.0
sCD3	14	57.1
CD4	15	60.0
CD5	14	92.9
CD7	15	100.0
CD8	15	80.0
CD4+/CD8+	15	53.3
CD4-/CD8-	15	13.3
CD10	12	16.7
CD34	9	11.1
Any B-Cell Marker	14	7.1
Any Myeloid Marker	9	11.1
Multiple Myeloid Markers	9	0.0

cCD3: Cytoplasmic CD3 expression, sCD3: Surface CD3 expression

**Conclusion.** Our study provides additional immunophenotypic data on childhood and adolescent T-LBL which may be diagnostically useful. Although it has been reported that myeloid antigens are often present in T-ALL/T-LBL, this was a distinctly uncommon occurrence in our study group. Of the myeloid markers studied, CD33 is the most likely to be aberrantly expressed. The TdT-negative subset is interesting, as these often represent diagnostically challenging cases. Although the absolute number of these cases is small (n=17 or 9.1% of total cases), TdT-negative T-LBLs appear less likely to express aberrant B-cell markers, myeloid markers, CD10, and CD34. They are also less likely to co-express CD4/CD8 but usually do retain expression of other mature T-cell markers, particularly cytoplasmic CD3, CD5, and CD7.

**062**  
**CLINICAL PRESENTATION, EVOLUTION AND PROGNOSIS OF PRECURSOR B CELL LYMPHOBLASTIC LYMPHOMA IN TRIALS LMT96, EORTC 58881 AND 58951**

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**Background.** In childhood, lymphoblastic lymphomas represent 30% of Non Hodgkin lymphoma (NHL). Among them, about 5% are precursor B-cell lymphoma

(PBCL). *Objectives.* The aim of our study was to evaluate the main clinical characteristics, the evolution and the prognosis of such lymphomas. *Material and method.* We have retrospectively included 57 children treated for PBCL between 1990 and 2008. These children have been included in three different international protocols which were LMT 96, EORTC 58881 and 58951. One patient was excluded because there were 25% of blasts in the bone marrow which is the limit to define an acute lymphoblastic leukaemia (ALL). These different protocols are BFM-derived ALL therapy with an induction, a consolidation, an interphase and an intensification followed by a maintenance phase for a total duration of 18 to 24 months. No patient received radiotherapy. *Results.* In our study, the median age of children suffering of PBCL was 7.75 (range 0.25; 16.7) years. Among the 56 patients, there were 26 girls. According to St Jude classification, there were 10 Stage I, 12 Stage II, 7 Stage III and 26 Stage IV. Clinical presentation is variable with nodal and extra nodal disease (subcutaneous forms (7), bone lesion (8), gonadal form (4), digestive localisation (1), pleural effusion (1) and bone marrow alone (2)). Less than half of the patients had a bone marrow disease (26/56) and only three patients had a central nervous system (CNS) involvement at diagnosis. The median follow up was 77.5 (IC 95% [56.2; 85.1]) months. At last follow up, 48 patients were in continuous complete remission whereas 8 had relapse (7 stages IV and 1 stage III) and died of lymphoma (5) or toxicity of treatment (3). Two others patients suffered from a secondary neoplasia (one melanoma and one glioblastoma) and are alive and well. *Conclusions.* The clinical presentation of PBCL is quite variable. Treatments with protocols derived from ALL therapy are giving good results with an 82% EFS (IC 95% [69; 90]) at 4 years and an 85% OS (IC 95% [72; 93]) at 5 years.

**063**  
**LYMPHOBLASTIC LYMPHOMA OF CHILDHOOD:**  
**RESULTS OF THE ITALIAN AIEOP LNH97**  
**PROTOCOL**

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*Background.* Pediatric lymphoblastic lymphomas (LBL) are mostly represented by T-cell lymphoblastic lymphoma (T-LBL) with a small percentage of pre-B cell lymphoblastic lymphoma. LBL respond to continuous leukemia-like treatment and significant improvements in

outcome have recently been achieved, particularly for T-cell LBL. *Objectives.* The AIEOP LNH97 protocol was designed with the aims to increase survival rates and reduce toxicity, particularly during early therapy phases, with respect to previous LNH92 preventing early relapses. *Methods.* AIEOP LNH97 protocol was based on the previous LSA2-L2 derived AIEOP LNH92. Modifications included an upfront use of cyclophosphamide, a different schedule of both L-asparaginase and anthracycline administration during Induction, the introduction of Reinduction in advanced stage disease, followed by Maintenance. Total therapy duration was 12 mo for stage I-II and 24 mo for stage III and IV disease. No cranial RT was administered as CNS prophylaxis. *Results.* From 1997 to 2005, 114 out of 138 children with newly diagnosed LBL were eligible for AIEOP LNH97 protocol. 24 patients were not eligible due to age >18 y (1), pre-treatment (6), blast infiltration >25% in BM (6) or other reasons (11 cases). Of the 114 patients, 84 were M (74%) and 30 F, median age 9.3 y (1.3-16.6). 18 patients (77%) were T-cell LBL; 26 were B-cell precursor LBL. Distribution according to St. Jude staging system was: 1 st-1, 10 st-2, 71 st-3 and 32 st-4 (1 with CNS involvement). CR was obtained in 111/114 children (97%). With a median follow-up of 5 y, 94 patients are alive and 20 died. OS was 83 % (SE 4%) and EFS 75% (SE 4%). We observed 3 remission failures (2 died of disease progression, 1 is alive in CR), 23 relapses at a median time of 16 mo, and 2 second malignancies (1 AML and 1 thyroid cancer, both alive). 18/23 relapsed patients died: 14 of disease progression, 4 of toxicity, in CR, after HSCT. By univariate analysis we could not identify any parameter with statistically significant impact on EFS. There was no early toxic death. W.H.O. toxicity was mostly grade III-IV hematological and gastro-intestinal subtypes. *Conclusion.* AIEOP LNH97 protocol achieved higher survival rates compared with AIOEOP LNH92 (OS 72%; EFS 69%) and showed mild toxicity.

**064**  
**TCR-BASED RQ-PCR ASSAY FOR MDD AND MRD**  
**ASSESSMENT IN T-CELL LYMPHOBLASTIC**  
**LYMPHOMA OF CHILDHOOD**

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*Background.* The outcome of T-cell lymphoblastic lymphoma (T-LBL) is rather favourable when treated with leukemia-type protocols. However, relapsed patients (pts) have a very poor outcome. In contrast to T-cell acute lymphoblastic leukemia, very few prognostic factors have been identified for T-LBL. Additionally, there is limited data on the role of minimal residual disease (MRD) in this lymphoma. *Objectives.* To evaluate the usefulness of the TCR-based RQ-PCR assay for minimal disseminated disease (MDD) and MRD analyses in T-

LBL of childhood, and to compare the sensitivity of such a method with flow-cytometric analysis (FCM). **Methods.** We studied 11 pts treated with AIEOP LBL protocols. Genomic DNA was prepared from tumor biopsies at diagnosis and amplified using various primer sets for TCRG, TCRD, TCRB and IGHD-J rearrangements. Clonal PCR products were identified by heteroduplex analysis and then sequenced, either directly or after cloning. Patient-specific oligonucleotides were designed on the junctional region identified. The RQ-PCR assay was performed according to the guidelines of the European Study Group on MRD in ALL. BM involvement at diagnosis was studied in all pts, whereas subsequent analysis was performed in 3 pts. The panel of monoclonal antibodies used for FCM were: CD4, CD8, CD7, CD5, CD3, CD99 and TdT. **Results.** A total of 38 TCR rearrangements were identified. In all pts, at least one target was detected and in 8/11 more than 3 targets were found. TCRG, complete TCRD, complete and incomplete TCRB and incomplete IGH rearrangements were found in 9, 3, 7, 6 and 2 pts, respectively. In 9/11 pts, 2 sensitive targets were available (detection limit  $\leq 10^{-4}$ ). For the remaining 2 pts, in one case the rearrangement detected was oligoclonal, in the other the sequencing reaction failed. The sensitivity level was 10<sup>-5</sup> for 9/18 selected targets. BM involvement at diagnosis was detected in all pts by both targets. In 8/11 children FCM was performed: in 3 cases BM was negative, although in one MDD was around 10<sup>-1</sup> by RQ-PCR. In 1/3 pts studied at end of induction, MRD remained positive (10<sup>-4</sup>). **Conclusions.** These preliminary results suggest that the TCR-based RQ-PCR assay could be successfully performed for MDD and MRD assessment in T-LBL. In our cases, the targets available were more than three in 8/11 pts and the sensitivity level achievable was 10<sup>-5</sup> for half of the targets.

#### 065

#### EXPRESSION OF MTOR PATHWAY PROTEINS AND NOTCH1 IN PEDIATRIC LYMPHOBLASTIC LYMPHOMA (LBL):

##### A CHILDREN'S ONCOLOGY GROUP REPORT

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**Background.** Acute lymphoblastic leukemia (ALL) and LBL are thought to represent a disease spectrum. Activating mutations in Notch1 are found in 50-60% of T-ALL. The downstream signaling pathways that are activated by Notch1 mutations are currently under investigation. Recent studies indicate that Notch1 activation results in upregulation of the mammalian target of rapamycin (mTOR) pathway. mTOR protein kinase controls a significant proportion of cellular translational activity, with resultant downstream effects on critical cellular functions such as growth and proliferation. Expression of the mTOR protein and its downstream targets has not been previously characterized in LBLs and may provide

a therapeutic target. **Objectives.** This study assesses the expression of mTOR pathway proteins and Notch1 in biopsy samples of pediatric precursor-T and B LBL. **Design.** We analyzed the prevalence of Notch1, phospho-mTOR (p-mTOR), phospho-70S6 kinase (p-p70), and phospho-S6 ribosomal protein (p-S6) by immunohistochemistry in 15 cases of T-LBL and 10 cases of B-LBL obtained from the Children's Oncology Group clinical trial CCG 5971 for treatment of disseminated lymphoblastic lymphoma. Immunohistochemical stains were independently analyzed by 3 individuals. Positive cases showed 25% expression. **Results.** High expression of p-mTOR, p-p70, and Notch1 were identified. Cytoplasmic expression of p-mTOR was weak to moderate in greater than 80% of cells in most positive cases. Weak to moderate cytoplasmic staining of p-p70 was observed in 30-80% of cells in the positive cases, and Notch1 showed moderate to strong nuclear staining in 100% of cells in all cases. The prevalence of p-S6 (cytoplasmic) expression was low with only 4/15 and 1/10 cases positive in T- and B-LBL, respectively. **Conclusions.** Our data demonstrate constitutive expression of p-mTOR, p-p70, and Notch1 in a majority of T-LBL and B-LBL, suggesting that both the Notch1 and mTOR pathways are active in these tumors. These results help to further characterize signaling pathways that are activated in LBLs, and support further studies in determining the potential therapeutic role of mTOR inhibitors in patients with LBL.

**Table. Results of Immunohistochemical Stains**

Protein	T-LBL	B-LBL
P-mTOR	12/15 (80%)	7/10 (70%)
P-p70	11/15 (73%)	8/10 (80%)
P-S6	4/15 (27%)	1/10 (10%)
Notch1	15/15 (100%)	10/10 (100%)

#### 066

#### NHL-BFM 90-BASED REGIMEN IMPROVED TREATMENT OUTCOME FOR JAPANESE CHILDREN WITH LYMPHOBLASTIC LYMPHOMA

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**Background.** To date, excellent outcome of children with lymphoblastic lymphoma (LBL) have been achieved with an acute lymphoblastic leukemia (ALL)-type treatments. The best results come from the Berlin-Frankfurt-Munster (BFM) group. In the NHL-BFM-90 study, the 5-year disease-free survival rate was 90%. However, the Tokyo Children's Cancer Study Group (TCCSG) NHL-T96-04 study which was an ALL-type treatment including high-dose intravenous cytarabine (HD-CA) resulted in event-free survival (EFS) rate of 65.7 $\pm$ 7.3%. **Objectives.** The

purpose of the TCCSG NHL-T0105 study was to test whether treatment with the NHL-BFM 90-based regimen could improve EFS rate for Japanese children with LBL. *Methods.* In TCCSG NHL-T0105, patients received NHL-BFM 90-based treatment with following modifications; reduced HD-MTX 3g/m<sup>2</sup> in protocol M, reduced L-asparaginase 6,000IU/m<sup>2</sup> in protocol I, and using mercaptopurine instead of thioguanine in protocol II. Patients with stage III or IV disease received prophylactic cranial radiotherapy as well as the original NHL-BFM 90. *Results.* From February 2001 to October 2004, thirty-three patients were enrolled onto TCCSG NHL-T0105. Nineteen were boys, and fourteen were girls. The median age was 9 years (range 1-15). The number of patients with stage II, III, and IV disease was 3, 21, and 9, respectively. According to the institutional diagnosis, 22, 9, and 2 patients were registered as having precursor T-cell LBL, precursor B-cell LBL, and others, respectively. The diagnosis was centrally reviewed in 30 of 33 patients. After the review, two diagnoses were revised other than LBL. One patient was lost to follow-up. At a median follow-up of 44 months, the estimated EFS rate at 3 years were 84.4±6.4%, and 82.8±7.0%, for evaluable 32 patients, and for 29 patients with stage III or IV disease, respectively. The estimated EFS rate for 27 patients with centrally reviewed LBL was 81.5±7.5%. One patient had not achieved a first complete remission. Four patients relapsed. All these five patients who had experienced tumor failure died. No treatment-related death during the first complete remission was documented. *Conclusion.* Treatment with the NHL-BFM 90-based regimen demonstrated efficacy and improved the EFS rate for Japanese children with LBL compared with the previous ALL-type treatment.

#### 067

#### **RESULTS OF ALL-MB 91/2002 PROTOCOL IN ADOLESCENTS AND YOUNG ADULTS WITH LYMPHOBLASTIC LYMPHOMA: SINGLE INSTITUTION EXPERIENCE IN RUSSIA**

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*Background.* Lymphoblastic lymphoma (LBL) is one of predominant non-Hodgkin's lymphoma (NHL) subtypes in children, adolescents and young adults (AYA) and consists 20-30%. LBL represents a distinctive entity with morphological features similar to those of acute lymphoblastic leukemia (ALL). LBL is usually treated with ALL-based chemotherapy. *Objectives.* The purpose of this study was to investigate outcomes with LBL treated with national pediatric protocols of ALL-MB 91/2002 versus BFM therapy for AYA. *Design.* 18 pts (m=14, f=4) were enrolled from July 1998 to December 2007. Eleven (67%) pts were treated with the ALL-MB 91/2002 and 6 (33%) pts – NHL-BFM 90 for non-B NHL. In ALL-MB 91/2002 the pts receive four drug induction with dexamethasone 6 mg/m<sup>2</sup> daily for 36 days, daunorubicin 45 mg/m<sup>2</sup> for 2 doses, vincristine 2 mg weekly for 5 doses and

intrathecal cytarabine/methotrexate/prednisolone weekly for 5 doses. Consolidation included L-asparaginase in a constant dose of 10000 ME/m<sup>2</sup> weekly for 18 doses and 6-merkaptopurine 50 mg/m<sup>2</sup> (100%) daily and methotrexate 30 mg/m<sup>2</sup> (100%) weekly with doses adjusted according to white blood cell count weekly. Cranial irradiation was performed only for pts with central nervous system (CNS) involvement at diagnosis. Maintenance was carried out up to 24 months. *Results.* Median age at time of presentation was 21.1 (range 15-42) years. 16 (89%) pts have a T-cell immunophenotype. All patients (100%) had advanced stage. The presenting sites of primary disease included mediastinal mass in 14 (78%) cases. The bone marrow was involved (25% blasts) in 8 (44%) pts. CNS involvements were found in 4 (22%) pts. 7 (39%) pts (ALL-MB 91/2002-5; NHL-BFM 90-2) previously received from 1 to 7 (median-4.5) regimes of therapy, such as one with B-NHL. Eleven (100%) pts are in complete remission (CR) on the protocols ALL-MB 91/2002 vs. 5/7 (71%) pts – NHL-BFM 90 with median time of observation 2.4 years. Only 1/7 (14%) pt had not responded (NHL-BFM 90). 2/11 (18%) pts relapsed after ALL-MB 91/2002. 6-years event free survival is 67 vs. 71% respectively, *p*>0.05. 6-years overall survival has 83 and 71% (*p*>0.05) respectively. *Conclusion.* The results of two protocols are comparable. Previous ineffectiveness of CHOP like regimens has not an absolute disadvantage prognosis for further ALL-like treatment.

**SCIENTIFIC SESSION 6:  
ANAPLASTIC LARGE CELL LYMPHOMA**

**THE ANAPLASTIC LYMPHOMA KINASE IN THE  
PATHOGENESIS OF ALCL**

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Anaplastic Lymphoma Kinase (ALK) is a Receptor Tyrosine Kinase first identified in chromosomal translocations associated with Anaplastic Large Cell Lymphoma (ALCL), a subset of T-cell Non Hodgkin Lymphomas. In ALCL, all translocations involving ALK produce fusion proteins with constitutive tyrosine kinase activity that derives from spontaneous auto-activation. This auto-activation is generally mediated by a dimerization of the ALK tyrosine kinase domain induced by the different fusion partners. The ALK fusion proteins provide a tonic tyrosine kinase activity to the lymphoma cells that induces cell transformation by controlling key cellular pathways. This tonic kinase activity largely compensates for the lack of T Cell Receptor (TCR) signalling in ALCL cells. In fact, the majority of ALCL cases lack TCR and most of the TCR-complex related signalling molecules, such as CD3, LAT, ZAP70 and SLP76. Most pathways activated by ALK have been characterized throughout the years, both in human-derived cell lines and in mouse models of ALCL lymphoma, and control fundamental cellular processes such as cell cycle progression, survival, cell migration and cell shape. Among these, the JAK-STAT3, Ras-MAPK-ERK, PI3K-AKT, VAV-Rho family GTPases pathways are the best characterized from both a molecular and biological point of view, and have been shown to be critical for lymphoma cell growth and survival. An overview on how ALK activated these pathways will be provided. From a therapeutic point of view, the deregulated tyrosine kinase activity of ALK has been proven to be essential for lymphoma growth and survival. ALK activity inhibition or its down-modulation via siRNA leads to cell cycle arrest and apoptosis of ALCL cells both *in vitro* and in mouse models. Therefore, ALK constitutes a good target for the development of specific inhibitors, in particular small molecules. Few compounds have already been tested *in vitro* and *in vivo* and others will be developed soon. Besides the small molecules inhibitors, the understanding of the molecular mechanisms involved in ALK-mediated transformation has generated additional potential targets for ALCL therapy, such as STAT3, AKT or Rho-family GTPases. As a complementary therapeutic strategy, the selective high expression levels of ALK protein in ALCL cells could be exploited to develop an active immune therapy against ALK as an oncoantigen. To this end, active vaccination strategies proved efficient in generating an immune response that controlled local and disseminated lymphoma growth in mouse models. Interestingly, ALCL patients spontaneously develop an immune response against the ALK protein, ranging from anti-ALK antibodies to ALK-specific CD4

and CD8 effector T cells. Vaccination strategies could boost this spontaneous immune response and contribute in the control of lymphoma relapses. In conclusion, most pathogenetic events that lead to ALK-mediated transformation in ALCL cells have recently been characterized and will serve as candidate targets for ALCL therapy.

**ANAPLASTIC LARGE CELL LYMPHOMA (ALCL):  
CURRENT MANAGEMENT**

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Optimal treatment (Tt) for pediatric ALCL is still under investigations. While most European countries recommend an intensive and short chemotherapy (CT) regimen derived from Tt for B-NHL, several other groups have treated patients (pts) with a less intensive but more prolonged CT. The relapse rate still attains 25 to 30% at 2 years (y) in the latest series: the ALCL99 trial, a large international trial for childhood ALCL, based on the NHL-BFM90 and aiming to compare efficacy and safety of 2 doses and modes of administration of high dose methotrexate (HDMTX) and to study the impact of adding vinblastine in pts at high risk of failure (Brugieres JCO2009), the POG9315 trial aiming to assess the effect of incorporating into APO (doxorubicin, prednisone, vincristine) 8 courses of HDMTX followed by HDARA-C (Laver JCO2005), the CCG5941 based on a 48 week intensive multiagent T-cell lineage CT (Lowe PBC2009). The ALCL 99 protocol is one of the most attractive current Tt given the good results obtained in terms of EFS, the short duration of the Tt and the lower cumulative doses of drugs known to be associated with a risk of long-term toxicity, such as alkylating agents, etoposide and anthracyclines, compared to other paediatric and adult protocols. The low frequency (less than 5%) of CNS involvement in ALCL ALK+ addresses the question of the general policy of intrathecal (IT) prophylaxis. Even though the CNS relapse rate is very low in all previous series of ALCL, most groups still recommend a CNS prophylaxis based on HDMTX and/or (IT). The ALCL R1 trial demonstrated that replacing MTX 1 g/m<sup>2</sup> in a 24h infusion + IT by MTX 3 g/m<sup>2</sup> in a 3h infusion is not associated with an excess of CNS-relapses. The question is raised whether this prophylaxis may be further reduced by reducing the number of HDMTX courses. One of the major developments in the recent years has been the identification of the high risk of failure associated with the presence of circulating cells in the blood and the bone marrow harboring the NPM-ALK fusion gene and detected by PCR at diagnosis (Mussolin Leuk2005, Damm-Welk Blood2007). This technique allows the definition of a group of pts at high risk of failure who might benefit of an early Tt intensification. Another important recent development is the description of both B and CTL immune response to ALK in ALK+ ALCL (Ait-Tahar K IntJC2006) and of the correlation of the presence of anti ALK antibody with the presence of circulating cells (Mussolin Leuk2009). This findings provides valuable information for developing future immunotherapeutic options for ALCL. The role of immunotherapy is also suggested by the emergence of allogeneic stem cell tran-

splantation which proved to be a potentially useful Tt for pts with refractory or early relapsed ALK<sup>+</sup> ALCL (Cesaro EJH2005, Woessmann BJH2006). In the future, it will also be important to look at the efficacy of innovative forms of therapy for ALK<sup>+/−</sup> ALCL such as anti-CD30 monoclonal antibodies conjugated to toxins or radioisotopes, vaccination strategies (Bartlett Blood2008) and drugs inhibiting NPM-ALK kinase activity or interfering with the downstream signaling pathways (Li MedRevRes2008).

**Table. Results of Immunohistochemical Stains**

	Period of inclusion	Nb of ps	Duration	CNS prophylaxis	EFS	OS
ALCL99	1999-2006	352	4,5–12 m (rand)	HDMTX + IT (rand)	74%+4%	92%+3%
POG9315	1994-2000	86	12 m	IT + HDMTX and HD Ara-C (rand)	71%+6%	86%+4%
CCG5941	1996-2001	86	48 w	IT + HD MTX and HD Ara-C	70%+10%	80+7%

**068  
ADVANCED-STAGE ANAPLASTIC LARGE-CELL LYMPHOMA IN CHILDREN AND ADOLESCENTS: RESULTS OF ANHL0131, A RANDOMIZED PHASE III TRIAL WITH STANDARD APO (DOXORUBICIN, PREDNISONE, VINCRISTINE) VERSUS CONSOLIDATION WITH A REGIMEN INCLUDING VINBLASTINE: A REPORT FROM THE CHILDREN'S ONCOLOGY GROUP**

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**Background.** Anaplastic large cell lymphomas (ALCL) comprise 10-15% of childhood lymphomas. Children with advanced-stage (stages III and IV) ALCL have a 2-year survival in the range of 70-80%. Vinblastine is an agent that has resulted in favorable responses in children with relapsed ALCL. **Objectives.** ANHL0131 examined whether a consolidation regimen including vinblastine to the standard APO (doxorubicin, prednisone and vincristine) regimen would result in superior long-term, event-

free survival (EFS) for children with advanced-stage ALCL. Advanced stage was defined as Murphy stage III or IV. **Design and Methods.** The study was open to Murphy stage III/IV with CD30<sup>+</sup> ALCL under 21 years of age. Induction was identical for both arms. Patients were randomized to either receive standard APO therapy with vincristine every 3 weeks during consolidation or a regimen that substituted weekly vinblastine for vincristine (APV). The duration of therapy was 1 year. Stringent criteria were followed to monitor and reduce the vinblastine dose if increased toxicity was observed. The initial starting dose of vinblastine was 6 mg/m<sup>2</sup> and was subsequently reduced to 5 mg/m<sup>2</sup> and then to 4 mg/m<sup>2</sup>. **Results.** From January 2004 to November 2008 119 eligible patients were enrolled with Murphy stage III/IV 30<sup>+</sup> ALCL. Interim analysis was performed in November 2008. The 2 year EFS for all patients was 77% (95% CI of 67-85%) and the 2-year OS was 88% (95% CI of 79-93%). For the patients randomized to the standard APO arm the 2 year EFS for was 75% (95% CI of 59-86%) and the 2-year OS was 90% (95% CI of 78-96%). The 2 year EFS was 80% (95% CI of 64-89%) and the 2-year OS was 86% (95% CI of 70-93%) for patients receiving the vinblastine arm (analysis included starting doses of 6, 5, and 4 mg/m<sup>2</sup>). There have been 8 deaths and 11 failures in the APO Arm and 7 deaths and 9 failures in APV. The APV arm was associated with more toxicity. Of those patients who completed therapy, 29/32 (91%) patients receiving APV experienced grade 3 or higher Neutrophils/granulocytes (ANC/AGC) toxicity during consolidation, which was significantly higher than the rate on APO with 16/36 (44%) patients experiencing Grade 3 or higher Neutrophils/granulocytes (ANC/AGC) toxicity (*p*<0.0001). **Conclusion.** Vinblastine administration was associated with increased myelosuppression when given weekly with conventional chemotherapy. The addition of vinblastine to APO therapy had no statistical improvement on EFS or OS.

**069  
CIRCULATING ANTIBODIES TO ALK INVERSELY CORRELATE WITH TUMOUR DISSEMINATION AND RELAPSE RISK IN CHILDREN AND ADOLESCENTS WITH ALK-POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA**

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**Background.** There is limited evidence that an autoimmune response contributes to lymphoma control. Circulating antibodies and T-cell responses to the tumour-associated antigen anaplastic lymphoma kinase (ALK) have been detected in patients with ALK-positive anaplastic large cell lymphoma (ALCL). Detection of low numbers of circulating tumour cells (CTCs) at diagnosis remains, however, the strongest poor prognostic factor. **Objective.** We

analyzed the magnitude of the autoantibody response to ALK and its association with a) tumor dissemination (stage and the levels of CTCs) and b) relapse risk. *Design/Methods.* ALK autoantibodies were analyzed in initial serum or plasma samples from 95 children and adolescents with nucleophosmin (NPM)-ALK-positive ALCL. NPM-ALK transcripts (CTCs) could be quantified in cells from bone marrow (BM) and/or blood of 69 and 59 of the patients (pts). The pts were treated according to the NHL-BFM95 and ALCL99 (BFM-type strategy) trials between 1996 and 2007. Antibody titres against ALK and copies of NPM-ALK were measured using ALK transfectants and quantitative RT-PCR as previously described (Pulford *et al.* 2000 Blood 96:1605; Damm-Welk *et al.* 2007 Blood 110:670). *Results.* Circulating antibodies to ALK were detected in 87/95 (92%) of pts. The median follow-up of the pts was 4.6 years (range 0.6-10.3). The magnitude of antibody titres correlated inversely with stage and mediastinal/visceral involvement. There was a significant inverse correlation between the magnitude of antibody titres and CTCs. None of the 24 pts with a high antibody titre ( $\geq 1/60750$ ) had more than 10 copies NPM-ALK/ $10^4$  copies ABL in BM compared to 8/27 pts with intermediate antibody titres ( $1/2025 < 1/60750$ ) and 6/12 pts with antibody titres  $< 1/2025$  ( $p = .001$ ). Amongst pts mounting an antibody response, higher antibody titres were significantly associated with lower cumulative incidence of relapses (CI-R): 19 pts with a titre  $< 1/2025$  had a CI-R of  $74 \pm 11\%$  compared to a CI-R of  $32 \pm 8\%$  of 39 patients with intermediate titres ( $1/2025 < 1/60750$ ) and a CI-R of  $11 \pm 6\%$  of 29 pts with titres  $\geq 1/60750$  ( $p < .001$ ). *Conclusion.* Our results provide the first clinical evidence that the strength of the immune response to ALK inhibits lymphoma dissemination and influences the relapse risk. These results support the use of ALK as an immunotherapeutic target in ALCL.

**070**  
**PROGNOSTIC IMPACT OF MORPHOLOGIC AND PHENOTYPIC FEATURES OF CHILDHOOD ALK-POSITIVE ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): RESULTS OF THE ALCL99 STUDY**

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*Objectives.* To assess the prognostic value of morphologic and phenotypic (i.e. ALK staining pattern and CD3 positivity) characteristics of systemic ALK-positive ALCL (sALK+ALCL). *Design/Methods.* Analysis of all patients registered in the ALCL99 study between 11/1999 and 06/2006 for a sALK+ALCL and reviewed by the international pathology panel. These cases were classified according to the patterns recognized by the 2001 WHO classification. The mandatory set of antibodies for immunophenotyping included ALK1, CD30, EMA, CD2, CD3, CD5, CD20, CD43, CD56, perforin, granzyme B. Cox models were used to assess risk of progression or relapse (failure) adjusted on clinical characteristics. *Results.* 355 of the 442 sALK+ALCL were reviewed by the international panel. These cases were classified as follows: 218 common pattern, 23 small cell (SC), 9 lymphohistiocytic (LH), 9 Hodgkin-like and 88 cases with several morphologic patterns observed in a single biopsy, including a SC component in 75 and a LH component in 11. Eight cases were unclassifiable. Overall, a SC or LH component (SC-LH) was noted in 118 cases (34%). A perivascular pattern of tumour cells was observed in 205/351 cases (42%), significantly more frequent in SC-LH subtypes than in others ( $p < .0001$ ). In 91% of cases, ALK staining was cytoplasmic and nuclear (c+n) and restricted to the cytoplasm (c) in the remaining cases. Perivascular pattern was more frequent in cases with ALK-staining restricted to the cytoplasm ( $p = .02$ ). The CD3 staining was negative in 65% of cases (193/298). CD3-positivity was significantly more frequent in SC-LH than in others ( $p < .0001$ ). SC-LH cases and perivascular pattern were significantly associated with a high incidence of skin lesions, mediastinal and visceral involvement. No correlation was found between clinical features and ALK staining pattern (c+n vs c). SC-LH pattern was significantly associated with a high risk of failure (HR=1.9,  $p = .004$ ) in multivariate analysis controlling for clinical characteristics, as well as the perivascular pattern (HR=2.1,  $p = .001$ ) whereas CD3 positivity was significant only in univariate analysis. *Conclusion.* sALK+ALCL with a small cell or a lymphohistiocytic component account for 34% of cases and represents a group of poor prognosis, independently of clinical risk factors.

071

**GENOMIC PROFILING OF PAEDIATRIC ALK-POSITIVE ALCL – A CHILDREN'S CANCER AND LEUKAEMIA GROUP UK STUDY**

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**Background and Objectives.** Anaplastic Lymphoma Kinase (ALK)-positive Anaplastic Large Cell Lymphoma (ALCL) is considered a T-cell malignancy in which ALK expression is a consequence of the t(2;5) or a variant translocation involving chromosome 2. For the most part this disease presents in the paediatric population and is of a good prognosis. Although the t(2;5) product Nucleophosmin (NPM)-ALK has been extensively studied for its transforming properties, very little is known regarding co-operative genetic mutations that may contribute to lymphomagenesis and may predict survival outcome, specifically in a purely paediatric population. We set out to determine the frequency and positions of genomic imbalances in this relatively rare disease. **Design and Methods.** We accumulated a collection of 17 paediatric ALK-expressing ALCL cases arising from biopsy specimens of patients enrolled mostly on the EICNHL ALCL99 clinical trial from within the UK. We performed array comparative hybridisation at a resolution of 1MB using DNA isolated from dissected tumour tissue. **Results.** Regions of genomic gain (n=121) were far more common than losses (n=11) and were detected in the majority on chromosomes 1-4, 5-12, 14 and 17 with chromosome 11 being the primary site for genomic imbalances. Patients with greater than or equal to 11 imbalances have a lower overall survival (85 to 40%). However, due to the small number of patients the confidence intervals vary widely but a hazard ratio of 4.7 suggests that the death rates of patients with  $\geq 11$  mutations is almost 5 times higher than those with less than 11 although this is not significant ( $p=0.18$ ). In addition patients demonstrating gains of genomic regions encoding either the DDB1 or BIRC5 genes have a poorer prognosis. Patients demonstrating gains of 11q12.2 (BAC RP11-286N22), containing the DDB1 gene (68% of total), display a lower overall survival (39%) suggesting some prognostic value. Likewise, in patients with gains of 17q25.3 (BAC RP11-141D1S) containing the BIRC5 gene (37% of the patient pool), only 33% survived. Interestingly, 71% of patients with BIRC5 gains also had a gain of the region encoding DDB1. **Interpretation and Conclusions.** A range of genomic imbalances exist in ALK-expressing ALCL of a paediatric origin with a greater number associated with poorer overall survival.

072

**CYTOGENETIC ANALYSIS OF PEDIATRIC ALCL: A SINGLE INSTITUTION EXPERIENCE AND A COMPARISON WITH ADULT SERIES REPORTED IN THE LITERATURE**

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**Background.** Anaplastic large cell lymphoma (ALCL) constitutes approximately 10 to 15% of all non-Hodgkin lymphomas (NHL) of childhood, but accounts for 3% of adult and 20-30% of pediatric large cell lymphomas. It is characterized by the reciprocal translocation t(2;5)(p23;q35). Most of the cytogenetic data available were obtained from adults. Only few data from pediatric patients were reported, mostly obtained from children treated with different regimens. **Objective.** To determine cytogenetic profiles in a series of pediatric ALCL and to compare the results with findings from children and adults reported in the literature. **Patients and methods.** A total of 18 children treated at our Institution were studied by cytogenetic analysis and RT-PCR for the specific t(2;5) translocation product. These results were compared with the karyotypes of 48 pediatric and 39 adult ALCL reported in the literature. **Results.** A total of 14 males and 4 females were analyzed (age range 4-15); 13 cases had T phenotype, 4 cases were null-cell and 1 was undetermined. The karyotype was obtained in 17/18 cases: 9 of them showed translocation t(2;5), 3 patients presented a variant form and 5 cases had normal karyotypes. In most cases involvement of chromosomes 6 and 7 was found. RT-PCR for NPM-ALK transcript derived from t(2;5) was performed in 10 patients and all, except the one ALK negative by immunohistochemical analysis, were positive; 2 cases positive for NPM-ALK by RT-PCR had a normal karyotype. In the comparison between pediatric and adult ALCL cytogenetic findings some differences emerged: I) karyotype with more than 59 chromosomes was observed in 28% of children and in 33% of adult cases, II) t(2;5), studied by cytogenetic analysis, accounts for 68% in children and 33% in adults, III) chromosome 7 aberration is preferentially found in association with t(2;5) in children, but rarely found in adults and usually without t(2;5) aberration, IV) chromosome 6 abnormalities were more frequent in adults compared to children (44% vs. 20%). **Conclusion.** Our data suggest that ALCL have different genetic characteristics in adults and in children. Whether this may have prognostic impact warrants further studies.

073

**SKP2 EXPRESSION IS MEDIATED BY E2F1 IN ANAPLASTIC LARGE CELL LYMPHOMA**

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**Background.** The majority of anaplastic large cell lymphomas (ALCL) are characterized by the chromoso-

mal translocation t(2;5)(p23;q35) leading to the expression of NPM/ALK. The constitutive activation of the NPM/ALK tyrosine kinase induces downstream mediators such as phosphoinositide 3-kinase (PI3-kinase)/AKT, JAK3 and STAT3 that result in increased cell proliferation and enhanced survival. Previous studies have shown that NPM/ALK-mediated PI3K/AKT activation is required for cell cycle progression and that inhibition of PI3K/AKT results in decreased p27Kip1 degradation and cell cycle arrest. We hypothesized that S-phase kinase protein 2 (SKP2) deregulation contributes to the oncogenic activity of NPM/ALK by regulating the degradation of p27Kip1. **Objective.** To analyze the molecular mechanism by which these pathways deregulate the cell cycle machinery in ALCLs. **Design/Methods.** The regulation of SKP2 by NPM/ALK was evaluated using quantitative RT-PCR and western blot analysis of cells ALCLs with and without shRNA ALK. Chromatin immunoprecipitations (CHIP) were performed to determine the role of E2F1 as a transcriptional regulator of SKP2. **Results.** Western blot analysis of five t(2;5)-positive ALCL-derived cell lines demonstrated an inverse pattern of expression of F-box protein SKP2 and p27Kip1. Inhibition of PI3K/AKT with Ly294002 (20uM) or JAK3 with WHI (10 uM) resulted in a dose and time-dependent decrease in cell viability (50% and 20% respectively at 24h). We performed quantitative RT-PCR and western blot analysis which demonstrated a decrease in both SKP2 transcript and protein levels after PI3K/AKT and JAK2 inhibition (33%; 47% at 24h respectively), with increase in the levels of p27 transcript and protein (47%, 71% at 24h respectively). Furthermore, the levels of E2F1 also decreased upon PI3K/AKT and JAK3 inhibition. Chromatin immunoprecipitation (ChIP) assays revealed that E2F1 binding to the SKP2 gene promoter was reduced after inhibition of PI3K/AKT and JAK3 while the binding to GAPDH gene promoter (control) was unaffected. **Conclusion.** The expression of SKP2 is regulated by PI3K/AKT and JAK3 which are known mediators NPM/ALK signaling. E2F1 mediates the transcriptional control of SKP2 expression. Our data support the role of SKP2-mediated regulation of p27Kip1 in ALCLs and implicate SKP2 and E2F1 as potential therapeutic targets in ALCLs.

**074**  
**PROTEOMIC ANALYSIS OF DENILEUKIN**  
**DIFTITOX (ONTAK) AS A POTENTIAL**  
**THERAPEUTIC AGENT FOR ALCL**

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**Background.** Constitutive expression of the NPM/ALK kinase is a key oncogene in anaplastic large cell lymphoma (ALCL). Our previous cDNA microarray analysis of

pediatric ALCLs demonstrated overexpression of IL-2R $\alpha$  in NPM/ALK-positive ALCLs relative to NPM/ALK-negative lymphomas. **Objective.** To determine the feasibility of targeting IL-2R by ONTAK for treatment of pediatric ALCLs, we assessed the prevalence of IL-2R expression in pediatric ALK positive ALCLs. **Design/Methods.** We used formalin-fixed paraffin-embedded tissues of ALCLs obtained from the CCG5941 clinical trial involving the pediatric population and determined the expression of ALK and CD25 (IL-2R) using immunohistochemistry. The effect of ONTAK on the viability of 5 cell lines derived from t(2;5)-positive ALCLs was assessed. Western blot analysis was performed to determine the effect of ONTAK on cell cycle proteins and apoptosis. We utilized an endoproteinase-catalyzed 16O/18O differential isotopic strategy to quantitatively determine the global proteomic sequelae of IL-2R inhibition using ONTAK. Proteins were collected from the cell lysates of treated and non-treated SUDHL1 cells and analyzed by quantitative mass spectrometry. **Results.** Analysis of 40 ALK positive ALCLs demonstrated strong membranous and cytoplasmic expression of CD25 in 27/40 (77%) cases while the reactive lymphocytes showed negligible or weak expression. Treatment of SUDHL-1 cells with ONTAK resulted in time and dose-dependent decrease in cell proliferation which was associated with induction of p27Kip1 and cleavage of PARP. A total of 253 proteins with 2 or more unique peptides were identified as being differentially expressed between treated and non-treated SUDHL-1 cells. Importantly a large number of proteins known to be altered by diphtheria toxin and important in protein translation were underexpressed in treated cells (eIF2C, hnRNP, BRCA1, CREG, NuMA, 60S ribosomal protein L8). In addition novel proteins with transcriptional and cell signaling activities were identified, representing unique pathways that may be affected by IL-2 signaling. **Conclusions.** Our studies demonstrate constitutive expression of CD25 in the majority of pediatric ALCLs. Our *in vitro* studies indicate that targeting of CD25 by ONTAK may represent a rational approach for treatment of pediatric patients with ALCL.

**075**  
**IDENTIFICATION OF PROGNOSTIC MARKERS BY**  
**GENE EXPRESSION PROFILING IN CHILDHOOD**  
**ALK-POSITIVE ANAPLASTIC LARGE CELL**  
**LYMPHOMA**

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**Background.** Clinical and histopathological characteristics have limited prognostic value for children with anaplastic large-cell lymphoma (ALCL). Gene expression profiling represents a powerful tool to identify prognostic subgroups of lymphomas. **Objective.** We aimed at iden-

tifying prognostic genes in children with ALK-positive (ALK+) ALCL by using genome-wide expression analysis. Subsequently, the differentially expressed genes were validated by using immunohistochemistry (IH) in ALCL biopsies from prospective trials of the Berlin-Frankfurt-Münster (BFM) study group. *Design/Methods.* 11 ALK+ ALCL biopsies from children and adolescents (age<18 years) were available for characterisation by tumor cell and reactive bystander cell content. 5 of the 11 children suffered a relapse. Gene expression analysis was performed using GeneChip U133 2.0 Plus arrays (Affymetrix). IH staining for selected differentially expressed genes was established on reactive tonsil sections and applied on formalin fixated and paraffin embedded material of ALCL. *Results.* 250 differentially expressed genes were identified between the ALCL of patients who relapsed and the ones of non-relapse patients. The vast majority of these candidate genes were down-regulated in the tumors of patients who relapsed compared to the ALCL from patients without relapse. There was little overlap between the identified differentially expressed genes and genes previously published to be associated with outcome. Moreover, most of the differentially expressed genes have not been reported to be associated with prognosis in ALCL yet. To validate the results, selected genes were analyzed for expression in an independent set of ALCL by immunohistochemistry. Selected data are presented. *Conclusion.* Gene expression profiling may help identifying genes differentially expressed among ALK+ ALCLs. However, using gene expression data to develop histological and immunohistochemical markers for prognosis in ALCL for routine diagnosis is challenging.

#### **076 CLINICAL CHARACTERISTICS AND OUTCOME OF CHILDHOOD ALCL TREATED IN A PEDIATRIC ONCOLOGY CENTRE**

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*Aim.* To study the clinical characteristics and outcome of children with anaplastic large cell lymphoma (ALCL) treated in our center. *Methodology.* This was a retrospective chart review of children with ALCL treated at the Pediatric Hematology-Oncology Unit of Christian Medical College, Vellore, India from 2004-2008. *Results.* Forty children were diagnosed to have Non-Hodgkin Lymphoma (NHL) in our center during this time period. In this group, 8 children, 3 females and 5 males were confirmed to have ALCL. The patients were aged between 2 to 14 years. One child had primary cutaneous ALCL. Two children had Stage II disease. The remaining children were classified as Stage III and IV and had B symptoms. Two children in this group had mediastinal disease and one of them had multiple lung lesions and tumor in the pericardium with intracardiac A 14 year old female had parameningeal disease with multiple cranial nerve palsies. Multiple bony involvement was present in 3 children. Histopathology showed CD30+, CD20 and CD15 negative in all patients. ALK was negative in 3 cases; one

of them had primary cutaneous lymphoma and the other two had stage III disease. All our patients were treated on modified BFM-NHL 90 protocol. Six of 8 children achieved complete remission and are well with follow up periods ranging from 10 months to 3 years after completion of treatment. Two children relapsed, one within 6 months of completing treatment and the other while on treatment. The first child completed treatment for relapse 2 years ago and the other child 2 months ago; both are currently in CR. All except one child tolerated chemotherapy well; the exception was a 7 year old boy treated on strategy III developed severe mucositis, septicemia, DIC and renal failure following CC-1 and BB-2 courses of chemotherapy needing hemodialysis and intensive care. *Conclusion.* In our study, 20% of NHL cases were found to be ALCL. Of this group, 60% had high risk disease. Three children had ALK negative ALCL. Two children with ALK negative systemic disease relapsed. Majority of our patients tolerated this treatment.

#### **077 VINBLASTINE IMPROVES OUTCOME IN CHILDREN WITH ANAPLASTIC LARGE CELL LYMPHOMA (ALCL) TREATED WITH A UNIFORM SHORT DURATION INTENSIVE PROTOCOL**

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*Background.* MCP 842 Protocol, a short duration intensive chemotherapy protocol was initiated at our centre in 1987 for treatment of ALCL. Vinblastine was substituted for Vincristine in our protocol for these patients from 2004 onwards (Modified-MCP842). *Objective.* To examine the clinical features, outcome and survival benefit of vinblastine for treatment of ALCL. *Methods.* A total of 27 previously untreated patients of anaplastic large cell lymphoma (ALCL) were prospectively enrolled on MCP-842 protocol between 1987 and 2006. Treatment consisted of eight alternating cycles of two regimens, A (Cyclophosphamide, Adriamycin, Vincristine and Cytosine-arabioside) and B (Etoposide, Vincristine, Methotrexate, and Ifosfamide). Intrathecal methotrexate and cytosine arabinoside were administered in the first 4 cycles. No radiotherapy or high dose methotrexate was given. From 2004, 8 patients received modified MCP-842 with Vinblastine in place of Vincristine. *Results.* The median age was 14 years (range 2.6-20 years). The male to female ratio was 4.5:1. Common disease sites included: Abdomen-33%; peripheral nodes-27%; Bones-19%, and mediastinum-10%. Four patients (15%) presented with stage I disease, 4 (15%) with stage II, 14 (52%) with stage III and 5 (18%) with stage IV. The response rate was 97% with complete response in 93% patients. There were 7 relapses (26%). 10 year EFS was 66.7% for localized stages (I & II) and 42% for advanced stages (III & IV) respectively with an overall EFS of 49.8% and OAS of 71%. The EFS increased from 43% to 75%, OS increased from 68% to 88% and relapse rate decreased from 31.5% to 12.5% after addition of Vinblastine in place of Vincristine in modified MCP-842 protocol. *Conclusions.*

Vinblastine has the potential to improve outcome of patients with ALCL and should be routinely incorporated in the treatment protocol of this disease.

**078**  
**NPM-ALK INHIBITS THE P53 TUMOUR SUPPRESSOR PATHWAY IN A JNK AND PI 3-KINASE DEPENDENT MANNER: MDM-2 IS A POTENTIAL THERAPEUTIC TARGET FOR THE TREATMENT OF ALK-EXPRESSING MALIGNANCIES**

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*Background and Objectives.* Anaplastic Lymphoma Kinase (ALK)-expressing Anaplastic large cell lymphoma (ALCL) is a T-cell malignancy characterised by the presence of the t(2;5)(p23;q35) or other translocations involving ALK on chromosome 2. The most common chromosomal translocation generates the Nucleophosmin-Anaplastic Lymphoma Kinase (NPM-ALK) fusion protein, a hyperactive kinase with transforming properties. We show that amongst these properties is the ability to inhibit the p53 tumour suppressor pathway. *Methods.* We employed biochemical techniques to examine the status of p53 in ALCL-derived cell lines, cell lines engineered to express NPM-ALK (BaF3) and primary tumour tissues. *Results.* NPM-ALK stabilises the p53 negative regulator MDM2 in a PI 3-Kinase dependent manner leading to p53 ubiquitination and degradation. Furthermore, we demonstrate that NPM-ALK activates JNK leading to sequestration and degradation of p53 again through the proteasome. However, JNK also targets MDM2 for ubiquitination and degradation resulting in a neutralising net effect. Antagonism of MDM2 activity with the specific inhibitor Nutlin-3 leads to stabilisation and activation of p53 resulting in apoptosis of tumour cells, an effect also observed to a lower level with JNK inhibitors. We also show that p53 is targeted to cytoskeletal elements in a PI 3-Kinase dependent manner and hence is excluded from the nucleus yet remains primed for nuclear entry following antagonism of MDM2. In proof, inhibition of PI 3-Kinase activity concomitant with MDM2 antagonism, disperses p53 throughout the cytoplasm whilst remaining excluded from the nucleus. Hence, maximal activity of p53 is observed following inhibition of MDM2 alone and not in combination with PI 3-Kinase inhibitors. However, inhibition of PI 3-Kinase, JNK and MDM2 have synergistic effects in the induction of apoptosis suggesting a combination of p53 dependent and independent activities in this process. *Conclusions.* We conclude that NPM-ALK regulates the activity of the p53 tumour suppressor pathway via sequestration by JNK and MDM2 leading to its degradation. NPM-ALK induced PI 3-Kinase activity also excludes p53 to the cytoplasm. Hence, MDM2 antagonists in combination JNK inhibitors may present targets for the treatment of ALK-expressing malignancies such as ALCL.

**079**  
**IS NPM HAPLOINSUFFICIENT IN THE DEVELOPMENT OF LYMPHOMA IN A TRANSGENIC MOUSE MODEL OF ALK-EXPRESSING MALIGNANCY?**

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*Background and Objectives.* Anaplastic Lymphoma Kinase positive (ALK<sup>+</sup>) Anaplastic Large Cell Lymphoma (ALCL) comprises a group of non-Hodgkin's Lymphoma, affecting mainly children and young adults. The defining feature of this disease is aberrant expression of ALK, which typically occurs as a result of a t(2;5) chromosomal translocation fusing the N-terminal region of Nucleophosmin (NPM) to the entire intracellular region of ALK. The product of this event, NPM-ALK, is a hyperactive tyrosine kinase which has been shown by us and others to be oncogenic *in vivo*. Whilst much is known about the signalling pathways activated by ALK, less is known about the role of NPM in ALCL. NPM is a ubiquitously expressed, multifunctional phosphoprotein that plays a key role in several cellular processes related to the control of cell growth and proliferation, and is haploinsufficient in the maintenance of genomic stability. This led us to investigate the impact of NPM heterozygosity resulting from the t(2;5) on lymphomagenesis. *Design and Methods.* We back-crossed our NPM-ALK transgenic mice to mice heterozygous for NPM and monitored them for the development of lymphoma/leukaemia. We developed a cohort of 50 NPM-ALK/NPM<sup>+/-</sup> and 50 NPM-ALK/NPM<sup>+/+</sup> genetically modified mice which were monitored for a two year period. Mice were analysed by post mortem and tumours taken for histological and FACS analyses. *Results.* We hypothesised that given the role of NPM in maintaining genomic stability lymphomagenesis would be accelerated in NPM-ALK transgenic mice on a genomically unstable NPM<sup>+/-</sup> background. In the event, loss of one allele of NPM had no impact; NPM-ALK+NPM<sup>+/-</sup> and NPM-ALK+NPM<sup>+/+</sup> mice display similar latencies to disease presentation (14 to 24 months) ( $p>0.05$ ) and tumour incidence (34% vs. 33%). However, the spectrum of tumours that develop in the mouse lines differs in that NPM-ALK expression on a NPM<sup>+/-</sup> background leads to a higher incidence of haematological tumours. We are yet to analyse the mice for the incidence of myelodysplasia/leukaemia. *Conclusion.* NPM is not haploinsufficient in the development of t(2;5)-induced lymphoma in transgenic mouse models but may contribute to the phenotype of the disease.

**080**

**PROGNOSTIC IMPACT OF HUMORAL RESPONSE TO ALK AND ITS RELATIONSHIP WITH MINIMAL RESIDUAL DISEASE IN PEDIATRIC ALCL**

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**Introduction.** Anaplastic large-cell lymphoma (ALCL) makes up approximately 15% of pediatric non-Hodgkin's lymphoma (NHL) and the vast majority of them harbour the t(2;5) translocation that results in expression of the hybrid oncogenic tyrosine kinase NPM-ALK. ALK overexpression may induce a host immune reaction, giving rise to autologous anti-ALK antibodies in patients with ALK-positive ALCL. **Objective.** To assess the levels of anti-NPM-ALK antibodies at diagnosis and at stop therapy (ST) in plasma of a relatively large series of children with ALCL treated according to the ALCL-99 protocol and evaluate the correlation with Minimal Disseminated Disease (MDD) at diagnosis. **Methods.** Cyto centrifuge preparations of COS-7 cells, transiently transfected with a plasmid cDNA encoding for NPM-ALK, or with empty vector, were prepared and stained using patient's plasma in an indirect immunoperoxidase assay. The MDD study in peripheral blood was conducted using a TaqMan-based quantitative real-time PCR (RQ-PCR) for NPM-ALK. **Results.** A total of 45 children affected by *de novo* ALCL were enrolled in this study. Circulating antibodies recognizing the NPM-ALK protein kinase were detected at diagnosis in 39 out of 45 samples (87%). PB samples were positive for NPM-ALK transcript in 49% of the cases. We found that MDD positivity for NPM-ALK in PB and antibody titer <1/2250 was significantly associated with risk of relapse by univariate analysis, as 64% of patients with these two characteristics relapsed compared with only 9% of the remaining patients (Fisher's exact test,  $p=0.02$ ). In particular, patients with a low antibody titer had a Relative Risk of relapse (RR) of 6.3 (1.3-29.7  $p=0.02$ ), patients with positive MDD had a RR of 4.2 (1.1-16.3,  $p=0.038$ ), but those with a combination of the two features showed a RR of 12.7 (3.2-49.8,  $p=0.0003$ ). Most of the patients who did not relapse maintained a high antibody titer at ST, whereas in all relapsed patients, except two, the NPM-ALK antibody titer decreased below 1/100 at stop therapy. **Conclusions.** Our results showed that the titer of anti-ALK antibody represents a prognostic marker in ALCL and that a combination of MDD and anti-ALK response may contribute to a better identification of patients with poor prognosis.

**081**

**DIFFERENTIAL EXPRESSION OF HSP70 IN ALK? AND ALK? ANAPLASTIC LARGE CELL LYMPHOMAS**

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**Background.** Molecular chaperones are proteins responsible for folding newly synthesized polypeptides under physiological conditions and misfolded proteins under stress. They function by preventing unfolded proteins to aggregate and be degraded, preserving growth and division and avoiding cell death, upon environmental insults. Their increased expression in tumors reflects the harsh settings of growth, as well as the exogenously applied environmental stress of chemotherapy. In these conditions, molecular chaperones prevent apoptosis of transformed cells, and their expression frequently correlates with a less favourable prognosis. **Design and Methods.** We evaluated the expression of Hsp70 in 2 ALK<sup>+</sup> (Karpas299 and SUDHL1) and in 1 ALK<sup>-</sup>(FEPD) ALCL cell lines, assessing its anti-apoptotic activity after drug treatment or cellular stress. We also assessed Hsp70 expression in ALCL tumors, by tissue microarray, and in healthy control and ALK<sup>+</sup> ALCL lymph nodes by RQ-PCR. **Results.** We found that Hsp70 is overexpressed in ALK<sup>+</sup> ALCL cell lines, and rapidly induced following stress-dependent phosphorylation of HSF1 transcription factor. When induced, Hsp70 binds and sequesters proapoptotic protein Bax into the cytoplasm. This prevents mitochondrial injury and lowers threshold for caspase activation. In contrast, induction of Hsp70 is not observed in ALK<sup>-</sup> cells under stress or after drug treatment, due to HSF1 inactivity. These results were compared with Hsp70 expression *in vivo*. Hsp70 was expressed in 20 (100%) of 20 ALK<sup>+</sup> ALCL tumors ( $p=0.0119$ , Fisher exact test). A statistical correlation between Hsp70 expression and patient outcome could not be demonstrated, possibly due to the small number of cases. However, 4/6 (67%) ALK<sup>+</sup> ALCL patients with Hsp70 overexpression (value 3) relapsed or died, compared to 3 (20%) of 13 patients with an expression value between 0 and 2. When Hsp70 mRNA was measured in reactive lymph nodes and ALK<sup>+</sup> lymph-node biopsies, overexpression of Hsp70 in tumors was confirmed ( $p=0.040$ , Kruskal-Wallis test), reflecting an increased gene transcription. **Conclusions.** We demonstrated the antiapoptotic role of Hsp70 in ALCL cells *in vitro* and its differential expression *in vivo*. However, such an analysis needs to be extended to a larger cohort of patients, to better define the role of Hsp70 in ALCL tumor growth and progression.

**082**

**THE IMPACT OF THE ASSOCIATION OF VINBLASTINE DURING INDUCTION CHEMOTHERAPY AND AS MAINTENANCE IN CHILDREN AND ADOLESCENTS WITH HIGH-RISK ANAPLASTIC LARGE CELL LYMPHOMA: RESULTS OF THE ALCL99-VLB TRIAL**

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*Objective.* To assess the impact of adding vinblastine (VLB) to a chemotherapy based on the NHL-BFM90 protocol in children with high-risk anaplastic large cell lymphoma (HR-ALCL). *Method.* The ALCL99-VLB trial is a randomized trial involving most European groups and the Japanese group. All patients (pts) received a prephase followed by 6 courses given every 21 days. Pts with HR-ALCL, defined as disease with mediastinal, lung, liver, spleen or biopsy proven skin involvement, were randomized before the 2nd course to receive either no VLB (No-VLB arm) or one VLB injection (6 mg/m<sup>2</sup>) during the 5 latter courses and then weekly for a total duration of treatment of one year (VLB-arm). The main end-point was the Event-Free survival (EFS). The main analysis was done on the intent-to-treat population. *Results.* Overall 217 pts (93% ALK<sup>+</sup>) were recruited from 11/1999 to 06/2006: 110 in the VLB-arm and 107 in the No-VLB-arm. In the VLB-arm, 100 pts started VLB maintenance treatment (median total treatment duration: 53 weeks, range, 21-87; median dose intensity during maintenance: 4.6 mg/m<sup>2</sup>/week). Median follow-up is 4.7y (2-8.4). Compared to No-VLB patients, VLB-pts had a significantly lower risk of event during the 1st year, Hazard Ratio of event, HR=0.31 [95%CI, 0.15-0.67], p=.002, whereas they had an increased risk of event after the 1st year, HR=4.9 [1.6-15], p=.003. Consequently, EFS rates

differed greatly at 1y (74% in the No-VLB-arm vs. 91% in the VLB-arm) but were quite similar at 2y (70% vs. 73%). Over the whole period, the EFS curves did not differ significantly, HR=0.91 [0.55-1.5], p=.69. Results were very similar after exclusion of the 7 pts for whom the diagnosis of ALCL was rejected after review. There was no significant impact of the addition of VLB on the complete remission rate (85% vs. 86%, p=.82) or on the overall survival (95% vs. 92% at 2y, p=.61). Toxicity during induction courses was very similar in both arms. Only 3 pts stopped maintenance treatment due to toxicity. *Conclusion.* The addition of VLB during induction and as maintenance for a total treatment duration of one year significantly delayed occurrence of relapses but did not reduce the risk of failure resulting in the absence of benefit when considering the overall EFS curves or the 2-year EFS.

**083**

**PROGNOSTIC FACTORS IN CHILDHOOD ANAPLASTIC LARGE CELL LYMPHOMA (ALCL): RESULTS OF THE ALCL99 STUDY**

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*Background.* ALCL99 study, opened in 1999 for pediatric ALCL, included a randomized trial testing the impact on event-free survival of the addition of Vinblastine maintenance to the NHL-90 BFM protocol in high-risk ALCL. Patients (pts) were classified as high risk if they had >1 risk factor defined as biopsy proven skin lesion, mediastinal, lung, liver or spleen involvement. All other pts with systemic ALCL (sALCL) were classified as standard risk, except pts with completely resected stage I disease or CNS involvement. Relevance of this stratification was

examined. *Objectives.* To study the prognostic value of clinical features and histological subtype in sALCL. *Methods.* The main analysis included all pts registered from 11/1999 to 06/2006 for a sALCL (N=463), excluding 33 pts for whom the diagnosis of ALCL was rejected after review and 3 pts with ALK-negative lymphoma with no histological review. We assessed the impact of clinical features and histological subtype (defined in 428/463 cases reviewed by the international panel or the national referent pathologist) on the risk of failure (progression or relapse) in Cox models. Stability of results was evaluated excluding 1) 32 pts with pre-existing pathology or pre-treatment, 2) 21 ALK-negative ALCL. *Results.* Median follow-up was 55m (13-103). 73% pts were in 1st CR at 3y. Considering only clinical characteristics, the risk of failure was significantly associated with skin lesions (Hazard Ratio, HR=1.6,  $p=.02$ ), mediastinal involvement (HR=1.5,  $p=.03$ ) and CNS disease (HR=2.5,  $p=.03$ ); it was not found to be associated with visceral or bone marrow involvement, stage 3/4 in St Jude or Ann-Arbor classification, B-symptoms or LDH elevation. Pts with small cell or lymphohistiocytic (SC-LH) pattern had a 2.7 fold risk of failure compared to others ( $p<10^{-4}$ ). When adjusted on the histological subtype, only CNS disease remained associated with the risk of failure. Pts with ALK-negative ALCL had a non significant increased risk of failure as compared to ALK-positive ALCL. Results were stable considering the different study populations. *Conclusion.* Only two factors were found to be associated with the risk of failure in this large cohort of pts with sALCL: the presence of a SC-LH component to the tumor and CNS disease. Other clinical features were not relevant for risk stratification when taking into account the histological subtype.

## SCIENTIFIC SESSION 7: MALIGNANT LYMPHOMA IN ADOLESCENTS AND YOUNG ADULTS

### AGE RELATED MOLECULAR BIOLOGICAL CHARACTERISTICS OF AGGRESSIVE B-CELL LYMPHOMAS IN CHILDREN, ADOLESCENTS AND YOUNG ADULTS

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Burkitt lymphomas (BL) and diffuse large B-cell lymphomas (DLBCL) are the most frequent aggressive B-cell lymphomas (B-NHL) in children and adults, respectively. We recently reported that BL does not differ molecularly between children and adults if analysed by gene expression profiling and matrix-CGH. However, little is known about age related differences between paediatric and adult DLBCL. We aimed to characterize BL and DLBCL to (i) evaluate similarities and differences between of mature aggressive B-NHL in children and adults and to (ii) determine a possible biological cut-off between "paediatric" and "adult" DLBCL within clinical trials. Lymphoma samples of 63 patients younger than 18 years were analysed by gene expression profiling, matrix-CGH, FISH and immunohistochemistry. The results were compared to a series of 188 adult patients (>18 years). Using gene expression profiling, paediatric DLBCL was predominantly characterized as the germinal center-like (GCB) subtype although a substantial proportion of lymphomas were classified as activated B-cell like (ABC) subtype. Using the recently developed method of pathway activation pattern (PAP), paediatric DLBCL almost exclusively belonged to the PAP-1 group, a subgroup of DLBCL which has previously been shown to be associated with a favourable prognosis in adult DLBCL. The analysis of the ABC/GCB subtypes and PAP-Groups indicates that DLBCL are a molecularly homogeneous group of lymphomas in children and adolescents. Although there are few data from DLBCL in children and young adults of 20-50 years, molecular heterogeneity seem to be predominantly a feature of DLBCL in patients older than 50 years. Molecular characterization of DLBCL in children and young adults might help to provide a biological rationale to delineate paediatric from adult DLBCL within clinical trials. However, more cases of DLBCL in children and young adults with an age up to 50 years have to be studied to finally understand the age-association of molecular DLBCL subtypes.

### TREATMENT AND OUTCOME OF ADOLESCENTS WITH NON-HODGKIN LYMPHOMA (NHL), POINT OF VIEW OF A PEDIATRICIAN

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The overall incidence and the frequency of the different histological subtypes of NHL vary according to age.

Adolescence is at the junction of childhood and adulthood with an incidence in between, with a majority of NHL seen in children (Burkitt and lymphoblastic), but with higher percentage of DLBCL as seen in adults. Concerning outcome of adolescents (ados) with NHL, there are 2 major questions: 1. do ados have a different prognosis than children when treated with the same protocols? If yes, what is the reason? 2. Treatment (Tt) strategy may be quite different in adult and pediatric settings, even for the same subtype of NHL: are results in ados in the same range when treated with these different strategies? First question: Answers were different depending on the series. In the small NCI series of 41 B-NHL, results were similar in patients (pts)±18 years (y) (Magrath, JCO 1996). In the LMB 89 study (Patte, Blood 2001), among 360 pts treated in group B, age >15y was a significant prognosis factor (19 pts, RR=6.7). In the International FAB LMB96 study (Cairo, Patte, abstract SIOP 2008), pts>15 y (166=15%) had also a worse outcome: their 5y EFS was 80±3.6% compared to 87±1.1% for the younger pts ( $p<0.045$ ) and 5y OS 85±3.2% vs. 91±0.03 ( $p<0.041$ ). To note, pts > 15y had more frequently mediastinal primary, LDH >Nx2, advanced stages. In the multivariate analysis, the difference in outcome disappeared after controlling for primary site and histology, LDH level and stages. In the BFM database of 2084 pts treated for NHL between 1986 AND 2002 (Burkhardt, BJH, 2005), the 15-18y had a 4y EFS of 81% compared to 87% for the 5-9y and 85% for th whole series. This was due to an inferior outcome of girls>15y with T-lymphoblastic (in fact this concerns also girls 10-14y) and with DLBCL. However, in the multivariate analysis for B-NHL, age and gender were not significant when LDH level >500 was taken into account. Second question: the review of the pooled data of 341 pts aged 15-20y and treated in the pediatric SFCE and adult GELA trials showed (Patte, Lugano 2005): better outcome than observed on the population based registry; similar outcome for Burkitt, but in France Tt is also LMB-based in adults; similar outcome for DLBCL, but with a weight of Tt heavier in the GELA studies; similar outcome in ALCL with a B-NHL like Tt; significant worse outcome for the lymphoblastic pts treated in the adult studies. In conclusion, in the pediatric studies, ados seem to have worse outcome than the younger pts, but this is correlated with a higher proportion of worse clinical prognostic factors in this age class. Ados have a better outcome when treated in clinical trials. In France where GELA treatment for adult B-NHL are more intensive than CHOP, results are similar for the 15-20y with B-NHL although weight of Tt is different. Ados with lymphoblastic NHL must be treated according "pediatric" leukemia-like protocols. The general agreement that ados <18y must be treated and included in pediatric protocols should be generalized in all countries. It would be interesting to see if in the adult trials designed for a wide range of age from 18 to 60y, clinical features, histological subtypes, and prognostic factors, are different for the ados and young adults compared to the older pts.

#### **CHARACTERISTICS, TREATMENT AND OUTCOME OF YOUNG ADULTS UP TO 30 YEARS OF AGE WITH AGGRESSIVE NHL IN THE GELA TRIALS ON ADULT NON-HODGKIN LYMPHOMA**

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Aggressive lymphomas in young adults (<30 years old) comprise diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), and lymphoblastic lymphoma (LL). BL and LL are mainly treated like those lymphomas arising in younger patients. So, mainly DLBCL will be covered in this presentation. Less than 15% of DLBCL are diagnosed in patients under 30 years and the incidence is 5 time less than at 60 years. Regarding histology, there is an excess of the subtype called primary mediastinal lymphoma. No really difference was described for clinical or biological characteristics except than more patients had a good performance status. Bone marrow involvement was less frequent in young patients than older ones. The International Prognostic Index depending on the age, IPI score is usually better in young patients than in older but there is not a difference for patients less than 30 years compared to those 30 to 50 years old. However, for age-adjusted IPI there is not difference for young and old patients. As in other age groups, outcome has dramatically changed with the combination of rituximab to CHOP regimen (R-CHOP). For patients without or few adverse prognostic factors, R-CHOP or R-CHOP-like regimens allowed >90% complete response (CR), >80% 5-year progression-free survival (PFS) and >90% 5-year overall survival (OS). However, these results are far less good for patients with intermediate- or high-risk score. Whether more intensive regimen must be used in these higher risk patients is currently under investigation.

#### **084**

#### **THE ADDITION OF RITUXIMAB TO DOSE-ADJUSTED EPOCH (DA-EPOCH) OBVIATES THE NEED FOR RADIATION TREATMENT IN YOUNG ADULTS WITH PRIMARY MEDIASTINAL B-CELL LYMPHOMA**

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*Background.* PMBL is a distinct clinicopathological entity with a predilection for young females. It clinically resembles classical HL(cHL) in the mediastinum and they share a third of their genes by molecular profiling. PMBL and cHL appear to reside at opposite ends of a disease continuum between which lies gray zone lymphoma(GZL)-diseases with overlapping morphologic and immunophenotypic features of both entities. As with mediastinal cHL, the risk of local failure after anthracycline-based therapy has led to the routine use of mediastinal RT in PMBL. Given the young age at presentation of PMBL and the potential for long-term serious toxicities after RT, strategies that avoid its use are needed.

**Objectives:** We set out to assess if DA-EPOCH-R could obviate the need for mediastinal RT in PMBL (incl. GZL) while maintaining high cure rates and to investigate the role of end of therapy FDG-PET in predicting outcome. **Design and Methods.** We prospectively evaluated DA-EPOCH-R for 6-8 cycles as previously described (JCO 2008; 28(16):2717) in 40 sequential pts with PMBL (32) and GZL (8). Most (30) pts had a PET scan at the end of therapy. **Results.** Enrolled pt characteristics were: median (range) age 32 (19-52); female sex 24 (60%); median ECOG PS 1 (1-3); stage III/IV 11 (28%); LDH > N 27 (68%); median mass size 10.7 (5-19.7) cm; extranodal sites 24 (60%). IHC profiling showed CD20+ in 100%, CD10 + 5%, BCL-6 + 81% and MUM-1 + 48%. At a median potential follow-up time of 4 years, EFS and OS were 90% and 100% for PMBL and 33% and 75% for GZL; 6% of PMBL and 37% of GZL pts required mediastinal RT. In a historical group (18) of PMBL pts treated with DA-EPOCH, EFS and OS were 65% and 77% at 12 years median potential follow-up. Adding R was associated with a significantly improved PFS ( $p=0.0031$ ) and OS ( $p=0.011$ ) by 2-tailed exact log-rank test in this non-randomized comparison. In total, 30 FDG-PET scans were performed at the completion of systemic therapy to determine need for RT. The positive and negative predictive value of FDG-PET for relapse was 62% and 90% respectively. **Conclusions.** DA-EPOCH-R is highly effective in PMBL with 100% OS and obviates the need for RT in >90% of pts. The addition of R likely improves outcome in PMBL. GZL appears more resistant to treatment and may require aggressive strategies including RT.

**085  
PRIMARY MEDIASTINAL B-CELL LYMPHOMA (PMBL) IN CHILDREN (C) AND ADOLESCENTS (A) IS ASSOCIATED WITH A SIGNIFICANTLY INFERIOR PROGNOSIS: REPORT OF THE FAB/LMB 96 STUDY COMMITTEE**

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**Background.** Single pediatric cooperative group studies have demonstrated EFS ranging from 65-75% in C & A with large cell lymphoma arising from the mediastinum (Lones/Cairo et al, JCO, 2000; Burkhardt/Reiter et al, BJH, 2005; Seidman/Reiter et al, JCO, 2003). Gene expression profiling has shown that PMBL more closely resembles classical Hodgkin disease than other DLBCLs and exhibits overexpression of NF- $\kappa$ B pathway genes

(Rosenwald et al, J Exp Med, 2003; Abramson et al, Blood, 2005). The optimal therapy for C & A with PMBL and the identification of subgroups with poorer outcomes are areas of active study. **Methods.** We analyzed the results of C & A with PMBL treated with Group B therapy on FAB/LMB 96 (Patte/Cairo et al, Blood 2007). Patients with histological evidence of disease after CYM1 intensification were switched to Group C therapy (Cairo/Patte, Blood, 2007). **Results.** There were 111 patients with stage III DLBCL (69 DLBCL non-PMBL, 42 PMBL) initially treated with Group B therapy on FAB/LMB 96. Among the PMBL patients, demographic data included: M/F: 26/16; 10-14 vs. 15-19 yrs: 28/14; and LDH <2 vs.  $\geq 2$  upper normal: 20/22. Two-year EFS for the stage III PMBL subgroup was 69% (CIs: 52-80%), significantly lower than two-year EFS of 91% (CIs: 82-96%) for the stage III non-PMBL DLBCL subgroup ( $p<.005$ ). OS for the PMBL subgroup was 73% (CIs: 56-84%). Seven (17%) of PMBL patients had less than a 20% response to initial COP therapy (non-response), while 1 achieved a complete response and 33 were intermediate responders (20-99% response). There was no significant difference in PMBL EFS with respect to age, gender, COP response and initial LDH. **Conclusion.** PMBL in C & A is associated with significantly inferior EFS compared with other stage III non PMBL DLBCL. Alternate treatment strategies including consideration of underlying biological differences need to be developed to improve EFS in C & A with this poor-risk sub-group of mature B-NHL.

**086  
OUTCOME OF ADOLESCENTS WITH NON-HODGKIN LYMPHOMA IN BFM STUDIES: RELEVANCE OF GENDER AND HISTOLOGICAL SUBTYPE**

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**Background.** Incidence, biology, treatment and outcome of Non-Hodgkin Lymphoma (NHL) differ considerably between children and adults, with adolescents forming a separate group that falls between these categories. Even though recent reports pay increasing attention to these patients, the impact of adolescent age on the characteristics and outcome of NHL remains yet to be determined. **Objectives.** The aim was to analyze the outcome of adolescents treated according to BFM protocols for childhood NHL. **Design/Methods.** Patients were treated according to the subsequent protocols NHL-BFM86, 90,

95, 04, ALCL99, EURO-LB02. Treatment subgroups were: 1) lymphoblastic lymphoma (LBL), 2) mature B-cell NHL including Burkitt lymphoma/leukemia (BL/B-AL), diffuse large B-cell lymphoma (DLBCL-CB) and primary mediastinal B-cell lymphoma (PMLBL), and 3) anaplastic large cell lymphoma (ALCL). *Results.* From 10/86 to 12/07, 2915 protocol pts were registered in the NHL-BFM study center. Of these, 378 pts (13%) were adolescents (15-18 years (y) of age) with BL/B-AL (101 pts), ALCL (74 pts), DLBCL-CB (55 pts), T-LBL (45 pts), PMLBL (24 pts), pB-LBL (13 pts) and other NHL (66 pts). The 5-year event free survival (pEFS) was 79±2% for adolescents compared to 85±1% in pts aged <15y (p 0.014). Regarding histological subtypes, pEFS was comparable for adolescents and children with T-LBL (87±6% vs. 82±2%, p 0.24), ALCL (70±6% vs. 70±3%) and PMLBL (57±10% vs. 68±8%, p 0.55) while pEFS was inferior in adolescents compared to children in BL/B-AL pts (82±4% vs. 88±1%, p 0.06) and in DLBCL-CB (pEFS 85±5% vs. 96±2%, p 0.002). Further analyses in adolescents revealed a higher frequency of advanced stages of disease (>stage II) in females (94/119) vs. males (177/259, p 0.04), but less frequent CNS disease in females (3/119 vs. 23/259; p 0.03). pEFS (5y) was inferior for adolescent females (70±5% vs. 84±2% in males, p 0.004). This was mainly due to the unfavorable pEFS for female pts with T-LBL (pEFS 57±17% vs. 97±3%, p 0.001) and DLBCL-CB (pEFS 71±9% vs. 97±3%, p 0.007). *Conclusion.* Adolescents with NHL treated according to pediatric NHL-BFM protocols have an acceptable outcome that is marginally inferior to that in children. Within the adolescent age-group, female pts are diagnosed more frequently with advanced stages of disease. In T-LBL and DLBCL-CB, female gender is associated with a worse prognosis.

**087**  
**DOSE-ADJUSTED EPOCH WITH RITUXIMAB (DA-EPOCH-R) HAS HIGH EFFICACY AND LOW TOXICITY IN YOUNG ADULTS WITH NEWLY DIAGNOSED BURKITT LYMPHOMA: A PROSPECTIVE STUDY OF 25 PATIENTS**

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*Background.* Burkitt lymphoma (BL) is a rare and aggressive lymphoma characterized by a very high tumor proliferation rate. While 'standard therapy' of BL is highly effective, it involves multi-agent intensive chemotherapy and this is associated with significant treatment related toxicity and mortality. *Objectives.* We hypothesized that DA-EPOCH-R may be very effective in BL, given its established efficacy and ability to overcome tumors with a high proliferation rate in diffuse large B-cell lymphoma, and its excellent tolerability in this group. We set out to investigate if this regimen could maintain the high cure rates of standard therapy in BL, while at the same time minimizing treatment related morbidity and mortality. *Design and methods.* Patients (25) eligible for this study

had untreated BL and could be HIV negative or positive. HIV negative patients (n=17) received 6 cycles of DA-EPOCH-R as previously described (JCO 2008: 28(16) 2717) and HIV positive patients (n=8) received 3-6 cycles of DA-EPOCH-R for a minimum of 3 cycles and 1 cycle beyond CR (Blood 2005: 106; #930). All patients received prophylactic intrathecal chemotherapy and outpatient therapy was instituted where possible. *Results.* Enrolled patient characteristics (n=25) are: median age 30 (18-66); male sex 20 (80%); median ECOG PS 1 (1-3); Murphy stage III/IV 15 (56%); LDH>N 14 (58%); extranodal sites 19 (76%) and ileocecal disease 15 (60%). No patients had CNS disease at diagnosis. 100% of patients achieved CR/CRu by clinical response criteria with 1 patient requiring consolidation radiation to a site of residual disease. At a median potential follow-up time of 35 months, OS and EFS are 100% and 96%. Notable toxicities were tumor lysis syndrome in 1 patient and fever/neutropenia complicating 16% of cycles. No treatment related deaths occurred. *Conclusion.* DA-EPOCH-R is a very effective regimen with low toxicity and low rates of tumor lysis syndrome in BL and represents a radical departure from 'standard' BL regimens. Given these results, a risk-adaptive strategy using DA-EPOCH-R in BL and accruing patients of 4 years and older is underway.

**088**  
**CHILDHOOD AND YOUNG ADULT NON HODGKIN LYMPHOMA IN OBAFEMI AWOLOWO UNIVERSITY TEACHING HOSPITAL COMPLEX, ILE-IFE NIGERIA: A 5 YEAR REVIEW**

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*Background.* Poor infrastructural base for rapid and accurate diagnosis, non availability of appropriate therapeutic options and high default rates constitute strong limitations to management of NHL in resource-limited countries like Nigeria. *Objective.* To evaluate the treatment outcome of childhood and young Adult NHL at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria. *Method.* We reviewed the prevalence as well as the spectrum of NHL in patients age 50 years and below (<50) seen between January 2003 and December, 2008 in OAUTHC. Patients' records were checked for Age, sex, Diagnosis, treatment and outcome of therapy. Overall survival (OS) was calculated by Kaplan Meier technique. Data were analyzed with SPSS Version 16.0 software. *Result.* Diagnoses were made by morphology (Fine needle aspiration cytology for Burkitt Lymphoma and histology for others). Ziegler's classification was adopted for Burkitt lymphoma (BKT) and Working Formulation classification was used for the NHLs. Patients with BKT had COM regimens plus intrathecal cytarabine and methotrexate. The Non Burkitt group had CHOP (aggressive histology), CVP (intermediate/indolent histology); all childhood NHLs were treated as high grade lymphomas. One Hundred and five

(105) cases of NHL were seen over the period under review; 84 patients (80.0%) had BKT (57 M, 27F, M:F=2.1:1, ages 2-24 years (median, 8 years)). Three patients (3.6%) had clinical stage A disease, 4(4.6%), 46(54.8%) and 31(36.9%) had clinical stages B, C, D, respectively. NHL was confirmed in 21 (20%) patients (13 M, 8F, ages 7 -50 (median, 24) years). Twelve (57.1%) of the NHL had aggressive lymphoma, while 5 (23.8) and 4 (19.0%) presented with intermediate and low grade histology, respectively. Immunophenotyping was not done for any patient and all of the patients tested negative for HIV/II antibodies. High default rate was a major problem for the patients, which improved significantly for BKT with the provision of drugs and oncology nurse/home visitor by the INCTR, so that only 55 (65.5%) of BKT were fully followed up; 44 (80%), 7 (12.7%) and 4 (7.3%) underwent CR, PR and NR after 1-3 cycles of chemotherapy, respectively. The OS was 51.6% at 12 months, 47.2% at 24 months, and 42% at 48 months. Treatment outcome was less remarkable for NHL because a large majority could not afford cost of chemotherapy, the patients had between 1 to 8 cycles (median 4 cycles) of chemotherapy. Treatment and follow up lasted between 1 to 27 months (median-3months). *Conclusion.* Burkitt Lymphoma is predominantly a disease of young children, while non Burkitt, non-Hodgkin lymphomas predominate in individuals over age 15 years. Without support, default rate was very high. Lack of immunohistochemistry is a strong limitation to proper disease characterization.

## 089

### **MODIFIED NHL-BFM 90 PROTOCOL FOR ADOLESCENTS AND YOUNG ADULTS WITH BURKITT LYMPHOMA (BL), MATURE B-CELL ACUTE LYMPHOBLASTIC LEUKEMIA (B-ALL) AND DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)**

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*Background.* The prognosis BL/B-ALL has improved since short, intensive, multi-agent chemotherapy regimens (like pediatric NHL-BFM 90), were introduced. Apparently, patients with the germinal center B-cell-like (GCB) DLBCL, frequently at a young age, may benefit of such intensive treatment. *Objectives.* The purpose of this study was to determine the activity and toxicity of the modified NHL-BFM 90 protocol in adolescents and young adults (AYA) with non-Hodgkin lymphoma (NHL). *Methods.* From 1994 to 2008, 40 pts (m=23, f=17) with *de novo* non-HIV BL (n=10), B-ALL (n=4) and DLBCL (n=26) were treated with 6 sequential chemotherapy cycles similar to those in the protocol NHL-BFM 90 for B-NHL. Before 2006, pts (n=28) received a modified treatment cycles with the reduction of methotrexate (1 g/m<sup>2</sup>/36h instead of original 5 g/m<sup>2</sup>/24h). Since 2006, pts (n=12) received therapy on a national pediatric protocol B-NHL-M 2004 (Samochatova E.V., 2004). This protocol differed from the original by adding Rituximab 375 mg/m<sup>2</sup> on the first day of each cycle and reduction of methotrexate doses only in the first 2 cycles (1 g/m<sup>2</sup>/24h instead of 5 g/m<sup>2</sup>/24h). *Results.* Median age including pts was 20.8 years (range, 15-37). Thirty three pts (83%) were diagnosed in advanced (III-IV) stages, bone marrow (BM) involvement observed in 10 (25%) and CNS infiltration in 6 (15%). Complete response (CR) was documented in 10 (71%) pts with BL/B-ALL and 19 (73%) with DLBCL. 6-years overall survival (6y-OS) was 0.64 (SE 0.14) for BL/BALL and 0.65 (SE 0.11) for DLBCL. 6-years event free survival (6y-EFS) was 0.64 (SE 0.14) and 0.60 (SE 0.10) relatively. Grade 3-4 non hematological toxicity was as follows: infection – 18%; elevation of ALT/AST – 16% and mucositis – 12%. Fourteen pts died on treatment: 8 pts (57%) - due to disease progression/relapse, 1 pt (7%) due to induction toxicity and 5 pts (36%) due to different type of toxicity in CR. The limited number and duration of observations cannot yet reveal the advantage of regime with Rituximab: CR (75 vs. 71%); 2y-OS (0.67 vs. 65; *p*>0.05) and 2y-EFS (0.58 vs. 0.63; *p*> 0.05). *Conclusions.* Modified NHL-BFM 90 protocol for B-NHL and B-NHL-M 2004 are active and well tolerated for AYA with mature B-cell NHL.

**SCIENTIFIC SESSION 8:  
RELAPSED/REFRACTORY LYMPHOMA**

**LYMPHOMA RELAPSE:  
MECHANISMS OF RESISTANCE  
AND HOW TO OVERCOME THEM**

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Mechanisms of resistance to conventional chemotherapy drugs are varied, and are now well recognized to go well beyond MDR or p-glycoprotein expression. Resistance of tumor cells to chemotherapy is generally defined as: (1) intrinsic or (2) acquired drug resistance. Intrinsic drug resistance accounts for the initial failure of the disease to respond to the initial line of chemotherapy. Acquired drug resistance describes the emergence of drug resistant clones of disease following multiple lines of chemotherapy for the relapsed state. At a molecular level, it is now widely recognized that dysregulation in apoptosis plays a major role in both intrinsic and acquired drug resistance. The apoptotic pathway involves the complex and coordinated inter-play between a variety of different proteins, all belonging to the Bcl-2 family. In general, these family members are sub-divided into pro-apoptotic (BAX/BAK) or anti-apoptotic proteins (Bcl-2, Bcl-xl, MCL-1, Bcl-w, for example) and BH3 only mimetics (NOXA, PUMA, bid, bik for example). The balance of pro- and anti-apoptotic proteins governs the likelihood of a cell to undergo apoptosis. Recently, a variety of new drugs targeting this biology have emerged. For example, some agents like proteasome inhibitors have been shown to increase the relative ratio of BH3 only mimetic proteins in a fashion that allows disassociation of the pro-apoptotic proteins like BAX/BAK, inducing programmed cell death. While the discrete mechanism of action of proteasome inhibitors is not yet entirely clear, increasing areas of research have begun to focus on their impact on the Bcl-2 family members, and how they may lower the threshold required for the induction of apoptosis. Other exciting new class of agents, the BH3 only mimetic small molecules, are now in early Phase 1 clinical trials. These drugs are designed to mimic the various BH3 only proteins and their specific interaction with anti-apoptotic proteins. In so doing, they liberate pro-apoptotic proteins, leading to mitochondrial transmembrane depolarization, cytochrome c release, and induction of apoptosis. Today, there are a number of small molecules in this class including AT-101 and ABT-263, both of which are now being studied in Phase 1 and 2 clinical trials. In this presentation, we will review the new pharmacologic strategies now emerging which appear to offer new opportunities to modulate this biology in a way that allows these drugs to play a critical role in overcoming both intrinsic and acquired drug resistance.

**ROLE OF HEMATOPOIETIC STEM CELL  
TRANSPLANTATION IN RELAPSED/REFRACTORY  
NON HODGKIN'S LYMPHOMA**

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*Background.* 10-25% of children and adolescent with Non-Hodgkin's lymphomas (NHL) suffer a relapse after current intensive front-line chemotherapies. The relapse rate differs according to NHL subtype: aggressive mature B-NHL – 10-15%; lymphoblastic lymphoma (LL) – 10-15%; anaplastic large cell lymphoma (ALCL) – 25-30%. Recommending treatment approaches for children and adolescents with relapsed NHL continues to be a challenge. *Objective.* To define the role of high dose chemotherapy with autologous blood stem cell rescue (autoSCT) and allogeneic hematopoietic stem cell transplantation (alloSCT) in the therapy of relapsed or refractory NHL after current intensive front-line treatments. *Design and methods.* A Pubmed-based literature search and an analysis of the relapse pts registered in the NHL-BFM-database from 1990 to 2005 served as a basis for this review. *Results.* Outcome in relapse differs according to NHL-subtype. It is dismal for pts with a relapse of a Burkitt's lymphoma or LL with reported survival rates of 10-30%. Survival for pts with a relapse of a diffuse large B-cell lymphoma (DLBCL) or ALCL reaches 50%. The prognosis of pts with a relapse of a primary mediastinal large B cell lymphoma (PMLBCL) and other rare NHL subtypes is about 25-40%. Relapses of LL occur very early (30% progression, 90% relapse during maintenance). More than half of the pts do not reach a second remission and die before planned SCT. The only surviving pts got consolidation with allogeneic SCT after obtaining a stable remission by intensive re-induction chemotherapy. Survival of pts with relapse of a mature B-NHL differs according to subtype and therapy. Pts treated without SCT have almost no chance to survive. Pts with DLBCL-relapse have a fair chance of >50% to reach a 2<sup>nd</sup> CCR by pulse-type re-induction chemotherapy followed by autoSCT. For Burkitt's lymphoma-relapse pts the major goal is achieving a stable second CR; relapses refractory to chemotherapy are not curable currently. Available data do not allow recommending the type of SCT for consolidation. The scarce data on PMLBCL-relapses suggest that consolidation by alloSCT may be more promising than by autoSCT. Despite comprising only 15% of the pediatric and adolescent NHL, pts with ALCL-relapse are the largest group of relapse pts allowing risk stratification in relapse. About 20% of relapses are progresses during front-line therapy. Survival of those pts has only been reported after alloSCT (OS 30-40%), even when transplant was performed with active disease. Pts relapsing after front-line therapy have a chance to survive by different strategies including VBL-maintenance, autoSCT or alloSCT. The low rate of tumor failure after alloSCT for first or further relapse of only 20-30% suggests the existence of a graft-versus-ALCL-effect. *Conclusion.* With increasing success of frontline treatment for childhood and adolescent NHL, relapses are highly refractory diseases. In LL and B-NHL-relapse pts, it is most crucial – and

challenging - to reach a stable second CR before any type of SCT for consolidation. Recommendations for SCT-type can currently be based on disease-entity and time of relapse/progression.

#### 090

### HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT) AS SALVAGE FOR NON-HODGKIN LYMPHOMA (NHL) IN CHILDREN AND ADOLESCENTS: A REPORT FROM THE CENTER FOR INTERNATIONAL BLOOD AND MARROW TRANSPLANT RESEARCH (CIBMTR)

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**Background.** The role of HSCT for pediatric NHL is difficult to establish due to small numbers of patients in published reports. **Objectives.** To define the efficacy of HSCT as salvage therapy for pediatric NHL in the era of "modern therapy" based on graft source (autologous vs. allogeneic) and histology – Burkitt (BL), diffuse large B cell (DLBCL), lymphoblastic (LL) and anaplastic large cell (ALCL) lymphoma. **Design/Methods.** Patients  $\leq 18$  years, with refractory or recurrent BL (n=41), LL (n=53), DLBCL (n=52) and ALCL (n=36), receiving autologous (n=90) or allogeneic (n=92-43 matched sibling and 49 unrelated donor) HSCT from 1990-2005 reported to CIBMTR were included in analyses. 5 yr progression-free survival (PFS) and probability of relapse/progression after HSCT were evaluated using stratified Cox regression. **Results.** Characteristics of allo and auto HSCT recipients were similar. Allo recipients were more likely to receive TBI-containing regimens, marrow stem cells, receive HSCT in more recent years, and have LL. Pt not in CR at time of HSCT did significantly worse with 5 yr PFS of 28% and 20% after auto and allo HSCT, respectively. Relapse rates in allo vs. auto HSCT were similar for DLBCL (35% vs. 32%) and BL (63% vs. 65%). For ALCL, the relapse rate was higher for auto (48% vs. 20%) but this did not reach statistical significance. For LL, there were significantly more relapses following auto vs. allo HSCT (86% vs. 23%). The 5-year PFS was similar after allo vs. auto HSCT for DLBCL (50% vs. 52%), BL (31% vs. 27%) and ALCL (46% vs. 35%). However, PFS was superior after allo vs. auto HSCT for LL (40% vs. 4%,  $p < 0.01$ ). Incidence of grade II-IV acute GVHD=43% and chronic GVHD=16%; therefore due to small numbers the effect of GVL could not be determined. **Conclusion.**

HSCT is effective as salvage therapy for pediatric NHL. Disease status at time of HSCT is a significant predictor of outcome. Outcome is similar using auto vs. allo HSCT for all histologies, except for LL where allo HSCT is far superior.

#### 091

### RELAPSE OF ALCL AFTER ALCL99-THERAPY: CHARACTERISTICS AND OUTCOME

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**Background.** 25-30% of patients (pts) with anaplastic large cell lymphoma (ALCL) suffer from progression or relapse. Data available on outcome after relapse are limited. **Objective.** To describe relapse characteristics and outcome after progression or 1st relapse of an ALCL in pts treated according to the ALCL99 front line protocol. **Patients and methods.** Among the 556 pts treated for a systemic ALCL with ALCL99 protocol between 11/1999 and 01/2008, 146 were reported to have progressed or relapsed. Present analysis of relapse treatment and further outcome was performed using a standardized documentation form completed for 141/146 pts. Progression-free survival (PFS) was computed from the date of progression/1st relapse to the date of 2nd relapse or death (failure). **Results.** Median time from start of initial therapy to relapse was 10 months (range, 0.7 to 60): 22 pts progressed during induction treatment, 55 relapsed within 3 months after end of treatment and 64 relapsed thereafter. Relapse was limited to sites initially involved in 43 pts. Salvage therapy was based upon HD-Ara C and Etoposide (CC or CYVE) in 66, vinblastine in 18, ICM/ICE in 10, CVA or CVB in 10, AA/BB in 9 and other in 20 (no treatment: 4; missing data: 4). 76% of pts reached a second complete remission (CR). A stem-cell transplant (SCT) was performed as part of the relapse strategy in 84 pts: allogeneic in 42, autologous in 42. With

a median follow-up of 40 months after 1st relapse, 95 pts are alive (61 CR2, 32 >CR2, 2 progressive disease) and 46 pts died (31 of disease and 15 of toxicity). PFS and overall survival (OS) 3 years after 1st relapse are 43±4% and 70±4%, respectively. The PFS differed significantly according to the time of relapse (3y-PFS: progression during treatment -23%, ≤3 months after end of treatment -35%, later -56%,  $p=.0001$ ). The role of therapy is difficult to assess as indications of SCT varied according to risk factors: 3-year PFS and OS were 42% and 90% after autologous SCT vs 65% and 74% after allogeneic SCT ( $p=.09$  and  $.22$ , respectively). Outcome of pts who did not receive SCT cannot be interpreted since it is a heterogeneous group of pts in whom the decision not to give SCT was based either on early progression or on good prognosis factors. **Conclusion.** Our data confirm that the time of relapse/progression is a strong prognostic factor for subsequent failure in ALCL.

## 092

### **OUTCOME OF CHILDREN AND ADOLESCENTS (C+A) WITH MATURE B-NHL WHO RELAPSE/PROGRESS ON FAB/LMB 96: SHORT TIME TO RELAPSE AND ELEVATED LDH ASSOCIATED WITH A SIGNIFICANT DECREASE IN OVERALL SURVIVAL (OS)**

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**Background.** We have previously reported on the excellent EFS of C+A with mature B-NHL treated with FAB/LMB 96 therapy with limited, intermediate and high-risk disease, respectively (Gerrard *et al*, BJH, 2008; Patte *et al*, Blood 2007; Cairo *et al*, Blood, 2007). In the past, the outcome, especially C+A who relapse/progressed, ranged between 20-30% (Cairo *et al*, BJH, 2003; Cairo *et al*, Am J Hem, 2003). **Objective.** To determine the probability of OS and the risk factors associated with OS in C+A who relapsed/progressed on the FAB/LMB 96 trial. **Methods.** C+A with newly diagnosed mature B-NHL were treated on FAB/LMB 96 as previously reported (Gerrard *et al*, BJH, 2008; Patte *et al*, Blood 2007; Cairo *et al*, Blood, 2007). A univariate log-rank analysis of post-recurrence survival was performed using the following risk factors: age <15 vs >15 yrs, sex, group, primary site, LDH< vs >2 NL, histology, BM/CNS, stage (St. Judes), cooperative group, and time to relapse <3 vs

3-6 vs. >6 mos. Variables with  $p < 0.05$  in the univariate analysis were included in a Cox multivariate regression analysis. Probability of OS was determined by Kaplan Meier method. **Results.** There were 1,111 patients entered on study with a 9.4% (N=104) incidence of relapse. The 1 yr OS was 28±4.4% (mean±SEM). Variables associated with a significant decrease in OS in the univariate analysis included: Group C vs. B: 17.5±5.5% vs. 38.6±6.6%,  $p=0.01$ ; LDH >2 NL: 19±4.6% vs. 58±9.7%,  $p < 0.001$ ; BM+: 18±5.8% vs. BM-36±6.2%,  $p=0.038$ ; CNS+: 19±7.0% vs. CNS-: 32±5.6%,  $p=0.043$  and < vs. > 6 months from diagnosis: 18±4.7% vs. 49±8.7%,  $p=0.0017$ . In a Cox multivariate regression analysis, only LDH > 2 NL,  $p=0.0094$  and <6 mo diagnosis to relapse ( $p=0.05$ ) remained significant. **Conclusion.** C+A with mature B-NHL who relapse/progress on FAB/LMB 96 therapy have a significantly poorer prognosis if they relapse early (<6 mo) and/or present with LDH >2 NL. Indicators of better outcomes were Group B disease, LDH <2NL, BM-, CNS- and longer time to relapse. These results will provide guidance into the development of risk adapted relapse strategies in C+A with mature B-NHL who relapse off modern short but intensive front-line therapies such as FAB/LMB 96.

## 093

### **OUTCOME AND PROGNOSIS OF RELAPSE IN THE LMB89, 96 AND 2001 PROTOCOLS OF SFOP/SFCE**

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**Background.** While survival of childhood B-Cell Non-Hodgkin's Lymphoma (B-NHL) has increased from 35 to 90% during the last years, especially in France with the LMB protocols, prognosis of relapses (R) after this intensive 1st line chemotherapy (1st CT) remains poor. **Objectives.** To study modalities, treatment (Tt) and outcome of R following the LMB 89, 96 and 2001 protocols in which patients (pts) were stratified in 3 risk groups (gr A,B,C) receiving Tt of progressive intensity. **Methods.** R registered in LMB database were reviewed (07/1989-03/2007). Additional data on Tt were collected by reviewing pts'clinical files. General guidelines were: 2<sup>nd</sup> line chemotherapy (2<sup>nd</sup> CT) and high dose chemotherapy (HDC) with stem cell transplantation (SCT) in 2nd CR. **Results.** 67 out of 1322 pts (5%) (27, 23 and 17 in LMB89, 96 and 2001 respectively) relapsed: 52 Burkitt (BL), 10 DLBCL and 5 non classified B-NHL. 3 were treated in grA, 38 in grB and 26 in grC. Median delay of

R after diagnosis was 145 days (70-361) in BL and 537 (116-981) in DLBCL. 33 pts relapsed in one site (12 primary, 1 bone marrow(BM) and 15 CNS) and 34 in multiple sites [including BM (25 pts) or CNS (10 pts)]. 65 pts received one or more lines of 2<sup>nd</sup> CT, depending on initial gr, with mostly CYVE in gr A and B and more heterogeneous Tt in gr C (MTXHD, ICE, ICN). 16 pts received Rituximab. After 2<sup>nd</sup> CT, 32 were considered in 2<sup>nd</sup> CR, 8 in PR and 2 in objective response. 21 pts progressed and 2 responses were not documented. 41 pts received HDCT with autologous (33) or allogeneic (8) SCT. The median follow-up was 6 years. 47 pts died, all during the 12 months (m) after R. 4 died of treatment-related toxicity. 20 pts were alive (2 grA, 14 B, 4 C). Survival rate at 12 m was 30% (95%CI=20%-42%). In multivariate analysis using Cox model, initial prognostic gr A or B with LDH level lower than 2 upper normal range ( $p=0.009$ ), histological diagnosis as DLBCL ( $p=0.007$ ), relapse in one site ( $p=0.003$ ) and CR obtained after relapse ( $p<0.0001$ ) were significantly associated with better survival. Rituximab and type of SCT had no impact, but numbers were low. *Conclusion.* Prognosis of R childhood B-NHL after intensive 1st CT is poor, in particular in case of multi-site R, and for pts of initial grB with high LDH level and grC. New combinations appear necessary to improve the CR rate before HDC and SCT. A double SCT rescue strategy should also be considered.

#### 094

##### **IMPACT OF THE AMOUNT OF CIRCULATING TUMOR CELLS AT INITIAL DIAGNOSIS ON THE RELAPSE CHARACTERISTICS AND OUTCOME AFTER RELAPSE OF PATIENTS WITH AN NPM-ALK POSITIVE ALCL**

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*Background.* 25-35% of children and adolescents with anaplastic large cell lymphoma (ALCL) suffer a relapse after BFM-type therapy. Quantification of circulating tumor cells (CTC) by RQ-PCR for NPM-ALK in initial blood or bone marrow allows identifying patients with a relapse risk of almost 70%. *Objective.* We analyzed the time of relapse of patients with high numbers of initial CTC (=relapse after initial high CTC; i.e., more than 10 copies NPM-ALK/104 copies ABL in PB or BM) as well as therapy and outcome of those pts in order to get hints whether to intensify first-line or relapse-therapy for those pts. *Design and methods.* Inclusion criteria: 1. NPM-ALK-positive ALCL, 2. RQ-PCR result for NPM-ALK in bone marrow and/or blood at diagnosis, 3. treatment according to the BFM-type protocols NHL-BFM95 or ALCL99 between 8/1998 and 10/2007, 4. relapse of the ALCL. 33 of the 112 patients fulfilling the criteria 1-3 relapsed. Relapse characteristics, therapy and outcome were analyzed for the 18 patients with a relapse after initial high CTC. *Results.* The median time of relapse/progression was 0.4 months (during therapy-13.7) after the end of intensive therapy in patients with a relapse after

initial high CTC. This was significantly earlier than the time of relapse of patients with initial low/no CTC (6.5 months, range 1.8-55;  $p<0.01$ ). 9/18 relapse pts after initial high CTC progressed during initial intensive therapy compared to none of the 15 relapse pts after initial low/no CTC ( $p<0.01$ ). Therapy and outcome could be evaluated for 17 of the 18 relapse pts after initial high CTC (1 still in therapy): Blood stem cell transplantation (SCT) was planned for 15/17 pts, 10 of these 15 progressed again before reaching SCT. All 4 pts who did not receive SCT died of disease (DOD); 2 of the 3 pts treated by autologous SCT (all without further progress before SCT) relapsed again; 7/10 pts treated by allogeneic SCT progressed again before SCT, 5 survived, 3 DOD and 2 died of complications. *Conclusion.* Half of the pts relapsing after initial high numbers of CTC progress during initial therapy. Half of these pts can be rescued by consolidation with allogeneic SCT in relapse/progression.

#### 095

##### **A PILOT STUDY OF MYELOABLATIVE (MA) AUTOLOGOUS STEM CELL (AUTOSCT) FOLLOWED BY REDUCED INTENSITY ALLOGENEIC TRANSPLANTATION (RI ALLOSCT) IN CHILDREN WITH RELAPSE OR REFRACTORY HODGKINS DISEASE (HD) AND NON-HODGKINS LYMPHOMA (NHL)**

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*Background.* AlloSCT may benefit patients with poor risk HD and NHL by providing a graft vs. lymphoma effect. In relapse/refractory (R/R) HD pts, Majhail *et al* (BBMT 2006) reported OS of 9%-37% post MA Auto SCT. Similarly, outcome for children with poor risk NHL remains dismal (Bradley/ Cairo, BMT 2008). Carella *et al* (JCO 2000) demonstrated the success of MA AutoSCT followed by RI AlloSCT in adults with R/R lymphoma. We investigated the feasibility of MA AutoSCT followed by RI AlloSCT in children with poor risk HD (Induction failure, early relapse [ $<1$ yr], late relapse [stage III-IV], 2<sup>nd</sup> and 3<sup>rd</sup> relapse) and NHL (induction failure, 1st, 2nd or 3rd PR and 2<sup>nd</sup> or 3<sup>rd</sup> CR), estimated OS 30%. *Methods.* MA conditioning prior to AutoSCT was cyclophosphamide 1500x4d mg/m<sup>2</sup>, carmustine 100x3d mg/m<sup>2</sup>, etoposide 800x3d mg/m<sup>2</sup>. AlloSCT conditioning was fludarabine 30x5d mg/m<sup>2</sup>, busulfan 3.2x2d mg/kg, and R-ATG 2x4d mg/kg (MUD). CD20<sup>+</sup> NHL pts received rituximab and HD pts received involved field radiotherapy prior to RI AlloSCT. *Results.* Fifteen patients received both AutoSCT and AlloSCT. Median time to RI AlloSCT after MA Auto SCT was 110 days (95-212). Disease status prior to AlloSCT 7 NHL (CR1-1, CR2-4, PR-1, SD-1) and 8 HD (CR1-2, CR-2-3, PR-3). Median age: 15yrs (3-18 yrs), median f/u: 737 days (100-3033). Donors: 4 MRD, 4MUD, 7 UCB. Neutrophil and platelet engraftment was achieved at a median of 12 days (9-25) and 15 days (9-40) respectively, after AutoSCT and 18 days (15-45) and 33 days (11-99), respectively, after AlloSCT. Donor chimerism reached

>95% in all eligible pts by d100. Cumulative incidence of acute GVHD: grade II-III aGVHD was 14% (CI-95 1.8 18.4) and cGVHD 33% (CI-95 14 52), respectively. Ten pts are alive and NED post RI AlloSCT. Five pts died: 2 transplant related mortality and 3 relapsed. The probability of 5yr overall survival was 64% (CI-95 35 83). **Conclusions.** MA AutoSCT followed by RI AlloSCT is feasible and well tolerated in children with poor risk HD and NHL. A larger study with longer follow up is required to determine if this approach will reduce relapse, long term toxicity and/or improve survival compared to MA AutoSCT alone.

**096  
CLINICAL FEATURES AND OUTCOME OF RECURRENT ANAPLASTIC LARGE CELL LYMPHOMA (ALCL) IN CHILDREN AND ADOLESCENTS**

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**Background.** Recent studies in ALCL in Pediatrics have shown an event free survival of approximately 60-80%. The second complete remission rate is 85%. However, 40% of such patients develop second relapse. Among important factors which may adversely affect the outcome of first relapse are: relapse while on the first line therapy, prior Vinblastine treatment and CD3 positivity. **Objectives.** To review the clinical course, pathological features and possible risk factors in patients who failed first line therapy in our institution in the last 10 years. **Design/Methods.** A retrospective review of the clinico-pathological features, and outcome of patients with relapsed ALCL from 3/1999 to 2/2009. **Results.** Four patients relapsed. They ranged from 13.2 to 17.0 years (median: 14.3). Of 4 patients who relapsed, 2 are alive and disease free after their first relapse. One patient is alive and disease free 3.5 year after 2<sup>nd</sup> relapse. This patient's second relapse was after autologous stem cell transplantation (ASCT). She was successfully treated with weekly Vinblastine for 2 years. The remaining patient had extensive disease at diagnosis with CNS and BM involvement. His lymphoma expressed CD3. He failed 2 lines of salvage therapy, had multiple complications and died of disease progression.

**Table 1. GRecurrent ALCL-Patients' characteristics**

#	Stage at Dx*	ALK1	CD3	Vinblastine in Initial therapy	Time to first relapse <sup>^</sup>	Time from first to second relapse <sup>^</sup>	Follow up form last relapse <sup>^</sup>
1	I	+	-	No	1.5	1.9	3.5
2	II	+	-	No	0.3		3.8
3	IV	+	+	Yes	0.6	0.1	Died of progression 1.1 years from 2 <sup>nd</sup> relapse
4	III	-	-	Yes	1.4		1.0

<sup>^</sup>In Years, \*Dx: Initial Diagnosis

**Conclusion.** Our small series of patients support some reported unique features of ALCL. Successful salvage of recurrent disease with long courses of Vinblastine is feasible after second relapse and after failing high dose chemotherapy. Extensive disease especially with CNS and BM involvement at diagnosis and CD3 positivity may predict poor outcome.

**097  
TREATMENT OF A CNS RELAPSE WHILE ON THERAPY FOR BURKITT LYMPHOMA**

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**Background.** Non-Hodgkin Lymphoma (NHL) continues to be one of the most common pediatric malignancies diagnosed in the United States with approximately 500 cases reported each year, of which greater than 1/3 are of the Burkitt Lymphoma (BL) subtype. Patients with BL who have combined CNS and bone marrow (BM) involvement at diagnosis have been identified as a particularly high-risk subgroup with 4-year EFS rates around 60%. With the present inability to de-intensify therapy for the Murphy Stage 4/Group C BL patients without compromising outcomes, regimens in the relapse setting may offer little more than added toxicity. **Objectives.** We are reporting on a patient with Stage 4/Group C BL who had an early CNS relapse while on therapy and was successfully treated using a concentration times time (C x T) model approach. **Design//Methods.** A salvage treatment regimen incorporating ommaya directed therapy using the concentration times time (CxT) model was integrated into systemic chemotherapy based on the Children's Oncology Group (COG) ANHL01P1 study as a backbone for a single relapsed patient with Stage 4/Group C BL. **Results.** The patient had no evidence of lymphoma in his CSF at the time of his first ommaya therapy, after receiving CYVE therapy at time of relapse, and remained negative for malignancy throughout his treatment course. The patient completed 11 months of integrated systemic/CxT therapy without any neurotoxicity. During his entire treatment course on the combined regimen he had a single non-chemotherapy admission, which was for fever and neutropenia following a cycle of cytarabine. At the time of this report, the patient is now 12 months off therapy (20 months from his CNS relapse) and remains in complete remission. **Conclusion.** In summary, we report a patient with advanced stage BL who had a CNS relapse on therapy and was successfully treated by integrating CNS directed ommaya (CxT) therapy to intensified systemic chemotherapy with minimal toxicity.

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**SURVIVAL OF INTRACRANIAL NHL RELAPSE TREATED WITHOUT INVOLVEMENT OF CRANIAL RADIOTHERAPY**

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**Background.** Isolated CNS relapse of mature B-cell Lymphoma is uncommon and cure is rarely achieved: radiotherapy is generally advised. We report an unusual patient, successfully treated with chemotherapy followed by allogeneic bone marrow transplant alone. **Objectives.** The aim of our curative treatment plan was to avoid cranial radiotherapy to minimize radiotoxic side effects. **Design/Methods.** A 6 year old boy presented with diffuse lymphadenopathy and extensive (90%) bone marrow involvement with mature B-cell lymphoma (CD79a, CD10, CD20, bcl16 and Ki 67 positive). CSF cytology demonstrated no evidence of lymphoma, and he was treated according to national guidelines, identical to FAB-LMB-96 protocol, Group C, CNS negative. Initial management was uncomplicated and remission confirmed after cycle CyVE 2 (high dose Cytarabine and Etoposide), but the patient developed severe headache after maintenance cycle 3, and an isolated CNS recurrence was confirmed by lumbar puncture. Reinduction was begun with high dose dexamethasone and triple intrathecal therapy, and subsequent therapy using Rituximab, Ifosfamide, Carboplatin and Etoposide (R-ICE) with ongoing triple intrathecal therapy. Remission was consolidated with two courses of high dose Methotrexate (8g/m<sup>2</sup>) and a BEAM-conditioned sibling (10/10) allogeneic transplant. **Results.** Engraftment was seen at day +22; chimerism has remained stable for whole blood and CD3 lineage (both 100%) for more than 12 months. The patient remains well and in remission 26 months from diagnosis and 21 months from relapse. **Conclusion.** High dose chemotherapy followed by allogeneic bone marrow transplant can be an effective treatment option for isolated CNS B-cell relapse. Routine use of CNS radiotherapy needs to be questioned. To date, apart from this child, only four other similar survivors have been reported in the literature.

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**SALVAGE THERAPY INCLUDING AUTOLOGOUS STEM CELL RESCUE (ABMT) FOR RELAPSING CHILDHOOD BURKITT-LIKE NON-HODGKIN'S LYMPHOMA (NHL)**

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**Background.** Since relapsing B-cells solid tumors are exceptionally rare pediatric malignancies, there are no generally approved salvage protocols existing.

**Objectives.** We report on a 12 years-old boy with an isolated lung relapse of a Burkitt-like NHL 9 months after completion of primary therapy subsequently salvaged by multidrug combined chemotherapy. **Design/Methods.** Primary treatment was accomplished between July and September 2007 according to B-NHL BFM 2004, Stage III, TG R2 for Burkitt-like NHL with initial disease involvement of the mediastinum and the neck lymph nodes. CR1 was achieved and surgical biopsy of the residual tumor after cessation of chemotherapy showed necrotic tumour only. Isolated tumor relapse occurred in the right lung 9 months after completion of therapy. Prophylactic CNS treatment was reinstated with intrathecal Depocyte® injections every 4<sup>th</sup> week and systemic therapy reintroduced according to the BFM protocol with "VorfaSe" including Rituximab (375 mg/m<sup>2</sup>), followed by 3 courses of ICE (Ifosfamide 1 800 mg/m<sup>2</sup> d1-5, Etoposid 100 mg/m<sup>2</sup> d1-5 and Carboplatin 400 mg/m<sup>2</sup> d1-2) with a total of 7 doses of Rituximab (375 mg/m<sup>2</sup>). CR2 was achieved and consolidated with high dose Busulfan, Etopofos and Cyclophosphamide followed by ABMT. The karyopreserved CD34<sup>+</sup> PBSC were harvested after nadir of the first ICE course. Supportive therapy included intravenous immunoglobulin substitution, prophylactic antifungals, aggressive antibiotic and antifungal therapy during episodes of neutropenic fever. No major nor unexpected toxicities occurred. Despite high total number of Rituximab doses, there were no acute nor delayed complications of a marked B-cell suppression. **Results.** Durable CR2 has hopefully been achieved (reported at abstract submission deadline) 6 months after salvage chemotherapy incl. ABMT for relapsing Burkitt like NHL occurring 9 months after cessation of primary treatment. **Conclusion.** Combined ICE-based chemotherapy with an addition of monoclonal Rituximab antibody consolidated with high dose chemotherapy and autologous stem cell rescue is an effective salvage treatment in achieving subsequent remission for relapsed B-cell lymphoid malignancy. Optimal dosage of Rituximab needs further investigation as well as the use of intrathecal Depocyte.®

**PATHOPHYSIOLOGY AND MANAGEMENT OF ACUTE TUMOR LYSIS SYNDROME (ATLS)**

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ATLS is an oncologic and metabolic emergency, secondary to rapid and massive breakdown of tumor cells, resulting in severe metabolic abnormalities including hyperuricemia, hyperkalemia, hyperphosphatemia, hypocalcemia and acute kidney injury (AKI) (Hochberg/Cairo et al, Haematologica 2008). We recently developed a classification and grading system for ATLS dividing the syndrome into a laboratory component (LTLS) and a clinical component (CTLS) (Cairo/Bishop, BJH 2004). LTLS is now defined as having two or more metabolic derangements (increased uric acid, potassium and/or phosphorus and/or decreased calcium) three days before or seven days after initiation of cytotoxic therapy. CTLS is defined as having LTLS and at least one clinical manife-

station that is probably or directly related to TLS including renal, cardiac and/or neurological toxicities. The grading of CLTS is based on the NCI CTCAE 3.0 grading system for renal, cardiac and neurological toxicities and is graded by the maximal organ toxicity. There are a number of risk factors that have been identified to be associated with developing ATLS including tumor type (i.e. Burkitt lymphoma, lymphoblastic lymphoma, acute leukemia), increased tumor burden (LDH/WBC/stage), rapid proliferation rate, high sensitivity to cytotoxic therapy, renal involvement, pre-existing renal dysfunction, dehydration, sepsis and concomitant nephrotoxic agents. We have recently subdivided patients into low, moderate and high risk of developing ATLS (Coiffier/Cairo *et al*, JCO 2008). Prophylaxis and management is based on risk and developing clinical manifestations. General principles include hydration and volume expansion, prevention and therapy of hyperkalemia, prevention and therapy of hyperphosphatemia and prevention and therapy of hyperuricemia. The latter is prophylaxed and/or managed by either allopurinol or rasburicase (Figure 1). Allopurinol, which is metabolically converted to oxypurinol, inhibits xanthine oxidase which prevents production of new uric acid formation and leads to increased levels of xanthine or hypoxanthine. Limitations of allopurinol include: 1) does not reduce pre-existing uric acid, 2) may increase levels of xanthine which is less soluble than uric acid, 3) requires urine alkalinization for maximal efficiency, 4) can result in skin reactions and hypersensitivity and 5) requires a 75% reduction of other purines (6MP, 6TG) if given concomitantly. Allopurinol is generally indicated for low to low moderate risk. Rasburicase is a recombinant urate oxidase that converts uric acid to allantoin which is 10 times more soluble in a urine acid pH (Figure 1). Rasburicase is limited by: 1) history or diagnosis of G6PD deficiency, 2) previous history of methoglobinemia. Rasburicase is indicated in high and moderate high risk TLS. In the only randomized study in children of high risk TLS, rasburicase compared to allopurinol resulted in a significant and more rapid lowering of uric acid (Goldman/Cairo *et al*, Blood 2001). In summary, early identification of risk factors in children with hematological malignancies will allow for the categorization of these patients into specific risk classifications and foster the use of appropriate general and specific treatments for the optimal prevention and management of ATLS.

MISSING FIGURE

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Figure 1. Missing figure

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**SCIENTIFIC SESSION 9: RARE NHL SUBTYPES**

**BIOLOGY OF T/NK LYMPHOMAS-SIMILARITIES AND DIFFERENCES TO ANAPLASTIC LARGE CELL LYMPHOMAS (ALCL)**

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Mature T-cell and NK cell neoplasms are uncommon accounting for approximately 10% of all non-Hodgkin's lymphomas. Because mature T cells and NK-cells share some immunophenotypic and functional properties, neoplasms arising from these cells are usually considered together. These lymphomas show a spectrum of clinical and morphological features. The clinical presentation may be leukemic, nodal, extranodal or cutaneous. The classification of T-cell and NK-cell neoplasms proposed by the WHO classification of 2008 emphasizes a multiparameter approach, integrating morphologic, immunophenotypic, genetic, and clinical features. Clinical features are of particular importance in the subclassification of these tumors, in part due to the lack of specificity of other parameters. Many T-cell and NK cell neoplasms shared a number of similarities with ALCL. All these tumors show a broad spectrum of morphologic features ranging from small cell neoplasms to tumors consisting of a predominant population of large anaplastic cells. There is no specific immunophenotypic profile associated with most T-cell lymphoma entities/subtypes and ALCL. All these lymphomas can express CD3, CD2, CD7, CD4, CD8, CD56, and cytotoxic proteins, including perforin, granzyme B, and TIA-1. CD30 is the key marker of ALCL but it can be expressed by other T/NK-cell lymphomas (i.e. PTCL-NOS, Enteropathy-associated T-cell lymphoma and Extranodal NK/T cell lymphoma, nasal type). In addition, a significant proportion of T/NK cells lymphomas and ALCL shows a clonal rearrangement of the T-cell receptor genes. With few exception, specific genetic abnormalities have not been identified for many of the T-cell and NK-cell neoplasms and the molecular pathogenesis of most of these lymphomas remains to be defined. Regarding all these features, ALK-negative ALCLs are more closely related to "non-anaplastic T/NK-cell lymphomas" than to ALK-positive ALCL. Thus, ALK-negative ALCL is considered a provisional entity in the WHO classification of 2008. In the latter, ALK-positive ALCL is a distinct entity with characteristic morphologic (5 morphologic patterns) and genetic features. ALK-positive ALCLs show a broad morphologic spectrum. However, all cases contain a variable proportion of large cells with eccentric horseshoe or kidney shaped nuclei referred to as "hallmark" cells. Five morphologic patterns are now recognised: common pattern (70%); lymphohistiocytic pattern (10%), small cell pattern (10%), Hodgkin-like pattern (1%-3%) and ALCL with "composite pattern" (10% to 20%) defined as having features of more than one pattern in a single lymph node biopsy. These tumors are associated with the ALK oncogene expression due to translocations involving ALK gene at

2p23. They also show a distinct molecular signature (CEBPB, BCL6, serpine A1). Moreover, the overall overall 5-year survival of ALK-positive ALCL is close to 80% compared to less than 50% for ALK-negative ALCL. Furthermore, PTCL-NOS with high CD30 expression, a group that can be difficult to differentiate histologically from ALK-negative ALCL, has a poorer prognosis and a 5y OS of 19%. In a recent study, we have found an ALK exon 27 deletion in 7/9 relapsing patients whereas it was never found in control patients. The presence of this deletion seems to be strongly associated with relapse.

**099**

**NON-ANAPLASTIC PERIPHERAL T-CELL LYMPHOMA IN PEDIATRIC PATIENTS: RETROSPECTIVE ANALYSIS IN JAPAN**

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*Background.* Peripheral NK/T-cell neoplasms are an uncommon group of diseases that show distinct racial and geographic variation. The prognostic significance of the T-cell phenotype has been clearly defined in recent studies by using modern lymphoma classification systems. Anaplastic large cell lymphoma, not rare in childhood, is also one of peripheral T-cell lymphoma (PTCL). However, reports of non anaplastic PTCL in pediatric patients are relatively rare. *Objectives.* The aim of this study is to examine frequency, the appearance of disease style, and prognosis of PTCL in childhood and adolescent in Japan. *Method.* We performed a retrospective analysis in patients with PTCL during a period of 18 years (1991-2008). In the 18 years study period, 55 patients were registered as PTCL and NK/T lymphoma. In 55 patients, we could analyze clinical data in 14 patients of non-anaplastic PTCL. *Results.* Of the 14 patients, male were seven, and female were seven patients. Median age of onset was 12.5 years old (range: 4-21 years). The pathology was confirmed by central review in seven of 14 patients. For other seven children, histopathology was performed at the treating centre only and confirmed from copy of the pathology report. There were six patients with PTCL-unspecified (PTCL-u), six with extranodal, NK/T cell lymphoma, nasal type, one with angioimmunoblastic T-cell lymphoma, and one with subcutaneous panniculitis-like T-cell lymphoma. Initial lesions were cervical lymph node in four patients, skin in three patients. In three patients, hemophagocytic syndrome was initial symptom. With regard to stage of disease at diagnosis, three patients were stage I and II, five were stage III and five stage IV. Chemotherapy had been received in 12 patients. Among two patients not received chemotherapy, one patient had died during receiving treatment for HPS. Moreover, one different patient has improved naturally. Stem cell transplantation had been received in five patients. Twelve out of 14 patients are alive without disease. Overall survival rate of 5 years was 85.7%. *Conclusion.* Generally, results of conventional chemotherapy for high-risk peripheral T-

cell lymphoma are poor in adult patients. Excellent result in our study shows that PTCL of childhood is quite different from that of adult. However, the number of patients was small in this study. We expected the better result in the research including many patients.

**100**  
**PEDIATRIC FOLLICULAR LYMPHOMA:  
DETAILED MOLECULAR, CLINICAL AND  
HISTOPATHOLOGICAL DATA OF 25 CASES**

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**Background.** Follicular lymphoma (FL) is a rare subtype of B-cell non-Hodgkin lymphoma (NHL) in childhood. The WHO 2008 lists pediatric FL as specific variant of FL acknowledging its differences from the adult counterpart. Current knowledge about this disease nevertheless is limited to few case series. **Objectives.** We present here detailed molecular, histopathological and clinical data of 25 pediatric FL which to our knowledge represents the largest series hitherto published. **Methods.** 25 pediatric FL registered in the trials NHL-BFM 90, 95 and B-NHL-04 were selected from the files of the Lymph Node Registry in Kiel. The expression of BCL2, BCL6 and CD10 was evaluated by immunohistochemistry. Fluorescence *in situ* hybridization (FISH) on paraffin sections was performed using IGH-BCL2 double fusion and IGH, BCL6, MYC and BCL2 break apart probes. One case with available fresh material was also subjected to array CGH, gene expression profiling and IGHV mutation analysis within the MMML Network (Klapper *et al.*, Blood, 2008). **Results.** Median age of FL diagnosis was 11.1 (range 1.9-17.7) years with a male to female ratio of 2:1. 76% of patients presented with limited stage disease (stage I or II) often within the tonsillar and cervical area. The estimated probability of event free survival was 96±4%. All FL showed a histological grade 3 histology with simultaneous DLBCL component in 10/25 cases. Expression of CD10, BCL6 and BCL2 was detectable in 81%, 94% and 55% of cases respectively. BCL2-positivity was not associated with higher stage, age or worse outcome. All cases studied were negative for t(14;18) (n=22) or other BCL2-breaks (n=18). In 5 cases chromosomal breakpoints were detected by FISH including IGH in 5/15 and BCL6 breaks in 1/18 cases. None of the FL showed aberrations of the MYC locus. In the case with available fresh material no chromosomal breakpoints or imbalances were identified and gene expression profiling showed a GCB signature. A clonal VDJ rearrangement with 7.1% mutations was identified. **Conclusion.** Pediatric FL is confirmed as predominantly high grade germinal center lymphoma with excellent clinical outcome. From the genetic point of view, pediatric FL displays a spectrum of aberrations different from adult FL with wide

absence of the hallmark translocations of germinal center B-cell lymphomas like FL, DLBCL and BL.

**101**  
**IDENTIFICATION OF ALTERED PROTEIN  
EXPRESSION IN PRIMARY MEDIASTINAL B-CELL  
LYMPHOMA (PMBL) AFTER TREATMENT WITH  
NF- $\kappa$ B INHIBITOR ML120B AND BORTEZOMIB  
(BTZ)**

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**Background.** Pediatric PMBL has a poor prognosis as compared to other DLBCLs (Lones/Cairo, JCO, 2000; Patte/Cairo, Blood 2007) and exhibits up-regulation of specific NF- $\kappa$ B signal pathway genes (Savage, Blood, 2003; Rosenwald, J Exp Med, 2003; Feuerhake, Blood, 2005). Significant increases in apoptosis occur in PMBL after treated with NF- $\kappa$ B inhibitor: bortezomib (BTZ) and/or ML120B (I B kinase, IKK, inhibitor) (Waxman/Cairo, *et al.*, Ann Onc, 2008a). Increased apoptosis is associated with inhibition of NF- $\kappa$ B family members (Waxman/Cairo, AACR, 2009). **Objective:** To investigate the proteomic differences following BTZ and ML120B treatment and to elucidate the mechanism by which NF- $\kappa$ B blockade contributes to cell death in PMBL. **Methods.** Karpas-1106P cells were incubated with ML120B (10 $\mu$ g/ml, generously supplied by Millennium Pharmaceuticals), BTZ (5ng/mL) or both for 24 hours. Proteomic studies were performed from cell lysates utilizing the iTRAQ labeling followed by LC-MS/MS. Ratios between untreated and treated were quantile normalized and log<sub>2</sub> transformed. False-discovery rates (FDR) were used to estimate the error among the selected proteins. Proteins having a ratio beyond the 40% area of the NULL distribution was selected as differentially expressed. Data were analyzed using Ingenuity Pathway Analysis software. **Results.** The levels of expression of 121 proteins were significantly altered by treatment with ML120B, BTZ or both. BTZ treatment resulted in a decrease ( $\geq 2$  Fold) in 36 proteins including c-myc target genes SNRPN (4.7F), RPL27 (4F), RNA polymerase II pathway SND1 (4F) and HSP70 pathway HSP90AA1 (4.4F). ML120B treatment led to decrease ( $\geq 2$  Fold) in 28 proteins including c-myc pathway SNRPN (3.3F) and RPL27 (2.9F), TRAF 6 pathway PFKP (2.6F) and ESD (3.3F) and TGF- $\beta$ 1 pathway SNRBP (3.3F) and GARS (3.9F). The combination of BTZ and ML120B appeared to be additive in decreasing NF- $\kappa$ B proteins SLC16A1 (4F), RNA polymerase II protein DDX39 (3.5F) and others OC1AD2 (4.1F) and DDX24 (4.2F). **Conclusions.** The results suggest that both BTZ and ML120B inhibit NF- $\kappa$ B and non NF- $\kappa$ B

pathways that contribute to promotion of apoptosis in PMBL. Clinical studies with these agents should be considered in the future.

**102**  
**CD20 EXPRESSION, BUT NOT EBV POSITIVITY, PREDICTS FOR EVENT FREE SURVIVAL IN CHILDREN WITH POST-SOLID ORGAN TRANSPLANT (SOT) LYMPHOPROLIFERATIVE DISEASE (PTLD)**

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**Background.** The role of CD20 expression and/or Epstein-Barr virus (EBV) as prognostic factors for development and survival of pediatric PTLD post-SOT in young patients is lacking (Orjuela/Cairo *et al*, CCR 2003). **Objective.** To examine the prognostic significance of expression of CD20 and EBV in children, adolescents and young adults with PTLD following SOT treated at a single institution (1990-2008). **Methods.** All patients (pts) age  $\leq 25$  diagnosed with PTLD were analyzed and classified according to presence of detectable EBV (by EBER ISH) and CD20 by immunohistochemistry (IHC). EBV and CD20 status were determined for all evaluable tumor sites. CD20 status was categorized as positive when all tumor sites expressed CD20. EBV status was categorized as positive when any tumor sites were EBV positive (by ISH). Overall survival (OS) and EFS were estimated using Kaplan-Meier, significance was assessed by t-test and Mantel-Cox. Time to development of PTLD was analysed using linear regression. **Results.** 45 cases of PTLD (1:1 F/M) at a mean onset of 45 mos post primary SOT (4-153). SOT varied (28 heart, 11 liver, 6 kidney); 3 had multiple SOT. Mean age at dx of PTLD was 118 mo (range 14 -287). Of 40 evaluable tumors (11 monomorphic, 19 polymorphic, 10 other), 28 (70%) were CD20<sup>+</sup>, 32 (80%) were EBV<sup>+</sup>. Pts whose PTLD was CD20<sup>+</sup> and/or EBV<sup>+</sup> had fewer months between last SOT and onset of PTLD (CD20<sup>+</sup> vs. neg (mean $\pm$ SD): 28 $\pm$ 31 vs. 64 $\pm$ 44 mos,  $p < 0.01$ , EBV<sup>+</sup> vs. neg: 29 $\pm$ 24 vs. 77 $\pm$ 59 mos,  $p < 0.02$ ). After controlling for age at last SOT, expression of either CD20 or EBV was significantly associated with the shorter length of time between SOT and PTLD. All pts had immunosuppression reduced, followed by chemo and immunotherapy as needed. Patients with CD20<sup>+</sup> vs. neg PTLD had a higher 5-year OS and PTLD related EFS (92 vs. 56%,  $p = 0.02$ ; 84 vs. 29%,  $p < 0.001$ , Chi-square test). EFS differed by CD20 status, log rank test,  $p < 0.01$ . **Conclusion.** Both expression of CD20 and EBV in children with PTLD post-SOT predict time to onset between SOT and PTLD. Organ transplant and morphology subtype are unrelated to OS or EFS. EBV expression does not predict OS or EFS. CD20<sup>+</sup> PTLD is associated with significantly improved 5-year OS and EFS for pediatric PTLD post-SOT. Alternative treatment strategies based on CD20 status at PTLD dx are needed.

**103**  
**THREE MYC-BREAK NEGATIVE PEDIATRIC AGGRESSIVE B-CELL LYMPHOMAS WITH FEATURES OF BURKITT LYMPHOMA PRESENT COMMON GENETIC ALTERATIONS**

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**Background.** Burkitt Lymphoma (BL) is an aggressive B-cell lymphoma that is characterized by typical morphological, immunophenotypic and molecular features. The genetic hallmark of this disease is the t(8;14)(q24;q32) and variants resulting in the juxtaposition of the oncogene MYC next to one of the immunoglobulin (IG) loci leading to deregulation of MYC. It is a matter of debate whether MYC-negative BL do exist. Here we have genetically characterized three pediatric aggressive-B-cell lymphomas in which a reference diagnosis of BL or atypical Burkitt lymphoma was made but which lacked a MYC aberration by cytogenetics and FISH. **Objectives.** The aim of this study was to perform a complete genetic characterization of three rare pediatric aggressive B-cell lymphomas with features of BL but lacking a MYC-break to elucidate their genetic profile and to determine common genetic alterations that could be associated to this phenotype. **Design/Methods.** Three pediatric patients (2 males/1 female, ages: 7, 7 and 16 years) morphologically diagnosed with BL (n=2) or atypical BL (n=1) were studied by conventional cytogenetics (R-banding), Fluorescence *in situ* Hybridization (FISH) and SNP array analyses (Affymetrix SNP array platform 6.0). One of the patients was also diagnosed with Ataxia Telangiectasia. **Results.** All three cases presented complexly aberrant karyotypes devoid of a t(8;14) or variant. Recurrent cytogenetic changes affected chromosomes 6, 11 and 18. Cryptic MYC translocations were excluded by FISH analyses. SNP array analyses revealed 21, 25 and 17 aberrations per case. In total, 40 chromosomal gains, 6 amplifications and 17 losses were observed. Several commonly altered regions were identified including losses of 6q14.2-q22.2 (2 cases), gains/amplifications of 11q12.2-q23.3 (2 cases), losses of 11q23.3-qter (3 cases) and gains/amplifications of 18q21.1-q22.1 (2 cases). Additionally several small regions of UPD were observed in these three cases but might in part be constitutional. FISH analysis with selected probes confirmed several alterations like gains of ATM and amplifications of BCL2 and MALT. **Conclusion.** The three MYC-break negative cases diagnosed as BL/atypical BL present common regions of alteration. These regions might harbor pathogenetically relevant target genes involved in the formation of a phenotype resembling Burkitt lymphoma in this rare group of mature aggressive B-cell lymphoma.

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**CLINICAL FEATURES AND TREATMENT OUTCOMES OF PERIPHERAL T-CELL LYMPHOMA A REPORT OF 9 CASES FROM POLISH PAEDIATRIC LEUCAEMIA/LYMPHOMA REGISTRY**

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**Background.** Peripheral T-cell lymphomas (PTCL) are rare group of neoplasms in young patients. While a high proportion of adults with PTCL have poor risk disease, paediatric PTCL is not well characterized. **Objectives and methods.** The purpose of this retrospective study was to investigate clinical features and outcomes of 9 patients with PTCL, aged from 3 to 17 ys, diagnosed between 1989-2006. The medical history prior diagnosis was very long, even up to 8 ys. In 4 children the primary site of lymphoma was skin, in others: peripheral lymph nodes – 1, abdominal cavity lymph nodes-1. Two patients presented with disseminated disease. One child suffered from arthritis with pleural and peritoneal effusions before the diagnosis of lymphoma. **Results.** All cases of PTCL based on histological and immunohistochemical examination of tumour tissue were centrally reviewed. Histological subtypes according the recent WHO classification included PTCL, unspecified (1), extra-nodal NK/T-cell lymphoma of nasal type (3), subcutaneous panniculitis-like T cell lymphoma (1), blastic plasmacytoid dendritic cell neoplasm (2), Sezary syndrome (1) and EBV positive T cell lymphoproliferative disease of childhood (1). As the first line of cancer treatment 8 patients received chemotherapy according to different protocols: CHOP-1, BFM regimens for T-cell-5, BFM regimen for ALCL-1, ALLIC-1. In case of Sezary syndrome the chemotherapy was intensified by monoclonal antibody therapy. One patient with subcutaneous panniculitis like T-cell lymphoma achieved spontaneous skin remission without any treatment. Three patients received unrelated allogeneic bone marrow transplantation. Eight of 9 patients achieved CR, 1 patient with advanced disease and Down syndrome died due to toxicity, 2 children relapsed; 7 patients survived, the 5-year OS for whole group was around 78% with median time of follow-up 49 months. **Conclusion.** The numbers of children with PTCL were too small to allow conclusions on best therapy, but the favourable results support the regimen for T-NHL in this series. The role of BMT in these patient is still under estimation.

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**SWITCHING HISTOPATHOLOGICAL APPEARANCE BETWEEN LARGE B-CELL LYMPHOMA AND HODGKIN'S LYMPHOMA AT DIAGNOSIS AND PROGRESSION: POOR TREATMENT RESPONSE IN THREE ADOLESCENTS WITH SO-CALLED "GREY ZONE" LYMPHOMA**

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**Background.** Recently, several studies suggested that at least some cases of Hodgkin's lymphoma (HL) and large B-cell lymphoma (LBCL) are closely related entities which are derived from B-cells at similar stages of differentiation and share common pathogenic mechanisms. However, to date, data are scarce with respect to the characteristics and outcome in patients with so-called grey zone lymphomas whether being treated as HL or non-Hodgkin's lymphoma (NHL). **Objectives and Design.** Herein, we report on the characteristics, clinical course and outcome of three adolescent patients with switching histopathological appearance between LBCL and HL at primary diagnosis and progression. **Results.** They comprised one female and two male patients presenting with an age between 16½ and 17½ years at initial diagnosis. At primary diagnosis, centrally reviewed histopathology showed an anaplastic LBCL in two cases (with stage III disease) and a nodular lymphocyte-predominant HL (with stage IV-B disease) in the other patient. Initial therapy followed the contemporary chemotherapeutic protocols for B-cell NHL (B-NHL BFM 2004) and HL (GPOH-HD 95), respectively. The female patient with anaplastic LBCL remained in complete remission (CR) for 12 months but recurred with a nodular-sclerosing HL. The other two patients progressed during front-line therapy and switched to LBCL (specified as T-cell rich B-cell lymphoma) and nodular-sclerosing HL, respectively. Histopathology was centrally reviewed in both cases. In all patients, second-line chemotherapy was directed to the histological entity at disease recurrence but stable 2nd remission could not be achieved due to several disease progressions. The male patient with LBCL died from disease 1½ years after primary diagnosis, the female patient with LBCL is in CR after high-dose chemotherapy with autologous stem cell rescue 2 years after initial diagnosis and the remaining patient with HL is in partial remission awaiting the last cycle of chemotherapy before eventual allogeneic matched sibling stem cell transplantation 1½ years from primary diagnosis. **Conclusions.** Our experience suggests that in adolescent patients cases of grey zone lymphoma are difficult to treat and may have an inferior prognosis.

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**TCR  $\gamma/\delta$  LGL PROLIFERATION  
PROCEEDING INTO FATAL HEPATOSPLENIC  
T-CELL LYMPHOMA IN AN ADOLESCENT GIRL 5  
YEARS AFTER RENAL TRANSPLANTATION**

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**Background.** Post-transplant lymphoproliferative disorders (PTLD) occur in 1-20% of recipients receiving solid organ transplantation. We describe a patient who suffered from hepatosplenic T-cell lymphoma occurring after previous PTLD in a renal transplant recipient. **Patient and Methods.** An 11-yr-old girl underwent kidney transplantation for end-stage Fanconi's nephronophthisis in 2002. In October 2006 significant neutropenia (<200/ $\mu$ L) was firstly detected, without any abnormality in bone marrow (BM) aspirate. Episodes of neutropenia resolved spontaneously or after enhanced immunosuppression and G-CSF. In January 2007 new episodes of neutropenia and newly significant "monocytosis" were detected in peripheral blood (PB) and BM. Percentage of "monocytes" corresponded with immunophenotypically atypical TCR  $\gamma/\delta$  positive T cells (CD7weakposCD5negCD3bright) in PB and BM. Clonal TCR  $\gamma$  and  $\delta$  rearrangements were identified which enabled qPCR minimal residual disease (MRD) assessment. No lymphadenopathy was present, slight hepatosplenomegaly was identified by sonography. Conventional and molecular cytogenetic analyses didn't reveal any chromosomal aberration in PB and BM. No increased levels of EBV and CMV load by PCR were found. Partial increase of granulocytes and slight decrease of atypical TCR  $\gamma/\delta$  T cells were detected after administration of corticosteroid bolus and mercaptopurin. Three months later she presented with fever, rapidly progressive hepatosplenomegaly and pancytopenia, clinically corresponding with hepatosplenic lymphoma. At this time, newly acquired isochromosome 7q was detected by FISH. **Results.** Initial therapy with campath and fludarabine was ineffective. She didn't respond to the 2<sup>nd</sup> line treatment (prednisone, vincristine, daunorubicine, asparaginase) and died 2 weeks later from lymphoma progression. Retrospectively, we identified identical clonal TCR rearrangements in the PB samples from March 2006 (~0.03% of lymphoma PB MRD level), when neither changes in PB count nor clinical symptoms were found. **Conclusion.** We detected a "pre-lymphoma" phase

with clonal expansion of atypical TCR  $\gamma/\delta$  T cells more than 1 year before lymphoma manifestation. The presence of isochromosome 7q was a late change during this lymphoma genesis.

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**ESTABLISHMENT AND CHARACTERISATION OF A  
T(2;17) CLTC-ALK POSITIVE DIFFUSE LARGE  
B-CELL LYMPHOMA CELL LINE**

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**Background.** Anaplastic lymphoma kinase (ALK) positive diffuse large B cell lymphoma (DLBCL) with expression of CD38 and lack of CD30 was first described in 1997. With few exceptions these ALK-positive DLBCL show a fine granular cytoplasmic ALK-staining characteristic for the fusion of CLTC with ALK caused by the reciprocal translocation t(2;17)(p23;q23). This rare but characteristic lymphoma subtype is associated with a poor outcome. **Objectiv.** We report the immunohistological and genetic characterization of the first t(2;17)/CTLC-ALK positive DLBCL cell line (LM1). **Patient and methods.** LM1 was established from the bone marrow of a 13 year old girl suffering from a systemic relapse of an ALK-positive DLBCL. The relapse occurred early after allogeneic bone marrow transplantation for progression during first line therapy. The cultured tumor cells were characterized immunocytologically, by cytogenetics, molecular cytogenetics, molecular genetics and western blotting. **Results.** The immunophenotype of the cells in culture was confirmed to be the same one as the primary tumor. LM1 cells express CD138, VS38c, CD38 and EMA. The cells show fine granular cytoplasmic ALK-staining and expression of the immunoglobulin kappa light chain as well as  $\gamma$  heavy chain. Negativity for CD30, T cell markers and CD20 and CD79a further confirmed the diagnosis. Multicolour FISH revealed the translocation t(2;17)(p23;q23). The expression of the CLTC-ALK fusion could be demonstrated by RT-PCR with specific primers and automated sequencing analysis in both the primary tumor and LM1 cells. Immunoblot analysis with an ALK antibody showed an exclusive cytoplasmically expressed protein of the expected molecular weight for CLTC-ALK. The tumor cells contain a complex near tetraploid karyotype including the t(2;17): 74~91<4n>,XXXX,del(1)(p10p35),t(2;17)(p23;q23)x2,add(2)(p11),der(4)t(4;15),add(q34-q35)x2,der(9)t(9;13)(q24;q12)x2,add(17p11)x2,inc[cp15]. **Conclusion.** The cell line provides opportunities to further study the cell of origin, pathogenesis and causes of chemoresistance of this rare lymphoma subtype.

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**BURKITT LYMPHOMA AFTER SOLID ORGAN TRANSPLANTATION IN CHILDREN**

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**Background.** Post-transplantation lymphoproliferative disorders (PTLDs) are a known complication of solid organ transplantation (SOT) in children. Among them, lymphomatous PTLDs remain rarely reported, not uniformly treated, and with a poor prognosis despite various treatment strategies. **Objectives.** Address the feasibility and efficacy of intensive chemotherapy in pediatric patients (pts) specifically presenting Burkitt lymphoma (BL) after SOT. **Design/methods.** After enquiry into French pediatric SOT centers and into the Registre National des Hemopathies de L'Enfant, 13 cases of histologically proven BL, <18 years (y) of age, were found to be reported between 1997 and 2007 and retrospectively analysed. **Results.** 11 cases were eligible [1 death before diagnosis (dg), 1 clinical file lost]: 9 boys, 2 girls. They had undergone liver (L) transplantation (transpl) (9 pts) or kidney (K) transpl (2 pts). Median (med) age at transpl was 14 months (m) (L), 8y7m(K). EBV seroconversion occurred at a med time of 9m after transpl and 20m before BL. BL occurred after a med time of 33m(L), 13m(K) after transpl. At BL dg, K graft recipients had a mean clearance of 50 mL/min/m<sup>2</sup>; 3 L graft recipients showed chronic rejection. There were 6 stages III, 1 stage IV, 4 L3 ALL. 10/11 pts had high EBV blood viral load at dg. Immunosuppressive treatment was reduced in all cases (no acute rejection observed), 4 pts received rituximab without sustained efficacy, it was therefore not carried on in association with chemotherapy. 10/11 pts were treated according to SFOP LMB96 or LMB2001 protocol. 3 pts underwent dialysis at dg (1 K), 1 pt(L) died of tumor lysis syndrome on day 2. The infectious complications included documented bacteriemias(5 pts), fungal infections(2 pts) and 1 lung abscess(1 pt). 1 pt presented a severe neurological toxicity due to high-dose aracytine. The 9 pts effectively treated by the LMB protocol are all in 1st complete remission with a med follow-up of 4 y. The pt treated with prednisone + cyclophosphamide died in early relapse. **Conclusions.** Lymphomas represent a continuous long-term risk after SOT. Post-transpl BL children can be cured by intensive treatment such as SFOP LMB. Infectious complications may be more frequent and severe. BL and its management seem to have little influence on the graft (direct toxicity/rejection). We thank physicians and surgeons from transpl centers who referred the pts.

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**A RARE CASE OF MARGINAL CELL LYMPHOMA IN A CHILD WITH HYPERIGM SYNDROME**

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**Background.** In childhood, indolent B cell non Hodgkin Lymphoma (NHL) is extremely rare because nearly all paediatric patients have aggressive NHL. The few paediatric reports of Marginal zone lymphoma (MZL) are generally associated to MALT type lymphoma. **Objective.** We describe a case of Marginal zone NHL, not MALT associated, in a 12 year-old girl with congenital immunodeficiency. **Design/Methods.** The patient was just followed in other Institution until she was five year-old for hyperIgM syndrome and hypogammaglobulinemia supported by periodic i.v. immunoglobulin. At the age of 12 she presented multiple erythematous skin lesions, subcutaneous nodules and diffuse lymphadenomegaly (nucal, cervical, submentoneous and axillary nodes). In the suspect of lymphoma, on January 2008 the patient was transferred to our Department. Hystological specimens of subcutaneous nodules biopsy showed infiltration by atypical lymphoid cells, of medium size, characterized by scant cytoplasm and irregularly shaped nuclei. The immunohistochemistry was positive for CD20, CD79a, CD43, bcl-2 and the diagnosis of Marginal zone NHL was performed. MRI and PET-CT scans showed mediastinic and abdominal lymph nodes, and subcutaneous nodules in various district. Owing to supra and infra diaphragmatic localizations, serum LDH <500 IU/L, absence of bone marrow and central nervous system involvement and negativity of EBV infection, the patient was enclosed in stage III NHL according to Murphy classification and enrolled to the AIEOP NHL97 risk group R-2, modified for reducing methotrexate dose (500 mg/m<sup>2</sup> instead of 1 gr/m<sup>2</sup>). Protocol consisted of four multiagent courses preceded by a pre-phase with dexamethasone and cyclophosphamide. Toxicities were evaluated according to National Cancer Institute Criteria. **Results and conclusion.** The patient achieved a complete remission and from May 2008 was off-therapy; the protocol was well tolerated without any severe adverse event. At the moment the child is under shortened follow-up but a more prolonged observation is necessary to exclude the recurrence of disease or secondary neoplasm due to chemotherapy or/and underground immunodeficiency. At the best of our knowledge, this is the first case of association of IperIgM syndrome and indolent marginal zone non Hodgkin lymphoma in a young girl, brilliantly treated with a relative aggressive chemotherapeutic protocol.

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**POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER WITH CENTRAL NERVOUS SYSTEM INVOLVEMENT POST-CORD BLOOD TRANSPLANTATION FOR X-LINKED ADRENOLEUKODYSTROPHY**

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*Background.* X-linked adrenoleukodystrophy (X-ALD) is a metabolic disorder caused by a defect in the gene ABCD1 on Xq28. HSCT (haematopoietic stem cell transplantation) at an early stage of childhood form has been reported to result in stabilization of the demyelination. We describe a boy who received HSCT and who developed EBV-related post-transplant lymphoproliferative disorder (PTLD) with central nervous system involvement (CNS). *Case.* The patient was diagnosed with Addison disease due to X-ALD at the age of 10 y. Neurological symptoms were restricted to unsteady gait and fatigue. Brain scan showed minimal signs of periventricular demyelination. He received a 10/10 matched cord blood HSCT ( $0.13 \times 10^6/\text{kg}$  CD34<sup>+</sup> cells) at the age of 11.5y with conditioning according to the EBMT inborn errors protocol (busulphan-cyclophosphamide). Graft versus host disease prophylaxis consisted of methylprednisolone and cyclosporine. Donorchimerism was complete at D+66. At D+117 he was admitted with fever. Physical examination showed enlarged lymph nodes and hepatosplenomegaly. EBV PCR in blood showed a copy load of 1281694/ml, further rising to  $>5000000/\text{mL}$  D+124. CT-PET-scan of abdomen and thorax showed multiple lymphoproliferative lesions in lung, mediastinum, liver, spleen and retroperitoneum. Brain scan showed no new findings. Lymph node biopsy showed evidence of EBV+ PTLD with plasma cell differentiation, CD20 being expressed only focally. Rituximab was initiated at D+124 immunosuppression was stopped. At D+146 EBV copies had declined to 173589. PET scan showed a partial response. However the patient became confused and showed signs of spasticity. New brain scan showed cerebral foci suggestive of lymphoma and involvement of medulla oblongata and cerebellum. Cerebrospinal fluid (CSF) EBV copies were 20482/mL. Two-weekly intrathecal injections of methotrexate (MTX) and methylprednisolone were initiated together with weekly cidofovir. Whereas blood EBV and CSF copy load declined to  $<500/\text{mL}$  and 0 D+164, brain imaging showed massive increase of PTLD lesions. Monthly CHOP chemotherapy (cyclophosphamide, doxorubicin, vincristine, prednisone) in association with monthly IT MTX was added to the therapy. PET scan at D194 showed complete remission of the peripheral PTLD and partial remission of central PTLD. Cidofovir was stopped, 2 more CHOP courses were given. This therapy resulted in stable complete remission at 5m follow-up. Now, at 2 yrs follow-up, the patient is still in CR, however with residual left hemiple-

gia and frontal signs. *Conclusion.* Therapy for central PTLD is difficult; no large studies are available. In this patient, combination of rituximab, cidofovir, IT MTX and CHOP led to CR, however with important sequelae.

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**POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) IN CHILDHOOD, ADOLESCENT AND YOUNG ADULT PATIENTS: CHARACTERISTICS AND OUTCOME IN A BELGIAN UNIVERSITY HOSPITAL**

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*Background.* PTLD is a life-threatening complication of both solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). *Aim.* We undertook a retrospective analysis of all childhood, adolescent and young adult pts age  $<26$  yrs diagnosed with PTLD following SOT or HSCT between January 1989 and December 2008 at the University Hospitals of Leuven, aiming to obtain information about incidence, pretreatment characteristics, treatment and outcome. *Methods.* Medical records of all pts were used for this observational study. Information was obtained with regard to baseline pts characteristics, transplant related characteristics, PTLD characteristics, treatment administered and response to treatment. Histological subtyping was based on medical records. *Results.* 16 pts were included. PTLD was biopsy proven in 15 pts, whereas in 1 HSCT pt diagnosis was made based on rapid increase of EBV viral load in peripheral blood combined with a positive PET/CT scan. Overall incidence of PTLD for all transplant types and in all ages in our centre was 1.96%. The highest incidence was reported in heart-lung transplantation (7.5%), followed by heart (4.9%), lung (2.9%), liver (2.67%), HSCT (1.45%) and kidney transplantation (1.34%), whereas no PTLD was seen in bowel transplantation so far. Most PTLD in childhood, adolescent and young adult population were monomorphic (80 %), with diffuse large B-cell non Hodgkin lymphoma being the most frequent subtype. The majority of cases occurred  $<1$  year post-transplantation (62.5%). 50% of the pts showed elevated LDH at diagnosis, 31% had an ECOG performance state of = or  $>2$ , 50% had involvement of more than 1 extranodal, whereas Ann Arbor stage III or IV was diagnosed in 81.3%. Age adjusted IPI scores were calculated for all patients, leading to low risk in 12.5%, low intermediate in 37.5%, high intermediate in 25% and high risk in 25%. At the moment of PTLD diagnosis immunosuppressive therapy included calcineurin inhibitors (100%), antimetabolites (68.8%) and low dose steroids (81.3%). Reduction of immunosuppression was performed in all cases. Other first line treatment modalities included rituximab (75%), chemotherapy (25%), surgery (6.3%), antiviral therapy (12.5%) and high dose steroids (6.3%). Following first line therapy overall response rate was 68.7% (62.5% CR, 6.3% PR). At last follow

up 75% of the pediatric and adolescent pts were alive whereas 12.5% of the pts lost their graft during follow up. *Conclusion.* We report a retrospective analysis of 16 childhood, adolescent and young adult PTLD following SOT or HSCT. Incidence at all ages in our centre was highest in heart-lung and lowest in kidney and bowel transplant pts. The majority of pts presented with monomorphic PTLD, ie. DLBCL.

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**UNUSUAL CASE OF FIVE IDENTICAL IMMUNOHISTOCHEMICALLY AND MOLECULARLY DLBCL EPISODES DURING 17 YEARS IN A BOY WITH NIJMEGEN BREAKAGE SYNDROME**

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*Purpose.* Nijmegen Breakage Syndrome (NBS) is a rare autosomal recessive DNA repair disorder characterized by primary microcephaly with typical facial appearance, progressed immunodeficiency, hypersensitivity to ionizing radiation and very high risk of malignancy at a young age, especially non-Hodgkin lymphoma (NHL). *Method.* We analyzed the unusual course of NHL with five consecutive episodes of DLBCL during 17 years in a boy with Nijmegen Breakage Syndrome. *Results.* The first episode of DLBCL stage III in 11-year-old boy, in 1991, was treated according to BFM 86 protocol. The second one in 1998, also stage III, with unusual localization in paravertebral region only, was treated according to the LMB 89 protocol with success. In 2004 the third episode, II stage, was treated according to CHOP protocol with Rituximab, and the fourth one (stage II) in 2005 year according to DHAP protocol. Presently, the 28-year-man is being treated with individual protocol due to progression of the fifth episode (stage II) which occurred in 2008. In the first two regimens the doses of drugs such as cyclophosphamide as well as methotrexate and epidophilotoxins were reduced, in the last three regimens the doses were full. Each tissue sample was characterized immunohistochemically and molecularly, the first two respectively. The initial DLBCL histopathology (with an activated B cell immunophenotype CD10- and BCL6-negative) was preserved in consecutive lymphomas. This assumption was confirmed by Ig/TCR clonality analysis, showing identical IGH and IGK/IGL rearrangements in the consecutive proliferations. Finally, all the proliferations represented true relapses. *Conclusion.* The unusual incidence of five consecutive episodes of DLBCL during 17 years in a patient with Nijmegen Breakage Syndrome can be caused by NBS predisposition to lymphoma development, together with the mild treatment regimen in view of the risk of genomic instability.

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**REPORT OF 3 EBV+ PTLD INTERMEDIATE BETWEEN BL AND DLBCL**

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*Background.* Childhood post-transplant lymphoproliferative disorders (PTLD) are frequently associated with EBV detection. They may represent an escape of EBV infected B-cells as a consequence of the recipient immunosuppression (IS) and of the large proportion of seronegative children at the time of graft. PTLT comprise a wide spectrum of proliferations ranging from early lesions and polymorphic PTLD (which are likely to regress with IS reduction) to monomorphic PTLD which are categorized as in non-immunosuppressed patients. Most of the EBV+ monomorphic PTLD have a late germinal / post-germinal centre phenotype. *Cases reports.* 3 monomorphic PTLD occurred at our institution (2004-2008). The recipients were 3, 5, 9 yr-old and displayed abdominal tumor 2, 4, 3, 5 years following liver transplantation, respectively. Two tumors displayed a mixture of small/medium size cells and large cells with irregular nuclear shape. The cells were CD10+, Bcl2-, Ki67>90%. MYC rearrangement with IGH locus was detected by FISH on both cases. One tumor was mainly represented by a diffuse proliferation of large cells with focal proliferation of smaller cells mimicking Burkitt lymphoma (BL). The cells were CD10+, Bcl2+, Ki67>90%. The karyotype detected a t(8;14)(q24;q32) translocation as the sole aberration and FISH confirmed the rearrangement of the MYC locus. The 3 PTLD expressed the EBV LMP1 protein. Due to the detection of morphological and genetic characteristics of both DLBCL and BL without meeting criteria for classical BL or DLBCL, we retrospectively subclassified these lymphomas as B-cell lymphoma, unclassifiable with features intermediate between DLBCL and BL, according to the latest WHO classification. Complete IS withdrawal (FK506) associated with intensive polychemotherapy used for childhood mature B-cell lymphomas (LMB2001/03 recommendations for group B) allowed a complete remission (follow-up : 9m, 3yr 9m, 5 yr). IS had to be reintroduced in 1 case to avoid graft rejection. *Conclusion.* We report the first description of 3 EBV+ BL/DLBCL PTLD. Their morphological characteristics reflect the heterogeneity of this new category of lymphoma. Although EBV+, they did not exhibit any plasmacytoid differentiation and their phenotype was consistent with a germinal centre origin. These observations suggest that these BL/DLBCL PTLD may have a good response to intensive polychemotherapy associated with IS withdrawal.

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**CASE STUDY OF TWO PATIENTS WITH COMPOSITE LYMPHOMAS**

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*Background.* Composite lymphomas (CL), of two distinctly different and well-delineated varieties of lymphoma in a single anatomic site or mass, are unusual and rare. *Objectives.* Evaluate the clinical features and treatment results of two cases with composite lymphomas. *Design and Patients.* We present two patients: eleven years old boy and thirty-seven years old female patient with diagnosis CL: Diffuse Large Cellular B Lymphoma and Hodgkin Lymphoma (DLBCL/LH). During 2008 patients were treated in Clinical Center Nis according to different protocols. *Results.* In both patients disease started year or two before with painless enlargement of peripheral lymph nodes on neck and underarm. Detailed examination began after inexplicable temperatures. After pathohistological examination of lymph nodes in both patients diagnosis was: DLCL (T-cell/Histiocyt rich, CD20<sup>+++</sup>)/LH classic type. At that moment with the boy disease showed progression (high LDH, ascites, abdominal tumor mass, hepatosplenomegaly), and was categorised as III CS and R4 group of AIEOP LNH97 protocole. Because of dominant clinical HL picture, female patient was categorised into IV CS and treated according to ABVD protocole. Response to chemotherapy with the boy was excellent, and six months after treatment he is in complete remission. However, the female patient shows disease progression after IV ABVD, so therapy according to R-CHOP protocole was continued to which she achieves partial remission. *Conclusion.* Composite lymphomas are rare kinds of lymphomas and represent diagnostic and therapeutic problem. With our patients in the beginning the disease had slow evolution and later became more aggressive. Better response to therapy was achieved by using more aggressive protocols clearly denoting that prognosis relates to recognising more aggressive disease components.

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**PRIMARY SKIN LYMPHOMAS—RARE FORM OF MALIGNANT LYMPHOMAS IN CHILDREN: CASE REPORT**

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*Background.* Non-Hodgkin lymphomas (NHLs) usually involve lymph nodes, thymus or bone marrow, but in some cases soft tissues, bones, central nervous system, kidney or skin may also be infiltrated. Rarely, skin infiltrations may be the only manifestation of lymphoma. *Objectives.* We describe two cases of primary skin lymphoma, the only skin lymphoma cases treated in our centre during last 20 years. The disease is very rare in

children and the diagnostic and therapeutic standards are not available, which usually makes the diagnosis and treatment difficult. *Case 1.* 17 years old boy with inflammatory-necrotising skin changes of the nose was referred to an oncologist after 10 months of anti-inflammatory treatment administered by a laryngologist and dermatologist. The biopsy revealed T/NK-cell NHL of the skin (nasal type). At the time of diagnosis oedema and reddening of the right eyelid, the right cheek, the upper lip and ulceration of the right part and infiltration of the left part of nose were observed. Four courses of chemotherapy (CHOP) brought about significant improvement. The patient refused the local radiotherapy due to risk of vision loss. Bexarotene (Targretin) was given for following 4 months as a maintenance treatment. Though the PET-CT scan was negative, NMR showed elevated signal in the right cheek. Since viable lymphoma cells were still present in the biopsy, next three CHOP courses were administered. The patient is in remission for 31 months, and currently is referred to the plastic surgeon for reconstruction of the nose. *Case 2.* 8 years old girl with prolonged fever and nodular erythemas of the subcutaneous tissue of cheeks, the left eyelid, trunk and the left thigh, developed pancytopenia. The skin biopsy showed panniculitis-like T-cell lymphoma. Due to signs of haemophagocytic syndrome (anaemia, leukopenia, elevated ferritin concentration, haemophagocytosis in bone marrow) intensive chemotherapy was administered. After four CHOP courses the symptoms of haemophagocytic syndrome disappeared and complete clinical and radiological remission (PET-CT) was achieved. *Conclusion.* Due to their rarity in children, primary skin lymphomas make diagnostic and therapeutic problems.

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**DIAGNOSTIC AND THERAPEUTIC PROBLEMS IN 7-YEARS-OLD BOY WITH PULMONARY LYMPHOMATOID GRANULOMATOSIS**

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*Case report.* The 5y5m old boy was admitted to our hospital in July 2007 with fever, seizures and pleuropneumonia. Brain CT: normal. Chest X-ray: bilateral disseminated opacities with pleural effusion and lung CT: diffuse nodular infiltrates, some of them were located close to pleura. Laboratory investigations: slightly elevated ESR, GOT and GPT; serologic test for Mycoplasma pneumoniae IgM(+) and Chlamydia pneumoniae IgM(+). We were looking for acquired or inherited immunological disorder but elevated levels of IgA and IgM were the only findings. Despite antibiotic treatment the clinical and radiological symptoms progressed. Open lung biopsy was performed on 23.09.2007, but at that moment the histopathological diagnosis was inconclusive (unspecific inflammation etc.) Because of signs of respiratory insufficiency

iciency steroid treatment was implemented (pulses, continuation with prednisone orally). We observe improvement in clinical status of the patient during steroid treatment (afebrile, respiratory symptoms regressed), but shortly after steroid discontinuation symptoms reappeared (fever, seizures, pneumonia, X-ray changes). Brain MRI (Jan 2008) revealed unspecific changes. Steroids were readministered and continued for next 15 months (tapered doses) with clinical effectiveness. Unfortunately, control brain MRI (June 2008 and Feb 2009) showed progressive CNS disease with multiple unspecific intracerebral lesions, the biggest located within left temporal lobe. Thorac surgeons decided to reevaluate lung tissue sample taken on September 2007, and our reference pathologist diagnosis is LYMPHOMATOID GRANULOMATOSIS (LYG) GRADE I, with positive result of EBER (EBV RNA *in situ* hybridization). Last lung CT done on 03.02.2009: very good regression of large nodules, but the lung tissue is heterogeneous. Current patient presentation: good clinical condition on 5mg/day prednisone, anisocoria on neurological examination as the only pathological sign. Because of unclear etiology of CNS changes the brain stereotactic biopsy was proposed by neurosurgeon, now the patient is waiting for this operation. Unsolved questions: 1. Should we take the brain tissue specimen or should the brain changes visible in MRI be regarded as lymphomatoid granulomatosis? 2. Why steroids were ineffective to stop CNS disease? 3. What kind of treatment options would be the most effective in a case of CNS LYG?

#### 117 LYMPHOPLASMACYTIC LYMPHOMA AND PRIMARY IMMUNE DEFICIENCY IN A PAEDIATRIC CASE

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**Background.** Lymphoplasmacytic lymphoma (LPL) is a rare malignancy, accounting for only 1 to 2 percent of all hematologic tumors. The median age at diagnosis is around 70 years, with a range between 24 and 91 years in most of LPL series. **Design and Methods.** We describe for the first time a LPL in a 3-year old boy with syndromic association. This child was born from first degree consan-

guineous parents. First manifestations associated a nystagmus with mild mental retardation and a severe autoimmune pancytopenia with livedo reticularis at 2 years of age. Laboratory investigations showed a mixed connective tissue disease profile with detection of antinuclear antibodies, positive ANCA, positive ferritin test, decrease in complement and Ig G oligoclonal hypergammaglobulinemia. Evans syndrome was treated with prednisolone and azathioprine with partial response. Six months later, acute HSV encephalitis occurred with a good response to aciclovir. Immunological studies showed a decrease TLR6/7 and HSV antigen response both in patient's PBSC and fibroblasts suggesting a primary immune deficiency. At 30 months of age, the child presented with persistent fever, poor general status, multiple cervical adenopathies, associated with severe refractory thrombocytopenia. Abdominal ultrasonography showed a large retroperitoneal mass (10x2 cm in size), involving renal vessels without evidence for a compression. Chest radiography showed a mild mediastinal enlargement. Biopsy was performed on cervical adenopathies. Microscopic examination was compatible with a LPL. Immunophenotype was CD 79a, MUM 1, CD 38 and CD 138 positive. Monoclonality was demonstrated by molecular methods. Caryotype was not performed. Hypergammaglobulinemia persisted, with two spikes on electrophoresis (Ig G kappa and IgM). Microscopic examination of bone marrow and CSF was normal. **Results.** The child was treated with rituximab (R) without response. (R)-COPADM was administered, with 30 % reduction of retroperitoneal mass, complete regression of mediastinal adenopathies and partial improvement of thrombocytopenia. After 2 cycles of (R)-CYM, 4/6 unrelated cord blood transplantation was done with reduced intensity conditioning regimen. The patient is actually in a good condition 30 days post-transplantation. **Conclusion.** Further investigations will be necessary to demonstrate the role of constitutional TLR-pathway defect both for autoimmune manifestations and for LPL pathogenesis.

#### 118 CD56-NEGATIVE EXTRANODAL NK/T-CELL LYMPHOMA, NASAL TYPE, IN A PEDIATRIC PATIENT: A CASE REPORT

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**Background.** With the exception of anaplastic large cell lymphoma, peripheral T-cell neoplasms are uncommon in pediatric patients. Extranodal natural killer (NK)/T-cell lymphoma, nasal type, is a lymphoma of cytotoxic lymphocytes characterized by vascular destruction, necrosis, and association with Epstein-Barr virus (EBV). It is more prevalent in Asians and Native Americans and occurs most often in adults. Review of the literature found rare pediatric examples, including a single report of a CD56 negative case in a pediatric patient. **Objectives.** The objective of this study was to review the clinical and pathologic features of an unusual aggressive lymphoid neoplasm in a pediatric patient. **Design/methods** We

reviewed the clinical information, pathology reports, and diagnostic biopsies for this case. *Results.* The patient was a 4-year-old Native American male who presented with fluctuating and progressive right facial swelling suggestive of cellulitis along with right cervical lymphadenopathy and fevers. Multiple biopsies over a six-month period were required for definitive diagnosis. The diagnostic biopsy specimens demonstrated an atypical lymphohistiocytic infiltrate with fat necrosis, fibrosis, and angioinvasion with surrounding coagulative necrosis. A skin biopsy demonstrated prominent pseudoepitheliomatous hyperplasia. The lymphoid cells were predominantly small with occasional large forms with large, folded, hyperchromatic nuclei. Immunohistochemistry showed expression of CD2, CD3, CD7, CD8, and TIA-1 without CD4, CD20, CD30, ALK-1, CD1a, TdT, or CD56. *In situ* hybridization for EBV-encoded RNA (EBER) was strongly positive. Flow cytometry was negative, but a clonal T-cell receptor re-arrangement was identified by polymerase chain reaction. There was no involvement of bone marrow, CSF, or other organs or lymph nodes. After a very brief initial response, the patient failed 2 lines of lymphoblastic lymphoma multiagent chemotherapy over 4 weeks. After he developed severe complications and disease progression, he was transitioned to palliative care and died. *Conclusion.* This case represents an extranodal NK/T-cell lymphoma, nasal type. The lack of CD56 is unusual, but the diagnosis can still be made in the appropriate setting if both cytotoxic molecules and EBV are positive. The clonal rearrangement of the T-cell receptor suggests cytotoxic T-cell derivation rather than NK.

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**GOOD RESPONSE OF PRIMARY B-CELL LYMPHOMA OF THE BONE TREATED WITH RITUXIMAB: A CASE REPORT**

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*Background.* Extra nodal localization of primary B-cell malignant lymphoma of bones is very rare and has been only occasionally reported. *Case report.* We present a case of a 17-year old girl diagnosed as B-cell lymphoma localized in the knee. The girl presented with intermittent pain in the right knee, following, after a while, with limited movements of the joint. Examinations included CT showing an expansive process in the metaphysis of the right femur and scintigraphy showing pathologic accumulation in the region of the right knee. After extensive imaging diagnostics no other localization of the tumor mass was found. Bone marrow examination did not show infiltration with malignant cells. Knee biopsy was performed, and the diagnosis was established after histopathological investigation of the lesion. Immunophenotypic profile showed CD 20 positivity. Due to the rarity of the lesion and uncertain prognosis, we decided to combine cytotoxic treatment with Rituximab, which proved to be superb in adult B lymphomas. Cytotoxic therapy consisted of Etoposid, Doxorubicin, Vincristine, Cyclophosphamide, and Prednisone, known as EPOCH-R protocol for NHL-B, CD20 positive. The therapy was

well tolerated and without serious myelosuppression. After the follow-up period of 5 years, our patient has been in continuous remission. *Conclusion.* Rituximab combined with cytotoxic therapy proved useful for rare pediatric primary bone B-cell lymphoma.

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**OBSTRUCTIVE JAUNDICE AS THE PRESENTING SYMPTOM OF INTRA-ABDOMINAL NON HODGKIN LYMPHOMA IN A TEENAGER**

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*Background.* Obstructive jaundice is an unusual manifestation of non-Hodgkin lymphomas in children. Previous reports in children demonstrated involvement of the porta hepatis or a mass in the head of the pancreas. *Objectives.* We describe a 15 year old girl with obstructive jaundice and abdominal pain. She underwent a surgical investigation. The ultrasound of the abdomen showed diffuse enlargement of the pancreas with wide hepatic ducts. PET-CT which was performed a week later demonstrated a diffuse disease involving the entire pancreas, a mass in the porta hepatic, a huge mass in the internal genital organs, peritoneal spared a huge mass in the sacrum with bone distraction and bone marrow involvement. Biopsy was performed from the sacral mass, and the diagnosis was "Large B Cell Lymphoma". Cytogenetic exam showed "Hypotetraploid karyotype (80-86 XXXX) with changes of chromosome number, and no c-myc translocation. *Results.* She was treated with chemotherapy according to LMB gr. C protocol. Within a few days, jaundice disappeared and there was shrinkage of the tumor. Neither surgery nor percutaneous draining were needed. There was no surgical morbidity or persistent cholestasis. After 4 years of follow up the girl is with no evidence of disease. In conclusion, obstructive jaundice due to non Hodgkin lymphoma is very rare. It can be treated effectively without any surgical procedure.

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**IDENTIFICATION OF BIOMARKERS AND THERAPEUTIC TARGETS IN NATURAL KILLER CELL LYMPHOMA USING QUANTITATIVE MASS SPECTROMETRY**

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*Background.* Natural killer cell lymphoma (NKL) is a highly aggressive malignancy. Early diagnosis of the tumor is critical however there are no biomarkers which are useful for the diagnosis and monitoring of patients with NKLs. *Objective.* We hypothesized that comprehensive analysis of differentially expressed proteins by NKL

and normal NK cells would be a rational strategy for the discovery of NKL biomarkers and lead to better understanding of pathogenetic mechanisms of NKL. *Design/Methods.* We used total cell lysates obtained from highly enriched peripheral blood NK cells (CD3-, CD56dim) and an Epstein-Barr virus negative, IL-2 dependent cell line (NK-92MI) for quantitative proteomic analysis. Isotope-coded affinity tags (ICATM)-labeling followed by liquid chromatography-tandem mass spectrometry analyses (LC-MS/MS) identified 114 differentially expressed proteins in NKL compared to the normal NK cells. Out of these, 80 were upregulated 1.5-fold or greater and 34 were downregulated 1.5-fold or greater in NKL. Proteins within diverse cellular functional categories including cell signaling, transcriptional regulation, DNA metabolism, and immune response were differentially expressed. Furthermore, known proteins relevant to the function of NK cells such as perforin, granzyme and defensins were downregulated in the NKL cell line. We selected eight proteins to validate the changes identified by ICAT- LC-MS/MS using Western blot analysis. HSP90, PCNA,  $\alpha$ -enolase, GSTP1, galectin-1, and PARK7 were upregulated, and lactoferrin and annexin A1 were downregulated by western blot analysis demonstrating high concordance with the results of ICAT- LC-MS/MS analysis. *Results.* We validated the functional significance of HSP90 as a potential therapeutic target in NKL by investigating the effect of inhibitors of HSP90 (geldanamycin) on the survival of an NKL cell line. Inhibition of HSP90 led to significant decrease in cell viability and apoptotic cell death as confirmed by western blotting demonstration of PARP-1 induction and detection of its cleavage products. *Conclusion.* This study represents the first global quantitative proteomic cataloging of proteins expressed by NKL and normal NK cells, and reveals potential candidate proteins that may be useful for the diagnosis and function as therapeutic targets in patients with NKL.

## SCIENTIFIC SESSION 10: IMMUNOSURVEILLANCE AND LYMPHOMA

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### POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN PEDIATRIC SOLID ORGAN TRANSPLANT RECIPIENTS – INTERIM ANALYSIS OF TRIAL PED-PTLD PILOT 2005

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*Background.* Epstein-Barr Virus (EBV) associated post-transplant lymphoproliferative disorders (PTLD) are severe side effects of immunosuppressive therapy after organ transplantation. So far, no standard diagnostic and therapeutic approaches have been defined. *Objectives.* The prospective multicenter trial Ped-PTLD Pilot 2005 aims at evaluating a standardized diagnostic procedure and response-adapted treatment protocol for pediatric patients with CD20-positive PTLD after solid organ transplantation. *Methods.* Patients with histologically confirmed CD20<sup>+</sup> PTLD were treated by weekly infusions of anti-CD20-antibody (Rituximab). Initial treatment response was assessed after 3 weeks by imaging of tumor sites and quantification of tumor mass. Patients showing >25% reduction in tumor volume received 3 further Rituximab infusions on a protracted schedule. All other patients were stratified to receive a chemotherapy regimen including vincristin, cyclophosphamide, low dose methotrexate and prednisone (mCOMP). *Results.* 24 Patients have been enrolled until Jan 31, 2009, and 22 patients have completed treatment. 16 patients were treated by CD20-antibody alone, one of whom relapsed after 3 months. 6 patients received additional mCOMP chemotherapy. Of these, four patients showed complete remission, including 2 patients with Burkitt lymphoma characterized by C-MYC translocation. 2 patients failed mCOMP therapy and were subsequently treated according to NHL/BFM protocol. Except for 1 patient who died of small bowel perforation we noticed no treatment related mortality. Two patients died of reasons unrelated to PTLD or treatment. Overall survival is 83% at 2 years with an event-free survival of 68%. Patients with diffuse large B cell histology had a higher cumulative incidence of relapse/progress (43% vs. 0%,  $p=0.02$ ). After 3 Rituximab doses, long-term responders showed significantly lower EBV load in peripheral blood ( $p=0.0003$ ) than non-responders. *Conclusions.* Early evaluation of treatment response may identify patients in whom Rituximab monotherapy is sufficient to control PTLD. Complete remission can be achieved by mCOMP chemotherapy in patients non responding to Rituximab including Burkitt-like PTLD. Interdisciplinary and international collaborations are warranted to enhance statistical power and improve prognosis for patients with this rare disease.

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**GIRLS HOMOZYGOUS FOR AN IL2-INDUCIBLE T-CELL KINASE MUTATION THAT LEADS TO PROTEIN DEFICIENCY DEVELOP FATAL EBV-ASSOCIATED LYMPHOPROLIFERATION**

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**Background.** In boys, familial fatal immunodeficiency to Epstein-Barr virus (EBV), lymphoproliferation and lymphoma development has been linked to mutations in two X-chromosome encoded genes, SH2D1A and XIAP. We report two girls from a Turkish family who developed severe immune dysregulation and therapy-resistant atypical EBV-positive B-cell proliferation after EBV-infection clinically resembling X-linked lymphoproliferative disease (XLP). **Objectives.** Consanguinity of the parents suggested an autosomal recessively inherited disorder. The aim was to identify the genetic basis of this novel immunodeficiency syndrome. **Design/Methods.** We performed SNP array based genome wide linkage analyses with 8 family members and sequencing of the positional and functional candidate gene. Functional implication of the detected mutation was studied by in silico prediction of protein stability, in patient material and in 293T cells transfected with wildtype and mutant ITK plasmids. Western blot and lymph node staining were used for detection of protein expression, RT-PCR for quantification of RNA expression and flow cytometry for the description of lymphocyte surface markers. **Results.** In both affected girls we found a homozygous missense mutation of a highly conserved residue (R335W) in the SH2 domain of IL-2 inducible T-cell kinase (ITK), a non-receptor tyrosine kinase acting downstream of the T-cell receptor and regulating thymic T-cell selection processes. The patients showed low ITK expression levels in lymph node tissue, a lack of NKT cells in peripheral blood and extremely high mRNA levels of the transcription factor eomesodermin in CD8<sup>+</sup> cells, which are characteristics of ITK-deficiency in mouse models. In transfected 293T cells similar mRNA levels of mutant and wildtype ITK were found, whereas there was no expression of mutant ITK protein. In silico modeling provided further evidence, that R335W causes a profound instability of the ITK protein. **Conclusion.** ITK-deficiency leads to fatal inadequate immune response to EBV-infection. This is the first molecular cause of an autosomal recessively inherited EBV-associated lymphoproliferative disease. Similar to XLP in boys, in all children with EBV-associated lymphoproliferation or lymphoma ITK-deficiency should be considered.

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**TOWARDS STANDARDIZATION OF EPSTEIN-BARR VIRAL LOAD QUANTIFICATION IN IMMUNOSUPPRESSED PEDIATRIC PATIENTS AFTER HEART TRANSPLANTATION**

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**Background.** Epstein-Barr-Virus (EBV) associated post transplant lymphoproliferative disorders (PTLD) can cause a high degree of morbidity and mortality in pediatric cardiac transplant recipients. EBV load measurement in peripheral blood supports the monitoring of patients; however, the optimal sample type and way of PCR quantification has not been standardized as yet. **Objective.** Aim of the study was to compare the EBV DNA levels in whole blood (WB), in plasma and in peripheral mononuclear cells (PBMC) without (PBMCw/o) or with (PBMCw) normalization towards an endogenous control. **Patients and Methods.** We analyzed the blood of 397 samples from 93 cardiac transplant recipients (median age 12 years; age range 3 to 34 years) by using real time duplex PCR (ABI Prism 7500) over a period of two years. **Results.** 65/93 (70%) patients showed detectable EBV levels in their peripheral blood at least once (243/397 [61%] samples being positive for EBV DNA). Comparing the four ways of EBV quantification, we found strongest correlations in PBMC with and without normalization, the correlation coefficient  $r$  being 0.98 ( $p < 0.0001$ ). The EBV DNA levels in WB also highly correlated with those in PBMCw/o or in PBMCw ( $r = 0.93$  [ $p < 0.0001$ ] or  $0.92$  [ $p < 0.0001$ ], respectively). EBV detection in plasma was less sensitive than in WB or PBMC. Thus, correlations between plasma and WB or between plasma and PBMC were lower (plasma versus WB:  $r = 0.71$  [ $p < 0.0001$ ]; plasma versus PBMCw/o:  $r = 0.66$  [ $p < 0.0001$ ]; plasma versus PBMCw:  $r = 0.66$  [ $p < 0.0001$ ]). In 4/93 patients, we detected 5 episodes of PTLD. In all compartments analyzed, the EBV levels were significantly elevated. However, we detected significant fluctuations of the EB viral load only in plasma samples specifically exceeding the value of  $>10,000$  EBV-copies/mL plasma exclusively at diagnosis and at the initiation of PTLD treatment. **Conclusions.** EBV load detection in whole blood is equivalent to the analysis in PBMC. Normalization of EBV DNA in PBMC towards an endogenous standard does not seem to be necessary. The EB viral load detected in plasma samples reflected the courses of PTLD better than in whole blood or in PBMC.

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**COMPARISON OF FOUR DIFFERENT WAYS OF EPSTEIN-BARR VIRAL LOAD QUANTIFICATION IN PERIPHERAL BLOOD OF PEDIATRIC PATIENTS AFTER ALLOGENEIC HEMATOPOIETIC STEM CELL TRANSPLANTATION**

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**Background.** Epstein-Barr-Virus (EBV) induced post transplant lymphoproliferative disorders (PTLD) are serious complications with a high degree of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). EBV load measurement in peripheral blood supports the monitoring of patients; however, EBV load quantification in peripheral blood has not been standardized as yet. **Objective.** Aim of the study was to compare the EBV DNA levels in whole blood (WB), in plasma and in peripheral mononuclear cells (PBMC) without (PBMCw/o) or with (PBMCw) normalization towards an endogenous control. **Patients and Methods.** For this purpose, we examined the blood of 213 paired samples from 37 children and adolescents (median age 10 years; age range 1 to 21 years) after allogeneic HSCT by using real time duplex PCR (ABI Prism 7500) over a period of two years. **Results.** 21/37 (56.8%) patients became EBV positive (EBV+) in peripheral blood following HSCT. The median day on which patients were EBV+ after HSCT for the first time was day +49 (range, day +11 to day +180). In 11/37 (29.7%) patients we observed a total of 14 significant EBV-reactivations, defined as >100 copies/ml plasma or >500 copies/∞g PBMC DNA. All reactivations were found in WB, but only in 11/14 (78.6%) patients in their plasma or in 10/14 (71.4%) patients in PBMCw/o. EBV reactivations were frequently earlier detectable in WB than in PBMC or plasma. Comparing the four ways of EBV quantification, we found strongest correlations in PBMC with and without normalization, the correlation coefficient  $r$  being 0.998 ( $p < 0.0001$ ). The EBV DNA levels in WB also correlated with those in PBMCw/o or in PBMCw ( $r = 0.81$  [ $p < 0.0001$ ] or  $0.81$  [ $p < 0.0001$ ], respectively). EBV detection in plasma was less sensitive than in WB or PBMC. Accordingly, correlations between plasma and WB or between plasma and PBMC were lower (plasma versus WB:  $r = 0.57$  [ $p < 0.0001$ ]; plasma versus PBMCw/o:  $r = 0.51$  [ $p < 0.0001$ ]; plasma versus PBMCw:  $r = 0.50$  [ $p < 0.0001$ ]). **Conclusions.** Whole blood, PBMC and plasma show different kinetics of their EBV DNA levels after allogeneic HSCT. Monitoring of EBV DNA levels in whole blood appears to be equivalent to PBMC. Normalization of EBV DNA in PBMC towards an endogenous standard does not seem to be necessary.

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**PEDIATRIC, REFRACTORY POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD) FOLLOWING SOLID ORGAN TRANSPLANTATION (SOT): A CHILDREN'S ONCOLOGY GROUP PHASE II STUDY**

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**Background.** Patients (pts) with PTLD following SOT experience more toxicity with chemotherapy. A previous study (Gross TG, *et al*, J Clin Oncol 23:6481, 2005) demonstrated that a low-dose chemotherapy regimen in children with refractory, EBV (+) PTLD resulted in 75% CR, 19% relapse, 6% treatment-related deaths and 8% allograft loss. All 4 pts with fulminant PTLD (F-PTLD) died of progressive disease. 2 yr OS=73% (95% CI: 58-88%) and 2 yr EFS (alive, without PTLD and functioning original allograft)=67% (95% CI: 52-82%). **Objectives.** Determine safety and efficacy of adding rituximab to a low-dose chemotherapy regimen in children with refractory PTLD following SOT in a cooperative group setting. **Methods/Design.** Eligibility required biopsy proven EBV (+), CD20 (+) PTLD and failure of at least one therapy, e.g. immunosuppression reduction (>1 week), or F-PTLD defined as fever, hypotension and > 2 organ system failure. CNS PTLD were excluded. Treatment consisted of cyclophosphamide (600 mg/m<sup>2</sup>) IVx1 day, prednisone (1 mg/kg) IV/po bid x 5 days every 3 weeks for 6 cycles and rituximab (375 mg/m<sup>2</sup>) IV weekly x6. **Results.** 54 patients (4 with F-PTLD) were enrolled. 31 (57%) have had central pathology review - polymorphic (n=7), monomorphic (n=24). Clonality was determined in 21 cases (39%) - monoclonal (n=14) and polyclonal (n=7). Median follow-up is 1.8 yrs (0.1-4.1 yrs). Overall response was 87% (74% CR). All F-PTLD patients are PTLD-free and alive >2 yr. There have been no allograft loss, but 3 relapses (8%), all within 1 year from diagnosis. There have been 8 deaths (5 to PTLD, 3 treatment-related). Excluding F-PTLD, the estimated 1 & 2 yr overall survivals are 83% (95% CI: 71-95%) and 80% (95% CI: 67-93%), respectively. The estimated 1 & 2 yr EFS (pts PTLD-free with original functioning allograft) are 77% (95% CI: 64%-90%). **Conclusions.** Though results are preliminary, this regimen appears to be effective for refractory, pediatric EBV (+), CD20 (+) PTLD following SOT and rituximab does not increase toxicity of this low-dose regimen. Though it is difficult to compare directly with previous results, it appears that adding rituximab may benefit patients with F-PTLD, and perhaps reduce relapses.

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**NON-HODGKIN'S LYMPHOMA (NHL) IN CHILDREN WITH CHROMOSOMAL BREAKAGE SYNDROMES (AT AND NBS): EXPERIENCE FROM THE NHL-BFM TRIALS**

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**Background.** Lymphoma are the commonest malignant diseases in patients with chromosomal breakage syndromes (Ataxia teleangiectasia (AT) and Nijmegen breakage syndrome (NBS)). With improved management of infections, malignant disease is more frequently diagnosed and has become one of the commonest causes of death in pediatric AT and NBS. The risk for the development of severe toxicity is higher and due to small number of these patients experience in their treatment is limited. **Objectives.** The aim of this study is to develop a standardized therapy approach. **Methods.** In four consecutive multicenter therapy trials for pediatric non-Hodgkin's lymphoma (NHL-BFM), 2936 patients with newly diagnosed NHL have been registered between 1986 and 2008. 25 patients with AT (n=11) and NBS (n=14) were identified and analysed. **Results.** Median age of patients with AT and NBS at diagnosis of NHL was nine years. NHL-entities differed from non-AT/NBS-patients: most of them were diffuse large B-cell lymphomas, n=12 (48%), and lymphoblastic T-cell lymphoma, n=5 (20%). Stages were: I and II in seven patients (28%), III in 17 patients (68%), IV in one patient (4%). All patients received polychemotherapy according to tumor-entity and stage, none received radiation. Dose reduction according to individual tolerance concerned mainly methotrexate, alkylating agents and epipodophyllotoxines. Three patients died of toxic complications, five patients relapsed and died, six patients suffered from secondary lymphoma. In 18 of the 25 patients the follow up was over five years. In this group the 5-year-survival was 60% and the 5-year-event-free-survival was 50%. In case of relaps the 1 year survival was only 20%. In case of secondary lymphoma the 5 year survival was 50%. **Conclusions.** Patients with AT and NBS suffer from rare entities of pediatric NHL. Curative treatment is possible and should be attempted. Further cooperative trials using standardized approaches are required.

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**CORD BLOOD (CB) AND PERIPHERAL BLOOD (PB) CD56<sup>dim</sup> NK CELLS HAVE SIMILAR IN-VITRO CYTOLYTIC ABILITY AGAINST NHL: IMPLICATIONS FOR UNRELATED CB GRAFT VS. LYMPHOMA EFFECT**

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**Background.** NK cells are classified into cytotoxic CD56<sup>dim</sup> and cytokine producing CD56<sup>bright</sup> subsets (Shereck/Cairo, *et al* PBC, 2007). NK subsets function is based on their NK receptor (NKR) repertoire (Moretta, *et al*. Ann Rev Immunol 2001). We have demonstrated that CB vs. PB NK 56<sup>dim</sup> subsets have increased c-lectin receptors expression (NKG2a, NKG2d), no difference in NKR expression and that there are 33 and 37 proteins over and underexpressed by  $\geq 2$  fold between CB vs. PB CD56<sup>dim</sup> NK cells (Shereck/Cairo, *ASH*, 2007). We have recently demonstrated a graft vs. lymphoma effect following UCBT (Rodriguez/Cairo *et al*. JCO 2009). There is a paucity of data on the in-vitro cytotoxicity of CB NK<sup>dim</sup> cells against NHL targets. **Objective.** To compare the cytolytic ability of CB vs. PB NK 56<sup>dim</sup> subsets against ALCL and DLBCL tumor targets. **Methods.** PB and CB MNCs were selected for CD56<sup>dim</sup> subsets via magnetic separation (Miltenyi Biotec). Cytotoxicity was assessed by europium release assay (Perkin Elmer) using ALCL (Karpas) and DLBCL (Toledo) tumor targets (ATCC) at 20:1, 10:1, 5:1 E:T ratios as we have previously described (Ayello/Cairo *et al* BBMT 2006). **Results.** Cytotoxicity was significantly decreased in CB vs. PB NK<sup>dim</sup> subsets with Toledo (DLBCL) tumor targets at all E:T ratios ( $p < 0.001$ ); yet IL-2 activated CB and PB CD56<sup>dim</sup> NK cells demonstrated no difference and were increased at the same rate as PB CD56<sup>dim</sup> NK cells (E:T 20:1: 80 $\pm$ 6 vs. 84 $\pm$ 3; 10:1: 56 $\pm$ 2 vs. 59 $\pm$ 5; 5:1: 36 $\pm$ 2 vs. 26 $\pm$ 3%, NS). Cytolytic activity of PB and CB NK<sup>dim</sup> subsets ( $p > 0.001$ ) with Karpas (ALCL) tumor targets at 20:1, 10:1, 5:1 E:T ratios was increased; yet, no difference in cytotoxicities when comparing PB vs. CB NK<sup>dim</sup> subsets (45 $\pm$ 2 vs. 39 $\pm$ 2, NS; 20 $\pm$ 1 vs. 18 $\pm$ 1, NS; 11 $\pm$ 1 vs. 10 $\pm$ 1%, NS), respectively. IL-2 addition demonstrated a significant increase in cytotoxicity in PB and CB CD56<sup>dim</sup> NK cells ( $p < 0.001$ ) at all E:T ratios; yet, there was no significant difference when comparing CB vs. PB NK CD56<sup>dim</sup> subsets (20:1: 76 $\pm$ 3 vs. 66 $\pm$ 3; 10:1: 46 $\pm$ 2 vs. 56 $\pm$ 4; 5:1: 29 $\pm$ 1 vs. 38 $\pm$ 8%). **Conclusion.** Activated CB compared to PB CD56<sup>dim</sup> NK cells demonstrated similar cytolytic capability with DLBCL and ALCL tumor targets at all E:T ratios. CB CD56<sup>dim</sup> cells may contribute significantly to the graft vs. lymphoma effect in NHL following UCBT.

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**EBV-RELATED POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN CHILDREN: HISTOGENETIC PROFILE AND ITS CORRELATION WITH CLINICOPATHOLOGICAL FEATURES AND PROGNOSTIC IMPACT**

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**Background.** PTLD is a heterogenous group, mostly associated with EBV infection and B cell origin. Studies in diffuse large B-cell lymphomas (DLBCL) suggested that histogenetic profile of B cells is associated with particular clinicopathological features and prognosis. The histogenetic profile of PTLD is not well studied, especially in children. **Objectives.** To study the histogenetic expression pattern in pediatric PTLD and its prognostic impact; and to evaluate the association between histogenetic pattern and clinicopathological features. **Design.** 29 cases of PTLD(EBV related and B cell origin only) were selected from file(1995-2005) of Cincinnati Children's Hospital Medical Center. All original slides, corresponding pathological reports and relevant clinical information were reviewed. One tissue block was selected in each case for immunohistochemical stains of CD10, BCL6, MUM1/IRF4, CD138, BCL2, and MIB1. Based on immunophenotypic profile, each case was assigned to one of the following B cell differential patterns: germinal center(GC) (BCL6<sup>+</sup>CD10<sup>+</sup>); Late/post GC (BCL6<sup>+</sup> MUM1/IRF4<sup>+</sup>); and post GC (MUM1/IRF4<sup>+</sup>CD138<sup>+</sup>). **Results.** According to WHO classification the 29 cases were classified into 4 groups (see table). All group A are GC pattern and BCL2<sup>+</sup>; In group B, 7/8 (88%) were late/post GC and 1/8(12%) post GC pattern, and all cases were BCL2<sup>+</sup>; In group C, 1/10 (10%) were late/post GC and 9/10(90%) post GC pattern, and 60% were BCL2<sup>+</sup>; In group D, expression profile was atypical, not classifiable into any groups. **Conclusions.** Virtually all monoclonal pediatric B cell PTLD show GC differentiation. Group A are of GC origin and clinically aggressive. Most group B are late GC/early post GC and most group C post GC origin. BCL2 expression is seen in all group B and most group C, and has been associated with more aggressive disease in de-novo DLBCL. The histogenesis of pediatric PTLD appears to correlate with clinicopathological features and provides prognostic value in our study.

**Table.**

PTLD Group	# of cases	Avg. age of tx	Interval (tx to PTLD)	Disease site	Clinical features	Treatment requirement
A: Burkitt lymphoma	5	10m	4.5y	GI/Abd.	Aggressive	Aggressive chemo
B: DLBCL	8	4y	5m	50% nodal	Various	chemo
C: Polymorphic -PTLD	10	2y	2y	40% nodal	localized	↓immun supp +Rituximab
D:Hodgkin lymphoma	6	6.5y	3.4y	33% nodal	Aggressive	Chemo

<sup>A</sup>In Years, <sup>\*Dx</sup>: Initial Diagnosis

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**GENETICALLY REENGINEERED K562 CELLS SIGNIFICANTLY EXPAND CORD BLOOD (CB) NATURAL KILLER (NK) CELLS FOR USE IN ADOPTIVE CELLULAR IMMUNOTHERAPY FOR NHL**

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**Background.** An allogeneic GVL effect has been demonstrated in pediatric NHL (LBL/ALCL) (Bradley/Cairo, BMT 2006). NK cells are a major component of both innate and adaptive immunity (Caligiuri *et al*, Blood 2008) but are limited for use in tumor immunotherapy due to lack of tumor recognition and limited numbers of functionally active NK cells (Shereck/Cairo, PBC 2007). However, NK cells play a significant role in reducing relapse in NHL following AlloSCT (Dunbar *et al*, Haematologica 2008). **Objective.** To determine the efficiency of ex-vivo expansion of CB NK cells following stimulation with genetically reengineered K562 cells. **Methods.** Freshly isolated CB mononuclear cells (CBMNC) were cultured with modified K562 cells expressing membrane bound IL15 and 4-1BB ligand (K562-mbIL15-41BBL). After irradiation with 100Gy, K562-mbIL15-41BBL cells were incubated in a 1:1 ratio with CBMNC + 10 IU/mL rhIL-2 for 7-14 days. CD3 and CD56 expression was determined by flow cytometry at Days 0, 7 and 14. **Results.** On Day 0, the population of NK cells was 3.9±1.3%. After 7 days of ex-vivo stimulation, there was a significant increase in CB NK cells compared to WT K562 and media alone (71.7±3.9% vs. 42.6±5.9% vs. 9.7±2.4%) ( $p<0.01$ ). Overall, this represented a 35-fold or 3374±385% increase of the input NK cell number. This was significantly increased compared to culture with WT K562 (1771±300%,  $p<0.05$ ). Concomitantly, there was a significant decrease in CB T-Cells vs. culture with WT K562 or media (15.1±1.7% vs. 35.7±2.4% vs. 51±7.1%, respectively) ( $p<0.001$ ). On Day 14, there remained a significant increase in NK cell populations (62.0±2.1% vs. 27.9±2.4% vs. 5.5±0.4%,  $p<0.001$ ). CD56bright vs. CD56dim NK populations were 66.6% vs. 33.4%, respectively. However, there was an increase in expression of the NK activation marker CD107a compared to WT K562 (51.1±0.7% vs. 31.6±1.1%,  $p=0.003$ ). **Conclusion.** These results suggest that CBMNC can be ex-vivo expanded by K562 cells expressing membrane bound IL15 and 4-1BB ligand (K562-mbIL15-41BBL) resulting in specific expansion of CB NK cells. Future studies will involve transduction of CB NK cells with a plasmid containing a chimeric antigen receptor against CD20 [anti-CD20-BB- $\zeta$ ] to enhance cytotoxicity against NHL.

**131****NON HODGKIN'S LYMPHOMA IN CHILDREN WITH PRIMARY IMMUNODEFICIENCIES. RESULTS OF TREATMENT IN 10 PATIENTS FROM A SINGLE INSTITUTION**

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**Background.** Patients with congenital immunodeficiencies are susceptible for malignant disease and non Hodgkin lymphoma is the most frequent tumor in this group. A high mortality is associated with NHL in patients with congenital immunodeficiencies and these patients usually don't tolerate intense treatments. There is little information on the outcome of these children with current therapies. The aim of this study is to report our experience in the treatment of 10 children with immunodeficiencies and NHL. **Methods.** Retrospective study of 10 patients with congenital immunodeficiencies treated at our center from 1990 to 2008. Treatment was decided on an individual basis considering the NHL subtype, the primary immunodeficiency and the existence of co-morbidities. **Conclusions.** Lymphoma was successfully treated in 6/10 patients with NHL associated to congenital immunodeficiencies after tailored chemotherapy associated with rituximab or radiotherapy when necessary. Infectious complications were common.

**Table.**

Case (immunodeficiency)	NHL subtype	Treatment	Outcome
1. Ataxia telangiectasia	Burkitt like B cell lymphoma	Rituximab and reduced BFM chemotherapy	Alive and disease-free (+120)
2. Ataxia telangiectasia	Burkitt lymphoma	Reduced dose BFM chemotherapy	Dead because of immunodeficiency after CR from lymphoma
3. Ataxia telangiectasia	Burkitt lymphoma	Standard dose BFM chemotherapy	Alive and disease-free (+48)
4. Ataxia telangiectasia	Large cell lymphoma	Reduced dose BFM chemotherapy	Dead on induction.
5. Variable common immunodeficiency	Burkitt like B cell lymphoma	Standard dose BFM chemotherapy	Alive and disease-free (+186)
6. Hair-cartilage hypoplasia	Burkitt like B cell lymphoma	Steroids and rituximab	Dead surgical complication
7. XLP	Burkitt like B cell lymphoma	CHOP, rituximab allogeneic bone marrow transplantation	Alive disease-free (+32)
8. Congenital hypogammaglobulinemia	Lymphoblastic lymphoma	Standard dose BFM chemotherapy	Alive and disease free (+38)
9. PNP deficiency	Large B cell lymphoma	CHOP and rituximab	Dead. Infectious complications
10. PNP deficiency	Large B cell lymphoma	No treatment	Detected at autopsy

**132****POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDERS IN CHILDREN AND ADOLESCENTS: A SINGLE INSTITUTION EXPERIENCE**

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**Background.** Post-transplant lymphoproliferative disorders (PTLD) have variable pathologic and clinical features. Current classification is based on histologic and immunophenotypic findings. The incidence and natural history of the subtypes is not completely understood, particularly in pediatric patients. **Objectives.** To document the pathologic features, clinical characteristics and outcomes of PTLD occurring in the pediatric population at the University of Michigan. **Design and Methods.** A database search was performed for pediatric PTLD from 1990-2009. Clinical and histologic features were reviewed. **Results.** Forty-three patients with PTLD were studied, including 20 females and 23 males, with a median age of 8 years at the time of diagnosis. The organ transplants included renal (20), liver (14), cardiac (7), allogeneic bone marrow (1), and small intestine/liver/pancreas (1). The lymphoproliferative disorders consisted of 26 monomorphic, 8 polymorphic, and 9 early lesions. The monomorphic cases were diagnosed as diffuse large B-cell lymphoma (DLBCL) (15), Burkitt lymphoma (3), classical Hodgkin lymphoma (6), and plasmacytoma (2). Sites of disease included abdomen, peripheral lymph nodes, tonsils/nasopharynx, and lung/mediastinum. Forty percent of cases were clinically "early", occurring within 18 months of transplant. Of the 34 cases in which EBER or LMP was performed for Epstein-Barr virus, 85% were positive. Twenty-six patients had staging bone marrow biopsies; 1 was positive. Treatment included reduction in immunosuppression and variable combinations of rituximab, prednisone, antiviral and chemotherapeutic agents. Clinical outcomes included 4 deaths, 2 attributable to PTLD. Survivors included 24 patients with 3 or more years of follow-up and 15 with shorter follow-up. **Conclusion.** Pediatric PTLD has variable histologic presentations. While DLBCL is the most frequent diagnosis, other monomorphic subtypes occur, including six Hodgkin lymphomas in our series. The head/neck region and abdomen are common sites of disease, while bone marrow involvement is rare. The majority of tumors are EBV-associated. Nearly half of cases occurred within 18 months, warranting surveillance in the early post-transplant interval. Our data suggest the overall prognosis is excellent in the pediatric population, even with conservative therapy.

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**HAEMATOPOIETIC STEM CELL**  
**TRANSPLANTATION CORRECTS THE**  
**IMMUNODEFICIENCY AND IMMUNE**  
**SURVEILLANCE DEFECTS ASSOCIATED WITH**  
**X-LINKED LYMPHOPROLIFERATIVE DISEASE**

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X-linked lymphoproliferative disease (XLP) is an inherited immunodeficiency involving primarily T- and NK-cells. It is characterised by fulminant infectious mononucleosis, lymphoproliferative disorders, dysgammaglobulinaemia, and malignant lymphoma. The gene responsible for XLP has been cloned and named SAP (for SLAM associated protein) or SH2D1A. SAP regulates several critical aspects of immune function including lymphocyte activation and proliferation, cytotoxicity, cytokine secretion, adhesion and induction of humoral immune responses. XLP patients have inactivating mutations in the SH2 domain of SAP and this results in a significant increased risk of malignant and non-malignant lymphoproliferative disorders - the former usually being extranodal involving the intestine and of mature B-cell origin. Haematopoietic stem cell transplantation (HSCT) is the only curative therapeutic intervention in these patients. A 5 year old boy presented with EBV-driven haemophagocytic syndrome. SAP protein expression was absent and molecular genetic studies identified a total gene deletion of SH2D1A, confirming a diagnosis of XLP. Remission of his EBV-driven haemophagocytic syndrome was achieved following treatment with intravenous immunoglobulin, dexamethasone, cyclosporine, ganciclovir and Rituximab. Six weeks later he developed neurological features that were found to be secondary to a cerebral T-cell lymphoma. Two years following sibling allogeneic HSCT he remains well with normal immune function. Serial brain MR imaging has shown regression of his cerebral lymphoma despite no specific targeted therapy. In summary, we present the simultaneous presence of two different typical manifestations of XLP in a 5 year old boy and have shown that HSCT can correct the immune function and tumour-surveillance defects in XLP.

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**PLASMABLASTIC LYMPHOMA FOLLOWING**  
**RENAL TRANSPLANTATION IN PAEDIATRIC AGE**

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*Background.* Plasmablastic lymphoma is a rare variant of diffuse large B cell lymphoma which may occur in HIV positive patients and also in post-transplant setting. A majority of reported cases involve middle-age adults. *Design and Methods.* We describe the case of a 14-year old and HIV-seronegative girl, who received renal transplant at 5 years of age for nephronophthisis. Immunosuppression for prevention of graft rejection consisted of corticosteroids, ciclosporin A plus azathioprine replaced further by mycophenolate mofetil. Nine years later, she was referred for a bilateral cervical mass associated with a hypertrophic left tonsil. Histology was compatible with an aggressive large B cell lymphoma with plasmablastic features. Immunophenotype was CD20, CD19, CD138 negative, and CD30, MUM1, CD38 positive. Monoclonality was demonstrated by molecular method. EBV was detected using EBER *in situ* hybridization. HHV8 was undetectable by PCR. Cytogenetic analysis of the tumour showed complex karyotype, including 17 p deletion. C-myc rearrangement was absent. LDH were at normal levels. FDG scintigraphy, CT scan, CSF and bone marrow studies confirmed a localized stage II disease. First line treatment consisted of reduction in immunosuppression associated with rituximab (R) with no response. *Results.* After 2 courses of (R)-CHOP, we observed good partial response followed by rapid re-progression, leading to start (R)-ICE then (R)-CYVE regimens with the same pattern of response. She finally died from a diffuse refractory disease 9 months following the diagnosis. *Conclusion.* This observation highlights the very poor prognosis of post-transplant plasmablastic lymphoma especially with complex karyotype. The best treatment for plasmablastic lymphoma is actually not well established, particularly in childhood.

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**NON-HODGKIN LYMPHOMA AFTER TREATMENT**  
**OF HODGKIN LYMPHOMA IN CHILD WITH**  
**NIJMEGEN BREAKAGE SYNDROME –**  
**CASE REPORT**

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*Background.* Nijmegen breakage syndrome (NBS) is a rare DNA repair disorder characterized by microcephaly, immunodeficiency and predisposition to malignancy, mainly non Hodgkin lymphoma (NHL). *Case report.* We report on 8 year old patient with NBS who presented with nodular sclerosing type of Hodgkin disease (HD) stage IV B. Chemotherapy consisting of 6 cycles COPP/ABV regimen with reduction at 75% of full doses was employed. Radiotherapy was omitted. Careful monitoring for infection and prophylactic use of immunoglobulins were also employed. During this treatment, no major toxic or infectious complications were observed.

Complete remission was achieved. After 20 month complete remission patient had lymph node enlargement of neck 3 cm in size without other symptoms. Histology of lymph node biopsy confirmed diffuse large B cell NHL. Patient treated with 4 CHOP cycles + Rituximab at 75% of full doses and achieved complete clinical and laboratory remission. During maintenance therapy patient received 4 doses of Rituximab every three months. In this moment patient is in complete remission one month after the end of complete therapy. *Conclusion.* NBS are prone to develop lymphomas of various types. Better survival may be achieved both with adopted disease-specific regimens, and individualized approach considering patient's clinical condition. Also, better recognition and treatment of infections that may occur during chemotherapy may reduce early deaths in patients with DNA repair disorders.

## SCIENTIFIC SESSION 11: NEW THERAPEUTIC OPTIONS

### NEW THERAPEUTIC TARGETS IN CHILDHOOD AND ADOLESCENT NHL

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The common pathological subtypes in childhood and adolescent NHL include mature B-cell lymphoma (60%) Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL) and primary mediastinal B-cell lymphoma (PMBL), lymphoblastic lymphoma (LL) (30%) and anaplastic large cell lymphoma (10%) (ALCL) (Hochberg/Cairo *et al*, BJH, 2009). The progress (5yr EFS) following multiagent chemotherapy for localized, intermediate and advanced childhood and adolescent NHL is approximately 95-100%, 80-95% and 60-80%, respectively (Cairo *et al*, PBC, 2005). This excellent survival, however, is associated with acute and long-term toxicities especially, febrile neutropenia, infection, mucositis, prolonged hospitalization and potential long-term morbidities such as cardiac and infertility. Furthermore, patients who relapse or progress during and following therapy have a dismal prognosis. Therefore new therapeutic targets resulting in alternative treatment strategies will hopefully reduce early and long-term toxicity and increase initial EFS and reduce the number of patients developing relapse or progressive disease. A number of new targets have been recently identified in each of the three pathological subgroups. In mature B-NHL surface receptors such as CD20 (Perkins/Cairo, Clin Adv Hem, 2003) and CD22 (Raetz/Cairo, JCO, 2008) have therapeutic antibodies that are already active in pediatric trials such as rituximab (Cairo, ASH, 2008) in mature B-NHL and epratuzumab (Raetz/Cairo, JCO, 2008) in pre-B ALL, respectively. Other potential targets in mature B-NHL include c-Myc (antisense oligos), BCL-6 (HDACIs), NFκB (rituximab, ML120b) and proteasome inhibitors (bortezomib). Future surface targets may include CD19, CD40, CD52, CD74 and CD80 (Miles/Cairo/Perkins *et al*, BJH, 2007). Conjugated antibodies to radioisotopes, toxins and/or chemotherapy are another potential targeted therapeutic strategy for childhood and adolescent mature B-NHL (Cooney/Cairo *et al*, CCR, 2007). There are also a variety of targets and therapeutics strategies in T-LBL including DNA (nelarabine, clofarabine), NOTCH-1 (γ-secretase inhibitors), mTOR (rapamycin, temsirolimus), surface receptors such as CD3, CD4, CD8, CD52 (ATG, denileukin diftitox, alemtuzumab, immunotoxins) (Miles/Cairo/Perkins *et al*, BJH, 2007 and Smock/Cairo/Perkins, PBC, 2008). Similarly, there are a number of targets in ALCL that have been identified that will translate into alternative treatment strategies. SGN30 and SGN35, designed to bind to CD30, are in trial or in development for childhood and adolescent ALCL. CD25 is highly expressed in childhood ALCL (Miles/Cairo/

Perkins, BJH, 2008) and denileukin diftitox is a potential immunotoxin that binds to CD25 that may be active in childhood ALCL and a multicenter U.S. trial is pursuing this treatment study alone and in combination with ICE in relapsed/refractory patients (Cairo, personal communication). There has been a recent increase in the development of ALK inhibitors and phase I trials in patients with relapsed ALCL about to get underway in the U.S. Other potential targets in ALCL include heat shock protein 90 (geldanamycin) and mTOR (rapamycin, temsirolimus). A summary of the potential targets and agents is depicted in Figure 1. The future is bright for the development and incorporation of new therapeutic strategies that incorporate selected targets that hopefully will increase EFS and decrease both acute and long-term toxicity in childhood and adolescents with NHL.

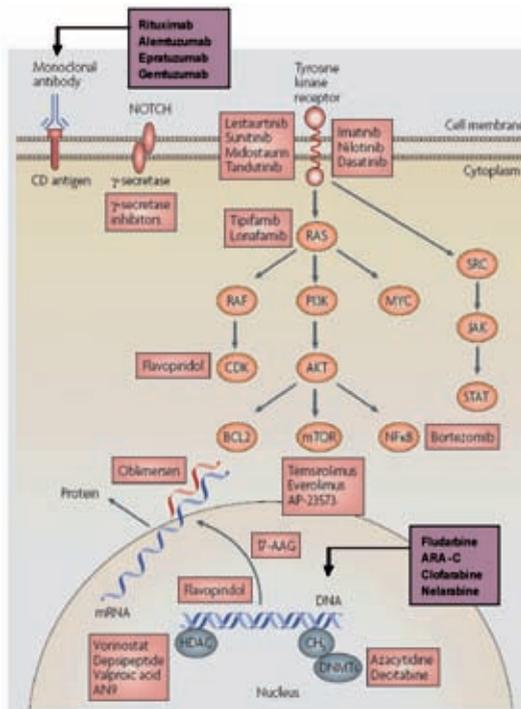


Figure 1. Target sites and Agents for Treatment of Hematological Malignancies (Adapted from Pui *et al*, Nature Reviews, 2007)

**ADOPTIVE T-CELL IMMUNOTHERAPY**

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Adoptive T-cell immunotherapy is increasingly used for the treatment of malignancies and viral-associated diseases. The use of donor lymphocyte infusions (DLI) for the successful treatment of hematological malignancies such as chronic myelogenous leukemia post hematopoietic stem cell transplantation (HSCT) has demonstrated the curative potential of T-cell therapies. However, the use of DLI is limited by potentially fatal complications such as graft versus host disease (GvHD) that arises from the presence of alloreactive T cells. To overcome this limitation, strategies have been developed to generate antigen-specific T-cell products that are devoid of alloreactivity. Our group has explored the use of antigen-specific T cells targeting Epstein Barr virus (EBV) antigens expressed in a diverse group of malignancies including EBV-associated lymphoproliferative disease (EBV-LPD) post HSCT, EBV-positive Hodgkin's disease (HD) and non-Hodgkin's lymphoma (NHL), and EBV-positive nasopharyngeal carcinoma. We have demonstrated that the adoptive immunotherapy with EBV-specific cytotoxic T cells (CTL) is an effective strategy after HSCT to reconstitute EBV-specific immunity, and prevent or treat EBV-LPD. For other EBV-associated malignancies the use of EBV-specific CTLs was less effective, however we observed several complete responses in heavily pretreated patients. To enhance the efficacy of EBV-specific CTLs we have developed strategies to target the subdominant EBV-antigens, LMP1 and LMP2, expressed in EBV-positive HD and NHL. Twenty-four patients with EBV-positive HD or NHL have been treated on our current dose escalation studies using LMP-specific CTLs. No immediate toxicity was observed. After CTL infusion, increased LMP-specific T cells were detected in the blood of 15/22 evaluable patients, persisting for up to 3 months. Additionally, two patients had lymph node biopsies 3-6 months post CTL, which showed selective accumulation of LMP2-specific CTL in lymph nodes. Twelve out of 13 high-risk and/or multiply relapsed patients who received LMP-specific CTL as adjuvant treatment after chemotherapy still remain in remission for a median of 2 years after CTL. Eleven patients had detectable disease at the time of CTL of whom 2 had progressive disease by 8 weeks and 9 had clinical responses. The median duration of the clinical responses is 1 year with one stable disease, one partial response and 7 complete responses. In preclinical models we have evaluated several genetic strategies to further enhance the antitumor activity of adoptively transferred T cells for EBV-positive malignancies. These include i) rendering CTL resistant to the immunosuppressive tumor microenvironment, ii) enhancing CTL trafficking to tumor sites, and iii) improving *in vivo* T-cell expansion and persistence. In addition, to broaden the application of T-cell therapies, we have used genetic approaches to target non-viral targets expressed on lymphomas. In the 1st part of the talk we will discuss our clinical experience using EBV-specific and LMP-specific CTL to treat patients with EBV-associated lymphomas. In the 2nd part of the talk we will review our current genetic strategies to enhance and broaden the applications of T-cell therapies.

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**A NOVEL CHEMOTHERAPY (ROMIDEPSIN) AND IMMUNOTHERAPY (NATURAL KILLER CELL) BASED APPROACH IN PEDIATRIC LEUKEMIA AND LYMPHOMAS: POTENTIAL FOR TARGETED ADOPTIVE CELLULAR IMMUNOTHERAPY (ACI)**

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**Background.** There is an urgent need for a novel therapeutic modality for children with relapsed leukemia and lymphoma (L/L) [Cairo *et al.* BJH, 2003]. NK cells play a significant role in tumor cytotoxicity mediated by NKG2D ligands (NKG2DL) [Shereck/Cairo *et al* PBC, 2007]. Surface expression of MIC A/B is significantly increased in various epithelial cancer cells after exposure to histone deacetylase inhibitor (HDACi) [Skov *et al*, Cancer Res, 2005]. **Objective.** To compare the expression of NKG2DL, MICA/B, in L/L cell lines and to determine NK cell mediated cytotoxicity after exposure to romidepsin (RM, HDACi). **Methods.** L/L cells were incubated for 24 hrs with varying concentrations (0, 5, 10 ng/mL) of RM. FACS staining was performed with MICA/B PE. NK cells (CD3<sup>+</sup>/56<sup>+</sup>) were purified via magnetic separation and activated with IL-2 (3000IU/mL) overnight. Target cells were cultured with 5-10 ng/mL of RM for 24 hrs. NK cell cytotoxicity was determined by a standard europium assay at the E:T ratio of 5:1, 10:1. **Results.** There was a significant increase in surface expression of MICA/B in cell lines when cultured with RM at 10ng/mL RS4:11 [MLL-ALL] 0.2% vs. 19.2%,  $p < 0.0001$ , REH [B-cell ALL] 0.2% vs. 46%,  $p = 0.0003$ , Jurkat [T-cell ALL] 1.12% vs. 44.7%,  $p < 0.0001$ , Toledo [DLBCL] 0.5% vs. 15.8%,  $p = 0.0001$ , Ramos [BL] 0.57% vs. 33.6%,  $p = 0.0003$  and DEL [ALCL] 1.2 vs. 67%,  $p = 0.0009$ . There was a significant increase in-vitro cytotoxicity in RS 4;11, Ramos and Jurkat cells at E:T ratio of 5:1 and 10:1. Mean specific release (MSR)( $\pm$ SEM) was measured for four conditions in the assay: NK cells (A) vs. NK cells with 10 ng/ml RM (C) vs. IL-2 activated NK cells (B) IL-2 activated NK cells +10 ng/ml of RM (D). At 5:1, MSR for RS 4;11 was 3.2 $\pm$ 2% vs. 78 $\pm$ 42% and 11 $\pm$ 3.3% vs. 123 $\pm$ 48%  $p < 0.01$  (A vs. C, B vs. D), and at 10:1 ratio-10 $\pm$ 3.6% vs. 108 $\pm$ 47% and 12 $\pm$ 4% vs. 145 $\pm$ 45%  $p < 0.05$  (A vs. C, B vs. D) for RS 4;11. 7 At 5:1, MSR for Jurkat was 22 $\pm$ 8.4% vs. 130 $\pm$ 14.6% and 51 $\pm$ 11% vs. 174 $\pm$ 27%  $p < 0.01$  (A vs. C, B vs. D). 7 At 10:1, MSR for Ramos was 7.6 $\pm$ 7.6% vs. 79 $\pm$ 15.6% and 35 $\pm$ 13% vs. 90 $\pm$ 22%  $p < 0.05$  (A vs. C, B vs. D). **Conclusion.** This data suggests MICA/B expression in L/L is significantly induced by RM thereby enhancing the susceptibility for NKG2D-mediated cytotoxicity by NK cells which forms the basis of future ACI.

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**GENOMIC SIGNATURES AND NETWORK OF CORD BLOOD (CB) VS. PERIPHERAL BLOOD (PB) CD56DIM NK CELLS**

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**Background.** Umbilical cord blood (UCBT) is a viable alternate source of hematopoietic stem cells for the treatment of NHL (Rodrigues/Cairo/Rocha JCO 2009). NK cells play important roles in both innate and adaptive immunity (Caligiuri *et al* Blood 2008). Rapid CD56<sup>+</sup> NK cell reconstitution following AlloSCT appears to reduce the risk of hematological relapse (Dunbar *et al* Haematologica 2008). CD56dim cells are primarily cytotoxic and make up 90% of PB NK cells (Shereck/Cairo PBC 2007). We demonstrated the ability to ex-vivo expand CB into NK phenotype with profound NK *in vivo* cytotoxicity against hematological malignances (Ayello/Cairo BBMT 2006). We observed differential protein expression including **?NKG2A, ?IP3R type 3, ?MAPKAPK5, and ?NOTCH 2 in CB vs. PB CD56dim** (Shereck/Cairo, ASH 2007; Shereck/Day/Cairo, ASBMT 2009). **Objective.** To investigate the genomic signatures in CB vs. PB CD56dim NK cells. **Methods.** CB and PB CD56<sup>+</sup> NK cells were isolated indirectly by flowcytometry and immuno-magnetic separation from non-NK cells. The CD56<sup>+</sup> CD16<sup>+</sup> NK cells (CD56dim) were then selected with CD16 (FCGR3) MicroBeads. Isolated RNA from CB and PB CD56dim was subjected to microarray studies (Affymetrix, U133A\_2). Signal intensities were normalized and presented as natural log values. Gene data were subjected to Agilent GeneSpring and Ingenuity pathway analyses. Welch test was used to perform statistical analysis and fold change of  $\leq 1.5$  and values of  $p < 0.05$  were considered to be significant. **Results.** CB vs. PB CD56<sup>dim</sup> NK cells were significantly over expressed at the genomic level in a variety of functional categories including: pro-apoptotic genes: CASP10 (3.1F), TNFSF11 (4.7F), CDC2 (3.0F), BCL2L1 (4.3F), NOTCH2 (1.5F); and cell/tissue development: PBX1 (7.6F), IL1RN (5.1F), CD24 (5.3F), CD34 (3.5F), CD55 (2.1F), CCL13 (2.2F). Conversely, there was significant under expression of NF1 (5.1F), MAP2K3 (1.7F), PIK3CD (2.1F), BAX (2.9F), and JUN (2.2F). **Conclusion.** CB vs. PB CD56dim NK cells demonstrate differential expression of genes involved in PI, NOTCH, and MAPK pathways which regulate apoptosis, cellular/tissue development and signaling. Further studies are required to determine the role of CB NK CD56<sup>dim</sup> cells and its effect on NHL in-vitro and in-vivo. (The first two authors contribute equally)

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**SAFETY AND KINETICS OF RITUXIMAB (R) FOLLOWING CHEMOIMMUNOTHERAPY (RITUXIMAB + FAB CHEMOTHERAPY) IN CHILDREN AND ADOLESCENTS WITH MATURE B-CELL NON-HODGKIN LYMPHOMA (B-NHL): A CHILDREN'S ONCOLOGY GROUP REPORT**

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**Background.** We previously demonstrated CD20, the receptor to the chimeric antibody R, is expressed in over 98% of all children with mature B-NHL (Perkins/Cairo *et al*, Clin Adv Hem, 2003). In adults (30-60 yrs) (Habermann *et al*, JCO, 2006) and elderly adults (>60 yrs) (Feugier *et al*, JCO, 2005) with DLBCL, the addition of rituximab to CHOP or CHOP like chemotherapy has significantly increased the EFS and OS (Pfreundschuh *et al*, Lancet Oncology, 2006 and Coiffier *et al*, NEJM, 2002, respectively). **Objectives.** To determine the safety and kinetics of R in pediatric patients with stage III/IV B-NHL receiving chemoimmunotherapy. **Methods.** Therapy consisted of FAB Group B4 therapy (adria 1 hr infusion) as we have previously described (Patte/Cairo *et al*, Blood, 2007). R 375 mg/m<sup>2</sup>/dose, generously supplied by Genentech, day -2 and day 0 in COPADM2 and day 0 in CYM 1+2 (4 doses, subpilot) and day -2 and day 0 in COPADM 1+2 and day 0 in CYM 1+2 (six doses, pilot), respectively. R levels were measured at baseline, 30-60 minutes after Day -2 and Day 0 in COPADM 1 (pilot) +2 (subpilot) and 1, 3, and 6 months after the last dose of R and measured by ELISA with a purified polyclonal goat anti-R antibody as the capture reagent and goat antibody to mouse IgG-conjugated to horseradish peroxidase as the detection reagent (detection limit of 0.5 mcg/mL). **Results.** Median age 11 (1-23 yrs), M/F (4:1), Burkitt (59%), DLBCL (24%), PMBL (5%), 11% NOS. The addition of R was safe and well tolerated with 274 infusions and there were no SAEs probably or definitely attributed to rituximab. The peak R level 30-60 minutes after the first infusion on day -2, day 0 in COPADM 1+2 was 220.4±10.5, 307.7±377.6, 267.3±66.0, and 402.1±38.6 mcg/mL, respectively. The trough levels prior to the second R dose in COPADM 1+2 was 128.9±16.9 and 208.5±15.3 mcg/mL, respectively. The levels 1, 3 and 6 months post the last dose of R was 75.0±14.7, 13.0±3.3 and 1.1±0.15 mcg/mL, respectively. There was no significant difference in R levels in BL vs. other mature B-NHL and age <8 vs. >8 yrs. **Conclusions.** In summary, the addition of R to FAB COPADM 1 + 2 and CYM 1+2 is well tolerated. R levels are comparable to levels in adults, and in the serum up to 3 months past the last dose.

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**CELLULAR LEVELS OF GLUTATHIONE S-TRANSFERASE II (GSTP1) PREDICT SUSCEPTIBILITY OF LYMPHOMA CELL LINES TO ETHACRYNIC ACID IN VITRO**

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**Background.** Relative abundance of specific proteins among lymphoid tumors can be indicative of unique pathogenetic pathways and novel therapeutic targets. We have used quantitative proteomics (iTRAQ) to identify differentially-expressed proteins among three cell lines of same lineage, but different biology: Primary Mediastinal Large B-cell Lymphoma (PMBL), classical Hodgkin Lymphoma (cHL) and diffuse large B-cell lymphoma (DLBCL). GSTP1, a detoxification enzyme and signaling protein, was over-expressed six-fold in PMBL (K1106P) vs. cHL (L428, NS-type) and virtually absent in the DLBCL cell line SUDHL-9. **Objectives.** In this study, we sought to investigate the functional significance and therapeutic potential of differential GSTP expression in lymphomas by using selective inhibition with ethacrynic acid (EA). **Design and Methods.** Several B-cell lymphoma cell lines expressing different degrees of GSTP1 expression by Western blotting (WB) were subjected to increasing concentrations (0-80  $\mu$ M) of EA for 24 or 48 hrs and the effects were studied using a viability assay (WST-1), flow cytometry for apoptotic fractions and WB for apoptosis and cell cycle progression-associated proteins. **Results.** WB screening of PMBL(2), DLBCL(16), cHL(2), ALCL(4), T-ALL(4), CTCL(2) and NK(1) lymphoma cell lines confirmed high GSTP1 expression in the PMBL cell line K1106P and its absence in the DLBCL cell line SUDHL-9. GSTP1 was also undetectable by WB in the cell lines SUDHL-8 (DLBCL) and Hut78 (CTCL). Cell lines with relatively high cellular GSTP1 (K1106P, KMH2, MedB1) were resistant to EA treatment up to 40  $\mu$ M concentrations, with minimal effects at 60  $\mu$ M, while the two DLBCL cell lines lacking GSTP1 (SUDHL-8 and -9) underwent significant cell death at the lowest (20  $\mu$ M) concentration, as evidenced by WST-1 and annexinV/PI staining. Giemsa staining showed morphologic features of apoptosis at 20  $\mu$ M EA and necrosis at 60  $\mu$ M for the latter. WB of several cell lines identified an EA-induced decrease in the S-phase Kinase-associated Protein 2 (p45SKP2), with concomitant increase in p27KIP1, a negative regulator of G1-S progression. **Conclusion.** The results of our study suggest an inverse relationship between cellular GSTP1 levels and susceptibility to EA-induced cell death *in vitro*, and identify GSTP1 as a putative therapeutic target in lymphomas with low GSTP1 expression.

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**TREATMENT OF PEDIATRIC ADVANCE STAGED MATURE B-NHL/B-ALL WITH INTENSIVE CHEMOTHERAPY+RITUXIMAB: RESULTS OF 4 YEARS MULTICENTER STUDY (04.2004-05.2008)**

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**Background.** Mature B-cell neoplasms in children are highly curable when treated with intensive dose-dense  $\dot{e}$ T. However such a therapy is toxic, especially during first cycles. Objectives To decrease the risk of delaying delivery of scheduled therapy due to toxicity not affecting efficacy we introduced rituximab in the protocol NHL BFM90 while reducing dose of Mtx in children with advance staged of mature B-NHL/B-ALL. According to preliminary analysis this strategy proved feasible and efficacious with decreased toxicity for a representative group of B-NHL/B-ALL pts in 8 Russian pediatric clinics. The final results of this trial with FU from 5 to 50 mo (med 30 mo) are presented. **Patients and Methods.** Newly diagnosed patients with III-IV St.Jude stages of diffuse large B-cell lymphoma (DLBCL), Burkitt lymphoma (BL) and B-ALL were enrolled in a trial and treated according to the protocol which differed from the original one by adding rituximab 375 mg/m<sup>2</sup> on day (-1) of each of the first 4 blocks of CT. Mtx dose was reduced to 1 g/m<sup>2</sup> in the first 2 blocks. **Results.** Of 61 pts (48M/13F), med age 8,7y (2-17), 26 had BL, 21-B-ALL, 14- DLBCL including 3 primary mediastinal BCL. All pts completed full protocol. Results were the follows. With a med FU of 29 (7,9-49) mo CCR rate is 88%, OS and EFS are 0,90 $\pm$ 0,04; RFS is 0,98 $\pm$ 0,02. Of note 14 pts (6 with DLBCL and 8 with BL) had residual mass on CT at the initial tumor localization after completion of therapy. Four of them with BL were FDG-PET-positive. They received additional therapy with course ICE followed by high dose CT and HSCT. Two of them proved refractory and died with progression whereas 2 pts were cured. Overall 12 pts with residual mass after completion of treatment are in CCR for a med 25,4mo (8,9-47). **Conclusion** Addition of rituximab to less intensive CT allowed to achieve good results while reducing immediate toxicity in representative group of children with advance staged mature B-NHL/B-ALL, especially with DLBCL. In spite of reduced dose of MTX relapse rate was low what is especially encouraging given the high rate of initial CNS involvement. Given the frequent detection of residual mass on CT, PET-CT has to be used to document remission state.

Table.

	B-NHL/B-ALL	B-ALL	DLBCL	BL
CNS	14	10	3	1
induction death	4	3	0	1
refractory	2	0	0	2
remission rate (abs/%)	55/90	18/86	14/100	23/88
Relapse, abs/%	1/1,6	1(CNS)/5,5	0	0
CCR abs/%	54/88,5	17/81	14/100	23/88
Med FU mo	30	25	20,3	27,7

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**RITUXIMAB COMBINED WITH FAB GROUP B4 THERAPY IN CHILDREN AND ADOLESCENTS WITH STAGE III/IV MATURE B-NHL: A CHILDREN'S ONCOLOGY GROUP REPORT**

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**Background.** The probability of 2 year EFS in children and adolescents (C+A) with stage III/IV mature B-NHL following Group B FAB therapy is 84 $\pm$ 1.6% (Patte/Cairo *et al*, Blood, 2007). We have previously demonstrated that CD20, the receptor to the chimeric antibody rituximab, is expressed in >98% of C+A mature B-NHL (Perkins/Cairo *et al*, Clin Adv Hem, 2003). The addition of rituximab to CHOP or MINT therapy improved the EFS and OS of adults with DLBCL (Coiffier *et al*, NEJM, 2002 and Pfredushuh *et al*, Lancet, 2006). **Objective.** To determine the safety and efficacy of rituximab combined with FAB Group B4 chemotherapy in C+A with stage III/IV B-NHL. **Methods.** Therapy consisted of FAB Group B4 (Patte/Cairo *et al*, Blood, 2007) (Adria 1 hour infusion) and rituximab (375 mg/m<sup>2</sup>) generously supplied by Genentech on days -2 and 0 in COPADM2 and day 0 in CYM 1+2 (4 doses-subpilot) and days-2 and 0 COPADM 1+2 and days 0 CYM 1+2 (6 doses-pilot). **Results.** Forty-eight pts (41 pilot, 7 subpilot); M/F (4:1); Age: median 11 yrs (1-23); stage III/IV (43/5); 59% BL, 24% DLBCL, 17% other; LDH < 2XULN 51%. Toxicity: grade III/IV febrile neutropenia/infections 47%, 29%, 9% 19% in induction 1+2 and consolidation1+2, respectively and grade III/IV mucositis 14 and 12%, respectively, induction 1+2. Probability of 2 yr EFS is 93% (CI95 86 - 100%). Among 4 relapses, 1 was ineligible and was a Group C pt treated as per Group C therapy, and two others were PMBL. Therefore, only one relapse among the remaining patients who had stage III/IV disease. There were 2 relapses and eventual disease related deaths among the 21 patients with elevated LDH>2XULN at

diagnosis. Excluding PMBL, pts with an LDH<2XULN had a 100% EFS (n=19). *Conclusion.* The addition of rituximab to Group B FAB therapy in C+A with stage III/IV mature B-NHL is well tolerated and is associated with an excellent EFS. Future randomized studies will be required to determine if rituximab added to FAB B4 therapy improves EFS in C+A with stage III/IV B-NHL (excluding PMBL). Finally, patients with stage III/IV (non PMBL) mature B-NHL with LDH<2 UNL are excellent candidates for future dose intensity reduction or substitution research studies.

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**THE EFFECT OF CYCLIN-DEPENDENT KINASE INHIBITOR FLAVOPIRIDOL ON ANAPLASTIC LARGE CELL LYMPHOMA CELLS AND RELATIONSHIP WITH NPM-ALK KINASE EXPRESSION AND ACTIVITY**

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*Background.* The loss of cell cycle regulation due to abnormal function of cyclin-dependent kinases (cdks) occurs in tumors and leads to genetic instability of chemotherapy-resistant cells. Deregulated cdk activity is a hallmark of human cancer, and a variety of genetic and epigenetic events, such as overexpression of cyclins, diminished levels of cdk inhibitors or gain-of-function mutations in cdks, have been described to cause overactivity of these enzymes and to provide a selective growth advantage in tumor cells. This renders cdks suitable targets for anti-cancer therapy and have prompted great interest in the development of specific inhibitors. *Objectives.* To investigate the effect of cdk inhibitor Flavopiridol in Anaplastic Large Cell Lymphomas (ALCL), in which unrestrained proliferation depends on NPM-ALK tyrosine kinase activity. *Design and Methods.* Effects of Flavopiridol were examined in ALK-positive and-negative ALCL cells by means of immunoblotting and immunofluorescence analyses to assess cdks expression and activity, QRT-PCR to measure drug-induced changes in transcription, and FACS analyses to monitor changes in proliferation and survival. *Results.* Treatment with Flavopiridol resulted in growth inhibition of ALCL cells, along with accumulation of subG1 cells and disappearance of S phase without cell cycle arrest. Consistent with Flavopiridol activity, phosphorylation at cdk2, cdk4, cdk9 sites on retinoblastoma (RB) protein and RNA polymerase II was inhibited. This correlated with induction of cell death through rapid mitochondria damage, inhibition of DNA synthesis, and downregulation of anti-apoptotic proteins and transcripts. Notably, Flavopiridol was less active in ALK-positive cells, as apoptosis was observed at higher concentrations and later time points, and resistance to treatment was observed in cells maintaining NPM-ALK signalling. NPM-ALK inhibition affected proliferation of ALCL cells, and resulted in a dramatic increase in apoptosis when combined with Flavopiridol. *Conclusions.* This work provides a demonstration that targeting cdks is an effective approach against ALCL

lymphoma cells, and proves the critical role of NPM-ALK in the regulation of responsiveness of tumor cells with cdks dysregulation.

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**TREATMENT RESULTS OF CHILDHOOD DISSEMINATED NON-HODGKIN'S LYMPHOMAS IN NATIONAL CANCER HOSPITAL FROM 6/2000 TO 12/2008**

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*Background.* Non Hodgkin's lymphomas (NHL) is the third most common malignancy followed by leukemias and brain tumors, constitutes 4.2% of all childhood cancers. NHL in children is generally considered to be widely disseminated from the outset. The appropriate protocols remain unclear. Therefore, we initiated this study with 2 following purposes: 1. Assess clinical and paraclinical characteristics of the children with disseminated NHL in National Cancer Hospital from 6/2000 to 12/2008. 2. Evaluate treatment results for these patients with ESHAP or NHL-BFM 90 regimens. *Objectives.* 59 children with stage III and IV NHL treated in National Cancer Hospital, Viet nam from 6/2000 to 12/2008. *Methods.* Describe, retrospective and prospective study. *Results.* The most common age: 9-12 y (35.6%). Male/female ratio: 3.54. The most common nodal area is in head and neck. The most common type is lymphoblastic lymphoma (18 pts-30.5%). Average rate of bone marrow lymphoblasts: 30.6%. Protocol ESHAP's treatment results: CR 73.3%, Median overall survival for all pts was 13.9 months ( $p=0.00026$ ). Median disease-free survival was 10.7 months ( $p=0.00412$ ). Protocol NHL-BFM 90's treatment results: All of 23 prospective pts who are treated with NHL-BFM 90 regimen attained CR. 95.7% of the pts are being alive and take part in the continuous phases of the regimen now. Median disease-free survival is  $19.957\pm 4.656$  months, median overall survival is  $22.5\pm 4.755$  months. *Conclusion.* NHL-BFM 90 is an active and effective regimen in disseminated childhood NHL.

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**SHOULD ADOLESCENTS WITH NHL BE TREATED AS OLD CHILDREN OR YOUNG ADULTS?**

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*Background.* The SEER (Surveillance, Epidemiology, and End Results) data show that children with non-Hodgkin lymphoma (NHL) have a better treatment outcome than do adults. *Methods.* Many factors may contribute to this age-related difference. Some factors are related to the patient whereas others pertain to tumor histology and biology. The spectrum of NHL subtypes is well known to differ in children and adults. From ages 5 through 14 years, Burkitt lymphoma is the predominant histologic subtype, whereas diffuse large B-cell lymphoma is most common in the 15- to 29-year age range. Because different treatment strategies are often used in children

and adults with NHL, the choice of therapy for adolescents and young is challenging and somewhat controversial. *Results.* It is reasonable to consider pediatric strategies for some adolescents and very young adults with NHL, and pediatric strategies are currently used to treat adults with certain subtypes of NHL. However, the use of pediatric strategies in adults does not guarantee a comparable outcome, as illustrated by trials for adult lymphoblastic lymphoma. *Conclusion.* There is clearly a need for further biologic study of NHL in children, adolescents, and young adults. Age-related differences in tumor biology have been demonstrated in anaplastic large-cell lymphoma (ALCL) and diffuse large B-cell lymphoma (DLBCL). Additional biologic data will not only improve prognosis and treatment stratification but, more important, will lead to the identification of specific molecular targets for therapy.

## SCIENTIFIC SESSION 12: QUALITY OF LIFE AND LATE RISKS

### LATE RISKS FOLLOWING NON-HODGKIN LYMPHOMA IN CHILDREN, ADOLESCENTS, AND YOUNG ADULTS

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Non-Hodgkin lymphoma accounts for 6% of cancers before 20 years of age in the U.S. Systemic chemotherapy, with or without radiation is the mainstay of treatment, and advances over the past three decades have resulted in 5-year survival rates exceeding 78%. Therapeutic exposures typically encountered in NHL survivors have included cyclophosphamide, vincristine, methotrexate, prednisone, epipodophyllotoxins, doxorubicin, intrathecal chemotherapy, radiation to bulk disease sites, and central system radiation therapy. The intensity of treatment, coupled with the excellent survival rates places a large population of NHL survivors at increased risk for long-term morbidity and mortality. The chronic disease burden in long-term NHL survivors is being increasingly reported. A recent study of a large cohort of NHL patients followed for an extended period of time revealed a 4-fold increased risk of late mortality, with elevated rates of death from solid tumors, leukemia, cardiac disease, and pneumonia. Risk for death remained elevated 20 years after diagnosis of NHL. Risk factors for death from causes other than NHL included female sex (3.4-fold increased risk), and exposure to radiation to the heart (1.9-fold). NHL survivors are at an increased risk of neurocognitive deficits, especially if they are treated at a young age with cranial radiation. Reduced fertility can follow exposure to cyclophosphamide. Exposure to doxorubicin may increase the risk of cardiomyopathy with a risk of progression to congestive heart failure. NHL survivors are at a 4-fold increased risk of developing second solid malignancies, with a cumulative incidence of 3% at 10 years. The most common solid malignancies include second breast cancer, thyroid cancer, oropharyngeal cancers, musculoskeletal and brain tumors. Risk factors include female sex (3-fold increased risk), mediastinal NHL (5-fold increased risk), and breast radiation (4.3-fold increased risk). Survivors of childhood NHL, particularly those treated with chest radiation are at a continued increased risk of premature death and second malignancies. NHL survivors exposed to alkylating agents or topoisomerase II inhibitors are also at increased risk of developing therapy-related myelodysplasia and acute myeloid leukemia (t-MDS/AML). The risk is modest with conventional treatment, but becomes magnified with high-dose therapy, followed by stem cell rescue (autologous hematopoietic cell transplantation [HCT]), and with increasing age. In fact, autologous HCT survivors carry an additional burden of morbidity, including an increased risk of cataracts, xerostomia, hypothyroidism, osteoporosis and avascular necrosis, congestive heart failure, and pulmonary fibrosis. A recent publication from the

Childhood Cancer Survivor Study reported the incidence of severe or life-threatening chronic health conditions or death due to these conditions to exceed 20% at 20 years from diagnosis. Given this high burden of morbidity, it is imperative that the survivors be monitored closely for early detection of these complications. The Children's Oncology Group has developed therapeutic- exposure related, risk-based clinical practice guidelines for follow up of childhood cancer survivors, available at [www-survivorshipguidelines.org](http://www-survivorshipguidelines.org). We believe that use of these or other similar guidelines would help reduce the morbidity in long-term NHL survivors.

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EDUCATIONAL ACHIEVEMENT, EMPLOYMENT,  
SMOKING, MARITAL AND INSURANCE STATUSES  
IN LONG-TERM SURVIVORS OF CHILDHOOD  
LYMPHOMAS**

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*Background.* Treatment advances have led to improved survival rates in childhood lymphomas and social, vocational and educational adjustments of survivors gained importance. Aim: To evaluate educational achievement, employment, smoking, marital, parenthood and insurance statuses in survivors of childhood lymphomas. *Patients and methods.* Records of children treated for Hodgkin (HL) or non-Hodgkin lymphomas (NHL) at our department between 1972-2006 were examined. Cases in complete remission with age  $\geq 18$  years were interviewed for their educational achievement, living situation, employment, marital, parenthood, smoking and social insurance statuses. Data were analyzed in relation with gender, age at diagnosis, stage of disease and follow-up time. *Results.* 99 patients were included with median ages at diagnoses and present study 10 (range: 3-19) and 24 (range:18-36) years, respectively. Median follow-up duration was 13 years (range: 3-31). 55/99 patients (55.6%) had HLs and 44 (44.4 %) had NHLs (M/F= 74/25). Educational statuses were: 16 (16.2%) had graduated from primary/secondary schools, 57 (57.6%) from high school, 26 (26.2%) from a university or equivalent. 53/99 patients (53.5%) had an occupation and 44/99 were working at a job; 89% had a social insurance. Independent habitation rate was 28.3%. Nineteen patients (19.2%) were married and 10 had ?one child. Rate of smoking was 9.1% in the whole group. There were no significant differences in having university education, employment, smoking, marital statuses or having a social insurance between the cases according to age at diagnosis ( $\leq 10$  or  $>10$  years) and gender. Occupational and marriage rates were higher in cases with a follow-up  $>13$  years ( $p$  values=0.001). Having a university degree, independent habitation and marriage rates were significantly higher in HL survivors ( $p=0.02$ ,  $p=0.001$  and  $p=0.005$ ). Survivors with early-stage diseases tended to have more university degrees, independent habitation and marriage rates ( $p=0.03$ ,  $p<0.001$ ,  $p=0.019$ ). *Conclusions.* The educational status of our patients was similar compared to their peers. HL survi-

vors had better outcomes in social life. Psychosocial support concerning education, employment, marriage and parenthood should be given to all survivors of childhood lymphomas. Attempts to reduce the smoking rates should be made in all patients.

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EFFECTIVE AND SAFE ACUTE TUMOR LYSIS  
SYNDROME (ATLS) PROPHYLAXIS AND  
TREATMENT (P+T) WITH RASBURICASE (R) AND  
NON-ALKALINE HYDRATION IN CHILDREN AND  
ADOLESCENTS (C+A) WITH HIGH-RISK FAB  
GROUP C MATURE B- NON-HODGKIN LYMPHOMA  
(B-NHL): A CHILDREN'S ONCOLOGY GROUP  
REPORT**

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*Background.* ATLS is characterized by hyperuricemia, hyperkalemia, hyperphosphatemia and hypocalcaemia. The incidence of TLS and renal insufficiency in C+A with Group C mature B-NHL with allopurinol was 26% and 27%, respectively (Cairo, Blood 2007). R is more effective than allopurinol in rapidly reducing hyperuricemia in C+A at high risk of ATLS. (Goldman/Cairo, Blood 2001). *Objectives.* To assess the toxicity, incidence of ATLS, renal insufficiency (RI) and assisted renal support (ARS) of R added to COP reduction in C+A with Group C B-NHL. *Design and Method.* COP was administered as previously described (Cairo, Blood, 2007). R was administered as a single dose of 0.2 mg/kg and as needed (max=5), and was generously supplied by Sanofi-Aventis. Hydration (without sodium bicarbonate) was administered a 3 L/m<sup>2</sup>/day. LTLS and CTLS were classified according to the Cairo-Bishop classification (BJH, 2004). Chemotherapy was initiated between 4 and 24 hours of the day 0 R dose. GFR (mL/min/1.73 m<sup>2</sup>) was calculated using the pediatric Schwartz formula. *Results.* 36 pts, median age 9.5 yrs, 82% Burkitt, 34% CNS +, 86% LDH  $> 2XULN$ . Initial UA was  $>8$  mg/dL in 14/31 pts (mean 13 (8.6-22) and  $<8$  mg/dL (mean 4.9) (0.9-7.6) in 17/31. 3/31 pts had K between 5-6 meq/L. 9/31 pts had PO<sub>4</sub>  $>6.5$  mg/dL (mean 8.3) (6.5-12.2) of which 2 required ARS. 5/31 pts (16%) had initial GFR  $<60$  of which 3 pts required ARS. 94% pts had an improved GFR at day 7. The initial mean GFR + SD vs. end of COP were 108 + 42 and 173 + 64 mL/min/1.73 m<sup>2</sup>, respectively ( $p<0.05$ ). All 4 pts who required ARS presented with RI at diagnosis and 3/4 patients were successfully taken off ARS by day 7. 17/36 (47%) pts received R before day 0 (median 1 dose). In pts without TLS at presentation, 19% and 0% developed LTLS and CTLS after R. There were no toxicities definitely or probably related to R therapy. *Conclusion.* R during COP reduction is well tolerated and highly effective in pts with FAB group C B-NHL. Initial elevated PO<sub>4</sub> and low GFR predicted pts who required

ARS. The addition of R to COP prevented RI in 89% of all Group C pts and completely prevented the development of RI in those patients who did not require ARS prior to R.

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**CARDIOMETABOLIC RISK IN PEDIATRIC SURVIVORS OF NON-HODGKIN'S LYMPHOMA**

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**Background.** It has been suggested that childhood cancer survivors have a higher than expected frequency of early cardiovascular disease (CVD) and type 2 diabetes. **Objectives.** To determine the cardiometabolic risk factors in childhood survivors (age<18 years) of Non-Hodgkin's Lymphoma (S-NHL) and compare them to healthy controls as part of a larger study of metabolic syndrome in childhood cancer survivors. **Methods.** Measures of insulin resistance (euglycemic hyperinsulinemic clamp adjusted for lean body mass-low Mlbm represents insulin resistance), fasting glucose, insulin and lipids, anthropometry, and blood pressure, were obtained in 16 pediatric S-NHL (10 with Burkitt's lymphoma and 6 with lymphoblastic lymphoma, ≥5 years from diagnosis) and 89 healthy controls. Linear regression analyses were used to evaluate risk factors for early CVD between groups after adjusting for age, gender, Tanner stage, and body mass index (BMI). **Results.** S-NHL children (age=15.9±2.1, 12 males) were older than controls (age 13.7±2.4, 50 males). There were no significant differences between S-NHL and controls for BMI, percent body fat, waist circumference, visceral fat, blood pressure, total- and LDL-cholesterol, and fasting glucose and insulin. However, S-NHL had lower HDL-cholesterol compared to controls (Table). **Conclusions.** These preliminary data suggest that children who have survived NHL may have at least one adverse cardiometabolic risk factor. This study is ongoing and accrual of additional NHL survivors will allow us to refine these findings

**Table.**

Variable	S-NHL (N=16)	Controls (N=89)	P-Value
BMI	23.5±1.3	21.4±0.7	0.12
Waist Circumference	76.4±3.2	70.1±1.6	0.06
HDL Cholesterol (mg/dL)	42.6±2.8	49.3±1.4	0.02
Triglycerides (mg/dL)	103.3±14.8	82.9±7.6	0.13
Mlbm (mg/kg/min)	13.6±1.2	14.1±0.6	0.7

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**OSTEONECROSIS IN CHILDREN WITH NON-HODGKIN LYMPHOMA (NHL)**

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**Background.** Osteonecrosis (ON) is a recognized and potentially debilitating complication emerging during and after treatment of childhood malignancies. **Objectives.** To describe the frequency and to analyze predisposing factors for the development of ON in a large cohort of children and adolescents of the NHL-BFM study group treated within the trials NHL-BFM 95, NHL-BFM 04, EURO-LB 02, or ALCL 99. **Design and Methods.** Patients (pts) diagnosed between 04/96 and 12/05 were included in the analysis. Pts with lymphoblastic lymphoma (LBL) were treated according to an ALL-type therapy (induction including 37 days of steroid administration - consolidation - reinduction including 30 days of steroid administration - maintenance). Pts with mature B-NHL and ALCL were treated according to B-type protocols with 2-8 chemotherapy courses, each including dexamethasone administration over 5-6 days. **Results.** Out of all 1504 pts, 39 pts (2.6%) were reported with ON. Among a total of 414 girls, 3.9% (n=16) and out of a total of 1090 boys, 2.1% (n=23) suffered from ON (p=0.0558). Mean age at diagnosis of NHL was significantly higher in pts with ON as compared to pts without ON (13.3+3.9 y vs. 9.8+4.3 y; p<0.0001). The incidence of ON was highest in pts with LBL, with 27 out of the 29 pts with ON diagnosed as stage III or IV. Further details are shown in the table below. **Conclusion.** Pts receiving an ALL-type therapy are at particular risk for ON, especially if a higher stage is diagnosed. We assume the cumulative corticosteroid dose as well as the schedule of corticosteroid administration to influence the risk for ON. Female gender and older age seem to be predisposing factors for ON. We speculate that the true incidence of ON might even be underestimated since follow-up in adolescents is usually shorter and ON may become symptomatic years after diagnosis.

**Table.**

Therapy type	N	Median time of follow after diagnosis (y)	Age at diagnosis of (NHL) (means±SD)	ON (n%)	Age at diagnosis in years (mean + SD): pts with vs. without ON	Females vs males with ON (n [%])
Whole group	ALL- and B-NHL	1504	4.7	39 (2.6)	13.3+3.9 vs. 9.8+4.3	16 (3.9) vs. 23(2.1)
LBL	ALL	345	5.0	29 (8.4)	14.0+3.3 vs. 8.4+4.4	12 (9.4) vs. 17 (7.8)
Mature B-NHL	B-NHL	953	4.4	6 (0.6)	10.0+5.4 vs. 10.0+4.2	3 (1.4) vs. 3 (0.4)
ALCL	B-NHL	206	5.2	4 (1.9)	12.8+3.6 vs. 11.0+4.4	1 (1.4) vs. 3 (2.2)

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**TREATMENT OF CHILDHOOD BURKITT LYMPHOMA IN NORTH AFRICA: A STUDY OF THE FRENCH-AFRICAN PEDIATRIC ONCOLOGY GROUP (G.F.A.O.P.)**

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**Background.** a first prospective multicentric study was performed by GFAOP in 2001-2004, aiming to assess the feasibility of adapted versions of LMB 89 protocol in Africa. Two schemes of different intensity were tested. One, MAT, was very similar to the model, and the other LB 2001, less aggressive without doxorubicin. Overall survival (OS) was 61%. (Harif, BPC, 2008) with better results with MAT regimen (74%). Treatment-related mortality declined from 27% to 10% during the first 3 years, the OS raising simultaneously from 54 to 73%. **Objectives.** For the second study, in order to improve outcome of patients with Burkitt, all centers in North Africa decided to treatment their patients (pts) with the MAT regimen, including the biggest center who used the LB scheme in the first study. **Methods.** All consecutive pts with Burkitt were stratified in 3 risk groups (A, B, C) as in LMB89, and treated with polychemotherapy of progressive intensity. All were prospectively registered in a common database in Casablanca and analysed in term of description of the disease and outcome. **Results.** From 04/05 to 03/09, 209 pts are evaluable: 9 stage (st) 1, 33 st 2, 134 st 3, 33 st 4 corresponding to 5 group A, 169 group B, 35 group C. Toxicity was observed in 353 pts about 1081 courses (32%). 40 pts died of toxicity, 14 of them (35%) during the pre induction course (COP): 23 (14%) in group B, 17 (48%) in group C. 28 pts relapsed (13%). The event free survival is 100% in st 1, 77% in st 2, 58% in st 3 and 33% in st 4. The overall survival is 60% for all pts, 100% in st 1, 80% in st 2, 63% in st 3 and 35% in st 4. **Conclusion.** Toxicity-related death rate is still too high, especially during the first week of treatment. Better management of initial complications is mandatory, including the possibility of using urate oxidase not yet available in North Africa.

**DEVELOPING COMMON INTERNATIONAL COOPERATIVE CLINICAL TRIALS**

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Developing common clinical trials for childhood cancer in a multi-national, multi-clinical research network environment requires a clear mission that the purpose of such an initiative

is to cure and prevent disease through scientific discovery and evidence-based improvements in standards of care. The vision to effect this mission requires an understanding of the distinction of clinical research from routine clinical management while accepting its close integration in clinical care. In addition, the clinical and biological subclassification of pediatric cancer diagnoses and the emphasis on risk-adjusted therapy results in difficulties in identifying appropriately sized patient populations for study when considering sample size requirements for adequately powered statistical methods planned to address clinical questions. This further subclassification of already relatively low incidence diseases mandates international cooperation and collaboration, despite possible regulatory, cultural, and operational differences experienced in such international endeavors and may result in distinct challenges. Creating effective infrastructure relationships which permit networks to relate in an interoperable fashion requires a uniform consensus with respect to the specific criteria of network membership including competence, commitment, and compliance for both individual investigator, regardless of discipline, as well as institutional or study site performance and participation. Effective network operation requires real time communication considerations, trust, shared ownership of design and planning, and delegated responsibility for oversight of study conduct codified in multi-lateral agreements. The optimal configuration of such international operations is likely best accomplished as a consortium of networks or groups model which limits to some extent possible cultural, but more importantly jurisdictional regulatory constraints. An attempt to assure that individual networks working together are similarly resourced and have compatible operational systems and procedures is necessary for success. Specific systems and procedures include protocol development and study conduct and monitoring (including external Data and Safety Monitoring Committee review), regulatory oversight and drug distribution, case report form development, data submission and management, information systems at a minimum. Trial development must include hypothesis-based primary objectives, limited secondary objectives and a critical review of all data elements requested for study participation. Most important is the requirement for robust scientific review by both internal and external peer review mechanisms with appropriate input from patient and parent advocates. In addition to sample size considerations, the rationale for international collaboration can extend to unique biology and epidemiology research opportunities and include not only specific gene-environment relationships on causation, but also explore genomic differences in study populations and investigate the impact of pharmacogenetics on treatment outcome. Such an approach to cooperation in pediatric cancer clinical research holds global promise for continual improvement in outcome for children.

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**DNA REPAIR DISORDERS AND CANCER: REDUCED VERSUS STANDARD DOSE CHEMOTHERAPEUTIC TREATMENT**

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**Background.** Two of the most common DNA repair disorders in childhood are Ataxia Telangiectasia (A-T) and Nijmegen Breakage Syndrome (NBS). A hallmark of both conditions is the increased incidence of cancer. Prevalence estimates range from 20-40% for classic A-T to almost 50% for NBS. The most commonly encountered malignancies are B and T cell Non-Hodgkin lymphoma (NHL), T-cell leukaemia, and Hodgkin lymphoma. Patients with atypical A-T (different ATM mutations compared to classic A-T) also have an increased cancer risk, however cancer develops at later age (mainly adulthood) and expresses a different subtype spectrum (epithelial malignancies, mature T-cell leukaemia's). There are no large follow-up studies on such patient groups, so exact figures still need to be generated. Three factors make treatment of cancer in patients with DNA repair disorders extremely difficult. The increased susceptibility to DNA damaging agents and radiotherapy resulting in increased treatment related toxicity (I), the underlying immunodeficiency possibly resulting in increased infection risk and impaired tumour immunosurveillance (II), and impaired lung function progressive with age (III), eventually leading to early death. In literature, two treatment strategies have been propagated. Reduced dose chemotherapy in order to minimise treatment related toxicity, yet accept the possibility of reduced Event Free Survival (EFS) and Overall Survival (OS). Or standard dose chemotherapy (according to general accepted treatment protocols) and accept the possibility of increased treatment related toxicity, yet maximise the EFS and OS. In order to develop insight, which strategy is the best, a European wide prospective clinical study for the most commonly occurring malignancies with special attention to supportive care and cautious toxicity monitoring, is needed. As a preparation for the design of such protocols we assembled all available data from literature concerning the key objectives of A-T and NBS patients with cancer supplemented with data on a large group of new patients. **Objectives.** 1. To study the spectrum of different (subtypes) of malignancies in patients

with A-T and NBS; 2. To study the treatment strategies; 3. To study the treatment related toxicity; 4. To study the outcome of patients in A-T and NBS. **Design/Methods.** In order to develop evidence based treatment protocols for malignancies in patients with A-T and NBS, we assembled clinical data related to cancer subtypes, treatment strategies, treatment related toxicity and outcome from patients with A-T and NBS from literature supplemented with data on new patients from different European countries. **Results.** 220 cases of cancer in A-T patients and 40 in NBS were assembled from literature. This group was completed with 70 new A-T and 44 NBS patients for a total of 290 cases of cancer in A-T patients and 84 in NBS patients. The most common malignancies in patients with A-T are NHL, leukaemia and Hodgkin lymphoma. The most common types of NHL (in descending order) are diffuse large B cell lymphoma (DLBCL), Burkitt lymphoma (BL) and lymphoblastic lymphoma (LBL). The majority of leukaemia cases are of (mature as well as immature) T-cell origin. NBS patients mainly develop NHL (DLBCL, BL and LBL), Hodgkin lymphoma (minority), and a small residual group of solid tumours. Most Hodgkin and non-Hodgkin lymphomas are high stage with B-symptoms. Treatment strategies vary from no treatment at all (considered upfront too toxic by patients, parents or treating physicians), to inclusion in regular (national) studies or individualized tailor-made therapy (by treating physician). Many patients who received standard dose chemotherapy completed the course with only minor dose modifications and short delays between courses. Chemotherapy related toxicity consisted mainly of bacterial and fungal infections, neutropenic episodes with fever, mucositis, massive enterocolitis, gastrointestinal bleeding, thrombosis, acute liver failure, multiple organ failure, renal insufficiency, hemorrhagic cystitis, progressive lung disease (bleomycine related), muscle weakness and second malignancies. The percentage of toxic deaths seem relatively small, however exact percentages are unknown. Radiotherapy results in severe mucositis and skin ulceration. The overall survival of cancer in patients with A-T and NBS varies with cancer subtype (e.g. survival of patients with Hodgkin lymphoma is extremely poor) and treatment strategy. Preliminary analysis of data shows that patients who receive upfront standard dose chemotherapy (minor modifications) achieve a higher complete remission rate, longer EFS and OS compared to patients who receive upfront-reduced dose chemotherapy. However, more data from a prospective study on the most common malignancies in patients with A-T and NBS is urgently needed. **Conclusions.** The occurrence of malignancies in patients with A-T and NBS is high (20-40% and 50%, respectively). The most commonly encountered malignancies are B and T-cell NHL, T-cell leukaemia, lymphoblastic lymphoma and Hodgkin lymphoma. Treatment related toxicity is high, however with close monitoring and adequate supportive care, the number of treatment related deaths seems relatively small. Upfront standard dose chemotherapy seems to result in a higher CR percentage, and a longer median EFS and OS compared to reduced chemotherapy. More data collected from prospective clinical trials on common malignancies in patients with A-T and NBS are necessary.

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**PRELIMINARY RESULTS OF A MULTICENTER BFM-BASED STUDY FOR B-CELL MALIGNANCIES IN CENTRAL AMERICA**

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**Background.** The outcome of children with mature B-cell malignancies (BCM) in our region was dismal before the creation of pediatric cancer units (PCUs) in the 1990s. Even after PCUs were developed, the use of unmodified protocols from developed countries resulted in unacceptable treatment-related morbidity and mortality. AHOPCA is a collaborative group that provides evidence based therapy to children with malignant diseases in Central America. **Objective.** The aim of this study was to increase survival of children with BCM and reduce toxicity by introducing a modified BFM-based regimen adapted to our local resources. **Methods.** Treatment intensity was adapted according to the risk of relapse using BFM risk groups. The methotrexate dose was reduced in blocks A and B to 1 or 3 g/m<sup>2</sup> in 3 hour infusion according to the patient's risk. The ifosfamide dose was reduced by half in block A. Block CC was omitted. Data were collected prospectively in www.POND4kids.org, a free web-based pediatric oncology database. All cases were presented weekly on www.Cure4kids.org, a free tool that provides web-conferencing technology, allowing for peer discussions among Central American colleagues and external experts. **Results.** From September 2004 to February 2009, 114 evaluable patients with a median age of 5.5 years were treated on the protocol. One hundred and eight had Burkitt (or Burkitt-like) lymphoma and 6 had large B-cell lymphoma. Thirteen patients had stage I-II, 86 stage III, and 15 stage IV (mature B-ALL). With a median follow up of 24 months, the 2-year overall survival for the whole group is 83% (4% SE) [(100% for stages I-II), 85% (4% SE) for stage III and 64% (13% SE) for stage IV]. Seven patients died of treatment-related toxicity, 10 relapsed (5 died of disease, one abandoned and 4 are still alive) and 8 abandoned therapy after 3 to 24 weeks. **Conclusions.** Survival rates of children with BCM in Central America have improved substantially with the implementation of a reduced-intensity BFM-based regimen, weekly on-line discussion of each patient, pathology support for second opinions and adequate data management. Current efforts focus on reducing toxic death and abandonment to further improve outcomes.

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**PRETREATMENT PLASMA FOLATE MODULATES THE PHARMACODYNAMIC EFFECT OF SYSTEMIC MTX IN CHILDREN WITH NHL AND ALL: "FOLATE OVER RESCUE" CONCEPT REVISITED**

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**Background.** Systemic MTX remains a key component of therapy for childhood NHL and ALL; however, the optimal MTX dose, and the need for folinic acid rescue remain an issue. CCG study 5971 (ASH 2008) showed that MTX at 5x4 g/m<sup>2</sup> in BFM protocol M was no better than oral MTX 20 mg/m<sup>2</sup>/wk for children with lymphoblastic NHL. **Objectives.** To evaluate the influence of pretreatment plasma folate concentrations in children with NHL and ALL treated with high-dose methotrexate, we assessed time profiles of plasma homocysteine, folate, in children treated HD MTX and leukovorin rescue. To compare pharmacokinetic data of HD-MTX to so called "Capizzi" MTX, time profiles of plasma MTX levels, homocysteine and folate were analyzed. **Methods.** We analyzed 100 treatment courses of HD MTX and 50 courses of low dose MTX given with no leukovorin rescue. The study endpoints were to determine how methotrexate exposure is translated to homocysteine accumulation and whether it is influenced by pretreatment plasma folate. **Results.** For children treated with HD MTX peak concentrations of homocysteine increased from the start of the intravenous infusion through cessation of MTX therapy up to time point t42, when this trend was reversed by administration of folinic acid. The area under the curve (AUC) for plasma homocysteine showed decreasing course-to-course tendencies with a statistically significant decrease only between courses 1 and 2, indicating decreased whole body homocysteine accumulation in response to unchanged exposure of consecutive MTX courses. Therapeutic courses with low initial folate concentrations (<10 nmol/L) gave significantly higher responses in homocysteine accumulation expressed both as hcysAUC0-66 h and the peak t42 homocysteine concentrations than did courses with initial folate >10 nmol/L. Escalating low doses of Capizzi MTX without leukovorin rescue showed antifolate effect measured by homocystein AUC comparable with HD MTX given with low initial folate, and subsequent courses lead to increased homocystein AUC. **Conclusion.** Endogenous pretreatment plasma folate modulates the magnitude of the MTX effect, providing support for a "folate over rescue" concept. The optimal dosing of systemic MTX for children with NHL and ALL, despite 6 decades of clinical experience, still remains to be established.

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**HSP90-DEPENDENT STABILIZATION OF ACTIVE NPM-ALK KINASE: STRUCTURAL INSIGHTS BY MUTAGENESIS OF THE ALK CATALYTIC DOMAIN**

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**Background.** Among the posttranslational modifications occurring in eukaryotic cells, phosphorylation is the most critical for proteins, as phosphate-mediated conformational switching of catalytic and non-catalytic motifs impact expression and activity through changes in structure stability. Activation by phosphorylation is the rule for protein kinases, including oncogenic protein tyrosine kinase NPM-ALK in Anaplastic Large Cell Lymphomas, though it renders the kinase prone to degradation. For several of them, but not all however, the maintenance of functional stability is provided by the molecular chaperone Hsp90, through the recognition of specific motifs within the catalytic domain. **Objectives.** To determine whether NPM-ALK kinase activity facilitated Hsp90 binding, and whether structural motifs within NPM-ALK catalytic domain favor or impair Hsp90 interaction. **Design/Methods.** To test this hypothesis we prepared a series of N- and C-terminal deletion mutants of wild-type NPM-ALK, as well as inactive point mutants by site-directed mutagenesis of the NPM-ALK ATP-binding site and activation loop. **Results.** Using NPM-ALK constructs in COS7 cells we showed that Hsp90 interacted with wild-type NPM-ALK, but failed to co-adsorb with inactive point mutants and with deletion mutants lacking the entire kinase domain, despite the comparable transcription and expression levels. In contrast, binding to Hsp90 family members Hsc70 and Hsp70 was independent of NPM-ALK activity, suggesting a greater dependence of active NPM-ALK on Hsp90 function. Accordingly, phosphorylated NPM-ALK associated with Hsp90 in ALCL cells Karpas299, and pharmacological inhibition of NPM-ALK interrupted such an interaction, as well as the association with the Hsp90 co-chaperone p50cdc37. However, deletions mutants unmasking the  $\alpha$ C-helix motif in the N-terminal lobe of the catalytic domain maintained the ability to bind Hsp90, whereas mutants lacking  $\alpha$ C-helix not. **Conclusions.** Our findings suggest a model in which, in addition to a role in folding nascent NPM-ALK, Hsp90 provides an "ongoing folding support" to the active kinase, by protecting it from degradation following recognition of specific unstable motifs ( $\alpha$ C-helix) within its catalytic domain.

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**POST ORGAN TRANSPLANTATION LYMPHOPROLIFERATIVE DISEASE (PTLD). A SINGLE INSTITUTION EXPERIENCE**

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**Background.** PTLD is a known complication of immunosuppressive therapy accompanying organ transplantations.

PTLD comprises a complex spectrum of lymphoid proliferation ranging from reactive lymphoid hyperplasia to polyclonal expansion to overt monoclonal malignant lymphoma. We report our institutional experience with PTLD over the last 10 years. **Objectives.** To study the clinico-pathological features, treatment and outcome of patients with PTLD who failed withdrawal of immunosuppression. **Methods.** Retrospective review of patients who were diagnosed with PTLD from 1/1999 to 12/2008 to determine the clinical, pathological features, treatment and outcome. Two patients who developed T-PTLD were excluded from this study. **Results.** Of 6 patients who failed withdrawal of immunosuppression for PTLD, 5 are alive without evidence of recurrence of recurrent PTLD and without graft rejection. All patients had EBV positive PTLD. Two patients had fulminant PTLD and both were successfully treated with combination of low dose chemotherapy and Rituximab. Patient number 5 had monomorphic B cell PTLD (Diffuse Large B cell Lymphoma). After initial response he progressed and failed aggressive chemotherapy and surgical resection. The other 5 patients had polymorphic-B PTLD. **Conclusion.** The outcome of fulminant PTLD cases in our series is excellent. Despite the small size of this experience this may suggest that combination therapy is effective in aggressive form of PTLD. Therapy was also successful in the 2 cases where Rituximab alone was used. The role of combination therapy in non fulminant PTLD cases is not determined.

**Table. PTLD-Patients' characteristics**

#	Organ transplanted	Age <sup>a</sup> at Transplant	Time <sup>a</sup> to PTLD	Follow up After PTLD <sup>a</sup>	Site of PTLD	Type of PTLD	Treatment of PTLD
1	Liver	1.0	1.8	5.9	Lung, LN	Fulminant	Combination
2	Heart	0.1	5.7	10.8	Lung		Rituximab
3	Liver	0.9	1.0	6.0	Lung		Rituximab
4	Liver	1.0	1.8	5.6	Lung, LN, Mediastinum	Fulminant	Combination
5	Kidney	10.7	4.0	Died of progressive PTLD	Allograft aorto-caval nodes		1. Combination 2. B-NHL therapy 3. Resection
6	Liver	1.0	6.8	7.4	Stomach, small intestine		Combination

<sup>a</sup> In years

\*Combination: Cytosan 600 mg/m<sup>2</sup> day1, Prednisone 2 mg/kg/day1-5. Both given every 21 days for a total of 6 cycles. Rituximab 375 mg/m<sup>2</sup> every week for 6 consecutive weeks.

\*\* Rituximab 375 mg/m<sup>2</sup> every week for 6 consecutive weeks.

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**MANAGEMENT OF THE AFRICAN BURKITT'S LYMPHOMA: THE KCMC EXPERIENCE**

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**Background.** Burkitt's Lymphoma was first identified in Africa, where there is every reason to believe that it has existed for millennia. It's presence prior to it's description by Europeans is attested to by wooden masks depicting jaw and orbital tumours. Denis Burkitt (1958), a missionary surgeon working in Uganda, reported on a clustering of lymphomas in central African children; the lymphomas followed the distribution of holoendemic malaria in these areas. When Denis Burkitt did his research in Uganda about 50 years ago

he travelled as well to neighbour countries like Tanzania. Burkitt's Lymphoma (BL) accounts for more than 50% of childhood cancer in this very poor sub-Saharan African country with a gross national product (GNP) per capita of 230 USD and a health expenditure of 3 USD per inhabitant per annum. But until now childhood malignancies are not of high priority within the Tanzanian health-system. And therefore there is paucity of diagnostic and treatment facilities. Patient's lack of money to pay and long distances to the respective centre means, that many cancers must remain undiagnosed and/or untreated. *Objectives.* When we started our project at KCMC there were no proper facilities and logistics for cancer treatment available. The biggest obstacles were: Lack of appropriate hospital infrastructure like diagnostic and therapeutic possibilities. No treatment protocols, no sustainable drug supply, no trained staff and a high number of treatment interruptions. *Methods.* According to the SIOP guidelines we started to establish a so called paediatric cancer unit (PCU) in our hospital. It starts with infrastructure and logistics, contains diagnostic and therapeutic procedures, encourages patients and staff in communication and training and last but not least gives academic staff a good opportunity to perform research and publications. *Results.* The implementation of a PCU in our hospital showed improvement in the following areas: time to establish diagnosis shortened; reduction of treatment related complications; raised adherence to therapy; good treatment results; raised staff and patients satisfactory; better qualified personnel; binding treatment protocols; pretherapeutic staging compulsory; professional medical and nursing care; better data management; collaboration with other centers in the region. *Conclusion.* There is good evidence for offering anti-cancer treatment for children in resource poor settings. Particularly BL is a good example for the effectiveness of a relatively simple cytotoxic treatment with acceptable side effects, low costs and a good outcome.

#### 156 THE ROLE OF FDG-PET/CT IN FOLLOW-UP OF CHILDREN OF CHILDREN WITH LYMPHOMA AND OSTEOSARCOMA

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*Background.* Positron emission tomography using 18F-fluorodeoxy-glucose (FDG-PET) is considered as an excellent tool for monitoring disease status in patients suffering from lymphoma. It can be able to detect occult disease missed by conventional methods. *Methods.* We retrospectively reviewed results of PET/CT scans performed after completing of therapy in 30 children with lymphoma (16 Hodgkin lymphoma and 14 non-Hodgkin lymphoma). 11 females and 19 males were examined. The median age of the patients at diagnosis was 13.7 years. The median follow up time after ending the treatment was 23.1 months. 69 examinations were carried out and analysed. *Results.* 42 scans showed a complete metabolic remission and all clinical investigations proved a complete remission of the malignant disease. From the 27 positive PET/CT scans 9 were false positive (histological examination negative or during closed follow up no progres-

sion). The other 18 positive scans detected residual tumor mass after therapy or relapse/progressive disease. The negative predictive value (NPV) of PET/CT was 100%. However, the positive predictive value (PPV) was 66%. *Conclusion.* According to our data a negative PET/CT scan during routine follow up for children with lymphoma strongly suggests absence of the malignant disease but a positive PET/CT scan has a lower PPV and should be interpreted with caution. Timing of scan in relation to chemotherapy and growth factors is of big importance. Large prospective studies are needed to appreciate the PET/CT's real impact on patient management.

#### 157 CLINICAL FEATURES AND TREATMENT RESULTS OF POST-TRANSPLANT LYMPHOPROLIFERATIVE DISORDER FOLLOWING ORTHOTOPIC LIVER TRANSPLANTATION IN CHINESE CHILDREN

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*Objectives.* To review the clinical features and treatment outcome of post-transplant lymphoproliferative disorder (PTLD) in Chinese children. *Design/Methods.* Retrospective review into clinical presentation and treatment results of consecutive cases of childhood PTLD diagnosed post-liver transplantation in an institutional liver transplantation centre of Hong Kong. *Results.* Ten cases of PTLD occurring in children under 18 years of age following liver transplantation were identified between 1999-2008. The cohort consisted of 5 males and 5 females with median age at diagnosis of 2.25 years (range 0.8 – 16.8 years). Nine children had congenital biliary atresia with failed Kasai operation and underwent orthotopic liver transplantation either from living related or cadaveric donor at or before one year of age. One adolescent boy (16.8 years) had refractory systemic lupus erythematosus and underwent autologous bone marrow transplantation. Time of transplantation to diagnosis of PTLD ranged from 2 to 22 months with median of 10 months. The time of onset of symptoms to date of diagnosis could be delayed for six months due to protracted clinical course and non-specific symptoms. The clinical presentation ranged from protracted infectious mononucleosis (IM)-like picture to frank aggressive lymphoma that developed within 3 months of liver transplantation. Histology of PTLD showed wide spectrum from IM-like or plasmacytic hyperplasia to polymorphous or monomorphous lymphoma-type lesions. All cases had B cell proliferations except one case with haemophagocytic lymphohistiocytosis (HLH) and detection of Epstein-Barr virus-infected CD8<sup>+</sup> T cells. All cases strongly expressed EBV-encoded small RNAs in the atypical lymphoid cells. Extranodal sites such as abdominal masses, gastrointestinal tract, nasopharynx and oral alveolar mucosa as well as lymph nodes and liver allograft could be involved. Rituximab was administered at 375 mg/m<sup>2</sup> IV at weekly interval for 4 consecutive weeks in 7 of 10 patients and all treated cases achieved CR. One case of frank DLBCL had additional COP chemotherapy. The other three cases died of rapidly progressive disease before any definitive treatment. Side effects mainly included infusion reactions which could present as severe

anaphylactoid reactions. *Conclusion.* PTLD in liver transplantation in children had diverse clinical presentation and histologic types and showed good response to treatment with rituximab.

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### **WAVE1 AS A NOVEL ANTI-APOPTOTIC PROTEIN IN LEUKEMIA CELLS**

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*Background and Objectives.* Apoptosis has now been widely accepted as a prominent tumor-suppression mechanism. Bcl-2 proteins are over-expression in many tumors, and are critically important for cell survival. The anti-apoptotic activities are determined by intracellular localization and post-translational modifications. WASP-family verprolin-homologous protein 1(WAVE1), a novel member of the actin regulatory protein, is a key regulator of actin polymerization and cytoskeleton reorganization in cell. Cytoskeleton reorganization is an important regulator of apoptotic cell death. The objective of this study was to investigate the relationship between WAVE1 and Bcl-2. *Methods.* 1. The expression of WAVE1 was detected in human blood cancer cell lines and non-blood cancer cell lines by Western blotting analysis. 2. The apoptosis of blood cancer cell lines was detected by morphologic analysis and flow cytometric analysis. 3. After enhanced expression of WAVE1 by gene transfection or suppression of WAVE1 expression by RNA interference in leukemia cells, the relationship between WAVE1 and Bcl-2 was investigated by Immunoprecipitation analysis, Caspase activity assay, Measurement of intracellular ROS and calcium. *Results.* We showed that WAVE1 was over-expressed in blood cancer cell lines, and functioned as a negative regulator of apoptosis. Further enhanced expression of WAVE1 by gene transfection rendered leukemia cells more resistant to anti-cancer drug-induced apoptosis; whereas suppression of WAVE1 expression by RNA interference restored leukemia cell's sensitivity to anti-drug-induced apoptosis. WAVE1 was found to be associated with mitochondrial Bcl-2, and its depletion led to mitochondrial release of Bcl-2, and phosphorylation of ASK1/JNK and Bcl-2. Furthermore, depletion of WAVE1 expression increased anti-cancer drug-induced production of reactive oxygen species in leukemia cells. *Conclusion.* The results suggest WAVE1 as a novel regulator of apoptosis, and potential drug target for therapeutic intervention of leukemia.

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### **NON-HODGKIN LYMPHOMA IN THAI PEDIATRIC PATIENTS: HISTOLOGICAL DISTRIBUTION, CLINICAL FEATURES, AND OUTCOME IN A SINGLE INSTITUTE**

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*Background.* Non-Hodgkin lymphoma (NHL) is the third most common childhood malignancy. In Thailand, there is no survival analysis report of childhood NHL yet. *Objective.* We would like to study clinical features and outcome of pediatric patients with NHL in a single institute. *Methods.* Newly diagnosed pediatric patients with NHL from Jan 1998 to Dec

2007 at our center were studied. Demographic data, pathological report, treatment protocol and clinical features were collected from the medical records. Stage I, II lymphoblastic lymphoma (LL) and large cell lymphoma (LCL) patients were treated with 6 cycles of cyclophosphamide (CTX), doxorubicin, vincristine and prednisolone (CHOP). LL patients were given orally with 6-mercaptopurine and methotrexate (MTX) for 6 months after CHOP. LL stage III, IV patients were treated with childhood acute lymphoblastic leukemia protocol. For stage III, IV LCL and all peripheral T-cell Lymphoma (PTCL) patients, they were treated with alternating A and B cycle; A: CTX, etoposide, dexamethasone and high dose MTX (HDMTX), B: ara-C, carboplatin, dexamethasone and HDMTX. Among B-cell lymphoma (BL) patients, LMB89 protocol was adopted for this group. Overall survival (OS) and event-free survival (EFS) were estimated by the Kaplan-Meier method and compared using the Wilcoxon test. **Results.** Sixty-one patients were enrolled; 67% male. Median age at diagnosis and follow-up time were 10.8 years (range 1.9 to 15.0) and 51.9 months (range 0.4-126.5), respectively. The pathological diagnosis distribution was 37.70% LCL, 22.95% PTCL, 19.67% LL and 19.67% BL. The 5-year OS and EFS of all patients were 67.72% (95%CI: 54.03-78.13%) and 61.00% (95%CI: 47.23-72.20%), respectively. The 5 year-OS of female patients; 84.38% (95%CI: 58.87-94.70%) was superior to male; 59.49% (95%CI: 42.39-73.03%),  $p=0.04$ . For BL and LL groups, 5-year OS were 91.67% (95%CI: 53.90-98.78%) and 83.33% (95%CI: 48.17-95.55%), respectively while those of LCL and PTCL groups were 55.34% (95%CI: 32.65-73.14%) and 57.14% (95%CI: 28.40-77.97%), respectively. However, there was no difference in survival among these groups,  $p=0.10$ . **Conclusion.** It seemed to be high prevalent in PTCL among patients in our center, a single tertiary care center in Thailand, compared to those from western countries. BL and LL groups might have better survival than LCL and PTCL groups.

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##### **DIFFUSE LARGE B-CELL LYMPHOMA IN SEVEN YEARS-OLD BOY WITH CYSTIC FIBROSIS TREATED WITH CHEMOTHERAPY AND RITUXIMAB**

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**Background.** Cystic Fibrosis (CF) is a rare hereditary disease affecting exocrine glands causing morbidity and mortality. CF patients have an increased risk to develop carcinoma of the gastrointestinal tract, whereas other malignancies occur at a similar rate as in the general population. More specifically, the risk for lymphoma is not increased in non lung-transplanted CF patients. **Objectives.** We present a case of diffuse large B-cell lymphoma in seven years-old boy with CF, and discuss this un-common case with the relevant literature. **Design and Methods.** A seven years old boy was admitted to our hospital with complaints of abdominal pain, nausea and vomiting. From his medical history it has been learned that the patient was diagnosed as CF when he was two-

months-old. The initial physical examination demonstrated hepatosplenomegaly and an abdominal mass. The radiological studies showed 7x8 cm lymphadenomegaly originating from the ileum, and multiple nodules at liver and kidneys. A HRCT of the chest revealed bronchiectasis but no evidence of mediastinal involvement. The patient underwent laparotomy and resection of intestine was performed. The histology revealed a diffuse large B-cell lymphoma. Extensive diagnostic workup showed no involvement of CNS or bone marrow. The patient was diagnosed as stage III and treated with NHL-BFM 90 with combination of 6 doses of intravenous rituximab at dose of 375 mg/m<sup>2</sup>. The patient vorphase and course AA and Course BB. After the planned chemotherapy the patient was evaluated as complete remission. **Results.** The co-existing of CF and Non-Hodgkin Lymphoma is rarely encountered. According to our knowledge this is the first case that has been successfully treated with chemotherapy in addition with rituximab in the English medical literature. **Conclusion.** The treatment related complication is naturally high in patients suffering cancer with another chronic disease. Targeted therapy regimes with close follow up seem safer for selective cases.

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##### **TREATMENT OF CHILDHOOD NON-HODGKIN'S LYMPHOMAS WITH NHL-BFM 90/95 PROTOCOLS**

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**Background.** Non-Hodgkin's lymphomas (NHL) account for approximately 10-12% of all malignancies in childhood and adolescents. Investigators of most clinical trials report survival estimates approaching 90%. **Method.** From 1994 through to December 2008, 224 children aged 2-18 with newly diagnosed NHL have received therapy based on BFM 90/95 protocols. Diagnosis of NHL was performed on biopsy material, which was studied for specific characteristics, including histology and immunophenotype. **Results.** In our study B-NHL was diagnosed in 148(66%) pts, in other cases-non-B-NHL (pre-T, T-cell, pre-pre-B, pre-B-cell). In 96(65%) cases B-NHL-III-IV stage, LDG level was more 500 ED/l in 65,5%. Distribution according BFM risk group was: R1 26(17,6%), R2 30(20,3%), R3 36(24,3%), R4 56(37,8%). Complete response (CR) was obtained in 129(87,2%) pts; 19pts were non-responder. Early relapse (within 6 months after finish of treatment) was noted in 10(7,8%) pts. 31(20,9%) pts died: 20(13,5%) due to progression of NHL, 11(7,4%) - infectious complications. The 5-year OS was 0,92±0,02. 5-RFS was 0,8±0,03. The main prognostic factors were stage, histology and response to treatment. 5-RFS rate was 0,96±0,02 for Burkitt's lymphoma and 0,86±0,06 for DLBCL ( $p=0,05$ ), for I and IIR stages - 1,0, III - 0,91±0,04, IV - 0,78±0,09 ( $p=0,01$ ), for pts who achieved CR after resection of tumor, prephase or block I - 1,0, after the second block - 0,9±0,05, after the third - 0,69±0,13, after the fourth - 0,67±0,27 ( $p=0$ ). Non-B-NHL was diagnosed in 76 (34%) pts, most of them (94,8%) had III-IV stages. Bone marrow involvement was diagnosed in 52,6%. Distribution according

BFM risk group was: SR 3(4%) pts, MR 58 (76,3%), HR 15 (19,7%). CR was achieved in 75 (98,7%) pts, in one child was noted progression of NHL after protocol M, in 8(10,7%) was diagnosed relapse. 9(11,8%) pts died from disease progression. 5-OS rate for non-B-NHL was  $0,90\pm 0,04$ , 5-RFS -  $0,88\pm 0,04$ . *Conclusion.* Therefore, NHL-BFM 90/95 protocols have confirmed its high cure rates in children with NHL. Further studies may consider new approaches to treat resistant and relapsing cases.

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### **NK/T CELL LYMPHOMA PROVOKING AN EMBRYONAL RHABDOMYOSARCOMA STATE**

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A 3 years old girl was hospitalized because of difficulty in breathing through her nose, exophthalmos of her right eye, suppurative secretion flew out of her right eye, the upper eyelid was bluish. Having made magnetic resonance imaging (MRI) of the paranasal sinus, the tumour was found in the right nasal meatus which was spreading to the sphenoid and to the right orbit. Histopathological findings were extranodal NK/T cell lymphoma nasal type. The patient was treated according to CHOP chemotherapy and after the first course exophthalmos decreased, the patient began to breathe through her nose easier, the tumour diminished. The patient received 6 CHOP chemotherapy courses. In spite of improvement, when the treatment was stopped, after a month and a half the disease was progressing. The tumor biopsy was made again, Histological findings: embryonal rhabdomyosarcoma. The patient was treated with chemotherapy according to CWS-2002P (High Risk Group) and radiotherapy, but the disease was progressing and the patient died. We suppose that the primary histological diagnosis was not precise and received changes were evaluated as reaction of the organism to oncological process (rhabdomyosarcoma).

