Session 9: pediatric lymphoma

088 WITH THE LMB AND BFM PROTOCOLS, CHILDREN AND ADOLESCENTS WITH B-CELL NON HODGKIN'S LYMPHOMA AND MATURE B-CELL LEUKEMIA HAVE SIMILAR SURVIVAL

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Purpose: Data of the 2 more recent French LMB and German-Austrian-Switzerland-Czech NHL-BFM studies were pooled to analyse and compare their results. In LMB, patients are stratified in 3 risk groups (A, B, C) depending on resection, stage and CNS involvement, and receive 2, 4/5 or 8 courses of chemotherapy. In BFM, patients are stratified in 4 groups (R1, R2, R3, R4) adding LDH level in the stratification and receive 2, 4, 5 or 6 courses. Although treatment schemes and drug dosages are different in the 2 regimens, drugs are the same: high dose methotrexate, corticosteroid, vincristine, cyclophosphamide (+/-ifosfamide), ara-C (HD in advanced stages (st)), doxorubicine, and +/-VP16.

Method: Data of the BFM 95 (Blood 2005), the ongoing 04 studies, the SFCE part of the FABLMB 96 (Blood 2007, BJH 2008) and the ongoing LMB 2001/03 studies were merged.

Results: There were 935 patients in the BFM (04/96-12/05) and 691 patients in the LMB (07/96-12/05) studies, and a total of 42 PMLBL. For the non PMLBL results are given for BFM and LMB in this order. 4y EFS was 89% (n=914) and 90% (n=670) respectively. By st, 4y EFS was: 97% (n=96) and 98% (n=58) for st1, 98% (n=228) and 96% (n=114) for st2, 88% (n=373) and 92% (n=285) for st3, 76% (n=71) and 85% (n=87) for st4, 81% (n=146) and 81% (n=126) for B-AL, 72% and 79% for the CNS+. For the higher risk patients, defined as st3 with high LDH level (>500 inBFM or >twice the upper normal value in LMB), st4 or B-AL, 4y EFS was 83% (n=406) and 85% (n=366). All results were NS.

Conclusion: These 2 regimens developed in parallel since 1981 using same drugs obtain similar results. This encourages an international collaboration, especially addressing the question of rituximab in higher risk patients.

089 IN CHILDHOOD B-CELL NON HODGKIN'S LYMPHOMA (B-NHL) AND MATURE B-CELL ACUTE LEUKEMIA (B-AL) WITH CNS DISEASE AT DIAGNOSIS, PATIENTS WITH BLASTS IN CSF ARE AT HIGHER RISK OF EVENT

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CNS involvement (CNS+) is known to be a factor of bad prognosis in B-NHL and B-AL. To investigate who are higher risk pts, data of the French CNS+ pts registered in LMB89, FABLMB96 and on going LMB2001 studies were analysed. CNS+ was defined by: any non doubtful blasts in CSF (CSF+), cranial nerve palsy, intracerebral mass or parameningeal extension, and intraspinal mass with cord compression symptoms. CNS+ pts were treated in groupC regimen: after a prephase, pts received 2 COPADM including HDMTX 8g/m^2, 2 CYVE consolidation with HDAra-C and VP16 and 4 maintenance courses. Cranial irradiation in LMB89 was further replaced by 2IT and one HDMTX in consolidation. In LMB96, early responding pts were randomised after COPADM2 to receive standard CYVE or reduced dose miniCYVE (arm C2). In LMB2001, HDMTX was infused in 24h vs 4h in previous studies.

Results: 189 pts were CNS+: 67 in LMB89, 53 in FABLMB96 and 69 in LMB2001 (12%, 14% and 13% of all pts). Of those, 37 (55%), 26 (50%) and 19 (28%) were CSF+ respectively. In pooled LMB89+2001 data, 3yEFS was significantly lower for CSF+ vs CSF- pts: 71% (n=56) vs 92% (n=80) (p=0.003). Because of significantly inferior results observed in arm C2 (Cairo, Blood 2007), in order to be able to analyse data of the 3 studies together, we selected the early responding pts receiving the standard groupC treatment: in the pooled data of the 3 studies, 3yEFS was 75% (n=62) for CSF+ vs 92% (n=87) for CSF- pts (p=0.02).

Conclusion: Among CNS+ pts, pts with blasts in the CSF are at higher risk of events and should benefit from new treatment modalities

090 SAFETY AND PHARMACOKINETICS (PK) OF RITUXIMAB (R) IN COMBINATION WITH FAB CHEMOTHERAPY IN CHILDREN AND ADOLESCENTS (C+A) WITH STAGE III/IV MATURE B-NHL: A CHILDREN'S ONCOLOGY GROUP REPORT

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Introduction/Background: The FAB/LMB96 trial demonstrated the safe reduction of chemotherapy intensity in C+A with intermediate risk Stage III/IV B-NHL (2 yr EFS 84%) (Patte/Cairo et al, Blood, 2007). R has significantly improved EFS and OS in adults with DLBCL (Coiffier et al, NEJM, 2002). Dose dense R dosing has been shown to result in sustained levels of R (Zwick et al, Semin Hematol, 2010) while demonstrating effective responses when compared to R dosed every 21 days (Habermann et al, J Clin Oncol, 2006). A recent murine model demonstrated an inverse relationship between tumor burden and R concentration (Dayde et al, Blood, 2009) suggesting pediatric B-NHL patients, who frequently present with high tumor burden, may benefit from a dose dense approach. The study objective was to determine PK and safety following addition of R to FAB B4 chemotherapy in a dose dense manner in C+A with Stage III/IV mature B-NHL.

Material/Methods: R (375mg/m^2), generously supplied by Genentech, was administered to patients with Stage III/IV mature B-NHL receiving FAB B4 chemotherapy. Subpilot patients received R (4 doses) on days -2 and 0 of COPADM2 and day 0 of CYM 1+2. Pilot patients also received R on days -2 and 0 of COPADM1 (6 doses). In a subset of patients, serum R levels were measured by ELISA using a polyclonal goat anti-R antibody conjugated to horseradish peroxidase (detection limit 0.5μg/mL) 1h prior (trough) and 30-60min after (peak) each R dose in COPADM 1+2 as well as 1, 3, 6 and 9 mo after the last R dose for estimating t½.

Results: Forty-eight patients received 274 R infusions. No SAE attributed to R occurred and no HACA were detected. PK samples were obtained for 22 patients. Serum R levels were as reported in Table 1.

Table 1. Serum R levels obtained 1h prior and 30-60min after each R dose during COPADM 1+2 as well as 1, 3, 6 and 9 months following the last R dose.

	N	Mean (μg/mL)	SEM
Trough 1	21	0	0
Peak 1	17	207.35	9.57
Trough 2	20	135.35	12.6
Peak 2	18	309.11	21.46
Trough 3	16	77.5	14.41
Peak 3	15	266	16.01
Trough 4	17	188.41	11.83
Peak 4	15	383.53	24.54
1mo	20	74.99	8.37
3mo	16	16.8	2.84
6mo	18	2.23	0.55
9mo	14	0.55	0.25

The t½ of R was 29±7 days. Younger children (age <13y) demonstrated higher peak values but similar trough levels and a shorter t½.

Conclusion: R can be safely added to FAB B4 chemotherapy with R peak/trough levels and t½ similar to those seen in adults. A dose dense approach can be safely utilized to achieve high R peak levels with sustained troughs despite high tumor burden. Our results also suggest younger children tend to achieve higher R peaks with a higher rate of clearance supporting the continued use of BSA based R dosing in pediatrics.

091 ANALYSIS OF TREATMENT FAILURE IN PATIENTS TREATED FOR NHL-B /B-ALL IN POLISH PEDIATRIC LEUKEMIA/LYMPHOMA STUDY GROUP

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Since B-NHL BFM 04 protocol was introduced in PPLLSG in 2004, lower EFS and OS results are obtained (NHL-B + B-ALL: OS=84,5±3,9%, EFS=79,3±4,1%; NHL-B: OS=89,9±3,8%, EFS=85,9±4,0%; B-ALL: OS=53,6±12,3%' EFS=40,2±12,4%), comparing to results reported by BFM (NHL-B + B-ALL: EFS=0,89%, B-ALL: EFS=0,81%) and LMB (NHL-B + B-ALL: EFS=0,90%, B-ALL: EFS=0,81%).

Since 2004, 131 pts were enrolled in the protocol, including 78 with Burkitt lymphoma (60%), 19 with DLBCL (15%), 2 with PMLBCL (1%), 20 with B-ALL (15%) and 12 with others (9%). The predominant primary site of the disease was abdomen - 51 (39 %) pts. 73% of patients presented with localized disease: st I – 5 (4%), st II – 29 (22%), st III – 61(47%) and 27% with disseminated disease: st IV –19 (14%) and B-ALL – 17 (13%). CNS involvement was diagnosed in 9 (7%) cases. Pre-treatment high LDH level was found in 57 (43%) cases. Pts were stratified in the following treatment groups: R1 – 2 (1%), R2 – 48 (37%), R3 – 26 (20%), R4 – 55 (42%).

CR was achieved in 103 patients (79%), 12 (60%) patients with B-ALL and 91 (82%) with NHL-B. Six patients (6%) relapsed - four with B-ALL, one with Burkitt lymphoma and one with PMLBCL. There were four isolated relapses (two CNS, one mediastinal, one testicular) and two with bone marrow involvement. In three cases of isolated relapses remission was achieved. Patients remain in RC (3 y, 2 y and 6 months respectively).

There were 15 deaths reported (11%) - seven in the NHL-B group and eight in B-ALL group. The main cause of death was lack of response to treatment (6 pts/4,5%); the other causes were the following: infectious complications (3 pts/3%), relapses (3 pts/3%), iatrogenic complications (venous thrombosis and pulmonary embolism post central catheterisation) (1pt/1%), late complications after completion of therapy (1pt/1%).

It seems that the main cause of treatment failure is a high number of patients not responding to chemotherapy. This group consists mostly of patients diagnosed with B-ALL and primary large tumor mass. It appears advisable to distinguish these patients as very high risk group, determine new prognostic factors for this group and establish more aggressive treatment.

092 EXCELLENT OUTCOME IN AGGRESSIVE NON HODGKIN'S LYMPHOMA IN ADOLESCENTS AND YOUNG ADULTS TREATED WITH CHOP-BASED REGIMENS IN DSHNHL TRIALS: AGE IS NOT A RISK FACTOR IN PATIENTS BELOW 50 YEARS

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Background: The IPI predicts outcome in aggressive lymphoma (aNHL); age > 60 yrs is a strong prognostic factor. It is not known if the age-dependen risk for OS (overall survival) and EFS (event free survival) increases in pts aged between 18 and 60 yrs. Adolescent pts are frequently treated with paediatric-type protocols, inducing a supposedly better outcome. However, biological differences such as the different proportion of non-mBL vs mBL (molecular Burkitts index) pts and genetic aberrations in different age groups (Klapper et al, 2009) may also account for these differences. In order to gain more insight into the age difference of outcome in young a NHL patients (pts), data from prospective trials of the DSHNHL were analyzed with a focus on age-dependent outcome.

Materials/Results: Patient data (age 18-60, n=2151) were retrieved from the following published DSHNHL trials: NHL-B1 (n=710,<60, LDH normal), MINT (n=821, IPI 0/1), High CHOEP phase II (n=119) and phase III (n=389, IPI 0/1), Mega CHOEP phase II (n=110, LDH elevated). 524 pts were < 35 yrs old. OS and EFS were calculated in pts 18-20 yrs (9.4%), and in 5-yr intervals > 20 yrs (21.6%, 31.9%, 37.2%). 82.4% had B cell histology. Mediastinal localization occured in 42% of pts <35 yrs. Treatment was CHOP/CHOEP based, rituximab was used in MINT only. In a multivariate analysis in NHLB1 pts < 35 yrs, stage III/IV (RR 3.4), ECOG>1 (RR 4.1) and etoposide use (RR 0.4) were significant risk factors, but none of the different age groups. This was similar for the other trials, and bulky disease was a risk factor in MINT. A larger dataset of 4165 pts aged 18-75 yrs was employed for a similar analysis, demonstrating that age dependent risk for similar IPI groups increases at age 50 approx. A martingale residual analysis in this cohort demonstrated that the risk for OS starts to increase at 45+ yrs, and the risk for EFS increases monotonously at 55+ yrs.

Conclusions: Outcome in all low-risk pts < 50 yrs is excellent with CHOP based protocols, with approx 90% OS @ 40 mths, and there is no difference in outcome between pts aged 18-20, 21-25, 26-30 and 31-40 yrs. Risk-dependent outcome seems similar to adolescent pts treated with paediatric protocols, e.g. in mediastinal lymphoma. Age dependent risk increases around 50 yrs of age. A matched pair analysis w/ paediatric-type treated pts, including biological risk factors as well as more rituximab data is warranted.

093 ALK TYROSINE KINASE INHIBITORS FOR THE TREATMENT OF NHL

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More than 15 different anaplastic lymphoma kinase (ALK) fusions (e.g., CLTC-ALK, multiple EML4-ALK variants, KIF5B-ALK, NPM-ALK, RanBP2-ALK, SEC31A-ALK, TFG-ALK, TPM4-ALK, and others) are now known to cause subsets of various malignancies including NHL (anaplastic large-cell lymphoma [ALK+ ALCL], diffuse large B-cell lymphoma [ALK+ DLBCL]), inflammatory myofibroblastic tumor (IMT), and non-small cell lung cancer (NSCLC). In addition, preliminary studies suggest that subsets of several other cancers may also express oncogenic ALK fusions that drive their development (e.g., breast, colorectal and esophageal cancers). Furthermore, point mutations that constitutively activate ALK have been identified as driver mutations in the pediatric malignancy neuroblastoma. Given the large variety of cancers caused by ALK deregulation, a number of pharmaceutical and biotech companies have ALK small-molecule inhibitors in development. Patients with ALK-driven cancers such as NSCLC and IMT treated with the ALK inhibitor PF-02341066 (crizotinib, Pfizer), which is currently in registration trials, have had marked antitumor responses including occasional complete remissions, indicating that these tumors are truly "addicted" to ALK. Preclinical ALK+ lymphoma models have demonstrated exquisite antitumor sensitivity to small-molecule inhibitors of the kinase, including apparent cures of even systemic lymphomatous involvement with ALK inhibitor monotherapy. To date, only a handful of ALK+ NHL patients have been treated with crizotinib but the responses reported in these few patients have also been quite marked, especially given that these are patients with lymphomas that have failed conventional therapies. Trials of crizotinib have recently begun recruitment of ALK+ NHL patients; thus, the efficacy of this ALK inhibitor in a controlled experimental clinical setting will soon be known. The availability of crizotinib – and of 2nd-generation ALK small-molecule inhibitors that are entering the clinic as well – promises to revolutionize the treatment of ALK+ NHL. However, considerable work will be required to determine the most beneficial role for such therapy including its timing, the preferred combination(s) of ALK inhibitors with conventional therapies, and the optimal strategy to combat the emergence of inhibitor resistance. Dr. Morris will present background regarding ALK and its pathogenic role in human cancers, an update on the current status of ALK inhibitor development, and will speculate as to the ultimate role for these inhibitors in the therapy of patients with ALK+ lymphomas.

094 ANTI-ALK ANTIBODIES IN CHILDREN WITH LYMPHOMA WITH VARIANT ALK TRANSLOCATIONS

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Introduction/Background: Autoantibody responses to the anaplastic lymphoma kinase (ALK) oncoantigen have been described in patients with nucleophosmin (NPM)-ALK positive anaplastic large cell lymphoma (ALCL). These antibody titres could be correlated with the risk of relapse (Ait-Tahar et al, Blood 2010). We now asked whether the presence of circulating ALK-specific antibodies was limited to patients with NPM-ALK-positive ALCL or could also be detected in those patients with ALK-positive ALCL or diffuse large B-cell lymphoma (DLBCL) patients whose tumours expressed variant ALK fusion partners.

Material and methods: 77 tumours from patients with the reference diagnosis of ALK-positive ALCL and 2 ALK-positive DLBCL were screened for the presence of *NPM-ALK* fusion by a *NPM-ALK*-specific RT-PCR technique or by fluorescence in situ hybridisation. Eleven patients lacked a detectable *NPM-ALK* fusion. Immunohistochemically, all displayed a characteristic cytoplasmically restricted ALK-staining pattern. The presence and magnitude of ALK-specific antibodies in their sera were analysed as previously described (Pulford et al, Blood 2000).

Results: The tumour cells of the 11 ALK-positive lymphoma-patients expressed the following variant ALK fusion partners: 9 ALCL with 2 ATIC-ALK, 1 MYH9-ALK, 3 TPM3-ALK, 3 variant ALK-partners not further classified; 2 plasmoblastic DLBCL with CLTC-ALK. Eight of the 9 patients with ALK-positive ALCL mounted an antibody response against ALK with titres ranging from 1/100 to 1/60750. No ALK autoantibodies were detected in the patient with the MYH9-ALK fusion. ALK-specific antibodies were detected in 1 of the 2 patients with CLTC-ALK positive DLBCL (titre: 1/6750). The 2 ALCL patients who relapsed among 8 patients with sufficient follow-up had low anti-ALK titres (1/100, 1/750). The DLBCL-patient with antibody response progressed during therapy.

Conclusion: Antibody responses against oncoantigenic ALK-fusion proteins are not restricted to NPM-ALK-positive ALCL but can be detected in patients with variant ALK-fusion partners in ALCL and DLBCL.

095 MOLECULAR CHARACTERIZATION OF MATURE B-CELL LYMPHOMAS IN CHILDREN: A COOPERATIVE STUDY OF MMML AND THE NHL-BFM GROUP

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Introduction/Background: Molecular profiling studies in follicular lymphoma (FL) and diffuse large-B-cell lymphoma (DLBCL) have been predominately performed in adult patients. Children with FL and DLBCL have a more favorable outcome but it is not known whether this is due to differences in host characteristics, treatment protocols or tumor biology. Immunoglobulin *(IG)* translocations are the hallmarks of several B-cell lymphoma types. The present study aimed at characterizing *IG* translocations in pediatric lymphomas.

Material and methods: In a collaborative study of the MMML network and the NHL-BFM study group pediatric mature B-cell lymphomas other than Burkitt lymphoma were investigated by FISH, LDI-PCR, immunohistochemistry, sequencing, gene expression profiling and array-CGH.

Results: FISH detected breakpoints in the *IGH* locus in 29/99 pediatric mature B-cell lymphomas, none carried an *IGH-BCL2* fusion. *IRF4* and *CBFA2T3* were identified as *IGH* partners by LDI-PCR in two and one of these lymphomas. FISH screening for chromosomal aberrations affecting these putative oncogenes revealed that these were recurrently involved in translocations, being *IG/IRF4* and *IGH/CBFA2T3* translocations present in 14 and 2 lymphomas. *IG/IRF4*-positivity was associated with a specific gene expression profile different from GCB and ABC. Moreover, the *CBFA2T3* gene was one of 123 genes significantly overexpressed in *IG/IRF4*-positive lymphomas.

Conclusions: Our data indicate that *IRF4* and *CBFA2T3* are novel recurrent oncogenic targets of *IGH* translocations. *IG/IRF4* positivity seems to define a novel subtype of lymphomas significantly associated with young age and a favorable outcome. Further studies have to show whether both, *IRF4* and *CBFA2T3*, interact in the pathogenesis of GC-derived lymphomas, particularly in children.

096 AMPLIFICATION OF CHROMOSOME 13Q31 IS ASSOCIATED WITH MIR-17 OVEREXPRESSION AND RELAPSE IN PEDIATRIC BURKITT LYMPHOMA

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Background: A subset of pediatric Burkitt lymphoma (pBL) patients have a deletion of the long arm of chromosome 13 [del(13q)], and these patients have a higher rate of relapse and a worse prognosis.The del13q region includes the microRNAs miR-15a and -16, but their role in the pathogenesis of pBL is not known.miR-17-92 at 13q31 is oncogenic in a mouse lymphoma model, amplified in a subset of human lymphomas, and also a target of MYC.We studied the relationship between copy number changes at these two loci, *MYC* expression, and miRNA expression in pBL.

Materials And Methods: Tumor (n=32) and germline reference (n=27, from staging bone marrows) DNA was isolated from archived formalin-fixed, paraffin-embedded (FFPE) tissue samples and submitted for Molecular Inversion Probe (MIP 330K Cancer Panel, Affymetrix) assay and data were analyzedfor genome-wide copy number. Relative levels of mRNA (also from FFPE) for miRNAs-15a, -16 and -17, and for *MYC*, were assayed by RT-PCR TaqMan assays. FISH using a probe targeting the 13q31 region was performed on FFPE sections of 9 cases of pBL.

Results: Genome wide copy number analysis was successfully performed on 28/32 samples. Amplification of 13q31 including the miR-17-92 locus was identified in 3/28 (11%) samples and was significantly associated with relapse (p=0.034). FISH confirmed the gain in 3/3 and was negative in 6 other samples that lacked this gain by MIP.2/28 samples (7%) showed copy number gain at 13q14.3, but deletions were not seen by MIP. Adequate RNA was isolated from 29/30 samples.miR-17 levels normalized to spleen RNA (1.0) ranged from 0.39-37.3 with a median of 9.96. The 3 samples with 13q31 locus gain had higher levels of miR-17 (29.9 vs. 10.4, $p<0.04$) than samples with normal copy number. However, 2 other samples had high levels of miR-17 (35.5 and 36.6) without copy number gain suggesting that there are other mechanisms for increased expression of miR-17. Relative levels of *MYC* mRNA varied over an approximately 27-fold range. Although miR-17-92 has been identified as a target of MYC in vitro, there was no correlation between *MYC* and miR-17 RNA in pBL tissues ($R^2=0.02$). Relative levels of miR-15a and miR-16 varied over an approximately 7-fold range and were strongly correlated with each other ($R^2=0.83$). One sample with copy number gain at 13q14.3 showed high levels of both miR-15a and -16 while the other did not.

Conclusions: Copy number gain of 13q31 occurs in a small subset of pBL cases and is associated with increased miR-17 expression and with relapse. Although described as a target of MYC, miR-17 levels did not correlate with levels of *MYC* message in pBL tissues. Gain of 13q14.3 was uncommon and not consistently associated with high levels of miR-15a and -16. Future studies will explore alternative mechanisms for regulation of the miRNAs and their roles in the pathogenesis of pBL, and will attempt to validate the association between 13q31 gain and relapse in a larger cohort of patients.

097 MINIMAL DISSEMINATED DISEASE/RESIDUAL DISEASE IN CHILDREN AND ADOLESCENTS WITH MATURE B-CELL NON-HODGKIN LYMPHOMA (B-NHL) MAY IMPACT THE RISK OF RELAPSE: A CHILDREN'S ONCOLOGY GROUP REPORT

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Background: Chromosomal aberrations and/or clonal immunoglobulin gene rearrangements found in minimal disseminated disease/residual disease (MDD/MRD) may identify children/adolescents with mature B-NHL as high-risk for relapse (Mussolin et al, JCO, 2007; Poirel/Cairo et al, Leukemia, 2009). A previously described method using tumor-specific primers effectively detected MDD/MRD (Shiramizu et al, JPHO, 2003) but a more efficient and clinically useful approach is warranted.

Methods: Children and adolescents with B-NHL were treated with FAB Group B4 (Patte/Cairo et al, Blood, 2007) and rituximab (Cairo/Goldman et al, ASCO 2010). Diagnostic and follow-up specimens (blood, marrow, or tumor) were assessed for MDD/MRD. Diagnostic/staging specimen DNA were screened for V_H family with the following primer pools: V_H1/V_H2; V_H3/V_H4; V_H5, V_H6/V_H7. For positive-MDD/MRD, individual V_H primers identified specific variable regions on follow-up specimens. If the follow-up specimen was negative, then rescreening with the other V_H family primers was performed to verify a true-negative result.

Results: Diagnostic tumor tissue was available from 12/35 Group B patients from whom each patient had initial staging specimens available; all screened positive and unique V_H family primers identified. Thirty-two of 33 patients were in clinical remission at end of therapy (EOT); had MDD/MRD-negative specimens; and confirmed on repeat screening with all V_H family primers. Two patients relapsed who had MDD/MRD-positive specimens (1 month and 3 months following induction therapy) suggesting lack of clearance of MDD/MRD prior to clinical relapse (4 and 36 months, respectively), p=0.002.

Conclusions: IgV_H primer pools were useful tools to assess MDD/MRD in children/adolescents with mature B-NHL specimens. Our findings expand upon B-cell malignancy subtypes that could potentially benefit from MDD/MRD assessment (Mussolin et al, Leukemia 2003). This study supports future investigations with a large cohort to assess the validity and clinical significance of MDD/MRD analysis with IgV_H primer pools in childhood and adolescent mature B-NHL. *(Supported by NIH CA121955; P20RR011091).*