Outcome and pathological classification of children and adolescents with mediastinal large B-cell lymphoma treated with FAB/LMB96 mature B-NHL therapy

Mary Gerrard1*, Ian M. Waxman2*, Richard Sposto3, Anne Auperin4, Sherrie L. Perkins5, Stanton Goldman6, Lauren Harrison7, Ross Pinkerton8, Keith McCarthy9, Martine Raphael10, Catherine Patte4**, and Mitchell S. Cairo7** on behalf of the FAB/LMB 96 International Study Committee11

1Sheffield Children’s Hospital, Sheffield, United Kingdom; 2Columbia University, New York, NY; 3Keck School of Medicine, University of Southern California, Los Angeles, CA; 4Institut Gustave Roussy, Paris, France; 5University of Utah Health Sciences Center, Salt Lake City, UT; 6Medical City Children’s Hospital, Dallas, TX; 7New York Medical College, Valhalla, NY; 8University of Queensland, Royal Children’s Hospital, Brisbane, Queensland, Australia; 9Gloucestershire Hospitals, National Health Service (NHS) Foundation Trust, Gloucestershire, United Kingdom; 10Centre Hospitalier Universitaire (CHU) Bicetre, Assistance Publique-Hopitaux de Paris (AP-HP, University Paris Sud 11, France; 11Children’s Oncology Group (COG), Arcadia, CA, USA; Societe Francaise d’Oncologie Pediatrique (SFOP), Paris, France; and the United Kingdom Children’s Cancer Study Group (UKCCSG), Leicester, United Kingdom

*Considered equal and primary first authors

**Considered equal senior authors

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Address correspondence and reprint requests to:

Mitchell S. Cairo, MD
Chief, Division of Pediatric Oncology, Hematology and Stem Cell Transplantation
Professor of Pediatrics, Medicine, Pathology, Microbiology and Immunology, and Cell Biology and Anatomy
Associate Chairman, Department of Pediatrics
New York Medical College
Munger Pavilion, Room 110
Valhalla, NY 10595
Phone: 914-594-3650
Email: mitchell_cairo@nymc.edu

For a complete list of FAB/LMB 96 International Study Committee members, please see the supplemental appendix.
Abstract

Mediastinal large B-cell lymphoma (MLBL) represents only 2% of mature B-cell non-Hodgkin lymphoma (B-NHL) in patients ≤18 years of age. Gene expression profiling demonstrates that MLBL in adults more closely resembles classical Hodgkin lymphoma than it does diffuse large B-cell lymphoma (DLBCL). We analyzed data from childhood and adolescent patients with Stage III MLBL (N=42) and non MLBL DLBCL (N=69) treated with Group B therapy on the FAB/LMB 96 study. Demographics of MLBL patients: M/F: 26/16; median age 15.7 yrs (12.5-19.7); LDH <2 vs. ≥2 ULN: 23:19. Six MLBL patients (14%) had <20% response to initial COP-therapy. Central pathology classification revealed approximately 50% with classical features of primary mediastinal B-cell lymphoma (PMBL). Five-year event-free survival (EFS) for Stage III MLBL and non-MLBL DLBCL groups were 66% (95% CI: 49-78%) and 85% (95% CI: 71-92%), respectively, p<0.001 (14%). The 5-year overall survival (OS) in the 42 MLBL patients was 73% (95% CI: 56-84%). MLBL in adolescent patients is associated with significantly inferior EFS compared with stage III non-MLBL DLBCL and can be of multiple histologies. Alternate treatment strategies should be investigated in the future taking into account both adult MLBL approaches and more recent biological findings in adult MLBL.

Keywords: MLBL, adolescent, chemotherapy, FABLMB96, event-free survival
Introduction

Mediastinal large B-cell lymphoma (MLBL) is a rare malignancy thought to arise from mature thymic B-cells. Although previously considered by the World Health Organization (WHO) to be a subtype of diffuse large B-cell lymphoma (DLBCL)\(^1\), primary mediastinal large B-cell lymphoma (PMBL) is now classified as a distinct mature B-cell neoplasm.\(^2\) According to the Murphy staging system for childhood non-Hodgkin’s lymphoma (NHL), MLBL, by virtue of its primary location in the mediastinum, must be at least stage III disease, even when tumor is localized above the diaphragm.\(^3\) The Children’s Cancer Group (CCG) and Berlin-Frankfurt-Munster (BFM) Group each reported results in children with mediastinal disease enrolled in series of completed multi-center mature B-NHL trials, and demonstrated 5-year event-free survival (EFS) of 75% ± 10% and 75% ± 8%, respectively.\(^4,5\) In contrast, a considerably higher 5-yr EFS of 85% ± 2% was reported for BFM patients with stage III non-primary-mediastinal B-cell NHL who received the same therapy as those with primary mediastinal disease, suggesting that MLBL may be an inherently different disease that requires alternative therapy than other identically grouped pediatric mature B-cell NHLs.

With the advent of gene expression profiling, it has become apparent that adult PMBL differs biologically from other mature B-cell NHLs, including DLBCL subtypes\(^1-3,6\) and Burkitt’s lymphoma (BL),\(^7\) and may in fact be a separate disease entity altogether. DNA microarrays have demonstrated similarities between PMBL and Hodgkin lymphoma (HL), suggesting that PMBL falls somewhere along the biological spectrum between DLBCL and HL.\(^6,8\) Recent studies have suggested that molecular hallmarks of PMBL include overexpression of genes in both NFκB and JAK/STAT pathways (Figure 1).\(^9\)
We report on the largest experience of pediatric patients with MLBL uniformly treated as part of the single international cooperative group study French-American-British/Lymphoma Malignancy B (FAB/LMB) 96, which enrolled pediatric patients with mature B-cell NHL from May 1996 to June 2001. Results for the low-risk, intermediate-and high-risk cohorts have been previously reported.\textsuperscript{10-12} This is the first report to focus specifically on the subset of 42 patients with Stage III MLBL enrolled on FAB/LMB 96, to describe their unique clinical and pathological characteristics, outcomes and risk factors for EFS and to compare them with patients with Stage III non-MLBL DLBCL treated with identical therapy.

**Materials and Methods**

**General**

The FAB/LMB 96 study was an open-label, randomized cooperative international study involving 161 pediatric cancer centers in 3 national groups: the Societe Francaise d'Oncologie Pediatrique (SFOP: France, Belgium and the Netherlands), Children’s Oncology Group (COG; United States, Canada and Australia), and the United Kingdom Children’s Cancer Study Group (UKCCSG). It was a planned 5-year study which enrolled patients from May 1996 until June 2001. The protocol was approved by each participating center’s institutional review board. Each national group was responsible for scientific, ethical, and administrative approvals, randomization, and data collection in a national database. Data was transferred every 6 months to the international database at Institut-Gustave-Roussy in France. Parents or patients over 18 years of age signed informed consent before randomization, in accordance with the Declaration of Helsinki.
Eligibility

Patients under 18 (SFOP, UKCCSG) or 21 (COG) years of age with newly diagnosed de-novo mature B-cell lymphoma classified as BL, Burkitt-like lymphoma (BLL), or DLBCL were eligible for the study.

Ineligibility criteria included immunodeficiency, HIV-positivity, prior solid organ transplant, previous malignancy, and/or prior chemotherapy.

Diagnosis

Staging was performed according to the Murphy classification system. Disease was categorized as low risk (Group A) for resected stage I and completely resected abdominal stage II, high risk (Group C) for bone marrow disease (≥25% L3 blasts) and/or central nervous system disease defined by one or more of the following: any L3 cerebral spinal fluid (CSF) blast, cranial nerve palsy, clinical spinal cord compression, isolated intracerebral mass, and/or cranial or spinal parameningeal extension, and intermediate risk (Group B) for all others, including MLBL.

The morphology and immunophenotype from the initial diagnostic material from each case was independently evaluated by each of the six hematopathologists from the three national cooperative groups (SFOP: M. Raphael, M.J. Terrier-Lacombe; COG: M.A. Lones, S.L. Perkins; UKCCSG: K. McCarthy, K., A. Wotherspoon) to establish a diagnosis of PMBL. All cases were classified according to the criteria described in the Revised European-American Lymphoma and WHO Classifications. An international consensus diagnosis was established for each case.
based on independent agreement by the group of hematopathologists or following review by the national groups on a multiheaded microscope.\textsuperscript{14} 

A protocol-specific standard immunophenotyping panel was performed on each case and included CD20, CD79a, CD3, CD45RO, TDT, CD30, and p80, as described previously.\textsuperscript{14} In addition, a subset of eleven cases had additional immunophenotypic analysis for germinal center differentiation markers CD10, BCL-6 and IRF4/MUM-1 as previously described.\textsuperscript{15} Retrospective pathologic review of all cases was performed to confirm a diagnosis of NHL and to attempt to sub-classify the lymphomas included in this analysis. The review was limited to morphology and the initial diagnostic immunophenotypic panels performed on the cases, as no additional case materials were available for further work-up. In particular, cases were evaluated for characteristic morphologic features of PMBL including compartmentalizing fibrosis, medium to large cells with abundant pale cytoplasm and round to oval nuclei lacking prominent macronucleoli and demonstrating strong, uniform expression of CD20 and CD79a with variable, weak expression of CD30. Only 10 cases had sufficient materials to allow for staging with IRF4/MUM1. No additional staining for characteristic markers such as CD23, I MAL, TRAF1 or REL were performed due to a lack of additional unstained slides or blocks. Some cases were considered indeterminant including “grey zone” lymphoma that is termed B-cell lymphoma, unclassifiable, with features intermediate between DLBCL and classical HL in the most recent WHO Classification.\textsuperscript{16}
Work-up included clinical examination, chest x-ray, abdominal ultrasound or computed tomography (CT), bone marrow aspiration, CSF cytology, and initial lactate dehydrogenase (LDH) level. CT scans and bone scintigraphy were performed as clinically indicated.

**Treatment and randomization**

Patients with Stage III MLBL and Stage III non-MLBL DLBCL were initially assigned to the group B treatment arm as we have previously described. Patients received a pre-phase consisting of low dose cyclophosphamide, vincristine, and prednisone (COP). Patients with a ≥20% response at Day 7 received a first induction course, COPADM1 (cyclophosphamide 1.5 g/m², vincristine, prednisone, doxorubicin, high-dose methotrexate [HDMTX 3 g/m² as 3-hr infusion with intrathecal MTX]). Upon recovery, patients received a second COPADM induction course (randomized to receive either cyclophosphamide 1.5 or 3 g/m²), 2 consolidation courses with CYM (cytarabine, HDMTX) and, depending upon randomization, either a single maintenance course M1 (cyclophosphamide [500 mg/m²], vincristine, prednisone, doxorubicin, HDMTX) or no maintenance. Response to treatment was measured at 3 time points. The first evaluation was performed after COP at day 7. Patients with a > 20% response to COP were considered responders to COP. Patients with <20% response to COP were switched to a more intensive high-risk (Group C) regimen including MTX (8 g/m²), high-dose cytarabine, and etoposide. The second evaluation was performed after the first COPADM course, and patients were randomized if there was no disease progression. The third evaluation was performed after the first consolidation CYM course (CYM1). Residual mass was recommended to be removed or biopsied. Patients with residual lymphoma after CYM1 were switched to the more intensive group C regimen at CYVE1 (consisting of high-dose (3g/m²) and continuous cytarabine [50
mg/m²] plus etoposide), and subsequently received a second course of CYVE and four maintenance cycles (M1: COPADM3 [same as M1 for group B]; M2: cytarabine plus etoposide; M3: cyclophosphamide, vincristine, prednisone, doxorubicin; M4: cytarabine plus etoposide) as we have previously described. Response criteria differed by time point. After the COP reduction phase, less then a 20% reduction in the product of the two largest diameters of measured lesions was considered a poor response and were switched to Group C therapy. Patients with an intermediate response was defined as a 20-99% reduction in the product of the two largest diameters of measurable lesions. Patients with a 100% reduction were considered to have a complete response. At the third evaluation a complete remission included patients with residual masses as long as an adequate biopsy demonstrated no viable tumor. Persistent disease was defined as histological proven residual mass while disease progression required a 25% or greater increase in the product of the two largest diameters of measurable lesions. Radiation therapy was not part of the primary treatment and was considered an off therapy event if administered.

**Statistical Methods**

The primary endpoint was EFS, defined as the time from randomization to disease progression, relapse, second malignancy, or death from any cause. Overall survival (OS) was a secondary endpoint. OS was defined as the time from randomization to death from any cause. For determination of EFS and OS, patients who did not experience an event were censored at the date of last follow-up contact. Probability of EFS and OS were determined using the Kaplan-Meier method. Comparisons of EFS and OS in patients with Stage III MLBL and Stage III non-MLBL DLBCL were performed using a log-rank test. Prognostic analyses of EFS according to
gender (male vs. female), age (10-14 vs. 15-21 yrs), COP response (response [incomplete or complete] versus no response [NR]), cooperative group, histology (Stage III MLBL vs. Stage III non-MLBL DLBCL), size (<10 cm vs. ≥10 cm) and LDH level (<2X upper limit of institutional normal [ULN] vs. ≥2X ULN) were performed using a log rank test.

**Results**

**Demographics**

Forty-two patients with MLBL primary arising out of the mediastinum were enrolled, constituting approximately 5% of the total number of patients registered in Group B therapy (n=762). Demographic information for the MLBL subset is summarized in Table 1. All patients were adolescents (median age 15.7 yrs) and the majority of patients were female (F:M, 3:2). Patients were enrolled from all 3 cooperative groups, with the majority receiving treatment at COG sites.

**Pathology**

All MLBL cases showed compartmentalizing sclerosis surrounding medium to large sized tumor cells (Figure 2A). The tumor cells had moderate amounts of clear cytoplasm with round to oval vesicular nuclei. Nucleoli were not prominent. Immunophenotypic analysis showed expression of the B-cell markers CD20 and CD79a in all cases. The tumor cells were negative for CD3, CD45RO, TdT and p80. All cases showed CD30 staining that was variable in intensity but usually weak and patchy in distribution in a predominantly cytoplasmic distribution. In the small subset of eleven cases stained for germinal center markers,15 it was noted that 2/11 cases expressed CD10 and BCL-6, 7/11 expressed BCL6 without CD10 (Figure 2B) and 2 cases
showed only MUM-1 without CD10 or BCL-6 expression. Interestingly, all cases (11/11), irrespective of germinal center marker expression, showed IRF4/MUM-1 staining (Figure 2C).

Limited retrospective pathologic review of the MLBL cases based on morphologic features and available immunohistochemical stains that were able to be performed on 32/36 cases. Four cases had no histologic or immunophenotypic slides available. Eighteen of 36 cases had morphologic and immunophenotypic features that were characteristic of PMBL whereas 5 cases had features that were more consistent with DLBCL (not otherwise specified [NOS]). The remaining cases (9/36 cases) had features that were indeterminant. Of the indeterminant cases, 5 cases had features that were indeterminant between classical HL and PMBL including thick bands of sclerosis, prominent macronucleoli, multinucleation of a subset of tumor cells and aberrant immunophenotypes with strong, uniform CD30 and variable expression of CD79a and CD30 and might be suspicious for a “grey zone” lymphoma, although this diagnosis could not be confirmed without additional immunophenotypic studies. Review of submitted pathology reports on these cases indicated that all tumor cells were strongly positive for CD45 and lacked definitive CD15 expression, although these findings could not be retrospectively confirmed. The remaining 4 cases had features that were indeterminate between DLBCL (NOS) and PMBL including large cell morphology with no discernable fibrosis and variable, weak CD30 expression.

**Response to Treatment**

The treatment course and response for each of the 42 MLBL patients is summarized in Figure 3. There were no toxic deaths on study. Of the 42 MLBL patients who were received COP therapy, 36 (84%) had at least a 20% response. No patient had evidence of disease progression at the
second evaluation (after COPADM1). Six patients transferred to Group C after COP therapy. Among the remaining 36 patients, 32 were randomized 36 according to the Group B therapy randomization as previously described.12 For patients were not randomized and treated on the B1 arm by physician choice. Eleven patients (7 randomized, 4 not randomized) were treated on the standard B1 arm, 8 were randomized and treated on arm B2, 10 on arm B3, and 7 on arm B4. Twenty-nine subjects on the B arms achieved a CR during the course of group B therapy, though one of these patients relapsed before the 3rd evaluation and was therefore no longer included among the CRs at CYM2 (Figure 3). At the end of CYM1, 29 of 36 patients had a residual mass on imaging. Only 23 of 29 patients who underwent a biopsy or excision and only 4 of 23 (17%) had residual viable tumor. Two additional patients treated on the B arms stopped therapy prior to achieving CR. One group B patient who came off-study due to disease progression after CYM1 achieved a CR after BEAM (carmustine, etoposide, cytarabine, melphalan) therapy but subsequently died later of relapse.

Five patients with evidence of residual disease (4 histologic disease, 1 PET-positive) switched to Group C therapy after the third evaluation (after CYM1) with one additional patient was transferred to Group C despite having achieved CR by physician choice (Figure 3). Excluding the 1 Group C subject already in CR at time of transfer, 8 of 11 patients in Group C attained CR. In group C, 3 patients failed to achieve a CR after CYVE2 (induction failure); all 3 subsequently died from progressive disease. Lastly, among the different histologies, within the group of 18 patients with PMBL histology, 13 remain in CCR; within the group of 5 with DLBCL histology, 3 remain in CCR; and within the group of 9 with indeterminate histology 7 remain in CCR.
EFS and OS

In the 42 MLBL patients, the probability of 5-yr EFS and OS were 66% (95% CI: 50-78%) and 73% (95% CI: 56-84%), respectively (Figure 4). At the time of final data analysis, a total of 28 patients remained in CR1. Among those who completed treatment on the 4 intermediate-risk Group B treatment arms (n=28), 21 remain in CR1 at the time of this analysis, while 7 patients have relapsed. Among the 6 patients who responded poorly to COP and were switched to Group C therapy prior at COPADM1 according to the protocol, 3 remained in CR1 after completion of therapy, while 3 others progressed. Among the 6 patients who were switched to Group C therapy after CYM1 according to protocol, 4 remained in CR1 at the time of final analysis, while another two died of disease progression (Figure 3). One patient who withdrew from therapy because of moving to a foreign country, died of intracranial hemorrhage unrelated to therapy. The majority of relapses and progression occurred within the first year of diagnosis with only a few events occurring in the second year of diagnosis.

The probability of 5-yr EFS in the MLBL group (n=42) was compared to the probability of 5-yr EFS in patients with Stage III non-MLBL DLBCL (n=69). The MLBL group had a significantly lower 5-yr EFS compared to non-MLBL DLBCL stage III subgroup, 66% (95% CI: 49-78%) vs 85% (95% CI: 71-92%), respectively, p<.001 (Figure 5).

Risk Factors Associated with EFS

In order to determine the effect of various risk factors on the probability of 5-year EFS, log-rank testing with stratification for gender, age, COP response, LDH level, and size of mass (>10cm versus ≤10cm) was performed. There was no difference in probability of 5-year EFS among each
of the risk factors ([complete response to COP vs. NO: 67.7 vs. 42.9%] [p=0.14], LDH ≥2 x ULN vs. <2 x ULN: [53.8 vs. 73.2%], p=0.2, >10cm ≤10cm: [53.8% vs 61.3%] p=0.07]). However, the numbers in each group were extremely low to have a high level of confidence. Large numbers would be required to determine the independent effect of each of these variables.

**Discussion**

MLBL is a rare subtype of B-cell NHL, accounting for approximately 2% of childhood and adolescent lymphomas.\(^{17}\) Despite its rarity, the treatment of adolescent MLBL is a topic of considerable interest, as childhood and adolescent patients with MLBL tend to have worse outcomes than do children and adolescent patients with other stage III mature B-cell NHLs.\(^5\) The BFM group was the first to report a decreased EFS in pediatric MLBL patients (75% vs. 85% 5-yr EFS), but the result was not statistically significant (p=.19) and included 30 patients treated during the course of three trials and an interim treatment period. More recently the BFM has reported a 34% relapse rate among 44 PMBL patients treated over a 23 year period on 4 different protocols NHL-BFM 86, 90, 95, and B-NHL BFM 04).\(^{18}\) The results from the current FAB/LMB 96 study confirm the findings reported by the BFM group, and provide important additional data to support the need for improved therapies for adolescent patients with MLBL. As our results come from the largest single uniform treatment study of adolescent MLBL and for the first time, show a highly significant decrease in 5-yr EFS compared to other stage III non-MLBL pediatric DLBCL patients, it is increasingly clear that the currently utilized treatment regimens, consisting of dose-intense courses of steroids, vincristine, methotrexate, cyclophosphamide (or ifosfamide), doxorubicin, cytarabine, etoposide and intrathecal chemotherapy, are not as effective therapy for
MLBL compared to other pediatric mature B-cell NHLs (BL and DLBCL) treated on similar
treatment regimens.

To further support the importance of studying new approaches for the treatment of adolescent
MLBL, we refer to recent improvements in the treatment of adult PBML. Various chemotherapy
regimens, including MACOP-B (methotrexate, doxorubicin, cyclophosphamide, vincristine,
prednisone, bleomycin) and VACOP-B (etoposide instead of methotrexate) have been studied in
adult patients with PMBL, and have been shown to offer a survival benefit over conventional
CHOP (cytoxan, doxorubicin, vincristine, prednisone) therapy.19-21 Additionally, in a small series
of adult patients with newly diagnosed PMBL, VACOP-B followed by autologous stem cell
transplant led to a disease-free survival rate of 93% after 35 months median follow-up.22

Rituximab, the CD20 monoclonal antibody known to inhibit the anti-apoptotic NF-kB pathway,
has also been extensively studied in adults with DLBCL. Rituximab is of interest in the treatment
of B-cell NHL as upregulation of subsets of NF-kB signaling genes in PMBL and activated B-
cell-like (ABC) DLBCL has been demonstrated by gene expression profiling.6,23 The addition of
rituximab to CHOP therapy (CHOP-R) has been shown to provide a benefit over CHOP alone in
adults with DLBCL.24-26 Similarly, the addition of rituximab to DA-EPOCH (dose-adjusted
etoposide, prednisone, vincristine, cyclophosphamide, doxorubicin) in adults has been shown to
benefit adults with DLBCL whose tumors overexpress Bcl-2.27 Although this latter study did not
include patients with PMBL, a separate study of 32 adult patients with PMBL (Bcl-2 status not
reported) treated with DA-EPOCH and rituximab demonstrated that the regimen was highly
effective, with 95% EFS. Among adult patients with PMBL, who also received an intensified CHOP regimen and rituximab, this strategy has also shown very encouraging results.

The pathology review, although limited by materials available, showed the need for careful clinical, morphologic and immunophenotypic examination to ensure that the mediastinal lymphoma is appropriately diagnosed. Although most cases of PMBL were relatively easily diagnosed, awareness of the “grey zone” lymphomas or those lymphomas with features that were indeterminant between classical HL and PMBL is necessary as these tend to be seen, albeit in low incidence, in adolescents and young adults, as was recently demonstrated in a recent retrospective study. We noted 5 cases that would need additional immunophenotypic work-up to distinguish between PMBL and “grey zone” lymphoma, although there were not sufficient materials available in this retrospective study to allow for this immunophenotyping to be performed, and this incidence of cases is similar to that seen in a previous study of mature B-cell lymphoma in children and adolescents. It is also important to recognize that all DLBCL that occur in the chest or mediastinum are not PMBL or “grey zone” lymphomas, but there are some cases of DLBCL, NOS that may initially present in this anatomic site although they often have evidence of disease in other sites. Both DLBCL, NOS and “grey zone” lymphomas may require different approaches to therapy than PMBL and have different prognostic implications and clinical behavior. Because of the diversity of lymphoid neoplasms that may present in the mediastinum, it is essential that a sufficient amount and quality of diagnostic materials be collected to allow for appropriate diagnostically important ancillary studies. In addition, some cases may require review by an experienced hematopathologist with appropriate immunophenotyping to meet the defined diagnostic criteria set forth in the 2008 WHO
Classification for accurate diagnosis and differentiation between PMBL, DLBCL (NOS) and “grey zone” lymphoma.18,31

Aside from rituximab, other inhibitors of the NF-κB pathway could be of benefit to patients with PMBL. NF-κB blocking agents such as proteasome inhibitors and small molecule inhibitors of the IKK protein may work synergistically with chemotherapy and rituximab and warrant further study. By blocking the pathway at various points in the NF-κB signal transduction pathway, it may be possible to overcome any compensatory up-regulation that may occur. Preliminary activity of proteasome inhibitors and small molecule IKK inhibitors has already been demonstrated in vitro in a PMBL cell line, with increases in apoptosis reported following incubation with a proteasome inhibitor, a small molecule IKK inhibitor or the combination of both. Additionally, these increases in apoptosis were shown to be associated with inhibition of multiple apoptosis-associated proteins, including members of the NF-κB family of transcription factors.32

The NF-κB signaling pathway is not the only pathway that is overexpressed in PMBL. Constitutive activation of the JAK-STAT signaling pathway due to recurrent gene mutations affecting the STAT6 DNA binding domain has been demonstrated in PMBL tumor samples, but not in DLBCL tumor samples.9,33 Targeted agents that block the JAK-STAT pathway may therefore also prove useful in the treatment of PMBL (Figure 1).9,33 Recent studies have demonstrated a consistent 9p24.1 amplification in adult PMBL and enhanced expression of PD-1 ligand.34 Lastly, reduced expression of MHC class II genes is an additional defining feature of adult PMBL with a strong correlation with expression of the master transcriptional regulator of
class II expression (CITA).\textsuperscript{35-37} Immunological approaches attempting to block PD-1 and/or increasing T-cell effector cells infiltration into the microenvironment may be additional alternative treatment strategies to consider in the future.

It is important to consider alternative chemotherapy regimens and the addition of targeted agents early in the treatment of all patients with adolescent MLBL, as patients who do not achieve a complete response with first-line therapy are difficult to retrieve with subsequent therapies. This is evidenced by the finding that all 3 of the patients switched to Group C therapy who were not in CR after the second CYVE2 intensification cycle went on to die of their disease. Although more intensive and targeted therapies may be important for all adolescent MLBL patients, they are most urgently needed for the treatment of patients without an early response to chemotherapy. Despite the fact that COP non-responders were switched to more-intensive Group C therapy, two of these patients still failed to achieve a complete response and subsequently died from disease progression. Furthermore, the single patient who did not respond to COP but remained on Group B therapy also went on to relapse after the completion of treatment and subsequently died.

Given the poorer outcome for adolescent patients with MLBL compared to pediatric patients with non-MLBL Stage III DLBCLs and taking into account the recent encouraging results in adults with MLBLs treated with rituximab and CHOP or DA-EPOCH like regimens, alternative therapeutic strategies should be explored in the future in this small subgroup of adolescent patients.\textsuperscript{38} Taking into account the difference in biology in adult PMBLs versus DLBCLs, targeted approaches, especially inhibitors of NF-kB and/or JAK/STAT pathways, should be
investigated in the relapsed setting.39-41 Additionally, this is a prototype disease that should be studied by both pediatric and adult cooperative groups together in adolescent and young adult (AYA) patient initiatives.42 Based on these results, an international pediatric trial of dose-adjusted EPOCH with rituximab in adolescents with MLBL has been initiated (personal communication, Catherine Patte).

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Authorship and Conflicts of Interest

M.G. and M.S.C. designed and performed the research, analyzed data, and wrote the manuscript; I.M.W. and S.G. analyzed data and wrote the manuscript; R.S. designed research and analyzed data; A.A. collected the data, and with C.P. designed, performed the research, analyzed the data and revised the manuscript; R.P. designed and performed the research; L.H. collected and analyzed data; S.L.P., K.M. and M.R. performed research and analyzed data.

I.M.W. is currently employed at Bristol-Myers Squibb. All other authors declare no conflict of interest.
References


37. Steidl C, Shah SP, Woolcock BW, et al. MHC class II transactivator CIITA is a recurrent

38. Cairo MS, Sposto R, Gerrard M, et al. Advanced stage, increased lactate dehydrogenase,
and primary site, but not adolescent age (≥ 15 years), are associated with an increased risk of
treatment failure in children and adolescents with mature B-cell non-Hodgkin's lymphoma:


40. Copie-Bergman C, Plonquet A, Alonso MA, et al. MAL expression in lymphoid cells:
further evidence for MAL as a distinct molecular marker of primary mediastinal large B-cell

41. Guiter C, Dusanter-Fourt I, Copie-Bergman C, et al. Constitutive STAT6 activation in

42. Hochberg J, Waxman IM, Kelly KM, Morris E, Cairo MS. Adolescent non-Hodgkin
Table 1: Summary of demographic data for all 42 MLBL patients enrolled on FAB/LMB 96.

<table>
<thead>
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<td>29 (69%)</td>
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<tr>
<td>SFOP</td>
<td>10 (24%)</td>
</tr>
<tr>
<td>UKCCSG</td>
<td>3 (7%)</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1. Main deregulated signaling pathways in PMBCL.** The main activation cascades of JAK-STAT and NF-κB signaling. Alternative pathway activation exists. Known gene alterations leading to constitutive pathway activity in PMBCL are shown in color.


**Figure 2A.** Hematoxylin and eosin histologic section of a mediastinal diffuse large B-cell lymphoma showing the characteristic large cell infiltrate with intervening bands of compartmentalizing fibrosis. The tumor cells form individual cells and small groups (400X magnification).

**Figure 2B.** CD20 immunoperoxidase staining demonstrating the mature B-cell large cell infiltrate (1000X magnification).

**Figure 2C.** IRF4/MUM 1 staining of a mediastinal B-cell lymphoma showing characteristic nuclear staining (100X magnification).

**Figure 3.** Treatment course for each MLBL patient treated on FAB/LMB 96. All MLBL patients received group B therapy initially. After COP reduction phase, patients with a complete response or intermediate response (20-99% reduction in the product of the two largest diameters of evaluable lesions) continued on group B therapy. At the third evaluation a complete remission included patients with residual masses as long as adequate biopsy demonstrated no viable tumor.
Patients with residual viable tumors (persistent disease) were switched to group C therapy beginning with CYVE1 consolidation. Nine patients in CR continued onto Group C M1-M4 therapy. One CR from Group B therapy and 8 CRs from Group C therapy. Patients with disease progression (>25% increase in the product of 2 largest diameters) were off study. Disease status at last follow-up for all patients completing therapy is included.

**Figure 4.** Probability of 5-year EFS and OS by Kaplan Meier method of MLBL patients treated on Group B therapy on FAB/LMB 96.

**Figure 5.** Probability of 5-year EFS by Kaplan Meier method for patients with MLBL versus stage III non-MLBL DLBCL patients treated on Group B therapy on FAB/LMB 96. 5-yr EFS: MLBL 66% (CI95 49-78%) vs. stage III DLBCL (non-MLBL) 85% (CI95 71-92%), p<0.001.)
Figure 1

JAK-STAT signaling

STAT dimers/oligomers

Interleukin receptors

STAT6

JAK2

SOCS1

RIP

TRAF

A20

TNF receptor superfamily

IKK complex

IkBa

IkBε

NFκB complex

IkB complex proteasomal degradation

p100

REL

p52

REL

Transcriptional regulation:
- Inflammation
- Cell proliferation
- Survival
- Differentiation
Figure 3
Figure 5

The graph shows the survival probability over time for two groups: DLBCL Non-MLBL (solid line) and MLBL (dashed line). The x-axis represents time in years from study entry, while the y-axis represents the probability. The data indicates a higher survival probability for DLBCL Non-MLBL compared to MLBL across the observation period.
Outcome and pathological classification of children and adolescents with mediastinal large B-cell lymphoma treated with FAB/LMB96 mature B-NHL therapy

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