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# Dose-Adjusted Epoch and Rituximab for the treatment of double expressor and double hit diffuse large B-cell lymphoma: impact of TP53 mutations on clinical outcome.

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**Running Head:** DA-EPOCH-R in Double Expressor Lymphoma

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## Contributions

A.D., A.G.designed research, analyzed data, and wrote the manuscript; F.M., A.T., A.R., M.B., A.C., M.P., L.D., F.R., F.C., and L.F. collected data; F.B. and R.M. performed statistical analyses; C.C., C.M., and D.R. performed TP53 mutations; A.C., D.R., V.M. and F.F. performed histological diagnosis and FISH analysis; P.C., and C.C-S.supervised the study.

## Abstract

Diffuse Large B-Cell Lymphoma (DLBCL) is a heterogeneous disease, including one-third of cases overexpressing MYC and BCL2 proteins (Double Expressor Lymphoma, DEL) and 5-10% of patients with chromosomal rearrangements of MYC, BCL2 and/or BCL-6 (Double/Triple-Hit Lymphomas, DH/TH). TP53 mutations are detected in 20-25% of DEL. We report the efficacy of dose-adjusted EPOCH and rituximab (DA-EPOCH-R) in a series of 122 consecutive patients, including DEL (n=81, 66%), DEL-MYC (n=9, 7%), DEL-BCL2 (n=13, 11%), or High-Grade Lymphomas (DH/TH) (n=19, 16%). Central nervous system (CNS) prophylaxis included intravenous methotrexate (n=66), intrathecal chemotherapy (IT) (n=40) or no prophylaxis (n=16). Sixty-seven pts (55%) had high-intermediate or high International Prognostic Index (IPI) and 30 (25%) had high CNS-IPI. The 2-year progression-free survival (PFS) and overall survival (OS) for the entire study population were 74% and 84%, respectively. There was a trend for inferior OS for DH/TH (2-year OS: 66%, p=0.058) as compared to all the others. The outcome was significantly better for the IPI 0-2 versus IPI 3-5 (OS: 98% vs. 72%, p=0.002). DA-EPOCH-R did not overcome the negative prognostic value of TP53 mutations: 2-year OS of 62% versus 88% (p=0.036) were observed for mutated as compared to wild-type cases, respectively. Systemic CNS prophylaxis conferred a better 2-year OS (94%) as compared to IT or no prophylaxis (76% and 65%, respectively; p= 0.008). DA-EPOCH-R treatment resulted in a favorable outcome in patients with DEL and DEL with single rearrangement, whereas those with multiple genetic alterations such as DEL-DH/TH and TP53 mutated cases still have an inferior outcome.

## Introduction

Diffuse Large B-Cell Lymphoma (DLBCL) is a clinically and biologically heterogeneous disease. Historically DLBCL patients have been uniformly treated with R-CHOP chemoimmunotherapy regimen (Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisone), leading to a long-lasting complete remission in approximately 60% of cases[1,2]. For these patients, the International Prognostic Index (IPI), is an easy and valid tool for prognostic stratification[3].

In 2000 Alizadeh et al. used Gene Expression Profiling (GEP) to identify the cell of origin (COO) and described prognostic subgroups according to diversity in gene expression patterns indicative of different stages of B-cell differentiation[4]. Immunohistochemical algorithms, used as surrogates of GEP for the identification of COO subgroups, have been extensively used but have a limited concordance with GEP when applied to patients with DLBCL treated with R-CHOP[5].

Among other possible prognostic indicators for patients with DLBCL, tumor protein p53 (TP53) mutations seem to represent simple and attractive biomarkers to be used in the daily routine clinical practice. TP53 gene is involved in maintaining genomic stability in response to DNA damage by activating DNA repair programs and by triggering cell-cycle arrest. The loss of TP53 is associated with lymphomagenesis and resistance to chemotherapy[6]. TP53 mutations are present in 10% of DLBCL patients and confer a poor prognosis in patients treated with R-CHOP[7,8]. Recently, Chapuy et al. performed whole genomic sequencing in 304 primary DLBCL patients and identified genetically different subgroups among GCB and ABC patients. Patients with TP53 mutations were described as a distinct cluster with a very poor prognosis[9].

MYC and BCL2 represent other possible poor prognostic markers when rearranged or overexpressed in DLBCL and lymphomas harboring the double or triple translocation (DH or TH) represent new entities in the recently updated 2016 WHO Lymphoma Classification. Most DH lymphomas are in the GCB category, they present with advanced stages and have a very poor prognosis when treated with the R-CHOP regimen[10-12]. Also, the BCL2 translocation alone has been shown to play a significant prognostic role in GCB DLBCL patients treated with R-CHOP[13,14]. Additionally, the presence of MYC rearrangements seems to be a poor prognostic marker, although its role in the absence of either BCL2 or p53 alterations remains controversial[15]. The concomitant overexpression of MYC and BCL2 on the tumor cell surface observed in double expressor lymphomas (DEL), could be the result of other mechanisms, different from translocation such as copy gain/amplification. The prognosis of DEL patients treated with standard R-CHOP is worse than that of non-DEL

patients[16,17], and these individuals have a risk of Central Nervous System (CNS) relapse of 9.7% at two years[18]. Moreover, a consensus on the optimal treatment for these patients has yet to be established.

Since 2013 at our Institution, DEL patients have been treated with the intensive chemotherapy regimen DA-EPOCH-R, achieving a promising 2-year PFS and OS of 62% and 85%, respectively. These survival rates were better than those reported with R-CHOP in a historical cohort[19].

The aim of the present study was to assess the incidence and prognostic role of TP53 mutations and BCL2, MYC translocations in a large cohort of DEL patients consecutively treated with DA-EPOCH-R. The analysis of the cumulative risk of CNS relapse as well as the effect of the different CNS prophylaxis applied in this selected cohort of DEL patients was also assessed.

## Methods

*Patients*

All consecutive patients with a diagnosis of DEL treated since 2013 with DA-EPOCH-R at four Hematological Divisions in Northern Italy were retrospectively identified. For inclusion in this observational study, the following inclusion criteria were required: 1) histologically proven diagnosis of DEL with an age ≥18 years; 2) availability of formalin-fixed, paraffin-embedded (FFPE) samples; 3) no exposure to previous therapy except for the first cycle of R-CHOP that was allowed while waiting for immunohistochemistry (IHC) and cytogenetic characterization. Exclusion criteria were HIV positivity, CNS involvement at diagnosis, and histology other than DEL. The ethics committees of the participating centers approved the study (INT 35/17). Written informed consent was obtained from all patients.

*Immunohistochemistry and Fluorescence in situ hybridization analysis*

Formalin-fixed, paraffin-embedded tissue samples were sectioned at 3-µm thickness. IHC was performed using the EnVision FLEX+, mouse, high pH method (Dako Denmark A/S Produktionsvej 42 DK-2600 Glostrup, Denmark) and a Dako Autostainer Link48 (Dako, Italia, SPA, Milano, Italy). Slides were stained with monoclonal antibodies against CD19, CD20, CD10, BCL2, BCL6, MUM1, MYC, and Ki67.

Fluorescence in situ hybridization (FISH) analyses for BCL2, BCL6, and MYC rearrangements were performed in all patients using “LSI BCL2 LSI BCL6 (ABR), C-MYC “break apart” probes (Vysis/Abbott Molecular, Illinois, USA) according to the manufacturer’s instructions. (detailed in Supplementary Methods).

*TP53 mutations*

TP53 is located on chromosome 17p13.1 and consists of 14 exons (1 - 11), 10 of which are coding sequences for the p53 protein[20,21]. TP53 mutations were assessed according to Institutional practice, Sanger sequencing was used for samples collected from patients at Humanitas Cancer Center while Next-Generation Sequencing followed by direct sequencing was used for all other samples (procedures described in the Supplementary Methods).

*Treatment and CNS prophylaxis*

Patients received the DA-EPOCH-R regimen therapy every 21 days for 6 cycles. In all patients dose adjustment based on cell counts between cycles according to the NCI algorithm was applied[22]. At diagnosis, all the patients performed lumbar puncture for cytology and flow cytometry analyses of cerebrospinal fluid (CSF). During this first procedure, all patients received intrathecal chemotherapy with Methotrexate (MTX) and Cytarabine. Neuro-imaging has been considered only in presence of neurological signs or symptoms. In absence of definitive clinical guidelines on CNS prophylaxis, the choice was determined by the treating physician. High-dose MTX (HD-MTX: 3 g/m2) was used as prophylaxis in 66 patients (for the majority at the end of the treatment). Intrathecal chemotherapy at day 1 of every cycle, including MTX/Cytarabine/dexamethasone, was given to 40 patients.

*Aim of the study and statistical analysis*

Objectives of the study were assessment of the effect of biological variables (TP53 mutations and BCL2 and MYC translocations) on survival analyses at 2-years in a large cohort of DEL patients consecutively treated with DA-EPOCH-R. Analysis of the cumulative risk of CNS recurrence in the whole population and PFS and OS impact of different CNS prophylaxis were also evaluated. Fisher's Exact Test was performed to assess the association between TP53 mutation status and patients' clinical characteristics. Statistical analyses were performed using R (version 3.5.0).(Detailed on Supplementary Methods).

## Results

*Patients' characteristics and treatment*

A total of 122 patients affected by DEL were consecutively treated with DA-EPOCH-R between November 2015 and March 2020. Patients' characteristics are summarized in **Table 1**. The median age was 59 years (range, 24-79), and 62% were male.

MYC and BCL2 rearrangements by FISH were evaluated in all cases. BCL6 rearrangement could be done for 95 out of 122 patients (78%). According to IHC and FISH testing, the study population was divided into three subgroups: 1) DEL only without any rearrangements (n=81, 66%); 2) DEL Single Hit (DEL-SH) with either MYC or BCL2 rearrangement (n=9 MYC, n=13 BCL2); and 3) high-grade lymphomas (DH/TH) (n=10 MYC/BCL2, n=3 MYC/BCL6, n=6 MYC/BCL2/BCL6). The COO assignment according to Hans algorithm was analyzed in 115/122 patients (94%) of whom 55 (45%) were GCB and 60 (49%) non-GCB.

Ninety-five (78%) patients had an advanced stage and 67 (55%) presented an IPI score of 3-5. Moreover, the number of patients with limited disease was low (n=27) with only four cases with stage I (all these cases presented with bulky extranodal disease) (Supplementary Table 1).

The median number of chemotherapy cycles was 6 (range, 1-6). Due to the aggressive clinical presentation requiring urgent treatment, 19 (15%) patients received the first cycle of R-CHOP while waiting for complete FISH analyses. Response assessment following DA-EPOCH-R treatment was feasible in 117 patients: of these 84 (72%) and 16 (14%) achieved a complete or partial remission, respectively. Seventeen patients (14%) showed progressive disease. Five patients were not evaluable for a response after DA-EPOCH-R for toxicity (n=1, death of pneumonia), de-escalation therapy (n=2), consolidation with high-dose therapy before the 6th cycle (n=2). Overall, 22 out of 122 (18%) patients (n=12 DE, n=3 SH-BCL2, n=1 SH-MYC, n=6 DH/TH) underwent autologous stem cell transplantation, in clinical remission, during treatment (n=2) and as consolidation after 6 cycles of DA-EPOCH-R (n=20) (Supplementary Table 2).

With the exclusion of one patient who died of pneumonia, other adverse events were manageable. We observed febrile neutropenia in 16/122 (13%) and infections requiring hospital admission (n=6 pneumonia, n=1 sepsis) in 7 patients (6%).

*Survival Outcome*

After a median follow-up of 24 months (IQR, 14-38 months), 110 patients were alive and 22 died (n=20 for disease progression, n=1 for toxicity, n=1 suicide). PFS (95% CI) and OS at 2-year were 74% (66-83%) and 84% (77-91%), respectively **(Figures 1A and 1B).** The 2-year OS and PFS were not significantly different between DEL, DEL-MYC, DEL-BCL2 and, DEL-DH/TH, with a trend for inferior survival in this last subgroup [OS: 66% (47%-92%), p=0,058) **(Figure 1C and 1D)**.

Age above 60 years did not affect outcome whereas the male sex was associated with a significantly shorter PFS (Supplementary Table 3). The COO did not show a significant impact either on PFS or OS. Isolated MYC (≥70%) or BCL2 (≥80%) as assessed by IHC did not impact PFS and OS. (data not shown).

As expected, patients with a limited disease had a significantly 2-year PFS 92% (81-100%) as compared to advanced stages [70%, (61-80%), p=0,048] **(Table 2).** The analysis of outcome by IPI score showed a 2-year PFS of 62% (51-76%) and OS of 71 % (61-85%) for high-intermediate and high-IPI score that was inferior compared to low-intermediate and low cases [88%, (79-98%) and 98% (94-100%); p=0,002 and p=0,002 respectively], **(Figures 1E,1F).** In those achieving a response, we did observe a significant difference in outcome between patients who received or not autologous transplantation (Supplementary Figure 1). Complete results of univariable Cox models for PFS and OS according to the patients and disease characteristics are reported in Supplementary Table 4.

*Evaluation of TP53 Mutation*

The TP53 mutation could be retrospectively evaluated in 69 out of 122 (57%) patients due to the absence of sufficient residual archival material or to poor quality of the genomic DNA extracted from paraffin-embedded tissues. The TP53 mutation status was assessed in 44 DEL (64%), 6 DEL-MYC (9%), 9 DEL-BCL2 (13%), and 10 DEL-DH/TH (15%). Overall a pathogenic TP53 mutation (as defined by the IARC TP53 database) was present in 16 patients (23%). We evaluated the outcome according to the presence or absence of TP53 mutation. The two groups were not statistically different for the main clinical characteristics (**Table 3**). The 2-year PFS was 58% (37-91%) and 80% (70-93%; p=0.033) and the 2-year OS was 62% (40-96%) and 88% (78-99; p=0.036), for mutated and wild-type cases respectively **(Figures 2A and 2B).**

*Multivariable Analysis.*

Cox multivariate models concerning the PFS and OS of the patients were performed as summarized in **Table 4.** TP53 mutation, IPI 3-5, and absence of CNS prophylaxis had a negative prognostic impact on OS whereas the female sex was associated with a significantly improved PFS.

*Outcome of relapsed patients*

Among 28 patients who relapsed [n=17 DEL, n=4 DEL-SH (only with BCL2 translocation), n= 7 DEL-DH/TH], nine-teen (68%) died of lymphoma whereas 9 patients are still alive (n=6 DEL, n=3 DEL/BCL2). 5 out of 9 patients are in complete remission after receiving different salvage therapies (n=2 auto-SCT, n=2 allo-SCT, n=1 lenalidomide in combination with radiotherapy).

*CNS Prophylaxis and CNS relapse*

At diagnosis, only two patients had cerebrospinal fluid involvement: one died early of systemic progressive disease and the other is still alive after therapy including high-dose methotrexate.

The CNS prophylaxis was chosen at the discretion of the treating physician. Sixty-six (54%) patients received systemic HD-MTX, 40 (33%) underwent intrathecal chemotherapy with methotrexate and cytarabine and 16 (13%) did not receive any CNS prophylaxis at all. In particular, patients not receiving CNS prophylaxis had less extranodal involvement at risk for CNS relapse. All characteristics are detailed in Supplementary Table 5.

Systemic Methotrexate-based CNS prophylaxis conferred a better 2-year OS (94%, 88-100%) as compared to intrathecal or no CNS prophylaxis [75%, (63-91%) and 65%, (42-100%) respectively; p=0.008) **(Figures 2C and 2D).** A significant advantage in OS was observed even after exclusion of DH/TH patients [2-year OS% 96% versus 81%, versus 63%, respectively, (p<0.001; **Figures 2E and 2F**)] that was the subgroup with the worst outcome.

Overall, we observed five CNS relapses, and the cumulative incidence of relapse at 1- and 2-year was 2% (1-9%) and 5% (2-13%) respectively in the entire cohort. All patients with CNS relapse were DEL only, all but one were non-GCB. Four out of five patients died of CNS lymphomas. The CNS relapse occurred even in patients who received CNS prophylaxis (3 out of 5 patients) and in 4 out 5 with low CNS-IPI.

## DISCUSSION

In the present retrospective study, we collected a large number of consecutive DEL patients (n=122) who were treated with the DA-EPOCH-R regimen to test the hypothesis that an intensive regimen could overcome poor clinical, and biological prognostic factors. To the best of our knowledge, this study includes the largest series of DEL patients exposed to an intensified regimen. All DEL patients were analyzed for MYC and/or BCL2 rearrangements and, partly, for TP53 mutational status. Indeed, the 2-year PFS and OS of 74% and 84%, respectively, for the entire cohort seem promising. Further, in patients characterized by IPI score of 3-5, the results are comparable to those achieved with other intensified treatments (R-CODOX/IVAC and R-ACVB)[23,24].

Currently, the treatment of DEL without any gene rearrangement remains an unmet clinical need. The phase III trial performed by the Alliance group comparing the R-CHOP and DA-EPOCH-R regimens in newly diagnosed DLBCL included only a limited number of DEL, thus preventing the possibility of drawing definitive conclusions on the role of DA-EPOCH-R in this subtype[25]. Our series of 81 DEL patients without any rearrangement showed a 2-year PFS and OS of 75% and 86%, respectively, suggesting a potential role of the intensive regimen. Recently, Morschhauser et al. evaluated the combination of venetoclax with standard R-CHOP or obinutuzumab-CHOP in DEL patients or those with high expression of BCL2, showing a similar 2-year PFS of 72% and 77%, respectively.

Patients with a DEL-DH/TH status showed mainly an intermediate and high-risk IPI (75%) and had a trend for inferior OS at 2 years (66%) as compared to DEL only (86%), whereas the observed 2-year PFS of 63% was not statistically different among these three subgroups. In addition, we have to consider that the prognosis of high-grade B-cell lymphomas (HGBCL) with overexpression of MYC or BCL2 proteins seems poor as compared to DH/TH, not DEL[27]. This finding suggests that more than 50% of DEL-DH/TH could be cured with an intensive regimen but that in case of relapse they have a trend for poor overall survival. Likely, the recent FDA and EMA approval of new therapies such as CAR T-cells will have an impact on the OS of relapsed DEL-DH/TH HGBCL [28,29].

A total of 27 patients with limited-stage disease (stage I, n=4 with extensive extranodal localization; stage II, n=23) (DEL, n=18; SH, n=6; DH/TH, n=3) were treated with DA-EPOCH-R and experienced an impressive 2-year PFS and OS of 92% and 95%, respectively. Interestingly, the majority of limited disease patients did not have TP53 mutation (mutated, n=2; wild-types, n=18; not tested, n=7). Only a handful of studies, mainly

of retrospective nature, have been focused on the prognosis of single hit or DH/TH lymphomas with limited-stage disease. Torka and colleagues analyzed the outcome of 81 patients carrying MYC rearrangement, including 40 DH, who received the standard R-CHOP regimen or intensive chemotherapy[30]. The authors did not find any statistical difference in PFS and OS across treatment strategies but in the subgroup of DH, the intensive therapy was associated with a higher CR rate.

Over the past years, the combination of CNS-IPI and biological factors has been considered the best way to estimate the CNS relapse risk. In a retrospective study of newly diagnosed DLBCL patients treated with R-CHOP, without any CNS systemic prophylaxis, Savage and colleagues reported that the DEL/non-GCB subgroup had a significantly higher risk of CNS relapse as compared to the non-DEL/non-GCB subgroup (15% vs 3%)[31]. In contrast, in the prospective Goya trial, COO and not MYC/BCL2 double expression impacted the risk of CNS relapse[32]. More recently, the Nordic group has suggested the efficacy of the early administration of HD-MTX in newly diagnosed DLBCL at high risk of CNS relapse[33]. Our study is the first in which the risk of CNS relapse has been evaluated following the administration of a DA-EPOCH-R regimen and HD-MTX in 66 out of 122 (54%) patients. Our population included 24% of patients with high-risk CNS-IPI. Despite the high-risk population analyzed, systemic CNS prophylaxis was associated with a very low cumulative incidence of CNS relapse (5%) and a significant advantage in PFS and OS. The advantage on the outcome can be influenced by other untested factors and should be confirmed in a larger trial.

Xu-Monette et al.[6] reported a 21% incidence of TP53 mutations in a large population of DLBCL patients treated with R-CHOP and demonstrated that TP53 disruption was associated to poor PFS and OS in both GCB and non-GCB subtypes. Following a better characterization of DLBCL beyond the COO, the frequency of this mutation was tested in DEL and did not result to be different from non DEL (25% vs 22%, respectively). The role of genomic alterations, including TP53 mutations, has recently been investigated in several studies by applying novel molecular techniques[9]. Interestingly, Meriranta et al. found a frequent association between TP53 alterations and the MYC overexpression or translocations and, in those with TP53 mutation, a worse outcome in DEL as compared to non-DEL was reported[34]. Recently, Song et al. reported a poor prognosis in DLBCL carrying double-hit signature and TP53 inactivation[35]. In our study, the prevalence of the TP53 mutation was in keeping with previous studies, and the PFS/OS following DA-EPOCH-R treatment in TP53-mutated patients remained significantly lower than that observed in non-mutated patients suggesting the failure of intensive therapy to overcome the mutation adverse effect. The observed 2-year OS of 62% seems better when compared to the OS of 48% reported by Xu Monette[6] with R-CHOP and of 27% described by Chiappella et

al.[36] following intensified therapy. These are indirect comparisons requiring a clinical study to be validated.

The intensified treatment with DA-EPOCH-R was well tolerated considering that 56% of patients were older than 60 yrs. We observed a limited incidence of severe toxicities, with one death for pneumonia and two patients de-escalated to R-CHOP for repeated infections. Other observed adverse events were manageable and did not compromise the completion of the therapeutic program.

Our data are promising, but we have to consider some limitations including: i) the retrospective nature; ii) the absence of information about MYC translocation partners (IG versus non-IG); iii) the determination of cell of origin performed according to the Hans algorithm and not by the nanostring technology; iv) the absence of a control series of non-DEL patients with a single rearrangement or with DH/TH genotype.

In conclusion, we show a good outcome for DA-EPOCH-R in combination with HD-MTX in DEL and DEL-SH lymphomas without TP53 mutations, but the lower survival of patients DEL-DH/TH subtype or DEL with TP53 mutations requires further clinical studies aimed at testing novel agents combined with chemotherapy.

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**Table 1.** Clinical Characteristics of 122 consecutive DLBCL patients overall and according to rearrangements

| | Overall N=122 | DEL N=81 | BCL2 N=13 | MYC N=9 | HG-BCLs N=19 |
|---|---|---|---|---|---|
| Age, continuous | | | | | |
| Median (years) | 59.0 | 57.0 | 64.0 | 57.0 | 62.0 |
| third/first quartile | (49.0; 65.0) | (46.0; 64.0) | (55.0; 67.0) | (37.0; 61.0) | (57.0; 67.0) |
| Age,categorical | | | | | |
| ≤60 years old | 66 (54.1) | 48 (59.3) | 5 (38.5) | 5 (55.6) | 8 (42.1) |
| >60 years old | 56 (45.9) | 33 (40.7) | 8 (61.5) | 4 (44.4) | 11 (57.9) |
| Rearrangements | | | | | |
| None | 81 (66.4) | 81 (100) | --- | --- | --- |
| DEL-BCL2 | 13 (10.7) | --- | 13 (100) | --- | --- |
| DEL-MYC | 9 (7.4) | --- | --- | 9 (100) | --- |
| DEL-DH/TH | 19 (15.6) | --- | --- | --- | 19 (100) |
| Ki67 (%)* | | | | | |
| Median | 90.0 | 90.0 | 90.0 | 90.0 | 75.0 |
| third/first quartile | (75.0; 90.0) | (80.0; 90.0) | (85.0; 95.0) | (81,3; 91.3) | (65.0; 87.5) |
| Sex | | | | | |
| Male | 75 (61.5) | 49 (60.5) | 7 (53.8) | 6 (66.7) | 13 (68.4) |
| Female | 47 (38.5) | 32 (39.5) | 6 (46.2) | 3 (33.3) | 6 (31.6) |
| Cell of origin | | | | | |
| GCB | 55 (45.1) | 22 (27.2) | 10 (76.9) | 5 (55.6) | 18 (94.7) |
| Non-GCB | 60 (49.2) | 52 (64.2) | 3 (23.1) | 4 (44.4) | 1 (5.3) |
| Not assessed | 7 (5.7) | 7 (8.6) | 0 (0.0) | 0 (0.0) | 0 (0.0) |
| Staging | | | | | |
| I-II | 27 (22.1) | 18 (22.2) | 2 (15.4) | 4 (44.4) | 3 (15.8) |
| III-IV | 95 (77.9) | 63 (77.8) | 11 (84.6) | 5 (55.6) | 16 (84.2) |
| IPI | | | | | |
| 0-2 | 55 (45.1) | 40 (49.4) | 4 (30.8) | 6 (66.7) | 5 (26.3) |
| 3-5 | 67 (54.9) | 41 (50.6) | 9 (69.2) | 3 (33.3) | 14 (73.7) |
| CNS-IPI | | | | | |
| 0-1 | 31 (25.4) | 21 (25.9) | 3 (23.1) | 5 (55.6) | 2 (10.5) |
| 2-3 | 61 (50.0) | 47 (58.0) | 5 (38.5) | 2 (22.2) | 7 (36.8) |
| 4-6 | 30 (24.6) | 13 (16.0) | 5 (38.5) | 2 (22.2) | 10 (52.6) |
| Extranodal site (at risk for CNS) | | | | | |
| Yes | 16 (13.1) | 10 (12.3) | 0 (0.0) | 4 (44.4) | 2 (10.5) |
| No | 106 (86.9) | 71 (87.7) | 13 (100.0) | 5 (55.6) | 17 (89.5) |
| CNS Prophylaxis | | | | | |
| None | 16 (13.1) | 12 (14.8) | 0 (0.0) | 1 (11.1) | 3 (15.8) |
| IT MTX | 40 (32.8) | 24 (29.6) | 5 (38.5) | 3 (33.3) | 8 (42.1) |
| IV MTX | 66 (54.1) | 45 (55.6) | 8 (61.5) | 5 (55.6) | 8 (42.1) |
| Autologous SCT | | | | | |
| Yes | 22 (18.0) | 12 (14.8) | 3 (23.1) | 1 (11.1) | 6 (31.6) |
| No | 100 (82.0) | 69 (85.2) | 10 (76.9) | 8 (88.9) | 13 (68.4) |

*Abbreviations:* DEL, double expressor lymphomas; HG-BCLs, High-Grade B-cell Lymphomas; DH/TH, double-hit/triple-hit; GCB, germinal center lymphomas; Non-GCB, Non Germinal Center Lymphomas; IPI, International Prognostic Index; CNS, Central Nervous System; IT, intrathecal; MTX, Methotrexate; IV, Intravenous.

*10 missing values, 8 in DEL group and 1 each in DEL/BCL2 and DEL/MYC rearrangements group

**Table 2.** Kaplan-Meier estimates of 2-year Progression-free (PFS) and Overall Survival (OS) according to patients and disease characteristics

| | 2-year PFS (95% CI) | P* | 2-year OS (95% CI) | P* |
|---|---|---|---|---|
| Age | | 0.138 | | 0.064 |
| ≤60 | 80.0 (70.4; 90.9) | | 88.4 (80.0; 97.7) | |
| >60 | 67.7 (55.6; 82.4) | | 78.2 (66.9; 91.3) | |
| Sex | | 0.012 | | 0.094 |
| Male | 66.3 (55.9; 78.7) | | 80.7 (71.3; 91.3) | |
| Female | 87.1 (77.0; 98.7) | | 88.9 (79.0; 100) | |
| Cell of origin** | | 0.544 | | 0.907 |
| Germinal central B-cell | 70.6 (59.1; 84.4) | | 81.0 (69.9; 93.9) | |
| Non-Germinal central B-cell | 74.6 (63.2; 87.9) | | 84.7 (75.4; 95.2) | |
| Rearrangements | | 0.203 | | 0.058 |
| DEL | 74.8 (64.9; 86.3) | | 85.9 (77.6; 95.2) | |
| DEL-BCL2 | 69.2 (48.2; 99.5) | | 90.9 (75.4; 100) | |
| DEL-MYC | 100 | | 100 | |
| DEL-DH/TH | 63.2 (44.8; 89.0) | | 66.2 (47.4; 92.5) | |
| Staging | | 0.048 | | 0.081 |
| I-II | 91.8 (81.6; 100) | | 95.0 (85.9; 100) | |
| III-IV | 69.8 (60.6; 80.4) | | 81.0 (72.6; 90.3) | |
| International prognostic index | | 0.002 | | 0.002 |
| 0-2 | 88.1 (79.6; 97.6) | | 97.8 (93.7; 100) | |
| 3-5 | 62.2 (50.7; 76.3) | | 71.8 (60.5; 85.2) | |
| CNS Prophylaxis | | 0.027 | | 0.008 |
| None | 50.8 (28.6; 90.2) | | 64.9 (41.7; 100) | |
| Intrathecal methotrexate | 69.5 (56.5; 85.5) | | 75.8 (63.2; 91.0) | |
| Intravenous methotrexate | 81.8 (72.0; 93.0) | | 94.3 (88.2; 100) | |
| TP53 mutation*** | | 0.033 | | 0.036 |
| Wild type | 79.9 (68.9; 92.8) | | 87.7 (78.0; 98.5) | |
| Mutated | 58.3 (37.3; 91.1) | | 61.7 (39.8; 95.6) | |

*Abbreviations:* PFS, progression-free survival; OS, overall survival; CI, confidence interval; DEL, double expressor lymphomas; DH/TH, double-hit/triple-hit; CNS, central nervous system.

* Log-rank test p-value;

**Excluding 7 not-assessed patients

***Excluding 53 not-assessed patients

**Table 3.** Clinical characteristics of 69 patients evaluated for TP53 mutation status.

| | Mutated TP53 N=16 | Wild type TP53 N=53 | P* |
|---|---|---|---|
| Rearrangements | | | 0.258 |
| None | 8 (50.0) | 36 (67.9) | |
| DEL-BCL2 | 3 (18.8) | 6 (11.3) | |
| DEL-MYC | 3 (18.8) | 3 (5.7) | |
| DEL-DH/TH | 2 (12.5) | 8 (15.1) | |
| Cell of origin** | | | 0.776 |
| Germinal central B-cell | 8 (53.3) | 25 (48.1) | |
| Non-Germinal central B-cell | 7 (46.7) | 27 (51.9) | |
| Staging | | | 0.124 |
| I-II | 2 (12.5) | 18 (34.0) | |
| III-IV | 14 (87.5) | 35 (66.0) | |
| International prognostic index | | | 0.161 |
| 0-2 | 5 (31.2) | 28 (52.8) | |
| 3-5 | 11 (68.8) | 25 (47.2) | |
| Systemic CNS therapy | | | 0.241 |
| None | 0 (0.0) | 5 (9.4) | |
| Intrathecal methotrexate | 7 (43.8) | 14 (26.4) | |
| Intravenous methotrexate | 9 (56.2) | 34 (64.2) | |
| Autologous stem-cell transplantation | | | 0.334 |
| Yes | 1 (6.2) | 11 (20.8) | |
| No | 15 (93.8) | 42 (79.2) | |
| Progression-free survival | | | 0.033*** |
| 2-year estimate (95% CI) | 58.3 (37.3; 91.1) | 79.9 (68.9; 92.8) | |
| Events | 6 (37.5) | 9 (17.0) | |
| CNS relapse-free probability | | | 0.782*** |
| 2-year estimate (95% CI) | 90.0 (73.2; 100) | 92.5 (84.6; 100) | |
| Events | 1 (6.3) | 3 (5.7) | |

*Abbreviations:* DH/TH, double/triple hit; CNS, central nervous system.

*Fisher Exact test p-value

**Excluding 7 not-assessed patients

***Log-rank test p-value

**Table 4.** Results of the multivariable Cox models for Progression-free and Overall survival

| Model | Progression-free survival | | Overall survival | |
|---|---|---|---|---|
| | Hazard ratio | P* | Hazard ratio | P* |
| Rearrangements | | 0.880 | | 0.408 |
| DEL-BCL2 vs DEL | 1.49 (0.48; 4.64) | | 0.16 (0.02; 1.48) | |
| DEL-MYC vs DEL** | --- | | --- | |
| DEL-DH/TH vs DEL | 1.15 (0.46; 2.87) | | 1.00 (0.37; 2.75) | |
| TP53 mutation | | 0.072 | | 0.002 |
| Mutated vs Wild type | 3.13 (1.04; 9.40) | | 8.90 (2.14; 36.99) | |
| Not performed vs Wild type | 0.98 (0.41; 2.35) | | 0.75 (0.22; 2.53) | |
| International prognostic index | | 0.063 | | 0.018 |
| 0-2 vs 3-5 | 0.36 (0.12; 1.06) | | 0.18 (0.04; 7.4) | |
| Systemic CNS therapy | | 0.062 | | 0.019 |
| None vs Intravenous MTX | 3.74 (1.22; 11.41) | | 8.49 (1.82; 39.57) | |
| Intrathecal MTX vs Intravenous | 1.99 (0.83; 4.76) | | 4.25 (1.2; 15.02) | |
| Staging | | 0.847 | | --- |
| III-IV vs I-II | 1.19 (0.21; 6.69) | | --- | |
| Sex | | 0.045 | | --- |
| Female vs Male | 0.36 (0.14; 0.98) | | --- | |
| Age*** | | --- | | 0.752 |
| 65 vs 49 | --- | | 1.52 (0.51; 4.56) | |

*Abbreviations:* CI, confidence interval; DEL, double expressor lymphomas; DH/TH, double-

*Wald test p-value

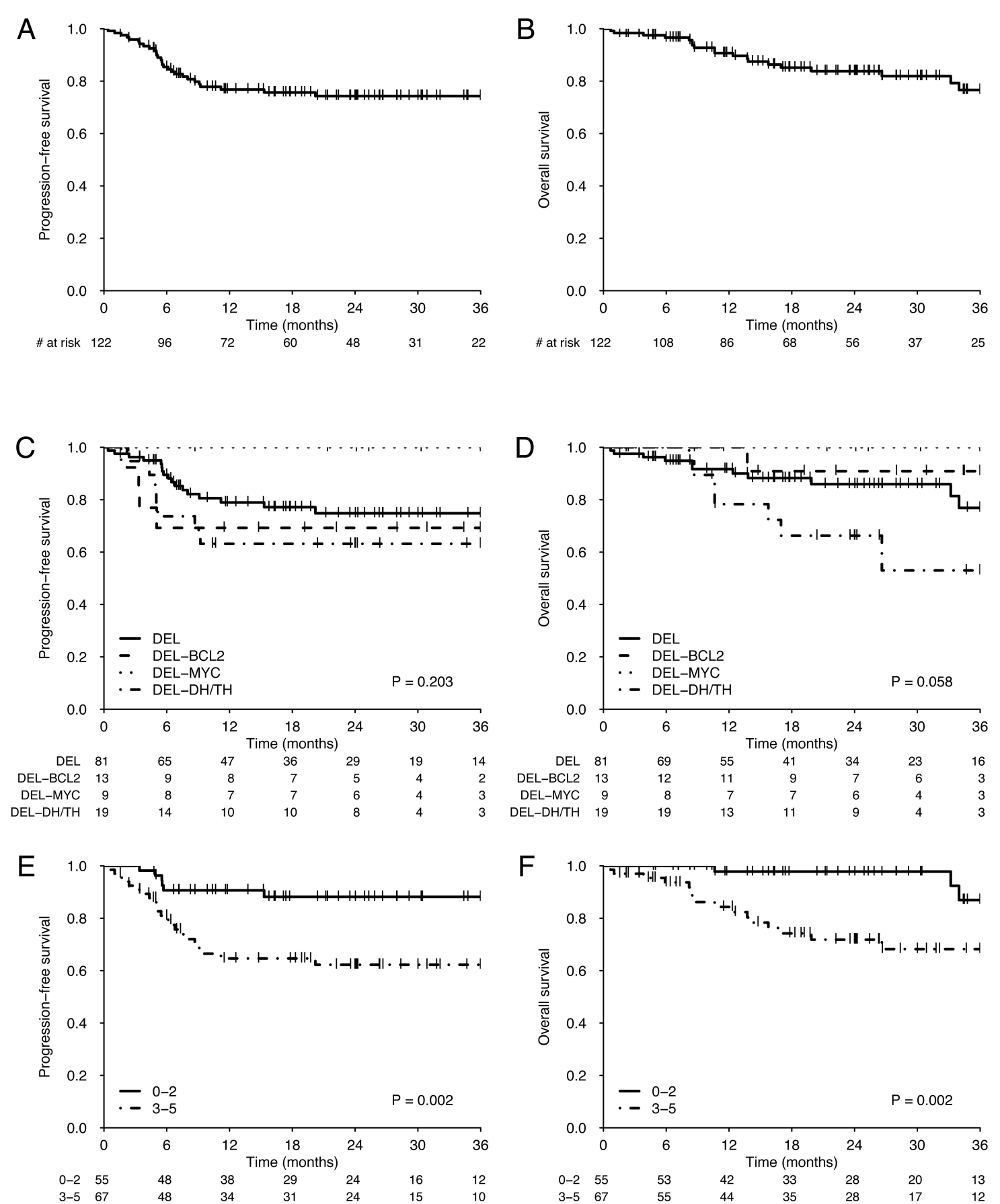
**No survival or progression events observed

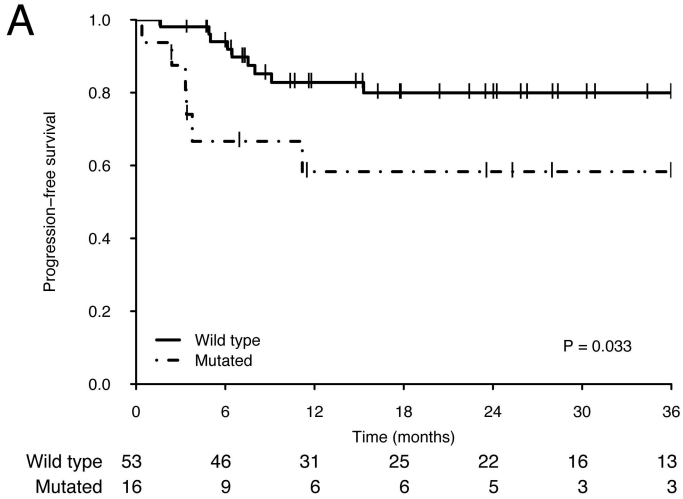
***Modeled as restricted cubic spline and reporteing result of 65 vs 49 years comparison

**Figures Legends**

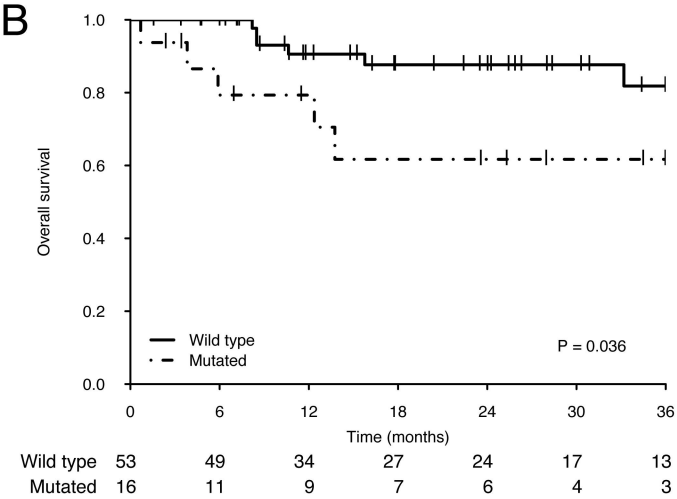
**Figure 1. Kaplan-Meier Estimates of Progression Free-Survival (PFS) and Overall Survival (OS):** PFS (panels A-C-E) and OS (panels B-D-F) for the whole cohort (panels A-B) and according to rearrangements [(panels C-D; DEL, double expressor lymphomas only; DEL-MYC, DEL-BCL2; High-grade lymphomas (DH/TH, double-hit/triple-hit)] and International Prognostic Index (panels E-F).

**Figure 2. Kaplan-Meier Estimates of Progression Free-Survival (PFS) and Overall Survival (OS) according to TP53 mutation and Central Nervous Prophylaxis:** PFS (panels A-C-E) and OS (panels B-D-F) according to TP53 mutational status (panels A-B) and central nervous system prophylaxis (none, intrathecal, intravenous methotrexate) including (panels C-D) and excluding (panels E-F) high-grade B-cell lymphomas (DH/TH).

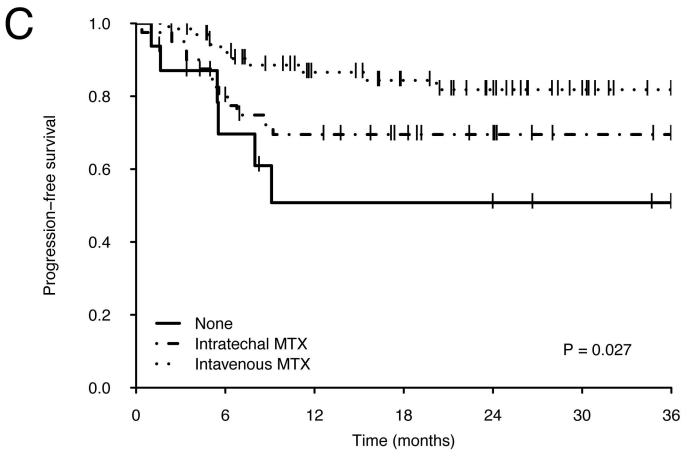

A
Progression−free survival
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30
36
Time (months)
# at risk 122 96 72 60 48 31 22
B
Overall survival
1.0
0.8
0.6
0.4
0.2
0.0
0
6
12
18
24
30
36
Time (months)
# at risk 122 108 86 68 56 37 25
C
Progression−free survival
DEL
DEL−BCL2
DEL−MYC
DEL−DH/TH
P = 0.203
Time (months)
DEL 81 65 47 36 29 19 14
DEL−BCL2 13 9 8 7 5 4 2
DEL−MYC 9 8 7 7 6 4 3
DEL−DH/TH 19 14 10 10 8 4 3
D
Overall survival
DEL
DEL−BCL2
DEL−MYC
DEL−DH/TH
P = 0.058
Time (months)
DEL 81 69 55 41 34 23 16
DEL−BCL2 13 12 11 9 7 6 3
DEL−MYC 9 8 7 7 6 4 3
DEL−DH/TH 19 19 13 11 9 4 3
E
Progression−free survival
0−2
3−5
P = 0.002
Time (months)
0−2 55 48 38 29 24 16 12
3−5 67 48 34 31 24 15 10
F
Overall survival
0−2
3−5
P = 0.002
Time (months)
0−2 55 53 42 33 28 20 13
3−5 67 55 44 35 28 17 12



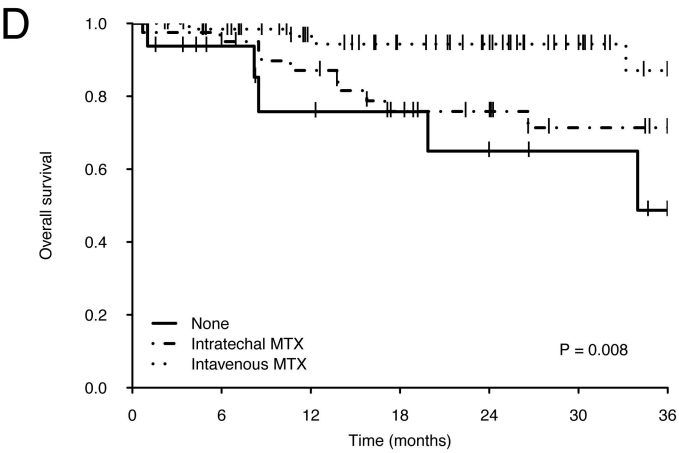

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Wild type | 53 | 46 | 31 | 25 | 22 | 16 | 13 |
| Mutated | 16 | 9 | 6 | 6 | 5 | 3 | 3 |



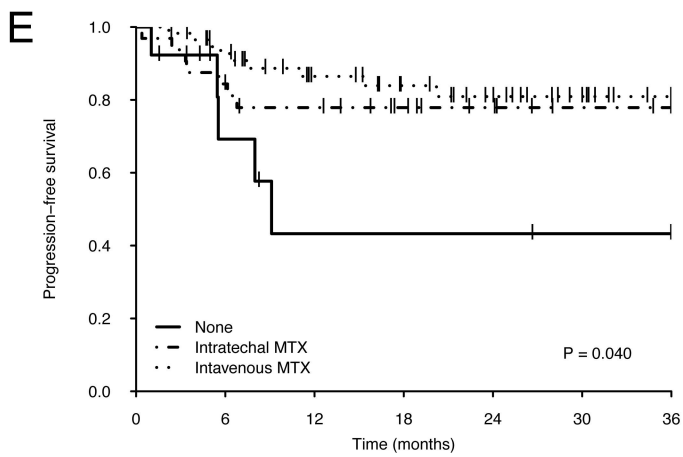

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Wild type | 53 | 49 | 34 | 27 | 24 | 17 | 13 |
| Mutated | 16 | 11 | 9 | 7 | 6 | 4 | 3 |



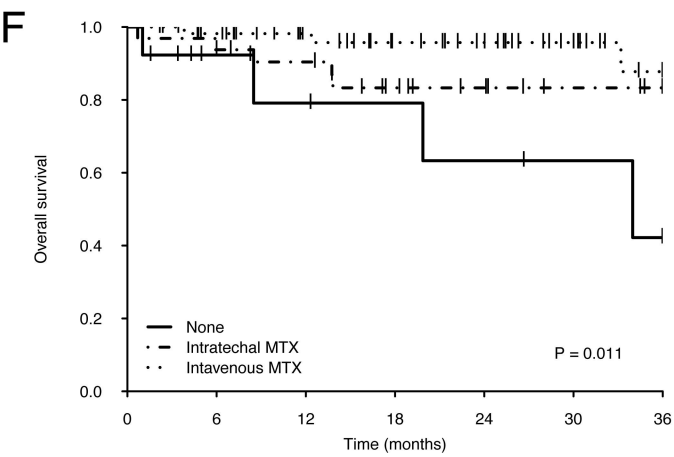

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| None | 16 | 8 | 5 | 5 | 5 | 3 | 2 |
| Intratechal MTX | 40 | 32 | 26 | 21 | 17 | 12 | 11 |
| Intavenous MTX | 66 | 56 | 41 | 34 | 26 | 16 | 9 |




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| None | 16 | 11 | 8 | 7 | 6 | 4 | 2 |
| Intratechal MTX | 40 | 38 | 33 | 24 | 20 | 14 | 12 |
| Intavenous MTX | 66 | 59 | 45 | 37 | 30 | 19 | 11 |




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| None | 13 | 6 | 3 | 3 | 3 | 2 | 2 |
| Intratechal MTX | 32 | 27 | 23 | 18 | 14 | 10 | 9 |
| Intavenous MTX | 58 | 49 | 36 | 29 | 23 | 15 | 8 |




| | | | | | | | |
|---|---|---|---|---|---|---|---|
| None | 13 | 8 | 6 | 5 | 4 | 3 | 2 |
| Intratechal MTX | 32 | 30 | 27 | 20 | 16 | 12 | 10 |
| Intavenous MTX | 58 | 51 | 40 | 32 | 27 | 18 | 10 |

## Supplementary

## Methods

*Immunohistochemistry and Fluorescence in situ hybridization analysis*

All steps were performed at room temperature. 3-3'-Diaminobenzidine tetrahydrochloride (DAB) was incubated for 10 min as a chromogen, and Mayer's hematoxylin was used for counterstaining for 10 min. The slides were dehydrated, cleared, and mounted with coverslips. We used CD10, BCL-6 , and MUM1 staining to divide all DLBCL cases into GCB or non-GCB subgroups according to the Hans[1].

Cases were considered positive for MYC if ≥40% of tumor cells were stained with the antibody. A cut-off level of 50% positive cells was used for BCL-2[2]. The cut-off values for BCL-6, MUM-1, and CD10 were set at 30%, according to recent literature. For FISH analyses, at least 100 nuclei were counted. Rearrangement was defined as the presence of break-apart signals in ≥15% of nuclei. DH and TH lymphomas were defined as the concurrent rearrangement of MYC and BCL2 and/or BCL6[3].

*Sanger Sequencing*

We performed the analysis of the TP53 mutation profile on FFPE specimens, using the Maxwell RSC, Promega for DNAs extraction ((Promega Corporation, Madison, Wisconsin, USA). Only a few biopsies were available as fresh tissues. Sanger's direct sequencing method was used for fresh biopsies. The analysis included coding sequences from exon 4 to exon 10, the most frequently involved regions). The cutoff for positivity of the mutation status was 20% of examined alleles, that is 10% of all cells. In order to establish the pathogenic role of our findings, we compared these results with the International Agency for Research on Cancer (IARC) TP53 mutation database. All the not pathogenic variants and silent mutations were considered as wild type.

*Next-Generation Sequencing*

TP53 mutations were analyzed using the Ion AmpliSeq™ TP53 Panel (Thermo Fisher Scientific, Inc, Waltham, Massachusetts, USA) designed to investigate all coding exons of

TP53 with 24 amplicons. Briefly, 40 ng of DNA extracted from FFPE diagnostic tissues were amplified, fragmented, ligated to adapters, barcoded, and clonally amplified onto beads to create DNA libraries. Following quality control analysis and quantification by the 4200 TapeStation System (Agilent Technologies, Inc., Santa Clara, CA, USA ), library mixtures were amplified and enriched. Finally, the library pool was sequenced with the Ion PGM™ Hi-Q™ sequencing kit (Thermo Fisher Scientific, Inc.). The mutation sites were analyzed by the IonTorrent variant caller plugin v5.12 according to the reference genome hg19 and the IARC TP53 database: http://p53.iarc.fr/TP53GeneVariations.aspx or TP53 web site: http://p53.fr/ and data reported as suggested. Identified mutations were confirmed by direct sequencing.

*Follow-up*

An intermediate disease assessment using CT was performed after 3 or 4 cycles of DA-EPOCH-R. Patients who exhibited less than partial response (PR) or progression of disease (PD) were shifted to second-line regimens according to institutional guidelines. Evaluation of final clinical response was performed at the end of cycle 6 using CT, PET , and bone marrow biopsy, when the biopsy was positive at disease onset. Disease assessment was performed during follow-up at 3-month intervals for the first 2 years, every six months until the 5th year, and annually thereafter. Response evaluation was assessed using the Lugano Revised Response Criteria[4].

*Statistical Analyses*

Progression-free Survival was defined as the time interval between diagnosis and disease progression or death due to any cause, whichever occurred first. Time was censored at the latest follow-up for living patients who were progression-free. Overall Survival was defined as the time interval between diagnosis to death due to any cause. Time was censored at the latest follow-up for living patients. The OS and PFS curves were estimated using the Kaplan-Meier method and the curves were compared using the log-rank test. Crude cumulative incidence of CNS relapse was estimated in a competing risk setting using cumulative incidence estimates and death without relapse was evaluated as a competing event. The

median follow-up was estimated with the reverse Kaplan–Meier method using OS data. All reported P values were two-sided.

Univariable and multivariable Cox models were performed to assess the association between the main patients and disease characteristics and the outcomes. Multivariable models included the statistically significant variables at univariable analysis.

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## Tables

**Supplementary Table 1.** Clinical Characteristics of 27 patients with limited disease at presentation

| | N=27 |
|---|---|
| Age, continuous (years) | |
| Median (third and first quartile) | 57.00 (45.00; 69.50) |
| Rearrangements | |
| DEL only | 18 (66.7) |
| DEL-BCL2 | 2 (7.4) |
| DEL-MYC | 4 (14.8) |
| DEL-DH/TH | 3 (11.1) |
| Ki67 (%)* | |
| Median (third and first quartile) | 87.50 (77.50; 90.00) |
| Sex | |
| Male | 17 (63.0) |
| Female | 10 (37.0) |
| Cell of origin | |
| Germinal central B-cell | 13 (48.1) |
| Non-Germinal central B-cell | 13 (48.1) |
| Not assessed | 1 (3.7) |
| CNS-International prognostic index | |
| 0-1 | 23 (85.2) |
| 2-3 | 4 (14.8) |
| 4-6 | 0 (0.0) |
| Systemic CNS therapy | |
| None | 2 (7.4) |
| Intrathecal methotrexate | 7 (25.9) |
| Intravenous methotrexate | 18 (66.7) |

*Abbreviations:* DH/TH, double/triple hit; CNS, central nervous system.
*3 missing values

**Supplementary Table 2.** Clinical Characteristics of 22 patients underwent Autologous Stem-cell Transplantation

| | N=22 |
|---|---|
| Age, continuous (years) | |
| Median (third and first quartile) | 59.00 (49.00; 62.00) |
| Age, categorical (years) | |
| ≤60 | 13 (59.1) |
| >60 | 9 (40.9) |
| Rearrangements | |
| DEL only | 12 (54.5) |
| DEL-BCL2 | 3 (13.6) |
| DEL-MYC | 1 (4.5) |
| DEL-DH/TH | 6 (27.3) |
| Ki67 (%)* | |
| Median (third and first quartile) | 80.00 (70.00; 86.25) |
| Sex | |
| Male | 10 (45.5) |
| Female | 12 (54.5) |
| Cell of origin | |
| Germinal central B-cell | 12 (54.5) |
| Non-Germinal central B-cell | 7 (31.8) |
| Not assessed | 3 (13.6) |
| Staging | |
| I-II | 3 (13.6) |
| III-IV | 19 (86.4) |
| International prognostic index | |
| 0-2 | 7 (31.8) |
| 3-5 | 15 (68.2) |
| CNS-International prognostic index | |
| 0-1 | 1 (4.5) |
| 2-3 | 13 (59.1) |
| 4-6 | 8 (36.4) |
| Extranodal sites risk CNS | |
| Yes | 3 (13.6) |
| No | 19 (86.4) |

*Abbreviations:* DH/TH, double/triple hit; CNS, central nervous system.

*2 missing values

**Supplementary Table 3.** Clinical characteristics of 122 consecutive Diffuse large B-cell lymphoma patients according to sex

| | Male N=75 | Female N=47 |
|---|---|---|
| Age, continuous (years) | | |
| Median (third and first quartile) | 61.00 (55.00; 65.00) | 56.00 (43.00; 63.50) |
| Age, categorical (years) | | |
| ≤60 | 36 (48.0) | 30 (63.8) |
| >60 | 39 (52.0) | 17 (36.2) |
| Rearrangements | | |
| DEL only | 49 (65.3) | 32 (68.1) |
| DEL-BCL2 | 7 (9.3) | 6 (12.8) |
| DEL-MYC | 6 (8.0) | 3 (6.4) |
| DEL-DH/TH | 13 (17.3) | 6 (12.8) |
| Ki67 (%)* | | |
| Median (third and first quartile) | 90.00 (72.50; 92.50) | 85.00 (80.00; 90.00) |
| Cell of origin | | |
| Germinal central B-cell | 35 (46.7) | 20 (42.6) |
| Non-Germinal central B-cell | 36 (48.0) | 24 (51.1) |
| Not assessed | 4 (5.3) | 3 (6.4) |
| Staging | | |
| I-II | 17 (22.7) | 10 (21.3) |
| III-IV | 58 (77.3) | 37 (78.7) |
| International prognostic index | | |
| 0-2 | 34 (45.3) | 21 (44.7) |
| 3-5 | 41 (54.7) | 26 (55.3) |
| CNS-International prognostic index | | |
| 0-1 | 21 (28.0) | 10 (21.3) |
| 2-3 | 36 (48.0) | 25 (53.2) |
| 4-6 | 18 (24.0) | 12 (25.5) |
| Extranodal sites risk CNS | | |
| Yes | 7 (9.3) | 9 (19.1) |
| No | 68 (90.7) | 38 (80.9) |
| CNS prophylaxis | | |
| None | 10 (13.3) | 6 (12.8) |
| Intrathecal methotrexate | 25 (33.3) | 15 (31.9) |
| Intravenous methotrexate | 40 (53.3) | 26 (55.3) |
| Autologous stem-cell transplantation | | |
| Yes | 10 (13.3) | 12 (25.5) |
| No | 65 (86.7) | 35 (74.5) |

*Abbreviations:* DH/TH, double/triple hit; CNS, central nervous system.

*10 missing values: 8 in male and 2 in female group

**Supplementary Table 4.** Results of the Univaraible Cox models for Progression-free and Overall Survival according to patients and disease characteristics

| | Progression-Free survival | | Overall survival | |
|---|---|---|---|---|
| | Hazard ratio (95% CI) | P* | Hazard ratio (95% CI) | P* |
| Age (continuous) | | 0,129 | | 0,205 |
| Linear | 1.51 (0.89; 2.57) | | 2.39 (0.87; 6.54)** | |
| Age | | 0,143 | | 0,073 |
| >60 vs ≤60 | 1.75 (0.83; 3.70) | | 2.35 (0.92; 5.99) | |
| Sex | | 0,018 | | 0,105 |
| Female vs Male | 0.31 (0.12; 0.81) | | 0.40 (0.13; 1.21) | |
| Cell of origin*** | | 0,545 | | 0,905 |
| GCB vs non-GCB | 1.26 (0.60; 2.64) | | 0.95 (0.38; 2.33) | |
| Rearrangements**** | | 0,188 | | 0,124 |
| DEL-BCL2 vs DEL | 1.69 (0.523; 4.38) | | 0.64 (0.07; 2.67) | |
| DEL-MYC vs DEL | 0.23 (0.00; 1.66) | | 0.32 (0.00; 2.46) | |
| DEL-DH/TH vs DEL | 1.83 (0.73; 4.17) | | 2.51 (0.95; 6.23) | |
| Staging | | 0,067 | | 0,117 |
| III-IV vs I-II | 3.84 (0.91; 16.14) | | 5.00 (0.67; 37.48) | |
| International prognostic index | | 0,004 | | 0,007 |
| 3-5 vs 0-2 | 3.71 (1.50; 9.14) | | 5.48 (1.59; 18.81) | |
| Systemic CNS therapy | | 0,037 | | 0,019 |
| None vs Intravenous MTX | 3.61 (1.31; 9.96) | | 6.47 (1.73; 24.17) | |
| Intrathecal MTX vs Intravenous MTX | 2.08 (0.90; 4.81) | | 3.56 (1.11; 11.36) | |
| TP53 mutation***** | | 0,042 | | 0,049 |
| Mutated vs Wild type | 2.93 (1.04; 8.25) | | 3.30 (1.01; 10.82) | |

*Abbreviations:* CI, confidence interval; GCB, Germinal central B-cell; DEL, double expressor lymphomas; DH/TH, double-hit/triple-hit; CNS, central nervous system, MTX, methotrexate.

*Wald test p-value

**Modeled as restricted cubic spline and reporteing result of 65 vs 49 years comparison

***Excluding 7 not-assessed patients

****Performed with Firth's penalized maximum likelihood bias reduction method

*****Excluding 53 not-assessed patients

**Supplementary Table 5.** Clinical characteristics of 122 consecutive Diffuse large B-cell lymphoma patients according to central nervous system prophylaxis

| | None N=16 | Intrathecal MTX N=40 | Intravenous MTX N=66 |
|---|---|---|---|
| Age, continuous (years) | | | |
| Median (third and first quartile) | 65.00 (61.25; 72.00) | 57.50 (48.75; 63.00) | 57.00 (46.25; 64.00) |
| Age, categorical (years) | | | |
| ≤60 | 4 (25.0) | 23 (57.5) | 39 (59.1) |
| >60 | 12 (75.0) | 17 (42.5) | 27 (40.9) |
| Rearrangements | | | |
| None | 12 (75.0) | 24 (60.0) | 45 (68.2) |
| DEL-BCL2 | 0 (0.0) | 5 (12.5) | 8 (12.1) |
| DEL-MYC | 1 (6.2) | 3 (7.5) | 5 (7.6) |
| DEL-DH/TH | 3 (18.8) | 8 (20.0) | 8 (12.1) |
| Ki67 (%)* | | | |
| Median (third and first quartile) | 90.00 (82.50; 90.00) | 90.00 (75.00; 95.00) | 85.00 (77.50; 90.00) |
| Sex | | | |
| Male | 10 (62.5) | 25 (62.5) | 40 (60.6) |
| Female | 6 (37.5) | 15 (37.5) | 26 (39.4) |
| Cell of origin | | | |
| Germinal central B-cell | 7 (43.8) | 20 (50.0) | 28 (42.4) |
| Non-Germinal central B-cell | 8 (50.0) | 17 (42.5) | 35 (53.0) |
| Not assessed | 1 (6.2) | 3 (7.5) | 3 (4.5) |
| Staging | | | |
| I-II | 2 (12.5) | 7 (17.5) | 18 (27.3) |
| III-IV | 14 (87.5) | 33 (82.5) | 48 (72.7) |
| International prognostic index | | | |
| 0-2 | 5 (31.2) | 15 (37.5) | 35 (53.0) |
| 3-5 | 11 (68.8) | 25 (62.5) | 31 (47.0) |
| CNS-International prognostic index | | | |
| 0-1 | 1 (6.2) | 8 (20.0) | 22 (33.3) |
| 2-3 | 9 (56.2) | 19 (47.5) | 33 (50.0) |
| 4-6 | 6 (37.5) | 13 (32.5) | 11 (16.7) |
| Extranodal sites risk CNS | | | |
| Yes | 0 (0.0) | 5 (12.5) | 11 (16.7) |
| No | 16 (100.0) | 35 (87.5) | 55 (83.3) |
| Autologous stem-cell transplantation | | | |
| Yes | 0 (0.0) | 8 (20.0) | 14 (21.2) |
| No | 16 (100.0) | 32 (80.0) | 52 (78.8) |

*Abbreviations:* MTX, Methotrexate; DH/TH, double/triple hit; CNS, central nervous system.

**10 missing values: 1, 2, and 7 not treated, intrathecal methotrexate, and intravenous methotrexate group, respectively*

**Supplementary Figure 1:** Progression-Free Survival (A) and Overall Survival (B) in patients who underwent or not Autologous Stem Cell Transplantation.