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MYD88 mutations identify a molecular subgroup of diffuse large B-cell lymphoma with an unfavourable prognosis

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Running heads

MYD88 mutational status improves classification and prognostication in DLBCL

Key words

MYD88, mutational status, classification, prognostication, DLBCL

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Abstract

The 2016 WHO classification defines diffuse large B-cell lymphoma subtypes based on EBV infection and oncogenic rearrangements of *MYC*/*BCL2*/*BCL6* as drivers of lymphomagenesis. A subset of diffuse large B-cell lymphoma, however, is characterized by activating mutations in *MYD88*/*CD79B*. We investigated whether *MYD88*/*CD79B* mutations could improve the classification and prognostication of diffuse large B-cell lymphomas.

In 250 primary diffuse large B-cell lymphomas, *MYD88*/*CD79B* mutations were identified by allele-specific PCR or next-generation-sequencing, *MYC*/*BCL2*/*BCL6* rearrangements were analyzed by FISH, and EBV was studied by EBER-ISH. Associations of molecular features with clinicopathologic characteristics, outcome, and prognosis according to International Prognostic Index were investigated.

MYD88 and *CD79B* mutations were identified in 29.6% and 12.3%, *MYC, BCL2,* and *BCL6* rearrangements in 10.6%, 13.6%, and 20.3%, and EBV in 11.7% of diffuse large B-cell lymphomas, respectively. Prominent mutual exclusivity between EBV positivity, rearrangements, and *MYD88*/*CD79B* mutations established the value of molecular markers for recognition of biologically distinct diffuse large B-cell lymphoma subtypes. *MYD88*-mutated diffuse large B-cell lymphoma had a significantly inferior 5-year overall survival than wild-type *MYD88* diffuse large B-cell lymphoma (log-rank;P=0.019). Diffuse large B-cell lymphoma without any of the studied aberrations had superior overall survival compared to cases carrying ≥1 aberrancy (log-rank;P=0.010). *MYD88* mutations retained their adverse prognostic impact upon adjustment for other genetic and clinical variables by multivariable analysis and improved the prognostic performance of the International Prognostic Index.

This study demonstrates the clinical utility of defining *MYD88*-mutated diffuse large B-cell lymphoma as a distinct molecular subtype with adverse prognosis. Our data call for sequence analysis of *MYD88* in routine diagnostics of diffuse large B-cell lymphoma to optimize classification and prognostication, and to guide the development of improved treatment strategies.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is characterized by substantial heterogeneity in tumor biology and clinical behavior.[1, 2] Currently, rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) is used as a 'one-size-fits-all' treatment. Unfortunately, a considerable percentage of patients will experience chemorefractory disease or relapse, resulting in a 5-year overall survival (OS) of approximately 60%.[3] Particularly, patients with chemorefractory disease or an early relapse have a poor prognosis. For optimal counseling, DLBCL patients are categorized in risk groups according to the International Prognostic Index (IPI).[4] The IPI consists of clinical and biochemical parameters, but does not include tumor biological characteristics or provide any indication for precision medicine.[5]

The recently updated World Health Organization (WHO) classification of lymphoid neoplasms (2016) recognizes this heterogeneity by including selected drivers of lymphomagenesis for subclassification of DLBCL, i.e. the delineation of high-grade B-cell lymphomas (HGBL) with *MYC* and *BCL2* and/or *BCL6* rearrangements, and of Epstein-Barr virus-positive (EBV+) DLBCL.[6] *MYC, BCL2,* and *BCL6* rearrangements are found in respectively 4-14%, 20-30%, and ~20% of DLBCLs.[7-9] HGBLs comprise approximately 5-10% of all DLBCLs.[9-11] It is thought that the combination of MYC-stimulated cell proliferation and anti-apoptotic effects of BCL2 in HGBL cause aggressive growth, relative resistance to therapy, and inferior OS.[12] In addition, Asian studies showed a frequency of 1-14% EBV positivity in DLBCLs and an association with inferior survival.[13, 14] EBV-associated viral proteins, such as latent membrane proteins (LMP)-1/2 and nuclear antigens, stimulate proliferation of B-cells via activation of nuclear factor-kappa-B (NF-κB), regulate immune evasion, and inhibit apoptosis.[13]

In the search for additional oncogenic drivers and to discriminate different molecular DLBCL subtypes, large next-generation-sequencing (NGS) studies have revealed specific mutational profiles that reflect the dysregulation of distinct intracellular pathways, including epigenetic regulation and NF-κB, Toll-like receptor (TLR), and B-cell receptor (BCR) signalling.[1, 2, 15, 16] Recurrent 'hotspot' mutations in *MYD88* (L265P) and *CD79B (*Y196) belong to the most prevalent sequence alterations in DLBCL. By altering the toll/interleukin-1 receptor domain of *MYD88,* the L265P increases interaction and consecutive phosphorylation of downstream targets, potentially without external stimuli from the TLR.[17] The connection of MYD88 with BCR signalling within the so-called 'My-T-BCR' supercomplex facilitates activation of the NF-κB pathway via TLR9.[2] Hotspot mutations, such as Y196, in the *CD79B* subunit of the BCR lead to increased BCR expression and inhibition of feedback in the BCR signalling pathway by attenuating downstream Lyn kinase. Therefore, *CD79B* mutations are thought to contribute to lymphomagenesis by enhancing chronic active BCR signalling.[18]

Both *MYD88* and *CD79B* mutations are more prevalent in the so-called non-germinal center B-cell (GCB)-type DLBCL according to the cell-of-origin (COO) concept, originally developed based on

gene expression profiling.[1, 2, 19] In addition, the prevalence of these mutations varies greatly among DLBCL originating at different anatomical sites. We recently described a high percentage of *MYD88* L265P and *CD79B* Y196 mutations in intravascular large B-cell lymphomas (44% *MYD88* and 26% *CD79B*).[20] A high frequency of these mutations has also been found in other extranodal DLBCL, such as primary cutaneous DLBCL, leg type,[21] orbita/vitreoretinal DLBCL,[22-24] primary breast DLBCL,[25] and DLBCL presenting at immune-privileged (IP) sites, i.e. primary testicular DLBCL (PTL)[26] and primary central nervous system B-cell lymphoma (PCNSL).[27-29] Several studies have shown that *MYD88* mutations are associated with inferior OS in DLBCLs compared to wildtype *MYD88*.[30, 31]

Despite the increasing knowledge of the landscape of genetic drivers in DLBCL, the clinical implications of different oncogenic driver mutations remain unclear,[32] and the R-CHOP regimen is used as a uniform treatment. Since patients with chemorefractory disease or relapses after R-CHOP have a poor outcome, global 5-year OS in DLBCL is approximately 60%.[3] While HGBL patients have been recognized as a particularly unfavorable subgroup, prognostication for the remaining DLBCLs is based on clinical and biochemical parameters that define the IPI as well as primary extranodal manifestations.[4, 5] In contrast, the prognostic significance and interaction of mutations in *MYD88* and *CD79B* with standard molecular aberrations (as designated by the WHO 2016) have not yet been conclusively elucidated. Therefore, the present study investigated whether assessment of the mutational status of *MYD88* and *CD79B* would improve classification and prognostication of DLBCL.

Methods

Patient cohort

This retrospective study investigated a cohort of 250 primary DLBCLs. DLBCL patients were diagnosed between 2000-2016 at the Amsterdam University Medical Center, location AMC (AUMC), the Leiden University Medical Center (LUMC), and their affiliated hospitals. In all cases, diagnosis was centrally revised following the WHO classification 2008. A subset of this cohort was previously published without survival analysis.[28, 29] As our academic hospitals are tertiary referral centers, this cohort is enriched for IP locations. Formalin-fixed and paraffin-embedded (FFPE) tissue samples were obtained during standard diagnostic procedures. The study was performed in accordance with the Dutch Code for Proper Secondary Use of Human Tissue in accordance with the local institutional board requirements and the revised Declaration of Helsinki 2008 and was approved by the medical ethics committees of both the AUMC (W15_213#15.0253) and the LUMC (B16.048). Patients were eligible in case tissue was available and *MYD88* mutational analysis was successful.

Histopathologic and molecular characterization

In the majority, immunohistochemistry was performed for CD20, CD10, BCL6, MUM1, and BCL2. The Hans' algorithm was used for COO classification.[33] EBV status was assessed by EBV-encoded RNA *in situ* hybridization. *MYC*, *BCL2,* and *BCL6* rearrangements were analyzed by fluorescence *in situ* hybridization (FISH) using break-apart probes. Antibodies and probes are depicted in supplemental table-1.[20, 29] In the AUMC, DNA was isolated using the QIAamp DNA Micro kit (Qiagen) and mutational status of *MYD88* and *CD79B* was established by allele-specific PCR, followed by mutation-specific primers and confirmed by Sanger sequencing, as described before.[28, 29] In the LUMC, DNA isolation was automatically performed with the TPS robot (Siemens Healthcare Diagnostics), as presented previously.[34] The Ampliseq Cancer Hotspot Panel V.2-V.4 (Thermo Fisher Scientific) was used for detection of variants in *MYD88* (exons 3&5) and *CD79B* (exons 5&6). The minimum coverage threshold was 100 on-target reads with a minimum variant allele frequency of ≥10% of the reads. Variants were analyzed using Geneticist Assistant NGS Interpretative Workbench (v.1.4.15, SoftGenetics, State College). As described, identified variants were classified into five classes based upon potential pathogenicity and only class 4 (possibly pathogenic) and class 5 variants (pathogenic) were reported.[35]

Statistical analysis

The correlation between clinicopathologic parameters and biological aberrations was examined with the Chi-square test or ANOVA. The Kaplan-Meier method was applied to estimate 5-year OS and progression free survival (PFS). The starting point for time-to-event analysis was date of histological diagnosis. An event for OS was defined as death by any cause. An event for PFS was

determined as relapse, disease progression, or death by any cause (whatever came first). If patients received palliative treatment and no remission evaluation was performed during follow-up, an event for progression was defined at 3 weeks before patients succumbed to their disease. Observational intervals of patients without any event at time of last follow up or at 5 years after diagnosis were censored. Median follow up time for the whole cohort was determined by use of reverse Kaplan-Meier.[36] The log-rank test was performed to compare risk groups. The Cox proportional-hazards model was used to estimate hazard ratios (HR) including 95% confidence intervals (95%-CI). Adjusted HRs were obtained in a multivariable Cox model. Competing risks analysis was used to estimate the cumulative incidences of relapse/progression, with non-relapse mortality considered as competing risk. Gray's test was performed to compare cumulative incidences, whereas a cause-specific Cox proportional-hazards model was used to estimate the impact of risk factors on them.[37] The incremental prognostic value of *MYD88* and/or *CD79B* was assessed by comparing Harrell's cross-validated C statistic for Cox models with and without *MYD88* and/or *CD79B*.[38] All statistical analyses were performed using SPSS software (version 23, IBM SPSS statistics) and RStudio (version 1.1442, RStudio, Inc. packages survival, prodlim, dynpred and cmprsk). P-values were two-sided and $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Table-1 depicts the baseline characteristics of the 250 DLBCL patients (AUMC N=224 patients and LUMC N=26 cases). The median age at diagnosis was 61.4 years (range 18.6-89.6). A total of 38 DLBCL patients were immune-compromised, due to inherited conditions (severe combined immunodeficiency disorder, common variable immunodeficiency disorder), HIV infection, or extended use of therapeutic immunosuppression necessitated by organ transplantation or auto-immune disorders. Based on anatomical locations, 75 patients (30.0%) had strictly nodal DLBCL and in 67 patients (26.8%) the lymphoma presented in IP sites: 33 patients with PTL and 35 patients with PCNSL of whom one patient had testicular and CNS locations synchronously. The remaining 108 patients (43.2%) had extranodal disease in non-IP sites (with or without nodal involvement). With respect to staging, PCNSL was considered as advanced disease equivalent to Ann Arbor Stage IV for assignment of the IPI and subsequent statistical analyses. With this definition, 83 patients (33.5%) were categorized as having regional disease (Ann Arbor stage I-II) and 165 patients (66.5%) had advanced disease (stage III-IV). Sixty-one patients (25.3%) had an IPI risk score of 0/1, 148 patients (61.4%) an IPI of 2-3, and 32 patients (13.3%) an IPI of 4-5. The IPI of 9 patients was unknown. The majority of (extra)nodal and testicular DLBCL patients were treated with R-CHOP (N=160), CHOP (N=25), or (R)CHOP-like treatments (N=5) with curative intent. Curative treatment regimens incorporating high-dose methotrexate were initiated for 23 patients with PCNSL. Because of older age, poor clinical Eastern Cooperative Oncology Group Performance Status (ECOG-PS), or patients' refusal of treatment, 34 patients received palliative care only, mainly with steroids or (local) radiotherapy. The median follow up time was 6.6 years (range 0.1-15.7).[36]

Molecular characterization: mutated *MYD88* discriminates a distinct DLBCL subgroup

According to the Hans' algorithm, DLBCLs were classified as GCB (N=100, 40.0%), non-GCB (N=130, 52.0%), or unclassifiable (N=20, 8.0%), with no statistical difference between nodal, extranodal, and IP locations (P=0.228)(table 2).[33]

In 198 patients (79.2%), molecular analysis for *MYD88* and *CD79B* mutations, *MYC, BCL2,* and *BCL6* rearrangements, and EBV infection was complete, whereas in 52 patients, partial data sets were available (figure-1; table-2). *MYD88* mutations were identified in 74 cases (29.6%), of whom 67 harbored the hotspot L265P mutation. The other *MYD88* variants were S219C (N=5) and S243N (N=2). In line with a published meta-analysis,[30] mutated *MYD88* was significantly correlated to older age (≥65 years), anatomical lymphoma location, and non-GCB subtype (P=0.006; P<0.001; P=0.042, respectively). *CD79B* mutations were detected in 29 patients (12.3%), including the hotspot Y196 mutation (N=28) and the L188 mutation (N=2, one patient had both mutations). *MYC, BCL2,* and *BCL6*

were rearranged in 23 (10.6%), 30 (13.6%), and 44 (20.3%) DLBCLs, respectively, with a total of nine HGBL patients (4.1%).

As suggested by previous reports and other studies, *MYD88* and *CD79B* mutations were significantly more common in IP-DLBCL (67.2% resp. 25.8%) compared to nodal (17.3% resp. 4.1%) and other extranodal sites (14.8% resp. 9.3%)(P<0.001 and P<0.001).[26, 29, 39] In contrast, *BCL2* rearrangements were more prevalent in nodal and extranodal DBLCL (P=0.001), whereas *MYC* and *BCL6* rearrangements were evenly distributed across the anatomical sites. EBV was positive in 28 patients (11.7%) and was not associated with anatomical location (P=0.091).

In the 198 cases with complete molecular analysis, hardly any overlap between the presence of oncogenic rearrangements, EBV positivity, or *MYD88* and/or *CD79B* mutations was observed (figure-2A), suggesting that they represent distinct DLBCL subgroups with different drivers of lymphomagenesis. *CD79B* mutations co-occurred with *MYD88* mutations in 18 of 23 cases (78.2%). In contrast, *MYD88* mutations co-occurred with any rearrangement in only seven of 60 patients (11.7%) and with EBV positivity in only one case (1.7%). EBV infection was detected in only three out of 71 cases (4.2%) with a rearrangement. In 51 patients (25,8%) with full molecular characterization, no aberrancy was detected.

Mutated MYD88 predicts inferior survival

All outcomes are reported at 5-year survival. For the entire cohort, OS was 61.0% (95%-CI 55.1-67.5) and PFS was 52.6% (95%-CI 46.6-59.3). Cumulative incidences of relapse/progression and non-relapse mortality were 37.2% (95%-CI 31.2-43.3) and 10.1% (95%-CI 6.4-13.9), respectively. Figure-3 shows survival outcomes presented for anatomical location, IPI-score, and *MYD88* status. Survival outcomes of COO and the other aberrations are outlined in supplemental figure-2 (none of these factors had a significant impact).

The IPI clearly predicted OS (figure-3): patients with IPI scores of 0/1, 2/3, and 4/5 had an OS of 84.9% (95%-CI 76.3-94.5), 58.0% (95%-CI 50.3-66.8), and 34.4% (95%-CI 21.3-55.5), respectively. IPI also showed a significant difference in cumulative incidences of relapses (Gray's; P=0.025) and non-relapse mortality (Gray's; P=0.006). In addition to the IPI, DLBCL with IP locations had inferior outcomes (OS 47.1%, 95%-CI 36.5-60.9; PFS 41.0%, 95%-CI 30.7-54.9) compared to nodal (OS 71.2%, 95%-CI 61.4-82.4; PFS 55.7%, 95%-CI 45.3-68.6) and other extranodal sites (OS 62.6%, 95%-CI 53.9-72.7; PFS 58.1%, 95%-CI 49.4-68.2) (log-rank; P=0.004 and P=0.024). This unfavorable prognosis was particularly associated with CNS location. Within the IP group, patients with CNS location had a significant inferior 5-year OS of 29.9% (95%-CI 17.7-50.5) compared to 65.5% (95%-CI 50.9-84.3%) for PTL (log-rank; P=0.003).

With respect to molecular markers, patients without any detected aberrancy demonstrated a good-risk profile with superior OS (78.0%, 95%-CI 67.2-90.4, versus 56.3%, 95%-CI:48.6-65.2; figure-

2B) (log-rank; P=0.010) and PFS (65.4%, 95%-CI 53.2-80.3, versus 48.2%, 95%-CI 40.6-57.3; figure-2C) (log-rank; P=0.031) compared to patients who had one or more aberration(s). The cumulative incidence of relapse/progression for this good-risk profile was 28.6% (95%-CI 15.8-41.4) compared to 39.3% (95%-CI 31.2-47.4) (Gray's; P=0.155). This good risk profile included patients with lower ECOG-PS, age<60 years, and more GCB subtypes (Chi square; P=0.012, P=0.001, and P=0.006, respectively) compared to patients with one or more aberrations. Patients in the good risk category seem to be susceptible for immune-chemotherapy with enduring responses, however, the molecular background of this subgroup remains unknown. In IP-DLBCL, a total of 93.8% of the patients were classified in the risk group with ≥1 aberrations.

MYD88-mutated DLBCLs had a significantly inferior 5-year OS compared to DLBCL with wildtype *MYD88* (log-rank; P=0.019; HR 1.64, 95%-CI 1.08-2.48) and significantly inferior 5-year PFS (log-rank; P=0.049; HR 1.46, 95%-CI 1.00-2.14). Employing competing risk analysis, *MYD88*-mutated DLBCLs revealed significantly higher relapse rates (46.6%, 95%-CI 35.1-58.1) than cases with wildtype *MYD88* (33.3%, 95%-CI 26.2-40.4)(Gray's; P=0.029; CSH 1.62, 95%-CI 1.06-2.48), while non-relapse mortality showed no significant difference (Gray's; P=0.832). Mutated *CD79B* showed higher cumulative incidence for relapse/progression (56.3%, 95%-CI 37.9-74.8) versus wildtype *CD79B* (35.1%, 95%-CI 28.5-41.8)(Gray's; P=0.019, CSH: 1.82, 95%-CI 1.06-3.14), whereas no significant difference was found for OS (HR 1.43, 95%-CI 0.81-2.53).

Despite relatively high HRs, none of the other molecular aberrations was a significantly adverse prognostic factor for OS (table-3), which can be explained by lack of power due to the low incidence of these aberrations. For these molecular data, univariate cause-specific hazards for relapse/progression showed similar results. The nine HGBLs had an OS of 50.0% (95%-CI 24.1-100) compared to 63.6% (95%-CI 57.3-70.6) (log-rank; P=0.628) for non-HGBLs.

Prognostic significance of *MYD88* mutations in multivariable analysis

To evaluate the prognostic impact of mutated *MYD88* on survival outcomes in addition to other molecular aberrations and the IPI, the initial multivariable Cox regression model included the standard individual IPI risk factors (Model 1, table-3A/3B). In the second model, the current WHO 2016 molecular aberrations (EBV and oncogenic rearrangements) were added. In the third model, also *MYD88* and *CD79B* mutations were included. *MYD88* mutations showed prognostic significance for OS (HR 1.87, 95%-CI 1.10-3.20) in addition to ECOG-PS (≥2) (HR 8.16, 95%-CI 4.90-13.59) and Ann Arbor stage (III/IV) (HR 1.84, 95%-CI 1.04-3.25). In this third model, oncogenic rearrangements, mutated *CD79B*, elevated LDH, and age (>65 years) did not have a significant impact. The performance of the IPI prognostic model was improved by adding all molecular aberrations and mutated *MYD88* and *CD79B* as risk factors, as indicated by an increase in cross-validated C-index (CVC) from 0.67 to 0.70. *MYD88* did not have significant impact on cause-specific survival (HR 1.42,

95%-CI 0.85-2.37), whilst ECOG-PS, Ann Arbor stage, and extranodal location were prognostic in this model.

Further multivariable analyses were performed to evaluate the prognostic significance of *MYD88* mutational status in comparison to COO subtype or anatomical lymphoma location. COO subtype did not improve the performance of models 2 and 3 (results not shown). However, the prognostic impact of model 2 was improved by adding anatomical lymphoma location (CVC index = 0.71, model 4, presented in supplemental table-1) and outperforms model 2 (table-3A, CVC index = 0.69, including the IPI factors and molecular aberrations of WHO 2016). Model 4 demonstrated a nearly identical prognostic performance when compared to model 3 (CVC index = 0.70, including the IPI factors, molecular aberrations of WHO 2016 and the mutational status of *MYD88* and *CD79B*). When adding the mutational status of *MYD88* and *CD79B* to model 4, the performance of this model 5 was not improved (CVC index 0.71, supplemental table-1). As such, the prognostic impact of the *MYD88* mutational status on mortality was not superior to anatomical lymphoma location.

Next, we explored whether mutated *MYD88* could improve the prognostic performance of the currently used IPI risk model (table-4). Inclusion of the IPI as continuous variable (0-5 points) and the *MYD88* status in the multivariable analysis demonstrated an independent and similar impact of mutated *MYD88* (HR 1.83, 95%-CI 1.19-2.80) and IPI (HR 1.77, 95%-CI 1.47-2.13) on OS. Similar effects were observed for cause-specific survival (table-4). For the models OS and relapse/progression, an increase in CVC-index was observed from 0.57 to 0.61 and 0.53 to 0.57, respectively. Altogether, these multivariable survival analyses demonstrated the significant prognostic importance of mutated *MYD88,* next to (genetic) aberrations and clinical/biochemical variables, and the improvement of adding mutated *MYD88* to the prognostic performance of the IPI.

To evaluate possible confounding of the impact of mutated *MYD88* and the outcomes by anatomical lymphoma location, we performed a sensitivity analysis for OS on the cohort stratified by anatomical lymphoma location, including CNS involvement. For patients with CNS involvement (N=35), *MYD88* had an unadjusted HR of 1.94 (95%-CI 0.77-4.90) in univariable analysis. For patients without CNS involvement (N=215), *MYD88* did not have a significant impact on OS with an adjusted HR of 1.81 (95%-CI 0.96-3.42), when applying multivariable analysis as described for model-3 (table-3B). Although not statistically significant, the adjusted HR for this subgroup was similar to the original HR for the entire cohort.

Discussion

To the best of our knowledge, this is the first study evaluating the clinical significance of mutated *MYD88* and *CD79B* in DLBCL, in addition to the oncogenic drivers that are currently included in the WHO classification 2016 (EBV status and *MYC, BCL2, and BCL6* rearrangements), the IPI risk factors, and well-defined anatomical locations.

The strength of this study is the large number of patients with good clinical annotation and complete molecular analysis (N=198). In addition, our study shows that the incorporation of mutational status of *MYD88* into a clinical/biochemical risk score as the IPI is feasible. An increase in the predictive performance of the IPI risk model as is illustrated by an increase in CVC-index, suggests that this model can be improved by the introduction of molecular aberrations. However, interpreting the results, we have encountered several limitations. *MYD88*-mutated DLBCLs more often had extranodal location, older age (and thus a high IPI), and non-GCB subtype. Therefore, these patients were more frequently subjected to palliative care. Possibly interaction between treatment and mutated *MYD88* has not been tested as more data is needed. We present an average effect over different treatment modalities. Since reported frequencies and survival outcomes are similar to previous literature, our cohort appears to be representative for the target population.[3, 7-9, 13] To investigate the prognostic significance of mutated *MYD88* adjusted for the IPI for the entire cohort, we considered PCNSL as advanced disease stage, although it is not common practice to apply the IPI in PCNSL patients. Additionally, our cohort is enriched for IP locations. Therefore, a sensitivity analysis was performed excluding PCNSL patients, demonstrating that the adjusted HR of *MYD88* for OS was similar to the entire cohort. This indicates that our results are not affected by confounding by CNS localisation. Hence, we believe that our data corroborate the clinical relevance of mutant *MYD88* for diagnostic classification and prognostication of DLBCL and support implementation of *MYD88* mutational analysis in routine diagnostics. The simplicity and accessibility to examine *MYD88* mutations and associated low costs permit an efficient timely implementation. In addition, *CD79B* mutations were prognostic in univariate analysis, but when adjusted for other aberrations in the multivariable analysis the prognostic importance disappeared. This finding may be explained by the prominent overlap between *MYD88* and *CD79B* mutations, as 78.2% of mutated *CD79B* had co-occurring *MYD88* mutations.

An important result of our study is the recognition of prominent mutual exclusivity between the presence of mutations in *MYD88* and/or *CD79B, MYC, BCL2,* and *BCL6* rearrangements, and EBV infection, indicating that *MYD88* and/or *CD79B*-mutated tumors present a distinct DLBCL subcategory. In accordance with a large meta-analysis and two other studies,[30, 40, 41] *MYD88* L265P mutations were preferentially found in specific anatomical sites (e.g. testis and CNS) and were significantly associated with non-GCB subtypes, older age, and poor OS. However, the published literature study did not explicitly analysed IP sites, nor evaluated the interaction of *MYD88* mutations

with EBV status or oncogenic rearrangements in multivariable analysis. Other NGS studies have recently demonstrated high frequencies of mutated *MYD88* (15-18%) in large cohorts of DLBCLs.[1, 2, 15, 42-44] Besides a certain association of mutated *MYD88* with poor OS (e.g. in non-GCB DLBCL), cluster analysis of multiple genes indicated distinct DLBCL subentities, including mutated *MYD88* as an important classifier for NF-κB pathway activation. Again, these NGS studies did not take into account specific anatomical sites or investigated the interaction and prognostic significance of mutated *MYD88* in correlation with EBV status or *MYC, BCL2,* and *BCL6* rearrangements.

In this context, our study adds important new knowledge by demonstrating *MYD88* mutations as an adverse prognostic factor for OS and relapse/progression in a multivariate analysis that takes all major known clinical and WHO classification-defined risk factors into account. This insight does not only show that the incorporation of mutational status of *MYD88* into a clinical/biochemical risk score as the IPI is feasible, but also highlights the importance of assessing *MYD88* at time of diagnosis for optimal classification and patient counselling. An increase in the predictive performance of the IPI risk model, as is illustrated by an increase in CVC-index, formally suggests that this model can be improved by the introduction of molecular aberrations. However, the prognostic impact of the *MYD88* mutational status on the presented multivariable models was not superior to anatomical lymphoma location. Whether the *MYD88* mutational status outperforms the predictive performance of anatomical lymphoma location in the described prognostic models needs further validation in an external cohort. Of note, no difference was found for non-relapse mortality, indicating that mutated *MYD88* is a lymphoma-specific poor prognostic factor. Routine diagnostic assessment of *MYD88* mutations is likely to gain decisive importance for DLBCL since several approaches may therapeutically target MYD88.[45] Several studies have indicated that DLBCLs with mutated *MYD88* and/or *CD79B* are more sensitive to Bruton's Tyrosine Kinase (BTK)-inhibitors.[46-48] As such, objective analysis of *MYD88* mutations will not only improve diagnostic classification and prognostication, but might also enable patient selection for precision medicine such as with BTK-inhibitors. However, the predictive significance of mutated *MYD88* with or without *CD79B* mutations needs to be validated in upcoming clinical trials, including precision medicine targeting the BCR and TLR cascades.

Finally, as a corollary of this study, we identified a novel good risk DLBCL group characterized by the absence of detected genetic aberrations. These DLBCLs appeared to be highly sensitive to standard immune-chemotherapy as first-line treatment. Future studies, employing a larger NGS targeted gene panel, may elucidate the genetic drivers in this group. We anticipate that there might be a parallel with the study of Chapuy *et al.,*[15] which identified a good-risk DLBCL group harbouring mainly aberrations in epigenetic pathways.

Studies by Rossi *et al.* and Kurtz *et al.*,[49, 50] have analysed liquid biopsies in DLBCLs demonstrating that the mutational load in circulating-free tumor DNA obtained by NGS technologies reliably mirror the mutational profiles of DLBCL tissues, including mutated *MYD88*. Additionally,

digital droplet PCR techniques enable the quantification of low amounts of mutated *MYD88* in any physiological fluid.[51] Further investigation is needed to determine whether the analysis of mutated *MYD88* in liquid biopsies prior to and during therapy will be significantly predictive for treatment response and to establish its specificity and sensitivity.

Conclusion

The present study demonstrates that the presence of *MYD88* and *CD79B* mutations is almost mutually exclusive with EBV infection and *MYC, BCL2,* and *BCL6* rearrangements, indicating distinctive molecular DLBCL subgroups that can be readily appreciated in clinical practice. Mutant *MYD88* showed its prognostic importance for inferior survival outcomes, even next to other genetic and clinical prognosticators and IPI. Additionally, patients lacking all analysed abberrancies represented a novel risk group with superior survival outcomes. Taken together and after validation in an independent cohort, these results provide a rationale for including *MYD88* mutational analysis in the routine diagnostics of DLBCL, to improve classification and prognostication, as well as to guide future treatment strategies.

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Table 1 – *Patient characteristics at time of diagnosis*

	All patients (N = 250)
Gender	
Male	168 (67.2 %)
Female	82 (32.8 %)
Median age in years (range)	61.4 (18.6-89.6)
History of immune deficiency	38 (15.2 %)
HIV	16 (6.4 %)
Organ transplantation with prolonged use of immune suppressive drugs	7 (2.8 %)
SCID/CVID	3 (1.2 %)
Other[a]	13 (5.2 %)
Anatomical lymphoma location	
Nodal	75 (30.0 %)
Extranodal[b] (with or without nodal location)	108 (43.2 %)
Immune-privileged	67 (26.8 %)
CNS location[c]	*35 (14.0 %)*
Testis location	*32 (13.2 %)*
Ann Arbor[d]	*(N = 248)*
I	51 (20.6 %)
II	32 (12.9 %)
III	26 (10.5 %)
IV	139 (56.0 %)
IPI[d]	*(N = 241)*
0	20 (8.3 %)
1	41 (17.0 %)
2	90 (37.3 %)
3	58 (24.1 %)
4	24 (10.0 %)
5	8 (3.3 %)
First line treatment	
R-CHOP	160 (64.0 %)
CHOP	25 (10.0 %)
Other chemotherapy[e]	5 (2.0 %)
Radiotherapy only	1 (0.4 %)
Surgery only	2 (0.8 %)
None / Palliative	34 (13.6 %)
High-dose methotrexate regimens (HD-MTX)[f]	23 (9.2 %)
Radiotherapy	77 (30.8 %)
With curative intent	60 (24.0 %)
Palliative care only	17 (6.8 %)
Response to first line treatment	
Complete response	166 (66.4 %)
Partial response	14 (5.6 %)
Stable disease	2 (0.8 %)
Progressive disease	67 (26.8 %)
Too early to call	1 (0.4 %)

Abbreviations: HIV – Humane Immunodeficiency Virus; SCID – Severe Combined Immunodeficiency Disorder; CVID – Common Variable Immunodeficiency Disorder; CNS – Central Nervous System; IPI – International Prognostic Index; (R-)CHOP – (rituximab), cyclophosphamide, doxorubicin, vincristine, prednisone.

[a] Others include inflammatory bowel disease, Sjögren, sarcoidosis, atopic dermatitis, and/or auto-immune haemolytic anaemia.

[b] Extranodal comprised lung, liver, spleen, bone marrow, breast, soft tissue, thyroid, bone, (ad)renal, orbital, stomach, skin, pancreas, bowel, bladder, ovary, and naso-/oropharynx locations.

[c] One patient experienced both CNS and testicular locations.

[d] PCNSL were classified as advanced stage (Ann-Arbor stage IV) and subsequently received one risk point for IPI.

[e] (R-)C(E)OP: (rituximab), cyclophosphamide, (etoposide), vincristin, prednisone.

[f] Specific regimens include HD-MTX + cytarabine + carmustine, HD-MTX + cytarabine, rituximab + HD-MTX + prednisone (RMP), cyclophosphamide + doxorubicin + teniposide + prednisone + vincristine + bleomycin (CHVmP/BV), HD-MTX + procarbazin + lomustin, HD-MTX + cytarabine + thiotepa + rituximab (MATRiX), HD-MTX + teniposide + carmustin + prednisone (MBVP) (+ rituximab).

Table 2 – *Hans' algorithm and molecular analysis at time of diagnosis*

	All patients (N = 250)	Nodal (N =75)	Extranodal with/without nodal (N = 108)	Immune-privileged (N = 67)	P*
Cell-of-origin, according to Hans' algorithm (N=250)					0.228
GCB	100 (40.0 %)	36 (48.0 %)	38 (58.3 %)	26 (38.8%)	
Non-GCB	130 (52.0 %)	35 (46.7 %)	63 (35.2 %)	32 (47.8 %)	
Unclassifiable	20 (8.0 %)	4 (5.3 %)	7 (6.5 %)	9 (13.4 %)	
MYD88 (N=250)					<0.001
Wildtype	176 (70.4 %)	62 (82.7 %)	92 (85.2 %)	22 (32.8 %)	
Mutated	74 (29.6 %)	13 (17.3 %)	16 (14.8 %)	45 (67.2 %)	
CD79B (N=236)					<0.001
Wildtype	207 (87.7 %)	70 (95.9 %)	88 (90.7 %)	49 (74.2 %)	
Mutated	29 (12.3 %)	3 (4.1 %)	9 (9.3 %)	17 (25.8 %)	
MYC (N=217)					0.434
Wildtype	194 (89.4 %)	59 (85.5 %)	89 (90.8 %)	46 (92.0 %)	
Rearranged	23 (10.6 %)	10 (14.5 %)	9 (9.2 %)	4 (8.0 %)	
BCL2 (N=221)					0.001
Wildtype	191 (86.4 %)	53 (74.6 %)	89 (89.9 %)	49 (96.1 %)	
Rearranged	30 (13.6 %)	18 (25.4 %)	10 (10.1 %)	2 (3.9 %)	
BCL6 (N=217)					0.675
Wildtype	173 (79.7 %)	57 (82.6 %)	78 (79.6 %)	38 (76.0 %)	
Rearranged	44 (20.3 %)	12 (17.4 %)	20 (20.4 %)	12 (24.0 %)	
High grade B-cell lymphoma *(N=221)*					0.686
Negative	212 (95.9 %)	66 (95.7 %)	98 (97.0 %)	48 (94.1 %)	
Positive	9 (4.1 %)	3 (4.3 %)	3 (3.0 %)	3 (5.9 %)	
EBV status *(N=239)*					0.091
Negative	211 (88.3 %)	65 (89.0 %)	88 (83.8 %)	58 (95.1 %)	
Positive	28 (11.7 %)	8 (11.0 %)	17 (16.2 %)	3 (4.9 %)	
Genetic aberrations *(N=198)*					0.002
None	51 (25.8 %)	21 (31.8 %)	27 (32.1 %)	3 (6.3 %)	
One or more	147 (74.2 %)	45 (68.2 %)	57 (67.9 %)	45 (93.8 %)	

Abbreviations: EBV – Epstein-Barr Virus.

* P-value indicating a difference in distribution between the three subgroups as calculated by Pearson's Chi Square test.

The number between brackets in the left-hand column represents the number of patients from whom this information was available.

Table 3A – *Prognostic impact of molecular aberrations and IPI risk factors on overall survival: univariable and multivariable analysis*

	Overall survival							
	Univariable		Multivariable Model 1 (IPI)		Multivariable Model 2 (IPI + molecular aberrations WHO 2016)		Multivariable Model 3 (IPI + molecular aberrations WHO 2016 + MYD88 + CD79B)	
	HR	95%-CI	HR	95%-CI	HR	95%-CI	HR	95%-CI
IPI: >2 Extranodal Yes (vs No)	1.37	0.91-2.07	1.41	0.90-2.22	1.49	0.94-2.37	**1.71**	1.07-2.74
IPI: Stage III/IV (vs I/II)	**2.33**	1.41-3.85	1.67	0.98-2.84	1.71	0.97-3.00	**1.84**	1.04-3.25
IPI: ECOG Performance Score >2 (vs ≤1)	**8.15**	5.23-12.7	**7.53**	4.67-12.15	**8.69**	5.23-14.45	**8.16**	4.90-13.59
IPI: Age ≥60 (vs <60)	**1.54**	1.00-2.37	1.35	0.85-2.13	1.38	0.87-2.19	1.33	0.83-2.12
IPI: LDH >Upper limit (vs Normal)	**1.53**	1.01-2.31	1.14	0.74-1.77	1.15	0.73-1.81	1.29	0.82-2.05
MYC Rearranged (vs Wildtype)	1.62	0.88-3.00			1.71	0.89-3.27	1.86	0.93-3.69
BCL2 Rearranged (vs Wildtype)	0.74	0.37-1.47			0.51	0.24-1.08	0.57	0.26-1.24
BCL6 Rearranged (vs Wildtype)	1.21	0.71-2.04			0.94	0.53-1.65	1.00	0.55-1.83
EBV Status Positive (vs Negative)	1.54	0.86-2.78			1.29	0.67-2.47	1.65	0.82-3.30
CD79B Mutated (vs Wildtype)	1.43	0.81-2.53					0.76	0.38-1.49
MYD88 Mutated (vs Wildtype)	**1.64**	1.08-2.48					**1.87**	1.10-3.20
Cross-validated C-index			***0.67***		***0.69***		***0.70***	

For the multivariable model, unknown was regarded as a separate category for these variables for which some data were missing (not reported).

Table 3B – *Prognostic impact of molecular aberrations and IPI risk factors on relapse/progression: univariable and multivariable analysis*

	Cause-specific hazards (CSH) for relapse/progression							
	Univariable		Multivariable Model 1 IPI		Multivariable Model 2 (IPI + molecular aberrations WHO 2016)		Multivariable Model 3 (IPI + molecular aberrations WHO 2016 + MYD88 + CD79B)	
	HR	95%-CI	HR	95%-CI	HR	95%-CI	HR	95%-CI
IPI: >2 Extranodal Yes (vs No)	1.57	0.99-2.41	1.55	0.99-2.41	**1.63**	1.04-2.57	**1.81**	1.14-2.86
IPI: Stage III/IV (vs I/II)	**2.76**	1.63-4.68	**2.12**	1.22-3.67	**2.06**	1.17-3.63	**2.14**	1.19-3.82
IPI: ECOG Performance Score >2 (vs ≤1)	**4.48**	2.58-7.78	**4.48**	2.58-7.78	**5.09**	2.86-9.05	**4.60**	2.57-8.22
IPI: Age ≥60 (vs <60)	1.14	0.75-1.74	1.11	0.71-1.72	1.14	0.73-1.79	1.12	0.71-1.77
IPI: LDH >Upper limit (vs Normal)	0.98	0.64-1.50	0.77	0.49-1.21	0.77	0.48-1.22	0.82	0.51-1.31
MYC Rearranged (vs Wildtype)	1.63	0.86-3.09			1.84	0.94-3.49	1.90	0.96-3.77
BCL2 Rearranged (vs Wildtype)	1.34	0.75-2.40			1.03	0.56-1.90	1.23	0.66-2.30
BCL6 Rearranged (vs Wildtype)	1.01	0.57-1.78			0.89	0.49-1.59	0.91	0.49-1.68
EBV Status Positive (vs Negative)	0.79	0.36-1.71			0.66	0.29-1.49	0.79	0.34-1.86
CD79B Mutated (vs Wildtype)	**1.82**	1.06-3.13					1.23	0.64-2.36
MYD88 Mutated (vs Wildtype)	**1.62**	1.06-2.48					1.42	0.85-2.37
Cross-validated C-index			***0.63***		***0.63***		***0.64***	

For the multivariable model, unknown was regarded as a separate group (not reported).

Table 4 – *Mutated MYD88 improved the prognostic performance of the IPI.*

	Overall survival				Cause-specific hazard (CSH) for relapse/progression			
	Univariable		Multivariable		Univariable		Multivariable	
	HR	95%-CI	HR	95%-CI	HR	95%-CI	HR	95%-CI
IPI-score								
As continuous variable	1.73	1.45-2.08	1.77	1.47-2.13	1.45	1.21-1.73	1.47	1.22-1.76
MYD88								
Mutated (vs Wildtype)			1.83	1.19-2.80			1.69	1.09-2.60
Cross-validated C-index	***0.57***		***0.61***		***0.53***		***0.57***	

Figure legends

Figure 1 – Oncoprint plot of the molecular analysis of 250 cases with diffuse large B-cell lymphoma (DLBCL).

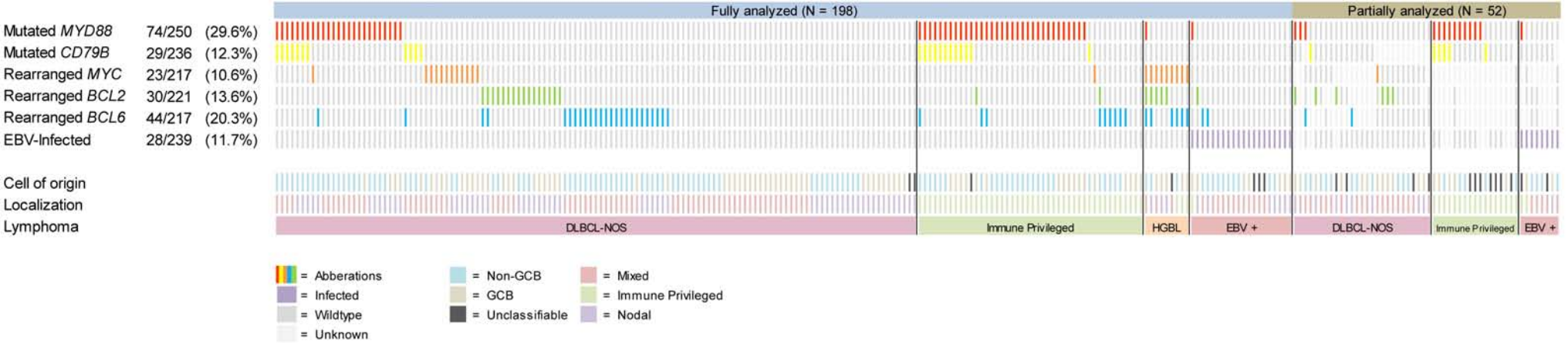
Abbreviations: EBV – Epstein-Barr virus, GCB – germinal center B-cell, IP – immune-privileged.
Of 52 cases, molecular analysis was not complete due to results that were not unambiguous to interpret or no FFPE material was left for subsequent analysis.

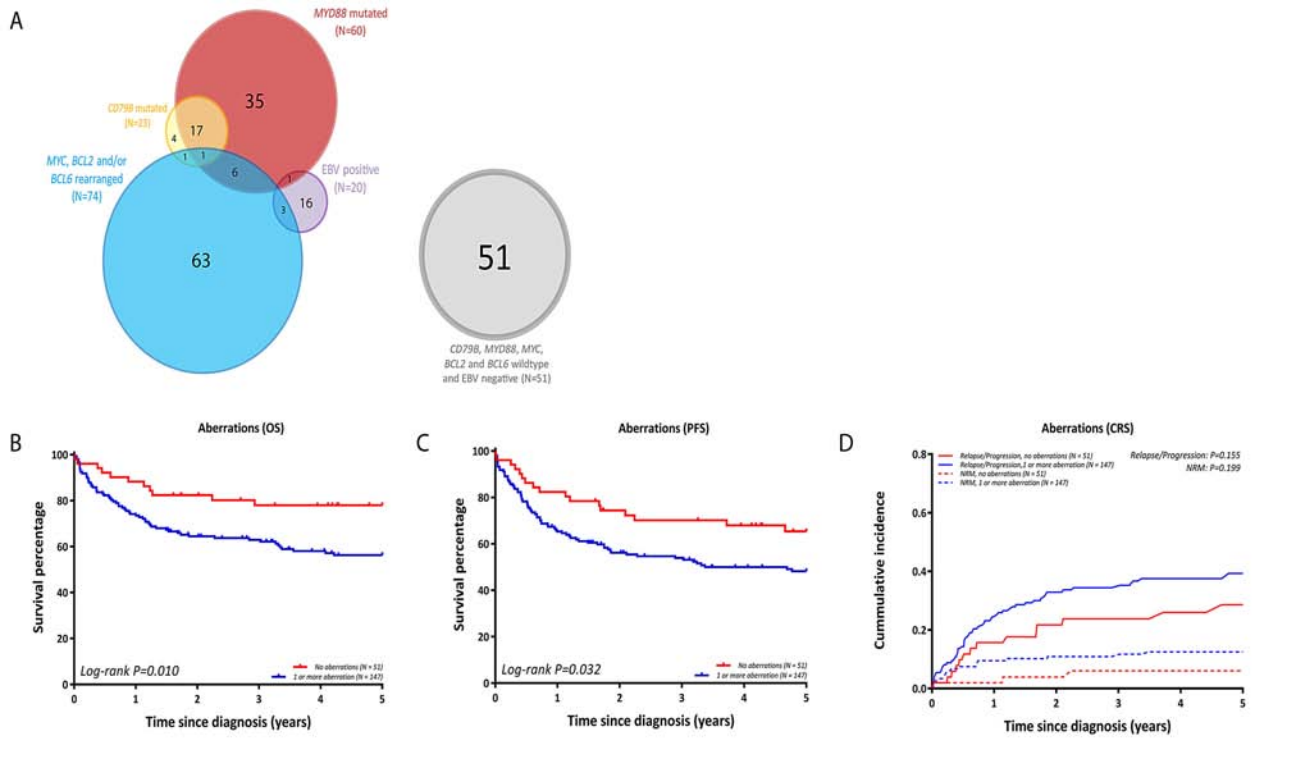
Figure 2 – Molecular characterization discriminates distinct DLBCL subgroups with prognostic impact.

(A) Venn diagram demonstrating the overlap of aberrations for 198 fully analysed DLBCLs. **(B)** DLBCLs without detected aberrations showed a superior overall survival compared to DLBCLs with ≥1 affected aberrations (for cases with complete aberration analysis), identifying a novel good-risk group. **(C)** Progression free survival of the novel identified risk group (for cases with complete driver analysis). **(D)** Cumulative incidences of novel identified risk group (for cases with complete driver analysis).
Abbreviation: CRS – competing risk.

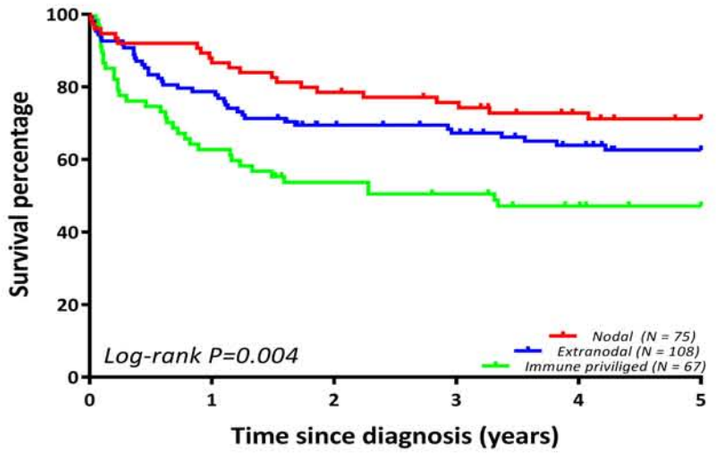
Figure 3 – Prognostic significance of anatomical location, IPI Score and *MYD88* in DLBCL.

Overall survival (OS), progression free survival (PFS), and cumulative incidence of relapse/progression compared to non-relapse mortality (NRM) (1^{st} row: Location, 2^{nd} row: IPI Score, 3^{rd} row: MYD88).
Abbreviation: CRS – competing risk.

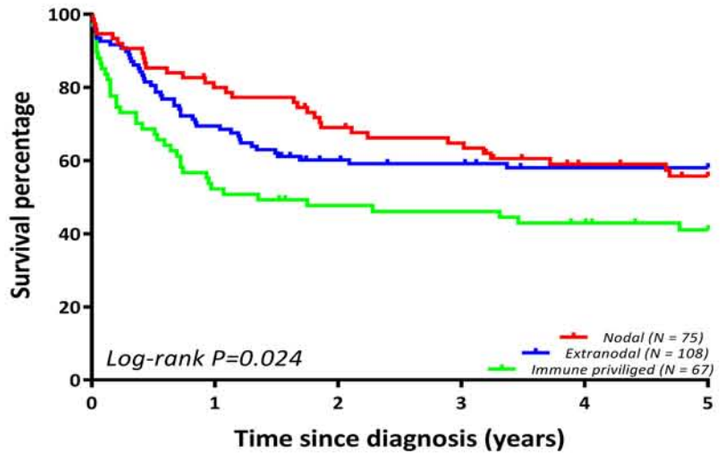

Fully analyzed (N = 198)
Partially analyzed (N = 52)
Mutated MYD88 74/250 (29.6%)
Mutated CD79B 29/236 (12.3%)
Rearranged MYC 23/217 (10.6%)
Rearranged BCL2 30/221 (13.6%)
Rearranged BCL6 44/217 (20.3%)
EBV-Infected 28/239 (11.7%)
Cell of origin
Localization
Lymphoma
DLBCL-NOS
Immune Privileged
HGBL
EBV +
DLBCL-NOS
Immune Privileged
EBV +
= Abberations
= Infected
= Wildtype
= Unknown
= Non-GCB
= GCB
= Unclassifiable
= Mixed
= Immune Privileged
= Nodal


A
MYD88 mutated
(N=60)
35
CD79B mutated
(N=23)
17
4
1
1
6
MYC, BCL2 and/or
BCL6 rearranged
(N=74)
63
1
EBV positive
(N=20)
3
16
51
CD79B, MYD88, MYC,
BCL2 and BCL6 wildtype
and EBV negative (N=51)
B
Aberrations (OS)
Survival percentage
Log-rank P=0.010
No aberrations (N = 51)
1 or more aberration (N = 147)
Time since diagnosis (years)
C
Aberrations (PFS)
Survival percentage
Log-rank P=0.032
No aberrations (N = 51)
1 or more aberration (N = 147)
Time since diagnosis (years)
D
Aberrations (CRS)
Relapse/Progression, no aberrations (N = 51)
Relapse/Progression, 1 or more aberration (N = 147)
NRM, no aberrations (N = 51)
NRM, 1 or more aberration (N = 147)
Relapse/Progression: P=0.155
NRM: P=0.199
Cummulative incidence
Time since diagnosis (years)

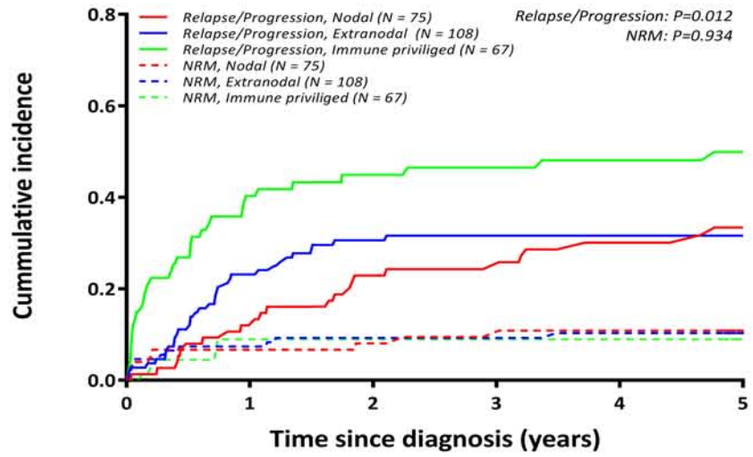
Localization (OS)



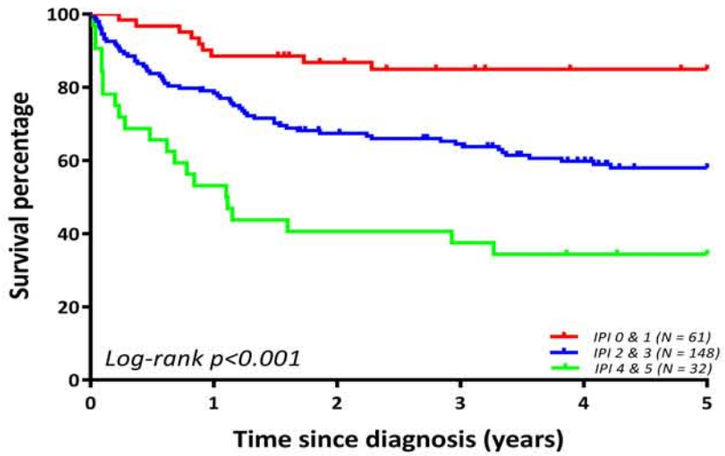
Localization (PFS)



Localization (CRS)



IPI Score (OS)



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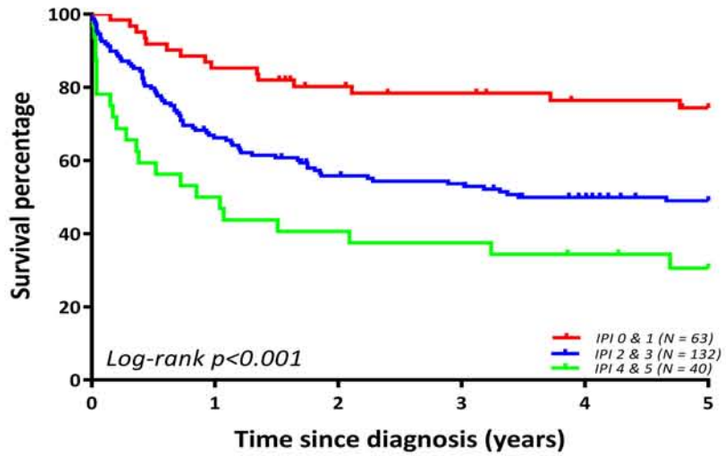
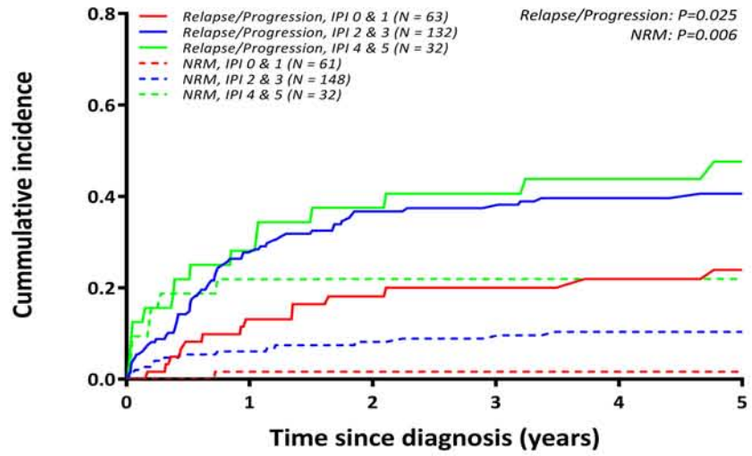
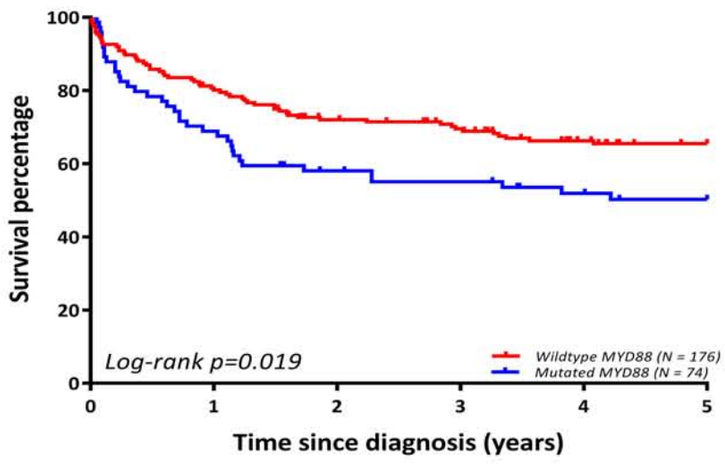


IPI Score (CRS)



MYD88 (OS)



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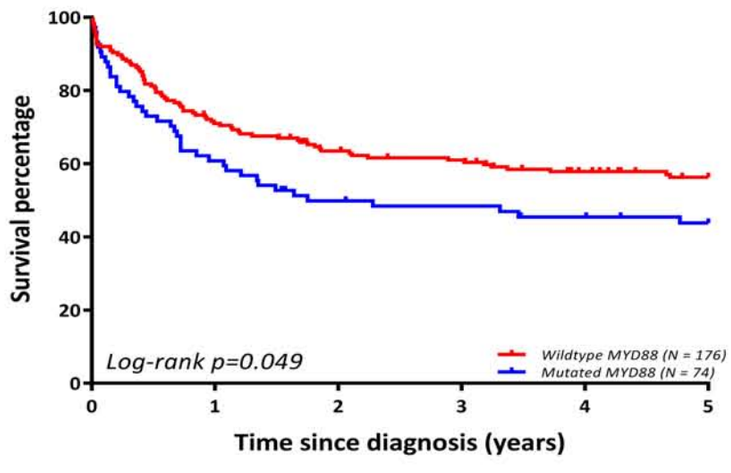

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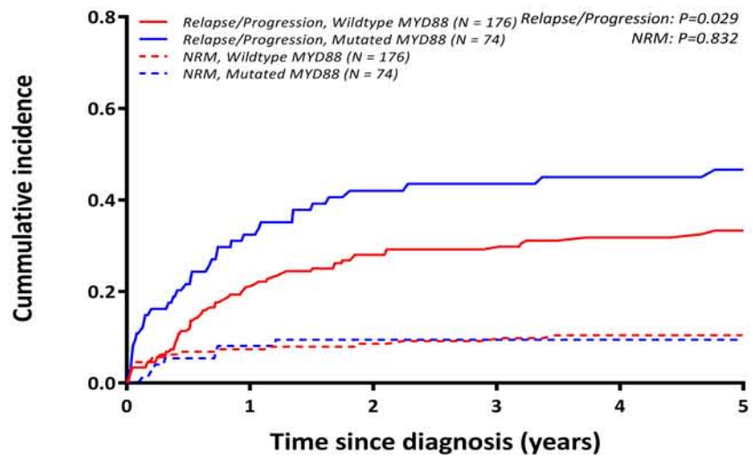

MYD88 mutations identify a molecular subgroup of Diffuse Large B-Cell Lymphoma with an unfavourable prognosis

Joost S. Vermaat[1,2,3], Sebastiaan F. Somers[3], Liesbeth C. de Wreede[4], Willem Kraan[2,5], Ruben A.L. de Groen[3], Anne M. R. Schrader[6], Emile D. Kerver[7], Cornelis G. Scheepstra[8], Henriëtte Berenschot[9], Wendy Deenik[10], Jurgen Wegman[1,11], Rianne Broers[12], Jan-Paul D. de Boer[13], Marcel Nijland[14], Tom van Wezel[6], Hendrik Veelken[3], Marcel Spaargaren[2,5], Arjen H. Cleven[6], Marie José Kersten[1,2] and Steven T. Pals[2,5]

Running heads:

MYD88 mutational status improves classification and prognostication in DLBCL

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E-mail: j.s.p.vermaat@lumc.nl

Supplementary information

Table of contents

Supplemental Methods - Antibodies for staining, EBV and FISH

Immunohistochemical staining - antibodies:
The following immunohistochemical stains were performed with the DAKO Autostainer Link 48, Agilent (LUMC) or the Labvision Autostainer 480S from Thermo Fisher Scientific (AUMC), according to the manufacturer's recommendations, with the antibodies as listed in table 1.

Table 1. Antibodies:

	AUMC	LUMC
CD20	Clone L26, DAKO, Glostrup, Denmark	Clone L26, DAKO, Glostrup, Denmark
CD10	Clone 56C6, Thermo Fisher Scientific, Rockford, IL, USA	Clone 56C6, DAKO
MUM1	Clone MUM1p, DAKO, Glostrup, Denmark	Clone MUM1p, DAKO,
BCL2	Clone 124, DAKO, Glostrup, Denmark	Clone 124, DAKO Glostrup, Denmark
BCL6	Clone PG-B6p, DAKO, Glostrup, Denmark	Clone PG-B6p, Invitrogen

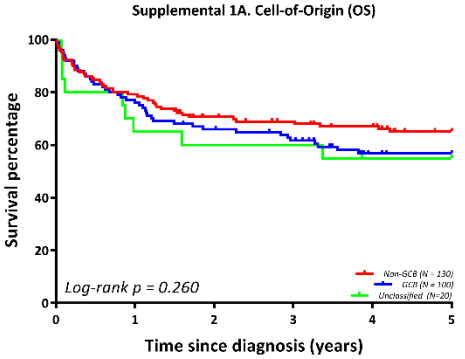
Epstein-Barr virus early RNA *in situ hybridization* (EBER-ISH)

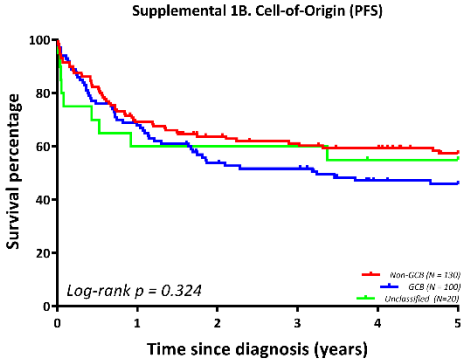
In situ hybridization for Epstein-Barr virus early RNA (EBER-ISH) was performed with EBER probes from Ventana (LUMC) or Biogenex (AUMC), according to the manufacturer's recommendations.

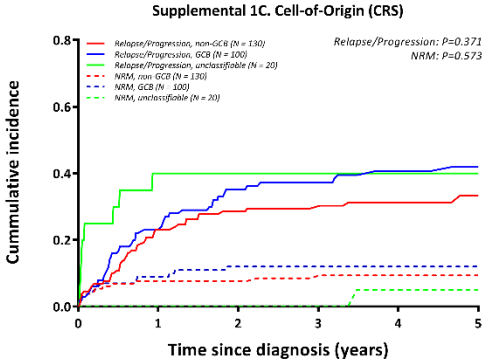
Fluorescence in situ hybridization (FISH) for *MYC*, *BCL2* and *BCL6*

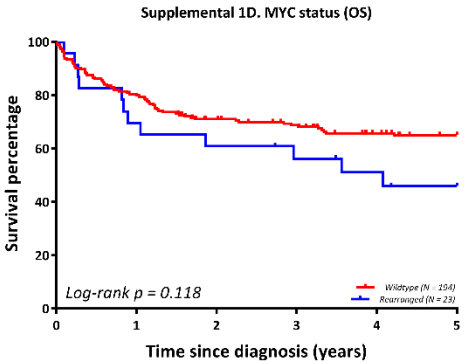
Fluorescence in situ hybridization was performed with break apart rearrangement probes for *MYC*, *BCL2* and *BCL6* from Abbott (LUMC) or DAKO (AUMC), with the DAKO Histology FISH Accessory Kit, Agilent, according to the manufacturer's recommendations.

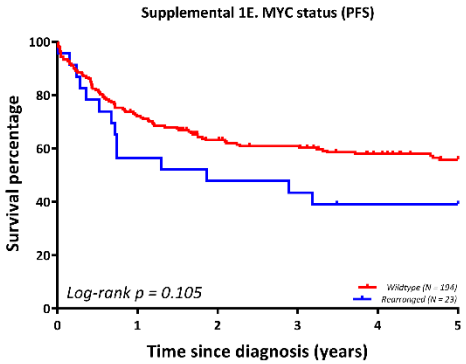
Supplemental figure 1 - Survival outcomes of COO and other aberrations

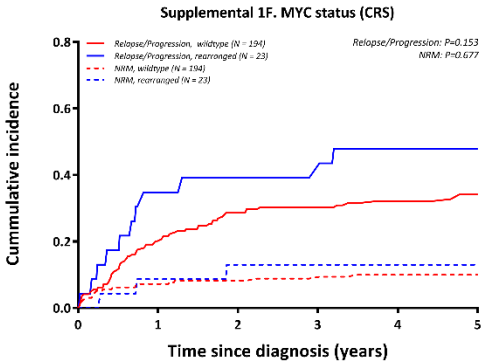


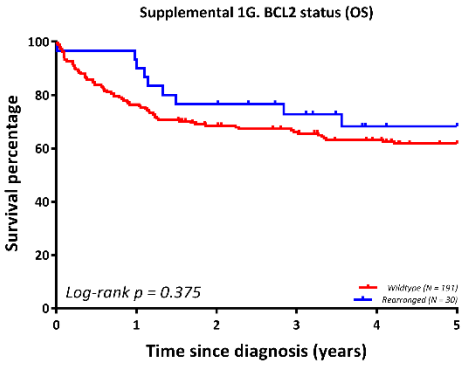


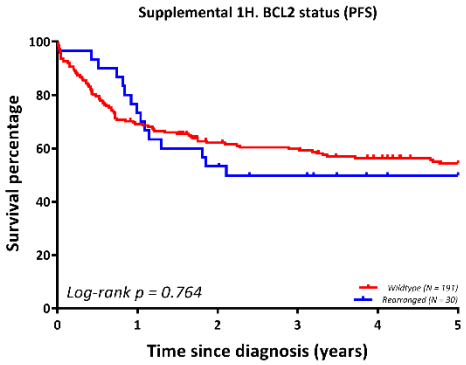


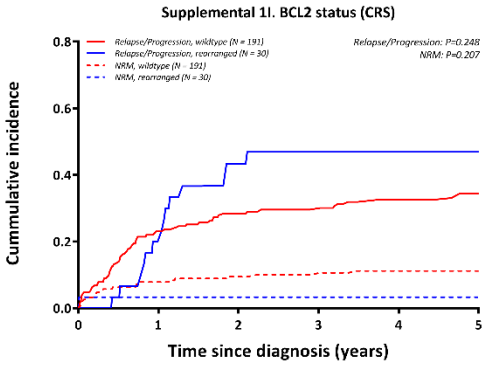


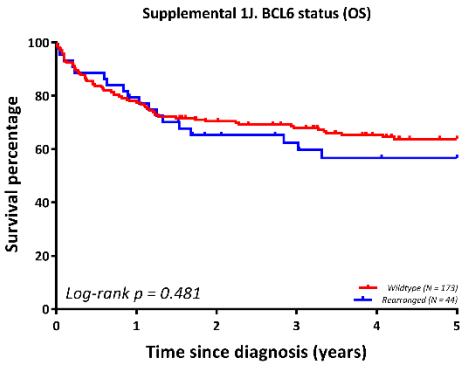


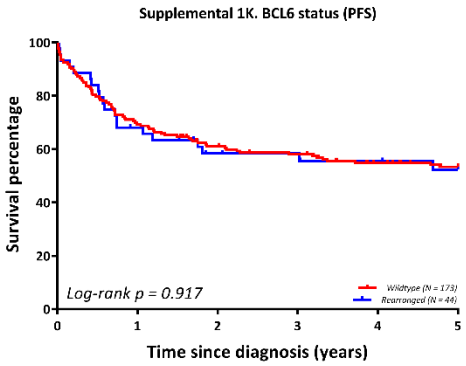


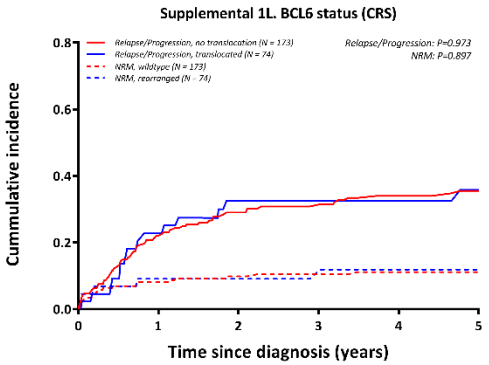






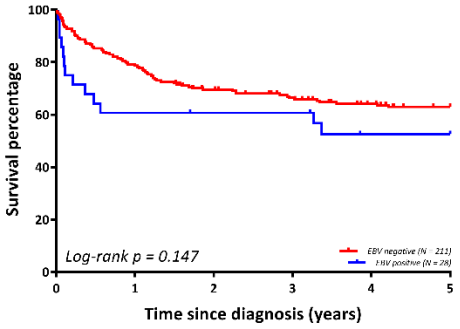




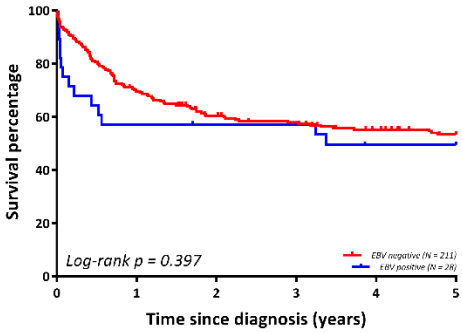


Supplemental figure 1 - (*continued*)

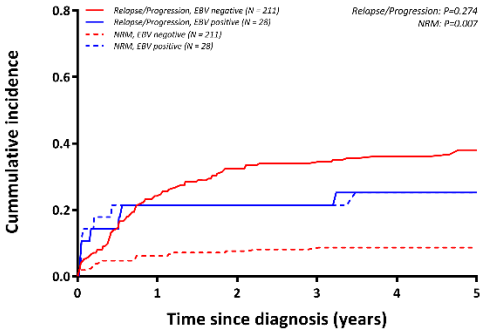
Supplemental 1M. EBV status (OS)



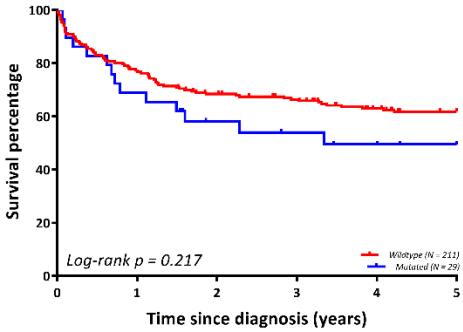
Supplemental 1N. EBV status (OS)



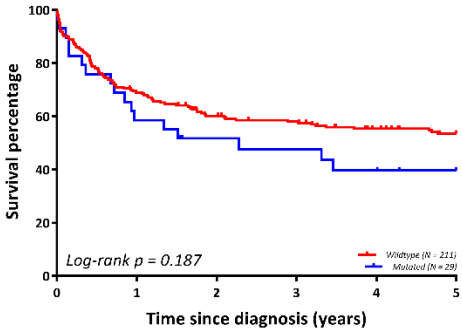
Supplemental 1O. EBV status (CRS)



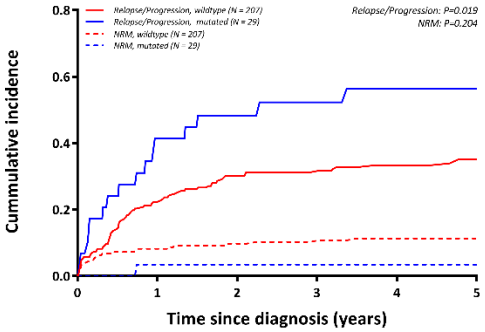
Supplemental 1P. CD79B status (OS)



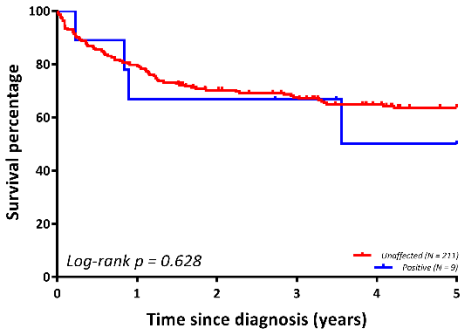
Supplemental 1Q. CD79B status (PFS)



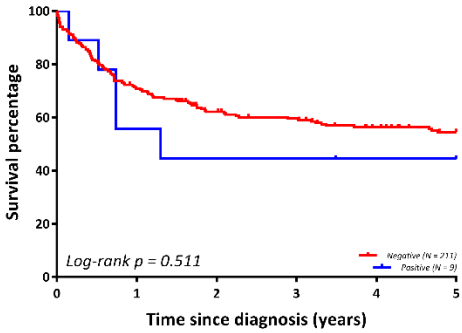
Supplemental 1R. *CD79B* status (CRS)



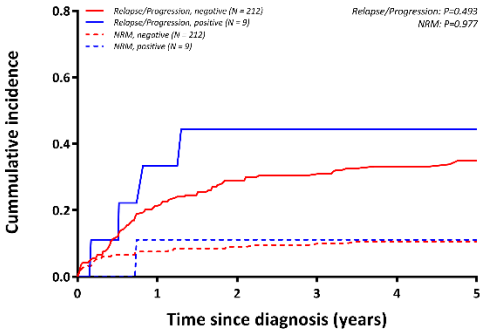
Supplemental 1S. High-grade B-cell lymphoma's (OS)



Supplemental 1T. High-grade B-cell lymphoma's (PFS)



Supplemental 1U. High-grade B-cell lymphoma's (CRS)



Supplemental table 1 - Prognostic impact of molecular aberrations, anatomical lymphoma location and IPI risk factors on overall survival: univariable and multivariable analysis

	Overall survival							
	Univariable		Multivariable Model 1 (IPI)		Multivariable Model 4 (IPI + anatomical localizations + aberrations WHO 2016)		Multivariable Model 5 (IPI + anatomical localizations + aberrations WHO 2016 + MYD88 + CD79B)	
	HR	95%-CI	HR	95%-CI	HR	95%-CI	HR	95%-CI
IPI: >2 Extranodal Yes (vs No)	1.37	0.91-2.07	1.41	0.90-2.22	1.59	0.92-2.74	1.64	0.96-2.80
IPI: Stage III/IV (vs I/II)	**2.33**	1.41-3.85	1.67	0.98-2.84	1.66	0.94-2.94	**1.87**	1.05-3.33
IPI: ECOG Performance Score >2 (vs ≤1)	**8.15**	5.23-12.7	**7.53**	4.67-12.15	**7.69**	4.65-12.72	**7.74**	4.64-12.92
IPI: Age ≥60 (vs <60)	**1.54**	1.00-2.37	1.35	0.85-2.13	1.25	0.78-2.00	1.24	0.77-2.00
IPI: LDH >Upper limit (vs Normal)	**1.53**	1.01-2.31	1.14	0.74-1.77	1.34	0.84-2.15	1.43	0.89-2.29
Anatomical localization Nodal Extranodal (+/- nodal) Immune-privileged	 1.42 **2.37**	 0.83-2.41 1.38-4.08			 1.39 **2.47**	 0.74-2.62 1.30-4.71	 1.55 **2.24**	 0.81-2.93 1.08-4.62
MYC Rearranged (vs Wildtype)	1.62	0.88-3.00			**2.00**	1.03-3.91	1.92	0.95-3.85
BCL2 Rearranged (vs Wildtype)	0.74	0.37-1.47			0.62	0.29-1.34	0.67	0.31-1.47
BCL6 Rearranged (vs Wildtype)	1.21	0.71-2.04			0.96	0.54-1.71	0.92	0.50-1.70
EBV Status Positive (vs Negative)	1.54	0.86-2.78			1.65	0.84-3.23	1.72	0.86-3.45
CD79B Mutated (vs Wildtype)	1.43	0.81-2.53					0.68	0.34-1.35
MYD88 Mutated (vs Wildtype)	**1.64**	1.08-2.48					1.45	0.78-2.71
Cross-validated C-index			***0.67***		***0.71***		***0.71***	

For the multivariable model, unknown was regarded as a separate category for these variables for which some data were missing (not reported)