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► **To cite this version:**

Wendy Cuccuini, Josette Briere, Nicolas Mounier, Hans-Ullrich Voelker, Andreas Rosenwald, et al.. MYC+ diffuse large B-cell lymphoma is not salvaged by classical R-ICE or R-DHAP followed by BEAM plus autologous stem cell transplantation.. Blood, American Society of Hematology, 2012, 119 (20), pp.4619-24. <10.1182/blood-2012-01-406033>. <inserm-00716894>

**HAL Id: inserm-00716894**

**<http://www.hal.inserm.fr/inserm-00716894>**

Submitted on 9 Sep 2012

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# **MYC+ diffuse large B-cell lymphoma is not salvaged by classical R-ICE or R-DHAP followed by BEAM plus autologous stem cell transplantation**

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## **Abstract**

Approximately 5–10% of diffuse large B-cell lymphomas (DLBCL) harbor a 8q24/*MYC* rearrangement (*MYC*<sup>+</sup>). We determined the prognostic significance of *MYC* rearrangement in patients with relapsed/refractory DLBCL prospectively treated by R-ICE or R-DHAP followed by high-dose therapy and autologous stem cell transplantation. Twenty-eight (17%) of the 161 patients analyzed presented a *MYC*<sup>+</sup> rearrangement, targeted as either simple hit (25%) or complex hits (n=75%) including *MYC/BCL2*, *MYC/BCL6*, and *MYC/BCL2/BCL6*. Results were statistically highly concordant in matched primary and relapsed biopsies (n=45). Compared to the *MYC*<sup>-</sup> DLBCL patients, the *MYC*<sup>+</sup> DLBCL patients presented with a more elevated lactico-dehydrogenase level (p=.0006) and a more advanced age-adjusted international prognostic index (p=.0039). The 4-year PFS and OS were significantly lower in the *MYC*<sup>+</sup> DLBCL patients than those in the *MYC*<sup>-</sup> DLBCL patients, with rates of 18% vs. 42% (p=.0322), and of 29% vs. 62% (p=.0113), respectively. Type of treatment, R-DHAP or R-ICE had no impact on survivals, with 4-year PFS rates of 17% vs. 19% and 4-year OS rates of 26% vs. 31%. In conclusion, *MYC* rearrangement is an early event in DLBCL. *MYC*<sup>+</sup> DLBCL patients have a significant inferior prognosis than *MYC*<sup>-</sup> DLBCL patients. Their outcome was not influenced by the proposed salvage therapy.

**MESH Keywords** Adult ; Aged ; Antibodies, Monoclonal, Murine-Derived ; administration & dosage ; adverse effects ; Antineoplastic Combined Chemotherapy Protocols ; administration & dosage ; adverse effects ; Carmustine ; administration & dosage ; adverse effects ; Chemotherapy, Adjuvant ; Cisplatin ; administration & dosage ; adverse effects ; Combined Modality Therapy ; Cytarabine ; administration & dosage ; adverse effects ; Dexamethasone ; administration & dosage ; adverse effects ; Etoposide ;

## INTRODUCTION

Diffuse large B-cell lymphomas (DLBCLs) are recognized as a heterogeneous group of aggressive lymphomas, with numerous clinical, morphological, immunohistochemical and molecular subtypes, as demonstrated by the last World Health Organisation (WHO) classification, which defines no less than 13 subentities[1]. Among the DLBCLs, between 4% to 14% harbor a *MYC* rearrangement (*MYC* DLBCL) as evaluated by fluorescence in situ hybridization (FISH)[2,6]. These cases differ from the defined “borderline” cases, which are considered unclassifiable B-cell lymphomas having features that are intermediate between DLBCL and Burkitt lymphomas [7,8] and presenting with a mixture of medium-to-large-sized cells, a high proliferation rate and an 8q24/*MYC* translocation in 35 to 50% of cases[7,8]. In contrast to the Burkitt lymphomas, *MYC* aberrations in DLBCL are usually associated with multiple cytogenetic abnormalities and other genetic lesions, such as concurrent *BCL2* and/or *BCL6* translocations, so-called “double-hit” or “triple-hit” lymphomas[5,9–13].

The clinical importance of the presence of an *MYC* aberration in DLBCL has been recently suggested in series of patients analyzed in first-line therapy. Patients with *MYC* DLBCL have been reported in one series[6] to present a disease similar to DLBCL but without an *MYC* aberration (*MYC* DLBCL) showing no differences in median age, LDH (Lactico-deshydrogenase), IPI (International Prognostic Index) or performance status. The only difference noted was a higher proliferative rate, as determined by Ki67 staining in excess of 80%, in patients harboring a *MYC* aberrations[6]. In other series, patients with *MYC* DLBCL present with a more aggressive disease including particular clinical features such as poor performance status and bone marrow involvement[5], or more advanced stage, higher IPI, and a higher age-adjusted IPI[2]. With respect to survival, all series agree in reporting that these patients with *MYC* DLBCL have poorer outcomes than patients with *MYC*<sup>-</sup> DLBCL[5,6], regardless of the treatment regimen included, such as cyclophosphamide, doxorubicin, vincristine, prednisone (CHOP), and CHOP plus etoposide (CHOEP)[4,5]. The presence of *MYC* aberrations retains its negative prognostic significance even in patients treated with rituximab and anthracycline-based immuno-chemotherapy[2,3,6]. However, one recent study reported that a regimen based on DA-EPOCH-R (Dose-adjusted etoposide, vincristine, and doxorubicin for 96 hours with bolus of cyclophosphamide and oral prednisone, plus Rituximab) in first-line may improve the outcome of these patients[14]. Importantly, the prognostic impact of the presence of *MYC* aberrations appears to be independent from other factors such as IPI, and bone marrow involvement[6]. Furthermore, the negative impact of *MYC* aberrations supersedes the favorable prognosis of GC DLBCL, with a significantly worse event-free and overall survivals compared with either the non-GC phenotype or *MYC*<sup>-</sup> DLBCL[4,6].

The purpose of this study was to screen for *MYC* aberrations in a selected population of patients with relapsed/refractory DLBCLs to determine the frequency of this occurrence and whether there were any defining phenotypic or clinical features in the *MYC*<sup>+</sup> group and to assess the prognostic impact of *MYC* aberrations in these relapsed/refractory DLBCL patients treated prospectively with R-DHAP (rituximab, dexamethasone, aracytine, cisplatin) vs. R-ICE (rituximab, ifosfamide, etoposide, carboplatin) that was followed by high-dose therapy plus autologous stem cell transplantation (HDT/ASCT).

## MATERIALS AND METHODS

The patients studied in the present biological analyses were a subset of the 477 patients analyzed in the CORAL study[15], which enrolled patients aged 18 to 65 years old who presented a relapsed/refractory CD20+ DLBCL to compare the efficacy of R-ICE and R-DHAP followed by HDT/ASCT (part 1) and to test maintenance with or without rituximab (part 2). The study was registered under European Union Drug Regulating Authorities Clinical Trials (EudraCT) No.2004-002103-32 and ClinicalTrials.gov NCT 00137995 and was conducted in accordance with Good Clinical Practice rules. All patients gave written informed consent to participate and to provide tissue material for biological studies.

### Fluorescent in situ hybridization (FISH) analysis

Histological material was available for a total of 161 patients at diagnosis (n=121 cases) and/or at relapse (n=87 cases) for FISH analysis. FISH analysis was performed on TMA or full slides of paraffin-embedded 2–3- $\mu$ m tissue sections using the break-apart probes for *c-MYC*/8q24 (Abbott, France and Germany). Further analyses were conducted using break-apart probes for *BCL2*/18q21 and *BCL6*/3q27 (Abbott, France and Germany). Samples were analyzed with an AxioImager.M1 epifluorescence microscope (Carl Zeiss, Germany). Images were captured with a 63X or 100X oil objective and analyzed with the Isis software (MetaSystems, Germany). The hybridization signal scoring was performed according to Haralambieva et al.[16], with a normal cutoff value of 10%.

### Morphology, immunohistochemistry and Cell of origin (COO) algorithms

A panel of five hematopathologists (JB, PG, HUV, CS, SC) conducted a central review to confirm the diagnosis of CD20+ DLBCL[1,17]. None of the 161 cases were intermediate cases between DLBCL and Burkitt lymphoma: all were diagnosed as DLBCL. The same panel of hematopathologists centrally evaluated the immunostainings and the FISH results. Immunostainings against CD10 (clone 56C6, dilution 1/50; Novocastra, Newcastle, United Kingdom), BCL2 (bcl-2 124; Dako, Glostrup, Denmark), IRF4/MUM1 (clone Mum1p, dilution 1/20; Dako A/S), BCL6 (clone P1F6, dilution 1/10; Dako A/S, Glostrup, Denmark), and FOXP1 (clone JC12, dilution 1/50; A.H. Banham, Oxford, United Kingdom) were performed using 3- $\mu$ m sections either from either full slides or from tissue microarrays (TMAs) containing two or three representative 0.6-mm cores of routinely FFPE (formaldehyde fixed-paraffin embedded) tissue from each cases. The results of each immunostaining trial were considered positive when greater than 30% of the lymphoma cells were stained. The tissue quality was morphologically evaluated on H-E staining. All evaluable cases were given a secondary classification according to the COO algorithms previously described by Hans et al.[18], Muris et al.[19], and Nyman et al.[20].

### Microarray procedures and analyses

A subset of 37 patients was selected with both the *MYC* FISH analysis and the GEP (gene expression profiling) analysis realized. A total of 47 samples (20 primary biopsies, 17 relapse biopsies and 5 matched cases) were then included. The microarray procedures are previously described[17]. Briefly, total RNA quantity and initial quality were estimated with a NanoDrop<sup>®</sup> ND-1000 spectrophotometer, and RNA quality was further assessed by electrophoresis (Agilent 2100 Bioanalyzer; Agilent Technologies, Mississauga, ON). The Agilent Whole Human Genome microarray (G4112F) and a gene-voting method were used to determine the COO based on the genes' discriminating GCB/ABC signatures previously reported by Alizadeh et al.[21] to define a GCB/ABC predictor similar to that described our previous work[17]. The samples were then classified based on this predictor. The microarray data were submitted to the Gene Expression Omnibus (GEO) (GSE26812).

### Statistical analysis

As previously reported[17], no statistical variations in each biological parameter analyzed by IHC obtained at diagnosis and at relapse were detected among the matched pairs (data not shown). This finding allowed us to analyze all data in a similar manner, irrespective of whether they were generated from diagnostic or relapse biopsies. All survival analyses were performed on an intention-to-treat basis. Patient characteristics and complete remission rates were compared by the chi-squared and Fisher exact tests. Progression-free survival (PFS) was defined as the time from study entry until disease progression or death. Overall survival (OS) was defined as the time from the start of treatment until death. Survival functions were estimated using the Kaplan-Meier method and compared with the log-rank test[22]. Differences between the results of comparative tests were considered significant at a 2-sided  $P < 0.05$ . Because the CORAL trial was not stratified by biological data, we controlled for the effects of prognostic factors on outcome due to sampling fluctuations in the treatment groups with a multivariate analysis of survival in a Cox model[23]. All statistical analyses were performed using SAS 9.13 (SAS Institute, Cary, NC) and S-Plus 6.2 (MathSoft, Cambridge, MA) software

## RESULTS

Overall, 161 of the 477 patients included in the CORAL trial were included based on a successful FISH analysis at defining the presence or absence of an *MYC* rearrangement using paraffin-embedded-tissue (TMA or full slides). One hundred and twenty-one cases were collected at diagnosis (primary biopsy), and 84 cases were collected at relapse (relapse biopsy), including 45 matched cases with the biopsies obtained at both diagnosis and relapse.

In total, 28 of the 161 (17%) cases disclosed an *MYC* rearrangement. Twenty-one of the *MYC*<sup>+</sup> cases (75%) had one or more concurrent translocations, implicating either *BCL2* in t(14;18), or *BCL6* in t(3;14), so-called "double-hits", or *BCL2* in t(14;18) and *BCL6* in t(3;14), so-called "triple-hits". The *MYC* rearrangements were mostly translocations (Table 1). In 2 cases, we observed multiple copies of *MYC*. Considering the 45 matched cases, the results for the primary and secondary biopsies were similar in 87% of the cases (Wilcoxon's paired ranked test  $p = .99$ ). At diagnosis, 6 (6/45, 13%) cases were *MYC*<sup>+</sup> cases, and 39 (39/45, 87%) were *MYC*<sup>-</sup> cases. At relapse 8 (8/45, 18%) cases were *MYC*<sup>+</sup> cases, and 37 (37/45, 82%) were *MYC*<sup>-</sup> cases.

### Clinical Characteristics

*MYC*<sup>+</sup> DLBCL patients were predominantly male (71%) with a median age of 55 years (range: 44–65 years). Compared with *MYC*<sup>-</sup> DLBCL patients, the *MYC*<sup>+</sup> DLBCL patients presented with more advanced disease, showing a high aaIPI in 54% of the patients ( $p = .0039$ ), and an elevated LDH level in 77% of them ( $p = .0006$ ) (Table 2). There was no difference in the frequency of extranodal site involvement. The number of patients with early relapse, defined as relapse less than 12 months from the end of first-line treatment, was identical in both groups (*MYC*<sup>+</sup> cases, 61% vs. *MYC*<sup>-</sup> cases, 50%,  $p = .3196$ ).

### IHC and cell of origin

*MYC*<sup>+</sup> DLBCL cases expressed significantly more CD10 than *MYC*<sup>-</sup> DLBCL cases (60% vs. 34%, respectively  $p=0.0137$ ) as shown in Table 3. Immunohistochemical expression of BCL6, MUM/IRF4, and FOXP1 in *MYC*<sup>+</sup> tumor cells were observed in 77%, 33%, 81% of the cases, respectively without significant differences compared with the *MYC* tumor cells.

The COO phenotype assignments by IHC based on Hans's algorithm, Muris's algorithm, and Nyman's algorithm, were available for 152 cases. Based on Hans's algorithm, *MYC*<sup>+</sup> DLBCL cases were classified as GC in 63% of the cases, whereas 46% were classified as GC in the *MYC*<sup>-</sup> DLBCL cases. Thirty-seven cases were assigned by GEP. Based on the GEP predictor for the GCB/ABC genotype, 3 *MYC*<sup>+</sup> DLBCL cases exhibited a GCB profile.

### **Impact on response and survival of *MYC* rearrangements in patients with relapsed/refractory DLBCL**

At salvage therapy, 83 patients were treated with R-DHAP and 78 were treated with R-ICE. After the induction treatment (R-ICE or R-DHAP), the overall response rate (ORR) was lower in the cohort of patients with *MYC*<sup>+</sup> DLBCL than in the cohort of patients with *MYC*<sup>-</sup> DLBCL (50% vs. 69%, respectively,  $p=0.0519$ ); see Table 2.

The complete response rate (CR) after induction treatment (R-ICE or R-DHAP) was significantly lower in patients with *MYC*<sup>+</sup> DLBCL than in those with *MYC*<sup>-</sup> DLBCL (25% vs. 45%,  $p=0.0497$ ). After the induction treatment, fewer *MYC*<sup>+</sup> DLBCL patients underwent HDT/ASCT than those *MYC*<sup>-</sup> DLBCL patients (43% vs. 60%,  $p=0.0928$ ).

The 4-year PFS and OS were significantly lower in the *MYC*<sup>+</sup> DLBCL patients than in the *MYC*<sup>-</sup> DLBCL patients (Figure 1), with rates of 18% vs. 42% ( $p=0.0322$ ), and 29% vs. 62% ( $p=0.0113$ ), respectively. The same result was observed for the *MYC*<sup>+</sup> DLBCL patients who underwent the HDT/ASCT. These *MYC*<sup>+</sup> DLBCL patients treated with HDT/ASCT had a 4-year PFS and OS rates of 14% and 23 %, respectively.

No difference was observed when we compared the group of cases with *MYC* rearrangements consisting of single chromosomal aberrations and the group of cases with *MYC* rearrangements presenting as complex aberrations with dual and triple translocations. The 4-year PFS (16% vs. 17%,  $p=0.8700$ ) and OS (25% vs. 33%,  $p=0.7677$ ) rates were similar in the single-hit and in the complex-hit groups.

### **Impact of R-ICE and R-DHAP treatment**

The type of treatment (R-ICE or R-DHAP) did not influence the outcomes of the *MYC*<sup>+</sup> DLBCL patients. The corresponding ORR and CR rates were 50% and 25% in the *MYC*<sup>+</sup> DLBCL patients, respectively, with no differences observed after R-ICE (ORR = 40%, CR 23%) or R-DHAP (ORR= 62%, CR = 26%) (ORR  $p=0.1607$ ; CR,  $p=0.8268$ ) (Table 4). In patients with *MYC*<sup>-</sup> DLBCL, the CR rates were 35 % and 54% with R-ICE and with R-DHAP respectively ( $p=0.0250$ ).

With respect to survival, R-ICE and R-DHAP yielded similar results in the cohort of patients with *MYC*<sup>+</sup> DLBCL. The 3-year PFS was at 19% in the R-ICE arm and 17% in the R-DHAP arm (Figure 2). The respective 3-year OS rates were 26% and 31% (Figure 2). In the cohort of patients with *MYC*<sup>-</sup> DLBCL, the 3-year PFS and OS rates were 31% and 51% in the RICE arm and 53% and 71% in the RDHAP arm, respectively.

### **Impacts of the GCB and non-GCB phenotypes**

When we classified the entire cohort of the 161 patients into subgroups based on the GCB vs. non-GCB phenotype using Hans's algorithm, the GCB DLBCL patients treated with R-DHAP had a significantly higher complete remission rate than the non-GCB DLBCL patients (49% vs. 31%,  $p=0.0348$ ), as previously reported[17](Table 4).

Multivariate analysis using a Cox proportional hazard model including the presence or absence of *MYC* aberrations, the type of induction treatment, R-ICE vs. R-DHAP, and the GC vs. non-GC phenotype based on Hans's algorithm confirmed that the only significant factor for both PFS and OS was the presence of a *MYC* aberration, with a relative risk [RR] of 1.8 for PFS ( $p = 0.0248$ ), and an RR of 2 for OS ( $p = 0.0162$ ).

## **DISCUSSION**

A limited number of studies evaluating the prognostic importance of *MYC* status have been reported to date but all were in the context of first-line treatment based on CHOP or CHOP-like regimens, with or without rituximab[2,4–6,11,13]. This report is the first study to analyze the impact of *MYC* aberration in patients less than 65 years old with relapsed/refractory DLBCL.

Several characteristics of the clinical presentation of the disease and the patients with *MYC*<sup>+</sup> DLBCL at relapse are interesting to highlight. First, the median ages *MYC*<sup>+</sup> DLBCL and *MYC*<sup>-</sup> DLBCL patients are similar. Second, the times to relapse between the end of first-line treatment and the time of relapse treatment, which correspond to the randomization in the CORAL trial, are similar in both patient cohorts. Third, the clinical presentation of *MYC*<sup>+</sup> DLBCL seems more aggressive than that of *MYC*<sup>-</sup> DLBCL, as reported previously in a series of patients undergoing first-line treatment[2,5,6]. *MYC*<sup>+</sup> DLBCL LDH levels were more often elevated, as were the IPI scores. However, we did not observe more patients with more than one extranodal site, representing 29% of the patients in our series.

All of the prior studies performed in first-line treatment using anthracycline-based regimens with or without rituximab found a strong prognostic impact of *MYC* gene rearrangement. In the context of relapsed/refractory DLBCL, the outcomes of the *MYC*<sup>+</sup> DLBCL patients were also found to be worse than those of the *MYC*<sup>-</sup> DLBCL patients. This was true regardless of the type of induction treatments, i.e. R-ICE or R-DHAP. Complete remission rates were low, accounting for approximately 25% of the *MYC*<sup>+</sup> DLBCL patients. Less than half of these responder *MYC*<sup>+</sup> DLBCL patients followed the complete course of treatment and received the high-dose treatment with autologous stem cell transplantation, compared with 60% in the *MYC*<sup>-</sup> DLBCL group.

With the caveat that this study examined a relatively small number of cases, the type of aberrations of *MYC* gene (single-, double- or triple-hit) had no impact on these results. The role of the *MYC* aberration appeared to be the same whether the aberration was extra copies of the gene or a chromosomal translocation implicating *MYC*. We observed no differences when *MYC* aberrations were associated as complex hits with other aberrations including *BCL2* and *BCL6* gene rearrangements.

Taking the opportunity to analyze the tumors at both diagnosis and relapse in the 45 matched cases, *MYC* aberrations were found to be present or absent in a similar way in the biopsies at diagnosis and those at relapse. This suggests that the occurrence of *MYC* aberration is probably an early event in the pathogenesis of this tumor.

Regarding the phenotypes of the cases, an *MYC* aberration was strongly associated with the GCB phenotype, showing significant associations with CD10 expression and a GCB phenotype based on a GEP analysis. The *MYC*<sup>+</sup> cases were more often classified as GCB based on Hans's algorithm. The outcome of the patients with GCB phenotypes based on Hans's algorithm who were treated with R-DHAP was better than the outcome of the patients with non-GCB phenotype, except when *MYC* gene was rearranged. *MYC* rearrangement was found to counteract the good prognosis associated with the GCB profile and R-DHAP treatment.

In conclusion, *MYC* rearrangements were found to be present in 17% of the patients with relapsed/refractory DLBCL, defining a small group of patients with a very bad prognosis independently from the known prognostic risk factors and regardless the type of treatment proposed, even in the case of HDT/ASCT. Moreover, our results suggest that this genetic event is probably an early event in the pathogenesis of the tumor and is associated with a GCB phenotype. Finally, our data together with those of previous studies highlight the fundamental role of *MYC* in the prognosis of DLBCL, particularly GCB-like DLBCL. This finding suggests the importance of performing FISH analysis for *MYC* rearrangement in highly proliferative DLBCL and emphasizes the need for new treatments for patients with *MYC*<sup>+</sup> DLBCL.

## Acknowledgements:

The study received a grant from the Programme Hospitalier de Recherche Clinique 2009, INCa, Institut National du Cancer, France (Grant PHRC AOM09271) and from F-Hoffmann La Roche Ltd. SC was sponsored by the Schweizerische Arbeitsgemeinschaft für Klinische Krebsforschung (SAKK). The authors would like to acknowledge GELARC (Groupe d'Etude des Lymphomes de l'Adulte – Recherche Clinique) for the clinical management of this work, GELAP (Groupe d'Etude des Lymphomes de l'Adulte – Pathology), specially Jerome Cuvillier, for the management of the lymphoma cases, Catherine Chevalier, Cecile Tuffier, and François Plassa for their excellent help in collecting the material and data as well as for their technical assistance.

## Footnotes:

**Author contributions** W. Cuccuini performed research, analyzed data and contributed to the writing of the paper J Briere designed and performed research, analyzed data, and wrote the paper N Mounier designed and performed research, analyzed data, and wrote the paper Hans-Ullrich Voelker performed research, analyzed data, and contributed to the writing of the paper A. Rosenwald analyzed data and contributed to the writing of the paper C. Sunstrom analyzed data and contributed to the writing of the paper S Cogliati analyzed data and contributed to the writing of the paper E Hirschfeld analyzed data and contributed to the writing of the paper L Ysebaert analyzed data and contributed to the writing of the paper D Bron analyzed data and contributed to the writing of the paper J Soulier analyzed data and contributed to the writing of the paper P Gaulard analyzed data and contributed to the writing of the paper R Houllgate performed research, analyzed data, and wrote the paper C Gisselbrecht analyzed data and contributed to the writing of the paper C. Thieblemont designed research, analyzed data, and wrote the paper

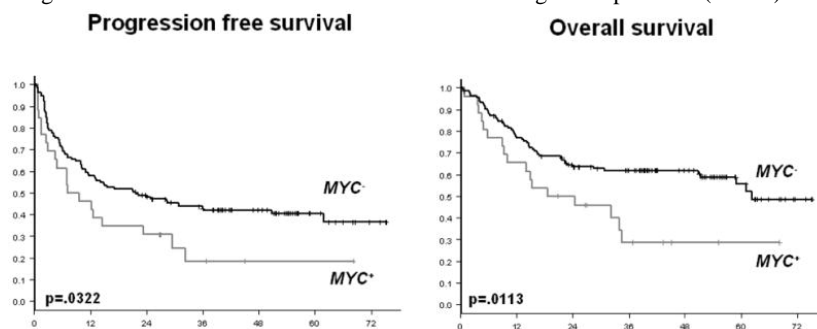
**Conflict of interest for this work:** None

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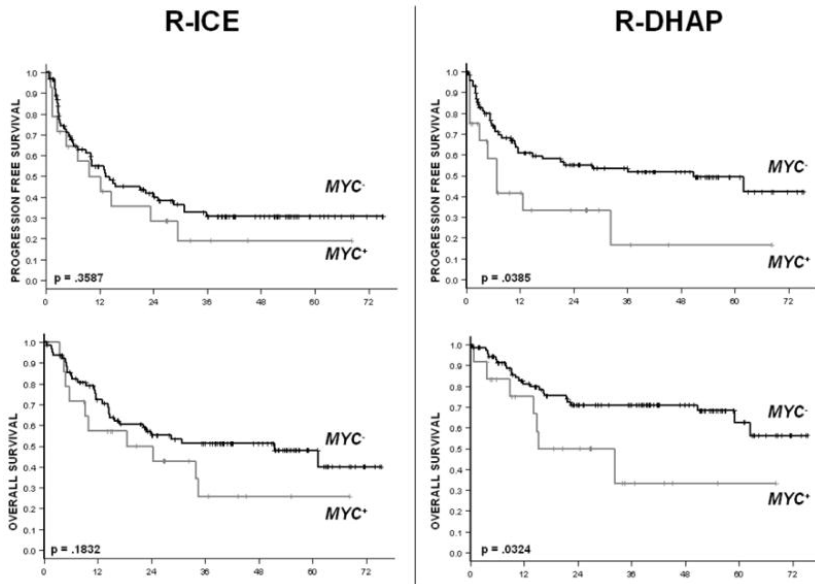
**Figure 1**

Progression-free survival and overall survival according to the presence (*MYC*+) or the absence (*MYC*-) of *MYC* aberration



**Figure 2**

Progression-free survival and overall survival according to the treatment and the presence (*MYC*+) or absence (*MYC*-) of *MYC* aberration





**Table 1**Type of *MYC*/8q24 aberration and its association with other rearrangements including *BCL2*/18q21 and *BCL6*/3q27 in the relapsed/refractory DLBCL

|   | n          | (%)          |
|---|------------|--------------|
| <b>Total of patients analysed</b>                             | <b>161</b> | <b>(100)</b> |
| <b>Presence of <i>MYC</i>/8q24 aberration</b>                 | <b>28</b>  | <b>(17)</b>  |
| <b>Simple hit : <i>MYC</i>/8q24 only</b>                      | <b>7</b>   |              |
| <b>Complex hits</b>   | <b>21</b>  |              |
| <b>Double hit</b>   |            |              |
| <i>MYC</i> /8q24 and <i>BCL2</i> /18q21                       | <b>13</b>  |              |
| <i>MYC</i> /8q24 and <i>BCL6</i> /3q27                        | <b>4</b>   |              |
| <b>Triple hit</b>   |            |              |
| <i>MYC</i> /8q24 and <i>BCL2</i> /18q21 and <i>BCL6</i> /3q27 | <b>4</b>   |              |
| <b>No rearrangement</b>                                       | <b>133</b> | <b>(83)</b>  |

**Table 2**Baseline characteristics of patients with either *MYC*<sup>+</sup> or *MYC*<sup>-</sup> relapsed/refractory DLBCL

| No. of patients              | <i>MYC</i> <sup>+</sup> DLBCL |    | <i>MYC</i> <sup>-</sup> DLBCL |    | P     |
|------------------------------|-------------------------------|----|-------------------------------|----|-------|
|                              | n =28                         | %  | n =133                        | %  |       |
| Sex                          |                               |    |                               |    |       |
| Male                         | 20                            | 71 | 77                            | 59 | .1835 |
| Female                       | 8                             | 29 | 56                            | 41 |       |
| Age                          |                               |    |                               |    |       |
| Median                       | 55                            |    | 54                            |    | .1135 |
| Range                        | 44–65                         |    | 19–65                         |    |       |
| PS(ECOG)                     |                               |    |                               |    |       |
| 0–1                          | 23                            | 82 | 122                           | 92 | .1128 |
| 2–3                          | 5                             | 18 | 11                            | 8  |       |
| Ann Arbor Stage              |                               |    |                               |    |       |
| I–II                         | 8                             | 29 | 63                            | 48 | .0200 |
| III–IV                       | 20                            | 71 | 70                            | 52 |       |
| Elevated LDH                 | 20                            | 77 | 53                            | 40 | .0006 |
| Extranodal site > 1          | 8                             | 29 | 33                            | 25 | .1821 |
| aaIPI                        |                               |    |                               |    |       |
| 0–1                          | 12                            | 46 | 94                            | 71 | .0081 |
| 2–3                          | 14                            | 54 | 38                            | 29 |       |
| Initial response             |                               |    |                               |    |       |
| CR-CRU                       | 16                            | 57 | 81                            | 61 | .3669 |
| CRU                          | 2                             | 7  | 18                            | 14 |       |
| PR                           | 5                             | 18 | 18                            | 14 |       |
| Stable disease               | 2                             | 7  | 4                             | 3  |       |
| Progression                  | 5                             | 18 | 12                            | 9  |       |
| Time to relapse              |                               |    |                               |    |       |
| < 12 months                  | 17                            | 61 | 67                            | 50 | .3196 |
| ≥ 12 months                  | 11                            | 39 | 66                            | 50 |       |
| Prior rituximab treatment    |                               |    |                               |    |       |
|                              | 20                            | 71 | 80                            | 60 | .2635 |
| Treatment at relapse         |                               |    |                               |    |       |
| RICE                         | 15                            | 54 | 63                            | 53 | .5505 |
| RDHAP                        | 13                            | 46 | 70                            | 47 |       |
| Response at induction        |                               |    |                               |    |       |
| CR/CRU                       | 7                             | 25 | 60                            | 45 | .0497 |
| PR                           | 5                             | 18 | 20                            | 15 |       |
| Overall response (CR/CRU/PR) | 14                            | 50 | 92                            | 69 | .0519 |
| Transplant                   |                               |    |                               |    |       |
|                              | 12                            | 43 | 80                            | 60 | .0928 |

**Table 3**Immunohistochemical staining results, cell of origin classification based on GC/nonGC algorithms, according the presence or the absence of *MYC* aberration

| Cases                          | <i>MYC</i> + |     | <i>MYC</i> - |     |       |
|--------------------------------|--------------|-----|--------------|-----|-------|
| Parameter                      | n            | (%) | n            | (%) | p     |
| Immunohistochemistry           |              |     |              |     |       |
| CD10                           | 27           |     | 127          |     |       |
| Positive                       | 16           | 60  | 84           | 66  | .0137 |
| Negative                       | 11           | 40  | 43           | 34  |       |
| BCL6                           | 26           |     | 128          |     |       |
| Positive                       | 20           | 77  | 88           | 69  | .4065 |
| Negative                       | 6            | 23  | 40           | 31  |       |
| MUM1/IRF4                      | 27           |     | 128          |     |       |
| Positive                       | 9            | 33  | 62           | 48  | .1523 |
| Negative                       | 18           | 67  | 66           | 52  |       |
| FOXP1 (Barrans)                | 27           |     | 128          |     |       |
| Positive                       | 22           | 81  | 86           | 67  | 1420  |
| Negative                       | 5            | 19  | 42           | 33  |       |
| BCL2                           | 27           |     | 128          |     |       |
| Positive                       | 22           | 81  | 31           | 76  | .5239 |
| Negative                       | 5            | 19  | 97           | 24  |       |
| GC/nonGC algorithm publication |              |     |              |     |       |
| Hans et al.                    | 27           |     | 125          |     |       |
| GC                             | 17           | 63  | 57           | 46  | .1017 |
| Non GC                         | 10           | 37  | 68           | 54  |       |
| Muris et al.                   | 27           |     | 126          |     |       |
| Group 1                        | 22           | 82  | 80           | 63  | .0719 |
| Group 2                        | 5            | 19  | 46           | 37  |       |
| Nyman et al.                   | 23           |     | 123          |     |       |
| ABC                            | 5            | 22  | 30           | 24  | .7846 |
| Others                         | 18           | 78  | 93           | 76  |       |
| Cell of origin based on GEP    |              |     |              |     |       |
|                                | 3            |     | 24           |     |       |
| GC                             | 3            | 100 | 13           | 54  | -     |
| ABC                            | 0            | 0   | 11           | 46  |       |

GC indicates germinal center; GEP gene expression profiling, and ABC, activated B-cell

**Table 4**  
 Complete response considering type of treatment (R-ICE vs R-DHAP) and phenotype characteristics including cell of origin based on Hans's algorithm and MYC aberration

| Treatment                        | Complete response |                    | p (X2) |
|----------------------------------|-------------------|--------------------|--------|
|                                  | R-ICE<br>n=78 (%) | R-DHAP<br>n=83 (%) |        |
| <b>Patients analysed by FISH</b> | 29 (37)           | 35 (42)            | .3187  |
| <b>Hans COO</b>                  |                   |                    |        |
| GC                               | 24 (31)           | 41 (49)            | .0348  |
| Non GC                           | 37 (47)           | 32 (38)            | .2931  |
| <b>MYC aberration</b>            |                   |                    |        |
| Presence                         | 20 (26)           | 19 (23)            | .8268  |
| Absence                          | 27 (35)           | 45 (54)            | .0250  |

GC = Germinal Center