MYC/BCL2 protein co-expression is associated with high-risk gene signatures and contributes to the inferior prognosis of activated B-cell subtype of diffuse large B-cell lymphoma: A report from The International DLBCL Rituximab-CHOP Consortium Program Study

SUPPLEMENTAL MATERIALS

MATERIALS AND METHODS

Patient selection and treatment

All cases were diagnosed as *de novo* DLBCL and all patients were treated with R-CHOP chemotherapy without upfront transplant. Patients had R-CHOP q3 weeks. All patients with advanced stage disease received 6 or 8 cycles, every 21 days, +/- RT for residual disease or initial bulk. Localized cases received 3 or 4 R-CHOP +/- RT or 6 cycles of R-CHOP without RT depending on the Centers. Both of these approaches are considered standard treatment for localized disease, until randomized trials would not tell us if any of the two is better than the other, in the Hematology Society.

Statistical analysis

Comparisons of clinical and laboratory features at time of presentation between different DLBCL subgroups were carried out using the χ^2 test and the Spearman rank correlation. Overall survival (OS) duration was calculated from the date of diagnosis to the date of last follow-up or death. Progression-free survival (PFS) duration was calculated from the date of diagnosis to the time of progression or death. Kaplan–Meier survival curves were used to estimate OS as well as PFS, and the log-rank (Mantel-Cox) test was used to assess differences in survival between groups. Multivariate analysis for survival of the study cohort was performed on IBM statistics SPSS 19 using the Cox

proportional hazards regression model. All differences with $P \leq 0.05$ were considered to be statistically significant.

Figure Legends for Supplemental Figures

Figure S1. Prognostic impact of MYC/BCL2 co-expression in DLBCL

(A, B) OS (A) and PFS (B) of patients with DLBCL with MYC/BCL2 co-expression (MYC^+BCL2^+) in the training set. **(C, D)** OS of patients with MYC^+ DLBCL in the presence (C) or absence (D) of BCL2 co-expression in the training set. **(E, F)** OS of patients with $BCL2^+$ DLBCL in the presence (E) or absence (F) of MYC co-expression in the training set. All analyses in this Figure were limited to the 411 cases classified by GEP results in the training set.

Figure S2. Prognostic impact of MYC/BCL2 co-expression in DLBCL risk-stratified according to clinicopathologic parameters

(A, B) OS (A) and PFS (B) of patients with MYC^+BCL2^+ DLBCL of the GCB subtype in the training set. **(C, D)** OS (C) and PFS (D) of patients with MYC^+BCL2^+ DLBCL of the ABC subtype in the training set. **(E, F)** OS (E) and PFS (F) of patients with MYC^+BCL2^+ DLBCL risk-stratified according to IPI risk scores in the training set. All analyses in this Figure were limited to the 411 cases classified by GEP results in the training set. DP: MYC/BCL2 double-positive; Non-DP: non-double positive.

Figure S3. Frequency of BCL2 and MYC expression in COO subtypes of DLBCL

(A) Relative frequency of the ABC vs GCB subtype in DLBCL positive for BCL2 expression, MYC expression, or MYC/BCL2 co-expression in the training set. **(B)** Frequency of BCL2 expression, MYC expression, or MYC/BCL2 co-expression (in the presence or absence of *MYC*/*BCL2* DH) in DLBCL of the ABC and GCB subtypes in the training set. All analyses in this Figure were limited to the 411 cases classified by GEP results in the training set. DH: double-hit.

Figure S4. MYC/BCL2 co-expression contributes to the inferior prognosis of ABC subtype of DLBCL

(A, B) OS (A) and PFS (B) of the ABC vs GCB subtype of DLBCL. **(C, D)** OS (C) and PFS (D) of the ABC vs GCB subtype of DLBCL after all MYC^+BCL2^+ cases were excluded. **(E, F)** OS (E) and PFS (F) of the ABC vs GCB subtypes in MYC^+BCL2^+ DLBCL. All analyses in this Figure were limited to the 411 cases classified by GEP results in the training set.

Figure S5. Prognostic impact of MYC/BCL2 co-expression in DLBCL is independent of *MYC/BCL2* co-rearrangement

(A, B) OS (A) and PFS (B) of patients with *MYC*/*BCL2* double-hit DLBCL. (**C, D**) OS (C) and PFS (D) of patients with MYC^+BCL2^+ DLBCL in the absence of *MYC*/*BCL2* double-hit. All analyses in this Figure were limited to the 411 cases classified by GEP results in the training set.

Figure S6. Prognostic impact of MYC/BCL2 co-expression in COO subtypes

(A, B) OS (A) and PFS (B) of patients with MYC^+BCL2^+ DLBCL of the GCB subtype in the training set. **(C, D)** OS (C) and PFS (D) of patients with MYC^+BCL2^+ DLBCL of the ABC subtype in the training set. The COO classification was achieved according to Choi algorithm in the cases unclassifiable by GEP or in which GEP was not performed.

Figure S7. Prognostic impact of MYC/BCL2 co-expression in DLBCL risk-stratified according to clinical parameters

Prognostic impact of MYC/BCL2 co-expression in DLBCL risk-stratified according to age (A), ECOG performance score (B), Ann Arbor stage (C), number of extranodal sites (D), serum LDH level (E), and B symptoms (F). DP: MYC/BCL2 double-positive; Non-DP: non-double positive.

Figure S8. Prognostic impact of MYC/BCL2 co-expression in DLBCL and COO subtypes in the validation cohort

Survival of patients with MYC^+BCL2^+ DLBCL in the validation set #1 overall (A), the GCB (B), or ABC (C) subtype of DLBCL. There are 234 cases of *de novo* DLBCL in this validation set. The COO classification is based on IHC. The clinicopathologic characteristics are as following:

Male: 131/234 (56%)

Median age (>60): 129/234 (55%) (Median: 62; range: 20-95)

ECOG performance ≥2: 35/161 (22%)

Ann Arbor stage III-IV: 77/148 (52%)

Extranodal sites ≥2: 40/197 (20%)

Elevated serum LDH: 121/186 (65%)

B-symptoms: 95/226 (42%)

ABC: 116 (50%); GCB: 118 (50%)

MYC^+: 127/234 (54%); $BCL2^+$: 120/234 (51%); MYC^+BCL2^+: 76/234 (32%)

Figure S9. COO stratification is not prognostically predictive in the absence of MYC/BCL2 co-expression in the validation cohort

OS (A) and PFS (B) of the ABC vs GCB subtype of DLBCL in the absence of MYC^+BCL2^+ cases in the validate set #2. This cohort was previously reported (*JCO.* 2012;30:3460-3467). In this cohort, 54 (29%) cases were MYC/BCL2 double-positive, 43 (23%) MYC/BCL2 double-negative, 88 (48%) were MYC or BCL2 single-positive. The overall (including double-positive and single-positive) MYC^+ rate was 54% and overall $BCL2^+$ rate 52%. Of the 131 non-double positive cases, the COO classification of 42 cases was based on GEP results and 89 based on Choi algorithm. Eighty-five were of the GCB subtype and 44 the ABC subtype. Two were undetermined.

Figure S1

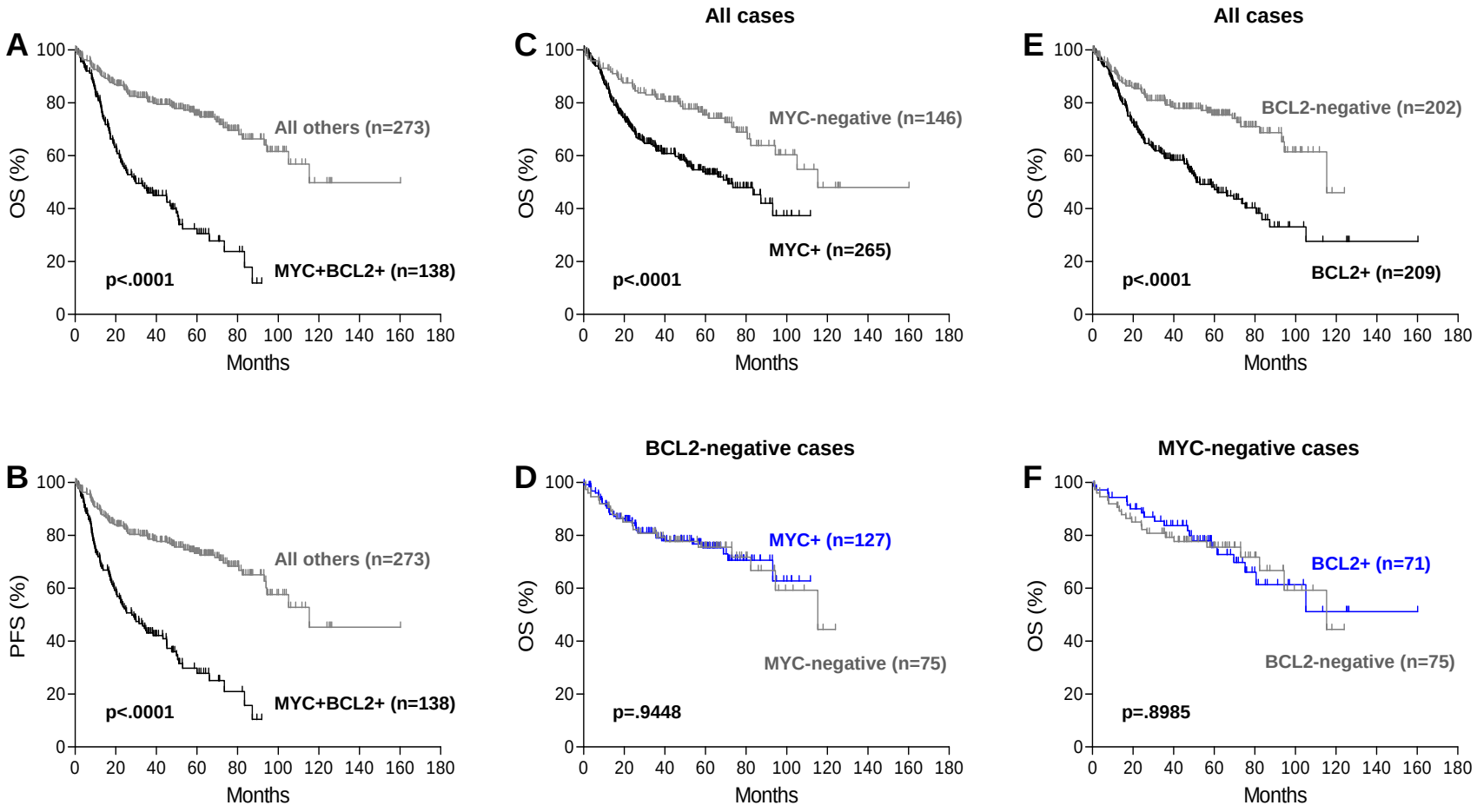

A
OS (%)
All others (n=273)
MYC+BCL2+ (n=138)
p<.0001
Months
B
PFS (%)
All others (n=273)
MYC+BCL2+ (n=138)
p<.0001
Months
All cases
C
OS (%)
MYC-negative (n=146)
MYC+ (n=265)
p<.0001
Months
BCL2-negative cases
D
OS (%)
MYC+ (n=127)
MYC-negative (n=75)
p=.9448
Months
All cases
E
OS (%)
BCL2-negative (n=202)
BCL2+ (n=209)
p<.0001
Months
MYC-negative cases
F
OS (%)
BCL2+ (n=71)
BCL2-negative (n=75)
p=.8985
Months

Figure S2

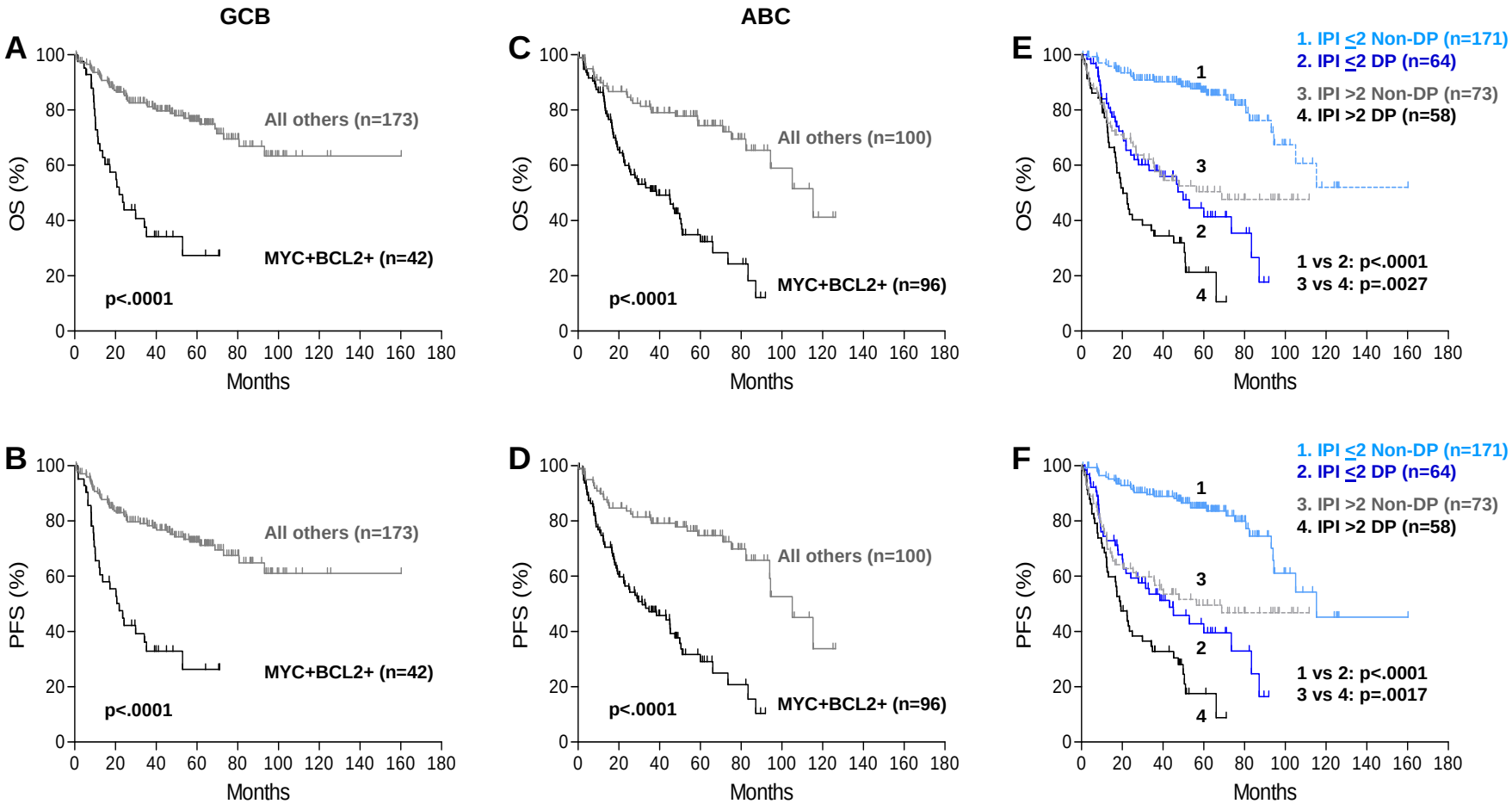

GCB
ABC
A
B
C
D
E
F
OS (%)
PFS (%)
Months
All others (n=173)
MYC+BCL2+ (n=42)
p<.0001
All others (n=100)
MYC+BCL2+ (n=96)
1. IPI ≤2 Non-DP (n=171)
2. IPI ≤2 DP (n=64)
3. IPI >2 Non-DP (n=73)
4. IPI >2 DP (n=58)
1 vs 2: p<.0001
3 vs 4: p=.0027
3 vs 4: p=.0017

Figure S3

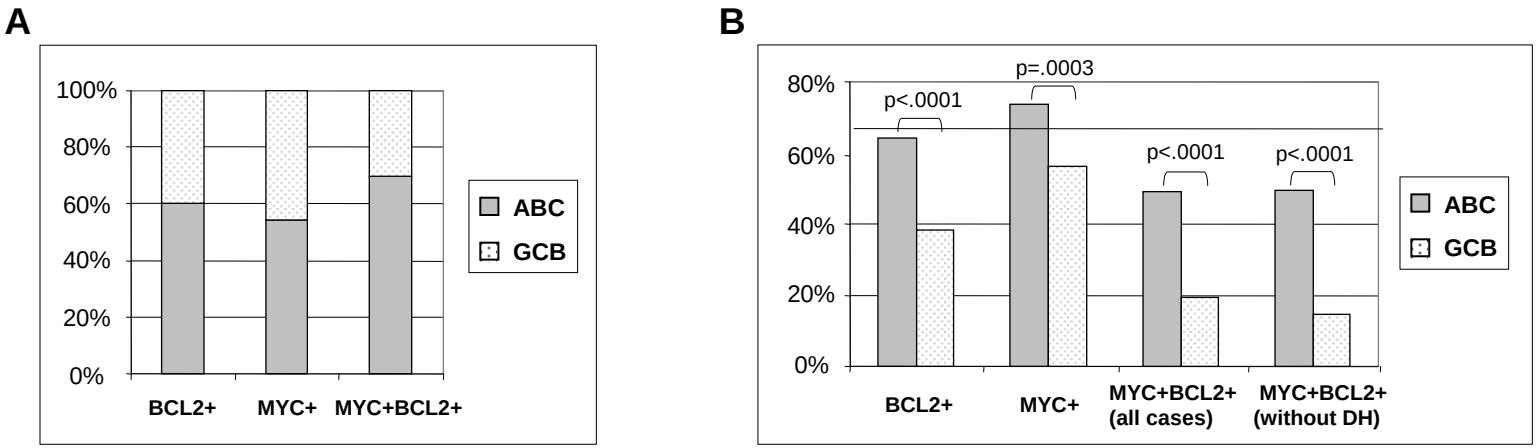

A
100%
80%
60%
40%
20%
0%
BCL2+
MYC+
MYC+BCL2+
ABC
GCB
B
80%
60%
40%
20%
0%
p<.0001
p=.0003
p<.0001
p<.0001
BCL2+
MYC+
MYC+BCL2+ (all cases)
MYC+BCL2+ (without DH)
ABC
GCB

Figure S4

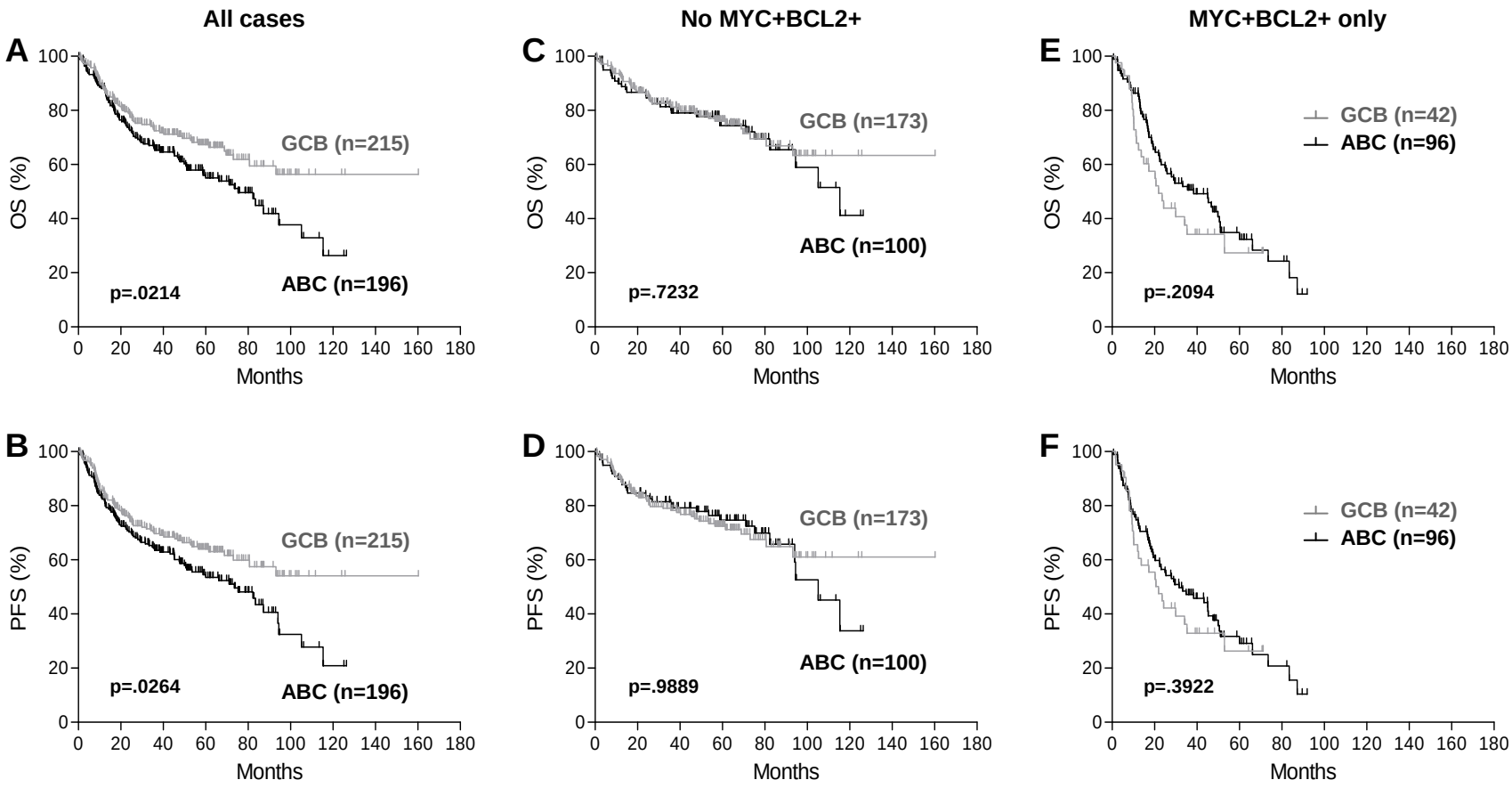

All cases
No MYC+BCL2+
MYC+BCL2+ only
A
B
C
D
E
F
OS (%)
PFS (%)
Months
GCB (n=215)
ABC (n=196)
GCB (n=173)
ABC (n=100)
GCB (n=42)
ABC (n=96)
p=.0214
p=.0264
p=.7232
p=.9889
p=.2094
p=.3922

Figure S5

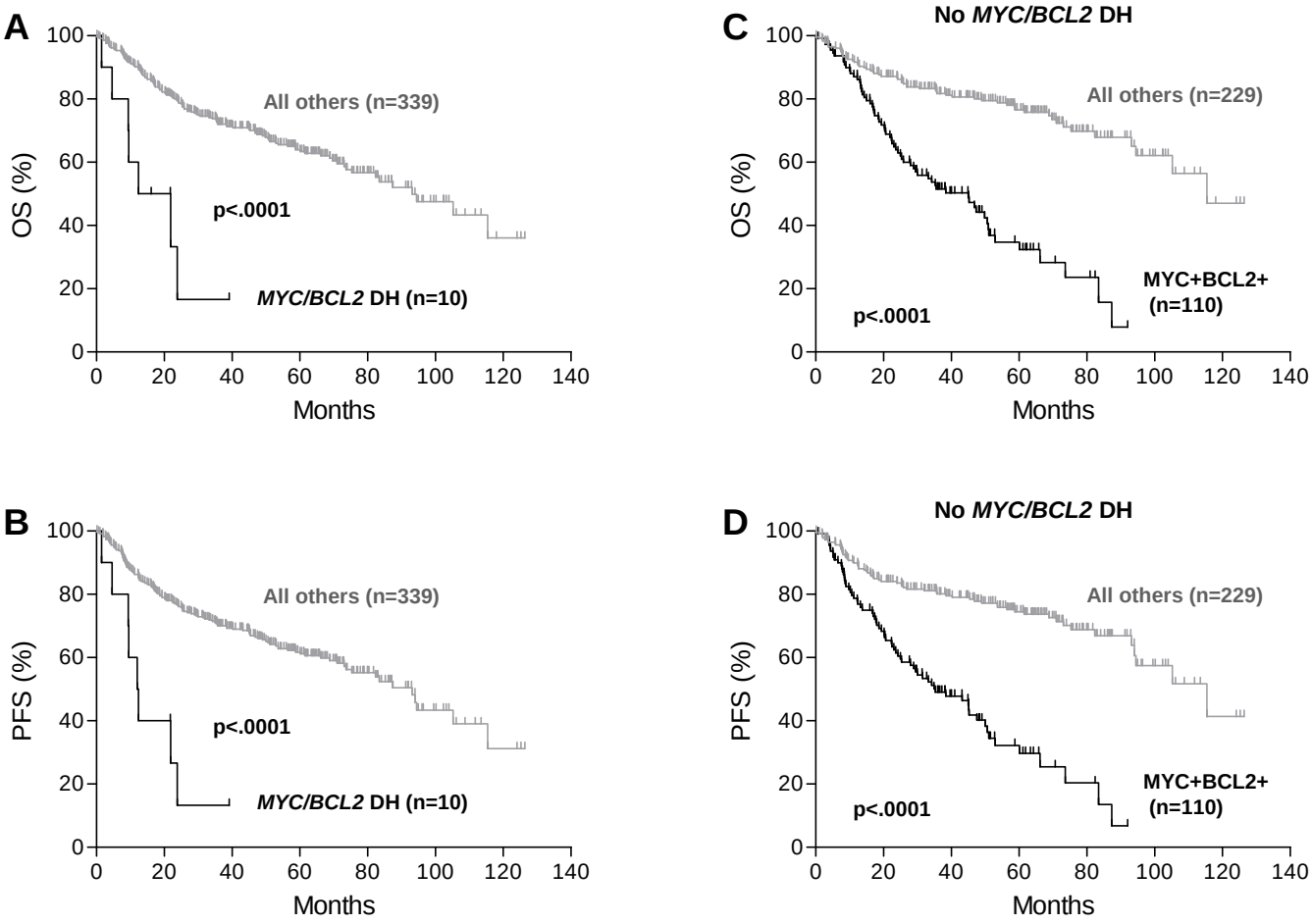

A
OS (%)
All others (n=339)
p<.0001
MYC/BCL2 DH (n=10)
Months
B
PFS (%)
All others (n=339)
p<.0001
MYC/BCL2 DH (n=10)
Months
C
No MYC/BCL2 DH
OS (%)
All others (n=229)
MYC+BCL2+ (n=110)
p<.0001
Months
D
No MYC/BCL2 DH
PFS (%)
All others (n=229)
MYC+BCL2+ (n=110)
p<.0001
Months

Figure S6

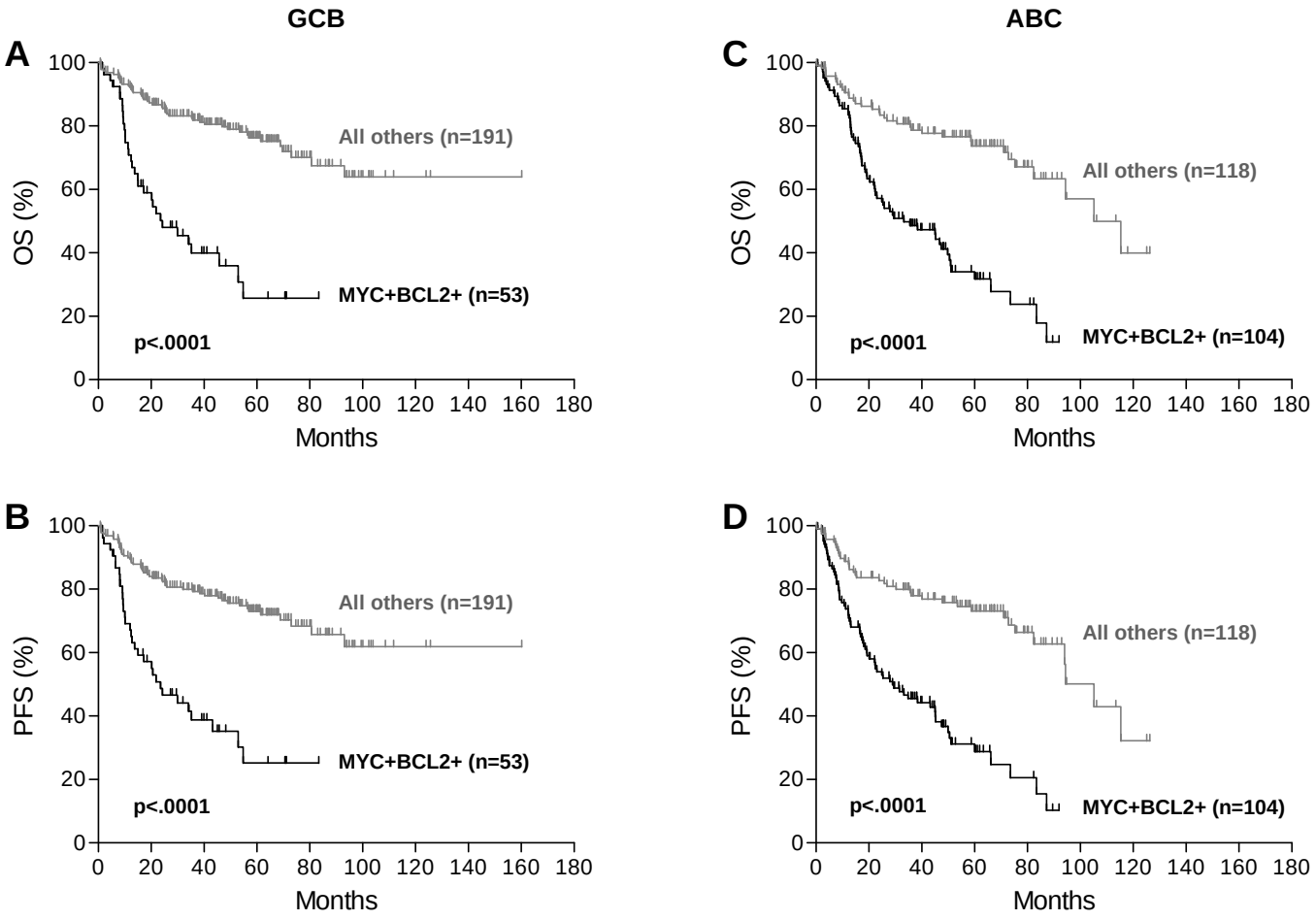

GCB
ABC
A
B
C
D
OS (%)
PFS (%)
Months
All others (n=191)
MYC+BCL2+ (n=53)
All others (n=118)
MYC+BCL2+ (n=104)
p<.0001
0
20
40
60
80
100
120
140
160
180

Figure S7

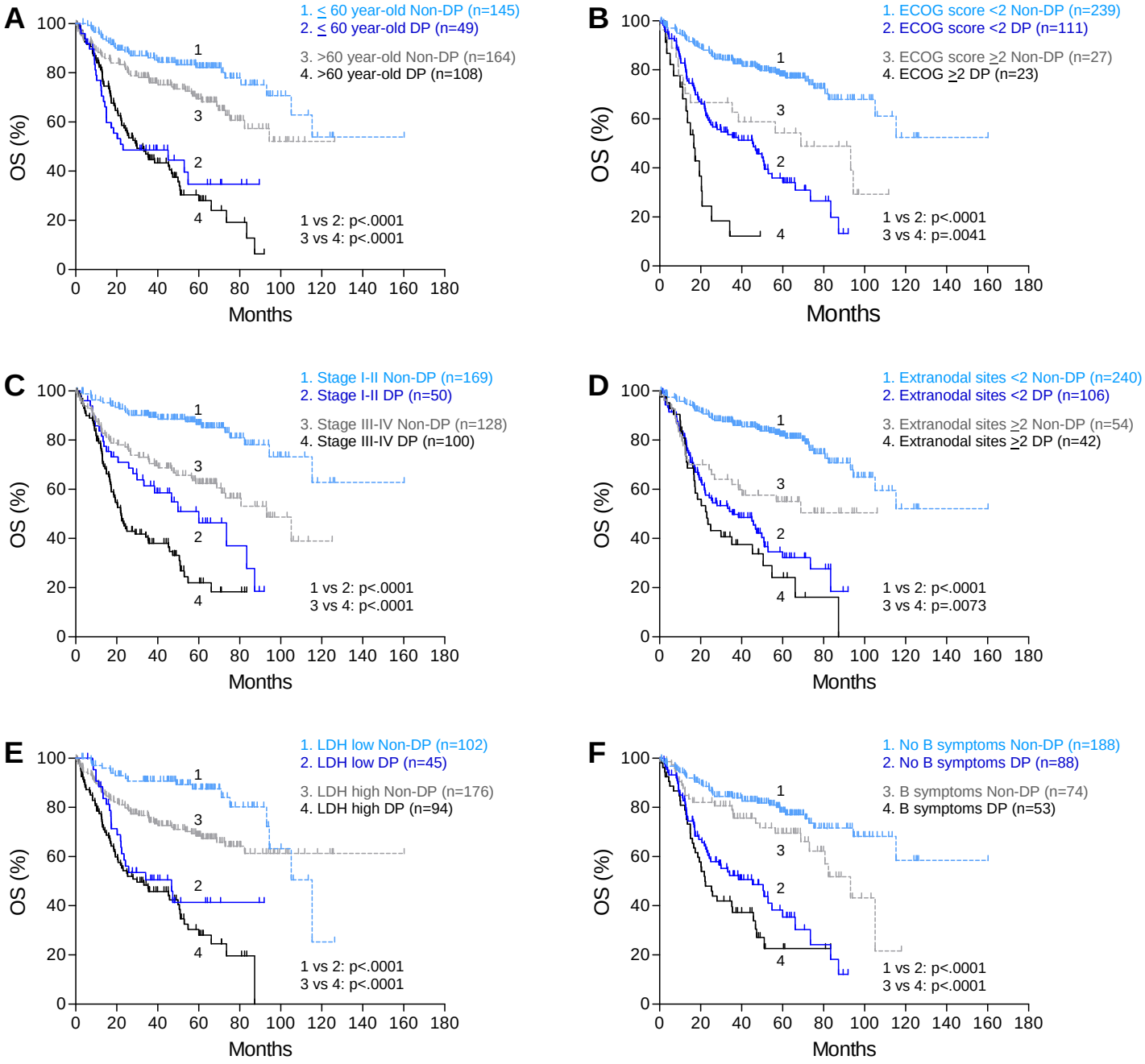

A
1. ≤ 60 year-old Non-DP (n=145)
2. ≤ 60 year-old DP (n=49)
3. >60 year-old Non-DP (n=164)
4. >60 year-old DP (n=108)
1 vs 2: p<.0001
3 vs 4: p<.0001
B
1. ECOG score <2 Non-DP (n=239)
2. ECOG score <2 DP (n=111)
3. ECOG score ≥2 Non-DP (n=27)
4. ECOG ≥2 DP (n=23)
1 vs 2: p<.0001
3 vs 4: p=.0041
C
1. Stage I-II Non-DP (n=169)
2. Stage I-II DP (n=50)
3. Stage III-IV Non-DP (n=128)
4. Stage III-IV DP (n=100)
1 vs 2: p<.0001
3 vs 4: p<.0001
D
1. Extranodal sites <2 Non-DP (n=240)
2. Extranodal sites <2 DP (n=106)
3. Extranodal sites ≥2 Non-DP (n=54)
4. Extranodal sites ≥2 DP (n=42)
1 vs 2: p<.0001
3 vs 4: p=.0073
E
1. LDH low Non-DP (n=102)
2. LDH low DP (n=45)
3. LDH high Non-DP (n=176)
4. LDH high DP (n=94)
1 vs 2: p<.0001
3 vs 4: p<.0001
F
1. No B symptoms Non-DP (n=188)
2. No B symptoms DP (n=88)
3. B symptoms Non-DP (n=74)
4. B symptoms DP (n=53)
1 vs 2: p<.0001
3 vs 4: p<.0001
OS (%)
Months

Figure S8

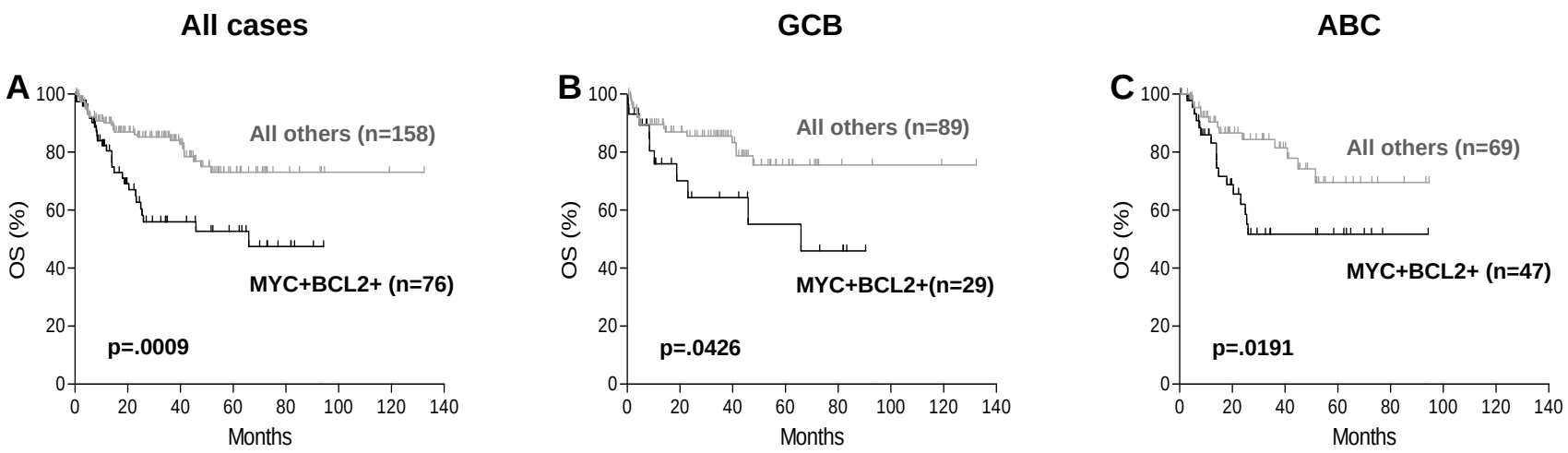
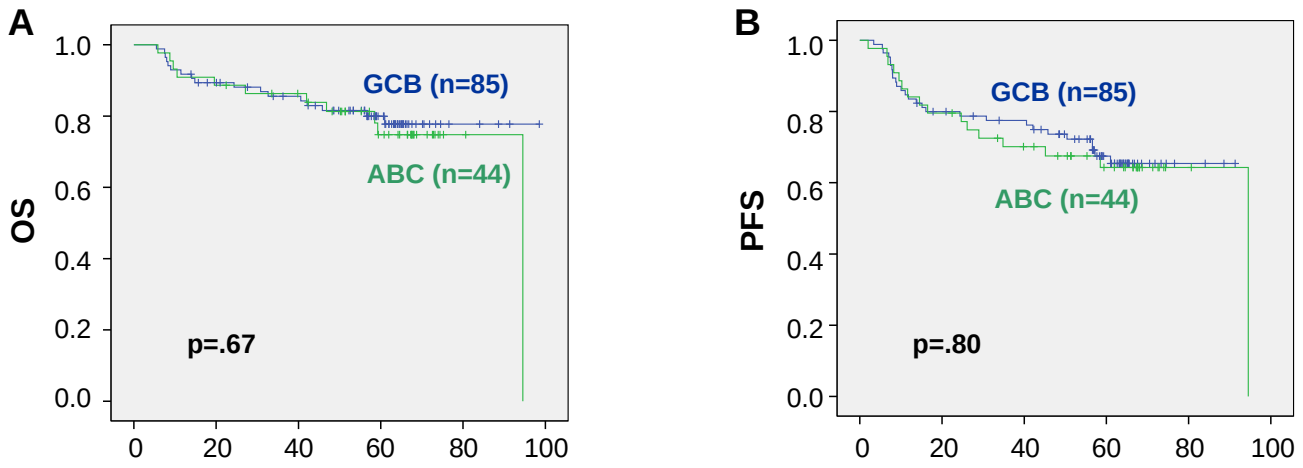

All cases
A
100
80
60
40
20
0
OS (%)
All others (n=158)
MYC+BCL2+ (n=76)
p=.0009
0
20
40
60
80
100
120
140
Months
GCB
B
All others (n=89)
MYC+BCL2+(n=29)
p=.0426
ABC
C
All others (n=69)
MYC+BCL2+ (n=47)
p=.0191

Figure S9


A
OS
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
GCB (n=85)
ABC (n=44)
p=.67
B
PFS
1.0
0.8
0.6
0.4
0.2
0.0
0
20
40
60
80
100
GCB (n=85)
ABC (n=44)
p=.80