Relapsed DLBCLs present frequent copy number variations of genes involved in lymphomagenesis with different pattern between early- and late-relapsed DLBCLs

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The authors are supported by fondation Nelia et Amedeo Barletta, Paris, France.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma. Despite major advance in frontline treatment, a significant proportion of patients will experience treatment failure.

- **Early relapses occur within the first year after the first-line treatment.**
- **Late relapses occur after 1 year or more.**

Prognosis is poor in both cases, and it is worse in early-relapsed DLBCLs.[1,2]

Study objectives

The present study aims at expanding our knowledge in the number and type of genetic alterations present in early- and late-relapsed DLBCLs by integrating structural variants with gene expression profiles.

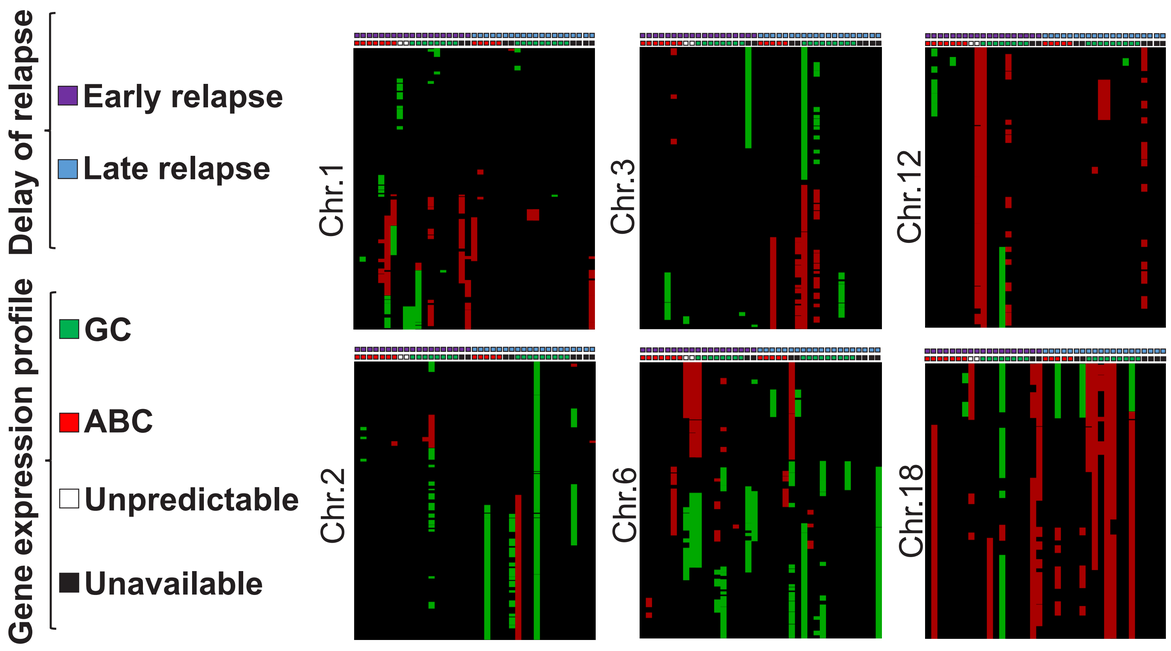

Figure 1: Genomic alterations in early- and late-relapsed DLBCLs. Only CNVs of a size ≥ 2 Mb are represented (losses in green and gains in red).

Results

- **The average total CNV number for the whole group was 15.**
- **Chromosomes 1, 2, 3, 6, 12 and 18 were the most frequently altered compared to other chromosomes ($p=6x10^{-4}$). We noted a great heterogeneity of CNV numbers between individuals (range 0-67 CNVs) but no difference between early-relapsed and late-relapsed DLBCLs (average total CNVs 15 and 16 respectively; p-value = 0.8).**
- **Frequent CNVs involved *ITPKB, XPO1, BCL-6, IRF4, IBTK, PRDM1, TNFAIP3, FOXO1, TP53* and *BCL2* genes but with no systematic difference between late-relapsed and early-relapsed DLBCLs.**
- **Deletions of *CDKN2A* was a common event in early-relapsed DLBCLs.**
- **Forty four genes showed significant CNV difference between early-relapsed and late-relapsed DLBCLs.**

Methods

- **From 396 patients with relapsed DLBCL included in the CORAL trial[(2)], frozen biopsies were available for a total of 39 patients (19 early and 20 late relapses).**
- **Copy Number Variants (CNV) of the 39 samples were determined by high-resolution array-based CGH using the Affymetrix SNP 6.0 platform, which interrogates 1,800,000 copy number probes spaced at mean 1.8 kb intervals throughout the human genome.**
- **Total copy numbers were computed according to the CRLMM algorithm[(3)]. In order to remove artifactual values, we calculated the median value of the signal within a sliding window of 18 kb.**
- **Gains and losses of copy number were defined by a median copy number ≥2.5 and ≤1.5, respectively.**

Gene	Function	Chr.	Early relapses			Late relapses			p-value
			Gains	Normal	Losses	Gains	Normal	Losses	
CD58	Immunity	1	0	16	3	2	18	0	0.07
ITPKB	BCR pathway	1	0	17	2	2	17	1	0.60
ID3	BCR pathway	1	0	17	2	1	19	0	0.35
NOTCH2	NOTCH pathway	1	0	18	1	0	20	0	0.48
XPO1	Cell cycle	3	2	16	1	2	17	1	1
MYD88	NFKB pathway	3	1	17	1	0	18	2	1
BCL6	Germinal center	3	0	18	1	2	17	1	0.73
PRDM1	Cell cycle	6	4	11	4	3	15	2	0.56
TNFAIP3	NFKB pathway	6	0	16	3	1	15	4	1
PIM1	NFKB pathway	6	2	16	1	0	20	0	0.10
IRF4	BCR pathway	6	3	15	1	1	19	0	0.21
EZH2	Epigenetic	7	0	19	0	1	18	1	1
BRAF	MAP kinase	7	0	19	0	0	20	0	1
CARD11	NFKB pathway	7	0	19	0	3	17	0	0.23
MFHAS1	Cell cycle	8	1	18	0	1	19	0	1
MYC	Cell cycle	8	1	17	1	2	18	0	1
NOTCH1	NOTCH pathway	9	2	16	1	1	19	0	0.40
CDKN2A	Cell cycle	9	3	8	8	3	14	3	0.13
CDKN2B	Cell cycle	9	3	8	8	3	14	3	0.42
SYK	BCR pathway	9	0	18	1	0	20	0	0.48
KLF4	Apoptosis	9	0	18	1	0	20	0	0.48
IBTK	BCR pathway	12	2	12	5	2	14	4	0.88
MLL2	Epigenetic	12	3	15	1	1	19	0	0.21
STAT6	JAK/STAT	12	3	16	0	1	19	0	0.34
FOXO1	BCR pathway	13	1	14	4	2	15	3	1
B2M	Immunity	15	1	18	0	1	15	4	0.10
CREBBP	Epigenetic	16	0	17	2	0	20	0	0.23
SOCS1	JAK/STAT	16	2	17	1	0	20	0	0.23
CIITA	Immunity	16	1	18	0	0	20	0	0.48
TP53	Apoptosis	17	1	16	2	0	15	5	0.40
GNA13	Cell cycle	17	1	18	0	0	19	1	1
CD79B	BCR pathway	17	1	18	0	3	17	0	0.60
BCL2	Cell cycle	18	3	14	2	5	15	0	0.45
TCF3	BCR pathway	19	2	17	0	1	18	1	1
TNFSF14	Immunity	19	0	18	1	1	17	2	1
CD79A	BCR pathway	19	0	18	1	2	18	0	0.48
EP300	Epigenetic	22	0	19	0	0	19	1	1

Table 1: Alterations in genes involved in lymphomagenesis.

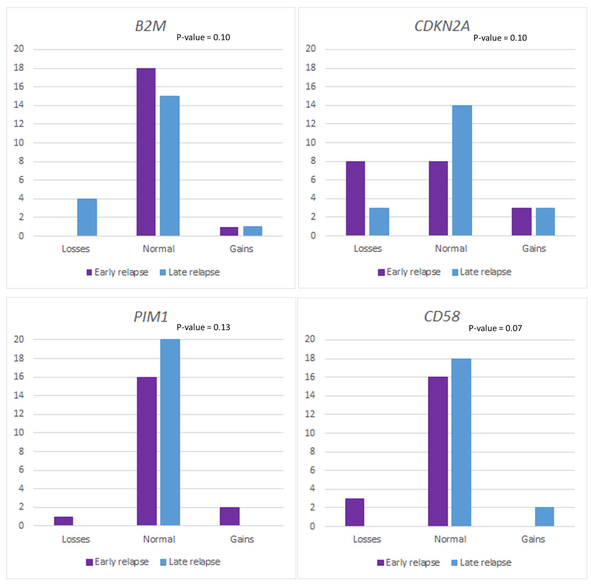

Figure 2: Examples of genomic alterations affecting genes involved in lymphomagenesis.

		Early relapse			Late relapse			
Gene	Function	Gains	Normal	Losses	Gains	Normal	Losses	p-value
LOC100507489	Antisense RNA	0	19	0	3	11	6	0.001
RAB11AP2	Ras oncogene family pseudogene	6	12	1	0	20	0	0.003
LGALS9C	Galectine family	9	9	1	2	9	9	0.004
LOC646214	P21 Protein (Cdc42/Rac)-Activated Kinase 2 Pseudogene	4	15	0	4	8	8	0.0059
CEHCR1	Keratinocytes proliferation and differenciation	6	10	3	0	18	2	0.0063
OR2A1	G-protein-mediated transduction of odorant signals	6	7	6	2	17	1	0.0087
OR2A9P	G-protein-mediated transduction of odorant signals	5	9	5	0	17	3	0.0130
KANSL1-AS1	Histone acetylation ; MLL and ACL complexes	8	10	1	1	16	3	0.0139
OR4K2	G-protein-mediated transduction of odorant signals	1	13	5	6	14	0	0.0145
KIAA1804	MAP kinase activity Negative regulator of TLR4 signaling	2	12	5	1	19	0	0.0176
IGHVII-2-1	Immunoglobulin pseudogene	0	18	1	5	12	3	0.0177
GPR133	G-protein coupled receptor activity	4	14	1	0	20	0	0.0201
RMST	Long non-coding RNA class	4	14	1	0	20	0	0.0201
RP11-110L15.2	LincRNA	4	14	1	0	20	0	0.0201
RP11-116D17.2	LincRNA	4	14	1	0	20	0	0.0201
RP11-749H20.1	LincRNA	4	14	1	0	20	0	0.0201
RPL7P38	Ribosomal protein L7 pseudogene 38	4	14	1	0	20	0	0.0201
STX2	Epithelial-mesenchymal interactions and epithelial cell morphogenesis and activation	4	14	1	0	20	0	0.0201
TBX5	DNA-binding domain : T-box Transcription factors involved in the regulation of developmental processes	4	14	1	0	20	0	0.0201
TMEM132C	Transmembrane protein	4	14	1	0	20	0	0.0201
TMEM132D	Transmembrane protein Oligodendrocyte differentiation	4	14	1	0	20	0	0.0201
GPLD1	Glycosylphosphatidylinositol (GPI) degradation Sodium channel regulator activity	4	15	0	0	18	2	0.0201
AKT3	Protein serine/threonine kinase activity Metabolism, proliferation, cell survival, growth and angiogenesis Downstream mediator of the PI 3-K pathway	2	13	4	1	19	0	0.0201
FUT9	biosynthesis of Lewis X antigen Expression of CD15 in mature granulocytes	3	10	6	0	17	3	0.0458
CCDC59	Component of the transcription complexes of the pulmonary surfactant-associated protein-B and C	4	15	0	0	20	0	0.0471
FCRL2	Normal and neoplastic B cell development	3	15	1	0	20	0	0.0412
FLJ37505	LincRNA	3	15	1	0	20	0	0.0412
HSPA8P5	Heat shock protein 8 pseudogene 5	3	15	1	0	20	0	0.0412
HSPE1P20	Heat shock protein 1 pseudogene 20	3	15	1	0	20	0	0.0412
MED13L	RNA polymerase II transcription cofactor activity	3	15	1	0	20	0	0.0412
NAV3	Cellular growth and migration	4	15	0	0	20	0	0.0412
PRB3	Proline-rich salivary protein Bacterial receptor	4	15	0	0	20	0	0.0412
RP11-781A6.1	LincRNA	4	15	0	0	20	0	0.0412
RP11-983C2.1	Known processed pseudogene	3	15	1	0	20	0	0.0412
RP4-765H13.1	LincRNA	3	15	1	0	20	0	0.0412
TMTC2	Transmembrane and tetratricopeptide repeat containing 2	4	15	0	0	20	0	0.0412
TRHDE	Thyrotropin-releasing hormone degrading enzyme Metallopeptidase and aminopeptidase activity	4	15	0	0	20	0	0.0412
ZNF177	Transcriptional regulation	0	19	0	2	15	3	0.0412
CDKN2B-AS1	Antisense RNA ; interact with polycomb repressive complex-1 (PRC1) and -2 (PRC2) Regulator for epigenetic transcriptional repression	2	11	6	1	18	1	0.0497

Table 2: Genes with differential distribution of CNVs between early and late relapses.

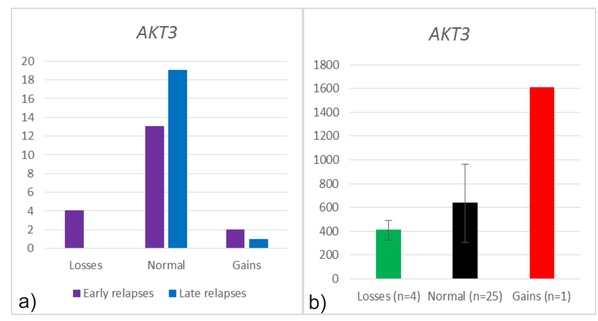

Figure 3. a) CNVs affecting *AKT3* are differentially distributed among early- and late-relapsed DLBCLs. b) Deletions and duplications have subsequent consequences on *AKT3* expression level.

Conclusion

In this series, we found an equivalent number of CNVs among early- and late-relapsed DLBCLs. Genes involved in lymphomagenesis frequently exhibited CNVs. *CDKN2A* showed a high frequency of deletions in early-relapsed DLBCLs. We identified a list of 44 genes, which abnormalities were differentially distributed among early- and late-relapsed DLBCL with subsequent consequence on expression level.

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13icml 164-P
Aggressive B-cell lymphoma
BROSÉUS Julien
DOI: 10.3252/pso.eu.13icml.2015
Poster presented at: 13-ICML
Printing supported by: Takeda ONCOLOGY
PosterSessionOnline.com