Mutational and copy number analysis at diagnosis and relapse of mantle cell lymphoma

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Abstract

Most patients diagnosed with mantle cell lymphoma (MCL) experience extended remissions following frontline chemoimmunotherapy, yet with extended follow-up, relapses seem nearly inevitable. This study aimed to define the genomic landscape of MCL at diagnosis and relapse and investigate the clonal evolutionary dynamics associated with progression of disease (POD). We conducted comprehensive genomic sequencing on 214 tumor specimens from 189 patients, including 144 treatment-naïve and 70 POD samples, with 25 patients providing longitudinal paired samples pre-treatment and at POD. Comparative analyses were performed on single nucleotide variants, insertions/deletions and copy number alterations to assess genomic differences between specimens from treatment-naïve and relapsed patients. Additionally, mutational signatures were evaluated in pre-treatment samples, stratified by time to progression (≤24 months *vs*. >24 months). One hundred patients who received standard frontline chemoimmunotherapy were included in the survival analysis. Genomic profiles of pre-treatment specimens from patients who ultimately relapsed were strikingly similar to those observed in POD, while distinctly different from profiles associated with prolonged remissions. This genomic 'stability' was further confirmed by analysis of 25 paired specimens, demonstrating a remarkable genomic concordance despite extended remission periods (median >3 years), without a clear pattern of acquired alterations. Our findings suggest that MCL relapse is predominantly driven by pre-existing malignant clones at diagnosis, rather than by new evolutionary events, underscoring the importance of early detection and eradication of resistant clones to improve long-term outcomes.

Introduction

Most patients diagnosed with mantle cell lymphoma (MCL) typically experience long remissions following frontline chemoimmunotherapy.[1,2] However, with extended follow-up, relapses appear almost inevitable, and are associated with progressively shorter remissions to subsequent therapies.[3-5] This ultimate shift in disease course suggests an acquired molecular event that promotes the emergence of a prevailing resistant clone.[6] Consequently, understanding the evolutionary processes that occur during these extended remissions is a priority.

Our understanding of the genomic evolution of MCL remains limited.[6-8] Similar to other lymphomas, two primary patterns of progression have been suggested.[6] The first includes primary refractoriness and early relapses, frequently characterized by persistent minimal residual disease.[9] The second pattern, is associated with prolonged minimal residual disease-negative remissions.[6,7] The only abnormalities that have consistently predicted inferior responses and shorter remissions across studies have been mutations in *TP53*. However, these account for fewer than half of POD events, leaving many cases unexplained.[10,11] Additional genomic alterations suggested to confer a poorer prognosis are mutations in *KMT2D* and deletions at the *CDKN2A* locus.[12,13] In particular, it appears, that copy number alterations (CNA) play a pivotal role in the disease course of MCL.[13] Notably, two prior studies on small cohorts of MCL with sequential samples before and after frontline treatment demonstrated a subset of patients acquiring CNA, primarily affecting *CDKN2A*. However, there was no definite acquisition of abnormalities to clearly support an

evolutionary process.[6,7]
In this study we sought to identify genomic alterations associated with progression in MCL and to evaluate whether an identifiable evolutionary process underlies lymphoma persistence along the continuum of care. To achieve this, we studied tumor specimens from a large cohort of MCL patients who were sequenced either prior to initiation of frontline treatment or at the time of progression, including a subset of patients with sequential samples.

Methods

This study analyzed data from patients diagnosed with MCL who underwent tumor specimen sequencing during their disease course. Sequencing was conducted in a CLIA-certified laboratory at Memorial Sloan Kettering Cancer Center using the institutional targeted sequencing platform, IMPACT-HEME. The study received Institutional Review Board approval, and all patients provided informed consent.

Targeted sequencing

Following consent, archival or new tumor biopsy samples were sequenced with saliva or nails used as a source of normal control (germline) DNA.[14] The MSK-IMPACT platform, a hybridization capture-based assay, was used to sequence all protein-coding exons of 399 cancer-associated genes on the Illumina HiSeq2500 (*Online Supplementary Table S1*). Average sequencing coverage across all tumors was 748-fold. Paired-sample variant calling identified single nucleotide variants (SNV) and small insertions/deletions (indels) (<30 bp) in tumor samples matched with normal controls.[14]

Identification of copy number alterations

CNA analysis used a modified version of Sequenza v2.1.2, optimized by BostonGene to minimize focal false-positive segments particularly in centromere regions. FACETS v0.5.14 single nucleotide polymorphism (SNP) database provided comprehensive extraction coverage. Given the limited heterozygous loci in the targeted sequencing approach, Strelka2 v2.8.2 germline caller was used to identify heterozygous variants, which were incorporated into individual reference files, improving statistical segmentation and CNA calling. CNA were limited to genes validated by SNP array and described in the literature (*Online Supplementary Figures S1* and *S2*). CNA were limited to genes validated by cases with available SNP array data (N=18; *Online Supplementary Table S2*) and those described in the literature.

Clustering, prognostic features and survival analyses

To identify tumors with shared genetic features we applied hierarchical clustering with Jaccard distances and Ward linkage. We selected this approach as it is particularly suited for asymmetric binary data and offers the added advantage of intuitive dendrogram visualization.[15] We compared genomic features between samples grouping on time of sequencing (pre-treatment *vs*. relapse) and stratified by Ki67 proliferation index (<30%, 30-59%, ≥60%). We further sub-divided the pre-treatment group into those who had POD ≤24 months after therapy (POD24) and those with a follow-up ≥24 months without progression (long remission) and compared these to samples at relapse. For sequential samples, a minimum of 3 months elapsed between frontline treatment initiation and the relapse sample. For patients with multiple relapse samples, the earliest specimen was selected. Only patients sequenced prior to standard frontline chemoimmunotherapy were included in survival analyses. Patients with concurrent malignancies (apart from low-grade or non-life-threatening cancers) were excluded.

Statistical analysis

Genomic abnormality frequencies were compared between groups using the Fisher exact test; paired samples were analyzed with McNemar and *t* tests. Kaplan-Meier survival analysis and the log-rank test evaluated progression-free and overall survival (PFS/OS), calculated from treatment initiation to progression, death, or censoring. *P* values and false discovery rate corrections by Bonferroni-Hochberg were reported, with false discovery rate significance set at $q < 0.1$. Analyses were conducted in R version 4.2.
Supplementary analyses included 125 additional samples excluded from the primary analysis because of lack of validated CNA data. Of these, 99 were omitted due to informed consent restrictions preventing CNA assessment and 26 sequenced by FoundationHeme without use of individual matched-normal data.

Results

We sequenced 334 samples from patients diagnosed with MCL. Of these, CNA data were available for 214 samples (189 unique patients; 88 cases excluded due to informed consent restrictions; 28 cases due to inadequate samples for CNA analysis). There were 144 patients sequenced prior to initiation of frontline therapy or while on expectant management and 70 patients were sequenced at the time of relapse (Table 1). Of these, 25 patients had samples sequenced prior to frontline treatment and at the time of relapse. One hundred patients were eligible for survival analysis (Figure 1; *Online Supplementary Figure S3* – expanded analysis N=321 without CNA data).
The most common genomic alterations were mutations in *ATM* (48%), *TP53* (28%), and *KMT2D* (22%). Gains or amplifications were observed in *TP63* (41%) and *MYC* (31%) and deletions were found in *RB1* (35%), *ATM* (34%), *CDKN2A* (28%), *ARID1B* (26%), and *TP53* (22%) (Figure 2; *Online Supplementary Tables S3-S4; Online Supplementary Figure S4* – expanded analysis without CNA data).

Deletions of *TP53* (17p13.1) co-occurred with mutations in *TP53* (65% of patients with mutated *TP53* also had deletions of *TP53*; and 52% of those with deleted *TP53* also had *TP53* mutations, *P*<0.001) and with amplification of *TP63* (3q28). The latter was also associated with mutations in *WHSC1* (*NSD2*) (Figure 3). Deletions of *ATM* (11q22.3) often co-occurred with mutations in either *ATM* or *BIRC3*, but not both (despite the proximity between the genes on the long arm of chromosome 11). Deletions of *TP53* and mutations of *TP53* were almost completely exclusive of deletions of

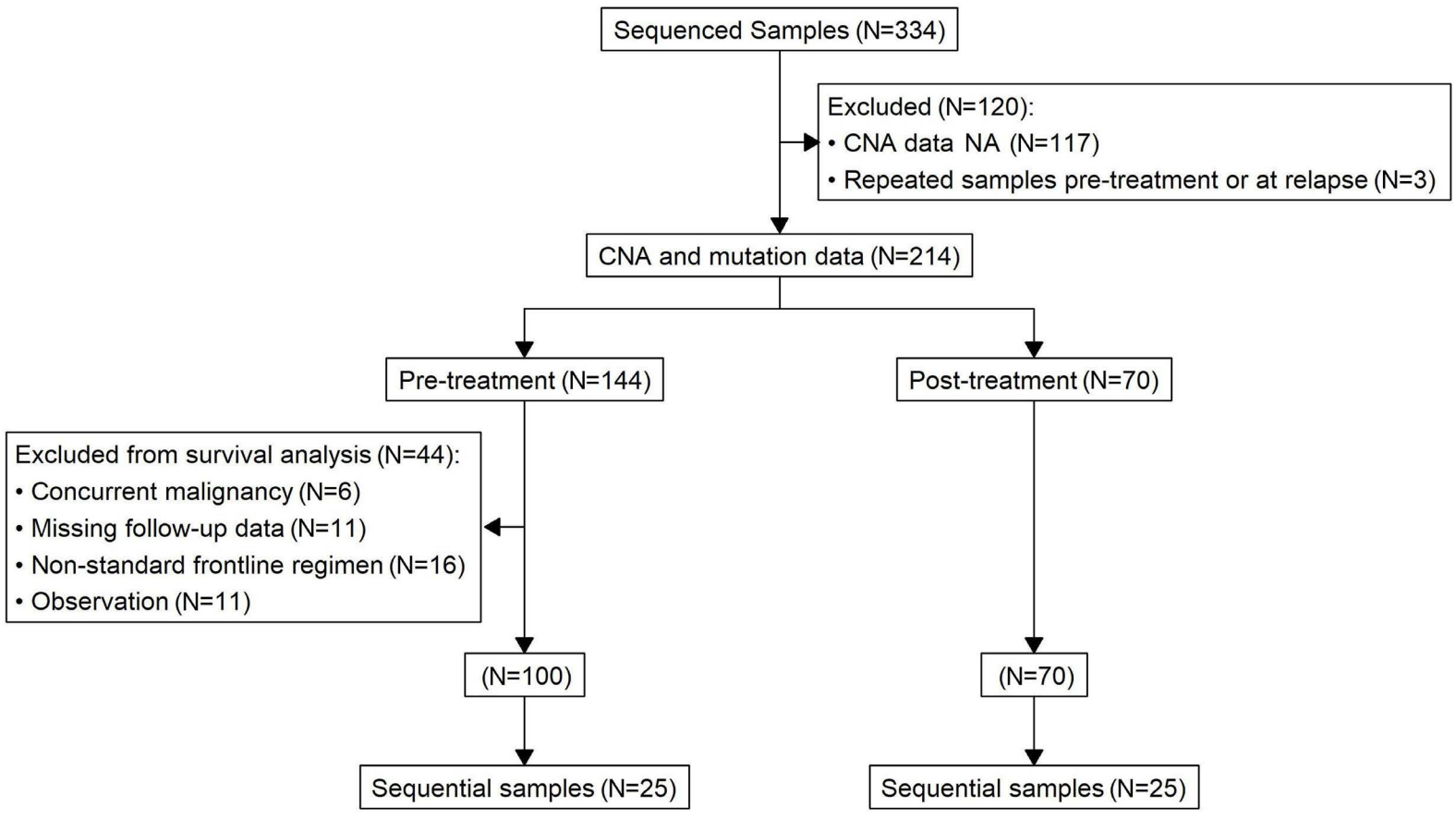


Figure 1. CONSORT (Consolidated Standards of Reporting Trials) diagram. We sequenced 334 samples from patients diagnosed with mantle cell lymphoma. Data on copy number alterations were available for 214 of these samples (189 unique patients): 144 patients were sequenced prior to initiation of frontline therapy or while on expectant management and 70 patients were sequenced at the time of relapse. One hundred patients were eligible for survival analysis. *25 patients were sequenced prior to frontline treatment and at the time of relapse. CNA: copy number alterations; NA: not available.

Table 1. Cohort characteristics.

Characteristics	All N=214	Pre-treatment N=144	POD N=70	*P*
Age, years, median (IQR)	66 (58-72)	64 (57-71)	68 (61-75)	0.006
Male sex, N (%)	168 (78.5)	108 (75.0)	60 (85.7)	0.11
Timing of sequencing, N (%)				
Prior to frontline	144 (67.3)	144 (100)	-	-
First relapse/second line	44 (20.6)	-	44 (62.9)	-
Second relapse/third line	18 (8.41)	-	18 (25.7)	-
Multiply relapsed	8 (3.74)	-	8 (11.4)	-
Features at the time of sequencing				
IGHV unmutated, N=77, N (%)	58 (75.3)	35 (79.5)	23 (69.7)	0.47
del(17p) by FISH/SNP, N=48, N (%)	22 (45.8)	11 (37.9)	11 (57.9)	0.29
Blastoid/pleomorphic, N (%)	42 (19.7)	17 (11.9)	25 (35.7)	<0.001
Ki67, N=167, N (%)				0.001
<30%	53 (31.7)	43 (41.3)	10 (15.9)	-
≥30%	71 (42.5)	42 (40.4)	29 (46.0)	-
≥60%	43 (25.7)	19 (18.3)	24 (38.1)	-

POD: progression of disease; IQR: interquartile range; FISH: fluorescence *in-situ* hybridization; SNP: single nucleotide polymorphism array; *IGHV*: immunoglobulin heavy chain; Ki67: proliferation index.

ATM and mutations in *ATM*, respectively. Deletions of *RB1* (13q14.2) co-occurred frequently with deletions of *ATM* (11q22.3), deletions of *CDKN2A* (9p21.3), deletions of *ARID1B* (6q25.3) and with mutations in *MEF2B*. *MEF2B* mutations were also mutually exclusive of amplifications of MYC (8q24.21) and mutations in *TP53*. Together, the above interactions generated three major clusters: the first converging around double-hit (deletion and mutation) in *ATM* (ATM-DH); the second around double-hit in *TP53* (TP53-DH) and the third characterized by absence of mutations in *TP53* and of CNA in *TP53* and *ATM*, but with common mutations in *ATM* (ATM-MUT) (Figure 3).

There were 164 patients with follow-up of at least 24 months from initiation of treatment. Of these, 70 remained disease-free after frontline systemic therapy (long remission) and 94 patients experienced a relapse (POD). Of the latter, 49 cases were sequenced prior to frontline therapy and 70 sequenced at relapse (25 patients contributed samples at 2 time-points). The median time from sequencing prior to frontline therapy and POD was 21 months. Samples from patients who had ultimately experienced a POD, whether sequenced prior to frontline treatment or later, had higher rates of *TP53* mutations (10% for those with long remission *vs*. 33% for progressors sequenced prior to frontline therapy, and 42% for samples sequenced at subsequent lines of therapy, $P \leq 0.001$), deletions of *CDKN2A* (19% *vs*. 27% and 35%, $P \leq 0.001$) and deletions of *TP53* (11% *vs*. 24% and 28%, $P \leq 0.001$) (Figure 4; *Online Supplementary Table S3*). Among the 49 patients sequenced before treatment whose lymphoma later progressed, there were 27 patients who experienced progression within the first 24 months from therapy initiation (POD24). Compared to samples sequenced prior to frontline therapy from patients who had long remissions, samples from patients who later experienced a POD24 had numerically higher rates of *TP53* (44% *vs*. 12%, $P=0.1$) and *SMARCA4* mutations (26% *vs*. 9%, $P=0.06$) (Figure 4; *Online Supplementary Figure S5* – expanded analysis mutation only). There was no significant difference in the variant allele frequency or number of mutations in any gene between patients sequenced at diagnosis and those at relapse (*Online Supplementary Table S3*).

Twenty-five patients had sequential samples from before treatment and at the time of POD/relapse. The median time between samples was 42 months (interquartile range, 18-51 months). Acquisition or disappearance of genomic abnormalities, mostly CNA, was seen in 24 out of 25 patients; however, these were sporadic changes that were each present in very few patients (Figure 5; *Online Sup-*

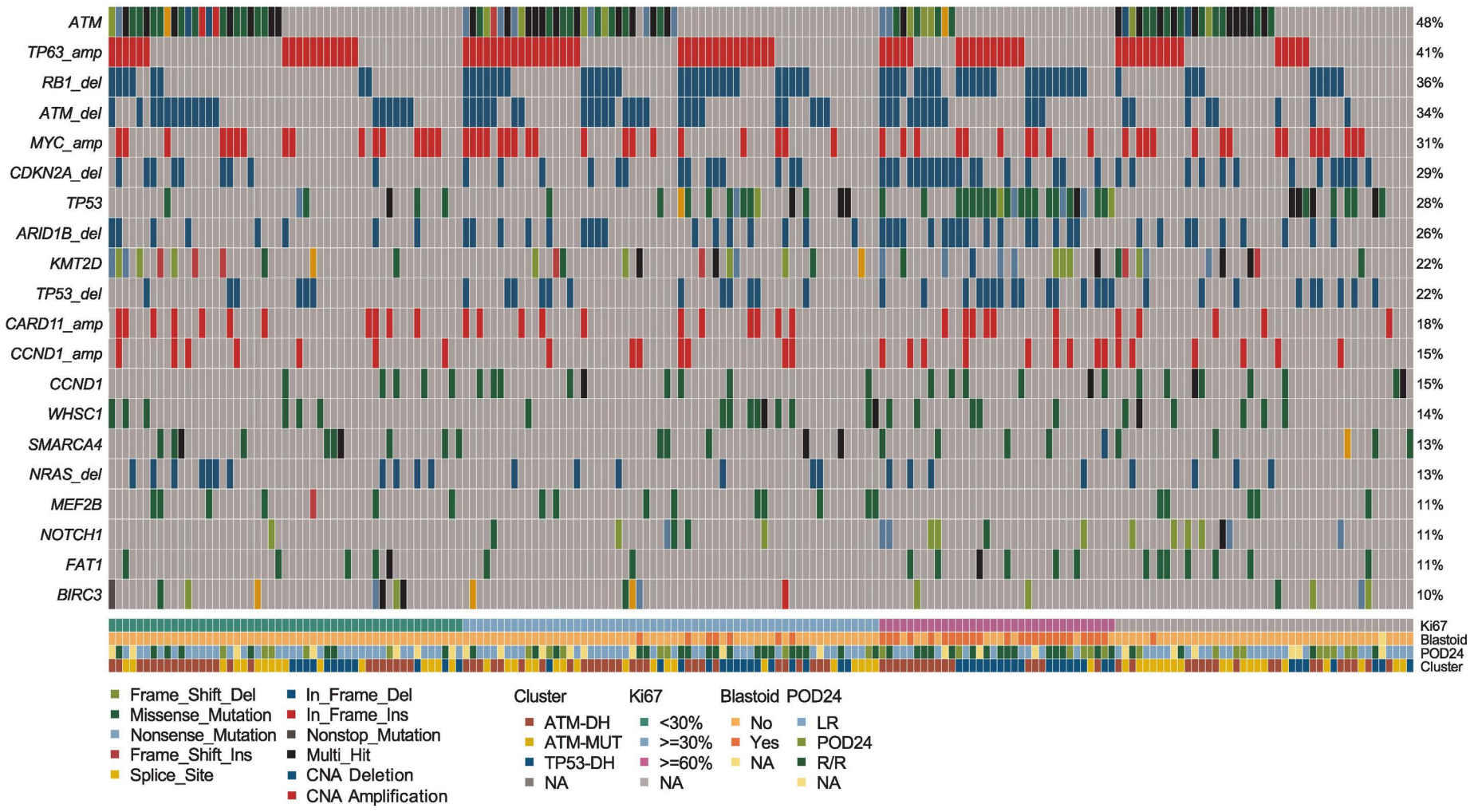
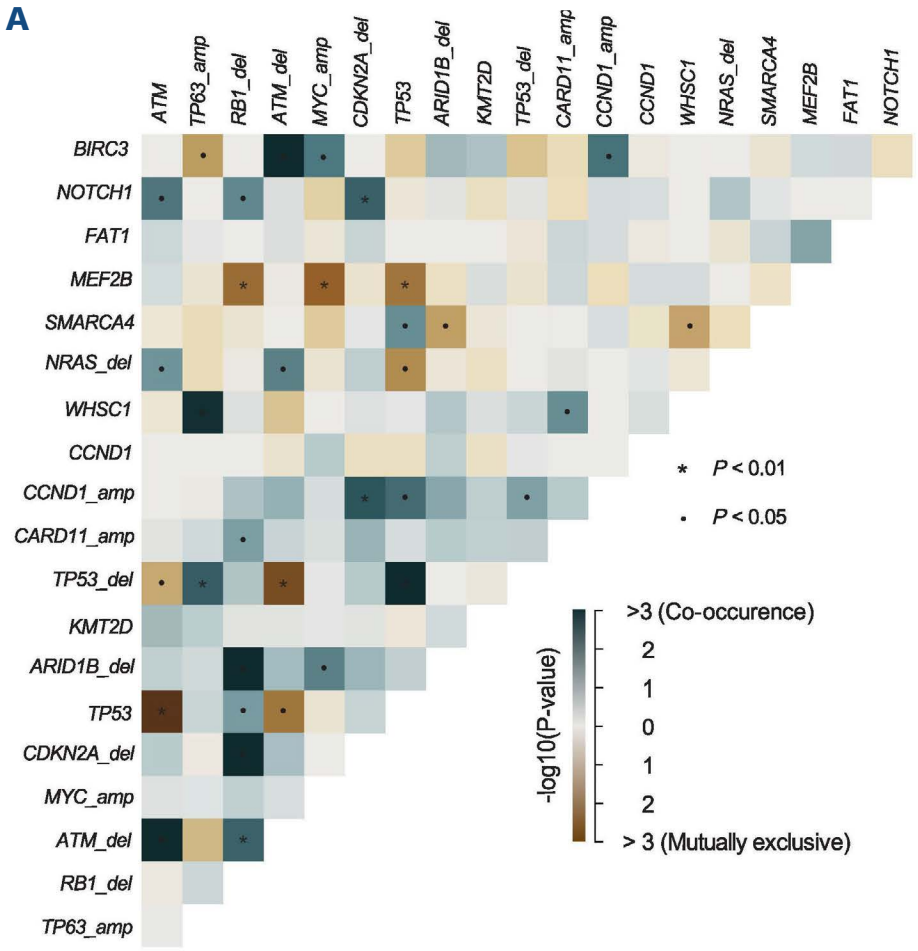


Figure 2. Genomic landscape of mantle cell lymphoma (grouped by Ki67 index). Genomic landscape of mantle cell lymphoma for 189 unique patients (excluding repeated samples). Higher Ki67 is associated with enrichment of *TP53* mutations and deletions in *RB1, CDKN2A, TP53* and *ARID1A* (q<0.05 after false discovery rate correction) (*Online Supplementary Table S4*). CNA: copy number alteration; ATM-DH: double-hit (deletion and mutation) in *ATM*; ATM-MUT: absence of mutations in *TP53* and of copy number alterations in *TP53* and *ATM*, but with common mutations in *ATM*; TP53-DH: double-hit (deletion and mutation) in *TP53*; NA: not available; Ki67: proliferation index; LR: long remission; POD24: progression of disease within 24 months; R/R: relapsed/refractory.

A



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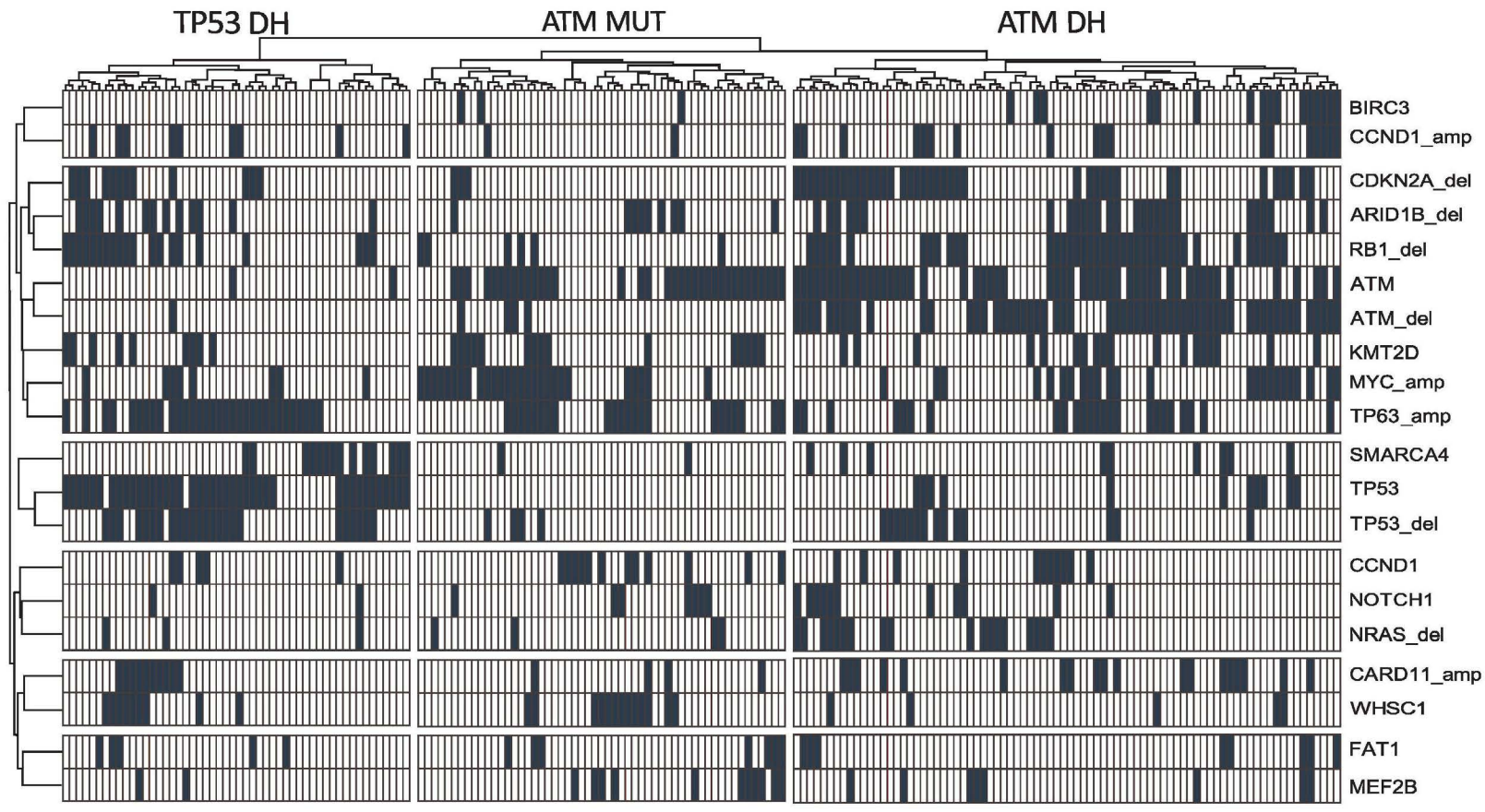

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Gene	Chromosome	Frequency	Gene	Chromosome	Frequency	Event	11	10	01	00	oddsRatio	p value	q value
TP53		0.28	ATM		0.48	Mutually_Exclusive	5	47	85	52	0.06	<0.001	<0.01
TP53_del	17p13.1	0.22	TP53		0.28	Co_Occurrence	27	15	25	122	8.58	<0.001	<0.01
RB1_del	13q14.2	0.35	ARID1B_del	6q25.3	0.26	Co_Occurrence	32	35	17	105	5.53	<0.001	<0.01
ATM_del	11q22.3	0.34	BIRC3		0.10	Co_Occurrence	16	47	3	123	13.63	<0.001	<0.01
CDKN2A_del	9p21.3	0.28	RB1_del	13q14.2	0.35	Co_Occurrence	33	21	34	101	4.58	<0.001	<0.01
ATM_del	11q22.3	0.34	ATM		0.48	Co_Occurrence	42	21	48	78	3.19	<0.001	<0.01
TP63_amp	3q28	0.41	WHSC1		0.14	Co_Occurrence	19	59	8	103	4.07	0.001	0.02
ATM_del	11q22.3	0.34	TP53_del	17p13.1	0.22	Mutually_Exclusive	6	57	36	90	0.26	0.003	0.03
MEF2B		0.11	MYC_amp	8q24.21	0.31	Mutually_Exclusive	1	20	58	110	0.09	0.005	0.05
CDKN2A_del	9p21.3	0.28	CCND1_amp	11q13.3	0.15	Co_Occurrence	15	39	14	121	3.27	0.006	0.06
MEF2B		0.11	RB1_del	13q14.2	0.35	Mutually_Exclusive	2	19	65	103	0.17	0.007	0.07
TP63_amp	3q28	0.41	TP53_del	17p13.1	0.22	Co_Occurrence	25	53	17	94	2.57	0.008	0.07
NOTCH1		0.11	CDKN2A_del	9p21.3	0.28	Co_Occurrence	11	9	43	126	3.52	0.009	0.08
ATM_del	11q22.3	0.34	RB1_del	13q14.2	0.35	Co_Occurrence	31	32	36	90	2.38	0.009	0.08
MEF2B		0.11	TP53		0.28	Mutually_Exclusive	1	20	51	117	0.11	0.01	0.08

Figure 3. Genomic abnormalities association matrix and cluster analysis. (A) Heatmap of co-occurrence and mutual exclusivity of mutations and copy number alterations. (B) Genomic clusters by hierarchical clustering. (C) Details of the co-occurrence and mutual exclusivity of mutations and copy number alterations including the number of samples with both alterations present ('11'), both absent ('00') and only one or the other present ('10'/'01'). TP53-DH: double-hit (deletion and mutation) in *TP53;* ATM-MUT: absence of mutations in *TP53* and of copy number alterations in *TP53* and *ATM*, but with common mutations in *ATM*; ATM-DH: double-hit (deletion and mutation) in *ATM*.

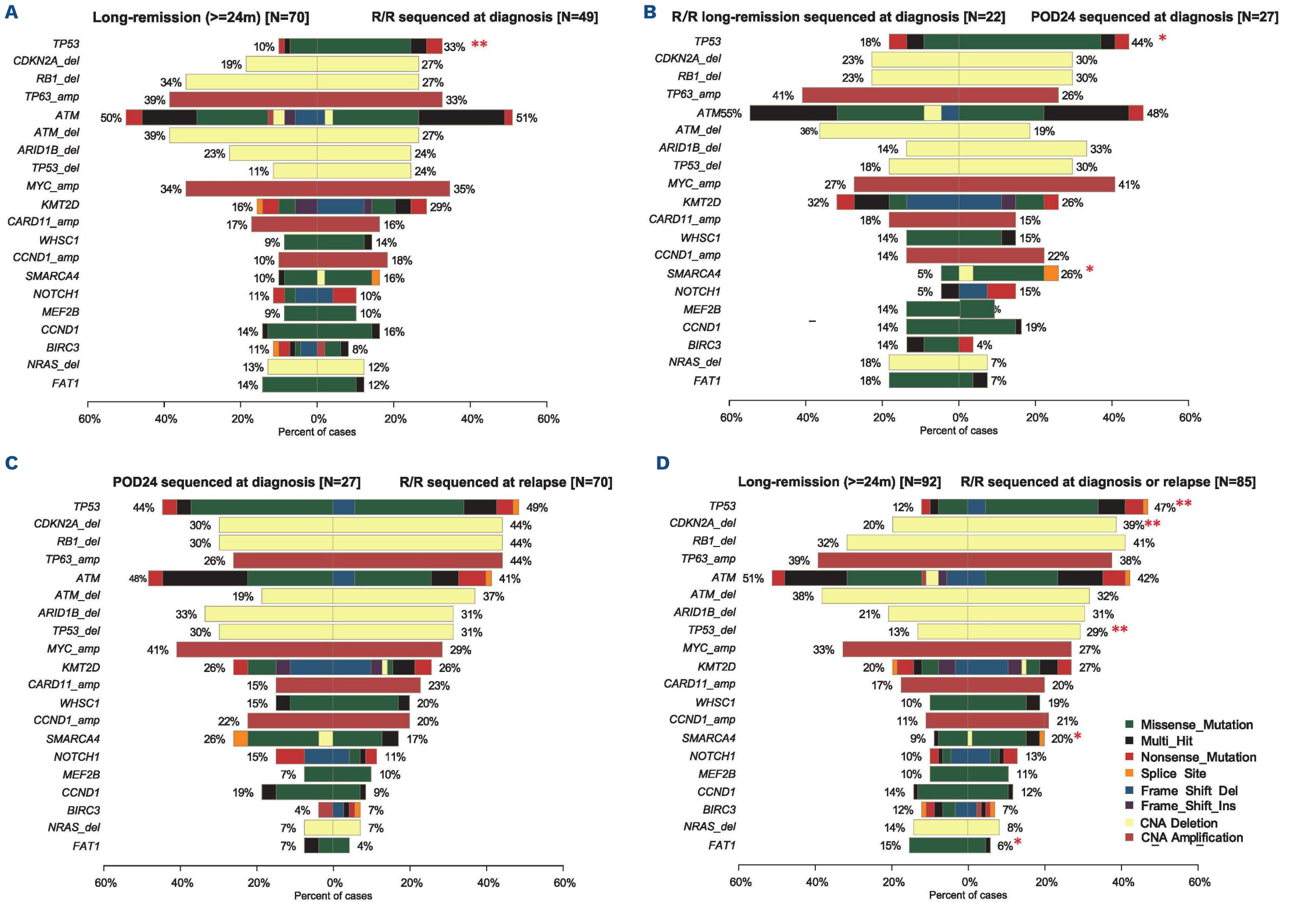

Figure 4. Genomic landscape at frontline and at later lines of treatment comparing patients with long remission to those with earlier progression of disease. Long remission indicates a follow-up of at least 24 months without progression of disease. Progression signifies disease relapse at any time (deaths without lymphoma not considered an event). (A) Samples prior to frontline therapy comparing patients with a long remission (>24 months) to those of patients who experienced a relapse at any time. (B) Samples prior to frontline treatment from patients who experienced progression of disease within 24 months (POD24) (N=27) had a trend towards higher rates of *TP53* and *SMARCA4* mutations (*$P \leq 0.1$; not meeting statistical significance due to sample size). (C) Samples sequenced at frontline from patients with POD24 were very similar to those sequenced at relapse. (D) Samples from patients sequenced at frontline who later experienced a POD24 and those sequenced at relapse had a higher rate of mutations in *SMARCA4* and a lower rate of mutations in *FAT1* (*$P<0.05$; insignificant after correction for false discovery rate). Samples (N=49) from patients with insufficient follow-up or not treated with chemoimmunotherapy are excluded. Plots C and E exclude sequential samples from the same patient. R/R: relapsed/refractory; CNA: copy number alteration.

plementary Figure S7A). Therefore, there was no statistically significant difference in the overall rate of genomic abnormalities between sequential samples nor was there a difference in the number of mutations per gene or in variant allele frequencies (*Online Supplementary Figures S6A* and *S7A; Online Supplementary Table S5*). The most common changes were the acquisition of a *CARD11* amplification (6 patients), deletion of *ATM* (4 patients), deletion of *TP53* (4 patients), and deletion of *CDKN2A* (4 patients). In six out of eight patients, amplification of *CCND1* seen at diagnosis was not identified at the time of POD as was the case for *BIRC* mutations in three of four patients. *TP53* mutations were seen as a new event in only two patients and there was no difference in the genomic loci, number, or variant allele frequencies between pre-treatment and progression samples (*Online Supplementary Figure S6B*; *Online Supplementary Table S5*). Of note, overall new events in the *TP53* gene were seen in five patients (2 of whom had *TP53* mutations at baseline and acquired a deletion).

There were 100 patients with samples sequenced prior to frontline treatment with chemoimmunotherapy, and eligible for survival analysis (Table 2). Of these, 37% (N=37) experienced POD, half of whom experienced POD24. Although not statistically significant, patients who later experienced a POD were slightly older and with a higher rate of blastoid/high-Ki67 MCL compared to patients with long remissions. There was also a lower use of high-dose chemotherapy with autologous stem cell transplantation (ASCT; 24% *vs*. 59%, *P*=0.03) in this group. Most patients (75%) were treated with the intent-to-use intensive regimens. Median follow-up was nearly 5 years (57 months; 95% confidence interval [95% CI]: 46-71 months) with 95% of patients followed for at least 24 months (5 patients in remission who were followed for <24 months were excluded from analyses of long

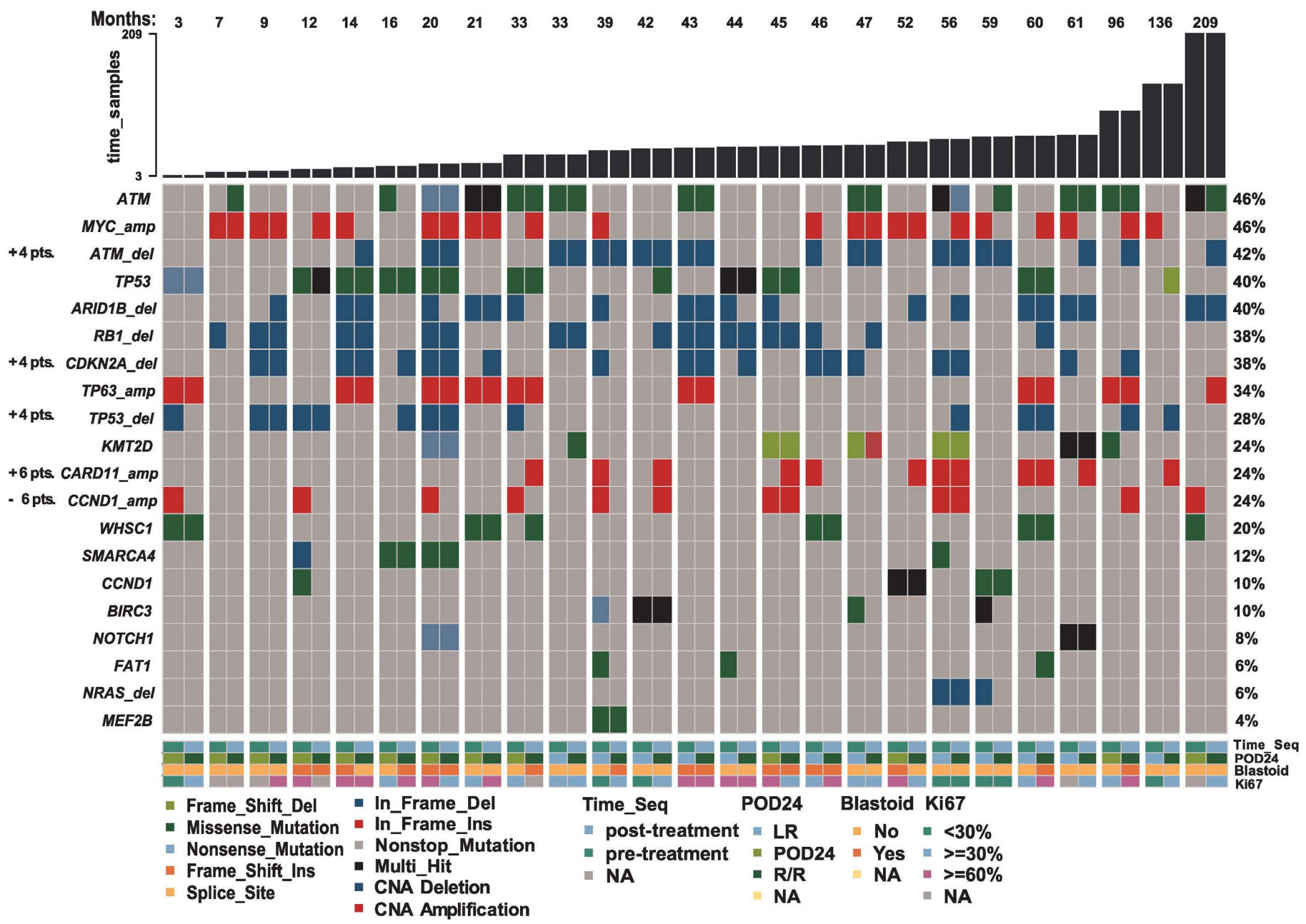

Figure 5. Genomic landscape prior to frontline treatment and at progression of disease (sequential samples). Sequential samples where Time Seq (top_bottom_row) represents the timing of sequencing pre-treatment (light green) or at the time of subsequent treatment, POD24 (second row) represents whether the first relapse occurred within 24 months (POD24 light green) or whether the relapse occurred after a longer remission (LR light blue). Thus, a combination of a light-blue box and a dark-green box on this line indicates a couplet of samples from before frontline treatment and after a considerable remission (the length of time between samples can be inferred from the bars at the top of the plot). Blastoid/Ki67 refers to disease status at the time of sequencing. CNA: copy number alteration; NA: not available; LR: long remission; R/R: relapsed/refractory.

remission). For patients with long remission the estimated 4-year PFS was 82% (95% CI: 73-93%).

There were 22 patients whose MCL harbored a mutation in *TP53* and 20 patients with a deletion in *TP53* (of these, 12 had both a mutation and a deletion). A mutation in *TP53* was the only genomic abnormality associated with a shorter PFS in univariable and multivariable analyses (4-year PFS 26% *vs*. 76%, 95% CI: 11-63% *vs*. 67-87%; HR=7.7; 95% CI: 3-21, *P*<0.0001; q=0.002) (*Online Supplementary Table S6*). This observation was also true for the subset of patients who proceeded to ASCT. The eight patients whose MCL harbored a deletion in *TP53* in the absence of a *TP53* mutation had a similar PFS as the *TP53*-wildtype population (sample too small for statistical significance) (Figure 6).

Table 2. Baseline characteristics of the survival-analysis cohort.

Characteristics	All N=100	Long-remission N=58	POD N=37	Insufficient follow-up N=5	*P*
Age, years, median (IQR)	64 (57-71)	64 (56-68)	68 (56-72)	66 (63-72)	0.21
Male sex, N (%)	66 (74.0)	42 (72.4)	28 (75.7)	4 (80.0)	0.93
ECOG PS >1, N (%)	96 (100)	56 (100)	35 (100)	5 (100)	-
MIPI, N (%) Low-risk Intermediate-risk High-risk	 34 (37.4) 37 (40.7) 20 (22.0)	 21 (39.6) 24 (45.3) 8 (15.1)	 11 (33.3) 11 (33.3) 11 (33.3)	 2 (40.0) 2 (40.0) 1 (20.0)	0.39 - - -
Stage, N (%) I II III IV	 1 (1.00) 7 (7.00) 13 (13.0) 79 (79.0)	 - 5 (8.62) 5 (8.62) 48 (82.8)	 1 (2.70) 1 (2.70) 8 (21.6) 27 (73.0)	 - 1 (20.0) - 4 (80.0)	0.16 - - - -
Spleen involvement, N (%)	44 (45.4)	26 (45.6)	17 (48.6)	1 (20.0)	0.55
Bone marrow involvement, N (%)	63 (69.2)	39 (68.4)	22 (73.3)	2 (50.0)	0.67
t(11;14) *IGH*:*CCND1*, N (%)	96 (98.0)	55 (96.5)	36 (100)	5 (100)	0.57
Blastoid/pleomorphic, N (%)	14 (14.0)	6 (10.3)	8 (21.6)	-	0.26
Leukemic non-nodal, N (%)	6 (6.00)	4 (6.90)	2 (5.41)	-	1.00
del17p by FISH/SNP, N (%)	6 (37.5)	2 (20.0)	4 (80.0)	-	0.06
IGHV unmutated, N (%)	26 (81.2)	10 (83.3)	16 (84.2)	-	0.24
Ki67, N (%) <30% 30-59% ≥60%	 33 (40.2) 34 (41.5) 15 (18.3)	 18 (38.3) 23 (48.9) 6 (12.8)	 12 (40.0) 9 (30.0) 9 (30.0)	 3 (60.0) 2 (40.0) -	0.22 - - -
Frontline systemic treatment, N (%) BR R-DHAX R2CHOP-RHDAC RBAC RCHOP RCHOP / RDHAX RCHOP / RHDAC RCHOP / RICE	 23 (23.0) 18 (18.0) 20 (20.0) 1 (1.00) 3 (3.00) 7 (7.00) 17 (17.0) 11 (11.0)	 10 (17.2) 11 (19.0) 12 (20.7) - - 4 (6.90) 13 (22.4) 8 (13.8)	 11 (29.7) 4 (10.8) 8 (21.6) 1 (2.70) 3 (8.11) 3 (8.11) 4 (10.8) 3 (8.11)	 2 (40.0) 3 (60.0) - - - - - -	 - - - - - - - -
Primary refractory, N (%)	11 (11.0)	-	11 (29.7)	-	<0.001
POD24, N (%)	19 (19.0)	-	19 (51.4)	-	<0.001
Frontline ASCT, N (%)	45 (45.0)	34 (58.6)	9 (24.3)	2 (40.0)	0.003

POD: progression of disease; IQR: interquartile range; ECOG PS: Eastern Cooperative Oncology Group performance status; MIPI: Mantle cell lymphoma International Prognostic Index; FISH: fluorescence *in-situ* hybridization; SNP: single nucleotide polymorphism array; *IGHV*: immunoglobulin heavy chain; Ki67: proliferation index; BR: bendamustine and rituximab; R-DHAX: rituximab, dexamethasone, high-dose cytarabine and oxaliplatin; R2CHOP: lenalidomide, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; RDHAC: rituximab and high-dose cytarabine; RBAC: rituximab, bendamustine and cytarabine; RCHOP: rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; RICE: rituximab, ifosfamide, carboplatin and etoposide; POD24: progression of disease within 24 months from initiation of frontline therapy; ASCT: autologous hematopoietic stem cell transplant.

Overall, there were 63 patients in the entire cohort whose lymphoma harbored a mutation in *TP53* (46% sequenced prior to frontline, 32% at first relapse and 22% at later lines of therapy). Having demonstrated that mutations in *TP53* were an early event in MCL, we analyzed data for this cohort collectively (i.e., regardless of the time of sequencing). Patients with *TP53*-mutated MCL had a lower response rate to frontline chemoimmunotherapy compared to those with wild-type *TP53*. Nonetheless most of these patients did display a response to treatment (overall response rate 76%; complete response 59%). There were 65 patients who underwent an ASCT in first complete response, of whom 16 had a *TP53* mutation. All but one patient with *TP53*-mutated MCL displayed a complete response after ASCT; however, their PFS was considerably shorter than that of patients with wild-type *TP53* (2-year PFS 63% and 4-year PFS 31% compared to 2-year PFS 98% and 4-year PFS 86%, *P*<0.0001) (Figure 6).

Discussion

We evaluated 214 samples from MCL patients sequenced either prior to frontline treatment (N=144) or at relapse (N=70). Samples from diagnosis from patients who ultimately progressed displayed a genomic profile similar to that of samples sequenced at relapse and distinct from that of patients with long remission. This trend was corroborated

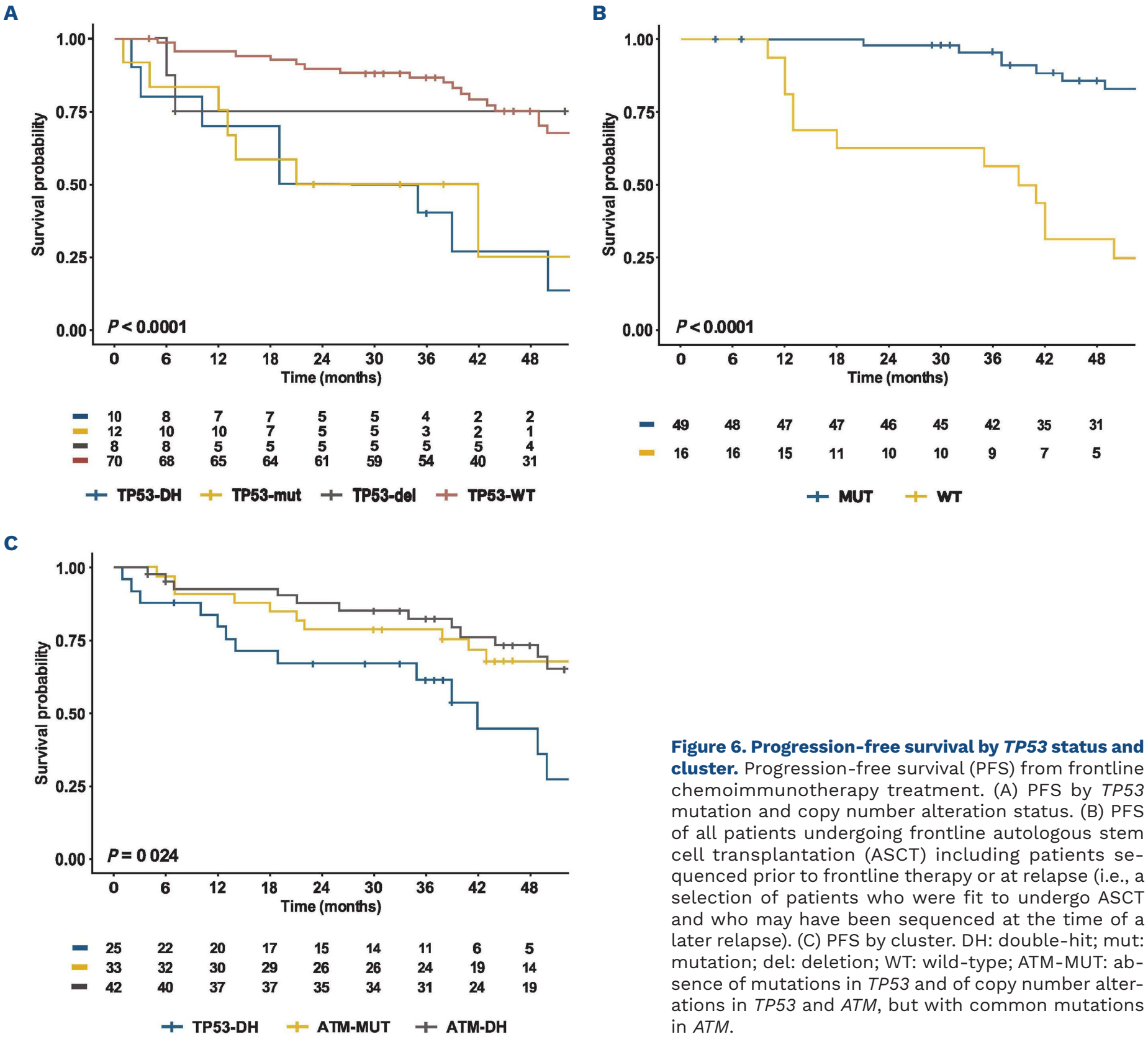

Figure 6. Progression-free survival by *TP53* status and cluster. Progression-free survival (PFS) from frontline chemoimmunotherapy treatment. (A) PFS by *TP53* mutation and copy number alteration status. (B) PFS of all patients undergoing frontline autologous stem cell transplantation (ASCT) including patients sequenced prior to frontline therapy or at relapse (i.e., a selection of patients who were fit to undergo ASCT and who may have been sequenced at the time of a later relapse). (C) PFS by cluster. DH: double-hit; mut: mutation; del: deletion; WT: wild-type; ATM-MUT: absence of mutations in *TP53* and of copy number alterations in *TP53* and *ATM*, but with common mutations in *ATM*.

in 25 sequential samples, which showed similar profiles between pre-treatment and relapse samples, even when separated by a prolonged remission period (median remission time was >3 years). Notably, these acquired alterations did not follow a common pattern, although a trend toward acquisition of CNA was observed. While recognizing the limitations of targeted sequencing and sample size, these observations suggest that in most MCL cases, relapse is not driven by a uniform genomic evolution but rather by the persistence of resistant clones present at diagnosis.

Observed rates of SNV, indels, and CNA were consistent with those previously reported in retrospective MCL cohorts.[6,16,17] Notably, the observed rates of *TP53* and *CDKN2A* associated abnormalities in prospective clinical trials have been lower.[11,12,18] Importantly, a considerable variability in CNA rates across studies suggests that algorithmic criteria used to infer CNA from next-generation sequencing data contribute to these differences. Consequently, we made extensive efforts to optimize our CNA calling pipeline.

In keeping with our observation, in a recent study of sequential samples sequenced prior to frontline treatment and at POD, nine out of 16 patients were noted to have newly acquired genomic abnormalities; however, these were primarily sporadic CNA, each present in a single case.[6] The only repeatedly acquired CNA (seen in 3 cases each) were deletions of 9p21.3-13.1 which includes *CDKN2A* and amplifications of 12q13.3-14.1 which includes *CDK4* (not covered by our panel). As with our data, acquired deletion or mutation of *TP53* was only seen in one patient each, indicating this is an early event.[6,7] Similarly, in our sequential cohort, though not statistically significant, non-sporadic acquired abnormalities were limited to CNA and included deletions of *CDKN2A* (9p21.3), *ATM* (11q22.3) and *TP53* (17p13.1) and amplifications of *CARD11* (7p22.2).[6,7] Interestingly, in six out of eight patients with an amplification of *CCND1* (11q13.3), the abnormality was no longer evident at relapse. Although not statistically significant, higher rates of deletions in *CDKN2A* and *ATM* were seen when comparing pre-treatment samples of patients who later relapsed *versus* relapsed samples (a similar trend was not observed for *CCND1* amplifications), suggesting chromosomal instability may be a key evolutionary process in MCL.[7,17] This would further explain why relapsed samples were enriched for deletions in *CDKN2A*, yet this chromosomal abnormality was not prognostic in the frontline setting.

Using hierarchical clustering we defined three major clusters which resemble those defined previously by Yi *et al*. The first cluster converged around double-hits (deletion and mutation) in *ATM* (corresponding to 'Cluster 2'); the second around double-hits in *TP53* (corresponding to 'Cluster 4') and the third characterized by common mutations in ATM in the absence of mutations in *TP53* and of CNA in *TP53* and ATM (corresponding to 'Cluster 3'). Cluster 1 identified by Yi *et al*. as representing non-nodal leukemic MCL was not captured, probably due to the smaller sample size and absence of *IGHV* status data in our clustering.

Samples from patients who ultimately experienced a POD, irrespective of whether the sequenced specimen was obtained at diagnosis or at relapse, exhibited considerably higher rates of genomic abnormalities in the apoptotic pathway, including mutations and deletions in *TP53* and *CDKN2A*. These abnormalities were particularly evident in cases with early progression (<24 months). As with prior studies, there was an incomplete overlap between *TP53* mutations and deletions, with *TP53* mutations emerging as the primary driver of dismal prognosis and accounting for nearly half of all relapses.[6,11,12] Similarly, the presence of a *TP53* mutation seems a more specific predictor of dismal outcome compared to the DH-TP53 cluster raising a question about the biological relevance of cluster allocation. There was no difference in the number or loci of *TP53* mutations between samples sequenced prior to frontline therapy or at POD. Although a large proportion of patients with mutations in *TP53* initially achieved remission with standard chemoimmunotherapy, with many subsequently proceeding to ASCT, responses were generally short-lived, with nearly all patients experiencing progression within 4 years. Notably, however, most patients did demonstrate a significant response to frontline chemoimmunotherapy, an outcome that is relevant when considering the applicability of chemotherapy as a temporizing measurement when rapid disease control is required.

The lack of a distinct pattern of acquired genomic abnormalities between diagnosis and relapse samples (noting the small proportion of cases showing acquisition of CNA) suggests two predominant patterns of progression for MCL. In the more common scenario, a resistant clone or sub-clone present at diagnosis may persist for years, even during so-called periods of remission, eventually driving relapse. In the second scenario, which may be more prevalent in later relapses, a divergent evolution occurs, in which a new clone drives progression, possibly originating from a precursor pre-malignant lymphoid clone.[19-21] The first scenario is supported by growing evidence linking undetectable minimal residual disease, as measured by ultra-sensitive techniques, with long-term remission.[9,22,23] The second scenario may be supported by findings that MCL-associated germline mutations occur in the general population and are linked to an increased risk of MCL development with acquired chromosomal abnormalities.[20,21,24] In this context, divergent evolution in patients with delayed relapses has also been recently suggested in diffuse large B-cell lymphoma.[25]

Several limitations that may impact the interpretation of our findings must be acknowledged. Firstly, the lack of a formal tumor evolutionary analysis, such as phylogenetic reconstruction or clonal trajectory inference, may restrict our ability to conclusively determine whether relapse is solely driven by pre-existing clones identifiable at diagnosis. Further, our use of a targeted sequencing panel, rather

than whole-exome or genome sequencing may have missed alterations outside the interrogated genes. Finally, the clinically diverse nature of our real-world patient population, which included individuals with varied backgrounds, disease presentations, and treatment regimens may have introduced a degree of heterogeneity that might confound specific associations between genotype and treatment response. Nonetheless, our findings are in keeping with those of previous cohorts of MCL in smaller more homogenous populations of patients sequenced with whole-exome or genome sequencing, while targeted panels allowed us to achieve high sequencing depths, supporting sensitive detection of low-frequency variants including subclonal mutations that might be missed by less sensitive approaches. Another limitation is that the size of our paired diagnosis-relapse cohort was limited (N=25), restricting statistical power for some evolutionary analyses. Notably, five out of 25 patients had new events in the *TP53* gene (2 of whom had *TP53* mutations at baseline and acquired a deletion) suggesting that, in a subset of patients, acquisition of new hits in the *TP53* gene may play a role in disease evolution under treatment pressure.[26] Notwithstanding, the striking genomic stability we observed in most pairs mirrors findings in chronic lymphocytic leukemia, diffuse large B-cell lymphoma and follicular lymphoma in which it has been shown that relapses are predominantly driven by selection of pre-existing resistant subclones.[27-30] We further recognize the absence of integrative multi-omic studies such as single-cell RNA sequencing or methylation analysis that could reveal non-genomic resistance mechanisms. In this regard, we invested substantial efforts into robust CNA calling, identifying accumulation of chromosomal changes that likely highlights the key role of genomic instability in MCL progression. Investigating other molecular mechanisms of progression would be of considerable interest but was unfortunately beyond the scope of our present study. In fact, accumulating evidence suggests that drug-tolerant states exist in the absence of genetic evolution through mechanisms of phenotypic plasticity to resistant or quiescent states.[31] In conclusion, in most patients with MCL, prognosis is primarily determined by malignant clones present at diagnosis, rather than by subsequent genomic evolution. In a subset of cases, however, acquisition of CNA may suggest a form of genomic evolution likely driven by intrinsic abnormalities in apoptotic pathways characteristic of this malignancy.

Disclosures

EJ has received honoraria from AbbVie and Janssen. MLP has received honoraria from Pharmacyclics, Celgene, Merck, Novartis, Regeneron and Juno Therapeutics, a Bristol-Meyers Squibb Company, and has received research funding from Genentech and Juno Therapeutics. AN has received research funding from Rafael Pharma, Pharmacyclics and the NIH and has provided consultancy services for Pharmacyclics, Medscape, Targeted Oncology, Morphosys, Pharmacyclics and Janssen. MM has provided consultancy services for and received honoraria and research funding from Genentech, Inc, Bayer, and Immunovaccine and has provided consultancy services for Merck, Bayer, Juno Therapeutics, F. Hoffmann-La Roche Ltd., Technologies, Daiichi Sankyo, Seattle Genetics, IGM Biosciences, Janssen, Pharmacyclics, Rocket Medical, Takeda, GlaxoSmithKline and Teva. PH has received research funding from J&J Pharmaceuticals, Portola, Molecular Templates and Incyte and has provided consultancy services for Celgene, Karyopharm and Juno Therapeutics. CB reports employment with Genetech. AK has received honoraria from Kite Pharmaceuticals, AstraZeneca, Celgene and Seattle Genetics and research funding from Pharmacyclics, Celgene, Adaptive Biotechnologies and AbbVie. AM has provided consultancy services for Seattle Genetics, Miragen Therapeutics, Imbrium Therapeutics, L.P. and Merck and has received research funding from Bristol-Myers Squibb, Incyte, Seattle Genetics and Merck. LF has received research funding from Genmab and Roche. GvK has provided consultancy services for and received honoraria from Merck. AD has provided consultancy services for Takeda and Roche and has received research funding from Physicians Education Resource, Corvus Pharmaceuticals, Seattle Genetics, EUSA Pharma, AbbVie and the National Cancer Institute. ADZ is a member of the Board of Directors or advisory committees for BeiGene, has provided consultancy services for Adaptive Biotechnology, Novartis, Amgen, Janssen, Celgene, Gilead and Genentech/Roche and has received research funding from Roche, Celgene, Sandoz, MorphoSys and MEI Pharma. VS, NK, EE, SD, AB and NF are employees of BostonGene.

Contributions

EJ, ADZ and AK designed the research. VS, NK, EE, SD, AB and NF developed and optimized the copy number alteration call algorithm. EJ, MU, SZ and KSB collected the data. EJ performed the biostatistical analyses and wrote the manuscript. EJ, CB, ZE-P, PG, PH, MM, AM, AN, MLP, GvK, LF, JY, GS, AK and ADZ enrolled subjects and provided clinical data. MA and AD provided targeted sequencing support. All authors reviewed and approved the manuscript.

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Data-sharing statement

Clinical and molecular data can be made available upon request.

References

1. Le Gouill S, Thieblemont C, Oberic L, et al. Rituximab after autologous stem-cell transplantation in mantle-cell lymphoma. N Engl J Med. 2017;377(13):1250-1260.
2. Hermine O, Hoster E, Walewski J, et al. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. Lancet. 2016;388(10044):565-575.
3. Rule S, Dreyling M, Goy A, et al. Outcomes in 370 patients with mantle cell lymphoma treated with ibrutinib: a pooled analysis from three open-label studies. Br J Haematol. 2017;179(3):430-438.
4. Eyre TA, Walter HS, Iyengar S, et al. Efficacy of venetoclax monotherapy in patients with relapsed, refractory mantle cell lymphoma after Bruton tyrosine kinase inhibitor therapy. Haematologica. 2019;104(2):e68-e71.
5. Visco C, Di Rocco A, Evangelista A, et al. Outcomes in first relapsed-refractory younger patients with mantle cell lymphoma: results from the MANTLE-FIRST study. Leukemia. 2021;35(3):787-795.
6. Yi S, Yan Y, Jin M, et al. Genomic and transcriptomic profiling reveals distinct molecular subsets associated with outcomes in mantle cell lymphoma. J Clin Invest. 2022;132(3):e153283.
7. Karolova J, Kazantsev D, Svaton M, et al. Sequencing-based analysis of clonal evolution of 25 mantle cell lymphoma patients at diagnosis and after failure of standard immunochemotherapy. Blood. 2022;140(Supplement 1):6409-6410.
8. Holte H, Beiske K, Boyle M, et al. The MCL35 gene expression proliferation assay predicts high-risk MCL patients in a Norwegian cohort of younger patients given intensive first line therapy. Br J Haematol. 2018;183(2):225234.
9. Ladetto M, Tavarozzi R, Pott C. Minimal residual disease (MRD) in mantle cell lymphoma. Ann Lymphoma. 2020;34(5):887-901.
10. Eskelund CW, Dimopoulos K, Kolstad A, et al. Detailed long-term follow-up of patients who relapsed after the Nordic mantle cell lymphoma trials: MCL2 and MCL3. Hemasphere. 2021;5(1):e510.
11. Eskelund CW, Dahl C, Hansen JW, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not benefit from intensive chemoimmunotherapy. Blood. 2017;130(17):1903-1910.
12. Ferrero S, Rossi D, Rinaldi A, et al. KMT2D mutations and TP53 disruptions are poor prognostic biomarkers in mantle cell lymphoma receiving high-dose therapy: a FIL study. Haematologica. 2020;105(6):1604-1612.
13. Le Bris Y, Magrangeas F, Moreau A, et al. Whole genome copy number analysis in search of new prognostic biomarkers in first line treatment of mantle cell lymphoma. A study by the LYSA group. Hematol Oncol. 2020;38(4):446-455.
14. Ptashkin RN, Ewalt MD, Jayakumaran G, et al. Enhanced clinical assessment of hematologic malignancies through routine paired tumor and normal sequencing. Nat Commun. 2023;14(1):6895.
15. Coombes CE, Liu X, Abrams ZB, Coombes KR, Brock G. Simulation-derived best practices for clustering clinical data. J Biomed Inform. 2021;118:103788.
16. Nadeu F, Martin-Garcia D, Clot G, et al. Genomic and epigenomic insights into the origin, pathogenesis, and clinical behavior of mantle cell lymphoma subtypes. Blood. 2020;136(12):1419-1432.
17. Hartmann EM, Campo E, Wright G, et al. Pathway discovery in mantle cell lymphoma by integrated analysis of high-resolution gene expression and copy number profiling. Blood. 2010;116(6):953-961.
18. Freeman CL, Pararajalingam P, Jin L, et al. Molecular determinants of outcomes in relapsed or refractory mantle cell lymphoma treated with ibrutinib or temsirolimus in the MCL3001 (RAY) trial. Leukemia. 2022;36(10):2479-2487.
19. Pararajalingam P, Coyle KM, Arthur SE, et al. Coding and noncoding drivers of mantle cell lymphoma identified through exome and genome sequencing. Blood. 2020;136(5):572-584.
20. Petrackova A, Savara J, Turcsanyi P, Gajdos P, Papajik T, Kriegova E. Rare germline ATM variants of uncertain significance in chronic lymphocytic leukaemia and other cancers. Br J Haematol. 2022;199(3):371-381.
21. Lampson BL, Gupta A, Tyekucheva S, et al. Rare germline ATM variants influence the development of chronic lymphocytic leukemia. J Clin Oncol. 2023;41(5):1116-1128.
22. Epstein-Peterson ZD, Batlevi CL, Caron P, et al. Frontline sequential immunochemotherapy plus lenalidomide for mantle cell lymphoma incorporating MRD evaluation: phase II, investigator-initiated, single-center study. Blood. 2020;136(Supplement 1):11-12.
23. Lakhotia R, Melani C, Dunleavy K, et al. Circulating tumor DNA predicts therapeutic outcome in mantle cell lymphoma. Blood Adv. 2022;6(8):2667-2680.
24. Zhang J, Jima D, Moffitt AB, et al. The genomic landscape of mantle cell lymphoma is related to the epigenetically determined chromatin state of normal B cells. Blood. 2014;123(19):2988-2996.
25. Hilton LK, Ngu HS, Collinge B, et al. Relapse timing is associated with distinct evolutionary dynamics in diffuse large B-cell lymphoma. J Clin Oncol. 2023;41(25):4164-4177.
26. Agarwal R, Chan YC, Tam CS, et al. Dynamic molecular monitoring reveals that SWI–SNF mutations mediate resistance to ibrutinib plus venetoclax in mantle cell lymphoma. Nat Med. 2019;25(1):119-129.
27. Zapatka M, Tausch E, Öztürk S, et al. Clonal evolution in chronic lymphocytic leukemia is scant in relapsed but accelerated in refractory cases after chemo(immune) therapy. Haematologica. 2022;107(3):604-614.
28. Landau DA, Tausch E, Taylor-Weiner AN, et al. Mutations driving CLL and their evolution in progression and relapse. Nature. 2015;526(7574):525-530.
29. Burger JA, Landau DA, Taylor-Weiner A, et al. Clonal evolution in patients with chronic lymphocytic leukaemia developing resistance to BTK inhibition. Nat Commun. 2016;7:11589.
30. Bai B, Wise JF, Vodák D, et al. Multi-omics profiling of longitudinal samples reveals early genomic changes in follicular lymphoma. Blood Cancer J. 2024;14(1):147.
31. Pérez-González A, Bévant K, Blanpain C. Cancer cell plasticity during tumor progression, metastasis and response to therapy. Nat Cancer. 2023;4(8):1063-1082.