INTRODUCTION

Mantle cell lymphoma (MCL) is an aggressive B cell neoplasm genetically characterized by the translocation t(11;14)(q13;q32), leading to CCND1 overexpression [1, 2]. Two molecular subtypes are currently recognized: (1) Classical MCL (cMCL) is composed of B cells with minimally mutated or unmutated immunoglobulin heavy chain variable (IGHV) region that express SOX11. Patients have generalized lymphadenopathy and the outcome is adverse. (2) In non-nodal MCL (nnMCL), B cells do not express SOX11 and often carry mutated IGHV. Here the involved organs are peripheral blood, bone marrow, and spleen. Cases are often clinically indolent [1]. The cells of origin are believed to be naïve B cells that do not undergo germinal center reactions as in the case of cMCL and memory B cells in nnMCL [3].

Frequent molecular alterations in MCL are found in ATM, TP53, NSD2, KMT2D, NOTCH1/2, UBR5, BIRC3, TRAF2, MAP2K14, CARD11, SMARCA4, and BTK [3]. The most characteristic alteration in MCL is, however, rearrangement of the CCND1 locus, which leads to the juxtaposition of the strong immunoglobulin heavy-chain enhancer on chr14, and results in CCND1 overexpression [4, 5]. The CCND1 transcript can also be stabilized by deletions or point mutations in the 3′UTR eliminating miRNA binding sites or creating premature polyadenylation sites [6, 7]. Stabilization of CCND1 at the protein level has also been observed in MCL. Alternatively, spliced isoforms lacking the T286 phosphorylation site needed for CCND1 degradation [8–10] and amino acid changes linked to increased protein stability have been described [11].

Widespread occurrence of CCND1 mutations caused by aberrant somatic hypermutation has first been detected by [12] using whole-transcriptome sequencing (WTS). Other screens have confirmed these findings [13–15]. However, the biological significance of these mutations has not been fully clarified. One reason may be found in the mechanism of aberrant somatic hypermutation (aSHM) generation by activation-induced cytidine deaminase (AID), whose activity is tightly regulated and restricted to 1–2 kb from the transcriptional start site of its target genes [16]. Another reason may be that the effects of aSHM are still incompletely understood.

Here we present the analysis of the mutation spectrum of 84 MCL patients whose genomes and transcriptomes have been fully sequenced. We report that CCND1 mutations generated by aSHM are particularly frequently observed in MCL as compared to other lymphomas carrying the t(11;14)(q13;q32) translocation. Our results are compatible with the hypothesis that CCND1 mRNA expression levels are a rate-limiting factor for MCL lymphomagenesis and that non-coding mutations generated by aSHM are selected for their impact on CCND1 mRNA expression.

MATERIALS AND METHODS

Patients and samples

The cohort includes 84 patients diagnosed with MCL among 4610 samples (5k data set) from a wide variety of hematological malignancies (Table 1 and Supplementary Table 1). The selection of samples for the 5k data set was based on the following criteria:
Library prep kit following the manufacturer’s recommendations (Illumina, San Diego, CA, USA) and processed as described previously [17, 18]. A so-called Tumor/Unmatched normal workflow was used for variant calling to reduce technical artifacts and germline calls. Each variant was queried against the gnomAD database (v2.1.1) and variants with global population frequencies >0.5% were excluded. The final analysis was performed only on PASS filtered variants. Mutational signatures were analyzed using the R package MutSignatures [19]. Oncoprint representations of mutation data were generated using the R package ComplexHeatmap [20].

**WTS and analysis**

For transcriptome analysis, the TruSeq Total Stranded RNA kit was used, starting with 250 ng of total RNA, to generate RNA libraries following the manufacturer’s recommendations (Illumina, San Diego, CA, USA). In all, 2×100 bp paired-end reads were sequenced on the NovaSeq 6000 with a median of 50 million reads per sample (Illumina, San Diego, CA, USA) and processed as described previously [17]. Counts were extracted with Cufflinks (v2.2.1) [21]. Counts were normalized applying the variance stabilizing transformation normalization method. Genes were kept if they were expressed (>5 CPM) in at least 66% of the samples. Gene expression differences were assessed using the DESeq2 package [22]. Model parameters included sequencing run, aSHM mutation status, material (peripheral blood or bone marrow), gender, chr11 breakpoint distance from CCND1 TSS, and mutation status of ATM, KMT2D, NSD2, and TP53. The significance of coefficients in a negative binomial generalized linear model was estimated using the Wald test. Gene set enrichment analysis was performed with the GSEA package [23, 24] using a CCND1 pre-ranked list based on DESeq2 normalized expression counts. Cluster analysis was carried out using the R package pheatmap (https://github.com/rainokolde/pheatmap).

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**Table 1. Overview of samples analyzed by whole-genome sequencing (WGS).**

| Abbreviation | Description | Samples | Median age | Female/male |
|--------------|-------------|---------|------------|-------------|
| aCML         | Atypical chronic myeloid leukemia | 77      | 74         | 25/52       |
| AML          | Acute myeloid leukemia             | 750     | 68         | 344/406     |
| AUL          | Acute undifferentiated leukemia     | 37      | 76         | 14/23       |
| BCP-ALL      | B cell precursor acute lymphoblastic leukemia | 285    | 54         | 137/148     |
| B-NHL        | B cell non-Hodgkin’s lymphoma       | 59      | 71         | 25/34       |
| BPDCN        | Blastic plasmacytoid dendritic cell neoplasm | 24    | 74         | 1/23        |
| CLL          | Chronic lymphocytic leukemia        | 306     | 67         | 110/196     |
| CML          | Chronic myeloid leukemia            | 110     | 57         | 47/63       |
| CMML         | Chronic myelomonocytic leukemia      | 208     | 77         | 69/139      |
| FL           | Follicular lymphoma                  | 64      | 54         | 32/32       |
| HCL          | Hairy cell leukemia                  | 92      | 64         | 19/73       |
| HGBL         | High-grade B cell lymphoma           | 65      | 70         | 33/32       |
| LPL          | Lymphoplasmacytic lymphoma           | 61      | 71         | 14/47       |
| MC           | Mastocytosis                         | 117     | 57         | 51/66       |
| MCL          | Mantle cell lymphoma                 | 84      | 69         | 29/55       |
| MDS          | Myelodysplastic syndrome             | 682     | 73         | 291/391     |
| MDS/MPN-RS-T | Myelodysplastic syndrome/myeloproliferative neoplasm with ring sideroblasts and thrombocytosis | 87    | 74         | 51/36       |
| MDS/MPN-U    | Myelodysplastic/myeloproliferative neoplasms, unclassifiable | 87      | 75         | 35/52       |
| MGUS         | IgM monoclonal gammapathy of undetermined significance | 20     | 61         | 5/15        |
| MLN_eo       | Myeloid or lymphoid neoplasms associated with eosinophilia | 47   | 52         | 6/41        |
| MM           | Multiple myeloma                     | 358     | 67         | 159/199     |
| MPAL         | Mixed-phenotype acute leukemia       | 38      | 64         | 16/22       |
| MPN          | Myeloproliferative neoplasm          | 355     | 68         | 146/209     |
| MZL          | Marginal zone lymphoma               | 80      | 71         | 33/47       |
| NK           | Natural killer cell neoplasm         | 110     | 69         | 44/66       |
| PNH          | Paroxysmal nocturnal hemoglobinuria  | 117     | 43         | 67/50       |
| PPBL         | Polyclonal B cell lymphocytosis       | 47      | 49         | 37/10       |
| T-ALL        | T cell acute lymphoblastic leukemia   | 121     | 37         | 37/84       |
| T-NHL        | T cell non-Hodgkin’s lymphoma        | 94      | 69         | 41/53       |
| vHCL         | Hairy cell leukemia variant           | 28      | 80         | 12/16       |
Variant enrichment analysis

Enrichment analysis using bipartite graph models was performed essentially as described [25, 26]. Briefly, bipartite graph models consisting of variant bins on one side of the graph and samples on the other side were constructed. The degree of bin vertices in this graph reflects the number of variants in that bin. Randomization of the graph yields a statistical null model. Two subgraphs were then constructed: one containing only MCL samples and the other containing only non-MCL samples. The significance of deviation of bin vertex degrees in the subgraphs from the predictions of the null model is then estimated using Poisson-binomial Z-scores and the bi-binomial approximation [26] of Poisson binomial P values.

Motif searches

Motif searches were performed using the AME and the Centrimo apps of the MEME suite [27] and by motif consensus matching to motifs listed in Jaaspar [28] and HOCOMOCO [29]. Motif matching results for all variants using wild-type and mutated sequences with 15 bases of padding to the left and to the right of the mutated position are listed in Supplementary Table 1.

IGHV mutation status

IGHV mutation status was analyzed by Sanger sequencing of corresponding PCR fragments, by IGCaller software [30] analysis of WGS data, or both.

In silico prediction of pathogenicity

The pathogenicity of amino acid changes was estimated using HePPY (https://doi.org/10.1182/blood-2019-128488).

RESULTS

The landscape of coding and non-coding variants in MCL

Our cohort of MCL patients comprises 84 cases. We performed enrichment analysis for the number of variants in bins of 10, 100, 1000, and 10,000 bases to identify genomic regions that carry significantly more variants in MCL patients as compared to our cohort of 4610 cases of leukemia and lymphoma (30 different entities, 5k data set, details listed in Table 1 and Supplementary Table 1) with whole-genome sequence data. We analyzed coding and non-coding variants separately.

Figure 1A shows the results for the analysis of coding variants. We detected three highly significant (−log10(P) ≥ 10) regions corresponding to CCND1, ATM, and NSD2. Coding mutations in these genes have been reported before for MCL [2] and this result validates our general approach. It is worth noting that our analysis is relative to other cohorts in our 5k data set (Table 1). Thus, genes like TP53 and KMT2D, although mutated not only in MCL but also in many other malignancies, will not score as enriched in MCL in this analysis. Mutation data for ATM, NSD2, KMT2D, and TP53 for all cohorts are listed in Supplementary Table 1.

The enrichment analysis for non-coding variants is shown in Fig. 1B. The complete list of genomic regions with significantly elevated densities of non-coding mutations in MCL can be found in Supplementary Table 2. The most significant region is found on chromosome 11 and corresponds to the CCND1 locus. We therefore turned our attention to the CCND1 locus for a more detailed analysis.

MCL samples are prone to mutagenesis in the CCND1 transcription regulatory region

We generated histograms for the number of non-coding variants in 100 bp bins covering the entire CCND1 locus and 5 kb upstream of the TSS (chr11:69455872, Fig. 2A). In this work, we refer to the 5 kb region upstream of the TSS as promoter region [31] whereas the region chr11:69450872-69458025 (hg19) encompassing the promoter, exon 1, and parts of intron 1 of CCND1 is denoted as transcription regulatory region. We noticed a strong increase of non-coding variants in the promoter region and the first intron in MCL samples as compared to other cohorts. The UCSC genome browser annotations of these regions show high levels of H3K27 acetylation, DNase 1 hypersensitivity, and transcription factor-binding sites (TFBSs). The bar plot at the bottom of Fig. 2A also shows that the variants in the first intron of the CCND1 gene are not uniformly distributed. Rather, they appear to be clustered in some regions while others remain essentially void of variants.

The accumulation of variants in the promoter, the first exon, and the first intron of CCND1 is not entirely specific for MCL. It is assumed that they are a byproduct of somatic hypermutation occurring in the germinal center microenvironment [12]. We detected such mutations in other B cell-derived malignancies such as multiple myeloma, albeit at a less pronounced level (see below).

However, MCL seems to be particularly prone to accumulate mutations in these regions. Figure 2B shows the percentage of...
samples with at least five non-coding mutations in the CCND1 transcription regulatory region for cohorts where such mutations were detected. The percentage of CCND1 mutated samples in the MCL cohort is two to three times higher than in other cohorts. The majority of these variants is found in intron 1 (Fig. 2A and Supplementary Table 2).

The nearly universal presence of the t(11;14)(q13;q32) translocation in MCL might be a confounding factor when comparing the percentage of CCND1 mutated samples between different types of lymphoma. Therefore, we analyzed only samples carrying a t(11;14)(q13;q32) translocation. Only samples with mutated IGHV were included to ensure that SHM has taken place. We found 28
MCL samples and 45 multiple myeloma samples satisfying these criteria. Also in this more stringent comparison, MCL samples consistently carry more than twice as many non-coding variants in the \textit{CCND1} locus as multiple myeloma samples (Fig. 2C). The majority of these variants are found in MCL samples with mutated \textit{IGHV}, as can be seen from the data in Supplementary Table S1. MCL samples with mutated \textit{IGHV} harbor nearly six times as many non-coding mutations than samples with unmutated \textit{IGHV}. We conclude that MCL samples are particularly prone to accumulate non-coding variants in the regulatory region of the \textit{CCND1} locus.

Mutational signature analysis
The higher propensity of MCL for mutations in the \textit{CCND1} locus might originate from differences in the mutational processes generating these mutations. We performed mutational signature analysis on mutations in the \textit{CCND1} locus to address this question. We included all MCL samples in this analysis. For comparison, we used samples carrying the t(11;14)(q13;q32) translocation. We refer to this artificial cohort as non-MCL. It is mainly composed of multiple myeloma samples (45) and some samples from our marginal zone lymphoma (12), IgM monoclonal gammopathy of undetermined significance (3), and B cell non-Hodgkin lymphoma (5) cohorts.

Figure 3 shows the results of extracting two mutational signatures by non-negative matrix factorization from the MCL and non-MCL cohorts. The domineering type of mutation is a C-T transition in both cohorts. This observation is compatible with the activity of AID during somatic hypermutation. Comparison of our signatures to known COSMIC signatures [32] confirms that the mutational processes at work in both cohorts are largely similar (Table 2). We conclude that differences in mutational processes are unlikely to account for a large number of mutations in the \textit{CCND1} locus in MCL.

Cohort-specific enrichment of variant clusters
Next, we addressed the question of whether the clustered appearance of variants bears cohort specificity, which might be a reflection of an underlying biological significance. We performed enrichment analysis for the number of non-coding variants in bins from size five to ten base pairs using bipartite graph models (see “Materials and methods” section for details) in the region covering the \textit{CCND1} promoter and the first intron. This analysis yields Z-score estimates for the significance of enrichment of variants in each bin for the MCL and the non-MCL cohorts. For each base

| SBS | MCL | non-MCL | Description |
|-----|-----|---------|-------------|
| sbs1 | x   |         | Spontaneous deamination of 5-methylcytosine (clock-like signature) |
| sbs3 | x   | x       | Defective homologous recombination DNA damage repair |
| sbs5 | x   | x       | unknown (clock-like signature) |
| sbs6 | x   | x       | Defective DNA mismatch repair |
| sbs9 | x   | x       | Polymerase eta somatic hypermutation activity |
| sbs15 | x   |         | Defective DNA mismatch repair |
| sbs23 | x   | Unknown |
| sbs31 | x   |         | Platinum chemotherapy treatment |
| sbs39 | x   | x       | Unknown |
| sbs40 | x   | x       | Unknown |
| sbs42 | x   | x       | Haloalkane exposure |
| sbs44 | x   | x       | Defective DNA mismatch repair |
| sbs84 | x   | x       | Activity of activation-induced cytidine deaminase (AID) |
| sbs87 | x   |         | Thiopurine chemotherapy treatment |

Table 2: Mutational signatures were compared to known signatures compiled by COSMIC [32] using cosine similarity plots.
The maximum Z-score was determined and plotted using a color scale. Figure 4A shows the results of this analysis. Some variant clusters are enriched in both the MCL and the non-MCL cohorts. However, the majority of clusters appear to display MCL cohort-specific enrichment.

We used motif searches to investigate whether the variant clusters co-localize with TFBS. The effect of mutations in TFBSs can be twofold: Some might serve to destabilize factor binding to a given site (destructive mutation) while others might change the binding specificity or increase the affinity of sites for some factors.
Mutual exclusivity of ATM and CCND1 mutations

Oncoprint representation of selected genes found mutated in MCL and CCND1 mutations. Mutated genes are depicted in rows, and cases are displayed in columns. Mutual exclusivity of ATM mutations and CCND1 coding (missense) \( p = 0.003 \), non-coding \( p = 0.044 \), and synonymous \( p = 0.009 \) mutations was detected (Fisher’s exact test).

To exclude confounding effects from MCL cases, we used the gene signature identified by [34] to classify samples as either cMCL or nnMCL as shown in Fig. 4D. Twenty-eight samples were identified as nnMCL. nnMCL samples with at least two mutations in MCL-specific TFBS mutation clusters and 10 or more non-coding mutations in the CCND1 regulatory region were assigned to the mutated group (nnMCL_MUT). The remaining nnMCL samples were assigned to the wild-type group (nnMCL_WT). Next, we used whole transcriptome data for two types of analysis: First, we tested for differential expression of CCND1 in the set of cMCL, nnMCL_MUT, and nnMCL_WT samples and, second, we performed gene set enrichment analysis using a list of nnMCL samples ranked according to CCND1 expression level. The results of these tests are shown in Fig. 4E, F. We observed that nnMCL_MUT samples carrying mutations in the transcription regulatory region of CCND1 have higher levels of CCND1 mRNA than nnMCL_WT samples.

**Mutual exclusivity of ATM and CCND1 mutations**

We investigated the relationship between CCND1 mutations and mutation patterns of known and potential driver genes in MCL with Oncoprint [35] (Fig. 5). Non-coding, missense, and synonymous CCND1 mutations were analyzed separately. All types of
CCND1 mutations are found mainly in samples without mutations in ATM ($P < 0.044$, Fisher’s exact test). While it is a possibility that mutual exclusivity between ATM and CCND1 mutations is based on biological differences in the cell of origin giving rise cMCL (naïve B cells) and nnMCL (memory B cells), we did detect ATM mutations also in nnMCL cases. Therefore, this mutation pattern may be a reflection of a common endpoint of ATM and CCND1 mutations, namely increased levels of CCND1 activity as ATM directly activates FBXO31 needed for CCND1 degradation [36].

The abundance of synonymous or benign amino acid changes in CCND1 exon 1
As a further test of the hypothesis that aSHM generated mutations of the CCND1 locus are driving CCND1 mRNA expression levels and that CCND1 is a rate-limiting factor for MCL lymphomagenesis, we analyzed the spectrum of coding mutations in the first exon of CCND1. The first exon as part of the CCND1 transcription regulatory region is potentially subject to selection pressure at the level of transcription as well as protein function. If protein function plays a crucial role, one would expect a mutation spectrum that is biased against synonymous mutations and mutations coding for benign amino acid changes. However, our results shown in Fig. 6A indicate that, in MCL, synonymous and benign mutations with low pathogenicity scores as estimated by HePPY (https://doi.org/10.1182/blood-2019-128488) are more abundant as compared to non-MCL samples, where deleterious mutations with high HePPY scores prevail.

Interestingly, among the most frequently occurring mutations are 11-69456186-G-A and 11-69456187-G-A. As can be seen in Fig. 6B, both mutations stabilize an E2F-binding site, and in three out of eight samples carrying these mutations, they co-occur (Supplementary Table 3). 11-69456186-G-A is a synonymous mutation. 11-69456187-G-A codes for the previously reported E36K mutation [11]. It is possible that E36K adds a selective advantage to cells carrying this change. However, the frequent occurrence of the nearby synonymous mutation supports the hypothesis that the efficiency of transcription of the CCND1 gene also plays a role. This notion is further supported by the significant enrichment of synonymous mutations observed in nnMCL samples as compared to cMCL and non-MCL samples shown in Fig. 6C.

**DISCUSSION**

We performed a genome-wide survey assessing the prevalence of non-coding mutations in MCL as compared to other types of leukemia and lymphoma. The CCND1 locus emerges as the major hotspot of non-coding mutagenesis. MCL is characterized by a near-universal presence of a t(11;14)(q13;q32) translocation bringing the CCND1 locus into close proximity with the immunoglobulin heavy-chain enhancer [4, 5], which can drive SHM in a subset of MCL cases [37]. SHM can occur outside of Ig loci. The first examples of SHM at non-physiological targets (aSHM) with a proven impact on target gene expression were MYC, BCL6, and CD95 [38–40]. aSHM at the CCND1 locus in MCL has first been reported by [12]. However, the restriction of AID activity mediating aSHM to about 1-2 kb downstream of the transcription start site [16] makes it difficult to distinguish bystander mutations from drivers on statistical grounds alone in the absence of mechanistic evidence of an impact on the transcription regulation of a candidate target of aSHM.

We addressed this difficulty in two ways. First, we analyzed relative enrichment of variants in the 5’ region of CCND1 as compared to other types of lymphoma carrying the t(11;14)(q13; q32) translocation with evidence for mutated IGHV. To exclude variants selected at the level of protein function, we restricted our analysis to the set of non-coding variants. We observed MCL-specific enrichment of mutations in TFBSs. Spatial clustering of non-coding mutations at the CCND1 locus in MCL samples has recently been reported by [15] using the tool OncodriveCLUST [41]. However, the mechanistic consequences of this clustering and the relative enrichment in MCL were not investigated in this study.

Second, we used motif searches to investigate the potential impact of clustered non-coding mutations on CCND1 expression. While the largely overlapping motifs for TFBSs belonging to the same family make it difficult to pinpoint the precise factor bound at a specific site, a consistent picture seems to emerge. We find that both destructive and constructive mutations can be identified. Destructive mutations prevent transcription factor binding that would normally occur. We detected destructive mutations in MEF2, STAT, and HOX motifs. A sizable fraction of these mutations occurs in sequence contexts that are compatible with c-AID mediated mutagenesis, whereas nc-AID mediated mutagenesis did not seem to play a major role. MEF2 transcription factors can activate or repress transcription depending on their association with activating or repressive co-factors [42]. MEF2B mutations have been reported in mantle cell lymphoma [13], STAT6 mutations in the DNA binding region have been described in Hodgkin lymphoma [43], HOX transcription factors are involved in T cell differentiation and are known as drivers of T-ALL [44, 45]. In mantle cell lymphoma, HOX genes are silenced by H3K27me3 chromatin methylation mediated by overexpression of EZH2 [46].

**Fig. 6** Prevalence of synonymous and benign mutations in the first exon of CCND1 in MCL. A The number of samples with mutations in exon1 of CCND1 by HePPY score is shown. HePPY is a predictor of missense variant pathogenicity (https://doi.org/10.1182/blood-2019-128488). Low HePPY scores indicate benign amino acid changes. Deleterious mutations are associated with HePPY scores close to 1. B The sequence coding for CCND1 L32 to S41 is shown. Two frequently observed mutations in the first exon of CCND1 are overlapping an E2F-binding site. The red guanine bases are often changed to adenine, which favors E2F binding. C Significance of the proportion of synonymous mutations in the sample groups displayed on the x-axis. $P$ values were calculated using the cumulative hypergeometric distribution. The negative decadic logarithm of $(1 - P)$ is shown. Values $> 2$ are considered significant.
We also observed constructive mutations that change the binding specificity of a site or create new binding sites. NFAT-binding sites were created in five different locations in the first intron of CCND1. NFAT proteins have long been known as major targets of antigen receptors expressed on T and B cells [47]. Recently, elevated levels of NFATC1 have been observed in CLL caused by DNA hypomethylation of the NFATC1 locus, and NFATC1 expression levels were shown to correlate with higher expression of CCND1 [48]. In the CCND1 promoter region as well as in the first intron, we observe the creation of CEBP binding sites. CEBP is known to be a major activator of CCND1 transcription [49]. Additionally, SOX binding sites are created in more than one location. SOX11 has been recognized as a major oncogene in mantle cell lymphoma [1, 3, 50]. In the 5′UTR an E2F site is created in close proximity to a pre-existing E2F binding site. E2F transcription factors are major drivers of cell cycle progression and are activated by CCND1 expression in a self-regulatory loop via CCND1 mediated phosphorylation of RB1 [51]. Interestingly, we observed co-occurrence of two mutations in the first exon (11-69456186-G-A, 11-69456187-G-A) that also create an E2F binding site. Both variants are among the most frequently observed mutations in the CCND1 locus. 11-69456186-G-A is a synonymous mutation while 11-69456187-G-A codes for E36K. The creation of an underlying E2F binding site may be an additional factor that increases CCND1 stability [11], is not a preferred mutation target in our MCL cohort, and that the Y44D mutation, which has been reported to increase CCND1 activity, was only found in one of the samples analyzed. The creation of CEBP binding sites. CEBP is known to be a major activator of CCND1 transcription level is increased by a-shm mediated mutagenesis, we observed more synonymous and benign amino acid changes in exon1 as compared to non-MCL samples with t11(14)(q13;q32) translocation and mutated IGHV.

We observed statistically significant differential regulation of CCND1 transcription depending on CCND1 mutation status in 28 unmcl cases present in our cohort. This number does not allow us to determine whether aberrations in the 3′UTR are mutually exclusive with a-shm-generated mutations in CCND1. Alternative polyadenylation [7], aberrations of miRNA target sites [6], or alternative splicing [52] could have an impact on CCND1 mRNA levels. All samples used in this analysis are lymphomas and express CCND1 mRNA at a sufficient level to sustain lymphoma genesis. This circumstance negatively influences the significance of a-shm-mediated upregulation of CCND1 expression levels. Furthermore, we have not been able to establish whether the CCND1 mutation status has an impact on the severity of the disease. The utility of non-coding CCND1 mutations as markers for the diagnosis of unmcl is another open question.

In conclusion, we find that non-coding mutations in the CCND1 locus are clustered to TFBSs in a MCL-specific manner. These mutations are associated with higher levels of CCND1 transcript. Assuming that CCND1 activity is a rate-limiting factor in MCL lymphomagenesis, a-shm-generated mutations at the CCND1 locus may be actively selected for their impact on transcription regulation. The selection process may be particularly pronounced in MCL, which could explain the abundance of non-coding CCND1 mutations in this disease.

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