CD6 expression is an independent predictor of time to first treatment in chronic lymphocytic leukemia

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Abstract

CD6 is a lymphocytic receptor expressed by all T cells and a subset of B and natural killer (NK) cells. It physically associates with the antigen-specific clonotypic receptor of T (TCR) cells, where it modulates the activation and differentiation signals delivered along lymphocyte development and upon peripheral antigen recognition. CD6 is also expressed in some B-cell malignancies (e.g., chronic lymphocytic leukemia [CLL]), though its biological role and clinical performance is largely unknown. To this end, we have evaluated the potential impact of *CD6* differential expression in a CLL patient cohort. 270 CLL patient case histories from the CLL-ES project with available RNA-Seq data have been analyzed. High *CD6* expression was found to be associated with mutated *IGHV* status and predictive of longer time to first treatment in a uni- and multi-variable model. Ten-year probability of receiving treatment was 33% *vs*. 55% in the $CD6^{hi}$ and $CD6^{lo}$ groups, respectively (P=0.0003), along with the lymphocyte count and the CLL International Prognostic Index. Further Gene Set Enrichment Analyses showed association of high *CD6* expression with downregulation of MYC-regulated, mitotic spindle-related, and RNA splicing-associated genes, all positively related to cancer progression. Interestingly, *CD38*, a widely studied adverse prognostic marker in CLL, was significantly down-regulated in the $CD6^{hi}$ group, in agreement with flow cytometry data. These results reinforce the notion that CD6 may play a pivotal role in neoplastic B-cell biology and lay the ground to further explore CD6 expression in the context of CLL prognoses.

Introduction

Chronic lymphocytic leukemia (CLL) is characterized by the neoplastic expansion of mature B cells ($CD19^+$ $CD23^+$ $CD5^+$) in the bone marrow, peripheral blood, and secondary lymphoid tissues.[1] Its biology and clinical course are heterogeneous: some patients never require treatment and others show rapid progression, including transformation to a large-cell lymphoma (Richter's transformation [RT]).[2] In order to predict disease progression, several biochemical, genetic, and clinical markers have been described: *IGHV* mutational status, *TP53* mutation, chromosomal abnormalities evaluated by FISH, ZAP70 or CD38 evaluated by flow cytometry.[3,4] The CLL International Prognostic Index

(CLL-IPI) is the most widely validated score, comprising *IGHV* status, presence of *TP53* mutation/del(17p), clinical Binet/Rai stages, serum β2-microglobulin, and age.[5]

CD6 is a type I transmembrane glycoprotein highly related at both structural and functional levels to CD5, a classical phenotypic marker of some B-cell neoplasms, such as CLL, mantle cell lymphoma, and hairy cell leukemia.[6,7] CD6 is normally expressed in thymocytes and mature T cells, as well as in a subset of B (B1a) and natural killer (NK) (CD-56^{dim} CD16^{+}) cells.[8,9] CD6 physically associates to the clonotypic antigen-specific receptor of T cells (TCR),[10] where it modulates activation and death signals upon antigen recognition, through interaction with its ligands (i.e., CD166/ALCAM, CD318/CDCP-1 and galectins 1 and 3).[11-13] Its role in signal transduction is still a subject of debate, showing both activation and inhibitory signals contingent on specific experimental circumstances. Thus, while initially reported as a co-stimulatory receptor, later evidence points to CD6 as a negative modulatory role in T-cell development and activation.[14] Accordingly, recent signalosome studies reveal an association of CD6 with both positive and negative intracellular signal transducers.[15]

In B-cell malignancies, CD6 is expressed by both poorly and well differentiated B-cell neoplasms.[16] Its involvement in normal B-cell physiology and leukemogenesis has not yet been fully unraveled. The limited information available in this regard shows that monoclonal antibody (mAb)-induced cross-linking of CD6 protects CLL cells from IgM-induced apoptosis by increasing the *bcl-2/baxa* ratio,[16] a system with a central role in apoptosis regulation.[17] This scarcity of information contrasts with that available for CD5, a highly homologous receptor evolved from the duplication of a common ancestor gene.[18,19] The role of CD5 in CLL progression, where it has been involved in IL-10 production as well as in apoptosis regulation, has been broadly studied.[20-22] Notably, CD5, which negatively modulates BCR signaling,[23] is unable to inhibit BCR-mediated signaling in CLL cells, potentially contributing to leukemic cell survival.[24] The close relationship of CD6 with CD5 further reinforces its impact in CLL.

This retrospective study explores the potential biological role and clinical performance of *CD6* expression in CLL. We have used RNA-seq data from a CLL patient cohort[20-22] for patient stratification based on *CD6* mRNA differential expression (DE) and its association with pre-defined clinical parameters and transcriptomic profiles based on two patient clusters. Our results provide new information on the involvement of CD6 in B-cell physiology and enhanced prognoses for CLL patients.

Methods

Study design and data collection

This retrospective cohort consisted of 294 patients diagnosed with CLL during the period 1988-2013, from the International Cancer Genome Consortium (ICGC).[25] While RNA-Seq data was available for all patients, only 270 included clinical data. CLL cells were purified and subsequently subjected to RNA-Seq analysis as previously reported.[26,27] All patients signed written informed consent before inclusion, and the project was approved by the Ethics Committee of Hospital Clínic de Barcelona (ref. number HCB/2021/0949). Criteria for treatment initiation followed local policy, which is in accordance with international guidelines,[28] and which have remained largely unchanged over time.

Flow cytometry analyses

Cryopreserved peripheral blood mononuclear cell (PBMC) samples (N=26) corresponding to the same day of RNA isolation were selected based on *CD6* mRNA expression levels (including *CD6* values, ranging from 210.61-95.17 [N=9], 86.85-74.83 [N=7], and 46.18-5.13 [N=10] TPM), and analyzed for CD6 surface expression by flow cytometry. Demographics data and disease features of the selected cases are shown in *Online Supplementary Table S1*. Staining mixes of fluorescent-labeled monoclonal antibodies (mAb), including CD19-FITC (HIB19, Biolegend) and CD6-PE (BL-CD6, Biolegend, Fluorophore-to-Protein ratio = 1.22), were prepared in 5% human AB serum in phosphate buffered solution. Quantitative flow cytometry was performed using the PE Quantibrite™ Beads (BD, 340495) as described elsewhere.[29] Briefly, forward (FSC) and side (SSC) scatter, and fluorescence intensity parameters were adjusted for PBMC, and 10,000 events of PE beads were collected with these same settings. CD6 expression was assessed on PBMC gated for CD19^{+} expression. A linear regression was created based on the known number of PE molecules and the mean fluorescence intensity (MFI) values of the beads. The molecule/cell value was inferred from the number of antibodies bound per cell, obtained upon normalization via the fluorophore-to-protein ratio of the mAb.

Statistical analysis

Maximally selected rank statistics (*maxstat* package, R software, Vienna, Austria)[30] was used to identify the best cut-off to predict TTFT. Based on normalized *CD6* gene expression (transcripts per million [TPM]), the result was rounded to 80 TPM to enable external applicability (Figure 1A). χ^2 or Fisher exact tests were used to compare categorical variables, and Student *t* test to compare quantitative variables. Kaplan-Meier curves were generated for overall survival (OS), and cumulative incidence was calculated (*cmprsk* package, R software, Vienna, Austria) to estimate time to first treatment (TTFT) and time to RT. Differences were assessed using the log rank and Gray test for survival and cumulative incidence endpoints, respectively. A Fine-Gray regression model was built for the multivariable analysis of TTFT. Considering the long inclusion period of the study and different treatment options across time, the analysis

of progression-free survival was omitted, to avoid finding differences attributable to therapy.

Differential gene expression and pathway enrichment analyses

Differential expression (DE) analysis was conducted on samples using DESeq2 based on the established cut-off of 80 TPM (details above). Sex, *IGHV* status, and CLL epigenetic status were included as co-variates. Given our statistically over-powered dataset, we applied a minimum $\log_2$ fold change (logFC) threshold of 0.2 in DESeq2 to filter out genes with limited biological relevance. Differentially expressed genes were identified using a false discovery rate (FDR) threshold of 10%.

These results were further analyzed to perform Gene Set Enrichment Analysis (GSEA) via the *fgsea*[31] and the *clusterProfiler*[32] R packages. The hallmarks annotation[33] and the gene ontology (GO) annotation[34] were tested against our DE results applying Benjamini-Hochberg (BH) correction. Pre-ranked gene lists based on $\log_2$ fold change and *P* value from the DE analysis were used for pathway enrichment analysis.

Results

Baseline clinicopathological characteristics and *CD6* expression

A summary of the most relevant baseline clinicopathological features of the 270 patients according to *CD6* expression is shown in Table 1. Eighty-three patients (31%) expressed *CD6* levels above the determined cut-off of 80 TPM (*CD6*^{hi}) (Figure 1A). For confirmatory purposes, surface CD6 expression levels were assessed via quantitative flow cytometry in a small sample of patients assigned to the *CD6*^{lo} (N=15) and *CD6*^{hi} (N=11) mRNA expression groups. *CD6* mRNA expression correlated with the number of membrane-associated molecules per cell, thus confirming the validity of the previously established mRNA cut-off (Figure 1A, B).

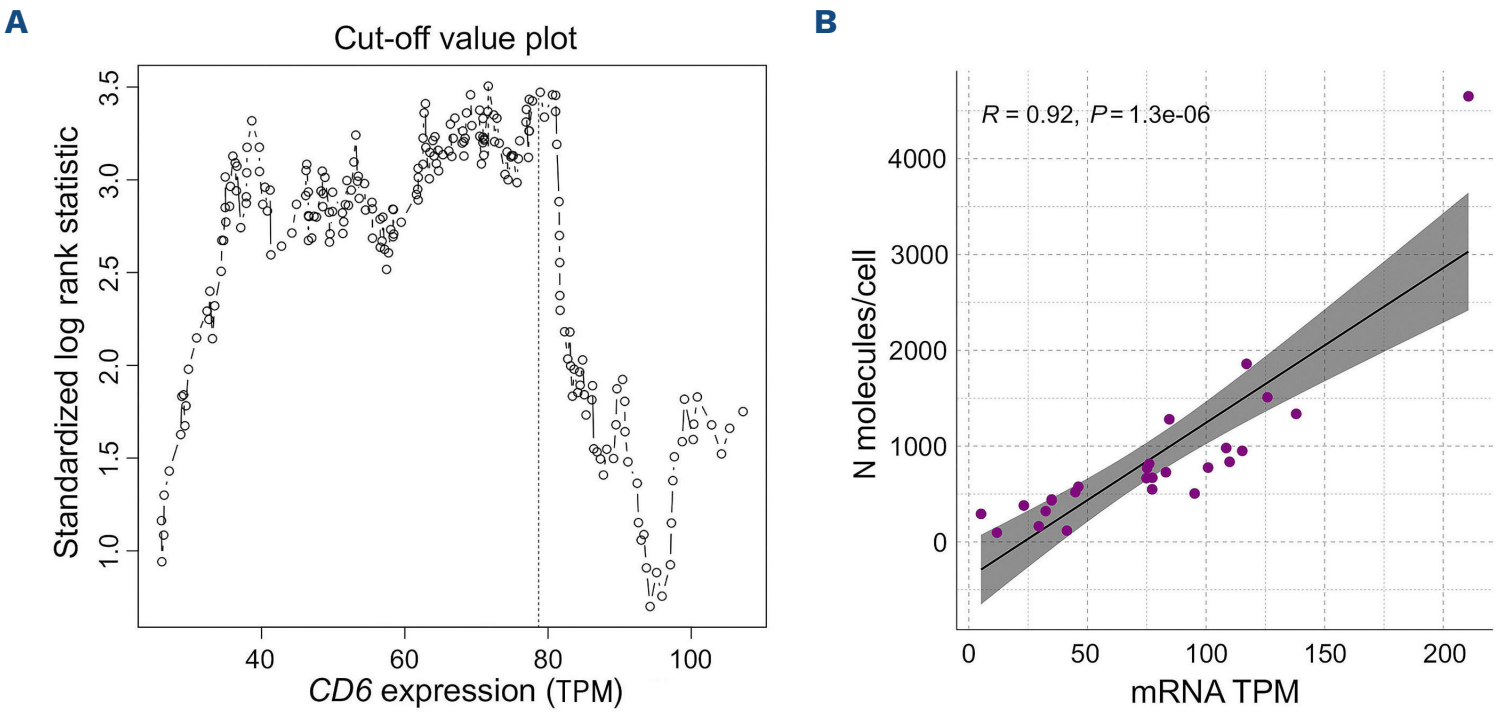

Figure 1. Assessment of CD6 mRNA and protein expression in *CD6*^{hi} and *CD6*^{lo} subgroups. (A) Standardized log rank statistics plots for the identification of the best cut-off value of mRNA CD6 expression to predict time to first treatment, with a peak at around 80 transcripts per million (TPM). (B) Surface CD6 expression assessed by quantitative flow cytometry in samples assigned to the *CD6*^{hi} and *CD6*^{lo} subgroups. Statistical significance assessed by the Spearman correlation test. (C) Spearman correlation of CD6 mRNA TPM versus the number of membrane CD6 molecules. Statistical significance assessed by the Mann-Whitney test. $^{****}P \leq 0.0001$.

Median age of the cohort was 62 years (range: 33-87 years); 223 (83%) patients had a diagnosis of CLL, 35 (13%) of CLL-type monoclonal B-cell lymphocytosis (MBL), and 10 (4%) of small lymphocytic lymphoma (SLL), with no differences according to *CD6* expression. The *CD6*^{hi} group was associated with a higher proportion of males (73% for *CD6*^{hi} *vs.* 57% for *CD6*^{lo}; *P*=0.016). Patients with *CD6*^{lo} had a higher frequency of unmutated IGHV status (41% *vs*. 23% for *CD6*^{lo} and *CD6*^{hi}, respectively; *P*=0.006). Neither of the CD6 groups showed any differences in ECOG performance status, Rai or Binet stages, absolute lymphocyte count (ALC), lactate dehydrogenase (LDH) or β_2-microglobulin levels, presence of *TP53* mutation/del(17p), or CLL-IPI score.

CD6 mRNA level is an independent predictor of time to first treatment

Median follow-up for the series was 12.8 years (range: 3 months-30.4 years). One hundred and thirty-seven patients (52%) received treatment during follow-up. The 10-year probability of needing treatment was 48% (95% Confidence Interval [CI]: 42-54%) and patients with *CD6*^{hi} showed a significantly longer TTFT compared to those with *CD6*^{lo} (10-year probability of receiving treatment 33 *vs*. 55%; *P*=0.0003) (Figure 2A). When the median CD6 mRNA level was considered (64 TPM), the *CD6*^{hi} group also showed a significantly longer TTFT (*Online Supplementary Figure S1*). Considering the factors that were statistically significant in the univariable models, and omitting those overlapping

Table 1. Overall baseline characteristics and according to *CD6* expression.

Characteristic	All patients N=270	*CD6* expression		
		CD6^{hi} >80 TPM, N=83, 31%	*CD6*^{lo} ≤80 TPM, N=187, 69%	*P*
Age, years, median (range)	62 (33-87)	61 (36-86)	63 (33-87)	NS
Male sex, N (%)	168 (62)	61 (73)	107 (57)	0.02
Diagnosis, N (%) CLL MBL SLL	 223 (83) 35 (13) 10 (4)	 69 (85) 11 (14) 1 (1)	 154 (82) 24 (13) 9 (5)	NS
ECOG PS ≥1, N (%)	23 (9)	5 (6)	18 (10)	NS
Binet stage C, N (%)	9 (3)	2 (2)	7 (4)	NS
Rai stage III-IV, N (%)	11 (4)	3 (4)	8 (4)	NS
Absolute lymphocyte count >15x10^9/L, N (%)	85 (34)	18 (25)	67 (39)	NS
LDH above ULN, N (%)	22 (8)	4 (5)	18 (10)	NS
β2-microglobulin >3.5 mg/L, 166 evaluated, N (%)	27 (16)	6 (11)	21 (19)	NS
IGHV unmutated, N (%)	96 (36)	19 (23)	77 (41)	0.006
FISH, 161 evaluated, N (%) Normal del(13)(q14.3) Trisomy 12 del(11)(q22.3)	 47 (29) 4 (46) 22 (14) 18 (11)	 16 (30) 28 (53) 3 (6) 4 (8)	 31 (27) 46 (40) 19 (17) 14 (12)	NS
TP53 mutated / del17p, N (%)	16 (6)	4 (5)	12 (6)	NS
CLL-IPI, 175 evaluated, N (%) Low Intermediate High / Very high	 82 (47) 49 (28) 44 (25)	 30 (54) 17 (31) 8 (15)	 52 (43) 32 (27) 36 (30)	NS
First-line treatment, 137 evaluated, N (%) Alkylating agents Purine analogs Novel agents and others	 45 (33) 76 (55) 16 (12)	 8 (27) 18 (60) 4 (13)	 37 (35) 58 (54) 12 (11)	NS

CLL: chronic lymphocytic leukemia; CLL-IPI: CLL International Prognostic Index; ECOG PS: Eastern Cooperative Oncology Group Performance Status; LDH: lactate dehydrogenase; MBL: CLL-type monoclonal B-cell lymphocytosis; N: number; NS: not statistically significant; SLL: small lymphocytic lymphoma; TPM: transcripts per million; ULN: upper limit of normal.

with the CLL-IPI score, a multivariable model for TTFT was built (Table 2). Only CLL-IPI risk groups, ALC >15x10^9/L and *CD6* expression retained statistical significance (*CD6*^{hi} Hazard Ratio [HR]=0.55 [95% CI: 0.33-0.9]; *P*=0.02) (Figure 2C). Due to the strong association between CD6 expression and *IGHV* status, we performed an additional bivariable model for TTFT only including these variables, and both retained their prognostic value (CD6hi HR=0.57 [95% CI: 0.38-0.86], *P*=0.007; unmutated IGHV HR=3.8 [95% CI: 2.7-5.4], *P*<0.001) (*Online Supplementary Figure S2*).

Eighty-five patients (31%) died during follow-up. Ten-year OS for the entire cohort was 79% (95% CI: 74-84%), with no significant differences according to CD6 expression (*P*=0.101) (Figure 2B). Front-line treatment regimens were similar between *CD6*^{hi} and *CD6*^{lo} groups (87% and 89% immuno-/chemotherapy, 13% and 11% targeted agents, respectively). Thirteen patients (4.8%) developed RT. For the entire series, the 10-year risk of RT was 3% (95% CI: 1.5-6%), with no significant differences according to *CD6* status.

Differential transcriptomic profile of *CD6*^{hi} and *CD6*^{lo} chronic lymphocytic leukemia patient groups

To explore the mechanisms behind the different clinical behavior of *CD6*^{hi} and *CD6*^{lo} groups, the same patient stratification was applied for transcriptomic analyses. Differential expression (DE) analysis of the *CD6*^{hi} group revealed a total of 26 up-regulated and 67 down-regulated genes (adjusted *P* value [Padj] <0.05, $\log_2$ fold change >0.6) (Figure 3A). In terms of biologically relevant genes, the *CD6*^{hi} subgroup showed downregulation of *MYC, IL15, CD38*, and *CD49D/ITGA4*. Decreased expression of both *CD38* and *CD49D/ITGA4* expression was later confirmed by flow cytometry

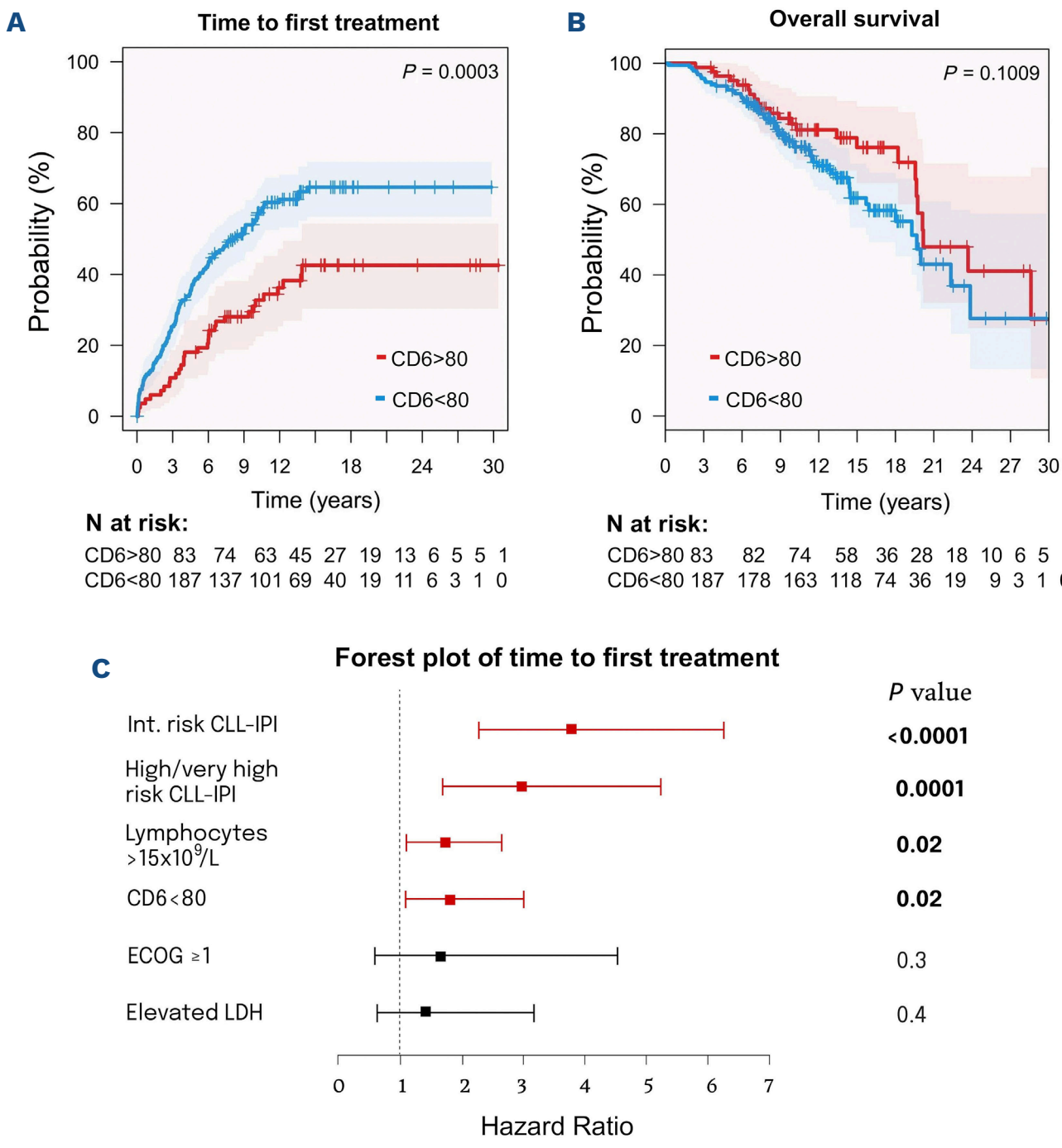

Figure 2. Survival analysis and predictive modeling for time to first treatment based on mRNA *CD6* dichotomized expression. (A) Time to first treatment (TTFT) according to *CD6* expression. (B) Overall survival (OS) according to *CD6* expression. (C) Model to predict TTFT. For OS and TTFT Kaplan-Meier curves, differences were assessed using the log rank and Gray's test for survival and cumulative incidence endpoints, respectively. A Fine-Gray regression model was built for the multivariable analysis of TTFT.

(*Online Supplementary Figure S3*).

Two different annotations were tested against our differential *CD6* expression results to provide context for their biological function based on predefined gene sets. Using the hallmarks annotation, three pathways down-regulated in the *CD6*^{hi} group were significant (P_{adj} <0.05), and no pathways up-regulated in the *CD6*^{hi} group were considered significant in our analysis (Figure 3B). The pathway with the lowest Normalized Enrichment Score (NES) value in the *CD6*^{hi} group corresponded to the v1 targets of the MYC protein (NES = -1.47, P_{adj}=0.0006). This annotation also showed decreased expression of genes involved in the mitotic spindle (NES = -1.41, P_{adj} =0.02) and in unfolded protein responses (NES = -1.41, P_{adj} =0.03) in the *CD6*^{hi} subgroup.

The gene ontology (GO) annotation was also tested against our DE results, showing 49 down-regulated pathways in the CD6lo group (Figure 3C). The most down-regulated pathway in the *CD6*^{hi} group corresponded to the ribonucleoprotein complex (NES = -1.5, P_{adj} =0.0153), followed by genes associated to RNA splicing (NES = -1.5, P_{adj} =0.0153). Deregulation of protein stability was noted when using both hallmark and GO annotations, maintaining a downregulation of this gene set in the *CD6*^{hi} group (NES = -1.49, P_{adj} =0.0153).

Discussion

Chronic lymphocytic leukemia prognostic markers have been explored in an effort to characterize its heterogeneous clinical course and gain insight into its biology.[35] While the CLL-IPI includes the main clinical, genetic and biochemical parameters, it still faces limitations concerning high-risk patients and predicting outcomes with new targeted drugs. Thus, the exploration of novel prognostic parameters can enhance risk stratification.

This study provides evidence of the clinical and biological impact of *CD6* expression, a gene expressed by both poorly and well differentiated B-cell neoplasms, on CLL progres-

Table 2. Predictors of time to first treatment in the uni- and multivariable analyses.

Parameter	Risk category	Univariate analysis		Multivariate analysis 142 cases, 101 events	
		HR (95% CI)	*P*	HR (95% CI)	*P*
Lymphocytes	>15 x10^9/L	2.4 (1.69-3.4)	<0.0001*	1.79 (1.11-2.66)	0.02*
LDH	Above ULN	2.56 (1.42-4.59)	0.02*	1.66 (0.6-4.54)	NS
ECOG PS	≥1	2.47 (1.47-4.15)	<0.0001*	1.42 (0.64-3.18)	NS
CLL-IPI risk group	Intermediate risk	4.01 (2.47-6.51)	<0.0001*	3.79 (2.29-6.26)	<0.0001*
	High/Very high risk	4.02 (2.58–6.26)	<0.0001*	2.99 (1. 17-5.24)	0.0001*
Binet	C	5.7 (1.59-20.39)	0.008*	NI[b]	
IGHV	Unmutated	4.05 (2.87-5.7)	<0.0001*	NI[b]	
del(17p) and/or *TP53* mut	Present	1.61 (0.93-2.82)	NS	NI[b]	
Age in years	>65	0.92 (0.67-1.29)	NS	NI[b]	
Sex	Male	1.36 (0.96-1.93)	NS	NI[a]	
β2-microglobulin	>3.5 mg/L	2.1 (1.35-3.26)	0.001*	NI[b]	
CD6 expression	>80 TPM	0.49 (0.33-0.73)	0.0003*	0.55 (0.33-0.9)	0.02*

CI: confidence interval; ECOG PS: Eastern Cooperative Oncology Group Performance Status; HR: hazard ratio; LDH: lactate dehydrogenase; NS: not statistically significant; TPM: transcripts per million; ULN: upper limit of normal. [a]Not included due to absence of statistical significance in the univariable analysis. [b]Not included to avoid overlapping with the Chronic Lymphocytic Leukemia International Prognostic Index (CLL-IPI). *Statistically significant.

sion.[16] For the entire cohort, the probability of receiving treatment was 32% at five years, and OS was 79% at ten years, which was in line with previously reported estimates (*Online Supplementary Figure S4*).[36-38] Our results show that the differential expression of *CD6*, measured by bulk RNA-Seq, predicts TTFT. *CD6*^{hi} expression levels (>80 TPM) led to lower 10-year probability of receiving treatment (33% *vs.* 55%; *P*=0.0003), and *CD6* expression predicted TTFT in both the uni- and multivariable analyses. In the multivariable model, only *CD6*^{hi} expression, CLL-IPI risk group, and ALC >15x10^9/L predicted TTFT. Dichotomized *CD6* expression retains its prognostic value even after considering well-established clinical prognostic markers. Furthermore, *CD6*^{hi} expression associates with a mutated *IGHV* status, one of the widely recognized indicators of better CLL prognosis. We are aware of the fact that the cutoff used as a TTFT predictor in our cohort (80 TPM) probably cannot be entirely generalized to other patient datasets; thus, future validation studies are warranted.

To unravel the mechanisms behind the different clinical progression of *CD6*^{hi} and *CD6*^{lo} patients, transcriptomic analyses on the same patient segregation were performed. Adverse prognostic markers in CLL such as *CD38* and *CD49D/ITGA4* expression were significantly down-regu-

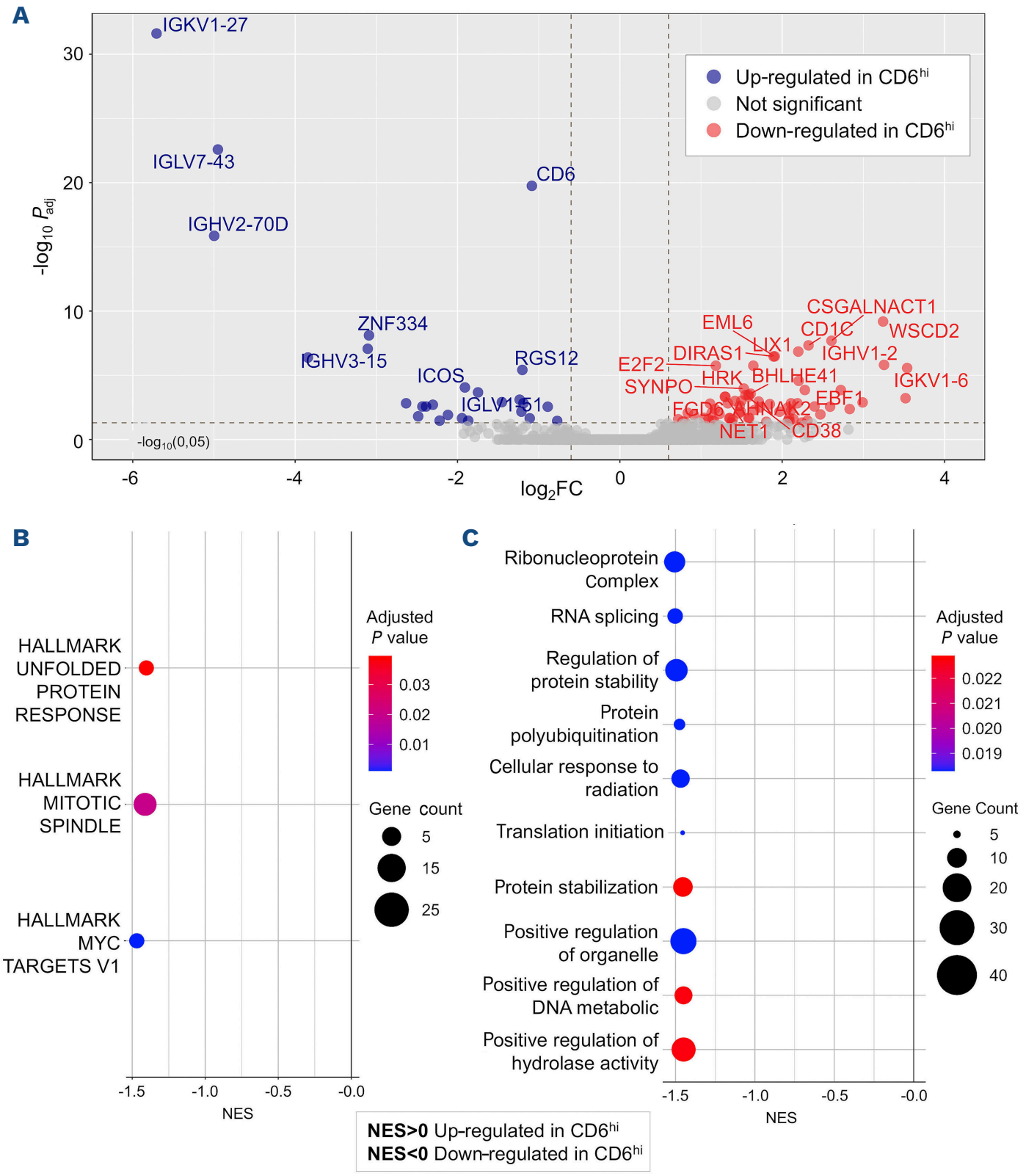

Figure 3. Transcriptomic profiling of *CD6*^{hi} and *CD6*^{lo} patients based on RNA-Seq data. Volcano plot for differentially expressed genes (A) and enrichment analysis via Gene Set Enrichment Analysis (GSEA), based on Hallmarks (B) or Gene Ontology (C) annotations, in the *CD6*^{hi} and *CD6*^{lo} subgroups. Differentially expressed genes were identified using a false discovery rate (FDR) threshold of 10%, and adjusted *P* value <0.05, log2fold change>|0.6|).

lated in the *CD6*^{hi} group, in line with TTFT findings. MYC proto-oncogene downregulation was also detected in the CD6^{hi} group, and its involvement confirmed in the GSEA analysis, as MYC targets were repressed in the same group. It is worth mentioning that MYC translocations are unusual in CLL patients, with about 5% of patients displaying 8q24 translocations.[39] This finding is associated with poorer outcomes and, more specifically, with RT.[40] Despite *MYC* identification as a possible contributor to better prognosis in the *CD6*^{hi} subgroup, no significant association with RT was detected based on *CD6* expression. The *CD6*^{hi} group also showed *IL15* downregulation, involved in CLL leukemogenesis in *in vitro* studies.[41,42]

Conversely, the *MYL9* and *RGS12* genes were up-regulated in the *CD6*^{hi} subgroup. The effects of *MYL9* on tumor progression are still a subject of debate, with different outcomes contingent on the specific cancer type,[43] where *RGS12* is consistently considered a favorable prognostic marker in osteosarcoma and oral cancer.[44,45]

Gene Set Enrichment Analysis revealed decreased expression of mitotic spindle-related genes in the *CD6*^{hi} subgroup, suggesting a general cell cycle and division deregulation linked to the increased proliferation of CLL cells. We also show RNA splicing-related gene downregulation, a hallmark of cancer suited for therapeutic intervention.[46] Genes associated to Unfolded Protein Response (UPR) (a general mechanism to promote cell survival in cancer and linked to BCR stimulation in CLL) were also down-regulated in the *CD6*^{hi} subgroup.[47]

Taken together, our results suggest that *CD6*^{hi} expression associates with better outcomes considering both clinical and transcriptomic data. How *CD6* expression influences normal and leukemic B-cell physiology is an open question. Studies in *Cd6*$^{-/-}$ mice, which harbor lower numbers of spleen B1a and marginal zone B cells, show lower titers of natural polyreactive antibodies.[48,49] This observation is particularly significant in the CLL setting, where B1a cells have been proposed as the healthy counterparts of these neoplastic cells.[50] Additional observations of CLL cells *in vitro* show that mAb-mediated CD6 ligation modulates the Bcl-2/Bax ratio and protects CLL cells from apoptosis induced by IgM crosslinking.[16] On this basis, the authors concluded that CD6 plays a relevant role in promoting CLL cell survival, contrary to our observation of faster CLL progression associated to lower *CD6* expression levels. It should be mentioned in this respect that CD6 interaction with both positive and negative intracytoplasmic signaling effectors is supported by signalosome studies.[15] Moreover, it is difficult to extrapolate the *in vitro* experimental conditions above to an *in vivo* clinical setting, where circulating CLL cells are unlikely to receive apoptotic signals via strong BCR crosslinking, and in which CD6 ligation by its counter receptors (i.e., CD166/ALCAM and CD318/CDCP-1), which are over-expressed on CLL cells, could reproduce the high affinity binding conditions mediated by anti-CD6 mAb. This warrants the exploration of CD6 ligation by such counter-receptors in the absence of BCR signaling in CLL cells.

T-cell malignancy patients, including T-cell acute lymphoblastic leukemia and T-cell lymphoma, show high *CD6* mRNA expression associated with better OS.[51] The same study reveals low *CD5* mRNA levels, the paralog gene of CD6, associated with higher OS. In the light of these results, we assessed whether *CD5* expression influenced TTFT and OS in our cohort, we have detected no significant differences based on dichotomized expression (*data not shown*). Therefore, the analysis of *CD5* impact was not pursued further.

In conclusion, our study identifies *CD6*^{hi} expression as an independent good prognostic marker in CLL patients, demonstrated by a longer TTFT, its association with a mutated *IGHV* status, and deregulation of specific pathways related to cancer progression.

Disclosures

FL is the founder and ad honorem scientific advisor of Sepsia Therapeutics S.L. All of the other authors have no conflicts of interest to disclose.

Contributions

LC-S, JAP, XSP, PM and FL designed the study. JAP, DC and PM collected and analyzed the clinical data. LC-S, PB-M, VP-R, LA-S, MV-dA, SC-L and XSP performed the cellular and genetic analysis. LC-S, JAP, PM and FL wrote the manuscript. All the authors reviewed the manuscript and approved the final version.

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Data-sharing statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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