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ORIGINAL RESEARCH ARTICLE

Plasma B-Cell Maturation Antigen Levels are Elevated and Correlate with Disease Activity in Patients with Chronic Lymphocytic Leukemia

Kyle A. Udd[1] · Sean Bujarski[1] · Eric Wirtschafter[2] · Tanya M. Spektor[3] · Matthew Ghermezi[3] · Laura Z. Rassenti[4] · Michael E. David[1] · Jason D. Nosrati[1,5] · Ashkon A. Rahbari[1] · James Wang[1] · Suzie Vardanyan[1] · Nika M. Harutyunyan[1] · Julia Linesch[1] · Mingjie Li[1] · Eric Sanchez[1] · Haiming Chen[1] · Thomas J. Kipps[4] · James R. Berenson[1,3]

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Abstract

Background Chronic lymphocytic leukemia (CLL) is a malignancy of late B cells. In another late B-cell malignancy (multiple myeloma), levels of solubilized B-cell maturation antigen (sBCMA) are elevated and predict outcomes.

Objective We sought to evaluate sBCMA as a possible prognostic factor and monitoring tool for patients with CLL.

Patients and Methods Using an enzyme-linked immunosorbent assay (ELISA), we assessed plasma (p) levels of BCMA in 171 CLL patients and compared them with levels in healthy individuals.

Results pBCMA levels were significantly higher among patients with CLL than those from healthy donors ($p < 0.0001$). Among patients with aggressive disease, pBCMA was elevated compared with patients with indolent disease ($p < 0.001$). Those with an initial pBCMA level in the highest quartile had a shorter time to first treatment compared with CLL patients with pBCMA levels in the lowest three quartiles ($p < 0.0001$). Among those in the highest quartile (pBCMA > 110.9 ng/mL), overall survival was shorter than those in the lowest three quartiles ($p = 0.0007$). Finally, among those patients who underwent serial pBCMA testing, changes in these levels correlated with changes in their clinical status.

Conclusions Together, our findings show that pBCMA is a promising new prognostic and predictive indicator for patients with CLL.

1 Introduction

Chronic lymphocytic leukemia (CLL) is the most common adult leukemia in Western societies [1]. It is characterized by an accumulation of monoclonal, mature B lymphocytes in the blood and lymphoid tissues. The disease course is highly heterogeneous [1, 2]. CLL primarily affects older individuals, with a median age at diagnosis ranging from 70 to 72 years [1].

B-cell maturation antigen (BCMA) is a tumor necrosis factor (TNF) superfamily protein receptor that is preferentially expressed on the surface of mature B lymphocytes. Binding of the agonist ligands B-cell activating factor (BAFF) and a proliferation-inducing ligand (APRIL) to BCMA promote B-cell survival and proliferation [3]. Surface BCMA is cleaved directly by γ-secretase, which inhibits its B-cell stimulatory activity and generates solubilized BCMA [4, 5].

We previously demonstrated that patients with multiple myeloma (MM) have increased levels of serum BCMA (sBCMA) compared with individuals with monoclonal gammopathy of undetermined significance and healthy controls [6]. Moreover, the level of sBCMA correlated directly with

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✉ James R. Berenson
jberenson@imbcr.org

1 Institute for Myeloma and Bone Cancer Research, 9201 Sunset Boulevard, Suite 300, West Hollywood, CA 90069, USA

2 UCLA-Olive View Medical Center in Sylmar, 14445 Olive View Drive, Sylmar, CA 91342, USA

3 Oncotherapeutics, 9201 Sunset Boulevard, Suite 317, West Hollywood, CA 90069, USA

4 Moores Cancer Center, University of California at San Diego, 3855 Health Sciences Drive, La Jolla, CA 92037, USA

5 College of Human Medicine, Michigan State University, 965 Fee Road A110, East Lansing, MI 48824, USA

Key Points

Plasma B-cell maturation antigen (pBCMA) levels are higher in patients with chronic lymphocytic leukemia (CLL) compared with healthy individuals.

High pBCMA levels are found in CLL patients with a short time from diagnosis to needed treatment, and vice versa.

Overall survival was shorter in CLL patients with the highest levels of pBCMA.

Changes in pBCMA levels correlate with patients' clinical status.

treatment response and was also shown to predict progression-free survival (PFS) and overall survival (OS) among patients with MM [6].

CLL is also thought to be critically dependent on BCMA signaling. In vitro data using CLL cell lines have demonstrated dependence on the canonical nuclear factor (NF)-κB pathway, which is mediated by upstream binding of BAFF and APRIL to BCMA on the surface of CLL cells. By contrast, normal B cells are more dependent on the alternative NF-κB signaling pathway [7].

Many prognostic and predictive markers have been identified for CLL patients. Classically, these include more heavily mutated immunoglobulin heavy chain variable (IGHV) regions (more favorable prognosis and responsiveness to chemoimmunotherapy), deletions of 17p and 11q (unfavorable prognosis with a poor response to chemoimmunotherapy), 13q deletion (favorable prognosis), ZAP70 expression of ≥ 20% (unfavorable prognosis), and serum β2-microglobulin > 4 mg/L (unfavorable prognosis) [8–10]. Because CLL represents a late B-cell malignancy, and because levels of solubilized BCMA were elevated and predicted outcomes among MM patients, we evaluated solubilized BCMA as a possible prognostic and predictive marker among patients with CLL from plasma samples obtained from the University of California at San Diego Tissue Bank.

2 Methods

2.1 Patient/Sample Characteristics

We analyzed plasma samples from 171 patients with CLL that had been archived at the University of California San Diego (UCSD) Moores Cancer Center Tissue Bank facilities, and compared them with 104 age-matched controls. Clinical data from 1999 to 2013 were extracted from these patients' medical records. The study was conducted in accordance with the principles of the Declaration of Helsinki and International Conference on Harmonization Good Clinical Practice guidelines.

Three of the 171 CLL samples were omitted from time to first treatment (TTFT) because patients had received therapy prior to their first plasma BCMA (pBCMA) assessment.

Patients were started on therapy if they satisfied International Workshop on CLL (IWCLL) criteria for initiation of treatment, but were not required to have been started on therapy in order to be included in the analysis [11]. A summary of the nature of treatments among those patients requiring CLL therapy is listed in electronic supplementary Table 1. For 21 patients, serial BCMA levels were available within 3 months of standard treatment response assessment, and, for this subset, we sought to correlate changes in pBCMA with clinical disease response using IWCLL criteria. Thirty-seven percent (62/167) of patients had samples taken within 6 months of CLL diagnosis. The time from diagnosis to sample date ranged from 1.3 to 275 months, with a median of 12 months. Thirty-four percent (59/172) of patients had a TTFT of < 6 months, including four subjects with a TTFT ≤ 0 (sample taken after the start of treatment).

2.2 Serum B-Cell Maturation Antigen (BCMA) Assay

We measured pBCMA with a polyclonal anti-BCMA antibody using an enzyme-linked immunosorbent assay (ELISA) from R&D Systems (Minneapolis, MN, USA; catalog #DY193E), as previously published [6].

2.3 Patient/Sample Stratification

We collected data regarding white blood cell (WBC) count, serum β2-microglobulin (β2 M), IGHV gene mutation status (≤ 98% homology to germline defined as mutated CLL [M-CLL] and > 98% homology defined as unmutated CLL [U-CLL]), ZAP70 expression on CLL cells (≤ or > 20% of cells), and CLL-related cytogenetic markers, including deletion of chromosomes 13q and 17p from information available in the UCSD CLL Tissue Bank database. We stratified patients into aggressive and indolent cohorts based on IGHV mutational status and ZAP70 expression. Aggressive characteristics were considered to be either ZAP70 expression on > 20% of CLL cells, IGHV gene with > 98% sequence homology to germline, or a combination of both. We also collected data on TTFT, time from sample collection to initiation of first treatment, and OS.

2.4 Minimal Residual Disease Determination Method

To determine minimal residual disease (MRD) status, first, specific flow cytometric analysis of bone marrow aspirate and/or peripheral blood is performed using a four-color panel designed to detect minimal residual CLL [11]. If the results reveal < 0.01% of the total nucleated cells have an immunophenotype consistent with CLL, the patient is considered to have a non-detectable level of MRD-negative; 0.01% is the limit of detection for this method, therefore < 0.01% is below the limit of detection of this method. Flow cytometric analysis was performed on a minimum of 100,000 cells for each antibody. The lysed bone marrow aspirate was stained with antibodies directed against the following antigens: CD3, CD5, CD10, CD11c, CD19, CD20, CD22, CD38, CD43, CD45, CD79b, CD81, FMC7, kappa, and lambda.

2.5 Data Analyses Examining Overall Survival (OS) and Time to First Treatment (TTFT)

The Mann–Whitney U test was used to examine the correlation between pBCMA levels and various groups of CLL patients requiring treatment or never treated, or healthy individuals (Figs. 1, 2). One-way analysis of variance (ANOVA) with Bonferonni's correction was applied to examine pBCMA levels among healthy individuals and CLL patients with indolent or aggressive disease (Fig. 1). Standard Kaplan–Meier analysis was applied to examine TTFT and OS based on pBCMA levels (Fig. 3). Kaplan–Meier analysis was also used to correlate changes in pBCMA levels with disease status (electronic supplementary Fig. 2).

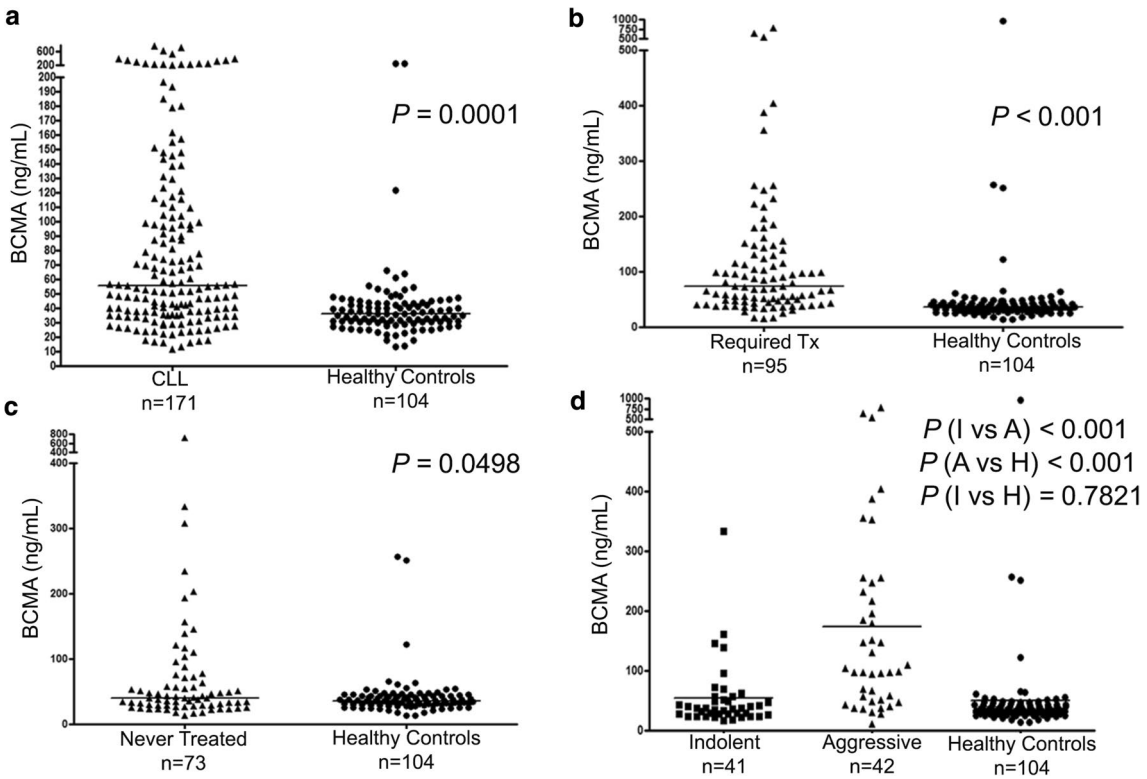


Fig. 1 Plasma BCMA levels are elevated in patients with CLL based on the Mann–Whitney *U* test. **a** 104 age-matched healthy donors (filled circle) had significantly lower pBCMA levels (median 36.03 ng/mL) than 171 CLL (filled triangle) patients (median 55.92 ng/mL; $p < 0.0001$). **b** pBCMA is significantly higher among untreated CLL patients ($p < 0.0001$) compared with CLL patients who went on to receive treatment (filled triangle; $n = 95$; median 73.80 ng/mL) and with age-matched healthy donors (filled circle; $n = 104$; median 36.03 ng/mL). **c** Patients who never went on to receive treatment (filled triangle; $n = 73$; median 40.59 ng/mL) showed significantly higher levels of pBCMA than age-matched healthy donors (filled circle; $n = 104$; median 36.03 ng/mL; $p = 0.0498$). **d** Patients with aggressive [**A**] CLL (filled triangle; $n = 42$; median 102.1 ng/mL) showed significantly higher levels of pBCMA than either indolent [**I**] CLL patients (filled square; $n = 41$; median 37.4 ng/mL; $p < 0.001$) or age-atched healthy donors [**H**] (filled circle; $n = 104$; median 36.03 ng/mL; $p < 0.001$). Indolent CLL patients did not show statistically significant higher pBCMA levels than age-matched healthy donors ($p = 0.7821$). *BCMA* B-cell maturation antigen, *pBCMA* plasma BCMA, *CLL* chronic lymphocytic leukemia, *Tx* treatment

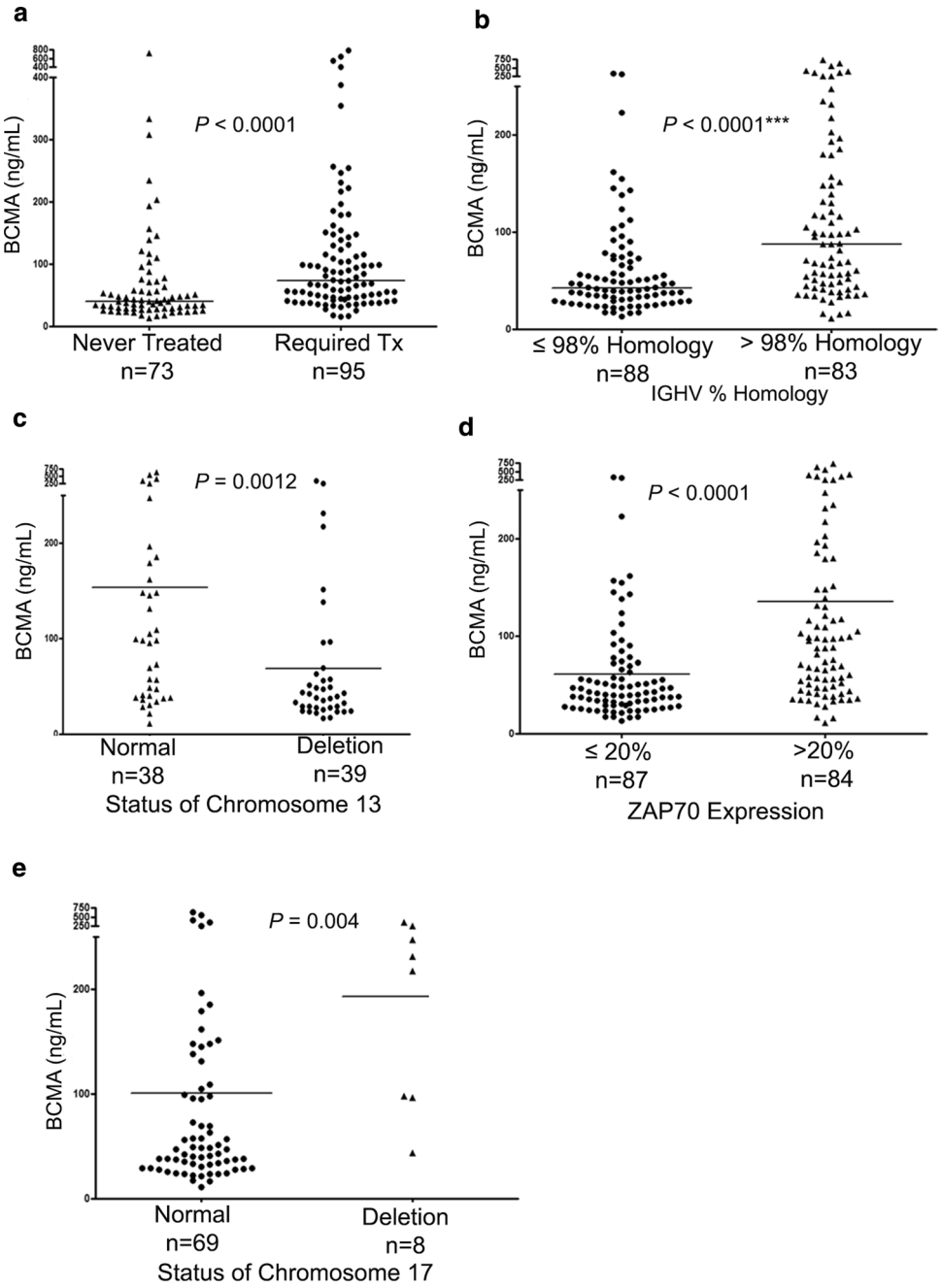
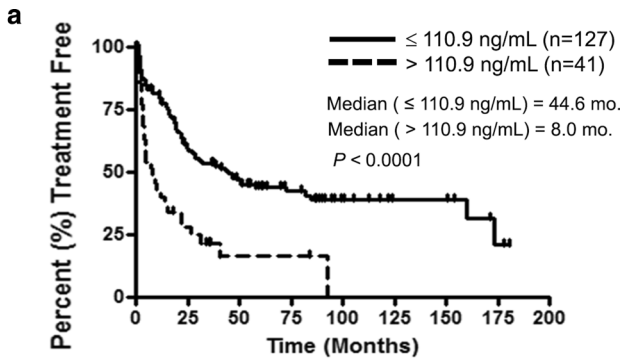


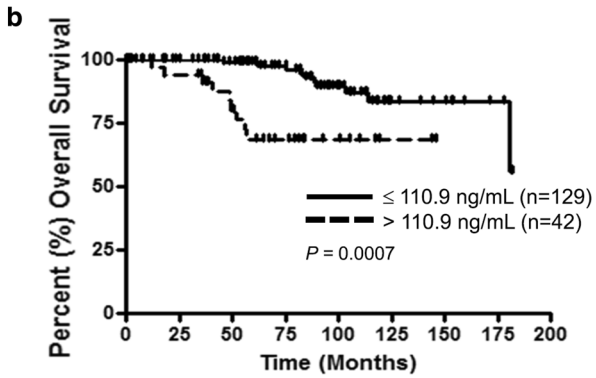
Fig. 2 Correlation of pBCMA levels and known prognostic markers for CLL. **a** CLL patients who eventually required treatment (filled circle; $n=95$; median 73.80 ng/mL) had significantly higher pBCMA levels than CLL patients who never required treatment (filled triangle; $n=73$; median 40.59 ng/mL; $p<0.0001$). **b** CLL patients were stratified into two groups based on sequence homology to IGHV germline sequences. pBCMA levels were significantly higher among patients with > 98% sequence homology to germline (filled triangle; $n=83$; median 87.54 ng/mL) than those with ≤ 98% sequence homology to germline (filled circle; $n=88$; median 42.59 ng/mL; $p<0.0001$). **c** CLL patients with a chromosome 13 deletion showed significantly lower levels of pBCMA (filled circle; $n=39$; median 39.90 ng/mL) than patients without a chromosome 13 deletion (normal; filled triangle; $n=38$; median 97.90 ng/mL; $p=0.0012$). **d** CLL patients with a greater proportion (> 20%) of ZAP70-expressing cells additionally had significantly elevated pBCMA levels (filled triangle; $n=84$; median 87.95 ng/mL) compared with CLL patients with lower levels (≤ 20%) of ZAP70-expressing cells (filled circle; $n=87$; median 42.13 ng/mL; $p<0.0001$). **e** CLL patients with a chromosome 17p deletion (filled triangle; $n=8$; median 224.40 ng/mL) showed higher pBCMA levels than those without chromosome 17p deletion (filled circle; $n=69$; median 47.60 ng/mL; $p=0.004$). *BCMA* B-cell maturation antigen, *pBCMA* plasma BCMA, *CLL* chronic lymphocytic leukemia, *Tx* treatment, *IGHV* immunoglobulin heavy chain variable

2.6 Statistical Analyses for BCMA Validation

The effect of each potential predictor on OS was investigated independently using Cox proportional hazards regression models (single regressor variable) at a 0.10 level of significance ($\alpha = 0.10$) (Table 1). The subset of variables for which the regression coefficient had a p value < 0.10 was identified and Spearman rank correlations were used to investigate the association among the potential predictor variables.



Months	0	25	50	75	100	125	150	175	180
Number at risk (n=127)	118	61	43	28	15	8	8	5	2
Number at risk (n=41)	36	10	3	3	1	1	1	1	1



Months	0	25	50	75	100	125	150	175	180
Number at risk (n=129)	122	105	88	66	41	15	11	5	4
Number at risk (n=42)	37	33	23	15	9	4	2	2	2

Fig. 3 Analysis of TTFT and OS of CLL patients based on pBCMA levels. **a** Patients with pBCMA values in the fourth quartile ($n = 41$, pBCMA range 110.9–782.97 ng/mL) had significantly shorter TTFT (from time of first measurement) compared with patients with pBCMA values below this threshold ($n = 128$, pBCMA range 11.57–109.39, $p < 0.0001$). **b** Patients in the highest quartile ($n = 42$; pBCMA range 110.9–782.97 ng/mL) had a significantly shorter OS compared with CLL patients with pBCMA values in the lower three quartiles ($n = 129$, pBCMA range 11.57–109.39 ng/mL, $p = 0.0007$). *TTFT* time to first treatment, *OS* overall survival, *pBCMA* plasma B-cell maturation antigen, *CLL* chronic lymphocytic leukemia, *mo.* months

Table 1 Summary of univariate regressions of potential predictor variables on overall survival

Predictor variable	p value	Hazard ratio	Number of subjects
BCMA	0.0047	1.003	171
ZAP70	0.0526	1.014	171
Log BCMA	0.0011	2.105	171
Chromosome 11 deletion	0.5374	1.908	77
Chromosome 12 trisomy	0.8553	0.868	77
Chromosome 13 deletion	0.2092	0.470	77
Chromosome 17 deletion	< 0.0001	14.970	77
IgG	0.9164	1.000	81
IgA	0.0680	0.993	81
IgM	0.1203	0.989	81
WBC	0.0189	1.004	83
Aggressive vs. indolent	0.0010	5.700	83
Age	0.5421	0.986	83
β2-microglobulin	0.0013	1.599	166

Cox proportional hazards regression model identifying the best potential predictor variables on overall survival. Variables with a p value < 0.1 are indicated in bold

Ig immunoglobulin, *WBC* white blood cell

A Cox proportional hazards regression model with stepwise variable selection was used to identify the variables within this subset that best predict OS. The p value for entering the model was < 0.10, and the p value for staying in the model was < 0.05. For each of the variables within this subset, a threshold value was identified that maximized the difference between the two groups defined by the threshold (value < threshold vs. value ≥ threshold). Specifically, the threshold was identified by plotting the p-value for the difference between the cohorts defined by a cut-off (or the hazard ratio itself) versus the cut-off as a type of receiver operating characteristic (ROC) curve (data not shown).

Kaplan–Meier curves were generated for the determination of OS for each of the subgroups defined by the threshold.

Table 2 provides a summary of statistics for the potential predictors with the most significant effect on OS. The mean (standard deviation), median (minimum–maximum), and 25th/75th percentile were calculated. The Spearman rank correlations of BCMA (and log BCMA) with the other seven variables were all significantly different from zero ($p \leq 0.0032$). These variables were used as potential predictor variables in Cox proportional hazards regression models with stepwise variable selection. Lastly, Table 3 summarizes the results of the multiple regression analyses of the effect of each potential predictor variable on time to treatment. A multiple regression analysis with all the significant predictors from the Spearman rank correlation was performed using a stepwise variable selection to get a set of significant

Table 2 Summary statistics for potential predictor variables on overall survival

Predictor variable	*n*	Mean (SD)	Median (min–max)	25th percentile 75th percentile
BCMA (ng/mL)	171	97.8 (117.80)	55.9 (11.6–783.0)	35.8 109.4
ZAP70 (% CLL cells w/ZAP70 expression)	171	30.0 (28.1)	19.7 (1–92.5)	6.3 46.8
Log BCMA	171	4.18 (0.83)	4.02 (2.45–6.66)	3.6 4.7
IgA (mg/dL)	81	120.3 (79.5)	99.0 (7–394)	60 167
WBC [^10^9/L]	83	41.5 (64.5)	21.9 (3.4–484)	11.8 40.2
β2-Microglobulin (mg/L)	166	2.48 (1.6)	2 (1.1–16.7)	1.7 2.8
	n	%		
Chromosome 17 deletion	171			
Deletion	8	5		
Normal	69	40		
Not performed	6	4		
Unknown	88	51		
Aggressive vs. indolent				
Aggressive	30	18		
Indolent	53	31		
Unknown	88	51		
Indolent				
BCMA (ng/mL)	53	75.5 (113.3)	42.13 (16.4–783.0)	28.7 83.8
ZAP70 (% CLL cells w/ZAP70 expression)	53	20.5 (28.5)	17.1 (1.0–92.4)	3.8 17.1
Log BCMA	53	1.9 (0.3)	1.8 (1.6–2.9)	1.7 2.0
IgA (mg/dL)	53	136.7 (83.8)	113.0 (7.0–394.0)	73.0 178.0
WBC (^10^9/L)	53	29.4 (29.7)	19.6 (6.9–164.)	10.9
β2-Microglobulin (mg/L)	53	2.3 (0.9)	1.9 (1.1–5.4)	37.5 1.7 2.6
Aggressive				
BCMA (ng/mL)	30	182.8 (161.3)	139.5 (11.57–642.9)	49.1 255.4
ZAP70 (% CLL cells w/ZAP70 expression)	30	51.3 (20.0)	45.4 (23.0–88.3)	33.6 65.5
Log BCMA	30	2.1 (0.4)	2.1 (1.1–2.8)	1.7 2.4
IgA (mg/dL)	30	89.3 (60.5)	71.0 (14.0–272.0)	51.2 114.0
WBC (^10^9/L)	30	62.9 (97.2)	28.5 (3.4–484.0)	13.9 70.6
β2-Microglobulin (mg/L)	30	2.4 (1.4)	2.0 (1.1–8.3)	1.7 2.6

Multiple regression analysis of potential predictor variables on overall survival are presented, with p values < 0.10 from the Cox proportional hazards regression model

SD standard deviation, *min* minimum, *max* maximum, *BCMA* B-cell maturation antigen, *Ig* immunoglobulin, *CLL* chronic lymphocytic leukemia, *WBC* white blood cell

Table 3 Results of regression analysis of potential predictor variables on TTFT

Predictor variable	*p* value	Hazard ratio	Number of subjects
BCMA	< 0.0001	1.003	168
ZAP70	< 0.0001	1.015	168
Log BCMA	< 0.0001	2.006	168
Chromosome 11 deletion	0.2397	1.867	74
Chromosome 12 trisomy	0.0079	2.764	74
Chromosome 13 deletion	< 0.0001	0.158	74
Chromosome 17 deletion	0.0003	4.369	74
IgG	0.2735	0.999	78
IgA	0.0003	0.990	78
IgM	0.3302	0.998	78
WBC	0.0491	1.003	80
Aggressive vs. indolent	< 0.0001	7.732	80
Age	0.7584	0.995	80
β2-microglobulin	< 0.0001	1.299	163

Cox proportional hazards regression model identifying the best potential predictor variables on TTFT. Variables with a *p*-value < 0.1 are indicated in bold

TTFT time to first treatment, *BCMA* B-cell maturation antigen, *Ig* immunoglobulin, *WBC* white blood cell

predictors and to eliminate highly correlated predictors. Variables associated with a *p*-value < 0.10 were highlighted.

3 Results

3.1 Plasma BCMA (pBCMA) Levels are Elevated among Chronic Lymphocytic Leukemia (CLL) Patients

We compared pBCMA levels between patients with CLL and age-matched controls without CLL. The median level of pBCMA in CLL patients ($n = 171$) at the time of first measurement was 55.92 ng/mL, compared with 36.03 ng/mL in the control group ($p < 0.0001$) (Fig. 1a). Those patients who went on to eventually receive treatment ($n = 95$) had higher baseline pBCMA levels (median 73.80 ng/mL) than those who did not require disease-specific treatment ($n = 73$) during the study period (median 40.59 ng/mL; $p < 0.0001$) and controls ($n = 104$, $p < 0.0001$). The pBCMA levels among CLL patients who did not require disease-specific treatment during the study period were significantly increased compared with controls ($n = 104$; 36.03 ng/mL; $p = 0.0498$) (Fig. 1c). pBCMA levels among patients with aggressive disease characteristics were also significantly higher than among healthy controls and patients with indolent disease ($p < 0.001$) (Fig. 1d). While there was a significant difference in pBCMA between patients with aggressive disease characteristics compared with patients with indolent disease and healthy controls, the difference between patients with indolent disease and healthy controls was not statistically significant ($p = 0.7821$) (Fig. 1d).

3.2 pBCMA Levels were Higher among CLL Patients with Prognostic Factors Reflecting an Aggressive Disease Course

We also compared pBCMA levels among patients with and without some widely accepted high-risk features. Prior studies have shown that CLL patients with adverse cytogenetics (deletion of 11q or 17p), unmutated IGHV, ZAP70 overexpression, and increased serum β2 M have shorter PFS and OS [2, 8, 9]. Therefore, patients who did not meet either of the aforementioned criteria for aggressive disease were stratified into either an aggressive cohort (ZAP70 expression on > 20% of CLL cells and/or an IGHV gene with > 98% sequence homology to germline) or a more favorable cohort. Among patients with aggressive disease, pBCMA was significantly elevated ($n = 42$; median 102.1 ng/mL) compared with patients with indolent disease ($n = 41$; median 37.4 ng/mL; $p < 0.001$) and the age-matched healthy donors ($p < 0.001$) (Fig. 1d).

Similar findings were observed in additional subsets based on known prognostic factors for CLL. Among patients with U-CLL ($n = 83$), pBCMA was higher (median 87.54 ng/mL) than among those with M-CLL ($n = 88$; median 42.59 ng/mL; $p < 0.0001$) (Fig. 2b). The median pBCMA level among patients ($n = 39$) with the favorable del(13q) was significantly lower (39.90 ng/mL) than among those who did not show del(13q) ($n = 38$; 97.90 ng/mL; $p = 0.0012$) (Fig. 2c). For CLL patients with ZAP70 expression > 20% ($n = 84$), the median pBCMA level was significantly higher (87.95 ng/mL) compared with those with ≤ 20% expression ($n = 87$; 42.13 ng/mL; $p < 0.0001$) (Fig. 2d). The median pBCMA among the eight patients with deletion 17p [del(17p)] was 222.40 ng/mL, which was higher than among the 69 patients without detectable del(17p), whose median pBCMA was 47.60 ng/mL ($p = 0.004$) (Fig. 2e). Altogether, these data show that pBCMA consistently correlates directly with disease aggressiveness, as assessed by multiple standard prognostic variables for determining outcomes among those with CLL.

3.3 Correlation between pBCMA and OS

Summary statistics of the potential predictive variables on OS are shown in Tables 1 and 2. Assessments of OS were performed based on the time of the initial pBCMA blood draw.

Table 1 summarizes the results of the independent univariate analysis of the effect of each potential predictor variable on OS. The potential predictors with the most significant effect on OS were log BCMA, β2 M, aggressive versus indolent, chromosome 17 deletion, and BCMA. Log BCMA, BCMA, and ZAP70 were available for all 171 subjects, however the other variables were missing some subjects and therefore a multiple regression model incorporating variables other than BCMA, log BCMA, and ZAP70 excluded a subset of subjects with the missing information.

Table 2 shows the results of multiple regression analysis (multivariate method) of potential predictive variables on OS. The Spearman rank correlations of pBCMA (and log pBCMA) with the other seven variables were all significantly different from zero ($p \leq 0.0032$). The correlation of pBCMA with ZAP70, β2 M, and WBC counts were positive, indicating that higher values of pBCMA were associated with higher levels of these variables. Higher values of pBCMA were also associated with aggressive disease and the presence of del(17p). Immunoglobulin (Ig)A seemed to have an inverse relationship with BCMA, where higher values of BCMA were associated with lower values of IgA.

Further multivariate analysis of these potential predictor variables was used in Cox proportional hazards regression models with stepwise variable selection. Since chromosome 17 deletion, IgA, WBC, β2 M, and type of disease (aggressive vs. indolent) were only recorded for the subset of subjects, three separate analysis were performed. One model included variables with the information available for all 171 subjects (BCMA, log BCMA, and ZAP70); the second model included BCMA, log BCMA, ZAP70, and β2 M; and the third model included all eight variables (BCMA, log BCMA, ZAP70, β2 M, chromosome 17 deletion, IgA, WBC, and type of disease). Based on the first Cox proportional hazards regression model, the most significant variable selected was log BCMA ($p < 0.001$). The results of the second model analysis demonstrated that both log BCMA ($p = 0.039$) and β2 M ($p = 0.001$) were selected as the significant predictor variables. The correlations of BCMA (and log BCMA) with B2 M was 0.4216 ($p < 0.0001$). Lastly, the results of the third model analysis showed the significance of chromosome 17 deletion ($p < 0.001$) and ZAP70 ($p = 0.029$). At the initial step of the stepwise model based on 76 subjects, the effect of log BCMA was significant ($p = 0.014$). However, once chromosome 17 deletion entered the model, the effect of log BCMA was no longer significant due to the high correlation of log BCMA with chromosome 17 deletion. For the 69 subjects with normal chromosome 17, nine subjects died (13%) and there were no deaths prior to 48 weeks after the BCMA assessment. For the eight subjects with chromosome 17 deletion, four subjects died (50%) and three of four deaths were on or before week 48. Unfortunately, due to the small number of subjects with chromosome 17 deletion assessment and the very small number of subjects with chromosome 17 deletion present (Table 2), the relationship between chromosome 17 deletion and BCMA was not able to be evaluated meaningfully.

OS ranged from < 1 month to ≥ 182 months. The median OS was not achieved, but the 25th percentile was 180.7 months. Using ROC curve prognostics, a threshold value for BCMA was identified that maximizes the difference between the two groups defined by the threshold (values < threshold vs. values ≥ threshold; data not shown). The 110.90 ng/mL cut-off separated the upper quartile of pBCMA values from the remaining three-quarters of patients, and was sufficient to identify two groups with different OS and TTFT outcomes. CLL patients with an initial pBCMA level in the highest quartile had a significantly shorter OS compared with CLL patients who had a pBCMA level in the lowest three quartiles ($p = 0.0007$) (Fig. 3b). Both BCMA and log BCMA were significantly associated with OS based on Cox regression models. Higher BCMA and log BCMA were associated with shorter survival.

3.4 pBCMA Correlates with TTFT

Table 3 shows the results of multiple regression analysis of potential predictive variables on TTFT. TTFT was calculated as the number of months from the date of the initial pBCMA sample collection to the post-collection treatment start date. In three patients, the initial pBCMA level was drawn following treatment initiation, therefore these patients were excluded from this analysis. Among the remaining 168 patients, treatment for their CLL occurred in 96 (57%) patients. TTFT ranged from < 1 month to 180.8 months. The median TTFT was 27.4 months (95% confidence interval 19.7–44.6 months). The TTFT (Fig. 3a) was longer among CLL patients with pBCMA levels in the lowest three quartiles (median 44.6 months; range of pBCMA 11.57–109.39 ng/mL) when compared with CLL patients with pBCMA in the highest quartile (median 8 months; range of pBCMA 110.90–782.97 ng/mL; $p < 0.0001$).

The potential predictors with the most significant effect on TTFT were BCMA, ZAP70, log BCMA, chromosome 12 trisomy, chromosome 13 deletion, chromosome 17 deletion, IgA, WBC, disease type (aggressive vs. indolent), and β2 M, as determined by independent univariate analysis (Table 3). Further multivariate statistical analysis of these 10 variables on TTFT using a Cox proportional hazards regression model with stepwise variable selection (multivariate method) was performed using three different models to account for the sample size differences of each potential predictor variable. The first model was performed on BCMA, log BCMA, and ZAP70, independent variables available for all 168 patients. Both log BCMA ($p < 0.001$) and ZAP70 ($p = 0.003$) were significantly associated with TTFT, therefore higher BCMA

and log BCMA levels were associated with shorter TTFT. The second model analyzed BCMA, log BCMA, ZAP70, and β2 M, while the third model encompassed all 10 variables. Consistent with the first model, log BCMA and ZAP70 maintained their significance ($p < 0.0001$ and $p = 0.004$, respectively) in all three types of analysis. Due to the small number of subjects with a disease status of aggressive versus indolent (80 subjects, including 28 with aggressive disease) (Table 3) and with chromosome 13 deletion (74 subjects, including 38 subjects with chromosome 13 deletion, of whom only nine had treatment), the assessment of the effect of these variables on TTFT requires further investigation.

A threshold value for BCMA was identified using ROC curve analysis that maximizes the difference between the two groups defined by the threshold (data not shown). Any threshold values of BCMA between 40 and 140 ng/mL resulted in hazard ratios > 2.5 and p values < 0.0001 for comparing the two groups defined by the threshold. This range included both the median BCMA (56 ng/mL) and the 75th percentile for BCMA (107 ng/mL) for the 168 subjects included in the TTFT analyses. This range also included BCMA of 110 ng/mL, which was the BCMA value defined as a threshold for OS analysis. Of the 41 subjects with a pBCMA value > 110.90 ng/mL, 29 (71%) subjects required treatment, and all except one of these patients began treatment within 1 year of the pBCMA assessment. In contrast, only 67 (52%) of the 128 subjects with pBCMA ≤ 110.90 ng/mL required treatment.

Alternative pBCMA threshold possibilities were explored. Among patients with pBCMA > 250.0 ng/mL, both OS and TTFT were shorter, but only 12 patients had pBCMA values above this level (data not shown). Patients with pBCMA > 56.09 ng/mL also showed a shorter TTFT, but did not show differences in OS (Electronic Supplementary Figs. 1a, b).

3.5 Changes in pBCMA Levels Correlate Directly with Disease Response

Serial pBCMA levels were available among 21 patients who underwent treatment. Blood levels were drawn within 3 months of a clinical response assessment, which was performed according to standard IWCLL criteria. One patient did not have sufficient follow-up information and was therefore excluded from the analysis. Of the 20 remaining patients, 2 showed disease progression from their treatment and 18 showed at least a partial response (PR) to therapy (11 PRs, six complete responses (CRs) with an MRD assessment, and one CR with undetectable MRD) (Electronic Supplementary Table 1). In 19 of the 20 evaluable cases (95%), pBCMA levels were concordant with IWCLL response criteria. Specifically, pBCMA levels decreased in patients achieving PR or CR, and increased in patients with disease progression (Electronic Supplementary Fig. 2). All patients who had a full assessment by IWCLL and had multiple draws, were included. Data for the 20 evaluable patients are shown in Electronic Supplementary Fig. 2. We identified one case of discordance that occurred in a CR patient who was considered to be MRD-negative by flow cytometry (TJK0687) (Electronic Supplementary Fig. 2a). The post-treatment pBCMA level of this patient remained relatively high (114.1 ng/mL) compared with the BCMA of the other six CR patients (< 25 ng/mL) despite a CR by other standard parameters. Interestingly, this patient relapsed only 9 months after the assessment of pBCMA.

4 Discussion

BCMA is expressed at high levels on the surface of malignant cells from MM patients [12]. We have previously shown that BCMA is elevated in the serum of these patients and correlates with disease burden and response to treatment [13]. We also demonstrated that plasma and serum show identical levels of BCMA [6]. CLL is another disease in which the malignant cells show high amounts of BCMA on their surface. The purpose of this study was to determine the levels of solubilized BCMA from a large plasma bank of CLL patients using an ELISA-based assay, and to assess whether circulating levels of this TNF receptor predicted for outcomes and treatment response in this B-cell malignancy.

Our data demonstrate that pBCMA levels were significantly higher among CLL patients compared with controls. Unfavorable prognostic factors, including lack of chromosome 13 deletion, presence of del(17p), U-CLL, and ZAP70 expression > 20% positively correlated with levels of pBCMA. In addition, those CLL patients requiring treatment showed higher pBCMA levels than those who did not. Using a pBCMA threshold of 110.9 ng/mL, we were able to distinguish 25% of patients who required earlier treatment intervention and had shorter OS.

To date, numerous prognostic biomarkers have been established for patients with CLL. The German CLL Study Group developed a prognostic model based on sex, age, performance status, del(17p), del(13q), IGHV mutational status, and serum thymidine kinase level. The model was able to separate patients into low-, intermediate-, high-, and very high-risk cohorts that had significantly different prognoses. The model was validated in an external cohort [14]. Similarly, in 2016, the International CLL-IPI Working Group performed a meta-analysis that included 3472 treatment-naive patients who were stratified according to age, Rai/Binet stage, β2 M, IGHV mutational status, and del(17p)/TP53 mutational status. Outcomes of these analyses also separated patients into four statistically distinct prognostic subgroups. A plethora of additional biomarkers

not mentioned above have also been described for CLL patients, including aberrant expression of microRNA-21 and -181b, BIRC3, and NOTCH1 mutations identified by next-generation sequencing, among many others [15–17]. Ig and IgG subclass deficiencies have also recently been identified as predictive and prognostic variables [18].

Our data add to the growing body of literature regarding prognostic and predictive biomarkers in this disease. There are a number of advantages for using pBCMA as a prognostic and predictive marker for CLL. Specifically, pBCMA is obtained from patients via simple and minimally invasive blood draw. Furthermore, pBCMA levels are measured using an inexpensive and easy ELISA-based assay, performed with a rapid turnover time, in contrast to some of the more complex and expensive immunophenotypic, chromosomal, and molecular markers that have been identified to date.

While our results demonstrate strong evidence for identifying pBCMA as a potentially novel biomarker for CLL, some of the drawbacks of our analysis are that it is restricted by the retrospective nature of the data collection and a small number of patients in the various subgroups that were analyzed. Moreover, while pBCMA levels were collected on pretreatment samples in all but three cases, they were not drawn at an identical, prespecified start point for all patients. This introduces the potential for lead- and lag-time bias, particularly in the analysis of TTFT and OS. In addition, another limitation of this study is that it was conducted prior to the introduction of the recently available novel CLL therapies, such as the BTK inhibitor ibrutinib and the Bcl-2 inhibitor venetoclax, therefore CLL patients treated with these novel therapies were not part of this study (Electronic Supplementary Table 1). The long OS of CLL patients precludes such an analysis among untreated CLL patients who have received these therapies. However, a recent study shows similar findings for the ability of solubilized BCMA to predict responses for 46 ibrutinib-treated CLL patients, but did not evaluate OS or PFS [19]. Thus, the role of pBCMA as a predictive and prognostic marker warrants prospective validation among CLL patients receiving these newer treatment options.

Another advantage of the pBCMA assay is that, based on our limited data, it appears to correlate directly with disease response as assessed by the more cumbersome IWCLL criteria. In the abstract by Sun et al. involving ibrutinib-treated CLL patients, pBCMA levels predicted responses and detected disease progression much more rapidly than conventional measures [19].

The half-life of pBCMA is only 24–36 h, therefore this analyte is capable of rapidly determining changes in clinical status, as we have recently reported among a large group of MM patients starting new therapies [20]. If pBCMA is shown to be a reliable surrogate for determining changes in clinical status in a prospectively validated cohort of CLL patients, it could similarly serve as a fast, non-invasive method to rapidly assess the impact of new treatments for these patients, as well as to determine changes in their clinical status.

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Compliance with Ethical Standards

Conflict of interest Dr. James R. Berenson is a director, employee, and shareholder in OncoTracker, Inc., a company that is involved in the development of BCMA as a marker. Haiming Chen, Eric Sanchez, and Mingjie Li are shareholders in OncoTracker, Inc. Kyle A. Udd, Sean Bujarski, Eric Wirtschafter, Tanya M. Spektor, Matthew Ghermezi, Laura Z. Rassenti, Michael E. David, Jason D. Nosrati, Ashkon A. Rahbari, James Wang, Suzie Vardanyan[1], Nika M. Harutyunyan, Julia Linesch and Thomas J. Kipps have no conflicts of interest to declare.

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