Review Article
Prognostic significance of CD38 for chronic lymphocytic leukemia: a meta-analysis

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Abstract: *Background and objectives:* Numerous studies have focused on the role of CD38 in chronic lymphocytic leukemia (CLL), but evidence regarding the prognostic value of CD38 with respect to overall survival (OS) in CLL remains controversial. The aim of this study is to gain a better insight about the direct relationship between CD38 expression and patients' survival status. *Materials and methods:* Relevant publications addressing the association between CD38 expression and OS in CLL patients were selected from PubMed, Embase, Web of Sience and the Cochrane library. Studies were pooled and summary hazard ratios (HR) were calculated. Sensitivity analyses and publication bias were also conducted. Statistical analysis was performed by STATA 12.0 software. *Results:* 18 studies met the inclusion criteria and were included in our meta-analysis. Combined HRs suggested that CD38 high expression had an unfavorable impact on CLL patients' survival (HR=1.92, 95% CI=1.41-2.6, *P*=0.000). Subgroup analyses according to the studies categorized by histological type, cutoff scores and follow-up period were also conducted, and all the above analyses supported the stability of the prognostic role of CD38. *Conclusion:* Our findings suggest that CD38 overexpression in CLL patients might be a poor prognostic factor. However, further large scale studies are needed to confirm these findings.

Keywords: Chronic lymphocytic leukemia, CD38, prognosis, meta-analysis

Introduction

Chronic lymphocytic leukemia (CLL) is the most prevalent adult leukemia, and despite the introduction of new therapies, remains a serious clinical challenge due to its heterogeneity. While some patients never require treatment and have a survival of several decades, others suffer from a much more aggressive, rapidly evolving form. Identification of these subgroups and insight into the prognosis for each individual patient will be crucial to determine individualized treatment strategies. With regard to the prognosis of CLL patients, the clinical staging systems proposed by Rai [1] and Binet [2], based on leukemia cell burden, are useful for assessing prognosis. However, the staging systems lack the ability to distinguish prospectively patients with early stage B-CLL that will rapidly progress to aggressive disease from patients who will remain in early stage for a long time [3].

In a continual effort to identify the patients with poor prognosis and to facilitate the clinical management of CLL, several prognostic markers have been identified during the last two decades [4]. CD38 is a diphosphate-ribose hydrolase that acts as a complex ecto-enzyme with adenosine diphosphate-ribosyl cyclase and cyclic adenosine [5]. The elevated CD38 expression is associated with inferior clinical outcomes of CLL [6]. Many laboratories now analyze CD38 expression as part of their routine diagnostic flow cytometric analysis of CLL patients. However, the clinical application of CD38 data has been clouded by a perception that major uncertainties remain in the literature concerning its prognostic value [7-9]. Some studies have reported that CD38 expression changed over time or that only relatively high levels of CD38 positivity were prognostic [8, 9], while other studies have shown that CD38 expression was stable over time and that the presence of even a small percentage of CD38-positive cells was associated with significantly poorer clinical outcomes [10, 11]. As meta-analysis is an essential tool for summarizing evidences accurately and reliably,

we conducted this meta-analysis to gain a better insight on the direct relationship between CD38 expression and patients' survival statuses, which, to the best of our knowledge, has not been previously performed.

Materials and methods

Literature search

Studies were identified via an electronic search of PubMed, Embase, Web of Sience and the Cochrane library (updated to January 31, 2016) using the following key words: chronic lymphocytic leukemia, CLL, CD38, prognostic, prognosis and survival. The language of published papers was restricted to English. Reference lists from retrieved documents were also searched.

Selection criteria

Studies had to meet the following criteria: (a) full text publication compared the OS between different expressions of CD38 in CLL; (b) measured CD38 expression in CLL with flow cytometry or fluorescence in situ hybridization (FISH); (c) hazard ratios (HRs) and their 95% confidence intervals (95% CIs) for OS according to CD38 status either had to be reported or could be computed from the data presented; (d) to avoid duplicated publications, the most recent report or the most informative one was included. Reviews, letters to the editor, conference abstracts or comments were excluded due to insufficient data. We also used a manual reference search for relevant articles, including original articles and reviews, to identify additional studies.

Data extraction

Data were extracted independently by two investigators (H. C and L. Z. G). The following data were collected from each article: first author, year of publication, country, number of patients, CD38 high/low expression cases, detecting methodology, cut-off, follow-up period, histological type and HR with 95% CI. Some published researches didn't provide HR and 95% CI directly. In that case, two reviewers (H. C and J. J) independently digitized and extracted the data through the Kaplan-Meier curves by using GetData Graph Digitizer 2.24 (http://getdata-graph-digitizer.com) and then reconstructed the HR and its variance (GraphPad Software, Inc, La Jolla, CA, USA). Disagreements between the reviewers were resolved by consensus.

Statistical analysis

The HR with its variance estimates (95% CI) was abstracted or calculated to quantitatively evaluate the association between CD38 expression and CLL prognosis. High expression of CD38 indicated poor prognosis in patients with CLL if HR>1 with the 95% CI did not overlap 1. To assess heterogeneity among the studies, Cochrane's Q test (Chi-squared test) and inconsistency (I^2) statistics were used. Where there is no heterogeneity (P>0.1; I^2<50%), the fixed effects model analysis was made, otherwise, the random effect model should be used. The possibility of publication bias was assessed using the Begg test and visual inspection of a funnel plot. We also performed the Duvaland Tweedie nonparametric "trim and fill" procedure to further assess the possible effect of publication bias in our meta-analysis [12]. This method considers the possibility of hypothetical "missing" studies that might exist, imputes their HRs, and recalculates a pooled HR that incorporates the hypothetical missing studies as though they actually existed. For all analyses, a two-sided *P* value less than 0.05 was considered as statistically significant. All analyses were performed using STATA software version 12.0 (Stata Corporation, College Station, TX).

Results

Selection and characteristics of studies

After the initial literature search, a total of 526 potentially relevant citations were retrieved. 72 articles were excluded after the first screening since they were reviews, abstracts, letters to editor, animal/in vitro studies. 426 were excluded based on abstracts or titles, which were duplications or studies irrelevant to the current topic, leaving 28 studies for detailed evaluation. After carefully reading the full text articles, 10 were excluded for the meta-analysis for lacking sufficient survival data. As a result, 18 eligible studies including 2975 CLL cases were included in this meta-analysis [6, 13-29]. The main characteristics

Table 1. Characteristics and results of eligible prognostic studies evaluating CD38

First author	Year	Country	N. of Patient	Method	Histological type	Duration of follow-up	N. of Positive	Cutoff value	OS		
									HR Estimate	HR	95% CI
Assem, M	2009	Egypt	50	Flowcytometry	B-CLL	120	9	30%	Sur. curve	2.96	0.33-26.33
Boonstra, J	2006	Netherlands	78	Flowcytometry	B-CLL	60	35	30%	Sur. curve	1.64	0.54-5
Chang, C	2003	USA	24	Flowcytometry	B-CLL	203	11	30%	Sur. curve	1.56	0.44-5.58
Chevallier, P	2002	France	122	FISH	B-CLL	157	32	30%	Sur. curve	2.38	1.30-4.35
Damle, R	1999	USA	36	Flowcytometry	B-CLL	240	17	30%	Sur. curve	1511.63	Not applicable
D'Arena, G	2001	Italy	61	Flowcytometry	B-CLL	180	22	30%	Sur. curve	1.01	0-283.35
Del Poeta, G	2001	Italy	168	Flowcytometry	B-CLL	132	50	30%	HR 95% CI	6.86	2.19-21.39
Domingo-Domenech, E	2002	Spain	155	Flowcytometry	B-CLL	129	30	30%	HR 95% CI	2.7	1.48-4.91
Dong, H	2014	China	194	Flowcytometry	CLL	82	74	30%	HR 95% CI	2.24	0.80-6.23
Hamblin, T.	2002	UK	145	Flowcytometry	B-CLL	428	61	30%	HR 95% CI	1.8	0.90-3.30
Hewamana, S	2009	UK	131	Flowcytometry	CLL	24	57	20%	HR 95% CI	2.8	0.96-9.82
Hock, B	2010	New Zealand	106	Flowcytometry	CLL	120	14	5%	Sur. curve	5.63	1.12-28.33
Hsi, E	2003	USA	131	Flowcytometry	CLL	103	92	100 (antibodies bound/cell)	Sur. curve	1	0.67-1.49
Ibrahim, S	2003	USA	120	Flowcytometry	B-CLL	NA	63	20%	Sur. curve	1.27	0.65-2.47
Kontos, C	2016	Germany	47	Flowcytometry	CLL	120	4	30%	HR 95% CI	1.02	0.98-1.06
Pepper, C	2012	UK	1035	Flowcytometry	CLL	96	343	20%	HR 95% CI	1.7	1.40-2.40
Pratt, G	2016	UK	225	Flowcytometry	CLL	48	170	7%	HR 95% CI	2.65	1.86-3.79
Xu, W	2009	China	147	Flowcytometry	B-CLL	184	45	30%	Sur. curve	2.67	0.16-44.35

N, number; CLL, chronic lymphocytic leukemia; B-CLL, B cell chronic lymphocytic leukemia; NA, no available; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

Table 2. Summarized HRs of subgroup analyses for CD38 on CLL survival

	N. of studies	Effect model	HR (95% CI)	Heterogeneity test	
				I²	P-value
Overall	16	Random	1.92 (1.41-2.6)	81.6%	<0.001
Histological type					
B cell CLL	9	Fixed	2.13 (1.61-2.82)	0.0%	0.44
Total CLL	7	Random	1.7 (1.13-2.54)	87.8%	<0.001
Cutoff value					
30%	10	Random	2.06 (1.3-3.28)	74.7%	<0.001
20%	3	Fixed	1.67 (1.31-2.13)	0.0%	0.49
Duration of follow-up					
<120 months	6	Random	2.28 (1.34-3.89)	63.4%	0.018
≥120 months	9	Random	1.78 (1.24-2.57)	78.4%	<0.001

N, number; HR, hazard ratio; 95% CI, 95% confidence interval.

of studies enrolled are summarized in **Table 1**. Briefly, sample sizes ranged from 24 to 1035. The mean follow-up period for the studies was 142.7 months, ranging from 24 to 428 months. Among the 18 studies, 7 focused on all types of CLL, while 11 reported B cell CLL only. All the studies investigated CD38 by flow cytometry, except one, which used FISH [16]. 6 of the 18 included studies identified CD38 high expression as a significant poor prognostic factor, whereas the other 12 studies reported that CD38 high expression had no significant association with CLL prognosis.

Meta-analysis results

The main results of this meta-analysis are summarized in **Table 2**. 16 studies had sufficient data for estimating HR and 95% CI, including 2878 patients. As shown in **Figure 1**, using random-effect model, CD38 high expression was a prognostic factor for poor survival in CLL patients (HR=1.92, 95% CI=1.41-2.6, P<0.001). Our results indicated that CD38 was an independent prognostic factor in patients with CLL.

We also conducted subgroup analyses based on histological type, cutoff scores and follow-up period. When grouped according to the histological type, the combined HR was 2.13 (95% CI: 1.61-2.82, P<0.001) for B cell CLL. Stratified by the positive threshold for CD38 expression, the combined HRs of 20% and 30% cutoff value were 1.67 (95% CI: 1.31-2.13, P<0.001)

and 2.06 (95% CI: 1.3-3.28, P=0.002), separately. Finally, in the subgroup analysis based on the follow-up period, the combined HR was 1.78 (95% CI: 1.24-2.57, P=0.002) for studies of shorter follow-up period (<120 months) and 2.28 (95% CI: 1.34-3.89, P=0.002) for those of longer follow-up period (≥120 months).

Sensitivity analyses and publication bias

Visual inspection of the Begg funnel plot revealed asymmetry (**Figure 2**). Although the Begg test was not statisitically significant (z=0.68; P=0.499), this raises the possibility of publication bias. Thus, we undertook a sensitivity analysis using the trim and fill method, which conservatively imputes hypothetical negative unpublished studies to mirror the positive studies that cause funnel plot asymmetry [30]. The pooled analysis incorporating the hypothetical studies continued to show a statistically significant association between CD38 and poor survival outcome of CLL (HR=1.69, 95% CI: 1.27-2.23, P<0.001).

Discussion

CD38 is a transmembrane glycoprotein that is widely expressed on a range of hematopoietic and non-hematopoietic cell types. There has been a considerable amount of interest and controversy in the perspective that CD38 expression in CLL is associated with short survival. Therefore, we performed the present meta-analysis to summarize all of the available researches on the impact of the CD38 on the prognosis of CLL, which to the best of our knowledge, has not been performed before.

By pooling all the studies which compared the survival outcomes of CLL patients according to expression status of CD38, our meta-analysis showed promising prognostic value of CD38 detected in tumor samples for OS of CLL. Patients with elevated CD38 expression had about 1.92 fold higher risk of poor progno-
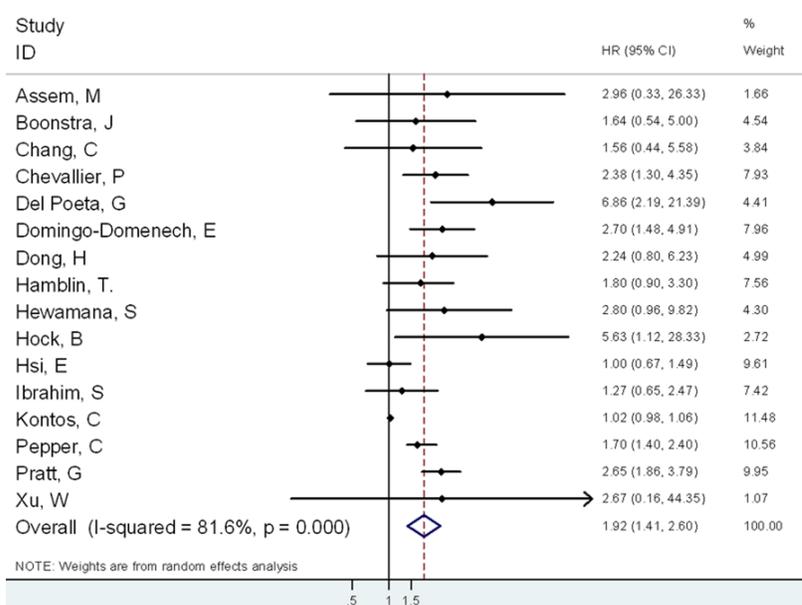
Figure 1. The association between CD38 overexpression and overall survival of CLL stratified by HR estimation. The summary HR and 95% CIs were shown (according to the random effect estimations).
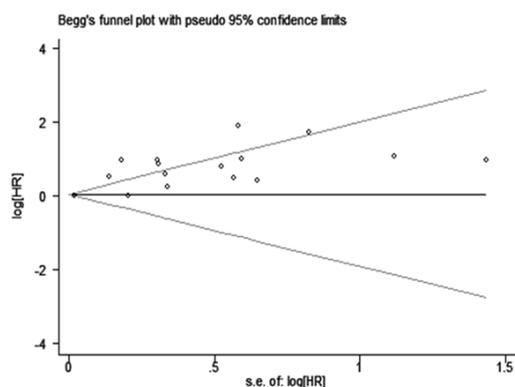

Figure 2. Funnel plots of Begg's to detect publication bias on overall estimate.

sis, compared with those without high CD38 expression. Significant heterogeneity was found in this meta-analysis (I^2=81.6%, P<0.001) for OS. Studies may have differed with regard to the baseline characteristics of the patients included (age, histological type, differentiation or disease stage), the duration of follow-up and adjustments for other cofactors, adding the heterogeneity between studies. Therefore, we performed the analysis using random-effects model which considered the between-study heterogeneity. Moreover, we attempted to perform a stratified subgroup analysis to reduce the heterogeneity. When histological type of CLL was taken into consideration, the pooled analysis results for B-CLL were similar to the overall results with increased HR, further proving the prognostic significance of CD-38 in B-CLL. Individual study selected various cutoff points, so we also stratified studies according to different cutoffs to increase the homogeneity, which would be meaningful in clinical application. A more dismal impact on survival was observed by using 30% cutoff value, indicating that a higher level of cutoff score, 30% for example, would be more helpful leading to a differential conclusion. Finally, we found that the association was more significant for studies of longer follow-up period (≥120 months), implying that CD38 might be more valuable on predicting long-term outcome of CLL.

CLL prognostic markers are often only obtained at diagnosis, and these single measurements do not reflect the changing nature of CLL over the disease trajectory. It is worth mentioning that CD38 expression often varies over time in CLL and repeated measurement of CD38 expression on peripheral blood CLL lymphocytes by flow cytometry appears to refine the initial information [31]. For the same reason, combinations of CD38 with other biomarkers are recommended to identify more aggressive CLL patient subgroups and to guide treatment decisions. It has been reported that patients with combined expression of ZAP-70 and CD38 had significantly shorter time to disease progression (TDP) and overall survival [13]. In patients with low CD38 expression, a subgroup with low CD31 expression had significantly longer survival compared with the survival for the entire group [25]. Further multi-factor analyses about the prognostic role of CD38 with other established prognostic factors for CLL patients are still needed.

The results should be interpreted cautiously since some limitations exist in present meta-analysis. First of all, the pooled HRs calculated in our study may be overestimated due to publication and reporting bias, while unpublished papers, abstracts and letters to the editor were not taken into account. Although the trim and fill sensitivity analysis did not change the general results, the possibility of publication bias is not fully excluded by this method. Second, the quality of the individual study was not always optimal, as shown by the general lack of information on blinding and recruiting of consecutive patients for all studies. Third, method used to extrapolate the HRs may bring potential bias. The HRs were extrapolated from the survival curves and recalculated if they were not reported in the study, which, in fact, seem to be less reliable than extracting HRs from published statistics because this strategy did not completely eliminate inaccuracy. Besides, Damle R and D'Arena G's studies [6, 17] did not provide sufficient OS data for meta-analysis. The missing information showed "positive" association of CD38 with CLL survival that might increase the significance of CD38 as a predictor of OS in CLL. Finally, CLL patients had received different treatments, which may influence the survival of CLL. Nevertheless, the majority of published studies lacked required data regarding patient treatment and all these sources of variability could produce additional inconsistencies and cause potential selection bias [32]. Therefore, appropriate multivariate analysis should be performed to examine whether CD38 is a prognostic factor, independently of as known clinical factors, such as age, sex, stages, and treatment.

In summary, despite of the above limitations, the present meta-analysis demonstrates a significant association between CD38 high expression and poor overall survival in patients with CLL. However, these findings had to be interpreted with caution when used in clinical practice, since the reported associations were diverse. Larger well-designed prospective cohort studies are still needed to further validate the prognostic role of CD38 in patients with CLL.

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Disclosure of conflict of interest

None.

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References

[1] Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, Pasternack BS. Clinical staging of chronic lymphocytic leukemia. Blood 1975; 46: 219-34.

[2] Binet JL, Auquier A, Dighiero G, Chastang C, Piguet H, Goasguen J, Vaugier G, Potron G, Colona P, Oberling F, Thomas M, Tchernia G, Jacquillat C, Boivin P, Lesty C, Duault MT, Monconduit M, Belabbes S, Gremy F. A new prognostic classification of chronic lymphocytic leukemia derived from a multivariate survival analysis. Cancer 1981; 48: 198-206.

[3] Keating MJ, Chiorazzi N, Messmer B, Damle RN, Allen SL, Rai KR, Ferrarini M, Kipps TJ. Biology and treatment of chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program 2003; 153-75.

[4] Shanafelt TD, Geyer SM, Kay NE. Prognosis at diagnosis: integrating molecular biologic insights into clinical practice for patients with CLL. Blood 2004; 103: 1202-10.

[5] Deaglio S, Mallone R, Baj G, Donati D, Giraudo G, Corno F, Bruzzone S, Geuna M, Ausiello C, Malavasi F. Human CD38 and its ligand CD31 define a unique lamina propria T lymphocyte signaling pathway. FASEB J 2001; 15: 580-2.

[6] Damle RN, Wasil T, Fais F, Ghiotto F, Valetto A, Allen SL, Buchbinder A, Budman D, Dittmar K, Kolitz J, Lichtman SM, Schulman P, Vinciguerra VP, Rai KR, Ferrarini M, Chiorazzi N. Ig V gene mutation status and CD38 expression as novel prognostic indicators in chronic lymphocytic leukemia. Blood 1999; 94: 1840-7.

[7] Van Bockstaele F, Verhasselt B, Philippe J. Prognostic markers in chronic lymphocytic leukemia: a comprehensive review. Blood Rev 2009; 23: 25-47.

[8] Rassenti LZ, Jain S, Keating MJ, Wierda WG, Grever MR, Byrd JC, Kay NE, Brown JR, Gribben JG, Neuberg DS, He F, Greaves AW, Rai KR, Kipps TJ. Relative value of ZAP-70, CD38, and immunoglobulin mutation status in predicting aggressive disease in chronic lymphocytic leukemia. Blood 2008; 112: 1923-30.

[9] Krober A, Seiler T, Benner A, Bullinger L, Bruckle E, Lichter P, Döhner H, Stilgenbauer S. V(H) mutation status, CD38 expression level, ge-

nomic aberrations, and survival in chronic lymphocytic leukemia. Blood 2002; 100: 1410-6.

[10] Gentile M, Mauro FR, Calabrese E, De Propris MS, Giammartini E, Mancini F, Milani ML, Guarini A, Foà R. The prognostic value of CD38 expression in chronic lymphocytic leukaemia patients studied prospectively at diagnosis: a single institute experience. Br J Haematol 2005; 130: 549-57.

[11] Boonstra JG, von't Veer MB, Gratama JW. The use of CD38 expression by monoclonal B lymphocytes as a prognostic factor in B-cell chronic lymphocytic leukemia. J Biol Reg Homeos Ag 2004; 18: 340-6.

[12] Higgins JP, Thompson SG. Quantifying heterogeneity in a meta-analysis. Stat Med 2002; 21: 1539-58.

[13] Assem M, Abdel Hamid T, Kohla S, Arsanyos S. The Prognostic Significance of Combined Expression of ZAP-70 and CD38 in Chronic Lymphocytic Leukemia. J Egypt Natl Canc Inst 2009; 21: 287-97.

[14] Boonstra JG, van Lom K, Langerak AW, Graveland WJ, Valk PJ, Kraan J, van't Veer MB, Gratama JW. CD38 as a prognostic factor in B cell chronic lymphocytic leukaemia (B-CLL): comparison of three approaches to analyze its expression. Cytometry B Clin Cytom 2006; 70: 136-41.

[15] Chang CC, Liu CZ, Cleveland RP. Relative importance of CD38 expression over myeloid-associated markers expression in predicting the clinical course of B-CLL patients. Leuk Lymphoma 2003; 44: 977-82.

[16] Chevallier P, Penther D, Avet-Loiseau H, Robillard N, Ifrah N, Mahe B, Hamidou M, Maisonneuve H, Moreau P, Jardel H, Harousseau JL, Bataille R, Garand R. CD38 expression and secondary 17p deletion are important prognostic factors in chronic lymphocytic leukaemia. Br J Haematol 2002; 116: 142-50.

[17] D'Arena G, Musto P, Cascavilla N, Dell'Olio M, Di Renzo N, Perla G, Savino L, Carotenuto M. CD38 expression correlates with adverse biological features and predicts poor clinical outcome in B-cell chronic lymphocytic leukemia. Leuk Lymphoma 2001; 42: 109-14.

[18] Del Poeta G, Maurillo L, Venditti A, Buccisano F, Epiceno AM, Capelli G, Tamburini A, Suppo G, Battaglia A, Del Principe MI, Del Moro B, Masi M, Amadori S. Clinical significance of CD38 expression in chronic lymphocytic leukemia. Blood 2001; 98: 2633-9.

[19] Domingo-Domenech E, Domingo-Claros A, Gonzalez-Barca E, Beneitez D, Alonso E, Romagosa V, De Sanjos S, Petit J, Grañena A, Fernández de Sevilla A. CD38 expression in B-cell chronic lymphocytic leukemia: association with clinical presentation and outcome in 155 patients. Haematologica 2002; 87: 1021-7.

[20] Dong HJ, Fang C, Wang L, Fan L, Xu J, Wu JZ, Lu TX, Li JY, Xu W. TP53 Pro72 allele potentially increases the poor prognostic significance of TP53 mutation in chronic lymphocytic leukemia. Med Oncol 2014; 31: 908.

[21] Hamblin TJ, Orchard JA, Ibbotson RE, Davis Z, Thomas PW, Stevenson FK, Oscier DG. CD38 expression and immunoglobulin variable region mutations are independent prognostic variables in chronic lymphocytic leukemia, but CD38 expression may vary during the course of the disease. Blood 2002; 99: 1023-9.

[22] Hewamana S, Lin TT, Rowntree C, Karunanithi K, Pratt G, Hills R, Fegan C, Brennan P, Pepper C. Rel a is an independent biomarker of clinical outcome in chronic lymphocytic leukemia. J Clin Oncol 2009; 27: 763-9.

[23] Hock BD, McKenzie JL, McArthur L, Tansley S, Taylor KG, Fernyhough LJ. CD38 as a prognostic marker in chronic lymphocytic leukaemia at a single New Zealand centre: patient survival in comparison to age- and sex-matched population data. Intern Med J 2010; 40: 842-9.

[24] Hsi ED, Kopecky KJ, Appelbaum FR, Boldt D, Frey T, Loftus M, Hussein MA. Prognostic significance of CD38 and CD20 expression as assessed by quantitative flow cytometry in chronic lymphocytic leukaemia. Br J Haematol 2003; 120: 1017-25.

[25] Ibrahim S, Jilani I, O'Brien S, Rogers A, Manshouri T, Giles F, Faderl S, Thomas D, Kantarjian H, Keating M, Albitar M. Clinical relevance of the expression of the CD31 ligand for CD38 in patients with B-cell chronic lymphocytic leukemia. Cancer 2003; 97: 1914-9.

[26] Kontos CK, Adamopoulos PG, Papageorgiou SG, Pappa V, Scorilas A. mRNA overexpression of kallikrein-related peptidase 14 (KLK14) is an independent predictor of poor overall survival in chronic lymphocytic leukemia patients. Clin Chem Lab Med 2016; 54: 315-24.

[27] Pepper C, Majid A, Lin TT, Hewamana S, Pratt G, Walewska R, Gesk S, Siebert R, Wagner S, Kennedy B, Miall F, Davis ZA, Tracy I, Gardiner AC, Brennan P, Hills RK, Dyer MJ, Oscier D, Fegan C. Defining the prognosis of early stage chronic lymphocytic leukaemia patients. Br J Haematol 2012; 156: 499-507.

[28] Pratt G, Thomas P, Marden N, Alexander D, Davis Z, Hussey D, Parry H, Harding S, Catovsky D, Begley J, Oscier D. Evaluation of serum markers in the LRF CLL4 trial: beta2m but not serum free light chains, is an independent marker of overall survival. Leuk Lymphoma 2016; 57: 2342-50.

[29] Xu W, Li JY, Wu YJ, Yu H, Shen QD, Tian T, Li L, Qiu HX. CD38 as a prognostic factor in Chinese patients with chronic lymphocytic leukaemia. Leuk Res 2009; 33: 237-43.

[30] Willi C, Bodenmann P, Ghali WA, Faris PD, Cornuz J. Active smoking and the risk of type 2 diabetes: a systematic review and meta-analysis. JAMA 2007; 298: 2654-64.

[31] Nipp RD, Volkheimer AD, Davis ED, Chen Y, Weinberg JB, Friedman DR. CD38 variation as a prognostic factor in chronic lymphocytic leukemia. Leuk Lymphoma 2014; 55: 191-4.

[32] Lou-Qian Z, Rong Y, Ming L, Xin Y, Feng J, Lin X. The prognostic value of epigenetic silencing of p16 gene in NSCLC patients: a systematic review and meta-analysis. PLoS One 2013; 8: e54970.