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Letter to Editor

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Can BCAT1 expression level help predict disease progression in chronic lymphocytic leukaemia

Steven J Coles[1]* and Alex J Wadley[2]

Institute of Science & the Environment, University of Worcester, UK

***Corresponding author**

Steven J Coles, Institute of Science and the Environment, University of Worcester, St Johns Campus, Henwick Grove, Worcester, UK, WR2 6AJ, Tel: 01905 542577; Email: s.coles@worc.ac.uk

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Letter to the Editor

Chronic lymphocytic leukaemia (CLL) is the most common blood cancer in the UK, with an incidence of >3500 newly diagnosed cases per year resulting in >1000 deaths. Disease prevalence increases with age, where the majority of patients are >65 years old [1]. CLL is a largely indolent disease and is routinely staged according to the Binet system as follows; stage A (involving 0-2 lymphoid sites), stage B (involving 2-5 lymphoid cites) and stage C (platelets < $1x10^{11}$/L or haemoglobin <10g/dL), the latter stage reflecting loss of bone marrow function [2]. Whilst for some stage A patients, the disease may remain stable for many decades (median life expectancy of 13 years), for others the disease progresses more rapidly [3]. This observation likely reflects the genetic and molecular heterogeneity of CLL. As such there are several prognostic risk factors used to stratify newly diagnosed patients, which include; trisomy-12, 13q/17p/11q23 deletion, advanced stage, males>females, unmutated VH Ig genes, raised lactate dehydrogenase activity and expression of Zap70 and CD38 [4]. CLL is traditionally treated with a combination of chemotherapeutic reagents, namely, Fludarabine/Cyclophosphamide/Rituximab (FCR), however treatment remains challenging within the elderly population [1]. Lately the Brutan's tyrosine kinase inhibitor, Ibrutinib, has shown great promise for the treatment of CLL [5]. However, exceptions are identified as well as treatment resistance prompting further research into CLL treatment strategies [6].

Recently upregulation of the cytosolic isoform of branched-chain amino transferase (BCAT1) has been implicated in the disease progression of chronic myeloid leukaemia (CML) [7]. Hattori demonstrated that BCAT1 expression was required for maintenance of CML cells in an undifferentiated state, an important hallmark of cancer [8]. This was achieved through metabolic reprogramming resulting in alterations in cellular α-ketoglutarate. Moreover, the authors showed that treating myeloid leukaemia cells with Gabapentin (BCAT1 inhibitor) [9] reduced cell proliferation in colony assays. Taken together, these findings suggest that BCAT1 has an important role in CML leukaemogenesis, which offers therapeutic potential for BCAT1 inhibitors, such as gabapentin to form part of new treatment strategies in CML. However, the implications of BCAT1 expression in CLL are currently unknown. Hence we investigated whether BCAT1 was over-expressed in CLL, and if so, was there any evidence to suggest that BCAT1 may be implicated in CLL disease progression.

To this end we analysed CLL patient microarray data available from the NCBI GEO (https://www.ncbi.nlm.nih.gov/geo/) for the expression of BCAT1 in peripheral blood or bone marrow mononuclear cells at point of diagnosis. The data set analysed herein was GEO accession GSE22762 (GPL570 platform), which consisted of 107 newly diagnosed censored CLL patients. The patient characteristics for this data set as stated in the original study published in [10] had a median age of 63 (33-85) years, of which 66.4% were male, 51.5% were stage A, 24.8% were stage B and 23.8% were stage C according to the Binet classification system. Initially we stratified the CLL patients according to time to first treatment as follows; <100 days (poor), 100–1,000 days (medium) and >1,000 days (good), and searched for the top 250 most dysregulated genes using the GEO2R online analytical tool. Of the 44,754 probes sets available on the GPL570 platform, probe set 226517_at which corresponds to BCAT1 features in the top 100 most dysregulated genes (P=0.0002) according to our risk stratification. A complete list of the top 100 genes generated by this analysis is summarised in Supplemental (Table S1). We next evaluated whether there was any significant difference in BCAT1 relative expression between our 3 risk groups. The data presented in Figure 1 illustrates a significantly higher relative BCAT1 expression in patients where time to first treatment was <100 days compared with patients where time to first treatment was >1,000 days (P<0.001). To corroborate this finding, the data was imported into Gene Spring (Agilent Technologies) for normalisation and background correction using the GC-RMA algorithm. The Gene Spring normalised analysis agreed with the relative expression analysis and displayed significantly higher BCAT1 expressing in the <100 days group compared with the >1,000 days group (P<0.001). Since expression of CD38 and Zap70 have major impact on disease progression in CLL, we wanted to

verify whether there was an association between CD38/Zap70 expression and BCAT1 using linear regression analysis. The data show no correlation between Zap70 expression and BCAT1 (R^2=0.028, P=0.087). However, a very weak positive correlation was detected for CD38 and BCAT1 expression (R^2=0.167, P<0.001). This suggests that BCAT1 expression is independent to Zap70 and CD38.

Supplemental Table S1: Top 100 dysregulated genes out of 44,754 probe sets from 107 CLL patients stratified according to time to first treatment. Data analysed from NCBI GEO accession GSE22762; GPL570 platform. Data originally published in [10]. Probe set 226517_at corresponding to BCAT1 is highlighted in red.

ID	P Value	Gene Symbol	ID	P Value	Gene Symbol	ID	P Value	Gene Symbol
"209772_s_at"	0.00000039	CD24	"230502_s_at"	0.0000577	no entry	"243931_at"	0.000126	no entry
"223649_s_at"	6.04E-07	SLC25A39	"210130_s_at"	0.0000615	TM7SF2	"209352_s_at"	0.000126	SIN3B
"223696_at"	0.00000118	ARSD	"229854_at"	0.0000632	OBSCN	"202600_s_at"	0.000131	NRIP1
"218903_s_at"	0.00000229	NABP2	"208243_s_at"	0.0000635	CNR1	"221725_at"	0.000132	WASF2
"210830_s_at"	0.00000474	PON2	"240493_at"	0.0000654	no entry	"208906_at"	0.000133	HNRNPUL 2-BSCL2///BSCL2
"243008_at"	0.00000487	RHEB	"208779_x_at"	0.0000656	MIR4640/// DDR1	"219065_s_at"	0.000138	DPY30///MEMO1
"229026_at"	0.00000492	CDC42SE2	"213757_at"	0.0000669	no entry	"236940_at"	0.00014	no entry
"225280_x_at"	0.00000508	ARSD	"229002_at"	0.0000686	FAM69B	"210344_at"	0.000143	OSBPL7
"223695_s_at"	0.00000592	ARSD	"202387_at"	0.0000704	BAG1	"203289_s_at"	0.000145	NPRL3
"211475_s_at"	0.00000597	BAG1	"238071_at"	0.0000713	LCN10	"218075_at"	0.000152	AAAS
"220172_at"	0.00000707	DCAF17	"239792_at"	0.0000742	UBL7-AS1	"201911_s_at"	0.000154	FARP1
"236917_at"	0.00000773	LRRC34	"200999_s_at"	0.0000773	CKAP4	"208748_s_at"	0.000157	FLOT1
"33646_g_at"	0.00000773	GM2A	"214720_x_at"	0.0000784	SEPT_10	"220068_at"	0.000157	VPREB3
"230131_x_at"	0.00000852	ARSD	"207793_s_at"	0.0000789	EPB41	"216807_at"	0.00016	CFAP74
"205554_s_at"	0.00000864	DNASE1L3	"206181_at"	0.000079	SLAMF1	"227547_at"	0.000166	no entry
"223732_at"	0.00000876	SLC23A1	"203796_s_at"	0.0000803	BCL7A	"209217_s_at"	0.000167	WDR45
"209151_x_at"	0.00000932	TCF3	"48659_at"	0.0000805	MIIP	"204454_at"	0.000178	LDOC1
"215891_s_at"	0.0000159	GM2A	"241520_x_at"	0.0000837	no entry	"211630_s_at"	0.000188	GSS
"210153_s_at"	0.0000169	ME2	"228588_s_at"	0.0000854	UBE2B	"210010_s_at"	0.000189	SLC25A1
"226364_at"	0.0000196	HIP1	"227820_at"	0.0000907	TBC1D25	"212563_at"	0.000193	MIR7112
"213048_s_at"	0.0000212	SET	"230618_s_at"	0.0000948	no entry	"200977_s_at"	0.000194	TAX1BP1
"235162_at"	0.0000244	MDM4	"220387_s_at"	0.0000968	HHLA3	"223565_at"	0.000198	MZB1
"213436_at"	0.0000267	CNR1	"217700_at"	0.000106	CNPY4	"201876_at"	0.000198	PON2

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"204262_s_at"	0.00003	PSEN2	"202599_s_at"	0.000112	NRIP1	"212698_s_at"	0.000199	SEPT_10
"225286_at"	0.0000321	ARSD	"230967_s_at"	0.000113	USP7	"210304_at"	0.000201	PDE6B
"205590_at"	0.0000392	RASGRP1	"238888_at"	0.000115	no entry	"226517_at"	0.000206	BCAT1
"234972_at"	0.000041	ARL16	"210401_at"	0.000115	P2RX1	"232792_at"	0.000207	TRIM69
"214182_at"	0.0000455	ARF6	"221286_s_at"	0.000116	MZB1	"225759_x_at"	0.000209	CLMN
"219207_at"	0.0000467	EDC3	"203795_s_at"	0.000116	BCL7A	"235006_at"	0.000217	CDKN2AI
"215749_s_at"	0.0000503	GORASP1	"229699_at"	0.00012	LOC1001	"201644_at"	0.000226	tissue
"206413_s_at"	0.000051	TCL1B	"214521_at"	0.000121	HES2	"37652_at"	0.00023	CABIN1
"202104_s_at"	0.000051	LOC101930112/// SPG7	"227299_at"	0.000123	CCNI	"224391_s_at"	0.000232	SIAE
"210154_at"	0.0000526	ME2	"210364_at"	0.000125	SCN2B	"217833_at"	0.000243	SYNCRIP

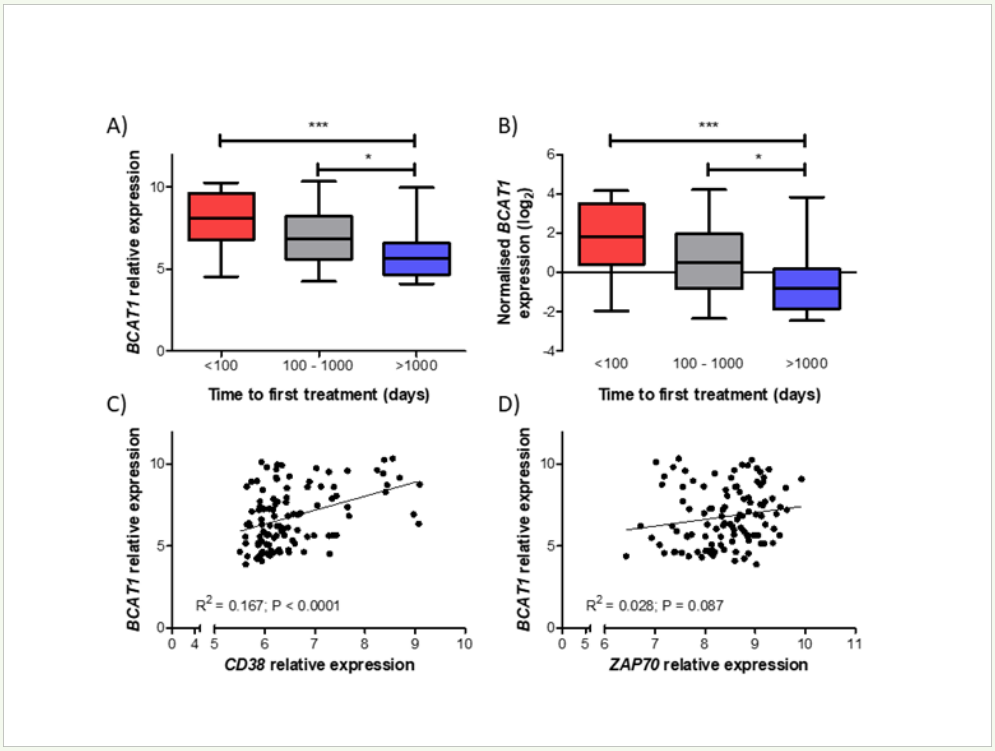

Figure 1: BCAT1 expression levels stratified according to time to first treatment. Microarray data from GEO accession GSE22762; GPL570 platform was stratified according to time to first treatment; <100 days (n=18), 100-1000 days (n=37) and >1000 days (n=40) and analysed for BCAT1 expression (226517_at probe set). The top 100 most dysregulated genes according to this stratification are summarised in Supplemental Table S1.

a) Relative BCAT1 expression level at each time point analysed using NCBI GEO2R online tool.

b) Normalised BCAT1 expression analysed in GeneSpring (Agilent Technologies) with CG-RMA background correction.

c) Linear regression analysis comparing BCAT1 relative expression with CD38 relative expression (205692_s_at probe set).

d) Linear regression analysis comparing BCAT1 relative expression with ZAP70 relative expression (214032_at probe set). n=95 for each plot. *P<0.05, ***P<0.001, data analysed by one way-ANOVA with Tukey's Multiple Comparison Test.

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In adults BCAT1 expression is restricted to the central nervous system, where it has a pivotal role in glutamate metabolism, however the structural homologue, mitochondrial branched-chain amino transferase (BCAT2) is ubiquitously expressed [11]. This suggests a developmental role for BCAT1, and 'housekeeping' role for BCAT2. Thus to further investigate BCAT1 expression in CLL, we compared expression levels between haematopoietic stem cells (HSC), CLL patient cells and healthy donor bone marrow (BM) for both BCAT1 and BCAT2 expression. Microarray data sets analysed were obtained from the NCBI GEO and included GEO accession GSE13496 (GPL96 platform) for HSC analysis and GSE4619 (GPL570 platform) for healthy BM analysis. Data sets were imported into Gene Spring for normalisation and CG-RMA correction prior to BCAT1/BCAT2 gene expression analysis. The data presented in (Figure 2) shows that both HSC and CLL cells express significantly more BCAT1 compared with BM from healthy age matched donors (P<0.01). This finding suggests that BCAT1 expression is lost during normal bone marrow differentiation, and supports the notion presented for CML that BCAT1 expression maintains the leukemic cell a less differentiated state [7]. These data are in contrast to BCAT2, where no significant difference in expression was detected between CLL and healthy BM (P>0.05). Furthermore, no correlation between BCAT1 and BCAT2 expression was detected in CLL (R^2=-0.016; P=0.202), suggesting that expression levels are independent. A summary of BCAT1 and BCAT2 normalised expression in CLL linked to our original risk stratification is presented in (Table 1). The data presented is the averaged normalised expression for each BCAT probe set and shows that BCAT1 expression (not BCAT2) is linked to disease progression, i.e. patients with time to first treatment <100 days have significantly higher BCAT1 expression levels.

Finally, Kaplan-Meier analysis was performed to evaluate whether BCAT1 expression level could predict CLL patient overall survival (OS) and time to first treatment (Figure 3). For this analysis, CLL patients were stratified according to normalised BCAT1 expression level; BCAT1(hi) 2.178 to 4.209 (top quartile) and BCAT1(lo) -1.187 to -2.686 (bottom quartile). There were a total of 27 CLL patients in each arm. The data presented in Figure 3 show that BCAT1(hi) CLL patients have a significantly worse OS compared with BCAT1(lo) (P=0.0015; Hazard Ratio=9.381) and a significantly shorter time to first treatment (P=0.0147; Hazard Ratio=3.283). This is in contrast to BCAT2, where no impact was observed when comparing BACT2(hi) with BCAT2(lo) expressers (P>0.05). It must be noted that the data presented here needs further risk stratification evaluation and assessment of BCAT1 protein expression at the cellular level.

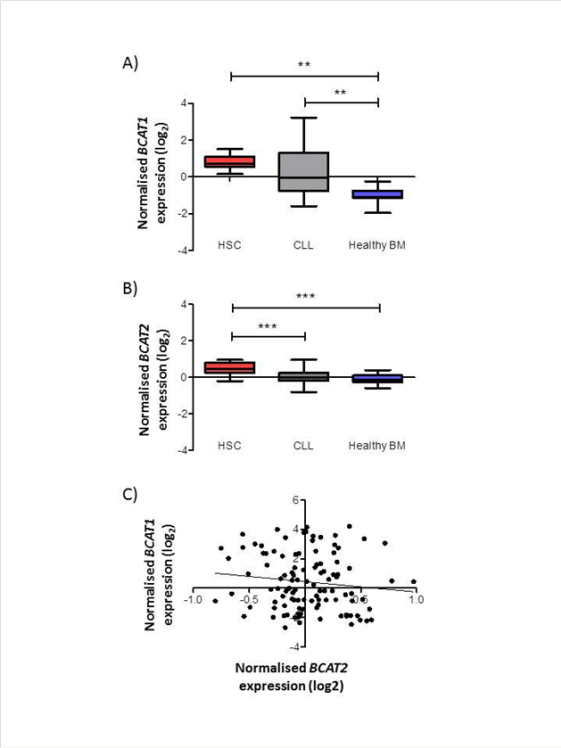

Figure 2: Comparison of normalised BCAT1 and BCAT2 expression level between CLL cells, haematopoietic stem cells (HSC) and healthy donor bone marrow (BM).

a) BCAT1 normalised expression level for 107 CLL patients (GEO accession GSE22762; GPL570 platform) was compared with 12 normal HSC samples (GSE13496; GPL96 platform) and 10 healthy BM samples (GSE4619; GPL570 platform).

b) Respective data comparing normalised BCAT2 expression level.

c) Linear regression analysis between normalised BCAT1 expression and normalised BCAT2 expression in CLL (R^2=-0.016; P=0.202). **P<0.01, ***P<0.001, data analysed by one way-ANOVA with Tukey's Multiple Comparison Test.

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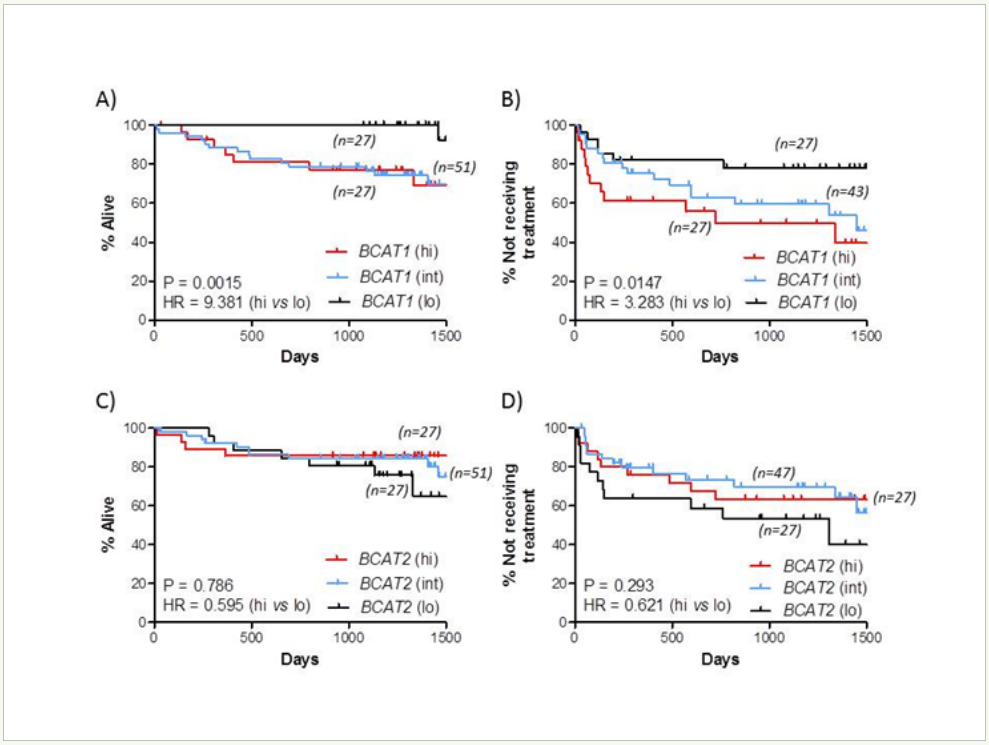

Figure 3: Kaplan-Meier analysis evaluating BCAT1 and BCAT2 expression in CLL. Patients were stratified according to BCAT1(hi); 2.178 to 4.209 (top quartile) and BCAT1(lo); -1.187 to -2.686 (bottom quartile), based on averaged $\log_2$ normalised expression values for each BCAT1 probe set (226517_at; 225285_at; 214452_at and 214390_s_at).

a) Charts show the impact of BCAT1 expression on overall survival and

b) Time to first treatment.

c) For comparison, the impact of BCAT2 was evaluated. Patients were stratified according to BCAT2(hi); 0.266 to 0.973 (top quartile) and BCAT2(lo); -0.183 to -0.802 (bottom quartile), based on averaged $\log_2$ normalised expression values for each BCAT2 probe set (215654_at; 203576_at). Charts show the impact on overall survival and

d) Time to first treatment. Patients that express intermediate levels of BCAT have been included for comparison: BCAT1(int); -1.132 to 1.790 (interquartile range) and BCAT2 (int); -0.182 to 0.255 (interquartile range) respectively.

Table 1: Risk stratification characteristics for BACT expression.

Patient Group (prognosis)	Days to First treatment	Gene	Probe Sets	Mean Normalised Expression (log2)*
Good (n=40)	>1000	BCAT1	226517_at; 225285_at; 214452_at; 214390_s_at	-0.427±1.77
		BCAT2	215654_at; 203576_at	0.058±0.306
Medium (n=37)	100 to 1000	BCAT1	226517_at; 225285_at; 214452_at; 214390_s_at	0.582±1.75
		BCAT2	215654_at; 203576_at	0.050±0.382
Poor (n=18)	<100	BCAT1	226517_at; 225285_at; 214452_at; 214390_s_at	1.67±1.86
		BCAT2	215654_at; 203576_at	-0.056±0.262

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Taken together, the data presented here using GEO microarray data sets demonstrates that BCAT1 is significantly dysregulated in CLL. Patients <100 days before first treatment expressed significantly higher levels of BCAT1 compared with patients >1,000 days before first treatment. We also showed that BCAT1 expression is lost through normal blood cell differentiation, suggesting that BCAT1 may have a functional role in normal haematopoiesis. Finally, disease progression may be predicted when CLL patients are stratified according to BCAT1 expression level. These findings align with recent studies evaluating the role of BCAT1 in CML and other cancers such as; glioma [12], breast cancer [13], hepatocellular carcinoma [14] and prostate cancer [15]. The clinically approved drug Gabapentin, which can inhibit BCAT1 activity, may therefore have a future role in the treatment of these cancers. Furthermore, stratification of patients according to BCAT1 expression level may be important clinically and help predict disease progression in CLL.

Conflict of Interest

The authors declare no conflict of interest.

Author Contribution

SC performed all data analysis. SC and AW contributed to the preparation of the final manuscript.

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