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Low CD49d expression in newly diagnosed chronic lymphocytic leukaemia may be associated with high-risk features and reduced treatment-free-intervals

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Abstract

This study was carried out to assess the prognostic power of low CD49d expression (≥10%) in newly diagnosed CLL patients using a previously described cohort. Eighty-five patients were included. Median age at diagnosis; 70 years (43–88); CD49d was expressed in 33/85 (38.8%); 23/33 (69.7%) at ≥30% referred to as 'HiCD49d' and 10/33 (30.3%) between 10 and 30% with a bimodal pattern on scatterplot analysis referred to as 'LoCD49d'. Eleven patients (12.9%) presented as Binet stage B, of whom 8 (72.7%) were CD49d+ (HiCD49d 7/8; LoCD49d 1/8). Seven of 81 patients (8.6%) were *NOTCH1* mutated and all were CD49d+ ($p \leq .01$). IgVH analysis was performed on 29 (87.8%) of the CD49d+ cases, of whom 21 (72.4%) were unmutated and 8 (27.6%) were mutated. CD38+/CD49d+ accounted for 11/20 (55%) (CD38+/HiCD49D: 9/11; CD38+/LoCD49D: 2/11). At 42 months, treatment had been initiated in 18/85 (21%) patients, of these 10/33 (30.3%) were CD49d+ versus 8/52 (15.4%) of the CD49d− group. The median treatment free interval for the CD49d+ group was 11 months (HiCD49d; 14.5 months, LoCD49d; 11 months) compared to 21.5 months for the CD49d− group. These findings suggest that the predictive value of CD49d expression is retained at expression levels down to 10%.

KEYWORDS
bimodal, CD49d, CLL, NOTCH1, treatment-free interval

Novelty statement

What is the new aspect of your work?

In newly diagnosed cases of CLL, we have demonstrated an association between mutated *NOTCH1* and low levels of CD49d expression (≥10 and <30%) which has previously only been reported at high levels of CD49d expression (≥30%).

What is the central finding of your work?

Low levels of CD49d expression in newly diagnosed CLL appears to be associated with high-risk disease displaying increased frequencies of mutated *NOTCH1* and unmutated IgVH with a median treatment-free interval of 11 months which is similar to those with high levels of CD49d expression.

What is (or could be) the specific clinical relevance of your work?

Identifying CLL cases with low levels of CD49d expression at diagnosis as a high-risk population would help to personalise patient education and disease surveillance plans.

1 | INTRODUCTION

Chronic lymphocytic leukaemia (CLL) is the most common form of adult leukaemia diagnosed in the western world and is characterised by a heterogenous clinical course; up to a third of patients are never treated while high-risk subtypes are chemo-resistant and require expensive targeted therapy.[1–3] This heterogeneity is related to the *IgVH* status as well as mutations of *TP53*, *NOTCH1*, *SF3B1*, *BIRC3* and *ATM* genes.[4] Furthermore, the complex interplay between these genes and adhesion molecules has been recognised as a mechanism for establishing niche microenvironments leading to high-risk CLL.[5–9]

NOTCH1 mutation is associated with upregulation of the integrin molecule CD49d.[10] CD49d is the α4 heterodimer of the α4β1 integrin molecule and plays a critical role in leucocyte trafficking, activation and survival through upregulation of BCL-2.[11] CD49d expression is detectable by flow cytometry in 35–40% of CLL cases and is clinically associated with bulky lymphadenopathy, reduced treatment-free intervals (TFI) and reduced overall survival (OS) times.[12–14]

Using a fluorochrome labelled anti-CD49d monoclonal antibody the standard cut-off for positivity in flow cytometry is ≥30%.[15] Subpopulations of CD49d+ CLL cells detectable below the 30% cut-off are identified by distinctive 'bimodal' patterns on scatter plot analysis and these small CD49+ subpopulations are reported to have the same prognostic implications as cases with high levels of CD49d expression levels of ≥30%.[12]

Up to a third of patients with CLL are never treated and do not require the detailed prognostic/treatment defining profiles including FISH, *IgVH* and *TP53* mutational status recommended in iwCLL guidelines prior to initiating treatment.[16] Nevertheless, predicting clinical outcomes at diagnosis in a cost effective, robust manner using flow cytometry would enable appropriate follow-up and facilitate accurate patient discussions regarding prognosis.

This multi-centre cross-sectional study aims to define the TFI, clinical and molecular features of newly diagnosed CD49d+ CLL. We aim to compare the findings of those with CD49d expression at the standard level of positivity (≥30%) which we have called 'HiCD49d' to cases that express low levels of CD49d (≥10 and <30%) and bimodal distribution patterns which we refer to as 'LoCD49d'.

2 | METHODS

2.1 | Study design

Newly diagnosed patients with CLL were recruited from the Trinity St. James's Cancer Institute, University Hospital Limerick and the Midlands Regional Hospital, Tullamore as part of a CLL epidemiology study between October 2017 and September 2018.[17] Ethics approval was obtained from institutional ethics committees and informed consent sought for clinical data, CD49d immunophenotyping, mutational analysis (*TP53*, *NOTCH1*) and biobanking. Consecutive newly diagnosed cases of CLL were identified by the central flow cytometry laboratory and were included in the study. Cytogenetic analysis was not performed at diagnosis.

2.2 | Laboratory characterisation

2.2.1 | Immunophenotyping

Performed by the regional flow cytometry service using a 3-laser, 8-colour BD FACS CANTO II flow cytometer with BD Biosciences and eBiosciences fluorochrome labelled monoclonal antibodies to CD19, CD22, CD79b, CD23, CD5, FMC7, SmIg, CD38 and CD49d and the modified Matutes scoring system identified CLL with a ≥4 score.[18] CD49d expression of ≥30% were classified as 'HiCD49d', CD49d expression between ≥10% and <30% triggered a scatterplot review for bimodal peaks and were referred to as 'LoCD49d'. 'CD49d+' referred to both HiCD49d and LoCD49d. CD49d expression levels of <10% or between ≥10% and <30% with no evidence of bimodality were classified as CD49d−.

TABLE 1 Demographics, Binet stages, CD38 expression and mutational status at diagnosis and numbers treated at 42 months

Variables	Total cohort $N = 85$	CD49d+ $N = 33$	HiCD49d $N = 23$	LoCD49d $N = 10$	CD49d− $N = 52$
Median age in years *(range)*	70 *(43–88)*	70 *(47–88)*	68 *(47–84)*	72.5 *(56–88)*	70 *(43–88)*
Male:female ratio	1.7:1	2.7:1	3.6:1	1.5:1	1.3:1
Binet stages A/B/C	72/11/2	24/8/1	16/7/0	8/1/1	48/3/1
CD38 positive (%)	20 (23.5)	11 (33.3)	9 (39.1)	2(20)	9 (17.3)
IgVH mutated (%)	8/29 (27.6)	8/29 (27.6)	6/21 (28.6)	2/8 (25)	N/A[b]
IgVH unmutated (%)	21/29 (72.4)	21/29 (72.4)	15/21 (71.4)	6/8 (75)	N/A[b]
NOTCH1 mutated (%)	7/81 (8.6)	7/31 (22.6)	3/22(13.6)	4/9 (44.4)	0/50 (0)
TP53 mutated (%)	7/83 (8.4)	4/33 (12.1)	2/23 (8.7)	2/10 (20)	3/50 (6)
[a]Dual TP53 and NOTCH1 mutations (%)	2/81 (2.5)	2/33 (6.1)	0	2/10 (20)	0
Treatment at 42 months (%)	18/85 (21.2)	10/33 (30.3)	6/23 (26.1)	4/10 (40)	8/52 (15.4)

[a]These were included separately in the *NOTCH1* and *TP53* figures.
[b]N/A – result not available.

TABLE 2 Characteristics of those requiring treatment within 42 months of diagnosis

Variables	CD49d+ $N = 10$	HiCD49d $N = 6$	LoCD49d $N = 4$	CD49d− $N = 8$
Median age in years, (range)	61 (56–77)	59 (56–66)	71 (56–77)	73 (64–77)
Male:female ratio	7:3	5:1	1:1	1:1
Binet A/B/C	5/4/1	3/3/0	2/1/1	5/2/1
CD38+ (%)	1 (10)	1 (16.7)	0	2 (25)
IgVH mutated	1 (10)	1 (16.7)	0	0
IgVH unmutated	9 (90)	5 (83.3)	4 (100)	3 (37.5)
Mutated *NOTCH1* (%)	2 (20)	0	2 (50)	0
Mutated *TP53* (%)	4 (40)	2 (33.3)	2 (50)	2 (25)
[a]Dual *TP53* and *NOTCH1* mutations (%)	2 (20)	0	2 (50)	0
Median TFI (months)	11	14.5	11	21.5

[a]Included separately in the *NOTCH1* and *TP53* numbers. One of these two cases displayed unmutated IgVH, the other, did not have IgVH analysis performed (sample not available).

2.3 | Molecular analysis

TP53 and *NOTCH1* mutation analysis was performed on all patients and IgVH analysis on CD49d+ patients. *TP53* was analysed using the ThermoFisher *TP53* community panel and sequenced on the ThermoFisher S5 next generation sequencer (NGS). All pathogenic variants with >5% variant allelic frequency were reported, as per European Research Initiative on CLL guidelines.[19] *NOTCH1* analysis was performed by PCR and reported as mutated if >10% mutant alleles were detected. IgVH mutational analysis was performed using the Invivoscribe IgVH Somatic Hypermutation assay kit v.2.0. A Bidirectional Sanger sequencing was performed. Consensus sequences were input into the IMGT/V-Quest database (http://www.imgt.org/IMGT_vquest) to determine mutational status.

2.4 | Patient follow-up

Patient charts were reviewed on the 1st of April 2021, 42 months after study initiation to determine if and when treatment was commenced.

2.5 | Statistics

Chi-square and Fisher's exact tests were used to determine an association between CD49d status and treatment requirement at 42 months, CD38 expression and *NOTCH1* and *TP53* mutational status. Data was checked for normality based on visual interpretation of histograms and Q–Q plot. For analysis of differences in time to treatment between groups, data was not normally distributed therefore Mann–Whitney test was applied. p values < .05 were considered significant.

3 | RESULTS

3.1 | Immunophenotype

Eighty-five newly diagnosed CLL case (modified CLL Matutes scores of ≥4) were included. In total 33/85 (38.8%) were CD49d+ comprising of; HiCD49d: 23/33 (69.7%) and LoCD49d: 10/33 (30.3%). 52/85 (61.2%) were CD49d−. Ten cases expressed CD49d between ≥10%

and <30% and on review of the scatterplots, all 10 had bimodal distribution patterns. 20/85 (23.5%) were CD38+, co-expression of CD38 and CD49d accounted for 11/20 (55%) (CD38+/HiCD49D: 9/11; CD38+/LoCD49D: 2/11) and 9/52 (17.3%) were CD49d− ($p = .87$). See Tables 1 and 2 for complete immunophenotypic data.

3.2 | Molecular results

NOTCH1 analysis was performed on 81/85 (95.3%) cases of whom 7/81 (8.6%) were mutated, all 7 were CD49d+ (HiCD49d: 3/7; LoCD49d: 4/7) ($p \leq .01$). IgVH results were available on 29/33 of the CD49d+ group, of whom 21/29 (72.4%) were unmutated and 8/29 (27.6%) were mutated. *TP53* mutational analysis was performed on 83/85 (97.6%) of whom 7/83 (8.4%) were mutated; 4/7 (57.1%) were CD49d+ (HiCD49d:2/7; LoCD49d:2/7) and 3/7 (42.8%) were CD49d− ($p = .461$).

3.3 | Treatment initiated at 42 months

At Forty-two months, 18/85 (21.2%) patients had commenced treatment of whom 10/33 (30.3%) were CD49d+ compared to 8/52 (15.4%) CD49d− patients ($p = .209$). CD49d+ patients had a shorter median TFI of 11 months, compared to 21.5 months for CD49d− patients ($p = .722$). See Table 2.

4 | DISCUSSION

Expression of CD49d at the standard positivity threshold of ≥30% has been established by numerous studies as an independent risk factor for an aggressive disease course in CLL, with patients shown to have shorter treatment free and overall survival times compared to those with CD49d negative disease.[13,15,20,21] Moreover, recent studies have suggested that the prognostic power of CD49d is preserved at levels of expression below the standard 30% cut-off.[12] This study concurs with the prognostic value of CD49d at both conventional and low levels of expression.[12] There were no significant differences found between the HiCD49d and LoCD49d groups with respect to age at diagnosis, gender and high-risk features. The CD49d+ patients displayed clinically aggressive disease with 30% requiring treatment within 42 months compared to 15% of the CD49d− group. The median TFI in the CD49d+ group was 11 months compared to 21.5 months in the CD49d− group. The LoCD49d patients did have some unique features including a lower level of Binet B disease (suggesting early CLL diagnosis) more mutated *NOTCH1* (see paragraph 2) and a shorter TFI which may reflect the small sample size.

A *NOTCH1* mutation was found in 8% of the cohort which is in keeping with the incidence of *NOTCH1* mutated cases in newly diagnosed CLL[22,29]; interestingly all of the *NOTCH1* mutated cases expressed CD49d (in both Hi and LoCD49d subgroups), whereas none of the CD49d− patients had a *NOTCH1* mutation. The association between *NOTCH1* and CD49d expression has been reported with the conventional CD49d expression levels of ≥30% but not in those expressing CD49d between 10 and 30%.[9] Although CD49d expression is not a surrogate marker for *NOTCH1* mutations they appear to be related. In vitro studies have demonstrated that mutated *NOTCH1* appears to upregulate CD49d through the NF-kB pathway.[9]

Unmutated IgVH is found in up to 40% of CLL patients at diagnosis and is a highly predictive, stable, prognostic factor for aggressive CLL but is not performed routinely because of assay complexity and cost.[23,24] The incidence of unmutated IgVH in our series of CD49d+ patients (Hi and loCD49d subgroups) was 72.4%. An association between with HiCD49d expression and unmutated IgVH has been previously reported although the underlying pathophysiology remains to be elucidated.[13,23,25]

CD38 is widely used as a prognostic marker in diagnostic CLL panels and its dual expression with HiCD49d has been reported in 20% of CLL cases.[26,30] CD38+/CD49d+ cases represented 33% of our CD49d+ series which was higher than the CD38+/CD49d− cohort of 17.3%, which may be accounted for by sample size.[26] Though CD38 and CD49d are biologically synergistic, they identify different patient populations with 15% of patients being reported as CD38−/CD49d+ in the literature, the relative merit and interaction of both markers' warrants further study.[27,28] Treatment was started at 42 months in 9.1% of the CD38+/CD49+ group compared to 40.9% of the CD38−/CD49d+ group suggesting that CD49d is the more useful prognostic indicator.

In conclusion, this study suggests that LoCD49d expression in newly diagnosed CLL patients identifies high-risk disease, displaying increased frequencies of mutated *NOTCH1* and unmutated IgVH. Forty percent of the LoCD49d group required treatment within 42 months and had a median TFI of 11 months, similar to those with conventional high levels of CD49d expression. Identifying the LoCD49d group as a high-risk population at diagnosis would help personalise patient education and follow up plans. The outcomes of newly diagnosed CLL patients with LoCD49d expression warrants further large-scale studies to confirm our findings.

AUTHOR CONTRIBUTIONS

Carmel Waldron and Elisabeth Vandenberghe designed the study, analysed and interpreted the results and critically reviewed the manuscript. Elizabeth Smyth and Aidan Kelly collated and analysed the data and wrote the manuscript. Sarah Brophy, Kanthi Perera, Gerard M. Crotty, Aileen Walsh, Aidan Kelly, Michelle Connolly, Ashique Khan, Ruth Clifford and Hilary O'Leary collected data and provided valuable assistance in the design of the study. Kanthi Perera, Gerard M. Crotty, Aileen Walsh, Michelle Connolly, Ruth Clifford, Hilary O'Leary, Christopher L. Bacon, David O'Brien, Deirdre Waldron, Emer Atkinson, Sarah Brophy and Fiona Quinn reviewed the manuscript. Emer Atkinson and Quinn Fiona did the molecular analysis while David O'Brien and Deirdre Waldron performed the flow cytometric analysis. Emily Smyth provided statistical analysis. Anthony M. Mc Elligott was responsible for biobanking.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data from this study is available from the corresponding author (ES) upon request.

PATIENT CONSENT

All patients that had molecular analysis (*TP53* and *NOTCH1* mutational status), CD49d immunophenotyping and biobanking performed gave written informed consent prior to inclusion in the study.

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