RESEARCH LETTER

blood advances

TO THE EDITOR:

No lymphocytosis increase after cBTKi is a rare phenomenon except for CD49d-positive CLL mainly expressed in trisomy 12

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Covalent Bruton tyrosine kinase inhibitors (cBTKi) are a class of drugs that have revolutionized the treatment of chronic lymphocytic leukemia (CLL).[1] They target BTK, which triggers pathways involved in cell survival, proliferation, and migration.[2] BTK inhibition results in the demarginalization of neoplastic lymphocytes from lymph nodes into peripheral blood.[3]

A few published series have reported abbreviated, although not absent, lymphocytosis after treatment initiation with cBTKi, especially ibrutinib in patients with trisomy 12. In 2014, Thompson et al[4] published data about abbreviated lymphocytosis in trisomy 12 (+12) CLL cells during ibrutinib treatment, noting that the mechanisms remain unknown.

Trisomy 12 cells show increased expression of integrins LFA-1, Mac-1, and VLA4-4; the upregulation of these integrins and their signaling enhances ITGA4/ITGB1-mediated motility and adhesion, promoting the retention of +12 CLL cells within tissues.[5,6]

The aim of this study was to describe patients with CLL treated with cBTKi who did not show any increase in lymphocytosis after treatment initiation and its impact on progression-free survival (PFS), time to next treatment (TTNT), and overall survival (OS).

We conducted a retrospective multicenter observational study including patients with previously untreated CLL who started first-line cBTKi treatment (ibrutinib, acalabrutinib, or zanubrutinib at approved doses) between 2016 and 2024 across 16 Italian centers. We enrolled 346 patients and studied their lymphocytosis kinetics, assessing each one for the median absolute lymphocyte count (ALC) at baseline and on days +15, +30, +60, +90, +120, +180, +270, and +360. Redistribution lymphocytosis was characterized as an increase in ALC from baseline within the first 30 days of therapy. Patients who did not show this initial peak on ALC were classified as having an absence of an increase in lymphocytosis.

In addition, clinical outcomes were assessed in 284 of 346 patients with sufficient follow-up (12 months). PFS, TTNT, and OS were calculated using the Kaplan-Meier method, with group

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The data sets generated and/or analyzed during this study are available from the corresponding author, Luca Laurenti (luca.laurenti@unicatt.it), on reasonable request.

Table 1. Baseline clinical and biological characteristics of patients with redistribution lymphocytosis vs no early ALC rise after cBTKi initiation

Characteristic	Redistribution lymphocytosis (n = 295)	No early ALC rise (n = 51)	*P* value
Male sex, %	62	61	0.92
Age, median, y	72 (66-78)	72 (63-82)	0.88
Binet stage A/B/C, %	12/43/44	20/39/41	0.28
Lymph nodes >5 cm, %	33	31	0.77
Splenomegaly, %	67.5	75	0.29
IGHV unmutated, %	59.3	71	0.34
del(17p) and/or TP53mut, %	21.9	18	0.54
del(11q), %	14.9	25	0.10
del(13q), %	44.1	35	0.23
Trisomy 12, %	13.2	45	<.001
CD49d-positive, % (available n)	44 (41/93)	73 (16/22)	0.016

del, delec; IGVH, Immunoglobulin Heavy Chain Variable gene; mut, mutated.

comparisons by the log-rank test. The study was conducted according to the Declaration of Helsinki and guidelines for Good Clinical Practice. All patients provided written informed consent.

Among 346 patients with treatment-naïve CLL receiving frontline cBTKi, 295 (85%) developed redistribution lymphocytosis, whereas 51 (15%) met the criteria for no early ALC rise (defined as no increase in ALC from baseline within 30 days). These patients showed an immediate and persistent reduction in circulating lymphocytes from day 15 through the 1-year follow-up. The frequency of no early ALC rise was comparable across cBTKi agents (17%, ibrutinib; 14%, acalabrutinib; and 12%, zanubrutinib). Baseline clinical characteristics were similar between the 2 groups, including median age, sex, Binet stage, bulky lymphadenopathy (>5 cm), splenomegaly, and immunoglobulin heavy chain variable gene (IGHV) status, without significant statistical differences (all with $P > .05$). (Table 1)

Analyzing the cohort according to the cytogenetic profile, we found that trisomy 12 was markedly enriched among patients with no early ALC rise (45% vs 13.2%; $P < .001$), which is higher than expected according to Döhner classification.[7] Other recurrent abnormalities, including del(17p) and/or TP53 aberration (18% vs 21.9%; $P = .54$), del(11q) (25% vs 14.9%; $P = .10$), and del(13q) (35% vs 44.1%; $P = .23$), did not differ significantly between the 2 groups (Table 1).

CD49d (VLA-4/ITGA4), an adhesion molecule implicated in leukemic cell homing and tissue retention, was assessed in a subset of patients by flow cytometry and considered positive when expressed on >30% of clonal B cells. CD49d status was available for 22 of 51 patients in the no early ALC rise group and for 93 of 295 patients in the redistribution lymphocytosis group. CD49d positivity was more frequent among patients with no early ALC rise (73% [16/22] vs 44% [41/93]; $P = .016$).

Patients without an early ALC rise showed an immediate and sustained reduction in circulating lymphocytes from day 15 through 12 months, without significant differences in ALC kinetics by cBTKi agent (Figure 1A) or IGHV status (Figure 1B). When stratified by cytogenetics, patients with trisomy 12 exhibited a more rapid decline in ALC during the first months of therapy (Figure 1C). Given the high prevalence (89%) of CD49d positivity in trisomy 12 patients, we explored the association between CD49d and ALC kinetics among patients without trisomy 12 (13/22 patients); within this subset, CD49d-positive cases (8/13 patients) showed a steeper ALC decline during the first 3 months than CD49d-negative cases (5/13 patients) (Figure 1D).

Among the 284 patients evaluable for time-to-event analyses (244 with redistribution lymphocytosis and 40 with no early ALC rise), there were no significant differences in PFS ($P = .9651$), TTNT ($P = .5346$), or OS ($P = .6848$) between groups.

In this multicenter real-world cohort of patients with treatment-naïve CLL receiving frontline cBTKi, patients with and without redistribution lymphocytosis were compared at the cohort level. Baseline clinical features were widely similar, whereas trisomy 12 and CD49d positivity were enriched among patients without an early ALC rise, supporting a biological basis for this phenotype.

Among patients who did not show a further rise of lymphocytosis after starting cBTKi therapy, we found no significant difference based on drugs used and IGHV mutational status. These data have not yet been investigated in literature for acalabrutinib and zanubrutinib in patients who did not show lymphocytosis after starting cBTKi therapy. In contrast, our group has published data showing differences in lymphocytosis kinetics due to mutated IGHV in patients during treatment with cBTKi of the first and second generation (ibrutinib vs acalabrutinib).[8]

We observed a more rapid lymphocyte decrease in patients with +12 CLL. This difference was statistically significant and confirmed, even in this setting, what is already known from literature, even if our study lacks day 7 lymphocyte counts to confirm the data of Thompson et al[4] about a speedy and brief lymphocytosis, that disappeared from day 15.

Almost all patients with +12 expressed CD49d, the α-subunit of integrin VLA-4 that represents the main adhesion molecule expressed by CLL cells, allowing neoplastic lymphocytes retention in lymph nodes[5,9]

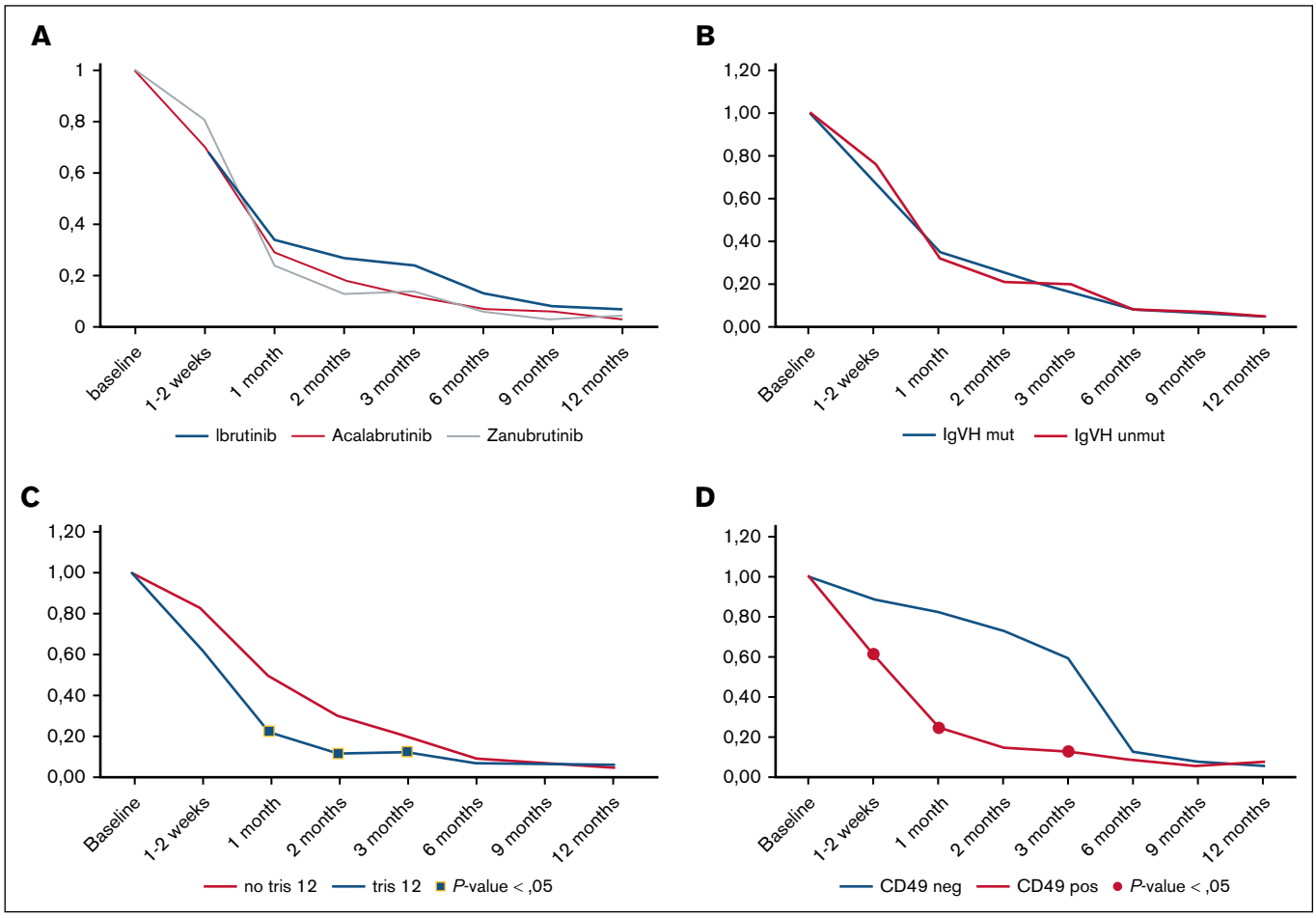

Figure 1. Relative change in ALC over time in patients with no early ALC rise (n = 51). Y-axis: relative ALC (baseline normalized to 1.0). (A) By cBTKi agent: ibrutinib (n = 23), acalabrutinib (n = 21), and zanubrutinib (n = 7). (B) By IGHV status: mutated (n = 15) vs unmutated (n = 36). (C) By trisomy 12: trisomy 12 (n = 23) vs no trisomy 12 (n = 28). Patients with trisomy 12 showed a more rapid decline at 1 month ($P < 0.001$), 2 months ($P = 0.02$), and 3 months ($P = 0.015$), as indicated. (D) Subgroup without trisomy 12 and with available CD49d data (n = 13): CD49d-positive (n = 8) vs CD49d-negative (n = 5). Patients with CD49d-positive CLL showed a faster decline at day 15 ($P = 0.045$), 1 month ($P = 0.003$), and 3 months ($P = 0.006$), as indicated. IGVH, Immunoglobulin Heavy Chain Variable gene; mut, mutated; neg, negative; pos, positive; tris, trisomy; unmut, unmutated.

Among patients without redistribution lymphocytosis, we further explored the association between CD49d expression and ALC kinetics in those without +12. We found that CD49d was expressed in the 62% and was negative in the 38% of patients with CLL, and those CD49d-positive patients showed a statistically significant faster decrease in ALC compared with that in CD49d-negative patients.

In addition to biological profiling, we observed no significant differences in PFS, TTNT, and OS between patients who experienced cBTKi-induced lymphocytosis and those who did not. These findings suggest that the absence of lymphocytosis does not adversely affect clinical outcomes, although the interpretation is limited by sample size and follow-up duration. Notably, this finding partially contrasts with previously published data on the role of CD49d in patients with CLL, in which reduced or absent lymphocytosis after ibrutinib therapy has been associated with shorter PFS. In our study, we compared patients with frontline CLL treated with first and second generation of cBTKi with or without lymphocytosis, but we did not stratify patients with CD49d expression. However, those findings may be influenced by biological stratification and treatment era. The novelty of our study lies in the inclusion of second-generation BTKi (acalabrutinib and zanubrutinib) and the real-world setting across multiple centers. CD49d status was available only in a subset of patients (22/51 patients without early ALC rise and 93/295 patients with redistribution lymphocytosis), a limitation related to the retrospective multicenter design.

In conclusion, our findings suggest that the adhesion molecule CD49d represents a biological correlate associated with the absence of early ALC rise in patients with cBTKi-treated CLL, whereas +12 plays an indirect role due to the high prevalence of CD49d expression in this subgroup, without affecting time-dependent parameters (PFS and TTNT). Future studies with larger cohorts and extended follow-up are warranted to clarify the prognostic impact of CD49d on long-term outcomes; prospective studies integrating chemokine dynamics and other microenvironmental correlates are also needed to elucidate mechanisms distinguishing patients with vs without redistribution lymphocytosis.

Contribution: L.L., F.G., A.M., and I.I. performed the research; C.V., M.C.M., G. Favrin, I.F., A.G., R.P., M.M., G.C., R. Murru, D.G., E.A.M., R. Moia, A.Z., E.T., F.A., A.T., L.S., T.Q., P.L.A., V.G., G.G., M.G., M.K., G. Farina, A.V., V.I., V.M., and M.C. collected the data; D.G. performed the data analysis; L.L. and F.G. wrote the manuscript; L.L., V.G., M.C., and I.I. supervised the study; and all the authors critically read, edited, and approved the final version of the manuscript.

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