

Letter to the Editor

IMPACT OF THE FUNCTIONAL *CD5* POLYMORPHISM A471V ON THE RESPONSE OF CHRONIC LYMPHOCYTIC LEUKAEMIA TO CONVENTIONAL CHEMOTHERAPY REGIMENS

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Chronic lymphocytic leukaemia (CLL) represents an abnormal clonal expansion of mature antigen-experienced CD5⁺ B1a cells (Chiorazzi *et al*, 2005), which present with a highly heterogeneous clinical course depending on associated chromosomal aberrations, somatic mutations within the immunoglobulin variable heavy chain genes (*IGHV*), and surface CD38 or intracytoplasmic ZAP-70 expression. Since key signalling components of the B-cell receptor (BCR) are relevant contributors to the variable clinical behaviour of CLL (Stevenson *et al*, 2011) we explored the influence of functionally relevant germline *CD5* variants on CLL prognosis. The rationale behind this is that CD5, a lymphocyte receptor normally expressed in all T cells and the B1a cell subset, is considered a negative modulator of intracellular signalling mediated by the antigen-specific receptor present on both T (TCR) and B1a (BCR) cells, to which physically associates (Soldevila *et al*, 2011). Moreover, in both normal and leukemic B cells, CD5 signalling is relevant for production of the B1a cell autocrine growth factor IL-10, and for acquisition of a common gene transcription signature (Gary-Gouy *et al*, 2007).

The existence of common nonsynonymous *CD5* single nucleotide polymorphisms (SNPs) has been recently reported (Carnero-Montoro *et al*, 2012). Two of them, rs2241002 (C>T) and rs2229177 (C>T), coding for amino acid changes at the extracellular (P224>L) and cytoplasmic (A471>V) regions of CD5, respectively, conform haplotypes relevant to CD5-mediated signal transduction. Accordingly, homozygous carriers of the ancestral P224-A471 (CC) haplotype are less efficient than the more recently derived P224-V471 (CT) haplotype in providing inhibitory signals to TCR-mediated activation responses and are associated with more clinically aggressive forms of autoimmune disease (Cenit *et al*, 2014). In light of this evidence, the putative influence of *CD5* SNPs rs2241002 and rs2229177 on clinical outcome of CLL patients from our Hospital was explored.

In total, 935 patients from our CLL database had DNA samples available for study. Median age was 63 years (range, 18-98 years) at CLL diagnosis. Binet stage was A in 83% of patients, B in 12% of patients, and C in 5% of patients. Results of FISH, CD38 expression, ZAP70 expression and *IGHV* mutation status were available in 81%, 77%, 83% and 79% of patients. Median follow-up was 34 months (range, 1-158) from frontline therapy and 93 months (range, 1-471) from diagnosis. Four hundred and seventeen (44%) patients required therapy at least once. The most commonly used chemotherapeutic agents were fludarabine, alone or in combination (37%),

chlorambucil (28%), COP/CHOP chemotherapy (17%) and cladribine (6%). Only 10% of these patients received rituximab as frontline therapy. The A471V genotypes CC, CT and TT were observed in 202 (22%), 432 (48%) and 269 (30%) patients, respectively. Regarding P224L, genotypes CC, CT and TT were documented in 575 (63%), 316 (34%) and 29 (3%) patients, respectively. There were no significant differences across all different A471V genotypes in terms of age, sex, prognostic factors, therapy administered or follow-up.

Median PFS from frontline therapy was 46 months (95% confidence interval: 38-52 months) for those patients who required therapy. Known adverse prognostic factors, such as high ZAP-70 expression ($P<0.001$), unmutated *IGHV* status ($P<0.001$), adverse FISH aberrations ($P=0.002$) and high CD38 expression ($P=0.007$) had a significant impact on PFS when the entire cohort was analysed. The A471V polymorphism had a trend towards a significant impact on PFS ($P=0.052$), while P224L had no significant effect on this outcome.

Next, only patients who required therapy but were homozygous for the most prevalent P224L genotype (P224P, CC) were selected for evaluation of the impact of the A471V polymorphism. Baseline characteristics of this selected population of patients are displayed in Table I. Age, sex, prognostic factors, therapy and follow-up were well balanced across all three genotypes. High ZAP-70 ($P=0.001$) and CD38 expression ($P=0.008$), and unmutated *IGHV* status ($P<0.001$) were equally significant in terms of PFS in this selected population, as was the A471V genotype. Indeed, patients carrying a CC or CT A471V genotype had a significantly prolonged median PFS compared to TT carriers (51 vs. 41 months, $P=0.024$) (**Fig. 1A**). This prognostic impact was particularly evident in patients with mutated *IGHV* (110 vs. 40 months, $P=0.028$), and not in patients with unmutated *IGHV* (36 vs. 47 months, $P=1.0$) (**Fig. 1B**). A multivariate Cox regression analysis comprising CD38 and ZAP-70 expression, A471V polymorphism and FISH aberrations was performed in this subgroup of patients with mutated *IGHV*, revealing that both the A471V polymorphism (hazard ratio [HR] = 3.7; 95% confidence interval = 1.21-11.3; $P=0.21$) and FISH aberrations (HR=3.5; 95% CI=1.08-11.3; $P=0.036$) had independent prognostic value in terms of PFS.

The overall survival was also analyzed but neither A471V, P224L nor the combined effect had any significant impact on that outcome, although there was a trend towards a significantly longer overall survival for patients carrying a CC or CT genotype for

A471V but only in patients with P224P (CC) genotype and mutated *IGHV* genes (P=0.073).

The present results indicate that CLL patients either homo- or heterozygous for the ancestral *CD5* allele A471 (C) have significantly prolonged PFS in less aggressive cases presenting mutated *IGHV*. They are reminiscent of a previous single report (Sellick *et al*, 2008) showing that the more recently derived V471 (T) allele was associated with shorter PFS independently of *IGHV* mutational status. In our study, this effect was particularly evident when we restricted the analysis to patients with the P224P (CC) genotype and mutated *IGHV*, and non-existent in patients with unmutated *IGHV*. Interestingly, most unmutated *IGHV* CLL cases contain mutations in driver genes, most of them conferring a bad prognosis and faster progression (Puente *et al*, 2015). By contrast, most mutated *IGHV* cases have mutations in better prognosis drivers such as *MYD88* or del(13q14), resulting in a less aggressive disease. These differences might allow the observation of the protecting effect of *CD5* polymorphisms only in mutated *IGHV* cases. On the other hand, only 10% of patients received a rituximab-based regimen, which may or may not abrogate the impact of the polymorphism, and is therefore the main limitation of our study.

How the *CD5* A471V variants influence the natural behaviour of CLL cells is an unresolved question. However, the lower signal transducing capability of the A471 variant (Carnero-Montoro *et al*, 2012; Cenic *et al*, 2014) could result in either 1) a lower capability for negatively modulating the antigen-receptor (BCR) mediated signalling, or 2) a lower anti-apoptotic signalling (Tibaldi *et al*, 2011). Whatever the case, the present results support the notion that *CD5* is not only a phenotypic marker but a relevant player in CLL cell biology as demonstrated by the influence of germline-defined functional *CD5* variants on CLL outcome.

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Competing interests

The authors have no competing interests.

Author contributions

All authors were involved in the design and approval of the final manuscript. FL supervised the work and wrote the manuscript. JD analyzed the data and wrote the manuscript. TB, LB, EC-M and EB performed the genetic analyses. XP, DC and EC provided the patient’s samples and clinical data.

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Table I. Baseline characteristics according to the A471V polymorphism of patients who required CLL-specific therapy and had a homozygous P224P (CC) genotype

| A471V genotype | CC (<i>n</i> = 156) | CT (<i>n</i> = 249) | TT (<i>n</i> = 141) | <i>P</i> value |
|---|----------------------|----------------------|----------------------|----------------|
| Age (y), median (range) | 65 (32-93) | 64 (28-98) | 63 (18-94) | .855 |
| Sex, % male/female | 58/42 | 53/47 | 60/40 | .280 |
| Binet stage B or C, <i>n</i> (%) | 26 (17) | 40 (16) | 14 (10) | .180 |
| Serum beta2-microglobulin (mg/l), median (range) | 1.9 (1.0-33) | 2.1 (1.0-17) | 2.2 (1.5-11) | .129 |
| Positive CD38 expression, <i>n</i> (%) | 33 (27) | 59 (31) | 27 (24) | .445 |
| Positive ZAP70 expression, <i>n</i> (%) | 34 (25) | 49 (24) | 36 (30) | .429 |
| Unmutated <i>IGHV</i> gene, <i>n</i> (%) | 47 (37) | 66 (33) | 45 (40) | .464 |
| FISH aberrations (Döhner's hierarchical model): | | | | .502 |
| Favorable (13q-,+12, none) | 105 (81) | 180 (86) | 96 (83) | |
| Unfavorable (11q-, 17p-) | 24 (19) | 29 (14) | 19 (17) | |
| Frontline therapy: | | | | .412 |
| Chlorambucil, <i>n</i> (%) | 21 (32) | 29 (28) | 14 (22) | |
| CHOP/COP, <i>n</i> (%) | 11 (17) | 14 (14) | 6 (10) | |
| Fludarabine mono/combo, <i>n</i> (%) | 22 (33) | 36 (35) | 21 (33) | |
| Cladribine monotherapy, <i>n</i> (%) | 5 (8) | 4 (4) | 6 (10) | |
| RCHOP, <i>n</i> (%) | 1 (1) | 0 (0) | 2 (3) | |
| RFC/RFCM, <i>n</i> (%) | 2 (3) | 10 (10) | 5 (8) | |
| Others, <i>n</i> (%) | 4 (6) | 9 (9) | 9 (14) | |
| Follow-up from frontline therapy (mo), median (range) | 49 (4-150) | 34 (3-158) | 28 (4-143) | .056 |
| Follow-up from CLL diagnosis (mo), median (range) | 101 (3-314) | 87 (4-463) | 86 (4-277) | .313 |

Abbreviations: FISH, fluorescent in-situ hybridization; B-CLL, B-cell chronic lymphocytic leukaemia; *IGHV*, immunoglobulin heavy chain variable region; CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; COP, cyclophosphamide, vincristine and prednisone; RCHOP, rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone; RFCM, rituximab, fludarabine, cyclophosphamide and mitoxantrone; RFC, rituximab, fludarabine and cyclophosphamide.

FIGURE LEGENDS

Fig. 1. (A) Progression-free survival according to the A471V polymorphism in patients who required CLL-specific therapy and had a homozygous P224P (CC) genotype ($P = 0.024$). (B) Progression-free survival according to the A471V polymorphism in patients who required CLL-specific therapy, and had a homozygous P224P (CC) genotype and mutated *IGHV* ($P = 0.028$).

