studies could probably be helpful in this setting. M component reductions to the IV formulation were brief and further studies are warranted to confirm these findings. Interestingly, a recent German Myeloma Group study (Merz *et al*, 2015) compared the IV *versus* the SC route retrospectively for VCD and PAD induction therapy in newly diagnosed multiple myeloma patients. The analysis of high quality responses revealed that IV-treated patients achieved higher rates of ≥very good partial response than SC-treated patients (42% vs. 29%, $P = 0{\cdot}02$) after 3 cycles of therapy. These data are in favour of a faster mechanism of action of the IV route, confirming a different pharmacokinetic profile at least in untreated patients, and multicentre studies are on-going to better clarify this aspect. In conclusion, to our knowledge, this is the first report regarding IV bortezomib efficacy after SC administration failure.

Author contributions

AG designed the study and wrote the manuscript; VC and GP were involved in patient treatment; BL and MD collected data; MB supervised the study and revised the manuscript. All authors approved the final version of the paper.

Disclosures

The authors declare no conflicts of interest.

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Impact of the functional CD5 polymorphism A471V on the response of chronic lymphocytic leukaemia to conventional chemotherapy regimens

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. Given that 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 function-

ally 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 it physically associates (Soldevila *et al*, 2011). Moreover, in both normal and leukaemic B cells, CD5 signalling is relevant for production of the B1a cell autocrine growth factor interleukin 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 the 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. Eighty-three per cent of patients were Binet stage A, 12% were stage B and 5% were stage C. Fluorescence *in situ* hybridization (FISH) results, 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 (COP, cyclophosphamide, vincristine, prednisone/cyclophosphamide, doxorubicin, vincristine, prednisone) 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 progression-free survival (PFS) from frontline therapy was 46 months [95% confidence interval (CI) : 38–52 months] for those patients who required therapy. Known adverse prognostic factors, such as high ZAP70 expression ($P < 0{\cdot}001$), unmutated *IGHV* status ($P < 0{\cdot}001$), adverse FISH aberrations ($P = 0{\cdot}002$) and high CD38 expression ($P = 0{\cdot}007$) had a significant impact on PFS when the entire cohort was analysed. The A471V polymorphism had a

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

FISH, fluorescence 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.

trend towards a significant impact on PFS ($P = 0{\cdot}052$), 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 ZAP70 ($P = 0{\cdot}001$) and CD38 expression ($P = 0{\cdot}008$) and unmutated *IGHV* status ($P < 0{\cdot}001$) 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, $P = 0{\cdot}024$) (Fig 1A). This prognostic impact was particularly evident in patients with mutated *IGHV* (110 vs. 40 months, $P = 0{\cdot}028$), and not in patients with unmutated *IGHV* (36 vs. 47 months, $P = 1{\cdot}0$) (Fig 1B). A multivariate Cox regression analysis comprising CD38 and ZAP70 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% CI = 1·21–11·3; $P = 0{\cdot}21$] and FISH aberrations (HR = 3·5; 95% CI = 1·08–11·3; $P = 0{\cdot}036$) had independent prognostic value in terms of PFS.

Neither A471V, P224L nor the combined effect had any significant impact on overall survival (OS), although there was a trend towards a significantly longer OS for patients carrying a CC or CT genotype for A471V but only in patients with P224P (CC) genotype and mutated *IGHV* genes ($P = 0{\cdot}073$).

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 which 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; Cenit *et al*, 2014) could result in either one) a lower capability for negatively modulating the antigen-receptor (BCR) mediated signalling, or two) 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.

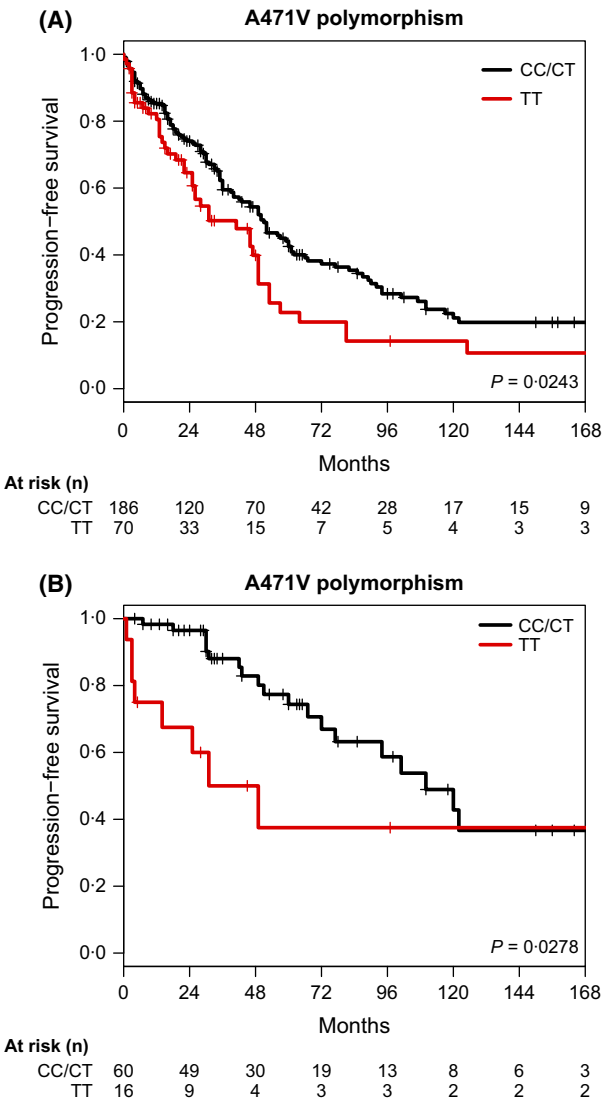

Fig 1. (A) Progression-free survival according to the *CD5* A471V polymorphism in patients with a homozygous P224P (CC) genotype ($P = 0{\cdot}024$) who required chronic lymphocytic leukaemia (CLL)-specific therapy. (B) Progression-free survival according to the *CD5* A471V polymorphism in patients a homozygous P224P (CC) genotype and mutated immunoglobulin variable heavy chain genes (*IGHV*) ($P = 0{\cdot}028$) who required CLL-specific therapy.

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Author contributions

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

Conflict of interest

The authors have no conflict of interest.

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