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Letter to the Editor

# CD200 and prognosis in chronic lymphocytic leukemia: Conflicting results

CD200 is a transmembrane type Ia glycoprotein belonging to the immunoglobulin superfamily [1]. It is expressed by various cell types, such as B cells, a subset of T cells including activated T cells, thymocytes, endothelial cells, and neurons. CD200 surface expression has been shown to have a relevant role in the differential diagnosis of B- cell chronic lymphoproliferative disorders (B-CLPDs) [2]. The major contribution of CD200 is the ability to discriminate between chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL), and between hairy cell leukemia and its variant form [3]. Furthermore, it has been also demonstrated a role of CD200 in the differential diagnosis of other forms of B-CLPDs, when incorporated in panels including other surface markers [4,5]. However, little is known about the prognostic significance of CD200 expression in CLL.

Here we report data on CD200 expression evaluated by flow cytometry in 105 patients with CLL seen at a single Institution, aiming at identifying a potential prognostic role of this surface antigen. The mean age of patients was 70 years (range 38–93 years), 59 were male and 46 female. All patients were evaluated at diagnosis. For this reason, as usual, the majority of them (92 patients; 88%) were found in Rai stage 0-II and 13 (12%) in Rai stage III-IV, reflecting the usual distribution of patients with CLL at diagnosis. Mean white blood cell count was $26.2 \times 10^9$/L (range $5.4–145 \times 10^9$/L); mean lymphocytes $20.6 \times 10^9$/L (range $23–135 \times 10^9$/L); mean hemoglobin level 12.9 g/dl (range 6–16.8 g/dl); mean platelet count $182 \times 10^9$/L (range $5–487 \times 10^9$/L). Patients were also classified on the basis of genetics abnormalities detected by FISH, according to Dohner categories [6], were available in 77 patients: negative in 29 (38%) patients; del13q in 24 (31%) patients; trisomy 12 in 12 (15%) patients; del11q in 6 (8%) patients, and del17p in 6 (8%) patients. IgVH mutation status was available in 68 patients, of which 29 (43%) were unmutated.

Immunophenotypic analysis of peripheral blood lymphocytes was performed by BD FacsCanto II flow cytometer using the FacsDiva software (BD Biosciences) and a 5–6 colors approach and the following antibody combinations: CD19 FITC (BD Biosciences)/CD5 PE (Beckman Coulter)/CD45 PerCP (BD Biosciences)/CD20 PE-Cy7 (Beckman Coulter)/CD23 APC (BD Biosciences); Kappa FITC/Lambda PE/CD19 PerCP-Cy5.5 (BD Biosciences)/CD20 PE-Cy7 (Beckman Coulter)/CD5 APC (BD Biosciences)/CD45 APC-Cy7 (Beckman Coulter); CD79b FITC (BD Biosciences)/CD200 PE (BD Biosciences)/CD45 PerCP (BD Biosciences)/CD19 APC (Beckman Coulter); FMC7 FITC (BD Biosciences)/CD22 PE (BD Biosciences)/CD45 PerCP (BD Biosciences)/CD19 APC (Beckman Coulter). For each sample, 100,000 events were collected. CD45+ lymphocytes were gated on CD45 vs SSC dot plot, then B cells were isolated by gating on CD19+ cells. A cut-off of CD30% on gated B-lymphocytes was used. We measured the mean fluorescence intensity (MFI) of CD200 antigen on CD19+ and CD19- lymphocytes, and then we calculated the relative fluorescence intensity (RFI) as the ratio of the MFI of CD200 on CD19+ lymphocytes and the MFI of CD200 on CD19- lymphocytes (Fig. 1).

Time-to-treatment (TTT) was defined as time from CLL diagnosis until the start of first treatment regimen, overall survival (OS) was defined as time from diagnosis until death for any cause. Receiver operating characteristic (ROC) curves were plotted in the attempt to find the best CD200 MFI or RFI cut-off that could predict TTT or OS. TTT and OS curves were plotted using Kaplan- Meier method, and log-rank test was performed to compare patients' groups. Mann-Whitney test was used for comparison of CD200 MFI or RFI values between subgroups of CLL patients. Differences were considered significant if $p < 0.05$. Statistical analyses were performed using GraphPad Prism version 6.01 for Windows (GraphPad Software, La Jolla, CA, USA).

In all studied cases, CD200 – evaluated on clonal B-cell surface – was found positive (mean 95%; range 37–100%). Mean CD200 MFI was 1986 (range 187–14,376) and mean CD200 RFI was 13 (range 2.4–49.5). No correlations were found between mutated or unmutated IgVH and CD200 MFI or CD200 RFI. Detection of del17p and trisomy 12 by FISH were found not correlated with CD200 MFI nor CD200 RFI. Patients with del11q displayed a significantly lower CD200 MFI value (522 ± 1534 vs 1436 ± 2085; $p = 0.04$) and lower RFI value (7.4 ± 1.9 vs 12.3 ± 7.6; p 0.0035). Patients with del13q14 had a significantly higher CD200 MFI value (but not CD200 RFI): 1958 ± 1493 vs 1189 ± 2263; $p = 0.02$.

Median follow-up was 45 months for the entire cohort. ROC analysis was not able to identify a cut-off value for the percentage of CD200 expression, CD200 MFI or CD200 RFI capable to discriminate patients with significantly different TTT or OS. We therefore considered the mean value of CD200 MFI and CD200 RFI in our cohort (2046 and 13, respectively) as cut-off. Patients with higher or lower CD200 MFI had superimposable TTT and OS (median TTT 104 months, 95% CI 29–179, vs 72 months, 95% CI 56–88, ns; estimated 36-month OS 97% vs 84%, ns) (Fig. 2A and B). Patients with a CD200 RFI > 13 did not differ from those with a CD200 RFI ≤ 13 in terms of TTT (median TTT 70 months, 95% CI 18–126, vs 72 months, 95% CI 55–89, ns) (Fig. 2C), but had a significantly longer OS (estimated 36-month OS 100% vs 76%, $p = 0.0065$) (Fig. 2D). The potential impact of infection or treatment related complications on OS were also not found.

The prognostic significance of CD200 expression has been clearly demonstrated in some hematological malignancies, such as acute myeloid leukemia [7] and multiple myeloma [8]. It is thought that CD200, through interaction with its receptor CD200R, acts directly suppressing memory T-cells function [9] and natural-killer cell function [10], thus influencing immune surveillance. However, little is known about CD200 prognostic role in CLL and conflicting results have been reported so far.

In 2014, Wang et al. identified two distinct groups of patients with CLL according to the expression of CD200 in bone marrow B cells (CD200 low group: < 50%; CD200 high group: ≥50%) [11]. A lower percentage of CD200-expressing cells was found to correlate with younger age, female gender, lower white blood cell count, lower

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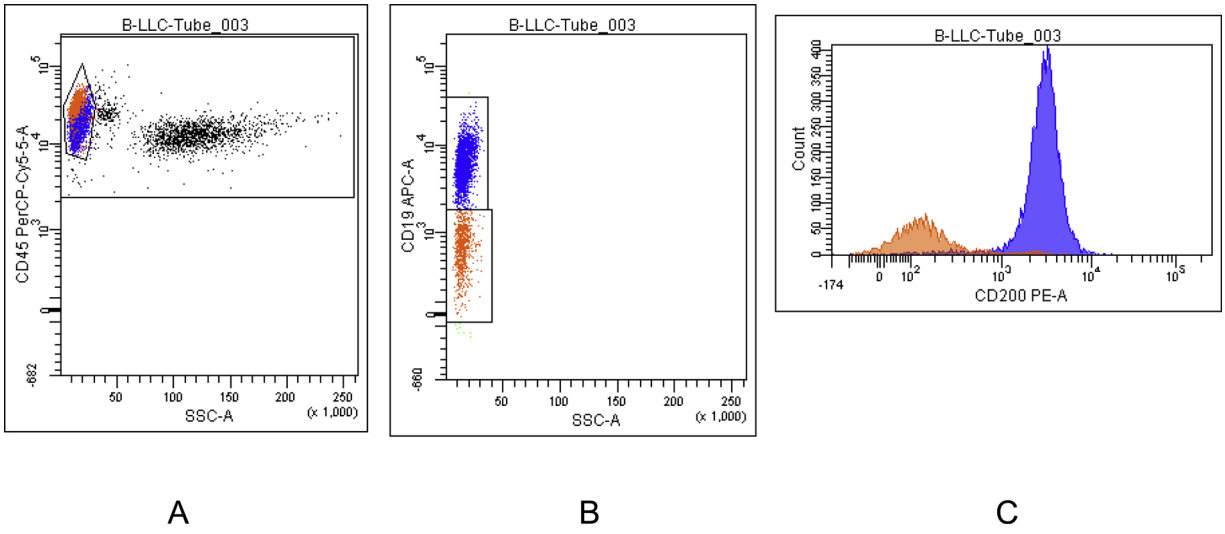


**Fig. 1.** Gating strategy and CD200 fluorescence intensity evaluation.
CD19+ (blue) and CD19- (red) cells were firstly gated (panels A and B). Histograms of CD200 fluorescence were evaluated on B-cell surface (blue) and non-B-cell surface (red). MFI and RFI were then calculated as ratio between MFICD200 and non B-cells (panel C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

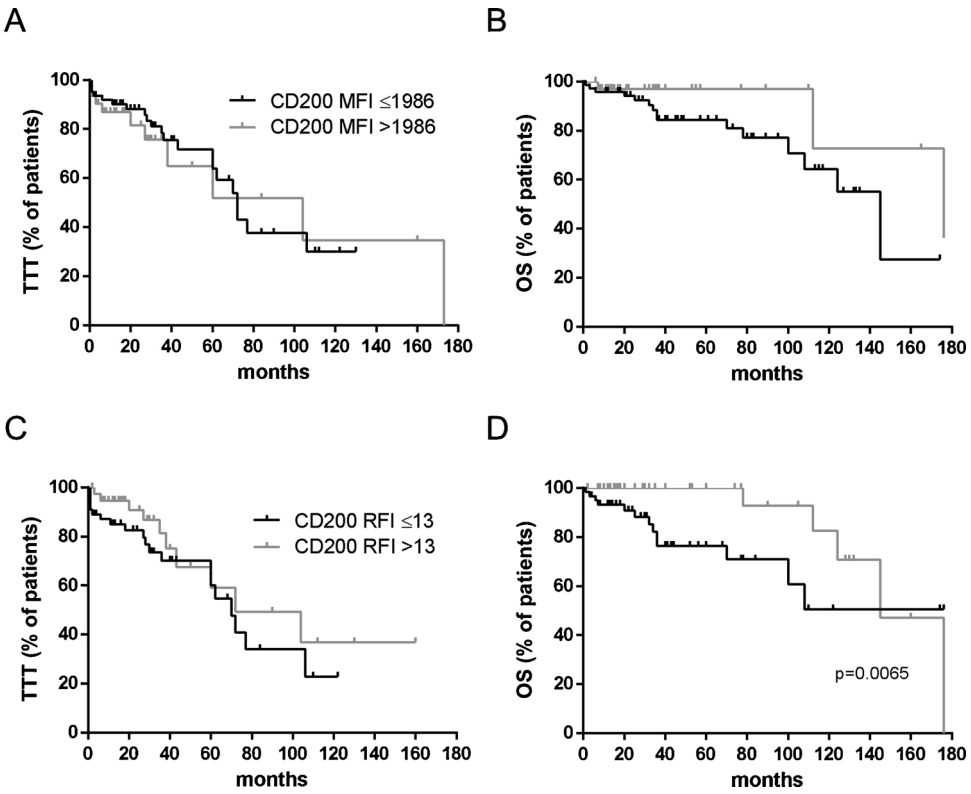


**Fig. 2.** TTT and OS according to CD200 MFI and RFI.
No differences in TTT and OS were found according to CD200 MFI (A and B). CD200 did not impact on TTT, but a significantly longer OS was found in patients with higherCD200 RFI.

absolute count and percentage of lymphocytes, lower lymph node involvement, and lower ZAP70 positive cells. Also, the majority of patients in the CD200 low group was in early disease stage. No data on TTT, response to therapy, or OS were reported.

Bahaa et al. later reported that in their cohort of 43 patients with CLL, high CD200 expression (> 50% of B cells) correlated with older age, lymphocytosis, hepatomegaly, splenomegaly, and a higher Rai and Binet stage [12]. On the contrary, no correlations were found with response to treatment and OS.

Miao et al. evaluated the MFI of CD200 in 307 patients with CLL, and identified two different CLL groups [13]: patients with lower CD200 MFI had a significantly shorter TTT, but no effect was found on OS, and CD200 MFI did not maintain its predictive value on TTT in multivariate analysis.

In our cohort, we found only an association between the presence of del11q and del13q14 with lower and higher CD200 expression, respectively. However, due to the small number of events a multivariate analysis could not be performed, and because of the small number of

patients with such abnormalities, this data must be evaluated with caution. Instead, CD200 MFI and RFI did not correlate with any other clinical or biological features evaluated at diagnosis. We also observed that a CD200 RFI superior to the mean value of the entire cohort characterizes patients with a significantly longer OS, but this data need to be confirmed with an extended follow-up.

In conclusion, conflicting results have been reported so far on the prognostic role of CD200 expression in CLL. Different methodologies and samples used (percentage of positive cells instead of MFI, bone marrow or peripheral blood cells, flow cytometry used and instrumental setting, gating strategy) could explain these differences. Further studies are needed to better understand this issue before to get definitive conclusions.

## Declaration of Competing Interest

All authors have no competing interests to declare.

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