Editorial

CD200 in hematological malignancies: just a diagnostic tool or more?

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Over the past two decades, the increased number of monoclonal antibodies and the constantly expanding availability of fluorescence probes significantly improved the efficiency and the accuracy of flow cytometric analysis, providing relevant information for the diagnosis, classification and follow up of various hematological malignancies. As a diagnostic tool, together with morphology and molecular genetics, flow cytometry has become a gold standard in the identification of acute and chronic leukemias, but is crucial also in the diagnosis of lymphomas and in the unusual "solid" presentation of hematological diseases. Consequently, it has gained a prominent position in the current WHO classification of hematological neoplasms (1). In particular, B peripheral lymphoproliferative diseases are considered the malignant counterpart of mature B cells and distinguished by their relationship with germinal center. So multiparameter flow cytometry (MFC) can often easily overcome the bias of an almost overlapping morphology, identifying follicular lymphoma (FL), chronic lymphocytic leukemia (CLL) and mantle cell lymphoma (MCL) by the expression of CD10, CD5 or CD23. More challenging remains the diagnosis of cases that lack the expression of those antigens, or in atypical CD5+ CLL, in which CD23 expression cannot discriminate between CLL and MCL (2). It's pleonastic to underline that a precise diagnosis has relevant clinical impact, as B chronic lymphoproliferative neoplasm comprises both indolent entities that may deserve a watchful waiting, and more aggressive diseases requiring an intensive approach. In volume 86B [98–105] of Clinical Cytometry, Sandes and colleagues tested the diagnostic utility of CD200 in differential diagnosis of 159 patients with mature B lymphoproliferative diseases (3). They not only added CD200 to a conventional MFC panel, evaluating its dichotomous expression, but also compared the fluorescence intensity in the tested cases, with the aim to create a reference pool of CD200 expression intensity that may further discriminate within the CD200-positive group. They conclude that CD200 identify CLL from MCL, even in case of atypical, CLL and propone a new diagnostic algorithm for the classification of CD5+ mature B neoplasm, including CD200. Moreover, they highlighted the role of CD200 in discriminating diseases of post-germinative origin, such as hairy cell leukemia, splenic marginal zone lymphoma and lymphoplasmacytic lymphoma, all expressing CD200 at different intensity. Similar pattern of expression was reported also by other groups, confirming the potential usefulness of adding CD200 into first-step MFC analysis (4-9). In our opinion, analysis should start from a limited number of "backbone" markers, sensitive enough to include the first physician's hypothesis and the principal alternative diagnoses, reserving a further characterization to a second time, not to be overwhelmed by un-interpretable results and excessive costs. This is particularly true if we want to evaluate antigens' intensity of expression, that is particularly influenced by hardware, software, fluorochrome choice and combination, instrument settings. The studies mentioned above employed different methods to define CD200 intensity (i.e., relative linear unit scaled 0 to 10^4; relative linear unit scaled in an arbitrary biexponential scale from 10^{-2} to 10^5; bright, dim, or moderate expression as compared to normal peripheral blood or bone marrow B cells; ratio of the mean fluorescence intensities of the tested antigen and its isotypic control; log shift in mean fluorescence intensity compared to isotype control), thus making interlaboratory

or longitudinal comparisons difficult, and suggesting the need of multicentric standardization trials.

Beyond diagnosis, in the past years emerged the potential role of CD200 as prognostic factor and as possible target of new engineered drugs. CD200 is a membrane glycoprotein, coded on chromosome 3q12, belonging to the immunoglobulin superfamily. In adults, CD200 is highly expressed in immune "sanctuaries", such as the central nervous system and the retina, and in resting and activated T cells, B cells and dendritic cells. Binding its specific receptor (CD200R) it is involved in the regulation of immune response and in maintenance of immune homeostasis (10). CD200 has also been proposed as a stem cell marker in many solid tumors and it has been hypothesized that cancer stem cells may evade immune system by inducing a tolerogenic response through CD200/CD200R interaction. Moreaux *et al.* reported a negative prognostic role of aberrant expression of CD200 in multiple myeloma plasma cells (11,12). Tazawa *et al.* observed a longer overall survival (OS) in CD200 negative, compared to CD200 positive, myeloma patients receiving bortezomib, lenalidomide and thalidomide therapy (13). In CLL, despite its clear diagnostic significance and *in vitro* evidence that CD200 overexpression generates an tolerogenic microenvironment by inhibiting T cell proliferation, suppressing tumor-specific T cell and expanding regulatory T cells, a negative role of CD200 on survival probability has not been demonstrated so far. Rather, Miao *et al.* reported a shorter time to treatment in CLL patients with low expression of CD200 (14).

Conversely, a negative impact of CD200 overexpression is emerging in myeloid neoplastic disease. Chen *et al.* reported a significant correlation between CD200 expression and WHO subtype and IPSS risk in a group of patients with myelodysplastic syndrome, and in multivariate analysis CD200 overexpression was found to have a negative prognostic role (15).

In 2007, Tonks *et al.* first reported on CD200 expression in 184 acute myeloid leukemia (AML). They found high frequency of CD200 and high intensity of expression in patients with core binding factor (CBF) associated translocations, that are generally associated with favorable response to therapy (16). Nonetheless, survival analysis stratified for CBF abnormalities demonstrated a lower OS probability in CD200 positive patients, indicating that CD200 have a negative prognostic value in AML. The same group later demonstrated that also in AML CD200 has the potential to induce the formation of Tregs, able to suppress the anti-leukemia response *in vitro* and thus regulating tumor immunity (17).

Our group evaluated the impact on survival and the association of CD200 with other prognostic factors in 244 patients with AML (18). CD200 aberrant expression was found in 56% of patients, with 30% of them displaying high intensity of expression. CD200 expression was more frequent in secondary AML, in CD34 positive cases, in Bcl2 overexpressing cases and in wild-type Flt3. Complete remission (CR) rate was significantly lower in CD200+ compared to negative ones (56% *vs.* 76%) and CD200 was associated to a shorter OS (3-year OS: 31% *vs.* 45%;). CD200 has an additive negative impact on survival in patients with unfavorable cytogenetic and in secondary leukemia; moreover, it exerted a worsening effect on prognosis of AML patients with favorable biological markers, such as mutated NPM, wild-type Flt3 and CD34 negativity.

In line with these results, we further analyzed CD200 expression in 139 patients with cytogenetically-normal (CN) AML (19). CD200 overexpression was again present in around half of the patients (48%), at high intensity in 28% of positive cases, and correlated with CD34 and Bcl2 expression. Also in the subset of CN-AML, CD200 expression was associated with a lower rate of CR (63% *vs.* 79%), with a further reduction in cases with high CD200 expression (50%). Three-year OS was 51% in CD200– and 27% in CD200+ patients, with a significant difference among cases with low or high CD200 expression (35% *vs.* 0%).

Moving from the observation of the frequent co-expression of CD200 and Bcl2, we investigated the role of concomitant aberrant CD200 and Bcl2 expression on outcome of 291 adult AML patients. The 94 patients (32%) displaying double positivity (DP) had the lower CR rate (57%, compared to 64% in the whole population and 77% in double negative patients) and the shorter survival (3-year OS 23%) (personal data, unpublished).

Taken together, this data points to CD200 as a direct or indirect target of new immunomodulating agents. There is evidence in CLL that the BTK/ITK inhibitor ibrutinib and the selective BTK inhibitor acalabrutinib increase effector and effector memory subsets and down-modulate expression of immunosuppressive CD200 and BTLA molecules, thus restoring immune reactivity (20), and recent *in vitro* data suggest its potential activity also in AML (21). Moreover, considering the linkage between PD1–PD1L1 and CD200/CD200R pathways in modulation of immune response, also PD1 inhibitors could be candidates to reverse the

immunosuppressive milieu induced by CD200. Finally, a monoclonal antibody anti CD200 (omalizumab, ALSX6000, Alexion Pharmaceuticals) is under investigation not only in lymphoproliferative disease but also in elderly, *de novo* AML patients.

In conclusion, although CD200 flow cytometric evaluation should be considered in selected patients, either for prognostication of if candidate for new target therapy involving CD200 pathway. Inter-laboratory trials should be planned to standardize analysis, make results comparable and find the appropriate cut-off level of expression.

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Footnote

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