Original Article

Flow Cytometry Assessment of CD26$^+$ Leukemic Stem Cells in Peripheral Blood: A Simple and Rapid New Diagnostic Tool for Chronic Myeloid Leukemia

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Background: Recent investigations in chronic myeloid leukemia (CML) have focused on the identification and characterization of leukemic stem cells (LSCs). These cells reside within the CD34$^+$/CD38$^-$/Lin$^-$ fraction and score positive for CD26 (dipeptidylpeptidase IV) a marker, expressed in both bone marrow (BM) and peripheral blood (PB) samples, that discriminates CML cells from normal hematopoietic stem cells (HSCs) or from LSCs of other myeloid neoplasms. CD26 evaluation could be a useful tool to improve the identification of CML LCSs by using flow-cytometry assay.

Methods: CD26$^+$ LSCs have been isolated from EDTA PB and BM samples of patients with leucocytosis suspected for CML. Analysis of LSCs CML has been performed by using custom-made lyophilized pre-titrated antibody mixture test and control tube and a CD45$^+$/CD34$^+$/CD38$^-$/CD26$^+$ panel as a strict flow cytometric gating strategy.

Results: The expression of CD26 on CD34$^+$/CD38$^-$ population was detectable in 211/211 PB and 84/84 BM samples of subsequently confirmed BCR-ABL$^+$ CP-CML patients. None of the 32 samples suspicious for CML but scoring negative for circulating CD26$^+$ LSCs were diagnosed as CML after conventional cytogenetic and

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molecular testing. To validate our results, we checked for PB CD26$^+$ LSCs in patients affected by other hematological disorders and they all scored negative for CD26 expression.

Conclusions: We propose flow cytometry evaluation of CD26 expression on PB CD34$^+$/CD38$^-$ population as a new rapid, reproducible, and powerful diagnostic tool for the diagnosis of CML.

Key terms: chronic myeloid leukemia; leukemic stem cells; CD26$^+$; flow cytometry; diagnosis; peripheral blood

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INTRODUCTION

Chronic myeloid leukemia (CML) is a myeloproliferative disorder characterized by increased proliferation and accumulation of immature myeloid cells in the peripheral blood (PB) and bone marrow (BM) of CML patients, without the loss of their capacity to differentiate. Its incidence is one to two cases per 100,000 adults and it accounts for approximately 15% of newly diagnosed cases of leukemia (1,2). The increase of myeloid precursors is due to a specific acquired genetic alteration in the DNA of the hematopoietic stem cells (HSCs) that behave as disease-initiating leukemic stem cells (LSCs), gaining a proliferative advantage and/or aberrant differentiation capacity over the normal counterpart to give rise to the expanded myeloid compartment (3,4).

CML is one of the best-characterized leukemias at a molecular level. The translocation t(9;22)(q34;q11), that leads to the formation of the Philadelphia chromosome (Ph) and of the BCR-ABL1 fusion protein, is found in up to 95% of patients affected by CML, and other additional complex rearrangements are found in 5–10% of the remaining patients (5,6). Criteria for an appropriate CML diagnosis consist of documenting, in the setting of persistent unexplained leukocytosis, the presence of the Ph chromosome by cytogenetic analysis, or the Ph-related molecular BCR-ABL1 abnormalities by fluorescence in situ hybridization (FISH) or by molecular studies (7). However, in some cases it may be difficult to differentiate CML from other myeloproliferative or myelodysplastic syndromes that could harbor the BCR-ABL1 fusion, and moreover, both cytogenetic and molecular analysis are costly and require several days to be completed.

New attempts for a fast and reliable CML diagnosis comprise the use of flow cytometry for the detection of the BCR-ABL transcript, by using antibodies able to directly bind to the leukemic BCR-ABL1 clone (8) or by quantifying leukocytes harboring the BCR-ABL1 fusion at the protein level (9). Nevertheless, those methods are time-consuming and thus could not substitute the cytogenetic or molecular tests as routine analysis; additionally being still focused on the search of the BCR-ABL product, they appear redundant with respect to standard assays for CML. A step forward in the development of a rapid CML diagnostic tool could be represented by flow cytometry direct evaluation of CML LSCs, so to overcome also the variability between CML patients on the BCR-ABL transcript level.

In CML, LSCs supposedly reside within the CD34$^+$/CD38$^-$/Lin$^-$ fraction; however, normal HSCs also exhibit this phenotype (10) so that additional markers are required to discriminate CML LSC from normal HSCs (11–13). Recent researchers have focused on the identification and characterization of LSCs, and Herrmann et al. identified CD26 (dipeptidylpeptidase IV) as a potential biomarker for the quantification and isolation of CML LSCs in BM samples of CML patients (14,15). In fact, in contrast to other tested antigens which are co-expressed on CML LSCs, acute myeloid leukemia (AML) LSCs, and normal HSCs, CD26 was the only marker, expressed in all tested bone marrow CP CML patients, which was not present on CD34$^+$/CD38$^-$ SC in normal BM or on LSCs of other myeloid neoplasms. The proof of stemness of CD34$^+$/CD38$^-$/CD26$^+$ population has been demonstrated by Hermann et al. in an NSG mice model where CD26$^+$ cells derived from CP CML patients were capable of inducing BCR-ABL$^+$ engraftment (14).

The work by Culen et al. further demonstrated that flow cytometry approach could be a useful tool for the identification of CML LSCs on BM samples by using a CD45$^+$/CD34$^+$/CD38$^-$/CD26$^+$ panel as a strict gating strategy (16).

We have recently demonstrated that in CML patients at diagnosis CD34$^+$/CD38$^-$/CD26$^+$ LSCs are easily measurable also in PB and that residual circulating CD26$^+$ LSCs persist, at lower level, in most patients during treatment with tyrosine kinase inhibitors (TKIs) and even after successful TKI discontinuation (17). Based on these premises, we here focus deeper on the feasibility and specificity of the detection of PB CD26$^+$ LSCs and propose flow cytometry as a new approach for a rapid screening of any suspicious leukocytosis as well as a reliable diagnostic tool for CML.

MATERIALS AND METHODS

Patients Cohort

Subjects with leukocytosis suspected for CML, referring to several Italian Hematology centers, entered this multicenter study (including 243 patients). All participating subjects provided an informed consent in accordance with their referred hospital policy and agreed upon having their blood or bone marrow assayed in Siena Flow Cytometry Laboratory in addition to the diagnostic tests performed locally. In order to validate our results, patients with blood disorders other than CML, and normal HSCs donors treated with granulocyte-colony stimulating factors (G-CSF), were included to participate as negative controls.

Sample Preparation

Three milliliters of EDTA PB samples (all 211 subjects) or 1 mL of EDTA BM sample (only 84 subjects) were centrally analyzed at Flow-cytometry lab in Siena within 24 h of collection, since in preliminary experiments it was shown that within 24 h cell viability was superior to 80%. BM and PB leukocytes and red blood cells count was performed using a Unicell DxH 800 Coulter (Beckman Coulter, Brea, CA). In all PB and BM samples, the presence of $CD34^+/CD38^-/CD26^+$ population was evaluated by multiparametric flow cytometry analysis using a four-color staining standardized protocol with lyse stain wash procedure. Red cells' lysis was performed with BD Pharm Lyse™ ammonium chloride (Ref 555899, BD Biosciences, San Jose, CA), 1:10 diluted in deionized water, using BD FACS™ Lyse Wash Assistant (LWA) instrument (BD Biosciences, San Jose, CA). After lysis, 2.0×10^6 leucocytes per mL were incubated with a custom-made lyophilized pre-titrated antibody mixture test and control tube (Ref 625183, BD Biosciences, San Jose, CA), containing CD34-FITC (clone 581), CD26-PE (clone M-A261), CD38-APC (clone HIT2), and CD45-V500 (clone 2D1) for tube test and CD34-FITC (clone 581), anti IGg1 PE, CD38-APC (clone HIT2), and CD45-V500 (clone 2D1) for control tube.

Flow Cytometry Analysis

Analysis of BM and PB samples was performed on two 3-lasers, 8-colors BD FACSCanto™ II flow cytometers using the FACSDiva 8 software version 8.0.1 BD™ (BD Biosciences, San Jose, CA) in order to reach a sensitivity of 10^{-5}, and the acquisition and analysis of at least 1.0×10^6 cells. Instruments setup were monitored daily and, to ensure reproducible results over time, we followed a standardized protocol that implied adjustments of FACS internal parameters, using the BD FACSDiva™ CS&T IVD Beads (Ref 656047; BD Biosciences, San Jose, CA), to keep constant the instrument performance by correcting wear of lasers and fluidic instability (18). For compensation setup, BD OneFlow™ Setup Beads (Ref 658620; BD Biosciences, San Jose, CA) and BD™ FC Beads 8-color Kit (Ref 658621; BD Biosciences, San Jose, CA) were used. The $CD26^+$ population was identified by sequential gates with the aim to exclude debris and doublets, using the analysis procedure described and published in our previous work (16). As shown in Figure 1, firstly, the

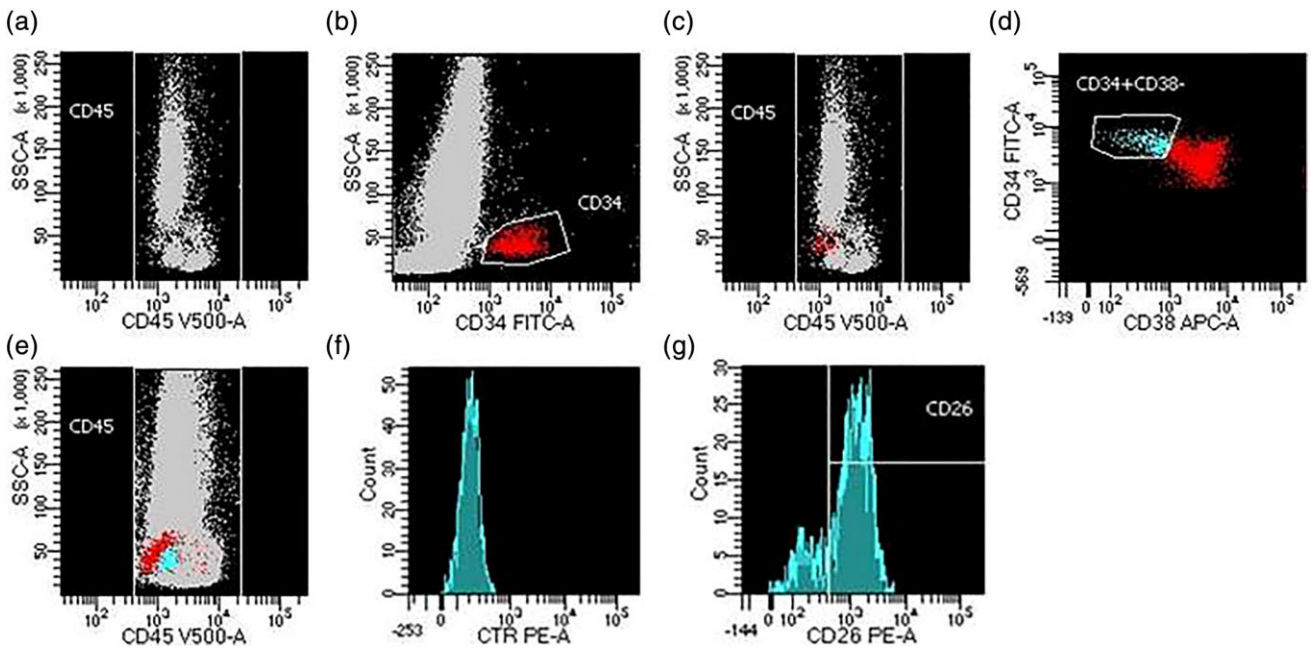

FIG. 1. Analysis of CD26 expression in PB $CD34^+$ fraction of suspected CML. **a**. CD45 versus SSC; **b**. Gate $CD34^+$ cells; **c**. $CD34^+$ cells display CD45 dim and SSC low; **d**. $CD34^+CD38^-$ cells in $CD34^+$; **e**. $CD34^+CD38^-$ cells display CD45 dim and SSC low; **f**. Expression of IgG isotipic control on $CD34^+$ $CD38^-$ gated cells; **g**. CD26 expression on $CD34^+CD38^-$ gated cells. [Color figure can be viewed at wileyonlinelibrary.com]

Table 1
CD26 flow cytometry evaluation in peripheral blood and bone marrow CML samples

	Total PB	PB untreated[1]	PB after cytoreductive treatment[2]	PB[3]	BM[3]
Samples	211	151	60	84	84
% $CD26^+$ cells within $CD34^+/CD38^-$ fraction	37,99	37,50	46,11	37,23	17,70
Median (range)	(1,11–99,85)	(1,33–99,85)	(1,11–98,57)	(5,59–98,57)	(5,12–98,18)
$CD26^+$ cells x 10^9/L	10,4625	8,53	19,2	15,54	13,17
Median (range)	(0,010–1037)	(0,010–1037)	(0,02–698)	(0,06–357)	(0,06–357)

[1]Untreated patients
[2]Patients treated with hydroxyurea
[3]Comparison between PB and BM CD26 evaluation

CD34 gate was performed on viable cells identified by FSC and SSC light properties (a–b); then, exclusively $CD34^+/CD38^-$ population was gated (d). Both $CD34^+$ and $CD34^+/CD38^-$ cells display $CD45^{dim}$ expression and low SSC (c–e). Figure 1f,g compare CD26 expression in control (f) and test tube (g).

RESULTS

Evaluation of $CD26^+$ CML LSCs

A total number of 243 patients with leukocytosis suspected of CML were tested for the presence of circulating PB $CD26^+$ LSCs. After flow cytometry evaluation 211/243 samples scored positive for the presence of the CD26 antigen and 211/211 (100%) were subsequently confirmed to be BCR-ABL1 positive by FISH and RT-PCR. None of the 32 samples resulting negative for the presence of $CD26^+$ LSCs was diagnosed as CML after conventional cytogenetic and molecular testing. In the 211 samples scoring $CD26^+$ LSCs positive, the median percentage of $CD26^+$ cells within the $CD34^+/CD38^-$ stem cell fraction was 37,99% (range 1,11–99,85) and the median absolute number of $CD26^+$ LSCs was $10{,}46 \times 10^9$/L (range $0{,}010–1037 \times 10^9$/L) (Table 1).

Comparison of $CD26^+$ CML LSCs Evaluation in PB versus BM Samples

In 84/243 cases the $CD34^+/CD38^-/CD26^+$ cells have been evaluated both in BM and PB samples. The results (Table 1) show that all BM samples of subsequently confirmed CML cases scored positive for the presence of $CD26^+$ LSCs. In particular, the proportion of $CD26^+$ cells within the $CD34^+/CD38^-$ cell fraction appeared to be lower in BM than that documented in PB (median value was 17,70%, range 5,12–98,18 vs 37,23%, range 5,59–98,57) but the absolute numbers in the BM and PB were superimposable (median value $13{,}17 \times 10^9$/L, range $0{,}21–365 \times 10^9$/L vs $15{,}54 \times 10^9$/L, range $0{,}06–357 \times 10^9$/L). Figure 2 compares CD26 expression in representative PB and BM samples. These results further confirmed the appropriateness to detect but also "quantify" LSCs directly from PB samples of CML patients.

Validation of $CD26^+$ CML LSCs Evaluation

As described above, 32/243 PB samples with suspicion of CML scored negative for $CD26^+$ LSCs. Most of them (23/32, 72%) turned out to be secondary inflammatory-related leukocytosis while 5/32 (16%) were subsequently concluded as myelodysplastic/myeloproliferative syndromes and 4/32 (12%) as myelofibrosis. In order to further validate our results, we checked for PB $CD26^+$ LSCs patients affected by other hematological disorders such as Ph^+ Acute Lymphoblastic Leukemia (5 cases), Ph^+ AML (2 cases), other chronic myeloproliferative neoplasms (18 cases) and normal HSCs donors treated with granulocyte-colony stimulating factors G-CSF (16 cases). In all these additional cases tested, different amount of circulating $CD34^+/CD38^-$ stem cells were detected but they always scored negative for the expression of CD26 antigen.

Eterogeneity of CML Patients Does Not Affect $CD26^+$ Evaluation

LSCs scored positive for CD26 antigen in all newly diagnosed CML cases, independently from the BCR-ABL transcript type of the patients. Indeed, from a molecular point of view, 207/211 patients had p210 BCR-ABL transcript, 3/211 had both p190 and p210 BCR-ABL transcript, while one patient had p230 transcript, yet all of them were positive for $CD26^+$ LSCs.

Moreover, 60/211 patients started hydroxyurea as a cytoreductive treatment before performing the flow cytometric analysis, but the drug seems not to affect the $CD26^+$ CML LSCs detection in the samples. Indeed, as showed in Table 1, the percentage and the absolute number of $CD26^+$ LSCs were very similar between treated and untreated patients (median value 46,11% range 1,11–98,57 vs 37,50% range 1,33–99,85, respectively; median value $19{,}2 \times 10^9$/L, range $0{,}02–698 \times 10^9$/L vs $8{,}53 \times 10^9$/L range $0{,}010–1037 \times 10^9$/L, respectively).

DISCUSSION

To our knowledge, this is the first study in which the feasibility and specificity of detecting $CD26^+$ LSCs in PB were investigated for diagnostic purpose in CML patients. Recent reports have shown that CML LSCs are

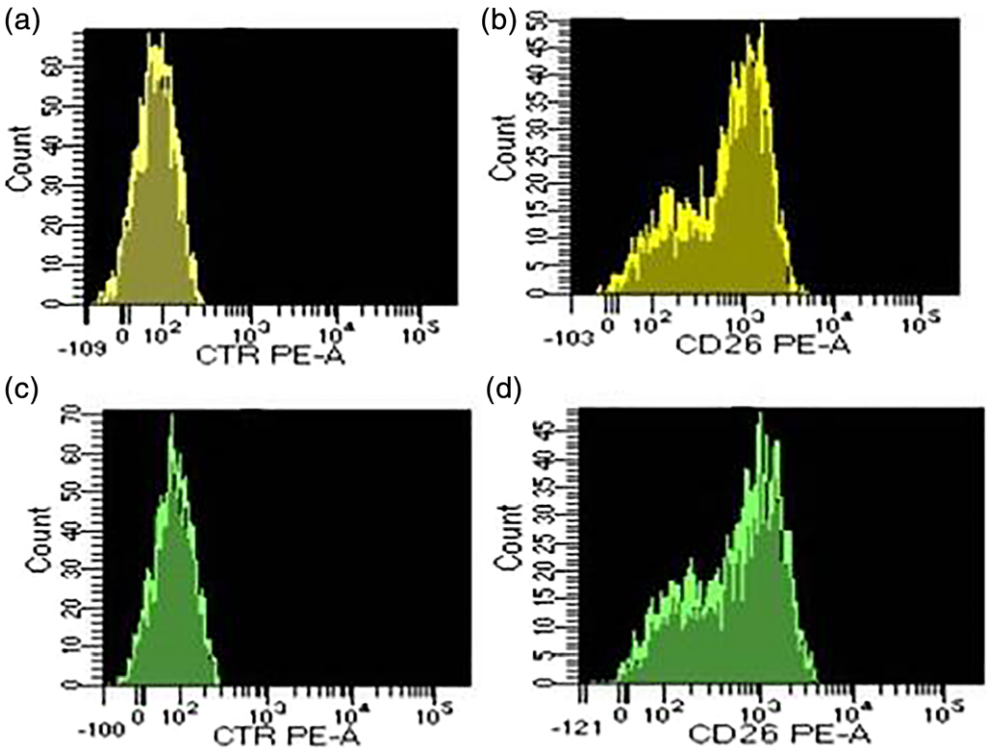

FIG. 2. CD26 expression in PB and BM $CD34^+$ fraction of suspected CML. **a**. Expression of IgG isotipic control on PB $CD34^+CD38^-$ gated cells; **b**, CD26 expression on PB $CD34^+CD38^-$ gated cells; **c**. Expression on IgG isotipic control on BM $CD34^+CD38^-$ gated cells; **d**. Expression on BM $CD34^+CD38^-$ gated cells. [Color figure can be viewed at wileyonlinelibrary.com]

restricted to the $CD34^+/CD38^-$ population and can be detected in BM of patients at the onset of the disease by co-expression of CD26 (dipeptidylpeptidase IV), an enzyme that disrupts LSC-niche interactions by degrading SDF-1 (15). CD26 may be considered a robust specific marker of bone marrow LSCs in CML because it is not detected on normal SC or in LSCs from other hematopoietic malignancies but the role of detecting PB $CD26^+$ LSCs as diagnostic tool for CML has not been rule out yet (14,15).

Our results clearly demonstrate that the expression of CD26 on $CD34^+CD38^-$ population was detectable in 211/211 PB samples of subsequently confirmed newly diagnosed CP-CML patients and was also demonstrable in 84/84 CML BM aspirates. None of the 32 samples suspicious for CML but scoring negative for circulating $CD26^+$ LSCs was diagnosed as CML after conventional cytogenetic and molecular diagnostic workout. These results not only confirm the specificity of the expression of CD26 as a marker of CML LSCs, but also clearly assess that this population is a robust and reliable PB hallmark for CML, to be assayed in a rapid, low-cost, patient-friendly, flow cytometry test to quickly rule out the suspicion of CML.

In fact, the determination of a $CD34^+/CD38^-/CD26^+$ population in PB by flow cytometry, using a four color staining with lyse stain wash procedure, is a simple, easy to perform, reliable, and accurate technique that provides results in a very short time. Having at disposition custom-made lyophilized pre-titrated antibody mixture tubes makes even easier and faster the analysis of CML LSCs and makes possible a high standardization of laboratory procedures. Moreover, the identification of $CD26^+$ LSCs retains its diagnostic value independently from the molecular BCR-ABL transcript of CML patients and it is to not influenced by pre-diagnosis cytoreductive treatment of samples.

The lack of invasiveness for the patient and the rapidity of the test (about 3 h from blood drawing to results) together with its demonstrated high specificity, suggest PB $CD26^+$ LSCs flow cytometry assay to be used as first line test in all cases of leukocytosis suspected for CML or other chronic myeloproliferative disorders helping the clinicians to better manage the patient in the short term. In fact, if conventional cytogenetics, FISH and RT-PCR analysis for BCR-ABL remain the gold standard assays for the diagnosis of CML, they suffer from a longer time of execution and results, a more relevant cost and may not be available everywhere or at the time the patients need to be evaluated. The possibility to easily confirm the diagnosis of CML by a simple and rapid blood test at any time could help clinicians to better timing subsequent specific and more invasive assays as well as to avoid useless and costly analysis. In the current era where health care costs are constantly increasing, we should forthcoming any effort to "target" also the diagnostic workout and to avoid waste of resources.

In conclusion, our data strongly suggest that circulating $CD26^+$ LSCs are a hallmark of CML and we propose flow cytometry evaluation of CD26 expression on PB $CD34^+/CD38^-$ population as a new powerful diagnostic tool for the diagnosis of CML. In addition, work is in progress to confirm $CD26^+$ LSC evaluation as an eligible test for MRD monitoring and for the identification of patients suitable for TKI discontinuation.

Literature Cited

1. Jabbour E, Kantarjian H. Chronic myeloid leukemia: 2016 update on diagnosis, therapy and monitoring. Am J Haematol. 2016;91: 252–265.
2. Huang X, Cortes J, Kantarjian H. Estimations of the increasing prevalence and plateau prevalence of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. Cancer. 2012;118: 3123–3127.
3. Faderl S, Talpaz M, Estrov Z, O'Brien S, Kurzock R, Kantarjian H. The biology of chronic myeloid leukemia. N Engl J Med. 1999;341: 164–172.
4. Melo JV, Barnes DJ. Chronic myeloid leukemia as model of disease evolution in human cancer. Nat Rev Cancer. 2007;7:441–453.
5. Rowley JD. Letter: A new consistent chromosomal abnormality in chronic myelogenous leukaemia identified by quinacrine fluorescence and Giemsa staining. Nature. 1973;243:290–293.
6. Luatti S, Baldazzi C, Marzocchi G, Ameli G, Bochicchio MT, Soverini S, Castagnetti F, Tiribelli M, Gugliotta G, Martinelli G, et al. Cryptic BCR-ABL fusion gene as variant rearrangement in chronic myeloid leukemia: Molecular cytogenetic characterization and influence on TKIs therapy. Oncotarget. 2017;8:29906–29913.
7. Jabbour E, Cortes JE, Kantarjian HM. Molecular monitoring in chronic myeloid leukemia: Response to tyrosine kinase inhibitors and prognostic implications. Cancer. 2008;112:2112–2118.
8. Lucas CM, Fagan JL, Carter A, Swale B, Evans C, Clark RE, Harris RJ. Rapid diagnosis of chronic myeloid leukemia by flow cytometric detection of BCR-ABL1 protein. Haematologica. 2011; 96:1077–1078.
9. Löf L, Arngården L, Olsson-Strömberg U, Siart B, Jansson M, Dahlin JS, Thörn I, Christiansson L, Hermansson M, Larsson A, et al. Flow cytometric measurement of blood cells with BCR-ABL1 fusion protein in chronic myeloid leukemia. Sci Rep. 2017;7:623.
10. Cimato TR, Furlage RL, Conway A, Wallace PK. Simultaneous measurement of human hematopoietic stem and progenitor cells in blood using multi-color flow cytometry. Cytometry B Clin Cytom. 2016;90:415–423.
11. Jiang X, Zhao Y, Smith C, Gasparetto M, Turhan A, Eaves A, Eaves C. Chronic myeloid leukemia stem cells possess multiple unique features of resistance to BCR-ABL targeted therapies. Leukemia. 2007;21:926–935.
12. Valent P. Emerging stem cell concepts for imatinib-resistant chronic myeloid leukaemia: Implications for the biology, management, and therapy of the disease. Br J Haematol. 2008;142: 361–378.
13. Kavalerchik E, Goff D, Jamieson CH. Chronic myeloid leukemia stem cells. J Clin Oncol. 2008;26:2911–2915.
14. Herrmann H, Sadovnik I, Cerny-Reiterer S, Rulicke T, Stefanzl G, Willmann M, Hoermann G, Martin Bilban M, Blatt K, Herndlhofer S, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. Blood. 2014; 123:3951–3962.
15. Valent P, Sadovnik I, Ráčil Z, Herrmann H, Blatt K, Cerny-Reiterer S, Eisenwort G, Lion T, Holyoake T, Mayer J. DPPIV (CD26) as a novel stem cell marker in Ph+ chronic myeloid leukaemia. Eur J Clin Invest. 2014;44:1239–1245.
16. Culen M, Borsky M, Nemethova V, Razga F, Smejkal J, Jurcek T, Dvorakova D, Zackoval D, Weinbergerova B, Semerad L, et al. Quantitative assessment of the $CD26^+$ leukemic stem cell compartment in chronic myeloid leukemia: Patient-subgroups, prognostic impact, and technical aspects. Oncotarget. 2016;7:33016–33024.
17. Bocchia M, Sicuranza A, Abruzzese E, Iurlo A, Sirianni S, Gozzini A, Galimberti S, Aprile L, Martino B, Pregno P, et al. Residual peripheral blood $CD26^+$ leukemic stem cells in chronic myeloid leukemia patients during TKI therapy and during treatment-free remission. Front Oncol. 2018;8:194.
18. Orrù V, Steri M, Sole G, Sidore C, Virdis F, Dei M, Lai S, Zoledziewska M, Busonero F, Mulas A, et al. Genetic variants regulating immune cell levels in health and disease. Cell. 2013;155: 242–256.