Investigating the Presence of Chronic Myeloid Leukemia Leukemic Stem Cells (CML LSCs) in Patients with Tyrosine Kinase Inhibitors Who Achieved Cytogenetic / Molecular Response

Zehra Narli Ozdemir1,2*, Atilla Uslu1,2, Selami Kocak Toprak1, Pervin Topcuoglu1, Klara Dalva4, Osman Ilhan1,2

1Department of Hematology, Faculty of Medicine, Ankara University, Ankara, Turkey
2Department of Turkish Stem Cell Foundation, Ankara, Turkey

*Corresponding author: Zehra Narli Ozdemir, Department of Hematology, Faculty of Medicine, Ankara University, Ankara, Turkey. Tel: +905559171054; Email: zehranarli@hotmail.com


Received Date: 12 July, 2018; Accepted Date: 17 August, 2018; Published Date: 27 August, 2018

Abstract

Background-Aim: In Chronic Myeloid Leukemia (CML), patients receiving treatment with tyrosine kinase inhibitors (TKIs) achieve complete cytogenetic response (CCyR); however, BCR-ABL-positive cells can be still detected. The interleukin-1 Receptor Accessory Protein (IL-1RAcP) was previously identified on candidate CML stem cells having CD34(+) and CD38(-) expressions. This study aimed to demonstrate the presence of CML LSCs by investigating IL-1RAcP expression in chronic phase CML patients who were at diagnosis or on first- or second-generation TKIs.

Methods: This study included bone marrow or peripheral blood samples collected from CML patients (median age, 50 years), of whom 7 were newly diagnosed patients and 8 were on TKI treatment with either first-generation (n=7) or second-generation (n=1) TKIs. The CML-LSC, CD34(+), CD38(-), and IL-1RAcP(+) cells were detected through flow cytometry.

Results: In all patients, the median number of LSCs and CML LSCs were 642 within 10⁶ cells (range, 37-6092) and 4 within 10⁶ cells (range, 0-35), respectively. The median number of CML LSCs was higher in the newly diagnosed patients (8 within 10⁶ cells [range, 1-35]) than in those on TKI treatment (3 within 10⁶ cells [range, 0-27]). In this cohort, all treated patients were in the chronic phase CML with CCyR. The median number of CML LSCs was 4 within 10⁶ cells (range, 0-27) in 7 of 8 patients who were on treatment and had CCyR and major molecular response. One of the patients providing peripheral blood sample was on imatinib therapy and lost major molecular response with 0 CML LSCs. The number of CML LSCs in the patient receiving second-generation TKI was 2 within 10⁶ cells.

Conclusion: This study demonstrated that CML stem cells persisted in the bone marrow despite TKI treatments. Monitoring CD34(+), CD38(-), and IL-1RAcP(+) CML LSCs with flow cytometry both in the peripheral blood and bone marrow samples may be practical to follow the disease response to TKIs in CML patients.

Keywords: Chronic Myeloid Leukemia, Flow Cytometry, IL-1RAcP, Leukemic Stem Cells, Tyrosine Kinase Inhibitors

Introduction

Chronic Myeloid Leukemia is characterized by robust marrow and extramedullary myeloid cell production due to constitutively active tyrosine kinase P210 BCR/ABL1. P210 BCR/ABL1, which is the product of Philadelphia (Ph) chromosome, is formed through a reciprocal translocation between chromosomes 9 and 22 [1, 2]. It has been shown that the Ph chromosome arises from the chronic myelocytic leukemia stem cells as it clonally can be found both in the myeloid and lymphoid cells [3]. CML is a disease that involves heterogeneous myeloid cells (at various maturation stages) that arise from CML stem cells with aberrant self-renewal capacity [4]. It is important to clarify the paradigm of both genetic and epigenetic happenings that drive aberrant stem/progenitor cell differentiation, self-renewal, and survival during both chronic and blastic phases of the disease [4].
Previous studies have suggested that quiescent CML leukemic stem cells (LSCs) are resistant to therapies targeting rapidly dividing cells [5, 6]. CML patients in deep molecular response (MR), which is defined as a ≥4-log reduction (MR4) or a ≥4.5-log reduction (MR4.5) with undetectable BCR-ABL1 transcripts, may have sustained MR after TKI withdrawal. On the other hand, MR4.5 has been defined as a novel molecular predictor for long-term outcome and survival in CML patients [7]; in the STOP second-generation (2G)-TKI study, 43.3% of the patients reported to experience a molecular relapse after TKI withdrawal [8]. Well-established predictive criteria to maintain treatment-free remission in CML are still missing. Molecular relapse probably occurs due to residual quiescent TKI-resistant LSCs that are not easily detectable by flow cytometry.

Interleukin-1 Receptor Accessory Protein (IL-1RAcP), which is a co-receptor for Interleukin (IL)-1 and IL-33 receptors, was previously identified on candidate CML stem cells having CD34(+) and CD38(-) expressions [9]. Considering that IL-1RAcP may be a promising novel therapeutic target for cellular therapies and therapeutic antibodies against the CML stem cells, the present study aimed to demonstrate the presence of CML LSCs in chronic phase CML patients who were at diagnosis or on first- or second-generation TKIs by investigating IL-1RAcP expression.

Methods

The present study included bone marrow or peripheral blood samples collected from CML patients (median age, 50 years) at Ankara University Faculty Of Medicine, Hematology Department between 2013 and 2015, of whom 7 were newly diagnosed patients and 8 were on TKI treatment with either first-generation (n=7) or second-generation (n=1) TKIs. This study was approved by the Local Ethics Committee and conducted in accordance with the Declaration of Helsinki. Informed consents of the patients were also obtained.

Bone marrow or peripheral blood samples of the patients were collected into EDTA containing tubes and analyzed within 4 hours of collection. In order to potentially detect CML-LSC, CD34(+), CD38(-), and IL-1RAcP(+) cells were selected from the CD34(+) cell population. In summary, 2x10^6 cells/sample were incubated with 10 µL of anti-human-IL-1RAcP-Biotin. After 30 minutes of incubation with this monoclonal antibody, 10 µL of other antibodies [CD45-PC5.5, CD34-APC, and CD38-FITC (Beckman Coulter, Navios, USA)] and the secondary antibody (Streptavidin-PE; R&D systems) were added to the tube and incubated for an additional 20 minutes at room temperature. Upon a lysis step of 10 minutes with Optlyse C, the cells were washed for 2 times (5 minutes each at 300×g) with Phosphate Buffered Saline (PBS) and then re-suspended in 500 µL of PBS. The acquisition of the cells was performed using the Navios Flow cytometer (3 laser-10 color, Beckman Coulter). Upon the daily checks of the instrument, 10^5 cells for each sample were acquired and the collected data was analyzed using the Kaluza software (Beckman Coulter, Navios, USA). After the selection of IL-1RAcP (+) cells within the CD34(+) cell population and these cells were also checked for CD38 expression. The CD34(+), IL-1RAcP (+), and CD38(-) cells were accepted as CML LSCs (Figure 1).

Results

The general characteristics and flow cytometry findings of the patients included in the present study (n=15) are presented in (Table 1). In all patients, the median number of LSCs was within 10^6 cells (37-6092) and the median number of CML LSCs was 4 within 10^6 cells (range, 0-35). As expected, the median number of CML LSCs was found to be higher in the newly diagnosed patients; 8 within 10^6 cells [range, 1-35]) and 3 within 10^6 cells [range, 0-27], respectively. In this cohort, all treated patients were in the chronic phase CML with complete cytogenetic response (CCyR). The median number of CML LSCs was 4 (range, 0-27) in 7 of 8 patients who were on treatment and had CCyR and major molecular response (MMR). One of the patients from whom peripheral...
blood was drawn was on imatinib therapy (400 mg/per day) and lost MMR with 0 CML LSCs. The number of CML LSCs in the patient receiving second-generation TKI was 2 within 10^6 cells and clinically, there was no difference in disease progress of this patient.

### Table 1: General characteristics and flow cytometry findings of the patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>CML patients (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>50 (25-79)</td>
</tr>
<tr>
<td>Patients on 1st/2nd-generation TKIs</td>
<td>7/1</td>
</tr>
<tr>
<td>Number of LSCs (n=15)</td>
<td>642 within 10^6 (37-6092)</td>
</tr>
<tr>
<td>Number of CML LSCs in the blood drawn</td>
<td>4 within 10^6 cells (0-35)</td>
</tr>
<tr>
<td>Patients on treatment (n=8)</td>
<td>3 within 10^6 cells (0-27)</td>
</tr>
<tr>
<td>Newly diagnosed patients (n=7)</td>
<td>8 within 10^6 cells (1-35)</td>
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</tbody>
</table>

1All treated patients were in the chronic phase CML with complete cytogenetic response.

CML, chronic myeloid leukemia; TKI, tyrosine kinase inhibitor; FCM, flow cytometry; LSCs, leukemic stem cells.

### Discussion

The present study aimed to evaluate blood circulating and bone marrow LSCs at diagnosis and during TKI treatment in CML patients who even had CCyR. Accordingly, by analyzing IL-1RAcP expression on the CD34(+) CD38(-) CML, it was demonstrated that the newly diagnosed CML patients had CML-specific IL-1RAcP(+) LSCs and that CML LSCs were also present in the patients on the first- or second-generation TKIs even they were in sustained MMR. In their study, Bocchia et al. [10], reported that there was no correlation between the type of TKI and the persistence of circulating LSCs and between the burden of PB CD26(+) LSCs and the depth of MR expressed as BCR-ABL1IS ratio or as MR categories. We suggested that flow cytometry performed for CD34(+), CD38(-), and IL-1RAcP(+) LSCs appeared feasible and suitable for monitoring residual stem cells in CML, for which predictive cut-off value for residual LSCs is an unmet need. Researchers try to fill the gap in determining the threshold value of residual CML stem or progenitor cells to predict disease clinical course. Stem Cell Monitoring for CML Patients Undergoing Nilotinib Therapy (NCT02353728) is one of the continuous clinical trials that may answer these issues.

Although the present study was conducted in a small CML cohort, its results are promising in developing treatments, particularly cellular therapies targeting the CML stem cells, which are likely to provide cure in CML. Targeting LSC expressing IL-1RAcP protein with chimeric antigen receptor (CAR)-T cell (NCT02842320) is an ongoing clinical trial and its results are highly anticipated.

Approximately 10% to 20% of all CML patients with MR are initiated to dose de-escalation and/or are discontinued from the treatment and 50% of these patients experiences relapse within 12 months [11,12]. Deeper MR obtained from the peripheral blood of CML patients is not predictive for treatment free response. In the present study, one of our patients in whom the measurements were performed from the peripheral blood sample lost MR with 0 CML LSCs. We are in the opinion that although CML LSCs could not be detected in the peripheral blood, bone marrow was still harboring residual quiescent LSCs. Deeper MR obtained from the bone marrow can prognosticate the eradication of CML LSCs [22].

Autophagy is an adaptive survival mechanism involved in degradation of intracellular material by lysosomal pathway in response to cellular stress such as nutrient deprivation or caloric restriction [13]. Previous studies have proved that impairment in autophagy has a role in the initiation and development of leukemia as well as in oncoprotein stabilization in preleukemic cells in order to maintain oncogenic fusion proteins such as PML-RARA or BCR-ABL that can be degraded in autophagic vesicles [14-17]. Ianniciello et al., [18], reported that CML progenitor cells required autophagy to survive in hypoxia-induced dormancy status. However, inhibition of autophagy is a novel strategy to overcome TKI resistance; TKI treatment may cause induction of autophagy in CML LSCs and this cause the CML stem cells to never be eradicated [19]. In the present study, the median number of CML LSCs in the patients on TKI treatment was 3 (range, 0-27); this result may be attributed to the hypothesis of induction of autophagy in CML LSCs with TKI treatment.

Interleukin-1 receptor accessory protein is emerging as a new therapeutic target due to being a surface molecule persistently overexpressed across myeloid malignancies particularly in CML [20] and in various subtypes of acute myeloid leukemia [21]. Therapeutic antibodies for blocking the intracellular signals from the IL-1RAcP target molecule and antibody-dependent cellular cytotoxicity against IL1-RAcP expressing tumor cells are the current topics for solid tumors in clinical trials (NCT03267316). It has been claimed that anti IL-1RAcP antibodies stimulates natural killer cells to attack cancer cells. We are in the opinion that the concept of antibody-mediated cytotoxicity of CML stem cells expressing IL-1RAcP may also have the synergistic effect with TKIs.

### Conclusion

Advances in CML therapy with TKIs have led to significant imp-
movements in survival rates for CML patients. On the other hand, the results of the present study demonstrated that CML stem cells persisted in the bone marrow despite TKI treatments and that monitoring CD34(+) CD38(-), and IL-1RAcP(+) CML LSCs with flow cytometry both in the peripheral blood and bone marrow samples may be practical to follow the disease response to TKIs in CML patients.

References


