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ORIGINAL ARTICLE

Prognostic Significance of Regulatory T-Cells and PD-1 + CD8 T-Cells in Chronic Myeloid Leukemia Patients Treated with Generic Imatinib

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Abstract

The impact of T-regulatory cells (Tregs), PD-1 + CD8 T-cells, and their dynamics during treatment with imatinib mesylate remains poorly understood in patients with chronic myeloid leukemia (CML). We conducted a prospective study on newly diagnosed, treatment-naïve adult (> 18 years old) patients with CML in the chronic phase (CP) and age- and sex-matched controls. Peripheral blood samples were collected at diagnosis and after three months of imatinib therapy to assess Tregs and PD-1 + CD8 T-cell levels using flow cytometry. The study comprised 57 patients with a median age of 39 years, including 27 males (47%). At baseline, the mean percentage of Tregs was significantly higher in CML patients (3.6 ± 0.32%) compared to controls (1.58 ± 0.21%) ($p < 0.0001$) but decreased significantly after three months of imatinib treatment (1.73 ± 0.35%) ($p < 0.0001$). Baseline Treg% exhibited positive correlations with Sokal (r = 0.29), Hasford (r = 0.33), EUTOS (r = 0.28), and ELTS (r = 0.31) risk scores ($p < 0.05$), as well as with the BCR-ABL transcript levels at three months ($p = 0.03$). Furthermore, the mean baseline percentage of PD-1 + CD8 T-cells was significantly elevated in CML patients (7.66 ± 0.36%) compared to controls (2.65 ± 0.32%) ($p < 0.0001$) and also decreased after treatment (3.44 ± 0.37%) ($p < 0.0001$). The baseline percentage of PD-1 + T-cells demonstrated positive correlations with Sokal (r = 0.26), Hasford (r = 0.27), and ELTS (r = 0.41) risk scores ($p < 0.05$). Our findings reveal a significantly higher proportion of Tregs and PD-1 + CD8 T-cells in patients with CML-CP compared to healthy controls, notably diminished following imatinib treatment. These observations suggest the potential for immunotherapy as a promising approach to managing immune exhaustion in CML patients.

Keywords CML · Imatinib · T-regulatory cells · PD1 + CD8 T-cells

Introduction

Chronic myeloid leukemia (CML) is a myeloproliferative neoplasm driven by the oncogenic BCR-ABL1 tyrosine kinase [1]. The immune system has been recognized as playing a crucial role in the initiation and progression of CML [2]. Among the immune cell subsets involved, CD4 + CD25 + FoxP3 + T-regulatory cells (Tregs) are a specific population of T-cells responsible for maintaining immune homeostasis and self-tolerance through the suppression of immune responses [3]. In the tumour microenvironment, the presence of Tregs contributes to the evasion of immune surveillance by malignant cells [4]. Another significant player in immune regulation is the programmed death receptor-1 (PD-1), an inhibitory immune checkpoint receptor expressed on activated cytotoxic CD8 T-cells [5]. Through interaction with its ligand, programmed death receptor-1 ligand (PDL-1), expressed on tumour cells, the PD-1 pathway induces T-cell silencing, leading to immune evasion and disease progression [6, 7].

Imatinib mesylate, a tyrosine kinase inhibitor (TKI), is widely used to treat CML in the chronic phase (CP) [8]. Beyond its primary effects, growing evidence suggests that TKIs may modulate the immunological microenvironment by influencing Tregs and the PD-1-PDL-1 interaction, ultimately contributing to treatment response in CML [9–13]. This study investigated the dynamics of Tregs and

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PD-1 + CD8 T-cells in newly diagnosed and treatment-naïve CML-CP patients before and after imatinib therapy. Furthermore, we compared their levels with those of age- and sex-matched healthy controls and explored their correlations with disease severity indices.

Methods

Study Population

Consecutive, newly diagnosed patients with CML in the chronic phase (CML-CP) who were initiated on a once-daily dose of 400 mg imatinib were included in this study after obtaining informed consent. The institutional ethics committee approved the study protocol, and it was conducted according to the principles outlined in the Declaration of Helsinki.

Diagnosis and Risk Assessment

CML-CP diagnosis was established based on bone marrow examination and detection of BCR-ABL1 transcript using real-time polymerase chain reaction (RT-PCR). The Sokal risk score, Hasford score, and European Treatment and Outcome Study (EUTOS) score were calculated using online tools to assess disease severity.

Sample Collection and Processing

Peripheral blood samples (3 ml) anticoagulated with EDTA were collected from patients at diagnosis and after three months of imatinib treatment. Age- and sex-matched healthy individuals from the community served as controls and underwent similar evaluations. Red blood cell lysis, cellular staining, and flow cytometry analyses were performed. Tregs were identified by staining with fluorochrome-conjugated monoclonal antibodies against human CD3, CD4, CD25, CD127, and FoxP3. PD-1 + CD8 T cells were analysed using anti-CD8 and CD279 antibodies. Flow cytometry data acquisition was performed using a triple laser BD FACS Canto II flow cytometer, and data analysis was conducted using BD FACS DIVA software (Becton Dickinson, USA). The percentage of CD4 + CD25highCD127lowFoxP3 + Tregs was determined relative to CD4 T lymphocytes (Fig. 1), while the percentage of CD8 + CD279high PD-1 + CD8 T cells was calculated relative to CD8 T lymphocytes.

Follow-Up

Patients underwent clinical examinations and regular blood counts every two weeks to monitor clinical and haematological remission. At the three-month follow-up, the percentage of BCR-ABL transcripts was determined using RT-PCR.

Statistical Analysis

Descriptive statistics, including mean (95% CI), median, standard error of the mean, and standard deviation, were used to summarise continuous variables. The Mann–Whitney U test was employed to compare the percentages of Tregs and PD-1 + CD8 T-cells between patients and healthy controls. Changes in the percentages of Tregs and PD-1 + CD8 T-cells before and after treatment in patients were analysed using parametric (paired t-test) or non-parametric (Spearman's rank test), depending on the data distribution. Pearson's test was utilised to assess correlations. Statistical analyses were performed using SPSS software version 22.0. The Chi-square test was employed to analyse qualitative variables. A p-value of less than 0.05 was considered statistically significant. Values were presented as mean ± SD.

Results

The study was conducted at a single centre from January 2017 to June 2018. During this period, a total of 60 cases of CML were screened. Three patients were excluded from the study due to being in the accelerated or blast phases, resulting in a final sample of 57 treatment-naive CML-CP patients. Age- and sex-matched healthy volunteers (n = 30) were included as controls.

The median age of the patients was 39 years, with 27 (47%) being males. The demographic details and disease characteristics are presented in Table 1. The mean Treg% in CML patients (3.61 ± 0.32%) was significantly higher than that in healthy controls (1.58 ± 0.21%, $p < 0.0001$). Higher-risk groups defined by Sokal, Hasford, EUTOS, and ELTS scores exhibited higher mean Treg% ($p < 0.05$).

After three months of treatment with imatinib, all 57 patients demonstrated a statistically significant reduction in Treg% ($p < 0.0001$). Furthermore, all but three patients achieved complete hematologic response (CHR) within three months. Although not statistically significant, patients who achieved CHR at six weeks (n = 34, 59.6%) had a numerically lower Treg% at presentation (3.57%) compared to those who did not achieve CHR (n = 23, 40%; 3.66% Tregs). Patients with an early molecular response (EMR), defined by BCR-ABL transcript levels of < 10% after three months of therapy, exhibited lower baseline Treg% than those with > 10% transcripts [14]. It was also observed that despite a decline with treatment, Treg% levels remained significantly higher than in controls

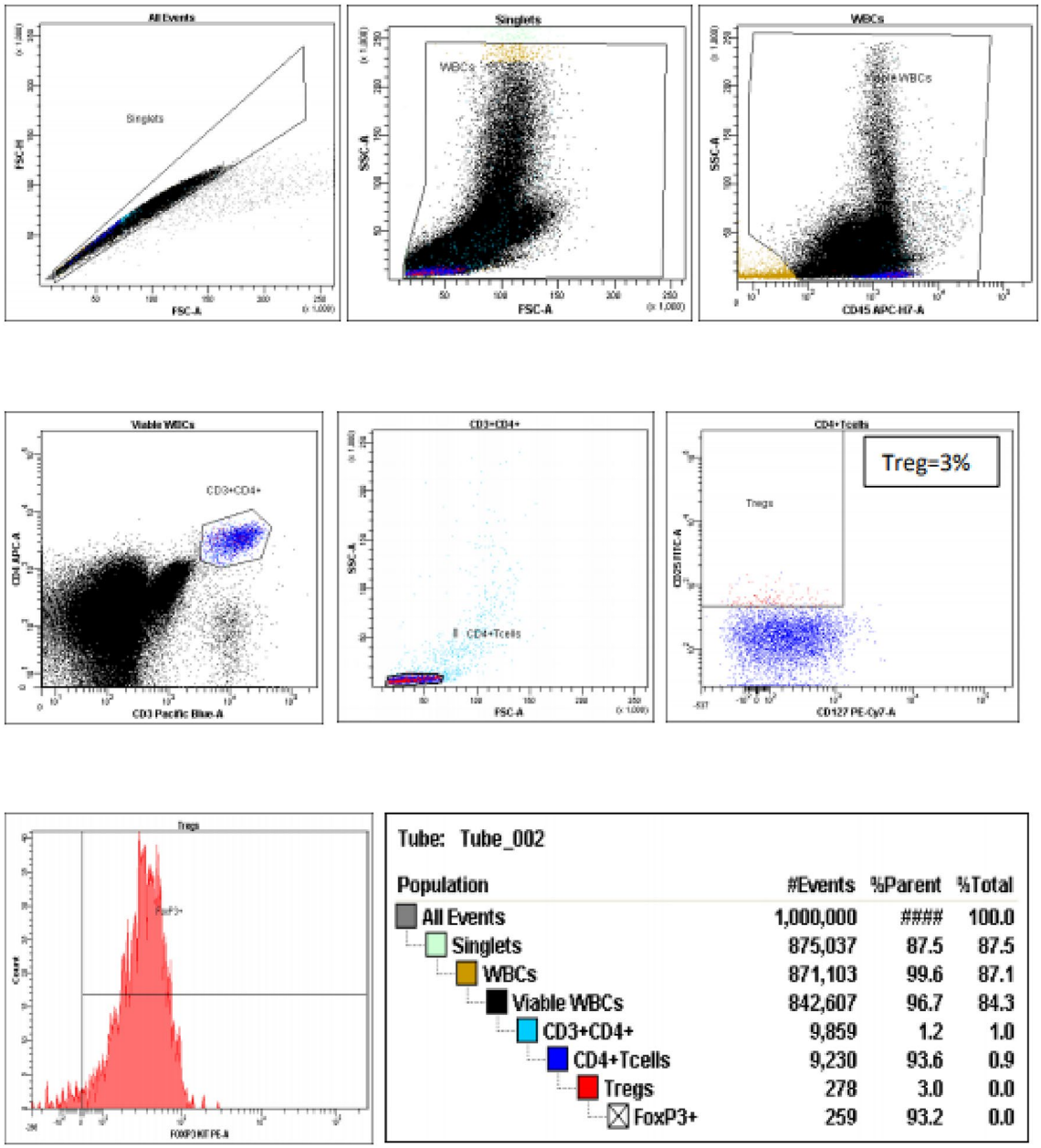

Fig. 1 Flowcytometric analysis for Tregs at diagnosis: Gating was done to remove doublets and debris. Then, the viable WBCs were gated for CD4 + cells. Tregs were identified as CD25high CD127low cells. Treg% at diagnosis was found to be 3% of CD4 lymphocytes

($p = 0.04$). Figure 2a is a box plot that suggests a notable difference in the baseline Treg% between the case and control groups, with the case group having higher baseline levels of Treg%. Figure 2b is a scatter plot depicting the significant correlation of baseline Treg% with BCR-ABL transcript levels at three months. Figure 2c is a scatter plot showing no significant correlation between the BCR-ABL transcripts and the percentage of Tregs at the three-month mark, given the flat trend and widening confidence interval. Figure 2d is a clustered bar chart that depicts the Treg% at baseline and after three months in individual patients, along with their Sokal scores. The graph provides a clear visual representation of how Treg% changes over time and across different risk categories.

The mean baseline percentage of PD-1 + CD8 T cells in CML patients ($7.66 \pm 0.36\%$) was significantly higher than that in controls ($2.65 \pm 0.32\%$) ($p < 0.0001$). The baseline percentage of PD-1 + T cells in patients correlated with the Sokal ($r = 0.26$), Hasford ($r = 0.27$), and ELTS ($r = 0.41$) scores ($p < 0.05$). Following three months of therapy, the mean percentage of PD-1 + CD8 T cells in CML patients ($3.44 \pm 0.36\%$) was significantly lower than their mean

Table 1 Patient and disease characteristics

Characteristic	Cases at baseline (n = 57)	Controls (n = 30)	Cases at 3 months (n = 57)	*p* value
Median age in years (range)	39 (16–64)	45 (23–63)		0.23
Gender (M:F)	27:30 (0.9:1)	30:30 (1:1)		0.84
Hemoglobin (g/dl)				
Mean	10.83 ± 1.83	13.62 ± 1.71	10.8 ± 1.72	
Range	6.9–15.1	10.1–16.8	9.4–12.8	
Total leukocyte count (cells/uL)				
Mean	178,573 ± 142,560	8187 ± 1603	6098 ± 1404	
Range	41,400–774,900	5200–11,100	5800–12200	
Platelet count (cells/μL)				
Mean	390,561 ± 226,490	264,567 ± 104,560	204,506 ± 101,360	
Range	143,000- 1064,000	136,500–468,460	104,00–398,460	
Sokal risk score				
Low (< 0.8)	17 (29.8%)			
Intermediate (0.8–1.2)	29 (50.9%)			
High (> 1.2)	11 (19.3%)			
Hasford risk score				
Low (≤ 780)	23 (40.4%)			
Intermediate (781–1480)	28 (49.1%)			
High (> 1480)	6 (10.5%)			
EUTOS risk score				
Low (≤ 87)	47 (82.5%)			
High (> 87)	10 (17.5%)			
ELTS risk score				
Low (≤ 1.5680)	27 (47%)			
Intermediate (1.5680–2.2185)	21 (37%)			
High (> 2.2185)	9 (16%)			
Achievement of CHR				
< 6 weeks	34 (59.6%)			
> 6 weeks	23 (40.3%)			
BCR-ABL transcript levels at 3 months with Treg% at baseline				
< 1	15 (26.3%)			
1–10	26 (45.6%)			
> 10	16 (28.1%)			

baseline value (7.66 ± 0.36%) ($p < 0.0001$). The details are presented in Table 2.

Discussion

In this study, we investigated the dynamics of immune cells in chronic myeloid leukaemia in chronic phase (CML-CP) patients undergoing imatinib treatment. Our findings demonstrated that the mean percentage of regulatory T cells (Tregs) at presentation was significantly elevated compared to the healthy population in CML-CP patients. Furthermore, we observed a significant correlation between the level of Tregs and the risk groups of the disease, which is consistent with previous literature [15–17]. These results support the hypothesis that Tregs play a role in the initiation and progression of CML by facilitating the evasion of immune surveillance by leukemic stem cells [9, 18].

After three months of imatinib therapy, we observed a significant decrease in the percentage of Tregs in all patients. Imatinib is known to suppress the survival and immunosuppressive function of Tregs by targeting cell surface receptors, signalling pathways, and transcription factors [10]. The previous studies in this regard are presented in Table 3. Thus, suppressing Tregs may represent one of the mechanisms by which tyrosine kinase inhibitors (TKIs) exert control over CML [10, 11, 19]. Notably, patients who achieved complete hematologic response at six weeks and early molecular response at three months had lower Treg percentages at presentation. This finding

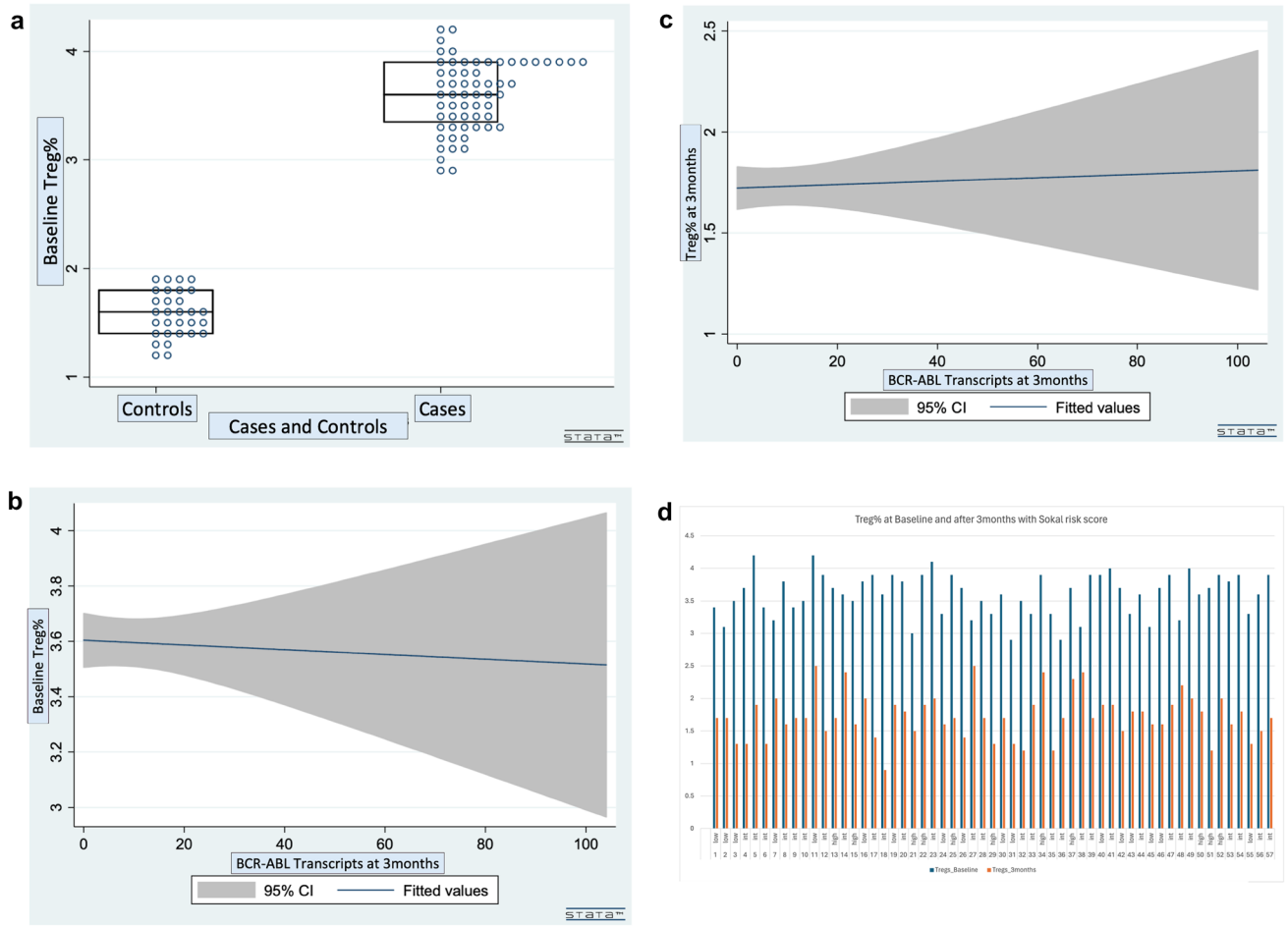

Fig. 2 **a** Plot representing baseline Treg% in cases and controls. **b** Plot correlating BCR-ABL transcript level at three months with baseline Treg%. **c** Plot correlating BCR-ABL transcript level at three months with Treg% at three months. **d** Treg% at baseline and after three months with Sokal risk score of patients

strengthens the hypothesis that the percentage of Tregs could be a potential biomarker for assessing treatment response and prognosis in CML [20].

Consistent with previous studies, our investigation revealed higher levels of programmed cell death protein 1 (PD-1) positive CD8 T-cells in CML patients compared to healthy individuals [12]. The elevated expression of PD-1 was associated with CD8 T-cell exhaustion and impaired anti-leukemia immune responses in myeloid malignancies [21]. Prior studies have reported a higher percentage of PD-1 positive CD8 T-cells in the bone marrow of CML patients with advanced disease stages and higher risk severity indices [2, 12, 22, 23]. In our study, we focused on peripheral blood samples, which are more easily accessible, and found a significant correlation between the percentage of PD-1 positive CD8 T-cells at presentation and risk severity indices. Consistent with previous findings, patients with a better response to therapy exhibited lower percentages of PD-1 + CD8 T-cells [13]. Longer follow-up is necessary to determine whether baseline PD-1 expression in peripheral blood can predict future outcomes.

Studies have demonstrated that leukemic stem cells persisting in the bone marrow during treatment can acquire BCR-ABL1 kinase domain mutations or accumulate genetic alterations, leading to disease relapse and progression [24, 25]. Preclinical models have shown that blocking PD-1 receptors, with or without adoptive cytotoxic T-cell transfer, can eliminate CML stem cells [7, 26]. Considering the immunomodulatory effects of imatinib, it is worth exploring whether the addition of immunotherapy could enhance the achievement of deeper molecular remission, either as an upfront treatment or in the relapsed-refractory setting. The ongoing Blast MRD CML 1 phase II trial aims to address this question [27].

One of the primary goals in managing CML is discontinuing TKIs, although studies have indicated that only 20–30% of newly diagnosed patients can achieve treatment-free remission [28–31]. Interestingly, our study revealed that

Table 2 Mean percentage of Tregs and PD-1 + CD8 T-cells compared with disease variables

Variable	N = 57	Mean Treg%	*p* value	Mean% of PD-1 + CD8 T cells	*p* value
CML-CP patients at baseline	57	3.61 ± 0.32	**< 0.0001**	7.66 ± 0.36	**< 0.0001**
Healthy controls	30	1.58 ± 0.21		2.65 ± 0.32	
Age (in years)					
< 30	15 (26.3%)	3.56 ± 0.23	0.79	7.59 ± 0.30	0.82
30–60	33 (57.9%)	3.61 ± 0.36		7.67 ± 0.39	
> 60	9 (15.7%)	3.66 ± 0.29		7.70 ± 0.43	
Sex					
Male	27 (47.4%)	3.57 ± 0.31	0.51	7.64 ± 0.39	0.68
Female	30 (52.6%)	3.63 ± 0.32		7.67 ± 0.34	
Sokal risk score					
Low (< 0.8)	17 (29.8%)	3.50 ± 0.32	**r = 0.29**	7.54 ± 0.25	**r = 0.26**
Intermediate (0.8–1.2)	29 (50.9%)	3.63 ± 0.21	***p* = 0.03**	7.66 ± 0.43	***p* = 0.04**
High (> 1.2)	11 (19.3%)	3.68 ± 0.26		7.77 ± 0.28	
Hasford risk score					
Low (≤ 780)	23 (40.4%)	3.54 ± 0.28	**r = 0.33**	7.54 ± 0.29	**r = 0.27**
Intermediate (781–1480)	28 (49.1%)	3.62 ± 0.31	***p* = 0.01**	7.62 ± 0.43	***p* = 0.03**
High (> 1480)	6 (10.5%)	3.77 ± 0.33		7.76 ± 0.19	
EUTOS risk score					
Low (≤ 87)	47 (82.5%)	3.58 ± 0.32	**r = 0.28**	7.61 ± 0.33	r = 0.20
High (> 87)	10 (17.5%)	3.72 ± 0.26	***p* = 0.03**	7.83 ± 0.41	*p* = 0.13
ELTS risk score					
Low (≤ 1.5680)	27 (47%)	3.51 ± 0.34	**r = 0.31**	7.50 ± 0.23	**r = 0.41**
Intermediate (1.5680–2.2185)	21 (37%)	3.59 ± 0.31	***p* = 0.01**	7.76 ± 0.38	***p* = 0.01**
High (> 2.2185)	9 (16%)	3.76 ± 0.26		7.87 ± 0.49	
Achievement of CHR					
< 6 weeks	34 (59.6%)	3.57 ± 0.35	0.29	7.61 ± 0.28	0.34
> 6 weeks	23 (40.3%)	3.66 ± 0.26		7.68 ± 0.19	
CML-CP patients at baseline	57	3.61 ± 0.32	**< 0.0001**	7.66 ± 0.36	**< 0.0001**
CML-CP patients after 3 months	57	1.73 ± 0.35		3.44 ± 0.37	
CML-CP patients after 3 months	57	1.73 ± 0.35	**0.04**	3.44 ± 0.36	**0.01**
Healthy controls at baseline	30	1.58 ± 0.21		2.65 ± 0.32	
BCR-ABL transcript levels at 3 months with Treg% at baseline					
< 1	15 (26.3%)	3.42 ± 0.33	**0.03**	7.61 ± 0.31	0.77
1–10	26 (45.6%)	3.55 ± 0.31		7.65 ± 0.37	
> 10	16 (28.1%)	3.69 ± 0.24		7.68 ± 0.32	

although the percentage of Tregs and PD-1 positive T-cells decreased significantly with treatment, their levels at three months remained significantly higher than those observed in healthy controls. Further research is necessary to investigate whether the kinetics of immune regulatory cells and the normalisation of their levels can serve as predictive markers for the successful discontinuation of TKIs [32, 33].

Our study's limitations include focusing solely on the percentage of Tregs without investigating their functional mechanisms. Additionally, we did not perform RQ-PCR to quantify BCR-ABL transcripts at baseline, which could have provided a more accurate understanding of the proportional decrease in BCR-ABL levels compared to the reduction in Treg percentages. We did not analyse Treg% in healthy controls at three months, which would have provided a more precise illustration of imatinib's effect on reducing Tregs in cases. Furthermore, more extended follow-up periods would be beneficial to establish a stronger correlation between Treg percentages and late responders.

Table 3 Existing literature on Tregs in CML

Study no	Author	Year	Number of CML-CP patients	Percentage of Tregs before treatment	Percentage of Tregs among healthy controls	TKI used, with duration	Percentage of Tregs after therapy
1	Rojas etal [16]	2010	39	2.54%	1.78% (significantly lower than cases)	Imatinib	1.45% (in patients in CCYR, significantly lower) 2.54% (in patients not in CCYR)
2	Hus etal [17]	2011	38	6.3%	Significantly lower than cases (% not mentioned)	Multiple TKIs (Imatinib, dasatinib, bosutinib), 6 months	No significant difference (% not mentioned)
3	Bachy etal [9]	2011	14	4.42%	4.04% (non-significant as compared to cases)	Imatinib, who were already on Imatinib	6.88%
4	Zahran etal [15]	2013	45	5.1%	3.54% (significantly lower than cases)	Imatinib, 1 year	0.09% (significantly lower)
5	Our Study	2018	57	3.61%	1.58% (significantly lower as compared to cases)	Imatinib, 3 months	1.73% (significantly lower than cases at baseline, yet higher than controls)

Conclusion

Our study revealed a significantly higher proportion of Tregs and PD-1 + CD8 T-cells in patients with CML-CP compared to healthy controls, notably diminished following imatinib treatment. The critical role of the immune system in the initiation and progression of CML and the modulation of the immune response through targeted agents and immunotherapy to achieve sustained drug-free remission in CML are subjects of further research.

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Declarations

Conflict of interest There are no relevant conflicts of interest to declare.

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