# Sequential Enumeration of CD26+ Leukemic Stem Cells and Its Correlation With *BCR::ABL1* Transcript Levels in Chronic Myeloid Leukemia at Diagnosis and During Tyrosine Kinase Inhibitor Therapy

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## INTRODUCTION

- CD26 has emerged as an explicit immunophenotypic marker for leukemic stem cells (LSCs) in chronic myeloid leukemia (CML).
- While many CML patients achieve an MMR with TKIs, only a small number reach PCR negativity. This is because TKI does not eliminate LSCs, which persist in the bone marrow stem cell niches and have the potential to cause relapse.
- Many studies have been published highlighting CD26 positivity on LSCs as a standalone immunophenotypic marker in the diagnosis of CML. However, there is paucity of literature on monitoring of CD26+ LSCs.

## AIM

1. To sequentially enumerate CD26+ LSCs by flow cytometry at diagnosis and during monitoring of Chronic myeloid leukemia i.e., +3, +6, and +12 months of Tyrosine Kinase Inhibitor therapy.
2. To correlate the absolute numbers of CD26+ CML leukemic stem cells with quantitative *BCR::ABL1* transcript levels at diagnosis.
3. To correlate the absolute numbers of CD26+ CML leukemic stem cells with quantitative *BCR::ABL1* transcript levels during monitoring of Chronic myeloid leukemia i.e., +3, +6, and +12 months of Tyrosine Kinase Inhibitor therapy.

## METHOD

- Patients with peripheral blood and/or bone marrow findings of CML (N=76), aged >12 years, and confirmed by demonstration of *BCR::ABL1* through molecular genetic studies were included.
- Of these, 25 patients were prospectively followed for $CD26^{+}$ leukemic stem cell (LSC) enumeration and *BCR::ABL1* quantification at 3, 6, and 12 months of TKI therapy.
- Additionally, 15 patients on random follow-up and 21 control samples were included.

## CONCLUSIONS

- CD26+ LSCs were detectable in all CML patients at baseline and showed significant log reductions during follow-up, indicating their value as a biomarker of 'stem cell response'.
- CD26+ LSCs persisted even in patients with undetectable transcripts, highlighting their role in residual disease and relapse risk.
- CD26+ LSC enumeration offers complementary insights beyond molecular monitoring and may serve as a future therapeutic target for deeper, durable responses.

## RESULTS

- In total, 197 peripheral blood samples and 41 bone marrow samples were tested.

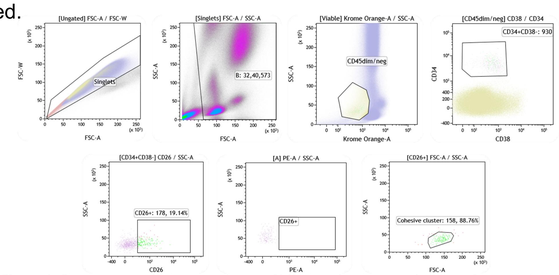


**Figure 1:** Flow cytometric dot plots from a CML chronic-phase patient at a 6-month follow-up illustrate the sequential gating strategy for identifying CD26+ leukemic stem cells (LSCs)

**Table 1: The baseline characteristics are shown below.**

| Parameters | De novo CML patients | Non-CML patients | CML on random follow-up |
|---|---|---|---|
| **Number of patients** | N = 76 | N = 21 | N = 15 |
| **Peripheral blood/bone marrow diagnosis** | CML-CP – 71<br>CML-AP – 03<br>CML-BC – 02 | Lymphoma staging with uninvolved BM (N = 4), Reactive BM (N = 4), AML (N = 4), Post-induction BM of Ph+ B-ALL (N = 3), Lymphoma infiltration (N = 1), T-ALL (N = 1), MPN (non-CML) (N = 2), MDS/MPN overlap (N = 2) | In CHR = 8<br>CML-CP = 2<br>CML-AP = 1<br>CML-BC = 4 |
| **Median age in years (Range)** | 43 (16–77) | 29 (3–76) | 55 (26–73) |
| **Male: Female** | 1.76:1 | 1.89:1 | 1.5:1 |
| **Median haemoglobin in g/dL (Range)** | 10.4 (3.4–15.4) | 9.75 (4.3–15.2) | 8.15 (5.7–12.4) |
| **Median total leukocyte count × $10^9$/L (Range)** | 118.2 (13.2–462.4) | 180.5 (3.0–358) | 11 (3.3–87.4) |
| **Median platelet count × $10^9$/L (Range)** | 300 (21–1532) | 163 (2–1343) | 158.5 (5–769) |
| **BCR::ABL1 transcript type** | p210 = 71, p190 = 1<br>Four cases diagnosed by FISH | Not detected | b2a2 = 2; b3a2 = 2;<br>e1a2 = 1 |
| **CD26 positivity** | 76/76 (100%) | Negative 21/21 (100%) | 8/15 (53.3%) |

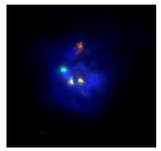

**Figure 2:** CD34+/CD38dim-/CD26+ LSCs were sorted, and FISH was performed using XL BCR/ABL1plus dual-color dual-fusion probe revealed a translocation involving the *BCR* and *ABL1* gene regions.

**Table 2: CD26+ LSC expression at diagnosis and at 3, 6 and 12 months.**

| N=25 | Diagnosis | 3 months | 6 months | 12 months | P-value |
|---|---|---|---|---|---|
| **Median CD26+ LSC % (Range)** | 0.25 (0.009-3.2) | 0.002 (0.0004-0.11) | 0.0009 (0.0001-0.01) | 0.0003 (0.00-0.02) | **<0.001 *** |
| **Median % of CD26+ LSC in CD34+/CD38- compartment** | 48.47 (28.35-97.1) | 17.18 (1.79-48.8) | 4.62 (1.48-34.9) | 5.03 (0.00-35.35) | **<0.001 *** |
| **Absolute no. of CD26+ LSC/µl** | **267.98 (5.92-3640.13)** | **0.15 (0.03-5.56)** | **0.05 (0.003-0.94)** | **0.02 (0.00-1.99)** | **<0.001 *** |

- Spearman correlation test was used to correlate the absolute CD26 levels at diagnosis with CHR Velocity, EMR and MMR. There was significant negative correlation between absolute CD26 levels at diagnosis and CHR Velocity (P=0.045).
- A significant reduction in the absolute CD26 LSC value was observed at 3, 6, and 12 months compared to baseline (P < 0.0001).
- At all-time points *BCR::ABL1* IS% was significantly lower in patients achieving MMR than those without MMR, P-value <0.01.
- No statistically significant differences in CD26+ LSC% between MMR achievers and non - achievers at 3, 6, and 12 months, P-value > 0.05.
- We had five patients with undetectable *BCR::ABL1* transcripts at 12 months. CD26+ LSCs were detected in 4/5 patients.

## ACKNOWLEDGEMENTS

1. Dr. Arnal Pal, Dept of Biochemistry for assistance in sorting of LSCs.
2. Intramural research grant by Postgraduate Institute of Medical Education and Research, Chandigarh, India

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