University of Glasgow

Munje, C. and Copland, M. (2017) Exploring stem cell heterogeneity in chronic myeloid leukemia. *Trends in Cancer*, 4(3):167-169 (doi:10.1016/j.trecan.2017.12.001)
This is the author's final accepted version.

There may be differences between this version and the published version. You are advised to consult the publisher's version if you wish to cite from it.

http://eprints.gla.ac.uk/155030/

Deposited on: 10 January 2018

Enlighten – Research publications by members of the University of Glasgow
http://eprints.gla.ac.uk

Title: Exploring stem cell heterogeneity in chronic myeloid leukemia (8 words)

Authors: Chinmay Munje and Mhairi Copland

Key words: hematopoietic stem cell, leukemia stem cell, chronic myeloid leukemia, single cell transcriptomics, heterogeneity

Abstract:

Until very recently, understanding the complexity of the stem cell compartment in both normal and leukemic hematopoiesis has been challenging due to the inability to separate and study normal and leukemic stem cells at the single-cell level. Recent advances in cell sorting techniques and single-cell technologies now make this possible, with the identification of a population of highly quiescent chronic myeloid leukemia stem cells that are enriched following therapy with tyrosine kinase inhibitors (73 words).

Main text:

The introduction of BCR-ABL tyrosine kinase inhibitors (TKI) has revolutionized management of chronic myeloid leukemia (CML) [1]. However, in CML, areas of unmet clinical need remain progression to aggressive advanced phase CML, resistance to multiple TKIs in chronic phase and low-level disease persistence which results in relapse on cessation of therapy [2]. Despite a molecularly targeted therapeutic approach, there remains a rare cohort of cells, termed leukemic stem cells, which are selectively resistant to TKI therapy and incompletely eradicated in most patients [1]. These LSCs are responsible for disease relapse post treatment discontinuation [2], and reside in the same microenvironment as their normal hematopoietic stem cell (HSC) counterparts, expressing the same $CD34^{+}CD38^{-}$ phenotype [3].

A better understanding of stem cell (SC) heterogeneity in CML will help devise new strategies to eradicate these cells and improve patient outcomes. To date, research has used “bulk” samples of unfractionated stem and progenitor cells. These samples likely contain a mixture of normal and leukemic cells, with different leukemic sub-clones and different levels of cell maturation. Recent advances in single-cell transcriptomic technologies have enabled researchers to study clonal evolution in different tumors [4, 5] including leukemia [6]. Recent work from Giustacchini *et al.* [7] characterizes distinct molecular signatures of SC subpopulations in CML from diagnosis through remission and disease progression (Figure 1).

The hallmark of CML is the presence of *BCR-ABL* fusion oncogene. As current single-cell RNA sequencing approaches, including Smart-seq2 [8], are not sensitive enough to consistently detect this fusion gene, Giustacchini *et al.* developed a *BCR-ABL*-targeted Smart-seq2 protocol (*BCR-ABL* tSs2). This approach enabled *BCR-ABL* detection with no evidence of bias in library quality and a strong correlation of RefSeq genes generated by Smart-seq2 or *BCR-ABL* tSS2 in CML cells. Comparison of *BCR-ABL*$^{+}$ and *BCR-ABL*$^{-}$ SCs from a CML patient identified differentially expressed genes and a strong correlation (P=0.87) was seen between single-cell RNA sequencing and qPCR data. Thus, the *BCR-ABL* tSs2 protocol allows sensitive and quantitative *BCR-ABL* detection with unbiased whole-transcriptome analysis.

Giustacchini *et al.* analysed SC data from 20 patients with CP-CML, and found *BCR-ABL*$^{-}$ SCs to be similar to normal HSCs in terms of number of genes detected, and expression patterns of genes involved in proliferation and quiescence (Figure 1.1). Contrastingly, *BCR-ABL*$^{+}$ SCs had a significantly higher number of reads detected compared to healthy normal donor HSCs and *BCR-ABL*$^{-}$ SCs from

CML patients; with a bias towards genes involved in proliferation and reduced quiescence-associated expression. Irrespective of patient profile, distinct clusters of normal HSCs, *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs were seen on t-distributed stochastic neighbour embedding (tSNE) analysis, a machine learning algorithm for dimentionality reduction, particularly suited to single-cell analyses [9]. The authors observed differential expression of genes previously implicated in CML pathogenesis, and identified several novel candidate genes that were differentially expressed between normal HSCs, *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs. Gene set enrichment analysis (GSEA) identified several gene sets enriched in *BCR-ABL*$^+$ SCs; none of which would have been identified through *in silico* bulk cell analysis. Given the importance of the tumor microenvironment, the authors also identified gene sets enriched in the *BCR-ABL*$^-$ SCs compared to normal HSCs, including those involved in IL6, TGFβ and TNFα pathways.

In further experiments by Giustacchini *et al.*, patient samples were stratified into good or poor responders to TKI. GSEA on diagnostic samples showed enrichment of signalling pathways, inflammation, TGF-β and TNF-α-associated genes in *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs from poor compared with good responders. In contrast, both *BCR-ABL*$^+$ SCs and *BCR-ABL*$^-$ SCs from good responders showed an enrichment of genes associated with increased proliferation such as E2F, MYC and G2M-checkpoint. These findings suggest that *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs from poorly-responding patients have enrichment of genes involved in quiescence, including TGF-β and TNF-α-associated genes, and this may be a mechanism of TKI resistance.

Single-cell sequencing of *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs was performed on paired patient bone marrow samples from diagnosis and following 3-6 months TKI therapy (Figure 1.2). Following TKI, fewer *BCR-ABL*$^+$ SCs were present, and *BCR-ABL*$^+$ and *BCR-ABL*$^-$ SCs had a similar average number of genes detected per cell. tSNE analysis revealed two distinct clusters of remission *BCR-ABL*$^+$ SCs; one enriched for quiescence and HSC-associated genes (group-A) and a second enriched for proliferation-associated genes (group-B). Group-A expressing cells were enriched with prolonged TKI treatment, indicating selective persistence of distinct and highly quiescent *BCR-ABL*$^+$ SCs (group-A) which are already present at diagnosis, rather than development of resistant CML-SCs, or stochastic persistence of heterogeneous CML-SCs. Further analyses demonstrated that, compared to normal HSCs, group-A *BCR-ABL*$^+$ SCs showed enrichment of Wnt/ β-catenin, TGF-β, TNF-α and IL-6-JAK-STAT-associated gene expression, while normal HSCs were enriched for MYC, E2F and G2M-checkpoint-associated genes. This single-cell transcriptomic analysis provides insights into pathways that are potentially involved in promoting selective persistence of distinct *BCR-ABL*$^+$ SCs following TKI treatment, and include quiescence and inflammation.

In separate experiments, tSNE analysis of blast crisis (BC)-CML-SCs from 3 patients demonstrated a separate cluster of *BCR-ABL*$^+$ SCs (Figure 1.3), clearly distinct from normal HSC, *BCR-ABL*$^+$ SCs from 18 patients with CP-CML and K562 cells. Two of the patients had progressed to BC-CML on TKI and had samples from diagnosis, presenting in CP, 12 and 3 months before transformation. One of the two patients developed lymphoid BC, and a minority of pre-BC SCs from that patient cluster with *BCR-ABL*$^+$ CP-CML-SCs. This demonstrates evolution from CP- to BC-CML within the SC compartment prior to any clinical or morphological evidence of BC. Further, exome sequencing of this patient's sample revealed a somatic *RUNX1* mutation and differential regulation of RUNX1 target genes between the pre-BC, CP-CML-SC and BC-CML-SC clusters, consistent with the acquisition of *RUNX1* mutation as a genomic event occurring pre-BC and driving BC transformation.

To conclude, single-cell gene expression approaches have tremendous potential to enhance our understanding of biological diversity and rare cell types. Giustacchini *et al.* used CML as the paradigm disease model, as the LSC compartment is well established and the persistence of rare

CML-SCs remains a key therapeutic challenge [1]. Single-cell transcriptomics were used to unravel LSC heterogeneity, and reveal insights that may help to predict and understand SC persistence in responding and disease progression in poorly-responding patients.

This novel single-cell approach to investigating cellular heterogeneity is an attractive avenue across not just other malignancies, particularly cancer stem cell disorders, or analysis of heterogeneity of circulating tumor cells (a relatively non-invasive approach), but also other areas of clinical interest, including studying virus-host interactions and neuronal transcriptional profiles in response to stimuli. This technology when backed with a robust computational pipeline and/or modelling has the potential to change our perspective of many diseases.

References

1. Gallipoli P, Abraham SA, Holyoake TL. Hurdles toward a cure for CML: the CML stem cell. Hematol Oncol Clin North Am. 2011;25(5):951-66.
2. Mahon FX, Réa D, Guilhot J, Guilhot F, Huguet F, Nicolini F, et al. Discontinuation of imatinib in patients with chronic myeloid leukaemia who have maintained complete molecular remission for at least 2 years: the prospective, multicentre Stop Imatinib (STIM) trial. Lancet Oncol. 2010;11(11):1029-35.
3. Herrmann H, Cerny-Reiterer S, Gleixner KV, Blatt K, Herndlhofer S, Rabitsch W, et al. CD34(+)/CD38(-) stem cells in chronic myeloid leukemia express Siglec-3 (CD33) and are responsive to the CD33-targeting drug gemtuzumab/ozogamicin. Haematologica. 2012;97(2):219-26.
4. Wang Y, Waters J, Leung ML, Unruh A, Roh W, Shi X, et al. Clonal evolution in breast cancer revealed by single nucleus genome sequencing. Nature. 2014;512(7513):155-60.
5. Kim KT, Lee HW, Lee HO, Song HJ, Jeong dE, Shin S, et al. Application of single-cell RNA sequencing in optimizing a combinatorial therapeutic strategy in metastatic renal cell carcinoma. Genome Biol. 2016;17:80.
6. Paguirigan AL, Smith J, Meshinchi S, Carroll M, Maley C, Radich JP. Single-cell genotyping demonstrates complex clonal diversity in acute myeloid leukemia. Sci Transl Med. 2015;7(281):281re2.
7. Giustacchini A, Thongjuea S, Barkas N, Woll PS, Povinelli BJ, Booth CAG, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. Nat Med. 2017;23(6):692-702.
8. Picelli S, Faridani OR, Björklund AK, Winberg G, Sagasser S, Sandberg R. Full-length RNA-seq from single cells using Smart-seq2. Nat Protoc. 2014;9(1):171-81.
9. Maaten Lvd, Hinton G. Visualizing Data using t-SNE. Journal of Machine Learning Research. 2008;9(Nov):2579-605.

Figure 1 legend.

Title: Exploring stem cell heterogeneity in chronic myeloid leukemia.

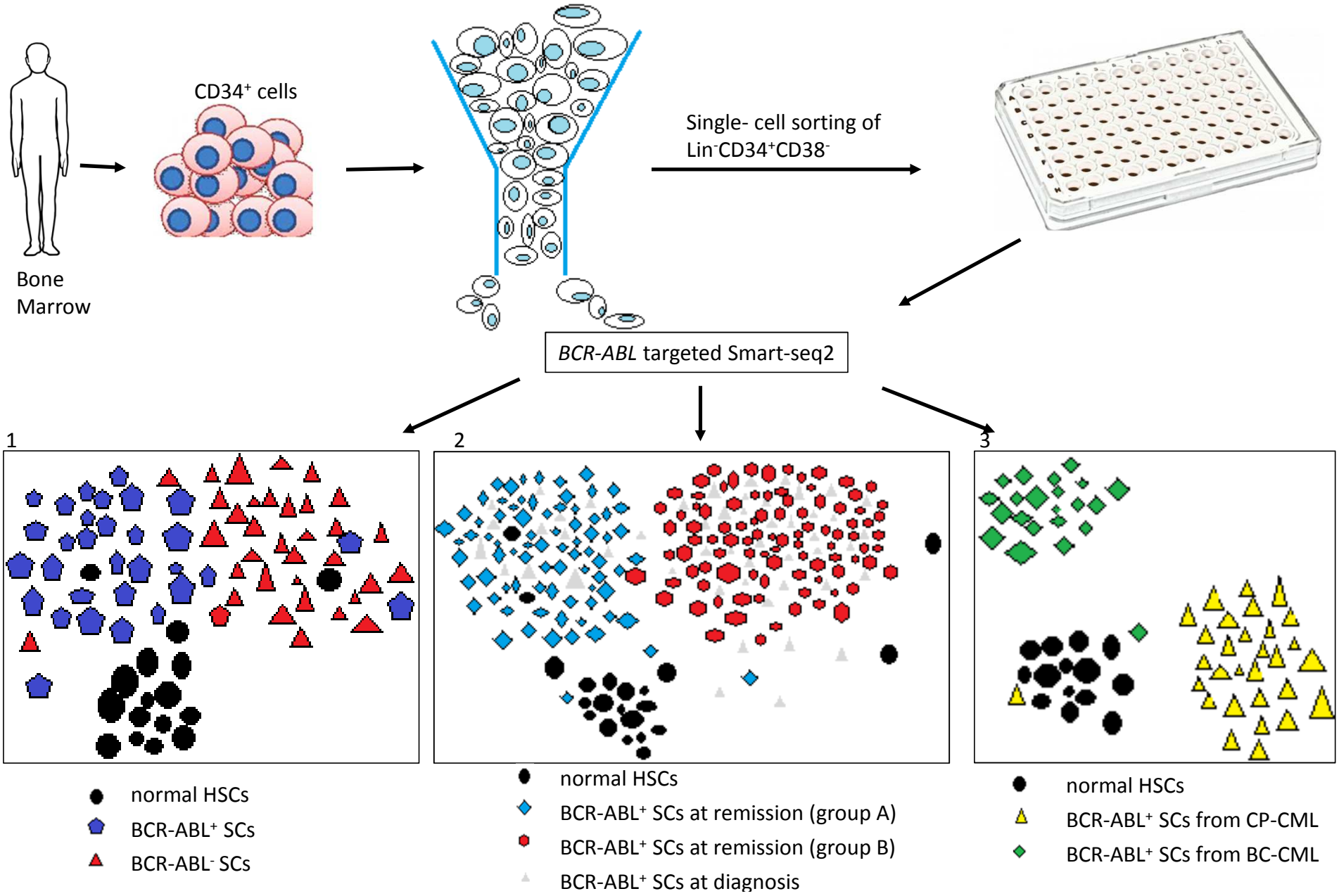
Single cells were FACS-sorted as $Lin^{-}CD34^{+}38^{-}$ from bone marrow samples of normal donors and CML patients. *BCR-ABL* tSs2 protocol was used to create single-cell cDNA libraries.

1) tSNE visualization of single normal HSCs (black circles), *BCR-ABL*$^{-}$ SCs (blue pentagons) and *BCR-ABL*$^{+}$ SCs (red triangles) shows distinct clustering of the 3 groups. Marked dysregulation of TGF-β and TNF-α pathways in *BCR-ABL*$^{+}$ SCs in patients with CML, associated with increase SC quiescence.

2) tSNE visualization of single normal HSCs (black circles), *BCR-ABL*$^{+}$ SCs from patients at diagnosis (grey triangles) and *BCR-ABL*$^{+}$ SCs from patients at remission: group A (blue diamonds) and group-B (red hexagons). Group-A *BCR-ABL*$^{+}$ SCs showed enrichment of TGF-β, TNF-α and IL-6 – JAK – STAT associated gene expression compared to normal HSCs or *BCR-ABL*$^{-}$ SCs. Group-A remission CML-SCs are characterized by marked quiescence-associated gene expression, selectively evading TKI eradication. Group-B remission CML-SCs showed enrichment of MYC, E2F and proliferation-associated gene sets and cluster with diagnostic *BCR-ABL*$^{+}$ SCs.

3) tSNE visualization from normal HSCs (black circles), CP-CML patients (yellow triangles) and BC-CML patients (green diamonds). Distinct clusters of normal HSCs, CP-CML and BC CML were observed; the authors also noticed a minority of pre-BC SCs from one patient to cluster with CP-CML SCs predicting imminent disease progression in cancer SC populations.

FACS; fluorescence activated cell sorting, CML; chronic myeloid leukemia, tSNE; t-distributed stochastic neighbour embedding, HSC; hematopoietic stem cells, SCs; stem cells, CP; chronic phase, BC; blast crisis.


Bone Marrow
CD34+ cells
Single- cell sorting of Lin-CD34+CD38-
BCR-ABL targeted Smart-seq2
1
2
3
normal HSCs
BCR-ABL+ SCs
BCR-ABL- SCs
normal HSCs
BCR-ABL+ SCs at remission (group A)
BCR-ABL+ SCs at remission (group B)
BCR-ABL+ SCs at diagnosis
normal HSCs
BCR-ABL+ SCs from CP-CML
BCR-ABL+ SCs from BC-CML