haematologica
Journal of the Ferrata Storti Foundation

Understanding drug resistance in chronic myeloid leukemia through the lens of leukemic stem cell states: insights from single-cell analyses

by Vaidehi Krishnan, Pavithra Shyamsunder and Tiong S. Ong

Received: July 4, 2025.
Accepted: April 1, 2026.

Citation: Vaidehi Krishnan, Pavithra Shyamsunder and Tiong S. Ong. Understanding drug resistance in chronic myeloid leukemia through the lens of leukemic stem cell states: insights from single-cell analyses. Haematologica. 2026 Apr 9. doi: 10.3324/haematol.2025.287767 [Epub ahead of print]

Understanding drug resistance in chronic myeloid leukemia through the lens of leukemic stem cell states: insights from single-cell analyses

Vaidehi Krishnan[1], Pavithra Shyamsunder[1], S. Tiong Ong[1, 2, 3]

[1]Cancer & Stem Cell Biology Signature Research Programme,
Duke-NUS Medical School, Singapore.
[2]Department of Haematology, Singapore General Hospital, Singapore.
[3]Department of Medicine, Duke University Medical Center, NC, USA.

Corresponding Author:
S Tiong ONG: email sintiong.ong@duke-nus.edu.sg

Disclosures: STO- Novartis Advisory Board, AUM Biosciences. STO serves on a Novartis Advisory Board, for which he has received consultancy fees. STO receives licensing fees from AUM Biosciences.

Author Contributions
STO and VK wrote the first draft of the manuscript. P.S revised and edited figures and text. All authors approved the final draft.

Acknowledgements
We would like to acknowledge Dr. John F. Ouyang for helpful discussions. We have made every effort to highlight and reference relevant publications. However, we apologize to any authors or teams whose work we may have inadvertently omitted. Due to space limitations and the focused scope of this review, which aims to present a case for viewing drug resistance through the lens of cell state, our discussion is illustrative rather than exhaustive.

Funding
This work was supported by grants from the Singapore National Medical Research Council (MOH-000059-00, MOH-001571-00, MOH-002028-00) and Agency for Science, Technology and Research Singapore (R23I2IR071).

ABSTRACT

Despite the advent of potent tyrosine kinase inhibitors (TKIs), resistance and disease persistence remain significant clinical challenges in chronic myeloid leukemia (CML). This perspective aims to synthesize concepts derived from recent advances in single-cell and multi-omics analyses, which have revealed profound heterogeneity among leukemic stem cells (LSCs). These findings augment traditional models that focus solely on clonal selection and resistance-conferring mutations. We discuss how LSCs, like normal hematopoietic stem cells (HSCs), exist in a spectrum of transcriptionally and epigenetically defined cell states, each governed by distinct gene regulatory networks (GRNs) that confer unique lineage biases and responses to therapy. Incorporating recent insights from single-cell analysis, our perspective highlights evidence for a conserved chronic phase (CP) LSC state characterized by lineage skewing, altered metabolic and environmental responsiveness, and epigenetic dysregulation, features that are likely to be underpinned by specific GRN configurations that collectively contribute to intrinsic TKI resistance. We explore how both intrinsic factors (such as germline polymorphisms and lineage bias) and extrinsic cues (including microenvironmental signals, immune interactions, and hypoxia) are likely to modulate GRN activity and LSC states, thereby affecting apoptotic thresholds, primary resistance, and the potential for treatment-free remission (TFR). Emerging data support the concept of GRN-defined LSC states at diagnosis that are predictive of TKI responses. Furthermore, multiple studies suggest that blast crisis (BC) converges on a common high-risk transcriptomic and GRN state that is agnostic to mutational diversity, and driven by polycomb and DNA methylation-dependent epigenetic reprogramming. Given that BCR::ABL1-independent mechanisms, regulated at the level of GRNs, may contribute to resistance and LSC persistence, these observations support placing greater emphasis in CML management on addressing GRN-defined cell-state vulnerabilities, with the goal of lowering the risk of blast crisis in high-risk patients and improving control of therapy-resistant CP LSCs.

I INTRODUCTION

Resistance to tyrosine kinase inhibitors (TKIs) in chronic myeloid leukemia (CML) remains a major barrier to durable control. Although CML is driven by a single genetic abnormality and responds to potent TKIs,[1, 2] most patients still require lifelong therapy.[3] Depending on the choice of frontline TKI, only between 30–60% achieve an optimal response,[4] precluding attempts at TKI cessation for almost half of patients, while roughly 25,000 deaths worldwide (≈1,290 in the US; SEER) still occur annually.[5]

Traditional models focus on leukemic stem cells (LSCs)* and resistance-conferring mutations arising in hematopoietic stem and progenitor cells (HSPCs).[6] Even so, recent multi-omics and single-cell analyses indicate that these frameworks may not fully capture the range of observed outcomes.[7] Recent studies uncover pronounced heterogeneity within the LSC compartment, with distinct cellular states that differentially fuel therapy resistance, disease persistence, and progression (**Table 1**).[8-15] This mirrors the diversity of normal hematopoietic stem cells (HSCs), where lineage tracing and single-cell work depict a continuum or "cloud" of overlapping states with varying lineage biases,[16-19] maintained by transcription factor-dependent epigenetic programs and their resulting gene expression signatures.[18, 20]

In this perspective, we synthesize emerging literature and advance updated models that explicitly incorporate discrete LSC states. Considering resistance from a cell-state perspective broadens the classic clonal selection model, centered on mutation-driven evolution, to also encompass the context-dependent plasticity of LSC epigenomes and transcriptional programs under therapeutic pressure. This integrated framework suggests new strategies to predict the emergence of TKI resistance, as well as novel approaches to prevent and eradicate drug-resistant CML cells.

II NORMAL AND LEUKEMIC STEM CELL STATES

Normal HSC states are characterized by self-renewal, multipotency, genomic stability, and regulated metabolism. These core features are preserved by intrinsic transcription factor (TF) networks (regulons) that establish epigenetic configurations and gene expression signatures essential for stem cell identity and function. These TF networks form gene regulatory networks (GRNs), which incorporate epigenetic modifiers, non-coding RNAs, and signaling molecules to integrate diverse intracellular and extracellular cues (**Figure 1**).[21]

GRNs are inherently robust, maintaining stable expression through feedback and redundancy, yet remain highly responsive. This dynamic adaptability, often reversible, allows HSCs to adjust to oxygen and nutrient fluctuations, inflammation, infection, blood loss, and other stresses, preserving identity, meeting physiological demands, limiting genomic and oxidative damage, and supporting organismal longevity.

LSCs and HSCs share stemness properties, including self-renewal, multipotency, and quiescence.[22] These features may be intrinsic to the cell of origin when oncogenic transformation does not itself induce self-renewal (e.g. with BCR::ABL1), or acquired directly from oncogenic mutations, such as certain leukemic fusion oncoproteins (e.g. MOZ::TIF2).[23] In both scenarios, oncogenic lesions disrupt normal GRNs to drive uncontrolled proliferation,

altered differentiation and lineage output, enhanced survival, and modified responses to extrinsic cues (**Figure 2**). [18, 24, 25] Thus, while LSCs may phenotypically resemble HSCs, their aberrant states fuel malignant transformation and therapy resistance. Viewing LSCs as a cell state underscores their dual robustness and plasticity: the capacity to maintain malignant properties while adapting to microenvironmental signals.

In CML, LSCs with these defining properties are present across the disease spectrum, from chronic phase (CP) to blast crisis (BC), underscoring their central role in disease maintenance and therapeutic resistance in both early and advanced stages [7, 9, 13, 22, 26-28] .

III EXISTENCE AND IMPORTANCE OF A CP LSC STATE

Single-cell technologies have revealed stable epigenetic cell states in acute myeloid leukemia (AML), often linked to mutations such as FLT3-ITD, RAS activation, and other leukemia drivers.[25] These pathological states are sustained by oncogenic signaling and transcriptional networks that reprogram how AML cells sense and respond to their environment, shaping differentiation, survival, and metabolism. Dissecting the origins and maintenance of these abnormal states has guided new therapies that disrupt them.[24]

Defining equivalent regulatory networks in non-CML myeloproliferative neoplasms (MPNs) has been harder, given striking phenotypic heterogeneity despite genetic simplicity (e.g., JAK2-mutated MPNs).[29] This variability may reflect differences in cells of origin and the long latency, often decades, between driver acquisition and disease presentation, allowing diverse genetic and epigenetic histories to accrue and generate phenotypic diversity.[20, 29] Even so, myelofibrosis studies have identified distinct states, including megakaryocyte-biased HSCs enriched for inferred GRNs regulating inflammation and megakaryocyte function, with features reminiscent of early fetal hematopoiesis, pointing to new therapeutic avenues.[20, 30]

In contrast, CML research, based on the clinical success of TKIs, has emphasized BCR::ABL1-driven signaling, with less attention to how this oncogenic signal reshapes the epigenome and rewires gene expression. Yet persistence and transformation in CML despite potent TKIs underscore the need to investigate these regulatory layers to uncover novel mechanisms of resistance and disease maintenance. A central question in CP CML is whether a conserved CP LSC state exists, which and whether it recurs across patients and cohorts. Although definitive proof is still emerging, several observations support a discrete, persistent LSC state common to most CP CML. Below, we describe diverse lines of evidence supporting the existence and importance of such a state:

1. **Conserved and Distinct Transcriptional Signature**. Multiple independent studies identify an immunophenotypically defined LSC population in CP CML with consistent gene expression signatures distinct from normal HSCs and CML progenitors.[9, 13, 27 31]
2. **Canonical BCR::ABL1-Driven Signaling**. In canonical BCR::ABL1 signaling, the fusion tyrosine kinase activates the STAT, PI3K-AKT, and RAS-MAPK pathways. These cascades transmit signals to the nucleus, where transcription factors remodel chromatin and epigenetic states, reprogramming cell identity and behavior.[32-50]
3. **Retention and Divergence from HSC Features**. Immunophenotypically defined CML LSCs preserve core HSC traits (e.g. quiescence and self-renewal) yet undergo

metabolic rewiring marked by elevated oxidative phosphorylation, increased reactive oxygen species (ROS), and altered mitochondrial dynamics. The coexistence of conserved stemness with non-HSC metabolic signatures delineates CP LSCs as a discrete cell state, implying unique functional behaviors and therapeutic vulnerabilities.[22, 39, 51-56]

4. **Stereotyped Hierarchy Remodeling**. Across CML, the hematopoietic hierarchy is reproducibly reshaped: primitive stem compartments are depleted, while progenitor output is skewed toward myeloid, erythroid, and megakaryocytic lineages. This consistent shift in lineage bias and compartment proportions indicates that CP LSCs are not merely HSCs under stress; they harbor an altered differentiation program at the stem level, defining a discrete LSC state with reprogrammed fate decisions.[57-60]
5. **Altered Environmental Responsiveness**. Compared with normal HSCs, CML LSCs interpret bone marrow niche signals differently, and exhibit atypical adhesion and chemokine responses, shifted sensitivity to cytokines and growth factors, and distinct reactions to hypoxia and inflammatory cues. This rewired niche sensing and signaling decouple CP LSCs from canonical HSC regulation, arguing for a discrete LSC cell state rather than a stressed HSC variant.[61-64]
6. **Epigenetic Dysregulation**. Genome-wide profiling reveals a reproducible, LSC-specific epigenetic landscape (distinct DNA methylation, histone mark patterns, and chromatin accessibility) compared with normal HSCs and CML progenitors, underscoring epigenetic foundations of a discrete CP LSC state. [26, 65-69]
7. **Characteristic Immunophenotype**. CML LSCs reproducibly express surface marker combinations such as $CD34^{+}CD38^{-}CD26^{+}CD35^{-}$ and $IL1RAP^{+}$, which are absent from normal HSCs and mature progenitors. This reflects rewired signaling and niche interactions at the stem level, again indicating a *bona fide* LSC state rather than an activated HSC variant.[8, 13, 70-73]

Together, these lines of evidence support conserved, distinct LSC states in CP CML, with unique molecular, functional, and phenotypic features that differentiate them from normal HSCs and other leukemic populations.

We anticipate that defining these states will have important clinical implications. As in AML, such states show distinct responses to environmental stressors compared to HSCs and can generate persistence or resistance programs. Notably, BCR::ABL1-driven CML is unique among MPNs, appearing to result from a single oncogenic event, with clinical manifestations typically arising 3-14 years after HSCs acquire BCR::ABL1, i.e. far sooner than the decades seen with JAK2 mutations.[1, 74] This shorter latency likely contributes to the relative phenotypic homogeneity in CML. Such clinical homogeneity, evident as TKI responsiveness, is likely mirrored molecularly in uniform CP LSC states and the GRNs sustaining them. Further characterization could illuminate persistence and guide novel therapies. Persistent LSC survival under potent TKIs suggests targeting LSC state properties and supportive microenvironments could drive faster deep molecular response (DMRs) and successful TFR.

IV DIFFERENTIAL CP LSC STATES AND PRIMARY TKI RESISTANCE

Effects of BCR::ABL1 on HSC function and cell state

A key question is how BCR::ABL1 reprograms the normal HSC state to drive functional alterations (**Figure 2**). Colony-forming assays point to three core effects on HSPCs: (1) a

reduction in long-term HSCs (LT-HSCs); (2) an expansion of proliferative progenitors; and (3) a bias in differentiation toward erythroid, megakaryocytic, and myeloid lineages.[58, 59] Taken together, these findings suggest that CML LSCs occupy a cell state with a lowered threshold for differentiation and a skewed lineage output-particularly toward erythroid and megakaryocytic fates-while maintaining myeloid production evident in peripheral blood. Complementary studies further characterize chronic-phase LSCs as: 1) more independent of extrinsic growth factors,[59] 2) more readily mobilized to the periphery, consistent with altered niche interactions,[60] and 3) reduced stemness with concomitant increased progenitor proliferation.

Building on foundational work, subsequent studies elucidate how BCR::ABL1 mediates lineage bias. The Lodish group showed BCR::ABL1 can directly induce erythroid differentiation without JAK2, generating burst-forming unit-erythroid (BFU-E) and colony-forming unit-erythroid (CFU-E) even without cytokines.[75] However, when the erythropoietin receptor is absent, cytokines are required for BFU-E formation. Moreover, BCR::ABL1 domain mutations (e.g., Y177F, SH2 domain deletions) render JAK2 indispensable for erythroid differentiation. Together, these findings indicate BCR::ABL1 supports erythroid commitment in HSPCs and functionally overlaps with physiologic JAK2 signaling.

Supporting lineage-bias observations, the Witte group used an inducible BCR::ABL1 in embryonic stem cell-derived multipotent progenitors (MPPs) and showed that higher BCR::ABL1 increased proliferation and biased output toward myeloid over erythroid progenitors reversible upon switching off BCR::ABL1.[76, 77] The Bhatia group found exogenous BCR::ABL1 expression in CD34+ cord blood generated both erythroid and myeloid progenitors, with a notable, dose-dependent increase in erythroid output, doubling the erythroid-to-myeloid ratio.[78] Whether JAK2 activity downstream of BCR::ABL1 mediated this was not assessed. Hematopoietic differentiation reflects both promotion and suppression of lineage programs (e.g., PU.1 vs TAL1). Perrotti and colleagues reported stronger BCR::ABL1 suppresses C/EBPa-driven myeloid differentiation via a MAPK-hnRNP-E2 pathway.[79] Collectively, these observation indicate that BCR::ABL1 drives lineage bias and progenitor proliferation.

Cellular context can also influence the effects of BCR::ABL1. Its impact on lineage output varies by system, implying the epigenetic landscape modulates function and necessitating frameworks that account for cell state. A clear example is AKT-FOXO signaling: in non-stem CML cells (e.g. murine c-Kit$^+$ Lin$^-$ Sca-1$^-$ and most CML lines), BCR::ABL1 activates AKT, phosphorylating and inactivating nuclear FOXO. In contrast, c-Kit$^+$ Lin$^-$ Sca-1$^+$ CML LSCs show AKT inhibition and FOXO activation, which is essential for LSC function.[80] Cell-state-dependent chromatin accessibility, e.g. differential FOXO binding site access, may further shape this pathway's distinct activities across cell contexts.

Germline and microenvironmental factors induce an apoptosis-resistant LSC state

A paradox in CML therapy is that while CML cells, including primary HSPCs, can be eradicated *in vitro* within 2–4 days, achieving similar effects in patients often requires months to years of TKI therapy. This discrepancy is unlikely to be fully explained by genetic or pharmacokinetic and pharmacodynamic factors alone. Instead, altered cell states,

particularly those elevating the apoptotic threshold, appear central to intrinsic resistance to TKI-induced cell death *in vivo*.

Both constitutional (germline) and microenvironmental influences contribute to these states, collectively raising LSC apoptotic thresholds and promoting relative TKI resistance. A well-characterized germline example is the *BIM* deletion polymorphism (BDP).[81] Identified in East Asian populations, the BDP confers partial resistance to targeted therapies in CML and EGFR- or ALK/ROS1-driven non-small cell lung cancer.[81-84] BIM, a pro-apoptotic protein essential for TKI-mediated killing via antagonism of BCL2 family proteins, is normally upregulated upon TKI exposure. The BDP favors non-apoptotic *BIM* splice isoforms, increasing the apoptotic threshold and rendering LSCs less sensitive. In humanized mice carrying the human BDP, this single variant enhances CML LSC competitive fitness and accelerates disease, implying that germline polymorphisms can modulate apoptotic thresholds, drug sensitivity, and disease potency.[85] Related variants affecting *BIM* expression in other populations further suggest broad modulation of LSC states and therapeutic response.[86]

Microenvironmental cues are equally critical. Extrinsic cytokine signaling can induce a BCR::ABL1 kinase-independent survival state, further elevating apoptotic thresholds.[87] Bone marrow stromal cells confer marked protection against TKI-induced apoptosis via contact and soluble factors that activate pro-survival pathways in LSCs.[63, 88] Physiologic hypoxia offers another example: at marrow oxygen levels, CML LSCs engage a HIF-1α dependent, BCR::ABL1-independent transcriptional program that confers relative imatinib resistance.[89] Under hypoxia, CML LSCs upregulate survival genes more than normal HSCs, illustrating a CML-specific, stress-induced, reversible LSC state driving non-genetic resistance. How BCR::ABL1 reshapes the epigenome to differentially interface with HIF-1α dependent transcription and other cues remains an important question.

Therapy itself can provoke resistant states. Studies, including primary CML samples, report TKI exposure activating PI3K/AKT/mTOR signaling, potentially contributing to resistance.[90] Whether this reflects intrinsic HSC features (e.g. c-KIT inhibition by imatinib) or LSC-specific circuitry remains unclear, but it underscores treatment-induced cell-state plasticity.

Together, germline polymorphisms, cytokine signaling, stromal interactions, hypoxic adaptation, and TKI exposure can dynamically reprogram LSC states, elevate apoptotic thresholds, and confer relative TKI resistance without additional mutations (**Figure 3**). These insights argue for targeting cell state-specific vulnerabilities to overcome persistence and achieve more durable responses.

Implications of HSC heterogeneity and origins of the CP LSC

Traditional models portrayed HSCs as homogeneous, with discrete progenitor populations progressively restricted in lineage potential.[18, 19] This view, imposed largely by technical limits, fostered an implicit assumption that the LSC pool is homogeneous and contributes little to clinical heterogeneity. It is now evident that HSCs and progenitors are functionally heterogeneous.[18] Recent models describe an HSC pool varying in self-renewal and differentiation properties, with restriction occurring further down the hierarchy. Multi-omics single-cell approaches, surveying whole transcriptomes and epigenomes, show this

heterogeneity exists along a continuum within both HSC and progenitor compartments.[16, 17] Within HSCs, subsets exhibit differing biases toward mature lineages. The molecular basis of these functional differences likely reflects combined transcriptional and epigenetic regulators that maintain each state. How these states arise remains unresolved, spanning intrinsic hematopoietic properties to stochastic and environmental influences. Current understanding suggests fate potential involves 'priming' toward particular lineages through opening regulatory elements of lineage-determining genes and low-level expression of lineage-defining transcription factors.[19] Thus, HSCs comprise both unbiased and lineage-biased populations, the latter with distinct epigenetic and gene expression states, including erythroid, myeloid, or pre-B cell biases. These models point to LSC heterogeneity as a potential contributor to the observed variability in TKI responses among patients. Consequently, it will be important to consider how BCR::ABL1 alters the HSC state and fate within pre-existing heterogeneity in the cell of origin. Conceptually, at least three variables may influence TKI resistance. First, the HSC cell-of-origin, i.e. where BCR::ABL1 first arises, as recent work links lineage fate to differential first-line TKI responses.[7] Second, the strength of BCR::ABL1 signaling, repeatedly shown to modulate lineage bias *in vitro*.[78] Third, the duration of unopposed signaling, which, if prolonged, may foster TKI-resistant epigenetic cell states, as seen in BC LSC.[26] Together, these considerations argue that decoding the interplay between baseline HSC diversity and oncogenic signal strength and duration will be essential for understanding CP LSC origins and for anticipating primary resistance.

Single-cell studies and the CP LSC state

Despite substantial progress, we still lack a complete understanding of how BCR::ABL1 reprograms normal HSCs into CML LSCs, the transcription factor networks that define the CML LSC state, and the implications of LSC heterogeneity. A seminal study from the Karlsson group linked single-cell immunophenotypic heterogeneity to molecular and functional differences, identifying among the most apical LSCs, a quiescent and relatively TKI-insensitive $Lin^-CD34^+CD38^{-/low}CD45RA^-cKIT^-CD26^+$ population.[8]

Consistent with LSC heterogeneity, the first single-cell omics analysis of CP LSCs demonstrated that within $CD34^+CD38^-$ HSPCs, CP LSCs exhibit a distinct gene expression program enriched for proliferative pathways (G2/M, MYC, E2F targets, oxidative phosphorylation), findings subsequently validated by other groups.[9, 27] Longitudinal profiling of CP LSCs from patients achieving a major molecular response (MMR) further showed the selection for a quiescent LSC population during effective TKI therapy. Notably, these quiescent LSCs differ from quiescent normal HSCs, particularly in JAK-STAT pathway expression.[9]

Differential LSC states and quality of TKI response

In a subsequent study, we used scRNA-seq on diagnostic bone marrow from CP CML patients to identify prognostic cell types.[27] Unbiased analysis across 22 detected cell types highlighted three with the greatest predictive accuracy for frontline imatinib response: LSCs (defined by canonical gene expression signatures), natural killer cells, and plasmacytoid dendritic cells. Focusing on LSCs, we applied the SCENIC pipeline[91] to infer transcriptional regulatory networks. LSCs from patients achieving DMR (ELN Optimal Response) showed activation of KLF1, TAL1, and GATA1 regulons, consistent with a canonical erythroid lineage

program. In contrast, LSCs from patients who progressed to BC exhibited activation of MYC and IRF1 regulons.

To validate functional consequences, we performed colony-forming assays with patient-derived $CD34^+$ HSPCs. Optimal responders displayed a significantly higher erythroid-to-myeloid progenitor ratio, which declined progressively in patients with suboptimal responses or who progressed to BC. Flow cytometry of diagnostic marrow corroborated an erythroid progenitor bias in patients with optimal TKI responses, diminishing with poorer responses. These findings demonstrate that differential lineage bias is an intrinsic feature of CP LSCs. The degree of erythroid versus myeloid bias is prognostic, can be evaluated *in vivo* at diagnosis, and represents a potential biomarker for predicting frontline TKI efficacy. Because these lineage programs have been described in the marrow compartment these biomarkers may not map one-to-one onto contemporaneous blood counts since a multiplicity of factors determine peripheral blood counts.[92 93] Prospective studies are needed to validate these markers and to assess whether peripheral blood can substitute for bone marrow. Critically, we further showed that erythroid progenitors are highly sensitive to imatinib, whereas myeloid progenitors exhibit relative resistance. This supports a model in which lineage-determining transcriptional programs in LSCs skew the hematopoietic hierarchy toward either TKI-sensitive erythroid or TKI-resistant myeloid progenitor populations, making patients with erythroid-biased LSC output more likely to achieve deep responses.

Supporting this model, the Karlsson group confirmed that robust TKI responses associate with expansion of the erythroid progenitor compartment.[13] Historical *in vitro* data similarly indicate erythroid progenitors are intrinsically more TKI-sensitive than myeloid progenitors.[57] This lineage-dependent sensitivity extends to other agents: in the pre-TKI era, increased marrow erythroid progenitors (and absence of fibrosis or splenomegaly) predicted improved survival with interferon-α, hydroxyurea, and busulfan.[94]

In summary, intrinsic CP LSC drug resistance underlies persistent CML. LSC functional heterogeneity, embodied in distinct transcriptional and epigenetic states, likely contributes to lineage bias and TKI response quality. Recent single-cell studies link erythroid versus myeloid LSC states to TKI sensitivity, underscoring the need to understand and target LSC states to overcome resistance and improve outcomes.

V LSC states and treatment free remission (TFR)

TFR is a key therapeutic goal in CML, yet outcomes after TKI cessation show a dichotomy: 40–60% maintain remission, while the rest relapse, often within months.[3] First observed in the French STIM trial and confirmed in TWISTER and EURO-SKI, TFR at six months was 61%.[95-97] Sustained TFR correlates with two clinical factors: duration of TKI therapy and duration of DMR (BCR-ABL1 ≤0.01% or MR4).[98] Each additional year of sustained DMR before withdrawal confers a 2–3% absolute increase in relapse-free survival.[97] These observations are consistent with gradual TKI-induced attrition of CML LSCs, while a residual pool persists and can fuel relapse after treatment cessation. We review TFR biology through the lens of CP LSC cell state (**Figure 4**), considering what clinical and biological observations imply about CP LSC states in patients who achieve DMR and are eligible for cessation.

TKI-resistant LSCs and TFR

TKI-resistant LSCs and TFR mathematical modeling supports gradual LSC attrition on TKIs but maintenance of a minimal reservoir that expands quickly after withdrawal.[99, 100] BCR::ABL1 levels double about every 9 days (~1-log monthly),[101] with most relapses occurring within six months. Some patients maintain remission despite fluctuating BCR::ABL1 transcripts below MMR (≤0.1%), while ~14% experience late relapse (≥2 years), necessitating retreatment.[102, 103] These findings suggest immune surveillance sustains remission in patients with low-level fluctuations, whereas late relapse may reflect loss of immune control.[104]

Isolating residual LSCs at cessation is challenging. Functional assays (e.g., long-term culture-initiating cells (LTC-ICs)) can readout for more primitive BCR::ABL1$^+$ progenitors but are poor predictors of TFR.[105] CD26 is a proposed CML LSC marker, yet levels fluctuate even in patients in durable remission.[106] Pagani et al. recently reported rare, differentiated BCR::ABL1(+) cells in granulocyte and T-cell lineages at cessation as powerful relapse predictors: 100% for granulocytes, 67% for T cells.[107] This supports a model where therapy-resistant, LSCs or multipotent progenitors persist and continue generating aberrant progeny in DMR. Such cells may pre-exist via intrinsic TKI-evasion pathways or arise during therapy through transcriptomic shifts to more resistant states, or both, requiring further study.

Intrinsic mechanisms of LSC persistence

To connect diagnostic LSC biology to relapse after cessation, key questions include: 1) Which GRNs drive LSC transcriptional heterogeneity and confer TKI-resistance to relapse-fated subclones; 2) Are there universal GRNs for persistence, or are they patient-specific; 3) Do BCR::ABL1$^-$ stem cells or myeloid populations support LSC persistence; and 4) Do BCR::ABL1 LSC states evolve from diagnosis to DMR and TFR relapse?

Single-cell RNA sequencing (scRNA-seq) begins to address these gaps. Giustacchini et al. identified two diagnostic LSC populations: Group A, marked by quiescence/stemness and expanded during TKI treatment; and Group B, dependent on inferred MYC/E2F proliferation programs and diminished on TKIs.[9] Group A likely constitutes a therapy-resistant reservoir maintained by GRNs conferring evasion. Clinically, Shanmuganathan et al. showed early BCR::ABL1::ABL1 halving times (<9.35 days) predict TFR success (80%), whereas slower kinetics (>21.85 days) associate with high relapse risk (96%).[108] Relapse potential may thus be intrinsically encoded at diagnosis, with BCR::ABL1 decline rates mirroring fundamental biological differences between patients with durable remission as against those that relapse.

Integrating these two studies into a unified model suggests that different LSC states may lead to distinct lineage outcomes, which are reflected in varying BCR::ABL1 halving times. Specifically, the halving time of BCR::ABL1 could be influenced by the LSC state present at diagnosis, either highly sensitive to TKI treatment or more resistant, consistent with the cellular states described by Giustacchini et al. Careful single-cell studies correlating halving kinetics with HSPC population dynamics and molecular signatures are needed to validate whether the initial LSC composition determines long-term therapeutic outcomes and TFR success.

These observations raise the prospect that diagnostic LSC transcriptional heterogeneity holds prognostic value for relapse years before cessation, enabling early intervention. Resolving whether LSC persistence is governed by universal or patient-specific GRNs, and clarifying regulatory roles of BCR::ABL1$^-$ non-malignant stem cells, could inform new therapies and combinations to improve TFR. Future work should employ targeted single-cell and clonal tracing studies in remission–relapse cohorts, including cellular indexing of transcriptomes and epitopes by sequencing (CITE-seq) with known CML markers (e.g., CD26, IL1RAP) alongside single-cell transcriptomics.

Extrinsic Regulators of LSC persistence: The effect of TKI therapy, immune control and niche Interactions

The CML therapeutic landscape is complicated by the dual effects of TKIs: they induce LSC quiescence, making cells less susceptible to TKI-induced death,[109] while restoring immune function via T/NK-cell activation and reduced monocytic myeloid-derived suppressor cells (MDSCs).[110] Thus, TKIs can both push LSCs into a quiescent resistant state and enhance immune clearance. LSCs may counter by upregulating immune evasion (e.g., PD-L1).[111] Functional co-culture studies during cessation, with or without TKIs, are essential but remain technically challenging.

Patients achieving sustained TFR exhibit distinct immune signatures at cessation: expanded cytotoxic NK and CD8$^+$ T cells, reduced regulatory T cells (Tregs) and MDSCs, and low CD86$^+$ plasmacytoid dendritic cells. [112-114] Mathematical models incorporating immune parameters improve TFR prediction, underscoring the immune-LSC balance. [115, 116]

Additional contributors include LSC–niche interactions (CXCR4, CD44, ITGA3), hypoxia adaptation (HIF-1α), inflammatory cytokines (IL1α, IL1β, TNFα, IL-6), and stromal signaling (TGFβ, SDF-1), all promoting TKI resilience. [117, 118] A recent study challenges the bone marrow as the sole relapse source, proposing the splenic pulp as an additional niche for CML LSCs. [119] Clarifying how LSCs, TKIs, immune control, and niche interactions intersect is central to understanding relapse biology.

Emerging single-cell technologies to understand TFR biology

Progress hinges on single-cell approaches defining transcriptomic/epigenomic signatures linked to relapse. Mutations in epigenetic regulators (ASXL1, DNMT3A, KDM6A) at diagnosis correlate with lower TFR,[120] suggesting epigenetic reprogramming sustains LSCs. Conversely, clonal hematopoiesis of indeterminate potential (CHIP) mutations at cessation correlate with better TFR.[121] Only single-cell methods can localize these mutations to LSCs versus non-malignant compartments. Combined with lineage tracing (e.g., mitochondrial DNA barcoding),[122] they could track relapse-prone LSCs from diagnosis through recurrence. Bone marrow spatial transcriptomics may reveal key LSC-immune and LSC-niche interactions shaping TFR. Ultimately, deciphering the dynamic interplay between persistent LSCs, immune surveillance, and niche protection should enable personalized strategies to predict and improve TFR.

VI EVIDENCE FOR A DISTINCT BC LSC STATE

A distinct BC LSC state is supported by several lines of evidence: 1. A reproducible, pan-BC gene-expression program (which we term the common BC signature or CBS) is detected across most $CD34^+$ BC samples regardless of the underlying mutational profile, establishing an empirically defined transcriptomic state; [26, 123, 124] 2. Functional LSC activity (serial xenotransplantation/long-term propagation) is demonstrated across multiple immunophenotypically-defined HSPC subsets [HSC-, lymphoid primed multipotent progenitor (LMPP)-, common myeloid progenitor (CMP)-, granulocyte-macrophage progenitor (GMP)-like], even within the same sample,[28] consistent with the notion that LSC activity is a property of cell state rather than a specific immunophenotype; 3. Most BC samples are clonal or oligoclonal with a dominant clone, indicating that the CBS is associated with clonal expansion;[125, 126] and 4. the CBS encompasses established LSC gene-expression signatures (e.g., quiescence/stemness modules, Wnt/β-catenin, HIF/stress-response, anti-apoptotic and niche/adhesion pathways), providing functional gene-expression evidence that links the BC program to stem-like behavior.[26] Collectively, these data are consistent with BC HSPCs representing a distinct state with a shared gene expression program and broad stemness features that can transcend genotype and immunophenotype.

Interpreting genetic diversity in the context of a common BC LSC state

A striking feature of BC is the pronounced genetic diversity among BC HSPCs arising against a surprisingly uniform transcriptomic backdrop shared by most cases. This common gene expression signature, first described by Radich et al.,[123] has been validated by others, including our previous studies.[26, 124]

As expected in advanced malignancy, extensive sequencing reveals a 'long tail' of acquired mutations, many clustering in leukemia-associated genes (e.g., RUNX1, IKZF1, ASXL1, BCORL1, GATA2, TET2), collectively accounting for over 70% of BC mutations.[6, 26, 125] Most encode transcription factors or epigenetic regulators, implicating cell-state shifts as central to BC evolution. Yet the diversity of mutated genes and the variable downstream programs they influence do not, on their own, explain the convergence on a shared BC transcriptomic state. Integrated multi-omics studies show both myeloid and lymphoid BCs exhibit this core profile, regardless of mutational landscape.[26] This raises a key question: How do biologically diverse mutations drive BC transformation if a uniform transcriptomic state predominates?

Emerging evidence suggests individual mutations are superimposed on a foundational CBS. For example, BC samples with RUNX1 mutations display an additional, mutation-specific signature with functional consequences, including altered responses to targeted therapies.[127, 128] A critical interpretive consideration is that some detected mutations may reside in non-leukemic clones. In CML, studies integrating BCR::ABL1 transcript quantification with mutational and single-cell analyses highlight the need to contextualize mutation calls with clonal architecture, tumor purity, and single-cell resolution to avoid overestimating the clinical relevance of mutations confined to minor or non-leukemic populations.

Collectively, these findings support a model in which a pool of HSPCs acquires an 'at-risk' cell state via a rewired GRN (**Figure 5**). This state, characterized by stemness, inflammation, impaired differentiation, and altered DNA damage/repair, creates cellular 'soil' in which

driver mutations accumulate and confer clonal advantage.[26] This model reconciles wide mutational heterogeneity with the convergent BC transcriptomic phenotype and explains heterogeneous outcomes when only genetic events are considered.[6, 129] Clinically, detecting this at-risk state in CP could signal a need for earlier or more targeted interventions to prevent progression and eradicate high-risk clones. Mechanistically, the CBS may also arise via convergent evolution, with heterogeneous genetic and epigenetic events selected for fitness traits embodied within the CBS, as described in solid tumors and select blood cancers.[130, 131]

Clinical implications of the CBS

The presence of the CBS across genetically diverse samples has possible therapeutic implications. By revealing shared gene-expression programs in BC cells (spanning stemness, inflammation, and DNA damage responses) it points to common, potentially targetable processes that could support more universal treatment approaches and lessen reliance on mutation-specific drugs. To translate this into practice, further work is needed to sharpen target specificity, limit toxicity (including to normal stem cell programs), and design rational combinations. In particular, CBS-directed therapies could be paired with mutation-targeted agents to enhance efficacy and prevent relapse from residual or resistant clones. Mechanistically, high-risk signature genes are enriched for BMI1-silenced targets and show aberrant DNA methylation; functionally, pharmacologic BMI1 inhibition with DNA methylating agents reduce BC cell survival and stemness, highlighting a potential therapeutic approach.[26] The shared transcriptomic signature also offers a basis for biomarkers to identify high-risk CP patients before progression; integrating transcriptomic and mutational data could improve sensitivity and specificity.[6, 129] Finally, preclinical models that reflect the CBS may better predict clinical outcomes than genetics-only models. Targeting and detecting the CBS in therapeutic and diagnostic strategies may enable more effective, broadly applicable treatments and earlier intervention, improving the durability of responses despite substantial genetic heterogeneity.

Evaluating predictions of the CBS: Biomarkers and Therapies

A key prediction from the CBS is that CBS-associated gene expression signatures could identify high-risk patients during CP, before overt transformation. This was substantiated by two independent studies analyzing $CD34^+$ cells from CP at diagnosis: both showed that specific transcriptomic signatures predict earlier progression to BC and clinical TKI resistance.[132] Notably, Zhang et al. directly compared signatures across cohorts and found significant overlap, underscoring the reproducibility and robustness of a common high-risk program in CP when the same starting cell types ($CD34^+$ HSPCs) are interrogated. [129]

These findings matter for two reasons. First, they suggest a shared gene expression signature can identify high-risk CP patients, independent of mutational heterogeneity. Second, they reveal that pre-existing biological features at diagnosis, rather than solely therapy-acquired mutations, contribute to clinical outcomes. Leveraging these insights, our group has developed a flow cytometry–based test to identify at-risk CP patients at diagnosis.[133]

Collectively, these studies point to a potential paradigm shift in CML: by deploying robust pretreatment biomarkers to detect high-risk clones at diagnosis, early intervention,

potentially with epigenetic modulators such as BMI1 inhibitors, could eliminate preleukemic or early leukemic stem cell populations and prevent progression to BC. This prevention-focused strategy could transform management by targeting vulnerable cell states before they acquire additional mutations and become clinically unmanageable.

VI OUTSTANDING QUESTIONS IN CML CELL STATE RESEARCH

In future work, a key objective will be the molecular dissection of clinically-relevant CML cell states at critical time points, including diagnosis, disease progression, achievement of DMR, and relapse following TKI discontinuation. It will be important to define GRNs and their component regulons, as well as the signaling pathways that induce these regulons. GRN activity should be related to cellular function in order to determine which networks are associated with therapeutic resistance, disease persistence, and progression. It is also important to understand how both somatic and germline genetic events influence GRNs and the resulting cell states. Another priority is to identify therapeutic vulnerabilities unique to specific cell states including targeting inflammation-related pathways in BC, as our understanding of these pathways remains limited. These discoveries can be translated into biomarker-based clinical studies and used to formulate strategies for targeting resistant and persistent disease states. Achieving these goals will require access to patient samples, particularly bone marrow collected at clinically-informative but rarely indicated time points, such as relapse after TKI cessation. If successful, these studies are expected to significantly advance our understanding of CML pathogenesis and persistence, paving the way for more precise, effective, and durable therapeutic interventions.

*For the purposes of this perspective, we have used the term LSC to encompass populations that are enriched for functional stem cells, i.e. have serial transplantation capacity, and which have other features of stemness. These include strong molecular, phenotypic, and clinical correlates, even without serial xenotransplantation.[9, 31, 51, 134] When single-cell studies are referenced, HSC/LSC populations are identified based on canonical HSC-specific gene expression markers.

REFERENCES

1. Kamizela AE, Leongamornlert D, Williams N, et al. Timing and trajectory of BCR::ABL1-driven chronic myeloid leukaemia. Nature. 2025;640(8060):982-990.
2. Braun TP, Eide CA, Druker BJ. Response and resistance to BCR-ABL1-targeted therapies. Cancer Cell. 2020;37(4):530-542.
3. Ross DM, Hughes TP. Treatment-free remission in patients with chronic myeloid leukaemia. Nat Rev Clin Oncol. 2020;17(8):493-503.
4. Shanmuganathan N, Branford S, Yeung D, Ross D, Hughes T. Frontline tyrosine kinase inhibitor selection influences eventual treatment-free remission eligibility and sustained treatment-free remission: A registry analysis of patients treated at CML referral sites in Australia. Blood. 2025;146(Supplement 1):2008.
5. Ning L, Hu C, Lu P, Que Y, Zhu X, Li D. Trends in disease burden of chronic myeloid leukemia at the global, regional, and national levels: a population-based epidemiologic study. Exp Hematol Oncol. 2020;9(1):29.
6. Branford S, Kim DDH, Apperley JF, et al. Laying the foundation for genomically-based risk assessment in chronic myeloid leukemia. Leukemia. 2019;33(8):1835-1850.
7. Thakur RK, Karlsson G. Insights from single-cell omics: cellular heterogeneity as a foundation of clinical outcome in chronic myeloid leukemia. Blood. 2026;147(5):485-496.
8. Warfvinge R, Geironson L, Sommarin MNE, et al. Single-cell molecular analysis defines therapy response and immunophenotype of stem cell subpopulations in CML. Blood. 2017;129(17):2384-2394.
9. Giustacchini A, Thongjuea S, Barkas N, et al. Single-cell transcriptomics uncovers distinct molecular signatures of stem cells in chronic myeloid leukemia. Nat Med. 2017;23(6):692-702.
10. Ma L, Pak ML, Ou J, et al. Prosurvival kinase PIM2 is a therapeutic target for eradication of chronic myeloid leukemia stem cells. Proc Natl Acad Sci U S A. 2019;116(21):10482-10487.
11. Pagliaro S, Desterke C, Acloque H, et al. Single-cell transcriptome in chronic myeloid leukemia: pseudotime analysis reveals evidence of embryonic and transitional stem cell states. Exp Hematol. 2020;85:47-56.e2.
12. Chen Y, Mobius S, Riege K, et al. Genetic separation of chronic myeloid leukemia stem cells from normal hematopoietic stem cells at single-cell resolution. Leukemia. 2023;37(7):1561-1566.
13. Warfvinge R, Geironson Ulfsson L, Dhapola P, et al. Single-cell multiomics analysis of chronic myeloid leukemia links cellular heterogeneity to therapy response. Elife. 2024;12:RP92074.
14. Scott MT, Liu W, Mitchell R, et al. Activating p53 abolishes self-renewal of quiescent leukaemic stem cells in residual CML disease. Nat Commun. 2024;15(1):651.
15. Huuhtanen J, Adnan-Awad S, Theodoropoulos J, et al. Single-cell analysis of immune recognition in chronic myeloid leukemia patients following tyrosine kinase inhibitor discontinuation. Leukemia. 2024;38(1):109-125.
16. Buenrostro JD, Corces MR, Lareau CA, et al. Integrated single-cell analysis maps the continuous regulatory landscape of human hematopoietic differentiation. Cell. 2018;173(6):1535-1548.e16.

17. Corces MR, Buenrostro JD, Wu B, et al. Lineage-specific and single-cell chromatin accessibility charts human hematopoiesis and leukemia evolution. Nat Genet. 2016;48(10):1193-1203.
18. Laurenti E, Gottgens B. From haematopoietic stem cells to complex differentiation landscapes. Nature. 2018;553(7689):418-426.
19. Velten L, Haas SF, Raffel S, et al. Human haematopoietic stem cell lineage commitment is a continuous process. Nat Cell Biol. 2017;19(4):271-281.
20. Roy A, Wang G, Iskander D, et al. Transitions in lineage specification and gene regulatory networks in hematopoietic stem/progenitor cells over human development. Cell Rep. 2021;36(11):109698.
21. Gottgens B. Regulatory network control of blood stem cells. Blood. 2015;125(17):2614-2620.
22. Vetrie D, Helgason GV, Copland M. The leukaemia stem cell: similarities, differences and clinical prospects in CML and AML. Nat Rev Cancer. 2020;20(3):158-173.
23. Huntly BJ, Shigematsu H, Deguchi K, et al. MOZ-TIF2, but not BCR-ABL, confers properties of leukemic stem cells to committed murine hematopoietic progenitors. Cancer Cell. 2004;6(6):587-596.
24. Assi SA, Bonifer C, Cockerill PN. Rewiring of the transcription factor network in acute myeloid leukemia. Cancer Inform. 2019;18:1176935119859863.
25. Assi SA, Imperato MR, Coleman DJL, et al. Subtype-specific regulatory network rewiring in acute myeloid leukemia. Nat Genet. 2019;51(1):151-162.
26. Ko TK, Javed A, Lee KL, et al. An integrative model of pathway convergence in genetically heterogeneous blast crisis chronic myeloid leukemia. Blood. 2020;135(26):2337-2353.
27. Krishnan V, Schmidt F, Nawaz Z, et al. A single-cell atlas identifies pretreatment features of primary imatinib resistance in chronic myeloid leukemia. Blood. 2023;141(22):2738-2755.
28. Kinstrie R, Karamitros D, Goardon N, et al. Heterogeneous leukemia stem cells in myeloid blast phase chronic myeloid leukemia. Blood Adv. 2016;1(3):160-169.
29. O'Sullivan JM, Mead AJ, Psaila B. Single-cell methods in myeloproliferative neoplasms: old questions, new technologies. Blood. 2023;141(4):380-390.
30. Psaila B, Wang G, Rodriguez-Meira A, et al. Single-cell analyses reveal megakaryocyte-biased hematopoiesis in myelofibrosis and identify mutant clone-specific targets. Mol Cell. 2020;78(3):477-492.e8.
31. Abraham SA, Hopcroft LE, Carrick E, et al. Dual targeting of p53 and c-MYC selectively eliminates leukaemic stem cells. Nature. 2016;534(7607):341-346.
32. Adnan-Awad S, Kim D, Hohtari H, et al. Characterization of p190-Bcr-Abl chronic myeloid leukemia reveals specific signaling pathways and therapeutic targets. Leukemia. 2021;35(7):1964-1975.
33. Horita M, Andreu EJ, Benito A, et al. Blockade of the Bcr-Abl kinase activity induces apoptosis of chronic myelogenous leukemia cells by suppressing signal transducer and activator of transcription 5-dependent expression of Bcl-xL. J Exp Med. 2000;191(6):977-984.
34. Cauchy P, James SR, Zacarias-Cabeza J, et al. Chronic FLT3-ITD signaling in acute myeloid leukemia Is connected to a specific chromatin signature. Cell Rep. 2015;12(5):821-836.
35. Sementino E, Hassan D, Bellacosa A, Testa JR. AKT and the hallmarks of cancer. Cancer Res. 2024;84(24):4126-4139.

36. Pellicano F, Scott MT, Helgason GV, et al. The antiproliferative activity of kinase inhibitors in chronic myeloid leukemia cells is mediated by FOXO transcription factors. Stem Cells. 2014;32(9):2324-2337.
37. Carra G, Torti D, Crivellaro S, et al. The BCR-ABL/NF-kappaB signal transduction network: a long lasting relationship in Philadelphia positive Leukemias. Oncotarget. 2016;7(40):66287-66298.
38. Hsieh MY, Van Etten RA. IKK-dependent activation of NF-kappaB contributes to myeloid and lymphoid leukemogenesis by BCR-ABL1. Blood. 2014;123(15):2401-2411.
39. Stein SJ, Baldwin AS. NF-kappaB suppresses ROS levels in BCR-ABL(+) cells to prevent activation of JNK and cell death. Oncogene. 2011;30(45):4557-4566.
40. Reuther JY, Reuther GW, Cortez D, Pendergast AM, Baldwin AS Jr. A requirement for NF-kappaB activation in Bcr-Abl-mediated transformation. Genes Dev. 1998;12(7):968-981.
41. Jin Y, Zhou J, Xu F, et al. Targeting methyltransferase PRMT5 eliminates leukemia stem cells in chronic myelogenous leukemia. J Clin Invest. 2016;126(10):3961-3980.
42. Sengupta A, Banerjee D, Chandra S, et al. Deregulation and cross talk among Sonic hedgehog, Wnt, Hox and Notch signaling in chronic myeloid leukemia progression. Leukemia. 2007;21(5):949-955.
43. Zhao C, Blum J, Chen A, et al. Loss of beta-catenin impairs the renewal of normal and CML stem cells in vivo. Cancer Cell. 2007;12(6):528-541.
44. Bennemann K, Galm O, Wilop S, Schubert C, Brummendorf TH, Jost E. Epigenetic dysregulation of secreted frizzled-related proteins in myeloproliferative neoplasms complements the JAK2V617F-mutation. Clin Epigenetics. 2012;4(1):12.
45. Staal FJ, Famili F, Garcia Perez L, Pike-Overzet K. Aberrant wnt signaling in leukemia. Cancers (Basel). 2016;8(9):78.
46. Zhao C, Chen A, Jamieson CH, et al. Hedgehog signalling is essential for maintenance of cancer stem cells in myeloid leukaemia. Nature. 2009;458(7239):776-779.
47. Dierks C, Beigi R, Guo GR, et al. Expansion of Bcr-Abl-positive leukemic stem cells is dependent on Hedgehog pathway activation. Cancer Cell. 2008;14(3):238-249.
48. Chaudhry P, Singh M, Triche TJ, Guzman M, Merchant AA. GLI3 repressor determines Hedgehog pathway activation and is required for response to SMO antagonist glasdegib in AML. Blood. 2017;129(26):3465-3475.
49. Shi X, Zhang Z, Zhan X, et al. An epigenetic switch induced by Shh signalling regulates gene activation during development and medulloblastoma growth. Nat Commun. 2014;5:5425.
50. Kuepper MK, Butow M, Herrmann O, et al. Stem cell persistence in CML is mediated by extrinsically activated JAK1-STAT3 signaling. Leukemia. 2019;33(8):1964-1977.
51. Kuntz EM, Baquero P, Michie AM, et al. Targeting mitochondrial oxidative phosphorylation eradicates therapy-resistant chronic myeloid leukemia stem cells. Nat Med. 2017;23(10):1234-1240.
52. Ianniciello A, Zarou MM, Rattigan KM, et al. ULK1 inhibition promotes oxidative stress-induced differentiation and sensitizes leukemic stem cells to targeted therapy. Sci Transl Med. 2021;13(613):eabd5016.

53. Zarou MM, Rattigan KM, Sarnello D, et al. Inhibition of mitochondrial folate metabolism drives differentiation through mTORC1 mediated purine sensing. Nat Commun. 2024;15(1):1931.
54. Khalaf A, de Beauchamp L, Kalkman E, et al. Nutrient-sensitizing drug repurposing screen identifies lomerizine as a mitochondrial metabolism inhibitor of chronic myeloid leukemia. Sci Transl Med. 2024;16(751):eadi5336.
55. de Beauchamp L, Himonas E, Helgason GV. Mitochondrial metabolism as a potential therapeutic target in myeloid leukaemia. Leukemia. 2022;36(1):1-12.
56. Pellicano F, Copland M, Jorgensen HG, Mountford J, Leber B, Holyoake TL. BMS-214662 induces mitochondrial apoptosis in chronic myeloid leukemia (CML) stem/progenitor cells, including CD34+38- cells, through activation of protein kinase Cbeta. Blood. 2009;114(19):4186-4196.
57. Jiang X, Forrest D, Nicolini F, et al. Properties of CD34+ CML stem/progenitor cells that correlate with different clinical responses to imatinib mesylate. Blood. 2010;116(12):2112-2121.
58. Eaves C, Cashman J, Eaves A. Defective regulation of leukemic hematopoiesis in chronic myeloid leukemia. Leuk Res. 1998;22(12):1085-1096.
59. Jiang X, Fujisaki T, Nicolini F, et al. Autonomous multi-lineage differentiation in vitro of primitive CD34+ cells from patients with chronic myeloid leukemia. Leukemia. 2000;14(6):1112-1121.
60. Sloma I, Jiang X, Eaves AC, Eaves CJ. Insights into the stem cells of chronic myeloid leukemia. Leukemia. 2010;24(11):1823-1833.
61. Moore S, Haylock DN, Levesque JP, et al. Stem cell factor as a single agent induces selective proliferation of the Philadelphia chromosome positive fraction of chronic myeloid leukemia CD34(+) cells. Blood. 1998;92(7):2461-2470.
62. Krause DS, Fulzele K, Catic A, et al. Differential regulation of myeloid leukemias by the bone marrow microenvironment. Nat Med. 2013;19(11):1513-1517.
63. Zhang B, Ho YW, Huang Q, et al. Altered microenvironmental regulation of leukemic and normal stem cells in chronic myelogenous leukemia. Cancer Cell. 2012;21(4):577-592.
64. Zhang B, Nguyen LXT, Li L, et al. Bone marrow niche trafficking of miR-126 controls the self-renewal of leukemia stem cells in chronic myelogenous leukemia. Nat Med. 2018;24(4):450-462.
65. Koschmieder S, Vetrie D. Epigenetic dysregulation in chronic myeloid leukaemia: A myriad of mechanisms and therapeutic options. Semin Cancer Biol. 2018;51:180-197.
66. Xie H, Peng C, Huang J, et al. Chronic myelogenous leukemia- initiating cells require polycomb group protein EZH2. Cancer Discov. 2016;6(11):1237-1247.
67. Scott MT, Korfi K, Saffrey P, et al. Epigenetic reprogramming sensitizes CML stem cells to combined EZH2 and tyrosine kinase inhibition. Cancer Discov. 2016;6(11):1248-1257.
68. Jelinek J, Gharibyan V, Estecio MR, et al. Aberrant DNA methylation is associated with disease progression, resistance to imatinib and shortened survival in chronic myelogenous leukemia. PLoS One. 2011;6(7):e22110.
69. Heller G, Topakian T, Altenberger C, et al. Next-generation sequencing identifies major DNA methylation changes during progression of Ph+ chronic myeloid leukemia. Leukemia. 2016;30(9):1861-1868.
70. Kinstrie R, Horne GA, Morrison H, et al. CD93 is expressed on chronic myeloid leukemia stem cells and identifies a quiescent population which persists after tyrosine kinase inhibitor therapy. Leukemia. 2020;34(6):1613-1625.

71. Agerstam H, Hansen N, von Palffy S, et al. IL1RAP antibodies block IL-1-induced expansion of candidate CML stem cells and mediate cell killing in xenograft models. Blood. 2016;128(23):2683-2693.
72. Jaras M, Johnels P, Hansen N, et al. Isolation and killing of candidate chronic myeloid leukemia stem cells by antibody targeting of IL-1 receptor accessory protein. Proc Natl Acad Sci U S A. 2010;107(37):16280-16285.
73. Herrmann H, Sadovnik I, Cerny-Reiterer S, et al. Dipeptidylpeptidase IV (CD26) defines leukemic stem cells (LSC) in chronic myeloid leukemia. Blood. 2014;123(25):3951-3962.
74. Van Egeren D, Escabi J, Nguyen M, et al. Reconstructing the lineage histories and differentiation trajectories of individual cancer cells in myeloproliferative neoplasms. Cell Stem Cell. 2021;28(3):514-523.e9.
75. Ghaffari S, Kitidis C, Fleming MD, Neubauer H, Pfeffer K, Lodish HF. Erythropoiesis in the absence of janus-kinase 2: BCR-ABL induces red cell formation in JAK2(-/-) hematopoietic progenitors. Blood. 2001;98(10):2948-2957.
76. Witte O. The role of Bcr-Abl in chronic myeloid leukemia and stem cell biology. Semin Hematol. 2001;38(3 Suppl 8):3-8.
77. Era T, Witte ON. Regulated expression of P210 Bcr-Abl during embryonic stem cell differentiation stimulates multipotential progenitor expansion and myeloid cell fate. Proc Natl Acad Sci U S A. 2000;97(4):1737-1742.
78. Modi H, McDonald T, Chu S, Yee JK, Forman SJ, Bhatia R. Role of BCR/ABL gene-expression levels in determining the phenotype and imatinib sensitivity of transformed human hematopoietic cells. Blood. 2007;109(12):5411-5421.
79. Chang JS, Santhanam R, Trotta R, et al. High levels of the BCR/ABL oncoprotein are required for the MAPK-hnRNP-E2 dependent suppression of C/EBPalpha-driven myeloid differentiation. Blood. 2007;110(3):994-1003.
80. Naka K, Hoshii T, Muraguchi T, et al. TGF-beta-FOXO signalling maintains leukaemia-initiating cells in chronic myeloid leukaemia. Nature. 2010;463(7281):676-680.
81. Ng KP, Hillmer AM, Chuah CT, et al. A common BIM deletion polymorphism mediates intrinsic resistance and inferior responses to tyrosine kinase inhibitors in cancer. Nat Med. 2012;18(4):521-528.
82. Takeuchi S, Hase T, Shimizu S, et al. Phase I study of vorinostat with gefitinib in BIM deletion polymorphism/epidermal growth factor receptor mutation double-positive lung cancer. Cancer Sci. 2020;111(2):561-570.
83. Zhang L, Jiang T, Li X, et al. Clinical features of Bim deletion polymorphism and its relation with crizotinib primary resistance in Chinese patients with ALK/ROS1 fusion-positive non-small cell lung cancer. Cancer. 2017;123(15):2927-2935.
84. Zhao M, Zhang Y, Cai W, et al. The Bim deletion polymorphism clinical profile and its relation with tyrosine kinase inhibitor resistance in Chinese patients with non-small cell lung cancer. Cancer. 2014;120(15):2299-2307.
85. Yu M, Nah GSS, Krishnan V, et al. The BIM deletion polymorphism potentiates the survival of leukemia stem and progenitor cells and impairs response to targeted therapies. Leukemia. 2024;39(1):134-143.
86. Augis V, Airiau K, Josselin M, Turcq B, Mahon FX, Belloc F. A single nucleotide polymorphism in cBIM is associated with a slower achievement of major molecular response in chronic myeloid leukaemia treated with imatinib. PLoS One. 2013;8(11):e78582.

87. Corbin AS, Agarwal A, Loriaux M, Cortes J, Deininger MW, Druker BJ. Human chronic myeloid leukemia stem cells are insensitive to imatinib despite inhibition of BCR-ABL activity. J Clin Invest. 2011;121(1):396-409.
88. Zhang B, Strauss AC, Chu S, et al. Effective targeting of quiescent chronic myelogenous leukemia stem cells by histone deacetylase inhibitors in combination with imatinib mesylate. Cancer Cell. 2010;17(5):427-442.
89. Ng KP, Manjeri A, Lee KL, et al. Physiologic hypoxia promotes maintenance of CML stem cells despite effective BCR-ABL1 inhibition. Blood. 2014;123(21):3316-3326.
90. Burchert A, Wang Y, Cai D, et al. Compensatory PI3-kinase/Akt/mTor activation regulates imatinib resistance development. Leukemia. 2005;19(10):1774-1782.
91. Aibar S, Gonzalez-Blas CB, Moerman T, et al. SCENIC: single-cell regulatory network inference and clustering. Nat Methods. 2017;14(11):1083-1086.
92. Moura MS, Benevides TCL, Delamain MT, et al. Evaluation of anemia after long-term treatment with imatinib in chronic myeloid leukemia patients in chronic phase. Hematol Transfus Cell Ther. 2019;41(4):329-334.
93. Yan K, Ding B, Huang J, Dai Y, Xiong S, Zhai Z. Normal platelet counts mask abnormal thrombopoiesis in patients with chronic myeloid leukemia. Oncol Lett. 2015;10(4):2390-2394.
94. Kvasnicka HM, Thiele J, Schmitt-Graeff A, et al. Bone marrow features improve prognostic efficiency in multivariate risk classification of chronic-phase Ph(1+) chronic myelogenous leukemia: a multicenter trial. J Clin Oncol. 2001;19(12):2994-3009.
95. Mahon FX, Rea D, Guilhot J, 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):1029-1035.
96. Ross DM, Branford S, Seymour JF, et al. Safety and efficacy of imatinib cessation for CML patients with stable undetectable minimal residual disease: results from the TWISTER study. Blood. 2013;122(4):515-522.
97. Saussele S, Richter J, Guilhot J, et al. Discontinuation of tyrosine kinase inhibitor therapy in chronic myeloid leukaemia (EURO-SKI): a prespecified interim analysis of a prospective, multicentre, non-randomised, trial. Lancet Oncol. 2018;19(6):747-757.
98. Saussele S, Richter J, Hochhaus A, Mahon FX. The concept of treatment-free remission in chronic myeloid leukemia. Leukemia. 2016;30(8):1638-1647.
99. Michor F, Hughes TP, Iwasa Y, et al. Dynamics of chronic myeloid leukaemia. Nature. 2005;435(7046):1267-1270.
100. Horn M, Loeffler M, Roeder I. Mathematical modeling of genesis and treatment of chronic myeloid leukemia. Cells Tissues Organs. 2008;188(1-2):236-247.
101. Branford S, Yeung DT, Prime JA, et al. BCR-ABL1 doubling times more reliably assess the dynamics of CML relapse compared with the BCR-ABL1 fold rise: implications for monitoring and management. Blood. 2012;119(18):4264-4271.
102. Rousselot P, Loiseau C, Delord M, Cayuela JM, Spentchian M. Late molecular recurrences in patients with chronic myeloid leukemia experiencing treatment-free remission. Blood Adv. 2020;4(13):3034-3040.
103. Atallah E, Sweet K. Treatment-free remission: the new goal in CML therapy. Curr Hematol Malig Rep. 2021;16(5):433-439.

104. Dulucq S, Nicolini FE, Rea D, et al. Kinetics of early and late molecular recurrences after first-line imatinib cessation in chronic myeloid leukemia: updated results from the STIM2 trial. Haematologica. 2022;107(12):2859-2869.
105. Chomel JC, Bonnet ML, Sorel N, et al. Leukemic stem cell persistence in chronic myeloid leukemia patients in deep molecular response induced by tyrosine kinase inhibitors and the impact of therapy discontinuation. Oncotarget. 2016;7(23):35293-35301.
106. Camacho MF, Pena M, Toloza MJ, et al. Evaluation of leukemic stem cell (CD26 +) in chronic myeloid leukemia patients with different molecular responses and in treatment-free remission. Clin Exp Med. 2025;25(1):93.
107. Pagani IS, Shanmuganathan N, Dang P, et al. Lineage-specific detection of residual disease predicts relapse in patients with chronic myeloid leukemia stopping therapy. Blood. 2023;142(25):2192-2197.
108. Shanmuganathan N, Pagani IS, Ross DM, et al. Early BCR-ABL1 kinetics are predictive of subsequent achievement of treatment-free remission in chronic myeloid leukemia. Blood. 2021;137(9):1196-1207.
109. Graham SM, Jorgensen HG, Allan E, et al. Primitive, quiescent, Philadelphia-positive stem cells from patients with chronic myeloid leukemia are insensitive to STI571 in vitro. Blood. 2002;99(1):319-325.
110. Hughes A, Clarson J, Tang C, et al. CML patients with deep molecular responses to TKI have restored immune effectors and decreased PD-1 and immune suppressors. Blood. 2017;129(9):1166-1176.
111. Bocchia M, Sicuranza A, Abruzzese E, 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.
112. Rea D, Henry G, Khaznadar Z, et al. Natural killer-cell counts are associated with molecular relapse-free survival after imatinib discontinuation in chronic myeloid leukemia: the IMMUNOSTIM study. Haematologica. 2017;102(8):1368-1377.
113. Schutz C, Inselmann S, Saussele S, et al. Expression of the CTLA-4 ligand CD86 on plasmacytoid dendritic cells (pDC) predicts risk of disease recurrence after treatment discontinuation in CML. Leukemia. 2018;32(4):1054.
114. Irani YD, Hughes A, Clarson J, et al. Successful treatment-free remission in chronic myeloid leukaemia and its association with reduced immune suppressors and increased natural killer cells. Br J Haematol. 2020;191(3):433-441.
115. Lai X, Jiao X, Zhang H, Lei J. Computational modeling reveals key factors driving treatment-free remission in chronic myeloid leukemia patients. NPJ Syst Biol Appl. 2024;10(1):45.
116. Hahnel T, Baldow C, Guilhot J, et al. Model-based inference and classification of immunologic control mechanisms from TKI cessation and dose reduction in patients with CML. Cancer Res. 2020;80(11):2394-2406.
117. Bhatia R. Targeting leukemia stem cell resistance in chronic myelogenous leukemia. Trans Am Clin Climatol Assoc. 2019;130:246-254.
118. Kumar R, Krause DS. Recent advances in understanding chronic myeloid leukemia: where do we stand? Fac Rev. 2021;10:35.
119. Buhrer ED, Amrein MA, Forster S, et al. Splenic red pulp macrophages provide a niche for CML stem cells and induce therapy resistance. Leukemia. 2022;36(11):2634-2646.
120. Adnan Awad S, Bruck O, Shanmuganathan N, et al. Epigenetic modifier gene mutations in chronic myeloid leukemia (CML) at diagnosis are associated with risk of relapse upon treatment discontinuation. Blood Cancer J. 2022;12(4):69.

121. Branford S, Fernandes A, Wadham C, et al. Longitudinal clonal tracking reveals that early and sensitive detection of blood cancer-related gene variants in patients with chronic myeloid leukemia predicts treatment failure. Blood. 2024;144(Supplement 1):996.
122. Weng C, Yu F, Yang D, et al. Deciphering cell states and genealogies of human haematopoiesis. Nature. 2024;627(8003):389-398.
123. Radich JP, Dai H, Mao M, et al. Gene expression changes associated with progression and response in chronic myeloid leukemia. Proc Natl Acad Sci U S A. 2006;103(8):2794-2799.
124. Zheng C, Li L, Haak M, et al. Gene expression profiling of CD34+ cells identifies a molecular signature of chronic myeloid leukemia blast crisis. Leukemia. 2006;20(6):1028-1034.
125. Ochi Y, Yoshida K, Huang YJ, et al. Clonal evolution and clinical implications of genetic abnormalities in blastic transformation of chronic myeloid leukaemia. Nat Commun. 2021;12(1):2833.
126. Branford S, Wang P, Yeung DT, et al. Integrative genomic analysis reveals cancer-associated mutations at diagnosis of CML in patients with high-risk disease. Blood. 2018;132(9):948-961.
127. Adnan Awad S, Dufva O, Ianevski A, et al. RUNX1 mutations in blast-phase chronic myeloid leukemia associate with distinct phenotypes, transcriptional profiles, and drug responses. Leukemia. 2021;35(4):1087-1099.
128. Lee KL, Ko TK, Saw NYL, et al. Validation and refinement of a RUNX1 mutation-associated gene expression signature in blast crisis chronic myeloid leukemia. Leukemia. 2022;36(3):892-896.
129. Krishnan V, Kim DDH, Hughes TP, Branford S, Ong ST. Integrating genetic and epigenetic factors in chronic myeloid leukemia risk assessment: toward gene expression-based biomarkers. Haematologica. 2021;107(2):358-370.
130. Konieczkowski DJ, Johannessen CM, Garraway LA. A convergence-based framework for cancer drug resistance. Cancer Cell. 2018;33(5):801-815.
131. Nuno K, Azizi A, Koehnke T, et al. Convergent epigenetic evolution drives relapse in acute myeloid leukemia. Elife. 2024;13:e93019.
132. Yong AS, Szydlo RM, Goldman JM, Apperley JF, Melo JV. Molecular profiling of CD34+ cells identifies low expression of CD7, along with high expression of proteinase 3 or elastase, as predictors of longer survival in patients with CML. Blood. 2006;107(1):205-212.
133. Yu M, Krishnan V, Shyamsundar P, et al. Single-cell transcriptomics-guided development of flow cytometric tests predicting chronic myeloid leukemia blast crisis transformation at chronic phase diagnosis. bioRxiv. 2025 Jan 15. doi: 10.1101/2025.01.09.632094 [preprint, not peer-reviewed].
134. Patterson SD, Copland M. The bone marrow immune microenvironment in CML: treatment responses, treatment-free remission, and therapeutic vulnerabilities. Curr Hematol Malig Rep. 2023;18(2):19-32.
135. Wilson NK, Foster SD, Wang X, et al. Combinatorial transcriptional control in blood stem/progenitor cells: genome-wide analysis of ten major transcriptional regulators. Cell Stem Cell. 2010;7(4):532-544.
136. Raitano AB, Halpern JR, Hambuch TM, Sawyers CL. The Bcr-Abl leukemia oncogene activates Jun kinase and requires Jun for transformation. Proc Natl Acad Sci U S A. 1995;92(25):11746-11750.

137. Walz C, Ahmed W, Lazarides K, et al. Essential role for Stat5a/b in myeloproliferative neoplasms induced by BCR-ABL1 and JAK2(V617F) in mice. Blood. 2012;119(15):3550-3560.

Table 1.

Study	Technology	Cell Type Assayed	Clinical Cohort	Key Findings
Warfvinge et al (2017)	Immunophenotypic screen (332 cell surface antibodies); single-cell gene expression (qPCR, 95 pre-selected genes)	$Lin^-CD34^+CD38^-$/low BM stem cells (~2,000 single cells)	CP CML (n=22 at diagnosis; n=10 at 3 months TKI: imatinib, bosutinib); normal BM (nBM, n=5)	• $CD26^+$ $Lin^-CD34^+CD38^-$/low$CD45RA^-$ $cKIT^-$ cells identified as a therapeutic target. • Myeloid/proliferative LSCs are more sensitive to TKIs than primitive/quiescent LSCs.
Giustacchini et al (2017)	BCR::ABL1 detection with scRNA-seq (Smart-seq technology)	$Lin^-CD34^+CD38^-$ BM stem cells (2,070 single cells)	CP CML at diagnosis (n=20); stratified by TKI response; paired pre-/post-TKI samples (n=19 total, 11 paired); BC-CML (n=3: 2 lymphoid BC, 1 myeloid BC)	• Quiescent BCR::$ABL1^+$ stem cells persist during TKI therapy. • BCR::$ABL1^-$ stem cells show signatures of TKI resistance. • BC-specific subclones are already present during CP
Ma et al (2019)	Single-cell nested PCR for BCR::ABL1; scRNA-seq	$CD34^+CD38^-CD90^+CD45RA^-$ cells	CP CML (n=3); ~48 BCR::$ABL1^+$ and ~48 BCR::$ABL1^-$ stem cells per patient	• PIM2, a pro-survival gene, is expressed in BCR::$ABL1^+$ CML stem cells. • PIM2 is regulated by BCR::ABL–dependent STAT5 and STAT4-independent pathways and represents a therapeutic target.
Pagliaro et al (2020)	Single-cell transcriptomics (87-gene panel); Fluidigm C1	Sorted $CD34^+$ cells	CP CML at diagnosis (n=3); 22 stem cells from a total of 256 cells	• Pseudotime analysis revealed embryonic-like and transitional stem cell states.
Chen et al (2023)	Single-cell mutation analysis	$Lin^-CD34^+CD38^-$/low $CD90^+CD45RA^-CD33^+CD26^+$ CML stem cells and ($Lin^-CD34^+CD38low/^-CD90^+CD45RA^-CD26^-$) normal HSCs	Colonies from CP-CML (n=2), genotyped for BCR::ABL1	• CD26/CD33 co-expression distinguishes CML stem cells from normal HSCs. • Patient-specific clonal mutation profiles are present in BCR::$ABL1^+$ CML stem cells.
Krishnan et al (2023)	scRNA-seq (10x Genomics); mass cytometry	Sorted CD34+ and CD34- cells from BM mononuclear cells	CP CML: optimal responders (n=9), imatinib failures (n=9), pan-TKI failures (n=6); healthy controls (n=8); ~163,000 cells analyzed	• Eight pretreatment BM features are linked to treatment response. • Prognostic signatures identified in LSCs, NK cells, pDCs, and monocytes. • Erythroid skewing and adaptive NK cell abundance associate with good prognosis; IFNγ signatures associate with poor prognosis.
Warfvinge et al (2024)	Single-cell multiomics (CITE-seq: 40 antibodies + scRNA-seq)	Lin^-CD34^+ and $Lin^-CD34^+CD38^-$/low cells from BM	CP CML: optimal, warning, and failure groups; 14,274 Lin-CD34+cells (n=9) and 6,779 Lin-CD34+CD38-/low cells (n=8)	• Failure patients show a higher proportion of primitive cells at diagnosis. • CD26 and CD35 distinguish BCR::$ABL1^+$ and BCR::$ABL1^-$ stem cells. • Increased $CD26^-CD35^+$ HSCs in optimal responders. • LSC/HSC ratio is increased in

				patients with prospective treatment failure.
Scott et al (2024)	scRNA-seq (10x Genomics)	$CD34^{+}CD38^{-}$ sorted HSCs/LSCs from PB	CP CML and healthy controls (n=5 each); 18,095 HSCs and 15,670 LSCs	• Quiescent LSCs in CP-CML exhibit gene networks similar to embryonic stem cells. • Wild-type p53 is involved in LSC self-renewal.
Huuhtanen et al (2024)	scRNA-seq; TCRαβ-seq	$CD45^{+}$ cells from PB; $CD34^{+}$, $CD34^{+}CD38^{-}$ cells	CP CML at diagnosis (n=7), before/after TKI cessation (n=6); healthy controls (n=7); CD34+ and CD34+CD38- samples (n=3)	• NK cell repertoire is active at diagnosis and matures after TKI therapy. • Post-TKI, CD56^dim NK cells in TFR upregulate GZMA/H, CXCR4, and IFNG. • NK cells interact with CML cells via LGALS9–HAVCR2 and PVR–TIGIT pathways.
Kamizela et al (2025)	Single-cell whole-genome sequencing	$CD34^{+}$ cells	1,013 hematopoietic colonies: CP CML (n=8), BC CML (n=1)	• BCR::ABL1 is confirmed as the principal CML driver. • $BCR::ABL1^{+}$ clones show annual growth rates exceeding 70,000%. • Clonal expansion inversely correlates with time to diagnosis and telomere length. • BC-CML exhibits further genomic evolution.

Abbreviations: CP CML-chronic phase chronic myeloid leukemia; BC CML-blast crisis CML; TKI -tyrosine kinase inhibitor; TFR-treatment-free remission; LSC-leukemia stem cell; HSC-hematopoietic stem cell; nBM-normal bone marrow; PB-peripheral blood; sc-single-cell

FIGURE LEGENDS

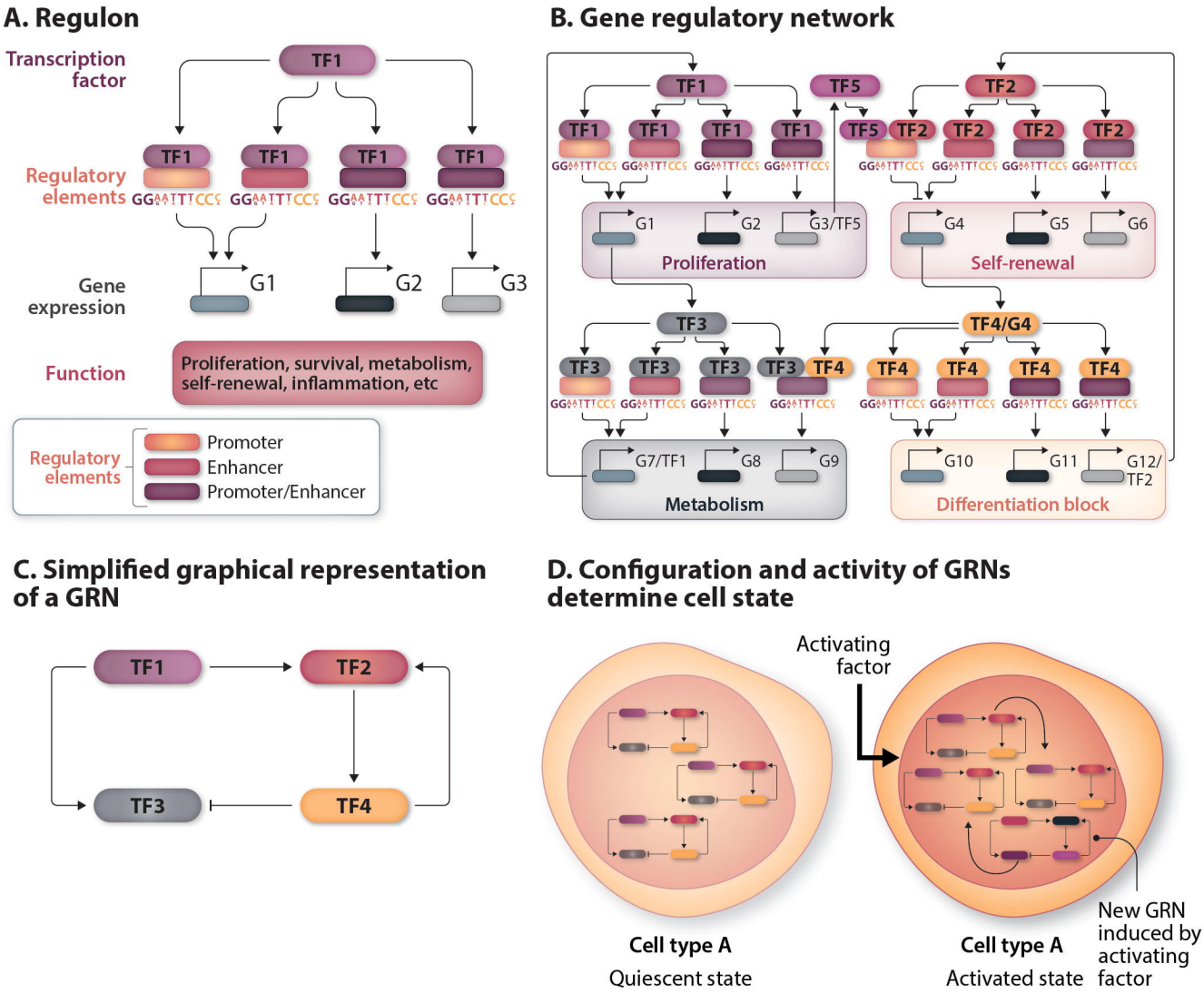
Figure 1. Gene regulatory networks comprise collections of interconnected regulons and determine cell type. A. Regulons are set of genes whose expression are controlled by a common transcription factor (TF). **B.** GRNs are made up of interconnected regulons which regulate global gene expression in a cell, determining cell identity and function. **C.** GRNs may be represented by simplified graphics. **D.** External and internal signals can induce changes in a cell's GRNs, and can alter a given cell type's state, e.g. from quiescent to activated. Significant changes in a cell's GRNs may change cell type, e.g. during differentiation and trans-differentiation.

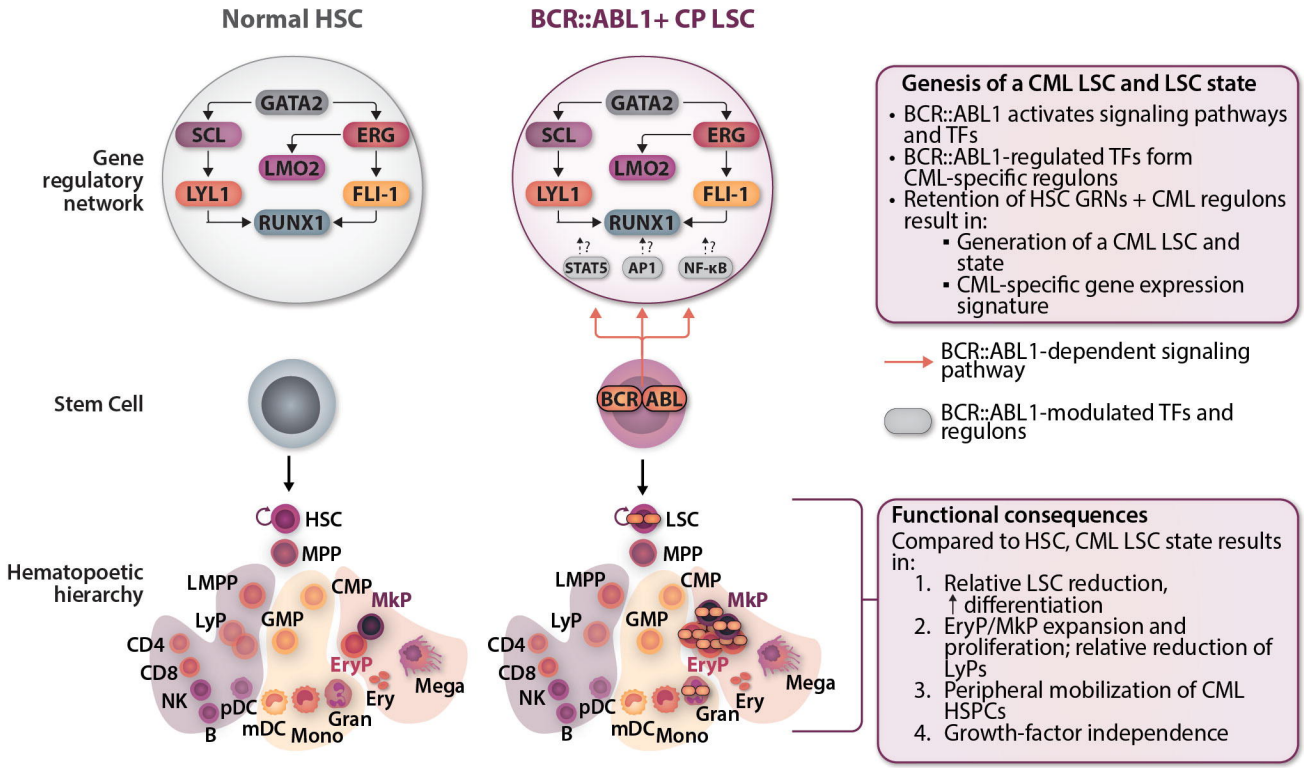
Figure 2. Gene regulatory networks maintain the normal and leukemic hematopoietic stem cell. The normal HSC state is maintained by a robust and conserved gene regulatory network. The GRN comprises a set of transcription factors, each regulating a set of genes, that establish the identity of the cell and its functions in response to both extracellular and intracellular signals. In the case of HSCs, such GRNs have been inferred and validated using a combination of genetic, epigenetic, and functional studies at both bulk and single-cell levels.[135] The presence of the BCR::ABL1 fusion protein in an HSC is sufficient to transform the HSCs into an LSC with altered gene expression programs, and functional responses to differentiation cues, and suggest BCR::ABL1-induced reprogramming of the HSC GRN to a CML LSC-specific GRN. We hypothesize that BCR::ABL1-dependent signaling engages canonical pathways that culminate in activation of STAT5, AP-1 and NF-κB transcription factors.[37, 136, 137] This TF activation reprograms gene expression to remodel the HSC gene regulatory network. This in turn results in skewing of the normal hematopoietic hierarchy as indicated. HSC= hematopoietic stem cell, LSC=leukemic stem cell, MPP=multipotent progenitor cell, LMPP=lymphoid primed multipotent progenitor, LyP=Lymphoid progenitor, GMP=granulocyte monocyte progenitor, EryP=erythroid progenitor, MKP=megakaryocyte progenitor, pDC=plasmocytoid dendritic cell, mDC=myeloid dendritic cell, Mono=monocyte, Gran=granulocyte, Ery=erythrocyte, Mega=megakaryocyte, TF=transcription factor, NBM=normal bone marrow, GF=growth factor.

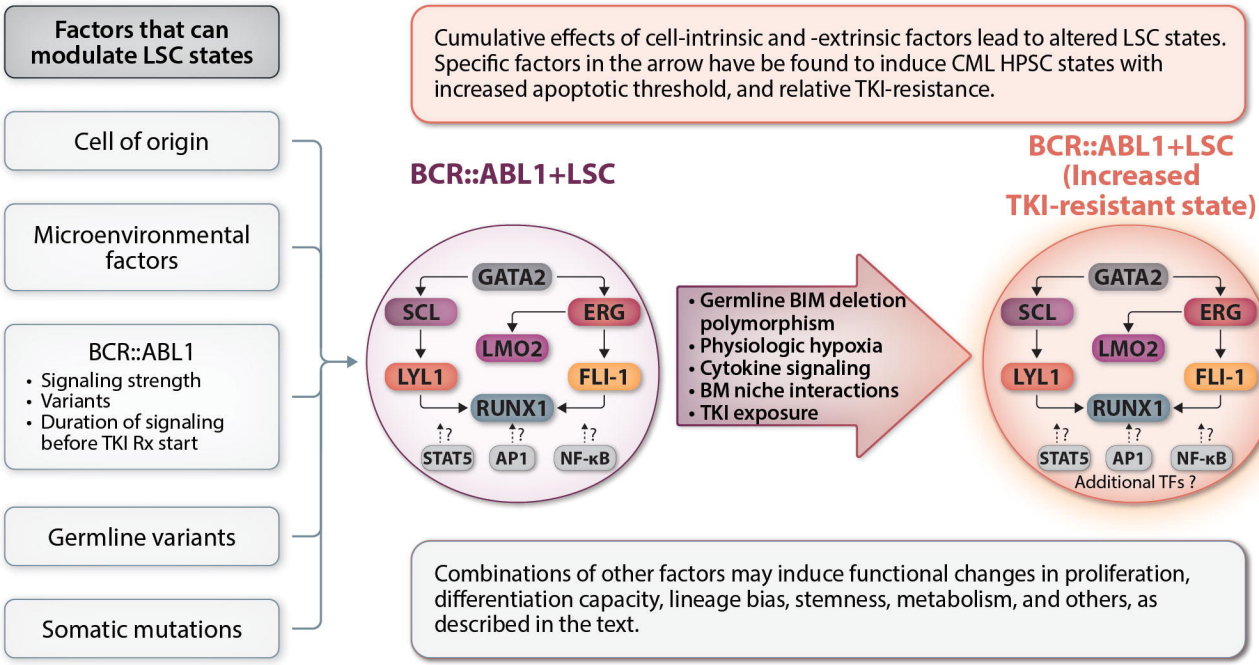
Figure 3. Putative factors that reshape CP LSC GRNs to modify gene expression, cell state, and apoptosis thresholds. Multiple factors are likely to interact with the LSC GRN to alter gene expression and cell state. The resulting gene expression and cell state changes may result in functional changes in the LSC. As described in the text, the *BIM* deletion polymorphism, physiologic hypoxia, extrinsic cytokine exposure, bone marrow stromal interactions, and TKI exposure itself may rewire the GRN to induce changes in the apoptotic threshold towards a more TKI-resistant state.

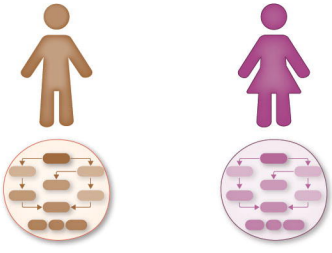
Figure 4. LSC states and treatment free remission. We anticipate considerable LSC heterogeneity at the time of diagnosis, and that factors noted in Figure 3 will continue to modulate LSC states. Additionally, ongoing treatment with TKIs, along with changes in the recovering bone marrow and immune system, may potentially alter LSC states from those present at diagnosis, even when the cells are clonally related. The absolute number of LSCs, and their gradual erosion during treatment, are not indicated but may be an additional factor that determines relapse.

Figure 5. A GRN-based model of CP to BC transition. BC HSPCs express a common BC signature (that encompasses hallmark features of BC). The existence of the CBS suggests the presence of a conserved GRN giving rise to the CBS. Elements of the BC GRN are likely to be present in samples from CP patients at-risk of transformation since they begin to express BC-like signatures. Functionally this results in an inflamed state with impaired differentiation. Rewiring of the CP GRN to a BC GRN is likely a combination of acquired epigenetic and genetic events superimposed on the GRN of the cell of origin.


A. Regulon
Transcription factor
TF1
Regulatory elements
GGAATTTCCS
Gene expression
G1
G2
G3
Function
Proliferation, survival, metabolism, self-renewal, inflammation, etc
Regulatory elements
Promoter
Enhancer
Promoter/Enhancer
B. Gene regulatory network
TF1
TF5
TF2
G1
G2
G3/TF5
G4
G5
G6
Proliferation
Self-renewal
TF3
TF4/G4
TF4
G7/TF1
G8
G9
G10
G11
G12/TF2
Metabolism
Differentiation block
C. Simplified graphical representation of a GRN
TF1
TF2
TF3
TF4
D. Configuration and activity of GRNs determine cell state
Activating factor
Cell type A
Quiescent state
Cell type A
Activated state
New GRN induced by activating factor


Normal HSC
BCR::ABL1+ CP LSC
Gene regulatory network
GATA2
SCL
ERG
LMO2
LYL1
FLI-1
RUNX1
STAT5
AP1
NF-κB
Stem Cell
BCR
ABL
Hematopoetic hierarchy
HSC
LSC
MPP
LMPP
CMP
MkP
LyP
GMP
CD4
CD8
EryP
NK
pDC
Ery
Mega
B
mDC
Mono
Gran
Genesis of a CML LSC and LSC state
• BCR::ABL1 activates signaling pathways and TFs
• BCR::ABL1-regulated TFs form CML-specific regulons
• Retention of HSC GRNs + CML regulons result in:
▪ Generation of a CML LSC and state
▪ CML-specific gene expression signature
BCR::ABL1-dependent signaling pathway
BCR::ABL1-modulated TFs and regulons
Functional consequences
Compared to HSC, CML LSC state results in:
1. Relative LSC reduction, ↑ differentiation
2. EryP/MkP expansion and proliferation; relative reduction of LyPs
3. Peripheral mobilization of CML HSPCs
4. Growth-factor independence

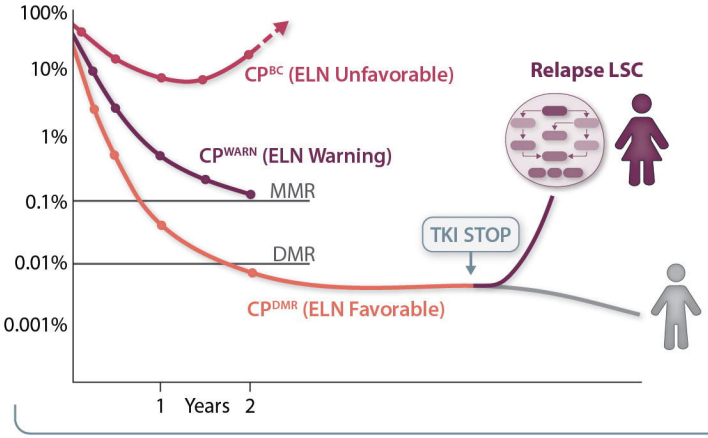

Factors that can modulate LSC states
Cell of origin
Microenvironmental factors
BCR::ABL1
• Signaling strength
• Variants
• Duration of signaling before TKI Rx start
Germline variants
Somatic mutations
Cumulative effects of cell-intrinsic and -extrinsic factors lead to altered LSC states. Specific factors in the arrow have be found to induce CML HPSC states with increased apoptotic threshold, and relative TKI-resistance.
BCR::ABL1+LSC
GATA2
SCL
ERG
LMO2
LYL1
FLI-1
RUNX1
STAT5
AP1
NF-κB
• Germline BIM deletion polymorphism
• Physiologic hypoxia
• Cytokine signaling
• BM niche interactions
• TKI exposure
BCR::ABL1+LSC (Increased TKI-resistant state)
Additional TFs ?
Combinations of other factors may induce functional changes in proliferation, differentiation capacity, lineage bias, stemness, metabolism, and others, as described in the text.



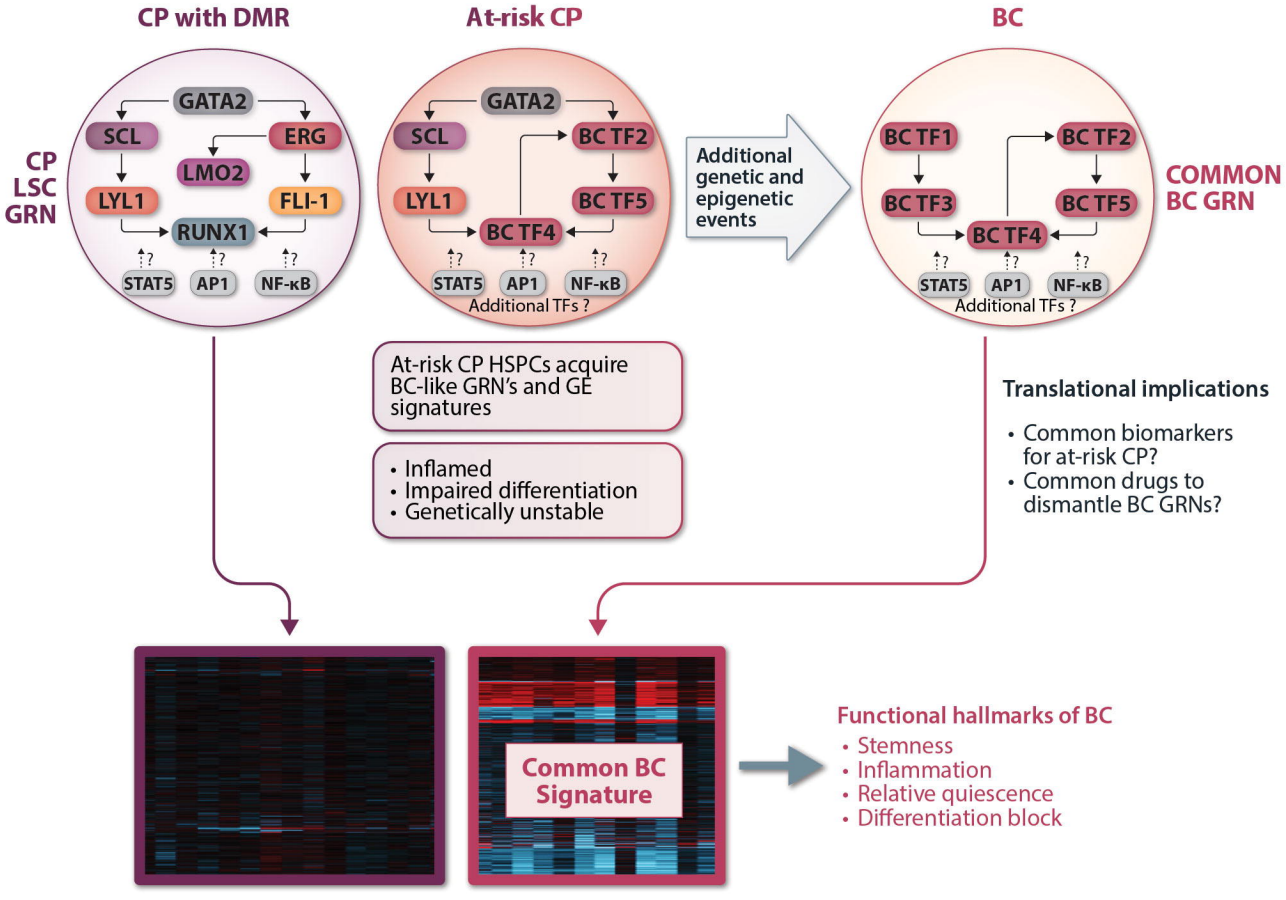
Diagnostic LSC (low relapse risk)

Diagnostic LSC (high relapse risk)

Cell of origin	BCR::ABL1 • Signaling strength • Variants • Duration of signaling before TKI Rx start
Microenvironmental factors	
Germline variants	Somatic mutations



- LSC numbers are thought to gradually decrease over time
- LSC states may also change with recovering bone marrow microenvironment and immune system
- The same LSC clone may be in a different state between diagnosis and DMR


CP with DMR
At-risk CP
BC
CP LSC GRN
GATA2
SCL
ERG
LMO2
LYL1
FLI-1
RUNX1
STAT5
AP1
NF-κB
BC TF2
BC TF5
BC TF4
Additional TFs ?
Additional genetic and epigenetic events
BC TF1
BC TF3
COMMON BC GRN
At-risk CP HSPCs acquire BC-like GRN's and GE signatures
• Inflamed
• Impaired differentiation
• Genetically unstable
Translational implications
• Common biomarkers for at-risk CP?
• Common drugs to dismantle BC GRNs?
Common BC Signature
Functional hallmarks of BC
• Stemness
• Inflammation
• Relative quiescence
• Differentiation block