

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

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## Abstract

Despite the advent of potent tyrosine kinase inhibitors (TKI), resistance and disease persistence remain significant clinical challenges in chronic myeloid leukemia. This review aims to synthesize concepts derived from recent advances in single-cell and multi-omics analyses, which have revealed profound heterogeneity among leukemic stem cells (LSC). These findings augment traditional models that focus solely on clonal selection and resistance-conferring mutations. We discuss how LSC, like normal hematopoietic stem cells, exist in a spectrum of transcriptionally and epigenetically defined cell states, each governed by distinct gene regulatory networks (GRN) that confer unique lineage biases and responses to therapy. Incorporating recent insights from single-cell analyses, our review highlights evidence for a conserved chronic phase 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. 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 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 GRN, may contribute to resistance and LSC persistence, these observations support placing greater emphasis in the management of chronic myeloid leukemia 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 chronic phase LSC.

## Introduction

Resistance to tyrosine kinase inhibitors (TKI) in chronic myeloid leukemia (CML) remains a major barrier to durable disease control. Although CML is driven by a single genetic abnormality and responds to potent TKI,<sup>1,2</sup> most patients still require lifelong therapy.<sup>3</sup> Depending on the choice of frontline TKI, only between 30-60% of patients achieve an

optimal response,<sup>4</sup> precluding attempts at TKI cessation for almost half of patients, while roughly 25,000 deaths worldwide ( $\approx$ 1,290 in the USA according to Surveillance, Epidemiology, and End Results [SEER]) still occur annually.<sup>5</sup> Traditional models focus on leukemic stem cells (LSC)\* and resistance-conferring mutations arising in hematopoietic stem and progenitor cells (HSPC).<sup>6</sup> Even so, recent multi-omics and single-cell analyses indicate that these

\*For the purposes of this perspective, we have used the term leukemic stem cell (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.<sup>9,31,51,135</sup> When single-cell studies are referenced, hematopoietic stem cell (HSC)/LSC populations are identified based on canonical HSC-specific gene expression markers.

frameworks may not fully capture the range of observed outcomes.<sup>7</sup> Recent studies have uncovered pronounced heterogeneity within the LSC compartment, with distinct cellular states that differentially fuel therapy resistance, disease persistence, and progression (Table 1).<sup>8-15</sup> This mirrors the diversity of normal hematopoietic stem cells

(HSC), among which lineage tracing and single-cell work depict a continuum or “cloud” of overlapping states with varying lineage biases,<sup>16-19</sup> maintained by transcription factor (TF)-dependent epigenetic programs and their resulting gene expression signatures.<sup>18,20</sup>

In this review, we synthesize emerging literature and ad-

**Table 1.** Studies using different technologies and examining various cell types have revealed pronounced heterogeneity within the leukemic stem cell compartment, with distinct cellular states that differentially affect therapy resistance, disease persistence, and clinical outcomes.

Study	Technology	Cell type assayed	Clinical cohort	Key findings
Warfvinge <i>et al.</i> (2017)	Immunophenotypic screen (332 cell surface antibodies); single-cell gene expression (qPCR, 95 pre-selected genes)	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-/low</sup> BM stem cells (~2,000 single cells)	CP CML (N=22 at diagnosis; N=10 at 3 months TKI: imatinib, bosutinib); normal BM (N=5)	<ul style="list-style-type: none"> <li>- CD26<sup>+</sup>Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>-/low</sup>CD45RA<sup>-</sup>cKIT<sup>-</sup> cells identified as a therapeutic target</li> <li>- Myeloid/proliferative LSC are more sensitive to TKI than primitive/quiescent LSC</li> </ul>
Giustacchini <i>et al.</i> (2017)	BCR::ABL1 detection with scRNA-seq (Smart-seq technology)	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-</sup> 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)	<ul style="list-style-type: none"> <li>- Quiescent BCR::ABL1<sup>+</sup> stem cells persist during TKI therapy</li> <li>- BCR::ABL1<sup>-</sup> stem cells show signatures of TKI resistance</li> <li>- BC-specific subclones are already present during CP</li> </ul>
Ma <i>et al.</i> (2019)	Single-cell nested PCR for BCR::ABL1; scRNA-seq	CD34 <sup>+</sup> CD38 <sup>-</sup> CD90 <sup>+</sup> CD45RA <sup>-</sup> cells	CP CML (N=3); ~48 BCR::ABL1 <sup>+</sup> and ~48 BCR::ABL1 <sup>-</sup> stem cells per patient	<ul style="list-style-type: none"> <li>- PIM2, a pro-survival gene, is expressed in BCR::ABL1<sup>+</sup> CML stem cells</li> <li>- PIM2 is regulated by BCR::ABL-dependent STAT5 and STAT4-independent pathways and represents a therapeutic target</li> </ul>
Pagliari <i>et al.</i> (2020)	Single-cell transcriptomics (87-gene panel); Fluidigm C1	Sorted CD34 <sup>+</sup> cells	CP CML at diagnosis (N=3); 22 stem cells from a total of 256 cells	<ul style="list-style-type: none"> <li>- Pseudotime analysis revealed embryonic-like and transitional stem cell states</li> </ul>
Chen <i>et al.</i> (2023)	Single-cell mutation analysis	Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-/low</sup> CD90 <sup>+</sup> CD45RA <sup>-</sup> CD33 <sup>+</sup> CD26 <sup>+</sup> CML stem cells and (Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>low/-</sup> CD90 <sup>+</sup> CD45RA <sup>-</sup> CD26 <sup>-</sup> ) normal HSC	Colonies from CP-CML (N=2), genotyped for BCR::ABL1	<ul style="list-style-type: none"> <li>- CD26/CD33 co-expression distinguishes CML stem cells from normal HSC</li> <li>- Patient-specific clonal mutation profiles are present in BCR::ABL1<sup>+</sup> CML stem cells</li> </ul>
Krishnan <i>et al.</i> (2023)	scRNA-seq (10x Genomics); mass cytometry	Sorted CD34 <sup>+</sup> and CD34 <sup>-</sup> 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	<ul style="list-style-type: none"> <li>- Eight pretreatment BM features are linked to treatment response</li> <li>- Prognostic signatures identified in LSC, NK cells, pDC, and monocytes</li> <li>- Erythroid skewing and adaptive NK cell abundance associate with good prognosis; IFN<math>\gamma</math> signatures associate with poor prognosis</li> </ul>
Warfvinge <i>et al.</i> (2024)	Single-cell multiomics (CITE-seq: 40 antibodies + scRNA-seq)	Lin <sup>-</sup> CD34 <sup>+</sup> and Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-/low</sup> cells from BM	CP CML: optimal, warning, and failure groups; 14,274 Lin <sup>-</sup> CD34 <sup>+</sup> cells (N=9) and 6,779 Lin <sup>-</sup> CD34 <sup>+</sup> CD38 <sup>-/low</sup> cells (N=8)	<ul style="list-style-type: none"> <li>- Patients with treatment cessation failure show a higher proportion of primitive cells at diagnosis</li> <li>- CD26 and CD35 distinguish BCR::ABL1<sup>+</sup> and BCR::ABL1<sup>-</sup> stem cells</li> <li>- Increased CD26-CD35<sup>+</sup> HSC in optimal responders</li> <li>- LSC/HSC ratio is increased in patients with prospective treatment failure</li> </ul>

Continued on following page.

Study	Technology	Cell type assayed	Clinical cohort	Key findings
Scott <i>et al.</i> (2024)	scRNA-seq (10x Genomics)	CD34 <sup>+</sup> CD38 <sup>-</sup> sorted HSC/LSC from peripheral blood	CP CML and healthy controls (N=5 each); 18,095 HSC and 15,670 LSC	<ul style="list-style-type: none"> <li>- Quiescent LSC in CP-CML exhibit gene networks similar to embryonic stem cells</li> <li>- Wild-type p53 is involved in LSC self-renewal</li> </ul>
Huuhtanen <i>et al.</i> (2024)	scRNA-seq; TCRαβ-seq	CD45 <sup>+</sup> cells from peripheral blood; CD34 <sup>+</sup> , CD34 <sup>+</sup> CD38 <sup>-</sup> cells	CP CML at diagnosis (N=7), before/after TKI cessation (N=6); healthy controls (N=7); CD34 <sup>+</sup> and CD34 <sup>+</sup> CD38 <sup>-</sup> samples (N=3)	<ul style="list-style-type: none"> <li>- NK cell repertoire is active at diagnosis and matures after TKI therapy</li> <li>- Post-TKI, CD56<sup>dim</sup> NK cells in TFR upregulate GZMA/H, CXCR4, and IFNG</li> <li>- NK cells interact with CML cells via LGALS9–HAVCR2 and PVR–TIGIT pathways</li> </ul>
Kamizela <i>et al.</i> (2025)	Single-cell whole-genome sequencing	CD34 <sup>+</sup> cells	1,013 hematopoietic colonies: CP CML (N=8), BC CML (N=1)	<ul style="list-style-type: none"> <li>- BCR::ABL1 is confirmed as the principal CML driver.</li> <li>- BCR::ABL1<sup>+</sup> clones show annual growth rates exceeding 70,000%.</li> <li>- Clonal expansion inversely correlates with time to diagnosis and telomere length</li> <li>- BC-CML exhibits further genomic evolution</li> </ul>

qPCR: quantitative polymerase chain reaction; BM: bone marrow; CP: chronic phase; CML: chronic myeloid leukemia; TKI: tyrosine kinase inhibitor; LSC: leukemia stem cell; sc: single-cell; seq: sequencing; BC: blast crisis; HSC: hematopoietic stem cell; NK: natural killer; pDC: plasmacytoid dendritic cells; IFN $\gamma$ : interferon gamma; CITE: cellular indexing of transcriptomes and epitopes; TCR: T-cell receptor; TFR: treatment-free remission.

vance 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.

## 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 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 (GRN), which incorporate epigenetic modifiers, non-coding RNA, and signaling molecules to integrate diverse intracellular and extracellular cues (Figure 1).<sup>21</sup>

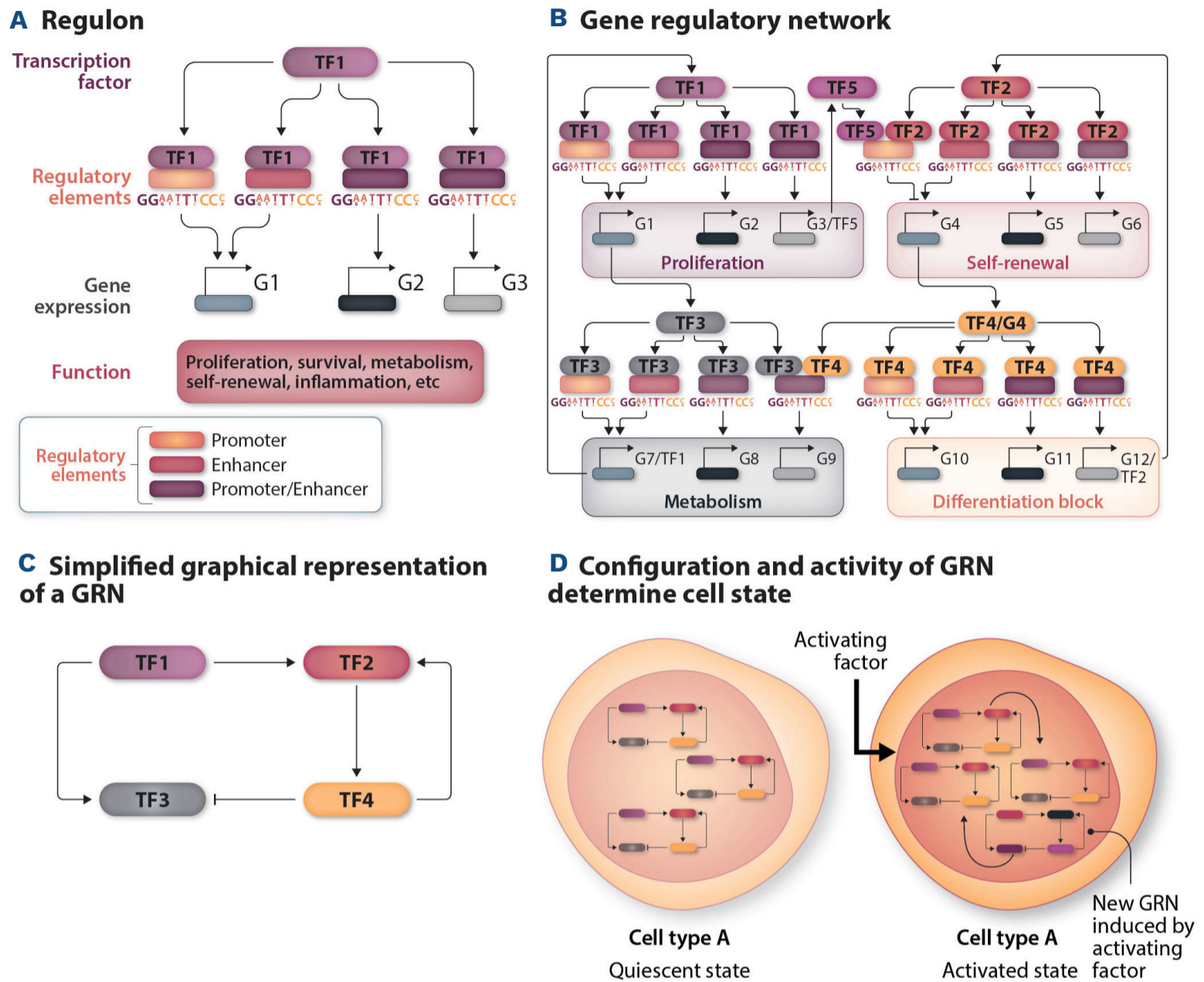
GRN are inherently robust, maintaining stable expression through feedback and redundancy, yet remain highly responsive. This dynamic adaptability, often reversible, allows HSC to adjust to oxygen and nutrient fluctuations, inflammation, infection, blood loss, and other stresses, preserving identity, meeting physiological demands, limiting genomic and oxidative damage, and supporting organismal longevity. LSC and HSC share stemness properties, including self-renewal, multipotency, and quiescence.<sup>22</sup> 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*).<sup>23</sup> In both scenarios, oncogenic lesions disrupt normal GRN to drive uncontrolled proliferation, altered differentiation and lineage output, enhanced survival, and modified responses to extrinsic cues (Figure 2).<sup>18,24,25</sup> Thus, while LSC may phenotypically resemble HSC, their aberrant states fuel malignant transformation and therapy resistance. Viewing LSC as a cell state underscores their dual robustness and plasticity: the capacity to maintain malignant properties while adapting to microenvironmental signals.

In CML, LSC 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.<sup>7,9,13,22,26-28</sup>

## Existence and importance of a chronic phase leukemic stem cell state

Single-cell technologies have revealed stable epigenetic cell states in acute myeloid leukemia, often linked to mutations such as *FLT3*-internal tandem duplication, *RAS* activation, and other drivers of leukemia.<sup>25</sup> These pathological states are sustained by oncogenic signaling and transcriptional networks that reprogram how acute myeloid leukemia cells sense and respond to their environment, shaping differentiation, survival, and metabolism. Dissecting the origins



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

and maintenance of these abnormal states has guided new therapies that disrupt them.<sup>24</sup>

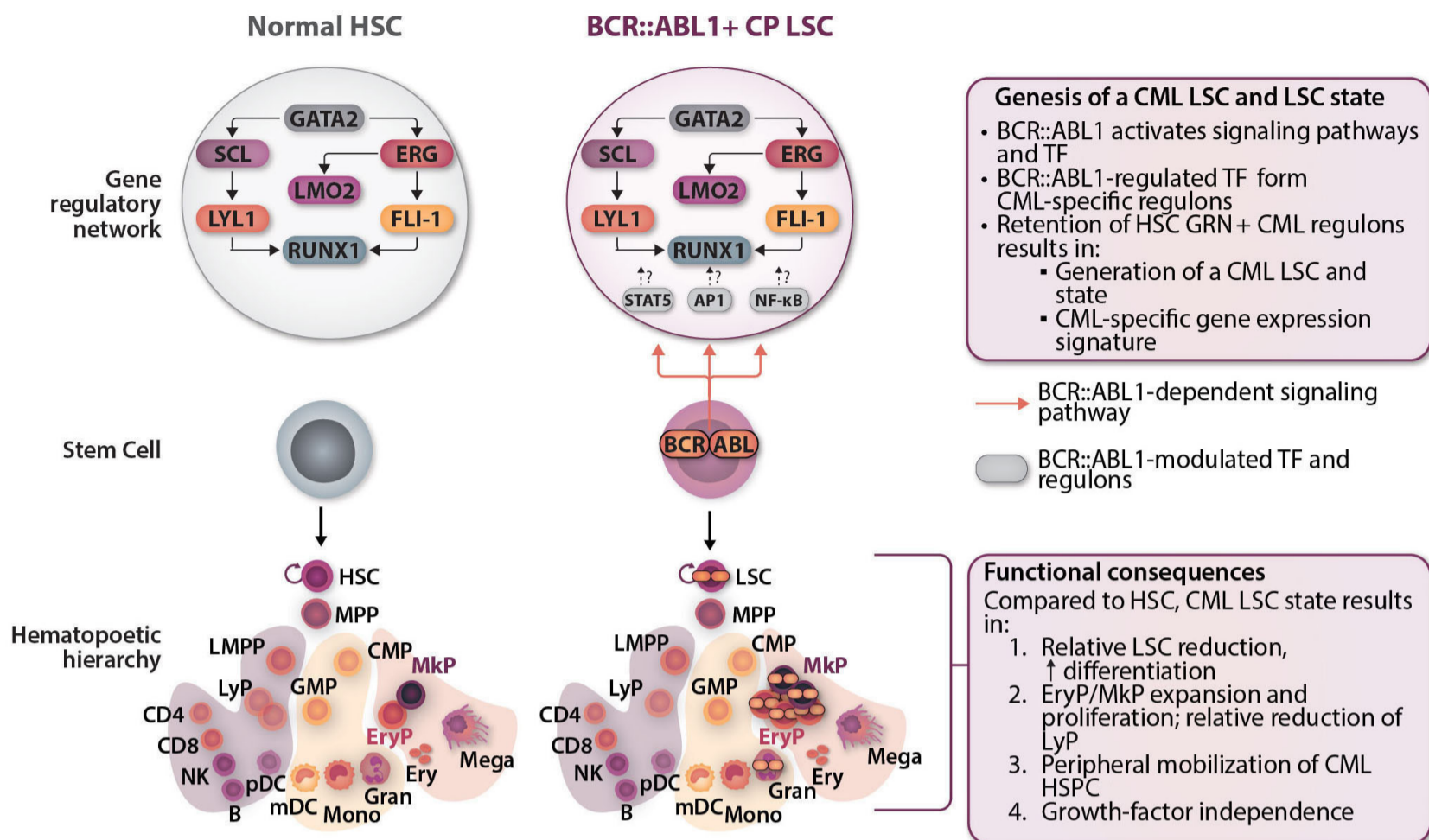
Defining equivalent regulatory networks in non-CML myeloproliferative neoplasms has been harder, given striking phenotypic heterogeneity despite genetic simplicity (e.g., *JAK2*-mutated myeloproliferative neoplasms).<sup>29</sup> This variability may reflect differences in cells of origin and the long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

In contrast, CML research, based on the clinical success of TKI, 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 TKI, 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, together with what it is 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.

**Conserved and distinct transcriptional signature**

Multiple independent studies identify an immunophenotypically defined LSC population in CP CML with consistent



**Figure 2. Gene regulatory networks maintain the normal and leukemic hematopoietic stem cell states.** The normal hematopoietic stem cell (HSC) state is maintained by a robust and conserved gene regulatory network (GRN). The GRN comprises a set of transcription factors, each regulating a set of genes, which establish the identity of the cell and its functions in response to both extracellular and intracellular signals. In the case of HSC, such GRN have been inferred and validated using a combination of genetic, epigenetic, and functional studies at both bulk and single-cell levels.<sup>136</sup> The presence of the BCR::ABL1 fusion protein in an HSC is sufficient to transform the HSC into a leukemic stem cell (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.<sup>37,137,138</sup> This TF activation reprograms gene expression to remodel the HSC GRN. This in turn results in skewing of the normal hematopoietic hierarchy as indicated. CP: chronic phase; MPP: multipotent progenitor cell; LMPP: lymphoid primed multipotent progenitor; LyP: lymphoid progenitor; GMP: granulocyte monocyte progenitor; EryP: erythroid progenitor; MkP: megakaryocyte progenitor; pDC: plasmacytoid dendritic cell; NK: natural killer; mDC: myeloid dendritic cell; Mono: monocyte; Gran: granulocyte; Ery: erythrocyte; Mega: megakaryocyte; CML: chronic myeloid leukemia; TF: transcription factor.

gene expression signatures distinct from those of normal HSC and CML progenitors.<sup>9,13,27,31</sup>

### 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 TF remodel chromatin and epigenetic states, reprogramming cell identity and behavior.<sup>32-50</sup>

### Retention and divergence from hematopoietic stem cell features

Immunophenotypically defined CML LSC preserve core HSC traits (e.g., quiescence and self-renewal) yet undergo metabolic rewiring marked by elevated oxidative phosphorylation, increased reactive oxygen species, and altered mitochondrial dynamics. The coexistence of conserved stemness with non-HSC metabolic signatures delineates

CP LSC as a discrete cell state, implying unique functional behaviors and therapeutic vulnerabilities.<sup>22,39,51-56</sup>

### 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 LSC are not merely HSC under stress; they harbor an altered differentiation program at the stem level, defining a discrete LSC state with reprogrammed fate decisions.<sup>57-60</sup>

### Altered environmental responsiveness

Compared with normal HSC, CML LSC 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 LSC from canonical HSC regulation, arguing for a discrete LSC cell state rather than a stressed HSC variant.<sup>61-64</sup>

### Epigenetic dysregulation

Genome-wide profiling reveals a reproducible, LSC-specific epigenetic landscape (distinct DNA methylation, histone mark patterns, and chromatin accessibility) compared with normal HSC and CML progenitors, underscoring epigenetic foundations of a discrete CP LSC state.<sup>26,65-69</sup>

### Characteristic immunophenotype

CML LSC reproducibly express surface marker combinations such as CD34<sup>+</sup>CD38<sup>-</sup>CD26<sup>+</sup>CD35<sup>-</sup> and IL1RAP<sup>+</sup>, which are absent from normal HSC 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.<sup>8,13,70-73</sup>

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 HSC and other leukemic populations.

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

## Differential chronic phase leukemic stem cell states and primary resistance to tyrosine kinase inhibitors

### Effects of BCR::ABL1 on hematopoietic stem cell 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 HSPC: (i)

a reduction in long-term HSC (LT-HSC); (ii) an expansion of proliferative progenitors; and (iii) a bias in differentiation toward erythroid, megakaryocytic, and myeloid lineages.<sup>58,59</sup> Taken together, these findings suggest that CML LSC 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 CP LSC as: (i) more independent of extrinsic growth factors;<sup>59</sup> (ii) more readily mobilized to the periphery, consistent with altered niche interactions;<sup>60</sup> and (iii) having reduced stemness with concomitant increased progenitor proliferation.

Building on foundational work, subsequent studies elucidated how BCR::ABL1 mediates lineage bias. Lodish's group showed that BCR::ABL1 can directly induce erythroid differentiation without JAK2, generating burst-forming units-erythroid and colony-forming units-erythroid even without cytokines.<sup>75</sup> However, when the erythropoietin receptor is absent, cytokines are required for burst-forming unit-erythroid formation. Moreover, BCR::ABL1 domain mutations (e.g., Y177F, SH2 domain deletions) render JAK2 indispensable for erythroid differentiation. Together, these findings indicate that BCR::ABL1 supports erythroid commitment in HSPC and functionally overlaps with physiological JAK2 signaling.

Supporting lineage-bias observations, Witte's group used an inducible BCR::ABL1 in embryonic stem cell-derived multipotent progenitors and showed that higher BCR::ABL1 levels increased proliferation and biased output toward myeloid over erythroid progenitors in a manner that was reversible upon switching off BCR::ABL1.<sup>76,77</sup> Bhatia's group found exogenous BCR::ABL1 expression in CD34<sup>+</sup> cord blood generated both erythroid and myeloid progenitors, with a notable, dose-dependent increase in erythroid output, doubling the erythroid-to-myeloid ratio.<sup>78</sup> 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 that stronger BCR::ABL1 suppresses CEBPA-driven myeloid differentiation via a MAPK-hnRNP-E2 pathway.<sup>79</sup> Collectively, these observations 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 that 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<sup>+</sup>Lin<sup>-</sup>Sca-1<sup>-</sup> and most CML lines), BCR::ABL1 activates AKT, phosphorylating and inactivating nuclear FOXO. In contrast, c-Kit<sup>+</sup>Lin<sup>-</sup>Sca-1<sup>+</sup> CML LSC show AKT inhibition and FOXO activation, which is essential for LSC function.<sup>80</sup> 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 leukemic stem cell state**

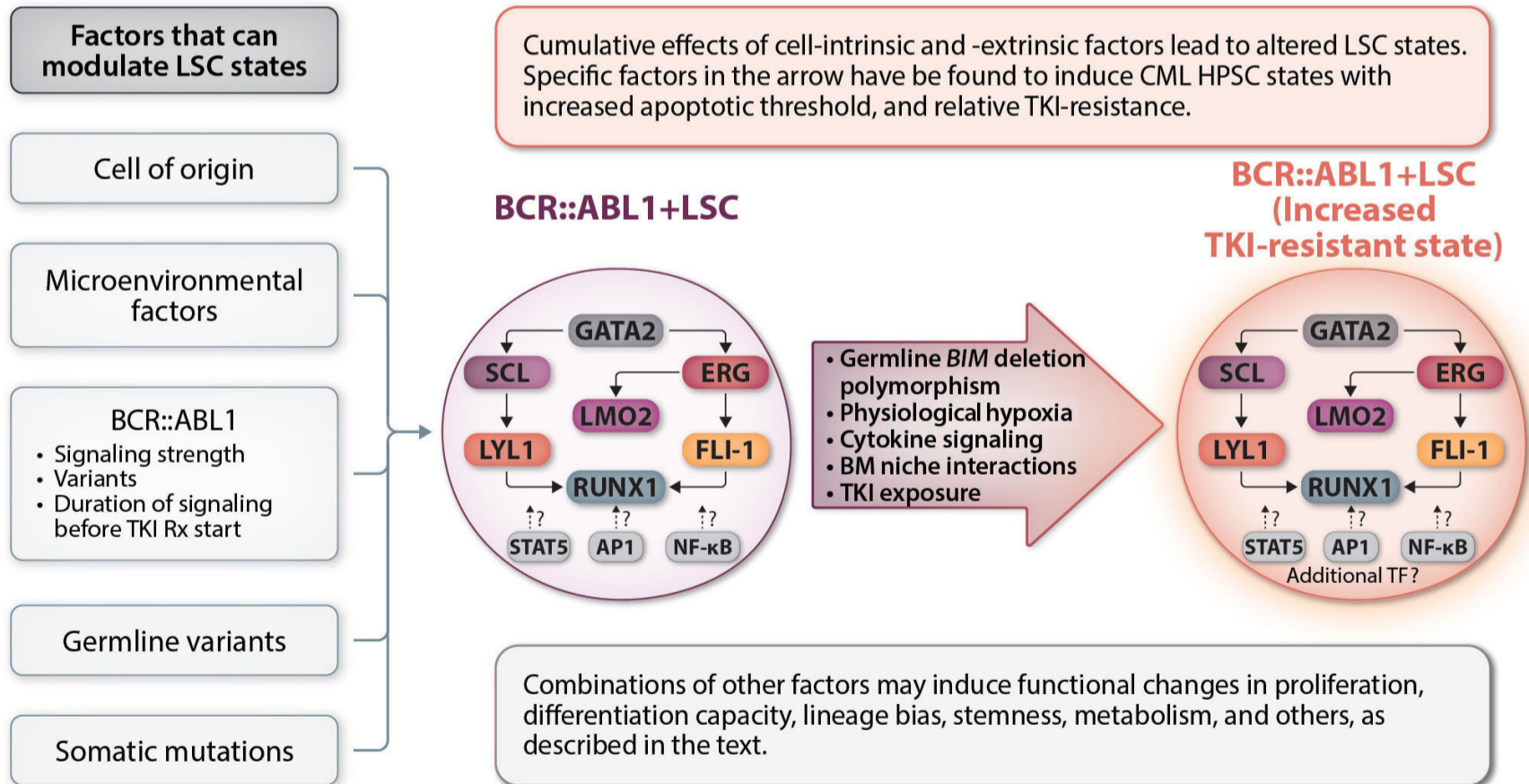
A paradox in CML therapy is that while CML cells, including primary HSPC, can be eradicated *in vitro* within 2-4 days, achieving similar effects in patients often requires months to years of TKI therapy. This discrepancy is unlikely to be fully explained by genetic or pharmacokinetic and pharmacodynamic 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).<sup>81</sup> 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.<sup>81-84</sup> *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 ren-

dering LSC 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.<sup>85</sup> Related variants affecting *BIM* expression in other populations further suggest broad modulation of LSC states and therapeutic response.<sup>86</sup>

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

Therapy itself can provoke resistant states. Studies, in-



**Figure 3. Putative factors that reshape chronic phase leukemic stem cell gene regulatory networks to modify gene expression, cell state, and apoptosis thresholds.** Multiple factors are likely to interact with the leukemic stem cell (LSC) gene regulatory network (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, physiological hypoxia, extrinsic cytokine exposure, bone marrow stromal interactions, and exposure to a tyrosine kinase inhibitor itself may rewire the GRN to induce changes in the apoptotic threshold towards a more TKI-resistant state. Rx: treatment; CML: chronic myeloid leukemia; HSPC: hematopoietic stem and progenitor cell; BM: bone marrow; TF: transcription factors.

cluding those on primary CML samples, have found TKI exposure activating PI3K/AKT/mTOR signaling, potentially contributing to resistance.<sup>90</sup> 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 hematopoietic stem cell heterogeneity and origins of the chronic phase leukemic stem cell

Traditional models portrayed HSC as homogeneous, with discrete progenitor populations progressively restricted in lineage potential.<sup>18,19</sup> 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 HSC and progenitors are functionally heterogeneous.<sup>18</sup> 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 that this heterogeneity exists along a continuum within both HSC and progenitor compartments.<sup>16,17</sup> Within HSC, 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 TF.<sup>19</sup> Thus, HSC 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.<sup>7</sup> Second, the strength of BCR::ABL1 signaling, repeatedly shown to modulate lineage bias *in vitro*.<sup>78</sup> Third, the duration of unopposed signaling, which, if prolonged, may foster TKI-resistant epigenetic cell states, as seen in BC LSC.<sup>26</sup> 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 chronic phase leukemic stem cell state

Despite substantial progress, we still lack a complete understanding of how BCR::ABL1 reprograms normal HSC into CML LSC, the TF networks that define the CML LSC state, and the implications of LSC heterogeneity. A seminal study from Karlsson’s group linked single-cell immunophenotypic heterogeneity to molecular and functional differences, identifying among the most apical LSC, a quiescent and relatively TKI-insensitive Lin<sup>-</sup>CD34<sup>+</sup>CD38<sup>-/low</sup>CD45RA<sup>-</sup>cKIT<sup>-</sup>CD26<sup>+</sup> population.<sup>8</sup>

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

### Differential leukemic stem cell states and quality of tyrosine kinase inhibitor response

In a subsequent study, we used single-cell RNA-sequencing on diagnostic bone marrow from CP CML patients to identify prognostic cell types.<sup>27</sup> Unbiased analysis across 22 detected cell types highlighted three with the greatest predictive accuracy for frontline imatinib response: LSC (defined by canonical gene expression signatures), natural killer cells (NK), and plasmacytoid dendritic cells (pDC). Focusing on LSC, we applied the SCENIC pipeline<sup>91</sup> to infer transcriptional regulatory networks. LSC from patients achieving a DMR (European LeukemiaNet optimal response) showed activation of KLF1, TAL1, and GATA1 regulons, consistent with a canonical erythroid lineage program. In contrast, LSC 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<sup>+</sup> HSPC. 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 bone 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 LSC. 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.<sup>92,93</sup> 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 LSC 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, Karlsson's group confirmed that robust TKI responses associate with expansion of the erythroid progenitor compartment.<sup>13</sup> Historical *in vitro* data similarly indicated that erythroid progenitors are intrinsically more TKI-sensitive than myeloid progenitors.<sup>57</sup> 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 in the case of treatment with interferon- $\alpha$ , hydroxyurea, and busulfan.<sup>94</sup>

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

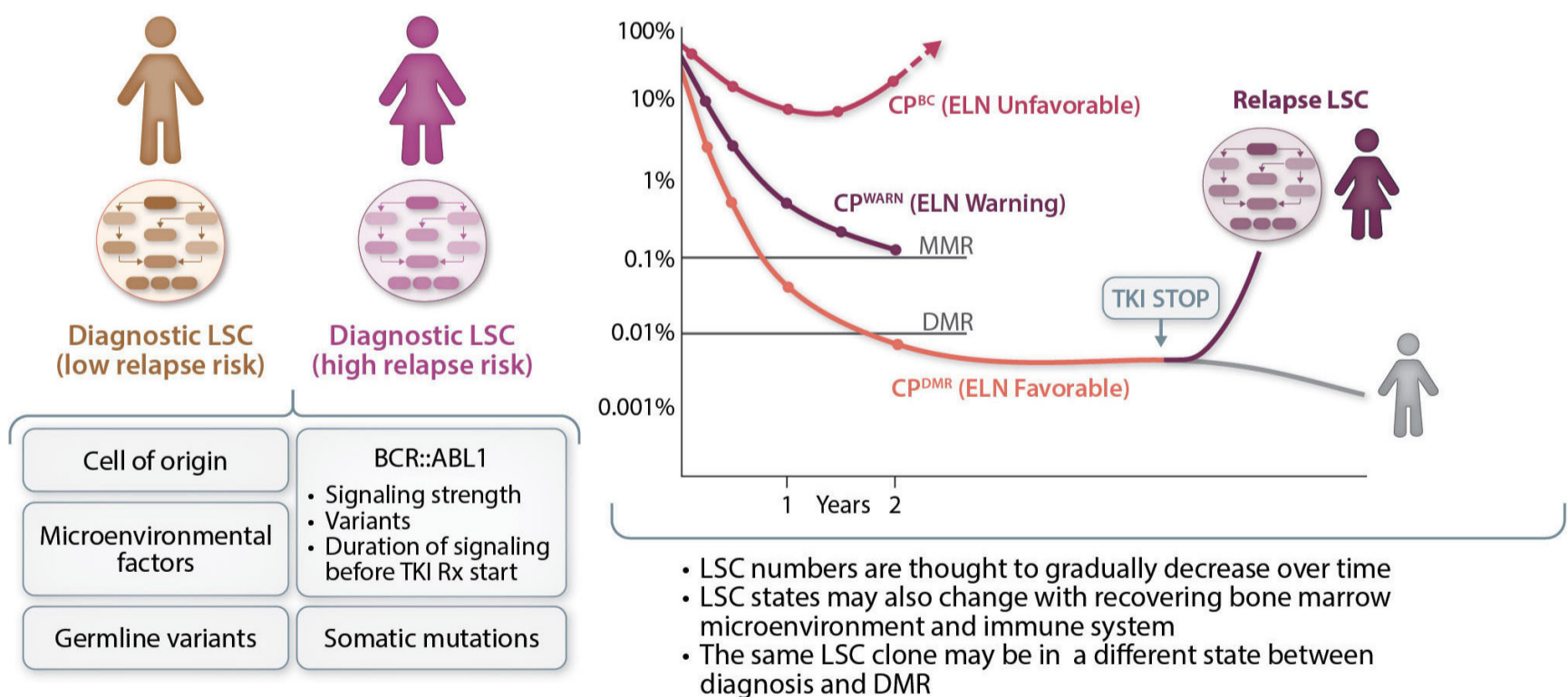
states to TKI sensitivity, underscoring the need to understand and target LSC states to overcome resistance and improve outcomes.

## Leukemic stem cell states and treatment-free remission

TFR is a key therapeutic goal in CML, yet outcomes after TKI cessation show a dichotomy: 40-60% of patients maintain remission, while the rest relapse, often within months.<sup>3</sup> First observed in the French STIM trial and confirmed in TWISTER and EURO-SKI, TFR at 6 months was 61%.<sup>95-97</sup> Sustained TFR correlates with two clinical factors: duration of TKI therapy and duration of DMR (BCR::ABL1  $\leq 0.01\%$  or MR4).<sup>98</sup> Each additional year of sustained DMR before TKI withdrawal confers a 2-3% absolute increase in relapse-free survival.<sup>97</sup> These observations are consistent with gradual TKI-induced attrition of CML LSC, 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.

### Tyrosine kinase inhibitor-resistant leukemic stem cells and treatment-free remission

TKI-resistant LSC and TFR mathematical modeling supports gradual LSC attrition while on TKI treatment but



**Figure 4. Leukemic stem cell states and treatment-free remission.** We anticipate considerable leukemic stem cell (LSC) heterogeneity at the time of diagnosis, and that factors noted in Figure 3 will continue to modulate LSC states. Additionally, ongoing treatment with tyrosine kinase inhibitors, 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 LSC, and their gradual erosion during treatment, are not indicated but may be additional factors that determine relapse. TKI: tyrosine kinase inhibitor; Rx: treatment; CP<sup>BC</sup>: chronic phase progressing to blast crisis; ELN: European LeukemiaNet; MMR: major molecular response; DMR: deep molecular response.

maintenance of a minimal reservoir that expands quickly after treatment withdrawal.<sup>99,100</sup> BCR::ABL1 levels double about every 9 days (~1-log monthly),<sup>101</sup> with most relapses occurring within 6 months. Some patients maintain remission despite fluctuating BCR::ABL1 transcript levels below that defining a major molecular response ( $\leq 0.1\%$ ), while ~14% experience late relapse ( $\geq 2$  years), necessitating re-treatment.<sup>102,103</sup> These findings suggest immune surveillance sustains remission in patients with low-level fluctuations, whereas late relapse may reflect loss of immune control.<sup>104</sup> Isolating residual LSC at treatment cessation is challenging. Functional assays (e.g., long-term culture-initiating cells can readout for more primitive BCR::ABL1<sup>+</sup> progenitors but are poor predictors of TFR.<sup>105</sup> CD26 is a proposed CML LSC marker, yet levels fluctuate even in patients in durable remission.<sup>106</sup> Pagani *et al.* recently reported rare, differentiated BCR::ABL1(+) cells in granulocyte and T-cell lineages at treatment cessation as powerful predictors of relapse: 100% for granulocytes, 67% for T cells.<sup>107</sup> This supports a model whereby therapy-resistant, LSC or multipotent progenitors persist and continue to generate aberrant progeny during a DMR. Such cells may pre-exist via intrinsic TKI-evasion pathways or arise during therapy through transcriptomic shifts to more resistant states, or both: this requires further study.

#### **Intrinsic mechanisms of leukemic stem cell persistence**

To connect diagnostic LSC biology to relapse after TKI cessation, key questions include: (i) which GRN drive LSC transcriptional heterogeneity and confer TKI-resistance to relapse-fated subclones; (ii) are there universal GRN for persistence, or are they patient-specific; (iii) do BCR::ABL1<sup>-</sup> stem cells or myeloid populations support LSC persistence; and (iv) do BCR::ABL1 LSC states evolve from diagnosis to DMR and TFR relapse?

Single-cell RNA sequencing 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 TKI.<sup>9</sup> Group A likely constitutes a therapy-resistant reservoir maintained by GRN conferring evasion. Clinically, Shanmuganathan *et al.* showed early BCR::ABL1 halving times (<9.35 days) predicted TFR success (80%), whereas slower kinetics (>21.85 days) associated with high relapse risk (96%).<sup>108</sup> Relapse potential may thus be intrinsically encoded at diagnosis, with rates of BCR::ABL1 decline mirroring fundamental biological differences between patients with durable remission as against those who 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 treatment cessation, enabling early intervention. Resolving whether LSC persistence is governed by universal or patient-specific GRN and clarifying the regulatory roles of BCR::ABL1<sup>-</sup> 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 leukemic stem cell persistence: the effect of tyrosine kinase inhibitor therapy, immune control and niche interactions**

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

Patients achieving sustained TFR exhibit distinct immune signatures at cessation: expanded cytotoxic NK and CD8<sup>+</sup> T cells, reduced regulatory T cells and myeloid-derived suppressor cells (MDSC), and low CD86<sup>+</sup> pDC.<sup>112-114</sup> Mathematical models incorporating immune parameters improve TFR prediction, underscoring the immune-LSC balance.<sup>115,116</sup> Additional contributors include LSC-niche interactions (CXCR4, CD44, ITGA3), hypoxia adaptation (HIF-1 $\alpha$ ), inflammatory cytokines (IL1 $\alpha$ , IL1 $\beta$ , TNF $\alpha$  IL-6), and stromal signaling (TGF $\beta$ , SDF-1), all promoting TKI resilience.<sup>117,118</sup> A recent study challenges the idea that the bone marrow is the sole source of relapse, proposing the splenic pulp as an additional niche for CML LSC.<sup>119</sup> Clarifying how LSC, TKI, immune control, and niche interactions intersect is central to understanding relapse biology.

#### **Emerging single-cell technologies to understand the biology of treatment-free remission**

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,<sup>120</sup> suggesting epigenetic reprogramming sustains LSC. Conversely, clonal hemato-

poiesis of indeterminate potential mutations at cessation correlate with better TFR.<sup>121</sup> Only single-cell methods can localize these mutations to LSC *versus* non-malignant compartments. Combined with lineage tracing (e.g., mitochondrial DNA barcoding),<sup>122</sup> they could track relapse-prone LSC 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 LSC, immune surveillance, and niche protection should enable personalized strategies to predict and improve TFR.

## Evidence for a distinct blast crisis leukemic stem cell state

A distinct BC LSC state is supported by several lines of evidence: (i) a reproducible, pan-BC gene-expression program (which we term the common BC signature or CBS) is detected across most CD34<sup>+</sup> BC samples regardless of the underlying mutational profile, establishing an empirically defined transcriptomic state;<sup>26,123,124</sup> (ii) functional LSC activity (serial xenotransplantation/long-term propagation) is demonstrated across multiple immunophenotypically-defined HSPC subsets (HSC-, lymphoid primed multipotent progenitor-, common myeloid progenitor-, granulocyte-macrophage progenitor-like), even within the same sample,<sup>28</sup> consistent with the notion that LSC activity is a property of cell state rather than a specific immunophenotype; (iii) most BC samples are clonal or oligoclonal with a dominant clone, indicating that the CBS is associated with clonal expansion;<sup>125,126</sup> and (iv) the CBS encompasses established LSC gene-expression signatures (e.g., quiescence/stemness modules, Wnt/ $\beta$  catenin, HIF/stress-response, anti-apoptotic and niche/adhesion pathways), providing functional gene-expression evidence that links the BC program to stem-like behavior.<sup>26</sup> Collectively, these data are consistent with BC HSPC 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 blast crisis state of leukemic stem cells

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

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.<sup>6,26,125</sup> Most encode TF or epigenetic regulators, implicating cell-state shifts as central to BC evo-

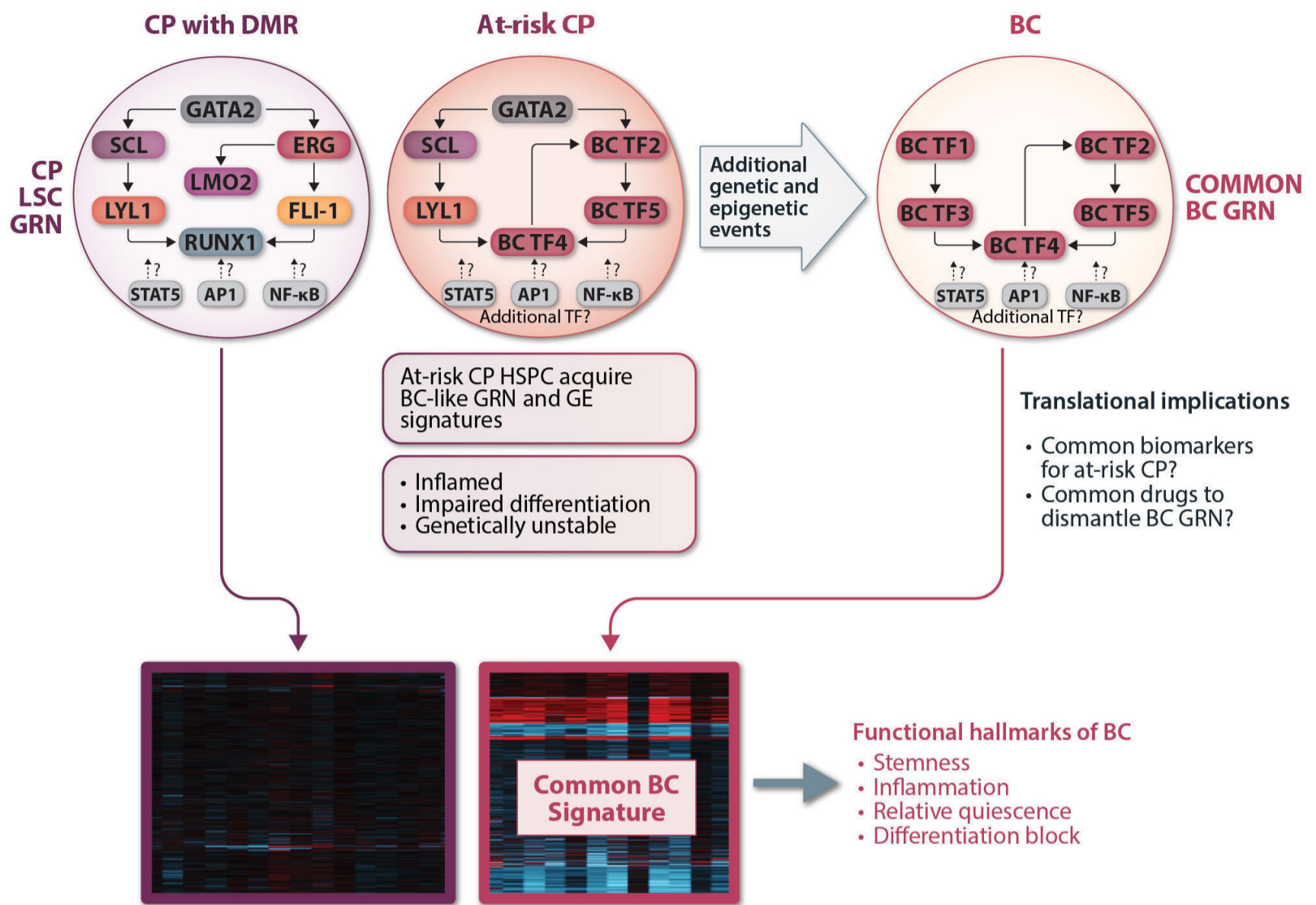
lution. Yet the diversity of mutated genes and the variable downstream programs they influence do not, on their own, explain the convergence on a shared BC transcriptomic state. Integrated multi-omics studies show both myeloid and lymphoid BC exhibit this core profile, regardless of mutational landscape.<sup>26</sup> This raises a key question: how do biologically diverse mutations drive BC transformation if a uniform transcriptomic state predominates?

Emerging evidence suggests that 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.<sup>127,128</sup> 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 HSPC 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.<sup>26</sup> This model reconciles wide mutational heterogeneity with the convergent BC transcriptomic phenotype and explains heterogeneous outcomes when only genetic events are considered.<sup>6,129</sup> 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 some blood cancers.<sup>130,131</sup>

### Clinical implications of the common blast crisis signature

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, pharmacological BMI1 inhibition



**Figure 5. A gene regulatory network-based model of chronic phase to blast crisis transition.** Blast crisis (BC) hematopoietic stem and progenitor cells express a common BC signature (CBS), which encompasses hallmark features of BC. The existence of the CBS suggests the presence of conserved gene regulatory networks (GRN) giving rise to the CBS. Elements of the BC GRN are likely to be present in samples from patients in chronic phase (CP) 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. DMR: deep molecular response; TF: transcription factors; HSPC: hematopoietic stem and progenitor cells; GE: gene expression.

with DNA methylating agents reduces BC cell survival and stemness, highlighting a potential therapeutic approach.<sup>26</sup> 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.<sup>6,129</sup> 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 common blast crisis signature: 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 substanti-

ated by two independent studies analyzing CD34<sup>+</sup> cells from CP at diagnosis: both showed that specific transcriptomic signatures predict earlier progression to BC and clinical TKI resistance.<sup>132,133</sup> 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<sup>+</sup> HSPC) are interrogated.<sup>129</sup>

These findings matter for two reasons. First, they suggest a shared gene expression signature can identify high-risk CP patients, independently 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.<sup>134</sup>

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.

## Outstanding questions in research on chronic myeloid leukemia cell state

In future work, a key objective will be the molecular dissection of clinically relevant CML cell states at critical timepoints, including diagnosis, disease progression, achievement of DMR, and relapse following TKI discontinuation. It will be important to define GRN 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 GRN 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 patients' samples, particularly bone marrow collected at clinically informative but rarely

indicated timepoints, 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.

### Disclosures

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

### Contributions

STO and VK wrote the first draft of the manuscript. PS revised and edited the figures and text. All authors approved the final draft.

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