Integrating genetic and epigenetic factors in chronic myeloid leukemia risk assessment: toward gene expression-based biomarker

by Vaidehi Krishnan, Dennis Dong Hwan Kim, Timothy P. Hughes, Susan Branford, and S. Tiong Ong

Received: July 16, 2021.
Accepted: September 28, 2021.


Publisher's Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors' final approval; the final version of the manuscript will then appear in a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.
Integrating genetic and epigenetic factors in chronic myeloid leukemia risk assessment: toward gene expression-based biomarkers

Vaidehi Krishnan,1,2 Dennis Dong Hwan Kim,2,3 Timothy P. Hughes, 2,4,5,6 Susan Branford,2,4,7,8 S. Tiong Ong,1,2,9,10,11

1Cancer and Stem Cell Biology Signature Research Program, Duke-NUS Medical School, Singapore, Singapore; 2International Chronic Myeloid Leukemia Foundation; 3Department of Medical Oncology and Hematology, Princess Margaret Cancer Centre, University Health Network, University of Toronto, Toronto, Canada; 4School of Medicine, University of Adelaide, Adelaide, Australia; 5South Australian Health & Medical Research Institute, Adelaide, Australia; 6Department of Haematology, Royal Adelaide Hospital, Adelaide, Australia; 7Department of Genetics and Molecular Pathology, Centre for Cancer Biology, SA Pathology, Adelaide, Australia; 8School of Pharmacy and Medical Science, University of South Australia, Adelaide, Australia; 9Department of Haematology, Singapore General Hospital, Singapore, Singapore; 10Department of Medical Oncology, National Cancer Centre Singapore; 11Department of Medicine, Duke University Medical Center, Durham, NC, USA;

Acknowledgments: VK and STO are supported by the National Medical Research Council Singapore (MOH-CSASI18may-0002, MOH-CIRG20nov-0003, NMRC/CIRG/1468/2017).

Author contributions: VK and STO conceived the topic for review, and wrote the first draft of the manuscript. DDHK, TPH, and SB contributed by the addition of new sections and critical discussions throughout the writing of the review.

Correspondence: STO, Cancer & Stem Cell Biology SRP, Duke-NUS Medical School, 8 College Road, Singapore 169857; email: sintiong.ong@duke-nus.edu.sg
Abstract

Cancer treatment is constantly evolving from a one-size-fits-all towards bespoke approaches for each patient. In certain solid cancers, including breast and lung, tumor genome profiling has been incorporated into therapeutic decision-making. For chronic phase (CP) chronic myeloid leukemia (CML), while tyrosine kinase inhibitor (TKI) therapy is the standard treatment, current clinical scoring systems cannot accurately predict the heterogeneous treatment outcomes observed in patients. Biomarkers capable of segregating patients according to outcome at diagnosis are needed to improve management, and facilitate enrolment in clinical trials seeking to prevent blast crisis transformation and improve the depth of molecular responses. To this end, gene expression (GE) profiling studies have evaluated whether GE signatures at diagnosis are clinically informative. Patient material from a variety of sources have been profiled using microarrays, RNA sequencing, and, more recently, single-cell RNA sequencing. However, differences in the cell types profiled, the technologies used, and the inherent complexities associated with the interpretation of genomic data, pose challenges in distilling GE datasets into biomarkers with clinical utility. The goal of this paper is to review previous studies evaluating GE profiling in CML, and explore their potential as risk assessment tools for individualized CML treatment. We also review the contribution that acquired mutations, including those seen in clonal hematopoiesis, make to GE profiles, and how a model integrating contributions of genetic and epigenetic factors in TKI resistance and BC transformation can define a route to GE-based biomarkers. Finally, we outline a four-stage approach for the development of GE-based biomarkers in CML.
Introduction
CML is a clonal disorder of the hematopoietic stem cell compartment defined and driven by the $BCR-ABL1$ gene rearrangement and the tyrosine kinase it encodes (1). Clinically, it is accompanied by an expansion of mostly myelo-erythroid progenitors that maintain the ability to differentiate terminally into neutrophils. Prior to the introduction of ABL1 tyrosine kinase inhibitors (TKI), most patients would progress to a terminal blast crisis (BC) stage marked by the acquisition of additional genetic abnormalities within an average of 5-7 years (2). Here, the clinico-pathologic features were the inexorable accumulation of either myeloid or lymphoid progenitors that had acquired aberrant self-renewal properties, broad resistance to cytotoxic therapies, and eventual patient demise from BM failure (2). The arrival of TKIs at the turn of the century resulted in remarkable responses, such that most individuals treated in chronic phase (CP) CML can expect to achieve near-normal life expectancies (3). Nevertheless, CML-related deaths are still reported, mainly due to resistance and progression to BC, especially in the first few years of treatment (4).

Current treatment aims and features of an ideal biomarker

Current therapeutic aims are directed to achieving sufficiently deep molecular responses that the risks of BC transformation are effectively negligible, and in the longer-term, increasing the rates of treatment-free remission (TFR) (5) (6, 7). Clinical guidelines toward achieving deep molecular responses (DMR) have been reviewed elsewhere (8), and at their core, prescribe the measurement of $BCR-ABL1$ transcript levels using the International Scale (IS) every three months as a readout of depth of TKI response. In turn, the depth of TKI response serves as a critical biomarker guiding patient management and prognostication (Fig. 1).
Given current treatment goals, an ideal biomarker would accurately predict patients who will achieve a DMR with first-line TKI, or require a switch to alternative therapy, and among those who achieve a DMR, those who will be able to stop TKI successfully (Fig. 1). The biomarker would be informative from the time of diagnosis and prior to TKI initiation, since this would enable early patient stratification for therapy with first-generation (1G) vs second/third generation (2/3G) TKI, allosteric BCR-ABL1 inhibitors, clinical trial, or preparation for allogeneic transplantation. Additionally, among patients who achieve TKI stoppage criteria, they would identify additional therapies that would enhance TFRs. Finally, GE-based biomarkers should be clinically robust, and widely available among centers and regions from both low and high Human Development Index countries (9).

**Why GE-based biomarkers?**

Contributions from genetic and epigenetic mediators to TKI resistance and BC transformation are well documented (10-14), and it is axiomatic that genetic or epigenetic factors mediating these outcomes will contribute to a cell’s GE signature. Accordingly, GE signatures offer a molecular profile that integrates risk factors encoded by both mutations and epigenetic states. However, faithfully extracting and interpreting GE-based information in clinical settings is challenging. Barriers to adoption include technical limitations, logistical factors, as well as differences in study design and data analysis, and are described in below.

Nevertheless, recent advances in technological and computational platforms are enabling the interrogation of patient samples at unprecedented scale, and are being translated into robust technical assays on patient-material reproducible in clinical laboratories (15). Such advances may eventually result in the identification of
pretreatment biomarkers that not only predict TKI resistance but suggest alternative non-BCR-ABL1-targeting therapies to preempt the emergence of clinical resistance. Accordingly, it is timely to review the results of GE studies using primary patient material annotated for clinical outcomes, and assess how genetic and epigenetic factors associated with treatment outcome contribute to GE signatures. In doing so, it is also important to develop models incorporating the interplay between genetic and epigenetic factors, and determine how best to use the resulting GE outputs to understand and predict CML drug resistance and transformation. Finally, it is incumbent on the CML community to develop the practical steps needed for the clinical development of GE-based biomarkers in CML.

GE signatures associated with TKI-resistance

Since the beginning of the TKI era, a variety of diagnostic material from CP patients have been used to discover TKI-resistance GE signatures (Table 1). Here, we review the key conclusions from these studies.

GE using peripheral blood

In the earliest studies by Kaneta et al. (16) and McLean et al. (17), microarray-studies were conducted on blood from imatinib responders and non-responders. Apart from CBLB which was downregulated in responders, there was no overlap between the two datasets. De Lavallade et al., conducted microarray studies on PBMCs to identify a 105 gene-set that was enriched in imatinib-non-responders, comprising mainly genes in cell cycle and DNA repair pathways (18). However, the GE signature could be validated only in an imatinib- but not in an interferon-α (IFN-α) treated cohort. As a targeted approach, the GE of 21 genes associated with TKI responses and disease progression were studied by Zhang et al (19). Increased
PTGS1 expression was the only gene that differentiated primary imatinib-resistant patients from responders, while 15 genes distinguished CP from BC. Twelve genes distinguished imatinib-responsive from secondary imatinib-resistant CML without BCR-ABL1 mutations, of which LYN, JAK2, PTPN22 and CEBPA downregulation was shared with BC samples. The study concluded that at least some features of secondary imatinib resistance overlap with BC transformation.

Most recently, Kok et al. conducted microarray-based analysis on diagnostic blood from 96 CP patients from the TIDEL-II trial to predict EMR failure (20), which correlates with inferior long-term outcomes (21, 22). 365 differentially expressed genes (DEGs) were identified which were enriched for ‘cell cycle’ and ‘stemness’ (MYC, HOXA9, β-catenin) but depleted for ‘immune-response’ categories in the EMR-failure group. A binary classification model was built to predict EMR failure based on 17 genes and the signature was validated in an independent cohort. Of these, eight genes IGFBP2, SRSF11, BAX, CDKN1B, BNIP3L, FZD7, PRSS57, and RPS28 intersected with previous CML TKI-resistance and progression studies. This study demonstrated that GE information from diagnostic samples could predict distal events in time, including MMR at 24 months, MR4.5 at five years, and BC transformation.

**GE using BM**

Independently, a series of studies used unselected BM for GE comparisons between patient responder groups. Frank et al. identified a 128 GE signature associated with imatinib resistance, specifically in an IFN-α pre-treated cohort. DEGs were involved in apoptosis (CASP9, TRAP1), DNA repair (MSH3, DDB2), oxidative stress protection (GSS, PON2, VNN1) and centrosomes (ID1) (23). Villuendas et al. (24) identified 46 DEGs of which a six-gene prediction score (BIRC4, FZD7, IKBKB, IL-
7R, TNC, VWF) that correlated with imatinib resistance after IFN-α failure was developed. DEGs were involved in cell adhesion (TNC and SCAM-1), drug metabolism (COX1 or PTGS1), protein tyrosine kinases (MKNK1), and phosphatases (BTK and PTPN22). Notably, the MKNK1/2 kinases have been shown by two independent groups to be involved in BC transformation (25, 26). In contrast to the prior studies, Crossman et al. found no DEGs between the imatinib responder categories. The use of mixed PB and BM samples, unselected WBCs and a heterogeneous patient cohort in late CP and heavily pre-treated, were suggested as potential reasons (27). The important conclusion was that GE comparisons should be made on purified CD34+ cells. Indeed, in a meta-analysis comparing six published GE studies in CML, DDX11, MSH5, and RAB11FIP3 were the only genes coincident between any two of the studies (28). The small differences in differential GE between responder groups, different GE platforms, different statistical methods and different sources of cells profiled were suggested reasons for the poor intersection. The disappointing results from unselected PB and BM provided the impetus to isolate and study CD34+ fractions.

**GE using CD34+ cells**

McWeeney et al. were the first group to use CD34+ cells from diagnostic BM (11). Cell adhesion genes were upregulated in imatinib-resistant patients suggesting that CD34+ cells may establish more adhesive interactions with the BM milieu. The enrichment for β-catenin binding targets suggested activated Wnt/β-catenin signaling in imatinib-resistant patients, a feature shared with CD34+ progenitors from BC (26, 29). The authors concluded that primary resistance to imatinib might reflect more advanced disease progression. A 75-probe minimal gene classifier predicted 88% of responders and 83% of non-responders in a validation cohort. Importantly, the
authors of this paper compared their GE signatures to those predicting early BC transformation, as discussed below, and provided an important resource for validation and comparison of other CD34+-based GE datasets.

**Single-cell-based GE analysis**

Recent advances in single-cell analysis have enabled novel GE-based insights on the roles of tumor cell heterogeneity and clonal evolution under the selective pressure of therapeutics, with obvious applications in biomarker development (30). LSC heterogeneity was characterized by Warfvinge et al. by combining high-throughput immunophenotyping with single-cell GE profiling with a defined panel of genes (31). LSC sub-fractions with more primitive and quiescent signatures had a higher persistence after TKI therapy with the most TKI-insensitive population identified as Lin-CD34+CD38low/-CD45RA-CD26+ stem cells. Giustacchini et al. used the Smart-seq2 platform to combine scRNA-seq analysis with BCR-ABL1 transcript detection using purified stem cells. A sub-population of BCR-ABL1+ quiescent stem cells enriched for hematopoietic stem cell (HSC) signatures were found to persist during TKI therapy (32). Intriguingly, the BCR-ABL1- cells in CML patients were enriched for inflammatory, TGF-β and TNF-α hallmarks and discriminated between the TKI responder groups.

In addition to LSC-derived signatures, the GE signature of immune cells can be equally instructive. For example, plasmacytoid dendritic cells (pDC), the major producers of IFN-α in vivo, promoted nilotinib resistance in CML patients (33). These studies imply that the cytokines released by immune cells in BM microenvironment, and the transcriptomic changes they bring about on the LSCs, may activate cytokine-dependent TKI resistance programmes (34). Together, these single-cell studies demonstrate that GE signatures within malignant and non-malignant compartments
in CML are prognostically informative. We anticipate the discovery of additional biomarkers among discrete cell types that have the potential to be assayed by platforms available in standard pathology laboratories, e.g. by flow cytometry or immunohistochemistry.

**GE signatures associated with BC progression**

Transcriptomic comparisons between the CP and BC stages have uncovered progression-signatures that can herald BC transformation (Table 2). In the pre-TKI era, the time to BC transformation from CP varied between patients, and to understand this difference, Yong et al. compared CD34+ cells from leukapheresis samples provided by patients who progressed to BC within 3 years (aggressive) or after more than 7 years (indolent) following diagnosis (13). The study identified that lower CD7 with higher PR3 and ELA2 expression at diagnosis were associated with longer survival. Intriguingly, when the Yong and McWeeney GE signatures were compared, a significant overlap was found (11). This important study demonstrated that biological processes associated with TKI-resistance and early BC transformation overlapped, and that CD34+ cells from different sources (BM vs PB) contained this information.

In the post-TKI era, a landmark study by Radich et al, identified distinct transcriptional programs during BC progression (25). About 3000 genes were associated with the BC stage with dysregulated WNT/β-catenin pathway, decreased Jun B and FOS, and higher PRAME expression. The Radich dataset was subsequently used to compute a six-gene signature comprising of NOB1, DDX47, IGSF2, LTB4R, SCARB1, and SLC25A3 to predict progression (35). Independently, Zheng et al isolated CD34+ cells and identified 34 DEGs as cells transited from CP to
BC. Amongst the misregulated genes, **SOCS2** and **CD52** were downregulated while HLA-related genes were overexpressed in BC (36).

To understand the biological mechanisms underlying GE changes in TKI-resistance and BC, a recent study tested the hypothesis that prognostically important genes were enriched for targets of the polycomb repressive complex (PRC; see below) (10). Importantly, target genes of PRC-associated silencing in BC progression were enriched for downregulated genes identified in both the McWeeney and Yong datasets (10). The cross-validation of these three independent datasets suggest important lessons for the development of GE-based risk assessment: 1. The discovery of reproducible GE-based biomarkers is possible when homogeneous CD34+ populations are used; 2. The processes of TKI resistance and BC transformation are biologically convergent despite genetic heterogeneity (10); and 3. PRC-regulated processes contribute to silencing of prognostically informative genes.

**Contribution of somatic mutations to GE signatures**

Recent reviews have described the range and frequency of specific genetic mutations in patients who developed TKI resistance and/or BC (37). For many of these genes, there is strong preclinical data that their associated mutations contribute to or are even sufficient to produce TKI resistance or transformation phenotypes (summarized in Table 3) (38-48). These studies imply that genetic mutations alter GE profiles, and here we review their contributions to GE changes in CML since these changes may represent useful GE-based biomarkers.

For **RUNX1** mutations, the Mustjoki group identified an accompanying GE signature in BC samples (49). They found that **RUNX1** mutations were associated
with the upregulation of stemness, B cell markers, interferon and immune signaling and transcription factors regulating pDC development.

In analogous work, the overexpression of an $IKZF1$ dominant-negative mutant in CD34$^+$ cells from CP patients increased STAT5 expression, a pathway associated with imatinib resistance (50), and enhanced transformation (51). $RAG$ expression status was recently assessed in diagnostic samples, given the role for RAG recombination as a mediator of $IKZF1$ deletions (52). Notably, $RAG1/2$ and $DNTT$ upregulation at diagnosis suggested imminent LBC transformation within 12 months (8/8 patients), demonstrating that GE signatures can reliably predict transformation.

Despite limited functional interrogation of $ASXL1$ using CML patient-material, insertion sites within $ASXL1$ promoted BC progression in a CP mouse model subjected to transposition-based mutagenesis (53). Transgenic expression of truncated protein $ASXL1^{aa1\text{-}587}$ in mice increased HSC self-renewal, Brd4 occupancy and chromatin accessibility around genes required for stemness, and predisposed mice to myeloid malignancies (54). However, the clinical relevance of diagnostic $ASXL1$ mutations is still unclear because some patients with $ASXL1$ variants at diagnosis can achieve MMR after TKI therapy (14). Also, $ASXL1$ mutation frequently disappeared when monitored in long-term during TKI therapy (personal observation by Dr. Dennis Kim). Meanwhile, direct evidence for contributions of other mutations to CML GE signatures is currently lacking, and we have to infer them from studies in other malignancies (Table 3).

**Lessons from clonal hematopoiesis**

Clonal hematopoiesis (CH) is the clinical phenomenon where populations of hematopoietic cells have expanded and carry a somatic mutation that is at least 2%
of the variant allele fraction (VAF) (55). The common genes comprise *DNMT3A*, *TET2*, and *ASXL1*, and others also found in CML individuals, including *RUNX1*, *BCORL1*, and *TP53* (56). Individuals with CH are at increased risk of developing hematologic malignancies, and it is therefore pertinent to ask if CH-related mutations also confer increased risk of TKI resistance or progression. Here, a study by Kim et al. has highlighted important features of CH-related mutations in CML (57). Firstly, they may occur in a non-Ph+ clone and predate the development of CML, and are unrelated to the CML clone. Secondly, even when a specific mutation occurs in the Ph+ clone, they only confer a relative risk of TKI resistance or progression. Indeed, patients with *RUNX1* mutations have been documented to achieve MMR (personal observation, Dr. Dennis Kim). Nevertheless, Kim et al. concluded that mutations in genes regulating epigenetic function (*TET2*, *ASXL1* among them) carried a higher risk of inferior TKI responses.

There is also strong preclinical data that CH-related mutation results in subtle but important changes in GE in HSCs. For example, *Dnmt3a* deficient HSCs show a loss of DNA methylation in regions enriched for self-renewal genes such as *Meis1*, *Evi1* and *HoxA9* (58). In Tet2-deficient mice, the loss of DNA demethylation is accompanied by an expansion of the stem and progenitor compartments, and eventual myeloproliferation (45). In *ASXL1*-deficient mice, an increase in self-renewal capacity of stem cells is observed, through the loss of PRC1-mediated gene repression (59). Another interesting aspect of HSPCs harboring inactivating mutations of *DNMT3A* and *TET2* is that they both led to increased cytokine production in peripheral myeloid cells, including IL-6 and IL-1β (60, 61). Furthermore, CH-associated mutations are frequently found in monocytes, granulocytes, and NK cells compared to B or T cells, suggesting that their effects may also be manifest in
multiple differentiated cell types within the hematopoietic compartment (62). Together, these observations are relevant to the search for prognostic GE signatures in CML for the following reasons: 1. Increased inflammation and cytokine production is associated with LSC persistence (32), and disease progression (10, 63); 2. Prognostic GE changes may be found in both CD34+ and CD34- fractions of PB or BM MNCs; and 3. Changes in NK cell function and number may predict TFR responses, and presumably contain informative NK GE signatures (64-66).

**Epigenetic contributions to GE signatures**

**PRC-associated GE changes**

Among well-studied epigenetic complexes in CML are the polycomb group (PcG) proteins (67). The PcG proteins assemble into two complexes, PRC2 and PRC1, which modify histones through repressive H3K27 trimethylation (H3K27me3) and H2AK119 monoubiquitination (H2AK119ub1) respectively, and in general repress gene expression (68).

From a mechanistic standpoint, the most commonly occurring mutations in CML appear to converge in their ability to interact with and function in conjunction with the PcGs. ASXL1 functions in transcriptional repression through its interaction with PRC2 and BAP1 (69). BCORL1 is a transcriptional co-repressor that interacts with PCGF1, a core complex of PRC1.1 complex (70). The RUNX1-CBF-β heterodimer mediates transcription by binding to RUNX sites, but also represses transcription by interacting and recruiting BMI1 of the PRC1 complex to target sites (71). IKZF1 regulates transcription by interacting with repressive epigenetic complexes such as HDAC1, HDAC2, CHD3, CHD4, and SWI/SNF complex, and also recruits PRC2 to target gene loci in T-cells (72). Thus, while the commonly
mutated genes in CML have their own exclusive roles in transcriptional regulation, they also share a striking commonality as modulators of the PRC complex. Whether mutated variants of *RUNX1*, *ASXL1*, *IKZF1*, and *BCORL1* drive aberrant PRC recruitment and GE in CML remains to be determined.

In this respect, a recent study has determined that LBC and MBC transcriptomes are highly congruent, and that both undergo a PRC-driven epigenetic reprogramming towards a convergent transcriptomic state (10). PRC-dependent epigenetic reprogramming was attributed to gain- and loss-of-function mutations in members of the PRC1 and PRC2 complexes, respectively. Of these, ongoing BMI1/PRC1 activity contributes to maintaining the BC transcriptome, while EZH2/PRC2 was instructional for DNA hypermethylation-dependent gene repression. Importantly, the integrative model proposed by the Ko et al., suggests that enrichment for PRC-dependent GE signatures at diagnosis can predict disease transformation and TKI resistance, as highlighted above (10).

We also note that dysregulated regulation of PRC has been identified as a key feature of TKI-resistant LSCs in CP. *EZH2* expression was higher in CML LSCs compared to normal HSCs, and CML LSCs have a higher dependence on the PRC2-EZH2 axis for survival and TKI resistance (73, 74). Likewise, higher *BMI1* levels at diagnosis correlated with disease progression from CP to BC (12), while *BMI1* overexpression in CP CD34+ cells increased proliferation and self-renewal (75), and transformed B-lymphoid progenitors *in vivo* (76).

**DNA methylation-associated GE changes**

Many studies have examined the role of DNA methylation as a regulator of aberrant GE in CML pathogenesis. In candidate-based approaches, genes involved in cell cycle regulation (*P16, P53, PLCD1, PER3, HIC1*), differentiation (*HOXA4, DLX4,
DDIT3, SPI1) proliferation (CDH13, DAPK1), apoptosis (BIM), Wnt regulation (sFRP1, CBY1), LSC maintenance (MTSS1), and cell signaling (Jun B, SOCS2) were identified as targets of DNA methylation (67) (77).

Recent unbiased genome-wide methylome analyses have solidified the concept of aberrant DNA methylation as a driver of resistance and transformation. The number of differentially methylated regions in CP increased from ~600 to ~6500 CpG sites in BC (78). BC was associated with heightened DNA hypermethylation, and to a lesser extent hypomethylation, around promoters of genes involved in stem cell fate, differentiation and leukemia-related functions (10). Mechanistically, differential DNA methylation patterns in CML have been attributed to underlying DNMT3A/TET2 mutations, PRC2-dependent epigenetic re-programming, and cytosolic sequestration of Tet2 by BCR-ABL1 (79). Notably, the physiological targeting of DNA hypermethylation using 5-aza-2'-deoxycytidine ameliorated disease phenotypes in a CP mouse model (80), while low-dose decitabine displayed clinical activity in patients refractory to imatinib (81), suggesting DNA methylation does indeed contribute to TKI resistance.

Based on the biological insights gleaned so far, it is possible that progression-related DNA methylation signatures may already be evident at diagnosis, particularly for patients presenting with advanced CP (10). The DNA methylation status of specific target genes might therefore be useful in the timely identification of such patients for more aggressive therapies. Further, given that DNA methylation is a relatively stable epigenetic and biochemical mark, there are practical advantages to develop DNA methylation-based biomarkers rather than transcript-based readouts, especially for the development of robust clinical-grade tests (Fig. 3).
GE profiles and mutations: ‘seed and soil’ revisited

As described above, it will be important to develop CML models that integrate the interaction between genetic and epigenetic factors in driving drug resistance and disease transformation. In this respect, the effects of specific mutations may be cell-context dependent, with differential effects on GE and function depending on the cell type being examined. In particular, for mutations affecting transcription factors where cell states, and their attendant chromatin accessibility profile, determine whether the mutated TF has access to its target genes.

To integrate contributions from both, we propose a model where the cell of origin, with its attendant epigenetic and transcriptional program, determines the ability of specific mutations to contribute to biologic and clinical outcomes (Fig. 2). This model is a derivative of the ‘seed and soil’ hypothesis of cancer initiation (82). The model will be useful for hypothesis testing, and likely explains an important feature of BCR-ABL1 itself. Here, it has been shown in murine models that only when expressed in HSCs, but not more committed progenitors, can BCR-ABL1 induce a myeloproliferative disorder. This is likely because BCR-ABL1 is incapable of conferring self-renewal capacity upon committed progenitors, indicating that CML cells rely on BCR-ABL1-independent mechanisms for stemness programs. These findings are in contrast to other leukemia fusion genes (e.g. MLL-ENL, MLL-AF9, MOZ-TIF2) which are capable of conferring self-renewal and transform progenitor cells (83). Relatedly, the model may also explain a naturally occurring phenomenon whereby normal individuals found to carry the BCR-ABL1 fusion in their PBMCs, apparently never develop CML (84). Here, the model would posit that the BCR-ABL1 fusion is occurring in a long-lived progenitor without self-renewal function.
Analogous to the situation with cancer initiation by leukemia fusion genes, mutations devoid of self-renewal function may only confer increased risk of BC transformation when they occur in a target cell that already possesses physiologic self-renewal function. According to this model, mutations in \textit{RUNX1} that are sufficient to induce BC-like disease in mice (Table 3) may be deemed a ‘strong’ biological seed that can transform many cell types within the hematopoietic hierarchy. Such mutations would be expected to induce disease progression in the majority of patients who harbor such mutations, which is indeed the case (37). However, a minority of CP patients with \textit{RUNX1} mutations continue to enjoy sustained DMRs (57), suggesting the existence of other important factors that modulate \textit{RUNX1} function. In a similar observation made recently, \textit{ASXL1} was the identified as the most frequently mutated gene at diagnosis in 9 patients, the majority (6) of whom eventually transformed to BC, while a minority (2) achieved MMR (14).

In contrast to the above examples, the prognostic impact of ‘weak’ seeds is even less clear. In a study by Kim et al., at least 4 different patterns were observed for \textit{TET2} mutations (57). One pattern is seen in patients with TKI resistance when both \textit{TET2} and \textit{ABL1} VAFs increased following TKI therapy, while another is seen when the \textit{TET2} VAF reduces after TKI treatment in patients with disease progression. In other cases, \textit{TET2} mutations were also detected within Ph$^-$ cells, and here, patients showed complex outcomes following TKI therapy, with some achieving MMR and others showing TKI resistance. There observations suggest that the effect of \textit{TET2} mutations are highly contextual.
Challenges ahead but room for optimism

As described above, the discovery of a limited and tractable set of genes that is prognostic across a majority of CML patients has been challenging for clinical, biologic, and technical reasons. Nevertheless, there is room for optimism. In the setting of breast cancer, GE panels comprising 21 genes that encompass various aspects of breast cancer biology have been found to be predictive of therapeutic response, and minimized the use of additional therapy without compromising survival (85). Among liquid tumors, a recent study in AML demonstrated that a parsimonious 17-gene GE score, derived from a larger set of stemness-conferring genes, predicts resistance to initial therapy (86). Interestingly, this score was independent of cytogenetic and mutational risk factors, and suggests that biologic factors (e.g. stemness) transcend traditional genetics-based groupings (87).

Encouragingly in CML, two recent reports suggest that it is possible, using PB samples taken at diagnosis or 3 months after diagnosis, to predict DMRs and also sustained TFRs. In the first study, the Adelaide group showed the rate of decline of \( BCR-ABL1 \) transcripts during first-line TKI therapy (calculated from baseline and 3-month \( BCR-ABL1 \) transcript levels) predicts TFR success (88). The time taken for \( BCR-ABL1 \) transcripts to halve was the strongest independent predictor of sustained TFR: 80% in patients with a halving time of <9.35 days versus 4% if the halving time was >21.85 days (P<.001). In a separate study, Radich et al, reported that GE signatures from PB taken prior to TKI initiation can identify individuals who achieve a DMR (MR4.5) at five years vs those with suboptimal responses (89). Thus, biologic information encoded in GE data can predict very long-term clinical outcomes in CML, and it is therefore conceivable that GE-based data will be able to identify patients in whom TKI therapy can be safely discontinued. More importantly, these early reports
suggest that despite the existence of diverse resistance mechanisms within the study populations, final common paths, readout either as dynamic measures of BCR-ABL1 transcript levels, or PB GE signatures are discoverable.

**Stages in developing GE-based risk assessment**

The stages of developing GE-based tests has been outlined in recent reviews and consensus statements, and comprise at least three phases that assess: analytical validity (reliably measuring the genotype of interest), clinical validity (ability to segregate patients into biologically- and clinically-important subsets), and clinical utility (ability to improve clinical decision making) (90, 91). In this section, we summarize the pertinent stages and highlight issues of particular relevance to GE-based biomarker development in CML (Fig. 3).

Stage 0 is the Discovery stage, which is where the field is currently. Here, we highlight three important components, which include the use of technical approaches for unbiased discovery, the simultaneous interrogation of leukemic and non-leukemic clones from the same sample (since both have been shown to be prognostic), and the use of robust statistical and computational pipelines to discover minimal prognostic genes sets. The advent of single-cell-based technologies and its application to well-annotated cohorts will facilitate this step.

In Stage 1, the minimal gene set has to be converted into a clinical test that accurately and reproducibly measures the GE phenotype. The test platform needs to be robust, as well as sensitive, specific and reliable. The assay should be developed for tissues that are collected as part of routine clinical care. Ideally, any additional processing of material beyond what is routine should be minimized, e.g. CD34+ selection, and should utilize standard procedures available in clinical
laboratories, e.g. flow cytometry (FC) and BM immunohistochemistry (IHC). An example would be detecting GE signatures of interest by a panel of antibodies for use in FC or IHC applications. It is preferred that the samples used for analytical validation are from well-characterized patients that are representative of ‘real world’ settings, and ideally, validated in at least one independent cohort. Sample size and power calculations should be determined prior to study start, and analytic sensitivity and specificity for the test available at the end of the study. At the end of Stage I, a locked down test should be evaluated in the Stage II, that of clinical validation.

In Stage II, the locked down test will be evaluated for its ability to differentiate between clinically meaningful outcomes in modern CML practice. The samples to be tested should be obtained from well-annotated cohorts representative of the broader population, and the test conducted on tissues in a blinded manner with respect to testing and result reporting. Ideal populations include patients who have been treated uniformly in clinical trials. At the end of this phase, the ability of the test to predict clinical outcome should be available as a test score, with clearly defined positive and negative predictive values.

The final stage, Stage III, will be the determination of clinical utility. This stage would entail the use of the GE-based test to improve clinical decision-making, and would require the study to demonstrate that meaningful outcomes are improved when the test is used compared to when the test is not used. Besides clinical outcomes such as improved PFS, OS, additional measures such as cost-effectiveness, avoidance of toxicities, quality of life and psychological measures are assessed. Such studies may also incorporate the contribution of pharmacologic factors (e.g. drug metabolism and side-effects, patient compliance) to overall
outcomes. Given the relative rarity of CML, it is envisaged that this will be a multi-center international study.

**Conclusion**

Genetic and epigenetic events contribute to the emergence of BCR-ABL1-independent clones that result in clinical TKI resistance and, if unopposed, BC transformation. Long-term TKI responses, including successful TKI stoppage, can be predicted by slower declines in *BCR-ABL1* transcript levels during first-line TKI therapy (88), suggesting that genetic and epigenetic factors contributing to TKI resistance are present at diagnosis. Recent studies describe a convergent GE signature common to the majority BC progenitors (10). Elements of this common or core transcriptome can be detected in CD34+ cells from CP patients at risk of TKI resistance or early transformation (11, 13), and specific mutations have been shown to contribute additional nuances to the core transcriptome (49). These observations are consistent with a seed and soil model that may be helpful for hypothesis generation (Fig. 2). Emerging technologies, particularly multimodal single-cell-based approaches, will facilitate the discovery of genetic and epigenetic biomarkers at presentation. This initial discovery phase has to be followed by the translation of GE-based information into validated analytical tests, and subsequently, the determination of clinical validity and utility. This process will be a multi-year, multi-institution international effort akin to that for the development of a genetic-based risk assessment (85, 90, 91). The integration of both gene mutation- and gene expression-based biomarkers into the care of CML patients will be an important step to achieving the ultimate goal of CML research: the cure of the majority of our patients.
References


### Table 1. Gene expression profiling studies comparing TKI responders with non-responders

<table>
<thead>
<tr>
<th>Study</th>
<th>Time sample taken</th>
<th>Platform</th>
<th>No. of DEGs</th>
<th>Time of predicted event</th>
<th>Biological insights</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kaneta et al, 2002</td>
<td>CP 18; AP 2; BC 2</td>
<td>cDNA Microarray</td>
<td>79</td>
<td>R=MCyR (&lt;35% Ph+); NR &gt;65% Ph+ at 5 months</td>
<td>First evidence that GE profiles can predict sensitivity to imatinib</td>
<td>79 DEGs were identified. 15 or 30 genes were used to develop a prediction score to separate TKI-responders from non-responders</td>
</tr>
<tr>
<td>McLean et al, 2004</td>
<td>CP 66</td>
<td>Microarray (Affymetrix HG_U95Av2)</td>
<td>55</td>
<td>R=CCyR (0% Ph); NR &gt;65% Ph+ at 12 months</td>
<td>Predictive genes enriched for cell adhesion, mitogenic signaling, apoptosis</td>
<td>No differentially expressed genes were used to develop a classifier to separate TKI-responders from non-responders</td>
</tr>
<tr>
<td>Crossman et al, 2004</td>
<td>CP 29, included patients previously on IFN-α</td>
<td>Microarray (Affymetrix HG_U95Av2)</td>
<td>-</td>
<td>R=CCyR within 9 months; NR &gt;35% Ph+ after 1 year</td>
<td>Gene expression profiling studies comparing TKI responders with non-responders</td>
<td>A 6-gene prediction model was constructed which could predict MCyR at 12 months</td>
</tr>
<tr>
<td>Villuendas et al, 2006</td>
<td>CP 32, 12 validation</td>
<td>Microarray (CNIO OncoChip)</td>
<td>46</td>
<td>MCyR at 12 months</td>
<td>Predictive genes associated with Wnt signaling, cell adhesion, NK4, apoptosis, DNA repair</td>
<td>A 6-gene predictor model was constructed which could predict MCyR at 12 months</td>
</tr>
<tr>
<td>Frank et al, 2006</td>
<td>CP 23 R; 11 NR</td>
<td>Microarray (Affymetrix HG_U133A)</td>
<td>128</td>
<td>R=MCyR (≤p35% Ph+); NR &gt;p35% Ph+ at 12 months</td>
<td>Predictive genes enriched for transcriptional regulation of apoptosis, oxidative stress, DNA repair, centrosomal genes</td>
<td>A 128-gene predictor of primary cytogenetic resistance to imatinib was identified</td>
</tr>
<tr>
<td>McWeeney et al, 2009</td>
<td>CP 12 R; 24 NR (discovery); CP17 R 6 NR (validation)</td>
<td>Microarray (Affymetrix HG-U133 Plus 2.0)</td>
<td>885</td>
<td>R=CCyR at 12 months; NR &gt;66% Ph+ at 12 months</td>
<td>Predictive genes enriched for cell adhesion and targets of the Wnt/β-catenin pathway</td>
<td>A 75-gene probe set classifier that separated the responder groups into primary TKI-R and non-responders</td>
</tr>
<tr>
<td>Zhang et al, 2009</td>
<td>Diagnostic PB &amp; BM</td>
<td>Microarray (Affymetrix HG-U133 Plus 2.0)</td>
<td>21</td>
<td>CCyR at 12 months</td>
<td>Predictive genes enriched for DNA repair by recombination</td>
<td>15 genes distinguished CP from BC -12 genes distinguished between secondary TKI-R vs optimal responders</td>
</tr>
<tr>
<td>de Lavallade et al, 2010</td>
<td>Diagnostic PB</td>
<td>TaqMan LDA</td>
<td>105</td>
<td>CCyR at 12 months; NR (failed to achieve any cytogenetic response)</td>
<td>Predictive genes overlapped with three independent datasets for BC genes (Zheng et al, 2006), genes predicting early BC transformation (Yong et al, 2006), PRC target genes in BC (Ko et al, 2020).</td>
<td>Identified a set of genes whose expression was differentially regulated in patients resistant to imatinib</td>
</tr>
<tr>
<td>Kok et al, 2019</td>
<td>Diagnostic PB</td>
<td>Microarray (Affymetrix HG_U95Av2)</td>
<td>365</td>
<td>EMR at 3 months</td>
<td>HR-GES had lower sensitivity in patients resistant to imatinib</td>
<td>A binary classification model based on 17 genes -HR-GES: 77% failure, but missed 2/9 -LR-GES: 95% did well, but missed 4/79 -44% sensitivity -97% specificity -HR-GES had lower rate of EMR failure with nilotinib</td>
</tr>
</tbody>
</table>

**Platform:**
- **cDNA Microarray:** Affymetrix HG-U95Av2
- **Microarray:** Affymetrix HG-U133A, Illumina HT-12v4

**No. of DEGs:**
- 79, 55, 46, 128, 885, 21, 105, 365

**Time of predicted event:**
- R=MCyR (<35% Ph+); NR >65% Ph+ at 5 months
- R=CCyR (0% Ph); NR >65% Ph+ at 12 months
- R=CCyR within 9 months; NR >35% Ph+ after 1 year
- MCyR at 12 months
- R=MCyR (≤p35% Ph+); NR >p35% Ph+ at 12 months
- R=CCyR at 12 months; NR >66% Ph+ at 12 months
- CCyR at 12 months; NR (failed to achieve any cytogenetic response)

**Biological insights:**
- First evidence that GE profiles can predict sensitivity to imatinib
- Predictive genes enriched for cell adhesion, mitogenic signaling, apoptosis
- Gene expression profiling studies comparing TKI responders with non-responders
- GE comparisons should be made on purified CD34+ cells
- Predictive genes associated with Wnt signaling, cell adhesion, NK4, apoptosis, DNA repair
- Predictive genes enriched for transcriptional regulation of apoptosis, oxidative stress, DNA repair, centrosomal genes
- Predictive genes enriched for cell adhesion and targets of the Wnt/β-catenin pathway
- Predictive genes involved in TKI influx/exflux, BC progression, BCR-ABL1 signaling
- Secondary TKI-R genes similar to BC genes but not primary TKI-R
- Predictive genes enriched for DNA repair by recombination

**Comments:**
- 79 DEGs were identified.
- 15 or 30 genes were used to develop a prediction score to separate TKI-responders from non-responders
- No differentially expressed genes were used to develop a classifier to separate TKI-responders from non-responders
- A 6-gene prediction model was constructed which could predict MCyR at 12 months
- A 128-gene predictor of primary cytogenetic resistance to imatinib was identified
- A 75-gene probe set classifier that separated the responder groups into primary TKI-R and non-responders
- 15 genes distinguished CP from BC -12 genes distinguished between secondary TKI-R vs optimal responders
- PTGS1, predicted primary TKI-R
- Identified a set of genes whose expression was differentially regulated in patients resistant to imatinib
- A binary classification model based on 17 genes -HR-GES: 77% failure, but missed 2/9 -LR-GES: 95% did well, but missed 4/79 -44% sensitivity -97% specificity -HR-GES had lower rate of EMR failure with nilotinib
### Table 2. Gene expression profiling studies comparing CP and BC

<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CP 68</td>
<td>CP 42; AP 17; BC 32</td>
<td>CP 11; AP 17; BC 34</td>
<td>CP 42; AP 17; BC 34</td>
<td>CP 16; MBC 13; LBC 5</td>
<td></td>
</tr>
<tr>
<td>Unselected/CD34+; PBMC/BM</td>
<td>CD34+; PBMC</td>
<td>Unselected; BM</td>
<td>CD34+; PBMC</td>
<td>Unselected; BM</td>
<td>CD34+; PBMC</td>
</tr>
<tr>
<td>Platform</td>
<td>Microarray</td>
<td>Microarray</td>
<td>Microarray</td>
<td>Microarray</td>
<td>Microarray</td>
</tr>
<tr>
<td>No. of genes</td>
<td>20</td>
<td>3000+</td>
<td>114</td>
<td>6</td>
<td>431 Upregulated LBC 522 downregulated</td>
</tr>
<tr>
<td>Comments</td>
<td>Identifies early (≤3y) vs late (≥7y) BC transformation Low CD7 &amp; high PR-3 predicts higher OS</td>
<td>Identifies TKI-R in CP (had BC-like signature)</td>
<td>Genes that distinguish CP and BP</td>
<td>Discriminates between early &amp; late CP</td>
<td>Identifies a core BC gene expression signature common to MBC and LBC. PRC-driven transcriptional reprogramming is enriched for poor prognostic genes in CP in the Yong et al, (2006) and McWeeney et al, (2009) CD34+ datasets.</td>
</tr>
<tr>
<td>Function</td>
<td>Mode of action and interactions</td>
<td>Interactions with other complexes</td>
<td>Aberration in CML</td>
<td>Effects on Gene expression</td>
<td>Effect of mutant protein/ gene knock out in vitro</td>
</tr>
<tr>
<td>---------------------------</td>
<td>---------------------------------</td>
<td>-----------------------------------</td>
<td>-------------------</td>
<td>----------------------------</td>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>Transcription factor</td>
<td>DNA binding via Runt domain.</td>
<td>P300, CBP, PRC1, NuRD, SWI/SNF, MLL/TxRg</td>
<td>Runt domain mutations, deletions and fusions</td>
<td>Up- interferon signaling, immune molecules, pDC-TFs, Down-DNA repair</td>
<td>Up-JAK-STAT signalling, self-renewal genes, Down- B-cell lineage and DNA repair genes</td>
</tr>
<tr>
<td>Transcriptional repression</td>
<td>DNA binding via zinc finger domain.</td>
<td>HDAC1, HDAC2, CHD3, CHD4, PRC2, CBP1, SWI/SNF</td>
<td>Deletions-exon Δ4–7 (IK6), exon Δ2–7</td>
<td>Up- interferon signaling, immune molecules, pDC-TFs, Down-DNA repair</td>
<td>Increased Brd4 occupancy and chromatin accessibility around genes</td>
</tr>
<tr>
<td>Represses transcription by binding to class II HDACs &amp; CTBP1</td>
<td>Represses transcription by binding to class II HDACs &amp; CTBP1</td>
<td>PRC2, BAPI complexes</td>
<td>Majority are frameshift and nonsense mutations in exon 12</td>
<td>Transcriptional repressor of E-cadherin</td>
<td>Transcriptional repressor of E-cadherin</td>
</tr>
<tr>
<td>DNA binding via zinc finger domain</td>
<td>DNA binding via zinc finger domain</td>
<td>PCGF1, the core PRC1.1 component</td>
<td>Frameshift, nonsense mutations</td>
<td>CML L359Y mutant inhibits transactivation by PU.1</td>
<td>CML L359Y mutant inhibits transactivation by PU.1</td>
</tr>
<tr>
<td>Conversion of 5mC to 5-hydroxymethylcytosine (hmC)</td>
<td>Conversion of 5mC to 5-hydroxymethylcytosine (hmC)</td>
<td>FOG1 through N-terminal zinc finger domain</td>
<td>Zinc finger domain variants</td>
<td>Impaired of 5-methylcytosine hydroxylation and decreased methylation at CpG sites in myeloid cancers</td>
<td>Impaired of 5-methylcytosine hydroxylation and decreased methylation at CpG sites in myeloid cancers</td>
</tr>
<tr>
<td>Transfer of methyl group to cytosines on DNA</td>
<td>Transfer of methyl group to cytosines on DNA</td>
<td>2-HG, vitamin C, OGT, WT1, VPRBP, IDAX</td>
<td>Missense, nonsense and frame-shift mutations</td>
<td>TET2 mutations in myeloid may be CHIP related or a part of the Ph+ clone</td>
<td>TET2 mutations in myeloid may be CHIP related or a part of the Ph+ clone</td>
</tr>
<tr>
<td>Methylation</td>
<td>Methylation</td>
<td>DNA binding via zinc finger domain</td>
<td>DNA binding via zinc finger domain</td>
<td>DNMT3A mutations are mostly CHIP mutations since they are also present in the Ph+ clone</td>
<td>DNMT3A mutations are mostly CHIP mutations since they are also present in the Ph+ clone</td>
</tr>
</tbody>
</table>

Table 3. Functional effects of frequently mutated genes in BC
FIGURE LEGENDS

FIGURE 1. Features of an ideal CML biomarker. Curves indicate changes in BCR-ABL1 transcript levels following initiation of TKI therapy in CP CML patients according to the BCR-ABL IS measurements. The corresponding MR value is provided next to the BCR-ABL IS value. Green, orange, and red curves are representative of the patients who experience ELN (European LeukemiaNet) 2020 Optimal, Warning, and Failure categories respectively. Major (MMR) and deep (DMR) molecular remissions are defined as 0.1% (MR 3) and 0.01% MR 4) BCR-ABL IS respectively. Green, orange, and yellow bullet points indicate guidelines for each category of response. Grey boxes describe predictive capabilities of an ideal biomarker. HCT, hematopoietic stem cell transplant. ‘R’-Responder; ‘NR’- Non responder; ‘PPV’- positive predictive value; ‘NPV’- negative predictive value; ‘TKI-R’- TKI resistance; ‘HR’- High-risk; ‘LR’- low-risk; ‘GES’- gene expression signature; ‘GSEA’- Gene set enrichment analysis.

FIGURE 2. Diagrammatic representation of CML ‘seed and soil’ model. The model proposes that both acquired mutations and the cell state of the mutation-acquiring cell contribute to the process of full transformation to BC. A ‘strong’ mutation is defined as being able to confer self-renewal function on a progenitor cell that does not possess inherent self-renewal capacity. A ‘weak’ mutation is unable to confer self-renewal function and can only transform a cell with native self-renewal ability, i.e. a stem cell. For both strong and weak mutations, it is likely that additional genetic and epigenetic events are necessary to confer the full suite of features required for BC transformation. The model is also based on the recent finding that BC progenitors which harbor different somatic mutations share a common or core transcriptome enriched for stemness, quiescence, and inflammatory GE signatures [20]. TKI-S/R denotes TKI-sensitive/resistance cells. HSC/LSC, hematopoietic/stem cell; MPP, multipotential progenitor; LMPP, lymphoid-primed multipotent progenitor; CMP, common myeloid progenitor; GMP, granulocyte-macrophage progenitor; Rx, treatment.

FIGURE 3. Stages of development of gene expression-based biomarkers. In CML, the development of GE-based biomarkers can be divided into three Stages following an initial Discovery Phase. These stages will each determine: Analytical Validity, Clinical Validity, and Clinical Utility of the tests in question. Examples of CML-specific issues or questions that are pertinent to each stage are outlined in boxes under each stage. IHC, immunohistochemistry; FC, flow cytometry; ISH, in-situ hybridization; LCM, laser capture microdissection; MNC, mononuclear cells; FFPE, formalin-fixed paraffin-embedded tissues; NK, natural killer cells; MDSC, myeloid-derived suppressor cells; NCCN, National Comprehensive Cancer Network; EFS, event-free survival; DFS, disease-free survival; PFS, progression-free survival; OS, overall survival.
Figure 1.

- **ELN 2020 Optimal**
  - Continue TKI
  - Evaluate for TKI stop after stable DMR

- **ELN 2020 Warning**
  - Alternate TKI
  - Increase TKI dose
  - Evaluate for HCT

- **ELN 2020 Failure**
  - Alternate TKI
  - Evaluate for HCT
  - Clinical trial

- **MMR loss after TKI stop**
  - BC transformation
  - MMR with 2nd/3rd TKI

**MR BCR-ABL**
- Identifies CP patients at high-risk of BC transformation
- Enables early clinical trial enrolment of at-risk patients

- Guides choice of anti-BCR-ABL1 therapy
- Identifies patients at-risk of pan-TKI failure

- Estimates duration of TKI Rx necessary for safe TKI stoppage
- Predicts relapse risk after TKI stoppage

**MMR**

**DMR**

3 months 6 months

- 1
- 2
- 3
- 4
- 5

- 100%
- 10%
- 1%
- 0.1%
- 0.01%
- 0.0032%
- 0.001%
- 0.0001%
Figure 2.