Curr Hematol Malig Rep (2013) 8:14–21
DOI 10.1007/s11899-012-0148-8

CHRONIC LEUKEMIAS (J GOLDMAN, SECTION EDITOR)

Targeting Chronic Myeloid Leukemia Stem Cells

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Published online: 20 December 2012

Abstract Chronic myeloid leukemia (CML) is a clonal myeloproliferative disorder that is characterized by the presence of the fusion oncogene *BCR-ABL* that encodes the tyrosine kinase BCR-ABL. Constitutive expression of BCR-ABL leads to the unregulated production of mature myeloid cells in the bone marrow and their subsequent release into the blood. Untreated, CML will progress from a chronic to accelerated phase over a number of years before quickly proceeding to a terminal blast crisis phase, reminiscent of acute leukemia. The advent of tyrosine kinase inhibitors has led to much improved management of the disease, but these drugs do not provide a cure as they are unable to eradicate the most primitive, quiescent fraction of CML stem cells. This review looks at recent research into targeting CML stem cells and focuses on major signalling pathways of interest.

Keywords Chronic myeloid leukemia · Leukemia stem cell · Oncogene addiction · WNT · Hedgehog · Self-renewal · Autophagy · SIRT1 · FOXO · JAK/STAT · Fatty acid metabolism · Stem cell microenvironment

Introduction

Chronic myeloid leukemia (CML) is a rare myeloproliferative disease arising from the acquisition of the Philadelphia chromosome (Ph) by hematopoietic stem cells (HSC). This is characterized by the reciprocal translocation between the long arms of chromosomes 9 and 22, t(9;22)(q34;q11) which in turn gives rise to a fusion of *c-ABL* and *BCR* to generate the *BCR-ABL* fusion gene that encodes the constitutively active tyrosine kinase BCR-ABL1 [1]. This results in the modulation of several key signalling cascades such as JAK/STAT, PI3K/FOXO, and RAS/MAPK amongst others, leading to increased cell proliferation, induction of cellular transformation and blockage of apoptotic pathways [1].

The development of tyrosine kinase inhibitors (TKI), such as imatinib mesylate (Gleevec™, Glivec™) has proven very effective in combating CML. These TKIs are enzymatically active against BCR-ABL and 5 years after commencing imatinib treatment, two-thirds of patients show a complete cytogenetic response (CCyR) [2]. However, discontinuation of imatinib is usually associated with disease relapse and it is currently recommended that patients should only stop TKI therapy in the context of a clinical trial. The majority of patients, therefore, are likely to receive TKI indefinitely to maintain remission [3••].

More potent second generation TKIs such as dasatinib (Sprycel™) and nilotinib (Tasigna™) have been effective in achieving CCyR in up to 50 % of patients failing to respond to imatinib [4, 5]. More recently, third generation TKIs, such as ponatinib, that target all clinically important BCR-ABL1 kinase domain mutants, [6••] have been used successfully in patients who fail to respond to dasatinib or nilotinib (clinical trials.gov identifier NCT01207440). Other BCR-ABL1 inhibitors such as DCC-2036 [7] and the pre-clinical HG-7-85-01 [8] have shown promising results in treating refractory CML.

The reasons for resistance to first and second generation TKIs are varied and poorly understood. Differing levels of BCR-ABL expression between patients, and the acquisition of mutations in BCR-ABL such as T315I are important factors. However, the major cause of resistance in CML is thought to be the existence of a fraction of primitive leukemic stem cells (LSC) that are not targeted by conventional TKIs [1]. LSCs are similar to normal hematopoietic stem cells (HSC) in that they are quiescent and have self-renewal

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capability, and evidence is emerging that they are not dependent on BCR-ABL for survival [9, 10]. Therefore, new targets need to be identified, which may, either alone or in combination with TKI therapy, result in eradication of the LSC. In this review we will aim to summarise the progress being made over the last 12 months in CML research with particular focus on targeting the LSC.

The CML Stem Cell

In chronic phase, CML stem cells are enriched within a small population of cells carrying the phenotype Lin^-, $CD34^+$, $CD38^-$, $CD90^+$. To characterize true stem cells, they must have self-renewal capacity in functional assays such as long term culture-initiating cell (LTC-IC) assays and the non-obese diabetic (NOD)/severe combined immunodeficiency (SCID) mouse repopulating cell (SRC) assay. Primitive Ph^+ LSCs exist in a quiescent state even in the presence of cytokines, whereas downstream progenitors are more proliferative and sensitive to cues that induce differentiation. Recent studies have shown that BCR-ABL^+ LSCs persist in patients that have undergone imatinib treatment for at least 4 years and have complete cytogenetic and molecular responses [11]. These cells retain long-term repopulating capacity after transplantation into recipient mice [12].

Oncogene Addiction

Although cancer cells can contain multiple genetic abnormalities, growth and survival of these can be impaired by the removal or inhibition of a single oncogene and are, therefore, said to be "oncogene addicted" [13]. It is still controversial in CML, whether the most primitive LSCs rely on pro-survival signals from BCR-ABL to evade TKI treatment and are thus oncogene addicted. To answer this fundamental, unresolved issue, Corbin et al. elegantly evaluated whether TKIs were capable of inhibiting BCR-ABL activity in immunophenotypically defined stem and progenitor cells and also in quiescent and cycling progenitors [9]. They showed that imatinib inhibited BCR-ABL activity to the same extent in all stem and progenitor cells tested and that even though expansion of these cells was reduced in the short term, growth factor supplementation allowed continued growth and survival even in the absence of BCR-ABL activity, suggesting primitive CML LSCs are not oncogene addicted [9]. In addition to this study, Hamilton et al. also aimed to investigate conclusively whether CML LSCs were dependent on BCR-ABL. They used an inducible BCR-ABL transgenic mouse model of CML to determine whether Bcr-Abl activity is required for the survival of transplantable murine CML-like and primary human CML LSCs. Following retransplantation, donor-derived CML LSCs that had tetracycline-regulatable Bcr-Abl turned on by withdrawal of tetracycline and then off with re-introduction of tetracycline were able to persist in the mouse and reinitiate CML-like disease in secondary recipients following Bcr-Abl re-expression [10]. This suggests that CML LSC survival is not dependent on BCR-ABL kinase and that eradication of the disease must focus on BCR-ABL kinase independent factors. However, a further study demonstrated that while imatinib treatment diminished CML disease load by targeting high BCR-ABL expressing stem and progenitor cells, more genetically stable clones with low levels of residual BCR-ABL expression were able to persist [14]. It was suggested that these BCR-ABL^{low} cells maintained a sufficient level of expression to generate a status of BCR-ABL oncogene dependence.

A number of major signalling pathways are implicated in the survival of CML LSCs and have been studied in detail as potential therapeutic targets [1]. A major focus of current interest is LSC self-renewal and aberrant self-renewal pathway signalling in CML.

Wnt/β-Catenin Signalling

The Wnt signalling pathway is important in embryonic development and cell proliferation and is one of several key signalling pathways involved in balancing the control of stem cell fate between self-renewal and differentiation [15]. β-catenin is the central effector molecule of the Wnt pathway and is negatively regulated by the multiprotein APC complex. Genetic deletion of β-catenin during fetal HSC development leads to impairment of self-renewal, but is dispensable in adult HSCs. It has been shown that Wnt signalling is aberrantly regulated in CML [16]. A recent study showed that deletion of β-catenin in a conditional mouse model of CML did not lead to significant increase in survival, but that this deletion synergised with imatinib to delay the onset of disease recurrence following discontinuation of imatinib treatment. This combination also eliminated CML stem cells [17]. Furthermore, mimicking this by treatment with the cyclooxygenase inhibitor, indomethacin, to block prostaglandin signalling led to a reduction in both β-catenin levels and LSC numbers. Use of a novel Wnt/ β-catenin inhibitor, AV65, inhibited proliferation and induced apoptosis in a number of CML cell lines, including those that harboured the T315I mutation. Combination with imatinib produced a synergistic inhibitory effect on the proliferation of primitive CML cells [18].

It is thought that a CML-specific immune response contributes to the control of the disease [19], but it is unclear whether the immune system promotes disease progression.

Using a mouse model of CML, Schurch et al. showed that LSCs and progenitors express the TNF family member CD27. Binding of its ligand, CD70, increased expression of Wnt target genes, resulting in increased proliferation and differentiation of LSCs. Blocking signalling through CD27 delayed disease progression and prolonged survival. In humans, CD27 signalling also promoted growth of LSCs by activating the WNT pathway [20].

Under normal circumstances, the serine threonine kinase glycogen synthase kinase 3B (GSK3β), a component of the WNT signalling cascade, exists in balanced active and inactive states and is localised between the cytoplasm and nucleus. However, a recent study has shown that GSK3β is constitutively phosphorylated at Y^{216} in CML stem and progenitor cells causing persistent relocation to the cytoplasm [21]. This was due to BCR-ABL dependent and independent activation of MAPK and p60-SRC/ GSK3β complex formation. The GSK3β inhibitor SB216763 caused almost complete suppression of primary CML stem and progenitor cells by acting to prime a pro-differentiation/ apoptotic program when combined with imatinib, but not dasatinib and did not target normal HSCs. The reason for this disparity could be explained by the fact that GSK3β pY^{216} levels were increased by imatinib, but reduced by dasatinib. CML cells integrate BCR-ABL and cytokine-activated signalling pathways that converge on GSK3β and β-catenin through MAPK and p60-SRC/GSK3β. It was suggested that BCR-ABL expression rather than tyrosine kinase activity may be enough to maintain active GSK3β pY^{216} levels through a growth factor-dependent mechanism that is annulled by dasatinib in addition to its kinase activity.

It is apparent that the WNT/ β-catenin/ GSK3β signalling cascade represents a good target for the development of new therapies for the eradication of CML LSCs, but β-catenin has proved a difficult therapeutic target thus far, with no clinical grade compounds available for clinical trials.

Hedgehog Signalling

Activation of the Hedgehog (Hh) signalling pathway is important in cancer formation and maintenance [22] and in the expansion of CML stem cells [23]. Hh proteins (ligands) bind to the receptor patched (PTCH), causing the release of inhibition by smoothened (SMO) and the subsequent activation of transcription factor GLI-1. GLI-1 then translocates to the nucleus where it can activate its target genes [24].

In a recent study using newly diagnosed cases of CML, differential activation of Sonic Hh (SHh) signalling was seen in 50 % of chronic phase, 70 % of accelerated phase and over 80 % of blast crisis cases. It was shown that autoactivation of SHh signalling provided survival and proliferative cues in CML progenitor cells through downstream β-catenin signalling and that by blocking the pathway with inhibitor or neutralising antibodies, apoptosis could be induced [25]. Silencing of GLI-1 by RNAi was accompanied by inhibition of BCR-ABL protein expression in K562 cells. This could be restored by purmorpharmine, a SMO agonist, or SHh peptide [26]. Resveratrol, a compound that has multiple intracellular targets that affect cell growth, inflammation, apoptosis, angiogenesis and metastasis and is also known to inhibit BCR-ABL, reduced GLI-1 activation and the viability of CML cells [26]. There are also a number of SMO inhibitors in clinical trials for CML currently, including LDE225 (NCT 01456676), BMS-833923 (NCT01357655 and NCT01218477) and PF-04449913 (NCT00953758) [27] and have shown some signs of promising results.

Other Potential Self-Renewal Targets

How myeloid progenitors acquire self-renewal capability to become LSCs is poorly understood. However, targeting self-renewal is seen as an attractive strategy for the eradication of LSCs. Continuous over-expression of Setbp1conferred self-renewal capability to myeloid progenitors in vitro and in the mouse. This was accompanied by increases in Hoxa9 and Hoxa10 expression. Suppressing either gene dramatically reduced self-renewal capability of myeloid progenitors [28]. Targeting SETBP1 activity in myeloid progenitors in CML may prove to be an attractive method of targeting self-renewal in these cells. The proto-oncogene MECOM (MDS1 and EVI1 complex) has an important role in normal development and is implicated in leukemogenesis. It is frequently expressed in CML blast crisis, implicating it in disease progression. However, its role in CML chronic phase is less well understood. Knockdown of MECOM was shown to reduce proliferation in CD34+ chronic phase CML cells independent of BCR-ABL kinase activity, and treatment with imatinib reduced MECOM expression in BCR-ABL+ cells. Therefore, it was proposed that BCR-ABL regulates MECOM expression in chronic phase cells and that BCR-ABL mediated its activity, in part, through MECOM giving selective advantage to cells expressing this gene [29].

Other Potential Targets in CML Stem Cells

By modulating the molecular pathways that regulate LSC function and not normal HSCs, it should be possible to specifically target those genes involved in forming cancer stem cells. The Src family tyrosine kinase, BLK was recently shown to be a key regulator in CML LSCs [30]. The study

showed that BLK acts as a tumour suppressor in CML and that it is downregulated by BCR-ABL in mouse and human CML cells. Suppression of BLK was maintained through all phases of the disease. A murine model showed that CML could be treated by restoring *Blk* expression or increasing expression of *Pax5* or *Cdkn1b*, or other members of the Blk pathway. Pax5 mediated down-regulation of *Blk* by Bcr-Abl through c-Myc, and p27 mediated the inhibitory effect of Blk on LSCs. Thus, inhibition of the Blk pathway accelerates CML development and increasing its activity can delay CML development.

JAK/STAT Signalling

The Janus kinases (JAK) are a family of intracellular, non-receptor tyrosine kinases that transduce cytokine mediated signals via the JAK/STAT pathway. Aberrant JAK/STAT signalling is widely implicated in various cancers, including CML. Modulating JAK2 activity by knockdown or chemical inhibition in mouse hematopoietic or human CML cell lines has been shown to reduce BCR-ABL signalling, leading to a decrease in oncogenic signalling of downstream pathways such as RAS, PI3K and STAT5. This suggests that CML cells are under the control of JAK2 [31]. Blockade of JAK2-mediated signalling by the inhibitors CYT387 or TG101209 led to the restoration of sensitivity of CML cells to TKIs, but also affected normal hematopoietic cells [32]. In addition, modulating STAT5 signalling activity by using derivatives of the cyclin-dependent kinase and GSK3β inhibitor indirubin led to induction of apoptosis in K562 cells, T315I expressing cell lines, TKI resistant cell lines and primary human CML cells [33]. Inhibition of JAK2-STAT5 signalling may prove to be a promising therapeutic target if the correct dosing can be achieved.

FOXO Signalling

One of the key pathways that BCR-ABL signals through is the PI3K pathway, leading to inhibition of the FOXO family of transcription factors. The FOXO proteins have diverse functions including cell growth, proliferation, differentiation and longevity. Inactivation of FOXO leads to increased proliferation and decreased levels of apoptosis in CML cells. In addition, FOXO transcription factors have been demonstrated to be important for the maintenance of CML LSCs, but the mechanism of FOXO-dependent leukemia initiation remains poorly understood [34]. Recent work has identified the proto-oncogene BCL6 as a key effector molecule downstream of FOXO that prevents CD34+ CD38- CML LSC self-renewal and depletion by transcriptional repression of Arf and p53 [35]. The authors argue that pharmacological inhibition of BCL6 using peptide-based inhibitors forces CML LSCs out of quiescence leaving them vulnerable to traditional therapies, such as imatinib. Resistance of CML cells to TKIs occurs through various mechanisms. Joha et al. showed that some imatinib-resistant BCR-ABL$^+$ cells had reduced expression of the glucocorticoid-induced leucine zipper protein (GILZ). Increasing expression of GILZ by glucocorticoid treatment overcame resistance to imatinib and suppressed tumour growth through mTORC2 inhibition. Interaction of GILZ with mTORC2 inhibited mTORC2 activity and inhibited pAKT causing activation of FOXO3a-mediated transcription of the pro-apoptotic factor BIM [36].

Targeting Stress Response Through Inhibition of SIRT1 in CML Stem Cells

Recently, a substantial amount of research has focused on the stress response gene SIRT1 and its role in promoting leukemogenesis [37–39]. SIRT1 promotes cell survival under metabolic, oxidative and genotoxic stress through deacetylation of numerous substrates including p53, Ku70 and FOXO proteins [40]. Overexpression of SIRT1 has been observed in solid tumours and hematopoietic malignancies and apoptosis can be induced by inhibiting SIRT1. Recent work from the Chen and Bhatia labs has shed some light on how SIRT1 promotes leukemogenesis [37–39]. They showed that BCR-ABL activates SIRT1 through STAT5 signalling and that this promotes CML cell survival and proliferation. Imatinib was able to partially inhibit SIRT1 expression and SIRT1 inhibition itself led to further sensitization of CML cells to imatinib-mediated apoptosis. Combination of gene knockout and imatinib treatment or treatment with the SIRT1 inhibitor tenovin-6 extends the survival of mice with CML-like disease [37]. Acquisition of mutations by BCR-ABL renders CML cells resistant to TKIs. Further studies showed that inhibition of SIRT1 blocks the acquisition of these BCR-ABL mutations. SIRT1 promoted acquisition of mutations by altering cellular DNA damage repair pathways and increasing error-prone DNA damage repair [38]. Restoring p53 function in cancer cells to inhibit proliferation or induce apoptosis or senescence is an ongoing area of research. As mentioned, increased SIRT1 expression has a clear inhibitory effect on the tumour suppressor p53. Inhibition of SIRT1 was shown to enhance targeting of CML LSCs by TKI treatment via activation of p53, indicating an essential role for SIRT1 in maintaining LSC growth and survival [39].

Genomic Instability in CML Stem Cells

Genomic instability is a key feature of cancer and usually results from an aberrant cellular response to increased

frequency of DNA damage [41]. In CML $CD34^+$ cells, DNA damage to a large extent is caused by the accumulation of intermediates of reactive oxygen species (ROS), either directly by ionizing radiation or following chemotherapy. BCR-ABL kinase activity itself can cause genomic instability in CML and TKI treatment has been shown to reduce DNA damage and genetic aberrations. Even so, mutations continue to be accumulated in patients receiving TKIs. Therefore, targeting genomic instability for a better therapeutic effect is a key area of research in CML.

CML LSCs and progenitor cells contain two to six times as much ROS and higher levels of oxidative damage than their normal counterparts [41–43]. A recent study has shown that high levels of ROS in LSCs and primitive progenitors was generated by alterations in mitochondrial membrane potential and electron flow through the respiratory chain complex III (MRC-cIII). This altered potential was shown to be caused by the GTPase Rac2. The resultant oxidative DNA damage triggered genomic instability and mutations in BCR-ABL that were resistant to TKIs. By inhibiting Rac2 through small molecule inhibitors or genetic deletion or by manipulating MRC-cIII, genomic instability was reduced [42]. Genomic instability also comes about by incorrect repair of double strand breaks (DSB). BCR-ABL was shown to promote RAD51 recombinase-mediated unfaithful homologous recombination repair (HomoRR). This is thought to contribute to accumulation of secondary chromosomal abnormalities that are responsible for CML relapse and progression [44].

Alterations in the Microenvironment

Proper regulation of the microenvironment within the bone marrow (BM) niche is critical to the homing and retention of HSC. The CXCR4/CXCL12 signalling axis is crucial to the maintenance of the BM stem cell niche and is critical for the homing and retention of HSC and for the maintenance of quiescence [45]. Interference in CXCR4/CXCL12 signalling by BCR-ABL, principally by down-regulation of CXCR4 is thought to alter the ability of CML LSC to home to and be retained within the BM niche [46]. These alterations in the microenvironment lead to a selective growth advantage of LSC over normal HSC.

A recent study [47] used an inducible transgenic BCR-ABL mouse model of CML [48] to characterize the microenvironmental regulation of CML LSC in detail. Long term engraftment and leukemia-initiating capacity was restricted to a subfraction of LSK cells that had a long-term HSC phenotype and this was related to a decrease in Cxcl12 expression, brought about by an increase in granulocyte colony-stimulating factor (G-CSF) production in leukemia cells. These results in the mouse model were validated using human CML cells and it was found that imatinib treatment could partially restore CXCL12 and other abnormally regulated cytokine levels [47]. In further mouse studies, cells deficient in the adhesion molecule P-selectin were shown to be better at repopulating the BM than wild type controls [49]. The authors argued that this was due to increased self-renewal potential rather than other factors such as increased homing capacity. These recipients of Bcr-Abl transduced BM from P-selectin-deficient donor mice developed a more aggressive form of CML with increased LSCs and progenitors.

A greater understanding of the regulation of the BM microenvironment and restoration of normal niche interactions could play a crucial role in leukemia control in a physiological setting.

Autophagy: Another Potential Clinical Target in CML

Autophagy is part of the normal catabolic process required for cellular homeostasis and is degradative in that it results in digestion of intracellular material as a source of energy in response to stress or nutrient deprivation [50]. It has previously been shown that inhibition of autophagy can improve the efficacy of TKIs and that combining TKIs with autophagy inhibitors such as chloroquine can effectively eliminate LSC in long term culture assays [51, 52•]. This hypothesis is currently being tested in a clinical trial (NCT01227135).

Several recent studies have aimed to shed more light on the role of autophagy in CML. The macrolide antibiotic clarithromycin was shown to be effective at increasing the sensitivity of CML cells to dasatinib-mediated cell death, by inhibiting late stage autophagy in K562 cells [53]. In addition, the small molecule TKI, lapatinib was demonstrated to induce autophagy and apoptosis in K562 cells [54]. Lapatinib targets ERBB1 and ERBB2 and has been the focus of a number of trials of solid tumours due to its efficacy and lack of serious adverse effects. The activity of lapatinib against K562 cells warrants further investigation in primary CML samples and in vivo.

High-mobility group box 1 protein (HMGB1) is an activator of autophagy and is involved in various human cancers by mediating resistance to chemotherapy. HMGB1 decreased the sensitivity of K562 cells to anti-cancer agents by upregulating autophagy pathways through JNK and ERK [55]. Autophagy has also been shown to be regulated by microRNAs (miRNAs), such as miR-101 [56]. miRNAs have roles in a variety of biological processes, but are also known to be deregulated in cancers including hematological malignancies. miR-30a has been shown to be a potent inhibitor of autophagy through the downregulation of Beclin 1 and ATG5 [57]. Yu and co-workers found that imatinib inhibits expression of miR-30a, but not miR-101

and that this leads to increased autophagy through Beclin 1 and ATG5 expression. This increase in autophagy led to a loss of imatinib-induced apoptosis. In contrast to this, increased miR-30a expression led to an increase in imatinib-mediated cytotoxicity [58]. The fact that imatinib not only activates cell death pathways, but also survival pathways such as autophagy means that the risk of initiating imatinib resistance will always be a potential problem with this TKI treatment [52•]. Inhibiting autophagy in conjunction with current CML treatments should overcome this problem and represents a novel therapeutic strategy that is currently being widely investigated.

Fatty Acid Metabolism

It has been reported that fatty acid metabolism has an important role in hematopoiesis and leukemogenesis [59]. Previous studies have shown that the lipid metabolic arachidonate 5-lipoxygenase (5-LO) gene (*Alox5*) is critical for functional regulation of LSCs, but not HSCs, possibly due to a failure of Alox5 null LSCs to upregulate β-catenin [60]. In a further study, Zhang et al. have characterised the role of stearoyl-CoA desaturase (Scd1) in CML[61]. Using the inducible BCR-ABL mouse model of CML [48], Scd1 was shown to be down-regulated in LSCs, but not normal HSCs and deletion accelerated CML disease progression. Scd1 regulated Pten, p53 and Bcl2 by an unknown mechanism. Scd1 was suggested to have a tumour-suppressive role in Bcr-Abl-driven leukemogenesis and that targeting LSCs by enhancing Scd1 function alongside inhibiting the function of Alox5 may be of therapeutic relevance [61].

Induced Pluripotent Stem Cells in CML

One novel, interesting area of research is the use of induced pluripotent stem cells (iPSCs) to model the pathogenesis of CML. Mouse models have proved useful in modelling human disease, but often don't accurately recapitulate certain aspects of the original disease. iPSCs can be generated from various types of normal or malignant cells by the transduction of defined transcription factors [62, 63]. A recent study established iPSCs from primary CML patient samples [64]. In this study, cells from a CML patient sensitive to imatinib were reprogrammed to generate iPSCs. Even though these cells expressed BCR-ABL, they were resistant to imatinib. Hematopoietic cells derived from these CML iPSCs recovered their sensitivity except the most primitive $CD34^{+}CD38^{-}CD90^{+}CD45^{+}$ cells, which went on to form disease. This would again back up the existence of the CML stem cell.

Conclusions

Cancer stem cell biology is a well studied area of research and CML remains the perfect system to study these cells. Despite the obvious clinical benefits of traditional TKIs in the management of CML, currently available drugs do not have the ability to completely eradicate the disease in the great majority of patients. Targeting the largely TKI-resistant quiescent LSC fraction in conjunction with traditional TKIs would appear to be the best hope of eradicating CML. The use of microarrays and other genomic and proteomic wide screens on LSC samples has shown us that there are vast differences in regulation of LSCs compared to normal HSCs. Targeting these changes should lead to the development of novel treatment strategies which will eliminate CML stem cells in the future.

Disclosure R. Kinstrie: none; M. Copland: received/pending grants from Bristol-Myers Squibb, Novartis; received honoraria from Bristol-Myers Squibb, Novartis, and Pfizer; travel accommodations/expenses covered or reimbursed by Bristol-Myers Squibb.

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