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De Novo Acquisition of BCR-ABL Mutations for CML Acquired Resistance

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1. Introduction

Chronic myelogenous leukemia (CML) is a rare and fatal neoplastic disease clinically presented as aberrant accumulation of myeloid cells in bone marrow, blood and spleen. CML occurs in 1 to 2 cases per 100,000 people, and is most common in older people with a median age at diagnosis of around 65. In the United States of America, there are 3500 to 5000 new cases per year (Jemal et al., 2010). If untreated, CML naturally progresses from initial chronic phase to accelerated phase and blast crisis that resembles acute myeloid leukemia. CML is one of the most extensively studied human cancers, and exemplifies that good scientific research can lead to successful treatment of a devastating disease.

In early 1960s, a characteristic small chromosome of CML was identified by Peter Nowell and David Hungerford who worked in University of Pennsylvania and Fox Chase Cancer Center in Philadelphia, respectively, and was subsequently called the Philadelphia (Ph) chromosome (Nowell and Hungerford, 1960; Nowell and Hungerford, 1961). Ph chromosome is found in 95% CML patients. In 1973, Janet Rowley identified that Ph chromosome was a product of reciprocal translocation of chromosome 9 and 22, the t(9;22)(q34;q11) (Rowley, 1973). In early 1980s, the genes involved in translocation were identified as proto-oncogene ABL (human homolog of Abelson leukemia virus gene) on chromosome 9 that was fused to BCR (break cluster region) on chromosome 22 (Groffen et al., 1984), revealing molecular insight of CML. In early 1990s, aberrant tyrosine kinase activity of BCR-ABL and its essential roles in transformation of cells were established by several groups (Daley et al., 1990; Heisterkamp et al., 1990; Lugo et al., 1990). These molecular discoveries provide crucial foundation for drug development for CML treatment. In 1996, Brian Druker and colleagues reported the first specific ABL kinase inhibitor imatinib mesylate (STI571, CGP 57148, or Gleevec) that effectively inhibited growth of BCR-ABL positive cells (Druker et al., 1996). In 1998, a phase I clinical trial of imatinib was initiated in three centers in the United States (Druker et al., 2001). Imatinib shows magnificent effect on bringing CML patients, especially those in chronic phase, into remission and improving long-term survival and disease management (Druker et al., 2006), and the drug is now the first line treatment for CML.

However, in contrast to chronic CML, patients at advanced phases (accelerated phase and blast crisis) generally have only transient response to imatinib, and relapse quickly (Deininger and Druker, 2003). Even for chronic phase patients, imatinib does not eradicate the disease and it relapses rapidly if imatinib is discontinued (Michor et al., 2005). Multiple

mechanisms, both BCR-ABL dependent and independent, have been described or proposed for CML drug resistance, including amplification of BCR-ABL, mutations of BCR-ABL, drug pump, leukemia stem cell quiescence, as extensively reviewed before (Apperley, 2007). For the scope of this chapter, we will focus on CML acquired resistance, the resistance developed after initial remission of the disease, through BCR-ABL genetic mutations.

2. Acquisition of genetic mutations of CML through drug selection of pre-existing mutants

Charles Sawyers and colleagues first described that acquired resistance to imatinib is mediated primarily by BCR-ABL mutations, and to a less extent, by amplification of BCR-ABL gene and other mechanisms (Gorre et al., 2001; Shah et al., 2002). These mutations confer various degree of resistance of CML cells to imatinib. Compiled from published data from 2001 to 2007, over 50 unique mutations have been identified in all CML patients, and they are mainly located at the BCR-ABL kinase domain (Apperley, 2007). We have updated this mutation profile by adding data published from 2008 to 2010 (Chien et al., 2008; Jones et al., 2008; Kim et al., 2009; Lewandowski et al., 2009; Markose et al., 2009; Press, 2010). The ten most common mutations representing 74% of total mutations (Fig. 1) exhibit similar patterns as shown before (Apperley, 2007). T315I remains as the most frequent mutation followed by Y253 and E255 mutations. Some minor changes are noticed: G250A/E moves to the 4th place ahead of M351T whereas M244V moves to the 10th place.

The four critical regions of BCR-ABL kinase domain are the prime targets for these mutations: T315I mutation for imatinib binding site, mutations on Y253, E255 and G250 for ATP binding site or P-loop, mutations on M351, E355 and F359 for catalytic domain and mutations on H396 for activation loop (Nagar et al., 2002; Schindler et al., 2000). M244V mutation occurs in close proximity to the P-loop and F317 mutations close to imatinib binding site. Although it is not functionally clear about the significance of every mutation detected, the profile highlights the importance of those high frequency mutations. T315I mutant BCR-ABL is resistant to inhibition by the second generation of tyrosine kinase inhibitors nilotinib (von Bubnoff et al., 2006; Weisberg et al., 2005) and dasatinib (Shah et al., 2004), which inhibit most other BCR-ABL mutations. T315I and P-loop mutations are associated with reduced survival and poor prognosis in patients (Nicolini et al., 2006). Y253F and E255K mutations increase BCR-ABL transformation capability whereas T315I mutation affects oncogenicity by altering P-loop phosphorylation (Griswold et al., 2006; Skaggs et al., 2006).

Progression of chronic phase to advanced phases may require additional alterations of CML genome beyond BCR-ABL (Perrotti et al., 2010). It is perceived that mutation mechanisms may be different in chronic phase from that in blast crisis CML cells. We have plotted ten most frequent mutations in chronic phase CML (Fig. 1) collected from data published from 2001 to 2009 (according to Table S2 in Klemm et al., 2009). The ten most common mutations representing 78% of total mutations in chronic phase are the same amino acids at the BCR-ABL kinase domain as seen in the all CML profile. Noticeably, M351T, instead of T315I, mutation is the most frequent mutation in chronic CML. It is evident that there is substantial increase of T315I mutation and reduction of M244V mutation in all CML profile, suggesting T315I is more favored and M244V is less favored towards the advanced phases. In addition, the most frequent mutations are clustered more tightly according to the functional regions of the kinase domain in all CML profile than in chronic CML profile. Although the above

difference may suggest the increased functional selection for drug resistance in advanced CML cells, mechanisms underlying such difference are unknown.

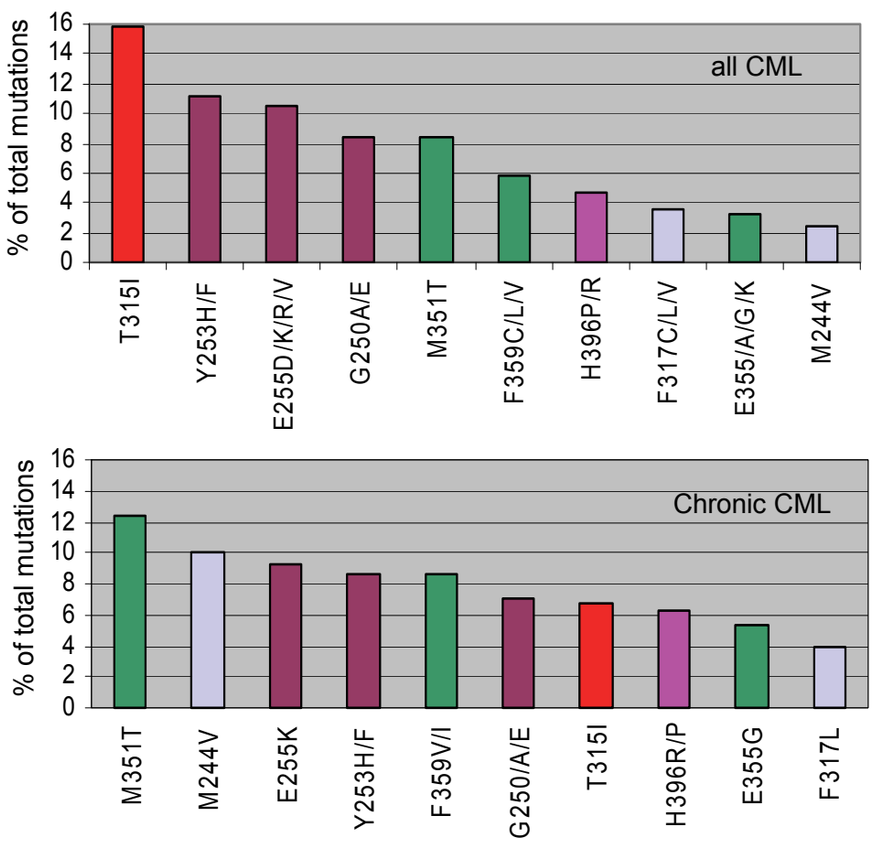


Fig. 1. The top ten mutations of BCR-ABL in all or chronic CML patients
Upper panel: Ten most frequent mutations in all CML. The profile was created by adding 344 mutation cases published between 2008 to 2010 to the previously published profile (Apperley, 2007). Lower panel: Ten most frequent mutations in chronic CML. The profile was created according to the previously compiled data containing 572 total mutation cases published from 2001 to 2009 (Klemm et al., 2009). Mutations in the regions of BCR-ABL kinase domain are color-coded: red for imatinib binding site, maroon for ATP binding site, green for catalytic domain, fuchsia for activation loop, and light blue for amino acids adjacent to those regions.

Charles Sawyers and colleagues were also among the first to identify mutations of BCR-ABL kinase domain in CML patients before imatinib treatment (Roche-Lestienne et al., 2002; Shah et al., 2002), and proposed that BCR-ABL mutations in relapsed CML patients upon imatinib treatment are a result of selection of pre-existing mutant clones (Shah and Sawyers, 2003). This model has been validated by subsequent studies from multiple groups identifying such mutations (Ernst et al., 2008; Roche-Lestienne et al., 2003; Willis et al., 2005). Mathematical modeling further provides support for rapid expansion of pre-existing mutant clones for fast relapse in CML patients (Michor et al., 2005). However, the rate of BCR-ABL mutations found in CML patients prior to imatinib exposure is around 21%, even using high sensitivity

methods for detection (Ernst et al., 2008; Roche-Lestienne et al., 2002; Willis et al., 2005), as compared to 40% to 90% in relapsed patients. This difference could be blamed to the insufficient sensitivity of detection methods. Alternatively, additional mechanisms for BCR-ABL mutations may exist and accentuate the mutations.

3. *De novo* acquisition of genetic mutations for CML drug resistance

To address mechanisms of CML acquired resistance, we have recently developed a novel tissue culture model using a naïve blast crisis CML cell line KCL-22 (Yuan et al., 2010). KCL-22 cells were derived from a female blast crisis CML patient, and were characterized as immature undifferentiated cells lacking lymphoid cell characteristics (Kubonishi and Miyoshi, 1983). KCL-22 cells are positive for myeloid cell markers including myeloid progenitor cell marker CD33. KCL-22 cells harbor two Ph chromosomes but do not have detectable BCR-ABL mutations (Yuan et al., 2010). In contrast to resistance of blast crisis CML in vivo, most blast crisis CML cell lines in culture are very sensitive to 1 μM imatinib with exception of KCL-22 (Deininger et al., 1997). Imatinib also kills KCL-22 cells, but it requires higher concentrations and takes longer time (Yuan et al., 2010). This unique feature of KCL-22 cell line perhaps made us successful in establishing it as a new model for acquired resistance of CML in vitro. The key characteristics of this model are summarized as follows:

1. High relevance: KCL-22 cells are incubated with in vivo therapeutic concentrations of imatinib, i.e. 2.5 to 10 μM. Cells undergo gradually increasing apoptosis for about a week followed by re-growing (relapse) with homogenous acquisition of T315I mutation.
2. Easy to use: KCL-22 cell line is commercially available from German Collection of Cell Culture. Cells are easy to grow in standard RPMI 1640 medium supplied with 10% fetal bovine serum, and no additional growth factors are needed. One single dose of imatinib treatment is sufficient.
3. Rapid turnaround: Relapse occurs in about two weeks with initial relapsed cells microscopically visible as small clusters of enlarged and sometimes irregularly shaped cells after around eight days of drug treatment.
4. High reproducibility: Multiple persons in the lab have used this model and it produces highly consistent results among them.

This model provides a novel tool for us to look into the process of acquisition of BCR-ABL mutations on the endogenous BCR-ABL in blast crisis CML cells, and has already provided valuable information in our initial study (Yuan et al., 2010) as summarized below.

1. Acquisition of BCR-ABL mutations does not require pre-existing mutant clones derived from the original patient, as clonal cells without an initial mutation can regenerate mutations and relapse on imatinib. The mutations do not derive from a fixed subpopulation of pre-mutant cells.
2. Acquisition of BCR-ABL mutations does not provoke additional chromosomal rearrangement, is not a result of mutator phenotype of KCL-22 cells, and can not be blocked by standard anti-oxidant treatment.
3. Acquisition of BCR-ABL mutations is a dynamic process influenced by culture and environmental conditions. This is supported by several lines of evidence. First, clonal KCL-22 cells exhibit great difference for BCR-ABL mutations whereas mutations on HPRT gene are relatively stable among clones. Second, clonal KCL-22 cells can acquire different single mutations for each clone, suggesting change of mutation hot spots after

cloning process. We have identified three clones that can acquire T315I, E255K and Y253H mutation, respectively. These three mutations have the highest frequency in human patients shown in Fig. 1. Third, the ability for acquisition of BCR-ABL mutations diminishes in parental KCL-22 cells after they are cultured continuously for more than 30 passages. Finally, we have found that the quality of fetal bovine serum can affect BCR-ABL mutation rate. We routinely use Hyclone characterized fetal bovine serum (Cat# SH30071.03) for mutation study, which typically produces consistent results among batches. However, when Hyclone serum was compared side by side with two other brands of fetal bovine serum, a noticeable reduction of BCR-ABL mutation rate was found in other brands (Fig. 2).

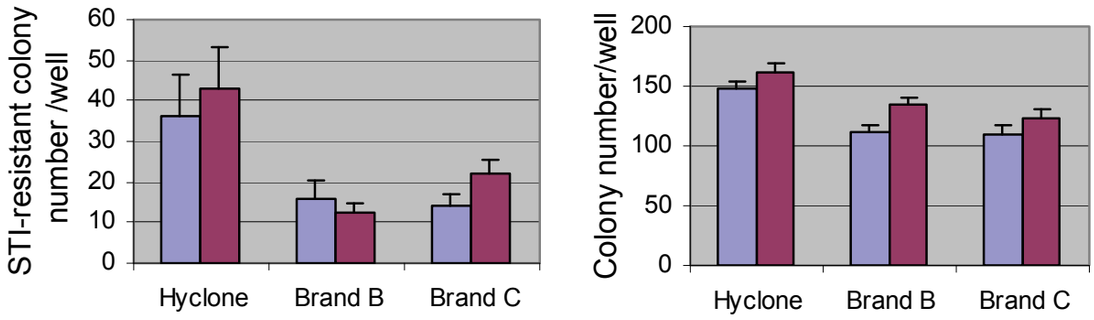


Fig. 2. Tissue culture conditions affect BCR-ABL mutations
Comparison of Hyclone characterized fetal bovine serum with two other brands of fetal bovine serum. KCL-22 cells were incubated with medium supplied with these sera, respectively, for two days, and then seeded onto soft agar for colony formation assay. Left panel, $1x10^6$ cells seeded per well with 2.5 μM imatinib. Right panel: plating efficiency with 500 cells seeded per well. Two independent batches of each serum (colored as blue and maroon) were tested.

4. Acquisition of BCR-ABL mutations depends on expression of BCR-ABL, since knockdown of BCR-ABL blocks KCL-22 cell relapse and mutations. By over-expression of kinase-inactive BCR-ABL, intriguingly, it was found that BCR-ABL kinase activity is not absolutely required for acquisition of mutations.
5. The endogenous BCR-ABL translocation locus significantly influences BCR-ABL mutations, as the mutation rate on the endogenous locus is nearly 10 times higher than that of the randomly integrated BCR-ABL cDNA as illustrated in Fig. 3. This finding reveals a previously unrecognized role of the translocation locus itself in driving BCR-ABL mutagenesis, suggesting possible involvement of the local epigenome for BCR-ABL mutations that will be discussed further below.

Our extensive analyses of BCR-ABL mutations in KCL-22 cells lead to the conclusion that mutations can be acquired *de novo* after imatinib treatment, although precise molecular mechanisms are yet to be uncovered. Such *de novo* acquisition of mutations may provide an alternative mechanism for clinical resistance of CML to tyrosine kinase inhibitors (Fig. 4). This is particularly important given that the majority of CML patients at the time of diagnosis do not harbor detectable BCR-ABL mutations. *De novo* acquisition of BCR-ABL mutations does not necessarily have to operate exclusively from selection of pre-existing mutations. It is anticipated that these two modes of acquisition of BCR-ABL mutations may

work in concert in certain patients leading to even faster relapse on the drug treatment. Therefore, we propose that *de novo* mutation acquisition and selection of pre-existing mutations are integrated processes for acquired resistance.

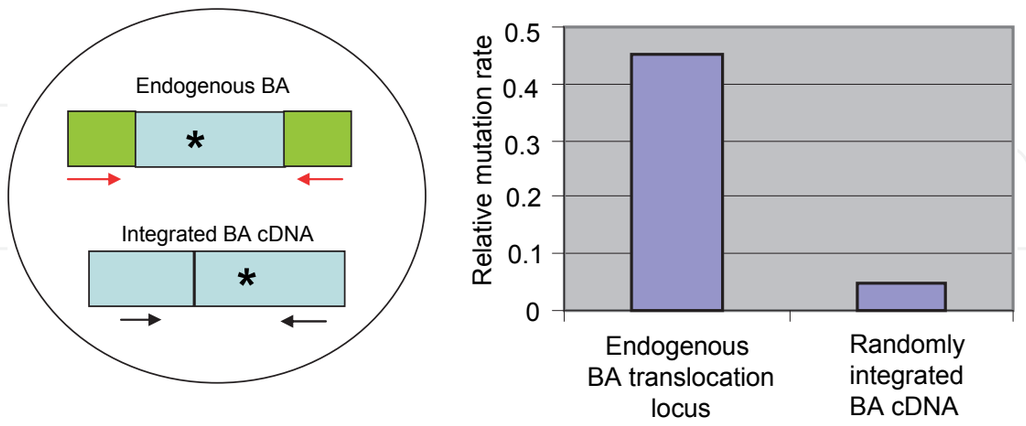


Fig. 3. Mutations on the endogenous BCR-ABL versus integrated BCR-ABL cDNA. Left, endogenous BCR-ABL (BA) was amplified by intron primers and integrated BCR-ABL was amplified by exon primers for mutation detection. Green for introns, blue for exons, asterisk for T315 mutation, and arrows for PCR primers. Right, relative mutation rate was the ratio of the number of clones bearing mutated endogenous BA or integrated BA cDNA divided by total number of clones, according to Yuan et al., 2010.

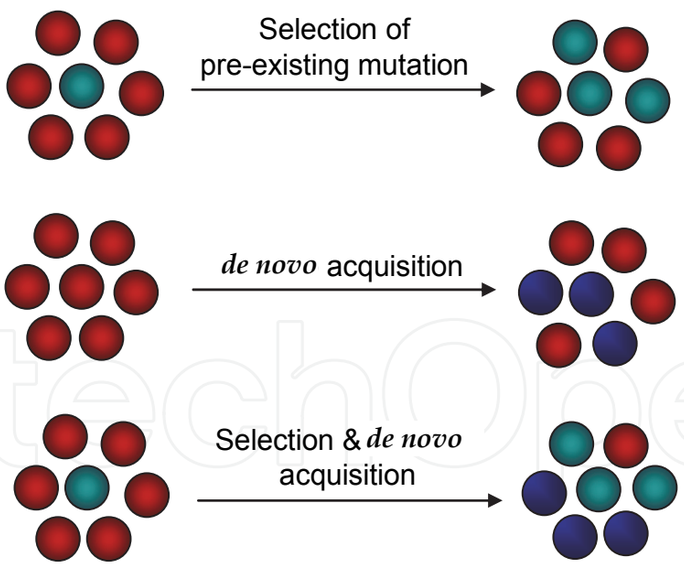


Fig. 4. Two modes of acquisition of BCR-ABL mutations for CML acquired resistance. Clinical relapse of CML on tyrosine kinase may be mediated by selection of pre-existing BCR-ABL mutant cells (green), *de novo* acquisition of BCR-ABL mutations (blue), or the combination of both. Non-mutant leukemia cells are in red. Two types of mutation acquisition could happen on the same amino acids.

Although the clinical readout for *de novo* acquisition and selection of pre-existing mutations are the same, i.e. relapse of the disease due to mutations, the implication for disease

management caused by these two modes of mutation acquisition could be different. Whereas strong tyrosine kinase inhibitors may be needed to suppress expansion of pre-existing mutant cells, prevention of *de novo* mutation acquisition by alternative strategies may help block relapse of CML in this category as detailed later.

4. Search for molecular evidence of *de novo* acquisition of mutations in human CML patients

So far, *de novo* acquisition of mutations has not yet been proved in patients. As described above, *de novo* mutation acquisition happens rapidly in the KCL-22 cell model in which early relapsed cells are microscopically visible after eight days of imatinib treatment. We have further found that mutations may likely form as early as two days after imatinib treatment (unpublished data). This rapid generation of mutations may prevent the appropriate use of mathematical models (Michor et al., 2005) to effectively distinguish between *de novo* mutation acquisition versus selection of pre-existing mutations. Separation of these two modes of mutation acquisition is further confounded by pre-existing mutations that may not be detected truly due to insufficient method sensitivity or by non-representative sample collection for analysis. It is currently unclear about the molecular signature of *de novo* mutation acquisition in CML patients or how to track this type of mutagenesis. However, mutations in clonal KCL-22 cells may provide a clue.

The mutation hot spots are different in three KCL-22 clones (clone L1 for E255K, clone L7 for Y253H and clone Ag 11 for T315I) (Yuan et al., 2010). When the codon changes are examined (TAC to CAC for Y253H, GAG to AAG for E255K, and ACT to ATT for T315I), a common molecular feature for these three mutations can be easily identified, namely, they are all transition mutations. It is important to note that both Y253 and E255 codons can acquire either transition or transversion mutation. Since these clonal cells are genetically identical, switching mutation hot spot from one transition mutation spot to another transition spot but not transversion, indicating that transition mutation is easier to be adapted on the BCR-ABL locus for kinase domain mutation. In line with this notion, frequencies for transition mutations for both Y253 and E255 codons are significantly higher than their transversion counterparts, Y253F and E255V in CML patients (Hochhaus et al., 2002; Press, 2010). Importantly, most of high frequency mutations shown in Fig. 1 are transition mutations. Further supporting this, a recent study shows that AID (activation-induced cytidine deaminase) promotes transition mutations in Ph^+ B lymphoid blast crisis CML (Klemm et al., 2009). However, AID is activated in B lymphoid CML, but not in myeloid blast crisis CML including KCL-22 cells (Klemm et al., 2009), there may be additional mechanisms to enhance transitional mutations in myeloid blast crisis CML that makes up about 60% of all blast crisis CML (Calabretta and Perrotti, 2004). On the other hand, genetic mutations tend to have bias towards transition mutations (Wakeley, 1996). Therefore, additional studies including rigorous analysis of clinical samples are needed to explore if transition mutations may indeed play a role in *de novo* acquisition of mutations in CML patients.

5. Mechanistic basis for integration of *de novo* mutation acquisition and selection of pre-existing mutations for drug resistance

A key question about *de novo* acquisition of mutations is how it arises and integrates with selection of pre-existing mutations. The selection model for drug resistance is rooted in

Darwinian genetics, in which mutations can randomly form without providing survival advantage until environmental stress is imposed. It has been well recognized that mammalian genome is under constant genotoxic assault from intrinsic or extrinsic source, and DNA damage contributes significantly to genetic mutations (Friedberg, 2003; Wiseman and Halliwell, 1996). Transformation of BCR-ABL in hematopoietic progenitor cells increases production of reactive oxygen species (ROS) and DNA damage (Koptyra et al., 2006). Expression of BCR-ABL alters functions of cellular DNA damage repair machineries, especially for double-strand break (DSB) repair, which promotes CML genetic instability (Melo and Barnes, 2007). It is reasonable to believe that increased DNA damage and genetic instability would predispose CML cells to form mutations prior to drug treatment.

In the past two decades, significant progresses have been made in the understanding of epigenetics and epigenomics for their roles in shaping gene functions, particularly in cancer development (Jones and Baylin, 2007). Unlike genetic changes, epigenetic/ epigenomic changes are versatile and reversible, which bring dynamics to genetic codes and bridge genetic alterations to environmental changes. Epigenomics plays important roles in DNA damage repair, best studied for DSB repair. Several chromatin remodeling complexes are participated in modifying local chromatin structure to facilitate DNA damage repair (Rossetto et al., 2010). Epigenetic regulation of chemoresistance is a burgeoning field, and little is understood how it is involved in acquisition of genetic mutations for drug resistance.

We propose the following model as illustrated in Fig.5: Acquisition of BCR-ABL mutation is a multi-step process involving at least three core processes: DNA damage initiation, DNA damage repair and survival of newly mutated cells. These core processes are bound together through local epigenome that may serve as a sensor for stress signals and influence either exposure of local DNA to damage, repair process or cell survival. BCR-ABL locus may sustain constant DNA damage and repair, and the status of such DNA damage and repair determines whether the eventual relapse is mediated by selection of pre-existing mutations or *de novo* acquisition of mutations under stress.

Based on this model, we predict that under the steady state, the BCR-ABL locus has ongoing DNA damage and repair, and most of cells do not harbor mutations because most of DNA damage is repaired correctly. Inappropriate repair will lead to low incidence of mutations and allow selection of such pre-existing mutations for cell survival and relapse during chemotherapy. On the other hand, chemotherapy, including but not limited to imatinib, may provide an extrinsic stress signal through epigenome to the locus, which may either increase local DNA damage load or interfere functions of repair machineries, accentuating the mutagenesis process and resulting in *de novo* acquisition of mutations.

The definition of pre-existing mutations or *de novo* acquisition of mutations is based on the readout of DNA sequencing. However, DNA sequencing only detects completed mutations but not DNA damage. Therefore, a damaged base undetectable as a mutation by sequencing may emerge as a mutation, if it is not repaired on time or is repaired improperly. Alternatively, a mutation may emerge as a result of new DNA damage elicited by extrinsic stress. Consequently, *de novo* mutation acquisition would not require a fixed subpopulation as we have shown (Yuan et al., 2010), and it would be a dynamic process if environmental changes, such as cell growth conditions and nutrients, affect local epigenome and subsequently interfere the repair process.

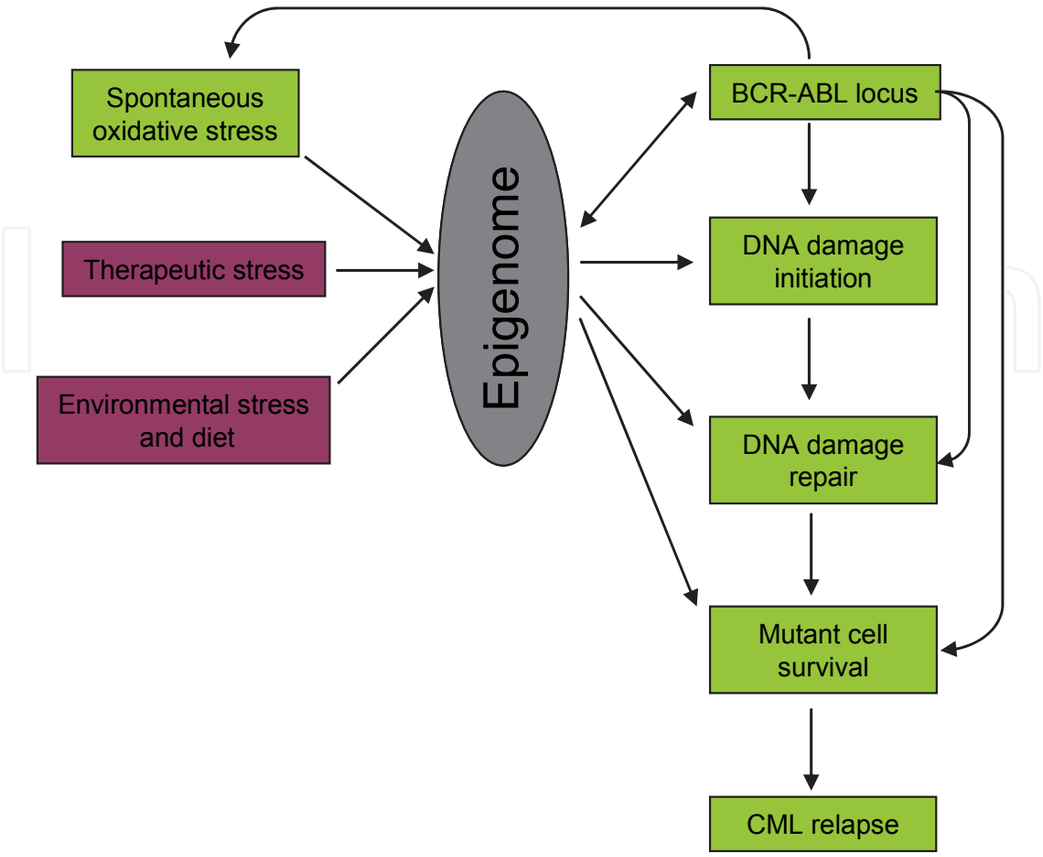


Fig. 5. An epigenome model for acquisition of BCR-ABL mutations
Acquisition of BCR-ABL mutations is a multi-step process that is regulated by BCR-ABL local epigenome. The epigenome is an important sensor of intrinsic oxidative stress signal (green) or extrinsic stress signal (red) including therapeutic or environmental stress, which in turn affects DNA damage, repair and cell survival, leading to maturation of genetic mutations for acquired resistance of CML.

6. New opportunities for studying molecular mechanisms of acquired resistance

In the coming years, it is anticipated that some steps of molecular regulation of mutation acquisition can be answered using the KCL-22 cell model. Here we discuss some steps that need obvious attention. Understanding these mutagenesis steps may help design new strategies to block BCR-ABL mutagenesis.

1. **Regulation of DNA damage initiation.** The very origin of mutations, where DNA damage is from, remains to be clearly defined. Many chemotherapeutic agents deliberately induce DNA damage to kill cancer cells. In contrast, imatinib treatment reduces bulk ROS production and DNA damage response measured by γH2AX (Yuan et al., 2010). It has been shown that anti-oxidants can inhibit BCR-ABL induced ROS production, DNA damage and mutations (Koptyra et al., 2006). However, anti-oxidants vitamin E and N-acetylcysteine, even in excessive amount, are unable to block acquisition of BCR-ABL mutations in KCL-22 cells (Yuan et al., 2010). There are several

possibilities for this observation: these anti-oxidants do not have sufficient potency, the DNA damage is too severe to be blocked, the anti-oxidants are inappropriate for the type of stress and damage in these cells, and the BCR-ABL locus is not accessible for these agents in KCL-22 cells. It is formally possible that there might be other sources of DNA damage.

One interesting but not surprising observation is that after imatinib treatment, ROS level in early apoptotic cells surges by more than 100 folds (Yuan et al., 2010) (Fig. 6). This high level of ROS may initiate cell death pathway. It is provocative to speculate that mutant cells might be derived from rare early apoptotic cells as they somehow abandon apoptosis process but sustain substantial DNA damage, which could not be blocked by treatment with standard anti-oxidants, and would thus lead to genetic mutations.

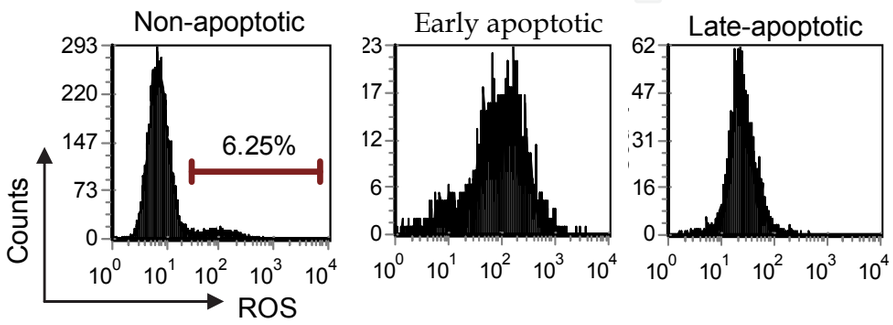


Fig. 6. ROS levels in different apoptotic fractions of KCL-22 cells after imatinib treatment.

2. **Regulation of DNA damage repair**. BCR-ABL alters functions of both homologous recombination (HR) and non-homologous end joining (NHEJ) repair machineries for DSB repair (Melo and Barnes, 2007). NHEJ is the major repair pathway in higher eukaryotes (Khanna and Jackson, 2001). NHEJ repair consists of both classic and alternative pathways. Classic NHEJ has core components KU70/86 and DNA-PKcs. When classic NHEJ is inhibited, alternative NHEJ that is independent of KU70/86 and DNA-PKcs will be activated. BCR-ABL inhibits classic NHEJ by degrading the key NHEJ factor DNA-PKcs (Deutsch et al., 2001). Recently, it has been found that BCR-ABL activates alterative NHEJ through upregulation of Werner Syndrome protein (WRN) and DNA ligase IIIa in CML cells (Sallmyr et al., 2008). For HR repair, BCR-ABL increases expression of RAD51, a central HR factor, which abnormally increases DNA repair and enhances CML drug resistance (Slupianek et al., 2001). In addition, BCR-ABL expression compromises repair fidelity and increases DNA repair errors in CML cells (Nowicki et al., 2004). It will be interesting to determine whether the above mentioned repair mechanisms and factors, and perhaps other repair mechanisms, may be involved in acquisition of BCR-ABL mutations for acquired resistance to imatinib.
3. **Regulation of survival of emerging mutant cells.** The newly relapsed KCL-22 cells exhibit morphological enlargement and irregularity with significant increase of G2/M fraction (Yuan et al., 2010). This indicates that newly relapsed cells may likely suffer mitotic crisis as they slowly progress through cell cycle. This is perhaps the last and critical step for the completion of mutagenesis process to allow full outgrowth of mutant cells. Molecular machineries involved in mitotic regulation might play a role in survival of newly relapsed CML cells. Among them, Aurora serine/threonine kinases are critically involved in regulating mammalian cell division (Keen and Taylor, 2004;

Marumoto et al., 2005). Aurora A over-expression overrides the mitotic spindle assembly checkpoint and promotes cancer cells resistance to chemotherapy (Anand et al., 2003). Interestingly, the pan-Aurora kinase inhibitor VX-680 not only inhibits Aurora kinases but also suppresses T315I BCR-ABL through distinct structural mechanisms (Carter et al., 2005; Young et al., 2006). Another mitotic kinase Polo-like kinase 1 (PLK1) is also known to mediate cancer chemoresistance (Luo et al., 2009). It remains to be determined if these and other mitotic regulators may play a role in CML acquired resistance.

4. **Regulation of epigenome.** Epigenome is a central component in our model. Identifying epigenetic factors involved in acquired resistance will significantly help us understand the dynamic mutation acquisition process. DNA methylation plays an important role in genetic mutations (Jones and Baylin, 2002). Methylation of cytosine at CpG sites significantly increases C to T transition mutation because of the spontaneous hydrolytic deamination of methylated cytosine. Cytosine methylation in gene coding regions is associated with mutation hot spots of certain tumor suppressor genes, such as p53, in cancer.

Histone modifications are essential for DSB repair. Within minutes of DSB, histone H2A variant H2AX is phosphorylated and recruited to damage foci, which is followed by accumulation of DNA repair and chromatin remodeling factors (Rossetto et al., 2010). Several types of chromatin remodeling occur on and surround DSB sites. ATP-dependent chromatin remodeling complex INO80 is rapidly recruited by γH2AX (Downs et al., 2004; Morrison et al., 2004; van Attikum et al., 2004). Recruitment of acetyltransferases and localized acetylation of histone H3 and H4 helps maintain open chromatin for repair (Tamburini and Tyler, 2005). Methylation of histone H4K20 also helps recruitment of repair factors through direct protein interaction (Botuyan et al., 2006; Sanders et al., 2004). When repair is completed, local deacetylation mediated by histone deacetylases restores chromatin structure. Importantly, the ability to modulate histone acetylation and deacetylation is essential for cell viability following HR repair, likely due to its signaling to prevent persistent activation of DNA damage checkpoint (Tamburini and Tyler, 2005). In addition, class III histone deacetylase SIRT1 is also recruited to DSB sites (O'Hagan et al., 2008) and regulates DNA damage response (Oberdoerffer et al., 2008; Yuan et al., 2007). It would be of great interest to define which and how chromatin modifiers response to therapeutic and environmental stress signals to mediate DSB repair and BCR-ABL mutagenesis.

7. Implication of *de novo* acquisition of genetic mutations in leukemia management

Blocking outgrowth of mutant BCR-ABL clones has clear therapeutic significance, regardless how the mutations may be acquired. However, strategies used to overcome resistance may be different for pre-exiting mutations and *de novo* acquired mutations. The former demands more and more potent BCR-ABL inhibitors, which could lead to stronger resistance each time. This has been proved with sequential use of kinase inhibitors, resulting in selection of compound drug-resistant mutations (Shah et al., 2007). More potent third generation of tyrosine kinase inhibitors have been developed (Quintas-Cardama et al., 2010), including AP24534 that inhibits all tested BCR-ABL mutations and blocks outgrowth of mutant clones (O'Hare et al., 2009). Clinical resistance to the third generation of tyrosine kinase inhibitors remains to be determined.

For mutations acquired *de novo*, however, a prevention strategy may be more appropriate and effective to block mutant cells from emergence at the beginning. With better understanding of mechanisms of mutations described above, it is expected that a new agent may be devised to modulate DNA damage initiation, repair, epigenome remodeling, or survival of newly emerged mutant cells, which will prevent CML acquired resistance in future. A combination therapy may be adapted with such a new agent and imatinib or other tyrosine kinase inhibitors. Alternatively, it is also reasonable to speculate that a multi-target agent can be developed to incorporate these features, for example, a drug that both inhibits BCR-ABL tyrosine kinase and modulates epigenome. We anticipate different ways to overcome resistance depending on which steps and factors the drug would target to block mutagenesis. We expect that such a strategy may also have broader implication for management of other types of leukemia to overcome drug resistance.

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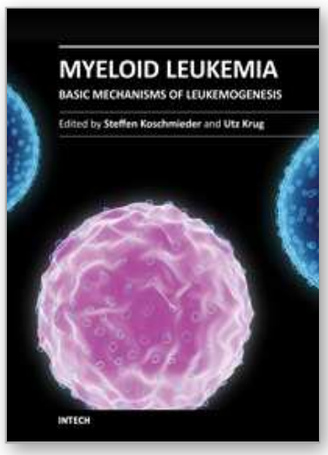
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The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.

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