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**Chronic Myeloid Leukemia: A look into how genomics is changing the way we treat Cancer.**

Until the late 1990s, nearly all treatment methods used for cancer (with the exception of hormone treatments) worked by killing cancerous cells using chemo or radiotherapy. While these techniques can kill cancerous cells in the body, they also kill normal cells resulting in numerous side effects ranging from immunosuppression to hair loss. Besides that, these methods target the killing of tumor cells rather than striking at the molecular cause of the disease. That has changed since the advent of the genome level understanding of the molecular causes of cancer. Understanding various mechanisms that lead to different types of cancer have given way to what is known as targeted therapy- a treatment method that blocks the growth of cancerous cells by interfering with targeted molecules that are needed for tumor growth. Apart from being more effective in preventing the proliferation of cancerous cells, these methods are also less hostile to normal cells.

A perfect example of how understanding the mechanism of carcinogenesis leads to effective drug development for targeted therapy is the development of targeted therapy for chronic myeloid leukemia (CML). CML is a clonal disorder of the pluripotent hematopoietic stem cell, characterized by an increased and unregulated growth of myeloid cells in the blood and bone marrow (Hehlmann, 2007). CML is linked to a single chromosomal abnormality – a translocation between chromosome 9 and chromosome 22, t (9;22) known as Philadelphia chromosome (Koretzky, 2007). Cytogenetically visible as a shortened chromosome 22, this translocation leads to a juxtaposition of the ABL gene from chromosome 9 and the BCR gene from chromosome 22 resulting in a BCR-ABL fusion that codes for fusion proteins with unusual tyrosine kinase activity. The fused BCR-ABL proteins are capable of interacting with the interleukin 3 beta receptor and activating a cascade of proteins which speed up cell

division. Also, since BCR-ABL tyrosine kinase activity does not require activation by cell signaling molecules, it is constitutively active unlike normal kinases. This means that white blood cells containing the fusion protein are able to proliferate without the presence of growth factors. In addition, BCR-ABL fusion proteins inhibit DNA repair, leaving the cell more susceptible to genetic abnormalities.

Cumulative effects of these molecular mechanisms make CML a progressive disease that develops in three stages. First stage called the chronic phase is clinically characterized by manifestations of excess white blood cells in the peripheral blood and bone marrow. After a median duration of 4-5 years in untreated patients, there is a transition from chronic phase CML to an accelerated phase or blast crisis. The accelerated phase is a transitional period with a median duration of 3-9 months and may either be asymptomatic, or be characterized by progressive enlargement of the spleen, bone pain, fever, night sweats, or weight loss. About 20%-40% of patients with CML progress to blast crisis without going through the accelerated phase. The malignant tumor loses the ability to differentiate terminally and leads to fatal leukemia in blast crisis (Kolibaba, 2000).

Going back to drug development, the discovery of the BCR-ABL-mediated pathogenesis of CML has provided the rationale for designing a drug that targets the kinase activity of BCR-ABL fusion protein. Gleevec (also known as imatinib mesylate or STI-571) is the targeted drug that unlike chemotherapy or radiation therapy acts on the molecular mechanism that leads to CML-the unusual tyrosine kinase protein coded for by BCR-ABL fusion. In order to carry out its kinase activity, BCR-ABL fusion protein contains a binding site for ATP, which is required for phosphorylation of its substrate. It can only successfully carry out its phosphorylating function if it can bind both ATP and substrate. With the knowledge of the structure of ATP binding site, Gleevec was developed with a shape complementary to the ATP binding site of BCR-ABL fusion protein. The shape complementarity allows Gleevec to bind to the ATP binding site of the fusion protein thereby competitively inhibiting its enzyme activity. This

prevents subsequent activation of transcription factors and cell cycle events, leading up to a reversal of the uncontrolled cell division.

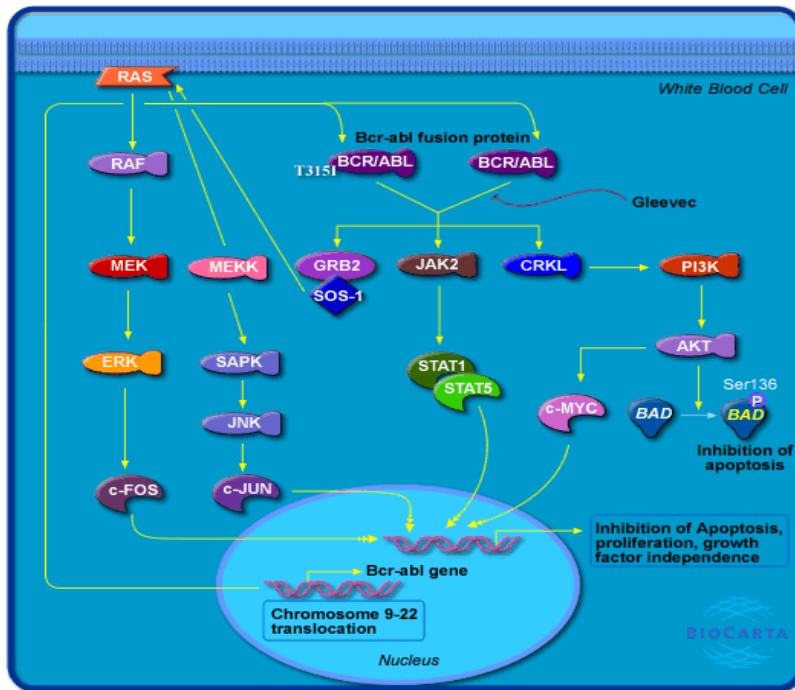


Figure 1: Inhibition of Cellular proliferation by Gleevec (Biocarta)

On the other hand, although Gleevec has produced very strong clinical responses in patients with early stage CML, patients with late stage disease have had an initial response followed by a relapse of drug resistant CML cells. Also, the degree of success of the treatment varies greatly among different people. In some patients, it can result in a complete elimination of all cells containing the Philadelphia chromosome (0% Ph<sup>+</sup> cells), a condition known as 'complete cytogenetic response'. In others, it leads to greater than 65% elimination of Ph<sup>+</sup> cells, a condition known as major cytogenetic response. In the worst cases, it shows no response at all. The question arises then about what leads to such variations in responses to the drug and how such variations could be addressed in drug designing techniques to make to drug more inclusive.

So, the next stage of targeted therapies focuses on finding patient-specific response to targeted therapies. Also known as stratified medicine or personalized medicine, this treatment seeks to incorporate the influence of genetic variation on drug response in patients by correlating gene

expression or single-nucleotide polymorphisms with a drug's efficacy or toxicity. In case of CML, the knowledge of a patient's genomic information has been used to understand the causes of resistance to imatinib, to determine patient-wise efficient dosage, and to identify other genes related to the disease that could act as potential drug targets.

As discussed above, although imatinib is representative of a critical advancement in CML treatment, nearly one-third of patients still have an inferior response to imatinib, either failing to respond to primary therapy or demonstrating progression after an initial response. Numerous genes associated with imatinib resistance have been identified with the use of complementary DNA (cDNA) microarray expression profiling. Research shows that a set of 46 genes were expressed differently in imatinib responders and non-responders (Villuendas, 2006). This set included genes involved in cell adhesion (TNC and SCAM-1), drug metabolism (cyclooxygenase 1), protein tyrosine kinases and phosphatases (BTK and PTPN22). From this set, a model based on six genes was created in order to accurately predict cytogenic response to the drug with an accuracy of 80%. Research done by Villuenda et al. has also identified BCR-ABL-independent mechanisms that could be involved in primary resistance to imatinib. For example, a batch of genes that this study associated with early imatinib resistance regulates drug metabolism. A gene known as CYP2B6, for example, encodes a member of the cytochrome P450 superfamily of enzymes which catalyze many reactions involved in drug metabolism. Although it has not been previously implicated in imatinib metabolism, CYP2B6 is also known to metabolize anticancer drugs such as cyclophosphamide. Another important gene involved in drug metabolism called cyclooxygenase 1(COX1) is also speculated to be involved in imatinib resistance (Villuenda , 2006). So, the inter-patient variation in drug resistance is a result of the interplay between wide numbers of genes. Given this involvement of many other genes in drug effectiveness, only a study of the genomic sequence of the patient will be able to accurately predict if a patient will respond effectively to the treatment

without any resistance. In addition, identification of these genes could also lead to research into the associated mechanisms as potential drug targets (McLean, 2004).

Through the study of Single Nucleotide Polymorphisms (SNPs) of resistant patients, recent studies have also established that resistance to tyrosine kinase inhibitors (TKIs) like imatinib is associated with particular genomic alterations. A large number of common deletions are found in tyrosine kinase inhibitor (TKI) resistant patients. More specifically, heterozygous deletions in the immunoglobulin lambda constant 1 (IGLC1) locus are detectable on chromosome 22q11 in resistant patients (Nowak, 2010). Chromosome 17 is also most heavily affected by secondary genomic alterations on development of TKI resistance (Nowak, 2010). In fact, genomic disruptions occurring on chromosome 17 are also one of the most commonly known changes arising during the progression of the disease from chronic phase to accelerated phase and blast crisis.

Genomic sequencing also provides important information on how to regulate dosage of the drug based on individual genetic differences. Often in cancer, fixed dosing is not the best approach to ensuring a good response. Similarly, while using imatinib, fixed dosage is not a good approach because the simple logic of tyrosine kinase inhibition is complicated by many other pharmacogenetic aspects of the drug. For instance, a number of genes coding for several of the cytochrome P450 enzymes affect imatinib because they are involved in metabolism of the drug. In order to get a good response with imatinib, knowledge of these specific genes needs to be used while treating CML patients with genetic variance in these specific gene areas (Li-Wan-Po, 2010). Another reason why the standard dose of imatinib fails to achieve the expected response in some patients is inter-individual variability in the expression of two types of transporters called human organic cation transporter 1 (hOCT1) and MDR1 (Thomas, 2004). Active transport processes by these two transporters mediate the influx and efflux of imatinib (Bixby, 2011). Differential expression of influx (hOCT1) and efflux (MDR1) transporters could be one of the

important factors to regulate intracellular drug levels (Li-Wan-Po, 2010). Genetic variations in these transporters need to be taken into consideration in deciding the efficient dosage for imatinib.

The development of imatinib for CML and the recent efforts to make it more personalized is representative of the direction towards which cancer medicine, in general, is heading. Advances in the understanding of molecular causes behind cancer and discoveries emerging from cancer genomics can be and are being translated into real clinical benefits for patients with cancer. Existing knowledge of genetic alterations is inadequate in addressing all the issues of diagnosis, progression and response to treatment. However, recent technological advances in high-throughput genomics have greatly accelerated gene discovery and facilitated the identification of molecular targets. Genomic approaches to cancer have taken targeted therapy to a whole new level of addressing individual genetic variations in treatment, an idea that is predicted to revolutionize cancer treatment.

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