Drug resistance in the cancer kinase BCR-ABL regarding Chronic Myeloid Leukemia

Grant Proposal

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Abstract

Cancer, a longstanding and lethal ailment, has garnered widespread attention, but one subtype often overlooked is leukemia, constituting approximately 1 in 10 cancer cases in the United States. Chronic Myeloid Leukemia (CML) emerges as a rare manifestation affecting the bone marrow, the sponge-like tissue responsible for blood cell production within bones. This condition leads to an increased presence of white blood cells in the bloodstream. A significant hurdle in cancer treatment is drug resistance, compromising the efficacy of anticancer drugs by hindering cancer cell responsiveness. Enzymes, particularly kinases, are pivotal in this resistance phenomenon, playing a crucial role in cellular signaling pathways governing processes like cell growth and survival. BCR-ABL, an enzyme targeted in CML treatment, is implicated in drug resistance, with mutations in its kinase domain identified as a primary cause of resistance to inhibitors. A potential strategy to counter drug resistance involves employing imaging software to construct inhibitors, preventing the activation of cancer kinases, and thereby reducing the occurrence of resistance in anti-cancer cells.

Keywords: Cancer, Chronic myelogenous leukemia (CML), BCR-ABL kinase, Drug resistance, Enzymes, Kinases, Structural biology

Understanding mutations that lead to drug resistance in cancer kinase BCR-ABL

Cancer:

Cancer is a disease where the body's cells grow and multiply uncontrollably. When your body is healthy cells will grow and divide at a normal rate in a controlled manner however cancer disrupts this process and leads to the creation of tumors. Possible cancer treatments include surgery, chemotherapy, radiation therapy, immunotherapy, and targeted therapies. Early detection through screenings and awareness of risk factors can significantly improve the prognosis for many types of cancer. Research continues to advance our understanding of cancer biology, leading to the development of new and more effective treatments. Chemotherapy has firmly established itself as a cornerstone of medical treatment.

Drug Resistance:

While we've witnessed significant strides in the development of chemotherapy, there's a pressing issue that casts a shadow over its effectiveness – the emergence of drug resistance to anti-cancer drugs in cancer cells. This development can lead to setbacks in our relentless battle against this formidable disease. The concept of chemotherapy resistance, often referred to as multidrug resistance (MDR), is not a mere theoretical concern. It's a stark reality that healthcare professionals and researchers confront daily as they work tirelessly to enhance patient outcomes. Drug resistance in the context of cancer cells and chemotherapy refers to the instances where cancer cells become less responsive or completely unresponsive to the

effects of certain anticancer drugs. Over time, cancer cells may adapt and develop mechanisms that allow them to survive and continue growing despite the presence of chemotherapy drugs that are designed to inhibit or kill them.

Enzymes and Kinases:

Enzymes play a crucial role in the development of anticancer drug resistance. Drug metabolism constitutes a biotransformation mechanism for drugs, facilitated by drug-metabolizing enzymes (DMEs), encompassing both phase I DMEs and phase II DMEs. Anomalies in the expression of DMEs manifest in various cancer stages, potentially influencing cancer development and introducing variability in individual drug responses through their impact on the metabolic processes of carcinogens and anticancer medications (Wang et al., 2020).

Certain enzymes called kinases have been known to be specifically targeted. Kinases are a type of enzyme that catalyze the transfer of phosphate groups from ATP to specific target molecules, often proteins, in a process known as phosphorylation (Ma et al., 2021). This process is a key mechanism in various cellular signaling pathways, regulating essential cellular processes such as cell growth and survival. In the context of cancer, activation, and deregulation of kinases are commonly observed. Cancer cells may exploit kinase signaling pathways to promote their uncontrolled growth and survival. Additionally, the overexpression or mutation of kinases can contribute to resistance against anticancer drugs (Manning et al, 2002).

CML:

Chronic myeloid leukemia (CML) is a type of cancer that originates in the blood-forming cells of bone marrow. The once relentless tide of this disease has been significantly stemmed by the advent of tyrosine kinase inhibitors (TKIs), which specifically target the aberrant signaling of the BCR-ABL tyrosine kinase (Deininger et al., 2000). These drugs bind competitively to the kinase domain, a highly conserved region within the BCR-ABL protein, effectively disrupting its oncogenic activity and halting uncontrolled cell proliferation (Druker et al., 2011).

BCR-ABL:

One specific enzyme that contributes to drug resistance in anticancer drugs is BCR-ABL, which is a cancer kinase targeted in the treatment of chronic myeloid leukemia (CML) a type of cancer mentioned above (Redaelli et all.) Mutations in the kinase domain (KD) of the BCR/ABL (Breakpoint Cluster Region protein/Abelson tyrosine-protein kinase 1) are commonly seen as a primary cause of resistance to tyrosine kinase inhibitors (TKI) in individuals with chronic myeloid leukemia (CML). These mutations have the potential to hinder TKI effectiveness by directly or indirectly affecting the drug's ability to bind to the protein (Gorre et al., 2007).

Structural Biology:

One way to possibly combat the effects of drug resistance is to utilize the field of structural biology. Structural biology is a scientific field that focuses on the study of the three-dimensional structures of biological molecules, such as proteins, nucleic acids, etc. The primary goal of structural biology is to understand the relationship between the structure and function

of these biomolecules. To help combat the effects of drug resistance in anti-cancer cells, one possible solution would be to utilize PyMOL software. PyMOL facilitates the visualization and analysis of protein structures, enabling researchers to explore how specific mutations affect the BCR-ABL kinase domain's conformation and TKI binding interactions (Laskowski et al., 2011).

Section II: Specific Aims

The long-term goal of this project is to use a form of imagery software to gain a developed understanding of why the activation of a certain cancer kinase leads to drug resistance taking place through structural biology. The rationale is that by using a form of 3D imaging software to understand the relationship between the structure and function of these biomolecules, further research would become a more feasible idea that would lead to possible future work to take place. What I propose here will help reduce the effect that drug resistance has on cancer cells and keep people from having to suffer through the disease. The expected result from this project will be that imagery software, most likely PyMOL, will be used to gain a further understanding of how this specific cancer kinase, BCR-ABL, contributes to drug resistance in CML.

Specific Aim 1: Identify and characterize BCR-ABL kinase mutations associated with drug resistance in CML.

Specific Aim 2: Conduct structural analyses to understand the impact of these mutations on the kinase's function.

Specific Aim 3: Investigate the correlation between structural changes and functional alterations leading to drug resistance.

The expected outcome of this work is that I will answer the question of why drug resistance in the cancer kinase enzyme BCR-ABL is affected by certain mutations in and kinase domain and in which mutations in by using imagery software and structural biology. This could lead to possible future research regarding the link between the structure and function of the molecule.

Section III: Project Goals and Methodology

Relevance/Significance: Chronic Myeloid Leukemia (CML), accounting for approximately 10% of cancer cases in the United States, warrants dedicated exploration and intervention. By delving into the intricate molecular landscape and focusing on crucial aspects like the enzymatic function of BCR-ABL, I'm aiming to illuminate the underlying contributors to drug resistance. This deeper understanding holds the potential to inform the development of more nuanced and effective treatment strategies for CML. Moreover, the project introduces an innovative approach by leveraging PyMOL imaging to meticulously craft targeted inhibitors. This novel technique not only offers a promising avenue for overcoming resistance specifically in CML but also holds broader implications for diverse cancer treatment approaches.

Methodology: The methodology beings with comparing wild-type BCR-ABL and its drug-resistant counterparts to identify key mutations. These mutations become markers on a map, helping to construct a graph that shows the strength of the drug concentration through IC50 values, which represents the drug concentration needed to hold back half the protein's function. By using Pymol I'm able to measure the distances between these mutations and the drug imatinib, within the kinase's crucial battleground. Through my research, I hope to understand the reasons behind drug resistance in this kinase so that future possibilities regarding new therapeutic strategies come to light.

IC50-fold increase (WT = 1)

		Imatinib	Bosutinib	Dasatinib	Nilotinib	Ponatinib	DCC-2036
	Parental	10.8	38.3	568.3	38.4	570.0	13.1
	WT	1	1	1	1	1	1
P-loop	M244V	0.9	0.9	2.0	1.2	3.2	1
	L248R	14.6	22.9	12.5	35.2	8.2	0.9
	L248V	3.5	3.5	5.1	2.8	3.4	1.0
	G250E	6.9	4.3	4.4	4.6	6.0	3.0
	Q252H	1.4	0.8	3.1	2.8	6.1	2.1
	Y253F	3.6	1.0	1.8	3.2	3.7	2.3
	Y253H	8.7	0.6	2.6	35.8	2.6	2.7
	E255K	6.0	9.5	5.8	6.7	8.4	3.5
	E255V	17.0	5.5	3.4	10.3	12.8	2.1
C-helix	D276G	2.2	0.6	1.4	2.0	2.1	4.5
	E279K	3.6	1.0	1.6	2.0	3.0	6.5
	E292L	0.7	1.1	1.3	1.8	2.0	1.9
ATP binding region	V299L	1.5	25.1	8.7	1.3	0.6	0.7
	T315A	1.7	6.0	58.9	2.7	0.6	0.4
	T315I	17.5	40.4	75.0	37.5	3.0	0.7
	T315V	12.2	29.3	738.8	57.0	2.1	0.8
	F317L	2.6	2.4	4.5	2.2	2.0	1.3
	F317R	2.3	20.5	114.8	2.3	4.9	21.0
	F317V	0.4	11.5	21.3	0.5	2.3	6.6
SH2-contact	M343T	1.2	1.1	0.9	0.8	0.8	1.0
	M351T	1.8	0.7	0.9	0.4	1.2	2.2
Substrate binding region	F359I	6.0	2.9	3.0	16.3	2.9	0.7
	F359V	2.9	0.9	1.3	5.2	4.4	0.6
A-loop	L384M	1.8	0.5	2.2	2.3	2.2	0.7
	H396P	2.4	0.4	1.1	2.4	1.6	1.1
	H396R	3.9	0.8	1.6	3.1	5.9	0.7
C-terminal lobe	F486S	8.1	2.3	3.0	1.9	2.1	0.6
	L248R + F359I	11.7	98.3	13.7	96.2	17.7	1.1
Sensitive	≤ 2						
Moderately resistant	2.1–4						
Resistant	4.1–10						
Highly resistant	>10						

Table I. Activity of Imatinib, Bosutinib, Dasatinib, Nilotinib, Ponatinib, and DCC-2036 Against Mutated Form of BCR/ABL. The relative IC50 increase over wild type BCR/ABL (RR) was calculated. We classified RR values in four categories: sensitive (RR ≤ 2), moderately resistant (RR between 2 and 4), resistant (RR between 4 and 10), or highly resistant (RR>10) (Redaelli et, all).

Specific Aim #1: **Identify and characterize BCR-ABL kinase mutations associated with drug resistance in CML.**

Justification and Feasibility. CML patients on TKIs develop resistance over time, significantly impacting treatment success. Identifying specific mutations responsible for resistance is crucial for understanding its mechanisms and informing targeted therapies. Existing also databases provide a wealth of BCR-ABL sequence data from resistant patients, offering a rich resource for identifying relevant mutations.

Specific Aim #2: Conduct structural analyses to understand the impact of these mutations on the kinase's function.

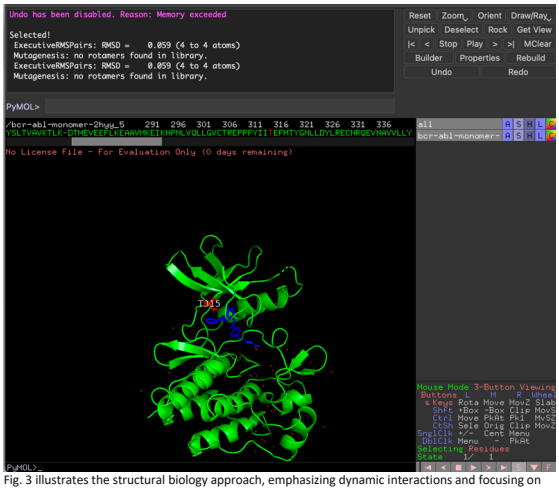

Fig. 3 illustrates the structural biology approach, emphasizing dynamic interactions and focusing on mutations impacting Thr315. In the 3D structural model, it displays the BCR-ABL kinase structure in green, the drug imatinib in blue, and the amino acid residue T315 where the mutations occur. In red.

Justification and Feasibility: BCR-ABL's kinase activity is critical for CML progression, and mutations can alter its structure and function. Understanding these changes is key to unraveling resistance mechanisms. PyMOL offers a powerful platform for visualizing and analyzing protein structures, allowing precise measurement of mutation-induced alterations in the BCR-ABL binding pocket. Investigating the spatial relationship between mutations and the drug-binding site can elucidate resistance mechanisms based on steric hindrance or altered drug interactions.

Specific Aim #3 Investigate the correlation between structural changes and functional alterations leading to drug resistance.

Justification and Feasibility: Linking structural changes with functional consequences reveals how mutations impact BCR-ABL activity and contribute to resistance. This insight can inform the development of targeted therapies that overcome resistance mechanisms based on specific mutations. Understanding the functional implications of structural changes could also lead to the identification of novel drug targets within the BCR-ABL kinase domain.

Section III: Resources and Equipment

To unravel the mechanisms of BCR-ABL mutation-induced drug resistance in CML, we will implement a robust methodological framework. I will utilize the **Protein database.org**, a comprehensive repository of protein sequences, to access and analyze wild-type and mutant BCR-ABL sequences from treatment-resistant patients. Employing advanced bioinformatics tools integrated within **Excel**, we will identify and spatially map key mutations within the kinase domain. Subsequently, we will leverage the powerful molecular visualization capabilities of **PyMOL** to precisely measure the distances between these mutations and the drug imatinib within the binding pocket. By using values calculated from Redaelli 2016, we will develop and implement a customized **IC50 calculation method** to quantify the drug concentration required to inhibit 50% of the kinase activity for each mutant, providing a quantitative measure of resistance.

Section V: Ethical Considerations

This project does not raise any ethical concerns as it uses publicly available data and established analytical techniques.

Section VI: Timeline

During the first weeks, my first task is hand to familiarize myself with the software program being used, the software to be used will most likely be PyMOL and I will make sure to understand how the software works and how it can be applied to my project. The next task is for me to Identify and characterize BCR-ABL kinase mutations associated with drug resistance in CM. Afterward, I would utilize the imagery software, to conduct structural analyses to understand the impact of these mutations on the kinase's function. I would consider the structural elements and interactions that take place to prevent kinase activation. Next, I would Investigate the correlation between structural changes and functional alterations leading to drug resistance. I would then present my findings at the February STEM fair.

Section VII: References

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