Determining the Role and Implications of Aurora A Kinase Inhibition In Acute Myeloid Leukemia

A Major Qualifying Project Report

Submitted to the Faculty of
WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the
Degree of Bachelor of Science
by

Eva Childers

Approved by:
Professor Amity L. Manning
Worcester Polytechnic Institute
Biology and Biotechnology

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Abstract

Acute Myeloid Leukemia (AML) is a cancer of overproliferative blood cells and is the most common type of blood cancer to occur in adults. AML patients are commonly treated with chemotherapeutic approaches. Although chemotherapy drugs are effective at limiting growth of leukemia cells, the pathways that they target are common in all proliferating cells and as a result, healthy proliferative tissue is also damaged. Currently, the identification and development of therapies that specifically or preferentially impact growth of leukemia cells is in high demand. Alisertib (MLN8237), an inhibitor of the mitotic kinase Aurora A is one potential drug that is currently being investigated clinically as a co-therapeutic for AML. AML cells have been reported to exhibit high levels of Aurora A and so are hypothesized to be exquisitely sensitive to inhibition of this mitotic regulator. However, the cell biological impact of Aurora A inhibition in AML cells has not been investigated, and it is unclear if Aurora A expression levels alone may be a good predictor of sensitivity to Alisertib.

Our studies have focused on exploring the molecular effects of Aurora A inhibition, and investigating cellular biomarkers, in addition to Aurora A levels, that may predict drug sensitivity in AML patients. Our initial correlative studies in a panel of AML cell lines, does not show a clear correlation between Aurora A levels and Alisertib sensitivity. Instead, our results suggest that an increased centriole number confers resistance of AML cells to Alisertib. We will continue to define centriole number as a potential biomarker through ongoing approaches that will test both the contribution of Aurora A levels and centriole number, both in isolation and in combination, on Alisertib sensitivity.

Acknowledgments

I would like to thank Dr. Amity Manning for providing us the opportunity to work on this project, and for all of the advice and support she's provided us throughout the project. In addition, I would like to thank Nicole Hermance, and Conor Herlihy for their continued guidance during this project.

1. Introduction

Acute Myeloid Leukemia (AML) is a type of blood cancer that occurs when the bone marrow makes abnormal myoblasts, platelets or red blood cells. These abnormal cells continue to develop and accumulate within the blood vessels which can result in anemia or easy bleeding. According to the American Cancer Society, AML is the most common type of blood cancer to occur in adults, and is generally seen in adults above the age of. The percent of individuals that will survive five years after AML diagnosis is approximately 27%. Most of the individuals will initially experience flu-like symptoms, including fever, sweats or body aches. According to the Seattle Cancer Care Alliance, in order to diagnose AML, several tests are needed, including a bone marrow biopsy, complete blood cell counts or tests for genetic abnormalities. Currently, chemotherapy is the most common treatment given to AML patients. This treatment is generally given in two steps, and the purpose of the treatment is to use chemotherapy drugs to eradicate all of the leukemic cells. However, chemotherapeutic approaches do not specifically target cancer cells but instead target all proliferating cells and cause side effects that impact the health of the patient as stated by the American Cancer Society. Targeted therapies are now being pursued, that may exclusively, or preferentially, target cancer cells through the use of an Aurora A kinase inhibitor called Alisertib.

Aurora A kinase is part of a family of serine/threonine kinases that are vital in cell cycle regulation and mitosis (Fu et al., 2007). Aurora A kinase's function is to control mitotic entry, and recruit components for centrosome maturation. Aurora A plays a critical role in the formation of a bipolar mitotic spindle, which is crucial for the proper separation of the sister chromatids to each of the daughter cells (Dutertre et al., 2002). When Aurora A kinase is overexpressed, centrosome amplification, cytokinesis inhibition and aneuploidy can result. Aurora A kinase has been found to be overexpressed in a variety of cancers, including breast, colon, and AML. Its role within the cell, and together with its overexpression profile in a range of cancers suggest that Aurora A is a promising drug target.

Previous studies have shown that the inhibition of Aurora A kinase function through depletion or inhibition results in mitotic spindle assembly defects (Manfredi, et al., 2011). Loss of Aurora A function disrupts mitotic spindle formation and results in spindles with one or multiple spindle poles. The Aurora A kinase inhibitor that this paper focuses on is Alisertib (or MLN8237). Alisertib has been shown to disrupt the growth of Acute Myeloid Leukemia (AML) cells, and a

common phenotype of Alisertib-treated AML cells is a monopolar spindle pole (Moore, A.S, et al, 2010). However, not all AML cells exhibit the same degree of Aurora A amplification, or spindle morphology defects and it remains unclear in which contexts Aurora A inhibition may have the greatest therapeutic value. In this Major Qualifying Project we aim to define the cellular implications of Aurora A kinase inhibition with Alisertib, and to investigate cellular biomarkers that predict drug response. In addition biomarkers that could suggest Alisertib would be more or less effective are considered. Lastly, this project explores the possibility that centriole number, independent of Aurora A level indicates the responsiveness of an AML cell to Alisertib.

2. Background

2.1 Acute Myeloid Leukemia (AML)

The National Cancer Institute defines hematopoietic stem cells, also known as blood stem cells, as immature cells that have the ability to self-renew and to differentiate into any type of blood cell. These cells are generally identified in the bone marrow, the soft, sponge-like tissue in the center of bones. Hematopoietic stem cells first differentiate into blood cells of two different lineages, the lymphoid and myeloid, which give rise to many other types of cells. The lymphoid stem cells further differentiate into T cells, B cells, and natural killer (NK) cells. The myeloid lineage gives rise to megakaryocytes, erythrocytes (MegE), as well as granulocytes (Iwaski and Akashi, 2007). Mature blood cells have short life-spans, and new blood cells are derived from hematopoietic blood cells (Robb, 2007).

According to the Johns Hopkins Comprehensive Cancer Center, blood cancers usually develop in the bone marrow, and it affects both the production and function of blood cells. Each type of blood cancer is due to defects in specific cell lineages. The hematopoietic stem cells replace the normal blood cells as they age and die. However, when an individual has blood cancer, this process is corrupted. Hematopoietic stem cells may not grow or differentiate normally, or the immune system will attack normal tissue. According to the American Society of Hematology, in all three different types of blood cancers - lymphoma, myeloma, and leukemia - the development of blood cells is hindered by the uncontrolled growth of a progenitor.

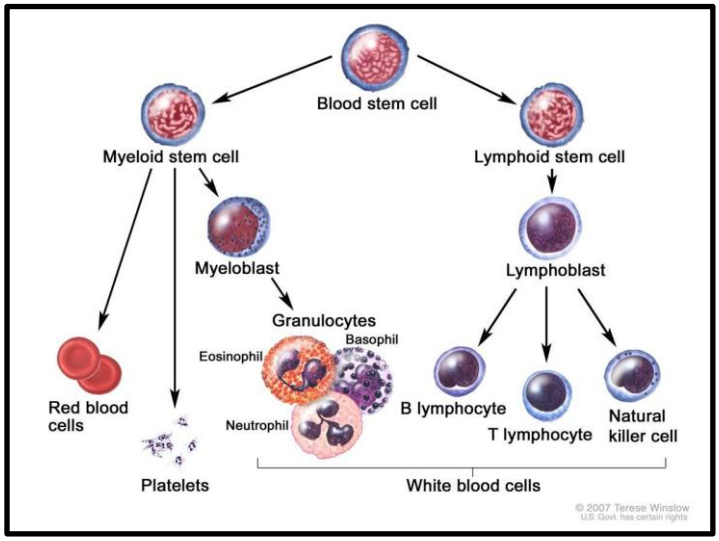

Figure 1. Differentiation of Hematopoietic Stem Cells

Blood stem cells can differentiate into two lineages of blood cells: Myeloid and Lymphoid. Each lineage is responsible for the production of different blood cell types

Retrieved from: Winslow, Terese. National Cancer Institute. 2007

The Mayo Clinic defines acute myeloid leukemia (AML), or acute myelogenous leukemia, as a type of blood cancer that occurs in bone marrow. According to the National Cancer Institute,

AML progresses rapidly and only affects the myeloid lineage of cells, therefore affecting the development of red blood cells, platelets and myeloblasts. These abnormal cells accumulate in the blood vessels and take the space of healthy blood cells. This might lead to anemia and easy bleeding.

2.1.1 Prevalence, Risk Factors and Symptoms

According to the World Health Organization, the incidence of AML worldwide in 2012 was 351,965 people. AML commonly occurs at older ages, with an average of 67 years old, and the lifetime risk for its occurrence is between 0.5 and 1%. According to the National Cancer Institute, the average 5-year survival rate for people with AML is 27%.

During the early stages of AML, patients will experience symptoms similar to the flu including fevers, sweats and body aches. The symptoms can vary based on the deficiency of various blood cell types. For example, patients will low white blood cells will suffer from bacterial or viral infections, and have occurrences of mouth inflammation or sores. To determine if the patient has AML, several tests have to be completed to accurately diagnose the patient. According to the Seattle Cancer Care Alliance, these can include bone marrow biopsies, complete blood counts, and a polymerase chain reaction to test for the presence of a certain chromosomal translocation. Other tests can be used to detect genetic abnormalities, like examining the FLT3 gene for example. The FLT3 gene, when abnormal, has been correlated with poor prognosis in AML patients. In addition to the FLT3 gene, Aurora A kinase has been observed to be overexpressed in AML compared with normal hematopoietic stem cells (Kim et al., 2012).

To date, a high risk factor associated with the development of AML is smoking. Substances present in tobacco do not only affect the cells that are in direct contact with them, such as the lung cells, but it also can affect the cells in the bloodstream, since the smoke diffuses from the lungs to the blood vessels (Lichtman, 2007). There are other risk factors that could increase the probability of AML development, including prolonged exposure to specific chemotherapy drugs such as alkylating agents, platinum agents, and topoisomerases II inhibitors. These can be used as treatments for other cancers. In addition, having blood diseases (i.e polycythemia vera, and idiopathic myelofibrosis) and genetic syndromes (i.e. down syndrome and fanconi anemia) have been linked to an increased risk of developing AML, especially when chemotherapy drugs have been used, as reported in the American Cancer Society. Although a few of the patients present

some of these risks factors, their presence alone is not sufficient to cause cancer. AML commonly develops after an accumulation of mutations in DNA over time, which can happen because of unknown reasons. While translocations are the most common chromosomal changes found in AML, many others can also occur, such as deletions, inversion, insertions or duplications.

2.1.2 Current Treatments

Currently, the most common type of treatment for AML is chemotherapy, and this can be followed by a stem cell transplant. The chemotherapy is conducted in two steps: induction and consolidation. Induction is the first phase, and aims to eliminate leukemic cells from the blood, get rid of all signs of disease for an extended time (also known as remission) and to increase the healthy blood count to within a normal range. Generally, doctors will use two or more chemotherapy drugs to treat AML, as each individual drug utilizes different methods to destroy the cancer cells. Therefore, combining drugs can strengthen the treatment's effectiveness. The second step, consolidation, is conducted after the patient has recovered from induction. The second phase is targeted at killing the small population of leukemia cells that may remain after induction. According to the Leukemia & Lymphoma Society, without consolidation, or "postremission therapy", the AML has a higher probability of returning.

Common chemotherapy drugs include cytarabine or anthracycline drugs. Cytarabine, once phosphorylated and is incorporated into DNA, will block DNA elongation by inhibiting DNA polymerase, and results in a decrease of DNA replication and repair (Fitzakerley, 2015). Anthracycline drugs primarily act through intercalation. Intercalation inserts an aromatic ring between DNA base pairs, compromises replication, and results in cytotoxicity (Barton et al, 1991). However, both of these chemotherapies can also harm other types of proliferative cells. According to the American Cancer Society, chemotherapy drugs can result in side effects including nausea, hair loss, mouth sores, fatigue, increased bruising and risk of infections.

Although the patient survival has increased over the years, the current treatments are not effective enough in patients older than 60 in which AML is more prevalent. Because of this, there is a need for the discovery of new targets for the development of new effective therapeutics.

2.2 Acute Myeloid Leukemia and Aurora A Kinase

In normal human cells, the cell cycle consists of four phases: S phase (chromosomal duplication), M phase (chromosomal separation) and two Gap phases (G1 and G2) that separate both S and M phases (see Figure 2)(van den Heuvel, 2005). The M phase consists of four stages: prophase, metaphase, anaphase, and telophase (O'Connor, 2008). During prophase, the chromosomes begin to condense, the nuclear envelope starts to break down, and the mitotic spindle begins to form. Following prophase, the cell proceeds to pro-metaphase where chromosomes attach to the spindle microtubules and begin to congress towards the center of the cell. Once all chromosomes have fully attached to a bipolar spindle and aligned at the spindle center, the cell is said to be in metaphase. The microtubules nucleated at the centrosomes attach to protein structures known as kinetochores. Attached chromosomes orient so that each replicated chromosome is associated with microtubules nucleated from a single centrosome/spindle pole. This attachment and alignment satisfies the spindle assembly checkpoint and enables the cell to enter anaphase. During this phase, cohesion between replicated chromosomes is lost and the sister chromatids are pulled apart and towards different cell poles. Following anaphase, nuclear envelopes reform around decondensing chromatin and cyotokinesis cleaves the dividing cell into two genetically identical daughters (O'Connor, 2008).

Centrosomes are organelles that organize microtubules and are involved in the process of cytokinesis (O'Connor & Adams, 2010). The centrosomes are composed of three parts: two centrioles (a mother and a daughter centriole), a matrix that connects the two centrioles, and pericentriolar material. As seen in Figure 2, the centrosomes are duplicated during S phase. The cell before mitosis contains four centrioles that are organized in two centrosomes. As the cell moves into mitosis, the centrosomes move apart towards opposite sides of the cell, where they nucleate and organize the microtubules of the mitotic spindle. After cytokinesis, each of the daughter cells contains a single centrosome with two centrioles.

2.2.1 Aurora Kinases Function and Regulation

Aurora kinases are a family of serine/threonine kinases that have been implicated in cell cycle control, and are vital during mitosis (Fu et al., 2007). These kinases are enzymes that control the functions of many substrates by phosphorylation. There are three members of the Aurora kinase family: Aurora A, Aurora B, and Aurora C (Fu et al., 2007). The activity of Aurora kinases is closely regulated, as disruption or deregulation of these kinases can lead to genetic instability due to defects in centrosome function, spindle assembly and chromosomal alignment. Aurora A plays a role in mitotic spindle assembly through facilitating centrosome maturation by recruiting various components such as y-tubulin (Fu et al., 2007). Aurora B regulates chromatid protein modification and separation and also known to play a role in cytokinesis. For example, when Aurora B kinase is depleted the cell will become polyploid (Fu et al., 2007). According to the National Center for Biotechnology Information, Aurora C has been known to play a role in microtubule organization in centrosome and spindle function during mitosis by forming complexes with Aurora B and centromere proteins. In many human cancers, the expression and activity of the Aurora kinases is increased, suggesting that they may play a role in tumorigenesis, and has become the focus of many anti-cancer drugs and treatments (Fu et al., 2007).

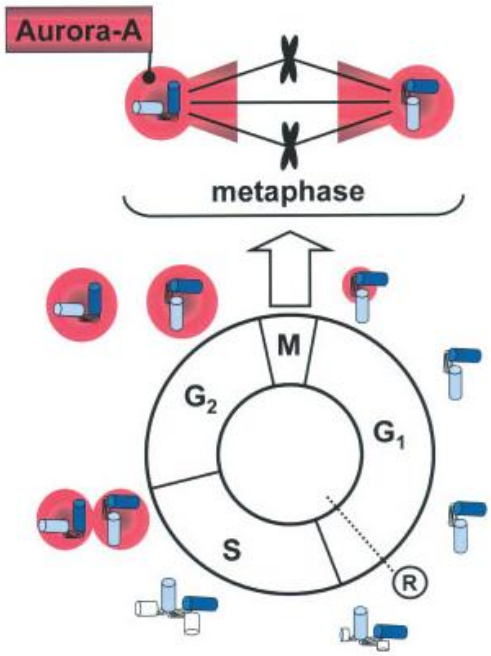

Figure 2. Aurora A Kinase and Centrosome Separation

Centrosomes are duplicated during S phase, move apart upon mitotic entry where they become microtubule nucleating centers that make up functional spindle poles

Retrieved from: Dutertre, Stephanie, Simon Descamps, and Claude Prigent. "On the Role of Aurora-A in Centrosome Function."*Nature.com*. Macmillan Publishers, 9 Sept. 2002. Web. 01 Feb. 2017.

The cell cycle is primarily regulated by cyclin-dependent kinases, also known as CDKs. CDKs are further regulated by phosphorylation, degradation of proteins that inhibit cyclin, and degradation of cyclins, among others (van den Heuval, 2005). The mitotic events of the cell cycle have other regulatory molecules besides CDKs, such as polo-like kinases, and aurora kinases. Aurora A kinase, for example, functions to control mitotic entry, which happens after the activation of CDK1 (Figure 3). This control is done indirectly, by interactions with Polo-like kinase 1 (Plk-1), a cell cycle kinase that regulates processes such as centrosome maturation,

spindle assembly and chromatin cohesion (Bruinsma et al., 2014). Plk-1 is located at the centrosomes and mitotic spindle. Plk-1 is switched on by phosphorylation at residue T210 during G2 phase, reaching its maximum activity during mitosis, after it is phosphorylated at residue T210 (Bruinsma et al., 2014). The phosphorylation of this residue is done by the Aurora A-Bora complex. The phosphorylation of residue T210 causes a change in the conformation of Plk-1 that provides Aurora A enhanced access to residue T210 (Fu, Jiang & Zhang, 2010). Inhibition of Aurora A has been shown to prevent the activation of Plk-1 and lead to the formation of monopolar spindles.

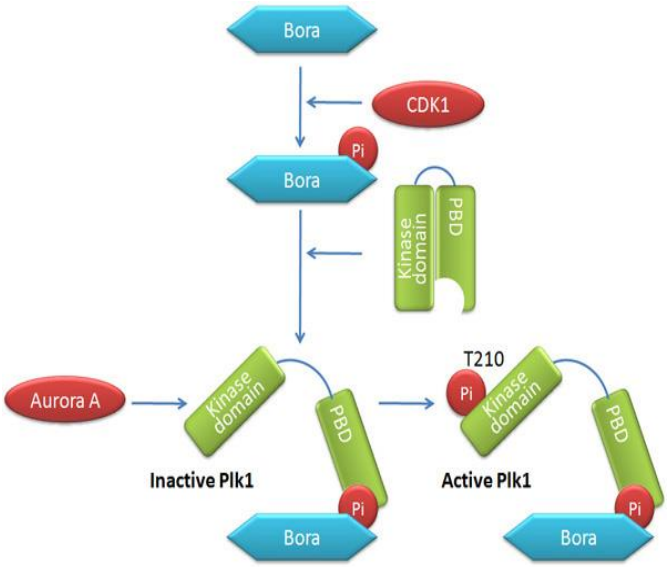

Figure 3. Role of CDK, Aurora A Kinase and Polo-Kinase 1

CDK1 activates the Aurora Bora complex, which leads to the phosphorylation and activation of PLK1. This results in the phosphorylation of residue T210

Retrieved from: Fu, J., Jiang, Q. & Zhang, C. (2010) Collaboration of Mitotic Kinases in Cell Cycle Control. *Nature Education* 3(9):82

During S phase, Aurora A localizes on duplicated centrosomes, and remains there until the beginning of G1 during the following cell cycle. While located on the centrosomes, Aurora A has three functions. First, as shown by experiments performed in *Xenopus* and in *Drosophila,* Aurora A contributes to centrosome separation. In these two animals, inactivation of Aurora A results in the formation of monopolar spindles, with the centrosomes failing to separate (Duterte et al., 2002). The mechanism by which Aurora A contributes to centrosome separation is through phosphorylation Eg5, a protein required for centrosome separation to occur. A second function of Aurora A at the centrosomes is to ensure that centrosome mature after they separate before mitosis. This maturation includes the recruitment of proteins, such as y-tubulin and centrosomin, to the centrosome to take part in its structure as well as to preserve its functionality during and after mitosis (Duterte et al., 2002).

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Lastly, Aurora A recruits and phosphorylates TACC3 at the centrosome. TACC3 is a protein that promotes microtubule stabilization (Lioutas & Vernos, 2013). This interaction contributes to the organization and stabilization of microtubules (Fu et al., 2007).

Both the localization and activity of Aurora A is carefully regulated to ensure that it correctly functions within the cells. In normal cells, Aurora A is down-regulated through APC/C-Cdh1 dependent, proteasome-mediated proteolysis. The degradation of Aurora A by APC/C-Cdh1 requires a destruction box in the C-terminal region and a motif in the N-terminus (D'Assoro et al., 2016). Aurora A is a major contributor to the proper segregation of the daughter cells during mitosis and Deregulation of its localization and activity can lead to abnormal spindle morphology and promote tumorigenesis (Duterte et al., 2002).

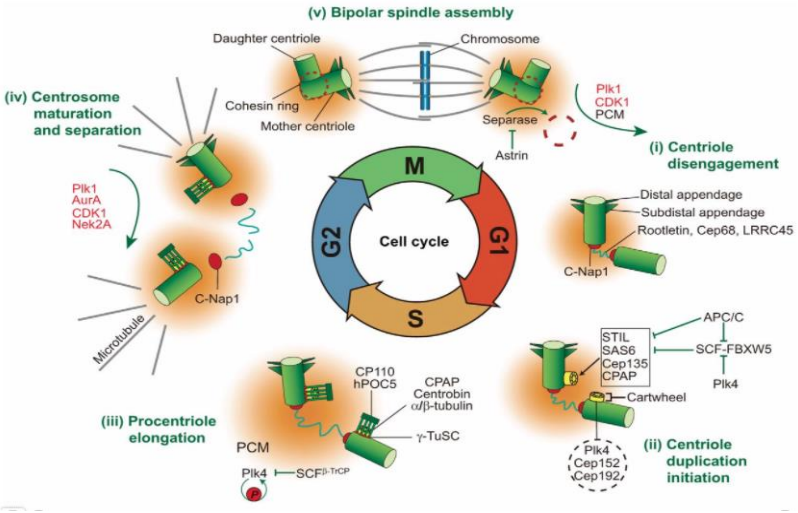

Figure 4. Diagram of Centrosome Duplication and Aurora A localization

Centrosomes duplicate during S phase, and move apart during G2/M phase. Aurora A contributes to centrosome separation during G2 phase.

Retrieved from: Wang, Gang, Qing Jiang, and Chuanmao Zhang. "The Role of Mitotic Kinases in Coupling the Centrosome Cycle with the Assembly of the Mitotic Spindle." Journal of cell science, vol. 127, no. Pt 19, 2014, pp. 4111-4122doi:10.1242/jcs.151753

2.2.2. Aurora A Kinase Overexpression and Effects in the Cell Cycle

The Aurora A gene is located in the 20q13 chromosome region, and is amplified in many cancers including breast, colon and ovarian cancers, leading to the overexpression of Aurora A kinase (Duterte et al., 2002). In a large majority of these cancers, the localization of Aurora A is diffused, being present in other parts of the cell such as the cytoplasm, and not being concentrated in the nucleus (Duterte et al., 2002).

Centrioles are duplicated during S phase concurrent DNA replication. The cell, therefore, contains four centrioles that are organized into two centrosomes throughout G2 and mitosis. Aurora A kinase activity is not observed until the G2 phase, which implies that the kinase activity of Aurora A is not needed in order to duplicate the centrioles (Duterte et al., 2002). While the activity of Aurora A is not required for centriole amplification, the overexpression of Aurora A has been shown to be sufficient for overamplification of centrioles (Meraldi et al., 2002). Recent

studies show that cells overexpressing Aurora A and containing increased number of centrioles also have multiple nuclei, suggesting that these cells experience abnormal mitosis. Overexpression of Aurora A causes cells to form aberrant mitotic structures, and defective anaphases including the presence of anaphase bridges and cytoplasmic connections (Meraldi et al., 2002). These aberrant anaphase defects cause the cells to fail cytokinesis, leading to the formation of tetraploid cells. Some cells that overexpress Aurora A kinase also overexpress Plk1 and Aurora B kinase, which have also been shown to cause an amplification of centrosomes (Meraldi et al., 2002). This amplification of centrosomes is also correlated with loss or mutation of p53, a tumor suppressor that plays a role during DNA damage response (Figure 5) (Goodsell, 2002). Cells that do not have a functional p53 protein have been shown to have a higher number of centrosomes when Aurora A, Aurora B or Plk1 are overexpressed. This could explain why the overexpression of Aurora A leads to the formation of multiple centrosomes, as p53 loss would abrogate the checkpoint for aberrant mitotic cells, and permit their continued cycling (Meraldi et al., 2002).

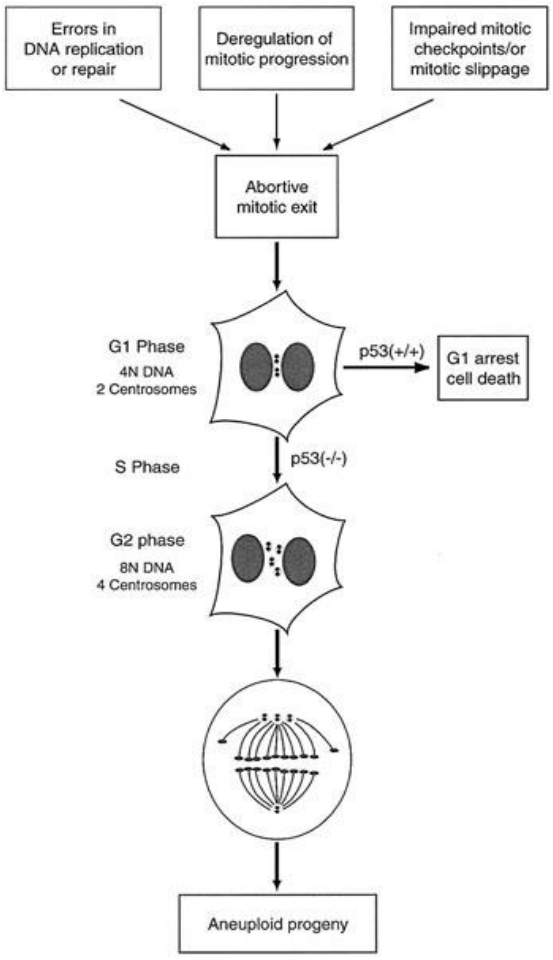

Figure 5. Diagram of Mitotic Errors

Errors in DNA replication, mitotic progression, or impaired mitotic checkpoints can result in an abortive mitotic exit. Cells lacking p53 can bypass G1 arrest cell death, and result in centrosome amplification

Retrieved from: Meraldi, Patrick, Reiko Honda, and Erich A.Nigg. "Aurora-A Overexpression Reveals Tetraploidization as a Major Route to Centrosome Amplification in P53−/− Cells." The EMBO Journal. EMBO Press, 15 Feb. 2002. Web. 28 Feb. 2017

2.2.3 Aurora A Kinase Inhibition and Implications

Currently, due to the role of Aurora A within the cell and its overexpression in many cancers, the effect of inhibition of Aurora A is being investigated. It has been observed that Aurora A inhibition can lead to mitotic spindle assembly defects, such as monopolar spindle poles which activate the spindle assembly checkpoint and induce mitotic arrest (Bavetsias & Linardopoulos, 2015). Following a prolonged mitotic arrest, some cells undergo mitotic catastrophe. Other cells that ultimately exit mitosis and enter G1 will senesce or apoptose. However, not all cancer cells respond similarly to Aurora A inhibition and it remains unclear what features of a cancer cell may promote the preferred mitotic catastrophe or apoptosis, over a G1 arrest. There are multiple Aurora A inhibitors in clinical trials, including AT9283, PF-03814735, and Alisertib (or MLN8237).

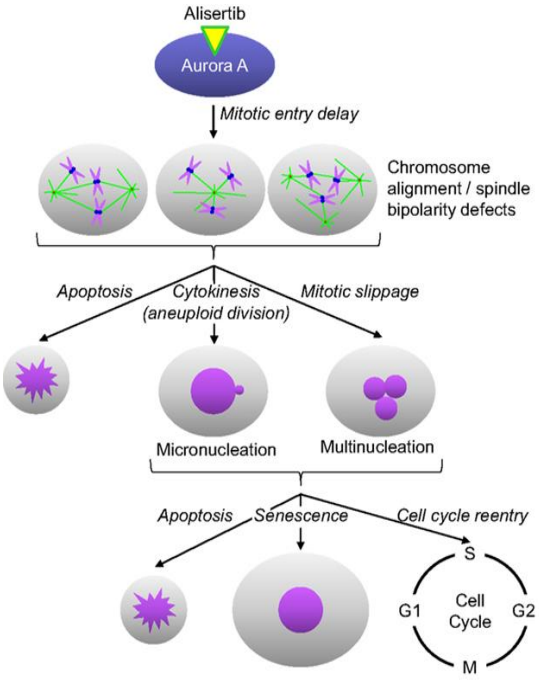

Figure 6. Alisertib Mechanism in vitro

Alisertib inhibits Aurora A kinase, resulting in mitotic entry delay. This leads to spindle bipolarity defects, which can lead to apoptosis or cell cycle reentry

Retrieved from: Niu, Huifeng, Mark Manfredi, and Jeffrey A. Ecsedy. "Scientific Rationale Supporting the Clinical Development Strategy for the Investigational Aurora A Kinase Inhibitor Alisertib in Cancer." Frontiers in oncology, vol. 5, 2015, pp. 189, doi:10.3389/fonc.2015.00189

First, AT9283 is a heterocyclic molecule that inhibits aurora kinases, including both Aurora kinase A and B. This inhibitor is used as a therapy for many solid tumors and leukemic cancers, as it shows a reduction in the proliferative profile of leukemic cancers, as well as an induction of aneuploidy and apoptosis (Qi et al., 2012). Second, PF-03814735 is a reversible inhibitor of both Aurora kinase A and B, and to a lesser extent FLT1, FAK, and TrkA which have been implicated in tumorigenesis in cancers such as leukemia and breast cancer. Small cell lung cancer and colon cancer are the most sensitive to PF-03814735, which works by blocking cytokinesis and, therefore, preventing cell proliferation and creating cells that are multinucleated (Jani et al., 2010). Lastly, MLN8237, also known as Alisertib, is the first oral and selective inhibitor of Aurora A kinase. It shows more than a 200-fold increased specificity for Aurora A

kinase than Aurora B kinase. Alisertib prevents the proliferation of cells, increases the number of cells in G2/M phase, and causes significant apoptosis and senescence (Figure 6) (Qi et al., 2013).

Previous work has shown that Alisertib, at clinically achievable concentrations, impairs the growth and survival of AML cell significantly more than normal Peripheral Blood Mononuclear Cells (PBMC) cells (Figure 7). Alisertib was also shown to increase the percentage of AML cells that experienced an induction of apoptosis (Kelly et al., 2012).

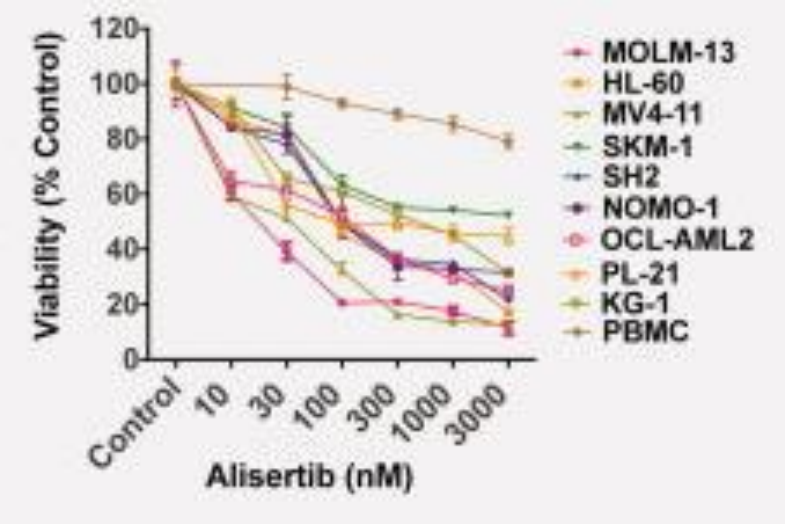

Figure 7. AML Cell Viability at Concentrations of Alisertib

All AML cell lines had significantly larger decreases of cell viability as the concentration of Alisertib increased compared with the control cell line PBMC.

Retrieved from: Kelly, Kevin R. et al. "Targeting Aurora A Kinase Activity with the Investigational Agent Alisertib Increases the Efficacy of Cytarabine through a FOXO-Dependent Mechanism." *International journal of cancer. Journal international du cancer* 131.11 (2012): 2693–2703. *PMC*. Web. 18 Jan. 2017.

Recently, a phase I trial conducted at Massachusetts General Hospital evaluated the safety and tolerability of Alisertib when combined with chemotherapy for patients diagnosed with AML. The treatment during this clinical trial involved infusions of cytarabine for 7 days, and another chemotherapy drug, idarubicin, for 3 days. After the cytarabine infusions on day 7, patients were administered oral doses of Alisertib for 7 days. Overall, the researchers observed that Alisertib was well tolerated. The results showed that overall 86% of the patients that participated in the study achieved complete remission. Within the patient group, 7 out of 8 patients that were over the age of 65 achieved a complete remission. In addition all patients that were diagnosed with high-risk AML achieved complete remission (Fathi et al., 2016).

Despite the success of the clinical trial, in vitro studies have demonstrated that the degree of sensitivity of AML cell lines varied, suggesting that the unique background of individual cell types may be a contributing factor in the cellular response to Alisertib (Kelly et al., 2012). Interestingly, previous research has failed to show a direct relationship between Aurora A expression levels and sensitivity to Alisertib. The reason for this remains unknown. The purpose

of this project is to explore biomarkers that indicate drug efficacy and explore the cellular implications of inhibiting Aurora A kinase by Alisertib.

3. Materials and Methods

3.1 Subculture of Human Tissue Culture

Table 1. AML and Control Cell Line Derivation

(Obtained from ATCC cell lines)

Cell Line	Tissue	Disease	Patient Details	Treatments	Category
RPE-1	Retina, eye	None	Female	None	Non-Transformed
PC-9	Lung	Adenocarcinoma	Unknown	None	Transformed
SAOS-2	Bone	Osteosarcoma	Female, 11 years old, Caucasian	RTG, methotrexate, Adriamycin, vincristine, Cytoxan and aramycin-C	Transformed
K562	Bone Marrow	Chronic Myelogenous Leukemia	Female, 53 years old	None	Transformed
HL60	Peripheral Blood	Acute Promyelocytic Leukemia	Female, 36 years old, Caucasian	None	Transformed
KG1a	Bone Marrow	Acute Myelogenous Leukemia	Male, 59 years old, Caucasian	None	Transformed
U937	Pleura effusion	Histiocytic Lymphoma	Male, 37 years old, Caucasian	None	Transformed
THP1	Peripheral Blood	Acute Monocytic Leukemia	Male, 1 year old	None	Transformed

In the experiments and results discussed below, a variety of AML and control cell lines are utilized. The control cell lines include RPE-1, PC-9 and SAOS-2. RPE-1 act as a negative control, as this cell line is non-cancerous and has not been reported to have any abnormal expression of Aurora A kinase. PC-9 and SAOS-2 both are cancerous tissues, and therefore, may have an overexpression of Aurora A. However, these will act as positive controls and help to determine cellular factors that may be specific to AML (Table 1).

Each cell line was subcultured at a ratio 1:5 every 72 hours. For adherent cell lines (RPE-1, RPE-PLK4, PC9, and SAOS2), the media was aspirated out, and 2 mL of 1XPBS was used to rinse the cells. The 1XPBS was then aspirated out and 2 mL of trypsin was added to the cells. The cells were left at 37C for 5 minutes. After the incubation period, 8 mL of media was added to the trypsinized cells. Two mL of this cell suspension was removed, placed into a new T75 flask, and the total volume increased to 10mL with media. All suspension cell lines (U937,

THP1, K562, and KG1a) were subcultured by moving 2 mL out of the current flask, transferring the 2mL to a new T75 flask, and bringing up the volume to 10mL with fresh media.

3.2 Fixing and Staining AML Cells for Immunofluorescence

Polylysine coverslip preparation

Coverslips were immersed in 10% acetic acid for 10 minutes in a shaking tray. Afterwards, the acetic acid was removed and the coverslips were washed with water twice by shaking for 10 minutes. Next, the coverslips were incubated in 10% polylysine (diluted in water) for 10 minutes. The 10% polylysine solution was removed and the coverslips were dipped briefly in water, and then air dried in a rack before use.

Immunofluorescence

In 2mL of media, 1.0 x 10^6 cells of each AML cell line and the RPE cells were plated. Each well received a polylysine coated coverslip. 24 hours after plating the cells, Alisertib was added so the final concentrations were 0nM (untreated), 25nM, 50nM, and 100nM. The cells were exposed to Alisertib for 18 hours. After exposure to Alisertib, the plates containing AML cells were centrifuged at 1000 rpm for 5 minutes to promote adherence of cells to the coverslip. Each coverslip was transferred to a dish containing 1mL ice cold methanol and was incubated at -20ºC for 15 minutes. The plates were then centrifuged again at 1000 rpm for 5 minutes. After centrifugation, the methanol was removed, and coverslips were washed with 1mL of 1x PBS. Coverslips were then blocked in 1mL TBS/BSA for 20 minutes at room temperature.

Primary antibodies were prepared by diluting dm1α (Santa Cruz Biotechnology, Reference Number: SC-32293) and centrin-2 (Santa Cruz Biotechnology, Reference Number: SC-27793R) 1:1000 and 1:200, respectively, in 1mL of TBS/BSA. The coverslips were placed in a humid chamber and 100 µL of primary antibody was added to each coverslip for 90 minutes. The coverslips were washed with TBS/BSA for 5-10 minutes. Secondary antibody was prepared at a 1:1000 dilution in DAPI/TBS. The coverslips were incubated with 100 µL of secondary antibody in a humid chamber in the dark for 45-60 minutes. The coverslips were then washed with TBS/BSA for 5-10 minutes, and then were mounted on a slide with Molecular Probe Prolong Gold Antifade Reagent (Reference Number: P36934).

Afterwards, each slide was viewed on a Nikon Ti at the objective 60X. To view the staining, channels DAPI, FITC, and TxRed were used to image the DAPI, centrin-2 and dm1-alpha staining respectively.

For this experiment, there were three biological replicates, each containing two technical replicates.

3.3 Observing Alisertib Impact on Acute Myeloid Leukemia Cells

Viability Assay

The five AML cell lines (U937, HL60, THP1, KG1a, and K562) and three control cell lines (RPE, PC9, and SAOS2) were treated with a range of Alisertib concentrations. 100 µL of media containing 6,000 cells were used in each well of a 96-well plate, and 100 µL of media or media with Alisertib concentrations of 10nM, 25nM, 50nM, 100nM, and 250nM were added. The viability assay contained three technical replicates in each plate and three biological replicates were done for each cell line. Resistant cell lines to Alisertib, U937 and K562, as well as the three controls were used for additional viability assays at Alisertib concentrations of 500nM, 750nM, 1µM, with the same volume and number of cells.

After three days of exposure to Alisertib, 20 µL of Thermo Fisher Presto Blue was added to each well. After 2h, the plate was read on a PerkinElmer 2030 Explorer at 600 A.

For this experiment, there were three biological replicates, each containing two technical replicates.

FACS Analysis

2.0 x 10^6 cells were plated in 10 cm dishes and media was added up to 10 mL. After 24 hours, the cells were treated with 100 ng/mL Nocodazole and with Alisertib concentrations of 100nM and 250nM for 16h. For only Alisertib treatment, the cells were plated and after 18 hours, were treated with 100 nM or 250 nM of Alisertib for 16h. Then 10uL of Thermo Fisher BrdU was added to the media for 1 hour. Afterwards, the media was collected in 15mL tubes and centrifuged at 1000rpm for 5 minutes, and the supernatant was aspirated. The pellet was resuspended in 150uL of PBS, followed by the addition of 350uL of cold 100% methanol. The 500uL solution was then collected in an Eppendorf tube and stored at -20C.

For analysis, the samples were spun at 800 rpm for 5 minutes, and the supernatant was aspirated. The pellet was washed twice in PBS, spinning and aspirating the supernatant between the two washes. 50 uL of 100 ug/mL ribonuclease and 200 uL of 50 ug/mL PI were added to each sample. The samples were run on the Accuri C6 Flow Cytometer, with a threshold of 50,000 events.

QPCR Protocol

RNA was extracted from AML cell lines using Trizol (Ambion, Life Technologies) and complementary DNA was synthesizes using the Superscript first-strand synthesis system (Applied Biosystems – Life Technologies, Austin, TX, USA). Aurora A expression was then analyzed using primers (Table 2). To establish target-gene expression levels, complementary DNA was quantified using the SYBR green kit (Applied Biosystems – Life Technologies, Austin, TX, USA), gene-specific primers, as well as GAPDH specific primers using relative quantification analysis. The copy number of the gene of interest was normalized to the copy number for GAPDH.

Table 2. qPCR Primer Sequences

GAPDH	**Forward**	5’ – CCCTCTGGTGGTGGCCCCTT – 3’
	Reverse	5’ – GGCGCCCAGACACCCAATCC – 3’
AURKA	**Forward**	5’ – TTTTGTAGGTCTCTTGGTATGTG – 3’
	Reverse	5’ – GCTGGAGAGCTTAAAATTGCAG – 3’

For this experiment, there were three biological replicates, each containing two technical replicates.

4. Results

4.1 Characterization of AML and Control Cell Lines

Several research studies have shown that Aurora A kinase is overexpressed in a wide variety of human cancers such as breast, colon, and cervical cancers (Fu et al, 2013). It has previously been demonstrated that AML cells frequently exhibit amplification or increased expression of the Aurora Kinase A gene (AURKA) (Kim et al, 2012).

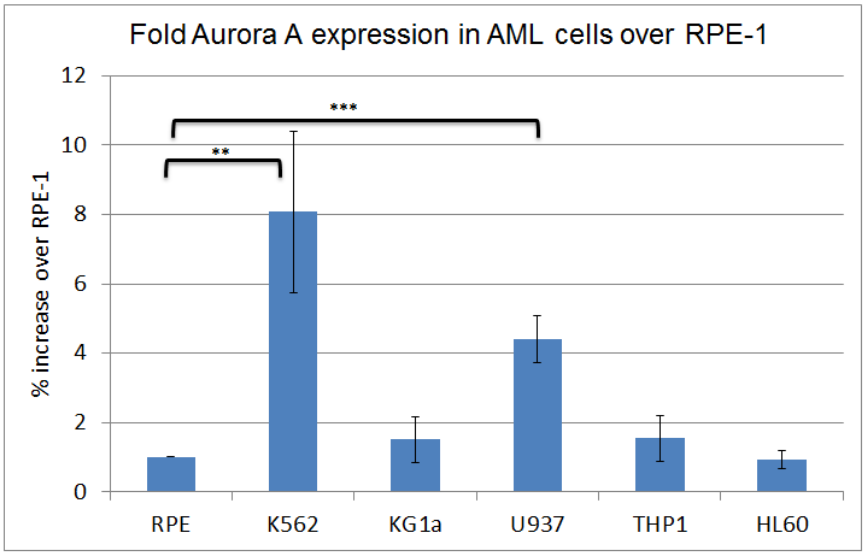

Figure 8. qPCR of Aurora A Expression in AML Cells over RPE-1

Quantitative PCR analysis of Aurora A kinase expression in AML and control cell lines. K562 and U937 scored had significantly higher fold expression over the control cell line, RPE-1. Standard Deviation is 1 way ANOVA, P value < 0.01 (**), P value < 0.001 (***)

We therefore investigated Aurora A kinase levels in AML cell lines using quantitative PCR. The RPE-1 cell line was used as the baseline for a normal expression of Aurora A kinase. KG1a, HL60 and THP1 all showed comparable Aurora A kinase expression to RPE-1 cells, whereas, the cell line U937 had a three-fold increase over RPE-1, and K562 had an eight-fold increase (Figure 8).

Given the well documented role for Aurora A in centriole duplication, regulation of mitotic spindle formation, and mitotic progression**,** Aurora A has been proposed to be a promising target for novel AML therapeutic approaches (Fu et all, 2007). However, it remains unclear to what extent Aurora A over-expression, or additionally, associated cellular phenotypes, may indicate a likely response of the patient to therapeutic approaches that inhibit Aurora A function.

To begin to explore the relationship between Aurora A overexpression related phenotypes and sensitivity to the inhibitor Alisertib, we initially characterized our panel of AML cell lines, using immunofluorescence microscopy, to assess spindle morphology, centriole number, ,and mitotic index. The RPE-1 cell line was utilized as a negative control (Table 1). Consistent with previous reports, our control RPE cells exhibit a mitotic index near 1%, with >70% of interphase cells containing a single pair of centrioles (Lambrus, 2015). The vast majority of mitotic RPE cells display a bipolar spindle and two pairs (four) of centrioles (Lambrus, 2015). Immunofluorescence analysis using alpha tubulin and DAPI (DNA) staining to identify mitotic nuclei indicated that AML cell lines exhibit a mitotic index between 0.5 and 2.0%, comparable to the percentage scored in the RPE-1 cell line (Figure 9). Previous research has shown that Aurora A overexpression can result in centrosome amplification, and lead to multipolar spindles and abnormal segregation of the chromosomes (Bavestias & Linardopoulos, 2015). However, only cell line K562, the one with the highest Aurora A levels, exhibit an increase in cells with >4

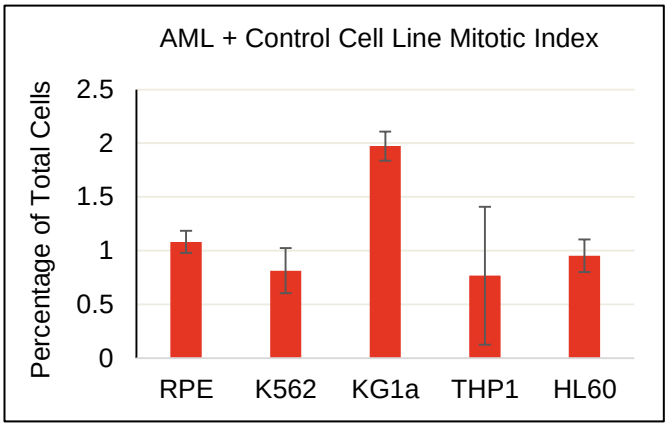

Figure 9. Mitotic Index of AML and Control Cell Lines

Immunoflouresence analysis was utilized to score mitotic index for AML and control cell lines. All AML cell lines had a mitotic index comparable to RPE-1

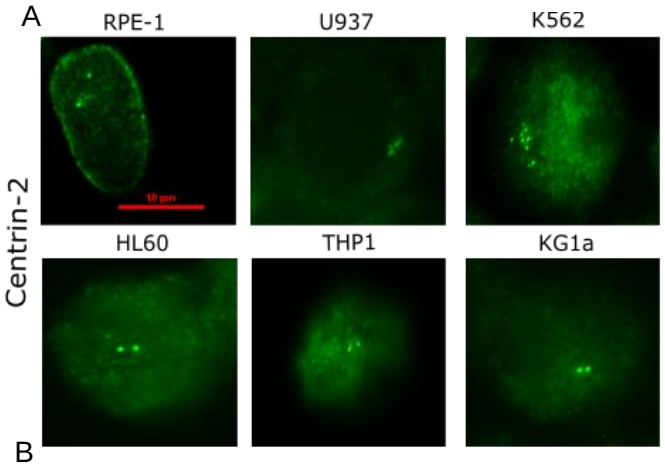

Figure 10. Centriole Number of AML and Control Cell Lines

Figure 8A shows immunoflouresence images of cells representative of each cell line's average centriole number. Figure 8B shows the scoring of each cell line's centriole number

centrioles (Figure 10). Consistent with measured centriole number, K562 cells but not those with normal centriole number, also exhibit an increase in multipolar mitotic spindles (Figure 11).

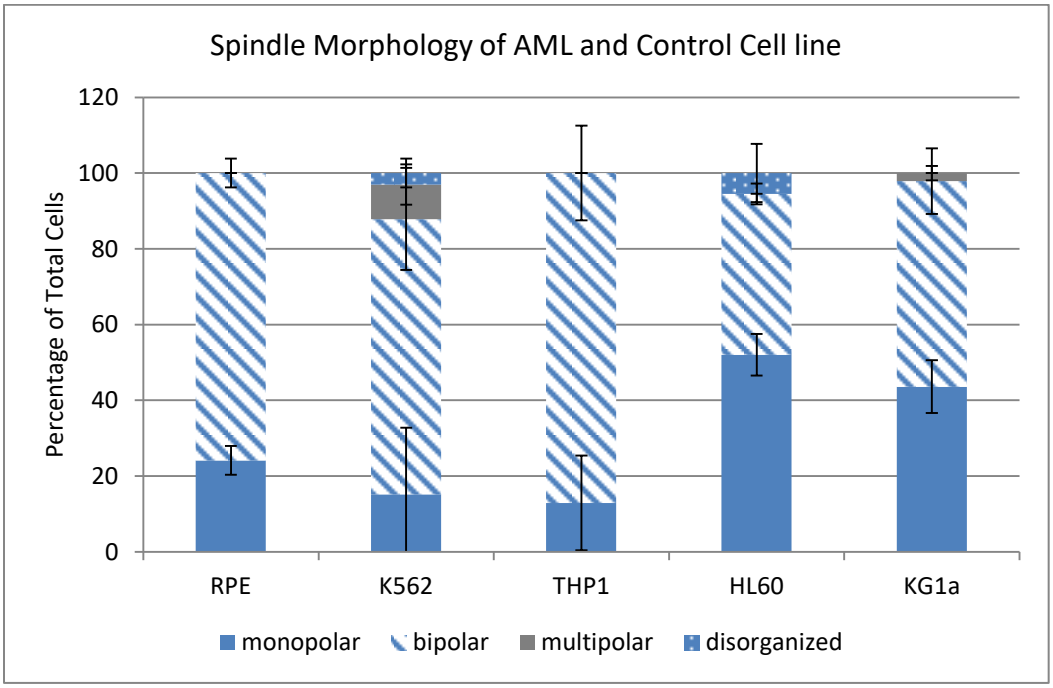

Figure 11. Spindle Morphology of AML and Control Cell Lines Untreated

Immunofluorescence analysis of the spindle morphology in the AML and control cell lines. K562 was scored to have a higher population of multipolar spindle poles in comparison to the control cell line.

4.2 Effect of Alisertib on AML and Control Cell Lines

To examine the susceptibility of the AML cells to mitotic arrest, and Alisertib-induced mitotic arrest, Fluorescence Activated Cell sorting (FACS) analysis was used to monitor DNA content as a readout of cell cycle progression following treatment with Alisertib alone, or Alisertib together with the microtubule poison Nocodazole. In figure 12A, 1.7% of PC9 cells were in mitosis, this percentage increased to 70.3% when treated with 250 nM of Alisertib and 100 ng/mL Nocodazole. For HL60 cells, both the 100 nM and 250 nM treatments were able to halt 50% of cells in mitosis. (Figure 12A). Immunofluorescence was used to score the mitotic index of AML cells after treatment with increasing concentrations of Alisertib. For AML cell lines K562, KG1a and HL60, all experienced significant increases in mitotic index at 50 and 100 nM of Alisertib (Figure 13). This analysis demonstrated that AML cells are responsive to conditions

that perturb mitotic progression such that Alisertib treatment, either alone or in combination with Nocodazole, leads to an enrichment of cells with 4N/mitotic DNA content.

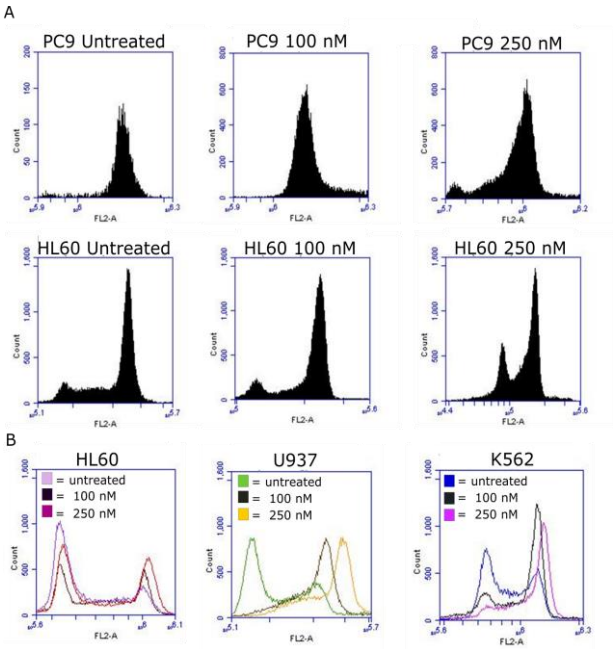

Figure 12. AML and Control Cell Lines Treated with Nocodozale and Alisertib FACS Analysis

FACS used to monitor cell cycle progression with Alisertib and Nocodazole (Figure 12A) or with increasing concentrations of Alisertib (Figure 12B). All AML cell lines were responsive to both treatments and halted during mitosis.

To explore the cause of this mitotic arrest, we performed immunofluorescence imaging analysis of mitotic structures in our AML cells with and without Alisertib treatment. In AML cells, Aurora A kinase inhibition by Alisertib can induce mitotic assembly defects, such as monopolar spindle morphology (Bavestias & Linadopoulos, 2015). We specifically examined the effect of Aurora A kinase inhibition on monopolar spindle formation in AML cells. The control cell line, RPE-1, experienced a dose-dependent increase of monopolar cells with the number of monopolar cells specifically increasing by 47% in response to 100 nM Alisertib (Figure 14). Three of the AML cell lines, KG1a, THP1 and HL60 showed a monopolar spindle formation increase comparable to RPE-1

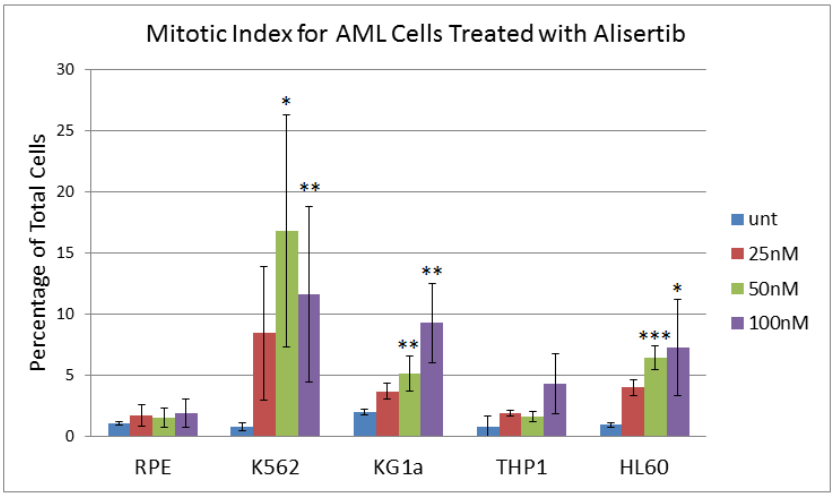

Figure 13. Mitotic Index for AML Cells Treated with Alisertib

Immunofluoresence was used to score mitotic index for AML cells treated with varying concentrations of Alisertib. K562, KG1a and HL60 experienced significant increases in mitotic index at 50 and 100 nM of Alisertib. Standard Deviation is 1 way ANOVA: P value < 0.05 (*), P value < 0.01 (**), P value < 0.001 (***).

(Data not shown). However, in response to 100 nM Alisertib, K562 displayed a 2% monopolar spindle formation (Figure 14).

Cells that experience Aurora A inhibition, and have formed monopolar spindle poles will experience a spindle checkpoint mitotic arrest. This arrest is temporary and can promote apoptosis (Bavetsias & Linardopoulous, 2015). To study the immediate effects of Aurora A inhibition on cell viability, and the sensitivity of the cells lines to Alisertib, we performed viability assays. Viability assays use Presto Blue, which is initially blue in color, and is rapidly take up in viable cells and converted into a red fluorescent dye. The number of viable cells can be measured by comparing the change in absorbance levels. We used this assay to determine the number of viable cells after being treated with Alisertib for 72 hours. The results for each cell line were normalized to its untreated condition. The sensitivity of the cells can be determined by their normalized Relative Expression Units (REU), where the value of 1 implies that the number of viable cells is the same as the untreated condition. The controls for these assays were the RPE-1, SAOS2 and PC9 cell lines. RPE-1 and SAOS2 experience significant decreases in cell viability

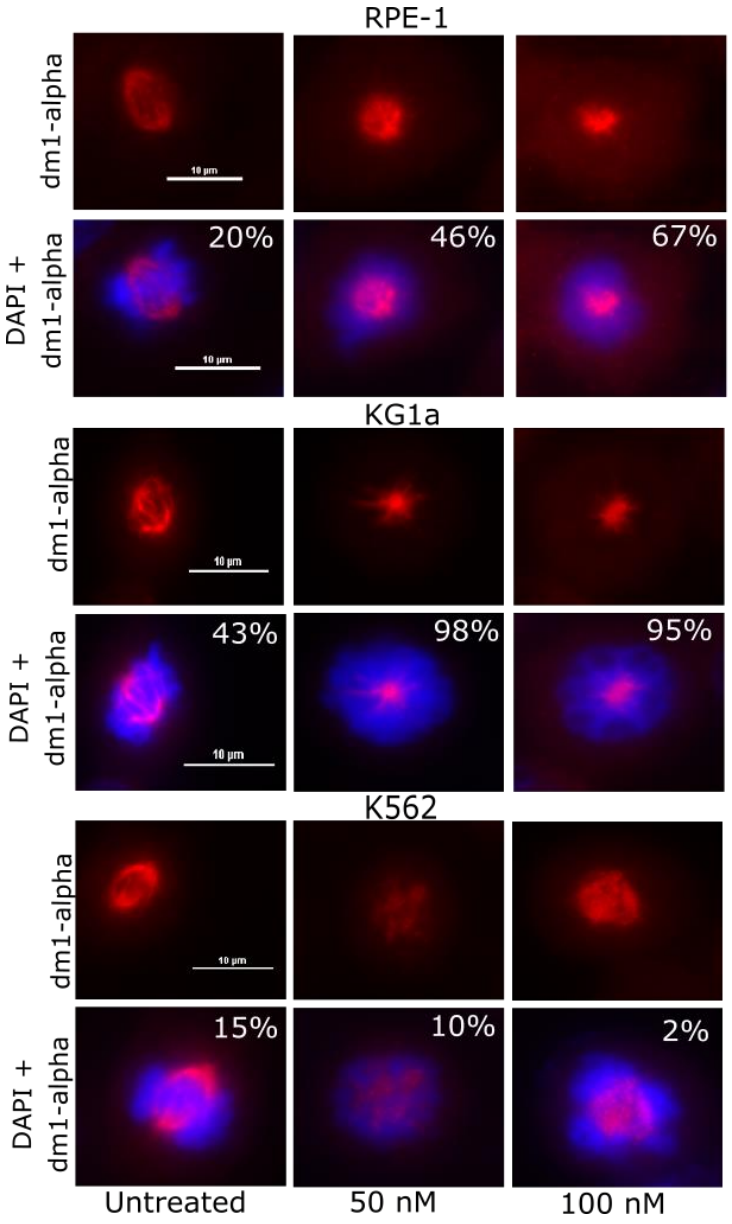

Figure 14. AML and Control Cell Line Spindle Pole Formation at Various concentrations of Alisertib

Immunofluorescent images of AML and control cell lines at untreated, 50 and 100 nM of Alisertib. The percentages represent the percentage of monopolar spindles observed at each concentration for each cell line

at 50 nM. PC9 had decreases in cell viability at 100 nM and 250 nM. HL60 had an increased sensitivity to Alisertib, as shown by the large reduction in cell viability at 10 nM. K562 and U937 had the lowest drug sensitivity, as neither of these cell lines had a significant decrease in cell viability until 250 nM (Figure 15). As described above, the K562 cell line overexpresses Aurora A kinase compared to controls and has a high incidence of extra centrosomes (Figures 9 & 10). This cell line may exhibit limited sensitivity to Alisertib due to inability to fully inhibit Aurora A activity. Alternatively, multipolar spindle morphology in these cell lines may render them resistant to forming monopolar spindles, maintaining a mitotic arrest, and ultimately undergoing apoptosis. To determine if high levels of Aurora A in K562 cells can be inhibited comparably to that In cell lines that are more responsive to Alisertib treatment, we are utilizing western blotting techniques to measure Aurora A levels together with autophosphorylation (as a readout of its kinase activity) in each AML cell line before and after Alisertib treatment.

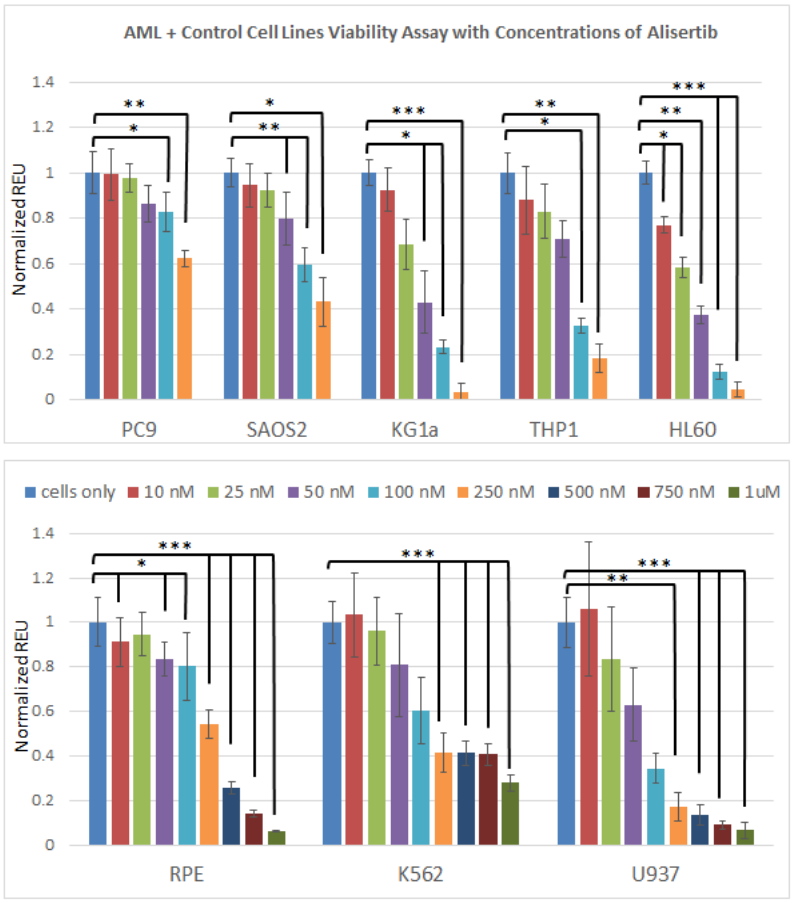

Figure 15. AML and Control Cell Lines Viability at Concentrations of Alisertib

Viability assays were used to assess cell viability in each cell line with concentrations of Alisertib. K562 and U937 both scored to have reduced drug sensitivity to Alisertib. Standard Deviation is 1 way ANOVA: P value < 0.05 (*), P value < 0.01 (**), P value < 0.001 (***).

4.3 Experimental Confirmation of Polo-Kinase 4 Overexpression to Amplify Centriole Number

In a second approach, to test if sensitivity to Alisertib might be impacted by centrosome number, independent of Aurora A levels, we utilized a cell line system with inducible PLK-4 expression. Polo-like kinase 4 (PLK-4) is a cell cycle kinase involved in centrosome maturation. When overexpressed, PLK-4 can induce centriole amplification by producing multiple procentrioles that adjoin to the parental centriole (Kleylein-Sohn et al, 2007). This centriole amplification is independent of increased Aurora A kinase levels. It is possible that the Alisertib sensitivity observed in the AML cell lines could be due centriole number and subsequent spindle morphology defects instead of, or in addition to increased Aurora A kinase levels. To test this, we obtained an RPE cell line engineered to carry a tet-regulated PLK-4 expression construct. By using this cell line, the specific effects of centriole amplification on drug sensitivity could be elucidated

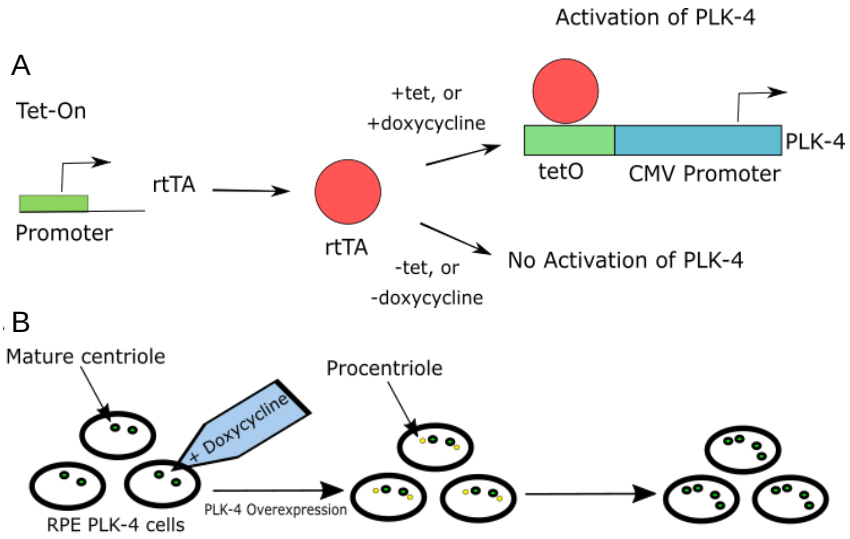

Figure 16. PLK-4 Overexpression Experimental Layout

Figure 16A shows the PLK-4 plasmid, and its transcription is activated by the addition of doxycycline. Figure 16B shows the experimental set-up of the induction of PLK-4 overexpression in RPE-1 cells. Doxycycline is added to RPE-PLK-4 cells, which results in the overexpression of PLK-4. This leads to the formation of additional procentrioles leading to the development of mature centrioles.

We used 2 µg/mL of doxycycline to induce PLK-4 overexpression and to induce the proliferation of centrioles within these cells (Figure 16). The PLK-4 plasmid is regulated by tetO, when doxycycline is added, it drives the expression of PLK-4. This overexpression leads to the amplification of centrioles (Figure 15). To monitor induction and function of PLK-4 overexpression, immunofluorescence analysis was used to observe centriole number in normal and induced cells. The PLK-4 RPE cells with normal PLK-4 expression retained 2 centrioles in non-mitotic cells, and 4 centrioles in mitotic cells (Figure 17). The cells that overexpress PLK-4,

had an increased number of centrioles in both non-mitotic and mitotic cells (Figure 17). 60% of mitotic cells overexpressing PLK-4 had a multipolar spindle formation. This data confirms that the induction of PLK-4 in the RPE-1 PLK-4 cell line can successfully induce the overduplication of centrioles.

4.4 Investigating Effect of Alisertib on Cells with PLK-4 Overexpression

Overexpression of PLK-4 will lead to the amplification of centrioles within the cell (Kleylein-Sohn, 2007), and our results suggest that centriole amplification could be a factor in reduced Alisertib sensitivity in some AML cell lines. To further explore this, mitotic index, spindle morphology and cell viability were scored in cells with and without PLK-4 overexpression following 18 hours of treatment with increasing amounts of Alisertib. Aurora A inhibition can cause cells to activate mitotic checkpoint and sustain mitotic arrest (Bavetsias & Linardopoulos, 2015), so treatment with Alisertib could result in an increased mitotic index. However, for cells with both normal and overexpressed PLK-4 , the mitotic index did not significantly increase as the concentrations of Alisertib increased

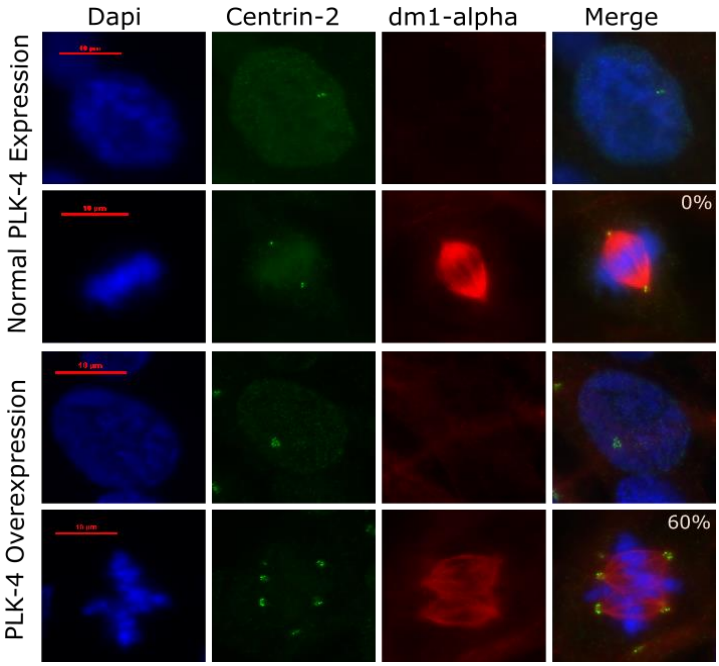

Figure 17. PLK-4 Overexpression and Centriole Amplification Confirmation
Immunoflourescence analysis of PLK-4 RPE-1 cells to confirm overexpression of PLK-4 and centriole amplification as a result.

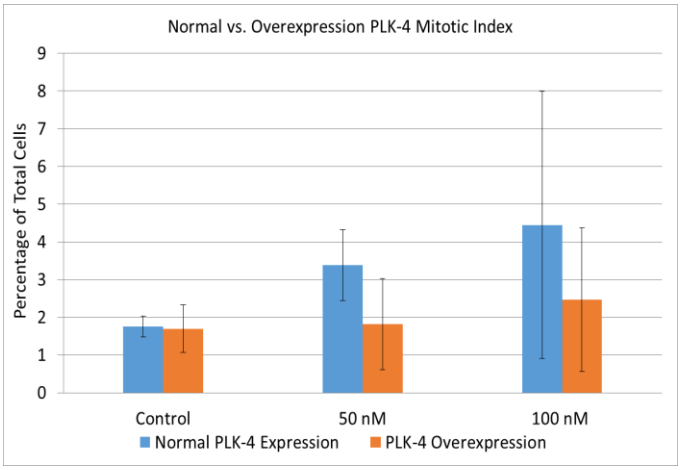

Figure 18. Mitotic Index of Normal and Overexpressed PLK-4 Cells

Mitotic index was scored in normal and overexpressed PLK-4 cells in 50 and 100 nM of Alisertib. Both normal and overexpressed PLK-4 cells showed an increased mitotic index as Alisertib concentrations increased.

(Figure 18). This could suggest that the Aurora A inhibition by Alisertib was not significant enough to result in increased mitotic arrest in RPE-1 PLK-4 cells.

We next examined the effect of centriole amplification on monopolar spindle formation using Alisertib to inhibit Aurora A kinase in the RPE-1 PLK-4 cells, with or without induction of PLK-4 overexpression. PLK-4 overexpression with increasing concentrations of Alisertib did not increase the percent of cells with monopolar spindles (Figure 19). However, approximately 40 – 60% of the overexpressed PLK-4 cells had a multipolar spindle structure (Figure 19).

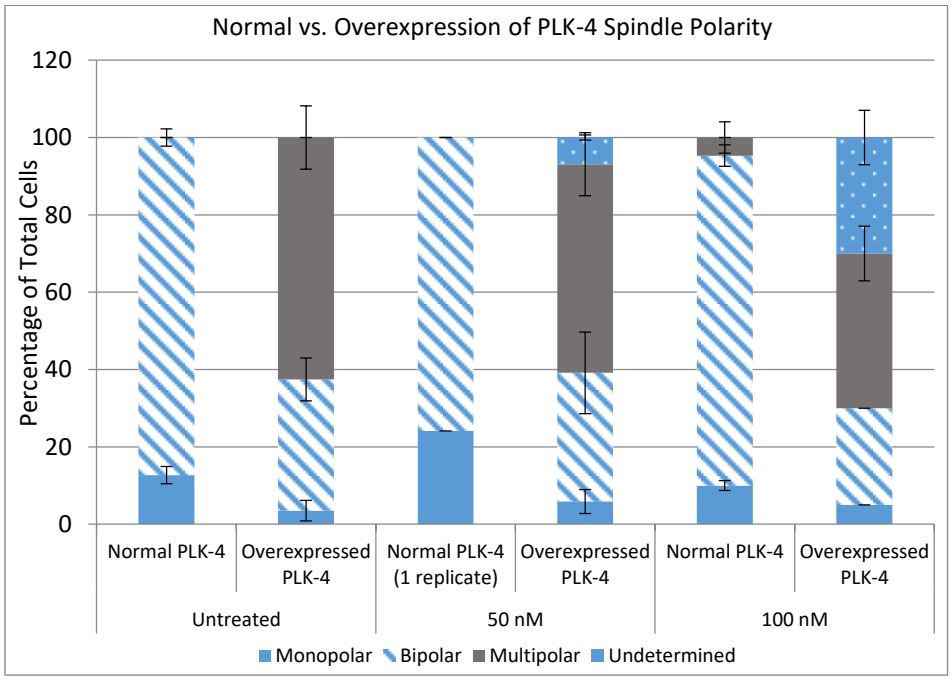

Figure 19. Spindle Morphology of Normal and Overexpressed PLK-4 cells

Immunofluorescent analysis of normal and overexpressed PLK-4 on spindle morphology at 50 and 100 nM of Alisertib. Cells with PLK-4 overexpression maintained multipolar spindle morphology even at 50 and 100 nM of Alisertib.

In one of the analyzed AML cell lines (K562) centriole amplification and reduced sensitivity to Alisertib were observed. To examine the role of centriole proliferation in Alisertib sensitivity, without additional factors like Aurora A overexpression as seen in the two AML cell lines, the RPE-1 PLK-4 cell line was used. The cells with regular levels of PLK-4 expression behaved comparably to the RPE-1 cells in Figure 12, showing a significant decrease in cell viability at 50 nM. In the cells with PLK-4 overexpression, there was not a significant decrease in cell survival until 250 nM (Figure 20). This is similar to what was observed in the K562 and U937 cell lines in

(Figure 20). These results demonstrate that an increase in centriole number could be a contributing factor in the decreased sensitivity of Alisertib.

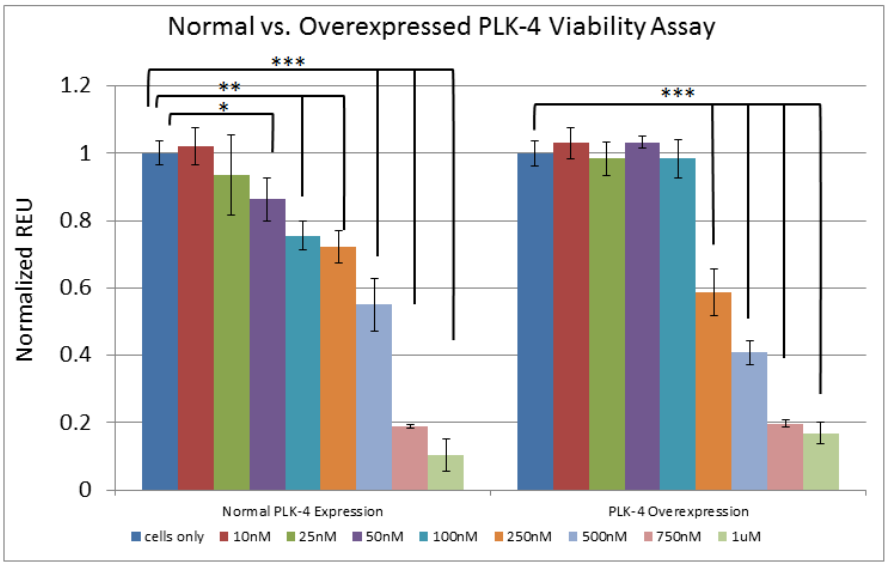

Figure 20. Normal and Overexpressed PLK-4 Cell Viability at Concentrations of Alisertib

Viability assays were used to assess the cell viability of normal and overexpressed PLK-4 cells at concentrations of Alisertib. Overexpressed PLK-4 cells did not show a significant decrease in cell survival until 250 nM. Standard Deviation is 1 way ANOVA: P value < 0.05 (*), P value < 0.01 (**), P value < 0.001 (***)

5. Conclusions and Future Work

In this study, we have utilized several methods to observe the effects of Aurora A kinase inhibition on AML cells and the role of centriole abundance in drug sensitivity. After assessing the AML and control cells for centriole number and spindle polarity, we examined the drug sensitivity of each cell line to increasing concentrations of Alisertib. We observed that three cell lines (K562, U937 and THP1) had a reduced sensitivity to Alisertib and two of these cell lines (K562 and U937) also had an increased centriole number. These results led us to question if centriole number was a contributing factor to drug sensitivity.

From these results, we investigated the potential relationship between centriole amplification and drug sensitivity. Using RPE-PLK4 cells, we were able to induce centriole amplification within the cells, and observed cellular changes when the cells were exposed to 50 nM, 100 nM and 250 nM of Alisertib. We observed that cells that had an overexpression of PLK-4 continued to have multipolar spindle poles, even at the concentration of 100 nM. However, more replicates of the spindle polarity experiment will have to be completed to verify these results. In addition, cells with PLK-4 overexpression had a reduced Alisertib sensitivity compared to the cells with normal levels of PLK-4. The cells without PLK-4 overexpression experienced a decrease in cell survival at 50 nM Alisertib, whereas the cells viability was not significantly affected in cells overexpressing PLK-4 until 250 nM. At Alisertib concentrations higher than 250 nM, both cell types were sensitive to the drug and cell survival decreased significantly. From these experiments, we conclude that centriole abundance may play a role in reduced drug sensitivity.

While centriole number may play a role in Alisertib sensitivity and monopolar spindle formation, the mechanism of this action and other cellular factors that may contribute were not investigated. Therefore, in future projects, it could be taken into consideration to overexpress Aurora A expression in RPE or non-AML cell lines and observe the effects on the cell's sensitivity to Alisertib. This would help to decipher if Aurora A overexpression has a significant impact on the monopolar spindle pole formation or the sensitivity of the cells to Alisertib. Loss of PLK-4 has been shown to prevent centriole duplication (Holland et al., 2012) Therefore, to further test the impact of centrosome number on Alisertib sensitivity, it would also be interesting to reduce the expression of PLK-4 in K562 (the cell line with an increase in centriole number), to observe the impact of reduced centriole number in these cell lines. This would provide additional insight into the relationship of centriole abundance and drug sensitivity.

To further understand the relevance of centriole abundance and drug sensitivity, in vivo models could be utilized to further assess what factors impact the effectiveness of Alisertib. For example, a cell line with centriole abundance such as K562 could be injected into mice, and another cell line that maintains normal centriole number, such as KG1a could be injected into another set of mice. Each set of mice would be divided into subgroups, and each subgroup would receive different concentrations of Alisertib. This experiment would be used to support previous experiments and also explore the potential effects of centriole amplifications in tumor growth and treatment in living models. If centriole amplification is found to be a cellular factor in reduced drug sensitivity in the in vivo model, another experiment could be completed with mice being injected with K562, followed by a treatment that reduces the expression of PLK-4, to reduce centriole number. During the experiment, these mice would also be treated with a single concentration of Alisertib. The tumor growth would be observed throughout the course of the experiment, to determine if drug sensitivity can be increased when centriole number is decreased.

These results could lead to further patient studies, where blood samples from each patient are taken and their cellular structure is analyzed. If their cells show signs of centriole abundance, physicians may instruct the patients to use higher dosing or even a treatment different from Alisertib or use a combination of therapies to target the leukemic cells in addition to Alisertib. This would be an initial step to creating patient profile that would indicate drug efficacy based on cellular structure.

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