EFFECTS OF NICOTINAMIDE AND NILOTINIB ON TELOMERASE ACTIVITY AND TELOMERE LENGTH ASSOCIATED WITH PARP-1 REGULATION IN K562 MYELOID CELL LINE

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By

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LIST OF SYMBOLS AND ABBREVIATIONS

-	Minus
%	Percentage
ºC	Degree Celsius
®	Registered
+	Plus
±	Plus minus
<	Less than
=	Equal
>	Greater than
36B4	Acidic ribosomal phosphoprotein
A_{260} A_{280}	Absorbance at 260 nm and 280 nm
A_{260} A_{230}	Absorbance at 260 nm and 230 nm
A_{450}	Absorbance at 450 nm
A_{690}	Absorbance at 690 nm
ABL	Abelson, the name of the leukaemia virus
ADP	Adenosine di-phosphate
ALL	Acute lymphocytic leukaemia
AML	Acute myeloid leukaemia
AP	Accelerated phase
ATCC	American type culture collection
ATP	Adenosine triphosphate
B-cell	Type of white blood cell
BAK	Bcl-2-antagonist killer
BAX	Bcl-2-associated X
BC	Blast chronic
Bcl-2	B-cell lymphoma 2
BCR	Breakpoint cluster region
BCRP	Breast cancer resistance protein
BD	Becton Dickinson company
BIK	Bcl-2-interacting killer
BIM	Bcl-2-interacting mediator

BLK	B-lymphoid tyrosine kinase
bp	Base pair
BP	Blast or blastic phase
cDNA	Complementary DNA
CI	Confidence interval
CLL	Chronic lymphocytic leukaemia
cm^2	Square centimetre
CML	Chronic myeloid leukaemia
CO_2	Carbon dioxide
CP	Chronic phase
C_t	Cycle threshold
DEPC	Diethyl pyro carbonate
DIG-POD	Digoxigenin-peroxidase
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide triphosphate
DTT	Dithiothreitol
E.coli	Escherichia coli
ELISA	Enzyme-linked immunosorbent assay
FACS	Fluorescence-activated cell sorting
FBS	Fetal bovine serum
FCS	Flow cytometry system
FDA	Food and drug administration
FITC	Fluorescein-5-isothiocyanate
g	Gram
g-force	Gravitational force
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GOI	Gene of interest
HK-60	Type of human leukaemia cell line
HKG	Housekeeping gene
HL60	Type of human leukaemia cell line
HPLC	High-performance liquid chromatography
hTERC	Human telomerase RNA component

hTERT	Human telomerase reverse transcriptase
hTP1	Human transition protein 1
IC_{50}	Half-maximal inhibitory concentration
K562	Chronic myeloid leukaemia cell line
kb	Kilobase
kDa	Kilodaltons
L-glutamine	Levo/left glutamine
LUX	Unit of illumination (Luminous intensity)
M	Molar
MDM2	Murine double minute 2
mg	Milligram
mL	Millilitre
mM	Millimolar
mol	Molecule
mRNA	Messenger ribonucleic acid
MRP1	Multidrug resistance-associated protein
MTP	Micro-titter plate
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
NAD	Nicotinamide adenine dinucleotide
NADP	Nicotinamide adenine dinucleotide phosphate
ng	Nanogram
ng/µL	Nanogram per microlitre
NHLS	Non-Hodgkin lymphoma
nm	Nanometre
nM	Nanomolar
NTC	Non-template control
OD	Optical density
P-glycoprotein	Permeability glycoprotein
p-value	Statistically significant value
p53	Tumour protein
PARP-1	Poly (ADP-ribose) polymerase 1
pBR233	Plasmid Bolivar Rodriguez
PBS	Phosphate buffer saline
PCR	Polymerase chain reaction

Pen-Strep	Penicillin and streptomycin
pg	Picogram
PGK1	Phosphoglycerate kinase 1
Ph	Philadelphia (chromosome)
PI	Propidium Iodide
PPIB	Peptidylprolyl isomerase B
PS	Phosphatidylserine
qPCR	Quantitative real-time polymerase chain reaction
R^2	Correlation coefficient
RF10	RPMI 1640 with the supplement of FBS and Pen-Strep
RIPK	Receptor interacting protein kinase
RNA	Ribonucleic acid
RNase	Ribonuclease
ROX	Fluorescent dye
Rpm	Revolution per minute
RPMI	Roswell Park Memorial Institute (media)
RT-PCR	Reverse transcription-polymerase chain reaction
RT-qPCR	Reverse transcription-quantitative real-time polymerase chain reaction
S	Svedberg
SCG	Single-copy gene
SD	Standard deviation
Sdn Bhd	Sendirian Berhad
SIRT1	Sirtuin (silent mating type information regulation two homolog) 1
SSIV	SuperScript IV
SV	Spin Vacuum method
SYBR	Synergy Brands
T25	25 cm^2 cell culture flask
TBE	Tris borate EDTA
TERT	Telomerase reverse transcriptase
TKI	Tyrosine kinase inhibitor
™	Trademark
TMB	3,3′,5,5′-Tetramethylbenzidine
TNF	Tumour necrosis factor

TNF-α	Tumour necrosis factor-alpha
TNFR	Tumour necrosis factor receptor 1
TRAF	TNF-R-associated factor
TRAP	Telomerase repeated amplification protocol
U	Unit
UK	United Kingdom
US	United State
The USA	The United States of America
USM	Universiti Sains Malaysia
UV	Ultraviolet
V	Volts
WHO	World Health Organization
WST-1	Water-soluble tetrazolium 1
WST-8	Water-soluble tetrazolium 8
x	Multiply
α	Alpha
Δ	Delta
µg	Microgram
µL	Microlitre
µM	Micromolar

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KESAN NIKOTINAMIDA DAN NILOTINIB KE ATAS AKTIVITI TELOMERASE DAN KEPANJANGAN TELOMER BERKAITAN DENGAN PENGAWALATURAN PARP-1 DI DALAM TITISAN SEL MIELOID K562

ABSTRAK

Leukemia mieloid kronik (CML) adalah salah satu daripada empat jenis penyakit leukemia utama yang menjejaskan sel mieloid. Fasa blas CML terus wujud sebagai penyakit yang mencabar walaupun terapi perencat tirosina kinase (TKI) yang maju telah diperkenalkan tetapi dengan batasannya dan kurang berkesan menyebabkan pesakit menjadi kurang responsif terhadap terapi. Dengan beralih kepada TKI yang sangat kuat seperti nilotinib juga tidak dapat meningkatkan kesihatan secara keseluruhan. Pada masa ini, tiada pilihan rawatan yang bertindak balas dengan berkesan dalam fasa krisis blas CML. Oleh itu, ia memerlukan pengenalpastian ubat terapi baharu untuk merawat pesakit CML ini. Dalam kajian ini, kami menyiasat kesan nikotinamida pada aktiviti telomerase dan panjang telomer yang dikaitkan dengan peraturan polimerase 1 (PARP-1) poli (ADP-Ribose) dalam titisan sel mieloid untuk meningkatkan rawatan semasa CML sebagai sebahagian daripada agen tambahan. Titisan sel K562 digunakan sebagai model yang mewakili CML dalam fasa blas telah dirawat dengan nikotinamida atau nilotinib sebelum gabungan kedua-dua bahan digunakan. Kajian ini telah menunjukkan kesan nikotinamida, nilotinib dan gabungan kedua-dua bahan dalam mempamerkan keupayaan anti-proliferasi pada titisan sel K562 selepas 48 jam. Mekanisme yang terlibat untuk mendorong kesan sedemikian masih belum jelas. Selain nikotinamida, nilotinib dan gabungan kedua-dua bahan pada titisan sel K562 telah menunjukkan sebagai positif-telomerase dengan aktiviti

telomerase yang jauh lebih tinggi. Analisis panjang telomer dinilai untuk menentukan hubungan dengan fungsi enzim telomerase untuk mengekalkan kep telomer kromosom. Data menunjukkan panjang telomer yang lebih panjang dalam semua kumpulan rawatan kecuali nikotinamida yang mempunyai sedikit penurunan di dalam aktiviti telomerase. Ekspresi gen *TERT* dalam kajian ini mencadangkan bahawa kesan bahan-bahan ini pada aktiviti telomerase dan panjang telomer semestinya bergantung kepada kesannya terhadap ekspresi *TERT*. Perencatan proliferasi sel pada titisan sel K562 mungkin dikaitkan dengan pengawal aturan penanda berkaitan PARP-1 (*BAX, RIPK1* dan *TRAF2*) dan kesan dalam ujian apoptosis yang tinggi. Kesan nikotinamida dan nilotinib pada peraturan PARP-1 termasuk mekanisme yang berkaitan dengan apoptosis memberikan bukti untuk penyelidikan masa depan dan lebih luas.

EFFECTS OF NICOTINAMIDE AND NILOTINIB ON TELOMERASE ACTIVITY AND TELOMERE LENGTH ASSOCIATED WITH PARP-1 REGULATION IN K562 MYELOID CELL LINE

ABSTRACT

Chronic myelogenous leukaemia (CML) is one of the four major types of leukaemia disease that affecting myeloid cells. The blast phase of CML has continued to exist as a challenging disease even though the advanced tyrosine kinase inhibitor (TKI) therapy has been introduced, but with its limitations and significantly less effective, causing the patient to be less favourably to respond to the therapy. Switching to highly potent TKI, such as nilotinib also not be able to improve the overall health. Currently, no treatment options are responding effectively in the blast crisis phase of CML. Therefore, it requires an identification of new drug therapies to treat these CML patients. In this study, we investigate the effect of nicotinamide on telomerase activity and telomere length associated with poly (ADP-Ribose) polymerase 1 (PARP-1) regulation in a myeloid cell line to enhance the current treatment of CML as part of the supplementary agent. K562 cell line was used as the model representing CML in the blast phase and was treated with nicotinamide or nilotinib before the combination of both substances was applied. This study has shown the effect of nicotinamide, nilotinib and combination of both substances in exhibit the anti-proliferation ability on the K562 cell line after 48 hours. The implicated mechanism involved in inducing such an effect is not yet clear. In addition of nicotinamide, nilotinib and combination of both substances on the K562 cell line have been indicated as telomerase-positive with significantly higher telomeres activity. Telomere length analysis was evaluated to

determine the relation to the function of the telomerase enzyme to maintain the telomere cap of the chromosome. Data showed in longer telomere length in all treatment groups except for nicotinamide with slightly decreases in telomerase activity. The expression of the *TERT* gene in this study suggests that the effect of these substances on telomerase activity and telomere length is necessarily dependent on their effect on *TERT* expression. Inhibition of the cell proliferation on the K562 cell line may be associated with the upregulation of PARP-1 related markers (*BAX, RIPK1* and *TRAF2*) and elevated effect in apoptosis assay. The effect of nicotinamide and nilotinib on PARP-1 regulation including mechanisms related to apoptosis, will provide evidence for future and wider research.

CHAPTER 1

INTRODUCTION

1.1 Information on research background

Cancer has been defined as a category of illnesses or diseases caused by the uncontrolled proliferation of aberrant cells or also called abnormal cells. Cancerous cells are also called as a malignant cell that begins when the cell in the body start to proliferate out of the control limit. These cells also could spread and affect other systems in the body. The ability of the cell to sustain rapid proliferation is the most fundamental trait of cancer cells (Hanahan, 2022). The likelihood of even worse results and capability to cause death will increases if the cell has migrated to other systems. Cancer cells have become the master of their own path by downregulating the growth-promoting signal used in cell division cycles in a normal cell.

About 19.3 million new cancer cases and almost 10.0 million cancer deaths have been recorded in 2020 around the world, where one of the leading causes of cancer deaths is lung cancer, followed by colorectal, liver, stomach and female breast cancers (Sung et al., 2021). According to Omar et al. (2006), in the Peninsular Malaysia, a total of 21773 cancer cases have been reported in 2006. The total number of cases has been increasing according to the World Health Organization (WHO), with a total of 48,639 new cancer cases recorded in Malaysia in 2021 (Yusof & Ishak, 2022). The chance of contracting the condition is influenced by both environmental and internal variables, including smoking, inherited genetic mutation, poor diet, or immunological disorder are all variables at play according to the National Cancer Institute (2015).

Leukaemia or also called as blood cancer is produced in a part of the bones known as bone marrow. The bone marrow is made up of a small amount of blood stem cells (such as lymphocytes and myeloid cells), fat cells, and more advanced and mature blood cells and tissue that will support to help the cells grows. The blood stem from the bone marrow will develop into new blood cells either lymphocytes which are a kind of white blood cell, or myeloid cells which are the other blood-forming cells. Rapid proliferation and cell differentiation that produce abnormal cells in the bone marrow form a malignant cell known as leukaemia. The first clinical use of chronic myeloid leukaemia treatment was by using tyrosine kinase inhibitor (TKI), which is imatinib has changed many prognoses for this disease for the better therapeutic strategies in the cancer study (Claudiani & Apperley, 2018). However, a large number of patients who did not achieve optimal responses in imatinib therapy led to the development of a new TKI (Bhamidipati et al., 2013).

Nilotinib is the current standard treatment for patients with resistance to first-line treatment in choric myeloid leukaemia. Nicotinamide as a part of the active form of vitamin B_3 has potential as a PARP-1 inhibitor and the ability to decelerate the rate of telomere shortening without causing any significant increase in telomerase activity. This association may help to enhance the effect of nilotinib, which has been shown to have a direct effect on telomerase activity.

1.2 The rationale for the study

Blast crisis remains the main challenge in the management of chronic myeloid leukaemia due to the more aggressive condition and genetic instability. Even the current treatment of CML also could not be able to improve the overall survival rate. While the original treatment for CML was meant to increase the life expectancy of the patients, slow the progression of the disease, and minimise the severity of side-effect. Consequently, it is necessary to determine another innovative treatment and require the identification of new pharmacological options to treat CML patients in a blast crisis. K562 cell line provides a proper model to represent CML in the blast phase with the positive-Philadelphia chromosome responsible for the continuous proliferation of myeloid leukaemia cells.

Multiple research projects on animal models suggest that nicotinamide may have an influence on the tumour by increasing the carcinogenesis in a dose-dependent approach and depending on the organ involved. Several different types of cancer cell lines also have been shown to respond favourably to the ability of nicotinamide to suppress cell proliferation in earlier studies. Besides that, the ability of nicotinamide has been pointed out to improve the replicative lifespan of normal human cells through its influence on telomere length. The amplified activity of telomerase in several cancers has been found to prevent telomere shortening, which enables the cancer cells to multiply beyond their usual limits. Since telomere and telomerase, which is an enzyme that stabilises telomere length play a role in contributing to the formation of a cancerous tumour in humans. It is hypothesised that nicotinamide may potentially affect telomere and telomerase activity in the K562 myeloid cell, which is one of the CML cell lines.

In addition, niacin deficiency is a possible side effect for cancer patients whose NAD^+ levels have been depleted by chemotherapy. Following that, PARP-1 regulation also required NAD^+ which is involved in a variety of biological processes ranging from DNA repair and cell death mechanisms. Hence, this has increased the necessity for the cancer patient to retain optimum amounts of nicotinamide, where several metabolic and cellular processes rely on NAD^+ as a vital energy source and cofactor.

Besides acting as an NAD^+ precursor in the PARP-1 mechanism, nicotinamide has also been demonstrated as an inhibitor of PARP-1, where inhibition of PARP-1 is currently being studied and explored as a potential antitumour agent. Thus, nicotinamide plays a vital and potentially important role in determining the fate of cell survival in response to a genetic mutation in the DNA. Previously published data also showed that telomere length might be affected by the PARP-1 regulation. Consequently, in the presence of nicotinamide may be able to demonstrate the association of telomere, telomerase, and PARP-1 mechanism in improving the therapeutic target for CML disease.

This study will be focused on the effect on telomerase activity and telomere length relation with PARP-1 mechanism after treatment with nicotinamide and/or nilotinib. Nilotinib is one of the current treatments used to treat CML patients in a more advanced phase of the disease and resistance development from previous treatment. However, no study has been done to explore the effect of nicotinamide and the combination of both nicotinamide and nilotinib on telomerase and telomere length associated with the PARP1 mechanism in the K562 cell line. Therefore, it is not quite understandable and has not been well discovered by any researcher. This research is

aimed to study the potential of nicotinamide as a supplementary agent in enhancing the sensitivity of nilotinib in treatment to inhibit tumour growth or promote the tumour cell death associated with the PARP-1 regulation. By understanding the role and potential of nicotinamide and/or nilotinib in telomere and enzyme telomerase related to PARP-1 might improve the treatment approach for CML for better management of this disease.

1.3 Objectives and aims of the study

The general and overall goal of this research project is to investigate and determine the impact of nicotinamide and nilotinib on telomerase activity and telomere length related to poly (ADP-Ribose) polymerase 1 (PARP-1) regulation in a myeloid cell line.

The study's specific objectives are as follows:

i. To determine the telomerase activity and telomere length in a myeloid cell line treated with nicotinamide and/or nilotinib.
ii. To determine the gene expression of telomerase component (*TERT*) in a myeloid cell line treated with nicotinamide and/or nilotinib.
iii. To determine the gene expression of PARP-1 related markers (*BAX, RIPK1*, and *TRAF2*) following treatment with nicotinamide and/or nilotinib.

CHAPTER 2

LITERATURE REVIEW

2.1 Leukaemia and its classification

Several kinds of cancer can be started by any cell from any part of the body, including the blood stem cells. They can be found in the inner part of the bones, known as bone marrow. Blood stem cells can differentiate into myeloid and lymphoid cells (Cairnie, 2012). Cell differentiation of the myeloid cells will develop various types of mature blood cells, such as platelets, erythrocytes, granulocytes, and monocytes. Meanwhile, lymphoid cells undergo cell development, progressing from lymphoblast to more mature cells, which are lymphocytes (Ogawa, 1993; Rohrbacher & Hasford, 2018). This cell differentiation is due to the activation of a group of genes that promote the production of a protein that will provide specific properties of the blood cells. Whenever this mechanism is disrupted, it will result in the formation of malignant cells in the bone marrow, known as leukaemia (Seita & Weissman, 2010; Weissman, 2005).

Leukaemia is a group of different types of blood cancer cells found in the blood or bone marrow. It is a malignant condition caused by a rapid production of aberrant white blood cells (Sell, 2005). A total of 474,519 new cases and 311,594 deaths were attributed to leukaemia in 2020, making it the 15th most common cause of cancer incidence and the 11th leading cause of cancer-related mortality globally (Sung et al., 2021). Acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), and chronic lymphocytic leukaemia (CLL) are the four primary kinds of leukaemia.

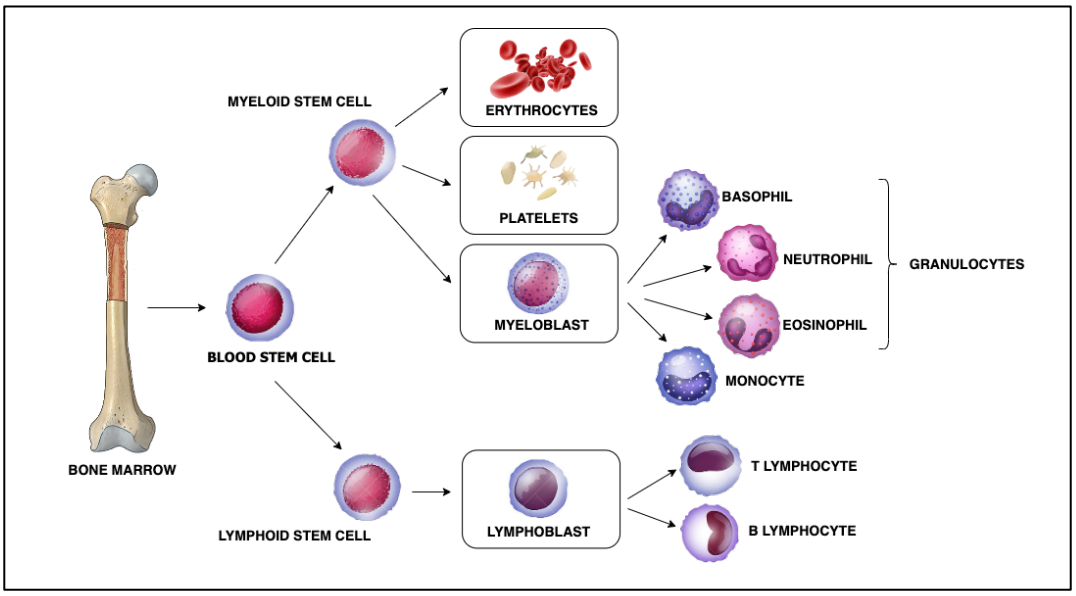

Figure 2.1: Standard representation of the differentiation of a multipotential hematopoietic stem cell into differentiated myeloid and lymphoid cells.

All four types of leukaemia were categorised by the speed of progression of the cancer cell and the type of cell that has been infected (Jaffe, 2008; Kampen, 2012). Speed progression by the cancer cell was divided into two, which are acute and chronic leukaemia (Aof et al., 2022). In acute leukaemia, immature blood cells, also known as blasts, are produced. The patient with this leukaemia required aggressive and timely treatment due to the rapid multiplication of blast cells, where the disease could worsen quickly. When compared to chronic leukaemia, it is involved with more mature blood cells. The cells can accumulate more slowly and patients with this leukaemia could go undiagnosed for years with no early symptoms (Murphy et al., 2017). All major types of leukaemia also can be summarised into two types of cells involved, which are lymphoblastic and myeloid cells.

The incidence of childhood acute lymphoid leukaemia is 80% higher than in adults, and the treatment outcome in children has shown consistent progress and improvement over the years (Pui, 2020; Pui & Evans, 2006; Pui et al., 2004). However, drug resistance decreased tolerance, and reluctance to endure transient adverse effects have all contributed to a decline in the efficacy of treatments for adult patients with ALL, leading to a less favourable and poorer result. Patients with ALL have standard treatments available, including chemotherapy that typically consists of vincristine, corticosteroids and an anthracycline (Gottlieb et al., 1984) with allogeneic stem cell transplantation for eligible candidates (Samra et al., 2020; Terwilliger & Abdul-Hay, 2017).

In contrast, chronic lymphoid leukaemia is more commonly diagnosed in adults with an average age of 72 years old. Since cases of CLL are more prevalent in the West than in Asia (Ghia et al., 2007), it has been hypothesised and speculated that either genetic or environmental factors play a role in the development of the CLL disease (Burger, 2020). Patients with CLL undergo chemotherapy that frequently uses alkylating agents such as chlorambucil, cyclophosphamide, and bendamustine.

However, results from treating acute myeloid leukaemia have been more encouraging and promising in younger adults than in older adults, who appear to be less prominent (Burnett et al., 2011). AML is the most frequent type of acute leukaemia in older adult with an average of 67 years old (O'Donnell et al., 2012). When dealing with AML, the patient's age is a major factor in determining the course of treatment and the potential for a successful outcome. Therapeutic options for AML have remained unchanged for over 30 years. where the therapy compromise of cytarabine with an anthracycline

(Dillman et al., 1991; Döhner et al., 2015; Rowe & Tallman, 1997; Tallman et al., 2005).

Du et al. (2022) have summarised that those males carried a greater risk proportion of the disease due to leukaemia compared to females. By type, the elderly was more likely to carry the risk and repercussions of AML, CML, and CLL than the younger generation. Nonetheless, ALL was found to have a more measurable influence on the younger age group. According to the study data, tobacco use was the single most important contributor to male mortality from leukaemia. While high body mass index (BMI) and smoking contributed to the same number of female leukaemia fatalities. It is still poorly understood the actual cause of leukaemia, where the source or factor of leukaemia may be from both hereditary and environmental factors (Apperley, 2015).

2.2 Chronic myeloid leukaemia (CML)

CML is likely one of the most thoroughly researched types of cancer in humans (Nowell & Hungerford, 2004; Rowley, 2004). It is one of the four main kinds of leukaemia disease that specifically affecting the myeloid cell (Shallis et al., 2019). CML is a slow-growing cancer, and it has three phases starting with the chronic phase, followed by further progress by the cancer cell in the accelerated phase, and lastly, the blast phase or blast crisis (Melo & Barnes, 2007; Rumpold & Webersinke, 2011). The immune system of patients with CML is compromised, making it difficult for them to fight against infections.

Chronic myeloid leukaemia cells are not fully mature as normal blood cells. When the bone marrow produces too many CML cells, it can interfere with normal cell production and pass through the bone marrow into the bloodstream. It will prevent the bone marrow from producing an appropriate quantity of red blood cells, normal white blood cells, and platelet-forming units that can cause the patient to be more susceptible to anaemia, infection, or easy brushing and bleeding.

Chronic myeloid leukaemia is a clonal disorder. It has been found that up to 95% of the patients have this hallmark of CML characteristic, which is the reciprocal translocation between the long arm of chromosome 9 and 22 (Faderl et al., 1999). It has been described since the first discovery until a recent study that the fusion will result in the Philadelphia chromosome (Ph) carried by the hematopoietic cell (Roskoski, 2022). The Philadelphia chromosome was the first chromosomal aberration consistently linked to a human malignancy of CML (Nowell & Hungerford, 2004; Nowell, 2007; Rowley, 2004).

This fusion between these two chromosomes causes the generation of protein that encodes the BCR-ABL with constitutively active protein tyrosine kinase as a molecular consequence of this translocation (Druker et al., 2001). The fusion forming BCR-ABL oncogene has caused an increasement in the proliferation rate of leukaemia cell, leading to uncontrolled cellular proliferation and inhibiting apoptosis (Deininger & Druker, 2003; Hazlehurst et al., 2009; Holyoake & Helgason, 2015).

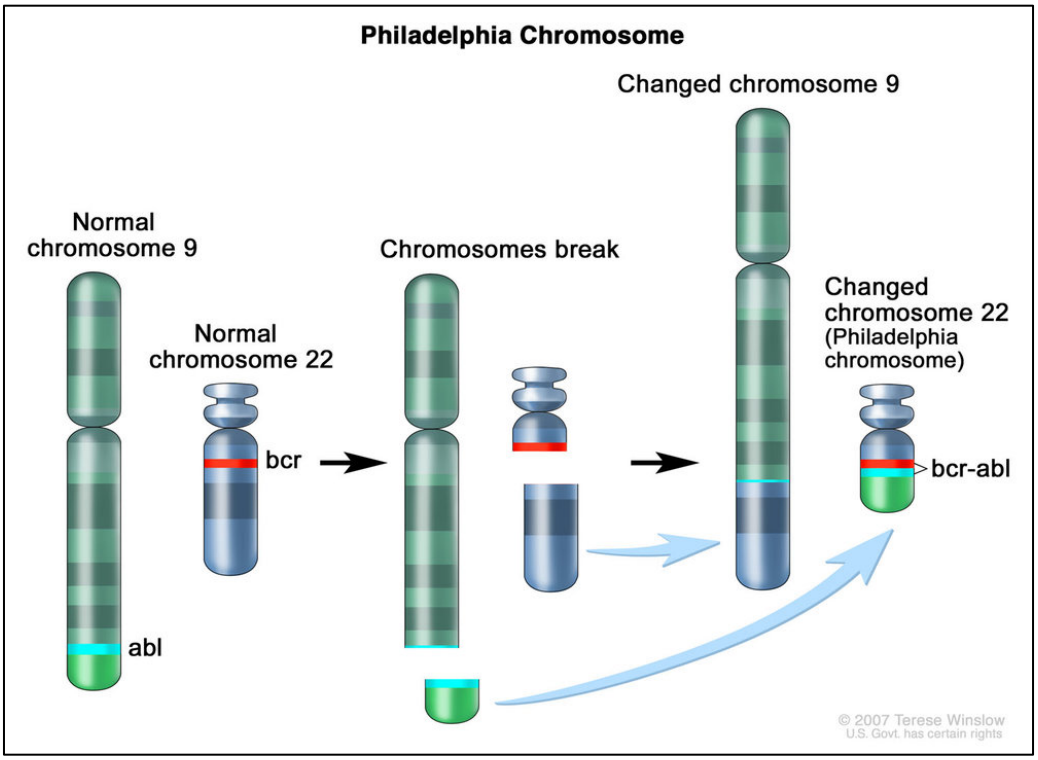

Figure 2.2: Illustration of reciprocal translocation of chromosomes 9 and 22 results in Philadelphia chromosome by Winslow (2007) for the National Cancer Institute (https://www.teresewinslow.com/#/cellular-scientific).

2.2.2 The experimental model of CML study

The pathogenesis of CML has been studied using various experimental settings such as fibroblast, hematopoietic cell line, primary cells, or animal study. CML fibroblast lines are more amenable to manipulation, but there are certain peculiarities in their protein interactions from those of other cell types (Heaney et al., 1997; Senechal et al., 1996). The introduction of BCR-ABL also caused a wide range of responses (Daley et al., 1987; Lugo & Witte, 1989), and there is also a possibility of growth inhibition in certain CML fibroblasts (Renshaw et al., 1992).

Meanwhile, research conducted using primary cells from patients with CML, especially in the chronic phase, has become the gold standard in CML studies. The drawback with this experimental setting is that the cell in the chronic phase in the culture condition tends to mature undoubtedly. This gives only a short amount of time for *in vitro* studies, thus giving a limited and narrow window of time (Pamies et al., 2018). Moreover, non-physiological modification is also possible in conducting primary cells (Garin et al., 2000), thus it leads to a considerable measure of variability between patients.

While it is clear that animal experiments have resulted in life-saving therapies, they have also revealed the crucial importance of physiological and genetic variations between human and animal cells and their contribution to the cost of regulating the study (Akhtar, 2015). They also have demonstrated that hematopoietic cell lines have contributed substantially to our knowledge of CML, as several BCR-ABL proteins have been found to be overexpressed and identified in these Ph-positive cell lines as

opposed to primary cells. It has become a great resource for preliminary testing of upcoming research.

When conducting scientific research on biological processes, cell lines are frequently utilised as a substitute for primary cells. They provide a continuous supply of material, are simpler to utilise, and eliminate the ethical difficulties involved with using animal and human tissue. Their benefits extend to a reduction in the overall application cost. Cell lines are also important because they make up a stable group of cells, allowing for repeatable experiments where reliable results can be obtained (Chaudhary & Singh, 2017). Vaccine manufacturing, medication metabolism and cytotoxicity testing, antibody development, gene function analysis, tissue engineering, chemical synthesis, and many other fields of study have all benefited greatly from the availability of cell lines (Dolskiy et al., 2020; Kaur & Dufour, 2012).

There have been numerous attempts to generate a chronic myeloid leukaemia cell line as a model for studying the differentiation of human leukaemia cells. The K562 cell line was the pioneer strain discovered in 1975. Following this, other studies have been conducted to generate a plethora of cell lines specifically designed to address CML cells, including KCL-22, LAMA84, KU812, and many others (Kishi, 1985; Kubonishi & Miyoshi, 1983). Various research also has employed these cell lines to represent as CML disease (Aalaei et al., 2019; Clapper et al., 2021; Fathi et al., 2019; Mahon et al., 2000; Tusa et al., 2020).

A previous study has exhibited a comprehensive analysis of 35 proteins with different levels of expression that showed LAMA84 cells were more likely to express proteins that led to an invasive behaviour in tumour progression and metastasis regulation. In contrast, drug-resistance-related proteins were most strongly expressed in K562 and KCL22 cells (Fontana et al., 2007). Although these CML cell lines share a similar pathogenic phenotype, the differences in their protein expression profiles support the notion that they constitute a distinct type of phenotypic leukaemia subclasses.

K562 cell line is originally from the human cell derived from 53 years old female patient with the blastic phase of chronic myeloid leukaemia. It has been reported to carry Ph chromosome marker (Lozzio & Lozzio, 1975). They have reported a strong proliferative ability of the K562 cell line that is able to grow effectively in suspension cultures while maintaining its original karyotype. It has been one of the common cell lines used to represent CML disease *in vitro*.

The K562 cell line also has served as a reliable 'workhorse' in the field of biomedical science since it was discovered (Butler & Hirano, 2014; Drexler et al., 2004; Grzanka et al., 2003; Zhou et al., 2019). In addition, it is one of the few extensively employed cell lines in high-throughput gene-targeting screening (Adamson et al., 2016; Arroyo et al., 2016; Han et al., 2017; Liu et al., 2017; Morgens et al., 2016; Wang et al., 2015). It has helped to advance the knowledge of fundamental human biological mechanisms and has been essential in both basic and translational cancer research.

2.2.3 Current treatment of CML

More than 90% of CML patients were found to have the BCR-ABL tyrosine kinase (TK). It can be as targeted molecular therapy in order to inhibit the oncogene activity from further proliferating out of control (Druker, 2008; Melo & Barnes, 2007; Yildirim et al., 2016). CML can be efficiently treated during the chronic phase (CP) by using a tyrosine kinase inhibitor (TKI). It is a type of drug that targets the mutant BCR-ABL protein by blocking its effects that have been found in CML cells. TKI therapy also has been approved in the clinical trial (Quintas-Cardama et. al., 2007). TKI was referred as molecular targeted therapy due to the specific approach to block the effect of a specific protein that causes the transformation of leukaemia cells. Improved treatment regimens over the past few decades have allowed exceptionally significant development in the field of haematological malignancy treatment with the development of targeted therapies in TKI (Antar et al., 2020; Rossari et al., 2018).

The therapy started with imatinib has widely used as the standard first-line treatment (Druker et al., 2001; Druker et al., 1996; Kantarjian et al., 2002; O'Brien et al., 2003). Imatinib have been tested for clinical investigation and application in 1998 before it continued to be used and approved by the Food and Drug Administration (FDA) in 2001 (Cohen et al., 2002; Sampaio et al., 2021). It has resulted in outstanding responses in patients with CML. Nonetheless, despite the excellent results with imatinib, a significant fraction of patients, notably those who have been treated in the accelerated phase and blastic stage, eventually acquire resistance to the drug (Druker et al., 2006; Hochhaus et al., 2017; Johansson et al., 2002; Quintas-Cardama et al., 2007). Even after discontinuing the imatinib treatment, molecular relapses were observed.

Therefore, all of these patients have to shift to the second-line therapy of TKI in intense conflict against this CML disease. Following the failure of imatinib, both nilotinib and dasatinib as the second generation of TKI have proved highly efficient to treat patients who becomes immunologically intolerant to imatinib (Hochhaus et al., 2007; Hughes & White, 2013; Leitner et al., 2011; Radich et al., 2018; Talpaz et al., 2006). It is debatably that this is the most prolific example of effective tailored therapy for targeted CML treatment to date.

Both of nilotinib and dasatinib have demonstrated equally effective, and no apparent difference was discovered in clinical therapy and studies (Garg et al., 2009; Mealing et al., 2013; Park et al., 2015). The efficacy of both secondary TKI was linked to significant high molecular response rates (Kantarjian et al., 2006; Talpaz et al., 2006), prolonged lifespan, and low rate of toxicity over long-term interval (Scalzulli et al., 2021). Nilotinib is also shown to be more cost-effective when taken into account the annual expenses, as well as after a follow-up period of one year (Adel et al., 2021). A study published by Li et al. (2017) also suggested that nilotinib's comparative effectiveness to dasatinib was associated with increased patient survival, improved quality of life, and fewer healthcare expenditures.

2.2.4 Nilotinib as the second-line treatment

Nilotinib is a new oral TKI that is used as a second-generation treatment which rationally designed to overcome imatinib resistance in CML (Giles et al., 2010). Nilotinib is a phenylaminopyrimidine derivative that was industrialised through the unification of ABI kinase and imatinib's crystalline structure in a complex (Weisberg et al., 2005). It has improved target specificity by demonstrating better selectivity and efficacy against BCR-ABL than imatinib. Specifically, it aims at the contributory oncoprotein in CML, which is the inactive form of BCR-ABL kinase. It has been used and approved since 2007 to treat patients in chronic and accelerated phase (Leitner et al., 2011; Tian et al., 2018).

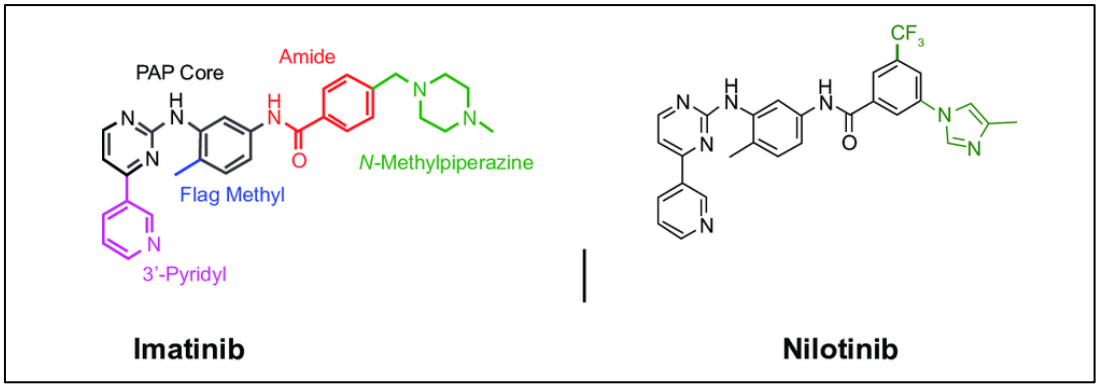

Figure 2.3: Chemical structure of imatinib and nilotinib used for the CML treatment (Martinelli et al., 2007).

A previous study also revealed that nilotinib is significantly more potent and substantially more effective than imatinib in suppressing the BCR-ABL kinase activity (Konig et al., 2008; Saglio et al., 2010). According to Rosti et al. (2009) and Swords et al. (2009), the increased affinity of the binding site causes nilotinib to be much more potent and efficient than imatinib in regulating BCR-ABL even though they are structurally similar, as exhibited in Figure 2.3. It also appears to be safer and in a better position to withstand toward resistance than imatinib. In 2007, the US FDA has

authorised and approved nilotinib for the treatment of patients with CML who have developed resistance or intolerance toward the imatinib (Neelakantan & Apperley, 2013).

These last few years, patients with CML have had significantly better prognoses due to TKI therapy. The aim of the treatment has shifted from prolonging the survival of life expectancy to enhancing the quality of life of the patient (Bower et al., 2016; Cortes et al., 2019; Hochhaus et al., 2020). However, it continues to exist as a challenging disease in order to treat the CML patient in a blast crisis phase even though advanced TKI therapy has been introduced (Pietarinen et al., 2015). Each of the therapy strategies has its own limitation and disadvantages. Whenever the TKI treatment is significantly less effective, it causes the patient to be less favourably to respond toward the therapy due to the more aggressive and combative characteristics of the advanced stage of CML.

Even the original TKI, such as imatinib, did not show any toxicity effect on the body after prolonged contact. Still, the intake of imatinib gives several side effects to the patients, and by switching the highly potent TKI such as nilotinib and dasatinib also could not be able to improve the overall survival (Bosi et al., 2019; Cortes & Lang, 2021; Hughes & White, 2013). As of right now, there are no treatment alternatives that are responding effectively in the blast crisis phase of CML with less negative consequences (Sampaio et al., 2021). Consequently, it is necessary to determine another innovative treatment and require identification of new pharmacological options to treat CML patients in a blast crisis.

Moreover, it is happened to be a target to treat the patient while they are at the chronic phase since no treatment currently has a significant impact in controlling or reversing the disease in the more advanced phase of the CML (Melo & Barnes, 2007). Research on CML is currently expanding beyond the existing mechanism that initiates the CML into other pathways (Cortes & Lang, 2021). It can be a possible treatment targeting mechanism in CML.

2.3 Nicotinamide in cancer therapy

Combination therapy is a keystone of cancer therapy where two or more therapeutic agents are combined to create a new or improved treatment for the disease (Blagosklonny, 2004; Gurunathan et al., 2018; Yap et al., 2013). It could potentially lower or minimise relapse and enhance the advantages of therapeutic anti-cancer, including decrease the rate of tumour growth or potential for metastasis. At the same time, it can cause or initiate apoptosis in the cancerous cell. In order to provide more targeted and efficient cancer therapy, newer approaches are required by not relying solely on a single treatment agent. Therefore, through the combination of treatments employing a naturally occurring chemical is an example of a new approach in treating malignancies (Cheng et al., 2020; White et al., 2001). Combination therapy also could prevent or reduce toxic effects on normal cells and yet, at the same time, also capable of inflicting cytotoxic effects on cancer cells. This will cause the toxicity to be significantly less because different pathways may be targeted (Mokhtari et al., 2017).

Usage of natural compounds and biological and synthetic agents to repress cancer initiation and progression have been involved in cancer chemoprevention which includes hormones, minerals, and vitamins (Benetou et al., 2015). Nicotinamide, as part of vitamin B_3, has been reported to exhibit the chemoprevention characteristics in skin cancer that is involved in a cell line, animal study, or human tissue (Nikas et al., 2020). Nicotinamide also elevated the reduction of ATP in UV-irradiated keratinocytes *in vitro* and, at the same time, was able to enhance the expression of enzyme protein to induce cell repair metabolism (Sivapirabu et al., 2009).

2.3.1 Nicotinamide

Nicotinamide, an active form of vitamin B_3, is the amide derivative of water-soluble niacin, often known as a nicotinic acid amide or niacinamide. Wherein various organisms, it able to provide many physiological and pharmacological functions and benefits (Song et al., 2019). It is a precursor for the synthesis of nicotinamide adenine dinucleotide (NAD) and phosphorylated derivative, nicotinamide adenine dinucleotide phosphate (NADP) (Audrito et al., 2011; Fricker et al., 2018). Both of them engaged in an extensive variety of biological and cellular mechanisms, such as in the energy metabolism (Li et al., 2004) and also in chemical reactions in the body. The reaction also includes ADP-ribosylation concerning the processes of DNA repair, apoptosis (cell death), transcription, cell cycle, and DNA replication (Ida et al., 2009).

The dietary sources of nicotinamide and niacin are widely accessible. It can be found naturally in plants and animals, such as in the liver, yeast, dairy product, bean, green vegetable, bread, coffee, and many more. Hydrolysis of NAD^+ and $NADP^+$ to nicotinamide can be enzymatically hydrolysed from the uncooked food during the process of cooking (Jacob & Swendseid, 1996). For years, nicotinamide also has been used within required doses with minimal side effects by countless of people. It was shown to have low toxicity and side effects that do not cause flushing, itching, hypotension, or headaches under doses of consumption (Hwang & Song, 2020; Knip et al., 2000; Ranchoff & Tomecki, 1986). Nicotinamide is also an inexpensive supplement that is widely accessible in any pharmacy and can be taken as an additional supplement for consumption.

2.3.2 Association of nicotinamide in cancer cell

Cancer patients were found to be at risk of niacin deficiency in a few studies due to the suppressed NAD^+ levels from chemotherapy. Therefore, maintaining an optimum level of nicotinamide is important to these patients (Surjana et al., 2010). Where NAD^+ is one of the important and essential energy sources and cofactor required in several metabolic and cellular processes (Belenky et al., 2007; Bieganowski & Brenner, 2004). Since its actual potential in the human body was revealed, the medical community has been increasingly focusing on NAD to better its patients' lives.

On the other hand, nicotinamide also functions as a sensitising agent. It counteracts chemotherapy and radiation therapy by increasing blood flow that will allow the elevated oxygen supply to the tumours (De Soto et al., 2006). Hence, it shows the clinical efficacy of nicotinamide against radiotherapy resistance due to tumour hypoxia (Tharmalingham & Hoskin, 2018). Thus, oxygen deprivation of tumour cells that referred as tumour hypoxia is reduced. Hypoxia in tumour happened caused by the aberrant growth of tumour cells, which leads to a reduction in the supply of oxygen and nutrients through the tumour’s blood streams (Muz et al., 2015; Vaupel & Harrison, 2004). The term "sensitising agent" refers to any substance that might cause an altered state of responsiveness that cause an immune system to overreact when been reintroduced.

According to a study by Ida et al. (2009), a nicotinic acid-related compound including nicotinamide, has the ability to inhibit the K562 cell proliferation by 50% to 90% and also particularly promotes and initiates the human acute promyelocytic leukaemia HK-60 cells to differentiate in the presence of those substances. More recently,

nicotinamide has shown to be able to suppress the proliferation as well as increase the apoptosis activity (Song et al., 2019) in breast cancer cell line (Jafary et al., 2014; T. Wang et al., 2013), liver cancer (Park et al., 2012), bile duct carcinoma *in vitro* (Wang et al., 2018) and also pancreatic cancer (Zhang et al., 2013) through various pathways.

Published data by Chen et al. (2015) demonstrated that this one type of vitamin B_3 also has the ability to preserve from the effects caused by the UV radiation and thus decrease the proportion of new premalignant skin cancer. They have found that nicotinamide has reduced and lowered the incidence of a variety of skin malignancies when treated with nicotinamide from 11% to 30% compared to other types of treatment, and there is no evidence of benefit after discontinuation of nicotinamide. They also have come to agree that the intake of oral nicotinamide as a therapeutic option was both safe and medically beneficial to be used in the treatment of high-risk patients with skin cancer.

UV radiation can cause damage to DNA, disturb the immunity system (Bernard et al., 2019; Yarosh, 2004), and deplete the cellular ATP, thereby inhibiting the DNA repair (Park et al., 2010; Roy, 2017). The production of ATP that can be used for DNA repair requires NAD^+, which can be obtained from nicotinamide that also acts as a precursor of NAD^+. Therefore, glycolytic blockade, ATP depletion, and the level of immunosuppression caused directly by UV radiation can be reduced and prevented by the nicotinamide (Malesu et al., 2020; Park et al., 2010; Surjana et al., 2013; Thompson et al., 2014; Yarosh, 2004).