EXPLORING CASPASE-INDEPENDENT APOPTOSIS MECHANISM OF NEW β-CARBOLINE DERIVATIVES IN CHRONIC MYELOGENOUS LEUKEMIA

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by

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TABLE OF CONTENTS

LIST OF TABLES

Page

LIST OF FIGURES

Page

LIST OF SYMBOLS

B	Beta
µl	Micro litre
Ml	Milli litre
°c	Degree Celsius
Mm	Milli molar
Mm	Micro molar
Δ	Delta
%	Percentage

LIST OF ABBREVIATIONS

CML	Chronic myelogenous leukemia
Cas	Caspases
AIF	Apoptosis inducing factor
Endo-G	Endonucleases G
GC-MS	Gas chromatography-mass spectrometry
AML	Acute myelogenous leukemia
ROS	Reactive oxidative species
MAPK	mitogen-activated kinases
TKIs	Tyrosine kinase inhibitors (TKIs)
DNA	Deoxyribonucleic acid
mRNA	messenger Ribonucleic Acid
APS	Ammonium Persulfate
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide

MENEROKA MEKANISMA APOPTOSIS-TIDAK BERGANTUNG PADA KASPAS YANG TERINDUKSI OLEH TERBITAN β-CARBOLINE BARU DALAM LEUKEMIA MIELOGENUS KRONIK

ABSTRAK

Kanser dikenali sebagai salah satu punca kematian yang serius dalam kalangan penduduk dunia. Salah satu rawatan yang paling boleh dipercayai untuk kanser adalah kemoterapi, walaupun rintangan dadah dan kesan sampingan yang teruk telah menyebabkan kemunduran besar untuk rawatan yang berkesan. Sejak kebelakangan ini, didapati bahawa memahami mekanisme tindakan agen kemoterapi mungkin membawa kepada rawatan yang lebih berkesan untuk penyakit ini yang dinamakan sebagai terapi sasaran. Dalam pencarian berterusan untuk alternatif ejen antikanser baharu, alkaloid β-karbolin telah dikenal pasti sebagai calon yang baik untuk ubat kemoterapi baharu. Sebatian β-karbolin M25, M56 dan M62 telah disintesis dan ketulenannya disahkan menggunakan analisis kromatografi gas-spektrometri jisim (GC-MS). Sitotoksisiti sebatian telah dinilai menggunakan ujian MTT terhadap garisan sel CML manusia K562 serta sel-sel bukan kanser Hs27 garisan sel kulup manusia dan garisan sel fibroblas tikus BALB/c3T3. Nilai IC50 untuk K562 ialah 0.8μM, 0.78μM dan 0.123μM untuk M25, M56 dan M62 masing-masing, dan nilai indeks selektiviti (SI) mereka dilaporkan berada dalam julat 2.9 dan 42.53 untuk kedua-dua saluran sel bukan kanser. Di samping itu, analisis apoptosis telah dijalankan menggunakan sitometri aliran dan ketiga-tiga sebatian menyebabkan apoptosis sebagai mod kematian sel

pada garisan sel K562. Sebelum menyiasat mekanisme tindakan sebatian ini, analisis dalam siliko telah dijalankan di mana kajian dok molekul mengesahkan pertalian pengikatan sebatian ini sebagai ligan dengan protein berkaitan apoptosis seperti Apoptosis Inducing Factor (AIF), Endonucleases G (Endo G), caspase 3 (CAS 3), caspase 9 (CAS 9) dan caspase 8 (CAS 8). Untuk mengesahkan ekspresi gen apoptosis, kajian RT-qPCR telah dijalankan dan dengan itu mengesahkan ekspresi gen ini. Tambahan pula, terjemahan dan ekspresi protein apoptosis yang terlibat dalam laluan kematian sel telah dinilai menggunakan kaedah western blot. Daripada kajian ini, boleh dikatakan bahawa sebatian yang baru disintesis mempunyai potensi untuk dibangunkan sebagai agen anti-leukemik yang selamat dan berkesan kerana ia mencetuskan laluan apoptosis dan pengawalan semula protein apoptosis. Potensi sebatian serta pemahaman tentang mekanisme apoptosis akan membuka jalan untuk pembangunan masa depan sebatian β-karbolin sebagai farmaseutik yang menjanjikan bukan sahaja untuk leukemia tetapi untuk kanser lain juga.

EXPLORING CASPASE-INDEPENDENT APOPTOSIS MECHANISM OF NEW β-CARBOLINE DERIVATIVES IN CHRONIC MYELOGENOUS LEUKEMIA

ABSTRACT

Cancer is known as one of the serious causes of mortality among the world population. One of the most reliable treatments for cancer is chemotherapy, though drug resistance and severe side effects have caused major setbacks to effective treatment. Lately, it has been found that understanding the mechanism of action of a chemotherapy agent might lead to a more effective treatment for this disease which is named as targeted therapy. In the constant search for new anticancer agent alternatives, β-carboline alkaloids have been identified as good candidates for a new chemotherapy drug. β-carboline compounds M25, M56 and M62 were synthesised, and their purities were confirmed using gas chromatography-mass spectrometry (GC-MS) analysis. Cytotoxicity of the compounds were evaluated using MTT assay against K562 human CML cell line as well as non-cancer cells Hs27 human foreskin cell line and BALB/c3T3 mouse fibroblasts cell line. The IC50 values for K562 were 0.8μM, 0.78μM and 0.123μM for M25, M56 and M62 respectively, and their selectivity index (SI) values were reported to be in the range of 2.9 and 42.53 for both non-cancer cell lines. In addition, apoptosis analysis was conducted using flow cytometry and all three compounds induced apoptosis as a mode of cell death on K562 cell line. Prior to investigating the mechanism of action of these compounds, in silico analysis was conducted whereby molecular docking study confirmed the binding

affinity of these compounds as ligands with apoptosis-related proteins such as Apoptosis Inducing Factor (AIF), Endonucleases G (Endo G), caspase 3 (CAS 3), caspase 9 (CAS 9) and caspase 8 (CAS 8). To confirm the expression of apoptotic genes, RT-qPCR study conducted and thus confirmed the expression of these genes. Furthermore, the translation and expression of apoptotic proteins involved in the cell death pathway were evaluated using the western blot method. From this study, it could be postulated that the newly synthesized compounds have the potential to be developed as a safe and effective anti-leukemic agent as it triggers an apoptotic pathway and the upregulation of apoptotic proteins such as AIF, Endo G, CAS 3 and Cas 9. The potential of the compounds as well as the understanding of the mechanism of apoptosis will pave the way for future development of β-carboline compounds as promising pharmaceutics for not only leukemia but for other cancers as well.

CHAPTER 1

INTRODUCTION

1.1 Background of study

Cancer has become one of the top common causes of death worldwide, and the number of cancer cases is growing at an alarming rate. The International Agency for Research on Cancer has been estimated that in 2020, 19.3 million new cancer cases and almost 10.0 million cancer deaths have occurred globally (Sung et al., 2021). A report by the World Health Organization (WHO) stated that in 2019, cancer is the leading cause of death globally for people of at least 70 years old.

Cancer is a kind of a disease which is characterized by cell growth that is uncontrollable and cell death that is inhibited. There are two variations in this disease which is known as haematological cancer and tumour. The general causes are mutation in genetic level due to environmental factors or inherited factors. Surgery or radiography is popularly known as the basic treatment which is applicable for non-metastatic cancer type. Anticancer drugs such as chemotherapy drugs are available as options for metastatic cancer. Even though surgery and radiography treatments are considered as the primary source of treatment and most efficient method for tumour cancer, but these may be ineffective to control metastatic cancer such as in myelogenous leukemia which is the type of cancer that spreads throughout the body. Therefore, chemotherapy drugs are playing an important role in successful treatment of both metastatic and non-metastatic types of cancer in patients (Pérez-

Herrero and Fernández-Medarde, 2015).

Chemotherapy drugs which are toxic to cancer cells can reach organs in the body through the blood stream, thus inhibiting uncontrolled cancer cell growth. However, chemotherapy drugs also kill normal cells which are essential in the body.

For instance, chemotherapy drugs kill rapidly growing cells of hair follicles, bone marrow and gastrointestinal tract, and this is considered as an adverse side effect of chemotherapy. In between the early 1940's and 1950's, the Food and Drug Administration (FDA) approved primary chemotherapy drugs which were antifolate drugs, nitrogen mustards and methotrexate. Since then, many chemotherapy drugs have evolved from scientific breakthroughs (Pérez-Herrero and Fernández-Medarde, 2015). The improvement of chemotherapy drugs can be clearly proved by the current successful treatment in cancer through adjuvant or combinatory chemotherapy and approval of important chemotherapy drugs such as cisplatin and paclitaxel. The success rate of cancer treatment using chemotherapy drugs were become well establish through the discovery of cell signalling pathway in 1990's which allows targeted therapy. This chemotherapy drugs generally blocks the uncontrolled cell proliferation by blocking specific biologic transduction pathways or cancer proteins that are involved in tumour growth and progression such as receptors, growth factors, kinase cascades or apoptosis and angiogenesis-related molecules related (Vasan et al., 2019).

For the past decades, chemotherapeutics is considered as the most reliable and effective treatment for cancer. However, drug resistance towards available

chemotherapy drugs has become a major problem in successful treatment rate. As a primary solution for this problem, novel treatment strategies were developed such as combining administration of chemotherapy agents with non-overlapping mechanisms of action, dose intensity, shorter-interval administrations of chemotherapy and higher doses of chemotherapy. Eventually those treatment strategies with chemotherapy drugs were led to achieve greater success rate in treating cancer. Lately, it been found that understanding the mechanism of action of a chemotherapy agent might lead to a much effective treatment for this disease which is named as targeted therapy (Herraiz et al., 2010).

β-carboline is an active alkaloid originally isolated from a plant called *Peganum harmala* L. family Zygophyllaceae which is structurally related to harmine. It has been traditionally consumed to treat various ailments, which triggered interest for its diverse pharmacological effects (Abdelsalam et al., 2018). The anticancer properties of β- carboline have been observed across various members such as harmane, harmine, harmaline, harmol and callophycin. Since the anticancer properties of β-carboline is well established, anticancer drugs derived from β-carboline scaffolds are gaining popularity. Developing a single drug of numerous biological targets is a novel therapeutic method gaining acceptance. This is because anticancer agents targeting a single biological target have limited practical relevance in the treatment of complicated diseases like cancer (Kumar et al., 2017). Commonly reported action to treat cancer cells are DNA intercalation, topoisomerase I AND II inhibition, blocking of cell mitosis and targeting specific cancer signalling pathways (Pistritto et al., 2016). Although this alkaloid is well known for its anticancer and anti-

proliferative properties, its mechanism of action as anticancer agent in the upstream and downstream pathways is less studied.

1.2 Problem statement

Conventional chemotherapeutics, regardless of their distinct targets and mechanisms, primarily induce cell death via caspase- dependent apoptosis, which is the best-known modality of programmed cell death (Pistritto et al., 2016). Meanwhile, cancer cells are usually sensitive to caspase-dependent apoptotic induction initially, but eventually they become drug-resistant due to the dysregulation of apoptotic machinery, manifested as the over-expression of anti-apoptotic proteins and the defects in pro- apoptotic factors (Mohammad et al., 2015). Therefore, developing new drugs and methods that can specifically treat drug-resistant cancers is an urgent task for saving lives. Preliminary study was originally reported by Kamaruzaman (2018) (Kamaruzaman et al., 2018). Three compounds have shown the highest activity and selectivity in killing human CML cells. However, unexpectedly, caspases were not upregulated, and this is unlike the conventional apoptosis mostly discussed in the literature as caspases were thought to be the key symbol of apoptosis.

This interesting finding prompted extensive literature search, that led to a unique non- classical route of apoptosis, known as caspase-independent apoptosis, which has been emerging gradually in the literature to denote a defined alternative apoptotic route. In mediating this alternative apoptotic pathway, most literature reported that AIF is the unique feature of this cell death pathway which enables

apoptosis in the absence of caspases activation. In addition, Endo G has also been reported to contribute to AIF- dependent apoptosis (Herraiz et al., 2010). However, research on AIF is limited, so precise role of AIF in the of promotion and regulation of apoptogenic processing and mechanism remains elusive. Therefore, this study will provide sufficient evidence of the mechanism regulating apoptogenic processing by AIF and its contributing protein, Endo G.

Conventional chemotherapeutics which mediates caspase-dependent apoptosis can sometimes be ineffective due to drug-resistance (Debatin, 2004). Therefore, finding new drug candidates with new alternative mechanisms should be a priority in cancer research.

A unique alternative apoptotic route, which follows a caspase-independent mechanism may be a viable option for an effective chemotherapeutic. Therefore, the caspase- independent apoptosis discovered in our laboratory could be a new unchartered pathway to finding an effective treatment for leukemia. Such discovery warrants for a detailed and specific set of experiments to determine the mechanism of action responsible for the leukemic activity of β-carboline. So far, there has been no reported study claiming that the induced apoptosis by β- carboline is caused independent of caspases in leukemia cells. Thus, this study will also be the first of its kind to discover this new potential of β- carboline in eliciting a unique pathway of apoptosis.

1.3 Objectives

Main objective: To determine the mechanism of apoptosis as induced by β-carboline compounds in CML cell line K562. In achieving this goal, the following objectives must be accomplished:

i) To determine the binding affinity between β-carboline compounds and apoptosis-related genes in inducing apoptosis through in silico study

ii) To evaluate anticancer effect of β-carboline compounds and mode of cell death in human CML cell line K562

iii) To identify apoptosis-related proteins such as AIF, Endo G, CAS 3, CAS 8 and CAS 9 that involved in inducing cell death in human CML cell line K562 by β-carboline compounds through gene and protein expression studies

CHAPTER 2

LITERATURE REVIEW

2.1 Introduction to Anticancer Research

The ultimate objective of cancer treatments is to successfully impede the growth and dissemination of cancerous cells while minimizing any potential harm to healthy cells. A noteworthy aspect of anticancer research revolves around the development of medications that possess the capability to either eradicate cancer cells or halt/impede their process of cell division. Many of these therapeutic drugs are comprised of active substances that effectively induce a process known as apoptosis within cancer cells. Apoptosis, a programmed cell death mechanism, is pivotal in regulating cancer and various physiological processes within cells. The essence of apoptosis lies in the fact that cancer cells undergo self-destruction. While apoptosis appears to bea pivotal factor in cancer therapies, it is crucial to acknowledge the existence of other types of programmed cell deaths, namely necroptosis, pyroptosis, and ferroptosis, which significantly contribute to the overall anticancer process.

These alternative pathways may work with apoptosis or serve as alternative mechanisms (Galluzzi et al., 2018; Park et al., 2023). Furthermore, scientific research has established that apoptosis and other programmed cell death can occur through two distinctive pathways, specifically intrinsic or extrinsic apoptosis. Any inhibition or impairments within these apoptotic pathways can lead to the development of cancer cell resistance against apoptosis, thus hampering the effectiveness of cancer

treatments (Green & Llambi, 2015).

Chronic myelogenous leukemia (CML) is a chronic malignant hematologic cancer of clonal myeloproliferative disorder with an acquired genetic abnormality affecting hematopoietic stem cells (HSCs) and caused by Bcr-Abl tyrosine kinase (LCR-ABL1) resulting from a reciprocal translocation between chromosomes 9 and 22 (Nowell & Hungerford, 1960; Soverini et al., 2018; Loscocco et al., 2019; Menger et al., 2024). By impairing cells' ability to differentiate into their proper counterparts in the bone marrow, the capacity of chronic phase CML cells to differentiate molecularly and morphologically disappears. CML progresses through two additional advanced phases: accelerated phase (AP) and blast phase (BP, with the appearance of two potential beneficiaries, lymphoid or frank myeloid blast crisis) (O'Brien et al., 2003; Zhao etal., 2022).

Although the LCR-ABL-targeted tyrosine kinase inhibitors (TKIs) such as imatinib mesylate have mostly converted CML from a generally late-fatal cancer to a manageable lifelong cancer, treatment issues, including cost, drug intolerance, and patient compliance, may also contribute to some patients who fail therapy or do not respond to therapy (Druker et al., 2006). A caspase- dependent or caspase-independent mechanism can mediate apoptosis. Some CML agents, such as imatinib, trigger caspase-independent apoptosis (Druker et al., 2006; Soverini et al., 2018; Menger et al., 2024).

2.2 Definition of Cancer

Cancer is a disease characterized by abnormal cell growth and the ability to spread to other body parts. Cancer is not a single disease but a group of related diseases, and many forms of cancer are treatable if detected early. Tens of millions of people survive, and many remain with a relatively good quality of life (Hanahan & Weinberg, 2011; Hanahan, 2022). Carcinogenesis is the multi- step process of cancer formation.

Each of these steps involves multiple changes at the genome level, including mutations, gene amplifications, deletions, and epigenetic modifications that cause oncogene activation and tumour-suppressor gene inactivation (Vogelstein & Kinzler, 2004). Once the oncogene is activated and the tumour suppressor gene is lost, the cells are no longer under the body's normal control, and their ability is inhibited.

One of the most aggressive forms of blood cancer is leukaemia, characterized by the uncontrolled development of circulating blood cells. Any suppression or prevention of differentiation (maturation) at the hemopoietic progenitor or stem cell level invariably causes a neoplasm, leukaemia (Faderl et al., 1999; Kana et al., 2024). Identifying the idiopathic neoplasm may reduce the characteristic heterogeneity based on the patient's disease progression. CML is a toxic malignant blood disease and is the third most common leukemia. CML is a clonal disorder characterized by the increased proliferation of immature and mature

granulocytes, erythrocytes, and platelet lines. CML can occur at any age, with 91 to 93% of cases occurring in adults (Cortes et al., 2021). More abnormalities, such as cyclin-dependent kinase (CDK) inhibitors, are related to CML. As hematopoietic stem cells feed and enhance certain foods, the stem cells also pass on the Ankarcinoma Center syndrome. Atypical tumour disease leads to the development of entire cells and the accumulation of myelobroma in the bone marrow. Apoptosis is now a retroactive step to the free hunting and eliminating the body's cells, including defective footprints. Extramedullary involvement is another feature of the CML development stage that occurs in about 12% of CML patients. Apoptosis serves as a quality control mechanism in addition to cell death regulation. Caspase enzymes are the initiators of apoptosis (Elmore, 2007).

2.3 Cancer Statistics

Based on the WHO Global Cancer Observatory, fewer data are available on less common blood cancers. In contrast, more common blood cancers such as lymphomas, myelomas, or leukaemia, except CLL, are better statistically represented in this registry. This initiative ensures the collection, methodology, and analysis of blood cancer data and accurate statistics on the incidence, prevalence, and outcomes of all blood cancers are available in the future. As many blood cancer countries have an overall prevalence of fewer than 5 people per 10,000, cancer registries should ideally provide data on new cases of the disease, data on stage, grading, molecular and chromosomal incidence, treatment modalities, patient survival and

the quality of life of patients, along with information on the prevalence of their condition.

Chronic Myelogenous Leukemia (CML) is reported in Europe by cancer registries annually, withan incidence rate of 1-1.5 per 100,000, with no major gender difference. The average age at diagnosis is around 60 years, although CML can occur at any age (Chen et al., 2013; Pfirrmannet al., 2016). There are few published European data on prevalence, mortality, and survival in CML, but this largely indicates patients with de novo CML and not those on tyrosine kinase inhibitors (TKIs). The new 2020 data report in the CML BloodNet Registry *(https://www.blood.gov.au/)* indicated relapse and resistance in 30% of CML patients, while the updated Hammersmith Hospital UK shows newly diagnosed chronic phase (CP) vs. accelerated and blast phase (BC) patients are maintaining life expectancy before CML diagnosis. Due to the discovery of the Ph chromosome in over 90% of CML cases, CML has been the first disease to be targeted by block and cytogenetically. This has led to advances, and now TKIs and high-dose chemotherapy followed by HSCT, or stem cell transplant, have enabled patients to achieve a similar prevalence age (Goldman & Melo, 2003; Kent & Pollyea, 2023).

2.4 Hallmarks of Cancer

For a long time, cancer was associated with an uncontrollable increase in the number of cells in the body. However, it is currently known that cancer is related to

both the unlimited replicative potential of cells and other fundamental changes, a series of characteristic traits that define the basic behaviours of cells that can develop into tumours.

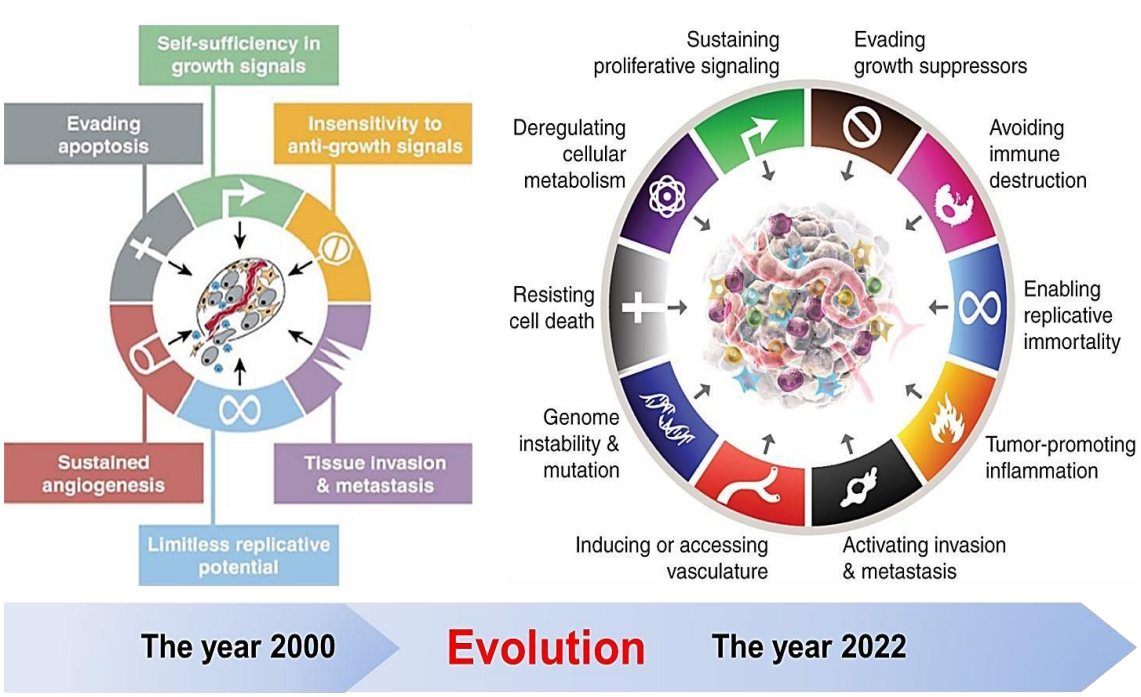

Figure 2.1 Evolution of the cancer cell

The essential characteristics or fundamental behaviors of cancer cells or disease drive the development and progression of primary cancers and their metastases. On Hanahan and Weinberg's initial list of six hallmarks in 2000 (**Figure 2.1**), the first three were considered essential characteristics required to enable the acquisition of the three new "emerging" metastatic hallmarks (the so-called 'Invasion-Metastasis Cascade') (Hanahan & Weinberg, 2000b, 2011a; Hanahan, 2022; Senga & Grose, 2021). The original hallmarks include 1) Sustaining proliferative signalling, 2)

Evading growth suppressors, and 3) Resisting cell death. Additionally, Hanahan and Weinberg presented the other three hallmarks as "Enabling replication". These characteristics have little relevance in most cancers' development and/or progression, with most of these proposed traits being of limited value. Therefore, the updated report further refined the acquired capabilities of cancer cells by adding the second of the two emerging hallmarks for a total of ten.

The acquired capabilities of cancer cells, according to a review of the original proposals for potential hallmarks. Few of these had been directly investigated in 2000 (Hanahan, 2022; Hanahan & Weinberg, 2000a). However, by 2011, enticing evidence of causative connections in the intravital had been discovered for most of the numeric ten. A Jekyll-Hyde and somewhat Heraclitean nature typify these seminal triggering mutations that presage treatment resistance and drive widespread clonal expansion (Hanahan, 2022).

2.5 Chemotherapy Drugs

Cancer is one of the most frequent causes of death globally. Generally, cancer may occur due to several factors, such as radiation, viruses, chronic infections, chemical exposure, or genetic predisposition. However, the principal common point is the existence of mutant cells that are created in different ways and escape from cell cycle regulation and cell death systems. Cancer treatment has several strategies, such as radiotherapy, surgery, and drug therapy. However, the most commonly used method is chemotherapy, which is very effective for rapidly growing cancers or leukaemia (DeVita & Rosenberg, 2012). Chemotherapy refers to drug treatment that

often requires the use of several drugs in combination. Similarly, chemotherapeutic agents can be classified into several groups, such as cytotoxic drugs, anti-metabolites, hormones, anti- angiogenesis, acute promyelocytic leukaemia therapy, monoclonal antibodies, bone resorption inhibitors, topoisomerase inhibitors, covalent-modifying drugs, and other cytotoxic drugs (**Table 2.1**). All chemotherapeutic agents somehow interfere with DNA or microtubules and the cell cycle (Chabner & Roberts, 2005; Mollaei et al., 2021). Developing new drugs through sufficient clinical trials is strictly regulated and arduous. But despite these limitations, many new chemotherapeutic agents are underway for clinical trials to overcome the limitations of existing drugs (Dancey & Chen, 2006).

Standard chemotherapy takes advantage of cancer cells' rapid cell division. However, no chemotherapeutic agent is entirely specific to cancer cells and may also possess some affinity for normal cells that generally divide more rapidly (Longley & Johnston, 2005; Dymova et al., 2021). This can produce some other side effects, such as gastrointestinal problems, bone marrow suppression, alopecia, nephrotoxicity, or peripheral neuropathy. Of course, the degree of general poisoning is also relative to the dose, frequency, patient's metabolism, and cancer cell growth adversely affected.

Table 2. 1 The category of chemotherapy drugs.

Category	**Drugs**	**Mechanisms of action**
Alkylating agents	Altretamine Busulfan Carboplatin Carmustine Chlorambucil Cisplatin Cyclophosphamide Dacarbazine Lomustine Melphalan Oxaliplatin Temozolomide Thiotepa 5-fluorouracil (5-FU) 6-mercaptopurine (6-MP) Capecitabine (Xeloda) Cytarabine (Ara-C)	Damage the DNA
Antimetabolite	Floxuridine Fludarabine Gemcitabine (Gemzar)	Substitute the RNA and DNA blocks

	Hydroxyurea Methotrexate Pemetrexed (Alimta) Epirubicin Idarubicin Daunorubicin Anthracyclines	
Anti-tumor Antibiotics	Doxorubicin (Adriamycin) Actinomycin-D Bleomycin Mitomycin-C Non-Anthracyclines Mitoxantrone	Interfere with the activity of DNA replication enzymes
Topoisomerase inhibitors	Topoisomerase inhibitor I Topoisomerase inhibitor II Topotecan Irinotecan (CPT-11) Etoposide (VP-16) Teniposide Mitoxantrone Docetaxel Estramustine	Interfere with the topoisomerase enzymes and incorporate the unwinding DNA in replication and transcription
Mitotic inhibitors	Ixabepilone	Hinder the cell

	Paclitaxel Vinblastine Vincristine Vinorelbine Prednisone Methylprednisone (Solumedrol)	proliferation and division
Corticosteroids	Dexamethasone (Decadron)	Palliate the chemotherapy side effects
EGFR inhibitors	Tarceva (Erlotinib) Erbitux (Cetuximab) Iressa (Gefitinib)	Blocks the epidermal growth factor receptors on tumor cells

Some cancer cells are resistant to chemotherapy from the beginning, while others develop resistance to chemotherapy after initial cancer cell killing. These non-random subsets of tumor cells, which generally progress based on genetic heterogeneity or genetic instability, including drug resistance, metastatic ability, stem cell self-fate, and maintenance features, are called cancer stem-like cells (CSC) or CSC models (Dean et al., 2005; Ou & Guo, 2007; Gao et al., 2023). These cells are responsible for treatment failure, thus leading to cancer recurrence.

In cancer, particularly in hematologic or leukemia, radiotherapy and chemotherapy can directly suppress the immune response and cause immunosuppression when their effect is beyond the maximum tolerability of white blood cells, called leukopenia. Furthermore, even if severe signs or symptoms disappear after chemotherapy, therapies should be continued to ensure further efficacy, the best overall outcome, and long-term survival with a high standard of living (Faiman & Faiman, 2017; Hoagland, 1982; Pignon et al., 2009).

Multiple drug resistance (MDR) and multidrug resistance protein family transporters, after genetic or epigenetic alteration, are key contributors to chemotherapy response with poor therapeutic effects and generally present suboptimum prognosis (Gottesman & Pastan, 1993; Wuet al., 2020). Therefore, further research is essential to develop more effective, standard, tailored therapy and better risk assessment. Clinical management of patients, therefore, begins with diagnosis.

2.6 Understanding Leukemia

A form of blood cancer, leukemia is a group of blood cell malignancies that result from the inappropriate and unregulated growth of blood cells (Hoffman, 2018). Leukemia commonly originates in the bone marrow, producing many transformed cells occupying a large portion of the bone marrow, leading to bone marrow failure. This disease can affect a person's health and make them more vulnerable to a range of diseases, so it is classified into several types depending on the rate of disease progression. Leukemia is classified into two major categories: acute and chronic. Acute leukemia progresses quickly when compared to chronic form, which indicates that it has a shorter life expectancy. It can also be divided into other types according to the cells affected and the disease subtypes (American Cancer Society, 2019; Society, 2020). The most recent study of leukemia cases reported that it remains in its place at number 10 worldwide, with some leukemia types being reported even more than 70 times annually.

Problems resulting from leukemia are associated with the excessive amount of ill-formed and inexperienced cells that cannot function appropriately. This may impact their action in defense of the body and in several other actions the body requires (Hallek et al., 2018). For instance, stopping bleeding, reducing the betters, and fighting the different charges and other hazards need enough blood cells in the organism. In addition, the skeleton depends on younger and newer cells that may not

be created to exchange older contraventions and keep the skeleton robust. If there were no bigger cells, several side effects might occur, including perfusion, dyspnea, physical weakness, and anemia. This disorder inside the body arises due to an accumulation of malignant and cancerous cells that cause or overrule ordinary work, ending in an irregular gain in numbers (Greaves, 1999).

2.6.1 Overview of Leukemia

Cancers result from uncontrolled growth and the spread of abnormal cells in various parts of the body. Leukemia or 'blood cancer' refers to a range of types of cancer with such likely origins (Ferlay et al., 2021). Leukemia is divided into two types: acute and chronic. Acute leukemia progresses rapidly with severe infection, bleeding, and extreme fatigue, eventually leading to death within a few months without chemotherapy, while chronic leukemia worsens slowly, mainly characterized by the proliferation of premature (blast) cells, increasing the number. In Korea, chronic myelogenous leukemia (CML) accounts for 15% of total cases of leukemia. CMLis caused by the abnormal fusion of two normal genes: BCR and ABL (Jabbour & Kantarjian, 2020).

These physiological changes elevate the ABL kinase activity. ABL kinase activity elevates several signalling proteins, including MAPK, PI3/AKT, mTOR, and STATs (Minciacchi et al., 2021). These intracellular signalling elicit effects on cell proliferation, survival, and angiogenesis in leukemia cells. Furthermore, cells having conditional resistance towards ABL kinase do not show tumor development. Thus, inhibition of ABL kinase is considered an optimal strategy. Patients with CML have substantially increased life expectancy because of the

discovery and clinical application of tyrosine kinase inhibitors, such as Glivec (imatinib), for the BCR-ABL oncogene (Druker et al., 2001). However, inappropriate apoptosis is causing remaining problems, including multidrug resistance (MDR). To solve these problems, studying apoptosis control mechanisms outside of the caspase system is necessary (Fulda & Debatin, 2006). This review discusses the apoptotic mechanisms of novel compounds that cause cell cycle arrest and caspase-independent apoptosis in K562 cells derived from CML.

2.6.2 Chronic Myelogenous Leukemia (CML)

Chronic myelogenous leukemia (CML) is a myeloproliferative disease that is a clonal disorder characterized by the presence of primitive multipotent hematopoietic stem cells. The genetic basis of this disease is the t (9;22) (q34; q11) translocation, which results in the formation of the so-called Philadelphia chromosome. This translocation converts c-ABL on chromosome 9 into the constitutively active oncoprotein BCR-ABL (Heisterkamp et al., 1983). This unique fusion gene product encodes the 210 kilodalton Bcr-Abl protein with a constitutive tyrosine kinase activity, leading to abnormal cell growth and the selective expansion of the myeloid cell line (Ben-Neriah et al., 1986; Waller et al., 1999). Three distinct phases characterize the natural history of CML. Approximately 95% of patients newly diagnosed with CML are in a chronic phase (CP). Over time, without effective treatment, patients enter the accelerated phase (AP) and the acute or blast crisis (BC) of CML, which is usually terminal. The currently available frontline treatment is tyrosine kinase inhibitors (TKIs)

(Druker et al., 2001; Minciacchi et al., 2021). Although the newest generations of TKIs are well-tolerated and have great efficacy, some patients still are not indicated for frontline TKI treatment. The treatments were referred to as "pre-TKI therapies," "TKI users" for individuals who were using TKIs before joining the studies, and "TKI resistant or intolerant" for those who had a documented intolerance or resistance to TKIs (O'Brien et al., 2003). The β-carboline skeleton has a wide range of biological activities, including anti-leukemic effects. A new mechanism of action has recently been suggested; some newly developed derivatives can cause apoptosis in CML cells through a caspase-independent mechanism (Luo & Song, 2021). This article provides an overview of the β-carboline class and its potential properties concerning treating CML.

2.7 Imatinib: The challenges associated with treating it in modern medicine.

Imatinib is a highly effective tyrosine kinase inhibitor that has completely revolutionized the treatment landscape for chronic myelogenous leukemia (CML), a cancer affecting the bone marrow and blood (Druker et al., 2006). However, despite its remarkable success, Imatinib has challenges that cannot be ignored. One of the foremost challenges associated with Imatinib treatment is the development of resistance over time (Shah et al., 2004). While many patients initially respond positively to Imatinib therapy, a significant number eventually develop resistance, rendering the drug ineffective in combating the progression of CML. This necessitates exploring alternative treatment strategies to overcome resistance and ensure improved patient outcomes. In addition to resistance, long-

term side effects pose another concern for individuals undergoing Imatinib therapy. Though generally well-tolerated, Imatinib has been associated with specific adverse effects that may significantly impact a patient's quality of life and overall well- being (Bauer et al., 2021). These side effects can range from mild to severe, encompassing issues like gastrointestinal disturbances, fatigue, muscle cramps, fluid retention, and even cardiotoxicity in rare cases. As a result, diligent monitoring and proactive management of these side effects become essential for optimizing treatment outcomes and maintaining patient safety (Bauer et al., 2021; Rea, 2015).

Despite these challenges, Imatinib remains an indispensable weapon in the fight against CML. With ongoing research, scientists and clinicians are dedicated to unraveling the underlying resistance mechanisms and developing novel therapeutic strategies to overcome this barrier. Moreover, advancements in personalized medicine and targeted therapies promise to mitigate the long-term side effects associated with Imatinib, improving patient compliance and overall treatment outcomes (Hochhaus et al., 2009: Adattini et al., 2022). In conclusion, while Imatinib has rightfully earned its status as a game-changer in treating chronic myelogenous leukemia, it must acknowledge and address its challenges. By striving for a comprehensive understanding of resistance mechanisms and employing innovative therapeutic approaches, healthcare professionals can continue to optimize Imatinib therapy and ensure the best possible care for individuals affected by CML.

Several problems can arise with imatinib treatment, commonly used for various medical conditions. While imatinib can be effective in treating certain diseases, it

has drawbacks. One major concern is the development of drug resistance, where the medication becomes less effective over time (Thompson et al., 2015). Additionally, imatinib can cause various side effects ranging from mild discomfort to severe complications. Some common side effects include nausea, fatigue, muscle pain, and gastrointestinal issues. In rare cases, imatinib has been linked to more serious complications such as liver toxicity and heart problems. Lastly, the cost of imatinib can be prohibitive for many patients, making it inaccessible for those who need it (Kanavos, 2006). All these factors contribute to the challenges associated with imatinib treatment. It is important for healthcare professionals to carefully monitor patients undergoing imatinib therapy and manage any issues that may arise to ensure the best possible outcomes.

2.7.1 K562 Cell Line

A human pathobiont is diagnosed and identified in 95% of cases by Quantitative Reverse Transcription Polymerase Chain Reaction using the BCR-ABL positive phenotype (Baccarani et al., 2009, 2013). Chronic Myeloid Leukemia (CML) originates in the bone marrow. It affects the trabecular stroma of long bones. Diagnosis includes the presence of abnormal liver cells and autoimmune antibodies. A key molecular marker is BCR/ABL, and there are additional chromosomes (EOS is +6) (Rowley, 1973). The clinical presentation is highly variable. In advanced stages, the disease can involve infiltration of the central nervous system, spleen, and skin and may lead to neuropathy, low platelet count, and enlarged spleen (Faderl et al., 1999; Maru, 2012). According to the World Health Institute of Hematology and Endocrinology (WIHE) prognosis, less than 60% of CML patients who are treated with allogeneic cell transplantation survive