

https://dspace.org/

High-throughput drug repurposing for precision medicine in the treatment of leukaemia in South African patients

Larissa, Kenmogne Vanelle

2025
University of the Witwatersrand, Johannesburg
https://hdl.handle.net/10539/50033
Larissa, Kenmogne Vanelle . (2025). High-throughput drug repurposing for precision medicine in the treatment of leukaemia in South African patients [PhD thesis, University of the Witwatersrand, Johannesburg]. WIReDSpace. SDG-3: Good health and well-being

Downloaded from a DSpace repository. https://dspace.org/

High-throughput drug repurposing for precision medicine in the treatment of leukaemia in South African patients



Kenmogne Vanelle Larissa

A Thesis submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Doctor of Philosophy.

Supervisors: Prof Pascaline Fru (WITS)
Dr Deepak Balaji Thimiri GovindaRaj (CSIR)
Prof Ekene Emmanuel Nweke (WITS)
Dr Mutsa Takundwa (CSIR)

DEPARTMENT OF SURGERY, FACULTY OF HEALTH SCIENCES,
UNIVERSITY OF WITWATERSRAND, JOHANNESBURG.
2025

DECLARATION

I, Kenmogne Vanelle Larissa, declare that this thesis entitled “High-throughput drug repurposing for precision medicine in the treatment of leukaemia in South African patients” is my own unaided work. It is being submitted for the Degree of Doctor of Philosophy at the University of Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other university.

Kenmogne Vanelle Larissa Date



signature August 26th, 2025

DEDICATION

To my dear husband and my precious daughter, you have been my refuge and my source of strength, through this journey. I am deeply grateful for your love and encouragement, which have helped me navigate this journey. I am relieved that this chapter has come to an end, and I look forward to the future with renewed hope.

PUBLICATIONS AND PRESENTATIONS

Publications

- **Kenmogne VL**, Takundwa MM, Monchusi B, Dube P, Nweke EE, Maher H, Toit JD, Philip-Cherian V, Fru PN, Thimiri Govinda Raj DB., The establishment of an ex vivo drug sensitivity screening and drug repurposing platform using South African leukemia patient samples (2025). Scientific Reports, 15(1), p.9160. DOI: 10.1038/s41598-025-93634-w
- **Vanelle Larissa Kenmogne**, Ekene Emmanuel Nweke, Mutsa M Takundwa, Pascaline N Fru, Deepak B Thimiri Govinda Raj. Application of Drug Repurposing-Based Precision Medicine Platform for Leukaemia Patient Treatment (2023). Adv Exp Med Biol. 1410:115-126. doi: 10.1007/5584_2022_744.

Conference Proceedings

- Mutsa Takundwa, **Vanelle Larissa Kenmogne**, Thudzelani Takalani Austin Malise, Bernice Monchusi, Phumuzile Dube, E. Nweke, June Fabian, H. Maher, Justin du Toit, Vinitha Philip-Cherian, P. Fru, A. Sajo, Arnold (Arrie) Mouton, M. C. van Aardt, C. Visser, Greta Dreyer, Deepak B. Thimiri Govinda Raj. 2D Tumor cell culture based Ex vivo drug sensitivity screening platform for South African patient samples (Leukemia, Multiple Myeloma, Ovarian and Uterine cancer) demonstrate potential for targeted cancer therapies. March 2024, Cancer research. DOI: 10.1158/1538-7445.AM2024-6890
- **Vanelle Larissa Kenmogne,** Mutsa Takundwa, Ekene Nweke, Pascaline Fonteh Fru Deepak B. Thimiri Govinda Raj. The First-in-Africa Ex vivo Drug Sensitivity Testing Platform identifies novel drug combinations for South African Leukaemia Patient Cohort**. Poster October 2024.** ***Conference: Nucleic Acids and Nanobiosystems for Cancer Theranostics***
- **Vanelle Kenmogne L**, Austin Malise Thudzelani Takalani , Ekene Emmanuel Nweke, Mutsa M Takundwa , June Fabian, Heather Maher, Justin Du Toit, Vinitha Philip-Cherian, Pascaline Fonteh Fru, Deepak Balaji Thimiri Govindaraj. Establishment of ex-vivo drug sensitivity screening platform for leukaemia and multiple myeloma using a South African patient cohort. *Hematology, Transfusion and Cell Therapy*. Volume 45, Supplement 3, October 2023, Pages S32-S33 (Abstract)

Poster Presentations

- **XIV Eurasian Hematology Oncology Congress, October 2024**
  Vanelle Larissa Kenmogne, Mutsa Takundwa, Ekene Nweke, Pascaline Fonteh FruDeepak B. Thimiri Govinda Raj. The First-in-Africa Ex vivo Drug Sensitivity Testing Platform identifies novel drug combinations for South African Leukaemia Patient Cohort.

ABSTRACT

Leukaemia, a heterogeneous group of haematological malignancies, presents significant challenges in diagnosis, treatment, and management, particularly in resource-constrained regions such as South Africa. Despite advancements in targeted therapies, drug resistance remains a major obstacle in leukaemia treatment, necessitating the exploration of alternative therapeutic approaches. Drug repurposing, which involves identifying new indications for existing drugs, offers a cost-effective and time-efficient strategy to expand treatment options. However, there is limited research on how leukaemia patients in South Africa respond to potential repurposed drugs and combination therapies. To address these gaps, forty-one leukaemia patients, including AML, CML, and CLL, were recruited, and their demographic, clinical, and treatment history profiles were analysed. Drug sensitivity screening was performed using 30 FDA-approved drugs on peripheral blood mononuclear cells (PBMCs) isolated from these patients. The effect of the ten promising single drug candidates and their combinations was further evaluated on cell viability and CD markers like CD3, CD8, CD19, CD38, and HLA-DR via multicolour flow cytometry, providing insights into the cell death mode, immune cell dynamics, disease progression, and treatment response. Finally, the molecular docking of irinotecan and nilotinib with proteins involved in key leukaemia signalling pathways was performed to evaluate the binding affinity. The result provided valuable insights into the disparities in the demographic, clinical, treatment history, and molecular landscape of South African leukaemia patients, highlighting the variability in drug responses among patients with different treatment histories. Irinotecan was found to be the most potent drug, with an average DSS score of 9.5. Among the three drug combinations tested, the combination of irinotecan with nilotinib was found to be the best, with a Loewe synergy score of -10.61. The flow cytometry results showed a moderate increase or activation of CD3+ cells (16.7%) upon treatment with irinotecan and nilotinib compared to the untreated condition (10.5%). Within the CD3+ population, patient cells treated with irinotecan and nilotinib showed a slight decrease in CD3+CD8- cells and an increase in CD3+CD8+ (24.9% vs 28.7% respectively) compared to the control sample. The molecular docking results demonstrated that irinotecan and nilotinib could act by inhibiting mTOR and PI3K by interacting with the amino acids at their docking sites through various covalent and non-covalent interactions.

In the context of drug repurposing for the treatment of leukaemia in South Africa, this research contributes to personalized medicine approaches by showing the feasibility of drug screening to identify potential drug candidates for patients who have exhausted treatment options.

ACKNOWLEDGEMENT

- I am extremely grateful to the Lord Jesus for his grace. Without his everlasting love, protection, and unmerited favour, I would not have achieved this milestone. The church of God from CMFI and Faith Bible Church South Africa. I did not have a family member in SA during this journey, but you have been more than family to me. Thank you for your unwavering love, support, and heartfelt prayers. They have been a source of strength and comfort throughout this endeavour.
- Words cannot fully express my gratitude to the Organisation for Women in Science for the Developing World (OWSD), the Swedish International Development Cooperation Agency (Sida), and the Council for Scientific and Industrial Research. Their generous financial support through a four-year full scholarship made it possible for me to fully dedicate myself to my academic journey through the supervision of Dr. Deepak B. Thimiri Govindaraj. I am deeply thankful for the opportunity they have given me to grow, learn, and make a meaningful impact. Additional funding for this project was through Prof Fru and Prof Nweke's MRC and NRF grants to cover the sampling and processing of blood samples used in the entire study and the costs associated with the immune cell surface marker response studies.
- I thank the Chris Hani Baragwanath Academic Hospital (CHBAH) and the Wits Donald Gordon Medical Centre (WDGMC) for granting permission to conduct this study. I also extend my appreciation to all the patients who consented to participate.
- I am grateful to Dr. Justin Du Toit from the Wits Donald Gordon Medical Centre and Dr. Vanitha Philip from the Chris Hani Baragwanath Academic Hospital for their assistance in providing clinical and demographic data, as well as for their collaboration in sample collection at their respective hospitals.
- I thank Dr. Deepak Balaji Thimiri Govindaraj for securing the funding necessary to support this study and for his assistance with data analysis. I also thank the CSIR for granting me access to their facilities, especially the drug screening platform.
- Dr Mutsa Takundwa, thank you for your invaluable assistance in the laboratory. I deeply appreciate the time and effort you dedicated to managing every experiment at the CSIR.
- Prof. Emmanuel Ekene Nweke, you exemplify the ideal supervisor every student aspires to have. Thank you for your unwavering patience, guidance, and support.

- Prof Pascaline Fru, thank you for being the one with an exceptional eye for detail, the perfecter of my writing, and a source of unwavering guidance and inspiration throughout this journey. Your support has been invaluable, and I am deeply grateful.
- I express my gratitude to Dr. Roxanne Adams, the BD Biosciences Application Specialist, for humbly taking the time to schedule meetings to help me analyse the flow cytometry data.
- To my colleagues and friends in the Surgery Department Laboratories and at CSIR, particularly Austin Malise, for his help in the flow cytometry experiment, I deeply appreciate each of you for your invaluable contributions and unwavering support. Your efforts and encouragement have played a significant role in the success of my study, and I am profoundly grateful. I also appreciate Dr Bernice Monchusi and Dr Phumzile Dube for their support during my time at the CSIR.
- My family, you are everything I have. You are my source of strength, joy, love, and unwavering support. Your encouragement has been my anchor, and your belief in me has fuelled my determination. I am forever grateful for your sacrifices, prayers, and unconditional love, which have carried me through every challenge of this journey.

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
PUBLICATIONS AND PRESENTATIONS iv
ABSTRACT vi
ACKNOWLEDGEMENT vii
TABLE OF CONTENTS ix
LIST OF FIGURES xiii
LIST OF TABLES xiv
LIST OF ABBREVIATIONS xv
CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW 1
1.1: General Introduction 1
1.2: Literature review 6
1.2.1: Characteristics of leukaemia subtypes and influence on therapy 7
1.2.2: The case for drug repurposing 9
1.2.3: The link between precision medicine and drug screening 10
1.2.4: Drug screening platforms 11
1.2.5: Clinical utility of drug repurposing 15
1.2.6: Modulation and inflammatory response in Leukaemia treatment 17
1.3: Rationale for the study 18
1.4: Aim 18
1.5: Objectives 19
CHAPTER 2: DEMOGRAPHIC AND CLINICAL PROFILING OF LEUKAEMIA PATIENTS 20
2.1: Introduction 20
2.1.1: Aetiology and risk factors of leukaemia 21
2.1.2: Demographics of leukaemia 21
2.1.3: Genetic abnormalities in leukaemia 22
2.1.4: Treatment of leukaemia 23
2.2: Material and Methods 23
2.2.1: Patient recruitment and sample collection 23
2.2.2: Data collection and statistical analysis 24

2.3: Results 24
2.3.1: Demographic and haematological profile 24
2.3.2: Drugs administered as a previous treatment to study participants. 27
2.3.3: Evaluation of patients' fitness status and treatment history 28
2.3.4: Evaluation of the treatment history of patients 29
2.3.5: Genetic characteristics of patients 31
2.4: Discussion 33
2.5: Conclusion 37
CHAPTER 3: DRUG SENSITIVITY AND RESISTANCE TESTING IN LEUKAEMIA SAMPLES 39
3.1: Introduction 39
3.1.1: Principle of drug screening and resistance testing: 39
3.1.2: Benefits of drug sensitivity testing for precision oncology 40
3.2: Aim 41
3.3: Materials and Methods 41
3.3.1: Cells and controls 41
3.4: Drug sensitivity and resistance testing 44
3.4.1: Selection of drugs 44
3.4.2: Optimising drug treatment duration 44
3.4.3: Preliminary drug screening with HeLa cells 45
3.4.4: Drug sensitivity testing of thirty drugs on patient samples. 46
3.5: Drug combination study on patient samples 46
3.5.1: Double drug combination 46
3.5.2: Full matrix drug combination 46
3.6: Data analysis 47
3.7: Results 48
3.7.1: Overall single drug efficacy on Leukaemia patient cells 48
3.7.2: Subtype-specific drug profile 51
3.7.3: Distribution of the effect of some drugs on CML cells 52
3.7.4: Overall drug combination effect 53
3.7.5: Molecular features affecting drug response 56
3.8: Discussion 58
3.8.1: Single drug response profile 58
3.8.2: Synergy of irinotecan and nilotinib 60

3.8.3: Impact of molecular abnormalities and generic compound 61
3.9: Conclusion 62
CHAPTER 4: EVALUATION OF IMMUNE CELL SURFACE MARKERS IN RESPONSE TO DRUG TREATMENT 63
4.1: Introduction 63
4.1.1: Mechanisms of drug-immune system interactions 63
4.1.2: Technologies for evaluating immune cell markers 64
4.1.3: Significance of cluster of differentiation (CD) markers in cancer management 65
4.2: Rationale 65
4.3: Materials and Methods 66
4.3.1: Sampling and processing 66
4.3.2: Cell surface marker analysis using multicolour flow cytometry 66
4.4: Data and statistical analysis 71
4.5: Results 71
4.5.1: Measurement of apoptosis and necrosis by annexin V and PI staining 72
4.5.2: Cell surface marker analysis 75
4.6: Discussion 78
4.7: Conclusion 80
CHAPTER 5: *IN SILICO* STUDY OF THE INHIBITION POTENTIAL OF IRINOTECAN ON KEY LEUKAEMIA PATHWAYS 82
5.1: Introduction 82
5.1.1: Dysregulated signalling pathways in leukaemia 82
5.2: Aim and rationale 83
5.3: Materials and methods 84
5.3.1: Hardware characteristics 84
5.3.2: Publicly available databases 84
5.3.3: Ligand preparation 84
5.3.4: Protein preparation 85
5.3.5: Molecular docking experiment 86
5.3.6: Visualisation of interactions 87
5.4: Results 87
5.5: Discussion 93
5.6: Conclusion 94
CHAPTER 6: GENERAL CONCLUSION AND PROSPECTS 96

6.1: Study limitations 97
6.2: Future Directions 98
REFERENCES 100
APPENDICES 128
Appendix 2.1 ECOG interpretation 128
Appendix 3.1 List of the 30 drugs tested 128
Appendix 3.2 List of drugs for cancer treatment 130
Appendix 3.3 list of drug combination arrangements 132
Appendix 3.4: Ex vivo Drug sensitivity testing is reproducible as demonstrated in HeLa cell lines 132
Appendix 3.5 Table of the drug cluster I 133
Appendix 4.1: Expression of surface markers on AML04 134
Appendix 4.2: Expression of surface markers on CML04 136
Appendix 5.1: 2D and 3D representation of the interactions established between proteins nilotinib and irinotecan 137
APPENDIX A: TURNITIN REPORT 141
APPENDIX B: ETHICS CLEARANCE 142
APPENDIX C: PERMISSION TO CONDUCT RESEARCH 143
APPENDIX D: DATA SHEET 144
APPENDIX E: INFORMATION SHEET 145
APPENDIX F: INFORMED CONSENT 146

LIST OF FIGURES

Figure 2.1: Age distribution by diagnosis and gender. AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia 25
Figure 2.2: Patient performance status. AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia. 0 indicating fully active and 1 means restricted in strenuous activity. 29
Figure 3.1:Illustrations of different drug combination designs. 47
Figure 3.2: Average drug sensitivity scores of thirty drugs tested on patient samples. Average DSS on the Y axis, drug name on the X axis. Thirty drugs tested on PBMC isolated from patients. 49
Figure 3.3: Drug response profile of patients. 50
Figure 3.4: DSS distribution among leukaemia subtypes. 52
Figure 3.5: Distribution of the effect of nilotinib, imatinib, and dasatinib on CML cells. DSS scores on the Y axis and CML samples on the X axis. 53
Figure 3.6 Overall drug combination response 54
Figure 4.1: Hierarchical gating strategy for flow cytometry analysis of BH30-treated stained cells. 72
Figure 4.2: Flow cytometry analysis using an Annexin V/PI double-staining assay 74
Figure 4.3 Surface marker analysis on BH30 (CLL) cells. 77
Figure 5.1: 2D and 3D structures of Irinotecan and Nilotinib. Downloaded from PubChem . 85
Figure 5.2: 3D (A) and 2D (B) representation of the interactions established between mTOR and irinotecan 90
Figure 5.3: 3D (A) and 2D (B) representation of the interactions established between mTOR and nilotinb. 92

LIST OF TABLES

Table 1.1: Examples of compounds with potent antileukemic activities identified by virtual screening. 13
Table 1.2: Examples of compounds from in vitro and ex vivo screening with potent antileukemic activities, their classes, and indications. 15
Table 2.1: Summary statistics of the subjects in the cohort 26
Table 2.2: Summary of the haematological profile of the cohort. 27
Table 2.3: A comprehensive list of the patients' prior treatment and commonly used chemotherapy drugs in the cohort. 28
Table 2.4: Treatment history of patients who received more than one chemotherapy drug. 30
Table 2.5: Genetic characteristics of patients. 32
Table 3.1: Variability of response among patients with similar characteristics. 57
Table 4.3: Expression of cell surface markers in AML04 and CML04 78
Table 5.1: Binding energies of nilotinib and irinotecan with STAT3, JAK2, PI3K, and mTOR 88

LIST OF ABBREVIATIONS

AML	Acute myeloid leukaemia
BCR-ABL	Breakpoint cluster region – abelson murine leukaemia viral oncogene
BTK	Bruton tyrosine kinase
CAR T-cells	Chimeric antigen receptor T-cells
CD	Cluster of differentiation
CHBAH	Chris hani baragwanath academic hospital
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
DSS	Drug sensitivity score
FDA	Food and drug administration
GLOBOCAN	Global cancer observatory
HLA-DR	Human leukocyte antigen – dr
IGVH	Immunoglobulin heavy chain variable region
IMiDs	Immunomodulatory drugs
LRP	Low-density lipoprotein receptor-related protein
MCV	Mean corpuscular volume
MDR1	Multidrug resistance 1
MRP	Multidrug resistance protein
mTOR	Mammalian target of rapamycin
NK cells	Natural killer cells
PDBQT	Protein data bank, partial charge (q), & atom type (t)
PI	Propidium iodine
PI3K	Phosphatidylinositol 3-kinas
STAT3	Signal transducer and activator of transcription 3
WBC	White blood count
WDGMC	Wits donald gordon medical centre
WHO	World health organization

CHAPTER 1: GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1: General Introduction

The immune system is a complex machinery made up of the innate and adaptive systems working together to protect the body against invaders. Some immune cells, like white blood cells, also called leukocytes, can undergo malignant modifications leading to cancer, the second cause of death worldwide. Haematological malignancies are cancers of the blood that often originate in the bone marrow from a myeloid or lymphoid lineage (Juliusson & Hough, 2016). GLOBOCAN, a worldwide cancer observatory, reported a global incidence of 474,519 cases with a death rate of about 3.2% (Chennamadhavuni et al., 2023). Leukaemia can affect people of all ages, but different age distributions can be observed depending on the acute or chronic stage of the disease (Juliusson & Hough, 2016). In developing countries including South Africa, the mortality rate of leukaemia is higher than the number of new cases (7.7% vs 6.6%) even though the incidence is far lower than in developed countries (Sung et al., 2021). Haematological cancers account for nearly 10% of the total cancer burden in Sub-Saharan Africa, with their incidence rising rapidly (Okello et al., 2021). Although the reported incidence of haematological malignancies in Sub-Saharan Africa is lower than in high-income countries, this discrepancy may be partially explained by underdiagnosis and underreporting. The World Health Organization (WHO) projects that by 2030, the incidence of haematological malignancies in low and middle-income countries will increase by 48% compared to 2018 (Okello et al., 2021). Cancers are genetically different even within patients living in the same environment, due to the molecular pathogenesis of the disease. Some factors like age, exposure to environmental pollutants, genetics, obesity, cancer progression, and treatment can be completely different (Nweke & Thimiri Govinda Raj, 2021). Additionally, during cancer progression and therapeutic intervention, clones and subclones may evolve in a patient's tumour. This phenomenon is called intra tumoural heterogeneity and is one of the causes of drug resistance, which occurs when cells are genetically different from those in the metastatic sites (Cajal et al., 2020). Precision oncology is a cancer treatment strategy that aims to find the most appropriate therapeutic options for the patient. Due to the high heterogeneity among patients, identifying the genetic profile of each patient before or during treatment enables a

more personalized treatment for cancer patients. However, genomic data alone are not sufficient to predict a successful drug; patients' demographic and clinicopathological history should be considered (Desai et al., 2022). Therefore, it is beneficial for both the physician and patients to establish an effective and precise platform that helps to identify and validate drug candidates for effective treatment and appropriate therapeutic regimens for the patients.

Just like solid cancers, the treatment of hematologic cancers such as leukaemia varies, including radiotherapy, stem cell transplant, chemotherapy, immunotherapy, and targeted therapy, and relies mostly on drugs. Furthermore, chronic lymphocytic leukaemia (CLL), chronic myeloid leukaemia (CML), and acute myeloid leukaemia (AML), which are distinct hematologic malignancies, also require different diagnostic approaches and treatment strategies. Selecting a treatment option needs careful consideration, given that there are currently multiple options. Drug combination therapy is becoming the standard of care for naive patients in place of conventional chemotherapy. Chemo-immunotherapy has improved patient responses and overall survival when compared to chemotherapy alone. However, a significant number of patients will still relapse and require additional treatment with more targeted therapies, often associated with more potential toxicity on the bone marrow precursors and frequency of infections after treatment completion (Tam et al., 2008). Thus, a standard regimen cannot be normalized due to the uniqueness of each patient and the heterogeneity of CLL (chronic lymphoid leukaemia) and or other blood cancers. Approved targeted agents for CLL treatment include an intracellular protein downstream of the B-cell receptor, Bruton tyrosine kinase (BTK) inhibitors like ibrutinib, which has become standard first-line chemotherapy. Ibrutinib is successful in monotherapy and has been used to treat previously untreated and relapsed/refractory patients with CLL but can also be used in combination therapy. This is proof that the choice of drug or drug combination should be assessed upfront based on patients' characteristics. For example, Woyach and colleagues found no significant difference in efficacy between ibrutinib taken alone or combined with rituximab in CLL patients aged ≥ 65 years (Woyach et al., 2019).

Many categories of drugs are used in cancer treatment. These include antitumor drugs like the anthracycline's family (doxorubicin, idarubicin) that inhibit DNA synthesis by insertion between DNA base pairs (Tabatabaei et al., 2024). Antineoplastic drugs include cell surface antigen-targeted monoclonal antibodies that bind to a specific antigen and facilitate the

destruction of the bound cells by the body's immune system. A good example is Rituximab, a monoclonal antibody that targets CD20 expressed on most B-cell malignancies. Antibody-drug conjugates (ADCs) is a term used for the combination of monoclonal antibodies with chemotherapeutic agents known to possess a more traditional cytotoxic mechanism. This category comprises alkylating agents that cause the breakage of the DNA strand by adding an alkyl group to the guanine base of the DNA molecule. With an increased understanding of the disease, modern therapies like immunotherapy with immunomodulatory drugs (IMiDs) and targeted therapy with proteasome inhibitors (PIs) have led to an improvement in patient survival rates (Bazarbachi et al., 2019). As the disease progresses with increasing genomic complexity, several signalling pathways known to be involved in treatment resistance are activated. These signalling pathways can eventually be targeted with a combination of signal transduction inhibitors and other approved drugs. Nonetheless, it is important to identify the responding patients upfront to treat them with pre-screened drugs to maximize health benefits and reduce the risk of relapse and resistance.

Leukaemia remains a significant clinical challenge, with drug resistance being one of the primary obstacles to effective treatment. Resistance arises when malignant cells fail to respond to chemotherapeutic agents, either due to inadequate drug delivery to target cells or because the cells develop mechanisms to evade the drug's effects. Several factors contribute to this phenomenon. These include cell-adhesion–mediated drug resistance, in which leukaemia cells anchor to the bone marrow stroma for protection; multidrug resistance, driven by efflux transporters such as multidrug resistance protein 1 (MDR1), multidrug resistance-associated protein (MRP), and low-density lipoprotein receptor-related protein (LRP) that actively pump drugs out of cells; the failure of apoptosis, allowing cancer cells to avoid programmed cell death; barriers to drug delivery that prevent compounds from reaching their intended targets; and genetic aberrations, where mutations alter drug targets and confer resistance (Calderon et al., 2024; Zhang et al., 2019). These multifactorial resistance mechanisms not only limit the efficacy of existing therapies but also underscore the urgent need for innovative strategies that can bypass or overcome them.

One promising strategy to address these challenges is drug repurposing, also known as drug repositioning. This approach involves identifying new therapeutic uses for existing, approved drugs beyond their original medical indications. In oncology, repurposing offers a unique

advantage: drugs that have already been approved for other diseases have known safety profiles, pharmacokinetics, and manufacturing protocols, which can significantly shorten the time and cost associated with drug development (Mag et al., 2024; Pushpakom et al., 2019). For leukaemia, drug repurposing holds particular promise because it allows researchers to screen widely available compounds—including non-oncology drugs—for anti-leukaemic potential. This not only accelerates the search for effective treatments but also provides options for patients who have exhausted conventional therapies due to drug resistance.

One promising strategy to address these challenges is drug repurposing, also known as drug repositioning. This approach involves identifying new therapeutic uses for existing, approved drugs beyond their original medical indications. In oncology, repurposing offers a unique advantage: drugs that have already been approved for other diseases have known safety profiles, pharmacokinetics, and manufacturing protocols, which can significantly shorten the time and cost associated with drug development (Mag et al., 2024; Pushpakom et al., 2019). For leukaemia, drug repurposing holds particular promise because it allows researchers to screen widely available compounds, including non-oncology drugs, for anti-leukaemic potential. This not only accelerates the search for effective treatments but also provides options for patients who have exhausted conventional therapies due to drug resistance.

To unlock the potential of drug repurposing, efficient and systematic evaluation methods are essential. High-throughput screening (HTS) has emerged as a critical technology for this purpose. HTS enables the rapid testing of thousands of compounds for their biological activity, using automated platforms that work at molecular or cellular levels (Boshuizen & Peeper, 2020). Unlike traditional biochemical and pharmacological screening methods, which are laborious and time-consuming, modern HTS employs microplate formats with microliter-scale assay volumes and minimal compound quantities (often around 1 μg), allowing simultaneous testing of multiple drug candidates (Nweke & Thimiri Govinda Raj, 2021).

In recent years, advancements in bioengineering—such as three-dimensional (3D) culture systems integrated with perfused microfluidic environments—have enhanced the ability to mimic the *in vivo* tumour microenvironment. These systems provide continuous nutrient supply, waste removal, and controlled oxygen levels, improving both the quality and reproducibility of microcultures (Jaafari et al., 2025). Thimiri Govinda Raj et al. (2018)

demonstrated a method that mimics the microenvironment of CLL and allows its proliferation for 5 days. The model uses *in vitro* luminescent assays to measure cell viability (cell Titer-Glo) and cell death (cellTox). The authors performed drug sensitivity screening for CLL patients in Norway and have been able to rank drugs by their selective drug sensitivity score (sDSS), generated based on variables such as the half-maximal inhibitory concentration (IC_{50}), the slope, and the area under the curve (AUC). The DSS for each CLL sample was then compared to the DSS of healthy samples for the whole patient sample cohort tested to create a selective DSS for each patient ($sDSS = DSS_{patient} - DSS_{healthy}$) (Thimiri Govinda Raj et al., 2018). Similarly, Skånland et al, (2020) suggested that phosphoflow be regarded as a potential technique for drug dosage and synergy prediction in CLL precision medicine. They investigated the effects of idelalisib, ibrutinib, and venetoclax on thirty-five protein targets using phosphoflow and identified drug combinations. Their results showed that the ordinary dosages of ibrutinib and venetoclax could be reduced without loss of effectiveness, possibly lowering medication costs and toxicities (Skånland et al., 2020).

Using the same technology as Thimiri Govinda Raj and colleagues (2018), this study aims to demonstrate the potential utility of a drug screening platform in a cohort of South African leukaemia patients. The identification of effective drug combination against leukaemia patient cells could help guide future personalised treatment and serve as a proof of concept for precision oncology in South Africa.

This thesis is structured into six chapters. Chapter 1 provides a general introduction and a comprehensive literature review, with sections of this chapter published as a review article (Kenmogne et al., 2023). Chapter 2 presents an analysis of the demographic and clinical characteristics of patients, including parameters such as age, gender, haematological profiles, and treatment history, based on data extracted from patient records. Chapter 3 details the drug sensitivity screening conducted on peripheral blood mononuclear cells (PBMCs) isolated from patient samples, where 30 FDA-approved drugs were screened, followed by drug combination studies focusing on the 10 most potent drugs, culminating in a full matrix combination analysis to determine optimal drug concentrations. Chapter 4 employs flow cytometry with a seven-colour panel to evaluate the impact of drug treatments on cell surface markers, including CD19, CD38, CD3, CD8, and HLA-DR, in selected patients. Chapter 5 explores the *in silico* molecular docking of irinotecan against key leukaemia-associated proteins, such as PIPK, mTOR, JAK,

and STAT3, with a comparative analysis against nilotinib. Finally, Chapter 6 provides a general conclusion and discusses future perspectives arising from the findings of this study.

1.2: Literature review

Blood cancers, like most malignancies, occur from genetic modifications resulting in neoplastic mutations in the DNA causing abnormal cell proliferation (Okello et al., 2021). The proliferation of the incompetent blood cells increases vulnerabilities to infections, making hematologic malignancies some of the most challenging cases of cancer to manage. Leukaemia is a blood cancer originating in the bone marrow from lymphoid or myeloid cell modification. Based on the duration of maturation and development of leucocytes, leukaemia is divided into acute and chronic subtypes. The response of patients to standard treatment varies considerably and the survival rate differs among subtypes. The 5 years survival rate of AML patients is 20% whereas CLL patients experience a better prognosis with an 86% survival rate (Andresen & Gjertsen, 2017; Ou et al., 2022). Although these metrics have improved with advances in medicine, many patients will relapse or become refractory to the treatment. The drug resistance phenomenon observed can be explained by the heterogeneous characteristic of the disease as well as the one-size-fits-all type of treatment options patients used. Therefore, new drugs or alternative treatments need to be identified to complement the existing ones or the unmet need for effective chemotherapeutic drugs.

Several studies have evaluated the possibility of redirecting the use of already approved drugs to emerging and heterogeneous diseases. The literature abounds with evidence of drugs successfully repurposed and released in the market with new indications for the prevention or the treatment of cancer (Jourdan et al., 2020; Walpole et al., 2024). The results from virtual, experimental studies and more importantly clinical trials have reported the efficacy of different classes of drugs against leukaemia subtypes. Additionally, many publications emphasize the benefit of using existing drugs for other indications. However, some studies have shown that only 75% of drugs can theoretically be repurposed, and the proportion is even smaller in practice (Nosengo, 2016). Many FDA-approved drugs for the treatment of cancer have been redirected and shown to have antileukemic activities. This is obvious as cancers share some similarities, and one anticancer agent indicated in treating a type of cancer is more likely to be active against another. For example, dasatinib, a kinase inhibitor used to treat certain types of

chronic myelogenous leukaemia has been identified as a targeted therapy for T-cell ALL (Laukkanen et al., 2017). Another example is Gefitinib, approved as an inhibitor of the epidermal growth factor receptor for the treatment of certain lung and breast cancers, has been repurposed for chronic myelogenous leukaemia treatment as a bcr-abl tyrosine kinase inhibitor (Singh et al., 2017).

1.2.1: Characteristics of leukaemia subtypes and influence on therapy

Leukaemia affects both children and adults, with a predominance in whites and males (Chennamadhavuni et al., 2023). The four major types of leukaemia are ALL which is more likely to occur in children, CLL, AML, and CML, which is more common in adults. The term acute means that the disease will progress rapidly in the absence of treatment whereas chronic leukaemia, requires observation and is harder to cure. On the other hand, lymphocytic and myelogenous refer to the type of bone marrow cells affected in the cancer type with lymphocytes for lymphocytic or lymphoblastic leukaemia and the granulocytes and monocytes for myelogenous leukaemia (Chennamadhavuni et al., 2023). The age factor is one of the elements to take into consideration when selecting a treatment for a particular patient. For example, the type of ALL found in children is different from the type that usually presents in adults. Additionally, younger ALL patients have a better prognosis than elderly patients, for which the outcome of intensive chemotherapy is poor and associated with higher toxicity (Chennamadhavuni et al., 2023).

Generally, the differences in leukaemia subtypes are found in the clinical, haematological, and molecular characteristics of the disease. Therefore, the subtypes and accompanying characteristics can affect the disease progression and treatment response, indicative of the need for personalised therapy.

Lymphocytic leukaemia

In acute lymphocytic leukaemia (ALL), there are three subtypes, namely: L1, L2, and L3. Patients classified as having the L1 subtype have relatively small lymphoblasts compared to those of subtypes L2 and L3. Blast cells are immature cells that develop into several types of blood cells. They have distinct characteristics that influence treatment (Faderl et al., 2010).

For example, blast cells with runt-related transcription factor 1 are more likely to respond to a treatment with purine analogues (Faderl et al., 2010). Patients with hyperdiploidy, who possess lymphoblasts particularly susceptible to cytotoxic drugs, are known to have a better prognosis than those who do not (Lee et al., 2021). Hyperdiploidy in ALL is defined as the presence of 51 to 65 chromosomes. This cytogenic abnormality is common in paediatric patients who comprise more than 25% of B lymphocyte-ALL compared to adults at 7-8%, giving children a favourable outcome and a better survival rate. However, some patients of this group may not respond to treatment due to the presence of additional cytogenic aberrations, particularly on chromosomes 1q and 17q (Chen et al., 2019).

Chronic lymphocytic leukaemia also called chronic lymphatic leukaemia (CLL) is characterised by a high level of expression of BCL-2, an oncoprotein that inhibits apoptosis. Intracellular heterogeneity is also observable in CLL with Immunoglobulin Heavy Chain Variable Region (IGVH) mutation status and the 17p and/or 11q deletion and the presence of a molecule called ZAP-70 affecting treatment outcome and overall survival (Montserrat & Dreger, 2016). Results of a study in Canada showed that first-line chemotherapy is urgently required in CLL patients with 11q deletion, however, this group of patients experienced a longer overall survival after treatment (Goy et al., 2017).

Myeloid leukaemia

There are two classification conventions for AML. The World Health Organisation (WHO) classifies AML into subtypes based on the type of genetic abnormality encountered and the French-American-British (FAB) classification that relies on the type of cells affected and their level of maturity i.e. M0-M7 (Walter et al., 2013). Phenotypic variations include AML with mixed phenotype consisting of two forms of leukaemia combined. These include AML with minimal differentiation designated AML-M0, in which there is no blast cell differentiation and the absence of myeloid markers, and AML with/without maturation (Juliusson & Hough, 2016). Myeloblastic leukaemia with maturation (M2) and myelomonocytic (M4) are both the major subtypes of AML with better and average prognosis, respectively.

The common types of genetic abnormalities encountered in acute myeloid leukaemia are translocations [t(8,21), t(9,11), t(6,9)] and inversions [inv(16), inv (3)]. AML patients with these abnormalities aged below 60 years old have a better prognosis after treatment with

cytarabine with 85 % rates of complete remission compared to patients with 20q deletion or patients with abnormalities involving chromosomes 3, 5, or 7 who only attain 30-50% complete remission. Furthermore, the correlation between the level of certain enzymes like lactate dehydrogenase (LDH) in patients' serum and shorter survival time has been demonstrated in a Chinese AML cohort (Xiao et al., 2021). The study revealed that LDH level was associated with a high risk for 60-day mortality among AML patients, especially for those with a concentration of 570U/L and greater. In the same study, patients with a distinct subset of AML (secondary AML: AML evolving from prior blood conditions like aplastic anaemia or myelodysplasia) were also observed to have poor prognosis. On the other hand, patients with a normal karyotype were classified as having a standard or intermediate risk with a 50-85% complete remission rate (Xiao et al., 2021).

A defining characteristic of CML is the presence of the Philadelphia (Ph) chromosome, resulting from a translocation between chromosomes 9 and 22 (Sampaio et al., 2021). This translocation leads to the formation of the BCR-ABL fusion gene, which drives leukaemogenesis by promoting uncontrolled cell proliferation and survival. Approximately 90% of CML patients are Ph-positive, characterised by an increased genetic instability, and only 5-10% are classified as Ph-negative but can possess the BCR-ABL fusion gene, and or other additional genetic mutations (Abdulmawjood et al., 2021). Genetic and clinical features in CML phases determine the sensitivity of patients to treatments. For example, the response of Ph+ AML patients to standard imatinib is reduced when the disease progresses from the chronic phase (CML-CP) to the accelerated phase (CML-AP) or to the blastic phase (CML-BP), which requires second-generation tyrosine kinase inhibitors (Deininger, 2015).

1.2.2: The case for drug repurposing

De novo drug discovery is a risky process that is long, labour-intensive, and highly expensive. It has about a 45% failure rate, chiefly due to safety or toxicity issues (Bhagat & Butle, 2021). The estimated cost for research and development of a new drug ranges from 1-2 billion dollars (DiMasi et al., 2016) and could take up to 15-17 years. Comparably, drug repurposing, repositioning, or reprofiling is a cost-efficient strategy to circumvent the difficulties associated with the de novo drug discovery process, as it can help to save 4 to 7 years in the development phases with a reduced risk of failure (Xue et al., 2018). Indeed, the

average cost to launch a drug using the repositioning approach is $1.6 billion compared to $12 billion for traditional strategy (Bhagat & Butle, 2021; Xue et al., 2018).

The concept of drug repurposing is based on two fundamental scientific principles, which stipulate that various diseases share similar targets and that drugs can have pleiotropic effects (Pushpakom et al., 2019). Target-based repositioning offers the advantage of facilitating the process of identifying specific targets and pathways using tools such as molecular docking and binding-site similarity that can be exploited in the drug development process (Jourdan et al., 2020; Pushpakom et al., 2019). Drug repurposing also offers the possibility to characterise diseases based on their molecular profile and link the profiles to drugs that are not used for the specific disease. In the era of precision medicine, drugs are administered based on the molecular profile of the patient, enhancing the effect of chemotherapy, which emphasises the potential of drug reprofiling and repurposing in patient treatment and management (Nweke & Thimiri Govinda Raj, 2021). The drug repurposing process involves a major step, which is drug sensitivity screening used to determine the effect of the drug(s) on the cancer cells. This screening not only helps identify existing drugs with potential anti-cancer activity but also provides insights into their efficacy across different patient samples. By systematically evaluating drug responses, this approach supports the selection of promising candidates for repurposing, guiding the development of more effective treatment strategies.

1.2.3: The link between precision medicine and drug screening

Precision medicine is a treatment strategy that focuses on identifying effective treatment approaches based on patients' genetic, environmental, and lifestyle features (Dugger et al., 2018). With the increased knowledge of molecular and genetic characterization of cancer cells and various cell-host interactions, precision medicine has emphasized the need for new drugs in the management of leukaemia. Both precision medicine and drug repurposing are relevant for leukaemia, and particularly for patients who have relapsed or are developing drug resistance and do not have any other treatment options (Li & Jones, 2012).

Common treatment options for leukaemia are based on cancer-fighting drugs like chemotherapy, biological and targeted therapies, and non-drug-based treatments like radiation therapy and stem cell transplant. Chemotherapy, a primary treatment approach, involves the administration of cytotoxic drugs that target rapidly dividing cancer cells (Sharma et al., 2024).

These drugs can be delivered through various routes, including intravenous infusion, oral administration, or intrathecal injection, depending on the type and stage of the cancer. Unfortunately, the need for new drugs has been exacerbated due to the high heterogeneity of the disease and the specificities of each patient. Therefore, precision medicine is used to reduce the lack of drug efficacy and the development of drug resistance by ameliorating patients' stratification and their sensitivity to new drugs (Dugger et al., 2018).

Drug screening can play a pivotal role in precision medicine by rapidly providing a drug sensitivity profile for patient-derived cells. This approach can identify compounds or drugs that may be effective against a patient's tumour.

1.2.4: Drug screening platforms

Drug screening platforms are an important aspect of the drug discovery and development process. Indeed, the platform developed for drug screening and resistance testing, the methods, and the models selected have an impact on the duration, the cost, and the outcomes of the process. Various platforms are used to screen drugs for their pharmacological effects on models of diseases. In oncology, chemosensitivity studies have evolved from *in vitro* cell lines-based screening to human primary cancer culture to better predict clinical responses. Advances in the development of drug screening platforms seek to reproduce the complexity of the interactions within the tumour microenvironment. Some examples of these platforms include microfluidic, *in silico* and computational modelling.

Microfluidic platforms

Microfluidic platforms for drug screening allow for the accurate processing and control of small volumes of fluid coupled with automated data collection of cell responses. The adoption of microfluidic technology provides significant benefits such as low reagent consumption, rapid mixing, quick operation and utilisation, and accurate control of the fluid properties (Liu et al., 2021). Types of microfluidic platforms used in drug screening include continuous-flow, successive droplet, and droplet array platforms (Serra et al., 2020). For drug evaluation and validation, microfluidic platforms utilise methods such as microfluidic single-cell, microfluidic cell spheroid, single-organ-on-a-chip, and multi-organ-on-a-chip (Liu et al., 2021). These

platforms offer the possibility to better understand cell heterogeneity, 3D cell culture that recapitulates cell-cell interactions and mimics organ functions *in vitro*.

In silico and computational modelling

With an appropriate virtual screening platform, researchers can identify potent anticancer hits by screening millions of drugs based on the structure or ligand-based approach. Screening databases for the identification of hit/lead compounds is a powerful approach to lower the cost of the drug screening process by identifying viable targets (Sliwoski et al., 2014). The pharmacological, structural, biochemical, and toxicological characterization and the archiving of key compounds have helped predict the exploitability and the development of new compounds (Rognan, 2017). Given the high volume of biological targets and drugs available, setting *in vitro* drug screening and testing for all drug candidates is nearly impossible. Thus, a virtual platform makes use of computer-aided screening to assess drug banks and molecular target databases to identify the best compound(s) which can further undergo *in vitro* testing for validation (Singh et al., 2017).

Gorgulla and colleagues have established an open-source drug discovery platform that allows ultra-virtual screening (Gorgulla et al., 2020). The highly automated platform called VirtualFlow incorporates each step required for the screening of billions of compounds using various molecular docking techniques with the use of software such as AutoDock, GOLD, or AutoDock Vina. The virtual platform features include linear scaling behaviour, cloud computing integration, and autonomous deployment for the ligand screen pipeline. Such platforms allow computer-aided drug screening using artificial intelligence, machine learning, and deep learning applications, which are currently being implemented in precision oncology and drug repurposing (Rafique et al., 2021). Regarding the use of machine learning algorithms to predict repurposing prospects using drug data, various types of machine learning algorithms, such as supervised and unsupervised machine learning, are useful in building and evaluating predictive models by identifying unique patterns in biological data connected to the medications (Vamathevan et al., 2019).

Although virtual screening has some limitations like the environment of drugs screened being different from the microenvironment of a living organism, the virtual screening has led to the identification of compounds of interest for the treatment of leukaemia (Table 1.1). For

example, Sohraby and colleagues (2017) used a set of computational analyses to predict the best inhibitor for CML drug-resistant T315I mutant and BCR-ABL1 mutation. The structure-based virtual screen (pharmacophore and E-pharmacophore virtual screening) and molecular dynamics study of 1590 FDA drugs led to the identification of new ligands (Table 1.1). The molecular docking revealed that chlorohexidine has a stronger effect on both T315I mutant and wild-type BCR-ABL1 than ponatinib, the reference drug used in the treatment of CML (Sohraby et al., 2017). Chlorhexidine is an antibacterial agent with a broad spectrum of antimicrobial activity. It was developed in 1950 and is used as an antiseptic and antibiotic in dermatology and dentistry to disinfect the skin, clean wounds, and treat yeast in the mouth (Mariotto et al., 2020; Rothwell et al., 2011). The antibacterial mechanism of action of chlorohexidine is due to its capacity to disrupt the cell membrane at high concentrations and stop bacteria growth at low concentrations. Other drug groups identified in Table 1.1 as tyrosine kinase inhibitors include heavy metal antagonists, aminoglycosides, and protease inhibitors.

Similarly, another study performed a virtual screening of 1424 compounds on a ligand-based virtual screening platform and showed that gefitinib, a compound used in the treatment of breast cancer, is a potential target of the allosteric pocket of ABL kinase (Singh et al., 2017).

Table 1.1: Examples of compounds with potent antileukemic activities identified by virtual screening.

Drugs	Drug class	Primary indication	New Indication	References
Gefitinib	Kinase inhibitor	Solid tumour	BCR kinase inhibitor (CML)	(Singh et al., 2017) (Natarajan et al., 2019)
Ovoflavin	Vitamins	Prevention of weight loss and skin lesions		
Hesperidin	Bioflavonoid	Bioflavonoid		
Butirosin	Aminoglycoside antibiotics	Active against Gram bacteria		
Chlorohexidine	Antimicrobial	Antibacterial	Antileukemic (CML) Tyrosine Kinase inhibitor	(Sohraby et al., 2017)
Paromomycin	Aminoglycoside antibiotics	Antibiotic		
Deferoxamine	Heavy metal antagonist	Anti-hemochromatosis		
Ritonavir	Protease inhibitor	Antiretroviral		
Hydrocarpin	Natural product	Flavonoids	Antileukaemia (AML) Inhibits AML receptors BCL-2, FLT3	(Bultum et al., 2022)
Bromocriptine	Dopamine receptor agonist	Hyperprolactinemia Parkinson's disease	Anti-AML: induces apoptosis and myeloid differentiation	(Lara-Castillo et al., 2016)

Pre-clinical models of drug screening

Complimenting virtual drug screening to testing in pre-clinical models enhances the drug repurposing process and can further lead to the identification of novel therapeutic agents that selectively target cancer cells like leukaemia cells. Examples of compounds from *in vitro* and *ex vivo* screening with potent antileukemic activities, their classes, and indications are listed in Table 1.2. One important relevance of the use of pre-clinical models is in demonstrating the synergistic effects of drugs on cancer cells. This combinatorial treatment can lead to enhanced efficacy in the treatment and reduction in toxicity. Importantly, *in vitro* drug screening offers the advantage that drugs can be tested in an environment that mimics the biological microenvironment. Based on their *in silico* results, one study undertook an *in vitro* test to validate the activity of gefitinib on K562 cells, which is a human CML immortalised cell line expressing BCR-ABL receptor (Kushwaha et al., 2015) . The combination of gefitinib (5 μM) and imatinib revealed a synergistic effect, suggesting that gefitinib can enhance the inhibitory effect of imatinib. Researchers have shown interest in screening drugs for leukaemia *in vitro* by medium to high throughput drug screening with encouraging results (Singh et al., 2017; Sohraby et al., 2017; Zapevalova et al., 2022) . Using high throughput screening *in vitro* on leukemic cancer cell lines as well as primary cells has led to crucial information necessary to improve the management of leukaemia. Through these screening processes, it has been reported that the standard dose of ibrutinib and venetoclax can be reduced thereby lowering costs and reducing toxicity in patients, while maintaining efficacy (Skånland et al., 2020).

Ex vivo drug screening on primary human leukemic cells has resulted in the identification of single agents or drug combinations. The advantage of *ex vivo* experiments over *in vitro* is the actionable or translational results that can match patient profiles for personalised diagnosis or treatment. Isoprenaline, a beta-adrenergic receptor inhibitor, has demonstrated antileukemic activity for the first time and has been combined with the purine analogue fludarabine and with ibrutinib, a Bruton's Tyrosine kinase inhibitor. The results showed a synergistic effect of isoprenaline and Ibrutinib on freshly isolated CLL primary cells, with a better synergistic effect for cells with higher Ibrutinib resistance (Nehdi et al., 2021).

In leukaemia research, specialised animal models, including xenograft mouse models using human leukaemia cells (e.g., NOD/SCID mice) and genetically engineered mouse models

(GEMMs) that recapitulate specific oncogenic mutations (e.g., MLL-AF9, BCR-ABL1), are commonly employed to mimic disease progression and treatment response. These models provide insight into tumour microenvironment interactions, drug resistance mechanisms, and toxicity, thereby bridging the gap between *in vitro* findings and clinical translation (Hauer et al., 2014)

Table 1.2: Examples of compounds from *in vitro* and ex vivo screening with potent antileukemic activities, their classes, and indications.

Drugs	Drug class	Primary indication	New Indication	References
Papaverine	Antispasmodics (Alkaloid)	Vasodilator	Antileukemic (CML) Promotes apoptosis and inhibits bcr-abl signalling	(Parcha et al., 2021)
Acriflavine	Antibiotic	Antiseptic	Antileukemic (AML, CML); modulate STAT3/5	(Hallal et al., 2020; Nel 2021)
Albendazole	Anthelmintic	Antiparasitic	AML inhibits colony formation	(Matchett et al., 2017)
Niclosamide	Anthelmintic	Antiparasitic	T-ALL inhibits GSH synthesis and NFAT signalling	(Hamdoun et al., 2017)
Moxidectin Ivermectin Milbemycin	Anthelmintic (Macrocyclic lactone)	Antiparasitic	Relapsed ALL, Induces cell death and synergises with BCL-2 agonists	(Mezzatesta et al., 2020)
Metformin	Biguanides	Antidiabetic (Type 2)	Activates p-AMPK, synergises with sorafenib in FLT3-mutated AML	(Shadman et al., 2015; F. Wang et al., 2015)
Quinacrine	Acridines	Antimalaria	Anti-AML	(Eriksson et al., 2015)

1.2.5: Clinical utility of drug repurposing

Virtual and laboratory drug screening are rapid and can lead to the unexpected discovery of new antileukemic drugs. However, the interaction of the body with the drug cannot be easily assessed *ex vivo*. Therefore, clinical trials are important to direct the final opinion on the efficacy of the compound being investigated. There is little clinical evidence of new drugs successfully repurposed in leukaemia and one possible explanation is the cost associated with undertaking clinical studies (Nipp et al., 2015). Another reason might be that a drug that has shown antitumour activity *in silico*, *in vitro*, or even *in vivo* with the use of animal models can be a failure once assessed clinically because of safety issues or inefficacy (Sun et al., 2022).

The complexity of the human organism and the patient's intrinsic and extrinsic features are some important points impacting the response of a patient to a certain treatment.

It should however be noted that there have been some instances resulting in the clinical utility of repurposed drugs in leukaemia treatment. One example is thioridazine, an antipsychotic drug from the phenothiazine family. It was previously widely prescribed for the treatment of depressive disorders and schizophrenia before it was withdrawn from the market globally in 2005 because of its adverse effect on cardiac rhythm. Thioridazine was later screened and confirmed to possess anti-inflammatory (Baig et al., 2018), and antibiotic activities (Van den Driessche et al., 2017). Its anti-inflammatory properties are particularly relevant in the context of HLA-DR expression, as HLA-DR serves as a marker of immune activation and inflammation (Baig et al., 2018). Elevated HLA-DR expression is often associated with immune dysregulation and inflammatory disorders, suggesting that thioridazine's ability to modulate inflammation could have implications for immune-related conditions, including leukaemia (Roerden et al., 2021). It was suggested as a potential drug to tackle drug resistance in acute myelogenous leukaemia (Jourdan et al., 2020). A phase-1 trial completed by Aslostovar and colleagues in 2018 evaluating the efficacy of Thioridazine in patients with refractory/relapsed acute myelogenous leukaemia demonstrated the safety and efficacy of thioridazine in combination with cytarabine (Aslostovar et al., 2018).

Another example is pyrimethamine, an antiparasitic drug indicated for the prevention and treatment of toxoplasmosis infection and malaria. A clinical trial evaluated the efficacy of pyrimethamine in patients with relapsed and refractory CLL after having demonstrated its capacity to drastically reduce the expression of the STAT 3 gene *in vitro*. Three cohorts of CLL patients who had received an average of six previous therapies were enrolled in a phase 1 trial. The results showed no objective response based on the guidelines of the International Workshop on CLLW-CLL in 2008, but half of the patients achieved stable disease, and a median overall survival of 22.2 months was observed (Byrd et al., 2014). Though the use of pyrimethamine as monotherapy did not lead to convincing results, important insights into the mechanism of action suggest that pyrimethamine can be combined with well-established signalling pathways inhibitors such as venotoclax, BTK, and PIK inhibitors (Brown et al., 2021).

Other combinational studies have demonstrated encouraging results for new regimens in the treatment of leukaemia. Statins, used to reduce the production of cholesterol have been proven to enhance the molecular response of CML patients when combined with tyrosine kinase inhibitors (Jang et al., 2021; Rizo-Liendo et al., 2020).

1.2.6: Modulation and inflammatory response in Leukaemia treatment

The immune system plays a critical role in leukaemia progression and treatment response, with immune cell dysfunction and chronic inflammation often contributing to disease pathogenesis (Sharma et al., 2024). Leukaemia disrupts normal haematopoiesis, leading to immune suppression, impaired antigen presentation, and altered cytokine signalling. Key immune cells, including T cells, natural killer (NK) cells, and dendritic cells, exhibit functional exhaustion or reduced cytotoxicity, limiting their ability to mount an effective anti-tumour response (Raskov et al., 2021). Additionally, increased expression of immune checkpoint molecules, such as PD-L1, and elevated levels of inflammatory cytokines create an immunosuppressive microenvironment that supports leukaemia cell survival and resistance to therapy (Laba et al., 2022). Given this immune dysregulation, drugs with immunomodulatory and anti-inflammatory properties, such as thioridazine, may offer therapeutic benefits. Thioridazine's ability to modulate inflammation and influence HLA-DR expression suggests a potential role in restoring immune function by reducing chronic immune activation while preserving anti-leukemic immunity (Roerden et al., 2021). In CLL for instance, CD19, CD20, CD23, and CD5 are commonly expressed on malignant B cells, distinguishing them from normal B-cell populations. The differential expression of CD38 and ZAP-70 is also linked to disease prognosis, with higher levels correlating with more aggressive disease (Zapevalova et al., 2022). Targeting CD markers has become a key therapeutic strategy, as seen with monoclonal antibodies such as rituximab (anti-CD20) and alemtuzumab (anti-CD52) (Hartinger et al., 2022), which enhance immune-mediated clearance of leukaemia cells. Additionally, chimeric antigen receptor (CAR) T-cell therapies targeting CD19 have revolutionized treatment for B-cell malignancies, offering durable remissions in relapsed/refractory cases (Haslauer et al., 2021). By targeting inflammatory pathways and enhancing immune cell activity, such drugs could be repurposed to improve leukaemia treatment outcomes, particularly in combination with existing immunotherapies or targeted agents.

1.3: Rationale for the study

Although there are some improvements in biomedicine, the treatment, and management of complex diseases of complex pathologies, especially blood cancers, continue to be a great challenge. Cancer incidence and mortality are still growing globally, and compared to developed countries there are high mortality rates found in developing countries like South Africa (Lortet-Tieulent et al., 2020). There are numerous cases of anticancer treatment failure due to first-line chemotherapy drug resistance to resulting from cancer inter and intra heterogeneity (Santiago et al., 2020). Precision medicine has been adapted as a solution to this problem toward more personalised cancer strategies based on the understanding of the clinical and biological features of patients with the goal to build the optimal therapeutic option for a specific patient. This approach can lead to the investigation of new drugs and drug combinations to meet the patients' unique characteristics. However, drug development is a costly process that does not always end up with a successful drug. For this reason, many studies are now focusing on repurposing or repositioning drugs. A major concern in the treatment of leukaemia is that patients rapidly develop drug resistance since most therapies are stratified and not personalised by identifying the responding patient through *ex vivo* drug screening. Medium-to-high-throughput drug screening with patient-derived cells allows for the systematic assessment of cellular responses to a diverse set of anticancer drugs, giving an unbiased strategy for individualized drug treatment selection. Although some studies have been published on drug repurposing for leukaemia precision medicine with some good examples of new drug indications (Fürstenau et al., 2019; Skånland et al., 2020; Thimiri Govinda Raj et al., 2018), leukaemia remains incurable. Furthermore, results from previously reported studies cannot be simply extrapolated to other populations due to the diverse genetic and environmental features. To the best of our knowledge, no study has been conducted to utilise drug screen to identify drugs with the potential of being repurposed for the treatment of South African leukaemia patients.

1.4: Aim

This project aims to utilize medium-to-high-throughput drug sensitivity screening to identify drugs that could be potentially repurposed for the treatment of leukaemia in South Africa.

1.5: Objectives

1. To describe the demographic and clinical profile (age, disease stage, type of mutation) of leukaemia patients
2. To evaluate the drug sensitivity of patient samples to a panel of thirty drugs using a medium- to high-throughput drug screening platform and identify effective drug combinations against patient cells.
3. To determine the effects of selected drug combinations on patient cells and mode of cell death by measuring cell viability using flow cytometry.
4. To evaluate the impact of selected drug combinations on cell surface markers using multicolour flow cytometry.
5. To study the inhibition potential of the most potent drug on the key proteins involved in leukaemia pathways with molecular docking technique.

CHAPTER 2: DEMOGRAPHIC AND CLINICAL PROFILING OF LEUKAEMIA PATIENTS

2.1: Introduction

Leukaemia, a group of malignant disorders characterised by the uncontrolled proliferation of abnormal leukocytes, represents a global public health challenge, affecting populations across various geographical regions. According to the Surveillance, Epidemiology, and End Results (SEER) database, an estimated 61,090 new leukaemia cases were reported in 2021, constituting 3.2% of all new cancer cases and ranking leukaemia as the 10th most prevalent cancer in the United States (Chennamadhavuni et al., 2023). In South Africa, the burden of leukaemia remains substantial. Though epidemiological data are limited, the South African National Cancer Registry report showed that leukaemia represents 357 males and 208 females per 100,000 population diagnosed histologically in 2020 (National Cancer Registry, 2020).

The major subtypes of leukaemia in adults include acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), and chronic lymphocytic leukaemia (CLL). These subtypes differ in the types of blood cells affected, the pace of disease progression, and the underlying genetic abnormalities. For instance, AML and ALL primarily affect immature white blood cells, whereas CML and CLL involve more mature cells. Moreover, each subtype may have unique risk factors contributing to its development, such as exposure to certain chemicals or genetic predispositions. Generally, the differences in leukaemia subtypes are found in the disease's clinical, haematological, and molecular characteristics. Clinical profiling encompasses a comprehensive assessment of patients' demographic characteristics, disease presentation, and prognostic factors, which guide treatment decisions and therapeutic strategies. Key parameters involved in clinical profiling include age at diagnosis, gender distribution, cytogenetic abnormalities, molecular markers, and comorbidities.

2.1.1: Aetiology and risk factors of leukaemia

The various biological subtypes of leukaemia each exhibit distinct causal mechanisms, with specific molecular abnormalities potentially associated with these causal factors. The processes leading to the initial mutation could differ from those promoting subsequent mutations.

The exact cause of leukaemia remains unknown. However, certain factors like ionising radiation, specific types of chemicals, particularly organic solvents like benzene, and some viruses have been identified as potential contributors to leukemogenesis (Richardson et al., 2009). Certain exposures consistently emerge as influential contributors to leukaemia risk factors. These encompass therapeutic, occupational, and wartime-related radiation, chemotherapy, familial predisposition, genetic syndromes, chemical exposures (both residential and occupational), and lifestyle elements like smoking (Bispo et al., 2020a). Notably, while specific exposures have been linked to subtypes of leukaemia, several prominent risk factors exert their influence across multiple subtypes (Bispo et al., 2020a). A case in point is the heightened mortality observed in individuals exposed to high doses of ionizing atomic bomb radiation in Japan (Richardson et al., 2009). This exposure showcases an increased risk for all non-CLL subtypes, including ALL, AML, and CML (Hsu et al., 2013; Richardson et al., 2009). Additionally, ionizing radiation exposure has been associated with the risk of non-CLL leukaemia in nuclear workers, radiologists, and patients subjected to therapeutic radiation for various medical conditions. Noteworthy is the robust link between occupational exposure to formaldehyde, a ubiquitous chemical found in building materials, household products, and industrial disinfectants, and the heightened incidence of myeloid leukaemia (Checkoway et al., 2012; Kang et al., 2021).

2.1.2: Demographics of leukaemia

Geographic Location: Geographic location and regional differences can influence the incidence and prevalence of leukaemia. Certain environmental exposures or risk factors may be more prevalent in specific geographic regions, leading to differences in leukaemia patterns across different areas.

The distribution of leukaemia burden exhibits a discernible geographic pattern closely tied to the level of development at the country level. Age-standardized incidence and mortality rates are notably higher in countries characterized by advanced development. The International

Agency for Research on Cancer (IARC) employs the GLOBOCAN database to categorize 185 nations based on the Human Development Index (HDI), a composite metric encompassing life expectancy, education, and standard of living (Sung et al., 2021). In 2018, the incidence rates of leukaemia in countries with high/very high HDI markedly surpassed those in low/medium HDI countries. A similar trend was observed in mortality rates, with high/very high HDI countries exceeding low/medium HDI countries (Bray et al., 2018).

Ethnicity and socioeconomic status

Current evidence suggests that leukaemia incidence rates in the United States vary among different racial groups. The highest incidence is seen in Whites at 15 cases per 100,000 people, followed by blacks at 11 per 100,000, and Hispanics at 10.6 per 100,000. Asians/Pacific Islanders (API) and American Indian/Alaskan Natives (AIAN) have lower incidence rates, with 7.8 and 8.3 cases per 100,000, respectively (Bispo et al., 2020b). A similar pattern is observed in mortality rates, which also differ among racial groups. Whites have the highest mortality rate at 7 per 100,000, followed by blacks at 5.6 per 100,000, and hispanics at 4.8 per 100,000. The mortality rates are lower for API and AIAN, at 3.8 and 3.3 per 100,000, respectively. Despite higher incidence and mortality rates in whites, survival rates are poorer for black patients across different age groups. For patients under 65 years at diagnosis, the five-year relative survival is 73% for whites and 63% for blacks. In the 65 and older age group, the five-year relative survival is 50% for whites and 43% for blacks (Bray et al., 2018; Siegel et al., 2024). Socioeconomic factors can impact access to healthcare, early diagnosis, and treatment options for adult leukaemia patients. Disparities in healthcare resources and socioeconomic status may lead to variations in disease outcomes and survival rates among different socioeconomic groups.

2.1.3: Genetic abnormalities in leukaemia

Leukaemia is characterised by genetic abnormalities that disrupt normal blood cell development and function. These include chromosomal abnormalities like translocations, deletions, and duplications, with the Philadelphia chromosome being a notable example, leading to the formation of the BCR-ABL fusion gene, implicated in CML (Sampaio et al., 2021). Mutations in genes such as *FLT3*, *TP53*, and *RAS* also play significant roles, contributing to uncontrolled cell growth and survival (Kloppers et al., 2021). FLT3 mutations

are common in AML, while *TP53* mutations are associated with several leukaemia types, including CLL and AML. Additionally, the BCR-ABL fusion gene, besides CML, is found in a subset of ALL cases, indicating its broader significance across leukaemia subtypes (Hallek et al., 2018; Jabbour & Kantarjian, 2018; Reikvam et al., 2011). Understanding these genetic aberrations is pivotal for accurate diagnosis, prognosis determination, and the development of targeted therapies tailored to the underlying molecular drivers of the disease.

2.1.4: Treatment of leukaemia

The treatment landscape of leukaemia has significantly evolved over the years. Conventional treatment approaches include chemotherapy, radiation therapy, and stem cell transplantation. Targeted therapies specifically target molecular abnormalities driving leukaemia growth, such as tyrosine kinase inhibitors CML targeting BCR-ABL fusion protein. Immunotherapies, such as monoclonal antibodies and chimeric antigen receptor (CAR) T-cell therapy, harness the immune system to recognize and eliminate leukaemia cells. During cancer progression and therapeutic intervention, clones and subclones may evolve in a patient. This phenomenon called intratumor heterogeneity is one cause of drug resistance (Desai et al., 2022). Considering tumour genetic features in clinical settings can help to overcome drug resistance linked to tumour heterogeneity and tailor personalized cancer therapy. The treatment modalities of leukaemia are extensively discussed in the previous chapter (Chapter 1).
This chapter aims to document the demographic and clinical profile (including treatment) of the adult leukaemia patients enrolled in this study and to analyse differences in treatment to support the need for precision medicine.

2.2: Material and Methods

2.2.1: Patient recruitment and sample collection

The University of the Witwatersrand, Human Research Ethics (REC-250208-004) granted ethical approval for the study (M211171). Permission to conduct the study was obtained from the Chris Hani Baragwanath Academic Hospital (CHBAH) and Wits Donald Gordon Medical Centre (WDGMC), both based in Johannesburg, South Africa. Sample and data collection were carried out at the Haematology Department of these hospitals from 1st June to 30th November 2022. Prior to sampling, voluntary informed consent (Appendix F) was obtained from all

participants. All patients aged 18 years and above, and diagnosed with AML, CML, and CLL, were included. Patients presenting with other cancers were excluded. Blood was drawn from forty-one patients and five healthy volunteers who were not on any chronic medication. Twenty millilitres (20 mL) of blood was collected in EDTA tubes from each participant once off. Leukaemia patients were selected based on clinical diagnosis. Demographic and clinical data were collected from medical reports, including age, gender, and ethnicity. Clinical and laboratory parameters collected from patients' files included the date of diagnosis, date of recruitment, previous treatment and duration, performance status, haematology parameters like haemoglobin (Hb), white blood cell count (WBC), lymphocytes, monocytes, disease stage, and cytogenic aberrations.
For more details on the parameters included, please refer to Appendix D.

2.2.2: Data collection and statistical analysis

Data were entered into a Microsoft Excel sheet and loaded in R version 4.3.2 for analysis. Descriptive statistics were calculated, including sums, proportions, percentages, means, and medians. The findings were also depicted using appropriate charts, tables, and figures. A p-value ≤ 0.05 was considered statistically significant. The Wilcoxon and Kruskal-Wallis rank-sum tests were employed to compare disparities in numerical covariates such as age, while Fisher's exact test was utilized to evaluate differences between categorical variables, such as gender or race.
The summary statistics were generated using the tbl_summary function of the gtsummary package in R, which automatically selects the appropriate summary statistics based on the variable type. For continuous variables, it calculates the mean and standard deviation if the data are approximately normally distributed, and the median and interquartile range (IQR) if the data are skewed. For categorical variables, it calculates the count and percentage in each category.

2.3: Results

2.3.1: Demographic and haematological profile

The age distribution by subtype and gender is illustrated in **Figure 2.1**. Female participants in the cohort were found to develop AML and CML at an early stage compared to male

participants. Specifically, the youngest participants were female, aged 19 and 21 years old, from the CML and AML subtypes, respectively, whereas in the male category, the youngest for the same subtypes were 31- and 58-years old Figure 2.1. The evaluation of age at disease onset and the age at the time of blood collection, gender, and race to gain a better comprehension of the population and variations in disease parameters is displayed in **Table 2.1**. A significant difference ($p = 0.02$) in age at enrolment was found among subtypes at the time of entry into the study. The median as a measure of central tendency revealed that the CML subtype had the smallest median age, followed by AML and CLL (45, 58, and 63 years, respectively).

The gender distribution in the cohort did not yield statistical significance. The study comprised in total a higher number of male participants (n=23) than female participants (n=18). Among the subtypes, females made up 71% of AML patients and 37% of CML patients (Table 2.1). Males, on the other hand, represented 29 % and 63 % of AML and CML subtypes, respectively. In the CLL subtype, the sex ratio was equal, with 50% of the cohort being female and 50% being male.

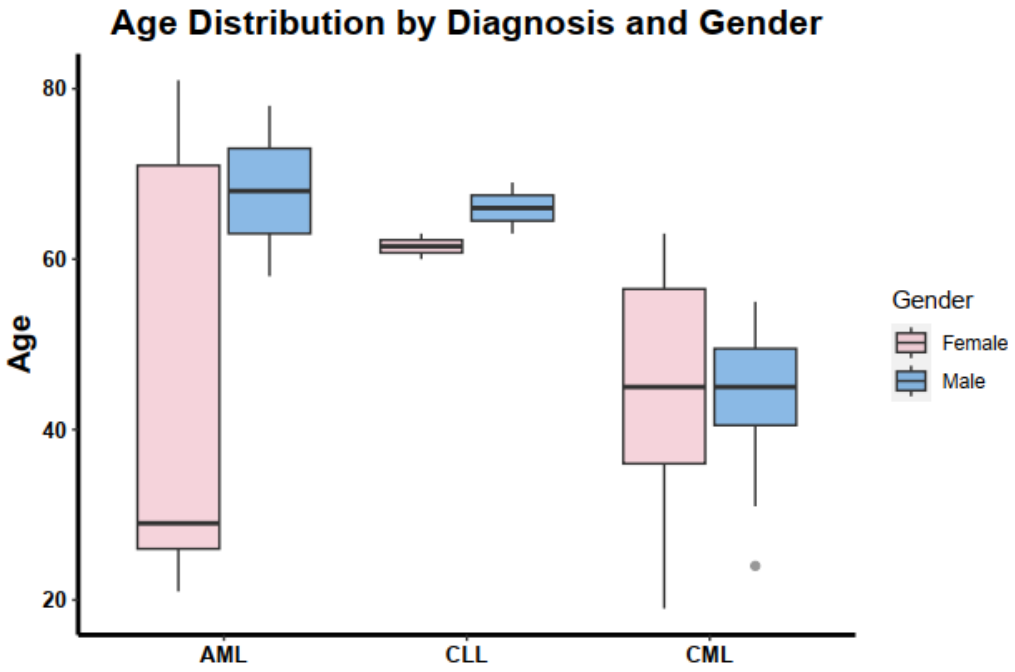


Figure 2.1: Age distribution by diagnosis and gender. AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia

Table 2.1: Summary statistics of the subjects in the cohort

Characteristics	AML, N = 7	CLL, N = 4	CML, N = 30	p-value
Age, median (IQR)	58 (28 - 74)	63 (62 - 64)	45 (40 - 52)	0.020
Onset Age, median (IQR)	38 (23, 64)	61 (60, 64)	40 (34, 48)	0.072
Unknown	1	1	9	
Gender				0.3
Female	5 (71%)	2 (50%)	11 (37%)	
Male	2 (29%)	2 (50%)	19 (63%)	
Weight (Kg)	64 (64, 71)	80 (76, 84)	74 (68, 79)	0.030
Ethnicity, *n (%)*				0.023
Black	3 (43%)	3 (75%)	25 (89%)	
White	4 (57%)	1 (25%)	3 (11%)	
Unknown	0	0	2	

AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia, IQR: interquartile range

The haematological characteristics of the cohort at the time of collection were matched and compared across the three subtypes (AML, CLL, and AML) and summarised in **Table 2.2**. No significant differences were observed among subtypes, except for the concentration of Haemoglobin, white blood cells (WBC), and neutrophils ($p \leq 0.05$). AML and CLL patients demonstrated abnormal values of haemoglobin (8.30 g/dL and 9.80 g/dL, respectively) outside the normal or reference range (12.4 g/dL – 16.7 g/dL) (BARC SA, April 2023). The same observation is true for the WBC and lymphocytes, where AML patients had median values of WBC and Lymphocytes (3 $x10^9$/L and 0.84 $x10^9$/L respectively) lower than their respective reference range. For CLL, the abnormal values of WBC (31 $x10^9$/L) and Lymphocytes (25.86 $x10^9$/L) are far higher than the maximum value of their respective reference range (4.0 $x10^9$/L – 12.0 $x10^9$/L and 1.0 $x10^9$/L – 4 $x10^9$/L). As compared to the reference ranges of the evaluated parameters, CML patients tend to have haematological parameters that fit into the normal range. Indeed, the mean corpuscular volume that measures the volume of the size of the red blood cells was within the normal range (79 fL – 100 fL) for AML (93.6 fL) and CML (90.9 fL), whereas CLL patients had a mean MCV higher than the normal range, with a MCV of 105.6 fL.

Overall, AML patients tended to have abnormal MCV values below the normal range whereas CLL patients had abnormal values higher than the reference range.

Table 2.2: Summary of the haematological profile of the cohort.

Characteristics	+Reference range	AML	CLL	CML	p-value
Haemoglobin (g/dL)	12.4 - 16.7	8.30 (8.10, 9.00)	9.80 (9.80, 9.80)	14.20 (11.23, 14.88)	0.014*
Mean Corpuscular Volume (fL)	79 – 100	93.6 (92.9, 94.5)	105.6 (105.6, 105.6)	90.9 (84.6, 95.1)	0.13
Platelets $x10^9$/L	150 -450	61 (28, 199)	92 (92, 92)	240 (205, 296)	0.071
WBC $x10^9$/L	4.0 – 12.0	3 (2, 4)	31 (31, 31)	6 (4, 7)	0.052*
Neutrophils $x10^9$/L	2.0 – 7.5	1 (1, 1)	4 (4, 4)	2 (2, 4)	0.053*
Eosinophils $x10^9$/L	0.00 – 0.50	0.02 (0.00, 0.06)	0.32 (0.32, 0.32)	0.05 (0.02, 0.17)	0.3
Basophils $x10^9$/L	0.00 - 0.30	0.01 (0.00, 0.02)	0.02 (0.02, 0.02)	0.03 (0.01, 0.19)	0.11
Lymphocytes $x10^9$/L	1.0 – 4.0	0.84 (0.28, 2.11)	25.86 (25.86, 25.86)	1.94 (1.57, 2.85)	0.089
Monocytes $x10^9$/L	0.2 – 1.0	0.52 (0.21, 0.89)	0.96 (0.96, 0.96)	0.40 (0.29, 0.51)	0.5

+ Reference range evaluated by Bio Analytical Research Corporation South Africa (BARC SA), https://www.barcsa.co.za/test-directory/test-reference-ranges/ Accessed on 8th April 2023. AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia. * p-value ≤ 0.05 considered statistically significant

2.3.2: Drugs administered as a previous treatment to study participants.

The treatment of leukaemia requires the use of drugs that vary depending on subtypes. As shown in Table 2.3, Imavec appeared to be the most frequent medication used in the cohort followed by Tasigna. These two drugs are commercial brand names containing the chemotherapy compounds imatinib mesylate and nilotinib respectively. Additionally, the comprehensive list (**Table 2.3**) showed the occurrence of different medications that can be grouped into different classes of drugs. Targeted therapies like dasatinib, imatinib, ponatinib, nilotinib, and asciminib, which are indicated for the treatment of chronic myeloid leukaemia as well as the class of drug called antimetabolites represented with the presence of hydroxyurea (HU). The presence of alkylating agents like venotoclax and chlorambucil confirms the presence of chronic lymphocytic leukaemia patients in the cohort. Furthermore, the results also

showed that some patients underwent bone marrow transplants (BM Transplant), which is a treatment strategy for acute myeloid leukaemia patients. Interestingly, non-cancer drugs such as tradozone (a serotonin modulator used for maintaining mental health) and antibiotics like fluconazole and andolex were also present in the corpus.

Table 2.3: A comprehensive list of the patients' prior treatment and commonly used chemotherapy drugs in the cohort.

Drug name	Frequency	Brand or active compound	Drug class
Imavec	11	Brand name (Imatinib)	Kinase inhibitors
Tasigna	7	Brand name (Nilotinib)	Tyrosine kinase inhibitor (TKI)
Nilotinib	6	compound	Kinase inhibitors
Vidaza	4	Brand name (Azacytidine)	Kemethylation agents
Dasatinib	3	compound	Kinase inhibitors
Gleevec	3	Brand name (imatinib)	Kinase inhibitors
Imatinib	3	compound	Kinase inhibitors
Ponatinib	3	compound	Kinase inhibitors
Venotoclax	3	compound	B-cell lymphoma-2 (BCL-2) inhibitors
Hydroxyurea (HU)	2	compound	Antimetabolites
Fluconazole	2	compound	Antifungals-triazoles
BM Transplant	2		
Thioguanine	1	compound	Purine analogues
Allopurinol	1	compound	Xanthine oxidase inhibitors
Andolex	1	Brand name	Anti-inflammatory
Asciminib	1	compound	Kinase inhibitors
Bendamustine	1	compound	Alkylating agents
Grantryl	1	compound	5-HT3 receptor-antagonists
Chlorambucil	1	compound	Alkylating agents

2.3.3: Evaluation of patients' fitness status and treatment history

Many cancer patients are given chemotherapy as a means to manage the disease, with a possible impact on their quality of life. The Eastern Cooperative Oncology Group (ECOG) Performance Status Scale (**Appendix 2.1**) is a widely used measurement tool that assesses a patient's level of functioning based on their ability to perform self-care, engage in daily activities, and maintain physical capabilities such as walking and working. It is used as a standardised criterion by multiple hospitals, cancer centres, and clinics to measure the effect of the disease and response to treatment on a patient's daily living abilities, commonly known as the patient's performance status (P.S) to physicians and researchers. Patients recruited in this study had a performance status of either 0 or 1 as shown in **Figure 2.2**. Just over half of the female participants with the CML subtype tended to have a performance status (P.S = 1) associated with restriction in strenuous physical activity compared to males with CML where the majority had a performance PS of 0, interpreted as fully active and able to carry on all pre-disease performance without restriction.

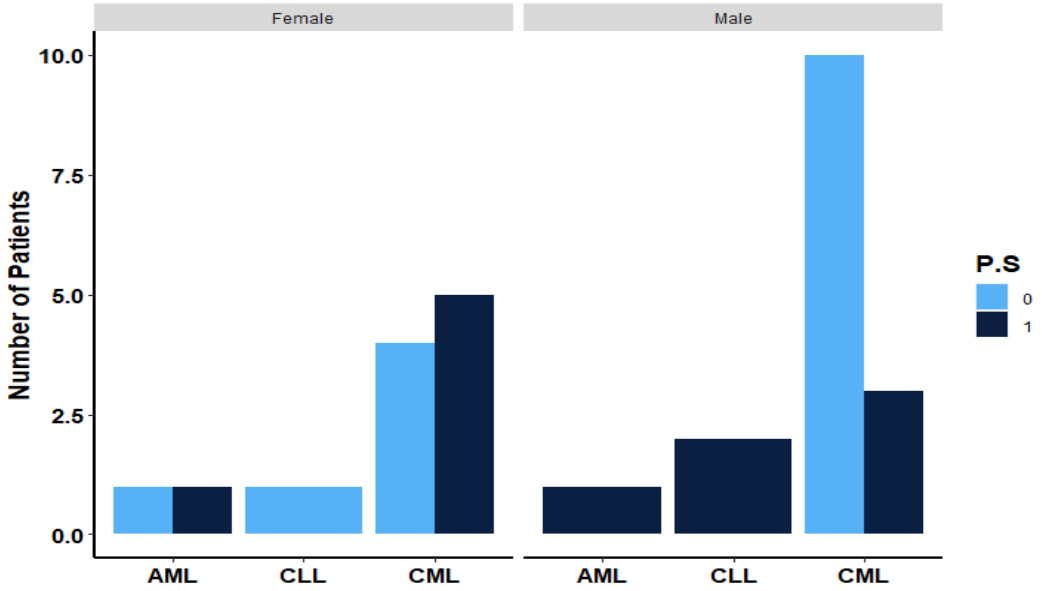


Figure 2.2: Patient performance status. AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia. 0 indicating fully active and 1 means restricted in strenuous activity.

2.3.4: Evaluation of the treatment history of patients

Overall, No CLL patient in the cohort underwent multiple treatments as CLL often requires a wait-and-see period and some patients do not require treatment. AML and CML are the subtypes that usually require immediate treatment at diagnosis to reduce the symptoms of the disease and to avoid disease progression and differentiation into another subtype. All CML patients had received at least one chemotherapy drug. **Table 2.4** shows the list of patients who underwent more than one treatment. For most CML patients, the first treatment regimen included a tyrosine kinase inhibitor like nilotinib or imatinib. Interestingly, the treatment response varies among patients and even among patients diagnosed in the same year. For example, patients BH14 and BH28 diagnosed in 2017 received imatinib as first-line chemotherapy but demonstrated different treatment responses over time. Patient BH14 started treatment in 2017 and received nilotinib in 2019, while patient BH28 only started nilotinib in 2022. Another notable observation of the treatment history is the difference in drug concentration of the various chemotherapeutic agents administered to patients. In only fourteen months of chemotherapy, BH22, who was diagnosed in 2021, has experienced a considerable treatment variation, starting with Imatinib for 6 months and then receiving Tasigna (nilotinib) in February 2022 (Table 2.4). The change in drug concentration is seen with the chemotherapeutic agent ponatinib, which was administered in September 2022 at 45 mg, reduced to 15mg in the same month, and stabilised at 30mg in October 2022.

A similar pattern of variability is observed in other patients with drugs like nilotinib, which was administered to BH08 from 400 mg to 200 mg but was increased from 400mg to 600mg on BH16 or from 200mg to 400mg on BH05 (Table 2.4).

Table 2.4: Treatment history of patients who received more than one chemotherapy drug.

Code	Subtype	Year of Diagnosis	Treatment history	Duration	PBMC (x 10^6)
BH22	CML	2021	July2021, Imavec (imatinib) February 2022, Tasigna (nilotinib) September 2022, ponatinib 45mg ponatinib 15mg, October 2022, ponatinib 30mg	6 months 6 months 1 month Current	5.3
BH08	CML	2021	April 2021 Tasigna (nilotinib) 400mg June 2021 Tasigna (nilotinib) 200mg	2 months 4 months Current	1.05
BH16	CML	2020	Tasigna (nilotinib) 400mg		5.7

			Imavec (imatinib) 600mg		
BH05	CML	2017	2017, imatinib 2021, Tasigna (nilotinib) 200mg April 2022, nilotinib 400mg	3 years 1 year	6.15
BH14	CML	2017	2017-2019, imatinib 2019-2021, Tasigna (nilotinib)	2 years 3 years	6.5
BH28	CML	2017	2017, Imavec 2022, nilotinib	5 years	3.1
BH29	CML	2013	2013, Gleevec (imatinib) 2022, Tasigna (nilotinib)	8 years	2.625
BH13	CML	2007	2007, Gleevec (imatinib) 2012, dasatinib	4 years	28
BH19	CML	2020	2020, Imavec (imatinib) 400mg Tasigna (Nilotinib)	2 years	6.1
BH20	CML	2019	2019, Imavec (imatinib) 400mg 2021, Imavec (imatinib) 600mg July 2021, nilotinib	2 years	2.25
BH23	AML	2017	asciminib, ponatinib 15mg		7.7
BH12	AML	2014	thioguanine relapsed twice		31
DGAML02	AML		2 cycles of Vidaza/Venetoclax		2.925

AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia. PBMC: Peripheral blood mononuclear cells, from healthy blood range from 0.5 to 3 x 10^6 cells/mL blood.

2.3.5: Genetic characteristics of patients

Leukaemia is characterised by a diverse range of cytogenetic abnormalities, which play a crucial role in disease classification, prognosis, and treatment stratification. These abnormalities often involve chromosomal translocations, deletions, and mutations affecting genes critical for haematopoietic differentiation and proliferation. Although the genetic characteristics of most patients were difficult to capture on our study sites some molecular aberrations were recorded and presented in **Table 2.5**. The most common cytogenetic abnormalities in AML include the t (8;21) translocations. This well-known translocation also known as AML-M2, was found in patient BH12. A relatively rare translocation, t (6,11) (q27; q23) was found in the patient DGAML02. Other recurrent abnormalities include mutations in genes such as FLT3, and NPM1. In this cohort, one out of the 5 AMLs presented this as an NPMI+/FLT3 ITD- abnormality (Table 2.5). This mutation involves the insertion or duplication of nucleotides within exon 12 of the NPM1 gene and the FLT3 ITD- indicating the

absence of internal tandem duplications in this patient. CML is characterized by the Philadelphia chromosome (Ph), resulting from a reciprocal translocation between chromosomes 9 and 22, and leading to the formation of the BCR-ABL1 fusion gene. This genetic abnormality among CML patients in this cohort was expressed at different percentages based on the International Scale (IS). The patient BH04 had a BCR-ABL fusion gene of 75% compared to BH18 with 91 %. The absence of Philadelphia chromosome (Ph-) was found in patients BH05 and BH29, with different percentages of BCR-ABL expressed 21.48% and 0.28% respectively (Table 2.5). CLL on the other hand is characterised by the accumulation of mature B lymphocytes with a characteristic immunophenotype (CD5+, CD23+, CD19+, CD20low/+). Cytogenic abnormalities generally include deletions of chromosomes 13q14, trisomy 12, and mutations affecting genes such as TP53. The patient DGCLL01 had del13q14.3 and trisomy 12, which are common genetic abnormalities associated with a good prognostic.

Table 2.5: Genetic characteristics of patients.

Code		**Diagnosis**	**Stage**	**Cytogenetic aberrations**	**BCR-ABL (%)**
	AML				
BH12		AML	M2	t (8,21)	*NA*
DGAML02		AML		t (6,11) (q27; q23)	*NA*
AML04		AML	Relapse	NPM1+/FLT3 ITD-	*NA*
	CML				
BH02		CML	AP	Ph+	
BH04		CML		Ph+	75%
BH05		CML	CP	Ph-	21.48%
BH07		CML	CP	t(9, 22) / Ph+	55%
BH18		CML		Ph+	91%
BH22		CML	CP	T315I	
BH29		CML		Ph-	0.28%
	CLL				
BH24		CLL	Rai1/BenetB		*NA*
DGCLL01		CLL		del13q14.3, trisomy 12	*NA*
CLL02		CLL			*NA*

	AML: Acute myeloid leukaemia, CLL: Chronic lymphocytic leukaemia, CML: Chronic myeloid leukaemia; AP: Acute Phase, CP: Chronic phase; NA: Not Applicable

2.4: Discussion

This chapter describes the demographic and clinical profiles of the adult leukaemia participants sampled from two hospitals in Johannesburg, South Africa. The investigation focused on sampling leukaemia subtypes, and we found three major subtypes prevalent in our cohort, namely AML, CML, and CLL.

Demographic profile

The median onset age of our sample of AML patients was 38 years, which is lower than the median age of onset of 67 years for AML globally (Kloppers et al., 2021). The patients in this cohort exhibited disease onset at a notably younger age. This observation is similar to the findings of a prior study conducted in South Africa, wherein adult AML patients manifested the disease with a median age of 38.5 (Kloppers et al., 2021). The reported median age for CLL was 61, somewhat lower than the median age generally reported in the literature, ranging from 66 to 72 years old (Cennamo et al., 2023; Kim et al., 2023). This confirms that CLL is an elderly disease affecting predominantly adults in their 60s and above. Overall, in this study, the majority of the study population is from black ethnicity, which is particularly true for CML (89%). These observations could be explained by an income factor and the study site, which included participants mainly from a public hospital in Johannesburg, compared to private hospitals, given that blacks constitute the majority of the SA population. Furthermore, due to historical reasons, a majority of this population still falls into a lower socioeconomic category. The influence of a patient's ethnicity on the clinical outcome of leukaemia has been demonstrated in many studies. Despite receiving equal standards of care, individuals of white ethnicity tend to experience a more favourable prognosis, characterized by longer event-free and overall survival compared to their black counterparts (Bhatia et al., 2002; Flowers & Pro, 2013). These findings highlight the significance of considering ethnic diversity when assessing clinical outcomes in leukaemia patients and underscore the need for equitable access to effective treatment strategies for all ethnic groups.

Clinical characteristics

Haematological parameters

The haematological data of the patients enrolled in this study included various markers such as white blood cell count (10^9/L), haemoglobin (g/dL), mean corpuscular volume (fl), lymphocytes (10^9/L), platelets (10^9/L), monocytes (10^9/L), mosinophils (10^9/L) and basophils (10^9/L). These data collectively provided valuable insights into the haematological status of the study population, facilitating the understanding of their blood-related health and potential indicators of underlying medical conditions. Significant differences were observed among the three subtypes (AML, CLL and CML) for haemoglobin, white blood count (WBC) and neutrophil levels. These differences weres found in the AML and CLL subtypes, which were generally out of the normal range (Table 2.2).

All the parameters evaluated were within the normal range for CML patients. This may be attributed to the efficacy of BTK inhibitors, which have revolutionised the treatment outcome for this subtype of leukaemia patients. On the other hand, CLL patients had a mean MCV and WBC higher than the normal ranges and a lymphocyte count of approximately 6 times higher than the normal range (Table 2.2). The results of the frequency of drugs in table 2.3 demonstrate that most of the CLL patients received less drugs. Indeed, CLL is a chronic subtype of leukaemia which generally requires a monitoring period and a third of patients never required treatment (Shadman, 2023). Despite having received chemotherapy and bone marrow transplantation, most clinical parameters for AML patients were lower than the normal ranges. A haemoglobin value less than 12.4g/dL indicates anaemia as seen in the AML and CLL subtype. Additionally, thrombocytopenia, characterised by a very low platelet count compared to normal ranges was found in AML and CLL patients (Table 2.2). This condition is life-threatening, placing these patients at risk of internal bleeding and impaired haemostasis which leads to difficulties in blood clotting and bleeding control (Hermsen & Hambley, 2023). Neutrophils and lymphocytes perform a crucial function in immune defence by phagocytising, eliminating, and digesting invading microorganisms, such as fungi and bacteria. The inability in fulfilling this role results in immunodeficiency, primarily characterized by the occurrence of recurrent infections (Vaillant & Zito, 2023). Neutropenia and Lymphopenia observed in the AML participants in this cohort could be signs of persistent leukaemia or the cytotoxic effect of the drugs administered. Chemotherapy-induced toxicity represents a prominent contributor to morbidity and mortality among cancer patients. Such adverse effects predominantly arise due to the inherent lack of specificity exhibited by chemotherapy agents, leading to direct damage to healthy tissues in addition to malignant cells (Vagace et al., 2011).

Cytogenetic abnormalities of patients

The identification of molecular abnormalities in leukaemia patients has significantly contributed to a more comprehensive understanding of the disease's biology. Moreover, this advancement has facilitated the incorporation of improved risk stratification into diagnostic algorithms (Roloff & Griffiths, 2018). Despite the significant progress in the understanding and management of leukaemia driven by these molecular discoveries, there have been limited publications specifically focused on the prevalence of these mutations in the South African leukaemia population. By investigating the frequency of these molecular abnormalities within the specific context of the South African population, this study can provide valuable insights into the genetic landscape of leukaemia in this region. This information may have implications for developing targeted treatment approaches and refining risk stratification methods that are more relevant and effective for patients in South Africa.

In this study, the *NPM1* mutation was present in patient AML04, unlike in the study of Kloppers and colleagues, who reported no NPM1 mutation for the study of 40 newly diagnosed AML patients recruited in an academic hospital in the Free State province of South Africa from November 2018 to December 2019 (Kloppers et al., 2021). Additionally, no NPM1 mutation was found in a study of 100 AML patients in Sudan (Elzain et al., 2021). In contrast, a study of 160 AML patients from Johannesburg, South Africa, showed that 7.5% of patients carry the mutation (Marshall et al., 2014). The variation of NMP1 mutation frequency may be attributed to the low prevalence or the age of the population. As reported by other studies, only approximately 20-30% of AML patients carry the NPM1 mutation, and the method used to determine this mutation varies, and one can be more sensitive than others (Raimondi et al., 2022; Sharma & Liesveld, 2023). However, due to limited available data, no definitive association can be established. The NPM1+/FLT3 ITD- genetic profile suggests favourable prognostic features for the patient. It implies the presence of NPM1 mutations, which are associated with a good response to chemotherapy and improved overall survival (F. Wang et al., 2015). Additionally, the absence of FLT3-ITD mutations further enhances the favourable prognosis associated with this genetic profile. Patients with AML harbouring the NPM1+/FLT3 ITD- genotype often respond well to standard induction chemotherapy and may have a lower risk of relapse compared to those with other genetic profiles. Another genetic

abnormality detected among AML patients is the presence of translocation t (8,21) and t (6,11) (q27; q23) which represents a distinct subgroup of AML patients. The t (8,21) translocation represents approximately 5% - 10% of all AML cases and is most common in younger patients (Reikvam et al., 2011). The t (6,11) (q27; q23) translocation is associated with a poor prognostic compared to t (8,21) (Benjamin et al., 2023; Sharma & Liesveld, 2023).

Common prevalent chromosomic abnormalities in chronic lymphocytic leukaemia (CLL) include the deletion of the short arm of chromosome 17 (deletion 17p), deletion of the long arm of chromosome 11 (deletion 11q), deletion of the long arm of chromosome 13 (deletion 13q), and trisomy of chromosome 12 (trisomy 12). These chromosomal alterations are collectively identified in approximately 80% of CLL patients (Kipps et al., 2017; Musaigwa et al., 2021). The CLL patients enrolled in this study were identified as having del13q14.3 and trisomy 12, which implies that they have a good prognostic factor.

The Philadelphia chromosome is a distinct chromosomal aberration arising from a reciprocal translocation event involving chromosomes 9 and 22. This rearrangement brings about the fusion of the BCR gene with the ABL1 gene located on chromosome 9, resulting in the formation of a novel fusion gene known as BCR-ABL1. The presence of Philadelphia chromosome (Ph-positive) is a defining characteristic of chronic myeloid leukaemia (CML) and is essential for diagnosis and treatment, and is present in more than 95% of CML patients are Ph-positive (Sampaio et al., 2021). In this study, CML patients were found to harbour this genetic feature with different levels of the BCR-ABL fusion gene. An interesting observation was the absence of Philadelphia chromosome (Ph-) in some CML patients, also with different percentages of BCR-ABL mutation (0.28 – 21.48%). Ph-negative patients constitute about 5% of CML cases. Despite the absence of t(9,22) rearrangement, Ph-negative CML may still exhibit similar clinical and haematological features as those with Ph-positive CML (Cross, 2020). The exact mechanisms and molecular abnormalities leading to Ph-negative CML are not yet fully understood. Some studies suggest that Ph(+) CML patients may develop Ph(-) CML during tyrosine kinase inhibitors (TKI) therapy (Ni et al., 2019; Yuan et al., 2019). These results not only highlight the heterogeneity of the disease but also suggest that personalised treatments should be considered.

Treatment history and performance status

Although patients were associated with immunodeficiency, in this study, they exhibited relatively good performance status, with all classified as PS 0 or 1 (Figure 2.2). This suggests

that despite underlying immune dysfunction, overall functional capacity was not significantly impaired.

The results of this study shed light on the medication profile and treatment strategies employed in the cohort of South African leukaemia patients. Targeted therapies, such as dasatinib, imatinib, ponatinib, nilotinib, and asciminib, were identified, reflecting their role in treating CML. These agents, known for their ability to selectively inhibit BCR-ABL1 fusion protein activity, have revolutionized CML treatment and improved patient outcomes (Cross, 2020; Natarajan et al., 2019). Although CML patients under TKI had an overall good performance status, the differences in reactiveness of patients were noticeable (Table 2.4). The duration of treatment varies considerably among patients, and the standard doses of some chemotherapeutic agents were decreased or increased depending on the response of a particular patient to the treatment. This result emphasises the fact that a one-size-fits-all treatment regimen is not the best approach for the treatment of leukaemia, especially for CML, where 20%-40% require a change of chemotherapy drug due to resistance or intolerance (Benjamin et al., 2023). Additionally, alkylating agents' efficacy in disrupting DNA replication highlights their significance in CLL therapy. Furthermore, the incorporation of bone marrow transplant as a treatment strategy for some patients showcases its potential in managing AML. The successful implementation of BM transplant can lead to remission and improved survival in AML cases (Mehdizadeh et al., 2022). The mortality rate for adults with AML remains high (Abbasi et al., 2019; Sasaki et al., 2021). The established treatment approach for AML involves administering remission induction chemotherapy, typically using a combination of anthracycline and cytarabine. Subsequently, the patient's further treatment pathway is determined based on their capacity to endure intensive therapy and the probability of achieving a cure solely through chemotherapy. Options for further treatment include consolidation chemotherapy or allogeneic stem cell transplantation (Kayser & Levis, 2023; Roboz, 2012).

2.5: Conclusion

The findings reported in this study provide valuable insights into the disparities in the demographic, clinical, and treatment history and molecular landscape of the cohort of South African leukaemia patients, underscoring the importance of tailoring personalised therapeutic approaches to improve patients' outcomes. Future research may delve deeper into the

underlying factors contributing to these variations, potentially leading to improved therapeutic interventions and better patient outcomes.

CHAPTER 3: DRUG SENSITIVITY AND RESISTANCE TESTING IN LEUKAEMIA SAMPLES

3.1: Introduction

The advent of advanced technologies has revolutionised the landscape of cancer research and treatment. Throughout the drug discovery and development phases, a large number of pharmaceutical compounds fail clinical trials because of factors such as formulation, safety, and distribution. A promising strategy that helps overcome these drug development challenges is drug repurposing, which involves medium to high throughput drug screening and requires a knowledge of the biochemical, physiological, and clinical significance of the effect of the drug(s) to be used (Boshuizen & Peeper, 2020). Drug repurposing or drug repositioning is a strategy of investigation and identification of new therapeutic uses for existing and authorized drugs other than their intended use. In the treatment of cancer, these already approved drugs can be commonly used in chemotherapy or compounds prescribed for diseases other than cancer (Pushpakom et al., 2019; Sleire et al., 2017). One of the primary goals of high-throughput screening technology is to speed up the drug development process for both infectious and non-communicable pathologies.

3.1.1: Principle of drug screening and resistance testing:

Drug screening plays a vital role in identifying potential treatments for leukaemia, whether through novel drug discovery or repurposing existing compounds. To ensure the accuracy and relevance of results, several critical factors must be considered. One of the most important is assay sensitivity and specificity. Sensitivity refers to the assay's ability to detect even small changes in leukaemia cells' response to treatment, which is particularly important when evaluating compounds that may exert subtle effects. Specificity, on the other hand, ensures that the observed effects are due to the intended interaction with the leukaemia cells, rather than non-specific interactions that could lead to false positives (Giliberto et al., 2022; Hughes et al., 2011). This is essential when testing drugs that might also modulate immune responses, such as immunomodulatory drugs, to ensure that the effects observed are truly related to their anti-

leukemic activity and not off-target immune activation (Sharma et al., 2024). Additionally, the choice of a compound library is critical. A diverse library of compounds increases the likelihood of identifying effective therapies, particularly for drug-resistant strains. By incorporating a wide range of chemical structures and mechanisms of action, researchers can identify novel treatments that may not have been considered through traditional drug discovery methods (Sleire et al., 2017). The selection of cell lines or disease models also plays a crucial role. Using models that reflect this genetic and phenotypic diversity is essential to ensure that the screening results are relevant and can inform treatment strategies for a broad range of patients. Furthermore, establishing the dose-response relationship allows for the identification of therapeutic windows and ensures that drugs are tested in clinically relevant concentrations (Giliberto et al., 2022). This is particularly important for drugs like tyrosine kinase inhibitors (TKIs), where the goal is to pinpoint effective doses that overcome resistance mechanisms in leukaemia cells (Yashiro et al., 2011). By incorporating these factors into drug screening, researchers can improve the chances of discovering treatments that will significantly impact leuk**a**emia therapy.

3.1.2: Benefits of drug sensitivity testing for precision oncology

Drug screening for precision oncology provides a comprehensive view of how individual samples respond to various drugs, allowing for the identification of the most effective treatments for each patient. This personalized approach minimizes the trial-and-error process in treatment selection, increasing the likelihood of achieving positive clinical outcomes. One of the key benefits of high-throughput drug sensitivity testing is its ability to address drug resistance, a major challenge in leukaemia treatment. By systematically exposing cells to a range of compounds, researchers can identify mechanisms underlying resistance to chemotherapy and targeted therapies. This, in turn, facilitates the discovery of alternative drugs or combination therapies that can overcome resistance and improve patient outcomes. Additionally, drug screening enables the identification of novel therapeutic targets by analysing leukaemia subtypes characterized by distinct genetic alterations. Exposing these cells to a diverse panel of compounds can reveal selective vulnerabilities, which, when integrated with genomic data, offer new insights into the molecular pathways driving drug responses (Kayser & Levis, 2023).

Beyond improving treatment precision, high-throughput drug sensitivity testing also plays a critical role in reducing treatment time and minimizing toxicity. Traditional drug selection methods often involve prolonged trial-and-error approaches that expose patients to ineffective or unnecessarily toxic treatments. By rapidly evaluating multiple drugs and their concentrations, high-throughput screening accelerates the selection of optimal therapies, sparing patients from unnecessary side effects and allowing for timely treatment adjustments (Szymański et al., 2011). Furthermore, this approach facilitates the identification of effective drug combinations, which is particularly important given the complex molecular alterations that characterize leukaemia. Many cases require combination therapies to enhance anti-leukemic effects and counteract resistance mechanisms. Additionally, high-throughput drug testing provides valuable guidance for clinical trial design by identifying patient subgroups that are most likely to benefit from specific treatments (Boshuizen & Peeper, 2020).

Drug sensitivity testing is performed on cells with a variety of drugs with a potential cytotoxic effect. While there exists a vast repertoire of available drugs, with a diverse range of mechanisms of action, target pathways, and therapeutic classes, the strategic selection of drugs enhances the feasibility of this study. In this study, a drug library composed of thirty FDA-approved drugs from various drug classes was selected.

3.2: Aim

This chapter aims to evaluate the effect of a drug library against leukaemia samples, specifically subtypes such as AML, CLL, and CML, and identify the best drug combinations.

This chapter has been accepted in Scientific Reports

3.3: Materials and Methods

3.3.1: Cells and controls

Two cell types were used in the screening process: a continuous cell line (HeLa) and primary cells, peripheral blood mononuclear cells (PBMCs). Dulbecco's Modified Eagle's Medium (DMEM) and Roswell Park Memorial Institute 1640 Medium (RPMI) were prepared by supplementing 445 mL of medium with 50 mL (10%) of heat-inactivated foetal bovine serum (FBS) and 5 mL (1%) of Penicillin-Streptomycin antibiotics (100µg/mL). This supplemented

medium was henceforth referred to as complete media. DMEM was used in the culturing and maintenance of HeLa cells, while RPMI was used for the primary cells (PBMCs)

A negative control of 0.1% dimethyl sulfoxide (DMSO) and a positive control of 100 µM BzCI were selected. The preparation of BzCI involved dissolving 0.448 g of benzethonium chloride powder in 10 mL of distilled water to create a 0.1 M solution in a 15 mL Falcon tube. Subsequently, 100 µL of the 0.1 M BzCI solution was pipetted into 900 µL of water in a 1.5 ml Eppendorf tube to obtain 10 mM BzCI, and 100 µL of this solution was further diluted with 900 µL of water to achieve 1 mM BzCI. Additionally, 1% DMSO was prepared in an Eppendorf tube by pipetting 2 µL of 99.5% DMSO into 198 µL of ddH20

3.3.2.: Revival and culture of HeLa cells

HeLa cell line (cervical carcinoma) obtained from Cellonex (Pty) Ltd (Separations Scientific) was used to assess the reproducibility of the platform in a preliminary experiment. Vials containing HeLa cells were retrieved from a -80°C storage unit and placed on ice. The vial was thawed by gentle agitation in a 37°C water bath for approximately 1 minute. After thawing, the vial was decontaminated with 70% ethanol, and the cells were then carefully transferred into a 50 mL Falcon tube containing 15 mL of pre-warmed DMEM after which centrifugation at 900 - 950 xg for 5 to 10 minutes was performed, and resuspended in complete DMEM media (DMEM supplemented with 5% foetal bovine serum (FBS) and 1% penicillin-streptomycin antibiotics). The resulting cell suspension was then transferred to a T-25 culture flask and incubated at 37°C with 5% CO2, with regular monitoring conducted to assess confluency. Trypsinization was performed at 70-90% confluency for further splitting and passaging of HeLa cells. The trypan blue exclusion method was used to perform the cell viability test, and cell counting was performed using the hemacytometer or automated cell counter system. The resulting single-cell suspension of HeLa cells was used to perform a primary screen to assess the cytotoxicity of the selected drugs.

3.3.3 Isolation of Peripheral blood mononuclear cells (PBMCs)

Whole blood was collected from patients at Wits Donald Gordon Medical Centre and Chris Hani Baragwanath Academic Hospital after ethics approval (M211171). Samples were drawn into BD Vacutainer® EDTA and transported immediately to the laboratory at ambient temperature. Approximately 30 min after collection, peripheral blood mononuclear cells

(PBMCs) were isolated from whole blood using the Ficoll-Paque™ technique (GE Healthcare, Illinois, USA) according to the manufacturer's instructions. The cells were adjusted to a concentration of 10^6 cells/ml and stored at -80°C and in liquid nitrogen until needed. Briefly, the plasma fraction was carefully removed from the EDTA-anticoagulated whole blood, and the remaining cellular component was diluted 1:1 with Roswell Park Memorial Institute (RPMI) medium without foetal bovine serum (FBS) (Sigma Aldrich, St. Louis, MO, USA). The diluted blood was layered onto Ficoll at a 2:1 ratio and centrifuged at 900xg for 30 minutes. The buffy coat containing the PBMCs was transferred to a new 50 mL Falcon tube, washed with 30 mL RPMI (Sigma Aldrich), and centrifuged at 900 xg for 10 minutes. Red blood cells were lysed with ammonium chloride potassium (ACK) buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA, pH 7.2) for 5 minutes, followed by washing with 15 mL RPMI and centrifuged at 900xg for 10 minutes. PBMCs were resuspended in a freezing medium consisting of 10% dimethyl sulfoxide (Sigma Aldrich, Missouri, USA) and 90% Gibco Foetal Bovine Serum (FBS, Thermo Fisher, Massachusetts, USA), placed in cryovials, and initially frozen at −80 °C. The vials were then transferred to the vapour phase of liquid nitrogen (−196 °C) for long-term storage until required for experiments.

3.3.4 Revival and culturing of patients' cells

All the following experiments described in this chapter were performed at the CSIR Centre for Synthetic Biology and Precision Medicine.

Vials containing patient samples were revived after approximately 4 months at -80°C by thawing and gentle agitation in a 37°C water bath for approximately one minute. After thawing, the vial underwent prompt decontamination with 70% ethanol, achieved through dipping or spraying. The cells were then carefully resuspended in 15 mL of pre-warmed Medium (RPMI) (Whitehead Scientific) supplemented with 10% of heat-inactivated FBS and 1% of Penicillin-Streptomycin (Sigma Aldrich) antibiotics, after which centrifugation was performed at 300 g for 10 minutes. Upon removal of the supernatant, 10 mL of fresh medium was added to resuspend the cell pellet. The cell suspension was transferred to a tissue culture flask or 96-well plate.

3.3.5 Revival, culturing, and the effect of incubation time on PBMCs viability isolated from patients.

The viability of PBMCs over time was evaluated to determine the incubation period for the experiment. PBMCs were revived as described in section **3.3.4.** Cells were cultured in T-25 culture flasks and incubated for 24, 48, and 72 hours. The viability for each duration was assessed by counting the live cells using trypan blue. The result showed the best viability at 24 and 48 hours. Single drug response was evaluated at 24 hours, and the combinations at 48 hours.

3.4: Drug sensitivity and resistance testing

3.4.1: Selection of drugs

The selection of thirty (30) drugs for screening in this experimental study was based on a strategic approach to optimise resources while ensuring meaningful insights. The drugs screened in this study were imatinib, simvastatin, bortezomib, vinblastine, thalidomide, cytarabine, ifosfamide, melphalan, dexamethasone, doxorubicin, gemcitabine, chlorambucil, fluorouracil, imiquimod, prednisolone, busulfan, dasatinib, fludarabine, lenalidomide, sorafenib, temsirolimus, bendamustine, cladribine, everolimus, irinotecan, nilotinib, tacrolimus, tretinoin, cyclophosphamide monohydrate, and 5-azacytidine. While there exists a vast range of available drugs, this subset was chosen to represent a diverse range of mechanisms of action, target pathways, and therapeutic classes (**Appendix 3.1**). In addition, the drugs were also selected based on the list of previous patients' treatment (Chapter 2, Table 2.3) and the list of oncologic drugs used to treat cancer in South Africa provided by the clinician involved in the study. (**Appendix 3.2).** The strategic selection of drugs enhances the feasibility of this study and enables a more in-depth exploration of potential candidates for repurposing in the context of precision medicine against leukaemia.

3.4.2: Optimising drug treatment duration

Drug treatment is typically conducted *in vitro* over a 48 to 72-hour period to observe changes in cell growth and viability; however, this extended incubation may overlook early cellular responses. For assessing cytotoxicity or immediate effects on cell signalling, shorter exposure times, such as 24 hours, are more effective, allowing the detection of acute drug effects without

the influence of prolonged exposure. In this study, a 24-hour readout was used to quickly identify the most effective drugs for combination experiments, while subsequent screenings were performed at 48 hours (Kumar et al., 2018).

3.4.3: Preliminary drug screening with HeLa cells

To evaluate the reducibility of the drug screening platform, an initial drug screen was performed on HeLa cells with the thirty drugs. The experiment was conducted twice on different days and times. Single-cell suspension was seeded at a seeding density of 15,000 cells per well into the drug-printed 96-well plate. The volume of cells needed was calculated using the formula below, where V (µL) represents the volume of the cell suspension in microliters, 15,000 cells is the desired cell number, and Total number of cells/mL refers to the cell concentration in the suspension. The factor 1000 is a unit conversion factor, ensuring that the volume is expressed in microliters (µL) rather than millilitres (mL).

$$\text{Volume (V)}\mu\text{L} = \frac{15{,}000 \text{ cells}}{\text{Total number of cells/mL}} * 1000$$

The required volume of cells was then pipetted into each well of the 96-well plates preseeded with drug in 1% DMSO, followed by the addition of media to bring the total volume to 100µL per well. The plates were incubated at 37°C for 72 hours. Following the incubation period, 10 µL of PrestoBlue™ Cell Viability Reagent (Invitrogen) was added directly to each well containing cells in the culture medium, excluding the media-only well. Subsequently, the plate was incubated for a minimum of 10 minutes at 37°C. The relative fluorescence unit (RFU) readings were obtained on the Tecan Infinite F5000 plate reader, with excitation at 535 nm (25 nm bandwidth) and emission at 615 nm (10 nm bandwidth). The viability percentage was calculated using the normalized relative fluorescence unit (RFU) for a given drug treatment, where the fluorescence intensity of drug-treated cells was compared to the DMSO control after subtracting the background fluorescence contributed by benzyl chloride (BzCl), a known cytotoxic agent or reference compound. This normalization ensures an accurate assessment of cell viability by accounting for non-specific fluorescence signals, thereby improving the reliability of the measurement.

$$\text{Viability (\%)} = \frac{\text{average RFU drug} - \text{average RFU BzCl}}{\text{average RFU DMSO} - \text{average RFU BzCl}} * 100$$

3.4.4: Drug sensitivity testing of thirty drugs on patient samples.

The same method and plate layout employed for HeLa cells was employed to evaluate the thirty drugs on the patient samples. The only difference was that the seeding density was 10,000 cells/ well and the incubation period was 24 hours for all the patients, compared to 15,000 cells/well and 72 hours incubation for the HeLa cells. The drugs were tested at four concentrations, 1nM, 10nM, 100nM, and 1000nM.

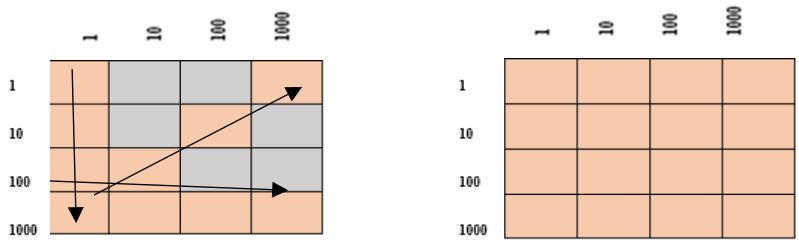
3.5: Drug combination study on patient samples

3.5.1: Double drug combination

Following single drug screening, the 10 most effective drugs (n=5 double combinations) were selected for dual combination studies. Based on clinical relevance to leukaemia and previous studies, combinations were arranged (**Appendix 3.3**) and tested on 35 patient samples (4 CLL, 5 AML, and 26 CML). Diagonal drug combinations were designed by pairing the first five most potent drugs with the next five most potent drugs in the ranking. Each dual combination was tested with each drug at the same concentrations ranging from 1 to 1000 nM. Five microliters (5 µL) of drug A were seeded into the 96-well plate at concentrations ranging from 1 to 1000 nM following the plate layout, and 5 µL of drug B was added at the same concentrations (1 to 1000 nM) into the respective wells. Cells were added to the plate at a density of 10,000 cells/well, and the plate was incubated for 48 hours.

3.5.2: Full matrix drug combination

Full matrix drug combination is a resource-intensive method. For this reason, we selected three of the best combinations from the drug combination experiment and performed a full matrix study as outlined by Ayuda-Durán and colleagues (Ayuda-Durán et al., 2023). **Figure 3.1** illustrates a typical layout of a full matrix-type DSS drug library design, where drugs are combined at various concentrations. The pink boxes highlight the concentrations that have been tested in the experiment.



Diagonal **Full matrix**

Figure 3.1:Illustrations of different drug combination designs.

In this example, two drugs (Drug 1 and Drug 2) were screened at four different concentrations. The drug combinations were tested using two distinct approaches:
Diagonal Design: Each drug was tested at the same concentration level as the other (e.g., both at 1 nM, both at 10 nM). Full Matrix Design: Each drug was tested at all possible concentration pairings, meaning Drug 1 and Drug 2 were combined at every included concentration. Drug A (5 µL) was added at concentrations ranging from 1 to 1000 nM, and Drug B (5 µL) was also added at concentrations ranging from 1 to 1000 nM, ensuring a comprehensive evaluation of drug interactions across all possible concentration pairs. In the accompanying figure, the pink boxes represent the concentration pairs that were tested, while the grey boxes indicate drug concentrations that were excluded from the experiment.

3.6: Data analysis

The drug sensitivity score values were calculated by uploading the raw fluorescence of samples and controls (DMSO and BzCl) in the Breeze open-source pipeline version 1.0 (Potdar et al., 2020).

The BREEZE data analysis tool is an integrated quality control and data analysis application for drug screening (Potdar et al., 2020). It combines comprehensive quality control and dose-response fitting and calculates the quantitative scoring parameters such as EC_{50}, IC_{50}, drug sensitivity score (DSS), and area under the curve (AUC).

The drug sensitivity score (DSS) is a metric employed to quantify cellular responses to specific drugs, integrating various aspects of drug activity such as potency and efficacy into a single, comprehensive value. Key components of the DSS include the $IC_{50,}$ which indicates the concentration at which 50% of the maximum response is achieved, with lower IC_{50} values signifying higher potency. By combining these factors, the DSS provides a robust assessment of drug effectiveness, capturing both the strength of the drug's action and the extent of its impact. This score is particularly valuable for comparing the performance of different drugs or evaluating the efficacy of drug combinations in preclinical studies, thereby offering a more nuanced and informative measure of drug activity than IC_{50} alone (Yadav et al., 2014). Therefore, the DSS metric was used for subsequent analyses.

Drug combination analysis

To assess the potential of a drug combination, mathematical and statistical models are required to establish the expected outcome, allowing for the formal quantification of synergistic effects. In this study, the Loewe additivity (LOEWE) was used to calculate the synergy score. In this study, the Loewe additivity was used to calculate the synergy score. (Ianevski et al., 2017).

3.7: Results

A primary screen on HeLa cells was conducted twice before drug screening on patient samples to assess the reproducibility of the platform. The results confirmed high reproducibility, with an R^2 value of 0.986 (**Appendix 3.4**).

3.7.1: Overall single drug efficacy on Leukaemia patient cells

PBMCs isolated from patients were screened against 30 drugs, with 24-hour viability drug responses assessed to evaluate the efficacy of individual drugs. The DSS integrates various aspects of drug activity calculated and drugs were ranked based on the average DSS on patients' cells. The drug screening results revealed that among the tested compounds, irinotecan emerged as the most potent drug, exhibiting the highest average DSS (**Figure 3.2**). Following irinotecan, everolimus, and bortezomib ranked as the next most effective drugs based on their average DSS values.

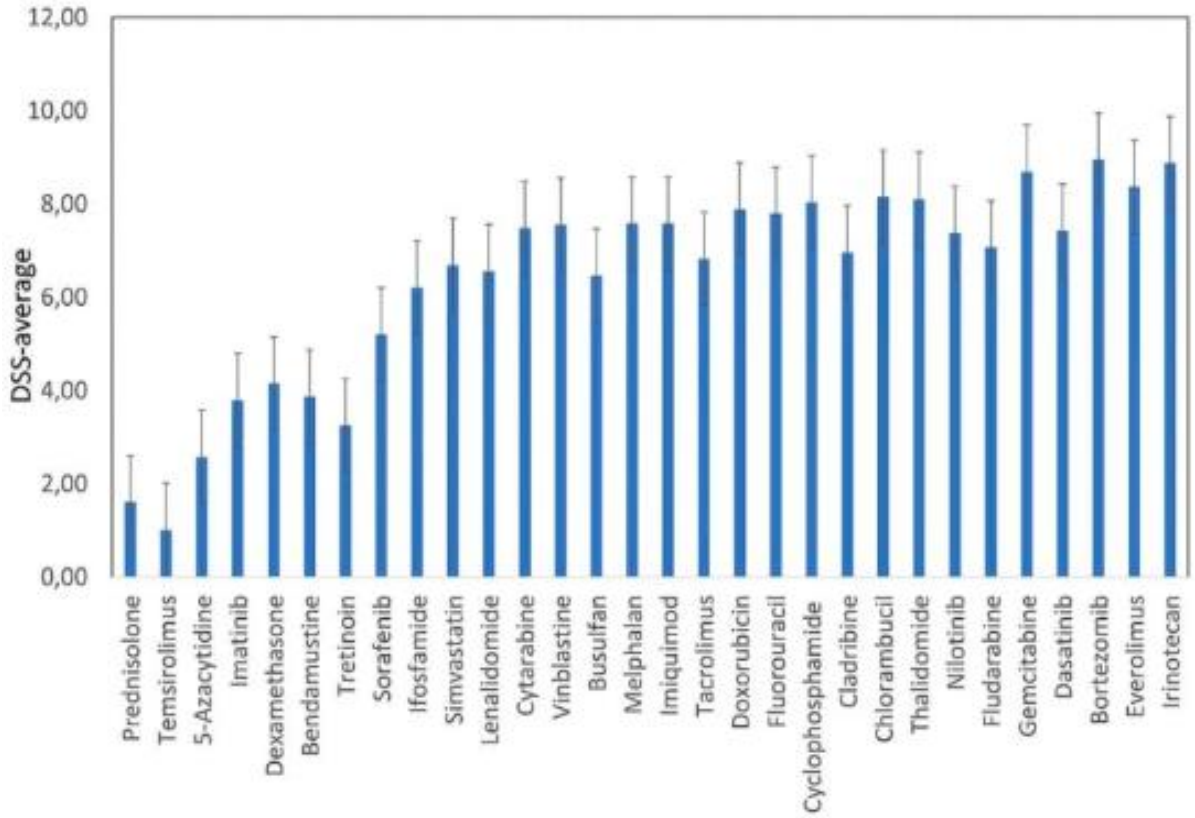


Figure 3.2: Average drug sensitivity scores of thirty drugs tested on patient samples. Average DSS on the Y axis, drug name on the X axis. Thirty drugs tested on PBMC isolated from patients.

Imatinib showed one of the lowest DSS among all compounds tested. In contrast, other tyrosine kinase inhibitors (TKIs) previously administered to patients, including dasatinib and nilotinib, displayed higher DSS values. Dasatinib ranked fourth overall in potency, and nilotinib ranked sixth.

The hierarchical clustering presented by a dendrogram in the heat map (**Figure 3.3**) represents the similarity between drugs (rows) or patients (columns) ordered based on cluster calculation. The result shows that the drugs can be classified into two main clusters, I and II, suggesting distinct patterns of drug sensitivity across the patient or sample profiles, highlighting two major groups. Cluster I (red box) represents patients who are more responsive to traditional chemotherapy, and cluster II represents those with a more variable response to targeted therapies like TKIs (green box).

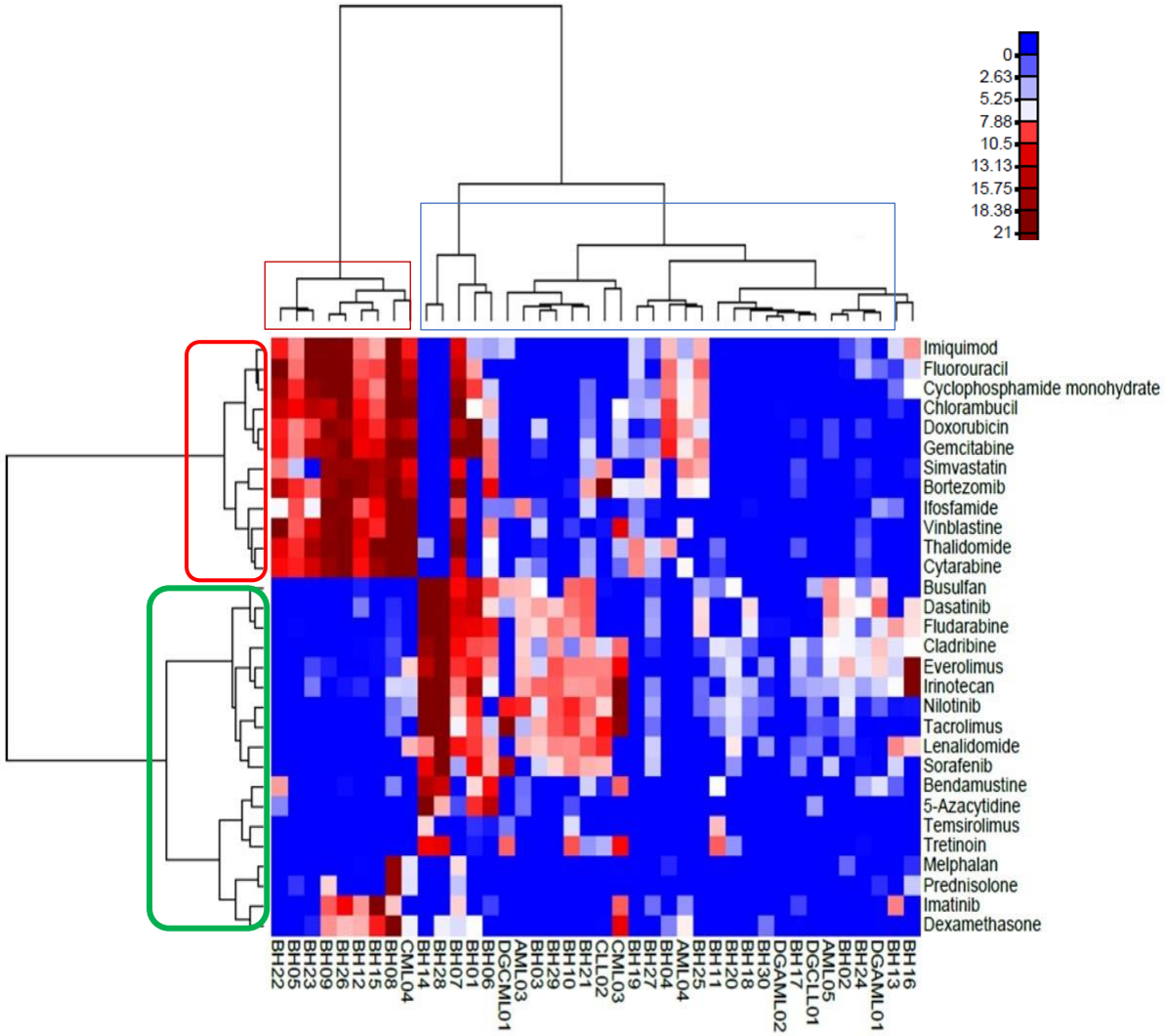


Figure 3.3: Drug response profile of patients.

Heatmap indicating patients' responses to 30 drugs and represented by DSS score. Samples (columns) were ordered according to clinical classification, and the drugs (columns) were ordered according to their activity. The DSS distribution range for each compound is shown on the lower panel at the left, forming drug clusters.

The upper drug cluster (highlighted in red box) includes drugs such as fluorouracil, cyclophosphamide, chlorambucil, and several others, which are primarily chemotherapy agents used in the treatment of various cancers, including haematological malignancies like CML and CLL (Figure 3.3). These are cytotoxic agents that target rapidly dividing cells, and the strong sensitivity here might be indicative of their effectiveness in treating the samples with aggressive disease profiles. The predominance of red hues in this region suggests that these drugs have higher efficacy or sensitivity in the clustered samples (Figure 3.3). This may

indicate that this group of patients is particularly responsive to these conventional chemotherapy drugs.

The lower cluster (Cluster II in green) contains drugs such as dasatinib, imatinib, nilotinib, sorafenib, and others, which are primarily tyrosine kinase inhibitors (TKIs) and immunosuppressive agents (Figure 3.3). Many of these are targeted therapies that act on specific molecular pathways critical for cancer progression, especially CML. The presence of more blue areas in this cluster suggests that these drugs are less effective or that the samples are more resistant to them, at least in this specific data set. However, certain drugs within this cluster may still show some effectiveness (as seen by occasional red patches). This cluster of dasatinib, imatinib, and nilotinib together indicates that these drugs may exhibit similar response patterns across the samples, likely due to their shared mechanism of action in inhibiting the BCR-ABL fusion protein, which is central to CML pathophysiology.

3.7.2: Subtype-specific drug profile

To further explore the impact of disease on drug response and identify disease-specific vulnerabilities, we compared the viability effects of each drug across different disease subtypes. The ranking shows that drugs like chlorambucil, cytarabine, gemcitabine, and fluorouracil have the highest DSS scores, indicating greater efficacy in AML patient cells (**Figure 3.4)**. TKIs such as nilotinib and imatinib, although lower in ranking, are known to be effective targeted therapies in CML, but here their lower DSS may reflect drug resistance in certain cases or variable patient responses. On the other hand, gemcitabine, irinotecan, thalidomide, and chlorambucil were the most potent drugs in CML based on their superior DSS. Everolimus, bortezomib, irinotecan, and chlorambucil ranked the highest in CLL, suggesting that these drugs are particularly effective against CLL cells. Interestingly, nilotinib also appears in the middle of the ranking for CLL, suggesting moderate sensitivity, but lower than in CML.

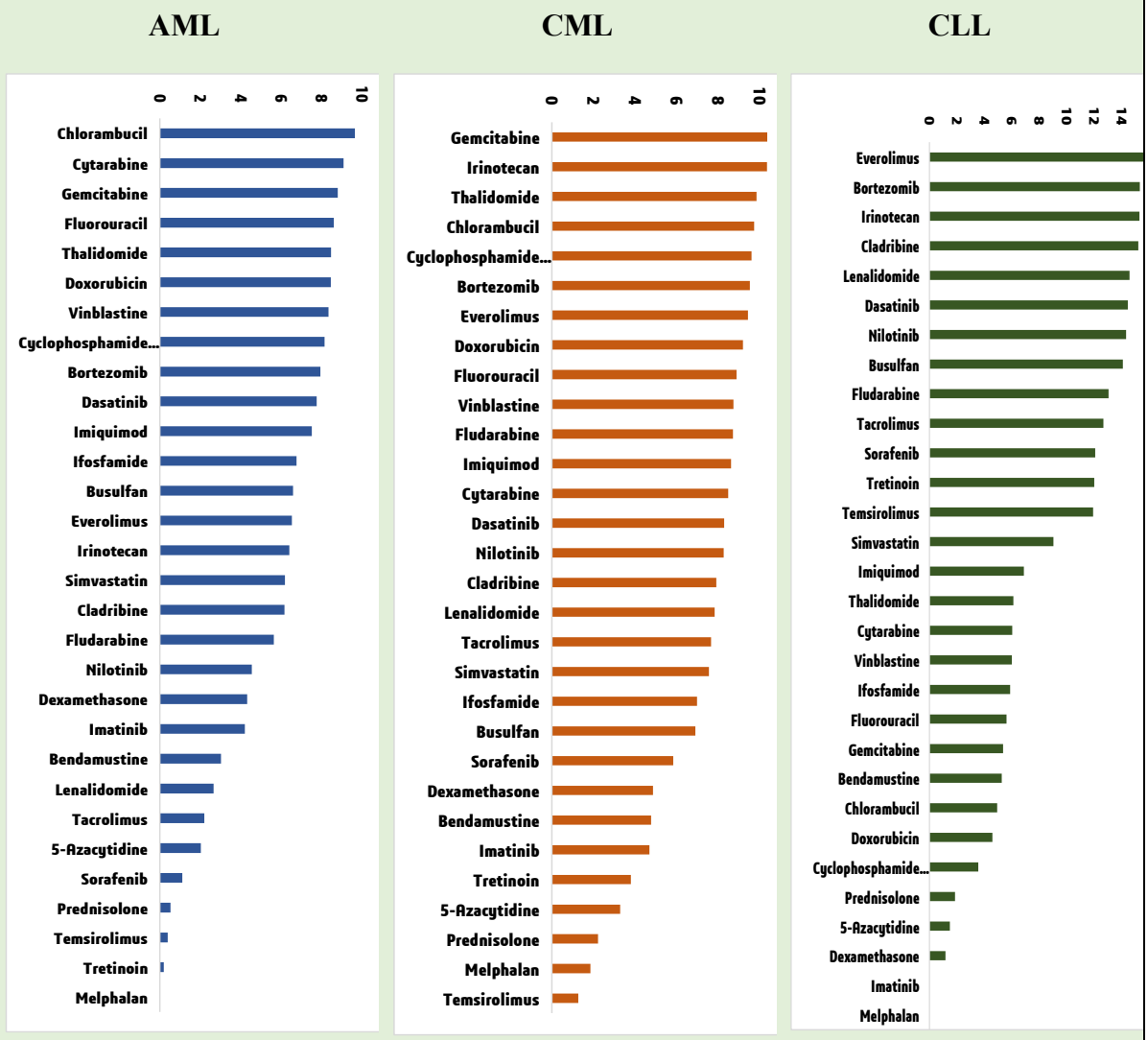


Figure 3.4: DSS distribution among leukaemia subtypes.
AML (Left chart, blue), CML (Middle chart, orange), CLL (Right chart, green)

3.7.3: Distribution of the effect of some drugs on CML cells

The boxplot illustrating the effect of nilotinib on CML patients shows a wide range of drug sensitivity, with most patients displaying moderate responses (as indicated by the interquartile range) but also significant outliers with very high or low DSS scores. On the other hand, the boxplot showing the distribution of imatinib's effect on CML patients reveals a similar pattern to the nilotinib plot, with most patients exhibiting a moderate response to the drug, as indicated

by the concentration of data points within the interquartile range (**Figure 3.5**). However, outliers with higher DSS scores showed the disparity among samples.

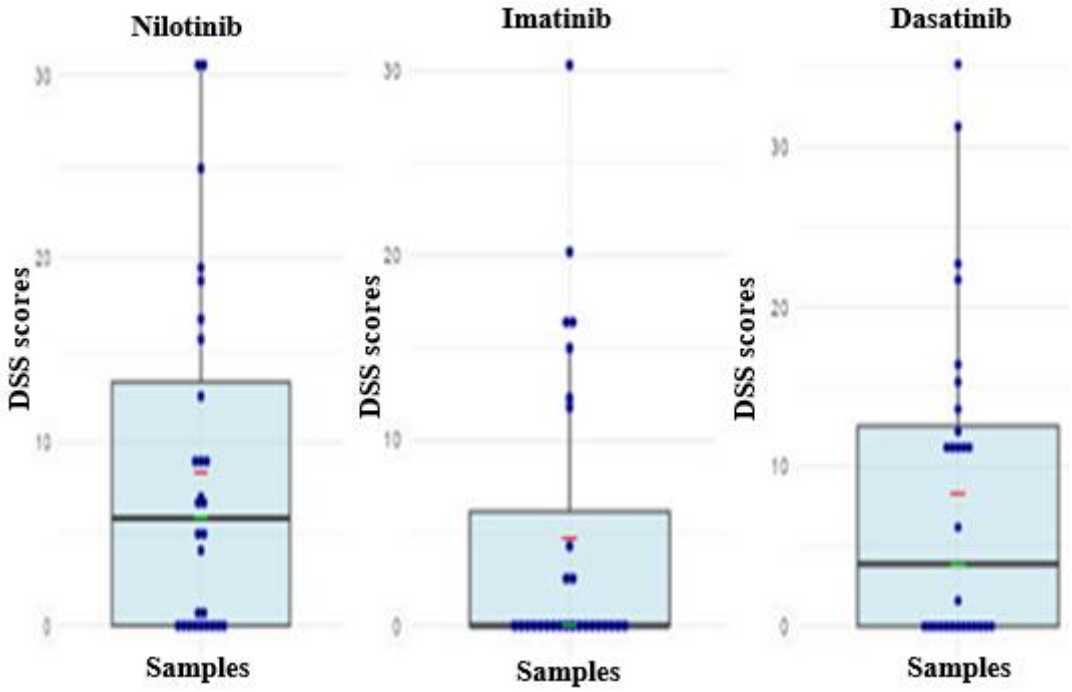


Figure 3.5: Distribution of the effect of nilotinib, imatinib, and dasatinib on CML cells. DSS scores on the Y axis and CML samples on the X axis.

3.7.4: Overall drug combination effect

The average drug sensitivity score (DSS) for various single drugs and drug combinations is illustrated in **Figure 3.6**. Among the evaluated treatments, only nilotinib-irinotecan exhibited a DSS higher than that of the single drug, suggesting potential synergistic effects when used together. Interestingly, an antagonist effect was found with the combination of everolimus and 5-azacytidine. There is a notable variation in the drug effect among the patients **Figure 3.7**.

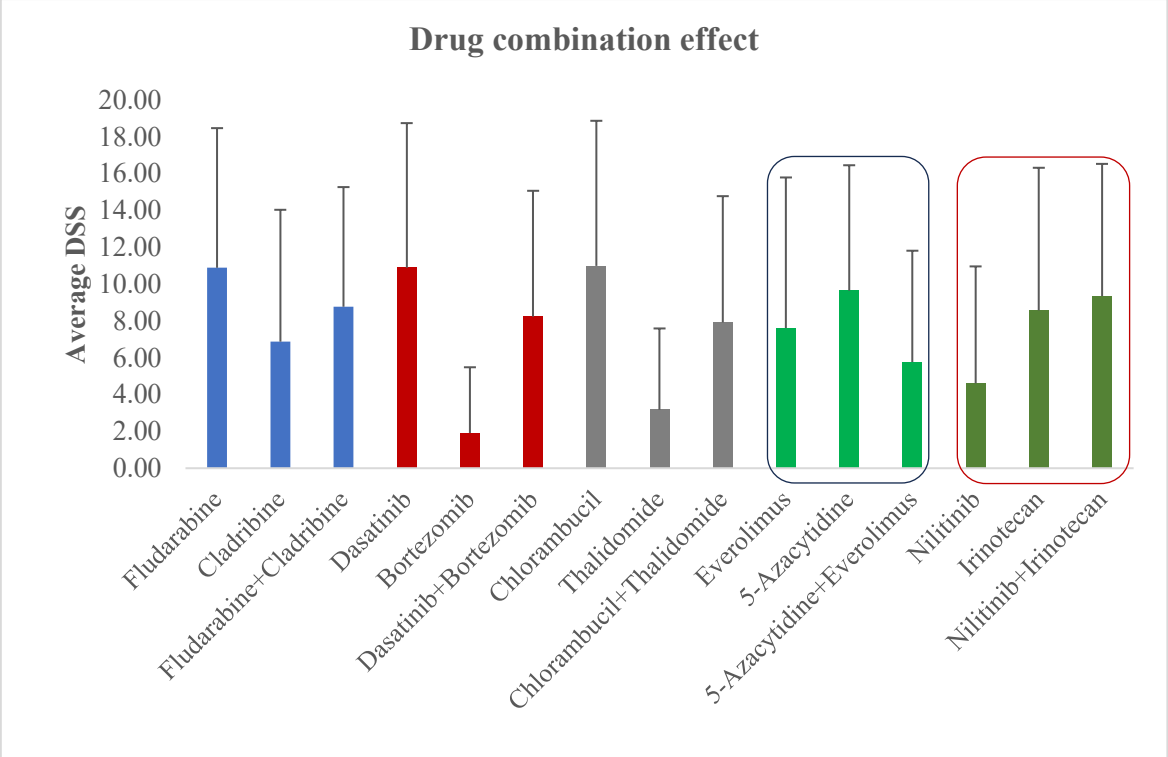


Figure 3.6 Overall drug combination response

The bar graph represents the average drug sensitivity score (DSS) for various single and combination drug treatments. Higher DSS values indicate greater drug efficacy. (A) The combination of nilotinib and irinotecan (orange bars) exhibits a synergistic effect, as the DSS is higher than either drug alone. (B) The combination of 5-Azacytidine and Everolimus (green bars) demonstrates an antagonistic effect, as the DSS is lower than the individual drugs.

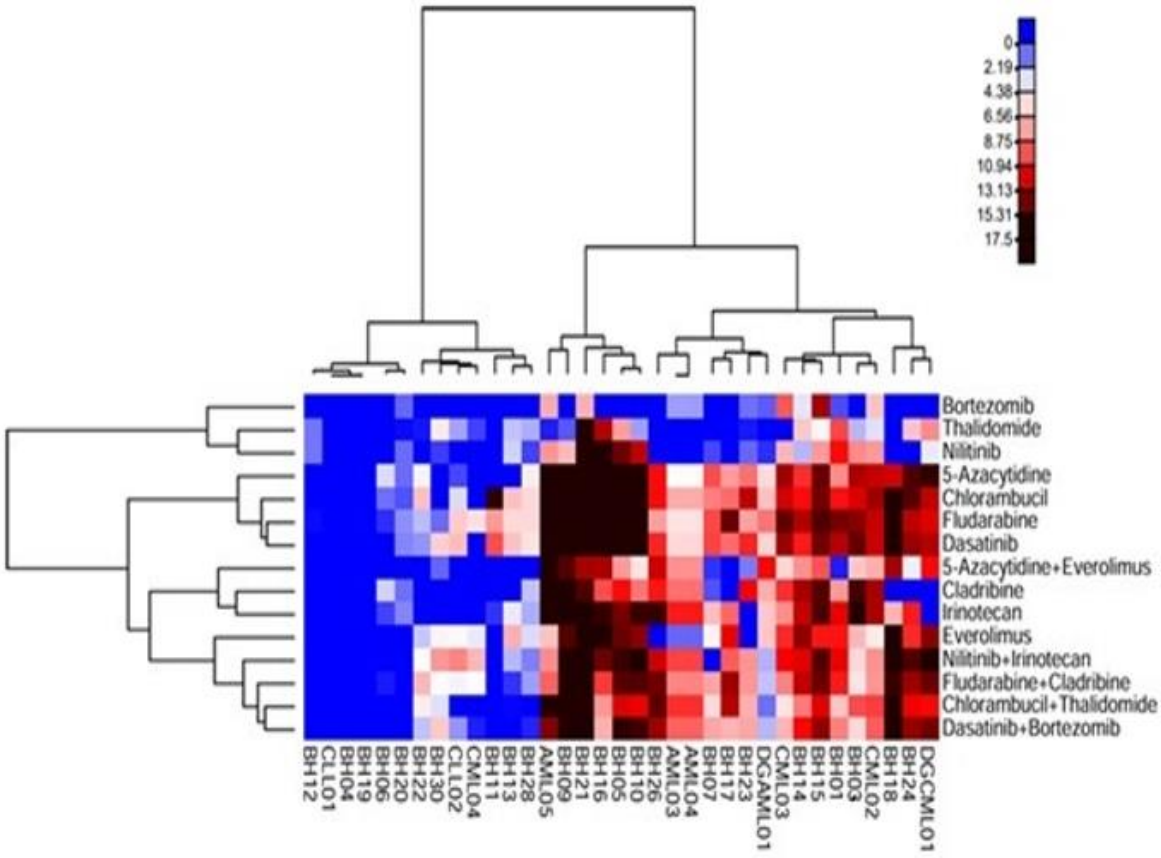


Figure 3.7: Drug combination response profile of patients.

Heatmap indicating patients' response to 10 compounds and their 2 by 2 combination represented by DSS score. Samples (columns) were ordered according to clinical classification, and compounds (columns) were ordered according to activity.

The Loewe synergy score is a measure used to determine the interaction between two drugs. A positive score indicates synergism (where the combined effect is greater than the sum of individual effects). In comparison, a negative score indicates antagonism where the combined effect is less than the sum of individual effects. Figure 3.8A and 3.8B represent the analysis of the synergy between irinotecan and nilotinib. The overall Loewe synergy score is -10.615, suggesting a general trend towards antagonism between irinotecan and nilotinib across the tested concentrations. Although the red area indicating synergy is limited, a strong synergistic effect is observed at the highest concentration of both drugs (1000 nM), coloured dark red, indicating a strong inhibition. A similar effect is observed with the combination of nilotinib at 1000 nM and 1 nM of irinotecan. This observation is consistent with the 3D plot (**Figure 3.8B**), which provides a visual representation of the interactions, showing a trough-like shape with

negative synergy scores dominating, while peaks in the surface plot indicate regions with higher synergy scores, though these are limited and relatively low.

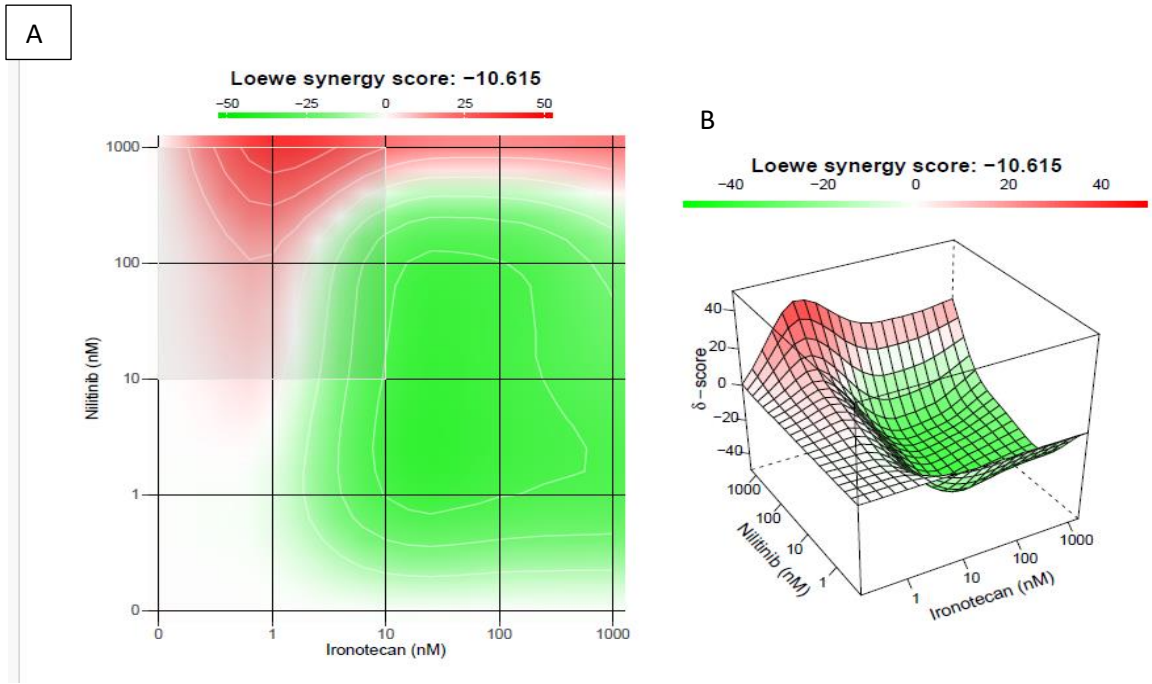


Figure 3.8: Drug response matrix of combined drug efficacy. Analysis of the synergy between irinotecan and nilotinib

3A shows the contour plot of the Loewe synergy scores across different concentrations of irinotecan and nilotinib. The colour green represents negative inhibition (potential growth promotion or lack of inhibition) and red represents positive inhibition (higher levels of inhibition). The Loewe synergy score, with green representing negative synergy scores (antagonism) and red representing positive synergy scores (synergism). The scale ranges from -50 to 50
3B shows the 3D surface plot: the tri-dimensional perspective of the Loewe synergy scores. The colour coding is consistent across the plots, with green representing negative synergy scores (antagonism) and red representing positive synergy scores (synergism). The x-axis represents the concentration of nilotinib (nM), ranging from 0 to 1000 nM, while the y-axis represents the concentration of irinotecan (nM) from 0 to 1000 nM.

3.7.5: Molecular features affecting drug response

Due to missing values on the genetic profile of patients, the comparison of the influence of genetic features on drug response was only done for the subtype CML. Various responses were observed among patient samples (Table 3.1). The table presents a detailed overview of six CML patients, highlighting their diverse clinical backgrounds, treatment histories, and cytogenetic profiles. The treatment regimens include a range of tyrosine kinase inhibitors (TKIs) such as nilotinib, dasatinib, imatinib (under the brand names Imavec and Gleevec), Tasigna (nilotinib), and ponatinib, reflecting personalized approaches based on individual

patient needs and disease characteristics. Notably, patient BH22, with a T315I mutation (Table 3.1), has been treated with ponatinib, a second-generation TKI effective against this resistant mutation, demonstrating the tailored nature of CML management in the presence of specific genetic abnormalities. The disease stage varies among patients, with some in the accelerated phase (AP) and others in the chronic phase (CP), suggesting a range of disease severities and progression states. The average treatment values, which may represent dosages or response levels, vary significantly, with BH18 showing the highest value (Table 3.1), potentially indicating a robust response to therapy or a need for higher dosages. This data underscores the complexity of CML treatment, influenced by factors such as mutation status, disease phase, and individual patient characteristics, highlighting the critical need for personalised medicine in managing this heterogeneous disease.

The average DSS values in this table were linked to specific cytogenetic abnormalities, primarily the presence of the Philadelphia chromosome (Ph+), which is a characteristic of CML. Patients BH02, BH04, BH05, and BH29 have Ph+ status and show varied DSS, with BH05 having a particularly high response to Nilotinib (9.1). This variation highlights that while Ph+ CML patients generally respond well to tyrosine kinase inhibitors (TKIs), individual sensitivity can differ significantly. Notably, BH22 has the T315I mutation, known for conferring resistance to first- and second-generation TKIs such as imatinib and nilotinib. This patient exhibited a low DSS for Imatinib (5.2) but a much higher sensitivity to Nilotinib (16.7) (Table 3.1), a third-generation TKI designed to overcome this resistance mutation. Therefore, the cytogenetic profile plays a critical role in determining the efficacy of TKIs, with mutations like T315I necessitating more advanced therapies for effective disease control.

Table 3.1: Variability of response among patients with similar characteristics.

CML patient	BH02	BH04	BH05	BH18	BH22	BH29

Previous treatment	Nilotinib	Dasatinib	Nilotinib 200mg	Imatinib *(Imavec)*	Imatinib *(Imavec)* 400mg	Imatinib (*Gleevec*)
			Nilotinib 400mg		Tasigna (nilotinib)	Tasigna (nilotinib)
					Ponatinib 45mg	
Average DSS	2.93	3.96	6.59	1.39	8.70	4.47
Dasatinib	11	0	0	11.4	0	12.2
Nilotinib	9.1	0	0	5.2	0	16.7
Imatinib	0	0	0	0	0	0
Stage	AP		CP		CP	
Cytogenic Abnormalities	Ph+	Ph+	Ph-	Ph+	T315I	Ph-

CML: Chronic myeloid leukaemia; DSS: Drug sensitivity score; AP: Accelerated phase; CP: Chronic phase; Ph+: Philadelphia chromosome positive; Ph-: Philadelphia chromosome negative; T315I: BCR-ABL mutation associated with resistance to tyrosine kinase inhibitors (TKIs). DSS represents the effectiveness of specific drugs in inhibiting leukaemia cell growth, with higher scores indicating greater sensitivity. Previous treatments and cytogenetic profiles are listed for each patient.

3.8: Discussion

3.8.1: Single drug response profile

The results of the drug sensitivity screening conducted on PBMCs isolated from leukaemia patients offer promising insights into the potential repurposing of existing cancer drugs for leukaemia treatment (Figure 3.2). Among the 30 drugs evaluated, irinotecan demonstrated the highest efficacy, achieving the top drug sensitivity score (DSS). Notably, irinotecan, a topoisomerase I inhibitor traditionally used to treat solid tumours (Chai et al., 2024), showed remarkable potency in reducing leukaemia cell viability. This unexpected finding suggests a broader therapeutic application for irinotecan beyond its conventional uses in solid tumours. The observed efficacy may be attributed to its ability to interfere with DNA replication and induce apoptosis, which could effectively target rapidly dividing leukaemia cells (Stenvang et

al., 2013). Similarly, bortezomib, a proteasome inhibitor used in treating multiple myeloma, reaffirmed its efficacy by achieving a high DSS in leukaemia samples. The bortezomib mechanism of action involves inhibiting protein degradation, leading to apoptosis in cancer cells, which may explain its effectiveness against leukaemia. This finding is consistent with the existing literature, which highlights the relevance of proteasome inhibition in hematologic malignancies (Robak & Robak, 2019).

The differential sensitivity observed among the tested drugs indicates that certain compounds, although traditionally used for other cancers, might possess significant activity in leukaemia (Figure 3.3). This suggests that drug repurposing, leveraging existing cancer therapies, could be an effective strategy in identifying new treatment options for leukaemia patients, particularly those who may not respond to standard therapies. Moreover, the study highlights the importance of personalized medicine approaches, as drug efficacy can vary among patients based on individual cellular responses.

The prevalence of blue areas in the TKI cluster (Figure 3.3 and Figure 3.7) suggests that resistance to these targeted therapies may be an issue for a subset of patients, which could warrant further investigation into alternative treatment strategies or combination therapies to overcome resistance. The heatmap (Figure 3.3) reinforces the importance of personalised medicine, where treatment regimens are tailored based on individual drug response profiles. Patients who exhibit resistance to one drug class may be more sensitive to another, and combining drugs from both clusters could lead to better therapeutic outcomes.

The clustering of nilotinib and dasatinib (Figure 3.7) together highlights their classification as second-generation TKIs, designed to overcome resistance and improve efficacy compared to Imatinib. Research has demonstrated that both nilotinib and dasatinib offer enhanced potency against BCR-ABL mutations that confer resistance to imatinib, leading to higher median DSS scores and broader response ranges in patients (Vener et al., 2020). For example, Kantarjian and colleagues (2021) have shown that nilotinib achieves faster and deeper molecular responses, which are crucial for long-term disease control and potential treatment-free remission (Kantarjian et al., 2021). Similarly, dasatinib has been effective in patients with imatinib-resistant or -intolerant CML, offering robust haematologic and cytogenetic responses (Kantarjian et al., 2012). In contrast, imatinib remains a cornerstone in CML treatment due to

its well-established safety profile and effectiveness in newly diagnosed patients. However, its lower median DSS scores in the cluster analysis (Figure 3.5) reflect its limitations in cases where resistance mutations are present or when patients require a more aggressive treatment approach. This underscores the importance of personalised medicine, where the choice of TKI is tailored based on individual patient profiles, including genetic mutations, disease stage, and prior treatment responses.

3.8.2: Synergy of irinotecan and nilotinib

The observed synergy at higher concentrations between irinotecan and nilotinib suggests potential benefits in combining these drugs for therapeutic purposes. However, the lack of inhibition at lower concentrations or in specific combinations (Figure 3.8) indicates that careful consideration of dosing strategies is crucial to maximize therapeutic efficacy while minimizing potential side effects.

The analysis of the Loewe synergy scores for irinotecan and nilotinib revealed a predominantly antagonistic interaction across the tested concentration ranges, as evidenced by an overall score of -10.61 (Figure 3.8) . These findings imply that the combination of irinotecan and nilotinib may not be optimal for therapeutic purposes within the tested concentration ranges. The observed antagonism suggests that the combined effect of these drugs is generally less effective than the sum of their individual effects. Future research should explore different concentration ranges or alternative drug combinations to identify potential synergies that could enhance therapeutic efficacy. This finding suggests that these two drugs may not be the most effective combination for therapeutic purposes, at least within the concentration ranges tested and for that particular patient. Further studies could explore different concentration ranges or combinations with other drugs to identify potential synergies.

In preclinical studies, many kinase inhibitors (KIs) exhibit synergistic effects with irinotecan, enhancing tumour cell death. However, clinical trials have shown poor tolerability of this combination due to overlapping toxicities, particularly severe diarrhoea and neutropenia. These adverse effects are linked to KIs influencing the metabolism of irinotecan's active metabolite, SN-38, by inhibiting UDP-glucuronosyl transferase 1A1 (UGT1A1) and impairing its transport, resulting in increased drug exposure and toxicity (Yashiro et al., 2011). While the combination enhances cancer cell death, the significant toxicity limits its clinical use

Studies have shown that certain TKIs, like dasatinib and erlotinib, demonstrate significant synergy with irinotecan in both *in vitro* and preclinical models (Chai et al., 2024; Xu et al., 2017). These combinations are often attributed to their ability to enhance irinotecan's cytotoxic effects through complementary mechanisms of action, such as inhibiting pathways involved in cell survival, proliferation, or repair. For instance, erlotinib, an epidermal growth factor receptor (EGFR) inhibitor, was found to synergize with irinotecan by disrupting cancer cell signalling, which further sensitizes the cells to DNA damage caused by irinotecan (Kichenadasse et al., 2017; Xu et al., 2017)

3.8.3: Impact of molecular abnormalities and generic compound

Imatinib is available under the brand name Gleevec and its generic forms such as Imavec. While the active ingredient is the same in both versions, there are potential differences in formulation, cost, and patient response. Gleevec, being the original branded version developed by Novartis, went through extensive clinical trials to establish its efficacy and safety profile (Abou Dalle et al., 2019). After its patent expired, generic versions like Imavec became available, offering a more affordable option to patients, which can significantly reduce treatment costs. However, minor differences in the excipients, or non-active ingredients, in the generic formulation could lead to variations in drug absorption or tolerability in some patients. Despite this, most studies show that generics perform similarly to Gleevec in terms of clinical outcomes for CML. The choice between the branded and generic often depends on availability, cost considerations, and individual patient response to treatment (Abou Dalle et al., 2019; Erçalışkan et al., 2021). Nonetheless, clinicians may prefer the original formulation in specific cases if a patient shows any unusual side effects or suboptimal responses to the generic version. Some studies have reported a loss of efficacy of imatinib mesylate when a patient switched to its generic version and responded again when they switched to the branded version of imatinib (Asfour & Elshazly, 2009; Mattar, 2010) suggesting the difference in clinical efficacy between the original version of imatinib (Gleevec) and its generic versions.

This study accurately replicated clinical observations on the efficacy of imatinib. As reported in Chapter 2, most CML patients were resistant to the first-generation TKI imatinib. This observation is confirmed by the drug screening, where most patients were resistant to imatinib but more sensitive to second-generation TKIs like nilotinib or dasatinib. The observed low

potency of imatinib in this context prompts a re-assessment of its clinical utility in CML and supports the exploration of alternative or additional therapies that exhibit higher DSS values. This finding reinforces the need for personalised medicine approaches, where treatment regimens are tailored based on robust *in vitro* and *in vivo* efficacy data, ensuring that patients receive the most effective and appropriate therapies for their specific condition. Further research could elucidate the reasons behind imatinib's reduced effectiveness in CML and potentially identify biomarkers that predict responsiveness to various treatments.

3.9: Conclusion

The results of the drug sensitivity screening conducted on PBMCs isolated from leukaemia patients provide valuable insights into the efficacy of various compounds, highlighting the potential of this approach for identifying more effective treatment strategies. While irinotecan demonstrated a high DSS, the primary significance of these findings lies in demonstrating the feasibility of drug screening to guide therapeutic decision-making. This framework not only helps identify potent individual drugs but also facilitates the development of optimised drug combinations, ultimately enhancing treatment efficacy in leukaemia.

Limitations

The identification of irinotecan as a promising therapeutic candidate was based on its superior drug sensitivity score (DSS). However, the study did not explore its detailed mechanism of action or validate its efficacy in combination with other drugs in preclinical or clinical settings. While the DSS framework is useful for prioritising candidates, follow-up studies are needed to confirm its therapeutic potential and assess its safety and tolerability *in vivo*.

CHAPTER 4: EVALUATION OF IMMUNE CELL SURFACE MARKERS IN RESPONSE TO DRUG TREATMENT

4.1: Introduction

The evaluation of immune cell surface markers in response to drug treatment is critical in understanding the complex interactions between the immune system and pharmacological interventions, particularly in oncology. Cell surface markers, which include proteins such as cluster of differentiation (CD) molecules, are essential in regulating immune cell function, activation, and differentiation (Raskov et al., 2021). These markers serve as identifiers of various immune cell subsets and play critical roles in mediating cellular responses to external stimuli, including therapeutic drugs (Kalina et al., 2019). With the advent of targeted therapies and immunomodulatory drugs, research on the impact of drug treatment on immune cell populations is rapidly expanding. Such treatments can induce changes in immune cell behaviour, either enhancing their activity to eradicate cancer cells or suppressing immune function to prevent autoimmune reactions (Sharma et al., 2024). For instance, drugs like tyrosine kinase inhibitors and monoclonal antibodies alter the expression of markers such as CD3, CD4, and CD8 on T-cells, thereby affecting their cytotoxic functions and regulatory capacities (Raskov et al., 2021).

4.1.1: Mechanisms of drug-immune system interactions

Various classes of drugs are known to alter immune cell functions by modulating cell surface markers (Laba et al., 2022). Chemotherapeutic agents, for instance, are widely used to target rapidly proliferating cells, including malignant immune cells. However, these agents can also affect normal immune cells, leading to changes in the expression of immune markers through immunomodulation, immune cell-mediated cytolysis, or apoptosis. Some drugs like alemtuzumab exert their cytotoxic activity by binding to the CD52 antigen at the surface of T and B lymphocytes causing depletion and repopulation of these cells (Li et al., 2018). Kinase inhibitors (TKIs), such as imatinib, have shown significant efficacy in the treatment of chronic myeloid leukaemia (CML) by specifically targeting the BCR-ABL fusion protein (Vener et al.,

2020). Interestingly, TKIs can also influence immune responses by altering the expression of surface markers on both leukemic and non-leukemic cells (Chaput et al., 2013). Several studies have demonstrated that TKIs can alter the expression of CD markers on immune cells, influencing their activation, exhaustion, or cytotoxic potential. For instance, certain TKIs downregulate inhibitory markers such as PD-L1 (also known as CD274) on leukemic cells, thereby restoring T-cell and NK-cell responses (Laba et al., 2022). Additionally, TKIs can increase the expression of activation markers such as CD69 and CD25 on T cells, promoting a more robust immune response (Cao et al., 2023). Conversely, some TKIs reduce CD4+ and CD8+ T-cell populations or impair antigen presentation by altering CD86 and HLA-DR expression on dendritic cells, potentially leading to immune suppression (Raskov et al., 2021). These immunomodulatory effects suggest that TKIs not only serve as targeted therapies for leukaemia but may also be repurposed in combination with immune checkpoint inhibitors or CAR-T cell therapies to enhance therapeutic efficacy.

4.1.2: Technologies for evaluating immune cell markers

Assessing immune cell surface markers post-treatment is critical for understanding immune responses, diagnosing diseases, and advancing therapeutic development. A range of technologies are available for this purpose, each offering distinct advantages. Flow cytometry enables evaluation of complex cell populations and their biological mechanisms. Flow cytometry presents compelling benefits such as radiation-free operation, simultaneous analysis of multiple cell subsets without necessitating separation, and the utilisation of various laser and colour combinations, thereby providing a broad spectrum of analytical possibilities (McKinnon, 2018). This method is widely used in immunology, oncology, and cell biology to study cell surface markers, intracellular proteins, apoptosis, the cell cycle (Landskron & Taskén, 2016). It involves several key steps, beginning with fluorescent labelling, where cells are stained with fluorochrome-conjugated antibodies or dyes specific to the target molecules. Each fluorochrome emits light at a specific wavelength when excited by a laser (McKinnon, 2018). Next, the stained cells are passed through a laser beam in a flow cytometer, where each fluorochrome is excited by a corresponding laser (such as blue, red, violet, or green) and emits fluorescence at its characteristic wavelength. Finally, optical filters are used to separate the emitted light, directing each wavelength to a specific detector, such as photomultiplier tubes or photodiodes (Adan et al., 2017; Schmit et al., 2021). Multicolour flow cytometers can

accommodate multiple detectors, allowing the analysis of fluorescence from several fluorochromes simultaneously.

4.1.3: Significance of cluster of differentiation (CD) markers in cancer management

Cluster of Differentiation (CD) are proteins found on the surface of leukocytes (white blood cells) and other cells. Each CD marker is assigned a unique number, such as CD4 or CD8, which helps in identifying specific cell types and their roles in immune responses. CD marker-specific antibodies are widely utilised for cell sorting, identification, and blood cancer diagnosis, playing a pivotal role in cancer treatment. Therapeutic antibody drugs have been developed to target cells expressing specific CD markers, such as rituximab targeting CD20 (Hanif & Anwer, 2024) for lymphoma and leukaemia treatment, and alemtuzumab targeting CD52 (Li et al., 2018) for chronic lymphocytic leukaemia and T-cell lymphoma treatment. The mechanism of action of these monoclonal antibodies includes binding to a receptor to prevent the binding of its corresponding ligand, as exemplified by cetuximab's interaction with the epidermal growth factor receptor (EGFR). This interaction inhibits the binding of soluble epidermal growth factor (EGF), thereby attenuating proliferation and survival signalling pathways in tumour cells (Marshall et al., 2017). In the case of CD20, the direct effects of monoclonal antibody (mAb) binding vary depending on the monoclonal antibody type. Type I mAbs induce a modest degree of apoptosis, which is thought to be associated with B-cell receptor (BCR) signalling, whereas Type II mAbs promote a non-apoptotic, lysosome-mediated cell death. The precise mechanisms underlying these effects remain a topic of ongoing investigation, though they are likely linked to the generation of reactive oxygen species (Cheadle et al., 2013). Beyond cancer diagnosis, CD markers aid in identifying optimal treatment strategies and assessing treatment efficacy by monitoring changes in relevant CD expression levels.

4.2: Rationale

The previous chapter demonstrated that irinotecan was the most effective among the 30 drugs tested, and its combination with nilotinib proved to be the most effective on the selected patients' samples. This chapter focuses on evaluating immune cell surface markers in response to drug treatment with the combination of irinotecan and nilotinib, using flow cytometry to analyse changes in marker expression across different immune cell subsets like CD3, CD8,

CD19, CD38, and HLA-DR. These CD markers provide critical insights into leukaemia immunophenotyping, disease progression, and treatment response. Specifically, CD3 and CD8 are key T-cell markers, essential for assessing cytotoxic T-cell populations and their role in anti-leukemic immunity (Raskov et al., 2021). Meanwhile, CD19 and CD38, hallmarks of B-lineage cells, are widely used to identify malignant B-cell populations in B-cell leukaemia and evaluate the impact of targeted therapies such as anti-CD19 monoclonal antibodies or CAR-T cell therapy (Haslauer et al., 2021). Furthermore, HLA-DR, a major histocompatibility complex class II molecule, functions as a marker of immune activation and is frequently overexpressed in leukemic blasts, providing insights into disease aggressiveness and immune evasion mechanisms (Roerden et al., 2021). By examining changes in these markers, we aim to elucidate the mechanisms by which specific drugs affect immune function, with potential implications for enhancing therapeutic efficacy and reducing adverse effects.

4.3: Materials and Methods

4.3.1: Sampling and processing

Blood samples were collected and processed as described in Chapter 3.3.1. Vials containing patients' cells were revived as described previously (Chapter 3.3.4). Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from whole blood using the Ficoll-Paque™ technique. Vials containing the cells were revived by thawing and gentle agitation in a 37°C water bath for approximately one minute, and the viability was assessed using trypan blue.
The three patient samples, each representing a leukaemia subtype —BH30 (CLL), AML04 (AML), and CML04, (CML)— and previously tested in the full matrix experiment (Chapter 3.7.4), were used to evaluate the effect of drug treatment on cell surface markers.

4.3.2: Cell surface marker analysis using multicolour flow cytometry

All the following experiments described in this chapter were performed at the University of Witwatersrand flow cytometry facility.

A seven-colour flow cytometry panel was designed for this experiment. This consisted of CD3 BD Horizon Brilliant^{TM} Ultraviolet 496 (BUV496), CD8 Brilliant Violet^{TM} 605 (BV605), HLA-DR Brilliant Violet^{TM} 650 (BV650), which were obtained from BD Biosciences (BD Biosciences, New Jersey, USA). RedFluor™ 710 CD19 and APC CD 38 were from BioLegend

(BioLegend, San Diego, California). Additionally, annexin V Fluorescein isothiocyanate (FITC) and propidium iodide (PI), detected on the phycoerythrin cyanide dye™ (PE) channel from BD Biosciences (BD Biosciences, New Jersey, USA) were used to assess apoptotic and necrotic cells, respectively, and hence identify viable cells once. Annexin V binds to phosphatidylserine, which is externalized on the outer leaflet of the plasma membrane during early apoptosis, whereas propidium iodide (PI) intercalates into DNA, indicating membrane permeability in necrotic or late apoptotic cells (Rieger et al., 2011). Before the surface markers analysis, these antibodies were optimised to determine the optimal concentration of each antibody that is required to give good separation between positive and negative populations and to determine the position of the negative population on the axis. Additionally, various experimental and instrument control experiments were performed. These optimisation assays are summarised in the next subsections

4.3.2.1 Antibodies titration

CD19 and CD38 were optimized by titration to optimally stain lymphocyte populations and their subpopulations using the antibodies mentioned in section 4.3.2. The other antibodies volumes, CD3, HLA-DR, CD8, and dyes annexin V, PI, were previously titrated by students in the laboratory (Maimela, 2024; Nalisa, 2021).

Peripheral Blood Mononuclear Cells (PBMCs) were thawed, washed with 2 mL of staining buffer, and centrifuged at 600 × *g* for 10 minutes. To determine the viability of the cells, trypan blue was used for counting. PBMCs from patient BH30 (multiple vials were available in storage, allowing for repeated experiments) were dispensed into a 24-well plate at a density of 10^6 cells per well and incubated for 48 hours without drugs. Post-incubation, suspension cells were harvested into Corning Falcon tubes (Corning Incorporated, New York, USA), and 50 µL of TrypLE™ Express (Gibco) was added to each well and incubated at 37°C for 5 minutes to detach any adherent cells such as macrophages. The detached cells were combined with suspension cells, centrifuged at 600 × *g* for 10 minutes, and the pellet resuspended in 1 mL of 1X phosphate-buffered saline (PBS) (Sigma-Merck, Missouri, USA). Antibodies were prepared by serial dilutions in seven Corning Falcon tubes (Corning Incorporated, New York, USA), starting with double the manufacturer's recommended volume in tube 1 (e.g., 10 µL antibody to 90 µL PBS for a 5 µL recommended dose), followed by transferring 50 µL sequentially between tubes to achieve a dilution series, leaving tube 8 as an unstained control.

The washed cells (50 µL) were added to all tubes, mixed thoroughly, covered with foil, and incubated in the dark at room temperature for 20 minutes. After incubation, the cells were washed with 1 mL staining buffer (1x PBS, 5%FBS, and 0.1% NaN3), centrifuged at 600 ×g for 10 minutes, resuspended in 300 µL staining buffer, and 100,000 events were recorded per sample on a BD LSR II Fortessa™ flow cytometer (BD Biosciences, New Jersey, USA). The data were analysed using the Stain Index (SI), which is defined as the difference between the Median Fluorescence Intensity (MFI) of the positive and negative populations divided by twice the standard deviation of the negative population. Based on these calculations, a plot of antibody volume versus SI was generated to determine the optimal volume corresponding to the highest SI value. The optimised and previously optimised titres for each antibody are presented in **Table 4.1**.

Table 4.1: Optimised multicolour panel used to analyse the cell surface markers

Specificity	Fluorochrome	Targeted cell type	Optimised volume
CD19	RedFluor™ 710	B lymphocytes	4
CD38	APC		4
CD3	BUV496	Lymphocytes	1
HLA-DR	BV650	Monocytes and CD8 cells	1.5
CD8	BV-605	T lymphocytes	1
Annexin V	FITC		2
PI	PE		2

Notes: BUV: BD Horizon Brilliant™ Ultraviolet; BV: Brilliant Violet™; PE: Phycoerythrin Cyanide dye™; FITC:Fluorescein isothiocyanate; HLA DR: human leukocyte D related

4.3.2.2 Flow cytometer set-up and tracking

To ensure consistent and reliable performance of the flow cytometer, we employed the BD™ Cytometer Setup and Tracking (CS&T) system in conjunction with BD FACSDiva™ software. One drop of BD Cytometer Setup and Tracking beads (CS&T) (BD Biosciences, New Jersey, USA) diluted in 350µL of BD FACSFlow^{TM} (BD Biosciences, New Jersey, USA) was used for instrument setup and tracking, following the manufacturer's protocol (BD^{TM} Tracking Beads and Cytometer Setup application guide, 2015). Flow cytometer setup and performance tracking were conducted using the BD™ Cytometer Setup and Tracking (CS&T) system with BD FACSDiva™ software. The CS&T workflow utilised BD™ CS&T beads to establish an initial cytometer baseline and maintain consistent performance over time. To define the baseline, CS&T beads were analysed across all fluorescence detectors, and their median fluorescence intensity (MFI) and robust coefficient of variation (%rCV) were measured. These parameters were used by the software algorithms to determine cytometer settings, including baseline photomultiplier tube (PMT) gains and target values for application-specific reproducibility. During this setup, laser delays, area scaling factors, and PMT voltages were optimised to ensure accurate signal detection and minimal variability.

4.3.2.3 Photomultiplier tube (PMT) voltage optimisation for patient samples

Photomultiplier tube voltage optimisation is performed to ensure optimal instrument performance during an experiment, particularly when conducted repeatedly. Voltage optimisation established negative and positive populations of target parameters and their linearity (Cossarizza et al., 2019). To provide reliable findings, the voltages of every fluorochrome were adjusted for the immunophenotyping test. Unstained and single-stained PBMCs (thawed and processed in a similar way to the titration experiment) were used to ensure that the cells were within the instrument's dynamic range.

4.3.2.4 Compensation controls and calculations

Compensation in multicolour flow cytometry is the technique of correcting fluorescence spillover across various fluorophores. Applying compensation allows for an adjustment in spillover to avoid false positives and negatives. One drop of negative and positive beads (Anti-Mouse Ig, κ/Negative Control Compensation Particles Set3) (BD Biosciences, New Jersey,

USA) were added separately to 5 mL Corning Falcon tubes (Corning Incorporated, New York, USA) with 50µL of 1X PBS (Sigma-Merck, Missouri, USA). The optimal pre-titrated antibody volumes were added in each Falcon tube and incubated in the dark for 30 minutes. The stained beads were rinsed with 2 mL of 1X PBS at 600 x g for 5 minutes. The supernatant was then decanted, and the pellet was resuspended in 300 µL of staining buffer. A total of 10,000 events were acquired and recorded using the compensation menu in the FACSDiva version 6 software (BD Biosciences, New Jersey, USA) on the BD LSR II Fortessa™ (BD Biosciences, New Jersey, USA) flow cytometer. The calculation, subtraction, and application of spillover to experimental samples were done automatically by the BD FACSDiva™ software.

4.3.2.5 Cell surface marker analysis

The drug response matrix of combined drug efficacy in chapter 3.7.4 (Figure 3.8) showed the best that the best effect was observed with the combination of irinotecan at 1 nm and 1000 nM for nilotinib. These concentrations of drugs were therefore used to evaluate their effect on surface markers, and the sample processing steps as described in 4.3.2.1 were followed but with the inclusion of treatment. PBMCs were subjected to drug treatment as per the plate layout (Table 4.2). Following an incubation period of 48 hours, harvested cells were stained with a solution of staining buffer containing the pre-titrated antibody concentrations, and the samples were incubated in the dark as described in 4.3.2.1. Untreated unstained and untreated stained controls were used to establish negative and positive populations for immunophenotyping.

Table 4.2: Plate layout

	1	2	3	4
	Untreated Unstained	**Untreated stained**	**Irinotecan + Nilotinib**	**Patient**
A			1:1000	BH30
B			1:1000	AML 04
C			1:1000	CML04

4.4: Data and statistical analysis

Flow cytometry standard (FCS) files linked to the compensation controls from FACSDiva™ (BD Biosciences, New Jersey, USA) software were uploaded and analysed on FlowJo LLC version 10 (BD Biosciences, New Jersey, United States). Cells were gated as singlets to exclude doublets using forward scatter height (FSC-H) and forward side scatter area (FSC-A). Forward scatter (FSC) versus side scatter (SSC) was applied to discriminate the white blood cell populations. Annexin V/PI staining was then analysed, and the surface markers were identified from a subset of viable cells. All populations were represented as percentages of the parent population. Cell differentiation included lymphocytes (CD3) and their subpopulations CD3+CD8+, CD3+CD8-, CD19, HLA-DR and CD38.

4.5: Results

For the immunophenotyping analysis by flow cytometry, three patient samples representing the three leukaemia subtypes, BH30 (CLL), AML04 (AML), and CML04 (CML), were analysed. Untreated unstained and treated stained cells were used as controls to set the negative and positive populations.

Figure 4.1 shows the hierarchical representation or gating strategy of stained cells showing the different target populations for the various markers. A similar picture applies to all fluorochromes used in the panel and all the samples. (A) Singlet events were gated on FSC/SSC to exclude doublets and aggregates. (B) The main cell population was identified, excluding debris. (C) Annexin V/PI staining showed reduced viability and increased early and late apoptotic/necrotic populations in BH30-treated cells. (D) $CD3^+$ T cells were identified within the live gate. (E) $CD3^-CD19^+$ B cells and $CD3^+CD19^-$ T cells were distinguished, with T cells predominating after treatment. (F) Among $CD3^+$ T cells, $CD4^+$ helper T cells represented 70.5% and $CD8^+$ cytotoxic T cells 29.5%.

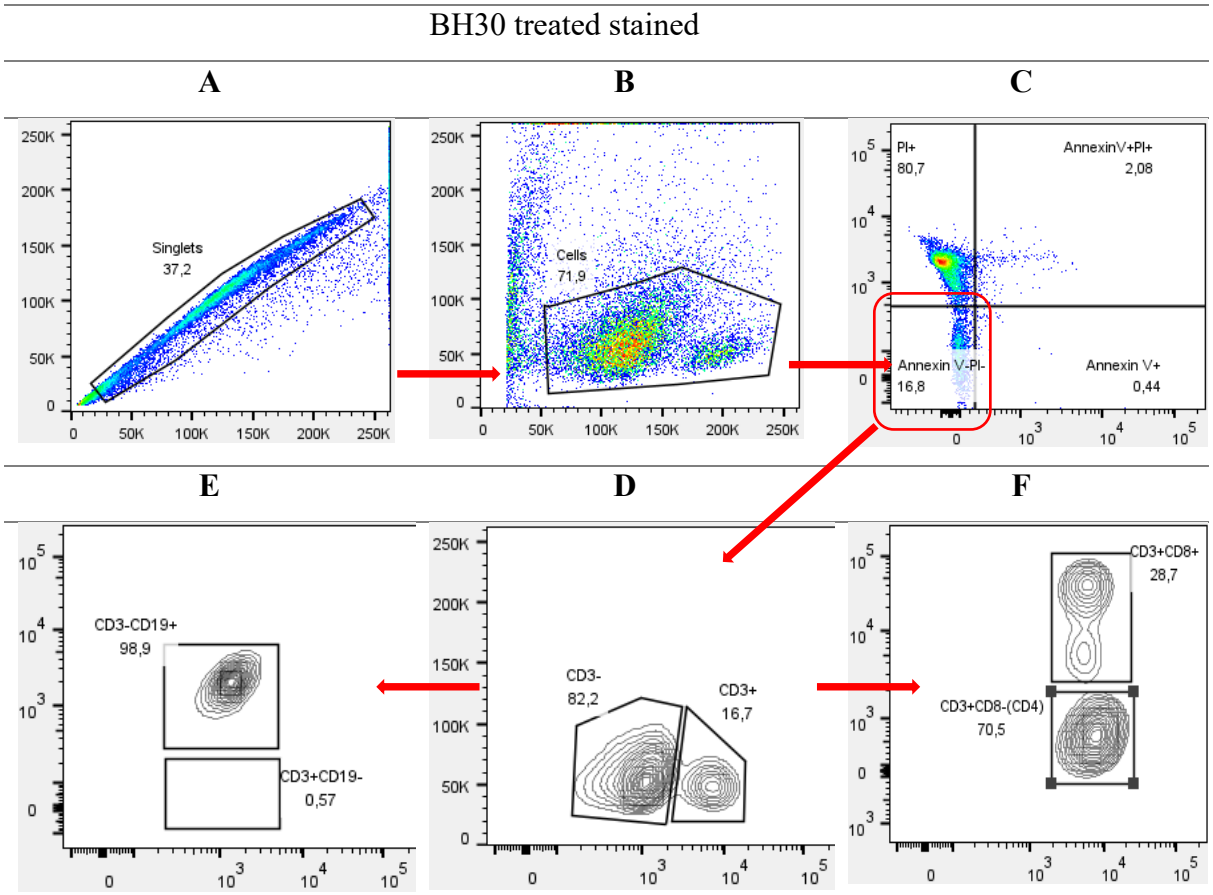


Figure 4.1: Hierarchical gating strategy for flow cytometry analysis of BH30 treated stained cells.

(A) Singlet gating: Selection of singlet events to exclude doublets and cell aggregates. The singlet population is defined based on forward scatter (FSC) and side scatter (SSC) characteristics. (B) Cells: Identification of the main cell population based on FSC and SSC to exclude debris. (C) Live cells gating: Annexin V and PI staining are used to differentiate live (Annexin V^-/PI^-), early apoptotic (Annexin V^+/PI^-), late apoptotic/necrotic (Annexin V^+/PI^+), and necrotic (Annexin V^-/PI^+) cells. (D) T-cell subset selection: Identification of $CD3^+$ T cells. . (E) B-cell and T-cell distinction: $CD3^-CD19^+$ B cells and $CD3^+CD19^-$ T cells are gated based on CD3 and CD19 expression. (F) $CD8^+$ and $CD4^+$ T-cell distinction: $CD3^+CD8^+$ cytotoxic T cells and $CD3^+CD4^+$ helper T cells are identified.

4.5.1: Measurement of apoptosis and necrosis by annexin V and PI staining

The combination of annexin V and PI staining allows for the differentiation between early apoptotic cells (annexin V positive, PI negative), late apoptotic or necrotic cells (both Annexin V and PI positive), and viable cells (both negative for annexin V and PI). Live cells are found in the lower left quadrant (annexin V and PI negative) **Figure 4.1C**, early apoptotic cells are indicated in the lower right quadrant (annexin V positive and PI negative), late apoptotic cells

are demonstrated in the upper right quadrant (annexin V and PI positive) and dead cells are shown in upper left quadrant (annexin V negative and PI positive).

Figure 4.2 represents the cell viability data obtained using an annexin V/PI double-staining assay. The untreated unstained cells represent the baseline without any treatment or staining. Most of the cells (98.6%, 95.0%, and 98.9%) of patients BH30, AML04, CML04 respectively fall into the live quadrant, indicating baseline characteristics in the absence of Annexin V and PI. On the other hand, when stained, all patients present a low viability percentage with patients showing viable cells below 20%. A high necrosis rate was observed among the tested patient samples. Over 60% of cells appeared to be dead in the untreated stained and treated stained groups. Cells were also present in Annexin V+PI+ quadrant, demonstrating the presence of cells in the late apoptosis stage. The treated stained group showed a higher percentage of late apoptotic cells (2.13%, 18.4%, and 16.1%) compared to the untreated stained group (0.60%, 9.33% and 12.7%) respectively for BH30, AML04, and CML04.

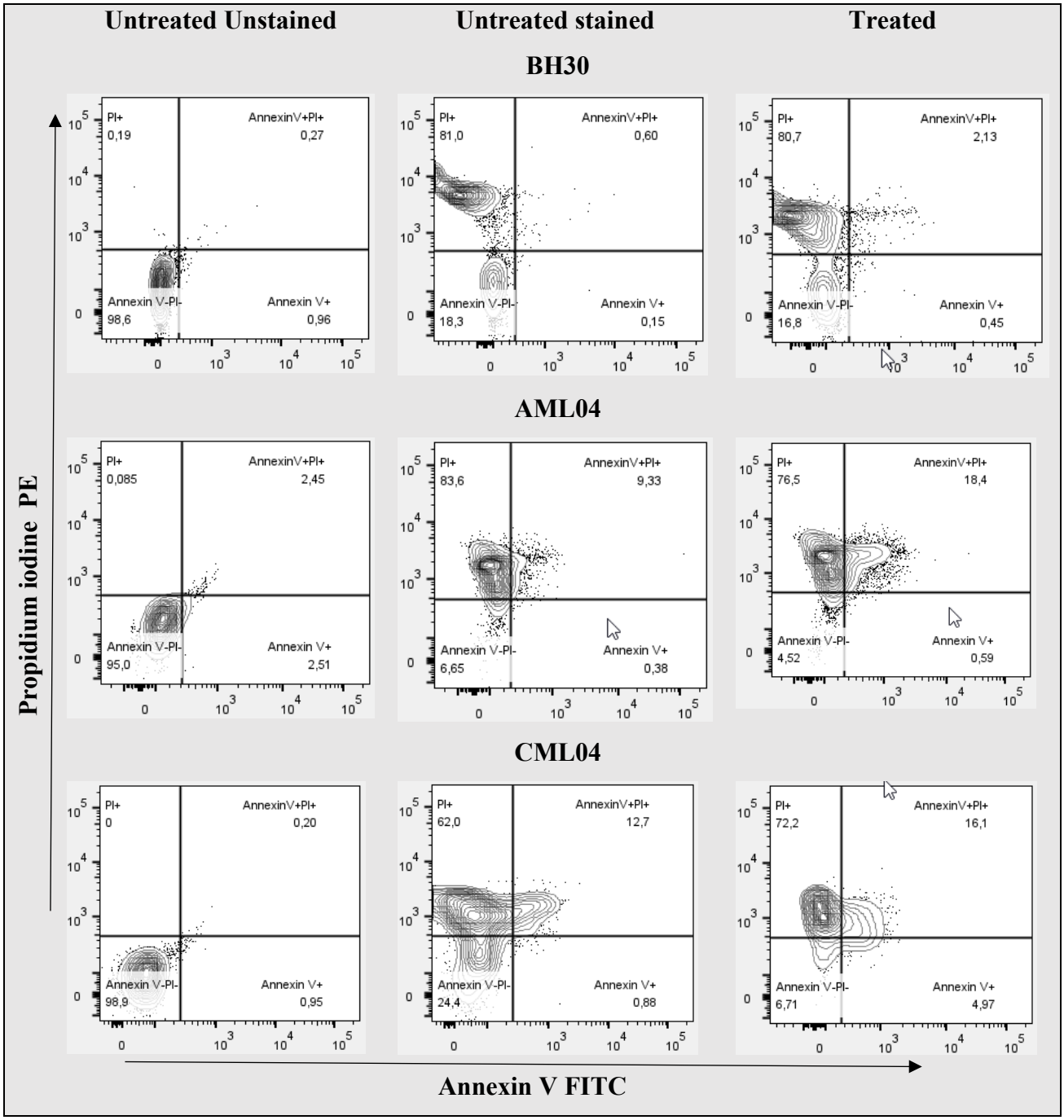


Figure 4.2: Flow cytometry analysis using an Annexin V/PI double-staining assay

The data represents findings from patients BH30 (CLL), AML04 (AML) AND CML04 (CML) analysed using an annexin v/pi double-staining assay. The plot was generated using FlowJo™ version 10 (Oregon, USA). The quadrant plot was used to separate live cells (Annexin V-P-), early apoptosis (Annexin V+PI-), late apoptosis (Annexin V+P+) and necrosis (P+Ann-).

Notes: PI: Propridium iodine, AML: Acute Myeloid Leukaemia, CML: Chronic myeloid Leukaemia

4.5.2: Cell surface marker analysis

4.5.2.1: Cell surface expression on BH30

The flow cytometry plots in Figure 4.3 represent markers associated with patient BH30 treated with irinotecan and nilotinib compared to the untreated. The comparison between untreated and treated BH30 cells, both fully stained, revealed notable differences in T-cell population dynamics. The proportion of CD3+ cells in the untreated stained condition is lower (10.5%) compared to the treated sample (16.7%), suggesting a slight increase or activation of T-cells upon treatment with irinotecan and nilotinib. Within the CD3+ compartment, the untreated sample compared to the treated sample showed a slight decrease in CD3+CD8- (probably CD4+) and an increase in CD3+CD8+ (24.9% vs 28.7% respectively).

The results showed a higher percentage of CD19+ cell population (98.2%) compared to CD3+ (16.7%) in the BH30 treated sample (Figure 4.3). This suggests that the majority of circulating lymphocytes in the sample consisted of B-cells rather than T-cells. This result confirms the leukaemia subtype found in BH30. The treatment with irinotecan combined with nilotinib showed almost no effect in the expression of CD19+ cells compared to the untreated sample with similar percentages (98.9% and 98.2% respectively). Further evaluation of HLA-DR marker provided insights into the activation status of B-cells. A similar percentage of HLA-DR and CD19 co-expression was found in the treated sample compared to the untreated sample (99.9% and 98.9% respectively).

In the sample AML04 representing acute myeloid leukaemia, the treated sample exhibited higher values across most parameters (**Table 4.3, plots showed in appendix 4.1)**. The T-cell population (CD3+) was upregulated (10.9%) compared to the untreated sample (6.12%). This trend was consistent in the CD3+CD8+ population, where more expression was seen in the treated sample (38.0%) versus the untreated sample 21.6%. However, an exception is observed in the CD3+CD8- population, where the untreated (63.7%) surpasses the treated sample (50.6%). The CD19+ population (CD3-CD19+) and HLA-DR+CD19+ subpopulations, showed an upregulation of these markers in the treated sample (22.1% and 15% respectively) compared to the untreated sample (6.98% and 2.7% respectively). On the other hand, the CD38+CD19+ population showed near-identical values for both, with samples at 100% and 99.3% for treated and untreated respectively.

CD3+ cells and their subpopulations were slightly upregulated in CML04 (**Table 4.3, plots showed in appendix 4.1**). The treated sample had slightly higher values of CD3+, CD3+CD8, CD3+CD8+, accounting for 38.0%, 56.1% and 40.4%, respectively compared to the untreated sample which accounted for 37.2%, 53.6% and 40.6 %, respectively. A very low CD19+ population was observed in the treated and untreated samples (1.12% and 0.3% respectively), and no expression of HLA-DR was found in them (0% HLA-DR+ CD19+). On the contrary, CD38 was expressed in all of them (100% CD38+CD19+).

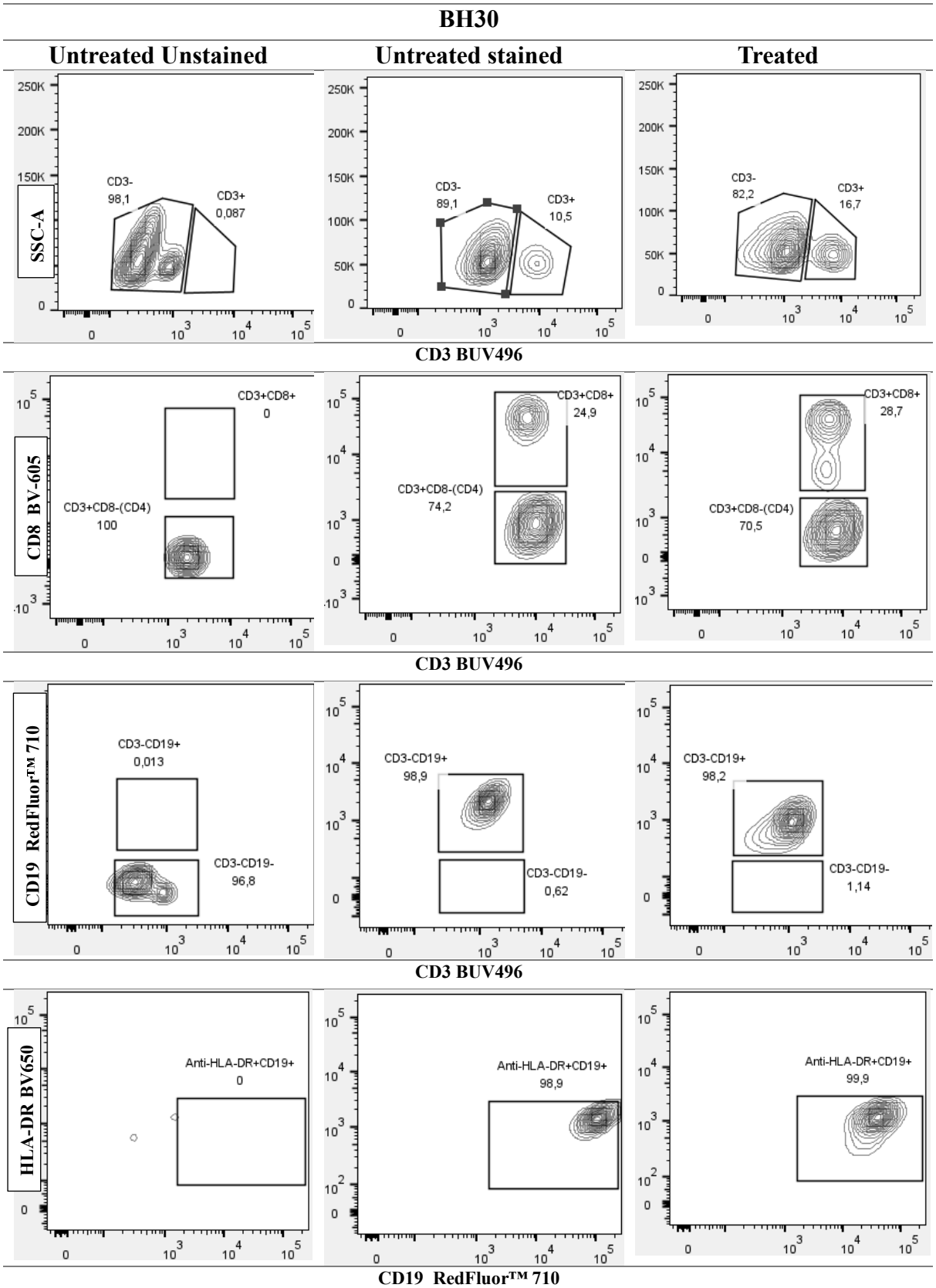


Figure 4.3 Surface marker analysis on BH30 (CLL) cells.

Notes: BUV: BD Horizon Brilliant™ Ultraviolet; BV: Brilliant Violet™; PE: Phycoerythrin Cyanide dye™; FITC:Fluorescein isothiocyanate; HLA DR: human leukocyte D related

Table 4.1: Expression of cell surface markers in AML04 and CML04

	AML_UU	AML04_US	AML04_TS	CML04_UU	CML04_US	CML04_TS
CD3+	0	6.12	10.9	0	37.2	38.0
CD3+CD8+	0	21.6	38.0	0	53.6	56.1
CD3+CD8-	0	63.7	50.6	0	40.6	40.4
CD3-	96.7	93.3	88.4	84	60.2	59.3
CD3-CD19-	99.4	65.9	61.9	98.9	93.4	87.6
CD3-CD19+	0.03	15	22.1	0	0.3	1.12
HLA-DR+ CD19+	0	2.7	6.98	0	0	0
CD38+CD19+	0	99.6	100	0	100	100

UU: Untreated unstained, US: Untreated stained, TS: Treated stained. All the values in the table are in percentage (%).

4.6: Discussion

Cluster of differentiation are crucial in markers in immune cell function, activation, and differentiation (Kalina et al., 2019). Their assessment provides valuable insights into the immunophenotypic changes associated with treatment response. Flow cytometry was utilised to analyse key markers, including CD3, CD8, CD19, CD38, and HLA-DR, across different immune cell subsets. In this chapter, the effect of irinotecan in combination with nilotinib was evaluated.

Cell death mechanism

The dual staining with annexin V and propidium iodide (PI) is particularly useful in studying cell death mechanisms and evaluating the effects of therapeutic treatments on cell viability. The result revealed that most of the untreated and treated cells were dead and that necrosis (Over 60%) was the most common form of cell death. This result suggests that cells were dying before treatment, therefore the higher cell death percentage noted was not attributable to the drug effect. However, as noted by Rieger et al. (2010) suggest that the ability of PI to stain RNA within the cytoplasmic compartment, makes conventional annexin V/PI protocols result in a high percentage of false positive events (Rieger et al., 2010). To reduce this risk, alternative dyes such as 7-aminoactinomycin D (7-AAD), which shows lower RNA binding and is less prone to false positives than propidium iodide, may be used (Cai et al., 2024). Additionally, the

multiple handling and processing steps involved in typical staining protocols—such as centrifugation, washing, and detachment—can induce mechanical stress and preferentially damage fragile or apoptotic cells, contributing to increased cell death that may occur independently of the treatment itself (Cossarizza et al., 2019)

Importantly, the increase in annexin V^+PI^+ cells in the treated group compared to the untreated stained group suggests an elevation in late apoptotic events following drug exposure. It was found that the percentage of late apoptotic cells varied across patients and was higher than early apoptosis. While apoptosis levels might appear relatively low, they remain significant within the context of this study, indicating that the combination of irinotecan and nilotinib contributes to programmed cell death. Irinotecan is a topoisomerase inhibitor that has been shown to induce apoptosis in colorectal cancer (Chai et al., 2024). In the present study, irinotecan exhibited the highest DSS against patient-derived CML cells, suggesting that its DNA-damaging mechanism may also be effective in haematological malignancies. This finding is critical as it suggests that the treatment effectively induces apoptotic pathways, which could be a crucial mechanism for targeting leukaemia cells.

Immune cell dynamics in response to the combination of irinotecan and nilotinib

T-cells play an important part in leukaemia treatment. The results (Figure 4.3) showed a slight increase in T-cell populations upon treatment with irinotecan and nilotinib where CD3+ CD8- cells (T helper) dominate over CD3+ CD8+ cells (Cytotoxic T-cells). These findings indicate that treatment shifts the immune balance toward a helper T-cell-dominant profile. CD3+CD8+ T-cells, commonly referred to as cytotoxic T-cells, play a crucial role in the immune system by identifying and eliminating harmful cells (Weigelin et al., 2021). These cells express two key surface proteins: CD3, which facilitates intracellular signalling and communication, and CD8, which enables the recognition and destruction of target cells (Trabelsi et al., 2019). The concentration of CD3+CD8+ T cells in the blood is an important indicator of immune system activity. Elevated levels of these cells may signify an ongoing immune response, such as combating an infection, managing an autoimmune disorder, or reacting to specific medications (Raskov et al., 2021). Changes in CD8+ T-cell frequencies, activation states could provide insights into immune competence and potential responsiveness to T-cell-based immunotherapies. Additionally, CD8+ T cells are key effectors of the adaptive immune system,

responsible for recognizing and eliminating malignant cells through cytotoxic mechanisms, including perforin-granzyme and Fas-FasL-mediated apoptosis (Wang et al., 2018). However, in leukaemia, immune evasion strategies such as upregulation of immune checkpoints like PD-1, and the immunosuppressive tumour microenvironment can impair CD8+ T-cell functionality, reducing their ability to effectively target leukemic cells (Haslauer et al., 2021). Emerging immunotherapies, such as monoclonal antibodies and chimeric antigen receptor (CAR) T-cell therapy, seek to overcome these immune evasion mechanisms by enhancing T-cell recognition and cytotoxicity (Haslauer et al., 2021). CAR T-cell therapy involves genetically engineering T-cells to express chimeric antigen receptors that specifically recognize leukaemia-associated antigens, such as CD19 in B-cell malignancies. Upon antigen binding, CAR-expressing CD8+ T-cells become activated, proliferate, and exert potent cytotoxic effects against leukaemia cells. This approach has shown promising clinical outcomes, particularly in relapsed or refractory leukaemia (Zhang et al., 2022).

The observed variability in CD3, CD19, and HLA-DR expression suggests dynamic changes in immune cell populations, potentially driven by treatment with irinotecan and nilotinib (Figure 4.3, Table 4.3). The increase in CD3+ T cells with treatment may indicate treatment-induced immune activation response, whereas the stability in HLA-DR+ B-cell populations reflects no changes in antigen presentation activity. The observed CD3+CD8− (helper T-cell) dominance over CD3+CD8+ (cytotoxic T-cell) populations in previous findings aligns with the notion that leukaemia treatments can shift the immune response balance (Jiang et al., 2024; Liu et al., 2025). The presence of antigen-presenting B cells is particularly significant, as HLA-DR upregulation is associated with enhanced immune modulation and response to leukaemia treatment (Chaput et al., 2013).

4.7: Conclusion

This study highlights important findings on cell death mechanisms and immune response during leukaemia treatment. The annexin V and propidium Iodide (PI) dual staining revealed high levels of necrosis in both untreated and treated cells, suggesting that the observed cell death may not be entirely due to drug effects. Though the apoptosis rate was low, treated cells underwent early and late apoptosis, highlighting the contribution of the combination of irinotecan and nilotinib to programmed cell death. However, possible limitations in the staining

method, such as false-positive results and potential stress from procedural steps, may have influenced the accuracy of these results.

Limitations of this aspect of work included a small number of cells, making it challenging to perform important controls like fluorescence minus one (FMO) controls or single stain controls.

CHAPTER 5: *IN SILICO* STUDY OF THE INHIBITION POTENTIAL OF IRINOTECAN ON KEY LEUKAEMIA PATHWAYS

5.1: Introduction

A hallmark of cancer cells is uncontrolled, rapid division, caused mostly by genetic and epigenetic changes in cancer genes or genes linked with signalling pathways, resulting in either over-proliferation or evasion of normal cell death mechanisms (Azizidoost et al., 2024). Thus, physiologic changes in cell signalling pathways are extensively studied in cancer to understand the initiation and progression of tumorigenesis (Hallal et al., 2020). Despite advances in treatment, drug resistance and disease relapse remain significant challenges. Signalling pathways such as PI3K/AKT/mTOR, JAK/STAT, and MAPK are critical in leukaemia pathogenesis and treatment response (Glaviano et al., 2023; Zhu et al., 2022). Developing new treatments for precision medicine in cancer, such as small molecule inhibitors and monoclonal antibodies targeting cancer-related proteins, is crucial. Irinotecan, a topoisomerase I inhibitor, has shown promise in targeting cancer cells, but its effects on leukaemia-specific signalling pathways are not fully understood (Kerstjens et al., 2021). In the previous chapters, the combination of irinotecan and nilotinib was found to be the most effective against patient cells demonstrated by the highest DSS. Irinotecan is a drug developed to target topoisomerase I in solid cancer. However, based on the principle of drug repurposing, a drug can have multiple targets. *In silico* studies offer a powerful tool to explore these mechanisms, enabling the identification of dysregulated pathways and potential therapeutic targets (Sohraby et al., 2017). This chapter aims to explore the inhibition potential of irinotecan on key proteins of leukaemia signalling pathways compared to nilotinib, a well-known kinase inhibitor in the treatment of leukaemia.

5.1.1: Dysregulated signalling pathways in leukaemia

The pathogenesis of leukaemia involves the dysregulation of multiple cellular pathways, which disrupt normal haematopoiesis and promote uncontrolled proliferation, survival, and differentiation of leukemic cells (Hallek et al., 2018). Key pathways implicated in leukaemia include signalling cascades, epigenetic regulators, and metabolic alterations, which collectively contribute to disease progression and therapeutic resistance. The PI3K/AKT/mTOR (PAM)

signalling pathway is a highly conserved signal transduction network found in eukaryotic cells that promotes cell survival, proliferation, and cycle advancement (Zhu et al., 2022). The PAM axis is the most often active signalling route in human cancer, and it is usually linked to anticancer drug resistance (Glaviano et al., 2023). Additionally, the PI3K/Akt/mTOR pathway is activated in up to 80% of AML cases and is linked to poor prognosis (Mirabilii et al., 2018).

Similarly, the JAK/STAT pathway plays a crucial role in enhancing cell survival through the activation of STAT proteins, particularly STAT3, which is often dysregulated in leukemic stem cells. STAT3 is known to be upregulated in a significant proportion of tumour types, including leukaemia (Brown et al., 2021) and is one of the most extensively studied signalling intermediaries in tumour development. It regulates the transcription of multiple genes involved in apoptosis inhibition and plays a role in various physiological processes, including cell proliferation, apoptosis, differentiation, haematopoiesis, and immune regulation (Ogiya et al., 2020). Continuous activation of STAT3 has been associated with imatinib resistance in leukaemia cells (Shao et al., 2014). Thus, the developing drug that targets STAT3 offers an alternative technique for treating imatinib-resistant CML. Additionally, the Notch signalling pathway contributes to the self-renewal and expansion of leukemic stem cells by activating downstream target genes. Phosphorylation of STAT5A at Ser779/780 (mouse/human) plays a critical role in regulating the proliferation and transformation/expansion of hematopoietic stem and progenitor cells (HSPCs), demonstrating greater potency compared to STAT5B (Ghanem et al., 2017). Additionally, other STAT family members are implicated in both normal and leukemic haematopoiesis. For example, STAT1 and STAT3 have been shown to regulate CD38 expression within the bone marrow microenvironment of multiple myeloma cells (Ogiya et al., 2020). Likewise, the Wnt/β-catenin pathway is essential for maintaining the stem cell pool and, when dysregulated, can drive increased proliferation and resistance to therapy in leukaemia (Azizidoost et al., 2024; Siveen et al., 2017). Together, these pathways create a complex network that supports leukemic cell survival and therapy resistance, highlighting their potential as therapeutic targets.

5.2: Aim and rationale

The previous chapter reported irinotecan as the most effective drug for patients in the cohort, and its combination with nilotinib was found to be found to be the most effective. Irinotecan is

a topoisomerase inhibitor, and nilotinib is a kinase inhibitor. Since dysregulated pathways in leukaemia involve many kinases, this study aimed at investigating the interaction of these two drugs on selected proteins and their binding mode. Knowing that nilotinib is already a kinase inhibitor clinically used in the treatment of leukaemia, the focus of the interpretation and conclusion is made on irinotecan.

5.3: Materials and methods

5.3.1: Hardware characteristics

The hardware configuration used for these docking studies consisted of an Intel® Core™ i7-8550 processor with 16 cores, each operating at a bootable frequency of 2.1 GHz. The system had 16 GB of random-access memory (RAM) and 500 GB of storage capacity (ROM). The software environment was based on the Windows 10, 64-bit operating system.

5.3.2: Publicly available databases

PubChem (pubchem.ncbi.nlm.nih.gov) is the world's largest database of freely available chemical-related information. Search for chemicals using their names, molecular formulas, structures, and other identifiers. Discover chemical and physical properties, biological activities, safety and toxicity data, patents, literature citations, and more. Protein data are generally stored in protein databases like the Protein Data Bank (https://www.wwpdb.org).

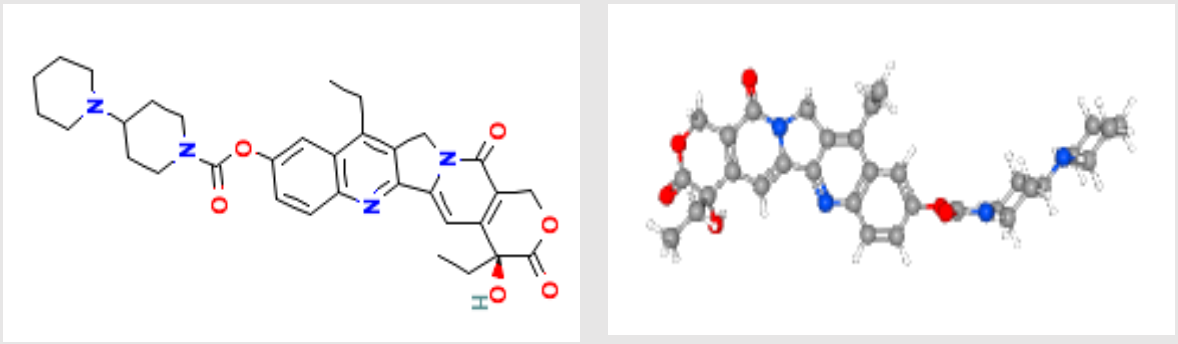
5.3.3: Ligand preparation

The 2D and 3D conformations of nilotinib (PubChem CID: 644241) and irinotecan (PubChem CID: 60838) were obtained from PubChem (Figure 5.1). Energy minimisation was performed using the conjugate gradient algorithm and the AMBER force field within AutoDockTools (Morris et al., 2009). This step aimed to relieve steric clashes and obtain low-energy conformations. The minimized structures were then saved in PDBQT format, a file format specifically designed for AutoDock. PDBQT format includes the information necessary for calculating molecular interactions, such as partial charges and atom types.

Irinotecan

PubChem CID: 60838

$C_{33}H_{38}N_4O_6$



Nilotinib

PubChem CID: 644241

$C_{28}H_{22}F_3N_7O$

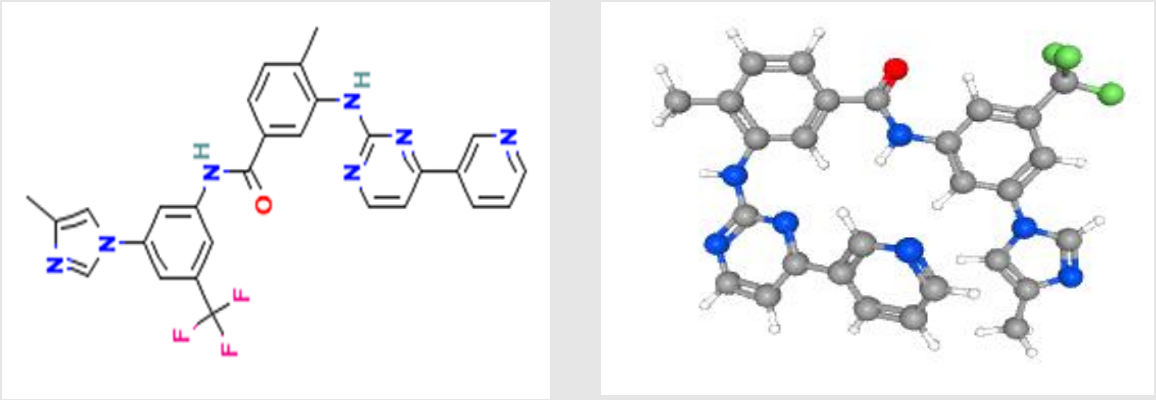


Figure 5.12D and 3D structures of Irinotecan and Nilotinib. Downloaded from PubChem

5.3.4: Protein preparation

The co-crystallised three-dimensional structures of the PI3K (4L23), JAK2 (4GFM), STAT3 (1BG1), and mTOR (4JSV) proteins were downloaded from the Protein Data Bank (PDB) (https://www.wwpdb.org). Once downloaded, they were imported into the Autodock interface,

where careful preparation was carried out to optimise their structures. Firstly, water molecules, co-crystallised ligands, and ions not directly involved in interactions with the ligand under study were removed. Hydrogen atoms were then added automatically to complete the molecular geometry of the protein. Partial charges were assigned to each atom using the AMBER force field, commonly used in molecular simulations, in order to represent the electronic distribution of the molecule and thus enable electrostatic interactions to be calculated accurately. Finally, the proteins were saved in PDBQT format, specifically designed for docking calculations with AutoDock, containing all the information needed for the simulations, such as atom types, hydrogen bonds, and partial charges.

5.3.5: Molecular docking experiment

A blind docking was performed on the co-crystallised proteins and the ligands using the grid box. The docking algorithm used was the Larckmakian genetic algorithm, and the poses generated were evaluated using the Autodock default score affinity function (Azmal et al., 2024; Rossino et al., 2020). The maximum number of poses retained per ligand was set at 10, to capture diverse and energetically favourable conformations. Once the parameters had been configured, the docking calculation was launched. Autodock4 generated several poses for nilotinib and irinotecan for each protein, each ranked according to its energy score, based on receptor binding affinity. The poses were ranked according to docking scores, with the lowest values corresponding to the most stable ligand-receptor complexes. To validate the molecular docking protocol and ensure the reliability of the docking results, a re-docking experiment was performed using the experimentally co-crystallized ligands of the target proteins. The original ligands were extracted from the crystal structures and then docked back into their respective binding sites using the same docking parameters as described above. The root mean square deviation (RMSD) between the heavy atoms of the co-crystallized ligand's original conformation and the re-docked conformation was calculated to assess the accuracy of the docking protocol. An RMSD value of less than 2.0 Å is generally considered indicative of a successful and accurate docking method that can reliably reproduce the experimentally observed binding mode (Azmal et al., 2024; Fathima et al., 2021).

5.3.6: Visualisation of interactions

Selected ligand-receptor complexes were visualized using Biovia Discovery Studio version 4.2 to identify key interactions, such as hydrogen bonds, hydrophobic interactions, and π-π interactions. Biovia's Ligand Interactions tool was used to map the residues involved in the interactions and generate 2D interaction patterns.

5.4: Results

The binding energy values of irinotecan and nilotinib with JAK, mTOR, PI3K, and STAT3 proteins (Table **5.1**) provide insights into their relative binding affinities and potential inhibitory interactions. Binding energy, expressed in kcal/mol, reflects the strength and stability of the ligand-protein complex, with more negative values indicating stronger binding.

Irinotecan exhibited variable binding affinities across all four proteins, with the most favourable binding energy observed for mTOR (-11.1 kcal/mol), followed closely by PI3K (-10.3 kcal/mol) and STAT3 (-10.0 kcal/mol). In comparison to irinotecan, nilotinib showed weaker binding affinity with mTOR (-10.2 kcal/mol), PI3K (-10.1 kcal/mol), and STAT3 (-9.2 kcal/mol). However, nilotinib exhibited slightly stronger binding affinity with JAK (-11.1 kcal/mol) compared to irinotecan (JAK (-10.1 kcal/mol).

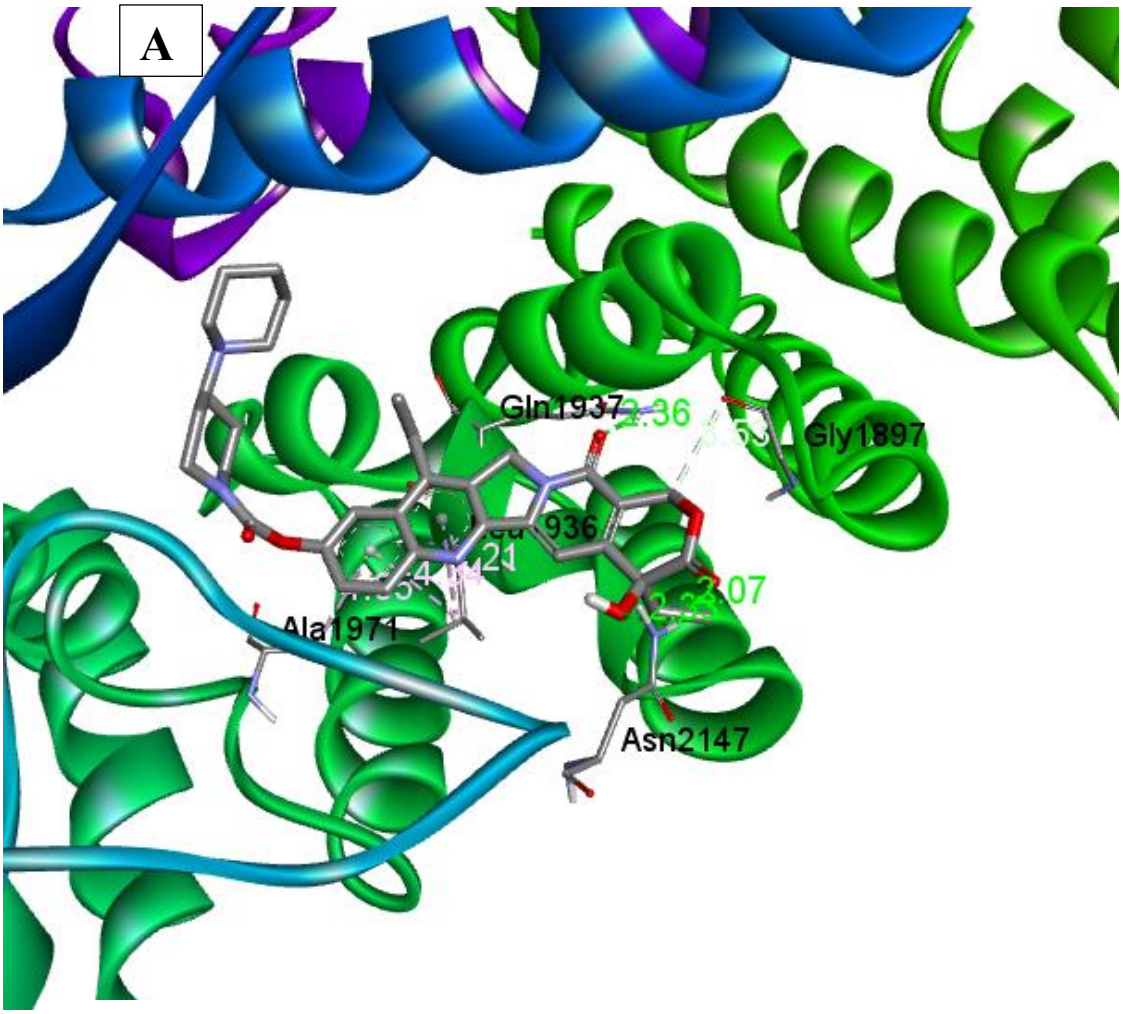
The 3D and 2D structure of the complex formed between mTOR and irinotecan (**Figure 5.2**) and nilotinib (**Figure 5.3**) revealed a complex network of interactions that stabilise the ligand within the active site of the protein. A key feature of the docking interaction is the presence of two conventional hydrogen bonds. These are formed between Irinotecan and asparagine (ASN B:2147) and glutamine (GLN B:1937). Additionally, a carbon-hydrogen bond was observed with leucine (LEU B: 1936), which may further stabilize the binding of irinotecan within the active site.

Van der Waals forces were evident with multiple residues, including arginine (ARG B:1896), aspartic acid (ASP B:2145), tyrosine (TYR B:2144), methionine (MET B:2199), isoleucine (ILE B:2228), and arginine (ARG B:2224). Furthermore, π-alkyl interactions were identified between irinotecan and leucine (LEU B:1936) and proline (PRO B:2146), suggesting additional stabilization via π-system interactions, which can enhance ligand retention in the active site.

The 3D and 2D structures of the interaction of irinotecan and nilotinib with the other proteins are given in (**Appendix 5.1**), their binding energy, the number of hydrogen bonds and amino acids involved in the interaction are summarized in Table 5.1

Table 5.1: Binding energies of nilotinib and irinotecan with STAT3, JAK2, PI3K and mTOR

Protein	Compound	Binding energy (Kcal/mol)	N^0 of H bonds (drug-protein)	RMSD
mTOR	Irinotecan	-11,1	2	1,1
	Nilotinib	-10,2	3	0,6
PI3K	Irinotecan	-10,3	0	0,6
	Nilotinib	-10,1	4	1,3
STAT3	Irinotecan	-10	5	2
	Nilotinib	-9,2	1	1,04
JAK2	Irinotecan	-10,1	2	2,4
	Nilotinib	-11,1	0	0,6


A
Gln1937
Gly1897
936
Ala1971
Asn2147


Interactions
van der Waals
Carbon Hydrogen Bond
Conventional Hydrogen Bond
Pi-Alkyl

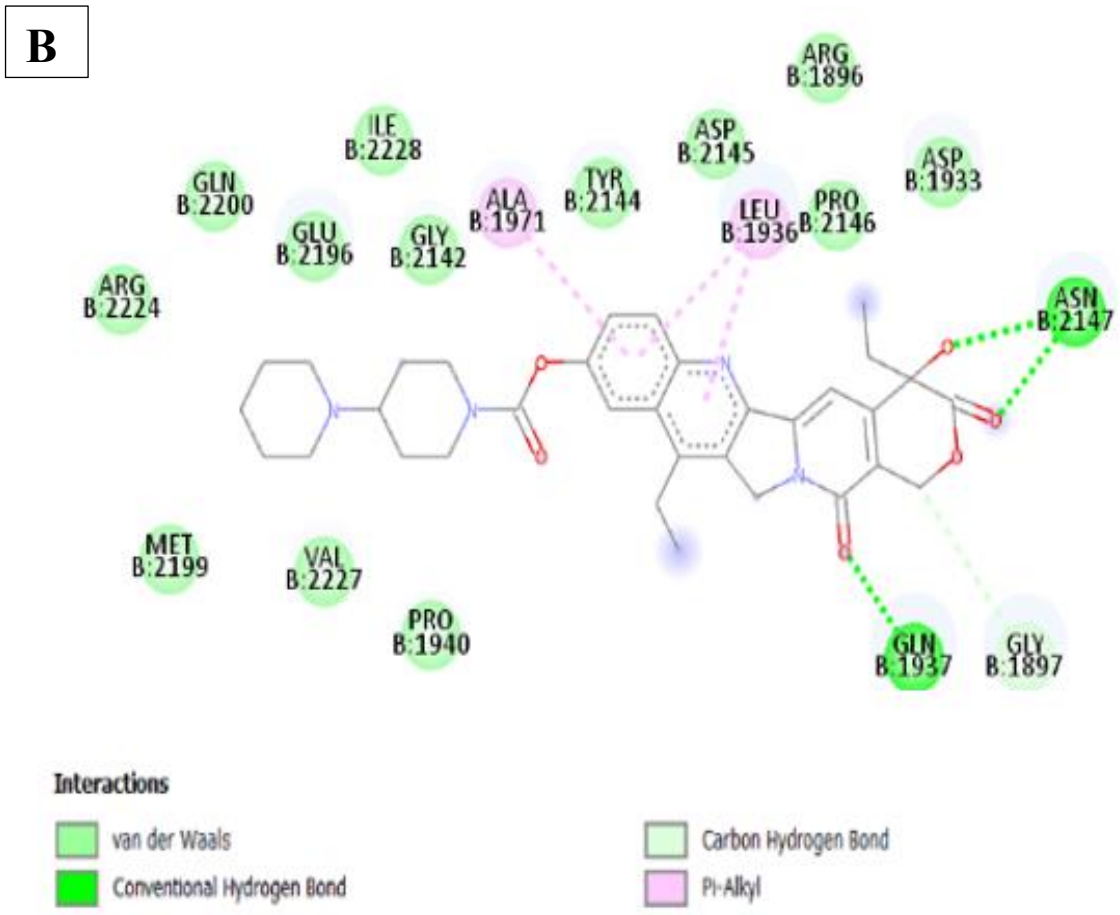
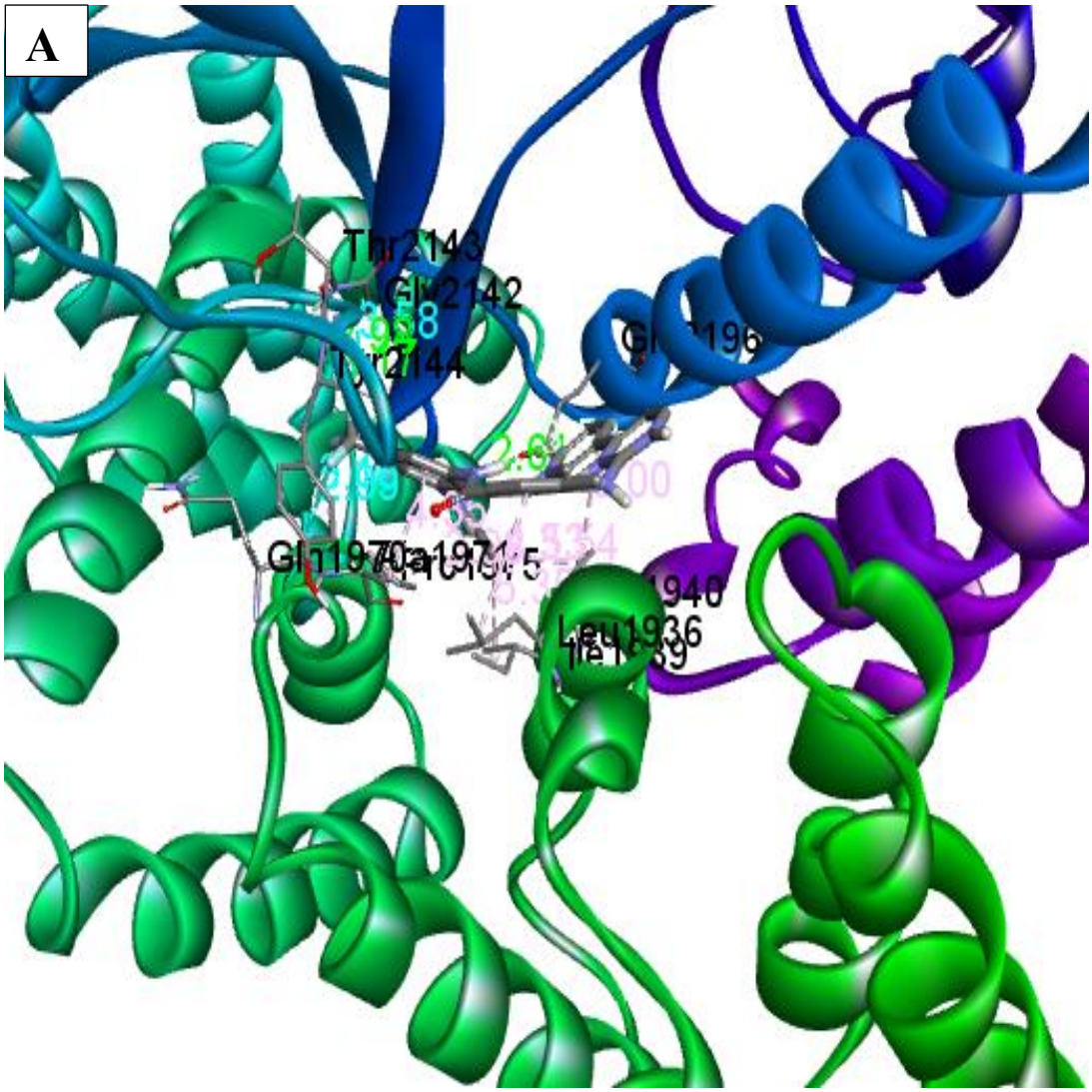


Figure 5.2: 3D (A) and 2D (B) representation of the interactions established between mTOR and irinotecan


A
Thr2143
Glu2142
Thr2144
Leu1936

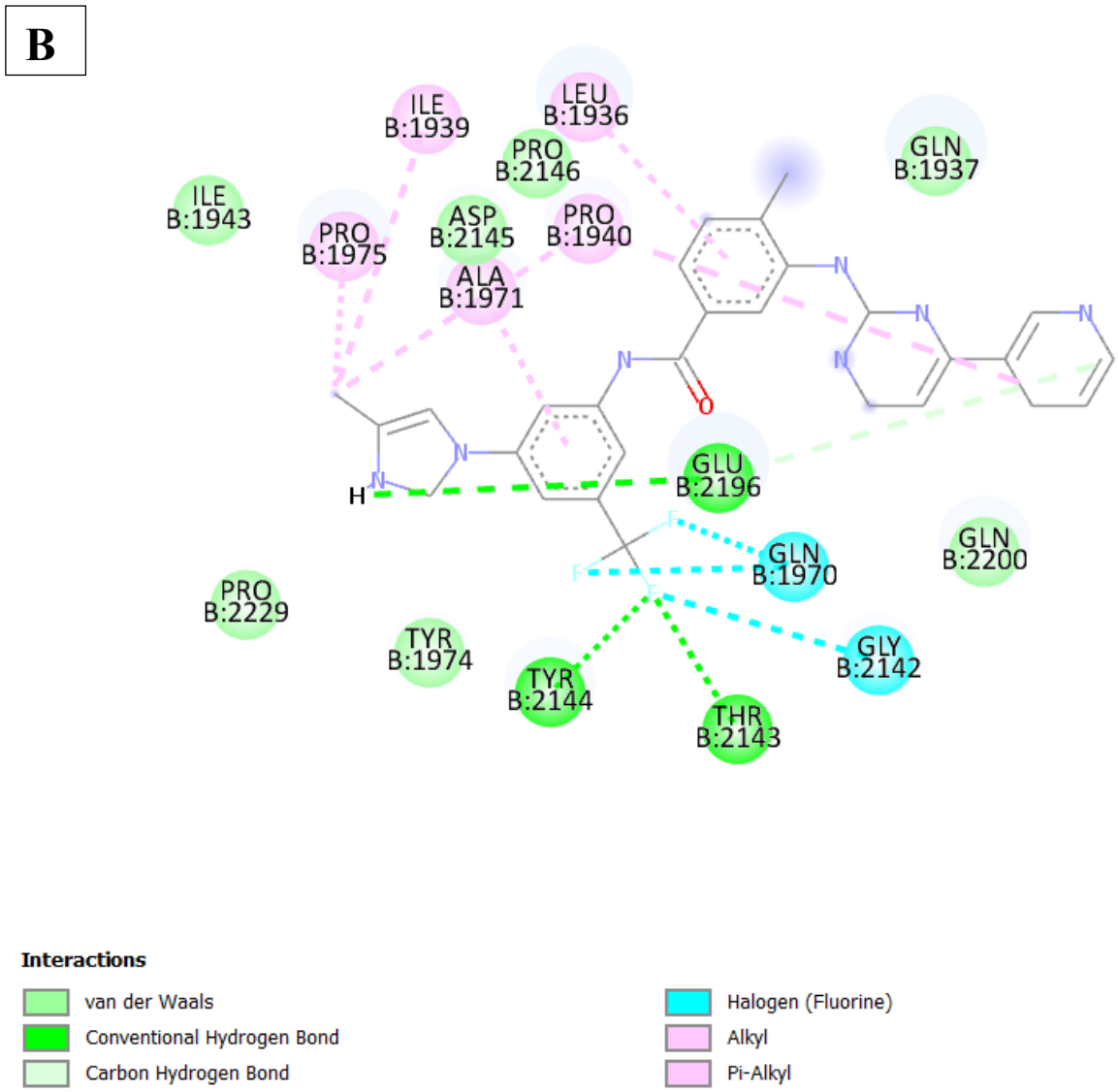


Figure 5.3 3D (A) and 2D (B) representations of the interactions established between mTOR and nilotinb.

5.5: Discussion

The docking simulation demonstrated irinotecan's strong binding affinity with key proteins associated with signalling pathways in leukaemia. Many compounds exert anti-cancer effects by interacting with the active sites of key proteins involved in signalling pathways.

Inhibition potential of irinotecan on PI3K/AKT/mTOR pathway

mTOR is one of the most important drug targets, which is overexpressed in many cancers, including leukaemia, and is closely related to the PI3K/Akt signalling pathway (Das et al., 2023). The results of this study predict an interesting inhibitory potential of irinotecan, particularly for mTOR and PI3K, where the binding energies were the lowest. Many studies have reported molecules capable of promoting apoptosis by binding to the catalytic site of mTOR. These include OSI-027 in lymphoma (Bhagwat et al., 2011). Another compound able to prevent the activation of mTORC2 is AZD8055 (Mirabilii et al., 2018). This finding emphasises the drug repurposing principle of polypharmacology, stating that drugs can have multiple biological targets. This finding adds up to the results obtained in previous chapters and suggests that irinotecan could be used in the treatment of leukaemia.

Analysis of hydrogen bonds

The widespread occurrence and adaptability of hydrogen bonds make them fundamental in bio-molecular interactions within aqueous environments. These interactions play a crucial role in various chemical and biological processes, including ligand binding and enzyme catalysis. Hydrogen bonds are weaker than covalent bonds but stronger than Van der Waals interactions (Bulusu & Desiraju, 2019). The presence of hydrogen bonds in the complex formed by irinotecan and the selected proteins highlights strong electrostatic interactions that contribute to ligand affinity. Additionally, carbon-hydrogen bonds were observed that may further stabilise the binding of irinotecan within the active site. The active site of an enzyme is a specialised region where substrate molecules bind and undergo a chemical reaction. It consists of amino acid residues that contribute to substrate binding (binding site) and catalytic activity (catalytic site). Structurally, the active site forms a three-dimensional cleft or pocket, composed of residues from different regions of the enzyme's amino acid sequence (Singh et al., 2022). Despite occupying only a small portion of the enzyme's total volume, the active site plays a crucial role in enzymatic function (Singh et al., 2022). Substrate binding is mediated by

multiple weak interactions, such as hydrogen bonds and van der Waals forces, ensuring specificity and stability (Bitencourt-Ferreira et al., 2019). Fathima and co-workers have reported that hydrogen bond interactions between paclitaxel and mTOR protein were observed at key residues with a binding energy of -6.8 kcal/mol, including GLY 2203, ARG 2224, and THR 2207 (Fathima et al., 2021). Another study reported interactions with distinctive mTOR residues TRP 2239, LEU 2185, and ASP 2375 for AZD2014 (ligand) (Naveed et al., 2017).

In this study, irinotecan established two hydrogen bonds with mTOR at ASN 2147 and GLN 1937 and five with STAT proteins (Table 5.1). The comparison with nilotinib, a well-known kinase inhibitor, showed weaker binding affinity with mTOR, even though there were more hydrogen bonds than irinotecan Table 5.1. This could be indicative of different types of hydrogen bonds in each of them. In biological systems, both specificity and reversibility are essential, as weaker hydrogen bonds facilitate transient interactions, allowing dynamic regulation of molecular functions. Additionally, their ability to form and dissociate more readily than stronger bonds is critical for maintaining biological flexibility and responsiveness (Bulusu & Desiraju, 2019).

A previous docking study has reported that N4, a STAT3 inhibitor, interacts with the STAT3-SH2 domain at multiple amino acid residues, including LYS591, SER636, VAL637, and GLU638 (Zapevalova et al., 2022). In this chapter, the mechanism of action of irinotecan involved binding to the active dimer formed by the tyrosine peptide, as observed in molecular docking analyses. Hydrophobic interactions play a significant role in the ligand's stabilization. The observations of the presence of Van der Waals forces with multiple residues suggest an additional stabilizing effect by facilitating close molecular contact between irinotecan and the surrounding amino acids. Furthermore, π-alkyl interactions identified between irinotecan and LEU B:1936 and PRO B:2146 in mTOR suggest additional stabilization via π-system interactions, which can enhance ligand retention in the active site.

5.6: Conclusion

The docking analysis suggests that irinotecan and nilotinib establish a well-defined interaction network within the binding site of the proteins of signalling pathways characterised by hydrogen bonds, carbon-hydrogen bonds, multiple Van der Waals contacts, and π-alkyl interactions. These findings predict the potential mechanism of action of irinotecan on

leukaemia pathways, suggesting mTOR as a new target aside from its primary indication as a topoisomerase I inhibitor for solid tumours.

Some limitations, such as *in silico* docking studies, only provide valuable preliminary insights but do not account for the dynamic nature of protein-ligand interactions in a biological system, which is why the results cannot be interpreted alone but must be linked to the *in vitro* studies you have already done. Additionally, the hydrogen bond length was not calculated, making it difficult to specifically differentiate and compare them.

CHAPTER 6: GENERAL CONCLUSION AND PROSPECTS

This thesis explores drug repurposing for leukaemia treatment as a pilot study for precision oncology in South Africa. The general introduction and literature review were covered in chapter 1. Chapter 2 talked about the demographic and clinical profile of patients. The demographic and clinical characteristics of patients were analysed based on data captured in patients' files. The data include age, gender, haematological parameters, and treatment history. The drug sensitivity screening on PBMC isolated from patients' samples was covered in chapter 3. Single drug screening of 30 FDA-approved drugs was conducted, and drug combinations based on the 10 most potent drugs were done. Additionally, a full matrix drug combination was carried out to evaluate the concentration at which each drug of the combination exerts the best effect. Chapter 4 focused on flow cytometry to evaluate the impact of drug treatment on cell surface markers on some patients. A seven-colour panel was designed to analyse CD markers like CD19, CD38, CD3, CD8, and HLA-DR. The *in silico* analysis of the binding affinity of irinotecan and nilotinib on some key leukaemia proteins was presented in Chapter 5, where molecular docking of irinotecan on proteins like PIPK, mTOR, JAK, and STAT3 was done and compared to nilotinib. This chapter represents the general conclusion and perspectives. The findings of this research offer critical insights into the disparities, challenges, and opportunities for improving leukaemia diagnosis and treatment.

Demographic and clinical characteristics

The demographic, clinical, and treatment history findings reported in this study highlight the substantial diversity in leukaemia subtypes across patient populations. By analysing leukaemia in the context of the South African region, this study uncovers disparities in the molecular landscape of leukaemia that underscore the importance of tailoring diagnostic and personalised therapeutic approaches. The research demonstrates how demographic and clinical differences among leukaemia subtypes, such as age, gender distribution, and treatment histories, significantly influence disease response to therapy. These findings emphasise the need for personalised, culturally sensitive healthcare models that account for local population-specific factors to improve patient outcomes.

Drug sensitivity testing

A panel of already approved drugs was tested on PBMCs isolated from a cohort of South African leukaemia patients. Irinotecan, a drug that is not clinically used in the treatment of leukaemia in South Africa, has been identified as the most potent drug in the cohort due to its superior drug sensitivity score (DSS). In the context of drug sensitivity screening, this research makes substantial contributions to leukaemia therapy by identifying effective compounds and strategies for their application. This screening approach also enabled the identification of potent drug combinations, providing a framework for optimising combination therapies to maximise therapeutic outcomes. The DSS framework employed in this study serves as a valuable tool for prioritising drug candidates and strategising treatment protocols for personalised medicine.

Expression of immune cell markers

The investigation into immune profiling through multicolour flow cytometry has expanded the understanding of the immune cell dynamics in leukaemia. The study examined markers such as CD3, CD8, CD19, CD38, and HLA-DR in peripheral blood mononuclear cells (PBMCs) isolated from leukaemia patients, revealing critical differences in immune cell composition and apoptotic responses across subtypes. These findings highlight the interaction between the immune system and leukaemia pathophysiology, offering potential targets for immune-based therapies.

Additionally, the *in silico* simulation showed the possible inhibitory effect of irinotecan on leukaemia key proteins like mTOR and PI3K, predicting potential new targets.

Overall, this finding indicates that many drugs that are currently not prescribed clinically for the treatment of leukaemia in South Africa can be repurposed to offer more options to patients who have exhausted the current drug regimen.

6.1: Study limitations

While this thesis provides valuable insights into drug repurposing for the treatment of leukaemia in South Africa, several limitations should be acknowledged to provide context for the interpretation of the findings and guide future research.

The limited sample size may limit the generalisability of the findings to broader populations. The data were primarily drawn from leukaemia patients in one city, with the majority from a public hospital, which may not capture the full spectrum of genetic, environmental, and clinical

variability observed in other cities or ethnic groups. A larger, more geographically and demographically diverse cohort would enhance the robustness and global applicability of the results.

Furthermore, the heterogeneity in patients' treatment histories, including variations in prior therapies, dosages, and treatment durations, introduced variability that could affect clinical and drug sensitivity outcomes, complicating direct comparisons across subgroups. Additionally, the *ex vivo* drug sensitivity screening, conducted on PBMCs isolated from leukaemia patients, did not fully replicate the complexities of the tumour microenvironment, as factors such as stromal interactions, cytokine signalling, and immune evasion mechanisms were not accounted for. While irinotecan emerged as a promising therapeutic candidate based on its overall superior drug sensitivity score (DSS), the absence of genetic and molecular correlation analyses limited insights into the underlying mechanisms driving drug sensitivity differences, highlighting the need for genomic and transcriptomic profiling in future studies as well as preclinical and clinical validation. Moreover, resource constraints in the South African research setting, including limited access to advanced technologies, reagents, and infrastructure, may have influenced the scope of experiments and the depth of findings. Despite these challenges, the study provides a valuable foundation for future research, underscoring the need for expanded cohort sizes, integration of advanced molecular techniques, and longitudinal studies. Translating the *ex vivo* and *in silico* findings into preclinical and clinical trials will be crucial to fully realize the therapeutic potential of the identified drug candidates.

6.2: Future Directions

The findings of this study open avenues for further research. Several future research directions can be pursued to enhance the clinical relevance and translational potential of the results. Expanding the scope of drug sensitivity screenings to include larger patient cohorts (taking into account their molecular/genetic characteristics) and a larger library of drugs could help refine therapeutic hierarchies and identify additional repurposable drugs. Furthermore, integrating co-culture systems or patient-derived xenograft (PDX) models into future studies will help to better replicate the tumour microenvironment, accounting for stromal interactions, cytokine signalling, and immune evasion mechanisms, thereby improving the predictive power of *ex vivo* drug sensitivity screening. Further investigation into the therapeutic potential of irinotecan is warranted. This includes elucidating its mechanism of action at the molecular level,

evaluating its efficacy in combination with other agents, and validating its effects in preclinical leukaemia models. Employing advanced screening methods such as high-throughput combinatorial drug testing and functional genomics could facilitate the identification of predictive biomarkers for precision medicine. Correlating drug response data with specific genetic alterations will enhance the personalisation of leukaemia treatment strategies and guide targeted therapeutic approaches. Longitudinal studies should also be prioritised to capture disease evolution, treatment responses, and changes in immune profiles over time. A multi-timepoint study design would enable the identification of dynamic biomarkers that can predict therapeutic outcomes and disease progression more effectively.

By expanding cohort sizes, integrating multi-omic analyses, and conducting preclinical and clinical trials, future research can build upon the foundation established in this study, advancing the field of leukaemia therapeutics and improving patient outcomes.

REFERENCES

Abbasi, S., Diab, O., Mohyuddin, G. R., & Singh, A. K. (2019). In-Hospital Morbidity and Mortality Among Acute Myeloid Leukemia Patients Admitted for Hematopoeitic Stem Cell Transplants between 2002-2014. *Blood*, *134*(Supplement_1), 2147. https://doi.org/10.1182/blood-2019-131832

Abdulmawjood, B., Costa, B., Roma-Rodrigues, C., Baptista, P. V., & Fernandes, A. R. (2021). Genetic Biomarkers in Chronic Myeloid Leukemia: What Have We Learned So Far? *International Journal of Molecular Sciences*, *22*(22), Article 22. https://doi.org/10.3390/ijms222212516

Abou Dalle, I., Kantarjian, H., Burger, J., Estrov, Z., Ohanian, M., Verstovsek, S., Ravandi, F., Borthakur, G., Garcia-Manero, G., Jabbour, E., & Cortes, J. (2019). Efficacy and safety of generic imatinib after switching from original imatinib in patients treated for chronic myeloid leukemia in the United States. *Cancer Medicine*, *8*(15), 6559–6565. https://doi.org/10.1002/cam4.2545

Adan, A., Alizada, G., Kiraz, Y., Baran, Y., & Nalbant, A. (2017). Flow cytometry: Basic principles and applications. *Critical Reviews in Biotechnology*, *37*(2), 163–176. https://doi.org/10.3109/07388551.2015.1128876

Andresen, V., & Gjertsen, B. T. (2017). Drug Repurposing for the Treatment of Acute Myeloid Leukemia. *Frontiers in Medicine*, *4*, 211. https://doi.org/10.3389/fmed.2017.00211

Asfour, I. A., & Elshazly, S. A. (2009). Changing therapy from Glivec® to a 'copy' imatinib results in a worsening of chronic myeloid leukemia disease status: Two case reports. *Cases Journal*, *2*, 9342. https://doi.org/10.1186/1757-1626-2-9342

Aslostovar, L., Boyd, A. L., Almakadi, M., Collins, T. J., Leong, D. P., Tirona, R. G., Kim, R. B., Julian, J. A., Xenocostas, A., Leber, B., Levine, M. N., Foley, R., & Bhatia, M. (2018). A phase 1 trial evaluating thioridazine in combination with cytarabine in patients with acute myeloid

leukemia. *Blood Advances*, *2*(15), 1935–1945. https://doi.org/10.1182/bloodadvances.2018015677

Ayuda-Durán, P., Hermansen, J. U., Giliberto, M., Yin, Y., Hanes, R., Gordon, S., Kuusanmäki, H., Brodersen, A. M., Andersen, A. N., Taskén, K., Wennerberg, K., Enserink, J. M., & Skånland, S. S. (2023). Standardized assays to monitor drug sensitivity in hematologic cancers. *Cell Death Discovery*, *9*, 435. https://doi.org/10.1038/s41420-023-01722-5

Azizidoost, S., Nasrolahi, A., Sheykhi-Sabzehpoush, M., Anbiyaiee, A., Khoshnam, S. E., Farzaneh, M., & Uddin, S. (2024). Signaling pathways governing the behaviors of leukemia stem cells. *Genes & Diseases*, *11*(2), 830–846. https://doi.org/10.1016/j.gendis.2023.01.008

Azmal, M., Paul, J. K., Prima, F. S., Talukder, O. F., & Ghosh, A. (2024). An in silico molecular docking and simulation study to identify potential anticancer phytochemicals targeting the RAS signaling pathway. *PloS One*, *19*(9), e0310637. https://doi.org/10.1371/journal.pone.0310637

Baig, M. S., Roy, A., Saqib, U., Rajpoot, S., Srivastava, M., Naim, A., Liu, D., Saluja, R., Faisal, S. M., Pan, Q., Turkowski, K., Darwhekar, G. N., & Savai, R. (2018). Repurposing Thioridazine (TDZ) as an anti-inflammatory agent. *Scientific Reports*, *8*, 12471. https://doi.org/10.1038/s41598-018-30763-5

Bazarbachi, A. H., Al Hamed, R., Malard, F., Harousseau, J.-L., & Mohty, M. (2019). Relapsed refractory multiple myeloma: A comprehensive overview. *Leukemia*, *33*(10), 2343–2357. https://doi.org/10.1038/s41375-019-0561-2

Benjamin, C., Murugan, S., Hoosen, S., & Rapiti, N. (2023). Chronic myeloid leukemia kinase domain mutations: A retrospective descriptive study on the therapeutic and prognostic significance in patients at King Edward VIII Hospital, KwaZulu-Natal, South Africa. *Health Science Reports*, *6*(7), e1376. https://doi.org/10.1002/hsr2.1376

Bhagat, R. T., & Butle, S. R. (2021). Drug Repurposing: A Review. *Journal of Pharmaceutical Research International*, 161–169. https://doi.org/10.9734/jpri/2021/v33i31B31704

Bhagwat, S. V., Gokhale, P. C., Crew, A. P., Cooke, A., Yao, Y., Mantis, C., Kahler, J., Workman, J., Bittner, M., Dudkin, L., Epstein, D. M., Gibson, N. W., Wild, R., Arnold, L. D., Houghton, P. J., & Pachter, J. A. (2011). Preclinical Characterization of OSI-027, a Potent and Selective Inhibitor of mTORC1 and mTORC2: Distinct from Rapamycin. *Molecular Cancer Therapeutics*, *10*(8), 1394–1406. https://doi.org/10.1158/1535-7163.MCT-10-1099

Bhatia, S., Sather, H. N., Heerema, N. A., Trigg, M. E., Gaynon, P. S., & Robison, L. L. (2002). Racial and ethnic differences in survival of children with acute lymphoblastic leukemia. *Blood*, *100*(6), 1957–1964. https://doi.org/10.1182/blood-2002-02-0395

Bispo, J. A. B., Pinheiro, P. S., & Kobetz, E. K. (2020a). Epidemiology and Etiology of Leukemia and Lymphoma. *Cold Spring Harbor Perspectives in Medicine*, *10*(6), a034819. https://doi.org/10.1101/cshperspect.a034819

Bispo, J. A. B., Pinheiro, P. S., & Kobetz, E. K. (2020b). Epidemiology and Etiology of Leukemia and Lymphoma. *Cold Spring Harbor Perspectives in Medicine*, *10*(6). https://doi.org/10.1101/cshperspect.a034819

Bitencourt-Ferreira, G., Veit-Acosta, M., & de Azevedo, W. F. (2019). Van der Waals Potential in Protein Complexes. *Methods in Molecular Biology (Clifton, N.J.)*, *2053*, 79–91. https://doi.org/10.1007/978-1-4939-9752-7_6

Boshuizen, J., & Peeper, D. S. (2020). Rational Cancer Treatment Combinations: An Urgent Clinical Need. *Molecular Cell*, *78*(6), 1002–1018. https://doi.org/10.1016/j.molcel.2020.05.031

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: A Cancer Journal for Clinicians*, *68*(6), 394–424. https://doi.org/10.3322/caac.21492

Brown, J. R., Walker, S. R., Heppler, L. N., Tyekucheva, S., Nelson, E. A., Klitgaard, J., Nicolais, M., Kroll, Y., Xiang, M., Yeh, J. E., Chaudhury, M., Giaccone, Z. T., Fernandes, S. M., Jacobsen, E. D., Fisher, D. C., Freedman, A. S., Davids, M. S., Supko, J. G., Wu, C., & Frank, D. A. (2021). Targeting constitutively active STAT3 in chronic lymphocytic leukemia: A clinical trial of the STAT3 inhibitor pyrimethamine with pharmacodynamic analyses. *American Journal of Hematology*, *96*(4), E95–E98. https://doi.org/10.1002/ajh.26084

Bultum, L. E., Tolossa, G. B., & Lee, D. (2022). Combining empirical knowledge, in silico molecular docking and ADMET profiling to identify therapeutic phytochemicals from Brucea antidysentrica for acute myeloid leukemia. *PLOS ONE*, *17*(7), e0270050. https://doi.org/10.1371/journal.pone.0270050

Bulusu, G., & Desiraju, G. (2019). Strong and Weak Hydrogen Bonds in Protein–Ligand Recognition. *Journal of the Indian Institute of Science*, *100*. https://doi.org/10.1007/s41745-019-00141-9

Byrd, J. C., Brown, J. R., O'Brien, S., Barrientos, J. C., Kay, N. E., Reddy, N. M., Coutre, S., Tam, C. S., Mulligan, S. P., Jaeger, U., Devereux, S., Barr, P. M., Furman, R. R., Kipps, T. J., Cymbalista, F., Pocock, C., Thornton, P., Caligaris-Cappio, F., Robak, T., … Hillmen, P. (2014). Ibrutinib versus Ofatumumab in Previously Treated Chronic Lymphoid Leukemia. *The New England Journal of Medicine*, *371*(3), 213–223. https://doi.org/10.1056/NEJMoa1400376

Cai, Y., Prochazkova, M., Kim, Y.-S., Jiang, C., Ma, J., Moses, L., Martin, K., Pham, V., Zhang, N., Highfill, S. L., Somerville, R. P., Stroncek, D. F., & Jin, P. (2024). Assessment and comparison of viability assays for cellular products. *Cytotherapy*, *26*(2), 201–209. https://doi.org/10.1016/j.jcyt.2023.11.008

Calderon, A., Han, C., Karma, S., & Wang, E. (2024). Non-genetic mechanisms of drug resistance in acute leukemias. *Trends in Cancer*, *10*(1), 38–51. https://doi.org/10.1016/j.trecan.2023.09.003

Cao, H., Wu, T., Zhou, X., Xie, S., Sun, H., Sun, Y., & Li, Y. (2023). Progress of research on PD-1/PD-L1 in leukemia. *Frontiers in Immunology*, *14*, 1265299. https://doi.org/10.3389/fimmu.2023.1265299

Cennamo, M., Sirocchi, D., Giudici, C., Giagnacovo, M., Petracco, G., Ferrario, D., Garganigo, S., Papa, A., Veniani, E., Squizzato, A., Del Vecchio, L., Patriarca, C., Partenope, M., & Modena, P. (2023). A Peculiar CLL Case with Complex Chromosome 6 Rearrangements and Refinement of All Breakpoints at the Gene Level by Genomic Array: A Case Report. *Journal of Clinical Medicine*, *12*(12), 4110. https://doi.org/10.3390/jcm12124110

Chai, Y., Liu, J.-L., Zhang, S., Li, N., Xu, D.-Q., Liu, W.-J., Fu, R.-J., & Tang, Y.-P. (2024). The effective combination therapies with irinotecan for colorectal cancer. *Frontiers in Pharmacology*, *15*. https://doi.org/10.3389/fphar.2024.1356708

Chaput, N., Flament, C., Locher, C., Desbois, M., Rey, A., Rusakiewicz, S., Poirier-Colame, V., Pautier, P., Le Cesne, A., Soria, J.-C., Paci, A., Rosenzwajg, M., Klatzmann, D., Eggermont, A., Robert, C., & Zitvogel, L. (2013). Phase I clinical trial combining imatinib mesylate and IL-2: HLA-DR+ NK cell levels correlate with disease outcome. *Oncoimmunology*, *2*(2), e23080. https://doi.org/10.4161/onci.23080

Cheadle, E. J., Sidon, L., Dovedi, S. J., Melis, M. H. M., Alduaij, W., Illidge, T. M., & Honeychurch, J. (2013). The induction of immunogenic cell death by type II anti-CD20 monoclonal antibodies has mechanistic differences compared with type I rituximab. *British Journal of Haematology*, *162*(6), 842–845. https://doi.org/10.1111/bjh.12427

Checkoway, H., Boffetta, P., Mundt, D. J., & Mundt, K. A. (2012). Critical review and synthesis of the epidemiologic evidence on formaldehyde exposure and risk of leukemia and other lymphohematopoietic malignancies. *Cancer Causes & Control*, *23*(11), 1747–1766. https://doi.org/10.1007/s10552-012-0055-2

Chen, Z., Sun, Y., Xie, W., Wang, S. A., Hu, S., Li, S., Tang, Z., Toruner, G., Medeiros, L. J., & Tang, G. (2019). Is hyperdiploidy a favorable cytogenetics in adults with B-lymphoblastic leukemia? *Cancer Medicine*, *8*(9), 4093–4099. https://doi.org/10.1002/cam4.2255

Chennamadhavuni, A., Lyengar, V., Mukkamalla, S. K. R., & Shimanovsky, A. (2023). Leukemia. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK560490/

Cossarizza, A., Chang, H.-D., Radbruch, A., Acs, A., Adam, D., Adam-Klages, S., Agace, W. W., Aghaeepour, N., Akdis, M., Allez, M., Almeida, L. N., Alvisi, G., Anderson, G., Andrä, I., Annunziato, F., Anselmo, A., Bacher, P., Baldari, C. T., Bari, S., … Zychlinsky, A. (2019). Guidelines for the use of flow cytometry and cell sorting in immunological studies (second edition). *European Journal of Immunology*, *49*(10), 1457–1973. https://doi.org/10.1002/eji.201970107

Cross, N. C. P. (2020). Update on CML-Like Disorders. *Clinical Lymphoma, Myeloma & Leukemia*, *20 Suppl 1*, S101–S102. https://doi.org/10.1016/S2152-2650(20)30477-8

Das, A., Swamy Purawarga Matada, G., Sanjay Dhiwar, P., Manjunathaiah Raghavendra, N., Abbas, N., Singh, E., Ghara, A., & Prasad Shenoy, G. (2023). Molecular recognition of some novel mTOR kinase inhibitors to develop anticancer leads by drug-likeness, molecular docking and molecular dynamics based virtual screening strategy. *Computational Toxicology*, *25*, 100257. https://doi.org/10.1016/j.comtox.2022.100257

Deininger, M. W. (2015). Diagnosing and Managing Advanced Chronic Myeloid Leukemia. *American Society of Clinical Oncology Educational Book*, *35*, e381–e388. https://doi.org/10.14694/EdBook_AM.2015.35.e381

Desai, R. H., Zandvakili, N., & Bohlander, S. K. (2022). Dissecting the Genetic and Non-Genetic Heterogeneity of Acute Myeloid Leukemia Using Next-Generation Sequencing and In Vivo Models. *Cancers*, *14*(9), 2182. https://doi.org/10.3390/cancers14092182

DiMasi, J. A., Grabowski, H. G., & Hansen, R. W. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. *Journal of Health Economics*, *47*, 20–33. https://doi.org/10.1016/j.jhealeco.2016.01.012

Dugger, S. A., Platt, A., & Goldstein, D. B. (2018). Drug development in the era of precision medicine. *Nature Reviews. Drug Discovery*, *17*(3), 183–196. https://doi.org/10.1038/nrd.2017.226

Elzain, E. A., Khalil, H. B., Elzain, E. A., & Khalil, H. B. (2021). Frequency and prognostic value of NPM1 mutations in Sudanese acute myeloid leukemia patients. *GSC Biological and Pharmaceutical Sciences*, *14*(2), Article 2. https://doi.org/10.30574/gscbps.2021.14.2.0055

Erçalışkan, A., Seyhan Erdoğan, D., & Eşkazan, A. E. (2021). Current evidence on the efficacy and safety of generic imatinib in CML and the impact of generics on health care costs. *Blood Advances*, *5*(17), 3344–3353. https://doi.org/10.1182/bloodadvances.2021004194

Eriksson, A., Österroos, A., Hassan, S., Gullbo, J., Rickardson, L., Jarvius, M., Nygren, P., Fryknäs, M., Höglund, M., & Larsson, R. (2015). Drug screen in patient cells suggests quinacrine to be repositioned for treatment of acute myeloid leukemia. *Blood Cancer Journal*, *5*, e307. https://doi.org/10.1038/bcj.2015.31

Faderl, S., O'Brien, S., Pui, C.-H., Stock, W., Wetzler, M., Hoelzer, D., & Kantarjian, H. M. (2010). Adult Acute Lymphoblastic Leukemia. *Cancer*, *116*(5), 1165–1176. https://doi.org/10.1002/cncr.24862

Fathima, J. S., Selvaraj, J., Sivabalan, V., Rekha, U. V., Ponnulakshmi, R., Vishnupriya, V., Kullappan, M., Sreekandan, R. N., Mohan, S. K., & Vijayalakshmi, P. (2021). Molecular docking of potential inhibitors with the mTOR protein. *Bioinformation*, *17*(1), 212–217. https://doi.org/10.6026/97320630017212

Flowers, C. R., & Pro, B. (2013). Racial differences in chronic lymphocytic leukemia. Digging deeper. *Cancer*, *119*(20), 3593–3595. https://doi.org/10.1002/cncr.28233

Fürstenau, M., Hallek, M., & Eichhorst, B. (2019). Sequential and combination treatments with novel agents in chronic lymphocytic leukemia. *Haematologica*, *104*(11), 2144–2154. https://doi.org/10.3324/haematol.2018.208603

Ghanem, S., Friedbichler, K., Boudot, C., Bourgeais, J., Gouilleux-Gruart, V., Régnier, A., Herault, O., Moriggl, R., & Gouilleux, F. (2017). STAT5A/5B-specific expansion and transformation of hematopoietic stem cells. *Blood Cancer Journal*, *7*(1), e514. https://doi.org/10.1038/bcj.2016.124

Giliberto, M., Thimiri Govinda Raj, D. B., Cremaschi, A., Skånland, S. S., Gade, A., Tjønnfjord, G. E., Schjesvold, F., Munthe, L. A., & Taskén, K. (2022). Ex vivo drug sensitivity screening in multiple myeloma identifies drug combinations that act synergistically. *Molecular Oncology*, *16*(6), 1241–1258. https://doi.org/10.1002/1878-0261.13191

Glaviano, A., Foo, A. S. C., Lam, H. Y., Yap, K. C. H., Jacot, W., Jones, R. H., Eng, H., Nair, M. G., Makvandi, P., Geoerger, B., Kulke, M. H., Baird, R. D., Prabhu, J. S., Carbone, D., Pecoraro, C., Teh, D. B. L., Sethi, G., Cavalieri, V., Lin, K. H., … Kumar, A. P. (2023). PI3K/AKT/mTOR signaling transduction pathway and targeted therapies in cancer. *Molecular Cancer*, *22*(1), 138. https://doi.org/10.1186/s12943-023-01827-6

Gorgulla, C., Boeszoermenyi, A., Wang, Z.-F., Fischer, P. D., Coote, P. W., Padmanabha Das, K. M., Malets, Y. S., Radchenko, D. S., Moroz, Y. S., Scott, D. A., Fackeldey, K., Hoffmann, M., Iavniuk, I., Wagner, G., & Arthanari, H. (2020). An open-source drug discovery platform enables ultra-large virtual screens. *Nature*, *580*(7805), Article 7805. https://doi.org/10.1038/s41586-020-2117-z

Goy, J., Gillan, T. L., Bruyere, H., Huang, S. J. T., Hrynchak, M., Karsan, A., Ramadan, K., Connors, J., Toze, C. L., & Gerrie, A. S. (2017). Chronic Lymphocytic Leukemia Patients With Deletion 11q Have a Short Time to Requirement of First-Line Therapy, But Long Overall Survival: Results of

a Population-Based Cohort in British Columbia, Canada. *Clinical Lymphoma, Myeloma & Leukemia*, *17*(6), 382–389. https://doi.org/10.1016/j.clml.2017.04.001

Hallal, R., Nehme, R., Brachet-Botineau, M., Nehme, A., Dakik, H., Deynoux, M., Dello Sbarba, P., Levern, Y., Zibara, K., Gouilleux, F., & Mazurier, F. (2020). Acriflavine targets oncogenic STAT5 signaling in myeloid leukemia cells. *Journal of Cellular and Molecular Medicine*, *24*(17), 10052–10062. https://doi.org/10.1111/jcmm.15612

Hallek, M., Shanafelt, T. D., & Eichhorst, B. (2018). Chronic lymphocytic leukaemia. *Lancet (London, England)*, *391*(10129), 1524–1537. https://doi.org/10.1016/S0140-6736(18)30422-7

Hamdoun, S., Jung, P., & Efferth, T. (2017). Drug Repurposing of the Anthelmintic Niclosamide to Treat Multidrug-Resistant Leukemia. *Frontiers in Pharmacology*, *8*. https://doi.org/10.3389/fphar.2017.00110

Hanif, N., & Anwer, F. (2024). Rituximab. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK564374/

Hartinger, J. M., Kratky, V., Hruskova, Z., Slanar, O., & Tesar, V. (2022). Implications of rituximab pharmacokinetic and pharmacodynamic alterations in various immune-mediated glomerulopathies and potential anti-CD20 therapy alternatives. *Frontiers in Immunology*, *13*, 1024068. https://doi.org/10.3389/fimmu.2022.1024068

Haslauer, T., Greil, R., Zaborsky, N., & Geisberger, R. (2021). CAR T-Cell Therapy in Hematological Malignancies. *International Journal of Molecular Sciences*, *22*(16), 8996. https://doi.org/10.3390/ijms22168996

Hauer, J., Borkhardt, A., Sánchez-García, I., & Cobaleda, C. (2014). Genetically engineered mouse models of human B-cell precursor leukemias. *Cell Cycle*, *13*(18), 2836–2846. https://doi.org/10.4161/15384101.2014.949137

Hermsen, J., & Hambley, B. (2023). The Coagulopathy of Acute Promyelocytic Leukemia: An Updated Review of Pathophysiology, Risk Stratification, and Clinical Management. *Cancers*, *15*(13), 3477. https://doi.org/10.3390/cancers15133477

Hsu, W.-L., Preston, D. L., Soda, M., Sugiyama, H., Funamoto, S., Kodama, K., Kimura, A., Kamada, N., Dohy, H., Tomonaga, M., Iwanaga, M., Miyazaki, Y., Cullings, H. M., Suyama, A., Ozasa, K., Shore, R. E., & Mabuchi, K. (2013). The incidence of leukemia, lymphoma, and multiple myeloma among atomic bomb survivors: 1950 – 2001. *Radiation Research*, *179*(3), 10.1667/RR2892.1. https://doi.org/10.1667/RR2892.1

Hughes, J. P., Rees, S., Kalindjian, S. B., & Philpott, K. L. (2011). Principles of early drug discovery. *British Journal of Pharmacology*, *162*(6), 1239–1249. https://doi.org/10.1111/j.1476-5381.2010.01127.x

Ianevski, A., He, L., Aittokallio, T., & Tang, J. (2017). SynergyFinder: A web application for analyzing drug combination dose–response matrix data. *Bioinformatics*, *33*(15), 2413–2415. https://doi.org/10.1093/bioinformatics/btx162

Jaafari, N., Kojabad, A. A., Shabestari, R. M., & Safa, M. (2025). Design and fabrication of novel microfluidic-based droplets for drug screening on a chronic myeloid leukemia cell line. *PLOS ONE*, *20*(1), e0315803. https://doi.org/10.1371/journal.pone.0315803

Jabbour, E., & Kantarjian, H. (2018). Chronic myeloid leukemia: 2018 update on diagnosis, therapy and monitoring. *American Journal of Hematology*, *93*(3), 442–459. https://doi.org/10.1002/ajh.25011

Jang, H.-J., Woo, Y.-M., Naka, K., Park, J.-H., Han, H.-J., Kim, H.-J., Kim, S.-H., Ahn, J.-S., Kim, T., Kimura, S., Zarabi, S., Lipton, J. H., Minden, M. D., Jung, C.-W., Kim, H.-J., Kim, J.-W., & Kim, D. D. H. (2021). Statins Enhance the Molecular Response in Chronic Myeloid Leukemia when Combined with Tyrosine Kinase Inhibitors. *Cancers*, *13*(21), 5543. https://doi.org/10.3390/cancers13215543

Jiang, Q., Lin, F., Li, Z., Duan, H., Jiang, C., Yu, X., Wang, C., Zhang, L., Sun, X., Zha, J., Liu, L., Lin, Z., & Xu, B. (2024). Changes of T cell subsets across treatments associated with prognosis in newly diagnosed follicular lymphoma. *Scientific Reports*, *14*, 27576. https://doi.org/10.1038/s41598-024-79173-w

Jourdan, J.-P., Bureau, R., Rochais, C., & Dallemagne, P. (2020). Drug repositioning: A brief overview. *Journal of Pharmacy and Pharmacology*, *72*(9), 1145–1151. https://doi.org/10.1111/jphp.13273

Juliusson, G., & Hough, R. (2016). Leukemia. *Progress in Tumor Research*, *43*, 87–100. https://doi.org/10.1159/000447076

Justiz Vaillant, A. A., & Zito, P. M. (2023). Neutropenia. In *StatPearls*. StatPearls Publishing. http://www.ncbi.nlm.nih.gov/books/NBK507702/

Kalina, T., Fišer, K., Pérez-Andrés, M., Kuzílková, D., Cuenca, M., Bartol, S. J. W., Blanco, E., Engel, P., & Zelm, M. C. van. (2019). CD Maps—Dynamic Profiling of CD1–CD100 Surface Expression on Human Leukocyte and Lymphocyte Subsets. *Frontiers in Immunology*, *10*, 2434. https://doi.org/10.3389/fimmu.2019.02434

Kang, D. S., Kim, H. S., Jung, J.-H., Lee, C. M., Ahn, Y.-S., & Seo, Y. R. (2021). Formaldehyde exposure and leukemia risk: A comprehensive review and network-based toxicogenomic approach. *Genes and Environment*, *43*, 13. https://doi.org/10.1186/s41021-021-00183-5

Kantarjian, H. M., Hughes, T. P., Larson, R. A., Kim, D.-W., Issaragrisil, S., le Coutre, P., Etienne, G., Boquimpani, C., Pasquini, R., Clark, R. E., Dubruille, V., Flinn, I. W., Kyrcz-Krzemien, S., Medras, E., Zanichelli, M., Bendit, I., Cacciatore, S., Titorenko, K., Aimone, P., … Hochhaus, A. (2021). Long-term outcomes with frontline nilotinib versus imatinib in newly diagnosed chronic myeloid leukemia in chronic phase: ENESTnd 10-year analysis. *Leukemia*, *35*(2), 440–453. https://doi.org/10.1038/s41375-020-01111-2

Kantarjian, H. M., Shah, N. P., Cortes, J. E., Baccarani, M., Agarwal, M. B., Undurraga, M. S., Wang, J., Kassack Ipiña, J. J., Kim, D.-W., Ogura, M., Pavlovsky, C., Junghanss, C., Milone, J. H., Nicolini, F. E., Robak, T., Van Droogenbroeck, J., Vellenga, E., Bradley-Garelik, M. B., Zhu, C., & Hochhaus, A. (2012). Dasatinib or imatinib in newly diagnosed chronic-phase chronic myeloid leukemia: 2-year follow-up from a randomized phase 3 trial (DASISION). *Blood*, *119*(5), 1123–1129. https://doi.org/10.1182/blood-2011-08-376087

Kayser, S., & Levis, M. J. (2023). The clinical impact of the molecular landscape of acute myeloid leukemia. *Haematologica*, *108*(2), Article 2. https://doi.org/10.3324/haematol.2022.280801

Kenmogne, V. L., Nweke, E. E., Takundwa, M. M., Fru, P. N., & Thimiri Govinda Raj, D. B. (2023). Application of Drug Repurposing-Based Precision Medicine Platform for Leukaemia Patient Treatment. *Advances in Experimental Medicine and Biology*, *1410*, 115–126. https://doi.org/10.1007/5584_2022_744

Kerstjens, M., Garrido Castro, P., Pinhanços, S. S., Schneider, P., Wander, P., Pieters, R., & Stam, R. W. (2021). Irinotecan Induces Disease Remission in Xenograft Mouse Models of Pediatric MLL-Rearranged Acute Lymphoblastic Leukemia. *Biomedicines*, *9*(7), 711. https://doi.org/10.3390/biomedicines9070711

Kichenadasse, G., Mangoni, A., & Miners, J. (2017). Combination of small-molecule kinase inhibitors and irinotecan in cancer clinical trials: Efficacy and safety considerations. *Translational Cancer Research*, *6*(Suppl 10). https://doi.org/10.21037/tcr.2017.10.07

Kim, J. S., Kim, T. M., Kang, M. J., Koh, S. A., Park, H., Nam, S.-H., Han, J. J., Lee, G.-W., Yuh, Y. J., Lee, H. J., & Choi, J. H. (2023). Treatment pattern of chronic lymphocytic leukemia/small lymphocytic lymphoma in Korea: A multicenter retrospective study (KCSG LY20-06). *The Korean Journal of Internal Medicine*. https://doi.org/10.3904/kjim.2022.408

Kipps, T. J., Stevenson, F. K., Wu, C. J., Croce, C. M., Packham, G., Wierda, W. G., O'Brien, S., Gribben, J., & Rai, K. (2017). Chronic lymphocytic leukaemia. *Nature Reviews Disease Primers*, *3*(1), Article 1. https://doi.org/10.1038/nrdp.2016.96

Kloppers, J. F., de Kock, A., Cronjé, J., & van Marle, A.-C. (2021). Molecular characterisation of NPM1 and FLT3-ITD mutations in a central South African adult de novo acute myeloid leukaemia cohort. *African Journal of Laboratory Medicine*, *10*(1), 1363. https://doi.org/10.4102/ajlm.v10i1.1363

Kumar, N., Afjei, R., Massoud, T. F., & Paulmurugan, R. (2018). Comparison of cell-based assays to quantify treatment effects of anticancer drugs identifies a new application for Bodipy-L-cystine to measure apoptosis. *Scientific Reports*, *8*(1), 16363. https://doi.org/10.1038/s41598-018-34696-x

Kushwaha, V. S., Gupta, S., Husain, N., Khan, H., Negi, M., Jamal, N., & Ghatak, A. (2015). Gefitinib, Methotrexate and Methotrexate plus 5-Fluorouracil as palliative treatment in recurrent head and neck squamous cell carcinoma. *Cancer Biology & Therapy*, *16*(2), 346–351. https://doi.org/10.4161/15384047.2014.961881

Laba, S., Mallett, G., & Amarnath, S. (2022). The depths of PD-1 function within the tumor microenvironment beyond CD8+ T cells. *Seminars in Cancer Biology*, *86*, 1045–1055. https://doi.org/10.1016/j.semcancer.2021.05.022

Landskron, J., & Taskén, K. (2016). Phosphoprotein Detection by High-Throughput Flow Cytometry. In L. von Stechow (Ed.), *Phospho-Proteomics* (Vol. 1355, pp. 275–290). Springer New York. https://doi.org/10.1007/978-1-4939-3049-4_19

Lara-Castillo, M. C., Cornet-Masana, J. M., Etxabe, A., Banús-Mulet, A., Torrente, M. Á., Nomdedeu, M., Díaz-Beyá, M., Esteve, J., & Risueño, R. M. (2016). Repositioning of bromocriptine for treatment of acute myeloid leukemia. *Journal of Translational Medicine*, *14*(1), 261. https://doi.org/10.1186/s12967-016-1007-5

Laukkanen, S., Grönroos, T., Pölönen, P., Kuusanmäki, H., Mehtonen, J., Cloos, J., Ossenkoppele, G., Gjertsen, B., Øystein, B., Heckman, C., Heinäniemi, M., Kontro, M., & Lohi, O. (2017). In silico and preclinical drug screening identifies dasatinib as a targeted therapy for T-ALL. *Blood Cancer Journal*, *7*(9), e604. https://doi.org/10.1038/bcj.2017.87

Lee, J. W., Kim, S., Jang, P.-S., Chung, N.-G., & Cho, B. (2021). Differing Outcomes of Patients with High Hyperdiploidy and ETV6-RUNX1 Rearrangement in Korean Pediatric Precursor B Cell Acute Lymphoblastic Leukemia. *Cancer Research and Treatment : Official Journal of Korean Cancer Association*, *53*(2), 567–575. https://doi.org/10.4143/crt.2020.507

Li, Y., & Jones, S. J. (2012). Drug repositioning for personalized medicine. *Genome Medicine*, *4*(3), 27. https://doi.org/10.1186/gm326

Li, Z., Richards, S., Surks, H. K., Jacobs, A., & Panzara, M. A. (2018). Clinical pharmacology of alemtuzumab, an anti-CD52 immunomodulator, in multiple sclerosis. *Clinical and Experimental Immunology*, *194*(3), 295–314. https://doi.org/10.1111/cei.13208

Liu, Y., Song, W., Song, Y., Zhu, J., & Xie, Y. (2025). Low levels of CD3 + and CD8 + T cells in peripheral blood can predict poor efficacy of first-line chemotherapy in patients with angioimmunoblastic T cell lymphoma. *Annals of Hematology*, *104*(3), 1705–1712. https://doi.org/10.1007/s00277-025-06314-0

Liu, Y., Sun, L., Zhang, H., Shang, L., & Zhao, Y. (2021). Microfluidics for Drug Development: From Synthesis to Evaluation. *Chemical Reviews*, *121*(13), 7468–7529. https://doi.org/10.1021/acs.chemrev.0c01289

Lortet-Tieulent, J., Georges, D., Bray, F., & Vaccarella, S. (2020). Profiling global cancer incidence and mortality by socioeconomic development. *International Journal of Cancer*, *147*(11), 3029–3036. https://doi.org/10.1002/ijc.33114

Mag, P., Nemes-Terényi, M., Jerzsele, Á., & Mátyus, P. (2024). Some Aspects and Convergence of Human and Veterinary Drug Repositioning. *Molecules*, *29*(18), 4475. https://doi.org/10.3390/molecules29184475

Maimela, M. G. (2024). *IL-6 and associated immune markers in acute pancreatitis: Potential for monitoring disease severity*. https://hdl.handle.net/10539/37689

Mariotto, A. B., Enewold, L., Zhao, J., Zeruto, C. A., & Yabroff, K. R. (2020). Medical Care Costs Associated with Cancer Survivorship in the United States. *Cancer Epidemiology, Biomarkers & Prevention: A Publication of the American Association for Cancer Research, Cosponsored by the American Society of Preventive Oncology*, *29*(7), 1304–1312. https://doi.org/10.1158/1055-9965.EPI-19-1534

Marshall, M. J. E., Stopforth, R. J., & Cragg, M. S. (2017). Therapeutic Antibodies: What Have We Learnt from Targeting CD20 and Where Are We Going? *Frontiers in Immunology*, *8*, 1245. https://doi.org/10.3389/fimmu.2017.01245

Marshall, R. C., Tlagadi, A., Bronze, M., Kana, V., Naidoo, S., Wiggill, T. M., & Carmona, S. C. (2014). Lower frequency of NPM1 and FLT3-ITD mutations in a South African adult de novo AML cohort. *International Journal of Laboratory Hematology*, *36*(6), 656. https://doi.org/10.1111/ijlh.12204

Matchett, K. B., Grishagin, I. V., Kettyle, L., Dowling, C., Ni Chonghaile, T., Mills, K. I., & Thompson, A. (2017). High-Throughput Screen Identification of Albendazole As a Novel Repurposed Drug in Acute Myeloid Leukaemia. *Blood*, *130*, 5062. https://doi.org/10.1182/blood.V130.Suppl_1.5062.5062

Mattar, M. (2010). Failure of copy Imatib (CIPLA, India) to maintain hematologic and cytogenetic responses in chronic myeloid leukemia in chronic phase. *International Journal of Hematology*, *91*(1), 104–106. https://doi.org/10.1007/s12185-009-0431-1

McKinnon, K. M. (2018). Flow Cytometry: An Overview. *Current Protocols in Immunology*, *120*, 5.1.1-5.1.11. https://doi.org/10.1002/cpim.40

Mehdizadeh, M., Bolourian, V., Zamani, G., Tavakoli-Ardakanii, M., Zamani, S., Tabarraee, M., & Hajifathali, A. (2022). Survival of Patients with Acute Myeloid Leukemia after Allogeneic Stem Cell Transplantation: An Experience in Developing Country. *International Journal of Hematology-Oncology and Stem Cell Research*, *16*(1), 55–65. https://doi.org/10.18502/ijhoscr.v16i1.8443

Mezzatesta, C., Abduli, L., Guinot, A., Eckert, C., Schewe, D., Zaliova, M., Vinti, L., Marovca, B., Tsai, Y.-C., Jenni, S., Aguade-Gorgorio, J., von Stackelberg, A., Schrappe, M., Locatelli, F., Stanulla, M., Cario, G., Bourquin, J.-P., & Bornhauser, B. C. (2020). Repurposing anthelmintic agents to eradicate resistant leukemia. *Blood Cancer Journal*, *10*(6), Article 6. https://doi.org/10.1038/s41408-020-0339-9

Mirabilii, S., Ricciardi, M. R., Piedimonte, M., Gianfelici, V., Bianchi, M. P., & Tafuri, A. (2018). Biological Aspects of mTOR in Leukemia. *International Journal of Molecular Sciences*, *19*(8), 2396. https://doi.org/10.3390/ijms19082396

Montserrat, E., & Dreger, P. (2016). Treatment of Chronic Lymphocytic Leukemia With del(17p)/TP53 Mutation: Allogeneic Hematopoietic Stem Cell Transplantation or BCR-Signaling Inhibitors? *Clinical Lymphoma, Myeloma & Leukemia*, *16 Suppl*, S74-81. https://doi.org/10.1016/j.clml.2016.02.013

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated Docking with Selective Receptor Flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791. https://doi.org/10.1002/jcc.21256

Musaigwa, F., Grewal, R., Abayomi, A., & Swanepoel, C. C. (2021). Chronic lymphocytic leukaemia trends and features at a tertiary hospital in South Africa (2011–2016). *South African Journal of Oncology*, *5*(0), Article 0.

Nalisa, M. (2021). *Early immune responses in acute pancreatitis and their role in predicting disease severity*. https://hdl.handle.net/10539/33585

Natarajan, A., Thangarajan, R., & Kesavan, S. (2019). Repurposing Drugs by In Silico Methods to Target BCR Kinase Domain in Chronic Myeloid Leukemia. *Asian Pacific Journal of Cancer Prevention : APJCP*, *20*(11), 3399–3406. https://doi.org/10.31557/APJCP.2019.20.11.3399

Nehdi, A., Samman, N., Mashhour, A., Alhallaj, A., Trivilegio, T., Gul, S., Reinshagen, J., Alaskar, A., Gmati, G., Abuelgasim, K. A., Mansour, F., & Boudjelal, M. (2021). A Drug Repositioning Approach Identifies a Combination of Compounds as a Potential Regimen for Chronic Lymphocytic Leukemia Treatment. *Frontiers in Oncology*, *11*, 579488. https://doi.org/10.3389/fonc.2021.579488

Nehme, R., Hallal, R., El Dor, M., Kobeissy, F., Gouilleux, F., Mazurier, F., & Zibara, K. (2021). Repurposing of Acriflavine to Target Chronic Myeloid Leukemia Treatment. *Current Medicinal Chemistry*, *28*(11), 2218–2233. https://doi.org/10.2174/0929867327666200908114411

Ni, H., Sun, X., Xu, Y., Lyle, D., Petersen, P., Zhao, X., Drum, H., You, B., Liu, D., Liu, C., & Jiang, J.-G. (2019). Clinical implications of clonal chromosomal abnormalities in Philadelphia negative cells in CML patients after treated with tyrosine kinase inhibitors. *Cancer Genetics*, *238*, 44–49. https://doi.org/10.1016/j.cancergen.2019.07.008

Nipp, R. D., Powell, E., Chabner, B., & Moy, B. (2015). Recognizing the Financial Burden of Cancer Patients in Clinical Trials. *The Oncologist*, *20*(6), 572–575. https://doi.org/10.1634/theoncologist.2015-0068

Nosengo, N. (2016). New tricks for old drugs. *Nature*, *534*(7607), 314–316.

Nweke, E. E., & Thimiri Govinda Raj, D. B. (2021). Drug Sensitivity and Drug Repurposing Platform for Cancer Precision Medicine. *Advances in Experimental Medicine and Biology*, *1326*, 47–53. https://doi.org/10.1007/5584_2021_622

Ogiya, D., Liu, J., Ohguchi, H., Kurata, K., Samur, M. K., Tai, Y.-T., Adamia, S., Ando, K., Hideshima, T., & Anderson, K. C. (2020). The JAK-STAT pathway regulates CD38 on myeloma cells in the bone marrow microenvironment: Therapeutic implications. *Blood*, *136*(20), 2334–2345. https://doi.org/10.1182/blood.2019004332

Okello, C., Niyonzima, N., & Marta Ferraresso, Sylivestor Kadhumbula, Henry Ddungu, Katherine Tarlock, Joyce Balagadde-Kambugu. (2021). *Haematological malignancies in sub-Saharan Africa: East Africa as an example for improving care*. *8: e756–69*.

Ou, Y., Long, Y., Ji, L., Zhan, Y., Qiao, T., Wang, X., Chen, H., & Cheng, Y. (2022). Trends in Disease Burden of Chronic Lymphocytic Leukemia at the Global, Regional, and National Levels From 1990 to 2019, and Projections Until 2030: A Population-Based Epidemiologic Study. *Frontiers in Oncology*, *12*. https://www.frontiersin.org/articles/10.3389/fonc.2022.840616

Parcha, P. K., Sarvagalla, S., Ashok, C., Sudharshan, S. J., Dyavaiah, M., Coumar, M. S., & Rajasekaran, B. (2021). Repositioning antispasmodic drug Papaverine for the treatment of chronic myeloid leukemia. *Pharmacological Reports*, *73*(2), 615–628. https://doi.org/10.1007/s43440-020-00196-x

Potdar, S., Ianevski, A., Mpindi, J.-P., Bychkov, D., Fiere, C., Ianevski, P., Yadav, B., Wennerberg, K., Aittokallio, T., Kallioniemi, O., Saarela, J., & Östling, P. (2020). Breeze: An integrated quality control and data analysis application for high-throughput drug screening. *Bioinformatics*, *36*(11), 3602–3604. https://doi.org/10.1093/bioinformatics/btaa138

Pushpakom, S., Iorio, F., Eyers, P. A., Escott, K. J., Hopper, S., Wells, A., Doig, A., Guilliams, T., Latimer, J., McNamee, C., Norris, A., Sanseau, P., Cavalla, D., & Pirmohamed, M. (2019). Drug

repurposing: Progress, challenges and recommendations. *Nature Reviews. Drug Discovery*, *18*(1), 41–58. https://doi.org/10.1038/nrd.2018.168

Rafique, R., Islam, S. M. R., & Kazi, J. U. (2021). Machine learning in the prediction of cancer therapy. *Computational and Structural Biotechnology Journal*, *19*, 4003–4017. https://doi.org/10.1016/j.csbj.2021.07.003

Raimondi, V., Ciotti, G., Gottardi, M., & Ciccarese, F. (2022). 2-Hydroxyglutarate in Acute Myeloid Leukemia: A Journey from Pathogenesis to Therapies. *Biomedicines*, *10*(6), 1359. https://doi.org/10.3390/biomedicines10061359

Raskov, H., Orhan, A., Christensen, J. P., & Gögenur, I. (2021). Cytotoxic CD8+ T cells in cancer and cancer immunotherapy. *British Journal of Cancer*, *124*(2), 359–367. https://doi.org/10.1038/s41416-020-01048-4

Reikvam, H., Hatfield, K. J., Kittang, A. O., Hovland, R., & Bruserud, Ø. (2011). Acute Myeloid Leukemia with the t(8;21) Translocation: Clinical Consequences and Biological Implications. *Journal of Biomedicine and Biotechnology*, *2011*, 104631. https://doi.org/10.1155/2011/104631

Richardson, D., Sugiyama, H., Nishi, N., Sakata, R., Shimizu, Y., Grant, E. J., Soda, M., Hsu, W.-L., Suyama, A., Kodama, K., & Kasagi, F. (2009). Ionizing radiation and leukemia mortality among Japanese Atomic Bomb Survivors, 1950-2000. *Radiation Research*, *172*(3), 368–382. https://doi.org/10.1667/RR1801.1

Rieger, A. M., Hall, B. E., Luong, L. T., Schang, L. M., & Barreda, D. R. (2010). Conventional apoptosis assays using propidium iodide generate a significant number of false positives that prevent accurate assessment of cell death. *Journal of Immunological Methods*, *358*(1–2), 81–92. https://doi.org/10.1016/j.jim.2010.03.019

Rieger, A. M., Nelson, K. L., Konowalchuk, J. D., & Barreda, D. R. (2011). Modified Annexin V/Propidium Iodide Apoptosis Assay For Accurate Assessment of Cell Death. *Journal of Visualized Experiments : JoVE*, *50*, 2597. https://doi.org/10.3791/2597

Rizo-Liendo, A., Sifaoui, I., Arberas-Jiménez, I., Reyes-Batlle, M., Piñero, J. E., & Lorenzo-Morales, J. (2020). Fluvastatin and atorvastatin induce programmed cell death in the brain eating amoeba *Naegleria fowleri*. *Biomedicine & Pharmacotherapy*, *130*, 110583. https://doi.org/10.1016/j.biopha.2020.110583

Robak, P., & Robak, T. (2019). Bortezomib for the Treatment of Hematologic Malignancies: 15 Years Later. *Drugs in R&D*, *19*(2), 73–92. https://doi.org/10.1007/s40268-019-0269-9

Roboz, G. J. (2012). Current treatment of acute myeloid leukemia. *Current Opinion in Oncology*, *24*(6), 711–719. https://doi.org/10.1097/CCO.0b013e328358f62d

Roerden, M., Märklin, M., Salih, H. R., Bethge, W. A., Klein, R., Rammensee, H.-G., Nelde, A., & Walz, J. S. (2021). Expression levels of HLA-DR in acute myeloid leukemia: Implications for antigenicity and clinical outcome. *Leukemia & Lymphoma*, *62*(8), 1907–1919. https://doi.org/10.1080/10428194.2021.1885659

Rognan, D. (2017). The impact of in silico screening in the discovery of novel and safer drug candidates. *Pharmacology & Therapeutics*, *175*, 47–66. https://doi.org/10.1016/j.pharmthera.2017.02.034

Roloff, G. W., & Griffiths, E. A. (2018). When to obtain genomic data in acute myeloid leukemia (AML) and which mutations matter. *Blood Advances*, *2*(21), 3070–3080. https://doi.org/10.1182/bloodadvances.2018020206

Rossino, G., Rui, M., Pozzetti, L., Schepmann, D., Wünsch, B., Zampieri, D., Pellavio, G., Laforenza, U., Rinaldi, S., Colombo, G., Morelli, L., Linciano, P., Rossi, D., & Collina, S. (2020). Setup and Validation of a Reliable Docking Protocol for the Development of Neuroprotective Agents by

Targeting the Sigma-1 Receptor (S1R). *International Journal of Molecular Sciences*, *21*(20), Article 20. https://doi.org/10.3390/ijms21207708

Rothwell, P. M., Fowkes, F. G. R., Belch, J. F., Ogawa, H., Warlow, C. P., & Meade, T. W. (2011). Effect of daily aspirin on long-term risk of death due to cancer: Analysis of individual patient data from randomised trials. *The Lancet*, *377*(9759), 31–41. https://doi.org/10.1016/S0140-6736(10)62110-1

Sampaio, M. M., Santos, M. L. C., Marques, H. S., Gonçalves, V. L. de S., Araújo, G. R. L., Lopes, L. W., Apolonio, J. S., Silva, C. S., Santos, L. K. de S., Cuzzuol, B. R., Guimarães, Q. E. S., Santos, M. N., de Brito, B. B., da Silva, F. A. F., Oliveira, M. V., Souza, C. L., & de Melo, F. F. (2021). Chronic myeloid leukemia-from the Philadelphia chromosome to specific target drugs: A literature review. *World Journal of Clinical Oncology*, *12*(2), 69–94. https://doi.org/10.5306/wjco.v12.i2.69

Santiago, R. y C., Sesé, M., Capdevila, C., Aasen, T., De Mattos-Arruda, L., Diaz-Cano, S. J., Hernández-Losa, J., & Castellví, J. (2020). Clinical implications of intratumor heterogeneity: Challenges and opportunities. *Journal of Molecular Medicine (Berlin, Germany)*, *98*(2), 161–177. https://doi.org/10.1007/s00109-020-01874-2

Sasaki, K., Ravandi, F., Kadia, T. M., DiNardo, C. D., Short, N. J., Borthakur, G., Jabbour, E., & Kantarjian, H. M. (2021). De novo acute myeloid leukemia: A population-based study of outcome in the United States based on the Surveillance, Epidemiology, and End Results (SEER) database, 1980 to 2017. *Cancer*, *127*(12), 2049–2061. https://doi.org/10.1002/cncr.33458

Schmit, T., Klomp, M., & Khan, M. N. (2021). The Application of Flow Cytometry for Simultaneous and Multi-parametric Analysis of Heterogenous Cell Populations in Basic and Clinical Research. *Methods in Molecular Biology (Clifton, N.J.)*, *2223*, 183–200. https://doi.org/10.1007/978-1-0716-1001-5_14

Serra, M., Mai, T. D., Serra, A. L., Nguyen, M.-C., Eisele, A., Perié, L., Viovy, J.-L., Ferraro, D., & Descroix, S. (2020). Integrated droplet microfluidic device for magnetic particles handling: Application to DNA size selection in NGS libraries preparation. *Sensors and Actuators B: Chemical*, *305*, 127346. https://doi.org/10.1016/j.snb.2019.127346

Shadman, M. (2023). Diagnosis and Treatment of Chronic Lymphocytic Leukemia: A Review. *JAMA*, *329*(11), 918–932. https://doi.org/10.1001/jama.2023.1946

Shadman, M., Mawad, R., Dean, C., Chen, T., Shannon-Dorcy, K., Sandhu, V., Hendrie, P. C., Scott, B. L., Walter, R. B., Becker, P. S., Pagel, J., & Estey, E. H. (2015). Idarubicin, Cytarabine and Pravastatin as Induction Therapy for Untreated Acute Myeloid Leukemia and High-Risk Myelodysplastic Syndrome. *American Journal of Hematology*, *90*(6), 483–486. https://doi.org/10.1002/ajh.23981

Shao, S., Yu, R., Yu, Y., & Li, Y. (2014). Dual-inhibitors of STAT5 and STAT3: Studies from molecular docking and molecular dynamics simulations. *Journal of Molecular Modeling*, *20*(8), 2399. https://doi.org/10.1007/s00894-014-2399-x

Sharma, A., Jasrotia, S., & Kumar, A. (2024). Effects of Chemotherapy on the Immune System: Implications for Cancer Treatment and Patient Outcomes. *Naunyn-Schmiedeberg's Archives of Pharmacology*, *397*(5), 2551–2566. https://doi.org/10.1007/s00210-023-02781-2

Sharma, N., & Liesveld, J. L. (2023). NPM 1 Mutations in AML—The Landscape in 2023. *Cancers*, *15*(4), 1177. https://doi.org/10.3390/cancers15041177

Siegel, R. L., Giaquinto, A. N., & Jemal, A. (2024). Cancer statistics, 2024. *CA: A Cancer Journal for Clinicians*, *74*(1), 12–49. https://doi.org/10.3322/caac.21820

Singh, Chang, H.-H., Kuo, C.-C., Shiao, H.-Y., Hsieh, H.-P., & Coumar, M. S. (2017). Drug repurposing for chronic myeloid leukemia: In silico and in vitro investigation of DrugBank database for allosteric Bcr-Abl inhibitors. *Journal of Biomolecular Structure and Dynamics*, *35*(8), 1833–1848. https://doi.org/10.1080/07391102.2016.1196462

Singh, K., Muttathukattil, A. N., Singh, P. C., & Reddy, G. (2022). pH Regulates Ligand Binding to an Enzyme Active Site by Modulating Intermediate Populations. *The Journal of Physical Chemistry. B*, *126*(47), 9759–9770. https://doi.org/10.1021/acs.jpcb.2c05117

Siveen, K. S., Uddin, S., & Mohammad, R. M. (2017). Targeting acute myeloid leukemia stem cell signaling by natural products. *Molecular Cancer*, *16*(1), 13. https://doi.org/10.1186/s12943-016-0571-x

Skånland, S. S., Cremaschi, A., Bendiksen, H., Hermansen, J. U., Thimiri Govinda Raj, D. B., Munthe, L. A., Tjønnfjord, G. E., & Taskén, K. (2020). An in vitro assay for biomarker discovery and dose prediction applied to ibrutinib plus venetoclax treatment of CLL. *Leukemia*, *34*(2), 478–487. https://doi.org/10.1038/s41375-019-0569-7

Sleire, L., Førde, H. E., Netland, I. A., Leiss, L., Skeie, B. S., & Enger, P. Ø. (2017). Drug repurposing in cancer. *Pharmacological Research*, *124*, 74–91. https://doi.org/10.1016/j.phrs.2017.07.013

Sliwoski, G., Kothiwale, S., Meiler, J., & Lowe, E. W. (2014). Computational Methods in Drug Discovery. *Pharmacological Reviews*, *66*(1), 334–395. https://doi.org/10.1124/pr.112.007336

Sohraby, F., Bagheri, M., Aliyar, M., & Aryapour, H. (2017). In silico drug repurposing of FDA-approved drugs to predict new inhibitors for drug resistant T315I mutant and wild-type BCR-ABL1: A virtual screening and molecular dynamics study. *Journal of Molecular Graphics and Modelling*, *74*, 234–240. https://doi.org/10.1016/j.jmgm.2017.04.005

Stenvang, J., Kümler, I., Nygård, S. B., Smith, D. H., Nielsen, D., Brünner, N., & Moreira, J. M. (2013). Biomarker-Guided Repurposing of Chemotherapeutic Drugs for Cancer Therapy: A Novel Strategy in Drug Development. *Frontiers in Oncology*, *3*. https://doi.org/10.3389/fonc.2013.00313

Sun, D., Gao, W., Hu, H., & Zhou, S. (2022). Why 90% of clinical drug development fails and how to improve it? *Acta Pharmaceutica Sinica B*, *12*(7), 3049–3062. https://doi.org/10.1016/j.apsb.2022.02.002

Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A., & Bray, F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians*, *71*(3), 209–249. https://doi.org/10.3322/caac.21660

Szymański, P., Markowicz, M., & Mikiciuk-Olasik, E. (2011). Adaptation of High-Throughput Screening in Drug Discovery—Toxicological Screening Tests. *International Journal of Molecular Sciences*, *13*(1), 427–452. https://doi.org/10.3390/ijms13010427

Tabatabaei, K., Moazzezi, S., Emamgholizadeh, M., Vaez, H., Baradaran, B., & Shokouhi, B. (2024). Improved Therapeutic Efficacy of Doxorubicin Chemotherapy With Cannabidiol in 4T1 Mice Breast Cancer Model. *Cancer Medicine*, *13*(21), e70395. https://doi.org/10.1002/cam4.70395

Tam, C. S., O'Brien, S., Wierda, W., Kantarjian, H., Wen, S., Do, K.-A., Thomas, D. A., Cortes, J., Lerner, S., & Keating, M. J. (2008). Long-term results of the fludarabine, cyclophosphamide, and rituximab regimen as initial therapy of chronic lymphocytic leukemia. *Blood*, *112*(4), 975–980. https://doi.org/10.1182/blood-2008-02-140582

Thimiri Govinda Raj, D. B. balaji, Cremaschi, A., Skånland, S. S., Gade, A., Schjesvold, F. H., Tjønnfjord, G. E., A Munthe, L., & Tasken, K. (2018). In-Vitro Drug Sensitivity Screening in Chronic Lymphocytic Leukemia (CLL) Primary Patient Samples Identifies Drug Candidates for Precision Cancer Therapy. *Blood*, *132*(Supplement 1), 4676–4676. https://doi.org/10.1182/blood-2018-99-110357

Trabelsi, M., Farah, F., Zouari, B., Jaafoura, M. H., & Kharrat, M. (2019). An Immunoscore System Based On CD3+ And CD8+ Infiltrating Lymphocytes Densities To Predict The Outcome Of

Patients With Colorectal Adenocarcinoma. *OncoTargets and Therapy*, *12*, 8663–8673. https://doi.org/10.2147/OTT.S211048

Vagace, J. M., Gervasini, G., Vagace, J. M., & Gervasini, G. (2011). Chemotherapy Toxicity in Patients with Acute Leukemia. In *Acute Leukemia—The Scientist's Perspective and Challenge*. IntechOpen. https://doi.org/10.5772/21239

Vamathevan, J., Clark, D., Czodrowski, P., Dunham, I., Ferran, E., Lee, G., Li, B., Madabhushi, A., Shah, P., Spitzer, M., & Zhao, S. (2019). Applications of machine learning in drug discovery and development. *Nature Reviews. Drug Discovery*, *18*(6), 463–477. https://doi.org/10.1038/s41573-019-0024-5

Van den Driessche, F., Brackman, G., Swimberghe, R., Rigole, P., & Coenye, T. (2017). Screening a repurposing library for potentiators of antibiotics against Staphylococcus aureus biofilms. *International Journal of Antimicrobial Agents*, *49*(3), 315–320. https://doi.org/10.1016/j.ijantimicag.2016.11.023

Vener, C., Banzi, R., Ambrogi, F., Ferrero, A., Saglio, G., Pravettoni, G., & Sant, M. (2020). First-line imatinib vs second- and third-generation TKIs for chronic-phase CML: A systematic review and meta-analysis. *Blood Advances*, *4*(12), 2723–2735. https://doi.org/10.1182/bloodadvances.2019001329

Walpole, I. R., Zaman, F. Y., Zhao, P., Marshall, V. M., Lin, F. P., Thomas, D. M., Shackleton, M., Antolin, A. A., & Ameratunga, M. (2024). Computational repurposing of oncology drugs through off-target drug binding interactions from pharmacological databases. *Clinical and Translational Medicine*, *14*(4), e1657. https://doi.org/10.1002/ctm2.1657

Walter, R. B., Othus, M., Burnett, A. K., Löwenberg, B., Kantarjian, H. M., Ossenkoppele, G. J., Hills, R. K., van Montfort, K. G. M., Ravandi, F., Evans, A., Pierce, S. R., Appelbaum, F. R., & Estey, E. H. (2013). Significance of FAB subclassification of "acute myeloid leukemia, NOS" in the 2008

WHO classification: Analysis of 5848 newly diagnosed patients. *Blood*, *121*(13), 2424–2431. https://doi.org/10.1182/blood-2012-10-462440

Wang, F., Liu, Z., Zeng, J., Zhu, H., Li, J., Cheng, X., Jiang, T., Zhang, L., Zhang, C., Chen, T., Liu, T., & Jia, Y. (2015). Metformin synergistically sensitizes FLT3-ITD-positive acute myeloid leukemia to sorafenib by promoting mTOR-mediated apoptosis and autophagy. *Leukemia Research*, *39*(12), 1421–1427. https://doi.org/10.1016/j.leukres.2015.09.016

Wang, S.-W., Chen, Y.-R., Chow, J.-M., Chien, M.-H., Yang, S.-F., Wen, Y.-C., Lee, W.-J., & Tseng, T.-H. (2018). Stimulation of Fas/FasL-mediated apoptosis by luteolin through enhancement of histone H3 acetylation and c-Jun activation in HL-60 leukemia cells. *Molecular Carcinogenesis*, *57*(7), 866–877. https://doi.org/10.1002/mc.22807

Weigelin, B., den Boer, A. T., Wagena, E., Broen, K., Dolstra, H., de Boer, R. J., Figdor, C. G., Textor, J., & Friedl, P. (2021). Cytotoxic T cells are able to efficiently eliminate cancer cells by additive cytotoxicity. *Nature Communications*, *12*(1), 5217. https://doi.org/10.1038/s41467-021-25282-3

Woyach, J. A., Ruppert, A. S., Heerema, N. A., Zhao, W., Booth, A. M., Ding, W., Bartlett, N. L., Brander, D. M., Barr, P. M., Rogers, K. A., Parikh, S. A., Coutre, S., Hurria, A., Brown, J. R., Lozanski, G., Blachly, J. S., Ozer, H. G., Major-Elechi, B., Fruth, B., … Byrd, J. C. (2019). *Ibrutinib Regimens versus Chemoimmunotherapy in Older Patients with Untreated CLL*. 18.

Xiao, Z., Gong, R., Chen, X., Xiao, D., Luo, S., & Ji, Y. (2021). Association between serum lactate dehydrogenase and 60-day mortality in Chinese Hakka patients with acute myeloid leukemia: A cohort study. *Journal of Clinical Laboratory Analysis*, *35*(12), e24049. https://doi.org/10.1002/jcla.24049

Xu, L., Zhu, Y., Shao, J., Chen, M., Yan, H., Li, G., Zhu, Y., Xu, Z., Yang, B., Luo, P., & He, Q. (2017). Dasatinib synergises with irinotecan to suppress hepatocellular carcinoma via inhibiting the

protein synthesis of PLK1. *British Journal of Cancer*, *116*(8), 1027. https://doi.org/10.1038/bjc.2017.55

Xue, H., Li, J., Xie, H., & Wang, Y. (2018). Review of Drug Repositioning Approaches and Resources. *International Journal of Biological Sciences*, *14*(10), 1232–1244. https://doi.org/10.7150/ijbs.24612

Yadav, B., Pemovska, T., Szwajda, A., Kulesskiy, E., Kontro, M., Karjalainen, R., Majumder, M. M., Malani, D., Murumägi, A., Knowles, J., Porkka, K., Heckman, C., Kallioniemi, O., Wennerberg, K., & Aittokallio, T. (2014). Quantitative scoring of differential drug sensitivity for individually optimized anticancer therapies. *Scientific Reports*, *4*, 5193. https://doi.org/10.1038/srep05193

Yashiro, M., Qiu, H., Hasegawa, T., Zhang, X., Matsuzaki, T., & Hirakawa, K. (2011). An EGFR inhibitor enhances the efficacy of SN38, an active metabolite of irinotecan, in SN38-refractory gastric carcinoma cells. *British Journal of Cancer*, *105*(10), 1522–1532. https://doi.org/10.1038/bjc.2011.397

Yuan, T., Wang, X. Y., Lai, Y. Y., Qin, Y. Z., Shi, H. X., Huang, X. J., & Jiang, Q. (2019). [Philadelphia chromosome-negative myeloid neoplasms in patients with Philadelphia chromosome-positive chronic myeloid leukemia during tyrosine kinase inhibtor-therapy]. *Zhonghua Xue Ye Xue Za Zhi = Zhonghua Xueyexue Zazhi*, *40*(7), 547–553. https://doi.org/10.3760/cma.j.issn.0253-2727.2019.07.003

Zapevalova, M. V., Shchegravina, E. S., Fonareva, I. P., Salnikova, D. I., Sorokin, D. V., Scherbakov, A. M., Maleev, A. A., Ignatov, S. K., Grishin, I. D., Kuimov, A. N., Konovalova, M. V., Svirshchevskaya, E. V., & Fedorov, A. Y. (2022). Synthesis, Molecular Docking, In Vitro and In Vivo Studies of Novel Dimorpholinoquinazoline-Based Potential Inhibitors of PI3K/Akt/mTOR Pathway. *International Journal of Molecular Sciences*, *23*(18), Article 18. https://doi.org/10.3390/ijms231810854

Zhang, H., Chen, S., & Xiao, Y. (2022). CAR-T Cell Therapy in Hematological Malignancies: Current Opportunities and Challenges. *Frontiers in Immunology*, *13*, 927153. https://doi.org/10.3389/fimmu.2022.927153

Zhang, J., Gu, Y., & Chen, B. (2019). Mechanisms of drug resistance in acute myeloid leukemia. *OncoTargets and Therapy*, *12*, 1937–1945. https://doi.org/10.2147/OTT.S191621

Zhu, K., Wu, Y., He, P., Fan, Y., Zhong, X., Zheng, H., & Luo, T. (2022). PI3K/AKT/mTOR-Targeted Therapy for Breast Cancer. *Cells*, *11*(16), 2508. https://doi.org/10.3390/cells11162508

APPENDICES

Appendix 2.1 ECOG interpretation

ECOG	Description
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light housework, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET, Carbone PP. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol. 1982 Dec;5(6):649-655. PMID: 7165009.

Appendix 3.1 List of the 30 drugs tested

	Drug name	Code	Class
A	Cladribine	7-B02	Anti-metabolite; Purine analog
B	5-Azacytidine	6-D04	DNA methylation
C	Bendamustine	6-E02	DNA-damaging
D	Bortezomib	3-A11	Proteasome inhibitor (26S subunit)
E	Busulfan	6-F05	Alkylating agent
F	Chlorambucil	3-H06	Alkylating agent
G	Cyclophosphamide monohydrate	4-A08	Alkylating agent
H	Cytarabine	4-A09	DNA synthesis

I	Dasatinib	7-D05	Abl, Src, c-Kit
J	Dexamethasone	2-F04	Interleukin receptor
K	Doxorubicin	2-H10	DNA topoisomerase II
L	Everolimus	8-A07	mTOR inhibitor
M	Fludarabine	8-B04	DNA synthesis, STAT1
N	Fluorouracil	4-C10	Antimetabolite
O	Gemcitabine	3-C10	Antimetabolite; Nucleoside analog
P	Ifosfamide	4-E06	Nitrogen mustard alkylating agent
Q	Imatinib	2-C04	Abl, c-Kit, PDGFRB inhibitor
R	Imiquimod	4-E07	Immune response
S	Irinotecan	8-E07	topoisomerase I inhibitor
T	Lenalidomide	8-F09	Immunomodulation
U	Melphalan	4-F09	Antineoplastic activity
V	Nilotinib	9-E08	BCR/Abl inhibitor
W	Prednisolone	5-A05	Immunomodulatory agent
X	Simvastatin	2-H02	HMG-CoA reductase
Y	Sorafenib	10-D09	Raf-1, B-Raf and VEGFR-2
Z	Tacrolimus	10-E07	FKBP12 (FK506 binding protein)
AA	Temsirolimus	10-F03	binds FKBP12, causes inhibition of mTOR
AB	Thalidomide	3-H09	Immunomodulation, E3 ubiquitin ligase
AC	Tretinoin	10-H07	Retinoic acid receptor agonist
AD	Vinblastine	3-G05	Mitotic inhibitor
AE	Pyrimethamine (Daraprim)	10-B06	
AF	Metformin	2-C09	

Appendix 3.2 List of drugs for cancer treatment

INN	Brand As Per Contract
Immunoglobulin, Antithymocyte (Rabbit)	ATG-Fresenius
Azathioprine	AZAMUN 50MG
Busulfan	MYLERAN 2MG TAB 100
Calcium Folinate	Rescuvolin 15mg
Chlorambucil	LEUKERAN 2MG TABS 25`S
Ciclosporin	Sandimmun Neoral 100mg
Ciclosporin	Sandimmun Neoral 25mg
Ciclosporin	Sandimmun Neoral 100mg/ml Oral solution
Ciclosporin	Sandimmun Neoral 50mg injection
Docetaxel	Docetere RTU 20mg 1's
Docetaxel	Docetere RTU 80mg 1's
Everolimus	Certican 0.25
Everolimus	Certican 0.75
Exemestane	Aromasin 25mg
Filgrastim	Filgrastim Teva 30MIU 5
Filgrastim	Filgrastim Teva 48MIU 5
Fludarabine	Fludara 10mg Oral Tabs 20's
Fludarabine	Fludara IV 50mg INJ Soln 5's
Fluorouracil	Efudix
Goserelin	Zoladex 3.6
Granisetron	Adco Granisetron 3MG
Imatinib	Sunmatin 400
Interferon Beta-1a	AVONEX SOLUTION FOR INJECTION
Irinotecan	Mylan Irinotecan 100mg/5ml
Irinotecan	Mylan Irinotecan 40 mg / 2ml
Medroxyprogesterone	Provera 100mg
Mercaptopurine	PURI-NETHOL 50MG TABS 25`S
Methotrexate	Abitrexate 1000mg/10ml
Methotrexate	Abitrexate 2.5mg tablets
Methotrexate	Abitrexate 5g
Mitoxantrone	Mitoxantrone Hexal 20mg /10ml Inj
Mycophenolate Mofetil	Mycocept 250mg Caps
Mycophenolate Mofetil	Mycocept 500mg Tabs
Mycophenolic Acid	Myfortic 180mg
Mycophenolic Acid	Myfortic 360mg
Nilotinib	Tasigna 200mg
Ondansetron	Zofer Rapitab 4
Ondansetron	ONDANSETRON HEXAL 4 mg INJECTION
Ondansetron	ONDANSETRON HEXAL 8 mg INJECTION

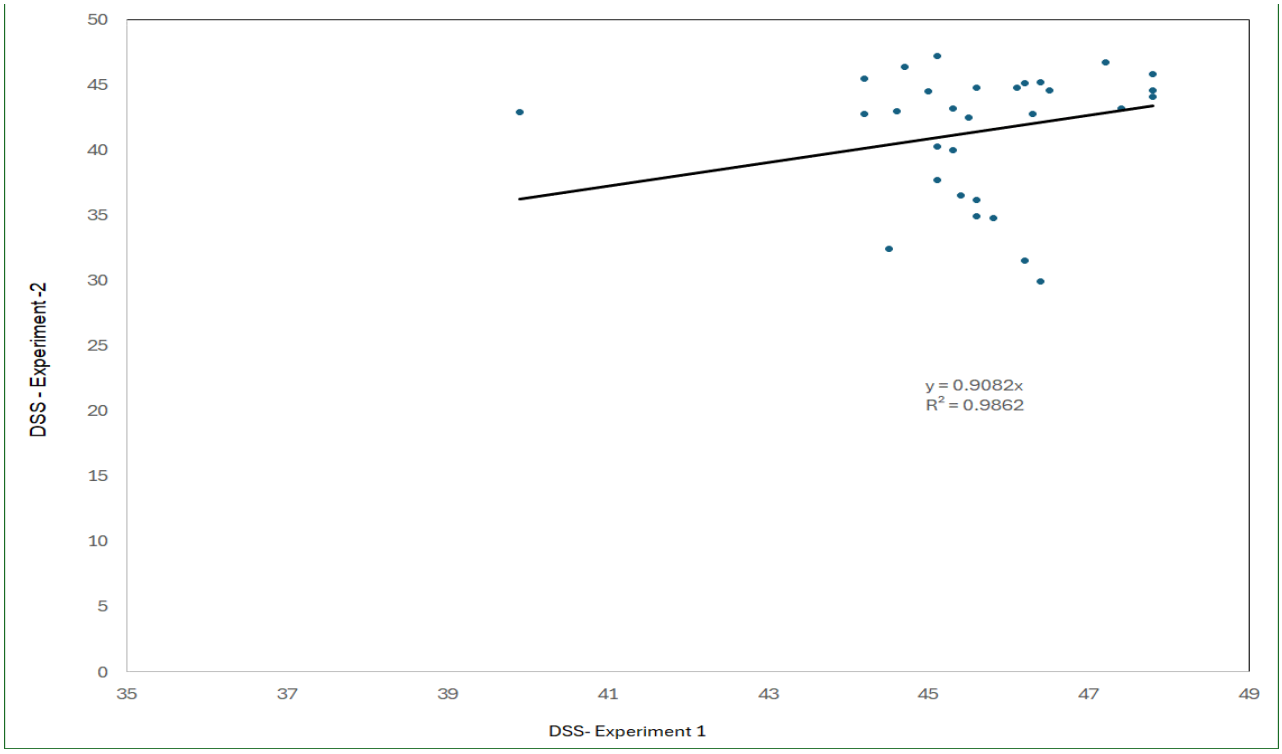
Ondansetron	Zofer 8
Oxaliplatin	Oxaliplatin PCH 100 mg/20 ml Conc
Oxaliplatin	Oxaliplatin PCH 50 mg/10 ml Conc
Sirolimus	Rapamune 1mg
Tacrolimus	Prograf 0.5mg
Tacrolimus	Prograf 1mg
Tacrolimus	Prograf 5mg
Thalidomide	Thalidomide Celgene 50 mg
Tioguanine	LANVIS 40MG TABS 25`S
Trastuzumab	Ogivri 440
Tretinoin	Vesanoid 10mg Capsules
Vinblastine	Vinblastine Teva 10
Vinorelbine	Sandoz Vinorelbine 10mg/1ml
Vinorelbine	Sandoz Vinorelbine 50mg/5ml
Zoledronic Acid	Zobone
Anastrozole	Accord Anastrozole 1mg
Bevacizumab	Avastin 100mg
Calcium Folinate	Abic Leucovorin 100mg/10ml
Calcium Folinate	Abic Leucovorin 300mg/30ml
Capecitabine	Capexa 150mg
Capecitabine	Capexa 500mg
Carboplatin	Carbosin 150
Carboplatin	Carbosin 450
Cisplatin	Cisacor 50mg
Cyclophosphamide	Endoxan Injection 1 g
Cyclophosphamide	Endoxan Injection 500 mg
Cyclophosphamide	Endoxan 50 mg tablets
Dacarbazine	Dacin 200
Doxorubicin	DOXORUBICIN -SOL PCH 10MG
Doxorubicin	DOXORUBICIN -SOL PCH 50MG
Gemcitabine	Accord Gemcitabine 1g
Gemcitabine	Accord Gemcitabine 200mg
Goserelin	Zoladex 10.8
Ifosfamide	Holoxan 1 g Injection
Ifosfamide	Holoxan 2 g Injection
Ifosfamide	Holoxan 500 mg Injection
Imatinib	Imatinib Accord 100mg
Interferon Beta-1b	Betaferon
Mesna	Uromitexan 400 mg Injection
Mycophenolate Mofetil	Cellcept OS Suspension
Paclitaxel	Cipla Paclitaxel 100 mg/16,7 ml
Paclitaxel	Cipla Paclitaxel 30 mg/5 ml
Rituximab	Mabthera 100mg
Rituximab	Mabthera 500mg
Tamoxifen	Nolvadex-D

Vincristine	Accord-Vincristine 1mg/ml

Appendix 3.3 list of drug combination arrangements

List A		List B	
Nilotinib	A	Irinotecan	F
Fludarabine	B	Cladribine	G
Dasatinib	C	Bortezomib	H
Chlorambucil	D	Thalidomide	I
5-Azacytidine	E	Everolimus	J

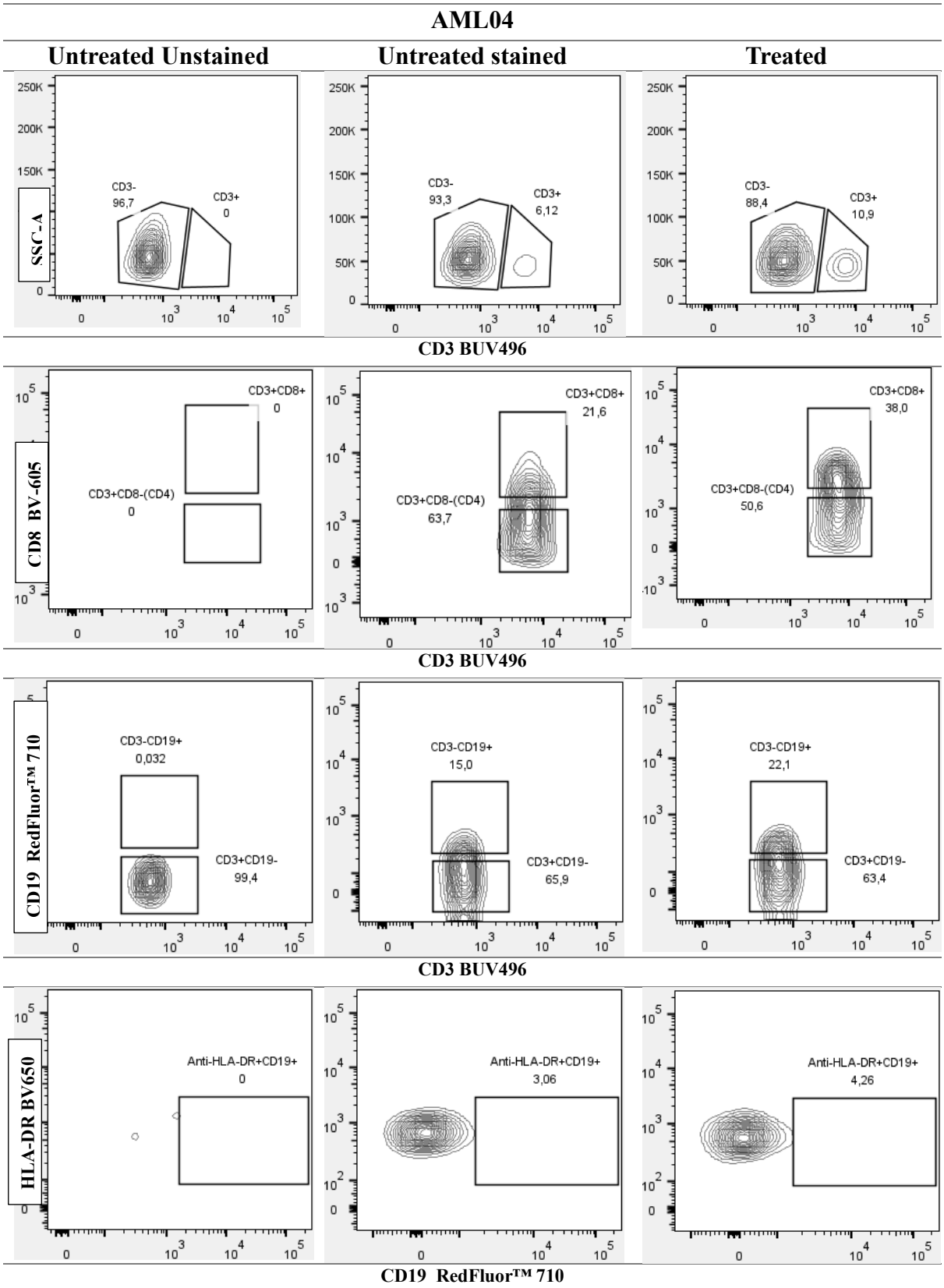
Appendix 3.4: Ex vivo Drug sensitivity testing is reproducible as demonstrated in HeLa cell lines

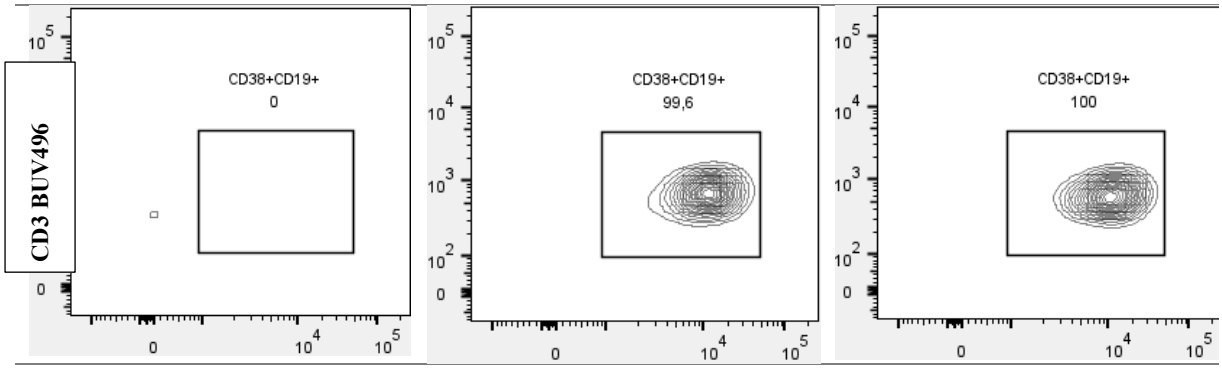


Appendix 3.5 Table of the drug cluster I

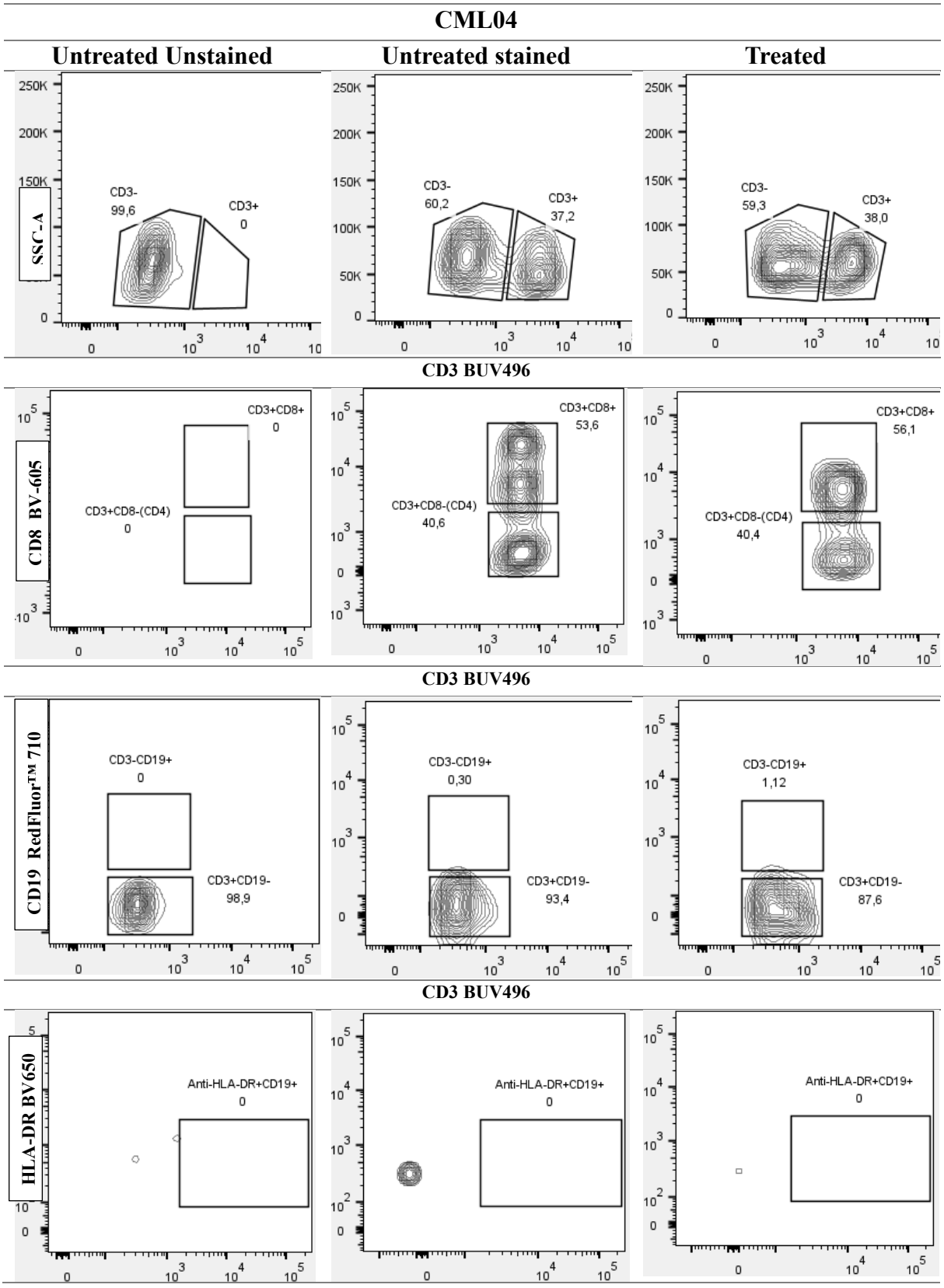
Drug of cluster I	class	Patient code/Subtype
Imiquimod	Immune response	CML04/CML
Fluorouracil	Antimetabolite	BH08/CML
Cyclophosphamide monohydrate	Alkylating agent	BH15/CML
		BH12/AML
Chlorambucil	Alkylating agent	BH26/CML
Doxorubicin	DNA topoisomerase II	BH09/CML
Gemcitabine	Antimetabolite; Nucleoside analogue	BH23/CML
Simvastatin	HMG-CoA reductase	BH05/CML
Bortezomib	Proteasome inhibitor (26S subunit)	BH22/CML
Ifosfamide	Nitrogen mustard alkylating agent	
Vinblastine	Mitotic inhibitor	
Thalidomide	Immunomodulation, E3 ubiquitin ligase	
Cytarabine	DNA synthesis	

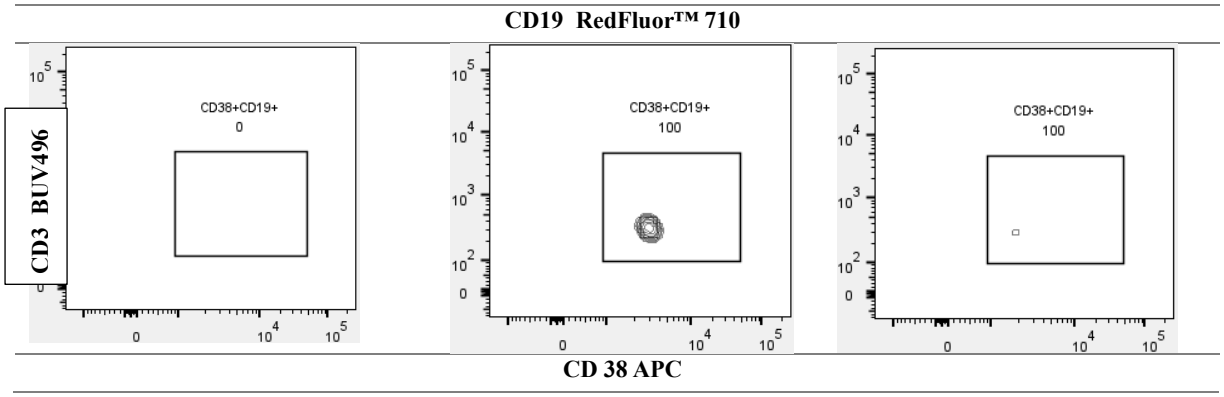
Appendix 4.1: Expression of surface markers on AML04



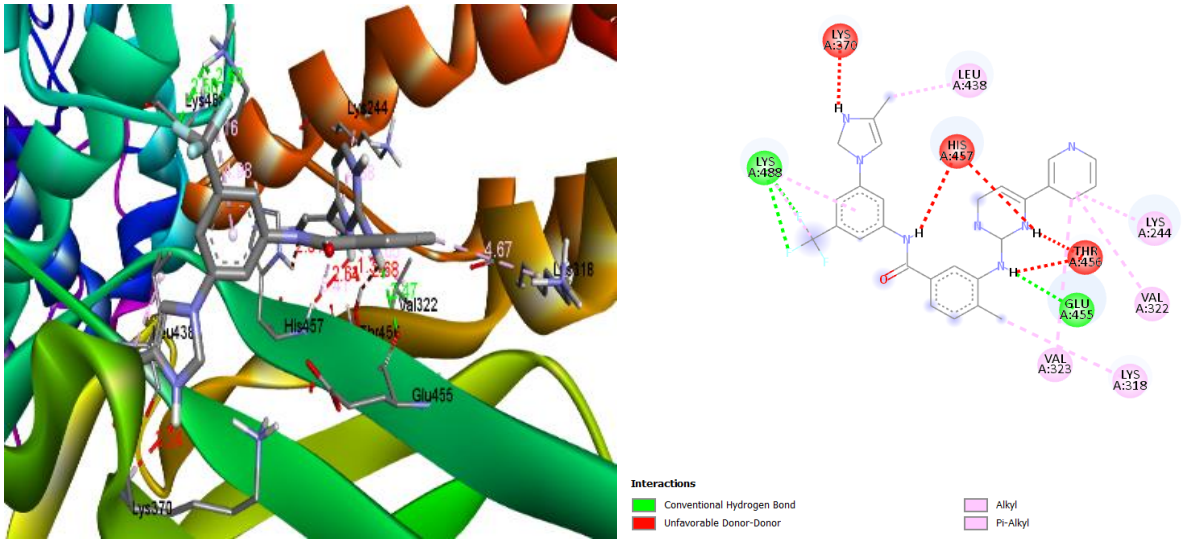

CD3 BUV496
CD38+CD19+
0
CD38+CD19+
99,6
CD38+CD19+
100
CD38 APC

Appendix 4.2: Expression of surface markers on CML04

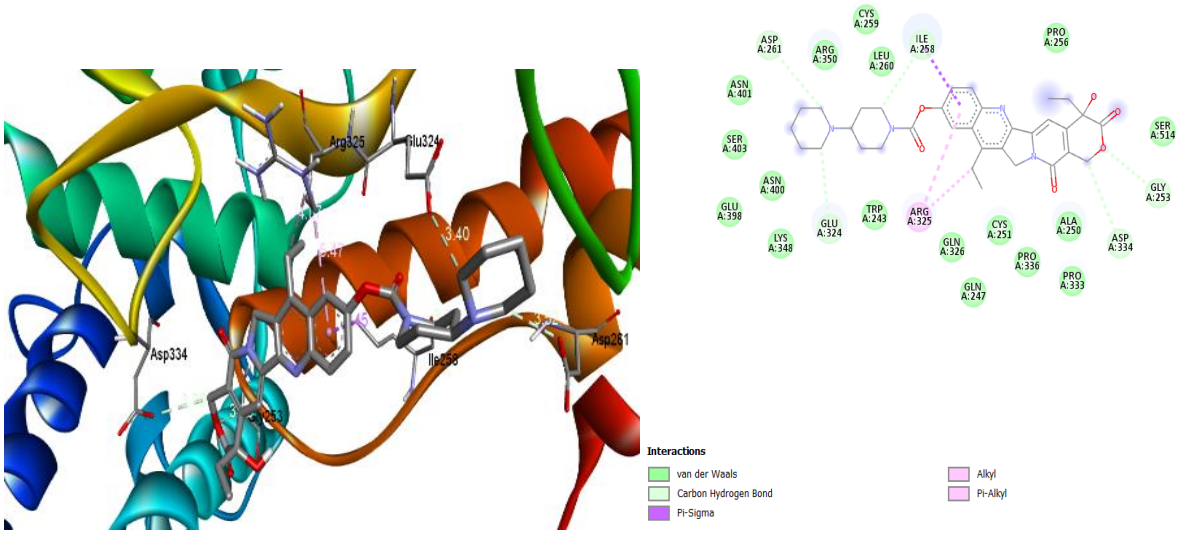




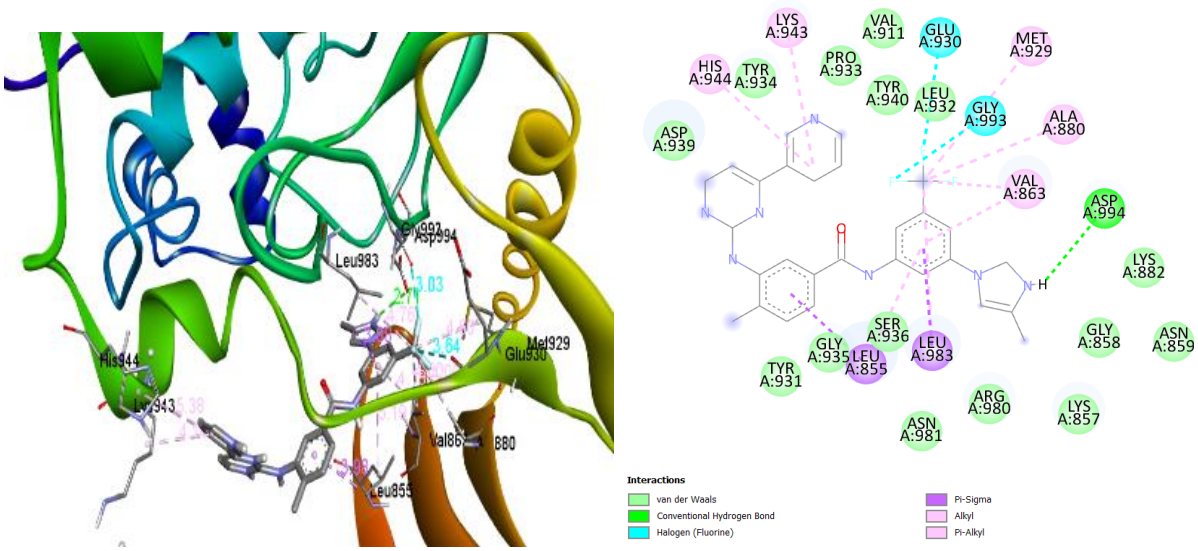
Appendix 5.1: 2D and 3D representation of the interactions established between proteins nilotinib and irinotecan



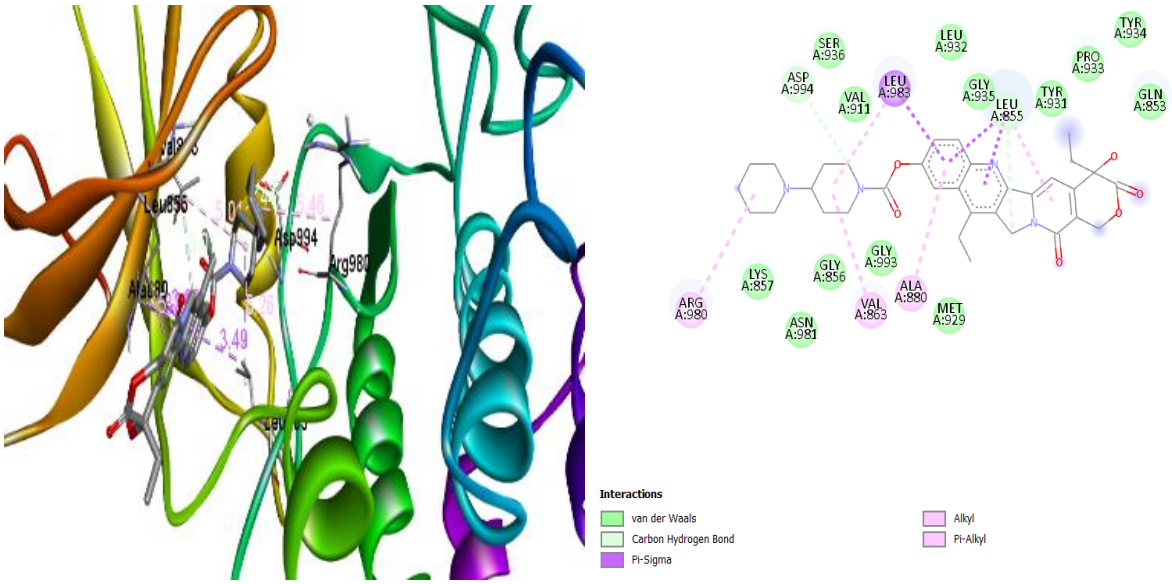
Nilotinib and STAT3



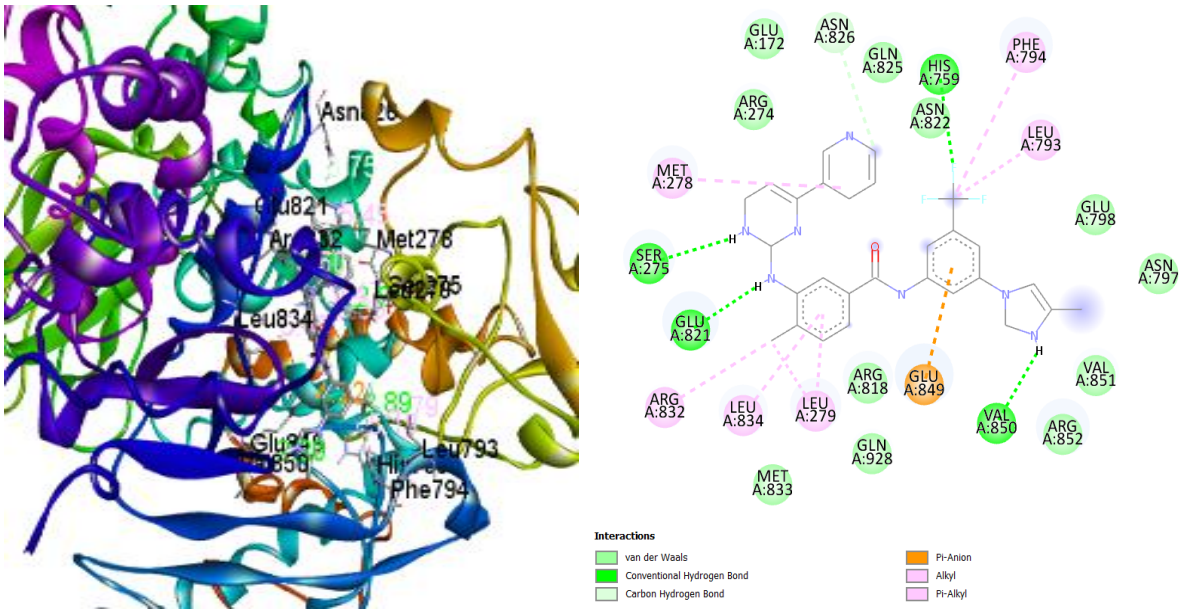
Irinotecan and STAT3



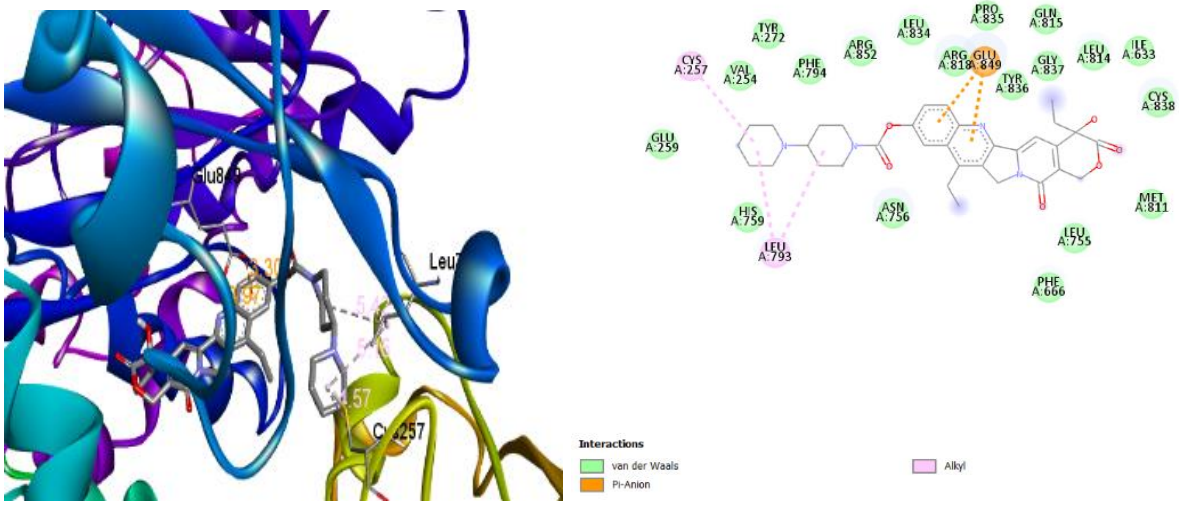
Nilotinib and JAK2



Irinotecan and JAK2

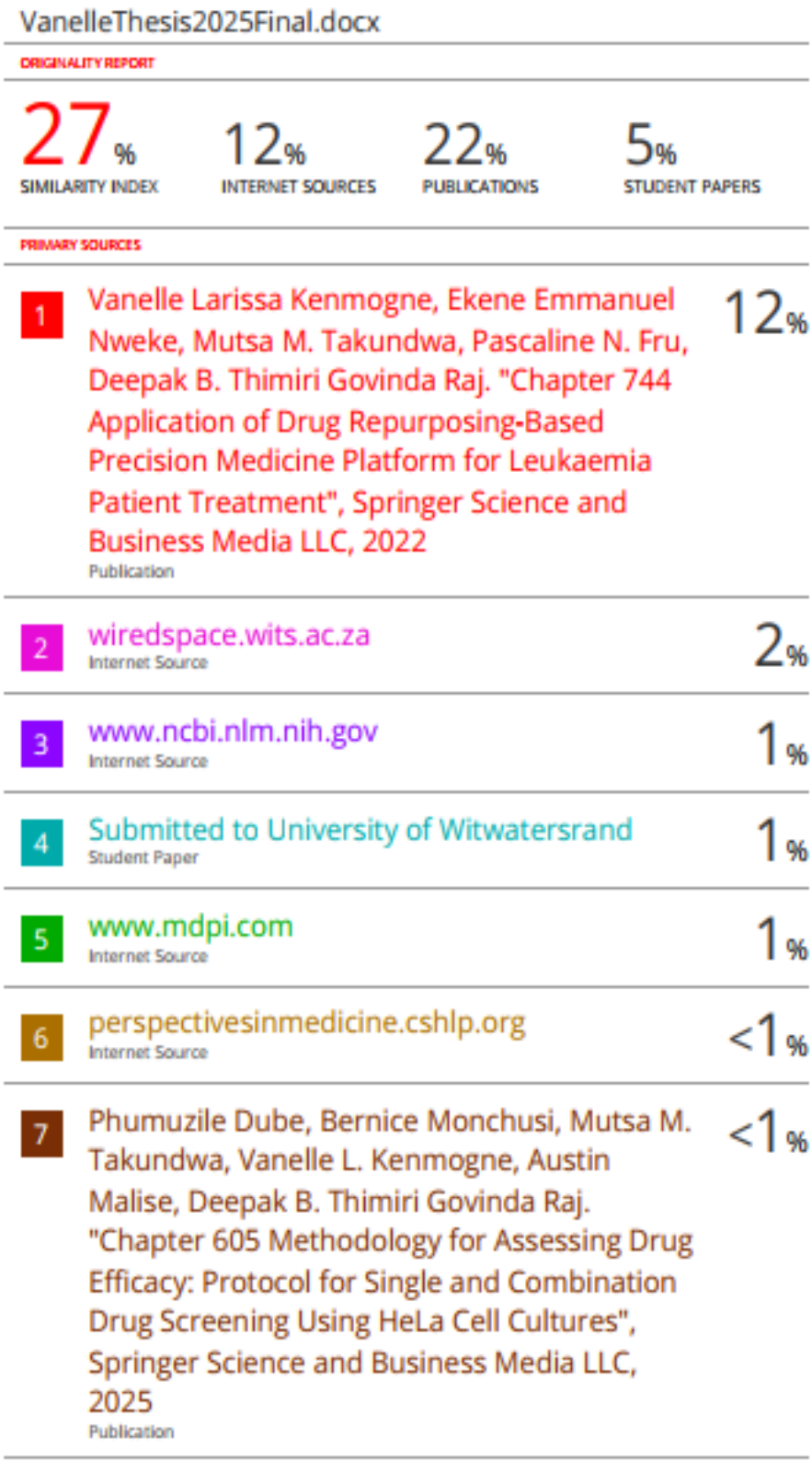


Nilotinib and PI3K



Irinotecan and PI3K

APPENDIX A: TURNITIN REPORT



VanelleThesis2025Final.docx

ORIGINALITY REPORT

27%	12%	22%	5%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	Vanelle Larissa Kenmogne, Ekene Emmanuel Nweke, Mutsa M. Takundwa, Pascaline N. Fru, Deepak B. Thimiri Govinda Raj. "Chapter 744 Application of Drug Repurposing-Based Precision Medicine Platform for Leukaemia Patient Treatment", Springer Science and Business Media LLC, 2022 Publication	12%
2	wiredspace.wits.ac.za Internet Source	2%
3	www.ncbi.nlm.nih.gov Internet Source	1%
4	Submitted to University of Witwatersrand Student Paper	1%
5	www.mdpi.com Internet Source	1%
6	perspectivesinmedicine.cshlp.org Internet Source	<1%
7	Phumuzile Dube, Bernice Monchusi, Mutsa M. Takundwa, Vanelle L. Kenmogne, Austin Malise, Deepak B. Thimiri Govinda Raj. "Chapter 605 Methodology for Assessing Drug Efficacy: Protocol for Single and Combination Drug Screening Using HeLa Cell Cultures", Springer Science and Business Media LLC, 2025 Publication	<1%

APPENDIX B: ETHICS CLEARANCE

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG

R49 Ms VL Kenmogne

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M211171

NAME: (Principal Investigator)	Ms VL Kenmogne
DEPARTMENT:	School of Clinical Medicine Department of Surgery Medical School University
PROJECT TITLE:	*High-throughput drug repurposing for precision medicine against Leukaemia in patients in South Africa*
DATE CONSIDERED:	2021/11/26
DECISION:	Approved unconditionally
CONDITIONS:	
NOTE:	If contact information regarding student study participants is required, please contact the Registrar's office - <Nicoleen.Potgieter@wits.ac.za>
SUPERVISOR:	Drs DBT Govindaraj, EE Nweke and P Fru
APPROVED BY:	Dr CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	2022/03/16

This Clearance Certificate is valid for 5 years from the date of approval. An extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office secretariat on the 3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated from the research protocol as approved, I/we undertake to submit details to the Committee. I agree to submit a yearly progress report. When a funder requires annual re-certification, the application date will be one year after the date when the study was initially reviewed. In this case, the study was initially reviewed in **November** and therefore reports and re-certification will be due in the month of **November** each year. Unreported changes to the study may invalidate the clearance given by the HREC (Medical).

Signature of Principal Investigator _____ Date _____

APPENDIX C: PERMISSION TO CONDUCT RESEARCH

Dr Clement Penny,
Chair
Wits Human Research Ethics Committee (Medical)
Wits Faculty of Health Sciences
2nd Floor
Phillip V Tobias Building
Faculty of Heath Sciences
Corner York Road and Princess of Whales Terrace
Parktown

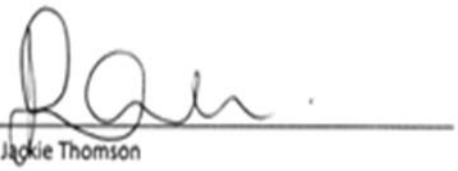
31st January 2022

Dear Dr Penny,

Permission to undertake research study at the Cellular and Immunotherapy Centre at WDGMC

This letter serves to confirm that *Vanelle Larissa Kenmogne* has our permission to access our patients for a research study entitled *High throughput drug repurposing for precision medicine against leukaemia patients in South Africa* at the Cellular and Immunotherapy Centre Wits Donald Gordon Medical Centre.

Kind regards



Dr Jackie Thomson
Director
Wits Donald Gordon Cellular and Immunotherapy Centre
Jackie@bonemarrowtransplant.co.za
0110531200



Dr Justin du Toit
Specialist Haematologist
Wits Donald Gordon Cellular and Immunotherapy Centre
Justin@bonemarrowtransplant.co.za
0110531200

Version 2 – 24th May 2018. Template – Dr H Etheredge

APPENDIX D: DATA SHEET

WITS UNIVERSITY

-APPENDIX III-

DATASHEET FOR CHRONIC LEUKAEMIA DIAGNOSIS

DEMOGRAPHICS

Sample No: _____

Hospital No:

_____

Sex: M ☐ F ☐

Age: _____

BMI _____kg/m²

_____

COMPLETE BLOOD COUNT

ON ADMISSION

WBC _____ul.
RBC _____Ul
Lymphocytes--------%
Platelets _____ul.
Immunoglobuline---------

IMMUNOPHENOTYPING

TYPE OF MUTATION (FISH)

IGVH status

STAGING

_____

DATE OF DIAGNOSIS

FIRST TREAMENT AND DURATION

Which drug has been prescribed to the patient?
History of previous treatment and duration
Current Treatment and duration

COMORBIDITY AND PATIENT OUTCOMES

Has the patient been diagnosed with any other disease apart from CLL (circle relevant option)?
Y N
If yes, state disease

_____

Is the patient HIV positive **Y N**

If HIV positive state whether they are on ARVs

_____

CD4 count_____ cell/mm³

Additional comments/observations
Additional comments/observations

_____
_____
_____
_____
_____

APPENDIX E: INFORMATION SHEET

WITS UNIVERSITY

-APPENDIX I-

INFORMATION SHEET: EXPERIMENTAL SAMPLE

HIGH THROUGHPUT DRUG REPURPOSING FOR PRECISION MEDICINE AGAINST LEUKAEMIA PATIENTS IN SOUTH AFRICA

Good day,

We are researchers from the University of the Witwatersrand doing research in precision medicine. Research is a process of learning and solving problems. In this study, we want to find out which treatment option will best suit people with leukaemia. How to prevent unnecessary prescriptions in order to reduce drug toxicity and avoid drug resistance.

We are inviting you to participate in this research study. This study seeking for at least 40 participants recently diagnosed with leukaemia (ALL, AML, CLL, or CML) or who have relapsed from their first treatment. You are invited to donate about 2 tablespoons (20 ml) of blood. The blood will be drawn from a vein in your arm using a syringe at the Haematology division of the academic hospital to which you are admitted (Wits Donald Gordon Medical Centre / Chris Hani Baragwanath hospital) and will be carefully transported by private means to the University of the Witwatersrand where the research will be done. The portion containing affected cells will be isolated from the blood and stored at -80C to preserve its properties for further use. A panel of approved drugs will be tested on the cells to determine the most effective ones.

Blood collection will be performed by a specialist (nurse or doctor), and you may be subjected to some pain or discomfort caused by the needle. The procedure will only take about 5 minutes and blood will be obtained from you with your consent and you will not be called to participate again.

Participating in this study is voluntary, free of charge and you may withdraw consent at any time without penalty or prejudice. Giving or refusing consent will not affect your treatment or care in any way. While it is unlikely the research will benefit you immediately, we hope that the results will help improve the treatment of patients in the future. Significant new discoveries developed during this research, which may relate to your willingness to continue participating in the study will be provided to you.

Blood samples will be collected anonymously to ensure confidentiality. Personal information may only be disclosed if required by law or organizations that inspect and/or copy research records for quality assurance and data analysis such as the Research Ethics Committee (REC). At the end of the study, leftover blood will be discarded in 10% bleach, sterilized by heating to 120 degrees Celsius, and disposed in bio-hazardous/ medical waste boxes.

For further information/ reporting study-related adverse effects/complaints contact:

Researchers:
-Kenmogne Vanelle Larissa (Research student)
Tel: 0619982772, 2505588@students.wits.ac.za
-Dr. Pascaline Fru; Dr Deepak Thimiri, Dr Emmanuel Ekene (Supervisors)
Tel: 0117172476, pascaline.fru@wits.ac.za; DGovindaraj@csir.co.za; Ekene.Nweke@wits.ac.za
- Dr. Justin Du Toit (Clinician, Wits Donald Gordon Medical Centre), justin@bonnemarrowtransplant.co.za
- Dr. Vinitha Philip (Clinician, Chris Hani Baragwanath Academic Hospital), Vinitha.philip-cherian@wits.ac.za
REC Chairperson – Prof Clement Penny: clement.penny@wits.ac.za
REC Administrators - Ms Zanele Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng
Tel 011 717 2700/2656/1234/1252, Email: HREC-Medical.ResearchOffice@wits.ac.za

APPENDIX F: INFORMED CONSENT

WITS UNIVERSITY

-APPENDIX II-

Consent Form: Use of Clinical Sample as control and Information for Research

Dear Sir/madam,

You are currently admitted to the Wits Donald Gordon Medical Centre / Chris H. Baragwanath academic hospital, Johannesburg. This hospital not only renders treatment but is also actively involved in conducting research aimed at improving the quality of care that is provided. From time to time such research involves the use of patient samples and records from which information is extracted. The use of such information is subject to approval from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand. We would like to obtain your consent to use information from your file for the purpose of research, subject to the aforementioned conditions.

1. I have been given a participant information Sheet which explains the nature and processes involved in this study, which is attached hereto.
2. I was given time to read it, or had it read to me, in the language I best understand.
3. I was given time to ask any questions I wanted to and found any answers given to me to be reasonable and satisfactory.
4. I believe I fully understand why the study is being conducted and what the intended outcomes will be;
5. I understand that there will be no immediate benefit to me, should I agree to participate, nor will I receive any payment; conversely, participation will not cost me anything but my time.
6. I understand that, even if I initially consent to take part in the study, I may subsequently withdraw at any time and would not be required to give any reasons; if that happened, any data collected about me for the purposes of the study would immediately be destroyed, unless I give consent for it to be retained.
7. I have been given a range of contact details, listed below. If I require further information or become concerned about any aspect of this study, I am free to speak to any of these contacts.

Should you wish to contact us at any stage regarding consent, phone:

Ms Vanelle Kenmegne at 0619982772, Dr Pascaline Fru at (011)717-2476 or Dr Emmanuel Nweke/Dr Deepak B. Thimiri Govindaraj (012 8412206)
Dr. Justin Du Toit (Wits Donald Gordon Medical Centre), justin@bonemarrowtransplant.co.za
Dr. Vinitha Philip (Chris Hani Baragwanath Academic Hospital), Vinitha.philip-cherian@wits.ac.za

---

A. Consent Given

I_____ hereby give consent for my records to be used as per the above-mentioned conditions for the purposes of research:

PATIENT SIGNATURE:_____ DATE: _____

B. Consent Not Given

I _____ do not give consent for my records to be used:

PATIENT SIGNATURE:_____ DATE: _____

2