DYSREGULATION OF TRANSCRIPTOMIC PROFILES OF MM1.S AND U266 MULTIPLE MYELOMA CELL LINES TREATED WITH EPIGENETIC INHIBITORS

NOR HAYATI BINTI ISMAIL

UNIVERSITI SAINS MALAYSIA

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by

NOR HAYATI BINTI ISMAIL

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LIST OF SYMBOLS

α	alpha
γ	gamma
β	beta
^{0}C	Celsius
<	less than
>	more than
Δ	delta
μ	micro
μM	micromolar
nM	nanomolar
mM	millimolar
mL	milliliters
μL	microlitres

LIST OF ABBREVIATIONS

BMI-1	Polycomb complex protein BMI-1
2OGXs	2-oxoglutarate (2-OG)
5-AZA	5-azacytidine
ABCG2	ATP-binding cassette transporter G2,
ADA	Adenosine deaminase
ADA2	Adenosine deaminase 2
ADP	Adenosine diphosphate
ANRIL	Antisense Non-Coding RNA in the INK4 Locus
anti-CTLA4	Anti-cytotoxic t-lymphocyte-associated protein 4
APC	APC regulator of WNT signaling pathway
ARHGDIB	Rho GDP dissociation inhibitor beta
ASCT	Autologous stem cell transplantation
ASPM	Abnormal spindle-like microcephaly-associated protein;
AURKA	Aurora kinase A
AURKB	Aurora kinase b
BCL2L11	BCL2 Like 11 (also known as BIM)
Bcl-XL	B-cell lymphoma extra large
BET	Bromodomain and extra-terminal domain
BID	Bh3 interacting domain death agonist
BIM	Bcl-2-like protein 11
BIRC5	Baculoviral iap repeat containing 5 (survivin)
BIRC5	Baculoviral IAP repeat-containing protein 5
BRAF	B-raf proto-oncogene, serine/threonine kinase
BUB1	Mitotic checkpoint serine/threonine-protein kinase BUB 1
CALCRL	Calcitonin receptor like receptor
CARM1	Coactivator-associated arginine methyltransferase 1
CASP1	Caspase 1
CBLN2	Cerebellin 2 precursor
CBP/p300	CREB-Binding Protein and E1A-Associated Protein P300
CCNA2	Cyclin A2
CCNB1	Cyclin B1

CCNB1	G2/mitotic-specific cyclin-B1
CCNB2	G2/mitotic-specific cyclin-B2
CCND1	Cyclin D1
CD4+ and CD8+	Cluster of Differentiation 4 and 8
CD47	Cluster of Differentiation 47
CDK1	Cyclin dependent kinase 1
CDC2	Cell Division Cycle 2, G1 to S and G2 to M
CDC20	Cell division cycle protein 20 homolog
CDCA8	Borealin
CDK1	Cyclin-dependent kinase 1
CDKN	Cyclin-dependent kinase inhibitor
CDKN2A	Cyclin-dependent kinase inhibitor 2a
CDKN2B	Cyclin-dependent kinase inhibitor 2b
CENPA	Histone H3-like centromeric protein A
CENPE	Centromere-associated protein E
CENPF	Centromere protein F
CEP55	Centrosomal protein of 55 kda
CHD1	Chromodomain-helicase DNA-binding 1
CIITA	Class II transactivator
circ-MYBL2:	Circular myb proto-oncogene like 2
circRNA	Circular rna (circular ribonucleic acid)
CKS1B	Cdc28 protein kinase regulatory subunit 1b
c-MTORC1	Mechanistic Target of Rapamycin Complex 1
c-Myc	Avian myelocytomatosis viral oncogene homolog
CNB1	Cyclin B1
CR2	Complement C3d receptor 2
CTLA-4	Cytotoxic t-lymphocyte-associated protein 4
CXCL9 and CXCL10	C-X-C Motif Chemokine Ligand 9 and 10
CXCR4	C-X-C motif chemokine receptor 4,
CYSLTR2	Cysteinyl leukotriene receptor 2
DAC	5-aza-2'-deoxycytidine
DAPK1	Death associated protein kinase 1
DCC	DCC netrin 1 receptor,
DDX3Y	ATP-dependent RNA helicase DDX3Y

DIS3	DIS3 Homolog, Exosome Endoribonuclease and 3'-5' Exoribonuclease
DKK3	Dickkopf WNT signaling pathway inhibitor 3,
DLGAP5	Disks large-associated protein 5
DNA	Deoxyribonucleic acid
DNMTs	DNA methyltransferases
DOCK10	Dedicator of cytokinesis 10
DOK2	Docking protein 2
EIF1AY	Eukaryotic translation initiation factor 1A, Y-chromosomal
ESMBL	European molecular biology laboratory
ETV5	ETS variant transcription factor 5
EZH2	Enhancer of zeste homolog 2
FADs	Flavin adenine dinucleotides
FAM46C	Family with sequence similarity 46, member c
FAT1	FAT atypical cadherin 1
FDA	United States Food and Drug Administration (FDA)
FHIT	Fragile histidine triad diadenosine triphosphatase,
FITC	Fluorescein isothiocyanate
FRMD6	FERM domain containing 6
G0/G1	Gap 0/gap 1
G2/M	Gap 2/ mitosis
GNAT	Gcn5-related n-acetyltransferases
GO	Gene ontology
GPX3	Glutathione peroxidase 3
GUARDIN	Gastric cancer associated rna, dna sequence
H2AC14	Histone H2A type 1-J
H2AC17	H2A clustered histone 17
H2BC17	Histone H2B type 1-O
H2BC4	Histone H2B type 1-C/E/F/G/I
H2BC5	Histone H2B type 1-D
H2BC7	H2B clustered histone 7
H3K27	Histone H3 lysine 27
H3K27me3	Histone h3 lysine 27 trimethylation
H3K36	Histone H3 lysine 36
H3K4	Histone H3 lysine 4

H3K79	Histone H3 lysine 79
H3K9	Histone H3 lysine 9
H4C3	H4 clustered histone 3
H4C5	H4 clustered histone 5
H4K20	Histone H4 lysine 20
HATs	Histone acetyltransferases
HDAC	Histone deacetylases
HDACi	Histone deacetylases inhibitor
HES6	Hes family bhlh transcription factor 6
HIF-1	Hypoxia-inducible factor 1
HJURP	Holliday junction recognition protein
HMGB2	High mobility group box 2
HMOX1	Heme oxygenase 1
IC_{50}	Half-maximal inhibitory concentration
IFI6	Interferon alpha inducible protein 6
IFIT1	Interferon induced protein with tetratricopeptide repeats 1
IFN	Interferon
IGF-1	Insulin-like growth factor 1
IGFBP7	Insulin like growth factor binding protein 7
IL-6	Interleukin 6
IMIDs	Immunomodulatory drugs
IRAG2	Inositol 1%2C4%2C5-triphosphate receptor associated 2
IRF4	Interferon regulatory factor 4
JAG2	Jagged canonical notch ligand 2
KDM3A	Lysine demethylase 3a
KDM5D	Lysine-specific demethylase 5D
KDM6A	Lysine demethylase 6a
KDM6B	Lysine demethylase 6b
KDMs	Histone lysine demethylases
KEGG	Kyoto Encyclopedia of Genes and Genomes
KIF11	Kinesin family member 11
KIF11	Kinesin Family Member 11 (also known as Eg5)
KIF20A	Kinesin-like protein KIF20A
KIF23	Kinesin-like protein KIF23

KIF2C	Kinesin-like protein KIF2C
KIF4A	Chromosome-associated kinesin KIF4A
KLF2	Kruppel-like factor 2
KRAS	Kirsten rat sarcoma viral oncogene homolog
LAPTm5	Transmembrane-5
LGALS1	Galectin 1
lncRNAs	Long non-coding rnas
mAbs)	Monoclonal antibodies
MAF	MAF BZIP transcription factor
MAGE	Melanoma antigen family
MALAT1	Metastasis-associated lung adenocarcinoma transcript 1
MAPK	Mitogen-activated protein kinase
MBD	Myeloma bone disease
MCC	MCC regulator of WNT signaling pathway
MCL-1	Myeloid cell leukaemia 1
MCM4	Minichromosome maintenance complex component 4
MEG3	Maternally expressed 3
METTL7A	Methyltransferase like 7A
MGMT	Methylguanine methyltransferase
MGUS	Monoclonal gammopathy of unknown significance
MHCII	Major histocompatibility complex class ii
MHCII and MCHI	Major Histocompatibility Complex I and II
miRNAs	Micrornas
MKI67	Marker of proliferation Ki-67
MLH1	Mutl Homolog 1
MM	Multiple myeloma
MORC1	MORC family CW-type zinc finger 1
mRNA	Messenger RNA
MX1	MX dynamin like gtpase 1
MYBPHL	MYBPHL: myosin binding protein H-like,
MYC	V-myc avian
MYST	MYST: MOZ, Ybf2/Sas3, Sas2, and Tip60
NCAM1	Neural cell adhesion molecule 1
NCBI	National Center for Biotechnology Information

ncRNAs	Non-coding rnas
NEAT1	Nuclear paraspeckle assembly transcript 1
NEK2	Serine/threonine-protein kinase Nek2
NELL2	Neural EGFL like 2
NFKB1	Nuclear factor kappa B subunit 1
NGS	Next-generation sequencing
NHP2L1	Nuclear protein, ataxia-telangiectasia locus-interacting protein 2-like 1
NLRC5	NOD-like receptor family CARD domain containing 5
NOP5/NOP58	Nucleolar protein 5/nucleolar protein 58
NOP56	Nucleolar protein 56
NRAS	Neuroblastoma ras viral oncogene homolog
NSD	Nuclear receptor binding SET domain
NUF2	Kinetochore protein Nuf2
OS:	Overall survival
OTOGL	Otogelin like
p21	P21^Cip1/Waf1 or Cyclin-Dependent Kinase Inhibitor 1A (CDKN1A)
p38	P38 Mitogen-Activated Protein Kinase
PAGE	Prostate-associated gene
PAN	Panobinostat
PANDA	P21 Associated ncrna DNA Damage Activated
PARP9	Poly(ADP-ribose) polymerase family member 9
PBK	Lymphokine-activated killer T-cell-originated protein kinase
PCDH1	Protocadherin 1
PCL	Plasma cell leukemia
PD-1	Programmed cell death protein 1
PD-L1	Programmed cell death 1 ligand 1
PD-L2	Programmed cell death 1 ligand 2
PFS	Progression-free survival
PI	Propidium iodide
PI3K/AKT	Phosphatidylinositol-3-kinase/protein kinase b
PIAS3	Protein Inhibitor of Activated STAT3
piRNAs	PIWI-interacting rnas
PIs	Proteasome inhibitors
PLK1	Serine/threonine-protein kinase PLK1

PPI	Protein-protein interaction
PRC2	Polycomb repressive complex 2
PRKCE	Protein kinase C epsilon type
PRM	Protamine
PS	Phosphatidylserine
PTMs	Post-translational modifications
PVT1	Plasmacytoma variant translocation 1
RAD54L	Rad54-like protein
RANKL	Receptor Activator of Nuclear Factor Kappa-Ligand
RASGRF2	Ras protein specific guanine nucleotide-releasing factor 2
RB52	Retinoblastoma protein
RBP1	Retinol binding protein 1
RNA	Ribonucleic acid
RNP	Ribonucleoprotein.
RPS4Y1	Ribosomal protein S4 Y-linked 1
RRM2	Ribonucleotide reductase regulatory subunit M2
rRNAs	Ribosomal rnas
S100A10 S100	Calcium binding protein A10
SAHA	Suberoylanilide hydroxamic acid
SATB1	SATB homeobox 1
SDCs	Syndecans
SELENOP	Selenoprotein P
SETD2	Set domain containing 2, histone lysine methyltransferase
SFR	SWI5 dependent homologous recombination repair protein,
shRNA	Short hairpin rna
SLC38A5	Solute carrier family 38 member 5
SLCO4A1	Solute carrier organic anion transporter family member 4A1
SMM	Smoldering MM
SNHG29	Small nucleolar RNA host gene 29
SNHG3	Small nucleolar RNA host gene 3
SNORNAs	H/ACA snornas
snRNAs	Small nucleolar rnas
SOCS	Suppressor of Cytokine Signaling
SOCS-1	Suppressor of Cytokine Signaling 1

SOCS3	Suppressor of Cytokine Signaling 3
SPAG5	Sperm associated antigen 5
SPANXSperm	Protein Associated with the Nucleus on the X Chromosome
SPARC	Secreted protein acidic & cysteine rich,
SQSTM1	Sequestosome 1
SSX	Synovial sarcoma, x breakpoint
STAT3	Signal Transducer and Activator of Transcription 3
TGFB1	Transforming growth factor beta-1,
TJP1	Tight junction protein 1
TMEM255B	Transmembrane protein 255B
TMSB10	Thymosin beta 10
TNFAIP8	Tumor necrosis factor alpha-induced protein 8
TOP2A	Dna topoisomerase ii alpha
TP53	Tumor protein p53
TPX2	Targeting protein for Xklp2
tRNAs	Transfer rnas (
TSA	Trichostatin A
TTK	Dual specificity protein kinase TTK
UBE2C	Ubiquitin-conjugating enzyme E2 C
UCSC	University of California, Santa Cruz
UHRF1	Ubiquitin like with PHD and ring finger domains 1
UHRF1	Ubiquitin like with PHD and ring finger domains 1
UNC13C	Unc-13 homolog C
USP9Y	Probable ubiquitin carboxyl-terminal hydrolase FAF-Y
UTX	Ubiquitously transcribed tetratricopeptide repeat, x chromosome
UTY	Histone demethylase UTY
WEE1	Wee1 g2 checkpoint kinase
WES	Whole-genome sequencing and protein-encoding exome sequencing
WHSC1	Wolf-Hirschhorn syndrome candidate 1
WIF1	Wnt inhibitory factor 1
WNT	Wingless-related integration site
WWOX	WW domain containing oxidoreductase

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NYAHPENGAWALATURAN PROFIL TRANSKRIPTOMIK TITISAN SEL MIELOMA BERBILANG MM1.S DAN U266 YANG DIRAWAT DENGAN PERENCAT EPIGENETIK

ABSTRAK

Multiple myeloma (MM) adalah hematologi malignan yang dicirikan oleh proliferasi sel plasma yang tidak terkawal dalam sumsum tulang. Nyahpengawalaturan epigenetik pula memainkan peranan penting dalam patogenesis MM, menjadikan perencat epigenetik sebagai sasaran terapeutik yang memberi harapan. Kajian ini mengkaji kesan tiga perencat epigenetik iaitu Trichostatin A (TSA), Panobinostat (PAN), dan 5- azacytidine (5- AZA) pada titisan sel MM1.S dan U266, yang memberi tumpuan kepada nyahpengawalaturan transkriptomik dan pengecaman gen teras yang berkaitan dengan hayat hidup pesakit MM. Lengkung tindak balas dos mendedahkan bahawa ketiga-tiga perencat epigenetik menghalang pergandaan sel dengan cara yang bergantung kepada dos, dengan PAN menunjukkan kesan anti-proliferatif yang paling kuat pada dos separuh kepekatan perencat maksimum (IC_{50}). Analisis sitometri aliran menunjukkan perubahan yang ketara dalam pengagihan kitaran sel selepas rawatan dengan perencat epigenetik. TSA, PAN, dan 5-AZA menginduksi penangguhan fasa G0/G1, penindasan dalam fasa S dan tiada perubahan yang diperhatikan dalam fasa G2/M dalam kedua-dua titisan sel MM1.S dan U266. Kajian apoptosis menunjukkan bahawa titisan sel MM1.S mengalami apoptosis fasa lewat dengan kesan tertinggi yang disebabkan oleh PAN. Sementara itu, titisan sel U266 pula menunjukkan apoptosis fasa awal selepas rawatan dengan perencat epigenetik dan kesan yang paling tinggi disebabkan oleh 5-AZA. Analisis pengayaan Ensiklopedia Genetik dan Genom Kyoto (KEGG) kedua-

dua titisan sel MM yang dirawat dengan perencat epigenetik ini telah mengenal pasti laluan penting yang melibatkan molekul lekatan sel, microRNA dalam kanser, dan interaksi protein virus dengan sitokin dan reseptor. Khususnya, kajian ini juga menunjukkan bahawa rawatan PAN dan 5-AZA menyebabkan kawal atur menaik gen histon teras tertentu (*H2A, H2B, H3, H4*), mempengaruhi struktur kromatin dan pengawalaturan gen, dengan itu mempengaruhi proses selular dan tindak balas terapeutik. Selain itu, analisis plot Kaplan-Meier mendedahkan bahawa gen teras yang dikaitkan dengan nyahpengawalaturan transkriptomik dikaitkan dengan peningkatan kadar kelangsungan hidup keseluruhan (OS). Bilangan tertinggi gen teras yang berkaitan dengan kelangsungan hidup ditemui dalam titisan sel yang dirawat dengan 5-AZA. Khususnya, rawatan 5-AZA dalam titisan sel MM1.S dan U266 meningkatkan pengekspresan gen teras yang serupa, menyebabkan pengawalaturan menurun *KIF20A, KIF4A*, dan *PLK*1, yang berkorelasi secara signifikan dengan peningkatan kadar OS (log-rank P: 1.4e-16). Dalam titisan sel MM yang dirawat dengan PAN, *ORC1, MCM2, MCM*5, dan *CXCL1* telah dikenal pasti sebagai gen teras dengan potensi terapeutik. Titisan sel U266 yang dirawat dengan TSA mendedahkan gen teras yang lebih tinggi daripada sel titik MM1.S, dengan *APOE* muncul sebagai gen utama yang dikaitkan dengan peningkatan kadar OS (log-rank P<1e-16). Secara keseluruhan, kajian ini memberikan pandangan komprehensif mengenai nyahpengawalaturan transkriptomik yang disebabkan oleh perencat epigenetik dalam titisan sel MM. Penemuan ini dapat meningkatkan pemahaman mengenai patogenesis MM dan menawarkan sasaran terapi yang berpotensi untuk merawat penyakit yang mencabar ini.

DYSREGULATION OF TRANSCRIPTOMIC PROFILES OF MM1.S AND U266 MULTIPLE MYELOMA CELL LINES TREATED WITH EPIGENETIC INHIBITORS

ABSTRACT

Multiple myeloma (MM) is a hematological malignancy characterized by the uncontrolled proliferation of plasma cells in the bone marrow. Epigenetic dysregulation plays a pivotal role in MM pathogenesis, making epigenetic inhibitors promising therapeutic targets. This study examines the effects of three epigenetic inhibitors—Trichostatin A (TSA), Panobinostat (PAN), and 5-azacytidine (5-AZA) on MM1.S and U266 cell lines, focusing on transcriptomic dysregulation and the identification of core genes associated with survival outcomes. Dose-response curves revealed that all three inhibitors inhibited cell proliferation in a dose-dependent manner, with PAN showing the most potent anti-proliferative effect at the half maximal inhibitory concentration ($IC_{50)}$ dose. Flow cytometry analysis indicated significant changes in cell cycle distribution upon treatment. TSA, PAN, and 5-AZA induced G0/G1 phase arrest, suppression in S phase and no changes observed in G2/M phase in MM1.S cells and U266 cells. Apoptosis assays demonstrated that MM1.S cell lines experienced late apoptosis with the highest impact induced by PAN. Meanwhile, U266 cell lines demonstrated early apoptosis event after treatment with epigenetic inhibitors and the most profound impact induced by 5-AZA. KEGG enrichment analysis of both MM cell lines treated with these epigenetic inhibitors identified significant pathways involving cell adhesion molecules, microRNAs in cancer, and viral protein interactions with cytokines and receptors. Notably, this study also demonstrated that PAN and 5-AZA treatments upregulated certain core

histone genes *(H2A, H2B, H3, H4*), co-impacting chromatin structure and gene regulation, thus influencing cellular processes and therapeutic responses. Furthermore, Kaplan-Meier plot analysis revealed that core genes linked to transcriptomic dysregulation were significantly associated with improved overall survival (OS) outcomes. The highest number of survival-associated core genes was found in 5-AZA-treated cell lines. Specifically, 5-AZA treatment increased the expression of similar core genes in both MM1.S and U266 cells, downregulating *KIF20A, KIF4A*, and *PLK1*, which correlated significantly with improved OS rate (log-rank P: 1.4e-16). In PAN-treated MM cell lines, *ORC1, MCM2, MCM5*, and *CXCL1* were identified as core genes with therapeutic potential. TSA-treated U266 cell lines revealed more significant core genes than MM1.S cell lines, with *APOE* emerging as a key gene linked to improved survival outcomes (log-rank P < 1e-16). Overall, this study provides comprehensive insights into the transcriptomic alterations induced by epigenetic inhibitors in MM cell lines. These findings enhance the understanding of MM pathogenesis and offer potential therapeutic targets for treating this challenging disease.

CHAPTER 1

INTRODUCTION

1.1 Multiple myeloma and epigenetic

Multiple myeloma (MM) is a haematological malignancy that involves the clonal expansion of plasma cells (Malard *et al.*, 2024). Interestingly, epigenetic dysregulation has been recognized as a crucial factor in the development and progression of MM, alongside genetic abnormalities (Alzrigat *et al.*, 2018c). Epigenetic modifications, such as DNA methylation, histone modifications, and non-coding RNAs, are essential in the development of MM (Ismail *et al.*, 2022). Epigenetic drugs, including histone deacetylase and DNA methylation inhibitors, have demonstrated significant potential in clinical trials for the treatment of MM (Issa *et al.*, 2017). This thesis aims to investigate the dysregulation of transcriptomic profiles in MM cell lines that have been treated with epigenetic inhibitors. The output of this study will provide insights into the impact of epigenetic modifications on MM pathobiology and explore the possibility of developing new treatment approaches.

1.2 Background to the study

The effects of epigenetic inhibitors, Trichostatin A (TSA), Panobinostat (PAN), and 5 Azacytidine (5-AZA), on transcriptomic dysregulation in MM cell lines were investigated in this study. Both TSA and PAN are classified as HDAC inhibitors (Zhang *et al.*, 2021), while 5-AZA is known as a DNA methylation inhibitor (Christman, 2002). The study was initially navigated by culturing the MM1.S and U266 cell lines and conducting an MTT assay to determine the IC_{50} values of each epigenetic inhibitor. The determination of IC_{50} values for epigenetic inhibitors is

crucial due to their potential as innovative therapeutic agents and their influence on diverse biological processes (Renner *et al.*, 2020). IC_{50} values are essential for determining the concentration of a drug needed to inhibit a biological process by 50% (Aykul and Martinez-Hackert, 2016). These values play a critical role in identifying safer epigenetic inhibitors with lower systemic toxicity, ultimately leading to potentially providing more effective treatments (Berrouet *et al.*, 2020). Understanding the effects of epigenetic inhibitors on cancer cells and predicting their response is crucial in the context of cancer, including MM. In this regard, the IC_{50} values of these inhibitors play a significant role. This groundbreaking effort is important for the development of effective treatment strategies and for understanding how epigenetic inhibitors work in MM.

Following this, the MM1.S and U266 cell lines were treated with the determined IC_{50} values. Subsequently, a range of assays, including apoptosis and cell cycle arrest assays, were conducted using a flow cytometry platform (Kluska *et al.*, 2023). The flow cytometry assay is a highly effective method for analyzing the induction of apoptosis in a cell population by detecting and quantifying apoptosis phases in a cell population (Telford, 2018). Through conducting an experiment wherein MM cell lines are treated with the inhibitor's IC_{50} value, followed by an apoptosis assay utilizing flow cytometry, an accurate assessment of the epigenetic inhibitor's capability to induce apoptosis in the MM cell lines can be obtained. It allows the analysis of individual cells in large quantities, rather than a heterogeneous population. Additionally, it has the capability to simultaneously assess cells that have been labelled with specific markers and indicators. This technique can assess the proportion of viable cells undergoing various stages of apoptosis, including early and late apoptosis, as well as cell necrosis induction (Telford, 2018) using the Annexin V

and propidium iodide (PI) staining method (Feng and Huang, 2021). In the context of apoptosis, Annexin V is a protein that exhibits a strong binding preference for phosphatidylserine (PS) (Reutelingsperger and van Heerde, 1997). This protein recognizes PS as it moves from the inner to the outer layer of the plasma membrane in the initial phases of apoptosis. PI, a fluorescent dye, is able to penetrate the membranes of late apoptotic and necrotic cells and bind to their DNA (Kari *et al.*, 2022). However, it is impermeable to live cells and early apoptotic cells. In practice, Annexin V is commonly labeled with a fluorophore, like fluorescein isothiocyanate (FITC), and PI fluorescence is detected in the phycoerythrin emission channel (Abbady *et al.*, 2017). Cells that exhibit Annexin V positivity and PI negativity are indicative of early apoptosis, whereas those that are double-positive indicate late-stage apoptosis or necrosis (Kari *et al.*, 2022). This study provides a quantitative analysis of apoptosis within a cell population, which is essential for investigating apoptotic cells. It allows for the identification of phosphatidylserine externalization and the assessment of membrane integrity. Consequently, this approach is valuable for evaluating the effectiveness of epigenetic inhibitors in inducing programmed cell death in MM cells.

The relationship between apoptosis and cell cycle regulation is intricately connected and considered a fundamental aspect of cellular physiology (Loftus *et al.*, 2022). In this study, the cell cycle arrest assay using flow cytometry was conducted after treating cells with the IC_{50} value of the epigenetic inhibitors. This assay enables the precise measurement of cell cycle phases, offering valuable insights into the distribution of cells across G0/G1, S, and G2/M phases. The quantitation of the S phase is crucial for comprehending the effects of the inhibitor on cell proliferation (Almalki, 2023), as it allows for a more precise assessment. In addition, flow

cytometry allows rapid and comprehensive analyses of the cell cycle, which is essential for investigating cell growth, differentiation, senescence, and apoptosis (Kim and Sederstrom, 2015). The integration of cell treatment, IC_{50} value determination, and cell cycle arrest assay through flow cytometry allows for the evaluation of the inhibitor's impact on cell cycle progression and proliferation. This analysis offers additional valuable insights into the potential therapeutic uses of the epigenetic inhibitor.

RT-qPCR was employed to assess the impact of epigenetic inhibitor treatment on the expression of HDAC subunits (Cheng *et al.*, 2021). The expression of HDAC subunits is one of key interest in epigenetics and MM research (Park *et al.*, 2021). HDACs are enzymes involved in chromatin modification, which plays a role in gene expression regulation (Nie *et al.*, 2024). They function by removing acetyl groups from histones and other protein regulatory factors (Milazzo *et al.*, 2020). The classification of HDAC subunits is based on their sequence homology and domain organization, resulting in four distinct classes. Class I HDACs (HDAC1, 2, 3, and 8) are predominantly found in the nucleus and play a role in gene expression regulation. Class II HDACs (HDAC4, 5, 6, 7, 9, and 10) are present in both the nucleus and cytoplasm and play a role in regulating cell differentiation and development. Class III HDACs, also known as SIRT1-7, rely on NAD+ and play a crucial role in controlling metabolism and the aging process. Class IV HDACs, specifically HDAC11, play a crucial role in gene expression regulation and cell differentiation (Curcio *et al.*, 2024). They share structural similarities with class I and II HDACs. There are considerable variation in the expression of HDAC subunits. HDAC inhibitors used in this study (TSA and PAN) have the ability to induce targeted alterations in gene expression and impact various cellular processes, such as growth

arrest, differentiation, cytotoxicity, and apoptosis induction (Wang *et al.*, 2017; El Omari *et al.*, 2023). HDAC inhibitors are crucial for the treatment of epigenetic cancer, especially in terms of inhibiting oncogenic signalling pathways (Jenke *et al.*, 2021). Additionally, several studies have demonstrated that HDAC inhibitors can modify the acetylation pattern of cytoplasmic proteins, thereby playing a role in their potential as anti-cancer agents (Li and Seto, 2016). Nevertheless, the impact of HDAC inhibitor treatment on HDAC subunit expression is intricate and necessitates additional research to comprehensively comprehend the molecular mechanisms that drive HDAC inhibitor function.

The RNA-Seq in Illumina platform was utilized to analyze the changes in the transcriptomic profile (Griffin *et al.*, 2023) of the treated MM cell lines. There are multiple reasons behind the pursuit of studying transcriptomic profiles in MM cells that exhibit significant epigenetic modifications. It is important to note that when epigenetic modulating agents are administered in vivo, they can cause changes in gene expression that are linked to prognosis and immunomodulation in MM (Cerchione *et al.*, 2022). This emphasizes the need to comprehend the effects of epigenetic modifications on the transcriptome in this particular disease. Furthermore, the DNA methylation patterns in MM exhibit significant diversity between patients, contributing to variations in gene expression. The presence of disrupted DNA methylation in MM is believed to be the cause of significant epigenetic variability (Derrien *et al.*, 2021c). Therefore, it is crucial to analyze transcriptomic profiles in order to fully understand the implications of these epigenetic changes.

The study explores the genetic and transcriptomic heterogeneity of MM, providing valuable insights into the coexisting transcriptional programs within

individual tumor cells and the underlying molecular mechanisms that drive disease progression. Gaining a comprehensive understanding of the transcriptomic landscape is crucial to unraveling the intricate nature of MM and emphasizing potential therapeutic targets. Furthermore, the investigation of transcriptomic and epigenomic changes plays a vital role in understanding the complex factors contributing to therapy resistance in MM. It is important to have a clear understanding of the existing epigenetic profiles of subclones that are linked to survival advantages, as well as the convergence of phenotypic adaptation among genetically distinct subclones. This highlights the necessity of incorporating multiomics analyses to monitor and describe mechanisms of therapy resistance. In this study, the analysis of mass RNA-Seq data has been conducted utilizing a a very broad range of bioinformatic tools including Cutadapt V1.9.1, FastQC V0.10.1, HISAT2 V2.2.1, HTSEQ V0.6.1, Stringtie V1.3.3b, DESeq2 V1.6.3, DESeq V1.18.0 , EdgeR V3.4.6, Cuffdiff V2.2.1, GOSeq V1.34.1, TopGO V2.18.0, Cufflinks V2.2.1, ASprofile V1.0.4, Cufflinks V2.2.1, samtools V0.1.19, Annovar V2016-05-11 and DEXSeq. These bioinformatic tools were used to detect genes that were expressed differently, as well as their corresponding gene ontology and significantly associated KEGG pathways. In addition, the protein-protein interaction (PPI) and the primary cluster and core genes implicated in the dysregulation of protein expression were predicted through the use of STRING and Cytoscape applications.

The overall survival (OS) analysis was conducted on the identified cluster of core genes that exhibit significant alterations in expression following treatment in my cell line models. The OS analysis is represents as a fundamental tool in cancer research, allowing for the assessment of patient outcomes over time (Goel *et al.*, 2010). This discovery underscores the potential significance of these genes in the

response to treatment and, consequently, patient survival outcomes. The rationale for proceeding with OS analysis of these core genes is multifaceted and compelling. Firstly, these genes have been identified as central players in pathways relevant to cancer progression, including cell cycle regulation, apoptosis, DNA repair, and immune response modulation. As such, their dysregulation may exert profound effects on tumor behavior and patient prognosis. Moreover, previous studies have implicated these genes in cancer development and progression, with evidence suggesting associations with treatment response and clinical outcomes across various cancer types. By investigating their impact on patient survival within the specific context of my treated cell lines, we can gain valuable insights into their clinical relevance and potential as prognostic biomarkers or therapeutic targets.

OS analysis offers a powerful means to explore the relationship between gene expression profiles and patient outcomes, providing a direct link between molecular alterations and clinical endpoints (Creighton, 2023). In the final stage, Kaplan-Meier plots and related statistical analyses were utilised to assess the impact of these core genes on survival probabilities, elucidating their prognostic significance and informing personalized treatment strategies. The identification of core genes in treated cell lines presented a unique opportunity to investigate their role in patient survival outcomes (Jiang *et al.*, 2021). By employing OS analysis techniques, this study aim to unravel the complex interplay between molecular alterations, treatment response, and clinical outcomes, ultimately advancing understanding of cancer biology and guiding the development of more effective therapeutic interventions. By uncovering how these core elements interact, this study will identify new biomarkers for MM diagnosis, prognosis, and disease monitoring, which may lead to more tailored and effective treatment strategies.

1.3 Statement of the problem

Despite significant notable progress in comprehending the genetic and epigenetic factors involved in the pathogenesis of MM, complete characterization regarding the precise influence of epigenetic inhibitors on the transcriptomic profiles of MM cell lines is still lacking. The aforementioned main mechanisms driving epigenetic dysregulation have been recognized as a significant factor in the development and progression of MM. However, the specific changes in the transcriptomic profiles of MM cell lines in response to epigenetic inhibitors have not been completely elucidated. It is essential to comprehend the dysregulation of transcriptomic profiles in MM cell lines that have been treated with epigenetic inhibitors. This understanding is crucial for uncovering the wider implications of epigenetic modifications on MM pathobiology and for identifying new potential treatment approaches. Therefore, this study is aimed to address a gap in knowledge by examining the precise modifications in gene expression caused by epigenetic inhibitors in MM cell lines. As a result, this study will enhance understanding of the relationship between epigenetics and the emergence and progression of MM.

1.4 Justification of the study

Studying the dysregulation of transcriptomic profiles of MM cell lines treated with epigenetic inhibitors is important due to the significant role of epigenetics in MM pathobiology and the potential for new treatment options. Multiple studies have emphasized the significance of epigenetic modifications in the progression of MM and the mechanisms by which DNA methylation, histone modifications, and noncoding RNAs influence disease biology. This study utilized the MM1.S and U266 cell lines due to their pivotal role in examining the impact of epigenetic inhibitors on

MM (Fulciniti et al., 2018). Threfore, these study anticipated to reveal alterations in their dysregulation of transcriptomic profiles associated with MM pathogenesis and progression.

The progress made in the field of epigenetic drugs, specifically histone deacetylase inhibitors, has demonstrated encouraging results in clinical trials focused on the treatment of MM. In addition, it is crucial to explore the specific transcriptomic changes caused by epigenetic inhibitors in MM cell lines (Caprio *et al.*, 2020a), considering the significance of identifying epigenetic drivers and understanding their impact on MM. This research also highlights the importance of studying the potential role of epigenetic dysregulation in MM.

In this study, Trichostatin A (TSA), Panobinostat (PAN) and 5-Azacytidine (5-AZA) were used as epigenetic ihhibitors on studying cellular changes and transcriptomic dysregulation in MM cell lines. TSA is a formidable inhibitor of histone deacetylases (HDACs), stands at the forefront of epigenetic research due to its potent and selective action. TSA functions by disrupting the deacetylation of histones, thereby inducing a hyperacetylated chromatin state. This chromatin remodeling facilitates the reactivation of previously silenced genes, providing a profound insight into the epigenetic landscape of MM (Li *et al.*, 2020) . The choice of TSA is driven by its well-established role in unraveling the complexities of HDAC-mediated gene repression and its capacity to unveil critical alterations in the transcriptomic profiles of MM1.S and U266 cell lines.

In addition, PAN represents a cutting-edge advancement in the realm of HDAC inhibition, possessing a broad-spectrum inhibitory profile that encompasses both class I and class II HDACs (Gillette, 2021). Its selection is not merely strategic

but transformative, given its clinical significance as a therapeutic agent for relapsed or refractory MM (Pan *et al.*, 2023). PAN's ability to induce profound changes in histone acetylation and gene expression (Moshref *et al.*, 2021) offers a unique opportunity to explore the dynamic and intricate mechanisms underlying transcriptomic dysregulation in MM. The integration of PAN into this study promises to yield unparalleled insights into the therapeutic potential and mechanistic actions of HDAC inhibitors in MM pathology.

Moreover, 5-AZA stands as a cornerstone of epigenetic intervention through its role as a potent DNA methyltransferase inhibitor. By specifically targeting and inhibiting the enzymes responsible for DNA methylation, 5-AZA disrupts aberrant DNA methylation patterns that contribute to gene silencing and genomic instability (Uddin and Fandy, 2021). Its application in this study is pivotal for dissecting the contributions of DNA methylation alterations to the dysregulation of transcriptomic profiles in MM1.S and U266 cell lines. The deployment of 5-AZA facilitates a comprehensive understanding of how demethylation can reverse pathological gene silencing and impact MM cell behavior.

1.5 Significance of the study

Understanding the specific transcriptomic changes induced by epigenetic inhibitors in MM cell lines is crucial for unraveling the broader impact of epigenetic modifications on MM pathobiology and for identifying potential novel treatment approaches. Therefore, this study's significance lies in its potential to contribute to the knowledge of MM pathogenesis and the development of new therapeutic strategies.

1.6 Objectives of the study

The general objective of this study is to investigate the dysregulation of transcriptomic profiles in MM cell lines treated with epigenetic inhibitors, shedding light on the impact of epigenetic modifications on MM pathobiology and the potential for novel treatment modalities.

Specifically, this study outlines the specific objectives as follows:

1. To quantify the IC_{50} for epigenetic inhibitors TSA, PAN and 5-AZA in MM1.S and U266 MM cell lines by MTT assay.
2. To evaluate the cells apoptosis induction in epigenetic inhibitors treated MM cells lines by flow cytometry.
3. To access the cell cycle progression arrest in epigenetic inhibitors treated MM cells lines by flow cytometry.
4. To determine the alteration of Histone Deacetylase (HDAC) subunit expression in both HDAC inhibitors (TSA and PAN) treated MM cells lines by RT-qPCR assay.
5. To determine the overall dysregulation of transcriptomic profiles of both epigenetic inhibitors-treated MM cell lines by RNASeq.

Figure 1.1 presents a comprehensive overview of the experimental process, along with a sequential arrangement of the specific objectives, to facilitate a deeper understanding of the study.

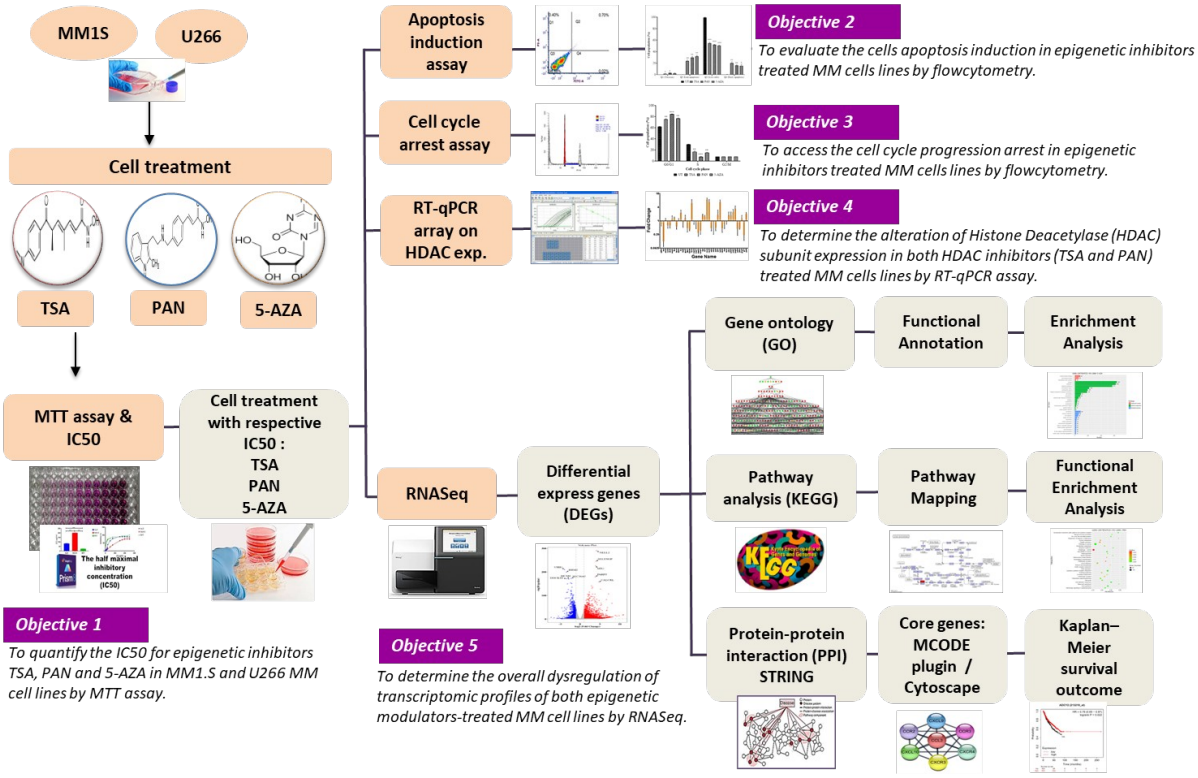

Figure 1.1 A comprehensive overview of the experimental process and sequential arrangement of the specific objectives

CHAPTER 2

LITERATURE REVIEW

2.1 Multiple Myeloma

Multiple myeloma (MM) was initially observed by William Macintyre in 1850 when he noticed proteinuria in samples from a patient experiencing intense chest and thoracolumbar pain for about a year. Renowned chemical pathologist Henry Bence Jones confirmed this discovery by analyzing the samples in 1848 (Kyle, 1994). In 1889, Otto Kähler published a follow-up study spanning eight years (1879-1887) of a 46-year-old patient exhibiting significant symptoms including bone pain, albuminuria, anemia, severe kyphosis, recurrent bronchial infections, and loss of height. Kähler acknowledged the protein in the patient's urine, noting its similarity to the one described by Bence Jones (Kyle, 1994).

MM is categorized as a B-cell malignancy affecting long-lived plasma cells residing in the bone marrow. These cells are non-dividing and terminally differentiated, with lifespans ranging from months to years. Normally, healthy plasma cells play a vital role in the immune system by producing antigen-specific immunoglobulins or antibodies to fight infections and diseases (Schirrmacher, 2023). B cells undergo stimulation and differentiation processes, eventually producing plasma cells that generate antibodies with high affinity for specific antigens. This process occurs within a timeframe conducive to effective host protection. However, rapid proliferation and somatic mutation of the B cell receptor can lead to oncogenic genetic variants, resulting in B cell malignancies such as MM (Barwick *et al.*, 2019a). Figure 2.1 illustrates the hematopoietic stem cell differentiation pathways and the generation of plasma cells from the B stem cell lineage.

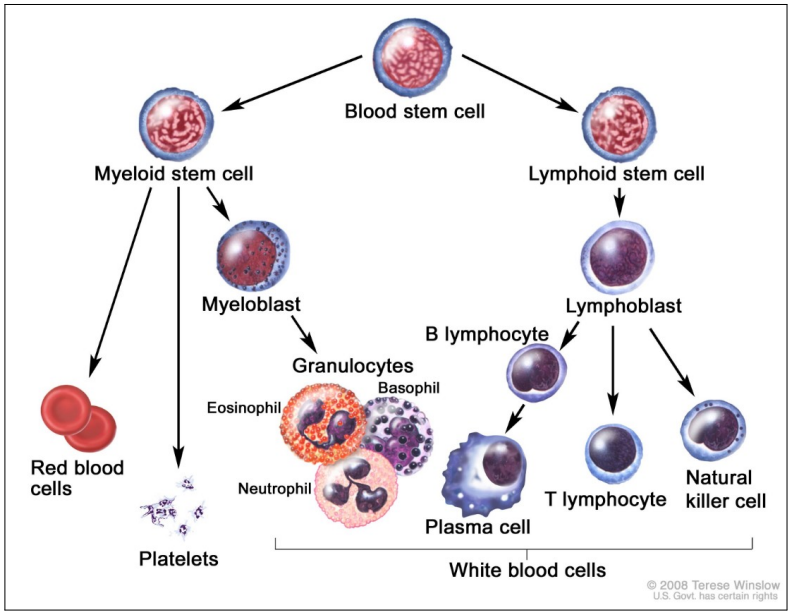

Figure 2.1 Haematopoietic stem cell differentiation pathways. (https://www.cancer.gov/publications/dictionaries/cancer-terms/def/plasma-cell**).**

MM, also referred to as Kähler's disease, myelomatosis, and plasmacytic myeloma, is a hematological malignancy characterized by abnormal and clonal proliferation of plasma cells within the bone marrow (Guedes *et al.*, 2023). These cells produce and secrete an abnormal monoclonal immunoglobulin, termed M protein, comprising a heavy chain (IgG γ, IgA α, IgM μ, IgD d, or IgE ε) and a light chain (kappa-κ and lambda-λ) (Jiang *et al.*, 2018). Clinical manifestations of MM stem from the increased presence of neoplastic plasma cells, excessive production of monoclonal immunoglobulin, and suppression of normal humoral immunity (Nobari *et al.*, 2022). Disease progression leads to various pathophysiological consequences, including hypercalcemia, bone deterioration, renal dysfunction, hematopoietic suppression, and compromised humoral immunity (Rajkumar, 2020a), heightening susceptibility to infections (Lin *et al.*, 2020). In advanced stages, a minority of MM patients (1% to 2%) present with extramedullary disease at diagnosis, with a slightly higher proportion (8%) developing it as the disease advances (Rajkumar, 2020b).

2.2 Epidemiology of MM

MM ranks as the second most prevalent hematologic malignancy globally, following non-Hodgkin lymphoma (Nikolaou *et al.*, 2022). It comprises 1.8% of all newly diagnosed cancer cases and 18% of hematologic malignancies in the United States (US). By 2022, an estimated 34,470 cases will be diagnosed, resulting in 12,640 fatalities (https://cancerstatisticscenter.cancer.org/). Worldwide, Malaysia (0.75/100,000), South Korea (0.54/100,000), the Philippines (0.86/100,000), and China (0.92/100,000) exhibit some of the lowest incidence rates, while Saudi Arabia (1.0/100,000) and India (1.0/100,000) closely follow. Conversely, New Zealand (5.3/100,000), Australia (5.0/100,000), the U.K. (4.3/100,000), Israel, and Norway (4.2/100,000) report the highest incidence rates. It was also reported that significant global variation in age-standardized incidence rates, ranging from 0.1 to 5.3 per 100,000 (Ludwig *et al.*, 2020).

Countries like Egypt, the Philippines, Thailand, Indonesia, Mexico, South Korea, and the United Arab Emirates exhibit low 5-year survival rates for MM, with mortality-to-incidence ratios of 9%, 10%, 13%, and 15%, respectively. Conversely, New Zealand (64%), Iceland (62%), the U.K. (60%), Belgium (59%), and Australia (57%) show higher survival rates (Ludwig *et al.*, 2020). The findings on the global incidence of MM, revealing a significant variation in age-standardized incidence rates ranging from 0.1 per 100,000 to 5.3 per 100,000. In addition, the study demonstrated that countries such as Egypt, the Philippines, Thailand, Indonesia, Mexico, South Korea, and the United Arab Emirates had low 5-year survival rates for MM, as indicated by their low mortality-to-incidence ratios. Egypt had the lowest rate at 9%, followed by the Philippines at 10%, Thailand at 13%, and Indonesia, Mexico, South Korea, and the

UAE all at 15%. On the other hand, New Zealand associated with the highest observed outcome (64%), followed by Iceland (62%), the U.K. (60%), Belgium (59%), and Australia (57%) (Ludwig *et al.*, 2020). Furthermore, there are several noteworthy risk factors that have been previously associated with MM, such as male gender (Fillmore *et al.*, 2019), other genetic variants (Chubb *et al.*, 2013), familial and ethnic background (VanValkenburg *et al.*, 2016), obesity (Marinac *et al.*, 2018), environmental factors and life style (Sergentanis *et al.*, 2015). Figure 2.2 depicts the estimated worldwide age-standardized incidence rate and the number of MM incidence cases and deaths in 2020.

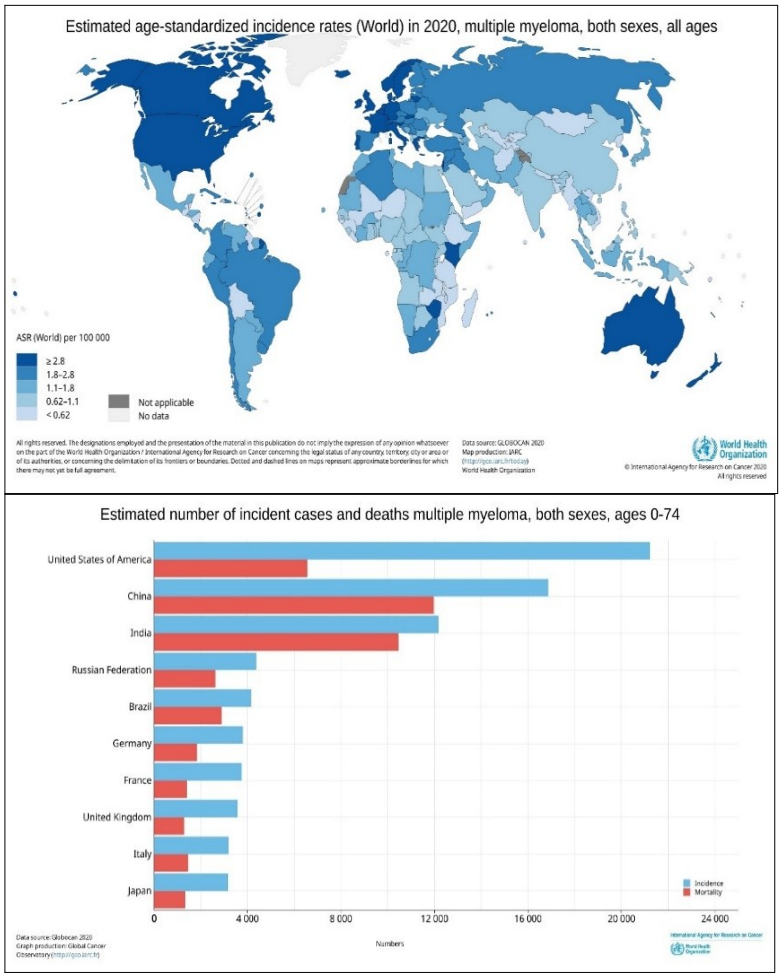

Figure 2.2 (Above) Worldwide estimation of age-standardized incidence rates of MM in 2020, across both sexes and all ages. (Below) Worldwide estimation number of incident cases and deaths MM, both sexes, ages 0-74 ((Sung *et al.*, 2021).

2.3 Etiology of MM

The exact cause of MM remains elusive, despite ongoing research . Disparities in MM occurrence and outcomes, including mortality rates, are influenced by various factors such as sociodemographic variables—age, gender, race/ethnicity, socioeconomic status—and geographic location. These factors affect healthcare utilization patterns, treatment access, and clinical trial participation, leading to differences in patient outcomes (Habr and Corsaro, 2022). Despite recent therapeutic advancements, progress in improving MM outcomes, especially among the elderly and diverse patient populations, remains limited (Kanapuru *et al.*, 2022). Disparities in MM healthcare extend to diagnosis, survival rates, and treatment access, with Black Americans experiencing a higher incidence of the disease, potentially twofold greater than other demographic groups (Bhutani *et al.*, 2023). Black American patients are often diagnosed with MM at more advanced stages and encounter barriers to accessing the latest and most effective treatments (Bhutani *et al.*, 2023).

2.4 Pathophysiology of MM

MM arises from abnormal B-cells and is characterized by the clonal expansion of plasma cells (PCs) and the overproduction of monoclonal proteins, primarily within the bone marrow (Abduh, 2024). This dysregulated process can lead to organ dysfunction over time (Palumbo and Anderson, 2011; Ji *et al.*, 2024). MM predominantly affects older individuals, with a median age at diagnosis of 69 years. This demographic is associated with a poorer prognosis, with only 55% of patients surviving for five years after diagnosis. One hypothesis proposes that the MM clone originates from post-germinal center B-cells, resulting in the abnormal proliferation of plasma cells capable of indefinite growth (Rizq *et al.*, 2023). Patients in the initial

stages of MM are identified with a non-symptomatic condition called monoclonal gammopathy of unknown significance (MGUS), which is a pre-cancerous stage that can progress to asymptomatic or smoldering MM (SMM) and inevitably to symptomatic MM (Landgren *et al.*, 2009; Rajkumar *et al.*, 2022).

2.4.1 Monoclonal gammopathy of unknown significance (MGUS)

Monoclonal gammopathy of undetermined significance (MGUS) represents a premalignant condition preceding MM, characterized by an abnormality in plasma cells (Dispenzieri, 2023; Mikhael *et al.*, 2024). This benign disorder is defined by a reduction in a specific blood protein known as monoclonal protein or M-protein (Kaseb *et al.*, 2024). The asymptomatic preneoplastic plasma cell disorder in MGUS is distinguished by a serum M-protein level <30 g/L, clonal plasma cells in the bone marrow <10%, and the absence of organ damage typically associated with plasma cell myeloma (such as hypercalcemia, renal insufficiency, anemia, or bone lesions) (Landgren, 2021). Additionally, MGUS is diagnosed only when there is no evidence of B-cell lymphoma or other disorders producing M-protein. Patients with MGUS do not manifest any symptoms indicative of an active pathological condition (Landgren *et al.*, 2009; Landgren, 2021).

The prevalence of MGUS increases with age, with the median age of diagnosis being 70 years. It affects 3.2% of individuals aged 50 and older, rising to 8.9% among those aged 85 and older (Atkin *et al.*, 2018). Annually, approximately 1% of individuals with MGUS progress to develop MM, with the chance of progression from MGUS to active myeloma being less than 1%. Only 20% of MGUS patients actually experience progression to active myeloma (Castaneda-Avila *et al.*, 2021). Genomic events observed in myeloma provide evidence for the transformation of mature B cells to MGUS. These

events encompass structural variants, changes in driver genes, clonal IGH translocations, alterations in genomic copy numbers, hyperdiploidy, and MYC translocation (Landgren, 2021; Oben *et al.*, 2021; Chojnacka *et al.*, 2022) (Figure 2). MGUS is typically detected incidentally during blood tests conducted for unrelated medical reasons, and there is currently no targeted intervention available to prevent its progression to MM. The appropriate course of follow-up care for individuals with MGUS is determined based on their initial risk assessment (Castaneda-Avila *et al.*, 2021). Low-risk MGUS carries a 2% risk of developing MM or a similar disorder within 20 years, while high-risk MGUS presents a greater likelihood of progression. Patients diagnosed with MGUS should receive ongoing monitoring from a specialist in blood disorders and cancer. Follow-up care may entail regular examinations of blood and urine samples to monitor M-protein levels (Castaneda-Avila *et al.*, 2021).

2.4.2 Smoldering MM (SMM)

SMM represents a preneoplastic condition characterized by an increased presence of plasma cells in the bone marrow and/or elevated levels of M protein in the bloodstream. It serves as a precursor to MM and carries the potential to evolve into active MM. While typically asymptomatic, individuals with SMM face a higher risk of progressing to MM compared to those with monoclonal gammopathy of undetermined significance (MGUS) (Rajkumar *et al.*, 2015; Vaxman and Gertz, 2022). In patients diagnosed with SMM, the annual risk of progression to MM is approximately 10%, significantly higher than the 1% annual risk observed in MGUS patients during the initial 5-year period post-diagnosis. While there are currently no definitive methods to prevent SMM, ongoing research is focused on identifying treatments capable of delaying or impeding MM development (Rajkumar *et al.*, 2022). SMM diagnosis is

established when patients meet specific criteria, including a spike in serum monoclonal protein (IgG or IgA) exceeding 3 g/dl, detected urinary monoclonal protein ≥500 mg per 24-hour collection, and/or 10-60% bone marrow clonal plasma cells during follow-up, along with no evidence of myeloma-defining events or amyloidosis (Rajkumar *et al.*, 2022).

The transition from SMM to MM involves complex genomic and transcriptional alterations. Previous studies have indicated a significant similarity in the genomic landscape, including mutational profiles and structural rearrangements, between SMM and MM. However, the progression to MM is characterized by diverse evolutionary patterns, distinct genetic mutations, notable changes in tumor suppressor genes, and alterations in specific genomic copy numbers. These mechanisms play pivotal roles in driving disease progression (Bolli *et al.,* 2018). Additionally, single-cell profiling has revealed that plasma cell subpopulations remain consistent during the transition from the pre-cancerous stage to MM. However, these subpopulations dynamically gain or lose characteristics as the disease advances, providing valuable insights into the stability and alterations in transcription associated with disease progression (Boiarsky *et al.*, 2022).

Close observation and monitoring of patients with SMM for signs of progression to active MM may prompt participation in clinical studies aimed at assessing more effective management strategies. It is important to note that not all patients with SMM will progress to active MM, and vigilant monitoring can effectively manage the condition (Rajkumar *et al.*, 2022). Genetic and epigenetic modifications acquired during MGUS and SMM influence genome integrity from the early stages of the disease and persist throughout its progression, shaping its trajectory (Kuehl and Bergsagel, 2002).

Figure 2.3 provides a comprehensive overview of the clinical model for tracking disease progression in MM, with a focus on the International Myeloma Working Group (IMWG) diagnostic criteria (Ho *et al.*, 2020b) .

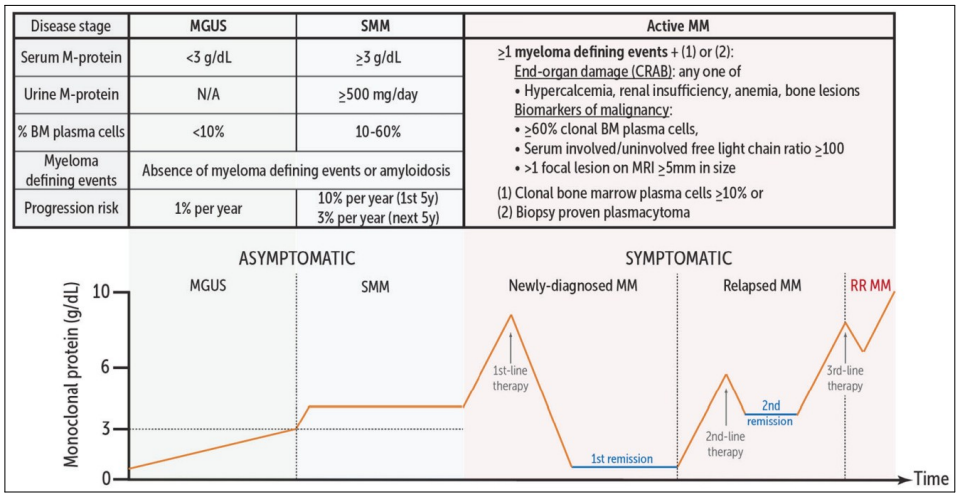

Figure 3.3 Clinical model of disease progression in MM based on the IMWG diagnostic criteria (Ho *et al.*, 2020b).

2.4.3 Plasma cell leukemia

Additionally, the bone marrow microenvironment emerges as a pivotal factor influencing MM progression and longevity (Mitsiades *et al.*, 2007). Notably, malignant plasma cells can acquire novel abnormalities that enable their survival outside the bone marrow niche. Consequently, they may disseminate via the bloodstream or migrate to other tissues, culminating in the development of more aggressive stages such as plasma cell leukemia (PCL) or extramedullary plasmacytomas (Palumbo and Anderson, 2011; Gundesen *et al.*, 2019a). CL, characterized by a significant quantity of aberrant plasma cells in the peripheral blood, is categorized into primary and secondary types. Primary PCL occurs in patients without a prior MM history, whereas secondary PCL arises in individuals with a known MM background (Gundesen *et al.*, 2019b). Recent updates to

the diagnostic criteria for PCL have lowered the threshold for the percentage of circulating plasma cells from 20% to 5% (Fernández de Larrea *et al.*, 2021). Primary PCL typically presents with aggressive clinical features and unfavorable molecular abnormalities compared to MM, often accompanied by significant symptoms and complications such as bone pain, fatigue, infections, bleeding, hypercalcemia, renal impairment, and hepatosplenomegaly (Chaulagain *et al.*, 2021). The etiology of PCL shares similarities with MM, with genetic alterations during plasma cell development implicated in cellular transformation (Sher *et al.*, 2010). While the exact cause of PCL remains incompletely understood, factors like age and exposure to industrial and environmental elements are believed to play significant roles (Wong *et al.*, 2016). reatment of PCL typically involves aggressive chemotherapy, with autologous stem cell transplantation recommended for eligible patients. Post-transplant maintenance therapy aims to reduce the incidence of early relapses; however, PCL is associated with an unfavorable prognosis, often resulting in early relapse (Jung and Lee, 2022).

2.4.4 Myeloma bone disease

The progression of MM may lead to the development of myeloma bone disease (MBD), a significant complication commonly observed in MM patients and a key clinical concern in disease management. MBD manifests as bone lesions that cause discomfort, fractures, mobility issues, and neurological deficits, contributing substantially to disability and morbidity among MM patients (Mukkamalla and Malipeddi, 2021). The pathophysiology of MBD involves an imbalance between bone destruction and formation, primarily driven by myeloma cells. These cells produce molecules that activate osteoclasts, leading to uncontrolled bone resorption and the

formation of lytic lesions, weakening bones and increasing susceptibility to fractures and skeletal-related events (Bernstein *et al.*, 2022).

Diagnosis of MBD usually entails a combination of imaging studies such as X-rays, along with bone marrow aspiration/biopsy and blood/urine tests to identify specific proteins associated with bone disease (Pop *et al.*, 2022). Management of MBD in MM patients focuses on preventing skeletal-related events, managing bone pain, and reducing fracture risk. Treatment strategies may involve chemotherapy, radiation therapy, surgical interventions, and medications aimed at preserving bone health (Pop *et al.*, 2022). Effectively managing MBD is paramount in providing comprehensive care to MM patients and addressing their clinical needs within the context of the disease.

2.5 Challenges in treating MM

Treating MM poses significant challenges, including drug resistance, relapse, and refractory disease (Rafae *et al.*, 2024), complicating the management of this condition. Relapse is a common occurrence, and in some instances, treatment resistance develops, exacerbating the difficulties in combating MM (Das *et al.*, 2022a). Moreover, determining the optimal sequencing strategy for various agents remains uncertain, leading to ambiguity regarding the most effective treatment approach (Walia *et al.*, 2023). Currently, pharmaceutical industries have proven notable advancements in the utilization of advanced treatments. Figure 2.4 illustrates the comprehensive timeline of global drug discovery and approval in MM treatment, showcasing the utilization of various medications with diverse modes of action. These include combining different medications with diverse modes of action, such as corticosteroids, proteasome inhibitors (PIs), anthracyclines, alkylating agents, monoclonal antibodies (mAbs), immunomodulatory drugs (IMIDs), nuclear export inhibitors, histone deacetylase

inhibitors (HDACis), and administering high doses of chemotherapy accompanied by autologous stem cell transplantation (ASCT) (Cejalvo and de la Rubia, 2017; Rajkumar, 2019). The meta-analysis found varying success rates for different HDACis in treating relapsed/refractory MM. Panobinostat showed the highest overall response rate at 64%, and it was particularly effective in patients who were refractory to lenalidomide, followed by vorinostat (51%) and ricolinostat (38%) (Gao *et al.*, 2019). Nevertheless, MM still stands as a highly relapse, incurable, and fatal disease, although it can be managed.

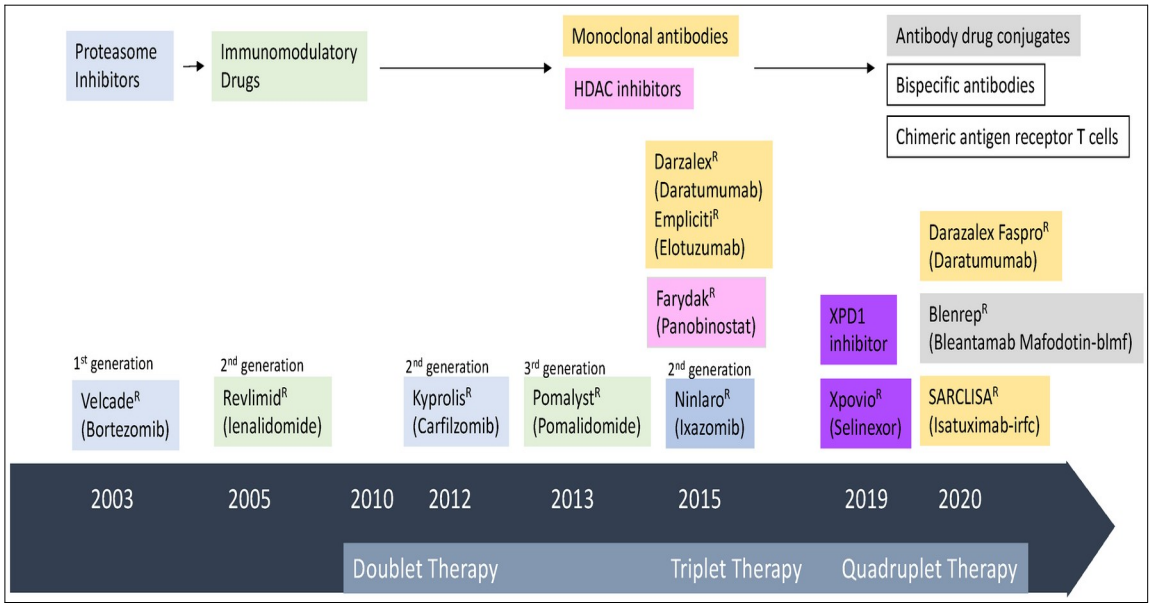

Figure 2.1 The comprehensive timeline established for global drug discovery and approval in MM treatment (Nishida, 2021).

The intricate genetic heterogeneity and variability among individual cancer cell populations in MM present substantial hurdles in developing successful treatments (Das *et al.*, 2022a). Additionally, the bone marrow microenvironment and the overexpression of RANKL by bone marrow stroma pose significant challenges in MM treatment, particularly in managing myeloma bone disease (Gau *et al.*, 2022). Furthermore,