UPPSALA UNIVERSITET

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1296

Targeted Inhibition of Polycomb Repressive Complexes in Multiple Myeloma

Implications for Biology and Therapy

MOHAMMAD ALZRIGAT

ACTA UNIVERSITATIS UPSALIENSIS UPPSALA 2017

ISSN 1651-6206
ISBN 978-91-554-9805-4
urn:nbn:se:uu:diva-312250

Dissertation presented at Uppsala University to be publicly examined in Rudbeck Hall, Rudbecklaboratoriet, 751 85 Uppsala, Sweden, Uppsala, Thursday, 16 March 2017 at 09:15 for the degree of Doctor of Philosophy (Faculty of Medicine). The examination will be conducted in English. Faculty examiner: Docent Kristina Drott (Lund University).

Abstract

Alzrigat, M. 2017. Targeted Inhibition of Polycomb Repressive Complexes in Multiple Myeloma. Implications for Biology and Therapy. *Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine* 1296. 53 pp. Uppsala: Acta Universitatis Upsaliensis. ISBN 978-91-554-9805-4.

Multiple myeloma (MM) is a hematological malignancy of antibody producing plasmablasts/ plasma cells. MM is characterized by extensive genetic and clonal heterogeneity, which have hampered the attempts to identify a common underlying mechanism for disease establishment and development of appropriate treatment regimes. This thesis is focused on understanding the role of epigenetic regulation of gene expression mediated by the polycomb repressive complexes 1 and 2 (PRC1 and 2) in MM and their impact on disease biology and therapy.

In **paper I** the genome-wide distribution of two histone methylation marks; H3K27me3 and H3K4me3 were studied in plasma cells isolated from newly diagnosed MM patients or age-matched normal donors. We were able to define targets of H3K27me3, H3K4me3 and bivalent (carry both marks) which are, when compared to normal individuals, unique to MM patients. The presence of H3K27me3 correlated with silencing of MM unique H3K27me3 targets in MM patients at advanced stages of the disease. Notably, the expression pattern of H3K27me3-marked genes correlated with poor patient survival. We also showed that inhibition of the PRC2 enzymatic subunit EZH2 using highly selective inhibitors (GSK343 and UNC1999) demonstrated anti-myeloma activity using relevant *in vitro* models of MM. These data suggest an important role for gene repression mediated by PRC2 in MM, and highlights the PRC2 component EZH2 as a potential therapeutic target in MM.

In **paper II** we further explored the therapeutic potential of UNC1999, a highly selective inhibitor of EZH2 in MM. We showed that EZH2 inhibition by UNC1999 downregulated important MM oncogenes; IRF-4, XBP-1, BLIMP-1and c-MYC. These oncogenes have been previously shown to be crucial for disease establishment, growth and progression. We found that EZH2 inhibition reactivated the expression of microRNAs genes previously found to be underexpressed in MM and which possess potential tumor suppressor functions. Among the reactivated microRNAs we identified miR-125a-3p and miR-320c as predicted negative regulators of the MM-associated oncogenes. Notably, we defined miR-125a-3p and miR-320c as targets of EZH2 and H3K27me3 in MM cell lines and patients samples. These findings described for the first time PRC2/EZH2/H3K27me3 as regulators of microRNA with tumor suppressor functions in MM. This further strengthens the oncogenic features of EZH2 and its potential as a therapeutic target in MM.

In **paper III** we evaluated the therapeutic potential of targeting PRC1 in MM using the recently developed chemical PTC-209; an inhibitor targeting the BMI-1 subunit of PRC1. Using MM cell lines and primary cells isolated from newly diagnosed or relapsed MM patients, we found that PTC-209 has a potent anti-MM activity. We showed, for the first time in MM, that PTC-209 anti-MM effects were mediated by on-target effects i.e. downregulation of BMI-1 protein and the associated repressive histone mark H2AK119ub, but that other subunits of the PRC1 complex were not affected. We showed that PTC-209 reduced MM cell viability via significant induction of apoptosis. More importantly, we demonstrated that PTC-209 shows synergistic anti-MM activity with other epigenetic inhibitors targeting EZH2 (UNC1999) and BET-bromodomains (JQ1). This work highlights the potential use of BMI-1 and PRC1 as potential therapeutic targets in MM alone or in combination with other anti-MM agents including epigenetic inhibitors.

Keywords: Multiple Myeloma, Epigenetics, Polycomb, PRC2, PRC1, EZH2, BMI-1, UNC1999, PTC-209, Epigenetic Therapy

Mohammad Alzrigat, Department of Immunology, Genetics and Pathology, Experimental and Clinical Oncology, Rudbecklaboratoriet, Uppsala University, SE-751 85 Uppsala, Sweden.

ISSN 1651-6206
ISBN 978-91-554-9805-4
urn:nbn:se:uu:diva-312250 (http://urn.kb.se/resolve?urn=urn:nbn:se:uu:diva-312250)

To my parents and my lovely little family

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I Agarwal, P. ‡, **Alzrigat, M**.‡, Párraga, AA., Enroth, S., Singh, U., Ungerstedt, J., Österborg, A., Brown, PJ., Ma, A., Jin, J., Nilsson, K., Öberg, F., Kalushkova, A.*, and Jernberg-Wiklund, H. * (2016) Genome-wide profiling of histone H3 lysine 27 and lysine 4 trimethylation in multiple myeloma reveals the importance of Polycomb gene targeting and highlights EZH2 as a potential therapeutic target. ***Oncotarget***. 7(6):6809-23.

I **Alzrigat, M.***, Párraga, AA., Agarwal, P., Zureigat, H., Österborg, A., Nahi, H., Ma, A., Jin, J., Nilsson, K., Öberg, F., Kalushkova, K., and Jernberg-Wiklund, H.* (2016) EZH2 inhibition in multiple myeloma downregulates myeloma associated oncogenes and upregulates microRNAs with potential tumor suppressor functions. ***Oncotarget***. doi: 10.18632/oncotarget.14378. [Epub ahead of print]

II **Alzrigat, M.,** Párraga, AA., Majumder, M., Ma, A., Jin, J., Nilsson, K., Heckman, C., Öberg, F., Kalushkova, A., and Jernberg-Wiklund, H. (2017) The polycomb group protein BMI-1 inhibitor PTC-209 is a potent anti-myeloma agent alone or in combination with epigenetic inhibitors targeting EZH2 and the BET bromodomains. (***Manuscript***)

‡: Indicates equal contribution as first authors
*: Indicates co-corresponding authorship

Contents

Abbreviations

BM	Bone marrow
BMI-1	B Lymphoma Mo-MLV Insertion Region 1 Homolog
CCND1	Cyclin D1
CCND3	Cyclin D3
CD	Cluster of differentiation
CDKN2A	Cyclin dependent kinase inhibitor 2A
CDKN2C	Cyclin dependent kinase inhibitor 2C
CpG	Cytosine-phosphate-guanine
DNMT1	DNA methyltransferase 1
DNMT3a	DNA methyltransferase 3a
DNMT3b	DNA methyltransferase 3b
ECM	Extra cellular matrix
EZH1	Enhancer of zeste homolog 1
EZH2	Enhancer of zeste homolog 2
FGFR3	Fibroblast growth factor receptor 3
H2AK119ub	Histone 2 A lysine 119 ubiquitination
H3K27me3	Histone 3 lysine 27 tri-methylation
H3K36me3	Histone 3 lysine 36 di-methylation
HDACis	Histone deacetylase inhibitors
IGF-1	Insulin like growth factor-1
IgH	Immunoglobulin heavy chain
IL-6	Interleukin-6
KDM6A	Lysine Demethylase 6A
KDM6B	Lysine Demethylase 6B
MAFB	Musculoaponeurotic fibrosarcoma oncogene homolog B
MAFC	Musculoaponeurotic fibrosarcoma oncogene homolog C
MGUS	Monoclonal gammopathy of undetermined significant
miRNA, miR	MicroRNA
MM	Multiple myeloma
MMSET	Multiple myeloma set domain
PCs	Plasma cells
PcG	Polycomb group protein
PCL	Plasma cell leukaemia
PRC1	Polycomb repressive complex 1
PRC2	Polycomb repressive complex 2
PTMs	Posttranslational modifications
SMM	Smoldering multiple myeloma

Introduction

Multiple Myeloma

Multiple myeloma (MM) is a malignancy of antibody producing plasmablasts/plasma cells (PCs). The MM clone is derived from a post-germinal center, isotype-switched, long living PC retaining the capacity to proliferate [1]. The disorder is characterized by clonal proliferation of the malignant PCs almost exclusively in the bone marrow (BM), monoclonal protein production, osteolytic bone lesions, renal failure and immunodeficiency [2-5]. MM accounts for 1% of neoplastic disorders and 13% of hematological malignancies [2-5]. The median age at diagnosis is approximately 70 years with males developing the disease more frequently than females [2-5]. Current treatments of newly diagnosed MM patients are based on combinations of novel therapeutics agents including proteasome inhibitors (bortezomib), immunomodulatory agent (IMiDS) (lenalidomide and thalidomide), corticosteroids (dexamethasone and prednisone) and alkylating agents (cyclophosphamide and melphalan) [6-8]. Despite the fact that these treatment strategies have improved the median survival time, the vast majority of MM patients eventually encounter refractory disease, and thus MM is still considered incurable and the need for improved therapy is imperative.

The Biology of Multiple Myeloma

MM is the last stage of a multistep process resulting in the malignant transformation of asymptomatic premalignant proliferative clonal PCs derived from post-germinal center B-cells. The current view is that nearly all MM cases are derived from a premalignant benign phase known as monoclonal gammopathy of undetermined significance (MGUS). MGUS may progress into a premalignant and asymptomatic smoldering multiple myeloma (SMM) and finally to the symptomatic MM (Figure 1). MM can eventually develop into a disseminated form known as plasma cell leukemia (PCL) [1, 9, 10]. The multistep development of MM is dependent on two main factors: 1) intrinsic factors i.e. genetic and epigenetic changes that occur in PCs during the course of the disease [11] and 2) extrinsic factors represented by the MM PCs interactions with the BM microenvironment [12].

The genetic abnormalities in MM are divided into two main categories: primary early chromosomal aberrations and secondary late-onset chromosomal translocations and gene mutations. These genetic lesions are important players in the development of MM as they affect the expression and function of important genes that regulate cell proliferation, survival, differentiation and transformation. Based on primary genetic events, MM can be divided into two different groups; hyperdiploid and non-hyperdiploid. The hyperdiploid group is characterized by trisomies of odd-numbered chromosomes, while the non-hyperdiploid group is known to harbor translocations involving the immunoglobulin heavy chain (IgH) loci [10, 13].

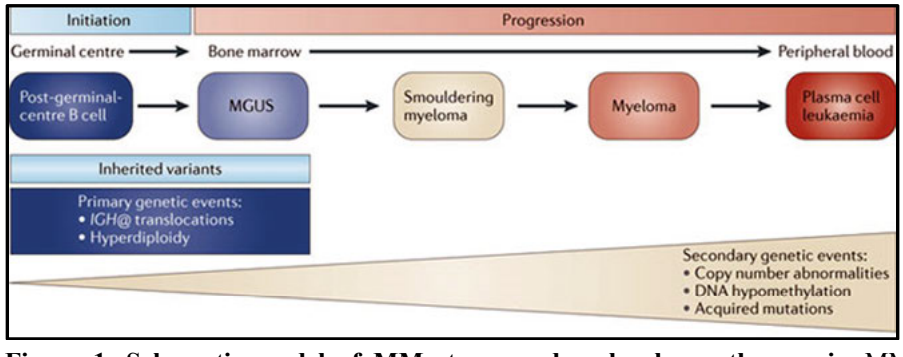

Figure 1. Schematic model of MM stages and molecular pathogenesis. MM develops from a germinal center B-cell. The current view is that MM is preceded by the pre-malignant condition MGUS, which could progress in some cases into the smoldering MM. MM is the symptomatic tumor that resides in the BM (intramedullary). MM tumor cells may obtain the capacity to grow in extramedullary places e.g. in the blood, which is termed plasma cell leukemia. Several oncogenic events are now identified in MM e.g., primary as well as secondary Ig translocations, numerical chromosomal abnormalities, epigenetic changes, acquired mutations in oncogenes and tumor suppressor genes. Reprinted with permission from Macmillan Publishers Ltd: Nature Review Cancer, (Morgan, GJ. et al.2012) [10].

In MM, hyperdiploidy is associated with a favorable prognosis, while non-hyperdiploidy has been correlated with bad prognosis. The IgH translocations lead to dysregulation of oncogenes (Table 1); such as cyclin D1 (*CCND1*) in case of t(11;14), cyclin D3 (*CCND3*) in t(6;14) [14] and the musculoaponeurotic fibrosarcoma oncogene homolog B (*MAFB*) in case of t(14;20) [15, 16]. These chromosomal translocations have been used for prognostic stratification in patients with MM (Table 1) [17]. It is, however, worth mentioning that IgH translocations are also frequently detected in the asymptomatic cases of MGUS and SMM and therefore may be regarded as necessary, but not sufficient to drive the transformation to the multiple myeloma [11, 14]. These findings highlight the importance of secondary or late genetic and epigenetic changes as well as BM microenvironment in MM pathogenesis. Consequently, the prognostic value of the primary IgH trans-

locations in MM as single events has been highly debated [18, 19]. Secondary late onset translocations and mutations involved in MM progression include complex immunoglobulin (Ig) and non-Ig translocations targeting the *MYC* oncogene, gain of function mutations in oncogenes such as *BRAF*, *NRAS* and *KRAS*, loss of function of tumor suppressors e.g. *P53*, *RB1*, *PTEN*, *CDKN2A* and *CDKN2C*. In addition, mutations in genes of the NF_kB pathway [20, 21] and epigenetic regulators such as the histone demethylases *KDM6A* (known as UTX) [22] and *KDM6B* (also known as JMJD3 or UTY) have been documented in MM [10, 20].

Table 1: Prevalence and prognostic value of primary IgH translocations in MM

IgH Transclocation	MGUS and SMM (%)	MM (%)	Upregulated oncogene	Prognostic value	Ref
t(4;14)(p16.3;q32)	**3-13**	**11**	***FGFR3* and *MMSET***	**Adverse**	**[16]**
t(6;14)(p21;q32)	**Unknown**	**4**	***CCND3***	**Unknown**	**[14]**
t(11;14)(q13;q32)	**15-30**	**16**	***CCND1***	**Favorable**	**[14]**
t(14;16)(q32;q23)	**3**	**3**	***MAFC***	**Adverse**	**[16]**
t(14;20)(q32;q12)	**1-5**	**1.5**	***MAFB***	**Adverse**	**[15, 16]**

Genetic lesions in MM alter the expression of cell surface markers on MM cells and that, in turn affects the interactions between the malignant PCs and BM cells or extracellular matrix (ECM) proteins. This plays an essential role in MM initiation and progression. Such interactions are mediated by cell surface receptors e.g. integrins, cadherins, selectins and the Ig superfamily of cell adhesion molecules. Interactions between the MM and BM cells alter

the BM fluid compartment, which consists of cytokines and growth factors secreted by the myeloma cells and BM cells (Figure 2). Interleukin 6 and 10 (IL-6 and 10), vascular endothelial growth factor (VEGF), insulin-like growth factor 1 (IGF1) and transforming growth factor beta 1 (TGFβ1) among others, are major cytokines and growth factors that enhance myeloma cell growth, survival, migration, drug resistance, myeloma cells homing to the BM and induction of osteolytic bone lesions [3, 4, 12, 23]. The end result of the accumulated genetic lesions in MM cells and changes in MM cells-BM interactions is the clonal expansion of the abnormal malignant plasma cells to occupy the BM hematopoietic stem cell niche.

Multiple Myeloma Clonal Heterogeneity

In 1967, Peter Nowell proposed the concept of clonal heterogeneity and evolution to cancer pathogenesis. He adapted the Darwinian theory of random genetic mutations and environmental driven selection for the generation of cancer clones described as the "survival of the fittest clones", which are responsible for disease progression and relapse [24]. The advances in DNA sequencing, gene expression and genomic hybridization technologies have proved that clonal heterogeneity is a typical feature of MM [10, 21, 25-27].

In 2012, Bergsagel and colleagues performed a cytogenetic study on 28 MM patients during the course of the disease. They revealed that the genomes of cytogenetically defined high-risk MM patients display extensive changes during disease progression and response to therapy [26]. Using array comparative genome hybridization to follow one high risk MM patient with t(4;14) translocation, Bergsagel and colleagues revealed the existence of two major sub-clones that compete and alternate in dominance in response to therapy [26]. Using massive parallel sequencing of paired tumor/normal plasma samples derived from 203 MM patients Lohr et al. showed that nearly all tumors analyzed in the study exhibited evidence of clonal heterogeneity, with most patients harboring a minimum of 3 sub-clones (not including the major clone) and some patients harboring as many as seven [21]. Later on, whole-exome and whole-genome sequencing have shown that intraclonal heterogeneity in MM is present at all stages of the disease from MGUS to PCL [28]. A recent study elegantly described this complex pattern of clonal heterogeneity in MM using exome sequencing coupled with copy number variation and cytogenetic analysis [27]. The authors revealed four diverse patterns of clonal evolution in MM patients i.e. no change in the clonal composition, linear evolution, differential clonal response, and branching evolution [27].

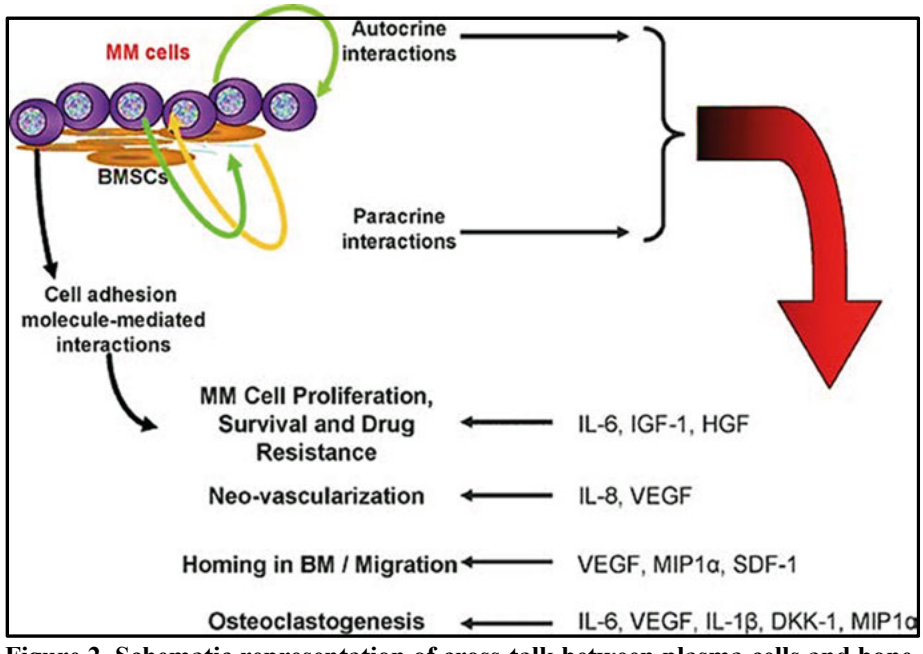

Figure 2. Schematic representation of cross-talk between plasma cells and bone marrow microenvironment in multiple myeloma. MM plasma cells interact with the BM cells and the ECM through cell adhesions molecules. These interactions increase the production of growth factors and cytokines, which operate through autocrine and paracrine actions. The ultimate aim of these interactions is to stimulate the MM PCs growth, drug resistance, migration, angiogenesis and the induction of osteolytic bone lesions. IL- (Interleukin)-6, 8 and 1β; IGF-1; insulin like growth factor-1; VEGF, vascular endothelial growth factor; MIP1α, macrophage inflammatory protein 1-alpha; SDF-1, stromal derived factor-1; DKK-1, dickkopf homolog-1. Reprinted with permission from John Wiley and Sons: Journal of Cellular Biochemistry (Mitsiades, CS. et al. 2007) [23].

The intra-tumor genetic and clonal heterogeneity in MM certainly limit the clinical benefits of personalized and targeted therapies. In most cases treatments induce partial response by suppressing drug sensitive clones, without affecting the resistant clones, allowing them to dominate the tumor [27]. These findings point toward the utilization of combination therapies targeting co-existing sub-clones, as well as the investigation of alternative mechanisms of importance for MM pathogenesis, i.e. epigenetic mechanisms, which could unravel new potential targets for pharmacological intervention. The contribution of epigenetic mechanisms to clonal heterogeneity in MM is becoming increasingly apparent. Recently, epigenetic mechanisms have been proposed as contributors to clonal heterogeneity and plasticity in MM [29]. In this study, epigenetic regulators were suggested to play essential roles in the bidirectional switch between a quiescent, less differentiated and low frequency subpopulation of malignant cells to the differentiated and highly proliferative dominant clone [29]. Collectively, these efforts underscore the

sophisticated and brilliant plastic nature of tumor cells and may explain the high relapse frequency commonly observed in MM patients.

Multiple Myeloma Tumor Initiating Cells

The current view on tumor initiation suggests the presence a subset of cells within the tumor, which display the main features of stem cells i.e. capacity of self-renewal, cytostatic drug resistance and induction of disease relapse [30]. Many studies have suggested that the MM initiating cell alternatively could belong to the hematopoietic stem/progenitor cells, B-cells or plasma cells [31, 32]. Different groups have also suggested the presence of two cell populations; $CD138^-$ (immature) and $CD138^+$ (mature) PCs in MM with clonogenic potential *in vitro* and *in vivo*. Matsui et al. demonstrated the clonogenic potential of $CD138^-$ fraction (including $CD34^-$, $CD19^+$ and $CD20^+$), but not the $CD138^+$ cells, in nonobese diabetic/ severe combined immunodeficiency (NOD/SCID) mice [33]. In contrast, the clonogenic ability of $CD138^+$ tumor cells derived from MM patients' specimens was demonstrated when implanted into SCID rabbit model [34]. Recently, our group provided evidence that both $CD138^-$ and $CD138^+$ MM PCs have clonogenic potential *in vitro* and *in vivo* [35]. We noticed, however, that $CD138^+$ PCs exhibited increased tumor formation capacity over the $CD138^-$ PCs in the 5T33 MM mouse model [35]. It is worth mentioning that discrepancies in these results could be reasoned to differences between the animal models and the bone marrow environment requirements of tumor establishment used in the different studies. Taken together, the data from these studies collectively suggest that the tumor initiating capacity of MM is not restricted to CD138 expression in the MM tumor clone.

Models of Multiple Myeloma

Multiple Myeloma research, utilizing different *in vitro* and *in vivo* models of MM, has largely increased our understanding of the biology of MM and aided in the development of different preclinical and clinical treatment strategies. The following is a brief overview of current relevant models that are being used in myeloma research.

In vitro Multiple Myeloma Models

Purified human primary MM cells and authentic MM cell lines are the preferred models for *in vitro* studies in myeloma research. One obstacle when using primary patient samples is that these cells do not proliferate and therefore cannot be maintained for long periods of time in cell culture. MM cell lines have been extensively used to understand the biology and targeted treatment of MM. Currently; there are about 80 authentic MM cell lines that

have been established in cultures from MM patients with advanced, often terminal, disease stage. MM cells derived from BM of non-progressive disease require the presence of a stromal feeder layer or conditioned medium with IL-6 to grow in culture [36]. Many MM cell lines such as RPMI-8226, LP-1, INA-6, U266, KMS-11, JJN3, KMS-12BM, OPM-2, and L363 are authenticated to be derived from MM patients and are now established tools used worldwide as *in vitro* MM models [36].

In vivo Multiple Myeloma Models

Current knowledge in MM has greatly benefited from the development of *in vivo* animal models for MM. A representative model for MM must reflect and imitate the biological and clinical characteristics of the disease in humans, i.e. it must exhibit the same genetic aberrations of myeloma cells, clinical symptoms of the patients and MM cell-BM interactions. There are some *in vivo* MM models that have been proven to represent many aspects of the human disease and are being widely used to understand the biology of MM and the development of anti-MM therapies. Of these models the 5T murine model, the SCID-human (SCID-hu) model and the MYC dependent Vk*MYC mouse model are most frequently used in MM research [37].

5TMM Mouse Model

The 5T murine model of MM is a syngeneic mouse model originally derived from aging C57BL/KaLwRij black mice which spontaneously develop MM [38]. The syngeneic 5TMM models, characteristically similar to the human condition, have two unique advantages: they are immune competent and they faithfully recapitulate BM homing [38]. The 5T model exists in several variants, two of which have been extensively characterized and utilized: 1) the 5T2MM model, which represents the slow progressing form of MM and, to a very large extent, resembles the human form of the disease [39]. 2) The 5T33MM model represents the aggressive rapidly growing form of MM [39]. Clinically, these two models largely resemble the human MM disease e.g. serum M-component, localization of MM cells to the BM, angiogenesis and osteolytic bone lesions [39, 40]. Thus, the 5TMM mice models are considered relevant in studying the etiology of human MM and can be used for treatment studies in large scale also combining drugs previously found relevant in *in vitro* studies.

SCID-human Mouse Model

The severe combined immunodeficient (SCID) mouse model is derived from the BALB/c C.B-17 inbred mouse strain, in which spontaneous point mutations in chromosome 16 have occurred. The SCID mice are characterized by

an intact innate immunity but lack the adaptive (T and B- cell) immunity [41] and therefore, are ideal animal models for studying human cancers. The SCID human mice model is a human MM model where the SCID mice serve as a host for the human MM cells and human fetal bone xenografts. Human fetal bone xenograft is intended to generate the human bone microenvironment to make it possible for primary human MM cells and cell lines to grow in the mice [42, 43]. Although, the model provides a suitable MM cells-BM network of interactions [42, 43], the SCID-human model has raised ethical concerns due to fetal bone xenografts.

The Vk*MYC Mouse Model

In the Vk*MYC mouse model a conditional activation of the *MYC* gene is achieved by the activation-induced deaminase (AID), which is required for Ig class switch recombination and somatic hyper-mutation in germinal center B-cells [44]. In human MM, *MYC* activation has previously been correlated with disease progression from MGUS to MM [45]. The Vk*MYC mice develop indolent MM, while control C57BL/6 mice develop MGUS with age. Vk*MYC mice displayed the biological and clinical features highly characteristic of the human disease [44]. Thus, the Vk*MYC MM mouse model could be used to study the biology of MM in its native microenvironment.

Epigenetic Regulation in Multiple Myeloma

Epigenetics describes the study of heritable changes in gene expression and cellular phenotypes independent of the DNA sequence. Epigenetic changes affect DNA templated processes such as DNA replication and repair, gene transcription and RNA splicing [46]. Several epigenetic mechanisms have been proven to be involved in gene regulation including: DNA methylation, histone protein posttranslational modification, chromatin remodeling and non-coding RNA [47]. Epigenetic mechanisms are important regulators of fundamental biological processes such as pluripotency, cellular differentiation and reprogramming [47, 48]. Deregulation of epigenetic mechanisms is evident in cancer with the current massive research efforts focusing on targeting aberrant epigenetic factors and profiles in the battle against cancer [49-51]. In addition to genetic abnormalities, the role of epigenetic mechanism is increasingly evident in the development of MM [52].

DNA Methylation in Multiple Myeloma

DNA methylation is the most studied epigenetic mechanism in mammals. It is defined as the covalent addition of a methyl group on the carbon-5 position of cytosine nitrogenous base, especially at CpG dinucleotide islands at

promoter regions, which leads to gene silencing [53]. DNA methylation is catalyzed by a set of enzymes called DNA methyltransferases 1, 3A and 3B (DNMT1, DNMT3a and DNMT3b). DNMT3a and DNMT3b are considered as de novo DNA methyltransferases, while DNMT1, also known as maintenance DNMT, is responsible for maintaining DNA methylation after replication. However, some studies have suggested that all DNMTs possibly possess both de novo and maintenance functions [54-56]. Initially, DNA methylation was suggested to be a permanent epigenetic mark; however, this idea has recently been challenged by the identification of an active process of DNA demethylation catalyzed by enzymes belonging to the ten-eleven translocation (TET) protein family [57-59].

Multiple Myeloma, as for most tumors, is characterized by global DNA hypomethylation and hypermethylation at the promoter regions of tumor suppressor genes. Several studies have reported on the hypermethylation of known tumor suppressor and cell cycle control genes in MM e.g. cyclin-dependent kinase inhibitor 2A and 2B (*CDKN2A*and *CDKN2B*) [60, 61], paired box 1 (*PAX1*), adenomatous polyposis coli (*APC*), suppressor of cytokine signaling 2 (*SOCS2*) [62], glutathione peroxidase 3 (*GPX3*), retinol-binding protein 1 (*RBP1)*, secreted protein, acidic, cysteine-rich (*SPARC*), and transforming growth factor β induced (*TGFBI)* [63]. All these studies suggest that promoter hypermethylation increases during the course of the disease, reaching its maximum in plasma cell leukemia and MM cell lines. However, a recent study using whole-genome bisulfite sequencing and high-density arrays reported that MM is characterized by regional DNA hypermethylation embedded in extensive hypomethylated regions [64]. In contrast to the common knowledge that DNA methylation is enriched at promoter CpG islands, these MM hypermethylated regions were located outside CpG islands and were enriched in intronic enhancer regions harboring binding sites of B cell-specific transcription factors [64]. Similar to other tumors, the global hypomethylation in MM leads to genomic instability, and correlates with disease progression [65] and poor prognosis [66]. The cause and consequences of abnormal DNA methylation pattern in MM is currently unknown; however, increased expression of DNMT1, DNMT3a [60, 62, 63] and miR-22 that negatively regulates TET2 [67] have been documented in MM, which may in part explain the deregulation in DNA methylation patterns observed in MM.

MicroRNA in Multiple Myeloma

MicroRNAs (miRNAs, miRs) are small (around 22 nucleotides) endogenous non-coding RNAs that regulate gene expression in a sequence specific manner. MiRNAs regulate the expression of target genes through the degradation of mRNA or translational inhibition depending on the sequence complemen-

tarity between miRNAs and their target mRNA molecules [68]. MiRNAs are important regulators of several biological processes including cell proliferation, differentiation and death [69]. Therefore, miRNAs have been described as tumor suppressors or oncogenes in various cancer types where the deregulation in miRNA expression could be linked to tumor initiation, progression and metastasis [70-72]. In MM, abnormal miRNA expression and function have been attributed to genetic lesions such as chromosomal translocations and copy number variations [73-76], as well as aberrant epigenetic mechanisms such as DNA methylation [77, 78]. Recent analysis of global miRNA expression in MM has stressed the important role that miRNAs play in MM by correlating deregulation of miRNA expression in MM to disease progression, molecular subtype, isotype, survival and response to treatment [73-76, 79, 80]. Consequently, miRNAs have now been assigned both oncogenic and tumor suppressor functions in MM.

Several studies have demonstrated the oncogenic role of miRNAs in MM. For example, IL-6 regulates the transcription of miR-21 in MM cell lines, and ectopic expression of miR-21 may sustain MM cell growth in the absence of IL-6, suggesting an oncogenic role for miR-21 in IL-6 dependent MM cell lines [81]. In addition, Pichiorri et al. reported an overexpressed oncogenic miRNA signature in both MGUS and MM in comparison to healthy donor plasma cells [82]. The MM signature included miR-21, miR-106b-25 cluster, miR-181a and b. In this study, miR-106b-25 cluster, miR-181a and b were shown target the P300/CBP-associated factor (PCAF) transcript, which is involved in regulating the activity of the tumor suppressor protein P53 [82]. Furthermore, the authors described a selective upregulation of miR-32 and miR-17-92 cluster in MM subjects and cell lines, but not in MGUS and normal plasma cells. MiR-17-92 cluster adopted oncogenic potential by targeting the suppressor of cytokine signaling 1 (*SOCS-1*) gene mRNA; a tumor suppressor acting as negative regulator of IL-6 signaling [82]. Others have reported the oncogenic properties of miR-17-92 cluster and Let-7 family members in MM by their role in enhancing angiogenesis [83], a hallmark of disease establishment and progression.

On the other hand, several studies have identified underexpressed miRNAs with tumor suppressor functions in MM. For example, underexpression of miR-196b, miR-135b, miR-320, miR-20a, miR-19b, miR-19a and miR-15a has been reported in MM, which is believed to enhance MM cell growth via overexpression of their predicted target oncogene cyclin D2 (*CCND2*) [84]. This hypothesis is supported by the fact that overexpression of pre-miRNA-15a in MM cells reduces MM cell growth *in vitro* and *in vivo* partly through downregulation of CCND2 protein levels [85]. Another example of tumor suppressor miRNAs in MM is miR-34a, which is reported to be underexpressed in MM [86]. Ectopic expression of miR-34a in MM cells demon-

strated anti-MM activity *in vitro* and *in vivo* via inhibition of cell growth and induction of apoptosis [87]. In a similar manner, the underexpression of miR-155, miR-198, miR-135a, miR-200c, miR-663and miR-483-5p was shown in MM and the ectopic expression of these microRNAs was found to reduce MM cell proliferation, migration and clonogenic capacity [88].

It is well known that interactions between MM cells and the BM microenvironment are important determinants in MM initiation, progression, dissemination, treatment and relapse [12, 23, 89]. Recent studies have demonstrated that MM cells and the BM microenvironment utilize miRNAs as novel routes for cellular cross-talk [90]. Roccaro et al. demonstrated that exosomes carrying miRNAs, released from BM mesenchymal cells, were actively transferred to MM cells resulting in sustained tumor growth *in vitro* and *in vivo* [91]. Similarly, miRNAs containing exosomes derived from MM cells were suggested to enhance MM tumor growth via inhibiting their targets in BM endothelial cells [92]. Although the impact of miRNA as inter-cellular communicators remains to be established, secreted miRNAs in MM have been investigated for their potential use as biomarkers. As such, analysis of miRNA expression in the plasma of MM patients has identified a set of miRNAs correlating with genetic sub-groups and survival in MM proposing the use of plasma miRNAs as potential clinical biomarkers in MM [93].

Histone Posttranslational Modifications

In eukaryotes, DNA forms a complex with histone proteins making up what is known as chromatin. Chromatin is made of building blocks called nucleosomes; each consisting of around 147 base pairs of DNA wrapped around a histone octamer, composed of 2 units of each the core histones (H2A, H2B, H3 and H4). Another histone called histone 1 (H1) links the nucleosome together and aids in the formation of high ordered chromatin structures. The N-terminal domains of the core histone proteins (also known as histone tails) are subjected to different posttranslational modifications (PTMs) that affect the chromatin structure and consequently all chromatin templated processes such gene transcription, DNA replication and repair [47]. This indicates the important role of histone PTMs in many cellular processes such as differentiation, proliferation and homeostasis. Aberrations in histone tail PTMs may have disastrous consequences, such as cellular transformation [94, 95].

Histone tails are prone to several covalent, reversible modifications including methylation, acetylation, phosphorylation, ubiquitination, and sumolyation. Histone acetylation, methylation and phosphorylation are the most studied posttranslational modifications due to their crucial role in regulating gene transcription [96, 97]. Histone acetylation occurs at lysine (K) residues and it is associated with open chromatin structure i.e. histone lysine acetyla-

tion is a mark of active gene transcription [98]. Similarly, histone protein phosphorylation is a mark of active transcription [99]. The situation is more complex with histone methylation as it has been documented that methylation of histone proteins at arginine and lysine residues marks both transcriptionally active and repressed regions. Whether a methylation mark is activating or repressing transcription is dependent on the amino acid modified and the state of methylation (mono, di or tri-methylation). For example, trimethylation of histone 3 lysine 4 (H3K4me3) and H3K36me3 is associated with gene activation, while H3K9me3, H3K27me3 and H4K20me3 signal transcriptional repression [96, 97, 100]. It is worth mentioning that histone PTMs form a chromatin language or what is known as "histone code" with which different histone PTMs communicate with each other and show a substantial cross-regulation, leading to complex regulatory networks [101]. Gene transcription is greatly influenced by the distribution of different histone PTMs in the regulatory regions and bodies of target genes, as shown in Figure 3 [97].

Polycomb Group Proteins in Multiple Myeloma

The polycomb group proteins (PcG) are a class of transcriptional regulators that mediate gene repression by several mechanisms including histone post-translational modifications [102, 103]. They were first discovered in the fruit fly Drosophila as critical regulators of the transcription of key developmental genes known as Hox genes [104]. PcG are highly conserved epigenetic regulators from Drosophila to Human. Therefore, their function as transcriptional regulators of key genes during mammalian embryonic development and cell differentiation is well recognized [105]. In order to regulate transcription, the PcG form multimeric protein complexes called polycomb repressive complexes (PRCs). Sofar two PRCs have been characterized; PRC1 and PRC2 [102, 103].

In mammals, PRC2 consists of core PcG components: embryonic ectoderm development (EED) subunit, suppressor of zeste 12 (SUZ12) and the enzymatic subunit enhancer of zeste 2 (EZH2) or its close homolog EZH1. Through EZH2 and EZH1, PRC2 can install the transcriptional repressive histone mark; tri-methylation of histone 3 lysine 27 (H3K27me3) at PRC2 target genes [102, 103].

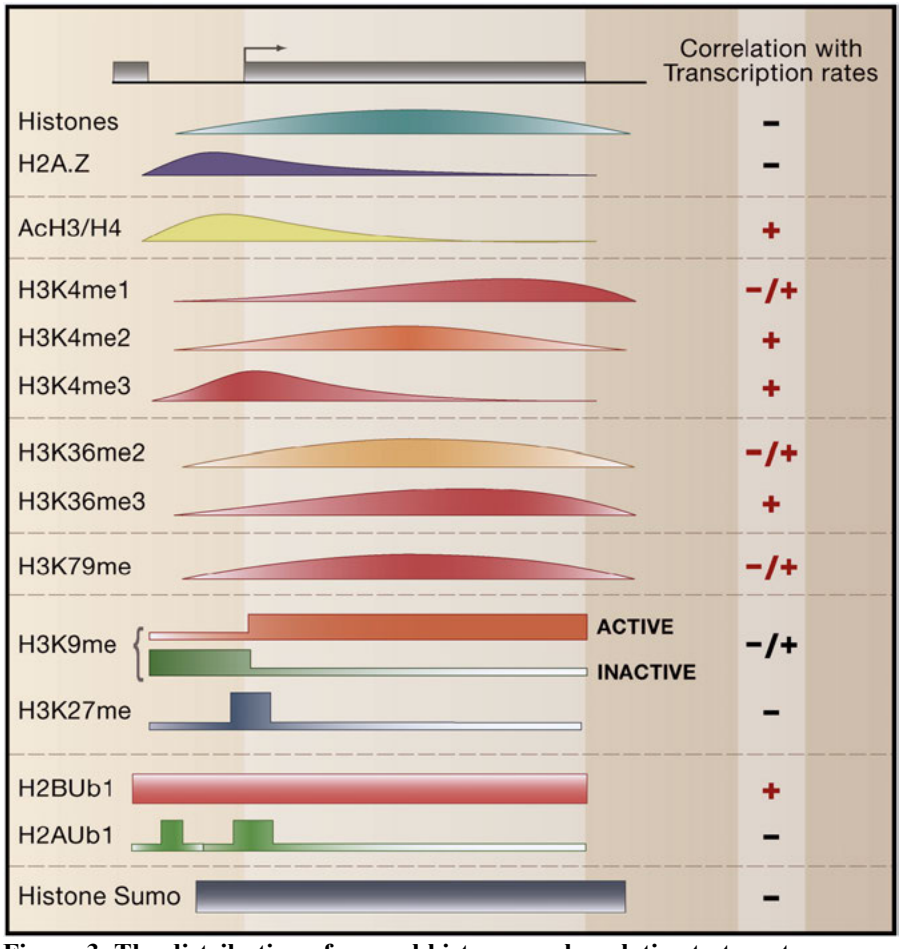

Figure 3. The distribution of several histone marks relative to target genes and their effects on transcription. Except for K27 and K9 methylation, the rest of the data is largely based on studies from yeast. Arrow indicates the transcription start site. The – and + signs indicate negative and positive regulation of transcription, respectively. Reprinted with permission from Elsevier: Cell (Li ,B. et al. 2007) [97].

The composition of PRC1 is more complex with two common components; RING finger protein 1A/B (RING1A/B) with B lymphoma Mo-MLV insertion region 1 homolog (BMI-1), polycomb group RING finger protein 2 (PCGF2) or PCGF1. RING1A/B possess an E3 ubiquitin ligase activity that catalyzes the formation of the transcriptional repressive histone mark; mono-ubiquitination of histone H2A at lysine 119 (H2AK119ub) at PRC1 target sites. It is worth mentioning that other components of both PRCs have been described [102, 103], which could be dependent on many factors; such as developmental stage, tissue and cell type and the nature of target genes.

Recently, two models have been proposed to explain the recruitment of PRC1 and PRC2 to their target genes (Figure 4). The canonical model suggests that PRC2 is first recruited to target genes by several ill-defined mechanisms, which possibly involve PRC2 specific response elements, long non-coding RNA and/or transcription factors. Once recruited, PRC2 installs the H3K27me3 mark, which acts as docking site for PRC1. PRC1 via one of the chromodomain-containing chromobox (CBX) subunits binds to H3K27me3 and subsequently stabilizes the repression of nearby genes through RING1A/B-mediated ubiquitination of histone H2A at lysine 119 (H2AK119ub) and subsequent chromatin condensation [106, 107]. The new model suggests a variant form of PRC1 complex contains ring 1 and YY1 binding protein (RYBP), lysine demethylase 2B (KDM2B) and polycomb group RING finger protein 1 (PCGF1) to be recruited first to unmethylated CpG islands. It ubiquitinates nearby H2AK119, leading to the recruitment of PRC2. Once recruited, PRC2 adds the H3K27me3 repressive mark, which in turn recruits the canonical PRC1 complex further spreading the H2AK119ub mark [107, 108]. However, it is important to mention that unique target genes to PRC1 or PRC2 also exist. To further enhance gene repression, PRC2 was reported to associate with DNA methylation leading to permanent gene silencing [109, 110]. However, the exact recruitment paths of these complexes remain elusive.

H3K27me3 is a mechanism that suppresses the transcription of genes proximal to the site of modification and play essential roles in many biological processes during embryonic development such as cellular pluripotency, growth, survival and differentiation [106, 111]. Deregulation of PRC2 components, especially EZH2 due to overexpression and mutations, has been described in tumors of different origins such as B-cell lymphomas [112, 113], ovarian cancer [114], prostate cancer [115], glioblastomas [116], and medulloblastoma [117]. This leads to the accumulation of H3K27me3 repressive mark and subsequent gene repression (Figure 5). Recently, the pathologic activation of EZH2 due to gain of functions mutations and the associated increase in H3K27me3 mark have been clearly demonstrated to be essential for the development of germinal center derived B-cell lymphomas [118, 119]. These data strengthen the role of PRC2, its enzymatic subunits EZH2/EZH1 and the associated H3K27me3 mark in tumor formation, and suggest the PRC2 as potential therapeutic target alone or in combination with current treatment regimes, in a wide range of tumors.

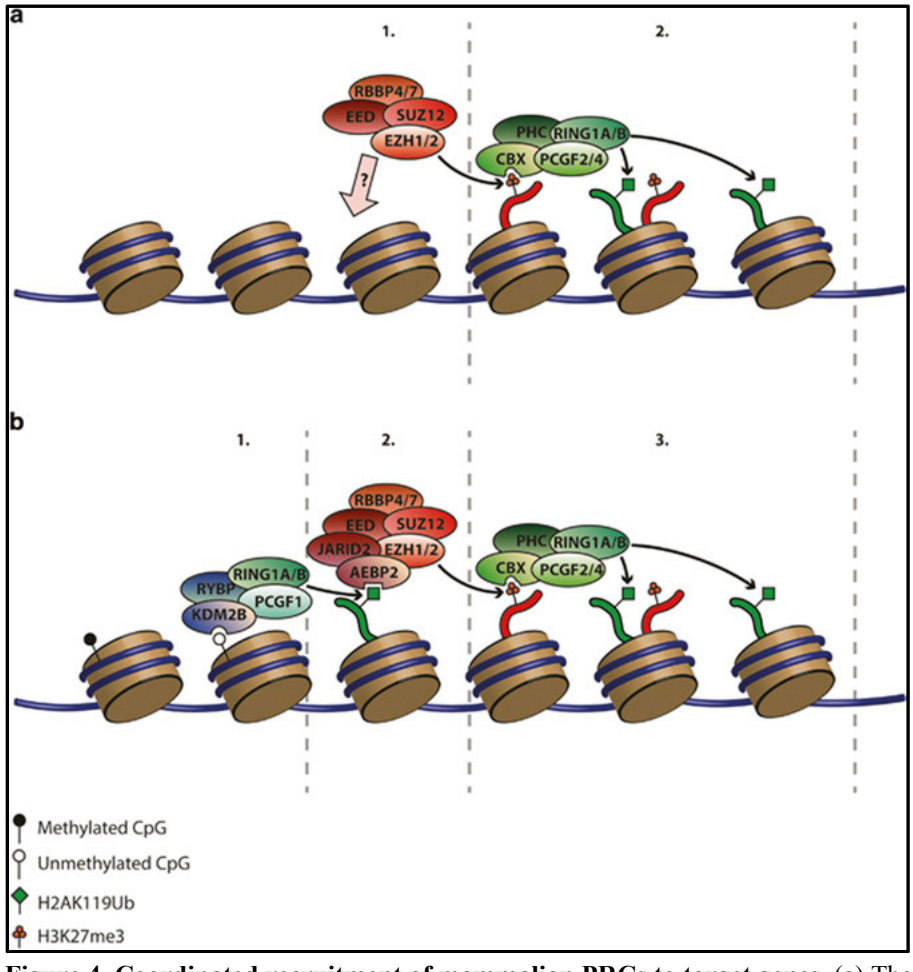

Figure 4. Coordinated recruitment of mammalian PRCs to target genes. (a) The canonical model supports the initial recruitment of PRC2 to target genes via several mechanisms and the installation of H3K27me3 (step 1), which followed by PRC1 recognition of H3K27me3 and recruitment (step 2), which allows the deposition of H2AK119ub further implying transcriptional repression. (b) The new model suggests that PRC1 is recruited first to unmethylated CpG sites followed by the addition of H2AK119ub (step 1), which followed by PRC2 binding to H2AK119ub and the installation of H3K27me3 mark (step 2). This allows the recruitment of canonical PRC1 to further repress gene transcription by further deposition of H2AK119ub (step 3). Reprinted with permission from Macmillan Publishers Ltd: Oncogene (Koppens, M and van Lohuizen, M. 2015) [107].

The role of PRC2 and H3K27me3 in MM pathogenesis has been suggested by us [120] and others [121]. In 2005, EZH2 was suggested to be an oncogene in MM as its overexpression and histone methyltransferase activity correlated to cellular transformation and tumor formation *in vitro* and *in vivo* [121]. Moreover, EZH2 was found to be overexpressed in the most aggressive myelomas [122]. It is worth mentioning that no mutations were found in

the *EZH2* gene in MM [123]. Recently, the overexpression of MMSET in MM with t(4;14) was described to cause global chromatin changes associated with increased H3K36me2 and redistribution of H3K27me3 [124]. Further highlighting the emerging role of H3K27 methylation in MM, loss of function mutations or deletions in the H3K27 demethylase *KDM6A* gene (also known as UTX) were reported in MM patients [22, 123]. In 2010, we aimed to identify mechanisms underlying gene silencing in MM by correlating publicly available gene expression data to previously defined literature concepts [120]. This integrative genomic analysis led us to the important discovery that genes commonly silenced in MM, when compared with normal plasma cells, significantly overlapped with genes targeted by polycomb in human embryonic cells [125]. Taken together, we hypothesized that polycomb gene targeting may have a pivotal role in MM tumorigenesis and proteins of the PRC2 may constitute potential therapeutic targets in MM.

Like PRC2, the PRC1 play important roles during embryonic development, stem cell maintenance, cell differentiation and reprogramming [105, 126]. However, several subunits of the PRC1 complex have been found to be deregulated in a wide range of tumors including hematological and solid tumors [107, 126]. Of particular importance, the PRC1 subunit BMI-1 reportedly confers stemness properties to cancer cells of solid and hematopoietic origins [127-129], correlating with therapy failure in various types of cancer [130-133]. In support of its function as an oncogene, BMI-1 was originally found to be an important co-factor partnering with the MYC oncoprotein in lymphomagenesis [134, 135]. Later, BMI-1 was found to be overexpressed in various human cancers with an important impact on tumor initiation, progression and response to anti-cancer therapy [136-138]. The role of BMI-1 in MM is becoming increasingly evident as it was found to be overexpressed in MM and to promote MM cell growth [139]. Recently, analysis of gene expression dataset of MM demonstrated the overexpression of BMI-1 in all stages of MM progression and suggested to be a predictor of poor survival in relapsed MM cases treated with bortezomib or dexamethasone [140]. BMI-1 knockdown was found to have anti-tumor effects in MM via upregulation of the pro-apoptotic gene *BIM* [139] and to sensitize MM cells to bortezomib treatment [141]. Taken together, these data highlight PRC1/BMI-1 as potential therapeutic targets in MM.

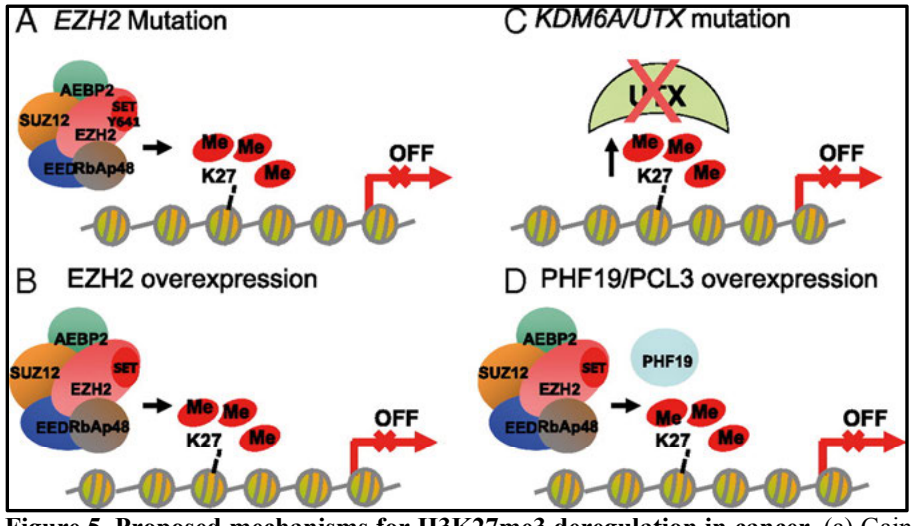

Figure 5. Proposed mechanisms for H3K27me3 deregulation in cancer. (a) Gain of function mutations in EZH2 (Y641). (b) Overexpression of EZH2. (c) Loss of function mutations in H3K27me3 demethylase UTX. (d) Overexpression in PRC2 component PHF19/PCL3 results in enhanced PRC2 recruitment to target genes. All scenarios lead to the accumulation of histone H3K27me3 at the promoter regions of PRC2 target genes and consequently transcriptional repression of key genes in cancer. Reprinted with permission from National Academy of Sciences, PNAS (Sneeringer, CJ. et al. 2010) [118].

Genetic-Epigenetic Cross-talk in Multiple Myeloma

Considering the large genetic heterogeneity of MM, several reports have documented a cross-talk between genetic lesions and aberrant epigenetic profiles such as DNA methylation [62, 142], histone modifications [124, 143] and non-coding RNA [67, 74, 76] in molecular pathogenesis and prognosis of MM.

The recurrent immunoglobulin translocation, t(4;14)(p16;q32) stands as a solid example of genetic-epigenetic cross-talk in MM. The t(4;14) translocation occurs in 15-20% of the patients and is associated with poor prognosis [1]. The t(4;14) translocation results in upregulation of the fibroblast growth factor receptor 3 (*FGFR3*) and multiple myeloma SET domain (*MMSET*) genes. It is worth mentioning that in 30% of MM tumors harboring the t(4;14), *FGFR3* is not expressed due to unbalanced t(4;14) and loss of derivative der(14) [144]. Importantly, the poor prognostic value of t(4;14) in these cases remains unaffected, suggesting an important role for *MMSET* in MM [144]. The *MMSET* (also known as *NSD2 /WHSC1*) gene encodes for a histone methyltransferase that catalyzes Histone 4 Lysine 20 di-and trimethylation (H4K20me2, 3) [145] and Histone 3 lysine 36 di-methylation (H3K36me2) [145, 146]. In MM, *MMSET* was suggested to function as an

oncogene as its overexpression restored the tumorigenicity of t(4;14) negative MM cells. This was associated with increased transcriptional activity of oncogenic programs that are dependent on H3K36me2 chromatin mark [146].

Another example of cross-talk between genetic and epigenetic mechanisms in MM is evident between copy number aberrations (CNAs) of 1q21 and DNA methylation status of the 1q12 pericentromeric heterochromatin [142]. The authors found that hypomethylation of 1q12 pericentromeric heterochromatin by 5-azacytidine induced copy number gains in the 1q21 region, which mimics CNAs found in the bone marrow of patients with high-risk disease [142]. Genetic-epigenetic cross-talk in MM is further documented by the global elevation of miRNA expression in high-risk myeloma due to overexpression of the miRNA master regulator of maturation and function; Argonaute 2 (*AGO2*) by copy number gains [67]. Furthermore, miRNA expression was found to correlate with number of DNA copies of their corresponding loci in primary plasma cell leukemia [73]. In addition, recent genomic analysis projects in MM identified the H3K27me3 demethylase *KDM6A* gene to harbor loss of function mutation [20, 22] or deletions [123]. In summary, these findings demonstrate the intricate relationship between genetic makeup and epigenetic mechanisms in MM and suggest genetic aberrations in MM as potential prime drivers of abnormal epigenetic profiles in MM.

Cross-talk between Epigenetic Mechanisms in Multiple Myeloma

Recent research in MM revealed that different epigenetic processes affect each other and may also cooperate to support MM cell growth. For example, the expression of miRNAs in MM has been extensively studied in the context of being targets for DNA methylation. Zhang et al. showed that DNA methylation mediate the repression of a set of tumor suppressor miRNAs; miR-152, miR-10b-5p and miR-34c-3p. Treatment with the DNA demethylating agent 5-aza-2´-deoxycytidine reactivated the expression of these miRNAs, resulting in the downregulation of their putative target oncogenes, induction of apoptosis and suppression of MM cell growth [77]. Similarly, miR-155, miR-198, miR-135a, miR-200c, miR-663 and miR-483-5p have been shown to be underexpressed in MM due to promoter hypermethylation. Treatment with the demethylating agent 5'azacytidine restored their expression with anti-MM effects [88].

Moreover, cross-talk between different histone modifiers to enhance the growth of MM cells has been documented in the silencing of the pro-

apoptotic gene *BIM*. We have previously reported that survival signaling via the IGF-1 receptor in MM leads to epigenetic silencing of the pro-apoptotic gene *BIM* via H3K9me3 [147]. Independently, Jagani et al. demonstrated the oncogenic function of the polycomb group protein (PcG) BMI-1 in MM through repression of the pro-apoptotic gene *BIM* [139]. A recently described example of cross-talk between histone modifiers and histone marks is the relationship between MMSET/H3K36me2 and EZH2/H3K27me3 in the context of t(4;14) MM. Martinez-Garcia and Popovic et al. showed that high MMSET activity is associated with increased levels of H3K36me2 and a striking decrease in H3K27me3 levels [124]. Later, the same groups showed that despite the decrease in H3K27me3 in MMSET overexpressing cells, H3K27me3 and EZH2 were enriched at specific genomic loci containing genes known to play roles in normal germinal center B-cells as well as genes known to be targets of c-*MYC* oncogene in B-cells [143]. Collectively, these data indicate that different epigenetic marks cooperate to establish a common oncogenic pathway to support MM cell growth.

Epigenetic Therapy in Multiple Myeloma

In the last decade, epigenetic factors, enzymes and regulators have become primary drug targets in the battle against cancer. A large number of agents, some of which are being currently used in clinical practice, have been developed to target several epigenetic processes; particularly DNA methylation, histone acetylation and methylation [49, 148]. For example, the DNA methyltransferases inhibitors (DNMTi) 5-azacytidine (Vidaza) and 5-aza-2`-deoxycytidine, (Dacogen or decitabine) are being used in the treatment of myelodysplastic syndrome [149, 150]. DNMTi exhibited anti-myeloma effects *in vitro* [151], while *in vivo* anti-myeloma effects need to be further examined in clinical trials. The development of miRNAs mimics and inhibitors have made miRNA based therapy a rapidly expanding field [152]. Sofar, overexpression of a few tumor suppressor miRNAs or their mimics has shown anti-MM activity *in vitro* [77, 88] and *in vivo* [87, 153]. The use of miRNA as a therapeutic procedure in clinical settings is very challenging as issues such as overcoming the endonuclease activity and delivery to target cells need to be addressed before miRNA can be introduced in clinical practice.

One class of epigenetic drugs used in MM treatment is the histone deacetylase inhibitors (HDACis). HDACis are classified into different groups based on their chemical structures (short chain fatty acids, mercaptoketones, cyclic tetrapeptides, hydroxamic acids and benzamides) and their HDAC protein subgroup targets (inhibitors of HDAC classes I, II or IV or multiple classes (pan-HDACis)) [154, 155]. Acetylation is a PTM of both histone and non-histone proteins and therefore enables HDACis to have pleiotropic effects

such as affecting chromatin, signaling pathways and protein stability [154, 155]. The anti-tumor role of HDACis in MM is the product of several consequences e.g. apoptosis, autophagy, inhibition of proteasomes, inhibition of angiogenesis, and reduced drug resistance [156, 157]. In clinical trials, the HDACi Panobinostat is now being used in combination with bortezomib and immuno-modulatory drugs, since single HDACis agent in treatment have not shown significant clinical benefits [158-160].

Recently, several small molecular inhibitors of EZH2/EZH1 enzymatic activity have been developed and proven to have anti-tumor effects *in vitro* and *in vivo* in EZH2 dependent tumors such as diffuse large B-cell lymphoma [119, 161-163], mixed lineage leukemia (MLL)-rearranged leukemia [164] and pediatric malignant rhabdoid tumors [165]. Currently, the EZH2 inhibitor Tazemetostat (EPZ-6438) is used in clinical trials on patients with hematopoietic and solids tumors. We and others have provided a proof-of-principle that targeting the PRC2 complex via 3-Deazaneplanocin A (DZNep) results in MM cell death *in vitro* [120, 166] and *in vivo* [166]. However, DZNep is a pan-methyltransferase inhibitor and it cannot be excluded that it exerts anti-tumor activity via non-PRC2 dependent pathways. Recently, EZH2 inhibition using the small molecule inhibitor GSK343 demonstrated anti-myeloma effects in the KMS-11 cell line with t(4;14) translocation [167]. Therefore, EZH2 inhibition in MM demands further investigation in pre-clinical models of MM. The BET bromodomain protein inhibitors such as JQ1 [168] has also been proven to have anti-myeloma activity *in vitro* and *in vivo* e.g. via downregulation of the MYC and its associated expression signature [169]. MYC is suggested to play an important role in MM pathogenesis [170] and therefore JQ1 or its improved version, the I-BET762 is considered to have potential as a new therapeutic agent in MM.

Summary of the Thesis

Background

Multiple myeloma (MM) is a malignancy of antibody producing plasmablasts/plasma cells (PCs). The MM clone is derived from a post-germinal center, isotype-switched, long living PC still retaining the capacity to proliferate. The disorder is characterized by clonal proliferation of the malignant PCs mainly in the bone marrow (BM), monoclonal protein production, osteolytic bone lesions, renal failure and immunodeficiency [3, 4]. Despite the fact that current treatment settings have improved disease management, the majority of MM patients eventually die of refractory disease. Several intrinsic factors e.g. genetic and epigenetic as well as extrinsic factors i.e. BM microenvironment are important players in MM pathogenesis, response to treatment and relapse [171]. Recent large scale genetic sequencing projects and gene expression studies in MM suggest deregulation in epigenetic modifiers activities and the associated epigenetic profiles as important contribution factors to MM pathogenesis [20, 22, 120, 123, 143]. Therefore, epigenetic modifiers and their profiles represent potential targets for therapy in MM.

One of the common epigenetic modifiers found to be upregulated in MM is the histone methyltransferase EZH2 [120-122]. EZH2 is the enzymatic subunit of PRC2, an important regulator of both normal development as well as disease [105, 107, 126]. PRC2 through EZH2 catalyzes the formation of H3K27me3 repressive histone mark, which regulates the expression of programs related to stem cell self-renewal, differentiation, but also cellular transformation [102, 111, 172]. In 2005, EZH2 was suggested to be a potential oncogene upregulated in MM, and being dependent on its methyltransferase activity [121]. In 2010, using integrative genomic approach our group has identified gene repression mediated by PRC2 as a common mechanism of gene silencing in MM. We showed that target genes of PRC2 are silenced in advanced stages of MM. Using PRC2 inhibitors available at that time Deazaneplanocin (DZNep) and the histone deacetylase inhibitor LBH589 (Panobinostat), we could show a reactivation in the expression of PRC2 target genes, which correlated with reduced tumor growth *in vitro* and *in vivo* [120].

In addition to PRC2, the polycomb repressive complex 1 (PRC1) mediates gene silencing by regulating chromatin structure via installing the transcriptional repressive mark histone H2A lysine 119 ubiquitination (H2AK119ub). PRC1 is indispensable for self-renewal of both normal and cancer stem cells [126]. The polycomb group protein BMI-1 of the PRC1 plays important roles in regulating several biological processes such as DNA damage response, cellular bioenergetics, and pathologies. Therefore, BMI-1 plays key roles in the development of different types of tumors [136-138]. Importantly, BMI-1 confers stemness properties to cancer cells of solid and hematopoietic origins [127-129]. In MM, BMI-1 is overexpressed in MM patients as compared with normal individuals, and was found to support MM cell growth by repressing the pro-apoptotic gene *BIM* [139]. BMI-1 has also been reported to be overexpressed during MM progression and has recently been suggested to be a predictor of poor survival in relapsed MM cases treated with bortezomib or dexamethasone [140]. Notably, BMI-1 knockdown was found to sensitize MM cells to bortezomib [141]. The wide spectrum of malignancies that implicate BMI-1 as a signature for stemness and oncogenesis make it a suitable candidate for therapy in cancer including MM.

Aims of the thesis

Provided the growing evidence concerning the importance of epigenetic mechanisms in MM, and based on our previous hypothesis that polycomb-mediated gene silencing could be an important factor in MM pathogenesis; the aims of my thesis work were:

1. To define the genome-wide distribution of H3K27me3 and H3K4me3 by ChIP-Seq in malignant PCs isolated from newly diagnosed patients with MM and in PCs derived from age-matched healthy donors. **(Paper I)**

2. To identify H3K27me3, H3K4me3 and bivalent targets common to MM patients and unique to MM when compared with targets in normal plasma cells. **(Paper I)**

3. To evaluate the effects and consequences of pharmacological inhibition of EZH2 enzymatic activity using high selective inhibitors (UNC1999 and GSK343) in MM using MM cell lines and patient primary cells and to describe the underlying mechanisms mediating the anti-MM effects upon EZH2 inhibition. **(Paper I and II)**

4. To evaluate the therapeutic potential of targeting PRC1 in MM using the highly selective inhibitor of BMI-1 (PTC-209). **(Paper III)**

Results

Paper I

We performed ChIP-Seq analysis to study the genome-wide distribution of H3K27me3 and H3K4me3 marks in $CD138^+$ plasma cells isolated from newly diagnosed MM patients or from age-matched hematologically normal individuals. We defined a set of H3K27me3, H3K4me3 and bivalent (carry both marks) target genes that are common to all MM patients used in this study and unique to MM when compared with targets in normal plasma cells. Using RNA-Seq, we confirmed that H3K27me3 and bivalency are associated with gene repression, while H3K4me3 mark is associated with active transcription. We used the Oncomine gene expression database to correlate MM specific H3K27me3 chromatin profiles generated in this study to gene expression in large cohort of MM patients. We found that MM unique H3K27me3 targets are repressed in advanced MM stages and in patients with poor survival. We also reported an increase in the number of bivalent genes in MM PCs compared with normal PCs, and found them to be silenced in gene expression studies performed in MM cell lines. In this study, we also evaluated the effects of two newly discovered highly selective inhibitors of EZH2, the UNC1999 and GSK343 on the viability of a panel of MM cell lines and primary cells derived from newly diagnosed MM patients. We found that both EZH2 inhibitors demonstrated anti-MM effects by reducing the viability of MM cell lines. Most importantly, the EZH2 inhibitor UNC1999 reduced the viability of $CD138^+$ PCs isolated from newly diagnosed MM patients. Flow cytometry analysis revealed that reduced MM cell viability following EZH2 inhibition was exclusively due to induction of apoptosis but not cell cycle arrest. Using western blot analysis, we demonstrated the on-target effect of both inhibitors by reducing the global levels of H3K27me3 mark in MM cell lines, leaving other histone methylation marks unaffected. To study the effect of pharmacological EZH2 inhibition on global gene expression in MM, we performed gene expression arrays in the MM INA-6 cell line treated with UNC1999 for 72 hours. We found EZH2 inhibition reactivates the expression of genes with anti-tumor activities such as genes involved in apoptosis e.g. ID1, ID2, ID3 and cellular differentiation such as SOX2. Notably, we found that the reactivated genes upon EZH2 inhibition were enriched among the MM unique bivalent genes, which was confirmed by ChIP-qPCR analysis of selected genes. We also reported that EZH2 inhibition in MM downregulated the expression of active genes (non-PRC2, H3K4me3 targets) involved in metabolism and signaling pathways.

Paper II

In this paper, we focused on the downregulated genes in MM upon EZH2 inhibition, which thus represent non-PRC2 and actively transcribed genes. We confirmed this notion by showing that the downregulated genes were only enriched in MM unique H3K4me3 targets defined by ChIP-Seq in **paper I**. Among the downregulated genes, we found that EZH2 inhibition reduced the expression of MM-associated oncogenes including IRF-4, XBP-1, BLIMP-1 and c-MYC at both the mRNA and protein levels. To provide molecular and mechanistic insights on how the inhibition of PRC2, a transcriptional repressive complex, leads to downregulation of active oncogenes, we investigated the possibility that PRC2 represses a set of non-coding RNA, mainly microRNAs (miRNAs), that may negatively modulate the expression of active oncogenes at a posttranscriptional level. To this end, we performed miRNA expression profiling in the MM INA-6 cell line treated with UNC1999 for 120 hours. We found that EZH2 inhibition upregulated a set of previously defined microRNAs with tumor suppressor functions. Using microRNA.org database we found that EZH2 inhibition reactivated the expression of miR-125a-3p and miR-320c that were predicted to target the 3' UTR regions of IRF-4, XBP-1 and BLIMP-1. We also found EZH2 inhibition led to upregulation in the expression of miR-494, which targets the 3' UTR of c-MYC. Using ChIP-qPCR we proved that these miRNAs are indeed targets of H3K27me3 and EZH2 in MM cell lines and patient primary cells. Furthermore, our ChIP-qPCR analysis showed that EZH2 inhibition using UNC1999 reduced H3K27me3 levels at genomic regions where these miRNAs are located.

Paper III

In this study, we investigated the therapeutic potential of targeting the polycomb group protein BMI-1 in MM using the recently developed specific inhibitor, PTC-209. Using cell viability assays we assessed the effect of PTC-209 treatment on the viability of MM cell lines and $CD138^+$ PCs isolated from newly diagnosed or relapsed MM patients. We found that PTC-209 demonstrated a potent anti-MM activity by reducing the viability of MM cell lines at concentrations less than 1.6 μM 48 hours following treatment. Notably, PTC-209 reduced the viability of primary MM cells purified from both newly diagnosed as well as from treatment refractory MM patients. Interestingly, PTC-209 demonstrated equal inhibitory efficacies on $CD138^+$ malignant PCs isolated from both categories of patients. To test whether PTC-209 demonstrates on-target effects in MM, we analyzed the effects of PTC-209 treatment on the expression of BMI-1 by qPCR and western blot. RNA analysis by qPCR after 48 hours treatment revealed that BMI-1 mRNA levels were not affected by PTC-209 treatment. However, western blot analysis

showed that PTC-209 downregulated BMI-1 protein levels. To further investigate the on-target specificity of PTC-209, we analyzed the protein levels of two other PRC2 core subunits; CBX-7 and the E3 ubiquitin ligase RING1B. We found that PTC-209 did not affect the protein levels of CBX-7 and RING1B in MM cells. We also revealed that PTC-209 mediated downregulation of BMI-1 reduced PRC1 chromatin modifying activity demonstrated by global reduction in the levels of the associated H2AK119ub histone repressive mark. Using FACS analysis we showed that PTC-209 led to MM cell death via induction of apoptosis, which further documented by downregulation of the anti-apoptotic protein MCL-1 and upregulation of the pro-apoptotic protein BIM. Analysis of gene expression by qPCR revealed that PTC-209 mediated inhibition of BMI-1 reactivated the expression of the pro-apoptotic gene *BIM*. To extend the therapeutic potential of PTC-209 in MM, we performed combination experiments in which we combined PTC-209 with the EZH2 inhibitor UNC1999 or with the BET bromodomains inhibitor JQ1. We found that combinations of PTC-209-UNC1999 and PTC-209-JQ1 demonstrated synergistic and additive inhibitory effects of MM cell lines growth as defined by combination index (CI) of < 0.8 for synergy and CI between 0.8-1.2 for addition using the **CompuSyn** software.

Scientific and clinical significance of the research

Multiple Myeloma (MM) is an incurable hematological malignancy characterized by the accumulation of malignant antibody producing plasmablasts/plasma cells in the bone marrow (BM). Disease associated clonal and interpatient heterogeneity has hampered identification of a common underlying mechanism for disease establishment and slowed the development of novel targeted therapies [3, 25]. Therefore, finding oncogenic processes/pathways common to all patients with potential therapeutic value is indeed imperative for improved MM therapy. Epigenetic modifiers and profiles represent promising therapeutic targets as they have proven to be amenable to re-programing during normal development and carcinogenesis without changing the underlying genetic makeup of cells [173, 174].

In the ***first study***, we defined by ChIP-Seq a common epigenetically repressed profile mediated by PRC2 in MM patients compared with normal individuals. This common epigenetically repressed profile in MM correlated with gene silencing in large cohorts of patients with advanced stages of the disease i.e. International Staging System (ISS) stage III and in patients with poor survival. Based on the ISS staging criteria of MM, patients with ISS stage III are characterized by high serum β_2-microglobulin level > 5.5 mg/L, high-risk chromosomal abnormalities or high serum lactate dehydrogenase level, which reflects high tumor load in MM patients [175]. MM patients

with poor survival were previously defined based on genetic lesions and gene expression profiling, in which 3 major genetic abnormalities and their associated gene expression defined high-risk MM patients with poor survival [17]. Here we suggest the PRC2 epigenetic profile common to all MM could be used as potential prognostic marker to predict advanced MM stages and to discriminate between low- and high-risk MM patients. Interestingly, we found H3K27me3 and bivalent targets common and unique to MM to be enriched among PRC2 targets in human embryonic stem cells [176] and human embryonic fibroblasts [125]. PRC2 has been suggested to induce stemness properties in tumors of different origins. Therefore, considering the fact that MM arise from terminally differentiated plasma cells it is an interesting and still open question whether PRC2 signature could define a population of MM cells with stemness properties in MM. In this study, we proposed the catalytic subunit of PRC2, the EZH2 as a potential therapeutic target in MM. We showed that EZH2 inhibition using selective inhibitors such as UNC1999 and GSK343 induced apoptosis in MM cell lines and most importantly reduced viability in MM cells derived from newly diagnosed MM patients. This is in agreement with the finding that EZH2 mediated knockdown by siRNA suppressed MM cells growth [121]. Our in-depth analysis revealed that EZH2 inhibitors mediated anti-MM properties is indeed due to on-target effects i.e. inhibition of EZH2 and H3K27me3 mark and upregulation of PRC2 targets in MM. In this study, we reported an increase in the number of bivalent genes in malignant PCs as compared with normal PCs. Strikingly, the EZH2 inhibitor UNC1999 predominantly reactivated the expression of bivalent genes with anti-tumor activity. Pathway analysis of reactivated genes revealed an enrichment of genes belonged to apoptosis and cell differentiation. Our findings were similar to previous reports in germinal center derived B-cell lymphoma, in which EZH2 hyperactivity was shown to promote lymphomagenesis via the formation of bivalent domains, which repressed the expression of genes required for germinal center exit [119]. Taken together, this study highlights the clinical significance of PRC2 repressed epigenetic profile in MM and suggest the methyltransferase EZH2 as a promising therapeutic target.

The therapeutic value of EZH2 inhibitors in MM is supported by our findings in the ***second study*** as we showed that pharmacological inhibition of EZH2 using UNC1999 downregulated the expression of important MM oncogenes; IRF-4, XBP-1, BLIMP-1 and c-MYC. The clinical relevance of these MM-associated oncogenes is well defined as several reports have shown the absolute requirement of these oncogenes for the survival of malignant PCs, disease establishment and progression in MM murine models and patients. For example, MM cell growth was found to be dependent on IRF-4 expression as IRF-4 knockdown suppressed the growth of myeloma cells independent of their genetic sub-type [177]. XBP-1 [178] and BLIMP-

1[179] were identified as drivers of MM pathogenesis in murine models. Deregulation in c-MYC expression is hallmark of disease progression from the benign phase MGUS to the malignant MM [45] and a requirement for MM cell growth [170]. Considering that EZH2 inhibition downregulated several MM-associated oncogenes, we propose EZH2 inhibitors as powerful anti-MM agents that demand urgent evaluation in clinical trials. In this study, we also reported for the first time that EZH2 represses the expression of microRNAs with tumor suppressor functions in MM. Most importantly, we found two microRNAs; miR-125a-3p and miR-320c to be repressed by EZH2 and H3K27me3 mark and predicted to negatively modulate the expression MM-associated oncogenes. The use of some microRNAs as anti-MM agents has shown success using MM relevant animal models [85, 87, 153]. Therefore, our work in this study proposes tumor suppressor microRNAs targeted by PRC2 in MM as potential anti-MM agents to be evaluated using MM relevant animal models. It is worth mentioning that EZH2 inhibitors are currently in phase I/phase II clinical trials in patients with B-cell lymphomas. Therefore, the work in this thesis (**studies I and II**) paves the way to use EZH2 inhibitors in pre-clinical models of MM in order to evaluate *in vivo* drug efficacy, toxicity and anti-tumor activity.

In the ***final study***, we evaluated the therapeutic potential of targeting the PRC1 using the specific inhibitor of BMI-1 subunit; PTC-209. BMI-1 is overexpressed in MM patients compared with normal individual and suggested to be a predictive of poor survival in MM [140]. In this study, we showed that the BMI-1 inhibitor PTC-209 is a potent anti-MM agent using MM cell lines and MM PCs cells isolated from newly diagnosed and relapsed MM patients. Notably, PTC-209 demonstrated on target effects in MM i.e. by downregulation of BMI-1 and H2AK119ub protein levels in MM. Our findings in this study showing the anti-MM activity of the BMI-1 inhibitor PTC-209 are in agreement with the finding that BMI-1 knockdown suppressed the growth of MM cells *in vitro* and *in vivo* [139]. We show that PTC-209 anti-MM activity is mediated by significant induction of apoptosis, which is in agreement with the anti-MM effects of BMI-1 knockdown [139]. BMI-1 promotes stemness properties in solid and hematological malignancies [127-129]. One major characteristic of MM is the extensive clonal heterogeneity and the presence of tumor cells that are resistant to different types of treatments with the capacity to re-populate the tumor. Here we could show that PTC-209 was equally efficient in reducing the viability of primary MM cells isolated from newly diagnosed or refractory patients. Therefore, BMI-1 represents a very attractive target for therapy in MM alone or in combinations with different treatment regimes. Recently, BMI-1 knockdown was shown to sensitize MM cells to bortezomib treatment [141]. In addition, PTC-209 has been reported to have synergistic and/or additive effects in MM when combined with dexamethasone, pomalidomide and carfilzomib

[140]. In this study, we suggest PTC-209 as a promising anti-MM agent in combination with epigenetic inhibitors targeting the PcG protein EZH2 (UNC1999) and the BET bromodomains (JQ1). PTC-209 demonstrated synergistic anti-MM effect when combined with the EZH2 inhibitor UNC1999 and the BET bromodomain inhibitor JQ1. Therefore, we propose combinations of epigenetic inhibitors as novel therapy in MM that would provide an efficient treatment to possibly target the MM resistant clones or even "MM tumor initiating cells".

In conclusion, the work presented in this thesis highlights the importance of epigenetic modifiers; mainly PRC2 and PRC1 and the associated chromatin signatures in MM pathogenesis. It further presents the potential use of highly selective inhibitors targeting EZH2 and BMI-1 as novel epigenetic therapeutic agents in MM alone or in combination with current treatment protocols. We further propose targeted epigenetic therapy as a novel strategy to treat MM.

Acknowledgments

First I want to thank all supervisors, colleagues, and co-authors who have helped and contributed to the work presented in this thesis. This work was carried out at the Department of Immunology, Genetics and Pathology, The Rudbeck Laboratory, Uppsala University. This research is supported by funding from the Swedish Cancer Society, the Hans von Kantzow Foundation, the Swedish Research Council and grant R01GM103893 (to Jian Jin) from the U.S. National Institutes of Health.

I want to thank my supervisor **Helena Jernberg-Wiklund** for giving me the opportunity to pursue my doctoral education in her group. This has given me the chance to grow as an independent researcher as I have learnt to constantly keep reading the literature, formulating ideas and projects, seeking interesting collaborations and improving my writing skills. I want to thank you for your patience, your knowledge, guidance and most importantly for the room of freedom to perform experiments during these 4.5 years. I also want to thank you for your efforts to help me bring my thesis into its current shape.

I want to thank my co-supervisor **Fredrik Öberg** for introducing me to the field of epigenetics as a master student and allowing me joining his lab to do my master degree project. I want to thank you for your knowledge and productive scientific discussions which were very helpful to my thesis.

I want to thank my doctoral education mentor **Kenneth Nilsson**. I appreciate your scientific knowledge, enthusiasm for research and most importantly your interests in my thesis work. You have always provided me with positivity and passion to do science. I very much enjoyed working with you.

I want to thank past and current members of Helena's group. **Inger**, **Pernilla**, **Lotta**, **Charlotte**, **Prasoon**, **Alba** and **Antonia**. It has been my pleasure to work with you guys. I really enjoyed working and sharing lots of nice moments with you, especially **Friday's Fika**. I especially want to thank **Lotta** for her great experimental assistance during the 4.5 years of my PhD work. We have done a lot of good work together, **Thank You Lotta**. I also want to thank **Prasoon** for all the late nights trying to finish the analysis of

gene/miRNA expression arrays, for helping me using the thesis template remotely from Canada and sharing lots of great moments.

I want to thank all collaborators for their important contributions to this work by providing epigenetic inhibitors, bioinformatics analysis, normal and patient plasma cells. I want to thank IGP for providing excellent facilities, which have indeed supported this work.

I also want to thank my supervisors in Jordan, **Dr. Adeeb Al-Zoubi**, and in Germany, **Dr. Peter Hemmerich**, who have significantly contributed to my scientific knowledge and experience.

I want to thank all staff at the administration department, especially **Christina Magnusson** and **Helene Norlin**. You have all the time been very positive and helpful. I want to thank many people at the Rudbeck lab for making it a wonderful working environment, **Anja**, **Diego**, **Emma**, **Larry**, **Lesley**, **Sara**, **Argyris**, **Soumi**, **Snehangshu**, **Marco**, **Sujata**, **Umash**, **Ivaylo**, and the whole **UU hematology network**. I also want to thank my friends outside IGP, especially **Hadaf** and **Nizar**. Thank you all for all the nice times that we spent together and my apologies if I have forgotten to mention other people.

I want to thank my parents in law, **Ulla and Sverker Praski**, for your love, care and support during our (Lisa's and mine) doctoral education especially the help with Adam. We are so grateful to you and lucky to have you.

I want to hugely thank my family and relatives in Jordan. I want to thank my loving parents, **Aida and Ahmad Alzrigat**, for all the support that they have given me during my whole life and during my education. Without your support, I would have not been able to go abroad and pursue my master and doctoral studies. I am so grateful to you; I love you and I miss you so much. I want to thank my siblings, you are so many to name, for their love and care. Not forgetting to thank my relatives **Yousef**, **Bashar**, and **Oqla** for encouraging me to pursue postgraduate studies.

Lastly, I want to thank my amazing loving little family, **Lisa** and **Adam**. You brought happiness and motivation into my life. **Lisa**, you have been very supportive and loving during these years. You have always pushed me forward and had a positive impact on my scientific career. **Adam**, my little lovely sunshine! You have been a great company during the whole time; your love and happy soul made me forget the toughest times that I have been through during the hectic times of PhD. **Jag älskar er**.

References

1. Kuehl WM and Bergsagel PL. Multiple myeloma: evolving genetic events and host interactions. Nat Rev Cancer. 2002; 2(3):175-187.
2. Chesi M and Bergsagel PL. Many multiple myelomas: making more of the molecular mayhem. Hematology Am Soc Hematol Educ Program. 2011; 2011:344-353.
3. Palumbo A and Anderson K. Multiple myeloma. N Engl J Med. 2011; 364(11):1046-1060.
4. Raab MS, Podar K, Breitkreutz I, Richardson PG and Anderson KC. Multiple myeloma. Lancet. 2009; 374(9686):324-339.
5. Rollig C, Knop S and Bornhauser M. Multiple myeloma. Lancet. 2014.
6. Suzuki K. Current therapeutic strategy for multiple myeloma. Jpn J Clin Oncol. 2013; 43(2):116-124.
7. Roussel M, Facon T, Moreau P, Harousseau JL and Attal M. Firstline treatment and maintenance in newly diagnosed multiple myeloma patients. Recent Results Cancer Res. 2011; 183:189-206.
8. Palumbo A, Bringhen S, Ludwig H, Dimopoulos MA, Blade J, Mateos MV, Rosinol L, Boccadoro M, Cavo M, Lokhorst H, Zweegman S, Terpos E, Davies F, Driessen C, Gimsing P, Gramatzki M, et al. Personalized therapy in multiple myeloma according to patient age and vulnerability: a report of the European Myeloma Network (EMN). Blood. 2011; 118(17):4519-4529.
9. Kyle RA, Therneau TM, Rajkumar SV, Offord JR, Larson DR, Plevak MF and Melton LJ, 3rd. A long-term study of prognosis in monoclonal gammopathy of undetermined significance. N Engl J Med. 2002; 346(8):564-569.
10. Morgan GJ, Walker BA and Davies FE. The genetic architecture of multiple myeloma. Nat Rev Cancer. 2012; 12(5):335-348.
11. Lopez-Corral L, Gutierrez NC, Vidriales MB, Mateos MV, Rasillo A, Garcia-Sanz R, Paiva B and San Miguel JF. The progression from MGUS to smoldering myeloma and eventually to multiple myeloma involves a clonal expansion of genetically abnormal plasma cells. Clin Cancer Res. 2011; 17(7):1692-1700.
12. Manier S, Sacco A, Leleu X, Ghobrial IM and Roccaro AM. Bone marrow microenvironment in multiple myeloma progression. J Biomed Biotechnol. 2012; 2012:157496.
13. Fonseca R, Bergsagel PL, Drach J, Shaughnessy J, Gutierrez N, Stewart AK, Morgan G, Van Ness B, Chesi M, Minvielle S, Neri A, Barlogie B, Kuehl WM, Liebisch P, Davies F, Chen-Kiang S, et al. International Myeloma Working Group molecular classification of multiple myeloma: spotlight review. Leukemia. 2009; 23(12):2210-2221.
14. Fonseca R, Barlogie B, Bataille R, Bastard C, Bergsagel PL, Chesi M, Davies FE, Drach J, Greipp PR, Kirsch IR, Kuehl WM, Hernandez JM, Minvielle S, Pilarski LM, Shaughnessy JD, Jr., Stewart AK, et al. Genetics and cytogenetics of multiple myeloma: a workshop report. Cancer Res. 2004; 64(4):1546-1558.

15. Boersma-Vreugdenhil GR, Kuipers J, Van Stralen E, Peeters T, Michaux L, Hagemeijer A, Pearson PL, Clevers HC and Bast BJ. The recurrent translocation t(14;20)(q32;q12) in multiple myeloma results in aberrant expression of MAFB: a molecular and genetic analysis of the chromosomal breakpoint. Br J Haematol. 2004; 126(3):355-363.
16. Ross FM, Chiecchio L, Dagrada G, Protheroe RK, Stockley DM, Harrison CJ, Cross NC, Szubert AJ, Drayson MT and Morgan GJ. The t(14;20) is a poor prognostic factor in myeloma but is associated with long-term stable disease in monoclonal gammopathies of undetermined significance. Haematologica. 2010; 95(7):1221-1225.
17. Zhan F, Huang Y, Colla S, Stewart JP, Hanamura I, Gupta S, Epstein J, Yaccoby S, Sawyer J, Burington B, Anaissie E, Hollmig K, Pineda-Roman M, Tricot G, van Rhee F, Walker R, et al. The molecular classification of multiple myeloma. Blood. 2006; 108(6):2020-2028.
18. Boyd KD, Ross FM, Chiecchio L, Dagrada GP, Konn ZJ, Tapper WJ, Walker BA, Wardell CP, Gregory WM, Szubert AJ, Bell SE, Child JA, Jackson GH, Davies FE and Morgan GJ. A novel prognostic model in myeloma based on cosegregating adverse FISH lesions and the ISS: analysis of patients treated in the MRC Myeloma IX trial. Leukemia. 2012; 26(2):349-355.
19. Avet-Loiseau H, Attal M, Moreau P, Charbonnel C, Garban F, Hulin C, Leyvraz S, Michallet M, Yakoub-Agha I, Garderet L, Marit G, Michaux L, Voillat L, Renaud M, Grosbois B, Guillerm G, et al. Genetic abnormalities and survival in multiple myeloma: the experience of the Intergroupe Francophone du Myelome. Blood. 2007; 109(8):3489-3495.
20. Chapman MA, Lawrence MS, Keats JJ, Cibulskis K, Sougnez C, Schinzel AC, Harview CL, Brunet JP, Ahmann GJ, Adli M, Anderson KC, Ardlie KG, Auclair D, Baker A, Bergsagel PL, Bernstein BE, et al. Initial genome sequencing and analysis of multiple myeloma. Nature. 2011; 471(7339):467-472.
21. Lohr JG, Stojanov P, Carter SL, Cruz-Gordillo P, Lawrence MS, Auclair D, Sougnez C, Knoechel B, Gould J, Saksena G, Cibulskis K, McKenna A, Chapman MA, Straussman R, Levy J, Perkins LM, et al. Widespread genetic heterogeneity in multiple myeloma: implications for targeted therapy. Cancer Cell. 2014; 25(1):91-101.
22. van Haaften G, Dalgliesh GL, Davies H, Chen L, Bignell G, Greenman C, Edkins S, Hardy C, O'Meara S, Teague J, Butler A, Hinton J, Latimer C, Andrews J, Barthorpe S, Beare D, et al. Somatic mutations of the histone H3K27 demethylase gene UTX in human cancer. Nat Genet. 2009; 41(5):521-523.
23. Mitsiades CS, Mitsiades NS, Richardson PG, Munshi NC and Anderson KC. Multiple myeloma: a prototypic disease model for the characterization and therapeutic targeting of interactions between tumor cells and their local microenvironment. J Cell Biochem. 2007; 101(4):950-968.
24. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976; 194(4260):23-28.
25. Bianchi G and Munshi NC. Pathogenesis beyond the cancer clone(s) in multiple myeloma. Blood. 2015.
26. Keats JJ, Chesi M, Egan JB, Garbitt VM, Palmer SE, Braggio E, Van Wier S, Blackburn PR, Baker AS, Dispenzieri A, Kumar S, Rajkumar SV, Carpten JD, Barrett M, Fonseca R, Stewart AK, et al. Clonal competition with alternating dominance in multiple myeloma. Blood. 2012; 120(5):1067-1076.
27. Bolli N, Avet-Loiseau H, Wedge DC, Van Loo P, Alexandrov LB, Martincorena I, Dawson KJ, Iorio F, Nik-Zainal S, Bignell GR, Hinton JW, Li Y, Tubio

JM, McLaren S, S OM, Butler AP, et al. Heterogeneity of genomic evolution and mutational profiles in multiple myeloma. Nat Commun. 2014; 5:2997.
28. Walker BA, Wardell CP, Melchor L, Brioli A, Johnson DC, Kaiser MF, Mirabella F, Lopez-Corral L, Humphray S, Murray L, Ross M, Bentley D, Gutierrez NC, Garcia-Sanz R, San Miguel J, Davies FE, et al. Intraclonal heterogeneity is a critical early event in the development of myeloma and precedes the development of clinical symptoms. Leukemia. 2014; 28(2):384-390.
29. Chaidos A, Barnes CP, Cowan G, May PC, Melo V, Hatjiharissi E, Papaioannou M, Harrington H, Doolittle H, Terpos E, Dimopoulos M, Abdalla S, Yarranton H, Naresh K, Foroni L, Reid A, et al. Clinical drug resistance linked to interconvertible phenotypic and functional states of tumor-propagating cells in multiple myeloma. Blood. 2013; 121(2):318-328.
30. Dean M, Fojo T and Bates S. Tumour stem cells and drug resistance. Nat Rev Cancer. 2005; 5(4):275-284.
31. Johnsen HE, Bergkvist KS, Schmitz A, Kjeldsen MK, Hansen SM, Gaihede M, Norgaard MA, Baech J, Gronholdt ML, Jensen FS, Johansen P, Bodker JS, Bogsted M and Dybkaer K. Cell of origin associated classification of B-cell malignancies by gene signatures of the normal B-cell hierarchy. Leuk Lymphoma. 2014; 55(6):1251-1260.
32. Vicente-Duenas C, Romero-Camarero I, Gonzalez-Herrero I, Alonso-Escudero E, Abollo-Jimenez F, Jiang X, Gutierrez NC, Orfao A, Marin N, Villar LM, Criado MC, Pintado B, Flores T, Alonso-Lopez D, De Las Rivas J, Jimenez R, et al. A novel molecular mechanism involved in multiple myeloma development revealed by targeting MafB to haematopoietic progenitors. EMBO J. 2012; 31(18):3704-3717.
33. Matsui W, Huff CA, Wang Q, Malehorn MT, Barber J, Tanhehco Y, Smith BD, Civin CI and Jones RJ. Characterization of clonogenic multiple myeloma cells. Blood. 2004; 103(6):2332-2336.
34. Yata K and Yaccoby S. The SCID-rab model: a novel in vivo system for primary human myeloma demonstrating growth of CD138-expressing malignant cells. Leukemia. 2004; 18(11):1891-1897.
35. Van Valckenborgh E, Matsui W, Agarwal P, Lub S, Dehui X, De Bruyne E, Menu E, Empsen C, van Grunsven L, Agarwal J, Wang Q, Jernberg-Wiklund H and Vanderkerken K. Tumor-initiating capacity of CD138- and CD138+ tumor cells in the 5T33 multiple myeloma model. Leukemia. 2012; 26(6):1436-1439.
36. Jernberg-Wiklund H and Nilsson K. (2002). Multiple Myeloma Cell Lines. In: Masters JW and Palsson B, eds. Human Cell Culture: Springer Netherlands), pp. 81-155.
37. Sanchez E, Chen H and Berenson J. (2013). Novel In Vivo Models in Myeloma. In: Munshi NC and Anderson KC, eds. Advances in Biology and Therapy of Multiple Myeloma: Springer New York), pp. 301-312.
38. Radl J. Multiple myeloma and related disorders. Lessons from an animal model. Pathol Biol (Paris). 1999; 47(2):109-114.
39. Asosingh K, Radl J, Van Riet I, Van Camp B and Vanderkerken K. The 5TMM series: a useful in vivo mouse model of human multiple myeloma. Hematol J. 2000; 1(5):351-356.
40. Vanderkerken K, Asosingh K, Croucher P and Van Camp B. Multiple myeloma biology: lessons from the 5TMM models. Immunol Rev. 2003; 194:196-206.
41. Bosma GC, Custer RP and Bosma MJ. A severe combined immunodeficiency mutation in the mouse. Nature. 1983; 301(5900):527-530.

42. Urashima M, Chen BP, Chen S, Pinkus GS, Bronson RT, Dedera DA, Hoshi Y, Teoh G, Ogata A, Treon SP, Chauhan D and Anderson KC. The development of a model for the homing of multiple myeloma cells to human bone marrow. Blood. 1997; 90(2):754-765.
43. Yaccoby S, Barlogie B and Epstein J. Primary myeloma cells growing in SCID-hu mice: a model for studying the biology and treatment of myeloma and its manifestations. Blood. 1998; 92(8):2908-2913.
44. Chesi M, Robbiani DF, Sebag M, Chng WJ, Affer M, Tiedemann R, Valdez R, Palmer SE, Haas SS, Stewart AK, Fonseca R, Kremer R, Cattoretti G and Bergsagel PL. AID-dependent activation of a MYC transgene induces multiple myeloma in a conditional mouse model of post-germinal center malignancies. Cancer Cell. 2008; 13(2):167-180.
45. Chng WJ, Huang GF, Chung TH, Ng SB, Gonzalez-Paz N, Troska-Price T, Mulligan G, Chesi M, Bergsagel PL and Fonseca R. Clinical and biological implications of MYC activation: a common difference between MGUS and newly diagnosed multiple myeloma. Leukemia. 2011; 25(6):1026-1035.
46. Zhang Y. Recent progress in the epigenetics and chromatin field. Cell Res. 2011; 21(3):373-374.
47. Kim JK, Samaranayake M and Pradhan S. Epigenetic mechanisms in mammals. Cell Mol Life Sci. 2009; 66(4):596-612.
48. Goldberg AD, Allis CD and Bernstein E. Epigenetics: a landscape takes shape. Cell. 2007; 128(4):635-638.
49. Dawson MA and Kouzarides T. Cancer epigenetics: from mechanism to therapy. Cell. 2012; 150(1):12-27.
50. Wilting RH and Dannenberg JH. Epigenetic mechanisms in tumorigenesis, tumor cell heterogeneity and drug resistance. Drug Resist Updat. 2012; 15(1-2):21-38.
51. Plass C, Pfister SM, Lindroth AM, Bogatyrova O, Claus R and Lichter P. Mutations in regulators of the epigenome and their connections to global chromatin patterns in cancer. Nat Rev Genet. 2013; 14(11):765-780.
52. Dimopoulos K, Gimsing P and Gronbaek K. The role of epigenetics in the biology of multiple myeloma. Blood Cancer J. 2014; 4:e207.
53. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. Nat Rev Genet. 2012; 13(7):484-492.
54. Bestor TH. The DNA methyltransferases of mammals. Hum Mol Genet. 2000; 9(16):2395-2402.
55. Kim GD, Ni J, Kelesoglu N, Roberts RJ and Pradhan S. Co-operation and communication between the human maintenance and de novo DNA (cytosine-5) methyltransferases. EMBO J. 2002; 21(15):4183-4195.
56. Liang G, Chan MF, Tomigahara Y, Tsai YC, Gonzales FA, Li E, Laird PW and Jones PA. Cooperativity between DNA methyltransferases in the maintenance methylation of repetitive elements. Mol Cell Biol. 2002; 22(2):480-491.
57. Williams K, Christensen J and Helin K. DNA methylation: TET proteins-guardians of CpG islands? EMBO Rep. 2012; 13(1):28-35.
58. Scourzic L, Mouly E and Bernard OA. TET proteins and the control of cytosine demethylation in cancer. Genome Med. 2015; 7(1):9.
59. Bagci H and Fisher AG. DNA demethylation in pluripotency and reprogramming: the role of tet proteins and cell division. Cell Stem Cell. 2013; 13(3):265-269.

60. Heuck CJ, Mehta J, Bhagat T, Gundabolu K, Yu Y, Khan S, Chrysofakis G, Schinke C, Tariman J, Vickrey E, Pulliam N, Nischal S, Zhou L, Bhattacharyya S, Meagher R, Hu C, et al. Myeloma is characterized by stage-specific alterations in DNA methylation that occur early during myelomagenesis. J Immunol. 2013; 190(6):2966-2975.
61. San-Miguel J, Garcia-Sanz R and Lopez-Perez R. Analysis of methylation pattern in multiple myeloma. Acta Haematol. 2005; 114 Suppl 1:23-26.
62. Walker BA, Wardell CP, Chiecchio L, Smith EM, Boyd KD, Neri A, Davies FE, Ross FM and Morgan GJ. Aberrant global methylation patterns affect the molecular pathogenesis and prognosis of multiple myeloma. Blood. 2011; 117(2):553-562.
63. Kaiser MF, Johnson DC, Wu P, Walker BA, Brioli A, Mirabella F, Wardell CP, Melchor L, Davies FE and Morgan GJ. Global methylation analysis identifies prognostically important epigenetically inactivated tumor suppressor genes in multiple myeloma. Blood. 2013; 122(2):219-226.
64. Agirre X, Castellano G, Pascual M, Heath S, Kulis M, Segura V, Bergmann A, Esteve A, Merkel A, Raineri E, Agueda L, Blanc J, Richardson D, Clarke L, Datta A, Russinol N, et al. Whole-epigenome analysis in multiple myeloma reveals DNA hypermethylation of B cell-specific enhancers. Genome Res. 2015; 25(4):478-487.
65. Bollati V, Fabris S, Pegoraro V, Ronchetti D, Mosca L, Deliliers GL, Motta V, Bertazzi PA, Baccarelli A and Neri A. Differential repetitive DNA methylation in multiple myeloma molecular subgroups. Carcinogenesis. 2009; 30(8):1330-1335.
66. Sive JI, Feber A, Smith D, Quinn J, Beck S and Yong K. Global hypomethylation in myeloma is associated with poor prognosis. Br J Haematol. 2016; 172(3):473-475.
67. Zhou Y, Chen L, Barlogie B, Stephens O, Wu X, Williams DR, Cartron MA, van Rhee F, Nair B, Waheed S, Pineda-Roman M, Alsayed Y, Anaissie E and Shaughnessy JD, Jr. High-risk myeloma is associated with global elevation of miRNAs and overexpression of EIF2C2/AGO2. Proc Natl Acad Sci U S A. 2010; 107(17):7904-7909.
68. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004; 116(2):281-297.
69. Tufekci KU, Meuwissen RL and Genc S. The role of microRNAs in biological processes. Methods Mol Biol. 2014; 1107:15-31.
70. Chan SH and Wang LH. Regulation of cancer metastasis by microRNAs. J Biomed Sci. 2015; 22(1):9.
71. Esquela-Kerscher A and Slack FJ. Oncomirs - microRNAs with a role in cancer. Nat Rev Cancer. 2006; 6(4):259-269.
72. Kent OA and Mendell JT. A small piece in the cancer puzzle: microRNAs as tumor suppressors and oncogenes. Oncogene. 2006; 25(46):6188-6196.
73. Lionetti M, Musto P, Di Martino MT, Fabris S, Agnelli L, Todoerti K, Tuana G, Mosca L, Gallo Cantafio ME, Grieco V, Bianchino G, D'Auria F, Statuto T, Mazzoccoli C, De Luca L, Petrucci MT, et al. Biological and clinical relevance of miRNA expression signatures in primary plasma cell leukemia. Clin Cancer Res. 2013; 19(12):3130-3142.
74. Lionetti M, Biasiolo M, Agnelli L, Todoerti K, Mosca L, Fabris S, Sales G, Deliliers GL, Bicciato S, Lombardi L, Bortoluzzi S and Neri A. Identification of microRNA expression patterns and definition of a microRNA/mRNA regulatory network in distinct molecular groups of multiple myeloma. Blood. 2009; 114(25):e20-26.

75. Corthals SL, Sun SM, Kuiper R, de Knegt Y, Broyl A, van der Holt B, Beverloo HB, Peeters JK, el Jarari L, Lokhorst HM, Zweegman S, Jongen-Lavrencic M and Sonneveld P. MicroRNA signatures characterize multiple myeloma patients. Leukemia. 2011; 25(11):1784-1789.
76. Chi J, Ballabio E, Chen XH, Kusec R, Taylor S, Hay D, Tramonti D, Saunders NJ, Littlewood T, Pezzella F, Boultwood J, Wainscoat JS, Hatton CS and Lawrie CH. MicroRNA expression in multiple myeloma is associated with genetic subtype, isotype and survival. Biol Direct. 2011; 6:23.
77. Zhang W, Wang YE, Zhang Y, Leleu X, Reagan M, Zhang Y, Mishima Y, Glavey S, Manier S, Sacco A, Jiang B, Roccaro AM and Ghobrial IM. Global Epigenetic Regulation of MicroRNAs in Multiple Myeloma. PLoS One. 2014; 9(10):e110973.
78. Wong KY, Huang X and Chim CS. DNA methylation of microRNA genes in multiple myeloma. Carcinogenesis. 2012; 33(9):1629-1638.
79. Bi C and Chng WJ. MicroRNA: important player in the pathobiology of multiple myeloma. Biomed Res Int. 2014; 2014:521586.
80. Seckinger A, Meissner T, Moreaux J, Benes V, Hillengass J, Castoldi M, Zimmermann J, Ho AD, Jauch A, Goldschmidt H, Klein B and Hose D. miRNAs in multiple myeloma - a survival relevant complex regulator of gene expression. Oncotarget. 2015; 6(36):39165-39183.
81. Loffler D, Brocke-Heidrich K, Pfeifer G, Stocsits C, Hackermuller J, Kretzschmar AK, Burger R, Gramatzki M, Blumert C, Bauer K, Cvijic H, Ullmann AK, Stadler PF and Horn F. Interleukin-6 dependent survival of multiple myeloma cells involves the Stat3-mediated induction of microRNA-21 through a highly conserved enhancer. Blood. 2007; 110(4):1330-1333.
82. Pichiorri F, Suh SS, Ladetto M, Kuehl M, Palumbo T, Drandi D, Taccioli C, Zanesi N, Alder H, Hagan JP, Munker R, Volinia S, Boccadoro M, Garzon R, Palumbo A, Aqeilan RI, et al. MicroRNAs regulate critical genes associated with multiple myeloma pathogenesis. Proc Natl Acad Sci U S A. 2008; 105(35):12885-12890.
83. Wu S, Yu W, Qu X, Wang R, Xu J, Zhang Q, Xu J, Li J and Chen L. Argonaute 2 promotes myeloma angiogenesis via microRNA dysregulation. J Hematol Oncol. 2014; 7:40.
84. Gutierrez NC, Sarasquete ME, Misiewicz-Krzeminska I, Delgado M, De Las Rivas J, Ticona FV, Ferminan E, Martin-Jimenez P, Chillon C, Risueno A, Hernandez JM, Garcia-Sanz R, Gonzalez M and San Miguel JF. Deregulation of microRNA expression in the different genetic subtypes of multiple myeloma and correlation with gene expression profiling. Leukemia. 2010; 24(3):629-637.
85. Roccaro AM, Sacco A, Thompson B, Leleu X, Azab AK, Azab F, Runnels J, Jia X, Ngo HT, Melhem MR, Lin CP, Ribatti D, Rollins BJ, Witzig TE, Anderson KC and Ghobrial IM. MicroRNAs 15a and 16 regulate tumor proliferation in multiple myeloma. Blood. 2009; 113(26):6669-6680.
86. Chim CS, Wong KY, Qi Y, Loong F, Lam WL, Wong LG, Jin DY, Costello JF and Liang R. Epigenetic inactivation of the miR-34a in hematological malignancies. Carcinogenesis. 2010; 31(4):745-750.
87. Di Martino MT, Leone E, Amodio N, Foresta U, Lionetti M, Pitari MR, Cantafio ME, Gulla A, Conforti F, Morelli E, Tomaino V, Rossi M, Negrini M, Ferrarini M, Caraglia M, Shammas MA, et al. Synthetic miR-34a mimics as a novel therapeutic agent for multiple myeloma: in vitro and in vivo evidence. Clin Cancer Res. 2012; 18(22):6260-6270.

88. Bi C, Chung TH, Huang G, Zhou J, Yan J, Ahmann GJ, Fonseca R and Chng WJ. Genome-wide pharmacologic unmasking identifies tumor suppressive microRNAs in multiple myeloma. Oncotarget. 2015; 6(28):26508-26518.
89. Misund K, Baranowska KA, Holien T, Rampa C, Klein DC, Borset M, Waage A and Sundan A. A method for measurement of drug sensitivity of myeloma cells co-cultured with bone marrow stromal cells. J Biomol Screen. 2013; 18(6):637-646.
90. Raimondi L, De Luca A, Morelli E, Giavaresi G, Tagliaferri P, Tassone P and Amodio N. MicroRNAs: Novel Crossroads between Myeloma Cells and the Bone Marrow Microenvironment. Biomed Res Int. 2016; 2016:6504593.
91. Roccaro AM, Sacco A, Maiso P, Azab AK, Tai YT, Reagan M, Azab F, Flores LM, Campigotto F, Weller E, Anderson KC, Scadden DT and Ghobrial IM. BM mesenchymal stromal cell-derived exosomes facilitate multiple myeloma progression. J Clin Invest. 2013; 123(4):1542-1555.
92. Umezu T, Tadokoro H, Azuma K, Yoshizawa S, Ohyashiki K and Ohyashiki JH. Exosomal miR-135b shed from hypoxic multiple myeloma cells enhances angiogenesis by targeting factor-inhibiting HIF-1. Blood. 2014; 124(25):3748-3757.
93. Huang JJ, Yu J, Li JY, Liu YT and Zhong RQ. Circulating microRNA expression is associated with genetic subtype and survival of multiple myeloma. Med Oncol. 2012; 29(4):2402-2408.
94. Ducasse M and Brown MA. Epigenetic aberrations and cancer. Mol Cancer. 2006; 5:60.
95. Lennartsson A and Ekwall K. Histone modification patterns and epigenetic codes. Biochim Biophys Acta. 2009; 1790(9):863-868.
96. Kouzarides T. Chromatin modifications and their function. Cell. 2007; 128(4):693-705.
97. Li B, Carey M and Workman JL. The role of chromatin during transcription. Cell. 2007; 128(4):707-719.
98. Verdone L, Caserta M and Di Mauro E. Role of histone acetylation in the control of gene expression. Biochem Cell Biol. 2005; 83(3):344-353.
99. Rossetto D, Avvakumov N and Cote J. Histone phosphorylation: a chromatin modification involved in diverse nuclear events. Epigenetics. 2012; 7(10):1098-1108.
100. Martin C and Zhang Y. The diverse functions of histone lysine methylation. Nat Rev Mol Cell Biol. 2005; 6(11):838-849.
101. Latham JA and Dent SY. Cross-regulation of histone modifications. Nat Struct Mol Biol. 2007; 14(11):1017-1024.
102. Di Croce L and Helin K. Transcriptional regulation by Polycomb group proteins. Nat Struct Mol Biol. 2013; 20(10):1147-1155.
103. Aranda S, Mas G and Di Croce L. Regulation of gene transcription by Polycomb proteins. Sci Adv. 2015; 1(11):e1500737.
104. Schuettengruber B, Chourrout D, Vervoort M, Leblanc B and Cavalli G. Genome regulation by polycomb and trithorax proteins. Cell. 2007; 128(4):735-745.
105. Aloia L, Di Stefano B and Di Croce L. Polycomb complexes in stem cells and embryonic development. Development. 2013; 140(12):2525-2534.
106. Simon JA and Kingston RE. Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put. Mol Cell. 2013; 49(5):808-824.
107. Koppens M and van Lohuizen M. Context-dependent actions of Polycomb repressors in cancer. Oncogene. 2015.

108. Kalb R, Latwiel S, Baymaz HI, Jansen PW, Muller CW, Vermeulen M and Muller J. Histone H2A monoubiquitination promotes histone H3 methylation in Polycomb repression. Nat Struct Mol Biol. 2014; 21(6):569-571.
109. Ohm JE, McGarvey KM, Yu X, Cheng L, Schuebel KE, Cope L, Mohammad HP, Chen W, Daniel VC, Yu W, Berman DM, Jenuwein T, Pruitt K, Sharkis SJ, Watkins DN, Herman JG, et al. A stem cell-like chromatin pattern may predispose tumor suppressor genes to DNA hypermethylation and heritable silencing. Nat Genet. 2007; 39(2):237-242.
110. Schlesinger Y, Straussman R, Keshet I, Farkash S, Hecht M, Zimmerman J, Eden E, Yakhini Z, Ben-Shushan E, Reubinoff BE, Bergman Y, Simon I and Cedar H. Polycomb-mediated methylation on Lys27 of histone H3 pre-marks genes for de novo methylation in cancer. Nat Genet. 2007; 39(2):232-236.
111. Margueron R and Reinberg D. The Polycomb complex PRC2 and its mark in life. Nature. 2011; 469(7330):343-349.
112. Majer CR, Jin L, Scott MP, Knutson SK, Kuntz KW, Keilhack H, Smith JJ, Moyer MP, Richon VM, Copeland RA and Wigle TJ. A687V EZH2 is a gain-of-function mutation found in lymphoma patients. FEBS Lett. 2012; 586(19):3448-3451.
113. Morin RD, Johnson NA, Severson TM, Mungall AJ, An J, Goya R, Paul JE, Boyle M, Woolcock BW, Kuchenbauer F, Yap D, Humphries RK, Griffith OL, Shah S, Zhu H, Kimbara M, et al. Somatic mutations altering EZH2 (Tyr641) in follicular and diffuse large B-cell lymphomas of germinal-center origin. Nat Genet. 2010; 42(2):181-185.
114. Chapman-Rothe N, Curry E, Zeller C, Liber D, Stronach E, Gabra H, Ghaem-Maghami S and Brown R. Chromatin H3K27me3/H3K4me3 histone marks define gene sets in high-grade serous ovarian cancer that distinguish malignant, tumour-sustaining and chemo-resistant ovarian tumour cells. Oncogene. 2013; 32(38):4586-4592.
115. Yu J, Yu J, Rhodes DR, Tomlins SA, Cao X, Chen G, Mehra R, Wang X, Ghosh D, Shah RB, Varambally S, Pienta KJ and Chinnaiyan AM. A polycomb repression signature in metastatic prostate cancer predicts cancer outcome. Cancer Res. 2007; 67(22):10657-10663.
116. Suva ML, Riggi N, Janiszewska M, Radovanovic I, Provero P, Stehle JC, Baumer K, Le Bitoux MA, Marino D, Cironi L, Marquez VE, Clement V and Stamenkovic I. EZH2 is essential for glioblastoma cancer stem cell maintenance. Cancer Res. 2009; 69(24):9211-9218.
117. Dubuc AM, Remke M, Korshunov A, Northcott PA, Zhan SH, Mendez-Lago M, Kool M, Jones DT, Unterberger A, Morrissy AS, Shih D, Peacock J, Ramaswamy V, Rolider A, Wang X, Witt H, et al. Aberrant patterns of H3K4 and H3K27 histone lysine methylation occur across subgroups in medulloblastoma. Acta Neuropathol. 2013; 125(3):373-384.
118. Sneeringer CJ, Scott MP, Kuntz KW, Knutson SK, Pollock RM, Richon VM and Copeland RA. Coordinated activities of wild-type plus mutant EZH2 drive tumor-associated hypertrimethylation of lysine 27 on histone H3 (H3K27) in human B-cell lymphomas. Proc Natl Acad Sci U S A. 2010; 107(49):20980-20985.
119. Beguelin W, Popovic R, Teater M, Jiang Y, Bunting KL, Rosen M, Shen H, Yang SN, Wang L, Ezponda T, Martinez-Garcia E, Zhang H, Zheng Y, Verma SK, McCabe MT, Ott HM, et al. EZH2 is required for germinal center formation and somatic EZH2 mutations promote lymphoid transformation. Cancer Cell. 2013; 23(5):677-692.

120. Kalushkova A, Fryknas M, Lemaire M, Fristedt C, Agarwal P, Eriksson M, Deleu S, Atadja P, Osterborg A, Nilsson K, Vanderkerken K, Oberg F and Jernberg-Wiklund H. Polycomb target genes are silenced in multiple myeloma. PLoS One. 2010; 5(7):e11483.
121. Croonquist PA and Van Ness B. The polycomb group protein enhancer of zeste homolog 2 (EZH 2) is an oncogene that influences myeloma cell growth and the mutant ras phenotype. Oncogene. 2005; 24(41):6269-6280.
122. Zhan F, Hardin J, Kordsmeier B, Bumm K, Zheng M, Tian E, Sanderson R, Yang Y, Wilson C, Zangari M, Anaissie E, Morris C, Muwalla F, van Rhee F, Fassas A, Crowley J, et al. Global gene expression profiling of multiple myeloma, monoclonal gammopathy of undetermined significance, and normal bone marrow plasma cells. Blood. 2002; 99(5):1745-1757.
123. Pawlyn C, Kaiser M, Heuck C, Melchor L, Wardell C, Murison A, Chavan S, Johnson DC, Begum DB, Dahir N, Proszek P, Cairns DA, Boyle E, Jones J, Cook G, Drayson MT, et al. The spectrum and clinical impact of epigenetic modifier mutations in myeloma. Clin Cancer Res. 2016.
124. Martinez-Garcia E, Popovic R, Min DJ, Sweet SM, Thomas PM, Zamdborg L, Heffner A, Will C, Lamy L, Staudt LM, Levens DL, Kelleher NL and Licht JD. The MMSET histone methyl transferase switches global histone methylation and alters gene expression in t(4;14) multiple myeloma cells. Blood. 2011; 117(1):211-220.
125. Bracken AP, Dietrich N, Pasini D, Hansen KH and Helin K. Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions. Genes Dev. 2006; 20(9):1123-1136.
126. Sauvageau M and Sauvageau G. Polycomb group proteins: multi-faceted regulators of somatic stem cells and cancer. Cell Stem Cell. 2010; 7(3):299-313.
127. Kreso A, van Galen P, Pedley NM, Lima-Fernandes E, Frelin C, Davis T, Cao L, Baiazitov R, Du W, Sydorenko N, Moon YC, Gibson L, Wang Y, Leung C, Iscove NN, Arrowsmith CH, et al. Self-renewal as a therapeutic target in human colorectal cancer. Nat Med. 2014; 20(1):29-36.
128. Mayr C, Wagner A, Loeffelberger M, Bruckner D, Jakab M, Berr F, Di Fazio P, Ocker M, Neureiter D, Pichler M and Kiesslich T. The BMI1 inhibitor PTC-209 is a potential compound to halt cellular growth in biliary tract cancer cells. Oncotarget. 2016; 7(1):745-758.
129. Raaphorst FM. Self-renewal of hematopoietic and leukemic stem cells: a central role for the Polycomb-group gene Bmi-1. Trends Immunol. 2003; 24(10):522-524.
130. Yang DD, Cui BB, Sun LY, Zheng HQ, Huang Q, Tong JX and Zhang QF. The co-expression of USP22 and BMI-1 may promote cancer progression and predict therapy failure in gastric carcinoma. Cell Biochem Biophys. 2011; 61(3):703-710.
131. Yin T, Wei H, Leng Z, Yang Z, Gou S, Wu H, Zhao G, Hu X and Wang C. Bmi-1 promotes the chemoresistance, invasion and tumorigenesis of pancreatic cancer cells. Chemotherapy. 2011; 57(6):488-496.
132. Wu Z, Min L, Chen D, Hao D, Duan Y, Qiu G and Wang Y. Overexpression of BMI-1 promotes cell growth and resistance to cisplatin treatment in osteosarcoma. PLoS One. 2011; 6(2):e14648.
133. Banerjee Mustafi S, Chakraborty PK, Naz S, Dwivedi SK, Street M, Basak R, Yang D, Ding K, Mukherjee P and Bhattacharya R. MDR1 mediated chemoresistance: BMI1 and TIP60 in action. Biochim Biophys Acta. 2016; 1859(8):983-993.

134. van Lohuizen M, Verbeek S, Scheijen B, Wientjens E, van der Gulden H and Berns A. Identification of cooperating oncogenes in E mu-myc transgenic mice by provirus tagging. Cell. 1991; 65(5):737-752.
135. Haupt Y, Alexander WS, Barri G, Klinken SP and Adams JM. Novel zinc finger gene implicated as myc collaborator by retrovirally accelerated lymphomagenesis in E mu-myc transgenic mice. Cell. 1991; 65(5):753-763.
136. Bhattacharya R, Mustafi SB, Street M, Dey A and Dwivedi SK. Bmi-1: At the crossroads of physiological and pathological biology. Genes Dis. 2015; 2(3):225-239.
137. Cao L, Bombard J, Cintron K, Sheedy J, Weetall ML and Davis TW. BMI1 as a novel target for drug discovery in cancer. J Cell Biochem. 2011; 112(10):2729-2741.
138. Wang MC, Li CL, Cui J, Jiao M, Wu T, Jing LI and Nan KJ. BMI-1, a promising therapeutic target for human cancer. Oncol Lett. 2015; 10(2):583-588.
139. Jagani Z, Wiederschain D, Loo A, He D, Mosher R, Fordjour P, Monahan J, Morrissey M, Yao YM, Lengauer C, Warmuth M, Sellers WR and Dorsch M. The Polycomb group protein Bmi-1 is essential for the growth of multiple myeloma cells. Cancer Res. 2010; 70(13):5528-5538.
140. Bolomsky A, Schlangen K, Schreiner W, Zojer N and Ludwig H. Targeting of BMI-1 with PTC-209 shows potent anti-myeloma activity and impairs the tumour microenvironment. J Hematol Oncol. 2016; 9:17.
141. Wu SQ, Xu ZZ, Niu WY, Huang HB and Zhan R. ShRNA-mediated Bmi-1 silencing sensitizes multiple myeloma cells to bortezomib. Int J Mol Med. 2014; 34(2):616-623.
142. Sawyer JR, Tian E, Heuck CJ, Johann DJ, Epstein J, Swanson CM, Lukacs JL, Binz RL, Johnson M, Sammartino G, Zangari M, Davies FE, van Rhee F, Morgan GJ and Barlogie B. Evidence of an epigenetic origin for high-risk 1q21 copy number aberrations in multiple myeloma. Blood. 2015; 125(24):3756-3759.
143. Popovic R, Martinez-Garcia E, Giannopoulou EG, Zhang Q, Zhang Q, Ezponda T, Shah MY, Zheng Y, Will CM, Small EC, Hua Y, Bulic M, Jiang Y, Carrara M, Calogero RA, Kath WL, et al. Histone Methyltransferase MMSET/NSD2 Alters EZH2 Binding and Reprograms the Myeloma Epigenome through Global and Focal Changes in H3K36 and H3K27 Methylation. PLoS Genet. 2014; 10(9):e1004566.
144. Keats JJ, Reiman T, Maxwell CA, Taylor BJ, Larratt LM, Mant MJ, Belch AR and Pilarski LM. In multiple myeloma, t(4;14)(p16;q32) is an adverse prognostic factor irrespective of FGFR3 expression. Blood. 2003; 101(4):1520-1529.
145. Marango J, Shimoyama M, Nishio H, Meyer JA, Min DJ, Sirulnik A, Martinez-Martinez Y, Chesi M, Bergsagel PL, Zhou MM, Waxman S, Leibovitch BA, Walsh MJ and Licht JD. The MMSET protein is a histone methyltransferase with characteristics of a transcriptional corepressor. Blood. 2008; 111(6):3145-3154.
146. Kuo AJ, Cheung P, Chen K, Zee BM, Kioi M, Lauring J, Xi Y, Park BH, Shi X, Garcia BA, Li W and Gozani O. NSD2 links dimethylation of histone H3 at lysine 36 to oncogenic programming. Mol Cell. 2011; 44(4):609-620.
147. De Bruyne E, Bos TJ, Schuit F, Van Valckenborgh E, Menu E, Thorrez L, Atadja P, Jernberg-Wiklund H and Vanderkerken K. IGF-1 suppresses Bim expression in multiple myeloma via epigenetic and posttranslational mechanisms. Blood. 2010; 115(12):2430-2440.
148. Rius M and Lyko F. Epigenetic cancer therapy: rationales, targets and drugs. Oncogene. 2012; 31(39):4257-4265.

149. Kantarjian H, Issa JP, Rosenfeld CS, Bennett JM, Albitar M, DiPersio J, Klimek V, Slack J, de Castro C, Ravandi F, Helmer R, 3rd, Shen L, Nimer SD, Leavitt R, Raza A and Saba H. Decitabine improves patient outcomes in myelodysplastic syndromes: results of a phase III randomized study. Cancer. 2006; 106(8):1794-1803.
150. Silverman LR, Demakos EP, Peterson BL, Kornblith AB, Holland JC, Odchimar-Reissig R, Stone RM, Nelson D, Powell BL, DeCastro CM, Ellerton J, Larson RA, Schiffer CA and Holland JF. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B. J Clin Oncol. 2002; 20(10):2429-2440.
151. Khong T, Sharkey J and Spencer A. The effect of azacitidine on interleukin-6 signaling and nuclear factor-kappaB activation and its in vitro and in vivo activity against multiple myeloma. Haematologica. 2008; 93(6):860-869.
152. Iorio MV and Croce CM. MicroRNA dysregulation in cancer: diagnostics, monitoring and therapeutics. A comprehensive review. EMBO Mol Med. 2012; 4(3):143-159.
153. Morelli E, Leone E, Cantafio ME, Di Martino MT, Amodio N, Biamonte L, Gulla A, Foresta U, Pitari MR, Botta C, Rossi M, Neri A, Munshi NC, Anderson KC, Tagliaferri P and Tassone P. Selective targeting of IRF4 by synthetic microRNA-125b-5p mimics induces anti-multiple myeloma activity in vitro and in vivo. Leukemia. 2015.
154. West AC and Johnstone RW. New and emerging HDAC inhibitors for cancer treatment. J Clin Invest. 2014; 124(1):30-39.
155. Marchion D and Munster P. Development of histone deacetylase inhibitors for cancer treatment. Expert Rev Anticancer Ther. 2007; 7(4):583-598.
156. Maes K, Menu E, Van Valckenborgh E, Van Riet I, Vanderkerken K and De Bruyne E. Epigenetic modulating agents as a new therapeutic approach in multiple myeloma. Cancers (Basel). 2013; 5(2):430-461.
157. New M, Olzscha H and La Thangue NB. HDAC inhibitor-based therapies: can we interpret the code? Mol Oncol. 2012; 6(6):637-656.
158. Richardson PG, Schlossman RL, Alsina M, Weber DM, Coutre SE, Gasparetto C, Mukhopadhyay S, Ondovik MS, Khan M, Paley CS and Lonial S. PANORAMA 2: panobinostat in combination with bortezomib and dexamethasone in patients with relapsed and bortezomib-refractory myeloma. Blood. 2013; 122(14):2331-2337.
159. Dimopoulos M, Siegel DS, Lonial S, Qi J, Hajek R, Facon T, Rosinol L, Williams C, Blacklock H, Goldschmidt H, Hungria V, Spencer A, Palumbo A, Graef T, Eid JE, Houp J, et al. Vorinostat or placebo in combination with bortezomib in patients with multiple myeloma (VANTAGE 088): a multicentre, randomised, double-blind study. Lancet Oncol. 2013; 14(11):1129-1140.
160. Offidani M, Polloni C, Cavallo F, Liberati AM, Ballanti S, Pulini S, Catarini M, Alesiani F, Corvatta L, Gentili S, Caraffa P, Boccadoro M, Leoni P and Palumbo A. Phase II study of melphalan, thalidomide and prednisone combined with oral panobinostat in patients with relapsed/refractory multiple myeloma. Leuk Lymphoma. 2012; 53(9):1722-1727.
161. McCabe MT, Ott HM, Ganji G, Korenchuk S, Thompson C, Van Aller GS, Liu Y, Graves AP, Della Pietra A, 3rd, Diaz E, LaFrance LV, Mellinger M, Duquenne C, Tian X, Kruger RG, McHugh CF, et al. EZH2 inhibition as a therapeutic strategy for lymphoma with EZH2-activating mutations. Nature. 2012; 492(7427):108-112.

162. Knutson SK, Wigle TJ, Warholic NM, Sneeringer CJ, Allain CJ, Klaus CR, Sacks JD, Raimondi A, Majer CR, Song J, Scott MP, Jin L, Smith JJ, Olhava EJ, Chesworth R, Moyer MP, et al. A selective inhibitor of EZH2 blocks H3K27 methylation and kills mutant lymphoma cells. Nat Chem Biol. 2012; 8(11):890-896.
163. Knutson SK, Kawano S, Minoshima Y, Warholic NM, Huang KC, Xiao Y, Kadowaki T, Uesugi M, Kuznetsov G, Kumar N, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, Waters NJ, Smith JJ, et al. Selective inhibition of EZH2 by EPZ-6438 leads to potent antitumor activity in EZH2-mutant non-Hodgkin lymphoma. Mol Cancer Ther. 2014; 13(4):842-854.
164. Xu B, On DM, Ma A, Parton T, Konze KD, Pattenden SG, Allison DF, Cai L, Rockowitz S, Liu S, Liu Y, Li F, Vedadi M, Frye SV, Garcia BA, Zheng D, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. Blood. 2015; 125(2):346-357.
165. Knutson SK, Warholic NM, Wigle TJ, Klaus CR, Allain CJ, Raimondi A, Porter Scott M, Chesworth R, Moyer MP, Copeland RA, Richon VM, Pollock RM, Kuntz KW and Keilhack H. Durable tumor regression in genetically altered malignant rhabdoid tumors by inhibition of methyltransferase EZH2. Proc Natl Acad Sci U S A. 2013; 110(19):7922-7927.
166. Gaudichon J, Milano F, Cahu J, DaCosta L, Martens AC, Renoir JM and Sola B. Deazaneplanocin a is a promising drug to kill multiple myeloma cells in their niche. PLoS One. 2014; 9(9):e107009.
167. Verma SK, Tian X, LaFrance LV, Duquenne C, Suarez DP, Newlander KA, Romeril SP, Burgess JL, Grant SW, Brackley JA, Graves AP, Scherzer DA, Shu A, Thompson C, Ott HM, Aller GS, et al. Identification of Potent, Selective, Cell-Active Inhibitors of the Histone Lysine Methyltransferase EZH2. ACS Med Chem Lett. 2012; 3(12):1091-1096.
168. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, Fedorov O, Morse EM, Keates T, Hickman TT, Felletar I, Philpott M, Munro S, McKeown MR, Wang Y, Christie AL, West N, et al. Selective inhibition of BET bromodomains. Nature. 2010; 468(7327):1067-1073.
169. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, Jacobs HM, Kastritis E, Gilpatrick T, Paranal RM, Qi J, Chesi M, Schinzel AC, McKeown MR, Heffernan TP, Vakoc CR, Bergsagel PL, et al. BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell. 2011; 146(6):904-917.
170. Holien T, Vatsveen TK, Hella H, Waage A and Sundan A. Addiction to c-MYC in multiple myeloma. Blood. 2012; 120(12):2450-2453.
171. Abdi J, Chen G and Chang H. Drug resistance in multiple myeloma: latest findings and new concepts on molecular mechanisms. Oncotarget. 2013; 4(12):2186-2207.
172. Cao R, Wang L, Wang H, Xia L, Erdjument-Bromage H, Tempst P, Jones RS and Zhang Y. Role of histone H3 lysine 27 methylation in Polycomb-group silencing. Science. 2002; 298(5595):1039-1043.
173. Onder TT, Kara N, Cherry A, Sinha AU, Zhu N, Bernt KM, Cahan P, Marcarci BO, Unternaehrer J, Gupta PB, Lander ES, Armstrong SA and Daley GQ. Chromatin-modifying enzymes as modulators of reprogramming. Nature. 2012; 483(7391):598-602.
174. Munoz P, Iliou MS and Esteller M. Epigenetic alterations involved in cancer stem cell reprogramming. Mol Oncol. 2012; 6(6):620-636.

175. Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, Richardson P, Caltagirone S, Lahuerta JJ, Facon T, Bringhen S, Gay F, Attal M, Passera R, Spencer A, Offidani M, et al. Revised International Staging System for Multiple Myeloma: A Report From International Myeloma Working Group. J Clin Oncol. 2015; 33(26):2863-2869.
176. Lee TI, Jenner RG, Boyer LA, Guenther MG, Levine SS, Kumar RM, Chevalier B, Johnstone SE, Cole MF, Isono K, Koseki H, Fuchikami T, Abe K, Murray HL, Zucker JP, Yuan B, et al. Control of developmental regulators by Polycomb in human embryonic stem cells. Cell. 2006; 125(2):301-313.
177. Shaffer AL, Emre NC, Lamy L, Ngo VN, Wright G, Xiao W, Powell J, Dave S, Yu X, Zhao H, Zeng Y, Chen B, Epstein J and Staudt LM. IRF4 addiction in multiple myeloma. Nature. 2008; 454(7201):226-231.
178. Carrasco DR, Sukhdeo K, Protopopova M, Sinha R, Enos M, Carrasco DE, Zheng M, Mani M, Henderson J, Pinkus GS, Munshi N, Horner J, Ivanova EV, Protopopov A, Anderson KC, Tonon G, et al. The differentiation and stress response factor XBP-1 drives multiple myeloma pathogenesis. Cancer Cell. 2007; 11(4):349-360.
179. D'Costa K, Emslie D, Metcalf D, Smyth GK, Karnowski A, Kallies A, Nutt SL and Corcoran LM. Blimp1 is limiting for transformation in a mouse plasmacytoma model. Blood. 2009; 113(23):5911-5919.

Acta Universitatis Upsaliensis

Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine 1296

Editor: The Dean of the Faculty of Medicine

A doctoral dissertation from the Faculty of Medicine, Uppsala University, is usually a summary of a number of papers. A few copies of the complete dissertation are kept at major Swedish research libraries, while the summary alone is distributed internationally through the series Digital Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine. (Prior to January, 2005, the series was published under the title "Comprehensive Summaries of Uppsala Dissertations from the Faculty of Medicine".)

Distribution: publications.uu.se
urn:nbn:se:uu:diva-312250

ACTA
UNIVERSITATIS
UPSALIENSIS
UPPSALA
2017