ANTI-CANCER EFFECT OF BIX01294 TOWARDS CML CELL LINES

LOH SHENG WEI

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE (EXCEPT MATHEMATICS & SCIENCE PHILOSOPHY)

INSTITUTE OF BIOLOGICAL SCIENCES
FACULTY OF SCIENCES
UNIVERSITY OF MALAYA
KUALA LUMPUR

2016

UNIVERSITY OF MALAYA

ORIGINAL LITERARY WORK DECLARATION

Name of Candidate: LOH SHENG WEI [redacted]

Registration/Matric No: SGR 130047

Name of Degree: MASTER OF SCIENCE (EXCEPT MATHEMATICS AND SCIENCE PHILOSOPHY)

Title of Project Paper/Research Report/Dissertation/Thesis ("this Work"):

ANTI-CANCER EFFECT OF BIX01294 TOWARDS CML CELL LINES

Field of Study: GENETICS AND MOLECULAR BIOLOGY

I do solemnly and sincerely declare that:

(1) I am the sole author/writer of this Work;
(2) This Work is original;
(3) Any use of any work in which copyright exists was done by way of fair dealing and for permitted purposes and any excerpt or extract from, or reference to or reproduction of any copyright work has been disclosed expressly and sufficiently and the title of the Work and its authorship have been acknowledged in this Work;
(4) I do not have any actual knowledge nor do I ought reasonably to know that the making of this work constitutes an infringement of any copyright work;
(5) I hereby assign all and every rights in the copyright to this Work to the University of Malaya ("UM"), who henceforth shall be owner of the copyright in this Work and that any reproduction or use in any form or by any means whatsoever is prohibited without the written consent of UM having been first had and obtained;
(6) I am fully aware that if in the course of making this Work I have infringed any copyright whether intentionally or otherwise, I may be subject to legal action or any other action as may be determined by UM.

[redacted]

Date: 7.4.2016

Subscribed and solemnly declared before,

[redacted]

Date: 7.4.2016

Name: Yap Lee Fah

Designation:

DR. YAP LEE FAH
Senior Lecturer
Dept of Oral Biology & Biomedical Sciences
Faculty of Dentistry
University of Malaya
50603 Kuala Lumpur
MALAYSIA

ABSTRACT

Background: H3K9 methylation is one of the essential histone post-translational modifications for heterochromatin formation and transcriptional repression. Recently, several studies have demonstrated that H3K9 methylation negatively regulates the type I interferon response.

Results: I report the application of EHMT1 and EHMT2 specific chemical inhibitors to sensitize CML cell lines to interferon and imatinib treatments. Inhibition of EHMT1 and EHMT2 with BIX01294 enhances the cytotoxicity of IFNα2a in four CML cell lines, K562, KCL22, BV173 and KT1. Chromatin immunoprecipitation assay shows that BIX01294 treatment enhances type I interferon response by reducing H3K9me2 at the promoters of interferon-stimulated genes. Additionally, BIX01294 treatment augments IFNα2a- and imatinib-mediated apoptosis in CML cell lines. Moreover, my data suggest that the expression level of EHMT1 and EHMT2 inversely correlates with the type I interferon responsiveness in CML cell lines.

Conclusions: My study sheds light on the role of EHMT1 and EHMT2 as potential targets in improving the efficacy of standard treatments of CML.

ABSTRAK

Latar Belakang: H3K9 metilasi adalah salah satu pengubahsuaian penting bagi histone selepas translasi untuk pembentukan heterochromatin dan penindasan transkripsi . Baru-baru ini , beberapa kajian telah menunjukkan bahawa tindakbalas Interferon jenis I dikawal oleh H3K9 metilasi secare negatif.

Hasil: Saya melaporkan Penggunaan inhibitor kimia EHMT1 dan EHMT2 yang khusus untuk merangsang sel CML terhadap rawatan interferon dan imatinib. Perencatan EHMT1 dan EHMT2 dengan BIX01294 meningkatkan sitotoksisiti sel CML terhadap IFNα2a termasuk sel K562, KCL22 , BV173 dan KT1 . Kromatin immunoprecipitation assay menunjukkan bahawa rawatan BIX01294 meningkatkan tindakbalas interferon dengan mengurangkan H3K9me2 di promoter "interferon-stimulated gene". Selain itu, rawatan BIX01294 mempercepatkan apoptosis sel CML dengan rawatan IFNα2a- dan imatinib. Akhir sekali, data saya mencadangkan bahawa hubung-kait tahap ungkapan EHMT1 dan EHMT2 dengan interferon jenis I adalah songsang.

Kesimpulan: Kajian saya mencadangkan peranan EHMT1 dan EHMT2 sebagai sasaran yang berpotensi dalam meningkatkan keberkesanan rawatan penyakit CML.

ACKNOWLEDGEMENT

I would like to express my deepest gratitude to my project supervisor Dr. Ea Chee Kwee for his kind guidance and selflessness in sharing his knowledge with me. His assistance has eased many problems encountered throughout the course of writing this dissertation as well as the research that lies behind. I am, also, grateful to Dr. Lim Yat Yuen for his guidance and advice that have refined my study. Thanks to my lab mates, Yeo Kok Siong and Ng Wei Lun, for their support and hard work throughout this study. They are dedicated teacher and friends to me, and have assisted me through my whole study.

I am grateful to the PIs, administrative staff and lab technologists from the HIR, University of Malaya for their help and the use of their excellent facilities throughout my project study.

Last but not least, I would like to express my deepest gratitude to my family for their support and care. They are my strongest motivation and inspiration as they have been supporting me with encouragement. I wish to dedicate this thesis to them as my appreciation to them.

In summary, I would like to thank everyone for putting up with me for the past few months.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF SYMBOLS AND ABBREVIATIONS

+	positive
–	negative or minus
<	less than
10X	ten times
1X	one times
Abl	Abelson murine leukemia viral oncogene homolog 1 gene
ADP	Adenosine diphosphate
APS	Ammonium Persulfate
ATP	Adenosine triphosphate
Bcr	breakpoint cluster region protein
cDNA	Complementary DNA
cm	centimetre
CO_2	Carbon dioxide
CML	Chronic myeloid leukemia
ddH_2O	double distilled water
DMSO	Dimethyl Sulfoxide
DNA	Deoxy Ribonucleic Acid
dNTP	deoxynucleoside triphosphate
DTT	Dithiothreitol
EDTA	ethylenediaminetetraacetic acid
EHMT1	euchromatic histone-lysine N-methyltransferase 1
EHMT2	euchromatic histone-lysine N-methyltransferase 2
ELISA	enzyme-linked immunosorbent assay
et al.	et all
EtOH	ethanol

FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
g	gram
H3K9	Histone 3 Lysine 9
H3K9me1	Histone 3 Lysine 9 monomethylation
H3K9me2	Histone 3 Lysine 9 dimethylation
H3K9me3	Histone 3 Lysine 9 trimethylation
hBMMS	human bone marrow-derived mesenchymal stem cells
HKMTs	Histone lysine methyltransferases
IFNα2a	Interferon alpha-2-a
IFNβ	Interferon beta
IFN-γ	Interferon gamma
IRF9	Interferon reglatory factor 9
Ig	Immunoglobulin
ISGs	Interferons stimulated genes
JAK/STAT	Janus Kinase/ signal transducers and activators of transcription
K	Lysine
kb	kilo base
kDa	kiloDalton
L	liter
mA	milliamperes
mg	miligram
mins	minutes
ml	milliliter
mm	millimeter
nm	nanometer

MOI	Multiplicity of Infection
MTT	3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide
^{o}C	degree Celsius
PAGE	polyacrylamide gel electrophoresis
PARP	poly-ADP-ribose polymerase
PBS	Phosphate Buffered Saline
PCR	Polymerase chain reaction
pH	Potential hydrogen
PI	Propidium iodide
PKMTs	Protein lysine methyltransferases
qPCR	Quantitative-PCR
RIZ	retinoblastoma protein-interacting zinc-finger methyltransferases
RNA	Ribonucleic acid
RNAi	RNA interference
rpm	revolution per minute
RT	Real-Time
RT-PCR	Reverse transcription polymerase chain reaction
SAM	S-Adenosyl methionine
SET	Su(var)3-9–Enhancer of zeste–Trithorax
SDS	Sodium dodecyl sulphate
SH2	Src homolog 2
shRNA	short hairpin RNA
TEMED	Tetramethylethylenediamine
Tris	Tris(hydroxymethyl)aminomethane
IU	International Unit
V	Volt

v/v	volume/volume
VSV	Vesicular Somatitis Virus
GFP	Green fluorescent protein
X	Times/multiplication
μm	Micrometer
μg	Microgram
μM	Micromolar

LIST OF APPENDIX

CHAPTER 1: INTRODUCTION

1.1 Epigenetics – An overview

In a multicellular organism, cells with the same genetic material may perform very diverse functions. For example, "Why are there skin cells and liver cells if each of human cells contains the same DNA materials?" This simple question clearly points out that there are additional factors that run in parallel to the genome that determine which sets of genes are expressed and which are suppressed at particular times, making a skin cell a skin cell and a liver cell a liver cell.

Cell-cell differences are determined by expression of different sets of genes with genes that are not needed being switched off during its development. This is crucial as it is the number of active genes that determined the cells capability to carry out their respective routine according to the cell type and location in the body. Therefore different types of cells, their roles and functions are discriminated by epigenetics.

The term epigenetics, which was coined by Conrad H. Waddington in 1942 (Waddington, 1942, 2012), was derived from the Greek word "epigenesis" which originally describes the influence of genetic processes on development. In Biology, epigenetics is defined by the heritable changes in gene activity and expression that do not involve changes to the underlying DNA sequence; or a change in phenotype without a change in genotype (A. Bird, 2007).

Different factors including age, lifestyle, health status and environment can influence epigenetics changes (Latham, Sapienza, & Engel, 2012). In some cases epigenetic changes may be inherited by future generations (Bossdorf, Richards, & Pigliucci, 2008).

Epigenetics marks dictate whether particular genes are expressed or not and form the molecular basis of epigenetics. Epigenetics marks including methylation of DNA and covalent modification of histone proteins are features not directly governed by the genetic code. DNA modification typically relates to long-term epigenetics memory: once methylated, genomic DNA remains methylated through generations (Adrian Bird, 2002). On the other hand, histone modifications provide short-term epigenetic memory and can be reversed after a few cell division cycles (Turner, 2002).

DNA methylation was first postulated in 1975 to be a heritable modification capable of influencing gene expression (Holliday & Pugh, 1975). Methylation of DNA at cytosine bases remains the best-studied DNA modification and the methylated genes are usually being silenced and are not transcribed.

Histones are highly alkaline proteins that package and order the DNA into nucleosomes. Histones can be tagged with acetyl, methyl, ubiquitin, phosphate, poly(ADP)ribose and other biochemical groups (Strahl & Allis, 2000). These groups and their particular pattern of protein modification modify the function of the tagged proteins and influence gene expression. For example, H3K9 di-methylation is an epigenetic determinant of interferon (IFN) and interferon-stimulated gene (ISG) expression. *Fang et al.* describe a reverse correlation between H3K9me2 occupancy at IFN and ISG promoters and the expression of the ISG genes (Fang et al., 2012).

1.1.2 Histone modification

Histone modification is a post-translational epigenetic modification mechanism for modifying chromatin structure (A. J. Bannister & Kouzarides, 2011). It alters the properties of the nucleosome and affects its interactions with other proteins.

Histones are highly basic proteins that act as spools around which DNA winds in chromatin, which allows the DNA to fit into a smaller space than it would otherwise. Within a nucleosome DNA are wrapped around a histone octamer (H3, H4, H2A and H2B) and the long N-terminal tails of histones which composed mainly of basic amino acids residues are subjected to modifications (B. E. Bernstein, Meissner, & Lander, 2007). Post-translational histone modifications usually take place on the basic side-chains in histone tails (Figure 1) in the form of methylation, acetylation and ubiquitinylation (A. J. Bannister & Kouzarides, 2011).

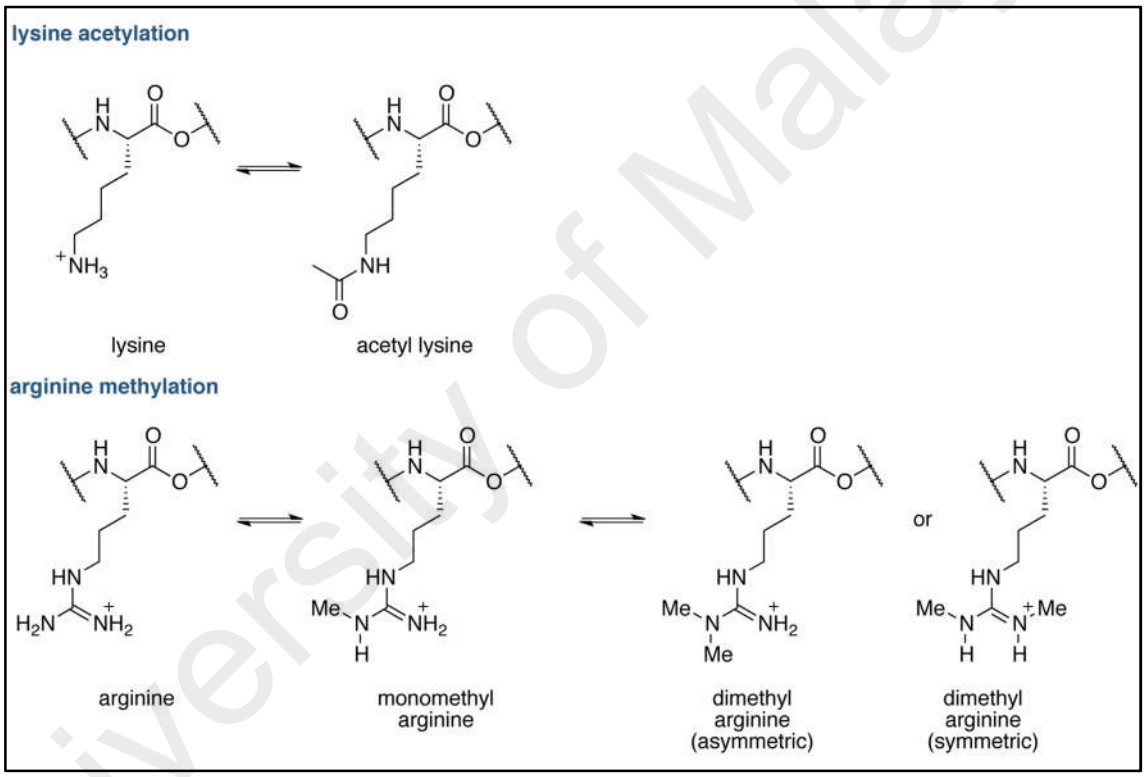

Figure 1.1: Acetylation and methylation of basic side-chain of histone tails. Retrieved November 8, 2014, from: http://www.atdbio.com/content/56/Epigenetics

Among the different histone modifications, histone methylation is one of the most well-studied histone modifications and involves the process of transferring methyl group(s) onto the amino acids of histone proteins (Figure 1.2). Depending on the target site, methylation and demethylation of histones may turn the gene in DNA "off" or "on" (Kouzarides, 2002a). For example, histone H3 lysine 4 di- (H3K4me2) and tri-methylation (H3K4me3) are associated with positive regulation of transcription and

recruitment of chromatin remodelling factors and histone acetyltransferases (Bradley E. Bernstein et al., 2002). Conversely, methylation at H3K9 and H3K27 are generally associated with heterochromatin formation associated with HP1 and silencing of transcription through the poly-comb group proteins (Lachner, O'Carroll, Rea, Mechtler, & Jenuwein, 2001). Methylation of histones regulates gene expression either through allowing or blocking transcription factors and other proteins to access the DNA.

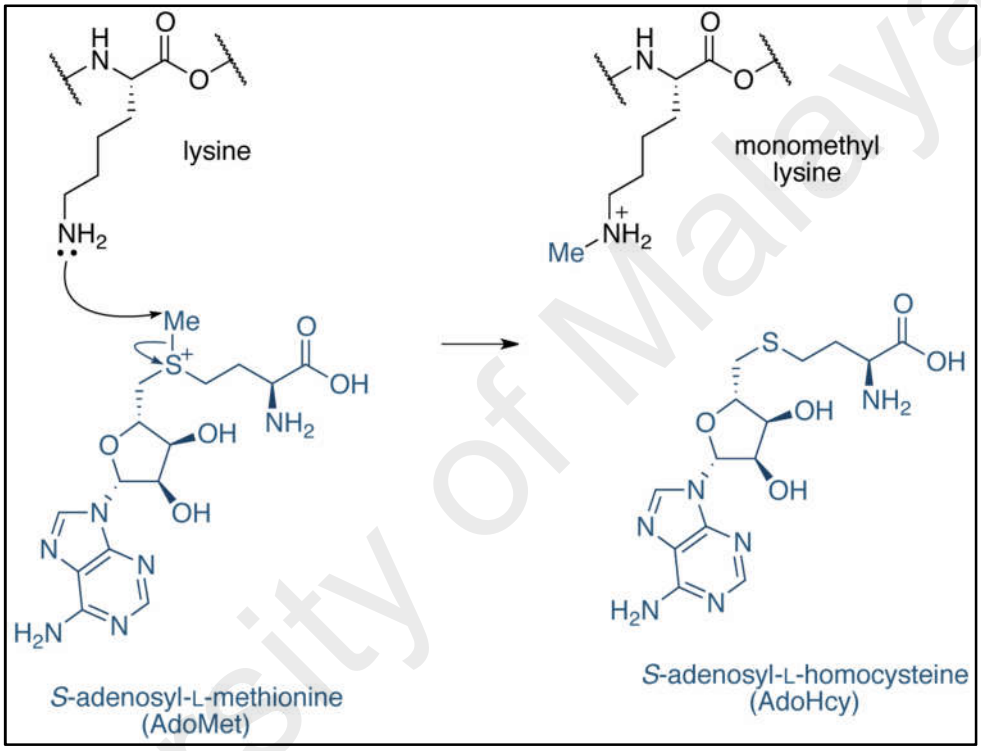

Figure 1.2: Chemical reaction of methylation of lysine. Retrieved November 8, 2014, from: http://www.atdbio.com/content/56/Epigenetics

While methylation of H3K4, H3K36, and H3K79 has been implicated in the transcriptional activation process; methylation of lysine 9 on histone 3 (H3K9) which is the major histone modification studied in this study is generally associated with repression (Krogan et al., 2003; Lachner et al., 2001). There are three types of H3K9 methylation (Figure 1.3). H3K9 methylation (H3K9me1) is linked to transcriptional repression. Nonetheless, H3K9 monomethylation represents a specific tag for HP1 protein recruitment to the methylated histones for epigenetic transcriptional repression (Jin, Li, & Robertson, 2011). H3K9 dimethylation (H3K9me2) is one of the prominent

marks of transcription repression (Wang et al., 2008). For examples, insulin is only expressed in beta cells and not in other cells, such as HeLa cells and human bone marrow-derived mesenchymal stem cells (hBMMS). It was shown that H3K9me2 is enriched at the promoter of insulin gene in HeLa cells and hBMMS cells but is depleted in beta cells (Mutskov, Raaka, Felsenfeld, & Gershengorn, 2007). H3K9 trimethylation (H3K9me3) also repress transcription and it is always linked to various disease conditions, including cancer and metabolic disorder (Muller-Tidow et al., 2010; Villeneuve et al., 2008).

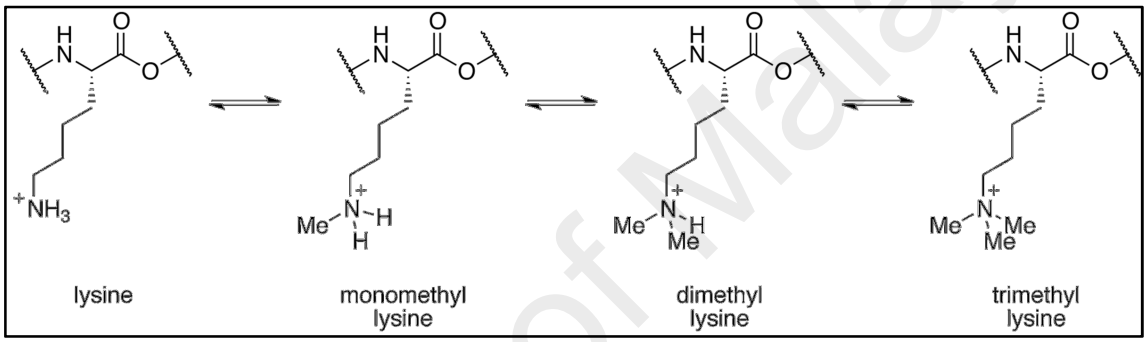

Figure 1.3: Three types of Lysine methylation. Retrieved November 9, 2014, from: http://www.atdbio.com/content/56/Epigenetics

1.1.3 Histone methyltransferases

Histone methyltransferases are the histone modifying enzymes, catalysing the histone methylation process by transferring one, two or three methyl groups from cofactor S-Adenosyl methionine (SAM) onto the lysine or arginine residues of histone H3 and H4 (Andrew J. Bannister & Kouzarides, 2005).

1.1.3.1 Histone lysine methyltransferases

Protein lysine methylation is one of the major post-translation modifications that regulate signal transduction in eukaryotic cells. This process has been intensively studied in the context of epigenetic regulation of gene expression through methylation of lysine residues of histones, but the discovery of a growing number of known non-

histone substrates also suggests that the impact of lysine methylation is not limited to chromatin biology.

Since the Su(var)3-9–Enhancer of zeste–Trithorax (SET) domain of Suv39h1/KMT1a was demonstrated to be the catalytic domain for lysine methylation (Rea et al., 2000), many SET domain-containing molecules have been found to be novel histone lysine methyltransferases (HKMTs) in various species, including viruses, bacteria, yeast, fungi, and other multicellular organisms (Qian & Zhou, 2006). All lysine-specific transferases, except DOT1L, have a SET domain (Min, Feng, Li, Zhang, & Xu, 2003; Qian & Zhou, 2006). Protein lysine methyltransferases (PKMTs) catalyse the transfer of a methyl group from SAM to the ε-amino group of lysine residues of proteins, including histones.

Histone methyltransferases that have methylation activity towards lysine 9 of Histone 3 are SUV39H1, SUV39H2, ESET, EHMT1, EHMT2, RIZ and SETDB1 (Qian & Zhou, 2006; Tachibana, Sugimoto, Fukushima, & Shinkai, 2001). Among them, EHMT1 and EHMT2 are likely the major euchromatic H3K9 histone methyltransferases in mammals. There is a drastic decrease of H3K9 methylation in euchromatic regions when and EHMT1 and EHMT2 gene were disrupted (Tachibana et al., 2002).

1.1.3.2 Euchromatin Histone Methyltransferases (EHMT)

EHMT1 and EHMT2 are members of the Suv39h subgroup of SET domain-containing molecules. Both EHMT1 and EHMT2 are the key HKMTs for H3K9me1 and H3K9me2 (Jenuwein, Laible, Dorn, & Reuter, 1998; Tachibana et al., 2002). EHMT2 was reported as the second HKMT and can methylate histone H1 and H3 (K9 and K27)

in vitro (Tachibana et al., 2001; Tachibana et al., 2002). EHMT2 is also involved in H3K9me3 modification in vivo (Shinkai & Tachibana, 2011). EHMT1 was originally described as a gene encoding a G9a-like protein (Shinkai & Tachibana, 2011). Other than H3K9, EHMT1 also weakly methylated Lysine-27 of histone H3. Others molecular functions of EHMT1 and EHMT2 include zinc binding and p53-methylation at K373 (Chen et al., 2010; Ueda, Tachibana, Ikura, & Shinkai, 2006). Since then, various biochemical studies have demonstrated that EHMT1 and EHMT2 possess the same substrate specificities on histones. Moreover, EHMT2 and EHMT1 form a homomeric and heteromeric complex via their SET domains (Tachibana et al., 2005).

Among the mammalian HKMTs, EHMT2 and EHMT1 have been extensively investigated at the biochemical and physiological levels and some specific inhibitors have been developed against these methyltransferases.

1.1.4 EHMT2 and EHMT1 inhibitors

Since the first PKMT was characterized in 2000, more than 50 human PKMTs have been identified (Rea et al., 2000). PKMTs show substantial variations in protein substrate selectivity and the degree of methylation on lysine, from mono- to di- to trimethylation. Selective pharmacological inhibition of individual PKMTs' catalytic activity in cellular systems is a useful strategy for deciphering the complex signalling mechanisms of histone and protein lysine methylation. However, very few small-molecule tools are currently available for probing the activity of individual PKMTs.

Growing evidence suggests that PKMTs are important in the development of various human diseases. In particular, EHMT2 (also known as KMT1C or G9a), which was initially identified as a histone H3 Lys9 (H3K9) methyltransferase, is

overexpressed in various human cancers including leukemia, prostate carcinoma, hepatocellular carcinoma and lung cancer (Vedadi et al., 2011). Thus, selective, potent and cell- active chemical probes for EHMT2 and EHMT1 would be extremely valuable tools for investigating the cellular role of these PKMTs, as well as for assessing their potential as therapeutic targets.

Chaetocin, a competitive inhibitor of SAM, was the first HKMT inhibitor discovered, and it specifically inhibits the enzymatic activities of HKMTs belonging to members of the SUV39 family, including SUV39H1, dSU(VAR)3-9, EHMT2, DIM-5, EHMT1, and ESET (Shinkai & Tachibana, 2011).

BIX01294, a small-molecule inhibitor of EHMT2 and EHMT1, is a selective protein lysine methyltransferases inhibitor and is non-competitive with the cofactor SAM (Figure 1.4) (Kubicek et al., 2007). BIX01294 was discovered through chemical library screening. BIX01294 inhibits the methyltransferase activity of EHMT1 and EHMT2 by binding to the substrate peptide groove at the location where the histone H3 residues N-terminal to the target lysine (Chang et al., 2009).

UNC0321, the next generation of BIX01294, is designed to improve the toxicity/function ratio. Even though UNC0321 is a selective chemical probe of EHMT1 and EHMT2, it is less potent than BIX01294 in cellular assay. The poor cell membrane permeability caused the poor cellular potency of UNC0321. Besides that, another variant, UNC0224 also faced similar difficulty to meet the need for a better probe. To improve the cellular potency of the probe, several new generation of this series of compounds have been designed. Another outstanding synthesized compound is UNC0638, which has balanced physicochemical properties and *in vitro* potency aiding

cell penetration, showing high potency in cellular assays and was less toxic to the cells compared to BIX01294. UNC0638 is a better tool for better understanding of epigenetic and cellular roles of EHMT1 and EHMT2 (Vedadi et al., 2011).

With the advancement of molecular technique and discovery of small molecule chemical inhibitor such as BIX01294, the functional roles of EHMT1 and EHMT2 have been vigorously studied. Studies have shown that EHMT1 and EHMT2 negatively regulate type I interferon response by promoting H3K9me2 at the promoters of ISGs (C. K. Ea, S. Hao, K. S. Yeo, & D. Baltimore, 2012; Fang et al., 2012). Thus, inhibiting the H3K9me2-specific methyltransferases, EHMT1 and EHMT2 via chemical inhibitors or RNAi approach could lift the repression off the ISGs promoter.

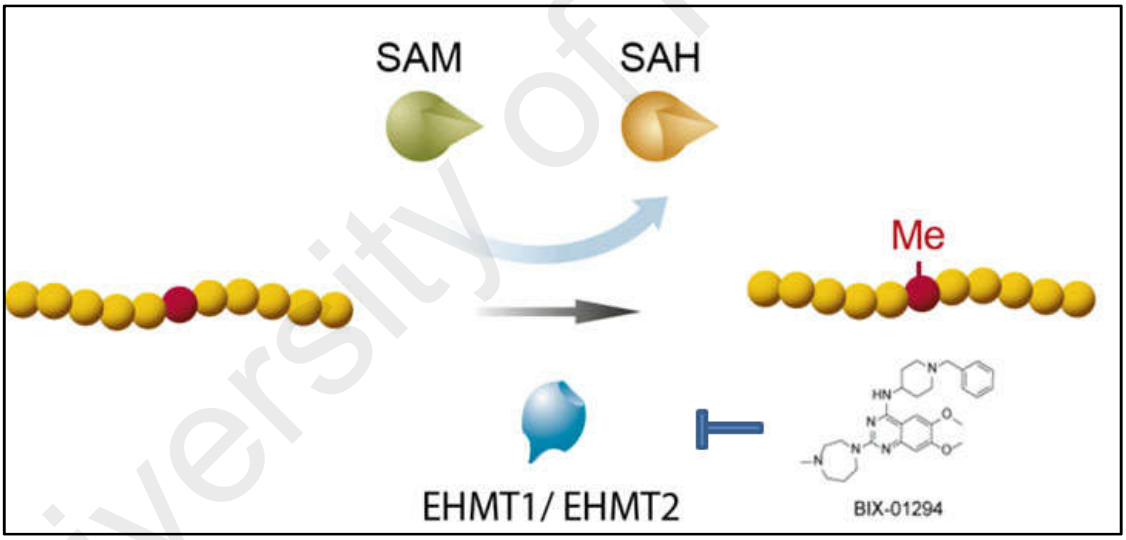

Figure 1.4: Biochemical action of BIX01294 mediated inhibition of EHMT1 and EHMT2. Retrieved November 9, 2014, from: http://www.atdbio.com/content/56/Epigenetics

1.2 JAK/STAT pathway

The Janus Kinase/ signal transducers and activators of transcription (JAK/STAT) pathway is a cascade used to transduce a multitude of signals for development and homeostasis in human (Rawlings, Rosler, & Harrison, 2004). The JAK/STAT pathway is the principle signalling mechanism for a wide array of cytokines and growth factors especially interferons.

Type I Interferons, including IFNα, IFNβ and Type II Interferons IFNγ are secreted glycoproteins that act as ligands for JAK/STAT pathway to stimulate gene expression, with anti-proliferative, anti-viral and immunoregulatory properties (Piehler, Thomas, Garcia, & Schreiber, 2012). In response to ligand binding, the JAK/STAT pathway receptors, Interferon (Alpha, Beta and Omega) Receptor 1 and Interferon (Alpha, Beta and Omega) Receptor 2 (IFNAR1 and IFNAR2) dimerize. The dimerization of receptors triggers activation of JAKs by bringing two JAKs into close proximity. Activated JAKs phosphorylate STAT1 and STAT2, which then dimerize through interaction of a conserved Src homology 2 (SH2) domain and interact with a third transcriptional regulator, IFN regulatory factor 9 (IRF9) to stimulate gene expression (Piehler et al., 2012) (Figure 1.5).

IFNα and IFNβ are the main innate anti-viral cytokine and is essential for effective immune response to viral infection (Price, Gaszewska-Mastarlarz, & Moskophidis, 2000; Samuel, 2001). IFNα is also an important therapeutic cytokine that exerts anti-tumor activity in a variety of tumor cells especially in chronic myeloid leukemia (CML) (Bonifazi et al., 2001).

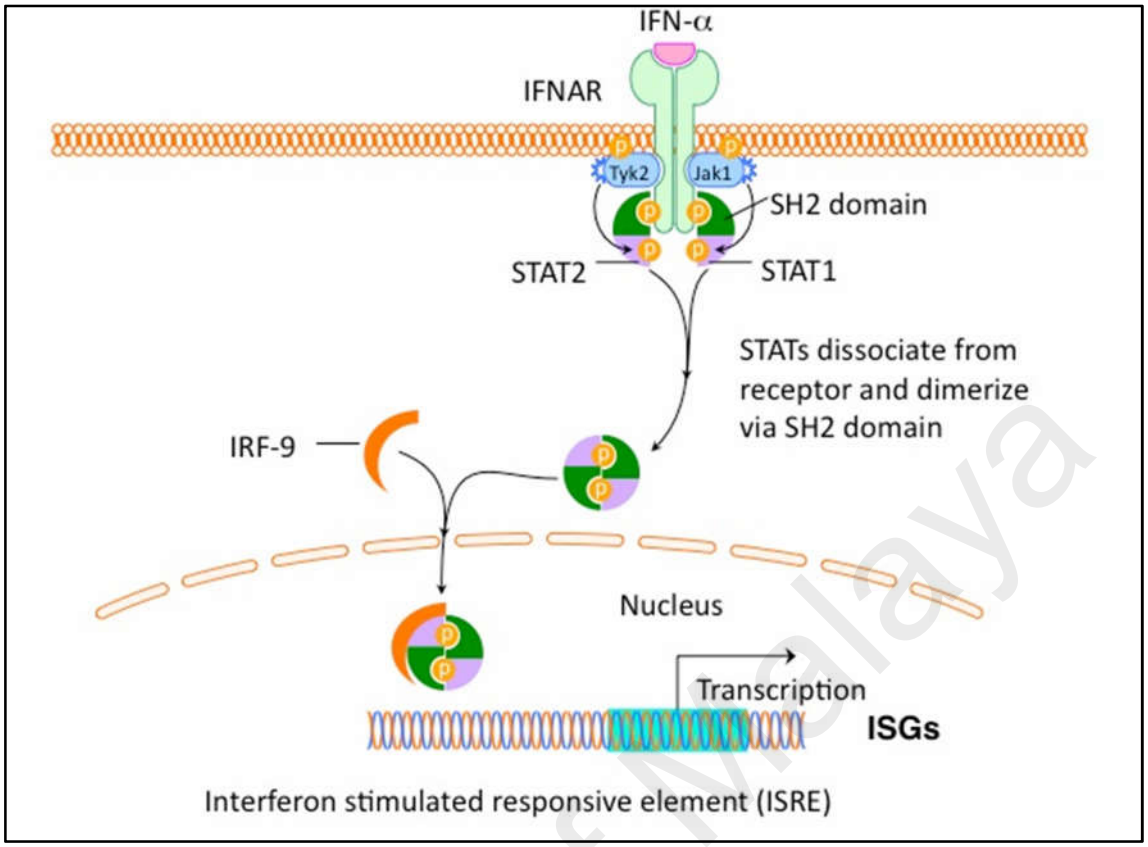

Figure 1.5: Type I interferons mediated JAK/STAT signalling. Adapted from Atsuko Masumi (2013). Hematopoietic Stem Cells and Response to Interferon, Stem Cell Biology in Normal Life and Diseases, Prof. Kamran Alimoghaddam (Ed.), ISBN: 978-953-51-1107-8, InTech, DOI: 10.5772/54689.

1.3 Chronic myeloid leukemia (CML)

CML is a cancer of white blood cells. At 2009, *Rohrbacher et.al* reported that the incidences rates of CML varied from 0.6 to 2.0 cases per 100 000 inhabitants, increased with age and is higher in men than women (Rohrbacher & Hasford, 2009). The most distinctive characteristic of CML is the presence of Philadelphia chromosome. The Philadelphia chromosome is formed when the Abelson murine leukemia viral oncogene homolog 1 gene (*Abl*) from chromosome 9 translocates and fuses to the breakpoint cluster region protein gene (*Bcr*) on chromosome 22 (Rowley, 1973). This event formed the oncogenic *Bcr-Abl* fusion gene, which encodes an abnormally regulated tyrosine kinase (Lugo, Pendergast, Muller, & Witte, 1990). Tyrosine kinase is a common signalling molecule that promotes cell division. The fusion of *Bcr-Abl* gene leads to a constitutively active tyrosine kinase, stimulate the hematopoietic stem cells to

proliferate at an abnormally high rate. This results in overproduction and accumulation of immature white blood cells. Unlike other cancer, these over-produced immature white blood cells do not form clumps. However, they may interfere with the production of red blood cells and functional immune cells.

There are 3 phases of CML including chronic phase, accelerated phase and blastic phase (Sessions, 2007). Most patients are present in the chronic phase and are usually asymptomatic until it is detected in a routine blood test or have only mild symptoms of fatigue, left side pain, joint and/or hip pain, or abdominal fullness. However, if left untreated, CML will eventually progresses to a blastic phase, often through the accelerated phase, with a fatal outcome. CML was treated with chemotherapy agents for years as these agents are able to control the clinical manifestations of the disease (Pavlovsky, Kantarjian, & Cortes, 2009). However, they are incapable of eliminating the malignant clone. Thus, the natural history of the disease was left unchanged until the introduction of recombinant IFNα, which has the ability to induce cytogenetic responses, and allogenic stem cell transplant which it is only suitable to small fraction of patients due to limiting factors such as stage of disease, treatment related morbidity and mortality and availability of donor (Maziarz & Mauro, 2003; Pavlu, Szydlo, Goldman, & Apperley, 2011). The use of tyrosine kinase inhibitors also improve the survival rates of CML patients dramatically (Huang, Cortes, & Kantarjian, 2012).

1.3.1 CML treatment

1.3.1.1 Interferon

Interferon alfa (IFNα) has been extensively studied as treatment for patients with CML since 1981, initially using partially purified IFNα (Talpaz, McCredie, Mavligit, &

Gutterman, 1983), followed by recombinant IFNα2a (Talpaz et al., 1986). Prior to the development of the tyrosine kinase inhibitor (TKI) imatinib, interferon was the non-transplant treatment of choice for most patients with CML. IFNα suppresses the proliferation of Philadelphia-positive CML cells, and induces both hematologic and cytogenetic remission with disappearance of Philadelphia clones (Borden & Ball, 1981). Several clinical trials suggested that interferon-alpha treatment was associated with a superior survival advantage over conventional chemotherapy agents (Urabe, 1997). However the use of recombinant-interferon incurs heavy cost. Moreover, the activity of single agent IFNα was found to be modest in late chronic and accelerated phase of CML (Kantarjian, O'Brien, Anderlini, & Talpaz, 1996).

1.3.1.2 Bone marrow transplant

Bone marrow transplant or stem cell transplant was a common and popular treatment option for CML (Maziarz & Mauro, 2003). Today, despite the breakthrough in treating CML patients with tyrosine kinase inhibitors or interferons, bone marrow transplant remains a viable offer to reverse the state of CML. There are two main types of stem cell transplants, which are allogeneic and autologous, with the difference in the source of the blood-forming stem cells.

Allogeneic stem cell transplants are the main type of transplant carried out for patients with CML (Copelan, 2006). In an allogeneic transplant, the stem cells come from a donor. The donor can be related, syngeneic or unrelated but whose tissues' human leukocyte antigens (HLAs) are almost identical to the patient's and has no other health problems. However the chance of getting a compatible donor is very low. The closer the donor's tissue matches the recipient, the better the chance the transplanted

cells will "take" and begin making new blood cells as well as limiting the risk of transplanted stem cell rejection or of severe graft-versus-host disease.

In an autologous stem cell transplant, the patient's own stem cells are removed from the bone marrow or peripheral blood and stored away (Santos, Yeager, & Jones, 1989). During this period, the patient receives high-dose chemotherapy and/or radiation treatment to the entire body to eradicate the malignant cells at the cost of destroying the patient's own bone marrow cells. Subsequently, the stored stem cells are given back to the patient via intravenous infusion. Compare to allogeneic stem cell transplant, autologous stem cell transplant is less commonly used to treat CML due to the possibility of collecting some leukemia cells along with the stem cells which are then given back to the patient after chemotherapy and/or radiation treatment. However, a process called purging can be used to maximally remove the leukemia cells from the collected stem cells (Baynes et al., 2000).

One of the most serious short-term complications from bone marrow/ stem cell transplant is the increased risk of infection from the environment. This is due to the low white blood cell count following the transplant. During this time, antibiotics are given and the patients are watched closely for fever or any signs of infection. Other side effects include low blood cell count, nausea, vomiting, loss of appetite, mouth sores and hair loss (Bellm, Epstein, Rose-Ped, Martin, & Fuchs, 2000). With allogeneic stem cell transplant, graft-versus-host disease occurrence which leads to fatality are possible even drugs is given to weaken the immune system to keep graft-versus-host disease under control. This happens when the donor immune system cells attack tissues of the recipient. Symptoms can include weakness, fatigue, dry mouth, rashes, nausea, diarrhoea, jaundice and muscle aches (Glucksberg et al., 1974). The expensive cost,

lack of suitable donors, stringent requirement for recipient, lengthy hospitalization and complex treatment make stem cell transplant last option to be considered even it did promise a permanent cure, theoretically.

1.3.1.3 Imatinib

Imatinib or its marketed name, Gleevec/Glivec, is a tyrosine kinase inhibitor that targets the *Bcr-Abl* fusion gene which encodes for an abnormal tyrosine kinase protein found in most CML patients (B. J. Druker, 2002). Imatinib synthesized in the lab enter clinical trials after its successful testing in cell culture which shows a 92%-98% decrease in the number of *Bcr-Abl* colonies formed, while causing no decrease in normal colony formation, suggesting that imatinib was effective, safe and did not harm healthy cells (Brian J. Druker et al., 1996). With its remarkable tolerance and excellent cytogenic remission data collected during clinical trials, imatinib have been approved by Food and Drug Administration (FDA) for its use in CML treatment. However imatinib resistance does exist in a proportion of patients particularly those in more advanced disease phases (Brian J. Druker, Sawyers, et al., 2001). Another significant limitation of imatinib is the inability of most CML patients to discontinue the imatinib therapy and maintain the remission due to imatinib halts the function of abnormally function tyrosine protein but the mutated *Bcr-Abl* gene is still present (Cortes, O'Brien, & Kantarjian, 2004; Rousselot et al., 2007). Therefore, there is an urge for a better treatment of advance phase CML. To this end, clinical trials have been carried out were reported significant and rapid response rates with the combination of imatinib and IFNα compared with the imatinib alone, suggesting that the addition of IFNα may broaden imatinib's therapeutic potential in CML treatment (Hehlmann et al.; Preudhomme et al.).

1.4 Rationale of study

Histone H3 lysine 9 methylation is an epigenetic signal which correlates with gene silencing (Kouzarides, 2002b; Snowden, Gregory, Case, & Pabo, 2002). Previous study from our group shows that EHMT1 negatively regulates type I interferon response (C.-K. Ea, S. Hao, K. S. Yeo, & D. Baltimore, 2012) and type I interferon have been approved and used for treating several kinds of cancer including chronic myeloid leukemia. Inhibition of EHMT1 will potentially enhance the interferon mediated cancer cell death (C.-K. Ea et al., 2012). Our group's preliminary results suggest that inhibiting EHMT1 and EHMT2 with BIX01294, an EHMT1 and EHMT2 specific inhibitor, enhances the interferon response in K562 cells (a CML cell line) and sensitizes K562 cells to IFNα2a treatment. These results warrant a more thorough investigation into the potential of EHMT1 and EHMT2 to serve as a new target for treating cancer and BIX01294 as a new anti-cancer drug.

1.5 Research objectives

To determine the anti-cancer efficacy of BIX01294 on CML cell lines by inhibiting EHMT1 and EHMT2.

CHAPTER 2: MATERIALS AND METHODS

2.1 Materials

List of Materials

Generic Name [Brand Name/Model]	**(Company, Country)**
Absolute Ethanol (Molecular Grade)	(Merck & Co., Germany)
Absolute Isopropanol	(Thermo Scientific, USA)
Acrylamide	(Sigma-Aldrich, Germany)
Adenosine 5'-triphosphate (ATP)	(Sigma-Aldrich, Germany)
BIX01294	(Sigma-Aldrich, Germany)
Dimethyl Sulfoxide (DMSO)	(Sigma-Aldrich, Germany)
Dithiothreitol (DTT) powder	(Sigma-Aldrich, Germany)
DNase I (RNase Free) (2000 units/mL)	(New England Biolabs, USA)
dNTP Mix (10 mM each)	(Thermo Scientific, USA)
Dulbecco's Modified Eagle's Medium (1X) [Gibco®]	(Life Technologies, USA)
Fetal Bovine Serum (FBS) [Gibco®]	(Life Technologies, USA)
Interferon-α2a	(Peprotech, USA)
Interferon-β	(Merck & Co., Germany)
Interferon-γ	(Merck & Co., Germany)
Lint-free Tissue [Kimtech Science Kimwipes]	(Kimberly-Clark, USA)
M-MuLV Reverse Transcriptase (200000 units/mL)	(New England Biolabs, USA)
M-MuLV Reverse Transcriptase Reaction Buffer (10X)	(New England Biolabs, USA)
Random Hexamer (50mM)	(Thermo Scientific, USA)

RNase Inhibitor, Murine (40000 units/mL)	(New England Biolabs, USA)
Penicillin-Streptomycin Mixture (10000 units/mL) [Gibco®]	(Life Technologies, USA)
Phosphate Buffered Saline (10X)	(Fisher Scientific, USA)
Primer	(1st BASE, Singapore)
GeneJET RNA Purification Kit	(Thermo Scientific, USA)
Roswell Park Memorial Institute Medium (1X) [Gibco®]	(Life Technologies, USA)
Sodium Dodecyl sulfate	(Thermo Scientific, USA)
SYBR® Select Master Mix [Applied Biosystems®]	(Life Technologies, USA)
Tris Base	(Thermo Scientific, USA)
Tryhan blue	(Life Technologies, USA)
Trypsin [TrypLE™ Express (1X), no phenol red (Gibco®)]	(Life Technologies, USA)
UNC0638	(Sigma-Aldrich, Germany)
Vesicular stomatitis Virus (VSV)	(Charles River, USA)
2-Mercaptoethanol	(Sigma-Aldrich, Germany)

List of Apparatus

Generic Name [Brand Name/Model]	**(Company, Country)**
Conical Centrifuge Tubes (15 mL)	(Thermo Scientific, USA)
Conical Centrifuge Tubes (50 mL)	(Thermo Scientific, USA)
Nalgene® Mr. Frosty Cryo 1 °C Freezing Containers	(Thermo Scientific)
Nunc™ CryoTube™ Vials	(Thermo Scientific, USA)
Cell Culture Dishes (10 cm)	(Corning, USA)
Glass Slides	(Sail Brand, China)
Haemocytometer	(Hecht, Germany)
Microcentrifuge Tubes (1.5 mL) [Axygen]	(Corning, USA)
Micropipettes [Pipetman]	(Gibson, USA)
Pasteur Pipettes (150 mm)	(Hurst Scientific, Australia)
PCR 8-well Tube Strip [Axygen]	(Corning, USA)
Pipette-Aid [Macroman™ Pipette Controller]	(Gilson, USA)
Pipette Tips [Axygen]	(Corning, USA)
Sealing Film [Microseal® 'C' Film]	(Bio-rad, USA)
Serological Pipettes (5 mL) [Fisherbrand™]	(Fisher Scientific, USA)
Serological Pipettes (10 mL) [Fisherbrand™]	(Fisher Scientific, USA)
6-well Plates	(Corning, USA)
12-well Plates	(Corning, USA)
Multiplate™ 96-well PCR Plate, Clear	(Bio-rad, USA)

List of Equipment

Generic Name [Brand Name/Model]	**(Company, Country)**
Biosafety Cabinet [Thermo Scientific 1300 Series A2]	(Thermo Scientific, USA)
Centrifuge [Heraeus™ Multifuge X1R]	(Thermo Scientific, USA)
CO_2 Incubator [Forma™ Steri-Cycle™ CO_2 Incubators]	(Thermo Scientific, USA)
Electronic Balance	(Ohaus, USA)
Inverted Microscope [Inverted Microscope CKX31]	(Olympus, Japan)
Microcentrifuge [Centrifuge 5424]	(Eppendorf, Germany)
PCR tube centrifuge [Sprout® Micro-Centrifuge]	(LabScientific)
Power Supply [PowerPac Basic Power Supply]	(Bio-rad, USA)
Real-Time Thermal Cycler [CFX Connect™ Real-Time PCR Detection System]	(Bio-rad, USA)
Sonicator [Bioruptor Plus UCD-300]	(Diagenode, Belgium)
Spectrophotometer [NanoDrop 2000c]	(Thermo Scientific, USA)
Thermal Cycler [Veriti® 96-well Thermal Cycler (Applied Biosystems®)]	(Life Technologies, USA)
Thermomixer [Thermomixer® Compact]	(Eppendorf, Germany)
Vacuum Pump [Fisherbrand® Oil-free]	(Thermo Scientific, USA)
Vortex Mixer [PV-1 Vortex Mixer]	(Grant, UK)
Water bath [Isotemp™ Digital-Control Water Baths]	(Fisher Scientific, USA)
-20 °C Freezer	(Thermo Scientific, USA)
-80 °C Freezer [Forma™ 88000 Series]	(Thermo Scientific, USA)

2.2 Methods

2.2.1 Cell culture conditions

K562, KCL22, BV173, KT1 and Jurkat cell lines were maintained in RPMI with penicillin (100 U/ml), streptomycin (100 µg/ml) and supplemented with 10% of fetal bovine serum (FBS). Cell passage was performed when the cells reached >70% confluency. HeLa S3, HEK293T and HaCat cell lines were cultured in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin G (100 U/ml), and streptomycin (100 µg/ml). The cells were incubated at 37^{o}C with 5% of carbon dioxide (CO_2).

2.2.2 Cell lines storage and recovery

Cells were cryopreserved in 90% of FBS and 10% of DMSO in liquid nitrogen.

2.2.3 Enumeration of cells

Cell counting was performed using a hemacytometer. A small amount of the cells suspension was mixed with tryphan blue at equal volume before counting. The viable cells appear as unstained cells and number of viable cells in 1.0 ml was estimated as follow.

Number of cells/ml = average number of cells per quadrant x dilution factor x 10^4

2.2.4 Cell proliferation assay

Cells were treated with or without various concentration of BIX01294 together with or without various concentration of IFNα2a in 96-well plates. After incubation for four days, 25 µl of 2mg/ml 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide

(MTT) in 1X PBS was added and cells were further incubated for three hours at 37℃ in a CO_2 incubator. The absorbance was measured with a M200 PRO microplate reader (Tecan) at the wavelength of 540nm.

2.2.5 Establishment of shRNA stable clone

2.2.5.1 Generation of shRNA carrying lentivirus

ShRNAs plasmids against human EHMT1 (sc-62261-SH), human EHMT2 (sc-43777-SH) and vector tet-pLKO-puro (Addgene) were purchased. Lenti-viruses were produced according to the Addgene's protocol.

2.2.5.2 Generation of K562 stable clone

One million K562 cells in 1 ml culture media was seeded into 6 wells plate and incubated with 1 ml of shRNA carrying lenti-viruses. Twenty-four hours post-incubation, the medium was topped-up to 10ml and transferred the cells into T-75 flask for puromycin selection.

2.2.6 Establishment of mEHMT1- and mEHMT2-expressing KT1 stable clone

Three hundred thousand KT1 cells in 2 ml of culture media were seeded into 6 wells plate and incubated overnight. pMSCV-FLAG-mEHMT1 and pCDNA3-HA-mEHMT2 plasmids were co-transfected into KT1 cells using lipofectamine 2000 according to manufacturer's protocol. Forty eight hours post-transfection, 1 µg/ml puromycin and 1.2 mg/ml G418 were added to the medium for two weeks to select cells stably expressing mEHMT1 and mEHMT2.

2.2.7 Determining ISGs expression level

2.2.7.1 Ribonucleic acid (RNA) extraction and quantification

RNA was harvested from the BIX01294 treated cells and control cells based on the GeneJET RNA Purification Kit's protocol (Thermo Scientific, UK). The concentration of RNA was determined using NanoDrop 2000c spectrophotometer (Thermo Fisher Scientific, USA). The purified RNA was kept at -80^{o}C.

2.2.7.2 Complementary DNA (cDNA) synthesis

Reverse transcription polymerase chain reaction (RT-PCR) was carried out for cDNA synthesis. Two microgram of RNA sample was used as starting materials. RT-PCR was performed with master mix including 10x M-MuLV RT buffer, DNaseI, 10 mM dNTP Mix, random hexamer, RTase, RNase Inhibitor and 10x RT M-MuLV buffer. Finally, each product was diluted with 40 µl of milliQ water for quantitative PCR.

2.2.7.3 Quantitative-PCR (qPCR)

Complementary DNA synthesized was analysed with Real Time-quantitative PCR to determine the expression of genes before and after drug treatment. Forward and reversed primers were design with Primer3 and Blast (sequences of the primers refer Appendix 1). After that, forward and reversed primers and SYBR® Select Master Mix (Life Technologies) was used to quantify the transcript as fluorescence signal is emitted when the non-saturating dye intercalates between the double stranded DNA amplified. qPCR was performed using CFX Connect^{TM} Real-Time PCR Detection System (BioRad, USA). Data was analysed by comparative quantification using BioRad CFX Manager 3.1 Software (BioRad, USA). The expression of each gene was normalized to a human housekeeping gene, *L32*. Histograms were derived from data which

representing the fold change induction against cDNA synthesize from cells that were treated with DMSO (negative control). Average and standard deviations were calculated between three biological replicates to ensure data accuracy and reliability of the results.

2.2.8 Cell cycle profiling

2.2.8.1 Flow cytometry

Flow cytometry was used for cell cycle analysis. Samples were prepared with ethanol fixation. Propidium iodide (PI) staining was used as a fluorochrome as it is capable of binding and labelling DNA and cell cycle profiling was done with MACSQuant Analyzer (Miltenyi Biotec) (Riccardi & Nicoletti, 2006). Data collected were further analysed with FlowJo software.

2.2.9 Chromatin Immunoprecipitation (ChIP)

ChIP assay was performed as previous described with some minor modifications (Attema et al., 2013). Antibodies included anti-histone H3 (ab1791; Abcam), and anti-dimethyl histone H3K9 (ab1220; Abcam), anti-RNA Polymerase II (05-623B; UpState) and normal mouse IgG (I5381; Sigma) were purchased from respective sources. Briefly, 12 million cells were cross-linked in 1% formaldehyde for 8 minutes at room temperature with gentle rocking or inversion every 2-3 minutes. Cells were pelleted by centrifugation (500xg for 5 minutes), and washed twice in ice-cold 1x PBS. The cells were lysed in 300 µl of lysis buffer (10 mM Tris pH 7.5, 1 mM EDTA, 1% SDS) containing protease inhibitor cocktail (PIC; Sigma) and incubated on ice for 10 minutes. After lysis, 900 µl of 1x PBS containing PIC was added and 300 µl was aliquoted into individual 1.5 ml tubes. Each 300 µl aliquot was sonicated by using a bioruptor plus® sonicator (Diagenode), which was empirically determined to give rise to genomic fragments ~200-500bp. The soluble chromatin was collected by 4°C ultracentrifugation

(13,000 rpm for 10 minutes) and pooled into a new 15ml falcon tube. The supernatant was diluted 2-fold with 2x RIPA buffer (10 mM Tris-HCl pH 7.5, 1 mM EDTA, 1% Triton X-100, 0.1% SDS, 0.1% sodium deoxycholate, 100 mM NaCl, PIC), 1/10 volume (40 µl) input was removed, and 400 µl of soluble chromatin (equivalent to 2 x 10^6 cells) was distributed to new 1.5 ml tubes. Each respective antibody was added at appropriate amount as tested in titration experiments using control promoters. Immunoprecipitations were performed overnight at 4°C with rotation, and antibody: protein:DNA complexes were then collected through binding to 15 µl of protein A and G magnetic beads (Thermo Fisher) for 4 hours of rotation. The beads were washed three times using 200 µl of RIPA buffer and once with TE (10 mM Tris-HCl, pH 8.0, 10 mM EDTA) buffer, then incubated with 200 µl of fresh elution buffer with proteinase K for 2 hours in a thermomixer (1300 rpm, 68°C) to reverse the protein:DNA cross-links. After incubation, eluates were collected into new 1.5 ml tubes. Genomic DNA was recovered by using phenol chloroform extraction and ethanol precipitation. Pellets were washed in 70% ethanol, briefly air-dried, and resuspended in TE buffer. Quantitation of ChIP DNA (relative enrichment) was performed using a CFX Connect™ Real-Time PCR Detection System (Bio-Rad) with SYBR® Select Master Mix (Life Technologies) and ChIP qPCR primer sequences as listed in Appendix. Enrichment of histone modifications at genomic regions were expressed as % input. % Input was calculated using the formula % (ChIP/ Total input)= 2^[(Ct(x%input) – log(x%)/log2) - Ct(ChIP)]x 100% to account for chromatin sample preparation differences. Ct (ChIP) and Ct (x%input) are threshold values obtained from exponential phase of qPCR for the IP'd DNA sample and input sample respectively; the compensatory factor (log(x%)/log2) is used to take into account the dilution 1:x of the input.

2.2.10 Immunoblotting

2.2.10.1 Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

The 15.0% SDS-PAGE resolving gel and 5% SDS PAGE stacking gel was prepared. The cells pallets were lysed using 50 µl nuclear lysis buffer (1% SDS, 10% glycerol, 10 mM Tris-Cl, pH 6.8, 1 mM ethylene diamine tetraacetic acid (EDTA), dithiothreitol (DTT), 1mM PMSF and protease inhibitor cocktail). The denatured protein samples in SDS-PAGE sample buffer (20 µl) was loaded into gel for electrophoresis. After the electrophoresis, the stacking gel was removed and the resolving gel was recovered for use in Western blotting.

2.2.10.2 Western blotting

Western blotting was performed using a wet blotting system. The transfer cassette was assembled the following sequence of 1 piece of Scotch Brite pad, 2 pieces of filter paper, 1 piece of nitrocellulose membrane, SDS PAGE gel, 2 pieces of filter paper and 1 piece of Scotch Brite pad. The assembly was placed into the transfer chamber filled with pre-chilled transfer buffer, with the nitrocellulose membrane towards anode (+) core and SDS PAGE gel towards cathode (–) core. The electrophoretic transfer was carried out at 250mA for 1 hour with an ice pad in the transfer tank. The nitrocellulose membrane was blocked with 5% skimmed milk and followed by respective primary antibody incubation. The membrane was washed 3 times with TBS-Tween 20 and incubated with suitable biotinylated secondary antibodies. The unbound antibodies were removed by washing 3 times with TBS-Tween 20 for 10 minutes each. The membrane was then incubated with freshly prepared 4-chloro-1-naphtol substrate solution. The dark purple bands were then photographed for record purposes.

CHAPTER 3: RESULTS

3.1 Inhibition of EHMT1 and EHMT2 with BIX01294 enhances type I interferon response in K562 cells

Previous research finding showed that inhibition of EHMT1 either with RNA interference (RNAi) or a chemical inhibitor enhances interferon response and protects cells from virus infection (C. K. Ea et al., 2012; Fang et al., 2012). Before any experiment was started, the effectiveness of an EHMT1 and EHMT2 specific chemical inhibitor, BIX01294 was determined. Hence, a simple Immunoblotting experiment was carried out. Immunoblotting result showed 83% reduction of the global level of H3K9me2 (Figure 3.1A) in K562 cells after being treated with BIX01294 for twenty-four hours. K562 cells used in this assay are human bone marrow chronic myeloid leukemia cells. This result confirmed the inhibition of EHMT1 and EHMT2 by BIX01294 in K562 cells.

To determine whether the inhibition of EHMT1 and EHMT2 in CML cells enhances their response to interferon, K562 cells were treated with IFNα2a in the presence and absence of BIX01294. The expressions of ISGs were measured using Real-Time qPCR, including *IFIT2, IFIT3, GBP1, GBP3, OAS2, OAS3 and IRF7* in a dose dependent manner (Figure 3.2A). In the presence of BIX01294, 100 IU/ml IFNα2a produced a comparable ISGs expression pattern and level to that of 1000 IU/ml IFNα2a-stimulated DMSO-treated K562 control cells. Moreover, IRF7 protein level increased in response to IFNα2a stimulation. Pretreating K562 cells with BIX01294 induce the protein level of IRF7 even in the absence of IFNα2a stimulation (Figure 3.2B).

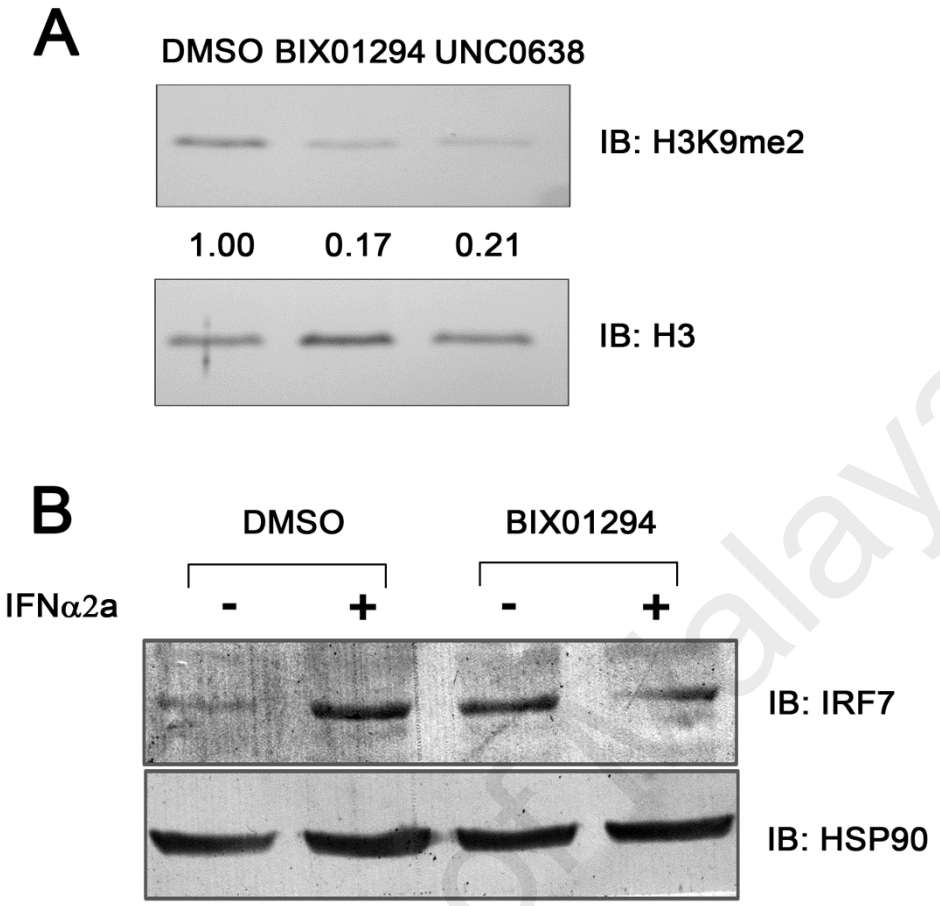

Figure 3.1: BIX01294 reduce the global level of H3K9me2, while increase the IRF7 protein production. (A) K562 cells were incubated with 2.5 μM BIX01294 and 5 μM of UNC0638 for 24 hours. The cells were then lysed and check for H3K9me2 protein level with Immunobloting technique. (B) K562 cells were pretreated with BIX01294 for 24 hours before stimulated with 1000 IU/ml IFNα2a. After two hours of IFNα2a stimulation, the protein level of IRF7 was check with specific antibody.

To test if BIX01294 treatment enhances the expression of ISGs induced by other interferons in K562 cells, K562 cells was pretreated with or without BIX01294 followed by IFNβ (a type I interferon) or IFNγ (a type II interferon) stimulation. Consistent with IFNα2a stimulation, pretreating K562 cells with BIX01294 enhanced IFNβ-induced, but not IFNγ-induced expression of IFIT2 and IFIT3 (Figure 3.2B).

Taken together, these results indicate that inhibition of EHMT1 and EHMT2 in K562 cells significantly enhances the expression of ISGs in response to type I interferons, but not type II interferon stimulation.

3.2 BIX01294 sensitizes CML cell lines to IFNα2a treatment

IFNα2a is approved by the US FDA as an anti-cancer drug against CML. However, K562 cells have been shown to be resistant to interferon treatment *in vitro* (IC_{50}> 10^5 IU/ml) (Colamonici, Domanski, Platanias, & Diaz, 1992; Sakai, Takeuchi, Yamauchi, Narumi, & Fujita, 2002). To test whether inhibition of EHMT1 and EHMT2 renders K562 cells sensitivity to interferon treatment, proliferation of K562 cells treated with or without IFNα2a in the presence or absence of BIX01294 was measured using MTT assays. Consistent with the previous reports, MTT assay result showed that K562 cells were resistant to IFNα2a treatment because K562 cells proliferation only reduced by 10% after treating with 10^5 IU/ml of IFNα2a alone (Figure 3.3A). On the other hand, in the presence of BIX01294, IFNα2a strongly inhibited the proliferation of K562 cells. For example, in the presence of 2 μM of BIX01294, 1000 IU/ml of IFNα2a achieved 40% inhibition of cell proliferation and 10^5 IU/ml of IFNα2a inhibited cell proliferation by 60% (Figure 3.3A). Moreover, treating K562 cells with 2 μM BIX01294 alone had no effect on their proliferation. These findings demonstrate the inhibition of EHMT1 and EHMT2 does not kill the cells but sensitizes K562 cells to interferon treatment.

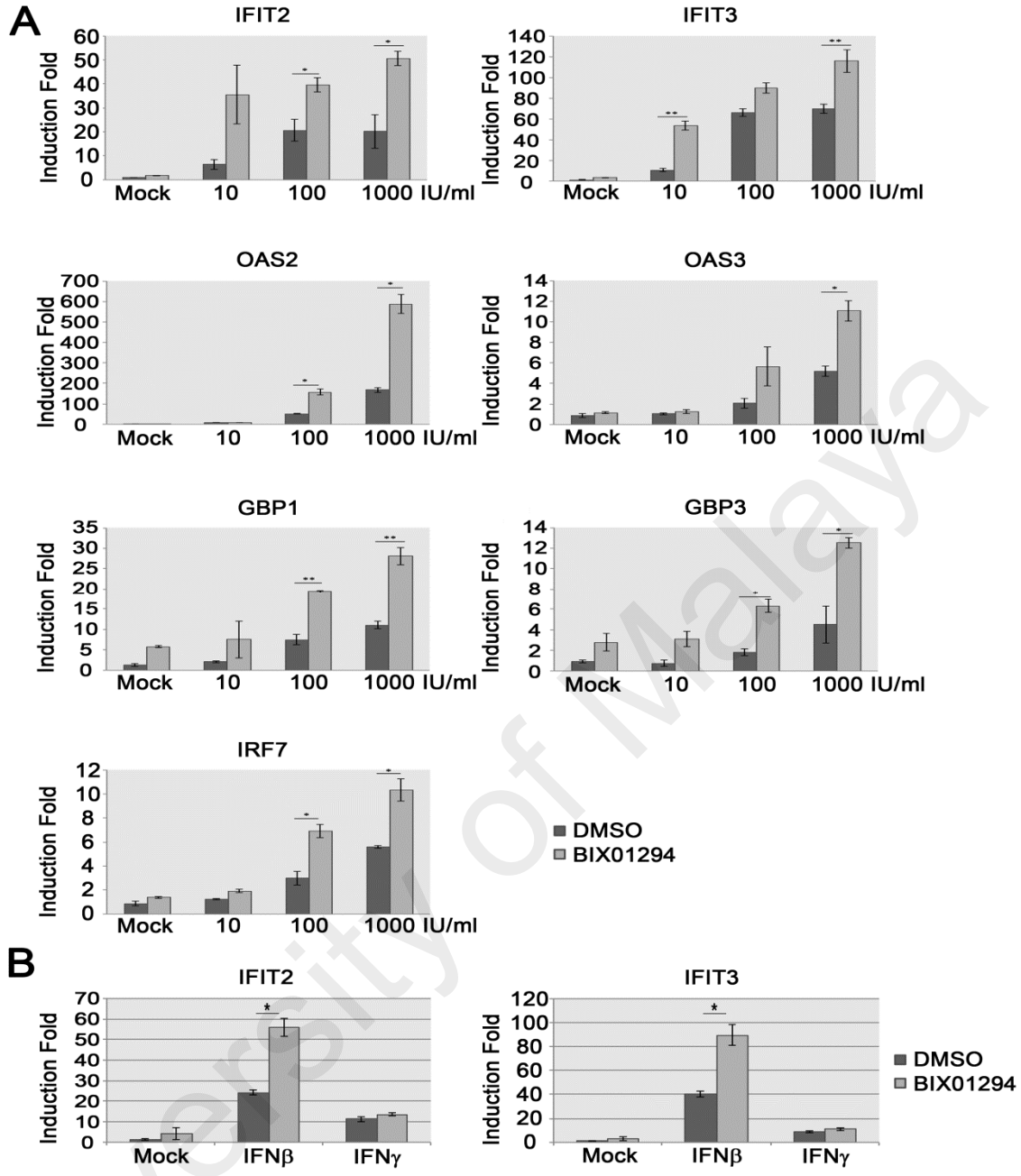

Figure 3.2: BIX01294 enhances the expressions of ISGs in K562 cells. (A) K562 cells were incubated with 2.5μM BIX01294 for 24 hours. The cells were then treated with various concentrations of IFNα2a as indicated. After two hours of IFNα2a stimulation, the expression of various ISGs was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. *: $p < 0.05$, **: $p < 0.01$. (B) K562 cells were incubated with 2.5μM BIX01294 for 24 hours. The cells were then treated with IFNβ or IFNγ for two hours. The expression of IFIT2 and IFIT3 was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. *: $p < 0.05$.

Three additional CML cell lines, KCL22, BV173 and KT1 were included to ensure the phenomenon observed is not unique to K562 cells. Among these cell lines, KT1 cells were sensitive to IFNα2a treatment, while KCL22 and BV173 cells were IFNα2a resistance (Figure 3.3B-D). A similar pattern of anti-proliferation was observed, BIX01294 treatment significantly sensitized KCL22 and BV173 cells to IFNα2a treatment. Furthermore, inhibition of EHMT1 and EHMT2 with BIX01294 moderately but still enhanced the cytotoxicity of IFNα2a in interferon-sensitive KT1 cells.

In additional to CML cell lines, three non-CML cell lines with different origins also included in the experiment. They are Jurkat (a T cell lymphoma), HeLa (a cervical cancer cell), and HaCat (an immortal human keratinocyte). None of these cells were sensitive to IFNα2a treatment as only about 20% inhibition of cell proliferation was observed at the highest concentration of IFNα2a treated (10^5 IU/ml) (Figure 3.4). Unlike responses observed in CML cell lines, inhibition of EHMT1 and EHMT2 in Jurkat, HeLa and HaCat cells had moderate or little effect on the sensitivity of these cells to interferon treatment.

In summary, these results suggest that inhibition of EHMT1 and EHMT2 with BIX01294 sensitizes CML cells, and to a lesser extent on Jurkat, HeLa or HaCat cells to interferon treatment.

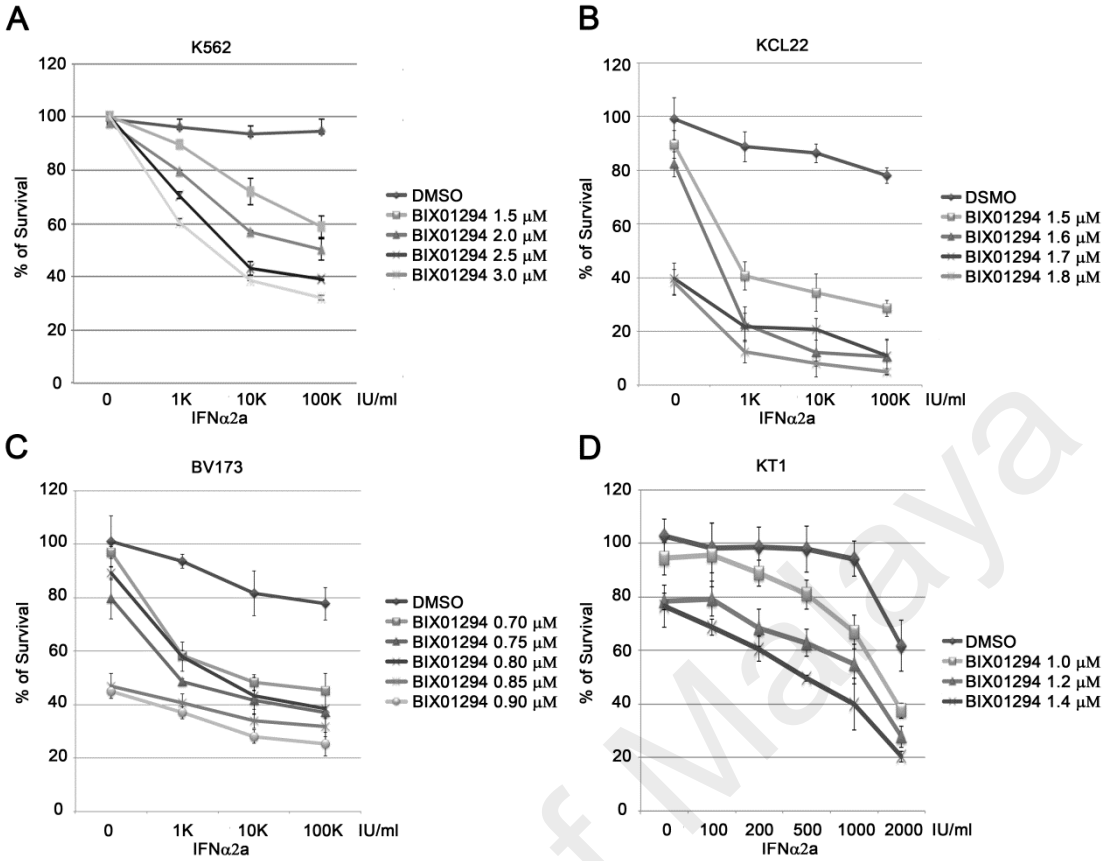

Figure 3.3: BIX01294 sensitizes CML cell lines to IFNα2a treatment. K562 (A), KCL22 (B), BV173 (C) and KT1 (D) cells were cultured with various concentrations of BIX01294 and IFNα2a as indicated. After four days, cell proliferation was measured with a MTT assay. Results represent the mean ± SD in quadruplicate experiments.

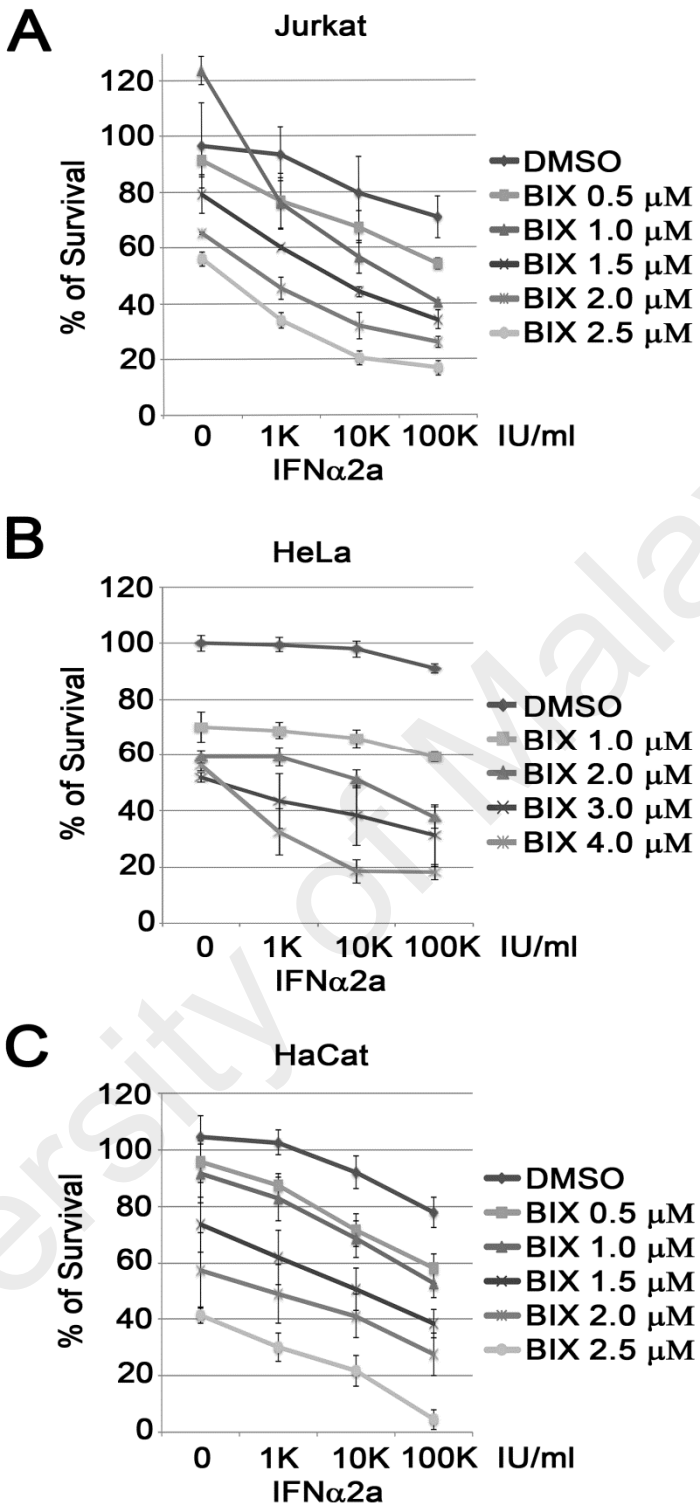

Figure 3.4: BIX01294 slightly enhance IFNα2a-induced anti-proliferation in non-CML cells. Jurkat (A), HeLa (B) and HaCat (C) cells were cultured with various concentrations of BIX01294 and IFNα2a as indicated. After four days, cell proliferation was measured with a MTT assay. Results represent the mean ± SD in quadruplicate experiments.

3.3 Inhibition of EHMT1 and EHMT2 with UNC0638 enhances Interferon response in K562 cells

A second commercially available EHMT1- and EHMT2-specific inhibitor, UNC0638 was included to minimize the possibility that the effect observed with BIX01294 treatment was caused by an off-target effect (Vedadi et al., 2011). Comparable to BIX01294, global level of H3K9me2 was reduced by 79% when K562 cells were treated with UNC0638 for twenty-four hours (Figure 3.1A).

Furthermore, the effect of UNC0638 on IFNα2a-mediated anti-proliferation was tested and similar phenomenon as in BIX01294 treatment was observed. The inhibition of EHMT1 and EHMT2 with UNC0638 sensitizes K562 cells to interferon treatment (Figure 3.5A). Nonetheless, Interferon response of K562 cells after UNC0638 treatment was measured with RT-qPCR. The result showed that treating K562 cells with UNC0638 enhanced the expression of several ISGs in response to IFNα2a stimulation. *IFIT2, IFIT3, OAS2, OAS3, GBP1, GBP3* and *IRF7* genes expression were all up-regulated (Figure 3.5B).

3.4 EHMT1 and EHMT2 RNAi knocked down enhanced Interferon response in K562 cells

In addition, the expression of either EHMT1 or EHMT2 was knocked down by stable transducing short hairpin RNAs (shRNAs) against EHMT1 and EHTM2 in K562 cells (Figure 3.6A). Infecting K562 cells with lenti-viruses carrying shRNAs against EHMT1 or EHMT2 achieved a 50-60% knockdown of the mRNA as well as the protein levels reduction of EHMT1 and EHMT2. It has been previously reported that EHMT1 and EHMT2 form functional heterodimer and knocked out of either EHMT1 or EHMT2 destabilizes EHMT2 or EHMT1 respectively (Tachibana et al., 2005). The similar

phenomena were observed as the expression of IFIT2 and IFIT3 were dramatically enhanced with the knocked-down of either EHMT1 or EHMT2 in shRNAs-transduced K562 cells upon IFNα2a treatment. Moreover, knocked down expression of either EHMT1 or EHMT2 in shRNAs-transduced K562 cells sensitized the cells towards interferon treatment (Figure 3.6B).

These results concluded that two different EHMT1- and EHMT2-specific chemical inhibitors, as well as knocking down the expression of either EHMT1 or EHMT2 enhance interferon response in CML cell lines, implying that inhibition of EHMT1 and EHMT2 sensitizes interferon resistant CML cell lines to interferon-mediated anti-proliferation.

3.5 EHMT1 and EHMT2 catalyze H3K9 methylation at the promoters of ISGs

To determine if EHMT1 and EHMT2 catalyze the H3K9 methylation at the promoters of ISGs, the levels of H3K9me2 at these promoters were determined in DMSO- or BIX01294-treated K562 cells stimulated with or without IFNα2a. In BIX01294 treated K562 cells, the basal levels of H3K9me2 was reduced by 75-80% at the *GBP3* and *IFIT3* promoters, and 36% at the *β-globin* promoter (Figure 3.7A). Accordingly, low level of H3K9me2 was detected at the promoter of highly expressed housekeeping gene, *GAPDH*. Furthermore, IFNα2a induced more polII recruitment to the *GBP3* and *IFIT3* genes in BIX01294 treated K562 cells than in DMSO treated K562 cells (Figure 3.7B). This is consistent with the observation that higher *GBP3* and *IFIT3* expression in BIX01294 treated K562 cells in response to IFNα2a stimulation (Figure 3.2).

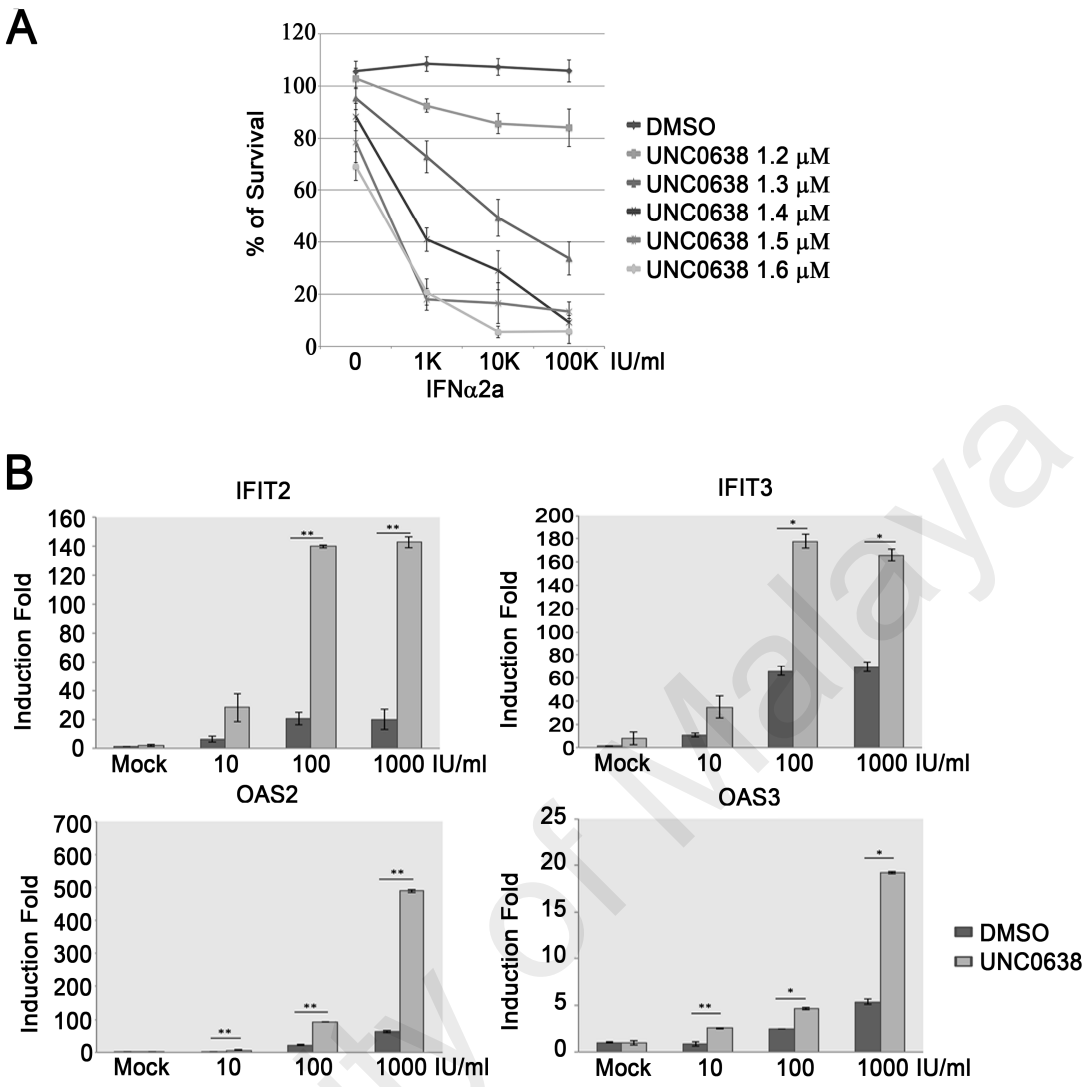

Figure 3.5: UNC0638 inhibits the proliferation of K562 cells and potentiates the expression of ISGs. (A) K562 cells were cultured with various concentrations of UNC0638 and IFNα2a as indicated. After four days, cell proliferation was measured with a MTT assay. Results represent the mean ± SD in quadruplicate experiments. (B) K562 cells were incubated with 5 μM UNC0638 for 24 hours followed with various concentrations of IFNα2a stimulation as indicated. After two hours of IFNα2a stimulation, the expression of various ISGs was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. *: $p < 0.05$, **: $p < 0.01$.

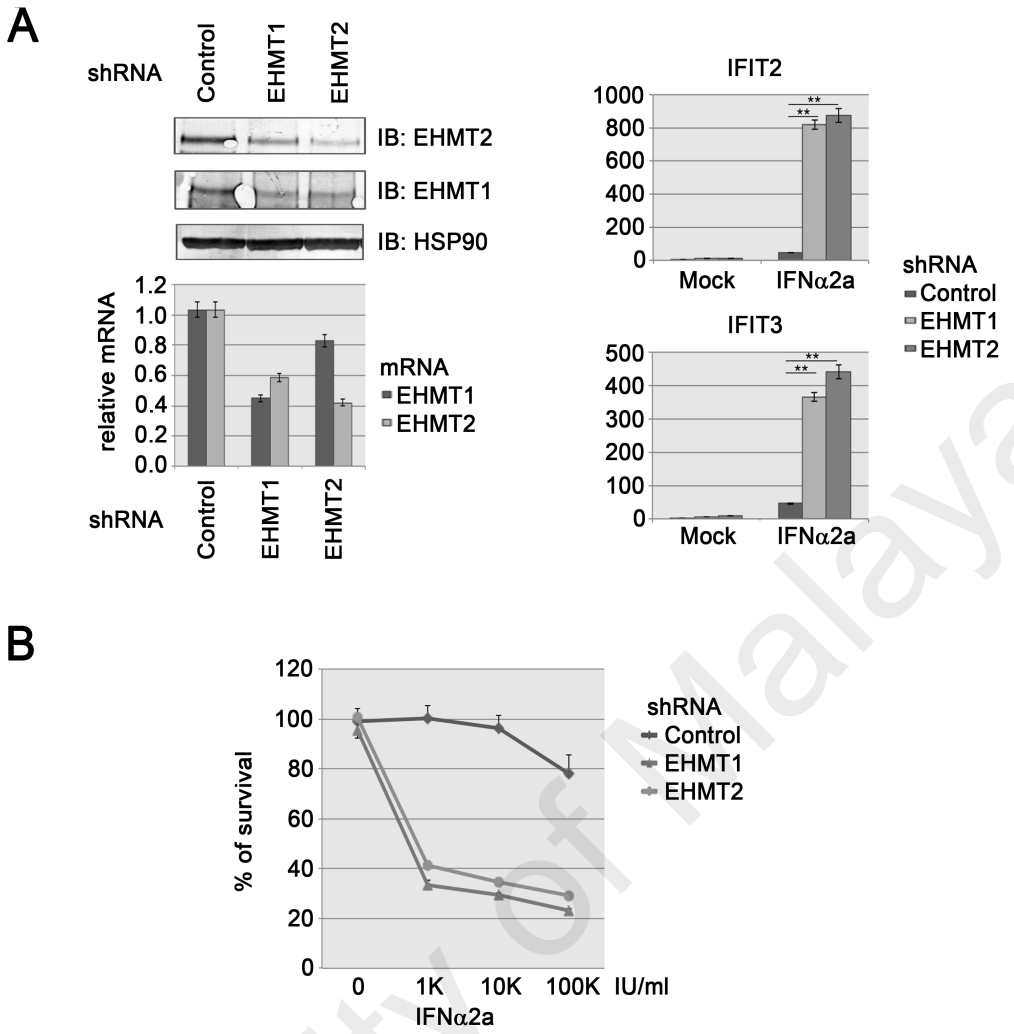

Figure 3.6: EHMT1 and EHMT2 RNAi knocked down enhanced interferon response in K562 cells. (A) Whole cell extracts or total RNA were generated from K562 cells infected with control or lentiviruses carrying EHMT1- or EHMT2-specific shRNAs (left). EHMT1 or EHMT2 protein levels were analysed by immunoblotting using indicated antibodies while mRNA levels were measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. The same cells were stimulated with 1000 IU/ml IFNα2a for two hours (right). The expression of various ISGs was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. **: $p < 0.01$. (B) K562 cells as in (A) were cultured with various concentrations of IFNα2a as indicated. After four days, cell proliferation was measured with a MTT assay. Results represent the mean ± SD in quadruplicate experiments.

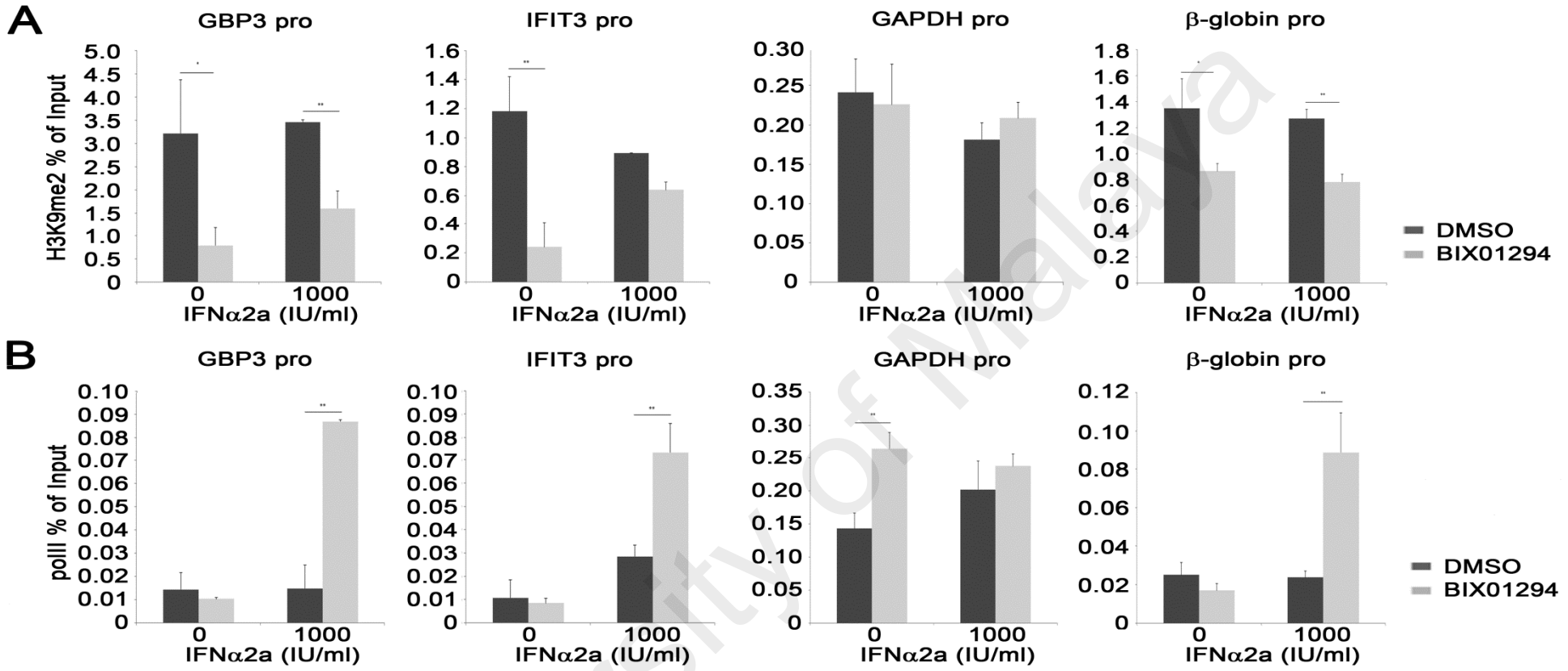

Figure 3.7: EHMT1 and EHMT2 promote H3K9 methylation at the promoters of GBP3 and IFIT3. (A-B) K562 cells treated with DMSO or BIX01294 were stimulated with IFNα2a for 2 hours, and were analysed by ChIP (H3K9me2 (A) and polII (B); mean ± SD; *: $p<0.05$;**: $p<0.01$; representative data of three independent experiment).

These results suggest that EHMT1 and EHMT2 are the major H3K9 HMT that catalyzes H3K9me2 at the *GBP3* and *IFIT3* genes.

3.6 Inhibition of EHMT1 and EHMT2 with BIX01294 enhances cytotoxicity of imatinib in K562 cells

CML is a unique disease, universally characterized by the presence of *Bcr-Abl* fusion genes, which encodes a constitutively active tyrosine kinase, and is considered responsible for the pathogenesis of CML (Lugo et al., 1990). Imatinib (Gleevec) is the first *Bcr-Abl* specific tyrosine kinase inhibitor approved by the FDA for treating CML (Henkes, van der Kuip, & Aulitzky, 2008).

To test if inhibition of EHMT1 and EHMT2 also enhances the anti-cancer effect of imatinib in CML, the cytotoxicity of imatinib is investigated in the presence or absence of BIX01294. K562 cells were very sensitive to imatinib treatment in that 150nM of imatinib reduced the proliferation of K562 cells by 57% (Figure 3.8A). More importantly, treating K562 cells with imatinib together with BIX01294 significantly enhanced the anti-proliferation effect of imatinib (Figure 3.8A).

3.7 Inhibition of EHMT1 and EHMT2 with BIX01294 enhances IFNα2a- and imatinib-induced apoptosis

To investigate if BIX01294 enhances the anti-proliferation effect of IFNα2a and imatinib by inducing cell cycle arrest or apoptosis, cell cycle analysis by FACS was performed for K562 cells treated with IFNα2a or imatinib in the presence or absence of BIX01294. The result showed S phase arrested when K562 cells were treated with IFNα2a, while imatinib treatment slightly increased the percentage of cells in the sub-G1 phase (Figure 3.8B). In the presence of BIX01294, cells in the sub-G1 phase were

significantly increased after prolonged IFNα2a or imatinib treatments ($p<0.05$). The increase of cell death was definitely not caused by BIX01294 itself, as treating K562 cells with BIX01294 alone did not increase the number of sub-G1 cells. However BIX01294 treatment did lead to a slight increase of cells in the G2 phase (Figure 3.7B). These results suggest that inhibition of EHMT1 and EHMT2 enhances IFNα2a- and imatinib-induced cell death.

To further test if BIX01294 enhances IFNα2a- and imatinib-induced cell death through apoptosis, the cleavage of the nuclear caspase substrate poly-ADP-ribose polymerase (PARP) and procaspase3, two biochemical markers for apoptosis was examined. DMSO treated K562 cells did not undergo apoptosis in response to IFNα2a or imatinib treatments as no cleaved PARP and activated caspase 3 were detected through immunoblotting (Figure 3.8C). On the other hand, combination of BIX01294 with IFNα2a or imatinib induced apoptosis in K562 cells as indicated by the generation of a shorter form of PARP (p85) and activated caspase 3 (p17 and p19) (Figure 3.8C). Therefore, by inhibiting EHMT1 and EHMT2, BIX01294 sensitizes K562 cells to IFNα2a- and imatinib-induced apoptosis.

3.8 CML cell lines show different amplitude of interferon response

In Figure 3.3, K562, KT1, KCL22 and BV173 cells are shown to exhibit different sensitivity to IFNα2a treatment. To determine if IFNα2a induces different expression levels of ISGs among K562, KT1, KCL22 and BV173, these cells were treated with 1000 IU/ml IFNα2a and the ISGs expression level were determined by RT-qPCR. The result demonstrates that KT1 cells expressed relatively higher basal and induced levels of IFIT2 and IFIT3 relative to K562, KCL22 and BV173 (Figure 3.9A-B).

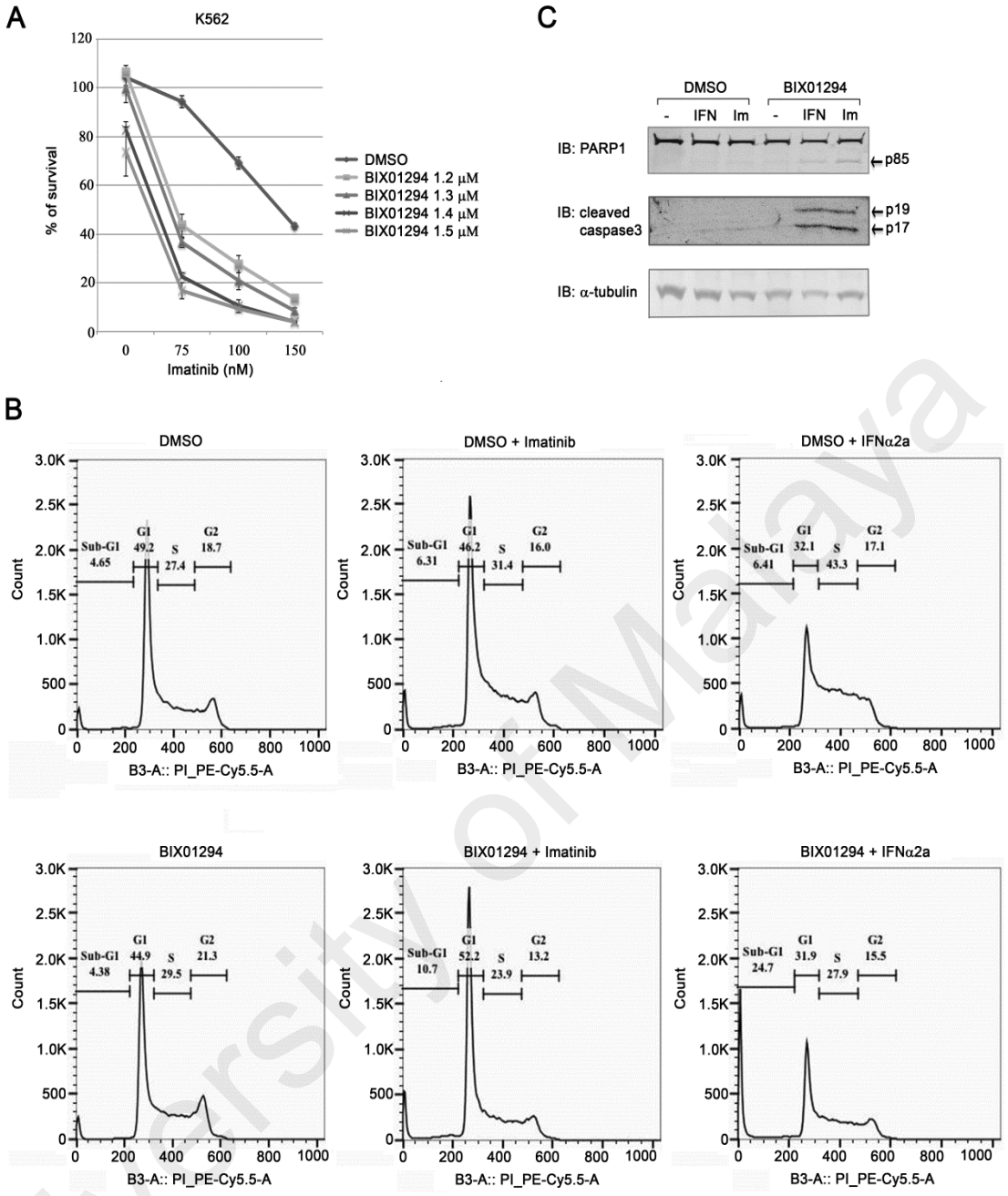

Figure 3.8: BIX01294 enhances imatinib- and IFNα2a-induced apoptosis in K562 cells. (A) K562 cells were cultured with various concentrations of BIX01294 and imatinib as indicated. After four days, cell proliferation was measured with a MTT assay. (B) K562 cells were stimulated with or without IFNα2 (15k IU/ml), or imatinib (150 nM) in the presence or absence of BIX01294 (2 μM) for 2 days. Cells were washed with PBS and fixed with 70% ethanol. Fixed cells were then stained with PI and analyzed with FACS. (C) K562 cells were stimulated with or without IFNα2a (10k IU/ml), or imatinib (75 nM) in the presence or absence of BIX01294 (2μM) for 2 days. Whole cell extracts were prepared and subjected to immunoblotting using the indicated antibodies. Results showed were representative data of three independent experiments.

Since type I interferons are potent anti-viral agents, these CML cell lines with different IFNα2a sensitivity profile could response differently to viral infection. To investigate if KT1 cells (with higher basal and ISGs expression level) are more resistant to viral infection, vesicular stomatitis virus carrying a GFP reporter (VSV-GFP) used to infect K562, KT1, BV173 and KCL22 cells at a multiplicity of infection (MOI) of 0.5. Twenty-four hours post-infection, infected cells were quantified by sorting the GFP positive cells using FACS. Consistent with the higher interferon response observed in KT1 cells, KT1 cells were more resistant to VSV-GFP infection compared to K562, BV173 and KCL22 cells (Figure 3.9B). At the MOI of 0.5, 40.9% of K562 cells, 20.8% BV173 cells and 78.3% KCL22 cells expressed GFP, while only 5.5% of KT1 cells were GFP positive at twenty-four hours post-infection.

It has been previously shown that the magnitude of type I interferon response is influenced by and inversely correlates with the expression level of EHMT1 and EHMT2. To test if the expression level of EHMT1 and EHMT2 contributes to different expression level of ISGs among KT1, BV173, KCL22 and K562 cells, the protein levels of EHMT1 and EHMT2 were compared among KT1, BV173, KCL22 and K562 cells (Figure 3.10). K562, BV173 and KCL22 cells expressed EHMT1 and EHMT2 at a slightly higher protein level compared to KT1 cells. Furthermore, the relative mRNA level of EHMT1 and EHMT2 in BV173, KCL22, K562 and KT1 cells were also measured with RT-qPCR. The results consistently showed that KT1 cells expressed less mRNA levels of EHMT1 and EHMT2 than those in BV173, KCL22 and K562 cells (Figure 3.10B).

To further test if the high ISGs expression in KT1 cells in response to interferon stimulation is due to the lower expression of EHMT1 and EHMT2, FLAG-mEHMT1 and HA-mEHMT2 were co-expressed in KT1 cells and the interferon response was again measured with RT-qPCR. The FLAG-specific and HA-specific antibodies and RT-qPCR were used to verify the expression of exogenous mouse EHMT1 and EHMT2 (Figure 3.10A-B). The results show that IFNα2a-induced expression of *IFIT2* and *IFIT3* were significantly reduced when EHMT1 and EHMT2 are overexpressed (Figure 3.10C).

Taken together, these results imply that intrinsic expression level of EHMT1 and EHMT2 correlates with the type I interferon responsiveness of CML cells.

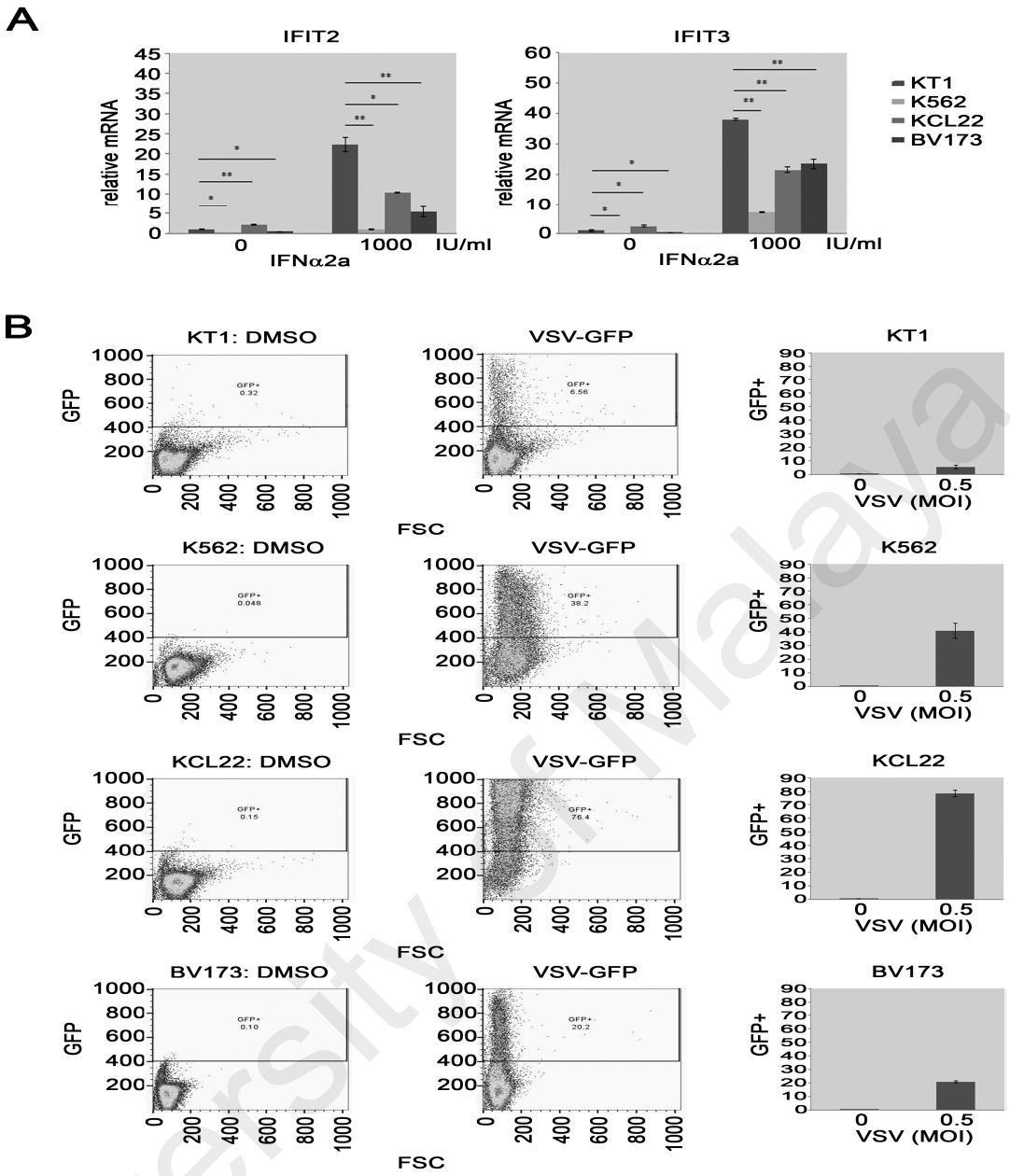

Figure 3.9: Expression level of EHMT1 inversely correlates with the sensitivity of CML cells to interferon. (A) KT1, K562, KCL22 and BV173 cells were treated with 1000 IU/ml IFNα2a for 2 hours, the expression of IFIT2 and IFIT3 was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. *: $p < 0.05$, **: $p < 0.01$. (B) KT1, K562, KCL22 and BV173cells were incubated with or without 2.5 µM BIX01294 for 24 hours. Cells were then infected with VSV-GFP at a MOI of 0.5 for 24 hours. GFP positive cells were sorted by FACS. Results represent the mean ± SD in triplicate experiments.

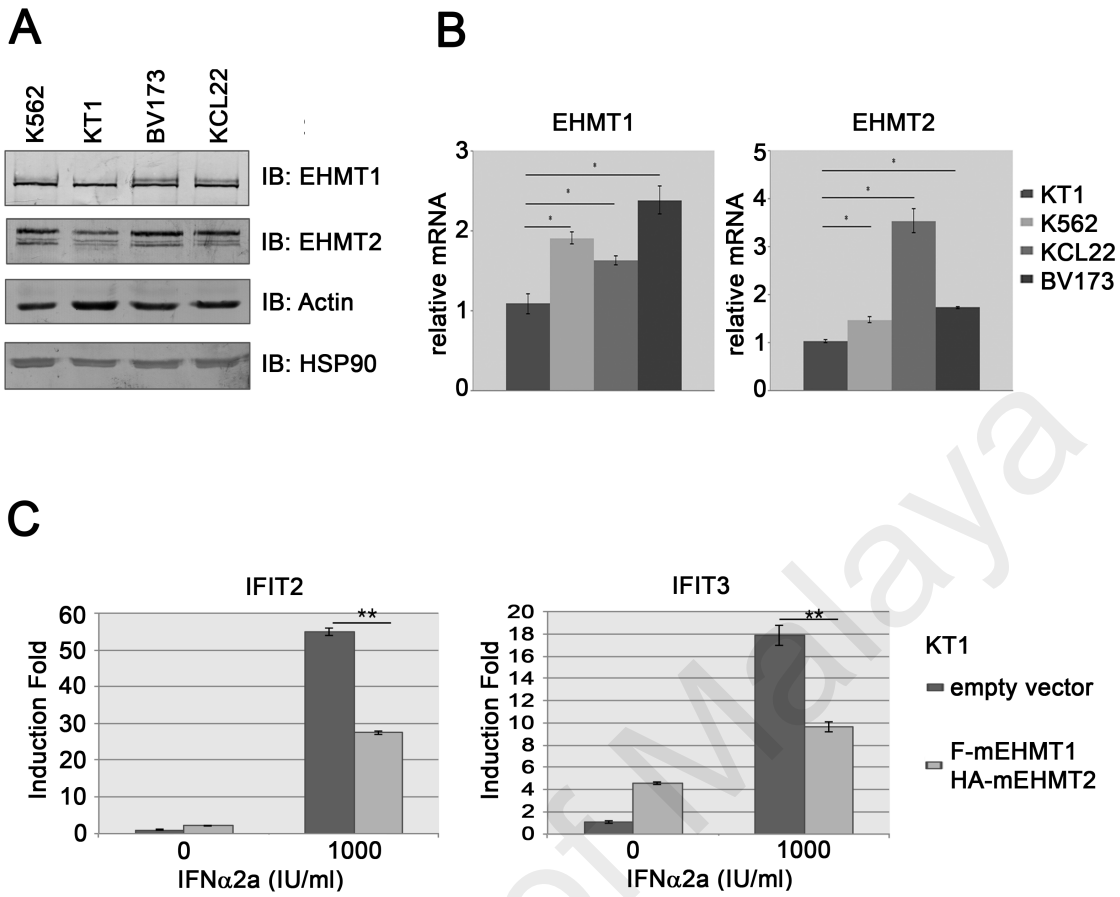

Figure 3.10: Different EHMT1 profile among CML cells. (A) Whole cell extracts were prepared from K562, KT1, BV173 and KCL22 cells, and examined by immunoblotting using the indicated antibodies. (B) The relative mRNA level of EHMT1 and EHMT2 was measured with RT-qPCR. Results represent the mean ± SD in quadruplicate experiments. *: $p < 0.05$. (C) KT1 empty vector or FLAG-mEHMT1-HA-mEHMT2 cells were treated with or without IFNα2a (1000IU/ml) for two hours, the expression of various IFIT2 and IFIT3 was measured with RT-qPCR. Error bars represent the variation range of duplicate experiments. *: $p < 0.05$, **: $p < 0.01$.

CHAPTER 4: DISCUSSION

Treatment of CML has changed from chemotherapy (busulfan, hydroxyurea) in the past to interferon, and finally to tyrosine kinase inhibitors such as imatinib. Interferon was previously given to CML patients based on the reasons that IFNα is capable of modulating gene expression, induces apoptosis, inhibits cell proliferation, and induces an immunomodulatory response (Goldman, 2010). It is also widely used in other cancer treatment (Guilhot, Roy, Saulnier, & Guilhot, 2009; Rubin, 1988). However, several studies in the past have pointed out that interferon treatment is only effective in treating patient with chronic phase CML and patients who developed accelerated and blastic phase tend to have poor prognosis and are irresponsive to interferon treatment (Guilhot et al., 2009). In addition, high dose of interferon may lead to severe toxicity, including neurotoxicity and depression especially after 3-5 years of treatments (Vial, Choquet-Kastylevsky, Liautard, & Descotes, 2000). Although interferons have now been dropped out from the first-line treatment regimens for CML, it still has a role in chemotherapy, bone marrow transplantation and for those tolerance to tyrosine kinase inhibitor treatment (Hoffman & Wadler, 1993).

Here, I showed that inhibiting the H3K9me2 specific methyltransferases, EHMT1 and EHMT2, either with a chemical inhibitor, BIX01294 or RNAi sensitizes CML cell lines to interferon treatment. EHMT1 and EHMT2 negatively regulates type I interferon response by promoting H3K9me2 at the promoters of ISGs. Using a second generation and commercially available EHMT1 and EHMT2 specific inhibitor, UNC0638, I managed to show that the sensitization of CML cell lines by BIX01294 to interferon treatment was not due to off-target effects. Doses of IFNα in clinical trials have ranged from 2 to 5 million IU/m^2. The current standard tolerated dose is 5 million IU/m^2, given daily by subcutaneous injection. The combination treatment of BIX01294

and IFNα would be more ideal as it could significantly reduce the dosage of interferon required to achieve therapeutic response. Therefore, the combined treatment regimen provides an alternative to reduce the toxicity side effects of the interferon mentioned earlier.

Before tyrosine kinase inhibitors are introduced, therapies for CML such as interferons and chemotherapeutic agents only provide temporary disease control but do not alter its progression to advanced disease. It is not until 1990 did the researchers linked the constitutive tyrosine kinase activity of *Bcr-Abl* to the pathogenesis of CML, as the tyrosine kinase promote the growth of CML cells (Lugo et al., 1990). This discovery spurred the development of the molecular-targeted therapy. Imatinib, the first approved tyrosine kinase inhibitor, functions by blocking the adenosine triphosphate (ATP) binding site on the *Bcr-Abl* kinase, is selective for killing cells expressing the *Bcr-Abl* kinase. The results of clinical trials with imatinib demonstrated impressive response rates in patients who had not responded to IFNα therapy (Brian J. Druker, Talpaz, et al., 2001). Moreover, successful large-scale clinical trials have proven imatinib has high and persistent efficacy in treating CML by maintaining patients in chronic phase and therefore improved long-term survival (Hochhaus et al., 2009). However, 25% of patients with imatinib monotherapy in Fausel's study show primary refractory disease and drug resistance (Fausel, 2007). The resistance is more often caused by the evolution of mutations in blocking imatinib interactions with the *Bcr-Abl* ATP binding site. Since then, several new generations of tyrosine kinase inhibitors were developed. However, imatinib and its kinds are also inhibitor of cytochrome P450 enzymes, thus requires careful monitoring of concomitant medications metabolized by these enzymes (Filppula, Laitila, Neuvonen, & Backman, 2012).

Despite all the side effects and inconsistent performances, some studies do provide evidence about the curative potential of the IFNα (Mahon et al., 2002). Mahon's study recorded 47% of the patients who received interferon treatment did not relapse after discontinuation of the treatment. This is important, especially for imatinib-resistance CML patients. Other than imatinib monotherapy, several clinical trials have shown some advantages for combining imatinib with IFNα treatment (Talpaz, Hehlmann, Quintas-Cardama, Mercer, & Cortes, 2013). One of the rationales for the combination therapy is that imatinib kills CML cells but not CML primitive progenitors while IFNα preferentially target CML stem cells. My study here shows that inhibition of EHMT1 and EHMT2 by the BIX01294 augments the cytotoxicity of imatinib. Therefore, inhibition of EHMT1 and EHMT2 will potentiate the efficacy of the imatinib and interferon combination therapy.

By comparing type I interferon response between interferon-resistant (K562, KCL22 and BV173) and interferon-sensitive (KT1) cells, I show that the levels of ISGs expression are important and may partially explained the different sensitivity of these cell lines to interferon treatment. For example, KT1 cells which contain higher ISGs expression upon IFNα2a stimulation are more resistant to viral infection and more sensitive to IFNα2a treatment. The reason behind the interferon sensitive may due to lower EHMT1 and EHMT2 profile in KT1 compare to interferon-resistance CML cells. With the result obtained, I further showed that EHMT1 and EHMT2 levels were inversely correlated with the expression of ISGs which was in agreement with other publications studying fibroblasts and dendritic cells (Fang et al., 2012). In a previous study by Ea and his co-workers, they reported that p50 recruits EHMT1 to the promoters of type I interferons response genes and repress the gene expression (C. K. Ea et al., 2012). They further showed that silencing and reduction of EHMT1 levels

enhances interferons production and strengthens the interferon-mediated inhibition of virus replication. Similarly, I showed that ectopic expression of EHMT1 and EHMT2 in KT1 cells reduced the type I ISGs expression in KT1 cells. Thus, cells that have low EHMT1 and EHMT2 profile are expected to be more resistant to viral infection and response better to interferon treatment. Therefore, the expression levels of EHMT1 and EHMT2 in CML cells might potentially be a biomarker to predict the interferon response of CML patients. Further experiments with the use of primary cells extracted from CML patients rather than commercial CML cell lines are required.

To further demonstrate the combined BIX01294 and interferon treatment's efficacy towards CML, it would be necessary to determine its effect *in vivo*. Previously, *in vivo* animal study was unable to be carried out due to some technical constraints: (1) poor pharmacokinetic property of BIX01294 limits the *in vivo* animal study. Several trials and routes of injections have been tested. BIX01294 have been injected into mice through peritoneal, subcutaneous and retro-orbital injection, but only less than 1% of total injected BIX01294 can be detected in the blood. (2) High dose of BIX01294 can kill CML cells through unknown mechanism, which is similar to the data obtained in Figure 3.3 and Figure 3.4, whereby I show that BIX01294 is toxic to all tested cell lines, with dosage higher than 2 μM. Hence, EHMT1 and EHMT2-specific chemical inhibitor's with better pharmacokinetic profile is required for future *in vivo* study.

In this study I found that BIX01294 treatment reduced the H3K9me2 level at the promoter of *β-globin* in K562 cells (Figure 3.7). These results imply that EHMT1- and EHMT2-regulated gene may play a role in *β-globin* expression in K562 cells. However, our group previous study showed that EHMT1 is dispensable for the H3K9me2 modification at the promoter of *β-globin* in *HeLa* cells (C. K. Ea et al., 2012). This

contradictive result may due to the expression of *β-globin* is restricted to erythrocytes. In 2015, Renneville's study also shows that EHMT1 and EHMT2 inhibition has the ability to induce *γ-globin* expression and HbF synthesis in primary adult human erythroid cells (Renneville et al., 2015). K562 cells are of the erythroleukemia type. Thus *β-globin* is considered a permissive gene in K562 and a non-permissive gene in other cell types, such as HeLa cells, a cervical cancer cell line. EHMT1 and EHMT2 are mainly associated with euchromatin where most of the permissive genes are located, while SUV39H1 and SUV29H2 are mainly present in heterochromatin that is enriched with non-permissive genes (Peters et al., 2001; Tachibana et al., 2002). Further study to test if *β-globin* is located within euchromatin in K562 cells while within heterochromatin in other cell types, and thus regulates the accessibility of *β-globin* by EHMT1 and EHMT2 in different cell types, would provide valuable insight on the mechanism of EHMT1- and EHMT2-mediated gene regulation.

CHAPTER 5: CONCLUSION

This study demonstrates the importance and role of histone methyltransferase, EHMT1 and EHMT2, in the treatment of CML. Experiment data presented also illustrated the relationship between the levels of EHMT1 and EHMT2 and the response of the CML cells towards type I interferon treatment. The cells with higher EHMT1 and EHMT2 profile tends to have lower ISGs expression and are less sensitive towards interferon treatment. Inhibition of EHMT1 and EHMT2 using chemical inhibitors or RNAi is capable to sensitize CML cells to type I interferon and imatinib treatments. Henceforth this study provides evidence and insight that targeting EHMT1 and EHMT2 is a potential new approach to improve existing CML treatments, including imatinib and interferon therapies.

REFERENCES

Attema, J. L., Bert, A. G., Lim, Y. Y., Kolesnikoff, N., Lawrence, D. M., Pillman, K. A., . . . Goodall, G. J. (2013). Identification of an enhancer that increases miR-200b~200a~429 gene expression in breast cancer cells. *PLoS One, 8*(9), e75517. doi:10.1371/journal.pone.0075517

Bannister, A. J., & Kouzarides, T. (2005). Reversing histone methylation. *Nature, 436*(7054), 1103-1106. Retrieved from http://dx.doi.org/10.1038/nature04048

Bannister, A. J., & Kouzarides, T. (2011). Regulation of chromatin by histone modifications. *Cell Res, 21*(3), 381-395. doi:10.1038/cr.2011.22

Baynes, R. D., Hamm, C., Dansey, R., Klein, J., Cassells, L., Karanes, C., . . . Peters, W. P. (2000). Bone Marrow and Peripheral Blood Hematopoietic Stem Cell Transplantation: Focus on Autografting. *Clinical Chemistry, 46*(8), 1239-1251. Retrieved from http://www.clinchem.org/content/46/8/1239.abstract

Bellm, L. A., Epstein, J. B., Rose-Ped, A., Martin, P., & Fuchs, H. J. (2000). Patient reports of complications of bone marrow transplantation. *Supportive Care in Cancer, 8*(1), 33-39. doi:10.1007/s005209900095

Bernstein, B. E., Humphrey, E. L., Erlich, R. L., Schneider, R., Bouman, P., Liu, J. S., . . . Schreiber, S. L. (2002). Methylation of histone H3 Lys 4 in coding regions of active genes. *Proc Natl Acad Sci U S A, 99*(13), 8695-8700. doi:10.1073/pnas.082249499

Bernstein, B. E., Meissner, A., & Lander, E. S. (2007). The mammalian epigenome. *Cell, 128*(4), 669-681. doi:10.1016/j.cell.2007.01.033

Bird, A. (2002). DNA methylation patterns and epigenetic memory. *Genes & Development, 16*(1), 6-21. doi:10.1101/gad.947102

Bird, A. (2007). Perceptions of epigenetics. *Nature, 447*(7143), 396-398. doi:10.1038/nature05913

Bonifazi, F., de Vivo, A., Rosti, G., Guilhot, F., Guilhot, J., Trabacchi, E., . . . Baccarani, M. (2001). Chronic myeloid leukemia and interferon-alpha: a study of complete cytogenetic responders. *Blood, 98*(10), 3074-3081.

Borden, E. C., & Ball, L. A. (1981). Interferons: biochemical, cell growth inhibitory, and immunological effects. *Progress in hematology, 12*, 299-339. Retrieved from http://europepmc.org/abstract/MED/6175994

Bossdorf, O., Richards, C. L., & Pigliucci, M. (2008). Epigenetics for ecologists. *Ecol Lett, 11*(2), 106-115. doi:10.1111/j.1461-0248.2007.01130.x

Chang, Y., Zhang, X., Horton, J. R., Upadhyay, A. K., Spannhoff, A., Liu, J., . . . Cheng, X. (2009). Structural basis for G9a-like protein lysine methyltransferase inhibition by BIX-01294. *Nat Struct Mol Biol, 16*(3), 312-317. doi:http://www.nature.com/nsmb/journal/v16/n3/suppinfo/nsmb.1560_S1.html

Chen, L., Li, Z., Zwolinska, A. K., Smith, M. A., Cross, B., Koomen, J., . . . Chen, J. (2010). MDM2 recruitment of lysine methyltransferases regulates p53 transcriptional output. *Embo j, 29*(15), 2538-2552. doi:10.1038/emboj.2010.140

Colamonici, O. R., Domanski, P., Platanias, L. C., & Diaz, M. O. (1992). Correlation between interferon (IFN) alpha resistance and deletion of the IFN alpha/beta genes in acute leukemia cell lines suggests selection against the IFN system. *Blood, 80*(3), 744-749.

Copelan, E. A. (2006). Hematopoietic stem-cell transplantation. *N Engl J Med, 354*(17), 1813-1826. doi:10.1056/NEJMra052638

Cortes, J., O'Brien, S., & Kantarjian, H. (2004). Discontinuation of imatinib therapy after achieving a molecular response. *Blood, 104*(7), 2204-2205. doi:10.1182/blood-2004-04-1335

Druker, B. J. (2002). Inhibition of the Bcr-Abl tyrosine kinase as a therapeutic strategy for CML. *Oncogene, 21*(56), 8541-8546. doi:10.1038/sj.onc.1206081

Druker, B. J., Sawyers, C. L., Kantarjian, H., Resta, D. J., Reese, S. F., Ford, J. M., . . . Talpaz, M. (2001). Activity of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in the Blast Crisis of Chronic Myeloid Leukemia and Acute Lymphoblastic Leukemia with the Philadelphia Chromosome. *New England Journal of Medicine, 344*(14), 1038-1042. doi:doi:10.1056/NEJM200104053441402

Druker, B. J., Talpaz, M., Resta, D. J., Peng, B., Buchdunger, E., Ford, J. M., . . . Sawyers, C. L. (2001). Efficacy and Safety of a Specific Inhibitor of the BCR-ABL Tyrosine Kinase in Chronic Myeloid Leukemia. *New England Journal of Medicine, 344*(14), 1031-1037. doi:doi:10.1056/NEJM200104053441401

Druker, B. J., Tamura, S., Buchdunger, E., Ohno, S., Segal, G. M., Fanning, S., . . . Lydon, N. B. (1996). Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of *Bcr-Abl* positive cells. *Nature Medicine, 2*(5), 561-566.

Ea, C.-K., Hao, S., Yeo, K. S., & Baltimore, D. (2012). EHMT1 Protein Binds to Nuclear Factor-kB p50 and Represses Gene Expression. *Journal of Biological Chemistry, 287*(37), 31207-31217. doi:10.1074/jbc.M112.365601

Fang, T. C., Schaefer, U., Mecklenbrauker, I., Stienen, A., Dewell, S., Chen, M. S., . . . Tarakhovsky, A. (2012). Histone H3 lysine 9 di-methylation as an epigenetic signature of the interferon response. *J Exp Med, 209*(4), 661-669. doi:10.1084/jem.20112343

Fausel, C. (2007). Targeted chronic myeloid leukemia therapy: Seeking a cure. *Am J Health Syst Pharm, 64*(24 Suppl 15), S9-15. doi:10.2146/ajhp070482

Filppula, A. M., Laitila, J., Neuvonen, P. J., & Backman, J. T. (2012). Potent mechanism-based inhibition of CYP3A4 by imatinib explains its liability to interact with CYP3A4 substrates. *Br J Pharmacol, 165*(8), 2787-2798. doi:10.1111/j.1476-5381.2011.01732.x

Glucksberg, H., Storb, R., Fefer, A., Buckner, C. D., Neiman, P. E., Clift, R. A., . . . Thomas, E. D. (1974). Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation, 18*(4), 295-304.

Goldman, J. M. (2010). Chronic myeloid leukemia: a historical perspective. *Semin Hematol, 47*(4), 302-311. doi:10.1053/j.seminhematol.2010.07.001

Guilhot, F., Roy, L., Saulnier, P. J., & Guilhot, J. (2009). Interferon in chronic myeloid leukaemia: past and future. *Best Pract Res Clin Haematol, 22*(3), 315-329. doi:10.1016/j.beha.2009.10.005

Hehlmann, R. d., Lauseker, M., Jung-Munkwitz, S., Leitner, A., MÃ¼ller, M. C., Pletsch, N., . . . SauÃŸele, S. Tolerability-Adapted Imatinib 800 mg/d Versus 400 mg/d Versus 400 mg/d Plus Interferon-Î± in Newly Diagnosed Chronic Myeloid Leukemia. *Journal of Clinical Oncology, 29*(12), 1634-1642. doi:10.1200/jco.2010.32.0598

Henkes, M., van der Kuip, H., & Aulitzky, W. E. (2008). Therapeutic options for chronic myeloid leukemia: focus on imatinib (Glivec, Gleevectrade mark). *Ther Clin Risk Manag, 4*(1), 163-187.

Hochhaus, A., O'Brien, S. G., Guilhot, F., Druker, B. J., Branford, S., Foroni, L., . . . Larson, R. A. (2009). Six-year follow-up of patients receiving imatinib for the first-line treatment of chronic myeloid leukemia. *Leukemia, 23*(6), 1054-1061. doi:10.1038/leu.2009.38

Hoffman, M. A., & Wadler, S. (1993). Mechanisms by Which Interferon Potentiates Chemotherapy. *Cancer Investigation, 11*(3), 310-313. doi:doi:10.3109/07357909309024857

Holliday, R., & Pugh, J. (1975). DNA modification mechanisms and gene activity during development. *Science, 187*(4173), 226-232. doi:10.1126/science.187.4173.226

Huang, X., Cortes, J., & Kantarjian, H. (2012). Estimations of the increasing prevalence and plateau prevalence of chronic myeloid leukemia in the era of tyrosine kinase inhibitor therapy. *Cancer, 118*(12), 3123-3127. doi:10.1002/cncr.26679

Jenuwein, T., Laible, G., Dorn, R., & Reuter, G. (1998). SET domain proteins modulate chromatin domains in eu- and heterochromatin. *Cell Mol Life Sci, 54*(1), 80-93.

Jin, B., Li, Y., & Robertson, K. D. (2011). DNA Methylation: Superior or Subordinate in the Epigenetic Hierarchy? *Genes & Cancer, 2*(6), 607-617. doi:10.1177/1947601910393957

Kantarjian, H. M., O'Brien, S., Anderlini, P., & Talpaz, M. (1996). Treatment of chronic myelogenous leukemia: current status and investigationnal options. *Blood, 87*, 3069-3081.

Kouzarides, T. (2002b). Histone methylation in transcriptional control. *Current Opinion in Genetics and Development, 12*(2), 198-209. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-0036532202&partnerID=40&md5=ea3e7351cd4f48367308810ecfeb24ec

Krogan, N. J., Kim, M., Tong, A., Golshani, A., Cagney, G., Canadien, V., . . . Greenblatt, J. (2003). Methylation of histone H3 by Set2 in Saccharomyces cerevisiae is linked to transcriptional elongation by RNA polymerase II. *Mol Cell Biol, 23*(12), 4207-4218.

Kubicek, S., O'Sullivan, R. J., August, E. M., Hickey, E. R., Zhang, Q., Teodoro, M. L., . . . Jenuwein, T. (2007). Reversal of H3K9me2 by a small-molecule inhibitor for the G9a histone methyltransferase. *Mol Cell, 25*(3), 473-481. doi:10.1016/j.molcel.2007.01.017

Lachner, M., O'Carroll, D., Rea, S., Mechtler, K., & Jenuwein, T. (2001). Methylation of histone H3 lysine 9 creates a binding site for HP1 proteins. *Nature, 410*(6824), 116-120. Retrieved from http://dx.doi.org/10.1038/35065132

Latham, K. E., Sapienza, C., & Engel, N. (2012). The epigenetic lorax: gene-environment interactions in human health. *Epigenomics, 4*(4), 383-402. doi:10.2217/epi.12.31

Lugo, T. G., Pendergast, A. M., Muller, A. J., & Witte, O. N. (1990). Tyrosine kinase activity and transformation potency of bcr-abl oncogene products. *Science, 247*(4946), 1079-1082.

Mahon, F. X., Delbrel, X., Cony-Makhoul, P., Faberes, C., Boiron, J. M., Barthe, C., . . . Reiffers, J. (2002). Follow-up of complete cytogenetic remission in patients with chronic myeloid leukemia after cessation of interferon alfa. *J Clin Oncol, 20*(1), 214-220.

Maziarz, R. T., & Mauro, M. J. (2003). Transplantation for chronic myelogenous leukemia: yes, no, maybe so. . . . An Oregon perspective. *Bone Marrow Transplant, 32*(5), 459-469. doi:10.1038/sj.bmt.1704163

Min, J., Feng, Q., Li, Z., Zhang, Y., & Xu, R. M. (2003). Structure of the catalytic domain of human DOT1L, a non-SET domain nucleosomal histone methyltransferase. *Cell, 112*(5), 711-723.

Muller-Tidow, C., Klein, H. U., Hascher, A., Isken, F., Tickenbrock, L., Thoennissen, N., . . . Serve, H. (2010). Profiling of histone H3 lysine 9 trimethylation levels predicts transcription factor activity and survival in acute myeloid leukemia. *Blood, 116*(18), 3564-3571. doi:10.1182/blood-2009-09-240978

Mutskov, V., Raaka, B. M., Felsenfeld, G., & Gershengorn, M. C. (2007). The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells, 25*(12), 3223-3233. doi:10.1634/stemcells.2007-0325

Pavlovsky, C., Kantarjian, H., & Cortes, J. E. (2009). First-line therapy for chronic myeloid leukemia: Past, present, and future. *Am J Hematol, 84*(5), 287-293. doi:10.1002/ajh.21380

Pavlu, J., Szydlo, R. M., Goldman, J. M., & Apperley, J. F. (2011). Three decades of transplantation for chronic myeloid leukemia: what have we learned? *Blood, 117*(3), 755-763. doi:10.1182/blood-2010-08-301341

Peters, A. H., O'Carroll, D., Scherthan, H., Mechtler, K., Sauer, S., Schofer, C., . . . Jenuwein, T. (2001). Loss of the Suv39h histone methyltransferases impairs mammalian heterochromatin and genome stability. *Cell, 107*(3), 323-337.

Piehler, J., Thomas, C., Garcia, K. C., & Schreiber, G. (2012). Structural and dynamic determinants of type I interferon receptor assembly and their functional interpretation. *Immunol Rev, 250*(1), 317-334. doi:10.1111/imr.12001

Preudhomme, C., Guilhot, J. l., Nicolini, F. E., Guerci-Bresler, A. s., Rigal-Huguet, F. o., Maloisel, F., . . . Guilhot, F. o. Imatinib plus Peginterferon Alfa-2a in Chronic Myeloid Leukemia. *New England Journal of Medicine, 363*(26), 2511-2521. doi:doi:10.1056/NEJMoa1004095

Price, G. E., Gaszewska-Mastarlarz, A., & Moskophidis, D. (2000). The Role of Alpha/Beta and Gamma Interferons in Development of Immunity to Influenza A Virus in Mice. *Journal of Virology, 74*(9), 3996-4003. doi:10.1128/jvi.74.9.3996-4003.2000

Qian, C., & Zhou, M. M. (2006). SET domain protein lysine methyltransferases: Structure, specificity and catalysis. *Cell Mol Life Sci, 63*(23), 2755-2763. doi:10.1007/s00018-006-6274-5

Rawlings, J. S., Rosler, K. M., & Harrison, D. A. (2004). The JAK/STAT signaling pathway. *J Cell Sci, 117*(Pt 8), 1281-1283. doi:10.1242/jcs.00963

Rea, S., Eisenhaber, F., O'Carroll, D., Strahl, B. D., Sun, Z. W., Schmid, M., . . . Jenuwein, T. (2000). Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature, 406*(6796), 593-599. doi:10.1038/35020506

Renneville, A., Van Galen, P., Canver, M. C., McConkey, M., Krill-Burger, J. M., Dorfman, D. M., . . . Ebert, B. L. (2015). EHMT1 and EHMT2 inhibition induces fetal hemoglobin expression. *Blood, 126*(16), 1930-1939. Retrieved from http://www.bloodjournal.org/content/126/16/1930.abstract

Riccardi, C., & Nicoletti, I. (2006). Analysis of apoptosis by propidium iodide staining and flow cytometry. *Nat. Protocols, 1*(3), 1458-1461. Retrieved from http://dx.doi.org/10.1038/nprot.2006.238

Rohrbacher, M., & Hasford, J. (2009). Epidemiology of chronic myeloid leukaemia (CML). *Best Pract Res Clin Haematol, 22*(3), 295-302. doi:10.1016/j.beha.2009.07.007

Rousselot, P., Huguet, F., Rea, D., Legros, L., Cayuela, J. M., Maarek, O., . . . Chronique, T. I. F. a. d. L. m. M. l. d. (2007). Imatinib mesylate discontinuation in patients with chronic myelogenous leukemia in complete molecular remission for more than 2 years. *Blood, 109*(1), 58-60. doi:10.1182/blood-2006-03-011239

Rowley, J. D. (1973). A New Consistent Chromosomal Abnormality in Chronic Myelogenous Leukaemia identified by Quinacrine Fluorescence and Giemsa Staining. *Nature, 243*(5405), 290-293.

Rubin, D. (1988). Cancer therapy with interferon: Google Patents.

Sakai, I., Takeuchi, K., Yamauchi, H., Narumi, H., & Fujita, S. (2002). Constitutive expression of SOCS3 confers resistance to IFN-alpha in chronic myelogenous leukemia cells. *Blood, 100*(8), 2926-2931. doi:10.1182/blood-2002-01-0073

Samuel, C. E. (2001). Antiviral actions of interferons. *Clin Microbiol Rev, 14*(4), 778-809, table of contents. doi:10.1128/cmr.14.4.778-809.2001

Santos, G. W., Yeager, A. M., & Jones, R. J. (1989). Autologous bone marrow transplantation. *Annu Rev Med, 40*, 99-112. doi:10.1146/annurev.me.40.020189.000531

Sessions, J. (2007). Chronic myeloid leukemia in 2007. *Am J Health Syst Pharm, 64*(24 Suppl 15), S4-9. doi:10.2146/ajhp070484

Shinkai, Y., & Tachibana, M. (2011). H3K9 methyltransferase G9a and the related molecule GLP. *Genes Dev, 25*(8), 781-788. doi:10.1101/gad.2027411

Snowden, A. W., Gregory, P. D., Case, C. C., & Pabo, C. O. (2002). Gene-Specific Targeting of H3K9 Methylation Is Sufficient for Initiating Repression In Vivo. *Current Biology, 12*(24), 2159-2166. doi:http://dx.doi.org/10.1016/S0960-9822(02)01391-X

Strahl, B. D., & Allis, C. D. (2000). The language of covalent histone modifications. *Nature, 403*(6765), 41-45. doi:10.1038/47412

Tachibana, M., Sugimoto, K., Fukushima, T., & Shinkai, Y. (2001). Set domain-containing protein, G9a, is a novel lysine-preferring mammalian histone methyltransferase with hyperactivity and specific selectivity to lysines 9 and 27 of histone H3. *J Biol Chem, 276*(27), 25309-25317. doi:10.1074/jbc.M101914200

Tachibana, M., Sugimoto, K., Nozaki, M., Ueda, J., Ohta, T., Ohki, M., . . . Shinkai, Y. (2002). G9a histone methyltransferase plays a dominant role in euchromatic histone H3 lysine 9 methylation and is essential for early embryogenesis. *Genes Dev, 16*(14), 1779-1791. doi:10.1101/gad.989402

Tachibana, M., Ueda, J., Fukuda, M., Takeda, N., Ohta, T., Iwanari, H., . . . Shinkai, Y. (2005). Histone methyltransferases G9a and GLP form heteromeric complexes and are both crucial for methylation of euchromatin at H3-K9. *Genes Dev, 19*(7), 815-826. doi:10.1101/gad.1284005

Talpaz, M., Hehlmann, R., Quintas-Cardama, A., Mercer, J., & Cortes, J. (2013). Re-emergence of interferon-[alpha] in the treatment of chronic myeloid leukemia. *Leukemia, 27*(4), 803-812. doi:10.1038/leu.2012.313

Talpaz, M., Kantarjian, H. M., McCredie, K., Trujillo, J. M., Keating, M. J., & Gutterman, J. U. (1986). Hematologic remission and cytogenetic improvement induced by recombinant human interferon alpha A in chronic myelogenous leukemia. *N Engl J Med, 314*(17), 1065-1069. doi:10.1056/nejm198604243141701

Talpaz, M., McCredie, K. B., Mavligit, G. M., & Gutterman, J. U. (1983). Leukocyte interferon-induced myeloid cytoreduction in chronic myelogenous leukemia. *Blood, 62*(3), 689-692.

Turner, B. M. (2002). Cellular memory and the histone code. *Cell, 111*(3), 285-291.

Ueda, J., Tachibana, M., Ikura, T., & Shinkai, Y. (2006). Zinc finger protein Wiz links G9a/GLP histone methyltransferases to the co-repressor molecule CtBP. *J Biol Chem, 281*(29), 20120-20128. doi:10.1074/jbc.M603087200

Urabe, A. (1997). Recent progress in therapy for chronic myelogenous leukemia. *Gan To Kagaku Ryoho, 24*(9), 1066-1073.

Vedadi, M., Barsyte-Lovejoy, D., Liu, F., Rival-Gervier, S., Allali-Hassani, A., Labrie, V., . . . Jin, J. (2011). A chemical probe selectively inhibits G9a and GLP methyltransferase activity in cells. *Nat Chem Biol, 7*(8), 566-574. doi:10.1038/nchembio.599

Vial, T., Choquet-Kastylevsky, G., Liautard, C., & Descotes, J. (2000). Endocrine and neurological adverse effects of the therapeutic interferons. *Toxicology, 142*(3), 161-172.

Villeneuve, L. M., Reddy, M. A., Lanting, L. L., Wang, M., Meng, L., & Natarajan, R. (2008). Epigenetic histone H3 lysine 9 methylation in metabolic memory and inflammatory phenotype of vascular smooth muscle cells in diabetes. *Proc Natl Acad Sci U S A, 105*(26), 9047-9052. doi:10.1073/pnas.0803623105

Waddington, C. H. (1942). The epigenotype. *Endeavour*(1), 18-20.

Waddington, C. H. (2012). The epigenotype. 1942. *Int J Epidemiol, 41*(1), 10-13. doi:10.1093/ije/dyr184

Wang, Z., Zang, C., Rosenfeld, J. A., Schones, D. E., Barski, A., Cuddapah, S., . . . Zhao, K. (2008). Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet, 40*(7), 897-903. doi:10.1038/ng.154

LIST OF PUBLICATION AND PAPER PRESENTED

Part of this study has been accepted for presentation in:

Poster presentation:

Loh SW, Ng WL, Yeo KS, Lim YY, Ea CK. Inhibition of Euchromatic Histone Methyltransferase 1 and 2 Sensitizes Chronic Myeloid Leukemia Cells to Interferon Treatment. MSMBB. 1st – 3rd October 2014, Monash University Malaysia, Selangor, Malaysia. (National).

Part of this study has been accepted for publication in:

Publication:

Loh SW, Ng WL, Yeo KS, Lim YY, Ea CK (2014) Inhibition of Euchromatic Histone Methyltransferase 1 and 2 Sensitizes Chronic Myeloid Leukemia Cells to Interferon Treatment. PLoS ONE 9(7): e103915. doi: 10.1371/journal.pone.0103915