Review

Histone Methylases and Demethylases Regulating Antagonistic Methyl Marks: Changes Occurring in Cancer

Joyce Taylor-Papadimitriou * and Joy M. Burchell

School of Cancer and Pharmaceutical Sciences, King’s College London, London SE1 9RT, UK; joy.burchell@kcl.ac.uk
* Correspondence: joyce.taylor-papadimitriou@kcl.ac.uk

Abstract: Epigenetic regulation of gene expression is crucial to the determination of cell fate in development and differentiation, and the Polycomb (PcG) and Trithorax (TrxG) groups of proteins, acting antagonistically as complexes, play a major role in this regulation. Although originally identified in Drosophila, these complexes are conserved in evolution and the components are well defined in mammals. Each complex contains a protein with methylase activity (KMT), which can add methyl groups to a specific lysine in histone tails, histone 3 lysine 27 (H3K27), by PcG complexes, and H3K4 and H3K36 by TrxG complexes, creating transcriptionally repressive or active marks, respectively. Histone demethylases (KDMs), identified later, added a new dimension to histone methylation, and mutations or changes in levels of expression are seen in both methylases and demethylases and in components of the PcG and TrX complexes across a range of cancers. In this review, we focus on both methylases and demethylases governing the methylation state of the suppressive and active marks and consider their action and interaction in normal tissues and in cancer. A picture is emerging which indicates that the changes which occur in cancer during methylation of histone lysines can lead to repression of genes, including tumour suppressor genes, or to the activation of oncogenes. Methylases or demethylases, which are themselves tumour suppressors, are highly mutated. Novel targets for cancer therapy have been identified and a methylase (KMT6A/EZH2), which produces the repressive H3K27me3 mark, and a demethylase (KDM1A/LSD1), which demethylates the active H3K4me2 mark, are now under clinical evaluation.

Keywords: epigenetics; cancer; Polycomb; Trithorax; histone methylases; histone demethylases

1. Introduction

Epigenetic regulation of gene expression plays a crucial role in the determination of cell phenotype and the functions of the Polycomb (PcG) and Trithorax (TrxG) groups of proteins, acting antagonistically, are core to the determination of cell fate in development and differentiation. In 1947, working with Drosophila mutants, the first Polycomb gene (PC) was identified [1] and twenty years later the aberrant positioning of embryonic segments which were caused by mutations of PC were documented and related to the ectopic expression of Homeotic genes (Hox) [2]. Identification of other genes showing the same phenotype upon mutation led to the specification of the Polycomb group of proteins. Subsequently, Trithorax was identified as a gene which suppressed the changes induced by Polycomb [3], and identification of other genes with the same function as Trithorax defined the Trithorax group of proteins. It is now clear that the PcG and TrxG complexes, acting antagonistically, play a central role in sustaining a balanced state of gene expression in numerous cellular processes.

It was later found that these complexes contain histone methylases [4], which can repress or activate transcription depending on the specific lysine being methylated. Thus, trimethylation of lysine 27 on histone 3 (H3K27me3) by the Polycomb complexes can repress gene transcription, including that of Hox genes [5], while methylation by Trithorax complexes at
lysine 4 (H3K4me2/3) and lysine 36 (H3K36me2/3) can activate transcription [6–8]. The Polycomb/Trithorax system is evolutionarily conserved and the components of these complexes which act antagonistically in mammalian development and differentiation are now largely defined [9–11]. There are two main sub-groups of TrxG, which generally promote an active chromatin state, the histone lysine methylases which, when methylating K4, act as components of the COMPASS family of complexes, and the SWI/SNF family, which are involved in ATP-dependent chromatin remodelling [12]. While the original Trithorax complexes with their respective methylases methylate K4, the methylases which methylate K36, producing the K36me2/3 active marks also antagonise Polycomb and regulate Hox genes [7]. One of these methylases (ASH1L) has been formally recognised as Trithorax, acting in a complex [8]. We will therefore in this review include the methyl transferases, which add methyl groups to K36 on histone 3, as well as those which methylate H3K4 and H3K27.

Enzymes which demethylate methylated histones were discovered more recently, the first being KDM1/LSD1 (lysine demethylase 1) [13]. LSD1 and LSD2 are amine oxidases which can demethylate H3K4me1/me2, while the other demethylases identified later use the jmjC domain to catalyse demethylation [14]. Unlike some of the methylases, including the KMT2 and KMT6 enzymes, which add methyl groups to K4 and K27, respectively, the histone lysine demethylases (KDMs) do not operate from specific complexes but are recruited to chromatin in various ways [15–17]. With the exception of ASH1L, which does operate from a complex, this is also true for the methylases which methylate H3K36 to produce H3K36me2 and H3K36me3.

The PcG and TrxG complexes and their component histone methylases have been the subject of several reviews [9,10] and separate reviews covering the histone demethylases are also available [15–17]. Here we review both the methylases and demethylases and their interaction in governing the methylation state of the H3K27me3 suppressive mark, and of the methylated H3K4 and H3K36 active marks, and describe the changes occurring in these interactions in cancer. We also focus on any enzyme independent actions that are relevant to cancer (see Box 1). Incorporating the methylases and demethylases for H3K36 has allowed a discussion of the important interaction of the KDM2B demethylase with a Polycomb complex. Figure 1 shows the products synthesized by the KMTs and the substrate specificity of the KDMs acting on lysines 4, 27 and 36 on histone 3.

![Figure 1. Action of the methylases and demethylases involved in the creation and removal of K36, K27, and K4 methyl marks on histone 3. Names in brackets are alternative names for the indicated enzymes. * KMT2A (MLL1) trimethylates a set of genes including HOX genes; KMT2B (MLL2) adds methyl groups to bivalent promoters in ESC; KMT2C (MLL3) and D (MLL4) can also add the monomethyl mark to H3K4 on active distal enhancers.](image-url)
Box 1. Enzyme dependent and independent function.

The KMT and KDM proteins have other functional domains in addition to their catalytic function. For example, acting independently of its enzyme activity, KDM6A has been shown to recruit the KTM2D complex and a histone acetyl transferase to chromatin to establish active enhancers. Since some of these proteins are being considered as targets for cancer therapy and inhibitors under development largely target the enzyme activity, we also focus on the relevance of enzyme activity to the changes occurring in carcinogenesis [18].

2. Regulation of Methylation of Lysine 27 on Histone 3

2.1. The PRC1 and PRC2 Complexes Methylating H3K27

In mammals, there are two Polycomb complexes: PRC1 and PRC2 (Polycomb Repressive Complexes 1 and 2). However, within these classes are subclasses, which differ according to the profile of the component proteins and/or the proteins they associate with [10,19,20]. Both PRC1 and PRC2 are required for repressive function. The methylase (KMT6A/EZH2) found in PRC2 complexes generates H3K27me3, while PRC1 complexes ubiquitinate lysine 119 on histone 2A. The pathway used to recruit the two complexes can be different, where the first complex recruited can be a PRC1 or a PRC2 complex as shown in Figure 2 [10,19–21]. However, recruitment of either complex to chromatin is not achieved directly by the core components of the complexes, but by associated proteins which can bind to unmethylated CpG-rich sequences.

**PRC1 complexes**: There are multiple PRC1 complexes in mammals. They are broadly classified as canonical or non-canonical, although all the complexes contain either RING1A or RING1B, which ubiquitinate H2A histone (H2AK119ub1), and one of the six PCGF proteins (Polycomb group really interesting new gene (RING) finger 1–6 paralogues).

The canonical complexes contain one of the chromodomain-containing proteins (CBX 2, 4, 6, or 8), which bind to H3K27me3, while the non-canonical complexes contain either RYBP (RING 1/YY1 binding protein) or its homologue, YAF2. The canonical complexes are only associated with PCGF2 or 4 and can be recruited following production of H3K27me3 by PRC2.1 [10,19,21–23] and see Figure 2 left hand pathway.

Some non-canonical PRC1 complexes can be recruited directly to chromatin, depending on the associated proteins. The ncPRC1.1 complex, containing PCGF1 and the H3K36me2 histone demethylase KDM2B, is recruited globally to unmethylated CpG islands by KDM2B, through its CxxC-ZF domain, independently of its enzyme activity [24–28]. KDM2B is also required for ubiquitination by RING1B and recruitment of PRC2.2 (see below) depends on this ubiquitination by PRC1.1 (see Figure 2 right hand pathway) [21,26–32]. PRC2.2 produces the H3K27me mark recognised by the CBX protein in a cPRC1 complex, which can be recruited to enhance ubiquitination [20,21,32].

**PRC2 complexes**: The KMT6A/EZH2 and KMT6B/EZH1 (enhancer of zeste homologues 1 and 2) methylases, found in PRC2 complexes, add the trimethyl group to lysine 27 on histone 3, creating the H3K27me3 suppressive mark [5]. In the complete PRC2 complexes, EZH2 can methylate H3K27 effectively and is expressed in the developing embryo and in proliferating cells, while the EZH1 PRC2 complex has low levels of methylase activity and is present in non-dividing cells in the adult [19]. However, the domain structure of the two KMT6 methylases is similar, and similar to all the methylases they include the catalytic SET domain (Su(var)3-9, Enhancer-of-zeste and Trithorax).

The SUZ12, EED, and EZH2 core components are present in stoichiometric amounts in the PRC2 complexes [19–21] and this core complex associates with one or more of several non-core components in sub-stoichiometric amounts to form complexes classified as PRC2.1 or PRC2.2 [21,33–35]. PRC2.1 complexes, through an interacting PCL protein, can be recruited to unmethylated CpG islands, thus initiating the pathway illustrated in Figure 2 (left hand pathway) which recruits cPRC1. The binding of the ncPRC1 complex described above recruits PRC2.2 complexes (Figure 2, right hand lane).

PRC2.1 and PRC2.2 are found together globally at the same genes, suggesting that the two recruitment pathways are coordinated.
Figure 2. Formation of the K27me3 and H2AK119ub1 marks by components of the Polycomb complexes. Polycomb complexes cooperate to form the H3K27me3 and H2A119ub1 marks. (Left-hand side) PRC2.1 is recruited to chromatin by PCL1-3 binding to unmethylated CpGs, allowing EZH2 (KMT6A) to methylate H3K27. cPRC1 is then recruited via CBX binding to the H3K27me3 mark, allowing H2AK119 ubiquitination via the E3 ligase RING1A/B. (Right-hand side) The ncPRC1.1 complex is recruited to chromatin by KDM2B binding to unmethylated CpGs, allowing ubiquitin to be deposited on H2A119 by RING1A/B. This allows PRC2.2 to be recruited via Jarid1B and AEBP2 and the formation of the H3K27me3 mark by EZH2.
2.2. Demethylation of H3K27me3

The two histone demethylases KDM6A/UTX (ubiquitously transcribed tetratricopeptide repeat, X chromosome) and KDM6B/JMJD3 (Jumonji Domain Containing 3) catalyse the demethylation of the repressive H3K27me3 mark, and both proteins play an important role in embryonic development and in regulation of Hox genes [36]. However, some functions operative in the early embryo are independent of the demethylase activity of these KDMs [37].

**KDM6A/UTX and UTY**: The gene coding for KDM6A is on the X chromosome and not subject to gene silencing. A gene on chromosome Y (UTY) codes for a protein which is similar to UTX but, because of mutations in the JmjC domain, it has no demethylase activity. Homozygous knock out of UTX leads to embryonic lethality in females, but males survive to term, indicating that a demethylase-independent function of UTY (probably also carried out by UTX) is important for embryonic development and survival [37]. As stated earlier, KDM6A/UTX has been studied as a component of the KTM2C and KTM2D trithorax complexes, playing a major role in establishing active enhancers carrying the H3K4me1 and H3K27Ac marks independently of its enzyme activity [18].

3. K27 Methylases and Demethylases in Cancer

3.1. The EZH2 Methylase: Expression, Mutations, and a Therapeutic Target

Both increases and decreases in H3K27 methylation are found in cancer, and EZH2 can act as an activator of transcription independently of Polycomb (see Figure 3).

**Figure 3.** Involvement of EZH2 and LSD1 in cancer and their use as potential therapeutic targets. (a) Involvement of EZH2 in cancer. (b) Involvement of LSD1 in cancer. Blue boxes indicate the different ways EZH2 or LSD1 are implicated in cancer; green boxes, potential targeting approaches.

* Governed by methylase dependent and independent pathways in CRPC.

Activating mutations in the SET domain of EZH2 (KMT6A) are seen in some lymphomas (see Table 1) and levels of EZH2 are increased in many carcinomas, including breast and prostate, and in multiple myeloma, where high expression is associated with poor prognosis [38–40].

The effects of loss-of-function mutations on other epigenetic factors which oppose EZH2 activity can also result in increased function of EZH2. Inactivating mutations affecting the enzyme activity of KDM6A/UTX [41], or of components of the SWI/SNF Trithorax complexes, which normally oppose Polycomb activity, are very common, and these mutations can confer sensitivity to EZH2 therapy ([42–44], see Figure 3). The action
of the SWI/SNF mutations in cancers relies on both methylase-dependent and independent effects, and the independent effects may involve stabilisation of the PRC2 complex [43].

Inactivating mutations of EZH2 are seen in myeloid disorders [45,46], while in 25% of T-ALL (T cell Acute Lymphoblastic Leukaemia), inactivating mutations are found, not only in EZH2, but also in SUZ12 [47]. These mutations are accompanied by activating mutations of NOTCH1, which opposes EZH2, indicating a Notch:PRC2 pathway interaction in tumorigenesis in TLL.

That EZH2 can act as an oncogene or as a tumour suppressor is seen in AML, where EZH2 expression is required for maintenance of the disease but acts as a tumour suppressor at the induction stage [48,49].

EZH2 acting independently of the Polycomb complex; EZH2 can activate expression of the androgen receptor (AR) independently of the PRC2 complex in methylase-dependent and independent ways and thus increases the expression of AR target genes ([50,51] see Figure 3).

Inhibitors of EZH2: Small molecular weight inhibitors of the EZH2 methylase activity have been developed and many Phase I/II trials are now ongoing to evaluate their efficacy against solid cancers, including castrate-resistant prostate cancer (in combination with an inhibitor of AR signaling), as well as cancers of the hematopoietic system. The inhibitor Tazemetostat has been most widely used and has FDA approval for treatment of patients with two types of follicular lymphomas, which can show activating mutations of EZH2 [52,53].

Table 1. Mutations and translocations of methylases and demethylases in cancer.

| Type of Mutation or Translocation | Gene                  | Cancer Type                                      | References            |
|----------------------------------|-----------------------|-------------------------------------------------|-----------------------|
| **Inactivating**                 | **Commonly found and high in bladder cancer** | **UTX/KDM6A** Affecting H3K27 methylation and activation of enhancers | Common in T-ALL [41] |
|                                  | **KMT2C and KMT2D** Affecting H3K4 mono-methylation at enhancers. | Bladder cancer ccRenal Carcinomas non-Hodgkin’s lymphoma neuro-ectodermal tumours | [54–56] |
|                                  | **SETD2/KMT3A** Inhibiting synthesis of H3K36me3 and therefore DNA damage response | ccRenal Carcinoma 32% Entero-pathy-associated T cell lymphoma (EATL) | [47,54,57–59] |
|                                  | **EZH2/KMT6A** Affects H3K27 methylation | Acute T cell leukaemia and myeloid disorders | [40,45–47] |
| **Activating mutations**         | **EZH2/KMT6A** Enhancing EZH2 function | Lymphomas | [48] |
|                                  | **NSD2/KMT3B** p.E1099K Increased enzyme activity | 10% of all ALL and especially paediatric B-ALL | [60,61] |
| **Duplication**                  | **MLL1/KMT2A** partial tandem duplication (PTD) of exons 5 to 11 | 4–7% of AML | [62] |
| **Translocations making fusion proteins** | **KMT2A-with 3' domain from multiple genes including SEC components** | 70% of infant leukaemia (ALL and AML) | [63,64] |
| Found in specific cancers        | **NUP98-NSD1/KMT3B** | 5% of AML | [65] |
|                                  | **NUP98-NSD3/KMT3F** | AML (rare) | [65] |
|                                  | **NSD2 placed under the control of the strong IGH intronic Em enhancer t** | 15–20% Multiple myeloma 5–10% Paediatric ALL | [58,60,66] |
|                                  | **SX18/SSX** | 90% Synovial sarcomas | [67] |
| **Mutations in non-canonical histones** | **H3F3A** K27K to M G34 to R/V/D G34 to W/V | Childhood glioblastomas | [68–71] |
| Found in specific cancers        | **H3F3B** K36 to Methionine | >90% Giant Cell tumours | |
Inhibitors destabilizing PRC2 are also available and cell lines harboring a SWI/SNF mutation, which were not sensitive to inhibition by an EZH2 methylase inhibitor, were inhibited by a compound that blocks the EZH2 interaction with EED (see Figures 2 and 3 and Box 2), and results in breakdown of the complex [48]. Therefore, demethylase-independent functions of EZH2 also contribute to its oncogenic effect. Moreover, EZH2, and EED interact directly with DNA methylases [72] and with HDACs [73], and combined targeting of EZH2 and HDACs has been recommended.

Interaction of EZH2 with HOTAIR: The EZH2 Polycomb complex can interact with the long non-coding RNA HOTAIR, which is highly expressed in breast cancer metastases, and a small molecular weight inhibitor of this interaction has been developed [74].

3.2. Mutations in Cancer Converting H3K27 to Methionine

Mutations occur in cancer in the non-canonical histone genes H3F3A or H3F3B, coding for histones 3.3 A and B [68], which are recruited to chromatin independently of mitosis by the ATRX/DAXX complex. The most striking mutation affecting Polycomb function is the change of lysine 27 in histone H3F3A to methionine, and is seen in 70% of paediatric glioblastomas ([69,70] and see Table 1). Moreover, although the mutation occurs in only one allele of H3F3A, the methylase activity of EZH2 is globally inhibited, resulting in considerably reduced levels of H3K27me3 [71]. However, the K27 methylase activity (although reduced) is required by the cancer cells to inhibit neural differentiation and allow proliferation. Therefore, EZH2 has been proposed as a target for therapy of these cancers [75].

3.3. KDM6A and KDM6B Demethylases in Cancer

The demethylase KDM6A/UTX is frequently mutated in cancer [41] and these mutations are not confined to the JmJC domain, so that demethylase-independent effects are possible (Tables 1 and 2). In such examples, UTY could be functional, but UTY mutations can also occur, and complete loss of the Y chromosome is not uncommon in males with a KDM6A mutation in cancer [41]. Where the mutation affects the demethylase activity of UTX, gender specific effects are seen.

T Cell Acute Lymphoblastic Leukaemia (T-ALL) and Acute Myeloid Leukaemia (AML): The role of both demethylases has been widely studied in T-ALL leukaemia, where they play opposing roles: KDM6A (UTX) acts as a tumour repressor, and mutations of KDM6A are seen dominantly in male patients, where there is a gender bias of 3:1 (male:female) in development of T-ALL (see Table 1). Moreover, ablation of KDM6A can lead to development of AML in specific mouse models [76]. KDM6B, on the other hand, interacts with NOTCH (which presents with activating mutations in T-ALL) in co-activating target genes, including oncogenes. Inhibition of KDM6B demethylase activity has been suggested as a possible therapy for this type of leukaemia [77] and the BET inhibitors, which inhibit myc expression, could be effective (see later section on K4 methylation).

Multiple myeloma (MM): In MM, loss of KDM6A resulted in increased proliferation, expression of c-myc, and loss of expression of E-cadherin [44]. Inhibition of EZH2 was effective in reversing these effects, suggesting that loss of UTX leads to abnormal PRC2-mediated repression, and that inhibition of EZH2 could be effective in MM.

Carcinomas: Work with pancreatic cancer (PANCS) supports the concept that KDM6A normally acts as a tumour suppressor, since ablation of KDM6A can lead to development of PANCS in a mouse model, and in patients, oncogenes are activated in females and in males with UTY loss [78]. In breast cancer, KDM6A is reported to inhibit the epithelial-to-mesenchymal transition (EMT) by silencing the expression of EMT transcription factors in collaboration with LSD1 and HDAC1 [79].
Box 2. Targeting EZH2 in cancer.

- Clinical trials targeting EZH2 methylase (KMT6A) activity for cancer therapy are ongoing. Pre-clinical studies indicate that inhibiting interactions of components of PRC2 to destabilise the complex could provide an alternative targeting strategy.
- The mutation of lysine 27 to methionine in a non-canonical histone is seen in 70% of paediatric gliomas and inhibitors of EZH2 or BET proteins are therapeutically options.
- The main body of data indicates that the KDM6A/UTX demethylase protein acts as a tumour suppressor, and that tumours lacking or carrying mutations in KDM6A may, in some cancers, be targeted with either EZH2 or BET inhibitors.

Table 2. Methylases and demethylases: functions independent of the enzyme activity of the two classes of enzymes.

| Functions in Normal Mouse Embryo Development and Viability Independent of Enzyme Activity | References | Enzyme Independent Effects in Cancer | References |
|---|---|---|---|
| KMT2A/MLL1 | [80] | EZH2/KMT6A | [50] |
| Progeny from mating of KMT2A heterozygous methylase null mice are produced at the expected Mendelian ratios. | Can activate transcription of genes targeted by the androgen receptor (AR) independently of the Polycomb complex which is required for methylase activity (Figure 3). |
| KDM6A(UTX) and UTY | [37] | LSD1/KDM1A | [81,82] |
| Male mice mutant for UTX can survive with UTY even though it has no demethylase activity. | Interaction of LSD1 with transcription factors activates expression of a network of genes favouring growth of CRPC prostate cancers (Figure 3). |
| KDM2B | [25,26] | KDM2B | [67] [83,84] |
| The ncPRC1.1 complex is recruited to chromatin in mouse ESCs by KDM2B through its CxxC-zinc finger (CxxC-ZF) domain, independently of its demethylase activity. | Synovial Cancer: KDM2B is required for proliferation independently of demethylase activity (>90% of these cancers). Pancreatic Cancer: KDM2B can activate genes involved in ribosomal and mitochondrial function. |
| KDM5B | [85,86] | KDM6A/UTX | [41] |
| The ∆ARID mice expressing KDM5B with no demethylase activity are viable and fertile. | Mutations in domains other than the SET domain are seen in cancer suggesting a role of these domains in the tumour suppressor function. |

4. Regulation of Methylation of Lysine 36 on Histone 3

4.1. H3K36 Methylation: Methylases Associated with SET2 Domain

Methylation of H3K36 opposes Polycomb repression [87–89]. In mammals, there are several enzymes that can dimethylate H3K36 but only one (KMT3A/SETD2) can add the third methyl group, even though all contain the SET2 domain originally identified in the yeast SET2 methylase [90].

Trimethylation of H3K36: SETD2/KMT3A is the only enzyme which can trimethylate H3K36 [90,91] and requires the H3K36me2 substrate formed by the dimethylases [92–94]. H3K36me3 regulates homologous recombination (HR), non-homologous end joining (NHEJ), as well as mismatch repair (MMR) [95]. Therefore, by producing the H3K36me3 mark, SETD2 protects the DNA damage response (DDR) and also regulates transcriptional elongation and splicing ([96] and see below).

Enzymes dimethylating H3K36: The NSD (nuclear receptor SET domain-containing) family of enzymes (NSD1 (KMT3B), NSD2 (KMT3G/F), and NSD3 (KMT3G/F)) are largely
responsible for the global dimethylation of H3K36, see Figure 1. The NSD1 protein (KMT3B), has been widely studied [92] and is required for embryonic development. Mutations in NSD1 are responsible for the childhood overgrowth condition known as Sotos syndrome and for some cases of Weaver syndrome [97,98]. Where the NSD proteins have been studied in disease, no reference is given to them being members of a specific complex, but these proteins do regulate Hox genes [89]. While the ASH1L (KMT2H) methylase, which does operate in a trithorax complex, also dimethylates K36, it is more selective than the NSD methylases, but the genes targeted include Hox genes [99,100].

Methylation of H3K36 and transcriptional elongation: In mammals, transcriptional elongation is accompanied by H3K36me3 deposition by SETD2 (KMT3A) towards the 3' end of transcription. The H3K36me3 mark recruits the Dnmt3b DNA methylase, which protects the gene body from cryptic transcription initiation [101]. Thus, the trimethylation of H3K36 ensures the fidelity of gene transcription initiation, as well as protects the function of the DDR [90,101].

4.2. Demethylation of Methylated H3K36

The two demethylase families which remove methyl groups from methylated H3K36 are KDM2 and KDM4.

KDM2A and KDM2B demethylases active on H3K36me2: KDM2A/(FBXL11) and KDM2B (FBXL10) specifically demethylate H3K36me2 and H3K36me1. KDM2A was the first Jumonji domain containing demethylase to be isolated and characterised [14], and KDM2B has been clearly shown to have the same activity by the Zhang laboratory [24]. Genetic ablation of KDM2B in mice results in early embryonic lethality, and the biological effects of KDM2B have been widely studied in mouse embryo fibroblasts (MEFs), and in mouse embryo stem cells (mESCs) [102,103]. Over expression of KDM2B results in immortalisation of MEFs, by repressing the Ink4a/Arf locus [24,104,105] and this effect is dependent on the demethylase activity.

KDM2B and the Polycomb complexes: As a component of the PRC1.1 Polycomb complex, KDM2B recruits the complex to unmethylated CpGs and through ubiquitination of histone 2A (H2AK119ub1), PRC2.2 is recruited. Studies with mESCs show that both PRC1 and PRC2 complexes can interact with KDM2B. However, while the actual recruitment of the PRC1.1 complex to chromatin by KDM2B is not dependent on the demethylase activity, the enzyme activity could be involved in other functions stimulated by these interactions. (See section on Polycomb complexes and Figure 2).

The KDM4 demethylases active on H3K36me3: The KDM4 demethylases KDM4A, B and C demethylate H3K36me3 but not H3K4me2, and can also demethylate H3K9me2/me3 [106,107]. The JmjN and JmjC domains are separated in the gene, but these domains lie adjacent in the protein and both domains are required for catalysis [108]. There appear to be no important functional domains in the sequence between the JmjN and C domains, in contrast to the KDM5 proteins, where functional domains are found to be separating JmjN and JmjC (see below). There is some functional redundancy in the KDM4A/KDM4C demethylases, as shown by KO experiments in mice [109].

5. Changes in Cancer Related to H3K36 Methylases

5.1. SETD2 (KMT3A): A Tumour Suppressor

Enteropathy-associated T cell lymphoma (EATL) is an aggressive, often lethal cancer, which can arise from celiac disease, and mutations in SETD2 are found in 32% of cases [57], while in clear cell renal carcinomas (ccRCC), deletions or mutations of SETD2 are also found [54,110,111]. Reduced levels of SETD2 are also seen in breast cancers and relate to poor prognosis [112]. The fact that SETD2 binds to p53 and is involved in p53 regulation of its downstream genes could relate to the function of SETD2 as a tumour suppressor [113].
5.2. Translocations and Mutations of NSD (KMT3B, G, F) Methylases

There are many reports of changes in the expression of the NSD methylases in a range of cancers, including lung, prostate, and breast cancer [65,66]. However, mutations and translocations are found in cancers deriving from haemopoietic cells. 

The E1099K mutations: In 10% of ALL, and especially in paediatric B-ALL (B-precursor ALL), a mutation of glutamic acid to lysine occurs in the SET domain of NSD2, resulting in increased activity of the enzyme, leading to increased levels of H3K36me2 and decreased levels of H3K27 trimethylation [60,61], see Table 1.

Translocations of the NSD methylases: In 15–20% of multiple myeloma patients, a t(4:14) translocation places NSD2 under the control of the strong IGH enhancer (see Table 1), and this translocation is also seen in ALL [60]. Through increased activity of the NSD2 methylase, this results in the loss of gene-specific H3K36me2 modifications, and this disruption of organised H3K36me2 marks leads to the expression of genes driving the oncogenic programme [58,66]. In addition, some AML patients exhibit translocations of NSD1 (5–10%) or NSD3 (rare) [t(5;11)(q35;p15.5)], which lead to the production of a fusion protein (see Table 1). The 5′ component is taken from NUP98 (nucleoporin 98), which can bind to a histone acetylase (CBP/300) and is fused to a carboxy sequence from NSD1 or NSD3, which retains the dimethylase active domain [114,115]. The expression of the NUP98-NSD1 fusion protein in myeloid stem cells leads to their continued proliferation, inhibition of differentiation, and to the expression of HOXA genes (A7, A9, A10) which are normally repressed by Polycomb [89].

5.3. ASH1L in Leukaemia

The synergistic action of two methylases: KMT2A/MLL1 (methylates H3K4, see next section) and ASH1L (KMT2H), is associated with leukemogenesis and with regulation of HOX gene expression in leukaemias with KMT2A translocations [116]. Therefore, ASH1L has been proposed as a possible therapeutic target in leukaemia. Very recently a small molecular weight inhibitor has been developed which not only effectively inhibits the growth of cells from leukaemias with a translocation of KMT2A but also reduces the expression of the fusion protein target genes [117]. ASH1L operates as a component of a Trithorax complex (currently termed hAMC or dAMC), and another component of AMC, the MRG15 protein, binds to ASH1L and liberates an auto-inhibitory loop to activate the enzymic activity of the SET domain [118,119]. The new inhibitor binds next to the auto-inhibitory loop and blocks the activation of enzymic activity. This will be very useful for studying the specific functions of ASH1L as it shows >100 selectivity for ASH1L when compared to the other H3K36 dimethylases.

5.4. Mutations in Non-Canonical Histones Affecting K36 Methylation

As previously discussed, mutations in H3F3A and H3F3B (coding for H3.3A or H3.3B) occur in cancer and mutations which affect H3K36 methylation are found in childhood glioblastomas, and in giant cell tumours and chondroblastomas, both of which develop in young adults (see Table 1).

Mutations in Glycine 34: In childhood glioblastomas, the mutations of glycine 34 in H3.3A (G34/RVD) [68,69], replacing glycine with an amino acid with extended side chains, inhibits the action of SETD2, and the lack of H3K36me3 results in the blocking of mismatched repair (MMR), leading to genome instability [120,121]. Moreover, the mutated histone inhibits the function of KDM4 demethylases [122]. Expressing the G/R mutant in mouse ES cells results in changes in chromatin and the transcriptome, which mimic the triple KO of the three KDM4 proteins, indicating a global effect of this mutation in one allele of the H3f3a histone.

Mutations of glycine 34 in H3.3A, affecting H3K36 methylation, are also found in over 90% of giant cell tumours, a rare tumour of osteoblast lineage where progression to malignancy is uncommon [123].
The mutation of H3K36 to methionine: In chondroblastomas, a rare bone tumour developing in the second decade of life, the driver mutation converting lysine 36 to methionine in histone 3.3B has been reported in 95% of cases [123]. The K36M mutation leads to a global inhibition of the methylation of H3K36, loss of H3K36me3, and a gain and redistribution of K27me3, blocking differentiation of mesenchymal progenitor cells [124]. It is proposed that the mutations affecting methylation of H3K36 lead to increased activity of PRC2 in silencing genes required for cell differentiation.

The mutations which result in inhibition of the function of the tumour suppressor SETD2 (KMT3A), thus inhibiting the production of the H3K36me3 mark, block DNA repair and create genomic instability. Inactivating mutations in ccRCC are also found in two histone demethylases known to be tumour suppressors: KDM5C (demethylates H3K4me3) and KDM6A /UTX [54]. The data illustrate the prevalence of mutations of tumour suppressors in cancer, as well as the interplay of the histone methylases and demethylases.

6. Changes in Cancer Related to H3K36 Demethylases

6.1. KDM2B Over-Expression in Cancer

This demethylase is highly expressed in lymphocytic leukaemia and in several carcinomas, including pancreatic, ovarian and breast, as well as in gliomas and synovial sarcomas [83,125].

Acute Lymphoblastic Leukaemia (AML): As seen in normal mouse and human haemopoietic stem and progenitor cells (HSPC), KDM2B is highly expressed in lymphoblastic leukaemic stem cells, and is required for their maintenance. Moreover, ectopic expression of KDM2B can transform HSPCs. Expression of KDM2B also enhances lineage commitment to T-ALL [103,126] where, working with Polycomb EZH2, developmental genes are repressed, through silencing of p15\(^{ink4b}\) [126].

Pancreatic cancer: High expression of KDM2B is seen in poorly differentiated pancreatic cancers (PANCS) and their metastases. Genomic analysis documenting genes affected by KDM2B expression and co-occupancy of promoters shows that KDM2B is associated with Polycomb in suppressing lineage-specific genes, and repression depends on the demethylase activity. In parallel, KDM2B, in association with KDM5A (demethylates H3K4me2/me3 see next section) and Myc, activates expression of genes involved in ribosomal and mitochondrial function [84]. The activation of genes by KDM2B is demethylase-independent (see Table 2).

Glioblastoma (GBM): KDM2B plays a major role in the maintenance of glioblastoma cancer stem cells (GSCs) and knockdown of KDM2B induces apoptosis and DNA damage [127]. The inhibitor GSK-J4 was found to decrease glioblastoma cell viability, as well as the self-renewal capacity of GSCs, and was said to be specific for KDM6A and B. However, it was later found not to be specific for demethylation of H3K27 [128]. Some of the inhibitory effect can be attributed to inhibition of the demethylase activity of KDM2B, since in GSK-J4 treated cells, levels of KDM2B were decreased and levels of H3K36me2, increased.

Synovial sarcoma: Synovial sarcomas are aggressive soft tissue sarcomas which affect children and young adults. They are often unresponsive to chemotherapy and can be lethal. Virtually 100% of synovial sarcomas express a fusion protein SX18/SSX. SS18 is a member of the human SWI/SNF chromatin remodeling complex and the SSX1, 2, and 4 proteins are transcriptional repressors interacting with Polycomb. KDM2B is required for proliferation of synovial sarcoma cells and recruits the fusion protein to chromatin [67], an action independent of its demethylase activity, to activate an aberrant differentiation programme normally subject to Polycomb repression (see Tables 1 and 2).

6.2. KDM4 A–C Proteins Demethylating H3K36me3

Over expression of one or more of the KDM4 demethylases are found in many carcinomas, in gliomas, and in head and neck cancers [106]. Since the H3K36me3 mark plays multiple roles affecting the chromatin state and gene expression, therapeutic targeting of a specific H3K36me3 demethylase, which removes this mark, has been recommended for
treatment of AML [129–131]. However, if the inhibitors target the demethylase activity, they would inhibit all the KDM4 proteins including those which only demethylate H3K9, and targeting demethylation of H3K9me2/3, as well as H3K36me3, could lead to side-effects in clinical application.

There are considerable data indicating that the KDM2B protein plays an important role in oncogenesis in a wide range of cancers, through maintenance of stem cell properties, inhibition of differentiation pathways and activation of oncogenic pathways. Suppression of developmental pathways appears to be dependent on the function of demethylase activity, while activation of oncogenic pathways can be independent of this activity.

7. Regulation of Methylation of Lysine 4 on Histone 3

There are many reviews on the enzymes that methylate and demethylate H3K4 and the reader is directed to references [9,10,15,16,55,132] as examples. Here we focus on the more recent developments and the association of changes in H3K4 methylation with cancer.

7.1. Methylases in COMPASS Complexes (Complex Proteins Associated with SET1)

COMPASS complexes methylate H3K4 and have evolved from the SET1 complex in yeast, to six complexes found in mammals [133]. The six methylases in humans are designated KMT2A, B, C, D, F, and G and have been also referred to as MLL1-4 (KMT2A-D) and SET1A and SET1B (KMT2F and G), see Figure 1. This nomenclature arose as the MLL1 protein (Mixed Lineage Leukaemia protein1), which undergoes translocation to form fusion proteins in a group of leukaemias, was found to be an analogue of Drosophila Trithorax (Trx) [134]. In the literature, the nomenclature for MLL2 and MLL4 has been used interchangeably and it is important to look for gene details and use the KMT nomenclature [55].

While all six methylases can methylate H3K4, different functions have been assigned to the different methylases [18,135,136], see Figure 1. Homozygous deletion of any one of the KMT2 methylases in mice results in embryonic lethality, each showing unique defects in embryonic development [11]. However, transgenic mice homozygous for expression of a methylase null mutant of the Kmt2a gene are viable, with some defects in formation of the spinal column and in Hox gene expression. Evidently, functions other than the catalytic SET domain of KMT2A/MLL1 are also important for embryonic development ([80] and see Table 2).

7.2. The KDM1 and KDM5 Demethylases Active on Methylated H3K4

As with the demethylation of methylated H3K36, there are two families of demethylases which demethylate methylated H3K4 (see Figure 1). The two enzymes of the KDM1 family (KDM1A/LSD1 and KDM1B/LSD2) remove methyl groups from dimethylated and monomethylated H3K4 and the four KDM5 proteins (KDM5A, KDM5B, KDM5C, and KDM5D) demethylate H3K4me3 and H3K4me2. KDM5A and KDM5B can also demethylate H3K4me1 as determined by knock down studies and immunohistochemistry of transfected stained cells [137–139]. Although primarily demethylating H3K4me1/me2, LSD1 was reported to demethylate the repressive methylated H3K9mark in prostate cancer and thus enhance the function of the androgen receptor (AR) [140].

LSD1 (KDM1A) and LSD2 (KDM1B): LSD1 was the first histone demethylase to be identified, acting as a component of the CoREST histone deacetylase repressor complex [13]. LSD1 is also found in the NURD repressive complex (Mi-2/nucleosome remodelling and deacetylase complex) [141]. LSD2 is a homologue of LSD1 but is structurally very different and associates with different factors or complexes [142].

Expression of LSD1 is high in mouse and human ESCs, where it contributes to the maintenance of pluripotency [143,144]. Indeed, LSD1 is essential for development, and knockouts in mice are early embryonic lethals. Through its involvement with the CoREST complex, LSD1 can act as a transcriptional repressor and, functioning within the NURD complex, LSD1 can decommission enhancers of pluripotent genes during differentiation of mESCs [145]. Interaction with other components of the CoREST complex are required
for LSD1 demethylase activity. There is an extensive literature covering the structure and biological functions of LSD1 and the reader is referred to a recent comprehensive review on this subject [146].

The KDM5 demethylases: The four KDM5 demethylases, KDM5A/B/C/D, can demethylate H3K4me3 and H3K4me2 in vitro assays and KDM5A and KDM5B (but not KDM5C and D) can also demethylate H3K4me1 in transfected cells [137–139,147–153]. While demethylation of H3K4me3 at promoters is involved in transcriptional repression, KDM5A and B could decommission enhancers by removing the H3K4me1 mark, and this has been confirmed for KDM5B acting on the FGFR4 enhancer [154].

The catalytically active domain of KDM5 demethylases is divided into the JmjN and JmjC domains and sequences separating the two domains code for the ARID/BRIGHT DNA binding domain [155,156] and the first PHD domain (PHD1), which binds to unmethylated H3K4 [157–159]. Approaches using mutational analysis or specifically deleting the ARID and/or the PHD domains have given conflicting results regarding the requirement of the ARID domain for demethylase activity [137–139,160]. However, there is agreement that the PHD1 domain can influence the re-modelling of the catalytic core by binding to H3K4me0 [15,19,161]. Our own data from the ∆ARID mouse expressing KDM5B with a deletion of the ARID domain, together with five amino acids from the JmjN domain (but with the PHD1 intact), show that this mutant has lost demethylase activity [85,86]. However, the fact that the ∆ARID mouse is viable and fertile indicates that domains other than the demethylase activity are important in development and viability (See Table 2 and below).

Effects of KDM5 mutations affecting neural activity: Mutations of KDM5 proteins are associated with intellectual disability and autism [151,153,162]. While KDM5B is found on chromosome 1 and KDM5A on chromosome 12, the KDM5C gene escapes silencing on the X chromosome and KDM5D is located on the Y chromosome. There has been some divergence of functions between KDM5C and KDM5D, but both proteins express KDM5 demethylase activity [163] and also play a role in cardiac function [164]. However, human males with mutations in KDM5C show cognitive abnormalities (X-linked intellectual disability: XLID) [153]. Clearly, the role of KDM5C in neural function is not rescued by WT KDM5D, even though it has demethylase activity [163]. In a mouse model expressing a demethylase null KDM5C protein, males show the behavioral defects seen in human males with XLID [152], suggesting that the demethylase activity of KDM5C plays a role in neural development. Furthermore, the dominance of mutations in the catalytic JmjC domain of KDM5B found in patients with ID also suggests that the KDM5 demethylase activity plays an important role in neural function [165]. However, both demethylase-dependent and -independent functions of KDM5 proteins may be involved in neural activity. It is important to ascertain the relevance of the demethylase activity of KDM5 proteins to neural function in the context of the development of inhibitors of this activity for potential therapeutic use.

Knocking out individual KDM5 genes in mice has allowed their function to be investigated, but their effects on cognitive functions have only recently been examined. The first KO of KDM5A was reported to have only minor changes in phenotype [148] but a more recent KDM5A KO was found to have cognitive and physical disabilities [162]. Different phenotypes have been reported for KO of KDM5B in C57Bl/6 with Catchpole et al. reporting early embryonic lethality [85], and Albert et al. finding perinatal death due to respiratory failure, resulting from neurological abnormalities [166]. Since the ∆ARID mouse, expressing a demethylase null mutant of KDM5B, is viable and fertile [85,86], it follows that functions other than demethylase activity are important in development. The ∆ARID mouse provides an appropriate model for examining the effect of the loss of KDM5B enzyme activity on neural activity and behaviour.

KDM5 protein interactions with repressor complexes: Many studies have focused on the demethylase activity of the KDM5 enzymes, acting as transcriptional repressors, but other domains of the KDM5 and components of the complexes can be involved in this activity. KDM5B binds to HDAC1 and to Class II HDACs and the PHD1 and PHD2 do-
mains in KDM5B are crucial for binding to HDAC4 [167]. Through binding to HDAC1, KDM5B binds to the repressive LSD1/NURD deacetylase complex and shows co-occupancy with the NuRD complex on chromatin [159,168]. KDM5A and KDM5C also bind to HDAC-containing complexes [169,170] and the activities of demethylation and acetylation are interlinked [171]. The combination of LSD1 and KDM5 proteins ensures the complete demethylation of H3K4 and, coupled with HDACs, this provides a very strong repressive signal.

The KDM5 demethylases can also bind to PRC2, suggesting coordinated removal of an active mark (H3K4me3/2) with the repressive action of Polycomb. In differentiation of mouse ES cells, interaction of the PRC2 complex with the KDM5A protein is required for repression of a significant number of Polycomb target genes [172] and a similar interaction of KDM5B with PRC2 is required to repress retinoic acid receptor target genes in the absence of retinoic acid [173].

8. Changes Associated with H3K4 Methylation in Cancer

Of the six Compass complexes that can methylate H3K4, KMT2A/MLL1, KMT2C, and KMT2D (MLL3 and 4) have been most widely implicated in cancer.

8.1. KMT2A/MLL1 and Development of BET Inhibitors

Largely due to the discovery of the translocations of KMT2A-producing fusion proteins in 70% of infant leukemias and 10% of adult leukemias [63,64], concentrated efforts have been applied to understanding the function of KMT2A in leukemogenesis. More than 80 partners have been identified as being linked in frame to a 5′ sequence of one allele of WT KMT2A to produce the oncogenic fusion proteins, designated as such by their ability to target the promoters of some oncogenes, as well as HOX genes [80,174,175]. The 5′ KMT2A sequence found in fusion proteins does not contain the catalytic SET domain but retains the ability to interact with PAFc (polymerase associated factor c) [176]. Importantly, most of the partners forming the 3′ end of the fusion proteins are components of or interact with components of the SEC complex (super-elongation complex) which contains pTEFb (positive elongation factor b).

The BET proteins (bromodomain and extra terminal BRD2,3 4), which bind to acetylated histones, also interact with PAFc and pTEFb and can recruit the fusion proteins to acetylated chromatin. BET inhibitors are now being evaluated for cancer therapy, not just in leukaemia, but for some solid cancers, in prostate cancer with androgen deprivation therapy, and in breast cancer, with fulvestrant hormone therapy [52]. Resistance can, however, develop to BET inhibitors in leukaemia patients [177] and other targets for drug development in leukaemia [174,178] are under investigation. The 5′ domain of KMT2A in fusion proteins retains the DNA binding site for menin and the leukemogenic activity of the fusion proteins is dependent on this interaction for recruitment to chromatin [63]. Moreover, menin is involved in the recruitment of the androgen receptor (AR) to target genes. Therefore, inhibitors of the menin interaction are being considered for therapy in leukaemic patients with KMT2A translocations and for patients with castration-resistant prostate cancer [52]. In the absence of translocations, in-frame partial tandem duplication (PTD) of exons 5 through to 11 occur in 4–7% of patients with AML [62], see Table 1.

8.2. Mutations in KMT2C and KMT2D

KMT2C (MLL3) and KMT2D (MLL4) are among the most frequently mutated genes in cancer and the mutations inactivate function [56]. KMT2C mutations are found in common carcinomas with mutations in bladder cancer being over 20%. KMT2D mutations are also found in carcinomas, as well as in non-Hodgkin’s lymphoma and some neuro-ectodermal tumours [55]. Pre-clinical studies have provided data demonstrating the important role these methylases play as tumour suppressors and the crucial role they play in activation of enhancers ([18,179] and see Table 1).
Although mutations in the SET domain of KMT2C and KMT2D are common in cancer (>25% of mutations), mutations are also found in other domains, including the PHD domains. Oncogenic mutations in KMT2C in breast cancer occur at hotspots in PHD domains which, in WT KMT2C, interact with the tumour repressor BAP1 (BRACA1-associated protein 1). BAP1 recruits KMT2C to chromatin and opposes the action of Polycomb by removing the ubiquitin added to H2A119 by the PRC1 complex. [180]. Mutations in these hot spots block the interaction of KMT2A with BAP1 and many genes become aberrantly down-regulated by the Polycomb complexes.

8.3. LSD1 (KDM1A) in Cancer

LSD1 is over-expressed in many cancers and a range of inhibitors targeting LSD1 have been developed based on a small molecular weight compound (TCP tranylcypromine), originally approved for treatment of anxiety disorders, see Figure 3. TCP only moderately inhibits (irreversibly) the amine oxidase activity of LSD1. However, using TCP as a scaffold, modified structures have been developed carrying different covalent modifications which are more effective in inhibiting the demethylase function of LSD1 and the proliferation of cancer cells. TCP and its derivatives operate by covalently binding to FAD, located within the active site of the demethylase, and the effects are not reversible. Preclinical studies with these inhibitors of LSD1 indicate that the most sensitive cell lines were derived from AML or small cell lung cancer (SCLC) [181], and clinical trials with LSD1 demethylase inhibitors are ongoing for patients with these cancers [52,81]. However, preclinical studies indicate that other cancers could be appropriate targets for LSD1 inhibitors, particularly if used in combination with other therapies such as HDAC inhibitors [52,81].

Oncogenic effects of LSD1 (KDM1A) independent of the demethylase activity, see Box 3: Important recent publications [82,182] have shown that in enhancing the growth of castration-resistant prostate cancer (CRPC cells), LSD1 activates a gene network dominated by cell cycle and DNA replication genes and that LSD1 ablation results in growth inhibition. This oncogenic effect is independent of the demethylase activity, and depends on the interaction with the ZNF217 protein, classified as an oncogene because of its increased expression in many cancers. An allosteric inhibitor has been developed which blocks the binding of ZNF217 to LSD1 and inhibits CRPC growth [82], (see Figure 3). Since ZNF217 can also form a complex with KDM5B, it is possible that interactions of KDM5B or other KDMs with ZNF217 occurs in other cancers [183].

LSD1 plays an important role in maintaining the oncogenic programme in leukaemias, showing a translocation of KMT2A and its inhibitors, which are in the clinic induce differentiation of the leukaemic blast cells [184–186]. However, pre-clinical studies with AML cell lines have shown that the action of the LSD1 inhibitors (developed to inhibit LSD1 demethylase activity) depends on inhibiting the interaction of LSD1 (in the CoREST complex) with the transcription factor GFI1B (or GF1) (see Figure 3). This disruption of the GFI1/LSD1 complex is independent of LSD1 demethylase activity, and leads to activation of expression of myeloid differentiation genes [187,188]. Vinyard et al. have used the technique of CRISPR–Cas9 mutagenesis to analyse the interaction of mutants of LSD1 with the small molecular weight inhibitors of LSD1 and drug resistance [189]. Their data confirm the crucial role of the GFI1B/LSD1 complex in AML and show that LSD1 mutants resistant to the GSK LSD1 inhibitor are enzymically inactive. CRISPR-suppressor scanning can detect differences in guidance RNAs and coding mutations selected with other inhibitors, giving new information on structure–activity relationships.

LSD1/EZH2/HOTAIR: The finding that the EZH2 Polycomb complex and the LSD1 complex can be associated by binding to different domains of the long non-coding RNA HOTAIR has added a new dimension to possible therapies based on this complex [190]. HOTAIR is over-expressed in many cancers, including breast and prostate carcinomas, and adult glioma [191,192], and the level of expression of HOTAIR determines to some degree the extent of LSD1/PRC2 binding. The expression of HOTAIR is very high in breast cancer metastases and already a small molecular weight inhibitor of the interaction of the EZH2
complex with the 5′ end of HOTAIR has been developed ([74] and see Polycomb section).
In gliomas, expression of HOTAIR has been shown to be driven by the BET protein BRD4,
and BET inhibitors can reduce expression of HOTAIR [192].

**Box 3. Targeting enzyme independent actions of LSD1.**

Of all the histone demethylases targeting methylated histones, progress in evaluating clinical
efficacy is most advanced with LSD1 and the development of inhibitors for LSD1 has been directed
to targeting the enzyme activity. However, pre-clinical studies show that in some cancers, an
interaction of LSD1 with tran-scription factors, acting independently of the enzyme activity, is
crucial for prolif-eration, and inhibitors of these interactions are under development (see Table 2
and Figure 3).

8.4. **KDM5 Demethylases and Cancer**

While it is abundantly clear that KDM5A, KDM5B, and KDM5C can be over expressed
in multiple cancers, defining their individual and separate roles in oncogenesis presents
a problem. There is an expansive literature describing preclinical studies with the KDM5
proteins, their known functions, and their expression in cancer (e.g., [15–17,193]). KDM5B,
has been—and is—the most widely studied KDM5 protein in cancer, and several reviews
covering KDM5B are also in print [194,195].

Since KDM5B was identified as being upregulated in breast and prostate cancer [137,138,149],
this member of the KDM5 family has been widely studied in these cancers. In breast
cancer, KDM5B is expressed most highly in the ER +ve subgroup, being classified as a
luminal lineage-driving oncogene [196]. However increased expression of KDM5A, B, and
C enzymes is seen in a wide range of cancers. Evidence that these enzymes can drive
cancer cell proliferation comes from observations using cell lines, which show that levels
of expression of the demethylase correlate with poor prognosis, and that knock-down
of expression results in inhibition of cell growth in these cell lines [59,197–199]. KDM5
inhibitors which target the demethylase activity can be used to ask if the changes induced
by KDM5 knock-down are demethylase-dependent [197].

**KDM5 proteins as tumour suppressors:** The KDM5C protein acts as a tumour suppressor
in clear cell renal cell carcinomas (ccRCC), where the incidence in males-to-females is 2:1,
and mutations in KDM5C are high [199]. KDM5C may also be involved in human papilloma
(HPV) malignancies, since the E2 tumour suppressor protein recruits KDM5C to repress
expression of the E6 and E7 oncoproteins [200]. KDM5D acts as a tumour suppressor in
the prostate, where it interacts with the androgen receptor to down-regulate expression of
AR target genes. In prostate cancer, downregulation of KDM5D is seen, thus enhancing
expression of AR target genes [201,202].

KDM5B inhibits initiation of leukaemogenesis in leukaemias expressing MLL fusion
proteins by reducing the high levels of H3K4me3 required by the leukaemic stem cells
(LSCs) for proliferation. KDM5B shows reduced expression in the more differentiated
leukaemic cells, so targeting KDM5B or other KDM5 proteins in leukaemia may not be an
option [203].

**KDM5 proteins and drug tolerance:** One finding which suggests that the KDM5 demethy-
lases could be cancer-therapeutic targets is that they play a significant role in the develop-
ment of “drug tolerance”. KDM5A and KDM5B have been shown to be required for, and
expressed by, a small population of reversibly drug-tolerant cells, (termed drug-tolerant
persisters: DTP), which express stem cell markers. Although this work has been done
largely with drugs used for lung cancer or melanoma, the principle has been found to apply
to a wide range of cancer types and therapeutic drugs [204,205]. The reversibility of
the drug-tolerant phenotype is evident when a cell line expanded from a drug tolerant clone
(drug tolerant expanded persister DTEP) is cultured in the absence of the drug and becomes
sensitive. This relates to the observation that patients who have acquired resistance to
a specific drug can respond to a second treatment after a period of absence from drug
treatment. DTPs have been found to show increased sensitivity to HDAC inhibitors, which
do not inhibit the growth of parental cells. Combining an HDAC inhibitor with ablation of KDM5A effectively kills DTPs and DTEPs developing from several cell lines [204]. Moreover, combined treatment of cancer cell lines with the pan KDM5 inhibitor CPI 455 and HDAC inhibitors, or other targeting agents, also inhibits the development of drug tolerance ([206] and see below).

**KDM5 Inhibitors:** The association of enzymes that could remove methyl groups from the active mark H3K4me3 and their increased expression in cancer has led to the development of selective inhibitors of KDM5 demethylases, which have been applied pre-clinically to evaluate their possible use in cancer therapy. These inhibitors (eg CP70, CPI455, and pBIT) have selectivity for KDM5 versus other JmjC-dependent demethylases, but are not selective for individual members of the KDM5 family, which share highly conserved sequences [206–210], see Box 4. CPI455 has been found to inhibit cell growth and the development of drug tolerance in melanoma and NSLC cell lines [206], and to enhance the biological efficacy of a DNA methylase inhibitor (5-Aza-2′-deoxycytidine) and of HDAC inhibitors [206,210]. Inhibition of cell growth of multiple myeloma cell lines by CP70 has also been demonstrated.

**Box 4. KDM5 inhibitors targeting the demethylase activity requires further research.**

Currently, there are no KDM5 inhibitors in the clinic. Until more information is available on the functions of the individual KDM5 demethylases in specific cancers, and more inhibitors targeting individual members are available, clinical application may have to wait. It is possible that oncogenic effects which are independent of de-methylase activity may present opportunities for selective inhibition of the individual KDM5 proteins. An important question is how important the functioning of the KDM5 demethylase activity in the adult brain is, in the context of inhibiting this function in cancer patients [86,152,211]. A comparison of behaviour in WT mice and the ∆ARID mice expressing a demethylase-null KDM5B could give some answers.

9. Perspectives

- There is considerable data arising from pre-clinical studies showing that disturbance of the methylation state of the histone lysines K4, K27, and K36 on histone 3 is a common event in cancer. These changes can occur as a result of mutations or translocations of the enzymes modifying the methylation state (Table 1), or to changes in their level of expression. While mutations in tumour suppressor genes, and changes of levels of expression, are found in a range of cancers, including the common carcinomas, other mutations and all translocations are associated with specific cancers, which are less common (see Table 1). Thus, inhibitors of the relevant KMTs or KDMs have been developed for potential clinical application in cancer therapy. However, only one methylase, EZH2 (KMT6A), and one demethylase, LSD1 (KDM1), are currently under extensive evaluation in the clinic for cancer therapy using inhibitors which target the enzyme activity.

- It is becoming clear that functions of the KMT and KDM proteins, which are independent of the enzyme activity, also play a role in development and in the changes occurring in some cancers (see Table 2 and Figure 3). However, pre-clinical research developing inhibitors for these histone methylases and demethylases has targeted the enzymatic activity. This approach makes it difficult to selectively inhibit specific homologues in families using the same chemical mechanism for enzymic activity. In evolution, the expansion of the number of genes with a common function from lower organisms to mammals (e.g., six homologues of the KMT2 methylase family, four dimethylating H3K36 and four of the KDM5 demethylases) is associated with increasing tissue complexity. This in turn emphasises the importance of cell phenotype in the expression and function of epigenetic factors. Obtaining more information regarding the differences in expression and function of the individual homologues in a family of KMTs or KDMs which may be seen in different tissues and in cancers developing from them is now a pressing area of research. Studies documenting the detailed structure of the homologues in a particular family could also give clues for identifying differential
targeting. Taking this approach, inhibitors of the ASH1L methylase have recently been developed [117] which are selective in inhibiting ASH1L enzyme activity, but not the other methylases, which dimethylate H3K36. Such studies could lead to the development of not only enzymatic inhibitors specific for a particular homologue, but also for a new class of inhibitors targeting a function which is independent of the enzyme activity, and may be operative in specific cancers.

**Author Contributions:** Writing, original draft preparation, J.T.-P.; writing, reviewing, and editing, J.T.-P. and J.M.B. All authors have read and agreed to the published version of the manuscript.

**Funding:** This review received no external funding.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. Lewis, P.H. New mutants report. *Drosoph. Inf. Serv.* 1947, 21, 69.
2. Lewis, E.B. A gene complex controlling segmentation in Drosophila. *Nature* 1978, 276, 565–570. [CrossRef] [PubMed]
3. Ingham, P.W. Differential expression of bithorax complex genes in the absence of the extra sex combs and trithorax genes. *Nature* 1983, 306, 591–593. [CrossRef] [PubMed]
4. Rea, S.; Eisenhaber, F.; O’Carroll, D.; Strahl, B.D.; Sun, Z.W.; Schmid, M.; Opravil, S.; Mecthtler, K.; Ponting, C.P.; Allis, C.D.; et al. Regulation of chromatin structure by site-specific histone H3 methyltransferases. *Nature* 2000, 406, 593–599. [CrossRef]
5. Cao, R.; Wang, L.; Wang, H.; Xia, L.; Erdjument-Bromage, H.; Tempst, P.; Jones, R.S.; Zhang, Y. Role of Histone H3 Lysine 27 Methylation in Polycomb-Group Silencing. *Science* 2002, 298, 1039–1043. [CrossRef]
6. Miller, T.; Krokan, N.J.; Dover, J.; Erdjument-Bromage, H.; Tempst, P.; Johnston, M.; Greenblatt, J.F.; Shilatifard, A. COMPASS: A complex of proteins associated with a trithorax-related SET domain protein. *Proc. Natl. Acad. Sci. USA* 2001, 98, 12902–12907. [CrossRef]
7. Yuan, W.; Xu, M.; Huang, C.; Liu, N.; Chen, S.; Zhu, B. H3K36 methylation antagonizes PRC2-mediated H3K27 methylation. *J. Biol. Chem.* 2011, 286, 7983–7989. [CrossRef]
8. Schnädising, S.; Meiler, A.; Lee, Y.; Mohammed, A.; Finik, K.; Tauscher, K.; Israel, L.; Wirth, M.; Philippou-Massier, J.; Blum, H.; et al. Regulation and function of H3K36 di-methylation by the trithorax group protein complex AMC. *Development* 2018, 145, dev163808. [CrossRef]
9. Piunti, A.; Shilatifard, A. Epigenetic balance of gene expression by Polycomb and COMPASS families. *Science* 2016, 352, aad9780. [CrossRef]
10. Schuettengruber, B.; Bourbon, H.M.; Di Croce, L.; Cavalli, G. Genome regulation by polycomb and Trithorax: 70 Years and Counting. *Cell* 2017, 171, 34–37. [CrossRef]
11. Geislee, S.J.; Paro, R. Trithorax and Polycomb group-dependent regulation: A tale of opposing activities. *Development* 2015, 142, 2876–2887. [CrossRef] [PubMed]
12. Cenik, B.K.; Shilatifard, A. COMPASS and SWI/SNF complexes in development and disease. *Nat. Rev. Genet.* 2021, 22, 38–58. [CrossRef] [PubMed]
13. Shi, Y.; Lan, F.; Matson, C.; Mulligan, P.; Whetstone, J.R.; Cole, P.; Casero, R.A.; Shi, Y. Histone demethylation mediated by the nuclear amine oxidase homolog LSD1. *Cell 2004*, 119, 941–953. [CrossRef] [PubMed]
14. Tsukada, Y.-i.; Fang, J.; Erdjument-Bromage, H.; Warren, M.E.; Borchers, C.; Tempst, P.; Zhang, Y. Histone demethylation by a family of JmjC domain-containing proteins. *Nature* 2006, 439, 813–816. [CrossRef]
15. Dimitrova, E.; Turberfield, A.; Klose, R.J. Histone demethylases in chromatin biology and beyond. *EMBO Rep.* 2015, 16, 1620–1639. [CrossRef]
16. Hojfeldt, J.; Agger, K.; Helin, K. Histone lysine demethylases as targets for anticancer therapy. *Nat. Rev. Drug Discov.* 2013, 12, 917–930. [CrossRef]
17. Pedersen, M.T.; Helin, K. Histone demethylases in development and disease. *Trends Cell Biol.* 2010, 20, 662–671. [CrossRef]
18. Wang, S.-P.; Tang, Z.; Chen, C.-W.; Shimada, M.; Koche, R.; Wang, L.-H.; Nakadai, T.; Chromiec, A.; Krirvost, A.V.; Armstrong, S.A.; et al. A UTX-MLL4-p300 Transcriptional Regulatory Network Coordinately Shapes Active Enhancer Landscapes for Eliciting Transcription. *Mol. Cell 2017*, 67, 308–321.e6. [CrossRef]
19. Aranda, S.; Mas, G.; Di Croce, L. Regulation of gene transcription by Polycomb proteins. *Sci. Adv.* 2015, 1, e1500737. [CrossRef]
20. Varlet, E.; Ovejero, S.; Martinez, A.-M.; Cavalli, G.; Moreaux, J. Role of Polycomb Complexes in Normal and Malignant Plasma Cells. *Int. J. Mol. Sci.* 2020, 21, 8047. [CrossRef]
21. Healy, E.; Mucha, M.; Glancy, E.; Fitzpatrick, D.J.; Conway, E.; Neikes, H.K.; Monger, C.; Van Mierlo, G.; Baltissen, M.P.; Koseki, Y.; et al. PRC2.1 and PRC2.2 Synergize to Coordinate H3K27 Trimethylation. *Mol. Cell 2019*, 76, 437–452.e6. [CrossRef] [PubMed]
22. Levine, S.S.; Weiss, A.; Erdjument-Bromage, H.; Shao, Z.; Tempst, P.; Kingston, R.E. The core of the polycomb repressive complex is compositionally and functionally conserved in flies and humans. *Mol. Cell. Biol.* 2002, 22, 6070–6078. [CrossRef] [PubMed]
23. Zhao, J.; Wang, M.; Chang, L.; Yu, J.; Song, A.; Liu, C.; Huang, W.; Zhang, T.; Wu, X.; Shen, X.; et al. RYBP/YAF2-PRC1 complexes and histone H1-dependent chromatin compaction mediate propagation of H2AK119ub1 during cell division. *Nat. Cell Biol.* 2020, 22, 439–452. [CrossRef] [PubMed]

24. He, J.; Kallin, E.M.; Tsukada, Y.L.; Zhang, Y. The H3K36 demethylase Jhdm1b/Kdm2b regulates cell proliferation and senescence through p15Ink4b. *Nat. Struct. Mol. Biol.* 2008, 15, 1169–1175. [CrossRef]

25. Farcas, A.M.; Blackledge, N.P.; Sudbery, I.; Long, H.K.; McGouran, J.F.; Rose, N.R.; Lee, S.; Sims, D.; Cerase, A.; Sheahan, T.W.; et al. KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands. *Elife* 2012, 1, e00205. [CrossRef]

26. He, J.; Shen, L.; Wan, M.; Taranova, O.; Wu, H.; Zhang, Y. Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes. *Nat. Cell Biol.* 2013, 15, 373–384. [CrossRef]

27. Wong, S.J.; Gearhart, M.A.; Taylor, A.B.; Narayes, D.R.; Ha, D.J.; Robinson, A.K.; Artigas, J.A.; Lee, O.J.; Demeler, D.; Hart, P.J.; et al. KDM2B recruitment of the Polycomb Group Complex PRC1.1, requires co-operation between PCGF1 and BCORL1. *Structure* 2016, 24, 1795–1801. [CrossRef]

28. Wu, X.; Vilstrup Johansen, J.; Helin, K. Fbxl10/Kdm2b recruits polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitination. *Mol. Cell* 2013, 49, 1134–1146. [CrossRef]

29. Blackledge, N.P.; Farcas, A.M.; Kondo, T.; King, H.W.; McGouran, J.F.; Hanssen, L.L.P.; Ito, S.; Cooper, S.; Kondo, K.; Koseki, Y.; et al. Variant PRC1 complex-dependent H2A ubiquitination drives PRC2 recruitment and polycomb domain formation. *Cell* 2014, 157, 1445–1459. [CrossRef]

30. Kalb, R.; Latwiel, S.; Baymaz, H.I.; Jansen, P.W.; Muller, C.W.; Vermeulen, M.; Muller, J. Histone H2A monoubiquitination promotes histone H3 methylation in Polycomb repression. *Nat. Struct. Mol. Biol.* 2014, 21, 569–571. [CrossRef]

31. Cooper, S.; Grijzenhout, A.; Underwood, E.; Ancelin, K.; Zhang, T.; Nanyes, D.R.; Ha, D.J.; Robinson, A.K.; Artigas, J.A.; Lee, O.J.; Demeler, D.; Hart, P.J.; et al. Variant PRC1 complex-dependent H2A ubiquitination drives PRC2 recruitment and polycomb domain formation. *Cell* 2014, 157, 1445–1459. [CrossRef]

32. Sugishita, H.; Kondo, T.; Ito, S.; Nakayama, M.; Yakushiji-Kaminatsui, N.; Kawakami, E.; Koseki, Y.; Ohi-nata, Y.; Sharif, J.; Harachi, M.; et al. Variant PRC1 complex-dependent H2A ubiquitination drives PRC2 recruitment with differentiation-associated transcriptional inactivation at target genes. *Nat. Commun.* 2016, 7, 13661. [CrossRef]

33. Agger, K.; Cloos, P.A.C.; Christensen, J.; Pasini, D.; Rose, S.; Rappsiilber, J.; Issaeva, I.; Canaani, E.; Salcini, A.E.; Helin, K. UTX and JMJD3 are histone H3K27 demethylases involved in HOX gene regulation and development. *Nature* 2007, 449, 731–734. [CrossRef]

34. Shpargel, K.B.; Sengoku, T.; Yokoyama, S.; Magnuson, T. UTX and UTY demonstrate histone demethylase-independent function in mouse embryonic development. *PLoS Genet.* 2012, 8, e1002964. [CrossRef]

35. Kleer, C.G.; Cao, Q.; Varambally, S.; Shen, R.; Ota, I.; Tomlins, S.A.; Ghosh, D.; Sewalt, R.G.A.B.; Otte, A.P.; Hayes, D.F.; et al. EZH2 is a marker of aggressive breast cancers and promotes neoplastic transformation of breast epithelial cells. *Proc. Natl. Acad. Sci. USA* 2003, 100, 11606–11611. [CrossRef]

36. Varambally, S.; Dhasekeran, S.M.; Zhou, M.; Barrette, T.R.; Kumar-Sinha, C.; Sanda, M.G.; Ghosh, D.; Pienta, K.J.; Sewalt, R.G.A.B.; Otte, A.P.; et al. The polycomb group protein EZH2 is involved in progression of prostate cancer. *Nature* 2002, 419, 624–629. [CrossRef]

37. Hernandez, H.; Gelato, K.A.; Lesche, R.; Beckmann, G.; Koebr, S.; Otto, S.; Steigenmann, P.; Stresemann, C. EZH2 Inhibition Blocks Multiple Myeloma Cell Growth through Upregulation of Epithelial Tumor Suppressor Genes. *Mol. Cancer Ther.* 2016, 15, 287–298. [CrossRef]

38. Wang, L.; Shilatifard, A. UTX Mutations in Human Cancer. *Cancer Cell* 2019, 35, 168–176. [CrossRef] [PubMed]

39. Bilzer, B.G.; Aird, K.M.; Garipov, A.; Li, H.; Amatangelo, M.; Kossenkov, A.V.; Schultz, D.C.; Liu, Q.; Shih, I.M.; Conejo-Garcia, J.R.; et al. Synthetic lethality by targeting EZH2 methyl transferase activity in ARID1A mutated cancers. *Mol. Cell Oncol.* 2015, 76, e1002964. [CrossRef]

40. Kim, K.H.; Kim, W.; Howard, T.P.; Vazquez, F.; Tsherniak, W.; Wu, J.N.; Wang, W.; Haswell, J.R.; Walensky, L.D.; Hahn, W.C.; et al. SWI/SNF mutant cancers depend upon catalytic and non-catalytic activity of EZH2. *Nat. Med.* 2015, 21, 1491–1496. [CrossRef]

41. Ezponda, T.; Dupéré-Richer, D.; Will, C.M.; Small, E.C.; Varghes, M.; Patel, T.; Nabet, B.; Popovic, R.; Oyer, J.; Bulic, M.; et al. UTX/KDM6A loss enhances the malignant phenotype of multiple myeloma and sensitizes cells to EZH2 inhibition. *Cell Rep.* 2017, 21, 628–640. [CrossRef]

42. Ernst, T.; Chase, A.J.; Score, J.; Hidalgo-Curtis, C.E.; Bryant, C.; Jones, A.V.; Waghorn, K.; Zoi, K.; Ross, F.M.; Reiter, A.; et al. Inactivating mutations of the histone methyltransferase gene EZH2 in myeloid dis-orders. *Nat. Genet.* 2010, 42, 722–726. [CrossRef]
52. Cheng, Y.; He, C.; Wang, M.; Ma, X.; Mo, F.; Yang, S.; Han, J.; Wei, X. Targeting epigenetic regulators for cancer therapy.

47. Ntziachristos, P.; Tsirigos, A.; Van Vlierberghe, P.; Nedjic, J.; Trimarchi, T.; Flaherty, M.S.; Ferres-Marco, D.; Da Ros, V.G.; Tang, Z.; Siegle, J.; et al. Genetic inactivation of the polycomb repressive complex 2 in T cell acute lymphoblastic leukemia. Nat. Med. 2012, 18, 298–302. [CrossRef]

50. Xu, K.; Wu, Z.J.; Groner, A.C.; He, H.H.; Cai, C.; Lis, R.T.; Wu, X.; Stack, E.C.; Loda, M.; Liu, T.; et al. EZH2 oncogenic activity in castration-resistant prostate cancer cells is polycomb-independent. Science 2012, 338, 1456–1459. [CrossRef]

51. Kim, J.; Lee, Y.; Lu, X.; Song, B.; Fong, K.W.; Cao, Q.; Licht, J.D.; Zhao, J.C.; Yu, J. Polycomb-and Methylation-Independent Roles of EZH2 as a Transcription Activator. Cell Rep. 2018, 25, 2808–2820. [CrossRef] [PubMed]

52. Cheng, Y.; He, C.; Wang, M.; Ma, X.; Mo, F.; Yang, S.; Han, J.; Wei, X. Targeting epigenetic regulators for cancer therapy: Mechanisms and advances in clinical trials. Signal Transduct. Target. Ther. 2019, 4, 62. [CrossRef] [PubMed]

53. Duan, R.; Du, W.; Guo, W. EZH2: A novel target for cancer treatment. J. Hematol. Oncol. 2020, 13, 104. [CrossRef] [PubMed]

54. Dalglish, G.L.; Furge, K.; Greenman, C.; Chen, L.; Bignell, G.; Butler, A.; Davies, H.; Edkins, S.; Hardy, C.; Latimer, C.; et al. Systematic sequencing of renal cancer reveals inactivation of histone modifying genes. Nature 2010, 463, 360–363. [CrossRef]

55. Rao, R.C.; Dou, Y. Hijacked in cancer: The KMT2 (MLL) family of methyltransferases. [CrossRef]

56. Krivtsov, A.V.; Armstrong, S.A. MLL translocations, histone modifications and leukaemia stem-cell development.

57. Moffitt, A.B.; Ondrejka, S.L.; McKinney, M.; Rempel, R.E.; Goodlad, J.R.; The, C.H.; Leppa, S.; Mannisto, S.; Kovanen, P.E.; Tse, E.; et al. Enteropathy-associated T cell lymphoma subtypes are characterised by loss of function of SETD2. J. Exp. Med. 2017, 215, 1371–1386. [CrossRef]

58. Kuo, A.J.; Cheung, P.; Chen, K.; Zee, B.M.; Kioi, M.; Lauring, J.; Xi, Y.; Park, B.H.; Shi, X.; Garcia, B.A.; et al. NSD2 links proliferation of hepatocellular carcinoma by regulation of cell cycle check-point proteins p15 and p27. J. Exp. Clin. Cancer Res. 2016, 35, 37. [CrossRef]

59. Jaffe, J.D.; Wang, Y.; Chan, H.M.; Zhang, J.; Huether, R.; Kryukov, G.V.; Bhang, H.-E.C.; Taylor, J.E.; Hu, M.; Englund, N.P.; et al. Global chromatin profiling reveals NSD2 mutations in pediatric acute lymphoblastic leukemia. Nat. Genet. 2013, 45, 1386–1391. [CrossRef]

60. Pierro, J.; Saliba, J.; Narang, S.; Sethia, G.; Saint Fleur-Leminy, S.; Chowdhury, A.; Qualls, A.; Fay, H.; Kilberg, H.L.; Moriyama, T.; et al. The NSD2p.E1099K Mutation is Enriched at Relapse and Confers Drug Resistance in a Cell Context-Dependent Manner in Pediatric Acute Lymphoblastic Leukemia. Mol. Cell. 2011, 44, 609–620. [CrossRef]

61. Dorrance, A.M.; Liu, S.; Yuan, W.; Becknell, B.; Arnoeczky, J.; Guimond, M.; Stout, M.P.; Feng, L.; Nakamura, T.; Yu, L.; et al. MII partial tandem duplication induces aberrant Hox expression in vivo via specific epigenetic alterations. J. Clin. Investig. 2006, 117, 2619–2716. [CrossRef] [PubMed]

62. Krivtsov, A.V.; Armstrong, S.A. MLL translocations, histone modifications and leukaemia stem-cell development. Nat. Rev. Cancer 2007, 7, 823–833. [CrossRef] [PubMed]

63. Dawson, M.A.; Prieur, R.K.; Dittmann, A.; Giotopoulos, G.; Bantschaff, M.; Chan, W.I.; Robson, S.C.; Chung, C.; Hopf, C.; Savitski, M.M.; et al. Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 2011, 478, 529–533. [CrossRef]

64. Morishita, M.; di Luccio, E. Cancers and the NSD family of histone methyltransferases. Biochim. Biophys. Acta 2011, 1816, 158–163. [CrossRef]

65. Lhoumaud, P.; Badri, S.; Rodriguez-Hernaez, J.; Sakellaropoulos, T.; Sethia, G.; Kloeugen, A.; Cornwell, M.; Bhattacharyya, S.; Ay, F.; Bonneau, R.; et al. NSD2 overexpression drives clustered chromatin and transcriptional changes in a subset of insulined domains. Nat. Commun. 2019, 10, 48431. [CrossRef]

66. Banito, A.; Li, X.; Laporte, A.N.; Roe, J.-S.; Sanchez-Vega, F.; Huang, C.-H.; Dancso, A.R.; Hatzis, K.; Chen, C.-C.; Tsaharganeh, D.F.; et al. The SS18-SSX oncoprotein hijacks KDM2B-PRC1.1 to drive synovial sarcoma. Cancer Cell 2018, 33, 527–541.e8. [CrossRef]

67. Nacev, B.A.; Feng, L.; Bagert, J.D.; Lemiesz, A.E.; Gao, J.; Soshnev, A.A.; Kundra, R.; Schultz, N.; Muir, T.W.; Allis, C.D. The expanding landscape of “oncohistone” mutations in human cancers. Nature 2019, 567, 473–478. [CrossRef]

68. Wu, G.; Broniscer, A.; McEachron, T.A.; Lu, C.; Paugh, B.S.; Becksfort, J.; Qu, C.; Ding, L.; Huether, R.; Parker, M.; et al. Somatic histone H3 alterations in pediatric diffuse intrinsic pontine gliomas and non-brainstem glioblastomas. Nat. Genet. 2012, 44, 251–253.
70. Schwartzzenbruber, J.; Korshunov, A.; Liu, X.Y.; Jones, D.T.; Pfaff, E.; Jacob, K.; Sturm, D.; Fontebasso, A.M.; Quang, D.A.; Tänjes, M.; et al. Driver mutations in histone H3.3 and chromatin remodelling genes in paediatric glioblastoma. Nature 2012, 482, 226–231. [CrossRef]

71. Lewis, P.W.; Müller, M.M.; Koletsky, M.S.; Cordero, F.; Lin, S.; Banaszynski, L.A.; Garcia, B.A.; Muir, T.W.; Becker, O.J.; Allis, C.D. Inhibition of PRC2 activity by a gain-of-function H3 mutation found in pediatric glioblastoma. Science 2013, 340, 857–861. [CrossRef] [PubMed]

72. Vire, E.; Brenner, C.; Deplus, R.; Blanchon, L.; Fraga, M.; Didelot, C.M.; Morey, L.; Van Eynde, A.; Bernard, D.; Vanderwinden, J.-M.; et al. The Polycomb group protein EZH2 directly controls DNA methylation. Nature 2006, 439, 871–874. [CrossRef] [PubMed]

73. Van der Vlag, J.; Otte, A.P. Transcriptional repression mediated by the human polycomb-group protein EED involves histone deacetylation. Nat. Genet. 1999, 23, 474–478. [CrossRef]

74. Ren, Y.; Wang, Y.; Zhang, J.; Wang, Q.-X.; Han, L.; Mei, M.; Kang, C.-S. Targeted design and identification of ACINOD4Q to block activity of HOXA11 by abrogating the scaffold interaction with EZH2. Clin. Epigenetics 2019, 11, 29. [CrossRef] [PubMed]

75. Mohammad, F.; Weissmann, S.; Leblanc, B.; Pandey, D.P.; Hoffeldt, J.W.; Comet, I.; Zheng, C.; Johansen, J.V.; Rapin, N.; Porse, B.T.; et al. EZH2 is a potential therapeutic target for H3K27M-mutant pediatric gliomas. Nat. Med. 2017, 23, 483–492. [CrossRef] [PubMed]

76. Gozdecka, M.; Meduri, E.; Mazan, M.; Tzelepis, K.; Dudek, M.; Knights, A.J.; Pardo, M.; Yu, L.; Choudhary, J.S.; Metzakopan, E.; et al. UTX-mediated enhancer and chromatin remodelling suppresses myeloid leukemogenesis through noncatalytic inverse regulation of ETS and GATA programs. Nat. Genet. 2018, 50, 883–894. [CrossRef] [PubMed]

77. Ntzniachristos, P.; Tsirigos, A.; Welstead, G.G.; Trimarchi, T.; Bakogianni, S.; Xu, L.; Loizou, E.; Holmfeldt, L.; Strikoudis, A.; King, B.; et al. Contrasting roles of histone 3 lysine 27 demethylases in acute lymphoblastic leukaemia. Nature 2014, 514, 513–517. [CrossRef]

78. Andricovich, J.; Perkail, S.; Kai, Y.; Casasanta, N.; Peng, W.; Tzatsos, A. Loss of KDM6A activates super-enhancers to induce gender-specific squamous-like pancreatic cancer and confers sensitivity to BET inhibitors. Cancer Cell 2018, 33, 512–526. [CrossRef]

79. Choi, H.-J.; Park, J.-H.; Park, M.; Won, H.-Y.; Joo, H.-S.; Lee, C.H.; Lee, J.-Y.; Kong, G. UTX inhibits EMT-induced breast CSC properties by epigenetic repression of EMT genes in cooperation with LSD1 and HDAC1. EMBO Rep. 2015, 16, 1288–1298. [CrossRef]

80. Terranova, R.; Agherbi, H.; Boned, A.; Meresse, S.; Djabali, M. Histone and DNA methylation defects at Hox genes in mice. J. Hematol. Oncol. 2019, 12, 129. [CrossRef] [PubMed]

81. Fang, Y.; Liao, G.; Yu, B. LSD1/KDM1A inhibitors in clinical trials: Advances and prospect. J. Hematol. Oncol. 2019, 12, 129. [CrossRef] [PubMed]

82. Sehrawat, A.; Gao, L.; Wang, Y.; Bankhead, A., 3rd; McWeeney, S.K.; King, C.J.; Schwartzman, J.; Urrutia, J.; Bisson, W.H.; Coleman, D.J.; et al. LSD1 activates a lethal prostate cancer gene network independently of its demethylase activity. Nat. Genet. 2018, 50, 474–478. [CrossRef]

83. Kuang, Y.; Lu, F.; Guo, J.; Xu, H.; Wang, Q.; Xu, C.; Zeng, L.; Yi, S. Histone demethylase KDM2B up-regulates histone methyltransferase EZH2 expression and contributes to the progression of ovarian cancer in vitro and in vivo. Onco. Targets Ther. 2017, 10, 3131–3144. [CrossRef] [PubMed]

84. Tzatsos, A.; Paskaleva, P.; Ferrari, F.; Deshpande, V.; Stoykova, S.; Contino, G.; Wong, K.K.; Lan, F.; Trojer, P.; Park, P.; et al. KDM2B promotes pancreatic cancer via Polycomb-dependent and -independent transcriptional programs. J. Clin. Investig. 2013, 123, 725–739. [CrossRef]

85. Catchpole, S.; Spencer-Dene, B.; Hall, D.; Santangelo, S.; Rosewell, I.; Guenatri, M.; Beatson, R.; Scibetta, A.G.; Burchell, J.M.; Taylor-Papadimitriou, J. PLU-1/JARID1B/KDM5B is required for embryonic survival and contributes to cell proliferation in the mammmary gland and in ER+ breast cancer cells. Int. J. Oncol. 2011, 38, 1267–1277.

86. Jamshidi, S.; Catchpole, S.; Chen, J.; So, C.W.E.; Burchell, J.M.; Rahman, K.M.; Taylor-Papadimitriou, J. The KDM5B protein expressed in the viable and fertile ARID mouse exhibits no demethylase activity. Int. J. Oncol. 2021, 59, 96. [CrossRef]

87. Schmitges, F.W.; Frusty, A.B.; Faty, M.; Stützer, A.; Lingaraju, G.M.; Aiwazian, J.; Hess, D.; Li, L.; Zhou, S.; et al. Histone Methylation by PRC2 is inhibited by active chromatin marks. Mol. Cell 2011, 42, 330–341. [CrossRef]

88. Huang, C.; Yang, F.; Zhang, Z.; Zhang, J.; Cai, G.; Li, L.; Zheng, Y.; Chen, S.; Xi, R.; Zhu, B. Mrg15 stimulates Ashl H3K36 methyltransferase activity and facilitates Ashl Trithorax group protein function in Drosophila. Nat. Commun. 2017, 8, 1649. [CrossRef]

89. Wang, G.G.; Bai, L.; Pasillas, M.P.; Kamps, M.P. NUP98–NSD1 links H3K36 methylation to Hox-A gene activation and leukemogenesis. Nat. Cell Biol. 2007, 9, 804–812. [CrossRef]

90. Strahl, B.D.; Grant, P.A.; Briggs, S.D.; Sun, Z.W.; Bone, J.R.; Caldwell, J.A.; Mollah, S.; Cook, R.G.; Sha-banowitz, J.; Hunt, D.F.; et al. Set2 is a nucleosomal histone H3-selective methyltransferase that medi-ates transcriptional repression. Mol. Cell. Biol. 2002, 22, 1298–1306. [CrossRef]

91. Wagner, E.J.; Carpenter, P.B. Understanding the language of Lys36 methylation in transcription: Antagonizing silencing and safeguarding transcription fidelity. Biophys. Rep. 2018, 4, 170–177. [CrossRef]
93. Venkatesh, S.; Workman, J.L. SET2 mediated H3 lysine 36 dimethylation: Regulation of transcription elongation and implications in organismal development. *Wiley Interdiscip. Rev. Dev. Biol.* 2013, 2, 685–700. [CrossRef]

94. Edmunds, J.W.; Mahadevan, L.C.; Clayton, A.L. Dynamic histone H3 methylation during gene induction: HYPB/Setd2 mediates all H3K36 trimethylation. *EMBO J.* 2008, 27, 406–420. [CrossRef]

95. Li, F.; Mao, G.; Tong, D.; Huang, J.; Gu, L.; Yang, W.; Li, G.-M. The histone mark h3k36me3 regulates human dna mismatch repair through its interaction with MutSα. *Cell 2013*, 153, 590–600. [CrossRef]

96. Sun, Z.; Zhang, Y.; Tang, Y.; Weu, H.; Fang, D. H3K36me3, message from chromatin to DNA damage repair. *Cell Biosci.* 2020, 10, 9. [CrossRef]

97. Douglas, J.; Hanks, S.; Temple, I.K.; Davies, S.; Murray, A.; Hughes, H.E.; Cole, R.T.; Rahman, N. NSD1 mutations are the major cause of sotos syndrome and occur in some cases of weaver syndrome but are rare in other overgrowth phenotypes. *Am. J. Hum. Genet.* 2003, 72, 132–143. [CrossRef]

98. Rayasam, G.V.; Wendling, O.; Angrander, D.; Song, M.; Niederreiter, K.; Song, L.; Lerouge, T.; Hager, G.L.; Chambon, P.; Lossos, I.N. NSD1 is essential for early post-implantation development and has a catalytically active SET domain. *EMBO J.* 2003, 22, 3153–3163. [CrossRef]

99. Klymenko, T.; Muller, J. The histone methyltransferases Trithorax and Ash1l prevent transcriptional silencing by Polycomb group proteins. *EMBO Rep.* 2004, 5, 373–377. [CrossRef]

100. Neri, F.; Rapelli, S.; Krepelova, A.; Incarnato, D.; Parlato, C.; Basile, G.; Maldotti, M.; Anselmi, F.; Oliviero, S. Intragenic DNA methylation prevents sporadic transcription initiation. *Nature 2017*, 543, 72–77. [CrossRef] [PubMed]

101. Boulard, M.; Edwards, J.R.; Bestor, T.H. FBXL10 protects Polycomb-bound genes from hypermethylation. *Nat. Genet.* 2015, 47, 479–485. [CrossRef] [PubMed]

102. Li, J.; Duns, G.; Westers, H.; Sijmons, R.; Berg, A.V.D.; Kok, K. SETD2: An epigenetic modifier with tumor suppressor functionality. *Proc. Natl. Acad. Sci. USA* 2008, 105, 1907–1912. [CrossRef] [PubMed]

103. Andricovich, J.; Kai, Y.; Peng, W.; Foudi, A.; Tzatsos, A. Histone demethylase KDM2B regulates lineage commitment in normal and malignant hematopoiesis. *J. Clin. Investig.* 2016, 126, 905–920. [CrossRef]

104. Tzatsos, A.; Paskaleva, P.; Lamper, S.; Contino, G.; Stoykova, S.; Chen, Z.; Wong, K.K.; Bardeesy, N. Lysine-specific demethylase 2B (KDM2B)-let-7 enhancer of zeste homolog 2 (EZH2) pathway regulates cell cycle progression and senescence in primary cells. *J. Biol. Chem.* 2011, 286, 33061–33069. [CrossRef] [PubMed]

105. Pfau, R.; Tzatsos, A.; Kampranis, S.C.; Serebrennikova, O.B.; Bear, S.E.; Tsichlis, P.N. Members of a family of JmjC domain-containing oncoproteins immortalize embryonic fibroblasts via a JmjC domain-dependent process. *Proc. Natl. Acad. Sci. USA* 2008, 105, 1907–1912. [CrossRef] [PubMed]

106. Lee, D.H.; Kim, G.W.; Jeon, Y.H.; Yoo, J.; Lee, S.W.; Kwon, S.H. Advances in histone demethylase KDM4 as cancer therapeutic targets. *FASEB J.* 2020, 34, 3461–3484. [CrossRef]

107. Hillringhaus, L.; Yue, W.W.; Rose, N.R.; Ng, S.S.; Gileadi, C.; Loenarz, C.; Bray, J.E.; Schofield, C.J.; Oppermann, U. Structural and Evolutionary Basis for the Dual Substrate Selectivity of Human KDM4 Histone Demethylase Family. *J. Biol. Chem.* 2011, 286, 41616–41625. [CrossRef]

108. Chen, Y.K.; Bonalidi, T.; Cuomo, A.; Del Rosario, J.R.; Hosfield, D.J.; Kanouni, T.; Kao, S.C.; Lai, C.; Lobo, N.A.; Matuszkiewicz, J.; et al. Design of KDM4 inhibitors with antiproliferative effects in cancer models. *ACS Med. Chem. Lett.* 2017, 8, 869–874. [CrossRef] [PubMed]

109. Pedersen, M.T.; Kooistra, S.M.; Radzisheuskaya, A.; Laugesen, A.; Johansen, J.V.; Hayward, D.G.; Nilsson, J.; Agger, K.; Helin, K. Continual removal of H3K9 promoter methylation by Jmjd2 demethylases is vital for ESC self-renewal and early development. *EMBO J.* 2016, 35, 1500–1506. [CrossRef]

110. Duns, G.; Berg, E.V.D.; Van Duivenbode, I.; Oisinga, J.; Hollema, H.; Hofstra, R.; Kok, K. Histone Methyltransferase Gene SETD2 Is a Novel Tumor Suppressor Gene in Clear Cell Renal Cell Carcinoma. *Cancer Res.* 2010, 70, 4287–4291. [CrossRef]

111. Li, J.; Duns, G.; Westers, H.; Sijmons, R.; Berg, A.V.D.; Kok, K. SETD2: An epigenetic modifier with tumor suppressor functionality. *Oncotarget* 2016, 7, 50719–50734. [CrossRef]

112. Al Sarakbi, W.; Sasi, W.; Jiang, W.G.; Roberts, T.; Newbold, R.F.; Mokbel, K. The mRNA expression of SETD2 in human breast cancer: Correlation with clinicopathological parameters. *BMC Cancer 2009*, 9, 290. [CrossRef]

113. Xie, P.; Tian, C.; An, L.; Nie, J.; Lu, K.; Xing, G.; Zhang, L.; He, F. Histone methyltransferase protein SETD2 interacts with p53 and selectively regulates its downstream genes. *Cell. Signal.* 2008, 20, 1671–1678. [CrossRef]

114. Rosati, R.; La Starza, R.; Veronese, A.; Aventin, A.; Schwienbacher, C.; Vallespi, T.; Negrini, M.; Martelli, M.F.; Mecucci, C. NUP98 is fused to the NSD3 gene in acute myeloid leukemia associated with t(8;11)(p11.2;p15). *Blood 2002*, 99, 3857–3860. [CrossRef] [PubMed]

115. La Starza, R.; Gorello, P.; Rosati, R.; Riezzo, A.; Veronese, A.; Ferrazzi, E.; Martelli, M.F.; Negrini, M.; Mecucci, C. Cryptic insertion producing two NUP98/NSD1 chimeric transcripts in adult refractor anaemia with an excess of blasts. *Genes Chromosomes Cancer 2004*, 41, 395–399. [CrossRef]

116. Zhu, L.; Li, Q.; Wang, S.H.; Huang, M.; Klein, B.J.; Shen, J.; Ikenouye, L.; Onishi, M.; Schneidawind, D.; Buechele, C.; et al. ASH1L links histone H3 lysine 36 dimethylation to MLL leukemia. *Cancer Discov.* 2016, 6, 770–783. [CrossRef]

117. Rogawski, D.S.; Deng, J.; Li, H.; Miao, H.; Borkin, D.; Purohit, T.; Song, J.; Chase, J.; Li, S.; Nidoj, J.; et al. Discovery of first-in-class inhibitors of ASH1L histone methyltransferase with anti-leukemic activity. *Nat. Commun.* 2021, 12, 2792. [CrossRef]

118. Hon, P.; Huang, C.; Liu, C.-P.; Yang, N.; Yu, T.; Yin, Y.; Zhu, B.; Xu, R.-M. Structural Insights into Stimulation of Ash1L’s H3K36 Methyltransferase Activity through Mrg15 Binding. *Structure 2019*, 27, 837–845. [CrossRef]
Lee, Y.; Yoon, E.; Cho, S.; Schmähling, S.; Müller, J.; Song, J.-J. Structural Basis of MRG15-Mediated Activation of the ASH1L Histone Methyltransferase by Releasing an Autoinhibitory Loop. Structure 2019, 27, 846–852.e3. [CrossRef]

Fang, J.; Huang, Y.; Mao, G.; Yang, S.; Rennert, G.; Gu, L.; Li, H.; Li, G.-M. Cancer-driving H3K34V/R/D mutations block H3K36 methylation and H3K36me3–Mut5a interaction. Proc. Natl. Acad. Sci. USA 2018, 115, 9598–9603. [CrossRef]

Jain, S.U.; Khaazaee, S.; Marchione, D.M.; Lundgren, S.M.; Wang, X.; Weinberg, D.N.; Deshmukh, S.; Juretic, N.; Lu, C.; Allis, C.D.; et al. Histone H3.3 G34 mutations promote aberrant PRC2 activity and drive tumor progression. Proc. Natl. Acad. Sci. USA 2020, 117, 27354–27364. [CrossRef]

Voon, H.; Udugama, M.; Lin, W.; Hii, L.; Law, R.; Steer, D.L.; Das, P.P.; Mann, J.R.; Wong, L.H. Inhibition of a K9/K36 demethylase by an H3.3 point mutation found in paediatric glioblastoma. Nat. Commun. 2018, 9, 3142. [CrossRef]

Bjehjati, S.; Tarpey, P.S.; Presneau, N.; Scheipl, S.; Pillay, N.; Van Loo, P.; Wedge, D.; Cooke, S.L.; Gundred, G.; Davies, H.; et al. Distinct H3F3A and H3F3B driver mutations define chondroblastoma and giant cell tumor of bone. Nat. Genet. 2013, 45, 1479–1482. [CrossRef]

Lu, C.; Jain, S.U.; Hoelper, D.; Bechet, D.; Molden, R.C.; Ran, L.; Murphy, D.; Venneti, S.; Hameed, B.R.; et al. Histone H3K36 mutations promote sarcomagenesis through altered histone methylation landscape. Science 2016, 352, 844–848. [CrossRef]

Yan, M.; Yang, X.; Wang, H.; Shao, Q. The critical role of histone lysine demethylase KDM2B in cancer. Am. J. Transl. Res. 2018, 10, 2222–2233.

Lee, Y.; Nguyen, A.T.; Zhang, Y. KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation and maintenance of acute myeloid leukemia. Blood 2011, 117, 3869–3880. [CrossRef]

Wang, Y.; Zhang, J.; Zhang, D.; Sun, Z.; Qiu, B.; Wang, X. KDM2B overexpression correlates with poor prognosis and regulates glioma cell growth. Oncotargets Ther. 2018, 11, 201–209. [CrossRef]

Heinemann, B.; Nielsen, J.M.; Hudlesbusch, H.R.; Lees, M.J.; Larsen, D.V.; Boesen, T.; Labelle, M.; Ger-lach, L.O.; Birk, P.; Helin, K. Inhibition of demethylase by GSK-11/J4. Nature 2014, 514, E1–E2. [CrossRef]

Mannironi, C.; Proietto, M.; Bufalieri, F.; Cundari, E.; Alagia, A.; Danovska, S.; Rinaldi, T.; Famiglini, V.; Coluccia, A.; La Regina, G.; et al. An high-throughput in vivo screening system to select H3K4-specific histone demethylase inhibitors. PLoS ONE 2014, 9, e86002. [CrossRef]

Agger, K.; Miyagi, S.; Pedersen, M.T.; Kooistra, S.M.; Johansen, J.V.; Helin, K. Jmdj2/Kdm4 demethylases are required for expression of Il3ra and survival of acute myeloid leukemia cells. Genes Dev. 2016, 30, 1278–1288. [CrossRef]

Agger, K.; Nishimura, K.; Miyagi, S.; Messling, J.E.; Rasmussen, J.; Helin, K. The KDM4/JMJD2 histone demethylases are required for hematopoietic stem cell maintenance. Blood 2019, 134, 1154–1158. [CrossRef] [PubMed]

Moreira, L.; Lübbert, M.; Jung, M. Targeting histone methyltransferases and demethylases in clinical trials for cancer therapy. Clin. Epigenetics 2016, 8, 57. [CrossRef]

Shilatifard, A. The COMPASS family of histone H3K4 methylases: Mechanisms of regulation in development and disease pathogenesis. Annu. Rev. Biochem. 2012, 81, 65–95. [CrossRef] [PubMed]

Milne, T.A.; Briggs, S.D.; Brock, H.W.; Martin, M.E.; Gibbs, D.; Allis, C.D.; Hess, J.L. MLL targets SET domain methyltransferase activity to hox gene promoters. Mol. Cell. 2002, 10, 1107–1117. [CrossRef]

Sze, C.C.; Shilatifard, A. MLL3/MLL4/COMPASS Family on Epigenetic Regulation of Enhancer Function and Cancer. Cold Spring Harb. Perspect. Med. 2016, 6, a026427. [CrossRef]

Denissov, S.; Hofemeister, H.; Marks, H.; Kranz, A.; Ciotta, G.; Singh, S.; Anas-Tassiadis, K.; Stunnenberg, H.G.; Stewart, A.F. Mll2 is required for H3K4 trimethylation on bivalent promoters in embryonic stem cells, whereas Mll1 is redundant. Development 2014, 141, 526–537. [CrossRef] [PubMed]

Yamane, K.; Tateishi, K.; Klose, R.J.; Fang, J.; Fabrizio, L.A.; Erdjument-Bromage, H.; Taylor-Papadimitriou, J.; Tempst, P.; Zhang, Y. PLU1 is an H3K4 demethylase involved in transcriptional repression and breast cancer cell proliferation. Mol. Cell. 2007, 25, 801–812. [CrossRef]

Xiang, Y.; Zhu, Z.; Han, G.; Ye, X.; Xu, B.; Peng, Z.; Ma, Y.; Yu, Y.; Lin, H.; Chen, A.P.; et al. JARID1B is a histone H3 lysine 4 demethylase up-regulated in prostate cancer. Proc. Natl. Acad. Sci. USA 2007, 104, 19226–19231. [CrossRef]

Christensen, J.; Agger; Cloos, P.A.; Pasini, D.; Rose, S.; Sennels, L.; Rappsilber, J.; Hansen, K.H.; Salcini, A.E.; Helin, K. RBP2 belongs to a family of demethylases, specific for tri-and dimethylated lysine 4 on Histone 3. Cell 2007, 128, 1063–1076. [CrossRef]

Metzger, E.; Wissmann, M.; Yin, N.; Müller, J.M.; Schneider, R.; Peters, A.H.F.M.; Günther, T.; Buettner, R.; Schüle, R. LSD1 demethylates repressive histone marks to promote androgen-receptor-dependent transcription. Nature 2005, 437, 436–439. [CrossRef]

Wang, Y.; Zhang, H.; Chen, Y.; Sun, Y.; Yang, F.; Yu, W.; Liang, J.; Sun, L.; Yang, X.; Shi, L.; et al. LSD1 Is a Subunit of the Nu RD Complex and Targets the Metastasis Programs in Breast Cancer. Cell 2009, 138, 660–672. [CrossRef] [PubMed]

Fang, R.; Chen, F.; Dong, Z.; Hu, D.; Barbera, A.J.; Clark, E.A.; Fang, J.; Yang, Y.; Mei, P.; Rutenberg, M.; et al. LSD2/KDM1B and its cofactor NPAC/GLYR1 endow a structural and molecular model for regulation of H3K4 demethylation. Mol. Cell 2013, 49, 558–570. [CrossRef] [PubMed]

Adamo, A.; Sesè, B.; Boue, S.; Castaño, J.; Paramonov, I.; Barrero, M.; Belmonte, J.C.I. LSD1 regulates the balance between self-renewal and differentiation in human embryonic stem cells. Nat. Cell Biol. 2011, 13, 652–659. [CrossRef] [PubMed]

Wang, J.; Hevi, S.; Kurash, J.K.; Lei, H.; Gay, F.; Bajko, J.; Su, H.; Sun, W.; Chang, H.; Xu, G.; et al. The lysine de-methylase LSD1 (KDM1) is required for maintenance of global DNA methylation. Nat. Genet. 2009, 41, 125–129. [CrossRef] [PubMed]
145. Whyte, W.A.; Bilodeau, S.; Orlando, D.A.; Hoke, H.A.; Frampton, G.M.; Foster, C.T.; Cowley, S.M.; Young, R.A. Enhancer decommissioning by LSD1 during embryonic stem cell differentiation. Nature 2012, 482, 221–225. [CrossRef] [PubMed]

146. Maiques-Diaz, A.; Somervaille, T.C. LSD1: Biologic roles and therapeutic targeting. Epigenomics 2016, 8, 1103–1116. [CrossRef]

147. Defeo-Jones, D.; Huang, P.S.; Jones, R.E.; Haskell, K.M.; Vuocolo, G.A.; Hanobik, M.G.; Huber, H.E.; Oliff, A. Cloning of cDNAs for cellular proteins that bind to the retinoblastoma gene product. Nature 1991, 352, 251–254. [CrossRef]

148. Klose, R.J.; Yan, Q.; Tothova, Z.; Yamane, K.; Erdjument-Bromage, H.; Tempst, P.; Gilliland, D.G.; Zhang, Y.; Kaelin, W.G. The Retinoblastoma Binding Protein RBP2 Is an H3K4 Demethylase. Cell 2007, 128, 889–900. [CrossRef]

149. Lu, P.; Sundquist, K.; Baecskstrom, D.; Poulsom, R.; Hanby, A.; Meier-Evert, S.; Jones, T.; Mitchell, M.; Pitha-Rowe, P.; Freemont, P.; et al. A Novel Gene (PLU-1) Containing Highly Conserved Putative DNA/Chromatin Binding Motifs Is Specifically Up-regulated in Breast Cancer. J. Biol. Chem. 1999, 274, 15633–15645. [CrossRef]

150. Barrett, A.; Madsen, B.; Copier, J.; Lu, P.; Cooper, L.; Scibetta, A.G.; Burchell, J.; Taylor-Papadimitriou, J. PLU-1 nuclear protein, which is upregulated in breast cancer, shows restricted expression in normal human adult tissues: A new cancer/testis antigen? Int. J. Cancer 2002, 101, 581–588. [CrossRef]

151. Iwase, S.; Lu, P.J.; Xia, Z.; Rincon-Arano, H.; Rothbart, S.B.; Peng, D.; Wen, H.; Larsson, C.; Zhang, X.; Zheng, X.; et al. The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. Cell 2007, 128, 1077–1088. [CrossRef] [PubMed]

152. Iwase, S.; Brookes, E.; Agarwal, S.; Badeaux, A.I.; Ito, H.; Vallianatos, C.N.; Tomassy, G.S.; Kasza, T.; Lin, G.; Thompson, A.; et al. A mouse model of X-linked intellectual disability associated with impaired removal of histone methylation. Cell Rep. 2016, 14, 1000–1009. [CrossRef] [PubMed]

153. Gonçalves, T.F.; Gonçalves, A.P.; Rodrigues, N.F.; dos Santos, J.M.; Pimentel, M.M.G.; Santos-Rebouças, C.B. KDM5C mutational screening among males with intellectual disability suggestive of X-Linked inheritance and review of the literature. Eur. J. Med Genet. 2014, 57, 138–144. [CrossRef] [PubMed]

154. Barrett, A.; Madsen, B.; Copier, J.; Lu, P.; Cooper, L.; Scibetta, A.G.; Burchell, J.; Taylor-Papadimitriou, J. PLU-1 nuclear protein, which is upregulated in breast cancer, shows restricted expression in normal human adult tissues: A new cancer/testis antigen? Int. J. Cancer 2002, 101, 581–588. [CrossRef] [PubMed]

155. Scibetta, A.G.; Santangelo, S.; Coleman, J.; Hall, D.; Chaplin, T.; Copier, J.; Catchpole, S.; Burchell, J.; Taylor-Papadimitriou, J. Functional analysis of the transcription repressor PLU-1/JARID1B. Mol. Cell Biol. 2007, 27, 7220–7235. [CrossRef]

156. Tu, S.; Teng, Y.-C.; Yuan, C.; Wu, Y.-T.; Chan, M.-Y.; Cheng, A.-N.; Lin, P.-H.; Tian, J.-L.; Tsai, M.-D. The ARID domain of the H3K4 demethylase RBP2 binds to a DNA CCGCCC motif. Nat. Struct. Mol. Biol. 2008, 15, 419–421. [CrossRef]

157. Torres, I.O.; Kuchenbecker, K.M.; Nnadi, C.I.; Fletterick, R.J.; Kelly, M.J.S.; Fujimori, D.G. Histone demethylase KDM5A is regulated by its reader domain through a positive-feedback mechanism. Nat. Commun. 2015, 6, 6204. [CrossRef]

158. Zhang, Y.; Yang, H.; Guo, X.; Song, Y.; Xu, Y.; Lan, W.; Zhang, X.; Liu, M.; Xu, Y.; et al. The PHD1 finger of KDM5B recognizes unmodified H3K4 during the demethylation of histone H3K4me2/3 by KDM5B. Protein Cell 2014, 5, 837–850. [CrossRef] [PubMed]

159. Klein, B.J.; Piao, L.; Xie, Z.; Rincon-Aranho, H.; Rothbart, S.B.; Peng, D.; Wen, H.; Larsson, C.; Zhang, X.; Zheng, X.; et al. The histone H3K4-specific demethylase KDM5B binds to its substrate and product through distinct PHD fingers. Cell Rep. 2014, 6, 325–335. [CrossRef]

160. Horton, J.R.; Engstrom, A.; Zoeller, E.L.; Liu, X.; Shanks, J.R.; Zhang, X.; Johns, M.A.; Vertino, P.M.; Fu, H.; Cheng, X. Characterization of a Linked Junomir Domain of the KDM5/JARID1 Family of Histone H3 Lysine 4 Demethylases. J. Biol. Chem. 2016, 291, 2631–2646. [CrossRef]

161. Longbotham, J.E.; Chio, C.; Dharmarajan, V.; Tinika, M.; Torres, I.O.; Goswami, D.; Ruiz, K.; Burlingame, A.L.; Griffin, P.R.; Fujimori, D.G. Histone H3 binding to the PHD1 domain of histone demethylase KDM5A enables active site remodeling. Nat. Commun. 2019, 10, 94. [CrossRef] [PubMed]

162. El Hayek, L.; Tuncay, I.O.; Nijem, N.; Russell, J.; Ludwig, S.; Kaur, K.; Li, X.; Anderton, P.; Tang, M.; Gerard, A.; et al. KDM5A mutations identified in autism spectrum disorder using forward genetics. eLife 2020, 9, e56883. [CrossRef] [PubMed]

163. Mizukami, H.; Kim, J.-D.; Tabara, S.; Lu, W.; Kwon, C.; Nakashima, M.; Fukamizu, A. KDM5D-mediated H3K4 demethylation is required for sexually dimorphic gene expression in mouse embryonic fibroblasts. J. Biochem. 2018, 165, 335–342. [CrossRef] [PubMed]

164. Kosugi, M.; Otani, M.; Kikkawa, Y.; Itakura, Y.; Sakai, K.; Ito, T.; Toyoda, M.; Sekita, Y.; Kimura, T. Mutations of histone demethylase genes encoded by X and Y chromosomes, Kdm5c and Kdm5d, lead to noncompaction cardiomyopathy in mice. Biochem. Biophys. Res. Commun. 2020, 525, 100–106. [CrossRef] [PubMed]

165. Wang, T.; Kim, C.; Bakken, T.E.; Gillentane, M.A.; Henning, B.; Mao, Y.; Gilissen, C.; The SPARK Consortium; Nowokowski, T.J.; Eichler, E.E. Integrated analyses of de novo mutations from 46,612 trios with autism and developmental disorders. bioRxiv 2021. [CrossRef]

166. Albert, M.; Schmitz, S.U.; Kooistra, S.M.; Malatesta, M.; Morales Torres, C.; Reiking, J.C.; Johansen, J.V.; Abarrategui, I.; Helin, K. The histone demethylase Jarid1b ensures faithful mouse development by protecting developmental genes from aberrant H3K4me3. PLoS Genet. 2013, 9, e1003461. [CrossRef]
167. Barrett, A.; Santangelo, S.; Tan, K.; Catchpole, S.; Roberts, K.; Spencer-Dene, B.; Hall, D.; Scibetta, A.; Burchell, J.; Verdin, E.; et al. Breast cancer associated transcriptional repressor PLU-1/JARID1B interacts directly with histone deacetylases. *Int. J. Cancer* **2007**, *121*, 265–275. [CrossRef]

168. Li, Q.; Shi, L.; Gui, B.; Yu, W.; Wang, J.; Zhang, D.; Han, X.; Yao, Z.; Shang, Y. Binding of the Jmjd demethylase JARID1B to LSD1/NuRD suppresses angiogenesis and metastasis in breast cancer cells by repressing chemokine CCL14. *Cancer Res.* **2011**, *71*, 6899–6908. [CrossRef]

169. Tahiliani, M.; Mei, P.; Fang, R.; Leonor, T.; Rutenberg, M.; Shimizu, F.; Li, J.; Rao, A.; Shi, Y. The histone H3K4 demethylase SMCX links REST target genes to X-linked mental retardation. *Nature* **2007**, *447*, 601–605. [CrossRef]

170. Nishibuchi, G.; Shibata, Y.; Hayakawa, T.; Hayakawa, N.; Ohhtani, Y.; Simmyozu, K.; Tagami, H.; Nakayama, J.-I. Physical and functional interactions between the histone H3K4 demethylase KDM5A and the nucleosome remodeling and deacetylase (NuRD) complex. *J. Biol. Chem.* **2014**, *289*, 28956–28970. [CrossRef]

171. Penterling, B.; Drexler, G.A.; Höhl, C.; Stamp, R.; Wilke, C.; Braselmann, H.; Caldwell, R.B.; Reindl, J.; Girst, S.; Greurl, C.; et al. Depletion of histone demethylase Jarid1A resulting in histone hyperacetylation and radiation sensitivity does not affect DNA double-strand break repair. *PLoS ONE* **2016**, *11*, e0156599. [CrossRef] [PubMed]

172. Pasini, D.; Hansen, K.H.; Christensen, J.; Agger, K.; Cloos, P.A.; Helin, K. Coordinated regulation of transcriptional repression by the RBIP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes Dev.* **2008**, *22*, 1345–1355. [CrossRef] [PubMed]

173. Zhang, Y.; Liang, J.; Li, Q. Coordinated regulation of retinoic acid signaling pathway by KDM5B and polycomb repressive complex 2. *J. Cell. Biochem.* **2014**, *115*, 1528–1538. [CrossRef] [PubMed]

174. Vedadi, M.; Blazer, L.; Eram, M.S.; Bartsyte-Lovejoy, D.; Arrowsmith, C.H.; Hajian, T. Targeting human SET1/MLL family of proteins. *Sci. Prog.* **2017**, *96*, 662–676. [CrossRef]

175. Ford, D.J.; Dingwall, A.K. The cancer COMPASS: Navigating the functions of MLL complexes in cancer. *Cancer Genet.* **2015**, *208*, 1780–1791. [CrossRef]

176. Muntean, A.G.; Tan, J.; Sitwala, K.; Huang, Y.; Bronstein, J.; Connelly, J.A.; Basrur, V.; Elenitoba-Johnson, K.S.; Hess, J.L. The PAF complex synergizes with MLL fusion proteins at HOX loci to promote leukemo-genesis. *Cancer Cell* **2010**, *17*, 609–621. [CrossRef]

177. Fong, C.Y.; Gilan, O.; Lam, E.Y.N.; Huang, X.; Wang, J.; Zhang, D.; Han, X.; Yao, Z.; Shang, Y. Binding of the JmjC demethylase JARID1B to LSD1/NuRD suppresses angiogenesis and metastasis in breast cancer cells by repressing chemokine CCL14. *Cancer Res.* **2011**, *71*, 6899–6908. [CrossRef]

178. Pasini, D.; Hansen, K.H.; Christensen, J.; Agger, K.; Cloos, P.A.; Helin, K. Coordinated regulation of transcriptional repression by the RBIP2 H3K4 demethylase and Polycomb-Repressive Complex 2. *Genes Dev.* **2008**, *22*, 1345–1355. [CrossRef] [PubMed]

179. Zhang, Y.; Liang, J.; Li, Q. Coordinated regulation of retinoic acid signaling pathway by KDM5B and polycomb repressive complex 2. *J. Cell. Biochem.* **2014**, *115*, 1528–1538. [CrossRef] [PubMed]

180. Wang, L.; Zhao, Z.; Ozark, P.A.; Fantini, D.; Marshall, S.A.; Rendleman, E.J.; Cozzolino, K.A.; Louis, N.; He, X.; Morgan, M.A.; et al. Resetting the epigenetic balance of polycomab and COMPASS function at enhancers for cancer therapy. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 8513–8518. [CrossRef]

181. Mohammad, H.P.; Smitheman, K.N.; Kamat, C.D.; Soong, D.; Federowicz, K.E.; Van Aller, G.S.; Schneck, J.L.; Carson, J.D.; Liu, Y.; Butticello, M.; et al. A DNA Hypomethylation Signature Predicts Antitumor Activity of LSD1 Inhibitors in SCLC. *Int. J. Cancer* **2017**, *140*, 1113–1125. [CrossRef] [PubMed]

182. Ellis, L.; Loda, M. LSD1: A single target to combat lineage plasticity in lethal prostate cancer. *Leukemia.* **2009**, *23*, 221–230. [CrossRef]

183. Banck, M.S.; Li, S.; Nishio, H.; Wang, C.; Beutler, A.S.; Walsh, M.J. The ZNF217 oncogene is a candidate organizer of repressive functional interactions between the histone H3K4 demethylase KDM5A and the nucleosome remodeling and deacetylase (NuRD) complex. *J. Biol. Chem.* **2014**, *289*, 28956–28970. [CrossRef]

184. Maes, T.; Tirapu, I.; Mascaró, C.; Ortega, A.; Estiarte, A.; Valls, N.; Castro-Palomino, J.; Arjol, C.B.; Kurz, G. Preclinical characterization of a potent and selective inhibitor of the histone demethylase KDM5A for MLL leukemia. *Epigenetics* **2009**, *4*, 100–106. [CrossRef] [PubMed]

185. Maes, T.; Tirapu, I.; Mascaró, C.; Ortega, A.; Estiarte, A.; Valls, N.; Castro-Palomino, J.; Arjol, C.B.; Kurz, G. Preclinical characterization of a potent and selective inhibitor of the histone demethylase KDM1A for MLL leukemia. *J. Clin. Oncol.* **2013**, *31*, e13543. [CrossRef]

186. Ishikawa, Y.; Gamo, K.; Yabuki, M.; Takagi, S.; Toyoshima, K.; Nakayama, K.; Nakayama, A.; Morimoto, M.; Miyashita, H.; Dairiki, R.; et al. A Novel LSD1 inhibitor T-3775440 disrupts GFI1B-containing complex leading to transdifferentiation and impaired growth of AML cells. *Mol. Cancer Ther.* **2015**, *14*, 2731–2738. [CrossRef]

187. Ishikawa, Y.; Gamo, K.; Yabuki, M.; Takagi, S.; Toyoshima, K.; Nakayama, K.; Nakayama, A.; Morimoto, M.; Miyashita, H.; Dairiki, R.; et al. A Novel LSD1 inhibitor T-3775440 disrupts GFI1B-containing complex leading to transdifferentiation and impaired growth of AML cells. *Mol. Cancer Ther.* **2015**, *14*, 2731–2738. [CrossRef]

188. Maiques-Diaz, A.; Spencer, G.J.; Lynch, J.T.; Ciceri, F.; Williams, E.L.; Amaral, F.M.; Wiseman, D.; Harris, W.J.; Sahoo, S.; et al. Enhancer Activation by Pharmacologic Displacement of LSD1 from GFI1 Induces Differentiation in Acute Myeloid Leukemia. *Cell Rep.* **2018**, *22*, 3641–3659. [CrossRef]

189. Vinyard, M.E.; Su, C.; Siegenfeld, A.P.; Waterbury, A.L.; Freey, A.M.; Gosavi, P.M.; Park, Y.; Kwan, E.E.; Senzer, B.; Doench, J.G.; et al. CRISPR-suppressor scanning reveals a nonenzymatic role of LSD1 in AML. *Nat. Chem. Biol.* **2019**, *15*, 529–539. [CrossRef]
190. Tsai, M.-C.; Manor, O.; Wan, Y.; Mosammaparast, N.; Wang, J.K.; Lan, F.; Shi, Y.; Segal, E.; Chang, H.Y. Long noncoding RNA as modular scaffold of histone modification complexes. Science 2010, 329, 689–693. [CrossRef]

191. Gupta, R.A.; Shah, N.; Wang, K.C.; Kim, J.; Horlings, H.M.; Wong, D.J.; Tsai, M.-C.; Hung, T.; Argani, P.; Rinn, J.L.; et al. Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis. Nature 2010, 464, 1071–1076. [CrossRef] [PubMed]

192. Pastori, C.; Kapranov, P.; Penas, C.; Peschansky, V.; Volmar, C.-H.; Sarkaria, J.N.; Bregy, A.; Komotar, R.; St Laurent, G.; Ayad, N.G.; et al. The Bromodomain protein BRD4 controls HOTAIR, a long noncoding RNA essential for glioblastoma proliferation. Proc. Natl. Acad. Sci. USA 2015, 112, 8326–8331. [CrossRef] [PubMed]

193. Harmeyer, K.M.; Facompre, N.D.; Herlyn, M.; Basu, D. JARID1 Histone Demethylases: Emerging Targets in Cancer. Trends Cancer 2017, 3, 713–725. [CrossRef] [PubMed]

194. Han, M.; Wu, Y.; Cheng, P.; Jin, H.; Wang, X. al Histone demethylase lysine demethylase 5B in development and cancer. Oncotarget 2017, 8, 8980–8991. [CrossRef]

195. Xhabija, B.; Kidder, B.L. KDM5B is a master regulator of the H3K4-methylome in stem cells, development and cancer. Semin. Cancer Biol. 2018, 57, 79–85. [CrossRef]

196. Yamamoto, S.; Wu, Z.; Russnes, H.G.; Takagi, S.; Peluffo, G.; Vasce, C.; Zhao, X.; Vollan, H.K.M.; Maruyama, R.; Ekram, M.B.; et al. JARID1B is a luminal lineage-driving oncogene in breast cancer. Cancer Cell 2014, 25, 762–777. [CrossRef]

197. Montano, M.M.; Yeh, I.-J.; Chen, Y.; Hernandez, C.; Kiselar, J.G.; De La Fuente, M.; Lawes, A.M.; Nieman, M.T.; Kiser, P.D.; Jacobberger, J.; et al. Inhibition of the histone demethylase, KDM5B, directly induces re-expression of tumor suppressor protein HEXIM1 in cancer cells. Breast Cancer Res. 2019, 21, 138. [CrossRef]

198. Dai, B.; Hu, Z.; Huang, H.; Zhu, G.; Xiao, Z.; Wan, W.; Zhang, P.; Jia, W.; Zhang, L. Overexpressed KDM5B is associated with the progression of glioma and promotes glioma cell growth via downregulating p21. Biochem. Biophys. Res. Commun. 2014, 454, 221–227. [CrossRef]

199. Ricketts, C.J.; Lineham, W.M. Gender Specific Mutation Incidence and Survival Associations in Clear Cell Renal Cell Carcinoma (CCRCC). PLoS ONE 2015, 10, e0140257. [CrossRef]

200. Smith, J.A.; Haberstroh, F.S.; White, E.A.; Livingston, D.M.; DeCaprio, J.A.; Howley, P.M. SMCX and components of the TIP60 complex contribute to E2 regulation of the HPV E6/E7 promoter. Virology 2014, 468–470, 311–321. [CrossRef]

201. Li, N.; Dhar, S.S.; Chen, T.-Y.; Kan, P.-Y.; Wei, Y.; Kim, J.-H.; Chan, C.-H.; Lin, H.-K.; Hung, M.-C.; Lee, M.G. JARID1D Is a Suppressor and Prognostic Marker of Prostate Cancer Invasion and Metastasis. Cancer Res. 2016, 76, 831–843. [CrossRef] [PubMed]

202. Komura, K.; Jeong, S.H.; Hinohara, K.; Qu, F.; Wang, X.; Hiraki, M.; Azuma, H.; Lee, G.S.; Kantoff, P.W.; Sweeney, C.J. Resistance to docetaxel in prostate cancer is associated with androgen receptor ac-tivation and loss of KDM5D expression. Proc. Natl. Acad. Sci. USA 2016, 113, 6259–6264. [CrossRef] [PubMed]

203. Wong, S.H.; Goode, D.L.; Iwasaki, M.; Wei, M.C.; Kuo, H.-P.; Zhu, L.; Schneidawind, D.; Duque-Afonso, J.; Weng, Z.; Cleary, M.L. The H3K4-Methyl Epigenome Regulates Leukemia Stem Cell Oncogenic Potential. Cancer Cell 2015, 28, 198–209. [CrossRef] [PubMed]

204. Sharma, S.V.; Lee, D.Y.; Li, B.; Quinlan, M.P.; Takahashi, F.; Maheswaran, S.; McDermott, U.; Azizian, N.; Zou, L.; Fischbach, M.A.; et al. A chromatin-mediated reversible drug-tolerant state in cancer cell subpopulations. Cell 2010, 141, 69–80. [CrossRef] [PubMed]

205. Roesch, A.; Fukunaga-Kalabis, M.; Schmidt, E.C.; Brafford, P.A.; Vultur, A.; Basu, D.; Gimotty, P.; Vogt, T.; Herlyn, M. A Temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell 2010, 141, 583–594. [CrossRef]

206. Vinogradova, M.; Gehling, V.S.; Gustafson, A.; Arora, S.; Tindell, C.A.; Wilson, C.; Williamson, K.E.; Guler, G.D.; Gangurde, P.; Manieri, W.; et al. An inhibitor of KDM5 demethylases reduces survival of drug-tolerant cancer cells. Cancer Cell 2016, 329, 531–538. [CrossRef]

207. Johannsson, C.; Velupillai, S.; Tumber, A.; Szykońska, A.; Hookway, E.S.; Nowak, R.; Strain-Damerell, C.; Gileadi, C.; Philpott, M.; Burgess-Brown, N.; et al. Structural analysis of human KDM5B guides histone demethylase inhibitor development. Nat. Chem. Biol. 2016, 12, 539–545. [CrossRef]

208. Sayegh, J.; Cao, J.; Zou, M.R.; Morales, A.; Blair, L.P.; Norcia, M.; Hoyer, D.; Tackett, A.J.; Merkel, J.S.; Yan, Q. Identification of small molecule inhibitors of jumonji AT-rich interactive domain 1B (JARID1B) histone demethylase by a sensitive high throughput screen. J. Biol. Chem. 2013, 288, 9408–9417. [CrossRef]

209. Bovetsias, V.; Lanigan, R.M.; Ruda, G.F.; Atrash, B.; McLaughlin, M.G.; Tumber, A.; Mok, N.Y.; Le Bihan, Y.-V.; Dempster, S.; Boxall, K.J.; et al. 8-Substituted Pyrido[3,4-d]pyrimidin-4(3H)-one Derivatives As Potent, Cell Permeable, KDM4 (JMJD2) and KDM5 (JARID1) Histone Lysine Demethylase Inhibitors. J. Med. Chem. 2016, 59, 1388–1409. [CrossRef]

210. Leadem, B.R.; Kagiampakis, I.; Wilson, C.; Cheung, T.K.; Arnott, D.; Trojer, P.; Classon, M.; Easwaran, H.; Baylin, S.B. A KDM5 Inhibitor Increases Global H3K4 Trimethylation Occupancy and Enhances the Bi-ological Efficacy of 5-Aza-2’-Deoxycytidine. Cancer Res. 2018, 78, 1127–1139. [CrossRef]

211. Madsen, B.; Spencer-Dene, B.; Poulsom, R.; Hall, D.; Lu, P.; Scott, K.; Shaw, A.T.; Burchell, J.M.; Freemont, P.; Taylor-Papadimitriou, J. Characterisation and developmental expression of mouse Plu-1, a homologue of a human nuclear protein (PLU-1) which is specifically up-regulated in breast cancer. Mech. Dev. 2002, 119, s239–s246. [CrossRef]