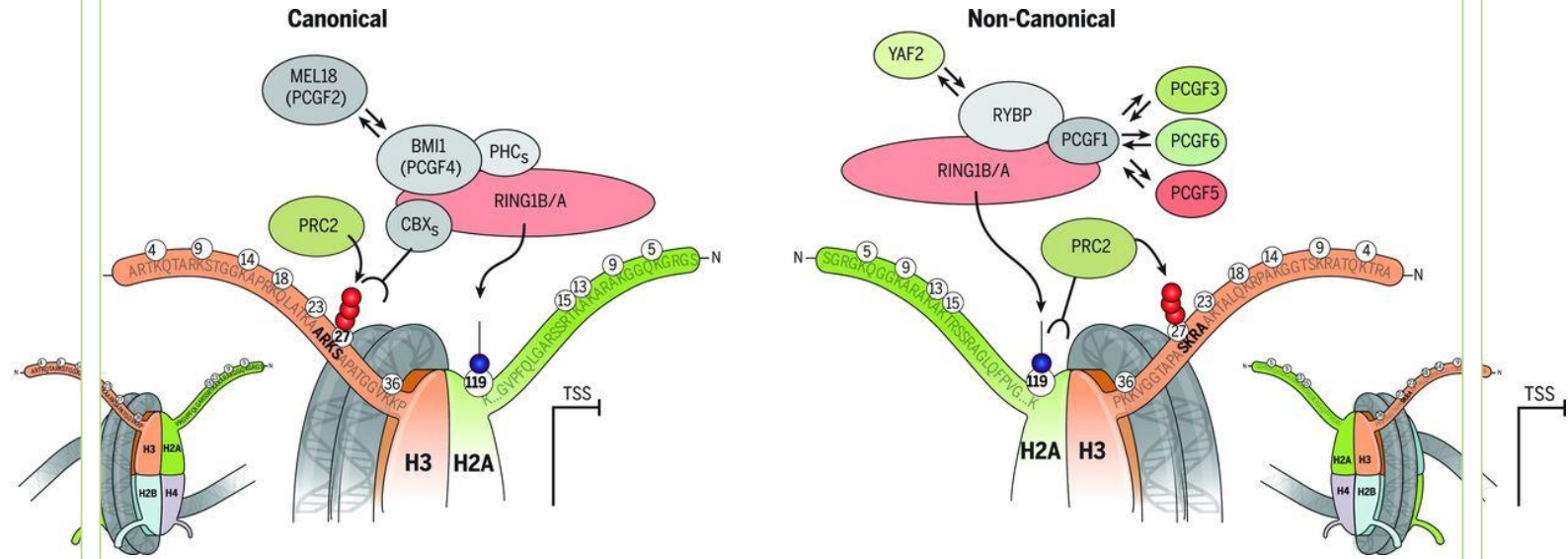
Drug-targeting the non-canonical PRC1.1 complex to delay leukemogenesis

Master essay at the department of Drug Design and Experimental Hematology

1-10-2017
Rijksuniversiteit Groningen
Anne de Groot



university of groningen / faculty of science and engineering

Drug-targeting the non-canonical PRC1.1 complex to delay leukemogenesis

Master essay at the department of Drug Design and Experimental Hematology

October 2017

Rijksuniversiteit Groningen (RuG)
Anne de Groot
S3258246

Supervisors: M. Groves (Drug Design)
J.J. Schuringa (Experimental Hematology)

[1] Reference figure at the front: A. Piunti, Epigenetic balance of gene expression by polycomb and COMPASS families, 2016

3

Contents

Introduction

Leukemic stem cells (LSCs) are responsible for continued proliferation and differentiation of clonal leukemia daughter cells. Currently, there are many treatment strategies for leukemic patients to remove leukemia cells from the body. However, many patients undergo relapse of the disease leading to an overall reduction in the survival rate of leukemia. This is because traditional therapies are focused on killing leukemic daughter cells without targeting the LSCs (1) (2).Therefore, it is suggested that targeting LSCs in combination with chemotherapy or tyrosine kinase inhibitors (TKIs) will result in the simultaneous eradication of LSCs and leukemic daughter cells within the body (3). LSCs can arise from normal hematopoietic stem cells (HSCs) or from normal progenitor cells with the capacity for self-renewal (4). Normally, HSCs provide for the differentiation of progenitor cells and matured blood cells. In addition to differentiation, HSCs also regulate self-renewal to maintain sufficient HSCs in the bone marrow. It is known that LSCs differ in their self-renewal capacity and that aberrant activation of self-renewal pathways, such as WNT, NOTCH, Hedgehog and BMP, are hallmarks of leukemia (5). Further, it is described that enhanced self-renewal activity leads to leukemic transformation (6).

Role of epigenetics in leukemia

Epigenetic mechanisms play an important role in the development of cancer and their regulators are frequently mutated in cancer. Mutated epigenetics lead to uncontrolled self-renewal of cancer stem cells (7). It is known that epigenetic modifiers like the polycomb group (PcG) protein family play an important role in the self-renewal capacity of stem cells (8). It is also known that overexpression and mutation of PcG proteins are related to the development of leukemia (9). Normally, PcG genes encode for different PcG proteins which form complexes and regulate the epigenetic silencing of genes by chromatin remodelling and histone modification (10). There are two main PcG protein complexes; polycomb repressive complex 1 (PRC1) and 2 (PRC2). PRC2 trimethylates histone 3 at lys27 which then recruits the PRC1 subunit CBX to chromatin. After binding of PRC1 to H3K27me3, the RING1A/B domain functions as a ubiquitin ligases and ubiquitinates lys119 on histone 2A (H2AK119ub) (11) (12). Ubiquitination leads to chromatin compaction and subsequently blocking of polymerase II, whereby transcription will be silenced (11) (13) (14) (figure 1).

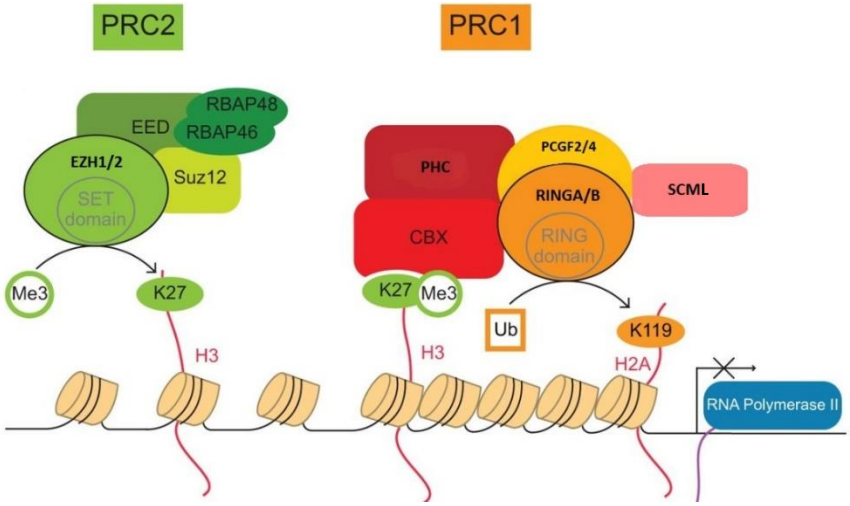


Figure 1 Interplay between PRC 1 and PRC2. PRC2 contains the subunits; EED, SUZ12 and EZH1/2. The SET domain of EZH1/2 trimethylates lys27 on histone H3 (H3K27me3). Then PRC1, consisting of the subunits PCGF2/4, PHC, CBX, SCML and RING1A/B, binds to H3K27me3 via its subunit CBX and ubiquitinates lys119 on histone 2A (H2AK119ub) through the RING1A/B subunit. H2AK119ub blocks ,polymerase II activation, which leads to transcriptional silencing **(15) (16)**.

However, several studies have demonstrated that PRC1 is able to add a ubiquitin group to H2AK119 in the absence of PRC2 (17) (18). Therefore, it is suggested that a non-canonical polycomb repressive complex 1 exists which consists of different subunits leading to targeting the DNA directly independently of H3K27 trimethylation.

Non-canonical PRC1

In total there are six PRC1 complexes. Two of those are canonical, known as PRC1.2 and PRC1.4 as they consist of the subunits PCGF 2 or 4. The other four PRC1 complexes, PRC1.1, PRC1.3, PRC1.5 and PRC1.6 are non-canonical. It is demonstrated that non-canonical PRC1.1 (ncPRC1.1) plays an important role in leukemogenesis and that knockdown of the ncPRC1.1 subunits results in delay of leukemia cell growth. It is also known that ncPRC1.1 is able to target[1] the DNA in the absence of PRC2 (15). Therefore, the role of canonical PRC1 and ncPRC1.1 subunits are described in tables 1 and 2 and their action is demonstrated in figure 2. First, the ncPRC1.1 subunit KDM2B recognizes and binds non-methylated CpG islands (15) (19). Therefore, ncPRC1.1 is in the position to bind to DNA in the absence of trimethylated H3K27 by the PRC2 complex. Next, the ubiquitin ligase activity of RING1A/B can add a ubiquitin group to H2AK119 (20) (21) (22) (23). However, the subunit USP7 acts as a de-ubiquitination enzyme (DUB) and it is suggested that USP7 indirect regulates the deubiquitination of H2AK119 (24). It is suggested that USP7 controls the ubiquitin levels of the polycomb proteins PCGF and RING1A/B. Therefore, a loss of the USP7 domain leads to too high ubiquitin levels of the polycomb complex members resulting nin dissembly of the complex (25) (26). Therefore, ncPRC1.1 activates transcription instead of blocking the polymerase II activation by the canonical PRC1 complex.

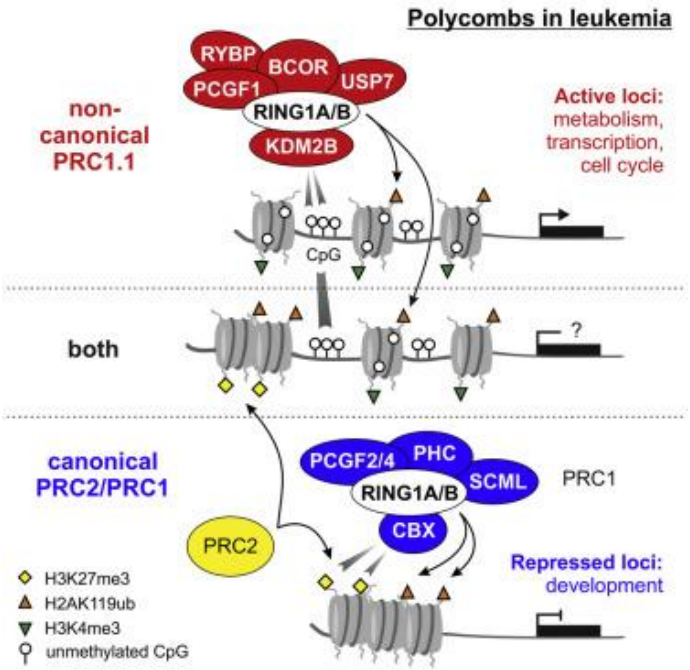


Figure 2 Non-canonical vs Canonical PRC1 complexes. Non-canonical PRC1.1 binds to non-methylated CpG islands with its KDM2B subunit. Thereafter, USP7 regulates deubiquitination of H2AK119ub which leads to an active locus. Canonical PRC1 binds to PRC2 trimethylated H3K27me3 with its CBX subunit. Thereafter, RING1A/B ubiquitinates H2AK119ub which leads to a repressed loci (15).

In this essay the research question is as follows;

- Is it possible to propose drug targets within the self-renewal pathway in leukemia stem cells and prevent relapse of the disease?

With the following sub-questions;

- Can we propose models in which inhibition of the polycomb repressive complex, an epigenetic modifier which regulates self-renewal of stem cells, lead to eradication of leukemia cells?

- Within these models, which subunits of the polycomb repressive complex are likely to be the best drug-targets to inhibit the function of the polycomb repressive complex?

Table 1 Canonical PRC complexes. Abbreviations: PCGF = polycomb group ring finger, PHC = polyhomeotic-like protein, CBX= polycomb chromobox, SCML= sex combs on midleg-like, RING = E3 ubiquitin-protein ligase, BMI = B-cell specific Moloney murine leukemia virus integration site. *RING1A/B is also present in the non-canonical PRC1.1 complex

Canonical PRC1 complexes

	Subunits	Function	Evidence
PRC1	PCGF2/4	- BMI1 (PCGF4) is required for correct formation of the PRC1 complex (12) (27) - BMI1 (PCGF4) is required for RING1A/B ligase activation (12) (27) - MEL18 (PCGF2) only stabilizes PRC1 but is not able to enhance RING1A/B ligase activation (12) (27)	- BMI1 (PCGF4) overexpression promotes lymphoma in mice (28) - BMI1 regulates self-renewal of leukemic stem cells (29) (30) - BMI1 enhances symmetrical cell division of HSCs (31) - BMI plays a role in bypassing senescence by repressing the INK4A/ARF locus (32)
	PHC	- Interacts with PCGF2/4 (33)	- B-cells maturation in splenocytes is blocked in the absence of PHC (34)
	CBX	- Recognizes PRC2 trimethylated H3K27 (11) (35) (36) (37) - Recruits PRC1 to chromatin (38) - Its chromodomain is a reader of methyl-lysine in histone tails (39)	- *In vivo*, H3K27me3 recruits CBX7 and CBX8 and not CBX2, CBX4 and CBX6 (40)
	SCML	- May regulates anatomical structure morphogenesis in embryo's (41)	- No relevant data found about the function *in vitro* or *in vivo*
	Ring1A/B*	- Ubiquitin E3 ligases (11) (42) - Guide transfer of a ubiquitin group on H2AK119 (H2AK119ub1) (11) (42) - Forms a dimer with PCGF (11) (42) - Its RAWUL domain can interact with Cbx members (43)	- Upregulated in AML cells (15) - Knockdown of RING1 did not impair MLL-AF9 translocation (44)

Table 2 Subunits of non-canonical PRC1 complexes. PCGF = polycomb group ring fingers, RYBP = Ring1 and YY1 Binding protein, YAF2 = YY1-associated factor, BCOR = BCL6 Corepressor, KDM2B = Lysine Demethylase 2B, USP = Ubiquitin Specific Protein, ES = embryonic stem cell, CLL = Chronic Lymphoblastic Leukemia, HDAC = Histone deacetylases.

Non-canonical PRC1 complexes

	Subunits	Function	Evidence
PRC1.1	PCGF1	- Supports histone H2A monoubiquitination (45) - Transcriptional activation (46) - Recruits RING1B to target genes (46) - Required for enzymatic activity of PCGF-RING1A/B (11) (42)	- Upregulated in AML cells (15) - Pcgf1 terminates the self-renewal program in hematopoietic progenitor cells (47) - In ES cells, Pcgf1 interacts with H2A and enhances H2A ubiquitination *in vivo* and *in vitro* (45) - Pcgf1 is a transcriptional activator for genes associated with mesoderm and ectoderm development and are related to differentiation in ES cells (46) - Knockdown of PCGF1 leads to reduced levels of AML cells *in vitro* (15)

RYBP (or its homolog YAF2)	- Binds to promotors with its DNA binding domains (48) - Interact with the RAWUL domain of Ring 1A/B (36) (48) (49) (50) - Recruits PcG complexes (51) - Transcriptional repressor (52) - RYBP/YAF2 stimulates H2A ubiquitination (specific for ncPRC1.1 complex) (53)	- RYBP interacts with repressor domain of E2E6 which leads to transcriptional repression *in vitro* (52) - RYBP reduces growth of clonal MLL-AF9 AML cells in vitro (54) - RYBP expression reduces leukemia and induces differentiation of hematopoietic cells in vivo (54) - In vitro, RYBP/YAF2 regulates ncPRC1.1 activation and thereby stimulate RING1B to enhance the ubiquitination of H2AK119 (53) - Knockdown of RYBP has a mild effect on reduction of MLL-AF9 AML cells *in vitro* (15) - RYBP/YAF2 interaction with RING1B-PCGF1 is responsible for the enzymatic activity of ncPRC1.1 *in vitro* (53)
BCOR(L1)	- Direct binding partner of PCGF (33) (55) - Corepressor when tethered to DNA (56) - Interacts with class-II HADCs (56)	- Upregulated in AML cells (15) - Knockdown of BCOR leads to reduced levels of AML cells *in vitro* (15) - BCOR loss of function mutant cells are linked to enhanced differentiation of myeloid cells (57) - Loss of BCOR is insufficient to induce myeloid malignancies *in vitro* (58)
KDM2B	- Binds to non-methylated CpG islands with CXXC domain (55) (59) (60) - It PHD domain binds to nucleosomes (61) (62) (63) and specific to H3K4 trimethylated nucleosomes (55) (64) - Recruits the ncPRC1.1 to the DNA (19) (65) - Its JmjC domain act as a histone demethylase (66)	- Knockdown of KDM2B leads to reduced levels of MLL-AF9 AML cells *in vitro* (15) - KDM2B maintains the human lymphoblastic leukemia cell population in vitro (67)
USP7	- De-ubiquitination of histone H2A (68).	- USP7 is overexpressed both at mRNA and protein level in CLL cells (69) (70) - *In vitro*, USP7 can remove ubiquitin from chromatin templates, mainly it de-ubiquitinates histone H2A and H2B (68) - USP7 ablation by RNAi treatment reduces H3K27 trimethylation (unpublished data in (71)) - USP7 knockdown decreases H3K27 trimethylation and overexpression increases H3K27me3 (72) - Loss-of-function mutations of USP7 are found in paediatric leukemia patients (73) - Targeting USP7 with a small molecule overcomes apoptosis resistance and induces apoptosis in CLL cells (69) (70)
RING1A/B	- Same as in canonical PRC1	

Non-canonical PRC1.1 complex as drug-target for leukemia treatment

It is known that the canonical PRC1 subunits, PCGF (28) (74) (75), CBX (76) and RING (75) are overexpressed in leukemia. Therefore, inhibiting those subunits could be supportive to get rid of the LSCs in leukemia. However, canonical PRC1 also plays an important role in the normal haematopoiesis and therefore we do not want to disturb this complex by targeting it. Identically, targeting RING1A/B is not the way to go as it is part of the canonical PRC1 complex as well.

As described in tables 1 and 2, the ncPRC1.1 subunits also play a role in the normal hematopoiesis. In addition, the ncPRC1.1 complex is associated with leukemia, as described by van der Boom et al. (15). Here we will describe which ncPRC1.1 subunits are promising targets to treat leukemia using small compounds to inhibit their activity or disturb the associated protein:protein interactions. First, van der Boom et al. showed that knockdown of PCGF1 leads to reduced levels of AML cells (15). However, Ross et al. found that PCGF1 knockdown leads to increased self-renewal activity of hematopoietic progenitor cells. Further, they showed that depletion of both PCGF1 and RUNX1 leads to constant self-renewal activity and loss of differentiating cells *in vitro* (47). Therefore, it is suggested that PCGF1 is needed for maturing hematopoietic cells and that it is blocking the clonal leukemia cell formation by terminating the self-renewal program. Ross et al. suggest that PCGF1 down-regulates the transcription of *HoxA* genes, by uniquitinating HoxA gene promoters, and thereby terminates self-renewal of hematopoietic cells. In addition, it is shown that *HoxA* overexpression ultimately leads to leukemia *in vivo* (77) (78). For this reason, PCGF1 is probably not a good target because PCGF is needed to terminate this self-renewal activity. However, another study suggested that ubiquitination of H2AK119 is not mediated by PCGF1, as the level of H2AK119ub1 does not change in PCGF1$^{-/-}$ embryonic stem (ES) cells[2]. They also found that PCGF1 does not regulate the self-renewal capacity of ES cells (46). They suggest that PCGF1 interacts with another ncPRC1.1 subunit, KDM2B, which then recruits the ncPRC1.1 complex to DNA for ubiquitination as described above. In figure 3, Yan et al. schematically demonstrated how they think PCGF1 interacts with KDM2B and regulates H2AK119 ubiquitination. They also found that lack of PCGF1 in ES cells reduces RING1B levels together with H2AK119ub1 levels (46). Therefore, we suggest that KDM2B together with PCGF1 is needed for the ubiquitination activity of RING1 in ncRC1.1. Therefore, it would be interesting to disturb this protein:protein interaction between PCGF1 and KDM2B[3]. This could prevent the formation of a functional ncPRC1.1 complex.

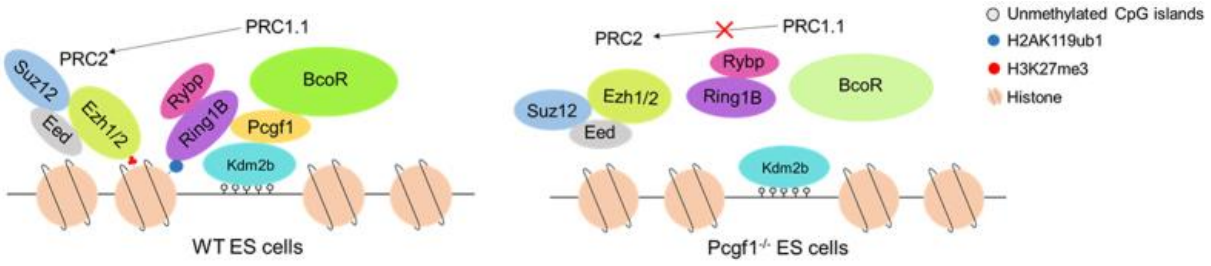


Figure 3 Function of PCGF1 within the non-canonical PRC1.1 complex in ES cells. KDM2B recruits ncPRC1.1 to non-methylated CpG islands in the presence of PCGF1. Subsequently, H2AK119 becomes monoubiquitinated (H2AK119ub1) which leads to activation of PRC2. In PCGF1$^{-/-}$ cells, only KDM2B binds to the DNA, without the other ncPRC1.1 subunits. Hereby, the PRC2 complex is not activated **(46)**. So, Yan et al. suggest that PCGF1 is needed to link KDM2B to the rest of the ncPRC1.1 complex and thereby without PCGF1 the ncPRC1.1 complex cannot ubiquitinate H2AK119 and subsequently silencing the *HoxA* genes.

To examine this, co-immunoprecipitation (Co-IP) experiments are needed to confirm if KDM2B alone does not lead to self-renewal termination in hematopoietic cells. With this experiments we should compare HSCs with LSCs and examine if PCGF1-KDM2B interaction is present in those cells. If KDM2B alone also leads to self-termination of hematopoietic cells, then PCGF1 is not a good target. This would suggest that KDM2B interacts with more ncPRC1.1 subunits then only PCGF1. If KDM2B alone does not lead to self-termination, this will suggest that only PCGF1 is needed to link KDM2B with the rest of the

ncPRC1.1 complex. PCGF1 is only a good target if KDM2B interacts with PCGF1 in LSCs and not in HSCs. So, this should be examined with Co-IP as well. From Co-IP experiments it is already known that PCGF1 interacts with BCOR and that the canonical PRC1 subunit PCGF4 does not interact with BCOR (79). From another study it is known that PCGF1 only interacts with KDM2B if it first did interact with BCOR (80). Figure 4 shows the interaction between KDM2B, PCGF1 and BCOR (figure 4A). In addition, figure 4 shows how the PCGF1/BCOR dimer interacts with the LRR domain of KDM2B (figure 4B).

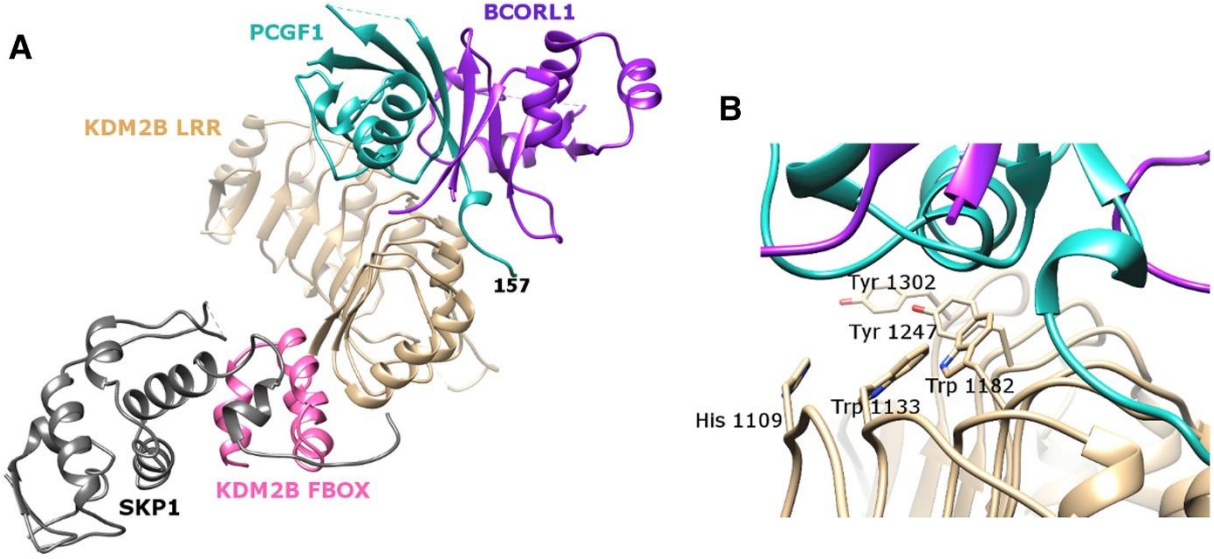


Figure 4 The crystal structures of BCOR, PCGF1 and KDM2B and their interactions. A) The interaction between the ncPRC1.1 subunits BCOR (purple), PCGF1 (green) and KDM2B (gold) (and also SKP1(black), which will not be further discussed in this essay). B) Alternating KDM2B LRR residues shows the binding site for the PCGF1/BCOR dimer **(80)**.

Furthermore, the enzymatic activity of ncPRC1.1 depends on the interaction of RING1B-PCGF1 with RYBP/YAF2 (53). It has been found that RYBP expression reduces proliferation and colony-forming of MLL-rearranged AML cells *in vivo*. Therefore, it has been suggested that RYBP is an suppressor of MLL-AML cells (54). In addition, knockdown of RYBP in MLL-AF9 AML cells did not have a major effect on the leukemia development *in vitro* (15). Further, when RYBP is removed from the ncPRC1.1 complex, the subunits BCOR and KDM2B can still interact with the RING1B-PCGF1 dimer (53). In conclusion, inhibition of RYBP with a small molecule is not likely to reduce the leukemia development, because probably the ncPRC1.1 complex can play its role without the RYBP subunit. That explains why knockdown of the RYBP complex has a mild effect on reduction of MLL-AF9 AML cells.

Furthermore, the subunit BCOR also is not a promising target for small molecules to slow down leukemia. Although, knockdown of BCOR leads to reduction of AML cells in vitro (15) and mutated BCOR leads to enhanced differentiation of myeloid cells (57), a study demonstrated that BCOR acts as a tumor suppressor in T-cell acute lymphoblastic leukemia (81). This study showed that BCOR knockdown did not change the levels of ubiquitinated H2A and trimethylated H3K27 in T cells. Further, by using ChIP-seq analysis they showed that BCOR binds to *Myc* and *Hes1* which are promotors of the NOTCH pathway. Moreover, they demonstrated that BCOR act as an antagonist for activation of oncogenes in the NOTCH1 pathway which thereby induces T-ALL (81).

Interestingly, KDM2B seems to be a promising subunit to inhibit. Van der boom et al. showed that knockdown of KDM2B leads to reduction of AML cells *in vitro* and *in vivo*. In addition, KDM2B knockdown blocks Hoxa9/Meis1-induced leukemia development (82). Moreover, KDM2B is needed to maintain the human lymphoblastic leukemia cell population and depletion of KDM2B leads to impaired lymphoid lineage *in vitro*. This appears to act by sustaining the NOTCH signalling pathway. In addition, KDM2B suppresses the WNT signalling pathway (67) which is, together with the NOTCH signalling pathway, a pathway for self-renewal in hematopoietic cells (6). Considering that KDM2B is the subunit which recruits the ncPRC1.1 complex to the DNA which then allows ncPRC1.1 to activate *Hox* genes

transcription, makes KDM2B a promising drug-target. In fact, it is suggested that transcription of *Hox* genes is linked to enhanced self-renewal activity of hematopoietic cells and subsequently to expansion of leukemia cell population (83) (84) (85). Therefore, drug-targeting KDM2B will probably lead to non-functional ncPRC1.1, as it cannot bind to the DNA, which blocks the *Hox* gene transcription and thereby leukemia development. This also suggest that targeting the CXXC DNA binding domain is the best way to block the function of KDM2B within the ncPRC1.1 complex. An earlier study already showed that the reprogramming program in induced pluripotent stem cells (iPSC) is dependent on the CXXC domain instead of the enzymatic activity domain, JMJC. This study used murine fibroblast and induced reprogramming with OSK factors. Further, they compared cells mutated for the KDM2B JmjC and CXXC domain. The CXXC domain seems to be more important for reprogramming without having an effect on the proliferation of fibroblasts (86) (figure 5).

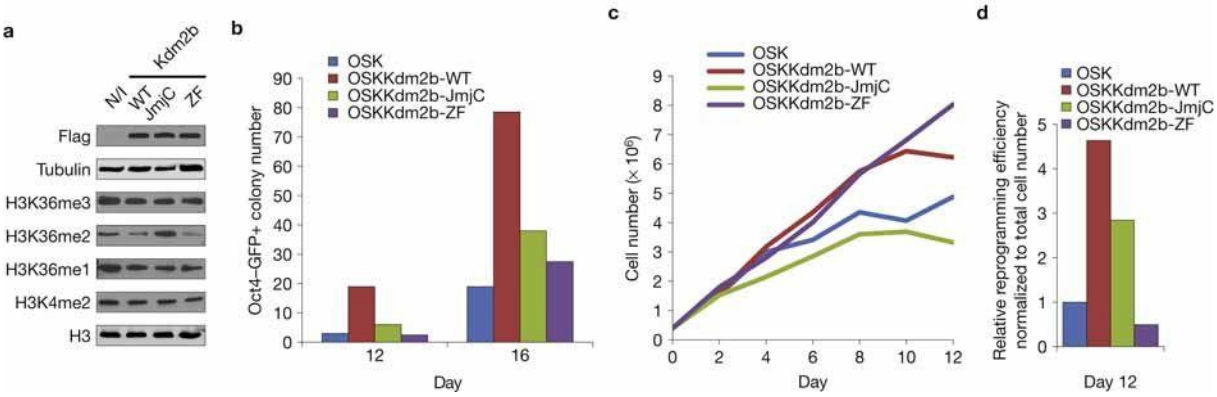


Figure 5 The effect of mutation in the CXXC (ZF) and JmJC domain of KDM2B on reprogramming capacity in iPSCs. A) WT KDM2B and mutated CXXC (ZF) KDM2B leads to decreased levels of H3K36me2, suggesting that the CXXC domain, and not the JmjC domain, plays a role in methylation of H3K36. B) Mutations of both CXXC (ZF) and JmjC lead to a reduced number of colonies, suggesting that both domains are necessary for enhanced reprogramming. C) Cell proliferation is increased in CXXC (ZF) mutants, suggesting that CXXC domain is not necessary for cell proliferation. D) Relative reprogramming efficacy is decreased in both CXXC (ZF) and JmjC mutants. However, the CXXC domain mutant shows the lowest reprogramming efficiency. Therefore, the CXXC domain seems to be more important in reprogramming **(86)**.

In addition, another study showed that the function of KDM2B depends on it CXXC domain and not its JmjC domain in ES cells. KDM2B regulates repression of the lineage-specific genes and is needed to maintain the ES cell pluripotent state. However depletion of KDM2B leads to expression of lineage-specific genes independent of its JmjC domain (87). The CXXC domain together with the PHD domain are named the zinc fingers of KDM2B and are demonstrated in figure 6. The CXXC domain is shown in red (figure 6).

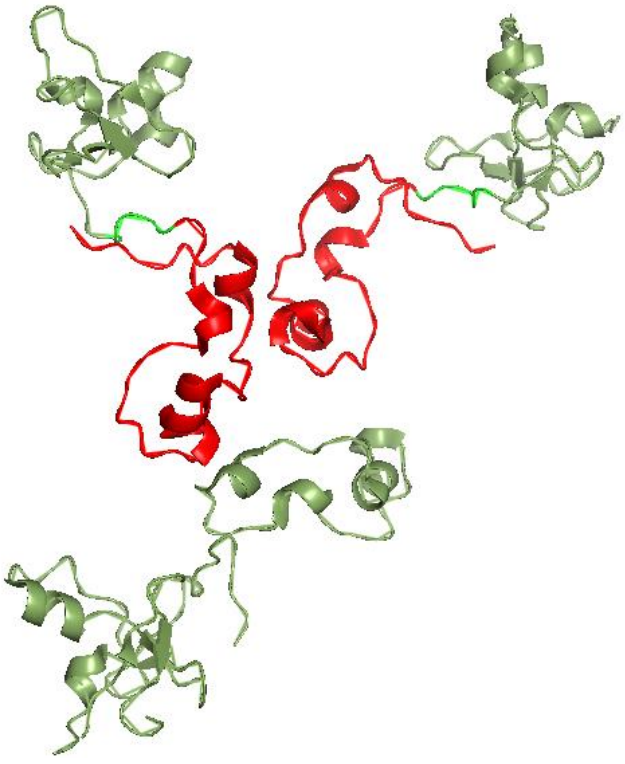


Figure 6 Zinc fingers of KDM2B. The zinc fingers consist out of two domain, the PHD domain and the CXXC domain (PBD ID: 4o64). The CXXC domain (608-652) is shown in red and the PHD domain (659-723) in green.

Finally, USP7 seems to play an important role in the development of leukemia and knockdown of USP7 overcomes apoptosis resistance in CLL cells (69) (70). Therefore, it could be suggested that inhibiting the function of USP7 within the ncPRC1.1 complex prevents leukemia relapsing. Chauhan et al. demonstrated that USP7 inhibition overcomes bortezomib, an anti-cancer drug, resistance in multiple myeloma cells (88)[4]. As histone 2A ubiquitnation is needed for PRC1 dependent gene repression (11) (14) and USP7 deubiquitinates histone 2A (68) [4] it is suggest that USP7 plays an important role for gene activation within the ncPRC1.1 complex. It is also suggested that inhibiting the catalytic domain of USP7 prevents USP7 to bind with ubiquitin, which therefore is a promising domain to inhibited. However, the same study also suggested that targeting protein interaction domains probably would be a better domain to target. Hereby USP7 is not able to form a complex, for example with the ncPRC1.1 complex. N-terminal TRAF-like domain (NTD) of USP7 is an example of a protein-protein interaction domain (89). Therefore, drug-targeting the USP7-NTD domain is a promising target to reduce leukemogenesis. The crystal structure of USP7 is demonstrated in figure 7. Both the catalectic domain (yellow) and the protein-protein interaction, TRAF-like domain (blue) are visible. The dark green structure is ubiquitin-aldehyde and thereby shows the ubiquitin biding site of the catalytic domain. A shallow groove on the surface of the NTD domain can interact with other proteins, in this case with the Epstein-Barr virus protein (EBNA1) (red). In addition, other proteins can interact with USP7 via its USP7-CTD domain, which consist of several Ubl domains (grey and pink) (90). Another study found that the Ubl domains 1 and 2 of USP7 interact with SRA-RING domain of UHRF1 (Ubiquitin-like, containing PHD and RING finger domains, 1). Moreover, they also found that Ubl1 alone was not able to interact with UHRF1 (91). This is an interesting finding regarding USP7-RING1A/B interaction within the ncPRC1.1 complex. Therefore, targeting the USP7-Ubl2 domain to disturb the formation of the USP7-PRC1.1 complex may inhibit the deubiquitination activity of ncPRC1.1. Several studies have shown that deubiquitination of H2AK119u1 is linked to transcriptional activation of *Hox* genes (92) (93) (94). Overexpression of *Hox* genes is linked to leukemia (77) (78) and therefore disturbing the USP7-PRC1.1 interaction maybe leads to better prognosis in leukemia.

In summary, ncPRC1.1 subunits KDM2B and USP7 are the most promising for drug-targeting. Knockdown of both subunit lead to reduction of leukemia cells. The CXXC domain seems to be the best domain to drug-target KDM2B as KDM2B cannot recruit the ncPRC1.1 complex to the DNA without this domain. Further, disturbing the interaction between KDM2B and PCGF1 with small molecules seems to be a promising treatment method to stop the transcription of leukemia related genes by ncPRC1.1. For USP7, the Ubl2 domain seems to be the best drug-targeting side as the Ubl2 domain probably interacts with the RING1A/B domain of the ncPRC1.1 complex and without this interaction ncPRC1.1 probably cannot activate transcription of leukemia related genes.

Specificity of drug-targeting non-canonical PRC1.1 subunits KDM2B and USP7

When designing a drug to target KDM2B or USP7, it is important that it only targets KDM2B in the leukemia cells and not somewhere else in the body as this can lead to side effects of the drug[5]. In table 3 it is summarized where KDM2B and USP7 are expressed at protein level in the body. KDM2B is mainly expressed in hematopoietic cells/organs which suggest that using a good therapeutic window would preferentially eradicate leukemia cells. However, KDM2B is also expressed in the liver, placenta, cervix, ovary and testis. Therefore, it is important to examine the consequences of KDM2B inhibition *in vivo*. Probably, inhibition of USP7 has a higher risk at side effects, as it is almost expressed everywhere in the body. However, the complex formation of USP7 via its Ubl2 is more specific. Besides binding the viral protein ICP0, Ubl2 can interact with the cellular proteins GMP synthase and UHRF1 (90). Therefore, specific drug-targeting the Ubl2 domain can reduce the leukemia cells without having a lot of off-target effects.

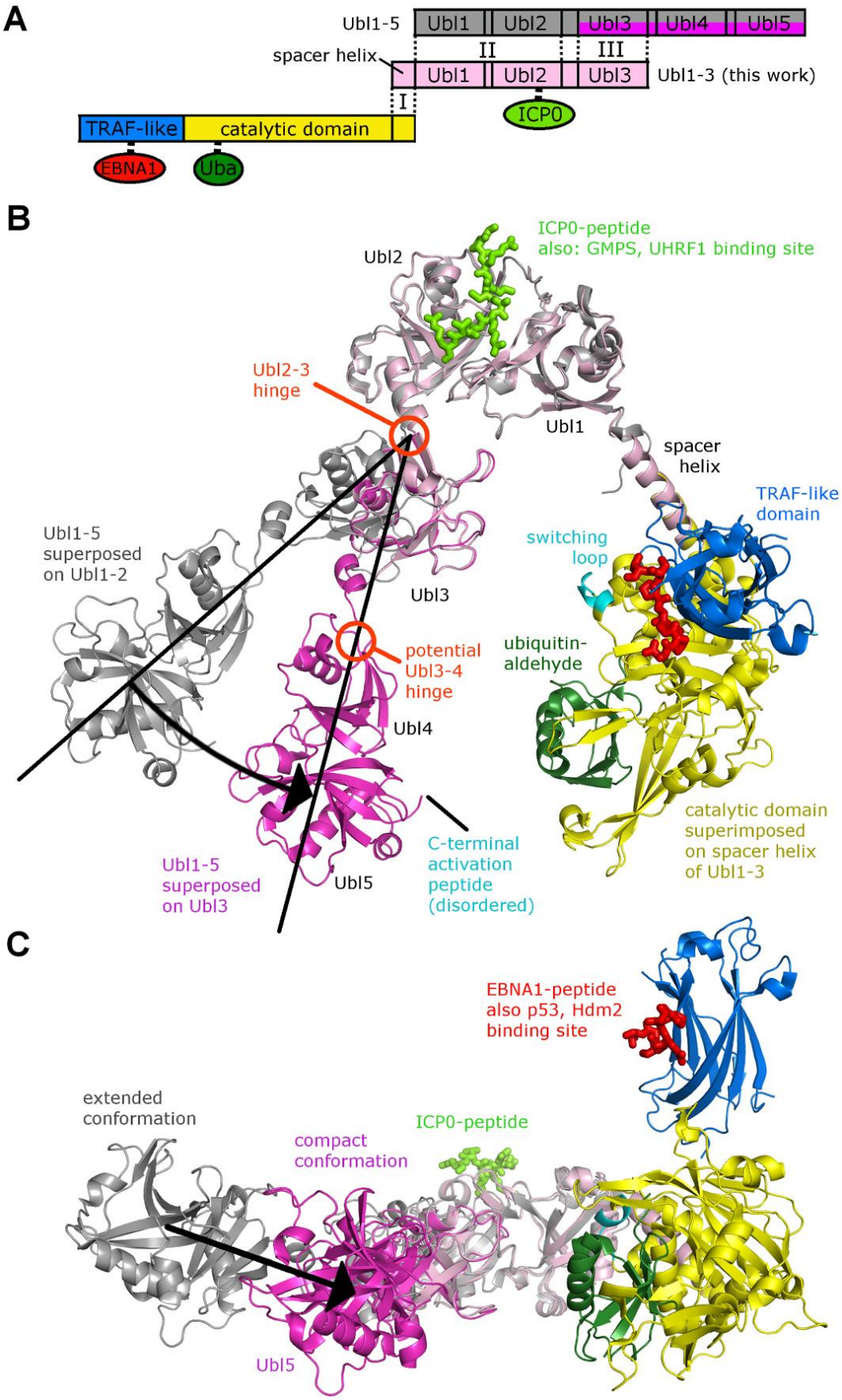


Figure 7 The crystalized structure of USP7. A) The different domains of USP7; the CTD domain, which contains 5 Ubl domains (grey/pink), the catalytic domain (yellow), which can bind ubiquitin-aldehyde (dark green) and the TRAF-like domain (blue), which can bound other proteins like EBNA1 (red). B, C) the crystalized structure of USP7 with the domains having the same color as shown in A **(90)**.

Table 3 Protein expression of PRC1.1 subunits KDM2B and USP7. Data obtained from Genecards, Weizmann Institute of Science 1996-2017.

PRC1.1 subunit	*Expression*
KDM2B	B-lymphocytes, CD4+ T-cells, CD8+ T-cells, platelets, bone marrow mesenchymal stem cell, bone, liver, spleen, placenta, cervix, ovary, testis.
USP7	monocytes, B-lymphocytes, T-lymphocytes, CD4+ T-cells, CD8+ T-cells, NK cells, peripheral blood mononuclear cells, platelets, lymph nodes, tonsils, brain, frontal cortex, spinal cord, retina, heart, colon muscle, oral epithelium, esophagus, stomach, cardiac, gut, colon, rectum, liver, kidney, spleen, lung, adipocyte, saliva, salivary gland, thyroid, adrenal, breast, pancreas, islet of Langerhans, Gallbladder, prostate, urinary bladder, skin, placenta, uterus, cervix, ovary, testis, seminal vesicle.

Conclusion

Recent studies showed us the important role of the canonical PRC1 complex and the ncPRC1.1 complex in normal hematopoiesis and leukemia development. It is shown that the ncPRC1.1 complex plays an important role in leukemogenesis (15). Therefore, drug targeting one of the ncPRC1.1 subunits probably delays the leukemogenesis. This essay summarizes the different studies which performed knockdown/depletion of the ncPRC1.1 subunits and subsequently studied the effect on hematopoiesis/leukemia development. Results of these studies suggest that drug-targeting the subunits KDM2B or USP7 is the most promising to treat leukemia. To answer the first sub-question; *Can we propose models in which inhibition of the polycomb repressive complex, a epigenetic modifier which regulates self-renewal of stem cells, lead to eradication of leukemia cells?*, we illustrated a proposed ncPRC1.1 complex in which we demonstrated the likely possible drug-target sites which thereby answers the second sub-question; *Within these models, which subunits of the polycomb repressive complex likely to be the best drug-targets to inhibit the function of the polycomb repressive complex?* (figure 8).

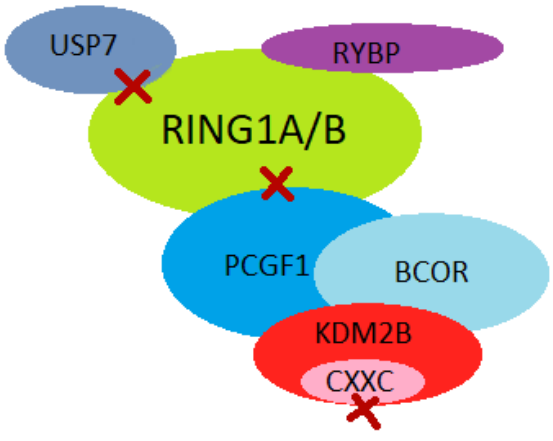


Figure 8 Proposed PRC1.1 model. RING1A/B forms a dimer with PCGF1 **(11) (42)**. PCGF1 also forms a dimer with BCOR to which KDM2B can bind **(80)**. The subunits RYBP **(36) (48) (49) (50)** and probably USP7 as well are also bound to RING1A/B. The red crosses indicate the possible drug-targeting sites.

First, KDM2B is the subunit which recruits ncPRC1.1 to non-methylated CpG islands domain (55) (59) (60). KDM2B is overexpressed in HSCs and this is linked to increased proliferation *in vitro* and *in vivo*.

Hence, it is suggested that KDM2B stimulates enhanced cell growth and induces leukemic transformation This confirmed by demonstrating that KDM2B overexpression leads to cells with high expression of the progenitor cell marker c-kit and low expression of myeloid markers. This also suggest that KDM2B blocks differentiation and keeps the cells in a progenitor like status by affecting the self-renewal capacity of those cells. Knockdown of KDM2B leads to reduction of Hoxa9/Meis1 induced leukemia which suggest that KDM2B activates *Hox* gene transcription. In addition, knockdown of KDM2B in LSCs leads to reduced self-renewal capacity and reduction of the clonal cell population. This suggest that KDM2B promotes *Hox* genes transcription whereby LCS undergo self-renewal which induces clonal daughter cell formation (82). Normally, canonical PRC1 represses *Hox* gene transcription (95) (96). Therefore, we suggest that blocking KDM2B recruitment to the Hox gene transcription regions will lead to eradication of leukemia daughter cells but also of prevent relapse of the disease as this is affecting the self-renewal capacity of leukemia stem cells. The best way to block the function of KDM2B is by targeting the CpG island recognizing domain, CXXC. This idea confirmed by He et al., showing that KDM2B regulates repression of the lineage-specific genes and promoting maintenance of the pluripotent state in ES cells with its CXXC domain (87). Besides hematopoietic cells and organs, KDM2B is known to be expressed in more organs. Therefore, when creating a drug targeting the CXXC domain, side effects should be avoided.

Therefore, disturbing the protein:protein interaction between KDM2B and the rest of the ncPRC1.1 complex is a better option to eradicate LSCs. As already described, it is suggested that PCGF1 links KDM2B to the rest of the ncPRC1.1 complex, therefore drug-targeting the KDM2B binding site of PCGF1 may eradicate LSCs. It is known that KDM2B interacts via its LRR domain with the BCOR-PCGF1 dimer (80). Another study showed that deletion of the F-box LRR domain disturbs the interaction between PCGF1 and RING1B (65). Also, when removing RING1B from the ncPRC1.1 complex, BCOR-PCGF1 can still assemble with KDM2B. It is demonstrated that BCOR alone cannot assemble with KDM2B but it is not yet known if PCGF1 alone can assemble with KDM2B (80). Therefore, it should be examined if PCGF1 alone can interact with KDM2B. It is known that the RAWUL domain of PCGF1 interacts with KDM2B. Therefore, drug-targeting the PCGF1-RAWUL domain could prevent the formation of a functional ncPRC1.1 complex and thereby reduce the amount of LSCs.

Finally, USP7 is the subunit which deubiquitinates histone 2A (68) and deubiqutination of lysine 119 at histone 2A is linked activation of *Hox* gene transcription (92) (93) (94). Therefore, we suggest that inhibiting the ncPRC1.1 subunit USP7 will lead to reduction of *Hox* induced leukemia. USP7 is known to maintain the neural stem/progenitor cell pool (97), indicating its important role in stem cell self-renewal. Zhang et al. found that the Ubl2 domain of USP7 interacts with the SRA-RING domain of UHRF1 (91). Therefore, we suggest that the Ubl2-USP7 domain also interact with the RING1A/B domain of ncPRC1.1. This makes the Ubl2 domain a good drug-target to block the function of USP7 within it ncPRC1.1 complex. When creating a drug which targets the Ubl2-USP7 domain, there should be examined if USP7 does not form a complex with other proteins via its Ubl2 domain. From literature it is known that the Ubl2-USP7 domain binds with the cellular proteins GMP synthase and URHF1 (90) but it is not yet known what the effect of blocking the binding of this proteins with USP7 is.

These findings help us to answer the research question of this essay; *Is it possible to propose drug targets within the self-renewal pathway in leukemia stem cells and prevent relapse of the disease?* It is known that the polycomb proteins play an important role in the self-renewal of LSCs and therefore we think that drug-targeting the polycomb repressive complex will lead to eradication of LSCs. To support this idea, knockdown studies of the subunits of the polycomb repressive complex already shows that drug-targeting this complex is promising in eradication of LSCs. To drug-target the non-canonical polycomb repressive complex, the subunits PCGF1, KDM2B and USP7 are the most promising. Therefore, future studies should focus on creating small molecules to target KDM2B or to disturb the protein:protein interaction between KDM2B and PCGF1 or between USP7 and RING1.

References

1. *Considerations for targeting malignant stem cells in leukemia.* **Guzman , M.L. en Jordan, C.T.** 2004, Cancer Control, pp. 97-104.

2. *Therapeutic targeting of acute myeloid leukemia stem cells.* **Pollyea, D.A. en Jordan, C.T.** 2017, Leukemic stem cells, pp. 1627-1635.

3. *Targeting self-renewal pathways in myeloid malignancies.* **Sands, W.A., Copland, M. en Wheadon, H.** 2013, Cell Communication & Signaling.

4. *Transformation from committed progenitor to leukaemia stem cell initiated by MLL–AF9.* **Krivtsov, A.V., et al.** 2006, Nature, pp. 818-822.

5. *Approaches for targeting self-renewal pathways in cancer stem cells: implications for hematological treatments.* **Horne, G.A. en Copland, M.** 2017, Expert Opinion on Drug Discovery, pp. 465-474.

6. *Hox genes in hematopoiesis and leukemogenesis.* **Argiropoulos, B. en Humphries, R.K.** 2007, Oncogene, pp. 6766-6776.

7. *Epigenetics and Cancer Stem Cells: Unleashing, Hijacking, and Restricting Cellular Plasticity.* **Wainwright, E.N. en Scaffidi, P.** 2017, Trends in Cancer, pp. 372-386.

8. *Polycomb-group proteins in hematopoietic stem cell regulation and hematopoietic neoplasms.* **Radulovic, V., de Haan, G. en Klauke, K.** 2013, Leukemia, pp. 523-533.

9. *The roles of Polycomb group proteins in hematopoietic stem cells and hematological malignancies.* **Takamatsu-Ichihara, E. en Kitabayashi, I.** 2016, International Journal of Hematology, pp. 634-642.

10. *Polycomb repressive complexes in hematological malignancies.* **Iwama, A.** 2017, Blood, pp. 23-29.

11. *Targeting Polycomb systems to regulate gene expression: modifications to a complex story.* **Blackledge, N.P., Rose, N.R. en Klose, R.J.** 2015, Progress, pp. 643-649.

12. *Epigenetic balance of gene expression by Polycomb and COMPASS families.* **Piunti, A. en Shilatifard, A.** 2016, Science.

13. *Native and recombinant polycomb group complexes establish a selective block to template accessibility to repress transcription in vitro.* **King, I.F., Francis, N.J. en Kingston, R.E.** 2002, Molecular Cell Biology, pp. 7919-7928.

14. *Roles of the Polycomb group proteins in stem cells and cancer.* **Richly, H., Aloia, L. en Di Croce, L.** 2011, Cell Death & Disease.

15. *Non-canonical PRC1.1 Targets Active Genes Independent of H3K27me3 and Is Essential for Leukemogenesis.* **van den Boom, V., et al.** 2016, Cell Reports, pp. 332-346.

16. *The emerging role of Polycomb repressors in the response to DNA damage.* **Vissers, J.H.A., van Lohuizen, M. en Citterio, E.** 2012, Journal of Cell Science, pp. 3939-3948.

17. *RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me.* **Tavares, L., et al.** 2012, Cell, pp. 664-678.

18. *Interdependence of PRC1 and PRC2 for recruitment to Polycomb Response Elements.* **Kahn, T.G., et al.** 2016, Nucleic Acids Research, pp. 10132-10149.

19. *KDM2B links the Polycomb Repressive Complex 1 (PRC1) to recognition of CpG islands.* **Farcas, A.M., et al.** 2012, eLIFE.

20. *Polycomb group proteins Ring1A/B link ubiquitylation of histone H2A to heritable gene silencing and X inactivation.* **Napoles, M., et al.** 2004, Developmental Cell, pp. 663-676.

21. *Role of histone H2A ubiquitination in Polycomb silencing.* **Wang, H., et al.** 2004, Nature, pp. 783-778.

22. *Structure and E3-ligase activity of the Ring-Ring complex of polycomb proteins Bmi1 and Ring1b.* **Buchwald, G., et al.** 2006, the EMBO Journal, pp. 2465-2474.

23. *Monoubiquitination of H2AX protein regulates DNA damage response signaling.* **Pan, M.R., et al.** 2011, Journal of Biological Chemistry, pp. 28599-28607.

24. *Regulation of USP7: A High Incidence of E3 Complexes.* **Kim, R.Q. en Sixma, T.K.** 2017, Journal of Molecular Biology.

25. *Regulation of the Polycomb protein RING1B ubiquitination by USP7.* **de Bie, P., Zaaroor-Regev, D. en Ciechanover, A.** 2010, Biochemical and biophysical research communications, pp. 389-395.

26. *USP7 cooperates with SCML2 to regulate the activity of PRC1.* **Lecona, E., Narendra, V. en Reinberg, D.** 2015, Molecular Cell Biology, pp. 1157-1168.

27. *Role of Bmi-1 and Ring1A in H2A ubiquitylation and Hox gene silencing.* **Cao, R., Tsukada, Y. en Zhang, Y.** 2005, Molecular Cell, pp. 845-854.

28. *Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF.* **Jacobs, J.J., et al.** 1999, Genes Development, pp. 2678-2690.

29. *Role of Bmi1 in H2A Ubiquitylation and Hox Gene Silencing.* **Wei, J., et al.** 2006, Journal of Biological Chemistry, pp. 22537-22544.

30. *miR-203 inhibits proliferation and self-renewal of leukemia stem cells by targeting survivin and Bmi-1.* **Zhang, Y., et al.** 2016, Scientific reports.

31. *Enhanced Self-Renewal of Hematopoietic Stem Cells Mediated by the Polycomb Gene Product Bmi-1.* **Iwama, A., et al.** 2004, Immunity, pp. 843-851.

32. *BMI1: A Biomarker of Hematologic Malignancies.* **Sahasrabuddhe, A.A.** 2016, Biomarkers in Cancer, pp. 65-75.

33. *Structure of the Polycomb Group protein PCGF1 (NSPC1) in complex with BCOR reveals basis for binding selectivity of PCGF homologs.* **Junco, S.E., et al.** 2013, Structure, pp. 665-671.

34. *Lack of the Polycomb-group gene rae28 causes maturation arrest at the early B-cell developmental stage.* **Tokimasa, S., et al.** 2001, Experimental Hematology, Journal of Hematology, Stem Cell Biology and Transplantation, pp. 93-103.

35. *Genome-wide mapping of Polycomb target genes unravels their roles in cell fate transitions.* **Bracken, A.P., et al.** 2006, Genes & Development, pp. 1123-1136.

36. *PCGF homologs, CBX proteins, and RYBP define functionally distrinct PRC1 family complexes.* **Gao, Z., et al.** 2012, Molecular Cell, pp. 344-356.

37. *Polycomb genes, miRNA, and their deregulation in B-cell malignancies.* **Wang, G.G., Konze, K.D. en Tao, J.** 2015, Blood, pp. 1217-1225.

38. *Transcriptional regulation by Polycomb group proteins.* **Croce, L.D. en Helin, K.** 2013, Nature Structural & Molecular Biology, pp. 1147-1155.

39. *Structure and Mechanisms of Lysine Methylation Recognition by the Chromodomain in Gene Transcription.* **Yap, K.L. en Zhou, M.** 2011, Biochemistry, pp. 1966-1980.

40. *Live-cell single-molecule tracking reveals co-recognition of H3K27me3 and DNA targets polycomb Cbx7-PRC1 to chromatin.* **Zhen, C.Y., et al.** 2016, Elife.

41. *Characterization of SCML1, a New Gene in Xp22, with Homology to Developmental Polycomb Genes.* **van de Vosse, E., et al.** 1998, Genomics, pp. 96-102.

42. *Occupying chromatin: Polycomb mechanisms for getting to genomic targets, stopping transcriptional traffic, and staying put.* **Simon, J.A. en Kingston, R.E.** 2013, Molecular Cell, pp. 808-824.

43. *Ring1B contains a ubiquitin-like docking module for interaction with Cbx proteins.* **Bezsonova, I, et al.** 2009, Biochemistry, pp. 10542-10548.

44. *CBX8, a Polycomb Group Protein, is Essential for MLL-AF9-Induced Leukemogenesis.* **Tan, J., et al.** 2011, Cancer Cell, pp. 563-575.

45. *Cooperation between EZH2, NSPc1-mediated histone H2A ubizuitination and DnmT1 in HOX gene silencing.* **Wu, X., et al.** 2008, Nucleic Acids Research, pp. 3590-3599.

46. *Loss of Polycomb Group Protein Pcgf1 Severely Compromises Proper Differentiation of Embryonic Stem Cells.* **Yan, Y., et al.** 2017, Scientific Reports.

47. *Polycomb group ring finger 1 cooperates with Runx1 in regulating differentiation and self-renewal of hematopoietic cells.* **Ross, K., et al.** 2012, Blood, pp. 4152-4161.

48. *RYBP, a new repressor protein that interacts with components of the mammalian Polycomb complex, and with the transcription factor YY1.* **Garcia, E., et al.** 1999, The EMBO Journal, pp. 3404-3418.

49. *Polycomb group targeting through different binding partners of RING1B C-terminal domain.* **Wang, R., et al.** 2010, Structure, pp. 966-975.

50. *RYBP-PRC1 complexes mediate H2A ubiquitylation at polycomb target sites independently of PRC2 and H3K27me3.* **Tavares, L., et al.** 2012, Cell, pp. 664-678.

51. *The E2F6 transcription factor is a component of the mammalian Bmi1-containing polycomb complex.* **Trimarchi, J.M., et al.** 2001, Proceedings of the National Academy of Sciences of the United States of America, pp. 1519-1524.

52. *YEAF1/RYBP and YAF-2 Are Functionally Distinct Members of a Cofactor Family for the YY1 and E4TF1/hGABP Transcription Factors.* **Sawa, C., et al.** 2002, Journal of Biological Chemistry, pp. YEAF1/RYBP and YAF-2 Are Functionally Distinct Members of a Cofactor Family for the YY1 and E4TF1/hGABP Transcription Factors.

53. *RYBP stimulates PRC1 to shape chromatin-based communication between Polycomb repressive complexes.* **Rose, N.R., et al.** 2016, Genes and Chromosomes.

54. *Polycomb Group Member Rybp Is a Functional Tumor Suppressor Repressed By Mir-9 in MLL-Rearranged AML.* **Price, C., et al.** 2014, Blood.

55. *Polycomb Group and SCF Ubiquitin Ligases Are Found in a Novel BCOR Complex That Is Recruited to BCL6 Targets.* **Gearhart, M.D., et al.** 2006, Molecular and Cellular Biology, pp. 6880-6889.

56. *A Novel Corepressor, BCoR-L1, Represses Transcription through an Interaction with CtBP.* **Pagan, J.K., et al.** 2007, Journal of Biological Chemistry, pp. 15248-15257.

57. *BCOR regulates myeloid cell proliferation and differentiation.* **Cao, Q., et al.** 2016, Leukemia, pp. 1155-1165.

58. *Role of Polycomb Gene BCOR in Hematopoiesis.* **Tanaka, T., et al.** 2014, Blood.

59. *Cloning of a mammalian transcriptional activator that binds unmethylated CpG motifs and shares a CXXC domain with DNA methyltransferase, human trithorax, and methyl-CpG binding domain protein 1.* **Voo, K.S., et al.** 2000, Molecular and Cellular Biology, pp. 2108-2121.

60. *The F-box protein Fb110 is a novel transcriptional repressor of c-Jun.* **Koyama-Nasu, R., David, G. en Tanese, N.** 2007, Nature Cell Biology, pp. 1074-1080.

61. *The PHD finger, a nuclear protein-interaction domain.* **Bienz, M.** 2006, Trends in Biochemical Science, pp. 35-40.

62. *The H3K36 demethylase Jhdm1b/Kdm2b regulates cell proliferation and senescence through p15(Ink4b).* **He, J., et al.** 2008, Nature Structural & Molecular Biology, pp. 1169-1175.

63. *The PHD finger: a versatile epigenome reader.* **Sanchez, R. en Zhou, M.M.** 2011, Trends in Biochemical Science, pp. 364-372.

64. *A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling.* **Wysocka, J., et al.** 2006, Nature, pp. 86-90.

65. *Fbxl10/Kdm2b recruits polycomb repressive complex 1 to CpG islands and regulates H2A ubiquitylation.* **Wu, X., Johansen, J.V. en Helin, K.** 2013, Molecular Cell, pp. 1134-1146.

66. *Histone demethylation by a family of JmjC domain-containing proteins.* **Tsukada, Y.I., et al.** 2006, Nature, pp. 811-816.

67. *Histone demethylase KDM2B regulates lineage commitment in normal and malignant hematopoiesis.* **Andricovich, J., et al.** 2016, The Journal of Clinical Investigation, pp. 905-920.

68. *Ubiquitin-specific proteases 7 and 11 modulate Polycomb regulation of the INK4a tumour suppressor.* **Maertens, G.N., et al.** 2010, The EMBO Journal, pp. 2553–2565.

69. *Therapeutic inhition of USP7-PTEN network in chronic lymphocytic leukemia: a strategy to overcome TP53 mutated/deleted clones.* **Carra, G., et al.** 2017, Oncotarget, pp. 35508-35522.

70. *USP7 inhibition alters homologous recombination repair and targets CLL cells independently of ATM/p53 functional status.* **Agathanggelou, A., et al.** 2017, Blood, pp. 156-166.

71. *GMP Synthetase Stimulates Histone H2B Deubiquitylation by the Epigenetic Silencer USP7.* **van der Knaap, J.A., et al.** 2005, Molecular cell, pp. 695-707.

72. *The stability of epigenetic factor ASXL1 is regulated through ubiquitination and USP7-mediated deubiquitination.* **Inoue, D., et al.** 2015, Leukemia, pp. 2257-2260.

73. *The landscape of somatic mutations in epigenetic regulators across 1000 pediatric cancer genomes.* **Huether, R., et al.** 2014, Nature Communications.

74. *The polycomb group BMI-1 gene is a molecular marker for predicting prognosis of chronic myeloid leukemia.* **Mohty, M., et al.** 2007, Blood, pp. 380-383.

75. *Overexpression of the EZH2, RING1 and BMI1 genes is common in myelodysplastic syndromes: relation to adverse epigenetic alteration and poor prognostic scoring.* **Xu, F., et al.** 2011, Annals of Hematology, pp. 643-653.

76. *Role of the chromobox protein CBX7 in lymphomagenesis.* **Scott, C.L., et al.** 2007, Proceedings of the National Academy of Science of the United States of America, pp. 5389-5394.

77. *Hoxa9 transforms primary bone marrow cells through specific collaboration with Meis1a but not Pbx1b.* **Kroon, E., et al.** 1998, the EMBO Journal, pp. 3714-3725.

78. *Overexpression of the myeloid leukemia-associated Hoxa9 gene in bone marrow cells induces stem cell expansion.* **Thorsteindottir, U., et al.** 2002, Blood, pp. 121-129.

79. *The variant Polycomb Repressor Complex 1 component PCGF1 interacts with a pluripotency sub-network that includes DPPA4, a regulator of embryogenesis.* **Oliviero, G., et al.** 2015, Scientific Reports.

80. *KDM2B Recruitment of the Polycomb Group Complex, PRC1.1, Requires Cooperation between PCGF1 and BCORL1.* **Wong, S.J., et al.** 2016, Structure, pp. 1795-1801.

81. *Internal deletion of BCOR reveals a tumor suppressor function for BCOR in T lymphocyte malignancies.* **Tanaka, T., et al.** 2017, the Jounal of Experimental Medicine.

82. *KDM2b/JHDM1b, an H3K36me2-specific demethylase, is required for initiation and maintenance of acute myeloid leukemia.* **He, J., Nguyen, A.T. en Zhang, Y.** 2011, Blood, pp. 3869-3880.

83. *Trithorax and polycomb cooperation in MLL fusion acute leukemia.* **Mereau, H. en Schwaller, J.** 2013, Haematologica, pp. 825-827.

84. *Epigenomic analysis of the HOX gene loci reveals mechanisms that may control canonical expression patterns in AML and normal hematopoietic cells.* **Spencer, D.H., et al.** 2015, Leukemia, pp. 1279–1289.

85. *MLL-AF9– and HOXA9-mediated acute myeloid leukemia stem cell self-renewal requires JMJD1C.* **Zhu, N., et al.** 2016, the Journal of Clinical Investigation, pp. 997-1011.

86. *Kdm2b promotes induced pluripotent stem cell generation by facilitating gene activation early in reprogramming.* **Liang, G., He, J. en Zhang, Y.** 2012, Nature Cell Biology, pp. 457-466.

87. *Kdm2b maintains murine embryonic stem cell status by recruiting PRC1 complex to CpG islands of developmental genes.* **He, J., et al.** 2013, Nature Cell Biology, pp. 373-384.

88. *A Small Molecule Inhibitor of Ubiquitin-Specific Protease-7 Induces Apoptosis in Multiple Myeloma Cells and Overcomes Bortezomib Resistance.* **Chauhan, D., et al.** 2013, Cancer Cell, pp. 345-358.

89. *Deubiquitinases and the new therapeutic opportunities offered to cancer.* **Pfoh, R., Lacdao, I.K. en Saridakis, V.** 2015, Endocrine-related Cancer, pp. 35-54.

90. *Crystal Structure of USP7 Ubiquitin-like Domains with an ICP0 Peptide Reveals a Novel Mechanism Used by Viral and Cellular Proteins to Target USP7.* **Pfoh, R., et al.** 2015, PLoS.

91. *An allosteric interaction links USP7 to deubiquitination and chromatin targeting of UHRF1.* **Zhang, Z., et al.** 2015, Cell Repress, pp. 1400-1406.

92. *Regulation of cell cycle progression and gene expression by H2A deubiquitination.* **Joo, H.Y., et al.** 2007, Nature, pp. 1068-1072.

93. *Deubiquitylation of histone H2A activates transcriptional initiation via trans-histone cross-talk with H3K4 di- and trimethylation.* **Nakagawa, T., et al.** 2008, Genes Development, pp. 37-49.

94. *Transcriptional activation of polycomb-repressed genes by ZRF1.* **Richly, H., et al.** 2010, Nature, pp. 1124-1128.

95. *Ring1B Compacts Chromatin Structure and Represses Gene Expression Independent of Histone Ubiquitination.* **Eskeland, R., et al.** 2010, Molecular Cell, pp. 452-464.

96. *Transcriptional repression by PRC1 in the absence of H2A monoubiquitylation.* **Pengelly, A.R., et al.** 2015, Genes Development, pp. 1487-1492.

97. *Deubiquitinase HAUSP Stabilizes REST and Promotes Maintenance of Neural Progenitor Cells.* **Huang, Z., et al.** 2011, Nature Cell Biology, pp. 142-152.