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REVIEW
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Rewiring the Epigenetic Networks in *MLL*-Rearranged Leukemias: Epigenetic Dysregulation and Pharmacological Interventions

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Leukemias driven by chromosomal translocation of the mixed-lineage leukemia gene (*MLL* or *KMT2A*) are highly prevalent in pediatric oncology. The poor survival rate and lack of an effective targeted therapy for patients with *MLL*-rearranged (*MLL*-r) leukemias emphasize an urgent need for improved knowledge and novel therapeutic approaches for these malignancies. The resulting chimeric products of *MLL* gene rearrangements, i.e., MLL-fusion proteins (MLL-FPs), are capable of transforming hematopoietic stem/progenitor cells (HSPCs) into leukemic blasts. The ability of MLL-FPs to reprogram HSPCs toward leukemia requires the involvement of multiple chromatin effectors, including the histone 3 lysine 79 methyltransferase DOT1L, the chromatin epigenetic reader BRD4, and the super elongation complex. These epigenetic regulators constitute a complicated network that dictates maintenance of the leukemia program, and therefore represent an important cluster of therapeutic opportunities. In this review, we will discuss the role of MLL and its fusion partners in normal HSPCs and hematopoiesis, including the links between chromatin effectors, epigenetic landscapes, and leukemia development, and summarize current approaches to therapeutic targeting of *MLL*-r leukemias.

Keywords: *MLL*-rearranged, leukemias, epigenetics, MLL, chromatin

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INTRODUCTION

In the 1980s, leukemia cases were observed that showed complete phenotypic lineage switching, i.e., patients initially diagnosed as acute lymphocytic leukemia (ALL) relapsed as acute myeloid leukemia (AML) during chemotherapy (Stass et al., 1984; Mirro et al., 1985; Gagnon et al., 1989). Accordingly, the term mixed-lineage leukemia was coined (Mirro et al., 1985; Slany, 2009). Mixed-linage leukemia was reported to be associated with translocation on the long arm (q) of chromosome 11 band q23 (11q23) (Hayashi et al., 1990). Recurring 11q23 translocations are also found in both ALL (Inaba et al., 2013; Roberts and Mullighan, 2015) and AML (Döhner et al., 2015; Papaemmanuil et al., 2016). Guided by these observations, researchers located and cloned an important gene that resides on 11q23 and drives leukemogenesis when it is translocated and fused with other gene partners (Ziemin-van der Poel et al., 1991; Djabali et al., 1992; Gu et al., 1992; Tkachuk et al., 1992). In the initial discoveries, the gene was given different names: *MLL* (myeloid/lymphoid, or mixed-lineage leukemia) (Ziemin-van der Poel et al., 1991), *ALL-1* (Gu et al., 1992), *HRX* (human trithorax, the human homolog of the *Drosophilia trithorax*, *trx*) (Tkachuk et al., 1992), and *trithorax-like* gene (Djabali et al., 1992). Subsequently, it was also re-named as *KMT2A* (lysine methyltransferase 2A), based on its lysine (Lys, K) methyltransferase enzymatic

activity. For consistent description of the gene, we will use *MLL* throughout this review article.

Accordingly, leukemias that involve chromosomal rearrangement of *MLL* are called *MLL*-rearranged (*MLL*-r) leukemias (Rowley, 1993; Arber et al., 2016; Krivtsov et al., 2017). In adults, *MLL*-r leukemia accounts for approximately 5% of ALL cases (Marchesi et al., 2011) and 5–10% of AML cases (Krivtsov and Armstrong, 2007; Chowdhury and Brady, 2008; Chen and Armstrong, 2015). *MLL*-r leukemias are more prevalent in infant patients (<1 year of age), in which approximately 70% of infants with ALL are diagnosed as having 11q23 rearrangements (Chen et al., 1993; Heerema et al., 1999; Muntean and Hess, 2012). AML is less common than ALL in infants, in which about 50–66% of infants with AML have 11q23 translocations (Sorensen et al., 1994; Martinez-Climent et al., 1995; Hilden et al., 1997; Satake et al., 1999). Prognosis is poor for infants with *MLL*-r ALL, who have worse outcomes than do older children (>12 months) (Pieters et al., 2007). The five-year event-free survival rate of non-*MLL*-r ALL can approach 60–96% in infants (<1 year of age), but is significantly lower in *MLL*-r infant ALL patients—only 34–39% depending on treatment protocols (Hilden et al., 2006; Tomizawa et al., 2007). In addition, approximately 2–15% of cancer patients receiving chemotherapeutic drugs that target DNA-topoisomerase II develop treatment-related AML with 11q23 translocations (Super et al., 1993; Broeker et al., 1996; Greaves, 1997; Felix, 1998). These situations emphasize the unmet need for a deeper understanding of the underlying biology and novel therapeutic approaches for *MLL*-r leukemia.

Surprisingly, comprehensive genomic studies revealed that the genomes of patient-derived *MLL*-r leukemia cells displayed remarkable stability with only a few genetic alterations (Mullighan et al., 2007; Radtke et al., 2009). This suggests that *MLL*-r leukemias are largely driven by epigenetic dysregulation (Radtke et al., 2009; Bernt and Armstrong, 2011). Epigenetics is the study of heritable and reversible control of gene expression that is not dependent on the DNA sequence. In eukaryotic nuclei, DNA molecules are wrapped around histone cores consisting of eight histone proteins—H2A, H2B, H3, and H4 (two copies of each protein are present in the core). Approximately 146 bp of DNA surrounds each octameric histone core to form a nucleosome, the basic structural unit of chromatin. The nucleosomal units are packed and condensed further to form chromatin. The N-terminal peptide tails of H3 and H4 protrude from the histone core, and can be post-translationally modified in various ways (e.g., acetylation, methylation, phosphorylation, ubiquitination, sumoylation) inside eukaryotic cells (Bannister and Kouzarides, 2011). These modifications can be added by epigenetic writers, interpreted by epigenetic readers, and removed by epigenetic erasers (Arrowsmith et al., 2012; Musselman et al., 2012; Seto and Yoshida, 2014; Zhang et al., 2015). Specific modifications or epigenetic histone marks have differential effects on gene expression. For example, acetylated histone marks (e.g., H3K9ac and H3K27ac) are usually associated with gene activation (Krejčí et al., 2009; Creyghton et al., 2010; Hawkins et al., 2011; Hezroni et al., 2011). In contrast, methylated modifications are context-dependent: for instance, methylation on H3K4 or H3K79 is associated with gene activation (Schübeler et al., 2004), whereas methylation on H3K9 or H3K27 is associated with gene silencing (Musselman et al., 2012). In this review, we will introduce the mechanistic roles of MLL in normal hematopoiesis and *MLL*-r leukemia, describe current therapeutic targets in *MLL*-r leukemia, with an emphasis on chromatin epigenetic regulators, and discuss the potential of using combined epigenetic targeting strategies to treat *MLL*-r leukemia.

MLL IN NORMAL HEMATOPOIESIS AND *MLL*-R LEUKEMIAS

MLL Protein Structure and Function

Expression of the developmentally important homeobox (*HOX*) cluster genes is mediated by MLL in normal hematopoietic stem/progenitor cells (HSPCs) (Kawagoe et al., 1999). Genetic knock-out of *Mll* in mice is embryonic lethal, with an altered *Hox* gene pattern, defects in yolk sac hematopoiesis, reduced proliferation and/or survival of hematopoietic progenitors, and defective HSPC activity in the aorta–gonad–mesonephros region (Yu et al., 1995; Hess et al., 1997; Yagi et al., 1998; Ernst et al., 2004). Using conditional *Mll* knock-out ($Mll^{-/-}$) mice, McMahon et al. (2007) demonstrated that Mll was not important for the production of mature adult hematopoietic lineages, but it was required for stem cell self-renewal in fetal liver and adult bone marrow. Furthermore, Mll plays an important role in regulating transcription initiation by RNA polymerase II via H3K4 methylation (Wang et al., 2009). Although Mll was shown to influence less than 5% of promoters that carry the H3K4me3 mark in mouse embryonic fibroblasts, these Mll-regulated promoters include important developmental regulators such as the *Hox* genes (Wang et al., 2009). In humans, the *MLL* gene encodes a protein product of 3,969 amino acids (**Figure 1A**). This product is post-translationally cleaved by threonine aspartase 1 (taspase1) into two distinct modules (MLL-N and MLL-C), then these two modules are assembled together via the FY-rich N- and C-terminal domains (FYRN and FYRC) (García-Alai et al., 2010; **Figure 1A**). A recent study showed that uncleaved MLL displays higher stability than the assembled dimer (MLL-N/MLL-C) (Zhao et al., 2018). Casein kinase II (CKII) phosphorylates MLL at a location proximal to the taspase1 cleavage site, which facilitates taspase1-dependent processing of MLL into MLL-N and MLL-C (Zhao et al., 2018). This finding suggested that pharmacological targeting of MLL to enhance its stability through inhibition of CKII may present a new therapeutic opportunity in *MLL*-r leukemia, as uncleaved MLL can displace leukemia-causing MLL-fusion proteins (MLL-FPs) from chromatin (Zhao et al., 2018).

MLL-N consists of a Menin-binding motif and a lens epithelium-derived growth factor (LEDGF)-binding domain (Yokoyama and Cleary, 2008); three DNA-binding AT-hook motifs (Zeleznik-Le et al., 1994); two nuclear-localization signals, SNL1 and SNL2 (Yano et al., 1997; Ayton et al., 2004); a pre-CxxC domain (Muntean et al., 2010); a non-methyl-CpG recognizing the CxxC domain (Birke et al., 2002; Ayton et al., 2004; Allen et al., 2006); a post-CxxC domain (Muntean et al., 2010);

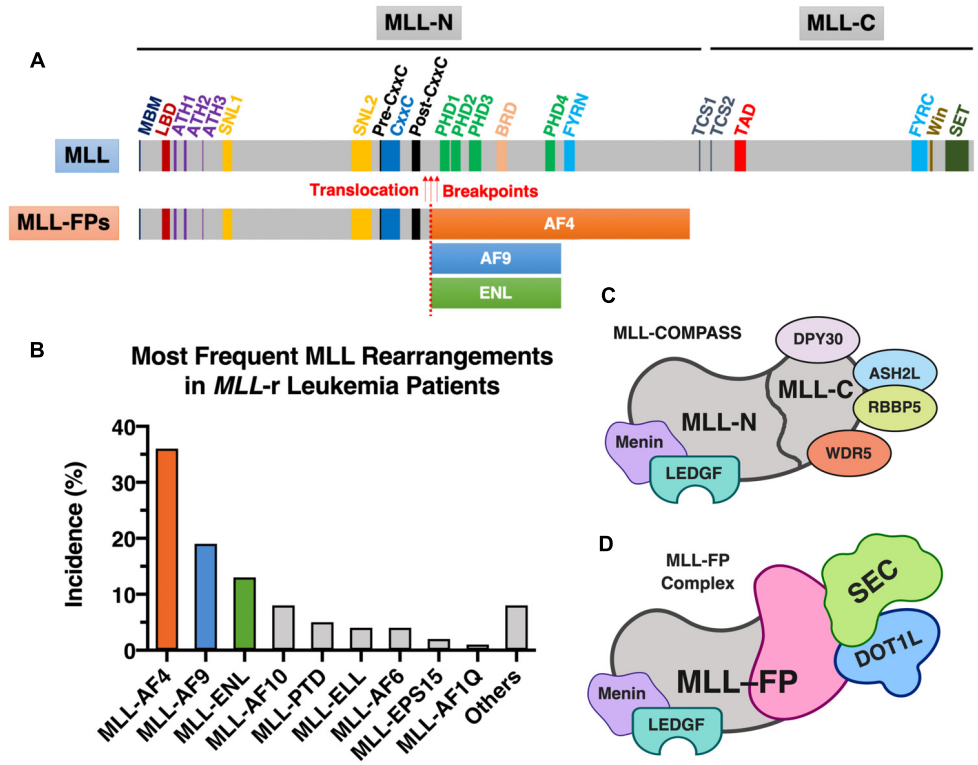

FIGURE 1 | MLL and MLL-FPs. **(A)** Schematic of domain architecture of wild-type MLL and MLL-FPs. The wild-type canonical form of human MLL protein (UniProt ID: Q03164) has a total of 3,969 amino acids in length and contains several functional domains and important sites (drawn to scale): high-affinity Menin-binding motif (MBM, residue 6–10) (Yokoyama et al., 2005), LEDGF-binding domain (LBD, residue 109–153) (Yokoyama and Cleary, 2008), AT-Hook1/2/3 (ATH1, residue 169–180; ATH2, residue 217–227; ATH3, residue 301–309; UniProt annotations of Q03164); nuclear-localization signal 1/2 (SNL1, residue 400–443; SNL2, 1008–1106) (Ayton et al., 2004), pre-CxxC region (residue 1149–1154) (Muntean et al., 2010), CxxC domain (residue 1147–1242) (Bach et al., 2008), post-CxxC (residue 1298–1337) (Muntean et al., 2010), plant homology domain 1/2/3/4 (PHD1, residue 1431–1482; PHD2, residue 1479–1533, PHD3, residue 1566–1627; PHD4, residue 1931–1978; UniProt annotations of Q03164), bromodomain (BRD, residue 1703–1748; UniProt annotations of Q03164), FY-rich N-terminal domain (FYRN, residue 2018–2074; UniProt annotations of Q03164), FY-rich C-terminal domain (FYRC, residue 3666–3747; UniProt annotations of Q03164), taspase1 cleavage site 1/2 (TCS1, residue 2666–2670, D/GADD; TCS2, residue 2718–2722, D/GVDD; the exact cleavage sites are indicated by forward slashes) (Hsieh et al., 2003), transactivator domain (TAD, residue 2829–2883) (Ernst et al., 2001), WDR5 interaction motif (Win; residue 3762–3773) (Patel et al., 2008), and Su(Var)3-9, enhancer-of-zeste, trithorax domain (SET, residue 3829–2945; UniProt annotations of Q03164). The most frequently observed translocation breakpoints (indicated by red arrows) are located in the region between CxxC and PHDs. The three most common MLL-FPs (MLL-AF4, MLL-AF9, and MLL-ENL) are illustrated (the translocation breakpoints and the size of FPs are partially drawn to scale). **(B)** The most frequent MLL rearrangements identified in *MLL*-r leukemia patients. The statistics shown in this figure was obtained from a study of 2,345 *MLL*-r leukemia patients dated from 2003 to 2016 (Meyer et al., 2018). **(C)** Components of MLL-COMPASS. **(D)** MLL-FP (e.g., MLL-AF4, MLL-AF9, or MLL-ENL) in complex with DOT1L and SEC. **Figure 1** was created with BioRender.com.

a bromodomain (BRD); four plant homology domains (PHD fingers) (Fair et al., 2001; Ali et al., 2014); and the homodimerization-facilitating domain FYRN (García-Alai et al., 2010; **Figure 1A**). The sequences flanking the CxxC domain—the pre-CxxC and post-CxxC domains—were demonstrated to be important for direct interaction of MLL with the polymerase associated factor complex (PAFc) (Muntean et al., 2010). PAFc promotes MLL and MLL-FP recruitment to target loci to activate transcription of target genes such as *HOXA9* (Muntean et al., 2010). Therefore, PAFc is a crucial cofactor for both transcriptional regulation by MLL and leukemogenesis mediated by MLL-FPs (Muntean et al., 2010). The BRD of MLL recognizes acetylated lysine residues, whereas the third PHD finger of MLL specifically interacts with H3K4me2/3 (Chang P.-Y. et al., 2010). Binding of the third PHD finger of MLL to H3K4me3 is required for MLL-dependent gene transcription (Chang P.-Y. et al., 2010).

MLL-C possesses two domains capable of modifying chromatin: a transactivator domain (TAD), followed by a SET [Su(Var)3-9, enhancer-of-zeste, trithorax] domain (**Figure 1A**). The MLL SET domain confers methyltransferase activity that catalyzes the transfer of a methyl group from S-adenosylmethionine to H3K4 (Milne et al., 2002). MLL-C is further assembled into a larger protein complex that contains several cofactors: WD repeat protein 5 (WDR5), retinoblastoma-binding protein 5 (RBBP5), Set1/Ash2 histone methyltransferase complex subunit ASH2 (ASH2L), and protein dpy-30 homolog (DPY30) (Rao and Dou, 2015). WDR5, RBBP5, ASH2L, and DPY30 form a core entity with the MLL SET domain, and enhance the H3K4 dimethylation activity of the MLL SET domain by ∼600-fold (Dou et al., 2006; Patel et al., 2009). Although complete deletion of the *Mll* gene in mice results in embryonic lethality (Yu et al., 1995), mice that harbor a

homozygous SET domain deletion (*Mll*Δ*SET*) survive into adulthood and maintain relatively normal hematopoiesis (Mishra et al., 2014). Because the profile of H3K4 methylation at the *Hoxa* loci remains normal in HSPCs isolated from *Mll*Δ*SET* mice, Mishra et al. (2014) speculated that MLL is not the dominant H3K4 methyltransferase that controls *Hox* gene expression. In addition to MLL, five more MLL family members of H3K4 methyltransferases (MLL2, MLL3, MLL4, SETD1A, and SETD1B) are found in mammals, and they associate with other protein factors to form larger macromolecular complexes called COMPASS (complex of proteins associated with Set1; named for the single yeast homolog) (Rao and Dou, 2015; Li et al., 2016; Slany, 2016; Meeks and Shilatifard, 2017). All of the MLL proteins physically associate with four conserved factors—WDR5, RBBP5, ASH2L, and DPY30 (**Figure 1C**), which stimulates the H3K4 methyltransferase activity of MLL proteins (Rao and Dou, 2015; Li et al., 2016). Among the six MLL proteins, MLL and MLL2 share two unique factors—Menin and LEDGF (**Figure 1C**), which mediate the recruitment of MLL/MLL2 to their gene targets (Rao and Dou, 2015). Using mouse embryonic fibroblasts as a cell model, Wang et al. (2009) showed that Menin-interacting Mll and Mll2 are key regulators of *Hox* genes, however, the loss of Mll3/Mll4 had little to no effect on H3K4 methylation of *Hox* loci and the expression of *Hox* genes. This suggests that individual MLL family member may play different functional roles. The MLL TAD interacts with the histone acetyltransferases CBP/p300, MOZ, and MOF, which transfer acetyl groups to H3K27, H3K9, and H4K16, respectively (Slany, 2016). These acetyltransferase activities are associated with *Hox* gene activation by the normal MLL protein (Ernst et al., 2001; Dou et al., 2005; Paggetti et al., 2010).

Common MLL-Fusion Proteins Associated With *MLL*-r Leukemias

As described above, the presence of *MLL* rearrangements at the 11q23 chromosomal location is associated with poor clinical prognosis, and certain subgroups of *MLL*-r leukemia are associated with worse therapeutic outcomes (Balgobind et al., 2009; Szczepański et al., 2010). As a result of different 11q23 chromosomal translocation events, a total of 135 different *MLL* rearrangements (of which 84 translocations generated in-frame MLL-FPs) were identified in patients with acute leukemia (Meyer et al., 2018). According to the report, the nine most frequent fusion partners of MLL are AF4 (∼36%), AF9 (∼19%), ENL (∼13%), AF10 (∼8%), PTD (∼5%), ELL (∼4%), AF6 (∼4%), EPS15 (∼2%), and AF1Q (∼1%), which together represent more than 92% of the MLL-FPs found in *MLL*-r leukemia patients (Meyer et al., 2018). We will focus on MLL-AF4, MLL-AF9, and MLL-ENL, the three most common MLL-FPs discovered in *MLL*-r leukemia patients (Meyer et al., 2018; **Figures 1A,B**).

Animal models of human disease are important for studying underlying disease mechanisms and testing therapeutic agents/approaches. Following discovery of numerous MLL-FPs in human patients, biomedical researchers attempted to recapitulate the leukemogenic effects driven by various MLL-FPs in mouse models (Milne, 2017). In various reports, the genetic introduction of MLL-AF4 (Chen et al., 2006; Metzler et al., 2006; Krivtsov et al., 2008; Tamai et al., 2011; Lin et al., 2016), MLL-AF9 (Corral et al., 1996; Dobson et al., 1999; Drynan et al., 2005; Barabé et al., 2007; Krivtsov et al., 2013; Buechele et al., 2015), or MLL-ENL (Lavau et al., 1997; Forster et al., 2003; Drynan et al., 2005; Barabé et al., 2007; Buechele et al., 2015), were demonstrated to be leukemogenic in mice. Further studies revealed the ability of these MLL-FPs to efficiently transform hematopoietic cells at different developmental stages (e.g., hematopoietic stem cells, common myeloid progenitors, and granulocyte and macrophage progenitors) into leukemic cells possessing stem-cell-like properties, such as being capable of self-renewal and leukemia initiation and maintenance (Cozzio et al., 2003; Krivtsov et al., 2006, 2008; Krivtsov and Armstrong, 2007).

MLL-Rearrangement Links Transcriptional and Epigenetic Abnormalities in Leukemia

MLL-FPs have different chromatin-modifying activities than normal MLL proteins. The C-terminal SET domain of wild-type MLL that harbors H3K4 methyltransferase activity is lost in MLL-FPs (**Figure 1**). Because multiple MLL fusion partners such as AF4/AF9/ENL/ELL are also components of the super elongation complex [SEC; composed of AF4 (or AFF4), AF9 (or ENL), EAF, ELL, and P-TEFb (positive transcription elongation factor b)] (Luo et al., 2012; Cucinotta and Arndt, 2016), chimeric MLL-FPs recruit the SEC (a crucial regulator of transcriptional elongation) (**Figure 1D**) and result in aberrant gene expression (Collins and Hess, 2016). Furthermore, several components of the SEC interact with the histone H3K79 methyltransferase DOT1L (disruptor of telomeric silencing 1-like) (Park et al., 2010; Biswas et al., 2011; Shen et al., 2013). Increased levels of H3K79 methylation by DOT1L are found at MLL-FP targeted genomic loci such as *HOXA9* and *MEIS1*, which are associated with leukemic transformation (Okada et al., 2005; Krivtsov et al., 2008; Bernt et al., 2011; Li and Ernst, 2014; Chen et al., 2015; Collins and Hess, 2016). Given that histone H3K79 methylation is associated with active gene transcription (Schübeler et al., 2004), this suggests that DOT1L activity and the altered H3K79 epigenetic signature observed at MLL-FP binding loci may also contribute to the expression of the oncogenic program in *MLL*-r leukemias (Bernt et al., 2011; Jo et al., 2011; Chen et al., 2013; Deshpande et al., 2013). Thus, current evidence suggests that MLL-FPs connect MLL to the SEC and the DOT1L-H3K79 methyltransferase complex (consisting of DOT1L, AF9, AF10, and ENL) (Bernt et al., 2011), thereby contributing to the ectopic expression of the leukemic program (**Figure 1D**).

THERAPEUTIC TARGETING OF THE MLL-FUSION PROTEIN EPIGENETIC NETWORK

In recent years, chemical inhibitors that target chromatin epigenetic regulators have undergone active development for treatment of various cancers (Filippakopoulos and Knapp,

2014; Shi and Vakoc, 2014; Cai et al., 2015; Fujisawa and Filippakopoulos, 2017; Ribich et al., 2017; Schiedel and Conway, 2018). As discussed above, MLL-FPs associate with cofactors and recruit epigenetic effectors, which eventually lead to aberrant gene expression and leukemic transformation. Therefore, pharmacological disruption of the key proteins in this MLL-FP epigenetic network represents a therapeutic opportunity for the treatments of *MLL*-r leukemias (**Figure 2**; bottom). Furthermore, *MYC* is a well-known proto-oncogene that is frequently over-expressed in cancer, including in ALL, AML, and *MLL*-r leukemias (Schreiner et al., 2001; Langenau et al., 2003; Luo et al., 2005; Delgado and León, 2010; Dang, 2012; Li L. et al., 2014; Sanchez-Martin and Ferrando, 2017). Indeed, the *MYC* gene is a direct transcriptional target of MLL-FPs (Dawson et al., 2011; Li and Ernst, 2014). Despite its known oncogenic role in *MLL*-r leukemias, direct inhibition of the MYC transcription factor (TF) is challenging, because of its difficult-to-drug three-dimensional structure. Thus, down-regulating *MYC* gene expression by targeting more easily druggable chromatin-binding domain structures on the regulatory proteins that affect *MYC* expression is an active area of research (**Figure 2**; top). In the following sections, we will discuss the latest approaches to therapeutic targeting of the MLL-FP epigenetic network.

Targeting Menin–LEDGF–MLL Interactions

Menin, encoded by the *MEN1* gene, acts as a molecular adaptor to tether both MLL and LEDGF and is required for the oncogenic transformation of *MLL*-r leukemia mediated by MLL-FPs (Yokoyama et al., 2005; Yokoyama and Cleary, 2008). Genetic knockout of *Men1* in mouse embryonic fibroblasts demonstrated that Menin is essential for H3K4 trimethylation at *Hox* loci and expression of nearly all *Hox* genes (Wang et al., 2009). Borkin et al. (2015) developed two potent small-molecule inhibitors (MI-463 and MI-503) that block the MLL-binding site on Menin, resulting in down-regulation of MLL-fusion targets (including *Hoxa9 and Meis1* genes), differentiation of leukemic blasts, and prolonged survival of mouse models of *MLL*-r leukemia. A study reported that MLL1 does not require interaction with Menin to sustain hematopoietic stem cell hematopoiesis (Li et al., 2013). This provides a good rational for developing inhibitors of the Menin–MLL interaction to treat *MLL*-r leukemia without affecting normal cells. Indeed, Borkin et al. (2015) revealed that Menin inhibition by MI-463 and MI-503 resulted in no impairment of normal murine hematopoiesis after 10 days of continuous Menin inhibition. Guided by the molecular scaffolds of the two Menin inhibitors, the same group recently reported the development of a more potent Menin inhibitor, MI-1481, which showed low nanomolar inhibition (IC_{50} = 3.6 nM, measured by fluorescence polarization assay using fluorescein-labeled MLL_{4-43}) against Menin–MLL interactions (Borkin et al., 2018). Compound MI-1481 showed potent activity in cells and *in vivo* models of *MLL*-r leukemias (Borkin et al., 2018).

Lens epithelium-derived growth factor is a transcriptional coactivator that specifically recognizes H3K36me2/3 (histone modifications that are associated with actively transcribed gene loci) through its PWWP domain (Pradeepa et al., 2012; Eidahl et al., 2013; Zhu et al., 2016). Through association with LEDGF, MLL is brought to chromatin to regulate transcription (Yokoyama and Cleary, 2008). MLL-ENL lacking the high-affinity Menin-binding motif (i.e., it cannot interact with Menin efficiently) failed to co-precipitate with endogenous LEDGF, suggesting that LEDGF interacts conjointly with MLL-ENL and Menin, but does not associate with either one of them separately (Yokoyama and Cleary, 2008). Recent reports suggest that LEDGF is dispensable for normal hematopoiesis but important for leukemogenesis; therefore, LEDGF is being considered as a potential drug target for *MLL*-r leukemias (Blokken et al., 2017; Ashkar et al., 2018).

Although wild-type MLL and MLL-FPs share the same MLL N-terminal and retain Menin/LEDGF-binding ability (**Figures 1C,D**), two studies suggested that MLL and MLL-FPs have different chromatin-tethering mechanisms (Wang et al., 2011; Xu et al., 2016). Wang et al. (2011) reported that MLL-FPs preferentially regulate only a small subset of wild-type MLL target genes using patient-derived leukemic cells and inducible MLL-ENL cellular system. Moreover, Xu et al. (2016) showed by ChIP-seq experiments that MLL and MLL-AF9 localized at different chromatin regions in a murine cell model of MLL-AF9, and they further demonstrated that these differences were due to the ability of MLL C-terminal domain to interact with WDR5. The C-terminal domain of MLL is lost in MLL-FPs, because of 11q23 translocations (**Figure 1**). These may explain why targeting either Menin or LEDGF could be a therapeutic approach in treating *MLL*-r leukemias, since MLL and MLL-FPs have differential dependence on Menin or LEDGF for activating gene expression.

Targeting the Enzymatic Core of DOT1L

Disruptor of telomeric silencing 1-like is a methyltransferase that catalyzes H3K79 mono-, di-, and tri-methylation (H3K79me1/2/3), which are associated with active gene expression (Barski et al., 2007; Jo et al., 2011). DOT1L binds to the nucleosomal disk surface and methylates H3K79 located in the globular histone core (Min et al., 2003). Because the aberrant methyltransferase activity of DOT1L is required for the leukemogenesis of *MLL*-r leukemia, DOT1L inhibition is a promising therapeutic intervention (Chang M.-J. et al., 2010; Bernt et al., 2011; Daigle et al., 2011; Nguyen et al., 2011). A number of small-molecule DOT1L inhibitors have been developed in recent years (Anglin and Song, 2013). One inhibitor, pinometostat (EPZ-5676), was identified as a highly potent and selective inhibitor of the DOT1L active site (Daigle et al., 2013). Pinometostat showed moderate to high clearance and low oral bioavailability in a non-clinical pharmacokinetic and metabolic study (Basavapathruni et al., 2014). A phase I clinical study recently revealed that administration of pinometostat was generally safe; however, its efficacy as a single-agent treatment was modest (Stein et al., 2018). Further studies are needed to investigate whether the combination of pinometostat with other pharmacological inhibitors can potentiate its therapeutic activity in patients with *MLL*-r leukemias.

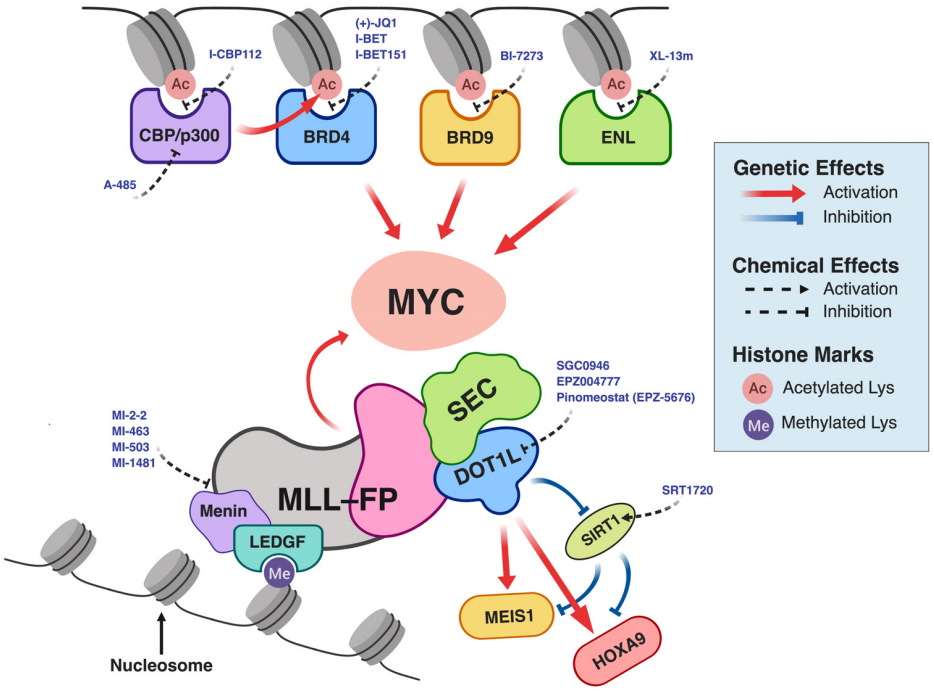

FIGURE 2 | Pharmacological intervention in the MLL-FP epigenetic network in *MLL*-r leukemia. For simplicity, the components of protein complexes interacting with CBP/p300, BRD4, BRD9, or ENL are not illustrated. BRD4 and MLL-FPs (MLL-AF4 or MLL-AF9) together form a complex with SEC and PAFc (Dawson et al., 2011), which directs *MYC* expression. This schematic was created using BioRender.com.

Targeting Lysine-Specific Demethylase 1 (LSD1)

Lysine-specific demethylase 1 (LSD1 or KDM1A), the first identified histone demethylase (Shi et al., 2004), is an epigenetic eraser of H3K4me1/2 and H3K9me1/2 histone marks (Shi et al., 2004; Forneris et al., 2005; Metzger et al., 2005; Amente et al., 2013). LSD1 is overexpressed in various solid cancers (bladder, breast, lung, and colorectal cancers) (Lim et al., 2010; Hayami et al., 2011), and in AML as well as lymphoid malignancies and myeloproliferative neoplasms (Lokken and Zeleznik-Le, 2012; Schenk et al., 2012; Niebel et al., 2014; Przespolewski and Wang, 2016). Harris et al. (2012) showed that LSD1 is required for sustaining the expression of the MLL-AF9 oncogenic program and identified LSD1 as a therapeutic target for *MLL-AF9* leukemia. Genetic knock-down of *Lsd1* by RNAi induced apoptosis and terminal macrophage differentiation of murine *MLL-AF9* cells, and reduced AML leukemia stem cell potential in mice transplanted with murine *MLL-AF9* cells (Harris et al., 2012). Moreover, Harris et al. (2012) found that pharmacological inhibition of LSD1 by OG-86 (also known as Compound B) (Maiques-Diaz and Somervaille, 2016) resulted in inhibition of colony formation and promotion of leukemic cell differentiation in murine *MLL-AF9* cells and primary human *MLL-AF6* and *MLL-AF9* leukemic blasts (Harris et al., 2012). In 2018, ORY-1001 was identified as a highly potent and selective LSD1 covalent inhibitor with good bioavailability after intra-peritoneal and oral administration (Maes et al., 2018). ORY-1001 binds irreversibly and rapidly to the cofactor flavin adenine dinucleotide (FAD) when it is covalently bound to LSD1, but not to free FAD. ORY-1001 showed nanomolar inhibition (IC_{50} = 18 nM) of recombinant LSD1 and low nanomolar activity (EC_{50} < 1 nM in a FACS-based differentiation assay) against cellular LSD1 in THP1 cells (a human *MLL-AF9* cell line) (Maes et al., 2018). ORY-1001 induced blast differentiation in leukemic cell lines (including *MLL*-r leukemic cell lines), primary AML samples, and two AML patients participating in a clinical trial of ORY-1001, and prolonged survival in murine models of acute leukemia (Maes et al., 2018). ORY-1001 is currently under ongoing phase I/IIa study in relapsed or refractory acute leukemia (EUDRACT no. 2013-002447-29) (Maes et al., 2018). Cusan et al. (2018) demonstrated that LSD1 inhibition using GSK-LSD1 (a LSD1 chemical inhibitor) induces an increase in chromatin accessibility on a global level. They also showed that the antileukemic effect exerted by GSK-LSD1 requires the TFs PU.1 and C/EBPα, as the reduction or depletion of PU.1 or C/EBPα expression level, respectively, rendered murine *MLL-AF9* leukemia cells resistant to GSK-LSD1 inhibition (Cusan et al., 2018).

Down-Regulation of *MYC* by BRD4 Inhibition

Bromodomains are specific epigenetic reader modules that recognize ε-N-acetylation of lysine motifs, the key epigenetic marks for maintaining open chromatin structure, which is associated with transcriptional activation. In the human genome, there are 46 known BRD-containing proteins. Some of these proteins possess more than one BRD, so there are a total of

61 different BRDs found in humans (Filippakopoulos et al., 2012). Large-scale structural and binding studies demonstrated that BRDs do not specifically read a particular acetylated lysine (Kac) sequence, but instead recognize a combination of different Kac motifs (Filippakopoulos et al., 2012). Although BRDs differ in the primary amino acid sequence, they all possess similar structural folds: a left-handed bundle of four α helices (designated αZ, αA, αB, αC) linked by loop regions of various lengths (ZA and BC loops) that contribute to specificity in Kac substrate recognition (Filippakopoulos et al., 2012; Filippakopoulos and Knapp, 2014; Fujisawa and Filippakopoulos, 2017). Large-scale co-crystallization of various Kac peptides and BRDs showed that Kac peptides bind in the central hydrophobic pocket and form hydrogen bonding with an asparagine residue, present in most BRDs (Filippakopoulos et al., 2012; Filippakopoulos and Knapp, 2014; Fujisawa and Filippakopoulos, 2017). Bromodomain-containing protein 4 (BRD4), a member of the bromodomain and extra-terminal domain (BET) family, is a chromatin reader that recognizes Kac residues through two BRDs. It plays key roles in the maintenance of epigenetic memory, cell cycle control, and transcriptional regulation (Dey et al., 2000, 2009; Yang et al., 2005; Devaiah et al., 2012). In 2010, two independent groups discovered the first BRD4 inhibitors, (+)-JQ1 (Filippakopoulos et al., 2010) and I-BET (Nicodeme et al., 2010). These two inhibitors also represent the first examples of small molecules that target epigenetic readers; before the discovery of (+)-JQ1 and I-BET, only epigenetic writers and erasers had been targeted in the field of epigenetics. This discovery opened up a new possibility of treating cancers by targeting epigenetic reader modules.

In Zuber et al. (2011) used an RNA interference (RNAi) genetic screen to identify BRD4 as a therapeutic target in AMLs, including *MLL-AF9*-induced AML. They demonstrated that the use of either short-hairpin RNAs (shRNAs) or (+)-JQ1 to induce knock-down or inhibition of Brd4, respectively, led to anti-leukemic effects in cells and *in vivo*. In a murine *MLL-AF9* AML cell model, shRNA or (+)-JQ1 treatments induced cell differentiation and led to depletion of leukemia stem cells and a global reduction in the expression of Myc target genes. Moreover, Zuber's group used ChIP-qPCR to show that Brd4 occupancy approximately 2 kb upstream of the *Myc* promoter was reduced after exposure to (+)-JQ1. These data suggest that the effects of Brd4 inhibition are linked at least in part to suppression of the Myc-dependent leukemia sustainment program. Indeed, ectopic over-expression of *Myc* cDNA rescued cell cycle arrest and terminal differentiation induced by *Brd4*-targeting shRNAs and (+)-JQ1 treatment. However, over-expression of *Myc* could not rescue cell death induced by (+)-JQ1, suggesting a Myc-independent function of Brd4 in regulating cell survival (Zuber et al., 2011). Consistent with this, another research group employed a large-scale global proteomic strategy to reveal that MLL-FPs that form part of the PAFc and SEC are associated with the epigenetic reader BRD4 (Dawson et al., 2011). Dawson et al. (2011) suggested that BRD4 may function to recruit leukemogenic MLL-FPs (e.g., MLL-AF4 or MLL-AF9) to chromatin for activating expression of oncogenic genes, and therefore they hypothesized that the displacement of BRD4 using chemical inhibitors may have anti-leukemic effect in *MLL*-r leukemias. The authors showed that I-BET151 displaced BRD4 from Kac substrates on the chromatin and resulted in down-regulation of the antiapoptotic gene *BCL2*, cell cycle regulator *CDK6*, and *MYC* (Dawson et al., 2011). Furthermore, I-BET151 showed anti-leukemic activity in cells and *in vivo*, and possessed better pharmacokinetic properties in mice than (+)-JQ1 (Dawson et al., 2011).

Mechanistically, the pronounced inhibition of *MYC* expression by (+)-JQ1 is thought to result from dissociation of BRD4 from MYC super-enhancers (Lovén et al., 2013). Super-enhancers are large clusters of transcriptional enhancers packed with TFs, cofactors, transcription apparatus, and chromatin regulators such as BRD4 (Hnisz et al., 2013). Sustainment of oncogenic *MYC* expression is associated with *MYC* super-enhancers in multiple myeloma (MM1.S) and *MLL*-r leukemia (*MLL-AF9*/*Nras*^{G12D}) cells (Lovén et al., 2013; Shi et al., 2013; Shi and Vakoc, 2014). BRD4 molecules densely bind to these active super-enhancers and promote transcriptional elongation through physical association with the Mediator coactivator complex and P-TEFb (Lovén et al., 2013; Shi et al., 2013; Xu and Vakoc, 2017). BRD4 recruits and activate P-TEFb, a multi-protein kinase complex that functions to promote transcriptional elongation (Slany, 2016; Xu and Vakoc, 2017). Chemical inhibition of BRD4 by (+)-JQ1 preferentially displaced BRD4 molecules, as well as Mediator and P-TEFb, from the super-enhancers, thereby down-regulating *MYC* and MYC-target gene expression (Lovén et al., 2013).

Down-Regulation of *MYC* by CBP/p300 Inhibition

Roe et al. (2015) provided evidence that lineage-specific TFs recruit the lysine acetyltransferases CBP or p300 to acetylate the TFs and histone lysine residues at lineage-specific promoters and enhancers in mouse *MLL-AF9*/Nras^{G12D} AML cells. BRD4 was shown to bind to hyperacetylated histone residues and acetylated TFs through its BRDs, then promote transcriptional activation via its association with Mediator and P-TEFb. This chromatin-based signaling cascade provides an additional mechanistic explanation for the rapid down-regulation of gene expression by (+)-JQ1, which functions to displace densely localized BRD4 molecules from transcriptionally activated cancer-promoting genes including *BCL2*, *CDK6*, and *MYC* (Roe et al., 2015; Xu and Vakoc, 2017). Given that CBP/p300 play important roles in maintaining *MLL*-r leukemia, this suggests that chemical inhibition of CBP or p300 may present a therapeutic strategy for treating *MLL*-r AMLs (Roe et al., 2015). Small molecules targeting the BRDs of CBP/p300 have been developed (Borah et al., 2011; Hay et al., 2014; Rooney et al., 2014; Brand et al., 2015; Picaud et al., 2015; Schiedel and Conway, 2018). For example, I-CBP112, a potent and selective CBP/p300 inhibitor with modest pharmacokinetic properties, was reported in 2015 (Picaud et al., 2015). I-CBP112 showed inhibitory effects on cancer cell growth in both human and mouse *MLL-AF9* AML cell lines and prolonged the survival of mice injected with *MLL-AF9* AML cells. Picaud et al. (2015) also reported that I-CBP112 sensitized MOLM-13 *MLL*-r leukemia cells to (+)-JQ1. The

combined use of both epigenetic inhibitors could achieve greater antileukemic effects than employing a single agent alone (Picaud et al., 2015). In Lasko et al. (2017), a major breakthrough was achieved in the development of a highly potent, selective, and cell-permeable CBP/p300 inhibitor. Instead of targeting BRDs, the new compound, A-485, binds and inhibits the catalytic core of CBP/p300 and shows inhibitory effects in various cancer cell lines, including MOLM-13 *MLL*-r leukemia cells. Importantly, A-485 was also found to robustly reduce *MYC* expression and show tumor-inhibitory effects in a prostate cancer mouse model.

Down-Regulation of *MYC* by BRD9 Inhibition

Bromodomain-containing protein 9 (BRD9) is a component of the SWI-SNF chromatin remodeling complex, also known as the BRG1-associated factor (BAF) chromatin remodeling complex in mammals. BRD9 is important for sustaining *MYC* expression *via* its BRD in diverse *MLL*-r leukemia cell lines, including MV4-11, MOLM-13, ML-2, EoL-1, and NOMO-1 (Hohmann et al., 2016). Hohmann et al. (2016) showed that application of RNAi or the small molecule BI-7273 (Martin et al., 2016) to achieve genetic knock-down or chemical inhibition of BRD9, respectively, induced cell growth inhibition and differentiation of *MLL*-r leukemia cell lines through down-regulation of MYC. The same team also devised a domain-swapping strategy to assess the on-target specificity of the chemical probe (BI-7273) on BRD9. In this method, they swapped the BRD of BRD9 with the first BRD of BRD4, to generate a new chimeric protein, BRD9-BET. The full biological function of BRD9 was preserved in BRD9-BET after the domain-swap; however, BI-7273 could no longer inhibit BRD9-BET. This validated the on-target specificity of BI-7273 for the BRD of BRD9.

Down-Regulation of *MYC* by ENL Inhibition

ENL (eleven-nineteen-leukemia protein or MTTL1) is the third most frequent MLL translocation partner identified in *MLL*-r leukemia patients (Meyer et al., 2018; **Figure 1B**). ENL is also a component of the SEC, which is observed in the MLL-FP complexes (Zeisig et al., 2005; Yokoyama et al., 2010; **Figure 1D**). The N-terminal of ENL possesses a YEATS domain. In additional to BRDs recognizing Kac (Dhalluin et al., 1999; Filippakopoulos et al., 2012), in 2014, YEATS domains were discovered to be epigenetic readers of Kac (Li Y. et al., 2014). YEATS domains were named after the five founding members of the YEATS-containing protein family—Yaf9, ENL, AF9, Taf14, and Sas5 (Li Y. et al., 2014). Structurally, the YEATS domains of ENL and AF9 adopt an eight-stranded β-sandwich fold (Li Y. et al., 2014; Wan et al., 2017), which is different from the four-α-helical fold of BRDs (Dhalluin et al., 1999; Filippakopoulos et al., 2012). ENL was found to be important in maintaining AMLs (including *MLL*-r leukemias) by two independent groups in 2017 (Erb et al., 2017; Wan et al., 2017). Depletion of ENL results in down-regulation of key leukemic drivers such as *MYC*, cell growth inhibition, reduced expression of the leukemia stem cell signature, and terminal differentiation of *MLL*-r leukemia cell models (Erb et al., 2017; Wan et al., 2017). Cas9-mediated depletion of ENL prolonged survival in leukemic mouse models, which were xenotransplanted with either MV4-11 or MOLM-13 cells transduced with single-guide RNA targeting the *ENL* gene (Erb et al., 2017; Wan et al., 2017). The two groups also demonstrated that the YEATS reader domain of ENL plays an important role in oncogenic expression and leukemia maintenance through YEATS-Kac interactions. This suggests that pharmacological inhibition of the ENL YEATS domain is a potential therapeutic target for AMLs such as *MLL*-r leukemias (Erb et al., 2017; Wan et al., 2017). Subsequently, another two research groups separately described small molecules capable of inhibiting the YEATS domains of AF9/ENL (Christott et al., 2018; Li et al., 2018). Christott et al. (2018) screened a library of 24,000 compounds using a peptide displacement assay and discovered a small molecule (XS018661) that binds to AF9 and ENL with equilibrium dissociation constant (K_d) values of 523 ± 53 and 745 ± 45 nM, respectively, as determined by isothermal calorimetry (ITC). Li et al. (2018), on the other hand, used structure-guided development to produce selective peptide-based AF9 and ENL inhibitors, termed XL-13a and XL-13m, respectively. They also showed that XL-13m down-regulated key leukemic driver genes including *MYC*, *MYB* (a transcriptional activator), *HOXA9*, and *MEIS1* in MOLM-13 cells (Li et al., 2018).

COMBINED EPIGENETIC THERAPIES FOR *MLL*-R LEUKEMIAS

Although the DOT1L methyltransferase inhibitor, pinometostat, has cell-inhibitory effect in cells and *in vivo* models of *MLL*-r leukemia (Daigle et al., 2013), recent results from a phase I clinical trial indicated that the small molecule has only modest efficacy in treating *MLL*-r leukemias (Stein et al., 2018). This suggests that combining DOT1L inhibitors with other pharmacological agents may be necessary to boost anti-leukemic efficacy. A genome-wide RNAi screen showed that SIRT1, an NAD^+-dependent deacetylase, is required to establish a chromatin-repressive state after inhibition of DOT1L (Chen et al., 2015). Consistent with this, a potent activator of SIRT1, SRT1720 (Milne et al., 2007; Mitchell et al., 2014), was demonstrated to synergize with EPZ004777, a DOT1L inhibitor, and enhance anti-proliferative activity against *MLL*-r leukemia cells (Chen et al., 2015). Another study by Gilan et al. (2016) showed that although DOT1L and BRD4 occupy distinct molecular complexes, they functionally cooperate with each other, and that such cooperation is particularly important for highly transcribed genes with proximity to super-enhancers. They found that combined targeting of both DOT1L and BRD4 using SGC0946 (a small-molecule inhibitor of DOT1L) and I-BET, respectively, resulted in growth inhibitory synergy against *MLL*-r cell lines, primary human leukemia cells, and mouse leukemia models (Gilan et al., 2016). In a more recent study, Dafflon et al. (2017) employed an epigenome-focused shRNA library to identify epigenetic regulators that sensitize *MLL*-r leukemia cells treated with EPZ004777. They reported that combination of EPZ004777

and MI-2-2 (an inhibitor of MLL–Menin interaction) enhanced the down-regulation of important leukemia genes (i.e., *MYC*, *HOXA9*, and *MEIS1)* and anti-proliferative effects against *MLL*-r leukemia cells, compared to either single-agent treatment (Dafflon et al., 2017). Maes et al. (2018) recently showed that the LSD1 demethylase inhibitor ORY-1001 could synergize with a retinoid derivative (ATRA), a nucleoside analog [cytosine arabinoside (ARA-C)], a FLT3 inhibitor (quizartinib), DOT1L inhibitors (EPZ-5676; SGC0946), DNMT1 inhibitors (decitabine; azacitidine), an HDAC inhibitor (SAHA), and a BCL2 inhibitor (ABT-737) to inhibit the proliferation of MV4-11 and MOLM-13 *MLL*-r leukemic cell lines.

In advance of identifying the first-in-class YEATS domain inhibitors, scientists used genetic and chemical biology tools to determine if ENL YEATS inhibition synergized with known inhibitors of epigenetic regulators (Erb et al., 2017; Wan et al., 2017). Wan et al. (2017) proved that Cas9-mediated depletion of ENL sensitized MOLM-13 cells to (+)-JQ1 treatment. In a proof-of-concept experiment, Li et al. (2018) used small-molecule chemical probes to demonstrate that the ENL YEATS domain inhibitor XL-13m synergized with either the DOT1L inhibitor pinometostat or the BRD4 inhibitor (+)-JQ1 in reducing the expression of *MYC* in MOLM-13 cells. Erb et al. (2017) applied a novel approach to induce proteasome-mediated acute degradation of ENL using degradation tag (dTAG) technology (Winter et al., 2015; Nabet et al., 2018) in leukemic cells. The dTAG system is a new technology that uses an $FKBP12^{F36V}$-fused target protein-of-interest and a heterobifunctional ligand degrader—a dTAG molecule (e.g., dTAG-13)—that binds $FKBP12^{F36V}$ and cereblon (CRBN) E3 ligase (Winter et al., 2015; Nabet et al., 2018; Mayor-Ruiz and Winter, 2019). This offers a generalized strategy to fuse any target protein to an engineered variant of the immunophilin FKBP12, then tag $FKBP12^{F36V}$-fused target proteins for acute proteasome-mediated degradation. The F36V mutation in $FKBP12^{F36V}$ affords a "bump-hole" strategy that allows specific tagging of the $FKBP12^{F36V}$-fusion proteins, without affecting endogenous FKBP12 proteins. The $FKBP12^{F36V}$-target fusion gene can be genetically introduced via transgene expression or CRISPR-mediated locus-specific knock-in Nabet et al. (2018). The dTAG-13 molecule induces dimerization of the $FKBP12^{F36V}$-fused target protein with CRBN E3 ligase, which leads to polyubiquitination of the target protein and proteasomal degradation. After degradation, the dTAG-13 molecules are recycled (i.e., without being degraded) for subsequent rounds of proteasome-mediated degradation of additional $FKBP12^{F36V}$-fused target proteins. Erb et al. (2017) demonstrated that combining dTAG-mediated acute degradation of ENL with pinometostat had additive inhibitory effects on the expression of *Myc* and *Hoxa9* in *MLL*-r cells (MV4-11, $Cas9^{+}$, $ENL\text{-}FKBP12^{F36V}\text{–}HA^{+}$, $ENL^{-/-}$).

CONCLUDING REMARKS

Chimeric MLL-FPs form complexes with different epigenetic regulators that are capable of rewiring the epigenetic networks and driving the leukemic programs in *MLL*-r leukemias. For example, MLL-FPs recruit DOT1L (an epigenetic writer), and cause aberrant expression of *HOXA9* and *MEIS1*, which leads to leukemic transformation. Moreover, MLL-FPs are able to form a molecular complex with BRD4 (an epigenetic reader), PAFc and SEC to sustain the oncogenic expression of *BCL2*, *CDK6*, and *MYC* in *MLL*-r leukemias (Dawson et al., 2011). Pharmacological intervention with small molecules disrupting the epigenetic networks rewired by the MLL-FPs (**Figure 2**), holds promises in treating *MLL*-r leukemias. However, in some circumstances, cancer monotherapies using single chemical agents may have sub-optimal efficacy in eliminating cancer cells and are likely limited by the emergence of resistant cell populations (Fong et al., 2015; Rathert et al., 2015; Doroshow et al., 2017). Recent studies have shown that combining epigenetic drug treatments offers improved therapeutic responses over monotherapies in treating various cancers including *MLL*-r leukemias (Doroshow et al., 2017). It is of crucial importance to understand the mechanistic actions of drugs, in order to build a comprehensive rationale for using the drugs in a combinatory setting. This is especially important in targeting context-dependent epigenetic regulatory proteins. The advent of massive parallel sequencing technologies and genetic screening technologies such as RNAi and CRISPR-Cas9 has enabled biomedical scientists to uncover cancer vulnerabilities and discover new therapeutic targets for cancer treatments in a high-throughput manner (Shalem et al., 2015; McDonald et al., 2017; Meyers et al., 2017; Tsherniak et al., 2017). With the emergence of dTAG technology, cancer researchers are equipped with an acute proteasome-mediated protein knock-out method for interrogating biological function and early validation of therapeutic targets, before a valid binding ligand is identified (Winter et al., 2015; Nabet et al., 2018; Mayor-Ruiz and Winter, 2019; Schiedel et al., 2019). With such genetic and chemical discovery tools in hand, we foresee that researchers are empowered to discover and validate important therapeutic targets to treat diseases driven by epigenetic abnormalities such as *MLL*-r leukemias.

AUTHOR CONTRIBUTIONS

Both authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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