EZH2 as a therapeutic target in solid tumors

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Introduction

The process of oncogenic transformation and tumor development is driven by both genetic and epigenetic changes [1] that allow tumor cells to bypass control of mitogenic signaling, evade apoptosis, invade surrounding tissue and eventually metastasize to distant sites within the body [2]. In addition to genetic mutations in driver genes like RAS, BRAF, and EGFR, cancer genomes are characterized by aberrant patterns of DNA and histone methylation. Underlying these tumor-specific changes is the deregulated expression or genetic mutation of epigenetic modifiers in many cancer types. In particular, the Polycomb group proteins (PcG) have been frequently implicated in cancer biology. PcG proteins modify histones to silence gene expression. Through this activity they serve an important role in normal development that greatly influences stem cell biology and cell fate decisions. However, PcG complexes are frequently co-opted by cancer cells to facilitate tumorigenesis [3].

The histone methyltransferase EZH2 is the catalytic subunit of Polycomb repressive complex 2 (PRC2) that methylates Histone H3K27 (mono, di or trimethylation) to repress gene transcription. EZH2 has been strongly linked to cancer biology, with early reports indicating that EZH2
expression is elevated in multiple tumor types [3] and correlates with metastatic growth in prostate and breast cancer [4, 5]. Further reports demonstrated that EZH2 expression is an independent prognostic indicator in multiple indications [6, 7]. In addition to EZH2 overexpression and correlation with disease outcome, genetic modifications also point toward an important role for this epigenetic modifier in cancer. EZH2 is amplified in multiple tumor types and this amplification correlates with increased expression [3, 8].

More recently, gain-of-function mutations of EZH2 have been identified in diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, acute lymphoblastic leukemia, and melanoma. These hotspot mutations confer enhanced methyltransferase activity to EZH2, resulting in dramatic increases in global H3K27me3 levels [9-14]. Together, these observations strongly suggest that EZH2 activity is important for tumorigenesis.

Early Target Validation Efforts

Based on the observations described above, many studies have sought to validate EZH2 as a target for anti-tumor therapies. Early studies that manipulated EZH2 levels using RNA interference or overexpression clearly demonstrated an important role for EZH2 in multiple settings. For example, EZH2 was shown to be essential for cellular proliferation in both normal and transformed cell lines [8]. Specifically, several reports indicated that EZH2 is important for the growth of prostate cancer cell lines. For example, silencing of EZH2 caused decreased proliferation, increased cell death and decreased xenograft tumor growth in prostate cell lines [15]. Additional work in prostate cell models confirmed these findings and forged new links between EZH2 and invasiveness [16, 17]. Further studies expanded these findings to additional models, such as renal cell carcinoma cell lines [18], bladder cancer cell lines [19], and colorectal cancer cell lines [20]. Specific roles for EZH2 beyond its role in proliferation were also demonstrated, implicating EZH2 in tumor metastasis [21], cell death responses [22] and inhibition of senescence [23]. Yet other studies defined roles for EZH2 in specific genetic backgrounds such as BRCA1 deficient breast tumors [24, 25]. While all of these studies have revealed important roles for the EZH2 protein in cancer biology, they failed to distinguish between enzymatic and non-enzymatic roles for EZH2.

3-Deazaneplanocin A (DZNep), an S-adenosylhomocysteine hydrolase inhibitor, was the first small molecule widely used to inhibit EZH2. DZNep was found to decrease EZH2 levels (as well as additional PRC2 components) and H3K27 methylation. Further, these effects correlated with increased cell death in cancer cells [26]. However, while DZNep was initially thought to preferentially affect EZH2 activity, it was later demonstrated to globally affect histone methylation patterns [27]. Therefore, conclusions made with this compound about the role of EZH2 enzymatic activity have to be viewed with some degree of caution. Regardless, multiple studies using DZNep essentially confirmed the conclusions drawn from the use of EZH2 RNAi [25, 26, 28, 29], reiterating the importance of this enzyme to cancer biology. However, because DZNep is a global methyltransferase inhibitor and depletes EZH2 protein, additional tools were needed to fully understand the role of EZH2 enzymatic activity in tumors. To this end, highly selective, catalytic inhibitors of EZH2 methyltransferase activity have recently been discovered and are beginning to shed new light on this important topic.

Identification of potent and selective inhibitors of EZH2 methyltransferase activity

A series of publications from 2012 to the present have reported the development of potent and selective inhibitors of EZH2 methyltransferase activity [30-35]. These compounds show variable selectivity against the EZH2-family member EZH1, but show tremendous specificity over other unrelated methyltransferases. Because these compounds inhibit EZH2 catalytic activity without decreasing EZH2 levels (or disrupting PRC2 complexes), they grant the ability to precisely probe the function of EZH2 methyltransferase activity in various systems. Using these inhibitors, several groups have demonstrated the central importance of EZH2 activity in a genetically defined subset of non-Hodgkin lymphoma (NHL) cell lines harboring heterozygous gain-of-function mutations in the EZH2 catalytic domain. Biochemical and cell-based data has shown that these mutations alter the substrate specificity of the enzyme, resulting in high levels of trimethylated H3K27 [13, 36]. Treatment of EZH2 mutant DLBCL cell lines of germinal center origin resulted in depletion of H3K27me3 and reactivation of EZH2 target genes involved in cellular differentiation and cell cycle control, suggesting that EZH2 mutations lock germinal center B-cells in an undifferentiated and highly proliferative state. Most importantly, pharmacological inhibition of EZH2 methyltransferase activity in EZH2 mutant cell lines lead to robust anti-tumor effects, suggesting a precision medicine approach for targeting this subset of NHL patients. Driven in large part by the robust pre-clinical data in EZH2-mutant DBLCl models, small molecule inhibitors of EZH2 are now in clinical development.

Moving beyond EZH2 mutant DBLCl: Efficacy of EZH2i in solid tumors

Despite the tremendous promise of selective EZH2
inhibitors in B-cell malignancies, much less is understood about the role of EZH2 methyltransferase activity in solid tumors. As described above, ablation of EZH2 protein strongly inhibits the growth of many cancer cell lines. However, until recently, there have been relatively few publications describing the use of selective EZH2 inhibitors in solid tumor cell models. These novel agents provide invaluable tools for distinguishing between the catalytic and scaffolding functions of the PRC2 complex. This was highlighted in a recent publication by Wee et al examining the effects of EZH2 inhibition on IFN-γ-JAK-STAT1 signaling in prostate cancer. In this study, the authors demonstrated that depletion of EZH2 by siRNA or DZNep treatment de-repressed the IFN-γ receptor, and when combined with IFN-γ, resulted in synergistic increases in apoptosis of MYC-driven prostate cancer cells [37]. In contrast, treatment with small molecule inhibitors of EZH2 (GSK343 and GSK126) did not de-repress the receptor and did not lead to increased apoptosis when combined with the ligand. Importantly, this inhibitor did decrease global and promoter-specific (INFGR1) H3K27me3, but did not decrease promoter-bound EZH2 nor increase INFGR1 mRNA levels. Thus, the presence of EZH2 (and likely the PRC2 complex) served to maintain repression of this gene independently of its catalytic activity on H3K27 [37]. These results call into question the role of EZH2 enzymatic activity in solid tumors and raise an important question—will the efficacy of EZH2 catalytic inhibitors be limited to B-cell malignancies bearing gain-of-function mutant EZH2? While the answer to this question must ultimately be resolved clinically, recent pre-clinical research performed by several groups have indeed established important roles for both wild-type and mutant EZH2 enzymatic activity in specific solid tumor settings (Figure 1).

Exploiting Epigenetic Vulnerabilities: Antagonism with SWI/SNF Complex

The PRC2 complex and the SWI/SNF nucleosome remodeling complex play antagonistic roles during development. This opens the door for synthetic lethal strategies that exploit genetic vulnerabilities caused by the loss of SWI/SNF family members. Malignant rhabdoid tumors (MRTs) are highly invasive soft-tissue malignancies that are characterized by frequent genetic loss of SMARCB1/INI1. As initial proof of the functional relationship between EZH2 and SMARCB1/INI1 in this

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**Figure 1. Opportunities for therapeutic intervention with catalytic inhibitors of EZH2.**

The clinical development of EZH2 inhibitors was initially driven by robust pre-clinical data in EZH2 mutant non-Hodgkin lymphoma models (highlighted in blue). More recently, EZH2 has emerged as a synthetic lethal target in solid tumors harboring deficiencies in SWI/SNF family members (highlighted in red). In addition, EZH2 inhibitors have also demonstrated pre-clinical activity in models where wild type EZH2 and other members of the PRC2 complex are aberrantly upregulated (highlighted in black). Importantly, several of these studies emphasize the need for alternative assay formats (e.g. 3D assays) and mouse models to uncover fundamental roles of EZH2 in cancer biology. Recent phase I clinical results and the emerging preclinical data suggest that the therapeutic utility of EZH2 inhibitors will not be limited to non-Hodgkin lymphoma patients harboring EZH2 gain-of-function mutations, and highlight the potential for broader utility in solid tumors and hematological malignancies in the context of both mutant and wild-type EZH2.
setting. Wilson et al demonstrated that conditional knockout of EZH2 impaired the growth of SMARCB1/INI1-deficient tumors [38]. A subsequent study by Knutson et al demonstrated that inhibition of EZH2 methyltransferase activity with the catalytic inhibitor EPZ-6438 induced differentiation and apoptosis in this defined genetic background, which lead to sustained, durable tumor regression [39]. A key aspect of this work was the use of longer-term assays (2 weeks) to achieve maximal phenotypic effects. These long time-points correlated with changes in differentiation status, rather than rapid effects such as cell cycle arrest or apoptosis that are associated with targeted therapy of classic oncogenic drivers. This suggests a new framework for the development of epigenetic inhibitors that must focus on alternative endpoints (i.e. differentiation status) and longer assay kinetics. Most importantly, this study established that EZH2 inhibitors can display efficacy in solid tumor settings with wild-type EZH2.

Genetic alterations in SMARCB1/INI1 are not prevalent outside of MRT. However, in synovial sarcoma, a t(X;18) translocation leads to expression of a SS18-SSX oncogenic fusion protein that displaces wild-type SMARCB1/INI1 from the SWI/SNF complex, resulting in its degradation by the proteasome [40]. Similar to the genetic inactivation of SMARCB1/INI1 in MRT, the loss of SMARCB1/INI1 protein in synovial sarcoma cells creates a potential vulnerability that may be targeted by EZH2 inhibition. To this end, Epizyme have reported that EPZ-6438 demonstrated anti-proliferative activity in both cell line and patient-derived models of synovial sarcoma [41]. Based on the positive pre-clinical data in both MRT and synovial sarcoma, Epizyme is pursuing the clinical development of EPZ-6438 in SMARCB1/INI1-deficient solid tumors [42].

The anti-proliferative activity of catalytic inhibitors of EZH2 in SMARCB1/INI1-deficient tumors raises the possibility that these agents could potentially be used to target cancer cells harboring deficiencies in other members of the SWI/SNF complex. Ovarian clear cell carcinomas have frequent mutation of the chromatin remodeler ARID1A (>50%) [43]. This genetic aberration was shown to be synthetically lethal with inhibition of EZH2 catalytic activity as GSK126-treated ARID1A mutant cells undergo cell death in culture and are inhibited in their ability to form tumors in vivo [44]. An important aspect of this work was the observation that catalytic inhibition of wild-type EZH2 did not have significant effects on ovarian cell growth in standard two-dimensional culture but did substantially decrease growth in three-dimensional culture [45]. This implies that in these cell lines EZH2 activity is important for cancer cell interaction with the microenvironment, but perhaps less important for proliferation of monocultures on plastic. As noted previously, these results highlight the requirement for novel assay formats to rigorously evaluate epigenetic inhibitors. To date, the synthetic lethality between EZH2 and SWI/SNF has only been demonstrated in the context of SMARCB1/INI1 and ARID1A deficient settings. However, it is entirely possible that this functional relationship will extend to other components of the SWI/SNF complex. With recent publications highlighting the spectrum of SWI/SNF mutations in human cancer, it opens the door for the expanded use of EZH2 inhibitors in additional SWI/SNF-deficient tumors [46, 47].

**EZH2 Overexpression**

The observation that EZH2 mRNA and protein are overexpressed in cancer has prompted several groups to investigate the role of EZH2 catalytic activity in solid tumor models. For example, EZH2 and its PRC2 subunits, EED and SUZ12, are highly expressed in small cell lung cancer (SCLC) [48, 49], and treatment with the EZH2 inhibitor GSK126 was shown to inhibit the growth of multiple SCLC cell lines in vitro [48]. A recent study by Poirier et al. linked EZH2 overexpression to high levels of CpG methylation in distinct subsets of SCLC [50]. Importantly, this group demonstrated that pharmacological inhibition of EZH2 with the small molecule EPZ-6438 inhibited tumor growth of a SCLC patient-derived xenograft model [50]. While it is unclear if sensitivity in this clinically relevant model is driven by high EZH2 levels and/or activity, this data provides additional rationale for targeting EZH2 in SCLC. Melanoma represents yet another indication where EZH2 catalytic activity has been shown to be important for multiple aspects of tumorigenesis. Initial observations revealed that EZH2 levels increase from nevi to in situ melanoma to metastatic melanoma, suggesting an important role for the enzyme in the pathogenesis and progression of this disease [51]. An elegant study published by Zing et al expanded upon these findings and delineated an important role for EZH2 catalytic activity in melanoma progression. The authors found that in a mouse model of metastatic melanoma both genetic ablation of EZH2 and small molecule inhibition of EZH2 (using GSK503) inhibited tumor growth and abolished metastasis formation without affecting normal melanocytes [52]. By applying EZH2 conditional knockout or GSK503 at different stages of progression within this model, the authors found that EZH2 catalytic activity contributes not only to melanoma initiation and growth, but also to the formation of distant metastases to the lymph nodes and lung. Similar results were also obtained in human melanoma cells as well. The authors then went onto show that EZH2 regulated gene clusters known to be involved in cytoskeletal rearrangements, extracellular matrix remodeling and genes
involved in the epithelial to mesenchymal transition (EMT). In addition to the biological insight provided by this study, these data establish that within this model, inhibition of EZH2 catalytic activity (without affecting EZH2 protein levels) recapitulated EZH2 loss. This strongly argues for further studies of specific EZH2 inhibitors in melanoma.

Gain-of-Function EZH2 Mutations

As previously mentioned, EZH2 gain-of-function mutations have been identified in several B-cell malignancies. Interestingly, EZH2 Y641 mutations also appear in a small subset of melanoma patients (~2%), but their role within this disease had not been characterized [12, 14]. In a recent study by our group, we examined the functional role of EZH2 gain-of-function mutations in melanoma by engineering A375 melanoma cell lines to express various EZH2 mutations, including Y641F, Y641N, A677G, A687V as well as wild-type EZH2 and loss-of-function EZH2 (Y726D). Despite different substrate affinities, all mutations resulted in substantial increases in global H3K27me3 levels [53]. While expression of these mutated forms of EZH2 had little effect on cell growth and morphology in standard tissue culture conditions (two dimensional, tissue-culture coated plastic), they imparted aggressive branching morphologies to cells grown in three-dimensional culture (3D). These morphologies were characterized by decreased cell contractility (i.e. down-regulation of ROCK activity/pMLC) and increased collective cell migration. The molecular underpinnings of these changes were revealed by gene expression analysis. These analyses revealed a conserved subset of genes regulated by multiple EZH2 mutants including gene families involved in interactions with the extracellular matrix as well as a novel pathway regulated by mutant EZH2- the axonal guidance pathway. Interestingly, these observations were corroborated by an independent study that revealed EZH2-dependent regulation of cell migration, integrin expression and downstream cofilin activity in colorectal cancer cells [54].

An important facet of our study is that the observed phenotypes were attributable to the enhanced catalytic activity of EZH2 mutants and not overall EZH2 protein levels, as neither EZH2 wild-type nor the EZH2 loss-of-function mutant gave rise to these phenotypes. Furthermore, inhibition of catalytic activity of mutant EZH2 by GSK126 dramatically decreased global H3K27me3, restored cell contractility, up-regulated axonal guidance genes, and attenuated aggressive 3D morphologies-collective cell migration. 3D-culture phenotypes and axonal guidance pathway genes were similarly affected by EZH2 catalytic inhibition (GSK126) in the IGR1 melanoma cell line which expresses the endogenous activating Y641N mutation. Relative to EZH2 mutant cell lines derived from B-cell malignancies, these cells were insensitive to EZH2 inhibition in standard 2D-culture. Thus, in both melanoma cell models (ectopically or endogenously expressing EZH2 mutations), 3D-culture phenotypes were more strongly impacted by EZH2 catalytic activity than growth/morphology in 2D-culture. This implies that EZH2 catalytic activity may play an important role in determining how melanoma cells interact with the tumor microenvironment. This was further evidenced by the observation that EZH2 hotspot mutants imparted an increase in in vivo tumor growth to the already rapidly growing A375 tumor xenografts, whereas WT or the loss of function mutant had little to no impact on tumorigenesis. Therefore this study reveals that the role of EZH2 catalytic activity may impact tumorigenesis in ways other than strictly driving proliferation as measured by standard assays. Importantly, this study echoes the findings of Zing et al and others in validating EZH2 as a target for melanoma, and as alluded to previously, suggests the need for alternative assays/endpoints that will help determine the importance of EZH2 catalytic activity to solid tumors and eventually the utility of EZH2 catalytic inhibitors for oncological indications outside of mutant EZH2 B-cell malignancies.

Potential for Combination Therapy

Given the fundamental role of EZH2 in regulating cellular fate, inhibition of EZH2 enzymatic activity in cancer cells is likely to have effects on processes such as differentiation and motility that alter the way in which these cells interact with the tumor microenvironment. While single agent anti-proliferative activity has been observed in specific settings, combination with standards of care and other targeted therapies may be needed to achieve the full therapeutic potential for EZH2 inhibitors.

To this end, a recent publication from Epizyme reported the synergistic activity of their clinical candidate EPZ-6438 and glucocorticoid agonists in NHL models. Mechanistic data suggests that this effect is due to the synergistic up-regulation of glucocorticoid target genes following treatment with EPZ-6438 and glucocorticoid agonists, such as prednisolone. Importantly, this robust anti-proliferative effect extended beyond DLBCL cell lines harboring EZH2 mutations to wild-type EZH2 cell lines that were refractory to EZH2 inhibitor alone [55].

Emerging data also suggests the potential for combination therapy may extend to solid tumors. For example, it has recently been reported that the EZH2 inhibitor GSK126 sensitizes non-small cell lung cancer (NSCLC) cell lines with
**BRG1** loss-of-function or **EGFR** gain-of-function mutations to topoisomerase II inhibitor treatment \[56\]. These data identify a potential precision medicine approach for the use of EZH2 inhibitors in NSCLC and also highlight the importance of genetic background to predicting sensitivity. Interestingly, the utility of an EZH2 inhibitor/topoisomerase inhibitor combination may be relevant in other solid tumor indications. Following up on the observation that EZH2 and TOP2A are both up-regulated in metastatic prostate cancer, Kirk et al., recently demonstrated that the combination of GSK126 and the topoisomerase II inhibitor etoposide significantly enhances the killing of prostate cancer cell lines \[57\]. While the full scope of potential combination partners remains to be fully elucidated, these data provide initial support for the pursuit of combination strategies for EZH2 inhibitors in solid tumors.

**Alternative Approaches: Targeting the PRC2 Complex**

Targeted therapies for EZH2 have focused on the development of small molecules that block the active site of the enzyme without disrupting the integrity of the PRC2 complex. However, emerging data suggest that the PRC2 complex may possess oncogenic activity that is independent of EZH2 methyltransferase activity. As referenced earlier, siRNA and shRNA inhibition of EZH2 have shown broader efficacy than enzymatic inhibitors and synergy with IFN-γ treatment was only observed upon disruption of the PRC2 complex, but not with catalytic inhibitors of EZH2 \[37\]. These inconsistencies suggest the potential for alternative strategies that target the stability of the PRC2 complex.

Taking advantage of an essential α-helical domain of EZH2 that engages the EED subunit, Stuart Orkin and colleagues designed stabilized α-helical EZH2 peptides (SAH-EZH2 peptides) that effectively block this key protein-protein interaction, resulting in disruption of the PRC2 complex and down-regulation of EZH2 protein levels \[58\]. In AML cells, treatment with SAH-EZH2 peptide selectively inhibits H3K27 methylation and induces a growth arrest phenotype that is accompanied by monocyte-macrophage differentiation. Importantly, the small molecule inhibitor GSK126, a potent inhibitor of H3K27me3, failed to mimic the effect of the SAH-EZH2 peptide. Selective inhibition by SAH-EZH2 peptide was also observed in breast and prostate cancer cell lines that are resistant to catalytic inhibitors of EZH2 \[37, 59\]. The disruption of the PRC2 complex and subsequent down-regulation of EZH2 represents a unique mechanism of action that may be particularly effective for targeting cancer cells that are driven by non-catalytic functions of EZH2 and the PRC2 complex.

While the development of SAH-EZH2 peptides represented the initial proof of concept for specifically targeting the PRC2 complex, peptide therapeutics have been traditionally hampered by poor drug properties. In an attempt to circumvent this liability, small molecule inhibitors are currently being used to exploit the drug-ability of protein-protein interactions. More recently, potent inhibitors of protein-protein interactions have been identified for WDR5-MLL, Menin-MLL, and WDR5-MYC \[60-62\]. To this end, virtual and biochemical screens have identified small molecule inhibitors that disrupt the interface between EZH2 and EED. Taking advantage of a crystal structure of EED in complex with EZH2, Kong and colleagues identified astemizole as a molecule that disrupts the EZH2-EED interaction and destabilizes the PRC2 complex, resulting in inhibition of H3K27me3 and growth arrest in PRC2-driven lymphoma cells \[63\]. The natural product wedelolactone was also identified in a screen for compounds that bind with high affinity to EED. This molecule was shown to disrupt the EZH2-EED interaction, resulting in destabilization of the PRC2 complex, degradation of PRC2 subunits, and de-repression of PRC2 target genes \[64\]. While additional work needs to be done to optimize the potency and selectivity of these early molecules, the initial studies provide proof of concept for alternative approaches to target EZH2 in the context of the PRC2 complex. Moving forward, it may also be possible to target the PRC2 complex for degradation by engineering chimeric molecules that bind to EZH2 and recruit E3 ubiquitin ligase complexes. This strategy has been used to successfully target BRD4, another epigenetic regulator that has been linked to several tumor types \[65, 66\]. Exploiting these unique mechanisms of action may broaden the clinical application of EZH2 targeted therapies.

**Conclusions and Perspectives**

The discovery of potent, selective inhibitors of EZH2 methyltransferase activity has provided the scientific community with invaluable reagents for validating this promising drug target. While early validation efforts with genetic tools and early small molecule inhibitors shed some light on the therapeutic potential of inhibiting this enzyme, these regents lacked specificity and were unable to distinguish between the catalytic and scaffolding roles of the PRC2 complex. With the appropriate tools in hand, recent preclinical studies have focused on elucidating the biological factors that confer sensitivity to EZH2 inhibitors in order to discover novel opportunities for therapeutic intervention. Emerging data suggest that the antagonism between the Polycomb and SWI/SNF complexes can be exploited as a synthetic lethal strategy to target SMARCB1/INI1- and ARID1A-deficient tumors. As the genes that encode SWI/SNF components are mutated in ~20% of all cancers, it is possible that the vulnerability to EZH2 inhibition will
extend to other SWI/SNF subunits\textsuperscript{[67]}. Moving beyond SWI/SNF, it has been suggested that a similar antagonism may occur between EZH2 and KDM6A/UTX, the enzyme involved in demethylation of H3K27, opening up the possibility of treating KDM6A/UTX-deficient tumors\textsuperscript{[68][69]}. In addition to genetic background, it has been proposed that tumor-specific overexpression of EZH2 creates a dependence on EZH2 methyltransferase activity. While EZH2 inhibitors are demonstrating efficacy in pre-clinical models with high EZH2 expression, it remains to be seen whether elevated EZH2 levels alone confer sensitivity in these settings. It is likely that overall sensitivity of a cancer cell to EZH2 inhibitors will depend on a multitude of intrinsic and extrinsic factors, including genetic context, differentiation status, interplay with the tumor microenvironment, and modulation of immune response pathways.

The early use of EZH2 inhibitors has highlighted the need for alternative assay formats that differ from those that have historically been used to assess the efficacy of cytotoxic drugs. Data suggest epigenetic reprogramming following EZH2 inhibition is a relatively slow process that requires multiple days to produce robust anti-proliferative phenotypes. Based on this observation, the evaluation of EZH2 inhibitors requires the use of long-term assays that measure traditional (proliferation, apoptosis) and non-traditional (differentiation, self-renewal) endpoints. In addition, the use of three-dimensional assays and mouse models has uncovered roles for EZH2 in cell migration, invasion, and metastasis that would not have been revealed in standard two-dimensional in vitro assay formats.

The first small molecule inhibitors of EZH2 have now entered the clinic (Table 1), and objective responses have been observed across multiple subtypes of NHL in the phase I dose escalation trial for EPZ-6438\textsuperscript{[42][70]}. Interestingly, the majority of responses reported to date have occurred in NHL patients with wild-type EZH2. While satisfying, this level of response would not have been predicted based on the available pre-clinical data. In addition, signs of clinical activity in SMARCB1/INI1-deficient MRT have also been reported, suggesting that the synthetic lethality observed in pre-clinical studies will potentially translate in the clinic\textsuperscript{[39][71]}. While it remains to be seen if superior responses will be achieved in NHL patients bearing EZH2 gain-of-function mutations, the early clinical data suggests that the efficacy of EZH2 inhibitors will not be limited to this patient population. To this end, emerging pre-clinical data in multiple solid tumor indications supports a broader therapeutic role for EZH2 inhibitors, both as single agents as well as in combination other therapies. Moving forward, we must continue to investigate the role of EZH2 in pre-clinical models that capture the essential roles of this enzyme in cancer biology and to incorporate this knowledge into future clinical development of novel therapies that target this key epigenetic regulator.

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Conflicting interests

The authors declare that they have no conflicts of interest.

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