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Treating lymphoma is now a bit EZ-er

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Tazemetostat represents the first epigenetic therapy approved for the treatment of follicular lymphoma (FL). It inhibits the activity of the enhancer of zeste homolog 2 (EZH2) histone methyltransferase, the first of a multitude of epigenetic regulators that have been identified as recurrently mutated in FL and germinal center diffuse large B-cell lymphoma. In this review, we discuss the initial discovery and ongoing exploration of the functional role of *EZH2* mutations in lymphomagenesis. We also explore the path from the preclinical development of tazemetostat to its approval for the treatment of relapsed FL, and potential future therapeutic applications. We discuss the clinical data that led to the approval of tazemetostat and ongoing research into the function of EZH2 and of tazemetostat in lymphomas that derive from the germinal center, which could increase the applicability of this drug in the future.

Introduction

Follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) are the most commonly occurring lymphomas worldwide. For decades, a growing list of common mutations that underlie these cancers has been emerging. Many of the genes mutated in both malignancies have roles in epigenetic regulation, the first described example being enhancer of zeste homolog 2 (*EZH2*) in 2010.[1] Motivated by the clear association between perturbed epigenetic regulation and malignancy, there were several attempts to manipulate the epigenome as a therapeutic vulnerability using existing epigenetic therapies, including histone deacetylase inhibitors and hypomethylating agents.[2-4] Disappointingly, these failed to show clinical benefit in FL and DLBCL and highlighted the need for rationally designed targeted therapies.

Tazemetostat represents the first therapy developed based on the unique genetic features of B-cell non-Hodgkin lymphoma (B-NHL) and the first specific inhibitor of EZH2 approved for clinical use. In June 2020, tazemetostat received accelerated approval by the US Food and Drug Administration (FDA) for the treatment of relapsed/refractory FL after 2 lines of therapy, along with a companion diagnostic test for identifying *EZH2*-mutant tumors. Here, we describe the initial discovery of *EZH2* mutations and the biological rationale for targeting EZH2 activity in B-NHL, beginning with the identification of a mutation pattern that is restricted to a subset of lymphomas arising from the germinal center of the lymphoid follicle. We also discuss the preclinical and clinical data that ultimately led to the approval of tazemetostat for the treatment of FL patients a decade later.

The molecular aberrations underlying germinal center B-cell lymphoma

Originally, the genes and mutations that contributed to lymphomagenesis had been identified using a combination of cytogenetic methods, array-based copy-number analysis, and candidate gene sequencing. These methods enabled early identification of oncogenes such as *MYC*, *BCL2*, and *BCL6*, which commonly acquire deregulated expression in B-cell lymphomas due to translocations with immunoglobulin loci. Similarly, by defining minimal common regions of deletion, well-known tumor-suppressor genes such as TP53 CDKN2A had been identified in FL and DLBCL.[5-7] Through targeted sequencing of regions affected by copy-number alterations, genes such as TNFRSF1[8] and SOCS1[9]

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were eventually found to be mutated in these lymphomas. This growing list of shared genetic features between FL and the germinal center B-cell (GCB) subgroup of DLBCL fostered an appreciation of biological similarities between these entities. Furthermore, the genes and mutation patterns that are restricted to these lymphomas highlighted the existence of context-specific cancer genes that are not commonly mutated in other malignancies.

The arrival of Illumina massive parallel sequencing afforded the opportunity to broadly search for additional lymphoma-related genes. An early demonstration of this potential was the combined application of whole-genome sequencing and RNA-Seq to a collection of DLBCLs, FLs, and composite lymphomas. This enabled the identification of a mutation hotspot in the gene *EZH2*.[1] The mutation pattern we observed was striking, with a single amino acid within the SET domain (Y646) commonly mutated to (mostly) 1 of 4 different residues. *EZH2* mutations were primarily observed in FLs and the GCB subgroup of DLBCLs, further affirming their shared molecular underpinnings.

At the time, hotspot mutations had been characterized in other oncogenes such as RAS family members and several kinases, where they typically exhibit a neomorphic effect. Although *EZH2* was considered an oncogene with transforming potential in some solid tumors,[10] its mutation had not been reported in cancer. Additional sequencing confirmed the same pattern of mutations and with no truncating mutations found. This supported the notion that these mutations cause a gain of function.[11,12] Although it had once been posited as a marker of proliferation in lymphomas,[13] there was sparse information regarding its function in B-cell biology.[14] Over the subsequent decade, countless large studies have explored the genetics of cancer and yet *EZH2*^{Y646} mutations have only rarely been observed in other cancers. The mutation appears to be rare even in most other germinal center–derived malignancies such as Burkitt lymphoma, highlighting a unique cellular context in which it can contribute to oncogenesis. In contrast, although gain-of-function mutations are observed in germinal center lymphomas, a pattern of inactivating *EZH2* mutations was being described as a feature of some myeloid cancers.

Functional insights in humans and flies

EZH2 is a histone methyltransferase and the catalytic subunit of the polycomb-repressive complex 2 (PRC2), which catalyzes a series of 3 methylation reactions that lead to trimethylation of lysine 27 on histone H3 (H3K27me3). *EZH2* is the namesake of the *Drosophila melanogaster* gene E(z). More than a hundred mutant alleles of this locus have been studied in the context of genetic interactions, with genes comprising the polycomb group.[15] Buried among these is a mutation orthologous to the *EZH2* hotspot mutation, an allele known as E(z)^{1}.[1] Interestingly, the phenotype of this mutation was observed as distinct from loss-of-function mutations in this gene.[16] Somewhat paradoxically, reconstituted polycomb complexes containing the mutant *Drosophila* E(z)^{1} protein were incapable of trimethylating H3K27 in vitro,[17] which suggested a more nuanced effect of this mutation.

In our study detailing the initial discovery of this hotspot, we performed functional characterization of *EZH2*^{Y646} mutations using recombinant PRC2. Similar to the observations of *Drosophila* ortholog, our experiments implied a reduction in catalytic activity in vitro[1] but this proved to be only a piece of a complex puzzle. Subsequent studies demonstrated that wild-type EZH2 efficiently catalyzes the mono- and dimethylation of H3K27, whereas *EZH2*^{Y646} produces an enzyme that more efficiently catalyzes the third methylation (Figure 1A).[18,19] Structural characterization of the catalytic domain of EZH2 revealed hydrogen bonding between Y646 and the substrate lysine that induces a conformational constraint thereby limiting the efficiency of the third methylation. All hotspot mutations remove this constraint and afford the active site with additional space to accommodate the third methyl group.[20] As a consequence, tumors and cell lines heterozygous for *EZH2*^{Y646} acquire a net gain of activity through the cooperation of mutant and wild-type PRC2.

EZH2 in normal and malignant B cells

By depositing the H3K27me3 epigenetic mark, the PRC2 complex is responsible for suppressing genes that promote the latter stages of cellular differentiation, thereby controlling cell-fate decisions in embryonic development and hematopoeisis.[21] At key developmental stages, many such genes harbor a distinct combination of H3K4me3 (active) and H3K27me3 (inactive) marks, which allows rapid induction upon removal of the latter. More than 1000 genes acquire this "poised" epigenetic mark in the transition from naive to GCB cells including several genes that are responsible for exit from the germinal center and terminal differentiation.[22] Based on this, *EZH2*^{Y646} mutations are predicted to maintain suppression of these prodifferentiation genes, thereby allowing retention of the phenotype of GCB cells. In keeping with this model, treatment of DLBCL cell lines with EZH2-inhibitory compounds caused plasma cell differentiation and proliferation arrest (Figure 1B). This was observed in GCB cell lines but not ABC cell lines and was not restricted to lines with *EZH2*^{Y646} mutations.[22] Further preclinical modeling of EZH2 inhibitors showed a similar effect on *EZH2*-mutant DLBCLs, firmly establishing EZH2 inhibition as a therapeutic option worthy of exploration in GCB lymphomas.[23-25]

There is a growing appreciation that EZH2 can reprogram malignant cells to affect interactions with the microenvironment. Conditional knock-in models exhibit an expanded population of centrocytes but no evidence of a differentiation blockade. The accumulation of centrocytes is instead attributed to enhanced proliferation and survival.[26] Additional sequencing confirmed that premalignant germinal B cells with *EZH2*^{Y646} become less reliant on T follicular helper cells for survival, instead relying on follicular dendritic cells. Another intriguing association with immune cell interaction is the observation that DLBCLs commonly lose expression of major histocompatibility complex (MHC) class I through mutation and epigenetic silencing of a variety of genes.[27] Treatment with EZH2 inhibitors can restore the expression of CD58,[28] another gene implicated in immune evasion. Similarly, reduced MHC class II is observed in a subset of DLBCLs and has been attributed to *EZH2* mutations.[29] Loss of expression of MHC due to these changes contributes to immune evasion. Because FL and DLBCL have distinct immune microenvironments, the utility of EZH2 inhibition in DLBCL and the optimal combination that exploits this feature remain to be explored (Figure 1B).

Targeting EZH2 became a viable consideration with the understanding of its structure and role in tumorigenesis. As a result, several EZH2 inhibitors are in clinical development, with tazemetostat receiving the first clinical approval for this class of agents. The lead compound was identified by high-throughput screening

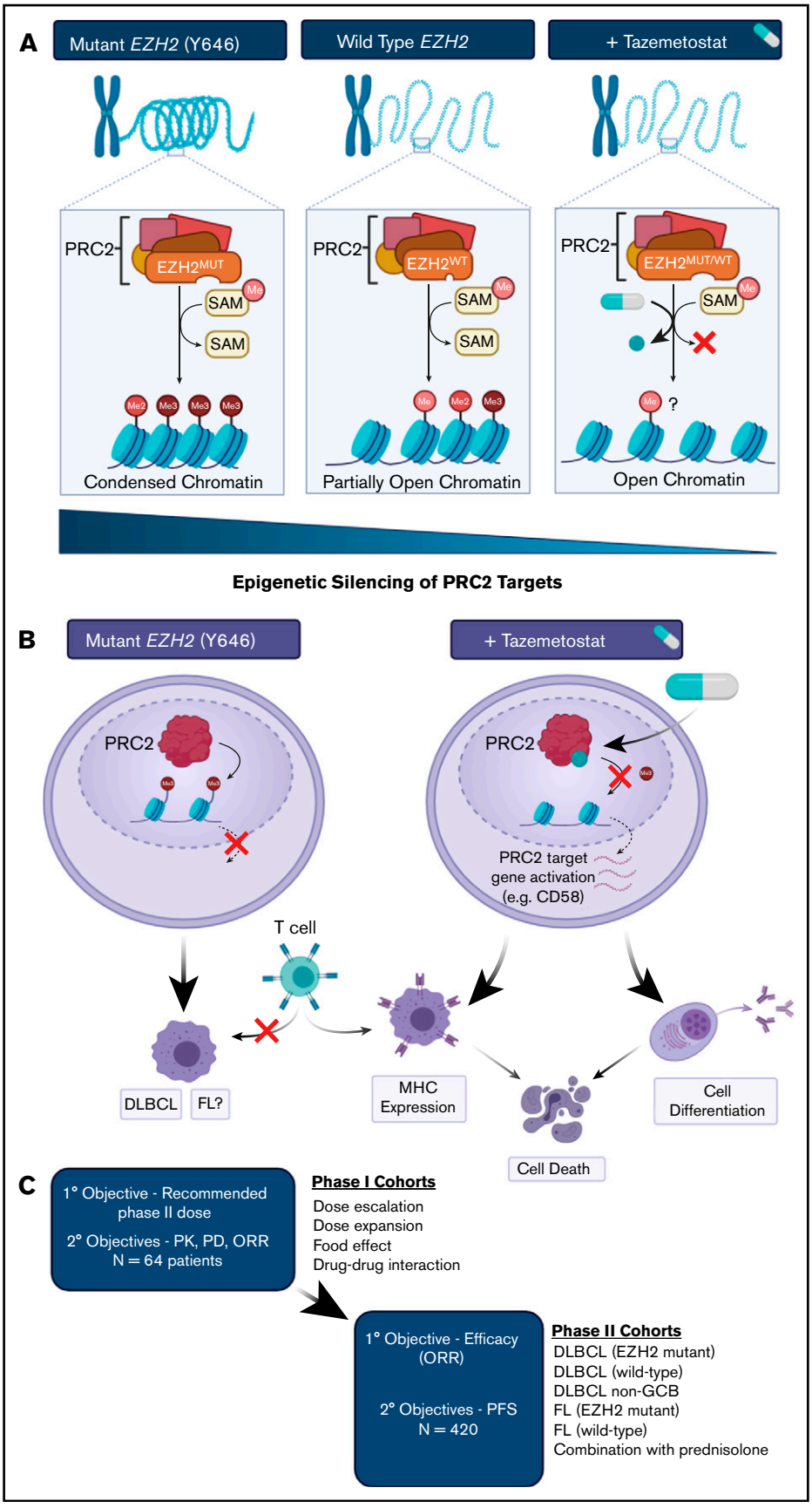

Figure 1. Targeting the neomorphic EZH2 function in lymphomas. (A) *EZH2* hotspot mutations promote the trimethylation of H3K27 leading to global H3K27me3 accumulation in mutant cells. This enhances suppression of PRC2 targets through epigenetic silencing. Treatment with tazemetostat blocks methylation of H3K27 leading to a global reduction in H3K27Me3. Question mark (?) denotes that the exact methylation state of H3K27 in cells treated with tazemetostat is currently unknown. (B) Malignant cells bearing *EZH2* mutations have reduced expression of genes associated with differentiation and major histocompatibility complex (MHC) expression. Treatment with tazemetostat can release these genes from epigenetic silencing and reverse both phenotypes, leading to differentiation and possibly cell death and/or reexpression of MHC, allowing recognition by T cells. This is known in DLBCL and hypothesized in FL. (C) Phase 1/2 trial design of tazemetostat in B-NHL. MUT, mutant; ORR, overall response rate; PD, pharmacodynamics; PK, pharmacokinetics; SAM, s-adenosyl methionine; WT, wild type. Illustration created with BioRender.com.

followed by iterative chemistry to produce an EZH2 inhibitor with strong potency and favorable pharmacokinetic properties. Tazemetostat is a small molecule competitive inhibitor of s-adenosyl methionine, the lysine methylation substrate for EZH2, and thereby inhibits the methylation of histone H3 lysine 27, resulting in lower levels of the fully methylated H3K27Me3. In vitro, the inhibition constant (*Ki*) is 2.5 ± 0.5 nM for wild-type EZH2 and is similar for all common mutants. Importantly, tazemetostat is highly selective for both wild-type and mutant EZH2, with limited effect on EZH1 and limited to no inhibitory activity against other lysine methyl transferases. Finally, tazemetostat shows good oral bioavailability in animals.[30]

Clinical development of tazemetostat in lymphoma

The clinical development program for tazemetostat includes both B-NHL and solid tumors. The first approval of tazemetostat was in unresectable epithelioid carcinoma with loss of integrase interactor 1 (INI1). The single-agent clinical data for tazemetostat in lymphoma was generated from 1 phase 1/2, first-in-human, dose-finding and efficacy study (NCT01897571) (Figure 1C). The phase 1 trial included 64 patients with solid tumors or NHL. A standard 3+3 design was used to determine maximum tolerated dose based on cycle 1 dose-limiting toxicity, with dose levels doubling from 100 mg up to 1600 mg orally twice daily. Ultimately, the recommended phase 2 dose of 800 mg twice daily was selected based on safety, pharmacodynamics, pharmacokinetics, and preliminary efficacy.[31]

The only dose-limiting toxicity was 1 episode of grade 4 thrombocytopenia, such that the maximum tolerated dose was never reached. The other common emergent adverse events (occurring in ≥10% of patients) were asthenia, anemia, anorexia, muscle spasms, nausea, vomiting, constipation, thrombocytopenia, dry skin, neutropenia, and diarrhea. Most of these adverse events were of grade 1 or 2 severity. Grade 3 or greater adverse events were uncommon and included thrombocytopenia, neutropenia, hypertension, and elevated bilirubin and transaminases.[31] A partial clinical hold was placed temporarily on the clinical program after 1 pediatric patient developed T lymphoblastic lymphoma while on tazemetostat. However, this patient was heavily pretreated with other DNA-damaging agents. Among 729 patients treated with tazemetostat, 0.7% developed myelodysplasia or acute myeloid leukemia and 2% of patients with FL stopped therapy due to a second primary malignancy. As a result, the drug label contains a warning for the development of second primary cancer.[32] Randomized, controlled studies will clarify the risk of second cancers with tazemetostat.

Global reduction of H3K27me3 in tissues serves as a pharmacodynamic measure of the cellular effect of tazemetostat on EZH2 inhibition.[31] In total, 32 patients with NHL or solid tumor in the phase 1 portion of the study were evaluated by skin-punch biopsy prior to and after 28 days of tazemetostat. A dose-dependent decrease in H3K27me3 was observed over the 100 to 800 mg dosing range, with an estimated 80% decrease at day 15 for the 800 mg orally twice-daily dose. An increase in dose to 1600 mg did not afford a much greater reduction in trimethylation. Similarly, in 3 of 4 paired tumor biopsies, reduction in H3K27 trimethylation was observed after 4 weeks of treatment. Moreover, in 1 patient with eventual disease progression, RNA-sequencing analysis demonstrated a fourfold reduction from baseline in *EZH2* expression and the differential expression of *EZH2* target genes following treatment with tazemetostat. Despite these molecular changes, this patient experienced disease progression.[31]

Pharmacokinetic studies demonstrate that tazemetostat is rapidly absorbed (time taken to reach maximum serum concentration, 1-2 hours) with a mean terminal half-life of 3 to 5 hours. Steady state is reached after 15 days of exposure when taken daily.[31] Tazemetostat has a mean bioavailability of 33%, with 88% being bound to plasma proteins. Because tazemetostat is metabolized by cytochrome P450 (CYP)3A, and eliminated in the feces, it should not be administered with moderate or strong CPY3A inhibitors, or dose-reduced accordingly.[32]

In total, 13 DLBCL, 7 FL, and 1 patient with marginal zone lymphoma were included in the phase 1 portion of NCT01897571. The median age was 62 years, 15 patients were male, and the median number of prior therapies was 3. These patients were treated at doses ranging from 100 mg to 1600 mg, with 8 treated at the recommended phase 2 dose of 800 mg. Objective responses were seen in 8 of 21 patients (38%; 95% confidence interval [CI], 18.1-61.6). Of these 8 responders, 3 patients (2 with FL and 1 with DLBCL) had a complete response (CR) as assessed by positron emission tomography/computed tomography scan. The remaining 5 patients (3 with DLBCL and 1 each with FL and marginal zone lymphoma) had a partial response (PR). One patient with a Y646H mutation had a durable PR lasting 16 months. Median time to response was 3.5 months. Three patients with an initial PR converted to a CR at 9, 22, and 24 months after therapy initiation. The median duration of response was 12.4 months (interquartile range, 2.5 to >18.3 months). Two patients, 1 each with FL and DLBCL, have remained on tazemetostat for 27.6 and 33.6 months, respectively, as of the reporting of these data.[31]

The phase 1 portion of NCT01897571 established the very good tolerability of continuous daily dosing of tazemetostat, defining 800 mg twice daily as the recommended phase 2 dose, and showed early signs of strong efficacy with complete and durable responses in both DLBCL and FL patients regardless of the presence of *EZH2* hotspot mutations.

Phase 2 study results: FL

The phase 2 portion of NCT01897571 included 5 treatment cohorts: GCB DLBCL with mutant *EZH2,* GCB DLBCL with wild-type *EZH2*, non-GCB DLBCL, FL with mutant and wild-type *EZH2*, and a combination cohort with prednisolone for all DLBCL subtypes. Tazemetostat obtained accelerated approval in the United States for the treatment of both mutant and wild-type *EZH2* relapsed FL, after 2 prior lines of therapy, based on the response rates of the FL subgroups within this study.

In total, 99 patients with FL were treated, including 45 with an *EZH2* mutation, which was determined centrally using archived or fresh tissue. The median age was 61 and 62 years, median prior number of therapies was 2 and 3, for mutated and unmutated *EZH2*, respectively. For mutant *EZH2* FL, the overall response rate (ORR) by independent review committee was 69% (95% CI, 53.4-81.1) and 35% (95% CI, 22.7-49.4) in wild-type *EZH2* FL. CRs occurred in 6 patients with mutant *EZH2* (13%) and 2 with wild-type *EZH2* (4%). Among patients with either transformed FL or grade 3b FL, 3 of 3 with mutant *EZH2*, and 2 of 6 with wild-type *EZH2* responded.

Impressively, among the 45 patients with mutant *EZH2*, all but 1 had a decrease in tumor size by bidimensional measurement. There was a lower but encouraging response rate among wild-type patients with 34 of 54 patients (65%) experiencing some tumor shrinkage.[33]

Patients with classical indicators of more aggressive FL responded equally well to tazemetostat as those with more favorable disease. For example, patients with progression of disease within 24 months of first-line therapy (POD24) had an ORR of 63% and 25%, in the mutant and wild-type groups, respectively[33] (more recent data suggest that the adverse prognosis of POD24 may be less than first estimated, with findings indicating that many patients with POD24 actually have transformed FL[34,35]). Double-refractory patients, to both rituximab and an alkylating agent, had ORRs of 78% and 27%, in the mutant and wild-type groups, respectively.[36] The high responses to tazemetostat in this cohort, particularly among the mutant patients, indicates the preserved pivotal role of *EZH2* in relapsed FL and the clinical value of the novel mechanism of action of tazemetostat. Whether the variant allele frequency or clonal burden of EZH2 mutation correlates with response has not been determined.

Consistent with the lymphoma patients in the phase 1 portion of the study, the time to response was 3.7 months in the FL patients. Relative to the 3 phosphatidylinositol 3-kinase (PI3K) inhibitors approved for third-line therapy of FL,[37-39] responses to tazemetostat appear to occur more slowly. Given that most patients eventually exhibit tumor shrinkage and that responses may improve over time, continuing therapy as long as it is tolerated and provides clinical benefit would seem reasonable, even in the absence of objective response.

The median duration of response was similar in mutant and wild-type *EZH2* FL: 10.9 vs 13 months. With the currently reported observation time, the longest duration of response has not yet been reached. For mutant and wild-type *EZH2*, respectively, the median progression-free survival (PFS) was 13.8 months (95% CI, 10.7-22) vs 11.1 (95% CI, 3.7-14.6).[33] The similar duration of response and PFS in responding patients with either mutant or wild-type *EZH2* suggests that dependence on EZH2 is equivalent whether acquired by direct mutation of *EZH2* or by alterations in other genes that result in increased EZH2 function.

The main treatment-related adverse events were nausea, asthenia, diarrhea, fatigue, and alopecia, as seen in the phase 1 study. Anemia related to therapy was seen in 9 patients, 2 of whom were grade 3 or higher. Therapy-related thrombocytopenia occurred in 8% of patients and was grade 3 or greater in 3 patients. Infections constituted the majority of serious adverse events but were mostly not considered related to therapy.[32,36]

A salient finding from this phase 2 study includes the tumor shrinkage observed in nearly all patients with mutated *EZH2* FL, which confirms the pivotal role of *EZH2* in driving tumor growth and survival in these cases. In contrast, in the wild-type *EZH2* group, responses occurred less consistently. Tazemetostat has a very favorable tolerability profile relative to other agents approved in relapsed FL, such as PI3K inhibitors, Revlimid (lenalidomide) and rituximab (R^2), and chemoimmunotherapy. Lastly, in this trial, patients with poorer-prognosis FL also benefit from tazemetostat. Tazemetostat could thus be considered a therapeutic option for a wide range of patients with relapsing FL, including those who are more fit as well as elderly or more frail patients, particularly among those with a mutation in *EZH2*.

Predictors of response in FL and DLBCL

Given the relative infrequency of *EZH2* mutations in B-NHL and the requirement for biopsy material for screening, it was a challenge to identify sufficient patients to complete enrollment for NCT01897571. The much higher response rate in the mutant *EZH2* cohort nonetheless confirms the value of this investment. On the other hand, including a wild-type cohort highlighted the importance of *EZH2* as an oncogene in some wild-type FL. The similar duration of response and PFS for the 2 cohorts supports the therapeutic value of tazemetostat in EZH2-dependent disease.

To date, data on the genetic alterations that are associated with tazemetostat response have been limited. Panel-based sequencing of archival tumor and circulating tumor DNA (ctDNA) from a subset of patients in NCT01897571 has provided candidate predictors of response in both wild-type and mutant *EZH2* lymphomas. In addition to testing for *EZH2* hotspot mutations using the Cobas assay, Illumina sequencing was used to identify somatic mutations, amplifications, and translocations. In the latest update of these results, *EZH2* and *STAT6* mutations were associated with response in FL and mutations in *BCL2*, *TNFAIP3*, *FOXO1*, and *MYD88* predicted for nonresponse. Only *EZH2* mutations were associated with response in both tissue and ctDNA.[40] In contrast, in this study of DLBCL, *EZH2* mutations were not associated with response, however, *MYD88*, *MEF2B*, *ETV6*, *MLH1*, *RECQL4*, and *RNF43* were. DLBCL with *PDL1*, *PDL2*, *BCL2*, and *SOCS1* mutations was less likely to respond to therapy. Mutations common to ctDNA and archived tissue were not found in DLBCL.[40]

Drawing firm conclusions from these mutation studies as patient numbers are small and multiple gene comparisons were undertaken is difficult. Furthermore, the massive diversity of genetic alterations that underlie DLBCL continues to be delineated. Recent studies indicate the existence of >100 recurrently mutated genes that potentially harbor driver mutations and many additional genes affected by recurrent somatic copy-number alterations.[41,42] Using a subset of these mutation and copy-number features, DLBCLs can now be categorized into more granular genetic subgroups including 1 subgroup, known as EZB or C3, which is strongly enriched for BCL2 translocations and *EZH2* mutations.[42-44] This genetic subgroup almost exclusively represents GCB cases but may represent a more homogeneous entity with shared molecular features. In the case of tazemetostat and other EZH2 inhibitors, tumors of the EZB/C3 subgroup are expected to have the greatest chance of clinical benefit. We anticipate confirmation of this through retrospective analysis of existing data. Moreover, we hope this will provide the impetus for novel clinical trial designs.

Rational drug combinations for tazemetostat

Both the excellent tolerability and unique mechanism of action of tazemetostat make it an ideal candidate for combination therapies to enhance rates of response and duration in B-NHL.

Interim results of tazemetostat in DLBCL show promise but point to the need for patient selection and rational drug combinations. Overall, 17% of patients responded, with 13 experiencing a CR (11 in patients with wild-type *EZH2*; N = 226).[45] Although synergy between tazemetostat and prednisolone has been observed in

preclinical models of DLBCL,[46] this did not translate into a higher response rate in patients with relapsed/refractory DLBCL where the ORR in this cohort was 9%.[45] Patient selection may have confounded the results as eligibility required neither mutated *EZH2* nor GCB DLBCL.

A phase 1 study of rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone (RCHOP) and tazemetostat in newly diagnosed DLBCL was recently reported. The regimen was tolerable and the recommended dose in combination was 800 mg orally, twice daily. Metabolic CR was achieved in 76.5% of patients (n = 17, 3 with mutant *EZH2*).[47] A larger, controlled trial would be needed to determine the superiority of this regimen over RCHOP.

EZH2-mediated histone H3K27 trimethylation represses the tumor production of T helper 1–-type chemokines and reduces T-cell trafficking to the tumor microenvironment. In an ovarian cancer model, inhibiting EZH2 enhanced T-cell trafficking to the tumor and improved the therapeutic efficacy of programmed death ligand 1 (PDL1) checkpoint blockade.[48] Based on these findings, a phase 1b study combined tazemetostat with atezolizumab, a PDL1 inhibitor, in patients with relapsed/refractory DLBCL. The best ORR was 16%, with 2 CRs. Among patients with mutant *EZH2* lymphoma, 3 of 5 responded. The observed efficacy did not justify further testing.[49] Overall, programmed cell death protein 1/PDL1 blockade in FL and DLBCL is low, and predictors of response are not known.[50,51] Combinations of tazemetostat with therapies that modulate immune evasion in lymphoma are warranted and may require separate exploration in FL and DLBCL.[45]

The combination of tazemetostat and the BCL2 inhibitor, venetoclax, has been tested in preclinical models the DLBCL subgroup of EZB/C3.[43] In cell lines and a patient-derived xenograft with these aberrations, exposure to tazemetostat and venetoclax resulted in synergistic cell killing. Tazemetostat increased proapoptotic proteins and venetoclax was postulated to enhance the apoptotic pathways thus triggered.[52] Patient selection for EZB subtype DLBCL and the addition of venetoclax represents a promising, targeted therapeutic avenue that leverages our emerging understanding of the molecular underpinnings of individual DLBCL subgroups.

Preclinical studies in mantle cell lymphoma cell lines have reported synergy with immunomodulatory drugs, venetoclax, and a variety of B-cell receptor pathway modulators.[53] These data support a currently enrolling study that compares tazemetostat and R^2 to R^2 in patients with FL in first relapse. The trial has a safety run-in followed by a cohort of *EZH2*-unselected FL patients, possibly followed by a third cohort of *EZH2*-mutated FL (NCT04224493).

Finally, EZH2 inhibitor-resistant cell lines have been generated using the EZH2 inhibitor GSK126. Mechanisms of resistance included upregulation of the PI3K, MAPK, and IGF-1R pathways and point mutations in *EZH2*, which prevented the binding of GSK126. Both pathways resulted in a reduction in apoptotic gene expression.[54] This would suggest that combinations with PI3K or BTK inhibitors could produce greater responses in lymphoma.

Conclusion

The development of tazemetostat represents an early example of how genome-wide cancer genomics can deliver on "precision medicine.. The superior response rate for FL patients with *EZH2* mutations highlights the growing need to screen patient tissue for such "actionable" driver mutations up front. Because FL is incurable, patients require multiple therapies over time, leading to inevitable cross-resistance and intolerance. The targeted nature of tazemetostat and its favorable toxicity profile make it an important addition to existing therapeutic options and knowledge of which patients harbor *EZH2*-mutant disease may enhance the appeal for selection of this drug. Postmarketing studies will determine when physicians choose to give this agent. Early data in DLBCL patients are also very encouraging, with some CRs observed with tazemetostat alone. Given the molecular commonalities between FL and GCB DLBCL, further combination trials are warranted and should be designed based on our understanding of the oncogenic roles of EZH2. Further work to identify FL and DLBCL subsets most likely to benefit, and to resolve, the molecular features of *EZH2* wild-type responders will help to define populations for further study. Furthermore, we feel that exploring the potential utility of tazemetostat in maintenance therapy for the elimination of minimal residual disease in both FL and DLBCL is warranted.

Authorship

Contribution: S.A., S.E.A., and R.D.M. wrote the paper and prepared the figure.

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