Mutations affecting key epigenetic modifiers tend to cluster in malignancies in which cells of origin lie in the germinal center (GC). EZH2, as transcriptional repressor, is mutated in high frequency in Chinese and Western patients with follicular lymphoma and may represent a rational target for GC-derived lymphomas. Clin Cancer Res; 20(12); 3047–9. ©2014 AACR.

In this issue of Clinical Cancer Research, Guo and colleagues report on two interesting findings (1). First, they report that the incidence of EZH2 mutations occurs in high frequency in Chinese patients with follicular lymphoma (FL), and second, that this incidence is similar to that found in Western cohorts.

B-cell lymphomas represent a very heterogeneous group of diseases. Central to the development of mature B-lymphocytes is the germinal center (GC) reaction. Within the GC, B-lymphocytes undergo somatic hypermutation (SHM) and isotypic switching to generate a repertoire of B cells with high affinity for diverse antigens. These GC reactions create a seemingly infinite spectrum of immunoglobulin gene rearrangements that provide the immune system the ability to recognize countless antigens. The GC represents an environment with a physiologically high rate of mutagenesis, and when unrestrained, can lead to aberrant SHM, generating a malignant genotype favoring lymphomagenesis. There are three main subtypes of GC-derived lymphomas: FL, diffuse large B-cell lymphoma (DLBCL), and Burkitt lymphoma, which range from profoundly indolent to incredibly aggressive diseases, respectively. Although each of these entities have distinct molecular features, they share characteristics of classic GC biology, in particular a transcriptionally repressed state. The proto-oncogene BCL6 is physiologically active during the GC reaction, leading to transcriptional repression of key tumor-suppressor proteins such as p53, cell-cycle regulators such as CDKN1A, and master regulators of terminal differentiation such as PRDM1 allowing for SHM (2). Recent gene expression profiling has further categorized select lymphomas as being derived from the GC, which are comparatively more dependent on Bcl6 or as being from post-GC lymphocytes, which are comparatively more dependent on NF-kB (3). Lymphomas of post-GC origin are driven by chronic signaling of the B-cell receptor pathway (including BTK, PI3 kinase, and AKT), converging on activation of NF-kB. These lymphomas have benefited from the development of therapies targeting this biology, including drugs such as ibritinib, the first-in-class inhibitor of BTK, and idelalisib, a PI3 kinase inhibitor (4). Although GC lymphomas historically have more favorable outcomes compared with post-GC lymphomas, some of the most substantial progress of late has been directed toward the post-GC DLBCLs.

As is the case for Bcl6, it has been found that enhancer of zeste homolog 2 (EZH2) is also implicated in maintaining the GC reaction by enforcing silencing of key tumor suppressors and cell-cycle regulators (5, 6). EZH2 is a subunit of the polycomb repressor 2 (PCR2) complex that enforces trimethylation of histone 3 lysine 27 (H3K4me3) via its SET domain containing histone methyltransferase. This epigenetic modification leads to chromatin condensation and transcriptional repression and is especially important for the regulation of bivalent genes such as those involved in differentiation and maturation (Fig. 1). The EZH2–PCR2 complex functions in cooperation with Bcl6, and like Bcl6, EZH2 is constitutively upregulated in the GC and acquires activating and gain-of-function mutations driving GC-derived lymphomagenesis (7). Mutations in EZH2 and BCL6 join a growing list of mutations affecting key epigenetic modifiers that tend to cluster in lymphomas of GC origin. These genes include loss of function of histone acetyltransferases (HAT) p300 and CEBBP and the mixed lineage leukemia MLL gene, which encodes another methyltransferase. Mutations or loss of expression of the HATs are found in 39% of GC-DLBCL and 41% of FL. This phenotype leads to a more aggressive course and is observed to a much lesser degree in post-GC–derived DLBCL (17%) and not at all in other B-cell lymphoma subtypes (8). MLL2 gene rearrangements are found in 24% of GC-DLBCL and may contribute to the oncogenic potential of GC lymphocytes (9). Together, these mutations lead to a GC-specific repressed state silencing proapoptotic factors, cell-cycle checkpoint regulators, and key modulators of...
lymphocyte maturation and differentiation. This phenotype allows for an amplified proliferative state, in which additional mutations can accumulate further contributing to malignant transformation. Interestingly, these mutations are found almost exclusively in GC-derived lymphomas, and these findings lend themselves toward building an epigenetic platform for the treatment of GC-derived malignancies.

In their article, Guo and colleagues further our understanding of the role of EZH2 in GC-derived malignancies. The authors demonstrate that EZH2 mutations are found in abundance in patients with FL and are associated with increased protein expression. The frequencies of these mutations and increased expression were similar to those reported previously and confirm that this is a global finding, demonstrating that patients from divergent ethnic backgrounds have similar patterns of expression and mutation frequencies (16.9%) compared with cohorts of Western FL patients (26.8%). The authors further define EZH2 mutations as being more prevalent in grades 1 and 2 as compared with grade 3 FL. They also demonstrate a significant association with BCL2 rearrangements in both Chinese and Western cohorts.

The notion of epigenetic targeting of GC lymphomas began with observations that the effects of both p53 and Bcl6 could be modified by acetylation. There is a critical inverse relationship between Bcl6 and p53, the functional status of which is linked to each transcription factor’s degree of acetylation. Deacetylation of Bcl6 is required for the transcriptional repressor effects of this oncogene. Conversely, acetylation activates the tumor suppressor, p53. One potential therapeutic strategy for targeting GC lymphomas involves the pharmacologic modification of Bcl6 and p53 using histone deacetylase (HDAC) inhibitors. Bcl6 and p53 are known to be acetylated in lymphoma cell lines treated with HDAC inhibitors in combination with the sirtuin inhibitor, niacinamide. This correlates with synergistic cytotoxicity that is restricted to GC lymphomas (10). This finding has been translated to the clinical setting for evaluation of vorinostat in combination with niacinamide for relapsed and refractory lymphomas. In a heavily pretreated cohort of patients, the combination led to a 24% overall response rate and an additional 57% of patients who achieved stable disease. This proof-of-principle study used two first-in-class drugs, which can be greatly improved upon, and despite this demonstrated that Bcl6 and p53 can be therapeutically modulated (10). Hypomethylating agents have also been studied in this context, both alone and in combination with HDAC inhibitors or combination chemotherapy. The HDAC inhibitor panobinostat is synergistic with the hypomethylating agent decitabine in preclinical models of DLBCL. The combination led to increased acetylation of histone 3 with unique effects on gene expression and gene-specific CpG methylation. The effects of this combination correlated with synergistic cytotoxicity in cell lines and tumor growth delay in in vivo models of DLBCL (11). Similar to these findings, preexposure with decitabine prior to chemotherapy led to enhanced chemosensitivity in cell lines refractory to doxorubicin and induction of hypomethylation, decreased growth rate, and reactivation of SMAD1, which plays a role in differentiation, proliferation, and apoptosis, as well as chemotherapy-induced senescence. Following a 5-day exposure to decitabine in lymphoma cell lines, SMAD1 expression was increased 5-fold. This finding was translated clinically in patients with de novo DLBCL who received a preexposure of
decitabine followed by R-CHOP. The preexposure led to decreased methylation marks in paired tissue samples and was well tolerated in this early-phase study (12).

These epigenetic strategies could potentially lead to a therapeutic effect by modulating the constitutively active EZH2–PCG2 complex in GC malignancies. In addition, new EZH2 inhibitors are presently in development by GlaxoSmithKline and Epizyme, which have demonstrated activity in GC lymphomas (13). These agents have shown potent activity in preclinical models in which EZH2 is either mutated or wild-type EZH2 is overexpressed, and they are now being studied in early-phase clinical trials. These findings support the potential benefit of EZH2 inhibitors for the treatment of GC lymphomas. EZH2 inhibitors may be potentially synergistic with other epigenetic modifying agents such as HDAC inhibitors and hypomethylating agents—an area yet to be studied. Hopefully, similar to the remarkable activity in preclinical models in which EZH2 is either mutated or wild-type EZH2 is overexpressed, and they are now being studied in early-phase clinical trials. These findings support the potential benefit of EZH2 inhibitors for the treatment of GC lymphomas. EZH2 inhibitors may be potentially synergistic with other epigenetic modifying agents such as HDAC inhibitors and hypomethylating agents—an area yet to be studied. Hopefully, similar to the remarkable development of therapeutic agents altering the natural history of post-GC lymphomas, these series of findings may lead to new treatment platforms, targeting the deranged epigenetic apparatus in GC lymphomas.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: J.E. Amengual, O.A. O’Connor
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): J.E. Amengual
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J.E. Amengual, O.A. O’Connor
Writing, review, and/or revision of the manuscript: J.E. Amengual, O.A. O’Connor
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): O.A. O’Connor

Grant Support
This work was supported by an RWJF-ASH-AMFDP Award, a Columbia University Provost Award (to J.E. Amengual), and the Leukemia & Lymphoma Society Translational Research Program (to O.A. O’Connor).

Received April 14, 2014; accepted April 15, 2014; published OnlineFirst May 23, 2014.

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Manipulating the Epigenome in Germinal Center Lymphomas: Is It Getting Easier and "EZier?"

Jennifer E. Amengual and Owen A. O'Connor