Bromodomains are conserved protein regions that function as epigenetic readers by recognizing specific histone modifications. The common association of bromodomains with enhancer and super-enhancer regions in diffuse large B-cell lymphoma contributes to its pathogenesis. Bromodomain inhibition reduces tumor growth largely through the disruption of transcriptional networks driven by oncogenic MYC. Clin Cancer Res; 21(1) January 1, 2015. CCR Translations

Bromodomain Inhibition in Diffuse Large B-cell Lymphoma—Giving MYC a Brake

Anja Mottok1,2 and Randy D. Gascoyne1,2

In this issue of Clinical Cancer Research, Trabucco and colleagues (1) report that JQ1, a small-molecule inhibitor of the bromodomain and extra-terminal (BET) protein family, is able to induce cell death, cell-cycle arrest, or senescence in diffuse large B-cell lymphoma (DLBCL) mediated through downregulation of MYC expression and subsequently suppression of MYC target gene transcription.

Over the past decades, it has become evident that epigenetic alterations play a major role in the pathogenesis of diseases, especially cancer. Targeting the epigenome has emerged as a promising strategy in the treatment of malignant lymphomas, some of the latter found in close proximity to MYC and CD79B (2). This in turn explains why lymphoma cells dependent on these super-enhancers and their downstream transcriptional networks are sensitive to treatment with BET inhibitors, particularly JQ1—a thieno-triazolo-1,4-diazepine derivative, which has high affinity to BRD4. JQ1 prevents the BET proteins from interacting with the chromatin by competitively occupying the binding pocket (3). Recently, it has been shown that JQ1 reduces proliferation/viability in multiple myeloma cell lines as well as in purified primary multiple myeloma cells and improves survival in a multiple myeloma mouse model (6). Similar results were obtained by Mertz and colleagues (7) in a large collection of cell lines, including Burkitt lymphoma (BL), multiple myeloma, acute myeloid leukemia, and some solid cancers. Furthermore, these studies emphasized that the effects seen resulted from the transcriptional repression of MYC and its downstream targets.

The helix-loop-helix leucine zipper protein MYC is characterized by its "omnipotency" to regulate important cellular functions, including growth, proliferation, apoptosis, metabolism, biosynthesis of ribosomal proteins and nucleic acids, as well as angiogenesis and differentiation downstream of signal transduction pathways. About 10% to 15% of human genes are believed to be regulated by the master transcription factor MYC, which forms a heterodimeric structure with MAX and is capable of activating or suppressing target genes (8). Given this broad spectrum of biologic functions, deregulated MYC is a feature of many different cancer types and is at least in part responsible for malignant transformation and tumor maintenance. Moreover, it is often associated with aggressive behavior in tumors and poor prognosis. Understandably, these features put MYC on top of the list of therapeutic targets, but for a long time, MYC seemed to be inviolable.

MYC translocations are a hallmark of BL, but are also detectable in multiple myeloma, plasmablastic lymphoma, rarely in acute lymphoblastic leukemia/lymphoma, and in approximately 10% of de novo DLBCL cases. Moreover, increased MYC protein expression can be observed in about one third of DLBCL samples by using immunohistochemistry, indicating that deregulated MYC protein expression can result from mechanisms other than translocations involving Ig or non-Ig genes.
A large proportion of patients diagnosed with DLBCL can be cured using the standard-of-care therapy regimen that consists of rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP). However, about 30% to 40% of patients are refractory to primary treatment or experience progression after initially responding. In either case, salvage therapies are suboptimal and outcomes are dismal (9). The intention to administer dose-intensified chemotherapy and autologous stem cell transplantation (ASCT) in the salvage setting can only be applied to roughly half of the patients because of advanced age and/or comorbidities, with even fewer having chemotherapy-sensitive disease deemed suitable for ASCT and only rare patients experiencing long-term cures.

Tumors showing a MYC rearrangement concomitant with translocations involving the BCL2- and/or BCL6-gene locus (so-called double-hit/triple-hit lymphomas) account for approximately half of the DLBCL cases harboring primary MYC translocations. These patients are at high risk of treatment failure and eventually succumb to their disease within a short period of time. Numerous studies have demonstrated that not only the rearrangement status but also high-level protein expression of MYC especially in concert with BCL2 positivity is associated with inferior survival in DLBCL independent of the International Prognostic Index and other clinical parameters (10–12). A somewhat surprising observation in one of the studies was the fact that those "dual-expressers" displayed similar outcomes despite being assigned to different cell of origin (COO) subgroups. In addition, the exclusion of MYC+/BCL2- cases eliminated the negative prognostic impact of the activated B-cell subtype in the remainder of the cohort (12). These data allow one to speculate that the combined assessment of MYC and BCL2 protein may trump the analysis of COO subtype in DLBCL. Whether this represents a robust prognostic tool remains to be established.

In the current study, Trabucco and colleagues (1) demonstrate that treatment with JQ1 significantly reduces the proliferation of DLBCL cell lines in a dose-dependent manner irrespective of the molecularly defined subtype. Furthermore, by investigating cell-cycle distribution and measuring caspase 3/7, as well as β-galactosidase activity, they were able to show that after an initial cell-cycle arrest in the G1 phase, cell lines either underwent apoptosis or were prone to senescence when treated for a period of 7 days. As previously shown in a variety of other hematologic malignancies and solid cancers, the antiproliferative effect of JQ1 in DLBCL cell lines is induced by interfering with MYC expression regardless of the underlying genetic aberration affecting the MYC gene locus. In a xenograft mouse model used in the study by Trabucco and colleagues (1), JQ1 treatment was significantly accompanied by increased survival time and decelerated tumor growth. It could therefore be reasonably expected that a clinical grade derivative of JQ1 might improve outcomes for the 25% to 30% of patients with DLBCL with concomitant expression of MYC and BCL2 proteins (Fig. 1).
Taken together, these new data suggest that bromodomain inhibitors could be added to the ever growing list of promising new drugs in the therapeutic management of DLBCL. Moreover, recent studies have pointed to possible synergistic effects of JQ1 and EZH2- or HDAC inhibitors. Clinical trials have to further elucidate the role of these novel agents in the management of patients with lymphoma with respect to combined treatment regimens and toxicity profiles. Correlative investigations are needed to identify patients who will benefit from this approach and to develop meaningful biomarkers to predict either sensitivity or resistance to this targeted therapy.

Disclosure of Potential Conflicts of Interest
R.D. Gascoyne is a consultant/advisory board member for Genentech. No potential conflicts of interest were disclosed by the other author.

Authors’ Contributions
Conception and design: A. Mottok, R.D. Gascoyne
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A. Mottok, R.D. Gascoyne
Writing, review, and/or revision of the manuscript: A. Mottok, R.D. Gascoyne

Acknowledgments
The authors thank Dr. David Scott for helpful discussion and contributions to the figure.

Grant Support
R.D. Gascoyne was supported by the Terry Fox Research Institute Program Project award (grant no.1023).

Received July 30, 2014; accepted August 18, 2014; published OnlineFirst August 27, 2014.

References
Bromodomain Inhibition in Diffuse Large B-cell Lymphoma—Giving MYC a Brake

Anja Mottok and Randy D. Gascoyne