Hijing the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4

Highlights
- BRD4 PROTAC achieved rapid and potent degradation of BRD4
- BRD4 PROTAC suppressed proliferation and induced apoptosis in BL
- BRD4 PROTAC provides a novel and effective strategy to target BRD4

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In Brief
Lu et al. designed a potent BRD4 degrader using the PROTAC technology by recruiting BRD4 to the E3 ligase cereblon. This study demonstrates the potential of hijacking cereblon, or other E3 ligases, through the PROTAC platform, for effectively targeting pathological proteins as a therapeutic approach.
Hijacking the E3 Ubiquitin Ligase Cereblon to Efficiently Target BRD4

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SUMMARY

BRD4, a bromodomain and extraterminal domain (BET) family member, is an attractive target in multiple pathological settings, particularly cancer. While BRD4 inhibitors have shown some promise in MYC-driven malignancies such as Burkitt’s lymphoma (BL), we show that BRD4 inhibitors lead to robust BRD4 protein accumulation, which may account for their limited suppression of MYC expression, modest antiproliferative activity, and lack of apoptotic induction. To address these limitations we designed ARV-825, a hetero-bifunctional PROTAC (Proteolysis Targeting Chimera) that recruits BRD4 to the E3 ubiquitin ligase cereblon, leading to fast, efficient, and prolonged degradation of BRD4 in all BL cell lines tested. Consequently, ARV-825 more effectively suppresses c-MYC levels and downstream signaling than small-molecule BRD4 inhibitors, resulting in more effective cell proliferation inhibition and apoptosis induction in BL. Our findings provide strong evidence that cereblon-based PROTACs provide a better and more efficient strategy in targeting BRD4 than traditional small-molecule inhibitors.

INTRODUCTION

BRD4 belongs to the bromodomain and extraterminal domain (BET) family of proteins, which is characterized by two bromodomains (BD) at the N terminus and an extraterminal domain (ET domain) at the C terminus (Belkina and Denis, 2012; Shi and Vakoc, 2014). The two BDs recognize and interact with acetylated lysine residues at the N-terminal tails of histones; the ET domain, which is not yet fully characterized, is largely considered to serve a scaffolding function in recruiting diverse transcriptional regulators (Belkina and Denis, 2012; Shi and Vakoc, 2014). Thus, BRD4 plays a key role in regulating gene expression by recruiting relevant transcription modulators to specific genomic loci. Several recent studies establish that BRD4 is preferentially located at super-enhancer regions, which often reside upstream of important oncogenes such as c-myc, bcl-xL, and bcl-6, and play a key role in regulating their expressions (Chapuy et al., 2013; Loven et al., 2013). Owing to its pivotal role in modulating the expression of essential oncogenes, BRD4 has emerged as a promising therapeutic target in multiple cancer types, including midline carcinoma, acute myeloid leukemia (AML), multiple myeloma (MM), Burkitt’s lymphoma (BL), and prostate cancer (Asangani et al., 2014; Delmore et al., 2011; French et al., 2008; Loven et al., 2013; Mertz et al., 2011; Wyce et al., 2013; Zuber et al., 2011). In particular, BRD4 is exciting as an alternative strategy for targeting c-MYC, which contributes to the development and maintenance of a majority of human cancers but has remained undruggable (Baratta et al., 2015; Delmore et al., 2011; Gabay et al., 2014; Mertz et al., 2011). Indeed, the development of small-molecule BRD4 inhibitors, such as JQ1, iBET, and OTX015, has quickly demonstrated their promising therapeutic potential in preclinical models of various cancers, including BL (Asangani et al., 2014; Baratta et al., 2015; Boi et al., 2015; Chapuy et al., 2013; Delmore et al., 2011; Loven et al., 2013; Mertz et al., 2011; Puissant et al., 2013). Almost all BL cancers contain a c-myc gene translocation that places it under control of a super-enhancer located upstream of IGH, thus driving an abnormally high level of c-MYC expression, crucial to tumor development and maintenance (Klapproth and Wirth, 2010). Excitingly, preclinical studies with BRD4 inhibitors demonstrate their value in suppressing c-MYC and proliferation in BL cell lines, albeit with IC50 (half maximal inhibitory concentration) values often in the range of 100 nM to 1 μM (Ceribelli et al., 2014; Mertz et al., 2011).

Small-molecule inhibitors, either by inhibiting an enzyme activity (such as kinase inhibitors) or by interfering with protein-protein interactions (such as BRD4 inhibitors) have been the cornerstone of drug development in oncology (Hoelder et al., 2012). However, given the reversible binding of most small-molecule inhibitors, large systemic drug concentrations and continuous exposures are often required to ensure sufficient functional inhibition (Johnson et al., 2010). In a number of cases it proves challenging to achieve and maintain a drug concentration high enough for efficacy in vivo. As an alternative, approaches to eliminate disease-causing abnormal proteins, either by RNAi or small-molecule-induced protein degradation, are evolving as novel strategies for targeting the undruggable or difficult targets (Burnett et al., 2011; Howell et al., 2004). We have developed an induced protein degradation strategy that utilizes Proteolysis Targeting Chimeras (PROTACs) that recruit targeted proteins...
to the E3 ubiquitin ligase VHL (Von Hippel-Lindau) for ubiquitination and subsequent proteasome-mediated degradation (Buckley and Crews, 2014; Raina and Crews, 2010; Sakamoto et al., 2001, 2003; Schneekloth and Crews, 2005; Schneekloth et al., 2004). This PROTAC technology provides great potential in harnessing the action of a single E3 ligase toward pathologically important proteins that are currently undruggable through conventional strategies for drug development.

In this study, we demonstrate that the small-molecule BRD4 inhibitors, JQ1 and OTX015, lead to significant BRD4 protein accumulation over time in all BL cell lines tested. Although both inhibitors initially suppress downstream c-MYC level, the suppression is incomplete and requires high drug concentrations. This robust accumulation of BRD4, together with the reversible nature of inhibitor binding to BRD4, may in part account for the modest effect of these inhibitors on downstream c-MYC suppression and cell proliferation inhibition. To circumvent these limitations, we designed a hetero-bifunctional molecule, ARV-825, by connecting a small-molecule BRD4 binding moiety (OTX015) to an E3 ligase cereblon binding moiety (pomalidomide) using PROTAC technology (Boi et al., 2015; Fischer et al., 2014; Ito et al., 2010). OTX015 is currently in phase I clinical trials and pomalidomide is a potent third-generation immunomodulatory drug (IMiD), which functions through interacting with the E3 ligase cereblon and inducing degradation of essential Ikaros transcription factors in MM (Boi et al., 2015; Lu et al., 2014). ARV-825 actively recruits BRD4 to cereblon, resulting in the rapid and efficient degradation of the former via the proteasome. We demonstrate that, compared with the BRD4 inhibitors, ARV-825 treatment results in a strikingly more pronounced effect on the levels of c-MYC, and downstream cell proliferation and apoptosis induction in BL cell lines. These findings strongly support the development of BRD4 PROTACs as a promising novel strategy to efficiently target BRD4. Moreover, this study is the first to describe a potent PROTAC that acts via the recruitment of the E3 ligase cereblon, Our study demonstrates that hijacking the E3 ubiquitin ligase cereblon, or other E3 ligases, through the PROTAC platform, holds great potential for pursuing effective therapeutics.

RESULTS

Small-Molecule BET Domain Inhibitors Lead to Significant BRD4 Protein Accumulation and Inefficient c-MYC Suppression

As recently discovered, BL cells are responsive to BRD4 inhibitors, mostly due to their dependence on the c-myc oncogene that is translocated and brought under the control of upstream \( \text{IgH} \) super-enhancers regulated by BRD4 (Klapproth and Wirth, 2010; Mertz et al., 2011). Interestingly, we found that both JQ1, the most frequently used inhibitor in published studies, and OTX015, the most advanced inhibitor in clinical development, led to significant BRD4 protein accumulation in a dose-dependent manner in all BL cell lines tested (Figure 1A and Figure S1). This is consistent with the previously reported observation that JQ1 treatment results in BRD4 upregulation in some lung cancer cell lines (Shimamura et al., 2013). Moreover, the accumulation of BRD4 is rapid, resulting in a multi-fold increase of BRD4 within 24 hr of treatment (Figure 1B). We reasoned that the BRD4 inhibitor-induced increase in BRD4 protein level may incur a higher barrier for BRD4 to be effectively suppressed. Indeed, even at concentrations up to 10-fold over their IC\(_{50}\) values, JQ1 and OTX015 were unable to suppress c-MYC levels fully (Figure 1C). Even though a higher BRD4 level does not necessarily warrant higher BRD4 nuclear localization under these conditions, this finding implies that cancer cells may quickly restore their transcriptional activation upon the withdrawal of bromodomain inhibitors. Indeed, c-MYC levels were rapidly restored to normal upon the withdrawal of JQ1 and OTX015 from the culture media (Figure 1D). This is consistent with previous findings in AML that c-MYC is repressed by JQ1 treatment, but rebounds quickly upon JQ1 withdrawal (Mertz et al., 2011).

Hijacking the E3 Ubiquitin Ligase Cereblon to Create PROTAC to Efficiently Degrade BRD4

We noted the rapid and robust accumulation of BRD4 associated with inhibitor treatments, and the resulting moderate effects on downstream c-MYC suppression and proliferation. To circumvent these limitations, we used PROTAC technology to design the chimeric small molecule ARV-825 (Figure 2A). ARV-825 consists of a classic BRD4 binding moiety of the triazolo-diazepine acetamide class seen in OTX015, and pomalidomide, a known cereblon binding moiety of the IMiD class (Fischer et al., 2014), connected by a flexible polyethylene glycol linker. This linker was chosen because of its high conformational flexibility that would maximize the chance of the PROTAC associating with both BRD4 and cereblon. We subsequently demonstrated that ARV-825 shows only slightly reduced binding affinities to bromodomains 1 and 2 compared with those of OTX015 (Table 1).

Next, we showed that treatment of BL cell lines with ARV-825 results in almost complete BRD4 protein degradation, with DC\(_{50}\) (50% of maximum degradation) below 1 nM (Figure 2B). Given that BRD4 and cereblon binding moieties in ARV-825 have \( \text{K}_D \)s of 28–90 nM and \( \sim 3 \mu\text{M} \) to their respective targets (Table 1) (Lopez-Girona et al., 2012), this suggests that ARV-825 acts in a substoichiometric way in mediating BRD4 degradation. Interestingly, we observed a bell-shaped dose dependence of BRD4 degradation by ARV-825 treatment, with some BRD4 protein remaining at the high concentration of 1 μM (Figure 2B). This phenomenon argues for a BRD4/ARV-825/cereblon trimer complex as the active species in driving efficient BRD4 degradation. High concentrations of ARV-825 would be predicted to result in the formation of non-functional BRD4/ARV-825 and ARV-825/cereblon dimers that compete with formation of the active trimer, resulting in lower BRD4 degradation (Figure S2). The BRD4 degradation induced by ARV-825 occurs rapidly, resulting in more than 50% of protein being lost within 2 hr of compound treatment (Figure 2B).

To confirm that BRD4 degradation induced by ARV-825 is mediated by cereblon, we treated BL cells with ARV-825 in the presence of an excess of the cereblon ligand pomalidomide. As expected, the excess pomalidomide was able to reduce ARV-825-induced BRD4 protein degradation, confirming a cereblon-mediated mechanism. Pomalidomide treatment by itself in the same experiment had no impact on BRD4 protein levels (Figure 2C). Furthermore, co-treatment with a proteasome inhibitor (MG132 or carfilzomib) completely blocked the BRD4
degradation induced by ARV-825 (Figure 2D). Taken together, these data demonstrate that ARV-825 leads to fast and efficient BRD4 degradation in a cereblon-mediated and proteasome-dependent mechanism.

**ARV-825 Leads to More Significant and Longer Lasting c-MYC Suppression than Small-Molecule Inhibitors**

Next, we performed a head-to-head comparison between ARV-825 and the small-molecule inhibitors JQ1 and OTX015, to determine their effects on BRD4 and c-MYC levels. JQ1 or OTX015 treatment consistently leads to robust BRD4 accumulation and inefficient c-MYC suppression (Figure 3A). In contrast, ARV-825 results in significant BRD4 degradation and a more pronounced downregulation of c-MYC when compared with that seen with JQ1 and OTX015 treatment at higher concentrations (Figure 3A). Moreover, the suppression of BRD4 and c-MYC protein levels by ARV-825 is long lasting (Figure 3B). Namalwa cells were treated with ARV-825, JQ1, or OTX015 for 24 hr, then washed with fresh medium three times to remove compounds. c-MYC recovers to the control level 4 hr after the removal of JQ1 and OTX015, whereas ARV-825 sustains its effect on SLC19A1 repression significantly longer (Figure 3C).

**ARV-825 Leads to a Superior Effect on Suppression of BL Cell Proliferation Compared with BRD4 Inhibitors**

BL cells are known to be sensitive to BRD4 inhibitors, which suppress c-MYC signaling and inhibit cell proliferation (Mertz et al., 2011). Because ARV-825 treatment results in prolonged BRD4 downregulation and downstream signaling suppression compared with BRD4 inhibitors, we hypothesized that it would
provide superior functional effects compared with the inhibitors. Indeed, ARV-825 leads to more pronounced suppression of proliferation than both JQ1 and OTX015 in all BL cell lines tested (Figure 4A). Moreover, the proliferation suppression effect of ARV-825 is sustained longer than that of JQ1 and OTX015 after the removal of compounds following a 24-hr treatment (Figure 4B). This is consistent with our earlier findings that ARV-825 provides a long-lasting effect on BRD4 degradation and downstream signaling repression (Figures 3B and 3C). As demonstrated in Figure 2C, the presence of excess cereblon ligand, pomalidomide, is able to prevent efficient BRD4 degradation induced by ARV-825 due to competition of cereblon binding. The presence of excessive pomalidomide rescued the proliferation suppression effect of ARV-825 in BL cells in a dose-dependent manner (Figure 4C). Importantly, pomalidomide alone did not show any significant effects on the proliferation of these cell lines (Figure 4D).

**DISCUSSION**

BRD4 has captured considerable attention from academia and the pharmaceutical industry alike due to its great potential as a novel target in multiple disease settings, particularly in cancer. One key advantage of BRD4 as an oncology target is that it is identified as preferentially clustering at super-enhancer regions in control of pivotal oncogenes such as c-myc, bcl-xl, and pax5 (Chapuy et al., 2013; Loven et al., 2013), and thus offers
an alternative strategy in targeting those oncogenes which are difficult to inhibit by traditional strategies. Moreover, BRD4’s distinct high occupancy of genomic loci proximal to specific oncogenes provides the potential for a therapeutic window that could allow specific targeting of tumor cells while sparing normal tissues. Indeed, BRD4 inhibitors have shown antitumor activities with good tolerability in different mouse tumor models (Asangani et al., 2014; Baratta et al., 2015; Boi et al., 2015; Ceribelli et al., 2014; Chapuy et al., 2013; Loven et al., 2013; Mertz et al., 2011; Shimamura et al., 2013; Wyce et al., 2013). Also, not surprisingly, high sensitivity to BRD4 inhibitors, such as JQ1, has been associated with high level of either c-MYC or n-MYC in different tumor types, including c-MYC-driven BL (Baratta et al., 2015; Loosveld et al., 2014; Mertz et al., 2011; Puissant et al., 2013). Also, not surprisingly, high sensitivity to BRD4 inhibitors, such as JQ1, has been associated with high level of either c-MYC or n-MYC in different tumor types, including c-MYC-driven BL (Baratta et al., 2015; Loosveld et al., 2014; Mertz et al., 2011; Puissant et al., 2013). Currently four BET bromodomain inhibitors are in phase I clinical trials with a focus largely on midline carcinoma and hematological malignancies (CPI-0610, NCT01949883; GSK525762, NCT01587703; OTX015, NCT01713582; TEN-010, NCT01987362). In this report, we found that the BRD4 inhibitors JQ1 and OTX015 lead to fast and robust accumulation of BRD4 protein in all BL cell lines tested. Similar observations have been found in a panel of lung and prostate cancer cell lines (Shimamura et al., 2013). One possible explanation is that the binding of inhibitors to BRD4 results in a conformational change, which leads to increased thermodynamic stability of the protein. Similarly, inhibitor binding could hinder BRD4 accessibility to the endogenous cellular degradation machinery, thus rendering it kinetically stable. Alternatively, the BRD4 inhibitors may be interrupting a BRD4-mediated negative feedback loop that regulates BRD4 protein levels. Nevertheless, this prominent increase of BRD4 levels, together with the reversible nature of inhibitor binding, could prevent efficient BRD4 inhibition. Indeed, both preclinical and clinical studies have shown that the effects of BRD4 inhibitors are largely cytostatic, with apoptosis limited to a few cell lines and tumors from phase I patients (Chapuy et al., 2013; Delmore et al., 2011; Shao et al., 2014). This could significantly limit the potential benefit of patients at clinically achievable concentrations of BRD4 inhibitors. One strategy to achieve more effective BRD4 inhibition is to design irreversible/covalent inhibitors, which have revived significant interest in recent years, as they may achieve the desired pharmacological effect at lower drug concentrations (Johnson et al., 2010). However, covalent inhibitors have their own limitations, most notably the potential immunogenicity of protein adduct and high hurdle of selectivity (Johnson et al., 2010).

Here, we designed a novel chimera molecule, ARV-825, using the PROTAC platform, to efficiently degrade BRD4, as an alternative strategy of targeting BRD4. In the process, we also demonstrated for the first time the incorporation of the E3 ligase cereblon into the PROTAC technology paradigm (Fischer et al., 2014). We successfully achieved rapid and prominent BRD4...
degradation by ARV-825, which leads to robust and long-lasting downstream c-MYC suppression. Most importantly, ARV-825 results in more significant proliferation suppression, and more robust apoptosis induction, than even high concentrations of both JQ1 and OTX015. The improved functional effects of BRD4 degrader over inhibitors could be partially attributed to the more complete and sustained suppression on c-MYC, a driver oncoprotein in BL. It is also possible that BRD4 possesses scaffolding functions, as it is a large protein with many binding partners, many of which remain to be identified and elucidated. Understandably, eliminating BRD4 would elicit a more profound effect than mere inhibition of its binding to acetyl lysine-containing partners. A comparison of phenotypes of BRD4 knockout or knockdown (such as by CRISPR or shRNAs) with that of BRD4 inhibition by inhibitors would help inform this possibility. Such an analysis, however, is beyond the scope of this report.

The more complete and longer lasting disruption of BRD4 function by PROTACs or other means, on the one hand, will benefit future patients because of the more effective targeting and less frequent dosing regimen. On the other hand, it could also be associated with more severe side effects, as BRD4 regulates diverse biological processes in a cellular context-dependent manner (Bolden et al., 2014; Fernandez et al., 2014). Recent findings using an inducible and reversible transgenic BRD4 targeting RNAi mouse model have elegantly shed some light on the potential limitations of potent and sustained BRD4 targeting, including epidermal hyperplasia, alopecia, and reduced cellular diversity and stem cell loss in the small intestine (Bolden et al., 2014). This represents a dilemma commonly seen in drug development, namely, determining whether and how an optimal therapeutic index can be achieved in the clinic. Importantly, these on-target side effects of BRD4 suppression appear to be reversible, giving hope that there will be a therapeutic window through better understanding and management of BRD4 targeted therapeutics (Bolden et al., 2014).

Due to the high homology of BET family BD1 and BD2 domains, current BET inhibitors in clinical development, including OTX015, are not selective in binding and inhibiting BRD2/3/4/T (Filippakopoulos et al., 2010; Noel et al., 2014). We also examined the effect of ARV-825 on BRD2 and BRD3 (BRDT is only expressed in testis). Not surprisingly, both BRD2 and BRD3, like
BRD4, can be degraded by ARV-825 (Figure S3) owing to the similar affinity of OTX015 to all BET family members, and a large number of overlapping surface lysines on all BET members. However, PROTACs can provide more opportunities to achieve better selectivity through the careful design of the “linker” region that could increase/decrease affinities to various binding targets, or could position its E3 ligase moiety for optimal surface lysine presentation of specific targets. These hypotheses need extensive investigation through both modeling and experimental testing. If true, PROTACs, as a new class of drug molecules which actively recruits an E3 ligase to target specific pathological proteins for degradation, thus rendering many drug targets refractory to traditional small-molecule approaches now druggable.

Figure 5. ARV-825 Leads to a Superior Effect on BL Cell Apoptosis Induction Compared with Small-Molecule Inhibitors

(A) Various BL cell lines were treated with ARV-825 (0.1 μM), JQ1 (1.0 μM), OTX015 (1.0 μM), or puromycin (10 mg/ml) as positive control of apoptosis induction, for 24 hr; caspase 3/7 activity was measured by Caspase-Glo 3/7 assay. Error bars represent the SEM.

(B) Ramos and CA-46 cells were treated with increasing doses of ARV-825 (up to 1.0 μM), or JQ1 and OTX015 (up to 10.0 μM) for 48 hr. Lysates were collected and analyzed by immunoblot for PARP cleavage with actin as loading control.

In this study, we designed a novel bifunctional PROTAC molecule, which contains a BRD4 recruiting moiety and an E3 ligase cereblon recruiting moiety. Our BRD4 PROTAC actively degrades BRD4, leading to significant and persistent downstream c-MYC suppression and, most importantly, resulting in robust proliferation inhibition and apoptosis induction in BL. BRD4 PROTAC represents a new strategy to efficiently block BRD4, which is a promising target in multiple cancers. Moreover, this study serves as an example that PROTAC-mediated protein degradation provides a promising strategy to target undruggable pathological proteins.

SIGNIFICANCE

In this study, we report a novel strategy to efficiently target BRD4 by creating a potent BRD4 degrader through PROTAC technology using the E3 ligase cereblon. This breaks ground for a new class of drug molecules which actively recruits an E3 ligase to target specific pathological proteins for degradation, thus rendering many drug targets refractory to traditional small-molecule approaches now druggable.

EXPERIMENTAL PROCEDURES

Reagents

Namalwa, Ramos, CA-46, and DAUDI cells were purchased from ATCC and maintained as instructed. Antibodies against BRD4 (#E2A7X), BRD2 (#S848), c-MYC (#D84C12), and PARP (#46D11) were purchased from Cell Signaling Technology. Actin (#A5441) antibody was purchased from Sigma-Aldrich. BRD3 antibody (#20985c3a) was purchased from Santa Cruz Biotechnology. Secondary antibodies (#7074, #7076) were purchased from Cell Signaling Technology. MG132 (#M7449) was purchased from Sigma-Aldrich. Carfilzomib (#S2853) was purchased from Selleck. JQ1 (Filippakopoulos et al., 2010), OTX015 (Sueoka et al., 1998), and pomalidomide (Ge et al., 2007) were synthesized according to methods published.

Western Blot Analysis

Cultured cells were collected in lysis buffer containing 40 mM HEPES (pH 7.4), 140 mM NaCl, 2.5 mM EDTA, 1% NP-40, 0.1% SDS, and protease inhibitor...
Based on high-performance liquid chromatography analysis. Purity of ARV-825 was more than 97% indicated concentrations. After 72 hr, 100,000 cells were seeded in 96-well tissue culture plates followed by addition of compound at 0.5 μM.

**Proliferation and Caspase 3/7 Assays**

To assess the effect of compounds on proliferation, cells (50,000/100 μl) were added to 96-well plates and treated with compounds for 72 hr. Proliferation and Caspase 3/7 Assays were performed following the manufacturer’s instructions.

**Compound Synthesis**

The synthetic scheme and detailed experimental procedure for the preparation of ARV-825 is described in Supplemental Experimental Procedures. The structure of ARV-825 was confirmed by 1H-nuclear magnetic resonance and liquid chromatography/mass spectrometry. Purity of ARV-825 was confirmed by reverse transcription PCR (qRT-PCR) using the manufacturer's instructions.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Supplemental Experimental Procedures, four figures and can be found with this article online at http://dx.doi.org/10.1016/j.chembiol.2015.05.009.

**AUTHOR CONTRIBUTIONS**

J.L. and C.K. designed the experiments; J.L., M.A., K.R., and J.H. performed the experiments; Y.M.Q., H.G.D., J.W., and A.P.C. designed and modeled compounds; J.L., K.C., J.D.W., A.P.C., and C.M.C. analyzed the data; J.L. wrote the manuscript; K.R., Y.M.Q., K.C., J.D.W., A.P.C., and C.M.C. edited the manuscript.

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Note Added in Proof
While this paper was in production, a report came out online describing the development of dBET1, a compound that utilizes the same PROTAC technology used to develop ARV-825, and induces selective cereblon-dependent BET protein degradation via the same mechanism as ARV-825 (http://dx.doi.org/10.1126/science.aab143). Although ARV-825 and dBET1 were not compared in the same assay, our data suggest that the increased potency of BRD4 PROTAC in comparison with JQ1 is more significant in ARV-825 (Figure 4) than in dBET1. Based on a study of several BRD4 PROTAC molecules, we observed that the linker connecting BRD4 and cereblon ligands can have significant effects on the potency of BRD degradation, downstream c-Myc suppression, and cell proliferation inhibition, which could explain the difference between ARV-825 and dBET1. More importantly, the identification of dBET1 further provides strong validation for the development of novel therapeutics using the PROTAC technology.