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Bromodomain inhibition overcomes treatment resistance in distinct molecular subtypes of melanoma

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Therapy of *BRAF*-mutant melanoma with selective inhibitors of BRAF (BRAFi) and MEK (MEKi) represents a major clinical advance but acquired resistance to therapy has emerged as a key obstacle. To date, no clinical approaches successfully resensitize to BRAF/MEK inhibition. Here, we develop a therapeutic strategy for melanoma using bromosporine, a bromodomain inhibitor. Bromosporine (bromo) monotherapy produced significant anti-tumor effects against established melanoma cell lines and patient-derived xenografts (PDXs). Combinatorial therapy involving bromosporine and cobimetinib (bromo/cobi) showed synergistic anti-tumor effects in multiple BRAFi-resistant PDX models. The bromo/cobi combination was superior in vivo to standard BRAFi/MEKi therapy in the treatment-naive *BRAF*-mutant setting and to MEKi alone in the setting of immunotherapy-resistant *NRAS*- and *NF1*-mutant melanoma. RNA sequencing of xenografts treated with bromo/cobi revealed profound down-regulation of genes critical to cell division and mitotic progression. Bromo/cobi treatment resulted in marked DNA damage and cell-cycle arrest, resulting in induction of apoptosis. These studies introduce bromodomain inhibition, alone or combined with agents targeting the mitogen activated protein kinase pathway, as a rational therapeutic approach for melanoma refractory to standard targeted or immunotherapeutic approaches.

melanoma | targeted therapy | bromodomain inhibition | drug resistance

Significance

Resistance to targeted and immunotherapeutic agents has emerged as a critical impediment to melanoma therapy. This study identifies the bromosporine/cobimetinib combination as a therapeutic strategy with broad-based activity in multiple settings, including in both treatment-naive and resistant *BRAF*-mutant melanoma and in *NRAS*- and *NF-1*-mutant melanoma following resistance to PD-1 blockade.

Melanoma is the fifth most common malignancy in the Unites States, with an estimated 106,110 new cases in 2021 (1). The death toll attributed to melanoma has decreased sharply, owing in part to the revolution that has taken place in the therapy of advanced disease, with significant advances both in immunotherapeutic and targeted interventions. In the realm of targeted therapy, the efficacy of small molecule inhibitors targeting mutant *BRAF* in metastatic melanoma (2, 3) represented a landmark in the targeted therapy of cancer. Subsequently, the combination of BRAF and MEK inhibition resulted in increased response rates and prolonged survival (4–6). More recently, durable responses have been reported with BRAF/MEK-targeted therapy (7). However, despite these clear improvements in patient outcome, many patients eventually progress. As a result, the development of acquired resistance to targeted agents constitutes a significant clinical obstacle. While numerous mechanisms of resistance to targeted therapy have been described (8), many of these mechanisms result in reactivation of the mitogen activated protein kinase (MAPK) pathway in which BRAF operates (9, 10). Therefore, new therapeutic approaches will be required to increase the proportion of responding patients, the durability of the responses observed, and to resensitize melanoma cells to BRAF inhibitors upon the development of acquired resistance. To date, few effective targets for combinatorial therapy with BRAF inhibitors (beyond MEK) have been identified, and none that have proved superior to the BRAFi/MEKi combination.

Previously, we identified an important role for the *BPTF* gene in melanoma progression, and as a potential therapeutic target (11). BPTF promotes melanoma progression by activating a cascade of gene expression including ERK, BCL2, and BCL-XL, resulting in promotion of cell-cycle progression and suppression of apoptosis. *BPTF* gene silencing resulted in abrogation of the proliferative and metastatic potential of melanoma cells and in sensitization to BRAF inhibition (11).

Recently, bromodomain inhibition has emerged as a novel approach to cancer therapy. Bromodomains are protein motifs that bind to acetylated lysine residues on histones, with a critical role in chromatin remodeling (12, 13). The development of ligands targeting the BET (bromodomain and extracellular-terminal) family member BRD4 (14) demonstrated the potential of small molecule inhibition of the bromodomain-acetyl-lysine interaction and is being pursued actively in the clinical arena. However, BET family bromodomains do not share significant sequence homology with that of BPTF (15),

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indicating that distinct inhibitors will be required to effectively target BPTF. Collectively, these observations suggest the potential utility of a bromodomain inhibitor, alone or in combination with MAPK pathway inhibition, as a rational therapeutic strategy for melanoma, which represents the focus of the current analysis.

Results

We aimed to explore the preclinical utility of bromosporine (bromo), a bromodomain inhibitor with demonstrated affinity for BPTF (16). Bromo treatment of several established *BRAF*-mutant melanoma cell lines in culture produced broad-based and dose-dependent cytotoxicity, with IC_{50}s ranging from 2 to 8 μM (*SI Appendix*, Fig. S1*A*). Bromo administration to multiple melanoma cell lines resulted in significant suppression of cell-cycle progression, as evidenced by reduced S phase and G1 arrest (*SI Appendix*, Fig. S1 *B–D*) and was accompanied by induction of apoptosis (*SI Appendix*, Fig. S1 *E–G*). Bromo treatment of melanoma cells resulted in down-regulation of pERK, BCL2, and BCL-XL (*SI Appendix*, Fig. S1*H*). Finally, bromo administration produced significant in vivo anti-tumor effects against LOX human melanoma cells (*SI Appendix*, Fig. S1*I*). Taken together, these studies demonstrate that bromo is active against *BRAF*-mutant melanoma. In addition, bromo treatment recapitulates several key effects of *BPTF* silencing in human melanoma cells. Specifically, the down-regulation of pERK following bromo treatment suggested its potential to resensitize to or synergize with MAP kinase pathway inhibitors (i.e., BRAFi or MEKi).

Next, we assessed the effects of bromo administration alone or in combination with MAPK pathway inhibitors against a panel of metastatic melanoma (MM) PDX lines harvested from patients who progressed on BRAFi therapy (Dataset S1). For our pharmacological analyses, we selected vemurafenib (vem) and cobimetinib (cobi) to represent standard-of-care BRAFi and MEKi therapy, respectively. Initially, we assessed whether administration of bromo could resensitize to BRAFi therapy using the MM-302 line, collected from a patient following progression on dabrafenib. While there was evidence of modest activity upon treatment with either vem or bromo alone in MM-302 cells, there was markedly improved activity of the vem/bromo combination (*SI Appendix*, Fig. S2*A*). In vivo drug treatment of MM-302 tumors demonstrated resistance to vem, whereas single agent bromo exhibited significant anti-tumor activity (Fig. 1*A*). Importantly, addition of bromo to vem restored the anti-tumor efficacy of BRAF inhibition.

We then aimed to confirm the activity of bromo in additional PDX models obtained from patients progressing on BRAFi-based therapy. In these in vivo analyses, we included assessment of the bromo/cobi combination, as well as comparison with the vem/cobi combination. MM-300 is a PDX line obtained from another patient progressing on dabrafenib (Dataset S1). Studies in culture indicated that neither vem alone nor combined with cobi showed significant antiproliferative activity, whereas all bromo-containing regimens produced marked cytotoxic effects (*SI Appendix*, Fig. S2*B*). The in vivo drug treatment of MM-300 cells revealed resistance to single-agent vem administration, while displaying evidence of single agent activity for bromo (Fig. 1*B*). The bromo/vem combination was more active than either agent alone, similar to that observed with MM-302 cells ($P < 0.00005$, ANOVA). MM-300 cells were sensitive to the standard vem/cobi combination. However, while both bromo-containing regimens were superior to the standard regimen, the bromo/cobi combination emerged as the most active treatment, with an 87% and 61% reduction in tumor volume when compared to vehicle and BRAFi/MEKi treatment, respectively ($P < 0.00005$ for each comparison).

Subsequently, we assessed the activity of bromo-based therapy against PDX lines obtained following progression on combined BRAFi/MEKi therapy using MM-337 and MM-358 (Dataset S1). When assessed in culture, BRAFi alone or with MEKi did not produce a high level of cell killing, whereas all bromo-containing regimens exhibited robust anti-proliferative activity (*SI Appendix*, Fig. S2 *C* and *D*). In vivo studies in the MM-337 (Fig. 1*C*, $P < 0.00005$, ANOVA) and MM-358 (Fig. 1*D*, $P < 0.001$, ANOVA) lines consistently demonstrated the superior performance of the bromo/cobi combination. In the MM-337 model, while BRAFi/MEKi produced marked antitumor activity, bromo/cobi treatment was significantly more effective, with a 93% and 59% reduction in tumor volume when compared to vehicle ($P < 1.3 \times 10^{-9}$) or BRAFi/MEKi ($P < 1.4 \times 10^{5}$), respectively, including evidence of tumor regression (Fig. 1*C*). In the MM-358 model, bromo monotherapy was as effective as BRAFi/MEKi therapy, with further improvements in anti-tumor efficacy observed with the bromo/cobi regimen (Fig. 1*D*).

Having established the activity of the bromo/cobi combination in the setting of resistance to BRAFi-based therapy, we aimed to assess its efficacy in the treatment-naive setting using the MM-313 line, which was obtained from an untreated *BRAF*-mutant metastatic melanoma patient. Studies in culture established sensitivity of MM-313 cells to bromo alone or in combination with cobi or vem (*SI Appendix*, Fig. S2*E*). In vivo analysis showed the superior anti-tumor activity of both bromo-containing regimens when compared with vem/cobi, which, as expected, was highly active in this untreated line ($P < 0.0003$, ANOVA, Fig. 1*E*). The bromo/cobi combination proved the most active in this setting, with a 37% reduction in tumor volume when compared with the standard combinatorial regimen ($P < 0.006$). Furthermore, given that the bromo/cobi combination does not require presence of a *BRAF* mutation for its anti-tumor activity, we assessed its efficacy in melanoma PDX lines harboring other mutational drivers, obtained following progression on PD-1 blockade. Bromo/cobi administration was highly active against both MM-386 (an *NRAS*-mutant line, *SI Appendix*, Fig. S2*F* and Fig. 1*F*) and MM-505 (a *NF1*-mutant line, *SI Appendix*, Fig. S2*G* and Fig. 1*G*) and proved superior to treatment with MEKi alone [$P < 0.002$ (Fig. 1*F*) and $P = 0.001$ (Fig. 1*G*)]. We also assessed the potential synergy of the bromo/cobi combination in several of these PDX lines using the combination index (CI) (17), where CI values <1, 1, and >1 indicate synergism, additivity, and antagonism, respectively. We found multiple synergistic interactions for inhibition of cell viability across concentration ranges in several melanoma lines (Fig. 1*H*). In the multiple in vivo studies, the bromo/cobi regimen was well tolerated, and laboratory analysis of complete blood count and serum chemistries conducted at the completion of a study in the MM-358 model did not identify any abnormalities in white blood cell count, neutrophil count, or liver or kidney function (Dataset S2).

In addition, we aimed to elucidate the signaling pathways altered in melanoma cells following bromo/cobi treatment. Studies in culture indicated that combinatorial therapy of MM-337 and MM-358 cells resulted in marked suppression of cell-cycle progression (involving reduced S phase and G1 arrest) (Fig. 2*A* and *SI Appendix*, Fig. S3 *A* and *B*), accompanied by induction of apoptosis (Fig. 2*B* and *SI Appendix*, Fig. S3 *C* and *D*). In

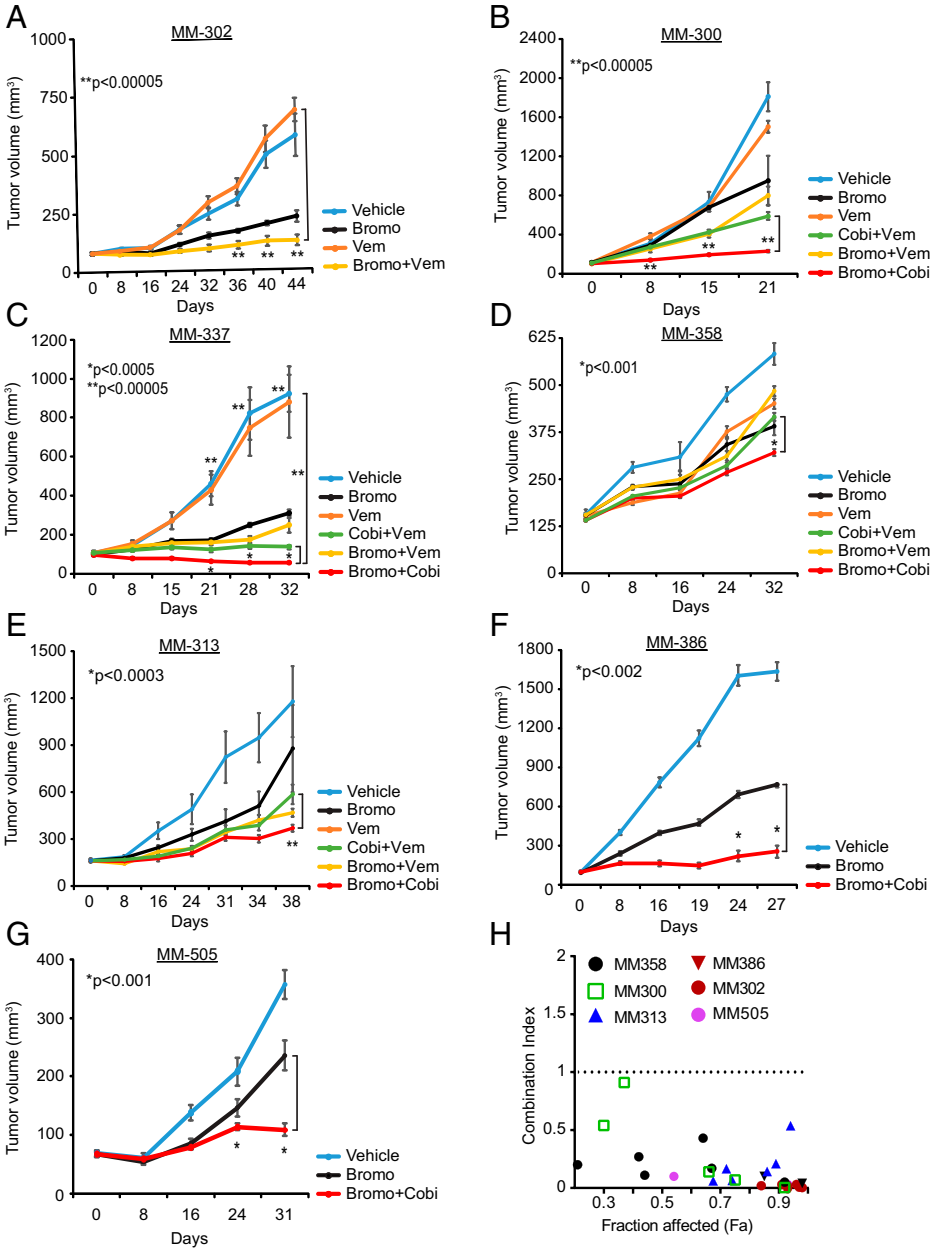

Fig. 1. Effects of single or combination drug treatments in various melanoma PDX models. Anti-tumor activity of various single drugs or drug combinations on the following PDX models in vivo, respectively: MM-302 (*A*); MM-300 (*B*); MM-337 (*C*); MM-358 (*D*); MM-313 (*E*); MM-386 (*F*); and MM-505 (*G*). (*H*) CI values for the bromo/cobi combination in different PDX models.

addition, bromo/cobi treatment resulted in suppression of pERK, BCL2, and BCL-XL (Fig. 2*C* and *SI Appendix*, Fig. S3*E*), similar to that observed with bromo administration alone. In vivo analysis of treated tumors similarly revealed down-regulation of pERK, BCL2, and BCL-XL (Fig. 2*D* and *SI Appendix*, Fig. S3*F*) and was accompanied by markedly decreased Ki-67 immunostaining (Fig. 2 *E* and *F* and *SI Appendix*, Fig. S3 *G* and *H*).

In order to identify genome-wide transcriptomic alterations associated with this effective drug combination, we compared the RNA sequencing (RNA-seq) profiles of in vivo MM-300 tumors treated with vehicle or with bromo/cobi. Supervised analysis of the RNA-seq profiles of the two groups revealed 523 significantly down-regulated and 506 up-regulated genes (Fig. 3*A*). Gene ontology analysis identified several biological processes altered following bromo/cobi treatment (Dataset S3). Strikingly, plotting the Z-score against *P* value of the down-regulated genes within this analysis indicated suppression of key biological processes, including cell division and cell cycle (including the M phase), microtubule organization and sister chromatid segregation, as well as hypoxia and angiogenesis (Fig. 3*B*). The differentially expressed genes included several cyclins (e.g., *CCNB1*), kinesins (e.g., *KIF4A*), kinetochore components (e.g., *CENPM*), as well as key regulators of mitosis (e.g., *CDK1*, *AURKA*, and *PLK1*), cytokinesis (e.g., *CDC20*), angiogenesis (e.g., *HIF1A* and *VEGFA*), and translational elongation (e.g., *EEF1A2*). The down-regulation of each of these genes was confirmed using quantitative reverse transcription–PCR (qRT-PCR) analysis in drug-treated in vivo specimens (Fig. 3*C*). Prompted by this gene expression profile, we assessed whether combinatorial drug treatment resulted in DNA damage and mitotic arrest. Bromo/cobi treatment of both MM358 and LOX cells resulted in activation of the DNA damage marker γH2Ax (Fig. 3*D* and *SI Appendix*, Fig. S4 *A–C*), as well as significantly elevated comet tail (Fig. 3*E* and *SI Appendix*, Fig. S4*D*). This was accompanied by profound

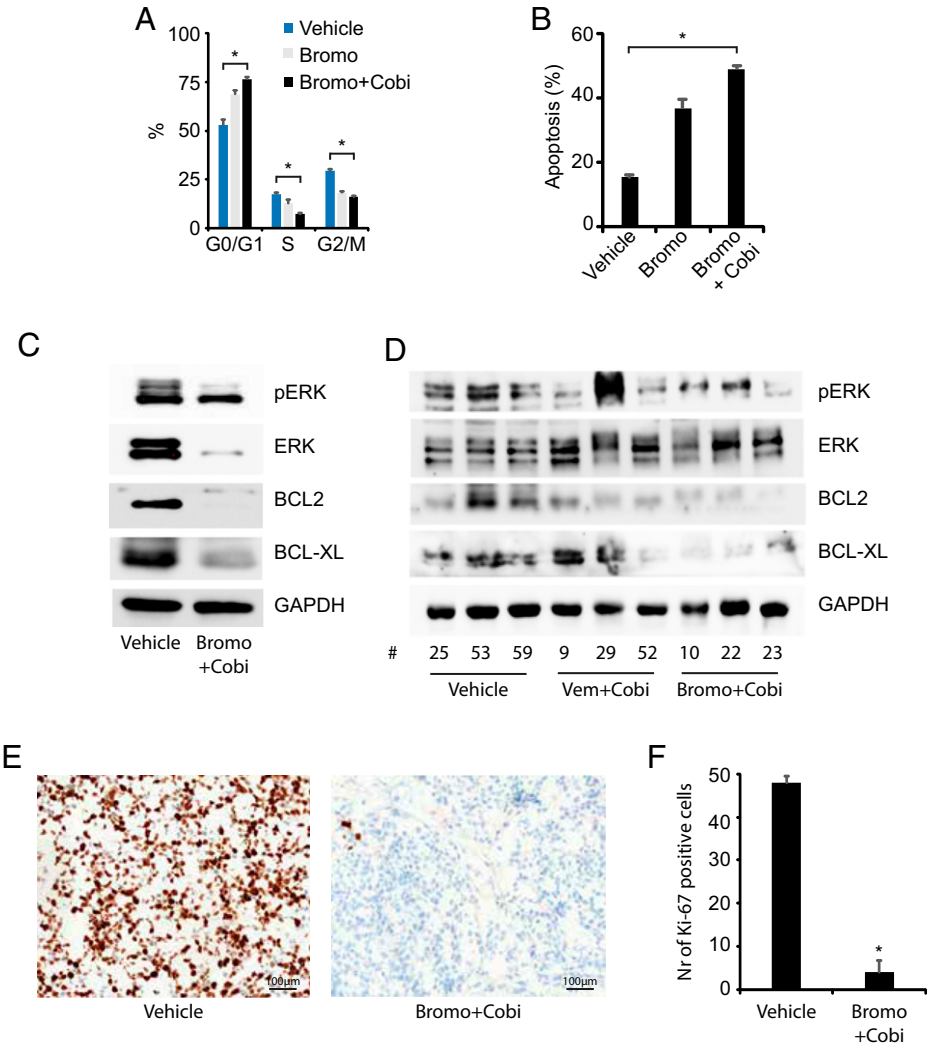

Fig. 2. Effects of bromo/cobi treatment on melanoma PDX lines. (*A*) Bar graph showing proportion of MM-337 cells in different cell-cycle phases following treatment with bromo alone and in combination with cobi. (*B*) Bar graph showing percentage of apoptotic MM-337 cells treated with bromo alone and in combination with cobi. (*C*) Western blot analysis of expression of various proteins in MM-337 cells in culture. (*D*) Western blot analysis of expression of various proteins following drug treatment of MM-337 tumors in vivo. (*E*) Ki-67 staining of in vivo MM-337 samples following bromo/cobi treatment. (*F*) Bar graph showing quantification of Ki-67 staining. (Scale bar: 100 µm; *$P < 0.05$.)

activation of two histone modifications, methylated H3K4 and H3K27, which are recruited to stabilize stalled replication forks (Fig. 3 *F* and *G* and *SI Appendix*, Fig. S4 *E–J*). These changes would usually be accompanied by activation of RPA and RAD51, which represent key mediators of stalled replication fork protection and restart. Importantly, however, bromo/cobi treatment resulted in marked suppression of both RPA (Fig. 3*H* and *SI Appendix*, Fig. S5 *A–C*) and RAD51 (Fig. 3*I* and *SI Appendix*, Fig. S5 *D–F*). qRT-PCR analysis extended the profound down-regulation of *RPA* and *RAD51* expression following bromo/cobi administration to the transcriptional level (*SI Appendix*, Fig. S5 *G* and *H*). Thus, the transcriptional repression mediated by a drug combination involving a bromodomain inhibitor results in exhaustion of two key DNA damage sensors, whose depletion has been shown to induce replication catastrophe (18, 19).

As replication stress itself has been shown to result in mitotic arrest (20), we further explored the consequences of drug treatment on the mitotic apparatus. As indicated, RNA-seq analysis identified significant down-regulation of several genes key to mitotic entry and progression, including *CDK1*, *CCNB1*, *PLK1*, and *AURK1*. The differential expression of these genes following combinatorial drug therapy was confirmed in multiple melanoma cell lines using qRT-PCR (Fig. 4*A* and *SI Appendix*, Fig. S6 *A* and *B*), and Western analysis (Fig. 4*B* and *SI Appendix*, Fig. S6*C*). In addition, bromo/cobi treatment resulted in activation of p21, which also plays an important role in inhibition of mitotic entry, in part by regulating activity of CCNB1 and CDK1 (Fig. 4*C* and *SI Appendix*, Fig. S6 *D* and *E*) (21, 22). Accordingly, analysis of in vivo drug-treated tumors revealed significantly suppressed expression of the mitotic marker phosphohistone H3 (pHH3) in melanoma PDX lines treated with bromo/cobi in vivo (Fig. 4*D* and *SI Appendix*, Fig. S6*F*).

Finally, also prompted by the RNA-seq analysis, we assessed the effects of bromo/cobi treatment on the angiogenic potential of melanoma cells. Combinatorial treatment of melanoma cells in culture was shown to suppress HIF1A expression (by immunofluorescence analysis) (Fig. 4*E* and *SI Appendix*, Fig. S6 *G–I*) and VEGFA secretion (using ELISA analysis) (Fig. 4*F*). Analysis of microvessel density using CD31 immunofluorescence analysis of in vivo PDX tumors treated with bromo/cobi demonstrated significant anti-angiogenic effects (Fig. 4*G* and *SI Appendix*, Fig. S6*J*).

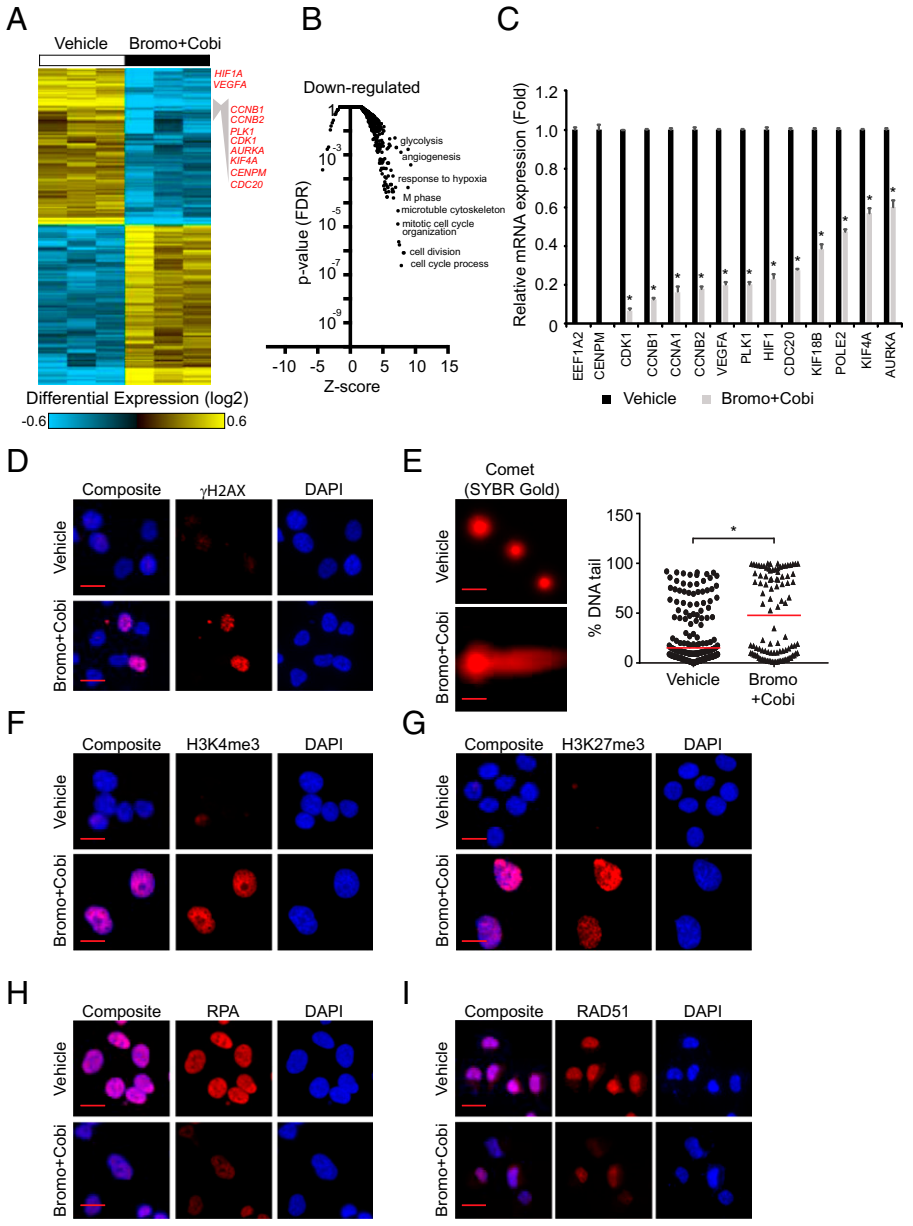

Fig. 3. RNA-seq analysis following bromo/cobi treatment. (*A*) RNA-seq profiles using supervised hierarchical analysis of MM-300 in vivo samples following treatment with vehicle or the bromo/cobi combination. (*B*) Z-score of down-regulated genes in various biological processes. (*C*) qRT-PCR analysis of various down-regulated genes from MM-300 in vivo samples. (*D*) Qualitative immunofluorescence analysis of expression of γH2AX in LOX cells treated with bromo/cobi. (*E*) Comet assay illustrating increased DNA strand breaks in LOX cells treated with bromo/cobi versus vehicle. Qualitative immunofluorescence analysis of expression of H3K4me3 (*F*), H3K27me3 (*G*), RPA (*H*), and RAD51 (*I*) in LOX cells treated with the bromo/cobi combination. (Scale bar: 20 μm; *$P < 0.05$.)

Discussion

In this study, we assessed the consequences of bromodomain inhibition with bromosporine as a rational strategy for the therapy of melanoma, both in the treatment-refractory and treatment-naïve setting. Bromosporine monotherapy was effective in culture against multiple established melanoma cell lines and PDX models. The combination of bromo with either BRAFi (vem) or MEKi (cobi) resensitized BRAFi-resistant PDX lines to MAPK pathway targeting. The bromo/cobi combination proved consistently superior in multiple treatment-refractory PDX models to the standard BRAFi/MEKi regimen, a phenomenon that was also demonstrated in a previously untreated *BRAF*-mutant melanoma line. The activity of bromo/cobi was also demonstrated in immunotherapy-resistant *NRAS*- and *NF-1*-mutant PDX lines. Finally, RNA-seq analysis identified key genes and pathways altered following bromo/cobi administration, providing evidence of profound DNA damage and cell-cycle arrest, resulting in apoptosis induction.

Several prior studies have examined the consequences of acquired resistance to BRAFi in efforts to identify therapeutic vulnerabilities to target treatment-resistant melanoma in vivo. These include the use of compounds that inhibit RAF, PAK, or histone deacetylation, as well as those triggering ferroptosis, autophagy, ER stress, or antioxidation (23–30). Nevertheless, the findings presented here are significant and unprecedented for a number of reasons. To begin with, they systematically demonstrate the ability of bromodomain inhibition to resensitize to MAPK pathway inhibition in vivo in multiple PDX models of acquired BRAFi resistance. This was a key hypothesis that prompted our analysis, and studies of multiple PDX lines derived following progression on BRAFi in cell culture and

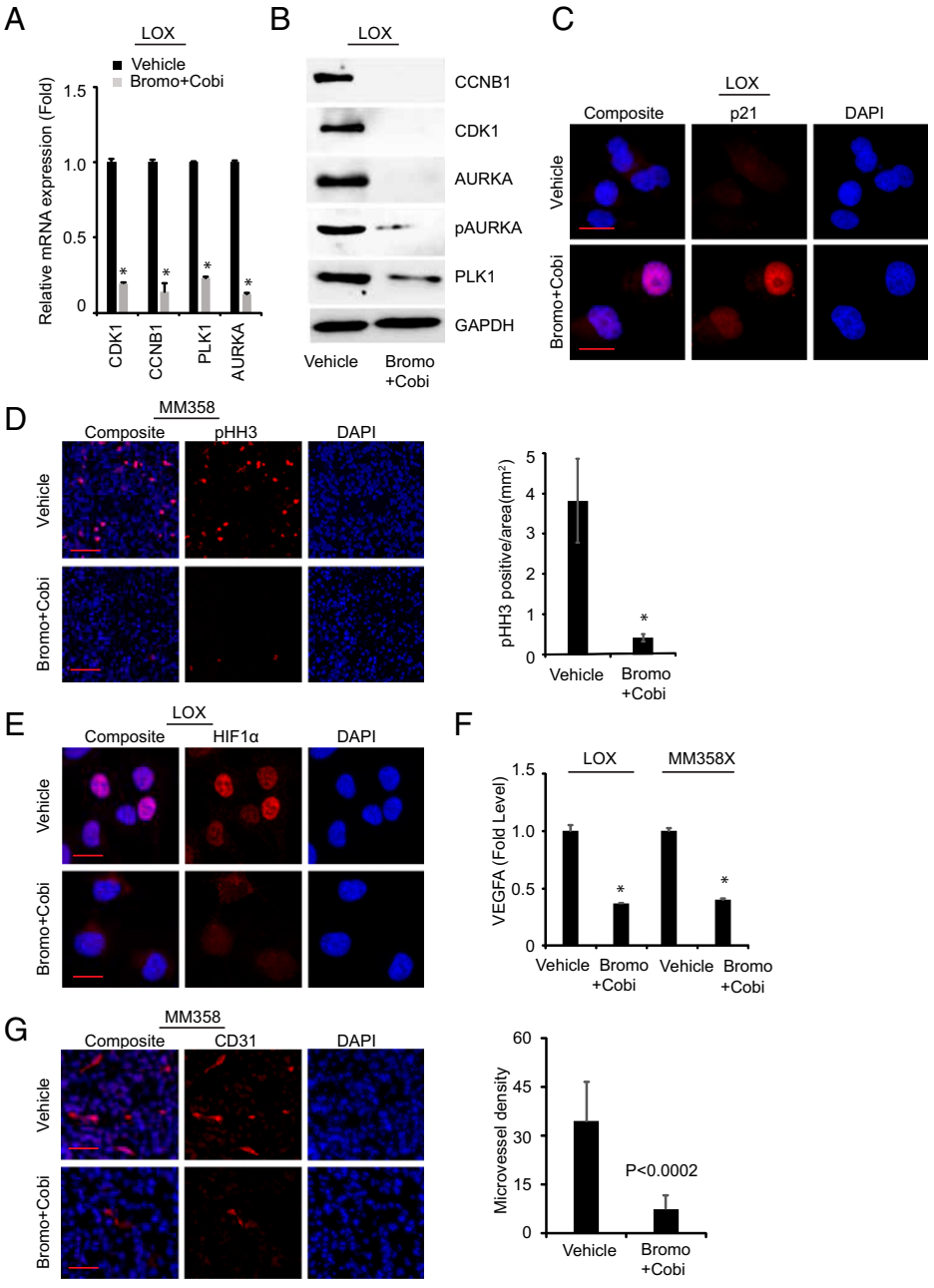

Fig. 4. Impact of bromo/cobi treatment on expression of target proteins and angiogenic potential. (*A*) qRT-PCR analysis of expression of various genes in LOX cells following bromo/cobi treatment. (*B*) Western blot analysis showing expression of various proteins following bromo/cobi treatment of LOX cells. (*C*) Qualitative immunofluorescence analysis of expression of p21 following bromo/cobi treatment of LOX cells. (*D*) Qualitative immunofluorescence analysis of expression of pHH3 in MM-358 in vivo samples (*Left*). Bar graph showing quantification of pHH3 levels (*Right*). (*E*) Qualitative immunofluorescence analysis of expression of HIF1α in LOX cells following bromo/cobi treatment. (*F*) Quantification of VEGFA expression by ELISA in LOX and MM-358 cells following bromo/cobi treatment. (*G*) Microvessel density, as determined by mean CD31 pixel intensity, of MM-358 tumors treated with bromo/cobi or vehicle. Representative images provided of immunofluorescence analysis of treatment and control groups. (Scale bar: 20 μm; *$P < 0.05$.)

in vivo provided consistent evidence of down-regulation of pERK levels following bromo/cobi treatment, providing a mechanism for resensitization to MAPK pathway inhibition. It is important to note that two other studies have shown the potential to sensitize to BRAFi therapy in vivo. Girotti et al. (31) demonstrated the improved activity of gefitinib plus vemurafenib in an A375 subline with acquired BRAFi resistance, while Vargas et al. (32) reported the activity of BETi and MEKi in a PDX model of acquired resistance to dabrafenib.

Beyond the resensitization strategy, our study of MM-313 cells provided evidence of superiority of the bromo/cobi combination to standard BRAFi/MEKi therapy in a treatment-naïve PDX line. These studies uniquely demonstrate the significantly improved activity of this targeted approach in vivo when compared with standard-of-care BRAFi/MEKi therapy in melanoma. In addition, our studies of BRAFi resistance are unique in their assessment of PDX lines that were also resistant to standard immunotherapeutic approaches in melanoma, including antibodies to either CTLA-4 or PD-1 (or both). Accordingly, we showed the activity of the bromo/cobi combination in the setting of *BRAF*-, *NRAS*-, and *NF-1*-mutant melanoma failing therapy with PD-1 blockade. Thus, our studies suggest bromo/cobi as a promising therapeutic approach with broad-based activity against distinct molecular subtypes of melanoma, both in the treatment-refractory and treatment-naive setting. As our studies assessed the consequences of bromo/cobi treatment in various PDX models for up to

38 d, whether these anti-tumor effects can persist beyond this interval awaits future studies of this combination.

While our analysis was motivated in part by prior studies on the role of BPTF in melanoma progression, it is important to stress that bromo targets several bromodomains, and as such, its anti-tumor effects go beyond those driven by BPTF inhibition alone. Future studies with more specific inhibitors of BPTF would address the issue of anti-tumor activity of targeting BPTF alone for the therapy of melanoma and other malignancies in which BPTF is activated. Nonetheless, our studies demonstrate compelling activity for bromosporine-based therapy in the setting of targeted therapy resistance. In addition, in contrast to BET inhibitors, which have been associated with significant toxicity in the clinic (33), the bromo/cobi regimen was well tolerated in NSG mice utilized for the PDX models.

Beyond the demonstration of effects on the MAPK pathway, our analysis identified previously unreported mechanisms of action for the bromo/cobi combination. Specifically, RNA-Seq analysis of drug-treated tumors in vivo identified numerous differentially expressed genes involving fundamental cellular processes such as cell division, chromosome segregation, and mitotic progression. Analyses in melanoma cell lines confirmed the differential expression of these genes following combinatorial drug therapy and enabled a broader understanding of the cellular effects of bromo/cobi administration. Specifically, bromo/cobi treatment produced profound DNA damage, ultimately resulting in exhaustion of cellular pools of RPA and RAD51, which protect replication forks from breakage, and whose depletion results in replication catastrophe (18, 19).

In addition to the effects of drug treatment on DNA replication, RNA-seq analysis of bromo/cobi-treated tumors also indicated down-regulation of several proteins essential to mitotic entry and progression, including CCNB1, CDK1, AURKA, and PLK1, accompanied by activation of p21. These results were consistent with the cell cycle analyses, in which drug treatment prevented mitotic entry. The CCNB1-CDK1 complex is critical for mitotic entry, whereas AURKA and PLK1 play key roles during chromosome segregation. In addition, given the regulation of BCL2 by CDK1 (34), suppression of CDK1 can directly result in inactivation of BCL-2, tipping the balance in favor of proapoptotic mechanisms. Taken together, the repressive transcriptional cascade mediated by bromo/cobi treatment (*SI Appendix*, Fig. S7) results in induction of DNA damage, cell-cycle arrest, and apoptosis, as well as reduced angiogenic potential, that together account for its significant anti-tumor activity.

In summary, our results identify bromodomain inhibition with bromosporine, either alone or in combination with MAPK pathway inhibitors, as a promising therapeutic approach for multiple molecular subtypes of melanoma, including in the setting of acquired resistance to both targeted and immunotherapeutic interventions.

Materials and Methods

Experimental details and methods can be found in *SI Appendix*, including sources of cell lines and cell culture conditions, assays of cell cycle, viability, apoptosis and animal studies. Methods for RNA-seq, Western blot, qRT-PCR, ELISA, and comet assays, as well as immunofluorescence and immunostaining of tumor specimens and statistical analyses are also described in *SI Appendix*. The PDX models utilized were developed after informed consent obtained from patients under the auspices of a study protocol approved by the Institutional Review Board at California Pacific Medical Center.

Data Availability. RNA-seq data that support the findings of this study have been deposited in GEO "Bromodomain targeting overcomes treatment resistance in distinct molecular subtypes of melanoma" (Dar *et al.*, 2021) and are accessible through GEO Series accession number GSE169656 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE169656) (35). All other study data are included in the article and/or supporting information.

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