Transcriptional programming drives Ibrutinib-resistance evolution in mantle cell lymphoma

Highlights

- Transcriptome reprogramming rewires kinome signaling in Ibrutinib-resistant MCL
- Super-enhancer remodeling contributes to transcriptional changes in IR MCL
- Targeting CDK9 or BRD4 compromises transcriptome and kinome rewiring to overcome IR
- The EMMA platform can be a tool to predict clinical response in primary MCL samples

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In brief
Zhao et al. conduct unbiased proteomic, enhancer, and transcriptional profiling in combination with high-throughput drug screening to identify super-enhancer and kinome remodeling as an Ibrutinib resistance mechanism. CDK9 and BRD4 are vulnerabilities for Ibrutinib resistance necessary to sustain super-enhancers, and inhibition of CDK9 or BRD4 prevents the emergence of resistance.
Transcriptional programming drives Ibrutinib-resistance evolution in mantle cell lymphoma

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SUMMARY

Ibrutinib, a bruton’s tyrosine kinase (BTK) inhibitor, provokes robust clinical responses in aggressive mantle cell lymphoma (MCL), yet many patients relapse with lethal Ibrutinib-resistant (IR) disease. Here, using genomic, chemical proteomic, and drug screen profiling, we report that enhancer remodeling-mediated transcriptional activation and adaptive signaling changes drive the aggressive phenotypes of IR. Accordingly, IR MCL cells are vulnerable to inhibitors of the transcriptional machinery and especially so to inhibitors of cyclin-dependent kinase 9 (CDK9), the catalytic subunit of the positive transcription elongation factor b (P-TEFb) of RNA polymerase II (RNAPII). Further, CDK9 inhibition disables reprogrammed signaling circuits and prevents the emergence of IR in MCL. Finally, and importantly, we find that a robust and facile ex vivo image-based functional drug screening platform can predict clinical therapeutic responses of IR MCL and identify vulnerabilities that can be targeted to disable the evolution of IR.

INTRODUCTION

Mantle cell lymphoma (MCL) demonstrates a poor prognosis due to the emergence of drug resistance and the rapid progression of relapsed disease (Jares et al., 2012). B cell receptor (BCR) signaling inhibitors have emerged as promising therapeutic agents for various B cell lymphomas. Ibrutinib, a bruton’s tyrosine kinase (BTK) inhibitor, showed high response rates in patients with MCL (Zucca and Bertoni, 2013; Wang et al., 2013). Unfortunately, despite dramatic clinical responses to Ibrutinib, resistance inevitably develops with treatment (Colomer and Campo, 2014). Once Ibrutinib-treated patients relapse, the 1-year survival is only 22% (Cheah et al., 2015; Wang et al., 2015). Thus, new therapeutics that augment the initial response and, more importantly, sustain remission are desperately needed.

Acquired drug resistance emerges following an initial period of drug responsiveness via evolution of drug-tolerant cancer cell populations, and this process is facilitated by the induction of a complex network of survival and proliferative pathways following exposure to therapy (Diaz et al., 2012; Russo et al., 2016). Acquired resistance can involve bypass mutations, loss of the original target, activation of key downstream effectors of the targeted pathway, and/or activation of alternative signaling pathways, all of which can render the malignant cell indifferent to the original therapy (Kobayashi et al., 2005; Pao et al., 2005). We modeled acquired resistance to Ibrutinib by generating Ibrutinib-resistant (IR) MCL cell lines and performing activity-based (ATP-binding) protein profiling (ABPP) (Zhao et al., 2017). These studies revealed that adaptive kinase reprogramming drives IR phenotypes in MCL, such as unrestrained proliferation (Zhao et al., 2017) and increased sensitivity to the BCL-2 inhibitor ABT-199 (Jiang et al., 2019). Collectively, our studies established that IR evolves via a series of responses in both MCL and stromal cells, creating a positive signaling feedback loop
that amplifies the pro-survival and growth signals, ultimately leading to the acquired IR phenotype.

Adaptive changes in the kinase also manifest in ABT-199-resistant MCL and large B cell lymphoma. We revealed that drug-resistance evolution was associated with reprogramming of super-enhancers (SEs) that activate the transcription of select genes (Zhao et al., 2019). CDK7 inhibition was shown to disable SE-dependent transcription and kinase programs of ABT-199-resistant lymphoma, leading to cell death ex vivo and tumor regression in vivo (Zhao et al., 2019). Importantly, CDK7 inhibitor also blocked the evolution of ABT-199 resistance, suggesting a combination strategy that could have a significant impact in the clinic (Zhao et al., 2019). Ser2 of RNA polymerase is phosphorylated by P-TEFb (positive transcription elongation factor b), which comprises cyclin-dependent kinase 9 (CDK9) and one of several cyclin subunits and associates with other factors such as BRD4 in a large complex of proteins coined the SE complex (Yang et al., 2005; Smith et al., 2011; He et al., 2010). Notably, BRD4 binding to P-TEFb provokes recruitment of the complex to SEs and productive transcriptional elongation (Yang et al., 2005; Gargano et al., 2007; Guo and Price, 2013; Peterlin and Price, 2006).

Given our findings that ABT-199 resistance in lymphoma was associated with CDK7-dependent SEs and kinase remodeling, and the established roles of CDK9 in controlling P-TEFb function, we reasoned that transcriptional targeting might also disable IR MCL. Using unbiased proteomic, enhancer, and transcriptional profiling as well as drug screening of a broad range of transcriptional inhibitors against IR MCL primary patient samples on an ex vivo imaged-based platform that mimics the tumor microenvironment (TME), we report that SEs and kinase remodeling also manifest in IR MCL; CDK9 and BRD4 are selective vulnerabilities for IR MCL that are necessary to sustain IR SEs; and loss or inhibition of CDK9 or BRD4 disables and prevents the emergence of IR. These studies support the need for the development of chromatin-targeting therapeutic strategies to improve MCL patient outcomes in response to Ibrutinib. Finally, we demonstrated that evaluating drug response in primary MCL patient samples with a robust and facile ex vivo image-based functional platform predicted clinical therapeutic responses of IR MCL and identified vulnerabilities that can be targeted to disable the evolution of IR.

**RESULTS**

**Transcriptome reprogramming rewires kinase signaling in IR MCL**

IR in MCL occurs via adaptive kinome reprogramming that leads to constitutive activation of the PI3K/AKT/mTOR pathway and increased levels of BCL-2 (Zhao et al., 2017; Jiang et al., 2019). To gain insights into mechanisms that might drive kinome reprogramming in IR MCL cells, we performed RNA sequencing (RNA-seq) analyses on two independent MCL IR derivatives (SP49 versus SP49-IR, Jeko-1 versus Jeko-1-IR) (Zhao et al., 2017). Analysis of significantly differentially expressed genes revealed both shared and selective transcriptomic changes in IR MCL, with 2,412 upregulated and 1,328 downregulated genes in SP49-IR cells and 1,619 upregulated and 1,832 downregulated genes in Jeko-1-IR cells (Figures 1A and S1A). Common differentially expressed genes including upregulated and downregulated genes between SP49 and Jeko-1 (IR versus Sen) were used as inputs for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and Gene Ontology (GO) term (molecular function) analyses through Enrichr (Table S1). As shown in Figures 1B and S1B, the BCR signaling pathway and mTOR signaling pathway, as well as the RNA polymerase II (RNAPII) core promoter proximal region sequence-specific binding, were on top of the significantly enriched pathways and molecular function terms. GO terms enriched in Jeko-1-IR cells were then mapped as a network of gene sets (nodes) related by mutual overlap (edges), where the color indicates the significance of the gene set (false discovery rate [FDR] q value). Notably, RNAPII, kinase cascade, and cell adhesion regulation are highly enriched in IR compared to parental MCL (Figures 1C and S1C), which were pathways previously identified by ABPP (Zhao et al., 2017). Further, integrating ABPP and RNA-seq data revealed a positive correlation between gene transcript changes and kinase alterations in IR cells (Figure S1C; Table S2). Thus, transcription reprogramming manifestation in IR MCL at least partially contributes to kinome remodeling.

**Increased RNAPII activity and dependence on CDK9 are a hallmark of IR MCL**

Recent studies have shown that activation of the transcriptional machinery underlies kinome reprogramming in tumors (Zawistowski et al., 2017; Flaherty et al., 2012; Erglu and Ribas, 2016; Ascieto et al., 2013; Long et al., 2014). To address this, we implemented our automated first-in-class combination of in silico ex vivo drug response assay, termed EMMA (ex vivo multi-ple myeloma [MM] and MCL advisor), which is capable of screening primary hematologic malignant cells against a panel of targeted and chemotherapeutic agents (Zhao et al., 2017, 2019; Silva et al., 2015, 2017; Ren et al., 2018). Briefly, this drug response assay is capable of screening primary lymphoma cells directly from biopsies against a panel of drugs in an ex vivo reconstruction of the bone marrow TME, including extracellular matrix, patient-derived soluble factors, and patient-derived stroma. Using this platform, we tested the relative sensitivity of MCL cell lines and patient specimens against inhibitors of CDK9 (NVP-2), CDK7 (THZ1), CDK12 (THZ531), and BRD4 (INCB054329 or JQ-1), along with other targeted drugs (Figures 2A and S2A; Table S3). This assay showed drugs targeting the transcriptional kinases CDK9, CDK7, CDK12, and BRD4 have potent and selective activity in IR MCL cell lines and primary MCL (Figures 2A–2C and S2B). The most potent of these agents in primary MCL samples was the CDK9 inhibitor NVP-2. Additionally, NVP-2 sensitivity was shown to be negatively correlated with Ibrutinib sensitivity in MCL samples, though NVP-2 is effective against both Ibrutinib-sensitive and IR MCL cell lines and primary samples (Figures 2C and 2D; Table S4).

Next, western blot revealed higher levels of RNAPII phosphorylation in IR compared to parental cells (Figure S2C). Further, treatment with a half maximal effective concentration (EC50) dose of NVP-2 led to more marked reductions in the phosphorylation of Ser2 on RNAPII and AKT, as well as the level of MCL-1, in IR versus parental MCL lines (Figure S2C). NVP-2 treatment
triggered apoptosis in IR cells and MCL primary patient samples, as judged by poly (ADP-ribose) polymerase (PARP) cleavage (Figure 2E). Given that BRD4 binds CDK9 as a transcriptional elongation complex to coordinately regulate transcription activities, BRD4 inhibitor was employed in parallel to validate the function of transcriptional activation in driving IR. Indeed, similar selective effects manifested following treatment of primary MCL patient samples with the BRD4 inhibitor (Figure S2D). Thus, transcriptional machinery key regulators CDK9 and BRD4 are vulnerabilities for IR MCL.

Targeting CDK9 or BRD4 compromises transcriptome and kinome reprogramming of IR MCL

Next, chemical proteomic ABPP was performed to test if targeting the transcriptional machinery with CDK9 and BRD4 inhibitors affected the kinome of IR MCL cells. Parental and IR
MCL cells were treated with low and high doses of NVP-2 for 24 h and BRD4 inhibitor for 24 and 48 h. Then, lysates were processed for ABPP, and the abundancies of enriched peptides were determined by mass spectrometry. We compared peptide abundancies between treated IR cells and untreated IR cells for each inhibitor and defined inhibitor-regulated kinases as those that had a 1.5-fold change or higher in peptide abundance upon treatment. These analyses identified rather profound effects on kinome activity, where shared kinases whose activity was dependent on CDK9 were upregulated in IR MCL (Figures 3A and S3A; Table S5). KEGG pathway analysis indicated that the NVP-2 or BRD4i-regulated kinases were involved in AKT-mTOR, extracellular-signal-regulated kinase (ERK), chemokine, and BCR pathways that were enriched in the IR MCL cells (Figures 3B and S3B). Thus, targeting CDK9 and BRD4 perturbs the IR-associated kinome.

To evaluate the possible transcriptome influences on the IR MCL kinome changes resulting from CDK9 and BRD4 targeting, RNA-seq studies were performed in paired parental and IR MCL lines treated with NVP-2, BRD4i, or Ibrutinib. Using 2-fold change as the cut-off, we identified 290 genes in Jeko-1-IR cells and 328 genes in SP49-IR cells that were both significantly upregulated in IR MCL and downregulated following NVP-2 treatment (for BRD4i treatment, the numbers of significantly regulated genes were 426 and 523) (Figures 3C and S3C; Table S5). Interestingly, nearly half of the NVP-2- and BRD4i-suppressed genes were upregulated in IR MCL cells when compared with parental MCL cells (Figures 3D, S3D, and S3E). Further, GO analysis for molecular function terms revealed that these genes are consistent with top IR-associated genes, such as RNAPII-activation-associated genes and pathways, and are negatively enriched by NVP-2 and BRD4i treatment (Figures 3E and S3F). Accordingly, principal-component analysis (PCA) revealed that NVP-2 treatment drives and reverses the IR MCL gene profile phenotypes toward those in...
Figure 3. CDK9 is required to sustain transcriptional and kinase reprogramming in IR MCL cells
(A) FC in activity of kinases in IR versus Sen MCL cells (x axes) and kinase upregulation in IR cells after treatment with NVP-2 (y axes) (left panel, 10 nM; right panel, 50 nM) in the SP49 cell line. Venn diagram shows overlap in shared protein kinases that are increased in SP49-IR cells versus Sen cells and that are significantly decreased by NVP-2 treatment in IR cells. These overlapped kinases are highlighted by the red box in each scatterplot. Cut-off, FC = 1.5. n = 3 biological replicates.

(B) KEGG pathway enrichment of kinases that are both increased in SP49-IR cells versus Sen cells and significantly decreased by NVP-2 treatment in IR cells. Top axis represents -log10(p value). P values were calculated as in Figure 1B.

(C) RNA-seq heatmap showing genes that are increased in IR versus Sen cells and that are decreased by NVP-2 treatment in Jeko-1 and SP49 IR cells. n = 3 biological replicates.

(D) Pie charts showing the percentage of gene expression changes (increase/conserved/decrease) in IR compared to Sen cells for NVP-2 decreased genes in IR cells. Around half (49.0% for Jeko-1 and 48.8% for SP49) of the NVP-2 decreased genes are increased in IR cells compared to sensitive cells.

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Ibrutinib-sensitive MCL cells (Figures 3F and S3G). In contrast, Ibrutinib treatment failed to shift and reverse the gene phenotypes of IR lines to those of Ibrutinib-sensitive cells (Figures 3F and S3G). Together, these results support the notion that transcriptome and associated kinome reprogramming characteristics of IR MCL are disabled by inhibition of the transcription machinery.

SE remodeling contributes to transcriptional changes in IR MCL To assess if the rewired signaling and transcriptional programs in IR MCL reflected remodeling by SE, chromatin immunoprecipitation sequencing (ChIP-seq) analysis of lysine-27 acetylated histone H3 (H3K27Ac), a mark of SEs and actively transcribed genes, was performed on paired parental and IR MCL cells (Bradner et al., 2017; Hnisz et al., 2013). ChIP-seq and ranking of enhancers by the amplitude and density of H3K27ac marks revealed distinct upregulated (gained) and downregulated (lost) SEs in IR cells (Figures 4A and S4A). As expected, increased expression of genes found in IR cells was associated with gained SEs, whereas SEs lost in IR cells were associated with decreased gene expression in IR cells (Figure 4B) (Zhao et al., 2017; Hnisz et al., 2013). Notably, gained SEs in IR MCL cells were associated with increased expression of known drivers of lymphoma, specifically BCL2, CXXCR4, PLCG, MYC, TRAF4, MIR-17, and CCND1, among others (Figure 4A). Gene set enrichment analysis (GSEA) confirmed that genes regulated by IR-associated SEs are positively enriched in IR cells relative to Ibrutinib-sensitive parental cells (Figure 4C), and enrichment analysis using the Genomic Regions Enrichment of Annotations Tool (GREAT) (McLean et al., 2010) established that IR-associated H3k27ac profiles were upregulated in several pathways that were significantly increased in IR MCL cells as determined by ABPP and RNA-seq profiling (Figures 4D and S4B).

Strikingly, the increased expression of genes associated with gained SEs in IR MCL was compromised by NVP-2 treatment (Figures 4E and S4C). Further, the suppressive effects of NVP-2 or BRD4i treatment on SE-regulated genes were more profound than on genes driven by typical enhancers (TEs) (Figures 4F and S4D). Finally, ChIP-seq and RNA-seq studies performed on primary MCL patient samples revealed that the increased expression of genes associated with SEs was suppressed following inhibition of CDK9 or BRD4 (Figure S4E). Thus, SE-associated transcriptome reprogramming in IR can be disabled by targeting CDK9 or BRD4.

Targeting CDK9 prevents emergence and overcomes IR in MCL Given that IR in MCL is associated with increased RNAPII phosphorylation and a heightened dependence on CDK9 and BRD4, we next tested the ex vivo efficacy of CDK9i and BRD4i against IR lines and primary IR MCL samples on our EMMA platform and combined these results with matched RNA-seq in these samples. RNA-seq analyses of primary IR MCL samples treated with and without NVP-2 or BRD4i revealed that IR-associated genes and pathways (e.g., mTORC1) are dramatically suppressed by these inhibitors (Figures 5A and S5A). Further, combined treatment by NVP-2 or BRD4i with Ibrutinib demonstrated increased compound effects, measured as decreased cell viability, when compared to any single agent alone (Figure 5B). Importantly, synergy and enhanced effect of the combined treatment with NVP-2 with Ibrutinib was also observed in primary MCL samples, where the combination induced more cell death and suppression of cell viability than did single agents alone (Figures 5C and S5B).

We next applied colony formation assays to monitor the emergence of drug resistance as described previously (Zhao et al., 2019) and to test if NVP-2 or BRD4i inhibition could impair the onset of IR ex vivo. As expected, following an initial and profound decrease of cell viability, IR emerged in both cell line models, as reflected by accelerated colonogenic growth following treatment with 1 μM Ibrutinib (Figure 5D). In contrast, co-treatment of these models with the combination of Ibrutinib and NVP-2 blocked this accelerated growth phase and nearly abolished colony formation (Figure 5D). To assess the efficacy of these combinations in vivo, immunocompromised NOD severe combined murine immune deficiency (scid) gamma (NSG) mice bearing parental Jeko-1 MCL cells were used. After tumors reached 100 mm³, mice were treated daily interperitoneally (i.p.) with Ibrutinib (25 mg/kg, daily), low-dose NVP-2 (2.5 mg/kg, twice a week), or both and followed for disease progression. Mice treated with Ibrutinib only experienced an initial transient response to the drug in the first 2 weeks of treatment. However, tumor volumes in mice treated with Ibrutinib alone exceeded those of mice in the vehicle group by week 3 after inoculation (Figure 5E).

Notably, the NVP-2 treatment was much more effective than the Ibrutinib treatment at preventing disease progression, and the combination was superior to both Ibrutinib and NVP-2 treatment alone (Figure 5E); thus, these data support that CDK9 inhibition also impairs the development of IR in vivo. CDK9 activity is also necessary for the emergence of IR in this model.

To further test the in vivo efficacy of the NVP-2/Ibrutinib combination, a four-armed study was performed using Jeko-1-IR cells. Again, there was also obvious synergy of the NVP-2 + Ibrutinib combination in NSG mice bearing Jeko-1-IR tumors in delaying tumor progression and in improving overall survival (Figures 5F and S5G). Thus, CDK9 inhibition can sensitize IR MCL to inhibitors of BCR signaling. Finally, in a luciferase-expressing patient-derived xenograft (PDX) IR MCL xenograft model, the NVP-2/Ibrutinib combination was again superior at preventing tumor progression (Figure 5H). Indeed, the NVP-2/Ibrutinib combination provoked nearly complete tumor regression (Figure 5H). No significant weight loss or movement disorders were observed after drug treatment (single or combined treatment). A complete blood count (CBC) evaluation revealed no significant changes of
Figure 4. SE remodeling drives transcriptional programming and drug sensitivity to CDK9 inhibition in IR MCL lines and primary samples

(A) ChIP-seq hockey-stick plot ranking enhancers by H3K27ac signal density. Number of SEs in each sample is labeled in plot. SE-associated genes with H3K27ac signal densities that surpass the inflection point are indicated in red font.

(B) Log2FC IR vs. Sen

(C) Enrichment score

(D) SP49-IR_H3K27ac signal GREAT enrichment MSigDB pathway

(E) Enrichment score

(F) Log2FC NVP2 vs. DMSO

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total red blood cell (RBC) count, hemoglobin, neutrophil count, platelet count, and reticulocyte cell numbers with any monotherapy (Ibrutinib or NVP-2) or combination treatments when compared with the vehicle control treatments in these NSG mice (Table S6). Overall, these results provide reassurance that the lower dose of NVP-2 that we chose to use to treat these mice was not toxic, though the potentially toxic off-target effects of using a larger dose of NVP-2 should not be ignored. Collectively, these studies provide proof-of-concept that co-targeting CDK9 or BRD4 with Ibrutinib is a rational therapeutic strategy that can prevent the development of IR and can re-sensitize IR MCL to Ibrutinib.

The EMMA platform as a tool to predict clinical responses and inform vulnerabilities in primary MCL samples

A daunting therapeutic challenge in treating MCL patients once IR develops is inter- and intra-tumoral heterogeneity and plasticity that limits the efficacy and duration of response. We again implemented the EMMA platform and examined 60 MCL patient specimens for their response to Ibrutinib, which were ranked by calculating the area under curve (AUC) of five serial diluted dose response curves, the EC$_{50}$, and the maximal efficacy at 96 h after treatment. Primary MCL samples displayed variable responses to Ibrutinib, where, for example, Pt61 was sensitive, Pt13 was resistant, and Pt31 was intermittently sensitive (Figure 6A).

To identify a gene expression signature that discriminated Ibrutinib-sensitive from IR samples, RNA-seq was performed on 32 of these patient specimens. Supervised hierarchical clustering of the gene expression of these 32 samples based on the 96-h maximal efficacy to Ibrutinib was performed. These analyses revealed a significant enrichment and correlation of differentially expressed genes between clinical IR and EMMA-defined IR (Figure 6B; Table S4). Pathway analysis and GSEA were performed on genes that were differentially expressed between Ibrutinib responders and non-responders. Consistent with the findings from IR MCL lines (Figure 2), IR primary samples exhibited significant positive enrichment for the mTORC1 HALLMARK signature (Figures 6C and S6A). These findings are in accord with the dependencies of primary IR to kinase and transcriptional inhibitors present in primary IR MCL samples.

To determine and compare the relative significance of the HALLMARK signatures identified in Ibrutinib-sensitive and IR primary MCL patient samples, single-sample GSEA (ssGSEA) was performed, and the resulting enrichment scores were ranked based on the responder and non-responder groups defined by our drug screen. In accordance with our MCL cell line studies, there were higher enrichment scores for MYC, E2F, nuclear factor κB (NF-κB), and mTOR pathways in IR MCL patient samples, while higher enrichment scores for wingless-related integration site (WNT) pathways were observed in Ibrutinib-sensitive MCL patient samples (Figure S6B). Notably, we validated these signatures by querying an independent dataset of Ibrutinib-treated MCL samples with clinically defined responses (Zhang et al., 2019a) and demonstrated that enriched HALLMARK signatures from IR patients as determined by the EMMA platform positively correlated with the findings from this MD Anderson study (Figures 6D and S6C). Thus, as in myeloma patients (Silva et al., 2017), the EMMA platform can predict drug response in primary MCL.

To determine how sensitive and specific our ex-vivo-based drug screen is at predicting clinical Ibrutinib responses, we compared the receiver operating characteristic (ROC) curve for AUC and maximum effect (Figure 6F). ROC curves for clinical response status over the maximum effect were the most predictive of clinical Ibrutinib responses (Figure 6F), where areas under the ROC curve and the 95% confidence intervals (CIs) were 0.830 (0.653–1.007). To assess the potential clinical usefulness of the EMMA maximum effect to predict clinical responses to Ibrutinib, the Youden-index-based cut-off value for this metric was determined and was calculated to be 63.2. Further, the associated positive predictive value (PPV; response rate above the cut-off) was 66.7%, and the negative predictive value (NPV; non-response rate below the cut-off) was 92.9%. This suggests a potential for a high discriminatory value of maximum effect from EMMA that enriches for response rates to Ibrutinib (i.e., increasing PPV) while preserving a high NPV. Having said this, a formal evaluation of clinical utility depends on cut-offs selected for implementation of a clinical-grade diagnostic device in developmental trials that is dependent on the goals of those trials, and such trials are needed for the EMMA platform. Nonetheless, these findings suggest that maximum effect from EMMA can be used to determine clinical responses to Ibrutinib, thus providing a means to stratify and tailor treatment of MCL patients.

DISCUSSION

Our findings are in accord with recent studies that have shown that adaptive kinome reprogramming and altered cancer cell states are due to transcriptional programs coordinated by chromatin and transcriptional regulators (e.g., BRD4, CDK7, CDK9, and RNAPII) that bind to and activate distinct SE sites.
Our findings that such SE-dependent adaptive signaling contributes to the evolution of drug resistance support the notion that clinical resistance to Ibrutinib can arise in the absence of new genetic mutations, as we and others have failed to identify recurrent mutations in IR MCL patients (Zhao et al., 2017; Zhang et al., 2020), and similar observations have been reported in other tumor types such as AML and solid tumors (Zawistowski et al., 2017; Rusan et al., 2018; Agarwal et al., 2019). Indeed, as we have shown, numerous kinase signaling networks are rewired and activated in MCL cells as they become IR (Zhao et al., 2017). Thus, combinations of two or more kinase inhibitors are unlikely to be sufficient to provide durable therapeutic responses. Given inter-tumor heterogeneity, especially in primary MCL samples, comparing gene expression between sensitive and resistant samples failed to detect any single gene or gene pair that clearly defined IR, suggesting more complex molecular processes. Genetic changes, such as mutations in BTK, NOTCH1, and KMT2D, and alternative NF-κB pathway pathways have been shown to be associated with primary and acquired IR in only a minority of cases (Chiron et al., 2014; Martin et al., 2016; Nomie et al., 2020; Rahal et al., 2014). Recent studies have approached overcoming IR by developing treatment strategies that combine Ibrutinib with additional inhibitors targeting proteins such as HSP90, ROR1, and XO1 as well as with inhibitors that target the oxidative phosphorylation pathway, with some of these strategies moving into early-phase clinical trials (Jacobson et al., 2016; Zhang et al., 2019a, 2019b; Hing et al., 2015). However, our global pharmacogenomic approaches instead identified common pathways associated with IR evolution and lead us to focus specifically on transcriptional machinery to disable global signaling reprogramming. Thus, the inhibitions of the transcriptional mechanisms that control kinase remodeling are a very attractive alternative strategy to overcoming drug resistance. Our translational results provide a foundation for CDK9 inhibitors, such as the recently developed agent AZD4573, which is currently in a phase of clinical trials, to be used in the treatment of aggressive and drug-resistant lymphomas (Cidado et al., 2020). Importantly, our studies strongly indicate that CDK9 or BRD4 targeting can be effective against MCL and can prevent the development of IR in initially Ibrutinib-sensitive tumors and can re-sensitize IR MCL to Ibrutinib, addressing two major clinical hurdles in the treatment of MCL patients. Thus, the findings presented fully support such combinations as strategies for the treatment of IR MCL patients that have few therapeutic options and dismal outcomes and as regimens that may prevent the emergence of resistance to improve outcomes of treatment-naive MCL patients.

Finally, we submit that platforms such as EMMA could be powerful clinical tools, as treatment of individual MCL patients with either conventional chemotherapy or an ever-increasing number of targeted agents remains highly empiric, and inter- and intra-tumoral heterogeneity and tumor plasticity limit their efficacy and durability. The EMMA platform is a considerable improvement for deciding drug choices over cell line studies, where by recapitulating the 3D lymphoma TME, it is possible to temporally and robustly assess the dynamics and magnitude of the response of primary lymphoma cells to single agents and drug combinations over a short interval to inform patient therapies. Most importantly, our studies showed a strong correlation of Ibrutinib response detected by EMMA with patient clinical Ibrutinib response. This first-in-kind automated in silico platform is an accurate predictor of the clinical responses of MCL patients and allows for patient-specific approaches for tailored therapies that are effective at eradicating their drug-resistant disease and at preventing the emergence of drug resistance in treatment-naive patients across a broad spectrum of hematological malignancies.

**STAR METHODS**

Detailed methods are provided in the online version of this paper and include the following:

- **KEY RESOURCES TABLE**
- **RESOURCE AVAILABILITY**
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  - Materials availability
  - Data and code availability

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**Figure 5. Targeting CDK9 prevents emergence and overcomes IR in MCL ex vivo and in vivo**

(A) GSEA of HALLMARK_MTORC1_SIGNALING pathway for NVP-2 treatment in the indicated IR MCL patient (Pt) specimens. (B) Cell-based imaging analysis for drug response in SP49-IR cells treated with NVP-2, Ibrutinib, or the NVP-2/Ibrutinib combination (left panel) or with BRD4i (NCBI0534329), Ibrutinib, or the BRD4i/Ibrutinib combination (right panel). (C) Drug response assay of primary MCL patient samples (n = 8) treated with Ibrutinib or NVP-2 alone or Ibrutinib/NVP-2 combination. AUC values calculated from cell-based imaging analysis. p values were calculated by one-way ANOVA. (D) Colony formation analysis revealed the Ibrutinib/NVP-2 and Ibrutinib/BRD4i combination markedly impairs colony formation in HBL-2 MCL cells. (E) Tumor volume in NSG recipient mice bearing Jeko-1 Sen tumors that were treated daily with Ibrutinib (25 mg/kg, i.p.) or biweekly with NVP-2 (2.5 mg/kg, i.p.) or the Ibrutinib/NVP-2 combination. First arrow, start of drug application; second arrow, onset of drug resistance evolution. p values were calculated by Student’s t test. (F) Tumor volume in NSG recipient mice bearing Jeko-1-IR tumors that were treated daily with Ibrutinib or biweekly NVP-2, or the Ibrutinib/NVP-2 combination. P values were calculated by Student’s t test. (G) Kaplan-Meier survival analysis of mice in (F). Mantel-Cox test was used for statistical analysis. For (E) and (F), dosages of the treatment are indicated in the figures. Black arrows indicate treatment start time, and red arrow in (E) indicates the time that Ibrutinib group tumor sizes are larger than vehicle group. (H), Representative images taken 6 weeks after transplant of NSG mice bearing IR MCL PDX tumors that were treated with vehicle, Ibrutinib daily, biweekly NVP-2, or NVP-2/Ibrutinib combination. n is at least 4 for each group. Dosages of the treatment are same as (F). For (E) and (F), data are shown as mean ± SD. For (E)–(G), n = 4 mice per treatment group. Data in (B) and (D) are representative of three independent experiments run in triplicate.

See also Figure S5 and Table S6.
EXPERIMENTAL MODEL AND SUBJECT DETAILS

- Patients and tumor specimens
- Mice
- Cell lines

METHOD DETAILS

- High-throughput small-molecule drug screens
- Cell-based imaging analysis of drug screening assay
- RNA-sequencing
- Chromatin immunoprecipitation followed by highly parallel sequencing (ChIP-Seq)
- ChIP-Seq data processing
- Identifying enriched regions
- Calculating read density
- Mapping typical enhancers and super-enhancers using H3K27ac enhancer definitions
- Activity-based protein profiling (ABPP)
- Xenograft studies
- PDX model and imaging
- Gene set enrichment analysis (GSEA)

Figure 6. The EMMA platform predicts clinical responses and informs vulnerabilities in primary and IR MCL

(A) Drug response assays of Ibrutinib sensitivity in primary patient samples with cell-based imaging analysis showing representative dose responses of sensitive, intermediate, and resistant primary MCL patients.

(B) Heatmap showing top differential genes between primary IR and Sen MCL samples separated by maximum Ibrutinib effect measured by cell-based imaging analysis. n = 32 primary samples.

(C) GSEA shows MTORC1_SIGNALING is positively enriched in primary IR MCL samples compared to Sen primary MCL samples. NES, normalized enrichment score; FDR, false discovery rate.

(D) Median NES of single-sample GSEA (ssGSEA) reveals the correlation of Moffitt and MD Andersen gene signatures of IR and Sen primary MCL samples.

(E) Shared positively enriched HALLMARK pathways of IR compared to Ibrutinib-sensitive primary MCL samples of Moffitt and MD Andersen datasets.

(F) Receiver operating characteristic (ROC) curves of AUC and maximum effect from cell-based imaging analysis compared to clinical response of patients. AUC and Youden cut-off indexes for maximum effect ROC curve are shown.

See also Figure S6 and Table S4.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.celrep.2021.108870.

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lymphoma failing ibrutinib are unlikely to respond to salvage chemotherapy
219–231.

Conception and design, J.T., X.Z., J.L.C., and J.Q.; performed experiments and collected and assembled the data, X.Z., M.Y.W., H.J., T.Linvi, J.G., Y.R., P.M.P., M.B.M., A.S.S., and T.Li; PDX MCL cell preparation, S.S. and L.S.; analyzed and interpreted the data, X.Z., M.Y.W., W.Z., J.S., N.A.F., X.W., J.T., J.L.C., and J.Q.; writing, review, and/or revision of the manuscript, J.T., X.Z., M.Y.W., K.S., B.D.S., J.L.C., and J.Q.; administrative, technical or material support, K.S., B.D.S., J.T., E.M.S., and M.W.; study supervision, B.D.S. and J.T.

DEVELOPMENT OF INTERESTS

The authors declare no competing interests.

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### Key Resources Table

| Reagent or Resource   | Source                          | Identifier          |
|-----------------------|--------------------------------|---------------------|
| **Antibodies**        |                                |                     |
| cPARP                 | Cell Signaling Technologies    | Cat# 5625; RRID: AB_10699459 |
| pAKT(Ser473)          | Cell Signaling Technologies    | Cat# 9271; RRID: AB_329825 |
| AKT                  | Cell Signaling Technologies    | Cat# 4685; RRID: AB_2225340 |
| RNA pol II CTD phospho-Ser2 | Millipore             | Cat# 04-1571-I; RRID: AB_11212363 |
| RNA pol II CTD phospho-Ser5 | Millipore             | Cat# 04-1572-I; RRID: AB_11213421 |
| RNA pol II CTD phospho-Ser7 | Millipore             | Cat# 04-1570-I; RRID: AB_2801298 |
| RNA pol II            | Cell Signaling Technologies    | Cat# 2629; RRID: AB_2167468 |
| MYC                  | Abcam                          | Cat# ab32072; RRID: AB_731658 |
| CDK7                 | Cell Signaling Technologies    | Cat# 2916; RRID: AB_2077142 |
| MCL-1                | Santa Cruz Biotechnology       | Cat# sc-819; RRID: AB_2144105 |
| Actin                | Santa Cruz Biotechnology       | Cat# sc-47778 HP; RRID: AB_2714189 |
| RNAPII               | Diagenode                      | Cat# C15100055; RRID: AB_2750842 |
| H3K27ac              | Abcam                          | Cat# ab4729; RRID: AB_2118291 |
| **Bacterial and virus strains** |                       |                     |
| pCDH-EF-eFFLY-T2A-mCherry Plasmid | Addgene             | Cat# 104833; RRID: Addgene_104833 |
| **Biological samples** |                               |                     |
| Primary Patient Specimens (MCL) | Moffitt Cancer Center | N/A               |
| **Chemicals, peptides, and recombinant proteins** |                       |                     |
| (+)-JQ1               | Cayman Chemical                | Item# 11187         |
| (R)-PFI-2             | Cayman Chemical                | Item# 14678         |
| A-1331852             | Selleckchem                    | Cat# S7801          |
| A-366                 | Cayman Chemical                | Item# 16081         |
| ABT-199               | Selleckchem                    | Cat# S8048          |
| ABT-263               | Selleckchem                    | Cat# S1001          |
| A-196                 | Cayman Chemical                | Item# 18317         |
| Alisertib             | Selleckchem                    | Cat# S1133          |
| AZD7762               | Selleckchem                    | Cat# S1532          |
| AZD8055               | Selleckchem                    | Cat# S1555          |
| BAY-598               | Cayman Chemical                | Item# 18233         |
| BEZ-235               | Selleckchem                    | Cat# S1009          |
| BI-9564               | Cayman Chemical                | Item# 17897         |
| Bendamustine          | TargetMol                      | Cat# T6095          |
| Bortezomib            | Selleckchem                    | Cat# S1015          |
| Carfizomib            | Selleckchem                    | Cat# S2653          |
| CPA7                  | DC Chemicals                   | Cat# DC12009        |
| CPD23                 | Dana-Farber Cancer Institute   | N/A                |
| Dinaciclib            | Selleckchem                    | Cat# S2768          |
| Doxorubicin           | Selleckchem                    | Cat# S1208          |
| GSK343                | Cayman Chemical                | Item# 14094         |
| GSK484                | Cayman Chemical                | Item# 17488         |
| GSK591                | Cayman Chemical                | Item# 18354         |
| GSK864                | Cayman Chemical                | Item# 18782         |
| GSK-J4                | Cayman Chemical                | Item# 12073         |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| GS1-LSD1            | Cayman Chemical | Item# 16439 |
| Irutinib            | Selleckchem | Cat# S2680 |
| I-CBP112            | Cayman Chemical | Item# 14468 |
| INCBO52793          | Incyte Corporation (Wilmington, DE) | N/A |
| INCBO54329          | Incyte Corporation (Wilmington, DE) | N/A |
| INCBO59872          | Incyte Corporation (Wilmington, DE) | N/A |
| Lenalidomide        | Selleckchem | Cat# 1029 |
| Lumpib              | Selleckchem | Cat# S1069 |
| MK-1775             | Selleckchem | Cat# S1525 |
| MS049               | Cayman Chemical | Item# 18348 |
| NVP2                | MedChemExpress | HY-12214A |
| OF-1                | Cayman Chemical | Item# 17124 |
| OICR-9429           | Cayman Chemical | Item# 16095 |
| Olaparib            | Selleckchem | Cat# 1060 |
| PFI-3               | Cayman Chemical | Cat# 15267 |
| PFI-4               | Cayman Chemical | Item# 17683 |
| PIK-75              | Selleckchem | Cat# S1205 |
| PRT1000220-005      | Incyte Corporation (Wilmington, DE) | N/A |
| R406                | Selleckchem | Cat# S2194 |
| Ruxolitinib         | Selleckchem | Cat# S1378 |
| S33845              | ApexBio | Cat# A8737 |
| SCH-772964          | Selleckchem | Cat# S7101 |
| SGC0846             | Cayman Chemical | Item# 13967 |
| SGC707              | Cayman Chemical | Item# 17017 |
| Silvestrol          | MedChemExpress | HY-13251 |
| THZ1                | Dana-Farber Cancer Institute Nathanael Gray Lab | N/A |
| THZ531              | Dana-Farber Cancer Institute Nathanael Gray Lab | N/A |
| Trametinib          | Selleckchem | Cat# S2673 |
| UNC0642             | Cayman Chemical | Item# 14604 |
| UNC1215             | Cayman Chemical | Item# 13968 |
| UNC1999             | Cayman Chemical | Item# 14621 |
| VE-821              | Selleckchem | Cat# S8007 |
| Volasertib          | Selleckchem | Cat# S2235 |

Critical commercial assays

| Universal Mycoplasma Detection Kit | ATCC | Cat# 30-1012K |
| Resazurin                     | R&D Systems | Cat# AR002 |
| Bovine Type I Atelo-Collagen Solution | Advanced BioMatrix | Cat# 5005-B |
| Lymphoprep                    | STEMCELL Technologies | Cat# 07851 |
| RNase Plus Mini               | QIAGEN | Cat# 74134 |
| TruSeq Stranded mRNA Library Prep Kit | Illumina | Cat# RS-122-2101/2 |
| 1.5 ml Bioruptor Plus TPX microtubes | Diagenode | Cat# C30010010 |
| Bioruptor                     | Diagenode | Cat# B01060010 |
| Dynabeads Protein G for Immunoprecipitation | ThermoFisher Scientific | Cat# 10007D |
| RNase A                       | Roche | Cat# 10109169001 |
| Proteinase K                  | Life Technologies | Cat# AM2546 |
| ThruPLEX DNA-seq Kit          | TaKaRa | Cat# R400675 |

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| REAGENT or RESOURCE | SOURCE | IDENTIFIER |
|---------------------|--------|------------|
| AMPure Beads (Agencourt AMPure XP) | Beckman Coulter Life Science | Cat# A63882 |
| Pipin Prep | SAGE Sciences | Cat# PIP0001 |
| KAPA Biosystems Library Quantification Kit | KAPA Biosystems | Cat# KK4824 |
| Pierce Kinase Enrichment Kit with ATP Probe | ThermoFisher Scientific | Cat# 88310 |
| Zeba Spin Desalting Columns, 7K MWCO, 5 mL | ThermoFisher Scientific | Cat# 89892 |
| Captisol | Selleckchem | Cat# S4592 |
| D-Luciferin | GoldBio | Cat# LUCK |

Deposited data

| RNaseq and CHIPseq | This Paper, Gene Expression Omnibus GEO: GSE141336 |
| ABPP Proteomics | This Paper, Proteome Xchange ProteomeXchange: PXD005734 |

Experimental models: cell lines

| Jeko-1 | ATCC | Cat# CRL-3006; RRID: CVCL_1865 |
| Jeko-1 IR | Tao Lab | N/A |
| SP49 | University of Pennsylvania Mariusz A. Wasik Laboratory | N/A |
| SP49 IR | Tao Lab | N/A |
| HK | Cellosaurus | Cat# CVCL_IY38; RRID: CVCL_IY38 |
| Autologous stromal cells | Tao Lab | N/A |

Experimental models: organisms/strains

Mouse: NOD-SCID (NOD-scid IL2Rnull) | The Jackson Laboratory | Cat# 001303; RRID: IMSR_JAX:001303 |

Software and algorithms

| Bamliquidator (version 1.0) | https://github.com/BradnerLab/pipeline/wiki/bamliquidator | N/A |
| ROSE2 | https://github.com/BradnerLab/pipeline/blob/master/ROSE2_main.py | Ref: Brown et al., 2014 |
| EMMA Platform Image Processing | Ref: Silva et al., 2015 |
| ImageJ | https://imagej.nih.gov/ij/ | RRID:SCR_003070 |
| Eclipse (Mars) | https://www.eclipse.org/mars/ | N/A |
| MATLAB R2014b | https://www.mathworks.com/products/matlab.html | RRID: SCR_001622 |
| MATLAB R2016b | https://www.mathworks.com/products/matlab.html | RRID: SCR_001622 |
| GraphPad Prism 7 | https://www.graphpad.com/ | RRID: SCR_002798 |
| R (version 3.6.1) | https://cran.r-project.org/ | RRID: SCR_003005 |
| R Studio (version 1.1.456) | https://rstudio.com/ | N/A |
| Tophat2 | https://ccb.jhu.edu/software/tophat/index.shtml | RRID:SCR_013035 |
| Cuffnorm | http://cole-trapnell-lab.github.io/cufflinks/cuffnorm/index.html | N/A |
| Bowtie2 (version 2.2.1) | http://bowtie-bio.sourceforge.net/bowtie2/index.shtml | RRID: SCR_016368, Ref: Langmead and Salzberg, 2012; Langmead et al., 2019 |
| MACS (version 1.4.1) | https://github.com/macs3-project/MACS | RRID:SCR_013291 |
| MaxQuant (version 1.2.2.5) | https://www.maxquant.org/ | RRID: SCR_014485, Ref: Cox et al.,2008 |
| KEGG | https://www.genome.jp/kegg/ | RRID: SCR_012773 |
| Enrichr | https://amp.pharm.mssm.edu/Enrichr | RRID: SCR_001575, Ref: Chen et al., 2013; Kuleshov et al., 2016 |
| GSEA | http://gsea-msigdb.org/gsea/index.jsp | RRID: SCR_003199, Ref: Subramanian et al., 2005 |

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RESOURCE AVAILABILITY

Lead contact
Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Jianguo Tao (jianguo.tao@moffitt.org).

Materials availability
This study did not generate new unique reagents.

Data and code availability
The accession number for the RNAseq and CHIPseq data reported in this paper is GEO: GSE141336.
The accession number for the ABPP proteomics data reported in this paper is ProteomeXchange: PXD005734.
Computational code used for ChIP-seq analyses can be obtained from the Jun Qi Laboratory github page. Namely, Bamliquidator was used to calculate read density which can be found at https://github.com/BradnerLab/pipeline/wiki/bamliquidator; and ROSE2, was used to identify enhancers and can be found at https://github.com/BradnerLab/pipeline/(ROSE2_main.py).
All algorithms and codes used to process images and perform downstream analyses for the EMMA platform were provided directly by the Silva Lab (Silva et al., 2015).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Patients and tumor specimens
The primary samples from MCL patients were obtained from fresh biopsy-derived lymphoma tissues (lymph nodes) and from peripheral blood following informed consent from patients and approval by the Moffitt Cancer Center/University of South Florida Institutional Review Board. For preparation of viable, sterile, single cell suspensions, the lymph node tissue was diced and forced through a cell strainer into RPMI-1640 tissue culture medium. Cells, obtained after low-speed centrifugation, were re-suspended in media. Lymphoma cells from peripheral blood were isolated by Ficoll-Plaque purification, and only lymphoma samples that had greater than 80% tumor cells were used for experiments. Age and gender of patients from which primary samples were obtained are not included in this publication as this information is not provided to research laboratories by the institution.
The human specimen studies presented were approved by the Moffitt/University of South Florida Institutional Review Board and patients provided signed informed consent forms.

Mice
Six- to eight-week-old male NOD/SCID mice were purchased from the Jackson Laboratory and used for xenograft experiments as described (Zhao et al., 2017).
Four- to eight-week-old male NOD/SCID mice were purchased from the Jackson Laboratory and used for PDX experiments as described (Zhao et al., 2017).
All animal studies were conducted in accordance with the NIH guidelines for animal care. All experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee of the Moffitt Cancer Center and the University of South Florida.

Cell lines
Mantle cell lymphoma cell line Jeko-1 was purchased from ATCC. SP49 was provided by Dr. M. Wasik from the University of Pennsylvania. Stromal cell line HK was purchased from Cellosaurus. These cells and their ibrutinib-resistant derivatives (Zhao et al., 2017) were cultured in RPMI-1640 ( GibCO-Invitrogen) with penicillin (100 U/ml) and streptomycin (100 µg/ml) and maintained at 37°C in 5% CO2. Cell lines were routinely tested for mycoplasma using the Universal Mycoplasma Detection Kit from ATCC.
METHOD DETAILS

High-throughput small-molecule drug screens
Using a semi-automated platform, we tested the potency of a 60 small molecule annotated library in Jeko-1/SP49 parental and Ibrutinib-resistant derivative IR cells. Cell viability was estimated by using Resazurin (R&D Systems, AR002). In brief, cells were seeded in 384-well plates with 2,000 cells per well in 25 μL medium. Cells were cultured in the presence of different compounds at serial three-fold diluted concentrations. After 2 hr (kinase inhibitors) or 6 days (epigenetic inhibitors) of treatment, 6 μL of Resazurin reagent was added into each well and incubated for 2 hr. Plates were read at 560/590 nm wavelength to estimate cell proliferation.

Cell-based imaging analysis of drug screening assay
Cells were seeded by a robotic pipettor in 384-well plates of a reconstructed lymphoma TME using the following components and concentrations: high physiological densities of primary patient cells (1-10 × 10^6 cells/ml) with lymphoma stromal cells (HK cells or autologous stromal cells, 2 × 10^5 cells/ml) suspended together in 600 μL RPMI 1640, 240 μL of 10X MEM, 240 μL of deionized H2O, 120 μL of 7.5% sodium bicarbonate solution, 600 μL of 1x RPMI 1640 and 1800 μL of 3.1 mg/ml Bovine collagen type I for 1 full 384-well plate, as detailed previously (Silva et al., 2015). After cell seeding, the 384-well plates were incubated at 37 °C in 5% CO2 for 1-2 hr before an additional layer of media was added on top of the initial TME/tumor cell layer. A panel of drugs at five serial diluted concentrations was then added to the media, and plates were continuously imaged every 30 mins for 4 days (for cell line) or 6 days (for primary samples) by the Evos Auto FL microscope. All images were analyzed using a digital image analysis algorithm in ImageJ to detect cell viability based on membrane motion (pseudo-colored in green), and changes in viability were quantified by area output; 30 s on, 30 s off). Sheared lysates were clarified by centrifuging at 20,000 x g for 1 min and supernatants were collected together, setting aside 50 μL of beads as an input sample. For all other ChIP-seq experiments, magnetic protein G beads (Dynabeads, ThermoFisher Scientific) were washed 3 times with cold blocking buffer, added to the diluted and clarified chromatin supernatant, and rotated overnight at 4 °C. The bound chromatin was then washed twice with 1-mL cold sonication buffer (1.5 mL per 50 million cells; 50 mM HEPES pH 7.3, 140 mM NaCl, 1 mM EDTA, 1% SDS, 0.1% Na-deoxycholate, 0.1% SDS, Roche protease inhibitor cocktail), Samples were divided into 1.5 mL Bioruptor Plus TPX microtubes (Diagenode, #C30010010) at 250 μL per tube and sheared at 4 °C using a water bath sonicator (Bioruptor, Diagenode; 22.5 minutes at high output; 30 s on, 30 s off). Sheared lysates were clarified by centrifuging at 20,000 x g for 4°C for 10 min and supernatants were collected together, setting aside 50 μL as an input sample. For all other ChIP-seq experiments, magnetic protein G beads (Dynabeads, ThermoFisher Scientific) were washed 3 times with cold blocking buffer, added to the diluted and clarified chromatin supernatant, and rotated overnight at 4 °C. The bound chromatin was then washed twice with 1-mL cold sonication buffer, once with 1-mL cold sonication buffer supplemented with 500 mM NaCl, once with cold LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate), and once with TE supplemented with 50 mM NaCl. Finally, beads were resuspended in 210 μL elution buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, and 1% SDS) and chromatin was eluted by vortexing every 5 min while incubating at 65 °C for 15 min. Beads were centrifuged at 20,000 x g for 1 min and the supernatant, together with input sample was placed at 65 °C overnight to reverse crosslinks. RNA was digested with 0.2 mg/mL RNase A (Roche, 10109169001) at 37 °C for 2 hr and protein was digested with 0.2 mg/mL proteinase K (Life Technologies, AM2546) at 55 °C for 30 min. DNA was isolated with phenol chloroform extraction and ethanol precipitation.

RNA-sequencing
All samples were prepared in biological triplicates. 1×10^6 cells were treated for 6 hr or 48 hr with either Ibrutinib, NVP-2, INCB054329 or DMSO at equal concentration as vehicle control. Total RNA was isolated using the RNeasy Plus Mini (QIAGEN Cat# 74134). Library prep was conducted using TruSeq Stranded mRNA Library Prep Kit (Illumina Cat #RS-122-2101/2) according to the manufacturer’s instructions. RNA sequencing was performed on HiSeq 2500v4 high output (50-bp, single-end reads). Tophat2 was used to align the Fastq files. TPM values were calculated and normalized using Cuffnorm. Genes that had a p < 0.05 and at least a two-fold change were considered to be significantly altered between treatments. Cutoff value for expressed genes was a TPM value equal to or higher than 1.

Chromatin immunoprecipitation followed by highly parallel sequencing (ChIP-Seq)
ChIP-seq was performed according to established protocols, with minor modifications. 50 million cells were used for ChIP-seq of H3K27ac. Crosslinking was performed in batches of 50 million cells in 50-mL tissue culture media by addition of one-tenth volume of 10X cross linking solution (11% formaldehyde, 50 mM HEPES pH 7.3, 100 mM NaCl, 1 mM EDTA pH 8.0, 0.5 mM EGTA pH 8.0). After 10 min of crosslinking at room temperature, formaldehyde was quenched with 125 mM glycine, cells were then washed three times in PBS pH 7.4, flash frozen in liquid nitrogen, and stored at -80 °C. Frozen pellets were thawed on ice, resuspended in cold lysis buffer 1 (LB1; 5 mL per 50 million cells; 50 mM HEPES pH 7.3, 140 mM NaCl, 1 mM EDTA, 10% glycerol, 1% Triton X-100, Roche protease inhibitor cocktail), and rotated for 10 min at 4°C. LB1 was removed and pellets were resuspended in cold lysis buffer 2 (LB2; 5 mL per 50 million cells; 10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA pH 8.0 and 0.5 mM EGTA pH 8.0, Roche protease inhibitor cocktail) and rotated for 10 min at 4°C. LB2 was removed and pellets were resuspended in cold sonication buffer (1.5 mL per 50 million cells; 50 mM HEPES pH 7.3, 140 mM NaCl, 1 mM EDTA, 1% EDTA, 1% Triton X-100, Roche protease inhibitor cocktail), Samples were divided into 1.5 mL Bioruptor Plus TPX microtubes (Diagenode, #C30010010) at 250 μL per tube and sheared at 4 °C using a water bath sonicator (Bioruptor, Diagenode; 22.5 minutes at high output; 30 s on, 30 s off). Sheared lysates were clarified by centrifuging at 20,000 x g for 4°C for 10 min and supernatants were collected together, setting aside 50 μL as an input sample. For all other ChIP-seq experiments, magnetic protein G beads (Dynabeads, ThermoFisher Scientific) were washed 3 times with cold blocking buffer, added to the diluted and clarified chromatin supernatant, and rotated overnight at 4 °C. The bound chromatin was then washed twice with 1-mL cold sonication buffer, once with 1-mL cold sonication buffer supplemented with 500 mM NaCl, once with cold LiCl wash buffer (20 mM Tris pH 8.0, 1 mM EDTA, 250 mM LiCl, 0.5% NP-40, 0.5% Na-deoxycholate), and once with TE supplemented with 50 mM NaCl. Finally, beads were resuspended in 210 μL elution buffer (50 mM Tris-HCl pH 8, 10 mM EDTA, and 1% SDS) and chromatin was eluted by vortexing every 5 min while incubating at 65 °C for 15 min. Beads were centrifuged at 20,000 x g for 1 min and the supernatant, together with input sample was placed at 65 °C overnight to reverse crosslinks. RNA was digested with 0.2 mg/mL RNase A (Roche, 10109169001) at 37 °C for 2 hr and protein was digested with 0.2 mg/mL proteinase K (Life Technologies, AM2546) at 55 °C for 30 min. DNA was isolated with phenol chloroform extraction and ethanol precipitation.
Libraries for Illumina sequencing were prepared using ThruPLEX DNA-seq Kit (Rubicon) using 50 pg of DNA or less and amplifying according to manufacturer instructions. Amplified libraries were size-selected first using AMPure beads (Agencourt AMPure XP) and subsequently, using a 2% gel cassette in the Pippin Prep (SAGE Sciences) to capture fragments of 200-700 bp. Libraries were quantified by qPCR using the KAPA Biosystems library quantification kit, multiplexed with equimolar DNA content, and sequenced on an Illumina NextSeq 500 (single end 75 bp reads).

**ChIP-Seq data processing**

**Sequence alignment**

All datasets were aligned using Bowtie2 (version 2.2.1). All default parameters, except for --N 1 (reads that mapped uniquely to the genome with one or fewer mismatches) were used to align to human genome build NCBI37/HG19.

**Identifying enriched regions**

The MACS version 1.4.1 (Model based analysis of ChIP-Seq) 67 peak finding algorithm was used to identify regions of ChIP-Seq enrichment over background. A p value threshold of enrichment of 1e-9 was used for all datasets.

**Calculating read density**

We calculated the normalized read density of a ChIP-Seq dataset in any genomic region using the Bamliquidator (version 1.0) read density calculator. Briefly, ChIP-Seq reads aligning to the region were extended by 200 bp and the density of reads per base pair (bp) was calculated. For ChIP-seq, the density of reads in each region was normalized to the total number of million mapped reads producing read density in units of reads per million mapped reads per bp (rpm/bp).

**Mapping typical enhancers and super-enhancers using H3K27ac enhancer definitions**

H3K27ac super-enhancers (SEs) and typical enhancers (TEs) were mapped using the ROSE2 software package that has been previously described (Brown et al., 2014). MACS defined peaks were considered for rank-ordering by the ROSE2 algorithm. ROSE2 optimizes a stitching parameter on a per-sample basis for combining nearby peaks. Briefly, the algorithm optimizes for the enriched fraction of stitched regions. Read density within these regions was then quantified as noted above and stitched regions were ranked by this metric. Super-enhancers were called by re-scaling both the signal values and the ranks to fall between 0 and 1 and plotting a curve with scaled ranks on the x axis and scaled signal on the y axis. The x coordinate of the intersection point of the line of slope 1 that is tangent to the curve was used to define a cutoff for super-enhancers such that all stitched enhancers with a scaled rank greater than this cutoff were considered super-enhancers. Default ROSE2 parameters for stitching and region filtering, including exclusion of TSS-proximal signal (within 2.5 kb), were used. ROSE2 was also used to rank-order regions disproportionately enriched for POLII ChIP-seq signal as described for H3K27ac ChIP-seq above, except that no peak stitching was used and without excluding ± 2.5 kb from each TSS.

**Activity-based protein profiling (ABPP)**

Briefly, cell pellets were sonicated in IP/Lysis buffer, desalted and then depleted of endogenous ATP with Zeba spin column, and incubated with 10 μM desthiobiotin-ATP probes at room temperature for 10 min. The labeled proteins were reduced, alkylated and trypsin digested at 37°C for 2 hr. The labeled peptides were purified with high capacity streptavidin agarose resin, washed, eluted and subjected to LC-MS/MS for peptide sequencing. The peptide identification and relative quantification were performed using MaxQuant software (Version 1.2.2.5). The procedures of ABPP were as detailed previously (Zhao et al., 2017). Fold-change distribution of the ATP binding proteome was performed by GraphPad software. GO enrichment analysis was performed on the ABPP profile using Enrichr. Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis was performed on the increased protein kinases (comparing DTEP cells to parental cells) from 2 out of 3 cell lines. Raw Data are available via ProteomeXchange with identifier PXD010193.

**Xenograft studies**

Ten million Jeko-1 parental or IR were injected into the lower flank of NOD/SCID mice in a volume of 0.1 mL PBS. Mice were then randomized into control and treatment groups when tumor volume reached 0.2 cm³ (4 mice per group). Tumors were measured with calipers and when tumor volume reached 100-200 mm³ mice were randomized for treatment with Ibrutinib, NVP-2 or vehicle. Ibrutinib and NVP-2 was formulated in 30% (w/v) Captisol (pH 3.0). NVP-2 2.5 mg/kg was given i.p. twice a week and Ibrutinib was 25 mg/kg. Mice were humanely sacrificed when the control tumor reached ~4,000 mm³ or after the loss of more than 10% of body weight.

**PDX model and imaging**

Primary cells from patient specimen were transduced with the Vector: pCDH-EF-eFFLy-T2A-mCherry. Irradiation (200 cGy) was performed within 24 hours prior to xenografting. 1x10⁶ of PDX cells were injected into the tail vain of NOD/SCID mice in a volume of 0.2 mL PBS. Mice were then randomized into control and treatment groups 4 days after tumor xenografting (4 mice per group). Drugs were given i.p. and the dose for NVP-2 was 2.5 mg/kg and Ibrutinib was 25 mg/kg. Mice were humanely sacrificed when the control...
tumor reached ~4,000 mm³ or after the loss of more than 10% of body weight. Images are taken 6 weeks after tumor xenografting. D-Luciferin (GoldBio Catalog # LUCK) was made as of 15 mg/mL in DPBS. 10 μL of Luciferin stock solution per gram of body weight (normally ~200 μL for a 20 g mouse for a standard 150 mg/kg injection) intraperitoneally was given about 10 mins before images were taken using in vivo imaging system IVIS200 (PerkinElmer).

**Gene set enrichment analysis (GSEA)**

Gene set enrichment analysis (GSEA) was performed as described (Subramanian et al., 2005). The most differentially expressed genes ranked by log2 fold change for each comparison were used to generate a signature for GSEA analysis. The input activated or inactivated SE gene sets were extracted from H3K27ac ChIP-seq data. GSEA estimates whether the members of IR-specific gene set are found at the top or bottom of the NVP-2/INCB054329 treatment list, and if genes are specific to either parental or resistant cells, indicating they are associated with a specific phenotype, rather than being distributed uniformly or randomly across the list. An enrichment score (ES) is calculated to quantify the degree to which a gene set is over-represented at the top or bottom of the entire ranked list. After calculation of the scores for a collection of gene sets, an empirical phenotype-based permutation test procedure is used to estimate P values. GSEA normalizes the ES for each gene set to account for the variation in set sizes, yielding a normalized enrichment score (NES) and a false discovery rate (FDR). The FDR gives an estimate of the probability that a set with a given NES represents a false positive finding; it is computed by comparing the tails of the observed and permutation-computed null distributions for the NES.

**ssGSEA (single sample GSEA)**

To determine the relative activity of cancer pathways between Moffitt and MD Anderson cohorts, ssGSEA (version gsea2-2.2.1) was applied using patients’ gene expression profiles. To eliminate batch effects, we normalized gene expression by calculating the z-score within each cohort. Then, for each sample, we ranked all genes on the basis of their expression values to create a .rnk file as input for the software GSEAPreranked. The enrichment score was computed for HALLMARK as the assessment. HALLMARK signatures were downloaded from the Molecular Signatures MD Anderson cohort was downloaded from EGA. We also compared the increased HALLMARK in Ibrutinib resistant patient compared to Ibrutinib sensitive patients.

**Enrichment map**

Enriched gene-sets are graphically organized into a network, where each set is a node and edges represent gene overlap between sets; gene sets map to specific biological processes/pathways involved in Ibrutinib resistance. The Cytoscape network software (version 3.7.1) and the plugin “EnrichmentMap (version3.1.0)” and “AutoAnnotate (version 1.2)” were used to build the network. Plugin and source code are available at http://baderlab.org/Software/EnrichmentMap. Node color encodes the enrichment q-value. Node size is proportional to the total number of genes belonging to the corresponding gene-set (Merico et al., 2010).

**QUANTIFICATION AND STATISTICAL ANALYSIS**

**Statistics**

P values of less than 0.05 were considered significant. Unless otherwise stated, comparison and statistical significance between two groups in this paper are based on two-sided t test. Analysis of variance (ANOVA) or the Kruskal-Wallis test was used for comparing data from multiple groups.
Supplemental information

Transcriptional programming drives

Ibrutinib-resistance evolution

in mantle cell lymphoma

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**Figure S1**

A. Heatmap showing relative expression of genes in Jeko-1 and SP49 cells under Sen and IR conditions.

B. GO Molecular Function of common DEG in IR vs. Sen:
- transcription factor activity
- phosphatase binding (GO:0019902)
- small GTPase binding (GO:0031267)
- RAGE receptor binding (GO:0050786)
- transcriptional activator activity
- racemase and epimerase activity, acting on carbohydrates and derivatives (GO:0016857)
- thiolester hydrolase activity (GO:0016790)
- cysteine-type endopeptidase activity involved in execution phase of apoptosis (GO:0097200)
- RNA polymerase II core promoter proximal region sequence-specific DNA binding (GO:0000978)
- cysteine-type endopeptidase activity involved in apoptotic process (GO:0097153)

1. RNA polymerase II core promoter proximal region sequence-specific binding (GO:0000982)
2. RNA polymerase II transcription regulatory region sequence-specific binding (GO:0001228)

C. Scatter plots showing correlation between RNAseq and ABPP Log2FC for Jeko-1_IR vs. Sen_kinases (r=0.49, P<0.001) and SP49_IR vs. Sen_kinases (r=0.31, P=0.002).

D. Network diagram illustrating gene pathways:
- Development morphogenesis
- Inflammatory coagulation
- Lymphocyte activation
- Kinase cascade ERK
- RNA Pol II
- Neuron development
- Chemotaxis
- Regulation of cytokine production
- Interferon gamma modification
- Cellular response hormone
- Alpha granule platelet
- Gated channel ion
- Regulation of transport production
- Regulation of cell adhesion
Figure S1 (Related to Figure 1). Transcriptome Reprograming Rewires Kinome Signaling in Ibrutinib Resistant (IR) Mantle Cell Lymphoma (MCL).

A, Heatmap showing common significant differential gene expression profiles in IR cells (Jeko-1-IR and SP49-IR) vs their Sen parental cell lines in biological triplicate. B, GO term Molecular function analysis of common differential expressed genes (from figure S1A). Bar length and top axis represent \(-\log_{10}(P\ value)\). Color bar intensity represents \(-\log_{10}(P\ value)\) where the darker colors are indicative of higher significance (lower P value).

C, Correlation between differential mRNA expression and kinase activities of IR vs. Sen Jeko-1 and SP49 cells. Log2 fold change of mRNA and kinase expression between paired IR and Sen Jeko-1 and SP49 cells are shown. RNA-seq data are from triplicate samples and ABPP data are from three or four replicates.

D, Enrichment map of IR-associated genes in SP49-IR cells. The map displays the enriched gene-sets in SP49-IR cells. Nodes represent gene-sets and edges represent overlap between gene-sets. Gene-sets that did not pass the enrichment significance threshold are not shown. Clusters of functionally related gene-sets were assigned a label using “AutoAnnotate” Add-in in Cytoscape; node color intensity is proportional to enrichment significance, clusters of biological and functional interest for their role in IR are highlighted in red.
Figure S2

A

Relative Cell Viability (%)

0 0.012 0.037 0.11 0.33 1
NVP-2 (µM)

B

Relative Cell Viability (%)

0 25 50 75
Pt19
Pt22
Pt24
Pt35

C

Relative Cell Viability (%)

0 25 50 75
Pt0448
Pt1888

D

Relative Cell Viability (%)

0 0.02 0.1 0.5
0.01 0.037 0.11 0.33
0 0.02 0.1 0.5
0 0.1 0.3 1

Ibrutinib
- 10.0 µM
- 3.33 µM
- 1.11 µM
- 0.37 µM
- 0.12 µM

BRD4i (µM)

120 100 80 60 40 20 0
Relative Cell Viability (%)

0 0.02 0.1 0.5
0.01 0.037 0.11 0.33
0 0.1 0.3 1

Jeko-1-Sen Jeko-1-IR

RNAPII CTDpSer2 RNAPII pAKT AKT MCL-1 cPARP β-Actin

RNAPII CTDpSer2 RNAPII pAKT AKT MCL-1 cPARP β-Actin

RNAPII CTDpSer2 RNAPII pAKT AKT MCL-1 cPARP β-Actin

RNAPII CTDpSer2 RNAPII pAKT AKT MCL-1 cPARP β-Actin

RNAPII CTDpSer2 RNAPII pAKT AKT MCL-1 cPARP β-Actin
Figure S2 (Related to Figure 2). Ibrutinib Resistant (IR) Mantle Cell Lymphoma (MCL) Cells Are Highly Sensitive to CDK9 Inhibition. A, Cell viability assay shows the dose response of NVP-2 (left) and INCB054329 (right) in paired IR and Sen parental cells. B, Drug response assessment of primary MCL specimens treated with the indicated doses of Ibrutinib. C, NVP-2 treatment induced more suppression of phosphorylation of the C-terminal repeat domain (CTD) of RNA polymerase II (RNAPII) at the large subunit of RNAPII on Ser2, pAKT, and MCL-1 levels, and induced more PARP cleavage in IR compared to Sen cells. D, INCB054329 induced suppression of pAKT and MCL-1 and increased PARP cleavage in primary MCL patient samples (Pt0448, Pt1888). Data in A, C and D are representative of 3 independent experiments.
Figure S3

A  
![Log2(FC_IR vs. Sen) vs. Log2(FC_DMSO vs. Sen)](image)

B  
KEGG pathway enrichment
- log (P value)
0 0.5 1.0 1.5 2.0 2.5 3.0 3.5
mTOR signaling pathway
MAPK signaling pathway
ErbB signaling pathway
Toll-like receptor signaling pathway
TNF signaling pathway

C  
![Heatmap of Jeko-1 and SP49 expression](image)

D  
![Venn diagrams showing changes in IR vs. Sen](image)

E  
Changes in IR vs. Sen for genes decreased by BRD4i
- Jeko-1
  - Increased 58.5%
  - Conserved 30.0%
  - Decreased 11.5%
- SP49
  - Increased 51.2%
  - Conserved 31.8%
  - Decreased 17.0%

F  
MF_Jeko_SP49_Common_Increased in IR and decreased by BRD4i
- Log10 (P value)
0 1 2 3 4 5 6
RNA polymerase II regulatory region sequence-specific DNA binding
kinase binding
RNA polymerase II regulatory region DNA binding
CD4 receptor binding
non-membrane spanning protein tyrosine kinase activity
protein kinase binding
transcription regulatory region sequence-specific DNA binding
RNA polII core promoter proximal region sequence-specific binding
RNA polII core promoter proximal region sequence-specific DNA binding
RNA polII transcription regulatory region sequence-specific binding

G  
![Principal Component Analysis](image)
**Figure S3 (Related to Figure 3). CDK9 Is Required to Sustain Transcriptional and Kinase Reprogramming in Ibrutinib Resistant (IR) Mantle Cell Lymphoma (MCL) Cells.**

**A,** Fold change in kinase activity in IR vs. Sen SP49 cells (x-axes), and in SP49-IR cells treated with BRD4i INCB054329 (y-axes; 24hrs [top] or 48hr [middle]). Venn diagram at bottom shows the overlap between kinases whose activity is increased in IR cells and decreased by INCB054329 treatment. These overlapped kinases are highlighted by the red box in each scatterplot. Cutoff, FC=1.5. n = 3 biological replicates.  

**B,** KEGG pathway enrichment of kinases whose activity is increased in IR compared to Sen MCL cells, and that also decreased by INCB054329 treatment in IR cells. Top axis and bar length represent -log10(P value).  

**C,** RNAseq heatmap of genes that are increased in IR compared to Sen and that are decreased by INCB054329 treatment. n = 3 biological replicates.  

**D,** Venn diagrams show the overlap between IR specific genes and genes decreased by NVP-2 or INCB054329 treatment in IR cells.  

**E,** Pie chart showing the percentage of gene expression changes (increase/conserved/decrease) in IR compared to Sen cells for INCB054329 decreased genes in IR cells. More than half (58.5% for Jeko-1 and 51.2% for SP49) of the INCB054329 decreased genes are increased in IR cells compared to sensitive cells.  

**F,** Molecular function enrichment results by Enrichr for genes in (D). Top axis and bar length represent -log10(P value).  

**G,** PCA analysis of RNA-seq data showing that IR cells are distinct from Sen cells and that Ibrutinib treated IR cells cluster with IR cells treated with vehicle (DMSO). In contrast, INCB054329 treatment of IR MCL cells provokes a shift towards Sen cell clusters. n = 3 biological replicates.
Figure S4

A

Jeko-1-H3K27ac_SE
IR
58
88
399
Sen
SP49-H3K27ac_SE
IR
469
176
138
Sen

B

Jeko-1-IR_H3K27ac signal GREAT enrichment MSigDB pathway
-log10(Binomial p value)
0
5
10
15
20
25
30

Genes involved in Cytokine Signaling in Immune system
Genes involved in Interferon gamma signaling
Fc gamma R-mediated phagocytosis
EPO signaling pathway
Genes involved in Signaling by the B Cell Receptor (BCR)
CXCR4-mediated signaling events
BCR signaling pathway
Class I PI3K signaling events
B cell receptor signaling pathway
p73 t transcription factor network
Genes related to PIP3 signaling in B lymphocytes
Validated targets of C-MYC t rapo transcriptional repression
Members of the BCR signaling pathway
Natural killer cell mediated cytotoxicity
TCR signaling in negative CD4+ T cells
Role of Calcineurin-dependent NFAT signaling in lymphocytes
HIV-1 Nef; negative effector of Fas and TNF
MAPK Signal ing Pathway
Genes involved in GAB1 signalosome

C

Enrichment score

DMSO
BRD4i
Jeko-1-IR

SP49-IR

NES=-1.84
FDR=0.0028

NES=-2.22
FDR<0.0001

P=1.36e-15

P=7.19e-10

GREATenrichmentMSigDBpathway

D

SP49-IR
Jeko-1-IR

Log2FC_BRD4i vs. DMSO

P=7.19e-10

GenesinvolvedinCytokineSignalinginImmunesystemGenesinvolvedinInterferongammaSingalingFcgammaR-mediatedphagocytosisEPOsignalingpathwayGenesrelatedtopIP3signalinginBlymphocytesValidatedtargets of C-MYC transcriptional repressionMembers of the BCR signaling pathwayNatural killer cell mediated cytotoxicityTCR signaling in negative CD4+ T cellsRole of Calcineurin-dependent NFAT signaling in lymphocytesHIV-I Nef: negative effector of Fas and TNFMAPKinase Signaling PathwayGenes involved in GAB1 signalosome
Figure S4 (Related to Figure 4). Super-Enhancer (SE) Remodeling Drives Transcriptional Programming and Drug Sensitivity to CDK9 Inhibition in Ibrutinib Resistant (IR) Mantle Cell Lymphoma (MCL) lines and Primary Samples. A, Venn diagram (left) and waterfall (right) plot showing overlap and specific SE in paired IR and Sen parental Jeko-1 (top) and SP49 (bottom) MCL cells. B, Genomic Regions Enrichment of Annotations Tool (GREAT) analysis of Jeko-1-IR H3K27ac enrichment. C, Gene Set Enrichment Analysis (GSEA) shows gained SE regulated genes are decreased by INCB054329 treatment in Jeko-1-IR and SP49-IR cells. D, Box plots showing Log2 fold changes of gene expression following INCB054329 treatment vs. vehicle (DMSO) in Jeko-1-IR and SP49-IR cells. Typical enhancer (TE) or SE associated genes are defined by the density and amplitude of H3K27ac marks from ChIP-seq analyses. Significance was determined using the Kruskal-Wallis test; SP49 $P = 1.36e-15$; Jeko-1 $P = 7.19e-10$. E, GSEA shows SE regulated genes are decreased by INCB054329 or NVP-2 treatment in primary IR MCL samples. NES, normalized enrichment score; FDR, false discovery rate.
IbrutinibCombBRD4i

\( P = 0.0021^* \)

\( P = 0.0003^{**} \)

\( P = 4.0 \times 10^{-10} \)

\( n = 31 \)

150

100

50

0

Sensitivity AUC

Figure S5

A

Top 200 upregulated genes in IR Pt

B

NES = -1.60

FDRq = 0.003

NES = -1.85

FDRq = 0.0

**

***

NES = -1.60

FDRq = 0.003

Enrichment score

DMSO Pt22_BRD4i

Enrichment score

DMSO Pt24_BRD4i

Top 200 upregulated genes in IR Pt

NES = -1.85

FDRq = 0.0

NES = -1.60

FDRq = 0.003

DMSO Pt22_BRD4i

DMSO Pt24_BRD4i

B

150

100

50

0

Sensitivity AUC

\( * P = 0.0021 \)

\( ** P = 0.0003 \)

\( *** P = 4.0 \times 10^{-10} \)

n = 31
Figure S5 (Related to Figure 5). Targeting CDK9 Prevents Emergence and Overcomes Ibrutinib Resistance (IR) in Mantle Cell Lymphoma (MCL) *ex vivo* and *in vivo*. A, Gene Set Enrichment Analysis (GSEA) of differential gene expression shows top 200 upregulated genes in IR primary MCL patient samples are negatively enriched by INCB054329 treatment. NES, normalized enrichment score; FDR, false discovery rate. B, Drug response assay of primary MCL patient samples treated with Ibrutinib or INCB054329 alone or the Ibrutinib/INCB054329 combination by AUC measured by cell-based imaging analysis. *P* values were calculated by one-way ANOVA. n = 31 primary MCL patient samples.
Figure S6

A

**HALLMARK_MTORC1_SIGNALING**

- NES = 7.434 (p.adj = 0.0062) for Pt 13
- NES = 6.539 (p.adj = 0.0024) for Pt 31

B

**Moffitt data set**

- Higher in Resistant
- OXIDATIVE PHOSPHORYLATION
- MTORC1 SIGNALING
- MYC TARGETS V1
- MYC TARGETS V2
- TNF SIGNALING VIA NFKB
- DNA REPAIR

**MD Anderson dataset**

- Higher in Sensitive
- MTORC1 SIGNALING
- DNA REPAIR
- WNT SIGNALING VIA NFKB
- G2M CHECKPOINT

C

**ssGSEA - MTORC1_SIGNALING**

| MD Anderson | Moffitt |
|-------------|---------|
| Sensitive   | Resistant | P = 0.006 |
| Sensitive   | Resistant | P = 0.01  |

**ssGSEA - OXIDATIVE PHOSPHORYLATION**

| MD Anderson | Moffitt |
|-------------|---------|
| Sensitive   | Resistant | P = 0.06 |
| Sensitive   | Resistant | P = 0.04  |
Figure S6 (Related to Figure 6). The EMMA Platform Predicts Clinical Responses and Informs Vulnerabilities in Primary and Ibrutinib Resistant (IR) Mantle Cell Lymphoma (MCL). A, Single-sample gene set enrichment analysis (ssGSEA) of representative primary MCL samples for mTORC1_SIGNALING Negative enrichment in Ibrutinib sensitive MCL samples (left panel, Pt 61), positive enrichment in IR MCL samples (right panel, Pt 13) and intermediate enrichment (middle panel, Pt 31). B, ssGSEA of enriched pathways in IR versus sensitive MCL patients from the MD Anderson (right) and Moffitt (left) datasets were performed, and the resulting enrichment scores were compared between IR and Sen patient groups. The Ibrutinib sensitivity groups were defined by maximal Ibrutinib effect measured by cell-based imaging analysis for Moffitt datasets and clinical response for MD Anderson datasets. Note the red highlighted circles indicate higher enrichment scores for MYC, NF-κB and mTOR pathways in IR MCL patient samples, and higher enrichment scores for WNT and DNA repair pathways in Ibrutinib sensitive MCL patient samples of both datasets. C, ssGSEA reveals higher enrichment score for the mTORC1 and OXIDATIVE PHOSPHORYLATION pathway HALLMARK signatures in resistant compared to sensitive primary patient sample for both MD Anderson and Moffitt datasets. Ibrutinib sensitivity groups are defined as in B.