Research Article

iMedPub Journals
www.imedpub.com

Journal of Medical Research and Health Education

2018
Vol.2 No.3:11

Efficacy and Mechanism of Action of the Novel Bromodomain Inhibitor, PLX51107, in B Cell Malignancies

Monica Mead[1*], Erika Von Euw[2], Dylan Conklin[2,3], Kanthinh Manivong[2], Eileen Do[4], Dennis J Slamon[5] and Sarah Larson[6]

[1]UCLA Medical Center, Los Angeles, USA

[2]Division of Hematology and Oncology, University of California, Los Angeles, USA

[3]Plexxikon, Berkley, USA

[4]NYP, Weill Cornell Medical Center, New York, USA

[5]Division of Hematology/Oncology, Department of Medicine, UCLA, Los Angeles, USA

[6]University of California, Los Angeles, USA

[*]**Corresponding author:** Dr. Monica Mead, Lymphoma Program, University of California, Los Angeles, 2825 Santa Monica Blvd, Suite 200, Santa Monica, 90404, USA, Tel: 310-315-8780; E-mail: mmead@mednet.ucla.edu

Received date: September 22, 2018; **Accepted date:** September 27, 2018; **Published date:** October 01, 2018

Citation: Mead M, Euw EV, Conklin D, Manivong K, Do E, et al. (2018) Efficacy and Mechanism of Action of the Novel Bromodomain Inhibitor, PLX51107, in B Cell Malignancies. J Med Res Health Educ. Vol.2 No.3:11.

Abstract

BRD4 functions as an important epigenetic regulator of transcription of pathways involved in oncogenesis. While down regulation of c-myc following BRD4 inhibition has been described, the exact mechanism of action of BRD4 inhibitors across B cell malignancies remains unclear. We demonstrate the *in vitro* efficacy of PLX51107 across a spectrum of B cell lymphoma (BCL) cell lines and the signaling pathways involved. PLX51107 demonstrated potent anti-tumor effects (median IC_{50} of 300 NM), primarily driven by cell cycle arrest and induction of premature senescence. Treatment of BCL cultures resulted in down regulation of c-myc, p-IRAK, and P-MAPK Erk 1/ 2. PLX51107 affected the expression of proteins in several critical cellular pathways in sensitive cell lines, while proteins involved in drug resistance were affected in resistant cell lines. These results demonstrate that PLX51107 has mechanisms of action beyond down regulation of the c-myc transcriptional program and is a promising targeted therapeutic agent.

Keywords: Lymphoma; Bromodomain inhibitors; Epigenetics; Proteins

Introduction

The epigenome is comprised of various molecules that bind to chromatin and regulate transcription of genes critical to vital cellular activities, including proliferation, self-renewal and differentiation [1]. In cancer, the epigenome can play a role in oncogenesis through anomalous alterations in histone modifications, DNA methylation and noncoding RNA expression levels [1]. Regulation of the epigenome is a dynamic process and some pathogenic alterations in this program can be reversed [2], making the epigenome an attractive target for the treatment of malignancies. Epigenetic readers are histone modifying enzymes that contain specialized domains which recognize distinct covalent alterations of the nucleosome and/or function as a scaffolding platform for additional epigenetic regulators [3]. Bromodomain and extraterminal domain (BET) proteins, including BRD2, BRD3, BRD4, and BRDT, are a family of epigenetic readers containing highly conserved motifs that bind acetylated chromatin and facilitate RNA polymerase 2-dependent transcription initiation through recruitment of the transcriptional elongation complex pTEFb [4-6]. BRD4 amplifies expression of genes already selected for expression through preferential occupation of enhancers and super-enhancers in cancer cells. BRD4 is distributed throughout the genome and found adjacent to genes known to be involved in B-cell lymphoma biology, notably MYC [7], BCL6, CD79B, PAX5 and IRF8 [8], providing the rationale for targeting BET proteins in the treatment of B cell lymphomas. Successful development of two BET protein inhibitors, JQ1 [9] and I-BET762 [10,11] were initially reported in 2010, representing the first effacious pharmacological targeting of an epigenetic reader. BET bromodomain inhibitors have demonstrated antitumor effects across a variety of hematologic malignancy tumor models, through G1 cell-cycle arrest, apoptosis and senescence [8,12-20]. Dysregulated cellular pathways resulting in development of B-cell malignancies vary based on the maturational stage of the transformed B-cell. Consequently, effects of BET inhibition vary across B-cell malignancies,

including DLBCL-ABC, DLBCL-GCB and mantle cell lymphoma (MCL).

Pre-clinical studies using a panel of 56 different human DLBCL and MCL models identified distinct groups of genes targeted by BET inhibition. The first group, identified in both the GCB and ABC DLBCL subtypes, includes myc and E2F-dependent genes, members involved in B- cell receptor signaling and the MYD88/toll ligand receptor (TLR) pathways [8]. The second group included lineage-specific groups of genes; IRF4, PIM1 and CCND2 found in the DLBCL-GCB subtype [8] and NFκB gene signatures found in the ABC subtype [20]. Inhibition of BET proteins in MCL tumor models has been shown to result in down regulation of myc and cyclin-dependent kinase 4/6, in addition to inhibition of nuclear RelA levels and expression of NFκB target genes [21]. In this study we evaluated the pre-clinical activity of a novel bromodomain inhibitor, PLX51107, in human B-cell lymphoma cell lines and report data on the potential mechanisms of action as well as on genetic features associated with sensitivity/resistance to the compound.

Materials and Methods

Bromodomain inhibitor and human lymphoma cell lines

The BRD inhibitor, PLX51107 was solubilized using 20.1 mg in 4.67 ml of DMSO to develop a stock solution with a concentration of 10 mm Fresh preparations of PLX51107 were prepared on the day of each experiment. The cell lines used for the studies presented in this report include DoHH2, DB (DLBCL-GCB subtype); Ly3, U2932 (DLBCL-ABC subtype); and Mino, GRANTA (MCL). Cells were grown and tested in RPMI (Corning) with 10% FBS (Omega Scientific), 1% L-Glutamine 100X (Gibco) and 1% Penicillin-Streptomycin 100X (Corning).

Measurement of cell growth and survival

Cells were transferred from T75 culture flasks to 50 ml tubes. Cell concentrations were determined with use of a Vi-CELL cell viability analyzer (Beckman Coulter, Brea, CA) to ensure viability of 85% or greater. Cells were diluted to a target concentration ranging from 5-50,000 cells per well and plated into 24 well plates. Each experiment was performed in duplicate and additional samples were seeded for determination of cell count control numbers for day 0 and day 5. Cells were incubated at 37°C for 24 hours. A day 1 control cell count was then determined using a Z1 particle counter (Beckman Coulter, Brea, CA). Each well was then treated with serial dilutions of PLX51107, at a starting concentration of 10 µM, followed by serial 1:5 dilutions. The treated cells were then incubated with drug at 37°C for 5 days. Cell counts were obtained for all remaining wells on day 5. Generational percent inhibition was determined through nonlinear regression curve-fitting.

Cell cycle and apoptosis measurements

Treated cells were incubated at 37°C for 72 hours, harvested by centrifugation and washed with 1X PBS. Cells were stained with propidium iodide (PI), incubated at room temperature in the dark and analyzed by fluorescence activated cell sorting (FACS).

Cells were exposed to PLX51107 at their IC_{50} or DMSO for 3 days. Cells were then harvested by centrifugation, washed with 1X PBS and stained with PI and fluorescein isothiocyanate (FITC) using the Annexin V FITC Apoptosis Detection Kit (BD Biosciences, San Jose, CA) according to the manufacturer's protocol and analyzed by FACS.

Measurement of cell senescence

Treated cells were incubated at 37°C for 7 days then harvested by centrifugation and washed with 1X PBS. Bafilomycin A was purchased from Millipore (Billerica, MA) and solubilized in DMSO according to the package insert, for a working solution concentration of 100 µM and added to appropriate media for a final concentration of 1:1000. One ml of this solution was added to each sample and incubated at 37°C for 60 minutes. C12FDG was purchased from Life Technologies (Grand Island, NY) and dissolved in DMSO according to the package insert for a stock solution of 2 mM. C12FDG was added to each sample for a final concentration of 33 µM and incubated at 37°C for 60 minutes. Cells were collected by centrifugation, washed twice with 1X PBS, re suspended in 150 µL of 1X PBS and analyzed by FACS.

Protein assessments using western blot and reverse phase protein array (RPPA) analyses

Cell cultures were exposed to DMSO or PLX51107 at their respective IC_{50}. After 4 and 24 hours, cells were collected by centrifugation and lysed with RIPA buffer (Millipore–Upstate, Temecula, CA) supplemented with a protease inhibitor cocktail, 0.5 mM PMSF, and 0.2 mM Na_3VO_4. Prior to loading, samples were adjusted to contain equivalent amounts of protein, ranging from 10 to 20 µg per sample. Proteins were separated in a 4-12% Tris glycine midi-gel SDS-PAGE (Life Technologies, Grand Island, NY) and transferred to nitrocellulose membranes with iBlot gel transfer (Life Technologies, Grand Island, NY). Membranes were blotted with antibodies from Cell Signaling Technologies (Danver, MA, c-myc, p65, p-Iκβα, p-AKT, p-MEK 1/ 2, p-p38 MAPK and GAPDH) and Sigma-Aldrich (St. Louis, MO; p-IRAK).

In order to more broadly assess the expression pattern of a wide variety of potentially critical proteins within the entire B-cell lymphoma cell line panel, each line was individually pelleted from cultures treated with PLX51107 or DMSO vehicle control were prepared. Cell cultures were exposed to PLX51107 at their respective IC_{50} for 24 hours. The treated cells were collected by centrifugation at 1,500 rpm, washed twice with 1X PBS and re suspended in 150 µL of 1X PBS. Cell suspensions were transferred to 1.5 mL microtubules and centrifuged at 4,000 rpms for 5 minutes at 4°C. The

supernatant was discarded and pellets underwent reverse phase protein array analysis according to protocol guidelines at MDAnderson.org.

Results

Antiproliferative activity of PLX51107 *in vitro*

A panel of 56 human B-cell lymphoma (BCL) cell lines, including DLBCL-ABC subtype, DLBCL-GCB subtype and MCL were evaluated for the antiproliferative activity of PLX51107. Treatment of cell cultures using increasing concentrations of drug allowed for determination of a subset of sensitive BCL cell lines defined by an IC_{50} of 300 nm or less which is a concentration that is feasible in the clinical setting. PLX51107 was active in a dose-dependent manner in all sensitive cell lines. Consistent with previous reports, the antiproliferative effects of PLX51107 did not vary significantly among the different BCL subtypes [19]. The median IC_{50} values for the sensitive DLBCL-GCB, DLBCL-ABC and MCL cell lines were 111 nm, 176 nm and 128 nm, respectively. **Table 1** outlines the characteristics of the cell lines selected for further evaluation.

Table 1 IC_{50} values* for patient-derived B cell lymphomas treated with PLX-51107. *IC_{50} values were calculated using a customized database generated at the Translational Oncology Research Laboratory, Santa Monica, CA.

Cell line	Histologic subtype	PLX-51107 IC_{50} (μM)
DoHH2	DLBCL-GCB	0.111
Mino	Mantle cell lymphoma	0.128
Ocl-Ly3	DLBCL-ABC	0.176
U2932	DLBCL-ABC	0.327
Granta	Mantle cell lymphoma	1.05
DB	DLBCL-GCB	2.04

Effects of PLX51107 on cell cycle arrest, apoptosis and senescence across the panel of human BCL cell lines

We exposed BCL cell lines in the panel to PLX51107 and assessed them for cell cycle arrest, induction of apoptosis and induction of senescence. When comparing effects of cell cycle perturbation between PLX51107- and DMSO-treated cells, a substantial increase in G1 arrest was observed in all 3 sensitive BCL cell lines as compared to matched resistant histologic counterparts **(Figure 1)**. A marked increase in apoptosis was observed in 2 of the 3 sensitive BCL cell lines; MCL (MINO) and DLBCL-ABC (Ly3) subtype. Apoptosis was not increased significantly in the sensitive DLBCL-GCB subtype (DoHH2) or the matched resistant BCL cell lines **(Figure 2)**, consistent with prior studies [17]. Interestingly, while exposure of DoHH2 to PLX51107 did not result in increased apoptosis, a substantial increase in premature senescence was observed. The DLBCL-ABC sensitive cell line (Ly3) and one of the three paired resistant cell lines, U2932, also exhibited an increase in premature senescence **(Figure 3)**.

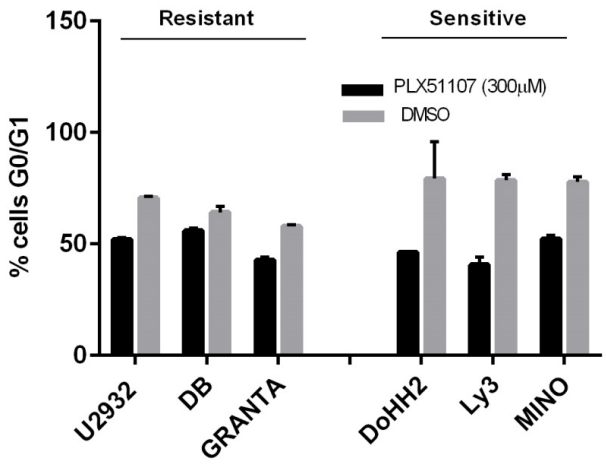

Figure 1 PLX51107 induces G1 cell cycle arrest in DLBCL-GCB (DoHH2), DLBCL-ABC (Ly3) and MCL (MINO) human cell lines. % cells in G0/G1in BCL treated with PLX51107 for 72 hours was compared with that in cells treated with DMSO. Error bars are SEM of 3 independent experiments.

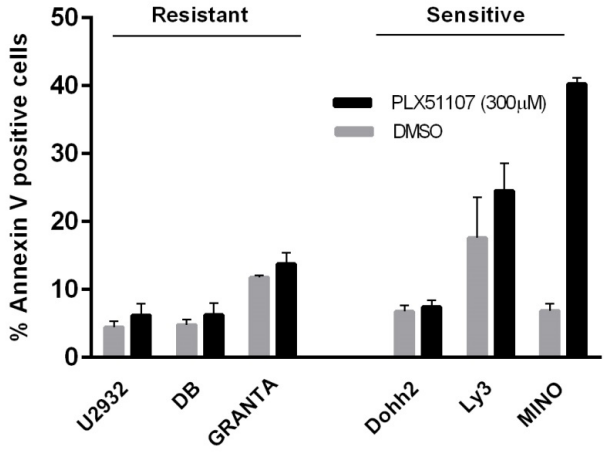

Figure 2 PLX51107 induces apoptosis in MCL (MINO) human cell lines. % Annexin V positive cells treated with PLX51107 for 3 days in BCL was measured and compared with that in cells treated with DMSO. Error bars are SEM of 3 independent experiments.

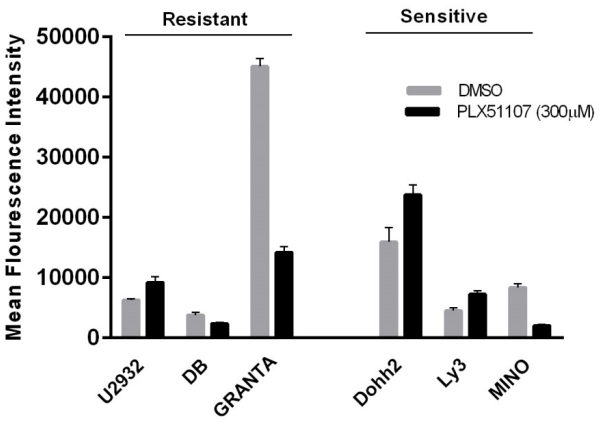

Figure 3 PLX51107 induces senescence in human DLBL-GCB (Dohh2) and DLBCL-ABC (Ly3) cell lines. % b-galactosidase activity in BCL cells treated with PLX51107 for 7 days was measured and compared with that in cells treated with DMSO. Error bars are SEM of 3 independent experiments.

Proteomic analysis of PLX51107 sensitive and resistant human BCL cell lines using reverse phase protein arrays (RPPA)

To compare the differential effects on protein expression induced by PLX51107 exposure among sensitive and resistant BCL cell lines, we performed RPPA on BCL cell lines treated with DMSO or PLX51107 at their respective IC_{50s}. PLX51107 affected the expression of proteins in several critical cellular pathways in the sensitive cell lines including: the cell cycle regulatory programmed cell death, B-cell receptor (BCR) signaling, and PI3K/AKT/mTOR pathways. Up regulated proteins in the sensitive BCL cell lines were primarily represented by proteins involved in apoptosis (that is p21, PARP1), Notch signaling (RBM1), cell cycle regulation (RB, cyclinD1) and DNA repair (ATM, MSH2); while down regulated proteins were represented by BCR signaling (syk), apoptosis (BCL2) and protein elongation (EEF2). Proteins affected by PLX51107 in the resistant cell lines are primarily known to be involved in drug resistance (PKCα, YB1). Phosphorylated PKCα is a protein kinase that mediates resistance to tyrosine kinase inhibitor (TKI) therapy in lung cancer [22] while YB1 has been shown to bind to multi-drug resistance (mdr1) proteins and to promote drug resistance in BCL [23]. Similar to findings of previous pre-clinical studies of bromodomain inhibitors [13,24,25], treatment with PLX51107 results in down regulation of c-myc expression in a time-dependent manner across the spectrum of sensitive BCL cell lines, irrespective of underlying subtype, immunophenotype or MYC aberrations, as assessed by immunoblotting **(Figure 4a-4c)**. A decrease in c-myc expression was observed in all three sensitive BCL cell lines at 4 and 24 hours of exposure to PLX51107, with the DLBCL-GCB and MCL cell line demonstrating near-complete down regulation at the earlier time point.

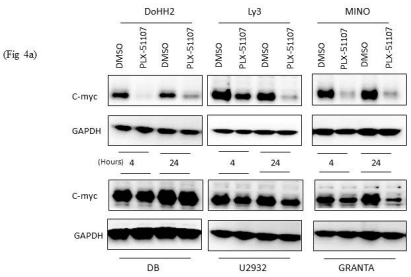

Figure 4a Human BCL cell lines were treated with PLX51107 (300 μM) at the indicated time points. Total protein lysates were loaded on a gel for western blot analysis using c-myc.

Two BCL cell lines, U2932 and GRANTA, deemed resistant by IC_{50}, also demonstrated decreased c-myc expression upon exposure to PLX51107 at 4 and 24 hours. To further investigate this observation, c-myc expression was assessed following exposure of five additional resistant BCL cell lines (Karpass422, RL, and SU-DHL-5 DLBCL-GCB; Ri-1 DLBCL-non-GCB, Jeko1-MCL) to PLX51107 for 4 and 24 hours. Down regulation of c-myc protein was confirmed in 3 of those 5 resistant cell lines (Karpass422, RL and SU-DHL-5 **(Figure 5)**).

Effects of PLX51107 on the MAPK signaling pathway in B-cell lymphoma models

The pro-survival mitogen-activated protein kinase (MAPK) pathway, which is downstream of BCR signaling, has been shown to play a role in lymphomagenesis of B-cell malignancies through activation of highly oncogenic transcription factors [26]. Previous data demonstrated that myc induces Ras/Mapk signaling [27]. Given that bromodomain inhibition results in decreased myc expression and transcriptional programming, we hypothesized that PLX51107 would disrupt MAPK signaling in a c-myc-mediated manner. We next performed western blot analyses on PLX51107 treated-BCL cell lines probed with activated phosphorylated MAPK Erk 1/2. All sensitive BCL subtypes demonstrated decreased p-MAPK Erk 1/2 expression, while no corresponding reduction was observed in the matched resistant cell lines **(Figure 4b)**. Down regulation of MAPK signaling correlated with c-myc down regulation in the sensitive cell lines, while MAPK signaling was independent of c-myc expression in the resistant cell lines.

(Fig 4b)

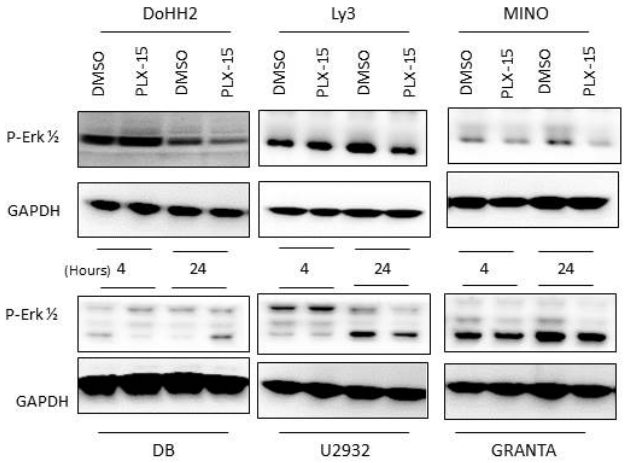

Figure 4b Human BCL cell lines were treated with PLX51107 (300 µM) at the indicated time points. Total protein lysates were loaded on a gel for western blot analysis using p-MAPK Erk ½.

Effects of PLX51107 on the MyD88-dependent TLR signaling and NFκB pathways in B-cell lymphoma models

Previous data have supported an impact of bromodomain inhibition on the TLR pathway in DLBCL [17]; however the effect in other BCL histologies, in particular MCL, is largely unknown. We evaluated the effect of PLX51107 on the TLR pathway through immunoblot assays probed with activated phosphorylated IRAK, a signaling intermediary of the MYD88-dependent pathway. Consistent with prior data, PLX51107 demonstrated an inhibitory effect on the TLR pathway in sensitive DLBCL cell lines (DoHH2, Ly3), irrespective of immuno phenotype, while the corresponding resistant cell lines were unaffected. Of note, the TLR pathway of the sensitive MCL cell line (MINO) was also negatively regulated by PLX51107, which has not been previously demonstrated **(Figure 4c)**.

The importance of the pro-survival NFκB pathway varies among different BCL histologies. In the DLBCL-ABC subtype, NFκB signaling is commonly constitutively activated; while DLBCL-GCB subtype generally has lower expression of NFκB target genes [28]. The oncogenic drivers of MCL are most commonly overexpression of the cyclin D1 gene [29] and to a lesser extent, Notch1 signaling [30]; while the role of the NFκB pathway is not well established. The constitutive activation of NFκB signaling observed in DLBCL-ABC is driven by somatic mutations in various genes, including CARD11, CD79 [31,32] or MYD88 [33]. Previous studies investigating the impact of bromodomain inhibition on the NFκB pathway in BCL have demonstrated negative regulation in DLBCL-ABC [17,20] and -GCB subtypes [17], while the effect on MCL is less clear. To investigate the effect of PLX51107 on the NFκB signaling pathway in BCL, we performed western blot analyses using antibodies directed against phosphorylated IκBα (p-IκBα) that is a cytosolic protein responsible for sequestering NFκB in an inactive state in the cytosol. Upon phosphorylation, translocation of NFκB to the nucleus occurs, followed by transcription of pro-survival genes [34]. In contrast to prior studies [28], expression of p-IκBα was unaffected following 4 or 24 hours of exposure to PLX51107 in the sensitive DLBCL-ABC cell line **(Figure 4c)**. Similar observations were seen in the sensitive MCL cell line. Interestingly, DoHH2, the DLBCL-GCB subtype, demonstrated a reduction in p-IκBα after 4 and 24 hours of exposure to PLX51107. These data lend additional support that the anti-proliferative effects of PLX51107 are mediated by subtype-specific pathways.

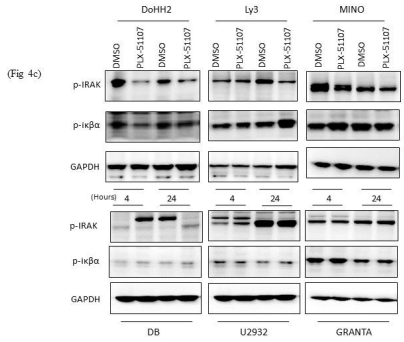

Figure 4c Human BCL cell lines were treated with PLX51107 (300 µM) at the indicated time points. Total protein lysates were loaded on a gel for western blot analysis using p-IRAK and p-IκBα.

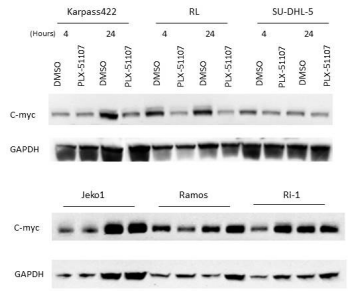

Figure 5 Resistant human BCL cell lines were treated with PLX51107 (300 µM) at the indicated time points. Total protein lysates were loaded on a gel for western blot analysis using c-myc antibody.

Discussion

In this study, we evaluated the *in vitro* efficacy of PLX51107 in a diverse panel of 56 human B-cell lymphoma cell lines and explored the potential underlying mechanisms of its anti-tumor activity. Our data shows that (i) PLX51107 has *in vitro* antiproliferative activity; (ii) PLX51107 displays lineage-specific anti-tumor effects and (iii) PLX51107 down regulates MYC, NFκB and MYD88-dependent TLR pathways in a lineage-specific pattern. PLX51107 resulted in antiproliferative effects at clinically achievable concentrations across a heterogeneous spectrum of B cell lymphomas, including DLBCL- GCB and ABC as well as MCL cell lines. In accordance with previous findings, PLX51107 induced apoptosis in DLBCL-ABC subtype [17] and MCL [21], while DLBCL-GCB predominantly displayed a senescence phenotype. At this point, our data reveal no clear known underlying predictive biomarker that correlates with these differential mechanistic findings.

It is well established that myc aberrations may contribute to oncogenesis in several hematologic malignancies and has been a challenging therapeutic target. Initial studies involving bromodomain inhibitors demonstrated that down regulation of myc and myc-target genes was an important mediator of their anti-proliferative effects in BCL [35]. However, more recent studies suggest bromodomain inhibition may have lineage specific anti-tumor effects beyond the global effect of c-myc attenuation. Consistent with previous bromodomain inhibitors, PLX51107 induced down regulation of the myc transcriptional program as well as almost complete attenuation of c-myc expression in all sensitive B cell lymphoma cell lines. Interestingly, treatment with PLX51107 resulted in down regulation of myc in a subset of resistant BCL cell lines including U2932 (DLBCL-ABC) and GRANTA (MCL), each with wild-type myc. This finding was further confirmed in DLBCL-GCB (SU-DHL-5, Karpass422 and RL).

Despite the decrease in c-myc expression in these resistant BCL cell lines, anti-proliferative effects were not observed following exposure to PLX51107. RPPA data is notable for the up regulation of Notch signaling in the PLX51107-treated resistant BCL cell lines, suggesting alterations in Notch signaling may explain this paradoxic finding. Notch1 has been shown to mimic the oncogenic functions of EBNA2, a viral protein that plays an important lymphoma genic role in EBV-positive BL [36] that includes B-cell immortalization and the ability to activate transcription of a number of cellular genes. Aberrant Notch signaling has been linked to B-cell malignancies and has been shown to impact myc activity [37-40]. These data suggest that notch signaling may be responsible for the resistance phenotype in BCL cell lines showing c-myc down regulation, while that of sensitive BCL cell lines is mediated through previously described epigenetic modifications [41]. This finding provides further support that c-myc down regulation is not the sole mediator of the antiproliferative effects of bromodomain inhibitors, and suggests that the combination of PLX51107 with a Notch1 inhibitor may result in synergistic antiproliferative effects and increased sensitivity among BCL cell lines.

While BRD4 inhibition resulted in decreased expression of p-IRAK, in all sensitive BCL cell lines, subsequent inhibition of the NFκB pathway was observed only in the DLBCL-GCB subtype. This finding contrasts with other bromodomain inhibitors that induced down regulation of the NFκB pathway in DLBCL-ABC cell lines [8,17]. This may be explained by the presence of an underlying mutation(s) in CARD11 and/or CD79 that would promote constitutive activation of the NFκB pathway independent of the effect of BRD4 inhibition on the MYD88-dependent pathway. Interestingly, a similar pattern to DLBCL-ABC was observed in the MCL cell line, despite lack of an established lymphoma genic role of the NFκB pathway in MCL. PLX51107 targets the MYD88-dependent pathway and

downstream signaling of NFκB in the DLBCL-GCB subtype, which may be explained by the low frequency of NFκB activating mutations in GCB subtype.

Anti-tumor effects of BRD4 inhibitors are not well characterized in MCL. PLX51107 demonstrates anti-proliferative effects in the sensitive MCL cell line characterized by a robust apoptotic phenotype, near complete attenuation of MYC expression and down regulation of MAPK signaling this suggests MYC may play an important role in the pathogenesis of a subset of MCL. Previous data has demonstrated MYC overexpression in approximately one third of patients with MCL [42] and MYC expression has been found to be an independent risk factor for chemotherapy resistance and poor clinical outcome [43,44]. Novel treatment strategies are clearly needed for these patients. Dai et al. have recently demonstrated MYC is regulated by MALT1, a component of the signaling intermediary complex between the BCR and NFκB pathway, in a subset of MCL cell lines [45]. Furthermore, interference of MALT1 in this subset of MCL results in cytotoxicity. These findings suggest future studies should explore the effects of PLX51107 exposure on MALT1 in MCL. Additionally, this data suggests novel combinations incorporating PLX51107 should be explored in MCL, particularly in the subset characterized by MYC overexpression.

Our data support multiple potential mechanisms in mediating resistance to PLX51107 in BCL cell lines. The protein (RPPA) data demonstrated that treatment with PLX51107 resulted in an attenuated down regulation of proteins involved in promoting cell cycle progression and programmed cell death in resistant as compared to sensitive BCL cell lines. Additionally, p-PKCα expression, which is linked to TKI resistance in lung cancer cell lines, was down regulated after exposure to PLX51107 in resistant BCL cell culture, suggesting it may also play a role in mediating resistance to PLX51107. This presents the possibility that combining PLX51107 with pharmacologic inhibition of PKCα may induce sensitivity of resistant BCL cell lines to BRD4 inhibition and this combination should now be evaluated in preclinical models. Lastly, treatment of resistance BCL cell lines with PLX51107 resulted in up regulation of YB1, a protein that binds to mdr1, an ATP-dependent drug efflux pump that contributes to drug resistance in a variety of malignancies. Previous studies have shown Mdr1 promotes drug resistance in BCL and furthermore, pre-treatment with a MAPK inhibitor prevents nuclear translocation of YB1 and expression of mdr1 [23]. These findings suggest that combination treatment with PLX51107 and a MAPK inhibitor may also result in increased sensitivity of BCL to PLX51107.

Conclusion

Taken together, the results of this study provide further support that PLX51107 is a promising new agent for the treatment of a spectrum of B-cell lymphomas. There is clear antiproliferative activity exhibited by the drug in three separate BCL subtypes; including DLBCL-ABC and GCB and MCL. Our data are consistent with the fact that the primary antiproliferative effects induced by PLX51107 vary based on the underlying maturational stage of the BCL. Down regulation of c-myc expression is a universal effect of PLX51107, irrespective of underlying histology or response, and may be mediated through lineage-specific mechanism. Novel signaling pathways are affected across PLX51107-treated B-cell malignancies including the MYD88-dependent TLR pathway. Finally, the data presented here provide support for additional investigation of PLX51107 in combination with targeted therapies in B-cell lymphomas.

Acknowledgements

Work is supported by The Aramont Foundation for Hematologic Malignancies

Disclosure of Interest

The authors report no conflicts of interest.

References

1. Jiang Y, Hatzi K, Shaknovich R (2013) Mechanisms of epigenetic deregulation in lymphoid neoplasms. Blood 121: 4271-4279.
2. Geutjes EJ, Bajpe PK, Bernards R (2012) Targeting the epigenome for treatment of cancer. Oncogene 31: 3827-3844.
3. Dawson MA, Kouzarides T, Huntly BJ (2012) Targeting epigenetic readers in cancer. N Engl J Med 367: 647-657.
4. Florence B, Faller DV (2001) A novel family of transcriptional regulators. Front Biosci 6: 1008-1018.
5. Malik S, Roeder RG (2010) The metazoan Mediator co-activator complex as an integrative hub for transcriptional regulation. Nat Rev Genet 11: 761-772.
6. Mochizuki K, Nishiyama A, Jang MK, Dey A, Ghosh A, et al. (2008) The bromodomain protein Brd4 stimulates G1 gene transcription and promotes progression to S phase. J Biol Chem 283: 9040-9048.
7. Loven J, Hoke HA, Lin CY, Lau A, Orlando DA, et al. (2013) Selective inhibition of tumour oncogenes by disruption of super-enhancers. Cell 153: 320-334.
8. Chapuy B, McKeown MR, Lin CY, Monti S, Roemer MG, et al. (2013) Discovery and characterization of super-enhancer-associated dependencies in diffuse large B cell lymphoma. Cancer Cell 24: 777-90.
9. Filippakopoulos P, Qi J, Picaud S, Shen Y, Smith WB, et al. (2010) Selective inhibition of BET bromodomains. Nature 468: 1067-1073.
10. Nicodeme E, Jeffrey KL, Schaefer U, Beinke S, Dewell S, et al. (2010) Suppression of inflammation by a synthetic histone mimic. Nature 468: 1119-1123.
11. Gosmini R, Nguyen VL, Toum J, Simon C, Brusq JM, et al. (2014) The discovery of I-BET726 (GSK1324726A), a potent tetrahydroquinoline ApoA1 up-regulator and selective BET bromodomain inhibitor. J Med Chem 57: 8111-8131.
12. Delmore JE, Issa GC, Lemieux ME, Rahl PB, Shi J, et al. (2011) BET bromodomain inhibition as a therapeutic strategy to target c-Myc. Cell 146: 904-917.

13. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, et al. (2011) Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proc Natl Acad Sci USA 108: 16669-16674.

14. Dawson MA, Prinjha RK, Dittmann A, Giotopoulos G, Bantscheff M, et al. (2011) Inhibition of BET recruitment to chromatin as an effective treatment for MLL-fusion leukaemia. Nature 478: 529-533.

15. Bhadury J, Nilsson LM, Muralidharan SV, Green LC, Li Z, et al. (2014) BET and HDAC inhibitors induce similar genes and biological effects and synergize to kill in Myc-induced murine lymphoma. Proc Natl Acad Sci USA 111: 2721-2730.

16. Emadali A, Rousseaux S, Bruder-Costa J, Rome C, Duley S, et al. (2013) Identification of a novel BET bromodomain inhibitor-sensitive, gene regulatory circuit that controls Rituximab response and tumour growth in aggressive lymphoid cancers. EMBO Mol Med 5: 1180-1195.

17. Boi M, Gaudio E, Bonetti P, Kwee I, Bernasconi E, et al. (2015) The BET Bromodomain Inhibitor OTX015 Affects Pathogenetic Pathways in Preclinical B-cell Tumor Models and Synergizes with Targeted Drugs. Clin Cancer Res 21: 1628-1638.

18. Coude MM, Braun T, Berrou J, Dupont M, Bertrand S, et al. (2015) BET inhibitor OTX015 targets BRD2 and BRD4 and decreases c-MYC in acute leukemia cells. Oncotarget 6: 17698-17712.

19. Trabucco SE, Gerstein RM, Evens AM, Bradner JE, Shultz LD, et al. (2015) Inhibition of bromodomain proteins for the treatment of human diffuse large B-cell lymphoma. Clin Cancer Res 21: 113-122.

20. Ceribelli M, Kelly PN, Shaffer AL, Wright GW, Xiao W, et al. (2014) Blockade of oncogenic IkappaB kinase activity in diffuse large B-cell lymphoma by bromodomain and extraterminal domain protein inhibitors. Proc Natl Acad Sci USA 111: 11365-11370.

21. Sun B, Shah B, Fiskus W, Qi J, Rajapakshe K, et al. (2015) Synergistic activity of BET protein antagonist-based combinations in mantle cell lymphoma cells sensitive or resistant to ibrutinib. Blood 126: 1565-1574.

22. Abera MB, Kazanietz MG (2015) Protein kinase Calpha mediates erlotinib resistance in lung cancer cells. Mol Pharmacol 87: 832-841.

23. Shen H, Xu W, Luo W, Zhou L, Yong W, et al. (2011) Up regulation of mdr1 gene is related to activation of the MAPK/ERK signal transduction pathway and YB-1 nuclear translocation in B-cell lymphoma. Exp Hematol 39: 558-569.

24. Chaidos A, Caputo V, Gouvedenou K, Liu B, Marigo I, et al. (2014) Potent antimyeloma activity of the novel bromodomain inhibitors I-BET151 and I-BET762. Blood 123: 697-705.

25. Ott CJ, Kopp N, Bird L, Paranal RM, Qi J, et al. (2012) BET bromodomain inhibition targets both c-Myc and IL7R in high-risk acute lymphoblastic leukemia. Blood 120: 2843-2852.

26. Turjanski AG, Vaque JP, Gutkind JS (2007) MAP kinases and the control of nuclear events. Oncogene 26: 3240-3253.

27. Gramling MW, Eischen CM (2012) Suppression of Ras/Mapk pathway signaling inhibits Myc-induced lymphomagenesis. Cell Death Differ 19: 1220-1227.

28. Davis RE, Brown KD, Siebenlist U, Staudt LM. (2001) Constitutive Nuclear Factor κB Activity Is Required for Survival of Activated B Cell–like Diffuse Large B Cell Lymphoma Cells. The Journal of Experimental Medicine 194: 1861-1874.

29. Rimokh R, Berger F, Delsol G, Digonnet I, Rouault JP, et al. (1994) Detection of the chromosomal translocation t(11;14) by polymerase chain reaction in mantle cell lymphomas. Blood 83: 1871-1875.

30. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, et al. (2012) Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood 119: 1963-1971.

31. Lenz G, Davis RE, Ngo VN, Lam L, George TC, Wright GW, et al. (2008) Oncogenic CARD11 mutations in human diffuse large B cell lymphoma. Science 319: 1676-1679.

32. Compagno M, Lim WK, Grunn A, Nandula SV, Brahmachary M, et al. (2009) Mutations of multiple genes cause deregulation of NF-kappaB in diffuse large B-cell lymphoma. Nature 459: 717-721.

33. Ngo VN, Young RM, Schmitz R, Jhavar S, Xiao W, et al. (2011) Oncogenically active MYD88 mutations in human lymphoma. Nature 470: 115-119.

34. Hayden MS, Ghosh S (2008) Shared principles in NF-kappaB signaling. Cell 132: 344-362.

35. Trabucco SE, Gerstein RM, Evens AM, Bradner JE, Shultz LD, et al. (2015) Inhibition of Bromodomain Proteins for the Treatment of Human Diffuse Large B-cell Lymphoma. Clinical Cancer Research 21: 113-122.

36. Strobl LJ, Hofelmayr H, Marschall G, Brielmeier M, Bornkamm GW, et al. (2000) Activated Notch1 modulates gene expression in B cells similarly to Epstein-Barr viral nuclear antigen 2. J Virol 74: 1727-1735.

37. Ortega M, Bhatnagar H, Lin AP, Wang L, Aster JC, et al. (2015) A microRNA-mediated regulatory loop modulates NOTCH and MYC oncogenic signals in B- and T-cell malignancies. Leukemia 29: 968-976.

38. Kridel R, Meissner B, Rogic S, Boyle M, Telenius A, et al. (2012) Whole transcriptome sequencing reveals recurrent NOTCH1 mutations in mantle cell lymphoma. Blood 119: 1963-1971.

39. Weng AP, Millholland JM, Yashiro-Ohtani Y, Arcangeli ML, Lau A, et al. (2006) c-Myc is an important direct target of Notch1 in T-cell acute lymphoblastic leukaemia/lymphoma. Genes Dev 20: 2096-2109.

40. Palomero T, Lim WK, Odom DT, Sulis ML, Real PJ, et al. (2006) NOTCH1 directly regulates c-MYC and activates a feed-forward-loop transcriptional network promoting leukemic cell growth. Proc Natl Acad Sci USA 103: 18261-18266.

41. Mertz JA, Conery AR, Bryant BM, Sandy P, Balasubramanian S, et al. (2011) Targeting MYC dependence in cancer by inhibiting BET bromodomains. Proceedings of the National Academy of Sciences 108: 16669-16674.

42. Hernandez-Ilizaliturri FJ, Deeb G, Zinzani PL, Pileri SA, Malik F, et al. (2011) Higher response to lenalidomide in relapsed/refractory diffuse large B-cell lymphoma in nongerminal center B-cell-like than in germinal center B-cell-like phenotype. Cancer 117: 5058-5066.

43. Yi S, Zou D, Li C, Zhong S, Chen W, et al. (2015) High incidence of MYC and BCL2 abnormalities in mantle cell lymphoma, although only MYC abnormality predicts poor survival. Oncotarget 6: 42362-42371.

44. Hernandez L, Hernandez S, Bea S, Pinyol M, Ferrer A, et al. (1999) c-myc mRNA expression and genomic alterations in mantle cell lymphomas and other nodal non-Hodgkin's lymphomas. Leukemia 13: 2087-2093.

45. Dai B, Grau M, Juilland M, Klener P, Horing E, et al. (2017) B-cell receptor-driven MALT1 activity regulates MYC signaling in mantle cell lymphoma. Blood 129: 333-346.