HHS Public Access

Author manuscript

Curr Res Transl Med. Author manuscript; available in PMC 2022 July 01.

Published in final edited form as:

Curr Res Transl Med. 2021 July ; 69(3): 103290. doi:10.1016/j.retram.2021.103290.

PHF19 inhibition as a therapeutic target in Multiple Myeloma

Carolina D. Schinke[1], **Jordan T. Bird**[2], **Pingping Qu**[3], **Shmuel Yaccoby**[1], **Valeriy V Lyzogubov**[1], **Randal Shelton**[1], **Wen Ling, PhD**[1], **Eileen M Boyle**[4], **Sharyu Deshpande**[1], **Stephanie D. Byrum**[2], **Charity Washam**[2], **Samuel Mackintosh**[2], **Owen Stephens**[5], **Sharmilan Thanendrarajan**[1], **Maurizio Zangari**[1], **John Shaughnessy Jr**[1], **Fenghuang Zhan**[1], **Bart Barlogie**[6], **Frits van Rhee**[1], **Brian A Walker**[7]

[1]Myeloma Center, Division of Hematology/Oncology, Winthrop P. Rockefeller Cancer Institute, University of Arkansas for Medical Sciences, Little Rock, AR

[2]College of Medicine, Department of Biochemistry and Molecular Biology, University of Arkansas for Medical Sciences, Little Rock, AR

[3]Cancer Research and Biostatistics, Seattle, WA

[4]Perlmutter Cancer Center, NYU Langone Health, New York, NY, USA

[5]The College of Medicine, Department of Biomedical Informatics, University of Arkansas for Medical Sciences, Little Rock, AR

[6]Division of Hematology, The Mount Sinai Hospital, New York, NY, USA Sinai

[7]Division of Hematology Oncology, Indiana University, Indianapolis, IN, USA.

Abstract

Epigenetic deregulation is increasingly recognized as a contributing pathological factor in multiple myeloma (MM). In particular tri-methylation of H3 lysine 27 (H3K27me3), which is catalyzed by PHD finger protein 19 (PHF19), a subunit of the Polycomb Repressive Complex 2 (PRC2), has recently shown to be a crucial mediator of MM tumorigenicity. Overexpression of PHF19 in MM has been associated with worse clinical outcome. Yet, while there is mounting evidence that PHF19 overexpression plays a crucial role in MM carcinogenesis downstream mechanisms remain to be elucidated. In the current study we use a functional knock down (KD) of PHF19 to investigate the biological role of PHF19 and show that PHF19KD leads to decreased tumor growth in vitro and in vivo. Expression of major cancer players such as bcl2, myc and EGR were decreased upon PHF19 KD further underscoring the role of PHF19 in MM biology. Additionally, our results highlighted the prognostic impact of PHF19 overexpression, which was significantly associated with worse survival. Overall, our study underscores the premise that targeting the PHF19-PRC2 complex would open up avenues for novel MM therapies.

Corresponding author: Carolina D. Schinke, cdschinke@uams.edu.

Introduction

Multiple myeloma (MM) is a hematopoietic malignancy of terminally differentiated plasma cells (PC) that reside within the bone marrow (BM). It is universally preceded by premalignant stages, including monoclonal gammopathy of undetermined significance (MGUS) and smoldering MM (SMM) and can transform into malignant MM over time [1,2]. Despite the advent of novel therapies in recent years, MM remains incurable for the majority of patients and the disease course is typically characterized by recurrent relapses and increasing drug resistance that will eventually lead to aggressive MM proliferation and patient death[3]. The mechanisms by which MGUS and SMM develop into MM remain incompletely understood, as are the pathways that lead to more resistant MM during the disease course. Overcoming this gap in knowledge will be crucial to open avenues for novel therapeutic strategies that will eventually lead to a cure in this disease. We and others have previously shown that PHF19 plays a crucial role in MM oncogenesis. [4–7] High expression levels of PHF19 correlate significantly with advanced and aggressive MM disease stages and functional *in vitro* and *in vivo* experiments have underscored the biological importance of PHF19 for cancer growth and drug resistance and its potential as a therapeutic target.

Previous work which was conducted primarily in embryogenesis has shown that PHF19 is an epigenetic regulator that activates the polycomb repressive complex 2 (PRC2). PRC2 employs the subunits EZH1 and EZH2 that contain methyltransferase activity and lead to tri-methylation of H3Lys27 (H3K27me3).[8–11] This process results in downstream gene repression and silencing of tumor suppressor genes. Aberrant EZH1/2 expression has shown to lead to increased tumor proliferation in diverse cancer models and the role of PHF19 in MM and cancer biology is just beginning to be understood. [12,13] Despite the mounting evidence of its pathological importance, there is a lack of understanding in downstream mechanisms. In the current study we underscore the functional importance of PHF19 in MM using a xenograft model and further elucidate pathways that are altered by PHF19 overexpression.

Methods

PHF19 expression and survival analysis in patients with myeloma

Specimens were obtained after institutional review board approved by the University of Arkansas for Medical Sciences in accordance with the Declaration of Helsinki. Gene expression analysis of isolated plasma cells (CD138+) from 870 newly diagnosed MM that had been enrolled into our Total Therapy (TT) 3–5 trials[14,15]. MM cells were enriched by CD138 immunomagnetic bead selection of mononuclear cells fractions of bone marrow aspirates (autoMACS; Miltenyi Biotec) and purity (≥80%) was assessed with flow cytometry. Gene expression was performed using U133 Plus 2.0 microarrays (Affymetrix) as previously described.[16]

Human myeloma cell lines and culture

The multiple myeloma cell lines JJN3 (DSMZ, ACC541) and ARP1 (established at the University of Arkansas)[17] were grown in Mycoplasma-free conditions and maintained in

complete culture medium (RPMI supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin) in tissue culture flasks, at 37 °C in a 5% CO_2 humidified incubator.

Lentiviral short hairpin RNA vectors and transduction

A *PHF19* targeting short hairpin RNA (shRNA, V2THS_21282, Dharmacon) and non-silencing control scrambled shRNA (RHS4743, Dharmacon) with tetracycline controlled transcriptional activation (TRIPZ) was used for knockdown studies as previously described[6]. In brief, the JJN3 and ARP1 MM cell lines were transduced with the shRNA-containing lentiviruses, which also included a red fluorescent protein (RFP) expressing plasmid to facilitate recognition of successfully induced cells. Puromycin at a concentration of 1 μg/ml was added 72 hours after transduction to select for transduced cells. After 2 weeks of puromycin selection, doxycycline (1 μg/ml) was added to the cells to induce the production of *PHF19* and control shRNA. *PHF19* KD efficiency was measured by real-time PCR and western blotting after 72 hours (JJN3) and 168 hours (ARP1) of doxycycline induction, as described below.

Colony formation assay

Colony formation assay was performed as previously described.[18] In brief, 2×10^3 cells were grown in 1 mL of semisolid methylcellulose medium (MethoCult H4100; StemCell Technologies Inc.) supplemented with complete medium, 10% FBS and 1 μg/ml doxycycline. Colonies were counted after 14 days using light microscopy. One colony was defined as an accumulation of at least 50 cells.

Cell lysis and western blotting

Cells were lysed in lysis buffer (Thermo Scientific) and western blotting was performed as previously described using a *PHF19* directed antibody (Invitrogen) [5,6]. To assess whether PHF19 knockdown would induce decrease of H3K27me3 via the EZH1/2 pathway as shown in previous studies we used an EZH2 inhibitor (Tazemetostat) at a concentration of 1μM as a negative control. EZH2, H3K27me2, H3 and beta actin directed antibodies were purchased from Cell Signalling.

Xenograft model—JJN3 cells carrying the PHF19 knock down plasmid or non-silencing shRNA plasmid were additionally transduced with a GFP+ luciferase reporter vector (Promega) for bioimaging. NOD-SCID mice (Jackson lab) were injected via tail vein with 2×10^5 JJN3 cells containing either the PHF19 KD plasmid (n=10) or scrambled shRNA control (n=10). Mice were gavaged with 0.2 mL of 20 mg/mL doxycycline daily to induce the TRIPZ plasmids starting on the first day after cell injection until death or end of study. In vivo MM growth was monitored via weekly chemiluminescene imaging of mice following intraperioteneal injection with luciferin using the Xenogen IVIS Spetrum System (Caliper Life Sciences). ELISA for detection of Kappa light chain excretion was performed weekly to measure overall tumor burden from mouse serum (Bethyl).

RNA sequencing and Mass Spectometry—RNA processing and sequencing as well as Mass Spectometry of in culture grown JJN3 and ARP1 cells, each containing the induced PHF19 KD plasmid or shRNA control were performed at the UAMS genomics and

proteomics core facilities. The procedure are detailed in the supplementary text. Each experiment was performed in triplicate.

Statistical Analysis—Data are presented as the mean ± standard deviation (SD) for 3 independent experiments unless otherwise noted. Student t-tests were used to test differences in continuous variables (e.g. colony formation in cell lines and tumor burden in treated mice). Logrank tests were used to compare survival outcomes. Methods for the RNAseq and proteomics analyses can be found in the supplemental material.

Results

PHF19 expression correlates with disease aggressiveness and is a prognostic marker of survival

We have previously shown that *PHF19* expression correlates significantly with MM disease stages.[4] Using gene expression microarray data we had shown that while *PHF19* expression levels were similar in healthy subjects and patients with MGUS and SMM, the levels increased significantly in patients with symptomatic MM and even more so in patients with primary plasma cell leukemia (pPCL), supplementary Figure 1[6]. To further underscore the prognostic impact of *PHF19* expression we investigated 870 newly diagnosed MM patients from our TT 3–5 cohort and randomly split them into two-third training and one-third as a validation set. Within the training set, logrank test identified a *PHF19* expression level of 10.46 as an optimal cut point to predict for worse overall survival (OS) in the patient population with high *PHF19* expressing group (≥ 10.46) compared to significant better OS in the patient group with a low PHF19 expression (<10.46) Figure 1A, $p<0.001$. These results were further confirmed in the validation set showing that high PHF19 expression (>10.46) predicts for significantly worse OS, Figures 1 B, $p<0.0001$. High *PHF19* expression was significantly associated with several adverse clinical parameters, such as higher ISS stage, lower Albumin and hemoglobin, higher beta-2-globulin and LDH values as well as higher percentage of cytogenetic abnormalities and GEP70 defined high risk, Table 1, $p<0.05$. We further performed univariate and multivariate cox regression analysis in a training and validation set to explore the prognostic impact of PHF19 expression on overall survival as an independent parameter, Supplemental Table 1 and 2. Other factors included in the analysis were age, albumin, beta-2-microglobulin, GEP70 high risk[16], ISS staging and various cytogenetic and molecular abnormalities. While several parameters appeared to have significant prognostic impact in the training set (including age, albumin beta-2-microglobulin, GEP70 high risk, PHF19 as well as the MAF, MMSET and PR molecular subgroups) only age, albumin, GEP70 high risk and high PHF19 expression were associated with worse prognosis, $p<0.1$, in the validation set.

Knockdown of *PHF19* leads to decreased tumor growth in *in vitro* and *in vivo* experiments

We have previously shown that lentivirally induced knockdown (KD) of *PHF19* lead to decreased viability in the MM cell lines JJN3 and ARP-1 due to a slowing of the cell cycle,[6] Supplementary Figure 2B + C. We further explored the functional effects of *PHF19* KD on colony formation – a marker of stemness - and show that colony formation was significantly reduced in MM cell lines carrying the *PHF19* KD compared to control (CTL), Figure 2 A

+B. *In vivo* testing was subsequently performed using NOD-SCID mice that were injected with JJN3 cell lines transduced with either the *PHF19* KD plasmid (n=10) or a non-silencing shRNA control plasmid (n=10), Figure 3A+B. Overall survival was substantially improved in the *PHF19* KD mice compared to CTL, Figure 3C, p=0.05 and tumor burden measured by ELISA was significantly reduced in the PHF19KD group compared to CTL, Figure 3D, p<0.05.

Major cancer players are involved in PHF19 downstream pathways

Expression analysis through RNAseq—Previous reports have shown that PHF19 interacts with PRC2 to regulate tri-methylation of H3K27 and thereby regulates gene expression through epigenetic mechanisms. The PRC2 subunits EZH1/2 have been shown to contain methyltransfarase activity and are the main source of trimethylation of H3K27[5,10,19]. We have previously shown that our sh-RNA mediated model was able to successfully reduce the amount of PHF19 within the JJN3 and ARP1 cell lines[6] and here we further show that similar to inhibiting EZH2 with Tazemetostat, PHF19 KD leads to decreased trimethylation of H3K27, Figure 4A. Trimethylation of H3K27 with subsequent gene silencing has been demonstrated to be the main mechanism of action of PHF19 regulation[5], yet there remains a lack of understanding of PHF19 regulated downstream mechanisms.

Here we report that shRNA mediated knockdown in the JJN3 and ARP1 cell line lead to significant changes in overall gene expression measured by RNA sequencing. Knockdown and control cell RNA was sequenced in triplicate and compared to identify genes with significant changes in expression. There were a total of 228 overlapping genes in both cell lines, which were up- or downregulated in the same direction by at least 0.5 fold change with a p-value of <0.05, suggesting that these genes are directly affected by PHF19 regulation, Supplemental Table 3. Of special interest is the downregulation of several molecules that have been previously described to be crucial in the biology of MM, notably BCL2 and myc, both known to play anti-apoptotic roles in MM[20–22], Figure 4 C+D. The downregulation of EGR1, which previously has shown to be responsible for drug resistance in MM[23] further underscores the therapeutic potential of targeting PHF19. On the other hand, SLAMF7 was significantly upregulated in both cell lines suggesting that targeting PHF19 overexpression could also enhance the efficacy of other therapeutic drugs, such as Elotuzumab, a currently FDA approved monoclonal antibody that targets SLAMF7. Pathway analysis demonstrated that mechanisms affected by the down- or upregulation of the overlapping 228 genes after PHF19 knockdown were associated with Cancer, organismal injury, cell movement and cell death and survival, Figure 4B (p<0.0001), further underscoring the pathological importance of PHF19 overexpression in Multiple Myeloma.

Additionally, we investigated PHF19 dependent alterations within the INF/JAK-STAT pathway. Previous reports have indicated a negative correlation between PHF19 and genes related to Interferon-JAK-STAT signaling. JAK1 and STATs have been shown to be directly bound by H3K27me3 and recent reports have highlighted the role of PHF19 in altering the expression of the JAK-STAT signaling genes in MM cell lines[5,24]. Using Ingenuity pathway analysis (IPA), our results show that similar to previous reports, PHF19 KD leads to increased activity of genes implicated in the JAK-STAT pathway (including JAK1 and 3,

STAT 2,4 and 6), which has shown to lead to decreased MM growth in in vitro models. However, this alteration was observed only in the JJN3 cell line, Supplemental table 4, $p<0.05$.

Protein analysis via Mass Spectrometry

Similarly, on a protein level we saw significant differences in protein transcription after PHF19 knockdown. Proteomics analysis revealed 33 proteins that were significantly up- or downregulated in the JJN3 and ARP1 cell lines with a 0.5 fold difference and p-value of <0.05 upon PHF19 knockdown, Supplemental Table 5. Of particular interest were proteins that were previously described to have tumor suppressor activity and were significantly upregulated after PHF19 knockdown. These include LHPP,[25] LSP1,[26] LZTFL1,[27] METTL7A[28] and MVP.[29] The altered expression of PHF19, bcl2, myc and EGR1 was not detected by proteomics which is likely due to the low abundance of these regulatory proteins within the cell.

When looking at overlapping genes from RNAseq and Proteomics analysis, we show that 7 genes with significantly up or downregulated RNA expression after PHF19 knockdown corresponded with their respective protein, Figure 4E. Genes and proteins that became downregulated included APBA2- previously described to be associated with amyloid production, and SLC3A2, an amino acid transporter which has been shown to promote worse prognosis in breast cancer and whose expression is thought to be driven by myc.[30,31] The remaining gene-protein pairs were upregulated and included regulators of immune function (CAMK1D[32,33], CASP1[34] and SIAE[35]) and glycolysis (PFKFB2[36]) as well as TCEAL4, a modulator of transcription elongation.[37] The role of these proteins in cancer and MM biology remains largely unexplored. Furthermore the associations between protein expression and their regulation through PHF19 expression or degradation needs to be further elucidated.

Discussion

Epigenetic regulation of mechanisms that modify growth and drug resistance have recently been recognized to play major roles in the pathophysiology of MM.[38] In particular the PHF19 regulated tri-methylation of H3K27 has gained increasing attention, as several recently published reports have shown that the targeting of PHF19 and/or its downstream pathway could open the door for novel therapeutic avenues in MM.[5,7] Additionally, apart from its evident biological role in MM, PHF19 has shown to have a powerful impact as a prognostic marker. In fact in a recent crowdsourcing study that included expression data from over 1500 MM patients, PHF19 expression was found to have most predictive value as a single gene and outperformed other putative adverse prognostic markers, such as MMSET expression.[6] Similar to previous studies, our results underscore the prognostic and biologic importance of PHF19 overexpression in MM. High PHF19 expression was a significant independent predictor of shorter overall survival. The functional *in vitro* and *in vivo* studies are in line with previous studies and highlight the therapeutic benefit of targeting PHF19 to decrease tumor growth. Yet, while there is mounting evidence describing the crucial biological and prognostic role of PHF19 in MM, the pathways and mechanisms regulated by

PHF19 require further exploration. Ren et al. showed that upregulations of genes within the JAK/STAT pathway was at least to some part responsible for the downstream effects of PHF19 knock down. However, this alteration was observed only in the JJN3 cell line in the present study, suggesting that PHF19 downstream targets could slightly vary depending on affected cell and tissue type. Furthermore we show that knockdown of PHF19 affects major players in MM tumorigenicity. In particular the anti-apoptotic bcl-2 protein plays a crucial role in MM biology and targeting bcl-2 has shown promising results in preclinical and clinical MM models.[22] Our results are further corroborated by recent reports in ovarian cancer where targeting of PHF19 likewise showed a decrease of bcl-2 expression.[39] Similarly, the downregulation of myc and EGR1 further underscore the regulatory role of PHF19 in myeloma biology. Myc deregulation and overexpression is one of the key features of disease progression and has been implicated to be a trigger of MGUS to MM transition [40] while targeting EGR1 has previously shown to counteract bortezomib resistance in MM cell lines.[23] The knockdown of PHF19 further lead to significant changes of protein expression, most notably the upregulation several previously described tumor suppressor proteins, including LHPP, LSP1, LZTFL1, METTL7A and MVP.[25,27,29,41–43] Currently no PHF19 inhibitors exist, however therapeutic strategies have been developed that aim at targeting the PHF19-PRC2 interaction or inhibiting EZH1/EZH2 downstream of PHF19.[44,45] Clinical trials have confirmed the effectiveness of these drugs in non-Hodgkin lymphomas and some solid tumors indicating promising potential also in MM.[44,46,47] It is of note though that the MM-promoting and gene-silencing functions of PHF19 not solely rely on its interaction with PRC2 and downstream involvement of EZH1/EZH2, but also on the conserved chromatin-binding domains (Tudor and EH) as previously described.[5] These findings suggest that direct inhibition of PHF19 and/or its upstream targets, which remain to be elucidated, could further increase the therapeutic potential and additional investigation is needed for developing PHF19-targeted strategies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Kyle RA, Therneau TM, Rajkumar SV, et al. Prevalence of monoclonal gammopathy of undetermined significance. N Engl J Med. 2006;354(13):1362–1369. [PubMed: 16571879]
2. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. Blood. 2009;113(22):5412–5417. [PubMed: 19179464]
3. Palumbo A, Anderson K. Multiple myeloma. N Engl J Med. 2011;364(11):1046–1060. [PubMed: 21410373]
4. Schinke C, Boyle EM, Ashby C, et al. Genomic analysis of primary plasma cell leukemia reveals complex structural alterations and high-risk mutational patterns. Blood Cancer J. 2020;10(6):70.
5. Ren Z, Ahn JH, Liu H, et al. PHF19 promotes multiple myeloma tumorigenicity through PRC2 activation. Blood. 2019.
6. Mason MJ, Schinke C, Eng CLP, et al. Multiple Myeloma DREAM Challenge reveals epigenetic regulator PHF19 as marker of aggressive disease. Leukemia. 2020.

7. Yu T, Du C, Ma X, et al. Polycomb-like Protein 3 Induces Proliferation and Drug Resistance in Multiple Myeloma and Is Regulated by miRNA-15a. Mol Cancer Res. 2020;18(7):1063–1073. [PubMed: 32312841]
8. Xu B, On DM, Ma A, et al. Selective inhibition of EZH2 and EZH1 enzymatic activity by a small molecule suppresses MLL-rearranged leukemia. Blood. 2015;125(2):346–357. [PubMed: 25395428]
9. Agarwal P, Alzrigat M, Parraga AA, et al. Genome-wide profiling of histone H3 lysine 27 and lysine 4 trimethylation in multiple myeloma reveals the importance of Polycomb gene targeting and highlights EZH2 as a potential therapeutic target. Oncotarget. 2016;7(6):6809–6823. [PubMed: 26755663]
10. Ballare C, Lange M, Lapinaite A, et al. Phf19 links methylated Lys36 of histone H3 to regulation of Polycomb activity. Nat Struct Mol Biol. 2012;19(12):1257–1265. [PubMed: 23104054]
11. Brien GL, Gambero G, O'Connell DJ, et al. Polycomb PHF19 binds H3K36me3 and recruits PRC2 and demethylase NO66 to embryonic stem cell genes during differentiation. Nat Struct Mol Biol. 2012;19(12):1273–1281. [PubMed: 23160351]
12. Kim KH, Roberts CW. Targeting EZH2 in cancer. Nat Med. 2016;22(2):128–134. [PubMed: 26845405]
13. Kalushkova A, Fryknas M, Lemaire M, et al. Polycomb Target Genes Are Silenced in Multiple Myeloma. Plos One. 2010;5(7).
14. van Rhee F, Szymonifka J, Anaissie E, et al. Total Therapy 3 for multiple myeloma: prognostic implications of cumulative dosing and premature discontinuation of VTD maintenance components, bortezomib, thalidomide, and dexamethasone, relevant to all phases of therapy. Blood. 2010;116(8):1220–1227. [PubMed: 20501894]
15. Jethava Y, Mitchell A, Zangari M, et al. Dose-dense and less dose-intense total therapy 5 for gene expression profiling-defined high-risk multiple myeloma. Blood Cancer J. 2016;6:e471. [PubMed: 27635734]
16. Zhan F, Huang Y, Colla S, et al. The molecular classification of multiple myeloma. Blood. 2006;108(6):2020–2028. [PubMed: 16728703]
17. Hardin J, MacLeod S, Grigorieva I, et al. Interleukin-6 prevents dexamethasone-induced myeloma cell death. Blood. 1994;84(9):3063–3070. [PubMed: 7949178]
18. Popovic R, Martinez-Garcia E, Giannopoulou EG, et al. Histone methyltransferase MMSET/NSD2 alters EZH2 binding and reprograms the myeloma epigenome through global and focal changes in H3K36 and H3K27 methylation. PLoS Genet. 2014;10(9):e1004566.
19. Aloia L, Di Stefano B, Di Croce L. Polycomb complexes in stem cells and embryonic development. Development. 2013;140(12):2525–2534. [PubMed: 23715546]
20. Jovanovic KK, Roche-Lestienne C, Ghobrial IM, Facon T, Quesnel B, Manier S. Targeting MYC in multiple myeloma. Leukemia. 2018;32(6):1295–1306. [PubMed: 29467490]
21. Slomp A, Peperzak V. Role and Regulation of Pro-survival BCL-2 Proteins in Multiple Myeloma. Front Oncol. 2018;8:533. [PubMed: 30524962]
22. Touzeau C, Maciag P, Amiot M, Moreau P. Targeting Bcl-2 for the treatment of multiple myeloma. Leukemia. 2018;32(9):1899–1907. [PubMed: 30076373]
23. Chen L, Wang S, Zhou Y, et al. Identification of early growth response protein 1 (EGR-1) as a novel target for JUN-induced apoptosis in multiple myeloma. Blood. 2010;115(1):61–70. [PubMed: 19837979]
24. Beira JV, Torres J, Paro R. Signalling crosstalk during early tumorigenesis in the absence of Polycomb silencing. PLoS Genet. 2018;14(1):e1007187.
25. Hou B, Li W, Li J, et al. Tumor suppressor LHPP regulates the proliferation of colorectal cancer cells via the PI3K/AKT pathway. Oncol Rep. 2020;43(2):536–548.
26. Flister MJ, Bergom C. Genetic Modifiers of the Breast Tumor Microenvironment. Trends Cancer. 2018;4(6):429–444. [PubMed: 29860987]
27. Wei Q, Chen ZH, Wang L, et al. LZTFL1 suppresses lung tumorigenesis by maintaining differentiation of lung epithelial cells. Oncogene. 2016;35(20):2655–2663. [PubMed: 26364604]
28. McKinnon CM, Mellor H. The tumor suppressor RhoBTB1 controls Golgi integrity and breast cancer cell invasion through METTL7B. BMC Cancer. 2017;17(1):145. [PubMed: 28219369]

29. Bai H, Wang C, Qi Y, et al. Major vault protein suppresses lung cancer cell proliferation by inhibiting STAT3 signaling pathway. BMC Cancer. 2019;19(1):454. [PubMed: 31092229]
30. El Ansari R, Craze ML, Diez-Rodriguez M, et al. The multifunctional solute carrier 3A2 (SLC3A2) confers a poor prognosis in the highly proliferative breast cancer subtypes. Br J Cancer. 2018;118(8):1115–1122. [PubMed: 29545595]
31. Kim J, Lee JH, Iyer VR. Global identification of Myc target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. PLoS One. 2008;3(3):e1798.
32. Volpin V, Michels T, Sorrentino A, et al. CAMK1D Triggers Immune Resistance of Human Tumor Cells Refractory to Anti-PD-L1 Treatment. Cancer Immunol Res. 2020;8(9):1163–1179. [PubMed: 32665263]
33. Lawson J, Dickman C, MacLellan S, et al. Selective secretion of microRNAs from lung cancer cells via extracellular vesicles promotes CAMK1D-mediated tube formation in endothelial cells. Oncotarget. 2017;8(48):83913–83924. [PubMed: 29137392]
34. Mariathasan S, Newton K, Monack DM, et al. Differential activation of the inflammasome by caspase-1 adaptors ASC and Ipaf. Nature. 2004;430(6996):213–218. [PubMed: 15190255]
35. Cariappa A, Takematsu H, Liu H, et al. B cell antigen receptor signal strength and peripheral B cell development are regulated by a 9-O-acetyl sialic acid esterase. J Exp Med. 2009;206(1):125–138. [PubMed: 19103880]
36. Ozcan SC, Sarioglu A, Altunok TH, et al. PFKFB2 regulates glycolysis and proliferation in pancreatic cancer cells. Mol Cell Biochem. 2020;470(1–2):115–129. [PubMed: 32415418]
37. Akaishi J, Onda M, Okamoto J, et al. Down-regulation of transcription elogation factor A (SII) like 4 (TCEAL4) in anaplastic thyroid cancer. BMC Cancer. 2006;6:260. [PubMed: 17076909]
38. Alzrigat M, Parraga AA, Jernberg-Wiklund H. Epigenetics in multiple myeloma: From mechanisms to therapy. Semin Cancer Biol. 2018;51:101–115. [PubMed: 28962927]
39. Ruan S, Zhang H, Tian X, et al. PHD Finger Protein 19 Enhances the Resistance of Ovarian Cancer Cells to Compound Fuling Granule by Protecting Cell Growth, Invasion, Migration, and Stemness. Front Pharmacol. 2020;11:150. [PubMed: 32180719]
40. Mouhieddine TH, Weeks LD, Ghobrial IM. Monoclonal gammopathy of undetermined significance. Blood. 2019;133(23):2484–2494. [PubMed: 31010848]
41. Zhang H, Wang Y, Liu Z, et al. Lymphocyte-specific protein 1 inhibits the growth of hepatocellular carcinoma by suppressing ERK1/2 phosphorylation. FEBS Open Bio. 2016;6(12):1227–1237.
42. Wang L, Guo J, Wang Q, et al. LZTFL1 suppresses gastric cancer cell migration and invasion through regulating nuclear translocation of beta-catenin. J Cancer Res Clin Oncol. 2014;140(12):1997–2008. [PubMed: 25005785]
43. Qi L, Song Y, Chan THM, et al. An RNA editing/dsRNA binding-independent gene regulatory mechanism of ADARs and its clinical implication in cancer. Nucleic Acids Res. 2017;45(18):10436–10451. [PubMed: 28985428]
44. Tremblay-LeMay R, Rastgoo N, Pourabdollah M, Chang H. EZH2 as a therapeutic target for multiple myeloma and other haematological malignancies. Biomark Res. 2018;6:34. [PubMed: 30555699]
45. Potjewyd F, Turner AW, Beri J, et al. Degradation of Polycomb Repressive Complex 2 with an EED-Targeted Bivalent Chemical Degrader. Cell Chem Biol. 2020;27(1):47–56 e15. [PubMed: 31831267]
46. Italiano A, Soria JC, Toulmonde M, et al. Tazemetostat, an EZH2 inhibitor, in relapsed or refractory B-cell non-Hodgkin lymphoma and advanced solid tumours: a first-in-human, open-label, phase 1 study. Lancet Oncol. 2018;19(5):649–659. [PubMed: 29650362]
47. Morschhauser F, Tilly H, Chaidos A, et al. Tazemetostat for patients with relapsed or refractory follicular lymphoma: an open-label, single-arm, multicentre, phase 2 trial. Lancet Oncol. 2020;21(11):1433–1442. [PubMed: 33035457]

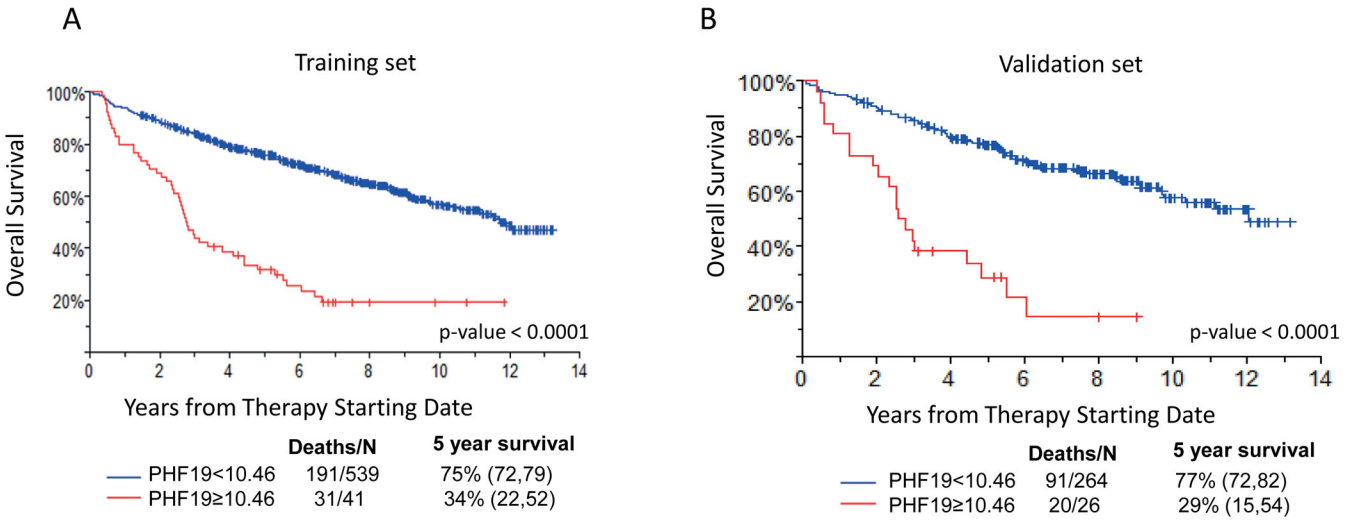

Figure 1.

Patients with high PHF19 expression (≥10.46) have significant worse overall survival compared to patients with low PHF19 expression (<10.46) shown here in the training set, **A,** and validation set **B**.

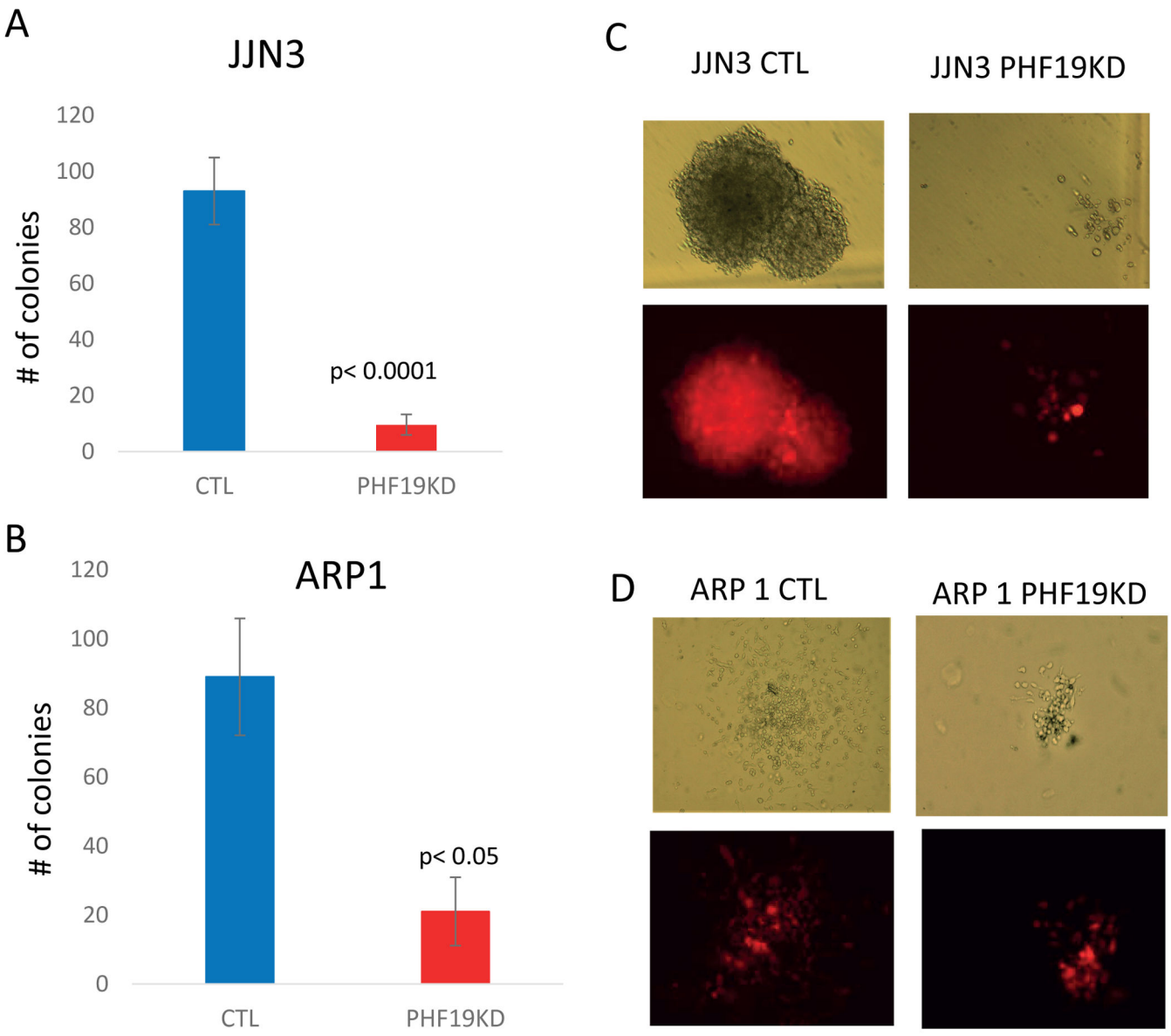

Figure 2.

Colony formation assay shows significant reduction in number of colonies in JJN3 and ARP1 cells with PHF19 KD compared to scrambled control (CTL), **A+B**, $p<0.05$. Furthermore, the colony sizes are both substantially reduced in both cell lines carrying the PHF19 KD compared to the control as seen under light- and fluorescent microscopy, **C+D**.

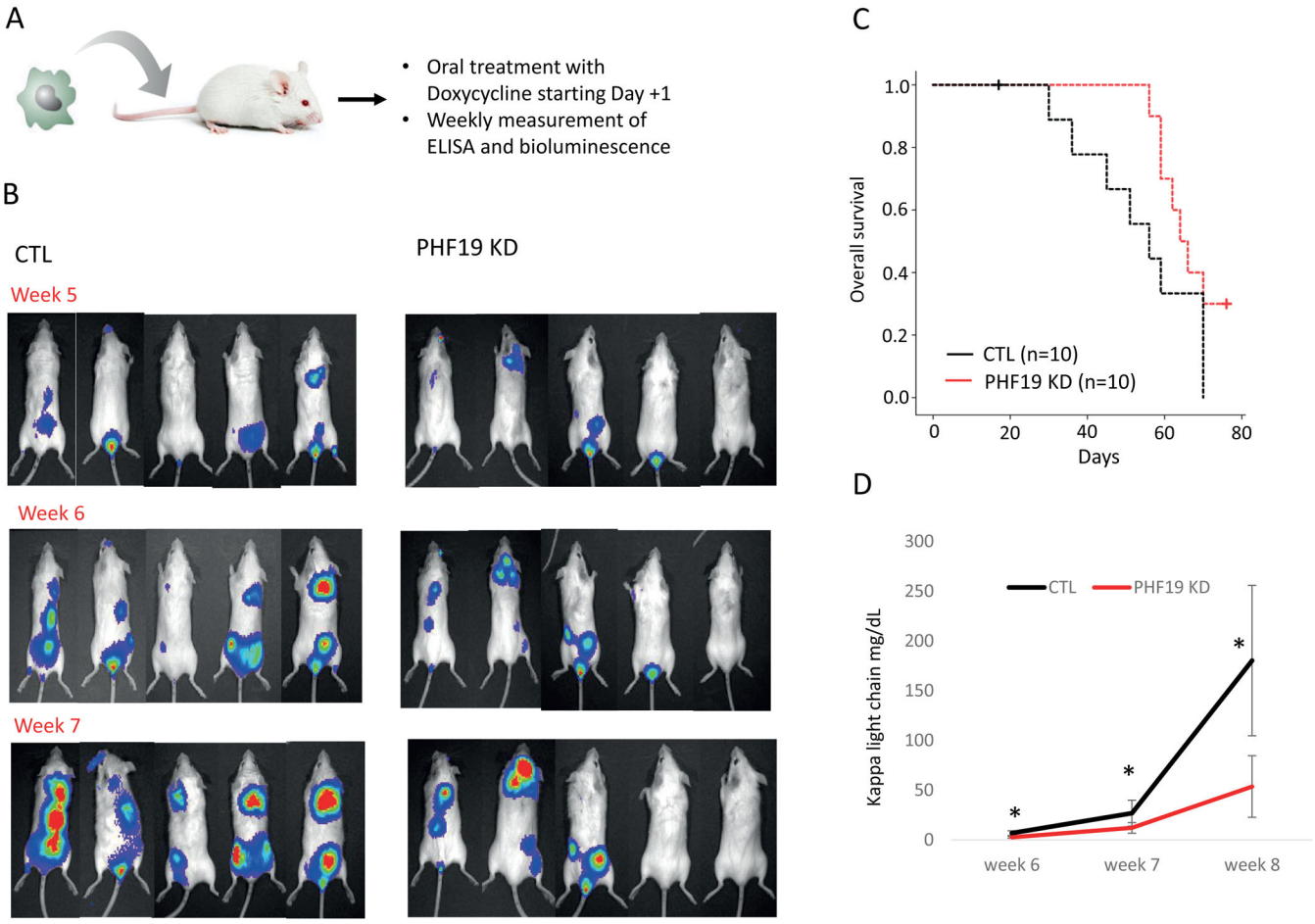

Figure 3.

Schematic overview of the xenograft model using the JJN3 cell line carrying either the PHF19 KD plasmid (n=10) or the CTL plasmid (n=10), **A**. Chemiluminescene imaging of 5 exemplary mice in the CTL vs PHF19KD group show more rapid tumor growth in the control group, **B**. Kaplan-Meier analysis shows that overall survival is worse in the mice with the CTL plasmid compared to the PHF19KD plasmid, **C**, p=0.05. Tumor burden measured by Kappa light chain (mg/dL) was significantly less in the PHF19 KD group compared to the control group, **D**,*= $p<0.05$.

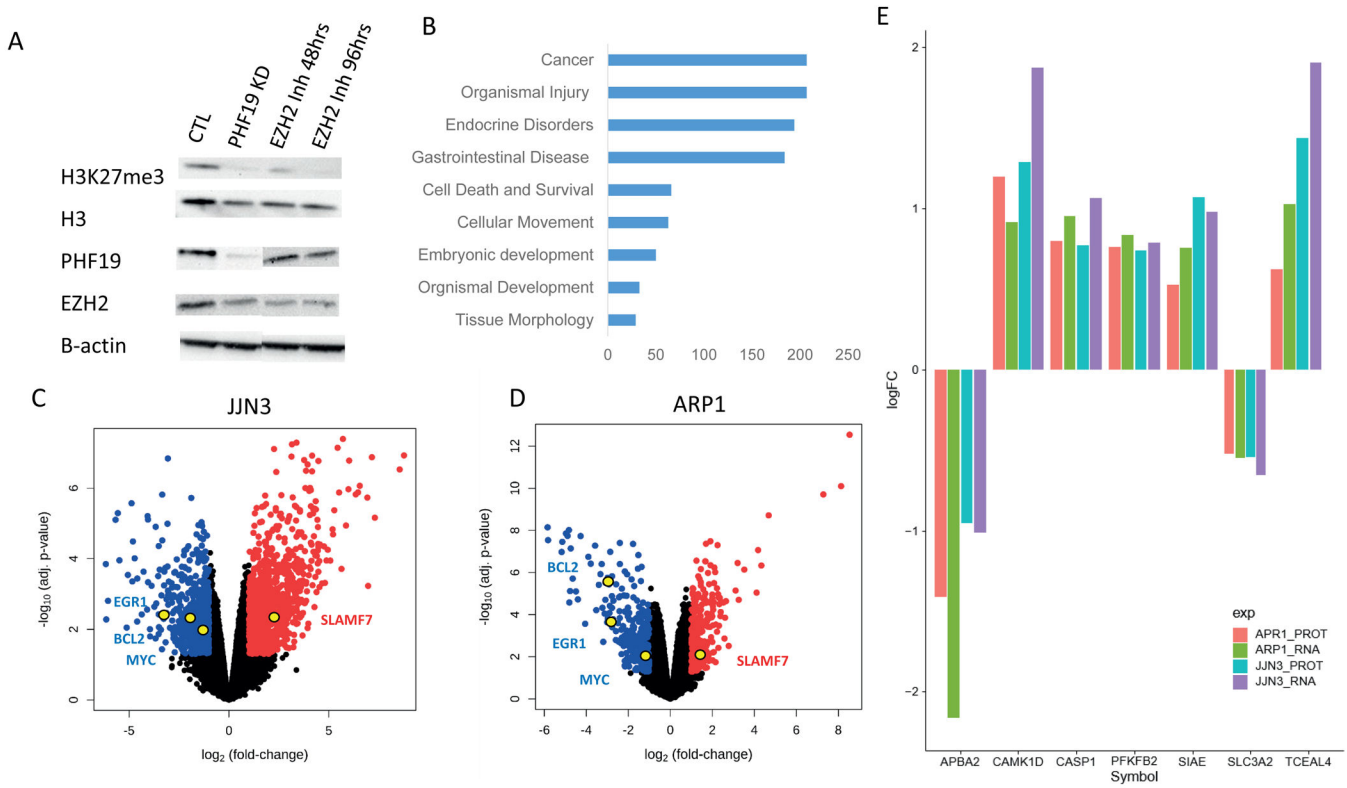

Figure 4.

PHF19 KD lead to a decrease of H3K27me3 compared to scrambled CTL. The EZH inhibitor (Tazemetostat) at a concentration of 1μM was used as a negative control, **A**. A total of overlapping 228 genes were up- or downregulated by PHF19 KD in both cell lines (JJN3 and ARP1) ingenuity pathway analysis (IPA) elucidated pathways that were significantly affected by dysregulation of these overlapping genes after PHF19 KD, **B** $p < 0.0001$. Expression analysis of these overlapping 228 genes in the JJN3, **C**, and ARP1 cell lines, **D**, shows that several genes that are crucial to MM pathophysiology are affected by PHF19 KD, including bcl2, myc, egr1 and slam7. Genes with their corresponding proteins that are up- or downregulated in the same direction after PHF19KD, **E**.

Table 1.

Patient characteristics for PHF19 high and low groups

Factor	All Patients	PHF19 low risk (<10.46)	PHF19 highrisk (≥10.46)	P-value
Age >= 65 yr	249/870 (29%)	224/803 (28%)	25/67 (37%)	0.110
Female	325/870 (37%)	293/803 (36%)	32/67 (48%)	0.071
White	764/870 (88%)	706/803 (88%)	58/67 (87%)	0.748
ISS Stage 1	288/867 (33%)	276/800 (35%)	12/67 (18%)	0.004
ISS Stage 2	344/867 (40%)	321/800 (40%)	23/67 (34%)	0.348
ISS Stage 3	235/867 (27%)	203/800 (25%)	32/67 (48%)	<.001
Albumin < 3.5 g/dL	347/870 (40%)	310/803 (39%)	37/67 (55%)	0.008
B2M >= 3.5 mg/L	468/867 (54%)	421/800 (53%)	47/67 (70%)	0.005
B2M > 5.5 mg/L	235/867 (27%)	203/800 (25%)	32/67 (48%)	<.001
Creatinine >= 2 mg/dL	56/870 (6%)	48/803 (6%)	8/67 (12%)	0.067*
Hb < 10 g/dL	325/870 (37%)	290/803 (36%)	35/67 (52%)	0.010
LDH >= 190 U/L	195/870 (22%)	162/803 (20%)	33/67 (49%)	<.001
GEP70 high risk	129/870 (15%)	81/803 (10%)	48/67 (72%)	<.001

n/N (%): n- Number with factor, N- Number with valid data for factor

ND: No valid observations for factor

*P-value from Fisher's exact test, otherwise chi-squared test.

P-values represent a comparison between groups, not against the overall population.