Unlocking the therapeutic potential of selective CDK7 and BRD4 Inhibition against multiple myeloma cell growth

by Yao Yao, Shuhui Deng, Jessica Fong Ng, Mei Yuan, Chandraditya Chakraborty, Vera Joy Weiler, Nikhil C. Munshi, and Mariateresa Fulciniti

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Unlocking the therapeutic potential of selective CDK7 and BRD4 Inhibition against multiple myeloma cell growth.

Authors: Yao Yao\textsuperscript{1,2,3}, Shuhui Deng\textsuperscript{1,4}, Jessica Fong Ng\textsuperscript{1}, Mei Yuan\textsuperscript{2}, Chandraditya Chakraborty\textsuperscript{1}, Vera Joy Weiler\textsuperscript{1}, Nikhil Munshi\textsuperscript{1,5}, Mariateresa Fulciniti\textsuperscript{1}

Author contributions:
MF designed and conducted the study and wrote the manuscript; YY performed the in vitro and in vivo experiments and analyzed the data; SD, JF, MY, CC and JW helped with the in vitro and in vivo experiments; NM provided critical evaluation of experimental data and manuscript.

\textsuperscript{1}Jerome Lipper Multiple Myeloma Disease Center, Dana-Farber Cancer Institute, Harvard Medical School, Boston, MA, USA; \textsuperscript{2}Blood Disease Institute, Key Laboratory of Bone Marrow Stem Cell, Xuzhou Medical University, Xuzhou, China; \textsuperscript{3}The Affiliated Hospital of Xuzhou Medical University, Xuzhou, China; \textsuperscript{4}State Key Laboratory of Experimental Hematology, National Clinical Research Center for Blood Diseases, Haihe Laboratory of Cell Ecosystem, Institute of Hematology & Blood Diseases Hospital, Chinese Academy of Medical Sciences & Peking Union Medical College, Tianjin 300020, China; \textsuperscript{5}VA Boston Healthcare System, Boston, MA, USA.

Running Title: Targeted CDK7 and BRD4 Inhibition in myeloma.

Correspondence: Mariateresa Fulciniti, Dana-Farber Cancer Institute, 440 Brookline Ave, Boston, MA 02115; e-mail: mariateresa_fulciniti@dfci.harvard.edu

Data-sharing statement:
The data that support the findings of this study are available on request from the corresponding author.

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Abstract

Multiple myeloma (MM) is a plasma cell malignancy considered incurable despite the recent therapeutic advances. Effective targeted therapies are therefore needed. Our previous studies proved that inhibiting CDK7 impairs the cell cycle and metabolic programs by disrupting E2F1 and MYC transcriptional activities, making it an appealing therapeutic target for MM. Given that CDK7 and BRD4 operate in two distinct regulatory axes in MM, we hypothesized that targeting these two complementary pathways simultaneously would lead to a deeper and more durable response. Indeed, combination therapy had superior activity against MM cell growth and viability, and induced apoptosis to a greater extent than single-agent therapy in both cell lines and patient cells. This synergistic activity was also observed in Waldenström’s Macroglobulinemia (WM) cells and with other inhibitors of E2F1 activity. Dual inhibition effectively impaired the MYC and E2F transcriptional programs and MM tumor growth and progression in xenograft animal models, providing evidence for combination therapy’s potential as a therapeutic strategy in MM and WM.
Introduction

Multiple myeloma (MM) is characterized by aberrant cell cycle regulation and enhancer alteration. Dysregulation of the cyclin-dependent kinase (CDK)–RB–E2F axis, which is the core transcriptional machinery controlling cell cycle progression and timing and fidelity of genome replication, is universally observed fostering uncontrolled malignant cell proliferation.

Additionally, besides cell cycle regulators, transcription factors, chromatin-associated factors that mark DNA and histone proteins with heritable, chemical modifications and other essential components of the transcriptional apparatus, are frequently disrupted in MM, manifesting as a notable dependency, as indicated by meta-analyses of pan-cancer genome-wide CRISPR screening data.

Bromodomains (BRDs) are protein interaction modules that specifically recognize ε-N-lysine acetylation motifs functioning as “readers” of the lysine acetylation state. Dysfunction of BRD proteins has been linked to the development of several diseases, including cancer. The bromodomain-containing protein 4 (BRD4) is a member of the bromodomain and extraterminal (BET) family proteins characterized by two N-terminal bromodomains and an extraterminal (ET) domain, which is involved in many processes including transcription elongation and activation of genes involved in cell growth and cell cycle progression. Many studies have been focused on the role of BRD4 in cancer, and BET inhibitors have shown significant activity against various types of tumors. BRD4 positively correlates with disease progression in MM patients and MM cells are sensitive to the treatment with the BRD4 inhibitor prototype JQ1, which causes preferential loss of transcription at super-enhancer-associated genes and induces cell cycle arrest and a senescent phenotype. Additional BET bromodomain inhibitors showed significant efficacy in preclinical MM models.

However, the emergence of intrinsic and acquired resistance to BRD4 inhibitors as well as their unwanted toxicities and side effects hampered their widespread clinical application. These problems may potentially be overcome by combining BRD4 inhibitors with complimentary therapies, enabling lower doses of BRD4
inhibitors and enhancing treatment specificity\textsuperscript{15}.

In our previous investigation, we delineated distinct regulatory axes controlled by E2F1 and BRD4 in MM, with E2F predominately regulating growth/proliferation genes at active promoters and BRD4 mainly affecting enhancer-regulated tissue-specific genes\textsuperscript{16}. Intriguingly, E2F1 genetic depletion potentiated the anti-MM effect of JQ1 \textit{in vitro} and \textit{in vivo} \textsuperscript{16, 17}. We recently uncovered CDK7, a kinase with a dual role in cell cycle progression and transcription\textsuperscript{16}, as a major driver of E2F1 activity in MM\textsuperscript{17, 18}. Its suppression with the small molecule YKL-5-124\textsuperscript{17, 19} indeed exerted therapeutic effects in MM by mitigating cell cycle CDK plasticity and perturbing the E2F1 transcriptional program in an Rb-dependent manner\textsuperscript{17}.

Given that E2F1 and BRD4 operate in two distinct regulatory axes in MM, we hypothesized that simultaneously targeting of E2F1 (via CDK7 or CDK4/6 small molecule inhibitors) and BRD4 (via BET inhibitors) would be more effective at reducing tumor growth compared to single agent. Indeed, the data reported in this study suggest that the combination therapy halts tumor cell growth and viability, offering a promising therapeutic strategy to improve outcomes for patients with MM while minimizing the risk of resistance and toxicity associated with the use of high doses of single agents.

\textbf{Methods}

\textbf{Cells.} The human MM (MM1s, JJN3, OPM2, LR5, ANBL6WT, H929, AMO1, SKMM1, XG1, KMS12BM and IM9) and WM (BCWM1, MWCL1 and RPCIWM1) cell lines were cultured in RPMI-1640 (Gibco or KeyGEN BioTECH) supplemented with 10\% fetal bovine serum (FBS, Gibco or Bio-channel), 2 mmol/L L-glutamine, 100U/mL penicillin, and 100mg/mL streptomycin, with 2.5ng/mL of IL-6 in ANBL6 and XG1 cells.

In accordance with the Declaration of Helsinki and under the oversight of the Ethics Consultation Service at Dana Farber Cancer Institute, primary MM cells and bone marrow mononuclear cells were isolated from BM aspirates of myeloma patients using Ficoll-Hypaque density gradient sedimentation and anti-CD138 microbead separation. Primary WM cells were separated from BM samples of WM patients with
Peripheral blood mononuclear cells (PBMCs) were isolated using Ficoll-hypaque density gradient sedimentation from healthy donors and activated with 20μg/mL PHA (InvivoGen, #inh-phap). Bone marrow stromal cells (BMSCs) were established from mononuclear cells isolated via Ficoll-Hypaque density gradient centrifugation from bone marrow specimens of MM patients, as previously described20, 21.

**Reagents.** YKL-5-124 was a kind gift from Nathanael S. Gray. JQ1, Palbociclib, ARV-825 and I-BET151 were purchased from MedChemexpress (MCE). Compounds were dissolved in dimethyl sulfoxide (DMSO) unless otherwise stated.

**Cell viability, cell cycle and apoptosis assays.** Cell viability was detected by CellTiter-Glo assay (CTG; Promega, G7572) or Cell Counting Kit-8 (CCK8, VICMED). The cell cycle was measured by flow cytometric assay following PI (BD Biosciences, 564907) staining and then analyzed by ModFit software. Apoptosis was evaluated by flow cytometric analysis following APC Annexin-V (BD Biosciences, 561012) and DAPI (BD Biosciences, 564907) staining.

**RNA-seq.** AMO1 cells were treated with YKL-5-124 (50nM) or JQ1 (200nM) alone or in combination for 24 hours. RNA extraction was performed using a RNeasy Plus Mini Kit (QIAGEN, #74136) according to the manufacturer’s instructions, and then subjected to bulk RNA sequencing. RNA-seq analysis was performed using the HG19 ERCC human reference genome with HG19 ERCC gene annotations. Gene set enrichment analysis (GSEA) was performed via the computational platform of the Broad Institute with Hallmark gene signature.

**Murine xenograft model of human MM or WM.** SCID mice were purchased from Charles River Laboratories. All animal studies were approved by and conducted according to the protocols of the Animal Ethics Committee of the Dana-Farber Cancer Institute. These mice were irradiated by 200 cGy and then inoculated subcutaneously in the right or left flank with 5×10⁶ MM or WM cells. Tumor growth was measured in two dimensions by caliper, and volume was calculated using the formula: \( V = 0.5 \times a \times b^2 \), where “a” and “b” represent the length and width of the tumor. The weight of the animal body was measured every week throughout the study to monitor the toxicity of
drugs.

**Statistical analysis.** All values are displayed as mean ± the standard deviation (SD). The significance of differences between experimental variables was analyzed using the Student’s t-test or ANOVA for multiple comparisons with Prism GraphPad software. The significance of the *P* value is *< 0.05, ** < 0.01, and *** < 0.001.

**Results**

**Dual E2F1 and BRD4 inhibition leads to synergistic anti-myeloma effects.** We evaluated the effect of dual inhibition of CDK7 and BET bromodomain proteins with low doses of YKL-5-124 and JQ1, respectively, in an extensive array of cell lines (n=11), to account for the molecular heterogeneity of MM cells and their varying response to therapy. We observed superior activity of the combination against all MM cell lines tested compared to single perturbation alone ([Figure 1A](#) and [Figure S1A](#)). Isobologram analysis revealed strong synergism of the combination as compared to single agents, with a combination index (CI) <1.0 at all tested doses ([Figure 1B](#)). On the other hand, PBMCs from healthy donors were less sensitive to the combination therapy ([Figure S1B](#)). We also tested the effect of the combination in XG1 MM cells, which have intrinsic resistance to both YKL-5-124 and JQ1 and confirmed dual inhibition to be synthetic lethal in these cells ([Figure 1C-D](#) and [Figure S1C](#)). These findings were validated with two additional BET inhibitors: ARV-825 and I-BET151 ([Figure 1E-F](#)).

Consistent with YKL-5-124 being a CDK7 inhibitor, we observed reduced phosphorylation of CDK4 and increased activation of Rb in both YKL-5-124 single agent and combination therapy ([Figure 2A-B](#)); early cell cycle arrest, with a higher percentage of cells in the G1 phase than with either agent alone ([Figure 2C](#)) and a moderate increase in apoptotic cell death and cleavage of caspase 3 ([Figure 2D and Figure S1D](#)).

In addition, we tested the combination of JQ1 with the FDA-approved CDK4/6 inhibitor Palbociclib, confirming its anti-MM effect compared to single drug administration ([Figure S1E](#)). The combination of low doses of JQ1 and Palbociclib was indeed synergistic with isobolographic analysis revealing a strong synergism in
most MM cell lines (**Figure S1F**). This synergism between BRD4 and cell cycle inhibitors was also observed in other cancer contexts ^22-24^.

We next assess if this combination regimen could be applicable to additional hematological malignancies, such as Waldenström’s Macroglobulinemia (WM). We observed that WM cells are more sensitive to the inhibition of CDK7 than BET (**Figure S2A**). Indeed, CDK7 inhibition robustly reduced the viability of WM cell lines (n=3) and primary WM cells in a dose- and time-dependent manner (**Figure S2B**), impairing E2F, MYC and cell cycle–associated gene set signatures, as observed in MM cells (**Figure S2C**). Moreover, combination treatment effectively killed WM cell lines and primary cells from patients, albeit the effect was greater in MM cells (**Figure S2D-E**).

**Transcriptomic analysis in MM with JQ1 and YKL-5-124 combination treatment.**

To gain deeper insight into the molecular basis of the observed drug synergism, we performed gene expression profiling after a 24h treatment with 200nM JQ1 and 50nM YKL-5-124 in the AMO1 cell line. RNA-seq analysis of cells treated with either a low dose of YKL-5-124 or JQ1 or a combination showed that, in general, combination therapy had a more profound impact on the transcriptome than single-agent therapy (**Figure 3A**). Canonical pathway analysis revealed that the upregulated pathways were enriched in pathways for autophagy and senescence, whereas cell cycle regulation, glycolysis, the unfolded protein response, and cholesterol biosynthesis were enriched among the downregulated pathways (**Figure 3B**). Moreover, analysis of the predicted transcriptional regulators using IPA software found that FOXM1, BRD4, E2F and MYC were among the inhibited regulators and CDKN1A(p21), TP53 and Rb were among the activated regulators (**Figure 3C**).

In line with this result, we observed a pronounced decrease in the expression of MYC upon combination therapy (**Figure 3D**). Moreover, dual targeting synergistically decreased the expression of additional genes important to the MM oncogenic state, including MYBL2 (b-Myb) (**Figure 3E**). MYBL2 is a cell-cycle and proliferation
gene frequently overexpressed in several cancers: here, we found MYBL2 to be a dependency in a panel of MM cell lines (Figure S3A), with its high expression associated with poor patient outcome in the MM patient datasets (Figure S3B-C).

**CDK7 inhibition synergizes with JQ1 against MM cells ex vivo and in vivo.** We finally tested the therapeutic potential of the tested drug combinations. We first confirmed the strong synergism observed in primary MM cells, where a combination index (CI) <1.0 was found at all tested doses, with the effect being considerably less significant in PBMCs derived from MM patients (Figure 4A). This was also confirmed in primary cells treated with a combination of JQ1 and Palbociclib (Figure S4A-B). We next evaluated the effect of the combination in the context of the bone marrow milieu. The combination of low doses of YKL-5-124 and JQ1 had a striking effect in inhibiting the proliferation of MM cells cultured for 24 hours with supernatant from BMSCs derived from MM patients (Figure 4B). Similarly, the combination regimen had a significant impact on the viability of primary CD138+ MM cells cultured in the presence of their bone marrow microenvironment, while CD138- bone marrow cells derived from the same patient were unaffected (Figure 4C). Altogether these data suggest a favorable therapeutic index.

The synergistic effects of combining YKL-5-124 with JQ1 in vitro prompted us to assess the efficacy of this combination in two in vivo models of MM. SCID mice were injected subcutaneously with MM1s or H929 cells. When tumors reached 100mm^3, mice were randomized to vehicle or a low dose of JQ1 (50mg/kg) or YKL-5-124 (2.5mg/kg) or combination treatment for 2 weeks. Combination therapy resulted in smaller tumors compared to those for single agents (Figure 4D and Figure S4C), without evident toxicity or weight loss (Figure 4E). This pattern was also noted in vivo when JQ1 was combined with Palbociclib, although it didn't achieve statistical significance, likely due to the limited sample size. (Figure S4D).

**Discussion.**

Alterations (loss- or gain-of-function) in factors and enzymes that control
transcription and genome stability are frequently associated with transformation, contributing to the “transcriptional addiction” observed in cancer. Myeloma cells are characterized by enhancer alterations, such as Ig enhancer translocations to key drivers including CCND2, MAF, MYC, and MMSET; and chromatin modifiers have been successfully exploited as novel targets against myeloma cells \(^1, 25-29\). Indeed, both genetic and pharmacological interventions aimed at BRD4 yield anti-myeloma effects in preclinical models\(^11, 13\). However, despite being a promising target in preclinical models with known c-MYC dependency, monotherapy trials assessing the efficacy and safety profile of BET inhibitors in MM fell short of expectations. In the OTX015 clinical trial, none of the 12 MM patients exhibited activity, with only two (17%) experiencing stable disease\(^13\). Moreover, BET inhibitors demonstrated a relatively high incidence of treatment-emergent adverse events, with thrombocytopenia, nausea, diarrhea, and fatigue ranking as the top four adverse events (AEs) of all grades\(^14\).

One of the main challenges of monotherapy is the potential for cancer cells to develop resistance over time. Cancer cells, including myeloma cells, can adapt and evolve, often rendering single-agent treatments ineffective in the long term. Additionally, targeting a single pathway may not be sufficient to completely halt tumor growth, as cancer cells can utilize alternative pathways to bypass the effects of the inhibitor. The therapeutic application of cell cycle kinases in MM, for example, has generally encountered obstacles due to a lack of efficacy as single agents, suggesting that targeting cell cycle regulation is insufficient to elicit a sustained response in MM\(^28\). This aligns with our investigations into YKL-5-124, a specific CDK7 inhibitor\(^19\), in MM cells, where we observed that selective perturbation of CDK7 activity alone was insufficient to consistently downregulate most super-enhancer-driven gene expression programs in MM cells.

We therefore tested whether combining inhibitors for CDK7 and BET bromodomain proteins would overcome each of their limitations as single agents. We found that low doses of YKL-5-124 and JQ1, respectively, proved highly synergistic and synthetically lethal in MM and WM cells, including in resistant settings. This drug combination robustly inhibited cell proliferation and triggered apoptosis. We also demonstrate that
the synergistic cytotoxicity observed *in vitro* translates into anti-tumor effects *in vivo*, supporting the therapeutic potential of targeting CDK7 and BRD4 in combination in MM. In line with our findings, recent reports have documented synergistic effects between JQ1 and THZ1 (a CDK7 inhibitor with additional inhibitory activity toward CDK11 and 12) against glioma, MYCN-amplified neuroblastoma and medulloblastoma\(^2\). Notably, the combination of CDK7 inhibition with JQ1 exhibited more pronounced antiproliferative activity compared to JQ1 combined with CDK4/6 inhibition. By targeting multiple nodes within the cell cycle machinery, CDK7 inhibition likely exerts a more comprehensive blockade of cell cycle progression and may more effectively bypass the plasticity and compensatory/adaptation mechanisms observed with CDK4/6 and CDK2 inhibitors\(^3\).

Recent analysis of CRISPR screen data in breast cancer has shown that deletion of positive regulators of G1-S progression (e.g., *CCND1* and *CDK4*) sensitizes cells to JQ1, whereas deletion of negative regulators (e.g., *RB1* and *CDKN1A*) increases JQ1 resistance\(^3\). Given that YLK-5-124 indirectly inhibits a positive regulator (CDK4) and activates a negative regulator (Rb), it effectively increases the sensitivity to JQ1 and may overcome the acquired and intrinsic resistance, as shown in the XG1 MM cells. This delicate balance likely underpins the observed synergistic cell death elicited by the combination therapy.

In summary, our study elucidates several mechanisms contributing to the heightened efficacy of the combination therapy in our MM system. These mechanisms include CDK7-mediated G1 arrest and activation of Rb, alongside the modulation of distinct and overlapping super-enhancer dependencies. Overall, our findings provide compelling preclinical evidence supporting the therapeutic strategy of targeting CDK7 in combination with epigenetic therapies relying on BRD4 inhibition for the treatment of patients with MM and WM.
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Figure Legends

Figure 1. Enhanced Suppression of multiple myeloma cell proliferation through dual inhibition strategy. A-B. A panel of 11 multiple myeloma cell lines were treated with YKL-5-124 or JQ1 alone or in combination, and cell survival was assessed by CTG uptake assay. Data from a representative cell line (MM1S) is shown as % of cell viability compared to untreated cells in (A). Mean ± SD are shown; n=3 (A). Synergism analysis was performed with the Calcusyn software. Combination Index (CI) = 1 means additive effect, CI < 1 means synergistic effect, and CI > 1 means antagonistic effect in (B). *** p< 0.001 and **** P< 0.0001. C-D. XG1 cells were cultured in the presence of different concentrations of YKL-5-124 with or without JQ1 for 72h, and cell survival was assessed by CTG uptake assay. Data are presented as % of cell viability compared to untreated cells. Mean ± SD are shown in (C); n=3. Synergism analysis was performed with the Calcusyn software and shown in (D). * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001. E-F. IM9, RPMI8226 and H929 were cultured in the presence of BRD4 inhibitor ARV-825 (E) or BRD4 degrader I-BET151 (F) with or without YKL-5-124. Cell viability was assessed by CCK8 assay. Data represent mean±SD; n=3. * p < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001.

Figure 2. Enhanced Suppression of multiple myeloma cell proliferation via cell cycle arrest and apoptosis. A-B. AMO1 cells were treated with 100nM YKL-5-124 or 100nM JQ1 alone or in combination for 24 hours, and WB analysis was performed using indicated antibodies against p-RB (s780, s795 or s801/811), RB, p-CDK4, and CDK4, with Tubulin as a loading control (left panel). The ratio of phosphorylation of RB/Tubulin (A) and p-CDK4/CDK4 (B) were analyzed by Image J software (right panel). C. Multiple myeloma cells were treated with 50nM YKL-5-124 or 50nM JQ1 alone or in combination for 24 hours. Cell cycle was evaluated by flow cytometric analysis following propidium iodide (PI) staining and analyzed with ModFit LT 5.0 software. Data represents mean of data obtained from n=2 cell lines (MOLP8 and SKMM1). D. MOLP8 and SKMM1 cells were treated with 50nM YKL-5-124 or 50nM JQ1 alone or in combination for 48 or 72 hours. Apoptotic cell death was assessed by flow cytometric analysis following Annexin V+/PI staining.

Figure 3. JQ1 and YKL-5-124 combination treatment induces transcriptomic alterations in multiple myeloma cells. A. AMO1 cells were treated with 200nM JQ1 and 50nM YKL-5-124 for 24 hours individually and in combination and then
subjected to RNA-seq analysis. Venn diagram showing overlap of genes in these three groups (Log2FC>1, Adjusted p <0.05 or Log2FC<1, Adjusted p <0.05). B. Canonical pathway analysis was done using ingenuity pathway analysis (IPA). Top predicted negative canonical pathways are shown in bubble plot. C. Biological upstream regulators associated with CDK7 and BET inhibition were identified using IPA. Top predicted upstream regulators for RNA-seq data in YKL-5-124 & JQ1 combination group is shown in table. (red= activated, green=inhibited). D. SKMM1 and XG1 cells were treated with 100nM JQ1 and 100nM YKL-5-124 for 24 hours individually and in combination, and WB analysis was performed using antibodies against MYC and Tubulin. E. MYBL2 log2 fold change following treatment with single agent or combination regimen compared to DMSO control.

**Figure 4. Therapeutic potential of combination therapy ex vivo in primary cells and in vivo in a humanized myeloma mouse model.** A. CD138+ primary cells and PBMCs were cultured in the presence of different concentrations of YKL-5-124 with or without JQ1 for 24h and cell survival was assessed by CTG uptake assay. Data are presented as synergy (combination index-CI) and cell death (Fraction affected-FA). B. Multiple myeloma cells were cultured with and without supernatant from primary BMSC cultures obtained from BMMNC from two myeloma patients, in the presence of YKL-5-124 or JQ1 alone and in combination for 24 hours. Data represents the mean value ±SD of one experiment performed in triplicate. * p <0.05, *** p < 0.001 and **** p < 0.0001. C. BMMNC cells from one myeloma patient were cultured in the absence or presence of 50nM YKL-5-124 or 100nM JQ1 alone and in combination for 24h. Cell viability of both CD138+ and CD138- cells was assessed and expressed as percentage of live cells compared to untreated cells. ** p <0.01 and *** p <0.001. D-E. Sub-lethally irradiated SCID mice were injected subcutaneously with MM1S cells. Mice were randomized to receive YKL-5-124 (2.5 mg/kg, i.p, 5 days/week), JQ1 (50mg/kg, 2 days/week) or a combination for 2 weeks. Tumor volume was evaluated by caliper measurement. Fold change increase of tumor volume compared to start of treatment (Day 1) (mean +/- SD) (D) and body weight (E) and are shown. p-values indicate significant differences between groups. * p <0.05, ** p <0.01 and **** p < 0.0001.
Figure 1: Cellular viability assays with varying concentrations of YKL-5-124 and JQ-1. The graphs show the combination index (CI) and the effect of YKL-5-124 and JQ-1 on cell viability compared to controls.
Figure 2

A. AMO1

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C. G2M, S, G1

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D. MOLP8, SKMM1

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Figure 2
Figure 3
Supplemental Methods

Western Blot. Treated MM cells were lysed using RIPA lysis buffer (Boston Bio Products, #BP-115) supplemented with a protease inhibitor cocktail (Thermo Fisher Scientific, #78440). Protein concentrations were measured by the BCA Protein Assay Kit (Thermo Fisher Scientific, #23227) and separated by SDS-PAGE. The following primary antibodies were employed in this study: Rb (sc-73598), phospho-Rb (s780) (CST, #8180), phospho-Rb (s795) (CST, #9301), phospho-Rb (s807/811), p-CDK4 (ABclonal, AP-0593), CDK4(CST, #12790), Cleaved-caspase3 (CST, #9661), MYC (sc-40), and Tubulin (CST, #2128).

Supplemental Figure Legends.

Figure S1. Dual inhibition synergistically inhibits MM cell growth. A. A panel of 10 MM cell lines was treated with YKL-5-124 or JQ1 alone or in combination, and cell survival was assessed by CTG uptake assay. Data are shown as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. B. PBMCs from healthy donors were treated with different concentrations of YKL-5-124 or JQ1 alone or in combination for 72 hours, and cell viability was assessed by CTG uptake. Data are presented as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. C. XG1 cells were cultured in the presence of different concentrations of YKL-5-124 with or without 50nM JQ1 for 72h, and apoptotic cells were assessed by flow cytometric analysis following Annexin V+/PI staining. D. H929 and JJN3 cells were treated with 100nM YKL-5-124 or 100nM JQ1 alone or in combination for 24 hours, and WB analysis was performed using indicated antibodies against cleaved caspase3, with Tubulin as a loading control. E-F. A panel of MM cells was treated with a low dose of Palbociclib or JQ1 alone or in combination, and cell viability was assessed by CTG. Data from a representative panel of cell lines are shown as % of cell viability compared to untreated cells (E). Data represent mean±SD; n=3. Synergism analysis was performed with the CalcuSyn software (F). The gray area delineates potent synergistic combinations. CI = 1 means additive effect, CI < 1 means synergistic effect, and CI > 1 means antagonistic effect.

Figure S2. Targeting CDK7, alone and in combination, halts WM cell growth. A. Three WM cell lines were treated with different doses of YKL-5-124 for 24-72 hours, and cell growth was assessed by CTG assay. Data represent mean ± SD; n=3. Data from these WM cells are shown as % of cell viability compared to untreated cells. B. Primary WM cells from patients were treated with low doses of YKL-5-24 for 24 hours, and cell
growth was assessed by CTG assay. Data from these WM cells were shown as % of cell viability compared to untreated cells. Data represent mean ± SD; n=3. **p <0.01 and **** p <0.0001. C. RPCIWM1 cells were treated with DMSO or YKL-5-124 for 24h, and then subjected to RNA-seq analysis. Gene Sets Enrichment Analysis of differentially expressed genes using Hallmark gene set in panel. D-E. BCWM1 and MWCL1 were treated with low doses of YKL-5-124 and JQ1 for 72 hours, and cell growth was assessed by CTG uptake assay (D). Data was shown as % of cell viability compared to untreated cells. Data represent mean±SD; n=3. Synergism analysis was performed with the CalcuSyn software (E). Data represent the average over 3 replicates. CI = 1 means additive effect, CI < 1 means synergistic effect, and CI > 1 means antagonistic effect.

**Figure S3. MYBL2 is oncogenic in MM.** A. CRISPR screen score (CSS) for MYBL2 in CRISPR-Cas9 KO screening in 16 MM cell lines. B-C. Correlation of MYBL2 with overall survival (OS) or event-free survival (EFS) in the MMRF CoMMpass (B) and GSE2685 patient datasets (C).

**Figure S4. Therapeutic potential of combination therapy ex vivo and in vivo.** A. Primary MM cells from patients were treated with a low dose of Palbociclib and JQ1 for 24 hours, and cell viability was assessed by CTG uptake assay. Data represent mean ±SD; n=3. ***p < 0.001 and ****p < 0.0001. B. PBMCs and primary MM cells were treated with a low dose of Palbociclib and JQ1 for 24 hours, and cell viability was assessed by CTG assay. Data represent mean±SD; n=3. C. Sub-lethally irradiated SCID mice were injected subcutaneously with H929 cells. Mice were randomized to receive YKL-5-124 (2.5 mg/kg, i.p, 5 days/week), JQ1 (50mg/kg, 2 days/week) or a combination for 2 weeks. Tumor volume was evaluated by caliper measurement. Fold change increase of tumor volume compared to start of treatment (Day 1) (mean +/- SD) are shown. p-values indicate significant differences between groups. * p <0.05. D. Sub-lethally irradiated SCID mice were injected subcutaneously with H929 cells. Mice were randomized to receive Palbociclib (25 mg/kg, i.p, 3 days/week), JQ1 (50mg/kg, 2 days/week) or a combination for 2 weeks. Tumor volume was evaluated by caliper measurement. p-values indicate significant differences between groups. *p<0.05.
**Figure S1**

**A**

Cell Viability (%) compared to control

YKL-5-124 (nM)

**B**

Cell Viability (%) compared to control

YKL-5-124 (nM)

**C**

% of Annexin V + cells

**D**

JQ-1 (nM) 0 100 0 100 0 100 0 100 0 100 0 100

YKL-5-124 (nM) 0 0 100 100 0 0 100 100 0 0 100 100

Cleaved caspase3

β-tubulin

**F**

Combination Index (CI)

Fa (growth inhibiton)
Figure S2
A. MYBL2 KO: CSS

B. CoMMpass

C. GEO2658

Overall survival probability

Follow up in months

Figure S3
Figure S4