RESEARCH PLAN A. Specific Aims

Multiple myeloma (MM) is one the most common hematologic malignancy that accounts for about 13% of all hematologic malignancies and 1% of overall cancer. Despite advances in treatment strategies, MM is still incurable and the lack of reliable biomarkers to predict development and better prognosis for further treatment is a critical barrier. MM disease progression begins with the transformation of normal plasma cells that develops into asymptomatic condition monoclonal gammopathy of undetermined significance (MGUS), then smoldering multiple myeloma (SMM), and finally into aggressive malignant MM. However, the molecular mechanisms underlying malignant evolution has not yet been delineated. Additionally, current RNA sequencing strategies were developed using bulk samples from primary tumors thereby lacking the resolution and accuracy for characterizing small tumorigenic subpopulations that are possibly driving MM. Long non-coding RNAs (lncRNAs) lack coding potential, have been shown to bind to proteins to regulate genes, but yet have been explored in MM tumorigenesis. Due to lncRNA tissue specificity, they show promise as both prognostic and diagnostic biomarkers. Therefore, elucidating their function and assessing their potential clinical applicability could significantly alter the management of cancer. **The long-term goal of my laboratory is to understand how lncRNAs promote the progression of MM for creation of better biomarkers for prognosis, to improve diagnostics, and development of novel therapies**. To address the critical knowledge gap of the malignant evolution of MM, we analyzed single-cell RNA sequencing (scRNA-Seq) data focusing on plasma and B cells taken from normal, MGUS, SMM, and MM patient samples including a validation cohort derived from MM patient samples from the Multiple Myeloma Research Foundation CoMMpass Study. We identified five differentially expressed lncRNAs comparing normal to MGUS samples, nine lncRNAs comparing MGUS to SMM samples, 26 lncRNAs comparing SMM to MM, and 25 lncRNAs comparing normal to MM, (27 unique lncRNAs) which we term *Multiple Myeloma Progression associated lncRNAs (MMPals)*. Our studies focused on *MMPal1,* known as *NEAT1,* as it was the most differentially expressed lncRNA in MM progression showing higher expression in MGUS and MM compared to normal. We silenced expression of *MMPal1* in MM cells and saw a decrease in proliferation and cell viability. To determine if *MMPal1* is associated with drug resistance, we treated cells with Melphalan, a standard treatment commonly used followed by autologous stem cell transplant for transplant-eligible patients with MM, and found Melphalan sensitive MM1.S cells showed less *MMPal1* expression when compared to Melphalan resistant U266B1 cells. We next assessed if *MMPal1* binds to Chromobox 4 (CBX4) protein, due to its similar cellular location in nuclear speckles, epigenetic regulation, and known binding to lncRNAs. We indeed detect that *MMPal1* binds to CBX4 in MM cell lines and Melphalan treated U266B1 resistant cells. *Our preliminary data serves as strong rationale for our hypothesis that MMPal1 may play a role as a master epigenetic regulator to promote MM progression and lncRNAs can be utilized as biomarkers for prognosis of MM disease progression.*
We will pursue our hypothesis with the following aims:

Aim 1 Validate the expression of MMPal1 as a prognostic biomarker. We will perform multiplexed Fluorescent RNA *In situ* Hybridization (mFISH) of *MMPal1* in a larger cohort of 12 normal and 24 patient bone marrow aspirates each from MGUS, SMM, and MM patient samples. This aim will validate our single-cell RNA sequencing data and provide evidence of *MMPal1* as a potential genetic biomarker.
Aim 2 Characterize MMPal1-dependant CBX4 regulation. First, we will generate a U266B1 *MMPal1* CRISPR/Cas9 knock-out (CRISPR KO) cell line to validate *MMPal1* function. Second, we will characterize changes in gene promoter occupancy of CBX4 upon *MMPal1* interaction in U266B1 wild type, *MMPal1* CRISPR KO, and Melphalan treated cells via chromatin immunoprecipitation sequencing and assess expression of gene targets via RNA sequencing. This aim will expand our knowledge of how aberrant *MMPal1* expression alters epigenetic regulation to promote MM progression.
Aim 3 Identify functionally important lncRNAs associated with promoting MM. A CRISPR interference (CRISPRi) platform targeting 16,401 known lncRNAs will be used to assess a genome-wide view of lncRNA cell viability, cytotoxicity, and apoptosis of sgRNA transduced U266B1 GFP-luciferase cells. DNA isolated from transduced cells will be sequenced to identify specific sgRNAs causing loss of function. The top two identified lncRNAs will be validated using siRNAs. This aim will identify the top functionally important lncRNAs associated in MM.

Overall this proposal will provide new molecular insight of lncRNAs and specifically *MMPal1* as a genetic biomarker to predict MM progression in selected populations and thus help to improve survival and longevity. Accomplishment of the proposed aims will provide the critical evidence and clinical significance for these lncRNAs in MM that will position us well for future *in vivo* work. In the long-term, this work has the potential to impact patient care by revealing novel targets for cancer therapy.

B. Scope of work and relevance of project

Despite advances in understanding the genetic progression of MM the molecular mechanisms remain poorly characterized. Identifying new biomarkers that may diagnose cancer earlier at pre-malignant states including prognosis is essential. However, the inter-patient heterogeneity and cell heterogeneity in MM makes it increasingly difficult to identify new biomarkers. LncRNAs tissue specificity and important biological functions show promise as potential new prognostic and diagnostic biomarkers. By taking advantage of scRNA sequencing (scRNA-Seq), we can determine if lncRNAs can serve as biomarkers and predict the progression of MM to improve survival and longevity.

Innovation

This proposal includes a number of unique and innovative features that increase the overall significance of the project:

1. Our preliminary data represents the first comprehensive lncRNA discovery effort in MM disease progression using scRNA-Seq of normal, MGUS, SMM, and MM.
2. This proposal will further characterize *MMPal1*, which we previously found to: (i) show increasing expression comparing normal to MGUS, SMM to MM, and normal to MM, (ii) has specific expression in Mature B cell neoplasm cells and is highly expressed in MM cancer cell lines, (iii) decreases proliferation and viability, (iv) is more highly expressed in Melphalan resistant cell lines, and (v) binds to CBX4 in Melphalan treated U266B1 resistant cells.
3. We will evaluate *MMPal1* as a potential biomarker of progression-free survival using difficult to obtain tissue samples from patients with MM progression which in the future can help clinicians strategize treatment to best improve MM survival and hence longevity.
4. We are the first to use a lncRNA CRISPRi genome-scale screen in MM to explore only functionally relevant lncRNAs.

Timeline outlined to achieve clinical application: One strength of our proposal is the use of scRNA-Seq data to identify lncRNAs that play a role in promoting MM progression. Our collaborations with Dr. Ravi Vij (Director of Multiple Myeloma Tissue bank) gives us access to more than 2,000 patient samples with associated clinical information, of which 84 will be used in this proposal as a validation cohort. The results generated in this proposal will spur subsequent clinical translation as we envision next targeting these lncRNAs directly with a new generation of antisense oligonucleotides[1,2] (ASOs), which have shown increasing promise based on recent successful human clinical trials. Further, using a global lncRNA knockout screen gives us an advantage to identify functionally important lncRNAs. These strengths give us an estimated timeline of only about two to three years for *in vivo* validation and use of lncRNAs as new biomarkers for diagnosing patients who may progress to malignant MM. Importantly, we have preliminary evidence to suggest that *MMPals* have been found to be deregulated in other cancer types thereby broadening the impact of this study across a much larger patient population.

C. Background and significance

Multiple myeloma (MM), an incurable malignancy of mature plasma cells, is the second most common hematologic malignancy accounting for ~13% of all hematologic malignancies and 1% of overall cancer. MM is always preceded by a premalignant phase called monoclonal gammopathy of undetermined significance (MGUS) and can then progress to smoldering multiple myeloma (SMM) and/or malignant MM. Although survival of MM patients has improved with new treatments, most patients suffer fatal relapse. The lack of a basic understanding of mechanisms and reliable biomarkers to predict which MGUS patients will develop MM is a critical barrier. In addition, interpatient heterogeneity, use of microarrays, and bulk RNA sequencing have prevented the identification of the molecular mechanisms that control malignant progression of MM plasma cells[3-6]. Long non-coding RNAs (lncRNAs) have shown to be deregulated in MM[4,7], however these studies have not yet studied lncRNAs in MM progression. lncRNAs are longer than 200 base pairs, are non-protein coding, and are transcribed by RNA polymerase II[8]. The GENCODE consortium and others have estimated there are 15,000-23,000 unique lncRNAs, many of which have yet to be characterized[9]. The few lncRNAs that have been characterized have been shown to have regulatory functions by binding to proteins[10,11]. Our previous work has proven these biological functions to play important roles in promoting tumorigenesis, metastasis, and drug resistance in metastatic colon and late stage relapse breast cancer[12-18]. Further, due to lncRNAs tissue specificity, they have shown promise as both prognostic and diagnostic biomarkers[19-22]. **Therefore, this proposal will validate and characterize our identified Multiple Myeloma Progression associated lncRNA1 (*MMPal1*) in patient samples, determine its mechanism, and identify all functional**

lncRNAs in MM that may play significant roles in altering the management of MM and serve as potential prognostics and diagnostics to improve survival and longevity.

D. Preliminary data

Discovery of Multiple Myeloma progression associated lncRNAs (MMPals)

We analyzed scRNA-Seq data from two cohorts to identify deregulated lncRNAs. The first cohort included data from a previous study by Ledergor et. al[23] containing four healthy control normal samples isolated from hip, seven MGUS and six SMM patients asymptomatic samples, and 12 MM patient symptomatic samples (>23,000 plasma cells)[23]. Figure 1 shows our scRNA-Seq analysis pipeline which begins by normalizing data, filtering low reads, removing dead cells, and mitochondrial genes. Next, we subset only Plasma and B cells, since these are the cells derived from MM, then compare individual patient samples by disease stage (p-value < 0.0005). These stringent parameters identify specific Plasma and B cell associated lncRNAs from differential expression that are not caused by differences in lncRNA tissue specificity, cell type abundance, or cellular state; an advantage of using scRNA-Seq data for this study. To increase our sample population, we included a second cohort of scRNA-Seq data of 18 CD138- sorted samples (>18,000 total cells) from the Multiple Myeloma Research Foundation (MMRF) CoMMpass Study through our collaboration with Dr. Li Ding. We identified five differentially expressed lncRNAs comparing normal to MGUS samples, nine lncRNAs comparing MGUS to SMM samples, 26 lncRNAs comparing SMM to MM, and 25 lncRNAs comparing normal to MM. We term the 27 unique lncRNAs, *Multiple Myeloma Progression associated lncRNAs (MMPals,* Figure1*)*.

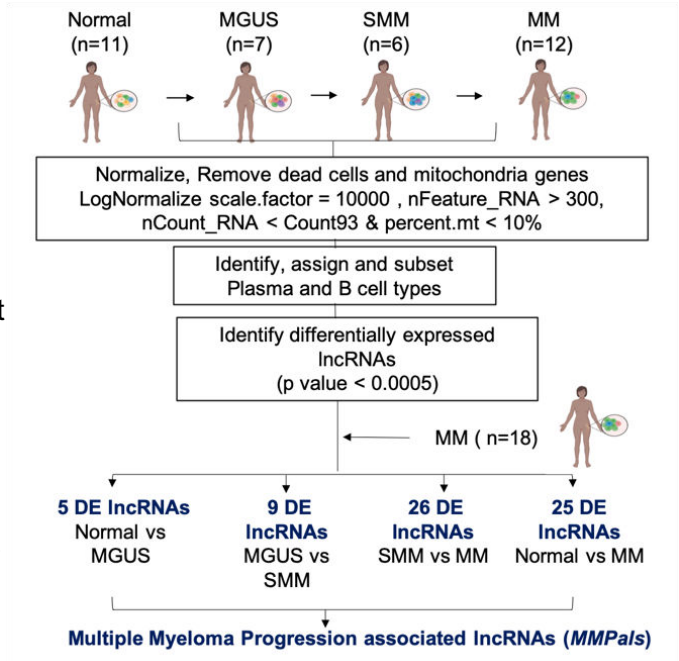

Figure 1: Discovery of Multiple Myeloma progression associated lncRNAs using single cell RNA Sequencing. Monoclonal Gammopathy of Undetermined Significance (MGUS), Smoldering Multiple Myeloma (SMM), Multiple Myeloma (MM)

MMPal1 is the top significantly expressed lncRNA in MM progression and promotes proliferation and cell viability

MMPal1, also known as *NEAT1,* was the most significantly upregulated lncRNA when comparing all cohorts. *MMPal1* had little to no expression in normal samples and gradually increasing expression with each progressive stage of MM (normal vs MGUS, *p* value = 1.92 e-14; SMM vs MM; *p* value= 9.35e-18, normal versus MM, *p* value = 1.10 e-12, Figure 2a and b). We next determined that *MMPal1* expression was specific to Mature B-cell neoplasm cell lines as compared to other blood cancer types (B-lymphoblastic leukemia, *p* value = 0.002; Hodgkin lymphoma, *p* value = 0.001; Leukemia, *p* value = 0.04; and T-Lymphoblastic leukemia, *p* value = 0.01) using cBioPortal (Figure 2c). Due to the heterogeneity and detection of hyperploidy in several chromosomes of MM patients, we assessed expression of *MMPal1* in genetic sub-types of MM patient RNA sequencing data from the MMRF CoMMpass study and internal Washington University patient cohorts (n=170) and saw no difference in expression (Figure 2d). We found that *MMPal1* had high expression in MM cell lines (Figure 3a) then decreased its expression using siRNAs in U266B1 cells to detect a decrease of

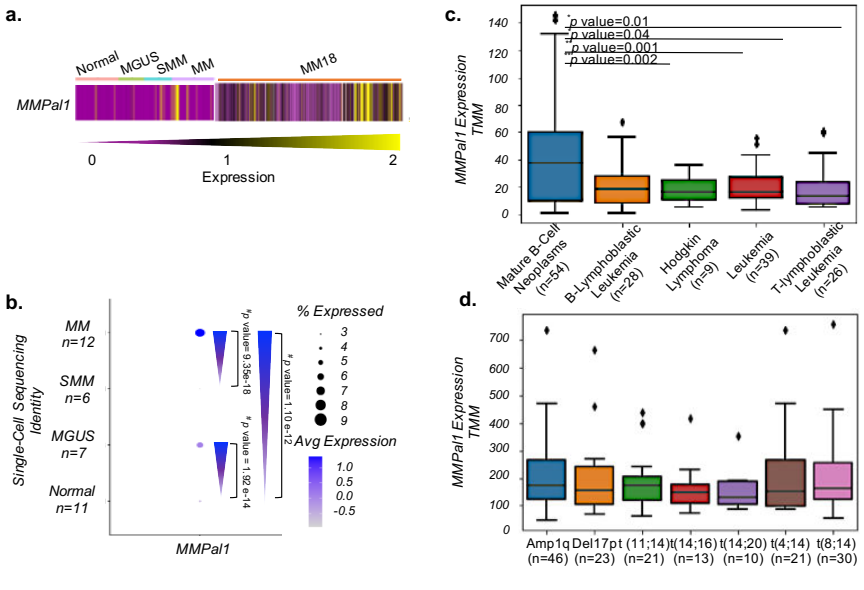

Figure 2: *MMPal1* expression in single-cell RNA sequencing cohorts shown as **a.** hierarchical and **b.** dot plot. **c.** RNA sequencing expression of *MMPal1* is significantly overexpressed in mature b-cell neoplasms compared to all other blood cancer cell lines from cBioPortal and **d.** no difference of *MMPAl1* expression comparing molecular characterization of myeloma patient RNA sequencing data

cellular proliferation (48 hours; *p* value = 0.005) and cell viability (24 hours; *p* value = 1.27e-06, 48 hours; *p* value = 0.004, Figure 3b-d). These results show that *MMPal1* promotes MM.

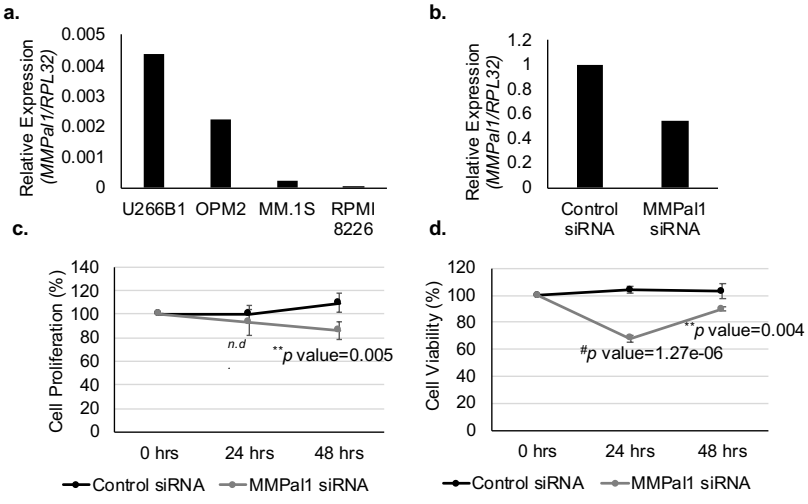

Figure 3: a. Expression of *MMPal1* in multiple myeloma cell lines by qPCR. **b.** *MMPal1* silenced expression in U266B1 cells using siRNAs. **c.** Decrease cell proliferation (Alamar Blue) and **d.** cell viability (Cell Citer Glo) in *MMPal1* silenced cells.

MMPal1 binds to CBX4 protein and is associated with Melphalan resistance

To determine if *MMPal1* is associated with drug resistance, we treated cells with Melphalan, a standard treatment commonly used followed by autologous stem cell transplant for transplant-eligible patients with MM[24], and found Melphalan sensitive MM1.S cells showed less *MMPal1* expression when compared to Melphalan resistant U266B1 cells (Figure 4). Next, we assessed if *MMPal1* binds to Chromobox 4 (CBX4) protein, due to its similar cellular location in nuclear speckles, epigenetic regulation, and known binding to lncRNAs [16,25].We performed RNA immunoprecipitation (RIP) qPCR of CBX4 in MM1.S and U266B1 MM cell lines and found that *MMPal1* has high enrichment of binding to CBX4 in both cell lines (MM1.S; fold enrichment = 39.73, U266B1; fold enrichment = 72.74, Figure 5a and b) as compared to IgG negative control. Next, we treated cells with Melphalan and conducted CBX4 RIP qPCR. Interestingly we found less *MMPal1* enrichment of binding to Melphalan treated MM1.S cells (*p* value = 0.008) as compared to non-treated cells (Figure 5c), but continued binding to CBX4 in Melphalan treated U266B1 cells (Figure 5d). We conclude that *MMPal1* is highly bound to CBX4 in MM cell lines and is highly bound in Melphalan resistant setting. These results indicate that *MMPal1* does indeed bind to proteins and the lncRNA-protein interaction may be a potential cause of regulation in promoting MM progression and therapy resistance.

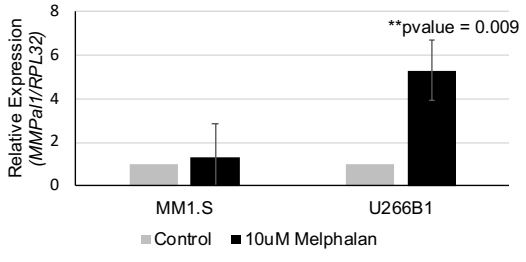

Figure 4: qPCR showing increased expression of *MMPal1* in U266B1 Melphalan resistant cells compared to MM1.S Melphalan sensitive cells when treated with Melphalan.

Our preliminary data serves as strong rationale for *our hypotheses that MMPal1 is highly expressed in MM progression and may function as a master epigenetic regulator in drug resistance.* We will test our hypotheses with the following aims (Figure 6):

E. Research design and methods

Specific Aim 1: Validate expression of MMPal1 as a prognostic biomarker associated with progression.

Rationale: Due to the increasing expression of *MMPal1,* we believe that it can serve as a novel biomarker for MM progression. Therefore, we chose to study *MMPal1* in a larger cohort of progression samples to predict prognosis in these patients.

Strategy and Analysis:

SA1.1 Sample processing and preparation from MM patients. Dr. Ravi Vij's (Director of Multiple Myeloma Tissue Bank), has collected bone marrow aspirates from patients treated at Washington University. We selected 84 samples from the following stages of disease: normal (n=12), MGUS (n=24), SMM (n=24), and MM (n=24). Samples are linked to a maintained database with complete clinical information, treatment, stage, and available follow-up data. The frozen bone

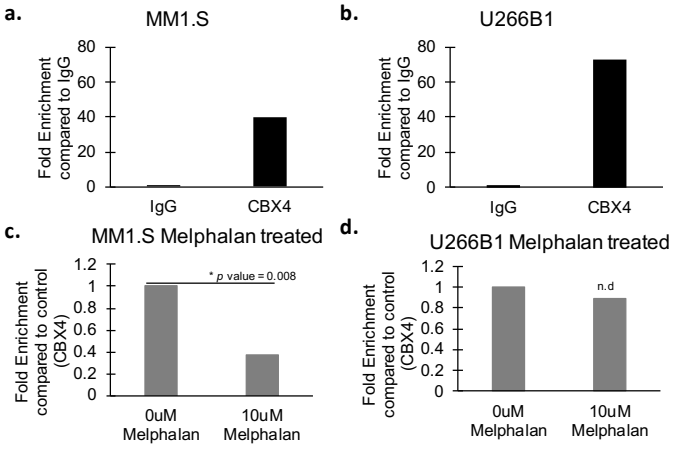

Figure 5: RNA immunoprecipitation (RIP) qPCR of *MMPal1* with CBX4 in **a.** MM1.S and **b.** U266B1 cells. CBX4 RIP qpcr of *MMPal1* in treated cells with Melphalan for 72 hours in **c.** MM1.S (Melphalan sensitive cell line) and **d.** U266B1 (Melphalan resistant cell line). * p value < 0.05, n.d (no difference)

marrow aspirates will be thawed and seeded in 8-well chamber slides (250,000 cells per well, in triplicate) as previously described[26] and are **available for this Aim**.

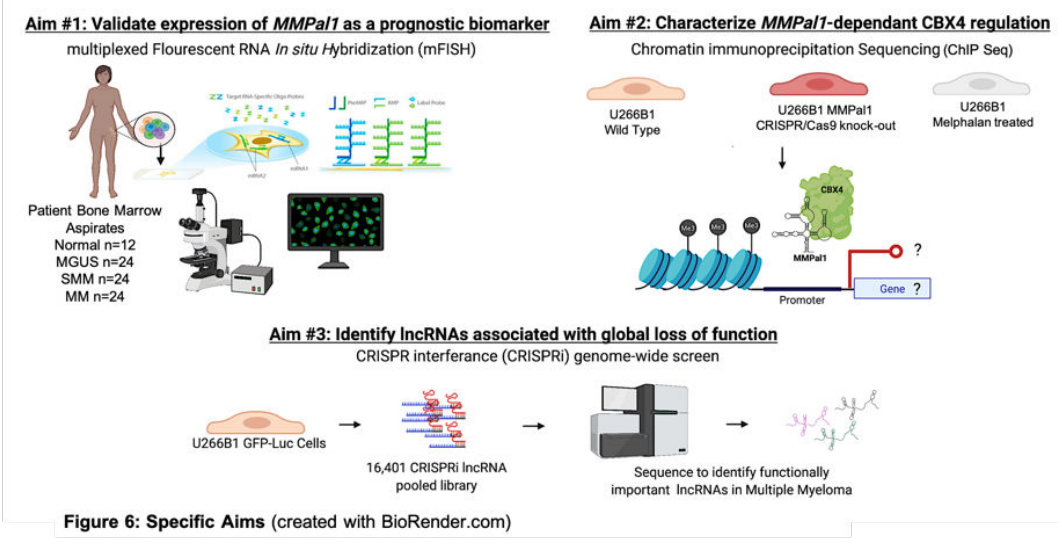

Figure 6: Specific Aims (created with BioRender.com)

SA1.2 Multiplexed Fluorescent RNA In situ Hybridization (mFISH) expression and data analysis. We will use Advanced Cell Diagnostics RNAscope Multiplex fluorescent assay to assess gene expression from the bone marrow aspirates. We will design probes to detect the expression of *MMPal1,* p54 *(*paraspeckle marker*),* CD38 (plasma cell marker), *MMPal25* (known as *MALAT1,* positive control) and internal negative control probes. 8-well chamber slides with patient's bone marrow aspirates will be processed using the standard protocol visualizing 4 different targets at one time. We will compare expression of *MMPal1* and *MMPal25* in the different cohorts and simultaneously verifying their localization in paraspeckles (p54) and plasma cells (CD38). We will visualize our target RNA molecules using an EVOS M5000 imaging system and will quantify targets with RNAscope Spot Studio Software to obtain cell-count per region and number of spots per cell analysis. We will spot count ~100 randomly selected cells to determine per-cell copy number by dividing total spot count by the number of cells counted. *Inclusion of Women and Minorities:* Women and Minorities are eligible for the proposed study. No gender, racial, or ethnic preferences are considered for participation in this study. The patient population will be reflective of the diversity of the general population in the St. Louis area.

***Pitfalls, alternative strategies, and expected outcomes*:** We have experience using mFISH so do not expect any issues in the technique however, if we are unable to conduct mFISH on fresh frozen bone marrow aspirates, we will utilize fresh frozen paraffin embedded patient samples, thereby avoiding any delays in our timeline. lncRNA probes for both *MMPal1 and MMPal2* have been pre-made and optimized. Successful completion of this Aim will enable use of *MMPal1* as a biomarker allowing for more informed treatment decisions for patients.

Specific Aim 2: Characterize MMPal1-dependant CBX4 regulation.
Rational: Our preliminary data suggest that *MMPal1* binds to CBX4 in MM and in Melphalan treated resistant cell lines, supporting our hypothesis of *MMPal1* as a master epigenetic regulator in drug resistance.

SA2.1a Create a MMPal1 CRISPR/Cas9 knock-out (KO) cell line. We will generate a robust stable U266B1 *MMPal1* KO cell line model*,* in collaboration with Dr. Xiaoxia Ciu (The Director of the Genome Engineering & Stem cell Center (GESC@MGI), as we previously only transiently decreased its expression with siRNAs (Figure 3b). We will use U266B1 myeloma cells as they showed highest endogenous expression of *MMPal1* (Figure 3a) as the use of myeloma primary patient cells or PDX models has not yet been developed or optimized for use with CRISPR/Cas9 system. We have previously successfully worked with GESC@MGI to generate a CRISPR/Cas9 system for lncRNAs[16].

SA2.1b Determine if modulating expression of MMPal1 affects CBX4 occupancy*:* We will conduct chromatin immunoprecipitation sequencing (ChIP-Seq) experiments, which directly map protein-DNA interactions in U266B1 wild type control, *MMPal1* CRISPR KO, and Melphalan treated cells in triplicate. We will use antibodies for CBX4 (Abcam), its known histone activation mark H3K4me3 (Activemotif) and histone repressive mark H3K9me3 (Abcam), compared to an IgG control, as done previously[16,18,27]. ***Authentication of reagents:*** We will first authenticate antibodies by validating size and expression by western blot. ChIP-qPCR for CBX4 and histone marks will be assessed using negative control primers (Activemotif) and positive control primers *GAPDH* gene for H3K4me3 and *SAT2* for H3K9me3. Once all steps have been validated, we will proceed with creating libraries and sequencing ChIP DNA.

SA2.2 Detect genome-wide altered chromatin states upon modulation of MMPal1 data analysis: We will use the in-house GTAC@MGI RNA-Seq pipeline for alignment to GRCh38/hg38 with annotations from Ensembl release 76 primary assembly with STAR version 2.7.9a[28]. Gene counts will be derived from the number of uniquely aligned unambiguous reads by Subread:featureCount version 2.0.3[29]. Isoform expression of known Ensembl transcripts will be quantified with Salmon version 1.5.2[30]. ChIP-Seq peaks will be identified using MACS v2.0[31]. We will identify differential peaks between the cell lines. The top 10 genomic regions will then be validated using ChIP-qPCR. We will also confirm that these regions mediate a transcriptional response by generating one lane of RNA-Seq reads, as previously described[15], using the same model as above. We will generate read counts for every Ensembl Transcript and subsequently use EdgeR 3.8.6[32] to identify differentially expressed transcripts. We will develop a *MMPal1* target gene signature comprised of genes with altered CBX4 promoter occupancy to confirm the association of CBX4 and Melphalan treated genes.

Pitfalls, alternative strategies, and expected outcomes: We do not foresee any significant pitfalls in the ChIP-Seq or RNA-Seq experiments as they are straightforward and we have expertise in these areas. Given our preliminary data, we expect to observe that *MMPal1* affects CBX4 occupancy with and without Melphalan treatment. We may also find lncRNA-dependent changes in genes promoting proliferation independent of CBX4 occupancy, which will spur new research directions to better understand how *MMPal1* promotes progression. We have focused our proposal on how *MMPal1* mediates CBX4 based on the strength of our preliminary data and to stay within the scope of the one-year funding period. However, as a subsequent strategy, we can identify two other associated CBX4 histone marks H2AK119ac and H3K27me3 occupancy. Completion of this aim will expand our understanding of *MMPal1*-dependant CBX4 occupancy and how it regulates chromatin states that may be associated MM progression and drug resistance.

Specific Aim 3: Identify lncRNAs associated with global loss of function.
Rationale: While few independent studies have shown that lncRNAs are important in promoting MM, we do not know which lncRNAs are the most important in terms of function. Given the potential role of *MMPal1* as a master epigenetic regulator in MM progression, lncRNAs may serve as ideal therapeutic targets.

Strategy and Analysis:
SA3.1 CRISPRi genome-scale lncRNA screen. We will conduct a CRISPRi genome-scale lncRNA screen in U266B1 GFP-luciferase (luc) cell lines to assess a genome-wide view of lncRNA loss of function. In collaboration with the GESC@MGI, we will create 16,401 targeted lncRNAs guides (with 10 sgRNAs per transcription start site) as previously described[33]. This method represses transcription of any gene via targeted recruitment of the nuclease-dead dCas9-KRAB repressor fusion protein to the transcriptional start site by a single guide RNA (sgRNAs). *MMPal1* guides will first be tested as proof-of principle approach to show viability of method. We will seed 20,000 U266B1 GFP-luc cells in triplicate into a 96-well plate, transduce with lncRNA sgRNAs and non-targeted negative control sgRNAs, add ApoTox-Glo Triplex assay reagents, and measure fluorescence using a Varioskan LUX microplate reader. Transduced U266B1 GFP-luc cells will be monitored weekly (3-6 weeks) for viability, cytotoxicity, and apoptosis from same cells via ApoTox-Glo Triplex assay. DNA from cells will be isolated weekly for assessment of guides that are affecting the associated phenotypes.

SA3.2 CRISPRi data analysis. We will detect intensity of fluorescence (relative fluorescence units) and luminescence (relative luminescence units) and plot data using log-based transformation to detect significance comparing lncRNA sgRNAs compared to non-targeted negative control sgRNAs. We use randomly sampled non-targeting sgRNA phenotypes to generate “negative control genes” and analyze them with lncRNA genes, enabling an empirical false discovery rate for each screen[33]. LncRNA genes are considered hits if combined phenotype effect size and p-value correspond to empirical false discovery rate of 5%. DNA from the same cells will be isolated and sequenced by our collaborators at MGI@GTAC to detect which guides are promoting loss of function. For validation of top two identified targets, we will decrease expression using targeted silencer RNAs in four cell lines in triplicate (U266B1 that showed phenotype and three cells of unknown phenotype) and re-screen with ApoTox-Glo Triplex assay. This screen will be highly useful in verifying the biological functions of all possible lncRNAs associated with progression.

Pitfalls, alternative strategies, and expected outcomes: We do not expect any issues with creating the guides for lncRNAs as the GESC@MGI are highly experienced in this area of CRISPR generation. In scope of awards one-year time frame, we will validate top two lncRNAs, but will also compare the list to our scRNA-Seq to assess differential gene expression of all *MMPals*. Completion of this Aim will identify functionally important MM lncRNAs.

PLANS FOR OBTAINING ADDITIONAL EXTRAMURAL FUNDING

Support from the Longer Life Foundation will allow my lab to begin this innovative preliminary work in identifying novel biomarkers in multiple myeloma and aid in my successful and competitive application for submitting an NIH NCI R01 in October 2022. The data from this project will serve as a great foundation to continue future *in vivo* work to show lncRNAs as novel therapeutic targets using LNA ASOs that have shown increasing promise based on recent successful human clinical trials.

REFERANCES:

1. Bianchini, D.*, et al.* First-in-human Phase I study of EZN-4176, a locked nucleic acid antisense oligonucleotide to exon 4 of the androgen receptor mRNA in patients with castration-resistant prostate cancer. *Br J Cancer* **109**, 2579-2586 (2013).
2. Yuan, Y.*, et al.* A Polyethylenimine-Containing and Transferrin-Conjugated Lipid Nanoparticle System for Antisense Oligonucleotide Delivery to AML. *BioMed research international* **2016**, 1287128 (2016).
3. Zhou, M.*, et al.* Identification and validation of potential prognostic lncRNA biomarkers for predicting survival in patients with multiple myeloma. *J Exp Clin Cancer Res* **34**, 102 (2015).
4. Meng, H., Han, L., Hong, C., Ding, J. & Huang, Q. Aberrant lncRNA Expression in Multiple Myeloma. *Oncol Res* **26**, 809-816 (2018).
5. Dong, H.*, et al.* Upregulation of lncRNA NR_046683 Serves as a Prognostic Biomarker and Potential Drug Target for Multiple Myeloma. *Frontiers in pharmacology* **10**, 45 (2019).
6. Ronchetti, D.*, et al.* A compendium of long non-coding RNAs transcriptional fingerprint in multiple myeloma. *Scientific reports* **8**, 6557 (2018).
7. Butova, R., Vychytilova-Faltejskova, P., Souckova, A., Sevcikova, S. & Hajek, R. Long Non-Coding RNAs in Multiple Myeloma. *Non-coding RNA* **5**(2019).
8. Rinn, J.L. & Chang, H.Y. Genome regulation by long noncoding RNAs. *Annual review of biochemistry* **81**, 145-166 (2012).
9. Derrien, T.*, et al.* The GENCODE v7 catalog of human long noncoding RNAs: analysis of their gene structure, evolution, and expression. *Genome Res* **22**, 1775-1789 (2012).
10. Angrand, P.O., Vennin, C., Le Bourhis, X. & Adriaenssens, E. The role of long non-coding RNAs in genome formatting and expression. *Frontiers in genetics* **6**, 165 (2015).
11. Kopp, F. & Mendell, J.T. Functional Classification and Experimental Dissection of Long Noncoding RNAs. *Cell* **172**, 393-407 (2018).
12. Silva, J. & Smith, D. *Long non-coding RNAs and Cancer* (Caister Academic Press, La Jolla, California, 2012).
13. Silva, J.M., Boczek, N.J., Berres, M.W., Ma, X. & Smith, D.I. LSINCT5 is over expressed in breast and ovarian cancer and affects cellular proliferation. *RNA Biol* **8**, 496-505 (2011).
14. Silva, J.M.*, et al.* Identification of long stress-induced non-coding transcripts that have altered expression in cancer. *Genomics* **95**, 355-362 (2010).
15. White, N.M.*, et al.* Transcriptome sequencing reveals altered long intergenic non-coding RNAs in lung cancer. *Genome Biol* **15**, 429 (2014).
16. Silva-Fisher, J.M.*, et al.* Long non-coding RNA RAMS11 promotes metastatic colorectal cancer progression. *Nature communications* **11**, 2156 (2020).
17. Cabanski, C.R.*, et al.* Pan-cancer transcriptome analysis reveals long noncoding RNAs with conserved function. *RNA Biol* **12**, 628-642 (2015).
18. Eteleeb, A.M.*, et al.* LINC00355 regulates p27(KIP) expression by binding to MENIN to induce proliferation in late-stage relapse breast cancer. *NPJ Breast Cancer* **8**, 49 (2022).
19. Sanchez, Y. & Huarte, M. Long non-coding RNAs: challenges for diagnosis and therapies. *Nucleic acid therapeutics* **23**, 15-20 (2013).
20. Cheetham, S.W., Gruhl, F., Mattick, J.S. & Dinger, M.E. Long noncoding RNAs and the genetics of cancer. *Br J Cancer* **108**, 2419-2425 (2013).
21. Ling, H., Fabbri, M. & Calin, G.A. MicroRNAs and other non-coding RNAs as targets for anticancer drug development. *Nature reviews. Drug discovery* **12**, 847-865 (2013).
22. Smith, J.S.a.D. Long Non-coding RNAs (lncRNAs) and Cancer. in *Non-coding RNAs and Epigenetic Regulation of Gene Expression: Drivers of Natural Selection | Book* (ed. Morris, K.) (Caister Academic Press, The Scripps Research Institute, La Jolla, California, USA, 2012).
23. Ledergor, G.*, et al.* Single cell dissection of plasma cell heterogeneity in symptomatic and asymptomatic myeloma. *Nature medicine* **24**, 1867-1876 (2018).

24. Poczta, A., Rogalska, A. & Marczak, A. Treatment of Multiple Myeloma and the Role of Melphalan in the Era of Modern Therapies-Current Research and Clinical Approaches. *J Clin Med* **10**(2021).
25. Martin-Perez, D., Piris, M.A. & Sanchez-Beato, M. Polycomb proteins in hematologic malignancies. *Blood* **116**, 5465-5475 (2010).
26. Tsang, M., Gantchev, J., Ghazawi, F.M. & Litvinov, I.V. Protocol for adhesion and immunostaining of lymphocytes and other non-adherent cells in culture. *Biotechniques* **63**, 230-233 (2017).
27. Klauke, K.*, et al.* Polycomb Cbx family members mediate the balance between haematopoietic stem cell self-renewal and differentiation. *Nature cell biology* **15**, 353-362 (2013).
28. Dobin, A.*, et al.* STAR: ultrafast universal RNA-seq aligner. *Bioinformatics* **29**, 15-21 (2013).
29. Liao, Y., Smyth, G.K. & Shi, W. featureCounts: an efficient general purpose program for assigning sequence reads to genomic features. *Bioinformatics* **30**, 923-930 (2014).
30. Patro, R., Duggal, G., Love, M.I., Irizarry, R.A. & Kingsford, C. Salmon provides fast and bias-aware quantification of transcript expression. *Nat Methods* **14**, 417-419 (2017).
31. Zhang, Y.*, et al.* Model-based analysis of ChIP-Seq (MACS). *Genome Biol* **9**, R137 (2008).
32. Robinson, M.D., McCarthy, D.J. & Smyth, G.K. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* **26**, 139-140 (2010).
33. Liu, S.J.*, et al.* CRISPRi-based genome-scale identification of functional long noncoding RNA loci in human cells. *Science* **355**(2017).