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TITLE: Defining Overexpression of MYBL2 as a Driver of Lethal Prostate Cancer

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14. ABSTRACT Due to inevitable therapeutic resistance, prostate cancer remains a lethal disease, so is critical to delineate mechanisms underlying therapeutic resistance to implement novel treatments that will extend patient survival and quality of life. We hypothesize MYBL2 over-expression facilitates resistance through a mechanism involving induction of lineage plasticity. We will test this hypothesis and determine novel treatment strategies for MYBL2 high prostate cancers and validate the power of MYBL2 expression/activity to predict ADT resistance in prostate cancer samples.					
15. SUBJECT TERMS MYBL2, castrate resistant prostate cancer, aggressive variant prostate cancer, tumor stemness, mouse models, chromatin					
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INTRODUCTION

Prostate cancer (PCa) is the second most frequently diagnosed cancer type among men in America. Currently, the cumulative five-year survival rate for patients with prostate cancer is over 98%. Unfortunately, the average survival for men with metastatic or recurrent prostate cancer drops considerably. Second-generation androgen deprivation therapies (ADT) have provided significant life-extending therapies for recurrent, or metastatic castration resistant prostate cancer (mCRPC) patients. A particular resistant, aggressive subset of these mCRPC tumors is independent of AR activity (CRPC-AI). CRPC-AI primarily adapt to ADT via lineage plasticity rather than a result of resistant mutations, adopting a phenotype no longer reliant on AR expression and signaling. These tumors may display neuroendocrine features, a stem or basal cell-like phenotype, altered kinase signaling, and characteristic epigenetic alterations, including upregulation of EZH2. Currently there is no therapeutic option to provide long term durable response to CRPC-AI patients. Therefore, a deeper knowledge of molecular mechanisms driving CRPC-AI will significantly move the field forward to provide discoveries for therapeutic vulnerabilities to successfully inhibit progression or treat CRPC-AI. From this, we have a novel candidate, MYBL2, that may act as a master-regulator transcription factor that upon RB1 LOF drives lineage plasticity and resistance to ADT.

KEYWORDS

MYBL2, castrate resistant prostate cancer, aggressive variant prostate cancer, tumor stemness, mouse models, chromatin

ACCOMPLISHMENTS

What were the major goals of the project?

To date the Ellis has accomplished the following major goals:

Major Task #1

1. Breed Mybl2 floxed alleles into DKO mice to generate models of heterozygous (DKO+Mybl2F/+) and homozygous (DKO+Mybl2F/F) Mybl2 loss.
2. Determine phenotype of Mybl2 knockout molecularly and pathologically.
3. Determine the dependence of MYBL2 in human models of CRPC-AI.

Major Task #2

1. Perform in vitro drug screening.

Major Task #3

1. Conduct gene-expression profiling in collaboration with Genome-Dx.
2. Perform gene-expression analysis based on MYBL2 quartiles.
3. Perform Kaplan Meier analysis towards MYBL2 predictive power towards therapy response.

What was accomplished under these goals?

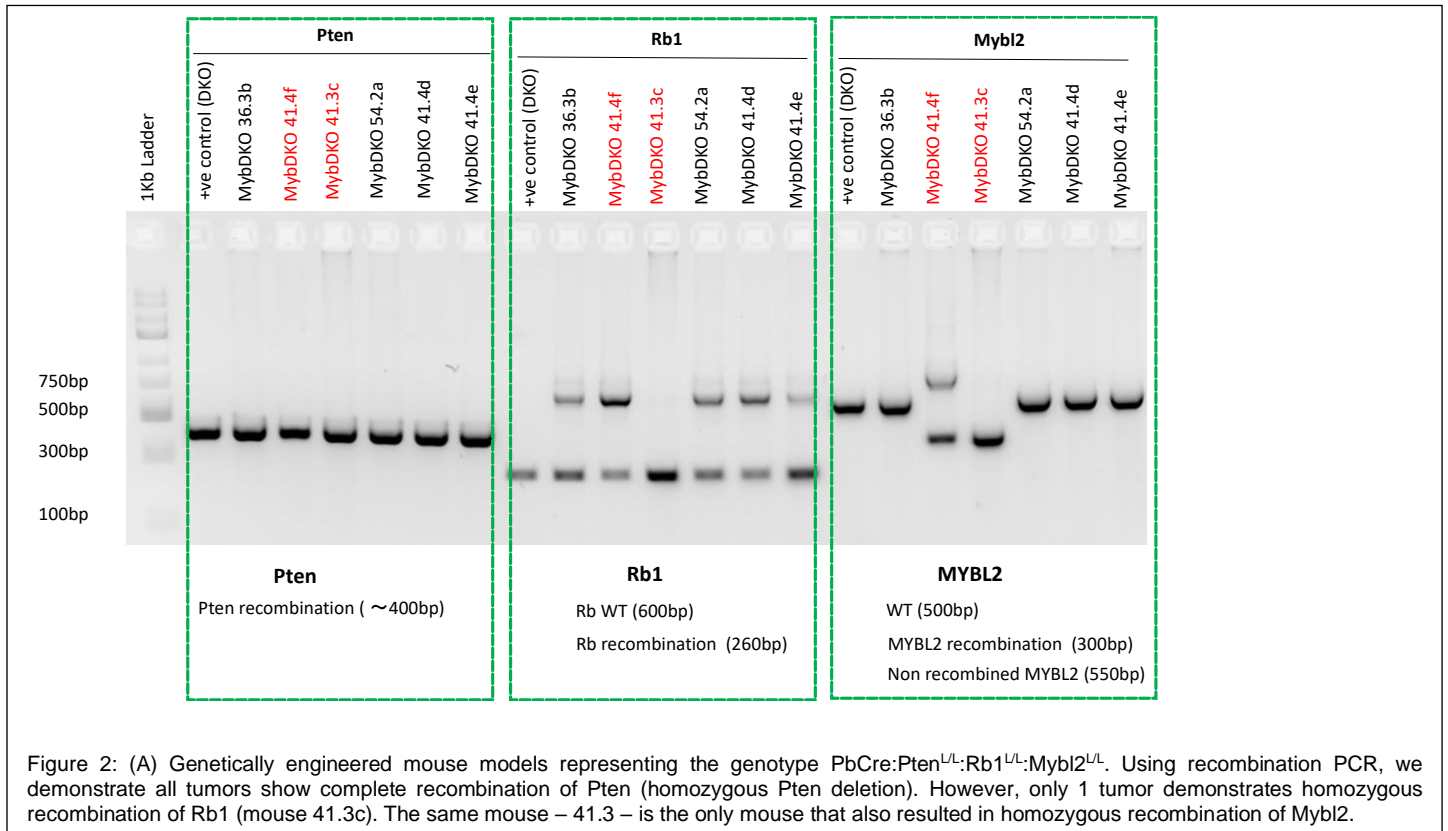
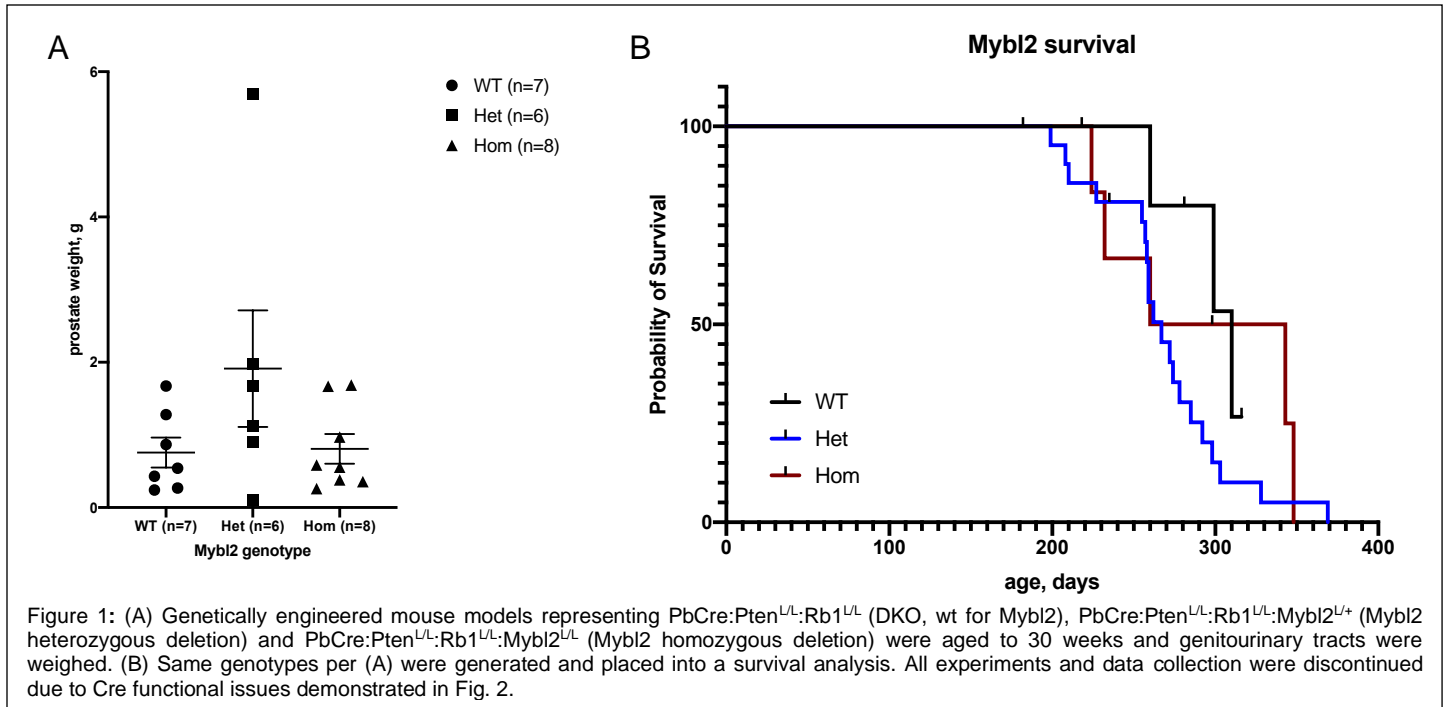
Major Task #1

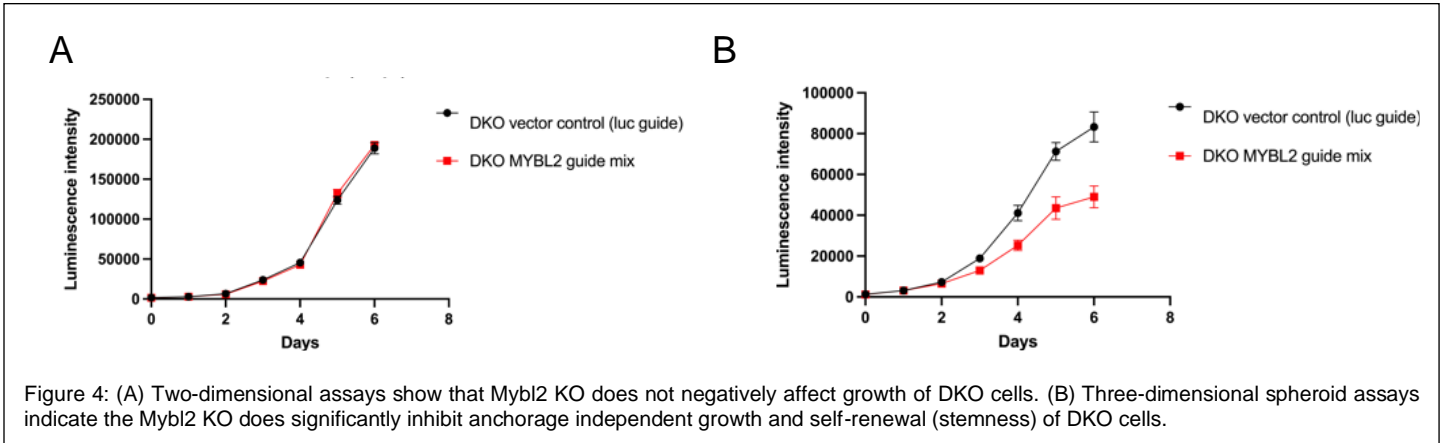
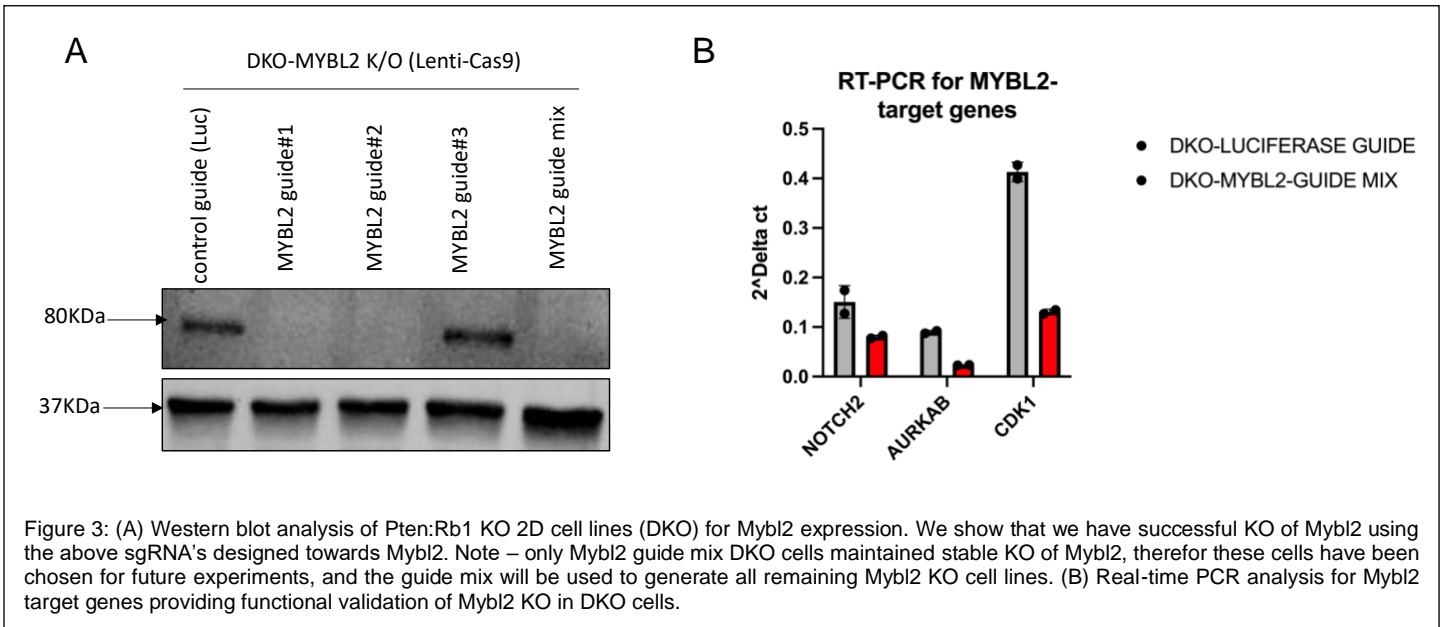
1. We successfully generated all required mouse genotypes (DKO, DKO:Mybl2+/-, and DKO:Mybl2-/-) and conducted both our 38-40 week endpoint and overall survival studies (Fig. 1).

Upon conclusion of the endpoint study and during our survival study we had performed regular validation of mouse genotypes that had been sacked prior to further analysis. During this, we had noticed that use of recombination PCR to ensure our target genes have been targeted correctly, we unfortunately observed in some of our mice that desired genes, especially Mybl2, had not been genetically knocked out completely (Fig.2). Due to this we will not move forward with collected samples and all mouse colonies have been ended and their generation has started again.

To compensate for this loss, we have been generating Mybl2 KO cell lines using our murine cell lines and Crispr technology. While the DOD award focuses of Mybl2 KO within the DKO cells/tumors we are expanding the generation of Mybl2 KO in multiple genotypes from our mouse cell lines. These include from the application SKO (Pten KO) and DKO (Pten:Rb1 KO). In addition we will use DKO-CR (Castrate Resistance

Pten:Rb1:Trp53^{mut}), and TKO (Pten:Rb1:Trp53 KO). We are also currently generating MYBL2 KO in our described human cell lines from the application. Currently, we have successfully generated and validated DKO:Mybl2 KO cell lines and have determined the dependence of Mybl2 for anchorage independent growth and self-renewal (stemness) of DKO cells (Fig. 3). This work continues and will be finalized in the very near future.





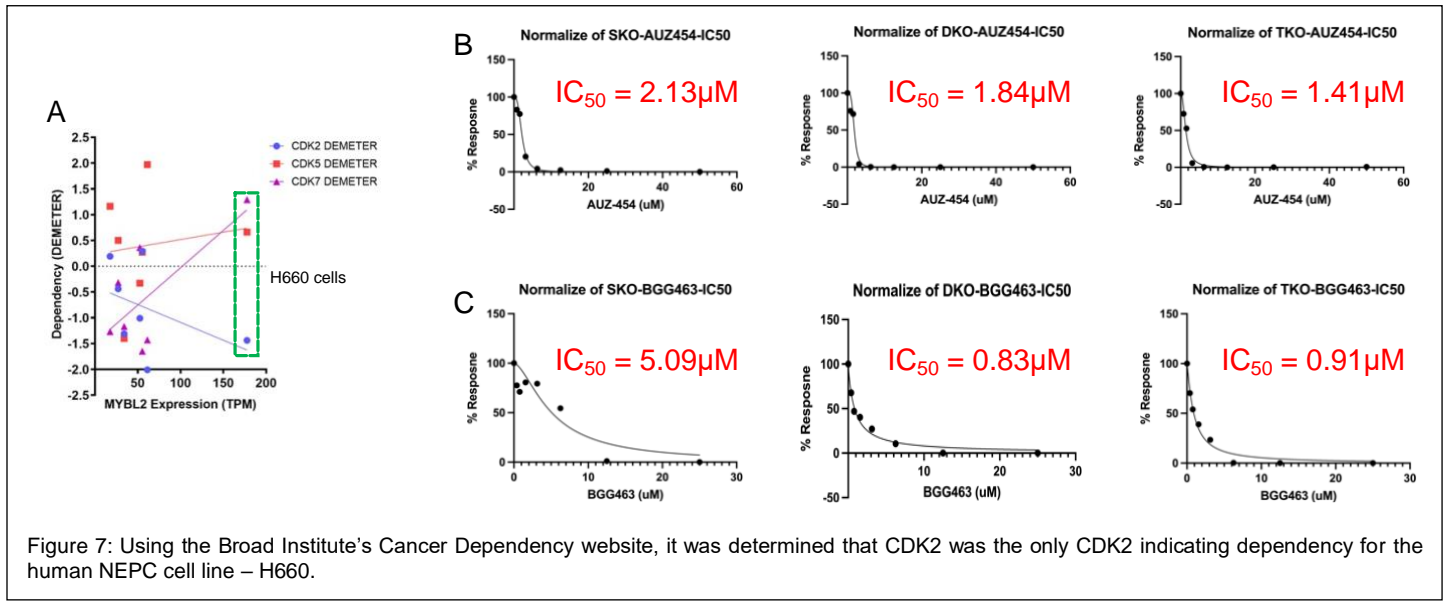
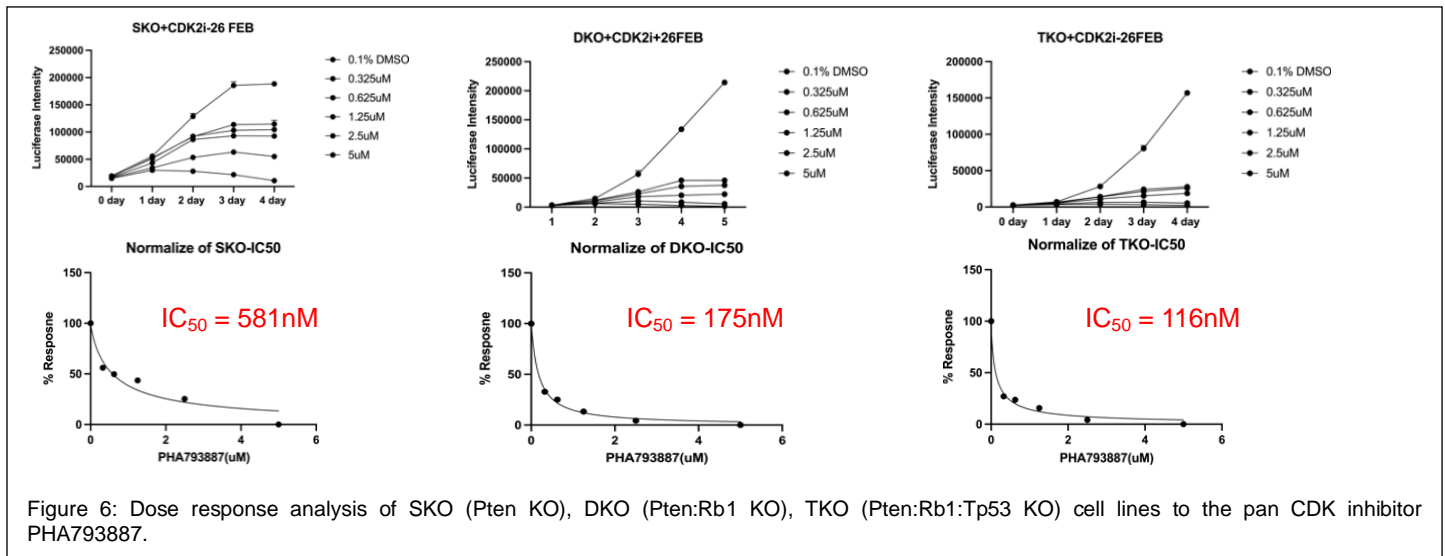
Major Task #2

1. Perform in vitro drug screening.

MYBL2_High_rowmedians	MYBL2_Low_rowmedians	delta	TARGET	TARGET_PATHWAY
0.92	0.51	↑ 0.41	PI3K (Class 1), MTORC1, MTORC2	PI3K/MTOR signaling
0.85	0.55	↑ 0.3	AKT1, AKT2, AKT3	PI3K/MTOR signaling
0.54	0.24	↑ 0.3	MTORC1, MTORC2	PI3K/MTOR signaling
0.90	0.66	↑ 0.24	PKCB	Other, kinases
0.97	0.73	↑ 0.24	ALK5	RTK signaling
0.92	0.71	↑ 0.21	HSP90	Protein stability and degradation
0.86	0.66	↑ 0.2	dsDNA break induction	DNA replication
0.89	0.70	↑ 0.19	MTORC1, MTORC2	PI3K/MTOR signaling
0.90	0.72	↑ 0.18	CDK2, CDK7, CDK5	Cell cycle
0.93	0.76	↑ 0.17	Antimetabolite (DNA & RNA)	Other

Figure 5: A Mybl2 target gene signature was uploaded to the Broad Institute Cancer Dependency Map to identify current therapeutic targets to be tested in NEPC models.

We had initially proposed to test response to numerous drug target families based off our data gained from the Broad Cancer Dependency Map database following the upload of a Mybl2 target gene signature (Fig. 5). It was our initial plan to test therapies targeting PI3K/mTORC, HSP90, DNA replication, and CDKs. With lost time due to the lab move and time to hire new staff, it was decided that our therapy focus would be towards CDKs. With a past of negative data in CRPC clinical trials including PI3K/mTORC, HSP90, and DNA replication, it appeared targeting CDKs would be the most novel and innovative direction to follow. As in Fig. 5 we proceeded to test our mouse cell line model response to the pan-CDK inhibitor PHA-793887.



Data in Fig. 6 clearly shows that all cell lines have a robust response to pan CDK inhibition, as expected for any cycling cell. However, from these initial results it was clear that cell lines with a NEPC genotype (Rb1 and/or Tp53 KO) displayed a more significant response to this inhibition. To investigate if all proposed CDKs from Fig.5 were necessary for therapeutic targeting, we further used the Broad's Dependency Map. Upon a specific look at all human prostate cancer cell lines available, it was clear that only CDK2 was a possible synthetic lethal dependency in the human NEPC cell lines – H660 (Fig. 7A). These data further encouraged us to focus on targeting CDK2 in our mouse cell lines. We tested 2 independent CDK2 inhibitors – AUZ454 and BGG463. First, AUZ454 did show inhibition in all cell lines indicating a potential low specificity for CDK2 and potential off target effects (Fig. 7B). Second, BGG463 exclusive sensitivity towards DKO and TKO cell lines compared to SKO. With this, we are continuing to generate both single agent and combination therapy results using our proposed mouse and human cell lines.

Major Task #3

1. Conduct gene-expression profiling in collaboration with Genome-Dx.
2. Perform gene-expression analysis based on MYBL2 quartiles.
3. Perform Kaplan Meier analysis towards MYBL2 predictive power towards therapy response.

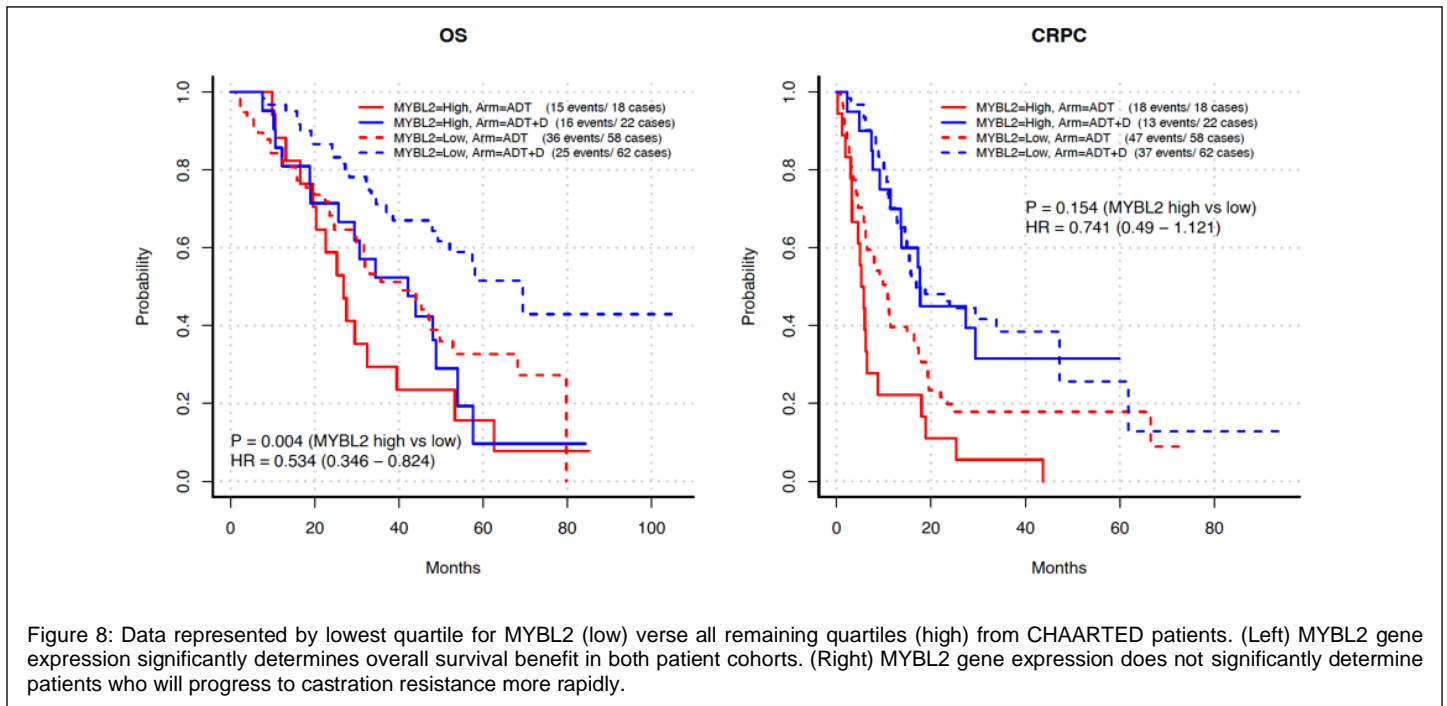


Figure 8: Data represented by lowest quartile for MYBL2 (low) versus all remaining quartiles (high) from CHARTED patients. (Left) MYBL2 gene expression significantly determines overall survival benefit in both patient cohorts. (Right) MYBL2 gene expression does not significantly determine patients who will progress to castration resistance more rapidly.

Using gene expression analysis performed from prostate cancer patient samples from the CHARTED trial led by Dr. Christopher Sweeney – MYBL2 gene expression was determined, and patients were ranked by quartile distribution. Kaplan-Meier analysis was performed based on patient overall survival and time to development of CRPC. There was no significant predictive value for determination of patients who would progress to CRPC more rapidly. However, for overall survival analysis, MYBL2 gene expression were a significant indicator of patients with a survival advantage in both treatment arms (low versus high). Even within the treatment groups, MYBL2 expression levels indicated patients had an overall survival advantage with ADT + docetaxel combination therapy (Fig. 8).

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

We will continue to conduct in vitro and in vivo experiments to determine the role of Mybl2 in driving tumor cell aggressiveness and resistance to ADT. Also, data should be at a mature enough level to start presenting our data at local, national, and international meetings. We should also, at a minimum, have a manuscript online with BioRxiv.

IMPACT

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

CHANGES/PROBLEMS

Changes in approach and reason for change.

Because of our loss of GEMMs with our move to LA we aim to compensate these experiments using in vivo transplant experiments and in vitro 3D assays using already established 2D lines from our GEMMs.

Actual or anticipated problems or delays and actions or plans or plans to resolve them.

Nothing to report.

Changes that had significant impact on expenditures.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

PRODUCTS

Publications, conference papers, and presentations

Journal publications

Nothing to report.

Books or other non-periodical, one-time publications

Nothing to report.

Other publications, conference papers, and presentations

Nothing to report.

Website(s) or other internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other products

Nothing to report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dr. Leigh Ellis
Project Role:	Co-PI
Researcher Identifier:	0000-0003-4739-5049
Nearest person month worked:	3
Contribution to project:	Co-PI
Funding Support:	NCI, DOD

Name:	Dr. Christopher Sweeney
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Project Role: Co-PI
Researcher Identifier:
Nearest person month worked: 3
Contribution to project: Co-PI
Funding Support: NCI, DOD

Name: Dr. Jagpreet Nanda
Project Role: Postdoc Scientist
Researcher Identifier:
Nearest person month worked: 6
Contribution to project: Postdoc Scientist
Funding Support:

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

SPECIAL REPORTING REQUIRMENTS

Nothing to report.

APPENDICES

Nothing to report.