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TITLE: Cotargeting the Trinity of DNA Damage Repair Sensing Kinases in Prostate Cancer

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CONTRACTING ORGANIZATION: UT Southwestern Medical Center

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14. ABSTRACT In this grant, we will systematically establish the preclinical utility of Compound B for clinical translation as combination therapy with cytotoxic therapies. Specifically, we will Specific Aim 1: Characterize effects of RUVBL1/2 inhibition on DNA damage repair and induction of apoptosis in prostate cancer cells in vitro and patient derived explants ex vivo Specific Aim 2: Evaluate the therapeutic efficacy of combining RUVBL1/2 inhibition with ionizing radiation on DNA damage repair and cell survival of prostate cancer xenografts in vivo Specific Aim 3: Characterize immunostimulatory effects of RUVBL1/2 inhibition in prostate cancer cells in vitro, ex vivo and in vivo using immunocompetent syngeneic tumor models. We expect that our data will establish the preclinical rationale for developing clinical trials with RUVBL1/2 inhibitors in prostate cancer.					
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TABLE OF CONTENTS

	<u>Page</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	9
5. Changes/Problems	9
6. Products	9
7. Participants & Other Collaborating Organizations	9
8. Special Reporting Requirements	9
9. Appendices	9

1. Introduction

Our overarching goal in this grant was to establish the importance of targeting the trinity of kinases involved in DDR in prostate cancer and to evaluate the utility of a RUVBL1/2 inhibitor to block all three kinases in enabling DDR in prostate cancer. This year, we first established the critical importance of targeting all three kinases using unbiased phosphoproteomics, CRISPR based knockdown and targeted inhibitors. We have shown that Compound B, the inhibitor of RUVBL1/2 targets effectively all three kinases in multiple models of prostate cancer and have established the bases for the animal studies in the year ahead. These studies have strengthened the bases for our studies and form the basis for

Specifically, we have:

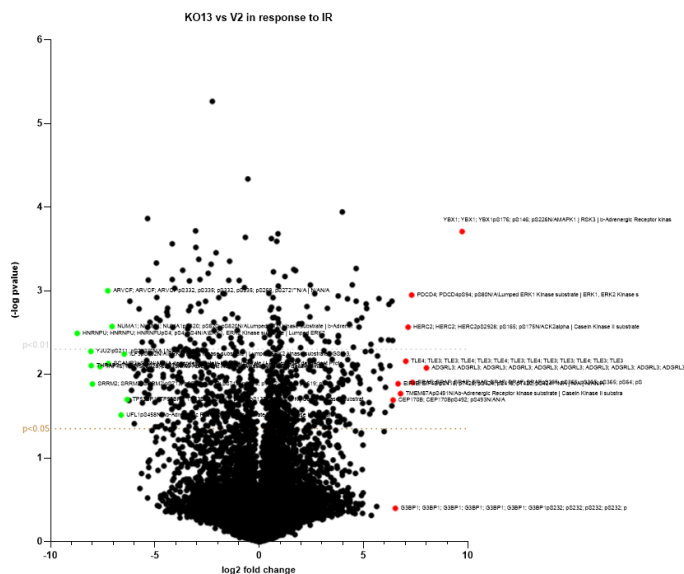
1. Shown using unbiased phosphoproteomics that the key kinase pathways activated in prostate cancer following IR involve ATM. In ATM knockout prostate cancer cells, we have shown that although ATM and its immediate downstream kinases are not activated that the ATM pathways is still intact. Interrogation of kinase pathways activating the downstream ATM pathways indicated that both DNA-PKc and ATR kinases were responsible for maintaining these pathways in ATM knockout cells. These data establish the central importance of DNA-PKc and ATR kinases as key compensatory drivers of DDR in ATM knock out cells. These studies performed in two distinct prostate cancer cell lines incontrovertibly establish the compensatory nature of the three kinases.
2. Using inhibitors of ATM, ATR and DNA-PKc in two different prostate cancer cell line models, we have established that if any of these three kinases is active, then the DDR proceeds to repair DNA damage caused by IR in prostate cancer cells. If all three kinases are blocked, then DDR does not occur and IR causes prostate cancer cell death.
3. Using inhibitors of ATR and DNA-PKc in two different ATM knockout prostate cancer cell line models, we have established that if any of these two kinases is active, then the DDR proceeds to repair DNA damage caused by IR in prostate cancer cells. If both ATR and DNA-PKc kinases are blocked, then DDR does not occur and IR causes prostate cancer cell death.
4. We have validated that Compound B, the inhibitor of RUVBL1/2 targets effectively all three kinases in multiple models of prostate cancer and have established the bases for the animal studies in the year ahead.

2. Keywords

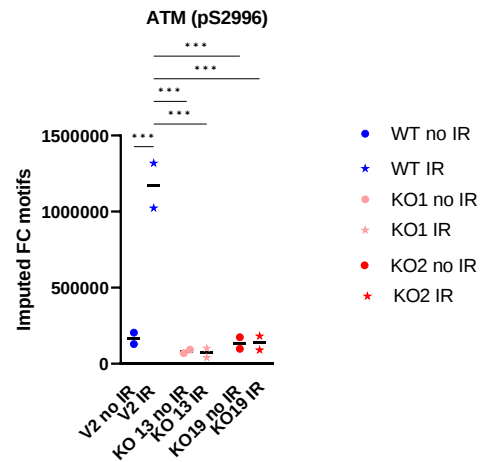
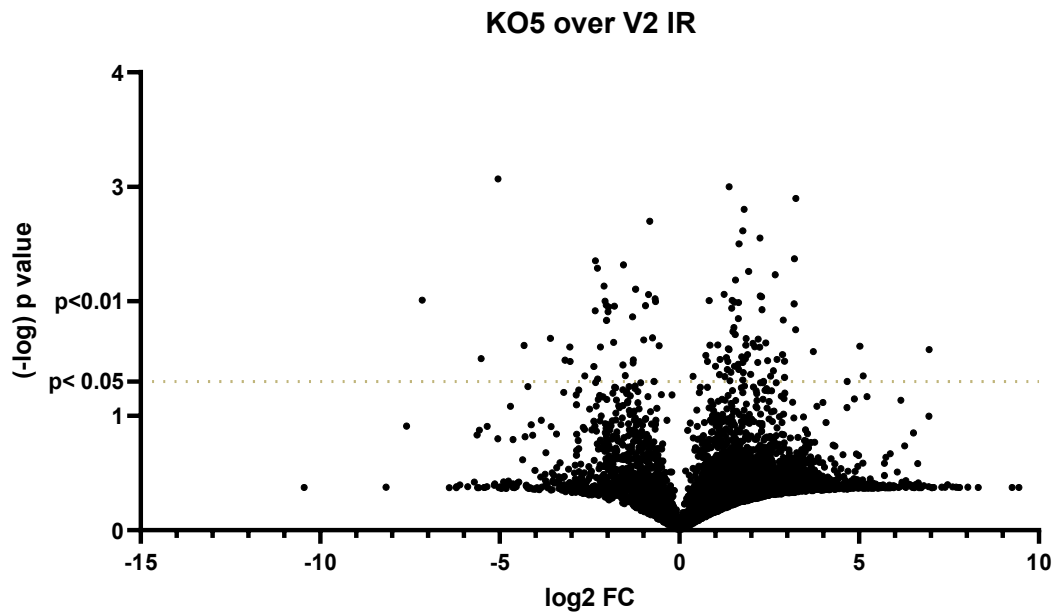
RUVBL1/2 inhibitor, ATR inhibitor, DNA-PKc inhibitor, DDR, DNA damage, Compound B.

3. Accomplishments

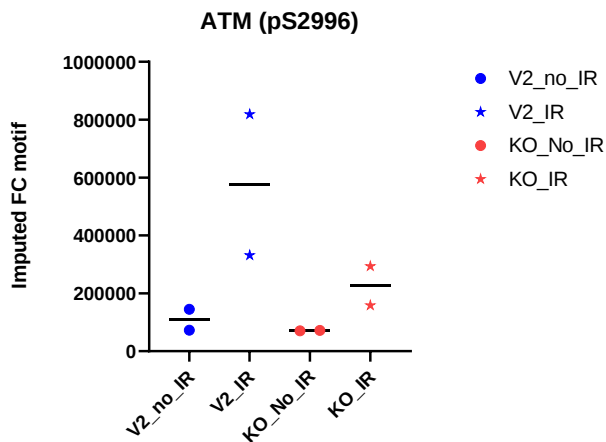
1. We have performed phosphoproteomic screen in response to IR in c4-2 wt and ATM k/o cells.



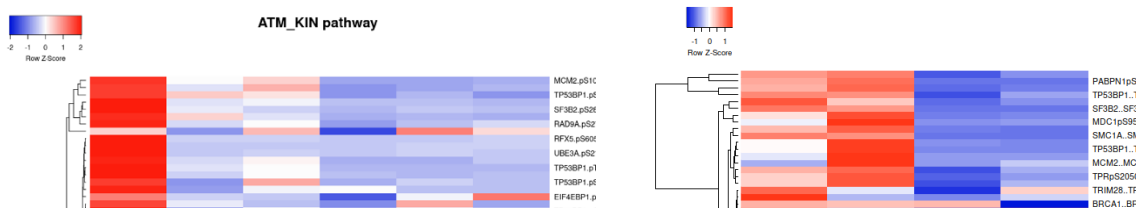
A similar screen in 22RV1 wt and ATM k/o cells shows a similar pattern



In both C4-2 and 22RV1 cells, we first validated ATM k/o

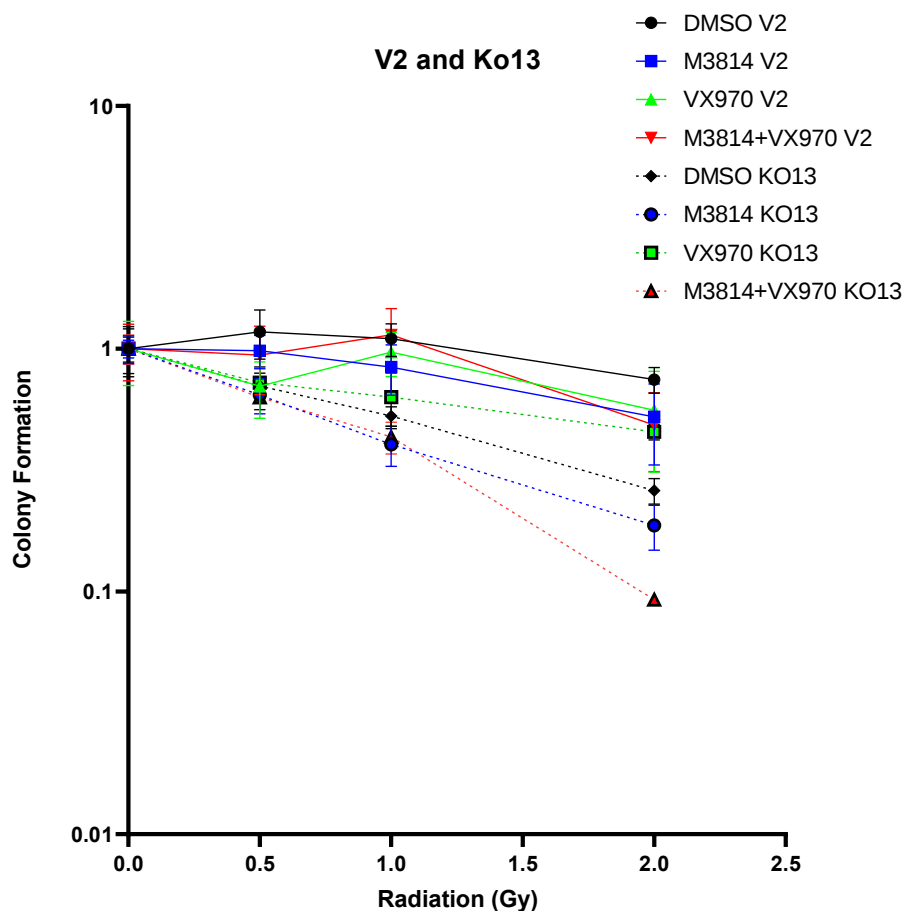


Importantly despite knockdown of ATM, downstream ATM kinase pathways were maintained in both ATM k/o cells similar to ATM wt cells

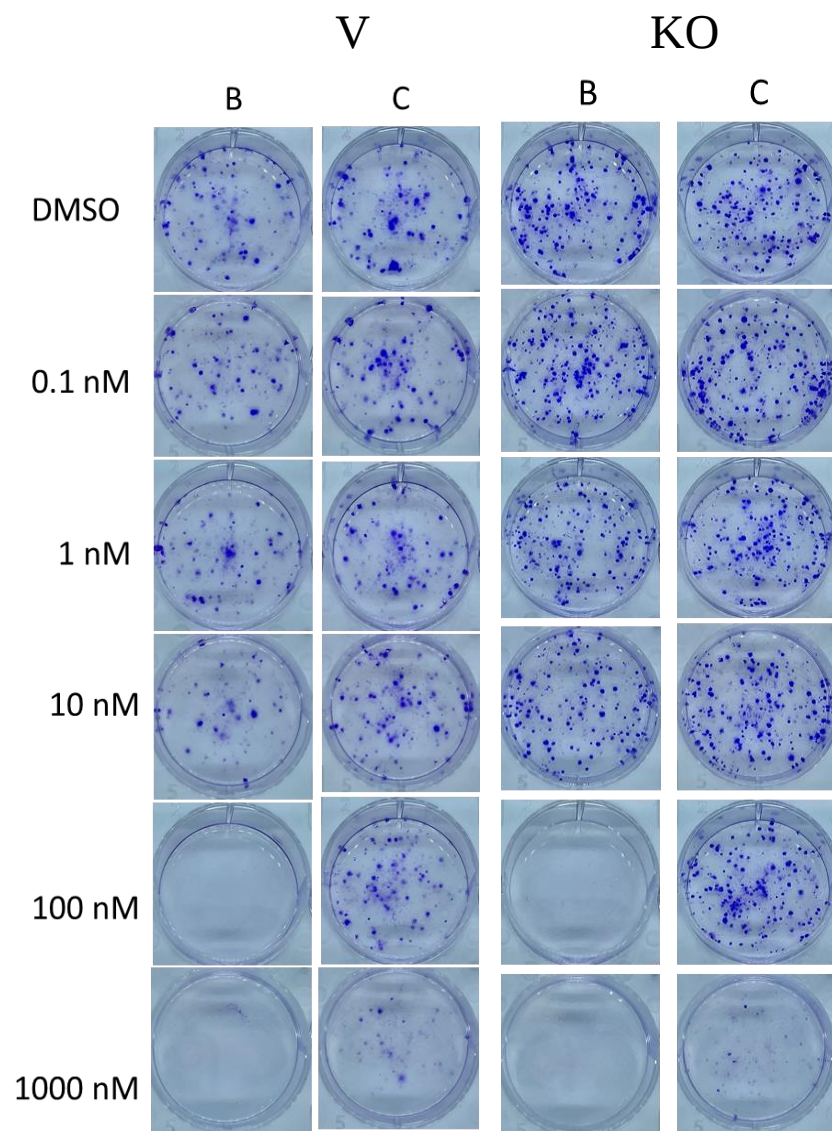


KSEA analyses indicates that DNA-PKcs (multiple motifs) 40/47 (14/16 significantly upregulated with IR) ATR kinase (multiple motifs) 15/47 (8/16 significantly upregulated with IR) were the top compensatory kinases in ATM k/o cells and were important in maintaining ATM downstream kinase pathways. This finding using unbiased phosphoproteomics has validated the primary hypothesis of the proposal that the three kinases play interdependent and compensatory roles in regulating DDR and when one is knocked down, the other two are able to step in and compensate for their activity. These technically challenging analyses have been invaluable in establishing the need for targeting all three kinases to overcome DDR in prostate cancer

2. Using multiple ATR and DNA-PKc inhibitors, we have validated the need for any one of the three kinases to be active in order to mediate DDR in prostate cancer cells. Our initial studies had been with Nu1774 (DNA-PKc inhibitor) and VX-970 (ATR inhibitor). Since data with single inhibitors is only suggestive, we clearly established the need for inhibition of all three kinases using alternative DNA-PKc inhibitors (M-3814 and SN0083) and alternative ATR inhibitors (BAY1895344). With each of these different inhibitors, the same additive effect was noted on clonogenic assays.



We have validated that Compound B, the inhibitor of RUVBL1/2 targets effectively all three kinases in multiple models of prostate cancer including in 22RV1 and C4-2 and with similar activities in wt and ATM knockout cells. Our data establish clearly that Compound B but not Compound C knocks out all three kinases in prostate cancer and with nearly equally efficacy in wt and ATM k/o cells. This finding is critical as it establishes a broader scope of utility of Compound B in prostate cancer that just for ATM k/o. The higher sensitivity in ATM k/o cells suggest that Compound B may be more potent for ATM k/o cells. These data are supported by effectively a similar level of knockdown of DNA-PKc and ATR kinases by Compound B in both cell lines.



In terms of Specific Aims and SOW

Specific Aim 1	SOW Timeline	Status	Site
Major Task: Characterize effects of RUVBL1/2 inhibition on DDR and induction of apoptosis in PCa.			
Subtask 1: Compare efficacy of RUVBL1/2 inhibition to PIKK inhibitors:	1-24	Ongoing, on schedule	R/K
Subtask 2: Compare efficacy of RUVBL1/2 inhibition on sensing, recruitment, kinase cascade and pathway used for DDR	1-24	Ongoing, on schedule	R, K
Subtask 3: Validate that Compound B activity is dependent on inhibiting RUVBL1/2 activity:	7-24	Ongoing, on schedule	K
Milestone(s) Achieved Completion of mechanistic understanding of RUVBL1/2 action on DDR in PCa.		Ongoing, on schedule	R,K
Specific Aim 2			
Major Task: Evaluate therapeutic efficacy of combining Compound B with IR on PCa xenografts in vivo			
Subtask 1: IACUC approval	3	Completed	R
Subtask 2: Evaluation in XDEs:	3-36	on schedule	R
Subtask 3: Evaluation in xenografts:	11-36	on schedule	K
Milestone(s) Achieved Milestone(s) Achieved: Evaluation of Compound B with IR in vivo and ex vivo		on schedule	R,K
Specific Aim 3			
Major Task: Evaluate therapeutic efficacy of combining RUVBL1/2 inhibition with chemotherapy			
Subtask 1: Effect on mCRPC ex vivo	3-26	On schedule	R
Subtask 2: Effect on mCRPC in vivo	13-30	on schedule	K
Subtask 3: Effect on innate immunity	28-36	on schedule	K,R
Milestone(s) Achieved Evaluation of effect of Compound B with chemotherapy		on schedule	R,K

4. Impact

Nothing to Report

5. Changes/Problems

Nothing to Report

6. Products

Nothing to Report

7. Participants & Other Collaborating Organizations

Ganesh Raj, PI – No change

Kittler, Ralf – No change

Ma, Shihong – No longer employed at UTSW – is replaced by Carlos Roggero

Huo, Xiaofang – No change

What other organizations were involved as partners? *-Nothing to Report*

8. Special Reporting Requirements

Nothing to Report

9. Appendices

Nothing to Report