BET inhibition sensitizes preclinical models of bladder cancer to DDR inhibitors

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BACKGROUND

- Bladder cancer (BC) remains a common and deadly malignancy, with a projected 83,190 new diagnoses and 16,840 deaths in the U.S. in 2024.
- According to the MSK/TCGA Bladder Cancer dataset, mutations in DNA damage response (DDR) genes that are part of the homologous recombination (HR) pathway occurred in up to 55% of patients, representing a potential therapeutic target in BC (Figure 1).
- In previous work, we used UNC's EpiG Diamond compound library to show that, as a class of compounds, inhibition of the methyl-lysine reader bromodomain and extra-terminal domain (BET) proteins potently abrogates BC cell line viability.
- Here, we evaluated mechanisms related to DDR inhibition that could explain pan-BET inhibitor OTX-015's potency in BC.

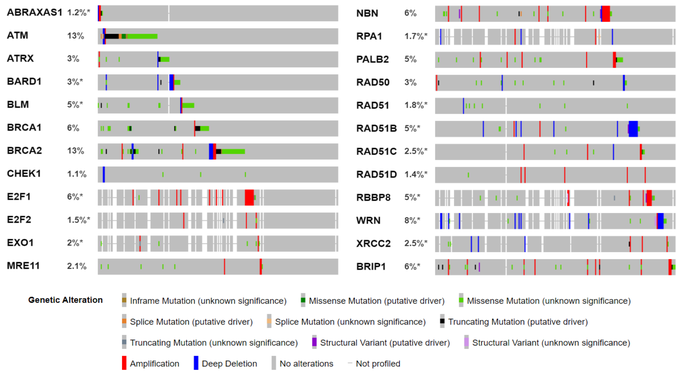

Figure 1: Mutational burden of HR genes in Bladder Cancer patient samples. Figure made using OncoPrint from cBioPortal for Cancer Genomics[1,2], V.4.1.3 (available at: https://www.cbioportal.org/)

METHODS

- 5637 and J82 BC cells were pretreated with OTX-015 for 24 h and then transfected via electroporation with a Cas9 ribonucleoprotein, a gRNA targeting *LBR2* and a 996 bp HR donor.
  - Transfected cells were treated with OTX-015 for 24 h, and DDR pathway function was assessed by digital droplet PCR.
- Cells were treated with increasing doses of OTX-015 (0.1 nM-100 μM), Olaparib, BO-2, RI-1, triapene (100 nM-200 μM), or a combination of the two individual doses.
  - CellTiter-Glo™ (Promega, Madison, WI) assessed viability after 72 h and 96 h incubation.
  - Compusyn v1.0 (ComboSyn Inc., Paramus, NJ) assessed relative synergy of the two compounds using the Chou-Talalay method.[3]
- BC cells were then synchronized to four points in the cell cycle prior to treatment:
  - Asynchronous cells were cultured in normal media for 24 h prior to treatment.
  - Serum starved cells were cultured in serum-free media for 24 h prior to treatment in order to stall progression at the G1/S transition.
  - Thymidine blocked cells were cultured in thymidine-containing media for 18 h, normal media for 9 h, and again in thymidine-containing media for 18 h prior to treatment in order to stall progression in S-phase.
  - Cells were cultured in 9 μM of the CDK1 inhibitor RO-3306 for 20 h prior to treatment in order to stall progression in G2/M.
- After synchronization, 5637 and J82 BC cells were treated with 1 μM OTX-015, 5 μM olaparib, a combination of the two, or 0.1% DMSO control for 48 h.
  - Asynchronous cells were also treated with 1 μM OTX-015 alone for 0-72 h.
  - *POLQ, RAD51,* and *RBBP8* expression were confirmed by RT-PCR in biological triplicates after 48 h incubation, normalized to an *SDHA* housekeeper, and compared to a 0.1% DMSO control.

REFERENCES

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AUTHOR DISCLOSURES

None of the authors of this presentation have any disclosures concerning possible financial or personal relationships with commercial entities that may have a direct or indirect interest in the subject matter of this presentation.

RESULTS

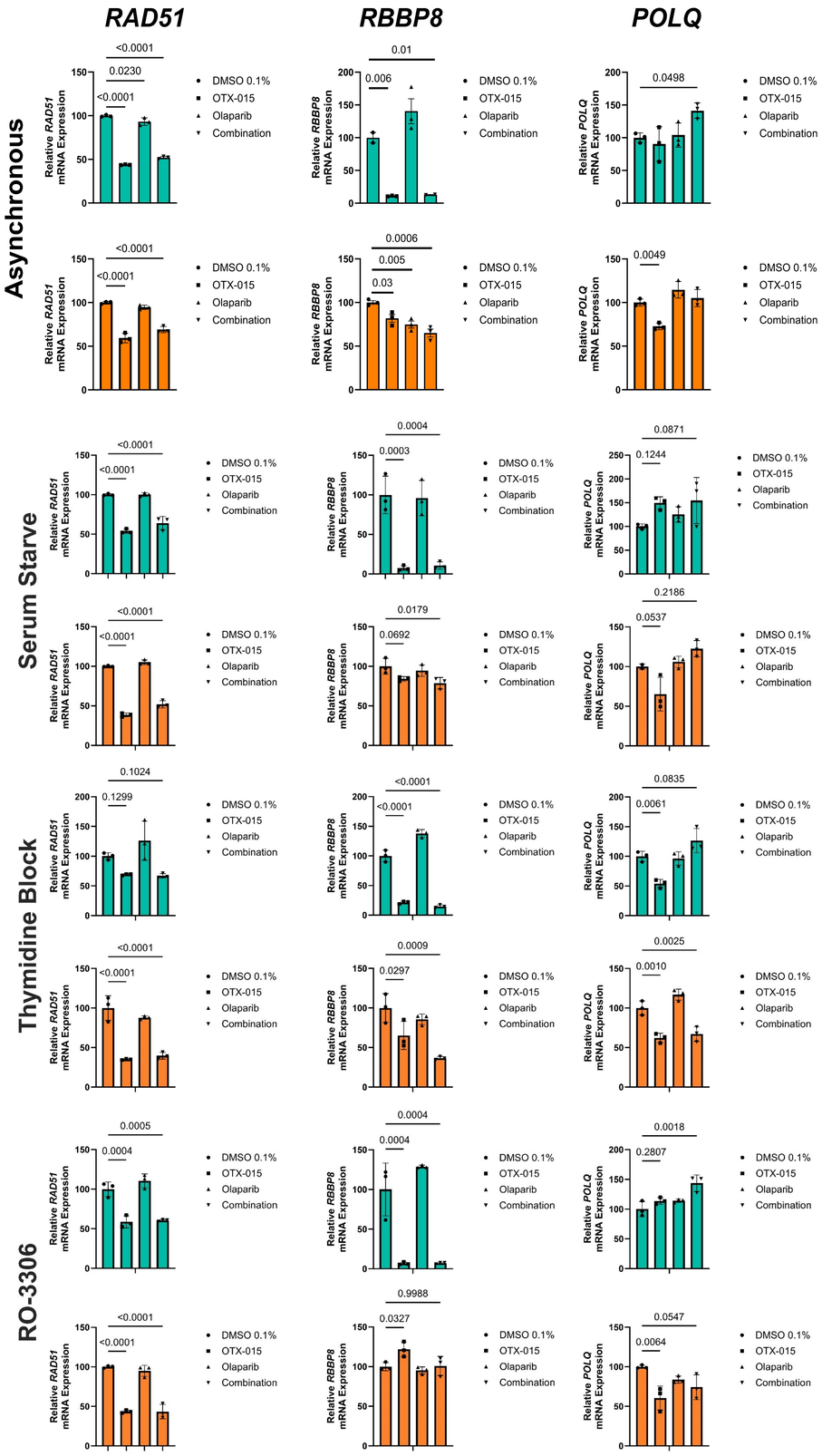

Figure 2: Gene expression changes of DNA Damage Repair genes *RAD51, RBBP8,* and *POLQ* after 48-hour treatment with 1 μM OTX-015, 5 μM olaparib, or combination in both asynchronous and cell-cycle synchronized BC cells. Data shown are mean ± SD, n=3. 5637 cells are represented in teal, J82 cells in orange.

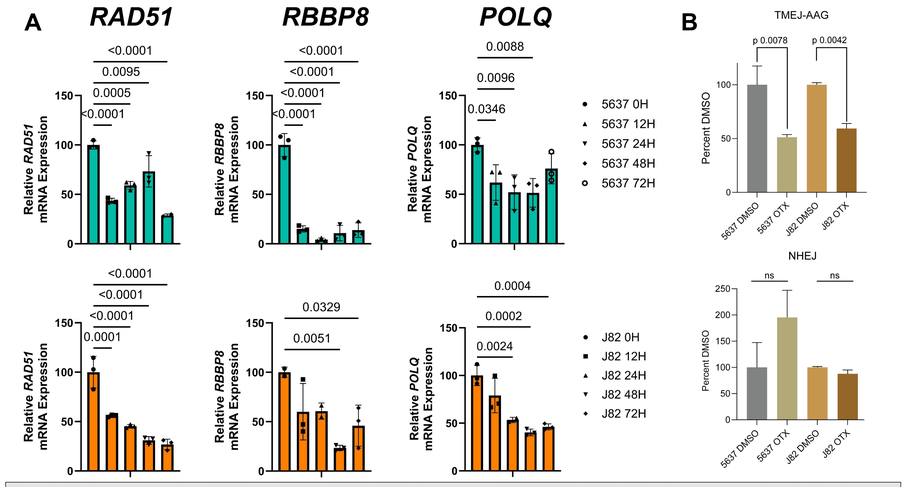

Figure 3: (A) Gene expression data of DDR genes *RAD51, RBBP8, POLQ* after treatment with OTX-015 for 0-72h. 5637 cells are represented in teal, J82 cells in orange. **(B)** Impact of OTX-015 treatment on mechanisms of DDR responses in BC cells. Data shown are mean ± SD, n=3.

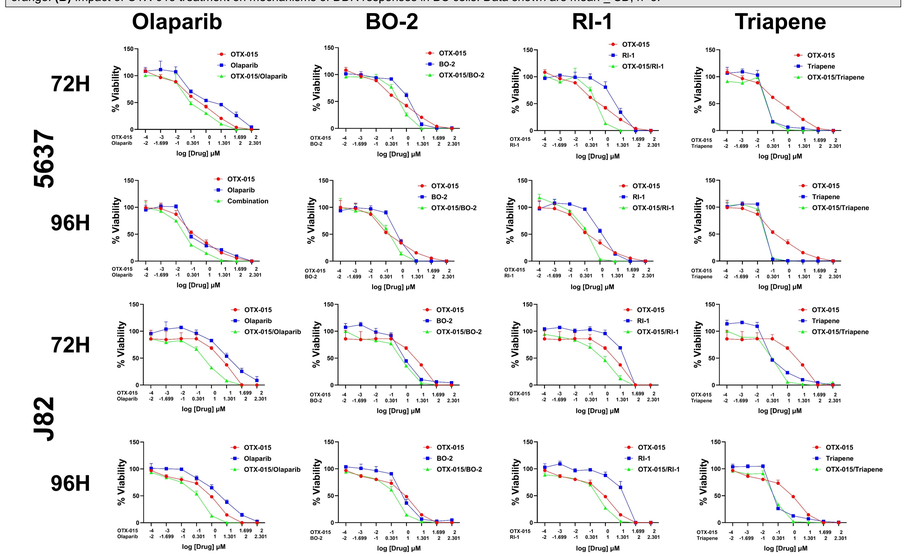

Figure 4: Cell viability data of BC cells after treatment with OTX-015, the PARP inhibitor olaparib, the RAD51 inhibitors BO-2 and RI-1, the CtIP inhibitor triapene, or the combination of OTX-015 and each inhibitor.

Average CI	Olaparib	BO-2	RI-1	Triapene
5637 72H	0.74	<2	<2	0.91
5637 96H	0.93	1.69	<2	<2
J82 72H	0.17	0.49	0.34	0.52
J82 96H	0.38	0.97	1.04	<2

Table 1: Average Combination Index values for each inhibitor combined with OTX-015. Values less than 1 represent synergism and are indicated in green, values greater than 1 represent antagonism, and are represented in red. CI values were calculated using the Chou-Talalay method[3] in Compusyn v1.0.

DISCUSSION AND CONCLUSIONS

- Synchronous and asynchronous BC cells displayed comparable gene expression changes to *RAD51, RBBP8* and *POLQ* after combined PARP+BET inhibition, suggesting a lack of cell-cycle specific effect.
- BET inhibition significantly reduced TMEJ in both 5637 and J82 BC cells but did not significantly impact HR or NHEJ.
  - Despite this, BET inhibition significantly reduced gene expression of HR pathway members *RAD51* and *RBBP8* in a time-dependent manner.
- Combined BET inhibition and PARP inhibition was the most synergistic combination across all time points.
- Asynchronous BC cells treated with combined OTX-015 and olaparib showed significant reduction in gene expression of *RAD51* and *RBBP8.*
  - Of note, adding olaparib caused the OTX-015 mediated *POLQ* repression to be lost.
- **Work is ongoing to characterize protein expression changes in synchronized BC cells.**

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