Synergistic activity of DNA damage response kinase inhibitors in combination with the targeted alpha therapy radium-223 dichloride for metastatic castration-resistant prostate cancer

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The 1st International Electronic Conference on Cancers: Exploiting Cancer Vulnerability by Targeting the DNA Damage Response

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Prostate Cancer - UK

- Prostate cancer is the most common cancer in men.
- More than 47,500 men are diagnosed with prostate cancer every year.
- Every 45 minutes one man dies from prostate cancer.
- 1 in 8 men will be diagnosed with prostate cancer in their lifetime.
- Approximately 400,000 men are living with and after prostate cancer.
223\(^{\text{Ra}}\) Treatment for mCRPC

- Metastatic castrate-resistant prostate cancer (mCRPC) stops responding to hormone treatment and is found in other parts of the body including the bones.
- Bone metastases often leads to pain or skeletal events and, therefore, may decrease the patients’ quality of life.
- Radium-223 (\(^{223}\text{Ra}; \ Xofigo^\circledR\)) is an \(\alpha\)-emitting radionuclide that can incorporate into newly formed bone in areas of osteoblast activity and increase bone turnover surrounding prostate cancer bone metastases.

Low LET Radiation versus High LET Radiation

- High LET particles have a 3-5 times greater relative biological effectiveness compared to X-rays, γ-rays or β-emitters.
- Low LET radiation produces sparse ionization and individual DNA lesions whereas high LET radiation produces clusters and more complex DNA damage.
- The high cytotoxicity of high-LET particle emitters is independent of the dose rate.
- Alpha particles have a high energy and an intermediate path length in biological tissues.

• The ALSYMPCA randomized Phase III trial compared $^{223}$Ra efficacy versus placebo in 921 patients with CRPC and symptomatic bone metastases.

• $^{223}$Ra showed an overall survival benefit in patients with CRPC and symptomatic bone metastases treated with $^{223}$Ra compared with patients who received placebo (14.9 months vs 11.3 months).

• Patients treated with $^{223}$Ra also had a longer time to symptomatic skeletal events (15.6 months vs 9.8 months) and a better biological response.
Relative prevalence of DNA repair mutations in prostate cancer patients

- Currently, it is reported that the prevalence of Homologous Recombination (HR) mutations in primary prostate cancer is likely between 8–10% and in mCRPC is likely between 20–25%.
- The HR pathway genes commonly mutated include BRCA1, BRCA2, CHEK2, ATM, RAD51D, AND PALB2.

<table>
<thead>
<tr>
<th></th>
<th>Localized PCa</th>
<th>MPCa</th>
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<tbody>
<tr>
<td>Homologous Recombination Pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td>2-3%</td>
<td>7-8%</td>
</tr>
<tr>
<td>ATM</td>
<td>2-4%</td>
<td>5-6%</td>
</tr>
<tr>
<td>PALB2</td>
<td>&lt;1%</td>
<td>1-2%</td>
</tr>
<tr>
<td>BRCA1</td>
<td>1%</td>
<td>1%</td>
</tr>
<tr>
<td>CHEK2</td>
<td>&lt;1%</td>
<td>1-2%</td>
</tr>
<tr>
<td>RAD51D</td>
<td>1-2%</td>
<td>3-4%</td>
</tr>
<tr>
<td>CDK12</td>
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<tr>
<td>Mismatch Repair Pathway</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>&lt;1%</td>
<td>1%</td>
</tr>
<tr>
<td>MSH2</td>
<td>&lt;1%</td>
<td>2-3%</td>
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<tr>
<td>MSH6</td>
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</tr>
<tr>
<td>PMS2</td>
<td>&lt;1%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Overall</td>
<td>8-10%</td>
<td>20-25%</td>
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</table>

Efficacy of $^{223}$Ra in mCRPC with and without Homologous Repair Gene Defects


- Patients who harbour homologous recombination mutations may have a greater clinical benefit from $^{223}$Ra.
- Patients with homologous recombination mutations showed greater ALP responses (80% vs 39%), longer time to ALP progression (median 10.4 vs 5.8 months) and longer overall survival (median 36.9 vs 19.0 months).
DNA damage response pathways and targets for inhibition
Aims

1. To determine the response of prostate cancer cell lines to different types of radiation (X-rays, and $^{223}$Ra) and whether an ATR inhibitor increases this response.

2. Determine the response of prostate cancer cells with HRD mutations to $^{223}$Ra and whether the addition of DDR inhibitors increases this response.
Radiosensitivity of prostate cancer cell lines to different doses of X-rays and $^{223}\text{Ra}$

**Graph Description:**
- **X-axis:** Dose (Gy)
- **Y-axis:** % Surviving Fraction
- **Graph Lines:**
  - PC3 X-ray
  - DU145 X-ray
  - PC3 $^{223}\text{Ra}$
  - DU145 $^{223}\text{Ra}$
- **RBE Calculations:**
  - $\text{RBE}_{0.5} = \sim 10.02$
  - $\text{RBE}_{0.5} = \sim 15.25$

**Textual Description:**
- **RBEs at 50% survival of Radium-223 (High LET) are > 5 times more effective in inducing cell death compared to X-rays (Low LET).**

**Acknowledgments:**
- Dunne *et al.*, in preparation
- Prostate Cancer UK
- LFT Charitable Trust
- The Patrick G Johnston Centre for Cancer Research
Radioisensitisation response to $^{223}$Ra or X-rays in combination with ATR kinase inhibition

**DU145**

**PC3**

**RWPE-1**

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>PC3</th>
<th>DU145</th>
<th>RWPE-1</th>
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<tbody>
<tr>
<td>Treatment Group</td>
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<tr>
<td>X-Rays vs X-Rays + AZD6738</td>
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<tr>
<td>$^{223}$Ra vs $^{223}$Ra + AZD6738</td>
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<td>$^{223}$Ra vs $^{223}$Ra + AZD6738</td>
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</tr>
<tr>
<td>RER</td>
<td>1.33</td>
<td>12.83</td>
<td>2.74</td>
</tr>
<tr>
<td></td>
<td>12.09</td>
<td>1.33</td>
<td>2.01</td>
</tr>
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Dunne et al., in preparation
DNA damage response to $^{223}$Ra or X-rays in combination with ATR kinase inhibition

**DU145**

**PC3**

**RWPE-1**

Dunne et al., in preparation
The effect of $^{223}$Ra or X-rays in combination with ATR kinase inhibition on cell cycle distribution

**DU145**
- Sub G1
- G1
- S
- G2/M

**PC3**
- Sub G1
- G1
- S
- G2/M

**RWPE-1**
- Sub G1
- G1
- S
- G2/M

Cleaved PARP

β-actin
The effect of $^{223}$Ra or X-rays in combination with ATR kinase inhibition on mitotic catastrophe

A

B

Kalamida et al.
Summary

- This study evaluates the impact of combining the ATR inhibitor AZD6738 with $^{223}$Ra to investigate whether a greater radiosensitisation response occurs in comparison to standard X-rays.

- DDR inhibitors in combination with $^{223}$Ra significantly enhanced radiosensitivity ($p < 0.001$) response in comparison to combined treatment with X-rays in prostate cancer cell lines and to a lesser extent in a normal epithelial prostate cell line. Additionally, a greater quantity of residual DSBs at 24 hours post combination treatment was observed after $^{223}$Ra exposure in comparison to X-ray exposure in PC3 and DU145 cells but not normal RWPE-1 cells ($p < 0.001$).

- Cell cycle analysis indicates that either X-ray or $^{223}$Ra treatment combined with AZD6738 abrogated the G2/M cell cycle checkpoint, with $^{23}$Ra in combination with AZD6738 inducing a large Sub G1. Further analysis indicates that cell death was apoptotic as evidenced by PARP cleavage.

- Our findings strongly support the combination of DNA damage induction by $^{223}$Ra with an ATR inhibitor as a novel potential treatment option for mCRPC patients in order to improve clinical outcome.
**Aim:** Determine the response of prostate cancer cells with HRD mutations to $^{223}$Ra and whether the addition of DDR inhibitors increases this response.

**In vitro**
- Clonogenics, DNA damage kinetics, cell cycle distribution, mitotic catastrophe

**In vivo**
- Subcutaneous implant to investigate response to different radiation modalities in combination with DDR kinase inhibitors

Dunne *et al.*, in preparation
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