Identifying Mechanisms Of Sensitivity And Resistance In Response To LuTate PRRT

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Aim 1: To identify genes related to sensitivity and resistance to PRRT
Aim 2: To validate target genes and identify novel combination treatments to overcome resistance to PRRT

Primary and secondary resistance to Peptide Receptor Radionuclide Therapy (PRRT) using Lu-177 DOTA-octreotate (LuTate) for neuroendocrine tumours (NET) limits its effectiveness and durability.
Hypothesis: Resistance to LuTate PRRT is mediated through enhanced recognition and repair of radionuclide-induced DNA damage - rather than loss of the PRRT target, somatostatin receptor-type 2 (SSTR2).
DNA-damage repair (DDR) pathways are critical for sensing and repairing radiation-induced DNA damage and ultimately directing cell fate. Accordingly, DDR inhibitors could provide for clinically relevant treatment options.
The combination of a PARP inhibitor (limiting repair of single-strand DNA breaks) with PRRT improved outcomes in animal models (Cullinane et al, 2020).
PRRT resistance does not result from loss of the target, SSTR2, as shown in our animal models of resistance generated through repeated LuTate treatment.

■ Whole-Genome CRISPR Screen ■ DNA Damage Repair Inhibition ■ LuTate Resistance ■

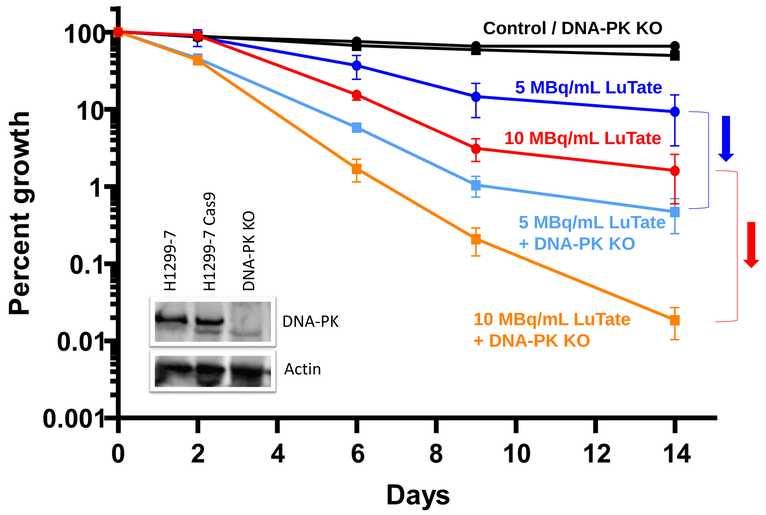
Using CRISPR to identify genes involved in Sensitivity and Resistance to PRRT

Genes important in the response to PRRT were identified through an unbiased whole genome knockout CRISPR screen. H1299-7 cells were treated with 5MBq/mL LuTate, and upon sequencing, at Day 21 post treatment, we were able to identify a selection of genes that when knocked out, resulted in sensitivity or resistance to LuTate. The DDR pathway was identified as the most significant pathway important to sensitivity and the most significant gene, DNA-PK. Fewer genes were identified as being involved in the development of resistance to LuTate, with the most significant gene being Beta-Arrestin 2, and no pathways identified.

Sensitivity – DNA-PK

DNA-PK is a crucial component of a cell's DDR pathway, essential in the activation of the non-homologous end-joining pathway (NHEJ) used to detect and repair DNA double-strand breaks. To validate whether loss of DNA-PK increased sensitivity to LuTate, a single gene knockout cell line (H1299-7 DNA-PK KO) was derived using CRISPR technology (see western blot, right).

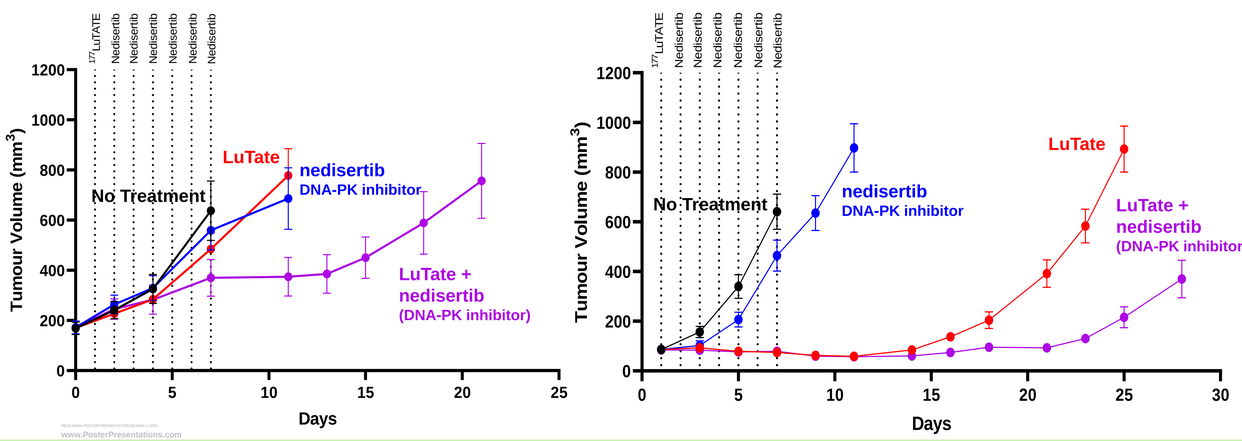
Treating the H1299-7 and DNA-PK KO cell lines with increasing concentrations of LuTate (5MBq and 10MBq/mL) and tracking cell growth over a 14 day period, showed that loss of DNA-PK significantly increased sensitivity to LuTate in a dose dependent manner (right).



Additionally we have taken two DNA-PK inhibitors, nedisertib and AZD-7648, and shown that inhibition of DNA-PK sensitizes cells to LuTate therapy to the same degree as seen above with the knockout cells.

Inhibiting DNA-PK Sensitizes Tumours to LuTate Therapy

In both the H1299-7 (below, left) and the AR42J (below, right) *in vivo* models the combination of nedisertib and LuTate increased survival and delayed tumour regrowth compared to either treatment alone. Tumour bearing mice were treated with a single dose of 6-8MBq of Lutate, followed by 6 days of 150mg/kg nedisertib starting 24hr post LuTate treatment.

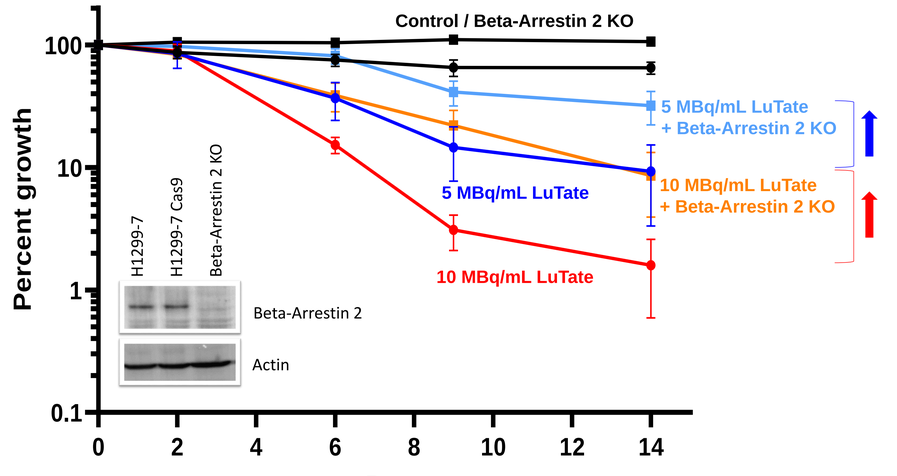


Resistance – Beta–Arrestin 2

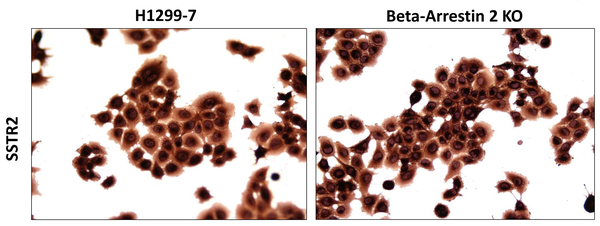
Beta-Arrestin 2 is a scaffolding protein involved in the regulation of the internalization and recycling of G protein-coupled receptors, including SSTR2, the receptor target for LuTate PRRT. In our CRISPR screen loss of Beta-Arrestin 2 resulted in resistance to LuTate. To validate this response, single gene CRISPR technology was used to derive the H1299-7 Beta-Arrestin 2 KO cell line (see western blot, below).

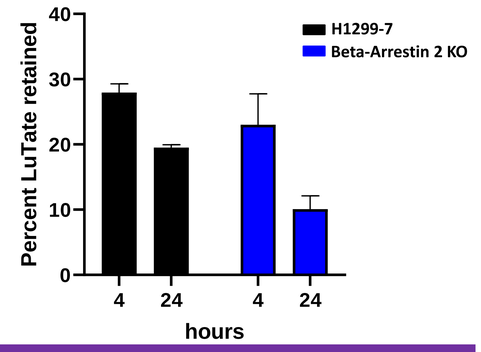
Response to LuTate therapy in the H1299-7 and Beta-Arrestin 2 KO cells was determined by treating the cells with increasing concentrations of LuTate (5MBq and 10MBq/mL) and tracking their growth over 14 days. The knockout of Beta-Arrestin 2 resulted in significant resistance to LuTate (right).

Given the role of Beta-Arrestin 2 in SSTR2 internalization and recycling we have assessed the expression of SSTR2 in both cell lines *in vitro*, and also the ability of the Beta-Arrestin 2 KO cells to retain LuTate after treatment.



No difference in SSTR2 expression was observed in the H1299-7 and Beta-Arrestin 2 KO cells (below). While the Beta-Arrestin 2 KO cells showed a reduced ability to retain LuTate, after cells were treated with 10MBq/mL LuTate for 4hrs, and collected at 4hr and 24hr (right).





Conclusions and Future Work

DNA-PK inhibition is a valid target for **increasing sensitivity** to LuTate PRRT. We are now testing this approach in tumours and cells that are resistant to LuTate therapy.

Loss of Beta-arrestin 2 results in **resistance** to LuTate *in vitro.* However, Beta-arrestin 2 loss doesn't impact SSTR2 expression. We are investigating its role in localization and recycling of the SSTR2 receptor after ligand binding as a possible mechanism of resistance.

Acknowledgements

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