Targeting Auger electrons to cellular nuclei via PARP-1
Short Title: Targeting Auger electrons to PARP-1

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INTRODUCTION: Auger emitting radionuclides have promising therapeutic properties due to their high linear energy transfer with nanometer ranges in tissue, although they have proven challenging to work with since they must be targeted to cellular nuclei to directly cause DNA damage. In the present study, we sought to utilize PARP-1 as a molecular target for the delivery of Auger electrons to induce local DNA damage without altering PARP-1 enzymatic activity. PARP-1 is an abundantly expressed nuclear protein involved in the DNA damage response and the primary target of emerging therapeutics for the treatment of homologous recombination deficient (HRD) ovarian cancers.

METHODS: In-vitro cytotoxicity studies were performed by treating BRCA1 deficient ovarian carcinoma cell lines (UWB.1289 and OVCAR8) with various doses of [125I]KX1 or [123I] KX1, a PARP inhibitor analogue. To understand the specificity of KX1, the same treatments were given to matching cell lines that either were engineered to have a restored HRD status via BRCA1 expression (UWB1.289 BRCA1-restored), or PARP1 knockout via CRISPR/cas9 genome editing (OVCAR8 PARP1-knockout). Next, cellular immunofluorescence was performed and DNA damage was assessed by measuring GH2AX, 53bp1, PAR, and PARP-1 levels at 2 or 24 hours after treatment with [125I]KX1 or [123I]KX1.

RESULTS: Cytotoxicity data revealed a dose dependent reduction in cell viability in both BRCA1 HRD cell lines after treatment with either Auger emitter. Cell death in UWB1.289 BRCA1-restored and OVCAR8 PARP1-knockout cell lines was reduced when compared to identical treatments in parent cell lines. Immunofluorescence analysis showed a dose dependent increase in GH2AX and 53bp1 foci as early as 2 hours post treatment in OVCAR8. Similar to the cytotoxicity data, GH2AX foci formation was reduced in OVCAR8 PARP1-knockout. Interestingly, treatment with radiolabeled [123I]KX1 did not reduce cellular ribosylation (PAR) as seen with the treatment of a commercially available PARP inhibitor, but resulted in an increase from control.

CONCLUSIONS: Auger emitting radionuclides delivered to nuclei via PARP-1 can cause significant DNA damage. Reduced cytotoxicity and GH2AX foci observed after treating OVCAR8 PARP1-knockout cell lines demonstrated that both Auger emitters caused DNA damage as a result of specifically targeting PARP-1. Furthermore, these agents did not inhibit the enzymatic activity of PARP-1 and may have the potential to elicit neo-antigenicity through DNA damage without altering the pro-inflammatory pathways regulated by ribosylation.