Title
Examining the efficacy of various novel radiotherapies on prostate cancer cell lines

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Publication Date
2021

Peer reviewed|Thesis/dissertation

Examining the Efficacy of Various Novel Radiotherapies on Prostate Cancer Cell Lines

by
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THESIS
Submitted in partial satisfaction of the requirements for degree of
MASTER OF SCIENCE

in

Biomedical Imaging

in the

GRADUATE DIVISION
of the
UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

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Acknowledgements

I am honored to have had the privilege of completing my thesis for MSBI. I had a wonderful time working at a cancer research lab for the summer and many people contributed to this delightful experience. Dr. Robert Flavell has been a terrific advisor and provided great guidance over the past few months. Dr. Anil Bidkar was my direct mentor and was an absolute pleasure to work with. The other members of the group, Dr. Kondapa Naidu Bobba, Dr. Sinan Wang, and Dr. Niranjan Meher, helped tremendously with my projects. I want to also give a big thanks to my committee members, Dr. Henry VanBrocklin, Dr. Youngho Seo, and Dr. Michael Evans for steering me in the right direction this summer.

Examining the Efficacy of Various Novel Radiotherapies on Prostate Cancer Cell Lines

Bhanu Bucchireddigari

September 1, 2021

Abstract

Current treatments for prostate cancer, such as chemotherapy and androgen receptor signaling inhibitors, have varying success rates with unpredictable outcomes. As a result, there is a need for the development of novel targeted therapies which can provide positive responses from patients. Radioligand therapy (RLT) in combination with poly (ADP-ribose) polymerase (PARP) inhibitors has shown efficacy in preliminary in vitro studies. In this study, we have used RLT with beta emitter (177-Lu) and alpha emitters (227-Th and 225-Ac) in combination with PARP inhibitors (Niraparib and Talazoparib). The cell viability assays for combination treatment of 225Ac-YS5 and Talazoparib showed a promising ZIP synergy score of 25.35, indicating that the two work synergistically to provide better therapeutic outcomes. However, these results are preliminary and will require further confirmation with both in vitro and in vivo models.

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1 Introduction

1.1 Prostate Cancer

After a diagnosis of prostate cancer, the primary treatment involves androgen deprivation therapy. The progression of prostate cancer relies on activating the androgen receptor (AR) gene, suppressing the ligands, androgens such as testosterone and dihydrotestosterone is crucial to slow down metastasis (Haapala, 2007). This can be done through procedures such as orchiectomy or luteinizing hormone-releasing hormone (LHRH) therapy. On average, this works for only about 18 - 36 months until resistance develops, leading to metastatic castration-resistant prostate cancer (mCRPC) (Kim, 2021). There are numerous treatment options for mCRPC, such as androgen signaling inhibitors (abiraterone/enzalutamide) and radionuclide therapy. However, these treatments have a very mixed success rate, and the patient response is unpredictable. As a result, there is an unmet need for a novel radioligand therapy for mCRPC, which measurably improves the outcomes for patients (Kim, 2021).

1.2 Radioligand Therapy (RLT)

RLT is an emerging therapeutic approach for prostate cancer that overcomes the limitations associated with conventional therapeutic approaches (Heck, 2017). Recently approved RLTs including 177Lu-DOTATATE, 131I-metaiodobenzylguanidine, and Zevalin have shown promising results for the treatment of other cancer metastases (Chan, 2020). For prostate cancer in particular, there have been recent identification of new targeting moieties which has further increased the efficacy of RLTs. In RLT, a cytotoxic radioisotope is precisely delivered to the tumor site. For instance, prostate-specific membrane antigen (PSMA) is a promising biomarker for prostate cancer, and thus a target for RLT. However, recent studies have shown that PSMA is not

uniformly expressed and may even be absent in some cases. As a result, a different target was identified, CD46, which seems to play a role in immune evasion, and is highly expressed in metastatic prostate cancer (Rosellini, 2021). Additionally, CD46 surface expression is upregulated after AR targeting therapies, which is advantageous because many patients will likely have undergone AR inhibition drug courses. One drawback, however, is that CD46 expression can also occur in background tissue, such as in the GI tract. To overcome this, the YS5 antibody, which binds specifically to a conformational epitope on CD46 from cancer cells, is used. In this study, this antibody will be labeled with beta emitter 177-Lu, 227-Th, or 225-Ac, forming our experimental radioligand.

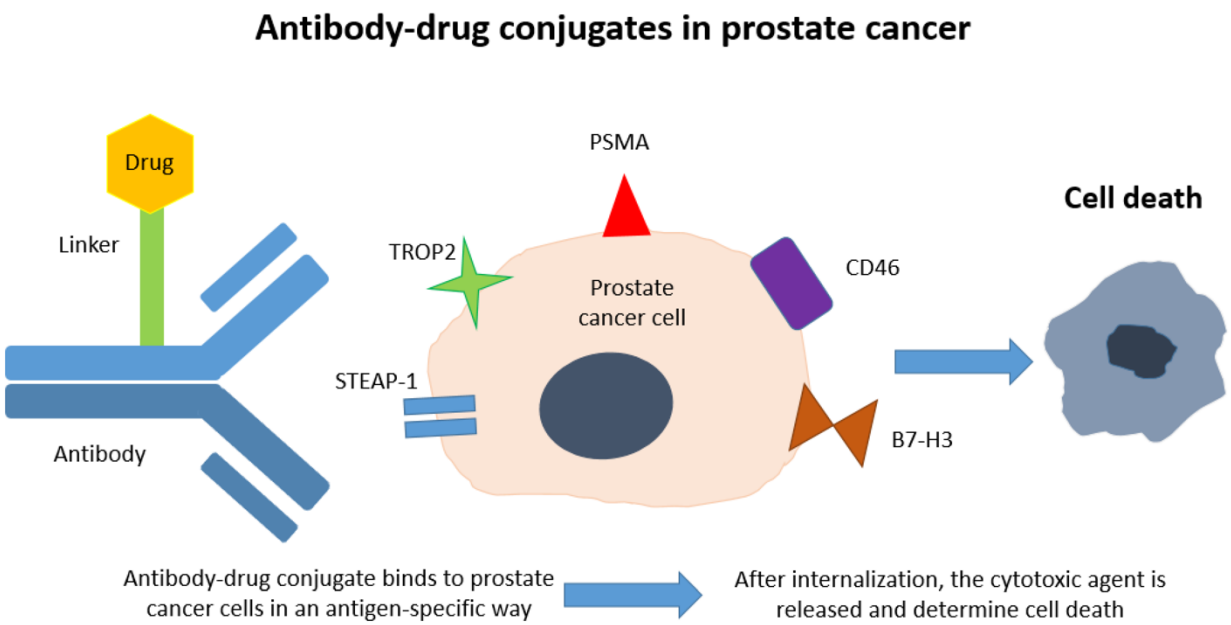

Figure 1: Schematic of Antibody-Drug/Radioisotope Conjugate (Rosellini, 2021)

1.3 Beta Emitters

The negatively charged electrons emitted from the nucleus of decaying radioactive atoms are called beta particles, which can have various ranges of energy of 50–2,300 keV. However, these beta particles have a low linear energy transfer (0.2 keV/μm) and have a relatively long range

(Kassis, 2008). As these beta particles travel through matter, they lose their kinetic energies, follow a twisted path, and eventually come to stop. During the course, beta particles can damage DNA by causing single or double stranded breaks, modify base chemicals and cause formation of protein crosslinks (Czerwińska, 2020). Because beta particles have a long range in tissue (1-10mm) they can cause cytotoxicity in surrounding healthy cells which were not targeted – known as the crossfire effect. This effect may bring some advantage in the sense that the radionuclide agent may not be required to enter the cancer cell to cause death. As a result, this will be useful in the treatment of large tumors. Ultimately, however, this will damage normal tissue as well. In this project, we are using Lutetium-177 as our beta-emitting radioisotope.

1.4 Alpha Emitters

Alpha emitters are positively charged and have the same mass and charge as helium. Alpha particles have an energy range of 5 to 9 MeV, which is much greater than the energy of beta particles, and have a high linear energy transfer of about 80–100 keV/µm (Kassis, 2008). As a result, they are heavier and only travel in a straight line a short distance of 50–100 µm, the length of a few cell diameters. Therefore, alpha emitters can be used to target smaller tumors. In these experiments, we used Thorium 227 and Actinium 225 as our alpha emitters.

1.5 PARP Inhibitors

Mutations in DNA damage repair pathways contribute to prostate cancer growth and progression. These mutations are known to be more incident in cases of metastatic prostate cancer. When DNA single-strand breaks occur, they are repaired by PARP enzymes (Jacob, 2020). PARP inhibitors (PARPi) block the catalytic activity of these enzymes and as a result, the single-strand breaks progress into double-strand breaks. Typically, double-strand breaks are repaired through homologous recombination in healthy cells. However, cancer cells which lack this capability

would not be able to repair, leading to cell death. Consequently, this drug allows us to target and kill cancer cells.

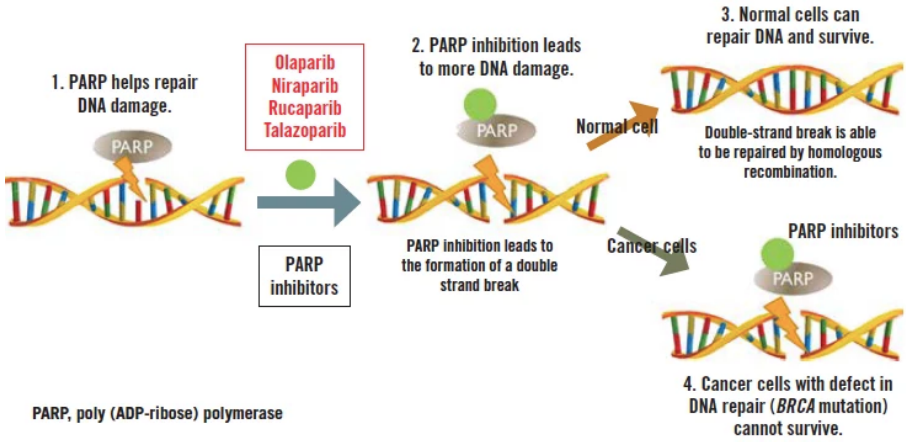

Figure 2: Mechanism of PARP inhibition (Jacob, 2020).

1.6 Combination of RLT and PARPi

Radioligand therapy (RLT) involves using radiopharmaceuticals conjugated to the targeting ligands or antibody. β, α, or Auger electron-emitting radionuclides exert their effect by damaging the target cells. PARPi or DNA damaging molecules have been shown to radiosensitize the target cells for RLT. Because the RLT mainly works by inducing DNA damage inside the nucleus, the effect can be significantly increased using PARPi (Chan, 2020).

Because preliminary data from previous studies suggest a synergistic combination, we aim to develop a combination of CD46 radioligand therapy and PARPi. In this study, we will investigate the cellular response of the PARPi alone, and then the therapeutic action of the radioligand alone. Finally, we will test the synergistic action of the combinations of both PARPi and radioligand.

1.7 External Beam Radiation Therapy (EBRT)

External beam radiation therapy is another method to treat patients with localized or locally advanced prostate cancer. Modern radiotherapy instruments allow for precise targeting of cells;

however, as with other therapies, it is still a challenge to effectively target the tumor without harming healthy tissue. EBRT induces DNA damage by causing double stranded DNA breaks. One study found an interesting correlation where irradiation led to an increase in surface CD46 levels in two cell lines (Pokrovska, 2020). We hypothesized that we would see a similar increase in our cell lines, 22Rv1 and DU145 after EBRT.

2 Methods

2.1 Cell Culture

Human embryonic kidney cells (22Rv1), lung cells (DU145) were maintained in RPMI medium supplemented with 10% (v/v) fetal bovine serum and 1% penicillin & streptomycin at 37°C in humidified air containing 5% CO_2.

2.2 Preparation and Characterization of Chelator

The three experiments with Lu-177, Th-227, and Ac-225 all had a very similar preparation process. For Lu-177, The YS5 antibody was conjugated to DOTA-SCN in the first step. Briefly, 5 mg of YS5 (in 0.1M NaHCO3, pH 9) was incubated with p-SCN-Bz-DOTA at 1:10; and 1:20 molar ratios for 30 min at 37°C. Following this, the reaction mixture was passed through the PD10 column to purify the DOTA conjugated YS5. This YS5-DOTA antibody will be used for 177-Lu labelling. The number of the DOTA molecules on antibody was determined by taking mass spectrometry of the YS5 and YS5-DOTA antibodies.

For the Th-227 and Ac-225 experiments, in 0.1M Na2CO3-NaHCO3 buffer pH 9, YS5 antibody was incubated with HOPO-NCS for Th-227, and DOTA-NCS for Ac-225, in 12 uL DMSO at 37 °C for 2h. The reaction was purified with PD10 eluting with 0.25 M sodium acetate solution at pH 6.0.

2.3 Labelling of YS-5 Antibody with Radioisotopes

The 177-Lu was mixed with YS5-DOTA 0.5M NH_4OAc (pH-5.5) buffer at 37˚C for 30 min. The labelling conditions for Th-227 with HOPO-YS5 and Ac-225 with DOTA-YS5 are 37 °C, 30 min – 1 hr, 1M NH_4OAc buffer, pH = 5.5 Afterward, an iTLC was performed to confirm the labeling of the YS5 with the radioisotope.

2.4 Cell Viability Assay

The cytotoxicity of the radioisotope conjugates, PARPi, and their combinations were studied with an MTT assay. Initially, the 22Rv1 and DU145 cells will be seeded at the density of 500 cells/well in 96 well plates. The cells will be treated with the following concentrations of the radioactivity and PARP inhibitor separately. Following the treatment for a duration of 96 hours, cells will be incubated with MTT dye. Thereafter, 150 µL of DMSO will be added to each well. The absorbance of each well was measured at 570 nm for dissolved formazan crystals. The cell viability was determined by assuming 100% viable cells in untreated wells. Inhibitory concentrations (IC) of the drugs were determined by fitting the data to the standard dose-response curve.

2.5 Combination Study and ZIP Analysis

ZIP analysis was performed from the cell viability results obtained in these experiments to confirm the synergy and calculate the combination index values.

2.6 External Beam Radiation Therapy (EBRT)

For colony forming assay, 22Rv1 cells were seeded at the density of 500 cells per well in six well plate. These six well plates were irradiated with 0, 2, 4, and 6 Gy with XRAD-320 irradiator. Subsequently, the treated cells were allowed to grow for two weeks. The colonies were fixed with formaldehyde, stained with crystal violet, and counted. The cell viability of the irradiated cells was also recorded using MTT assay separately. For viability assay, cells were treated with 2, 4, 6, 8, 10, 12, 15, 20, 25 Gy, and the MTT assay was carried out after 96 hour incubation.

3 Results

3.1 Conjugation of the Antibody and Chelator:

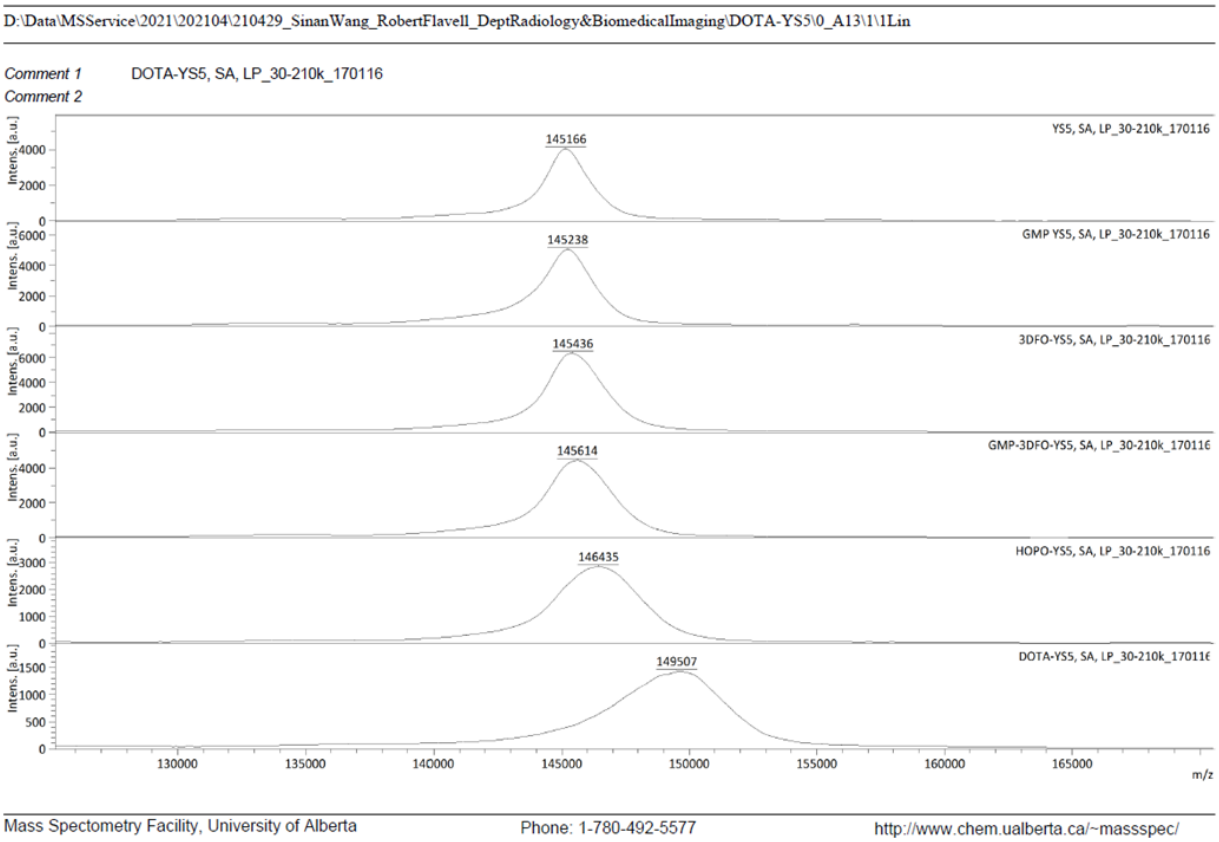

Figure 3: Mass spectrometry of YS5-HOPO and YS5-DOTA conjugation

This mass spectrometry result allows for the confirmation of the conjugation of the chelator to the YS5 antibody. The ratio of mass-to-charge indicates whether the conjugation occurred. We can see that the HOPO-YS5 sits at 146435 m/z while DOTA-YS5 is at 149507 m/z.

3.2 Confirming Labelling of Radioisotope to Antibody Conjugate

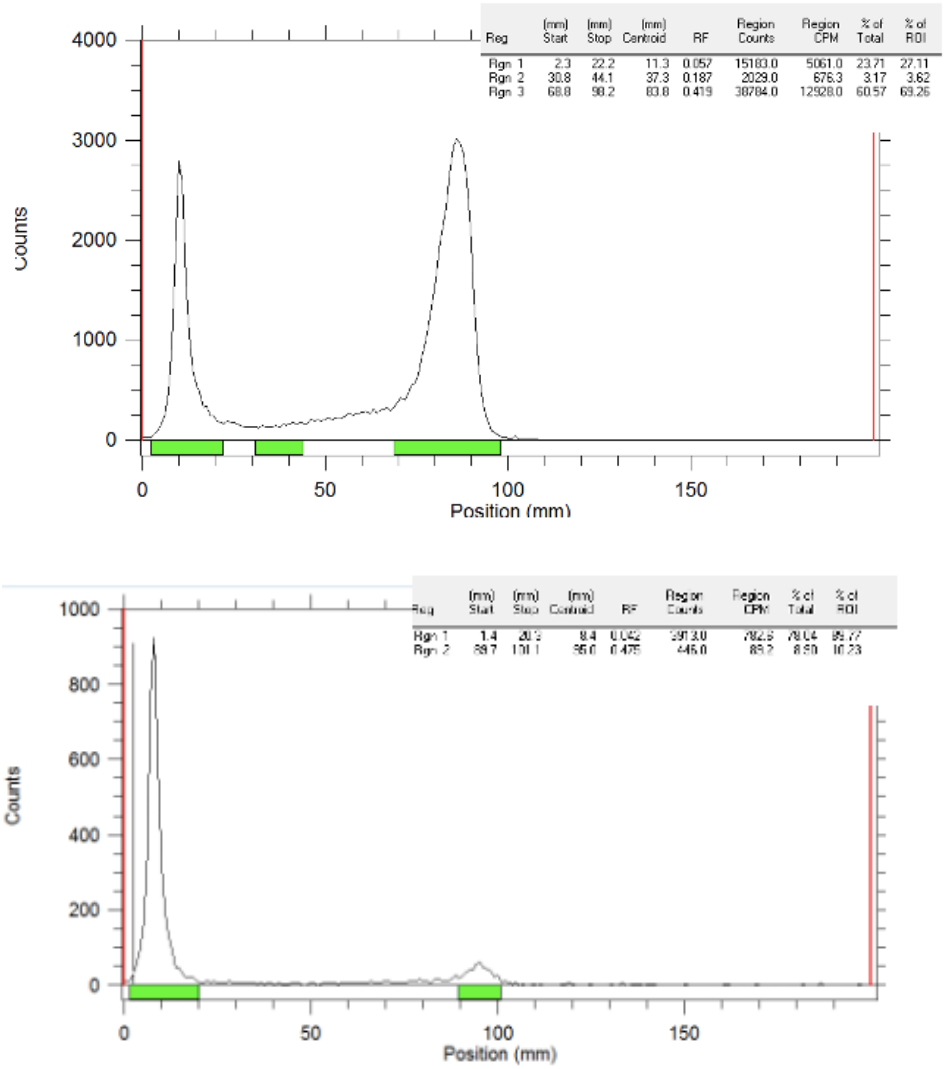

Figure 4: iTLC: Radiolabeling of YS5-HOPO Conjugate to 227-Th

These iTLC results indicate whether the antibody was bound to the radioactivity or not. The first graph is before purification and the second graph is after purification. In the second graph, the large peak on the left (lower distance) indicates a bound antibody with radioisotope. The smaller wider peak on the right (higher distance) is the result of the free antibody. We have observed a percent labeling of 70%.

3.3 Lutetium-YS5 and PARPi, Individual Treatments

The study started with a cell treatment of Lutetium labelled YS5 antibody (177Lu-YS5). The viability of the cells treated with 177Lu-YS5 was determined by MTT assay. Figure 5 shows the cell viability curve for 177Lu-YS5 in 22Rv1 and DU145 cells, with IC50 values of 1.2 µCi and 0.92 µCi, respectively. As expected, the dose dependent decrease in cell viability was observed. Similarly, the treatment of the Niraparib also decreased the viability of both cells DU145 and 22Rv1 (Figure 6). It was observed that the cell viability considerably decreased after treatment with 1 uM of Niraparib. IC50 values for the 22Rv1 and DU145 cell lines were 9.3 µM and 12.6 µM, respectively.

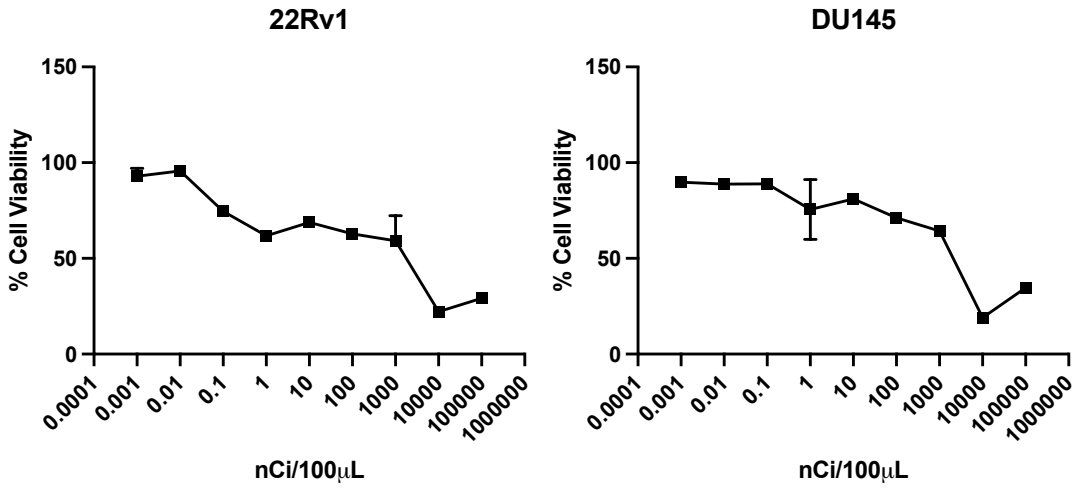

Figure 5: Lu-YS5 MTT Assay

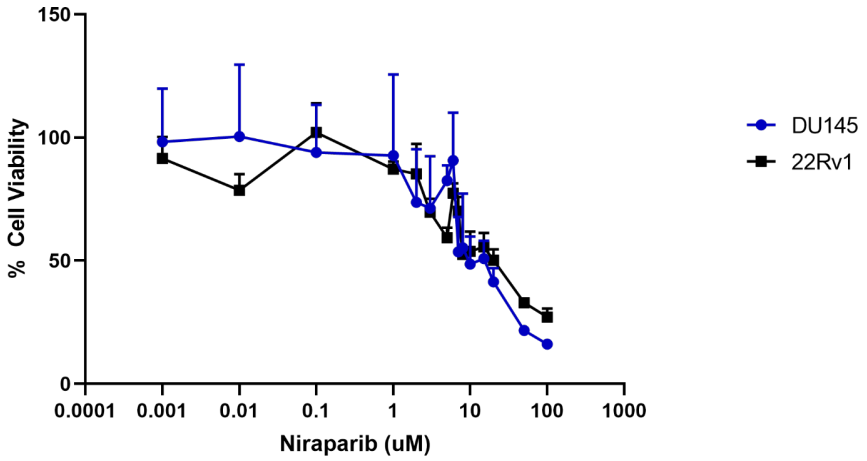

Figure 6: MTT assay for Niraparib

3.4 Combinations of Lutetium-YS5 and PARPi,

To test the effect of the combination therapy, the 22Rv1 and DU145 cells were treated with 177Lu-YS5 and PARPi. However, the combination treatment did not show a decrease in the cell viability. We did not see a clear sigmoidal curve even at a higher concentration of the 177Lu-YS5 and PARPi. The possible reason for these results could be a lower range of the concentrations of the 177Lu-YS5 and PARPi. Increasing the number of concentrations of the 177Lu-YS5 and Niraparib could help to find the effective concentration for inhibition of the cell growth.

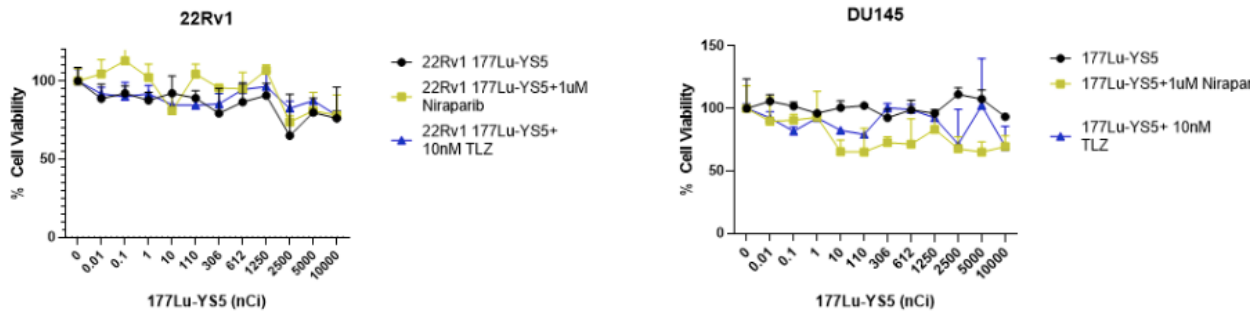

Figure 7: Combination treatment of 177-Lu and Niraparib

3.5 Thorium-YS5 and PARPi, Combined Treatment

In the next step, we utilized the Thorium-227 labelled YS5 antibody (227Th-YS5). The preliminary MTT assays were carried out to find the effectiveness of 227Th-YS5 and PARPi on 22Rv1 cells (Figure 8). Similar to the 117Lu-YS5 combination experiment, the dose dependent decrease in cell viability was not observed. There were mixed and erratic response curves of Thorium and PARPi leading to no conclusion regarding their synergy.

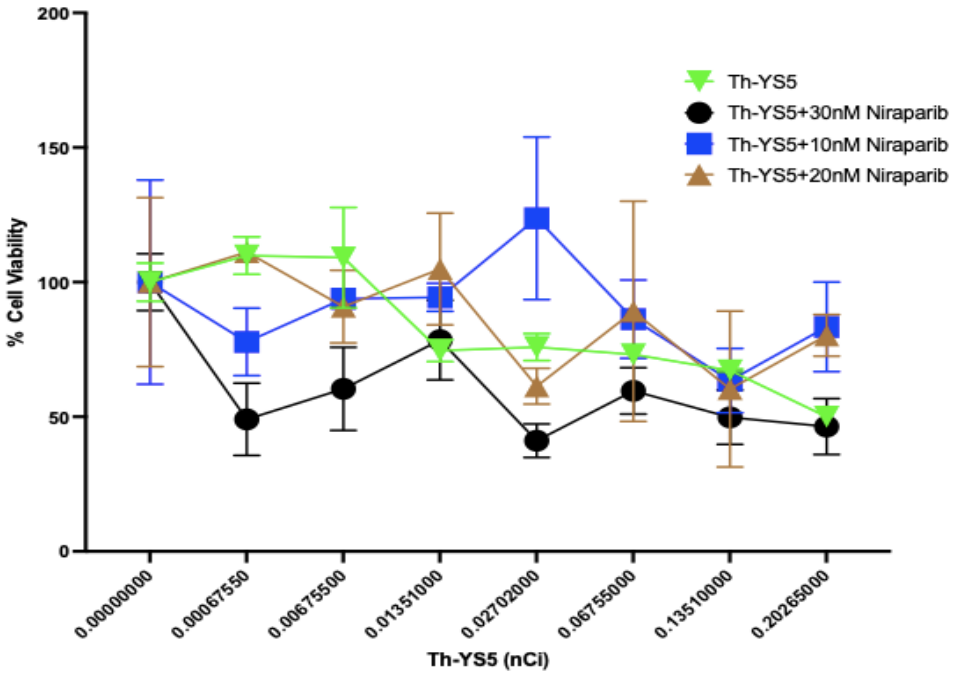

Figure 8: Combination treatment of 227-Th and Niraparib

3.6 Combination of the Actinium labelled YS5 (225Ac-YS5) and PARPi:

The combination effect of the Actimium-225 and the TLZ was also studied in 22Rv1 cells. Figure 9 shows the dose response curve for the 225Ac-DOTA-YS5 and TLZ, respectively. With increasing concentration of actinium, we saw increased inhibition and even observed a sigmoidal curve. Interestingly, with talazoparib, the PARP inhibitor, we did not see any significant cell death.

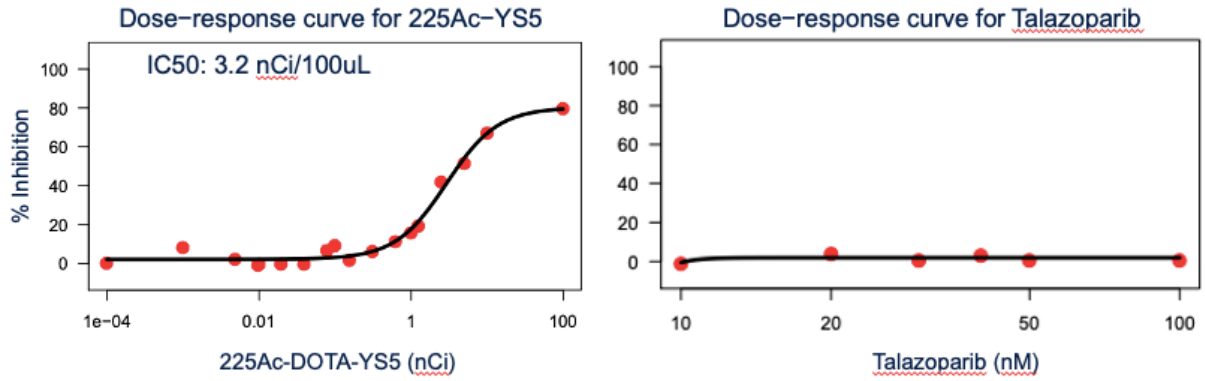

Figure 9: Combination treatment of 225-Ac and Talazoparib

To confirm the synergistic action of the 225Ac-DOTA-YS5 and TLZ, a ZIP analysis was performed from the viability assay results of the combination studies. After performing ZIP analysis, we obtained a ZIP synergy score of 25.35, which indicates that the two drugs work well together.

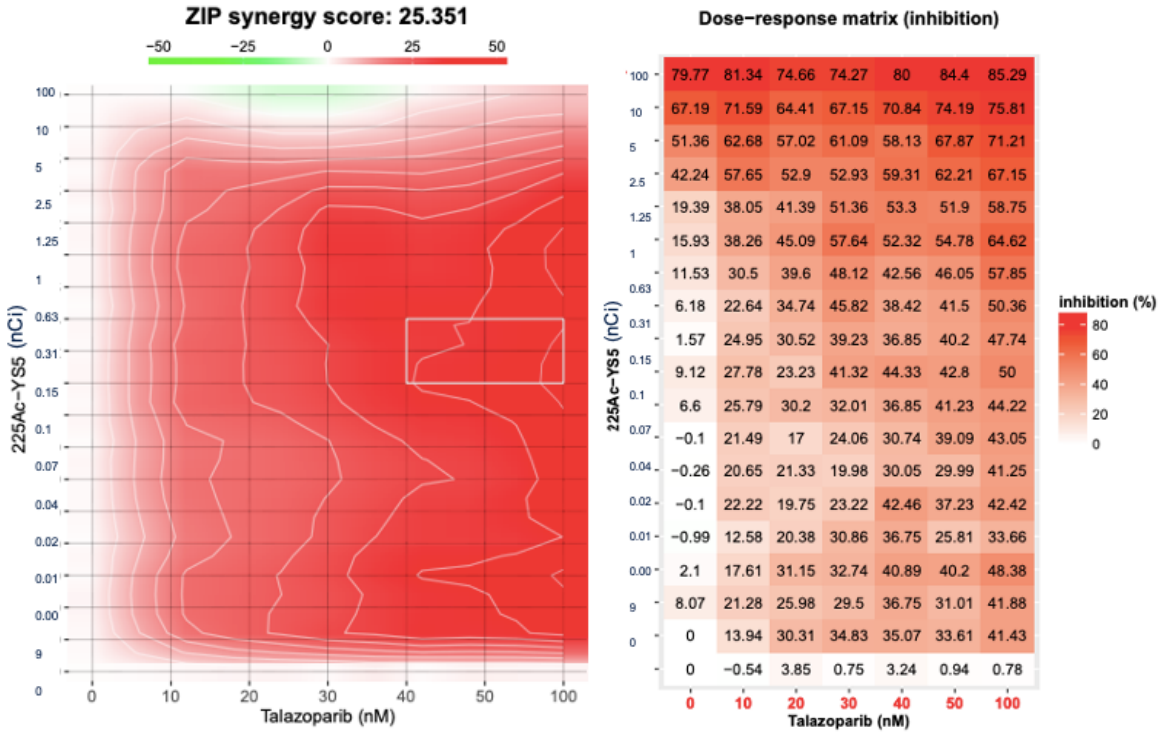

Figure 10: Synergy analysis of 225-Ac and Talazoparib

3.7 External Beam Radiation Therapy

Here, we performed two experiments: colony forming assay and MTT assay. For the colony forming assay we plated 500 cells/well and treated and incubated for 2 weeks. Then, stained with crystal violet dye to visualize the colonies and count them. With increased dosage of the external beam radiation, we see a decreased in the percent of colonies formed. Similarly, with the MTT assay we can see a decrease in cell viability after some amount of radiation. However, we do not see a downward trend with increasing dosage of radiation.

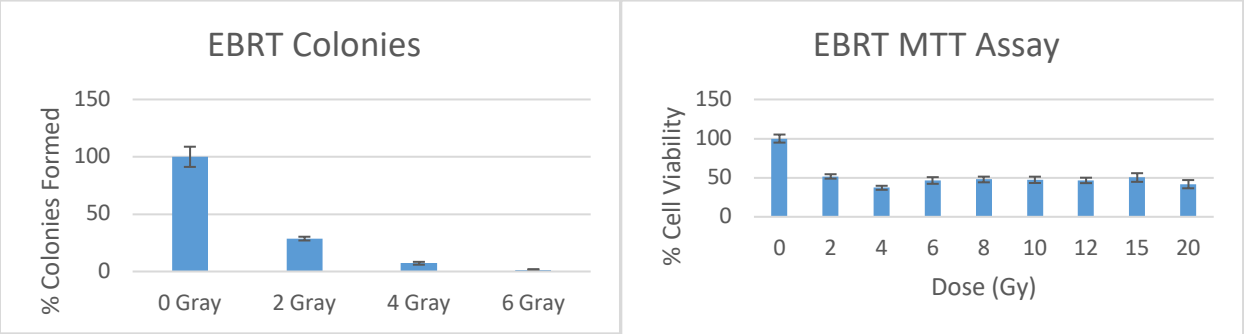

Figure 11: EBRT Assays

4 Discussion

4.1 Limitations

For sources of error during the thorium experiment, we believe that the incubator which was used was malfunctioning and did not maintain a 5% CO_2 level. Furthermore, there could have been bacterial contamination during the treatment of the cells with radioactivity. Because we had to perform the pipetting inside of a lead shield, we did not treat the cells within the confines of a sterilized laminar flow hood. As a result, bacteria could have entered the wells. In such cases, the MTT assay data will not be accurate because we will observe an increased cell activity in the wells with contamination.

There are some interesting remarks regarding the synergy results of the combination of 225-Ac and Talazoparib. There was a clear response curve associated with the Actinium alone, but no response from the Talazoparib alone. However, when combined, there was increased effectiveness compared to Actinium alone, indicating a good synergy between the two drugs.

When we saw the dose response curve for the Talazoparib, which showed no efficacy on its own, we noticed that the dosage used in the treatment was too low. Perhaps, if we had used a range of higher concentrations, there would have been a clear efficacy curve. Furthermore, this could have led to an even better synergy score. We will test this by using the higher range of concentrations for both Talazoparib and 225-Ac and performing another ZIP analysis. We can expect to have good synergy with combination of the IC 50 doses from each drug.

4.2 Drawbacks

The development of radionuclides for therapy involves the consideration of many factors, which makes the process very challenging. Firstly, we must consider the physical properties of the radionuclide. Then, the interaction between the radionuclide and the biological environment inside the body must be studied. Finally, we must identify the proper chelator molecules for conjugation with a ligand.

The way energy from ionizing radiation gets deposited in mammalian cells is a random process. Although intranuclear localization in cancer cells is the ideal outcome, this energy can also be absorbed by normal cells, leading to molecular modifications and cell death. Although therapeutic radiopharmaceuticals are aimed to target a solid tumor or a specific cancer biomarker, the distribution is heterogenous. This issue could be a result of tumors having dissimilar regions, high interstitial pressure, and nonuniform binding-site densities (Kassis, 2008).

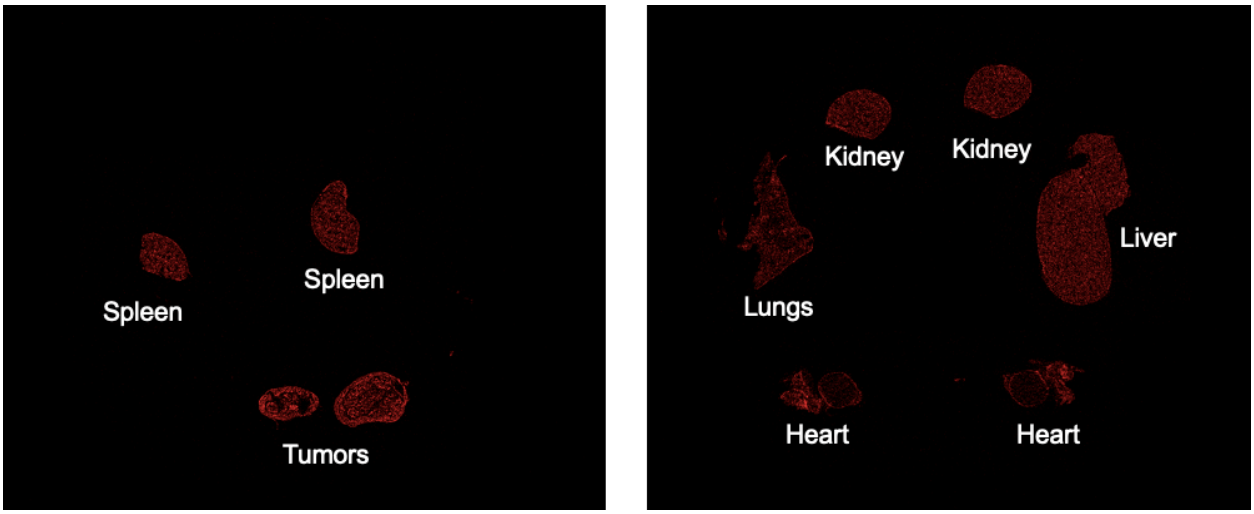

Figure 12: Autoradiography results

Figure 12 illustrates autoradiography results from mice treated with Th227-YS5 which were injected with 0.5 uCi activity. The tumors and other organs were collected 72hrs after injection, kept in OCT medium, and sectioned. Then, these slices are placed into the autoradiography machine: images were taken from an ionizing radiation Quantum Imaging Detector (iQID) camera, an instrument consisting of a scintillator coupled to an image intensifier. The light generated by emitted radioactive particles is amplified and is captured by a CCD/CMOS camera sensor (Miller, 2014). This takes place over a span of 24 hours. During this time, image analysis algorithms are used to generate the above results. These images show that the targeting of the radioactivity reached the destination of the tumor because we can see a bright red color in the tumor slice. However, it also went into the other organs. This shows that targeting antibodies are not very specific, and there remains quite a way until we can identify a method to target only cancer tissue.

5 Conclusion

The results from the Actinium and Talazoparib combination show promise of efficacy. Their synergy further confirms the theory that PARP inhibitors can radiosensitize target cells. This combination can prove to be an effective therapeutic regimen in advanced prostate cancer patients. In order to get to a stage of clinical trials, after seeing further in vitro efficacy, we will continue forward and test in vivo, in murine models.

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