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Development of a Voxel-Based Monte Carlo Radiation Dosimetry Methodology for a Targeted Alpha Particle Therapy

by

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy
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DEDICATION

This dissertation is dedicated to Betty Tichacek Cottrell and Carl Junior Thrush.
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# TABLE OF CONTENTS

LIST OF TABLES ........................................................................................................................... iv
LIST OF FIGURES .......................................................................................................................... v
ABSTRACT ....................................................................................................................................... x

CHAPTER ONE: INTRODUCTION .................................................................................................. 1
1.1 Overview .................................................................................................................................. 1
1.2 Objectives ................................................................................................................................. 2
1.3 Outline .................................................................................................................................... 3

CHAPTER TWO: BACKGROUND ................................................................................................. 5
2.1 Mechanism of Action ............................................................................................................... 5
2.2 Alpha Emitting Radionuclides ................................................................................................. 10
2.3 Internal Radiation Dosimetry .................................................................................................. 17

CHAPTER THREE: A MONTE CARLO METHOD FOR DETERMINING THE RESPONSE RELATIONSHIP BETWEEN TWO COMMONLY USED DETECTORS TO INDIRECTLY MEASURE ALPHA RADIATION .................................................. 23
3.1 Introduction ............................................................................................................................. 23
3.2 Materials and Methods .............................................................................................................. 26
  3.2.1 Mathematical Model ......................................................................................................... 26
  3.2.2 Monte Carlo Model ............................................................................................................ 27
  3.2.3 Monte Carlo Simulations ................................................................................................... 30
  3.2.4 Experimental Measurements ......................................................................................... 31
3.3 Results .................................................................................................................................... 33
3.4 Discussion ............................................................................................................................... 40
3.5 Conclusions ............................................................................................................................. 43

CHAPTER FOUR: MELANOCORTIN 1 RECEPTOR TARGETED ALPHA-PARTICLE THERAPY FOR METASTATIC UVEAL MELANOMA ................................................................. 45
4.1 Introduction ............................................................................................................................. 45
4.2 Materials and Methods ............................................................................................................. 47
  4.2.1 Compound Synthesis and Loading with Lanthanide ......................................................... 47
  4.2.2 Cell Culture and Characterization .................................................................................... 48
  4.2.3 Radiochemical Synthesis and Characterization ............................................................... 48
  4.2.4 Animal Studies ................................................................................................................. 49
6.2.2.1 CT to MCNP Conversion Algorithm ........................................ 81
6.2.2.2 ROI Extraction Algorithm ...................................................... 82
6.2.2.3 SPECT to MCNP Conversion Algorithm .................................. 83
6.2.3 In vivo Imaging Studies ............................................................... 84
6.3 Results .......................................................................................... 86
   6.3.1 Biodistribution and Mean Organ Dosimetry .............................. 86
   6.3.2 Phantom SPECT/CT imaging ................................................. 89
   6.3.3 In vivo Imaging Studies ............................................................. 90
6.4 Discussion .................................................................................... 101

CHAPTER SEVEN: CONCLUSIONS ......................................................... 105

REFERENCES .................................................................................. 107

APPENDIX A: PUBLICATIONS AND CONFERENCE PRESENTATIONS ........ 117

ABOUT THE AUTHOR ........................................................................ END PAGE
LIST OF TABLES

Table 1. Physical characteristics of alpha emitting radioisotopes investigated for clinical use. .................................................................................................................................................. 13

Table 2. Physical characteristics of the radioactive sources used for NaI(Tl) detector energy response measurements. .................................................................................................................. 30

Table 3. Ion chamber and NaI(Tl) measured activities. .......................................................................................................................................................... 36

Table 4. In vitro serum stability of $^{225}$Ac-DOTA-MC1RL. ................................................................................................................................................. 54

Table 5. Radiation dosimetry and clearance kinetics parameters for $^{225}$Ac-DOTA-MC1RL in non-tumor bearing BALB/c mice. .......................................................................................................................... 60

Table 6. Radiation dosimetry and clearance kinetics for $^{225}$Ac-DOTA-MC1RL in SCID mice bearing MEL270 tumors. .................................................................................................................................. 61

Table 7. PK parameter estimates. .................................................................................................................................................................................. 76

Table 8. The materials and corresponding densities and elemental compositions used in dosimetric calculations. ........................................................................................................................................ 82

Table 9. The SPECT derived ROI activities compared to organ activities measured using the ion chamber. .................................................................................................................................................. 93
LIST OF FIGURES

Figure 1. Survival of a human kidney T-cell culture irradiated with ionizing particles of different kinds. ................................................................. 8

Figure 2. Decay chain of Actinium-225 ($^{225}$Ac). ................................................................. 24

Figure 3. System of equations describing the decay of $^{225}$Ac to the very long-lived $^{209}$Bi. .......... 27

Figure 4. Geometry of the Monte Carlo model wipe test scintillator detector. Image generated using the Visual Editor software for MCNP. 2: NaI; 3: MgO; 4: Al; 6: polyethylene; 7: air; 8: H$_2$O with $^{225}$Ac, $^{221}$Fr, $^{213}$Bi source distributions. ......................... 28

Figure 5. Solutions to system of decay ordinary differential equations of for an initial activity of $3.7 \times 10^4$ kBq of $^{225}$Ac. ................................................................................................. 34

Figure 6. Comparison of measured and simulated spectra of $^{137}$Cs........................................ 35

Figure 7. NaI(Tl) measured gamma spectrum with Gaussian fitting................................. 36

Figure 8. High definition Monte Carlo simulated gamma spectra of (A) $^{225}$Ac, (B) $^{221}$Fr, (C) $^{213}$Bi, and (D) all three superimposed. ................................................................. 37

Figure 9. The mean percent difference between measured spectra and simulated spectra using the ion chamber reading measurement as input for the Monte Carlo model................................................................. 38
Figure 10. Measured vs. simulated $^{225}$Ac spectra for (A) 70.3 kBq (0.17%), (B) 144.3 kBq (0.5%), (C) 222.0 kBq (0.19%), (D) 299.7 kBq (0.99%), (E) 370.0 kBq (0.11%), and (F) 447.7 kBq (0.02%). The values in parentheses are the mean percent differences between the two spectra. .......................................................... 39

Figure 11. Activity response relationship between ion chamber reading and Monte Carlo-NaI(Tl) corrected spectra activity. .......................................................... 40

Figure 12. Radiochemical synthesis of $^{225}$Ac-DOTA-MC1RL .......................................................... 49

Figure 13. MTD study for non-tumor bearing mice: (A) Percent weight gain, (B) BUN and (C) blood creatinine. .......................................................... 57

Figure 14. Plot of rat blood clearance. Exponential decay non-linear regression line fit of $^{225}$Ac alpha activity in rat blood over time, following intravenous administration of $^{225}$Ac-DOTA-MC1RL (n=4 rats). .......................................................... 57

Figure 15. Biodistribution of $^{225}$Ac-DOTA-MC1RL. $^{225}$Ac, $^{221}$Fr and $^{213}$Bi activities in tissues from (A) non-tumor bearing BALB/c mice (n=6 per time point) and (B) SCID mice bearing MEL270 human uveal melanoma tumors (n=5 per time point). .......................................................... 58

Figure 16. Biodistribution of (A) $^{225}$Ac-DOTA-MC1RL and (B) $^{225}$Ac-DOTA-SP in bilateral A375 and A375/MC1R tumors (n=5 per time point). .......................................................... 59

Figure 17. Efficacy study in mice bearing MEL270 tumors: (A) Representative images of tumors (outlined); (B) initial tumor growth volumes; (C) Kaplan-Meier plots. ....... 63

Figure 18. (A-B) Metastasis study in MEL270 uveal melanoma mouse model and (C-D) MC1R expression in tumors reaching endpoints from each treatment group. (A) Representative H&E staining and corresponding threshold segmentations of sections containing liver and lung metastases (Cold=La-DOTA-MC1RL, Scrambled=untargeted, treated=$^{225}$Ac-DOTA-MC1RL), blue=normal tissue and
(B) quantified metastasis burden. (C-D) MC1R IHC staining of MEL270 tumors after reaching endpoints.

Figure 19. Efficacy study in mice bearing A375/MC1R tumors: (A) Representative images of tumors (outlined); (B) initial tumor growth volumes; and (C) Kaplan-Meier plots.

Figure 20. Scheme showing synthetic route to metal chelation.

Figure 21. A) Schematic of the multi-compartmental pharmacokinetic model. B) The system of ODE’s that represent the change in compartmental radioactive concentrations.

Figure 22. Time activity plots generated for A) Ahx and B) di-D-Glu.

Figure 23. Biodistribution results for A) $^{225}$Ac-DOTA-Ahx-MC1RL and B) $^{225}$Ac DOTA-di-D-Glu-MC1RL.

Figure 24. Optimized fitting of compartment solution: Ahx. Note: Compartments were solved and fit simultaneously but plotted separately for visualization.

Figure 25. Optimized fitting of compartment solution: di-D-Glu. Note: Compartments were solved and fit simultaneously but plotted separately for visualization.

Figure 26. Affine transformation to map to voxel location. Pxyz are the Cartesian coordinates of the voxel, Sxyz are the Cartesian coordinates of the voxel in the top left corner of the dataset, ijk are the voxel indices, Δijk are the pixel spacing and slice thickness, and XYZ define the directional cosines.

Figure 27. The clinical workflow to perform patient 3D specific dosimetry.
Figure 28. A) Monte Carlo simulated gamma spectrum of 148 kBq of $^{67}$Ga. B) Representative gamma spectrum and fitting of $^{67}$Ga acquired with the NaI(Tl) scintillation detector. .................................................................................................................. 87

Figure 29. Biodistribution of $^{67}$Ga-DOTA-MC1RL ................................................................. 88

Figure 30. Mean $^{67}$Ga-DOTA-MC1RL absorbed dose ............................................................... 88

Figure 31. Electron Density Phantom. A) CT-image of middle axial slice, B) visible light image and C) segmented CT image. .................................................................................................................. 89

Figure 32. A) The MCNP converted EDP dataset. B) The TMM ROI’s in voxel coordinates. .................................................................................................................. 89

Figure 33. Sphere filled with known activity. SPECT images were acquired and used to determine the absolute activity calibration factor for the scanner ................................................. 90

Figure 34. Coronal CT slice of mouse with manually drawn kidneys and liver. The external contour was generated by thresholding ............................................................................... 91

Figure 35. Kidneys and liver volumes in voxel coordinates .......................................................... 91

Figure 36. Coronal views of SPECT/CT images acquired A) 24 hours, B) 48 hours, and C) 96 hours post injection of 18.5 MBq of $^{67}$Ga-DOTA-MC1RL in SCID mice. ........ 92

Figure 37. For 24, 48, and 96 hours: A) Mouse-specific phantom obtained from CT. B) Absorbed dose map for $^{67}$Ga-DOTA-MC1RL. C) Corresponding absorbed dose map for $^{225}$Ac-DOTA-MC1RL. Note the difference in scales. Spatial dimensions are in centimeters. ................................................................................................. 94
Figure 38. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the right kidney at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters. ................................................................. 95

Figure 39. Exponential fitting of the mean dose rate as a function of time for $^{225}$Ac-DOTA-MC1RL in the right kidney. ................................................................. 96

Figure 40. Dose volume histogram for $^{225}$Ac-DOTA-MC1RL in the right kidney. ..................... 96

Figure 41. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the left kidney at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters. ................................................................. 97

Figure 42. Exponential fitting of the mean dose rate as a function of time for $^{225}$Ac-DOTA-MC1RL in the left kidney. ................................................................. 98

Figure 43. Dose volume histogram for $^{225}$Ac-DOTA-MC1RL in the left kidney. ..................... 98

Figure 44. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the liver at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters. ................................................................. 99

Figure 45. Exponential fitting of the mean dose rate as a function of time for $^{225}$Ac-DOTA-MC1RL in the liver. ................................................................. 100

Figure 46. Dose volume histogram for $^{225}$Ac-DOTA-MC1RL in the liver. ..................... 100
ABSTRACT

The use of targeted ligands to deliver radioisotopes directly to tumor cells is a promising therapeutic strategy. Because of the short path length and high LET of alpha particles, targeted alpha particles are ideal for treating metastatic disease while minimizing damage to non-targeted tissues. However, clinical methods for determining 3D radiation dosimetry in patients with multiple metastatic lesions are needed to support clinical translation of novel targeted alpha particle therapies for personalized treatment, especially in patients that have previously received radiotherapy. Recently interest in novel radiopharmaceutical development has grown significantly. However, compared to external beam radiation therapy, progress in customizing radiopharmaceutical treatments specific to the patient has remained stagnant for decades. Currently, therapies are given using fixed dose administrations and dosimetry is performed using outdated simplistic representations of a standard human. The potential benefits of targeted alpha therapies cannot be taken advantage of until pretreatment planning is employed to optimize each patient’s therapy on an individual basis. Dose response relationships need to be analyzed post-treatment to assess tumor control probabilities and normal tissue complications. With new developments in medical imaging and instrumentation, along with the continuously increasing computational power available, personalized targeted alpha therapies can be achieved.

In this work, a novel targeted alpha therapy for treatment of metastatic uveal melanoma, $^{225}$Ac-DOTA-MC1RL, is developed and thoroughly tested pre-clinically. The therapy showed
rapid eradication of tumors with no normal tissue toxicity with a single administration. Radiation
detection instrumentation is improved upon by developing a method to more accurately quantify
radioactivity for administration, biodistribution, pharmacokinetics, and dosimetry. A voxel-based
Monte Carlo dosimetry methodology is developed using a novel companion imaging agent in
both phantom and in vivo pre-clinical imaging studies. From these studies, a clinically
translatable workflow is described and tested. 3D dosimetry calculations were performed
enabling volumetric dose analysis for the novel therapy.
CHAPTER ONE:
INTRODUCTION

1.1 Overview

Advances in the understanding of molecular characteristics of cancerous cells have paved the way for targeted radiopharmaceutical therapy. Identifying a malignant target and designing a vehicle to deliver high doses of radiation has become a promising modality to treat cancer. Recently, interest in using alpha emitting radioisotopes to selectively destroy the DNA of cancer cells has increased. This is due to their short path length and large linear energy transfer. The range of an alpha particle is three to six cell lengths and only one cell traversal is needed to cause irreparable damage. Ideally the drug will bind to the targeted cells without causing damage to surrounding healthy cells.

The use of radiation to treat cancer is well established and has been a strategy for over 100 years. During this time, external beam therapy has become the most common form of radiation therapy. Advances in geometrical tumor targeting, treatment planning, optimization, dose calculation, delivery, guidance, verification, and disease control have made external beam therapy the standard delivery of therapeutic radiation. For a modern external beam radiation treatment, the radiation therapy team may spend weeks designing and tailoring the optimal plan by choosing variations in beam type, energy, treatment time, and geometry. Radionuclide therapy has been a cancer treatment approach since the 1940’s with the use of $^{131}$I for the
treatment of thyroid disease. However, patient specific treatment design and workflow innovation has severely lagged behind the advancements made in external beam therapy. In radionuclide therapy it is common to prescribe fixed-dose non-specific administrations to all patients. This leads to “consciously choosing that patients be treated with a lower standard of care than external beam patients”. These fixed administrations are based on calculations performed on a stylized fixed geometry model of a standard human. Since radionuclide therapies are systemic, toxicities are not only a function of administration but also functional and anatomical variations.

With the growing interest in developing new radiopharmaceuticals that target specific cells which previously could not be optimally treated, the field has made progress in entering the era of personalized medicine. Developments in modeling biological endpoints and pharmacokinetic analysis have led to the emergence of patient specific internal dosimetry. Improvements in quantitative imaging and computational power have led to work in modeling an individual patient’s anatomy and physiology and computing the 3D radiation dose distribution specific to each patient using Monte Carlo.

1.2 Objectives

In this work, a novel targeted alpha therapy is developed and tested. The principal goal is to develop a methodology for 3-dimensional patient specific internal radiation dosimetry for the targeted alpha therapy that can be implemented clinically. The methodology should be quick, straight forward and accurate. The workflow is initially developed using phantom studies and then it is applied to a mouse experiment. Patient specific models are created using computed tomography (CT) and spatial and temporal activity distribution maps are created using single
photon emission tomography (SPECT). Dosimetric calculations are performed using the Monte Carlo N-Particle (MCNP) package. As a benchmark, the results of the dosimetric workflow will be compared to conventional internal dosimetry methods.

**1.3 Outline**

This work is structured into six chapters. Chapter 2 starts by describing the radiation biology and mechanism of action of alpha particles. The chapter continues with a summary of the clinically relevant alpha emitting radionuclides. The final portion of this chapter is a summary of internal radiation dosimetry methods.

Chapter 3 describes the identification of an issue that is commonly overlooked in targeted alpha therapy development. Many alpha emitting radionuclides have complex decay chains consisting of alpha emitting daughters. The radioactivity of these daughters must be quantified for a complete analysis of the therapy. If the daughter species emit gamma rays, a scintillation detector can be used and spectrum analysis can be performed to separate the daughter activities. Scintillation detectors however suffer physical limitations with increasing activity. Therefore a method is described that corrects the scintillation detector’s readings at higher activities using Monte Carlo to permit the analysis of daughter products.

Chapter 4 describes the preclinical testing of the novel metastatic uveal melanoma targeted alpha therapy. The chemistry, biology, and physics considerations are presented. The physics portion includes the description of the indirect measurement of alpha activity for administration analysis, blood pharmacokinetics, biodistribution, and conventional radiation dosimetry.
Chapter 5 describes the development of a multi-compartment pharmacokinetic model for the targeted alpha therapy and a derivative that was developed and tested. The model can be used to implement patient specific therapy by optimizing administration activities from simulated biodistribution obtained from imaging and/or blood and urine samples.

Chapter 6 describes the development of the voxel-based 3D internal dosimetry methodology. A companion imaging agent to the targeted alpha therapy is developed and tested. Phantom studies are conducted to develop the image-to-Monte Carlo conversion algorithms. 3D absorbed dose is generated for both the imaging agent and the therapeutic agent in mice and validated by comparing to conventional methods.

Chapter 7 summarizes the results of previous chapters.
CHAPTER TWO:
BACKGROUND

2.1 Mechanism of Action

Since the discovery of radioactive materials, the effect of radiation on the properties of matter has been of significant interest in the disciplines of material science, geology and astrophysics. For example the first large-scale effect of radiation on solid material was observed by E.P. Wigner from exposure to a nuclear fission reactor and was thus named the Wigner effect, or "Wigner’s disease". Since then, the large spread of medical technology involving sources of ionization radiation and the development of nuclear weapons have caused a spike in studies of the effect of radiation on living tissue. That work increased our understanding of the concept of radiation risk and created new fields of scientific study, for example, radiation health safety, radiation dosimetry, and radiation oncology.

Because of the high energy of alpha particles and stochastic nature of ionization radiation, their effects may be observed on all levels of a biological system. Any molecule, cell, tissue or organ can display alpha decay radiation damage and such damage can be localized, or occur throughout the entire body of any multicellular organism.

The first step in producing radiation effects is the generation of a primary recoil atom and alpha particles by a radioactive decay. Such events take place very rapidly in much less than 1fs. In the case of alpha decay of radiopharmaceutical isotopes of interest, the average kinetic energy
per recoil atom is ~100 keV, and the average kinetic energy deposited within the range of a single alpha particle is on the order of 5 MeV. It is clear that the relatively high levels of energy deposited by the combination of the fast moving heavy ions and high energy alpha particles can cause large amounts of damage to solid matter. There are many methods to estimate the effect of radiation in solid materials, i.e. the stopping power theory based on Coulombic interactions, molecular dynamics, transport theory, etc. However, unlike solid materials, biological tissues do not consist of solid crystalline structures, and this significantly increases the complexity of estimating the effects of alpha decay (as well as other ionization radiations), making the application of the aforementioned methods impossible or extremely difficult numerically. To overcome such limitations, a semi-quantitative approach has been applied. First, the biological effects observed in irradiated subjects were separated into one of two categories\textsuperscript{4,6}: Deterministic effects, which have a practical threshold absorption dose below which effects are negligible or not evident; and stochastic effects, where the relationship between dosage and severity of effect is either less evident or absent.

Maintaining the integrity of many different types of macromolecular structures is important to cell viability and all cellular organic molecules are subject to damage by ionizing radiation. However, the genomic DNA molecules are considered to be the most critical target for the biological effects of ionizing radiation because intact DNA is required for cellular replication, and damaged but improperly repaired DNA can result in fixation of genetic mutations that can affect normal cellular function and viability.\textsuperscript{7} Ionizing radiation interacts with DNA either by directly transferring energy to the biological material or indirectly by creating reactive free radicals from the radiolysis of water. These interactions result in damage to the DNA structure as a result of broken covalent bonds. Linear energy transfer (LET) is an approach
to describe the spatial distribution of ionization and excitation produced by direct or indirect effects of different types of radiation along a linear path.\textsuperscript{8} Alpha particles are high LET radiation because they create dense ionizations and excitations in matter due to coulombic interactions with atoms. Being a heavy charged particle, an alpha particle will continuously slow down along its track with minimal deflection. Through the process of slowing down, the interaction cross-section towards the end of travel increases, resulting in increased LET, which is known as the Bragg Peak.\textsuperscript{9,10}

Relative biological effectiveness (RBE) is the ratio of the dose of a reference radiation and the dose of a test radiation that produces the same biological effect. Some of the most common biological effect measurements are necrotic and apoptotic (programmed) cell death, DNA damage, chromosomal aberrations and genetic mutations. The RBE of alpha particles can range from 3.5 to 4 for cell killing, 6 to 12 for mutation, and 10 for cell transformation.\textsuperscript{8} As a comparison, the RBE for low LET electrons and photons is 1.

An important biological endpoint is cell killing. Damage to cells can be classified as either sub-lethal events or lethal events. Sub-lethal events are due to the accumulation of damage that has potential to be repaired, typically as a result of exposure to lower doses, and lethal events typically result from irreparable damage due to exposure to higher doses.\textsuperscript{4} The ability to repair these events is seen as a shoulder on the cell survival curve and is characteristic of low LET radiation (Figure 1).\textsuperscript{11} A single event of high LET radiation can be lethal. The cell survival curve for a lethal event does not have a shoulder, indicating the inability to repair.\textsuperscript{12}
Figure 1. Survival of a human kidney T-cell culture irradiated with ionizing particles of different kinds: 1) particles with $E = 2.5 \text{ MeV}$, $\text{LET} = 165 \text{ keV/m}$; 2) particles with $E = 25 \text{ MeV}$, $\text{LET} = 25 \text{ keV/m}$; 3) deuterons with $E = 3.0 \text{ MeV}$, $\text{LET} = 20 \text{ keV/m}$; 4) X-rays with $E = 20 \text{ keV}$ and $\text{LET} = 6 \text{ keV/m}$; 5) X-rays with $E = 250 \text{ keV}$ and $\text{LET} = 2.5 \text{ keV/m}$; and 6) particles with $E = 2.2 \text{ MeV}$, $\text{LET} = 0.3 \text{ keV/m}$. This figure and legend were reproduced from Kudryashov, Y.B., *Radiation Biophysics (Ionization Radiation)*. Nova Science Publishers Inc., 2006.

The DNA double strand break is the most biologically significant type of damage, which occurs as a result of two single strand breaks in close proximity or a rupture of the double strand at the site of interaction.\(^{13}\) Cell survival is highly dependent on the spatial distribution of double strand breaks.\(^8\) Given the same dose, high LET radiation can create up to four times more double strand breaks than low LET radiation. Also, the formation of high LET double strand breaks are more complex compared to low LET in that they are less randomly distributed and form clustered DNA damage to multiple base-pairs.\(^9\) It has been widely accepted that high oxygen levels play a large role in a cell’s sensitivity to ionizing radiation and, hence, tumor hypoxia is an established factor in resistance to radiation therapy.\(^{14}\) This is due to enhancement of free radical production by the presence of oxygen. Free radical production occurs as a result of indirect action, or low LET interactions. Since alpha particles interact directly with the DNA, the level of
oxygen becomes irrelevant, hence, eliminating a major mechanism of resistance to therapy. See Figure 1.

After exposure to radiation that results in DNA damage, the cell cycle can be stopped at cell-cycle checkpoints which allow the cell to repair the damage via multiple repair mechanisms in order to preserve genomic integrity. In the case of irreparable damage, the cell will eventually undergo cell-death by apoptosis or necrosis. The two main repair mechanisms of double strand breaks are homologous recombination and non-homologous end joining. Homologous recombination occurs in the late S and G2 phases of DNA synthesis where an intact DNA template is available, resulting in a more efficient and high fidelity repair. Non-homologous end joining occurs throughout the cell cycle but is the only means of repair in G1 and early S phase. In this error prone repair method, DNA ends are rejoined with no sister template. In this case chromosomal aberrations can occur as a result of recombining incorrect DNA ends, i.e. combining a loose end to some other molecule, and truncation of ends. If incorrect repair occurs prior to DNA replication, these errors can be replicated in daughter DNA which can lead to mitotic cell death or can lead to the generation of genomic mutations without cell death. There is also the situation where double strand breaks are not repaired and the dividing cell enters mitosis, leading to mitotic catastrophe and eventual cell death. A higher proportion of double strand breaks remain un-rejoined after exposure to high LET radiation. When it comes to damage from high LET alpha particles in close proximity to the cells being irradiated, the main radiobiological effect is complex and irreparable DNA damage resulting in cell death by either apoptosis or necrosis.

In the last few years, successful attempts have been made to explain the bystander effect. The bystander effect is defined as effects that are observed in cells that have not been
directly irradiated following the irradiation of other nearby cells. Two mechanisms were proposed, one is the transfer of genomic instability through p53-mediated pathways and the other suggests that irradiated cells secrete cytokines or other factors that transit to other cells that were not irradiated and signal increased levels of intracellular reactive oxygen species.\textsuperscript{16} One of the defined sub-classes of bystander effect is the “abscopal effect,” in which radiation treatment of a tumor propagates to tumors outside the irradiated volume.\textsuperscript{16} A more recent publication demonstrated an effect that might explain the abscopal experience.\textsuperscript{17} It was demonstrated that alpha-particle treatment of prostate cancer cells generated an adaptive antitumoral immune response, as has been previously reported for other forms of radiation. The combination of bystander effects and the abscopal (likely immune) response in vivo are potential mechanisms of efficacy for tumors that are not avid for the targeted α-emitter radiotherapy in a patient with heterogeneous target expression.

2.2 Alpha Emitting Radionuclides

Using radiation as a method of cancer therapy requires delivering the maximum dose to the tumor while minimizing dose to healthy tissues. Targeted radionuclide therapy is advantageous in that it seeks molecular and functional targets within patient tumor sites.\textsuperscript{18} Beta emitting radionuclides (\textsuperscript{90}Y, \textsuperscript{131}I, \textsuperscript{177}Lu, \textsuperscript{186}Re) are used for cancer-targeted therapy but have problems with cross fire irradiation of normal tissues due to their relatively long range in tissue which is in the range of 0.5-12 mm. In contrast, alpha particles deposit higher energy over a much shorter range (40-90 µm), potentially causing higher cytotoxicity to tumor cells while delivering a lower dose to normal adjacent tissues.\textsuperscript{19} Alpha emission is the process by which an unstable nucleus ejects a highly energetic heavy charged particle consisting of two protons and
two neutrons. Alpha particles have a higher LET (100keV/μm) compared to beta particles (0.2keV/μm) which results in dense ionization along its track in matter. The short range of alpha emission provides specificity to the target cell population with minimal effect on surrounding normal cells, and the high LET leads to a high frequency of irreparable DNA double strand breaks. This limits cytotoxic effects to within a small distance from the location of decay. It has been estimated that only one cell traversal by an alpha particle track is necessary to kill the cell while thousands of beta traversals are required for the same effect. Because of the long range of beta particles and the need of a high number of hits for cell killing, a large portion of the dose deposited is outside of the intended target. Hence, beta-emitting radionuclides are typically used for targeted treatment of non-solid or circulating types of cancer, i.e. leukemias and lymphomas, where target cells are not stationary in order to minimize damage to surrounding normal tissues. Regardless, use of alpha particles for targeted treatment of circulating disease could be an improvement due to potentially reduced damage to normal tissues. Use of targeted alpha particle therapy has been considered for killing isolated cancer cells in transit in the vascular and lymphatic systems, in regressing tumors by disruption of tumor capillary networks and in treatment of micrometastatic foci. In particular, targeted alpha particle therapy may be ideal for treatment of solid metastases as the short range will primarily kill tumor cells with little deleterious impact on surrounding normal tissue. Additionally, heterogeneity of target expression has been observed within a given tumor or metastasis and this is thought to be a mechanism of developing resistance to targeted therapies, where non-target expressing cells survive treatment and clonally expand into a resistant population. In this case, alpha emissions from a targeted cell will serve to kill surrounding untargeted cells within the effective range, potentially reducing the development of resistance.
A number of factors need to be considered in choosing an alpha emitting radionuclide for therapy. These include proper nuclear characteristics, ease of radiochemical incorporation, specific activity, synthesis yields, chemical and biological stability, availability, and cost.\textsuperscript{19}

The physical half-life of the radionuclide should be long enough to allow for radiosynthesis preparation and be compatible with the pharmacokinetics of tumor localization.\textsuperscript{23} The decay pathway of the alpha emitter should be carefully analyzed. Due to the conservation of energy and linear momentum a daughter nuclide which subsequently decays by alpha emission could detach from the radioimmunoconjugate, see for example.\textsuperscript{24} These free products could travel away and deposit dose to healthy tissue. A decay chain that is long and complicated, having many different decay types could also present an issue dosimetrically especially if the daughter products are metabolized differently than the parent. A possible way to overcome this issue is to use \textsuperscript{225}Ac as an in vivo generator in which the delivery system is designed to be internalized into the target cell where the toxic daughter elements may detach from the targeting vector but remain trapped in the cell.\textsuperscript{25}

Another important nuclear characteristic is having a large number of alpha emissions per decay. The radiotoxicity associated with having multiple emissions could be high enough to kill a tumor cell in a single decay. An accompanying gamma photon emission with energy suitable for \textit{in vivo} imaging is also beneficial to assist with pharmacokinetic and dosimetric evaluation.\textsuperscript{23}

Another important element for radionuclide selection is availability. Alpha emitters are produced either in cyclotron bombardment or by reactor irradiations, incorporated into a generator, and eluted from a parental source. Obtaining radionuclides in pure form with high specific activity and large quantities is essential for adequate therapeutic evaluation. High specific activity is important to avoid receptor saturation by the unlabeled targeting agent.\textsuperscript{26} If
membrane antigenic density is low, insufficient binding to tumor cells will occur.\textsuperscript{26} Transportation of these radionuclides safely and economically is also a key issue in selection.

While there are over 100 alpha emitting radionuclides, only several have been investigated in preclinical and clinical studies. This is mostly due to radionuclides lacking nuclear properties, the absence of viable chemistry, complicated decay chains, and production and economic issues.\textsuperscript{27} Therefore radionuclides meeting the criteria for therapeutic use have been limited to \isotope{225}{Ac}, \isotope{212}{Bi}, \isotope{213}{Bi}, \isotope{211}{At}, \isotope{212}{Pb}, \isotope{223}{Ra}, and \isotope{227}{Th}. The physical characteristics of these isotopes can be seen in Table 1.

<table>
<thead>
<tr>
<th>Isotope</th>
<th>Half-Life</th>
<th>Max Energy (MeV)</th>
<th>Emissions Per Decay</th>
</tr>
</thead>
<tbody>
<tr>
<td>\isotope{225}{Ac}</td>
<td>10.1 d</td>
<td>5.83</td>
<td>4 (\alpha), 2(\beta)-</td>
</tr>
<tr>
<td>\isotope{211}{At}</td>
<td>7.2 h</td>
<td>5.87</td>
<td>1 (\alpha), 1 EC</td>
</tr>
<tr>
<td>\isotope{212}{Bi}</td>
<td>1.01 h</td>
<td>6.09</td>
<td>1 (\alpha), 1(\beta)-</td>
</tr>
<tr>
<td>\isotope{213}{Bi}</td>
<td>45.6 min</td>
<td>5.87</td>
<td>1 (\alpha), 2(\beta)-</td>
</tr>
<tr>
<td>\isotope{212}{Pb}</td>
<td>10.6 h</td>
<td>6.09</td>
<td>1 (\alpha), 2(\beta)-</td>
</tr>
<tr>
<td>\isotope{223}{Ra}</td>
<td>11.4 d</td>
<td>5.87</td>
<td>4 (\alpha), 2(\beta)-</td>
</tr>
<tr>
<td>\isotope{227}{Th}</td>
<td>18.7 d</td>
<td>6.04</td>
<td>5 (\alpha), 2(\beta)-</td>
</tr>
</tbody>
</table>

The first alpha emitter to be used in human clinical trials for therapy was \isotope{213}{Bi} in 1997, when it was labeled to the anti-leukemia antibody HuM195.\textsuperscript{28} \isotope{213}{Bi} is available through generator based \isotope{225}{Ac} and decays with a 45.6 min half-life by emission of 1 alpha (8.37MeV) and 2 betas. The generator is produced at Oak Ridge National Laboratory in the US and at the Institute for Transuranium Elements in Karlsruhe Europe. In the decay of \isotope{213}{Bi}, there is an emission of a 440keV isomeric gamma which is beneficial for imaging studies.
\(^{211}\)At decays with a half-life of 7.2 h and emits 2 alphas through a split decay pathway with energies of 5.87 and 7.45 MeV. One path is to \(^{207}\)Bi by alpha emission followed by electron capture to \(^{207}\)Pb and the other is by electron capture to \(^{211}\)Po followed by alpha emission to \(^{207}\)Pb. An advantage of this decay path is that \(^{211}\)Po emits 77-92 keV characteristic x-rays which can be used for imaging.\(^27\) The main disadvantages are availability and purity. Conventionally the production of \(^{211}\)At requires an alpha particle cyclotron, which there are only a few worldwide, to produce the \(^{209}\)Bi(alpha,2n)\(^{211}\)At reaction with minimal \(^{210}\)At contamination.\(^29\) Astatine has significant metallic characteristics that lead to complications in standard antibody labeling and results in rapid release of free \(^{211}\)At.\(^{27,30,31}\) To resolve this problem, approaches have been developed in several research groups based on small linker molecules that create an aryl carbon–astatine bond involving an astatodemetallation reaction using tin, silicon, or mercury precursors.\(^32\)

\(^{225}\)Ac is a radiometal with a half-life of 10.1 days and produces six radionuclide daughters in the decay path to stable \(^{209}\)Bi. For each decay event of \(^{225}\)Ac, there are successively four alpha and two beta emissions with high energy (alpha 8.38 MeV, beta 1.42 MeV). In the decay of \(^{225}\)Ac and its daughters there are several isomeric gamma emissions with energy suitable for imaging studies. The relatively long half-life allows for a centralized production site that can ship \(^{225}\)Ac to users.\(^33\) The main method for generating \(^{225}\)Ac for clinical studies is through the decay of \(^{220}\)Th which originates from \(^{233}\)U. In the world there are the three main sources of \(^{229}\)Th: Oak Ridge National Laboratory (USA), The Institute of Physics and Power Engineering (Russia), and The Institute for Transuranium Elements (Germany). The quantities produced are not enough for global application of \(^{225}\)Ac. To keep up with the increasing demand for \(^{225}\)Ac for clinical applications, it has been found that large scale quantities can be produced.
through high energy proton irradiation of $^{232}$Th.$^{34,35}$ To address the shortage the US Department of Energy formed a Tri-lab collaboration of Los Alamos (LANL), Brookhaven (BNL) and Oak Ridge (ORNL) National Laboratories with the goal of developing an alternative route for production of Ac.$^{36}$ Another limitation for this radionuclide has been with the radiochemical stability of the attachment to immunoconjugate. McDevitt et al.$^{25}$ state that instability of these attachments is due to the high classical recoil energy (100-200keV) of the daughter product which breaks the molecular bonds of the chelator. Significant advances have been made in developing chelators that form thermodynamically stable and kinetically inert complexes with $^{225}$Ac. Khabibullin et al., recently calculated the chelation stability of $^{225}$Ac and daughters in the 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) chelator.$^{37}$

As one potentially abundant starting material, $^{212}$Bi (1.01 h half-life) can be obtained from $^{226}$Th and decays via a branched pathway to $^{208}$Tl (36% α) and $^{212}$Po (64% β), then both decay to stable $^{208}$Pb.$^{23}$ However, $^{212}$Bi has several disadvantages that potentially limit its use. First, its short half-life can be problematic if the production and shipping processes are lengthy. This issue can be solved by using $^{224}$Ra as a generator to locally produce $^{212}$Bi. Another complication is the high energy gamma emission (2.6 MeV) which requires a considerable amount of shielding to minimize exposure. This along with the short half-life makes shipping difficult, resulting in an availability problem.

$^{223}$Ra is found naturally in trace amounts following the decay of $^{235}$U, but it is mainly produced artificially by the decay of $^{227}$Th (T$_{1/2}$ = 18.7 days), which is produced from $^{227}$Ac (T$_{1/2}$ = 21.77 years). Since $^{227}$Ac is found only in traces in uranium and thorium ores, it is mainly synthesized by $^{226}$Ra (T$_{1/2}$=1600 years) irradiation in a nuclear reactor.$^{38}$ $^{223}$Ra has a half-life of 11.4 days and emits 4 alpha particles, 2 beta particles and gamma rays on the path to stable
While the emission of 4 alpha particles is advantageous for tumor toxicity, $^{219}$Rn in gaseous form is also emitted which can redistribute in the body and dose non-targeted cells.

The major challenge using $^{223}$Ra is finding a suitable ligand for in vivo sequestration of radium. $^{223}$Ra has cationic charge similar to Ca$^{++}$ and because of this it is readily taken into bone. Because of this characteristic, $^{223}$Ra-dichlorde (Xofigo®) has demonstrated significant efficacy in the palliative treatment of bone metastases in castration resistant prostate cancer and has FDA approval for use in this application. However, this same property raises concerns about toxicity to normal bone if used for targeting cancer cells not associated with bone.

$^{227}$Th can be produced continuously from $^{227}$Ac and decays with a half-life of 18.7 days to $^{223}$Ra$^{39}$. The long half-life is beneficial for radiolabeling and targeting. $^{227}$Th also decays with accompanying gamma emissions of 236keV and 50keV which can be used for imaging but are low enough to avoid the need for patient shielding.

$^{212}$Pb has a 10.6 h half-life and is produced either from the decay chain of $^{228}$Th or by the $^{224}$Ra generator. Because of its long half-life and the fact that $^{212}$Pb is a β emitter that decays to $^{212}$Bi, one approach has been to use this radionuclide as an in vivo generator to compensate for the short half-life of $^{212}$Bi. $^{212}$Pb can deliver over ten times the dose per unit of administered activity compared to $^{212}$Bi or $^{213}$Bi. The major issue with $^{212}$Pb is the electron capture and auger electron emissions which can cause significant recoil of the $^{212}$Bi daughter. The free $^{212}$Pb has been shown to cause severe bone marrow toxicity while the free Bi has shown to cause kidney toxicity. By co-injecting DTPA or EDTA chelating agents, rapid release of free $^{212}$Bi can be achieved.
2.3 Internal Radiation Dosimetry

Dosimetry provides a means for evaluating the efficacy of a radiation therapy modality.\(^4\) Because the goal is to deliver high dose to tumor cells with minimal dose to normal tissues, it is of great importance to quantify accurately where, when, and how dose is being deposited. The absorbed dose assessment involves the calculation of the total energy per unit mass of the volume of interest.\(^{45}\) These volumes can be whole organs, tissue subregions, voxelized tissue structures, or individual cellular compartments.\(^{46}\) Internal radiation dosimetry requires the knowledge the radionuclide's physical properties (i.e. type of particles, emission energies, abundances, half-lives) and the measurements of biological properties (i.e. uptake, clearance, and tissue masses). Internal dosimetry can be divided into two categories: fixed-geometry and 3D image based dosimetry.

The conventional method of internal dosimetry was developed by the Medical Internal Radiation Dose Committee (MIRD) in the 1960’s. The MIRD method falls into the fixed geometry category. In this method, the dose calculation is dependent on the amount of activity as a function of time in a source organ, the energy emitted per disintegration and the fraction of emitted energy absorbed by a target organ.\(^{47}\) The fraction of energy absorbed from a source organ to a target organ for a given radionuclide is derived from a fixed geometry using a standard anatomical model.

The fundamental equation for calculating absorbed dose using the MIRD schema is given in Equation 1.

\[
D_x(r_T, T_D) = \tilde{A}(r_S, T_D) \frac{\sum \Delta_i^x \varphi(r_T \rightarrow r_S; E_i^x)}{M(r_T)}
\]  

(1)

where \(\tilde{A}(r_S, T_D)\) is the total number of nuclear transitions in the target region, \(\Delta_i^x\) is the mean energy emitted per disintegration for the \(i^{th}\) emission of type \(x\), \(\varphi(r_T \rightarrow r_S; E_i^x)\) is the fraction of
energy emitted per nuclear transition in the source region that is absorbed in the target region by the \(i^{th}\) emission of type \(x\) that is emitted with initial energy \(E\), and \(M(r_T)\) is the mass of the target region. \(\bar{A}(r_S, T_D)\), known as the cumulated activity, describes the kinetic portion of the dose and depends on the spatial and temporal distribution of the radionuclide. Serial activity measurements are taken over time post-administration and a time activity curve (TAC) is generated. The TAC is then fitted with a kinetic compartmental model and integrated over time to determine the total number of disintegrations in the source organ:

\[
\bar{A}(r_S, T_D) = \int_0^{T_D} A_s(t) dt
\]  

(2)

The mean energy emitted per emission \(\Delta_i\) is a physical property of the radionuclide and can be obtained from nuclear decay tables. The absorbed fraction for each decay type, \(\varphi\), is dependent on the reference phantom fixed geometry and obtained through Monte Carlo calculations. One can define an “S” value as:

\[
S = \sum \Delta_i \varphi(r_T \rightarrow r_S; E_i^x) / M(r_T)
\]  

(3)

Reducing Equation 1 to:

\[
D_x(r_T, T_D) = \sum r_s \bar{A}(r_S, T_D) \times S
\]  

(4)

The S value is pre-calculated and tabulated from the fixed geometry and tabulated for each particular radionuclide.

The value of the MIRD schema is its simplicity in that the physical aspects of the dose calculation are separated and all that needs to be obtained experimentally is the temporal kinetic information. The disadvantages of this method are in the broad assumptions that are made. This conventional method for the calculation of mean absorbed dose assumes uniform activity within the source volumes as well as assuming that the result will be predictive of biological effects. The spatial distribution of activity cannot be determined. The anatomical model is based on a
very primitive mathematical geometry consisting uniform density shapes. The most extensive software that has adapted the MIRD S factors is OLINDA/EXM which has calculated internal dose for over 200 radionuclides, including alpha emitters, in 10 different phantoms. The predetermined S values from these phantoms are unrealistic for human application. As a result of these assumptions it has been estimated that errors range from 30-100%. In therapeutic applications, where tumor control probability and normal tissue toxicity are of utmost importance, this standardized fixed geometry is unacceptable. In radionuclide therapy, especially alpha therapy, nonuniform activity distributions arise over temporal and spatial dimensions. Using the mean absorbed dose will under- or over estimate subregions in the volume of interest. The outcome of a therapy is then limited by the normal tissue toxicity.

With the development of high quality 3-dimensional imaging capabilities and increasing computational power, patient specific internal dosimetry has become possible. Using 3D computed tomography (CT) images, a phantom specific to the patient’s anatomy can be generated. Accurate organ volumes, location and densities are obtained through CT imaging. These voxelized volumes are segmented into regions of interest (ROI’s) to define source and target regions. Functional imaging such as single photon computed tomography (SPECT) or positron emission tomography (PET) is then used to obtain spatial and temporal biodistribution, at the voxel level, in the ROI’s. The SPECT/CT images are registered together and integrated voxel by voxel over time to obtain the 3D time integrated activity. A Monte Carlo package is then employed to model particle transport and energy deposition in each voxel.

Three methods of 3D voxel-based dosimetry using Monte Carlo can be used: (1) the voxel S value method, (2) the point dose kernel convolution method, and (3) full direct Monte Carlo calculation.
The fastest is the voxel S value method. This method extends on the original MIRD schema by developing voxelized phantoms to better match standard human anatomy. For example, Christy and Eckerman developed phantoms that represented a male, a female, and children.\textsuperscript{51} Later, as computational power increased, voxelized phantoms like VoxelMan were created based off of 3D imaging.\textsuperscript{52} MIRD Pamphlet No. 17 describes a method to extend the S value formalism to the voxel level to account for nonuniform distributions of activity.\textsuperscript{53} While accuracy is improved, the voxel S value method is still a model based approach to dosimetry.

In the point dose kernel convolution method, Monte Carlo is used to generate radionuclide specific absorbed dose per decay maps at radial distances from an isotropic point source in a homogeneous medium. The patient specific activity distribution obtained from SPECT imaging is then convolved with the dose kernel to obtain a 3D dose distribution. The major disadvantage of this method is that since the dose kernels are obtained in homogeneous media, tissue heterogeneity is not modeled.

The most accurate method for calculating 3D internal absorbed dose at the voxel level is direct Monte Carlo calculation. With full Monte Carlo simulation, all emission types can be simulated, tracked, and tallied to generate 3D dose distributions specific to the patient’s anatomy accounting for tissue heterogeneity. The patient’s CT dataset is converted into a voxelized phantom. Each voxel is defined to have a specific elemental composition and density creating an attenuation map. The patient’s SPECT provides the voxelized activity distribution map for the Monte Carlo source definition. Full Monte Carlo simulations are performed and energy deposition is tallied in every voxel within the patient derived phantom. Several software packages are available for 3D patient specific dosimetry including MCID \textsuperscript{54}, OEDIPE \textsuperscript{55}, and SCMS \textsuperscript{56} which are based on MCNP, 3D-RD \textsuperscript{57} and DOSIMG \textsuperscript{58} which are based on EGS \textsuperscript{59}.\textsuperscript{59}
RAYDOSE $^{60}$ and RAPID $^{61}$ which are based on GEANT4 $^{62}$, and DPM $^{63}$ which is not a public domain code. Both MCNP and GEANT4 can simulate alpha particles while EGS can only transport photons and electrons. While direct Monte Carlo methods are the most accurate means to perform 3D image based internal dosimetry, their use in the clinic has been limited due to complexity and expensive computational requirements.

While many radionuclides in radiopharmaceutical therapy have accompanying photon emissions, the energies and abundances are not large enough for practical 3D biodistribution studies using SPECT or PET. To overcome this obstacle, companion imaging agents have been developed. This theranostic approach involves developing a SPECT or PET tracer compound that will have similar biodistribution and pharmacokinetics as the therapeutic. For optimized treatment planning, the imaging agent is administered, the 3D accumulated activity distribution is obtained, and one of the above Monte Carlo approaches is employed to calculate what the absorbed dose will be in organs of interest following administration of the therapeutic agent. This approach has been used for pretreatment dosimetric analysis for many radiopharmaceuticals. Examples of using the theranostic approach include using $^{123}$I/$^{131}$I-metaiodobenzylguanidine (mIBG) in neuroblastomas, pheochromocytomas, and paragangliomas $^{64}$, $^{99m}$Tc/$^{188}$Re-hydroxyethylidene diphosphate (HEDP) for osteoblastic bone metastases $^{65}$, $^{68}$Ga/$^{177}$Lu-DOTA-TATE for peptide receptor radionuclide therapy of metastatic neuroendocrine neoplasia $^{66}$, $^{18}$F/$^{131}$I-F-ICF15002 for metastatic melanoma $^{67}$, and $^{111}$In/$^{90}$Y-ibritumomab tiuxetan for B-cell non-Hodgkin’s lymphoma $^{68}$. For image based dosimetry of the $^{223}$Ra-dichloide alpha emitting radiopharmaceutical, $^{99m}$Tc-methyl diphosphonate has shown to be a suitable companion imaging agent in the treatment of treatment of bone metastases $^{69}$. The $^{68}$Ga/$^{225}$Ac-Prostate-specific membrane antigen-617 (PSMA-617) $^{70}$ and the $^{203}$Pb/$^{212}$Pb-DOTA-
MCiL$^{71}$ pairs have also been used in dose calculations for the targeted alpha therapy of metastatic castration resistant prostate cancer and metastatic melanoma respectively.

The use of companion imaging agents for dose calculations improves on conventional methods. However, in most cases, the kinetic data obtained from the images are input into OLINDA and only mean organ doses are reported. For optimized treatment planning, the dosimetry calculations need to be performed at the voxel level. In this work I will expand on the conventional method of internal dose calculation used by OLINDA by developing a methodology using a companion imaging agent to calculate absorbed dose at the voxel level for our targeted alpha therapy.
Note to Reader

This research was originally published in *Molecules*. A Monte Carlo Method for Determining the Response Relationship between Two Commonly Used Detectors to Indirectly Measure Alpha Particle Radiation Activity. Christopher J. Tichacek, Mikalai Budzevich, Thaddeus J. Wadas, David L. Morse, Eduardo G. Moros. Molecules 2019, 24(18), 3397. © 2019 by the authors. Licensee MDPI, Basel, Switzerland.

3.1 Introduction

The use of targeted ligands to deliver alpha particles directly to cancer cells has become a promising strategy to treat tumors.\(^{70,72-74}\) This is because of their short path length of 40–80 µm and high linear energy transfer (LET~100 keV/µm).\(^{75}\) These two properties make alpha particles highly cytotoxic to targeted cells, with little damage to surrounding normal cells.

Owing to the large amount of energy deposited by alpha particles, it is important to quantify the radioactivity of the targeted radiopharmaceutical initially injected and its subsequent biodistribution, pharmacokinetics, and radiation dose deposited in various tissues. As a result of its short path length, the direct measurement of alpha particles’ activity in tissues is not possible in most, if not all, preclinical scenarios. If a parent metastable radioisotope also emits gamma rays, indirect methods of detection such as gamma spectroscopy can be used to estimate activity
using scintillation detectors and well-type ion chambers that are commonly available in research laboratories and nuclear medicine clinics.

Actinium-225 ($^{225}\text{Ac}$) was chosen as the radionuclide for alpha-particle therapy (TAT) targeted to the melanocortin 1 receptor for treatment of metastatic uveal melanoma$^{76}$, the prostate-specific membrane antigen (PSMA) for metastatic prostate cancer$^{72}$, and CD33 for acute myeloid leukemia.$^{77}$ Clinical studies have been carried out for the PSMA and CD33 TATs and others in pre-clinical development will soon go to clinical trials.$^{76}$

$^{225}\text{Ac}$ has a 10-day half-life and decays via six daughter radionuclides resulting in a net of four alpha particles per Actinium disintegration (Figure 2).$^{20,78}$ $^{225}\text{Ac}$ and two of its daughters, $^{221}\text{Fr}$ and $^{213}\text{Bi}$, also decay with accompanying isomeric gamma photons. Using gamma detection systems, such as ion chambers or scintillation detectors, it is possible to indirectly determine the alpha activity by gamma ray abundance per decay conversions.

![Decay chain of Actinium-225 ($^{225}\text{Ac}$).]$^{79}$

In the clinic, ion chambers are readily available and their use is the standard of practice for checking activities for diagnostic and therapeutic agents. An ion chamber reading does not
discriminate the collected charge between the parent and daughter radionuclides because it does not provide energy identification. In these situations, gamma spectroscopy using scintillation or semiconductor detectors, such as a sodium iodide doped with thallium scintillation detector \([\text{NaI(Tl)}]\) or a high purity germanium (HPGe) detector, can be used. With this approach, it is possible to determine the activity of each gamma emitting daughter, which gives more information on how the decay species behave.

While HPGe seems to be the attractive option owing to its superior energy resolution, its performance suffers as a result of its low detection efficiency, cost, and requirement of sophisticated cooling systems. Despite the advantages of the NaI(Tl), dead time losses at higher activities and poor energy resolution may also provide incorrect activity measurements, leading to underestimation or overestimation of radiation dose.\(^{80}\)

In pre-clinical and clinical biodistribution studies, activity measurements of collected blood and tissues are used to calculate pharmacokinetics and radiation dosimetry. Therefore, accurate measurements for \(^{225}\)Ac and daughters are needed. Hence, scintillation detector measurements are needed to generate these spectra. Alpha radiation dosimetry is performed using the methods recommended by the Committee on Medical Internal Radiation Dose.\(^4^{46}\) It is essential that these measurements are accurate because the dosimetry estimates are then extrapolated to human estimations and, therefore, serve as the fundamental basis for all patient safety measures.

Scintillation detectors are calibrated by analyzing three aspects of the detector: energy, resolution, and efficiency.\(^81\) Once the detector is fully calibrated, it can be used to perform gamma spectroscopic measurements in order to determine the activity of radioactive samples.\(^82^{87}\) Ion chambers are calibrated by measuring a sample of the radiopharmaceutical in question with known activity, correcting for decay, and applying a calibration factor. The American
National Standards Institute (ANSI) recommends that the applied calibration factor adjusts the measurement to within ±10% of the known activity. Although reference standards have been developed for isotopes used in internal radiotherapy, for example, $^{177}\text{Lu}$ $^{89}$ $^{225}\text{Ac}$ standards are currently under development by NIST and are not yet available. For this study, $^{225}\text{Ac}$ provided by Oak Ridge National Lab was used as a cross-reference source.

While there are evident limitations in making clinical predictions when translating a radiopharmaceutical from mice to humans, it is important to minimize these limitations that are the result of instrumentation. Herein, a threshold is identified above which the scintillation detector cannot accurately measure the activities of $^{225}\text{Ac}$ and daughters. An activity response relationship between the ion chamber and scintillation detector measurements is reported and used in a method to improve activity determination above the threshold by correcting scintillation detector measurements via Monte Carlo simulations. These corrections improve the dosimetry of pre-clinical work and ultimately facilitate the translation of the new radiopharmaceutical to the human clinic.

3.2 Materials and Methods

3.2.1 Mathematical Model

The $^{225}\text{Ac}$ decay chain can be characterized by a system of ordinary differential equations that describe the activities and abundances of each species as a function of time (Figure 3).
In Figure 3, $N_X$ and $\lambda_X$ denote the number of atoms of radionuclide and the decay constant of radionuclide $X$, respectively. These equations were simultaneously solved in Mathematica with the initial (time zero) $^{225}\text{Ac}$ activity set to $3.7 \times 10^4$ kBq and all daughters’ activities set to 0 kBq to determine the times at which daughter products reach equilibrium with the parent.

### 3.2.2 Monte Carlo Model

The Monte Carlo N-Particle version 6.1 (MCNP6.1) package was used to simulate the pulse height gamma distribution from $^{225}\text{Ac}$. A computational model of the 2” × 2” NaI(Tl) 4π detector was built using the material compositions and dimensions given in the manufacturer’s manual, as illustrated in Figure 4.
Figure 4. Geometry of the Monte Carlo model wipe test scintillator detector. Image generated using the Visual Editor software for MCNP. 2: NaI; 3: MgO; 4: Al; 6: polyethylene; 7: air; 8: H₂O with \(^{225}\)Ac, \(^{221}\)Fr, \(^{213}\)Bi source distributions.\(^{79}\)

A standard 5 mL polyethylene test tube filled with 1 mL of water was placed in the well of the detector. The source was defined to be uniformly distributed in the 1 mL water volume. All 75 photon emissions from \(^{225}\)Ac, \(^{221}\)Fr, and \(^{213}\)Bi were defined by energy and abundance.\(^{78}\) The photomultiplier tube was modeled as a 30 mm diameter aluminum cylinder to account for backscatter.\(^{93}\)

Photon transport was conducted using the mcplib84 photoatomic data library and detailed photon physics interactions.\(^{78}\) These include Thomson scattering, Compton scattering, and photoelectric absorption with the creation of fluorescence photons or Auger electrons. Full photon and electron simulations were performed and spectra were compared to those obtained from only photon transport. Photon and electron cell importances were set to 1 for all cells in the geometry. A mean percent difference of less than 1\% between photon/electron transport and photon only transport was observed in benchmark generated spectra of \(^{137}\)Cs. There were also less than 1\% differences in the Compton edge and Compton regions. Therefore, the computationally expensive
tracking of electrons was omitted in subsequent simulations and electron cell importances were set to 0. In this case, the electrons generated are assumed to deposit their energy locally. Any electron induced bremsstrahlung photons produced are accounted for in the thick-target bremsstrahlung model.\textsuperscript{94} This model skips the electron tracking step and transports the generated photon in the direction of the parent electron.

The pulse height distribution tally (F8) was used to calculate the deposited energy in the NaI(Tl) crystal. This tally accumulates the kinetic energy lost by local photon-induced secondary electrons, per history, in a specified volume. The tally was divided into 64 bins, each with a 12.5 keV width to match the energy resolution of the detector. To relate the simulated response to activity, values for the number of gamma emissions per disintegration for each isotope, scaled by the activity, were placed on the source probability card in the source definition. The resulting tallies were then multiplied by the sum of these values, the live acquisition time, and an activity conversion factor containing photon abundance per decay. For each simulation, $2 \times 10^8$ histories were run to achieve less than 1\% error under each photopeak.

MCNP6.1 models an ideal detector and thus does not take into account the Gaussian shape of experimental NaI(Tl) energy resolution resulting from intrinsic light collection inefficiency, statistical fluctuation in charge multiplication in the photomultiplier tube, and electronic noise.\textsuperscript{95} To account for the statistical variance of physical spectra, a Gaussian energy broadening function (MCNP6.1 FT8 GEB) was applied to the simulated pulse height distribution. The GEB function was obtained experimentally from measurements of the detector’s specific full width at half maximum (FWHM) from a set of isotopes of differing energies. Spectra from five isotopes were measured and the FWHM of each peak was calculated. The isotopes used and their physical characteristics are listed in Table 2.
Table 2. Physical characteristics of the radioactive sources used for NaI(Tl) detector energy response measurements.\textsuperscript{78,79}

<table>
<thead>
<tr>
<th>Source</th>
<th>Half-Life</th>
<th>Energy (keV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{129}$I</td>
<td>1.6 yr</td>
<td>39.5</td>
</tr>
<tr>
<td>$^{241}$Am</td>
<td>432.6 yr</td>
<td>59.5</td>
</tr>
<tr>
<td>$^{57}$Co</td>
<td>271.7 d</td>
<td>122.0</td>
</tr>
<tr>
<td>$^{68}$Ga</td>
<td>67.7 m</td>
<td>511.0</td>
</tr>
<tr>
<td>$^{137}$Cs</td>
<td>30.1 yr</td>
<td>661.6</td>
</tr>
</tbody>
</table>

A curve of FWHM as a function of energy was fit with the following nonlinear function shown in Equation 5:\textsuperscript{91}:

$$FWHM = a + b\sqrt{E + cE^2},\quad (5)$$

where \(E\) is the incident energy of the incident photon in keV; and \(a\), \(b\), and \(c\) are parameters that were determined by least squares fitting and input in the FT8 command.

### 3.2.3 Monte Carlo Simulations

There are no standard NIST-traceable calibration sources for $^{225}$Ac to validate the detector model. Instead, the spectrum of a NIST-traceable 155.4 kBq $^{137}$Cs (662 keV photopeak) source was measured in the NaI(Tl) detector and compared to a simulated spectrum of the same activity for validation.

After the model was validated, a high definition version of the F8 pulse height tally (1 keV bin width) was simulated with only $^{225}$Ac defined as the source. This was followed by separate high definition simulations of $^{221}$Fr and $^{213}$Bi. These simulations were done to see what radiations contribute to each peak, which are masked by the poor energy resolution of the NaI(Tl) detector.
3.2.4 Experimental Measurements

The $^{225}$Ac used in this work was obtained from Oak Ridge National Laboratory (ORNL). The Atomlab™ Wipe Test Counter (Biodex Medical Systems, Inc., Shirley, New York, USA) was used to perform gamma spectroscopy measurements with $^{225}$Ac. The wipe test counter is a 2” × 2” NaI(Tl) well-type scintillation detector with 1.2 cm of lead shielding. The multichannel analyzer associated with the wipe test NaI(Tl) detector has 64 12.5 keV channels ranging from 0 to 800 keV. Spectra obtained with the NaI(Tl) detector for 30 s acquisition time and full energy window to include gamma counts from $^{225}$Ac, with composite peak at 99.8 keV, and from its two gamma-emitting daughters, $^{221}$Fr ($T_{1/2} = 4.9$ min) with a peak at 218.1 keV and $^{213}$Bi ($T_{1/2} = 46$ min) with a peak at 440.5 keV. Background spectrum measurements were performed and subtracted from all obtained $^{225}$Ac spectra. The net background readings for the NaI(Tl) detector were in the range of 100 to 200 net counts per minute. The net counts for an $^{225}$Ac sample of 45 kBq activity was 444,000 per minute.

Dead time measurements and calculations were performed according to the two-source method. Two different sources of $^{22}$Na with different activities were each measured individually and then in combination.

In-house software was developed using MATLAB to calculate the net number of gamma counts, N, by fitting each peak with a Gaussian fit and integrating. Alpha particle activity for each species was calculated using factors for gamma abundance (Y) per alpha decay: $^{225}$Ac (1%), $^{221}$Fr (11.4%), $^{213}$Bi (25.9%).

The detector efficiency, $\varepsilon$, and energy calibration for the NaI(Tl) detector were determined by performing measurements of NIST-traceable $^{137}$Cs, $^{133}$Ba, and $^{57}$Co sources. These radionuclides
have numerous photopeaks covering the gamma energy spectrum of $^{225}$Ac. The activity is then
given by the following Equation 6:

$$A = \frac{N}{t \cdot \varepsilon \cdot Y'} \tag{6}$$

where $t$ is live time. The activity of $^{225}$Ac determined using this approach was compared to a decay
corrected sample of known activity from ORNL. A factor of 0.082 was applied to Equation 2 to
obtain $^{225}$Ac activity values to within 5% of the ORNL specified $^{225}$Ac activity. The uncertainty in
the NaI(Tl)-determined activity was calculated by propagating individual uncertainties in the net
number of counts and the efficiency measurements, as is typically done in nuclear counting
measurements.

The Atomlab™ 500 Dose Calibrator is a well-type pressured ion chamber filled with argon
gas that is commonly used in the clinic. This instrument was calibrated for energy and efficiency
with the following NIST-traceable sources: $^{137}$Cs (6.63 MBq), $^{57}$Co (39.26 MBq), and $^{133}$Ba (5.71
MBq) by the vendor. Lower activity NIST-traceable sources provided by Eckert & Ziegler were
also used: $^{137}$Cs with 158.73 kBq and $^{57}$Co with 391.09 kBq activities. No significant variation in
the calibration coefficient as a function of photon energy was observed. An ion chamber linearity
test was performed with $^{18}$F in the activity range of 1.2 GBq to 266.0 kBq and a variance of 1.4%
was observed. The dial number was set at 38.2 for $^{225}$Ac, as indicated by the vendor and confirmed
by our comparison with two decay corrected samples of different activities obtained from Oak
Ridge. Measurements of these samples in the ion chamber agreed with the Oak Ridge
measurements to within 7%. In order to determine the relationship between this ion chamber and
the NaI(Tl) detector, six different activities of $^{225}$Ac samples were measured using the ion
chamber: 70.3 ± 7.0, 144.3 ± 14.4, 222.0 ± 22.2, 299.7 ± 30.0, 370.0 ± 37.0, and 447.7 ± 44.7 kBq.
Each sample was measured and placed in the NaI(Tl) detector, and a spectrum was obtained and
compared. Using the activity reading from the ion chamber as the initial parent–daughter equilibrium activity input for the Monte Carlo model, simulated spectra were calculated and compared to the measured spectra from the NaI(Tl) detector. Mean absolute percent differences at analogous bins between the experimental and simulated spectra were calculated. Using the ion chamber measured activity as input to the Monte Carlo simulations underestimated the readings from the NaI(Tl), so several successive runs were completed using different activities as input in symmetrical range around the initial point in order to match the spectra. The percent differences were plotted against input activity and fitted with a linear model. The x-intercept was found in order to determine the minimum mean percent difference between the measured and simulated spectra. This process was repeated for each sample and an activity response function relationship was determined between the ion chamber and the NaI(Tl) detector. The uncertainty in the activity relationship determined using this methodology was analyzed by adding the uncertainties in the NaI(Tl) measurements, \( \sigma_{NaI}^2 \); the ion chamber measurements, \( \sigma_{IC}^2 \); and the Monte Carlo calculations, \( \sigma_{MC}^2 \), in quadrature (Equation 7):

\[
\sigma_A = \sqrt{\sigma_{NaI}^2 + \sigma_{IC}^2 + \sigma_{MC}^2}.
\]  

3.3 Results

The system of ordinary differential equations describing the \(^{225}\text{Ac}\) decay chain was solved to determine the daughters’ activities as a function of time; these results are plotted in Figure 8 for \(^{225}\text{Ac}\), \(^{221}\text{Fr}\), and \(^{213}\text{Bi}\). For an initial activity of \(3.7 \times 10^4\) kBq of \(^{225}\text{Ac}\), \(^{221}\text{Fr}\) reaches secular equilibrium with \(^{225}\text{Ac}\) in 55 minutes, while \(^{213}\text{Bi}\) takes 380 minutes. Therefore, after 380 minutes (6.37 h), the parent and all daughter nuclides are in secular equilibrium.
To account for the statistical variance of physical spectra, a Gaussian energy broadening function was integrated with the ideal Monte Carlo simulation model. The parameters of this function were determined by nonlinear fitting of the measured FWHM versus energy. These parameters were determined to be $a = 0.005616$, $b = 0.0521$, and $c = 2.027$ with an R-squared value of 0.9984. This allowed the benchmark validation of the Monte Carlo model using the $^{137}$Cs standard source (Figure 6). Simulating 155.4 kBq, the known source activity, showed a 1.02 mean percent difference at analogous bins between measured and simulated spectra.
Figure 6. Comparison of measured and simulated spectra of $^{137}$Cs.\textsuperscript{79}

The measured NaI(Tl) gamma spectrum and Gaussian peak fitting of $^{225}$Ac and its daughters can be seen in Figure 10. The two source method was used to determine the dead time of the scintillation detector to characterize the saturation of the signal with increasing activity. The value was determined to be $4.6 \times 10^{-5}$ s\textsuperscript{-1}. The dead time value was incorporated into the gamma spectrum fitting method that was used to determine the scintillation detector activities of $^{225}$Ac, $^{221}$Fr, and $^{213}$Bi, and these values were compared to the ion chamber measurements (Table 3). All samples were in secular equilibrium during measurement, as shown in Figure 8. The ion chamber manufacturer error is reported as 3%. Measurements using NIST-traceable sources within the activity range of 5.705 to 39.257 MBq had 3% uncertainties. However, when measuring NIST-traceable sources at the lower activities of 158.73 and 391.09 kBq, the uncertainties were 9.7% and 9.6%, respectively.
Figure 7. NaI(Tl) measured gamma spectrum with Gaussian fitting.\textsuperscript{79}

Table 3. Ion chamber and NaI(Tl) measured activities. Single measurements were taken in each detector. Notice that as the ion chamber readings increase, the discrepancies with the NaI(Tl) increase. All values in kBq. Note that the uncertainties in the ion chamber readings are expressed as 10\% in accordance with the calibration measurements in the activity range (above). The NaI(Tl) uncertainties are expressed as the propagation of uncertainties in all steps of the calculation. The difference between the ion chamber readings and the NaI(Tl) determined activity for $^{225}$Ac are indicated in the last column to the right.\textsuperscript{79}

<table>
<thead>
<tr>
<th>Ion Chamber Reading</th>
<th>$^{225}$Ac</th>
<th>$^{221}$Fr</th>
<th>$^{213}$Bi</th>
<th>$^{225}$Ac Percent Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>70.3 ± 7.0</td>
<td>57.35 ± 7.57</td>
<td>66.97 ± 8.18</td>
<td>63.64 ± 7.98</td>
<td>20.23</td>
</tr>
<tr>
<td>144.3 ± 14.4</td>
<td>96.20 ± 9.81</td>
<td>115.07 ± 10.73</td>
<td>111.74 ± 10.57</td>
<td>40.00</td>
</tr>
<tr>
<td>222.0 ± 22.2</td>
<td>124.69 ± 11.17</td>
<td>152.81 ± 12.36</td>
<td>143.93 ± 12.00</td>
<td>56.14</td>
</tr>
<tr>
<td>299.7 ± 30.0</td>
<td>140.23 ± 11.84</td>
<td>177.97 ± 13.34</td>
<td>165.39 ± 12.86</td>
<td>72.50</td>
</tr>
<tr>
<td>370.0 ± 37.0</td>
<td>152.07 ± 12.33</td>
<td>198.32 ± 14.08</td>
<td>182.41 ± 13.51</td>
<td>83.49</td>
</tr>
<tr>
<td>447.7 ± 44.7</td>
<td>140.23 ± 11.84</td>
<td>211.64 ± 14.55</td>
<td>187.59 ± 13.70</td>
<td>104.59</td>
</tr>
</tbody>
</table>

The spectra acquired by the scintillation detector (Figure 8) have overlapping multiple peaks that are further masked by the poor resolution of the detector. Monte Carlo simulations with 1 keV resolution were run for the parent and gamma emitting daughter radioisotopes. As observed in Figure 5, $^{225}$Ac, $^{221}$Fr, and $^{213}$Bi all exhibit many peaks in the 90 to 200 keV range. Monte
Carlo simulations allowed for the accounting of contributions from each individual radioisotope to the overlapping gamma spectra.

![Graphs showing simulated gamma spectra of different isotopes.](image.png)

**Figure 8.** High definition Monte Carlo simulated gamma spectra of (A) $^{225}$Ac, (B) $^{221}$Fr, (C) $^{213}$Bi, and (D) all three superimposed.

Monte Carlo simulations were run using the bin width of the NaI(Tl) detector and ion chamber readings. The resulting spectra were compared to the corresponding scintillation detector spectra and it was observed that as the readings increased, the percent difference between the measured and simulated spectra increased. The percent differences ranged from 19% at 70.3 kBq up to 156% at 447.7 kBq. This is illustrated in Figure 9. The readings that
minimized the mean percent differences were used as the equilibrium input activities in the Monte Carlo simulations. Figure 10 shows the matching of simulated spectra to measured spectra for all six of the samples of different quantities. This procedure was repeated for all ion chamber readings. Figure 11 shows the ion chamber reading and the simulated activity that matches to within 1% of the NaI(Tl) spectra for all of the samples. At low readings, the measured and simulated quantities agree reasonably well: 70.3 kBq measured in the ion chamber matched with 62.9 kBq equilibrium Monte Carlo activity and 144.3 kBq measured in the ion chamber matched with 107.3 kBq Monte Carlo activity. For larger ion chamber readings, the matched response began to plateau.

Figure 9. The mean percent difference between measured spectra and simulated spectra using the ion chamber reading measurement as input for the Monte Carlo model.
Figure 10. Measured vs. simulated $^{225}$Ac spectra for (A) 70.3 kBq (0.17%), (B) 144.3 kBq (0.5%), (C) 222.0 kBq (0.19%), (D) 299.7 kBq (0.99%), (E) 370.0 kBq (0.11%), and (F) 447.7 kBq (0.02%). The values in parentheses are the mean percent differences between the two spectra.
3.4 Discussion

The purpose of this work is to develop a method to determine the activities of $^{225}$Ac and daughters in samples using standard clinical instruments. This is important for the determination of blood clearance pharmacokinetics and tissue biodistribution, both of which are used for radiation dosimetry calculations. In this work, an activity response relationship is determined between two radiation detectors commonly used for pre-clinical and clinical applications, a well-type NaI(Tl) scintillation detector and an ion chamber, respectively, where $^{225}$Ac activities between the range of 70 to 450 kBq are not accurately measured by the scintillation detector. This relationship was used to inform Monte Carlo simulations that corrected scintillation detector measurements for accurate readings within this range. Although the error of the ion chamber measurements increased to $\sim$10% within this activity range, compared with errors of 3% observed when measuring activities as low as 6 MBq, the error within this range for the
scintillation detector as compared with the benchmarked Monte Carlo simulations ranged from 20% to 160% (Figure 9).

Several preclinical studies have reported using a NaI(Tl) gamma counter to measure $^{225}$Ac injection and biodistribution activities.$^{25,96-99}$ Ion chambers are commonly used in nuclear medicine departments to prepare radiopharmaceuticals for patient injection. Measuring the activity of radioisotopes with complicated decay pathways, such as in the case of $^{225}$Ac, the readings are not only the result of the parent’s activity, but also from the contributions from the daughters’ activities. From the solution of the system of decay equations, it was determined that $^{225}$Ac and its daughters reached secular equilibrium in less than seven hours. While the daughters emit alpha particles and/or beta particles, the ranges of these particles are not penetrative enough to enter the ion chamber. Therefore, the ion chamber reading is a result of the gamma rays emitted from $^{225}$Ac, $^{221}$Fr, and $^{213}$Bi. For example, a recent human $^{225}$Ac therapy study reported injection activities for $^{225}$Ac, but it was not clear if the reported values were from bulk measurements or if spectra were acquired and only $^{225}$Ac emissions were considered.$^{78}$

Several groups have built Monte Carlo models of NaI(Tl) detectors to simulate response functions based on standard benchmark simulations and measurements of $^{137}$Cs without description of further extensions of their models.$^{93,100-104}$ Herein, this procedure was extended to include the detector response for $^{225}$Ac and its daughters. Implementation of the adjustment coefficients obtained by the nonlinear fitting into the Monte Carlo model adequately described the Gaussian spread of the detector pulses and was evident in benchmark testing. For all simulations, it was assumed that $^{225}$Ac, $^{221}$Fr, and $^{213}$Bi were in secular equilibrium and thus had equal activities. The high-definition Monte Carlo simulations showed that both $^{221}$Fr and $^{213}$Bi exhibit peaks in the energy range of $^{225}$Ac gamma pulse height distribution. Because of the 12.5
keV energy resolution of the NaI(Tl) detector, these peaks are summed to form a larger composite photopeak resulting in greater net counts, and thus over-calculated activities.

Gamma spectroscopic measurements were performed using the NaI(Tl) scintillation detector. All samples were measured after the calculated time to reach secular equilibrium, but the resulting activities were not equal. Further, the sum of activities for all three species greatly overestimated the ion chamber readings. Although the activities determined for the three isotopes should theoretically be identical, discrepancies are the result of the low energy bin resolution of the detector and the dead time count saturation. Using the measured ion chamber readings as input to the Monte Carlo simulations (Figure 9) largely overestimated the measured NaI(Tl) spectrum.

After minimizing the difference between each sample spectra and the Monte Carlo output, an activity response function relationship was determined for the two detectors (Figure 11). Both the Monte Carlo activity and the ion chamber reading represent the sum of the activities of $^{225}$Ac, $^{221}$Fr, and $^{213}$Bi. The plot shows a curve with a decreasing positive slope as the ion chamber measurements increased beyond 148 kBq. This is a result of the NaI(Tl) dead time losses. As the activity of a sample increases, more pulses are lost, leading to errors in counting. The discrepancy between the low energy measured and simulated peaks in Figure 10 is also a result of dead time effects. The ratio of simulated to measured peaks increases with increasing activity because the Monte Carlo model does not account for dead time. Further, the disagreement in the simulated and measured spectra in the low count region arises from the fact that the model represents an idealized detector and does not account for the metallic impurities of a real detector.
Standard NIST-traceable samples of $^{225}$Ac are currently unavailable, so $^{225}$Ac obtained from Oak Ridge was used as a surrogate calibration reference. These samples did not come with uncertainty estimations. As measurements using the recommended dial number agreed with the Oak Ridge surrogate reference samples to less than 10%, the readings of the ionization chamber were considered reliable for the study carried out in this work. The scintillation detector’s measured energy resolution and detection efficiency over the energy range of $^{225}$Ac gamma emissions (40-662 keV) agreed with other published studies and reports. Consequently, this detector was also considered to be calibrated in efficiency and energy for $^{225}$Ac analysis. The calibration sources used were all less than 185 kBq. When calibrating the ion chamber with sources with activity above 5.71 MBq, the error was less than 3%. When testing with sources in the lower activity range, error in the measurements increased to 10%, which is still within ANSI recommended tolerance. The measurement results showed that the responses from these two detectors do not correlate well in the activity range of 70 to 450 kBq, even though both detectors were calibrated for efficiency and energy resolution according to their respective recommended methods. The Monte Carlo model developed herein corrects NaI(Tl) measurements, allowing for a more accurate estimate of $^{225}$Ac and daughter activities over a range of activities useful for both pre-clinical and clinical use.

3.5 Conclusions

In this work, the $^{225}$Ac activity response function between two commonly used instruments was investigated, a range of activities (70 to 450 kBq) was identified, and a method was developed using Monte Carlo simulations to correct scintillation detector measurements within this range. The scintillation detector was calibrated in energy and efficiency using NIST-
traceable sources covering a broad energy range, while the ion chamber was calibrated in efficiency using a surrogate calibration reference. The error of ion chamber measurements increased with decreasing activity, but remained within the ANSI recommended value of 10%. The NaI(Tl) performed poorly from 70 to 450 kBq. Therefore, a Monte Carlo model was built to correct the NaI(Tl) measurements, thereby providing improved accuracy. The corrections provided by the Monte Carlo model will provide a useful tool to make corrections to the activity values measured by the well-type NaI(Tl) detector. This allows for the better quantification of alpha-particle emitting radiopharmaceuticals and daughter byproducts in pre-clinical and clinical studies. In this case, use of scintillation gamma spectroscopy will be the preferred method.
CHAPTER FOUR:

MELANOCORTIN 1 RECEPTOR TARGETED ALPHA-PARTICLE THERAPY FOR METASTATIC UVEAL MELANOMA

Note to Reader


4.1 Introduction

Uveal melanoma is the most common primary intraocular malignancy and differs from the more common cutaneous melanoma in terms of risk factors, primary treatment, anatomic spread, molecular changes and response to systemic therapy\textsuperscript{107,108}. Patients that develop uveal melanoma metastases, primarily in the liver, have a very poor prognosis, with a median survival of about one year. Because uveal melanomas have different characteristic mutations compared to cutaneous melanomas, targeted therapies that have been effective for cutaneous melanoma, e.g. BRAF, are not indicated\textsuperscript{109}. Immune checkpoint inhibition therapies that are successful in cutaneous melanoma have had poor efficacy in ocular melanoma with less than 10% of patients responding and with rapid recurrence\textsuperscript{109}.
The melanocortin 1 receptor (MC1R) is highly expressed in uveal melanoma metastases\(^{110}\). MC1R is a member of a family of five G protein coupled melanocortin receptors, four of which bind melanocyte-stimulating hormone (MSH) and related ligands (MC1R, 3R, 4R & 5R)\(^{111}\). Unlike the other members of this G protein family, MC1R is not expressed in most normal human tissues\(^{112}\), lessening concern for therapy-related toxicity. Although expression is found in the brain\(^{113}\) and normal melanocytes\(^{114}\), this is not a major concern as conjugates can be designed to not cross the blood brain barrier and in the most severe cases of melanocyte loss, the most serious symptom is vitiligo\(^{115}\). MC1R expression has been reported on activated monocytes, macrophages and dendritic cells derived from monocytes\(^{116}\). This is also not a significant concern since the population of activated monocytes and macrophages can be replenished within days and lymphoid dendritic cells, which do not express MC1R, will not be depleted. MC1R is highly polymorphic\(^{117}\), but the wild-type frequency is ~50%\(^{114}\) and the most common mutations occur with a frequency of 21.5% in cytoplasmic domains, 19.7% in transmembrane domains and 0% in the extracellular domain\(^{117}\). Hence, a large majority of patients will have an MC1R isoform that is suitable for ligand binding. A MC1R specific ligand (MC1RL) and conjugates were previously developed with high specificity (>200 fold) and affinity (0.2-0.4 Nm K\(_i\)) for MC1R\(^{118,119}\). A fluorescent-dye conjugate was rapidly internalized by MC1R expressing tumor cells, does not cross the blood brain barrier and is rapidly cleared from circulation\(^{113}\).

Herein is reported the preclinical development and testing of a novel MC1R targeted radiopharmaceutical, \(^{225}\)Ac-DOTA-MC1RL, for targeted \(\alpha\)-particle therapy (TAT)\(^{120,121}\) of uveal melanoma. Alpha-particle emissions consist of di-cationic helium nuclei (He\(^{2+}\)) that have high linear energy transfer and a short mean free path of only a few cell diameters (< 100 \(\mu\)m) in
tissue. $^{225}$Ac is an $\alpha$-particle emitting radionuclide that has a 10 day half-life, four $\alpha$-emissions in its decay chain and high (28 MeV) total energy release.

### 4.2 Materials and Methods

#### 4.2.1 Compound Synthesis and Loading with Lanthanide

MC1RL was synthesized according to a conventional N$^\alpha$-Fluorenylmethyloxycarbonyl (Fmoc) peptide synthesis strategy, except the Fmoc-Lys(Alloc)-OH was coupled to allow orthogonal alloc deprotection of the linker on the epsilon amino group of the lysine following the linear peptide synthesis. The alloc group is removed and Fmoc-aminohexanoic acid linker and tri-t-butyl-1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetate (DOTA, TCI) were coupled sequentially using O-(1H-6-chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU) activation. The DOTA-MC1RL peptide was cleaved from the resin with a cocktail of trifluoroacetic acid (TFA, Chem-Impex International), water ($H_2O$), and triisopropylsilane (TIS, Sigma-Aldrich) (95:2.5:2.5, v/v), precipitated in cold diethyl ether, pelleted/decanted, and lyophilized. The crude white powder was purified by a reverse-phase high-performance liquid chromatography (HPLC, Agilent) and characterized by both MALDI-TOF mass spectroscopy (JEOL) and analytical HPLC. A scrambled peptide ligand (DOTA-SP) was synthesized by changing the order of amino acids (sequence: 4-Phenylbutyric Acid-Trp-Gly-His-Arg-(D)-Phe-Lys(Aminohexanoic Acid-DOTA)-CONH$_2$). The Eu-DTPA-MC1RL was synthesized as described before except that MC1RL was used as the binding ligand. Competition binding assays were performed as previously described using the Eu-DTPA-NDP$_\alpha$-MSH ligand. The Eu-DTPA-MC1RL binding affinity was determined using saturation binding assays. To determine MC1RL binding affinity for murine MC1RL, saturation
binding assays were performed using the Eu-DTPA-MC1RL and B16-F10 murine melanoma cells with high expression of murine MC1R118.

4.2.2 Cell Culture and Characterization

Uveal melanoma cell lines were acquired: (OCM1, OCM3 and OCM8 from Dr. Kan-Mitchel, University of Southern California; OMM1 from Dr. Gregorius P. Luyten, University Hospital at Rotterdam; and MEL270, MEL290 and OMM2.3 from Timothy Murray, Bascom Palmer Eye Institute), and grown in RPMI medium, 10% fetal bovine serum, 100 units/ml penicillin, 100 mg/ml streptomycin, 1% 200 Mm L-Glutamine, 1% 100 Mm sodium Pyruvate, 1% MEM Essential Vitamin Mixture (100X), 1% NEAA Mixture (100X) and 1% 1M HEPES in 5% CO2 at 37°C. A375, A375/MC1R human cutaneous melanoma cells and Hek293/MC1R cells were obtained and grown as before118,126. Cells were authenticated per American Type Culture Collection (ATCC) guidelines127, monitored for original morphology, tested for mycoplasma (MycoAlert kit, Lonza) and only passage numbers <25 cells were used. MC1R expression and receptor number were determined as previously described118,119 except that Eu-DTPA-MC1RL was used for saturation binding.

4.2.3 Radiochemical Synthesis and Characterization

DOTA-MC1RL or DOTA-SP (10 μg/10 ml water),225Ac(NO3)3 (3.4 MBq), 90 ml of water and 10 ml of 20% L-ascorbic acid were added to a 1.5 ml tube followed by Ph adjustment to 5.5-6 (1 M Tris buffer; 10-12 ml) and incubation at 60°C for 1 h (Figure 15).
Specific activity was calculated using a standard method\textsuperscript{128}. Radiochemical purity was assessed 24 h after collection by gamma counter and \textit{in vitro} serum stability was determined by adding 50 µL of $^{225}$Ac-DOTA-MC1RL (2072 kBq) to 1 ml of human serum (n=4), incubated at 37°C for 10 days and quantified at multiple time points by TLC scanner and gamma-counter using established methods\textsuperscript{129}.

\textbf{4.2.4 Animal Studies}

All protocols were approved: University of South Florida IACUC protocol IS00000805 and Wake Forest University Health Sciences IACUC protocol A11-144. Male and Female animals were used. Sprague-Dawley rats, 10-12 weeks old, 200-250 g were purchased with jugular vein catheters installed (Charles River). Non-tumor studies used BALB/c mice (10-12 weeks, 18-22 g, Charles River). Severe combined immunodeficient (SCID) mice (6-8 weeks, 15-20 g, Charles River) were used for xenografting cell lines. Tail vein catheters were used for agent administration to mice.
For xenografting, $1 \times 10^6$ cells in 80 µl of PBS and 20 µl of Matrigel (phenol red free, Corning) were injected subcutaneously into the flank. Tumor volumes were determined by caliper using the formula: volume = $(\text{length} \times \text{width}^2)/2$ for A375 and A375/MC1R, and volume = $(\text{length} \times \text{width} \times \text{height})/2$ for MEL270 tumors, which were initially flat with a gradual shift to a rounded shape.

### 4.2.5 Histology and Immunohistochemistry (IHC)

Excised tissues were prepared for histology, hematoxylin and eosin (H&E), MC1R IHC staining, and slide scanning were performed as previously described. Metastasis burden was determined using images of 3 sections (25%, 50% and 75%) through each liver and lung. Metastasis area was determined by segmentation using intensity and size threshold classifiers on the triple red channel (Visiopharm software version 6.7.0.2590, Denmark), total tissue area was determined with an intensity threshold classifier on the I intensity channel and the percent metastasis calculated.

To quantify MC1R expression in tumors, images from serial H&E and IHC sections were analyzed using Visiopharm 2017.7. Each serial section pair (H&E and IHC) were aligned using the tissue align module and viable tumor segmented by thresholding the hematoxylin channel. A multi-threshold marker area analysis was then performed within the viable tumor region on each IHC image. Each pixel was placed into 4 categories (Negative, Weak, Moderate, Strong) based on thresholds set by a pathologist and percentages of each category were normalized by total area of interest.
4.2.6 Maximum Tolerated Dose (MTD)

The MTD study was performed as previously described.\textsuperscript{129}

4.2.7 Measurement of Activity

Since $\alpha$-particles from $^{225}$Ac cannot be directly measured in tissue due to the short mean free path,\textsuperscript{124} $^{225}$Ac $\alpha$-activities were estimated using measurements of related gamma emissions. For the initial MTD study, syringes were prepared with a range of activities as determined by the gamma counter (Wallac 1470 Wizard, Perkin-Elmer). For subsequent studies, syringes were prefilled with 148 kBq $\pm$10\% (per appendix E of the BioDex manual) of $^{225}$Ac-conjugate activity using a dose calibrator (BioDex Atomlab 500). Activities were measured for 2 mins using dial number 38.2 as recommended by Biodex. Activities of $^{225}$Ac, and the $^{221}$Fr and $^{213}$Bi daughter products\textsuperscript{124} were measured by acquiring isomeric gamma spectra (Figure 9) prior to administration using a 4$\pi$ well-type wipe-test gamma counter (BioDex Atomlab 500). Activities ($^{225}$Ac) were calculated using factors for gamma ray abundance per $\alpha$-decay using calibration parameters and correction coefficients from Appendices A and E of the instrument manual. A full energy window (0-800 keV) was used for spectra acquisition that included gamma counts from $^{225}$Ac (99.8 keV peak, 1\% abundance), and two gamma emitting daughters, $^{221}$Fr (218.1 keV peak, 11.4 \% abundance) and $^{213}$Bi (440.5 keV peak, 25.9 \% abundance)\textsuperscript{130}. The $\alpha$-activities were determined by fitting each peak with a multi-Gaussian fit and integrating to determine the net number of counts while incorporating the acquisition time. Spectra were acquired $\geq$24 h post-radiosynthesis or tissue rendering ensuring that $^{225}$Ac and daughters were in secular equilibrium\textsuperscript{131}. Activity remaining in the syringe and catheter post-injection were calculated and subtracted to determine net administered activity.
4.2.8 Blood Pharmacokinetics

Sprague-Dawley rats were weighed prior to injection with radioactivity and injected with 148 kBq (±10%) of $^{225}$Ac-DOTA-MC1RL in the syringe. Serial blood draws (45 µL) were taken from 5 min to 24 h post-injection (p.i.). $^{225}$Ac α-activity was calculated as described above. Data were fitted using an exponential decay nonlinear regression.

4.2.9 Biodistribution

Non-tumor bearing BALB/c mice, or SCID mice bearing MEL270 xenografts (160-650 mm³), or A375 and A375/MC1R bilateral xenograft tumors (189-1680 mm³) were intravenously administered 148 kBq (±10%) of $^{225}$Ac α-activity in the syringe. Tissues were rendered and weighed at multiple time-points between 24 h to 3 weeks p.i.. For each tissue, $^{225}$Ac, $^{221}$Fr and $^{213}$Bi α-activities were calculated as described above and reported as percent injected activity per gram (%IA/g).

4.2.10 Radiation Dosimetry (RD)

Tissue BD data for the different tissues were fitted using an exponential decay nonlinear regression and dosimetry calculations were performed for $^{225}$Ac, $^{221}$Fr, $^{217}$At, $^{213}$Bi, and $^{213}$Po using the generalized internal dosimetry schema of the Medical Internal Radiation Dose (MIRD) Committee for α-particle emitters. The β⁻ decay branching ratio for $^{217}$At to $^{217}$Rn is only 0.01%, therefore it was assumed that all decays of $^{217}$At were by α-emission to $^{213}$Bi. The branching ratios for decay of $^{213}$Bi to $^{213}$Po (98%) or $^{209}$Tl (2%) were included in the calculation. Due to the relatively low linear energy transfer and the small dimensions of the target tissues, the β⁻ emissions from $^{217}$At, $^{213}$Bi, $^{209}$Tl and $^{209}$Pb were assumed negligible and were not included
in the calculations. The following assumptions were made: 1) uniform distribution of activity in the tissue volume; 2) no α-particles escaped from the source tissue due to the short range; and 3) electron and photon contributions were assumed to be negligible compared to α-particle energy deposition. It was also assumed that α-particles from $^{221}$Fr (4.9 min T$_{1/2}$), $^{217}$At (32.2 ms T$_{1/2}$), $^{213}$Bi (46 min T$_{1/2}$) and $^{213}$Po (4.2 µs T$_{1/2}$) were deposited in the same location as $^{225}$Ac (10 d T$_{1/2}$) due to the relatively shorter half-lives of these daughter isotopes. Although $^{217}$At and $^{213}$Po do not have detectable gamma emissions, under the assumption that the decay chain had reached secular equilibrium, the accumulated activity of these two daughters would equal that of $^{221}$Fr and $^{213}$Bi, respectively. The total absorbed α-particle dose was calculated from the summation of doses from $^{225}$Ac, $^{221}$Fr, $^{217}$At, $^{213}$Bi and $^{213}$Po.

4.2.11 Anti-tumor Efficacy

Tumor bearing mice (n=11/group) were injected with activities of $^{225}$Ac-DOTA-MC1RL or $^{225}$Ac-DOTA-SP, cold La-DOTA-MC1RL, or saline solution (0.9%, Cardinal Pharmaceuticals). Surpassing 2000 mm$^3$ tumor volume was the experimental endpoint unless clinical endpoints, e.g. 20% weight loss, tumor ulceration, hunched back, lack of grooming or lethargy were observed. Metastasis formation was identified by necropsy.

4.2.12 Statistical Analysis

The T-test was used for the MTD study. The following analyses were used for comparison of the efficacy study groups: Kaplan-Meier for time to endpoint, a mixed model analysis for tumor growth change, a paired Wilcoxon Signed Rank test for initial decrease in tumor volume, the Fisher’s Exact test with corrections for multiple testing using the Holm
stepdown method for metastasis burden, and non-parametric Kruskal-Wallis test for IHC staining.

4.3 Results

4.3.1 Synthesis and Characterization of Parent Compound and Lanthanide Chelates

The unmetallated DOTA-MC1RL was synthesized and, since there are no non-radioactive isotopes of Ac, the analogous La-DOTA-MC1RL chelate was prepared for use as a non-radioactive control. Both DOTA-MC1RL and La-DOTA-MC1RL had high binding affinity for human MC1R, 0.24 ± 0.20 and 0.23 ± 0.18 Nm K_i respectively (Supplemental Fig. 9A). The binding affinity of Eu-DTPA-MC1RL to human MC1R was determined to be 4.4 ± 2.3 Nm K_d. Lower, 1.3 µM K_d affinity was observed for Eu-DTPA-MC1RL binding to murine MC1R. The scrambled peptide controls, La-DOTA-SP and Eu-DOTA-SP, did not bind.

4.3.2 Radiosynthesis and Characterization of ²²⁵Ac Radiopharmaceutical

Radiochemical purity of 99.8% and specific activity of 181.3 ± 92.5 kBq/µg and 140.6 ± 55.5 µkBq/µg for ²²⁵Ac-DOTA-MC1RL and ²²⁵Ac-DOTA-SP, respectively, were observed. In vitro serum stability was high, with 90% intact after 10 days (Table 4).

Table 4. In vitro serum stability of ²²⁵Ac-DOTA-MC1RL.

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<th>Day</th>
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<td>2</td>
<td>97.3 ± 0.5</td>
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<td>4</td>
<td>95.6 ± 1.1</td>
<td>95.1 ± 0.8</td>
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<tr>
<td>6</td>
<td>93.5 ± 0.8</td>
<td>93.2 ± 1.3</td>
<td></td>
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<tr>
<td>8</td>
<td>91.4 ± 1.2</td>
<td>91.0 ± 0.9</td>
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<td>10</td>
<td>90.2 ± 0.7</td>
<td>89.9 ± 1.3</td>
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4.3.3 MC1R Expression on Uveal Melanoma Cell Lines and Xenograft Tumors

MC1R mRNA and protein expression were confirmed in a set of uveal melanoma cell lines. Only MEL270, OMM2.3 and OMM1 cells carry the GNAQ or GNA11 mutations found in nearly all uveal melanomas. The MEL270 and OMM1 cells formed tumors in immunocompromised mice and all xenografts had high and uniform MC1R protein expression.

4.3.4 Receptor Number for Tumor Cell Lines

MEL270 cells were selected for the in vivo studies and it was determined that MEL270 cells have 410,000 receptors per cell, which is a higher level of endogenous expression than the engineered A375/MC1R cells that have 75,000 receptors per cell. The parental A375 melanoma cell line has extremely low expression with 400 ± 93 MC1R per cell.

4.3.5 In vitro MC1R-specific Cytotoxicity

Cytotoxicity assays were performed with the goal of demonstrating target-specific cytotoxicity. Assay conditions were not optimized to demonstrate maximal toxicity. Significantly reduced proliferation (p < 0.0001) was observed in uveal melanoma cells and the engineered A375/MC1R cells treated with Ac-DOTA-MC1RL relative to the untargeted Ac-DOTA-SP or PBS controls. All cell lines also had a significant (p < 0.001) response to incubation with Ac-DOTA-SP relative to PBS. However, there was no significant difference in A375 cell proliferation (extremely low MC1R) when treated with either the targeted or untargeted radiopharmaceutical. These results demonstrate MC1R-specific cytotoxicity. Assay replicates yielded comparable results.
4.3.6 Maximum Tolerated Dose (MTD)

The MTD was evaluated in immune-competent non-tumor bearing BALB/c mice (n = 5/cohort). Cohorts received a single i.v. injection of $^{225}$Ac-DOTA-MC1RL over the range of 0-148 kBq in the syringe. At completion of the study (>11 $^{225}$Ac half-lives, 118 days post injection) serum and tissues (adipose, bone, cecum, colon, duoden, esophageal, heart, ileum, kidney, liver, lung, lymph nodes, muscle, pancreas, small intestine, spleen and stomach) were collected for histology and then blindly examined by a veterinary pathologist to assess radiation-induced tissue damage. There was no remarkable damage observed in any of the tissues, e.g. the control kidneys had minimal multifocal interstitial fibrosis and minimal medullary protein in tubules which were both considered to be incidental findings. The incidental minimal medullary protein was also found in some kidneys from the groups that received treatment activities, but each treatment group also included kidneys that were within normal limits of all types of damage. The renal cortex of one kidney from the group with the highest administered activity had focal extracellular cortical hyaline substance which was healing and was considered to be an incidental finding. Blood urea nitrogen (BUN) and creatinine, which are important indicators of renal function, were also determined and were not significantly elevated among the groups (Figure 16 B&C). All animals had gained weight by the end of the study, albeit less weight was gained by animals at the highest dose level relative to the lowest (Figure 13A).
Figure 13. MTD study for non-tumor bearing mice: (A) Percent weight gain, (B) BUN and (C) blood creatinine.

4.3.7 Pharmacokinetics and Biodistribution

In rats, $^{225}$Ac-DOTA-MC1RL rapidly cleared (<15 min p.i.) from blood circulation (Figure 14).

Figure 14. Plot of rat blood clearance. Exponential decay non-linear regression line fit of $^{225}$Ac alpha activity in rat blood over time, following intravenous administration of $^{225}$Ac-DOTA-MC1RL (n=4 rats).

Following administration to non-tumor bearing BALB/c mice, $^{225}$Ac activities were observed primarily in clearance tissues. At 24 h p.i. of $^{225}$Ac-DOTA-MC1RL, the liver, kidneys, spleen and intestine had 21.2 ±2.8, 6.9±0.9, 2.9±0.8 and 2.9±2.0 %IA/g, while negligible activity
was observed in the other tissues measured. Activity had largely cleared from the tissues in 1-3 weeks (Figure 15A).

Figure 15. Biodistribution of $^{225}\text{Ac}$-DOTA-MC1RL. $^{225}\text{Ac}$, $^{221}\text{Fr}$ and $^{213}\text{Bi}$ activities in tissues from (A) non-tumor bearing BALB/c mice (n=6 per time point) and (B) SCID mice bearing MEL270 human uveal melanoma tumors (n=5 per time point). For tumor-bearing animals, activity was retained in MC1R positive tumors, i.e. MEL270 (Figure 15B) and A375/MC1R tumors (Figure 16A) that had 3.6±1.2 and 2.8±0.8 %IA/g, respectively,
compared to the nominal 0.30±0.1 %IA/g in the MC1R negative A375 tumors at 24 h post-injection. The clearance tissues in tumor bearing animals had lower activities compared to clearance tissues in the non-tumor bearing mice, e.g. 14.4±1.7 %IA/g in the livers of MEL270 tumor-bearing mice at 24 h (Figure 15B), compared to the 21.2 ±2.8 %IA/g observed in the non-tumor bearing mice (Figure 15A). The $^{225}$Ac-DOTA-SP tumor distribution in the bilateral A357 and A375/MC1R model was also determined and, as expected, uptake was minimal and did not differ among the positive and negative A375 tumors (Figure 16B).

![Figure 16. Biodistribution of (A) $^{225}$Ac-DOTA-MC1RL and (B) $^{225}$Ac-DOTA-SP in bilateral A375 and A375/MC1R tumors (n=5 per time point).](image)

The distribution of $^{221}$Fr and $^{213}$Bi were also determined (Figures 15 & 16). However, since $^{225}$Ac and daughters are at secular equilibrium by 24 h p.i. and the $^{221}$Fr and $^{213}$Bi atoms present
during injection will be mostly decayed, the $^{221}$Fr and $^{213}$Bi detected in the samples are from decay of the $^{225}$Ac taken into the tissues.

### 4.3.8 Radiation Dosimetry

BD data were fitted, and clearance kinetics, tissue biological half-life, accumulated activity, and absorbed dose/injected activity (Gy/kBq) were estimated for each radionuclide in each tissue for non-tumor bearing and MEL270 tumor bearing mice (Tables 5&6).

Table 5. Radiation dosimetry and clearance kinetics parameters for $^{225}$Ac-DOTA-MC1RL in non-tumor bearing BALB/c mice.\(^7\)

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<th>Parameter</th>
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The effective decay half-lives ($T_{\text{eff}}$) calculated for $^{225}$Ac in tissues, e.g. 7.2 d in liver, were shorter than the radiodecay half-life of $^{225}$Ac (10 d) indicating biological clearance. The calculated total absorbed dose per injected activity (Gy/kBq) for $^{225}$Ac-DOTA-MC1RL was minimal in all tissues except clearance organs and positive tumor. Since the positive tumors shrank rapidly in response to the treatment and the total absorbed dose values were extrapolated from data collected over a two week period, the dose values for the tumors are likely subdued relative to the clearance organs which did not have appreciable cellular toxicity at the administered activities. The total absorbed dose in the liver was generally lower in mice with tumors compared to non-tumor mice, e.g. 0.284 Gy/kBq and 0.704 Gy/kBq, respectively.

Table 5. (Continued)

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Table 6. Radiation dosimetry and clearance kinetics for $^{225}$Ac-DOTA-MC1RL in SCID mice bearing MEL270 tumors.  

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<td>0.6167</td>
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<td>547.425</td>
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<td>3.3617</td>
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<td>0.3859</td>
<td>3.3617</td>
<td>2.4736</td>
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<td>0.0056</td>
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4.3.9 Anti-tumor Efficacy

SCID mice bearing MEL270 tumors (124±36 mm$^3$ pre-treatment tumor volumes) were injected with a single administration of $^{225}$Ac-DOTA-MC1RL (92.5±9.3 kBq), $^{225}$Ac-DOTA-SP (99.9±9.9 kBq), La-DOTA-MC1RL (1 pmol/mouse) or saline. Representative images show much smaller tumors in treated mice relative to controls (Fig. 17A) and tumor volumes decreased immediately after treatment relative to controls (p=0.001) prior to eventual regrowth (Figure 17B).

![Figure 17](image)

Figure 17. Efficacy study in mice bearing MEL270 tumors: (A) Representative images of tumors (outlined); (B) initial tumor growth volumes; (C) Kaplan-Meier plots.
Treated mice had a significantly delayed time to experimental or clinical endpoint (p < 0.001) with a median survival of 148 days, compared to the median survival of control groups (79-108 d) and differences among the controls were not significant (Figure 17C). In this study, some animals were euthanized due to reaching clinical endpoints instead of the experimental endpoint. Some of the animals that reached clinical endpoints had metastases in the liver or lungs and metastasis burden was significantly lower in the $^{225}$Ac-DOTA-MC1RL treated group relative to the controls (p = 0.024) (Figure 18 A&B).

![Figure 18](image)

Figure 18. (A-B) Metastasis study in MEL270 uveal melanoma mouse model and (C-D) MC1R expression in tumors reaching endpoints from each treatment group. (A) Representative H&E staining and corresponding threshold segmentations of sections containing liver and lung.
metastases (Cold=La-DOTA-MC1RL, Scrambled=untargeted, treated=\(^{225}\)Ac-DOTA-MC1RL), blue=normal tissue and green=metastasis; and (B) quantified metastasis burden. (C-D) MC1R IHC staining of MEL270 tumors after reaching endpoints.\(^7\)\(^6\)

Mice bearing A375/MC1R tumors (240±110 mm\(^3\) pretreatment volume) were also injected with either sterile saline, La-DOTA-MC1RL, 107.3±11.1 kBq of \(^{225}\)Ac-DOTA-SP or 59.2±5.9 kBq of \(^{225}\)Ac-DOTA-MC1RL and significant decreases in tumor volume (p = 0.005) and tumor growth delay (p < 0.0001) were observed. Some tumors that disappeared did not recur and those mice lived their natural lifespan.

After tumors reached an endpoint, MC1R staining was quantified and the level of MC1R expression was not significantly different in treated tumors that responded by shrinking prior to
regrowth relative to control tumors (p = 0.60 MEL270 and p=0.82 A375/MC1R) (Figure 18 C&D).

4.4 Discussion

We have developed and evaluated a novel MC1R-targeted radiopharmaceutical, $^{225}\text{Ac}$-DOTA-MC1RL, for TAT of metastatic uveal melanoma. The choice of using a peptide targeting ligand is reinforced by the recent pre-clinical and clinical successes of TAT radio-peptides $^{99,120,121,137,138}$. Another group has also reported the development of a peptide-based TAT, $^{212}\text{Pb}$-CCMSH, that is targeted to melanocortin receptors for treatment of melanoma $^{139}$. However, $^{212}\text{Pb}$-CCMSH was associated with renal toxicity. This is likely due to the use of an $\alpha$-MSH (melanocyte stimulating hormone) derivative targeting ligand as $\alpha$-MSH has specificity for multiple melanocortin receptor isoforms, including MC5R which is expressed in the human kidney and lungs $^{112}$. The MC1RL targeting moiety used in the current work has specificity for the MC1R isoform $^{119}$, greatly reducing the potential for renal toxicity. Another advantage of $^{225}\text{Ac}$-DOTA-MC1RL compared to the $^{212}\text{Pb}$-TAT agent, is that $^{225}\text{Ac}$ has greater cell killing potential through generation of four $\alpha$-particle emissions per radionuclide compared to the single $\alpha$-emission of $^{212}\text{Pb}$ in their relative decay chains $^{25,139}$.

$^{225}\text{Ac}$-DOTA-MC1RL has high affinity for MC1R, high radiochemistry yield and purity, high biostability and MC1R specific cytotoxicity in vitro. In vivo studies demonstrated low toxicity, rapid blood clearance and uptake into MC1R positive tumors and clearance organs. Biodistribution studies demonstrated that $^{225}\text{Ac}$ remains in the compartments where $^{225}\text{Ac}$-DOTA-MC1RL was initially distributed, i.e. tumors and clearance organs, and the corresponding clearance kinetics parameters and radiation dose delivered by all $\alpha$-particle emitting
radioisotopes in the decay chain were calculated. Considering the 10 d half-life of $^{225}$Ac, most of the administered $^{225}$Ac-DOTA-MC1RL will have either been taken into tumor cells or cleared from the blood prior to decay. Hence, $^{225}$Ac-DOTA-MC1RL likely functions as an \textit{in vivo} $\alpha$-particle generator, concentrating $\alpha$-emissions in the target tumor tissues with limited translocation of daughter isotopes. This is consistent with the recent observations of efficacy with low toxicity observed for an $^{225}$Ac-PSMA targeting small-molecule conjugate.

\textit{In vivo} efficacy studies demonstrated significant tumor and metastasis growth delay, and prolonged survival in human uveal and cutaneous melanoma xenograft models in mice following a single treatment of $^{225}$Ac-DOTA-MC1RL, including some cures. Tumors that shrank and regrew following treatment had the same MC1R expression levels as controls, suggesting that multiple treatment regimens would increase efficacy.

\textbf{4.5 Conclusion}

We have developed and evaluated a novel MC1R-targeted radiopharmaceutical for TAT of metastatic uveal melanoma. \textit{In vivo} studies demonstrated low toxicity, rapid blood clearance, uptake into MC1R positive tumors and clearance organs, significant tumor and metastasis growth delay, and prolonged survival in human uveal melanoma xenograft models in mice following a single treatment of $^{225}$Ac-DOTA-MC1RL. This novel radiopharmaceutical has strong potential to benefit patients with metastatic uveal melanoma, which has had no significant improvement in treatment in the last 20 years.
CHAPTER FIVE:

BIODISTRIBUTION AND MULTI-COMPARTMENT PHARMACOKINETIC ANALYSIS OF TWO TARGETED ALPHA PARTICLE THERAPIES

5.1 Introduction

Receptor-targeted cancer therapy using alpha-emitting radionuclides has become an area of significant interest for both preclinical and clinical investigation.\textsuperscript{141} The interest in the use of alpha particles to treat metastatic cancer arises from their favorable physical interactions in tissue, i.e., short path length (50-100µm) and high linear energy transfer (LET) (80-100keV/µm), which are the result of their large kinetic energy and 2+ charge.\textsuperscript{4} These properties allow for the potential to target disseminated cancer cells through ligand conjugation and create irreparable DNA double strand breaks and other intracellular damage while minimizing radiation dose to non-targeted tissues.

The most common ocular malignancy, uveal melanoma, accounts for 85-95\% of all ocular melanoma cases.\textsuperscript{142} Despite favorable outcomes for local control, the five year survival after metastasis is less than 1\%, with a median survival time of 3.6 months post diagnosis.\textsuperscript{143} The melanocortin-1 receptor (MC1R) belongs to the melanocortin family of G protein coupled receptors and has been shown to be predominantly expressed by melanoma and melanocytes.\textsuperscript{144,145} MC1R has been shown to be overexpressed in malignant primary and metastatic uveal melanoma cell lines with insignificant expression in other normal
tissues.\textsuperscript{112,116,146} α-Melanocyte stimulating hormone (α-MSH), a tridecapeptide, is the most potent naturally occurring melanotrophic peptide for the activation of MC1R\textsuperscript{147} and is a potent stimulator of the differentiation of pigmented melanoma cells.\textsuperscript{144} Radiolabeled peptide ligands based on the sequence of α-MSH have been investigated, however these do not show selectivity for MC1R.\textsuperscript{146} An MC1R specific ligand, MC1RL, with high specificity and affinity for MC1R has been developed and tested.\textsuperscript{148,149} An MC1R targeted alpha emitting radiopharmaceutical, Ac-DOTA-MC1RL, has been synthesized and tested \textit{in vivo} showing promising results for clinical translation.\textsuperscript{76}

The quantity of radiopharmaceutical to be administered and resulting absorbed dose may be determined based on a prediction of the biodistribution (BD), uptake, and clearance of the drug over time in organs and tumors.\textsuperscript{150} Optimization of a radiotherapy consists of maximizing tumor control while minimizing normal tissue complications. Pharmacokinetic (PK) modeling using pre-clinical and clinical distribution data is a useful tool that can enable optimized treatment by estimating transport properties of the radiopharmaceutical.\textsuperscript{151} By segmenting the body into compartments which are related through differential equations, and placing measured radioactivity concentrations in them, one can make predictions on the effectiveness of the radiopharmaceutical. Typically, blood PK measurements are taken over time and a two compartment model is constructed and solved with acquired samples.\textsuperscript{152} A novel multi-compartmental PK model for a targeted alpha therapy could help to compare the BD, predict the relative effectiveness of different TAT compounds and provide a basis for rejection early in the developmental process.\textsuperscript{153} Many alpha emitting radionuclides cannot be directly imaged for biodistribution analysis. The model could be used alongside a companion imaging agent for patient specific treatment planning.
Two versions of a therapeutic alpha radiopharmaceutical are being investigated: $^{225}$Ac-DOTA-Ahx-MC1RL (Ahx) and $^{225}$Ac-DOTA-di-D-Glu-MC1RL (di-D-Glu). In this study, the BD in mice from each was assessed and a computational multi-compartment pharmacokinetic PK model was developed to describe drug distribution and elimination rates.

5.2 Materials and Methods

5.2.1 MC1RL Peptide Synthesis

The MC1RL peptide with di-D-Glu linker was synthesized on Rink Amide resin (initial loading: 0.568 mmol/g) using N$^\alpha$-Fluorenylmethyloxycarbonyl (Fmoc)-protecting amino acids and O-(1H-6-Chlorobenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HCTU)/Diisopropylethylamine (DIEA) strategy. After the resin was swollen in Dichloromethane (DCM) for 30 minutes, the Fmoc protecting group was removed with 2% 1,8-Diazabicyclo(5.4.0)undec-7-ene (DBU) in Dimethylformamide (DMF) (2 × 5 min). The resin was washed with N-methyl-2-pyrrolidone (NMP) and DCM three times each and the first amino acid was coupled using HCTU and DIEA in NMP (4 equiv of N$^\alpha$-Fmoc amino acid, 4 equiv of HCTU, and 8 equiv of DIEA). The double coupling was performed at all steps under the same coupling condition due to the sequence deletion and slower coupling rate in longer sequences. After coupling, the resin was washed with NMP (× 3) and DCM (× 3) and any unreacted free amine groups on the resin were treated using 50% acetic anhydride in pyridine for 5 min. After the resin was washed with NMP (× 3) and DCM (× 3), the same procedure was repeated for the next amino acid coupling until every residue was coupled.
5.2.2 Alloc Deprotection

The Alloc protecting group of C-terminal Lys was deprotected with piperidine (5-10 drops) and Pd(Ph₃)₄ (0.2 equiv) in anhydrous chloroform. Under the nitrogen gas, the reaction mixture was stirred for 15 min then repeated. After the resin was washed with chloroform, NMP and DCM, the following sequence D-Glu-D-Glu-DOTA was coupled to the free amine via HCTU coupling as described above.

5.2.3 Cleavage of Peptide from the Resin

The peptide and protecting groups were cleaved using cleavage cocktail (88 % Trifluoroacetic acid, 5 % water, 5 % phenol, and 2 % triethylsilane) for 4 h. The crude peptide was isolated from the resin by filtration, the filtrate was concentrated, and the peptide was precipitated in ice cold diethyl ether, dissolved in water, and lyophilized. The off-white crude powder was purified by reverse-phase chromatography. See Figure 20.

Figure 20. Scheme showing synthetic route to metal chelation.

The complexation of $^{225}$Ac with DOTA-di-D-Glu-MC1RL was performed identically to what has been previously described for previously described for $^{225}$Ac-DOTA-Alx-MC1RL.⁷⁶
5.2.4 *Radiosynthesis of* $^{225}$Ac *Radiopharmaceutical*

Radiochemical synthesis and characterization of $^{225}$Ac-DOTA-Ahx-MC1RL was performed as previously described.\(^{76}\)

5.2.5 *Animal Studies*

A375 and A375/MC1R human cutaneous melanoma cells were obtained and grown as before.\(^{118}\)

Two groups of 16 severe combined immunodeficient (SCID) mice bearing A375 and A375/MC1R bilateral xenograft tumors (189-1680 mm\(^3\)) were intravenously administered 148 kBq (±10%) of Ahx or di-D-Glu. After injection, 4 groups (n=4) were euthanized at 24, 96, 144 and 288 hour time points for each cohort. Tumors and 11 other organs including: blood, bone, brain, heart, intestine kidney, liver, lung, muscle, skin, and spleen, were harvested at each time point. Activities were determined for organs as described previously.\(^{76}\) Briefly, isomeric gamma spectra were acquired using a 4\(\pi\) well-type wipe-test gamma scintillation detector (BioDex Atomlab 500). Activities were calculated using factors for gamma ray abundance per alpha decay using calibration parameters and correction coefficients from Appendices A and E of the instrument manual.\(^{92}\) A full energy window (0-800 keV) was used for spectra acquisition that included gamma counts from $^{225}$Ac (99.8 keV peak, 1% abundance).\(^{130}\) The alpha activities were determined by fitting the $^{225}$Ac peak with a Gaussian fit and integrating to determine the net number of counts while incorporating the acquisition time of 30 seconds. BD was then reported as percent injected activity per gram (%IA/g). Time activity curves were then calculated for each organ. A 5-compartment PK model was built with the following compartments: blood, tumor, normal tissue, kidney, and liver (Figure 21 A).
This model is characterized by a system of 5 ordinary differential equations (ODE’s) using mass action kinetics which describe uptake, inter-compartmental transitions and clearance rates (Figure 21 B). $C_i$ represents radioactive volume concentration in different compartments in Bq/mL. The $C_{normal}$ compartment represents the sum of radioactive volume concentrations in non-clearance and non-MC1R specific tissues. $k_{ti}, k_{to}, k_{ni}, k_{no}, k_{ki}, k_{ko}, k_{li}$, and $k_{lo}$ are the forward and reverse first order transfer rate constants for the tumor, normal tissue, kidney, and liver compartments, respectively. $k_{ke}$ and $k_{le}$ are the first order elimination rate constants from the kidney and liver compartments. A bolus injection was assumed at time zero in the $C_{blood}$ compartment. Using Matlab, the ODE’s were simultaneously numerically solved and fit to experimental time activity curves using a genetic optimization algorithm.

Figure 21. A) Schematic of the multi-compartmental pharmacokinetic model. B) The system of ODE’s that represent the change in compartmental radioactive concentrations.
5.3 Results

5.3.1 Biodistribution

The time activity curves and biodistribution data were generated and can be seen in Figure 22 and Figure 23 respectively.

![Figure 22. Time activity plots generated for A) Ahx and B) di-D-Glu](image)

![Figure 23. Biodistribution results for A) $^{225}$Ac-DOTA-Ahx-MC1RL and B) $^{225}$Ac DOTA-di-D-Glu-MC1RL.](image)

Both compounds have minimal distribution to organs at risk other than kidney and liver, which are clearance. The Ahx and di-D-Glu had similar kidney uptake at 24 hours of 4.01 %IA/g and 5.33 %IA/g respectively. The di-D-Glu however had much lower uptake at 24 hours in the liver at 1.55 %IA/g compared to 11.49 %IA/g for the Ahx.
5.3.2 Pharmacokinetic Analysis

The fitting of the model solutions to the experimental Ahx biodistribution and di-D-Glu biodistribution data with optimization by the genetic algorithm can be seen in Figure 24 and Figure 25 respectively.

Figure 24. Optimized fitting of compartment solution: Ahx. Note: Compartments were solved and fit simultaneously but plotted separately for visualization.
Figure 25. Optimized fitting of compartment solution: di-D-Glu. Note: Compartments were solved and fit simultaneously but plotted separately for visualization.

The calculated pharmacokinetic transfer and elimination rates for investigated compounds can be seen in Table 7.

Table 7. PK parameter estimates. All values are [1/hour].

<table>
<thead>
<tr>
<th>Compound</th>
<th>kti</th>
<th>kto</th>
<th>kni</th>
<th>kno</th>
<th>kki</th>
<th>kko</th>
<th>kke</th>
<th>kli</th>
<th>klo</th>
<th>kle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ac-DOTA-Ahx-MC1RL</td>
<td>0.0031</td>
<td>0.0008</td>
<td>0.0130</td>
<td>0.0025</td>
<td>0.0034</td>
<td>0.0035</td>
<td>0.0008</td>
<td>0.1193</td>
<td>0.0227</td>
<td>0.0002</td>
</tr>
<tr>
<td>Ac-DOTA-di-D-Glu-MC1RL</td>
<td>0.0026</td>
<td>0.0003</td>
<td>0.0010</td>
<td>0.0057</td>
<td>0.0015</td>
<td>0.0071</td>
<td>0.0002</td>
<td>0.0022</td>
<td>0.0081</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

All of the calculated PK parameter estimates had less than 5% error. From these data, the Ahx showed larger and faster uptake in the liver. Both compounds had comparable uptake and clearance rates for other compartments.
5.4 Discussion

Several multi-compartment pharmacokinetic models have successfully been designed for therapeutic radiopharmaceutical analysis; however these have been limited to applications using beta emitters. In this work, the biodistribution and pharmacokinetic behavior for two targeted alpha-emitting radiopharmaceuticals targeted to MC1R were investigated.

Both Ahx and di-D-Glu were preferentially taken into high expressing MC1R tumors while negligibly taken into non-clearance organs and low expressing MC1R tumors. While the liver showed large uptake of Ahx at 24 hours, these values rapidly decreased to negligible levels by the final timepoint. Although lower, the di-D-Glu remained nearly constant in the liver during the course of the study. Both compounds showed similar renal uptake at 24h hours, however Ahx was more rapidly eliminated from the kidney. Two other peptide MC1R targeted alpha therapies have been developed and tested in vivo, $^{212}$Pb-DOTA-Re(Arg$^{11}$)CCMSH, and $[^{212}$Pb]DOTA-MC1L. In the study of both of these compounds, relatively large concentrations of activities were found in the kidneys and varying degrees of nephrotoxicity were observed. In the current study, neither Ahx, nor di-D-Glu treatment resulted in renal toxicity.

A PK model was previously developed to analyze the PK of the MC1RL conjugated to a near infrared fluorescent dye. The model from that study was adapted for analysis of the targeted alpha therapy described in this work. The findings of the biodistribution analyses were reflected in the optimized fitting of the multi-compartment pharmacokinetic model. When the obtained transfer and elimination rates were input into the model, the resulting fits to experimental data agreed with less than 5% error. The liver uptake rate for the Ahx derivative was calculated to be >5 times larger than the intercompartmental transfer rate and 600 times greater than the elimination rate. The decrease in activity in the liver then is a result of physical
radioactive decay with a small portion being returned to the blood compartment. It was previously shown through histology studies, that despite the large uptake in the liver for the Ahx compound, no remarkable damage was observed.76

On the other hand, solutions for the di-D-Glu derivative showed much slower uptake, of about 50 times less than the Ahx, and much slower reverse transfer rate. This is in agreement with the BD analyses. The calculated MC1R tumor uptake rates were similar between the compounds. The reverse transfer rate for each was much slower indicating favorable long residence times in these tissues. The PK calculations indicate that since there are comparable distributions of radioactivity between the compounds, with much less liver uptake for di-D-Glu, the injected dose of this derivative could possibly be increased to increase the uptake into MC1R positive tumor tissues without toxicity.

In conclusion, the two targeted alpha therapies tested in this study, $^{225}$Ac-DOTA-Ahx-MC1RL and $^{225}$Ac-DOTA-di-D-Glu-MC1RL showed favorable uptake into MC1R positive tissues with no toxicity to other organs of interest. Since the multi-compartment model’s solutions agreed with the measured biodistribution data with low error, therapeutic optimizations can be considered. The kidney and liver have been identified as the primary dose limiting organs for the two derivatives. The PK model can be used to simulate the uptake rates and accumulation rates, clearance rates, and absorbed radiation doses to the organs of interest for varying initial injection activities and multiple dosing regimens. The model could be used alongside a companion imaging agent to predict biodistribution for patient specific treatment planning. This analysis will aid in the accurate prediction of the effectiveness of the therapies in patients.
CHAPTER SIX:

DEVELOPMENT OF A THREE DIMENSIONAL VOXEL-BASED MONTE CARLO RADIATION DOSIMETRY METHODOLOGY FOR A TARGETED ALPHA PARTICLE THERAPY

6.1 Introduction

The goal of this work is to enable individualized 3D voxel based dosimetry of the $^{225}\text{Ac}$-DOTA-MC1RL radiopharmaceutical. While there are detectable gamma emissions in the $^{225}\text{Ac}$ decay chain, SPECT/CT acquisition times required to obtain acceptable counting statistics as a result of low gamma emission abundance would be unrealistic in the clinical setting. Therefore, a companion imaging tracer needs to be developed with comparable BD properties and computational methods need to be developed to convert tracer activity measured to alpha-emission activities from the $^{225}\text{Ac}$ decay chain, followed by dose calculations. In the case of the $^{225}\text{Ac}$-DOTA-MC1RL radiopharmaceutical, $^{67}\text{Ga}$ is an ideal SPECT imaging tracer for the voxel-based dosimetry application, as it is chemically similar to $^{225}\text{Ac}$ in terms of charge, is readily chelated by DOTA and has a long 3.26 d half-life so that longitudinal post-administration acquisitions can be made. MCNP was used to calculate the volume-specific absorbed dose of the $^{67}\text{Ga}$-DOTA-MC1RL tracer. Corresponding ex vivo $^{67}\text{Ga}$-DOTA-MC1RL and $^{225}\text{Ac}$-DOTA-MC1RL BD/PK activity measurements and MIRD-calculated dosimetry data from the same
animal models and time-points were then used to optimize the simulations and evaluate its accuracy.

6.2 Materials and Methods

6.2.1 Biodistribution and Mean Organ Dosimetry Studies

Sixteen SCID mice were intravenously injected with 18.5 Mbq of $^{67}$Ga-DOTA-MC1RL in the syringe. The method of net injected activity determination was conducted as described for $^{225}$Ac-DOTA-MC1RL. Three groups (n=4) were euthanized at 24, 48, and 96 hour time points. Thirteen organs were harvested at each time point. Organ activities were determined using the Biodex scintillation detector. Spectra were obtained and fitted with multi Gaussian fits. The computational model of the NaI(Tl) detector described in Section 2.1.2 was used to simulate the $^{67}$Ga gamma spectrum and determine the detector correction factor. The activity at each timepoint was calculated by integrating each photopeak, applying the corresponding detector efficiency and abundance at the energy of each photopeak, and summing. The activities at each time point were averaged, and the biodistribution was calculated and presented a %IA/g. Tissue BD data for the different tissues were fitted using an exponential decay nonlinear regression and mean absorbed dose calculations were performed using the MIRD methodology. Since $^{67}$Ga is a gamma emitter, the assumption that all emissions remain in source location fails and absorbed fractions need to be considered. Monte Carlo pre-calculated S-values obtained from the Mouse Whole-Body (MOBY) phantom for $^{67}$Ga were used to in these calculations. The absorbed dose in each target organ was calculated as the summation of contributions from all source organs, including the target organ as a source organ.
6.2.2 Development of Voxel-Based Dosimetry

6.2.2.1 CT to MCNP Conversion Algorithm

In order to develop the voxelized dosimetric algorithm, a preliminary CT dataset was acquired on a clinical CT scanner of the Electron Density Phantom (EDP, Sun Nuclear Corp.). The EDP consists of a zero HU Solid Water® disk the size of an average human pelvis. Sixteen insert chambers in the disk are designed to be used with interchangeable sets of Tissue Mimicking Materials (TMM), including 13 different materials with a wide range of electron density values (lung, breast, zero solid water, liver, brain, inner bone, bone, cortical bone, true water, adipose, and optional bones with different mineral composition). To inform the Monte-Carlo model of the elemental composition of a given volume that corresponds to a specific HU value, electron densities of specific volumes were linked to HU values determined in the TMM’s and HU-ED calibration curves were generated. EDP inserts have well known electronic densities and material composition. A material library containing elemental composition and electron densities was created. ROIs in the phantom CT images were contoured using Mirada Medical software. The external boundary of the whole EDP was also segmented so that radiation dose to the rest of the phantom can be calculated and the photon and electron scattering will be properly simulated.

The CT dataset and DICOM tags were read and stored using MATLAB. MCN6.2 has the capability to define repeated structures as a lattice. The lattice defines an array of indexed hexahedra prisms that can fill any structure in the geometry. Lattice elements with similar properties, such as materials can be grouped together into “universes”. Therefore, the geometry of the entire CT dataset can be defined using several DICOM tags found in the headers of the CT. The ImagePositionPatient tag defines the Cartesian coordinate of the center of the upper-left
most voxel of the first slice of the dataset. PixelSpacing and SliceThickness define the
dimensions of each voxel, while Height, Width, and Slices define the number of voxels in the x,
y and z directions in the dataset. Using these tags, the boundaries of the dataset were defined in
MCNP which was then filled with a lattice describing the voxels.

The dataset was stored in MATLAB as a 3D matrix where each element is a voxel
containing its grayscale intensity value. To interpret these intensities in a physical manner, the
values were linearly transformed to Houndsfield Units (HU) using the RescaleSlope and
RescaleIntercept DICOM tags. The HU scale was segmented into ranges that represent the
radiodensities of the TMM of the EDP phantom. The material definitions are shown in Table
8.

Table 8. The materials and corresponding densities and elemental compositions used in
dosimetric calculations:  

<table>
<thead>
<tr>
<th>ID</th>
<th>Material</th>
<th>HU Range</th>
<th>Density (g/cm³)</th>
<th>H (Z=1)</th>
<th>C (Z=6)</th>
<th>N (Z=7)</th>
<th>O (Z=8)</th>
<th>Na (Z=11)</th>
<th>Mg (Z=12)</th>
<th>P (Z=15)</th>
<th>S (Z=16)</th>
<th>Cl (Z=17)</th>
<th>Ar (Z=18)</th>
<th>K (Z=19)</th>
<th>Ca (Z=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Air</td>
<td>&lt;800</td>
<td>0.001225</td>
<td>-</td>
<td>-</td>
<td>0.755636</td>
<td>0.231475</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.012842</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Lung</td>
<td>-799 –  -200</td>
<td>0.26</td>
<td>0.103</td>
<td>0.105</td>
<td>0.031</td>
<td>0.749</td>
<td>0.002</td>
<td>-</td>
<td>0.002</td>
<td>0.003</td>
<td>0.003</td>
<td>-</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Fat</td>
<td>-199 –  -50</td>
<td>0.9</td>
<td>0.114</td>
<td>0.598</td>
<td>0.007</td>
<td>0.278</td>
<td>0.001</td>
<td>-</td>
<td>0.001</td>
<td>0.001</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Water</td>
<td>-49 – 50</td>
<td>1</td>
<td>0.111</td>
<td>-</td>
<td>-</td>
<td>0.889</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Muscle</td>
<td>50-280</td>
<td>1.05</td>
<td>0.102</td>
<td>0.143</td>
<td>0.034</td>
<td>0.71</td>
<td>0.001</td>
<td>-</td>
<td>0.002</td>
<td>0.003</td>
<td>0.001</td>
<td>-</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Bone</td>
<td>&gt;281</td>
<td>1.6</td>
<td>0.034</td>
<td>0.155</td>
<td>0.042</td>
<td>0.435</td>
<td>0.001</td>
<td>0.002</td>
<td>0.103</td>
<td>0.003</td>
<td>-</td>
<td>-</td>
<td>0.225</td>
<td></td>
</tr>
</tbody>
</table>

The entire CT dataset was defined as integer values representing each material. Each element of
the same material designation was defined as a universe, which then filled the defined lattice.
The geometrical and material definitions were written to a file compatible for MCNP input.

6.2.2.2 ROI Extraction Algorithm

After the CT dataset was acquired, each TMM was segmented into 13 regions of interest
(ROI’s) in Mirada. The resulting DICOM structure set (RTSS) file was imported into MATLAB.
This file contains the series of points in Cartesian space that define each ROI on each CT slice.
An affine transformation was applied to map the points of each ROI to specific voxels of the corresponding CT dataset.

\[
\begin{bmatrix}
P_x \\
P_y \\
P_z \\
\end{bmatrix} = \begin{bmatrix}
X \Delta i & Y \Delta j & Z \Delta k & S_x \\
X \Delta i & Y \Delta j & Z \Delta k & S_y \\
X \Delta i & Y \Delta j & Z \Delta k & S_z \\
0 & 0 & 0 & 1
\end{bmatrix} \begin{bmatrix}
i \\
j \\
k \\
\end{bmatrix}
\]

Figure 26. Affine transformation to map to voxel location. \(P_{xyz}\) are the Cartesian coordinates of the voxel, \(S_{xyz}\) are the Cartesian coordinates of the voxel in the top left corner of the dataset, \(ijk\) are the voxel indices, \(\Delta ijk\) are the pixel spacing and slice thickness, and \(XYZ\) define the directional cosines.

A binary mask was applied to each voxelized ROI with the parameter that if greater than half of a given voxel was covered by the line of shortest distance between neighboring points, that voxel would return true.

6.2.2.3 SPECT to MCNP Conversion Algorithm

The absolute activity quantification must be determined for a particular SPECT system and isotope to perform image based internal dosimetry. This calibration factor is used to convert the reconstructed SPECT voxel values to activity.\(^{163}\) A 5 mL polyethylene sphere was filled with activity of \(^{67}\)Ga and measured in the Biodex ion chamber. SPECT images were immediately obtained of the filled sphere in air using the Siemens Inveon two head SPECT scanner with 5-MWB-1.0 collimators. The SPECT data were reconstructed using the OSEM3D reconstruction algorithm with the point spread function model. Effective isotropic voxel size was 0.5 mm. The dataset was imported into Mirada where the sphere was contoured into an ROI. The ROI extractor was used to voxelize the ROI and sum the SPECT values within the volume. The absolute activity calibration factor (in units of Bq/counts) was determined by dividing the activity measured in the ion chamber by the sum of the SPECT voxel values. This value was
applied to all voxels in the dataset. The indices of each element and corresponding activity value of the dataset were then incorporated into the voxelized source definition of the MCNP input.

### 6.2.3 In vivo Imaging Studies

After developing the image conversion algorithms using the phantom studies, a clinically translatable workflow was designed (Figure 27).

![Workflow Diagram](image)

**Figure 27.** The clinical workflow to perform patient 3D specific dosimetry.

This workflow was applied to a preclinical in vivo experiment described below.

μSPECT/CT images were acquired for three SCID mice at 24, 48, and 96 hour time points. CT images were obtained using the Siemens Inveon conebeam CT scanner. Effective pixel resolution was 88.0 μm. CT data were reconstructed using Siemens’ OSEM3D
reconstruction protocol with no binning. SPECT images were obtained using the Siemens Inveon two head SPECT scanner with 5-MWB-1.0 collimators. SPECT data were reconstructed using the OSEM3D reconstruction algorithm with point spread function model. Effective isotropic voxel size was 0.5 mm. The CT and SPECT datasets were registered manually using Mirada. The CT dataset was then resampled to match the 120x120x240 matrix size of the SPECT dataset. ROI’s for the kidneys, liver, and external were created for each mouse. The ROI extraction algorithm was employed and the whole organ SPECT-calculated $^{67}$Ga activity was compared to measurements in the ion chamber. For each time point, the CT and SPECT conversion algorithms were executed to generate the MCNP input. A full Monte Carlo run was performed for each time point with all $^{67}$Ga gamma emissions above 0.5% abundance defined. Detailed photon physics treatments were employed. The detailed physics treatment includes coherent scattering, fluorescent photons post photoelectric absorption, and Compton profiles to account for electron binding effects$^{91}$. Secondary electrons were produced but assumed to deposit their energy locally using the thick target bremsstrahlung model. Energy deposition (F6) tallies were placed in every voxel in the geometry within the external contour. The Monte Carlo generated dose rate maps for $^{67}$Ga-DOTA-MC1RL were integrated to obtain total absorbed dose. This was compared to total body absorbed dose calculated using the MIRD fixed geometry schema.

Therapeutic absorbed dose was calculated for $^{225}$Ac in the kidneys, liver, and total body. The $^{67}$Ga-to-$^{225}$Ac activity conversion was performed using the SPECT derived activity per voxel and assuming that the number of $^{67}$Ga atoms was equal to the number of $^{225}$Ac atoms at each location in the geometry:

$$A_{225Ac} = A_{67Ga} \frac{\lambda_{225Ac}}{\lambda_{67Ga}} \quad \text{Equation (9)}$$
resulting in a 3D $^{225}$Ac activity map. Monte Carlo simulations were performed for the entire spectrum of $^{225}$Ac alpha particle emission. Alpha particles were tracked with the FermiLab angular deflection model with Vavilov straggling.\textsuperscript{91} Delta ray production was turned on with and tracked with a 1 keV energy cutoff. Bremsstrahlung production and transport were turned on also with a 1 keV energy cutoff. The alpha particle tracking energy cutoff was set to 10 keV. Absorbed dose calculations were performed for the total body for $^{67}$Ga and $^{225}$Ac for each time point. Voxelized organ absorbed dose simulations were also performed for $^{225}$Ac-DOTA-MC1RL considering only each specific organ resulting in absorbed dose rates at each time point. The dose rates were fitted with exponential nonlinear regression and integrated to obtain the total absorbed dose in each voxel. The image based mean absorbed dose in each organ was compared to the MIRD mean absorbed doses previously calculated. Dose volume histograms (DVH) were generated to analyze the 3D dose distributions. All Monte Carlo simulations were performed on the high performance computing cluster at Moffitt Cancer Center. The number of histories for each simulation was chosen to give tally errors of less than 10% in each voxel.

### 6.3 Results

#### 6.3.1 Biodistribution and Mean Organ Dosimetry

The computational Monte Carlo model of the NaI(Tl) detector described in Chapter III was used to simulate the $^{67}$Ga gamma spectrum and determine the detector correction factor (Figure 28 A). The gamma spectrum fitting algorithm was applied to acquired $^{67}$Ga measurements (Figure 28 B).
Figure 28. A) Monte Carlo simulated gamma spectrum of 148 kBq of $^{67}$Ga. B) Representative gamma spectrum and fitting of $^{67}$Ga acquired with the NaI(Tl) scintillation detector.

The NaI(Tl) correction factor was determined to be 5.65. The harvested organs were measured in the NaI(Tl) detector and activities determined using the spectrum fitting algorithm. The biodistribution is shown in Figure 29.
The tissue BD data were fitted using an exponential nonlinear regression and integrated in order to obtain the cumulated activity in each organ. Using the pre-calculated S values from the MOBY phantom and the MIRD schema, estimations for mean organ absorbed doses were made (Figure 30).

Figure 29. Biodistribution of $^{67}$Ga-DOTA-MC1RL.

Figure 30. Mean $^{67}$Ga-DOTA-MC1RL absorbed dose.
6.3.2 Phantom SPECT/CT imaging

CT images of the EDP phantom were acquired with a matrix size of 512x512x28 (Figure 31). Contours were manually drawn around all 13 TMM’s and the external on each relevant slice.

![Electron Density Phantom](image)

Figure 31. Electron Density Phantom. A) CT-image of middle axial slice, B) visible light image and C) segmented CT image.

Using the CT to MCNP algorithm, the EDP dataset was converted from DICOM format to the MCNP lattice geometry (Figure 32 A). The voxel values were replaced with material/universe integer ID values.

![MCNP Converted EDP Dataset](image)

Figure 32. A) The MCNP converted EDP dataset. B) The TMM ROI’s in voxel coordinates.
The ROI extraction algorithm was implemented on the segmented EDP dataset (Figure 32 B).

The absolute activity quantification was determined for the Inveon scanner with $^{67}$Ga. The Biodex ion chamber reading of the $^{67}$Ga activity filled sphere was 234.95 kBq. SPECT images of the sphere were acquired and segmented creating an ROI (Figure 33). The ROI extraction algorithm was applied and the total counts within the voxels of the ROI were 9339018 counts. The resulting activity calibration was 0.0252 Bq/count.

![Figure 33](image.png)

Figure 33. Sphere filled with known activity. SPECT images were acquired and used to determine the absolute activity calibration factor for the scanner.

### 6.3.3 In vivo Imaging Studies

Three mice were injected with 18.5 MBq of $^{67}$Ga-DOTA-MC1RL in the syringe. SPECT/CT’s were acquired at 24, 48, and 96 hour timepoints. The CT datasets were downsampled to match the resolution of the SPECT datasets. The SPECT datasets were then manually registered to the CT datasets. ROI’s were drawn for the kidneys, liver and external on
the CT (Figures 34, 35, 36). The ROI extraction algorithm voxelized the ROI’s and converted them into a format readable by MCNP.

Figure 34. Coronal CT slice of mouse with manually drawn kidneys and liver. The external contour was generated by thresholding.

Figure 35. Kidneys and liver volumes in voxel coordinates.
The SPECT-based activity was determined by calculating the total activity in each ROI. These values were compared to whole organ activity measured in the ion chamber (Table 9). The image based activity calculations generally underestimated the ion chamber measurements. For the ion chamber kidney measurements it was assumed the activities of the left and right kidneys were equal as they were measured together. The average percent differences across all three timepoints for the right kidney, left kidney, liver, and body were $11.46 \pm 3.31$, $12.14 \pm 3.26$, $13.59 \pm 6.03$, and $50.64 \pm 10.42\%$ respectively. The percent difference between the methods remained less than $\sim 20\%$ for the organs. The differences in total body activity estimations was large reaching up to $63\%$ difference at the 96 hour timepoint.
Table 9. The SPECT derived ROI activities compared to organ activities measured using the ion chamber.

<table>
<thead>
<tr>
<th></th>
<th>24 hrs</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney_R</td>
<td>Kidney_L</td>
<td>Liver</td>
<td>Body</td>
</tr>
<tr>
<td>SPECT Activity (kBq)</td>
<td>23.45 ± 10.81</td>
<td>22.44 ± 8.33</td>
<td>136.00 ± 9.49</td>
<td>254.02 ± 4.55</td>
</tr>
<tr>
<td>IC Activity (kBq)</td>
<td>25.90 ± 2.59</td>
<td>25.90 ± 2.59</td>
<td>120.95 ±12.09</td>
<td>444.00 ± 4.44</td>
</tr>
<tr>
<td>% Difference</td>
<td>9.48</td>
<td>13.38</td>
<td>12.44</td>
<td>42.79</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>48 hrs</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney_R</td>
<td>Kidney_L</td>
<td>Liver</td>
<td>Body</td>
</tr>
<tr>
<td>SPECT Activity (kBq)</td>
<td>17.93 ± 11.77</td>
<td>13.28 ± 10.68</td>
<td>59.73 ± 9.25</td>
<td>166.24 ± 4.85</td>
</tr>
<tr>
<td>IC Activity (kBq)</td>
<td>15.55 ± 1.55</td>
<td>15.55 ± 1.55</td>
<td>65.08 ± 6.51</td>
<td>311.73 ± 31.17</td>
</tr>
<tr>
<td>% Difference</td>
<td>15.28</td>
<td>14.60</td>
<td>8.22</td>
<td>46.67</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>96 hrs</th>
<th></th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kidney_R</td>
<td>Kidney_L</td>
<td>Liver</td>
<td>Body</td>
</tr>
<tr>
<td>SPECT Activity (kBq)</td>
<td>6.58 ± 9.97</td>
<td>5.50 ± 8.42</td>
<td>29.73 ± 9.51</td>
<td>150.67 ± 3.75</td>
</tr>
<tr>
<td>IC Activity (kBq)</td>
<td>6.01 ± 0.60</td>
<td>6.01 ± 0.60</td>
<td>37.21 ± 3.72</td>
<td>401.45 ± 40.15</td>
</tr>
<tr>
<td>% Difference</td>
<td>9.61</td>
<td>8.44</td>
<td>20.12</td>
<td>62.47</td>
</tr>
</tbody>
</table>

The CT to MCNP conversion algorithm was performed on the three datasets. The defined materials were air, lung, fat, water, muscle, and bone. The SPECT to MCNP conversion algorithm was performed for the $^{67}$Ga-DOTA-MC1RL and the converted $^{225}$Ac-DOTA-MC1RL distributions for the three timepoints. Monte Carlo absorbed dose calculations were performed for the whole body for both radionuclides at each time point. Voxelized organ absorbed dose simulations were also performed for $^{225}$Ac-DOTA-MC1RL considering only each specific organ. The converted CT, and corresponding slices of the resulting whole body absorbed dose distribution from $^{67}$Ga-DOTA-MC1RL and converted $^{225}$Ac-DOTA-MC1RL can be seen in Figure 37.
Figure 37. For 24, 48, and 96 hours: A) Mouse-specific phantom obtained from CT. B) Absorbed dose map for $^{67}$Ga-DOTA-MC1RL. C) Corresponding absorbed dose map for $^{225}$Ac-DOTA-MC1RL. Note the difference in scales. Spatial dimensions are in centimeters.
The mean whole body absorbed dose for $^{67}$Ga-DOTA-MC1RL was 2.6085E-06 mGy/MBq. This underestimated the MIRD calculated whole body dose of 4.9E-04 mGy/MBq.

Mean absorbed doses for the right kidney and liver were calculated. Voxelized dose maps for $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL can be seen in Figure 38.

![Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the right kidney at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters.](image)

Figure 38. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the right kidney at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters.

The dose rates were fitted with an exponential regression and integrated to obtain the total absorbed dose to the kidney from $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL (Figure 39). The mean absorbed dose to the right kidney from $^{67}$Ga-DOTA-MC1RL was calculated to be $0.0084 \pm 0.0022$ mGy/MBq. Using the MIRD schema, the mean absorbed dose to the right
kidney was found to be 0.0079 ± 0.0016 mGy/MBq (Figure 30). The same procedure was performed to calculate the mean dose from $^{225}\text{Ac}$-DOTA-MC1RL to the right kidney.

![Graph showing exponential fitting of mean dose rate as a function of time for $^{225}\text{Ac}$-DOTA-MC1RL in the right kidney.](image)

**Figure 39.** Exponential fitting of the mean dose rate as a function of time for $^{225}\text{Ac}$-DOTA-MC1RL in the right kidney.

The mean absorbed dose to the right kidney was calculated to be 0.0213 ± 0.0082 Gy/kBq. Using the MIRD schema, the mean absorbed dose to the right kidney was found to be 0.0150 ± 0.0031 Gy/kBq (Table 5). The DVH for the kidney can be seen in Figure 40.

![Graph showing dose volume histogram for $^{225}\text{Ac}$-DOTA-MC1RL in the right kidney.](image)

**Figure 40.** Dose volume histogram for $^{225}\text{Ac}$-DOTA-MC1RL in the right kidney.
The absorbed dose maps for the left kidney can be seen in Figure 41.

Figure 41. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the left kidney at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters.

The dose rates were fitted with an exponential regression and integrated to obtain the total absorbed dose to the left kidney from $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL (Figure 42). The mean absorbed dose to the left kidney from $^{67}$Ga-DOTA-MC1RL was calculated to be $0.0075 \pm 0.0011$ mGy/MBq. This is compared to the MIRD calculated dose of $0.0079 \pm 0.0016$ mGy/MBq.
Similarly, the voxel-based mean absorbed dose was calculated for $^{225}$Ac-DOTA-MC1RL resulting in $0.0110 \pm 0.0002$ Gy/kBq as compared to $0.0150 \pm 0.0031$ Gy/kBq found conventionally. The DVH can be seen in Figure 43.
Absorbed dose maps for the liver can be seen in Figure 44.

Figure 44. Absorbed dose maps for $^{67}$Ga-DOTA-MC1RL in the liver at 24, 48, and 96 hour time points and corresponding dose maps for $^{225}$Ac-DOTA-MC1RL. The spatial dimensions are in centimeters.

The mean organ absorbed dose to the liver was calculated for $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL by fitting the dose at each timepoint with an exponential fit and integrating (Figure 45).
Figure 45. Exponential fitting of the mean dose rate as a function of time for $^{225}$Ac-DOTA-MC1RL in the liver.

The total absorbed dose to the liver from $^{67}$Ga-DOTA-MC1RL was 0.0016 ± 0.0001 mGy/MBq. The MIRD calculated absorbed dose was 0.0031 ± 0.0006 mGy/MBq. The resulting absorbed dose from $^{225}$Ac-DOTA-MC1RL was found to be 0.0633 ± 0.0082 Gy/kBq compared to 0.1485 ± 0.0283 Gy/kBq. The dose volume histogram for the absorbed dose in the liver can be seen in Figure 46.

Figure 46. Dose volume histogram for $^{225}$Ac-DOTA-MC1RL in the liver.
6.4 Discussion

In this work a method was developed to calculate the absorbed radiation dose at the voxel level for a targeted alpha particle therapy. The companion imaging agent, $^{67}$Ga-DOTA-MC1RL, was developed and tested and compared to the therapeutic agent. Other groups have developed and used companion imaging agents to perform image-based dosimetry for alpha emitting radiopharmaceuticals. For example, the $^{68}$Ga/$^{225}$Ac-Prostate-specific membrane antigen-617 (PSMA-617)\(^{70}\), the $^{203}$Pb/$^{212}$Pb-DOTA-MC1L\(^{71}\), and the $^{99m}$Tc-methyl dipiphosphonate/$^{223}$Ra-dichloide\(^{69}\) pairs have been used in dose calculations for the targeted alpha therapy of metastatic castration resistant prostate cancer, metastatic melanoma, and bone metastases respectively. While this approach results in patient specific prediction of the biodistribution and pharmacokinetics of the targeted alpha therapy, the treatment planning will be suboptimal. OLINDA is a model based approach using pre-calculated S factors obtained from a fixed geometry phantom to calculate mean organ level absorbed dose.

The spectrum fitting algorithm was updated for $^{67}$Ga-DOTA-MC1RL biodistribution analysis using the Monte Carlo detector model that was previously developed. The BD of a companion imaging agent should ideally mimic the BD of the therapeutic agent in order to implement a treatment planning procedure. The BD of $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL showed that these agents distribute differently throughout the body. At 24 hours, $^{67}$Ga-DOTA-MC1RL showed increased uptake into the kidneys with slightly lower uptake in the liver. This is much different than the liver uptake of $^{225}$Ac-DOTA-MC1RL which was shown to be nearly 3.5 times greater than kidney uptake at 24 hours. Despite this difference, there was some uptake into intestine, lungs, and spleen and minimal uptake into other tissues for both $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL. Dosimetry estimates were performed based on the
\textsuperscript{67}Ga-DOTA-MC1RL BD using the MIRD methodology and fixed geometry S values. Since \textsuperscript{67}Ga is a gamma emitter, the assumption that cross organ dose contribution is negligible fails. Using the S values generated from the standard mouse phantom it was found that the mean dose to the kidneys was over 5 times greater than the dose to the liver. This is in contrast with the \textsuperscript{225}Ac-DOTA-MC1RL calculated dose to the liver which was nearly 5 times greater than the dose to the kidneys. This discrepancy can be accounted for by applying a factor that corrects the \textsuperscript{67}Ga-DOTA-MC1RL biodistribution to that of the therapeutic.

Using the EDP phantom, an algorithm was created that converted acquired CT images into the MCNP lattice geometry. An algorithm was created that voxelized the segmented ROI’s drawn around the TMM’s. The EDP phantom has 13 TMM’s however only 6 materials were modeled in this work. This was necessary because of the complexity of the image derived geometries and computationally expensive tracking of charged particles through different materials.

The absolute activity calibration factor was obtained by applying the ROI algorithm to a spherical phantom filled with \textsuperscript{67}Ga in air. As a result, the SPECT based organ activity estimations could be compared to ion chamber based whole organ measurements. The image based activity estimations for the liver and kidneys agreed with the IC measurements well. The whole body estimates however were not in agreement with IC measurements with percent differences of up to ~60%. At all three time points the image based whole body activities underestimated the ion chamber. A first step to address these differences would be to fill the spherical phantom with pure \textsuperscript{67}Ga instead of the whole \textsuperscript{67}Ga-DOTA-MC1RL compound. This would allow for uniform activity distribution \textsuperscript{67}Ga in the phantom and avoid aggregation of the solution as seen in Figure 33.
Voxelized absorbed dose maps were successfully generated from the in vivo CT and SPECT datasets. The whole body absorbed doses did not correlate well with the MIRD calculations. This was to be expected given the large discrepancies in the image-based activity estimates and ion chamber measurements. Also, in the conventional calculation of total body absorbed doses, the assumption is made that the activity not used for organ calculation activity is uniformly distributed throughout the remainder of the body. The doses calculated for the kidneys and liver on the other hand agreed with MIRD calculations to within an order of magnitude for both $^{67}$Ga-DOTA-MC1RL and $^{225}$Ac-DOTA-MC1RL. This suggests that the developed methodology is valid. Discrepancies are likely the result of the many sources of error in the methodology. The major source is error in activity estimation. ROI’s were drawn manually on downsampled CT datasets that were manually registered to SPECT datasets. Since the mice were sacrificed after imaging for tissue harvesting, each timepoint involved a single different mouse. To reduce error, serial images the same mouse with multiple mice per timepoint should be performed.

It is evident from the voxel based dose calculations that the uptake of $^{67}$Ga-DOTA-MC1RL in the liver and kidneys is not uniform. In the case of the kidney, the dose accumulated more in the outer cortex region. Using the conventional dosimetry formalism, it is not possible to observe this result. DVH plots are one of the most utilized tools in external beam therapy to evaluate treatment plan quality. DVH’s were generated for ROI’s with the developed voxelized methodology enabling capability of volumetric analysis.

Due to the complexity of performing patient specific internal dosimetry, clinical applications have been limited. Several other voxel-based platforms have been developed, however the fixed geometry software OLINDA remains the only FDA approved system. The
most notable voxel-based systems include 3DRD\textsuperscript{57}, SCMS\textsuperscript{56}, DPM\textsuperscript{165}, VIDA\textsuperscript{166}, and RAPID\textsuperscript{61}. Similarly to the work presented in this thesis, all of these systems have been benchmarked against conventional calculations and show good agreement. These systems use the point kernel convolution method for Monte Carlo simulation and therefore don’t account for tissue heterogeneities. This method was chosen to make these systems more clinically applicable by speeding up the calculation, however, none have seen wide acceptance for clinical use. The methodology developed in this thesis performs full Monte Carlo calculations by tracking all relevant particles accounting for material compositions. The length of time to perform a full dose calculation, including all pre and post processing, at a single time point takes \textasciitilde 30 hours. This is faster than the all the aforementioned platforms. This is noteworthy because these platforms have mostly been used for photon dose calculations, while the methodology developed here tracks and tallies not only alpha particles, but delta rays and resulting bremsstrahlung productions. While several fundamental aspects of the methodology developed here align with these systems, this is the only one specifically designed to perform targeted alpha therapy dosimetry.
CHAPTER SEVEN:
CONCLUSIONS

In this work, an alpha emitting radiopharmaceutical was thoroughly assessed. Methods for improving the accuracy of alpha particle radioactivity determination were developed. This is important for pharmacokinetic evaluation and patient safety as many aspects of activity measurement have been overlooked both pre-clinically and clinically. The alpha therapeutic, $^{225}$Ac-DOTA-MC1RL, was tested in several experiments and was shown to have low toxicity, rapid blood clearance, uptake into MC1R positive tumors and clearance organs, significant tumor and metastasis growth delay, and prolonged survival in human uveal melanoma xenograft models in mice following a single administration. Conventional radiation dosimetry analysis showed 10x greater absorbed doses in high MC1R expressing tumors than in low MC1R expressing tumors as well as low doses to non-clearance organs. A multi-compartment pharmacokinetic model was developed with the aim of increasing the personalization of the treatment by optimizing administration activities for the patient and predicting biodistribution through the use of a companion imaging agent. Ultimately, the goal of this work was to develop a patient specific image based dosimetric methodology for $^{225}$Ac-DOTA-MC1RL treatment planning. Most therapeutic radiopharmaceutical therapies use fixed administrations with no personalization. A companion imaging agent, $^{67}$Ga-DOTA-MC1RL was developed and tested. $^{67}$Ga-DOTA-MC1RL has somewhat similar biodistribution to $^{225}$Ac-DOTA-MC1RL allowing
for the visualization and prediction of the spatial and temporal distribution that the therapeutic will have. By acquiring several SPECT images post administration of $^{67}$Ga-DOTA-MC1RL, cumulated voxelized activity maps can be generated, converted to $^{225}$Ac-DOTA-MC1RL activity and submitted for Monte Carlo dose calculation. The result is a voxelized 3D absorbed dose distribution. This information can then be used to optimize a treatment plan for patients with the goal of improving their outcome.
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APPENDIX A:

PUBLICATIONS AND CONFERENCE PRESENTATIONS

Publications


†Co-first author

Conference Presentations

Christopher J. Tichacek, Mikalai M. Budzevich, Narges Tafreshi, Brian Miller, Hyun Joo Kil, Thaddeus J. Wadas, Mark L. McLaughlin, David L. Morse, Eduardo G. Moros
Spatial Distribution of Targeted Alpha Particle Therapy in Rat Kidney
AAPM Annual Meeting 2019: ePoster Presentation; San Antonio, Texas; July 16th
Narges K. Tafreshi, Hyun Joo Kil, Darpan N. Pandya, **Christopher J. Tichacek**, Michael L. Doligalski, Mikalai M. Budzevich, Nella C. Delva, Michael Langsen, Eduardo G. Moros, Thad J. Wadas, Mark L. McLaughlin, David L. Morse

*Lipophilicity Determines Route of Clearance of a Melanocortin 1 Receptor Targeted Radiopharmaceutical*

Society of Nuclear Medicine & Molecular Imaging; Anaheim, California; June 22-25th

**Christopher J. Tichacek**, Mikalai M. Budzevich, Narges K. Tafreshi, Thaddeus J. Wadas, Mark L. McLaughlin, David L. Morse, Eduardo G. Moros

*Biodistribution and Pharmacokinetic Analysis of a Targeted Alpha Particle Therapy*

AAPM Annual Meeting 2018: Oral Presentation; Nashville, Tennessee; August 1st

Eduardo G. Moros, **Christopher J. Tichacek**, Mikalai M. Budzevich, David L. Morse

*Voxel-based Radiation Dosimetry for Clinical Translation of Targeted Alpha Particle Therapy*

National Cancer Institute Workshop on Dosimetry of Systemic Radiopharmaceutical Therapy Poster Presentation; Bethesda, MD; April 19-20th

**Christopher J. Tichacek**, Mikalai M. Budzevich, David L. Morse, Eduardo G. Moros

*A Monte Carlo Method for Determining the Response Relationship Between Two Commonly Used Detectors to Indirectly Measure Alpha Particle Activity*

National Cancer Institute Workshop on Dosimetry of Systemic Radiopharmaceutical Therapy Poster Presentation; Bethesda, MD; April 19-20th

**Tichacek CJ**, Budzevich MM, Tafreshi NK, Wadas TJ, McLaughlin ML, Moros EG, Morse DL

*Multicompartment Pharmacokinetics Modeling of 225Ac-based Targeted Alpha Particle Therapy*

National Cancer Institute Workshop on Dosimetry of Systemic Radiopharmaceutical Therapy Poster Presentation; Bethesda, MD; April 19-20th

Narges K. Tafreshi, Hyun Joo Kil, Darpan N. Pandya, Michael L. Doligalski, **Christopher J. Tichacek**, Mikalai M. Budzevich, Nella C. Delva, Bhatt NB, Eduardo G Moros, Thaddeus J. Wadas, Mark L, McLaughlin, David L. Morse

*Lipophilicity Determines Route of Clearance of a Melanocortin 1 Receptor Targeted Radiopharmaceutical*

2018 Moffitt Cancer Center Scientific Symposium; Tampa, FL May 9th

Winner: Best Translational Science Poster

**Christopher J. Tichacek**, Mikalai M. Budzevich, Gary V. Martinez, David L. Morse, Eduardo G. Moros

*Precise Monte Carlo Simulations of NaI(Tl) Spectra for Measurement of Actinium-225 Targeted Alpha Particle Bio-Distribution*

AAPM Annual Meeting 2017: Electronic Poster Presentation; Denver, Colorado; July 30th

**Christopher J. Tichacek**, Narges K. Tafreshti, Michael L. Doligalski, Mikalai M. Budzevich, Epifanio Ruiz, Nella C. Delva, Thaddeus J. Wadas, Mark L, McLaughlin, David L. Morse, Eduardo G. Moros

*Dosimetry of a Novel Targeted Alpha Particle Therapy for Metastatic Uveal Melanoma*
AAPM Annual Meeting 2017: Electronic Poster Presentation; Denver, Colorado: July 30th

Narges K. Tafreshi, Nella C. Delva, Michael L. Doligalski, Christopher J. Tichacek, Darpan N. Pandya, Hyun Joo Kil, Mikalai M. Budzevich, Epifanio, Ruiz, Thaddeus J. Wadas, Mark L. McLaughlin, David L. Morse

Targeted Alpha Particle Therapy for Uveal Melanoma

AACR Annual Meeting 2017: Poster Presentation; Washington D.C. April 1-5th

Christopher J. Tichacek, Narges K. Tafreshi, Mikalai M. Budzevich, Epifanio Ruiz, Thaddeus J. Wadas, Mark L. McLaughlin, Eduardo G. Moros, David L. Morse

Radiodosimetry of a Novel Alpha Particle Therapy Targeted to Uveal Melanoma: Absorbed Dose to Organs in Mice

AAPM Annual Meeting 2016: Oral Presentation; Washington D.C. August 3rd
ABOUT THE AUTHOR

Christopher Tichacek was born and raised in Richmond, Virginia by John and Brenda Tichacek. He has two younger sisters, Morgan and Nichole. He received his Bachelor of Science degree in applied physics with a minor in mathematics from Bridgewater College in 2010. While there, he played college football for four years, was a member of the team’s leadership council and earned several awards as a student athlete. He earned his Master of Science degree in medical physics in 2013 from the Georgia Institute of Technology in Atlanta, Georgia. He then worked as a clinical research physicist at the Winship Cancer Institute of Emory University. In 2015 he moved to Tampa, Florida to begin pursuing a Ph.D. in applied physics with emphasis in medical physics at the University of South Florida. While there, he has worked as a medical physicist in training at the H. Lee Moffitt Cancer Center & Research Institute. He enjoys spending time with his family. He also enjoys sports, staying active, and exploring the Tampa Bay area.