**Research Article**

**Long-term 227Ac Dose of Accelerator Generated 225Ac Constructs in Mice**

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**Abstract:**

The US DOE Tri-Lab effort to create accelerator-produced Ac-225 is a welcome solution to the low output from reactor-produced Ac-225. To estimate potential safety of accelerator-produced Ac-225 radiotherapeutics containing the small fraction of inseparable Ac-227 contaminants, we performed a biodistribution study, and estimated worst-case scenario short and long-term biological doses. This was performed on mice with three different Ac-225 constructs, and dose was modeled out to one Ac-227 half-life of 21.77 years for several organs of interest. We found that while the Ac-227 dose is not entirely negligible from a chronic radiation syndrome standpoint, it does not likely pose a significant risk to outweigh treatment with Ac-225 radiotherapeutics in the first place. Further, there appeared to be some form of isotopic fractionation with variable biological Ac-225/Ac-227 localization ratios which may be investigated further.

**INTRODUCTION**

Ac-225 radiotherapeutics researchers are ripe with enthusiasm over the exceptional results being produced in both the lab and in experimental patient treatments 1–4. And yet, they still face numerous challenges ahead, from finding an improved chelator for superior radiolabeling efficiency 5–9, to solving recoil effects 1011, to the simple lack of availability of the isotope to begin with 12. Fortunately, the US DOE Tri-Lab efforts in accelerator produced Ac-225…. \*\*INSERT REBECCA on production, difference in number of potential patients treated, lack of separation\*\*

Both Ac-225 and Ac-227 have multiple daughters that emit alpha particles, with Ac-227 releasing approximately 20% more total energy from each parent atom. Contrastingly, 21.77 years, or 7946 days is the half-life of Ac-227 compared to 10 days for Ac-225 (**Figure 1**). As is readily apparent, the biodistribution of Ac-227 contamination is vital in determining long-term toxicity. If the Ac-227 complex is excreted at the same rate as Ac-225, then the risk of long term dose is lower. However, if bioaccumulation occurs, radiosensitive tissues such as the kidney, liver, bone marrow etc. may impart chronic radiation sickness. This could impart a long-term dose limitation beyond the acute radiation received from a primary Ac-225 complex bolus.

Creating a pseudo-worst case biodistribution scenario, we produced constructs using accelerator-generated Ac-225 which contained up to 0.5% Ac-227 contaminant. From modeling based upon Bateman dose calculations for the primary daughter series of Ac-225 and Ac-227, we extrapolated the future cumulative dose and dose per day for each tissue in our mouse biodistribution data set.

**RESULTS**

After injection of 2 nCi/g of Ac-225 construct with up to 0.5% Ac-227 contaminant to either 1) female Swiss-Webster mice with DOTA-Ac or HOPO-Ac control, or 2) female NOD SCID PDX mice with Trastuzumab-DOTA-Ac, biodistribution of Ac-225 and Ac-227 was investigated over several time points. DOTA was used as the ligand for actinium due to its *in vivo* stability and commercial availability as a bifunctional chelator 13. Biodistribution was compared via recovered dose per gram (RD/g) or localization ratio (LR) of Ac-225/Ac-227 RD/g values to determine any distribution effects.

**Biodistribution**

*Recovered Dose per Mass*

DOTA-Ac-225 biodistribution in healthy mice was typical with activity locating heavily in the kidneys initially compared to other organs, and rapidly dropping activity across all collected tissues due to renal clearance (**Figure 2A**). DOTA-Ac-227 biodistribution, however, indicated lingering carcass content over 6 days, but at low content (**Figure 2C**). In NOD SCID HER2-positive patient derived xenograft mice treated with targeted Trastuzumab-DOTA-Ac, RD/g biodistribution was also typical, where blood circulation content decreased over time more slowly than DOTA-Ac due to extended circulatory half-life antibodies provide, and with increasing spleen uptake as the largest %RD/g (likely due to immune opsonization), followed by liver and tumor uptake (**Figure 2B/D**). As a control for a predominantly hepatic-based clearing system, HOPO-Ac was also tested. As anticipated in native mice, liver uptake was high for both Ac-225 and Ac-227, with lower biodistribution to other organs throughout 6 days after injection (**Figure S3**).

*Isotopic Localization Ratio*

Interestingly, even though both Ac-225 and Ac-227 samples were counted at secular equilibrium using the same method (liquid scintillation counting of ashed tissue samples, Ac-227 equilibrium starts at roughly 100 days, Ac-225 after roughly 13 hours), Ac-225 appears to localize differently to Ac-227 depending on the initial parent construct. LR data in **Figure 3** are based upon ratios from **Figure 2** biodistribution recovery results. Across the board for all tissues, the LR of DOTA-Ac (**Figure 3A**) shows rapid clearance from the body of Ac-225 compared to Ac-227, with LR well below 1 for all tissues other than the abdominal remaining tissues (ART). With Trastuzumab-DOTA-Ac (**Figure 2B**), however, the Ac-227 clears faster at longer time points for the spleen. As the blood LR increased for Ac-225, so did spleen, but with only a single significant difference at the final 10-day time point for the spleen LR (n=5, mean = 2.94 ± 1.43 SD, one-tailed P-value = 0.02 vs. unity). The 10-day time point for Trastuzumab-DOTA-Ac LR is beyond the scale due to extremely low activity in the Ac-227 blood sample, and there is no significance. HOPO-Ac LR for the heart, lungs, kidneys, liver, and carcass tended to be at or greater than 1 (**Figure S4**). LR also trended a decrease from above unity in earlier time points to at or below unity in later time points for all organs except the spleen and ART.

**Dose Modeling**

After dose interpolation and extrapolation of each organ’s RD/g assuming an equal 200 nCi Ac-225 and 1 nCi Ac-227 (0.5%), output measurements of dose vs time, dose ratio (Ac-227/Ac-225) vs time, and dose/day vs time were calculated. **Figures 4** and **5** show extrapolated future dose (**Figure S9** and **S10** are in log scale), and **Figure S2** shows dose at each collected time point. HOPO doses are found in **Figures S5** and **S6**, and time taken to reach dose equivalency is found in **Figure S7**.

*DOTA-Ac*

DOTA-Ac had the greatest cumulative Ac-225 dose to the kidneys, with a dose of 0.0167 Gy (95% CI 0.0150-0.0183 Gy) at 100 days (**Figure 4A**), where Ac-227 only showed roughly 20% the dose at 0.00331 Gy (95% CI 0.00315-0.00347 Gy) (**Figure 4B**). However, the estimated Ac-227 cumulative dose continued to increase to 0.311 Gy (95% CI 0.296-0.326 Gy) after 7946 days (**Figure 4B**) for a Ac-227/Ac-225 dose ratio of roughly 20 times at 7946 days (**Figure 4C**). Compared to the kidneys, the carcass for Ac-227 showed roughly 2x higher cumulative dose. The dose per day for DOTA-Ac-225 in the kidneys showed a maximum at the initial time point of 1 hour, with 3.02E-3 Gy/day (95% CI 2.95E-3 - 3.09E-3 Gy/day) (**Figure 5A**), with a DOTA-Ac-227 maximum at 200 days with only 5.35E-5 Gy/day (95% CI 5.09E-5 – 5.60E-5 Gy/day) (**Figure 5B**), and the DOTA-Ac-227 carcass showing roughly twice the dose per day.

*Trastuzumab-DOTA-Ac*

Trastuzumab-DOTA-Ac showed uptake primarily in the spleen, with a 125-day dose maximum of 20.4 Gy (95% CI 17.0-23.8 Gy) for Ac-225 (**Figure 4D**), and after 7946 days Ac-227 showed a slowing cumulative dose of 33.7 Gy (95% CI 29.6-37.7 Gy) (**Figure 4E**) for a dose ratio of Ac-227/Ac-225 of roughly 1-5:1 for all samples but blood (**Figure 4F**). The dose per day, however, showed a lower peak dose rate for the spleen of 0.88 Gy/day (95% CI 0.71-1.1 Gy/day) for Trastuzumab-DOTA-Ac-225 at 7 days (**Figure 5C**), and at 200 days 0.058 Gy/day (95% CI 0.051-0.065 Gy/day) for Trastuzumab-DOTA-Ac-227 (**Figure 5D**). Cumulative dose to the kidneys was found to be low for both Ac-225 and Ac-227, at 0.54 Gy (95% CI 0.49-0.58 Gy) and 1.42 (95% CI 1.35-1.49) respectively, as was the kidney dose per day, with Ac-225 at 3 mGy/day (95% CI 2.9-3.1 mGy/day) and Ac-227 at 54 µGy/day (95% CI 51-56 µGy/day).

**DISCUSSION**

This study was set up as an initial estimate of the long term toxicity for trace Ac-227 present in radiopharmaceuticals utilizing accelerator-produced Ac-225. Mice in each group were given a target dose of 2 nCi/g Ac-225 with up to 0.5% Ac-227 contaminant. At the final time point of biodistribution sampling, it was assumed that distribution was now static in time, disallowing future distribution, with only decay occurring. Also, all samples were left to sit to secular equilibrium for Ac-225 and Ac-227 counting (see **Figure 1**). Estimates of future cumulative and dose per day were based on a real injection into mice of 8 nCi/g Ac-225 constructs (40% maximum tolerated dose (MTD) of antibody-DOTA-Ac-225 conjugates 14,15. Considering these MTD are based on acute effects of Ac-225 (no Ac-227) out to 35 days, it does not speak for long term dose effects of Ac-227, and is taken only as an acute guideline.

In general, for radiotherapies, as well as targeted alpha therapies, the dose limiting factor tends to be renal toxicity 16–18. In humans, short half-life radiotherapies typically present a delayed toxicity well beyond the decay of the radioisotopes, and is known to occur between months and years after radiation treatment 1,19. Based upon the literature, we can create ballpark estimates to determine for both Ac-225 and Ac-227 if, 1) is the cumulative dose to vital organs at or below the acute threshold, and if not, 2) are the dose-per-day values reasonable due to cumulative dose occurring over an extended period.

Considering small molecule therapies with Ac-225, in human patients off-trial using experimental Ac-225-PSMA-617 peptide conjugate, the maximum cumulative dose to the kidneys was tolerated at 16.8 SvRBE5 (3.36 Gy) from 3x fractionated ~7.4 MBq doses (200 nCi each, 5.6 Gy/µCi) 1,2, with an approximate 27 SvRBE5 as the MTD (5.4 Gy) 20. For protein based Ac-225 radioimmunoconjugates, clinicaltrials.org shows six recruiting/active/completed phase I studies in the United States, however dose estimate data could not be found. Nonetheless for an estimate, in mice, there have been a few studies reporting a variety of ranges for radioimmunoconjugate dosimetry. In one study with Ac-225-HuM195 antibody conjugates, an upper-end kidney dose of 27.6 Gy (138 SvRBE5) was tolerated with the additional administration of a kidney protectant from 350 nCi administration to SCID mice, giving (79 Gy/µCi) 21. In another with a lower end range, Ac-225-DOTA-anti-PD-L1-BC conjugates delivered to *neu*-N mice at a 15 kBq dose (405 nCi) created absorbed doses of 9.2 Gy kidneys (22.7 Gy/uCi, 46 SvRBE5), 11.1 Gy liver (27.4 Gy/µCi, 55.5 SvRBE5), and 2 Gy spleen (4.9 Gy/µCi, 10 SvRBE5) 15. These values are, as is typical, markedly higher for mice than for humans.

In our study, Trastuzumab-DOTA-Ac conjugates administered to NOD SCID mice showed the anticipated biodistribution, considering antibodies are often distributed to immune-functioning organs 22 such as the spleen, liver, as well as the expressing tumor (**Figure 2**). Also typical was DOTA-Ac complex biodistribution in healthy Swiss-Webster mice, where DOTA displayed rapid renal clearance 18 (**Figure 2**). While there is higher kidney distribution than other organs, the rapid renal clearance resulted in over an order of magnitude lower kidney dose (**Figure 4**) due to the long circulatory half-life of Ac-225 and Ac-227 antibody conjugates. Contrasting to DOTA, our HOPO control group typically shows primarily hepatic clearance 23, and accordingly the liver showed the largest portions of distribution (**Figure S3**). Comparing our cumulative mouse dose modeling data to the literature values, the cumulative Ac-225 dose maximum to the liver after 100 and 125 days for DOTA-Ac-227 and Trastuzumab-DOTA-Ac-225 respectively are significantly lower than the clinical threshold of 3.36 Gy found in the Ac-225-PSMA-617 trial (see **Table 1** for a data compilation).

While acute doses are typical for radiotherapies, long term dose effects from alpha radiation is less studied. Long term doses often involve reduction in immune function due to chronic bone marrow function decay, as over time there tends to be a depletion of the stem cell compartments 19,24. Thus, Ac-227 effects from this study may be compared to what literature is available, often involving long-term gamma irradiation. In an estimate to correlate to external beam studies, where 100% of injected Ac-227 was retained in the mouse regardless of carrier (4% RD/g total body for a 25 g mouse at 10 days), after 7946 days, Ac-227 would show 2.25 Gy cumulative total body dose, with a peak of 388 µGy/day, or 142 mGy/year after 200 days, lessening to 79 mGy/year after 7946 days. The RBE for whole body alpha is generally considered to be 10:1 alpha:gamma, and so that would give 1.4 SvRBE10/year, which is over the 0.4 Gy/year threshold for depression of hematopoiesis 25, but much lower than the lethal bone marrow dose maximum of >4.5 Gy/year However, looking at the actual carcass (ashed remainder of animal, including bones) values for Trastuzumab-DOTA-Ac-227 show 197 uGy/day maximum (95% CI 194-200 uGy/day) which equates to 72 mGy/year on average or 0.36 SvRBE5/year for bones which is just below the 0.4 Gy/year threshold.

Considering the spleen is not as vital an organ as the kidneys or liver, spleen chronic toxicity has been shown to appear in rats and mice exposed to 0.01–0.5 Gy/day of gamma radiation, which calculates out to 0.05-2.5 SvRBE5/day alpha 19. While the Trastuzumab-DOTA-Ac-225 rate per day reaches beyond this level, it is only for a short term, but Ac-227 does get within the toxicity range with an equivalence mean of 0.29 SvRBE5/day.

It is important to keep in mind that this study is limited to counting only activity due to direct parent-daughter activity after tissue resection. This would likely result in undercounting effects due to daughter re-distribution to organs after recoil effects 10,11. Daughter isotope re-distribution has been observed in other studies, and redistribution of Bi-213 has been shown to cause dose-limiting renal toxicity in Ac-225 therapies 26. As an example, modeling the fraction of activity produced from decays prior to the first alpha decay in each Ac-225 and Ac-227 decay chains produces **Figure S8**, and exemplifies how if the first alpha decay were to release the metal from the ligand carrier due to recoil, isotopic daughter distribution variance would also occur. Within the first few days of administration of pure Ac-225 and pure Ac-227 into a ligand, Ac-227 and Th-227 daughter would remain largely intact, whereas Ac-225 would rapidly reach secular equilibrium, with more activity coming from freed daughters than parent. This effect may be measured, if desired, via gamma counting, but it was not deemed necessary considering we were more interested in long-term dose effects from Ac-227.

Nonetheless, we did observe interesting localization ratio (LR) effects of Ac-225/Ac-227. As can be seen in the LR for all three tested constructs, while LR is calculated via %RD/g ratios, LR is not directly dependent upon the absolute %RD/g between constructs. For instance, while DOTA-Ac shows much lower activity than Trastuzumab-DOTA-Ac and the DOTA-Ac LR is below 1 and Trastuzumab-DOTA-Ac at or greater than 1, the HOPO-Ac control has several tissues with sub-10 %RD/g that are greater than 1 (blood, heart, lungs). Therefore, this is likely due to a factor other than activity counting conditions that is creating isotopic localization, post-equilibrium. The trend these LR data do seem to follow is the rate of excretion from the animal. DOTA-Ac is the quickest to clear, HOPO-Ac the second quickest, and Trastuzumab-DOTA-Ac is the slowest. Since %RD/G is not based on the initial injected amount but the amount recovered in total at the final time point, this is also not simply due to rapid decay of Ac-225 vs Ac-227. It is possible that changes in LR may be due to biomolecular actinium scavenging of constructs directly, pre-decay. However, that would require a difference in chemical potential between isotopes in the same constructs and/or scavenging molecules. While this occurs naturally in numerous systems 27–29, most notably δ13C differential sequestration in ecology , where kinetic fractionation occurs due to the added neutron mass, the added mass in Ac-227/Ac-225 (+0.88%) is a much smaller fraction than with C-13/C-12 (+8.3%). If this is the case, then perhaps the DOTA-Ac complexes are more easily scavenged than Trastuzumab-DOTA-Ac due to less steric hindrance, and Ac-225 more easily scavenged compared to Ac-227 and cleared more rapidly from the body. Otherwise, there are some cases of mass-independent fractionation due to differences in nuclear magnetic moment in heavier elements, such as in paramagnetic iron isotopes in magnetotactic bacteria 30. Search for differences could be tested through further experimentation in mice with variations of Ac-225 / Ac-227 dose in a weak ligand, such as citrate, to promote biomolecular scavenging.

**CONCLUSIONS**

Considering the limitations of this study only collecting raw data out to 6-10 days, the dose extrapolation appears promising for utilization of accelerator generated Ac-225 with longer and large scale studies. While Ac-227 constructs do seem to create a measurable absorbed dose over extended periods of time, the cumulative dose does not become significant for years, and is likely lower risk than avoiding Ac-225 radiotherapy for older or late-stage patients. The observation of isotopic localization of Ac-225 vs Ac-227 is an interesting and unexpected outcome, and will be investigated further.

**MATERIALS AND METHODS**

**Materials**

3,4,3-LI(1,2-HOPO), referred to as HOPO, was XXXXXpurchased or synthesized?XXXXXXX. IgG1 antibody was purchased from Sigma-Aldrich. Trastuzumab was XXXdonated or purchased?XXXXXXX. 1,4,7,10-Tetraazacyclododecane-1,4,7-tris-acetic acid-10-maleimidoethylacetamide (DOTA-MMA) was purchased from Macrocyclics, Bradford reagent was purchased from Bio-rad, and tris(2-carboxyethyl)phosphine hydrochloride (TCEP HCl), L-glutathione reduced, and all other chemicals were purchased from Sigma-Aldrich.

**Activity counting**

All activity was counted with a Perkin-Elmer Tri-Carb 2910 TR. Dilutions of radiolabeled solution activity for injection were diluted with 10 mL of Ultima Gold LLT scintillation cocktail. For biodistribution, samples were ashed in a furnace, dissolved in nitric acid, and diluted into 10 mL Ultima Gold LLT scintillation cocktail.

**Radiolabeling**

*Caution: Ac-225 and Ac-227 are radioactive isotopes that may present serious health risks to the user. Experiments were performed in facilities specially designed for the safe-handling of radioactive materials at the Lawrence Berkeley National Laboratory (LBNL).*

For DOTA and HOPO radiolabeling sans antibody, a dry heating block was used to heat ligands to 60 ºC for 2 hours in pH 7.4 10 mM phosphate buffered saline (PBS) at 200:1 excess Ligand:Metal. For antibodies-DOTA conjugates, a dry heating block was warmed to 45 ºC and antibody-DOTA conjugates (cysteine sites) were pre-incubated for 5 minutes, dissolved in 0.1M pH 5.4 ammonium acetate. Radionuclide in 0.05N HCl was added at 200x excess Ligand:Metal for 2 hours. Starting activity was based on an aliquot of the stock solution at equilibrium upon radiolabeling. These radiolabeled solutions were washed and buffer exchanged (10x volume 5 times) into PBS of pH 7.4, and aliquots of filtrate and retentate were taken for final activity and yield verification.

**Animal Handling**

All procedures and protocols used in the described *in vivo* studies were reviewed and approved by the LBNL Institutional Animal Care and Use Committee (IACUC) and were performed in AAALAC accredited facilities.

**Animal Injection**

Contamination is achieved by intravenous injection in a warmed lateral tail vein of the challenge chelated isotope. Animals are housed in metabolism cages, per randomization group (n = 3). Urine and fecal pellets are collected daily until necropsy. Blood, liver, kidneys, spleen, heart, lungs, thymus, abdominal remainder tissue (ART), skeleton, and soft tissue remainder samples collected at scheduled necropsy and processed for analysis. Counting is done on a gamma counter and on an alpha/beta LSC counter. Samples are counted promptly after processing and repeatedly over 100 days to allow for equilibration of Ac-227 daughter products.

**Biodistribution**

Biodistribution results are reported as percent recovered dose per mass (%RD/g) as excreta was collected alongside the organs and tissues. Xxxxxxxx\*\*\*Insert Dahlia?\*\*\*xxxxxxxxxx

**Dose Modeling**

Utilizing the kinetic dose results, we created an estimated future dose for DOTA-Ac, Trastuzumab-DOTA-Ac, and HOPO-Ac as a control, using accelerator-generated actinium constructs. The input dose bolus was 200 nCi for Ac-225 and 1 nCi for Ac-227 (0.5% Ac-227 contamination), and assumed to be pure actinium without daughters (all activity is only actinium at time = 0 of the injection). Dose modeling was performed across two regimes: 1) interpolation within the actual recorded data set (0-10 days), and 2) extrapolation of future dose assuming no further change in final time point. Between real recorded time point values of 1 hr, 4 hr, 24 hr, 4 days, 6 days (and 10 days for Trastuzumab-DOTA only), small step changes were added for interpolation. Numerically solving the standard Bateman ordinary differential equations produced an activity per time correlation (**Figure 1**). Next, using the energy output for each decay along the daughter series’, a moving average of power per count of activity (MeV/minute / CPM) for each time step was found, to get MeV/count (**Figure S1**). 100% efficiency of counting was assumed per decay. For time points within the real recorded time points, a monotonic cubic spline was used to gather activities, where activity past the real data assumes no change in biodistribution, and only decay occurs. Power per mass (MeV/(g\*day)) was found per time step based on organ masses, and was numerically integrated to convert to energy per mass for conversion into units of Grey. Unequal variance standard deviation was also modeled via cubic spline interpolation and numerical integration for real data points with error propagation, and 95% confidence intervals were determined via assuming n=5 for each future time point beyond the real data of n=5. To determine the time of unity for Ac-227/Ac-225 ratios for dose per day, an exponential fit was used, and for cumulative dose a linear fit was used. Each organ/tissue point of unity was determined from a cubic spline interpretation, along with the error.

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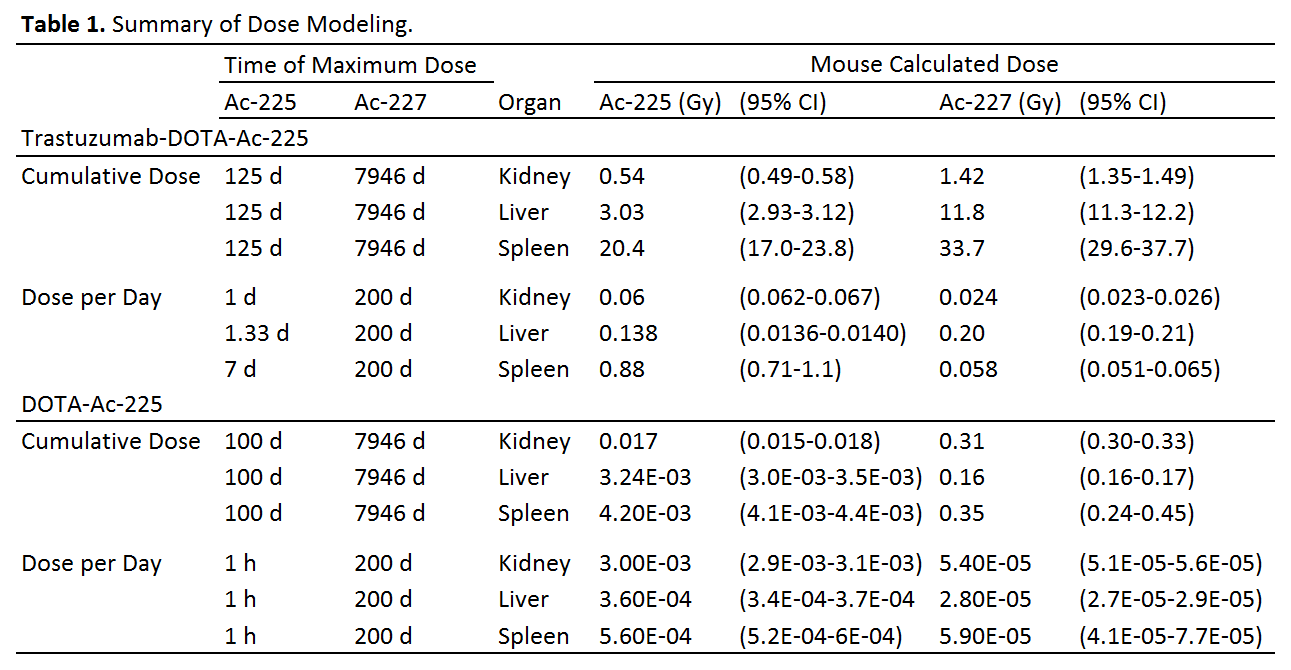
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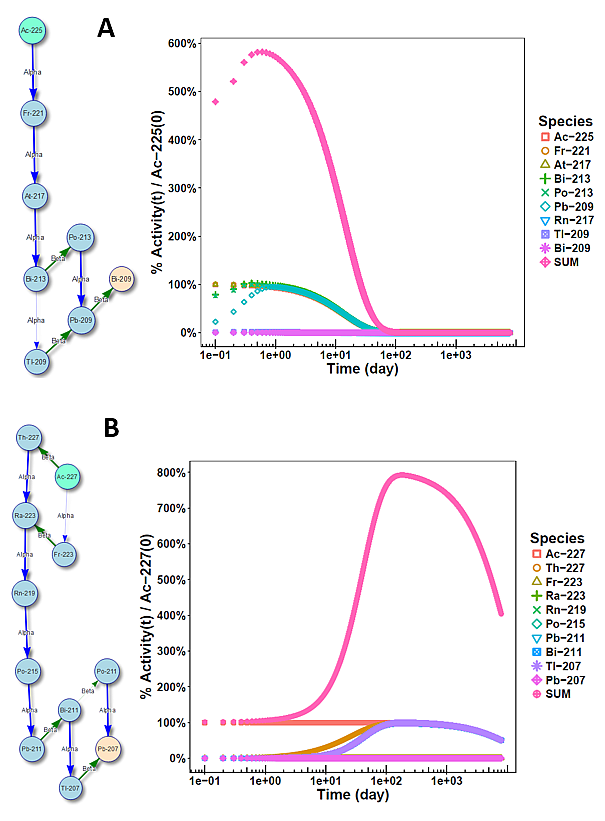
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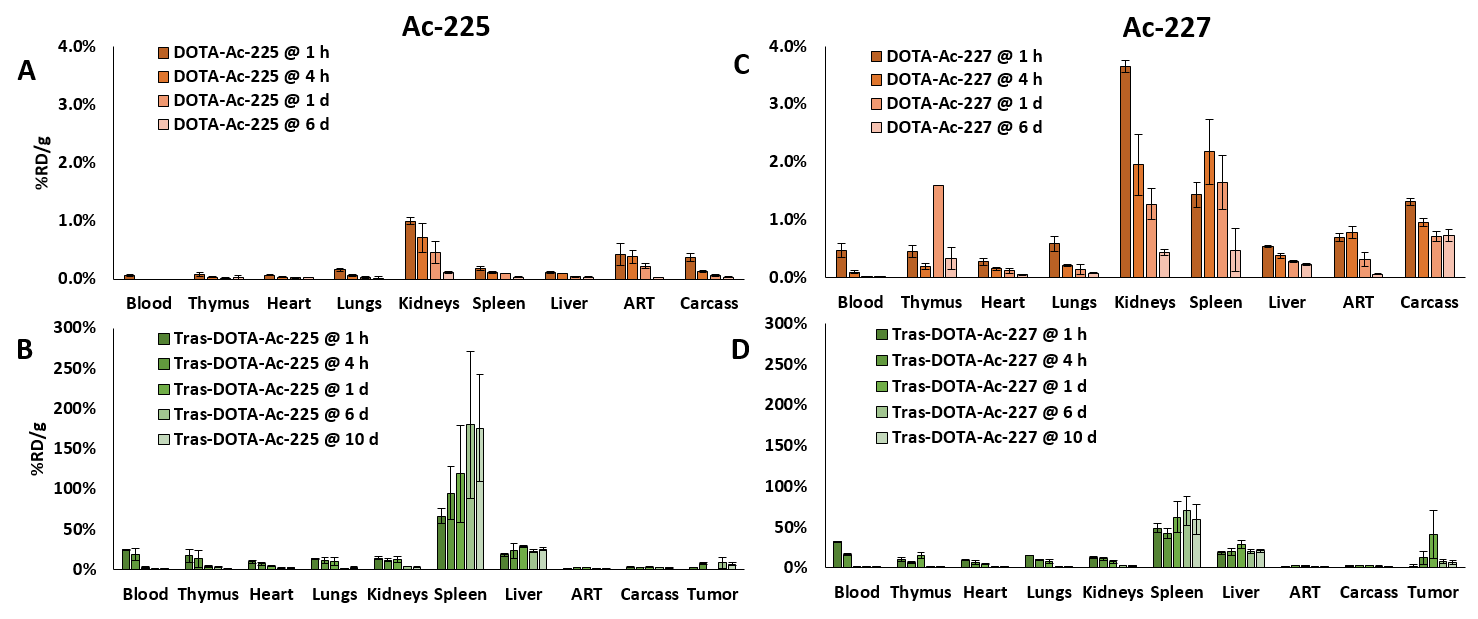
**TABLES**



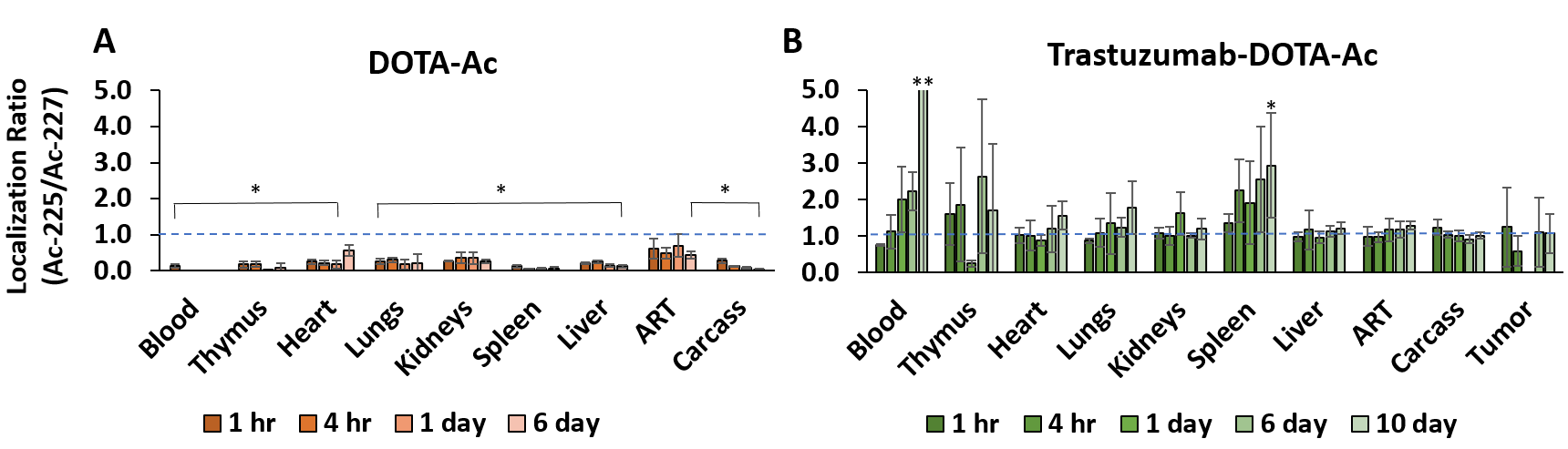
**FIGURES**



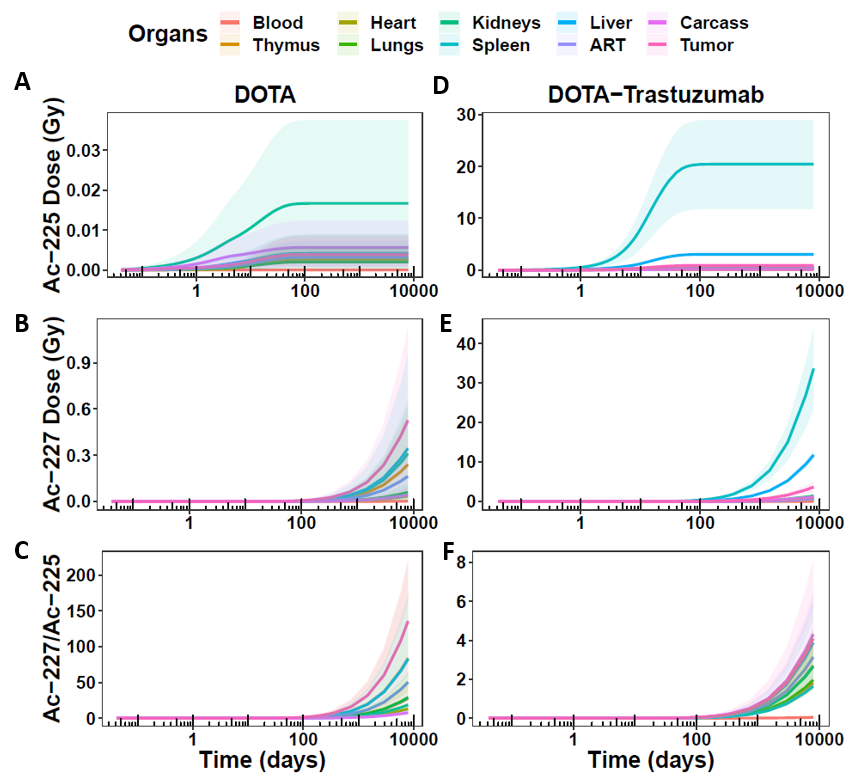
**Figure 1.** Comparison of A) Ac-225 and B) Ac-227 decay. Left: Vertical placement is in relation to proton count. Parent is in teal, final daughter is in salmon, intermediate species with >0.1% incidence are blue. Line thickness indicates probability (thicker is greater probability). Right: Species activity in relation to pure actinium parent at t=0 [% Activity of Species(t) / Ac-22X(0)]. SUM is total of all species.



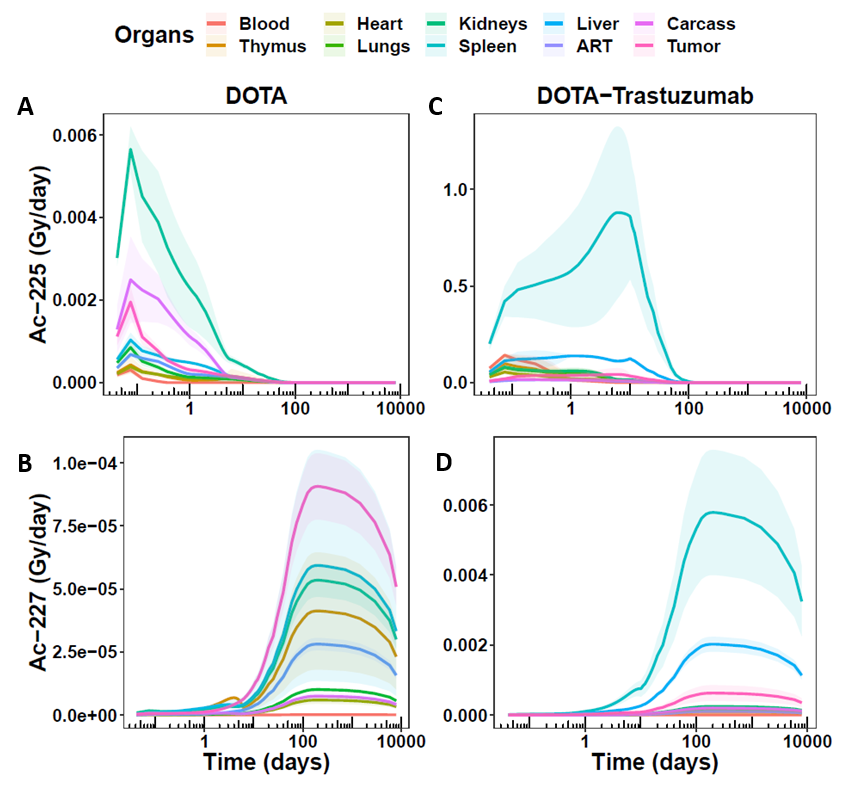
**Figure 2.** %Recovered dose per mass (%RD/g) of A/B) Ac-225 and B/C) Ac-227 %RD/g. Mean ± SD, n=5.



**Figure 3.** Localization ratio (recovered dose per mass of Ac-225/Ac-227) for A) DOTA-Ac, and B) Trastuzumab-DOTA-Ac. Mean ± SD, n=5. \*P-value < 0.05 with Bonferroni correction. \*\*value out-of-scale, mean 10.8 ± 19.0 SD.



**Figure 4.** Cumulative dose extrapolation modeling over one Ac-227 half-life (7946 days) for A/B/C) DOTA-Ac and D/E/F) Trastuzumab-DOTA-Ac. Solid lines are a mean from n=5, and shading indicates standard deviation.



**Figure 5.** Dose per day extrapolation modeling over one Ac-227 half-life (7946 days). A/B are DOTA-Ac, C/D are Trastuzumab-DOTA-Ac. Solid lines are a mean from n=5, and shading indicates standard deviation.

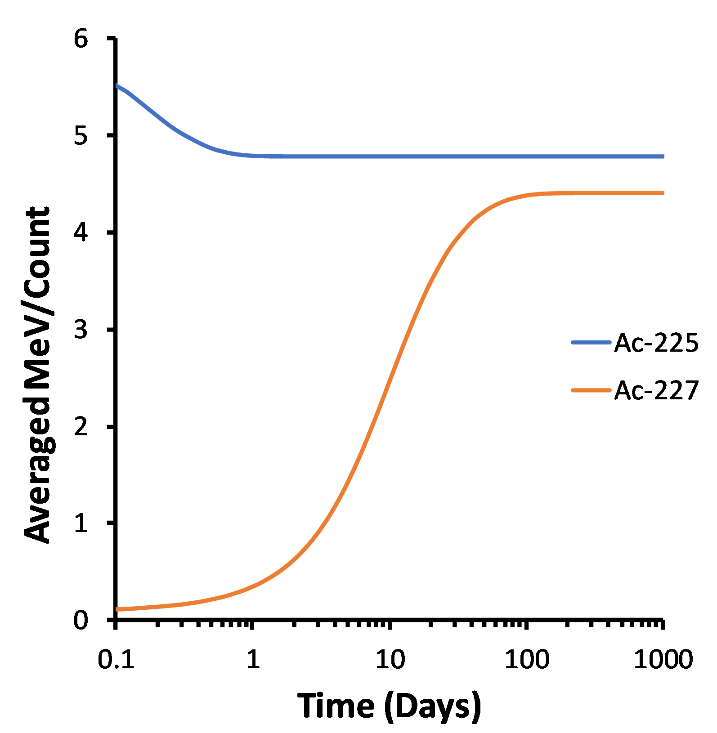
**DISCLOSURE**

The authors have no disclosures.

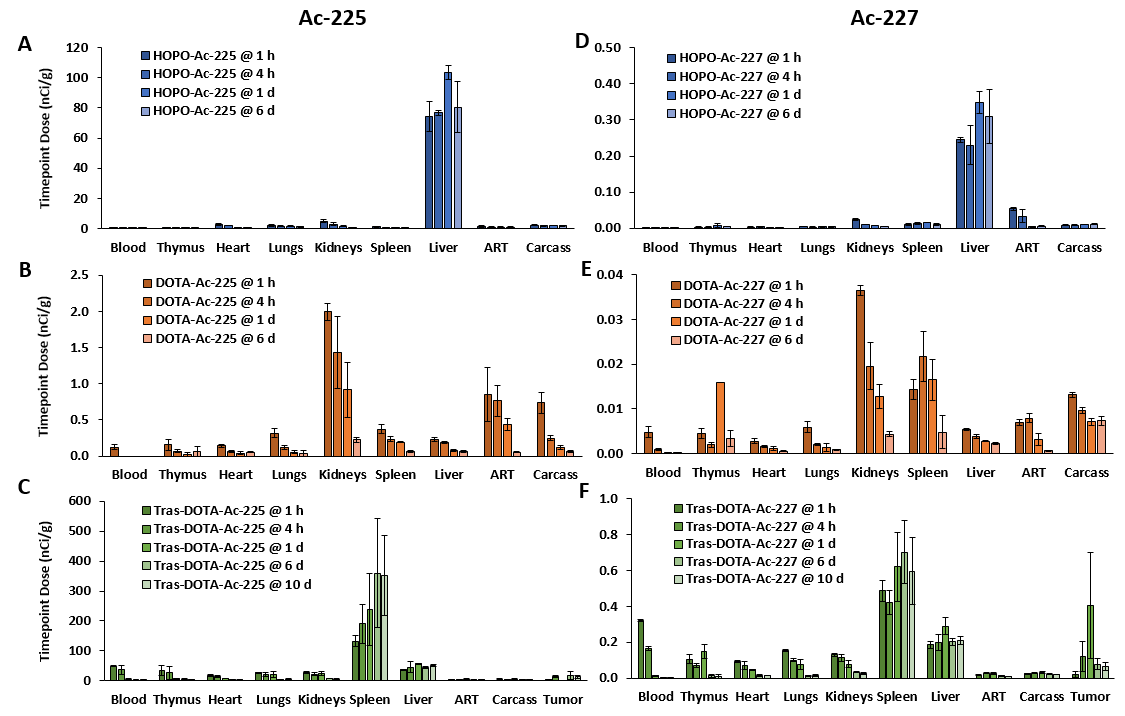
**ACKNOWLEDGEMENTS**

This work was supported by xxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxxx

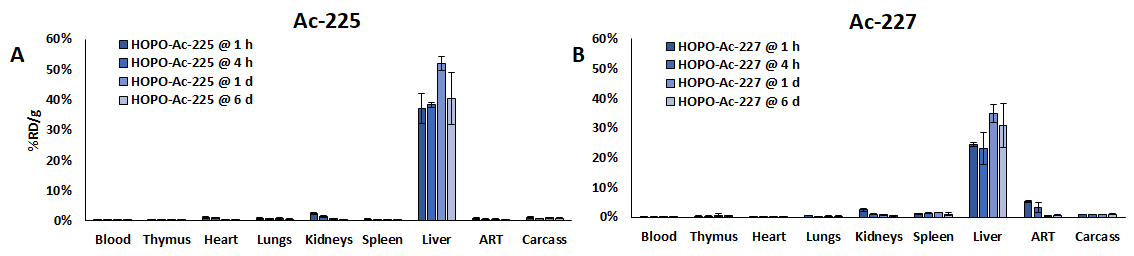
**SUPPLEMENTAL INFORMATION**



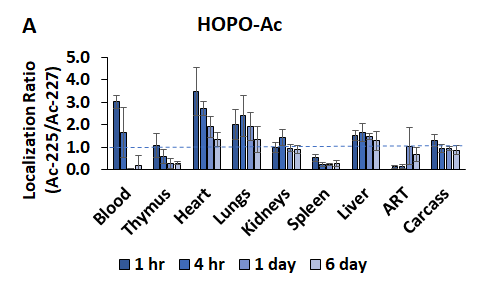
**Figure S1.** Starting with pure actinium species without daughters, equilibrium of average energy per destruction (counting both alpha *and* beta species) occurs rapidly within one day for Ac-225, and only after 100 days for Ac-227.



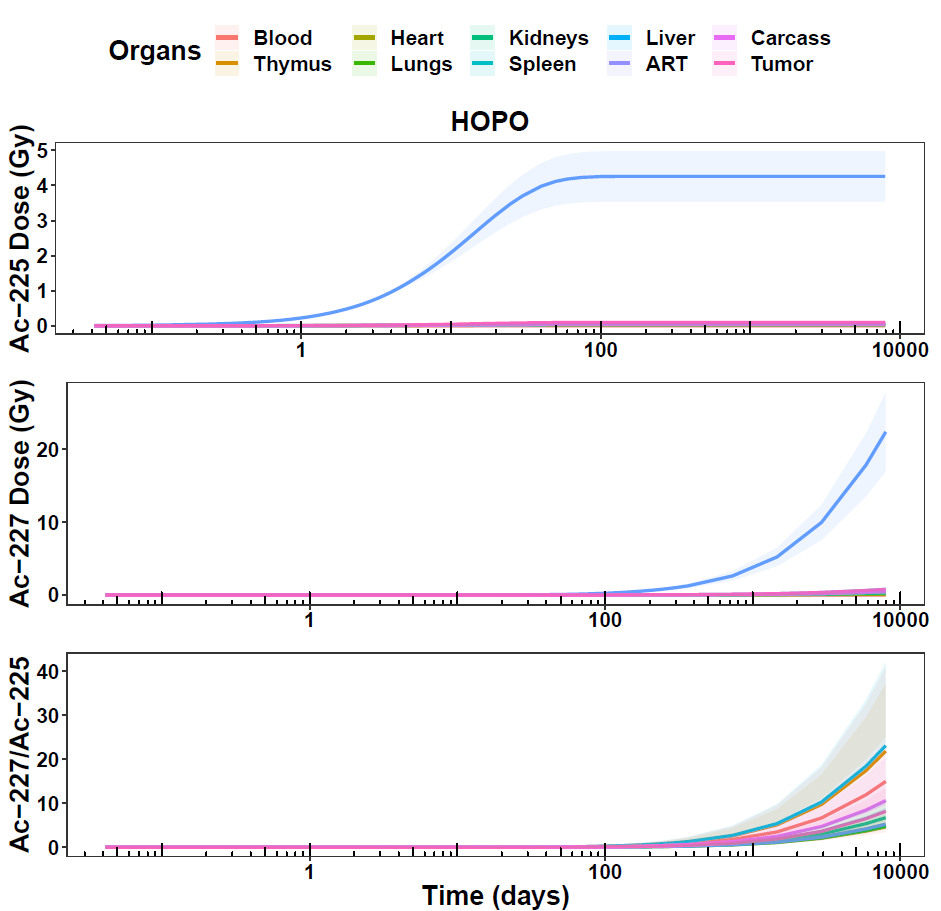
**Figure S2.** Dose biodistribution with exactly 200 nCi Ac-225 and 1 nCi Ac-227 per mouse, based on %RD/g plots in Figure 2. Mean ± SD, n=5.

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**Figure S3.** HOPO %RD/g. Considering the RD/g values from Figure 2 are proportional to relative organ dose, HOPO showed greatest dose to the liver for both Ac-225 and Ac-227, with a cumulative Ac-225 mean dose of 4.26 Gy (95% CI 3.97-4.54 Gy) which remained steady after 125 days (A). HOPO-Ac-227 at 125 days showed an increasing mean dose of 0.323 Gy (95% CI 0.30-0.36 Gy), and after 7946 days, a mean of 22.4 Gy (95% CI 20.3-24.5 Gy) (B). Mean ± SD, n=5.

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**Figure S4.** HOPO localization ratio. Mean ± SD, n=5.

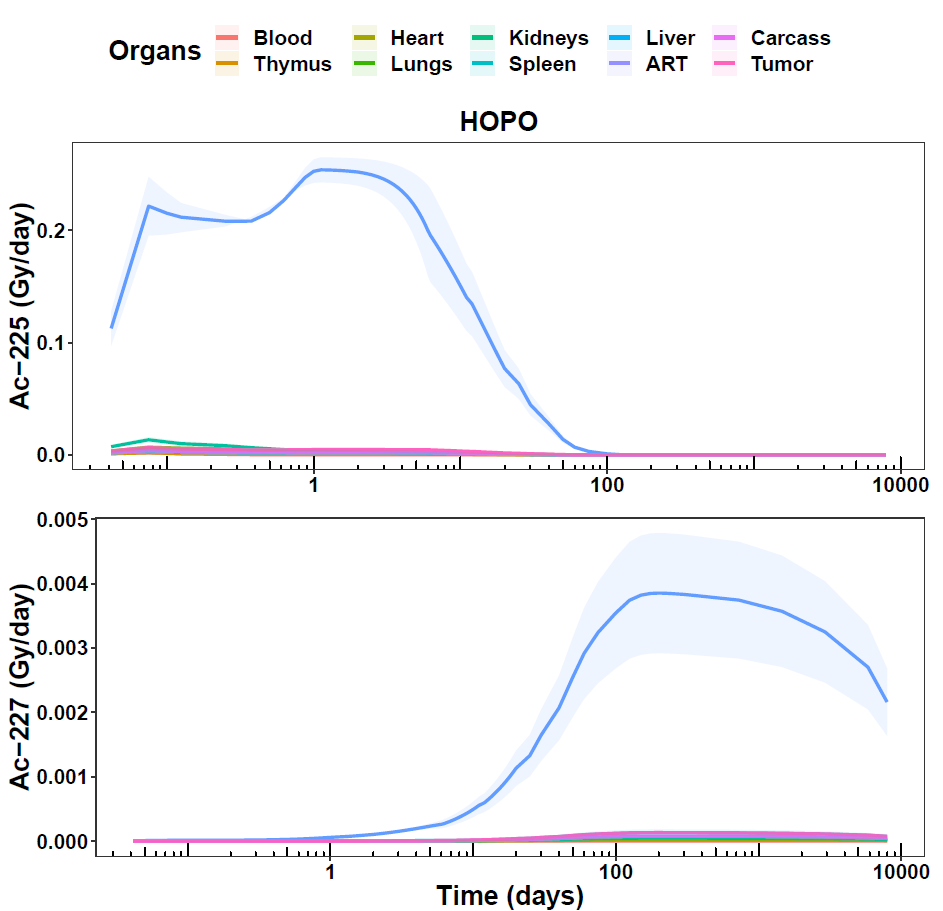
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**A**

**B**

**C**

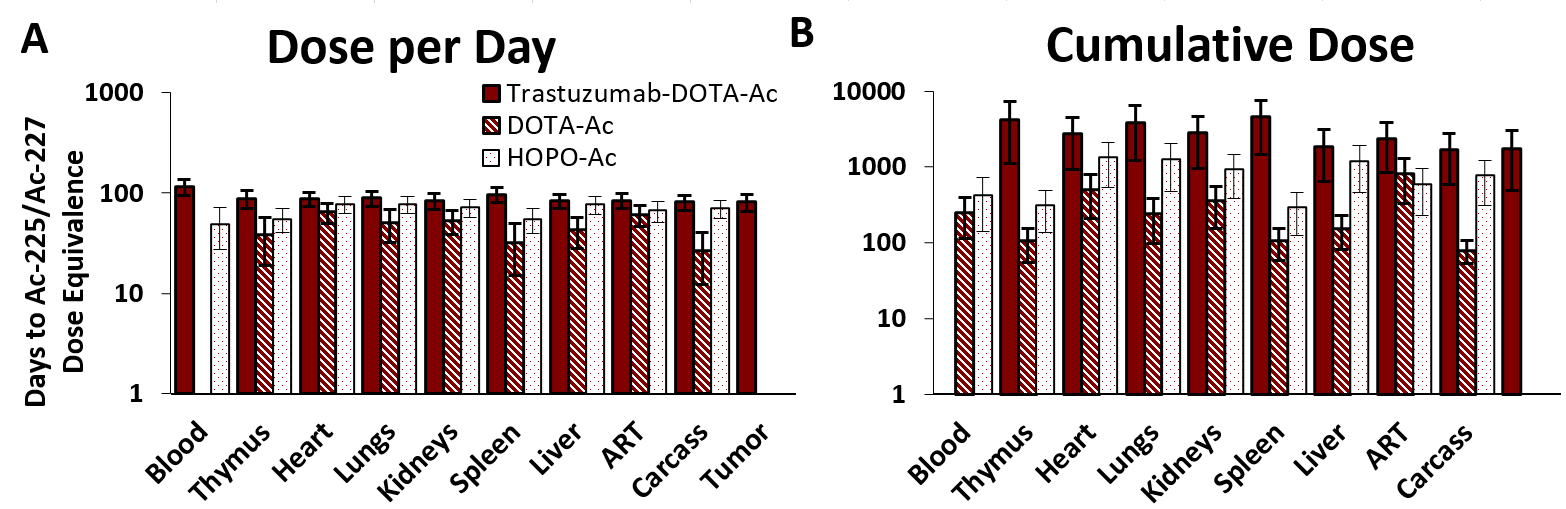
**Figure S5.** HOPO cumulative dose modeling. Due to the increasing values of Ac-227 cumulative dose, the dose ratios were initially small, and increased to a range of roughly 5-20 at 7946 days (C). Solid lines are a mean from n=5, and shading indicates standard deviation.

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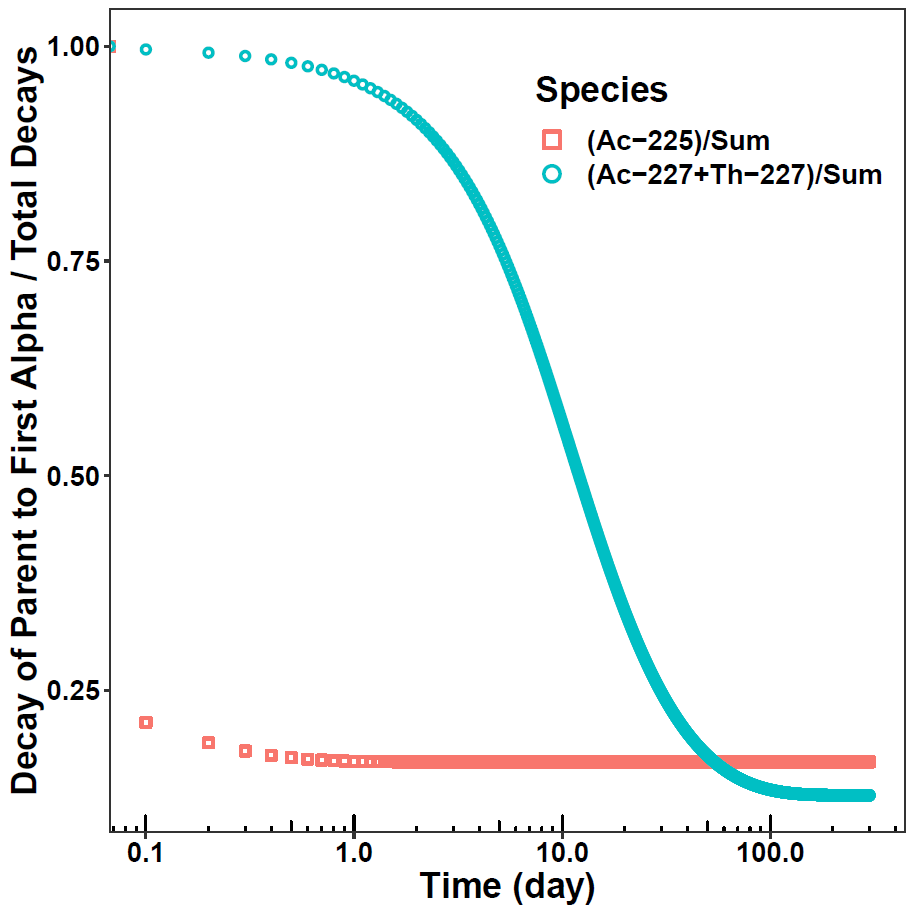
**A**

**B**

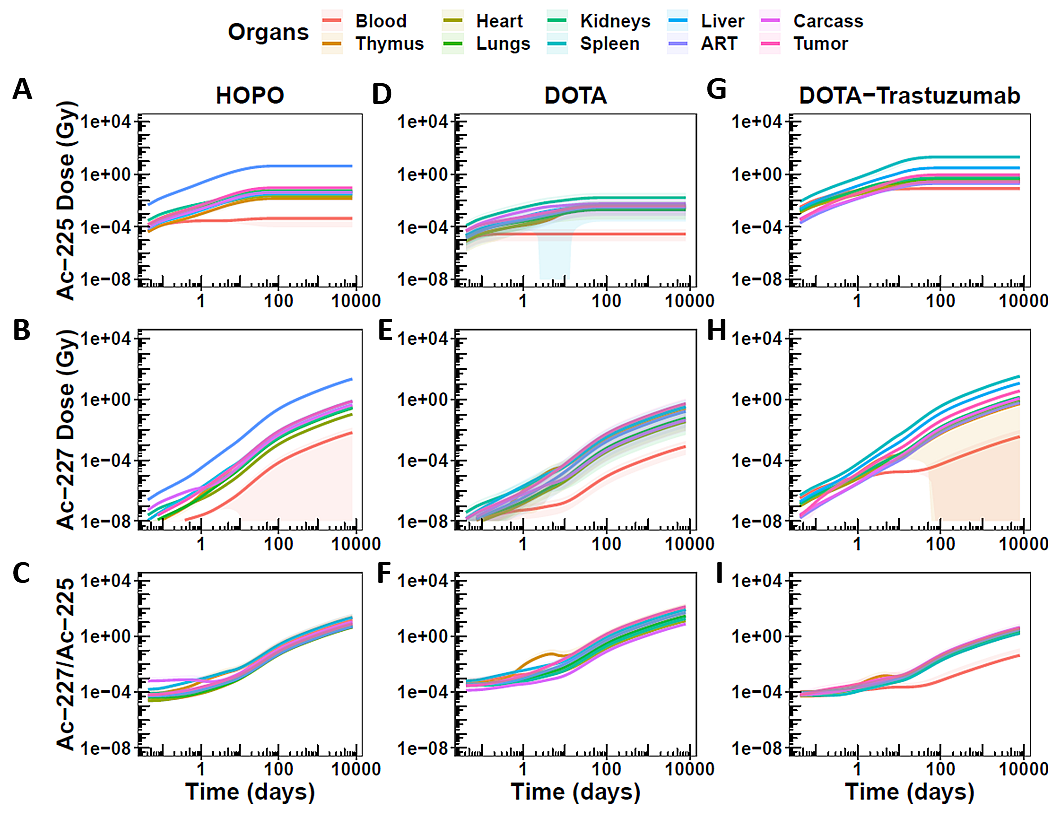
**Figure S6.** HOPO dose per day modeling. HOPO-Ac-225 showed a maximal mean of 0.254 Gy (95% CI 0.249-0.258) at 1.33 days (A) in the liver, whereas HOPO-Ac-227 showed a maximal mean of 0.039 Gy (95% CI 0.035-0.042) at 200 days in the liver and continued to decrease as Ac-227 decayed (B). Solid lines are a mean from n=5, and shading indicates standard deviation.



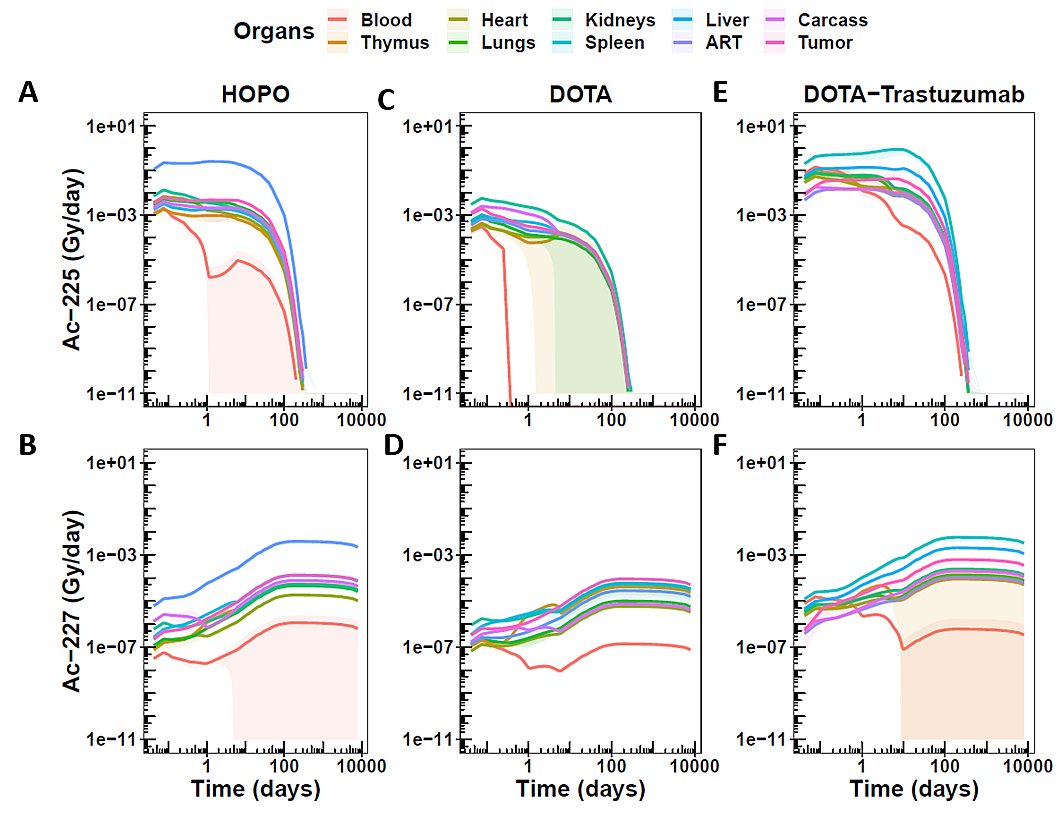
**Figure S7.** Number of days until dose equivalence between extrapolated Ac-227/Ac-225 dose ratios. In A), DOTA-Ac does not reach dose equivalence for the blood, and in B), Trastuzumab-DOTA-Ac does not reach dose equivalence for the blood. Mean ± SD, n=5.

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**Figure S8.** Activity ratio of Ac-225’s first daughter decay to Fr-221 over the sum of all daughter decay activities (blue circles), compared to the activity ratio of Ac-227’s decay to Th-227 plus Th-227’s decay to Ra-223 activity over the sum of all daughter decays (orange squares).



**Figure S9.** Cumulative dose extrapolation modeling over one Ac-227 half-life (7946 days). Solid lines are a mean from n=5, and shading indicates standard deviation.



**Figure S10.** Dose per day extrapolation modeling over one Ac-227 half-life (7946 days). Solid lines are a mean from n=5, and shading indicates standard deviation.

extra letters (DELETE)

**A**

**B**

**C**

**D**

**E**

**F**

**G**

**H**

**I**

**A**

**B**

**C**

**D**

**E**

**F**