Low Dose chemotherapy Empowers α-particle radionuclide therapy against Metastatic TNBC

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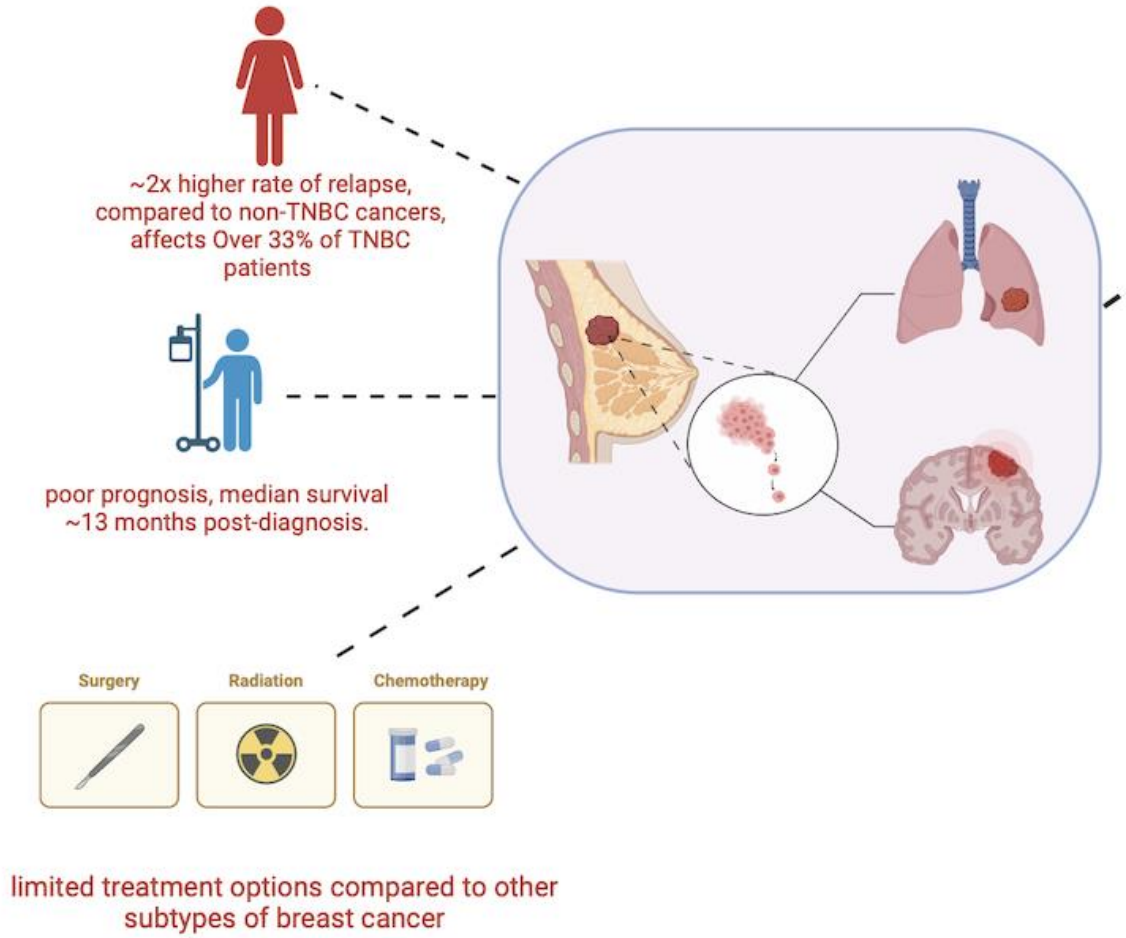
Metastatic Breast Cancer: High Risk, Poor Prognosis



Challenges of treating established (vascularized) TNBC Metastasis:

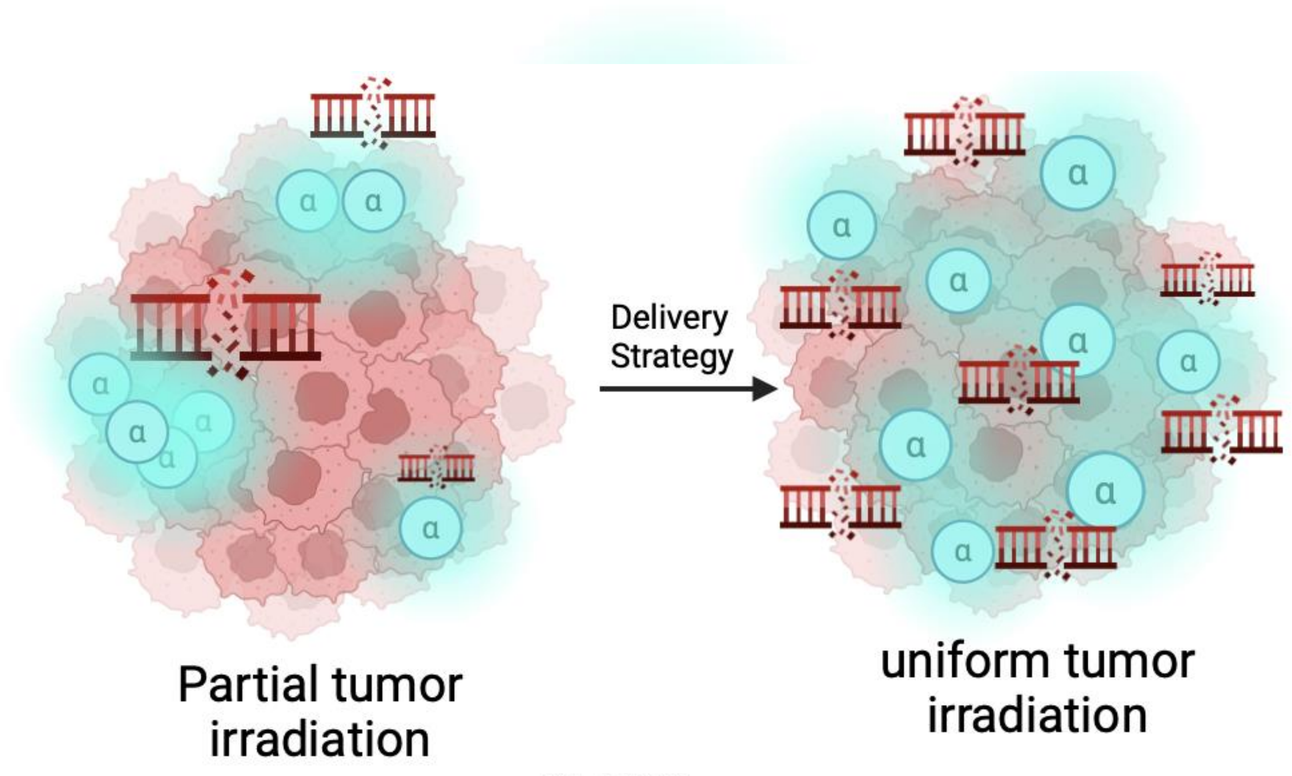
- **Uneven drug distribution** within the tumor
- Dose limitations due to **toxic effects on nearby healthy tissues**
- Development of **resistance**

Urgent Unmet Need: for an effective therapy to selectively and effectively kill Metastatic soft tissue TNBC solid tumors, to improve the quality of life and to prolong survival of patients

Yin, L et al., Breast Cancer Res, 2020
National Cancer Institute. (n.d.). *Cancer stat facts: Female breast cancer subtypes*
O Reilly et al., World J Clin Oncol. 2021

Images created on biorender

alpha-particles: what makes them ideal for hard-to-treat cancers



Emitted from Radioactive atoms, can be injected in blood

High Energy, Causes irreparable double strand breaks

The tumor regions not being hit by the delivered α-particles will likely not be killed.

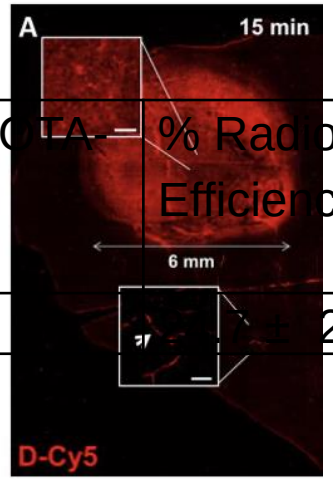
α-particles' short range 50-100μm needs uniform distribution in desired location

Sofou et al. J Nucl Med, 2004
Sgouros G, Cancer Res. 2019
Sgouros G,Semin Nucl Med. 2020

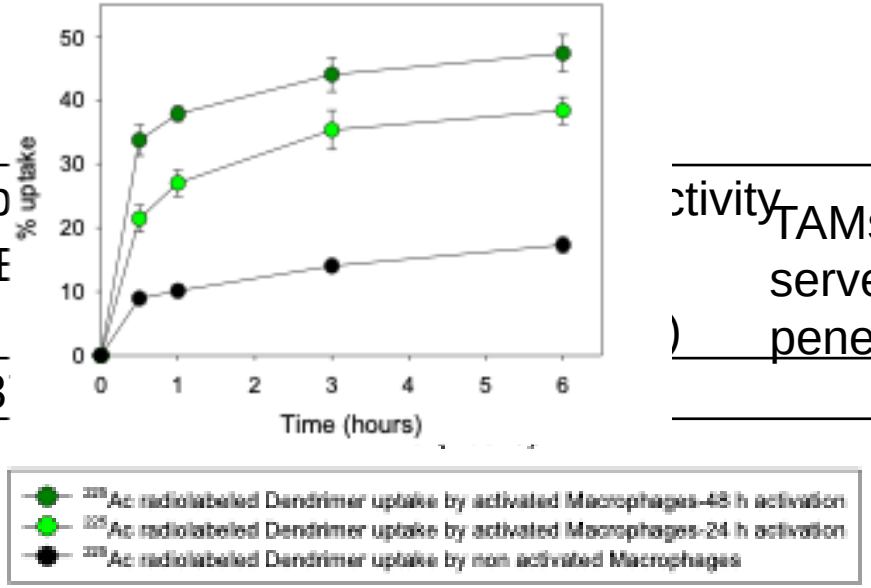
Achieving uniform distribution of α-particle therapy in solid tumors using PAMAM-OH dendrimers

Ideal delivery carrier

- Small, has near neutral charge to cross even the Blood Brain Tumor Barrier 6.7 nm
- Selectively targets the tumor, avoiding nearby healthy tissue
- Because dendrimers are taken up by TAMs (Tumor associated macrophages) without any additional targeting ligands



Zhang F, et al., *Biomaterials*. 2015

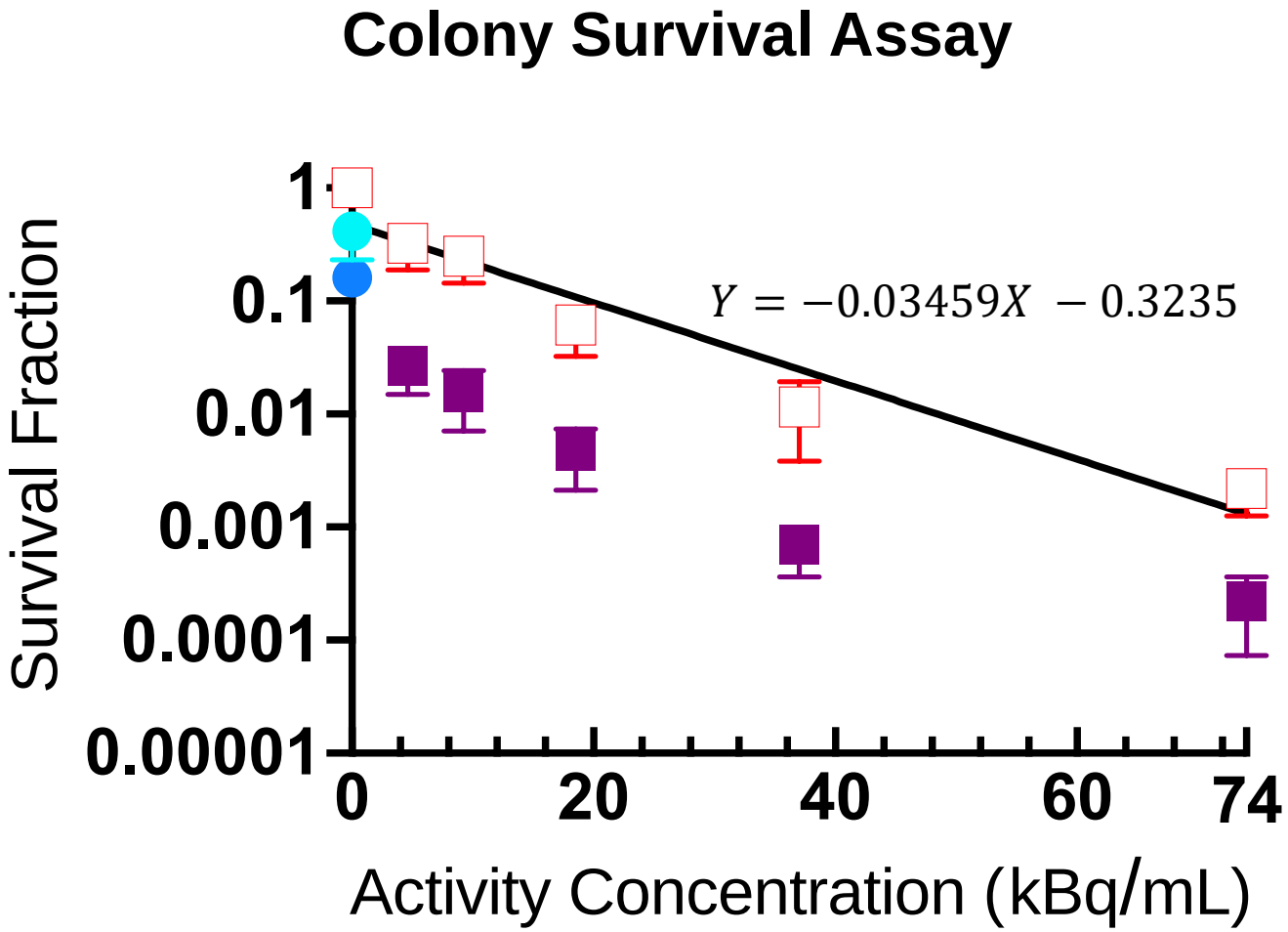


Nair R, Sarkar A, et al., *Eur J Nucl Med Mol Imaging*, 2025

[^{225}Ac]Ac-DOTA-dendrimer	% Radiolabeling Efficiency	Sp (kE	ctivity)	% Radiochemical Purity	% Stability
N=11	7 ± 2.2	13		94.4 ± 0.9	92.5 ± 1.1

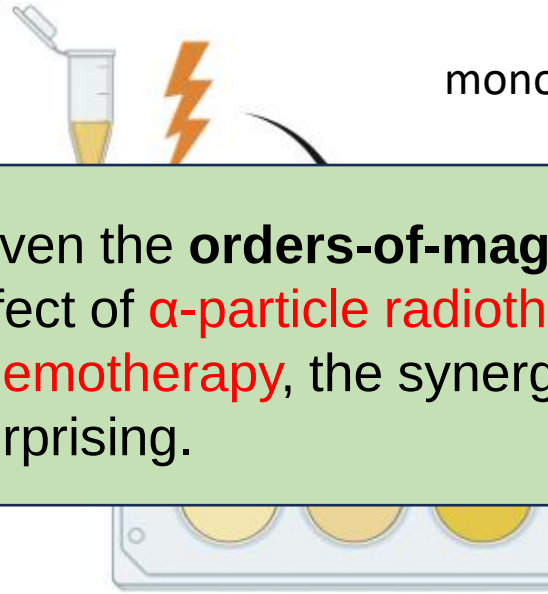
TAMs make up 30-50% of tumor mass; serves as delivery strategy to improve penetration of αRPT

Rationale for combining α-RPT with Cisplatin



Cell line used – 4T1

1 Becquerel – one disintegration per second

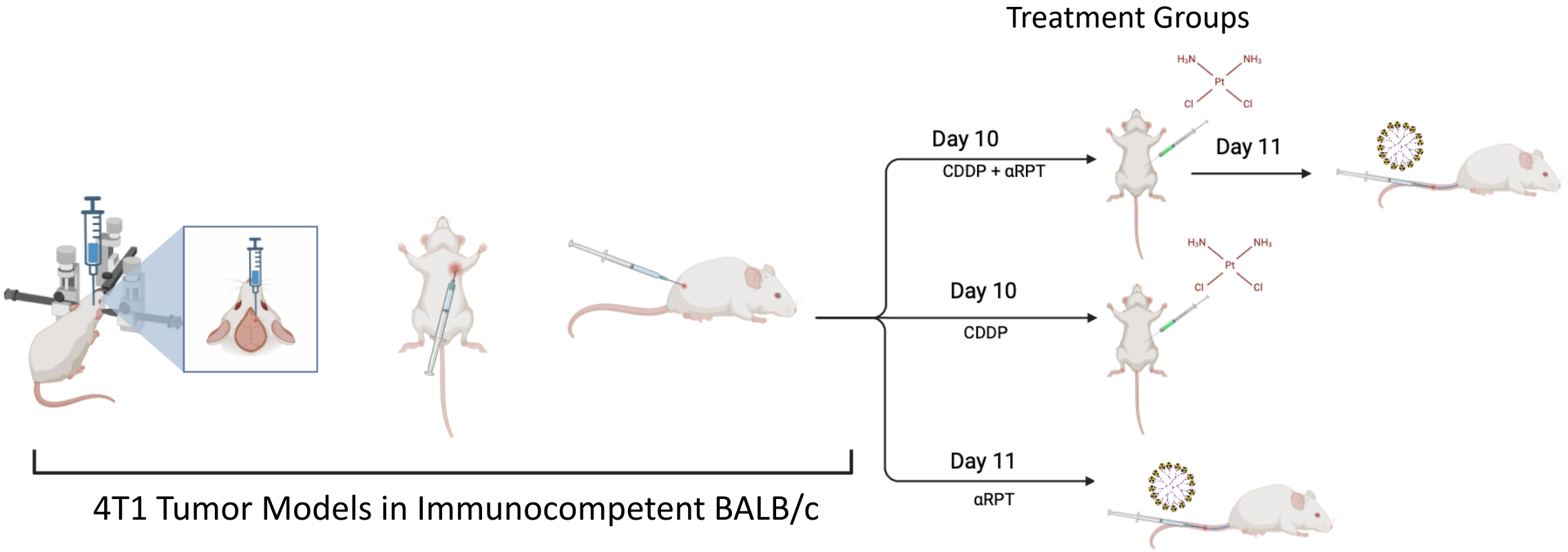


monolayers

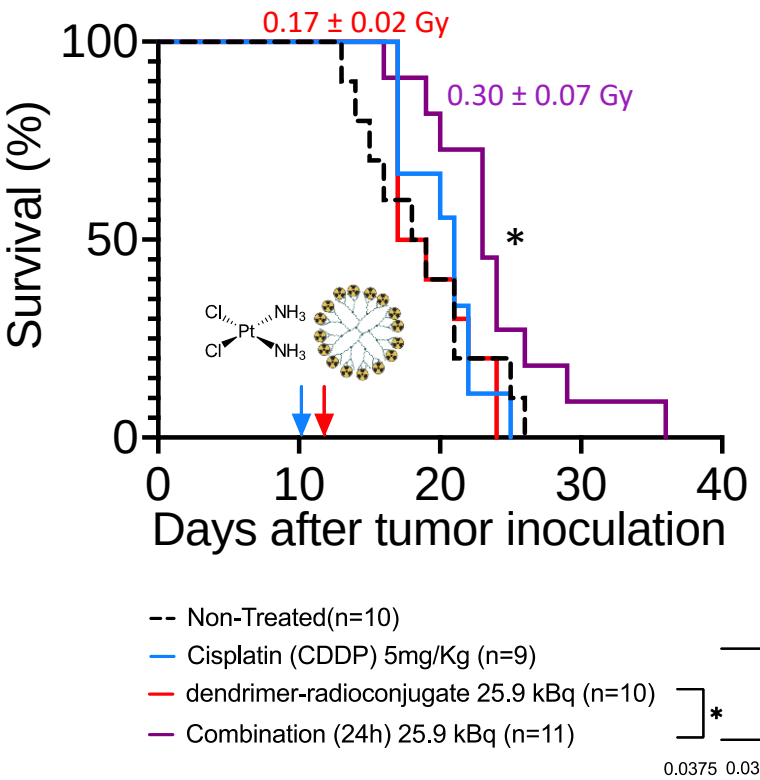
Given the **orders-of-magnitude** greater killing effect of α-particle radiotherapy compared to chemotherapy, the synergy in efficacy is surprising.

- Cisplatin (CDDP) (0.2mg/mL) (n=2)
- CDDP (0.4mg/mL) (n=2)
- dendrimer-radioconjugate(n=5) + CDDP (0.2µg/mL) (n=2);
- + CDDP (0.4mg/mL) (n=2);

Does combination therapy outperform individual therapies *in vivo?*



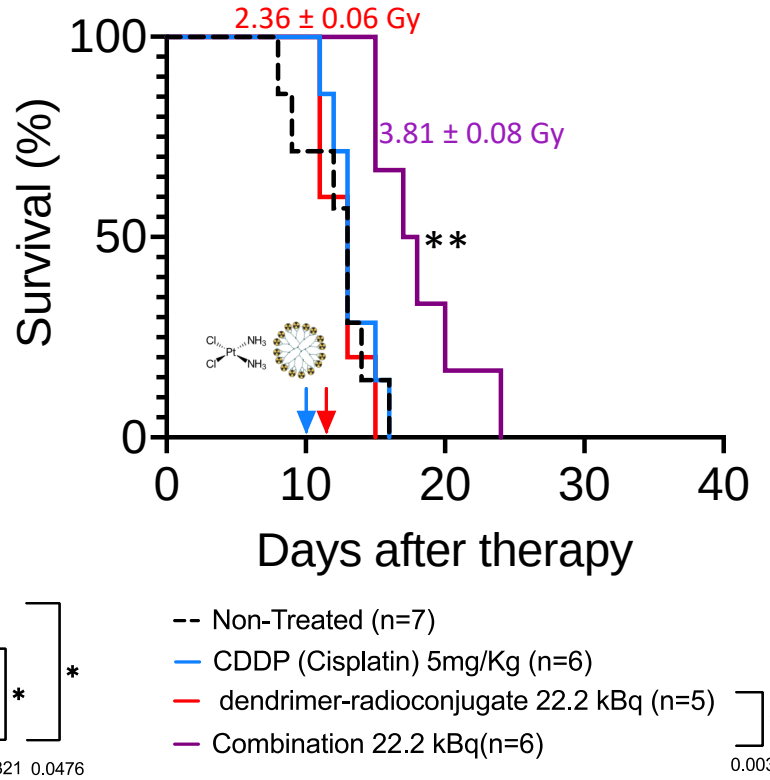
α-RPT injected AFTER low-dose Cisplatin improves survival *in vivo* independent of (metastatic/recurrent) tumor location in the body



Intracranial

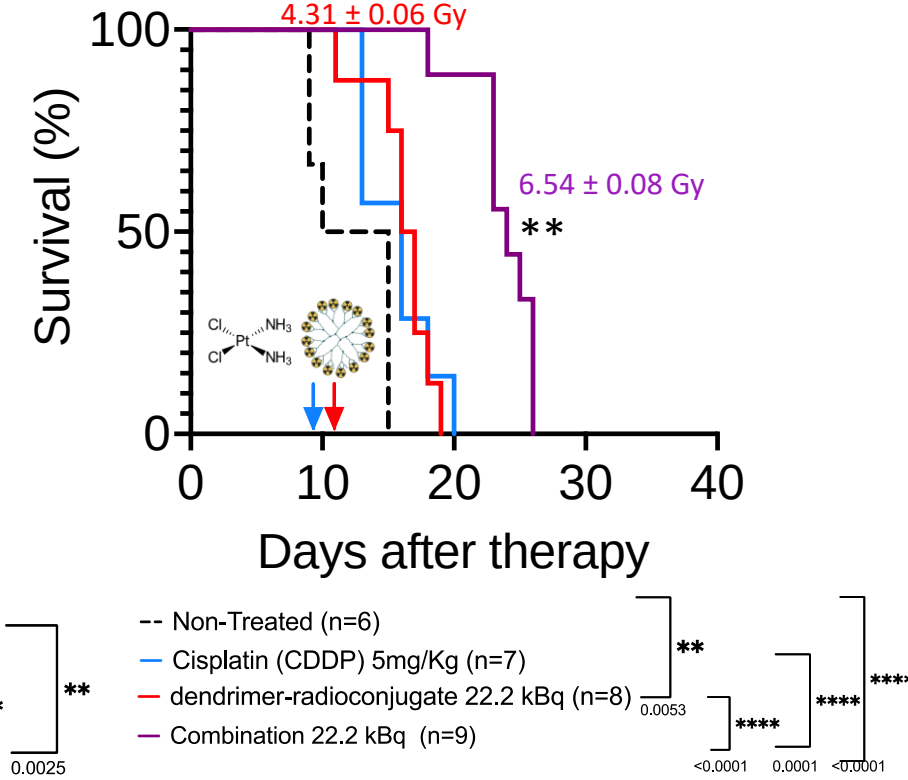
Tumor volume*– 26.5 ± 5.33mm^3

Endpoint: Weight loss ≥ 20%



Orthotopic

Endpoint – Average diameter ≥20mm



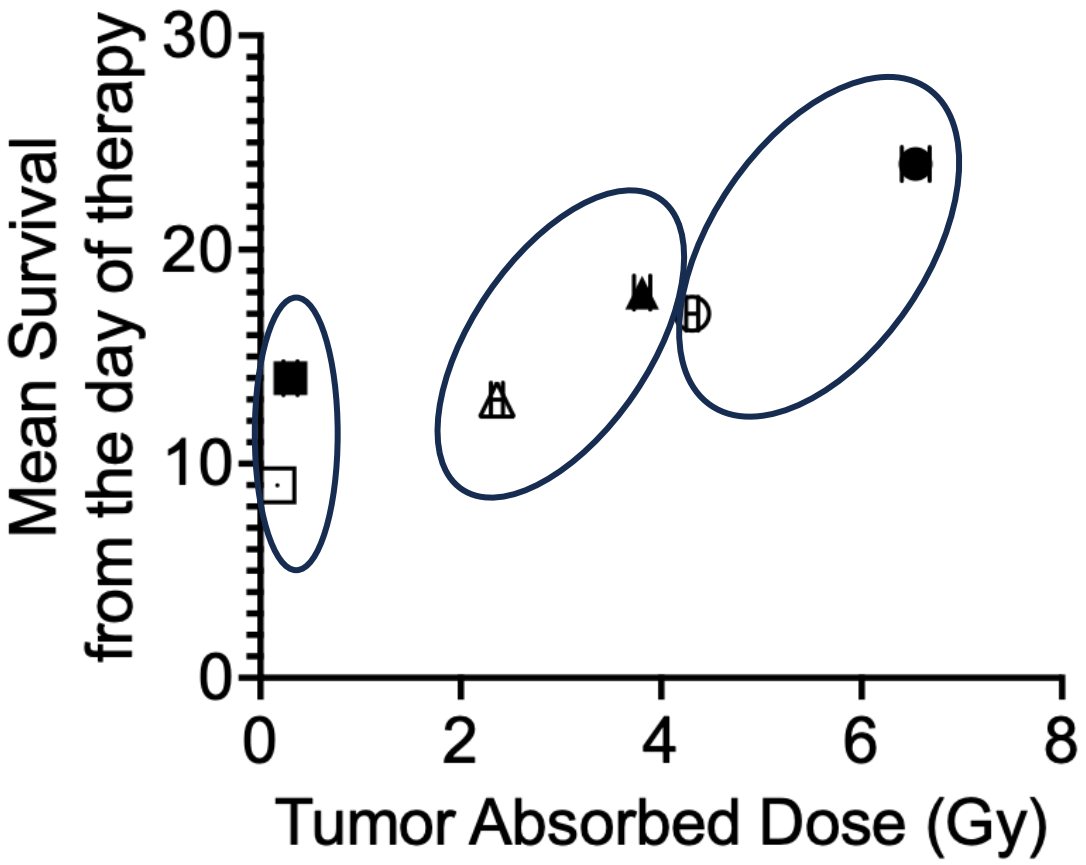
Subcutaneous

Tumor volume* – 100mm^3

Endpoint – Immobility, weight loss ≥ 20%

*Tumor volume at initiation of treatment

Mean survival scales strongly with tumor absorbed dose independent of tumor site in the body



Within the same tumor model

Tumor absorbed dose increases in the presence of cisplatin

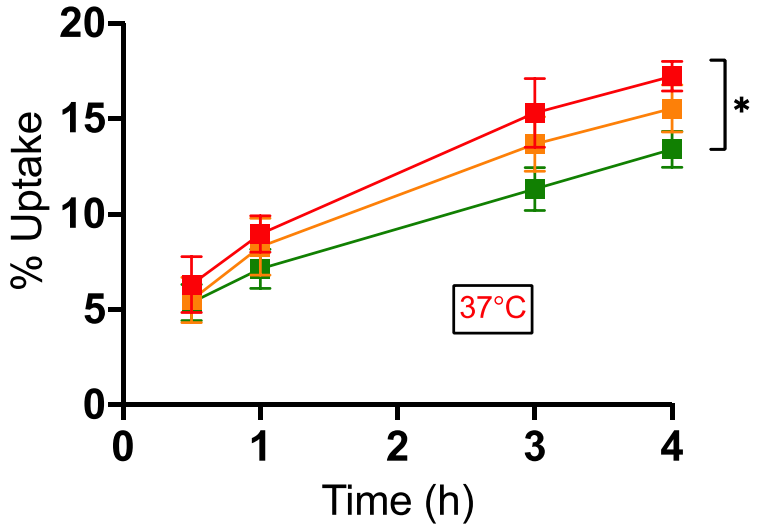
Across tumor models

Mean Survival increases with increase in tumor absorbed dose

- □ Intracranial dendrimer-radioconjugate
- ■ Intracranial combination
- △ Orthotopic dendrimer-radioconjugate
- ▲ Orthotopic combination
- ○ Subcutaneous dendrimer-radioconjugate
- ● Subcutaneous combination

Low dose of Cisplatin boosts 1) cellular uptake of dendrimer, 2) increases extent of dendrimer localization in the tumor

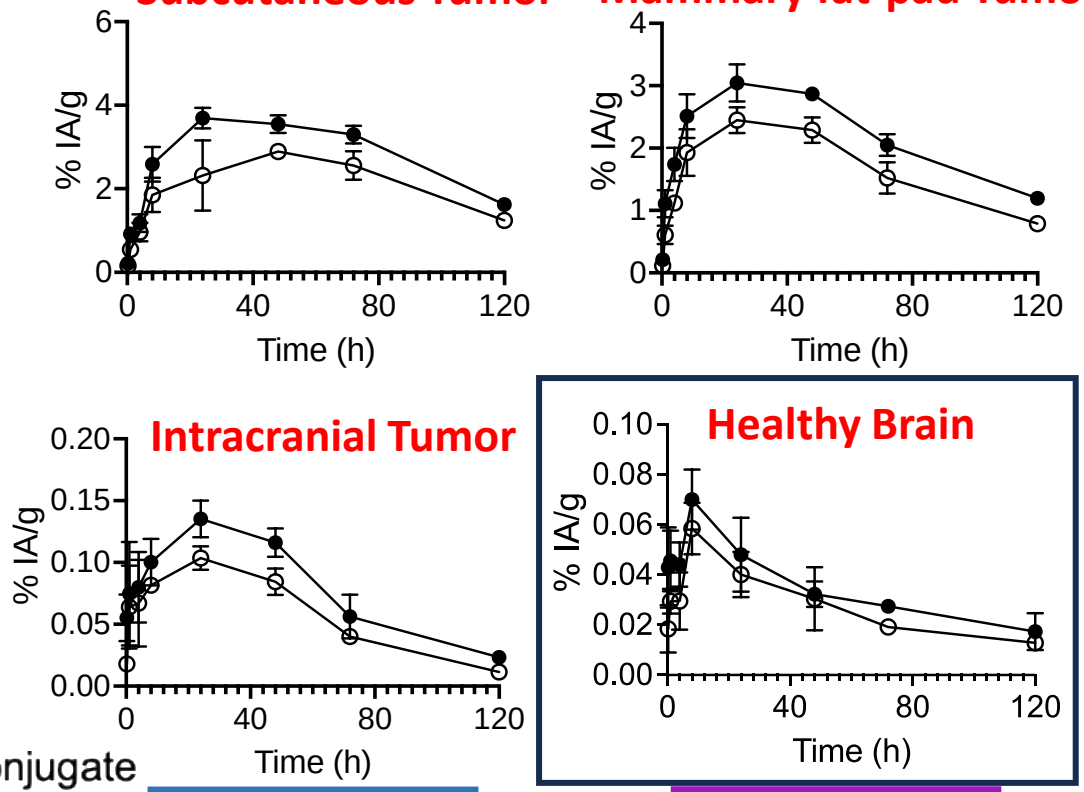
1.



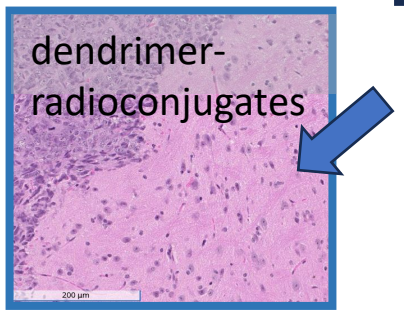
- Dendrimer + CDDP Pre-exposure (2 hours)
- Dendrimer + CDDP Co-exposure
- Dendrimer only

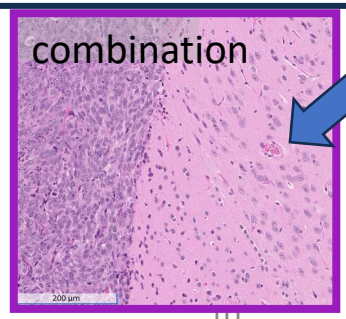
n = 3 runs

2

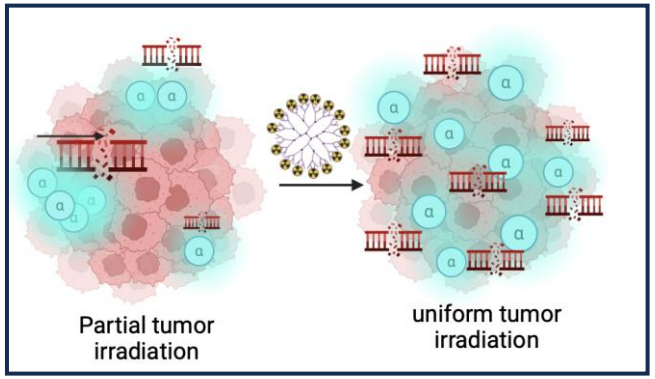


- Combination
- dendrimer-radioconjugate

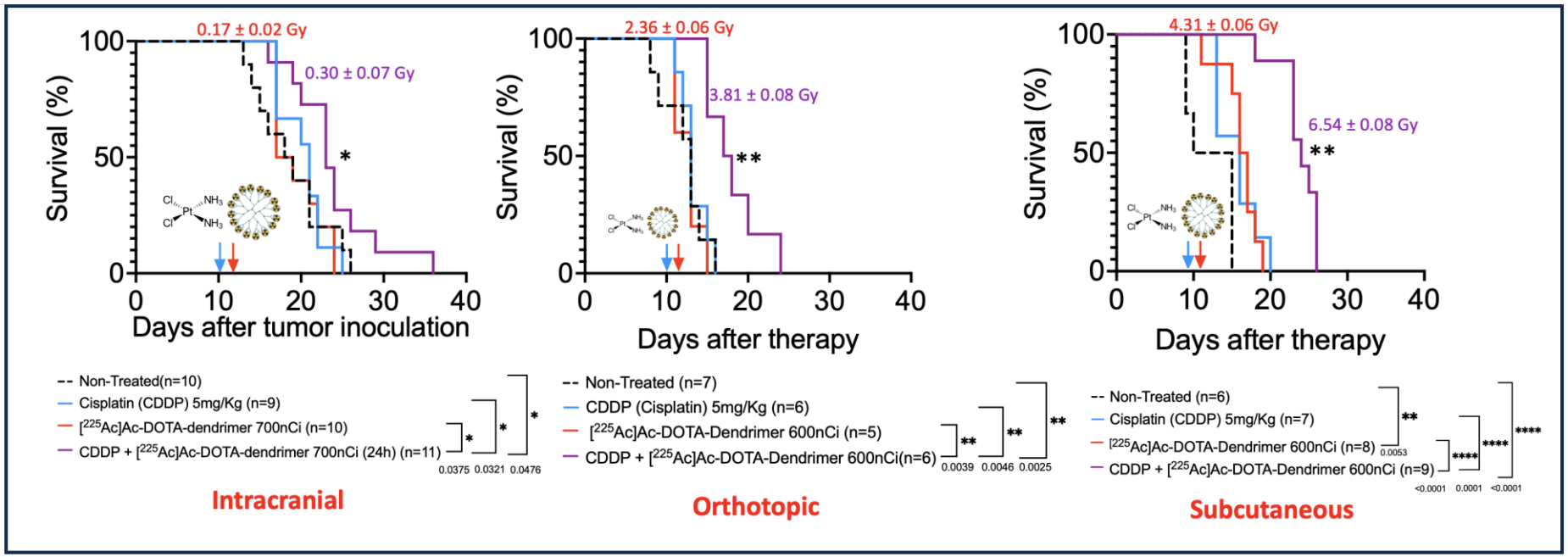


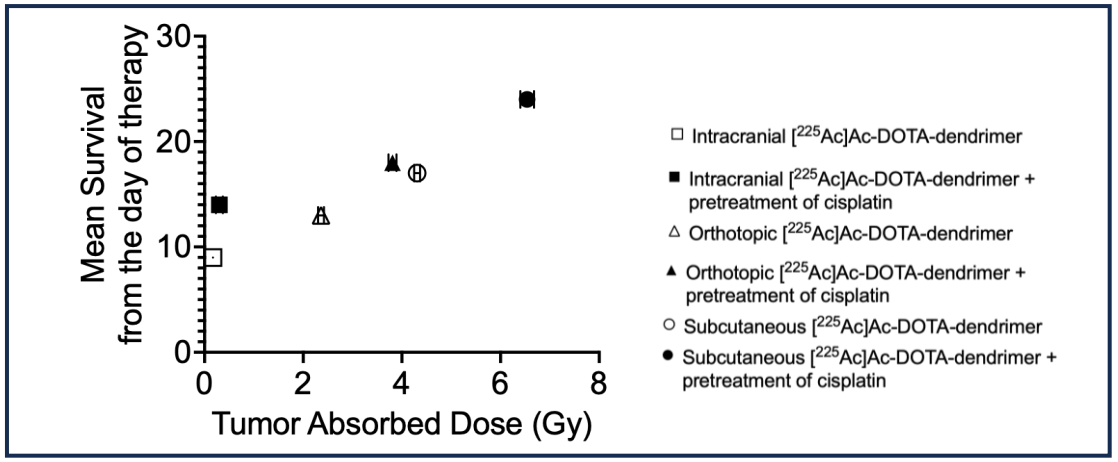


Conclusion



Improved distribution of αRPT in metastatic TNBC tumors irrespective of location, using TAM targeted dendrimers





αRPT + low dose chemotherapy is more effective than any of the parts alone

Mean survival strongly scales with tumor absorbed dose independent of tumor location

Acknowledgements

Members/Collaborators

- **Prof. Stavroula Sofou – Principal Investigator**
- Prof. Kannan Rangaramanujam
- Prof. Daniele Gilkes
- **Dr. Rajiv Ranjit Nair**
- **Aira Sarkar**
- Dr. Aprameya Ganesh Prasad
- Sumana Peddibhotla
- Jenny Zhang
- Hyeongmin Cho

Funding

Allegheny Health Network-JH Cancer Research Fund

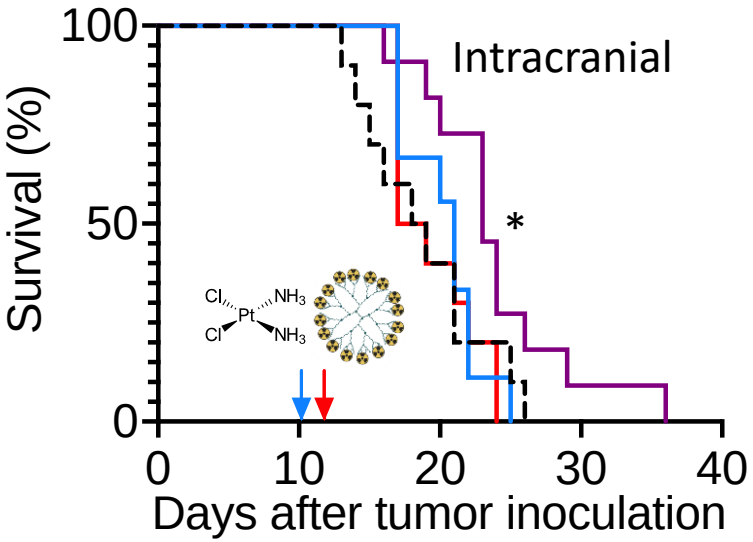
WW Smith Charitable Trust

Oak Ridge National Laboratory

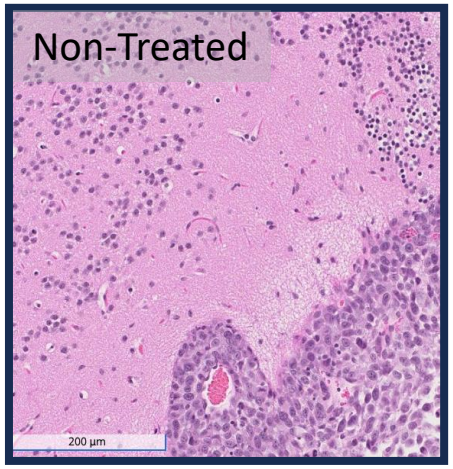
The actinium-225 used in this research was supplied by the U.S. Department of Energy Isotope Program managed by the Office of Isotope R&D and Production.

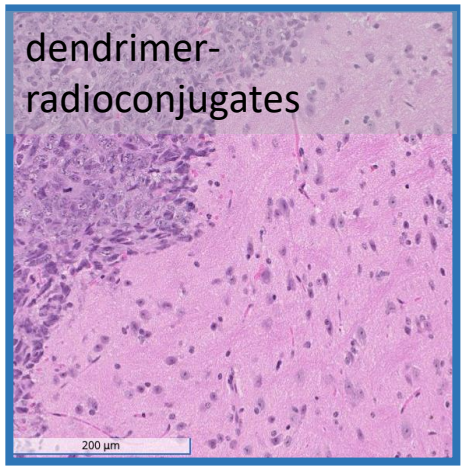
Thank You!

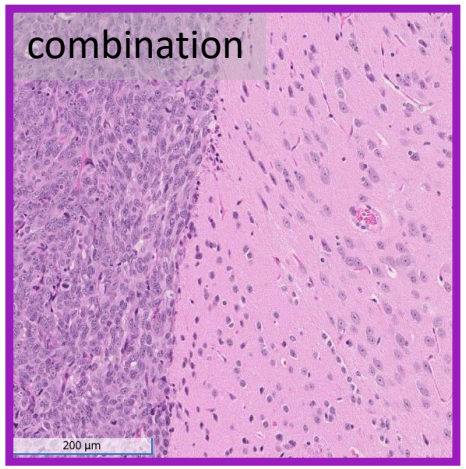
TNBC Brain Metastasis: Sparing of the nearby brain



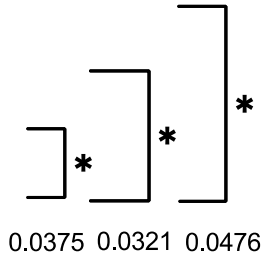
Brain-Tumor Interface





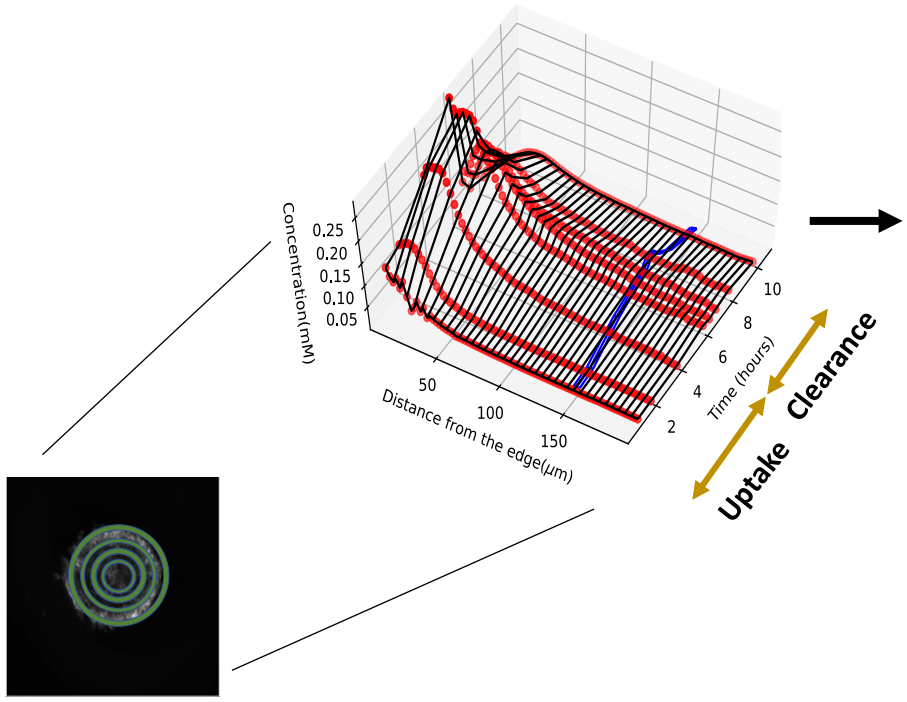


- Non-Treated(n=10)
- Cisplatin (CDDP) 5mg/Kg (n=9)
- dendrimer-radioconjugate 25.9 kBq (n=10)
- Combination (24h) (n=11)

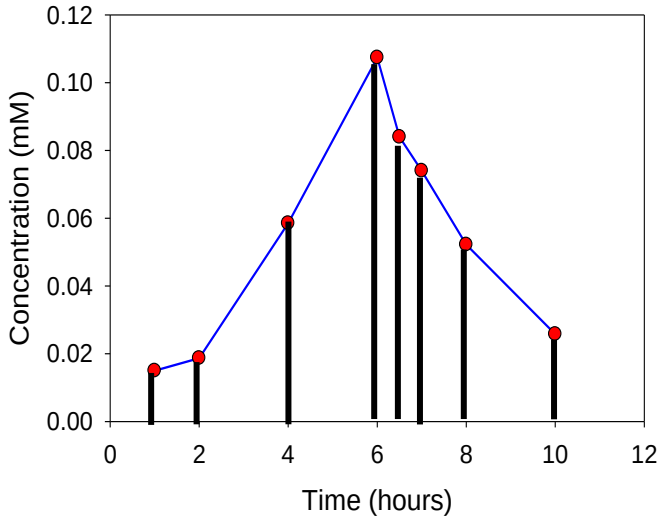


Obtaining AUC curve

Spatiotemporal profile of carrier

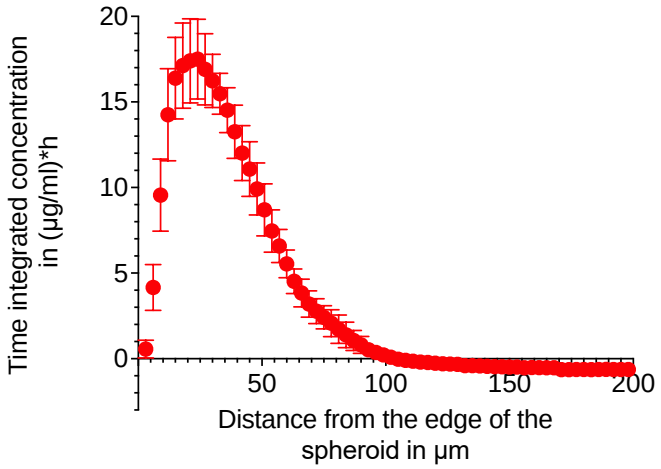


Using Trapezoid rule to integrate over time



Representative temporal profile for the highlighted (in blue) location in the spheroid

Total amount of exposure to a drug that a certain physical region in the spheroid experiences over a period of time.

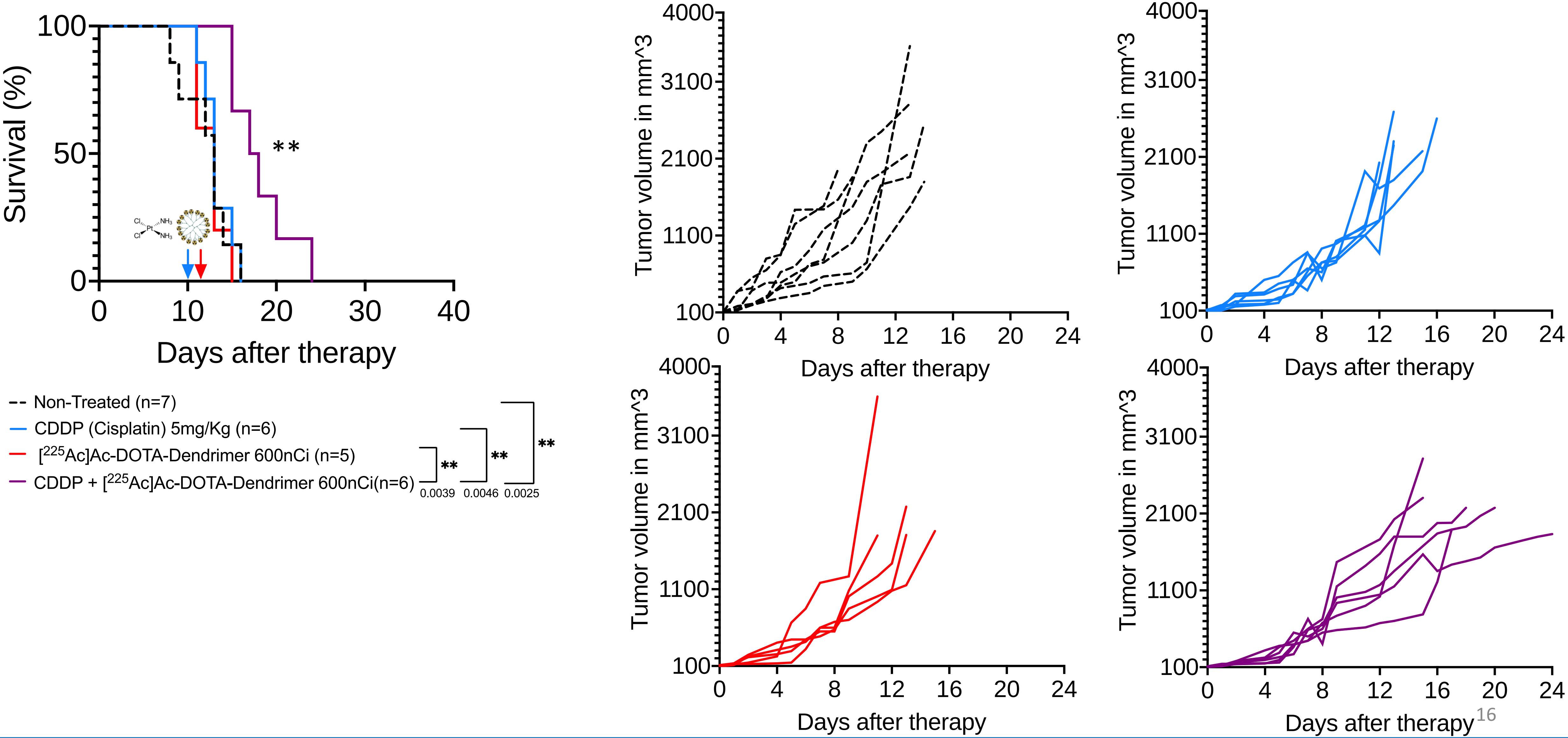


Repeating the integration for each location in the spheroid

Images from Dr. Omkar Bhatavdekar

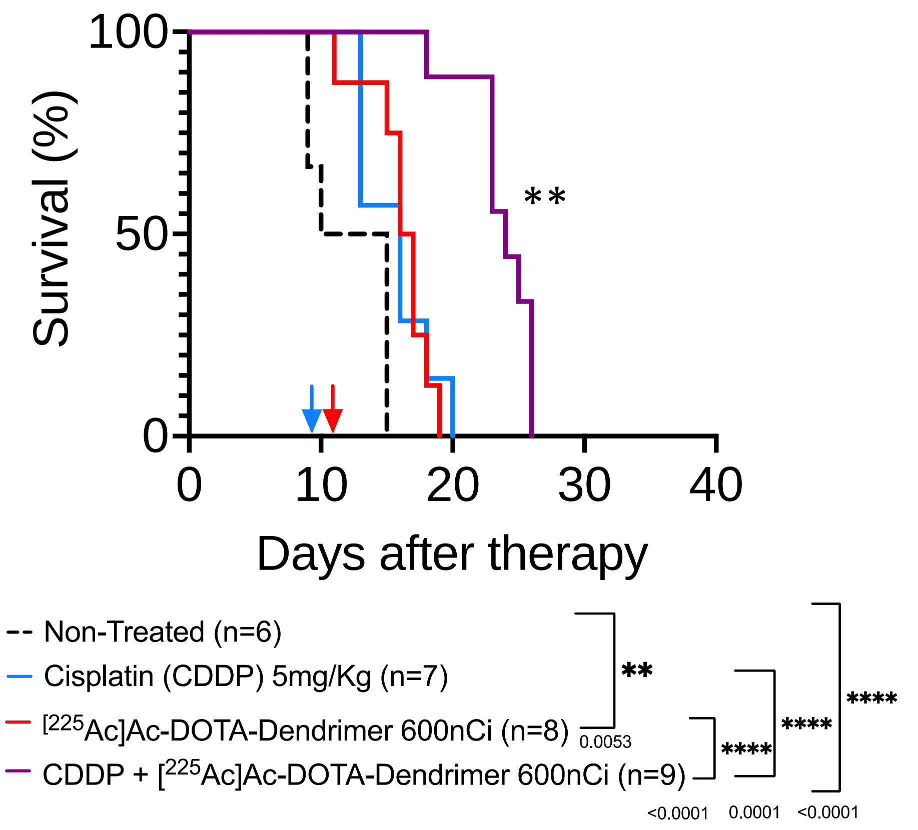
The dendrimer was localized more near the edge of the spheroid and did not penetrate deeper into the tumor spheroid model (in the absence of TAMs)

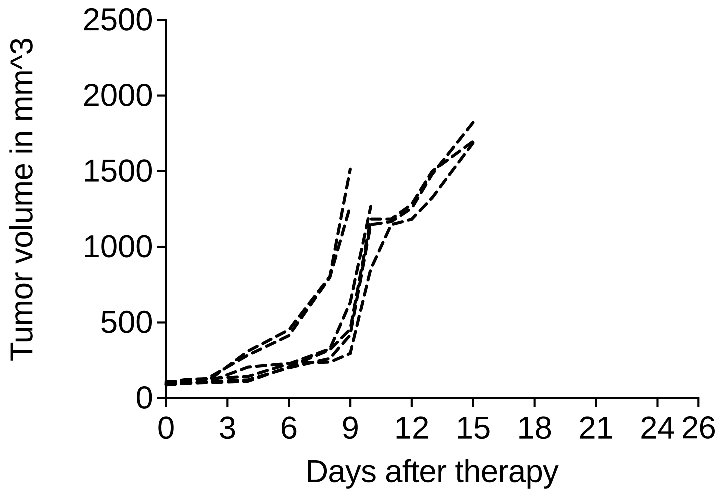
Tumor volumes inform survival in orthotopic model

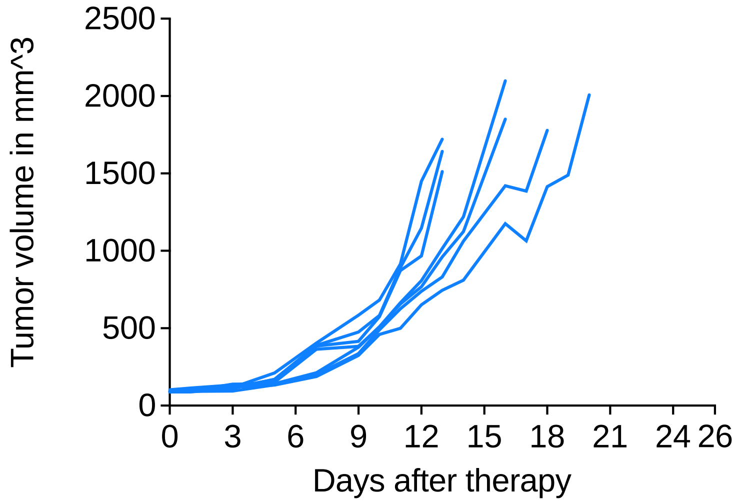


Objective #2 – In Vivo Tumor Volumes

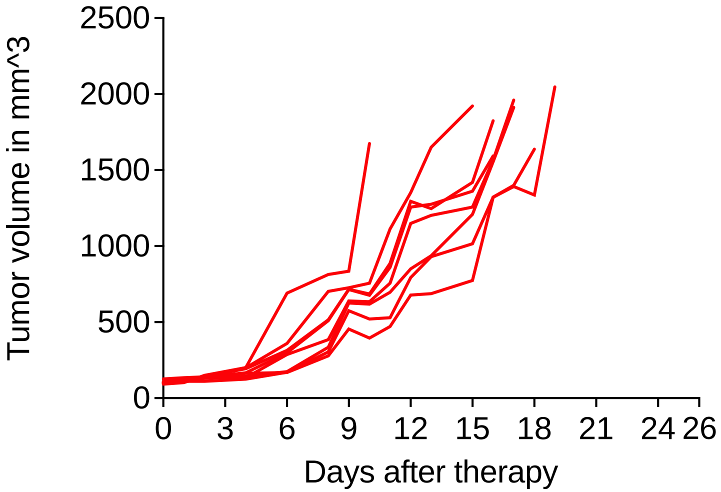
Tumor volumes inform survival in subcutaneous model

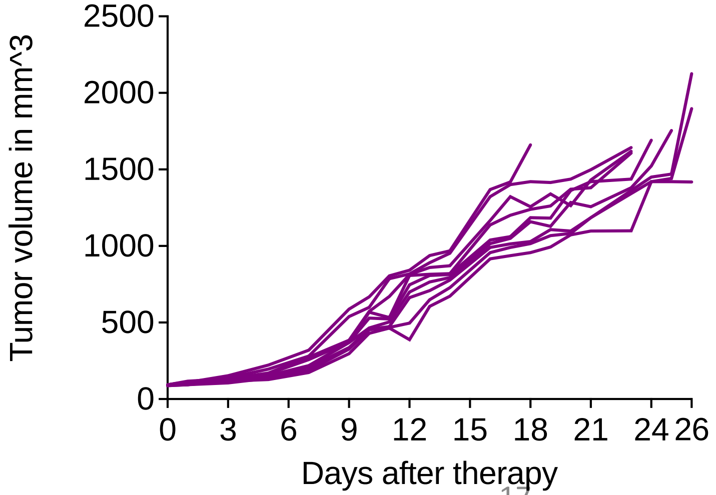






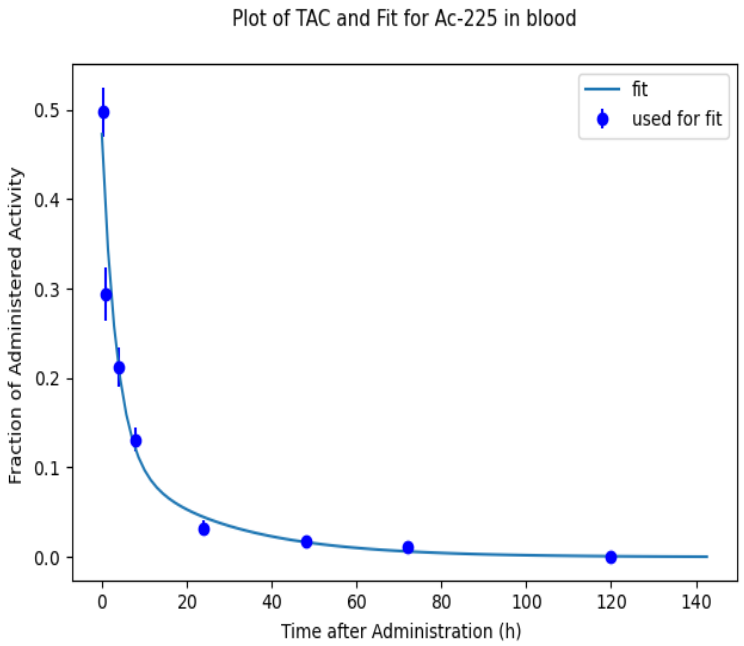
4.31 ± 0.06





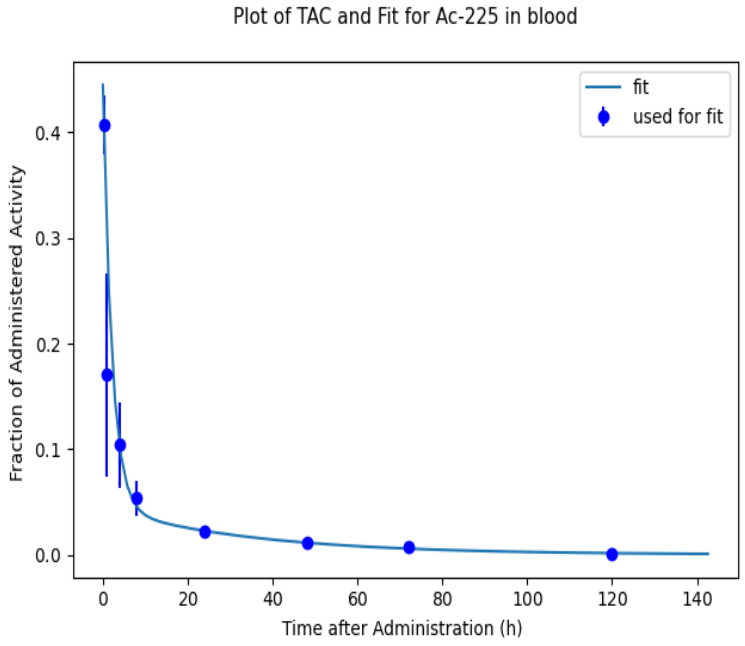
SUBCUTANEOUS MODEL

Appendix



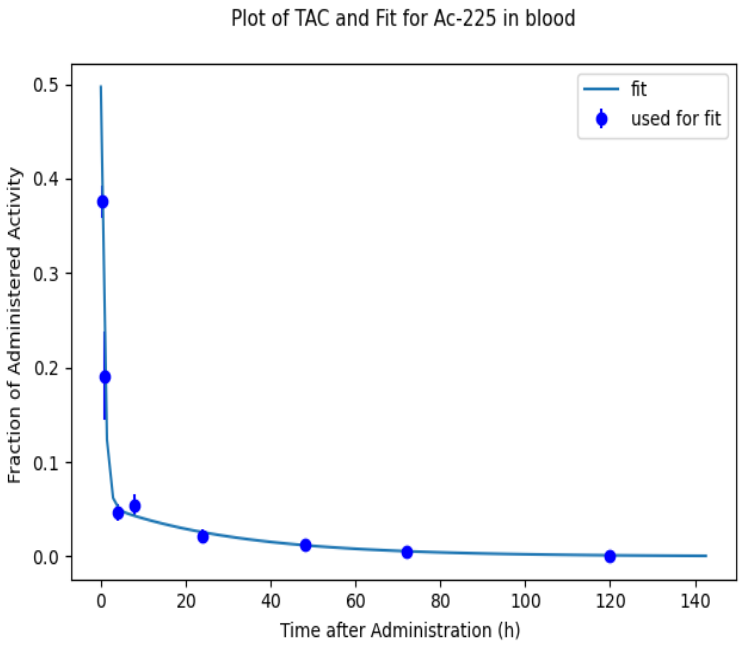
Intracranial

$$0.35e^{\left(-\frac{0.693t}{2.37}\right)} + 0.11e^{\left(-\frac{0.693t}{16.48}\right)}$$



Orthotopic

$$0.4e^{\left(-\frac{0.693t}{1.48}\right)} + 0.04e^{\left(-\frac{0.693t}{24.17}\right)}$$



Subcutaneous

$$0.44e^{\left(-\frac{0.693t}{0.545}\right)} + 0.05e^{\left(-\frac{0.693t}{21.55}\right)}$$

Dosimetry Data

Intracranial Dendrimer only

Organ	Absorbed Dose (Gy)
brain	0.05 ± 0.00
kidney	0.94± 0.05
lung	0.00 ± 0.00
liver	0.24 ± 0.01
heart	0.77 ± 0.05
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.08 ± 0.00
tumor	0.17± 0.00

Intracranial D+C

Organ	Absorbed Dose (Gy)
brain	0.07 ± 0.00
kidney	1.24 ± 0.09
lung	0.00 ± 0.00
liver	0.36 ± 0.01
heart	0.76 ± 0.18
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.16 ± 0.00
tumor	0.30 ± 0.07

Orthotopic Dendrimer only

Organ	Absorbed Dose (Gy)
brain	0.03± 0.00
kidney	0.71 ± 0.02
lung	0.00 ± 0.00
liver	0.09 ± 0.00
heart	0.42 ± 0.02
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.04 ± 0.00
tumor	2.36 ± 0.06

Orthotopic D+C

Organ	Absorbed Dose (Gy)
brain	0.05 ± 0.00
kidney	0.76 ± 0.02
lung	0.00 ± 0.00
liver	0.11± 0.01
heart	0.99 ± 0.14
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.06 ± 0.00
tumor	3.81 ± 0.08

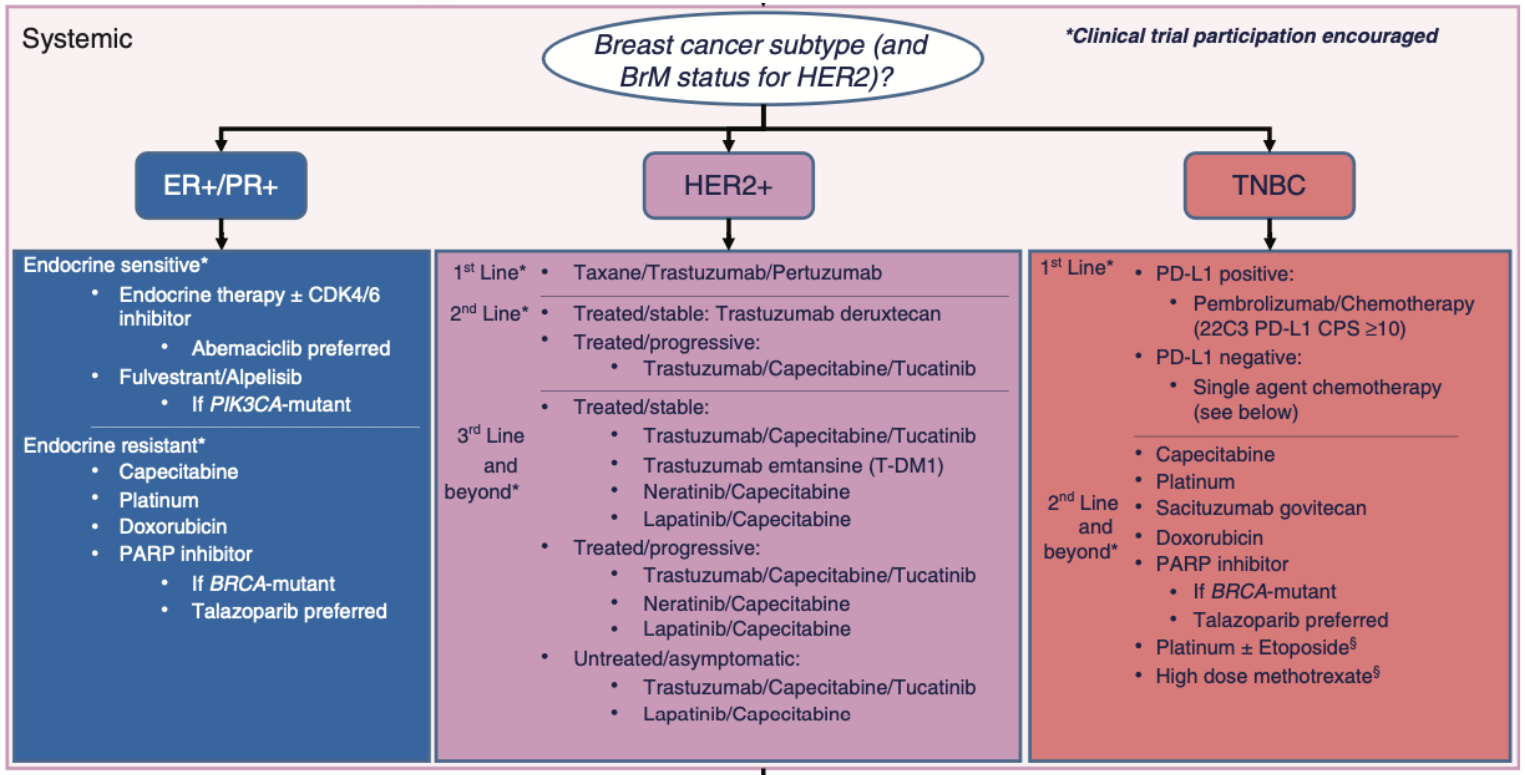
Subcutaneous Dendrimer only

Organ	Absorbed Dose (Gy)
brain	0.06 ± 0.01
kidney	0.90 ± 0.02
lung	0.00 ± 0.00
liver	0.21 ± 0.01
heart	0.65 ± 0.05
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.05 ± 0.00
tumor	4.31 ± 0.06

Subcutaneous D+C

Organ	Absorbed Dose (Gy)
brain	0.02 ± 0.00
kidney	1.17 ± 0.03
lung	0.00 ± 0.00
liver	0.34 ± 0.02
heart	0.57 ± 0.02
stomach	0.00 ± 0.00
intestine	0.00 ± 0.00
spleen	0.05 ± 0.00
tumor	6.54 ± 0.14

Breast Cancer Brain Metastases treatments are limited



Sammons S, Neurooncol Adv. 2021

Brain Metastases of TNBC subtype is currently treated with cocktails of chemotherapy

Limited Treatment Options

Gold Standards

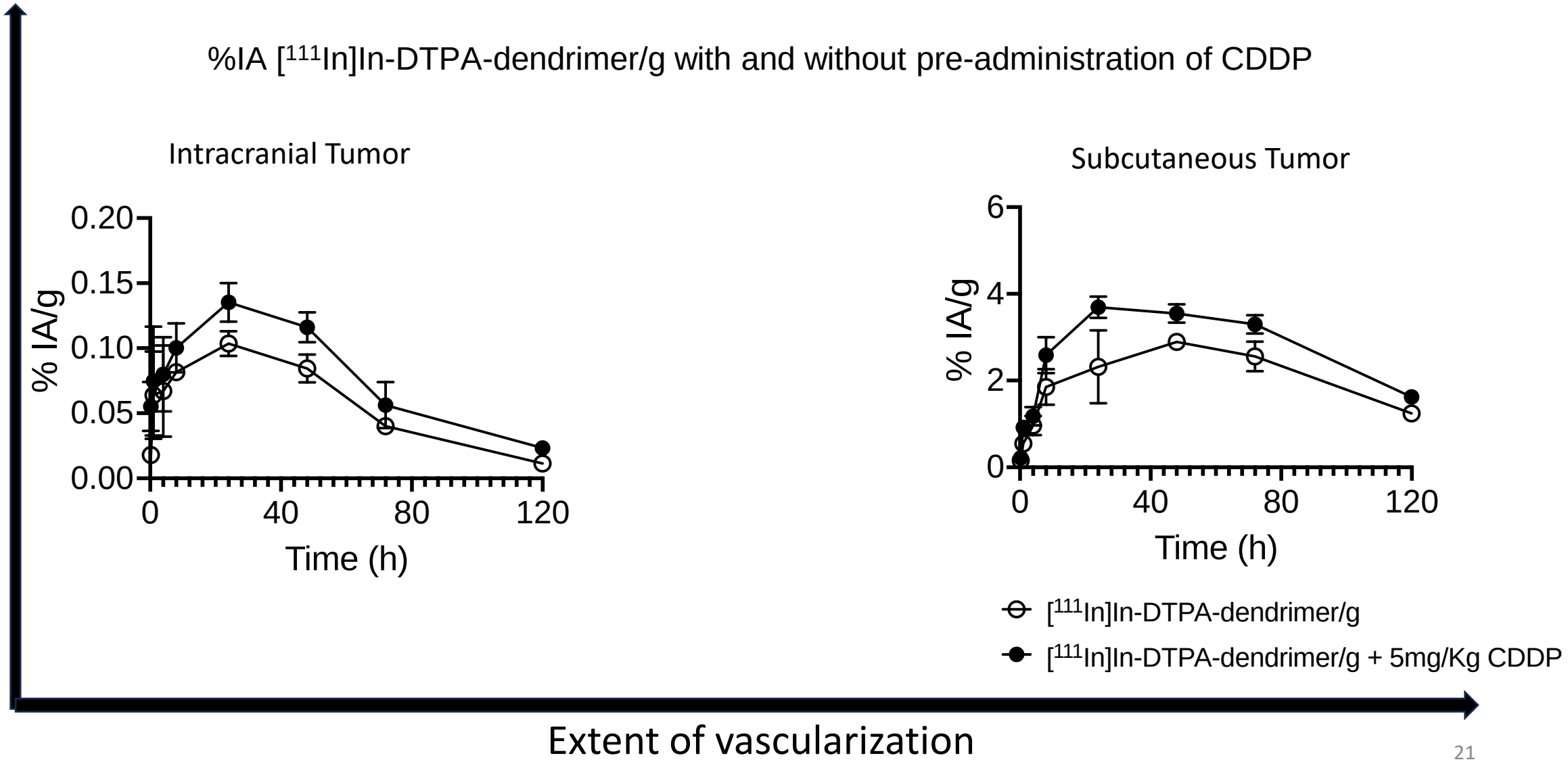
Emerging Therapies

- Surgical Resection
  for Isolated Metastases
- Targeted Therapies
  Primarily for HER2+, ER+ subtypes
  - Whole Brain Radiation Therapy
    for >10 BM
  - **Tucatinib – FDA Approved for treating HER2+ BCBM**
    Leads to Neuro-cognitive impairments, local brain necrosis
  - **Immune checkpoint inhibitors and PARP inhibitors explored for TNBC**
    Can lead to brain edema, local brain necrosis
  - Stereotactic Radiosurgery
    for <10 BM
- Systemic Chemotherapy
  Resistance, poor permeability of BBTB

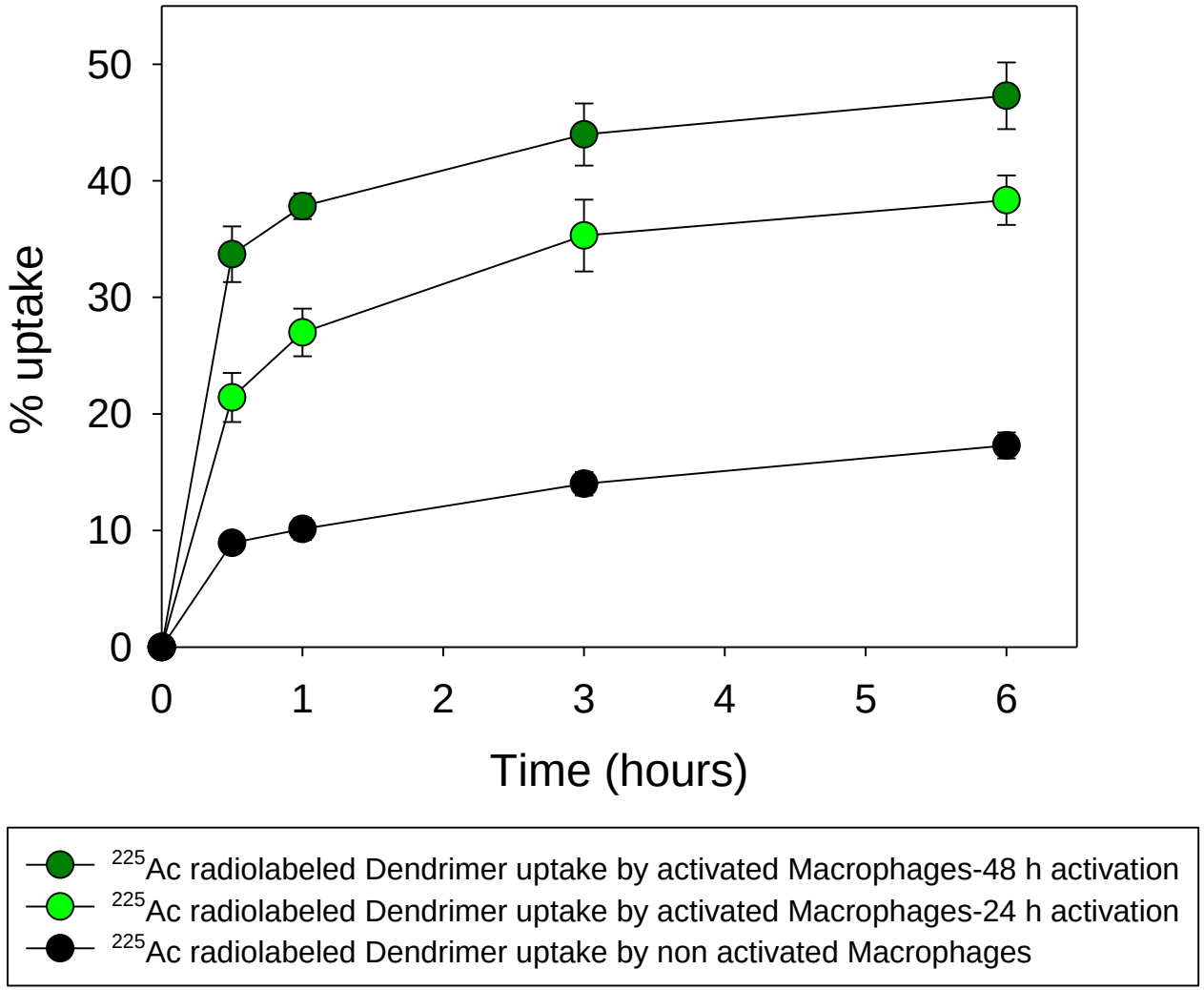
Watase C et al., Cancers (Basel), 2021
Leone JP, Leone JP, *Exp Hematol Oncol*, 2015
Bailleux C et al., Br J Cancer, 2021

Extent of uptake of dendrimer increases with vascularized model

%IA [^{111}In]In-DTPA-dendrimer/g with and without pre-administration of CDDP



Uptake of Dendrimers by M1 activated Macrophages



Cell concentration	**2 x10^6 cells/mL**
Dendrimer Concentration	**10.2 µg/mL**
Dendrimer to Cell Ratio	**50x10^6 : 1**

Data from Mr. Rajiv Nair

Activated by LPS treatment

90µl LPS to 30 ml media (1:1 10%FBS : 0% FBS)

We use 4T1 cell line which is a murine tnbc cell line used as a model to study BCBM.

We performed clonogenic assay wherein we incubated these cells with different radioactivity concentrations, with and without chemotherapy, and seeded them in low densities and allowed for surviving cells to form colonies. We then plotted the % survival as colonies formed divided by cells seeded and accounted for plating efficacy.

On the left

We have profiles of clonogenic assay done with alpha, alpha + chemo , we can see that cell kill increases with addition of chemo. On the right we have the clongenic assay of cisplatin and see the curved profile, and we see that curving on the right in addition with chemo as well