ICTR-PHE 2016
Uniting physics, biology and medicine for better healthcare

ODSH as a countermeasure for radiation induced thrombocytopenia

Stephen Avery, Ph.D.
Assistant Professor of Radiation Oncology

PENN RADIATION ONCOLOGY
Penn Medicine

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NIAID Radiation/Nuclear Medical Countermeasures Development Program

- HHS assigned NIH/NIAID with the responsibility to identify, characterize and develop new medical countermeasure products against radiological and nuclear attacks that may cause a public health emergency.
- Research priority areas of the program are to develop:
  - Drugs to treat or mitigate radiation injury
  - Drugs to remove radioactive materials from the body
  - Biodosimetry tools to determine levels of radiation exposure received by an individual

The need

- **The release of nuclear material at Fukushima raised concerns about US preparedness to treat large-scale exposure of citizens and military personal to ionizing radiation**

- **Post-exposure interventions are needed that can be delivered as late as possible following exposure while still ensuring survivability against the acute effects of ionizing radiation exposure**

The need

- **To date there is no agent with FDA approval for use as a radiation countermeasure:**
  - **G-CSF**: An advisory committee convened in May 2013
  - Based on animal studies such as our proposed studies, recommended 17-1 that leukocyte stimulating factors like G-CSF could be helpful
    - Only affects WBC
    - No effect on platelets or survival
  - **TPO:** has been studied as well but seems to work best if given prior to injury.

Radiation Toxicity

- **Hematopoietic: As low as 0.25 Gy, but may not be felt below 1 Gy**
  - drop in the number of blood cells
    - Infections: low white blood cells; bleeding: low platelets; and anemia: low red blood cells.
    - Conventional trauma and burns resulting from a bomb blast are complicated by the poor wound healing caused by hematopoietic syndrome, increasing mortality
- **Gastrointestinal: 6–30 Gy (600–3000 rad)**
  - Nausea, vomiting, loss of appetite, and abdominal pain
  - Vomiting within first 4 hours is marker for whole body exposures that are in the fatal range above 4 Gy
  - The death is generally due to infection
- **Neurovascular: >30 Gy (3000 rad)**
  - dizziness, headache, or decreased level of consciousness, occurring within minutes to a few hours, and with an absence of vomiting and is invariably fatal

Radiation Toxicity

- **Acute effects of ionizing radiation exposure:**
  - The prodrome (early symptoms) of Acute Radiation Syndrome (ARS): as low as 0.35 Gy (35 rad)
    - nausea and vomiting, headaches, fatigue, fever and short period of skin reddening.
    - These symptoms are common to many illnesses and may not, by themselves, indicate acute radiation sickness
    - Having a low risk therapy that can be administered to individuals easily within 24 hours of exposure that impacts outcomes is important

Radiation-induced Thrombocytopenia (RIT)

- **Condition where you do not have enough platelets in your blood.**
  - Platelets help the blood to clot, which stops bleeding.

- **Patients receiving Chemotherapy or Radiation Therapy can have damage to their platelets which leads to thrombocytopenia.**
  - Patients receiving both are at greater risk

- **Patients whose levels are very low may require a transfusion and/or delay in treatment.**

Our model: RIT

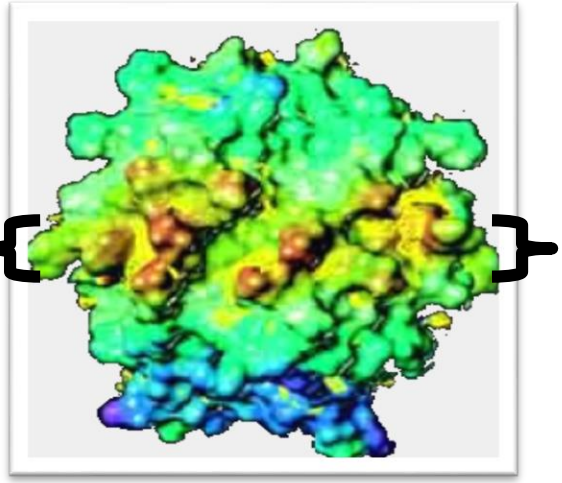
Exploits a series of transgenic and knockout mice with a protein important in megakaryopoiesis:

Platelet Factor 4 (PF4)

Platelet Factor 4

- **Binds avidly to heparin and cell surface heparan sulfates.**
- **Synthesized almost exclusively by megakaryocytes.**
- **Released by megakaryocytes following their injury such as by irradiation .**
- **Negative feedback: inhibits megakaryopoiesis in the setting of bone marrow injury.**
- **Only known protein clinically linked to platelet count.**

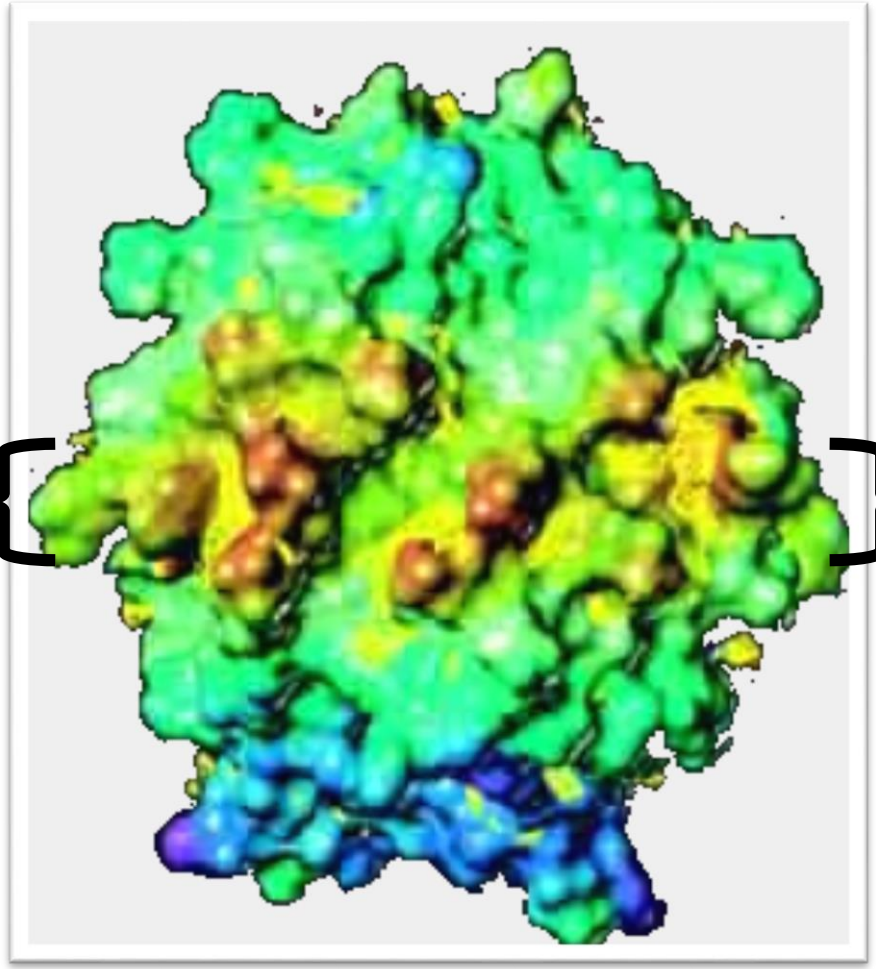
Positively charged ring



Positively charged ring

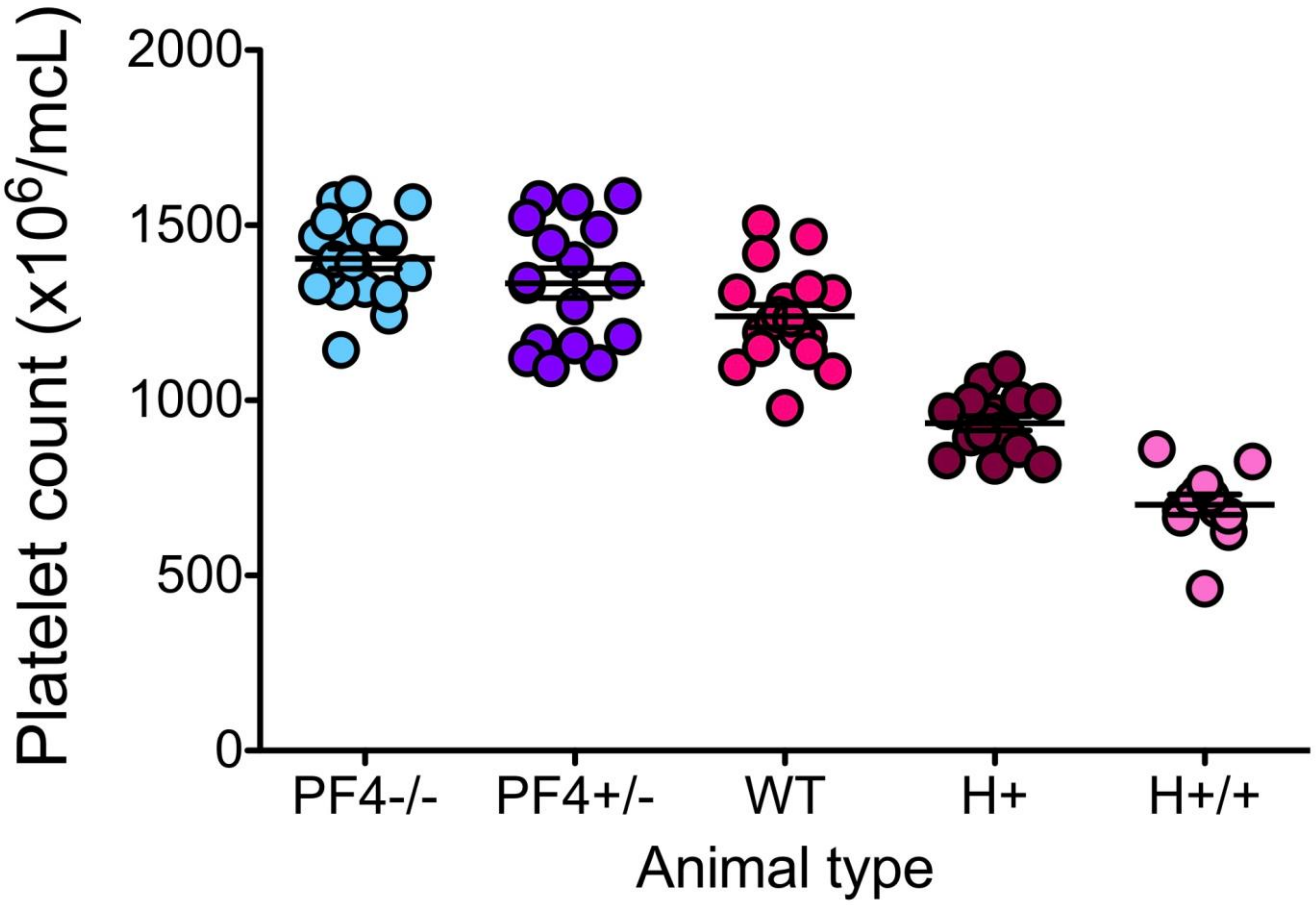
Platelet Factor 4

Positively charged ring



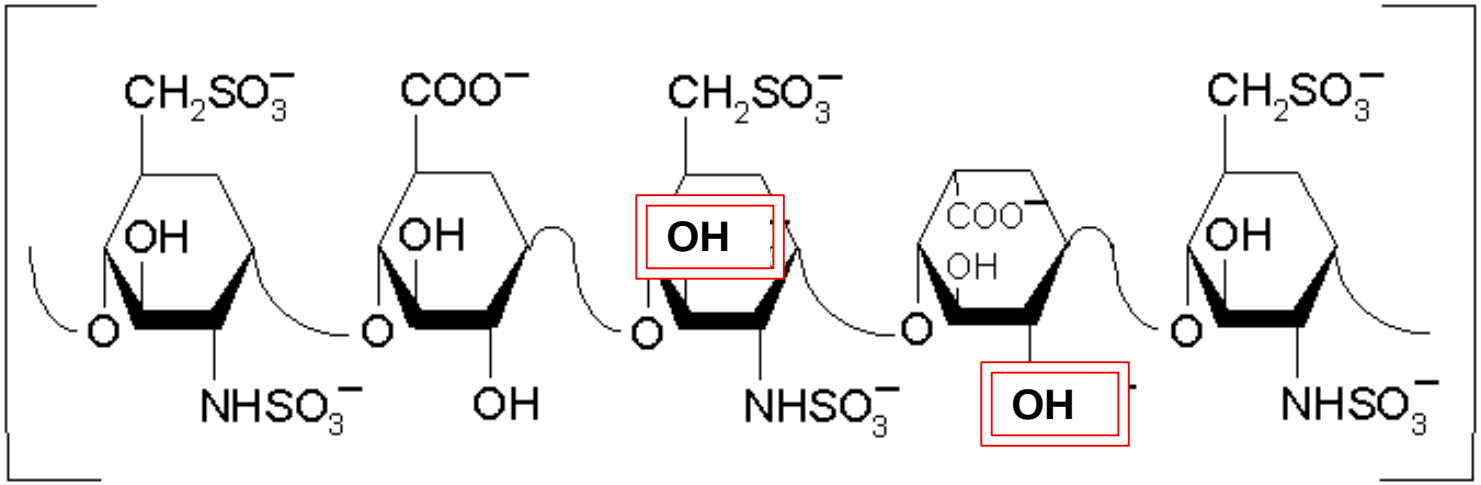
Positively charged ring

Background

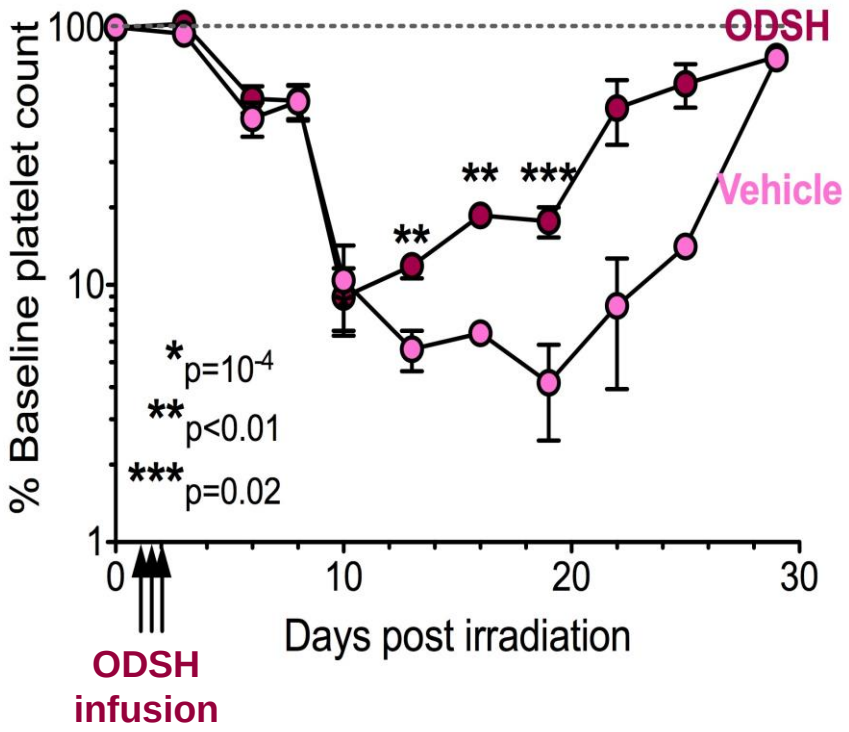

Platelet count (x10^6/mcL)
2000
1500
1000
500
0
PF4-/-
PF4+/-
WT
H+
H+/+
Animal type

2-O, 3-O Desulfated Heparin (ODSH)

- **Modified heparin with significantly reduced anticoagulant activity.**
- **Retains interaction with PF4.**
- **Retains anti-inflammatory properties.**

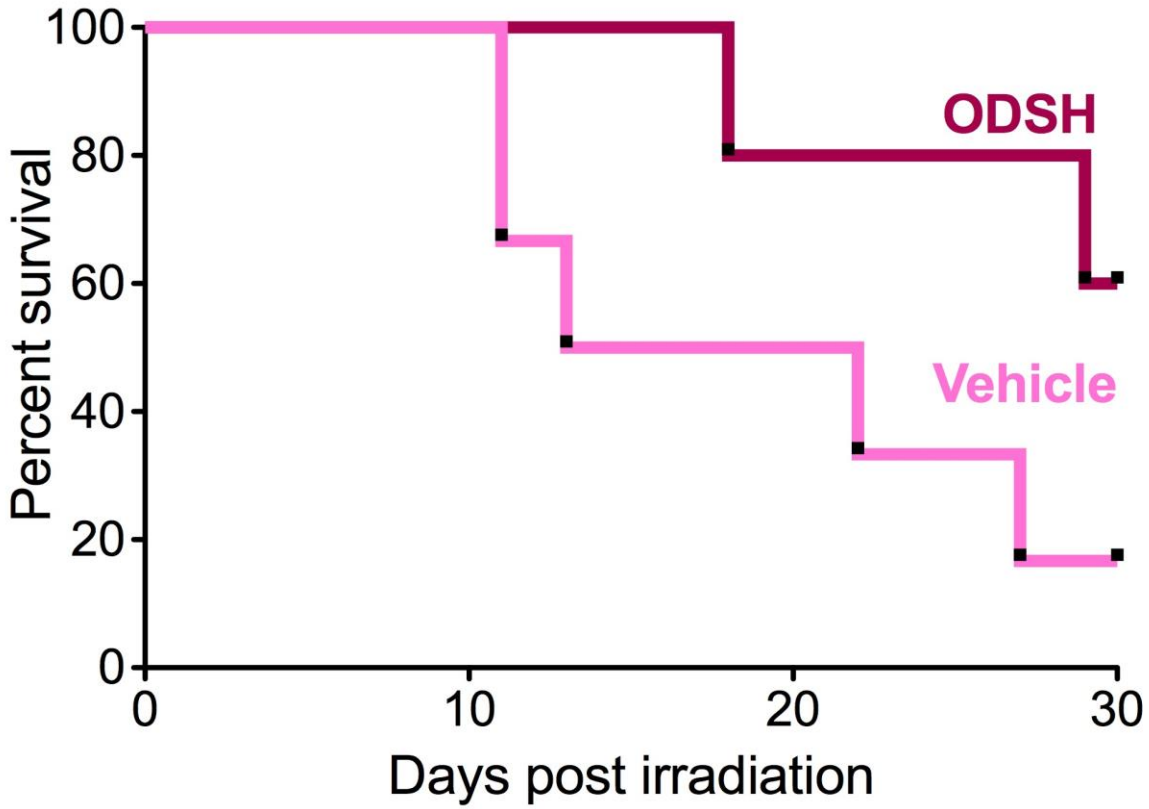


ODSH improves recovery in RIT in C57Bl6 mice over expressing human PF4



n=9 animals per arm

ODSH improves survival in RIT



n=9 animals/arm
p=0.04

This Project Goals

- **Develop a Ca^{2+}-ODSH formulation for use in humans as a radiation countermeasure**
- **Define the optimum dose and schedule for administration in case of significant radiation exposure or suspected ARS**
- **Pre-clinical testing of Ca-ODSH in mice, dogs and rats to allow for FDA IND filing**

Lack of Dosimetry Detail in Radiation Biology Studies

The following lists approximate rate of occurrence of specific information within previous 15 issues of Radiation Research journal:

Animal/Cell type	100%	Dose (rel to water, tissue?)	94%
Animal/Cell strain	100%	Dose Rate (fractionated?)	81%
Irradiator Manufacturer/Model	80%	Location of Detector	20%
Source (nuclide, HVL, filtering)	100%	Dose Reference Location	7%
Radiation Energy	78%	Published Standards/Guides Used	7%
Irradiation Geometry*	48%	Uncertainty in Dose	4%
Dosimetry Method	37%		

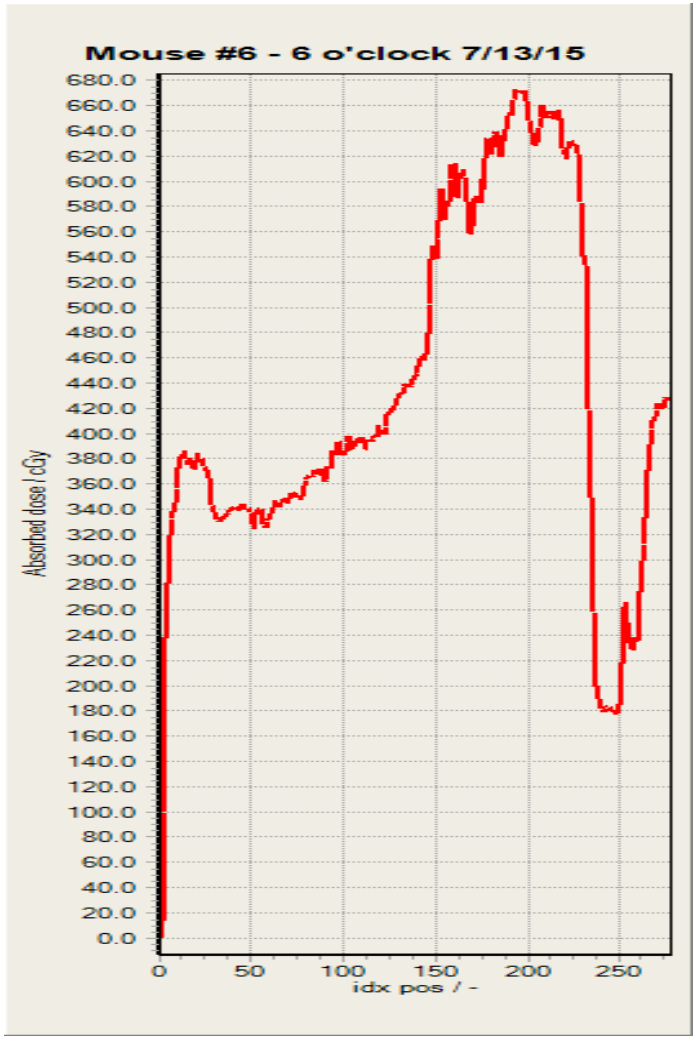
* "TBI" or "WBI" was only given partial credit.

Small Animal Irradiator

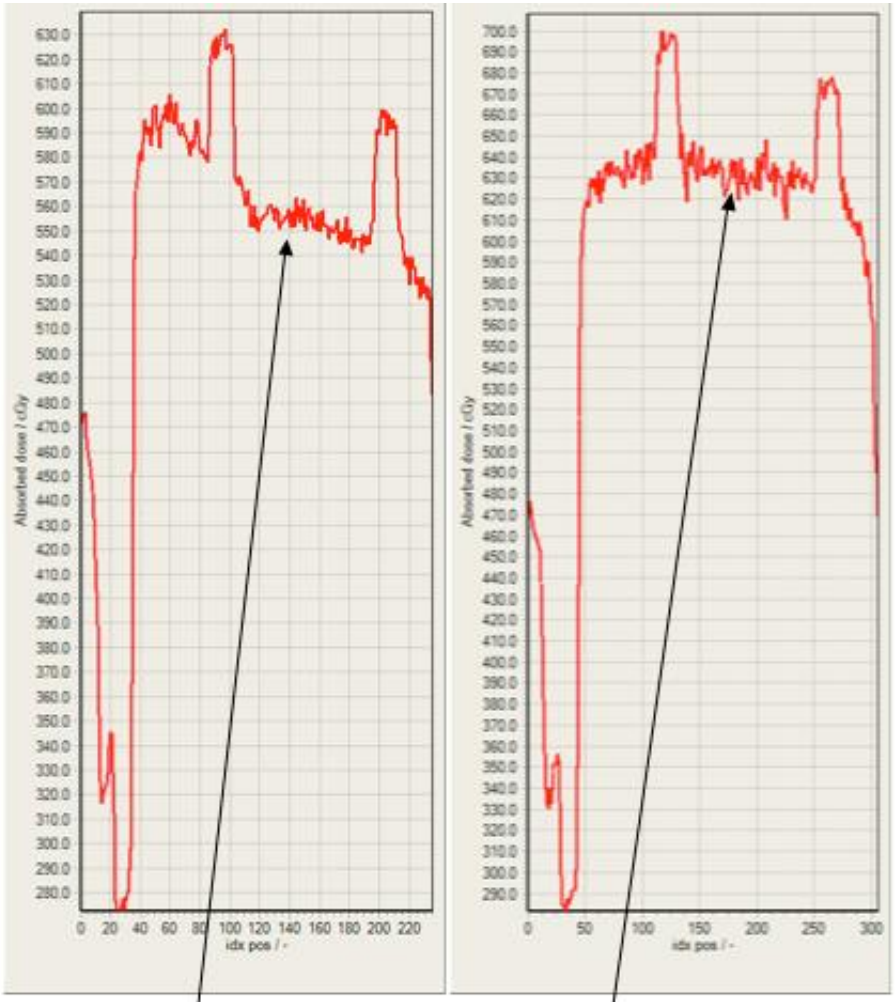
Small Animal Irradiator

- In order to answer whether the uncertainty in dose measurements across the radiation biology community is significant to effect studies, a dosimetric standard should be applied.
- AAPM protocol for 40–300 kV x-ray beam dosimetry in radiotherapy and radiobiology (TG-61) will be used to calibrate irradiator.
- Monthly measurements with be used to monitor dose constancy

FilmQA Pro

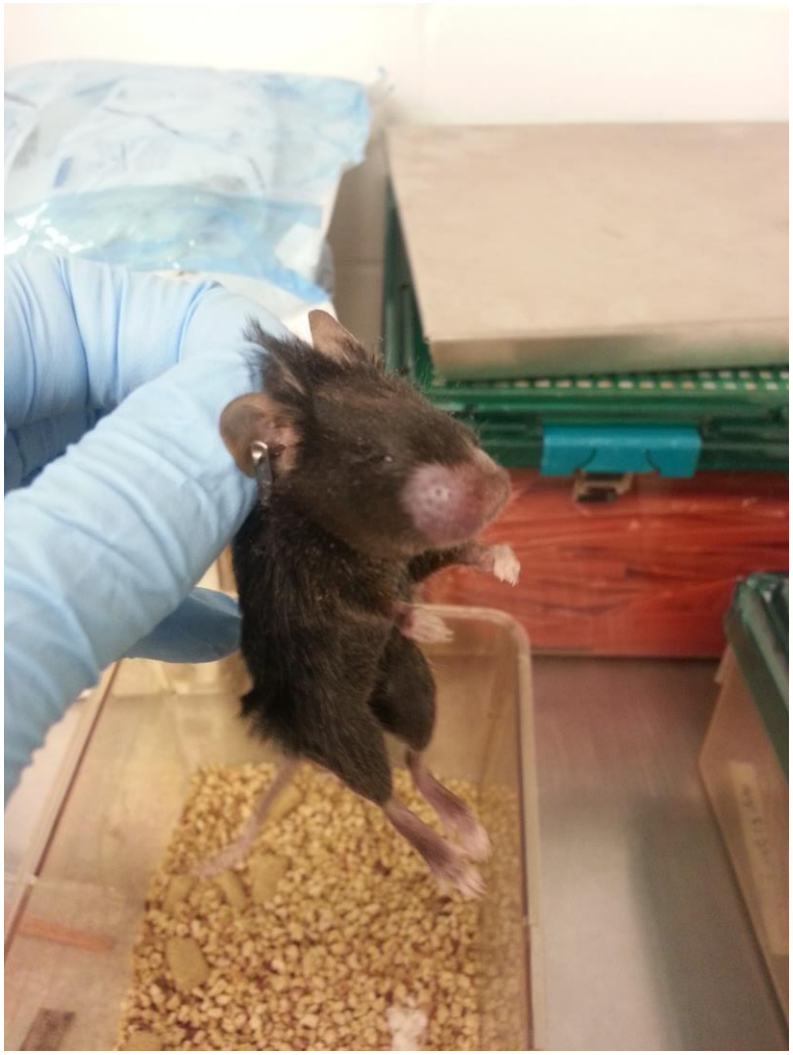

Mouse #6 - 6 o'clock 7/13/15
Absorbed dose / cGy
680.0
660.0
640.0
620.0
600.0
580.0
560.0
540.0
520.0
500.0
480.0
460.0
440.0
420.0
400.0
380.0
360.0
340.0
320.0
300.0
280.0
260.0
240.0
220.0
200.0
180.0
160.0
140.0
120.0
100.0
80.0
60.0
40.0
20.0
0.0
0
50
100
150
200
250
idx pos / -

Hotspots

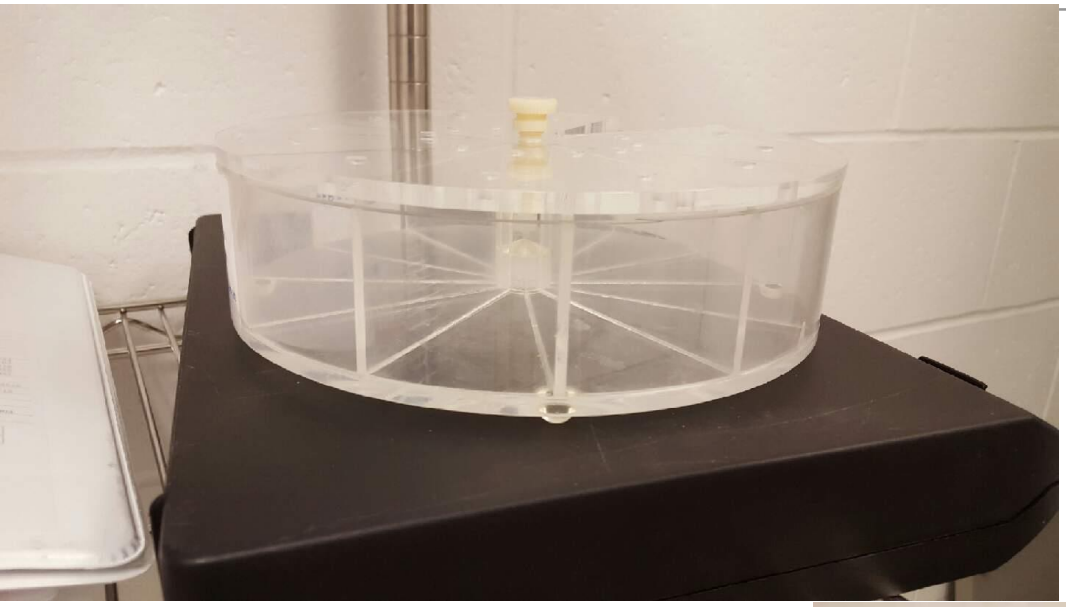


Profile with no mouse shows that the distribution is uniform some areas and not uniform in others. Also see the effect of "hotspots" due to holes in the lid. Certain areas of the mouse are receiving more dose.

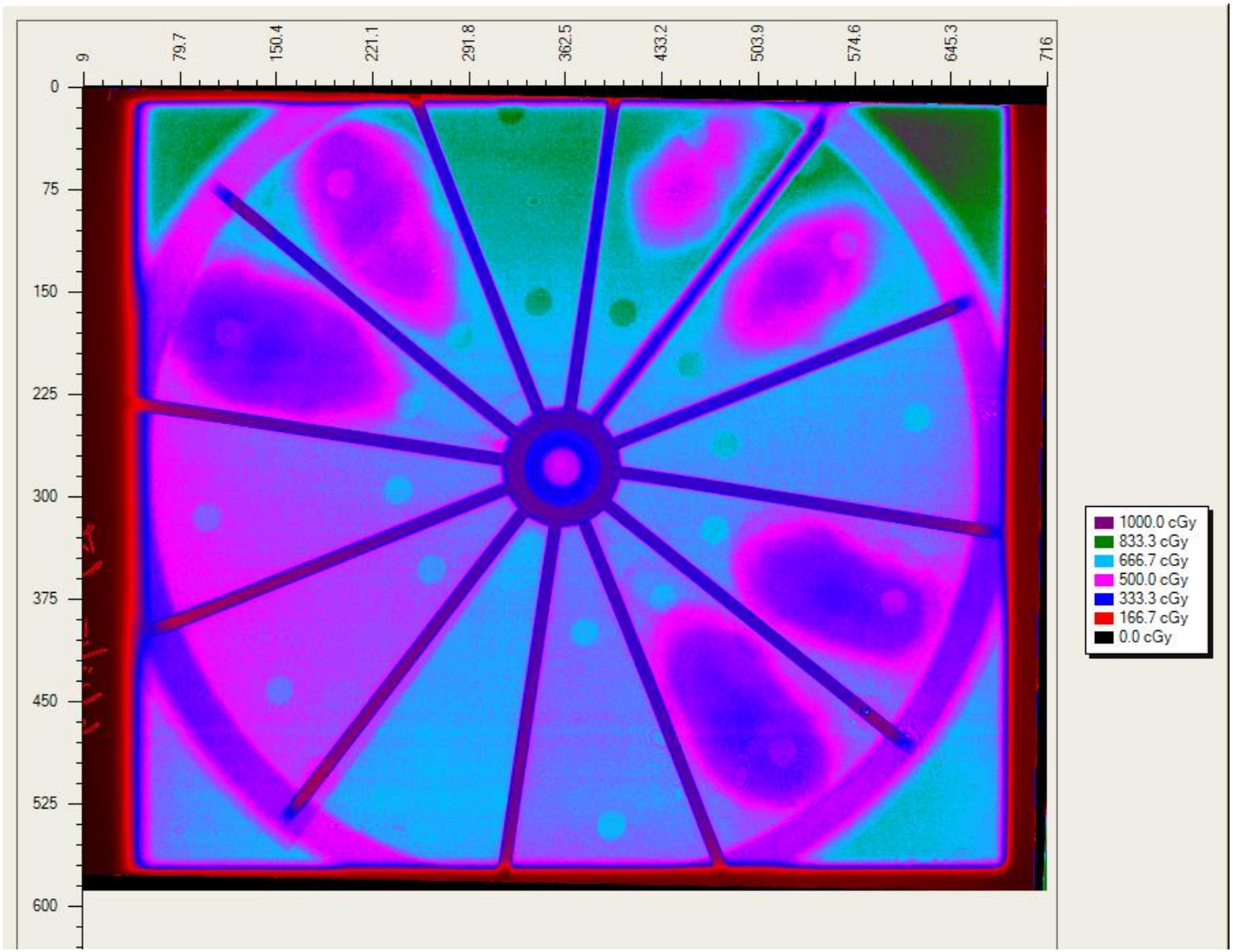
Swollen Muzzle



Modified Container



Dose Map


9
79.7
150.4
221.1
291.8
362.5
433.2
503.9
574.6
645.3
716
0
75
150
225
300
375
450
525
600
1000.0 cGy
833.3 cGy
666.7 cGy
500.0 cGy
333.3 cGy
166.7 cGy
0.0 cGy

Conclusions

- **In murine models of radiation toxicity:**
  - ODSH has shown great promise for decreasing thrombocytopenia.
  - Significant improvement in survival.
- **New formulation of ODSH in development which can be used for clinical trials**
- **Radiation biology experiments should have physics support to assure accuracy of results**

NIAID/NIH Contract

- **Development of Medical Countermeasures to Enhance Platelet Regeneration and Survival following Radiation Exposure from a Radiological/Nuclear Incident.**

- **Principal Investigator: Michele P. Lambert, M.D**

Children's Hospital of Philadelphia (CHOP)
Department of Pediatrics
Division of Hematology/Oncology

Solicitation Number: BAA-NIAID-DAIT-NIHA1203166

Thank You