UNIVERSITY of MARYLAND
SCHOOL OF PHARMACY
MASS SPECTROMETRY CENTER

Biomarker development to assess radiation-induced injury

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Biomarkers: Pathway from Discovery to Validation

Well-Defined Models — *NHP, Mouse*

↓

Discovery Omics — *Metabolomics, lipidomics, proteomics*

↓

Identification of candidate biomarkers — *Radiation injury-responsive*
- *Tissue*
- *Plasma*

↓

Validation of candidate biomarkers

- *Cross species utility*
- *Circulating markers ability to inform on tissue damage*
- *Kinetics: time- and dose-dependency of change in abundance*
- ★ ***Biomarker-Clinical Endpoint relationship: Ability to reflect syndrome and correlation with organ specific injury***

Biomarker discovery, development and multi-omic analysis of IR-induced injury

Mechanisms of Injury, Biomarker Identification and Characterization

Proteomics

Global, label-free proteomic analysis via LC-MS/MS

Huang et al. PMID: 32947488	Plasma, NHP
Huang et al. PMID: 34546219	Lung, NHP
Muller et al. PMID: 34546218	Lymph node, NHP
Zalesak et al. PMID: 34546217	Heart, NHP
Huang et al. PMID: 34546216	Kidney, NHP
Huang et al. PMID: 32947489	GI, NHP
Huang et al. PMID: 30652977	Lung, mouse
Huang et al. PMID: 30624357	GI, mouse
Yu et al. PMID: 34546221	Plasma, lung, heart, jejunum, NHP

Mass Spectrometry Imaging

(spatial metabolomics)

Muller et al. PMID: 34546218	Lymph node, NHP
Carter et. al. PMID: 32665567	Lung, NHP
Carter et al. PMID: 30681424	GI, NHP
Carter et al. PMID: 28871103	Lung, NHP
Carter et al. PMID: 26425906	Lung, NHP, + MCM

Metabolomics

Targeted metabolomic analysis via LC-MS/MS

Jones et al. PMID: 30681425	Plasma, mouse, M/F
Kumar et al. PMID: 34546220	Plasma, NHP
Zalesak et al. PMID: 34546217	Heart, NHP
Jones et al. PMID: 30624349	GI, plasma, mouse
Jones et al. PMID: 28971289	Lung, mouse, + MCM
Muller et al. PMID: 34546218	Lymph node, NHP
Jones et al. PMID: 27557409	Lung, mouse

Biodosimetry & Biomarkers

Biodosimetry utilizes changes induced in the individual by ionizing radiation to:

- estimate the dose

- predict or reflect the clinically relevant response
  - i.e., the biological consequences of the dose

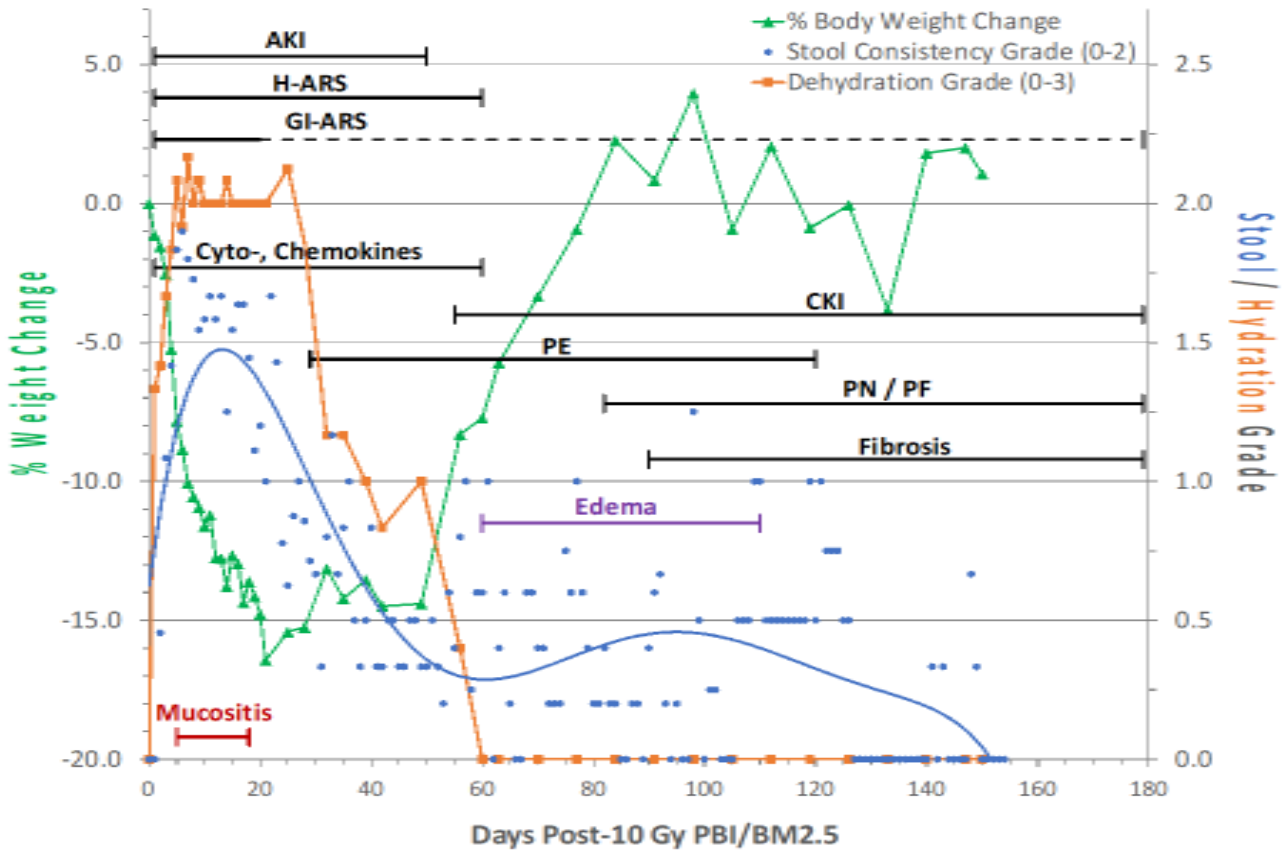
Metabolite/lipid/protein biomarkers

Biological consequences of radiation dose

Radiation injury has acute and delayed sequelae

- ARS: GI-ARS
- DEARE: lung DEARE

- Clinically relevant response
  - Survival
  - Histological assessment of tissue injury



GI-ARS Lung-DEARE

Study design and samples

Model

Non-human primates consisted of male rhesus macaques (Macaca mulatta)

- 10 to 12 Gy of partial body irradiation with either 2.5% or 5% bone marrow sparing (PBI/BM5 or PBI/BM2.5)
- with a peak 6MV linear accelerator (LINAC)-derived photons with an average energy of 2 MV at 0.80 Gy min^{-1}.
- Bone marrow sparing was accomplished with tibiae outside the beam field

Study	Radiation Dose	Exposure	Matrix	ImmPort Accession Number
AXR16	10, 11, 12 Gy	PBI/BM5	Plasma	SDY1997
AXR23	10 Gy	PBI/BM5	Plasma	SDY1854
AXR24	10 Gy	PBI/BM2.5	Plasma	SDY2058
AXR26	12 Gy	PBI/BM2.5	Plasma	SDY2002

https://www.immport.org

Longitudinal plasma samples between **d0 to d180** post-radiation

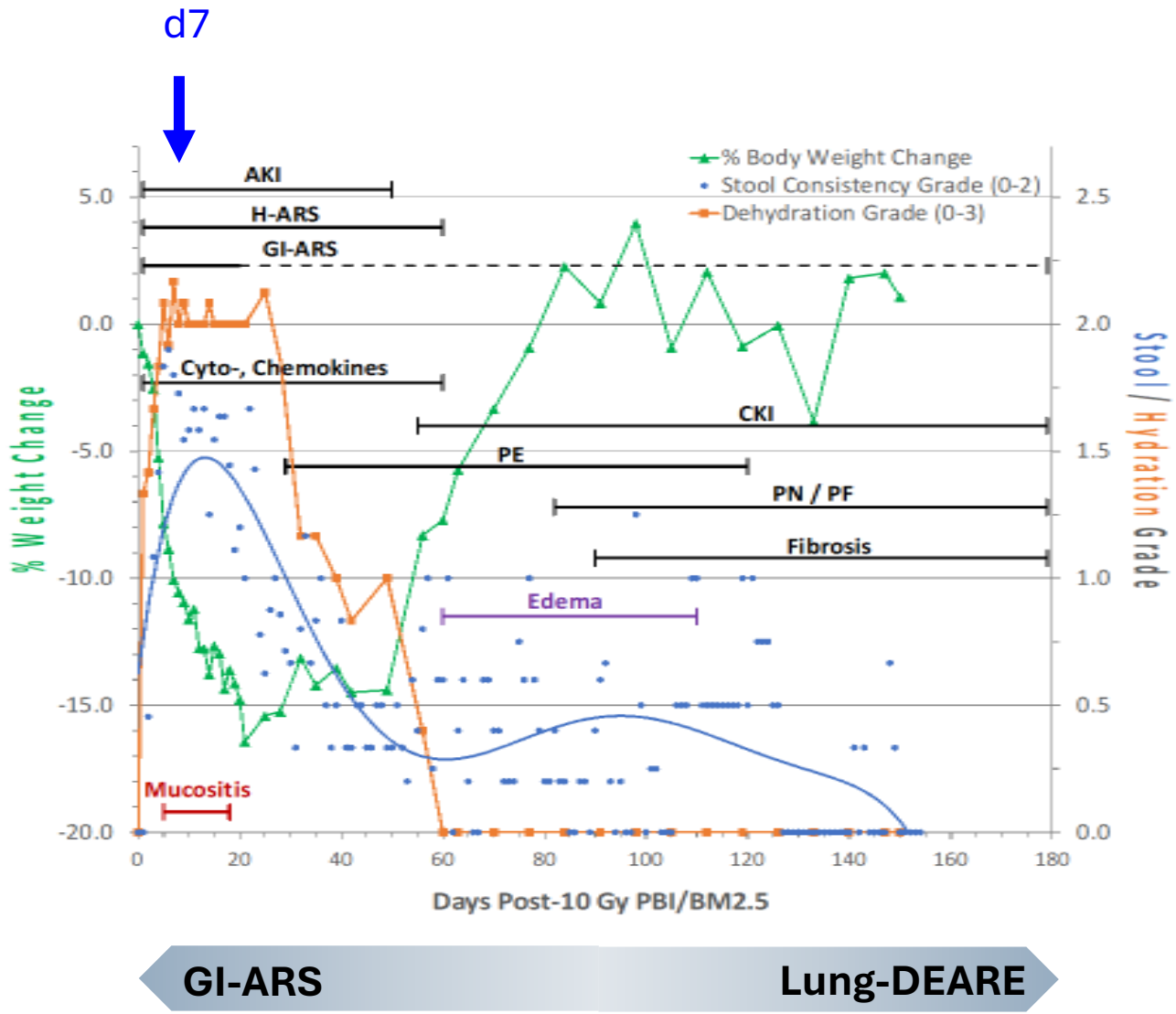
Select time points shown here

Metabolomics

- **LC-MS/MS.** Targeted, quantitative metabolomics was performed using Biocrates AbsoluteIDQ p180, MxP Quant 500, or MxP Quant 500 XL kit (Biocrates, Life Science AG, Innsbruck, Austria).
  - Performed on a ACQUITY UPLC coupled to a TQ-XS or TQ-S (Waters Corporation)
- **Data analysis via:** MetIQ software (Biocrates), MetaboAnalyst 5.0, GraphPad Prism (v 7.03)

Biological consequences of radiation dose

- **ARS: GI-ARS**

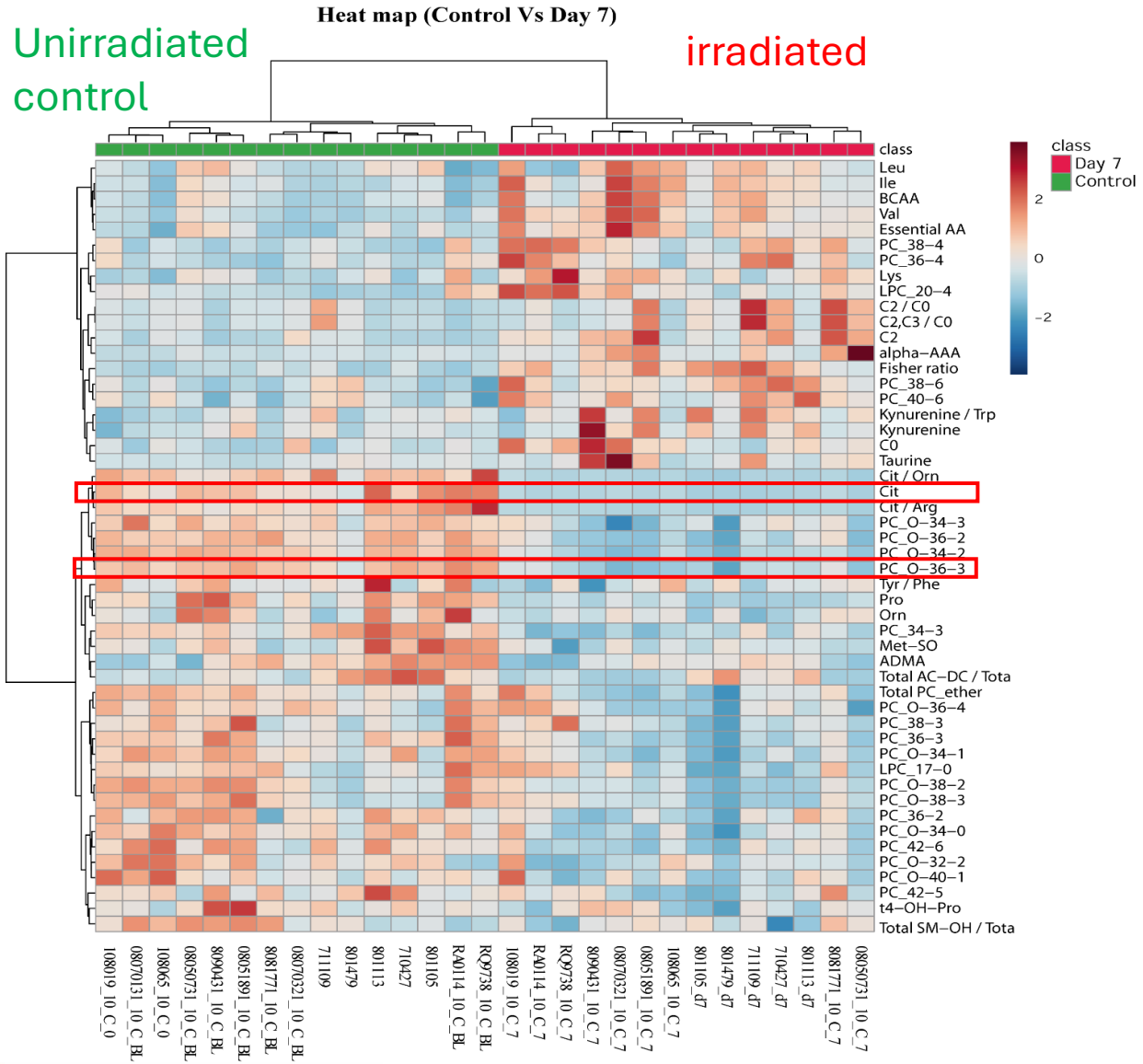


- Clinically relevant response
  - Survival
  - Histological assessment of tissue injury

GI-ARS: Correlation of D7 plasma biomarker candidates with survival

Unirradiated control

irradiated

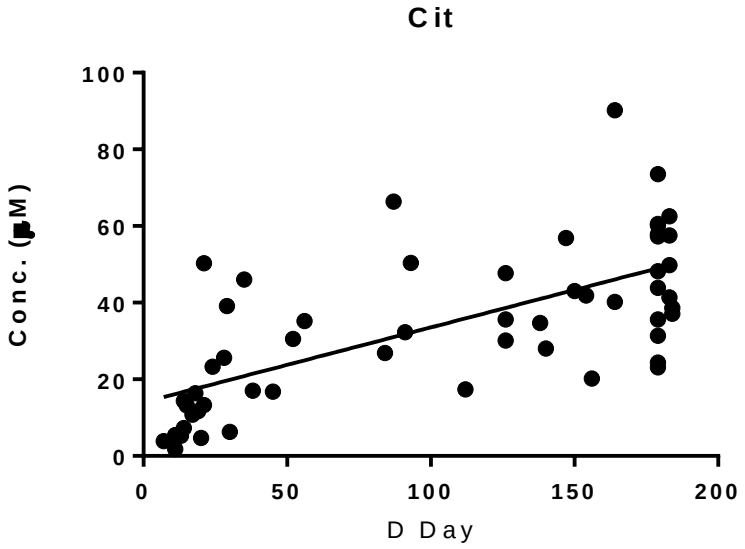


Pearson's correlation (r)

Correlation of GI-ARS candidate plasma biomarkers with survival		
Metabolites	r	p value
PC_36-3	0.73	<0.0001
PC_O-36-3	0.7	<0.0001
Cit	0.68	<0.0001
PC_34-3	0.64	<0.0001
PC_O-36-2	0.61	<0.0001
Cit/Arg	0.59	<0.0001
Cit/Orn	0.59	<0.0001
PC_36-2	0.57	<0.0001
PC_O-32-2	0.52	<0.0001
Tyr/Phe	0.44	0.005
PC_O-38-3	0.36	0.005
PC_42-6	0.36	0.005
PC_O-38-2	0.31	0.01
Met SO4	0.31	0.01
PC_38-6	-0.31	0.01
BCAA	-0.36	0.004
Fisher ratio	-0.53	<0.0001

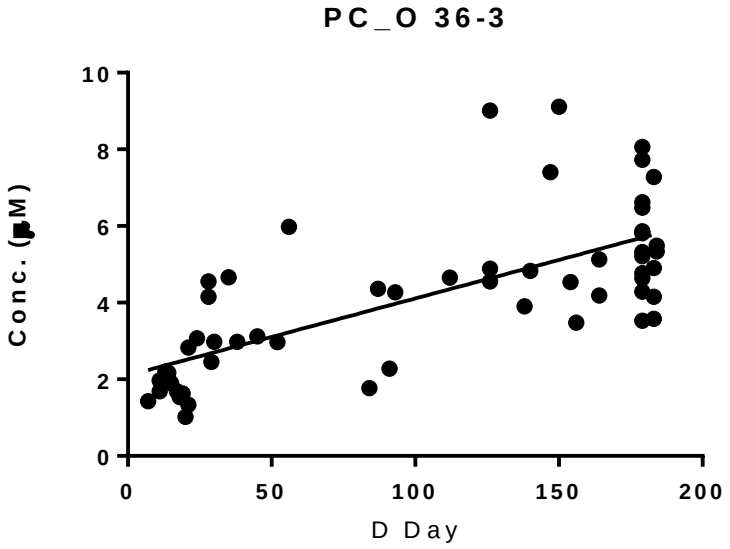
D7 irradiated data is from AXR23, n=5; AXR24, n=10 (n=15 total D7 irradiated).
Unirradiated / naïve, n=14. (10 Gy PBI/BM5 (AXR23) or 10 Gy PBI/BM2.5 (AXR24)).

GI-ARS: Correlation of D7 plasma biomarker candidates with survival



Pearson r	
r	0.6827

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes



Pearson r	
r	0.7096

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes

Correlation of GI-ARS candidate plasma biomarkers with survival

Metabolites	r	p value
PC_36-3	0.73	<0.0001
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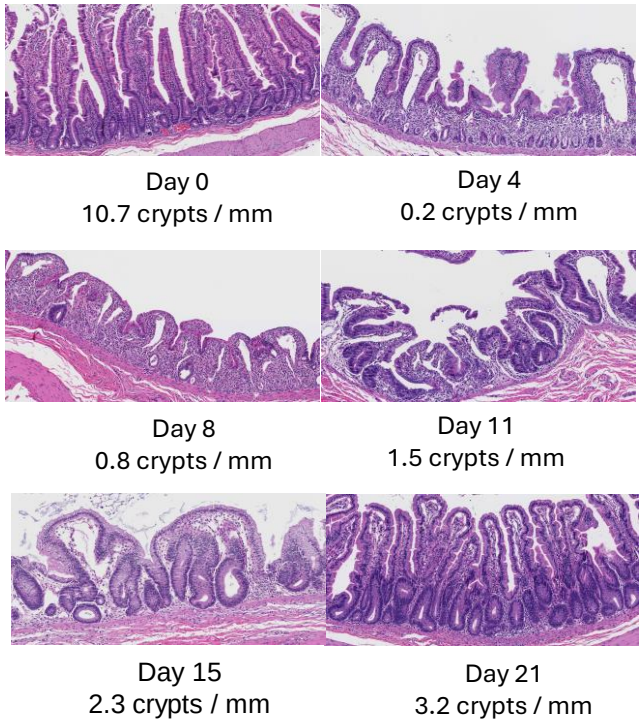
Cit/Arg	0.59	<0.0001
Cit/Orn	0.59	<0.0001
PC_36-2	0.57	<0.0001
PC_O-32-2	0.52	<0.0001
Tyr/Phe	0.44	0.005
PC_O-38-3	0.36	0.005
PC_42-6	0.36	0.005
PC_O-38-2	0.31	0.01
Met SO4	0.31	0.01
PC_38-6	-0.31	0.01
BCAA	-0.36	0.004
Fisher ratio	-0.53	<0.0001

GI-ARS: biomarker correlation with histological scoring of injury severity

Histological Staining: H&E



Day 0
10.7 crypts / mm

Day 4
0.2 crypts / mm

Day 8
0.8 crypts / mm

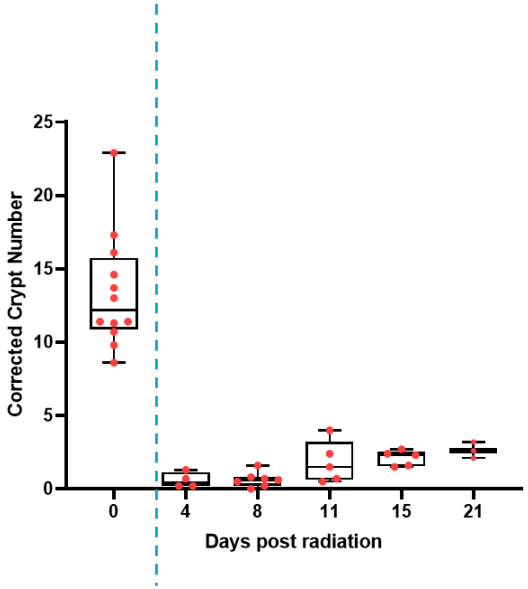
Day 11
1.5 crypts / mm

Day 15
2.3 crypts / mm

Day 21
3.2 crypts / mm

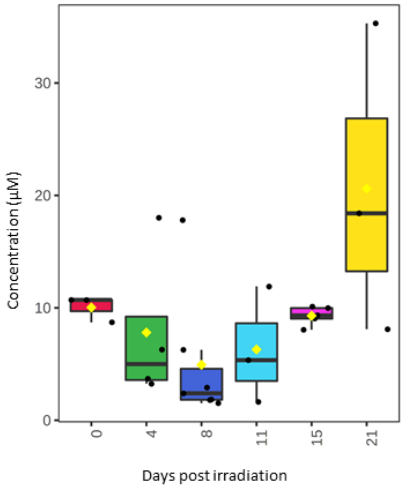
Histological Scoring of Injury Severity

corrected crypt number



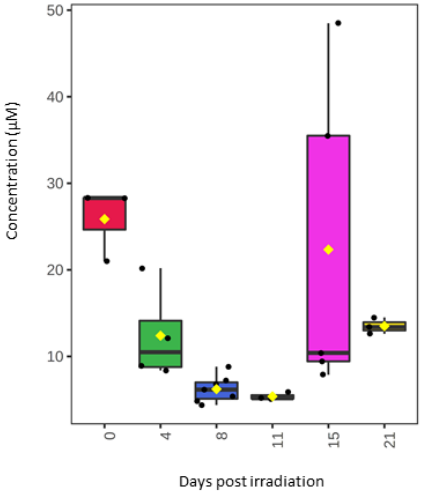
Biomarker Quantification in Injured Tissue

Jejunum Citrulline



Biomarker Quantification in Accessible Biofluid

Plasma Citrulline



Kumar et al. Health Phys. 2020; 119: 594-603. PMID: 32947487

GI-ARS: biomarker correlation with histological scoring of injury severity

A. **Jejunum – CCN correlation**

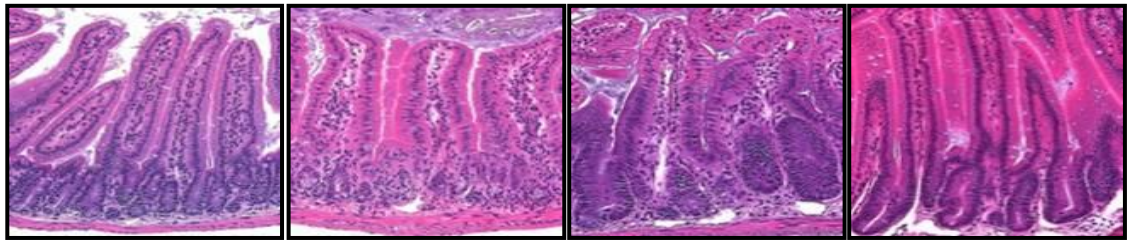
Correlation with corrected crypt number		
Analyte	**R**	**p-value**
PC ae C36:2	0.62	0.0010
PC ae C38:1	0.60	0.0015
PC ae C38:2	0.52	0.0073
PC ae C34:3	0.35	0.0902
PC ae C34:2	0.66	0.0004
Serotonin	0.42	0.0344
Acylcarnitine C18	0.30	0.1451
PC ae C36:3	0.76	<0.0001
Citrulline	0.54	0.0051

B. **Plasma – CCN correlation**

Correlation with corrected crypt number		
Analyte	**R**	**p-value**
PC ae C36:2	0.18	0.3850
PC ae C38:1	0.11	0.6119
PC ae C38:2	0.20	0.3481
PC ae C34:3	0.35	0.0839
PC ae C34:2	0.23	0.2783
Serotonin	-0.07	0.7532
Acylcarnitine C18	0.05	0.7965
PC ae C36:3	0.28	0.1747
Citrulline	0.67	0.0003

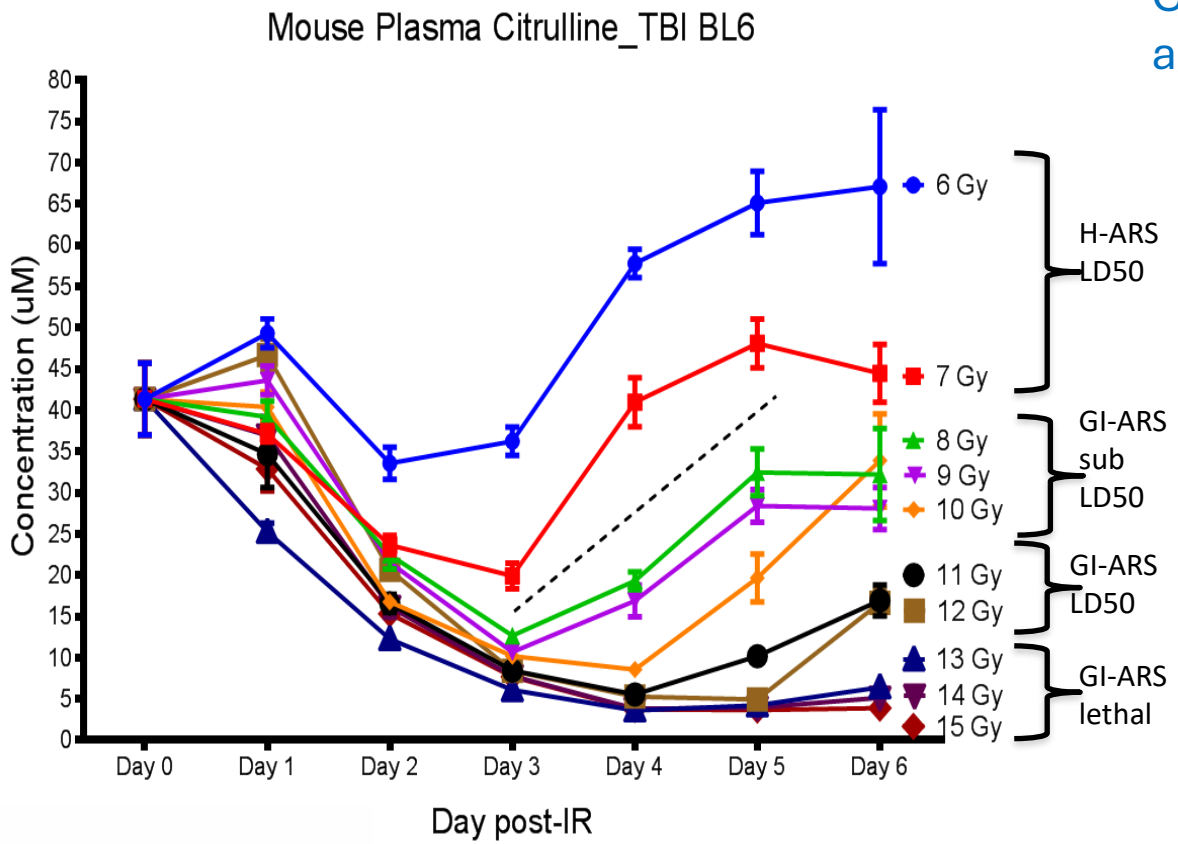
Kumar et al. Health Phys. 2020; 119: 594-603. PMID: 32947487

Cross-species utility: Correlation of biomarker with histological scoring in mouse

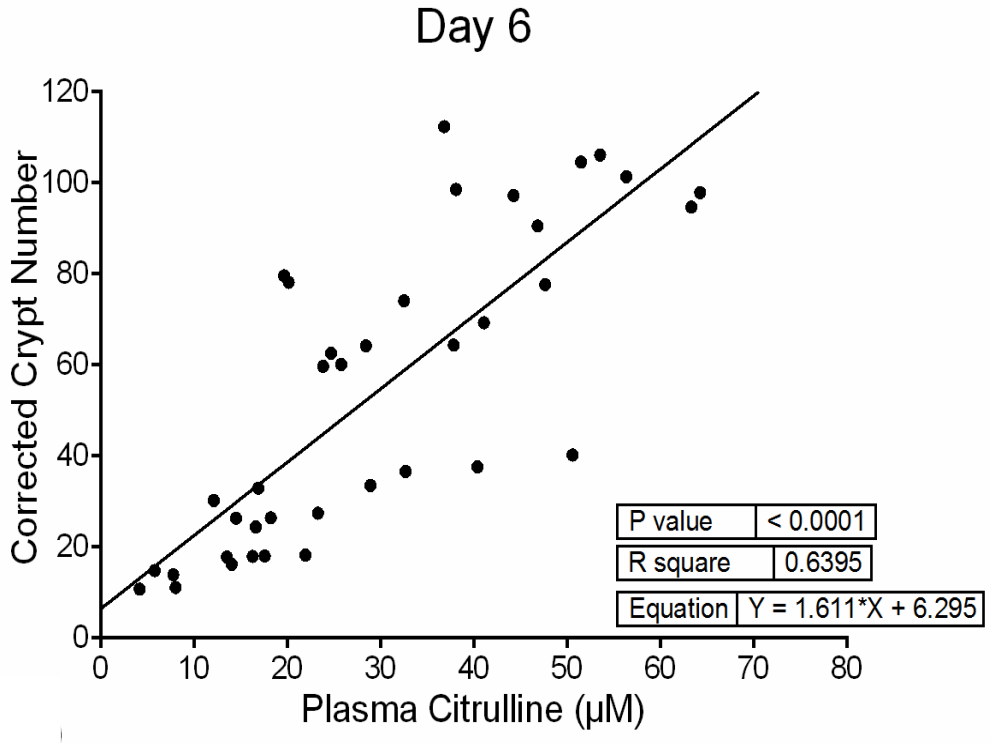


Un-irradiated | Day 2 | Day 4 | Day 6

10GyTBI



Correlation in mouse agrees with results in NHP



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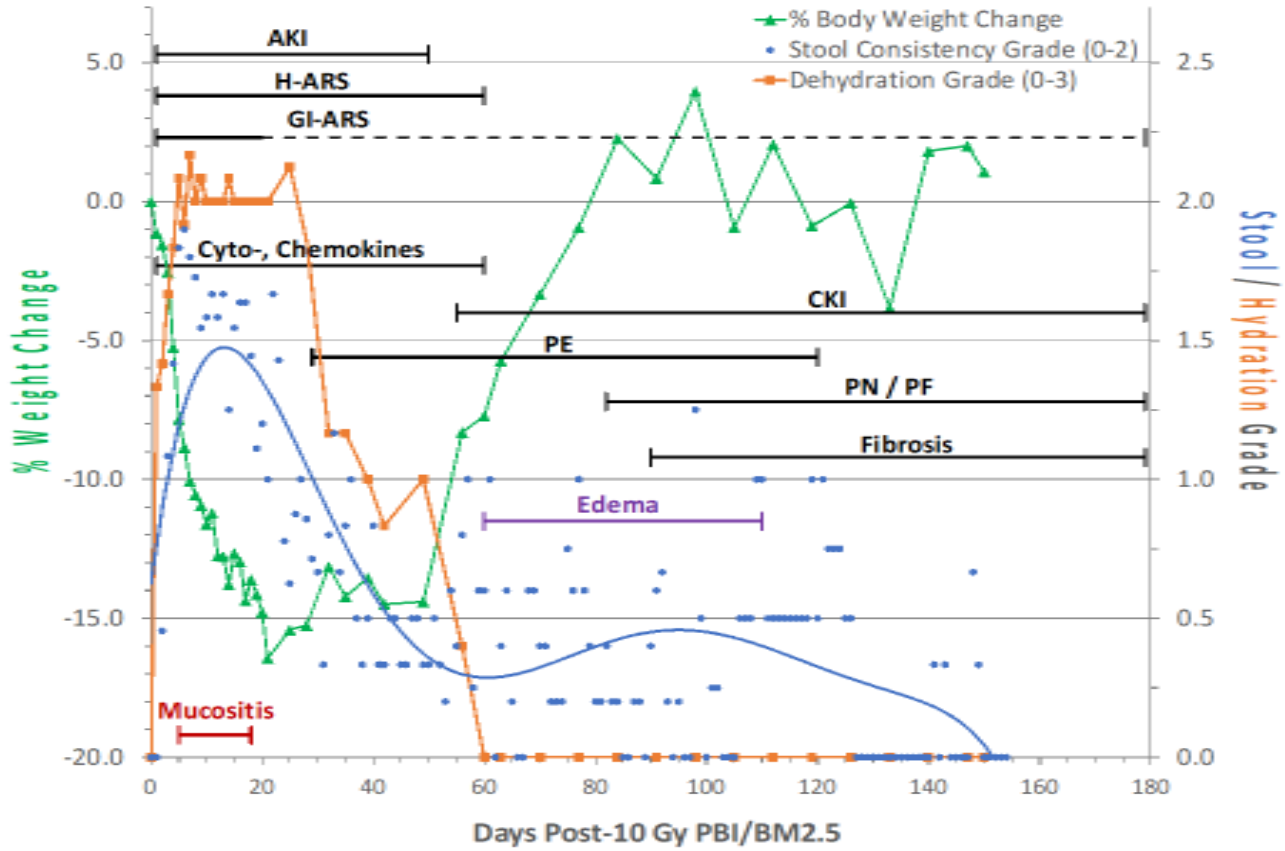
Biological consequences of radiation dose

- **DEARE: lung DEARE**

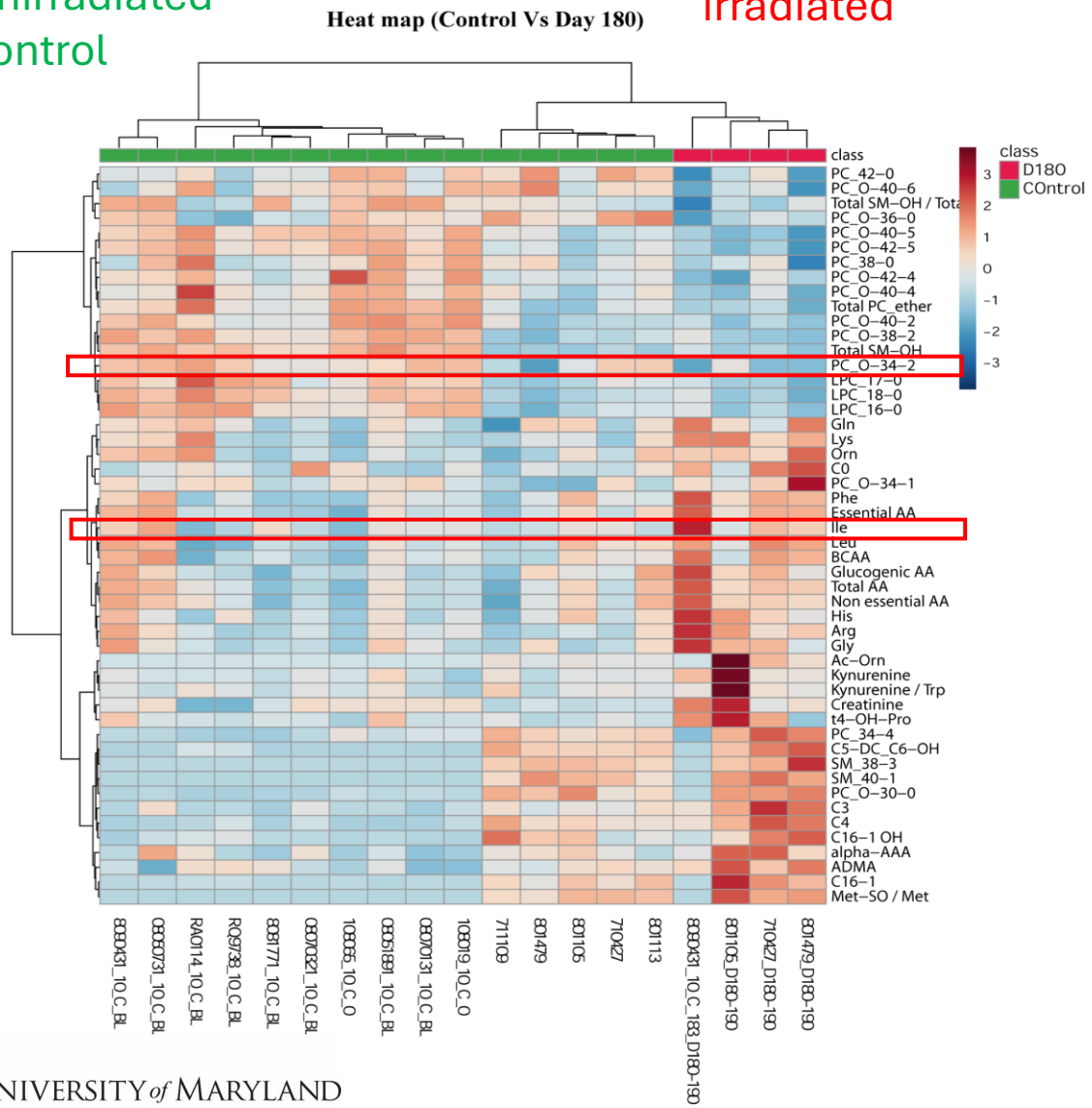
- Clinically relevant response
  - Survival
  - Histological assessment of tissue injury



Lung-DEARE: Correlation of D180 plasma biomarker candidates with survival

Unirradiated control

irradiated

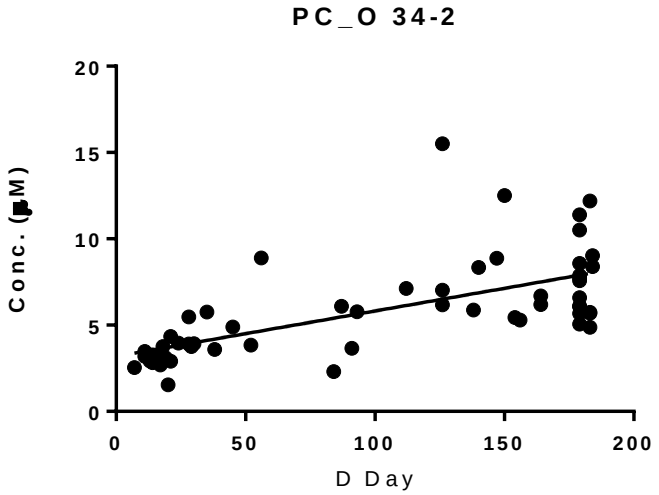


Pearson's correlation (r)

Correlation of lung-DEARE candidate plasma biomarkers with survival		
Metabolites	r	p value
PC_O-34-2	0.65	<0.0001
PC_O-34-3	0.61	<0.0001
PC_42-0	0.39	0.002
PC_O-36-0	0.36	0.004
Gln	0.31	0.01
PC_O-42-5	0.28	0.03
Ile	-0.35	0.006

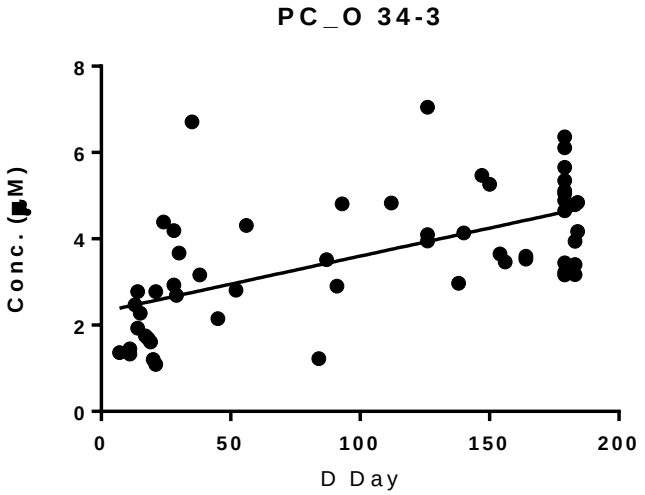
Lung-DEARE: Correlation of D180 plasma biomarker candidates with survival

Correlation of lung-DEARE candidate plasma biomarkers with survival		
Metabolites	r	p value
PC_O-34-2	0.65	<0.0001
PC_O-34-3	0.61	<0.0001
PC_42-0	0.39	0.002
PC_O-36-0	0.36	0.004
Gln	0.31	0.01
PC_O-42-5	0.28	0.03
Ile	-0.35	0.006



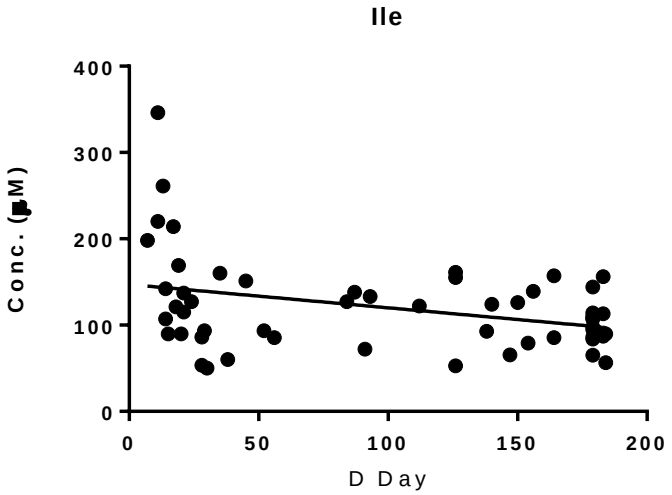
Pearson r	
r	0.6531

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes



Pearson r	
r	0.6147

P value	
P (two-tailed)	< 0.0001
P value summary	****
Significant? (alpha = 0.05)	Yes



Pearson r	
r	-0.3596

P value	
P (two-tailed)	0.0060
P value summary	**
Significant? (alpha = 0.05)	Yes

Lung-DEARE: Correlation of histological scoring of injury severity

Sixteen different histological assessments

Histological Endpoints Assessed in Lung
CTGF-positive staining, alveolus/duct
TGFB-positive staining, pleural
TGFB-positive staining, interstitial
Collagen-1 IHC interstitium;increased staining
Collagen-1 IHC pleural surface;increased staining
MPO IHC- pos. staining
CD206-alveolar macrophages
CD163-alveolar macrophages
CD163-interstitial macrophages
Congestion
Edema, alveolar
Inflammation, interstitial
Infiltration, lymphocytic
Infiltration, neutrophilic
Accumulation, alv macrophages
Trichrome-fibrosis, interstitial

Biomarker	TGFB-positive staining, interstitial	Collagen-1 IHC pleural surface;increased staining	Edema, alveolar	Trichrome-fibrosis, interstitial	Infiltration, neutrophilic
PC_O-34-2	ns	** (r = 0.7997)	* (r - 0.7378)	*(r- -0.5187)	ns
PC_O-34-3	*(r - -0.7712)	* (r - 0.6455)	*(r - 0.7482)	** (r - -0.5846)	ns
Ile	ns	ns	ns	ns	*(r - 0.7986)
PC_O-38-4	* (r - -0.7781)	ns	** (r- 0.7769)	ns	*(r - -0.5286)
PC_38-4	* (r -0.7559)	*(r - 0.6398)	*(r - 0.7615)	ns	*(r - -0.5303)
C0	ns	ns	ns	*(r - 0.7843)	ns
PC_38-6	ns	*(r --0.6818)	ns	*(r - 0.7839)	ns

Conclusions

- Both ARS and DEARE biomarker candidates correlate with survival
- Correlation of histological endpoints shows biomarkers may be useful toward assessment of injury severity
- Correlations with clinically relevant endpoints
  - Inform on triage decisions
  - Allow for stratification into treatment groups
  - Inform treatment decisions

Acknowledgements

Funding

- NIH/NIAID: 75N93020D00011 (SRI International, Chang (PI))
  - subcontract number: #62395 (Kane (PI))
- NIH/NIAID: HHSN272201500013I (SRI International, Chang (PI))
- NIH/NIAID: HHSN272201000046C (UMB, MacVittie (PI))
- University of Maryland School of Pharmacy Mass Spectrometry Center (SOP1841-IQB2014)

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CRL
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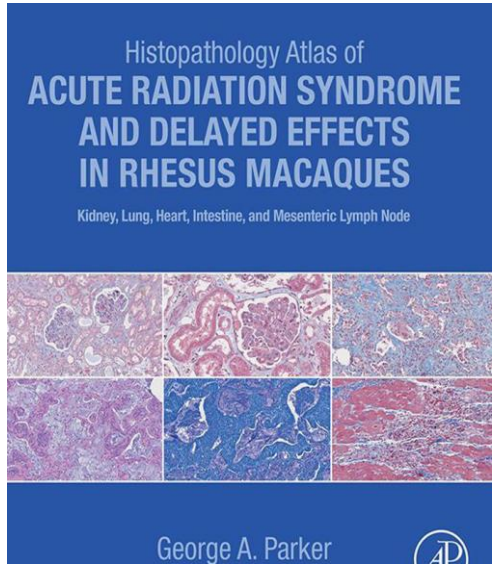

ISBN: 978-0-323-91393-5.