Cardiovascular responses to simulated spaceflight: molecular signatures and surrogate outputs to measure CVD risk

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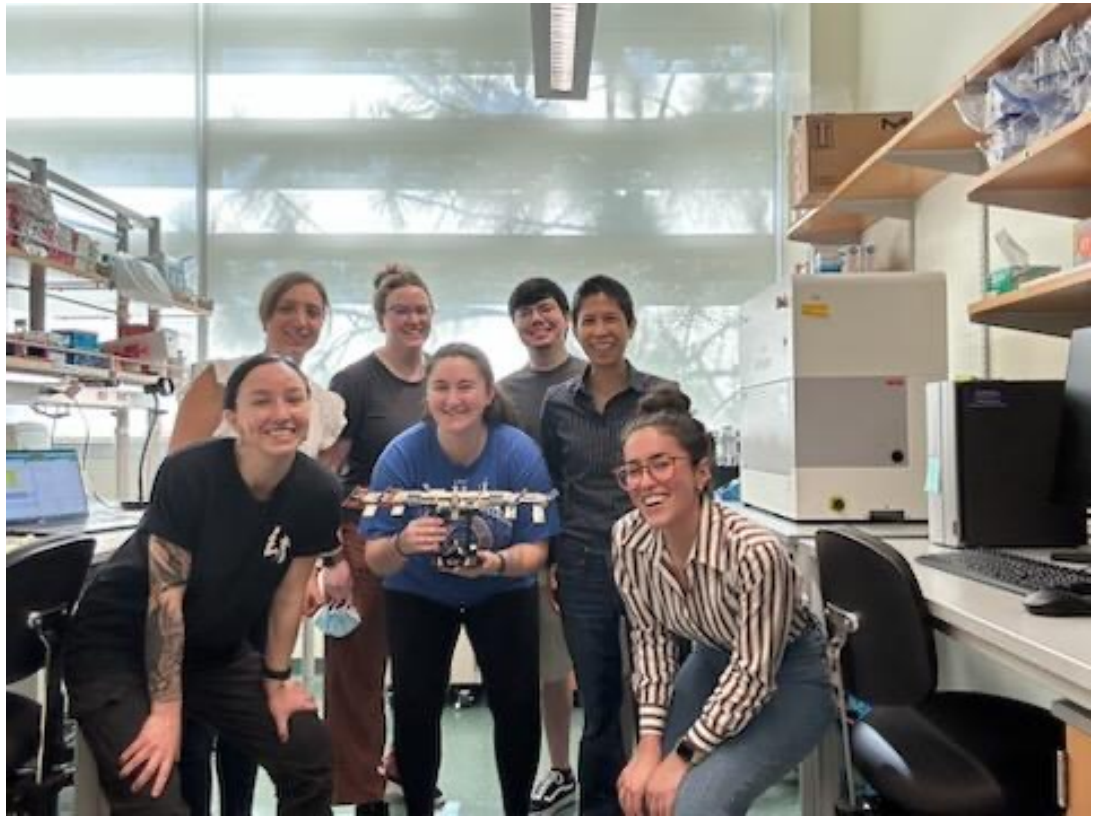


Spaceflight-induced cardiovascular changes

- Increased left atrial volume after 6 months in ISS (Khine et al. 2018)
- Reduced left ventricular mass 10 days after STS-55 mission (Perhonen et al. 2001)

In some astronauts returning from ~6 month stay in ISS
38 H post-flight vs preflight

- 17–30% increase in arterial stiffness (Hughson et al. 2016)
- Aging of arteries by 10-15 years after 6 months of spaceflight

- Abnormal retrograde blood flow in jugular vein (6/11 astronauts) at day 50 and 150 in flight
- Thrombus formation in 1 astronaut which resolved using anticoagulants

Marshall-Goebel et al 2019

- Appears to be transient (resolves after re-adaptation to Earth)

Cardiovascular changes in rodent models of simulated space radiation exposure

Latent effects in animals exposed to lower doses (3 – 50 cGy) of simulated space radiation

- Decline in select measures of cardiovascular function, increased oxidative damage in heart at 1-3 months post exposure (Yan et al. 2014)
- In hearts, altered expression of genes linked to cardiovascular and degenerative diseases at 16 months post exposure (Garikipati et al. 2021)

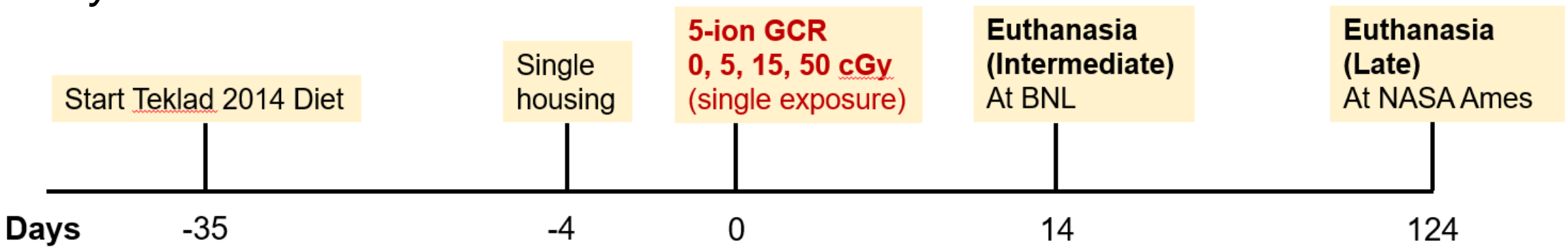
Goal: Gain insight on the effects of simulated space radiation singly or in combination with microgravity on the cardiovascular system
→ Inform on risks and countermeasures for deep space missions

Hypotheses

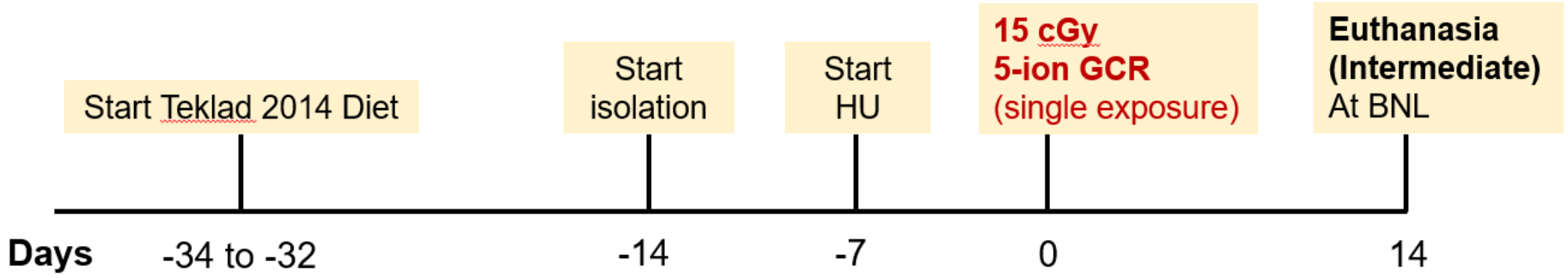
- Space radiation alone or with simulated microgravity
  → changes in CVD markers, structure, transcriptome, and cytokine milieu of heart and vasculature
    → decreased CV performance, aging and increased CVD risk
- Correlation between measures of cardiovascular and systemic immune signatures
  → Less invasive measures of cardiovascular health
    → informative in extrapolating cardiovascular health and performance

Experiment design

Study 1



Study 2



Genes linked to CVD progression are differentially expressed in females vs males (0 cGy groups) at 124 days post-GCR exposure

Disease	FDR	No. of DEGs	DEGs
Chronic heart failure	0.007	10	PENK, LEPR, ADIPOQ, TIMP4, LOX, CD34, NFKB1, VEGFD, DKK3, SOD2
Congestive heart failure	0.018	26	CYP1A1, PDK4, PENK, ACTB, XIST, LEPR, AKAP12, SULT1E1, FZD4, PTN, CCN5, ADIPOQ, TIMP4, LOX, SPON1, CD34, CNTN5, NFKB1, PAPPA, HADH, HSD11B1, LUM, VEGFD, DKK3, SOD2, CA3
Coronary Artery Disease	0.039	23	CYP1A1, MGAM, ACTB, KDM5D, GDF10, LEPR, SULT1E1, SERPINE2, ADIPOQ, TIMP4, EGR3, LOX, CD34, NFKB1, PRKAB2, COL14A1, PAPPA, CCL11, HSD11B1, LUM, CXCL5, DKK3, SOD2
Hypertensive disease	0.041	27	CYP1A1, MGAM, ACTB, LEPR, SULT1E1, FZD4, MEST, ADIPOQ, EFEMP1, CEP20, SLC24A2, AMOT, LOX, CD34, CNTN5, NFKB1, KCNN3, FGF10, CCL11, BMPER, HSD11B1, CXCL5, GSTO1, WNK3, PRNP, MX1, SOD2
Dilated cardiomyopathy	0.045	9	PDK4, ACTB, ANO5, ADIPOQ, TIMP4, NFKB1, KCNN3, FBXO32, SOD2

RNAseq analysis of hearts (left ventricle)
Thresholds: FRD<0.05; log2FC<-0.25 or >0.25. N=9/group

GCR-dependent changes in the heart transcriptome at 124 days post-GCR exposure

Adamtsl3: KO mice develop cardiac dysfunction and dilatation after pressure overload

CD81: extracellular vesicle marker; EVs play a role in cardiac Ca^{2+} cycling and disease

Group	Symbol	Gene name	Log2 FC
F 15 cGy	Zfp236	zinc finger protein 236	1.42
	Septin2	septin 2	1.26
	Afg3l2	AFG3-like AAA ATPase 2	-0.27
	Cd81	CD81 antigen	-0.35
	Capzb	capping protein (actin filament) muscle Z-line, beta	-0.45
	Adamtsl3	ADAMTS-like 3	-0.50
	Zc3h7b	zinc finger CCCH type containing 7B	-0.97
F 50 cGy	Rab26os	RAB26, member RAS oncogene family, opposite strand	1.74
	Adamtsl3	ADAMTS-like 3	-0.49
	Zc3h7b	zinc finger CCCH type containing 7B	-0.79
	Ccdc152	coiled-coil domain containing 152	-1.98
M 15 cGy	Pdzd9	PDZ domain containing 9	-2.13
M 50 cGy	Surf4	surfeit gene 4	-0.30
	Cd81	CD81 antigen	-0.31
	Maea	macrophage erythroblast attacher	-0.38
	Wdr60	WD repeat domain 60	-1.17
	Polr3h	polymerase (RNA) III (DNA directed) polypeptide H	-1.76
	Pdzd9	PDZ domain containing 9	-2.52

RNAseq analysis of hearts (left ventricle)
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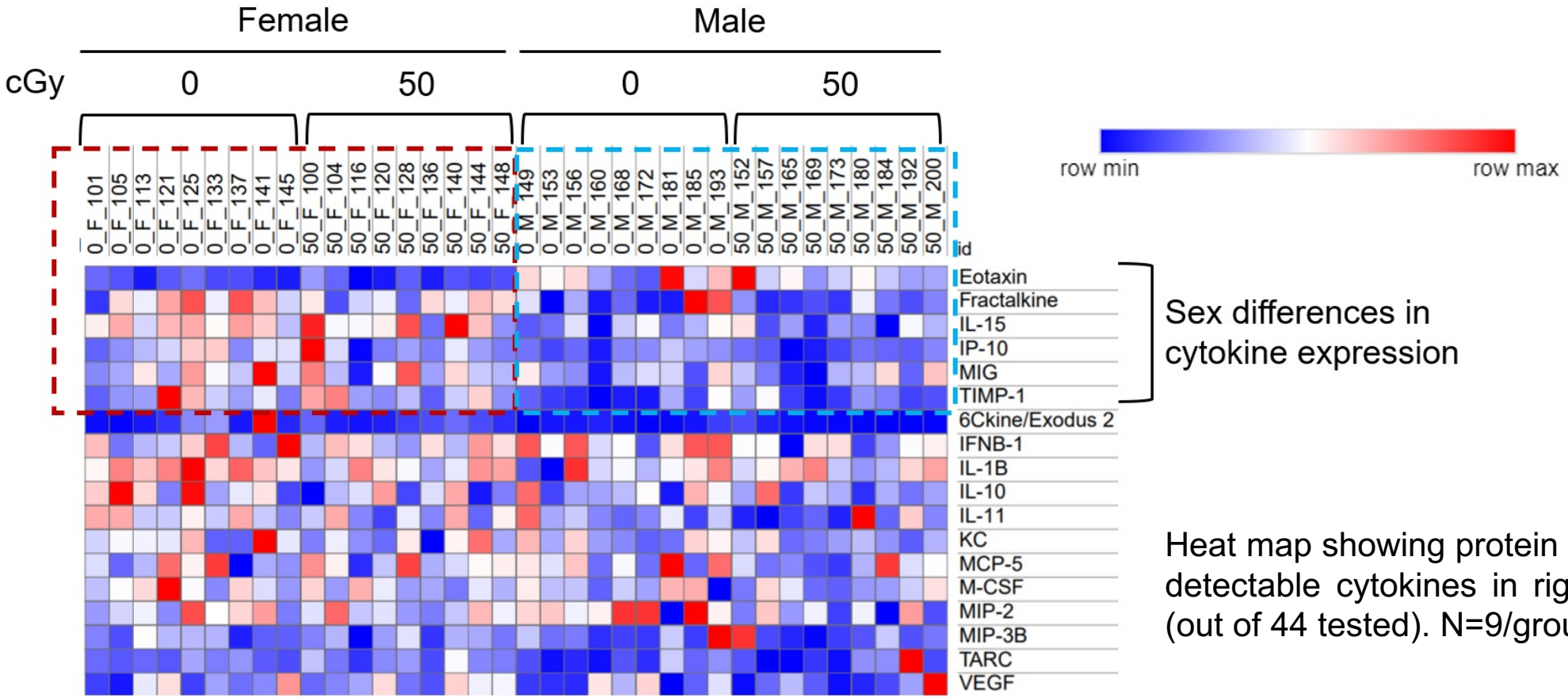
Sex differences in immune-modulating cytokines in the heart

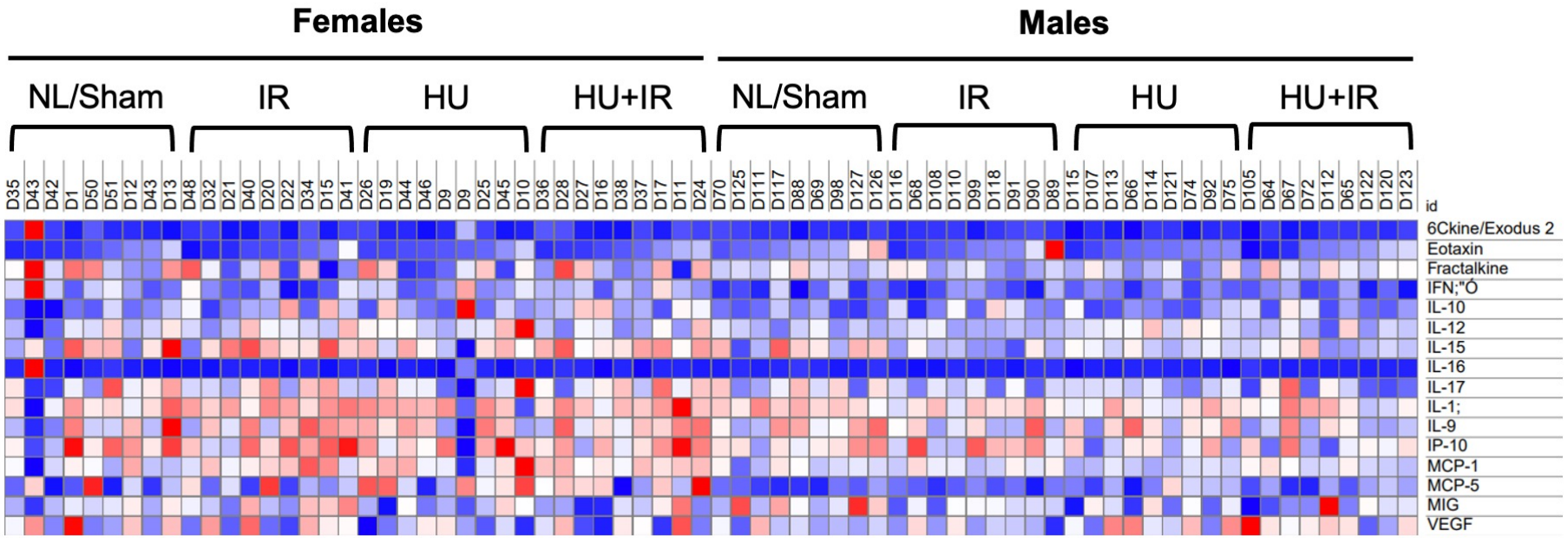
No differences between 0 vs 50 cGy groups at 124 days post-GCR exposure



Sex differences in cytokine expression

Heat map showing protein levels of 18 detectable cytokines in right ventricle (out of 44 tested). N=9/group.

HU and GCR exposure did not alter protein cytokine expression, no combined effects at 14 days post-GCR exposure



Heat map showing protein levels of 16 detectable cytokines in right ventricle (out of 44 tested)

Legend:

NL/Sham: normally loaded, sham controls

IR: 15 cGy GCR

HU: Hindlimb unloaded

Correlation analysis: plasma vs heart cytokines at 124 days post-GCR

Plasma vs heart cytokines

- Female, 0 cGy
- Female, 50 cGy
- Male, 0 cGy
- Male, 50 cGy

Spearman correlation at $p<0.05$

Representative results: Female, 50 cGy

Plasma Cytokine	Heart Cytokine	Spearman Correlation
IL-10	TIMP-1	0.85
IL-1B	IL-15	0.7848
IP-10	IP-10	0.7628
IL-1B	TARC	0.7563
IL-1B	MIG	0.7479
IL-10	Eotaxin	0.7167
IL-9	IL-11	0.7143
MIP-1B	VEGF	0.6891
IP-10	Eotaxin	0.6833
IL-10	IL-11	0.6807
IL-10	IP-10	0.6781
IP-10	IL-11	0.6723
IL-9	MCP-5	0.6667
MCP-5	VEGF	-0.6833
IL-16	6Ckine/Exodus 2	-0.8452
IL-16	Fractalkine	-0.7983
TARC	IL-1B	-0.7833
Fractalkine	VEGF	-0.7833
IL-1B	IFNB-1	-0.7394
IP-10	M-CSF	-0.7

Correlation analysis: plasma vs heart cytokines at 124 days post-GCR

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Spearman correlation at $p<0.05$

Generate pairwise interaction networks

Representative results: Female, 50 cGy

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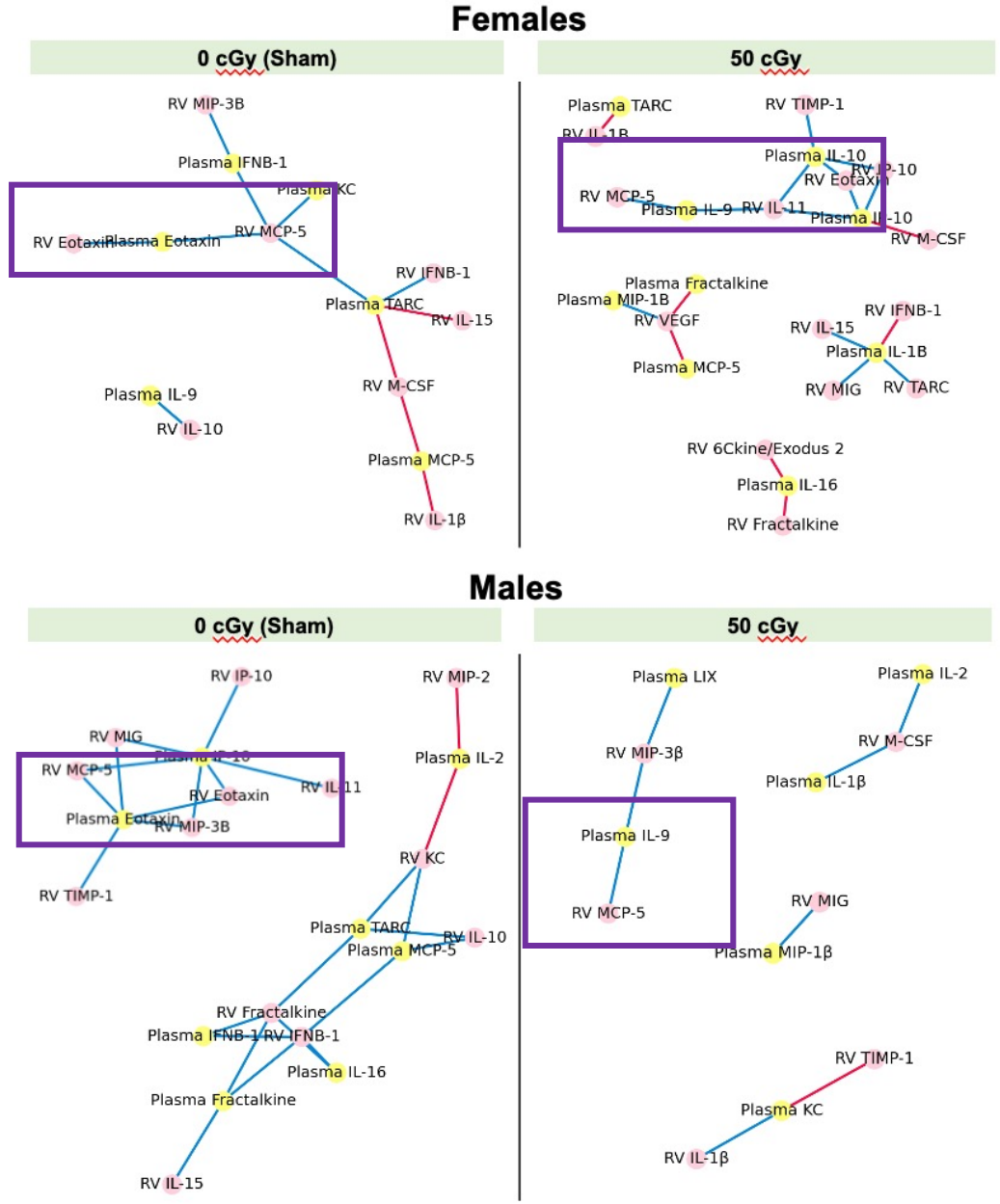
GCR exposure leads to long-term alterations in correlations between plasma and heart cytokine protein levels

Pairwise interactions networks between plasma (PL) and heart cytokines (RV) at 124 days post-GCR exposure

GCR exposure leads to long-term alterations in cytokine-cytokine interactions and regulation in a sex-dependent manner.



Positive correlation

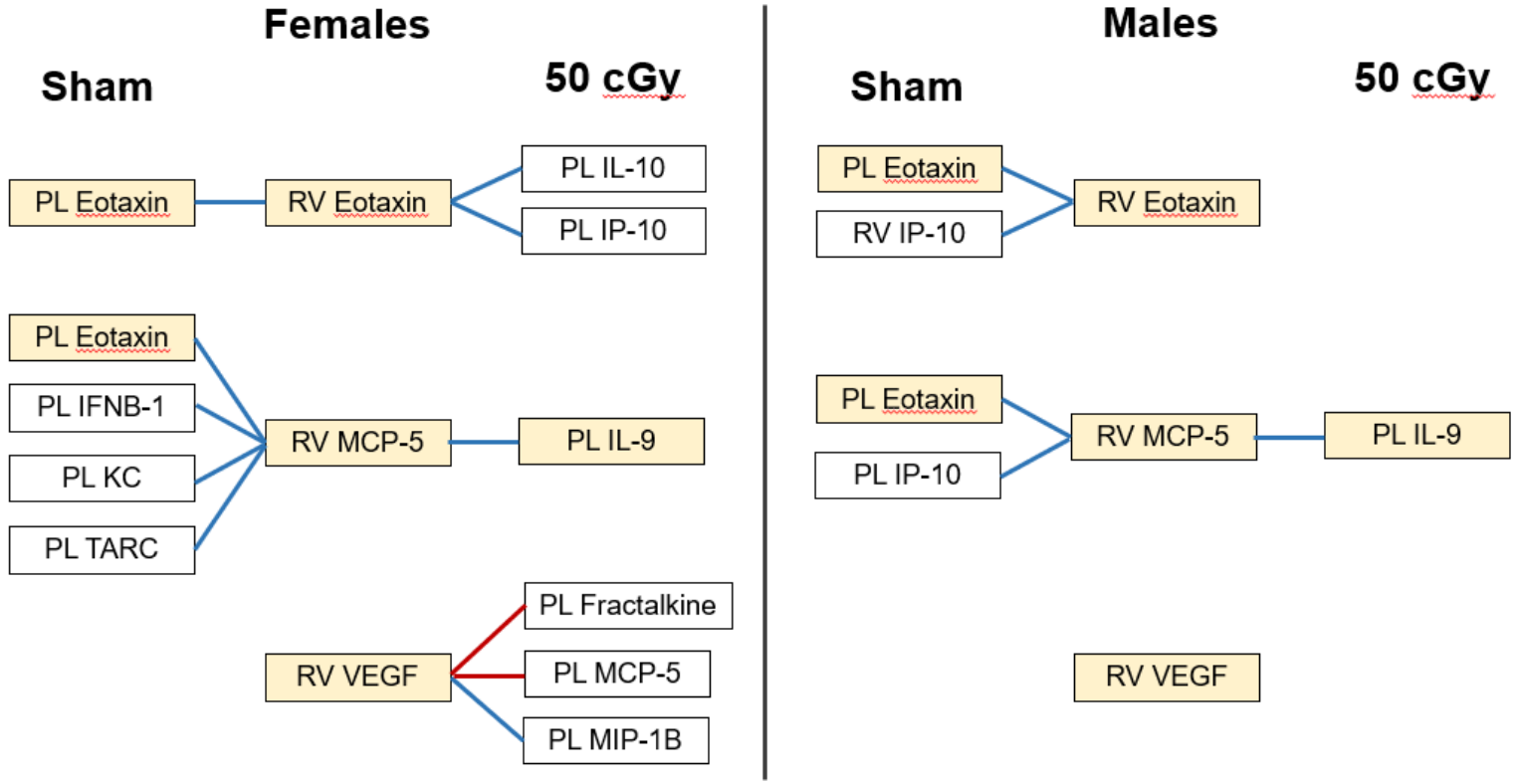
Negative correlation

Circulating and heart cytokines implicated in CVD show show altered correlations after 124 days post-GCR exposure

Eotaxin (CCL11)

- Upregulated in eosinophilic myocarditis
- Plasma and heart cytokine levels show + correlation in sham groups
- Correlation lost after 50 cGy exposure

- + correlation with heart MCP-5 levels in sham groups
- Correlation with MCP-5 lost after 50 cGy exposure



GCR exposure leads to long-term alterations in interactions of cytokines implicated in CVD and inflammatory disease.

Sex differences in markers for aging and mitochondrial function but no GCR effects in heart and aorta

Time-dependent differences also observed (14 vs 124 day post GCR groups)

Factors	Mitochondrial DNA copy number		Telomere length	
	Aorta*	Heart	Aorta*	Heart
Dose	Yes (50 cGy > 5 cGy)	No	No	No
Sex	No	Male > Female	No	No
Time	Yes (Intermediate > Delayed)	No	Yes (Intermediate > Delayed)	No
Dose*Sex	No	No	No	No
Dose*Time	No	No	No	No
Sex*Time	No	No	No	No

qPCR-based assays; Two-way ANOVA: when main effects were observed, a t-test was used to compare between groups within that main effect; N=8-9/group

Summary

Sex differences in molecular signatures

Transcriptome: differential expression of CVD-related and antioxidant genes

Cytokine milieu: generally higher in female hearts

- Consistent with sex differences in cardiovascular disease susceptibility in humans

Long-term effects of GCR on molecular signatures

Transcriptome: changes in small number of genes linked to development of CVD

Cytokine milieu: no changes in protein levels; altered correlations between circulating and heart cytokines

- Long-term changes in regulation and interactions between heart and circulating cytokines; differ between the sexes

➜ informative in assessing inflammatory signaling in cardiovascular system?

- Sex differences should be considered in the development of strategies to maintain cardiovascular health of mission crew
- Rodent models are useful tools in anticipating human responses to spaceflight and underlying mechanisms

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