RIP 1 kinase inhibition for acute ischemic kidney injury

Kevin Gallagher
MRC/KRUK/GSK PhD fellow
University of Edinburgh
Supervisors: Ewen Harrison, Jeremy Hughes, Jim Ross, Lorna Marson, Stephen Wigmore, Allison Beal

Ischemia reperfusion injury: Acute kidney injury in transplant

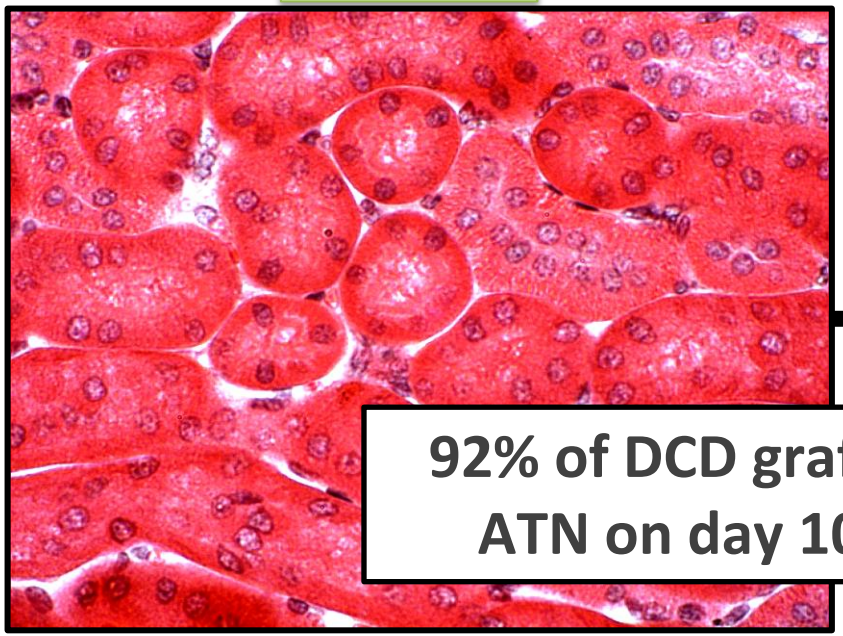
DCD transplantation

40% get delayed graft function

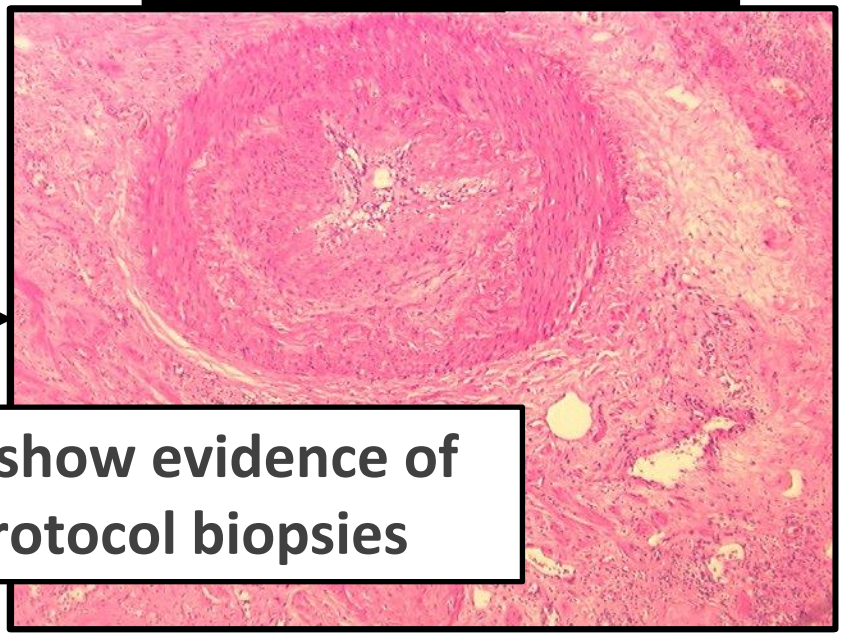
Increased rate of graft loss

The pathology: Acute tubular necrosis

Healthy



Fibrosis leading to graft loss



92% of DCD grafts show evidence of ATN on day 10 protocol biopsies

Bank et al *Nephrology Dialysis Transplantation*, December 2017

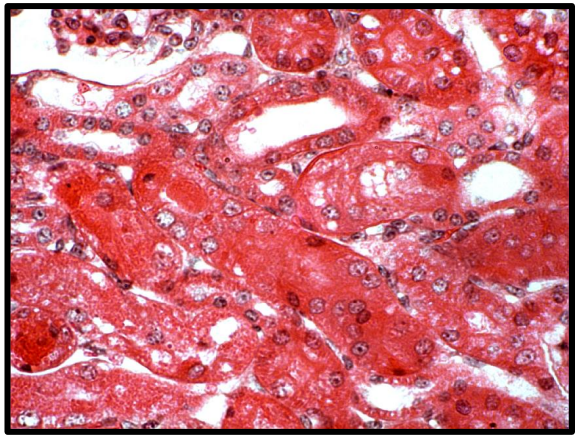
The pathology: Acute tubular cell necrosis

How do cells die and can we stop them?

Programmed cell suicide:

APOPTOSIS

NECROSIS



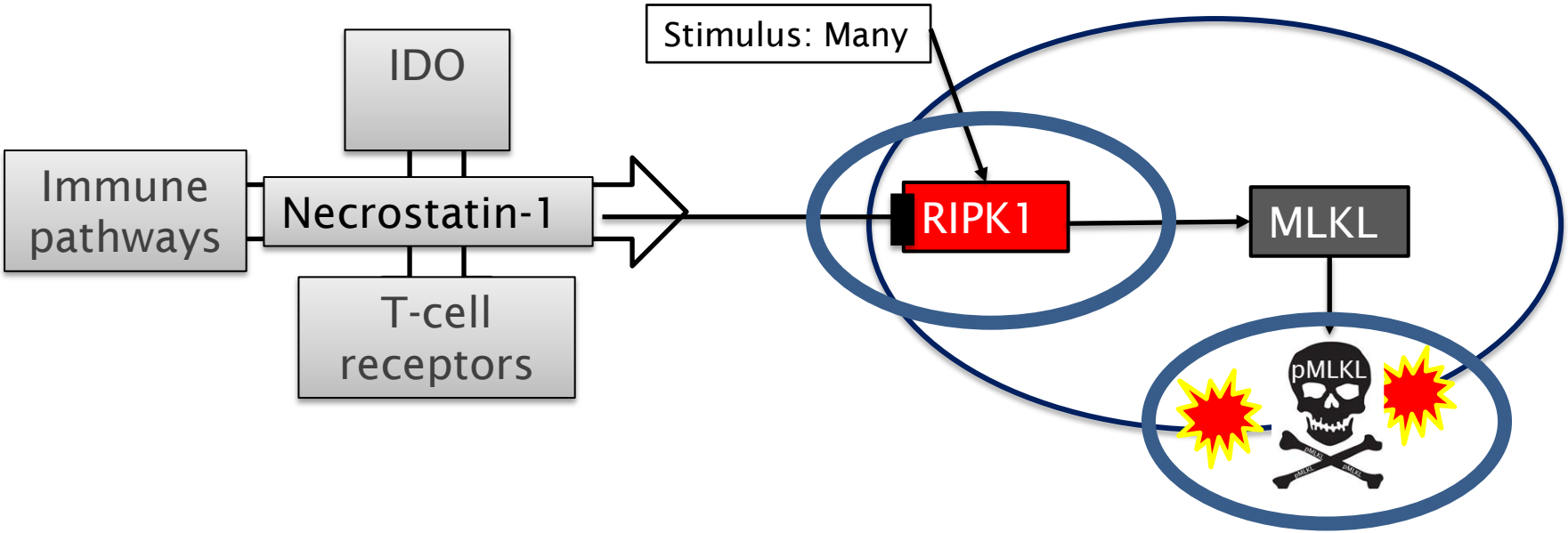
PROGRAMMED NECROSIS?

NECROPTOSIS

Can we stop tubular cells from necrosing with a drug?

Necroptosis – a wild fire of programmed necrosis.

Definition? Caspase independent cell death dependent on **MLKL**



Overall aim:

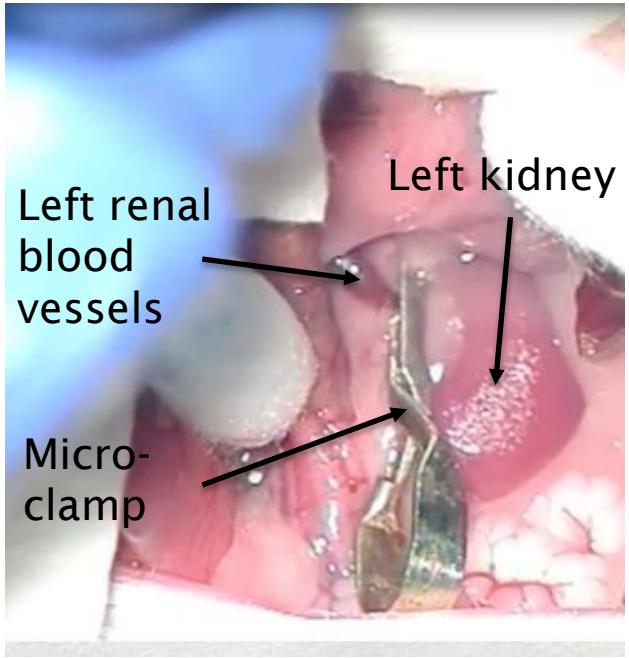
To prove that tubular necrosis can be prevented by inhibiting RIPK1/*NECROPTOSIS* in ischemia reperfusion injury

And determine if this is beneficial even if drug is given *AFTER* the injury

Methods: Mouse and human cell ischemic injury

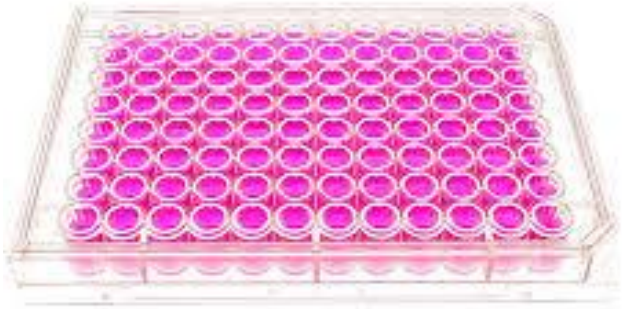
The drug: World first, highly specific and potent RIPK1 inhibitor (human ready)

Mouse: IRI

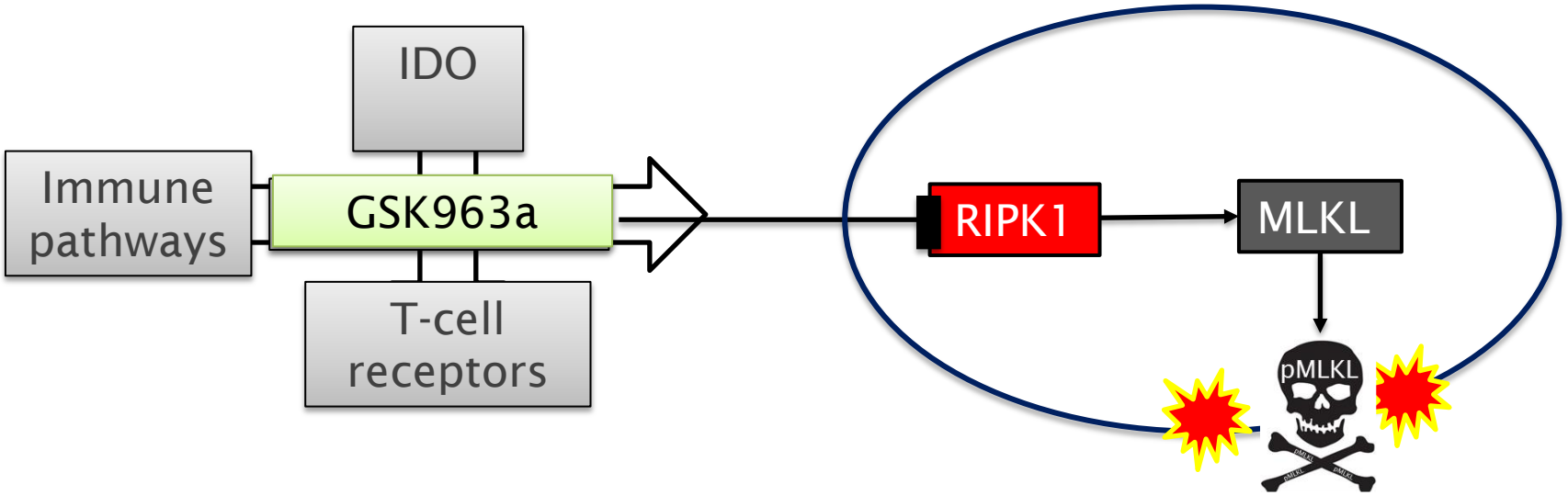


Human: proximal tubular cell

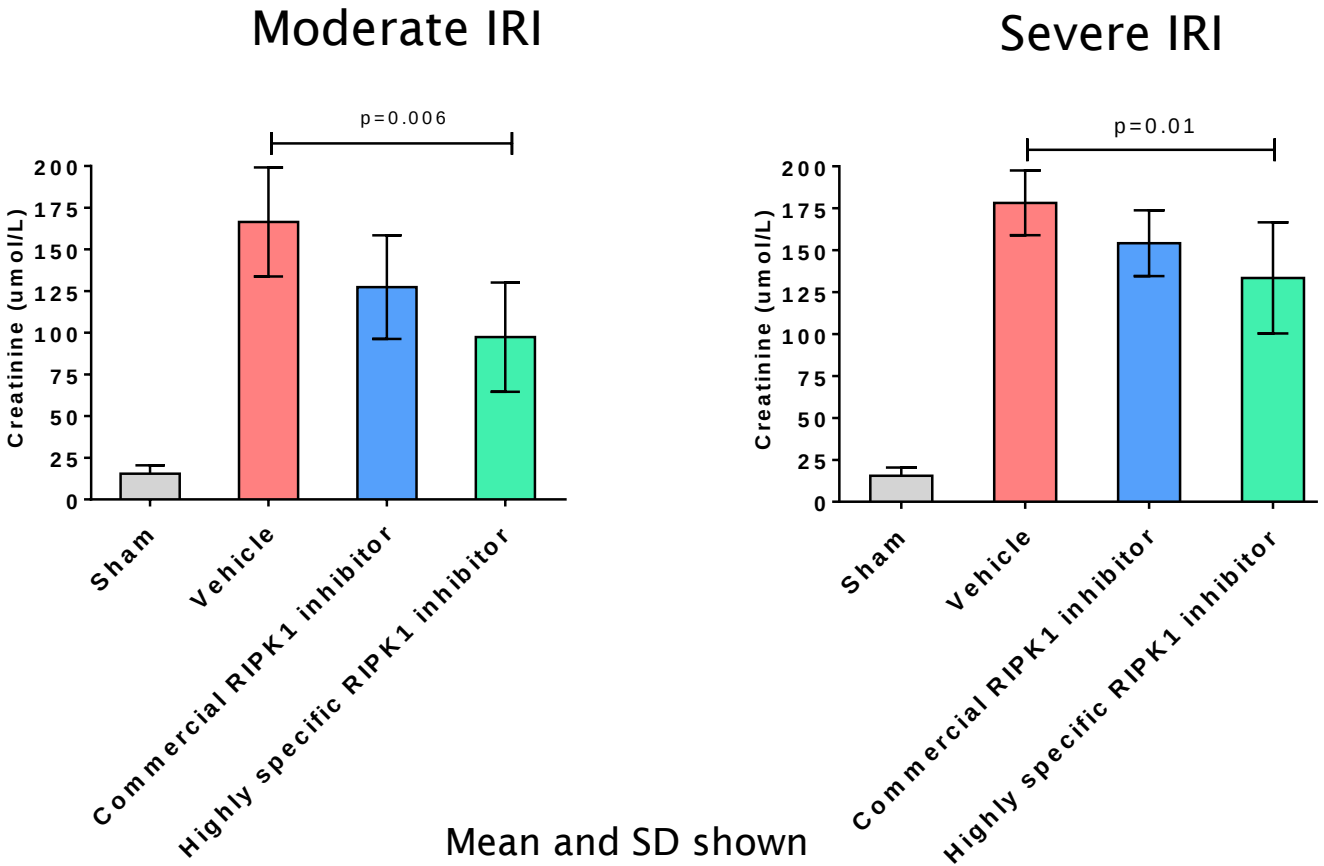
In-vitro ischemic models



Does *specific* RIPK1 inhibition improve kidney function and reduce cell death in IRI?



Highly specific RIPK1 inhibition reduces creatinine in IRI



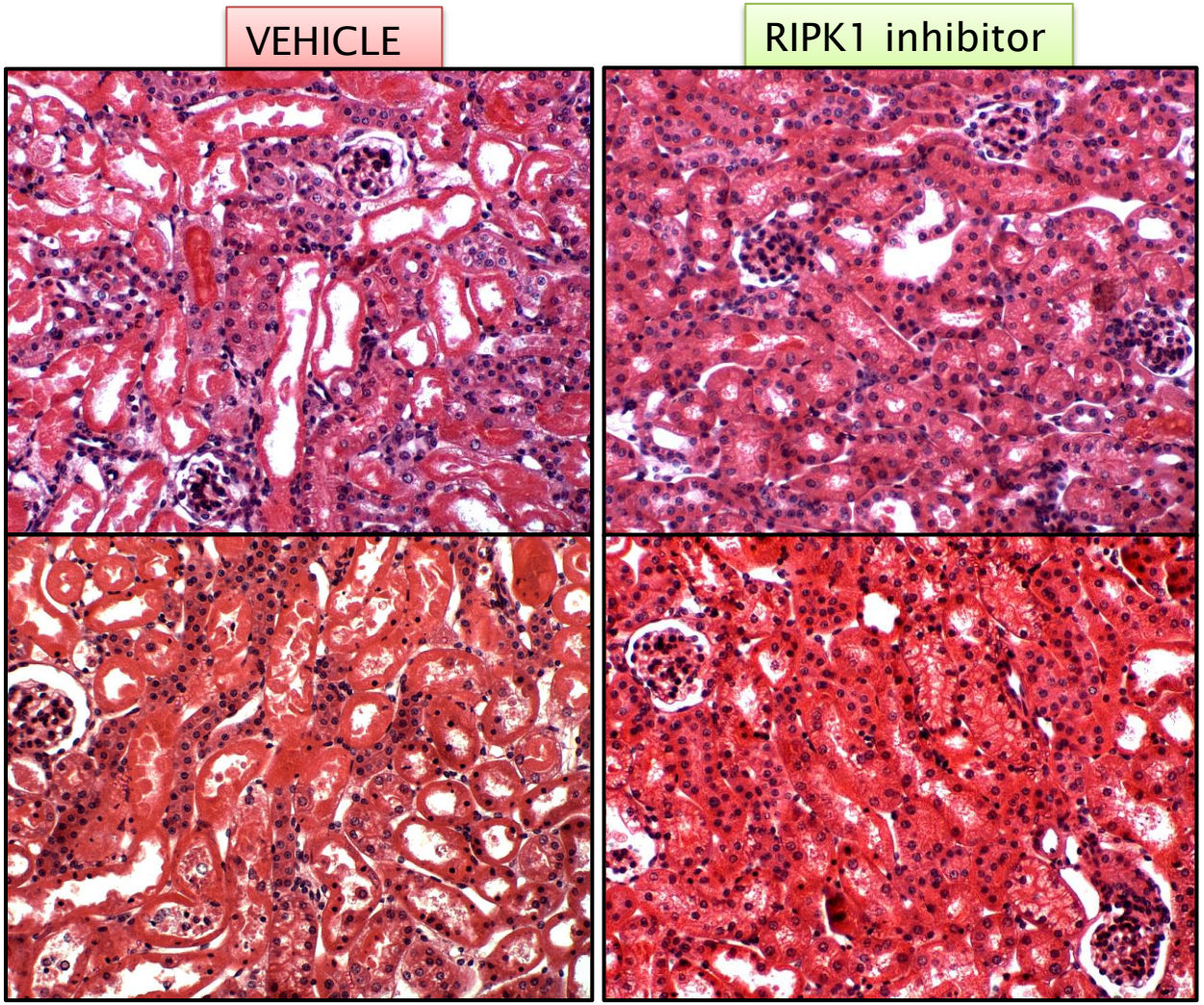
Mean and SD shown

N=8

x200

Moderate injury 50-60% CMJ tubular necrosis

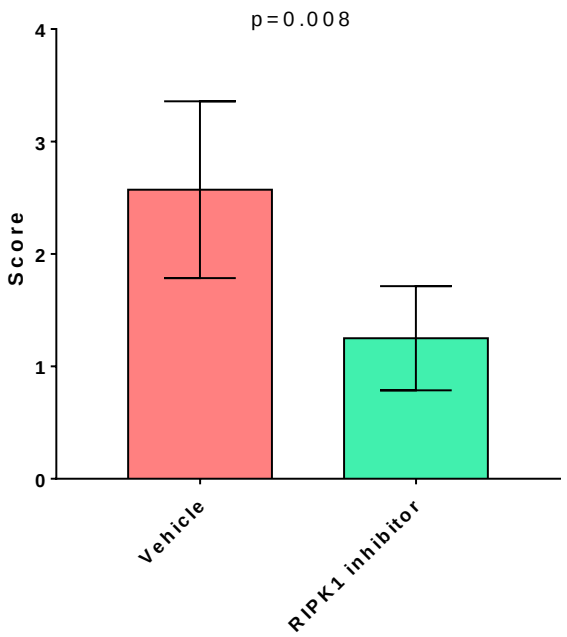
Severe injury 80-90% CMJ tubular necrosis



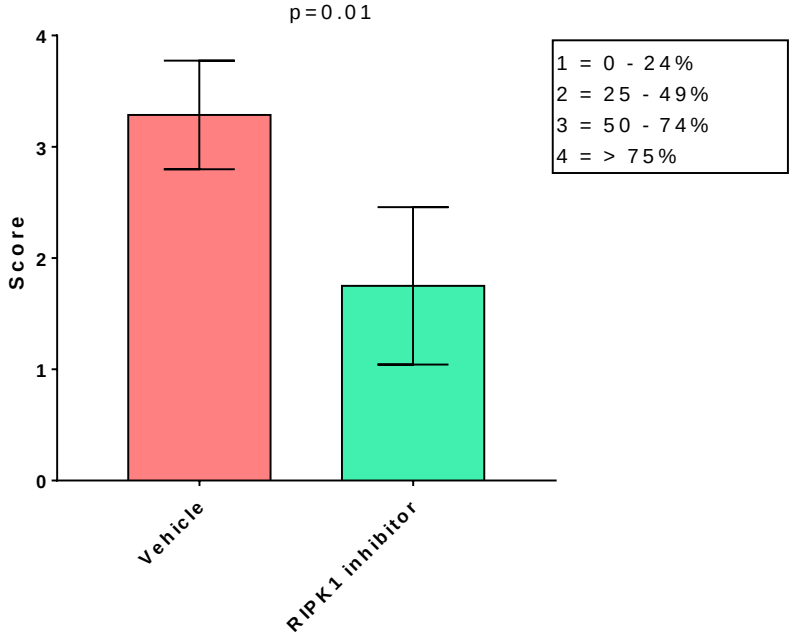
RIPK1 inhibition significantly reduces tubular necrosis in IRI

Tubular necrosis scores:

Moderate IRI



Severe IRI



Median (25th, 75th) shown

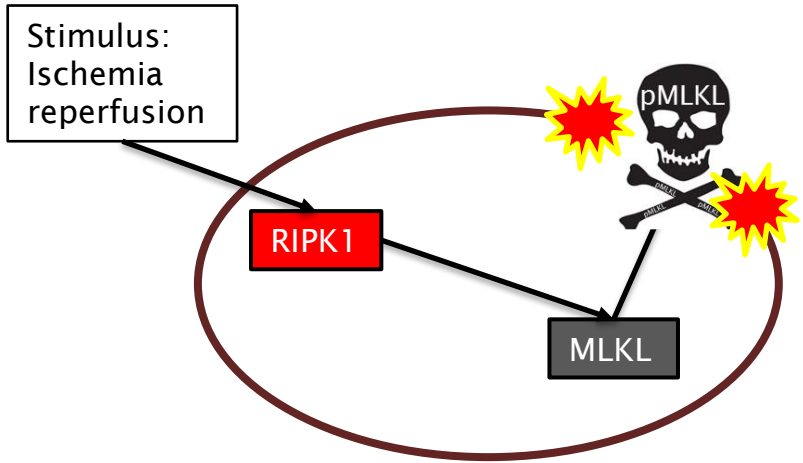
Does RIPK1 inhibition reduce tubular cell necroptosis?

Improves renal function ✓

Reduces *necrosis* ✓

Do tubular cells undergo *necroptosis*?

Need to show evidence of MLKL ***phosphorylation:***



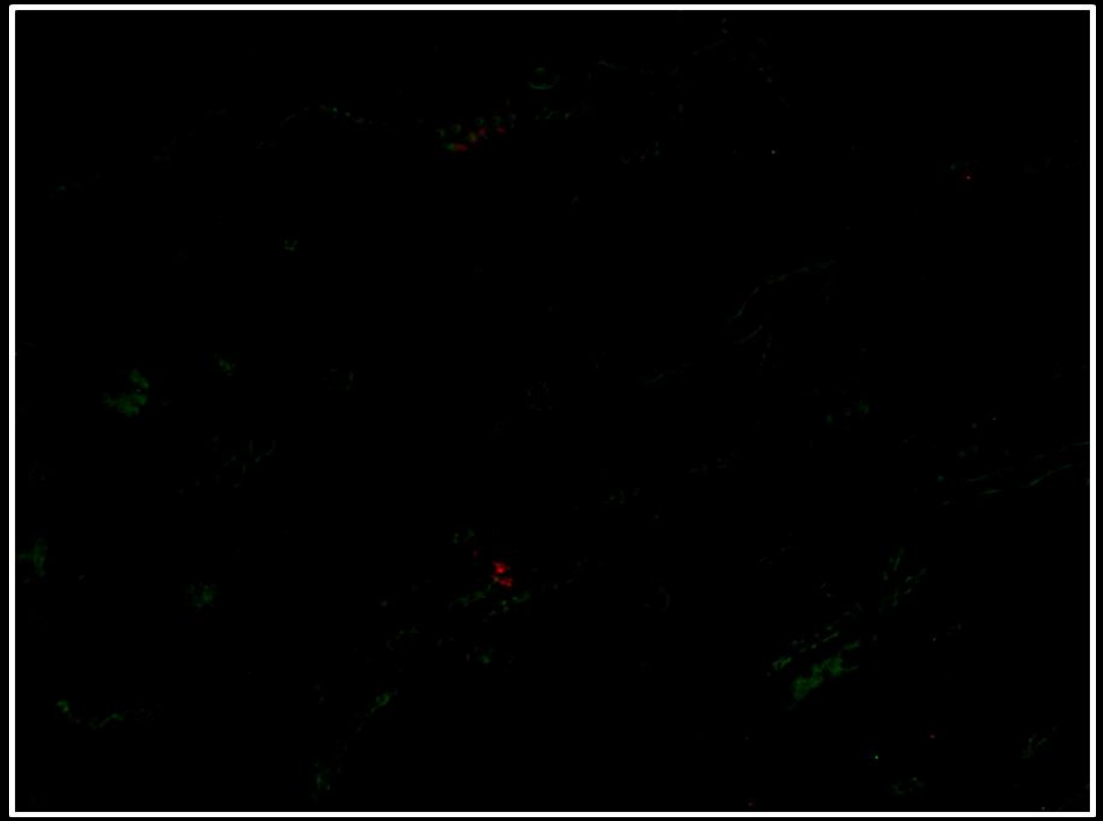
?

Healthy kidney (sham): No dead cells (red), no phosphorylated MLKL (green)

Green: Activated MLKL (phosphorylated)

Red: Dead cells (TUNEL)

x100



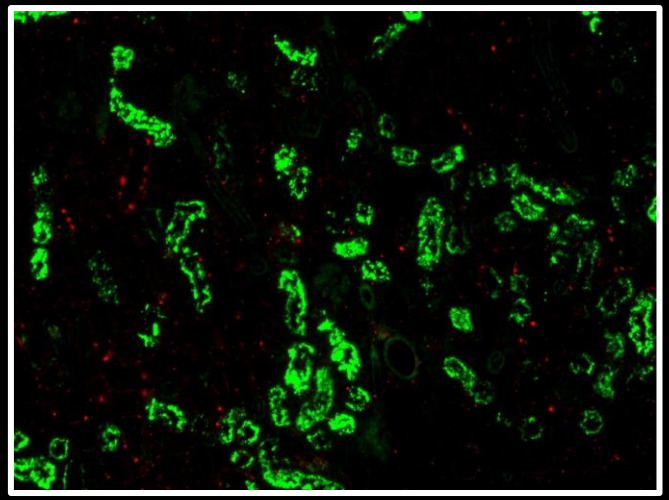
Green: Activated MLKL (phosphorylated)
Red: Dead cells (TUNEL)

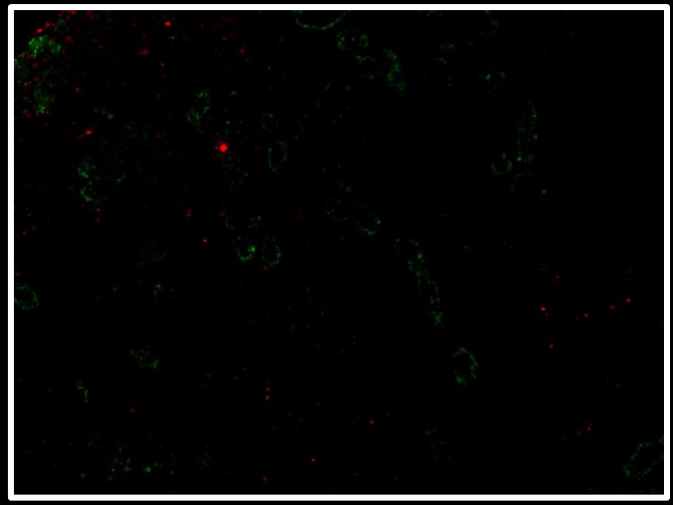
x100

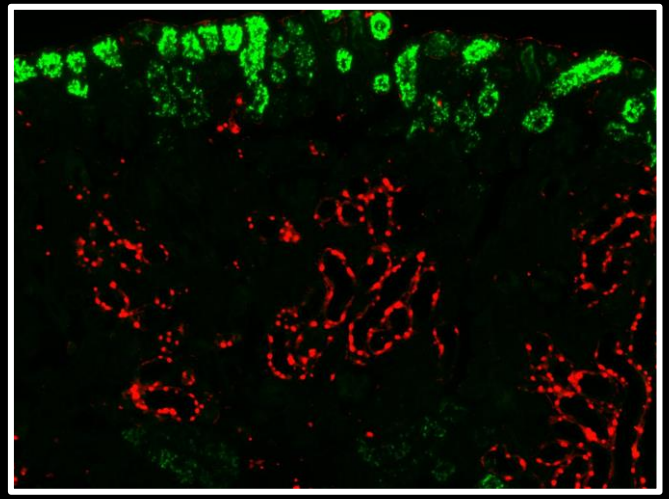
Moderate injury

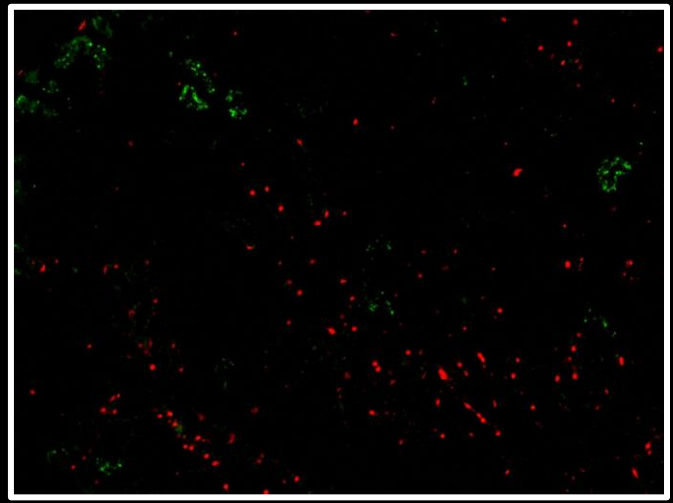
Renal ischemic injury is associated with MLKL phosphorylation

Severe injury

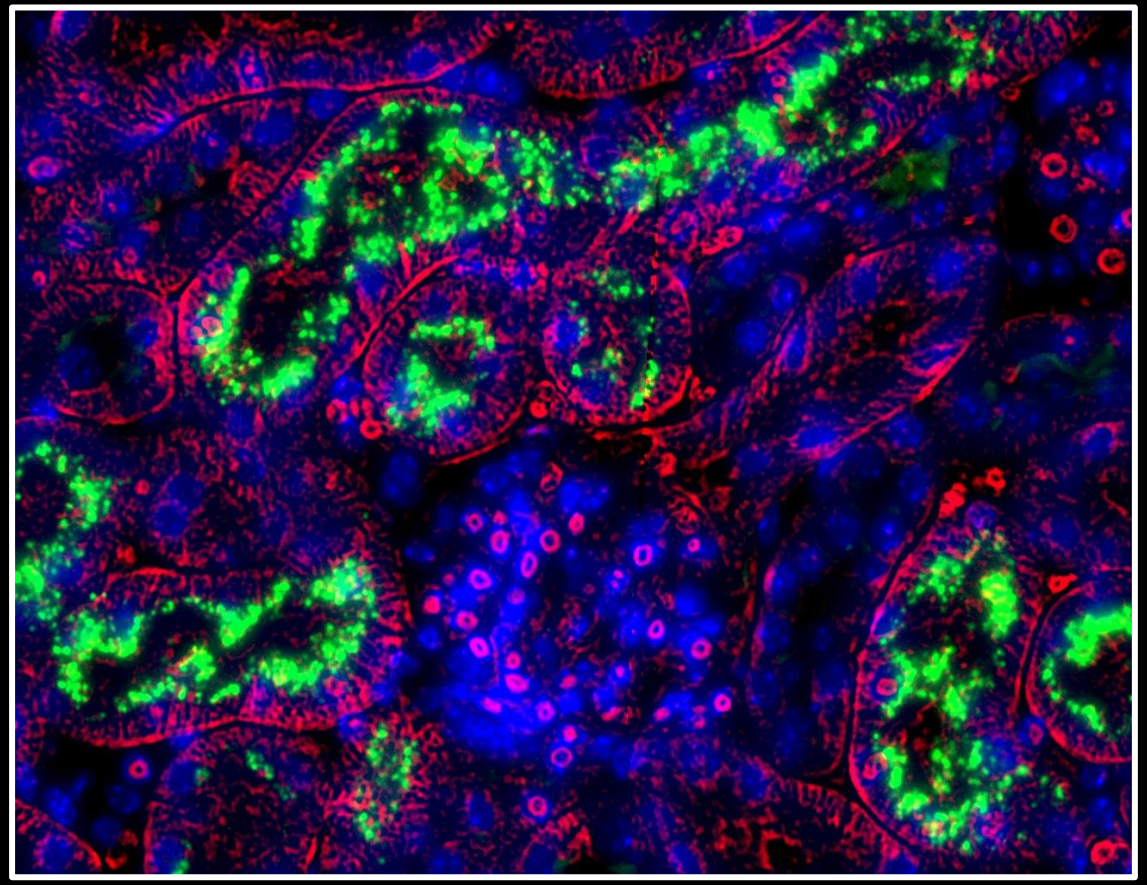








Activated MLKL is found on the apical boarder of IRI injured tubules:



Green: Activated MLKL (phosphorylated)

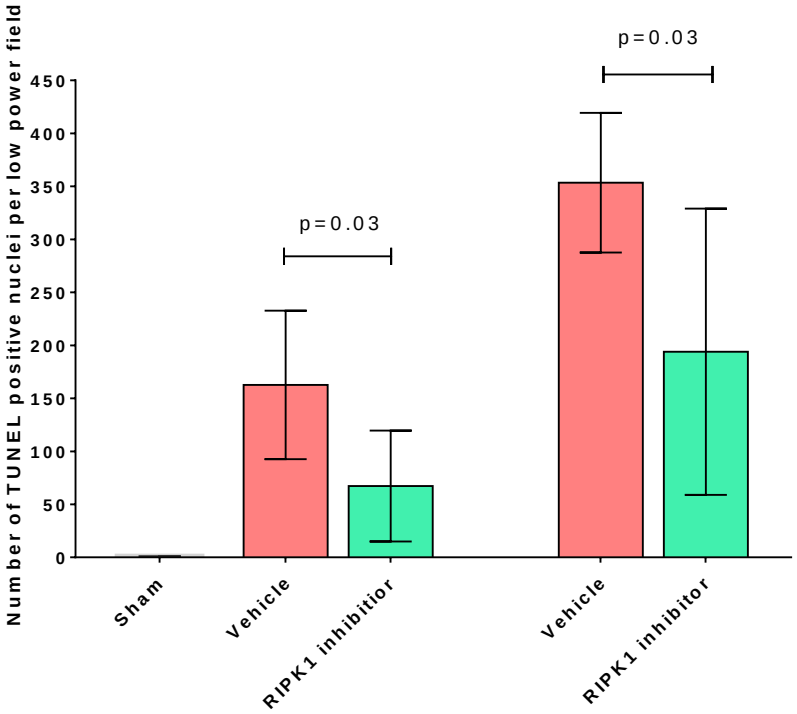
Red: Brightfield pseudocolour

Blue: DAPI (nuclei)

X400 magnification

TUNEL and Phosphorylated MLKL: Replicated and quantified

TUNEL positive cells

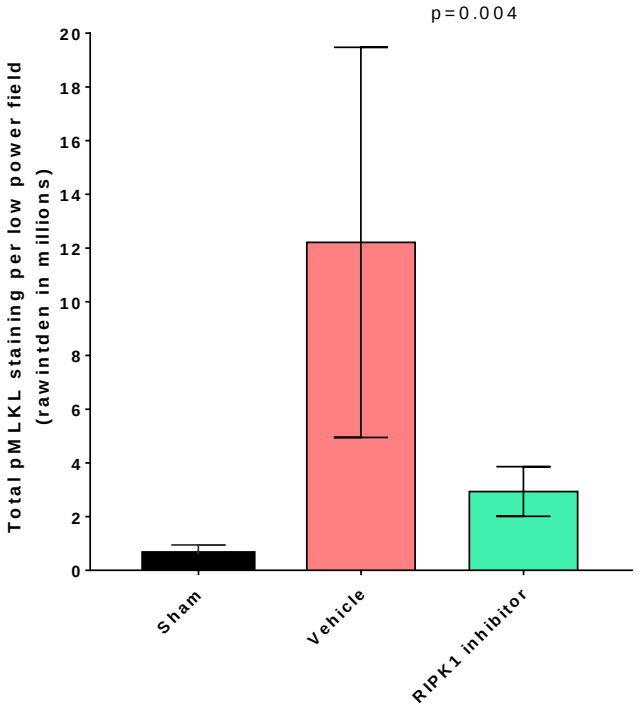


N=6

Moderate IRI

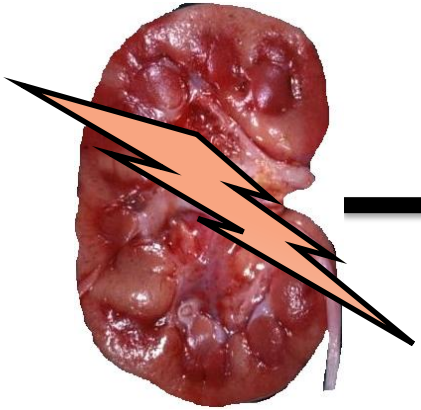
Severe IRI

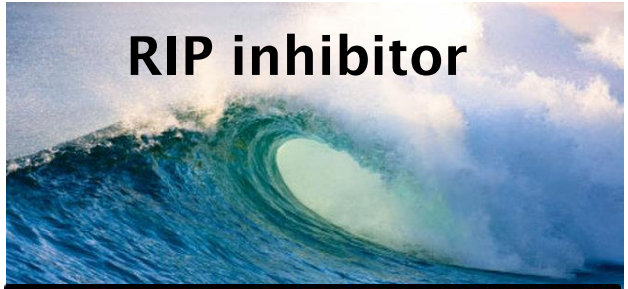
pMLKL quantification

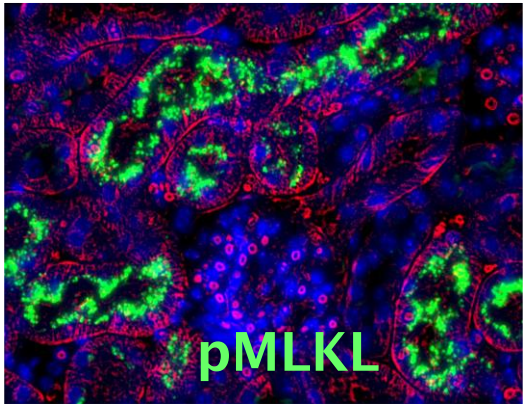


Moderate IRI

Can the benefit be maintained with drug given after the injury?







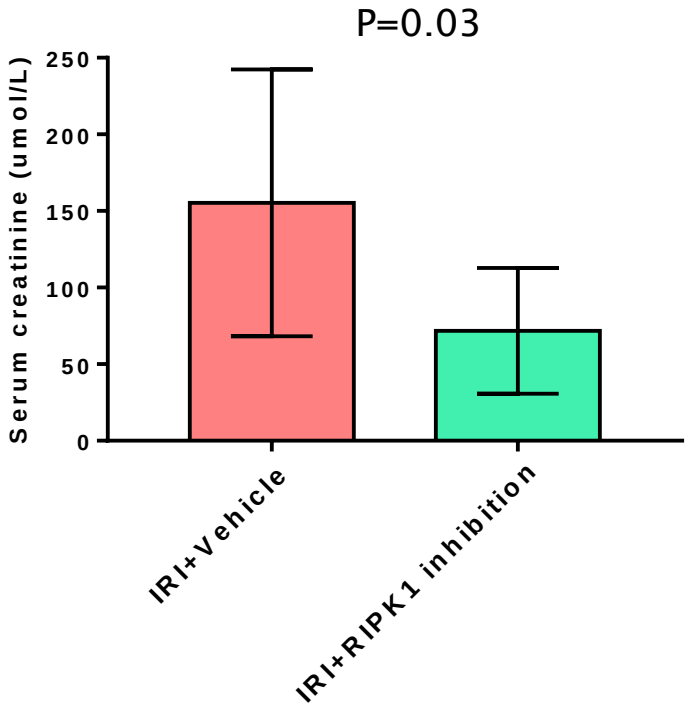
T=0

T=24

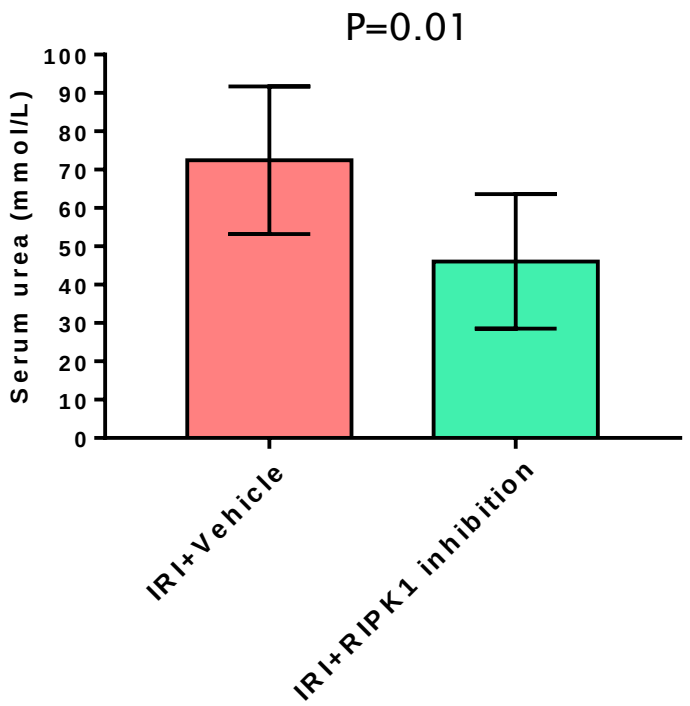
RIPK1 inhibition still effective when given AFTER the injury

Drug first given after 4 hours of reperfusion

Creatinine 48 hours after injury



Urea 48 hours after injury



N=8

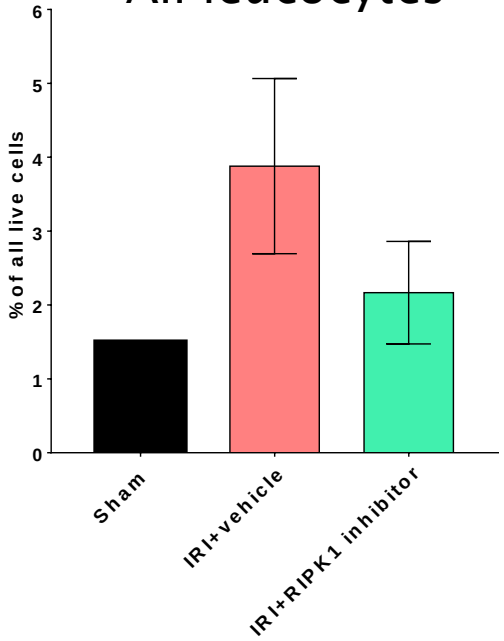
Are there effects on the ensuing immune cell influx?

RIPK1 inhibition reduces the number of inflammatory macrophages in the kidney at 48 hours

Whole kidney flow cytometry macrophage panel (8 antibodies)

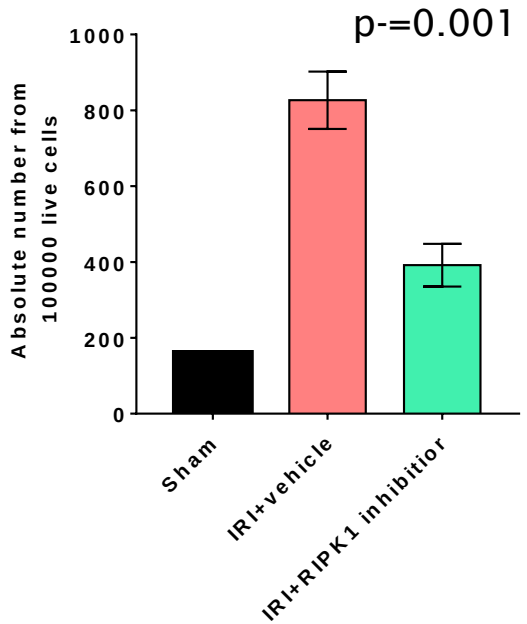
CD45+ve

All leucocytes



CD45+ve/F480+ve/Ly6c+ve/CD206-ve

Inflammatory macrophages



IRI with 48 hours reperfusion: Drug given 4 hours after injury

N=3

RIPK1 inhibition reduces renal inflammatory macrophage numbers 48 hours after IRI

Each dot = a macrophage. Split by: Inflammatory activation.

SHAM

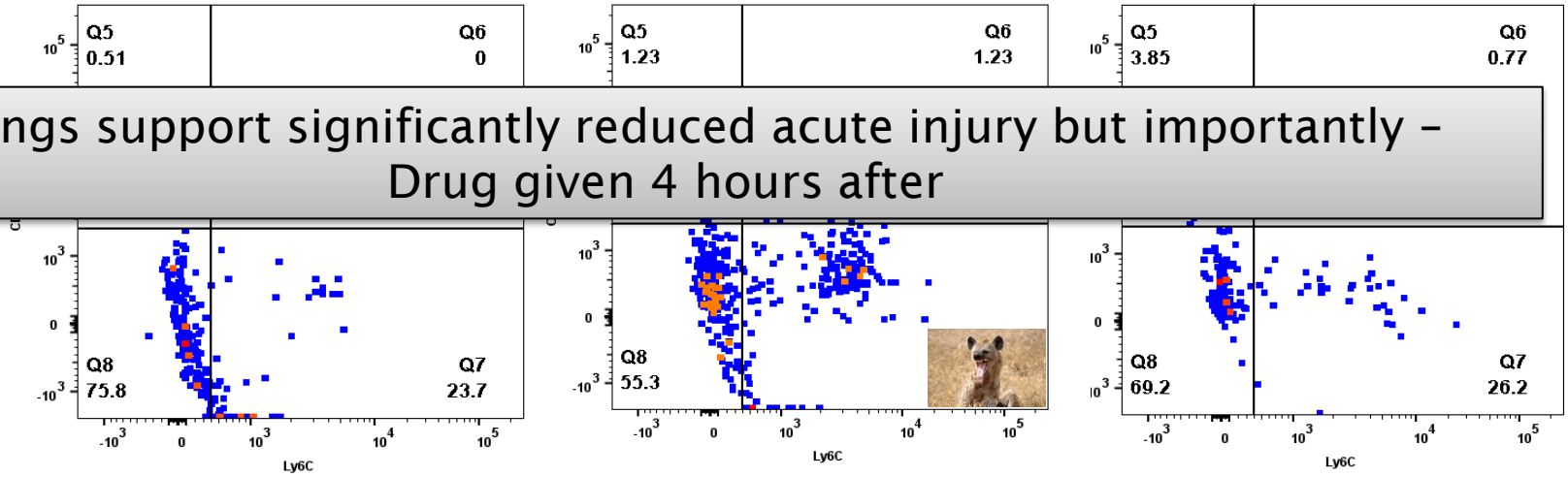
Sample Name	Subset Name	Count
IRI kid_1_001.fcs	CD45>F480 Positive	198

IRI+Vehicle

Sample Name	Subset Name	Count
IRI kid_6_012.fcs	CD45>F480 Positive	405

IRI+RIPK1 inhibitor

Sample Name	Subset Name	Count
IRI kid_5_010.fcs	CD45>F480 Positive	130

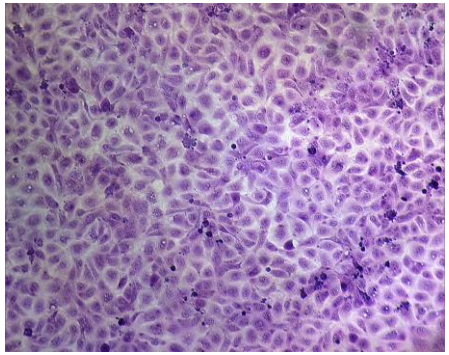


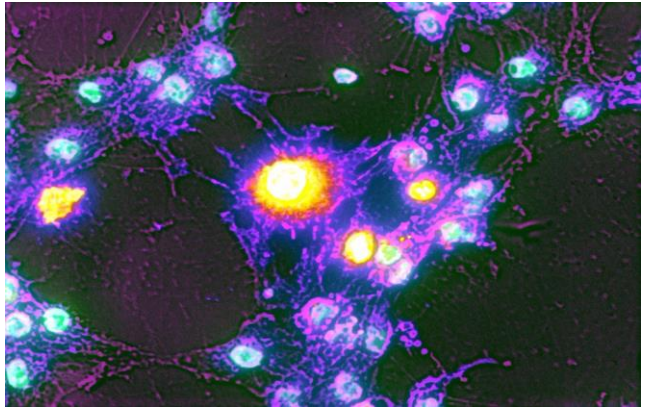
Findings support significantly reduced acute injury but importantly – Drug given 4 hours after

CD206 positive: anti-inflammatory

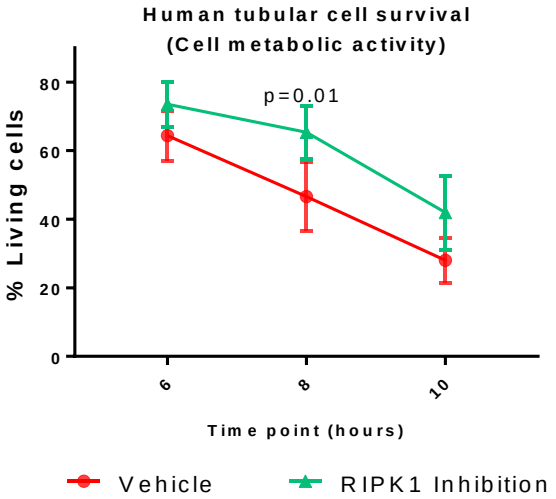
Ly6C positive: highly inflammatory

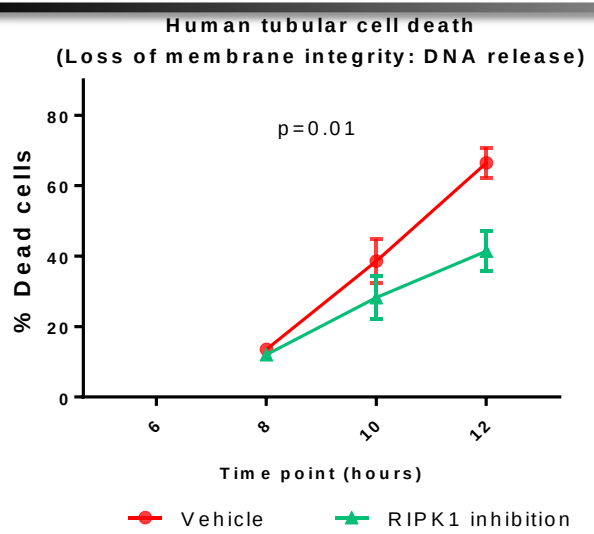
Is RIPK1 inhibition beneficial in **human** ischemic tubular cell injury?

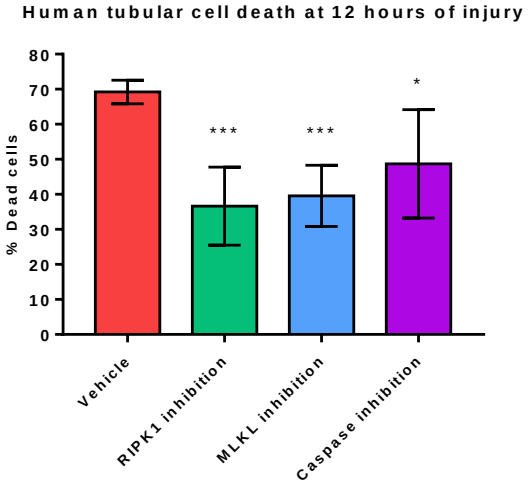




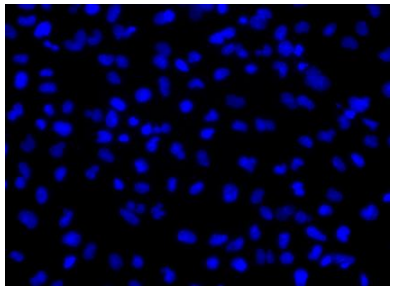
Human tubular cells are protected from chemical anoxia by RIPK1 inhibition



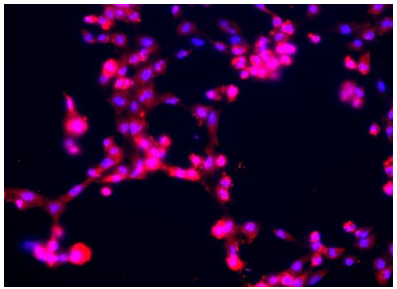




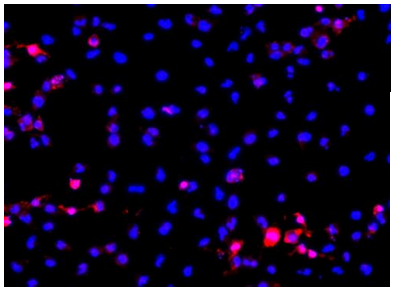
Negative control



Vehicle



RIPK1 inhibition

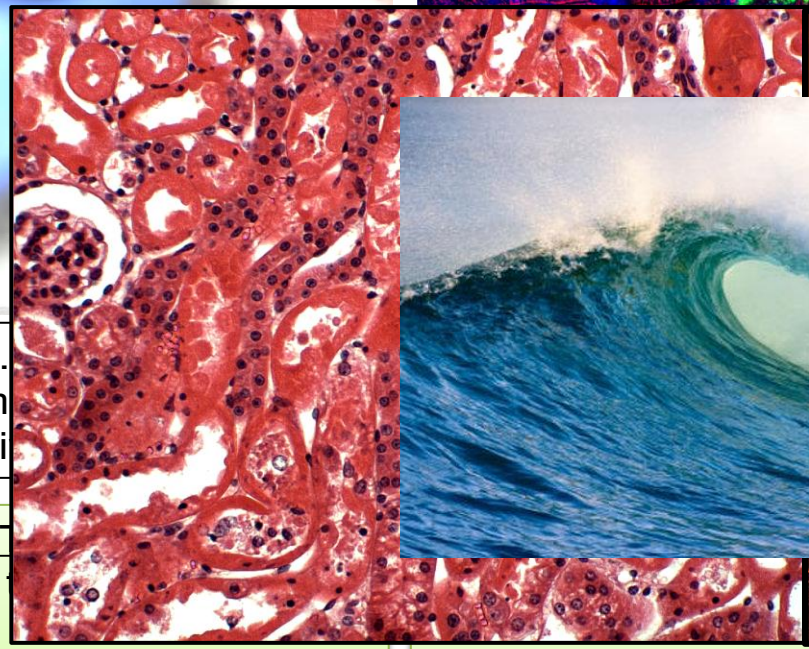


Blue = live cell Red/Pink=dead cell

Injury = Inhibit ATP production (antimycin A) and glucose deprivation.
N = 6

.

Summary: *Highly specific* RIPK1 inhibition



beneficial in IRI

RIPK1 inhibition reduces this

kidney cells

Ackowledgements

- MRC, KRUK and GSK
- My supervisors
- Jim Black, Kathryn Sangster, Jyoti Nanda, Anwar Palakka , Gary Borthwick, Laura Denby, Carolynn Cairns