

Ashvattha

THERAPEUTICS

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Poster: PO0940

Systemic Therapies Targeted to Ischemia in a Model of Diabetic Acute Kidney Injury

October 22, 2020

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1. Ashvattha Therapeutics, Inc., Redwood City, CA, United States

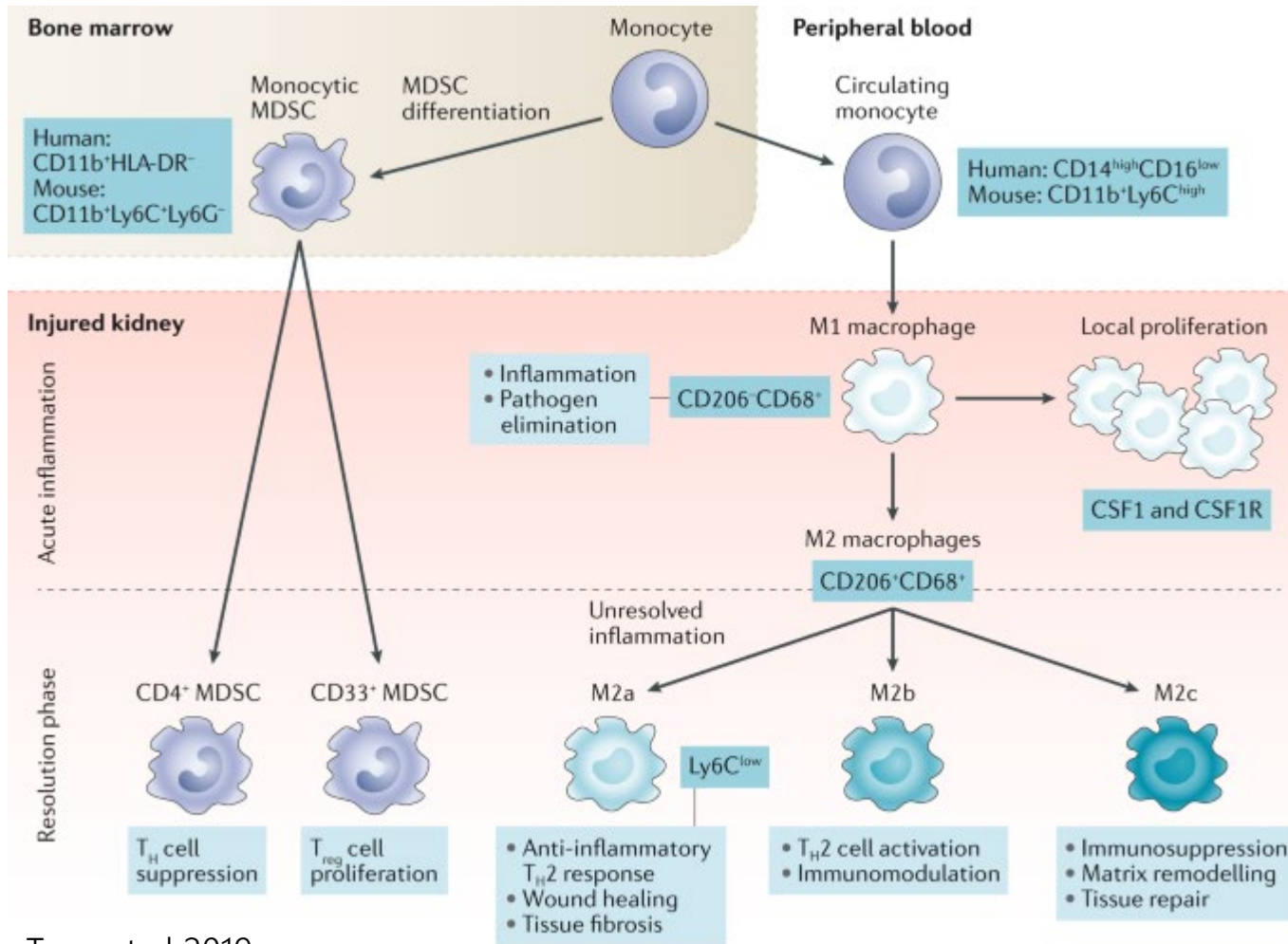
2. Creative Biolabs, Shirley, NY, United States

3. Johns Hopkins School of Medicine, Baltimore, MD, United States

Disclosures

- Rishi Sharma, PhD, Santiago Appiani and Jeffrey Cleland, PhD are employees of Ashvattha Therapeutics which funded the research
- Jinglei Zhang and Audrey Chang are employees of Creative Biolabs which was contracted by Ashvattha for the animal studies
- Sujatha Kannan, MD, and Kannan Rangaramanujam, PhD are employees of Johns Hopkins School of Medicine and co-founders of Ashvattha Therapeutics

Role of Macrophages in Kidney Disease



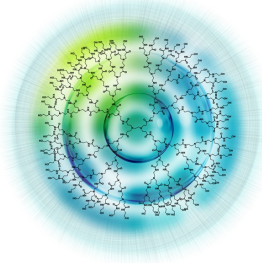
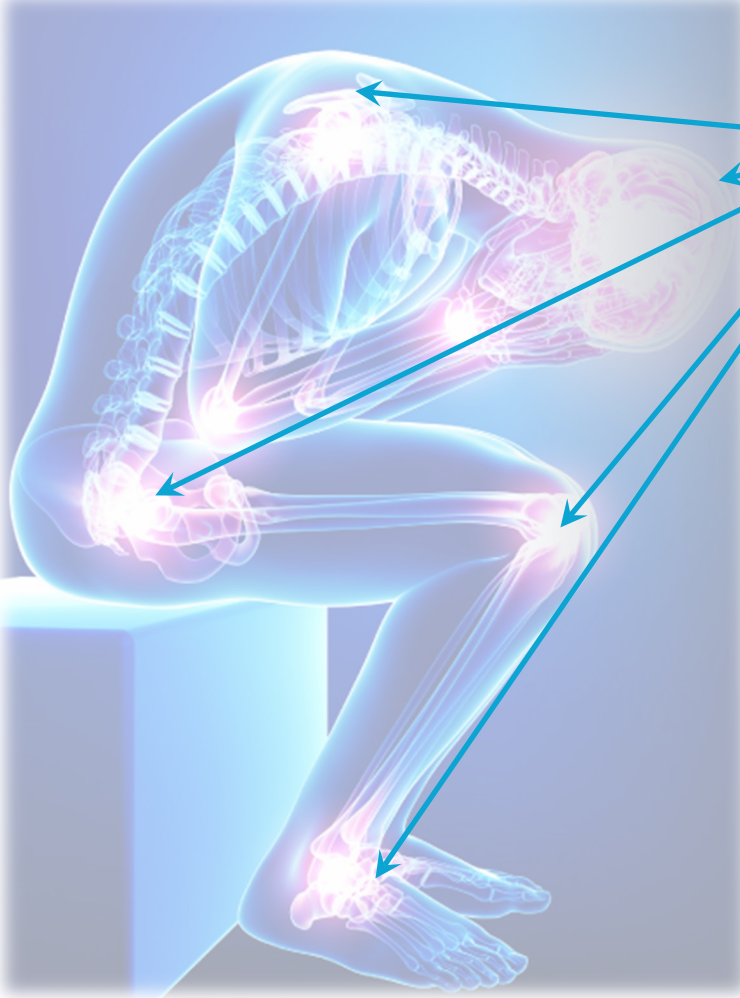
Tang et al 2019

Insult Leads to Chronic Activation

- Injury (AKI) or disease (diabetes) leads to chronic inflammation and macrophage activation
- M1 macrophage cause direct tissue damage
- M2 macrophage facilitate resolution of injury and repair
- Chronic activation of M1 macrophage leads to severe tissue damage and loss of renal function
- Key objective: Determine when reactive M1 macrophage are present and facilitate conversion to M2 macrophage

HD Technology Enables Treatment for Broad Range of Diseases

Hydroxyl Dendrimer (HD)



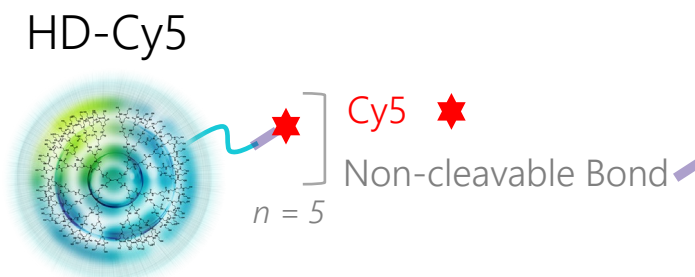
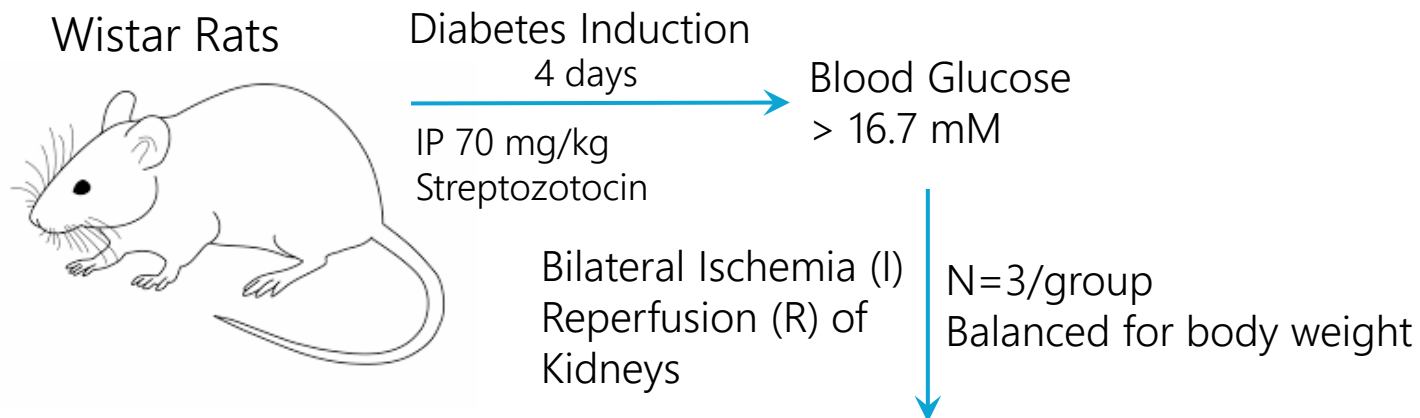
- Water-like Surface (novel finding)
- Targets Key Cells
- No Ligand Needed

Key Attributes

- Only taken up by **reactive inflammatory cells** in diseased tissues (broad range of diseases)
- Targeted systemic therapy (**Oral or injectable**)
 - **Crosses tissue** barriers (BBB, retina, tumor)
 - **Safe at high doses** in animals & humans
 - **Sustained** duration of effect
- **Low cost** manufacturing, rapid discovery process (**Over 65 HDTs** to date)
- Broad license to technology from **Johns Hopkins University (JHU)**
(>15 yrs, >\$30 M NIH, >30 JHU collaborators, >70 papers, 22 issued & 50 pending patents)

Study Design for Targeting Reactive Renal Macrophages

Diabetic Rat Model of Acute Kidney Injury



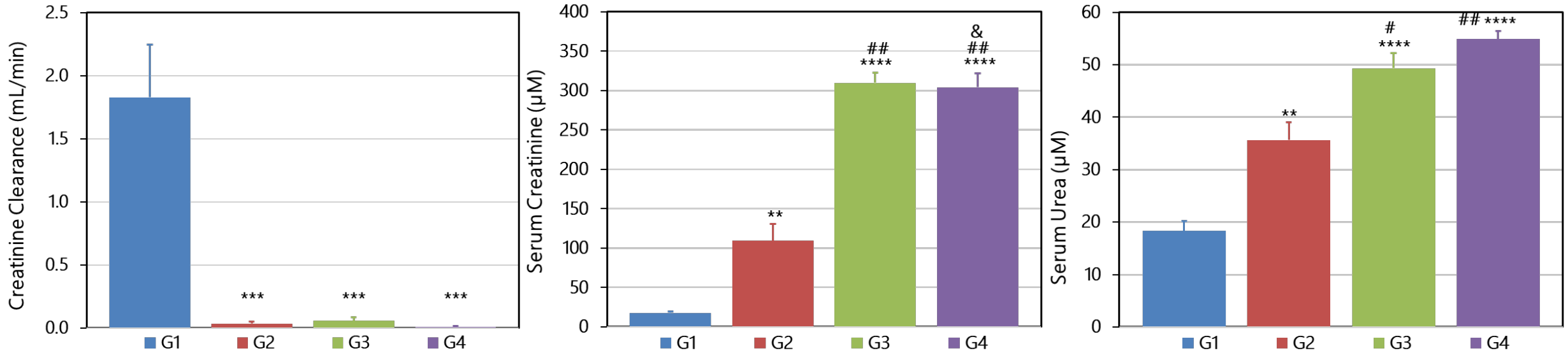
Group	I / R Timing	HD-Cy5 IP Dosing Time	Euthanization
1	Sham surgery	1 hr after surgery	24 hr after surgery
2	60 min I / 6 hr R	1 hr after start of reperfusion	6 hr after reperfusion
3	45 min I / 24 hr R	1 hr after start of reperfusion	24 hr after reperfusion
4	45 min I / 24 hr R	12 hr after start of reperfusion	24 hr after reperfusion

Assessments:

- Renal Function
- Tubular Damage
- Tubular Necrosis
- Macrophage

Renal Function Post-I/R

Blood glucose ~ 30 mM across all groups



p<0.01, *p<0.001, ****p<0.0001, compared to G1 (one-way ANOVA, Dunnett's multiple comparison test)

#p<0.05, ##p<0.01, ###p<0.0001, compared to G2 (one-way ANOVA, Dunnett's multiple comparison test)

&p<0.01, compared to G3 (one-way ANOVA, Dunnett's multiple comparison test)

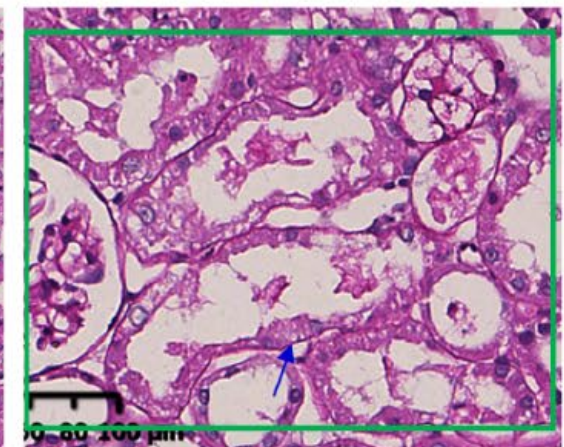
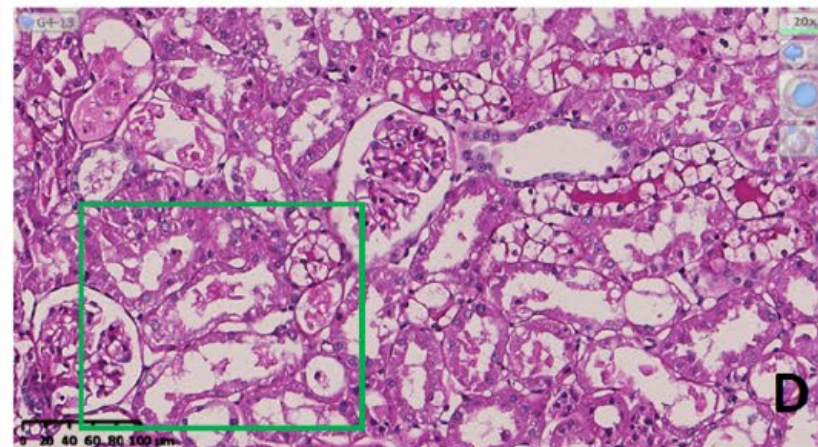
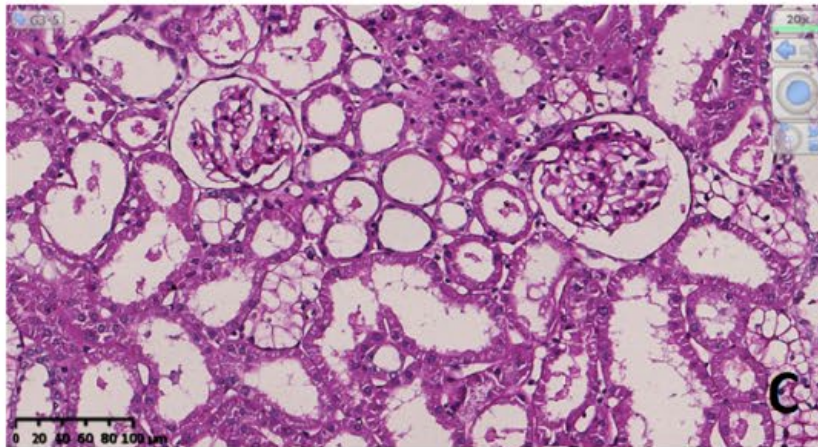
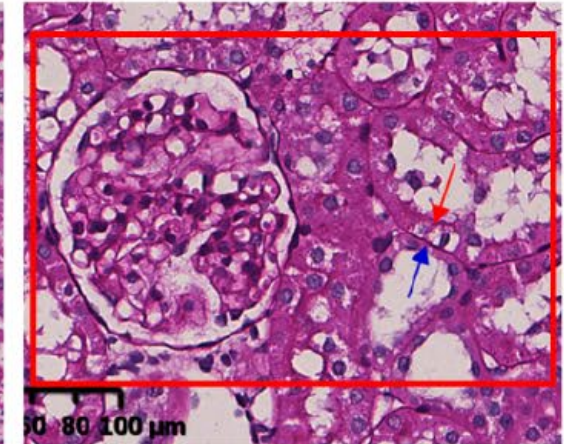
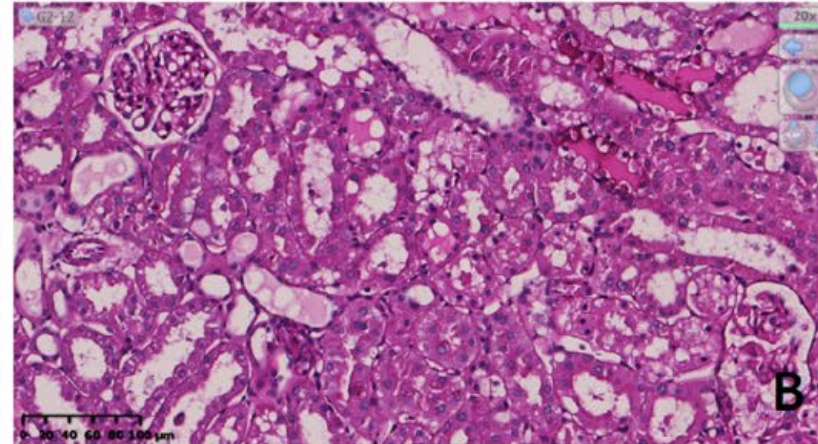
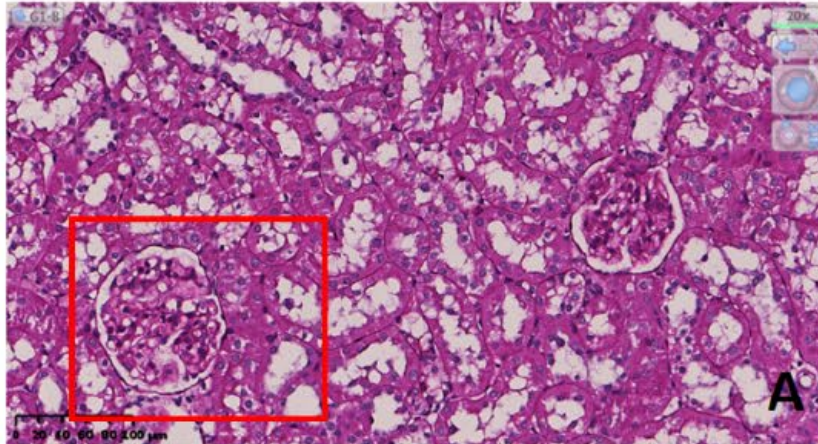
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Longer Reperfusion Resulted in Greater Loss of Function

Histological Evidence of Kidney Injury

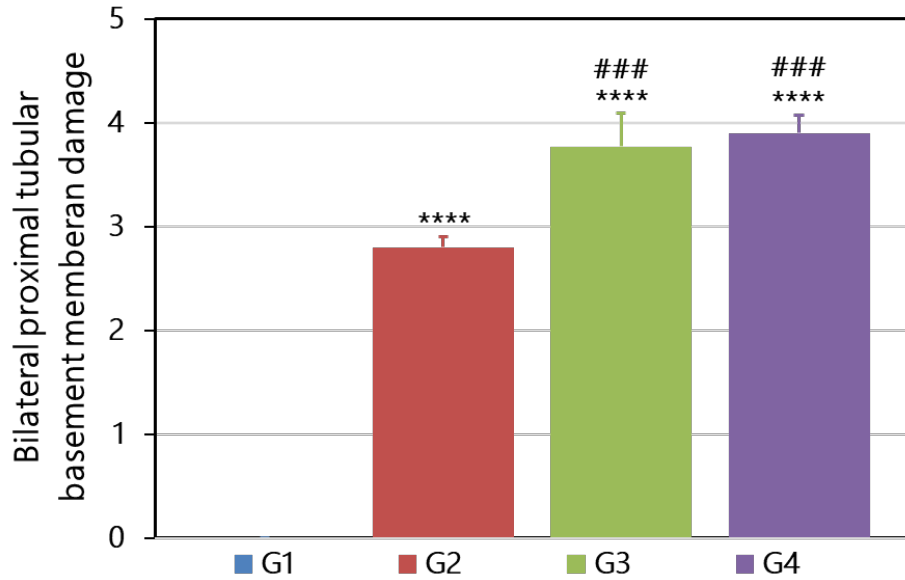
PAS Staining

G1: A G2: B G3: C G4: D

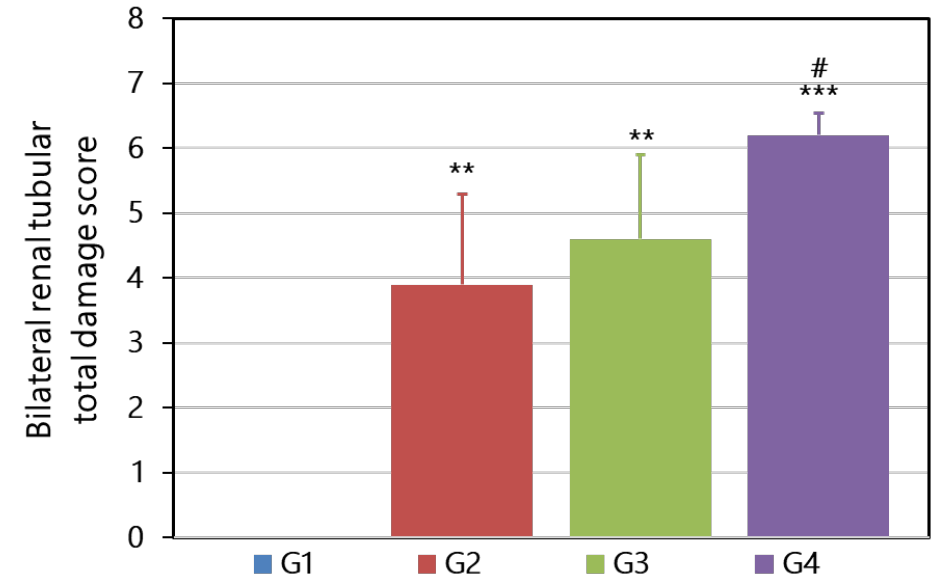


I/R Induced tubular dilation, detached brush border, & damaged basement membrane

Quantitation of Kidney Injury Post-I/R



Grade	% tubular epithelial flattening and tubular dilation
0	0
1	0-10
2	10-25
3	25-50
4	50-75
5	>75



Proximal tubular brush border damage was comparable across Groups 2-4 (~4), and significantly greater than Group 1 (~1; $p < 0.0001$)

Total damage score = Degeneration + Necrosis Scores
(1 = Mild; 2 = Moderate; 3 = Severe)

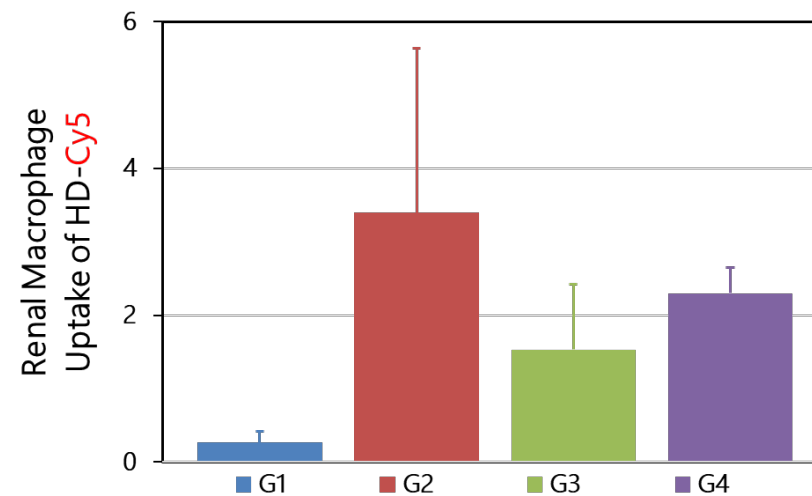
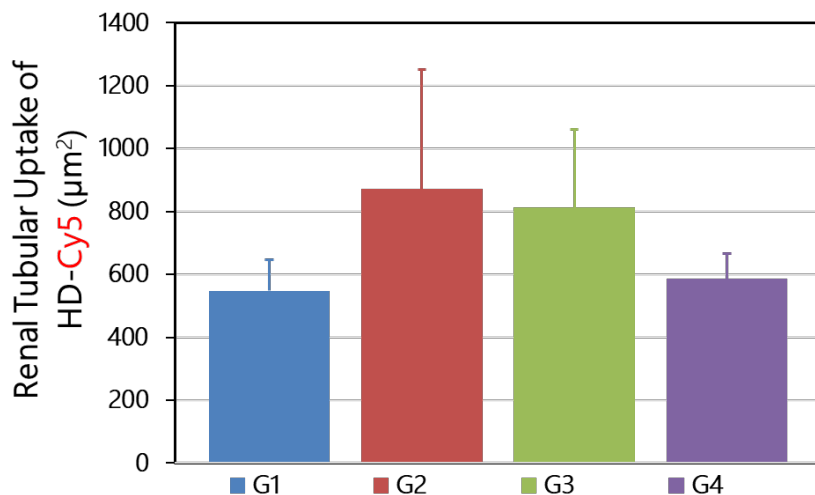
** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, compared to G1 (one-way ANOVA, Dunnett's multiple comparison test)

$p < 0.05$, ## $p < 0.01$, ### $p < 0.001$, compared to G2 (one-way ANOVA, Dunnett's multiple comparison test)

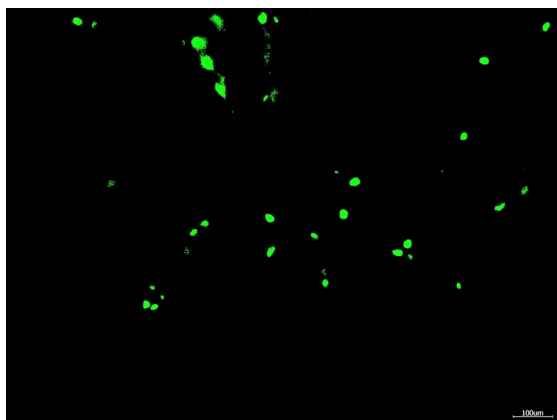
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Longer Reperfusion Resulted in Greater Injury

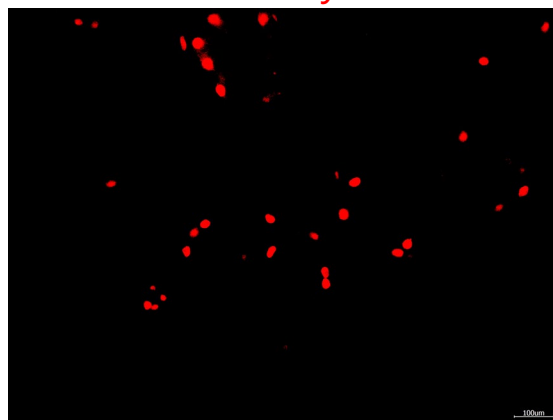
HD-Cy5 Uptake in Renal Macrophages



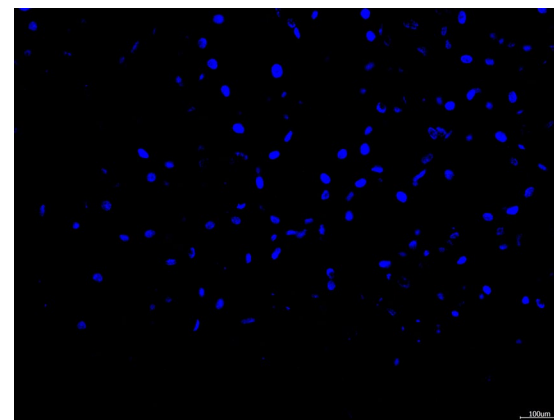
G2 ED1 (Macrophage)



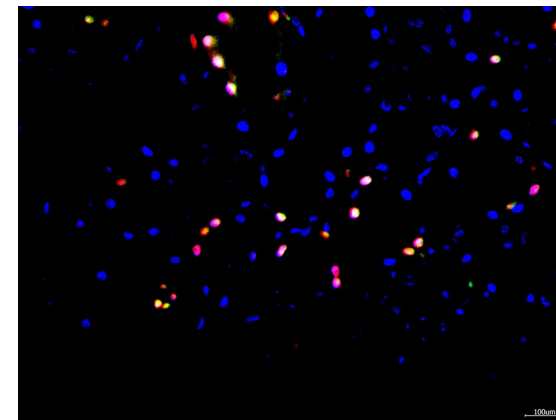
HD-Cy5



DAPI



ED1+HD-Cy5+DAPI



Renal Macrophage Activation Occurs <1 hr post-R Resulting in HD Uptake

Summary: Targeted Uptake in Renal Macrophages in Diabetic AKI

- Diabetic rats with AKI have loss of renal function and kidney damage
 - Degree of function loss and damage increases with reperfusion time
- Renal macrophages become activated within 1 hr after reperfusion
 - G2 & G3 – ED1 positive cell levels
- HDs are selectively taken up by renal macrophages in areas of injury
 - No significant HD uptake observed in other cells or tissues
- HDs provide a platform for targeting therapies to treat kidney diseases
 - Drugs to modulate renal function
 - Drugs to treat renal cell carcinomas (supporting data of HDs targeting tumor associated macrophages)

Hydroxyl Dendrimers Selectively Target Renal Macrophages Activated by Injury

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