Intravenous Gemini™ for Prevention of Acute Kidney Injury

Prophylactic Administration of Gemini (intravenous PHAD®) Reduces Tissue Damage and Improves Kidney Function in a Rat Model of Bilateral Ischemic Reperfusion Induced Acute Kidney Injury (AKI)

Presented by Robin Marsden, Senior Vice President, Biology

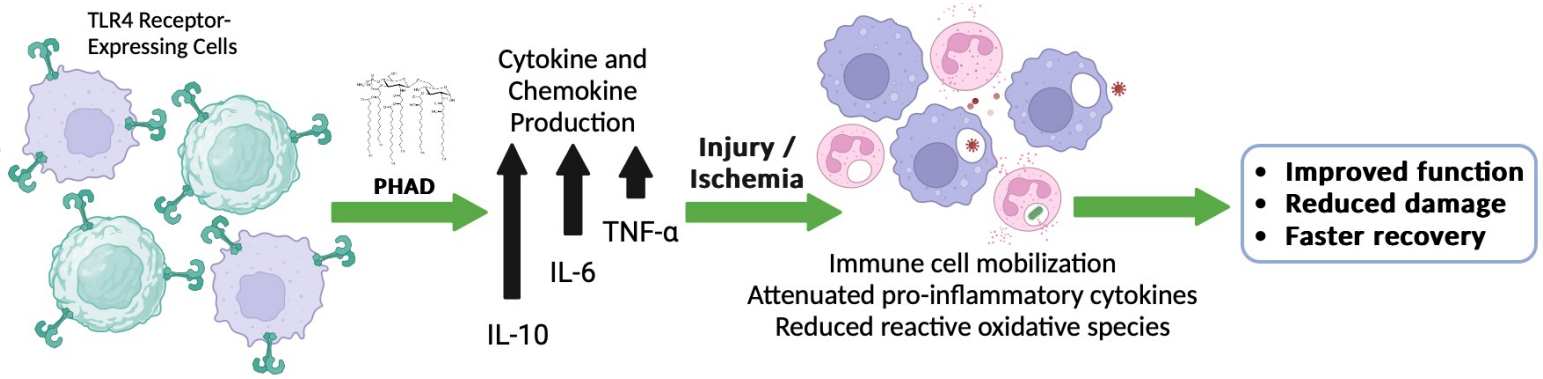
REVELATION BIOSCIENCES

Oral Presentation at the 29th International Conference on Advances in Critical Care Nephrology: AKI and CRRT - March 2024

Gemini Reprograms Toll-Like Receptor 4 Signaling

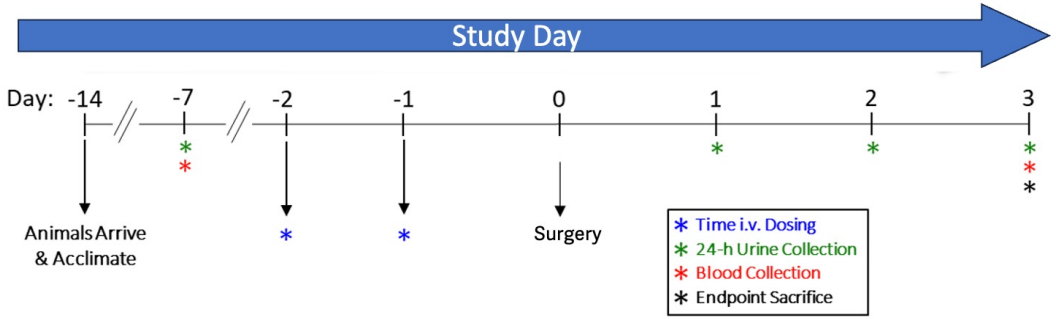
Introduction

- Gemini is a proprietary formulation of phosphorylated hexaacyl disaccharide (PHAD), a synthetic, detoxified version of lipopolysaccharide (LPS).
- PHAD stimulates TLR4 to precondition immune cells, including early, robust induction of IL-10, followed by an attenuated induction of IL-6, and TNF-α.



- Following injury (e.g. ischemia), immunostimulatory preconditioning contributes to mobilization of innate immune cells, a reduced pro-inflammatory response, and a reduction in reactive oxidative species.
- Immunostimulatory preconditioning was shown to provide significant improvement in function, less tissue damage, and accelerated resolution of injury in multiple models of kidney injury (I/R and UUO).

Methods: Ischemia/Reperfusion AKI Model[1]



- Rats (n=8-28 per group) were administered vehicle or Gemini 24 and/or 48 hours prior to I/R surgery (30-minute ischemia) at different dose levels (0.07 and 0.35 mg/kg).
- In addition to clinical observation, rats were evaluated for kidney function and functional biomarkers at 24 and 72 hours post surgery and kidney damage at 72 hours (sacrifice) post surgery.

[1] Skrypnyk NI, et. al. Ischemia-reperfusion model of acute kidney injury and post injury fibrosis in mice. J Vis Exp. 2013 Aug 9;(78):50495. doi: 10.3791/50495. PMID: 23963468; PMCID: PMC3854859.

Gemini Preserves Kidney Function and Reduces Injury

Kidney Function at 24 and 72 hours

Figure 1a. Change in Creatinine[1]

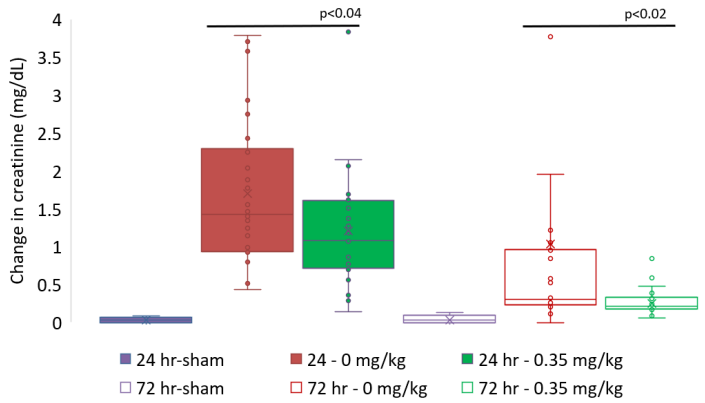
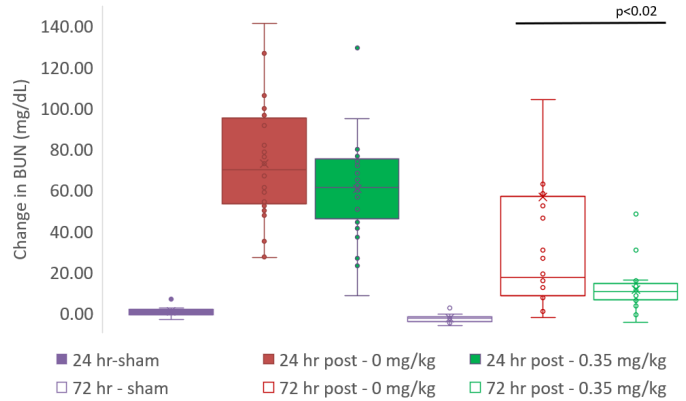


Figure 1b. Change in BUN[1]



- Gemini pretreatment (0.35 mg/kg) significantly preserved kidney function with return to base line faster relative to untreated control animals (Figure 1a and 1b). A similar trend was observed for 0.07 mg/kg (data not shown).
- Additional renal function findings (data not shown) included improved creatinine clearance and excretion relative to the untreated group

Kidney Injury at 72 hours

Figure 2a. Acute Cortical Tubular Necrosis[2]

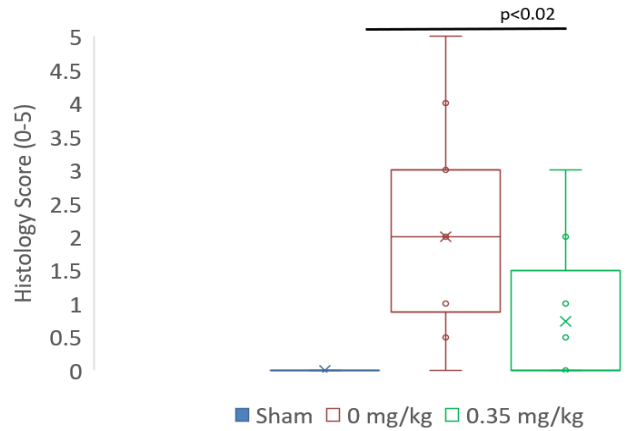
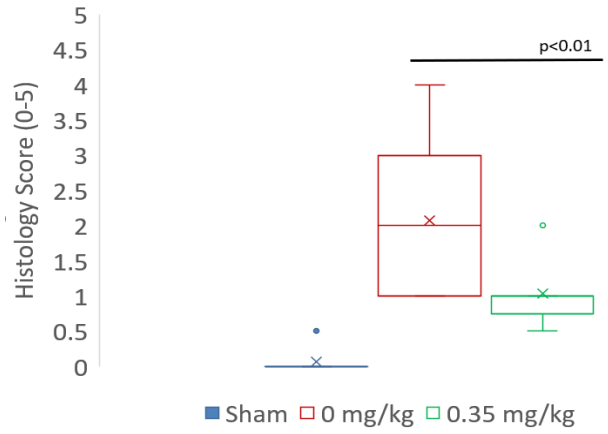


Figure 2b. Medullary Tubular Necrosis[2]



- Gemini reduced injury to the cortical and medullary tubules as measured by histopathology (Figure 2a and 2b). A similar trend was observed for 0.07 mg/kg (data not shown).
- Additional findings: Gemini did not significantly lower cortical or medullary tubular degeneration or tubular protein casts included (data not shown) relative to the untreated group.

[1]N=16-28, dosed 24 and/or 48 hours prior to surgery. [2]N=8-14, dosed 24 hours prior to surgery.

Gemini Significantly Attenuates Inflammatory Response

Figure 3a. Urinary CRP at 24 hours[1]

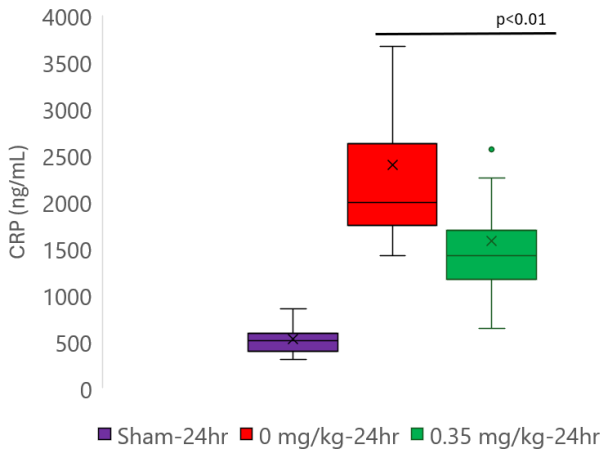


Figure 3b. Urinary IL-6 at 24 hours[1]

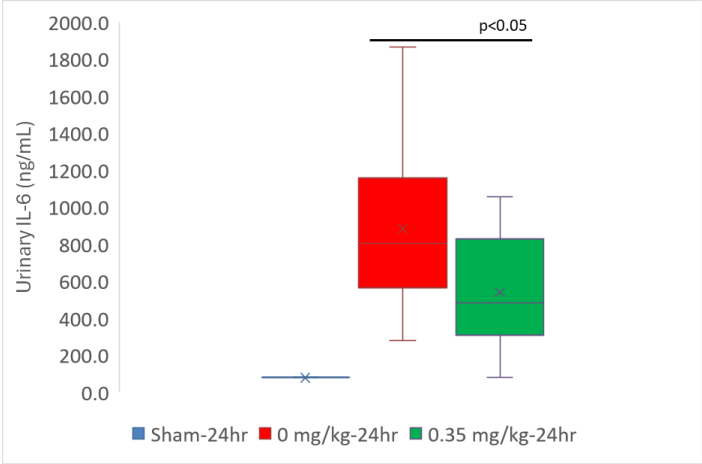


Figure 3c. Serum CRP at 72 hours[1]

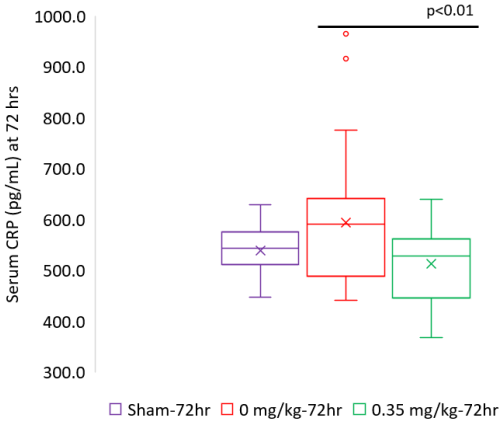
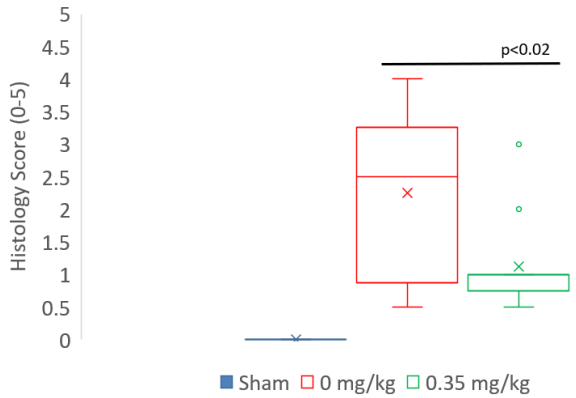


Figure 3d. Neutrophilic Inflammation at 72 hours[2]



- Pretreatment with Gemini (0.35 mg/kg) significantly reduced multiple markers of local inflammation at 24 and/or 72 hours in urine (Figure 3a and 3b, 72-hour data not shown).
- Pretreatment with Gemini (0.35 mg/kg) significantly reduced a key marker (c reactive protein, CRP) of systemic inflammation at 72 hours in serum (Figure 3c).
- Pretreatment with Gemini also significantly reduced markers of cellular inflammation as observed via reduced neutrophilic inflammation (Figure 3d).
- Additional markers of reduced cellular inflammation observed (data not shared).

[1]N=16-28, dosed 24 and/or 48 hours prior to surgery. [2]N=8-14, dosed 24 hours prior to surgery.

Gemini Clinical Evaluation Initiated

Discussion

- Improved kidney function and reduced necrosis likely due to reduction in pro-inflammatory activities, as observed in reduced neutrophil inflammation and reduced local and systemic CRP and IL-6 levels
- Gemini may halt necroinflammation feedback loop by reducing proinflammatory signals that contribute to cellular necrosis, effectively improving organ health
    - Additional indications of improved kidney health include local and systemic changes in NGAL and HO-1 (results not reported here)
- Study limitations: rodent to human translation, sample timing and volume (limited), magnitude of effect of treatment corresponds to AKI severity

Conclusions

- Immunostimulatory preconditioning with Gemini reduces tissue damage and improves kidney function in a rat model of bilateral ischemic reperfusion induced acute kidney injury
- Phase 1 clinical studies with Gemini initiated in February 2024

Intravenous Gemini™ for Prevention of Acute Kidney Injury

Prophylactic Administration of Gemini Reduces Tissue Damage and Improves Kidney Function in a Rat Model of Bilateral Ischemic Reperfusion Induced Acute Kidney Injury

Robin Marsden[1], James Rolke[1], Jackson Stephens[1], and Allie Zygmont[1], [1]Revelation Biosciences

Abstract

Purpose and Background

The purpose of this study was to evaluate Gemini pretreatment in an ischemia reperfusion model of AKI. Due to its severe nature, AKI represents a significant health problem, especially in patients with co-morbidities such as diabetes. Approximately 1% of all hospitalized patients present with AKI upon admission. Phosphorylated hexaacyl disaccharide (PHAD), the active ingredient in Gemini, is a synthetic small molecule that preconditions the innate immune response via a more selective activation of toll-like receptor 4 (TLR4), characterized by an attenuated pro-inflammatory response relative to traditional TLR4 agonists such as lipopolysaccharide (LPS), while retaining the capacity to engage the innate immune response. We hypothesize that Gemini preconditioning will elicit an attenuated proinflammatory response following ischemic reperfusion (IR).

Methods

Rats were administered vehicle or Gemini intravenously at 0.07 and 0.35mg/kg at 24 and/or 48 hours prior to undergoing bilateral IR to induce acute kidney injury (AKI). A surgical sham group was also included. Blood and urine were collected pre-dose, and post-surgery at 24 and 72 hours (termination), and serum was assessed for BUN and creatinine levels. Urine was evaluated for c-reactive protein (CRP). Kidneys were evaluated for histological changes at 72 hours.

Results

Pretreatment with Gemini reduced serum BUN, serum creatinine and urine CRP in a dose dependent manner at 24 and 48 hours post-dose with significance at 0.35 mg/kg ($p<0.05$) relative to IR control. A single dose of 0.35mg/kg Gemini also significantly reduced the total degree of acute necrosis in cortical and medullary tubules ($p<0.05$). Neutrophil inflammation was significantly reduced ($p<0.05$) with a single pretreatment of Gemini at 0.35mg/kg.

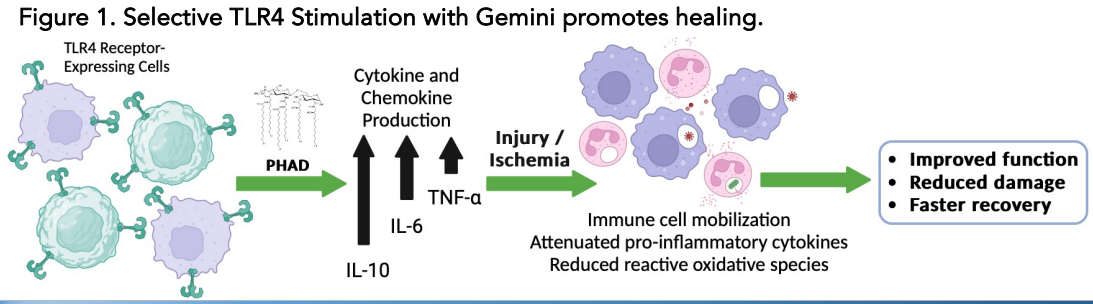
Conclusion

Pretreatment with Gemini significantly improved kidney function as demonstrated by reductions in BUN, creatinine and CRP, and reduced kidney injury as evident in the reductions in cortical and medullary tubular necrosis. Gemini also reduced AKI-related inflammation attributed to neutrophils in the kidney. These results demonstrate the premise of trained immunity as a means of prevention of AKI. The collective improvement in renal function along with the reductions in cellular necrosis and neutrophilic inflammation demonstrate Gemini pretreatment attenuates the severity of IR AKI.

Introduction

- Gemini is a proprietary formulation of phosphorylated hexaacyl disaccharide (PHAD), a synthetic, detoxified version of lipopolysaccharide (LPS).
- PHAD stimulates TLR4 to precondition immune cells, including early, robust induction of IL-10, followed by an attenuated induction of IL-6, and TNF-α.
- Following injury (e.g. ischemia), immunostimulatory preconditioning contributes to mobilization of innate immune cells, a reduced pro-inflammatory response, and a reduction in reactive oxidative species.
- Immunostimulatory preconditioning was shown to provide significant improvement in function, less tissue damage, and accelerated resolution of injury in multiple models of kidney injury (I/R and UUO).

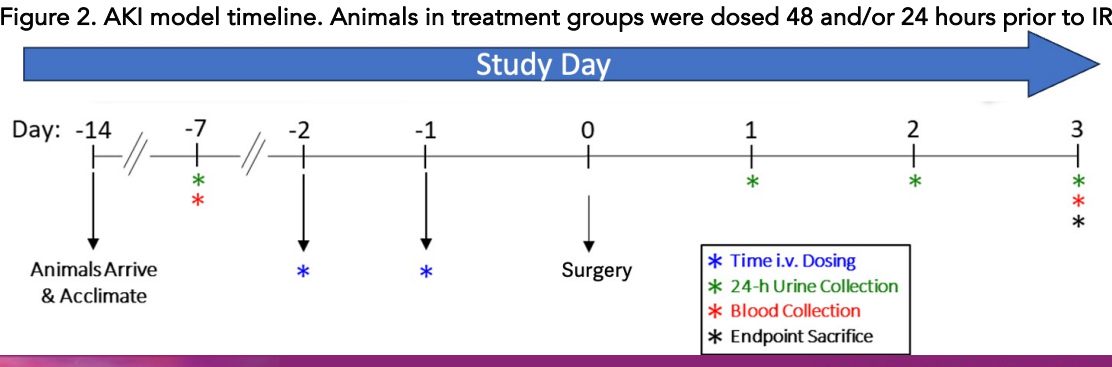
Figure 1. Selective TLR4 Stimulation with Gemini promotes healing.



Study Design and Timeline

- Rats (n=8-28 per group) were administered vehicle or Gemini 24 and/or 48 hours prior to IR surgery (30-minute ischemia) at different dose levels (0.07 and 0.35mg/kg).
- In addition to clinical observations, rats were evaluated for kidney function and functional biomarkers at 24 and 72 hours post surgery, and kidney damage at 72 hours (sacrifice) post surgery.

Figure 2. AKI model timeline. Animals in treatment groups were dosed 48 and/or 24 hours prior to IR.



Results

Administration of Gemini 24 hours prior to IR reduced kidney injury as demonstrated in the significantly reduced tissue injury, improved renal function, and attenuation of inflammatory markers. Specifically, as CRP is a general marker of inflammation, the reduction in urinary and serum CRP demonstrate an overall reduction of local and systemic inflammation.

The 0.07 mg/kg dose group also demonstrated non-statistically significant dose dependent reduction in Creatinine and BUN – data not shown.

Figure 3a. Acute Cortical Tubular Necrosis.

Figure 3b. Acute Medullary Tubular Necrosis.

Figure 4a. Change in Serum Creatinine.

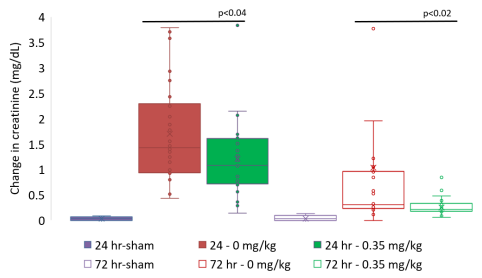


Figure 4b. Change in Serum BUN.

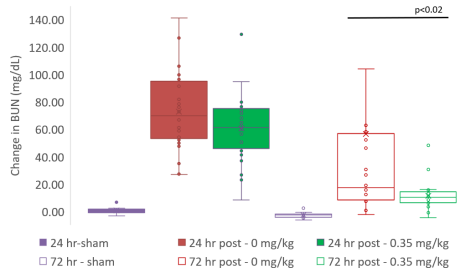


Figure 5a. Urinary CRP at 24 hours.

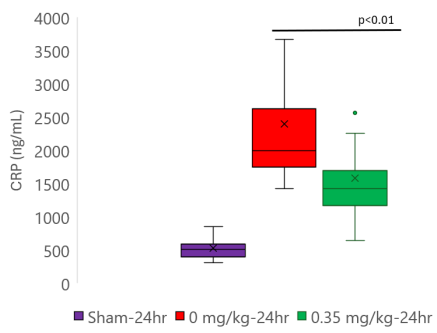


Figure 5b. Serum CRP at 72 hours.

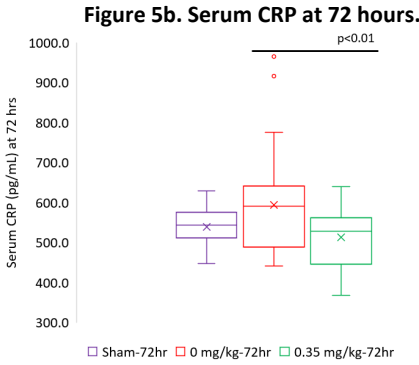


Figure 5c. Urinary IL-6 at 24 hours.

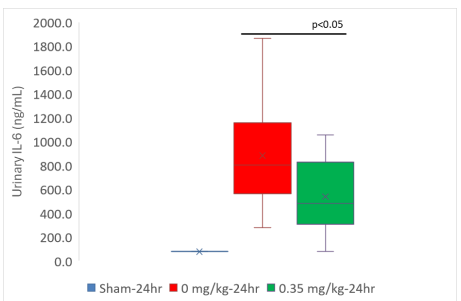
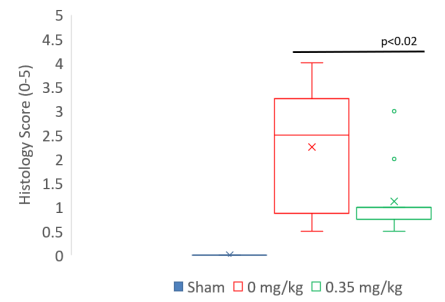


Figure 5d. Neutrophilic Inflammation in Kidney.



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