

# Cholesterol-siRNA Conjugates With Enhanced Stabilization Chemistry (ESC) Show Efficient Myostatin Silencing in NHP

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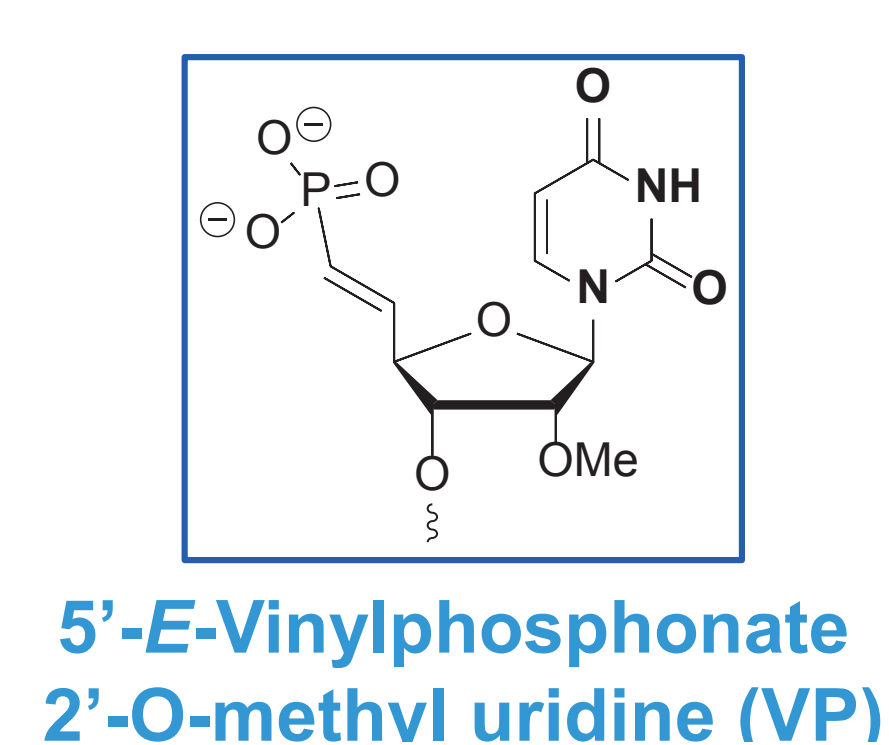
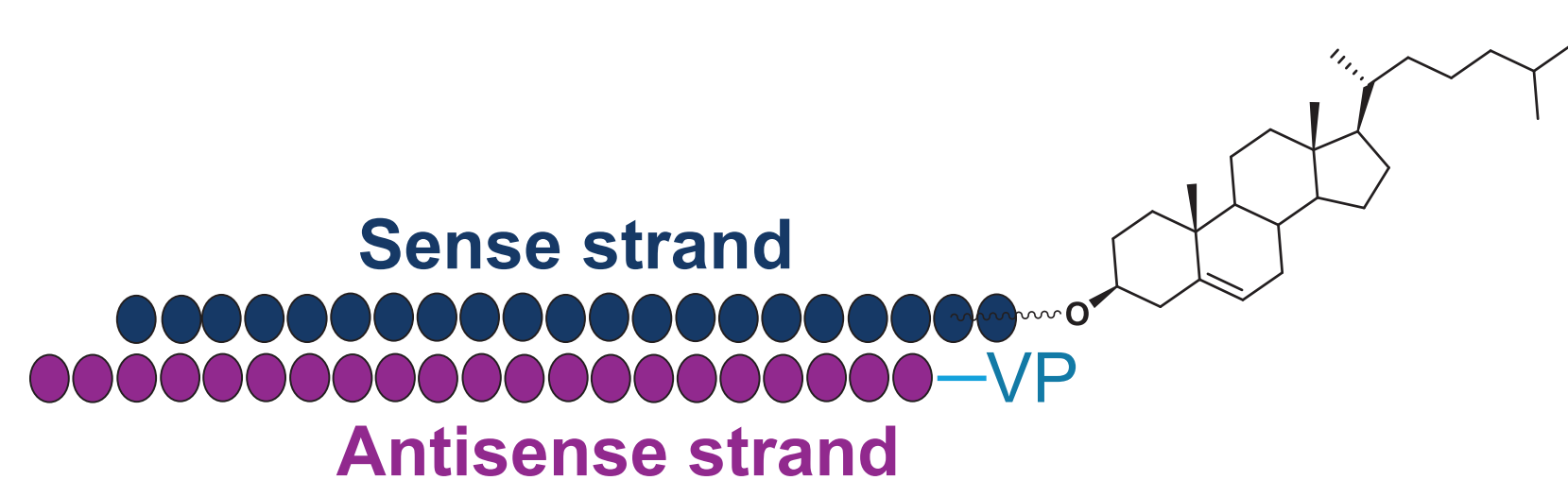
## Background

Significant progress has been made for hepatic delivery with N-acetylgalactosamine (GalNAc)-siRNA conjugates using enhanced stabilization chemistry (ESC). This delivery approach has been successfully translated into humans with demonstrated silencing of therapeutically relevant targets across multiple clinical programs. Efficient delivery of siRNAs to extra-hepatic tissues such as ocular, brain, tumour and muscle have been achieved *in rodent models*, with limited success in clinic. The successful application of siRNA for extra-hepatic therapy requires the development of conjugates or delivery systems that results in appropriate exposure levels in the target tissues, while maintaining optimal potency, specificity and safety. Recently, we reported the silencing of myostatin gene in muscle using cholesterol-siRNA conjugates following single intravenous injection in mice<sup>1</sup>. Here we describe refined conjugate designs, incorporating ESC chemistry that translated favourably to non-human primates, suggesting the potential use of these conjugates in treating muscle disorders.

### Selection of Myostatin (Mstn) siRNA-Conjugate

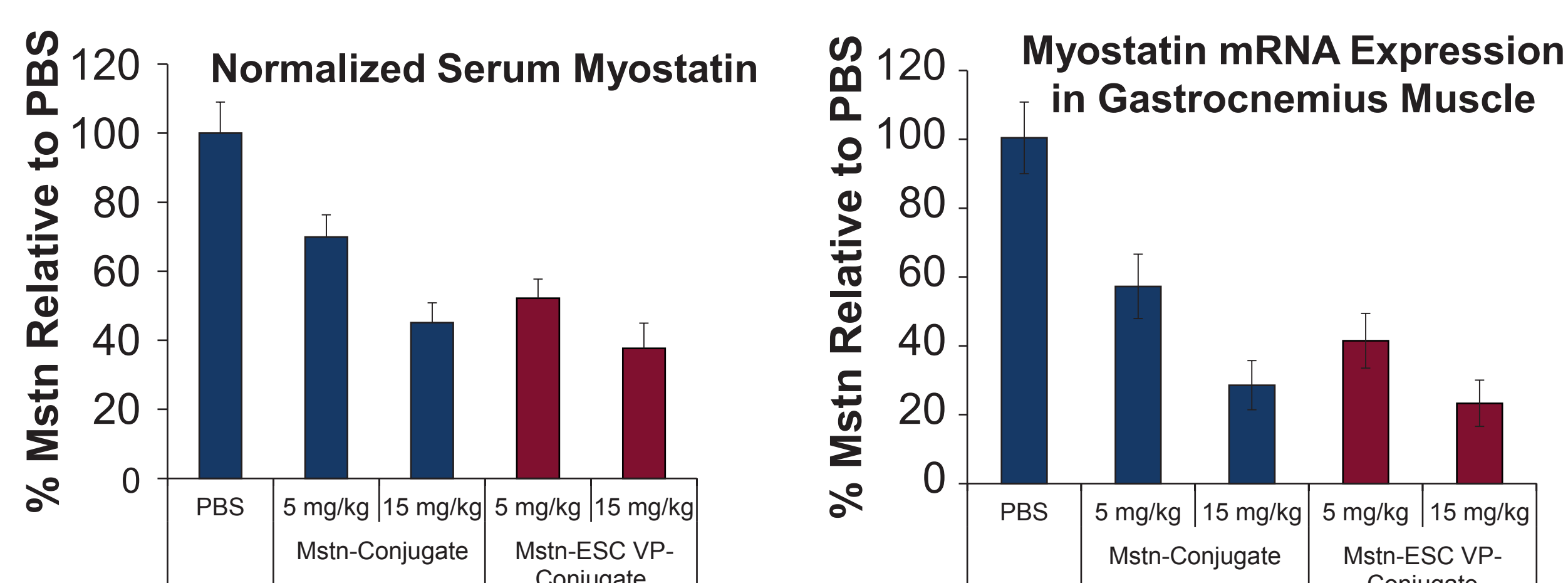
- Myostatin, a gene expressed at high levels in muscle, was chosen to evaluate siRNA delivery to muscle<sup>2</sup>
- To identify lead siRNAs a myostatin dual luciferase reporter assay in Hepa1-6 cells was used under standard lipid transfection conditions
- siRNA leads were converted to cholesterol conjugates and evaluated *in vivo* by IV injection<sup>1</sup>
  - Cholesterol was conjugated to the 3' end of the sense strand by a non-cleavable linker
  - Vinylphosphonate (VP), a stable phosphate mimic was incorporated at the 5' end of antisense strand to improve RISC loading<sup>3</sup>
- Initial *in vivo* POC was achieved using Merck's siRNA chemistry
  - 75% mRNA myostatin silencing was observed in multiple muscles at 72 h post a single 15 mg/kg IV dose, accompanied by 50 % decrease in myostatin protein serum levels<sup>1</sup>
- Further siRNA-cholesterol conjugate optimization was achieved by incorporating enhanced stabilization chemistry (ESC)

### Myostatin (Mstn) siRNA Cholesterol Conjugate



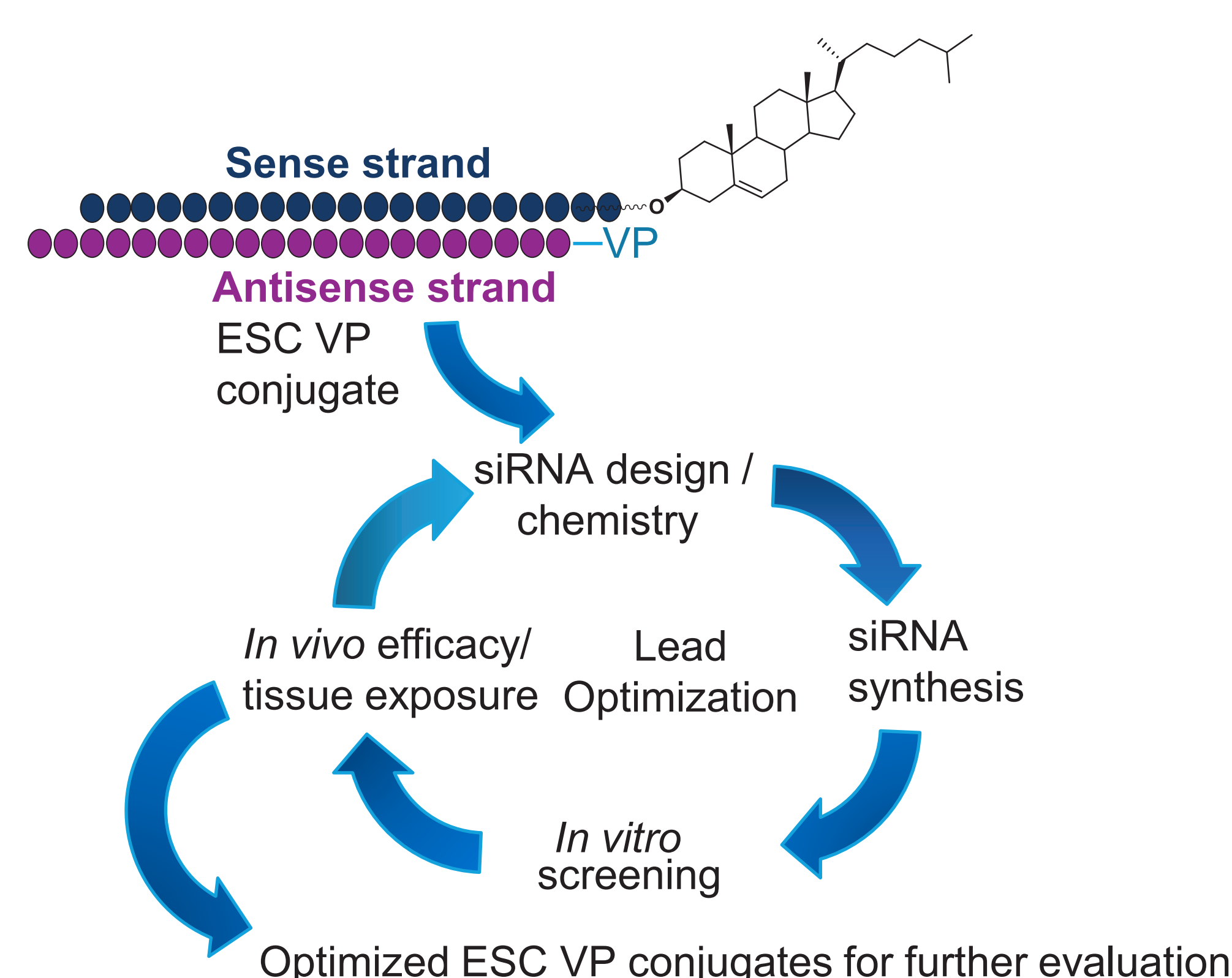
### Mouse Myostatin Knockdown by Cholesterol siRNA Conjugates

#### Single IV Dose of Mstn-siRNA Cholesterol Conjugates in Mice (Day 7)



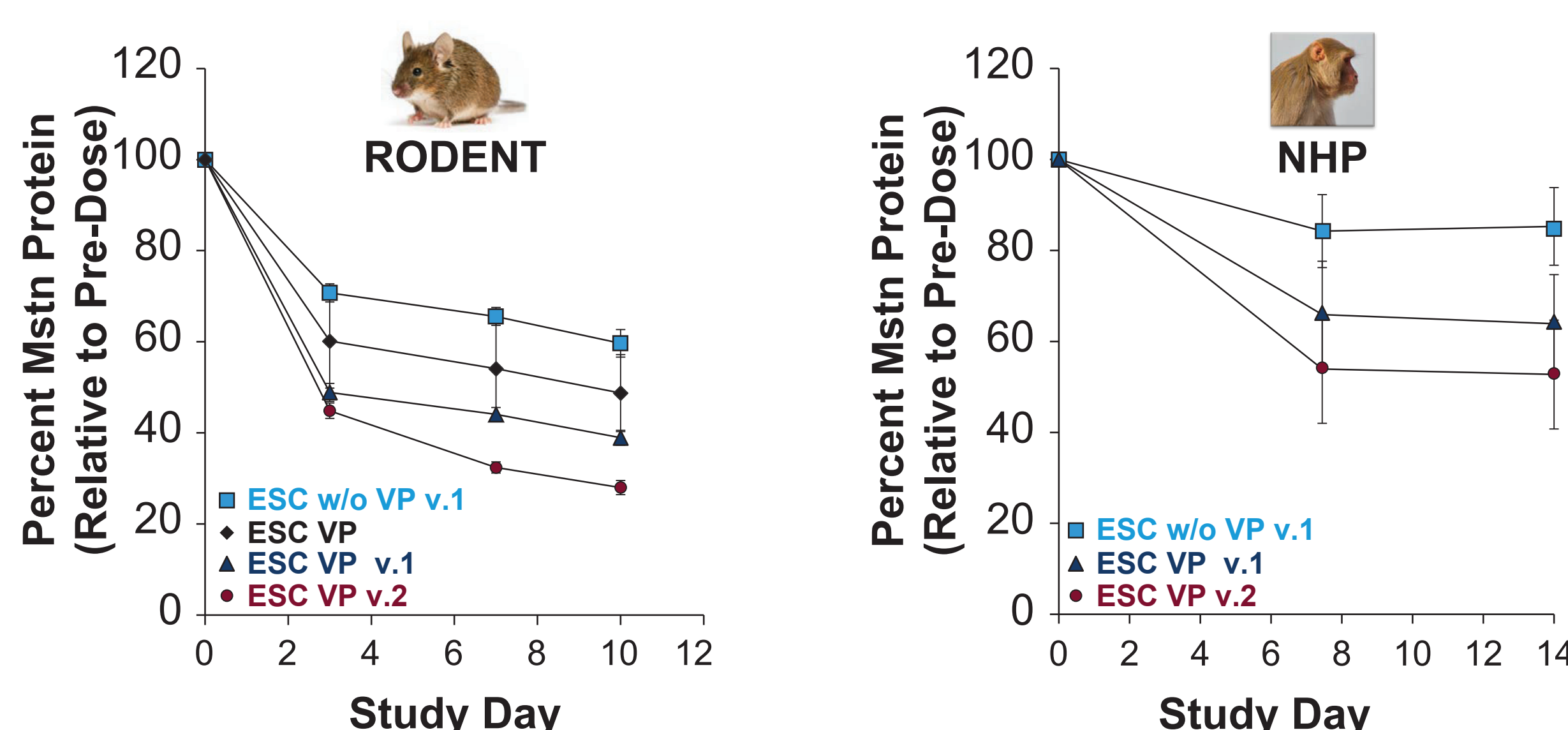
- Mstn-conjugate indicates siRNA with Merck chemistry design<sup>1</sup>
- Mstn-ESC VP-conjugate indicates siRNA design containing enhanced stabilization chemistry with vinylphosphonate modification at 5' end of antisense strand

### Further Optimization of Mstn-ESC VP-Conjugate

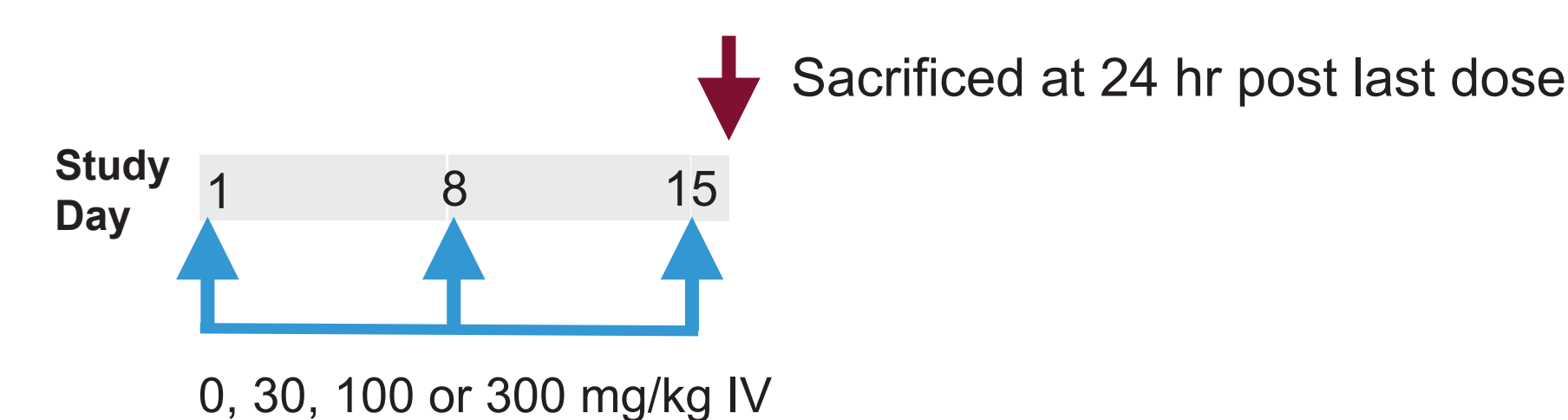


### Muscle Potency Gains in Mice and NHP by Further Chemistry Optimization

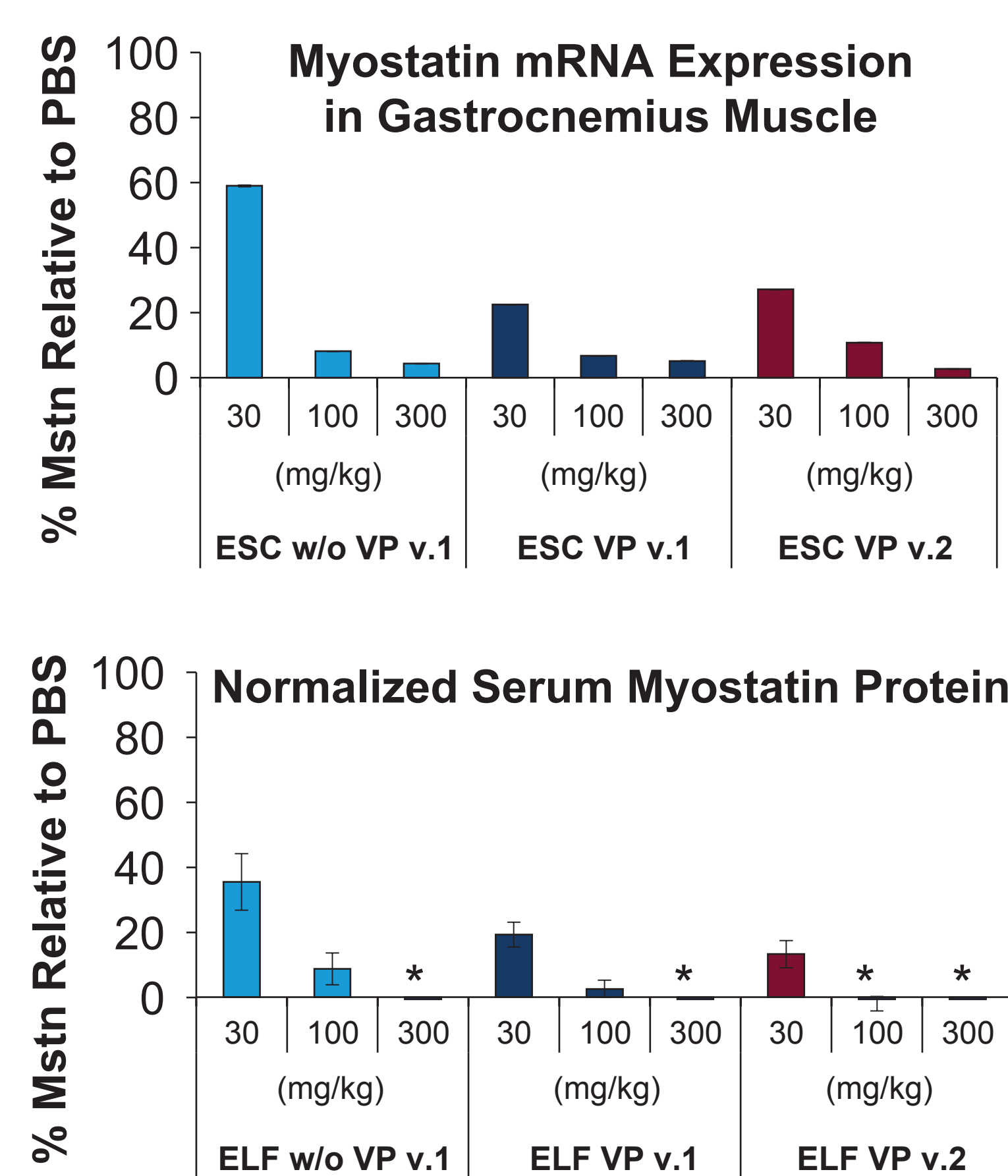
#### Serum Myostatin Levels After a Single 5 mg/kg IV Dose



### Rat Toxicokinetic Study of Myostatin-siRNA Conjugates



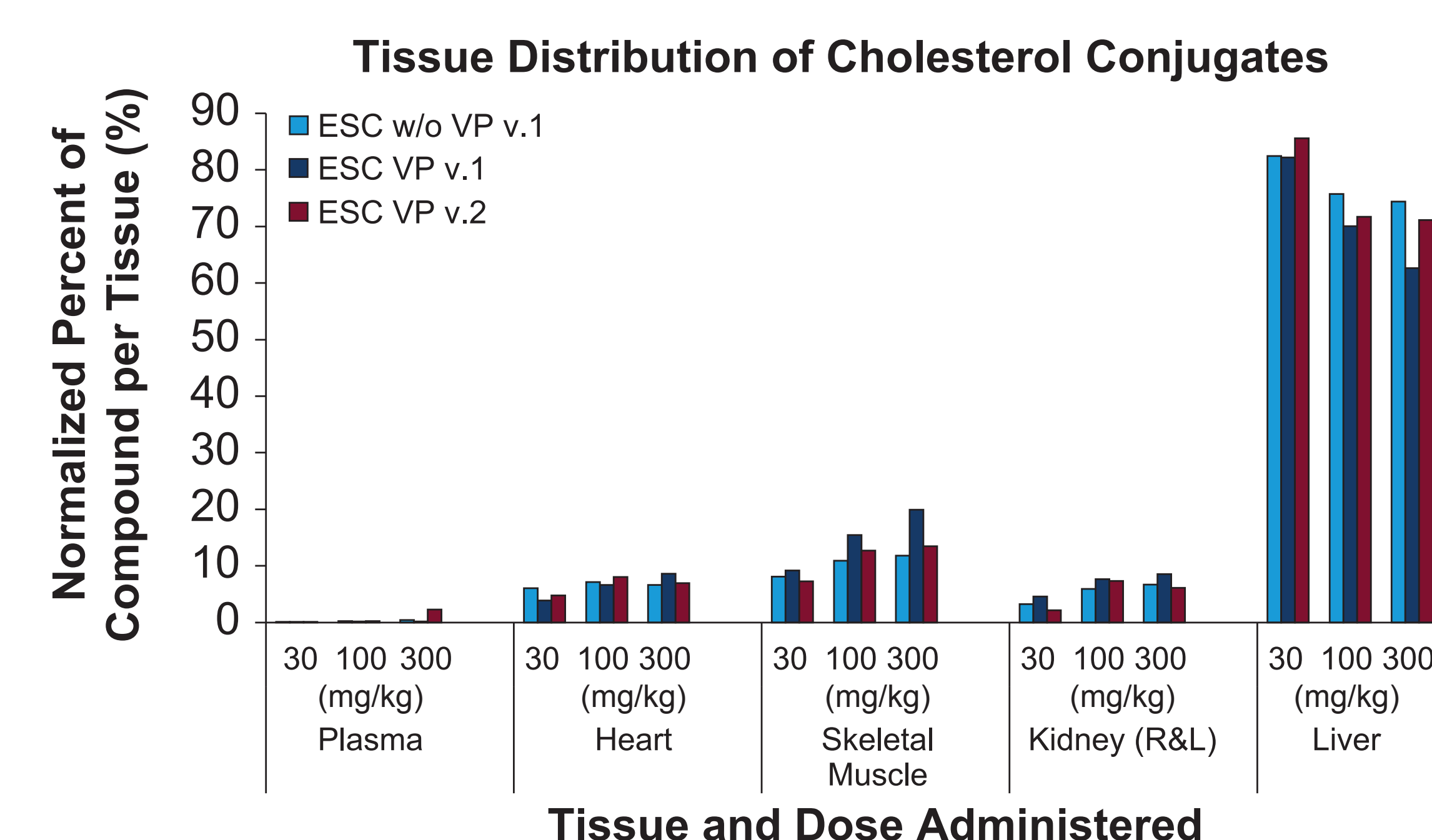
#### A Robust Myostatin Knockdown at Day 16 Following qwx3 IV Doses



\* = Below detection limit

### Rat Toxicokinetic Study of Myostatin-siRNA Conjugates (cont'd)

#### B Percentage of Injected siRNA Dose



- Highest accumulation of cholesterol conjugates observed in liver followed by kidney
- Cholesterol conjugates are known to associate with lipoproteins, which mediate tissue distribution including muscle and cellular uptake<sup>4</sup>

#### C Toxicology Summary

- Target organ across compounds included liver, kidney, heart and injection site
  - Main findings were vacuolated macrophages in the liver, kidney, and heart and injection site inflammation (minimal to moderate)
  - No test article related liver, skeletal muscle or heart necrosis or ALT/AST increases
- Most of the toxicity findings were limited to the high dose groups at 300 mg/kg

## Conclusions

- First time demonstration of muscle specific gene knockdown in non-human primates using cholesterol-siRNA conjugates targeting myostatin
- Further optimization of siRNA chemistry yielded approximately 3-fold increase in potency
- Robust target silencing observed despite relatively low siRNA accumulation in muscle compared to liver
- Preliminary data from preclinical toxicology studies suggest favorable safety profile for further development

## References

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