

# Annexin V-6L15, A Novel Injury-Site Directed Anticoagulant Ameliorates Ischemic-Reperfusion Injury and Promotes Survival of Ischemic Rat Abdominal Fasciocutaneous Flaps

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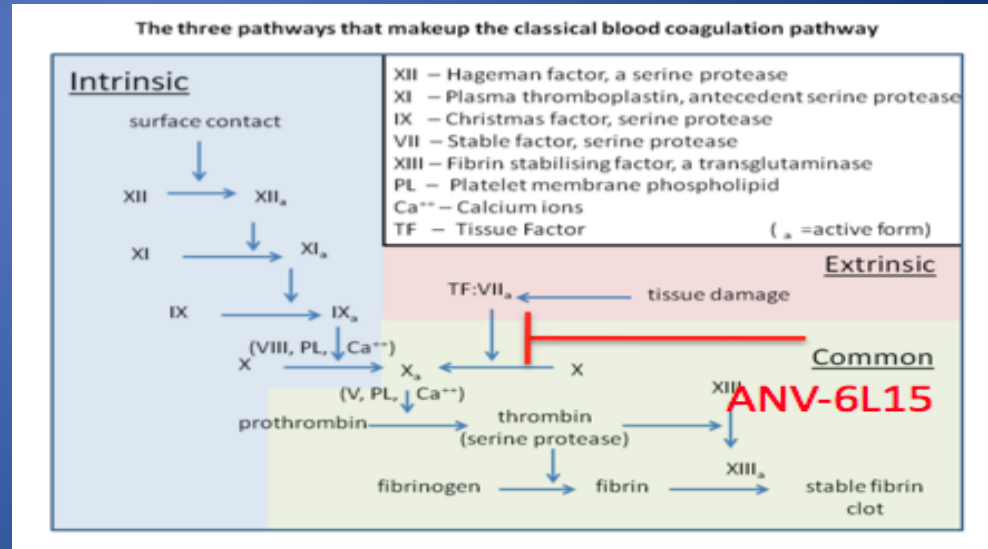
•Disclosures: The authors have no financial interests or other conflicts of interest to disclose

# Background

- Imperfect technique, inappropriate anticoagulation or perfusion facilitation, extended ischemia time are key reasons for partial flap failure
- Current anticoagulants: aspirin, warfarin, heparin, LMWH, direct thrombin inhibitors  
→ systemic effects may lead to bleeding risk

# Design of Annexin V-6L15

- Fusion protein: Annexin V (ANV) + 6L15
- Acts as Tissue factor-VIIa pathway inhibitor



# Mechanism of action

- Annexin V: targets phosphatidylserine (PS) binding sites, which are expressed in apoptotic/injured endothelial cells
- 6L15: aprotinin mutant (KPI domain) with inhibitory activity towards VIIa-TF complex

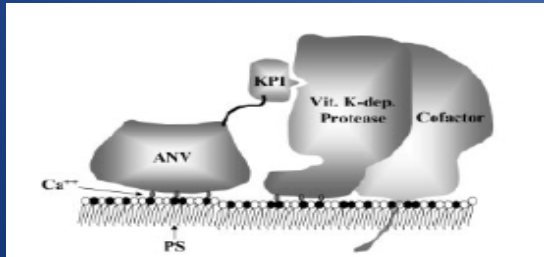
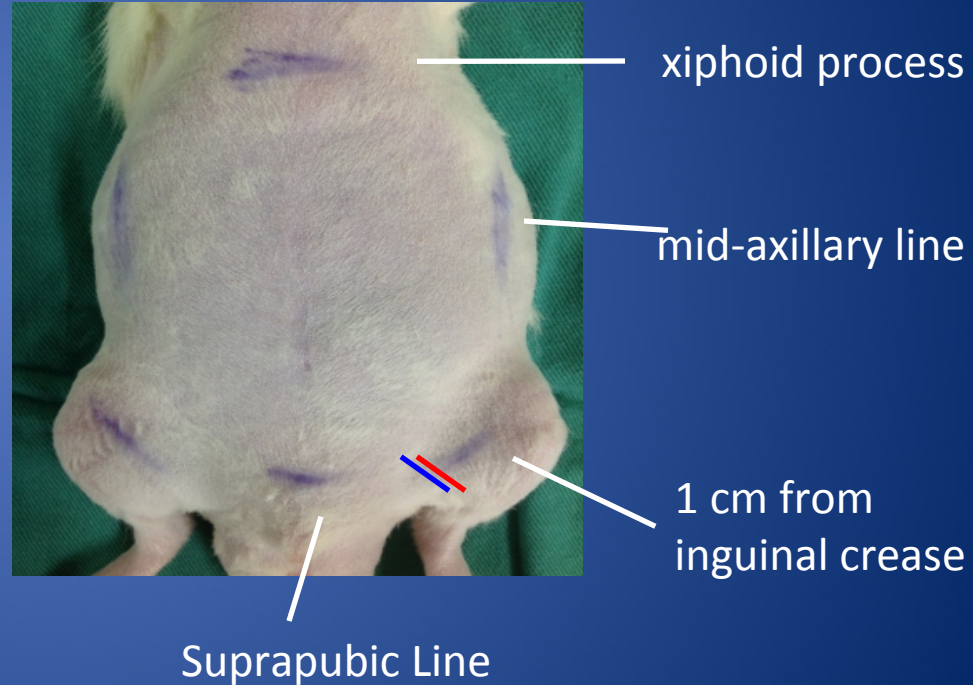


Image source: Chen HH, et al. *Blood* (2005)

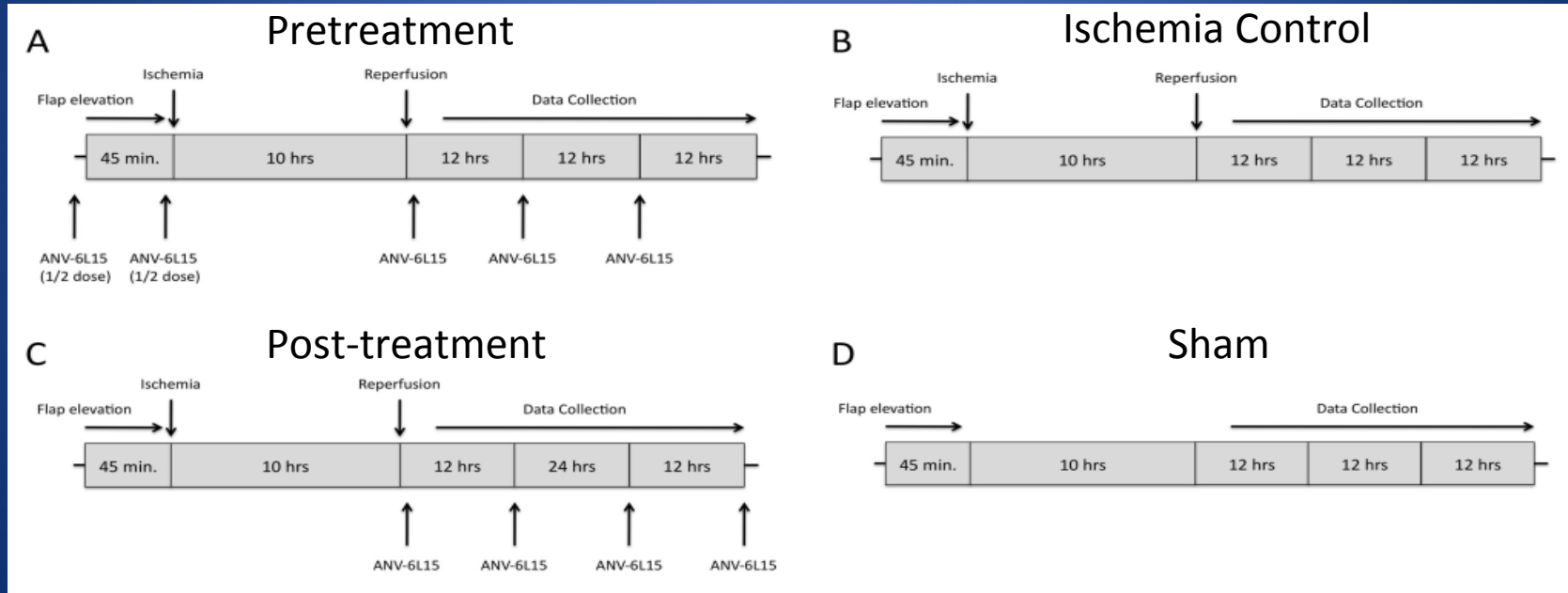
# Abdominal Fasciocutaneous Flap Model

- Based on unilateral superficial inferior epigastric artery (SIEA)
- Pedicle extension to proximal femoral artery/vein
- Artery clamped, No anastomosis



# Treatment Protocols- Flap model

- Drug administered through penile vein (n=6)
1. Dosage: 200 $\mu$ g/kg, total 4 dosages

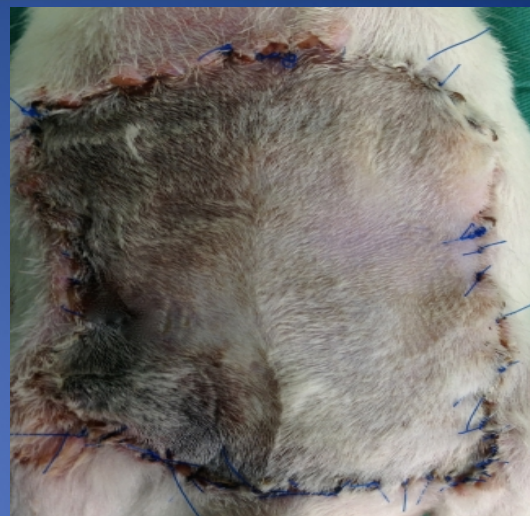


Sham



84.7±4.6%

Control



48%±8.7%

Day 5  
Survival

Post-Rx

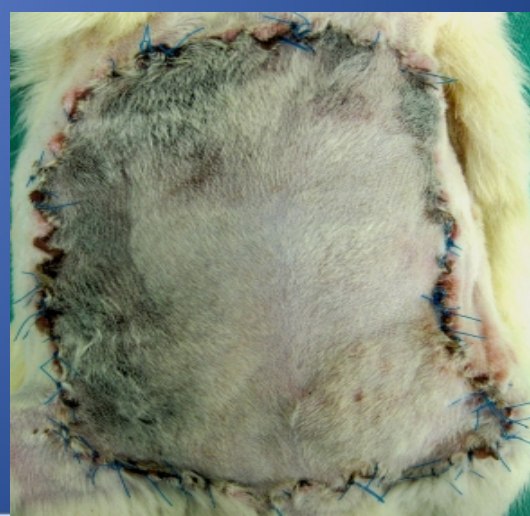


61.2±6.9%

ANOVA:  
 $p < 0.0001$

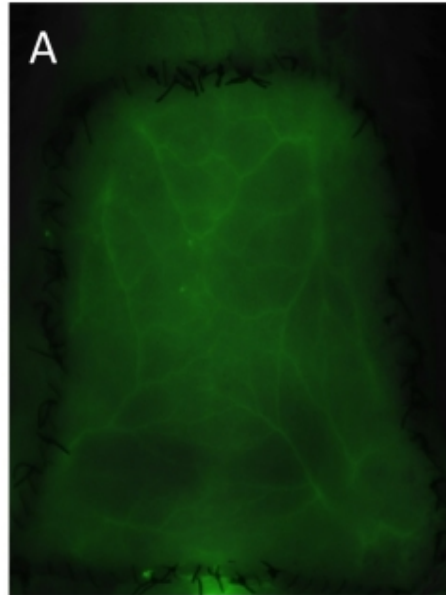
Post-hoc test:  
All vs. Sham  
Post vs. Control  
Pre vs. Control  
 $p < 0.05$

Pre-Rx

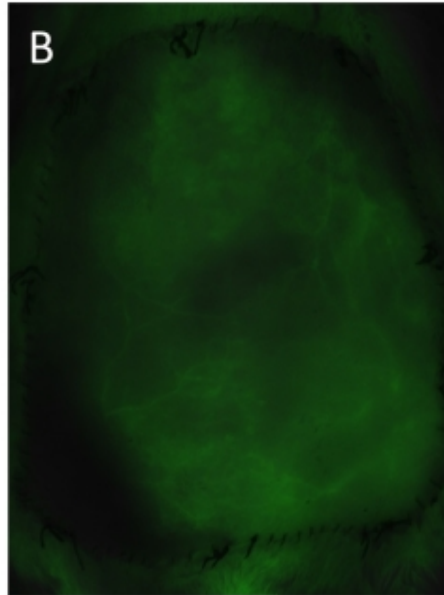


59.3±6.9%

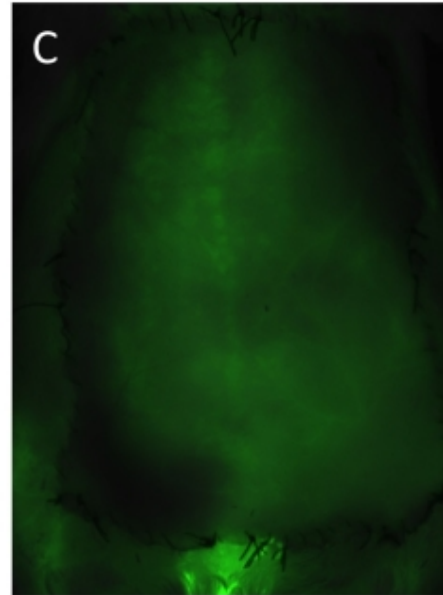
# Near-IR Perfusion Imaging



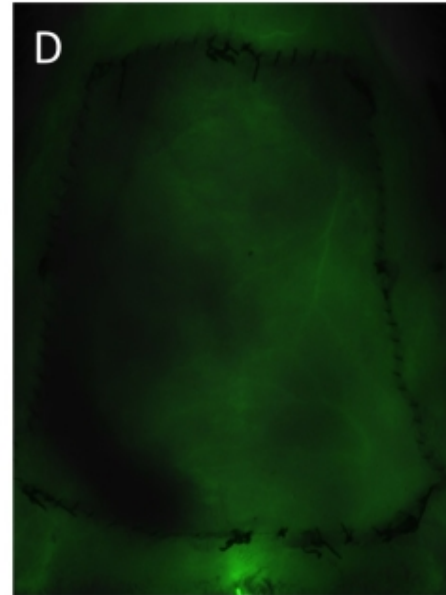
Sham



Post-treatment



Pre-treatment

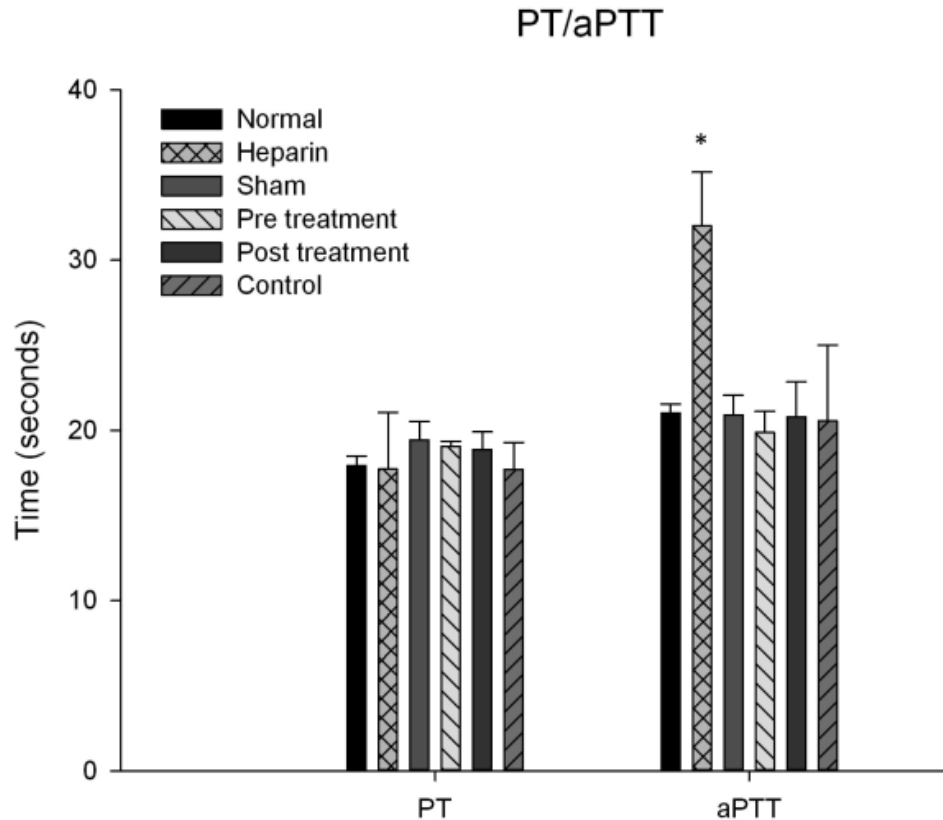


Control

Near-IR perfusion imaging with novel imaging agent at 36 hours post op  
\*Dark zones are poorly perfused



# PT/aPTT Assay

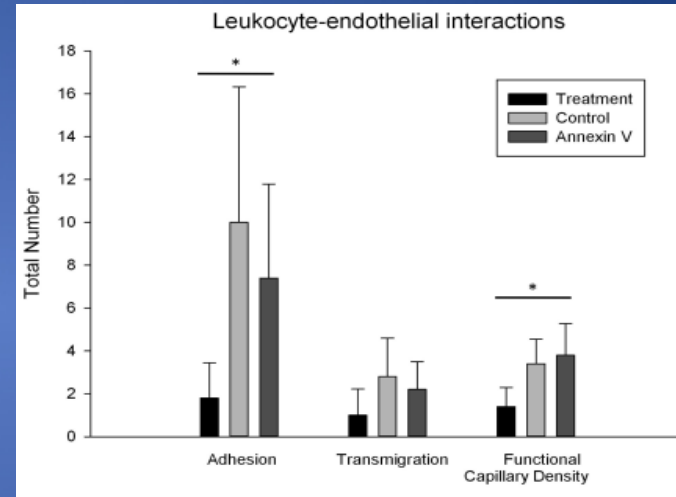


- Samples: Day 2 (n=5~6)
- Positive control: unfractionated heparin (300u/kg)
- Blood samples taken 5 min. post Rx
- The Ceveron alpha (Technoclone GmbH, Austria) automated blood coagulation analyzer was used. Reagents: Technoplastin<sup>®</sup> HIS for PT and Siron LS for aPTT.

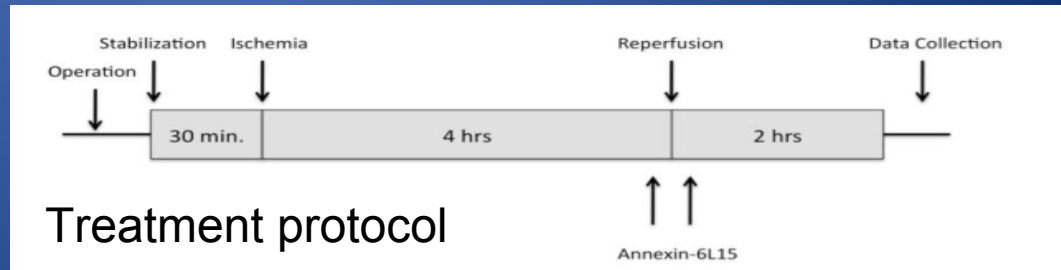
# Cremaster model

- Drug administered through right JV cannula
- Dosage: 200 $\mu$ g/kg
- Groups: Treatment vs. Ischemia vs. ANV

1. Post stabilization baseline taken

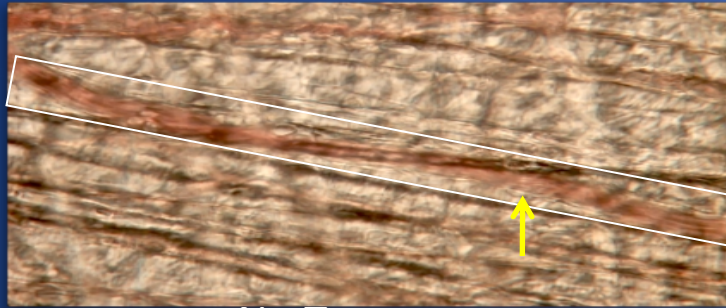


Intravital microscope observations (n=5)



# Leukocyte-endothelial Interaction

Baseline

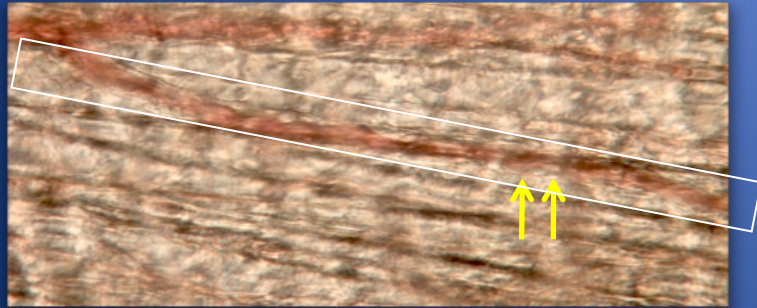


No Treatment



Treatment

Post I-R



Increased number of leukocytes



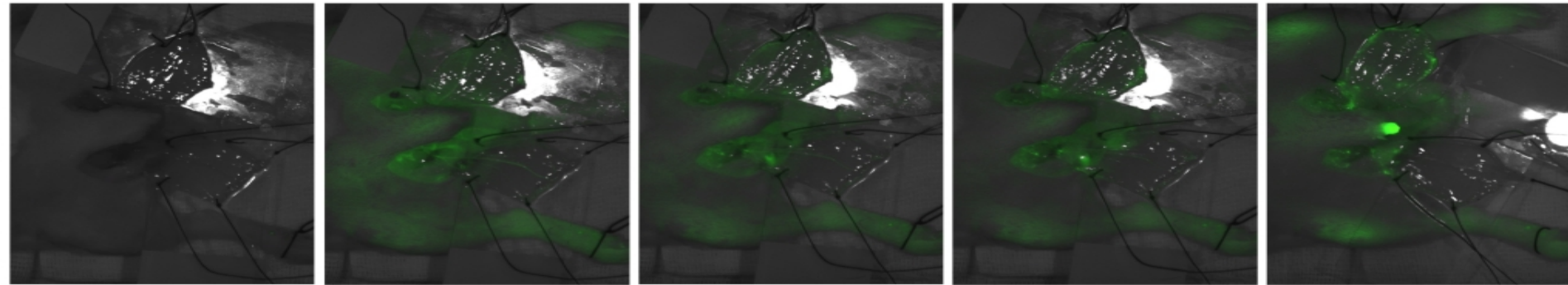
No increase



# Distribution and Excretion



Near-IR fluorescent probe conjugated ANV-6l15 study



blank

1 min

5 min

10 min

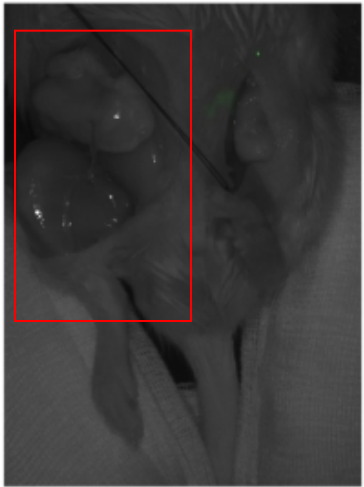
2 hrs

1. Instantaneous distribution of ANV-6l15 into cremaster circulation
2. Fluorescence begins to fade by 10 minutes
3. Excretion of ANV-6l15 by 2 hrs (penis)

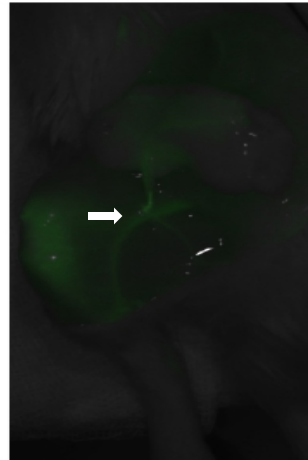
# Specific Binding & Duration

Near-IR fluorescent probe conjugated ANV-6I15 study

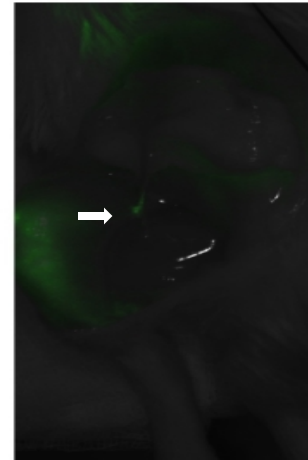
- Specific binding at SIEA vessel trauma site (white arrows)
  1. Binding for up to 24 hours



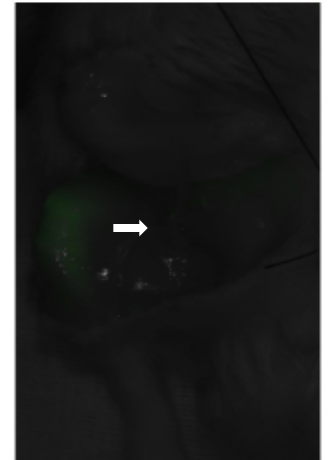
Blank



5 min



1 hour



24 hours

# Conclusion & Significance

- ANV-6L15, a site-targeted, novel anticoagulant demonstrates effects in ameliorating ischemic injury within flaps
- May work through promoting perfusion, preventing thrombosis, and amelioration of ischemic-reperfusion injury related leukocyte adhesion and capillary dysfunction
- ANV-6L15: new class of anticoagulants with no effects on PT/APTT at dosages that promote flap survival