

Nanotechnology in Cerebrovascular Care

Conflict: Founder/Co-founder
AxoNeural Therapeutics, Inc.
ProTransit Nanotherapy, LLC
Advanced NanoTherapies, Inc.
Issued and Pending Patents

Vinod Labhasetwar, Ph.D.
Department of Biomedical Engineering
Lerner Research Institute
Taussig Cancer Institute
Cleveland Clinic, Cleveland, OH



What is nanomedicine?

- Exploring nanotechnology for therapy and/or imaging
- Small size, typically 100-300 nm in diameter.
- Made of polymers/lipids/metals



Lyophilized
Nanoparticles



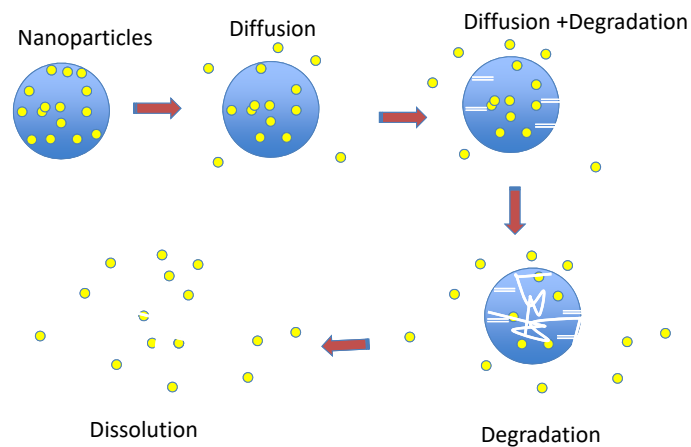
TEM Picture

Bar= 100 nm

Nanoparticle-mediated drug delivery

- Stabilize therapeutic agents (proteins, enzymes)
- Sustain drug effect
- Alter biodistribution
- Achieve targeted drug delivery
- Reduce dosing frequency/better compliance
- Reduce toxicity
- Enhance cellular/tissue drug uptake and retention

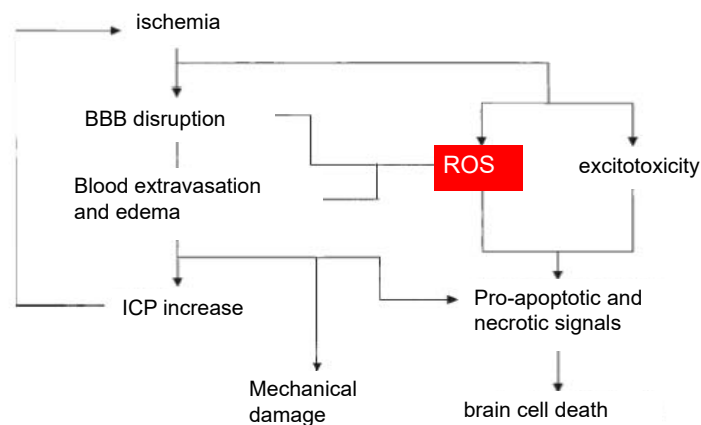
Sustained Release Mechanism (Weeks to Months)



CNS injury: Cascade of Degenerative Events

- Under normal conditions, CNS contains high levels of endogenous antioxidants
- To counteract reactive oxygen species (ROS) formed due to metabolism of neurotransmitters and excitatory amino acids
- This balance is lost in neuronal injury due to overproduction of ROS; rapid consumption of antioxidants, downregulation of genes.
- Loss of redox balance causes oxidative stress
- Triggering a cascade of degenerative events
 - inflammation,
 - excitotoxicity, apoptosis, lipid peroxidation, DNA damage
 - hyperglycemia,
 - breakdown of blood-brain barrier/blood-spinal barrier,
 - edema formation
- Neuronal cells are more susceptible to oxidative damage because they are rich in unsaturated lipids

Cascade of Degenerative Events Following Ischemic Stroke



Rationale and Hypothesis

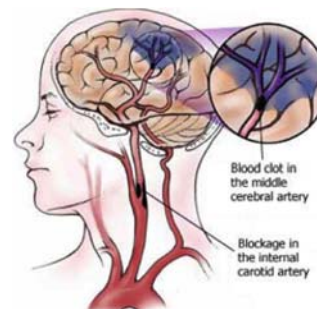
- Effective delivery of antioxidant(s) at the injured site can neutralize excessive ROS formed
- Antioxidant enzymes such as superoxide dismutase (SOD) or catalase (CAT) are ineffective because of their short-half life (half-life, 8-11 min)
- PEGylated enzymes (half life, ~40 h) or lecithinized SOD half-life (~25 to 31 h) but are ineffective because of their poor tissue/cellular uptake
- Neuronal Protective Nanoparticles (SOD and CAT) encapsulated in PLGA polymer with sustained release of active enzymes (Pro-NPs)

Hypothesis: Effective delivery of antioxidant enzyme loaded nanoparticles would mitigate the oxidative stress related cascade of degenerative events and stimulate the endogenous repair mechanism(s) involving neuronal and circulating progenitor cells.

Stroke, Spinal Cord Injury and Traumatic Brain Injury (TBI, blast associated)

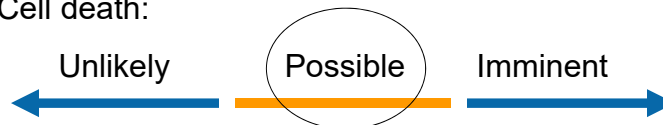
Ischemic Stroke

- 85 to 90% of cases are ischemic stroke
- Results in rapid onset of neurological impairment
- Third leading cause of death and disability in adults
- Significant brain damage occurs following restitution of blood flow, known as reperfusion injury



<http://usd316.k12.ks.us/projectfolder/valerie/pictureofstroke.jpg>

Cell death:



Current Therapy/Intervention

- Fibrinolytic agent, tissue plasminogen activator (t-PA) is the only approved treatment in the US.
 - Window of treatment 3 - 4.5 hrs after the stroke,
 - Only ~5% of patients can receive t-PA therapy.
 - t-PA is neurotoxic and aggravates reperfusion injury
- Endovascular thrombectomy (EVT)
 - Large vessel occlusion only
 - Does not prevent reperfusion injury
 - Most effective within ~4.5 hr post stroke
 - Must be performed by trained neurointerventionalists,
 - Associated with device-related vessel injury

Objectives

- **Reduce t-PA associated complications**
- **Extend the window for treatment**

Middle Cerebral Artery Occlusion Model

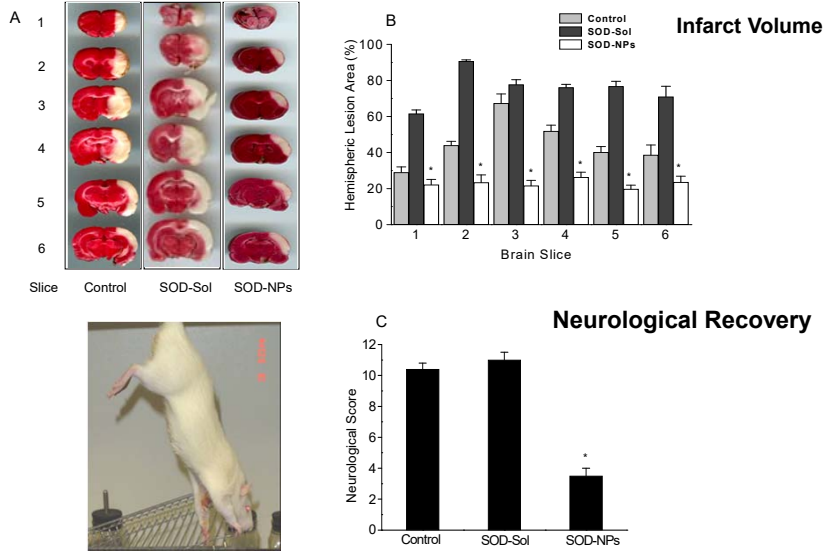


Infracted rat showing fore limb and hind limb paralysis



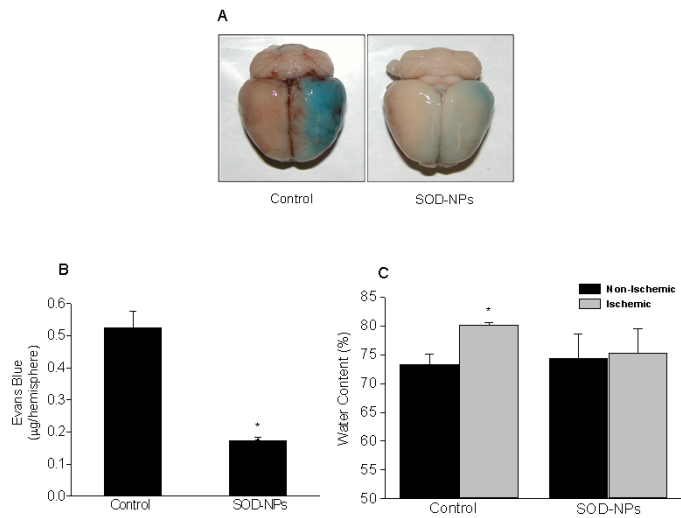
Circling movements

Efficacy of Pro-NPs in Stroke Model (Injection via Carotid Artery)

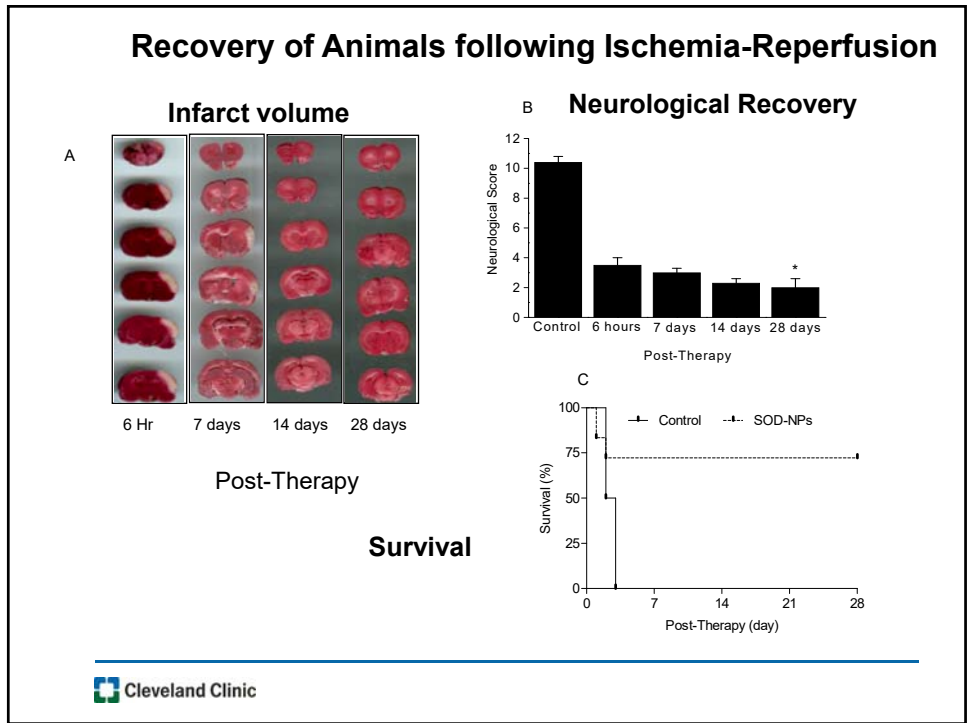
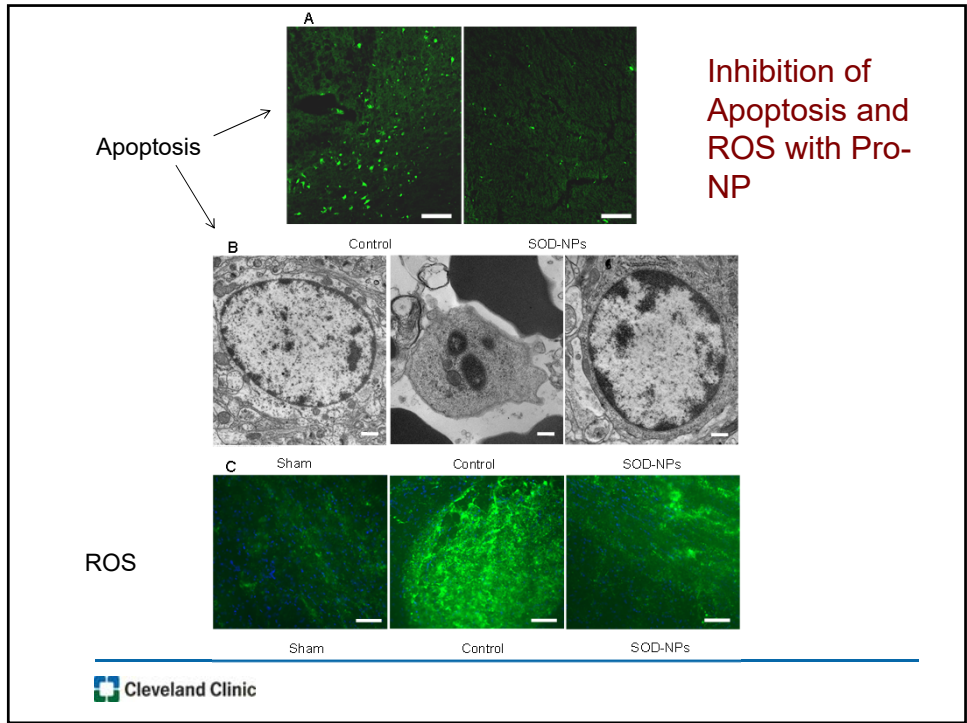


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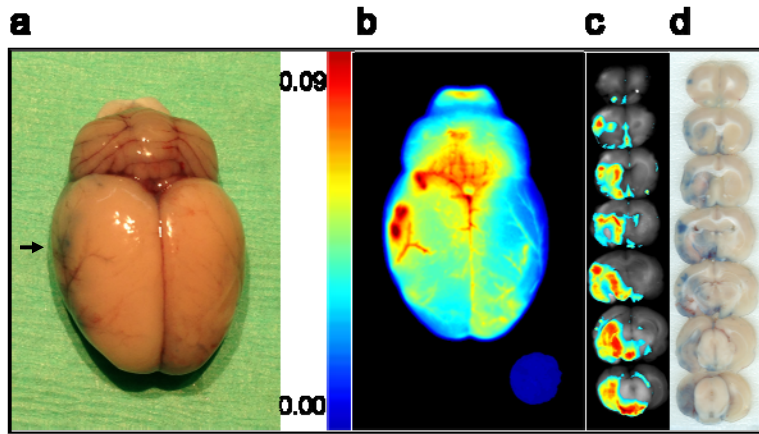
Protection of Blood-Brain Barrier (BBB) with Pro-NP



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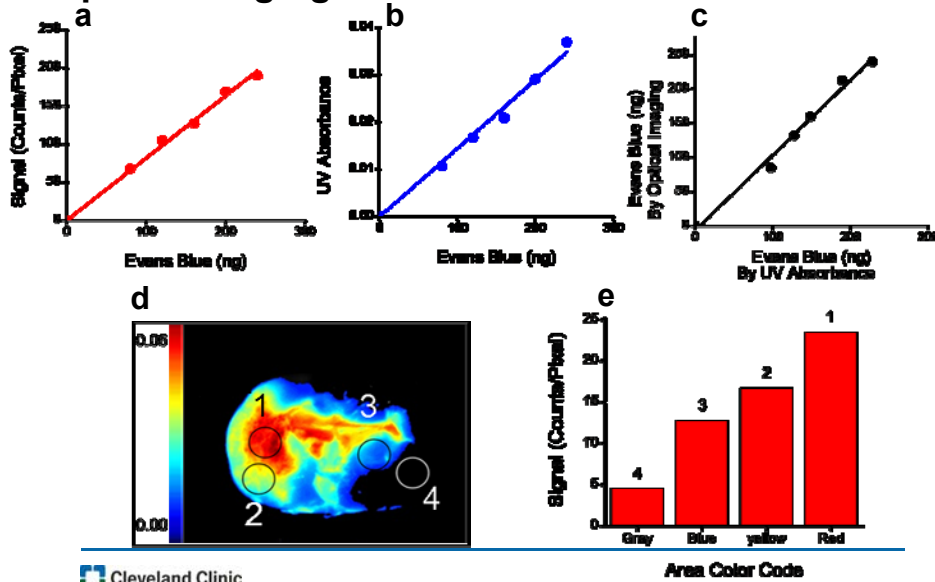


Thromboembolic stroke model (Optical Imaging of Evans Blue)

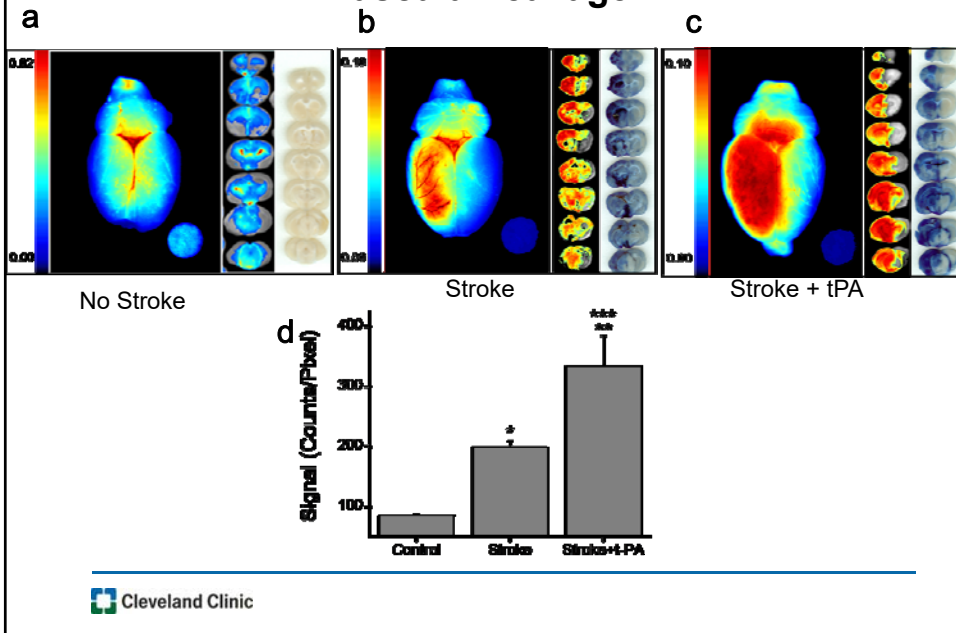


Sci Rep. 2013

Thromboembolic stroke model: Vascular Leakage Optical imaging method 1000 fold more sensitive

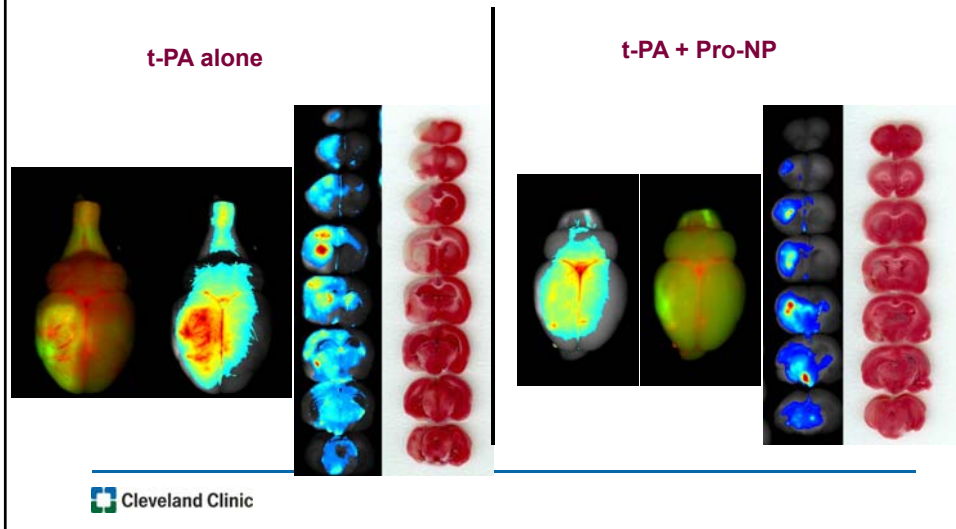


Thromboembolic stroke model: t-PA causes vascular leakage

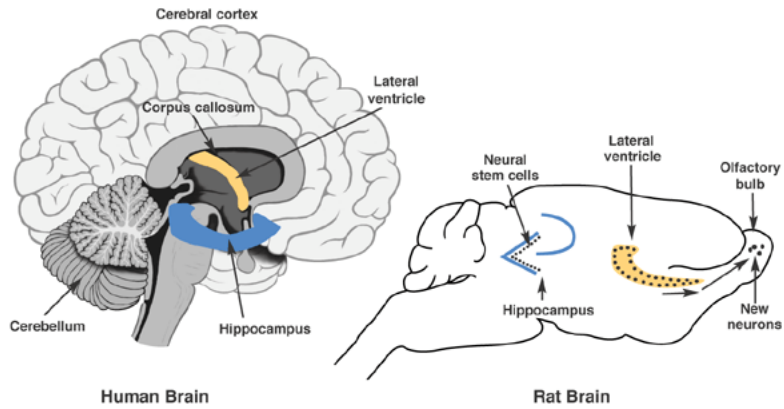


Vascular Leakage at 7-days Post-stroke

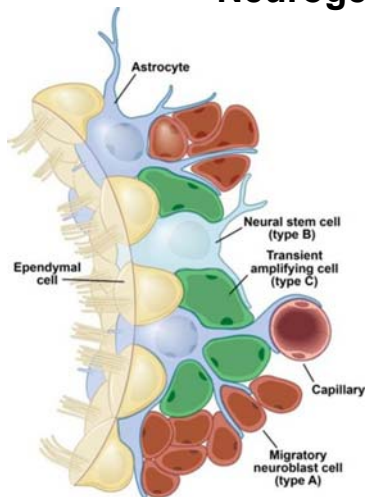
Treatment 6 Hr Post-embolism
 Sequential: t-PA followed by Pro-NPs via Carotid Artery



Neurogenesis in Adult Brain

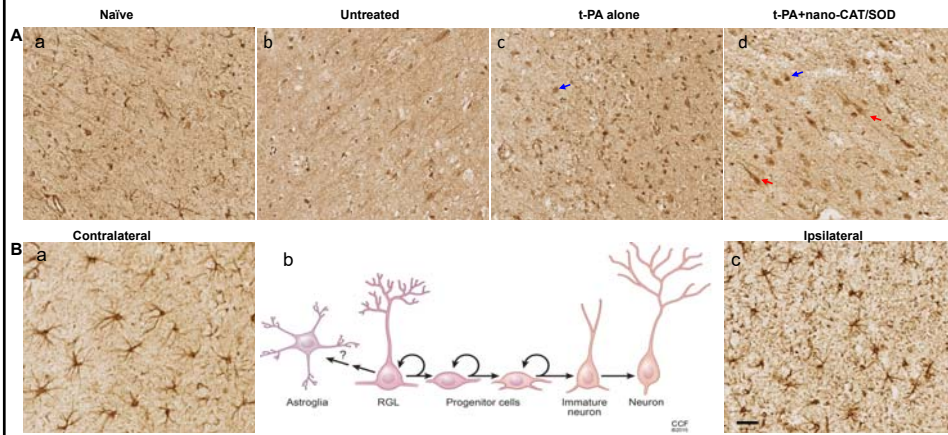


Neurogenesis in Adult Brain



Schematic representing various stem and glial cells associated with the inherent process of neurogenesis in post-stroke conditions.

Neurogenesis Post-Stroke Treatment

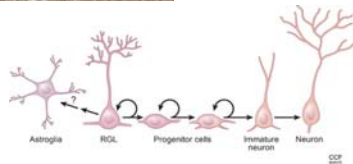
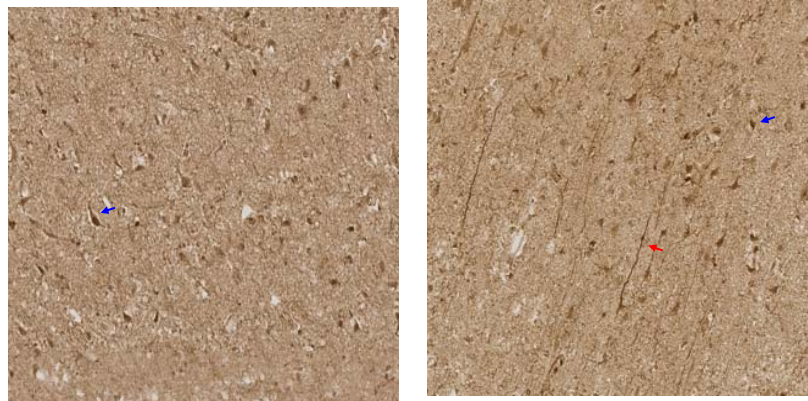


Immunohistochemical analysis of brain sections for GFAP-expressing astrocytes, progenitor cells (blue arrows) and radial glia-like (RGL) cells (red arrows).

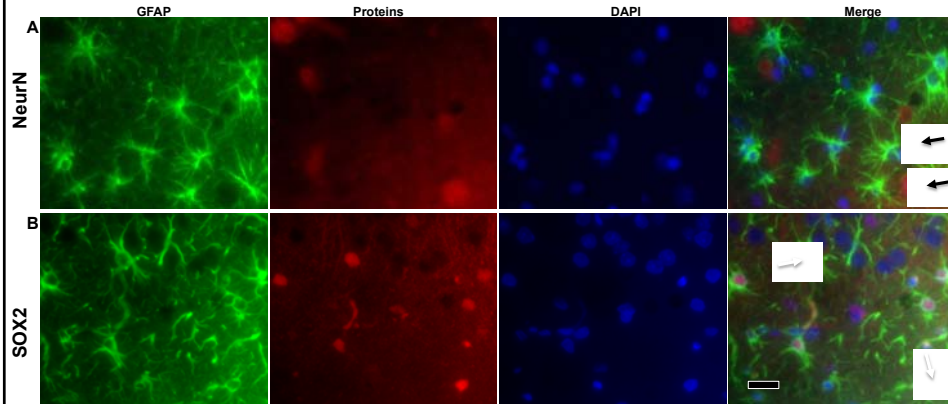
Neurogenesis Post-Stroke Treatment

t-PA alone

t-PA+nano-CAT/SOD



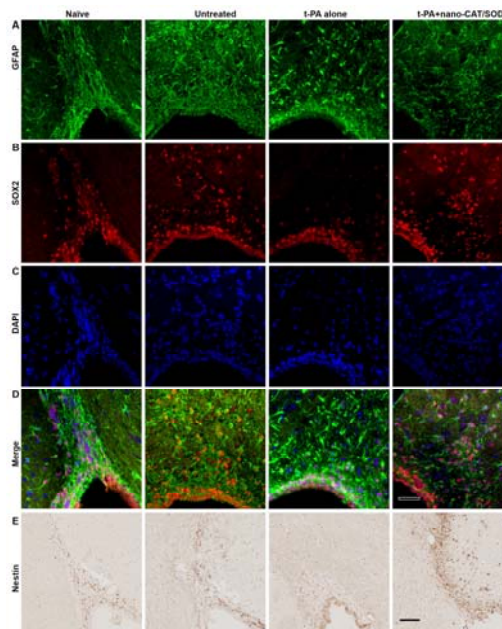
Neurogenesis Post-Stroke Treatment



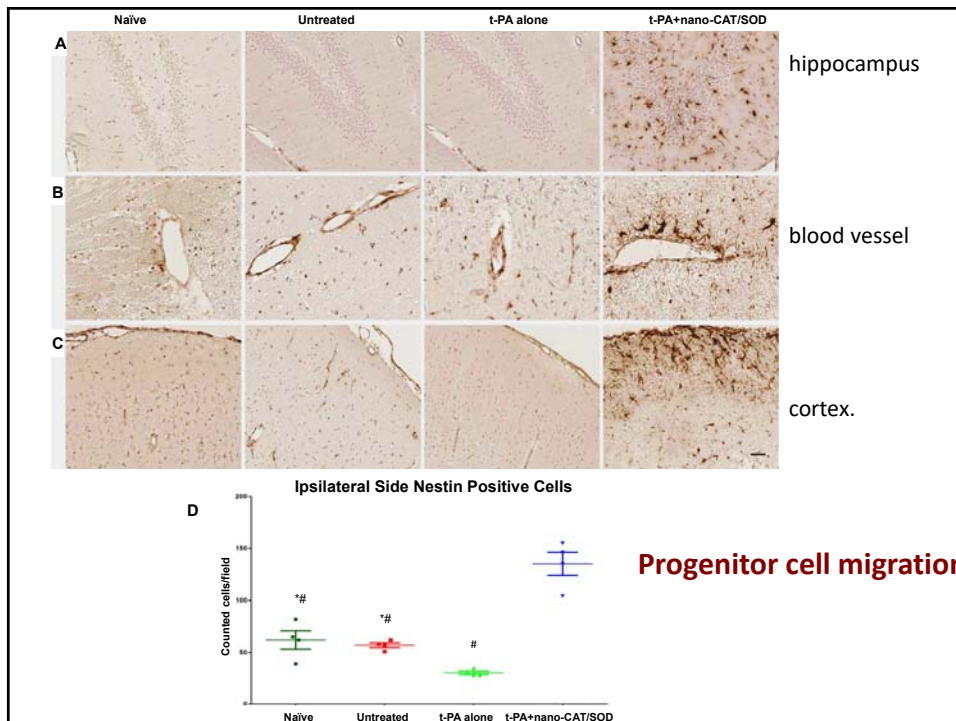
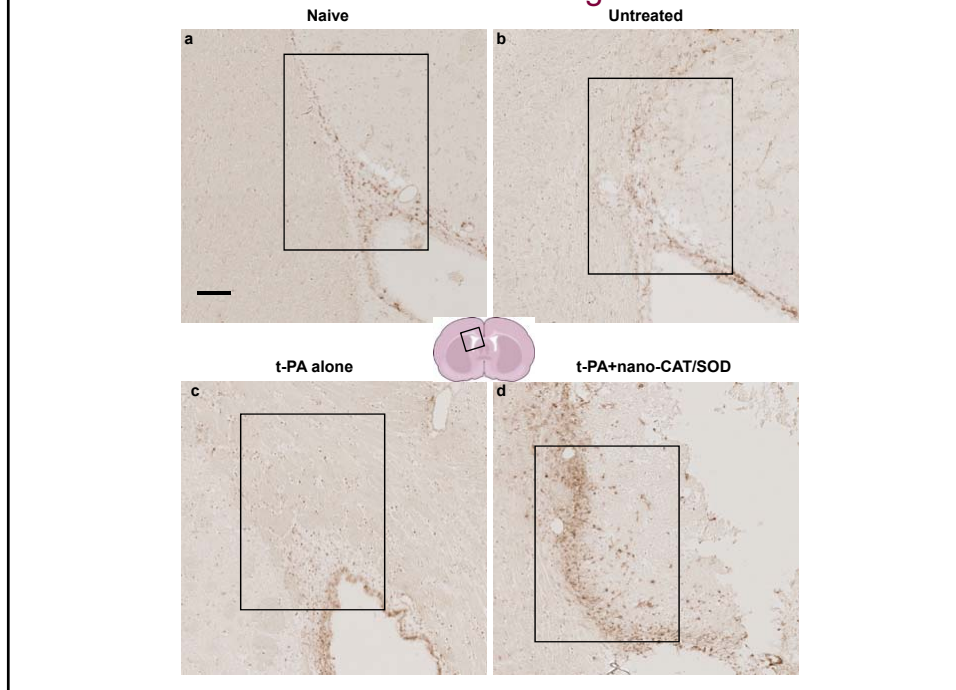
Black Arrows: mature neuron (NeuN)
White arrows: neural progenitors (SOX2)



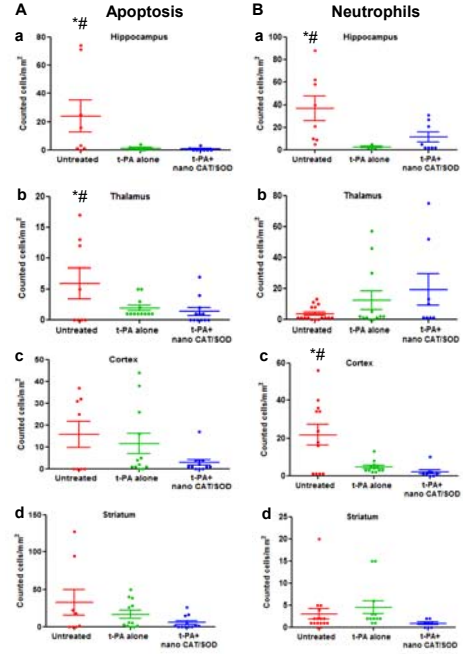
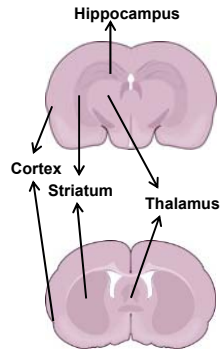
Evidence of Neurogenesis



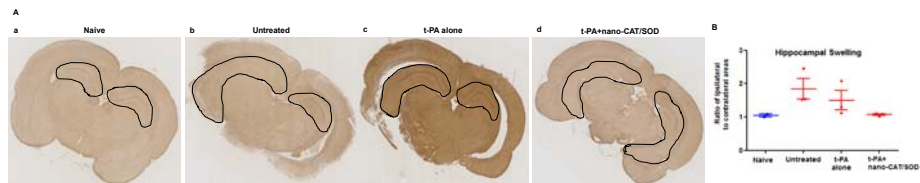
Evidence of Neurogenesis



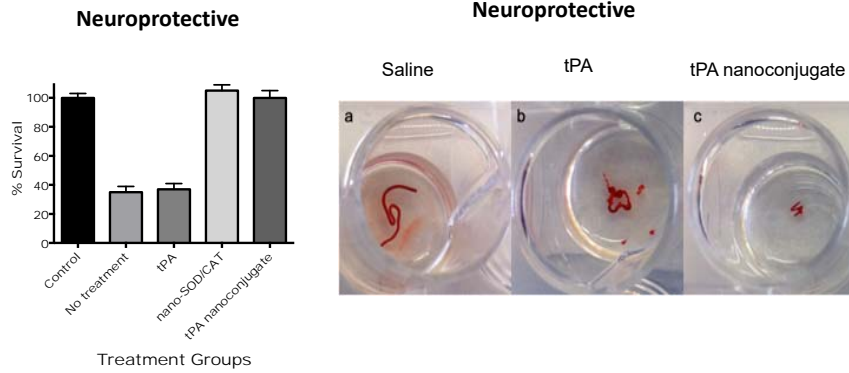
Reduced inflammatory cell migration and apoptosis



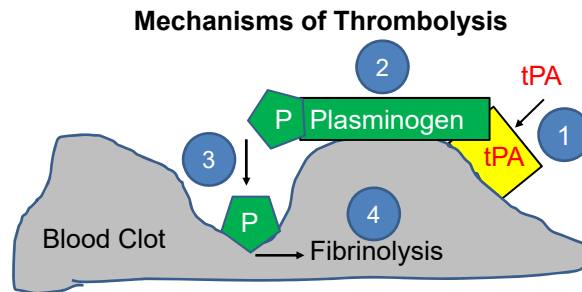
Reduced Hippocampus Swelling in t-PA +Pro-NP



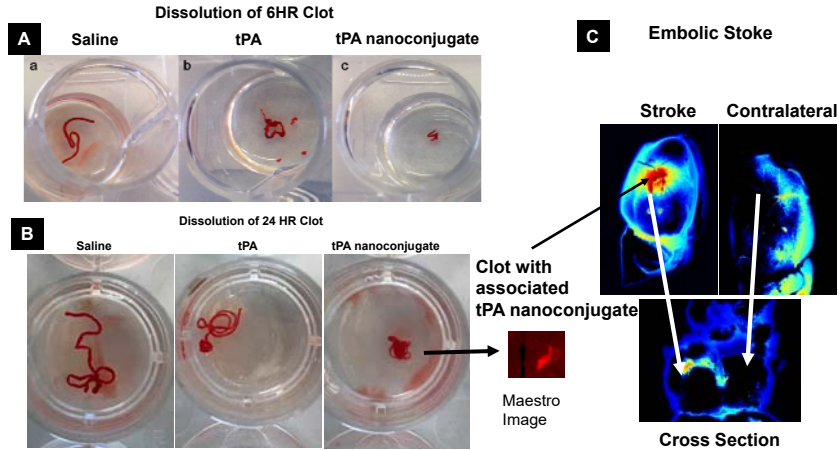
tPA Nanoconjugate (Nano-T-Lytic) Both thrombolytic and neuroprotective



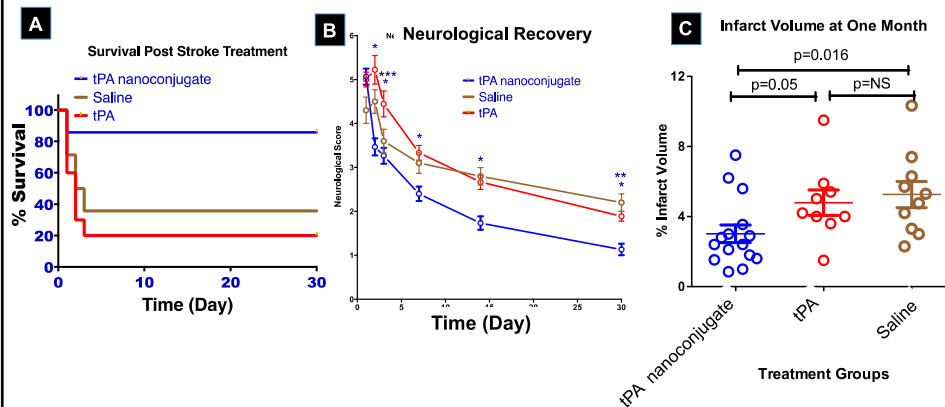
tPA as a targeting ligand to thrombus



tPA as a targeting ligand to thrombus

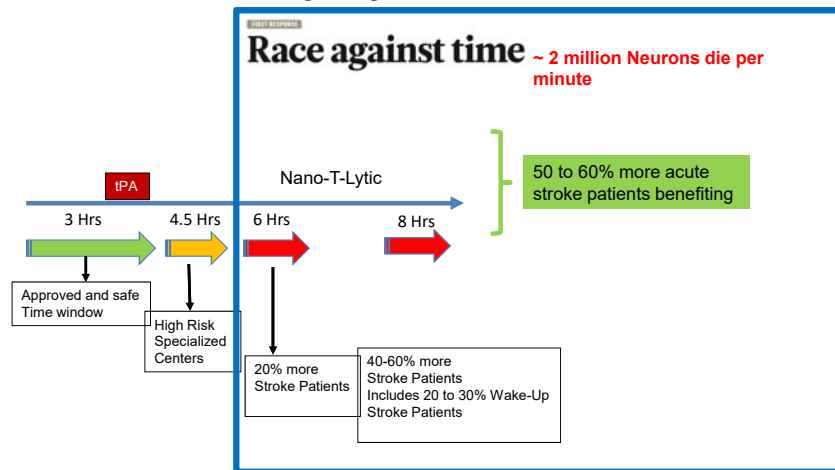


Efficacy of tPA nanoconjugate (6 hr post-stroke, tail vein injection, Male Rats)



i-Corp Program

a-ha



Conclusions

- Neutralization of excess ROS inhibits the cascade of degenerative events
- Neuronal regeneration occurs under oxidative stress free environment

Future Studies

- Neuronal connectivity leading to functional recovery
- Window of treatment after injury
- Long-term survival

Acknowledgements

- Lab Members and collaborators
- Funding from National Institutes of Neurological Disorders and Stroke (NINDS).