Lipid Vesicles Offer a Selective Splenic Targeting Strategy for Peripheral Immunomodulation Following Ischemic Stroke

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CONTROLLED RELEASE SOCIETY
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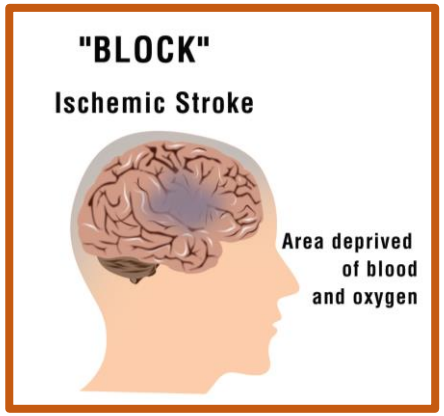
INTEGRATING
Delivery Science
ACROSS DISCIPLINES

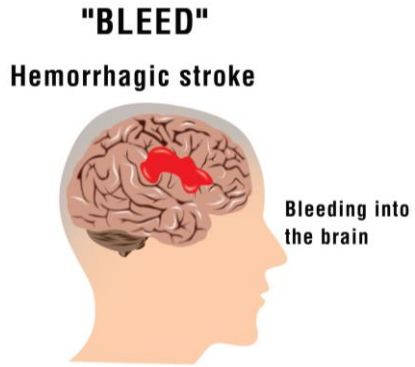
Stroke and Stroke Associated Infections

Stroke

- Stroke is a 2nd leading cause of death worldwide



85% cases



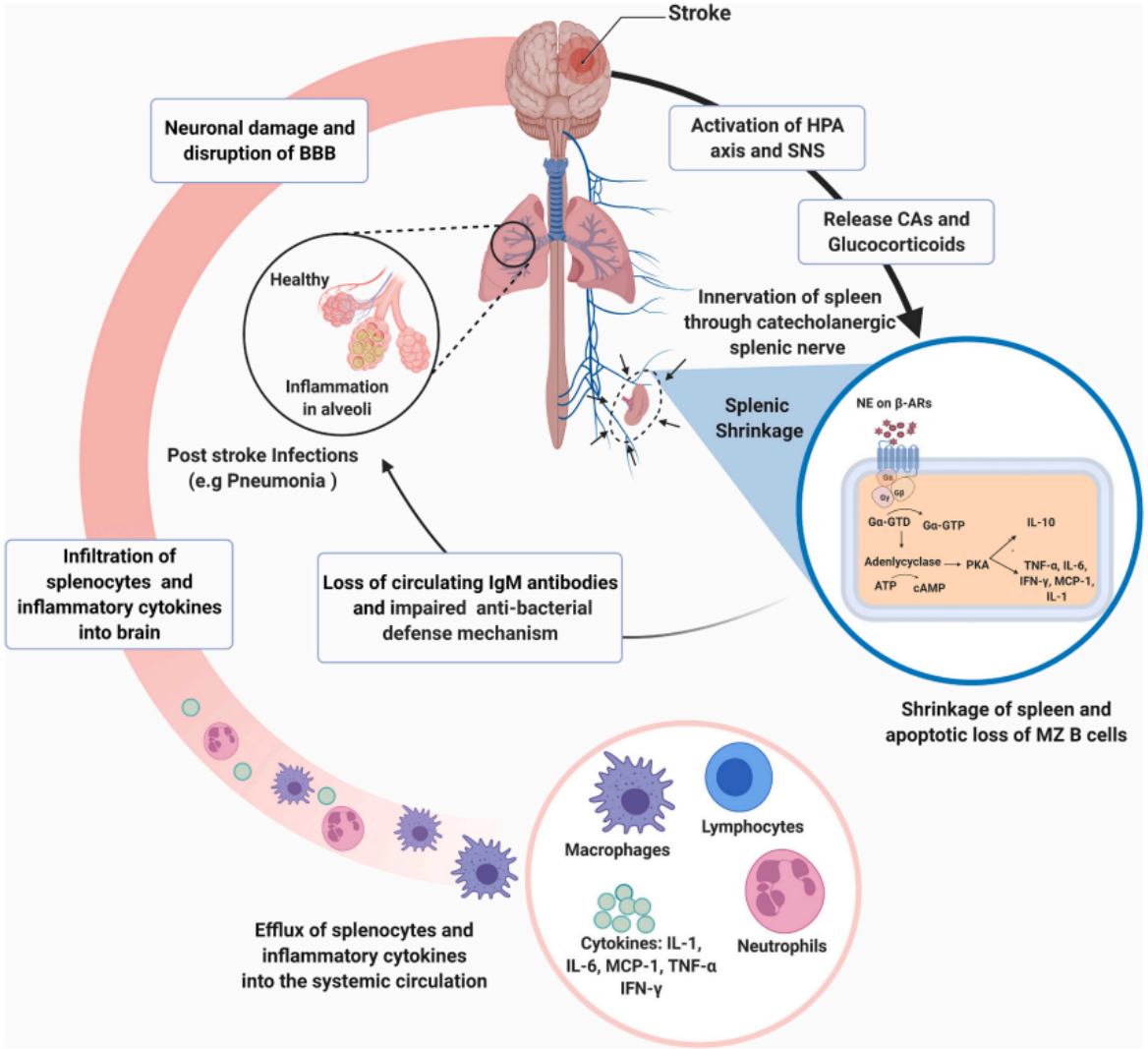
- 7.4 million deaths attributable to stroke worldwide
- Someone died of stroke every 3 minutes 14 seconds

- Immediate cell death, brain damage, Neurological deficits

Post-Stroke Complications: Infections,

- Stroke associated infections effect up to one third of patients
- Respiratory infections(18%) and urinary track infections (29%)
- Pneumonia associated with high rates of mortality

Splenic Response to Acute Brain Injury



Apoptosis of

- B-cells & MZ B cells
- T-cells & NK-cells

Kaur et.al, JCR, 2022

Available Therapies: Clinical & Preclinical

Clinical

- **Prophylactic Antibiotics**


Cochrane Library
Trusted evidence.
Informed decisions.
Better health.

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[Intervention Review]

Antibiotic therapy for preventing infections in people with acute stroke

Jan-Dirk Vermeij[1], Willeke F Westendorp[1], Diederik WJ Dippel[2], Diederik van de Beek[1], Paul J Nederkoorn[1]

- **Beta-Blockers**


Pre-Stroke Use of Beta-Blockers Does Not Lower Post-Stroke Infection Rate: An Exploratory Analysis of the Preventive Antibiotics in Stroke Study

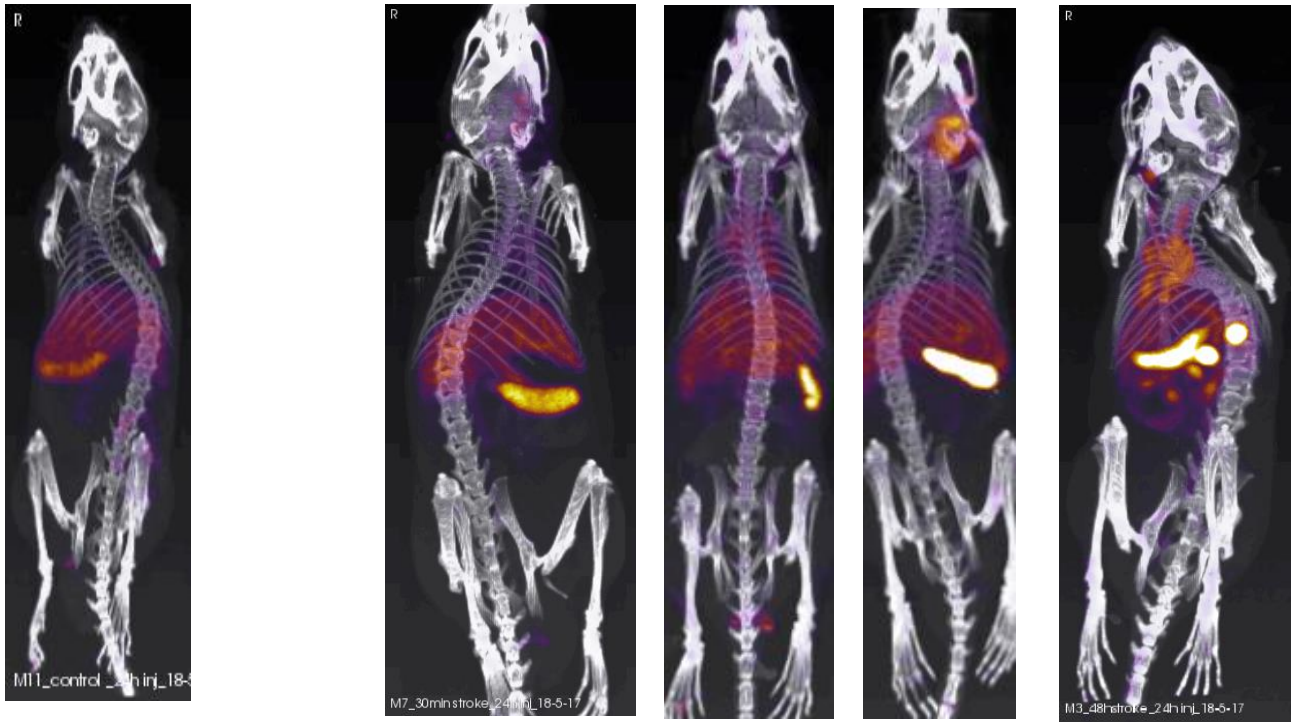
Willeke F. Westendorp[a] Jan-Dirk Vermeij[a] Matthijs C. Brouwer[a, b] Y.B.W.E.M. Roos[a] Paul J. Nederkoorn[a] Diederik van de Beek[a, b] for the PASS Investigators

Pre-clinical

- **Caspase Inhibitor, Immunomodulatory or splenectomy**
- **Showed some promising results but could not prevent Post-Stroke infections**
- **Amount of drug reaching to spleen is inefficient?**

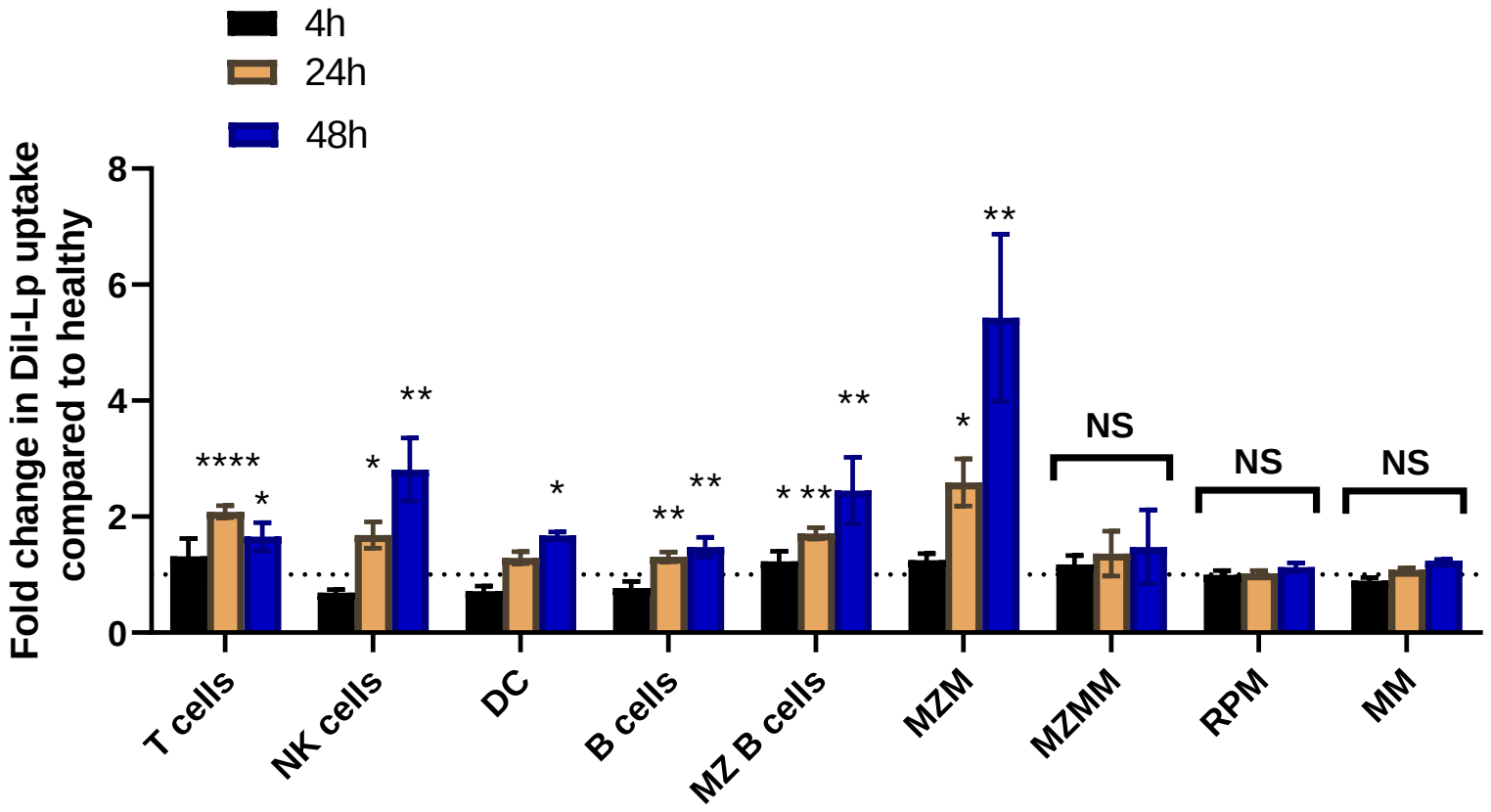
❖ **Emphasising on selective splenic targeted approaches**

Selective Splenic Targeting Post-Stroke Using Lipid Nanoparticles (MCAo mice model)



- **>90% of ID reaching spleen 24-48h post-stroke**

Stroke Significantly Alters Cellular Liposomal Splenic Localization

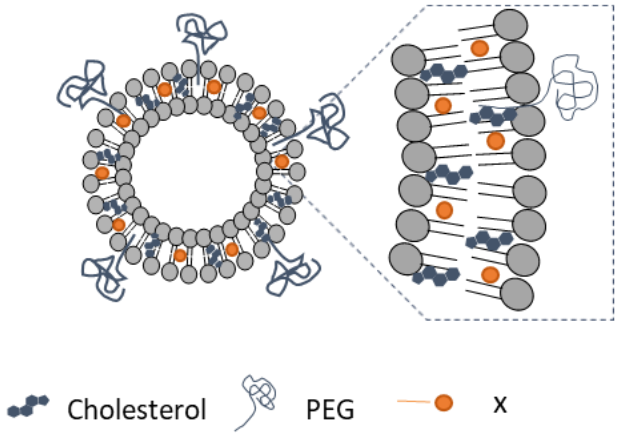


- Significant enhancement in DiI-Lp uptake by WP and MZ cells as early as 2h after I.V injection

Targeting Spleen with Therapeutic drug to Reverse Apoptosis of Splenocytes After Stroke

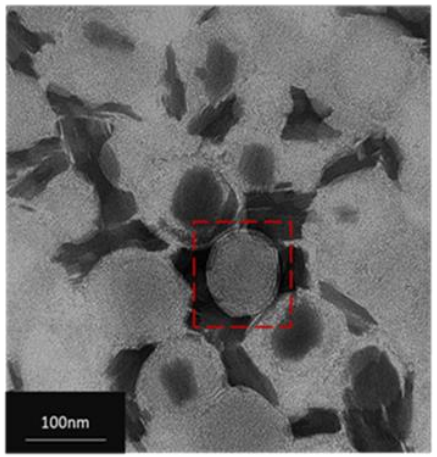
Therapy Study: Development of Lipid Nanoparticles-X

High-Chol. liposomes-X

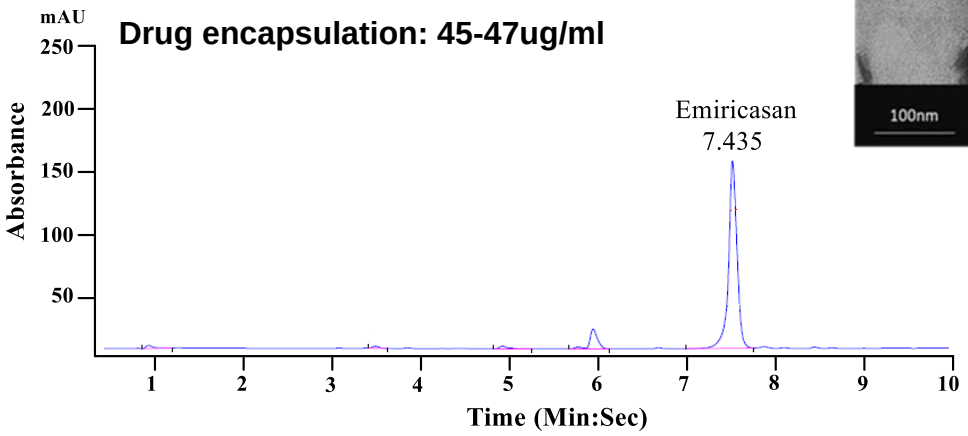


Size: 110.6±5 nm
Zeta Potential: -23.23
PDI: 0.104

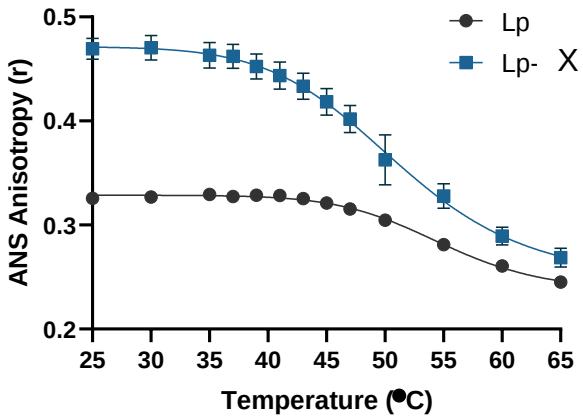
<20% Drug encapsulation
Drug stability: 14 days



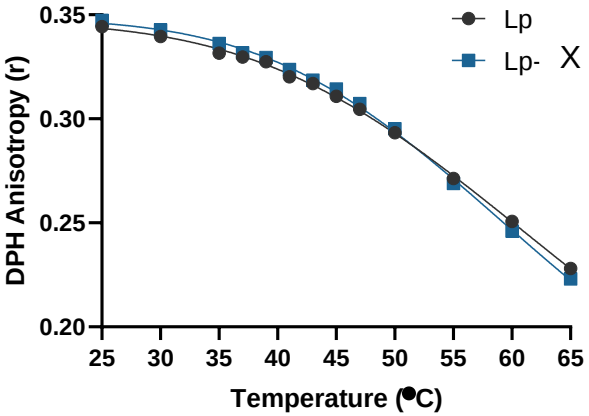
Drug encapsulation: 45-47ug/ml



ANS

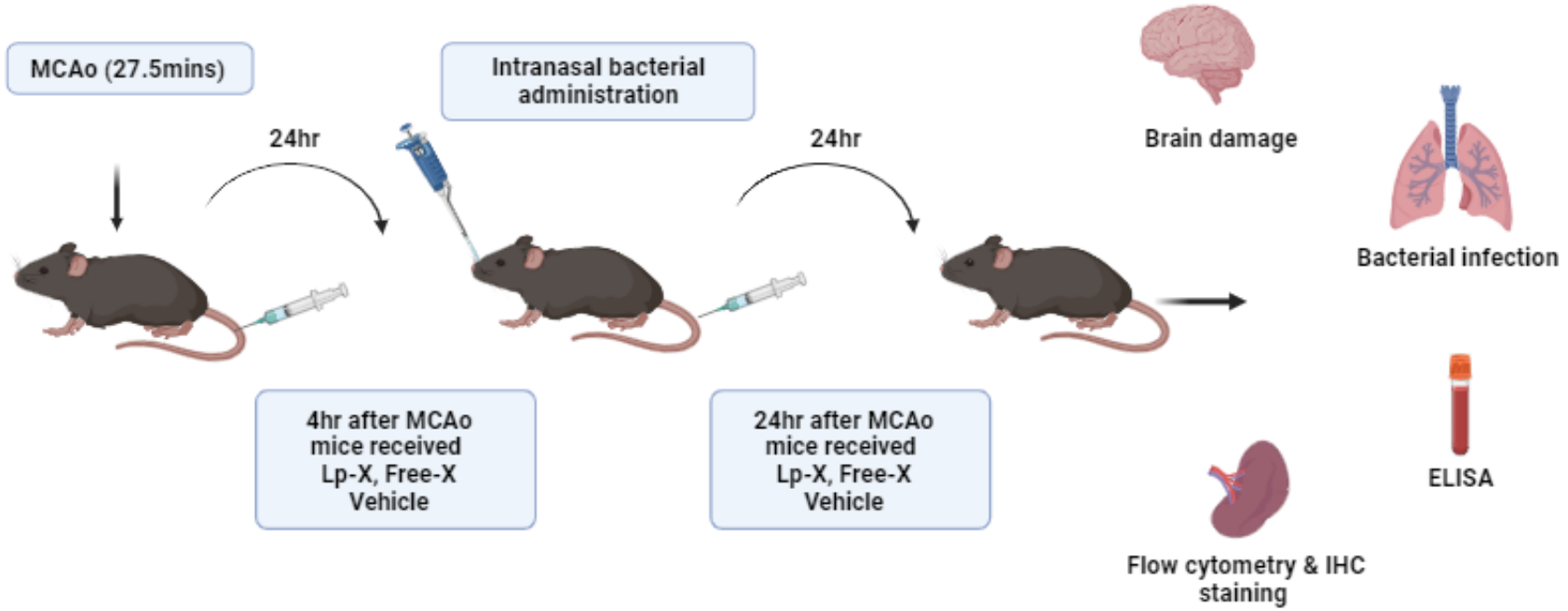


DPH



- **Drug resides in close proximity to hydrophilic region of lipid vesicles**

Effect of Lp-X on Induced Infection MCAo Mice Model



Power analysis: n=12/group

- C57BL/6 male mice (10-12 weeks)
- Lp-X
- Free-X
- Vehicle

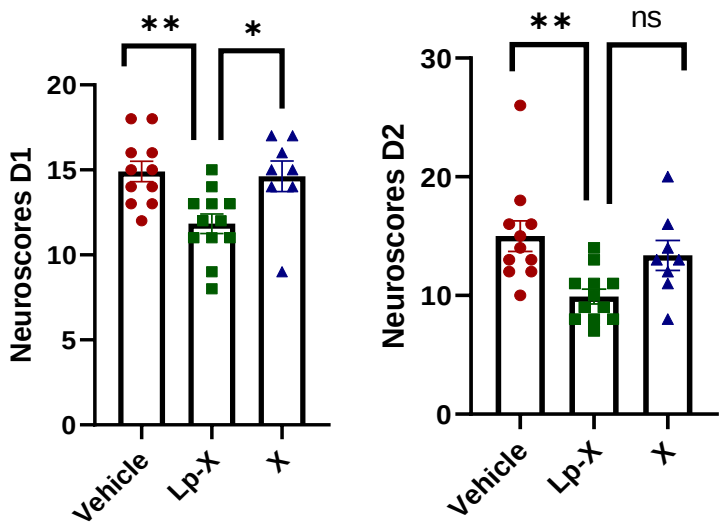
} 0.9mg/kg

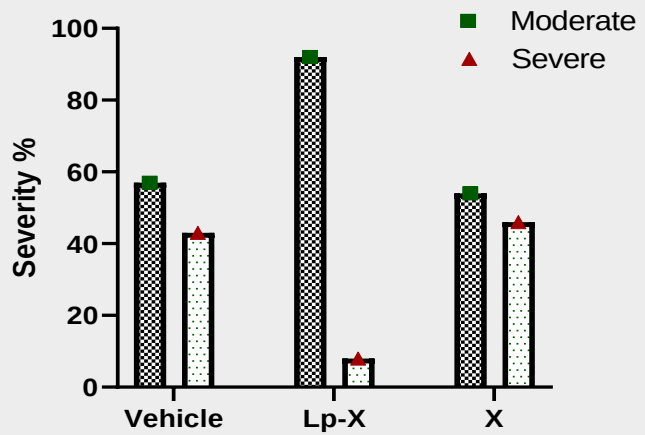
Randomized-blind intervention trail

- Intranasal dose: $1*10^6$CFU/ml

Therapy Study: Neuronal Damage Post-Stroke

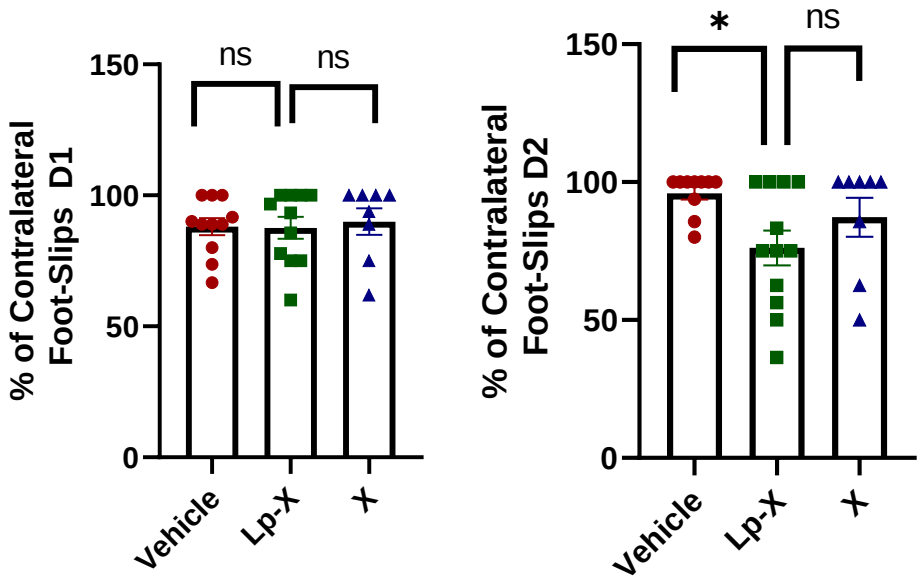
Neuroscore (0-28)	0	1	2	3	4
Body Symmetry	Normal	Slight asymmetry	Moderate asymmetry	Prominent asymmetry	Extreme asymmetry
Gait	Normal	Stiff	Limping	Trembling, drifting, falling	Does not walk
Climbing (45° angle)	Normal	Climb with strain, limb weakness	Just Hold onto slope	Slide down the slope	Slide immediately
Circling Behaviour	Not present	Predominatel y one-sided turns	Tendency to circle one side	Circle constantly to one side	Pivoting, no movement
Front limb symmetry	Normal	Light asymmetry	Marked asymmetry	Prominent asymmetry	No body/limb movement
Compulsory circling	Not present	Tendency to turn to one side	Circle to one side	Pivots to one side sluggishly	Does not advance
Whisker response	Symmetrica l response	Light asymmetry	Prominent asymmetry	Absent response ipsilaterally, diminished contralaterally	Absent response bilaterally





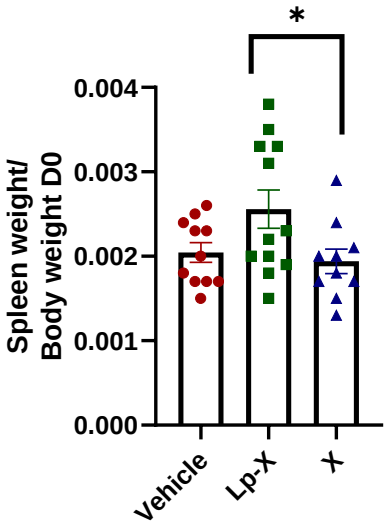
Therapy Study: Neuronal Damage Post-Stroke

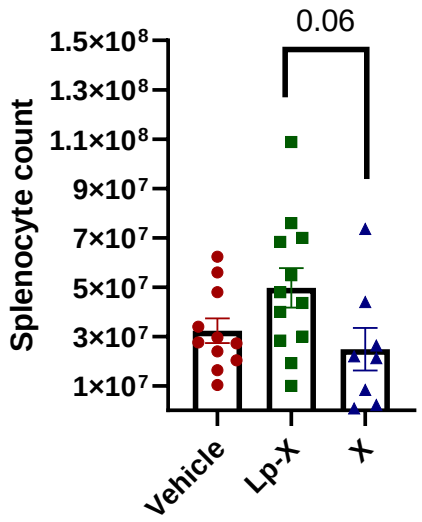
Foot-Fault Test

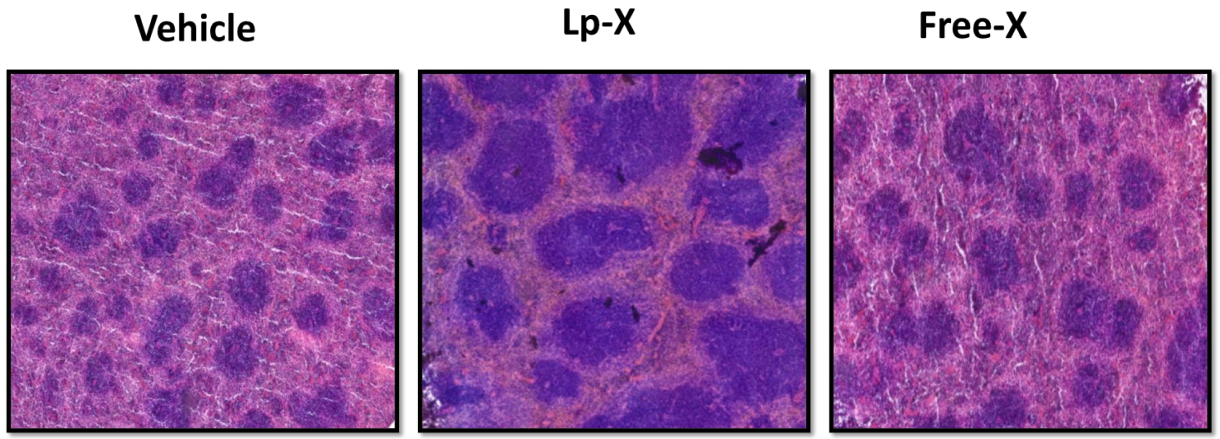


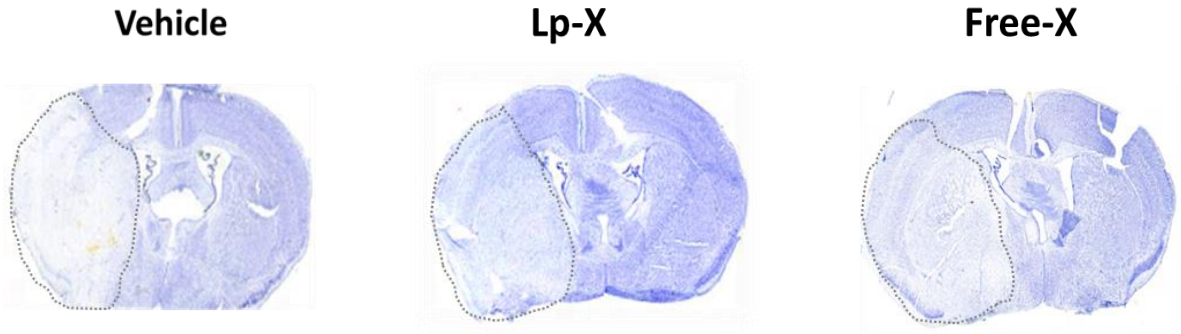
- **Lp-encapsulated therapeutic agent X increases survival rate of animals after 24-48h of MCAo**

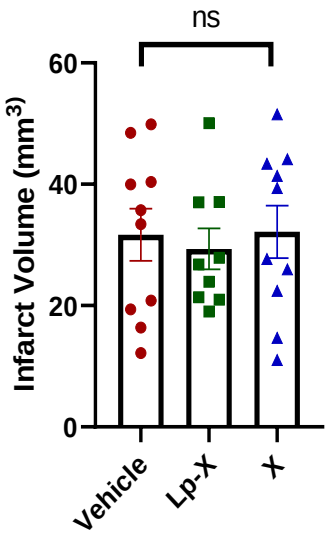
Therapy Study: Splenic Response to Brain Injury



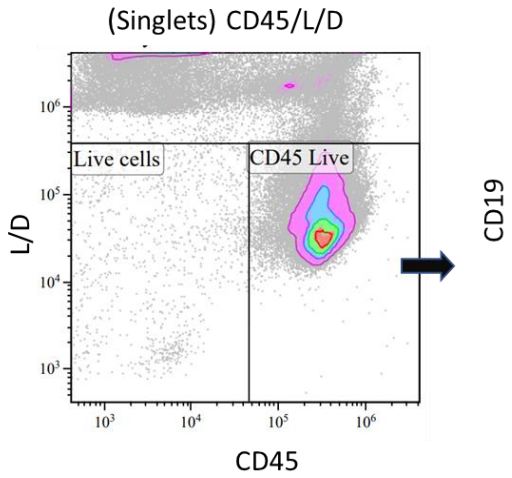


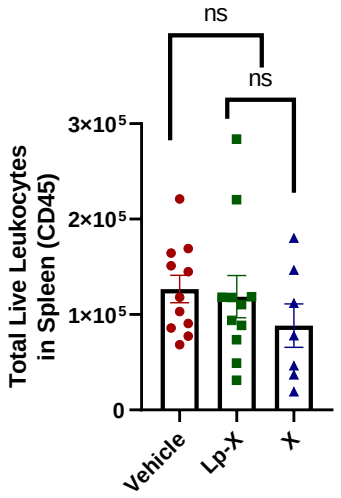


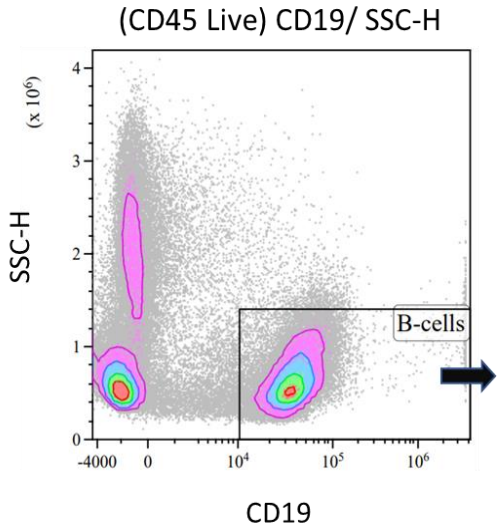




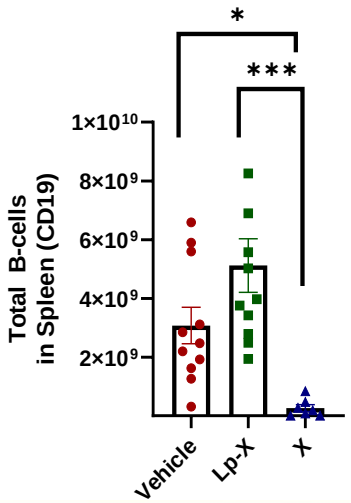
Therapy Study: Apoptotic Loss of Splenocytes Post-Stroke



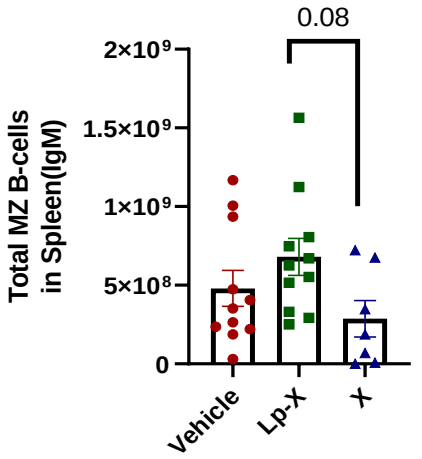




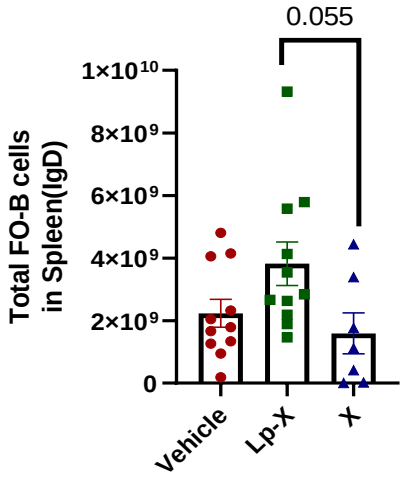
B-cells



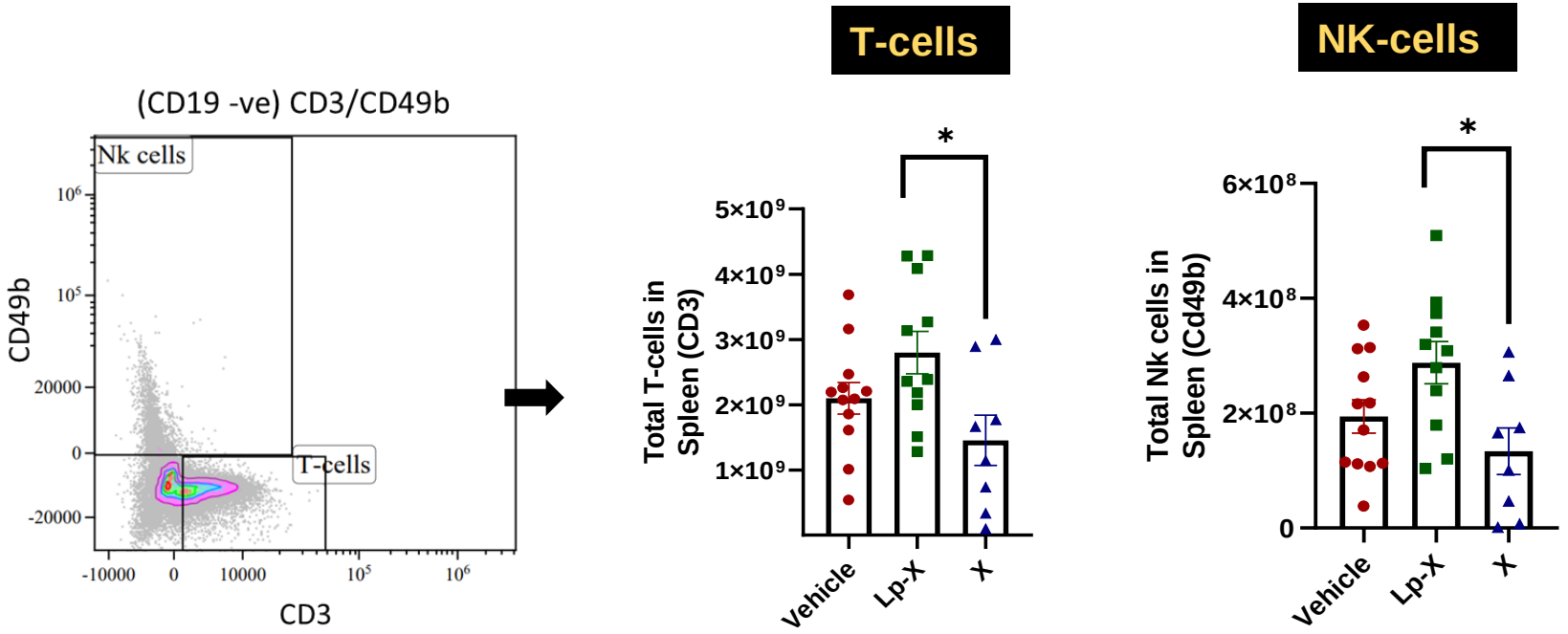
MZ B-cells



FO B-cells

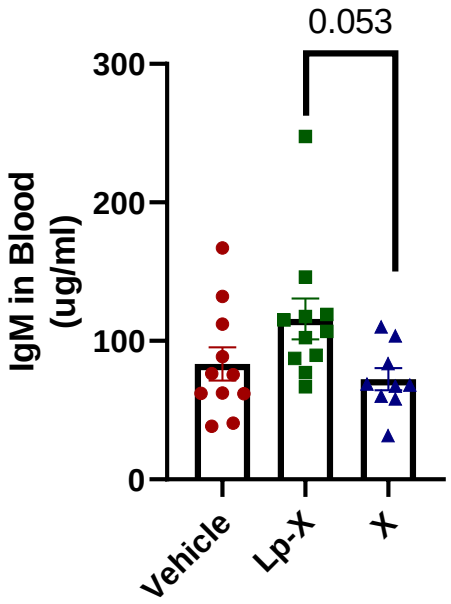


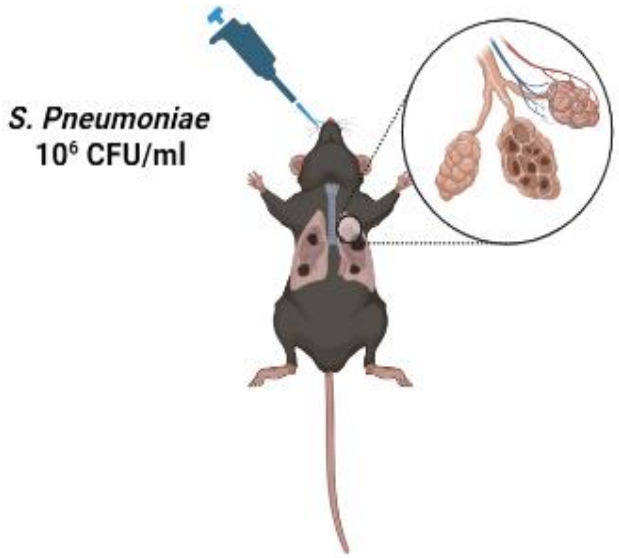
Therapy Study: Apoptotic Loss of Splenocytes Post-Stroke

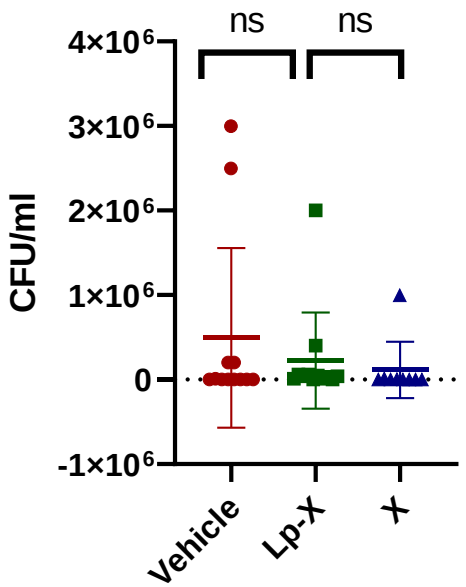


❖ **Lp-X significantly prevent the apoptosis of splenic lymphocytes populations within 24-48h of MCAo**

Therapy Study: IgM Levels in Serum and Lung Infections





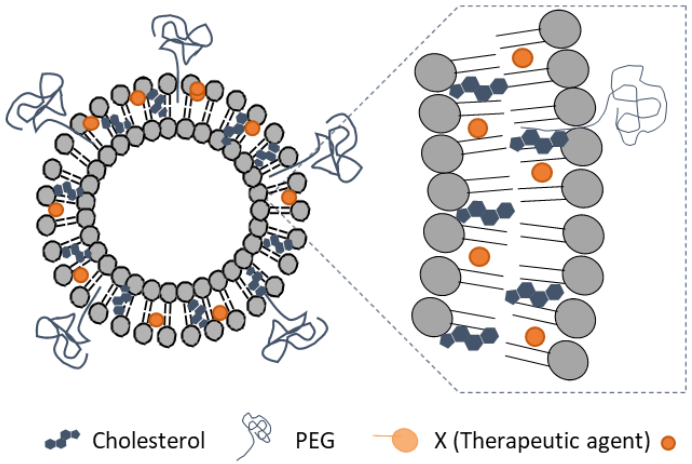


- **Lp-X has tendency to maintain blood IgM levels and reduce bacterial lung infections after 24-48h of MCAo**

Summary

Lipid Nanoparticles-X

- Improved overall well-being of animals after stroke
- Significant improvement in spleen size and splenocyte count
- Reverse apoptotic loss of splenic B-cells, T-cells and NK cells
- Maintain high IgM levels in serum and reduce bacterial infections



❖ **Patent Submitted**

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