Development and Clinical Translation of Dendrimer Nanomedicines for CNS and Retinal disorders

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CRS 2025 ANNUAL MEETING & EXPOSITION
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JULY 13-18, 2025

NEXT-GENERATION DELIVERY INNOVATIONS

Disclosures

- Patents related to this technology are currently licensed to and owned by Ashvattha Therapeutics (clinical stage company) and Samata Therapeutics- JHU start up. Sujatha Kannan and Rangaramanujam Kannan are co-founders of these start-ups and co-inventors on the patents.
- S. Kannan, RM Kannan and JHU are entitled to royalty distributions and share equity related to the dendrimer platform and the off-label products discussed here
- All COI is managed by Johns Hopkins University.

Principles of treating acute neurological disorders: Broadly remain the same

Trephination: From Neolithic period

Egyptian papyrus: 3000-1600 BC

Ayurveda/Sushruta samhita: 4000-600 BC

- Make a hole in the skull to relieve the pressure
- Rest
- Nutrition to promote healing

Challenges in CNS Targeting with Systemic Delivery

- Crossing the BBB
- Traversing the brain parenchyma
- Targeting specific cells

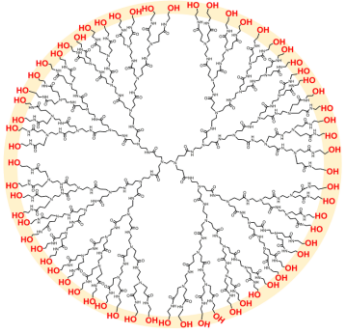
The power of disease-cell-targeted drug delivery

Total number of cells in the human body	30 Trillion
Number of macrophages/microglia/immune	2 Trillion (7%)
Number of neurons	80 Billion (0.25%)
Number of disease cells requiring	30 Million (0.0001%)

- Only one cell out of a million may need therapy.
- Delivering drugs specifically into target disease cells is a transformative opportunity
- Even a very low potency drug (N-acetyl cysteine, mM) conjugated to dendrimer can outperform antibodies (nM) in the clinic[1]

[1]Gusdon, A. M., et al., Science translational medicine, 14(654), eabo2652

Broad therapeutic potential from 20 years of focused research into 2 cells and 2 dendrimers

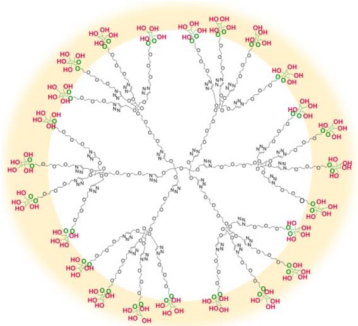


Hydroxyl Dendrimer

Targeting based on enhanced endocytosis at site of inflammation

Microglia/Macrophages

Evaluated in 50 models
$50+ M grant funding
Multiple clinical trials

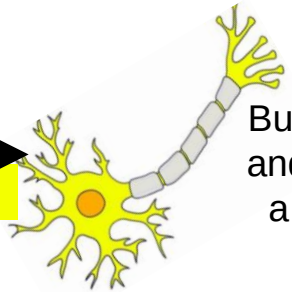


Glucose Dendrimer

Based on dysregulated glucose metabolism, glut-receptor mediated uptake at site of pathology

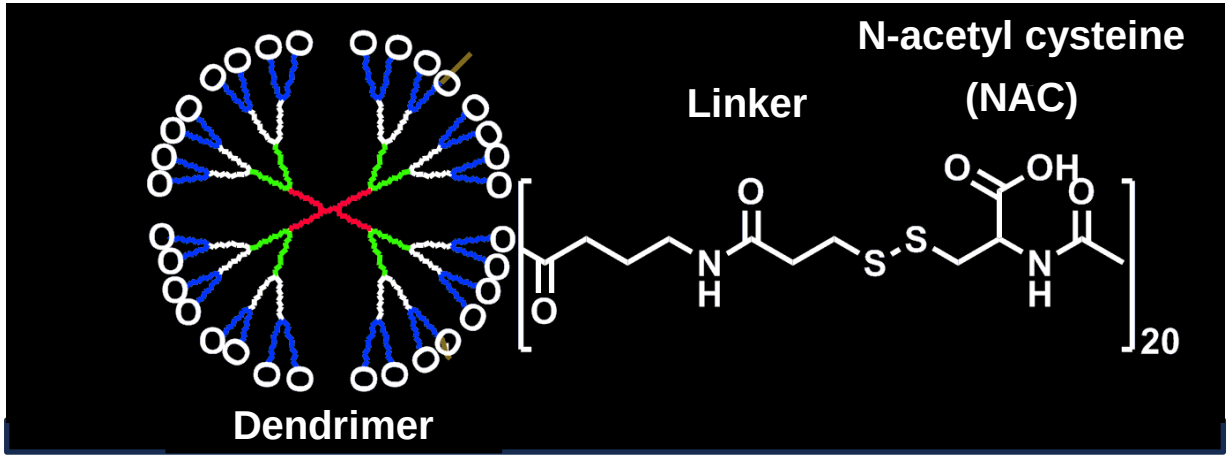
Neurons, Ganglion cells

Adipocytes, adipose macrophages



Builds on prior validation and learnings to address a new set of disorders

Example clinical validation of hydroxyl dendrimer platform: dendrimer-*N*-acetyl cysteine conjugate



Disulfide link enables intracellular drug release via glutathione, resulting in minimal release in systemic circulation (validated in humans)

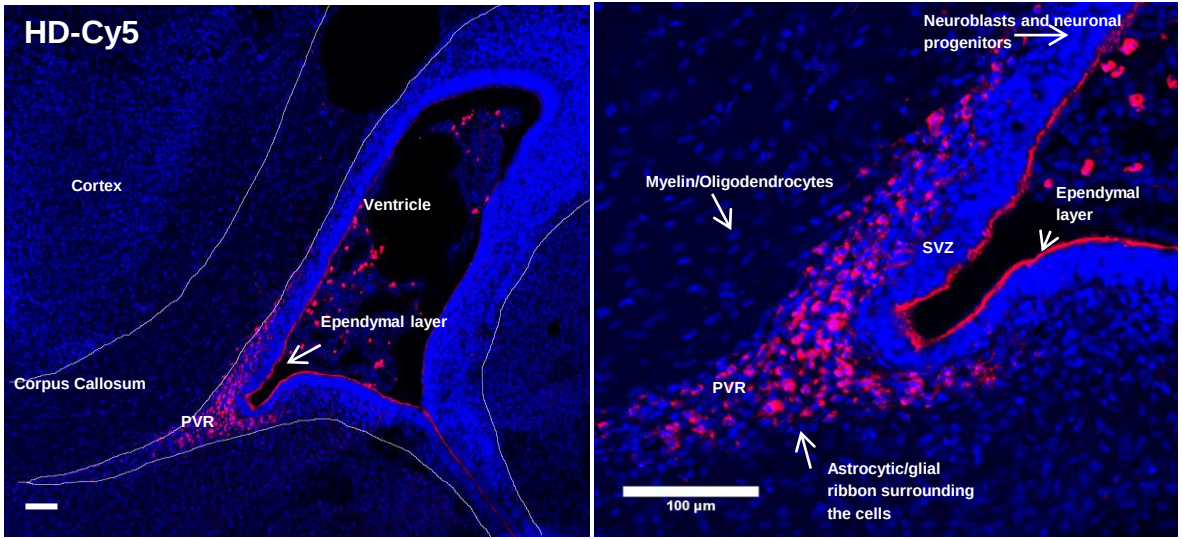
Payload: 20 NAC molecules per dendrimer
Size: 20.1 kDa; 5 nm diameter
Highly soluble in water

GMP production at kg scale and shown to be safe in multiple Phase I trials, by our first start-up Ashvattha Therapeutics.

Gusdon, et al. Science Trans. Med. (2022)

Preclinical: Systemically administered hydroxyl dendrimer rapidly localizes in activated microglia in brain regions affected in cerebral palsy

Hydroxyl dendrimers target reactive microglia and macrophages at site of pathology in rabbit cerebral palsy model



No uptake in the subventricular zone (SVZ) predominantly comprised of neuronal progenitors

Hydroxyl dendrimer is found in regions of inflammation and significant microglia/macrophage activation

Extent of uptake is dependent on degree of neuroinflammation

Agnostic to species and etiology of neuroinflammation

Kannan, et al, Science Trans Med. (2012)

Cerebral Palsy: single systemic dose of dendrimer-NAC leads to symptom-free survival up to adulthood

Site-specific delivery + timed release = increased efficacy + reduced side effects

Saline-treated CP kit
100% mortality and severe disability

Rabbit model: injury in utero followed by single systemic treatment postnatal

D-NAC-treated CP kit
Low mortality and near-normal function

- A single, systemic dose of 10 mg/kg D-NAC dramatically improves motor function, survival, and cognition into adulthood. Free NAC at 10-100x higher doses is not effective.
- D-NAC has been validated in 10+ models across diverse indications (16+ publications demonstrating efficacy of D-NAC in different neuroinflammation models).

Kannan, Sci Transl. Med. 2012; Nino et al., Science Trans. Med., 2018; Turk, Ann of Neurol, 2018

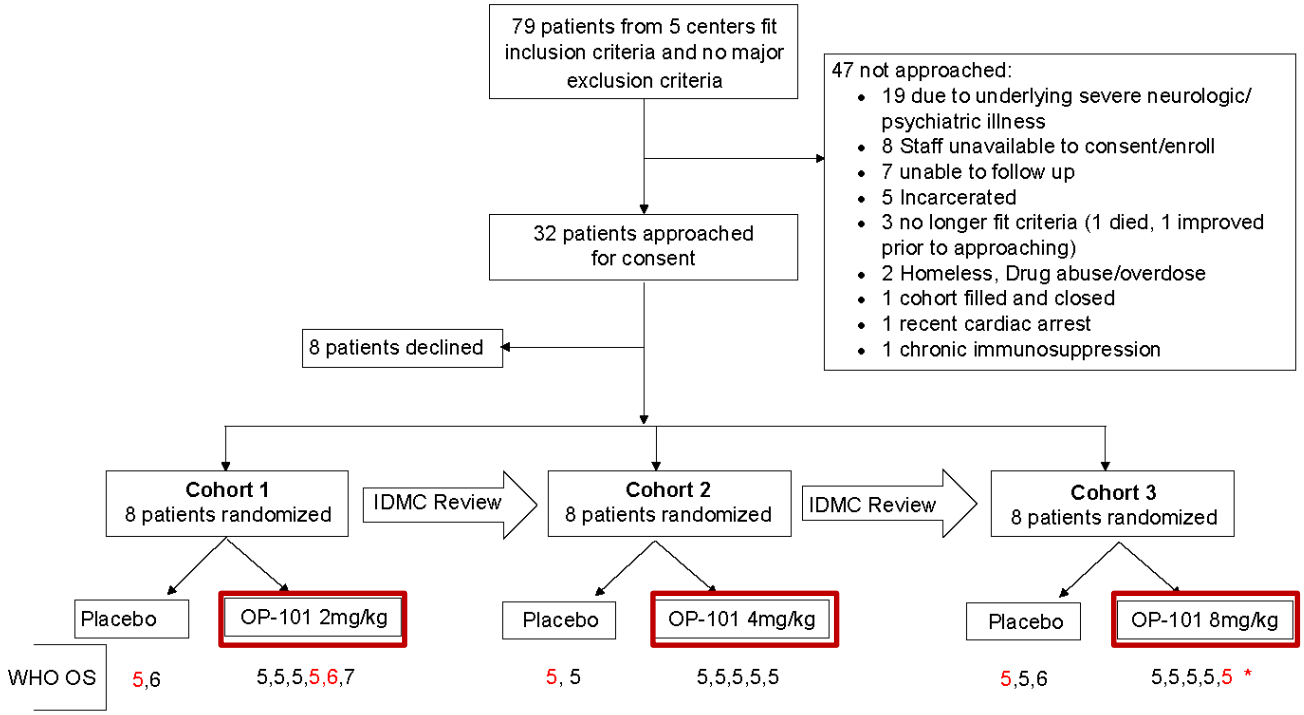
Clinical efficacy of Dendrimer-NAC

Phase 2 proof of concept results for hydroxyl dendrimer-NAC conjugate (OP-101) for treatment of severe COVID-19 in hospitalized patients: Single IV dose of ~ 4mg/kg

Targeting hyper-inflammation for improved survival, and preventing neurological damage

(Ashvattha Therapeutics): Gusdon, A. M., et al., Science translational medicine, 14(654), eabo2652

Trial Design: CONSORT Diagram



24 patients enrolled in 3 cohorts in 5 centers; Phase 2a Adaptive trial design with 3 escalating doses (placebo at each time point)
Patients received standard of care (incl. Steroids and Remdesivir)

No drug related adverse events were noted at all doses

Predictable PK: OP-101 was cleared intact through the urine by 48 hours

Science Transl Med. 2022

Summary of findings

- **14/17 patients (82%) who received D-NAC were alive and ventilator free at 60 days vs 2/7 placebo patients (29%)**
- By 3 days after treatment >70% of patients who received D-NAC saw a 50% or more decrease in CRP levels
- The rate of drop in CRP was ~6 fold higher than that with standard therapy (steroids)
- Greater rate of drop in cytokine levels with D-NAC
- Decrease in brain injury biomarkers (NfL, Tau, GFAP) seen with OP-101 while they continued to rise for standard of care pts. ***Indicates that it penetrates the brain and prevents neuroinflammation.***

Gusdon et al Sci TM 2022

Clinical evidence for the significance and importance of drug delivery

D-NAC trial by our first start-up Ashvattha Therapeutics[1], : DNAC performed better than gold-standard therapies

Therapy **IV dose to hospitalized patients**	**Single IV dose** **OP-101 (D-NAC)[1]** **2, 4, 8 mg/kg**	**Tocilizumab (Toci)** **(placebo controlled trials)[2] 8 mg/kg**	**All IL-6 antagonists** **All studies[2]**
Endpoint Mortality (28 day all cause)	Therapy vs Control 3/17 vs 4/7 17.6% vs 57% **40% reduced mortality**	Therapy vs Control 182/1171 vs 92/581 15.5% vs 15.8% **0.3% reduced mortality**	Therapy vs Control 1407/6449 vs 1169/4584 21.8% vs 25.5% **3.7% reduced mortality**

Dendrimer-NAC treated neurological consequences of COVID-19: Blood biomarker of brain injury (NfL)

NfL (pg/mL)	**OP-101 (4, 8 mg/kg)[1]**	**Placebo + Dexamethasone[1]**	**Tocilizumab + Dexamethasone[3]**
Day 1	22.3	24	16.5
Day 14	22.3	223	58.9
Fold change	**No increase**	**9.3x increase**	**3.6x increase**

Increase in NfL at 14 days correlated strongly with neurocognitive deficits at 6 months

No increase in brain injury biomarker

[1]Gusdon, A. M., et al., *Science translational medicine*, *14*(654), eabo2652
[2]Daniel A et al., Clinical Microbiology and Infection, 2024; 1198-743X
[3]Duindam, HB et al., 2024:117, 510–520.

Phase 2 study of HD-4517 for advanced retinal disease: Systemic Rx for wet AMD and DME

Study Eye Results

Subjects Completing 24 Weeks from Day 1	Wet AMD (N=11)	DME (N=8)	Total (N=19)
Mean number of IVT 24 Weeks prior to screening	3.18	3.75	3.42
Mean number of supplement IVT 24 Weeks after Day 1	0.64	0.75	0.68
Reduction in treatment burden vs. 24 weeks prior (%)	79.9%	80.0%	80.1%
Fold Reduction in treatment burden	5.0	5.0	5.0

Fellow Eye Results

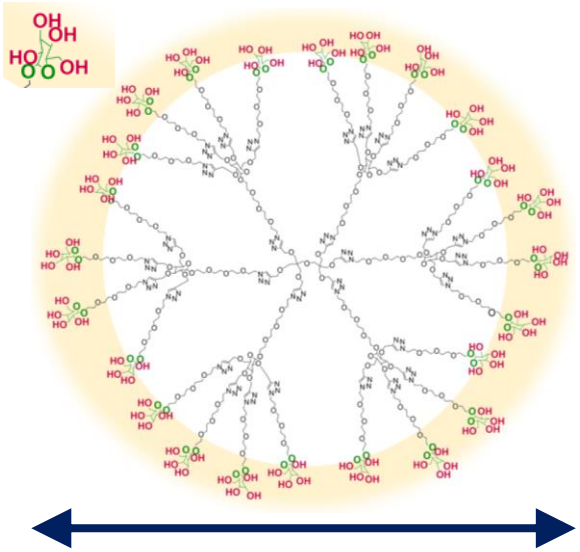
Subjects Completing 24 Weeks from Day 1	Wet AMD (N=3)	DME (N=3)[1]	Total (N=7)
Mean number of injections 24 Weeks prior to screening	3.00	2.33	2.67
Mean number of injections 24 Weeks after Day 1	1.00	0.33	0.57
Reduction in treatment burden vs. 24 weeks prior (%)	66.7%	85.5%	74.9%
Fold Reduction in treatment burden	3.0	7.1	4.0

From Ashvattha Therapeutics (published on X)

- Patients on IVT Eylea Rx recruited
- Rx with D-4517 (Tyrosine kinase inhibitor) once every 2 weeks or 4 weeks for 24 weeks
- On average patients were getting Eylea once every 2 months pre-Rx
- After D-4517, frequency of D-Eylea was once every 9-12 months
- Substantial reduction in Rx burden in fellow eye also

Novel dendrimer platform incorporates surface glucose moieties to target neurons

Glucose dendrimers target injured/metabolically dysregulated neurons and glia at the site of pathology



Glucose Dendrimer

PEG building blocks with glucose surface groups maintain surface OH density while adding glucose interactions

While retaining surface hydroxyl density, we added **glucose** moieties to further improve targeting of neurons in addition to microglia and macrophages

Targeted systemic therapy **(oral, subcutaneous, intravenous)**

- **Safe at high doses** in animals and humans
- **Sustained** duration of effect

Low-cost manufacturing and rapid discovery process

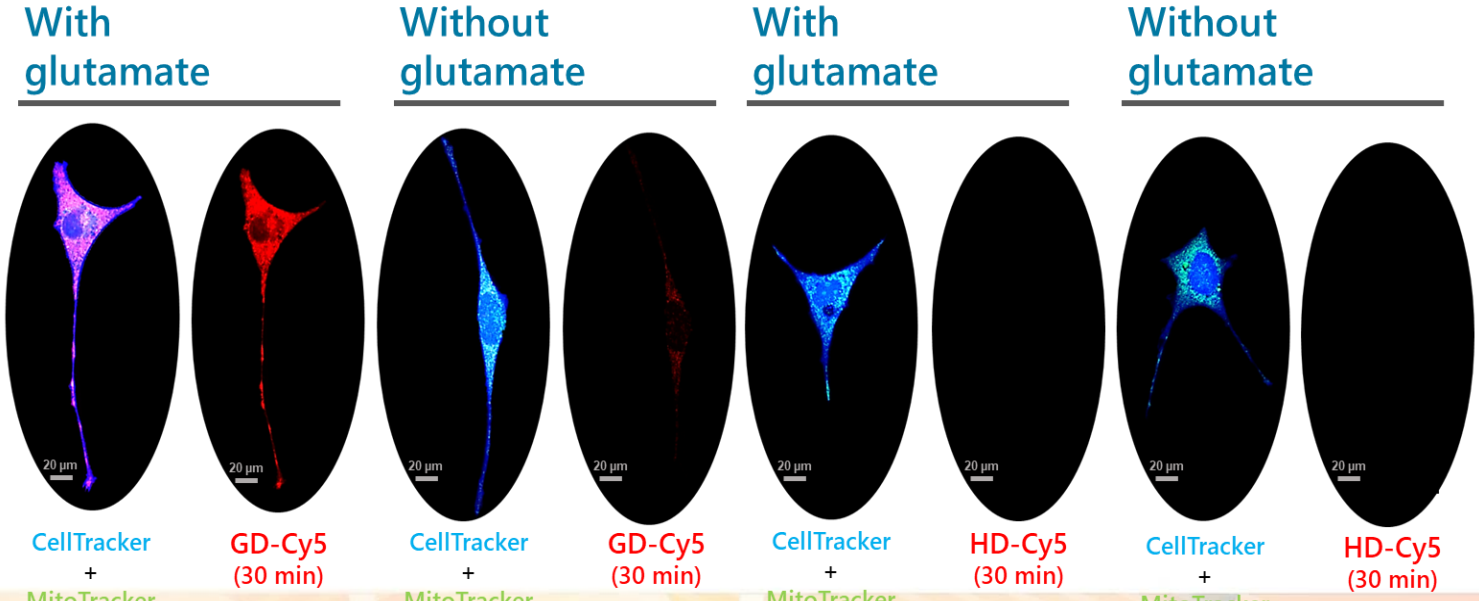
NEXT-GENERATION DELIVERY INNOVATIONS

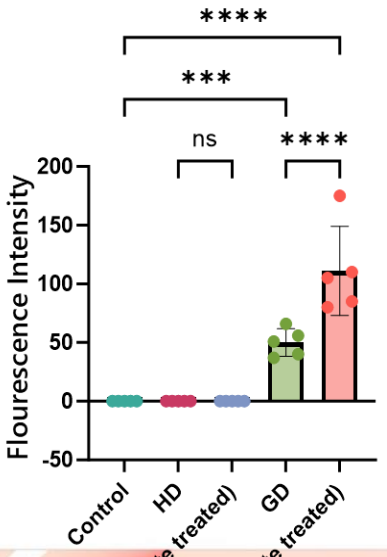
Sharma, et al, Bioengg Trans. Med. (2024)

Glucose dendrimers, but not hydroxyl dendrimers, rapidly enter injured neurons *in vitro*

Glucose Dendrimer

Hydroxyl Dendrimer

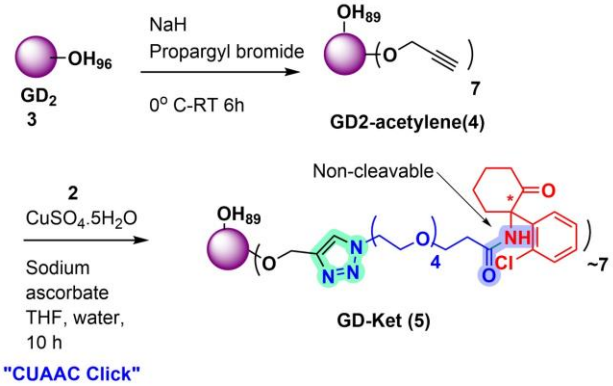




Synthesis and characterization of GD-Ketamine

(a) Synthesis of clickable ketamine

(b) Synthesis of glucose dendrimer conjugate



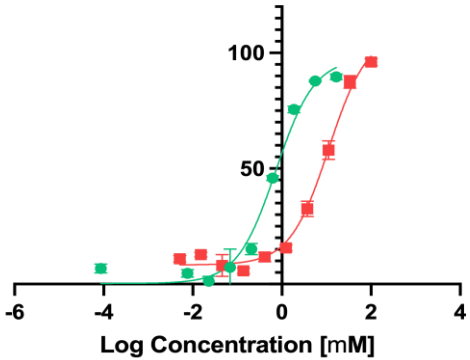
In vivo testing in multiple disease models

Physiochemical Characterization	
Size	6.2 nm
Zeta Potential	-0.41 mV
No. of attachments	7
Drug Loading	~ 10%
HPLC purity	> 99%
Mol. Weight	16,690 Da

Stable at intracellular and extracellular conditions

Not metabolized by liver enzymes unlike ketamine and norketamine

NMDAR 1A/2B Antagonist Assay

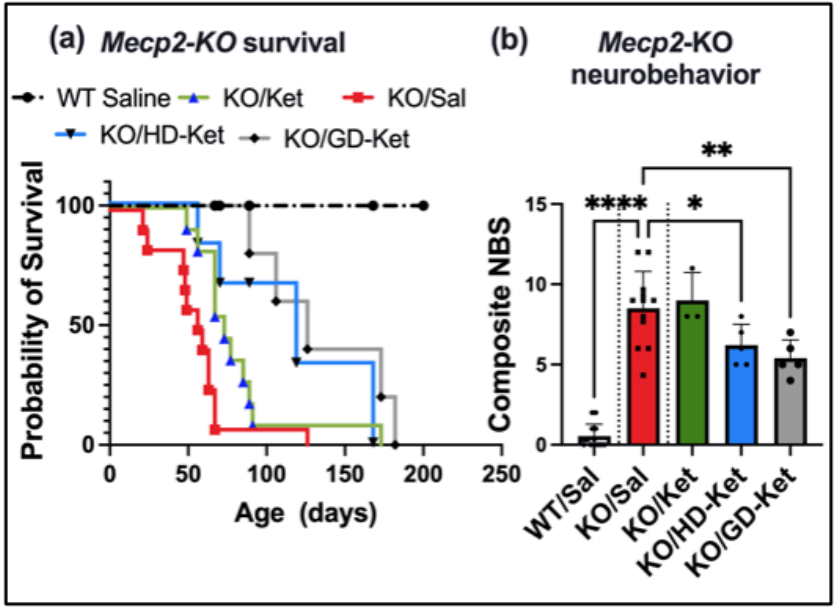


More potent than Ketamine, superior IC_{50}

Activity at Sigma 2 receptor (novel)

Dendrimer-norketamine is more effective in improving survival and neurobehavior in *Mecp2*-KO mice

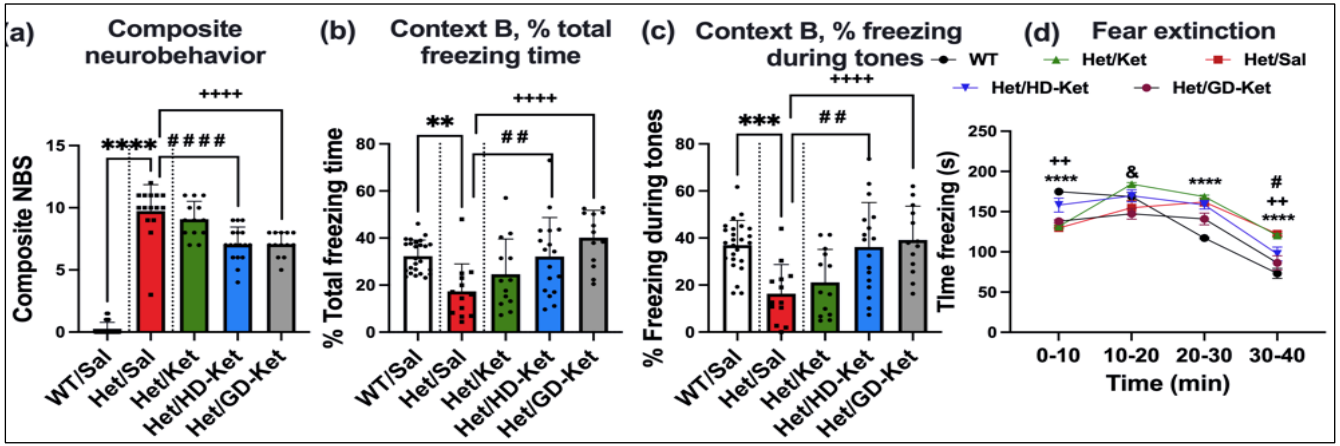
Dendrimer-ketamine (GD-ket and HD-ket) improves NBS score and survival in MeCP2 KO mice



- ❑ Dendrimer-norketamine conjugate is more effective than ketamine in improving survival and NBS in KO mice (Rx from PND15)
- ❑ Total dose of ketamine in conjugate was 40mg/kg (2.5mg/kg IP twice weekly)
- ❑ Previous studies showed efficacy at total dose of 448mg/kg (8mg/kg/day)
- ❑ AUC for survival **GD-ket>HD-ket>Ketamine>Saline**
- ❑ **Cellular targeting of ketamine leads to greater efficacy at lower doses**

Dendrimer-norketamine is more effective in improving NBS and fear memory in *Mecp2*-Het mice

Dendrimer-norketamine improves fear conditioning memory and extinction in MeCP2 Het mice



Ketamine dose: 2.5 mg/kg Intraperitoneal biweekly for 8 weeks starting from 4 weeks of age

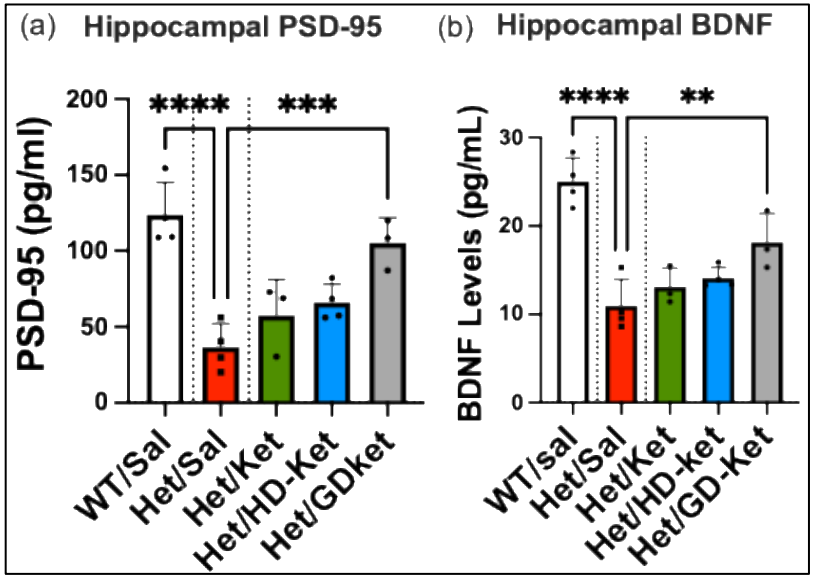
GD-ket: Glucose dendrimer-norketamine (targets Mecp2 null neurons)

HD-ket: Hydroxyl dendrimer-norketamine (targets glia)

- ❑ Dendrimer-norketamine conjugate is more effective than ketamine in improving NBS and learning/memory in Mecp2-het mice.
- ❑ Treatment started at ~4 weeks of age till 12 weeks. (2.5mg/kg twice weekly for 8 weeks)
- ❑ Efficacy GD-ket>HD-ket>Ketamine>Saline
- ❑ **Cellular targeting of ketamine leads to greater efficacy at lower doses in Het mice.**

Dendrimer-norketamine improves synaptic plasticity in *Mecp2*-Het mice

Dendrimer-norketamine (GD-ket and HD-ket) improves BDNF and PSD-95 in hippocampus in MeCP2 Het mice

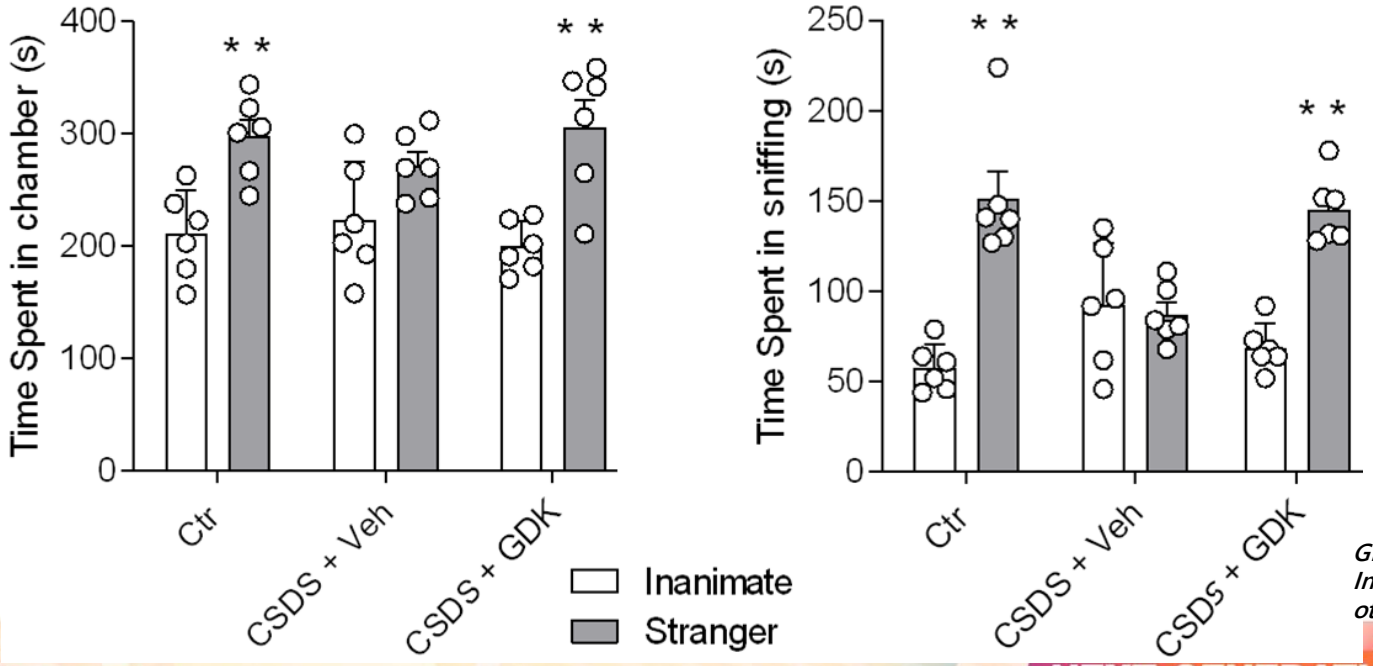


- ❑ GD-Ket conjugate is more effective than ketamine in BDNF and PSD-95 levels in the hippocampus and cortex (data not shown)
- ❑ GD-ket is more effective than HD-ket and ketamine alone.
- ❑ **Neuronal targeting** of ketamine is more efficacious in improving synaptic markers and plasticity

Ketamine dose: 2.5 mg/kg Intraperitoneal biweekly for 8 weeks starting from week 4
GD-ket: Glucose dendrimer-norketamine (targets Mecp2 null neurons)
HD-ket: Hydroxyl dendrimer-norketamine (targets glia)
Brain tissue analyzed at Week 12

GD-norketamine is effective in alleviating depression in a mouse chronic stress model

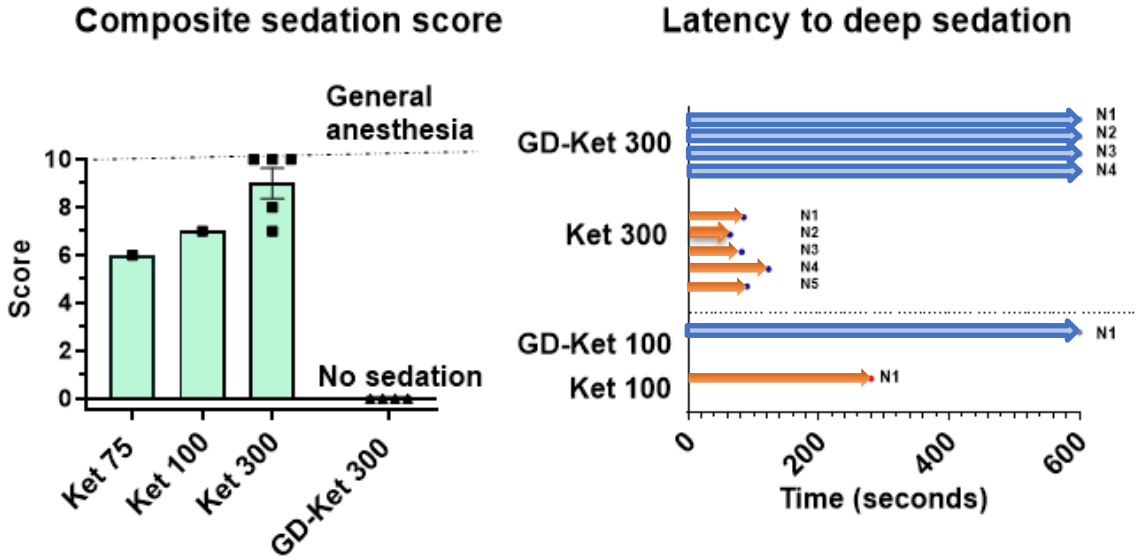
Dendrimer-norketamine (GDK) improves social interaction in chronic social defeat stress model



GD-ket dose: 2.5 mg/kg
Intraperitoneal every other day (10 days)

Dendrimer-norketamine conjugates do not lead to side effects such as sedation and respiratory depression

Dendrimer-norketamine is non-sedating even at very high doses



- Dendrimer-norketamine conjugates are non sedating even at 300mg/kg of ketamine equivalent (lethal doses in mice).
- Conjugation with dendrimer leads to increased safety and improves therapeutic index
- Dendrimer ketamine conjugates have potentially decreased abuse liability

Our Cell-targeting Technology: Next-Generation, Ligand-free Dendrimers

Kannan Labs
Johns Hopkins Medicine

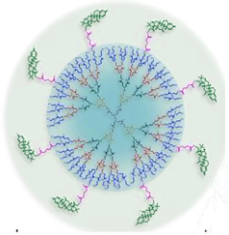
20+ years of dendrimer platform technology development including:

- **50** collaborators at Johns Hopkins
- **7 Species/50+ Models** Preclinical and Clinical Validation
- **$50+ M** in NIH funding
- **150** patents

Three dendrimer platforms,
100's of conjugates,
drugs, genes and antibodies,
Many indications

A subset of several Hydroxyl dendrimer small drug compositions for inflammation licensed to the first clinical stage start-up

Ashvattha THERAPEUTICS



Hydroxyl Dendrimer-drug conjugates

Macrophage/Microglia Targeting

- **Ophthalmology**
  Migaldendranib
  Ph.2a success: Wet AMD/DME
- **Inflammation**
  OP-101
  Ph.2a success: Severe COVID-ARDS
- **Neurology**
  OP-801
  Ph.1/2 ongoing, imaging

6 positive Phase I clinical trials, 2 positive Phase II clinical trials,
$150 M raised for **Hydroxyl Dendrimer-drug conjugates for inflammation**

New dendrimer conjugates and IP targeting neuronal cells, fat tissue, new drug/gene/antibody payloads, Licensed to Avatar Precision Therapeutics, LLC

Samata Therapeutics (epilepsy, pain, depression, mental health)
Mandara Therapeutics (cardiometabolic disorders)
Pediatric CNS, superior antibodies, gene therapy, longevity

CRS 2025 ANNUAL MEETING & EXPOSITION PHILADELPH JULY 13-18, 2025

Acknowledgements/Collaborators

Anesthesiology/Critical Care Medicine

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Related presentations by Kannan Labs' group members

1) Tech Session II: Nanomedicine and Nanoscale Delivery (Focus: Nervous)
Dr. Durgadas Cherukaraveedu, Targeted Glucose Dendrimer-Cannabidiol Conjugates: Safe and Effective Treatment of Pain and *Status Epilepticus*
Tuesday, July 15, 2025 3:49 PM - 4:00 PM EDT, Location: 120 B/C

2) Tech Session IV: Immuno Delivery II
Dr. Milan Paul, Dendrimer-Antibody Conjugates: An Innovative Approach for Targeted Intracellular Antibody Delivery
Wednesday, July 16, 2025 3:05 PM - 3:16 PM EDT , Location: 119 B

3) Tech Session VI: Oral Delivery III
Mr.Jose Diego Almodiel, Orally administered macrophage targeted dendrimer-Tesaglitazar for obesity and atherosclerosis
Thursday, July 17, 2025 , 5:35 PM - 5:46 PM EDT , Location: 125

4) Tech Session VII: Delivery to the Nervous System II
Dr. Preeti Vyas, Targeted glucose dendrimer ketamine therapy protects against refractory status epilepticus in mice.
Friday, July 18, 2025 8:54 AM - 9:05 AM EDT , Location: 119 A

5) Tech Session VII: Nanomedicine and Nanoscale Delivery (Focus: Bioengineering)
Dr. Narendra Kale, Novel glucose dendrimer approach to target hyperactive neurons in vivo: mechanistic insights
Friday, July 18, 2025 , 9:16 AM - 9:27 AM EDT , Location: 121 B/C