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Poster: 4927 - B0132

Targeted Sustained Intracellular Delivery to Choroidal Neovascular Lesions After a Single Systemic Administration as Demonstrated by Imaging

April 30, 2020

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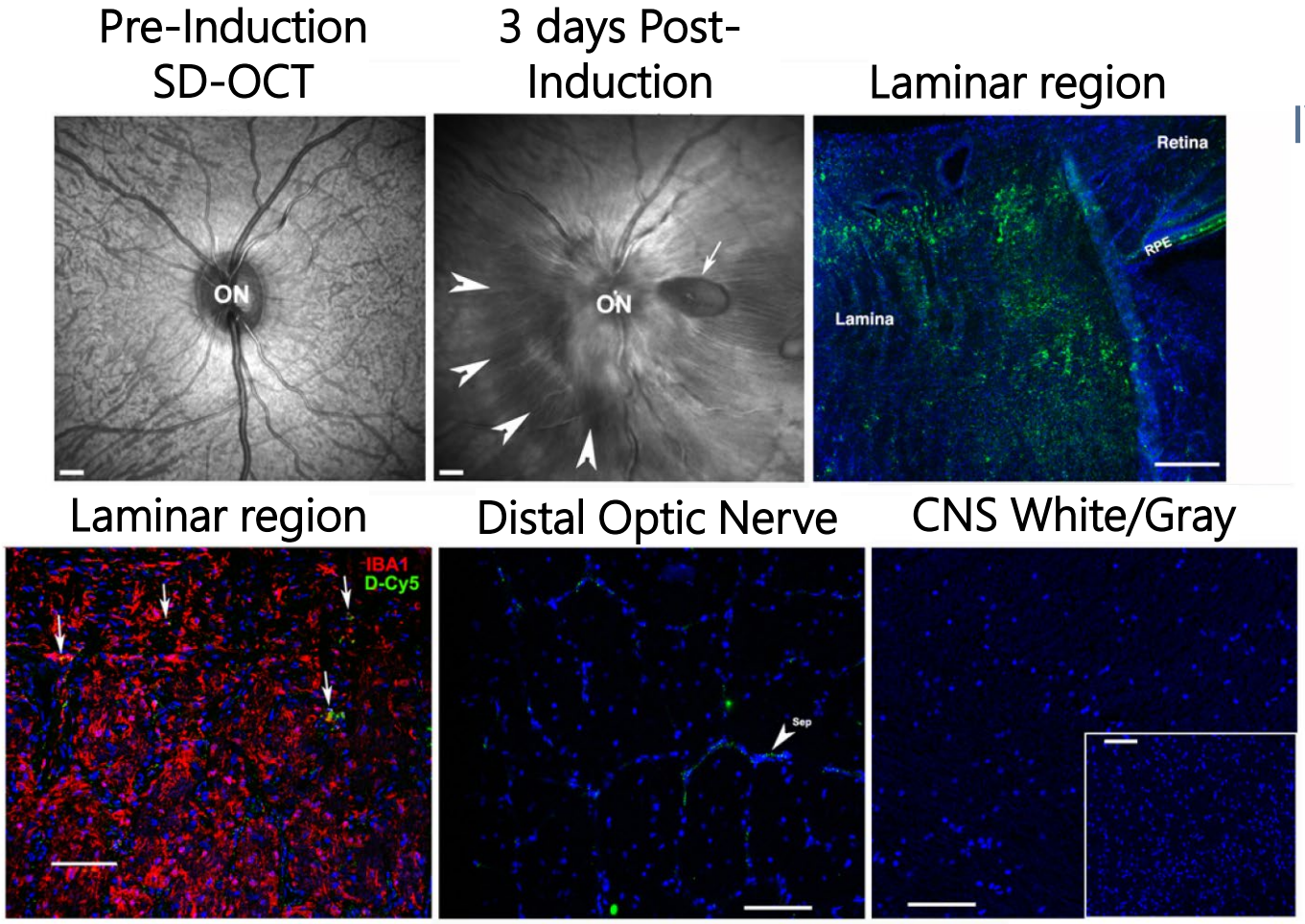
Disclosures

- Rishi Sharma, PhD, Santiago Appiani and Jeffrey Cleland, PhD are employees of Ashvattha Therapeutics which funded the research
- Justin Prater, M. Grazia Spiga, and David Culp are employees of Powered Research which was contracted by Ashvattha for the animal studies
- Kannan Rangaramanujam, PhD is an employee of Johns Hopkins School of Medicine and a co-founder of Ashvattha Therapeutics

Background – Targeting across Ocular-Blood Barrier

Non-human Primate Optical Nerve Injury Model



IV dose of Fluorescent Dendrimer (D-Cy5)

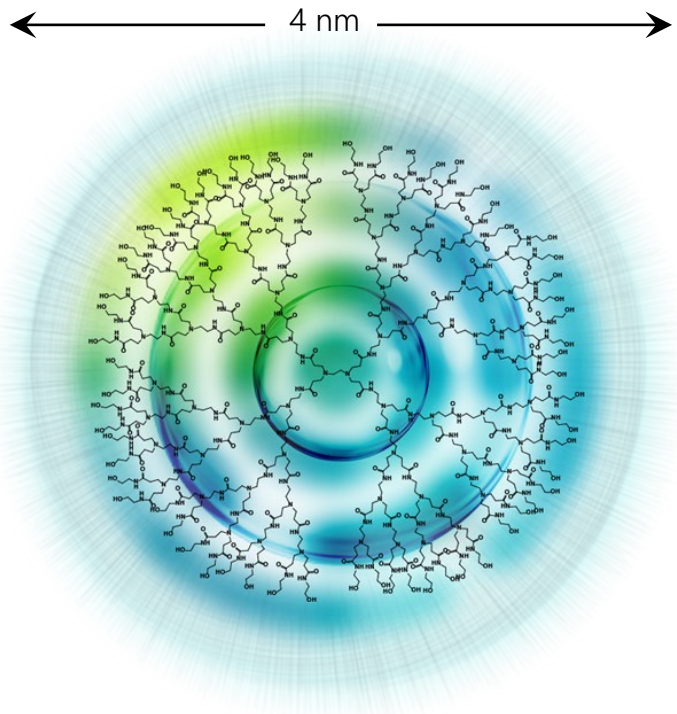
- Selective update in retinal pigment epithelia (RPE) cells, reactive microglia and reactive macrophage
- Minimal dendrimer in non-injured region of optic nerve or CNS
- Systemic dosing (IV) led to greater uptake in injured region than IVT dosing (rat studies)

Guo et al, 2016 PLoS ONE, 11(4): e0154437

Hydroxyl Dendrimer for Hyper-Targeted Delivery

- Selective targeting to activated cells (cells actively endocytosing) at sites of inflammation
- Proof of concept in 30+ animal models, 6 species including dogs and monkeys
- No off-target toxicity – no toxicity of platform at 1000 mg/kg
- Human safety (up to 40 mg/kg) – no clinical adverse events
- Flexible dosing – oral or injectable
- Wide range of drugs (>65) – small molecules, proteins, RNA/DNA
- Tunable functionality – multiple linkers and chemistries, different size dendrimers to alter PK/distribution
- Inexpensive to manufacture at large scale (>1 kg) (1 kg GMP lots)

Kannan et al. 2012, Science Transl Med; Sharma et al. 2017, BioEng Trans Med



~40 hydroxyl ends
~5-20 drug molecules per dendrimer

Tunable drug loading
Drug conjugates = NCEs

Hydroxyl Dendrimer Constructs

Building Blocks

Hydroxyl dendrimer

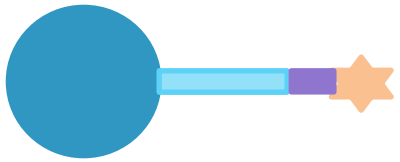
Bonds between dendrimer-drug
- Cleavable bond
- Non-cleavable bond

Linker Arm

Fluorescent tag

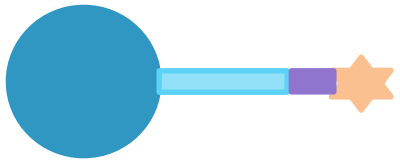
Constructs

Dendrimer-Isocyanine Green (D-ICG) (non-cleavable)



2-3 ICG/dendrimer

Dendrimer-tetramethylrhodamine (D-TRITC) (non-cleavable)



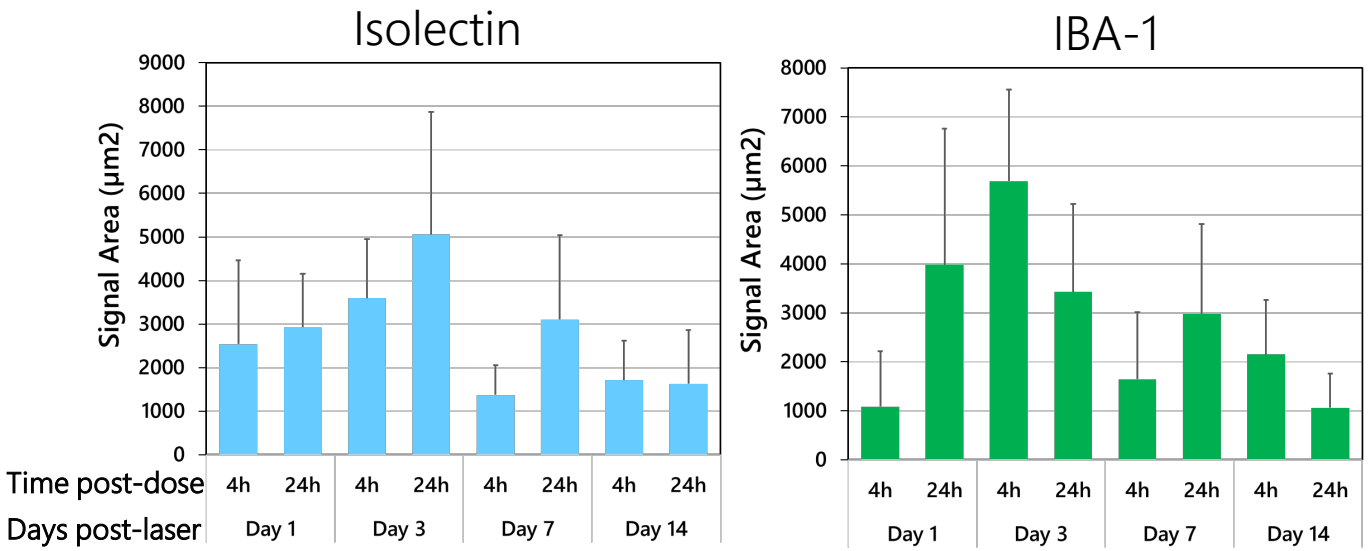
2-3 TRITC/dendrimer

In vitro release
- Intracellular conditions: pH 5.5, esterase, 37°C
- Extracellular conditions: PBS, pH 7.4, 37°C

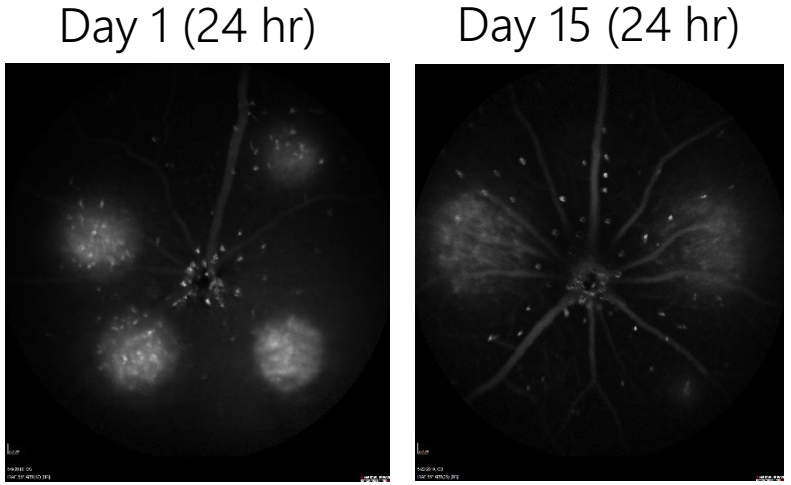
No Significant Release of ICG or TRITC from Dendrimers

Study 1: Timing of D-ICG uptake in CNV model

- Laser induced choroid neovascularization C57BL/6 mouse model (n=5/group)
- Administered IV 100 µL of D-ICG or vehicle control at 1, 3, 7, or 14 days post-laser
- Eyes analyzed by optical coherence tomography (OCT) with ICG imaging at 4 or 24 hr post-dose.
- Flat-mounts of the sclera-choroid/retinal pigment epithelial (RPE) complexes were stained by fluorescently tagged isolectin and IBA-1



IBA signal increases over the first 24-48h post-laser
Isolectin increases slightly later at the 48h post-laser



Early stage of CNV lesion greater uptake consistent with efficacy studies (24 hr post-laser)

Reactive Macrophage and Microglia Endocytose Dendrimer (24 hr post-laser)

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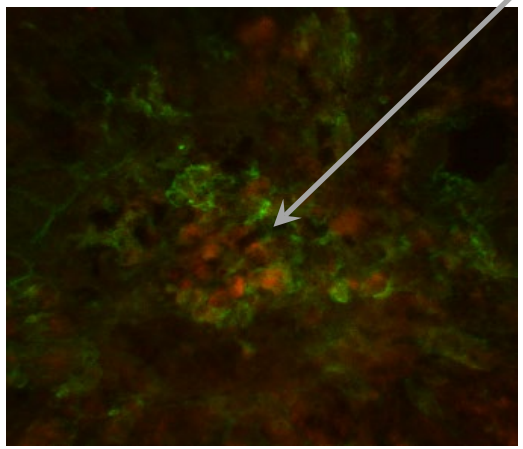
Study 2: Localization and Persistence in CNV lesions

- Laser induced choroid neovascularization C57BL/6 mouse model (n=5/group)
- Administered IV 100 µL of D-ICG and 100 µL of D-TRITC (1 hr after D-ICG) or vehicle control at 24 hr post-laser
  - Mice analyzed and sacrificed at 4, 7, 14, 21, and 28 days post-dose (n=5/group)
- Control group: free ICG (1.23 mg/mL), 100 µL, IV dosed 24 hr post-laser
  - Mice analyzed and sacrificed at 2, 4, 7, and 14 days post-dose (n=5/group)
- Eyes analyzed by optical coherence tomography (OCT) with ICG imaging
- Flat-mounts of the sclera-choroid/RPE complexes were stained by fluorescently tagged IBA-1

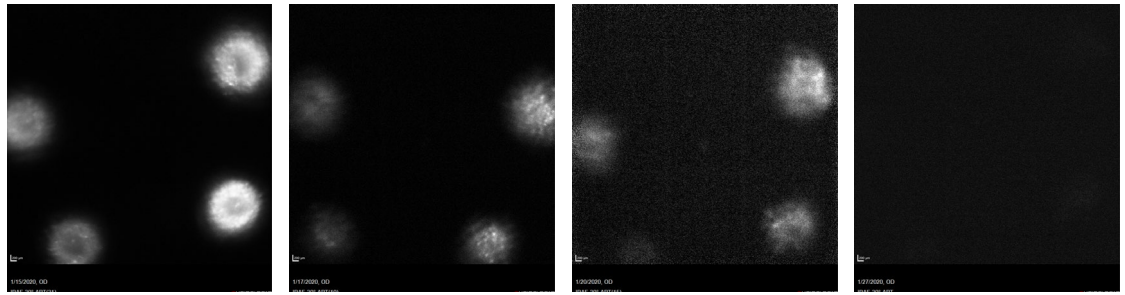
Green = IBA-1
Red = Dendrimer

Co-localization



Dendrimer localized in macrophages in CNV lesions

Free ICG Control Group:



Day 2 4 7 14

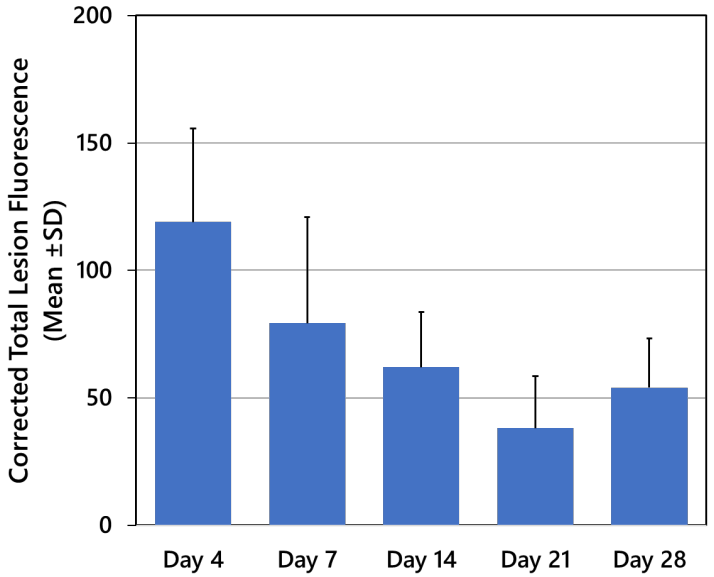
Free ICG no longer in lesions between 7-14 days

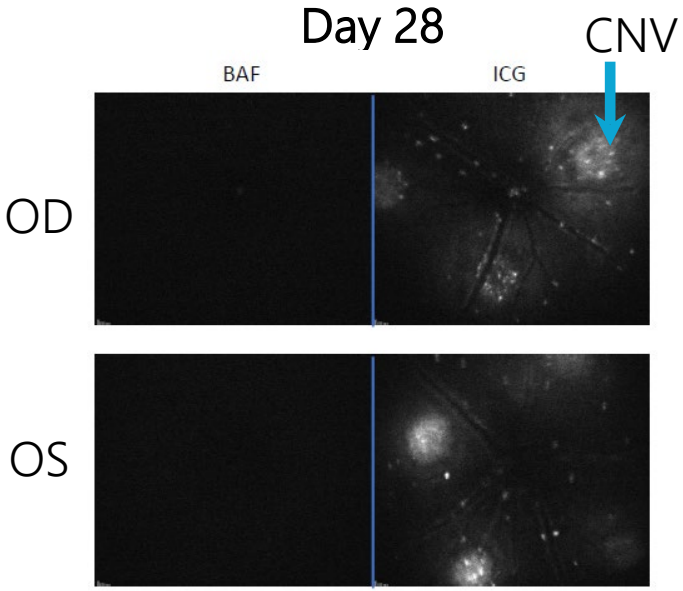
Sustained localization at CNV Lesions

Single IV dose of Dendrimer – ICG (D-ICG)

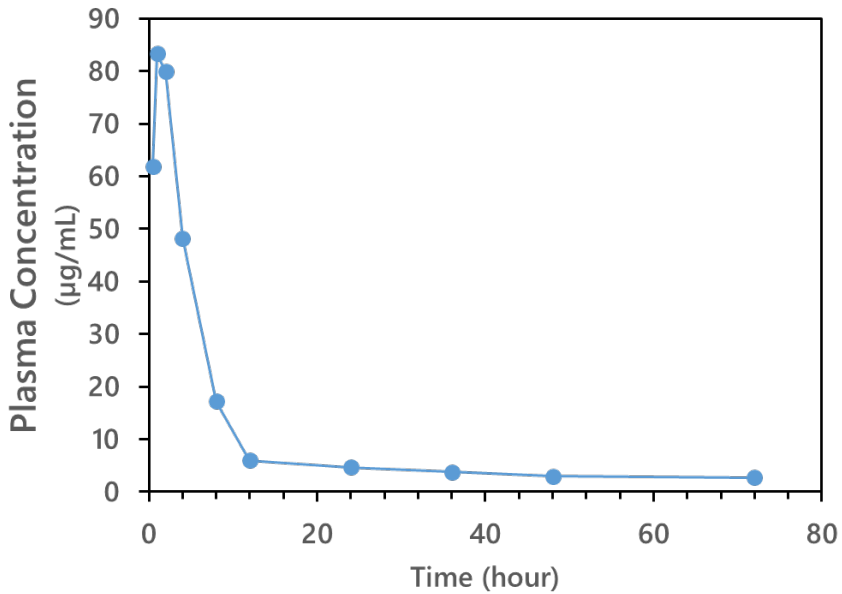
Mouse laser-induced choroidal neovascularization (CNV) model
(dosing 24 hr post-laser)

Targets & Persists in CNV area for >28 days





Systemic Clearance < 2 days



Potential for once per month systemic (subcutaneous or oral) treatment for retinal diseases with minimal systemic exposure

Summary: Targeted Sustained Localization from Single Systemic Dose

- Hydroxyl dendrimers target CNV lesions from systemic administration and are retained in lesions for >28 days
- Hydroxyl dendrimers co-localize with reactive macrophages in choroids, microglia/macrophages in retina, and RPE cells at the site of inflammation/neovascularization
- Macrophage, microglia, and RPE cells express VEGF receptors (VEGFR) in CNV animal models and patients with age-related macular degeneration[1-3]
- Conjugation of VEGF receptor tyrosine kinase inhibitors to hydroxyl dendrimers may enable drug targeting to ocular inflammation after systemic administration
  (see Presentation Number: 3974)

Hydroxyl Dendrimers Target and Persist in Cells Expressing VEGFR

1. McLeod et al, 2016 Retinal Cell Biology, 57: 5843-5854
2. Couturier et al, 2014 Molecular Vision, 20: 908-920
3. Liu et al, 2017 Oncotarget, 8: 979-987

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