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Suppression of Murine Choroidal Neovascularization After Systemic Administration of a Targeted Anti-VEGF Therapy

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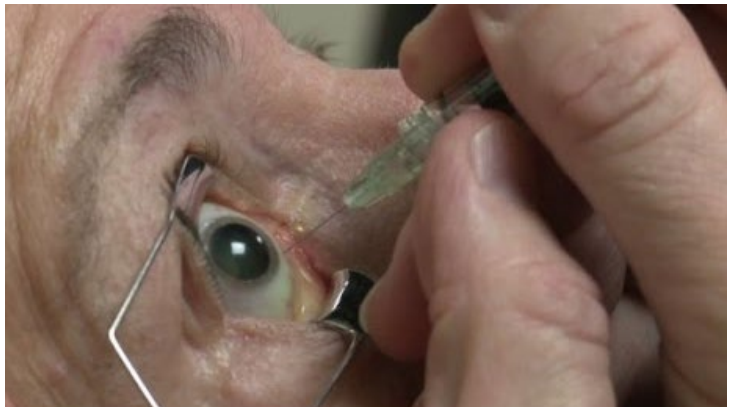
Disclosures

- 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

Background Rationale

- Current treatments for retinal and choroidal neovascularization require intravitreal injections as often as every 4 weeks
- Systemic (injectable or oral) administration every 4 weeks is a more convenient treatment option

Current Approved Therapies



Our Approach

Dose Route:	Intravitreal	Subcutaneous
Dose Frequency:	4 – 12 weeks	≥ 4 weeks
Drug MOA:	Anti-VEGF	VEGF RTKi

Challenges of Systemic Administration

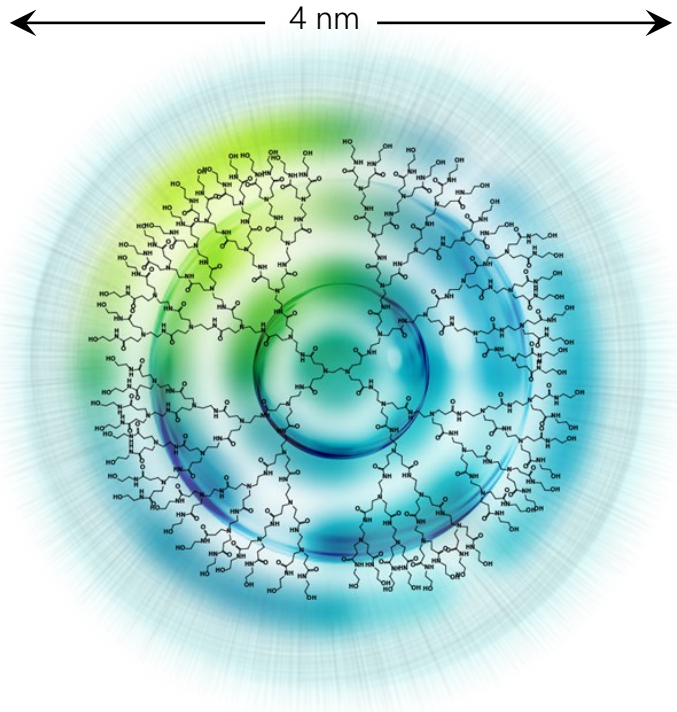
- Lack of drug targeting to ocular tissues
  - High systemic doses required
  - Off target toxicity
- Available anti-VEGF therapies
  - Antibodies and antibody fragments - long half-life (days) with systemic side-effects (Black Box warnings)
  - Small molecule receptor tyrosine kinase inhibitors (RTKi, e.g. sunitinib) - frequent dosing with systemic side-effects (Black Box warnings)
  - Systemically administered sunitinib is effective in treating laser induced choroidal neovascularization (CNV) in mouse models[1]
  - Sunitinib in an intravitreal depot formulation has demonstrated efficacy in CNV animal models[2] and in patients with wet age-related macular degeneration (AMD)[3]
- *Goal*
  - *Develop a systemically administered anti-VEGF therapy with selective targeting to CNV to achieve efficacy with minimal side effects*

1. Takahasi et al, J Ocul Pharmacol Ther 2006
2. Tsujinaka et al, Nat Commun 2020
3. Boyer et al, Hawaiian Eye & Retina annual meeting, 2019

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



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

Tunable drug loading
Drug conjugates = NCEs

Sunitinib Analog Hydroxyl Dendrimer Constructs

Building Blocks

Hydroxyl dendrimer

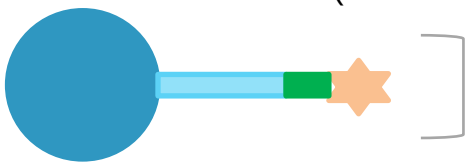
Bonds between dendrimer-drug
- Cleavable bond
- Non-cleavable bond

Linker Arm

Sunitinib analog

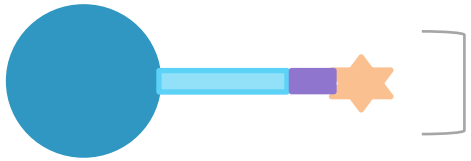
Constructs

D-CSA (cleavable)



5 (10.5% w/w drug)

D-NSA (noncleavable)



7 (12.6% w/w drug)

In Vitro Release of Sunitinib Analog:

D-CSA
- 6 days (timepoint of CNV analysis): ~65% cleaved at pH 5.5 with esterase (intracellular conditions)
- 24 hr (clearance of dendrimer): ~15% cleaved at pH 7.4 (plasma conditions)

D-NSA
- No detectable release

Binding Affinities (Kd, VEGFR2):

Free sunitinib analog released from D-CSA: 0.13 nM
Sunitinib analog noncleavable with linker: 1 nM
D-NSA: 27 nM

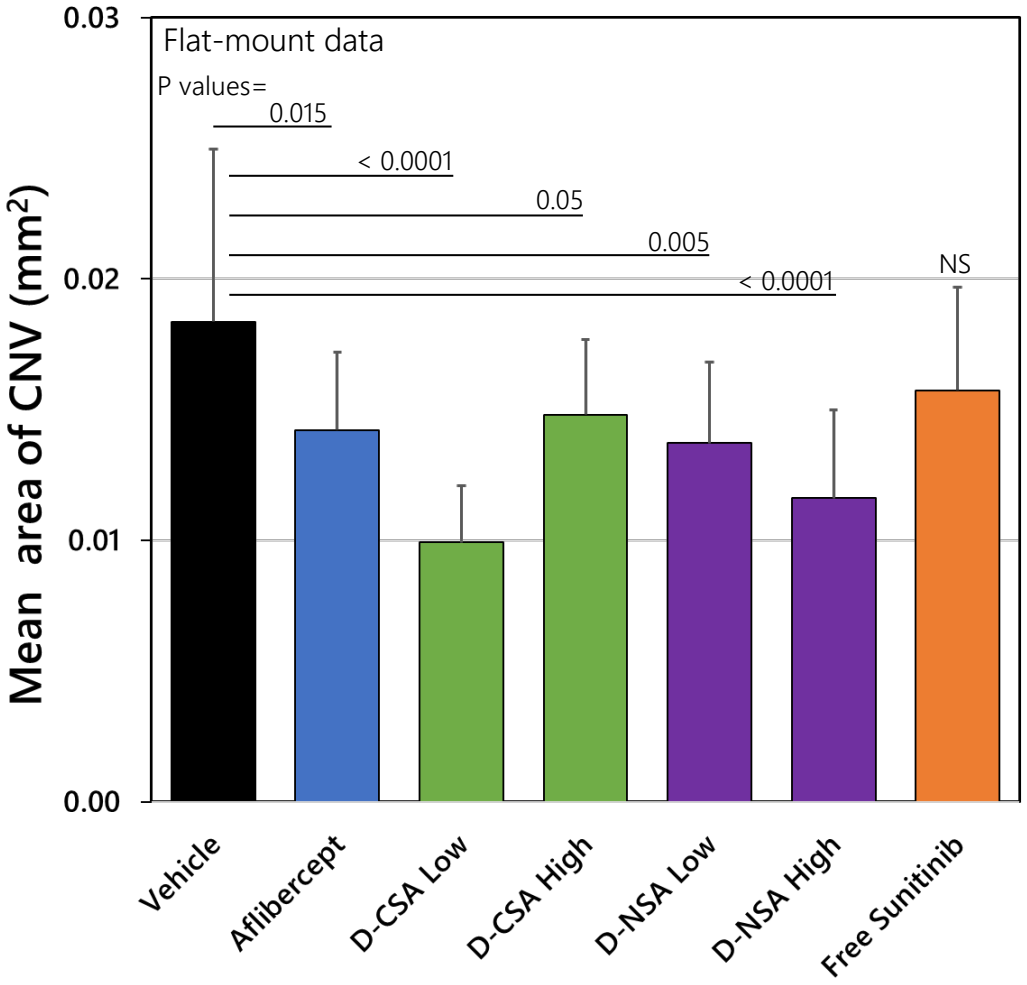
High Binding Affinity Retained in D-NSA

Preclinical Efficacy from Systemic Dosing

- Laser-induced CNV mouse model
  (Dosed 24 hrs post-laser; N=8/group (both eyes used))

- Study 1:
  - **SINGLE** IV dose (100 µL):
    D-CSA (5.25 (low) or 26.25 (high) mg/kg SA equivalent)
    D-NSA (6.3 (low) or 15.75 (high) mg/kg SA equivalent)
    Free sunitinib (32.5 mg/kg)
    Vehicle control
  - Intravitreal dosing of aflibercept (Eylea®; 1 µL, 40 µg)
  - The CNV area measured 7 days after laser treatment by both fluorescein angiography and flat-mounts of the sclera-choroid/RPE complexes stained with isolectin IB4

- *Release of sunitinib analog not required*
- *Efficacy comparable to IVT Aflibercept*



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Duration of Efficacy and Drug Systemic Clearance

- Laser-induced CNV mouse model

  (Dosed 24 hrs post-laser; N=8/group (both eyes used))

- Study 2 – Study Duration of Effect:
  - **SINGLE** IP dosing (100 µL):

    D-CSA: 5.25 mg/kg SA equivalent

    D-NSA: 6.3 mg/kg SA equivalent

    Free sunitinib 6.5 mg/kg

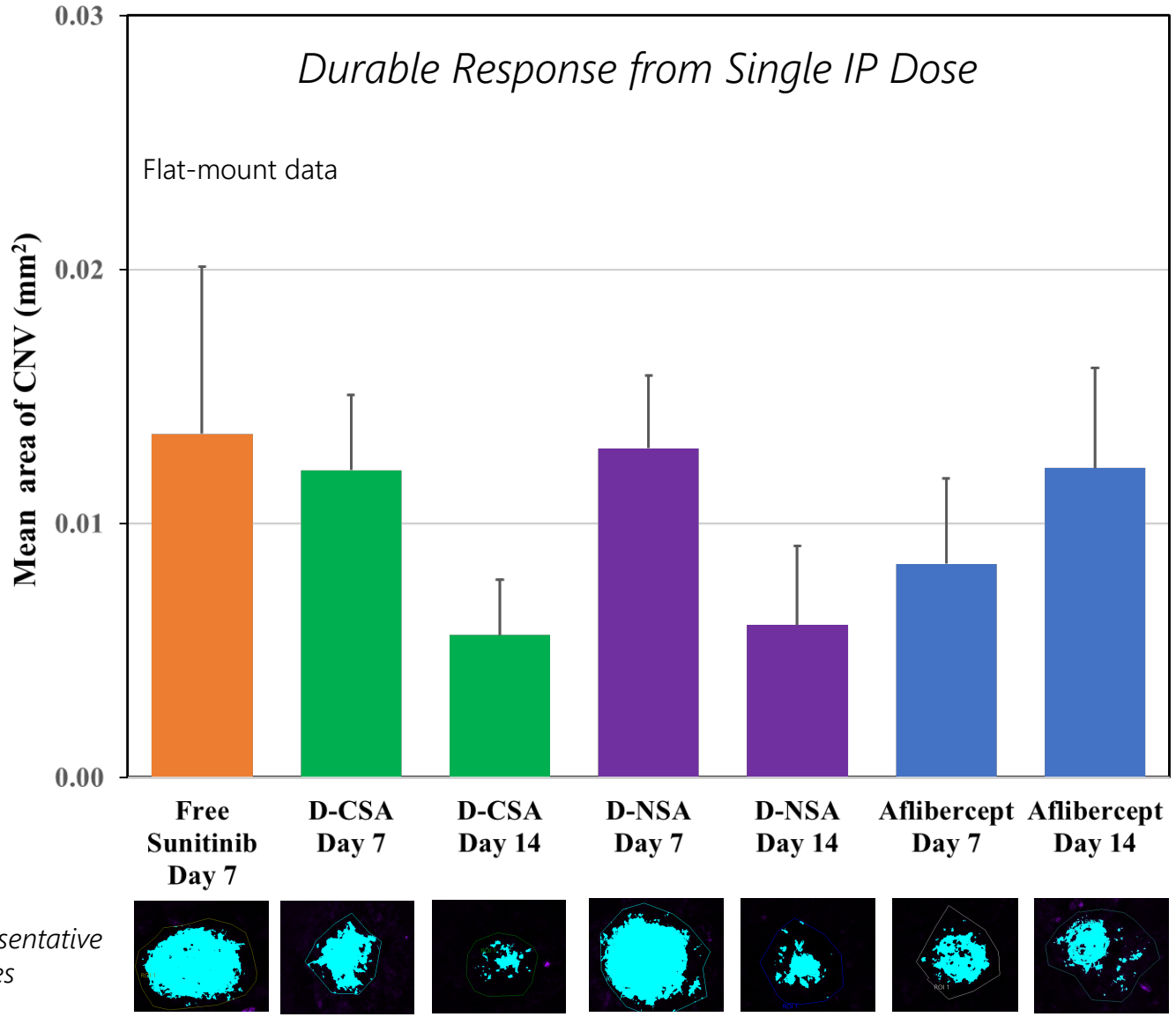
  - Intravitreal dosing of aflibercept (Eylea®; 1 µL, 40 µg)
  - CNV area evaluations in 2 Phases:

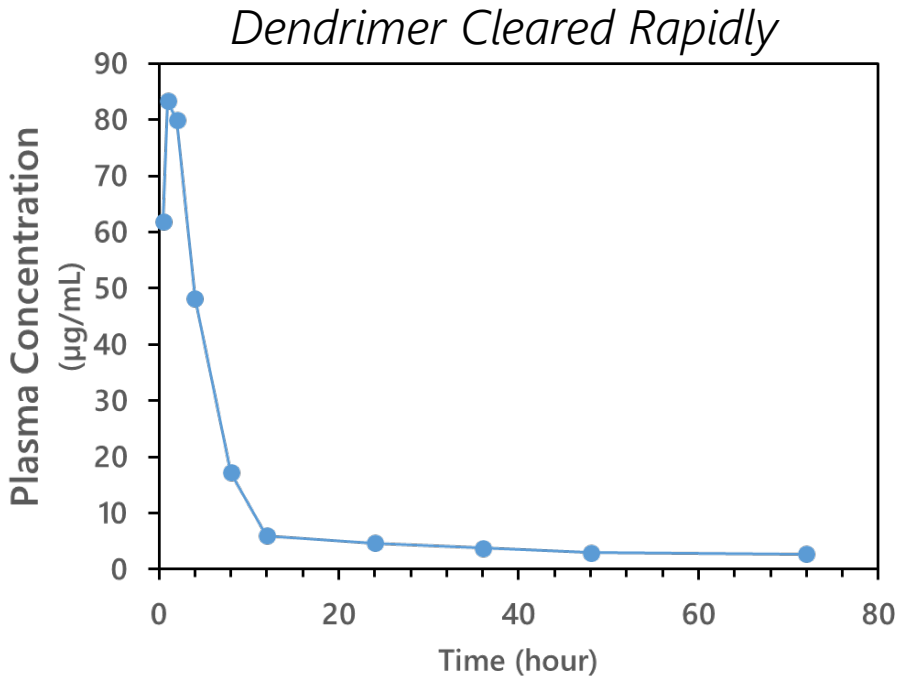
    1: Day 7 analysis with FA and flat-mounts for all D-CSA and D-NSA groups, vehicle and aflibercept (n=8/group)

    2: Day 14 analysis with FA and flat-mounts for high dose D-CSA and D-NSA groups, vehicle and aflibercept (n=8/group)

- Plasma PK study:
  - same dendrimer labelled with Cy5, single IP injection in mice followed by plasma collection up to 72 hr

Study 2: Durability of Response vs Clearance





- *D-CSA/D-NSA cleared within 2 days*
- *Prolonged local effect on CNV lesions*
- *Lesion size continues to decrease at Day 14*

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Summary: Targeted Systemic Anti-VEGF Therapy

- Hydroxyl dendrimers target CNV lesions from systemic administration and are retained in lesions for >28 days
  (see Poster # 4927-B0132)
- Conjugation of a sunitinib analog to hydroxyl dendrimers maintains nanomolar potency for VEGF RTK
- Single doses of D-CSA/D-NSA administered in laser-induced CNV mouse model demonstrated efficacy comparable to aflibercept
- Response to D-CSA/D-NSA is durable and effect increased with time in CNV model
- Toxicology studies are ongoing to demonstrate safety of systemic dosing

Dendrimer-sunitinib analog conjugates may enable systemic treatment of CNV

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