Single Subcutaneous (or Oral) Dose of Anti-Angiogenesis Drug Safely Suppresses Choroidal Neovascularization Comparable to Intravitreal Aflibercept

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Disclosures

- Rishi Sharma, PhD, Santiago Appiani, Minghao Sun, PhD, Siva Kambhampati, PhD, and Jeffrey Cleland, PhD are employees of Ashvattha Therapeutics which funded the research
- Justin Prater and David Culp are employees of Powered Research which was contracted by Ashvattha for the animal studies

Background

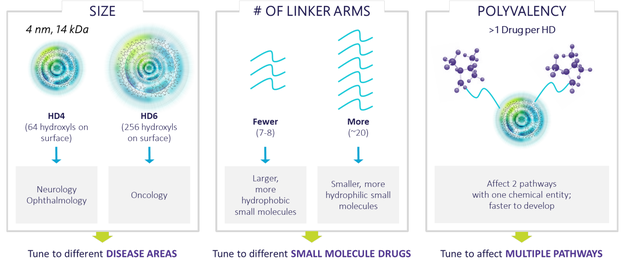
- Hydroxyl dendrimers (HDs) selectively target choroidal neovascular (CNV) lesions after a single systemic dose[1]
- HDs are taken up by macrophages, microglia and hypertropic retinal pigment epithelial (RPE) cells[1]
- HDs are retained in these cells in CNV lesion for at least 1 month after a single systemic dose[1]
- Sunitinib has been shown to suppress neovascularization in animals and humans[2], but has significant off target toxicity when administered systemically[3]
- Covalently linking a sunitinib analog to the HD creates a new chemical entity that solves the toxicity issues while retaining the potency
- Previous studies demonstrated a sustained inhibition (14 days) of CNV formation after a single systemic dose of HD-sunitinib analog (D-4517) comparable to an intravitreal (IVT) dose of aflibercept[4]

Hydroxyl Dendrimer (HD) Technology

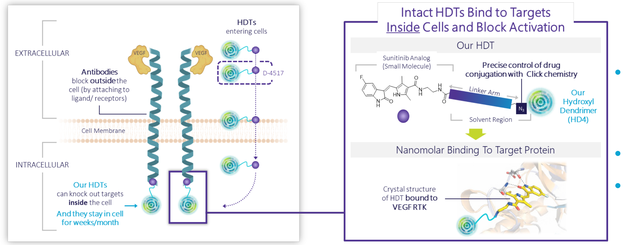
A Differentiated, Superior Approach to Targeted Intracellular Therapy

Attribute	HDs	Antibody (MAb/Fab) Drug Conjugates (ADCs)	Other Nanoparticles	Exosomes
Selective Uptake	✓	X — Targets receptors (reactive and normal cells)	X	X
Crosses Tissue Barriers	✓	X — Requires transport /restricted to vasculature	X	X
Lack of Off-target Toxicity	✓	X — All cells with receptors	X	X
Sustained Intracellular Effects	✓	X — Payload released within days	X	X
Tunable for Each Indication	✓	X — Same PK/distribution	X	X
Low-Cost Manufacture	✓	X — High – very high COGS	X	X

Tunable for Each Indication and Small Molecule Drug



D-4517: Mechanism of Action & Design



- No detectable degradation *in vitro* or metabolism *in vivo*
- No labile bonds
- Renal clearance

Nanomolar binding to RTKs:
10 nM (VEGFR1), 14 nM (VEGFR2), 11/7.5 nM (PDGFRA/PDGFRB), 41 nM (CSF1R), 3 nM (KIT)
Each D-4517 has 7-8 sunitinib analogs conjugated to the **HD4**

D-4517: Preclinical Proof of Concept

Study Design
- 24 hr prior to dosing, laser-induced rupture of Bruch's membrane in both eyes of C57BL/6 mice (n=8/group)

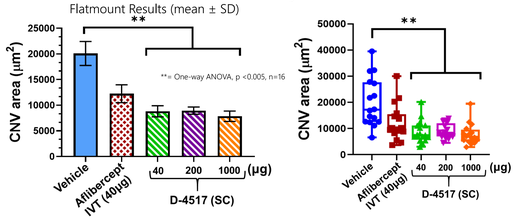
Study 1:
- Single subcutaneous dose of D-4517 at 40, 200 or 1000 µg.
- Positive control of 40 µg aflibercept IVT
- Negative control of vehicle (PBS) IVT

Study 2:
- Single subcutaneous dose of D-4517 at 10 or 40 µg.
- Single oral dose of D-4517 at 1000 or 5000 µg.
- Positive control of 40 µg aflibercept IVT
- Negative control of vehicle (PBS) IVT

CNV area measured 14 days after laser treatment by both fluorescein angiography and flat-mounts of the sclera-choroid/RPE complexes stained with Isolectin

Study 1 Results

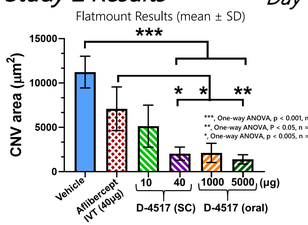
Day 14

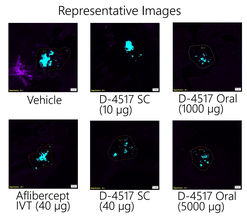


Consistent Response to D-4517 SC Doses

Study 2 Results

Day 14





Persistent and Significantly Greater Response to Single oral or SC dose of D-4517 compared to Aflibercept

D-4517: Toxicology

Study Design
- Non-GLP toxicology study with Sprague Dawley rats (10 male/10 female per group for toxicology and 5 male/5 female per group for toxicokinetics)
- 4 Groups
  - Vehicle control (daily IP) x 14 days
  - 30 mg/kg sunitinib (daily PO) x 14 days
  - 12 mg/kg (~3 mg) D-4517 (daily IP) x 14 days
  - 168 mg/kg (~42 mg) D-4517 x 1 dose
- Study conducted by Charles River Laboratories
- Toxicokinetics, clinical evaluation, pathology and histology on all animals

Daily Sunitinib (30 mg/kg, PO)	Daily D-4517 (12 mg/kg, IP)	Single Dose D-4517 (168 mg/kg, IP)
4 of 15 animals died on study (bone marrow toxicity)	No deaths	No deaths
Generalized bone marrow toxicity	No observed effect	No observed effect
Liver toxicity (decreased size/increased ALT/AST)	No observed effect	No observed effect
Proteinuria, decrease in kidney size (Males)	No observed effect	No observed effect
Hypoglycemia (Females)	No observed effect	No observed effect
Decrease in heart size (Males)	No observed effect	No observed effect

Sunitinib toxicity consistent with previous studies[3] and Sutent® label

No observed adverse effects of D-4517 at >80 fold above the effective dose

Conclusions & Next Steps

- **Single subcutaneous dose** of D-4517 (10 or 40 µg) resulted in comparable or better inhibition of CNV to a single IVT aflibercept dose (40 µg)
- **Single oral dose** of D-4517 resulted in significantly better inhibition of CNV compared to a single IVT aflibercept dose (40 µg)
- Repeat dose (12 mg/kg daily) or high single dose (167 mg/kg) of D-4517 did not result in any observed adverse effects in rats, whereas a comparable dose of sunitinib cause significant toxicity and mortality
- No observed toxicity at >80 fold higher dose than effective dose in preclinical efficacy studies
- GLP toxicology ongoing in rats and dogs to support IND
- Phase 1 study (safety) to be completed by end 2021
- Initial Phase 2 data in mid-2022 (wet AMD patients)

References

1. Sharma et al, 2020, ARVO Poster: 4927 - B0132
2. Jackson et al, 2017, JAMA Ophthalmology 135(7): 761-767
3. Patyna et al, 2008, Toxicologic Pathology 36: 905-916
4. Cleland et al, 2020, ARVO Presentation 3974, Session 429

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