Safety and Tolerability of a Single Subcutaneous Dose of Anti-Angiogenesis Drug to Treat Neovascular Age-related Macular Degeneration (wet AMD) and Diabetic Macular Edema (DME)

Jeffrey L. Cleland[1], Rishi Sharma[1], Santiago Appiani[1], Jerome Moore[2], Brian Rogers[2]

1. Ashvattha Therapeutics, Inc, Redwood City, CA; 2. Pacific BioDevelopment, Davis, CA

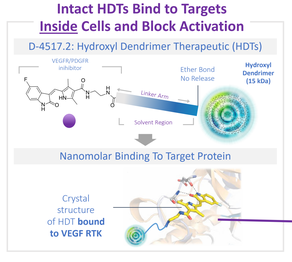
Disclosures

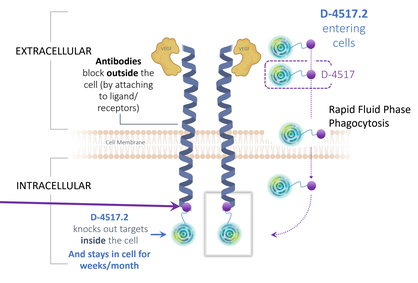
- Rishi Sharma, PhD, Santiago Appiani, and Jeffrey Cleland, PhD are employees of Ashvattha Therapeutics which funded the research
- Jerome Moore and Brian Rogers are employees of Pacific BioDevelopment, a consulting firm, which was contracted by Ashvattha for the design and analysis of the animal studies

Background

- **Hydroxyl dendrimers (HDs) are a new class of compounds to enable disease cell targeted precision medicine with superior targeting compared to antibodies**
- HDs are metabolically stable in animals and humans, smaller than antibodies, and renally excreted intact (no liver uptake).
- Chemical conjugation of small molecule drugs to HDs create HD therapeutics (HDTs) that are new chemical entities having the distribution, clearance and safety profile of the core HD.
- HDTs reduce or eliminate off target toxicity and increase efficacy in preclinical models when compared to small molecule therapeutics.
- Hydroxyl dendrimers (HDs) selectively target choroidal neovascular (CNV) lesions after a single systemic dose.[1-4]
- HDs are taken up by macrophages, microglia and hypertropic retinal pigment epithelial (RPE) cells only in areas of neovascularization or inflammation in the choroid and retina.[1,4]
- HDs are retained in these cells in CNV lesion for at least 1 month after a single systemic dose.[1]
- Previous studies demonstrated a sustained (14 days) inhibition of CNV formation after a single systemic dose of D-4517.2 comparable to an intravitreal (IVT) dose of aflibercept[3]
- A study in Oxygen-Induced Retinopathy (OIR) mouse model demonstrated comparable efficacy between subcutaneously dosed D-4517.2 and aflibercept (data not shown).

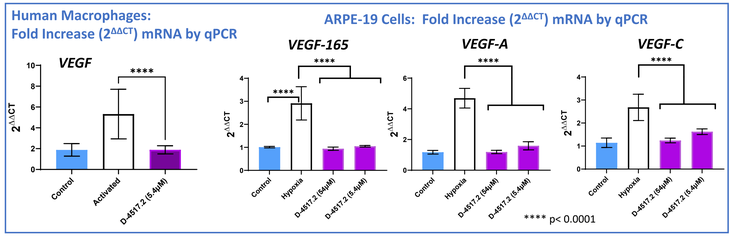
D-4517.2: Systemic Therapy for Macular Disease





Nanomolar binding to RTKs:
10/14 nM (VEGFR1/2), 11/7.5 nM (PDGFRA/PDGFRB), 41 nM (CSF1R), 3 nM (cKIT); Each D-4517.2 has 7-8 drug arms

Restores VEGF Expression to Normal



GLP Toxicology

- Toxicology studies were conducted by Charles River Laboratories in accordance with ICH M3 (R2) and per FDA requirements to support an IND.
- In vitro data demonstrated that D-4517.2 was stable in human, rat and dog plasma with no detectable metabolism.
- Single dose and multiple dose (28 day) GLP toxicology studies were conducted in rats and dogs with comprehensive clinical evaluations and anatomical pathology evaluations that included the examination of a full list of tissues.

Single Dose & Repeat Dose (28 day) Sprague-Dawley Rat Studies (subcutaneous)

- Clinical and anatomical pathology alterations attributable to changes in the kidney (clearance organ).
- No-observed-adverse-effect level (NOAEL) of 40 mg/kg.

Single Dose & Repeat Dose (28 day) Beagle Dog Studies (subcutaneous)

- Minimal tubular degeneration in the kidney associated with the pigment accumulation; NOAEL of 50 mg/kg.

Genetic Toxicology

- No evidence of genotoxicity was observed in in vitro bacterial (Ames) and mammalian (micronucleus) assays or the in vivo micronucleus test in rats.

Local Tolerance

- Reversible dose-responsive gross and microscopic changes at the site of injection were noted in rats and dogs.

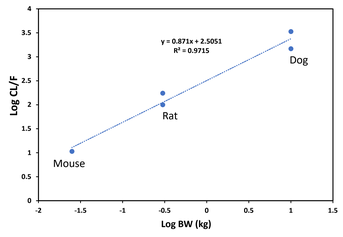
Comparison of Animal NOAEL Doses and Highest Anticipated Clinical Dose

Species	NOAEL Following Weekly Administration 4-Week Study (mg/kg)	HED[a] (mg/kg)	Multiple of Highest Anticipated Bi-Weekly Clinical Dose[b]
Rat	40	6.5	**3.25**
Dog	50	28	**14**

a. HEDs are calculated using a factor of 6.2 for Sprague Dawley rats and 1.8 for Beagle dogs.
b. When compared to the 2 mg/kg human dose.

Pharmacokinetics - Allometric Scaling

- Pharmacokinetics of D-4517.2 was evaluated in mice, rats, and dogs at Charles River Laboratories
- Plasma concentrations were determined by LC/MS/MS
- Allometric plot of Log CL/F (Clearance, h-μg/mL/Bioavailability) vs Log BW (Body Weight, kg) indicates a linear relationship



Translation to Human Clinical Trials

- Efficacy in CNV mouse model at 0.5 and 2 mg/kg[4] yield exposures of 1.2 to 4.7 h-μg/mL
- Proposed human doses of 0.25 to 1 mg/kg projected to yield comparable exposure

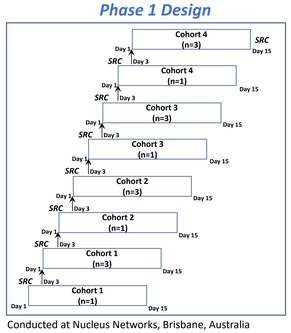
Phase 1 Healthy Volunteer Study Design

- **Single Ascending Subcutaneous Dose Phase 1 in Healthy Volunteers**

- **Objectives**:
  - Primary: Evaluate safety and tolerability of D-4517.2 after single subcutaneous (SC) doses in healthy subjects
  - Secondary: Determine the pharmacokinetic (PK) profile of D-4517.2 after single SC doses in healthy subjects

- **Summary Design**:
  - Sentinel (1) subject at each dose level evaluated for safety and tolerability at Day 3 by Safety Review Committee (SRC)
  - Additional 3 subjects enrolled at same dose level
  - After all subjects at a dose level achieve Day 3, SRC evaluates safety and tolerability to escalate to next dose level
  - Dose Levels: 0.25 mg/kg (Cohort 1), 0.50 mg/kg (Cohort 2), 1.0 mg/kg (Cohort 3), 2.0 mg/kg (Cohort 4)
  - For additional details see https://www.clinicaltrials.gov/ct2/show/NCT05105607

Phase 1 Design



Conducted at Nucleus Networks, Brisbane, Australia

Phase 1 Demographics

Parameter	Statistics	D-4517.2 (0.25 mg/kg) (N=4)	D-4517.2 (0.50 mg/kg) (N=4)	D-4517.2 (1.0 mg/kg) (N=4)	Overall (N=12)
Age (years)	Mean	27.1	44.2	41.0	37.5
	Std Dev	5.0	16.6	23.9	31.1
	Median	26.4	46.4	39.7	17.2
	Min, Max	22.5, 33.2	24.4,59.5	22.4, 65.9	22.4, 65.9
Male	n (%)	0 (0)	1(25)	1(25)	2 (16.7)
Female	n (%)	4 (100)	3 (75)	3 (75)	10 (83.3)
Asian	n	1[a]	0	0	1
White	n	1[a]	4	4	9
Other	n	2	0	0	2
Unknown	n	1	0	0	1
Hispanic or Latino	n	2	0	0	2
Not Hispanic or Latino	n	2	4	4	10

a. One subject identified as part White and part Asian

Phase 1 Safety & Pharmacokinetics

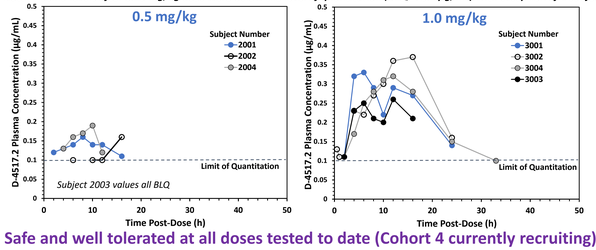
OVERALL SAFETY	D-4517.2 (0.25 mg/kg) (N=4) n (%)	D-4517.2 (0.50 mg/kg) (N=4) n (%)	D-4517.2 (1.0 mg/kg) (N=4) n (%)	Overall (N=12) n (%)
Intent-to-Treat Set	4 (100)	4 (100)	4 (100)	12 (100)
Subjects Discontinued from Study	0	0	0	0
Adverse Events (Total)	0	5	2	7
Death	0	0	0	0
Subjects with TEAE	0	2 (50)	1 (25)	3 (25)
Drug-related TEAE	0	4	1	5
Serious TEAE	0	0	0	0
Drug-related Serious TEAE	0	0	0	0

0.50 mg/kg: 1 subject with two mild transient injection site reactions (ISR) & 1 subject with mild transient ISR and mild transient fatigue; 1.0 mg/kg: 1 subject with mild transient ISR; No abnormalities on Safety Labs (N=12)

Pharmacokinetics

Dose (mg/kg)	C_{max} (μg/mL)	C_{max}/D (kg*μg/mL/mg)	T_{max} (h)	AUC_{last} (h*μg/mL)	AUC_{last}/D (h*kg*μg/mL/mg)	CL/F (mL/hr/kg)	$T_{1/2}$ (h)
0.50	0.171	0.342	11.333	1.679	3.359	114.960	14.755
1.00	0.320	0.320	11.500	5.430	5.430	124.578	11.298

C_{max} = Maximum plasma concentration; D = Dose; T_{max} = time to maximum plasma concentration;
AUC_{last} = Area under the curve to last time point measured; CL = Clearance; F = bioavailability; $T_{1/2}$ = plasma half-life
Note: Plasma levels after 0.25 mg/kg dose were below the level of quantitation (BLQ, < 0.1 μg/mL) with exception of 1 subject



- **Safe and well tolerated at all doses tested to date (Cohort 4 currently recruiting)**
- **Mild transient injection site reactions to be addressed with injection method changes**
- **Exposures consistent with allometric scaling indicating 0.50 to 1.0 mg/kg in humans may provide comparable effect to that observed in CNV mouse model**

Conclusions & Next Steps

- **Single subcutaneous doses of D-4517.2 were safe and well tolerated in healthy subjects**
- **D-4517.2 exposure** in healthy subjects translates to efficacious doses in CNV mouse model[4]
- **Phase 2 study initiating in wet AMD and DME patients**
  - Stage 1: Crossover design to evaluate IVT aflibercept vs SC D-4517.2 in same patient (n = 30)
  - Stage 2: Randomized, double masked, sham/placebo non-inferiority study compared to aflibercept (n = 270)

References

1. Sharma et al, 2020, ARVO Poster: 4927 - B0132
2. Kambhampati et al, 2021, J. Controlled Release 335: 527-540
3. Cleland et al, 2020, ARVO Presentation 3974, Session 429
4. Cleland et al, 2021, ARVO Poster: 3540583

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