#2039 Beagle Dog Studies for ANG1005, a Novel Antimicrotubule Agent that Targets the Brain

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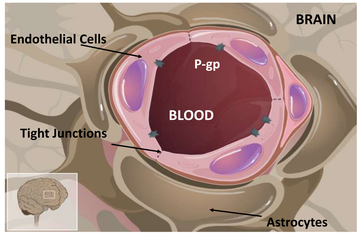
ABSTRACT

ANG1005 is an antimicrotubule agent that contains the proprietary sequence of amino acids responsible for receptor-mediated transcytosis across the blood brain barrier (BBB). Angiochem's proprietary Engineered Peptide Compound (EPiC) platform targets the low-density lipoprotein receptor-related protein (LRP) receptor family. ANG1005 has been tested in Sprague-Dawley rats in safety pharmacology and toxicology studies. The toxicity profile of ANG1005 in Beagle dogs has been investigated in a series of intravenous (IV) infusion toxicology studies. ANG1005 was administered at single IV dose levels of 0, 100, 200, or 400 mg/m^2 with both 400 mg/m^2 animals not surviving past Day 4. Reversible decreases observed for platelets, reticulocytes, and WBCs (all types) at 100 or 200 mg/m^2. The maximum tolerated IV dose was 200 mg/m^2. The mean half-life for all ANG1005-treated animals following a single dose was 2.76 0.53 h. In a repeated-dose IV toxicity study, ANG1005 was administered at 0, 15, 45, or 90 mg/m^2 twice weekly for 4 weeks. Modest hematological changes were observed in animals administered 90 mg/m^2 ANG1005, with the effects fully recovered by the end of the recovery period. Microscopic changes related to ANG1005 included unilateral and bilateral degeneration of the seminiferous tubules in the testes of males (a known effect of paclitaxel) in either the 45 mg/m^2 (1/4) or 90 mg/m^2 (1/6) treatment groups, with the effect reversible for the 90 mg/m^2 males. Plasma concentrations decreased mono-exponentially with an overall half-life of 2.68 0.49 h. The TK profiles of ANG1005 were similar at all dose levels, with no apparent gender differences. There were no treatment-related findings for the testes at the lowest dose (15 mg/m^2) and the NOAEL in this study was 15 mg/m^2. Based on the lack of severe findings in this study, the highest non-severe toxic dose (HNSTD) is 90 mg/m^2. The ANG1005 dog toxicity studies supported in part the initiation of 2 Phase 1/2 clinical trials in brain cancer patients.

INTRODUCTION

Angiochem is a clinical-stage biotechnology company discovering and developing new breakthrough drugs that are uniquely capable of crossing the blood brain barrier (BBB) to treat brain diseases and related disorders. These proprietary Engineered Peptide Compounds (EPiC), consisting of small and large molecules, have the potential to address significant medical needs, many of which cannot be effectively addressed due to the fundamental physiological challenge the BBB presents.



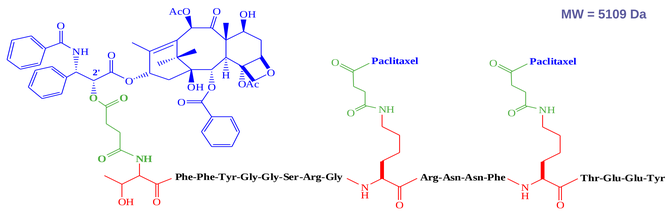
The BBB is a selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. The BBB is important as it provides an insulated environment for stable neuronal function. Endothelial cells forming the BBB are not only able to form tight junctions, but also possess the following characteristics that further protect the brain, they:

- ✓Lack fenestra;
- ✓Lack transendothelial channels;
- ✓Lack pinocytic vesicles; and
- ✓Express high levels of the active efflux pump (P-gp).

ANG1005, an antimicrotubule EPiC, is the first drug candidate of this platform to reach the clinical stage of development. Preclinical studies have shown that ANG1005 enters the brain through targeting the low-density lipoprotein receptor-related protein (LRP). This endogenous transcytosis system has a number of inherent biochemical advantages for drug transport across the BBB, including high expression, rapid turnover, numerous ligands of varying sizes, and limited down-regulation. LRP has been shown to be upregulated in primary brain tumors, brain metastases from lung cancers, a human hepatocellular carcinoma cell line, breast cancers, and melanomas (Bu *et al*., 1993; Moestrup *et al*., 1995; Grimsley *et al*., 1997; Orlando *et al.,* 1997; Gliemann, 1998; Hussain, 2001). It is postulated that following intravenous administration, ANG1005 binds to the LRP and is transported across the BBB *and* reaches the cancer cells in the brain where it is internalized, with subsequent esterase cleavage to release paclitaxel.

In vitro studies have demonstrated that ANG1005 maintains numerous mechanisms comparable to paclitaxel including: inhibition of tumor cell proliferation (cytotoxicity), blockage of tumor cells in G2/M phase, and induction of β-tubulin polymerization.

Nonclinical pharmacology studies have demonstrated ANG1005 to have a safe and effective profile to date; and the results suggest that it is promising as an innovative treatment for brain cancers.



In Vitro Cytotoxicity Study

The in vitro antitumor activity of ANG1005 has been tested in a series of human cancer cell lines cells. The IC_{50} values for ANG1005 were comparable to paclitaxel in most of the cells lines; data from this study are shown below.

Cell Line	IC_{50} (nM)			
	Paclitaxel		ANG1005	
	Mean	SD	Mean	SD
U-87 (human glioblastoma)	12.94	2.49	17.75	10.82
CGL-3 (human glioblastoma)	>100	-	>100	-
CGL-9 (human glioblastoma)	>100	-	>100	-
NCI-H460 (human lung carcinoma)	6.61	3.35	12.68	10.08
Hep G2 (human hepatocarcinoma)	>100	-	42.98	-
SK-HEP-1 (human hepatocarcinoma)	8.84	-	7.83	-
MCF-7 (human breast carcinoma)	>100	-	>100	-
HCC1954 (human breast ductal carcinoma)	6.12	-	8.26	3.37
MDA-MB-231 (human breast adenocarcinoma)	17.61	-	28.16	-
MDA-MB-468 (human breast adenocarcinoma)	13.52	-	1.41	-
BT-474 (human breast carcinoma)	62.86	-	40.22	-

In Vitro Safety Pharmacology

Effects of ANG1005 on hERG currents from transfected HEK293 cells were tested.

ANG1005 at concentrations up to 25 µM (the maximum feasible dose) did not result in significant inhibition on hERG tail current density in transfected HEK293 cells, suggesting that ANG1005 does not interact with the protein that encodes the hERG gene and is responsible for the IK_r-like current.

METHODS FOR TOXICOLOGY STUDIES

Toxicology studies of ANG1005 were conducted in Beagle dogs and in Sprague-Dawley rats (Abstract No. 2038).

Study	Number of Animals	Treatment and Dose(s)	Comments
Toxicology			
Single Dose	1M and 1F per group	IV (4-hour infusion) 0, 100, 200, or 400 mg/m^2	14-day observation period
Repeated Dose	Main: 4M/4F per group Recovery: 2M/2F per group	IV (4-hour infusion) 0, 15, 45, or 90 mg/m^2 on Days 1, 4, 8, 11, 15, 18, 22, and 25 (twice weekly for 4 weeks)	3-day observation period for main study animals and a 14-day observation period for recovery animals

M = Male; F = female

*All studies were conducted using the same route of administration, IV (4-hour infusion) and drug formulation (ANG1005 and 7% Solutol).

The general non-rodent toxicity studies included standard endpoints such as mortality, clinical observations, toxicokinetics (TK), clinical pathology, body weight and food consumption parameters, ophthalmoscopy (repeated-dose), electrocardiography (repeated-dose), clinical pathology, necropsy, organ weights, macroscopic, and microscopic (repeated-dose) examinations.

RESULTS

Single Dose

The high-dose (400 mg/m^2) animals (1M/1F) did not survive past Day 4 (male died and female sacrificed moribund). Clinical signs observed for high-dose (400 mg/m^2) animals were similar to other treated animals and vehicle control (7% Solutol) animals (*e.g.,* swelling, salivation, redness of the skin, hypoactivity); suggesting that many of these findings were primarily due to the vehicle and not considered treatment related. Body weight losses were observed in high-dose (400 mg/m^2) animals and the mid-dose (200 mg/m^2) female. Reversible decreases were observed for platelets, reticulocytes, and white blood cells (WBC) (all types) at 100 or 200 mg/m^2.

For 100 or 200 mg/m^2 animals, organ weight changes were observed for the spleen and adrenal glands of females and the thymus of males and females. The maximum tolerated dose was 200 mg/m^2.

Repeated Dose

Many clinical signs were observed in this study; however, there was no difference in the onset, duration, or severity between groups and the effects were attributed to the vehicle (7% Solutol).

Increases in heart rates ranging from mild to marked were noted in all animals, including control animals, following dose administration on Days 1 and 25. These effects were considered related to the vehicle (7% Solutol) as it is known to cause histamine release resulting in hypotension and compensatory tachycardia in dogs (BASF, 2002; EMEA, 2003).

Modest hematological changes were observed in animals administered 90 mg/m^2 ANG1005, with the effects fully recovered by the end of the recovery period.

Microscopic changes related to ANG1005 included unilateral and bilateral degeneration of the seminiferous tubules in the testes of males (a known effect of paclitaxel) in either the 45 mg/m^2 (1/4) or 90 mg/m^2 (1/6) treatment groups, with the effect reversible for the 90 mg/m^2 males. There were no treatment-related findings for the testes at the lowest dose (15 mg/m^2).

The NOAEL in this study was 15 mg/m^2. Based on the lack of severe findings in this study, the highest non-severe toxic dose (HNSTD) was 90 mg/m^2.

Toxicokinetics

TK of ANG1005 were evaluated as a component of the single-dose and repeated-dose toxicity studies. Data are shown in the following tables.

Single Dose

Dose (mg/m^2)	Sex	C_{max} (µg/mL)	$AUC_{(0\text{-}last)}$ (µg•h/mL)
100	M	68.7	351.7
	F	57.4	328.5
200	M	131.7	712.3
	F	117.5	639.6
400	M	188.8	1,262.8
	F	258.1	1,634.9

Repeated Dose

Dose (mg/m^2)	Sex	Day 1		Day 25	
		C_{max} (µg/mL)	$AUC_{(0\text{-}last)}$ (µg•h/mL)	C_{max} (µg/mL)	$AUC_{(0\text{-}last)}$ (µg•h/mL)
15	M	5.3	23.5	4.8	23.5
	F	5.9	26.3	5.2	28.2
45	M	15.1	77.6	18.3	99.2
	F	14.8	79.1	14.6	76.9
90	M	30.4	164.1	31.6	172.9
	F	29.3	167.7	33.8	203.8

Abbreviations: $AUC_{(0\text{-}last)}$, area under the concentration-time curve at steady-state over time t; C_{max}, maximum serum concentrations measured

Single Dose:
✓ Overall half-life of 2.76 0.53 h, low volume of distribution (Vd= 1,138 256 mL/m^2 or ~65 mL/kg), and low clearance (Cl= 286 26 mL/h•m^2 or ~ 0.28 mL/min•kg);
✓ No sex-related differences in the TK profiles; and
✓ Plasma C_{max} and AUC rose in proportion to the dose even when the maximum tolerated dose (MTD) of 200 mg/m^2 was exceeded.

Repeated Dose:
✓ Overall half-life of 2.68 0.49 h, low volume of distribution (Vd= 2,050 507 mL/m^2 or ~110 mL/kg), and low clearance (Cl= 540 146 mL /h•m^2 or ~0.50 mL /min•kg);
✓ No accumulation of ANG1005; TK profiles were similar on Days 1 and 25;
✓ No sex-related differences in the TK profiles; and
✓ Dose-proportional increases in C_{max} and AUC_0.

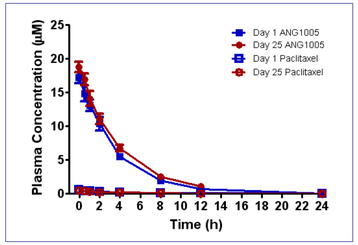


Figure illustrates the combined M+F plasma profile on Days 1 and 25 of both ANG1005 and free paclitaxel following repeated administrations of ANG1005 at **90 mg/m^2**

✓ Free paclitaxel represented only a small fraction of the plasma levels attributed to ANG1005;
✓ Both C_{max} and AUCs for paclitaxel were dose-proportional, and remained less than 5% of those for ANG1005; and
✓ Half-life of paclitaxel was approx. 5.5 h, slightly higher than ANG1005.

CONCLUSIONS

✓ The preclinical pharmacology, pharmacokinetics/toxicokinetics, and toxicology of ANG1005 have been well characterized;
✓ The results of the observed studies are consistent with the proposed mechanism of action; ANG1005 targets the LRP receptor and enables transcytosis across the BBB. This suggests that ANG1005 will be effective in the treatment of brain cancers;
✓ Based on the favourable preclinical toxicity profile, ANG1005 is currently being tested in two Phase 1/2 clinical studies in patients with brain cancers.

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Creating breakthrough drugs to treat brain diseases