#2038 Nonclinical Rodent Studies for ANG1005, a Novel Antimicrotubule Agent that Targets the Brain

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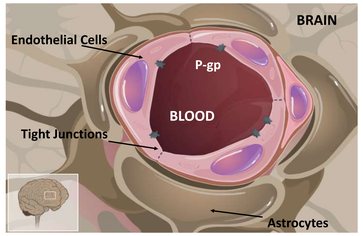
ABSTRACT

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. Following single intravenous (IV) doses of ANG1005 up to 300 mg/m^2, no behavioral/central nervous system effects were observed in male rats. In a single-dose IV toxicity study, ANG1005 was administered at 0 to 850 mg/m^2. Effects on white blood cells (and related parameters) and platelet counts were observed at dose levels ≥200 mg/m^2. Macroscopic findings and changes in organ weights were observed for the spleen at ≥400 mg/m^2 and for the testes at 850 mg/m^2. The maximum tolerated single IV dose was 400 mg/m^2. In a repeated-dose IV toxicity study, ANG1005 was administered at 0, 25, 50, or 100 mg/m^2 twice weekly for 4 weeks. ANG1005-related mortality was observed at 100 mg/m^2 after ~2 weeks (3/24 M, 2/24 F); and the dose lowered to 75 mg/m^2. Moderate dose-related decreases in testicular weights were observed in 50 or 100/75 mg/m^2 males, with partial recovery by Day 40. Corresponding macroscopic and microscopic changes were observed, with soft, small testes noted and minimal to moderate degeneration of the seminiferous tubules observed in 6/9 males at 100/75 mg/m^2 and in 1/10 males at 50 mg/m^2. These microscopic findings were not resolved during the recovery period and secondary effects were observed in recovery animals (e.g., aspermia in the epidymides; a known paclitaxel effect). The NOAEL in this study was 25 mg/m^2. Based on mortalities observed in this study [5/48 (10.4%) at 100/75 mg/m^2], the severely toxic dose in 10% of the animals (STD_{10}) was estimated to be 75 mg/m^2. The ANG1005 rodent 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.



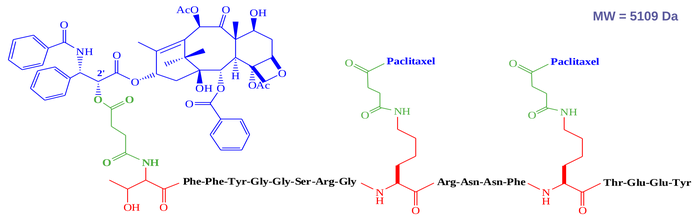
The BBB is a selective barrier formed by tightly packed endothelial cells that line the cerebral capillaries. The BBB is important as it provides an insulated environment for stable neuronal function. Endothelial cells forming the BBB are not only able to form tight junctions, but also possess the following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



METHODS

In vivo xenograph studies were conducted in mice and rats. Toxicology studies of ANG1005 were conducted in Sprague-Dawley rats and in Beagle dogs (Abstract No. 2039).

Study	Number of Animals	Treatment and Dose(s)	Comments
Safety Pharmacology			
hERG Assay	In vitro	0.5 to 2.5 µM	Effects on hERG currents from transfected HEK293 cells were tested
Functional Observation Battery	8M per group	IV (4-hour infusion) 0, 30, 100, or 300 mg/m^2	Mortality, clinical observations , body weight and general behavioral observations were assessed using a FOB that was performed on 5 separate occasions
Toxicology			
Single Dose	3M/3F per group	IV (4-hour infusion) 0, 100, 200, 400 or 850 mg/m^2	14-day observation period
Repeated Dose	Main: 10M/10F per group Recovery: 5M/5F (control and high-dose) TK: control (3M/3F), ANG1005 (9M/9F)	IV (4-hour infusion) 0, 25, 50, or 100 mg/m2 on Days 1, 4, 8, 11, 15, 18, 22, and 25 (twice weekly for 4 weeks)	3-day observation period for main animals and 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 rodent toxicity studies included standard endpoints such as mortality, clinical observations, toxicokinetics (TK), clinical pathology, body weight and food consumption parameters, ophthalmoscopy (repeated-dose), clinical pathology, necropsy, organ weights, macroscopic, and microscopic (repeated-dose) examinations.

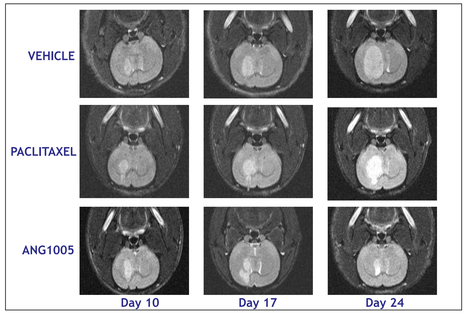
RESULTS

In Vivo Xenograft Study

Anti-tumor activity of ANG1005 showed better efficacy than paclitaxel in a series of experiments with nude mice and rats xenograft models. Data from the in vivo study in rats are shown on the right:

✓ Human U87 glioblastoma cells intracranially implanted into rats;
✓ ANG1005, paclitaxel or vehicle were administered (IP, twice weekly for 2 weeks); and
✓ Tumor size was examined on first day of treatment (Day 10), Day 17 and Day 24 by magnetic resonance imaging.

In both vehicle and paclitaxel treatment groups, tumors grew significantly over the 2-week treatment period, virtually engulfing an entire cerebral lobe by Day 24. In contrast, ANG1005 prevented any further tumor growth beyond that present at Day 10. Furthermore, no tumor was detected macroscopically in 5/8 ANG1005-treated mice at Day 24.



Safety Pharmacology

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.

Following single intravenous doses of ANG1005 up to 300 mg/m^2 in male rats, no behavioral effects, neurological/neuromuscular effects, or autonomic effects were seen that were attributed to ANG1005 when compared to control animals or values obtained at predose.

Single Dose Toxicity

Clinical signs were observed for 850 mg/m^2 animals (*e.g.*, dull, ungroomed, and matted fur, piloerection, thin body condition, hunchback posture, ptosis, salivation, weakness) and effects on body weight noted at dose levels ≥400 mg/m^2. Decreases in white blood cells (WBC), and related parameters (neutrophils, lymphocytes, monocytes, and/or eosinophils), and platelet counts were observed at dose levels ≥200 mg/m^2. Decreases in red blood cells, hemoglobin, and hematocrit were observed at ≥400 mg/m^2 on Day 8, with partial recovery by Day 15. By the end of the 14-day observation period, clinical signs had not fully recovered, body weight effects displayed a full recovery, and by Day 8 effects on WBC were reversible.

Macroscopic findings and changes in organ weights were observed for the spleen at ≥400 mg/m^2 and for the testes at 850 mg/m^2. A decrease in thymus weight at 850 mg/m^2 in both males and females was also observed.

The maximum tolerated dose was 400 mg/m^2.

Repeated Dose Toxicity

The high dose level of 100 mg/m^2 was reduced to 75 mg/m^2 after the 4th or 5th dose (Days 15 to 19) based on the deteriorating condition of these animals. ANG1005-related mortality was observed for 3 of 24 males and 2 of 24 females were sacrificed moribund at 100 mg/m^2.

Clinical signs such as dull, ungroomed, and matted fur, wet, brown stained fur, the presence of liquid brown material on the urogenital area, ptosis, thin body condition, hypoactivity, hunched back, and cold to touch were observed preceding the deaths of these high-dose animals (similar clinical signs were observed for the surviving 100/75 mg/m^2 animals). Marked weight loss accompanied by reduced food intake was also observed in these animals. Marked decreases in body weight gain at Day 17 were observed in 100/75 mg/m^2 males and females, with full recovery on Day 40.

Moderate dose-related decreases in absolute and relative testicular weights were observed in males administered 50 or 100/75 mg/m^2, but there was not a full recovery by Day 40. Corresponding macroscopic and microscopic changes were observed for the effects on testes weight, with soft and small testes noted and minimal to moderate degeneration of the seminiferous tubules observed in 6/9 males administered 100/75 mg/m^2 and in 1/10 males administered 50 mg/m^2. These microscopic findings were not resolved during the recovery period and secondary effects were observed in recovery animals (*e.g.,* aspermia in the epidydmides; a known paclitaxel effect).

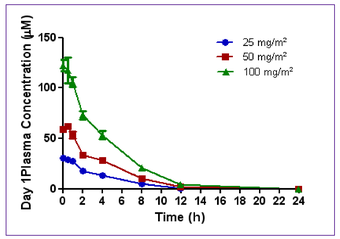
The NOAEL in this study was 25 mg/m^2. Based on mortalities observed in this study [5/48 (10.4%) at 100/75 mg/m^2], the severely toxic dose in 10% of the animals (STD_{10}) was estimated to be 75 mg/m^2.

Toxicokinetics

TK of ANG1005 were evaluated as a component of the repeated-dose toxicity study. The combined M+F plasma concentrations on **Day 1** are shown.

Dose (mg/m^2)	Sex	Day 1 C_{max} (µg/mL)	Day 1 $AUC_{(0\text{-}last)}$ (µg•h/mL)	Day 25 C_{max} (µg/mL)	Day 25 $AUC_{(0\text{-}last)}$ (µg•h/mL)
25	M	30.8	195	22.9	132
	F	30.2	193	25.4	105
50	M	61.8	392	54.1	286
	F	62.8	379	56.2	315
100/75	M	122.2	752	79.0	322
	F	122.0	797	75.8	374

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



✓ Overall half-life of 2.27 0.57 h, low volume of distribution (Vd= 522.0 197.5 mL/m^2 or ~73 mL/kg), and low clearance (Cl= 157.8 37.7 mL /h•m^2 or ~0.37 mL /min•kg);
✓ No accumulation of ANG1005; TK profiles were similar on Days 1 and 25;
✓ No sex-related differences in the TK profiles;
✓ Dose-proportional increases in C_{max} and AUC_0; and

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