Chapter from the book *Tumors of the Central Nervous System - Primary and Secondary*
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1. Introduction

4-Demethyl-4-cholesteryloxy carbonylpenclomedine (DM-CHOC-PEN), 1, is a polychlorinated pyridine cholesteryl carbonate, (Fig. 1) that is a derivative of 4-demethylpenclomedine (DM-PEN, 2) [1-4]. The latter is a non-neurotoxic metabolite of penclomedine (PEN, 3, NSC 338720, 2-trichloromethyl-3,5-dichloro-4,6-dimethoxy pyridine), that was identified during the NCI sponsored Phase I clinical trials with 3 [4-8]. 3 was found to be active vs. advanced cancers but possessed unacceptable neurotoxicity and discontinued from further study [5-10].

Where – 1. DM-CHOC-PEN: \( R=\text{CO}_2\)-cholesteryl, 2. DM-PEN: \( R=\text{H} \), 3. PEN: \( R=\text{OCH}_3 \)

Figure 1. Penclomedine (PEN) and Analogs
was synthesized at DEKK-TEC as part of a series of polychlorinated pyridine carbonates and carbamates that are lipophilic, non-neurotoxic alkylators of human xenograft brain and breast tumors implanted intracranially (IC) in mice [1-3].

Anticancer activity for 1 has been well documented in vivo vs. IC implanted human xenograft glioblastoma (U-251 and D-54) and breast cancer (MX-1) mouse models at doses lower or equivalent to its LD_{10} [1-3]. Over 25 carbonate and carbamate analogs of 2 have been synthesized and evaluated in vivo and 1 was the most active of the analogs vs. the above IC implanted human xenograft cancer models [1-3].

X-ray crystallography studies with 1 describe a perfectly linear configuration (Fig. 2) that includes a neutral heterocyclic ring linked through a stable carbonate group to a lipophilic cholesteryl moiety [2]. These basic characteristics plus the neutralizing effects (electrophilic) of the polyhalogenated substitutions on the pyridine ring probably contribute to 1’s ability to form micelle particles that penetrate the blood brain barrier, and accumulate in CNS tumor tissues [1, 11].

A mechanism of action has been proposed for 1 that involves cross-linking across the trichloromethyl group with tumor DNA in the major groove via N^7-guanine cross linking in a G-X-C sequence [1]. This mechanism of action would allow 1 to be administered in combination with many of the clinically significant DNA major groove-alkylating drugs, that include methylaing agents [e.g. dacarbazine and temozolomide (Temodar®)] and chloroethylating agents [e.g. bis(chloroethyl)-nitrosourea (BCNU) and clomesone] – all of which form carbonium ion-mediated adducts via O^6-guanine in contrast to binding with N^7-guanine, as does 1 [12].

The pharmacokinetics, metabolic fate and toxicology of 1 in three animal species (mice, rats and dogs) are reviewed here.

Cognitive/behavioral studies have been conducted in rats and dogs and are reviewed in depth.
2. Materials and methods

2.1. Drug formulation and chemicals

1 and 2 were synthesized by DEKK-TEC, Inc., using GLP/GMP guidelines, as previously described [1]. 1 is very stable in the solid state under ambient temperature and was administered in various vehicle media for the animal studies. For the rat studies, 1 was formulated as a buffered emulsion of soybean oil (20%), egg yolk lecithin (10%), glycerin (3%), histidine (3.1%) and water emulsion (containing 2-7 mg/mL of 1) [IND 86,876]. For the mouse and dog IV studies, 1 was formulated as a 0.3% Klucel+0.3-3.3% Tween® 80, saline suspension and for the oral mouse study an emulsion of 1 in a 8% Tween-80 Neobee®-1053 (Squibb) solution was used.

2.2. High pressure liquid chromatography (HPLC) analysis of 1 and a metabolite, 2

HPLC analysis was performed using an Agilent Technologies (New Castle, DE) 1200 model HPLC fitted with a diode array UV detector set at a wavelength of 244 nm (λ_max for 1). A Rheodyne Model 7725 injection port (Cotati, CA, USA) with a 20µL sample loop was used to inject the samples. Chromatograms were recorded with an Agilent Technologies integrator. Samples were chromatographed using an Alltech 150 x 4.6 µm column that contains Luna C8 (2) 100A packing (diameter of particles = 5 µm).

The mobile phase for 1 consisted of 80% THF: 20% water and for 2, was 45% THF: 47% water, which was degassed, filtered through a 0.45 µm Rainin filter (Woburn, MA, USA) and delivered at a flow rate of 1.0 mL/min.

Plasma and erythrocyte samples were stored at -74°C until analyzed. Standard solutions were prepared by dissolving 6 mg of 1 or 2 in 20 mL THF. Internal standards were 20 mg of cholesteryl benzoate (ChB, Sigma Aldrich Co) or phenol (P, Sigma Aldrich Co) each in 20 mL THF. All solutions were stable for at least 2 months at 5°C. Standard assays for 1 and 2 consisted of 0.25 mL of plasma, 25 µL of 1 or 2 and internal standard (ChB or P – 2 µL) and 2 mL dichloromethane. The samples were vortexed for 10 min and frozen to separate the layers. The bottom organic layer was removed with a 25 gauge needle and glass syringe, filtered through a 0.45 mm Acrodisc syringe filter and evaporated to dryness under vacuum, reconstituted with 100 µL of THF and analyzed as below.

Control dog and rat whole blood and plasma samples were spiked with 1 in the concentration range 5-1000 ng/mL, plus 20 µL of the above ChB. Similar controls were prepared for 2 (ng/mL) and its int. std. – P. Peak-area ratios of 1/ChB or (2/P) vs. the concentration of 1 or 2 (ng/mL) were subjected to linear regression analysis. Retention times for 1 and ChB were 6.41 and 4.63 min., respectively and for 2 and P were 6.6 and 18.5 min., respectively.

Verification of the HPLC assays included calibration curves derived from the assay of five (5) erythrocyte and eleven (11) plasma standards in duplicate prepared each with 1 and 2 (0.5 ng/mL-600 µg/mL). Plasma and erythrocyte samples were obtained from healthy rats and dogs and spiked with 1 or 2 and their respective internal standards. Drug concentrations in all
samples were calculated using the results of linear regression analysis. Reproducibility was higher than 85%. Limit of quantitation for 1 and 2 was 0.2 ng/mL.

2.3. P-glycoprotein (P-gp) transport studies

**Cell lines**-three (3) human cancer cell lines-A549: lung; MCF7: breast; and HeLa: ovarian were obtained from ATCC, Manassas, MD, and maintained in culture in a temperature controlled (36 °C), 5% carbon dioxide, and humidity controlled incubator system. All culture transfers were in a laminar flow hood under sterile conditions. Tissue culture medium used was RPMI 1640 containing 10% FBS and penicillin/streptomycin/fungizone (1%) – all purchased from InVitrogen.

**Material preparation**-Rho was prepared in distilled water as a stock solution (1 mg/mL), stored frozen at-22 °C. Rho (0.2μg/mL) was added to the culture medium in the presence or absence of Vpml and/or 1. Vpml (2-23 µg/10 mL) was prepared in PBS solution and 1 (0.5-73 µg/10 mL) in PBS+5% DMSO.

A **typical assay**-contained: 1-7.3 µg/mL, Vpml-2.5 µg/mL and Rho-0.2 µg/mL. Cells per test system were 0.8-1.2 x 10⁶/mL. Incubations occurred in the above incubator conditions for the times and schedules discussed below. Experiments were conducted in triplicate and at times as specified in the Results. Reaction incubations were 15-60 minutes in length as discussed in the Results section.

**Post incubation**-After treatment, cells were rapidly trypsinized (~2 min), washed with cold PBS and kept on ice until analyzed. Assay conducted with a Becton-Dickinson FACS-ARIA II flow cytometer with CELLQUEST software. The Rho fluorescence was measured at laser excitation 488 nm with emission at 530 nm and is expressed in arbitrary units compared to control cells (untreated). Measurements were conducted on 10,000 cells per assay.

2.4. Animals

Adult Sprague-Dawley mice [Crl: CD1(ICR) BR] (males 20-25 g and female 18-25 g) and rats [Crl: CD1(ICR) BR] (males 300-350 g and female 225-250 g) were obtained from Harlan Industries (Indianapolis, IN), housed in groups of three-five per cage in light-controlled (12 h/day) and temperature-controlled (24°C) animal isolators with filtered vents and exhausts. They were fed a diet of Purina Laboratory Chow (Purina Feed) and received tap water ad libitum. The rats were fasted and 3-4 mL of blood was drawn via the jugular vein. The order of bleeding was alternated (one animal from each dose group, then repeating) to reduce handling and time biases.

Adult male and female beagle dogs (6.5-7.5 mo. of age, 6.5-9.49 kg) were raised and maintained at MPI Research (Mattawan, MI). They were fed a diet of Purina Dog Chow (Purina Feed), received tap water ad libitum and exercised per IACUC protocol. Mouse, rat and dog were euthanized with phenobarbital/ketamine anesthesia and/or carbon dioxide inhalation. The chest cavities were exposed to insure death. Institutional animal care and use committees (IACUC) reviewed and approved all the studies.
All mice, rat and dog studies were conducted at MPI Research (Mattawan, MI) under GLP regulations as described in the Guide for the Care and Use of Laboratory Animals, Office for Laboratory Animal Welfare, NIH, Bethesda, MD.

For mouse toxicity studies, 1 was administered IV bolus via the tail vein or per oral gavage; for the dog studies, 1 was administered as an IV bolus via the femoral vein and for rats, administration was during a 3 h IV infusion via indwelling femoral vein catheters. The observation period for all studies was 14-days – followed with euthanasia. For the rat and dog studies, complete necropsies with complete blood pharmacokinetic parameters, hematology, coagulation profiles, clinical chemistry and urine analyses were performed. For the mouse studies physical/gross necropsy examinations were conducted.

For the acute behavioral studies, rats received drugs and controls to identify/verify gross behavioral patterns employing a Morris modified water maze (4’ x 3’ x 1.5’) with a single layer of white polyethylene peanuts that floated (in 6” of water) and covered a single mounting stage [13, 14]. Adult female rats (Hsd:SD, 175-225 g.) were grouped 3-6 animals per drug arm. The test agents were dissolved in or suspended in 5% aqueous Tween® 80-hydroxypropyl cellulose in 5% saline and administered intraperitoneally (IP). Controls received the vehicle only. Swimming trials began – 1, 2, 3 and 20 hours post-dosing. For each time period post-dosing, the rats were challenged on six (6)-back-to-back swim trial events to find the platform. The daily swimming times and ranges were compared to vehicle controls vs. chemotherapeutics alone in rats. The data (latency to find the platform) was analyzed by variance (ANOVA). Body weights and water temperature-prior to each dosing and during each FOB assessment were monitored.

2.5. Pharmacokinetic studies

Groups of rats (5/sex) were administered 100, 200, or 300 mg/kg of 1 as a 3 h timed infusion and samples of blood collected (in EDTA tubes) at various time points IPEOI:-15,+10,+45 min, 1.5, 3, 6, 8, 12, 24 h and 14-days. Each animal possessed an indwelling femoral catheter for ease of the study. The catheters were flushed after each blood draw. The plasma and erythrocytes were separated and stored separately at -74°C until analyzed.

Adult beagle dogs (8 M) were administered 1 as a slow bolus injection once through a femoral vein in doses of 10, 20, and 30 mg/kg. A 20-gauge venous catheter was inserted into a saphenous vein for bleeding and samples were withdrawn at 0, 5, 15, and 30 min. and 1, 1.5, 2, 4, 8, 12 and 24 h into EDTA containing tubes. Plasma was separated and stored at -70°C until assayed. Animals were anesthetized with ketamine.

2.6. Animal pharmacokinetic data analysis

Model parameters were estimated using Micropharm software and nonlinear least squares regression was performed using Simplex and Gauss Newton algorithms []. An open two-compartment model provided the best fit. Clearance, volume of distribution and half-lives were derived from estimates of the model parameters.
2.7. Data analysis

Data analysis was performed on all plasma and in vitro studies and analyzed via non-linear regression using a non-weighed quasi-Newtonian/simplex fitting algorithm (Statistical software available from Stat soft, Tulsa, OK).

3. Results

3.1. Stability studies

Bulk drug product, 1, as crystals, has been observed to remain stable for > four (4) years under ambient conditions [1]. The final clinical product (2 mg/mL) was stored at refrigerator temperatures (4-8°C) for 1.5 years without deterioration [15].

To further document product stability a 50:50 mixture of 1 (as the clinical product) with 10% Intralipid® (Fresenius Pharmaceuticals) was infused at room temperature over 8 h into a sterile container. Aliquots were withdrawn at 0, 0.5, 1, 2, 3, 4 h and analyzed by HPLC as described above. Decomposition of 1 during an 8-hour infusion at room temperature was 6% (mean for 4-runs). The breakdown product, 2, could not be identified during this extended simulated infusion study [11].

3.2. Toxicity

Acute oral and IV toxicity study results for 1 in mice, rats and dogs are presented in Table 1 which presents median lethal dose values observed. The oral study with mice failed to produce toxicity at maximal administered doses of 0.8-2 g/kg. Volume restrictions prevented higher escalations. The drug (oral) was not active in the xenograft models, thus additional oral administration route studies were terminated. Two separate single IV mouse-dosing studies calculated a LD$_{10/50}$ of 136/385 mg/kg (for both sexes; with 95% confidence limits) – Table 1. Specific lethal/sublethal values and details are discussed in Table 1.

Clinical signs generally reflecting the deteriorating state of both mice and rats post dosing were observed in both sexes for 1 in a dose-dependent manner and included body surface staining, decreased activity, lethargy, loss of appetite, decreased defecation, tremors, and/or whole-body edema. The lethal experiences were sedation followed by respiratory arrest. No seizures or loss of coordination was observed for the survivors.

3.3. Acute single dose intravenous studies in mice

Adult male and female mice, 10 animals per sex per dose level, were intravenously dosed with 50, 100, 200, 400 and 600 mg/kg. No animals died at 0 or 100 mg/kg, 1 animal died at both 50 and 200 mg/kg doses, 7 of 10 animals died at 400 mg/kg and 8 of 10 animals died at 600 mg/kg (Table 1). Various clinical signs reflecting treatment-related effects were noted in both sexes, oftentimes in a generally dose-dependent manner. These clinical signs included decreased activity, rapid/difficult/slow/shallow breathing, limbs splayed, tremors and skin
cold to touch. No seizures or loss of coordination was noted. The deaths at 400 and 600 were of a very immediate nature, occurring within minutes or less post-dose, with no clinical signs exhibited prior to death. While transient incidences of rapid breathing were also noted in a couple of control animals, a definitive relationship to the vehicle was unclear. No definitively clear treatment-related body effects were noted in those mice surviving the 14-day observation period when compared with controls. No macroscopic findings were noted in any animal at necropsy.

Neither aplastic bone marrow nor splenic depletion of lymphocytes was noted.

<table>
<thead>
<tr>
<th>Species</th>
<th>No/ Sex</th>
<th>Doses (mg/kg)</th>
<th>Method</th>
<th>LD_{10/50} (mg/kg)</th>
<th>Time* (Days)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mice</td>
<td>36 M</td>
<td>50-600</td>
<td>IV</td>
<td>132/385</td>
<td>2-14 days</td>
<td>Deaths were erratic</td>
</tr>
<tr>
<td>Mice</td>
<td>36 F</td>
<td>50-600</td>
<td>Bolus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>30 M</td>
<td>800-2000</td>
<td>Oral–</td>
<td>0.8 – 2.0 g/d x</td>
<td>21 days</td>
<td></td>
</tr>
<tr>
<td>Mice</td>
<td>30 F</td>
<td>800-2000</td>
<td>Gavage</td>
<td>5d – no deaths</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rat</td>
<td>73 M</td>
<td>IV</td>
<td>100</td>
<td></td>
<td></td>
<td>1-Rat died @ 100 mg/kg; cholesterol – elevated, but acceptable</td>
</tr>
<tr>
<td>Rat</td>
<td>73 F</td>
<td>50-300</td>
<td>Infusion</td>
<td>(LD_{10})</td>
<td>15 days</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>8 M</td>
<td>IV</td>
<td>&gt;30.0</td>
<td></td>
<td>10 days</td>
<td>No deaths</td>
</tr>
<tr>
<td>Dog</td>
<td>8 F</td>
<td>10-30</td>
<td>Bolus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>4 M</td>
<td>IV</td>
<td>&gt;30.0</td>
<td></td>
<td>10 days</td>
<td>No deaths</td>
</tr>
<tr>
<td>Dog</td>
<td>4 F</td>
<td>10-30</td>
<td>Bolus</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*The time of the last death.

Table 1. Medial dose summary for 1

Based on the conditions and findings of this study, the intravenous LD_{10} for 1 was calculated to be 136 mg/kg (95% confidence limits could not be calculated) in mice (combined sexes), while the intravenous LD_{50} was calculated to be 385 mg/kg (95% confidence limits).

3.4. Sub-chronic oral mouse toxicity (Table 1)

A study was conducted in groups of 10 male/10 female mice per dose. The study evaluated 1 administered daily for five days at doses of 0, 800, 1000, 1200, 1500 and 2000 mg/kg per gavage to mice. Only one death occurred at 800 mg/kg on day 2 after dosing. All animals demonstrated some degree of lethargy and unkempt appearance. Similar body appearances were noted with the controls. No seizures were noted.
3.5. Acute single dose intravenous studies in rats (Table 1)

A rat study was conducted with the objectives to evaluate and characterize acute toxicity, maximum tolerated dose (MTD), and evaluate pharmacology (including pharmacokinetic parameters) of 1 when intravenously (IV) administered over 3-hours, as an emulsion to rats. The same formulation that is being administered to patients via IV infusion in clinical trials was used for the rats (see Materials). A detailed clinical examination of each animal was performed daily and included evaluations of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, respiratory and circulatory effects, autonomic effects such as salivation, nervous system effects, including tremors, convulsions, reactivity to handling, and psychological behavior.

Initially, a dose range finding (DRF) study was conducted that consisted of four (4) treatment groups – each group included 3-M/3-F that were single dosed IV infusions and monitored – no deaths were observed. The IV doses of 1 administered were – 50, 100, 150 and 200 mg/kg/dose.

The main study phase consisted of a control group (10-M/10-F) that each received the vehicle only and three groups (10-M/10-F) that each received a single IV infusion of 1 at dose levels of 100, 200 or 300 mg/kg. One male (1) rat in the 100 mg/kg group became moribund and was euthanized. No animals died in the 200 or 300 mg/kg dosed groups. No external related body effects were noted in the rats surviving the 14-day observation period. Both liver and spleens were target organs noted to be enlarged and evaluated in detail as discussed below.

The rats were divided into 2-groups that were euthanized either on Day-2 or Day-15. Complete macroscopic/microscopic examinations and complete clinical chemistry, hematology, coagulation studies and urinalysis were completed on all animals.

There were no meaningful hematological effects noted. On Day-2, erythrocytes, hemoglobin, and hematocrit tended to be higher in the 300 mg/kg/dosed group. These changes were most likely a result of fluid imbalances relative to reduced water intake. Monocytes were increased in both sexes at 200 and 300 mg/kg/dose and lymphocytes were decreased in males at 300 mg/kg/dose. Neutrophils were elevated in all groups on Days-2 and 15 and were attributed to stress and/or route of administration. All other changes were resolved by Day-15 and all values returned to normal pre-drug limits.

There were no test article-related effects on coagulation parameters or on urine analysis values.

The most significant findings were 1’s related effects on the clinical chemistry analytes in the lipid profile studies (Table 2). Both alterations in the cholesterol and triglyceride profiles were significantly affected post dosing with 1. Cholesterol and 2 are formed following the metabolism of 1 by the liver and/or peripherally (Scheme 1). The total cholesterol levels significantly increased in all groups vs. the control vehicle group. Increased levels of LDL-cholesterol were the pre-dominant variant observed on Day-2. However, by Day-15 the total cholesterol levels had returned to within normal limits and the total cholesterol was predominantly accounted for as a HDL-variant.
### Table 2. Summary of lipid profiles in rats dosed with 1 as an IV infusion (Day 1)

The triglycerides also increased in the 200 and 300 mg/kg groups on Day-2 post-dosing, which resolved by Day 15. The females demonstrated the most significant elevations in both triglycerides and LDL-cholesterol. On Day 15 the profiles for both total cholesterol and triglycerides had returned to WNL. Table 2 reviews cholesterol and triglyceride trends.

Alanine aminotransferase (ALT) in males, and γ-glutamyl transferase (GGT), and alkaline phosphatase in females were minimally to mildly elevated at the 300 mg/kg/dose on Day 2. All of the findings noted on Day-2 had resolved by Day-15.

Various clinical signs reflecting treatment-related effects were noted, mostly lethargy that cleared. No behavioral alterations were noted.

Macroscopic/microscopic examinations revealed increased sizes of the livers and spleens. On Day-2, the 200 and 300 mg/kg groups possessed vacuolated macrophages in the liver (Kupffer cells) and spleen. By Day-15 the macrophages contained smaller oil aggregates and clusters.

**Scheme 1. Metabolism of 1**
within hepatic sinusoids. The findings in both the spleen and the liver showed trends toward resolution by Day-15 with both biliary hyperplasia in the liver and splenic focal necrosis resolving; vacuoles were smaller and cell cytoplasm had a more eosinophilic tint seen in both livers and spleens, albeit the vacuoles still expanded the cytoplasm of the cells. The latter changes are artifacts resulting from the extraction of drug/lipids/cholesterol from hepatic/splenic macrophages during fixation/preparation of tissues for microscopic examination. The changes seen on Day-2 in the spleens and the livers trended towards resolution by Day-15. Controls (vehicle alone) did not demonstrate the above changes.

Transitory changes in the hepatic profile are considered 2° to stasis of 1 in hepatic sinusoids with biliary congestion that results in cysts and shunting of blood to the spleen resulting in splenic cysts and fatty deposits.

Although the above findings resolved by Day-15, they must be considered adverse – based on the degree of elevation in triglycerides (3-fold) and LDL-cholesterol (30-fold in females) seen in some groups. The control group received the vehicle alone – soybean oil and egg yolk lecithin – both rich in triglycerides and did not demonstrate abnormal lipid profiles or the liver/spleen changes.

Based on the conditions and findings of this study, the intravenous LD\textsubscript{10} of 1 in rats was calculated to be 100 mg/kg (95% confidence limits could not be calculated) – Table 1.

3.6. Acute dog IV toxicity (Tables 1, 3)

A single IV dose study was performed in adult Beagle dogs, which consisted of 1 administered once as an IV bolus. Sixteen (16) adult beagle dogs (8 male and 8 female) divided into three groups received a single intravenous injection of 1. The experimental design and results are presented in Table 3.

No treatment related effects on survival, hematology, urinalysis, or macroscopic and microscopic evaluations were noted during the study. Numerous clinical signs reflecting treatment-related effects were noted in both sexes of all groups, including the control group, and exhibited no dose-dependent pattern, clearly suggesting that the effects were attributable to the 0.3% Klucel+1.92% Tween 80 vehicle rather than 1. Pertinent clinical signs noted included decreased activity, emesis, impaired righting reflex, limb function impaired, breathing slow/shallow, red skin discoloration (entire body, ears, or face), swelling (face and/or nose/muzzle), skin cold to touch, eyes swollen, slow gum capillary refill time, feces-mucoid/soft/discolored/watery, lacrimation, salivation, sclera injected, vocalization, tremors, and urination decreased. The effects were of immediate onset (within one hour post dose), with most of the signs clearing by Day 2 of the study. However, decreased activity persisted for Days 2, 3, or 4 in some of the animals and through the remainder of the study. No clear treatment-related body weight effects were noted during the study when comparing treated groups vs. controls. Slight body weight losses were noted in some animals which were non-dose related. The latter observations were in all probability attributable to the vehicle.
<table>
<thead>
<tr>
<th>Route/Schedule</th>
<th>Dose (mg/kg)</th>
<th>Number and Sex</th>
<th>Observations</th>
</tr>
</thead>
<tbody>
<tr>
<td>IV once</td>
<td>0</td>
<td>2M 2F</td>
<td>No deaths</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>2 M 2 F</td>
<td>No deaths</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>2 M 2 F</td>
<td>No deaths</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>2 M 2 F</td>
<td>No deaths</td>
</tr>
</tbody>
</table>

Table 3. Acute IV toxicity in the dog

There were marked elevations of alanine aminotransferase (ALT), and sorbitol dehydrogenase (SDH) in males in both controls and all treated animals on Day 2. Increased aspartate aminotransferase (AST) was observed in one (1) male with remarkably increased values for AST, ALT and SDH. The other male in this group exhibited only mildly increased values on Day 2 for these parameters. Similar trends were noted in females. ALT and SDH were highest on Day 2 for all dose levels, including controls. This acute and transient effect on liver enzymes exhibited no dose-dependent pattern and attributable to the vehicle. The latter finding was not observed to this extent in the rat study which used a different vehicle. The above was verified in a second group of 4M/4F.

The dog hematological studies confirmed that the 10-30 mg/kg doses do not produce myelosuppression, thrombocytopenia or anemia.

Treatment-related neurotoxicity was not observed following the single IV bolus administration of 1 to dogs. A second opinion review was obtained (RT), who conducted silver stains and confirmed MPI’s observation that there were no microscopic pathological CNS changes present in the brains of dogs treated with 1 (FDA IND – 68,876) [14].

3.7. Summary Median Lethal Dose (Single Dose)

Table 1 summarizes the toxic effects of single IV dose administrations of 1 in mice, rats and dogs. Three intravenous studies were conducted under FDA GLP guidelines. The summary of the median lethal single dose (LD₅₀) values were calculated by combining the data from the acute single dose studies according to species and are available only for mice and dogs.

3.8. Acute rat behavioral studies

Rats in groups (5-females) received 1, 2, or 3 in a dose range finding (DRF) screen to identify and verify gross behavioral patterns (Table 4). Documentation of drug cognitive/learning abilities were conducted in a Morris modified water maze with adult female rats (Hsd:SD, 175-225 g.) which were grouped 3-6 animals per drug arm (Table 5).

Impaired learning behavior has not been observed for 1 or 2 in contrast to observed data for 3 during the first 1-3 h and at 20 h periods post-dosing (Table 5). A vehicle and a 5-FU control were included for comparison. The observations noted for 3 and 5-FU support the literature reports that both drugs impair memory in patients receiving the drug [16-18]. The described
assay is a simple, reproducible quantitative assessment of impaired visuospatial cerebellar-learning/memory and performance functions via swimming and navigating a water maze. The treated and control rats were timed to navigate to find a hidden platform – Figures 3 and 4.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg) IP</th>
<th>1 - 4 Hours</th>
<th>5th Hour</th>
<th>7th Hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle (Controls)</td>
<td>0.5 mL</td>
<td>Alert; normal behavior</td>
<td>Alert; normal behavior</td>
<td>Alert; normal behavior</td>
</tr>
<tr>
<td>Mk-801 (control)</td>
<td>0.05 once</td>
<td>Lethargic</td>
<td>Lethargic</td>
<td>Lethargic</td>
</tr>
<tr>
<td>5-FU (Chemo Control)</td>
<td>78</td>
<td>Lethargy, eyes closed</td>
<td>More alert</td>
<td>Normal behavior</td>
</tr>
<tr>
<td>3</td>
<td>400-800 once</td>
<td>Eyes closed; spastic; lethargy</td>
<td>Less lethargy; spastic; lethargy</td>
<td>Normal behavior</td>
</tr>
<tr>
<td>2</td>
<td>350-600 once</td>
<td>No acute toxicity; no spasms</td>
<td>Normal behavior</td>
<td>Normal behavior</td>
</tr>
<tr>
<td>1</td>
<td>100 &amp; 300 once</td>
<td>No acute toxicity; no spasms</td>
<td>Normal behavior</td>
<td>Normal behavior</td>
</tr>
</tbody>
</table>

**Table 4.** Rats - gross behavior patterns (5 female rats per group, 160-168 g)

A control memory impairment agent, MK-801, is included to demonstrate complete impalement. In contrast, 1 and 2 had little or no influence on learning/memory, while 3 produced long lasting impairment.

**Figure 3.** A rat on the water maze platform.
3.9. Brain/tumor penetration

Adult male mice (athymic NCr-nu/nu – NCI-Frederick Production Area, NCI) were sedated and intracerebrally (IC) implanted with U251 glioma cells ($10^6$) from tissue culture. The mice were divided into 5-control and 5-treated with 1. The latter group was administered 1 (135 mg/kg/day) IP daily for two consecutive days (q1d x 2) beginning 4-days post inoculation of cells. Four hours after the second treatment the animals were sacrificed and the brains removed intact (cerebellum included), flash frozen in liquid nitrogen and stored at $-77 \, ^\circ \text{C}$ until assayed.

The intact frozen brains (~1.3 g) were coronal sliced into three sections in a mouse brain blocker (Kopf). The encapsulated gliomas were easily identified and separated readily from normal brain tissue with a scalpel and using microscopic ‘touch finger printing’ – separation verified.

The tumor tissues were weighed, pooled and homogenized in 10 mL saline at $5^\circ \text{C}$. This process was repeated for the normal brain tissue. The cold homogenates were separately extracted with 10 mL dichloromethane, the organic layer separated and evaporated to dryness.

The residues were dissolved in dichloromethane and underwent preparative TLC on silica gel plates (Sigma-Aldrich, Milwaukee, WI) with a mobile phase – hexane/dichloromethane:10/30. The respective spots for 1 and 2 were extracted with dichloromethane, concentrated and analyzed by HPLC (for procedure, see-Methods). 1 and 2 were present in the gliomas extracts -62 and 11 ng/g, resp. (avg.) were present in the gliomas, but none in the normal brain tissue.
Five (5) control mice bearing IC implanted U251 cells (non-treated) were used as the dissection and extraction controls. No chemicals were identified in the above extraction assays or in the brains from the control tumor/normal mice.

### 3.10. Normal brain penetration

Adult male rats [Crl: CD1(ICR) BR] (325-350 g wt) in groups of 5 animals were dosed intraperitoneally with 50 mg/kg of DM-CHOC-PEN in 0.3% Klucel/Tween80/saline daily x 2 days. On the 3rd day the rats were sacrificed and the intact brains removed (~1.9 g) and each homogenized in 10 mL saline at 5°C. To the cold homogenates, 20 mL dichloromethane was added and shaken for 30 minutes. The organic layer was removed and evaporated to dryness under vacuum at room temperature. The residues were dissolved in 1 mL of tetrahydrofuran.

---

### Table 5. Evaluation of the behavioral activity of penclomedine analogs in female rats

<table>
<thead>
<tr>
<th>TREATMENTS</th>
<th>LEARNING - IMPROVEMENT (MEAN) [*DIFFERENCE BETWEEN 1ST AND 6TH TEST – SAME TIME PERIOD]</th>
<th>MEMORY – IMPROVEMENT/IMPAIRMENT [Differences Between 1 – 20 hrs]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Agent</td>
<td>Dose (mg/kg/dose) #Rats Schedule</td>
<td>1 Hr</td>
</tr>
<tr>
<td>Control</td>
<td>Saline</td>
<td>24</td>
</tr>
<tr>
<td>MK-801</td>
<td>0.05</td>
<td>3</td>
</tr>
<tr>
<td>5-FU</td>
<td>78</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>400</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>6</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>6</td>
</tr>
</tbody>
</table>

*Rats were tested – six (6) separate trials – 1, 2, 3 and 20 hrs post dosing. The rats are timed to swim to the stage. The fold improvements are recorded in the table. For hours 1, 2, 3, and 20 post dosing, two values are given for each drug. The first number (X) for each hour is the difference (in seconds) between the initial and final attempts (first – sixth run). A negative number indicates complete lack of learning throughout six runs – taking a longer amount of time on the sixth try than the first. The next value (indicated with *) is X fold performance improvement. This is calculated by initial/final time. <1 indicates complete lack of improvement (taking longer on the last try than the first). Doses used were the therapeutic values as determined from the tumor models.

**MK-801 is a NMDA (N-methyl-D-aspartate) inhibitor that produced a solemn effect that prevented the rats from swimming and learning. After 3 days the rats were equivalent vs. control. 5-FU has been associated with memory loss in patients treated with chemotherapy (17).
and 100 µL chromatographed on silica gel plates with hexane:dichloromethane (1:1) as solvent. DM-CHOC-PEN was identified at \( R_f \) 0.74 with an additional spot – \( R_f \) 0.51. All spots were cut out, extracted with THF and analyzed by HPLC. DM-CHOC-PEN and a polar metabolite were identified by HPLC (see below).

DM-CHOC-PEN was calculated to be present-100 ng/g (avg.) of whole brain. The more polar peak (\( R_f \) 0.51) was not DM-PEN and present at 20 ng/g of whole brain. \(^1\)H-NMR of the latter fraction identified a pair of peaks at \( \delta \) 5.68 & \( \delta \) 5.75-consistent with loss of a methylene chlorine and binding to an NH-group, possible adduct. The material possessed a cholesteryl carbonate moiety. Normal brain tissue was used as a control.

3.11. Pharmacokinetic studies in rats and dogs (Table 5)

Plasma concentration-time profiles for 1 in adult rats post a 3-hour single dose IV infusion of 100, 200 and 300 mg/kg are presented in Fig. 5. Mean pharmacokinetic parameters for rats summarized in Table 6, Figs. 5 and 6 were – \( T_{1/2a} \) 15+/−7 h, \( T_{1/2b} \) 19.1+/−1.3 h and CL 22.2+/−6.5 L/h for 1, which could be detected 24 h post infusion. As mentioned previously and outlined in Scheme 1, 1 is metabolized to 2 and cholesterol, which are compared in Fig. 6 for the 100 mg/kg dose. The levels of 2 and cholesterol paralleled each other, as expected.

![Figure 5. Mean plasma levels for 1 IPEOI – 4-rats per group.](http://dx.doi.org/10.5772/58353)
The shifts noted in the bioavailability for 1 vs. 2 are suggestive of an enzyme overload – a Michaelis Menten effect (Fig. 6). For rats, the AUC and C\(_{\text{max}}\) values vs. doses of 1 administered were linear – Figs. 7 & 8. Dog pharmacokinetics for 1 are compared to the rat values in Table. For dogs, plasma clearance was constant between 10 and 30 mg/kg. The plasma clearance is 328.8 L/h vs. 346.8 L/h; demonstrating the PK linearity of 1. Differences seen between rats and dogs are due to the fact that rats received 1 during a 3-hr IV infusion and dogs via an IV bolus injection – thus not comparable.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Specie (N)</th>
<th>(T_{1/2\alpha}) (h)</th>
<th>(T_{1/2\beta}) (h)</th>
<th>AUC (mg*h/L)</th>
<th>CI (L/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mg/kg</td>
<td>Dog (4)</td>
<td>1.23 (Mean)</td>
<td>21.6 (Mean)</td>
<td>0.42 (Mean)</td>
<td>328.8 (Mean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.53 (SD)</td>
<td>16.00 (SD)</td>
<td>0.17 (SD)</td>
<td>221.2 (SD)</td>
</tr>
<tr>
<td>30 mg/kg</td>
<td>Dog (4)</td>
<td>0.63 (Mean)</td>
<td>18.7 (Mean)</td>
<td>1.12 (Mean)</td>
<td>346.8 (Mean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.09 (SD)</td>
<td>10.7 (SD)</td>
<td>0.09 (SD)</td>
<td>54.5 (SD)</td>
</tr>
<tr>
<td>100 mg/kg</td>
<td>Rat (5)</td>
<td>0.51 (Mean)</td>
<td>2.48 (Mean)</td>
<td>1.05 (Mean)</td>
<td>30.4 (Mean9.99)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05 (SD)</td>
<td>0.8 (SD)</td>
<td>0.53 (SD)</td>
<td>(SD)</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>Rat (5)</td>
<td>0.25 (Mean)</td>
<td>6.94 (Mean)</td>
<td>3.46 (Mean)</td>
<td>16.9 (Mean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1 (SD)</td>
<td>2.1 (SD)</td>
<td>0.46 (SD)</td>
<td>4.04 (SD)</td>
</tr>
<tr>
<td>300 mg/kg</td>
<td>Rat (5)</td>
<td>0.12 (Mean)</td>
<td>4.0 (Mean)</td>
<td>5.17 (Mean)</td>
<td>19.40 (Mean)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.06 (SD)</td>
<td>1.2 (SD)</td>
<td>1.74 (SD)</td>
<td>9.13 (SD)</td>
</tr>
</tbody>
</table>

(Males & females combined)

Table 6. Comparative PK parameters of 1 in rats and dogs.
3.12. P-glycoprotein (P-gp) transport (Table 6)

The results of incubating Rho in the presence or absence of Vpml and/or 1 are summarized in Table 7.

Six reaction conditions (1-6) are reviewed, where:
1 – is the result from incubating cell lines with Rho for 15 minutes.

2 – is the result from incubating cell lines with Vpml for 1 hour and then adding Rho during the last 15 minutes of incubation.

3 – is the result from incubating the cell lines with Vpml for 15 minutes, adding 1 after an incubation time of 30 minutes and then adding Rho during the last 15 minutes – total incubation time, 60 minutes.

4 – is the result from treating the cell lines simultaneously with Vpml and 1 for 45 minutes and then adding Rho and continuing the incubations for an additional 15 minutes – total incubation time – 60 minutes.

5 – is the result from incubating the cell lines with 1 for 15 minutes and then adding Vpml for additional 45 minute incubation. Rho is added during the last 15 minutes of the incubation.

Finally, 6 is the result of incubating each of the cell lines for 1 hour with 1 alone and then adding Rho during the last 15 minutes of incubation.

The results summarized in Table 7 for the 3 sensitive cell lines are coherent: The rate of incorporation of Rho is lower when cells are treated by the mixture of Vpml and DM-CHOC-PEN or Vpml alone but not when the cells are treated with DM-CHOC-PEN alone (mean fluorescence intensity of 6 roughly the same for control cells). This is interpreted as meaning that DM-CHOC-PEN has no effect on the function of P-gp transport.

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>Mean Fluorescence Intensity for Total Cells (Mean) (x 10^3)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HeLa</td>
<td>19.3**</td>
</tr>
<tr>
<td>A549</td>
<td>23.2</td>
</tr>
<tr>
<td>MCF-7</td>
<td>10.5</td>
</tr>
</tbody>
</table>

*Cell concentration per each assay. Average of triplicate assays.

Table 7. P-glycoprotein transport of 1

4. Discussion

The rationale for the pre-clinical development of 1, a polychlorinated pyridine cholesteryl carbonate, was based on observed antitumor activity vs. IC implanted human xenografts growing in mice, in comparison with standard therapy [1,2]. 1 was synthesized during an attempt to design and develop polychlorinated pyridine carbonates that could penetrate the BBB, with cytotoxic activity vs. intracranially growing brain tumors and without neurotoxicity [1,2].
We report here the results of acute toxicity and pharmacology studies with single intravenous injections of 1 in groups of rodents and dogs. The end-point of all the studies was to identify drug toxicity and an acceptable starting dose for a Phase I clinical trial in humans with advanced cancer.

The IV LD$_{10}$ single-dose value for mice (sexes combined) was calculated as 139 mg/m$^2$. The mouse study generally displayed a typical dose-response effect (with the exception of one death at 50 mg/kg), with 1 being slightly more toxic in males than in females at the two highest doses.

A sub-chronic oral mouse toxicity study was conducted at MPI Research, Mattawan, MI, under GLP conditions in male/female mice. The study evaluated 1 in an emulsion administered per oral gavage daily for five days at doses of 0, 800, 1000, 1200, 1500 and 2000 mg/kg per gavage to mice. Only one death occurred at 800 mg/kg on day 2 after dosing. All animals demonstrated some degree of lethargy and unkempt appearance, but no seizures. Similar body appearances were noted with the controls.

Adult rats were treated once with single IV infusions of 1 in doses of 50, 100, 150, 200, and 300 mg/kg. One (1) death occurred at the 100 mg/mL dose level. No deaths occurred in the treated vehicle group. There were no meaningful effects on hematology parameters. On Day-2 erythrocytes, hemoglobin, and hematocrit tended to be higher at the 300 mg/kg/dose. These changes were most likely a result of fluid imbalance. Monocytes were increased in both sexes at 200 and 300 mg/kg/dose and lymphocytes were slightly decreased in males at 300 mg/kg/dose level. Neutrophils were elevated relative to expected ranges in all groups on Days-2 and 15 and were attributed to stress and/or route of administration. All other changes were resolved by Day 15 and all values returned to normal pre-drug limits. There were no test article-related effects on either coagulation or on urinalysis parameters.

The most significant abnormal findings were the statistically increased plasma values for cholesterol and triglycerides in the 200 and 300 mg/kg treated groups. LDL-cholesterol was significantly elevated in females – increased from 5.4 to 142 and 156 mg/dL for the 200 and 300 mg/kg groups, resp. This elevation is significant and considered a SLT (CTEP.v4). The triglycerides were increased by 4-fold in the 300 mg/kg group females, however, they return to normal values by Day-15. Hepatic and splenic deposits of fats were also noted on gross and microscopic examinations which cleared by Day-15.

Cholesterol is released during metabolism of 1 (Table 2 & Scheme 1). The early formation of LDL-cholesterol is not a surprise since the formation of the LDL-variant is the initial natural method to ‘initially encase cholesterol molecules’. The cholesterol is cleared through the liver as the HDL-variant. Triglycerides were elevated secondary to increased cholesterol and the lipid character of the emulsion vehicle, which also rapidly reversed [3].

Although the above cholesterol and triglyceride findings resolved by Day-15, they must be considered adverse – triglycerides (3-fold) and LDL-cholesterol (30-fold in females). The control group received the vehicle alone – soybean oil and egg yolk lecithin – both rich in triglycerides and did not demonstrate abnormal lipid profiles.
Alanine aminotransferase (ALT) in males, and γ-glutamyl transferase (GGT), and alkaline phosphatase in females were minimally to mildly elevated in the 300 mg/kg group on Day-2. All of these findings on Day-2 resolved by Day-15. Transient elevations in transaminases were considered to be due to hepatic clearance of the drug. Neither gross nor microscopic evidence of toxicity (other than hepatic cysts) was noted at autopsies, including CNS. Table 7 compares calculated starting therapeutic doses for humans [19].

A single IV dose administration study was performed in adult beagle dogs employing single doses (10 – 30 mg/kg) of 1. No treatment related fatalities occurred. Numerous clinical signs reflecting treatment-related effects were noted in both sexes of all groups. Pertinent clinical signs noted included decreased activity, autonomic hyperactivity – vomiting, decreased urination, salivation, and lacrimation. The effects were of immediate onset (within one hour post dose), with most of the signs clearing by Day-2 of the study. However, decreased activity persisted for Days 2, 3, and 4 in some of the animals and through the remainder of the study. Slight body weight losses noted in some animals were not dose-dependent and it could have been attributable to the clinical signs (and associated stress) caused by the vehicle. There were marked elevations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and sorbitol dehydrogenase (SDH) noted in controls and all treated groups and apparently attributable to the vehicle – a Klugel/Tween mixture that will not be used in the clinical studies. All liver functions reversed by Day-15. This acute and transient effect on liver enzymes exhibited no dose-dependent pattern and was also apparently attributable to the vehicle. The latter finding was not observed in the rat study.

No hematological deficiencies were noted in any group. Drug-related neurotoxicity was not observed. This was confirmed by second opinion (RT), who conducted silver stains and confirmed MPI’s observation that there were no microscopic pathological CNS changes present in the brains of dogs treated with 1 [19].

Based on the conditions and findings of this study, a single bolus intravenous injection of 1 to groups of beagle dogs at dose levels of 10, 20 and 30 mg/kg produced no effects that were directly related to the test article; instead they were probably attributable to the 0.3% Klucel +1.92% Tween® 80 vehicle used.

Pharmacokinetic studies were conducted in two species – rats and dogs. Parameters were obtained from Gauss Newton algorithm modeling [3]. The values are compared in Table 6. No statistical differences between male and female rats were noticed. The differences in half life can be explained in reference to administration routes – dog-IV bolus vs. rat – IV infusion over 3 hrs. Similarly, the clearance is higher in the bolus studies as expected with a surge of drug being filtered.

In the learning/cognitive screening study, rats treated with 3 took a longer period of time to find the pedestal vs. 1, 2 and the controls. Despite normal gross appearance of the rats after 7 hr (Table 4), the 3 treated animals demonstrated impaired learning (Table 5). There were no signs of learning impairment noted in the rats treated with 1 or 2, as was seen for 3. 2 is a polychlorinated 4-hydroxypyridine (Fig. 1) and exists as a zwitterion that is too polar to cross the BBB. This behavior has been observed for other 4-hydroxypyridines [20]. The water
swimming maze assesses impaired visual spatial processing, as well as memory. The observations for 5-FU (drug control) confirmed literature reports of learning/memory impairment post dosing in humans [16].

The drug penetrated human glioblastoma tumor tissue growing IC in mice, with none detectable in the normal tissue. This only reinforced our interest in using the drug to treat patients with cancers involving the CNS.

1 does not have effects on the P-gp transport system. In the current study, Rho was selected as an indicator of 1’s interaction/inhibition with the P-gp protein transport system in three (3) human cancer cell lines-A549: lung; MCF7: breast; and HeLa: ovarian. Verapamil (Vpml) is a known inhibitor of the P-gp pathway and was included as a positive control, providing additional support that 1 is not a substrate for the P-gp transmitter system and not rejected via the high energy ATP-binding cassette (ABC) transport systems [11].

Both 1 and 2 bind to erythrocytes and could penetrate the blood brain barrier (BBB) and IC growing cancers attached to rbcs via breaks in the BBB (penetration routes of metastatic cancers) or sites of neo-angiogenic networks. However, as previously published, 2 alone is not active vs. IC implanted human breast and glioma tumors implanted in a mouse model; the presence of 2 in the IC implanted tumors (see Results-Brain/tumor penetration) must be due to IC steroid esterase hydrolysis of 1 [21].

Thus, preclinical studies, conducted under GLP guidelines are presented as support for 1 to enter Phase I clinical trials as treatment for advanced cancer with CNS involvement. Table 8 reviews calculated starting doses and data satisfied the FDA’s requirements for an IND (IND 68,876), which has completed a Phase I clinical trial – DTI-021 [19]. The phase I clinical trial is nearing completion with acceptable toxicities and responses noted in patients with advanced cancer involving the CNS system [15].

<table>
<thead>
<tr>
<th>Drug</th>
<th>Species</th>
<th>Acute IV LD_{10}</th>
<th>Comparable Human IV Dosage*</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM-CHOC-PEN</td>
<td>Mouse</td>
<td>136 mg/kg/d</td>
<td>39 mg/m²/d (10% of LD_{10})</td>
</tr>
<tr>
<td>DM-CHOC-PEN</td>
<td>Rat</td>
<td>100 mg/kg/d</td>
<td>60 mg/m²/d (10% of LD_{10})</td>
</tr>
<tr>
<td>DM-CHOC-PEN</td>
<td>Dog</td>
<td>&gt;30 mg/kg/d</td>
<td>&gt;100 mg/m²/d** (1/6th HNSTD)</td>
</tr>
</tbody>
</table>

* Standard conversion **Based on the highest dose – 30 mg/kg used in the dog studies
The initial level of dosing in the Phase I clinical trial has been established as 39 mg/m² (IND 86,876) [19].

Table 8. Estimated comparable human intravenous dosages

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