CuATSM / H_2ATSM (NSC-729307)

PRECLINICAL TOXICOLOGY SUMMARY

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A. MUTAGENICITY ASSAYS

In Vitro Salmonella Reverse Mutation Plate Incorporation Assay (IITRI Project No. 2073-002-001-002)

The potential mutagenic activity of CuATSM/H_2ATSM (NSC-729307) was investigated in the Salmonella Reverse Mutation Plate Incorporation Assay, which is an *in vitro* test designed to detect point mutations in bacterial tester strains induced by chemical agents. The mutagenic events are reverse mutations that cause histidine-requiring mutants to revert to their prototrophic (non-histidine requiring) state. The study was conducted in two parts such that an initial mutagenicity/cytotoxicity assay was conducted first, which was followed by a confirmatory assay. For both assays, CuATSM/H_2ATSM was tested in *S. typhimurium* strains TA98, TA100, TA102, TA1535, and TA1537 in the presence and absence of S9 metabolic activation. Concentrations of 0, 10, 25, 30, 40 and 100 μg/plate were used for the initial mutagenicity/cytotoxicity assay, whereas 0, 10, 40, 100, 500 and 1000 μg/plate were used in the confirmatory assay. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

In the range-finding assays, CuATSM/H_2ATSM did not exhibit a dose-related mutagenic response in any of the five tester strains in the presence or absence of S9 metabolic activation, and there was no evidence of cytotoxicity for any strain in the presence or absence of S9. Visible precipitate was not observed with any concentration. With the lack of cytotoxicity and visible precipitate with concentrations up to 100 μg/plate, the concentration range was increased to include 500 and 1000 μg/plate in the confirmatory assay. At these concentrations, precipitation was observed both visually and microscopically. Precipitation was also observed microscopically at 100 μg/plate. A modest (two-fold) increase of revertants relative to the vehicle control was observed for strain TA100 in the absence of S9 at 1000 μg/plate. Since this concentration was 10 times the limit of solubility of the CuATSM/H_2ATSM, it is likely that the response was due to soluble impurities in the CuATSM/H_2ATSM preparation and not from the CuATSM/H_2ATSM. Nevertheless, considering that each plate had a volume of 2.7 mL, the 1000 μg/plate (370 μg/mL) concentration is approximately 70,000-fold higher than the maximum possible plasma concentration that can be achieved in humans, based on a 70-kg individual

receiving 15 μg of CuATSM/H_2ATSM. The results of this study indicate that CuATSM/H_2ATSM is non-mutagenic in the *Salmonella* Reverse Mutation Plate Incorporation Assay.

***In Vitro* L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutation Assay** (IITRI Project Number 2073-002-001-001)

An *in vitro* L5178Y/TK$^{+/-}$ Mouse Lymphoma Mutation Assay was conducted to evaluate the mutagenic potential of CuATSM/H_2ATSM (NSC-729307) in a mammalian cell system. The L5178Y/TK$^{+/-}$ cell line is sensitive to the cytotoxic effects of the pyrimidine analog, trifluorothymidine (TFT), and when treated *in vitro* with mutagenic or carcinogenic agents, TK$^{+/-}$ is mutated to the TK$^{-/-}$ genotype which confers TFT-resistance. The mutant cells then proliferate and form colonies when cloned in selective medium containing TFT. The assay was done in three parts. The cytotoxicity experiment was conducted first to determine the concentration range for the mutagenicity experiments. The initial mutagenicity experiment was performed next, and to confirm negative or positive results from the initial mutagenicity experiment, a confirmatory (definitive) experiment was performed. Test article exposures were for 4 hours in the presence and absence of an S9 activation system (Aroclor 1254-induced rat liver S9) for the cytotoxicity and initial mutagenicity experiments. For the confirmatory experiment, exposures were for 4 hours in the presence of S9 and 24 hours in the absence of S9. Cultures from the initial and confirmatory mutagenicity experiments which demonstrated a relative suspension growth (RSG) of more than 10% were cloned in triplicate in restrictive medium to select for the mutant phenotype, following a two day expression period. After a 12-15 day selection period, mutant colonies for positive controls, solvent controls and positive test article responses (should any exist) were enumerated. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

Limited solubility of CuATSM/H_2ATSM/DMSO stock solution in culture media and the requirement to keep the final concentration of vehicle to 1% or less restricted the top dose to 100 μg/mL in the cytotoxicity experiment. The results of this first experiment showed that concentrations ≥25 μg/mL in both the presence and absence of S9 were excessively toxic (>90% toxic relative to control). No cultures were cloned from this experiment. In the initial mutagenicity experiment, concentrations of 0, 3 (without S9 only), 5, 7, 10, 12, 15, 18, 20 and 25 μg/mL were tested in the presence and absence of S9. The results of this experiment indicated excessive cytotoxicity with CuATSM/H_2ATSM concentrations ≥15 μg/mL in the

absence of S9, and ≥20 µg/mL in the presence of S9. CuATSM/H_2ATSM was non-mutagenic in both the absence and presence of metabolic activation, as the mutation frequency was not elevated by a factor of two or more times the vehicle mutation frequency at any of the concentrations tested. In the confirmatory assay, CuATSM/H_2ASTM concentrations of 0, 0.5, 1, 3, 5, 10, 12 and 15 µg/mL were tested without S9 (24 hr exposure), and 0, 10, 12, 15, 18 and 20 µg/mL were tested with S9 (4 hr exposure). In the absence of S9, excessive cytotoxicity was achieved with concentrations ≥10 µg/mL, whereas, the remaining concentrations exhibited a range of cytotoxic effects (41-88% toxic), relative to control. Excessive cytotoxicity was not observed in the presence of S9 in the confirmatory assay, instead the cytotoxicity ranged from nontoxic to 72% toxic, relative to control. No statistically significant ($p<0.05$) or dose-related increase in mutation frequency was observed at any of the CuATSM/H_2ATSM concentrations tested, and none of the concentrations produced a mutant frequency that was 2-fold or higher than the solvent control. The positive controls (methyl methanesulfonate and Benzo[a]pyrene) exhibited statistically significant increases in mutation frequency which were 2-fold or higher than the solvent control, showing that the assay was sensitive to detecting mutagenicity.

Under the conditions of this study, CuATSM/H_2ATSM (NSC-729307) was negative in the L5178Y/$TK^{+/-}$ Mouse Lymphoma Mutagenesis Assay.

In Vivo Micronucleus Assay in Rats

The potential for CuATSM/H_2ATSM (NSC-729307) to induce chromosomal aberrations and spindle malformations *in vivo* was evaluated using the Micronucleus Assay in conjunction with the 14-Day Toxicity Study of CuATSM/H_2ATSM (NSC-729307) in Rats (IITRI Project No. 2073-002-002). Fischer 344 rats (15/sex/group) were treated with intravenous doses of 0, 0.075 and 0.150 mg/kg/d (0, 0.450 and 0.900 mg/m²/d) CuATSM/H_2ATSM once a day for 14 consecutive days. Bone marrow was collected on study days 15 (10 rats/sex/group) and 29 (5 rats/sex/group) for preparation of bone marrow smears, however only the day 15 smears were evaluated. Cytogenetic damage is indicated by the presence of micronuclei in polychromatic erythrocytes. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

No statistically significant ($p<0.05$) or dose-dependent increase in micronucleated polychromatic erythrocytes was noted for rats treated with CuATSM/H_2ATSM (NSC-

729307) relative to the vehicle control groups on day 15. Rats treated with cyclophosphamide (positive control; males only) exhibited a statistically significant increase in micronucleated polychromatic erythrocyte counts on day 15, as compared to the vehicle control group. This demonstrated that the assay was sensitive to detecting mutagenicity. Since there was no apparent test article-related effect at the end of the treatment period, bone marrow slides that were prepared from rats (recovery animals) on day 29 were not evaluated. CuATSM/H_2ATSM (NSC 729307) was concluded to be negative in the rat micronucleus test.

B. SAFETY PHARMACOLOGY STUDIES

Cardiovascular and Pulmonary Safety Testing of CuATSM/H_2ATSM (NSC-729307) in Beagle Dogs (Battelle Study G465535A). The objective of this study was to evaluate the cardiovascular and pulmonary safety of CuATSM/H_2ATSM (NSC-729307) when administered as a single intravenous bolus dose in Beagle dogs (2/sex/dose group). CuATSM/H_2ATSM was dissolved initially in DMSO and then diluted with ethanol and 0.9% sodium chloride, USP such that the final concentration of the test article, DMSO, ethanol and sodium chloride, USP were 0.03 mg/mL, 0.3% (v/v), 7% (v/v), and 92.7% (v/v), respectively. A targeted dose of 0.300 mg/kg (6.00 mg/m^2) was administered to treated dogs, while control animals received an equal volume (10 mL/kg) of vehicle. Cardiovascular data (systemic arterial blood pressures, heart rate, and ECG waveforms) were collected for up to 60 hours post dosing via implantable radiotelemetry units. ECG interval measurements were made on the ECG waveforms. Pulmonary data (respiratory rate, tidal volume and minute volume) were collected continuously for approximately 4-5 hours post dosing. Clinical observations, body temperatures and clinical pathology were also evaluated. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

Mortality/Clinical Observations: No deaths occurred in this study. There were no test-article related abnormal clinical observations. However, excessive salivation was observed for one control dog and one test-article treated dog which may have been due to the DMSO component of the formulation.

Body Temperatures: There were no test article-related changes in body weights or body temperatures.

Clinical Pathology: There were no test article-related changes in hematology or clinical chemistry, including C-reactive protein, serum amyloid A and troponin T.

Cardiovascular Effects: There were no alterations in heart rate, blood pressure, or ECG that could be attributed to the administration of CuATSM/H_2ATSM. However, vehicle-related acute blood pressure and heart rate alterations were observed during the first 10 minutes post dosing. Blood pressures were increased up to 15 mmHg, and heart rates were increased up to 30 BPM, for both vehicle-dosed and drug-dosed animals. On average, blood pressures returned to near baseline values after 10 minutes, whereas, heart rates gradually returned to baseline values over the first 1.5 hours post-dose. These cardiovascular effects may have be due to the DMSO component in the formulation.

Evaluation of ECG interval data revealed no apparent or statistically significant differences in the test article group as compared to vehicle, and there were no alterations in ECG rhythm or morphology that could be attributed to the test article.

Respiratory Effects: There were no apparent or statistically significant alterations in respiratory rates, tidal volume or minute volume in this study.

Conclusions: No treatment-related effects on cardiovascular or respiratory function were observed in dogs dosed at 0.3 mg/kg (6 mg/m^2). This dose is approximately 750-fold higher than the maximum intended human dose. Acute vehicle-related increases in blood pressures and heart rates were observed.

Neurological Safety Assessment in Rats. Neurotoxicology assessments [functional observational battery (FOB) evaluations] for rats were done in conjunction with the 14-Day Toxicity Study of CuATSM/H_2ATSM (NSC-729307) in Rats (IITRI Project No. 2073-002-002). Rats (15/sex/group) were treated intravenously with CuATSM/H_2ATSM at doses of 0, 0.075 and 0.150 mg/kg (0, 0.450 and 0.900 mg/m^2) once a day for 14 consecutive days. Neurological toxicity evaluations were conducted on rats prior to dosing (pre-study), and on days 14 (10/sex/group) and 28 (recovery rats; 5 /sex/group). The following FOB parameters were monitored: home cage observation (e.g. tremors, convulsions, biting, vocalizations, posture, fur appearance), arousal/anxiety when handheld, open field mobility/gait, reactivity to sensory stimulation (e.g. visual, auditory, tactile, pain), hindlimb extension, catalepsy, forelimb and hindlimb grip strength, righting reflex,

footsplay, body temperature and body weights. The study was conducted in compliance with GLP regulations (21 CFR, Part 58).

No treatment-related, toxicologically significant or otherwise noteworthy changes were observed for any of the FOB parameters evaluated at pre-study (baseline) or on days 14 and 28.

C. IND-DIRECTED TOXICITY STUDIES OF CuATSM/H_2ATSM (NSC-729307) IN RATS AND RABBITS

14-Day Toxicity Study of CuATSM/H_2ATSM (NSC-729307) in Rats (IITRI Project No. 2073-002-002). The objective of this study was to evaluate the target organ toxicity of CuATSM/H_2ATSM and its reversibility when administered intravenously to rats. Male and female Fischer 344 rats (15/sex/group) were administered CuATSM/H_2ATSM (NSC-729307) intravenously at target dose levels of 0.075, and 0.150 mg/kg (0.450 and 0.900 mg/m^2) once a day for 14 consecutive days. Control rats received an equal volume (5 mL/kg) of vehicle (mixture of DMSO, ethanol and 0.9% sodium chloride for injection USP at final concentrations of 0.3% v/v, 7% v/v and 92.7% v/v, respectively) once a day for 14 days. Cageside clinical observations were performed and recorded daily, and hand-held physical and clinical observations were performed during pretest, and weekly throughout the treatment and recovery periods. Body weights were measured twice weekly during the treatment and recovery periods. Hematology and clinical chemistry parameters were evaluated on days 8, 15 and 29. Functional observational battery (FOB) tests to determine neurological toxicity were performed during pretest, and on days 14 and 28. Organ weights were determined on days 15 and 29. A bone marrow micronucleus assay was conducted using bone marrow from rats euthanized on day 15. Microscopic histopathology was performed on tissues from rats in the high dose (0.150 mg/kg/d) and control groups which were euthanized on day 15. The study was conducted in compliance with GLP regulations (21 CFR, Part 58). The results are summarized in Table 1.

Mortality/Clinical Observations: No deaths occurred, and no drug-related adverse clinical signs were observed after 14 days of dosing.

Functional Observational Battery: The results of the neurotoxicology assessments are summarized in the Neurological Safety Assessment in Rats.

Body Weights: There were no treatment-related changes in body weights or body weight gain during the treatment and recovery periods.

Hematology: Minimal changes in hematology parameters occurred that were not toxicologically relevant.

Clinical Chemistry: Minimal changes in clinical chemistry parameters occurred that were not toxicologically relevant.

Organ Weights: No treatment-related effect on organ weights was observed in this study.

Micronucleus Assay: The results of the micronucleus assay are summarized in the *In Vivo* Micronucleus Assay in Rats.

Pathology: No treatment-related gross lesions were observed in tissues of rats on day 15 or 29, and no treatment-related microscopic lesions were observed on day 15 in the tissues of rats that were dosed with 0.150 mg/kg/d. Tissues from recovery rats were not evaluated microscopically.

Conclusions: Intravenous administration of combined CuATSM/H_2ATSM (NSC-729307) at target dose levels of 0.075 or 0.150 mg/kg/d for 14 consecutive days did not result in any clearly dose-related, treatment-related and/or toxicologically significant effects on mortality, clinical observations, mean body weights, mean body weight gains, hematology, clinical chemistry, neurotoxicity (as measured by FOB), mutagenicity (bone marrow micronucleated erythrocytes) or organ weights. No treatment-related gross or microscopic lesions were observed in any of the tissues evaluated. Based on these findings, the no-observed-adverse-effect level (NOAEL) for the study was 0.150 mg/kg/d (0.900 mg/m^2/d). This dose is approximately 110-fold higher than the maximum intended human dose.

14-Day Toxicity Study of CuATSM/H_2ATSM (NSC-729307) in Rabbits (IITRI Project No. 2073-002-003). The objective of this study was to determine target organ toxicity of CuATSM/H_2ATSM and its reversibility when given intravenously to rabbits twice daily (BID) for 14 consecutive days. Male and female New Zealand White rabbits were administered CuATSM/H_2ATSM (NSC-729307) intravenously twice a day for 14 days at target dose levels of 0.030 and 0.060 mg/kg/d (0.360 and 0.720 mg/m^2/d). Control rabbits received an equal volume (1 mL/kg) of vehicle

(mixture of DMSO, ethanol and 0.9% sodium chloride for injection, USP at final concentrations of 0.3% v/v, 7% v/v and 92.7% v/v, respectively) twice a day for 14 days. Clinical signs of toxicity were monitored daily, and body weights were measured twice weekly during the treatment period and once weekly during the recovery period. Hematology and clinical chemistry parameters were evaluated on days 8, 15 and 29. Microscopic histopathology was performed on tissues from all rabbits in the high dose (0.06 mg/kg/d) and control groups that were euthanized on day 15. The study was conducted in compliance with GLP regulations (21 CFR, Part 58). The results are summarized in Table 1.

Mortality/Clinical Observations: No deaths occurred in this study. All rabbits were described as clinically normal at all observation times.

Body Weights: There were no treatment-related alterations in body weights or body weight gain during the dosing or recovery periods.

Hematology: No toxicologically significant changes in hematology were observed which could be attributed to CuATSM/H_2ATSM.

Clinical Chemistry: No toxicologically significant changes in clinical chemistry were observed which could be attributed to CuATSM/H_2ATSM.

Organ Weights: No treatment-related effect on organ weights was observed in this study.

Pathology: No treatment-related gross lesions were observed in tissues of rabbits on day 15 or 29, and no treatment-related microscopic lesions were observed on day 15 in the tissues of rabbits that were dosed at 0.060 mg/kg/d. However, minimal to mild pathological effects were observed (microscopically) which were considered incidental findings or associated with vehicle administration. These included, inflammation of the lung and hemorrhage at the injection site for males and females in the control and 0.060 mg/kg/d groups. Tissues from recovery rats were not evaluated microscopically.

Conclusions: Intravenous injection of CuATSM/H_2ATSM (NSC-729307) at target dose levels of 0.030 and 0.060 mg/kg/d to male and female rabbits for 14 days resulted in no premature or unscheduled deaths. No adverse clinical signs were observed, and no treatment-related effects on body weight, body weight gain,

hematology, clinical chemistry, or absolute and relative organ weights were seen. No drug-related gross lesions were observed in any male or female rabbit, and no drug-related histopathological effects were observed in any rabbit dosed at 0.060 mg/kg/d at the end of the 14 day dosing period. The no-observed-effect level for CuATSM/H_2ATSM was 0.060 mg/kg/d (0.720 mg/m^2/d), which is 90-fold higher than the maximum intended human dose. However, the vehicle may have produced minimal to mild effects on the lung and skin (at the injection site) tissues.

D. SUMMARY

A single dose of 0.015 mg/person (0.008 mg/m^2, based on a 70 kg/person) CuATSM/H_2ATSM is proposed for the clinical trials. In the cardiovascular and respiratory safety pharmacology study, dogs receiving a single dose of CuATSM/H_2ATSM at 0.300 mg/kg (6.00 mg/m^2), a 750-fold higher dose than the proposed clinical dose, exhibited no treatment-related effects. In the 14-day rat and rabbit toxicity studies, targeted maximum doses of 0.150 mg/kg/d (0.900 mg/m^2/d) for rats, and 0.060 mg/kg/d (0.720 mg/m^2/d) for rabbits, did not produce any drug-related effects. These doses were approximately 110-fold (rats) and 90-fold (rabbits) higher than the maximum intended dose when compared on a daily dose basis, and 1500-fold and 1200-fold higher when the total doses administered were compared. This demonstrates that CuATSM/H_2ATSM has an appropriate margin of safety for clinical use. However, one or more of the components in the formulation preparation produced a modest (two-fold) increase of bacterial revertants, relative to the vehicle control, for one tester strain (TA100). It is important to note however, that the concentration that produced this modest positive response is 70,000-fold higher than the maximum possible concentration that can be achieved in human plasma. The vehicle may have produced acute increases in blood pressures and heart rates of dogs, and minimal to mild effects on the lung and skin (at the injection site) tissues of rabbits.

TABLE 1. SUMMARY OF 14-DAY TOXICITY STUDIES WITH CuATSM/H_2ATSM (NSC-729307) IN RATS and RABBITS

Study Type	Lab/ Project No.	Vehicle	Species/ Strain	#/Sex/ Dose	Route/ Schedule	Daily Dose		Total Dose		Target Organs/Systems of Toxicity
						mg/kg/ d	mg/m^2/ d	mg/kg	mg/m^2	
Toxicity GLP	IITRI Project No. 2073-002-002	DMSO: Ethanol: Saline 0.3% v/v: 7% v/v : 92.7% v/v	Fischer 344 rats	15	i.v./ d x 14	0	0	0	0	Toxicity: none
						0.075	0.450	1.05	6.30	Toxicity: none Lesions: histopathology analysis was not conducted
						0.150	0.900	2.10	12.6	Toxicity: none Lesions: none
Toxicity GLP	IITRI Project No. 2073-002-003	DMSO: Ethanol: Saline 0.3% v/v: 7% v/v : 92.7% v/v	New Zealand White rabbits	8	i.v./ d x 14	0	0	0	0	Toxicity: one Lesions: minimal to mild inflammation of the lung and minimal hemorrhage at the injection site
						0.03	0.36	0.42	5.04	Toxicity: none Lesions: histopathology analysis was not conducted
						0.06	0.72	0.84	10.08	Toxicity: none Lesions: vehicle-related minimal to mild inflammation of the lung and minimal hemorrhage at the injection site