FINAL REPORT

Study Title

Bacterial Reverse Mutation Assay of AGI-19675

Test Article

AGI-19675

Author

Emily Dakoulas, BS

Study Completion Date

28 March 2016

Testing Facility

BioReliance Corporation
9630 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AE37RY.502ICH.BTL

Sponsor

Agios Pharmaceuticals
88 Sidney Street
Cambridge, MA 02139

Sponsor Number

AG120-N-074

1. STATEMENT OF COMPLIANCE

Study No. AE37RY.502ICH.BTL was conducted in compliance with the following regulations: US FDA Good Laboratory Practice Regulations as published in 21 CFR Part 58. This regulation is compatible to non-US regulations, OECD Principles of Good Laboratory Practice (C(97)186/Final); Japanese Ministry of Health, Labor and Welfare Good Laboratory Practices (Ordinance Nos. 21 and 114, if applicable); Japanese Ministry of Agriculture, Forestry and Fisheries Good Laboratory Practices (No. 11 Nousan-6283); Japanese Ministry of Economy, Trade and Industry Good Laboratory Practices, and allows submission of the report under the Mutual Acceptance of Data (MAD) agreement with applicable OECD member countries. The following exception was noted:

1. The identity, strength, purity, stability and composition or other characteristics to define the test article were determined by Shanghai SynTheAll Pharmaceutical Co., Ltd. However, the characterization documents do not indicate the regulations under which the analyses were conducted.

   Study Director Impact Statement: The impact cannot be determined because the appropriate information was not provided to the Study Director. The study conclusion was based on the test article as supplied.

Emily Dakoulas, BS
Study Director

28MAR2016
Date

2. QUALITY ASSURANCE STATEMENT

BioReliance by SAFC

Quality Assurance Statement

Study Information

Number: AE37RY.502ICH.BTL

Compliance

Procedures, documentation, equipment and other records were examined in order to assure this study was performed in accordance with the regulation(s) listed below and conducted according to the protocol and relevant Standard Operating Procedures. Verification of the study protocol was performed and documented by Quality Assurance.

US FDA Good Laboratory Practices 21CFR 58

Inspections

Quality Assurance performed the inspections(s) below for this study.

Insp. Dates (From/To)		Phase Inspected	To Study Director	To Management
16-Dec-2015	16-Dec-2015	Protocol Review	16-Dec-2015	16-Dec-2015
17-Dec-2015	17-Dec-2015	Dose Sample Analysis	18-Dec-2015	18-Dec-2015
12-Jan-2016	13-Jan-2016	Data/Draft Report - Analytical Dose Sample Analysis	14-Jan-2016	14-Jan-2016
14-Jan-2016	14-Jan-2016	Data/Draft Report	14-Jan-2016	14-Jan-2016
28-Feb-2016	28-Feb-2016	Data/Draft Report - Analytical Dose Sample Analysis	28-Feb-2016	28-Feb-2016
23-Mar-2016	23-Mar-2016	Final Report	23-Mar-2016	23-Mar-2016

The Final Report for this study describes the methods and procedures used in the study and the reported results accurately reflect the raw data of the study.

For a multisite study, test site QA Statements are located in the corresponding contributing scientist report.

E-signature

Quality Assurance: Luleayenwa Aberra-Degu 25-Mar-2016 1:24 pm GMT

Reason for signature: QA Approval

Printed by:Luleayenwa Aberra-Degu
Printed on:25-Mar-16

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4. STUDY INFORMATION

Study Conduct

Sponsor:	Agios Pharmaceuticals 88 Sidney Street Cambridge, MA 02139
Sponsor's Authorized Representative:	Andrew Olaharski, PhD, DABT 88 Sidney Street Cambridge, MA 02139
Testing Facility:	BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850
BioReliance Study No.:	AE37RY.502ICH.BTL
Sponsor No.:	AG120-N-074

Test Article

Identification:	AGI-19675
Batch No.:	15051502
Purity:	100%
Molecular Weight:	112.11 g/mol
Description:	White crystalline powder (as identified by BioReliance) Pale yellow crystals (as provided in the Testing Report of Raw Material)
Storage Conditions:	Room temperature, protected from light and with inert gas (nitrogen)
Receipt Date:	05 November 2015

Study Dates

Study Initiation Date:	04 December 2015
Experimental Starting Date (first day of data collection):	16 December 2015
Experimental Start Date (first day test article administered to test system):	17 December 2015
Experimental Completion Date:	21 December 2015

Key Personnel

Study Director:	Emily Dakoulas, BS
Testing Facility Management:	Rohan Kulkarni, MSc, Ph.D. Director, Genetic Toxicology Study Management
Laboratory Supervisor:	Jessica Heavin, M.S.
Report Writer:	Gayathri Jayakumar, MPS
Contributing Scientist (Analytical Chemistry Analysis):	Philip Atkins, MChem
Analytical Laboratory (Analytical Chemistry Analysis):	BioReliance Corporation 9610 Medical Center Drive Rockville, MD 20850

5. SUMMARY

The test article, AGI-19675, was tested to evaluate its mutagenic potential by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system. Dimethyl sulfoxide (DMSO) was used as the vehicle.

In the mutagenicity assay, the dose levels tested were 15.0, 50.0, 150, 500, 1500 and 5000 µg per plate. Neither precipitate nor toxicity was observed. No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

These results indicate AGI-19675 was negative for the ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system.

6. PURPOSE

The purpose of this study was to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* strain WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system.

Historical control data are found in Appendix I. A copy of the study protocol is included in Appendix II.

7. CHARACTERIZATION OF TEST AND CONTROL ARTICLES

Shanghai SynTheAll Pharmaceutical Co., Ltd has determined the identity, strength, purity and composition or other characteristics to define the test article and the stability of the test article. A copy of the Testing Report of Raw Material is included in Appendix III. Based on the retest date provided in the Testing Report of Raw Material, the test article is considered stable through 14 May 2016.

All unused test article was returned to the Sponsor prior to report finalization.

The vehicle used to deliver AGI-19675 to the test system was DMSO.

Vehicle	CAS Number	Supplier	Lot Number	Purity	Expiration Date
DMSO	67-68-5	Sigma-Aldrich	SHBG2668V	99.99%	October 2018

Test article dilutions were prepared immediately before use and delivered to the test system at room temperature under filtered light.

Positive controls plated concurrently with the mutagenicity assay are listed in the following table. All positive controls were diluted in dimethyl sulfoxide (DMSO) except for sodium azide, which was diluted in sterile water. All subdivided solutions of positive controls were stored at -10 to -30°C.

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98, TA1535	Rat	2-aminoanthracene (Sigma Aldrich Chemical Co., Inc.) Lot No. STBD3302V Exp. Date 31-Jul-2017 CAS No. 613-13-8 Purity 97.5%	1.0
TA100, TA1537			2.0
WP2 *uvr*A			15
TA98	None	2-nitrofluorene (Sigma Aldrich Chemical Co., Inc.) Lot No. S43858V Exp. Date 31-Mar-2016 CAS No. 607-57-8 Purity 99.4%	1.0
TA100, TA1535		sodium azide (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBH5113V Exp. Date 30-Jun-2016 CAS No. 26628-22-8 Purity 99.6%	1.0
TA1537		9-aminoacridine (Sigma Aldrich Chemical Co., Inc.) Lot No. 09820CEV Exp. Date 31-Mar-2016 CAS No. 52417-22-8 Purity 99.4%	75
WP2 *uvr*A		methyl methanesulfonate (Sigma Aldrich Chemical Co., Inc.) Lot No. MKBR6050V Exp. Date 31-Oct-2017 CAS No. 66-27-3 Purity 100.0%	1,000

The negative and positive control articles have been characterized as per the Certificates of Analysis on file with the Testing Facility. The stability of the negative and positive control articles and their mixtures was demonstrated by acceptable results that met the criteria for a valid test.

Dose Formulation Collection and Analysis

Dose formulation samples were collected from the mutagenicity assay as follows:

Vehicle Sampling	
Number of Samples[A]	Volume
2	0.500 mL

[A] One sample was used for analysis and the other served as the backup

Solution Sampling		
Dose Level	Number of Samples[B]	Volume
High Dose	2	0.500 mL
Low Dose	2	5.00 mL

[B]One sample was used for analysis and the other served as the backup

All samples were submitted to the analytical chemistry laboratory at BioReliance for analysis. Backup samples were discarded upon acceptance of the analytical results by the Study Director. A copy of the analytical report is included in Appendix IV.

8. MATERIALS AND METHODS

Test System

The tester strains used were the *Salmonella typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al*. (1975) and *Escherichia coli* WP2 *uvr*A as described by Green and Muriel (1976).

Tester strains TA98 and TA1537 are reverted from histidine dependence (auxotrophy) to histidine independence (prototrophy) by frameshift mutagens. Tester strain TA1535 is reverted by mutagens that cause basepair substitutions. Tester strain TA100 is reverted by mutagens that cause both frameshift and basepair substitution mutations. Specificity of the reversion mechanism in *E. coli* is sensitive to basepair substitution mutations, rather than frameshift mutations (Green and Muriel, 1976).

Salmonella tester strains were derived from Dr. Bruce Ames' cultures; *E. coli* tester strains were from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland.

Solubility Determination

DMSO was the vehicle of choice based on the solubility of the test article and compatibility with the target cells. The test article formed had previously been demonstrated to be soluble in DMSO at concentrations up to 100 mg/mL (Bioreliance Study No. AD98CM.502ICHNGLP.BTL).

Preparation of Tester Strain

Overnight cultures were prepared by inoculating from the appropriate frozen permanent stock into a vessel, containing 30 to 50 mL of culture medium. To assure that cultures were harvested in late log phase, the length of incubation was controlled and monitored. Following inoculation, each flask was placed in a shaker/incubator programmed to begin shaking at 125 to 175 rpm and

incubating at 37±2°C for approximately 12 hours before the anticipated time of harvest. Each culture was monitored spectrophotometrically for turbidity and was harvested at a percent transmittance yielding a titer of greater than or equal to $0.3x10^9$ cells per milliliter. The actual titers were determined by viable count assays on nutrient agar plates.

Identification of Test System

Each plate was identified by the BioReliance study number and a code system to designate the treatment condition, dose level and test phase, as described in detail in BioReliance's Standard Operating Procedures.

Exogenous Metabolic Activation

Aroclor 1254-induced rat liver S9 was used as the metabolic activation system. The S9 was prepared from male Sprague-Dawley rats that were injected intraperitoneally with Aroclor™ 1254 (200 mg/mL in corn oil) at a dose of 500 mg/kg, five days before sacrifice. The S9 (Lot No. 3560, Exp. Date: 02 December 2017) was purchased commercially from MolTox (Boone, NC). Upon arrival at BioReliance, the S9 was stored at -60°C or colder until used. Each bulk preparation of S9 was assayed for its ability to metabolize benzo(a)pyrene and 2-aminoanthracene to forms mutagenic to *Salmonella typhimurium* TA100.

The S9 mix was prepared on the day of use as indicated below:

Component	Final Concentration
β-nicotinamide-adenine dinucleotide phosphate	4 mM
Glucose-6-phosphate	5 mM
Potassium chloride	33 mM
Magnesium chloride	8 mM
Phosphate Buffer (pH 7.4)	100 mM
S9 homogenate	10% (v/v)

The Sham mix, containing 100 mM phosphate buffer at pH 7.4, was also prepared on the day of use.

Frequency and Route of Administration

The test system was exposed to the test article via the plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983).

Mutagenicity Assay

The mutagenicity assay was used to evaluate the mutagenic potential of the test article. TA98, TA100, TA1535, TA1537 and WP2 *uvr*A were exposed to the vehicle alone, positive controls and six dose levels of test article, in triplicate, in the presence and absence of Aroclor-induced rat liver S9.

Treatment of Test System

Media used in the treatment of the test system were as indicated below.

Component	Medium			
	Minimal top agar	Minimal bottom agar	Nutrient bottom agar	Nutrient broth
	Concentration in Medium			
BBL Select agar (W/V)	0.8% (W/V)	--	--	--
Vogel-Bonner minimal medium E	--	1.5% (W/V)	1.5% (W/V)	--
Sodium chloride	0.5% (W/V)	--	--	--
L-histidine, D-biotin and L-tryptophan solution	50 mM each	--	--	--
Sterile water	25 mL/100 mL agar (when agar not used with S9 or Sham mix)	--	--	--
Oxoid Nutrient Broth No. 2 (dry powder)	--	--	2.5% (W/V)	2.5% (W/V)
Vogel-Bonner salt solution	--	--	--	Supplied at 20 mL/L

To confirm the sterility of the S9 and Sham mixes, a 0.5 mL aliquot of each was plated on selective agar. To confirm the sterility of the test article and the vehicle, all test article dose levels and the vehicle used in each assay were plated on selective agar with an aliquot volume equal to that used in the assay. These plates were incubated under the same conditions as the assay.

One-half (0.5) milliliter of S9 or Sham mix, 100 µL of tester strain (cells seeded) and 50.0 µL of vehicle or test article dilution were added to 2.0 mL of molten selective top agar at 45±2°C. When plating the positive controls, the test article aliquot was replaced by a 50.0 µL aliquot of appropriate positive control. After vortexing, the mixture was overlaid onto the surface of 25 mL of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for 48 to 72 hours at 37±2°C. Plates that were not counted immediately following the incubation period were stored at 2-8°C until colony counting could be conducted.

Scoring

The condition of the bacterial background lawn was evaluated for evidence of test article toxicity by using a dissecting microscope. Precipitate was evaluated after the incubation period by visual examination without magnification. Toxicity and degree of precipitation were scored relative to the vehicle control plate using the codes shown in the following table. As appropriate, colonies were enumerated either by hand or by machine.

Code	Description	Characteristics
1 or no code	Normal	Distinguished by a healthy microcolony lawn.
2	Slightly Reduced	Distinguished by a noticeable thinning of the microcolony lawn and possibly a slight increase in the size of the microcolonies compared to the vehicle control plate.
3	Moderately Reduced	Distinguished by a marked thinning of the microcolony lawn resulting in a pronounced increase in the size of the microcolonies compared to the vehicle control plate.
4	Extremely Reduced	Distinguished by an extreme thinning of the microcolony lawn resulting in an increase in the size of the microcolonies compared to the vehicle control plate such that the microcolony lawn is visible to the unaided eye as isolated colonies.
5	Absent	Distinguished by a complete lack of any microcolony lawn over greater than or equal to 90% of the plate.
6	Obscured by Particulate	The background bacterial lawn cannot be accurately evaluated due to microscopic test article particulate.
NP	Non-Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye but any precipitate particles detected by the automated colony counter total less than or equal to 10% of the revertant colony count (e.g., less than or equal to 3 particles on a plate with 30 revertants).
IP	Interfering Precipitate	Distinguished by precipitate on the plate that is visible to the naked eye and any precipitate particles detected by the automated colony counter exceed 10% of the revertant colony count (e.g., greater than 3 particles on a plate with 30 revertants). These plates are counted manually.

Tester Strain Verification

On the day of use in each assay, all tester strain cultures were checked for the appropriate genetic markers.

Criteria for Determination of a Valid Test

The following criteria must be met for the mutagenicity assay to be considered valid:

All *Salmonella* tester strain cultures must demonstrate the presence of the deep rough mutation (*rfa*) and the deletion in the *uvr*B gene. Cultures of tester strains TA98 and TA100 must demonstrate the presence of the pKM101 plasmid R-factor. All WP2 *uvr*A cultures must demonstrate the deletion in the *uvr*A gene.

All cultures must demonstrate the characteristic mean number of spontaneous revertants in the vehicle controls as follows (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60.

To ensure that appropriate numbers of bacteria are plated, tester strain culture titers must be greater than or equal to $0.3x10^9$ cells/mL.

The mean of each positive control must exhibit at least a 3.0-fold increase in the number of revertants over the mean value of the respective vehicle control.

A minimum of three non-toxic dose levels is required to evaluate assay data. A dose level is considered toxic if one or both of the following criteria are met: (1) A >50 % reduction in the mean number of revertants per plate as compared to the mean vehicle control value. This reduction must be accompanied by an abrupt dose-dependent drop in the revertant count. (2) At least a moderate reduction in the background lawn (background code 3, 4 or 5).

Evaluation of Test Results

For each replicate plating, the mean and standard deviation of the number of revertants per plate were calculated and are reported.

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

Strains TA1535 and TA1537

Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 3.0-times the mean vehicle control value.

Strains TA98, TA100 and WP2 *uvr*A

Data sets were judged positive if the increase in mean revertants at the peak of the dose response was equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response was evaluated as negative if it was neither positive nor equivocal.

Electronic Data Collection Systems

The primary computer or electronic systems used for the collection of data or analysis included, but were not limited to, the following:

System	Purpose
LIMS Labware System	Test Article Tracking
Excel 2007 (Microsoft Corporation)	Calculations
Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments)	Data Collection/Table Creation
Kaye Lab Watch Monitoring system (Kaye GE)	Environmental Monitoring
BRIQS	Deviation and audit reporting

Records and Archives

All raw data, the protocol, pertinent study email correspondence and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years, unless otherwise requested by the Sponsor. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained by the BioReliance archives in accordance with the applicable SOPs. The raw data, reports and other documents generated at locations other than BioReliance will be archived by the test site.

Deviations

No deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

9. RESULTS AND DISCUSSION

Sterility Results

No contaminant colonies were observed on the sterility plates for the vehicle control, the test article dilutions or the S9 and Sham mixes.

Tester Strain Titer Results

Experiment	Tester Strain				
	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
	Titer Value (x 10^9 cells per mL)				
B1	2.7	2.6	1.7	3.3	3.6

Mutagenicity Assay

The results of the mutagenicity assay conducted at dose levels of 15.0, 50.0, 150, 500, 1500 and 5000 µg per plate in DMSO are presented in Tables 1 and 2. The maximum dose of 5000 µg per plate was achieved using a concentration of 100 mg/mL and a 50.0 µL plating aliquot. The test article formed clear solutions in DMSO at concentrations of 0.300 to 100 mg/mL.

Neither precipitate nor toxicity was observed.

No positive mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation.

A copy of the Common Technical Document Tables is included in Appendix V.

Dose Formulation Analysis

Dose formulations were sent to the analytical chemistry laboratory at BioReliance for analysis. A copy of the analytical report is included in Appendix IV. The results of the analysis indicate that the actual mean concentrations of the analyzed formulation samples (0.300 and 100 mg/mL) were 101.3 and 106.7% of target, respectively, with S/L ratios of > 0.925. This indicates that the formulations were accurately prepared. No test article was detected in the vehicle control sample. Additionally, AGI-19675 in DMSO, at concentrations of 0.304 and 107 mg/mL, was stable at room temperature for at least 3 hours. Owing to longer dosing time for the Ames study, additional stability analysis was performed. The additional stability results indicated that, AGI-19675 in DMSO, at concentrations of 0.286 and 97.3 mg/mL, was stable at room temperature for at least 4 hours.

10. CONCLUSION

All criteria for a valid study were met as described in the protocol. The results of the Bacterial Reverse Mutation Assay indicate that, under the conditions of this study, AGI-19675 did not cause a positive mutagenic response with any of the tester strains in either the presence or absence of Aroclor-induced rat liver S9. The study was concluded to be negative without conducting a confirmatory (independent repeat) assay because the results were clearly negative; hence, no further testing was warranted.

11. REFERENCES

Ames, B.N., J. McCann and E. Yamasaki (1975) Methods for Detecting Carcinogens and Mutagens with the *Salmonella*/Mammalian Microsome Mutagenicity Test, Mutation Research, 31:347-364.

Green, M.H.L. and W.J. Muriel (1976) Mutagen testing using trp+ reversion in *Escherichia coli*, Mutation Research 38:3-32.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. S2(R1) document recommended for adoption at step 4 of the ICH process on 9 November 2011. Adopted at Step 5 in Europe by CHMP December 2011 (issued as EMA/CHMP/ICH/126642/2008). Adopted at Step 5 in US by FDA on June 7, 2012 (issued as 77 FR 33748 pages 33748-33749). Adopted in Japan at Step 5 on September 20, 2012 (issued as PFSB/ELD Notification No. 0920-2).

Maron, D.M. and B.N. Ames (1983) Revised Methods for the *Salmonella* Mutagenicity Test, Mutation Research, 113:173-215.

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, adopted July 21, 1997.

12. DATA TABLES

TABLE 1
Mutagenicity Assay without S9 activation

Study Number: AE37RY.502ICH.BTL
Study Code: AE37RY
Experiment: B1
Date Plated: 12/17/2015
Exposure Method: Plate incorporation assay
Evaluation Period: 12/21/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**AGI-19675**	5000 µg	*13*	*2*	*0.9*	11^{A}, 13^{A}, 14^{A}
		1500 µg	*15*	*3*	*1.0*	18^{A}, 13^{A}, 15^{A}
		500 µg	*14*	*2*	*0.9*	15^{A}, 15^{A}, 11^{A}
		150 µg	*15*	*2*	*1.0*	16^{A}, 13^{A}, 15^{A}
		50.0 µg	*13*	*2*	*0.9*	14^{A}, 10^{A}, 14^{A}
		15.0 µg	*14*	*2*	*0.9*	16^{A}, 13^{A}, 13^{A}
	DMSO	50.0 µL	*15*	*4*		10^{A}, 17^{A}, 18^{A}
TA100	**AGI-19675**	5000 µg	*97*	*9*	*0.9*	99^{A}, 88^{A}, 105^{A}
		1500 µg	*94*	*5*	*0.9*	99^{A}, 93^{A}, 89^{A}
		500 µg	*88*	*6*	*0.9*	82^{A}, 93^{A}, 89^{A}
		150 µg	*109*	*10*	*1.1*	100^{A}, 107^{A}, 119^{A}
		50.0 µg	*95*	*15*	*0.9*	86^{A}, 87^{A}, 112^{A}
		15.0 µg	*96*	*5*	*0.9*	96^{A}, 92^{A}, 101^{A}
	DMSO	50.0 µL	*103*	*14*		119^{A}, 93^{A}, 96^{A}
TA1535	**AGI-19675**	5000 µg	*9*	*1*	*0.8*	8^{A}, 10^{A}, 8^{A}
		1500 µg	*14*	*3*	*1.2*	10^{A}, 16^{A}, 15^{A}
		500 µg	*11*	*3*	*0.9*	14^{A}, 9^{A}, 10^{A}
		150 µg	*14*	*2*	*1.2*	15^{A}, 15^{A}, 11^{A}
		50.0 µg	*15*	*3*	*1.3*	17^{A}, 16^{A}, 11^{A}
		15.0 µg	*11*	*2*	*0.9*	13^{A}, 11^{A}, 9^{A}
	DMSO	50.0 µL	*12*	*3*		9^{A}, 14^{A}, 14^{A}
TA1537	**AGI-19675**	5000 µg	*5*	*2*	*0.8*	5^{A}, 7^{A}, 3^{A}
		1500 µg	*5*	*1*	*0.8*	5^{A}, 5^{A}, 6^{A}
		500 µg	*6*	*1*	*1.0*	7^{A}, 5^{A}, 5^{A}
		150 µg	*5*	*3*	*0.8*	6^{A}, 8^{A}, 2^{A}
		50.0 µg	*7*	*3*	*1.2*	5^{A}, 10^{A}, 6^{A}
		15.0 µg	*6*	*2*	*1.0*	6^{A}, 8^{A}, 5^{A}
	DMSO	50.0 µL	*6*	*1*		7^{A}, 5^{A}, 6^{A}

Key to Automatic Count Flags

^{A}: Automatic count

TABLE 1 (CONT.)
Mutagenicity Assay without S9 activation

Study Number: AE37RY.502ICH.BTL
Study Code: AE37RY
Experiment: B1
Date Plated: 12/17/2015
Exposure Method: Plate incorporation assay
Evaluation Period: 12/21/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2uvrA	**AGI-19675**	5000 µg	*22*	*5*	*0.8*	27^{A}, 18^{A}, 21^{A}
		1500 µg	*27*	*8*	*1.0*	35^{A}, 19^{A}, 26^{A}
		500 µg	*24*	*2*	*0.9*	22^{A}, 26^{A}, 23^{A}
		150 µg	*23*	*5*	*0.9*	29^{A}, 19^{A}, 22^{A}
		50.0 µg	*21*	*4*	*0.8*	23^{A}, 17^{A}, 24^{A}
		15.0 µg	*21*	*4*	*0.8*	22^{A}, 17^{A}, 25^{A}
	DMSO	50.0 µL	*27*	*5*		30^{A}, 21^{A}, 29^{A}
TA98	**2NF**	1.0 µg	*133*	*29*	*8.9*	114^{A}, 119^{A}, 166^{A}
TA100	**SA**	1.0 µg	*564*	*54*	*5.5*	540^{A}, 626^{A}, 526^{A}
TA1535	**SA**	1.0 µg	*711*	*33*	*59.3*	693^{A}, 749^{A}, 690^{A}
TA1537	**9AAD**	75 µg	*362*	*43*	*60.3*	374^{A}, 315^{A}, 398^{A}
WP2uvrA	**MMS**	1000 µg	*375*	*58*	*13.9*	333^{A}, 441^{A}, 350^{A}

Key to Positive Controls

2NF	2-nitrofluorene
SA	sodium azide
9AAD	9-Aminoacridine
MMS	methyl methanesulfonate

Key to Automatic Count Flags

^{A}: Automatic count

TABLE 2
Mutagenicity Assay with S9 activation

Study Number: AE37RY.502ICH.BTL
Study Code: AE37RY
Experiment: B1
Date Plated: 12/17/2015
Exposure Method: Plate incorporation assay
Evaluation Period: 12/21/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
TA98	**AGI-19675**	5000 µg	*14*	*2*	*0.7*	16^{A}, 14^{A}, 13^{A}
		1500 µg	*23*	*4*	*1.1*	25^{A}, 19^{A}, 26^{A}
		500 µg	*24*	*3*	*1.1*	21^{A}, 25^{A}, 26^{A}
		150 µg	*20*	*9*	*1.0*	26^{A}, 24^{A}, 10^{A}
		50.0 µg	*20*	*5*	*1.0*	16^{A}, 18^{A}, 25^{A}
		15.0 µg	*19*	*4*	*0.9*	18^{A}, 15^{A}, 23^{A}
	DMSO	50.0 µL	*21*	*4*		21^{A}, 17^{A}, 24^{A}
TA100	**AGI-19675**	5000 µg	*114*	*2*	*0.9*	116^{A}, 115^{A}, 112^{A}
		1500 µg	*106*	*20*	*0.8*	122^{A}, 84^{A}, 111^{A}
		500 µg	*103*	*16*	*0.8*	112^{A}, 112^{A}, 84^{A}
		150 µg	*121*	*4*	*0.9*	116^{A}, 122^{A}, 124^{A}
		50.0 µg	*126*	*5*	*1.0*	127^{A}, 130^{A}, 121^{A}
		15.0 µg	*115*	*12*	*0.9*	129^{A}, 105^{A}, 111^{A}
	DMSO	50.0 µL	*129*	*9*		140^{A}, 124^{A}, 124^{A}
TA1535	**AGI-19675**	5000 µg	*11*	*6*	*0.8*	9^{A}, 17^{A}, 6^{A}
		1500 µg	*8*	*2*	*0.6*	9^{A}, 6^{A}, 8^{A}
		500 µg	*11*	*2*	*0.8*	13^{A}, 11^{A}, 10^{A}
		150 µg	*13*	*3*	*1.0*	15^{A}, 10^{A}, 14^{A}
		50.0 µg	*12*	*4*	*0.9*	11^{A}, 9^{A}, 16^{A}
		15.0 µg	*12*	*1*	*0.9*	13^{A}, 13^{A}, 11^{A}
	DMSO	50.0 µL	*13*	*3*		14^{A}, 10^{A}, 16^{A}
TA1537	**AGI-19675**	5000 µg	*6*	*3*	*0.9*	7^{A}, 3^{A}, 8^{A}
		1500 µg	*5*	*1*	*0.7*	5^{A}, 5^{A}, 6^{A}
		500 µg	*10*	*4*	*1.4*	14^{A}, 7^{A}, 10^{A}
		150 µg	*10*	*3*	*1.4*	8^{A}, 9^{A}, 13^{A}
		50.0 µg	*6*	*2*	*0.9*	5^{A}, 5^{A}, 8^{A}
		15.0 µg	*7*	*1*	*1.0*	8^{A}, 6^{A}, 7^{A}
	DMSO	50.0 µL	*7*	*2*		6^{A}, 7^{A}, 9^{A}

Key to Automatic Count Flags

^{A}: Automatic count

TABLE 2 (CONT.)
Mutagenicity Assay with S9 activation

Study Number: AE37RY.502ICH.BTL
Study Code: AE37RY
Experiment: B1
Date Plated: 12/17/2015
Exposure Method: Plate incorporation assay
Evaluation Period: 12/21/2015

Strain	Article	Dose level per plate	Mean revertants per plate	Standard Deviation	Ratio treated / solvent	Individual revertant colony counts and background codes
WP2uvrA	**AGI-19675**	5000 µg	*28*	*8*	*1.3*	19[A], 30[A], 35[A]
		1500 µg	*26*	*5*	*1.2*	22[A], 32[A], 24[A]
		500 µg	*24*	*3*	*1.1*	25[A], 26[A], 21[A]
		150 µg	*21*	*4*	*1.0*	21[A], 17[A], 25[A]
		50.0 µg	*18*	*4*	*0.8*	17[A], 15[A], 23[A]
		15.0 µg	*26*	*5*	*1.2*	31[A], 21[A], 25[A]
	DMSO	50.0 µL	*22*	*5*		23[A], 17[A], 27[A]
TA98	**2AA**	1.0 µg	*199*	*13*	*9.5*	212[A], 187[A], 197[A]
TA100	**2AA**	2.0 µg	*637*	*75*	*4.9*	552[A], 668[A], 692[A]
TA1535	**2AA**	1.0 µg	*818*	*25*	*62.9*	808[A], 846[A], 799[A]
TA1537	**2AA**	2.0 µg	*461*	*79*	*65.9*	458[A], 384[A], 542[A]
WP2uvrA	**2AA**	15 µg	*480*	*20*	*21.8*	457[A], 491[A], 491[A]

Key to Positive Controls

2AA 2-aminoanthracene

Key to Automatic Count Flags

[A]: Automatic count

13. APPENDIX I: Historical Control Data

Historical Negative and Positive Control Values 2014 Revertants per plate											
Strain	Control	Activation									
		None					Rat Liver				
		Mean	SD	Min	Max	95% CL	Mean	SD	Min	Max	95% CL
TA98	Neg	16	5	5	42	6-26	24	7	5	53	10-38
	Pos	232	258	57	2691		400	165	109	1382	
TA100	Neg	94	14	66	152	66-122	102	18	63	164	66-138
	Pos	681	176	213	1767		681	259	186	2793	
TA1535	Neg	11	4	2	31	3-19	13	5	2	36	3-23
	Pos	586	226	16	2509		117	99	23	1060	
TA1537	Neg	7	3	1	19	1-13	9	4	1	23	1-17
	Pos	411	355	32	2921		72	52	10	562	
WP2 *uvr*A	Neg	25	7	7	62	11-39	28	8	10	55	12-44
	Pos	376	123	99	1026		302	102	91	687	

SD=standard deviation; Min=minimum value; Max=maximum value; 95% CL = Mean ±2 SD (but not less than zero); Neg=negative control (including but not limited to deionized water, dimethyl sulfoxide, ethanol and acetone); Pos=positive control

14. APPENDIX II: Study Protocol

BioReliance by SAFC

Protocol

Study Title	**Bacterial Reverse Mutation Assay of AGI-19675**
Study Director	Emily Dakoulas, B.S.
Testing Facility	BioReliance Corporation 9630 Medical Center Drive Rockville, MD 20850
BioReliance Study Number	AE37RY.502ICH.BTL
Sponsor Number	AG120-N-074

1. KEY PERSONNEL

Sponsor Information:

Sponsor	Agios Pharmaceuticals 88 Sidney Street Cambridge, MA 02139
Sponsor Number	AG120-N-074
Sponsor's Authorized Representative	Andrew Olaharski, PhD, DABT 88 Sidney Street Cambridge, MA 02139 Phone:617-649-2035 Email: andrew.olaharski@agios.com

Test Facility Information:

Study Director	Emily Dakoulas, B.S. BioReliance Corporation Phone:301-610-2153 Email: emily.dakoulas@bioreliance.com
Contributing Scientist (Analytical Chemistry Analysis)	Philip Atkins, MChem BioReliance Corporation Phone: 301-610-2114 Email: philip.atkins@bioreliance.com
BioReliance Quality Assurance Representative	Karen Westray, RQAP-GLP BioReliance Corporation Phone: 301-610-2856 Email: karen.westray@bioreliance.com

2. TEST SCHEDULE

Proposed Experimental Initiation Date	08 December 2015
Proposed Experimental Completion Date	04 January 2016
Proposed Report Date	18 January 2016

3. REGULATORY REQUIREMENTS

This study will be performed in compliance with the following Good Laboratory Practices (GLP) regulations.

- US FDA Good Laboratory Practices 21 CFR Part 58

The regulation listed is compatible to non-US regulations, OECD Principles of Good Laboratory Practice (C(97)186/Final); Japanese Ministry of Health, Labor and

Welfare Good Laboratory Practices (Ordinance Nos. 21 and 114, if applicable); Japanese Ministry of Agriculture, Forestry and Fisheries Good Laboratory Practices (No. 11 Nousan-6283); Japanese Ministry of Economy, Trade and Industry Good Laboratory Practices, and allows submission of the report under the Mutual Acceptance of Data (MAD) agreement with applicable OECD member countries.

At a minimum, all work performed at US test site(s) will comply with the US GLP regulations stated above. Non-US sites must follow the GLP regulations governing their site. The regulations that were followed will be indicated on the compliance statement in the final contributing report.

4. QUALITY ASSURANCE

The protocol, any amendments, at least one in-lab phase, the raw data, draft report(s), and final report(s) will be audited by BioReliance Quality Assurance (QA) and a signed QA Statement will be included in the final report.

Test Site Quality Assurance (where applicable)

Test Site QA is responsible for performing an in-lab phase inspection, auditing raw data and final report(s), and providing the inspection results to the Principal Investigator, Study Director, and their respective management (Email Testing Facility Management at RCK-Tox-TFM@bioreliance.com). A signed QA Statement documenting the type of audit performed, the dates it was performed, and the dates in which the audit results were reported to the Study Director, Principal Investigator and their respective management must be submitted by the test site QA.

5. PURPOSE

The purpose of this study is to evaluate the mutagenic potential of the test article by measuring its ability to induce reverse mutations at selected loci of several strains of *Salmonella typhimurium* and at the tryptophan locus of *Escherichia coli* WP2 *uvr*A in the presence and absence of an exogenous metabolic activation system. The assay design is based on the OECD Guideline 471, updated and adopted 21 July 1997 and the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use (2011).

6. TEST ARTICLE INFORMATION

Identification	AGI-19675
Synonym	3-amino-5-fluoropyridine
CAS #	210169-05-4
Storage Conditions	Room Temperature Protect from light Blanketed with Nitrogen
Purity	100% (no correction factor will be used for dose formulations)

Molecular Weight 112.11 g/mol

Characterization of Test Article
Characterization of the Test Article is the responsibility of the Sponsor.

Test Article Reserve Sample
Since the in-life portion of this study is less than four weeks in duration, a reserve sample will not be retained

Characterization of Dose Formulations
Dose formulations will be analyzed by BioReliance using a method validated under BioReliance study number AE37RY.GTCHEM.BTL

Disposition of Test Article and Dose Formulations
All unused Test Article will be returned to the sponsor prior to report finalization unless the test article is used on another study. Residual dose formulations will be discarded after use.

Collection of Dose Formulation Samples
Samples will be collected on the day of preparation as follows. Samples will not be collected for any portion of the assay used to assess only toxicity. The sampling plan will be determined by the final dose formulation mixture (solution or suspension):

Vehicle Sampling	
Number of Samples[A]	Volume
2	0.5 mL

[A] One sample will be used for analysis and the other will serve as the backup

Solution Sampling		
Dose Level	Number of Samples[B]	Volume
High Dose	2	0.5 mL
Low Dose	2	0.5 mL

[B] One sample will be used for analysis and the other will serve as the backup

If necessary, alternate volumes or aliquots may be collected, as requested by Contributing Scientist, Study Director or designee. Submitted samples that are below the validated range will not be analyzed. The lowest sample, within the validated range, will be analyzed instead. All samples collected for analysis or as backups will be held at room temperature, or under the conditions of use for the dosing formulations, until delivered for analysis. Upon receipt, the samples designated for analysis will be maintained at the conditions of receipt until the analysis is performed; however back-up samples may be stored -10 to -30°C. If the analysis is performed on subsequent days, all samples will be stored at -10 to -30°C until required for analysis. After analysis, all samples and backups will be stored at -10 to -30°C. Unused samples will be discarded upon acceptance of the analytical results by the Study Director.

Stability
In the absence of confirmed stability in the vehicle at concentrations bracketing those used within this study, stability of the dose formulations will be confirmed by analyzing the appropriate samples from one set of dose formulations after at least 3 hours storage at conditions that mimic the handling of the dose formulations during dosing. If the samples are not analyzed on the day of dosing, additional stability will be performed to cover the conditions and duration of storage, at a minimum. The concentrations obtained must be 90 to 110% of the original concentrations to be considered stable. Alternatively, the chemistry laboratory may establish stability on independently prepared samples. Stability will also be assessed on the back up samples if they are used owing to a failure of the initial analysis.

Acceptance Criteria
For test article formulation samples that are solutions:

- Samples must be in the concentration target range of 85.0% to 115.0% of nominal and the ratio of the small/large obtained concentrations for the duplicate dilutions of each solution must be > 0.925.

The vehicle control sample must confirm the absence of test article such that the concentration of the test article in the vehicle formulations must be below the Limit of Detection of the analytical method.

In the event that a sample is outside of the acceptable specification range, the Study Director will justify the acceptability of the results or suggest re-analysis of the backup samples or retest the affected portion of the study.

Data Collection and Analysis System
Data will be collected and analyzed using Agilent ChemStation.

Reporting
A draft report that summarizes the methods, analysis, and results carried out by the Analytical Chemistry laboratory will be provided to the Study Director and Sponsor and/or Authorized Representative. The final report will be included in the main study report as an appendix.

7. TEST SYSTEM

The tester strains will include the *S. typhimurium* histidine auxotrophs TA98, TA100, TA1535 and TA1537 as described by Ames *et al.* (1975) and the *E. coli* tester strain WP2 *uvr*A as described by Green and Muriel (1976). The genotypes of strains are as follows:

Histidine Mutation			Tryptophan Mutation	Additional Mutations		
*his*G46	*his*C3076	*his*D3052	*trp*E	LPS	Repair	R-factor
TA1535	TA1537	-	-	*rfa*	Δ*uvr*B	-
TA100	-	TA98	-	*rfa*	Δ*uvr*B	+R

Histidine Mutation			Tryptophan Mutation	Additional Mutations		
*his*G46	*his*C3076	*his*D3052	*trp*E	LPS	Repair	R-factor
-	-	-	WP2 *uvr*A	-	Δ*uvr*A	-

The *S. typhimurium* tester strains were from Dr. Bruce Ames, University of California, Berkeley. The *E. coli* tester strain was from the National Collection of Industrial and Marine Bacteria, Aberdeen, Scotland (United Kingdom). The tester strains may also be obtained from Molecular Toxicology Inc. (Moltox).

8. EXPERIMENTAL DESIGN AND METHODOLOGY

The test system will be exposed to the test article via the plate incorporation methodology originally described by Ames *et al.* (1975) and updated by Maron and Ames (1983). This test system has been shown to detect a wide range of classes of chemical mutagens (McCann *et al.*, 1975; McCann and Ames, 1976).

If the Sponsor is aware of specific metabolic requirements (e.g., azo compounds), this information will be utilized in designing the assay.

Solubility Determination

AGI-19675 has previously been demonstrated to be soluble in DMSO at concentrations up to 100 mg/mL (Bioreliance Study No. AD98CM.502ICHNGLP.BTL).

Preparation of Tester Strain

Each tester strain culture will be inoculated from the appropriate frozen stock, lyophilized pellet(s), or master plate. To ensure that cultures are harvested in late log phase, the length of incubation will be controlled and monitored. Each inoculated flask will be placed in a shaker/incubator programmed to begin shaking at 125 to 175 rpm and incubating at 37±2°C.

All cultures will be harvested by spectrophotometric monitoring of culture turbidity rather than by duration of incubation since overgrowth of cultures can cause loss of sensitivity to some mutagens. Cultures will be removed from incubation at a density of approximately 10^9 cells/mL.

Identification of Test System

Each plate will be identified by the BioReliance study number and a code system to designate at least the treatment condition, dose level, and test phase.

Exogenous Metabolic Activation

Liver Homogenate

Liver homogenate (S9) will be purchased commercially (MolTox; Boone, NC). It is prepared from male Sprague-Dawley rats that have been injected intraperitonealy with Aroclor™ 1254 (200 mg/mL in corn oil), at a dose of 500 mg/kg, 5 days before sacrifice.

Sham Mix
100 mM phosphate buffer at pH 7.4

S9 Mix
S9 mix will be prepared on the day of use as indicated below:

Component	Final Concentration
β-nicotinamide-adenine dinucleotide phosphate	4 mM
Glucose-6-phosphate	5 mM
Potassium chloride	33 mM
Magnesium chloride	8 mM
Phosphate Buffer (pH 7.4)	100 mM
S9 homogenate	10% (v/v)

Controls
No analyses will be performed on the positive control articles or the positive control dose formulations. The neat positive control articles and the vehicles used to prepare the test article and positive control formulations will be characterized by the Certificates of Analysis provided by the Supplier(s). Copies of the Certificates of Analysis will be kept on file at BioReliance.

Vehicle Control
The vehicle for the test article will be used as the vehicle control for each treatment group. For vehicles with no historical control data, an untreated control will be included.

Sterility Controls
At a minimum, the most concentrated test article dilution and the Sham and S9 mixes will be checked for sterility.

Positive Controls
The positive controls that will be plated concurrently with the assay are listed below. Results obtained from these articles will be used to assure responsiveness of the test system but not to provide a standard for comparison with the test article.

Strain	Positive Control	S9	Concentrations (μg/plate)
Salmonella strains	2-aminoanthracene[B]	+	1.0 – 2.0
WP2 *uvr*A	2-aminoanthracene[B]	+	10 – 20
TA98	2-nitrofluorene[B]	–	1.0
TA100, TA1535	sodium azide[A]	–	1.0
TA1537	9-aminoacridine[B]	–	75
WP2 *uvr*A	methyl methanesulfonate[B]	–	1,000

[A]Prepared in water
[B]Prepared in DMSO

Frequency and Route of Administration
The test system will be treated using the plate incorporation method.

Verification of a clear positive response will not be required (OECD Guideline 471). Negative results will not be retested when justification can be provided. Equivocal results will be retested in consultation with the Sponsor using an appropriate modification of the experimental design (e.g., dose levels, activation system or treatment method).

Mutagenicity Assay
TA98, TA100, TA1535, TA1537 and WP2 *uvr*A will be exposed to vehicle alone and at least five concentrations of test article, in triplicate, in both the presence and absence of S9. Unless limited by solubility, the test article will be evaluated at a maximum concentration of 5000 µg/plate. Unless indicated otherwise by the Sponsor, the dose levels will be 5000, 1500, 500, 150, 50 and 15 µg per plate. If limited by solubility in the vehicle, the test article will be evaluated at the highest concentration permissible as a workable suspension. If a retest of the mutagenicity assay is needed, a minimum of five dose levels of test article will be used in the retest. These dose levels will be documented in the raw data. A range-finding assay will not be performed.

Treatment of Test System
Unless specified otherwise, test article dilutions will be prepared immediately prior to use. All test article dosing will be at room temperature under filtered light. One half milliliter (0.5 mL) of S9 mix or Sham mix, 100 µL of tester strain and 50 µL of vehicle, test article dilution or positive control will be added to 2.0 mL of molten selective top agar at 45±2°C. When necessary, aliquots of other than 50 µL of test article or vehicle or positive control will be plated. When plating untreated controls, the addition of test article, vehicle and positive control will be omitted. The mixture will be vortex mixed and overlaid onto the surface of a minimal bottom agar plate. After the overlay has solidified, the plates will be inverted and incubated for 48 to 72 hours at 37±2°C. Plates that are not counted immediately following the incubation period will be stored at 2-8°C.

Scoring
The condition of the bacterial background lawn will be evaluated for evidence of test article toxicity and precipitate. Evidence of toxicity will be scored relative to the vehicle control plate and recorded along with the revertant count for that plate. Toxicity will be evaluated as a decrease in the number of revertant colonies per plate and/or a thinning or disappearance of the bacterial background lawn. Precipitation will be evaluated after the incubation period by visual examination without magnification. As appropriate, colonies will be enumerated either by hand or by machine.

Tester Strain Verification
On the day of use in the mutagenicity assay, all tester strain cultures will be checked for the appropriate genetic markers.

9. CRITERIA FOR DETERMINATION OF A VALID TEST

The following criteria must be met for the mutagenicity assay to be considered valid. If one or more of these parameters are not acceptable, the affected condition(s) will be retested.

Tester Strain Integrity

To demonstrate the presence of the *rfa* mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to crystal violet. To demonstrate the presence of the *uvr*B mutation, all *S. typhimurium* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the *uvr*A mutation, all *E. coli* tester strain cultures must exhibit sensitivity to ultraviolet light. To demonstrate the presence of the pKM101 plasmid R-factor, tester strain cultures of TA98 and TA100 must exhibit resistance to ampicillin.

Vehicle Control Values

Based on historical control data, all tester strain cultures must exhibit characteristic numbers of spontaneous revertants per plate in the vehicle controls. The mean revertants per plate must be within the following ranges (inclusive): TA98, 10 - 50; TA100, 80 - 240; TA1535, 5 - 45; TA1537, 3 - 21; WP2 *uvr*A, 10 - 60. Untreated controls, when part of the design, must also be within the ranges cited above.

Tester Strain Titers

To ensure that appropriate numbers of bacteria are plated, all tester strain culture titers must be equal to or greater than 0.3×10^9 cells per milliliter.

Positive Control Values

Each mean positive control value must exhibit at least a 3.0-fold increase over the respective mean vehicle control value for each tester strain.

Toxicity

A minimum of three non-toxic dose levels will be required to evaluate assay data. A dose level is considered toxic if it causes a >50% reduction in the mean number of revertants per plate relative to the mean vehicle control value (this reduction must be accompanied by an abrupt dose-dependent drop in the revertant count) or a reduction in the background lawn. In the event that less than three non-toxic dose levels are achieved, the affected portion of the assay will be repeated with an appropriate change in dose levels.

10. EVALUATION OF TEST RESULTS

For the test article to be evaluated positive, it must cause a dose-related increase in the mean revertants per plate of at least one tester strain over a minimum of two increasing concentrations of test article as specified below:

Strains TA1535 and TA1537

Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 3.0-times the mean vehicle control value.

Strains TA98, TA100 and WP2 *uvr*A
Data sets will be judged positive if the increase in mean revertants at the peak of the dose response is equal to or greater than 2.0-times the mean vehicle control value.

An equivocal response is a biologically relevant increase in a revertant count that partially meets the criteria for evaluation as positive. This could be a dose-responsive increase that does not achieve the respective threshold cited above or a non-dose responsive increase that is equal to or greater than the respective threshold cited. A response will be evaluated as negative, if it is neither positive nor equivocal.

11. ELECTRONIC DATA COLLECTION SYSTEMS

Electronic systems used for the collection or analysis of data may include but not be limited to the following (version numbers are maintained in the system documentation):

System	Purpose
LIMS Labware System	Test Article Tracking
Excel (Microsoft Corporation)	Calculations
Sorcerer Colony Counter and Ames Study Manager (Perceptive Instruments)	Data Collection/Table Creation
Kaye Lab Watch Monitoring system (Kaye GE)	Environmental Monitoring
BRIQS	Deviation and audit reporting

12. REPORT

A report of the results of this study will accurately describe all methods used for generation and analysis of the data. The report will include, but not limited to information about the following:

- Test article
- Vehicle
- Strains
- Test conditions
- Results
- Discussion of results
- Conclusion
- Appendices: Historical Control Data (vehicle and positive controls with ranges, means and standard deviations), copy of protocol and any amendment, contributing reports (if applicable), and, if provided by the Sponsor, copies of the analyses that characterized the test article, its stability and the stability and strength of the dosing preparations.
- Statement of Compliance
- Quality Assurance Statement
- CTD Tables (unless otherwise requested)

The report will be issued as a QA-audited draft. After receipt of the Sponsor's comments a final report will be issued. A GLP Compliance Statement signed by the Study Director will also be included in the final report and will note any exceptions if

the characterization of the test article and/or the characterization of the dose formulations are not performed or provided. Four months after issuance of the draft report, if no communication regarding the study is received from the Sponsor or designated representative, the draft report may be issued as a final report. If all supporting documents have not been provided, the report will be written based on those that are provided.

13. RECORDS AND ARCHIVES

All raw data, the protocol, pertinent study email correspondence, and all reports for procedures performed at BioReliance will be maintained in the archives at BioReliance, Rockville, MD for at least five years, unless otherwise requested by the Sponsor. At that time, the Sponsor will be contacted for a decision as to the final disposition of the materials. All study materials will first be copied and the copy will be retained by the BioReliance archives in accordance with the applicable SOPs. Slides and/or specimens (as applicable) will be archived at EPL Archives and indexed as such in the BioReliance archive database. The raw data, reports, and other documents generated at locations other than BioReliance will be archived by the test site.

14. REFERENCES

Ames, B.N., McCann, J. and Yamasaki, E. (1975). Methods for detecting carcinogens and mutagens with the *Salmonella*/mammalian-microsome mutagenicity test. Mutation Research 31:347-364.

International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use. ICH Harmonised Tripartite Guideline. S2(R1) document recommended for adoption at step 4 of the ICH process on 9 November 2011. Adopted at Step 5 in Europe by CHMP December 2011 (issued as EMA/CHMP/ICH/126642/2008). Adopted at Step 5 in US by FDA on June 7, 2012 (issued as 77 FR 33748 pages 33748-33749). Adopted in Japan at Step 5 September 20, 2012 (issued as PFSB/ELD Notification No. 0920-2).

Green, M.H.L., and Muriel, W.J. (1976). Mutagen testing using trp^+ reversion in *Escherichia coli*. Mutation Research 38:3-32.

McCann, J. and Ames, B.N. (1976). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals: discussion. Proc. Natl. Acad. Sci. USA 73:950-954.

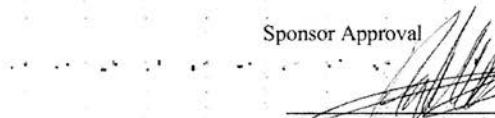
McCann, J., Choi, E., Yamasaki, E. and Ames, B.N. (1975). Detection of carcinogens as mutagens in the *Salmonella*/microsome test: assay of 300 chemicals. Proc. Natl. Acad. Sci. USA 72:5135-5139.

Maron, D.M. and Ames, B.N. (1983). Revised Methods for the *Salmonella* Mutagenicity Test. Mutation Research 113:173-215.

OECD Guideline 471 (Genetic Toxicology: Bacterial Reverse Mutation Test), Ninth Addendum to the OECD Guidelines for the Testing of Chemicals, adopted July 21, 1997.

APPROVALS

Sponsor Approval



Sponsor Representative

24-Nov-2015

Date

Study Director and Test Facility Management Approvals

BioReliance Study Director	04 DEC 2015 Date
BioReliance Study Management	04 DEC 2015 Date

15. APPENDIX III: Testing Report of Raw Material

Testing Report of Raw Material

Product Name	3-AMINO-5-FLUOROPYRIDINE		
Batch Number	15051502	Record Number	RW-751-03
Re-testing Date	05/14/2016	Batch Size	15KG*5
Material Code	186498A	QS Number	QS-RM-1864.02

Testing Items	Testing Result	Quality Standard
Appearance	Pale yellow crystals	Pale yellow crystals
Identification（NMR）	Conforms	Conforms to structure
Purity(HPLC)	100%	NLT 98%
Use test	Pass	Pass

Conclusion	Pass		
Version/Change	01/Original issuance		
Issued by Date	06/11/2015	Reviewed by Date	06/12/2015

16. APPENDIX IV: Dose Formulation Analysis and Stability

FINAL ANALYTICAL REPORT

Study Title

Bacterial Reverse Mutation Assay of AGI-19675

Report Title

Determination of AGI-19675 in DMSO Dosing Formulations

Test Article

AGI-19675

Author

Philip Atkins, MChem

Final Analytical Report Date

09 March 2016

Analytical Laboratory

BioReliance Corporation
9610 Medical Center Drive
Rockville, MD 20850

BioReliance Study Number

AE37RY.502ICH.BTL

Sponsor

Agios Pharmaceuticals
88 Sidney Street
Cambridge, MA 02139

Sponsor Number

AG120-N-074

1. COMPLIANCE STATEMENT

This portion of the study, AE37RY.502ICH.BTL, was conducted in compliance with the following regulations: US FDA Good Laboratory Practice Regulations as published in 21 CFR Part 58. This regulation is compatible to non-US regulations, OECD Principles of Good Laboratory Practice (C(97)186/Final); Japanese Ministry of Health, Labor and Welfare Good Laboratory Practices (Ordinance Nos. 21 and 114, if applicable); Japanese Ministry of Agriculture, Forestry and Fisheries Good Laboratory Practices (No. 11 Nousan-6283); Japanese Ministry of Economy, Trade and Industry Good Laboratory Practices, and allows submission of the report under the Mutual Acceptance of Data (MAD) agreement with applicable OECD member countries.

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09 Mar 11
Date

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3. ANALYTICAL CONDITIONS

The analysis of the test article formulations, AGI-19675 in DMSO, for study AE37RY.502ICH.BTL, was performed by high performance liquid chromatography (HPLC) using a method validated under BioReliance Study Number AE37RY.GTCHEM.BTL. The formulations were also analyzed in accordance with BioReliance SOPs "Dose Formulation Analysis" and "Dose Formulation Stability Determination". The analytical conditions used in this study are summarized in Table 1. The solvent standards were prepared per Table 2.

Table 1: Analytical Conditions

Instrument:	Agilent 1100/1200 HPLC
Detector:	UV @ 210 nm, bandwidth 6 nm, Reference off
Software:	Agilent ChemStation with Open Lab CDS
(MPA) Mobile Phase A:	2.5 mM Ammonium Phosphate Dibasic and 2.5 mM Ammonium Phosphate Monobasic Buffer in Deionized Water
(MPB) Mobile Phase B:	Acetonitrile
Diluent:	Acetonitrile: Deionized Water, (70:30, v/v)
Vehicle:	DMSO
(SS) Stock Solution:	100 µg/mL Test Article in Diluent
TA Correction Factor:	1.00
Column:	Waters Xbridge C8, 150 mm x 3 mm, 3.5 µm particles with a pre-column filter
Column Temperature:	40°C
Autosampler Temperature:	Ambient
Injection Volume:	5 µL
Flow Rate:	0.638 mL/min
Retention Time:	~6.084 minutes
Injections / Sample:	1
Run Time:	25 minutes
Calibration Curve:	y = Ax + B (not weighted)
Elution mode:	Gradient (see below)

Time (mins)	0	6	11	16	20	20.1	25
% MPA	98	98	55	15	15	98	98
% MPB	2	2	45	85	85	2	2

Table 2: Preparation of the Solvent Standard Solutions

Standard ID	SS (mL)	Final Volume with Diluent (mL)	Final TA Concentration (µg/mL)
S-0	0	10	0
S-1	1	10	10
S-2	2	10	20
S-3	3	10	30
S-4	4	10	40
S-5	5	10	50
S-6	6	10	60

4. DOSING FORMULATION ANALYSIS

Dosing formulations of AGI-19675 in DMSO were collected on the day of preparation and analyzed by HPLC to assess accuracy of the preparation (per Table 3). A sample of vehicle dosing solution was also analyzed to verify that it did not contain test article.

The dosing formulations were diluted to bring the test article concentration to a suitable level within the calibration range. The concentration of AGI-19675 was calculated by reference to the solvent standard solutions prepared (per Table 2) and analyzed concurrently with the dosing formulations. All solvent standard curves met the acceptance criteria (Table 4).

The formulation analysis was started on the day of formulation preparation (17 December 2015), but was stopped due to over pressure in the instrument and was restarted on 18 December 2015. However, it was determined that the mobile phases had been switched, and the analysis was restarted again on 21 December 2015.

All dosing formulations analyzed met the acceptance criteria of 85.0 – 115.0% of target concentration and the ratio of the small/large (S/L) obtained concentrations for the duplicate dilutions of each solution was found to be > 0.925 (Table 5). No test article was detected in the vehicle control (VC) samples.

Table 3: Summary of the Dosing Formulations

Experiment No./Phase	Date of Preparation	Date of Analysis (Start/End)	Concentration (mg/mL)	Homogeneity Testing (Y) Yes or (N) No
B1	17 December 2015	17 December 2015 / 21 December 2015	0	N
			0.300	N
			100	N

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Table 4: Solvent Standards for the B1 Dosing Formulation and Stability Analyses

Item	Value	Acceptance Criterion
Slope	28.41	NA
Intercept	-14.89	NA
Correlation Coefficient	0.99992	≥0.99
Recovery % (Range)	98.6 – 101.3	90-110

Table 5: B1 Dosing Formulation Analysis

Formulation ID	Conc. of Form. (mg/mL)	Conc. of Sample (µg/mL)	TA Peak Area	Mean Peak Area	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	S/L
VC (0)	0	0	ND	ND	NA	NA		NA	NA
0.300 A	0.300	30	849.9						
0.300 B	0.300	30	846.9	848.4	30.39	101.3	10	0.304	0.996
100 A	100	40	1184						
100 B	100	40	1211	1198	42.69	106.7	2500	107	0.978

5. STABILITY OF AGI-19675 IN DMSO DOSING FORMULATIONS

Stability of the dosing formulations was determined by storing the B1 dosing formulations at room temperature for 3 hours and reanalyzing as described above. The acceptance criterion of 90-110% of the concentration determined at T=0 was met (Table 6).

AGI-19675 in DMSO, at concentrations of 0.304 and 107 mg/mL, was stable at room temperature for at least 3 hours.

Table 6: Stability T=3 Hours Analysis

Formulation ID	Conc. of Form.[1] (mg/mL)	Conc. of Sample[1] (µg/mL)	TA Peak Area	Mean Peak Area	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	S/L
0.300 A T=3	0.304	30.39	840.9						
0.300 B T=3	0.304	30.39	810.8	825.9	29.59	97.4	10.00	0.296	0.964
100 A T=3	107	42.69	1171						
100 B T=3	107	42.69	1166	1169	41.67	97.6	2506	104	0.996

[1]Concentration determined at T=0 (Table 5)

Owing to longer dosing time for the Ames study, additional stability analysis was performed by preparing independent formulations in the Analytical Chemistry laboratory at concentration of 0.300 mg/mL and 100 mg/mL. The formulations were analyzed immediately and then stored at room temperature for 4 hours and reanalyzed as described above. The results met the acceptance criterion of 90-110% of the concentration determined at T=0 (Table 9).

AGI-19675 in DMSO, at concentrations of 0.286 and 97.3 mg/mL, was stable at room temperature for at least 4 hours.

Table 7: Solvent Standards for Stability Analyses

Item	Value	Acceptance Criterion
Slope	31.67	NA
Intercept	3.973	NA
Correlation Coefficient	0.99997	≥0.99
Recovery % (Range)	99.4-100.7	90-110

Table 8: Stability T=0 hours Analysis

Formulation ID	Conc. of Form. (mg/mL)	Conc. of Sample (µg/mL)	TA Peak Area	Mean Peak Area	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	S/L
0.300 A	0.300	30	913.6	910.0	28.61	95.4	10	0.286	0.992
0.300 B	0.300	30	906.3						
100 A	100	40	1232	1236	38.90	97.3	2500	97.3	0.994
100 B	100	40	1240						

Table 9: Stability T=4 Hours Analysis

Formulation ID	Conc. of Form.[1] (mg/mL)	Conc. of Sample[1] (µg/mL)	TA Peak Area	Mean Peak Area	Mean Conc. (µg/mL)	Target (%)	x Dilution Factor	Final Mean Conc. (mg/mL)	S/L
0.300 A T=4	0.286	28.61	904.2	921.5	28.97	101.3	9.997	0.290	0.963
0.300 B T=4	0.286	28.61	938.7						
100 A T=4	97.3	38.90	1265	1276	40.17	103.3	2501	100	0.983
100 B T=4	97.3	38.90	1287						

[1]Concentration determined at T=0 (Table 8)

6. CONCLUSION

The results of the analysis indicate that the actual mean concentrations of the analyzed formulation samples (0.300 and 100 mg/mL) were 101.3 and 106.7% of target, respectively, with S/L ratios of > 0.925. This indicates that the formulations were accurately prepared. No test article was detected in the vehicle control sample. Additionally, AGI-19675 in DMSO, at concentrations of 0.304 and 107 mg/mL, was stable at room temperature for at least 3 hours. In addition, AGI-19675 in DMSO, at concentrations of 0.286 and 97.3 mg/mL, was stable at room temperature for at least 4 hours.

7. DEVIATIONS

No deviations from the protocol or assay-method SOPs occurred during the conduct of this study.

8. ABBREVIATIONS AND CALCULATIONS

Calc. = Calculated
Conc. = Concentration
DMSO = Dimethyl sulfoxide
Form = Formulation
HPLC = High Performance Liquid Chromatography
M = Matrix
NA = Not Applicable
ND =Not Detected
S/L = Small/Large
T = Time
TA = Test Article
UV = Ultra-Violet
VC = Vehicle Control

The following formulas were used for the calculations:

1. Mean Concentration (µg/ mL) = (Mean Peak Area - Intercept) / Slope

   Intercept and slope calculated using linear regression analysis

2. Final Mean Conc. (mg/mL) = (Mean Conc. (µg/ mL) x Dilution Factor) / 1000

3. % of Target, % Recovery = $\frac{\text{Mean Calculated Concentration}}{\text{Concentration of Sample}} \times 100$

4. S/L = $\frac{\text{Small TA Peak Area}}{\text{Large TA Peak Area}}$

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17. APPENDIX V: Common Technical Document Tables

2.6.7.8 Genotoxicity: In Vitro

Report Title: Bacterial Reverse Mutation Assay of AGI-19675

Test Article: AGI-19675

Test for Induction of: Reverse mutation in bacterial cells

No. of Independent Assays: 1

Study No.: AE37RY.502ICH.BTL

Species/Strain: *S. typhimurium* TA98, TA100, TA1535, TA1537; *E. coli* WP2 *uvr*A

No. of Replicate Cultures: 3

No. Cells Analyzed/Culture: 1.7 to 3.6 x 10^8 cells per plate

Metabolizing System: Aroclor-induced rat liver S9

GLP Compliance: Yes

Vehicle for Test Article: DMSO

Vehicle for Positive Controls: DMSO, except sterile water for sodium azide

Treatment: Plate incorporation

Date of Treatment: 17 December 2015

Cytotoxic Effects: None

Genotoxic Effects: None

Metabolic Activation	Test Article	Dose Level (µg/plate)	Revertant Colony Counts (Mean ±SD)				
			TA98	TA100	TA1535	TA1537	WP2uvrA
Without Activation	DMSO	50.0 µL/plate	15 ± 4	103 ± 14	12 ± 3	6 ± 1	27 ± 5
	AGI-19675	15.0	14 ± 2	96 ± 5	11 ± 2	6 ± 2	21 ± 4
		50.0	13 ± 2	95 ± 15	15 ± 3	7 ± 3	21 ± 4
		150	15 ± 2	109 ± 10	14 ± 2	5 ± 3	23 ± 5
		500	14 ± 2	88 ± 6	11 ± 3	6 ± 1	24 ± 2
		1500	15 ± 3	94 ± 5	14 ± 3	5 ± 1	27 ± 8
		5000	13 ± 2	97 ± 9	9 ± 1	5 ± 2	22 ± 5
	2NF	1.0	133 ± 29				
	SA	1.0		564 ± 54	711 ± 33		
	9AAD	75				362 ± 43	
	MMS	1000					375 ± 58
With Activation	DMSO	50.0 µL/plate	21 ± 4	129 ± 9	13 ± 3	7 ± 2	22 ± 5
	AGI-19675	15.0	19 ± 4	115 ± 12	12 ± 1	7 ± 1	26 ± 5
		50.0	20 ± 5	126 ± 5	12 ± 4	6 ± 2	18 ± 4
		150	20 ± 9	121 ± 4	13 ± 3	10 ± 3	21 ± 4
		500	24 ± 3	103 ± 16	11 ± 2	10 ± 4	24 ± 3
		1500	23 ± 4	106 ± 20	8 ± 2	5 ± 1	26 ± 5
		5000	14 ± 2	114 ± 2	11 ± 6	6 ± 3	28 ± 8
	2AA	1.0	199 ± 13		818 ± 25		
	2AA	2.0		637 ± 75		461 ± 79	
	2AA	15					480 ± 20

Key to Positive Controls

SA	sodium azide
2AA	2-aminoanthracene
9AAD	9-Aminoacridine
2NF	2-nitrofluorene
MMS	methyl methanesulfonate