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TITLE: A First-in-Human, Phase I Clinical Trial of Mitochondrial-Targeted Hsp90 Inhibitor, Gamitrinib, in Advanced and Metastatic Prostate Cancer

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14. ABSTRACT

Research supported by the present application is designed to conduct a first-in-human, phase I clinical trial of the novel, mitochondrial-targeted small molecule Hsp90 inhibitor, Gamitrinib in patients with advanced cancer, including advanced, castration-resistant and metastatic prostate cancer. The clinical trial will be conducted at the Phase I Developmental Therapeutics Program of Fox Chase Cancer Center under the leadership of Anthony Olszanski, M.D., Director of the Program. These studies will be complemented by analysis of pharmacodynamics and biochemical characterization of target engagement of Gamitrinib therapy in an expansion cohort at maximal tolerated dose (MTD) in patients with advanced prostate cancer. Accomplishments obtained during the last reporting period have significantly advanced the fulfillment of the stated specific aims. Accordingly, a new formulation development of Gamitrinib has been successfully completed to include an innovative step of microfluidization designed to reduce particle size of the nanosuspension, and thus enable terminal sterilization for use in humans. Validated methods and three-month stability testing have also been established to support the large-scale manufacturing of GMP-grade Gamitrinib for clinical use, and protocols for drug substance identification and validation have been finalized. An Institutional Review Board (IRB) approval of the Gamitrinib clinical trial has been secured as well as Department of Defense (DoD) HRPO concurrence on human subject designation. Altogether, the program is on-track for submission of full Investigational New Drug (IND) application to the US Food and Drug Administration by December 2019 with patient enrollment scheduled to begin by March 2020. Although this is a single-site clinical trial, we expect a rapid accrual rate consistent with the timeline approved in the original Statement of Work. These studies will bring to the clinic a uniquely innovative therapeutic approach in the management of patients with advanced cancers, including prostate cancer.

15. SUBJECT TERMS
Mitochondria, cancer therapy, molecular chaperones, Heat Shock Protein-90, advanced and metastatic prostate cancer, tumor metabolism, first-in-human trial, pharmacodynamics

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1. INTRODUCTION:

Research supported by the present application is designed to conduct a first-in-human, phase I clinical trial of the novel, mitochondrial-targeted small molecule Hsp90 inhibitor, Gamitrinib in patients with advanced cancer, including advanced, castration-resistant and metastatic prostate cancer. The clinical trial will be conducted at the Phase I Developmental Therapeutics Program of Fox Chase Cancer Center under the leadership of Anthony Olszanski, M.D., Director of the Program. These studies will be complemented by analysis of pharmacodynamics and biochemical characterization of target engagement of Gamitrinib therapy in an expansion cohort at maximal tolerated dose (MTD) in patients with advanced prostate cancer.

2. KEYWORDS:

Mitochondria, cancer therapy, molecular chaperones, Heat Shock Protein-90, advanced and metastatic prostate cancer, tumor metabolism, first-in-human trial, pharmacodynamics

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Specific aim 1. A first-in-human, phase I clinical trial of Gamitrinib in patients with advanced and metastatic prostate cancer. This will be an open-label, phase I clinical trial of weekly IV administration of Gamitrinib to (i) identify the maximum tolerated dose (MTD); (ii) determine the dose-limiting toxicities (DLT); and (iii) characterize the pharmacokinetics profile. The clinical protocol uses an accelerated dose titration scheme, a 3+3 dose escalation phase and a twelve-patient expansion cohort at MTD. The trial will be conducted at the Phase I Developmental Therapeutics Program at Fox Chase Cancer Center under the leadership of Anthony J. Olszanski, M.D., Director of the Program.

Specific aim 2. Characterize the pharmacodynamics profile of Gamitrinib. For exploratory pharmacodynamics studies, paired pre- and post-treatment prostate cancer biopsies and peripheral blood mononuclear cells harvested from the patient expansion cohort at MTD will be examined for a signature of “cellular starvation” as surrogate biomarker of Gamitrinib target inhibition, in vivo. This will involve global metabolomics profiling of 301 biochemicals intercalated in multiple mitochondrial bioenergetics pathways, evaluation of differential AMPK phosphorylation, induction of autophagy, modulation of a mitochondrial-Endoplasmic Reticulum (ER) unfolded protein response and suppression of mTOR signaling, by immunohistochemistry and quantitative Western blotting.

Specific Aim 1: A first-in-human, phase I clinical trial of Gamitrinib in patients with advanced and metastatic prostate cancer	**Timeline**	**Site 1**	**Site 2**
Major Task 1	Months		
Submission to Sponsor's IRB of documentation of research proposal, consent form, and clinical trial advertising material	1	Dr. Altieri	N/A
Submission of Gamitrinib Human Subject Research Protocol to USAMRMC ORP	1	Dr. Altieri	N/A
Sponsor's IRB approval of Gamitrinib clinical protocol	1	Sponsor's IRB	N/A
Manufacturing of GMP-grade Gamitrinib including microfluidization of API emulsion for I.V. injection	2	Dr. Altieri (contract with Axia Pharmaceuticals)	N/A
USAMRMC ORP HRPO Approval	2	Sponsor's IRB	N/A
Full Investigational New Drug (IND) submission to US Food and Drug Administration	2	Dr. Altieri	N/A
FDA approval of IND submission	3	Sponsor's IRB	N/A
Completed chemical synthesis, quality control CMC validation and shipment of 250 g of GMP-grade Gamitrinib	3	Dr. Altieri (contract with Arcinova, Inc.)	N/A
Milestone(s) Achieved: Enrollment of the first subject in Gamitrinib phase I clinical trial at Developmental Therapeutics Program, Fox Chase Cancer Center	4	N/A	Dr. Olszanski
Major Task 2			
Enrollment of 25% of projected recruitment (6 patients)	4 – 6	N/A	Dr. Olszanski
Enrollment of 50% of projected recruitment (12 patients)	7 – 9	N/A	Dr. Olszanski
Enrollment of 75% of projected recruitment (19 patients	10 – 12	N/A	Dr. Olszanski
Enrollment of 100% of projected recruitment (25 patients)	13 – 15	N/A	Dr. Olszanski

Completion of dose escalation, PK profile and exploratory pharmacodynamics in a 12-patient expansion cohort at MTD	16 – 18	N/A	Dr. Olszanski
Milestone Achieved: Completed patient accrual of Gamitrinib clinical trial	19	N/A	Dr. Olszanski
Specific Aim 2: Characterize the pharmacodynamics profile of Gamitrinib			
Major Task 3			
High-throughput metabolomics profiling of pre- and post-treatment biopsies harvested from the 12-patient expansion cohort	19 – 21	Dr. Altieri	N/A
Immunohistochemical staining of a cellular starvation signature in pre- and post-treatment biopsies from the 12-patient expansion cohort	22	Dr. Altieri	N/A
Western blot characterization of a cellular starvation signature in pre- and post-treatment peripheral blood mononuclear cells from the 12-patient expansion cohort	22	Dr. Altieri	N/A
Completion of primary and secondary endpoint data analysis	22 – 24	Dr. Altieri	Dr. Olszanski
Completion of final report of the primary outcome	22 – 24	Dr. Altieri	Dr. Olszanski
Milestone Achieved: Completion of Gamitrinib pharmacodynamics studies and primary and secondary endpoints of data analysis	24	Dr. Altieri	Dr. Olszanski

What was accomplished under these goals?

Major Activities

Specific Aim 1 – Major Task 1. Complete chemical synthesis and validation of clinical grade Gamitrinib (Drug Substance). The stepwise, combinatorial chemical synthesis of Gamitrinib containing the Hsp90 inhibitor 17-AAG linked to the mitochondrial-targeting carrier, triphenylphosphonium via an hexylamine linker was optimized during the performance period of the present award and is shown in Figure 1.

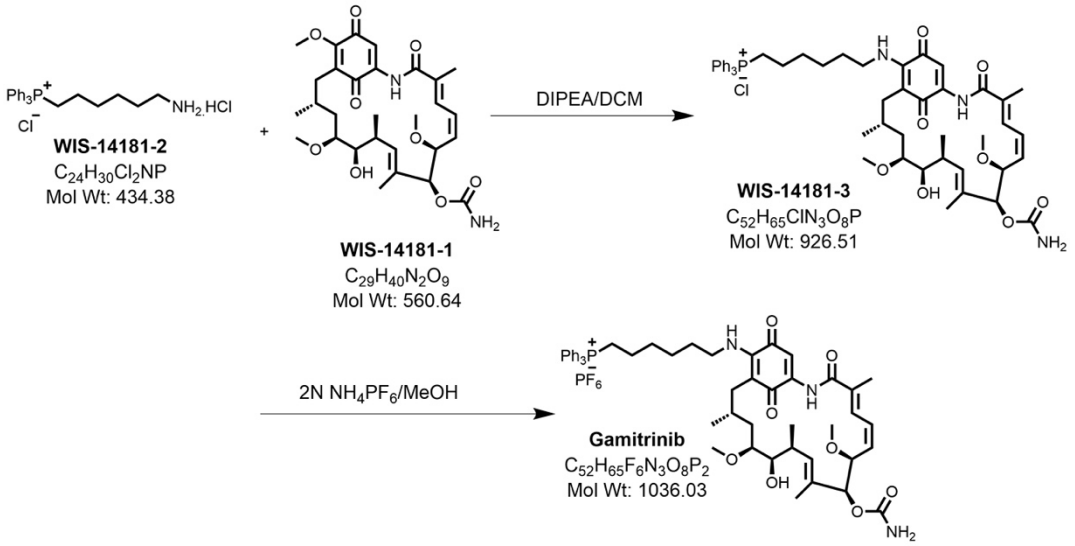


Figure 1. Stepwise chemical synthesis of Gamitrinib linking the Hsp90 ATPase inhibitor 17-AAG to the mitochondrial import carrier, triphenylphosphonium via a hexylamine linker.

Clinical-grade (GMP compliant) Gamitrinib synthesized as described in Figure 2 has the chemical formula $C_{52}H_{65}F_6N_3O_8P_2$ (>99.5% purity by UPLC), is purple solid (TM.795) and crystalline by X-ray powder diffraction, with a molecular weight of 1036.03. 500 MHz ^{1}H NMR spectrum (DMSO-*d6*), 125 MHz ^{13}C NMR spectrum (DMSO-*d6*) and 282 MHz ^{19}F NMR spectrum (DMSO-*d6*) are all consistent with structure. The water content is 0.7% (Karl Fischer analysis) and the residual solvent concentrations (methanol, DCM, MTBE and DIPEA) are all below limit of quantification (BLOQ, <3000, <600, <5000 and <3000 ppm, respectively).

Specific Aim 1 – Major Task 1. Manufacturing of GMP grade Gamitrinib (Drug Product). A sequential three-step process was optimized during the performance period of the award to prepare Good Laboratory Practice (GLP) working solutions of Gamitrinib (5 mg/mL) for preclinical studies. The formulation workflow is as follows: Step 1 – Solubilization of Gamitrinib powder in DMSO (2.5%); Step 2 – Dilution in 1.25% (w/v) Polysorbate 80, 0.31% (w/v) Lecithin (Lipoid S100) and 12.5% (w/v) Sucrose (10%) in sterile water for injection; Step 3 – Dilution in 5% dextrose (87.5%). Therefore, the final Gamitrinib formulation is ~ 5 mg/mL Gamitrinib, 2.5% DMSO, 0.125% Polysorbate 80, 0.031% Lecithin, 1.25% Sucrose and 4.375% Dextrose.

For Good Manufacturing Practice (GMP) studies, a Gamitrinib Injectable Suspension (GIS) is prepared by microfluidization. Gamitrinib stock solutions prepared as above are passed through a Dyhydromatics microfluidizer with rate of flow set at low, medium, and high. At the end of GIS processing, microfluidization is continued at reduced pressure (~2000 psi) for 1-2 min. The parameters for GIS microfluidization as are follows: ratio of organic to aqueous phase (DMSO:aqueous vehicle 1:40 v/v); filter membrane materials (PTFE for DMSO, cellulose acetate for aqueous vehicle); microfluidizer pressure during mixing organic and aqueous phase (28,000 psi); post-mixing pressure in microfluidizer (2000 psi); temperature inside interaction chamber (0 to -10ºC before initiating microfluidization). The final GIS after microfluidization is 4.86 mg/mL, with average particle size of 154 nm, D(0.9) size of 229 nm, pH 6.0. The workflow for GIS manufacturing established during the performance period of the award is shown in Figure 2.

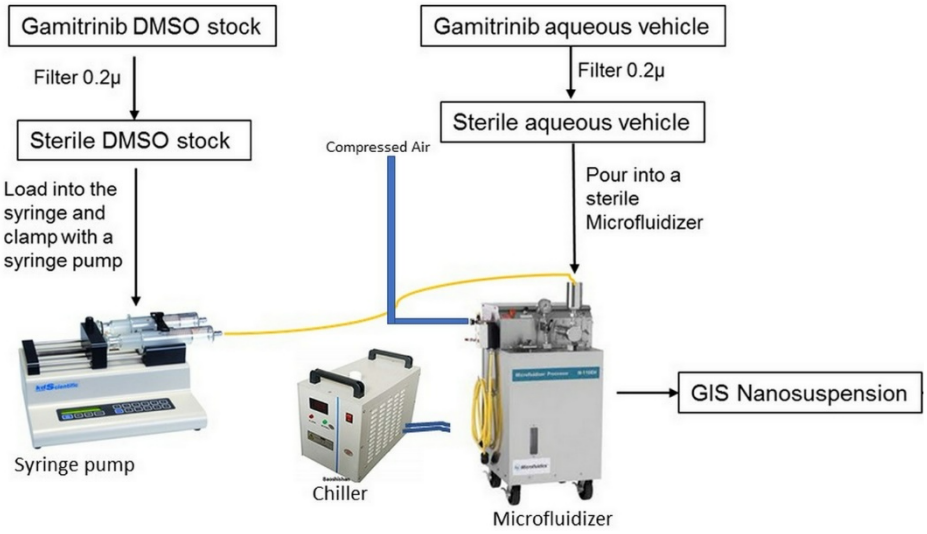


Figure 2. Workflow of Gamitrinib drug product with preparation of a GMP Gamitrinib Injectable Suspension (GIS, particle size, <200 nm) by microfluidization.

When stored frozen at -20ºC, the GIS shows no significant changes in stability or particle size distribution upon analysis at 1, 2, 4, 8, 12 and 24 weeks after manufacturing (Table 1).

	Time of storage (2 ml GIS in 4 ml sterile glass vials at -20°C						
	0	1 wk	2 wk	4 wk	8 wk	12 wk	24 wk
Appearance	Purple susp	Purple susp	Purple susp	Purple susp	Purple susp	Purple susp	Purple susp
Particle size (nm)	200	198	204	203	205	203	212
F/T particle size (nm)		197	209	207	216	208	225
Assay (mg/ml)	4.41	4.80	4.57	4.43	4.47	4.47	4.61
Recovery (% over T0)	100	109	103	100	101	101	104

Table 1. Stability and particle size distribution of Gamitrinib Injectable Suspension (GIS) throughout 24 weeks (wk) of storage.

Specific Aim 1 – Major Task 1. Sponsor's IRB approval of first-in-human, phase I clinical trial of Gamitrinib in patients with advanced cancer.

FOX CHASE CANCER CENTER
TEMPLE HEALTH

Institutional Review Board
Federal Wide Assurance #00003846

APPROVAL OF PROTOCOL – NEW STUDY SUBMISSION
July 27, 2021

Anthony Olszanski, MD, RPh
Office of Clinical Research

On 7/27/2021, the convened IRB reviewed the following protocol:

Type of Review:	Initial Study
Title:	PH-139: A Phase I Safety and Pharmacokinetic Study of Gamitrinib Administered Intravenously to Patients with Advanced Cancer
Investigator:	Anthony Olszanski, MD, RPh
IRB ID:	21-1045
Funding:	National Institutes of Health
Funding Source ID:	1 R01 CA225913-01A1
Grant Office ID:	Wistar-25151-01-359;
IND, IDE or HDE:	IND #132453
Documents Reviewed:	* 21-1045 Initial Application * 18-1053Change of Sponsor Approval Letter.pdf * 21-1045_HIPAA_2021-07-27 * 21-1045_Protocol_2021-07-14.docx * 21-1045_Treatment Consent_2021-07-27 * FDA MAY PROCEED LETTER 4-19-2021.pdf * Gamitrinib Investigators Brochure - FINAL.docx * NIH-EApplication_CA225913_01A1.pdf

Initial Approval.

Upon review, the IRB determined the submission meets the criteria for the approval of research outlined in 45 CFR 46.111 and 21 CFR 56.111.

The IRB approved the protocol from **7/27/2021 to 7/26/2022 inclusive**. Before 7/26/2022 or within 45 days of final study closure, whichever is earlier, you should submit a completed Continuing Review Progress Report and required attachments to request continuing approval or final study closure (termination).

If continuing review approval is not granted before the **expiration date of 7/26/2022**, approval of this study expires on that date.

Specific Aim 1 – Major Task 1. HRPO approval of Gamitrinib clinical protocol.

From: Nancy Englar
To: Dario Altieri
Cc: Odam, Kimberly L CIV USARMY MEDCOM USAMRMC (US); Bennett, Jodi H CIV USARMY MEDCOM USAMRMC (US); Frederick, Margaret M CTR USARMY MEDCOM USAMRMC (USA); Englar, Nancy E CIV USARMY MEDCOM USAMRMC (USA); Mishra, Nrusingha CIV USARMY MEDCOM CDMRP (USA); Shankle, Jennifer E CIV USARMY MEDCOM USAMRAA (US)
Subject: E00364.1a - HRPO Concurrence Memorandum (Proposal Number PC170275, Award Number W81XWH-18-1-0334)
Date: Friday, May 31, 2019 10:29:23 AM

SUBJECT: HRPO Concurrence With the Determination of Research Not Involving Human Subjects for the Protocol, "A Phase I Safety and Pharmacokinetic Study of Gamitrinib Administered Intravenously to Patients with Advanced Cancers," Submitted by Dario C. Altieri, MD, Wistar Institute, in Support of the Proposal, "A First-in-Human, Phase I Clinical Trial of Mitochondrial-Targeted Hsp90 Inhibitor, Gamitrinib, in Advanced and Metastatic Prostate Cancer," Submitted by Dario C. Altieri, MD, Wistar Institute, Philadelphia, Pennsylvania, Proposal Log Number PC170275, Award Number W81XWH-18-1-0334, HRPO Log Number E00364.1a

1. The subject protocol and supporting documents received on 18 December 2018 in the U.S. Army Medical Research and Materiel Command, Office of Research Protections (ORP), Human Research Protection Office (HRPO) have been reviewed for applicability of human subjects protection regulations.

2. The research involves the analysis of de-identified tissue biopsies and peripheral blood mononuclear cells collected from subjects with advance metastatic prostate cancer before and after the IV administration of the investigational drug, Gamitrinib.

3. The Wistar Institute Institutional Review Board (IRB) Office determined that the project is research not involving human subjects as it does not involve living individuals about whom an investigator conducting research obtains data through intervention or interaction with the individual or identifiable private information.

4. As required by DOD Instruction 3216.02, encl 3, paragraph 4.c(1), the ORP HRPO concurs with the Wistar Institute IRB Office's determination of research not involving human subjects. The project may proceed with no further requirement for review by the HRPO. The HRPO protocol file will be closed. If additional projects under this award involve non-exempt research, the HRPO protocol files for these projects will remain open.

5. In the event that there is a change to the subject research or statement of work (SOW), the Principal Investigator must notify the Contracting Officer's Representative (COR) or Grant Officer's Representative (GOR) and send a description of the change to the HRPO at usarmy.detrick.medcom-usamrmc.other.hrpo@mail.mil referencing both the proposal log number and the HRPO log number listed in the "SUBJECT" line above. The HRPO will re-open the protocol file if necessary.

Any changes to the SOW that the COR or GOR determines could involve research with human subjects (as defined above) must be reviewed by the HRPO prior to approval by the Contracting Officer/Grants Officer.

6. Do not construe this correspondence as approval for any contract or grant/cooperative agreement funding. Only the Contracting Officer/Grants Officer can authorize expenditure of funds by notice of official award documentation. It is recommended that you contact the appropriate contract/grants specialist or Contracting/Grants Officer regarding the expenditure of funds for your project.

7. Further information regarding this review may be obtained by contacting Margaret M. Frederick, PhD, CIP, at 301-619-7418 or Margaret.m.frederick.ctr@mail.mil <mailto:Margaret.m.frederick.ctr@mail.mil> .

Specific Aim 1 – Major Task 1. Full Investigational New Drug (IND) application to US Food and Drug Administration FDA).

a. Plasma protein binding, stability, microsome clearance and intestinal penetration.

Studies conducted during the performance period of the present award demonstrated that Gamitrinib is heavily bound to plasma proteins (99.3±0.07%) with an average free fraction of 0.7±0.07%, comparable to control warfarin (bound, 98.3±0.22%, free fraction, 1.68±0.22%). The stability of Gamitrinib in human plasma is 91.4% with an average recovery of 82.8±3% (warfarin, 88.3±2.9%). At a concentration of 0.5 μM, the elimination rate constant (k) of Gamitrinib in phase I, cytochrome P450-mediated human liver microsome metabolism is 0.041 (control Midazolam, k=0.04) with half-life (t½) of 16.7 min (Midazolam, t½=17 min) and intrinsic clearance (CLint) of 3.30 mL/min/g

(Midazolam, 3.23 mL/min/g). Gamitrinib shows negligible penetration across a monolayer of Caco2 intestinal cells with an apparent permeability coefficient (P_{app},) of 1.90 nm/s in the A-to-B direction and 10.94 nm/s in the B-to-A direction with a P_{app} Efflux Ratio (ER) of 5.77.

b. In vitro toxicity.

Concentrations of Gamitrinib that trigger tumor cell killing in culture (IC_{50} ~1-4 μM) do not inhibit cytochrome P450 isoforms CYP1A2 (IC_{50}, 32.9 μM), CYP2A6 (IC_{50}, 24 μM), CYP2B6 (IC_{50}, 16 μM), and CYP2C8 (IC_{50}, 8 μM). Conversely, Gamitrinib inhibits CYP2C9 (IC_{50}, 1.1 μM) and CYP3A4 (IC_{50}, 0.12-0.2 μM) (Figure 3).

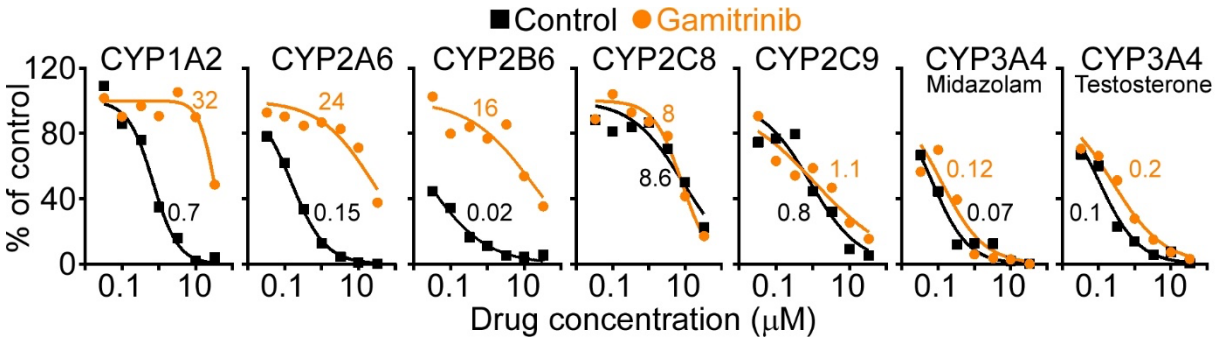


Figure 3. CYP inhibition. Increasing concentrations of Gamitrinib or relevant control were incubated with the indicated CYP isoforms and analyzed for % inhibition. The individual IC_{50} values of Gamitrinib (yellow) or control (black) are indicated per each CYP tested.

When analyzed for ion channel conductance, high concentrations of Gamitrinib (10 μM) inhibit Nav1.5 currents by 22.3±5.3% (control, 80.3±0.5%; n=3, pulse 26), Kv4.3/KChIP2 by 6.8±2.2% (control 50.5±2; n=13), Cav1.2 by 12.2±1.5% (control, 46.8±0.7%; n=34, pulse 2), Kv1.5 by 6.6±1.3% (control 61±1%; n=15), KCNQ1/mink by 22.5±1.1% (control 55.7±2.4%; n=16), hERG by 37.9±1.7% (control 42.9±1.2%; n=16), HCN4 by -0.2±3.7% (control 60.8±1.2%; n=16) and Kir2.1 by -7.3±3.4% (control 79.2±1.9%; n=15, pulse 10) (Figure 4A). A potential effect of Gamitrinib on hERG currents was evaluated in patch-clamp experiments in HEK293 cells stably transfected with hERG cDNA (Figure 4B). In this evaluation, concentrations of Gamitrinib of 0.5, 1, 5 and 10 μM inhibited hERG currents by -0.68±3.95%, 11.71±6.51%, 65.95±6.78%, and 81.83±1.88%, respectively (mean±SD) (Figure 4B). This results in a Gamitrinib IC_{50} of hERG inhibition of 3.5 μM (terfenadine IC_{50} 21.7 nM) (Figure 4C).

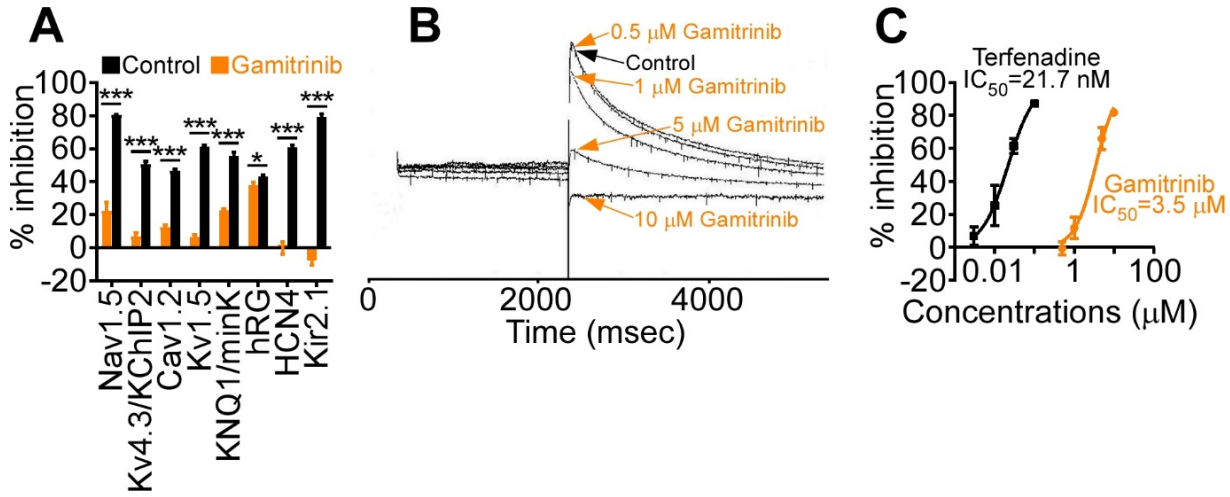


Figure 4 (*preceding page*). Ion channel activity. (A) Gamitrinib (10 μM) or relevant control is incubated with the individual channel-containing samples and the % inhibition of conductance compared to control is quantified (mean±SD). *, p=0.02; ***, p<0.0001. (B) Recording of hERG currents in the presence of control or the indicated increasing concentrations of Gamitrinib. Representative experiment. (C) HEK293 cells stably transfected with hERG cDNA are analyzed for inhibition of hERG currents in the presence of increasing concentrations of Gamitrinib or control terfenadine. The IC_{50} values for each compound tested are indicated.

To further characterize a potential cardiac toxicity of Gamitrinib, electrocardiography studies were conducted in beagle dogs administered IV Gamitrinib at dose levels of 1.25, 3.3, and 6.25 mg/kg twice weekly for 36 d plus a 14-d recovery period (Figure 5). In this analysis, one out of 5 male dogs administered Gamitrinib at 6.25 mg/kg/dose exhibited a small (7%) prolongation of QTc interval (17 msec) on d 32 of the dosing phase, which reversed during the recovery phase. No Gamitrinib-related prolongation of QTc interval was observed in female dogs administered 6.25 mg/kg/dose or in both sexes administered 1.25 or 3.33 mg/kg/dose (Figure 5). No Gamitrinib-related ECG changes in PR interval, QRS duration, QT interval, or heart rate were observed on d 32 of the dosing phase in animals administered 1.25, 3.33 or 6.25 mg/kg/dose or on d 11 of the recovery phase in animals administered 6.25 mg/kg/dose. No other rhythm abnormalities or qualitative ECG changes were observed in control or Gamitrinib-treated dogs (Figure 5).

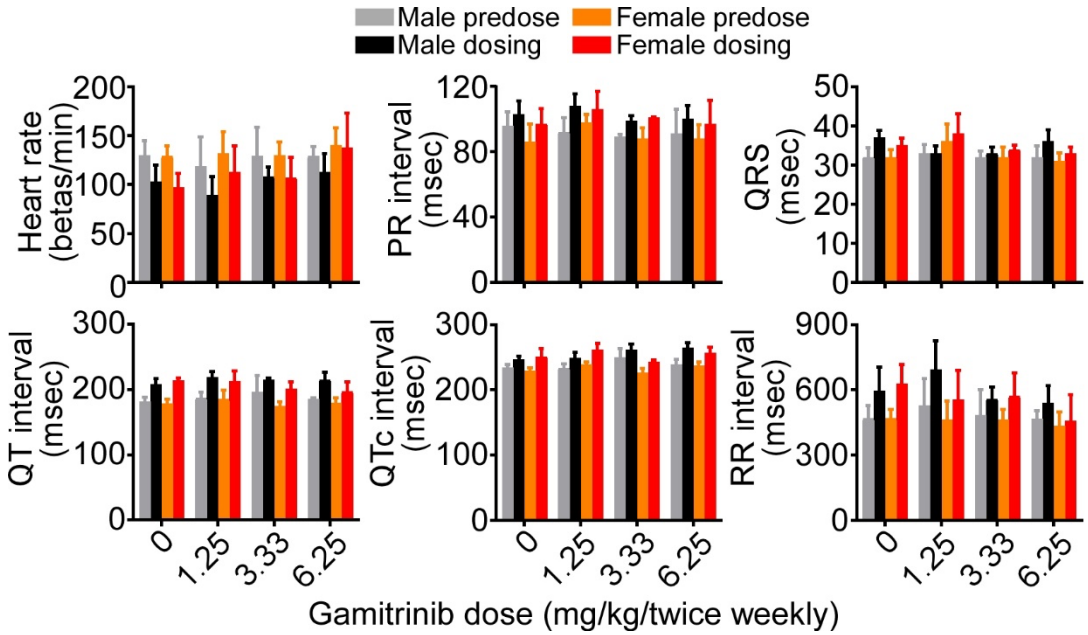


Figure 5. Male and female beagle dogs were administered IV Gamitrinib at 1.25, 3.33 and 6.25 mg/kg/dose twice weekly and electrocardiograms (ECGs) were obtained from unanesthetized animals once during the predose phase (predose) and 1 to 2 h post end of infusion on d 32 of the dosing phase (dosing). Mean±SD.

c. PK studies.

After IV administration (5 mg/kg) to Sprague-Dawley rats (n=3), the mean Gamitrinib C_{max} is 1175.807 ng/mL (Figure 6A), with mean volume of distribution at steady state (V_{ss}) of 65.471 L/kg, medium to slow clearance at 85.656±5.856 ml/min/kg and mean terminal phase half-life ($t_{1/2}$) of

12.25±1.55 h. Mean AUC_{0-t} and AUC_{INF} values are 783.199 and 976.002 hr•ng/mL, respectively (Table 2).

PK Parameters	Unit	Rat 1	Rat 2	Rat 3	Mean	SD	CV
$t_{1/2}$	h	11.623	11.107	14.022	12.250	1.555	12.7
C_{max}	ng/mL	1153.712	829.668	1544.041	1175.807	357.699	30.4
CL	mL/min/kg	79.233	87.035	90.700	85.656	5.856	6.8
MRT	h	5.539	5.411	5.524	5.492	0.0702	1.3
Vz	L/Kg	79.715	83.678	110.085	91.159	16.509	18.1
Vss	L/Kg	58.230	60.052	78.131	65.471	11.001	16.8
AUC_{last}	h•ng/mL	851.441	789.045	709.112	783.199	71.344	9.1
AUC_{INF}	h•ng/mL	1051.752	957.472	918.784	976.002	68.394	7.0

Table 2. Gamitrinib (5 mg/kg IV) PK in Sprague-Dawley rats.

Gamitrinib metabolism in rats does not generate detectable levels of 17-(amino)-17-demethoxygeldanamicyin (17-AG) (Figure 6B), a key metabolite of 17-AAG processing, in vivo. IV administration of Gamitrinib to Sprague-Dawley rats at 1, 10 or 25 mg/kg/dose twice weekly for 29 d results in increased C_{max} values from 1 to 25 mg/kg/dose followed by bi-exponential decline (Figure 6C). CL_{SS} values range from 84.83 to 131.33 mL/min/kg and V_{SS} values from 12.2 to 90.0 L/kg for d 4 and d 29. Gamitrinib C_{max} and AUC_{0-24} values were similar on d 4 and d 29, indicating no drug accumulation after multiple doses. Accumulation ratio values ranged from 0.045 to 1.04 for C_{max} and from 0.344 to 1.33 for AUC_{0-24}.

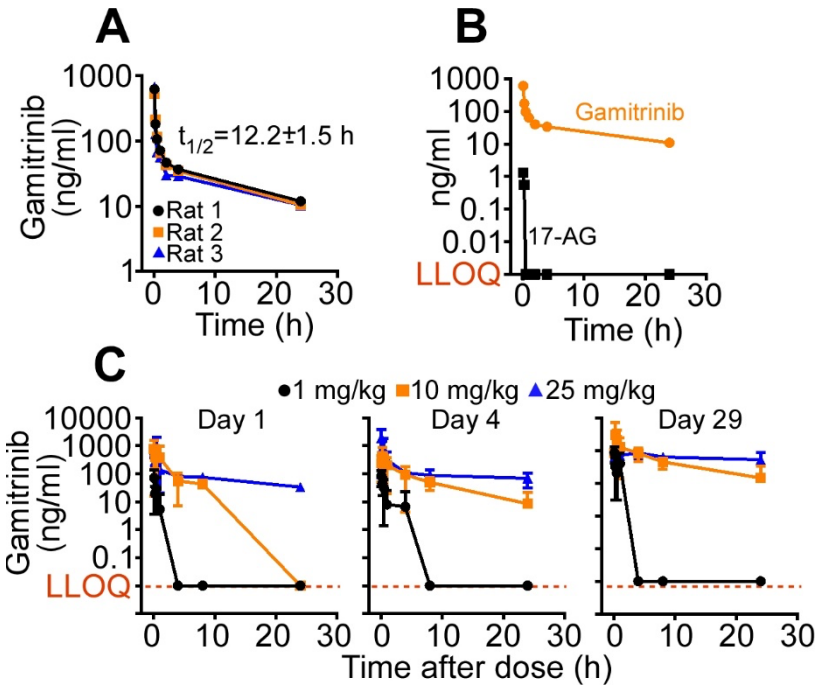


Figure 6. Gamitrinib PK in rats. (A) Gamitrinib (5 mg/kg) was injected IV in Sprague-Dawley rats and blood samples collected at the indicated time intervals were analyzed for Gamitrinib concentrations (C_{max}). Data from three individual animals and $t_{1/2}$ values (mean±SD) are shown. (B) The conditions are as in (A) and plasma samples from rats administered IV Gamitrinib were analyzed for Gamitrinib or 17-AG concentrations. (C) Male and female Sprague-Daley rats administered Gamitrinib IV at 1, 10 and 25 mg/kg/dose twice weekly were analyzed for Gamitrinib concentrations (C_{max}) on d 1, 4 and 29 of the dosing phase (mean±SD). LLOQ, lower limit of quantification.

d. Gamitrinib toxicity in Sprague-Dawley rats.

Male rats administered Gamitrinib IV (1 h infusion) at 1, 10 or 25 mg/kg/dose twice weekly on d 1, 4, 8, 11, 15, 18, 22, 25, and 29 (dosing phase) exhibited a small, fully recoverable and not adverse reduction in mean body weights at 10 (-5.5%) or 25 (-5.7%) mg/kg/dose (Figure 7). Gamitrinib-related clinical observations involved animals administered ≥10 mg/kg/dose, and included inguinal swelling, piloerection, hypoactivity, and sensitivity to touch at the infusion site. This correlated with microscopic findings of mixed cell inflammation at the catheter/infusion site, which increased in incidence and/or severity in animals administered ≥10 mg/kg/dose (both sexes) and persisted through recovery.

Alterations in clinical chemistry parameters, such as mildly to moderately higher neutrophil and platelet (Plts) counts, minimally prolonged partial thromboplastin time (PT), lower albumin, higher globulin, and alkaline phosphatase concentrations were observed at the highest Gamitrinib dose level tested of 25 mg/kg/dose (Figure 7) and likely related to inflammation. Consistent with systemic inflammation, these findings were accompanied by histologic evidence of spleen and liver extramedullary hematopoiesis.

Minimally to mildly higher serum urea nitrogen (UN) and creatinine concentrations were observed in animals receiving Gamitrinib at 25 mg/kg/dose (Figure 7). This correlated with increased kidney weight and microscopic findings of tubular degeneration/regeneration, which persisted to the end of the recovery phase. No effects on urinalysis or ophthalmic changes were identified. Gamitrinib-related mortality due to severe inflammation and marked hemorrhage at the infusion site occurred in two males and one female administered 25 mg/kg/dose and one male administered 10 mg/kg/dose. All other toxicity animals survived to their scheduled sacrifice. Gamitrinib-related mortality also occurred in two toxicokinetic females administered 10 mg/kg/dose and one toxicokinetic female administered 25 mg/kg/dose.

Based on these findings, the no observed adverse effect level (NOAEL) of Gamitrinib in rats as reported to the FDA during the performance period of this award is 1 mg/kg/dose, corresponding to C_{max} and AUC_{0-24} values of 87.1 ng/mL and 174 ng•hr/mL, respectively, on d 29 of dosing. Due to non-severely toxic effects or mortality in fewer than 10% of the animals administered Gamitrinib at 10 mg/kg/dose, the severely toxic dose in 10% of the animals (STD 10) is 10 mg/kg/dose. This corresponds to C_{max} and AUC_{0-24} values of 311 ng/mL and 1300 ng•hr/mL, respectively, on d 29.

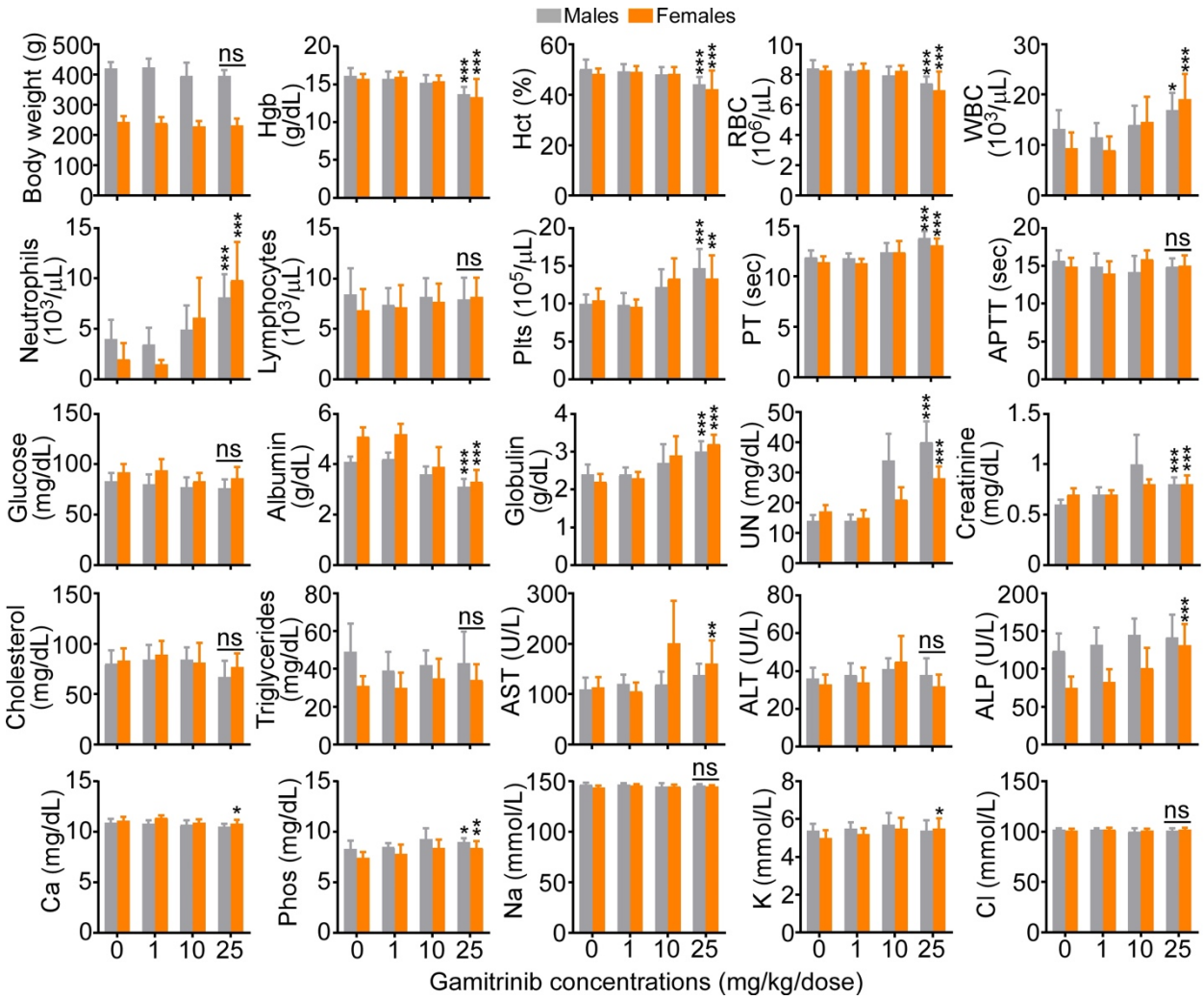


Figure 7. Gamitrinib toxicity in rats. Males and females Sprague-Dawley rats were administered IV Gamitrinib at 1, 10 and 25 mg/kg twice weekly for 29 d (dosing phase) and body weight values or blood samples collected at the end of the dosing phase were analyzed for the indicated clinical-chemistry parameters (mean±SD). *, p=0.01-0.03; **, p=0.001-0.002; ***, p=0.0004-<0.0001; ns, not significant.

e. Toxicity in beagle dogs.

Male and female beagle dogs administered IV Gamitrinib at dose levels of 1.25, 3.33 and 6.25 mg/kg/dose on d 1, 8, 15, 22, 29, and 36 of the dosing phase showed no reportable clinical observations. Bone marrow and liver parameters were unremarkable in all group levels, and only a trend of increased serum urea nitrogen and creatinine was observed in animals (both sexes) receiving the highest dose level of Gamitrinib of 6.25 mg/kg/dose (Figure 8). This correlated with microscopic finding of slight to moderate kidney tubular degeneration/regeneration, which was reversible during the recovery period. In addition, similar findings were present in one recovery sacrifice control male making their relationship to Gamitrinib uncertain.

No Gamitrinib-related changes in organ weights were observed and electrolyte, calcium and phosphorus levels were unchanged in the various groups (Figure 8). Catheter and infusion site findings were similar in control and Gamitrinib-treated animals. Based on these findings, the NOAEL of Gamitrinib in dogs as reported to the FDA during the performance period of this award

was 3.33 mg/kg/dose (C_{max}, 560±404 ng/mL; AUC_{0-24}, 1740±713 ng•h/mL on d 36 of dosing, both sexes). In the absence of effects on the overt well-being of the animals and evidence of reversibility of Gamitrinib-related findings, 6.25 mg/kg/dose is considered the highest non-severely toxic dose (HNSTD). This dose level corresponds to C_{max} and AUC_{0-24} values of 1260±556 ng/mL and 3290±1090 ng•h/mL, respectively (both sexes), on d 36 of the dosing phase.

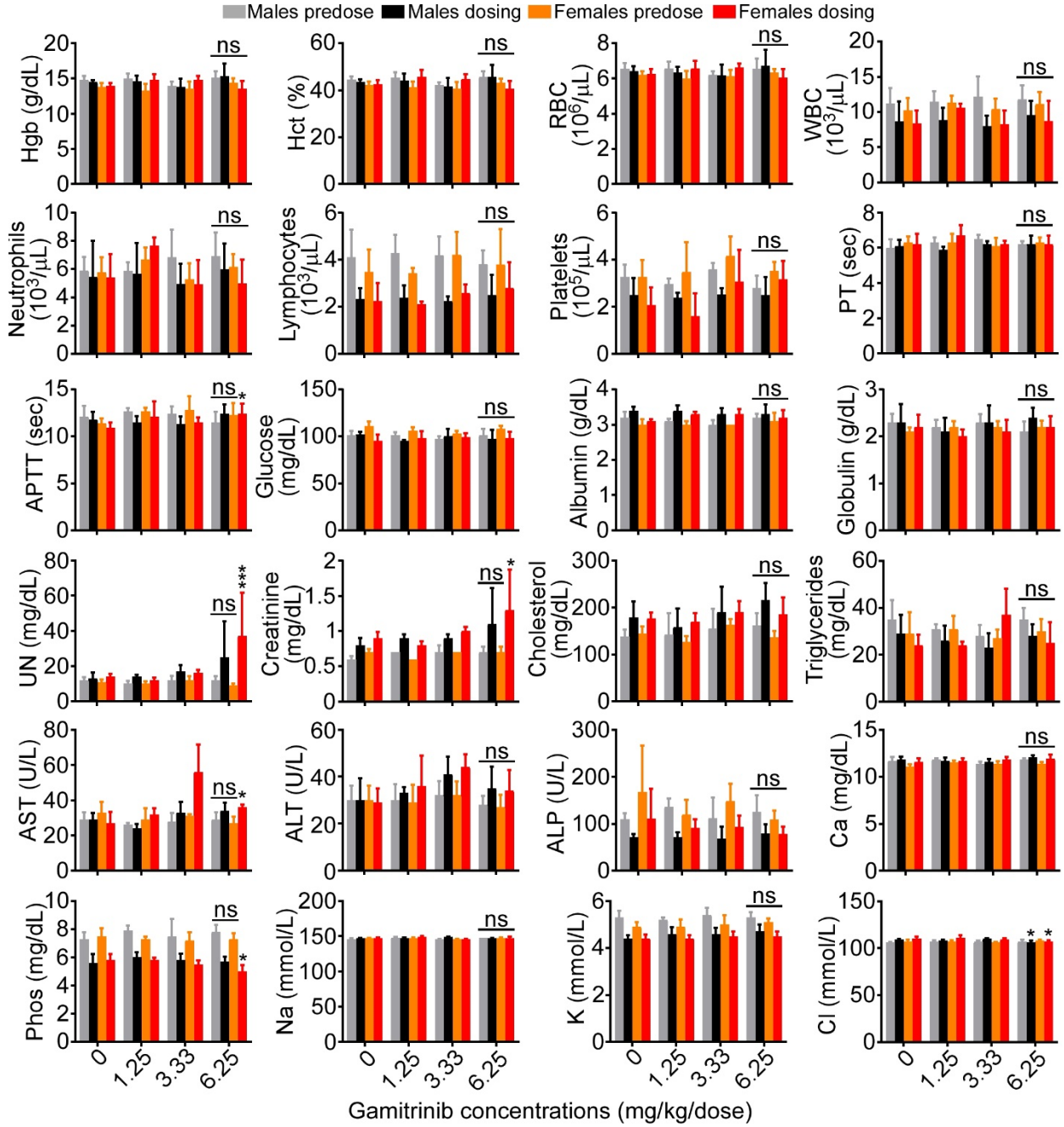


Figure 8. Gamitrinib toxicity in dogs. Male and female beagle dogs were administered IV Gamitrinib at 1.25, 3.33 and 6.25 mg/kg twice weekly for 36 d and blood samples collected prior to the initiation of dosing (predose) and on d 36 of the dosing phase (dosing) were analyzed for the indicated parameters (mean±SD). *, p=0.01-0.04; ***, p=0.001; ns, not significant.

In addition, no Gamitrinib-related changes in dog body weight (males and females) were observed at any dose level throughout the dosing phase (Table 3).

Days	**MALES Gamitrinib doses (IV infusion)**			
	0 mg/kg	1.25 mg/kg	3.33 mg/kg	6.25 mg/kg
1	10.1±0.83 (N=5)	10.3±0.91 (N=3)	10.1±1.1 (N=3)	9.9±0.83 (N=5)
8	10±0.75 (N=5)	10.2±1.04 (N=3)	10±1.04 (N=3)	9.8±0.85 (N=5)
15	9.9±0.66 (N=5)	10.1±0.93 (N=3)	9.9±1.15 (N=3)	9.5±0.57 (N=5)
22	10.1±0.73 (N=5)	10.3±0.69 (N=3)	10.2±1.21 (N=3)	9.2±0.57 (N=5)
29	10±0.66 (N=5)	10.3±0.75 (N=3)	10.1±1.21 (N=3)	9.2±0.46 (N=5)
36	10.1±0.76 (N=5)	10.5±0.52 (N=3)	10±1.36 (N=3)	9.1±0.58 (N=5)
Days	**FEMALES Gamitrinib doses (IV infusion)**			
	0 mg/kg	1.25 mg/kg	3.33 mg/kg	6.25 mg/kg
1	7.5±0.92 (N=5)	7.9±1.34 (N=3)	7.6±0.9 (N=3)	7.7±0.57 (N=5)
8	7.4±0.89 (N=5)	7.8±1.49 (N=3)	7.6±0.9 (N=3)	7.7±0.62 (N=5)
15	7.3±0.93 (N=5)	7.8±1.64 (N=3)	7.6±1.06 (N=3)	7.4±0.54 (N=5)
22	7.3±1.03 (N=5)	8±1.53 (N=3)	7.5±0.75 (N=3)	7.2±0.6 (N=5)
29	7.5±0.94 (N=5)	8.2±1.42 (N=3)	7.6±0.7 (N=3)	7.3±0.58 (N=5)
36	7.6±0.97 (N=5)	8.4±1.55 (N=3)	7.5±0.8 (N=3)	7.3±0.59 (N=5)

Table 3. Dog body weight changes (males and females) assessed during the Gamitrinib dosing phase throughout d 37 (mean±SD).

Specific Aim 1 – Major Task 1. FDA approval of IND submission.
The data summarized above were finalized during the performance period of the present award and submitted to the FDA as a full IND submission on March 15, 2021. Upon amendments requested by the Agency, the FDA approved the Gamitrinib IND (IND 132453) with *study may proceed* letter dated April 19, 2021.

U.S. FOOD & DRUG ADMINISTRATION

IND 132453

STUDY MAY PROCEED

The Wistar Institute
Attention: Dario C. Altieri, MD
3601 Spruce Street
Philadelphia, PA 19104

Dear Dr. Altieri:[1]

Please refer to your investigational new drug application (IND) submitted under section 505(i) of the Federal Food, Drug, and Cosmetic Act (FDCA) for gamitrinib.

We have completed our safety review of your application and have concluded that you may proceed with your proposed clinical investigation for advanced cancer.

In addition, we have the following non-hold comments for your consideration:

Product Quality

As development proceeds,

1. Revise your drug product strength to be consistent with the USP salt policy and express the gamitrinib drug substance strength as the free base form for your drug product. We refer you to the following FDA guidance "Naming of the Drug Products Containing Salt Drug Substances".

2. Tighten the acceptance criteria for and provide a risk based justification for the impurity, RRT 1.19 in the gamitrinib drug substance specification.

3. Use of an overage to compensate for the loss of drug substance during the drug product manufacture process is discouraged. It is noted that the clinical drug product label strength is revised for the current protocol. Moving forward, such revision to the drug product label strength is not recommended.

ADDITIONAL IND RESPONSIBILITIES

As the Sponsor of this IND, you are responsible for compliance with the FDCA (21 U.S.C. §§ 301 et. seq.) as well as the implementing regulations [Title 21 of the Code

[1] We update guidances periodically. For the most recent version of a guidance, check the FDA Guidance Documents Database https://www.fda.gov/RegulatoryInformation/Guidances/default.htm.

Specific Aim 1 – Major Task 1. Shipment of 250 g of clinical grade Gamitrinib

Clinical grade Gamitrinib formulated as a sterile, stable injectable suspension for administration to patients with advanced cancer was successfully shipped and received by the research pharmacy at Fox Chase Cancer Center, the clinical site conducting the Gamitrinib trial (email acknowledgment from Dr. Needleman) on May 7, 2021.

From: Needleman, Richard L
To: Umar Hayat Ph.D.; Romasko, Ryan J; Erdwin Orellana
Cc: Olszanski, Anthony J; Dario Altieri
Subject: RE: [EXT] CTM Shipment to clinical site
Date: Friday, May 7, 2021 9:23:03 AM
Attachments: image001.png

Hi Umar,

I just received the shipment of gamitrinib and placed the vials in the -20 freezer. The shipment did not contain a temperature monitoring device. The FDA is focused on temperature control during chain of custody. Moving forward you MUST include this type of device or I fear that this could be a major issue if this study is audited.

Best,

Rich

Richard Needleman, RPh.
Investigational Drug Services Pharmacist
Fox Chase Cancer Center
333 Cottman Avenue
Philadelphia, PA 19111
Phone- 215-728-3075
Fax- 215-728-3875
Email: Richard.needleman@fccc.edu

FOX CHASE CANCER CENTER
TEMPLE HEALTH

Specific Aim 1 – Major Task 2. Patient accrual on the *first-in-human* Gamitrinib clinical trial in patients with advanced cancer

The *first-in-human* Gamitrinib clinical trial (***ClinicalTrials.gov NCT04827810***) was opened during the performance period of the present award on July 27, 2021, and announced by Dr. Olszanski, principal investigator on the trial at Fox Chase Cancer Center with email to clinical staff (see below). The criteria for patient enrollment, which started on July 27, 2021 are as follows: potential participants in the Gamitrinib clinical trial are registered from 8:00 AM to 4:00 PM EST excluding holidays by emailing the Investigator-Sponsored Research Unit (ISRU) at: **FCCC.MONITOR@fccc.edu.** Eligible participants are entered on study centrally once the following items have been received by email:

- Completed registration form
- Copies of signed consent and HIPAA forms
- Eligibility checklist

Following registration, participants begin protocol treatment within 14 calendar days of registration. Issues that would cause treatment delays must be discussed with the Sponsor-Investigator. If a registered participant does not receive protocol therapy following registration, the participant will be recorded as withdrawn from study. The Study Monitor must be notified as soon as possible if a participant does not begin protocol treatment as scheduled. Participants must be registered and have received a sequence number prior to the initiation of treatment. As approved by the FDA during the performance period of the present award, the inclusion/exclusion criteria for patient screening and selection for the *first-in-human* Gamitrinib trial at Fox Chase Cancer Center are as follows:

Inclusion Criteria

Patients with advanced, castration-resistant and metastatic prostate cancer as well as grade IV glioma, i.e. glioblastoma, will be especially encouraged to participate in the study, given the preclinical evidence of Gamitrinib activity in these tumors.

- Histologically confirmed diagnosis of advanced cancer refractory to standard of care therapy, or for whom no standard of care therapy is available. Any numbers of prior therapies are allowed.
- Dose escalation phase: Solid tumors and lymphoma may have measurable or evaluable disease as per Response Evaluation Criteria in Solid Tumors (RECIST v. 1.1) or as per RECIL 2017 criteria
- Dose expansion phase:
    i. All patients must have at least one site of measurable disease as defined by RECIST v. 1.1. or RECIL 2017, for solid tumors and lymphoma, respectively
    ii. Patients in the expansion cohort must have at least one non-target lesion deemed safe to biopsy, in the opinion of the investigator, and be willing to undergo mandatory core biopsies. This includes pre-treatment and an on-treatment biopsy. Biopsies at the time of progression are highly desired, but optional.
    iii. The lesion(s) which will be used for response assessment may not be biopsied
    iv. Target lesions that have been previously irradiated will not be considered measurable unless increase in size is observed following completion of radiation therapy

- All previous therapies of cancer, including radiotherapy major surgery and investigational therapies must be discontinued for ≥14 days (≥ 28 days for mitomycin C or nitrosoureas) before Cycle 1 Day 1 (C1D1), and all acute effects of any prior therapy must have resolved to baseline severity or Grade ≤ 1 Common Terminology Criteria for Adverse Events (CTCAE v5), except alopecia or parameters defined in this eligibility list.
- Age ≥ 18 years.
- ECOG performance status 0- 2
- Patients must have normal organ and marrow function as follows:

Absolute neutrophil count	**≥1,500/mm^3 without growth factor use ≤ 7 days prior to C1D1**
Platelets	≥85,000/mm^3 without platelet transfusion ≤ 7 days prior to C1D1

Hemoglobin	>8.5 mg/dL without red blood cell transfusion ≤ 7 days prior to C1D1
Total serum bilirubin	<1.5 X upper limit of normal (ULN) (except for patients with documented Gilbert's syndrome)
AST (SGOT)/ALT (SGPT)	≤2 X ULN; ≤ 5 X ULN if liver dysfunction is felt to be secondary to tumor burden
Serum creatinine	≤ 1.5 X ULN (OR creatinine clearance ≥ 60 mL/min)
Serum or urine pregnancy test (WOCBP only)	negative ≤7 days of C1D1

- Ability to understand and willingness to sign a written informed consent, HIPAA consent document and comply with the study scheduled visits, treatment plans, laboratory tests and other procedures.
- Female patients must be surgically sterile or be postmenopausal or must agree to use effective contraception during the period of the trial and for at least 90 days after completion of treatment. Male patients must be surgically sterile or must agree to use effective contraception during the period of the trial and for at least 90 days after completion of treatment. The decision of effective contraception will be based on the judgment of the principal investigator or a designated associate.

Exclusion Criteria

- Patients with symptomatic brain metastases are excluded. Patients with asymptomatic and treated CNS metastases may participate in this trial. The patient must have completed any prior treatment for CNS metastases > 28 days prior to study entry, including radiotherapy or surgery. Concurrent use of steroids for the treatment of brain metastasis are not permitted.
- Current treatment on another (therapeutic) clinical trial
- History of ventricular tachycardia, torsade des pointes, complete left bundle branch block or third degree heart block or QTcF > 470 milliseconds, regardless of gender, on a 12-lead ECG during the screening period (based on mean of triplicate EKGs).
- Hypertension not adequately controlled with medications (>150/100 mm Hg despite optimal medical therapy)
- Active bacterial fungal or viral infection including hepatitis B (HBV), hepatitis C (HCV), requiring treatment with IV antibiotic, IV anti-fungal, or anti-viral (Testing is not required for eligibility).
    - Patients with treated hepatitis B or C, with no evidence of continued infection or requirement for antiviral medications, are permitted
- Known human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS)-related illness (testing is not required for eligibility).
- Any of the following in the previous 6 months: myocardial infarction, severe/unstable angina, coronary/peripheral artery bypass graft, symptomatic congestive heart failure, cerebrovascular accident, transient ischemic attack or symptomatic pulmonary embolism.

- Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration, or may interfere with the interpretation of study results, or in the judgment of the investigator would make the patient inappropriate for entry into the study
- Receiving or requiring the continued use of medications that are known to strongly inhibit or induce CYP3A4/5 as well as strong Pgp inhibitors. To participate in this study, such subjects should discontinue use of such agents for at least 2 weeks before cycle 1 day 1.
- Avoid the use of strong inhibitors and inducers of major cytochrome P450 enzymes and strong inhibitors of renal, hepatic and promiscuous transporters.

Email from Dr. Olszanski to Fox Chase Cancer Center staff announcing the opening of the Gamitrinib clinical trial (***ClinicalTrials.gov NCT04827810***) on July 27, 2021.

From: Olszanski, Anthony J
To: Sabella, Victoria; Haagen, Dana M; Romasko, Ryan J; Dario Altieri
Cc: George, Riad J
Subject: [EXT] Fw: 21-1045 has been approved
Date: Tuesday, July 27, 2021 7:02:53 PM

Dear Vicky,
Thank you so much for your help and support in getting this important trial this far! I am excited to have the SIV and get patients on and continue this amazing collaboration with Dr. Alteri and the Wistar institute. Thank you too, for making sure we met this extremely important timeline!

Dear Ryan,
You have been outstanding to work with. Your pleasant and charismatic personality and dedicated work ethic has been absolutely instrumental in moving this project forward. Thank you so very much for all of your unfailing hard work!

Dear Dana,
We are ready for the SIV, which I know you are getting scheduled. I am grateful to have you and your patient-centric attitude lead us in treating patients in need. With your keen eye for detail and hawk-like oversight, I am confident that this trial will offer great hope to so many! Let's go!

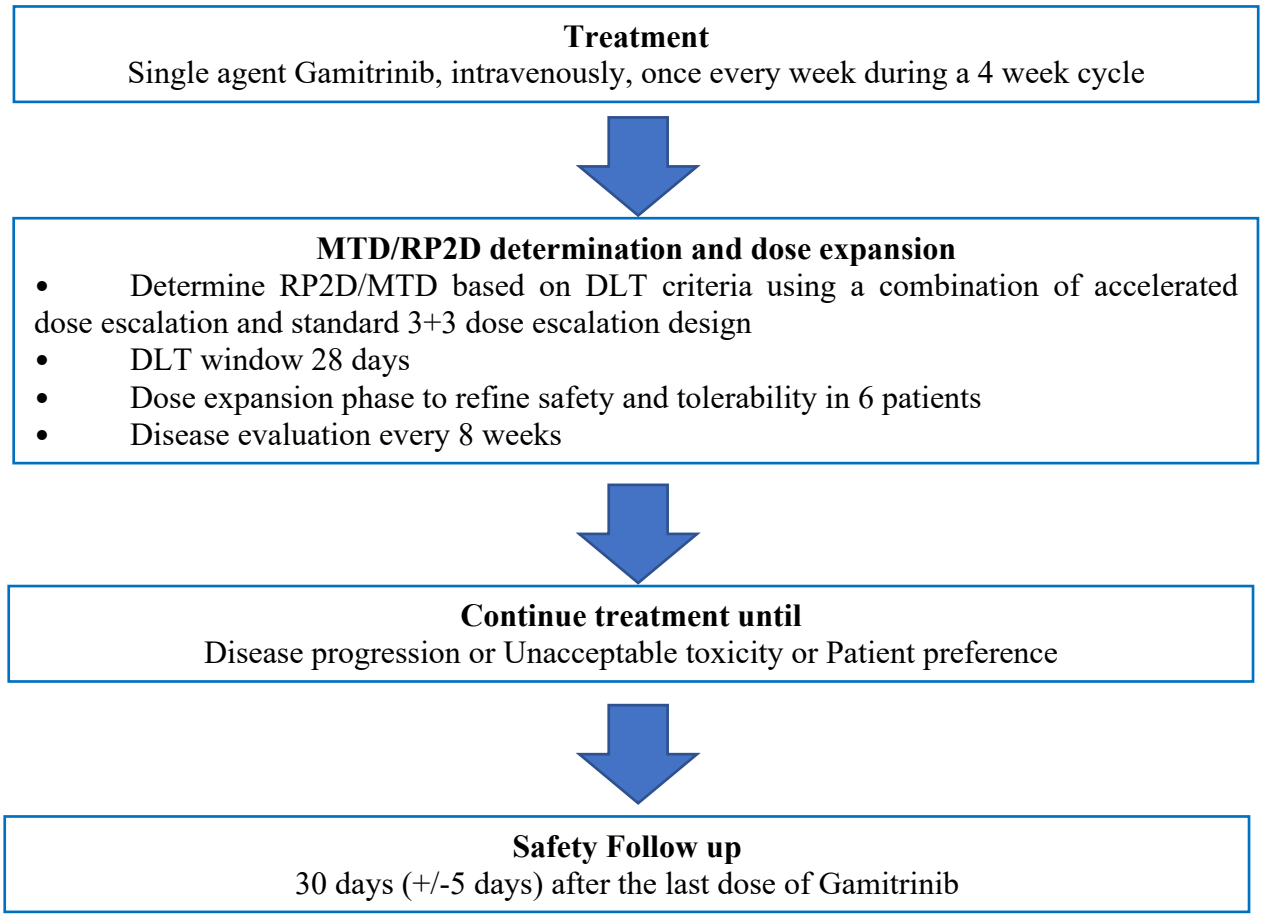
Dear Dr. Altieri,
After an incredibility complicated and frustrating pre-clinical to clinical development phase, we are finally poised to bring your idea and your discovery to the patient. Thank you for your amazing diligence and sheer will in moving this project forward after so many unexpected hurdles from animal tox, pharm sci, the RO1 grant that almost wasn't, rescinded FDA approvals and misinformation , among many other issues that surfaced! Your leadership has been instrumental and without it, this concept would not have made it to the patient. While the road ahead is unknown and will likely have its own set of challenges, I am both humbled and grateful to have worked alongside you in this important endeavor and I truly look forward to the future.

Thank you all for this amazing work. Now it's time to bring this medication to where it is needed. We will start off by providing many patients with another option that they did not know existed, and by that virtue alone we will bring great hope to those in great need. As we help these patients, God willing we will also witness their flawless altruism and understated bravery rewarded with objective responses!

Sincerely,
Tony

Anthony J. Olszanski, RPh, MD
Fox Chase Cancer Center
Vice Chair, Department of Hematology/Oncology
Director, Phase 1 Developmental Therapeutics Program
Director, Medical Oncology Melanoma Program
333 Cottman Avenue
Philadelphia, PA 19111
(215) 728-5673
Anthony.Olszanski@FCCC.edu

As indicated in the documentation above and consistent with the experimental objectives of Major Task 2, **the clinical trial was opened during the performance period of the present award**. The full Gamitrinib clinical trial design (***ClinicalTrials.gov NCT04827810***) is as follows:



The specific trial objectives are as follows:

Primary Objective

Determine the MTD and/or RP2D of Gamitrinib when administered once weekly.

Primary Endpoints

First cycle dose-limiting toxicities

Secondary Objectives

1) Evaluate the overall safety profile of intravenously administered single-agent Gamitrinib
2) To evaluate the PK profile of Gamitrinib
3) Assess the pharmacodynamic effects of Gamitrinib
4) Document any anti-tumor activity of single agent Gamitrinib

Secondary Endpoints

1) Overall, safety profile of Gamitrinib as characterized by type, frequency, severity, timing and relationship to study therapy of adverse events and laboratory abnormalities according to NCI CTCAE v5.0.
2) Plasma concentrations and PK parameters following IV administration of single-dose Gamitrinib
3) Assessment of Gamitrinib effects on pharmacodynamic markers
4) Objective tumor response, as assessed using RECIST 1.1 and RECIL 2017 criteria

Study Plan

This is a phase 1, open label, dose dose-escalation, safety, pharmacokinetic, and pharmacodynamic study of single agent Gamitrinib in sequential cohorts of adult patients with advanced solid tumors or lymphoma, for whom no standard therapy is available.

Once the maximum tolerated dose MTD or RP2D has been established, a six-patient expansion phase will ensue for the purpose of refining the safety assessment, exploring pharmacodynamics in paired biopsies, and the assessment of preliminary efficacy.

Throughout the study, AEs, SAEs, laboratory assessments, vital signs, physical examination findings, ECOG performance status, and EKGs will be obtained to evaluate the treatment. Toxicity will be evaluated according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE), version 5. Study drug may be discontinued if a patient experiences study treatment-related toxicity.

Serial blood samples to measure plasma concentrations of Gamitrinib, and potentially relevant biomarkers, will be collected at pre-specified time points as indicated in the Schedule of Activities table, and described in section 9. For solid tumors the underlying disease status will be assessed by the investigator per RECIST (version 1.1) using radiological evaluations (e.g. computed axial tomography [CT] scan or magnetic resonance imaging [MRI], as clinically indicated). For lymphoma, the Response Evaluation Criteria in Lymphoma (RECIL 2017) will be used for response assessment.

Number of Patients

It is anticipated that 18-36 evaluable patients in the dose escalation and an additional 6 patients in the expansion phase, will be enrolled into this study over the course of 18 months. Because an accelerated titration and a 3 + 3 dose escalation design will be used, the actual sample size will depend on the number of cohorts and the number of patients enrolled in each cohort.

Duration of Treatment

During the treatment period, patients will receive Gamitrinib, intravenously, once every week. 28 days will be considered one cycle of therapy. Dosing will continue until disease progression, unacceptable toxicity, or the patient discontinues for any other reason.

Starting Dose and Planned Scheme

Gamitrinib will be administered intravenously, over 1 hour, once weekly. The starting dose will be 10 mg. Treatment will be given in the outpatient setting. Each treatment cycle is 28 days.

Derivation of starting dose (cohort 1):

- Rat STD10 = 10 mg/kg
- HED conversion factor: multiply by 0.16
- HED=1.6 mg/kg
- Divide by safety factor of 10
- Max starting dose = 0.16 mg/kg
- Apply to adult 60 kg patient: Starting dose = 10 mg

Dose escalation

An accelerated dose titration scheme will be used in this study. Single patient cohorts with 100% dose increments will be incorporated until one patient experiences a DLT or ≥2 patients experience any NCI CTCAE ≥Grade 2 treatment-related toxicities, possibly, probably or definitely related to the treatment. Toxicity as defined above will result in using a standard 3+3 modified Fibonacci dose escalation design in sequential cohorts. For clarity, the modified Fibonacci dose increments are (approximately) 2, 1.67, 1.5 and then 1.33 thereafter.

Table 4: Planned Dose Escalation Cohorts

Cohort Assignment	**Dose Level* (mg)**	
	Accelerated Phase	**Standard Phase**
-1	--	5 mg
1 (starting dose)	10 mg	10 mg
2	20 mg	20 mg
3	40 mg	35 mg
4	80 mg	50 mg
5	160 mg	65 mg
6	320 mg	85 mg

*Additional cohorts may be tested based on PK and PD data, or emerging safety or efficacy data.

Specific Aim 2 – Major Task 3 –Characterize the pharmacodynamics profile of Gamitrinib

The planned pharmacodynamics profile of Gamitrinib treatment to be carried out on biopsies collected at MTD in the proposed patient expansion cohort could not be completed during the performance period of the present award. The reason is that the Gamitrinib trial is currently ongoing at Fox Chase Cancer Center and MTD dose levels in a patient expansion cohort have not yet been identified.

The deviation in the timeline proposed in the original Statement of Work for this award is due to an 18-month delay in the production of GMP-grade Gamitrinib, which was originally under contract at Arcinova, Inc. Despite having successfully synthesized an initial *demonstration* batch of 10 g of Gamitrinib, Arcinova failed to then execute the validated methods for drug synthesis, inordinately delayed the scaled-up manufacturing process and did not complete the agreed-upon contractual agreement with The Wistar Institute that called for the production of 250 g of GMP-compliant, clinical-grade Gamitrinib. Despite repeated and direct inquiries, including biweekly conference calls to review project status, Arcinova continued to postpone the drug synthesis process without truthful or convincing explanations to the sponsor (The Wistar Institute).

After repeated and unsuccessful attempts to restart due process, the contract with Arcinova was terminated on February 10, 2020, and a new contract for the synthesis of clinical-grade GMP-compliant Gamitrinib was established with Albany Molecular Research International (AMRI, now Curia). Arcinova's failure to execute the drug substance requirements of the present award set back the originally planned timeline for completion of Major Task 3 by at least 18 months.

The Principal Investigator on this award dutifully notified the CDMRP Science Officer assigned to the present award, Dr. Mishra Nrusingha of the delays incurred with Arcinova as early as early as June 19, 2019, and then again on October 16, 2019, and January 14, 2020, explaining in detail the nature of the problem encountered and Arcinova's failure to execute On February 10, 2020, the Principal Investigator informed Dr. Nrusingha that a new manufacturer for the production of Gamitrinib drug substance had been successfully identified, reviewed budgetary allocations to accommodate the new expenditures on February 11, 2020, and provided additional project updates on April 1, 2020 and November 6, 2020. On June 12, 2020, the Principal Investigator again wrote to Dr. Nrusingha to request a one-time, one-year no-cost extension of the present award, provided detailed project updates, and shared the successful method validation of Gamitrinib production at the new manufacturing site (AMRI) on January 13, 2021. On February 19, 2021, the Principal Investigator provided Dr. Nrusingha a new project update, revised timeline for completion, financial allocations, and then again on March 16, 2021, and April 19, 2021, with news of the successful IND submission to the FDA and Agency approval, respectively.

Although it is regrettable that the studies proposed in Major Task 3 have not been completed during the performance period of the present award, additional federal funding from the NIH/NCI (1 R01 CA225913-01A1) has now been secured by the Principal Investigator. The new award will ensure that the proposed correlative studies of Gamitrinib target engagement in the patient expansion cohort at MTD will be successfully carried out upon completion of patient accrual in the ongoing clinical trial at the Fox Chase Cancer Center.

Specific Objectives –

1. Completion of AMRI-Wistar quality agreement for the synthesis of 250 g of GMP-grade API (Gamitrinib)
2. Approval of AMRI Test Methods
3. Acquisition of CoA-supported bulk raw materials for Gamitrinib synthesis (Geldanamycin, triphenylphosphonium, linker)
4. Issue of demonstration batch CoA for Gamitrinib synthesis
5. Successful completion of all IND-enabling studies of Gamitrinib: drug substance; drug product, in vitro toxicity, including heart function liabilities, PK studies, toxicity in rats and toxicity in dogs
6. Successful formulation development and optimization of a new GMP-compliant Gamitrinib Injectable Suspension for IV administration in humans
7. HRPO concurrence letter
8. Completion of Gamitrinib method validation and reference standard
9. Completion of 3-recrystallization protocol for synthesis of GMP-grade Gamitrinib
10. Completion of microbial suitability report and elemental impurity of GMP API
11. Issue of CoA for GMP-grade API and shipment of drug substance to compounding pharmacy

12. Full IND submission to FDA
13. FDA approval of IND upon modification of clinical protocols and "*Study May Proceed*" letter
14. Delivery of clinical grade Gamitrinib for injection in patients to the Fox Chase Cancer Center
15. Transfer of IND from The Wistar Institute to the Fox Chase Cancer Center
16. IRB approval of modified clinical protocol by Fox Chase Cancer Center
17. Final submission of updated Gamitrinib CoA to FDA
18. Opening of first-in-human, phase I clinical trial of Gamitrinib in patients with advanced cancer (***ClinicalTrials.gov NCT04827810***)
19. Submission of collaborative manuscript reporting the preclinical development of Gamitrinib as supported by the present award.

Significant Results or Key Outcomes –
As described above, results obtained during the performance period of the present award led to the successful complete preclinical characterization of Gamitrinib as a first-in-class, subcellularly directed inhibitor of mitochondrial proteostasis and new anticancer therapy. Key outcomes of the performance period of the award included the (1) successful production, manufacturing and formulation development of Gamitrinib as a sterile, stable injectable suspension; (2) submission of full IND application to the FDA and Agency approval of the first-in-human Gamitrinib clinical trial in patients with advanced cancer; (3) regulatory approvals for clinical investigation by HRPO and Fox Chase Cancer Center IRB; (4) opening of the Gamitrinib clinical trial at Fox Chase Cancer Center under the direction of Dr. Olszanski (***ClinicalTrials.gov NCT04827810***).

Although Major Tasks 1 and 2 of the proposed statement of work were successfully met during the performance period of the award, the studies proposed in Specific Aim 2 – Major Task 3 of the original statement of work could not be completed before the closeout of the no-cost extension extension of the present award. As discussed above in detail, the deviation in the proposed timeline for completion of these studies was due to an 18-month delay incurred by Arcinova, Inc that failed to manufacture 250 g of clinical grade, GMP-compliant Gamitrinib per contractual agreement with The Wistar Institute. Although an alternative vendor for the manufacturing of clinical-grade Gamitrinib drug substance was later successfully identified and the study drug was produced to support the clinical trial now opened at Fox Chase Cancer Center as detailed in Major Task 2, the 18-month delay incurred early on in the performance of the present award significantly extended the timeline for completion of the clinical trial. Accordingly, the biopsy samples from the expansion patient cohort at MTD to be investigated in Major Task 3 have not yet been collected. Although it is regrettable that this aspect of the study could not be completed during the duration of the present award, additional federal funding recently obtained by the Principal Investigator (NIH/NCI CA225913-01A1) will ensure that these studies will be successfully completed once patient accrual on the Gamitrinib trial is completed and the patient expansion cohort at MTD has been finalized.

What opportunities for training and professional development has the project provided?

Nothing to report

How were the results disseminated to communities of interest?

Nothing to report

What do you plan to do during the next reporting period to accomplish the goals?

This award is terminated with the present final closeout report.

4. **IMPACT:**

What was the impact on the development of the principal discipline(s) of the project?

The findings reported above establish the feasibility and safety of targeting mitochondrial protein folding with Gamitrinib for cancer therapy. A first-in-human, publicly funded phase I clinical trial of Gamitrinib in patients with advanced cancer based on the results supported by the present award is currently ongoing (***ClinicalTrials.gov NCT04827810***) at the Fox Chase Cancer Center

What was the impact on other disciplines?

The clinical development of Gamitrinib as summarized in the current report validates a new strategy of subcellularly-targeted drug delivery that has not been used before for the development of anticancer agents. Functionally, this results in global inhibition of mitochondrial functions exploited for tumor progression, metabolic reprogramming and acquisition of metastatic competence. As demonstrated during the performance period of the present award, subcellularly-directed cancer therapy with Gamitrinib is feasible, safe in two animal species and without overt organ and tissue toxicities. While the first-in-human clinical trial of Gamitrinib therapy in patients with advanced cancer is currently ongoing, these studies may already open new possibilities for targeting other specific cancer pathways compartmentalized in subcellular organelles that escape inhibition by "untargeted" anticancer agents.

What was the impact on technology transfer?

Nothing to report

What was the impact on society beyond science and technology?

Nothing to report

5. CHANGES/PROBLEMS:

There have been no significant changes in direction of the approved SOW. Major Tasks 1 and 2 have been successfully met during the performance period of the present award. The 18-month delay incurred by Arcinova in the chemical synthesis of GMP-compliant Gamitrinib has delayed the completion of Major Task 3 proposed in the original statement of work, as the Gamitrinib clinical trial is currently still accruing at Fox Chase Cancer Center and the patient expansion cohort, required to complete the studies in Major Task 3 has not yet been established. As described above in detail, the delay incurred in drug manufacturing did not reflect problems in drug (Gamitrinib) synthesis, method validation or release, but, unfortunately, Arcinova's failure to execute its contractual agreement with The Wistar Institute. As indicated in detail in this report, the Principal Investigator had dutifully informed the CDMRP Science Officer assigned to this award of the delay incurred with Arcinova as early as June 19, 2019 and provided detailed updates on progress made to fulfill the objectives of the original statement of work throughout the duration of the award.

Actual or anticipated problems or delays and actions or plans to resolve them

Additional federal funding obtained by the Principal Investigator on the present award (NIH/NCI CA225913-01A1) will support the completion of the correlative studies of Gamitrinib target engagement in the patient expansion cohort at MTD proposed in Specific Aim 2 – Major Task 3. These studies could not be completed during the performance period of the present award due to the 18-month delay incurred by Arcinova, Inc in the manufacturing of clinical-grade, GMP-compliant Gamitrinib.

Changes that had a significant impact on expenditures

Nothing to report

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Significant changes in use or care of human subjects

No significant changes in use or care of human subjects. Nothing to report

Significant changes in use or care of vertebrate animals

No significant changes in use or care of vertebrate animals. Nothing to report

Significant changes in use of biohazards and/or select agents

No significant changes in use of biohazard and/or select agents. Nothing to report

6. PRODUCTS:

- **Publications, conference papers, and presentations**

Journal publications.

Ayat U, Elliott GT, Olszanski, AJ and Altieri DC (2021) Feasibility and safety of targeting mitochondria for cancer therapy – Preclinical characterization of Gamitrinib, a first-in-class, mitochondrial targeted small molecule Hsp90 inhibitor. Manuscript submitted for publication.

Books or other non-periodical, one-time publications.

Nothing to report

Other publications, conference papers and presentations.

Nothing to report

- **Website(s) or other Internet site(s)**

Nothing to report

- **Technologies or techniques**

Nothing to report

- **Inventions, patent applications, and/or licenses**

Nothing to report

- **Other Products**

Nothing to report

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Dario Altieri
Project Role:	Principal Investigator
Nearest whole person month worked:	1
Contribution to Project:	No Change
Funding Support:	No Change
Name:	Ekta Agarwal
Project Role:	Post-Doctoral Fellow
Nearest whole person month worked:	2
Contribution to Project:	No Change
Funding Support:	No Change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report

What other organizations were involved as partners?

Nothing to report

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS:

QUAD CHARTS:

9. APPENDICES: