CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

206162Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

LYNPARZA (olaparib)

Date: December 2, 2014
To: File for NDA 206162
From: John K. Leighton, PhD, DABT
Acting Director, Division of Hematology Oncology Toxicology
Office of Hematology and Oncology Products

I have examined pharmacology/toxicology supporting review for Lynparza conducted by Drs. Ricks and Chiu, and secondary memorandum and labeling provided by Dr. Palmby. I concur with Dr. Palmby's conclusion that Lynparza may be approved and that no additional nonclinical studies are needed for the proposed indication.

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/s/

JOHN K LEIGHTON
12/02/2014

MEMORANDUM

Date: December 1, 2014
From: Todd R. Palmby, Ph.D.
Pharmacology/Toxicology Supervisor
Division of Hematology Oncology Toxicology (DHOT)
Office of Hematology and Oncology Products (OHOP)
To: File for NDA 206162 Lynparza (olaparib)
Re: Approvability for Pharmacology and Toxicology
Indication: Monotherapy in patients with deleterious or suspected deleterious germline *BRCA* mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy

Non-clinical pharmacology and toxicology literature and original reports for studies to support Lynparza (olaparib) NDA 206162 monotherapy in patients with deleterious or suspected deleterious germline *BRCA* mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy were reviewed by Tiffany Ricks, PhD and Haw-Jyh Chiu, PhD. Studies conducted with olaparib and submitted in this NDA include pharmacology, pharmacokinetics and ADME, safety pharmacology, general toxicology, genetic toxicology and reproductive and developmental toxicology.

Olaparib is a poly (ADP-ribose) polymerase (PARP) inhibitor, which has in vitro activity against PARP1, PARP2 and PARP3. There are currently no poly (ADP-ribose) polymerase (PARP) inhibitors approved by the US FDA, which makes olaparib the first in this class. Original reports submitted to this NDA contained data demonstrating that olaparib inhibits growth of selected tumor cell lines in vitro and decreases tumor growth in mouse xenograft models of human cancer. Cell lines containing deficiencies in BRCA and treated with olaparib resulted in increased cytotoxicity and anti-tumor activity in in vitro studies and in vivo mouse tumor models. Multiple published articles and original study reports describing olaparib's putative mechanism of action were reviewed in support of this NDA. Based on the totality of data, olaparib-induced cytotoxicity in vitro may involve inhibition of PARP enzymatic activity and increased formation of PARP-DNA complexes, resulting in disruption of cellular homeostasis and cell death.

The Applicant provided a rationale for their selection of 400 mg twice daily as the clinical dose level to use in trials to support this NDA based on an exploratory analysis that included integration of data from non-clinical PK/PD studies. Based on a simulation conducted by the Applicant, a significant increase in single-strand DNA breaks (SSB) was only observed when poly (ADP-ribose) (PAR) levels were reduced by ≥ 90%. In a simulation of clinical unbound steady-state trough concentrations in patients who received 100, 200 and 400 mg of olaparib twice daily compared to the estimated IC_{90} values from non-clinical studies, only the

400 mg twice daily dose level resulted in all patients with trough concentrations exceeding the IC_{90} values.

General toxicology studies were conducted with oral olaparib administration for up to 26 weeks in both rats and dogs. Studies of 3-months duration would generally support marketing of a pharmaceutical intended to treat patients with advanced cancer. The studies of 26 weeks were not required or requested by the US FDA to support submission of an NDA for this indication. In general toxicology studies, the major target organ of toxicity was the hematopoietic system, which is consistent with adverse reactions reported in patients treated with olaparib. In animals, reduced circulating red blood cells and leukocyte populations correlated with microscopic findings in the bone marrow, thymus, spleen and liver. Additional toxicities in the gastrointestinal tract noted in animals were consistent with adverse reactions reported in olaparib-treated patients.

Olaparib is clastogenic, as it was positive in an in vitro mammalian chromosomal aberration assay in mammalian CHO cells and in an in vivo rat bone marrow micronucleus assay. Olaparib was not mutagenic in an in vitro bacterial reverse mutation (Ames) assay. PARP enzymes are involved in normal cellular homeostasis, such as DNA transcription, cell cycle regulation and DNA repair. Olaparib's mechanism of action involves an interaction with the enzymatic repair machinery that carries out SSB detection and repair. The Applicant's rationale for conducting clinical trials that supported this NDA submission was that combining the activity of olaparib with a deficiency in the double-strand DNA break (DSB) repair pathway, such as mutations in *BRCA*, may lead to an enhanced accumulation of double-strand DNA breaks, eventually leading to tumor cell death. Olaparib's mechanism of action is consistent with the clastogenicity observed in genetic toxicology studies, and has the potential to translate into an increased risk of developing secondary malignancies in patients treated with Lynparza. This risk may be further increased in patients with deleterious or suspected-deleterious mutations in *BRCA*. Olaparib's anti-tumor activity and its potential activity to induce secondary malignancies may be through the same mechanism. Cases of myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML) were reported in patients treated with olaparib in clinical trials. At this time it is unclear whether Lyparza treatment may increase a patient's risk for developing secondary malignancies; however, no additional non-clinical studies are warranted to support marketing for this indication.

The Applicant conducted fertility and early embryonic development studies with olaparib in rats, which were not required or requested by the US FDA to support an NDA for this indication. There were no adverse effects on mating and fertility rates in treated female rats at exposures below the human exposure at the recommended dose. Olaparib is embryotoxic and teratogenic to rats at exposures lower than human exposures at the recommended dose. In a fertility and early embryonic development study, olaparib administered to female rats prior to mating and through the first week of pregnancy caused an increase in

post-implantation loss. In an embryo-fetal development study, pregnant rats were administered oral doses of olaparib during the period of organogenesis. A dose of 0.5 mg/kg/day resulted in post-implantation loss and major malformations of the eyes, vertebrae/ribs, skull and diaphragm and additional abnormalities or variations including incomplete or absent ossification in the vertebrae/sternebrae, ribs and limbs and other findings in the pelvic girdle, lung, thymus, liver ureter and umbilical artery. Some of these findings in the eyes, ribs and ureter were observed at a dose of 0.05 mg/kg/day at lower incidence. These dose levels resulted in maternal systemic exposures significantly lower than those reported in patients receiving the recommended dose of olaparib. Based on these findings and the mechanism of action, pregnancy category D was assigned to Lynparza. If contraception methods are being considered during treatment with Lynparza, the package insert includes a recommendation to use them during treatment and for at least one month following the last dose. The duration of one month following the last dose was recommended by the Applicant based on cyclical hormone changes in females of reproductive potential.

When the primary review for NDA 206162 was completed by Drs. Ricks and Chiu, the drug product acceptance criterion for the degradant (b) (4) was not finalized. On 11/20/2014, the late-cycle meeting background package was sent to the Applicant, which included the following CMC review issue: "The proposed NMT (b) (4)% acceptance criteria for the content of the degradant, (b) (4), in olaparib capsules at end of expiry is not qualified. FDA advises that the acceptance criteria should be (b) (4)%." On 11/25/2014, the Applicant submitted a response in which they stated that a revised drug product specification would be submitted to the NDA including an acceptance criterion for (b) (4) of (b) (4)%. This is compliant with the qualification threshold in ICH Q3B and is acceptable from the pharmacology/toxicology team.

Recommendation: I concur with Dr. Ricks' and Dr. Chiu's conclusion that submitted pharmacology and toxicology data support the approval of NDA 206162 for Lynparza. There are no outstanding non-clinical issues that would preclude the approval of Lynparza for the proposed indication.

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/s/

TODD R PALMBY
12/01/2014

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application number:	206162
Supporting document/s:	1, 53
Applicant's letter date:	February 3, 2014
CDER stamp date:	February 3, 2014
Product:	Lynparza (olaparib)
Indication:	As monotherapy in patients with deleterious or suspected deleterious germline *BRCA* mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.
Applicant:	AstraZeneca Pharmaceuticals LP 1800 Concord Pike P.O. Box 8355 Wilmington, DE 19803-8355
Review Division:	Division of Hematology Oncology Toxicology (DHOT) for Division of Oncology Products 1 (DOP1)
Reviewer:	Tiffany K. Ricks, PhD Haw-Jyh Chiu, PhD
Supervisor/Team Leader:	Todd R. Palmby, PhD
Division Director:	John K. Leighton, PhD, DABT (DHOT, acting) Amna Ibrahim, MD (DOP1, acting)
Project Manager:	Rajesh Venugopal, MPH, MBA

Disclaimer

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1 Executive Summary

1.1 Introduction

Lynparza (olaparib) is an orally bioavailable poly (ADP-ribose) polymerase (PARP) inhibitor. Reports and literature for nonclinical pharmacology, pharmacokinetics, and toxicology studies were submitted and reviewed to support the original New Drug Application (NDA 206162) for Lynparza (olaparib) for the proposed indication of monotherapy for patients with deleterious or suspected deleterious germline *BRCA* mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.

1.2 Brief Discussion of Nonclinical Findings

PARP enzymes are involved in normal cellular homeostasis, such as DNA transcription, cell cycle regulation, and DNA repair. In vitro studies showed that olaparib inhibits various isoforms of PARP, including PARP1, PARP2, and PARP3. Olaparib inhibits growth of select tumor cell lines in vitro and decreases tumor growth in mouse xenograft models of human cancer both as monotherapy or following platinum-based chemotherapy. Increased cytotoxicity and anti-tumor activity following treatment with olaparib were noted in vitro and in mouse tumor models with cell lines with deficiencies in BRCA. In vitro studies have shown that olaparib-induced cytotoxicity may involve inhibition of PARP enzymatic activity and increased formation of PARP-DNA complex, resulting in disruption of cellular homeostasis and cell death. Based on the pharmacology data submitted in the NDA, the Established Pharmacology Class (EPC) of “poly (ADP-ribose) polymerase inhibitor” was determined to be both clinically meaningful and scientifically valid for olaparib.

General toxicology studies evaluated the effects of daily oral olaparib doses in rats and dogs for up to 26 weeks. The major target organ was the hematopoietic system. Reduced circulating red blood cells and leukocyte populations were reported in rats and dogs at ≥ 2% and 4% of clinical exposures at the recommended dose. Associated toxicological findings were observed in the bone marrow (atrophy, reduced hematopoiesis), thymus (atrophy, involution), spleen (pigmented macrophages) and liver (hemosiderin pigmented cells). In rats, reticulocytes and platelets were elevated at the end of dosing or recovery period, indicating a regenerative response to myelosuppression during the dosing period. Generally, the hematopoietic system was fully or partially recovered by end of the non-dosing period. In humans, olaparib caused hematological toxicities, including anemia, neutropenia, thrombocytopenia, and lymphopenia at the recommended clinical dose of 400 mg twice daily.

Additional nonclinical toxicities were reported primarily in dogs and considered minimal in severity at the doses tested. Gastrointestinal (GI) toxicities (discoloration, congestion, hemorrhage, inflammation) were noted in 4- and 26-week repeat-dose studies in dogs at ≥ 4% of the human AUC at the recommended clinical dose. Adverse reactions

reported in patients receiving olaparib in clinical trials included GI-related toxicities, such as nausea, vomiting, diarrhea, abdominal pain, and constipation. Additional nonclinical findings in the liver (congestion, hemorrhage), kidney (congestion, hemorrhage, pyelitis), and urinary bladder (congestion, hemorrhage, cystitis) were minimal in severity and without adverse correlates that would suggest there was an effect on organ function. Toxicity findings in nonclinical studies were consistent with the frequent adverse events observed in clinical trials.

Olaparib was not mutagenic in a bacterial reverse mutation (Ames) assay but was clastogenic. Consistent with the mechanism of action, olaparib induced chromosomal aberrations in mammalian CHO cells and was positive in the in vivo rat bone marrow micronucleus assay at all dose levels tested. The Applicant did not conduct carcinogenicity studies with olaparib due to its intended use in patients with advanced cancer.

The Applicant conducted fertility and early embryonic development studies with olaparib in rats and assessed reproductive organs in general toxicology studies. At the highest dose tested, oral olaparib had no adverse effects on female reproductive organs in general toxicology studies conducted in rats and dogs (approximately 18% and 26% of human AUC at recommended dose). There were also no adverse effects on mating and fertility rates in female rats when olaparib was administered for at least 14 days prior to mating through Day 6 of pregnancy (approximately 11% of human AUC at recommended dose).

In embryo-fetal development studies, olaparib was embryotoxic and teratogenic when administered to pregnant rats during the period of organogenesis. Olaparib caused 100% loss of pregnancy at maternal exposures that were approximately 2% of human AUC at the recommended dose. Major fetal malformations of the eyes, vertebra/ribs, skull, and diaphragm and minor skeletal and visceral abnormalities were reported at maternal exposures of less than 0.3% of human AUC at the recommended clinical dose. Based on these results and the mechanism of action, Pregnancy Category D is recommended, and females of reproductive potential should be advised to avoid pregnancy during treatment with olaparib.

1.3 Recommendations

1.3.1 Approvability

Recommended for approval. The reports and literature for nonclinical studies submitted to this NDA provide sufficient information to support the indication as a monotherapy in patients with deleterious or suspected deleterious germline BRCA mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy.

1.3.2 Additional Non Clinical Recommendations

Degradant of concern (see section 2.5 of this review for details):
The proposed drug product acceptance criterion for the [redacted] degradant of [redacted]% is not acceptable from a pharmacology/toxicology perspective. This level of [redacted] was not qualified by nonclinical studies or clinical trials. Based on manufacturing capabilities and discussions with the CMC review team, an information request was sent to the Applicant on 10/23/2014 to reduce the proposed drug product acceptance criterion for the degradant [redacted].

On 11/5/2014, the Applicant submitted a response to this information request, in which they proposed to lower the drug product acceptance criterion for [redacted] to [redacted]%. This level is higher than the ICH Q3B qualification threshold of [redacted]% and not qualified by nonclinical studies or clinical trials. The final acceptance criterion for [redacted] in the Lynparza drug product has not been established at this time, and discussions are ongoing with the Pharmacology/Toxicology and CMC review teams and the Applicant. This issue will be finalized subsequent to completing this review, and the conclusion will be documented in a later amendment.

1.3.3 Labeling

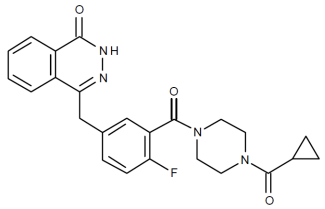
The content for the labeling of Lynparza (olaparib) is contained in this review. Based on the pharmacology data submitted in the NDA, the EPC of "poly (ADP-ribose) polymerase (PARP) inhibitor" was determined to be both clinically meaningful and scientifically valid for olaparib.

Based on analysis provided by the clinical pharmacology reviewer, the human $AUC_{(0-12)}$ of 42-44 μg·h/mL was doubled to estimate $AUC_{(0-24)}$ of 84-88 μg·h/ml in order to calculate the animal to human exposure ratios in sections 8.1 and 13.1 of the label for Lynparza.

2 Drug Information

2.1 Drug

CAS Registry Number	763113-22-0
Generic Name	Olaparib
Code Names	AZD2281, KU-0059436
Chemical Name	4-[(3-{[4-(cyclopropylcarbonyl)piperazin-1-yl]carbonyl}-4-fluorophenyl)methyl]phthalazin-1(2*H*)-one
Molecular Formula	$C_{24}H_{23}FN_4O_3$
Molecular Weight	434.46

Structure	
Pharmacological Class	Poly (ADP-ribose) polymerase (PARP) inhibitor

2.2 Relevant IND/s, NDA/s, and DMF/s

IND 75918

2.3 Drug Formulation

Olaparib is formulated in 50 mg white, opaque hard capsules.

Figure 1. Drug product composition.

Components	Quantity (mg per unit)	Function	Standard
Olaparib	50	Active ingredient	AstraZeneca
Lauroyl polyoxyl-32 glycerides[a]	(b) (4)		NF[e]
Hypromellose capsule shell[b]			AstraZeneca
Hypromellose			USP[f]
Titanium dioxide			USP
Gellan gum			NF
Potassium acetate			USP
Printing ink for capsule shell			
Shellac			NF
Ferrosoferric oxide			NF
(b) (4)			USP
Isopropyl alcohol			USP
N-butyl alcohol			NF
Propylene glycol			USP

Components	Quantity (mg per unit)	Function	Standard
[redacted]	[redacted]	[redacted]	NF
Total	595		

[a] Also known as lauroyl macrogol-32 glycerides (LMG) Ph Eur.
[b] Nominal capsule weight of [redacted] mg.
[c] [redacted]
[d] [redacted].
[e] National Formulary (NF)
[f] United States Pharmacopeia (USP)

2.4 Comments on Novel Excipients

The drug product contains [redacted] mg/capsule of a single excipient, lauroyl macrogol-32 glycerides (LMG), which was not present in olaparib formulations used in toxicology studies. At the recommended clinical dose of 400 mg twice daily, patients will receive 16 capsules containing a total daily intake of [redacted]. The Applicant cross-referenced the current LMG Drug Master File (DMF) [redacted] to provide reports for nonclinical toxicology studies conducted with LMG including genetic toxicology studies and a GLP-compliant 13-week repeat-dose toxicity study in Beagle dogs.

In genetic toxicology studies, LMG was not mutagenic in an in vitro bacterial reverse mutation (Ames) assay or in an in vitro L5178Y mouse lymphoma cell $TK^{+/-}$ forward gene mutation assay (Study No. PH 301-GAF-001-95 and PH 313-GAF-001-95). Additionally, LMG was not clastogenic in a mouse bone marrow micronucleus assay in vivo (Study No. PH-309-GAF-001-95).

In a GLP repeat-dose toxicity study, LMG was administered to Beagle dogs (3/Sex/Group) at doses of 0, 400, 1000, or 2500 mg/kg/day given as oral capsules for 13 weeks (Study No. [redacted]-261002). Toxicological endpoints included clinical signs, body weight, food consumption, hematology, clinical chemistry, urinalysis, ophthalmology, ECG, gross pathology, and histopathology. The only test article-related finding was an increased incidence of soft stool, mucoid feces, and diarrhea at the 2500 mg/kg/day dose group, consistent with the lipid composition of LMG. The no observed adverse effect level (NOAEL) was 2500 mg/kg/day, or a human equivalent dose of 81 g/day, which is well above the amount patients will receive each day at the recommended clinical dose. Therefore, the LMG excipient is adequately qualified by the submitted nonclinical toxicology studies at the level present in the Lynparza drug product.

2.5 Comments on Impurities/Degradants of Concern

The proposed specifications for two impurities were set at levels above those outlined in the ICH Q3A and Q3B guidances. [redacted] is a drug substance impurity with proposed acceptance criterion of no more than [redacted]% w/w. [redacted] was

detected in toxicology lot # C436/4 at (b) (4)%. The amount of this impurity administered to patients at the recommended clinical dose of 400 mg twice daily would be approximately (b) (4) than the levels of this impurity given at a dose level in a 4-week repeat-dose toxicity study in rats that did not result in severe toxicity (Study No. 2229/037). Therefore, the proposed drug substance acceptance criterion for (b) (4) is qualified for the recommended clinical dose.

(b) (4) is a drug product degradation product. Nonclinical toxicology studies were conducted with olaparib formulated for oral gavage, rather than the clinical capsule formulation. The (b) (4) degradant was not detected in the olaparib formulation used in toxicology studies. The proposed acceptance criterion of no more than (b) (4)% w/w is above the qualification threshold of (b) (4)% outlined in the ICH Q3B guidance at a recommended clinical dose of 400 mg twice daily. The degradation product was detected in 3 release batches at (b) (4)%. Following 36 months of storage, the degradation product was detected at (b) (4)%. As justification for the high acceptance criteria, the Applicant noted that (b) (4) is structurally similar to olaparib and referenced the ICH S9 guidance. The Applicant reported that more than 700 patients have received capsules at the recommended clinical dose of 400 mg twice daily and have been exposed to the degradation product although probably at lower levels than detected in 36-month stability studies.

2.6 Proposed Clinical Population and Dosing Regimen

The proposed clinical population is patients with deleterious or suspected deleterious germline BRCA mutated (as detected by an FDA-approved test) advanced ovarian cancer who have been treated with three or more prior lines of chemotherapy. The recommended dose is 400 mg orally, twice daily (800 mg/day).

2.7 Regulatory Background

Olaparib is a first-in-class, new molecular entity which has not been previously approved or marketed in the United States or any other country. A pre-NDA meeting was held on October 2, 2013 between the Applicant and FDA.

3 Studies Submitted

3.1 Studies Reviewed

Pharmacology

Study Number	Study Title	eCTD Section
Pharmacology Report 27	A study of PK, PD and efficacy using different olaparib (AZD2281) doses in the TNBC PTX in vivo model HBCx-10.	4.2.1.1.
Pharmacology Report 28	A study examining the effect of dose and scheduling of olaparib (AZD2281) on both initial	4.2.1.1.

Study Number	Study Title	eCTD Section
	tumour regression and progression treatment using the TNBC PTX in vivo model HBCx-10.	
Pharmacology Report 01	Profiling the KuDOS cell line panel for in vitro sensitivity to PARP inhibitor olaparib (AZD2281; KU-0059436) using a colony formation assay (CFA).	4.2.1.1.
Pharmacology Report 01 1b	The in vitro sensitivity of cell lines in response to PARP inhibitor AZD2281 (KU-0059436) in combination with DNA-damaging chemotherapeutic agents.	4.2.1.1.
Pharmacology Report 12	Summary of inhibition of PARP activity by AZD2281 (KU-0059436) in SW620 colorectal cell lines in vitro using an ex vivo electrochemiluminescence assay.	4.2.1.1.
Pharmacology Report 22	Correlation of olaparib and platinum agent response in vitro and in vivo.	4.2.1.1.
Pharmacology Report 24	In vitro activity of olaparib across a panel of cancer cell lines.	4.2.1.1.
Pharmacology Report 31	Activity of PARP inhibitors AZD2281 (olaparib) and AZD2461 against PARP-3.	4.2.1.1.
0818SY	AZD2281: Selectivity screening in radioligand binding, enzyme and electrophysiological assays in vitro.	4.2.1.2.
8157	In vitro pharmacology: phosphodiesterase assays.	4.2.1.2.
8234	In vitro pharmacology: (b) (4) – study of KU0059436 and (b) (4).	4.2.1.2.
0242SZ	AZD2281: Effects on Human Ether-a-go-go-related Gene (hERG) encoded potassium channel in vitro.	4.2.1.3.
2229/047	KU0059436: Effects on general activity and behavior in the rat following oral administration.	4.2.1.2.
2229/053	KU0059436: Cardiovascular and respiratory effects in the anesthetized dig following intravenous administration.	4.2.1.3.

Pharmacokinetics

Study Number	Study Title	eCTD Section
D2281 KKR007	[^{14}C]-KU-0059436 – a study of absorption, metabolism and excretion following oral and intravenous administration to the rat.	4.2.2.2.
D2281 KMD008	[^{14}C]-KU-0059436 – a study of absorption and excretion following oral and intravenous administration to the dog.	4.2.2.2.
D2281 KPM035	AZD2281: Pharmacokinetic study in the mouse following intravenous injection and oral (gavage) administration.	4.2.2.2.

Study Number	Study Title	eCTD Section
KMM016	[^{14}C]-KU-0059436 – Quantitative whole body autoradiography study in tumour bearing nude mice.	4.2.2.3.
KMR004	Tissue distribution of radioactivity in the rat following oral administration of [^{14}C]-KU-0059436 by quantitative whole-body autoradiography.	4.2.2.3.
KMR017	[^{14}C]-KU-0059436 (b) (4): Preliminary study to investigate absorption, distribution, metabolism and excretion following oral administration in the rat.	4.2.2.3.
D2281 KMR027	AZD2281: The disposition of [^{14}C]-AZD2281 in the rat.	4.2.2.3.
D2281 KPJ043	The binding of [^{14}C]-AstraZeneca AZD2281 to proteins in human plasma from healthy volunteers before and following multiple dosing with (b) (4)	4.2.2.3.
ADME_186	Metabolite characterization: Correction report for data interpretation of metabolites M11b, M18, M39 and M15.	4.2.2.4.
ADME_426	Metabolite characterization: Correction report for data interpretation of metabolites M17 and M18.	4.2.2.4.
KMN018	KU0059436 Metabolite investigation.	4.2.2.4.
D2281 KMX033	AstraZeneca AZD2281. The profiling and characterization of metabolites in plasma following multiple oral administration of AstraZeneca AZD2281 to patients.	4.2.2.4.

Toxicology

Study Number	Study Title	eCTD Section
TII0011	26 Week oral (gavage) toxicity study in the dog.	4.2.3.2.
2229/38	KU-0059436: 28 Day oral (gavage) administration toxicity study in the dog followed by a 4 week treatment-free period.	4.2.3.2.
TII0012	26 Week oral (gavage) toxicity study in the rat.	4.2.3.2.
1858KR	AZD2281: One month compound-batch comparison oral toxicity study in the rat.	4.2.3.2.
2229/037	KU-0059436: 28 Day oral (gavage) administration toxicity study in the rat followed by a 28 day treatment free recovery period.	4.2.3.2.
KUD-BIO-2229040	Determination of KU-0059436 in rat plasma samples and PK analysis from study 2229/040.	4.2.3.2.
TII0007	Bacterial reverse mutation test for KU-0059436 (b) (4) (b) (4).	4.2.3.3.
TII0008	In vitro mammalian cell cytogenetic test: Chinese	4.2.3.3.

Study Number	Study Title	eCTD Section
	Hamster Ovary cells.	
775498	Ku-0059436 [redacted]: Micronucleus test in bone marrow cells of CD rats 0 h + 24 h oral dosing and 48 h sampling.	4.2.3.3.
1557GR	AZD2281: Oral fertility and early embryonic development study in the female rat.	4.2.3.5.
1558GR	AZD2281: Oral fertility and early embryonic development study in the male rat.	4.2.3.5.
1555RR	AZD2281: Oral dose range finding embryofetal development study in the rat.	4.2.3.5.
1556TR	AZD2281: Oral embryofetal development study in the rat.	4.2.3.5.
[redacted]-261002	13-week oral subchronic toxicity study of [redacted] in dogs	LMG DMF [redacted]
PH 301-GAF-001-95	Ames/Salmonella-E. coli reverse mutation assay on [redacted]	LMG DMF [redacted]
PH 313-GAF-001-95	L5178Y mouse lymphoma cell $TK^{+/-}$ forward gene mutation assay on [redacted]	LMG DMF [redacted]
PH-309-GAF-001-95	In vivo micronucleus test with [redacted] in mouse bone marrow erythropoietic cells	LMG DMF [redacted]

3.2 Studies Not Reviewed

Pharmacokinetics

Study Number	Study Title	eCTD Section
D2281 KPV001	Validation for the determination of KU-0059436 (AZD2281) in rat plasma using solid phase extraction and liquid chromatography with tandem mass spectrometric detection together with an investigation into the frozen matrix stability at nominal -20°C.	4.2.2.1.
2229/042	Validation of an analytical procedure for the determination of KU-0059436 in dog plasma samples using manual solid phase extraction and liquid chromatography with tandem mass spectrometric detection.	4.2.2.1.
2229/043	Validation of an analytical procedure for the determination of KU-0059436 in rat plasma samples using manual solid phase extraction and liquid chromatography with tandem mass spectrometric detection.	4.2.2.1.
D2281 KPV034	AZD2281: Validation of an analytical procedure for the determination of AZD2281 in mouse plasma	4.2.2.1.

Study Number	Study Title	eCTD Section
	using solid phase extraction and liquid chromatography with tandem mass spectrometric (LC-MS/MS) together with an investigation of the stability of AZD2281 in mouse plasma stored at nominal -20°C.	
D2281 KMX045	AstraZeneca AZD2281. An investigation into the absorption of (b) (4) following multiple oral dosing to healthy human volunteers.	4.2.2.2.
Olaparib SimCYP1	Simulating the induction of CYP3A4/5 by rifampicin and the effect on the exposure of olaparib using SimCYP (version 12) based on data from in vitro studies and clinical studies.	4.2.2.7.
Olaparib SimCYP2	Simulating the inhibition of CYP3A4/5 by itraconazole and the effect on the exposure of olaparib using SimCYP (version 12) based on data from in vitro studies and clinical studies.	4.2.2.7.

Toxicology

Study Number	Study Title	eCTD Section
2229/036	(b) (4) (Ku-0059436): Single dose intravenous toxicity study in the mouse.	4.2.3.1.
2229/035	(b) (4) (Ku-0059436): Single dose oral toxicity study in the mouse.	4.2.3.1.
2229/034	(b) (4) (Ku-0059436): Single dose intravenous toxicity study in the rat.	4.2.3.1.
2229/033	(b) (4) (Ku-0059436): Single dose oral toxicity study in the rat.	4.2.3.1.
2229/044	(b) (4): Single dose oral toxicity study in the rat.	4.2.3.1.
2229/39	KU-0059436: Bioavailability study and maximum tolerated dose (MTD) followed by a 7 day fixed dose oral (gavage) administration toxicity study in the dog.	4.2.3.2.
2229/046	KU-0059436:7 Day oral (gavage) administration study in the dog followed by a 21 day treatment free recovery period.	4.2.3.2.
2229/040	KU-0059436: 7 Day oral (gavage) administration study in the rat followed by a 21 day treatment free recovery period.	4.2.3.2.
11296	Myelotoxicity studies with a variety of experimental compounds.	4.2.3.7.
ASU0117	AZD2281: 14 Day oral (gavage) tolerability and toxicokinetic study in the mouse.	4.2.3.5.

3.3 Previous Reviews Referenced

None

4 Pharmacology

4.1 Primary Pharmacology

In vitro, olaparib has been shown to inhibit PARP enzymatic activity as measured by inhibition of PAR synthesis using enzyme assays or in vitro cellular assays (Menear, et al., 2008). In these assays, olaparib inhibited PARP1 and PARP2 with IC_{50} values of 5 nM and 1 nM, respectively. In a separate experiment, olaparib inhibited PARP3-mediated PAR expression (IC_{50} = 4 nM) using an in vitro enzymatic assay followed by immunoblotting (Pharmacology Report 31).

Study title: Profiling the KuDOS cell line panel for in vitro sensitivity to PARP inhibitor olaparib (AZD2281; KU-0059436) using a colony forming assay (CFA).

Study no.:	Pharmacology Report 01 (KDRI No. KTS00101)
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	KuDOS Pharmaceuticals Ltd.
Date of study initiation:	October 1, 2007
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib, Batch No. 9 (% purity not reported)

The objective of the study was to evaluate the effect of olaparib on cell viability in a panel of 95 cells lines of colorectal, breast, ovarian, pancreatic, head and neck squamous cell carcinoma, and non-small cell lung cancer origin. In brief, cell lines were incubated in 10% (v/v) DMSO (vehicle control) or 0.123, 0.370, 1.111, 3.333, 10.00 μM olaparib at 37° C until > 50 colonies have formed (6 - 28 days). Seeding cell density was determined from preliminary experiments and is defined as seeding density producing linear cell growth approximately 7 – 14 days after cell incubation in 10% (v/v) DMSO (vehicle control). Cell viability was determined using the colony formation assay by counting Giemsa-stained colonies using Colcount software (Oxford Optronix). IC_{50} values were defined as the olaparib concentrations producing 50% cell viability when compared to vehicle control cells (100% cell viability).

Summary of results:
Olaparib inhibited colony formation (based on an Applicant-specified cut-off IC_{50} value of 1.5 μM) in a variety of cells lines, including breast, ovarian, pancreatic, non-small cell lung, and colorectal cancer, and head and neck small-cell carcinoma.

Figure 2. Effect of olaparib on cell viability in a panel of human cancer cell lines (Pharmacology Report 01).

Tumour Type	Cell Line Name	Comments	Cell Seeding number	Incubation time (days)	n=1	n=2	n=3	Mean AZD2281 IC50 (μM)	SD	AZD2281 Sensitivity [1.5μM] cut-off
Breast	MDA-MB-436		500	13	0.033	0.015	0.007	0.018	0.013	Responder
Breast	HCC1395		5000	18	0.031	0.009		0.020	0.016	Responder
Ovarian	59M		2000	20	0.013	0.019	0.030	0.021	0.009	Responder
Pancreatic	CAPAN-1	scored by eye	4500	19	0.014	0.018	0.041	0.024	0.015	Responder
Breast	SUM1315MO2		2000	13	0.043	0.023	0.030	0.032	0.010	Responder
Breast	HCC1187		14000	14	0.171	0.044	0.023	0.079	0.080	Responder
Non-Small Lung	NCI-H1838		4000	12	0.075	0.047	0.135	0.086	0.045	Responder
Non-Small Lung	HOP-92		1000	8	0.146	0.057	0.128	0.110	0.047	Responder
Breast	SUM149PT		1000	7	0.142	0.064	0.169	0.125	0.055	Responder
Colorectal	SKCO1		2000	13	0.182	0.132	0.157	0.157	0.025	Responder
Colorectal	HCT116		1250	7	0.190	0.221	0.182	0.198	0.021	Responder
Ovarian	OVCAR-3		1000	7	0.159	0.279	0.226	0.221	0.060	Responder
Head&Neck SCC	PE/CA-PJ41	scored by eye	1000	15	0.343	0.426		0.385	0.059	Responder
Ovarian	IGROV-1		1000	7	0.598	0.358	0.218	0.391	0.192	Responder
Breast	MDA-MB-453		2000	14	0.554	0.327	0.316	0.399	0.134	Responder
Head&Neck SCC	RPMI2650		1500	13	0.434	0.335	0.647	0.472	0.159	Responder
Head&Neck SCC	KYSE-30		600	7	0.453	0.897	0.206	0.519	0.350	Responder
Head&Neck SCC	Hs840.T	judged by eye	2000	9	0.300	1.000	0.300	0.533	0.404	Responder
Ovarian	TOV-21G		750	6	0.464	0.716	0.523	0.568	0.132	Responder
Breast	HCC1569		1500	16	0.566	0.501	0.655	0.574	0.077	Responder
Ovarian	OAW28		2000	9	0.590	0.782	0.415	0.596	0.184	Responder
Non-Small Lung	NCI-H23		2000	9	0.733	0.673	0.472	0.626	0.137	Responder
Ovarian	A2780		1000	7	0.774	0.523	0.590	0.629	0.130	Responder
Ovarian	OV90		1500	13	0.978	0.591	0.493	0.687	0.256	Responder
Colorectal	LS180		1250	9	0.494	0.590	1.110	0.731	0.331	Responder
Colorectal	HCA7		1500	8	0.748	0.609	0.851	0.736	0.121	Responder
Non-Small Lung	NCI-H1755		1500	13	0.307	1.017	1.080	0.801	0.429	Responder
Breast	MDA-MB-468		4000	9	0.635	1.094	0.958	0.896	0.236	Responder
Non-Small Lung	DMS114		2500	12	0.743	0.506	1.474	0.908	0.505	Responder
Breast	BT-20		8000	18	0.848	0.768	1.143	0.920	0.198	Responder
Colorectal	LOVO		2000	6	0.681	1.349	1.060	1.030	0.335	Responder
Head&Neck SCC	PE/CA-PJ34		2000	8	1.242	0.888	1.233	1.121	0.202	Responder
Breast	MDA-MB-361		3000	28	0.994	1.411	1.062	1.156	0.224	Responder
Head&Neck SCC	Detroit 562	scored by eye	750	28	1.460	1.957	0.311	1.243	0.844	Responder
Colorectal	SW620		2000	9	1.444	1.269	1.203	1.305	0.125	Responder
Head&Neck SCC	FaDu		750	9	1.195	1.120	1.654	1.323	0.289	Responder
Head&Neck SCC	CAL27		500	7	1.436	1.366	1.493	1.432	0.064	Responder
Pancreatic	T3M4		3500	9	1.799	1.932	0.659	1.463	0.700	Responder

Tumour Type	Cell Line Name	Comments	Cell Seeding number	Incubation time (days)	n=1	n=2	n=3	Mean AZD2281 IC50 (μM)	SD	AZD2281 Sensitivity [1.5μM] cut-off
Breast	MCF7		1500	6	1.584	1.197	2.589	1.790	0.719	Non-Responder
Non-Small Lung	CALU-3		4000	16	1.509	2.721	1.735	1.988	0.644	Non-Responder
Non-Small Lung	NCI-H522		1500	6	1.961	1.477	2.830	2.089	0.686	Non-Responder
Breast	HCC38		8000	13	2.211	1.709	2.861	2.260	0.578	Non-Responder
Breast	HCC1806		3000	8	2.603	2.112	2.190	2.302	0.264	Non-Responder
Ovarian	OVCAR-8		750	7	2.040	2.569	2.414	2.341	0.272	Non-Responder
Pancreatic	PSN-1		1500	6	2.457	2.281	2.297	2.345	0.097	Non-Responder
Pancreatic	PANC-1		1000	7	2.814	2.011	2.346	2.390	0.403	Non-Responder
Pancreatic	BxPC3		2000	10	2.895	2.606	1.812	2.438	0.561	Non-Responder
Head&Neck SCC	Hep-2		750	7	2.456	2.858	2.165	2.493	0.348	Non-Responder
Breast	CAL51		500	6	2.833	3.290	1.657	2.593	0.842	Non-Responder
Ovarian	PA-1		1500	6	3.109	2.767	2.471	2.782	0.319	Non-Responder
Ovarian	TOV-112D		750	9	3.479	2.476	2.466	2.807	0.582	Non-Responder
Breast	HCC1937		1500	10	3.302	2.680	2.478	2.820	0.429	Non-Responder
Head&Neck SCC	SW579		500	8	1.353	3.181	3.941	2.825	1.330	Non-Responder
Breast	MDA-MB-435S		3000	9	2.332	2.324	4.191	2.949	1.076	Non-Responder
Breast	T47D		750	12	3.343	2.212	3.518	3.024	0.709	Non-Responder
Non-Small Lung	HOP-62		2000	8	2.220	3.415	3.564	3.066	0.737	Non-Responder
Colorectal	SW948		2000	13	3.304	3.990	3.005	3.433	0.505	Non-Responder
Non-Small Lung	NCI-H322M		4000	14	3.941	2.966	3.703	3.537	0.508	Non-Responder
Non-Small Lung	H460		750	6	4.731	3.172	3.550	3.818	0.813	Non-Responder
Head&Neck SCC	KB		300	7	3.546	5.040	4.566	4.384	0.763	Non-Responder
Breast	BT549		1500	9	3.138	4.967	5.410	4.505	1.204	Non-Responder
Colorectal	BE		750	6	4.127	4.950	4.890	4.656	0.459	Non-Responder
Breast	HCC1954		2000	12	5.676	3.832	4.878	4.795	0.925	Non-Responder
Breast	SK-BR-3		1500	13	5.461	4.240		4.851	0.863	Non-Responder
Ovarian	OVCAR-4		2500	8	4.960	5.063	4.514	4.852	0.299	Non-Responder
Colorectal	HT29		1250	9	4.869	5.147	4.918	4.978	0.148	Non-Responder
Breast	HCC1143		4000	13	4.589	6.022	4.845	5.152	0.764	Non-Responder
Head&Neck SCC	PE/CA-PJ15		500	6	5.968	4.669	5.038	5.225	0.669	Non-Responder
Ovarian	SKOV3		500	6	5.624	5.731	5.170	5.508	0.298	Non-Responder
Colorectal	RKO		700	7	7.341	4.681	4.910	5.644	1.474	Non-Responder
Colorectal	SW1417		1500	14	3.996	6.884	6.109	5.663	1.495	Non-Responder
Breast	Hs578T		1500	9	5.685	5.820	5.547	5.684	0.137	Non-Responder
Colorectal	SW403	judged by eye	4000	21	6.000	6.000		6.000	0.000	Non-Responder
Pancreatic	ASPC-1		1500	10	5.395	7.540	5.745	6.227	1.151	Non-Responder
Ovarian	OAW42		400	6	6.819	6.129	6.009	6.319	0.437	Non-Responder
Non-Small Lung	NCI-H810		500	6	5.548	6.972	6.958	6.493	0.818	Non-Responder
Pancreatic	HUPT4		1500	8	5.062	7.516	7.360	6.646	1.374	Non-Responder

Tumour Type	Cell Line Name	Comments	Cell Seeding number	Incubation time (days)	n=1	n=2	n=3	Mean AZD2281 IC50 (μM)	SD	AZD2281 Sensitivity [1.5μM] cut-off
Ovarian	OVCAR-5		750	7	5.835	6.662	7.635	6.711	0.901	Non-Responder
Non-Small Lung	CALU-6		4000	6	7.128	6.289	7.585	7.001	0.657	Non-Responder
Pancreatic	PANC-89		2000	6	7.108	8.372	6.993	7.491	0.765	Non-Responder
Head&Neck SCC	HN5 (LICR-LON-HN5)		1000	7	9.143	7.135	7.243	7.840	1.129	Non-Responder
Pancreatic	CFPAC-1		1000	9	8.499	7.690	7.806	7.998	0.437	Non-Responder
Non-Small Lung	A549		500	6	8.423	7.161	8.676	8.087	0.811	Non-Responder
Non-Small Lung	NCI-H226		1500	8	8.025	8.636	7.626	8.096	0.509	Non-Responder
Breast	MDA-MB-231		1000	6	8.202	9.134	7.503	8.280	0.818	Non-Responder
Colorectal	HCT15		1000	7	10.000	10.000	6.260	8.753	2.159	Non-Responder
Non-Small Lung	NCI-H1299		500	6	8.025	9.415	9.945	9.128	0.992	Non-Responder
Breast	BT474		3000	16	9.736	8.635		9.186	0.779	Non-Responder
Head&Neck SCC	OE21		750	6	9.020	10.000	10.000	9.673	0.566	Non-Responder
Colorectal	SW480		1500	8	10.000	10.000	10.000	10.000	0.000	Non-Responder
Breast	ZR-75-1		1000	13	10.000	10.000		10.000	0.000	Non-Responder
Pancreatic	Hs766T		1000	10	10.000	10.000	10.000	10.000	0.000	Non-Responder
Pancreatic	HPAF-II		750	9	10.000	10.000	10.000	10.000	0.000	Non-Responder
Pancreatic	MIAPACA-2		500	6	10.000	10.000	10.000	10.000	0.000	Non-Responder
Head&Neck SCC	PE/CA-PJ49		750	9	10.000	10.000	10.000	10.000	0.000	Non-Responder

[Excerpted from Applicant's submission]

Conclusions:
In vitro, treatment with olaparib resulted in decreased cell viability, as measured by the colony formation assay, in cell lines from breast, ovarian, pancreatic, non-small cell lung, and colorectal cancers, and head and neck small-cell carcinoma.

Study title: The in vitro sensitivity of cell lines in response to PARP inhibitor AZD2281 (KU-0059436) in combination with DNA-damaging chemotherapeutic agents.

Study no.:	Pharmacology Report 11b (KDRI No. KTS0048)
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	KuDOS Pharmaceuticals Ltd.
Date of study initiation:	December 9, 2004
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib (lot # and % purity not reported)

The objective of the study was to evaluate the effect of olaparib in combination with methanemethylsulfate (MMS) on cell viability of SW620 human colorectal adenocarcinoma cell line. MMS is an alkylating agent which causes cell death in cells with defects in homologous-recombinant repair mechanism. Briefly, SW620 were first treated with 0, 1, 3, 10, 30, or 300 nM olaparib one hour prior to treatment with 0, 3, 5, 7, 10, 12, or 15 μg/mL MMS. Following 4-day incubation, cell viability (including IC_{50} and PF_{50} values) was analyzed using a sulphorhodamine-B (SRB) dye-based assay. SRB dye binding to protein components of cells which indirectly correlates with viable cell number. IC_{50} values were defined as the olaparib concentrations producing 50% cell viability when compared to vehicle control cells (100% cell viability). PF_{50} (potentiation factor at 50% cell viability) was defined as the ratio of the IC_{50} for MMS alone vs MMS in combination with a single concentration of olaparib.

Summary of results:

- Olaparib treatment alone did not have any effects on cell growth of SW620 cells.
- MMS treatment either alone or in combination with olaparib resulted in concentration-dependent decreases in cell growth of SW620 cells.
- Olaparib pre-treatment resulted in increased MMS-mediated decreases in cell growth.

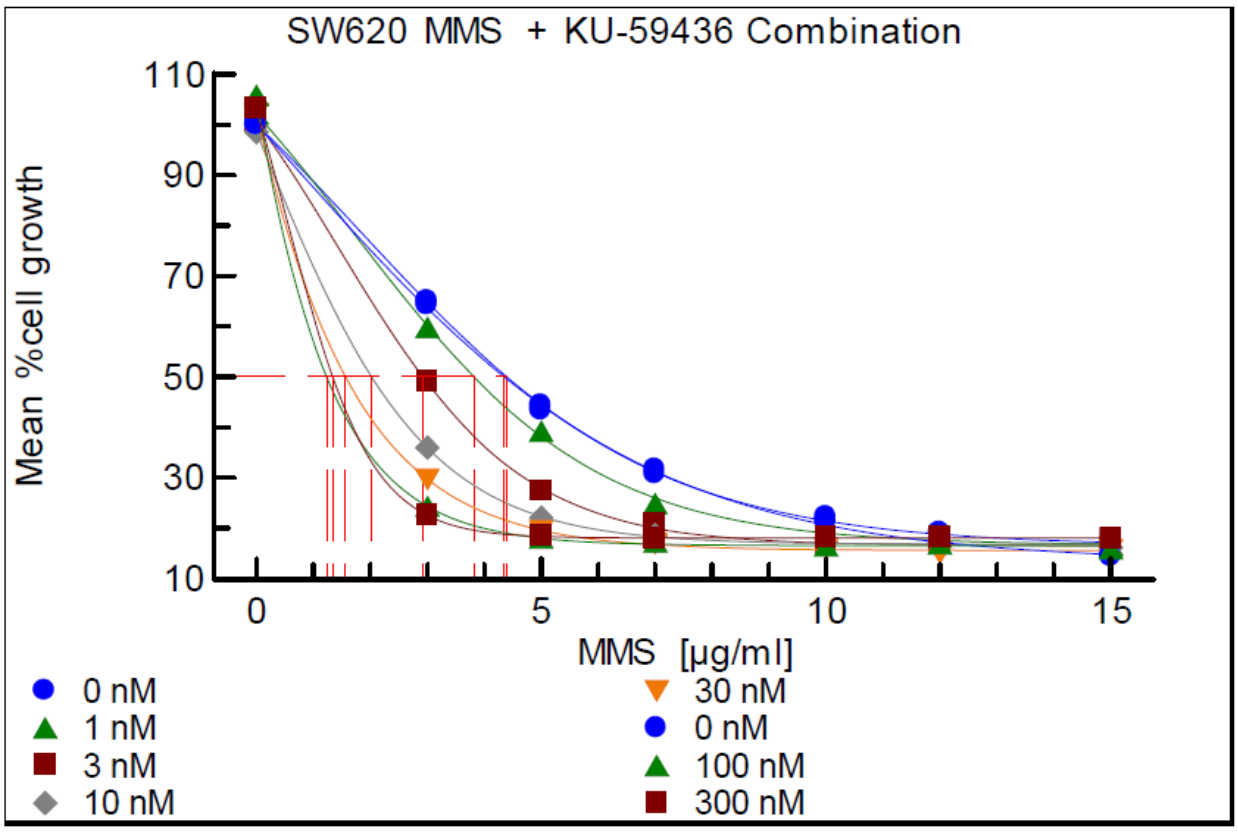
Figure 3. Effect of olaparib (KU59436), MMS, or olaparib + MMS on cell growth in SW620 cells (Pharmacology Report 11b).

Mean %cell growth

MMS [µg/ml]	KU59436 [nM]			
	0	1	3	10
0.00	100.000	102.085	100.592	98.548
3.00	65.049	59.792	48.937	35.973
5.00	44.345	39.185	27.357	22.067
7.00	30.732	24.826	20.866	18.595
10.00	22.181	19.098	16.842	17.199
12.00	19.114	18.238	16.842	17.199
15.00	16.534	16.534	16.177	16.096

Mean %cell growth

MMS [µg/ml]	KU59436 [nM]			
	0	30	100	300
0.000	100.000	102.201	105.785	103.166
3.000	64.232	29.710	24.300	22.526
5.000	43.362	19.505	18.003	18.396
7.000	31.621	16.980	17.253	17.867
10.000	21.160	15.990	16.433	18.191
12.000	17.270	15.000	16.809	18.191
15.000	14.386	15.717	15.904	17.935



PARP [nM]	MMS IC50 [µg/ml]
0	4.395
1	3.814
3	2.919
10	2.023

PARP [nM]	MMS IC50 [µg/ml]
0	4.349
30	1.563
100	1.232
300	1.339

PF50 for MMS @ [nM] KU-0059436	PF50
0	1.000
1	1.152
3	1.506
10	2.172
30	2.783
100	3.530
300	3.248

[Excerpted from Applicant's submission]

Conclusions:
In vitro, olaparib treatment alone did not have any effects on cell growth of SW620 cells, which is a cell line not deficient in BRCA homologous recombination-mediated DNA repair. However, olaparib potentiated the cell growth inhibitory activity of the alkylating agent MMS, under the testing conditions in this study.

Study title: Summary of inhibition of PARP activity by AZD2281 (KU-0059436) in SW620 colorectal cell lines in vitro using an ex vivo electrochemiluminescence assay.

Study no.:	Pharmacology Report 12 (KDRI No. KTS0049)
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	KuDOS Pharmaceuticals Ltd.
Date of study initiation:	March 26, 2007
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	AZD2281 (lot # and % purity not reported)

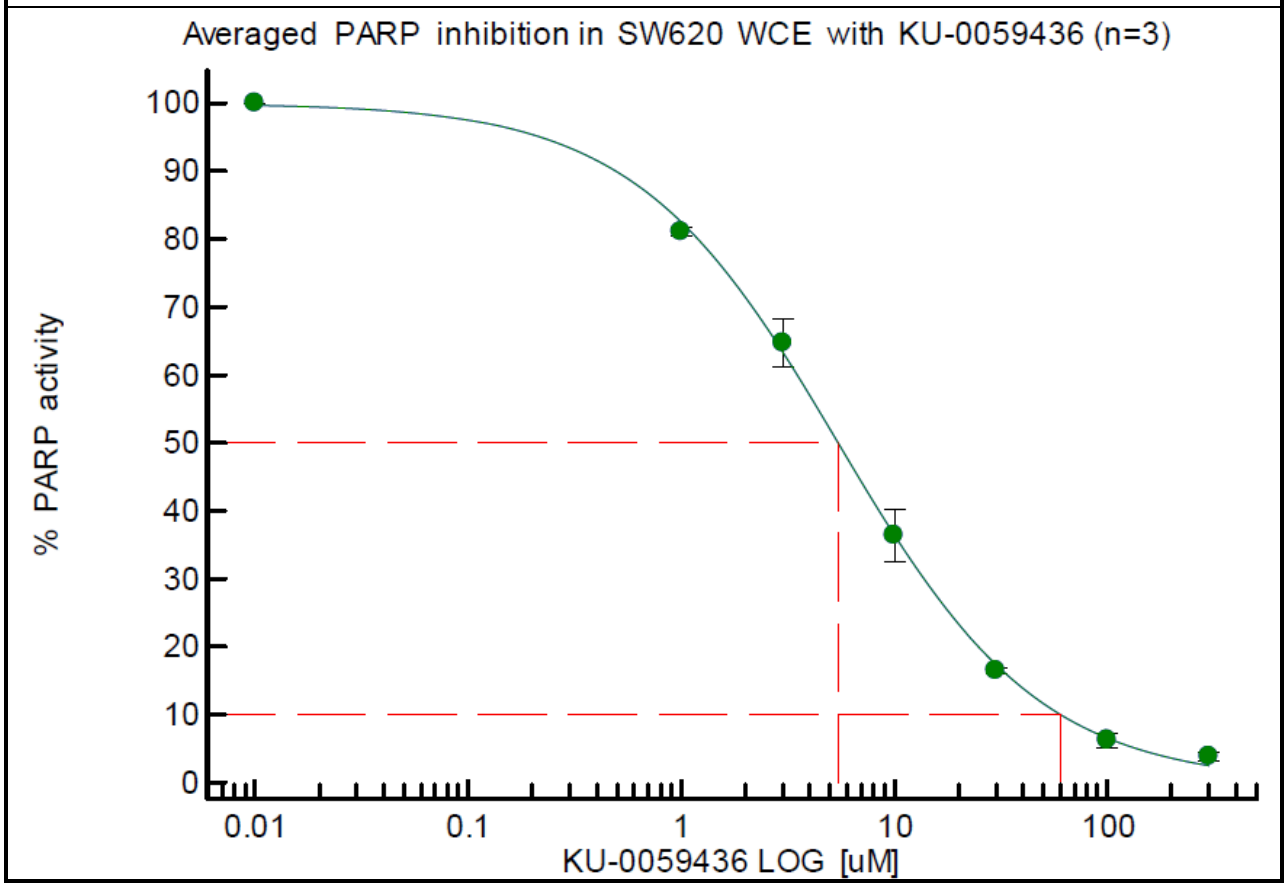
The objective of the study was to evaluate the PARP inhibitory activity of olaparib in SW620 human colon adenocarcinoma cell line. Briefly, SW620 extracts (lysates) were incubated with 0 – 300 nM olaparib for 5 minutes at 30°C in the presence of deoxyribonucleic acid. The activity of PARP was measured using an immunoassay detecting formation of poly ADP-ribose (PAR), a downstream product of PARP activation.

Summary of results:
- Olaparib inhibited formation of PAR in SW620 extracts in a dose-dependent manner.
- IC_{50} and IC_{90} values were determined to be 5.438 nM and 60.08 nM, respectively.

Figure 4. Inhibition of PARP activity by olaparib in SW620 cell extracts (Pharmacology Report 12).

Average % PARP activity (n=3)

KU-59436 [nM]	average	SD	SEM	%CV
0	100.00	0	0	0
1	81.05	0.606244	0.350015	0.747981
3	64.69	3.558911	2.054738	5.501309
10	36.36	3.809246	2.199269	10.47558
30	16.46	0.367593	0.21223	2.233009
100	6.22	1.104589	0.637735	17.74673
300	3.80	0.5621	0.324528	14.79657



[Excerpted from Applicant's submission]

Conclusions:
Olaparib inhibited PARP activity in SW620 cell extracts in a dose-dependent manner, with IC_{50} and IC_{90} values of 5.438 nM and 60.08 nM, respectively.

Study title: Correlation of olaparib and platinum agent response in vitro and in vivo

Study no.:	Pharmacology Report 22 (KDRI No. KTS0049)
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	AstraZeneca, Alderley Park (in vitro) [redacted] (b) (4) (in vivo) [redacted] (b) (4) (in vivo)
Date of study initiation:	September 1, 2008
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	AZD2281, Batch No. 2 (in vitro study; % purity not reported), Batch No. [redacted] (b) (4) KU-0059436, C463/4 (in vivo study; % purity not reported)

The objective of this study was to evaluate whether cell lines and tumors which were sensitivity to platinum-based chemotherapies were also sensitive to olaparib. Briefly, in vitro, cell viability was assessed in a panel of breast, head and neck squamous cell carcinoma, non-small cell lung cancer and ovarian cancer cell lines using CFA and, in vivo, anti-tumor activity was evaluated in patient derived mouse tumor models of NSCLC and triple negative breast cancer.

Figure 5. In vivo patient-derived tumor models (Pharmacology Report 22).

Patient-derived tumor	Summary of phenotype	Chemotherapy sensitivity
HBCx-10 (Breast ductal adenocarcinoma)	Mutated BRCA2, mutated TP53, no HER2 overexpression and no PR/ERa overexpression.	Responsive to adriamycin, cyclophosphamide, capecitabine and cisplatin. Resistant to docetaxel.
HBCx-17 (Breast ductal adenocarcinoma)	Mutated BRCA2, mutated TP53, no RB expression, no HER2 overexpression and no PR/ERa overexpression.	Responsive to adriamycin, cyclophosphamide, capecitabine and cisplatin. Resistant to docetaxel.
HBCx-9 (Breast ductal adenocarcinoma)	Mutated TP53, no HER2 overexpression and no PR/ERa overexpression.	Responsive to adriamycin, cyclophosphamide, capecitabine and docetaxel, and cisplatin.
Lu7433	Mutated TP53 and EGFR.	Responsive to paclitaxel

Patient-derived tumor	Summary of phenotype	Chemotherapy sensitivity
(Lung squamous cell carcinoma)		and cisplatin.
Lu7414 (Lung squamous cell carcinoma)	Not reported.	Responsive to gemcitabine, paclitaxel, erlotinib, and cetuximab. Resistant to cisplatin.

Summary of results:

- In vitro, statistically significant correlation in sensitivity to olaparib, cisplatin, and carboplatin in a panel of cancer cell lines.
- In vivo, anti-tumor activity of olaparib, either alone or in combination with platinum agents, was noted in patient-derived TNBC tumor models HBCx-10 and HBCx-17, but not HBCx-9.
- In vivo, anti-tumor activity was noted for NSCLC patient-derived tumor models Lu7433, but not Lu7414.

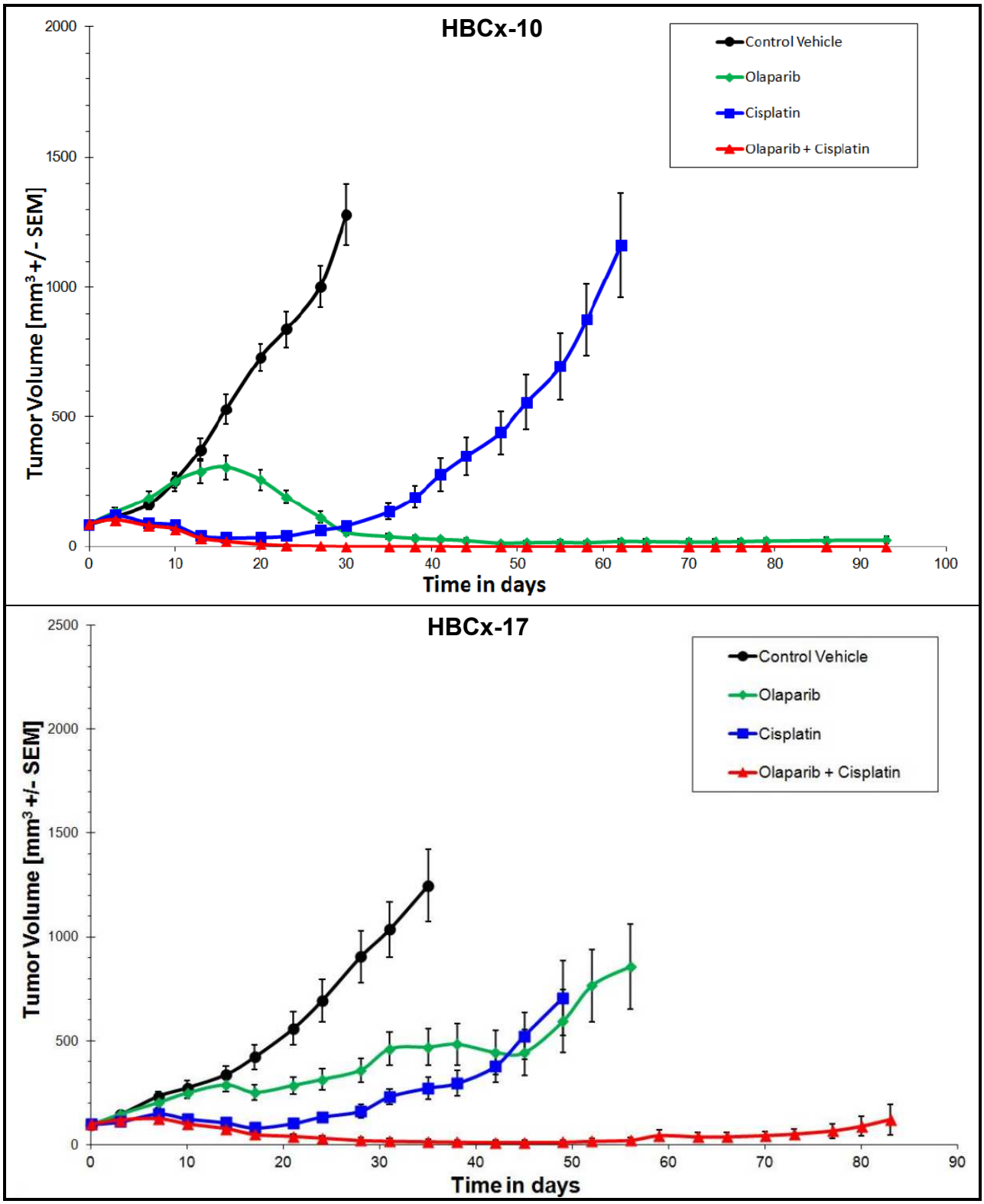
Figure 6. Correlation matrix between olaparib, cisplatin, and carboplatin, in vitro (Pharmacology Report 12).

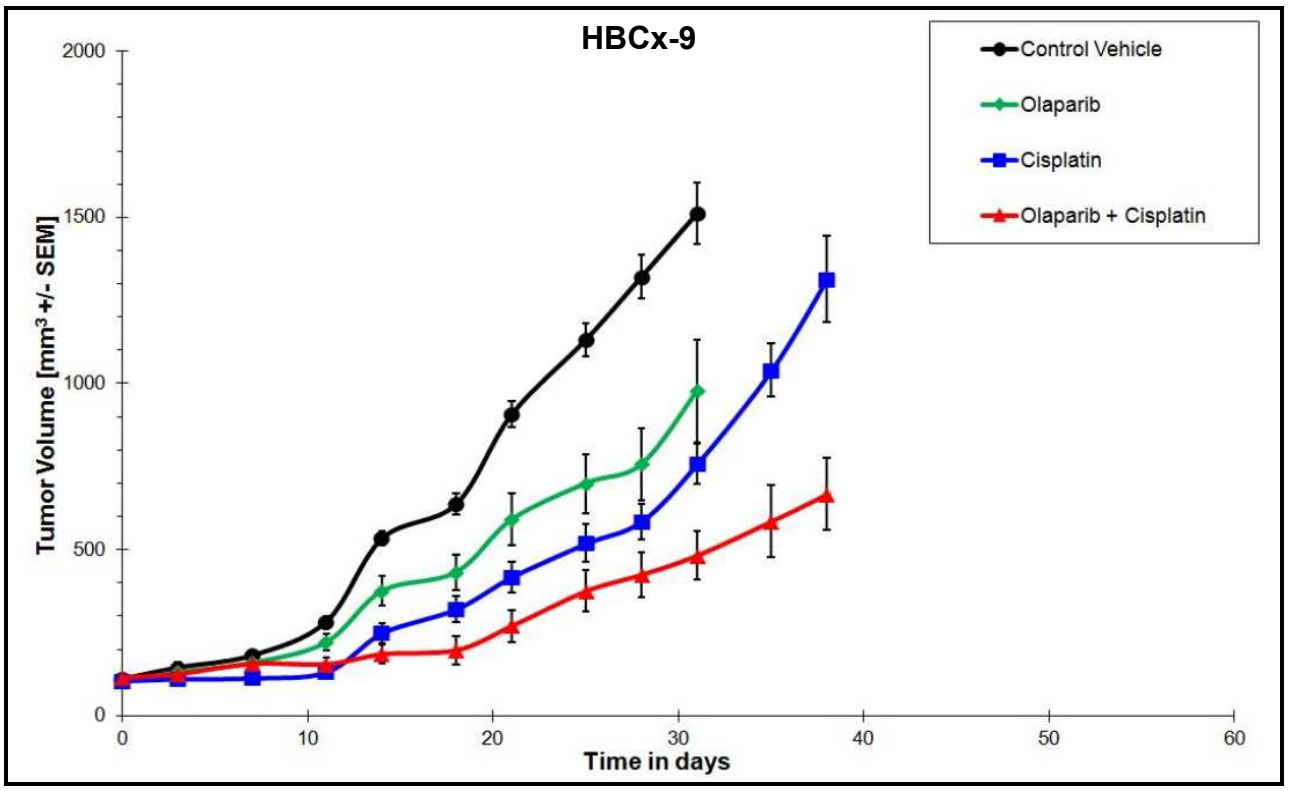
	HNSCC	NSCLC	Ovarian	Breast
Olaparib Cisplatin	0.873 p=0.00044	0.908 p=0.00028	0.739 p=0.0093	NT
Olaparib Carboplatin	0.889 p=0.00025	0.958 p=0.00001	0.856 p=0.0007	0.842 p=0.0006
Cisplatin Carboplatin	0.942 p=0.00001	0.952 p=0.00002	0.969 p=0.0000008	NT

NT =Not tested

[Excerpted from Applicant's submission]

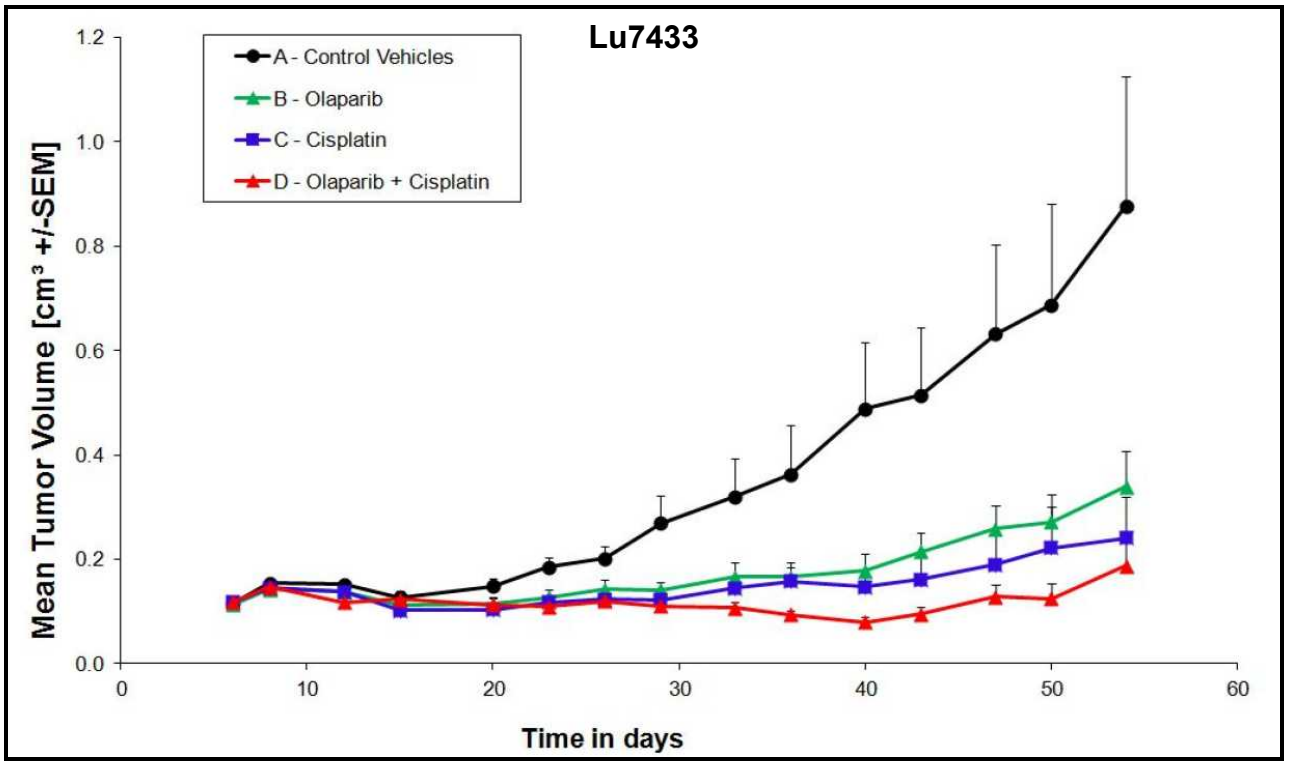
Figure 7. Effect of olaparib in patient-derived mouse tumor models of triple negative breast cancer (Pharmacology Report 12).

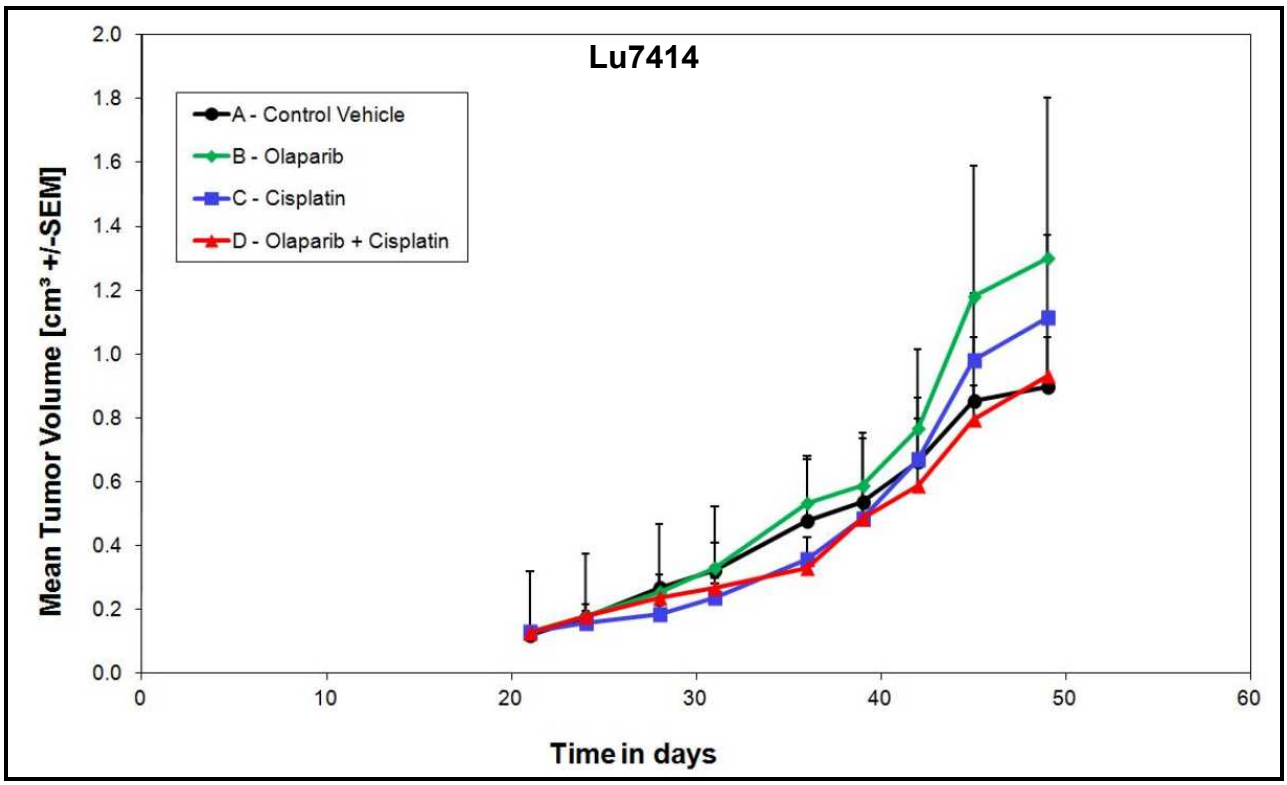




[Adapted from Applicant's submission]

Figure 8. Effect of olaparib in patient-derived mouse tumor models of lung cancer (Pharmacology Report 12).





[Adapted from Applicant's submission]

Conclusions:

- In vitro, statistically significant correlation was noted between olaparib and platinum agent sensitivity across a panel of breast, HNSCC, NSCLC, and ovarian cancer cell lines.
- In vivo, olaparib showed anti-tumor activity in breast and lung patient-derived tumor models which were also sensitive to cisplatin treatment. In the cisplatin-sensitive breast tumor model, co-treatment with olaparib and cisplatin resulted in greater anti-tumor effects when compared to either agent alone.
- Increased anti-tumor activity by olaparib correlated with patient-derived tumors, which were reported to have either mutated BRCA2 and/or TP53.

Study title: In vitro activity of olaparib across a panel of cancer cell lines.

Study no.:	Pharmacology Report 24
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	KuDOS Pharmaceuticals Limited, 410 Cambridge Science Park Milton Road, Cambridge, UK CB4 0PE
Date of study initiation:	October 1, 2007
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	AZD2281, Batch No. 9 (AZ12627831-002/SN1065179476), % purity not reported.

The objective of this study was to evaluate the growth inhibitory activity of olaparib (as well as carboplatin, camptothecin, doxorubicin, and paclitaxel) in a panel of 95 cancer cell lines using the colony formation assay (or clonogenic assay). The panel includes colorectal, breast, ovarian, pancreatic, head and neck squamous cell carcinoma and non-small cell lung cancer cell lines. Gene and protein expression for a number of DNA damage response genes such as BRCA1, BRCA2, ATM, ATR, MDC1 and MRE11A were also determined by qRT-PCR and western blotting.

Summary of Results and Conclusions:

- Positive growth inhibitory activity correlation was noted between olaparib, carboplatin, and campthothecin.
- Cell lines with BRCA1/2 mutations or low expression of homologous recombination repair (HRR) genes/proteins were more sensitive (IC_{50} < 1 μM) to growth inhibitory activity by olaparib.

Figure 9. A summary of olaparib activity (IC_{50}) and HRD classification across 95 cell line panel (Pharmacology Report 24).

			HR deficiency (HRD)				Putative resistance gene expression	
Cell Line Name	Tumour Type	Olaparib IC50 (μM)	BRCA1 or BRCA2 Mutation	Low BRCA1 or BRCA2 gene expression	Low HRR protein expression (non-BRCA)	Low HRR gene expression (Non-BRCA)	Low PARP1 expression	High ABCB1 (P-gp) expression
MDA-MB-436	Breast	0.018	BRCA1 (5396 + 1G>A)		ATM, MDC1	SHFM1		Low
HCC1395	Breast	0.02	BRCA1 (R1751X)	BRCA1	MRE11A, CHK1		High	
59M	Ovarian	0.021				ATR		
CAPAN-1	Pancreatic	0.024	BRCA2 (6174delT)		CHK2			
SUM1315MO2	Breast	0.032	BRCA1 (185delAG)		CHK1			Low
HCC1187	Breast	0.079			ATM, ATR	ATM		Low
NCI-H1838	Non-Small Lung	0.086						Low
HOP-92	Non-Small Lung	0.11		BRCA2		MRE11A, CHEK1, CHEK2		Low
SUM149PT	Breast	0.125	BRCA1 (2288delT)				High	
SKCO1	Colorectal	0.157			ATM			Low
HCT116	Colorectal	0.198		BRCA1. BRCA2		MRE11A, CHEK1		Moderate
OVCAR-3	Ovarian	0.221						
PE/CA-PJ41	Head&Neck SCC	0.385			ATM, CHK1, CHK2	ATM		
IGROV-1	Ovarian	0.391		BRCA1		MRE11A, CHEK2	Low	Moderate
MDA-MB-453	Breast	0.399		BRCA1	MDC1	SHFM1	High	
RPMI2650	Head&Neck SCC	0.472						
KYSE-30	Head&Neck SCC	0.519				CHEK2		Low
Hs840.T	Head&Neck SCC	0.533		BRCA1, BRCA2	All (may be techincal errors)	MDC1, CHEK1	Low	High
TOV-21G	Ovarian	0.568			ATM, MRE11A	ATM, MRE11A	Low	Low
HCC1569	Breast	0.574		BRCA2	ATM			
OAW28	Ovarian	0.596						
NCI-H23	Non-Small Lung	0.626			ATM			Low
A2780	Ovarian	0.629					High	
OV90	Ovarian	0.687				CHEK2		Low
LS180	Colorectal	0.731			MRE11A, ATR, CHK1	MRE11A		High
HCA7	Colorectal	0.736		BRCA1	ATM	ATM, CHEK2, SHFM1		
NCI-H1755	Non-Small Lung	0.801			ATM			High

MDA-MB-468	Breast	0.896				ATM, MRE11A		Low
DMS114	Non-Small Lung	0.908					High	Low
BT20	Breast	0.92			ATR	SHFM1		
LOVO	Colorectal	1.03		BRCA2		MDC1		High
PE/CA-PJ34	Head&Neck SCC	1.121			ATM, CHK1	ATM		Low
MDA-MB-361	Breast	1.156			ATM	CHEK2, SFHM1		
Detroit 562	Head&Neck SCC	1.243			CHK2			
SW620	Colorectal	1.305				CHEK2		High
FaDu	Head&Neck SCC	1.323						Low
CAL27	Head&Neck SCC	1.432		BRCA2	CHK1	ATM, MDC1, CHEK1, CHEK2, SHFM1	Low	
T3M4	Pancreatic	1.463						Moderate
MCF7	Breast	1.79				ATM		
CALU-3	Non-Small Lung	1.988		BRCA1, BRCA2	MDC1	ATM, MDC1		High
NCI-H522	Non-Small Lung	2.089				ATM, MDC1		
HCC38	Breast	2.26					High	Low
HCC1806	Breast	2.302			MDC1			Low
OVCAR-8	Ovarian	2.341		BRCA1		ATR		Moderate
PSN-1	Pancreatic	2.345				MRE11A, CHEK2		
PANC-1	Pancreatic	2.39						
BxPC3	Pancreatic	2.438			ATM, MDC1	MRE11A		
Hep-2	Head&Neck SCC	2.493				ATR, SHFM1		High
CAL51	Breast	2.593						Moderate
PA-1	Ovarian	2.782			MRE11A		Low	Low
TOV-112D	Ovarian	2.807						
HCC1937	Breast	2.82	BRCA1 (5382InaC)				High	
SW579	Head&Neck SCC	2.825			MDC1, MRE11A, ATR	SHFM1	Low	Low
MDA-MB-435S	Breast	2.949						
T47D	Breast	3.024				SHFM1		
HOP-62	Non-Small Lung	3.066				CHEK2		Moderate
SW948	Colorectal	3.433					Low	High
NCI-H322M	Non-Small Lung	3.537						
NCI-H460	Non-Small Lung	3.818						Moderate
KB	Head&Neck SCC	4.384				SHFM1		
BT549	Breast	4.505			ATR, CHK1	MRE11A		
BE	Colorectal	4.656		BRCA2	CHK2	ATM		High
HCC1954	Breast	4.795			ATM, MDC1, CHK1	ATM		
SK-BR-3	Breast	4.851		BRCA1		CHEK2		

OVCAR-4	Ovarian	4.852		BRCA1		ATM	High	
HT29	Colorectal	4.978			ATR			
HCC1143	Breast	5.152			ATM, CHK1	MRE11A, SHFM1		
PE/CA-PJ15	Head&Neck SCC	5.225			ATM, CHK1			Low
SKOV3	Ovarian	5.508				CHEK2		Low
RKO	Colorectal	5.644		BRCA2		MRE11A		
SW1417	Colorectal	5.663		BRCA2		BRCA2		Moderate
Hs578T	Breast	5.684			MDC1, CHK1, CHK2		Low	Low
SW403	Colorectal	6						Moderate
ASPC-1	Pancreatic	6.227			MDC1			High
OAW42	Ovarian	6.319		BRCA2		CHEK1		High
NCI-H810	Non-Small Lung	6.493				MRE11A		
HUPT4	Pancreatic	6.646						High
OVCAR-5	Ovarian	6.711				CHEK2, SHFM1		Low
CALU-6	Non-Small Lung	7.001						Low
PANC-89	Pancreatic	7.491						Low
HN5 (LICR-LON-HN5)	Head&Neck SCC	7.84						High
CFPAC-1	Pancreatic	7.998		BRCA2	ATM	MDC1, CHEK2		High
A549	Non-Small Lung	8.087						Low
NCI-H226	Non-Small Lung	8.096						Low
MDA-MB-231	Breast	8.28				CHEK2		
HCT15	Colorectal	8.753			CHK2			High
NCI-H1299	Non-Small Lung	9.128					Low	High
BT474	Breast	9.186					High	
OE21	Head&Neck SCC	9.673						Low
ZR-75-1	Breast	10				MRE11A		Low
SW480	Colorectal	10						Moderate
PE/CA-PJ49	Head&Neck SCC	10			CHK1, CHK2		Low	Low
HPAF-II	Pancreatic	10			MDC1			
Hs766T	Pancreatic	10			MDC1			
MIAPACA-2	Pancreatic	10			MDC1			

HRD classification is based on low (less than half / 50% change from mean) protein expression and mRNA gene expression analysis or published deleterious loss-of-function BRCA1 or BRCA2 mutations for each cell line. Data cells were coloured green if classified as HRD or red if they had low levels of PARP1 target gene/protein expression or high/moderate levels of ABCB1 (P-gp) drug transporter which might contribute to drug resistance (lower than expected activity).

[Excerpted from Applicant's submission]

Figure 10. Summary of chemotherapeutic agents activity across the breast cancer cell line sub-panel (Pharmacology Report 24).

Cell line	Mean Camptothecin IC50 (μM)	SD	Mean Carboplatin IC50 (μM)	SD	Mean Doxorubicin IC50 (μM)	SD	Mean Paclitaxel IC50 (μM)	SD
MDA-MB-436	0.000047	0.000062	0.066	0.069	0.004443	0.000958	0.002804	0.001340
HCC1395	0.000788	0.000020	0.396	0.037	0.018950	0.002240	0.004795	0.000002
SUM1315MO2	0.001092	0.000064	0.100	0.028	0.007331	0.002294	0.000594	0.000520
HCC1187	0.001761	0.000515	0.391	0.413	0.003474	0.002074	0.000407	0.000024
SUM149PT	0.001353	0.000046	0.328	0.008	0.005566	0.000168	0.000967	0.000750
MDA-MB-468	0.004833	0.000356	0.709	0.199	0.014726	0.000532	0.001614	0.000148
MCF7	0.001846	0.000157	2.903	0.689	0.016330	0.003621	0.000231	0.000327
HCC1937	0.011598	0.002217	0.483	0.166	0.016809	0.000749	0.001251	0.000397
T47D	0.006194	0.000595	3.361	0.871	0.004835	0.000481	0.001739	0.000476
BT549	0.005355	0.000294	1.915	0.023	0.008013	0.000453	0.001305	0.000423
MDA-MB-231	0.025920	0.003244	29.730	0.383	0.100000	0.000000	0.000782	0.000030

[Adapted from Applicant's submission]

Figure 11. Correlation matrix between olaparib, carboplatin, camptothecin, doxorubicin and paclitaxel chemotherapeutics agent activities (Pharmacology Report 24).

	Olaparib (AZD2281)	Camptothecin	Carboplatin	Doxorubicin	Paclitaxel
Olaparib (AZD2281)	-	0.80	0.84	0.50	-0.11
Camptothecin	0.80	-		0.81	-0.11
Carboplatin	0.84	0.81	-	0.66	-0.00
Doxorubicin	0.50	0.53	0.66	-	0.10
Paclitaxel	-0.11	-0.11	0.00	0.10	-

[Adapted from Applicant's submission]

Study title: A study of PK, PD and efficacy using different olaparib (AZD2281) doses in the TNBC PTX in vivo model HBCx-10.

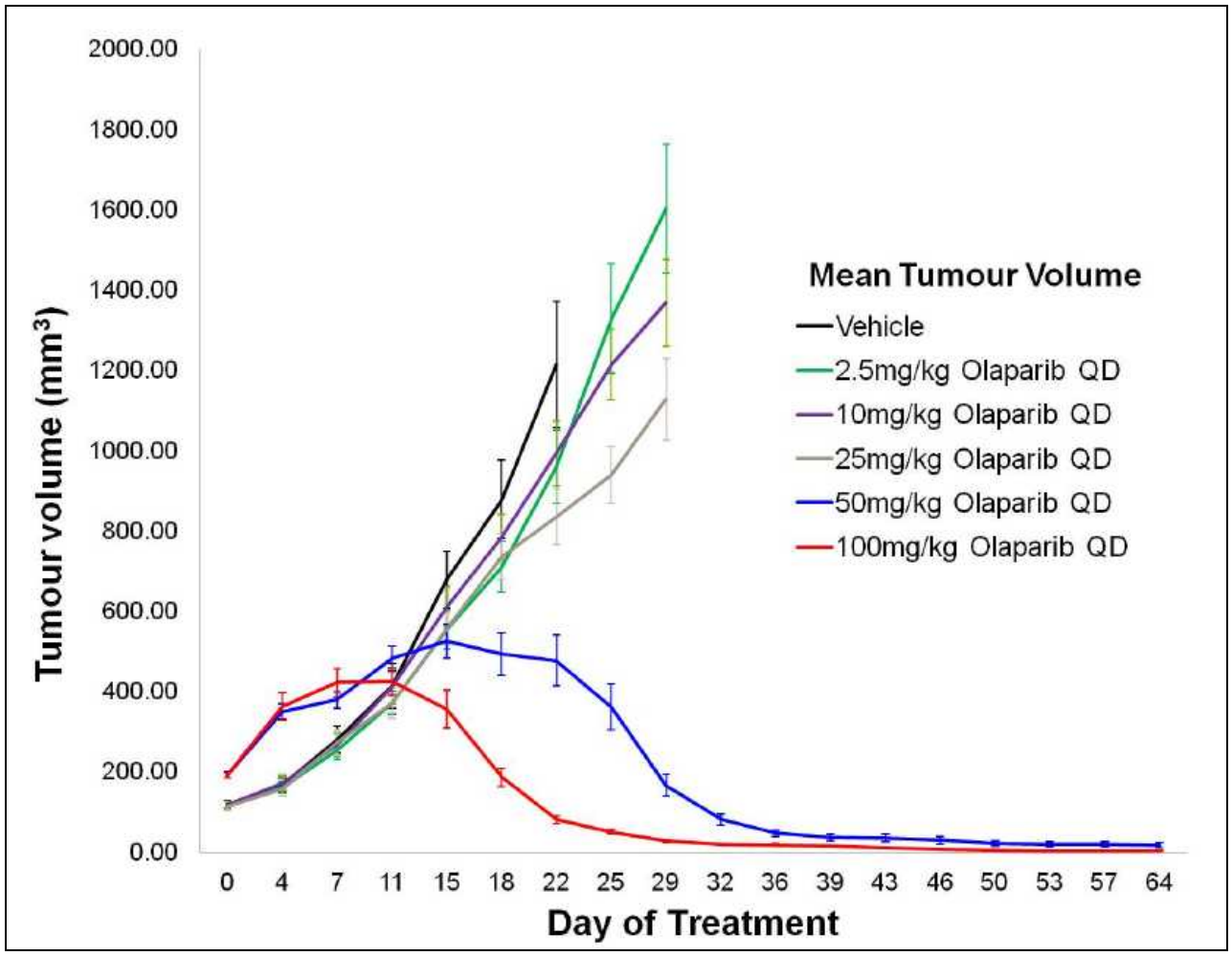
Study no.:	Pharmacology Report 27
Study report location:	eCTD Section 4.2.1.1.
Conducting laboratory and location:	iMed Oncology AstraZeneca, Alderly Park, England
Date of study initiation:	November 25, 2011
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib, Batch No. 002 (in vitro) and micronized KU-0059436, C463/4 (in vivo), % purity not reported

The objectives of this study were to evaluate anti-tumor activity of olaparib in the HBCx-10 patient-derived tumor explant model and to build a nonclinical PK/PD/activity relationship model. In brief, in the in vivo portion of this study, tumor-bearing mice were administered olaparib orally, once-daily. The concentration of olaparib in plasma and tumor was measured by LC-MS/MS. The concentration of PAR in tumor, a measure of pharmacodynamic effect of olaparib, was evaluated using the HT PARP in vivo Pharmacodynamic Assay II ELISA kit (Trevigen).

Summary of results:

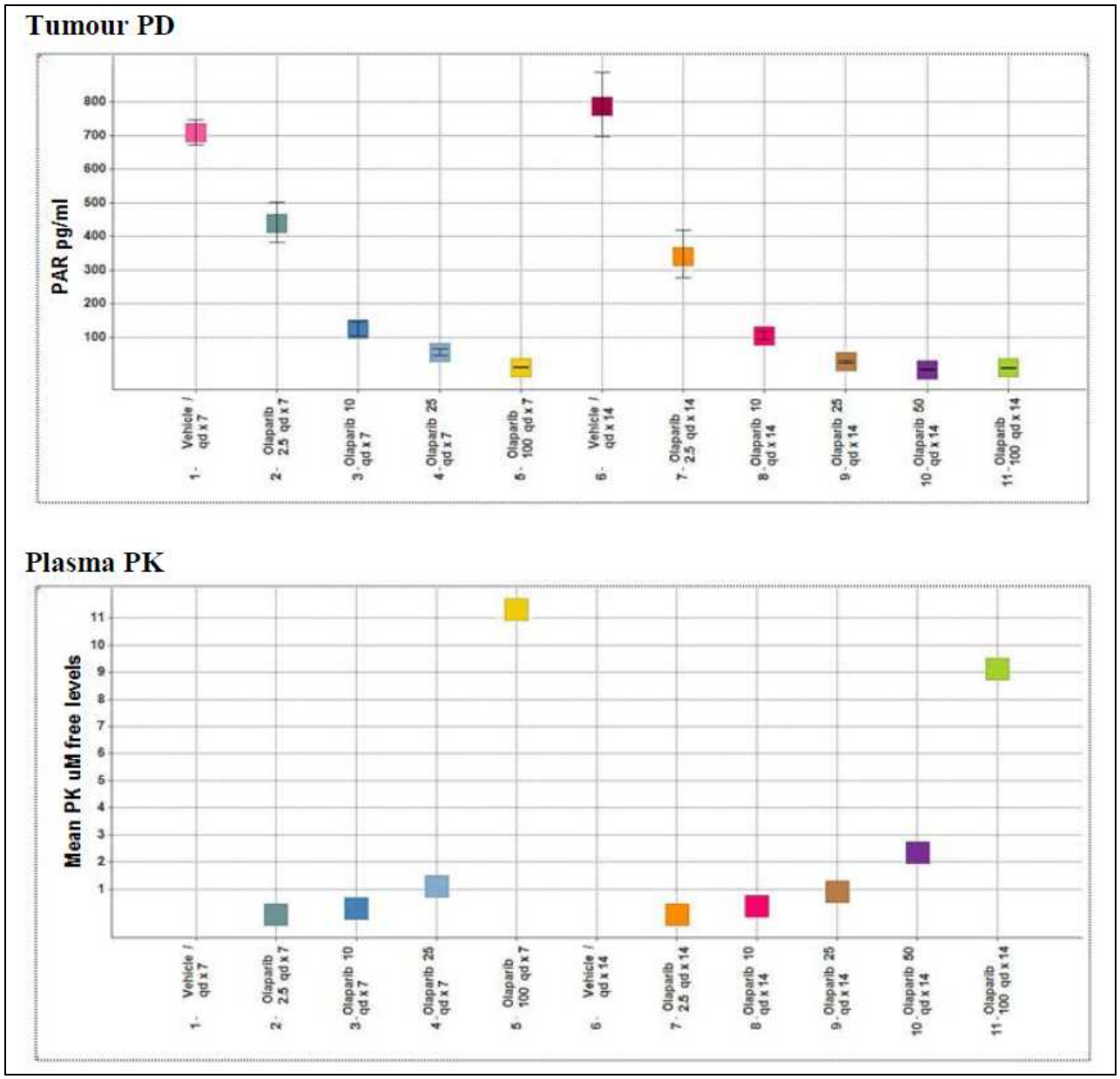
- 50 and 100 mg/kg/day olaparib, once-daily resulted in decreased tumor volume (8.6% and 1.4% of the starting volume by Day 30, respectively) when compared to vehicle control.
- Treatment with olaparib resulted in decreases in tumor PAR levels, inversely correlating with increasing doses and plasma free olaparib concentrations.
- Based on a mechanistic modeling simulation, significant increases in SSB numbers are only observed when PAR is reduced by 90% or more.
- Clinical unbound steady state trough concentrations in patients who received 200 and 400 mg BID olaparib exceeded the estimated IC_{90} values from nonclinical studies.

Figure 12. Anti-tumor activity of olaparib in the HBCx-10 patient-derived tumor model (Pharmacology Report 27).



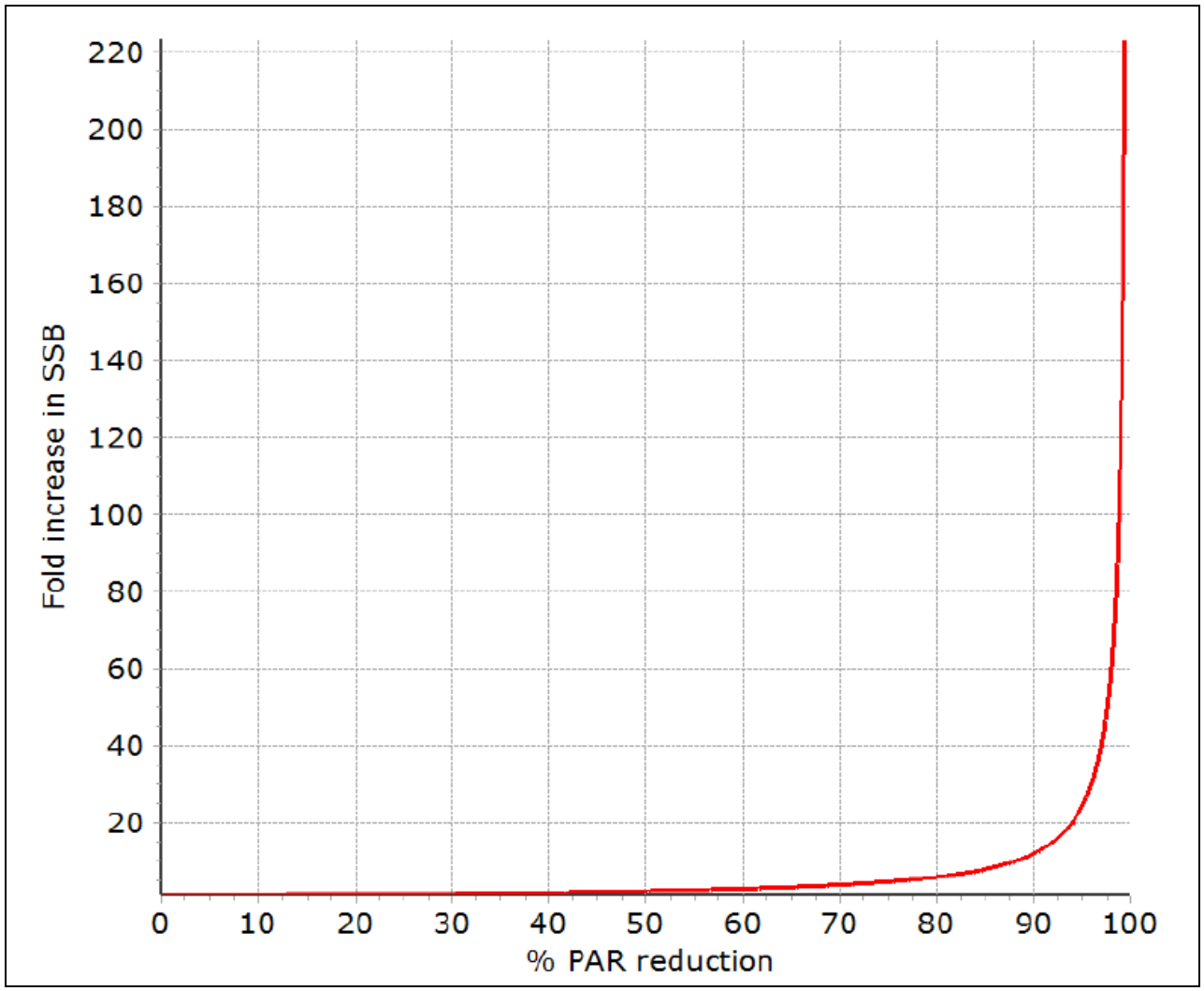
[Excerpted from Applicant's submission]

Figure 13. Summary of olaparib PK/PD relationship in HBCx-10 patient-derived tumor model (Pharmacology Report 27).



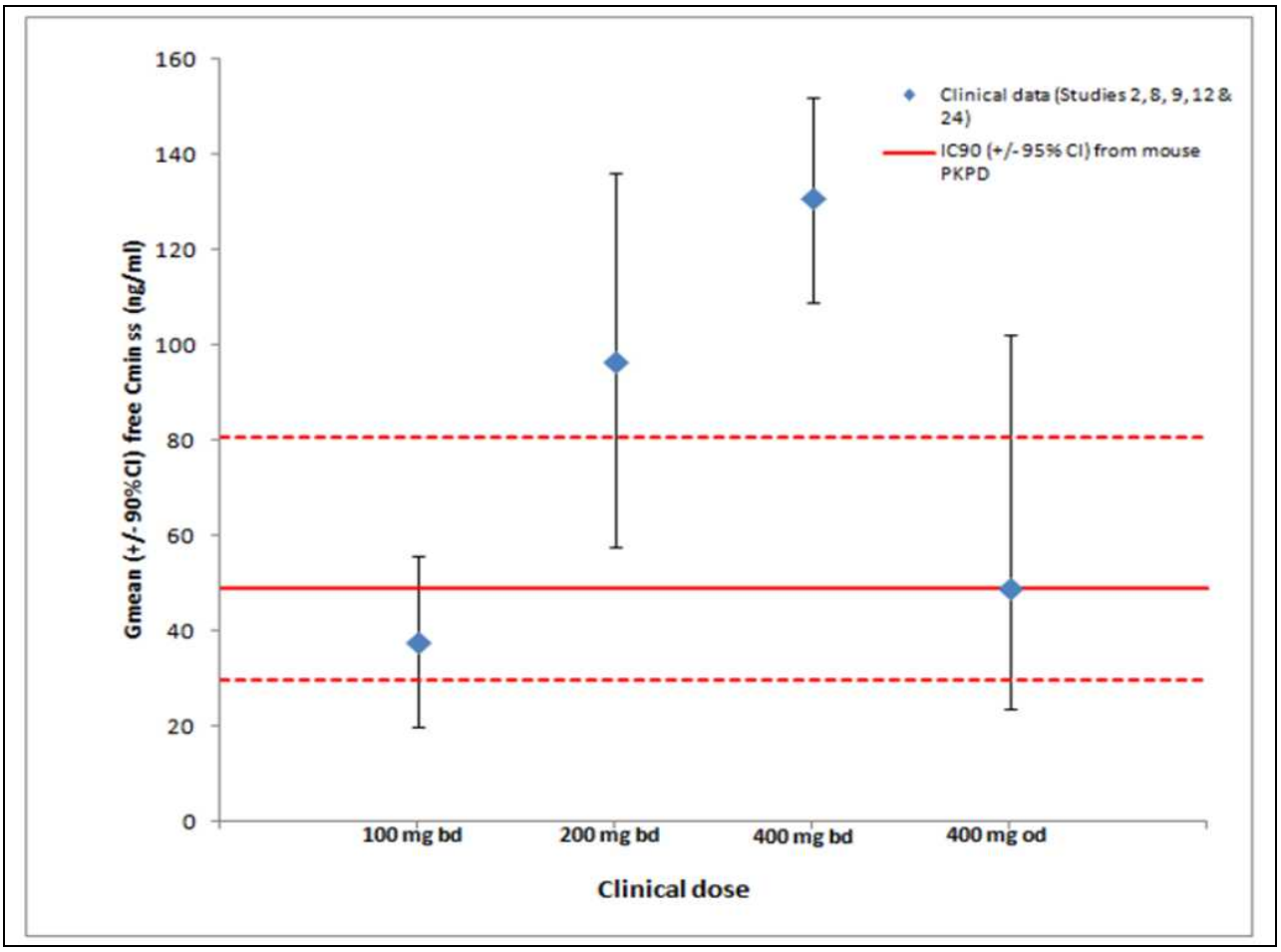
[Excerpted from Applicant's submission]

Figure 14. Steady-state simulation: relationship between PAR reduction and the increase in DNA SSBs (Pharmacology Report 27).



[Excerpted from Applicant's submission]

Figure 15. Steady-state simulation: relationship between estimated IC90 values from mouse PK/PD experiments and clinical unbound steady state trough concentration of olaparib (Pharmacology Report 27).



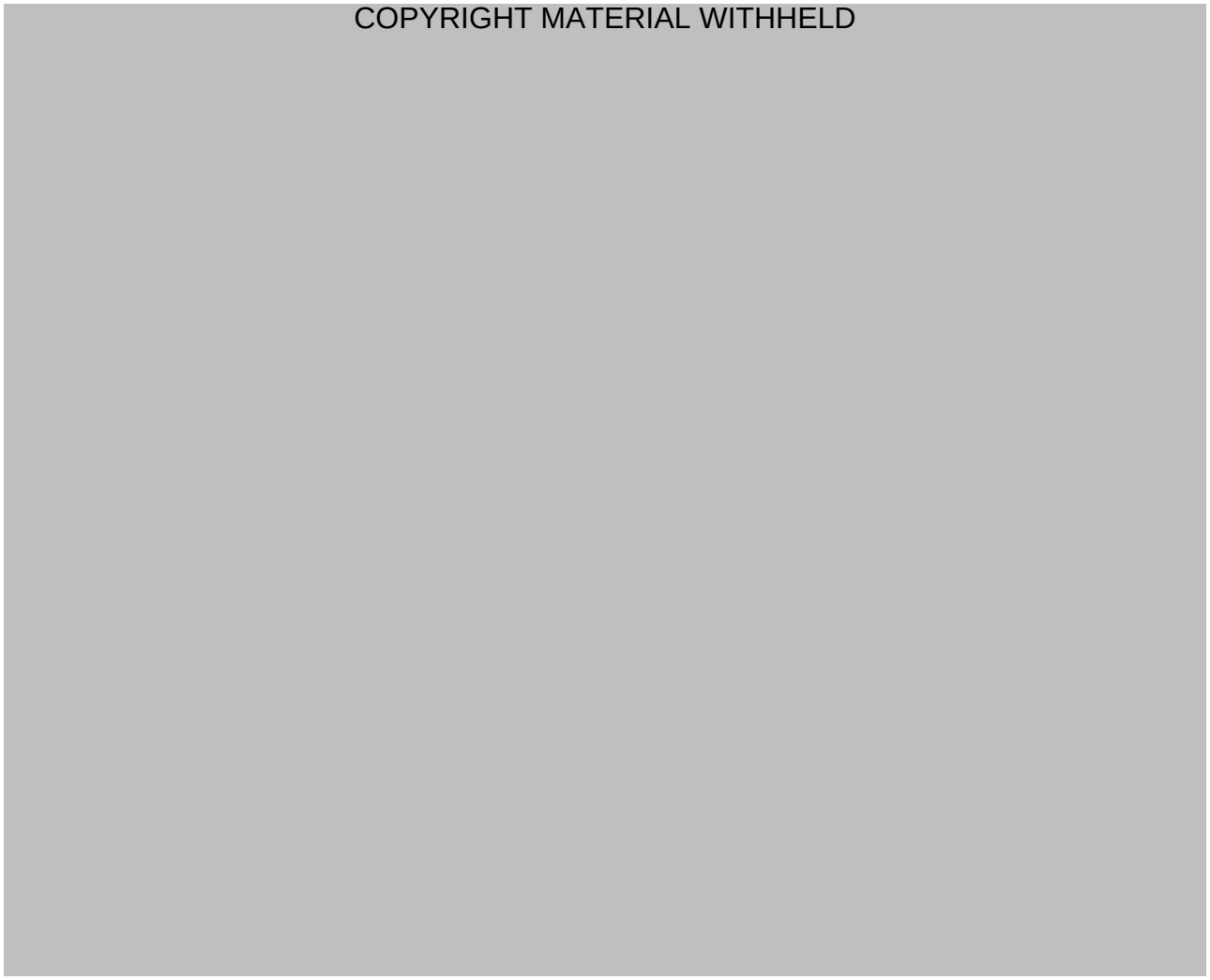
[Excerpted from Applicant's submission]

Conclusions:
The activity of olaparib appears to be both time and dose-dependent and modeling data suggest that unbound steady state trough concentrations of olaparib achieved by the 400 mg BID clinical dose exceed the IC_{90} value needed to cause significant reductions in PAR levels and SSBs in tumors.

Additional literature references were provided in this original NDA submission in support of the proposed mechanism of action of olaparib. Key findings from the literature references are summarized below.

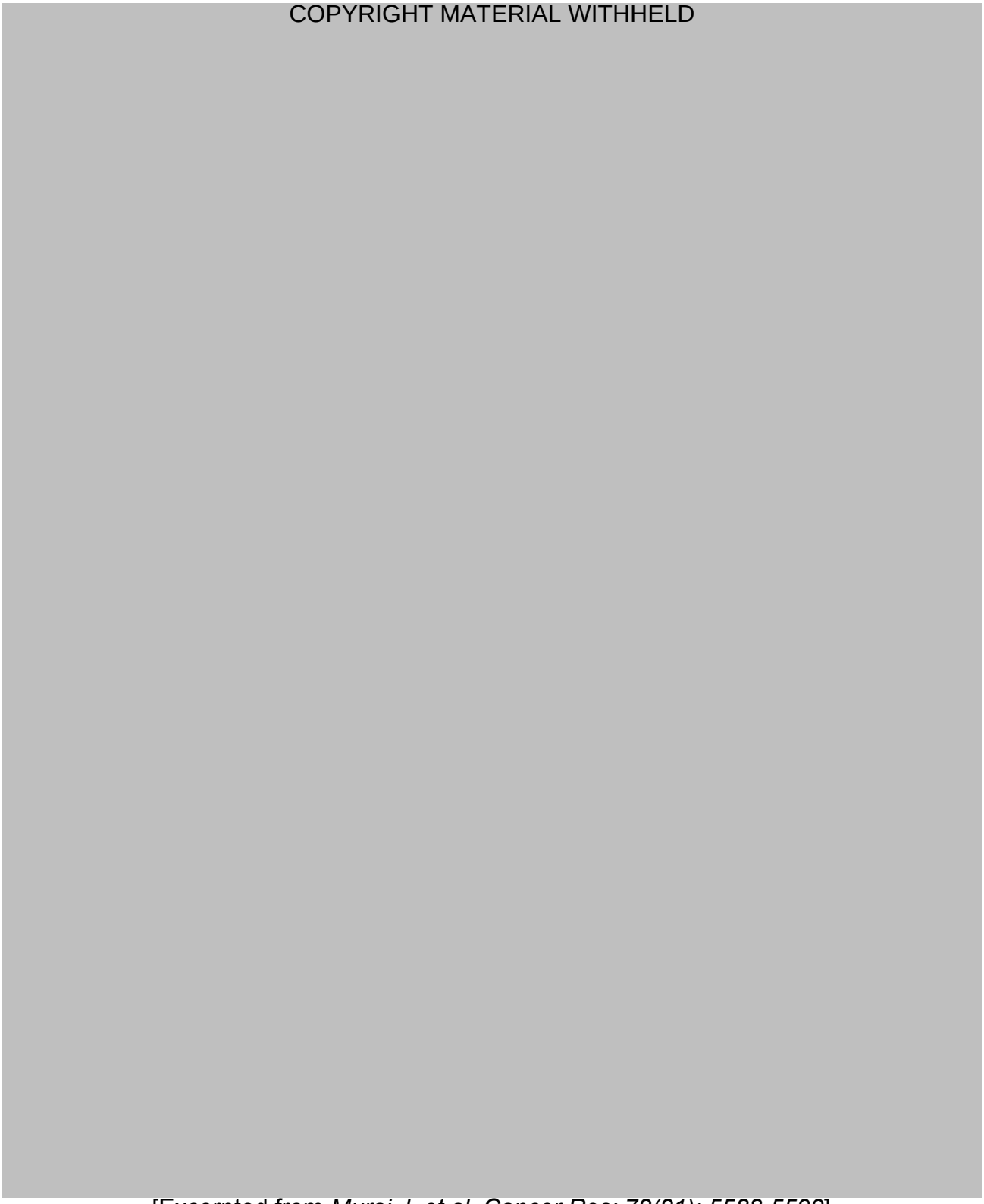
- **"Trapping of PARP1 and PARP2 by clinical PARP inhibitors".** *Murai J, et al. Cancer Res; 72(21): 5588-5599.*
  - Treatment of avian B-lymphoblast DT40 cells with olaparib resulted in PARP1-dependent decreases in cell viability, which correlated with increased γ-H2AX foci. A BRCA truncated mutant (BRCA2tr/-) cell line was more sensitive to olaparib-induced cell death when compared to the wild type cell line. Cell cycle analyses showed that olaparib had no impact on cell cycle of $PARP1^{-/-}$ cells, whereas olaparib induced G_2 accumulation in wild type and BRCA2tr/- cells.
  - In the presence of the alkylating agent MMS, olaparib induced PARP1 accumulation in the chromatin-bound fraction in wild type DT40 cells and increased cell death. Consistent with the role of PARP in DNA repair, both wild type cells treated with olaparib and PARP1-/- cells showed increased sensitivity to MMS-induced cell death. However, MMS induced greater cytotoxicity and cell cycle arrest with sub-G_1 accumulation in wild type cells in combination with olaparib when compared to its effects in $PARP1^{-/-}$cells. Overall, data suggest inhibition of PAR synthesis alone (without accumulation of chromatin-bound PARP1) does not explain the increased sensitivity of olaparib-treated cells to MMS-induced cell death.
  - In the presence of MMS, olaparib treatment resulted in PARP1 and PARP2 binding to chromatin in human DU145 prostate cancer, OVCAR4 ovary carcinoma, and SF295 glioma cell lines. Knockdown of PARP1 by siRNA transfection reduced sensitivity to olaparib in DU145 cells. In vitro, binding of PARP1 and PARP2 to chromatin is reversible following removal of olaparib.
  - In vitro, olaparib was a more potent inhibitor of PARP catalytic activity when compared to veliparib and niraparib, two other PARP inhibitors. However, in terms of cytotoxicity, niraparib was more potent than olaparib and veliparib. The potency to induce cytotoxicity correlated with increased and tighter binding of PARP to DNA, as shown by immunoblotting, fractionation, and fluorescence anisotropy DNA-binding assays.
  - Overall, the data suggest that olaparib causes inhibition of PARP1 and PARP2 catalytic activity by decreasing PAR production and increases binding of PARP1 and PARP2 to DNA. In vitro, both of these activities appear to contribute to olaparib-induced cell death.

Figure 16. PARP1 is required for cell killing by olaparib.



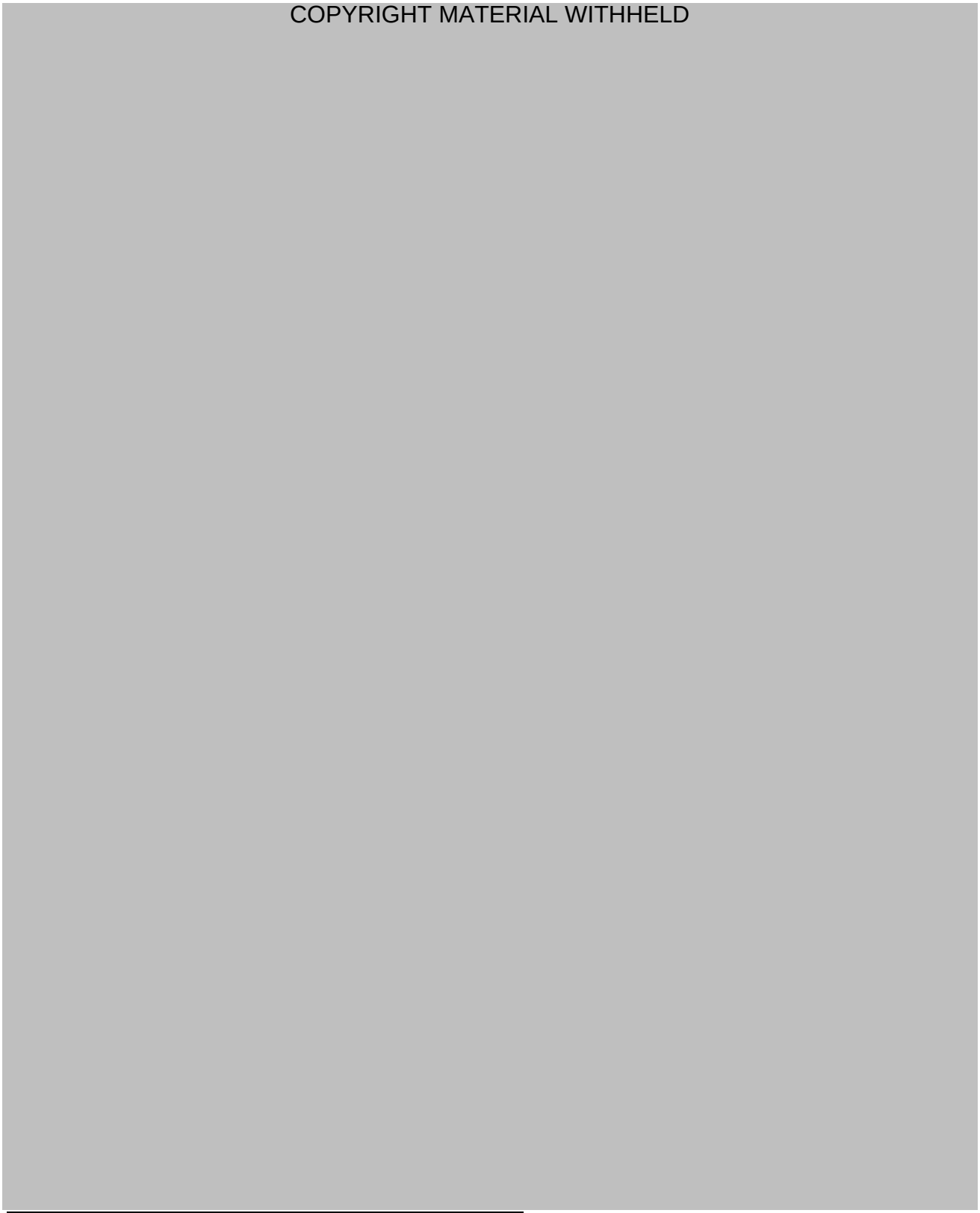
[Excerpted from *Murai J, et al. Cancer Res; 72(21): 5588-5599*]

Figure 17. Olaparib stabilizes PARP1-DNA complexes that are more toxic than unpaired SSBs.



[Excerpted from *Murai J, et al. Cancer Res; 72(21): 5588-5599*]

Figure 18. Effects of olaparib in human cancer cell lines.



[Excerpted from *Murai J, et al. Cancer Res; 72(21): 5588-5599*]

4.2 Secondary Pharmacology

Study title: AZD2281: Selectivity screening in radioligand binding, enzyme and electrophysiological assays in vitro.

Study no.:	0818SY
Study report location:	eCTD Section 4.2.1.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 21, 2007
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib, Batch No. C463/2 and AZ12627831-001, % purity not reported

The objective of this study was to evaluate the pharmacological activity of olaparib in a panel of cellular enzymes, receptors, transporters, and ion channels. Briefly, a single concentration of 10 μM olaparib was tested in in vitro radioligand binding and enzyme assays. Electrophysiological assays in a panel of 7 types of human recombinant voltage-gated cardiac ion channels (including $hCa_V1.2$, $hNa_V1.5$, $hK_V4.3$/hChIP2.2, $hK_V7.1$/hCNE1, $hCa_V3.2$, $hK_V1.5$, hHCN4) were conducted in a concentration-response mode up to the top concentration of 31.6 μM olaparib.

Summary of results:

- Olaparib had no significant activity (defined as > 50% inhibition) in any of the 220 in vitro radioligand binding and enzyme assays when tested at a concentration of 10 μM.
- Olaparib was inactive (active/inactive was defined as whether or not the % inhibition at the top test concentration could be distinguished from the variance in the assay baseline) in all 7 of the human recombinant voltage-gated cardiac ion channels.

Conclusions:
In vitro pharmacology screening radioligand, enzyme, or electrophysiological assays showed that olaparib did not have any significant off-target effects, under the conditions tested.

Study title: In vitro pharmacology: phosphodiesterase assays.

Study no.:	8157
Study report location:	eCTD Section 4.2.1.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	March 5, 2004
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib (b) (4)), Batch No. and % purity not reported

The objective of this study was to evaluate the effect of olaparib in phosphodiesterase enzyme assays in vitro, using bovine phosphodiesterase 1 and 6, and human phosphodiesterase 2, 3, 4, and 5.

Summary of results and conclusions:
Olaparib (10 μM) did not have any significant effects on phosphodiesterase enzyme activity, under the conditions tested.

Table 1. Study No. 8157: Summary of effects of olaparib (b) (4)) on phosphodiesterases.

(b) (4)

(b) (4)

[Excerpted from Applicant's submission]

Study title: In vitro pharmacology: (b) (4) – Study of KU-0059436 and (b) (4).

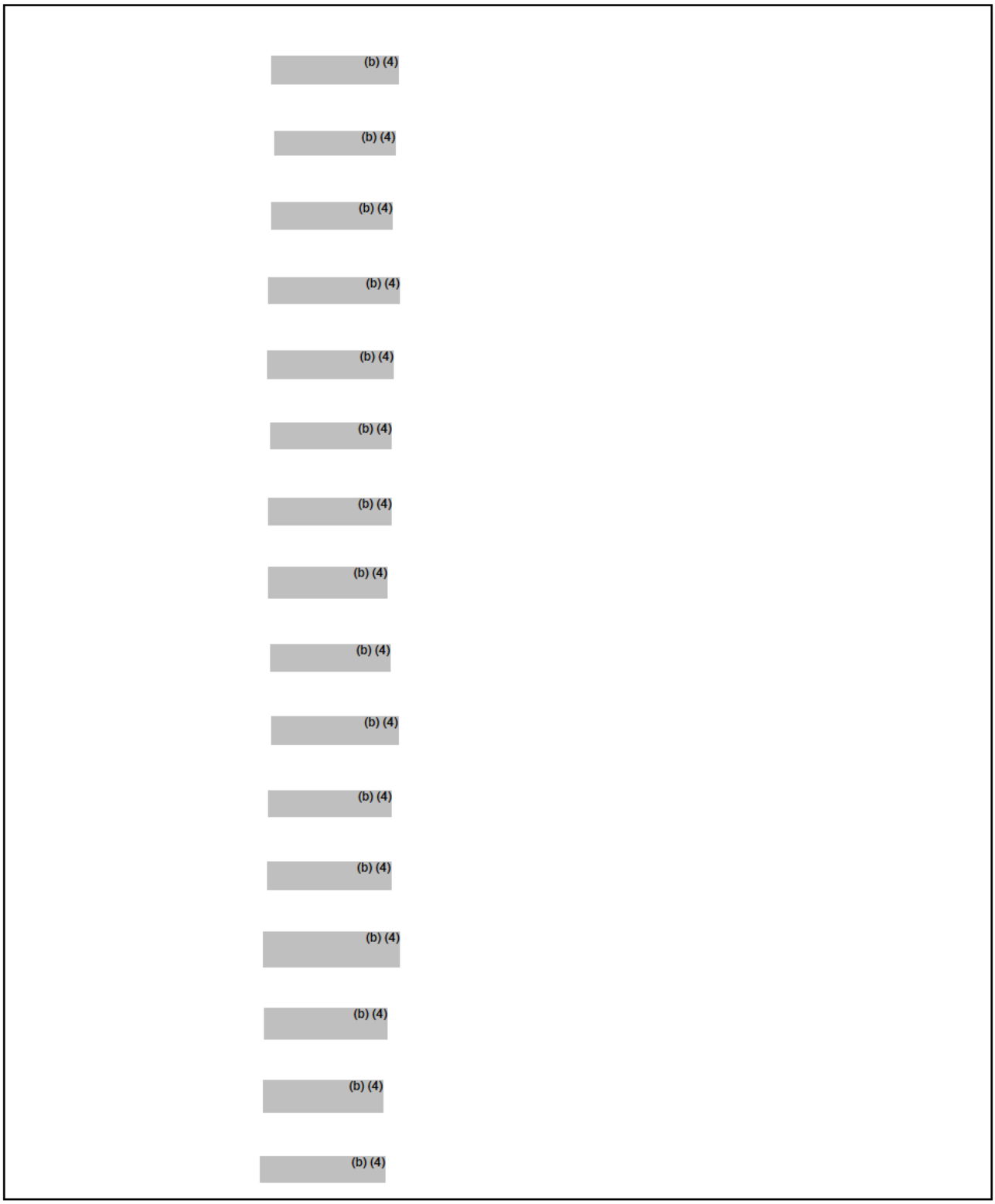
Study no.:	8234
Study report location:	eCTD Section 4.2.1.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 21, 2007
GLP compliance:	Non-GLP
QA statement:	No
Drug, lot #, and % purity:	Olaparib (KU-0059436), Batch No. 4 (% purity not reported) (b) (4), Batch No. 03 ((% purity not reported)

The objective of this study was to evaluate the effects of KU-0059436 and (b) (4) in a panel of in vitro receptor binding assays. Binding detection was measured by scintillation counting. The specific ligand binding to the receptors is defined as the difference between the total binding and the nonspecific binding determined in the presence of an excess of unlabeled ligand. The results are expressed as a percent of control specific binding and as a percent inhibition of control specific binding obtained in the presence of test compounds.

Summary of results and conclusions:
No significant inhibitory activity of olaparib was noted in a panel of *in vitro* receptor binding assays, under the conditions tested.

Table 2. Study No. 8234: Summary of effects of olaparib in a panel of in vitro receptor binding assays.

(b) (4)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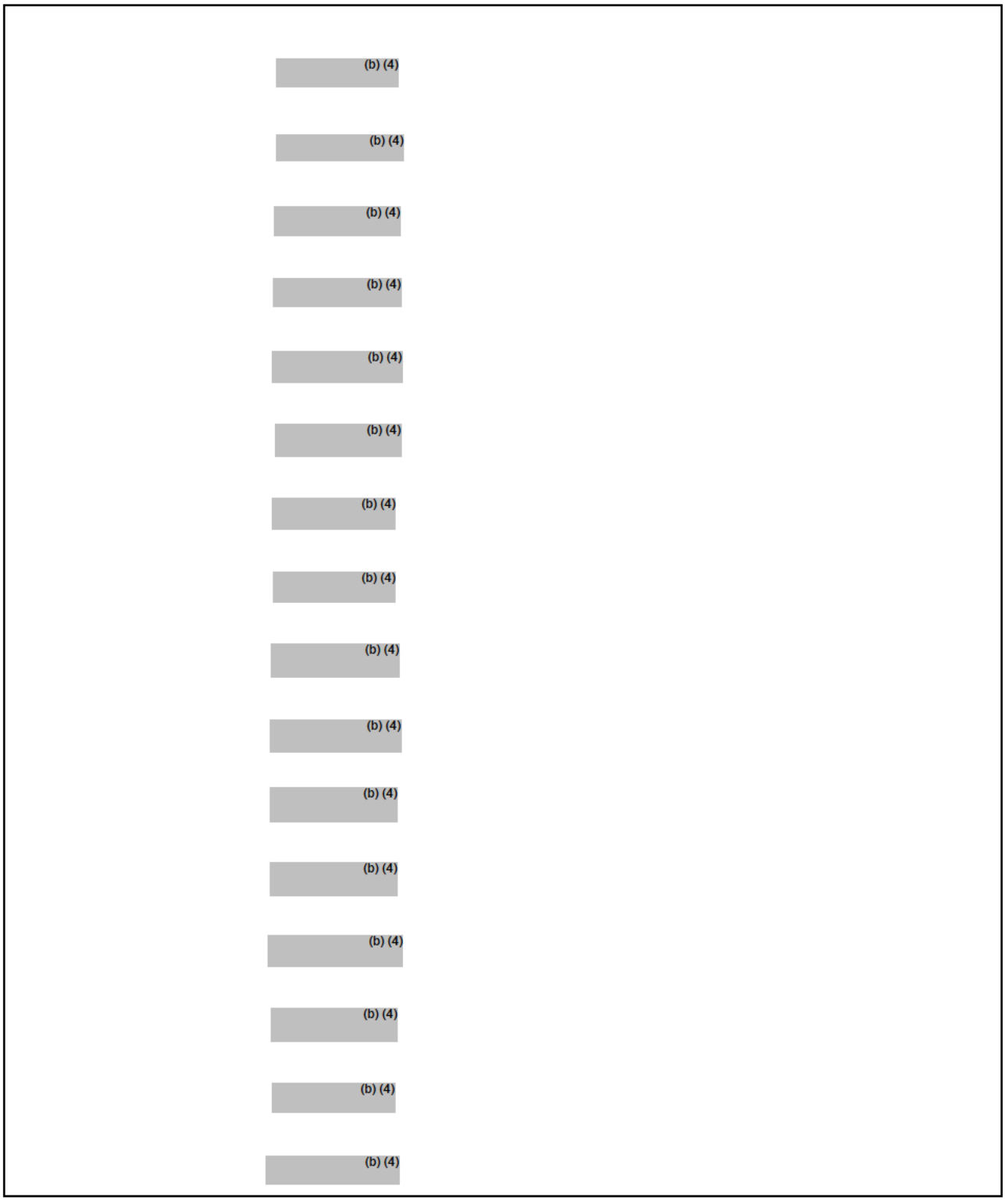
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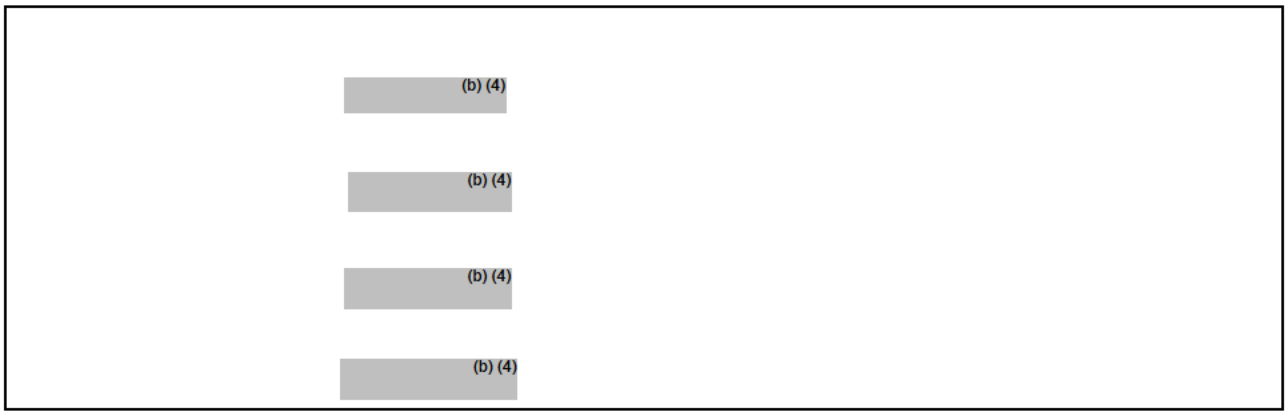

[Excerpted from Applicant's submission]

4.3 Safety Pharmacology

Study title: AZD2281: Effects on human Ether-a-go-go-related gene (hERG) encoded potassium channel in vitro.

Study no.:	0242SZ
Study report location:	eCTD Section 4.2.1.3.
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D
Date of study initiation:	August 21, 2007
GLP compliance:	Yes (OECD GLP principles and UK GLP regulation)
QA statement:	Yes
Drug, lot #, and % purity:	AZD2281, batch no. 060344, 99.52% purity

Key Study Findings

- Olaparib inhibited the hERG tail current with an IC_{50} of 226 μM, making it a low-potency or ineffective blocker.

Summary of Methods

Briefly, the effect of 1 - 300 μM AZD2281 on hERG tail current was evaluated in four hERG expressing CHO cells. Each cell was treated with ascending concentrations of olaparib to allow determination of a cumulative concentration-effect curve, according to the following table. hERG encoded currents were recorded using whole-cell patch clamp.

Table 3. Study No. 0242SZ: Test concentrations.

Addition	Compound	Concentration
1	DMSO	0.1%
2	AZD2281	1 μM
3	AZD2281	3 μM
4	AZD2281	10 μM
5	AZD2281	30 μM
6	AZD2281	100 μM
7	AZD2281	300 μM
8	DMSO	0.1%
9	Cisapride	3 μM

Summary of Results

- The concentrations of hERG buffer perfusates ranged from 80.3% to 111.0%.
- Treatment of hERG expressing CHO cells resulted in a concentration-dependent decrease in hERG tail current (IC_{50} = 226 μM).

Table 4. Study No. 0242SZ: Summary of inhibitory effects (normalized to vehicle control values) of olaparib on hERG tail current.

		Peak hERG tail current (% of control)			
Experimental condition	**Nominal concentration (μM)**	**Cell B**	**Cell F**	**Cell H**	**Cell K**
Vehicle	0.1%	100.0	100.0	100.0	100.0
AZD2281	1	93.5	97.5	98.4	98.3
AZD2281	3	95.1	100.9	106.6	99.6
AZD2281	10	92.6	97.4	111.5	100.0
AZD2281	30	87.7	98.9	114.7	98.2
AZD2281	100	63.3	80.5	86.6	79.5
AZD2281	300	37.4	48.1	56.1	50.3
Cisapride	3	0.0	0.0	0.0	0.0

[Excerpted from Applicant's submission]

Study title: KU0059436: Effects on general activity and behaviours in the rat following oral administration.

Study no.:	2229-047
Study report location:	eCTD Section 4.2.1.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 19, 2004
GLP compliance:	Yes
QA statement:	Yes
Drug, lot #, and % purity:	KU0059436 (olaparib), batch no. 040502, 97.35% purity.

Key Study Findings

Oral administration of 20, 115, or 250 mg/kg olaparib did not result in any test article-related behavioral, autonomic, and motor changes in male Wistar rats based on Irwin's method.

Methods

Doses:	0, 20, 115, 250 mg/kg olaparib
Frequency of dosing:	Single dose
Route of administration:	Oral/gavage
Dose volume:	25 mL/kg
Formulation/Vehicle:	10% dimethyl sulfoxide (DMSO) and 10% (w/v) hydroxypropyl b-cyclodextrin (HPBC) in phosphate buffered saline (PBS).
Species/Strain:	Rat/Wistar
Number/Sex/Group:	6 male animals/group
Age:	6 Weeks
Weight:	174 - 205 g
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None

Table 5. Study No. 2229-047: Treatment groups.

Group	Oral treatment	Dose level (mg/kg)	Formulation concentration (mg/mL)	Number of animals
1	Vehicle (10% DMSO + 10% HPBC in PBS)	-	-	6
2	KU0059436	20	0.8	6
3	KU0059436	115	4.6	6
4	KU0059436	250	10	6

[Excerpted from Applicant's submission]

Justification of doses and route of administration
The high dose selection was based on expected safety margin to human exposure. Olaparib was administered oral gavage because it is the same as the clinical route of administration.

Observations

Irwin observations were performed at 30, 60, 90, 180, and 300 minutes post-dose. Animals were kept for an additional 7 days following the day of dosing for general observations and mortality checks.

Results

Mortality

None.

Clinical Signs

Two 250 mg/kg group animals produced soft feces at 30 minutes post-dose.

Irwin Observation

No test article-related effects were noted.

Study title: KU0059436: Cardiovascular and respiratory effects in the anesthetized dog following intravenous administration.

Study no.:	2229-053
Study report location:	eCTD Section 4.2.1.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 3, 2004
GLP compliance:	Yes (UK GLP and OECD Principles on GLP)
QA statement:	Yes
Drug, lot #, and % purity:	KU0059436 (olaparib), batch # 040502, 97.35% purity

Key Study Findings

No statistically significant changes in cardiovascular or respiratory parameters were noted following a single intravenous administration of 1.5 or 5.0 mg/kg olaparib to anesthetized beagle dogs when compared to vehicle control-treated animals.

Methods

Doses:	0, 1.5, 5 mg/kg
Frequency of dosing:	3 doses administered at intervals of at least 45 minutes
Route of administration:	Intravenous infusion (10 min) via a cannula place in the jugular vein
Dose volume:	See table below
Formulation/Vehicle:	10% dimethyl sulphoxide (DMSO) and 10% (w/v) hydroxypropyl b-cyclodextrin (HPBC) in phosphate buffered saline (PBS)
Species/Strain:	Dog/Beagle
Number/Sex/Group:	2/sex/group
Age:	8 months
Weight:	9.25 – 13.9 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	None deemed to have had a significant impact on the integrity or interpretation of study results.

Table 6. Study Number: 2229-053: Dose groups.

Group number	Group description	Dose number	Dose concentration mg/mL	Dose volume mL/kg	Dose level mg/kg
1	Vehicle treated group	1	0	0.20	0
		2	0	0.67	0
		3	0	2	0
2	KU0059436 treated group	1	7.5	0.20	1.5
		2	7.5	0.67	5
		3	7.5	2	15

[Excerpted from Applicant's submission]

Observations

Cardiovascular Parameters	Blood pressure (systolic, diastolic and mean arterial), heart rate, left ventricular pressure and its derivative, dP/dtmax, mean femoral artery blood flow, ECG (RR, QRS, PR, QT and QT_{CF} intervals, and heights of the R, P and T-waves)
Respiratory Parameters	Peak inspiratory and expiratory flow, tidal volume, minute volume and rate respiration
Dosing Formulation Analysis	Three samples from the 7.5 mg/mL dosing solution on Day 2.

Results

Cardiovascular parameters

No toxicologically significant changes in cardiovascular parameters were noted.

Respiratory parameters

No statistically significant test article-related changes in respiratory parameters were noted.

Dosing Formulation Analysis

The actual concentration of the dosing formulation was 102% of the target levels, which was within the range of 90% to 110% of nominal concentration.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

5.1.1. Absorption

Study Title: AZD2281: Pharmacokinetic study in the mouse following intravenous injection and oral (gavage) administration.

Study No.: D2281 KPM035 ((b) (4) No. 8221942)
Report Date: July 2010
Study report location: eCTD 4.2.2.2.
Conducting Laboratory: (b) (4)
GLP: No

Pharmacokinetic parameters of olaparib were evaluated in male and female CD-1 mice following a single administration of 20 mg/kg olaparib IV (solution) or 80 mg/kg olaparib orally (nanosupension, microsuspension, or solution).

Results:

Table 7. Pharmacokinetic parameters following a single dose administration of olaparib to male and female mice (Study No. D2281 KPM035).

Parameter	Dose and route							
	Group 1 (iv 20 mg/kg)		Group 2 (Nanosuspension, po 80 mg/kg)		Group 3 (Microsuspension, po 80 mg/kg)		Group 4 (Solution, po 80 mg/kg)	
	Male	Female	Male	Female	Male	Female	Male	Female
C_{max} (µmol/L)	NA	NA	4.72	9.35	3.20	2.63	28.6	29.0
C_0 (µmol/L)	94.4	82.4	NA	NA	NA	NA	NA	NA
t_{max} (h)	NA	NA	0.750	0.500	0.500	0.333	0.500	0.333
$AUC_{(0-t)}$ (h.µmol/L)	14.9	12.0	9.66	15.2	6.42	6.68	33.0	28.6
$AUC_{(0-\infty)}$ (h.µmol/L)	14.9	12.0	9.87	15.5	6.58	6.84	33.1	28.9
$t_{½}$ (h)	0.648	0.969	2.05	1.75	2.35	1.93	1.74	2.12
Cl (L/h/kg)	1.34	1.66	NA	NA	NA	NA	NA	NA
V_{ss} L/kg	0.356	0.428	NA	NA	NA	NA	NA	NA
Bioavailability (%)	NA	NA	16.5	32.1	11.0	14.2	55.4	59.9

[Excerpted from Applicant's submission]

Study Title: [^{14}C]-KU-0059436 – A study of absorption, metabolism and excretion following oral and intravenous administration to the rat.

Study No.: D2281 KKR007
Report Date: July 25, 2008
Study report location: eCTD 4.2.2.2.
Conducting Laboratory: (b) (4)
GLP: No

PK/ADME parameters of [^{14}C]-KU-0059436 were evaluated in Wistar rats following a single 5 mg/kg oral or 1 mg/kg intravenous administration of [^{14}C]-KU-0059436. A biliary excretion investigation was also conducted following a single 1 mg/kg intravenous administration of [^{14}C]-KU-0059436 in rats, to evaluate the rates and routes of excretion. Metabolite profiles in plasma and feces were determined by mass spectrometry.

Results and Conclusions:

- Overall, sex-dependent differences in systemic exposure, metabolism, and routes and rates of excretion were noted in Wistar rats.
  - Systemic exposure of drug-related radioactivity and that of unchanged parent drug were lower in plasma from male rats compared to female rats.
  - Metabolism of [^{14}C]-KU-0059436 was reduced in female animals compared to male animals, with plasma, urine and bile from female animals exhibiting substantially higher proportions of unchanged [^{14}C]-KU-0059436 than was observed in corresponding male animals.
  - Recoveries of total radioactivity were essentially complete by 24 hours and 48 hours post-dose in male and female animals, respectively.
  - Renal excretion of radioactivity was the predominant route of elimination from female rats, while biliary excretion was the primary route of elimination from male rats.
  - Biliary excretion of radioactivity was more rapid and more extensive in male rats when compared to female rats.
  - Unchanged [^{14}C]-KU-0059436 accounted for > 10% of biliary excreted sample radioactivity at 2 hours from female animals, but < 1% from male animals.
  - Direct elimination of unchanged [^{14}C]-KU-0059436 was more prominent in female urine.
- Phase 1 hydroxylations and oxidations of olaparib were the predominant products of metabolism.

Table 8. Mean pharmacokinetic data after single oral and intravenous olaparib doses to rats (Study No. D2281 KKR007).

Parameter (mean)	Intravenous olaparib (1 mg/kg)		Oral olaparib (5 mg/kg)	
	Male	Female	Male	Female
C_{max} (µg/mL)	1.08	1.34	0.162	0.408
t_{max} (h)	0.5	0.25	1.0	2.0
AUC (µg.h/mL)	0.590	2.52	0.494	2.38
$t_{1/2}$ (h)	0.8	8.9	2.5	3.0
Plasma clearance (L/h/kg)	NC	NC	NC	NC
Volume of distribution (L/kg)	NC	NC	NC	NC
Bioavailability (%)	NA	NA	17.2	19.2

a Pharmacokinetic parameters for the rat were determined from composite profiles based on 3 animals/sex/timepoint
NC Not calculated

[Excerpted from Applicant's submission]

Study Title: [^{14}C]-KU-0059436 – A study on the absorption and excretion following oral and intravenous administration to the dog.

Study No.: D2281 KMD008
Report Date: December 17, 2008
Study report location: eCTD 4.2.2.2.
Conducting Laboratory: (b) (4)
GLP: No

The PK/ADME parameters of [^{14}C]-KU-0059436 were evaluated in male beagle dogs following a single 5 mg/kg oral and 1 mg/kg intravenous administration of [^{14}C]-KU-0059436. Each animal received the single oral and single intravenous doses separated by a four-week wash-out period.

Results and Conclusions:

- The pharmacokinetics of total radioactivity and unchanged parent [^{14}C]-KU-0059436 were similar following intravenous and oral administration of [^{14}C]-KU-0059436, with relatively short (< 5 hours) mean elimination half-lives of unchanged KU-0059436.
- Absorption of drug-related radioactivity was rapid, with maximum plasma concentrations observed at 1 h post-dose following oral administration.

- The mean bioavailability of drug related material was estimated to be 79%, based upon areas under the plasma concentration-time curves. Absorption was estimated to be approximately 64%, based upon renal elimination of drug-derived radioactivity.
- Recoveries of total radioactivity were essentially complete by 48 hours post-dose following both routes of administration, indicating rapid elimination of [^{14}C]-KU-0059436-related material from the animals.
- Biliary excretion was the predominant route of elimination following intravenous administration of [^{14}C]-KU-0059436, accounting for approximately three quarters of administered radioactivity.
- The direct elimination of unchanged [^{14}C]-KU-0059436 was approximately 2-fold higher in urine following intravenous administration when compared to oral administration.
- Metabolism of KU-0059436 was not extensive, with hydroxylation and oxidations of KU-0059436 the predominant Phase 1 biotransformations.

Table 9. Mean pharmacokinetic data after single oral and intravenous olaparib doses to male dogs (Study No. DD2281 KMD008).

Parameter (mean ± SD)	Intravenous olaparib (1 mg/kg)	Oral olaparib (5 mg/kg)
C_0 (µg/mL)	1.52 ± 0.421	NA
C_{max} (µg/mL)	1.41 ± 0.394	2.13 ± 0.175
t_{max} (h)	0.083	1.0
AUC (µg.h/mL)	2.75 ± 0.788	10.3 ± 2.01
$t_{1/2}$ (h)	1.71 ± 0.30	4.61 ± 1.66
Plasma clearance (L/h/kg)	0.39 ± 0.13	NC
Volume of distribution (L/kg)	0.93 ± 0.14	NC
Bioavailability (%)	NA	78.9 ± 11.5

NA Not applicable

[Excerpted from Applicant's submission]

5.1.2. Distribution

In vitro plasma protein binding and blood/plasma distribution of [^{14}C]-olaparib was evaluated in the mouse, rat, dog, and human plasma. Higher plasma protein binding of olaparib was noted in human plasma samples when compared to those from the mouse, rat, and dog. Similar blood/plasma distribution for olaparib was noted in mouse, rat, and human plasma. Higher association of olaparib to blood cells was noted in dog plasma when compared to mouse, rat, and human plasma.

Table 10. In vitro plasma protein binding of olaparib (Study No. KPJ019).

Concentration tested (µg/ml)	Fraction drug unbound (%)			
	Mouse	Rat	Dog	Human
0.010	30.0	26.7	40.4	8.9
0.100	28.4	26.5	38.1	8.8
1.00	29.3	26.6	40.7	9.1
10.0	30.6	27.3	45.3	18.1

[Excerpted from Applicant's submission]

Table 11. In vitro blood/plasma distribution following incubation of [^{14}C]-olaparib in male mouse, rat, dog, and human plasma.

Species/Strain	[^{14}C]-olaparib concentration (µg/ml)	Blood/plasma ratio	Partition coefficient (%)	Association with blood cells (%)
Mouse/CD1	0.100	0.67	75.9	24.1
	10.0	0.67	75.0	25.0
Rat/Han Wistar	0.100	0.71	76.1	23.9
	10.0	0.71	75.8	24.2
Dog/Beagle	0.100	0.79	66.0	34.0
	10.0	0.92	56.3	43.7
Human	0.100	0.60	87.8	12.2
	10.0	0.74	70.4	29.6

Mouse and rat data generated using pooled blood. Dog and human data is mean of 3 individual assays.

[Excerpted from Applicant's submission]

Study Title: The binding of [^{14}C]-AstraZeneca AZD2281 to proteins in human plasma from healthy volunteers before and following multiple dosing with (b) (4)

Study No.:	D2281 KPJ043
Report Date:	March 24, 2011
Study report location:	eCTD 4.2.2.3.
Conducting Laboratory:	Clinical Pharmacology & DMPK AstraZeneca UK Limited Alderley Park Macclesfield Chesire, SK10 4TG United Kingdom
GLP:	No

The objective of this study was to evaluate the in vitro protein binding of olaparib in human plasma samples obtained from healthy volunteer subjects administered 2000 or 8000 mg/day (b) (4) twice-daily for 4.5 days.

Results and Conclusions

- No statistically significant differences in in vitro plasma protein binding of olaparib were noted in plasma samples from subjects administered either at 2000 or 8000 mg/day olaparib.

Study Title: [^{14}C]-KU-0059436: Quantitative whole body autoradiography study in tumour bearing nude mice.

Study No.:	KMM016
Report Date:	June 28, 2005
Study report location:	eCTD 4.2.2.3.
Conducting Laboratory:	(b) (4)
GLP:	No

The objective of this study was to evaluate the distribution of KU-0059436 in mice following a single oral administration of 30 mg/kg [^{14}C]-KU-0059436 to female BALB/c nu/nu mice bearing subcutaneous heterotopic HCT-116 colorectal carcinoma.

Results and Conclusions:

- The highest concentrations of radioactivity were observed in the gastro-intestinal tract and liver after 6 hours post-dosing. At 96 hours, radioactivity was detected only in the gastrointestinal contents, liver, and tumor tissues.
- The apparent tissue half-lives were 25.7 hours in the liver and 36.0 hours in the tumor.

Table 12. Concentration of radioactivity in tissues following a single oral administration of [^{14}C]-KU-0059436 to tumor bearing mice (Study No. KMM016).

Results expressed as µg equivalents KU-0059436/g

Tissue	001F 002 6 Hours	F 0 24 Hours	03F 0 48 Hours	04F 72 Hours	005F 96 Hours
Adrenal gland	0.086	0.107	BLQ	BLQ	BLQ
Aorta	BLQ BL	Q BL	Q BL	Q	BLQ
Blood 0	.053	0.052	BLQ	BLQ	BLQ
Bone	BLQ BL	Q BL	Q BL	Q	BLQ
Bone marrow	0.200	0.103	BLQ	BLQ	BLQ
Brain	BLQ BL	Q BL	Q BL	Q	BLQ
Caecum contents	238[a]	48.4 1.	74	NS	2.30
Caecum mucosa	21.3	2.90	0.125	NS	BLQ
Ex-orbital lachrymal gland	BLQ B	LQ B	LQ B	LQ	BLQ
Eye	BLQ BL	Q BL	Q BL	Q	BLQ
Fat (brown)	0.088	0.052	BLQ	BLQ	BLQ
Fat (white)	BLQ B	LQ B	LQ B	LQ	BLQ
Intra-orbital lachrymal gland	BLQ B	LQ B	LQ B	LQ	BLQ
Kidney	0.443	0.131	BLQ	BLQ	BLQ
Large intestine contents	274[a]	70.7 5.	30	NS	4.16
Large intestine mucosa	7.99	9.41	0.595	NS	BLQ
Liver	12.9 8.	33 4	.57 1	.51	1.34
Lung 0	.060	0.061	BLQ	BLQ	BLQ
Muscle	BLQ 0.	056 B	LQ	BLQ	BLQ
Myocardium 0	.061	0.061	BLQ	BLQ	BLQ
Pancreas 0	.195	0.141	BLQ	BLQ	BLQ
Skin	0.059	BLQ B	LQ B	LQ	BLQ
Small intestine contents	167[a]	35.1 0	.803	0.926	0.768
Small intestine mucosa	14.4	3.93	0.455	BLQ	BLQ
Spinal cord	BLQ BL	Q BL	Q BL	Q	BLQ
Spleen 0	.443	0.203	BLQ	BLQ	BLQ
Stomach contents	193[a]	23.5 0	.883	0.428	0.501
Stomach mucosa	15.8 1.	46 1	.98	BLQ	BLQ
Thyroid gland	0.176	NS	BLQ	BLQ	BLQ
Tumour	0.339 0.	208 0.	116 0.	103	0.053

BLQ Below limit of quantification (0.041 µg equivalents/g)
NS Sample not sectioned
[a] Above upper limit of quantification (139 µg equivalents/g). Extrapolated value reported.

[Excerpted from Applicant's submission]

Study Title: Tissue distribution of radioactivity in the rat following oral administration of [^{14}C]-KU-0059436 by quantitative whole-body autoradiography.

Study No.: KMR004 (0088/446)
Report Date: April 2007
Study report location: eCTD 4.2.2.3.
Conducting Laboratory: (b) (4)
GLP: No

The objective of this study was to determine the tissue distribution of radioactivity in male and female Lister hooded rats following a single oral administration of [^{14}C]-KU-0059436 using quantitative whole-body autoradiography.

Results and Conclusions

- The highest levels of radioactivity were observed in the liver, uveal tract, and kidney.
- Similar tissue distribution of olaparib was noted in male and female animals.

Study Title: [^{14}C]-KU-0059436 (b) (4) Preliminary study to investigate absorption, distribution, metabolism and excretion following oral administration in the rat.

Study No.: KMR017
Report Date: April 14, 2005
Study report location: eCTD 4.2.2.3.
Conducting Laboratory: (b) (4)
GLP: No

The objective of this study was to evaluate the absorption, distribution, excretion, and metabolism of olaparib following a single oral administration of 15 mg/kg olaparib in Sprague-Dawley rats.

Results and Conclusions

- The majority of radioactivity was excreted via feces (65 - 87% of administered dose) within 48 hours.

- Using quantitative whole-body autoradiography, the greatest concentrations of radioactivity were observed in the kidney, liver, stomach, small intestine, large intestine, cecum, and urinary bladder 1 hour following administration of [^{14}C]-KU-0059436. By 48 hours, minimal radioactivity was observed in these tissues.
- Olaparib was the major component noted in urine and feces. Two major metabolites were also detectable – a monohydroxy metabolite (molecular weight 450) and a ring opened product (molecular weight 452). The later was only found in feces.

5.1.3. Metabolism

In vivo metabolism of olaparib was evaluated in rat, dog, and human. Olaparib is the major compound detected in plasma samples from the rat, dog, and human (~ 70 - 100%). Three major metabolites - M12, M15, and M18 – were noted in rat and human plasma samples. No unique human metabolites or metabolites which were present at disproportionately higher levels in humans than in the rat were identified.

Table 13. Quantitative rat, dog, and human plasma metabolite profiles following single oral or intravenous doses of [^{14}C]-olaparib.

Component name	Component identity	Proportion of circulating radioactivity (%)						
		Rat (oral)		Dog (IV)		Dog (oral)		Human (oral)
		Male	Female	Male		Male		Female
		Pool	Pool	1 h	4 h	1 h	8 h	Pool
M12	Ring-open hydroxyl-cyclopropyl (or isomer)	12.3	√	NI	NI	NI	NI	9.3
M15	Mono-oxygenated	9.4	√	NI	NI	NI	NI	10.3
M18*	Dehydrogenated piperazine	7.9	√	NI	NI	NI	NI	13.7
Olaparib	Parent	70.4	100	86.6	86.4	90.9	85.8	70.0

NI Not identified (no quantitative or qualitative identification completed)
√ Component detected by HPLC-MS but not quantifiable by radio-HPLC
* Component M18 was derived from M39 during MS analysis
Data from rat (KMR027 analysed in KMX032), dog (KMD008) and human (clinical trial D0810C00010 analysed in KMX032). Rat plasma samples also analysed in KKR007; less detailed analysis so data not included in table. Components only detected by HPLC-MS not included

[Excerpted from Applicant's submission]

Figure 19. Human and rat plasma metabolites.

*: Present in trace quantities only. Arrows show possible biotransformation routes. Circled structures are main human plasma metabolites.
Bracketed structures observed as HPLC-MS degradation products

[Excerpted from Applicant's submission]

Figure 20. Human and rat excreta metabolites.

*: Present in trace quantities only. Arrows show possible biotransformation routes. Circled structures are main human plasma metabolites.
Bracketed structures observed as HPLC-MS degradation products

[Excerpted from Applicant's submission]

5.1.4. Excretion

Study Title: AZD2281: The disposition of [^{14}C]-AZD2281 in the rat.

Study No.:	D2281 KMR027
Report Date:	November 3, 2008
Study report location:	eCTD 4.2.2.3.
Conducting Laboratory:	(b) (4)
GLP:	No

The main objective of this study was to evaluate the rates and routes of excretion of radioactivity following a single oral administration of [^{14}C]-AZD2281 to male and female Wistar rats.

Results and Conclusions

- Approximately 92% of the dosed radioactivity was excreted 24 hours post-dosing.

- Radioactivity was excreted predominantly in the feces (89% and 73% of the dose in males and females, respectively).

5.2 Toxicokinetics

Refer to toxicokinetic analyses within reviews of individual general toxicology studies.

6 General Toxicology

6.1 Single-Dose Toxicity

Single-dose toxicity studies with olaparib were not needed to support this NDA, so study reports from these studies were not reviewed at this time.

6.2 Repeat-Dose Toxicity

Study title: KU-0059436: 28 Day Oral (Gavage) Administration Toxicity Study in the Rat Followed by a 28 Day Treatment Free Recovery Period

Study no.:	2229/037
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 28, 2004
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436), lot # 040502, purity 97.35%

Key Study Findings

- The major target organs were the bone marrow, spleen, thymus, liver, and kidney.
- The 40 mg/kg dose reduced peripheral red and white blood cells, primarily in female rats. Elevated reticulocyte and platelet counts correlated with microscopic findings of bone marrow and splenic hematopoiesis and were indicative of a regenerative response.
- C_{max} and AUC values were 3 to 8-fold higher in females compared to males.
- No remarkable findings were noted at the end of recovery.

Methods

Doses:	0, 5, 15, 40 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Crl (GLX/BRL.Han)IGSBR rats
Number/Sex/Group:	10/Sex/Group
Age:	42 to 46 days old
Weight:	Males – 155.0 to 188.0 g Females – 127.3 to 151.4 g
Satellite groups:	3/Sex for control (TK study) 6/Sex/Group 2-4 (TK study) 3/Sex/Group (Hematology)
Unique study design:	None
Deviation from study protocol:	Deviations were not considered to affect the study design or interpretation of results.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Twice daily for routine health checks, weekly physical examinations
Body weights	Pre-test, weekly, and prior to necropsy
Food Consumption	Weekly, only 3 cages/sex/group
Ophthalmology	Pre-test (all animals) and Week 4 (control and high dose level)
Hematology[1]	Week 4 (main study) and Day 8, 15, 22, 29, and Week 8 (hematology study)
Coagulation and Clinical chemistry[2]	Week 4 (main study) and Week 8 (hematology study)
Urinalysis[3]	Week 4 (main study) and Week 8 (hematology study)
Bone marrow smear[4]	At end of necropsy for all groups
Toxicokinetics	Day 1 and Week 4 (0.25, 0.5, 1, 2, 4, 8, 12, and 24 h)

[1]Hematology parameters: hemoglobin concentration, packed cell volume, mean cell volume, mean cell hemoglobin concentration, red cell distribution width, plateletcrit, platelet distribution width, red bloods, reticulocytes, mean cell hemoglobin, hemoglobin distribution width, platelets, mean platelet volume, leukocytes, lymphocytes, neutrophils, monocytes, eosinophils, basophils

[2]Coagulation and clinical chemistry parameters: prothrombin time, activated partial thromboplastin time, aspartate aminotransferase, alkaline phosphate, potassium, inorganic phosphorus, total protein, globulin, total cholesterol, urea, creatinine, alanine aminotransferase, sodium, calcium, chloride, albumin, albumin/globulin ratio, glucose, total bilirubin

[3]Urinalysis parameters: color, microscopy of sediment, specific gravity, protein, ketones, blood, reducing substances, turbidity, volume, pH, glucose, bilirubin, urobilinogen

[4]Bone marrow smear parameters: proerythroblasts, early erythroblasts, intermediate erythroblasts, late erythroblasts, myeloblasts, promyelocytes, myelocytes, metamyelocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, megakaryocytes, plasma cells, reticulum cells, total erythropoietic cells, total myelopoietic cells, myeloid/erythroid ratio

Dose Justification

The dose levels were selected based on results from a 7-day repeat-dose study (Study No. 2229/040). Olaparib caused marked hematological toxicities at dose of ≥ 100 mg/kg/day that appeared reversible following a 21-day non-dosing period. According to the Applicant, a high dose level of 40 mg/kg was anticipated to cause target organ toxicity or non-specific toxicity. The mid dose level of 15 mg/kg was selected because it was the geometric mean of the low and high dose levels and equivalent to the expected dose in humans. The low dose level of 5 mg/kg was expected to be a pharmacologically active dose with no adverse effects.

Mortality

All animals survived to scheduled necropsy.

Clinical Signs

- Clinical signs of salivation and paddling were reported for all mice in the 40 mg/kg dose group during the last week of dosing.

Body Weights

- Male rats in the 40 mg/kg dose group showed a slight reduction in absolute body weight (↓7%) and body weight gain (↓18%) compared to controls. At end of recovery, absolute body weight was still reduced by 14% compared to control.
- No remarkable differences were observed in female rats.

Feed Consumption

No remarkable changes in food consumption were noted.

Ophthalmoscopy

No treatment-related effects were noted in the eye.

Hematology

- The majority of findings were observed in 40 mg/kg female animals. Olaparib slightly reduced hemoglobin, red blood cells, packed cell volume, and leukocyte populations. A statistically significant increase was noted in reticulocytes, platelets, plateletcrit, mean corpuscular volume (MCV), hemoglobin distribution width (HDW), and red cell distribution width (RDW). Collectively, these findings correspond to microscopic observations of bone marrow hematopoiesis and an apparent regenerative response at end of dosing necropsy.
- At end of recovery, total leukocytes and lymphocytes were slightly reduced.

Table 14. Summary of Week 4 hematology findings in rats administered oral olaparib daily (% change relative to controls male/female)

Parameters	15 mg/kg/day		40 mg/kg/day		40 mg/kg/day (recovery)
	Main study animals	Hematology animals	Main study animals	Hematology animals	Hematology animals
Number of animals	10	4	10	4	4
Hemoglobin			1.8/-10.2***		1.8/1.2
Red blood cells			-0.3/-14.8***		-1.8/-0.8
PCV			0.2/-10.4***		-1.2/2.5
Reticulocytes			-19.0/45.0*		-12.5/-15.8
MCV			0.5/5.3***		0.6/3.3
MCH			2.1/4.9*		4.6/2.1
HDW			5.4/18.7***		-2.4/15.1
RDW			1.9/37.8*		-5.0/13.3*
Platelets			1.8/40.5***	-3.1/46.4***	-7.5/-6.6
PCT			2.8/39.7	-4.4/51.6***	-12.6/-5.3
Leukocytes	2.6/-16.4		-2.6/-21.8	-25.9/-17.9	-28.2/-36.6
Lymphocytes			-6.2/-22.2*	-27.1/-22.2	-33.8/-37.5
Neutrophils		-33.3/-30.0		-25.0/0.0	0.0/-37.5

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Clinical Chemistry

No remarkable changes in clinical chemistry were observed.

Urinalysis

- Animals in the 40 mg/kg dose group had 40-50% increase in urine volume at the end of dosing and end of recovery period. No remarkable findings were noted in pH or specific gravity.

Gross Pathology

There were no test article-related macroscopic findings.

Organ Weights

There were no remarkable changes in organ weights.

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings

- At end of dosing necropsy, microscopic findings were observed in the bone marrow, liver, spleen, kidney, and thymus.
- Liver staining with Perl's Prussian Blue showed the presence of iron containing pigment hemosiderin in hepatocytes, correlating with hemorrhage and loss of red blood cells.

- No remarkable findings were noted in recovery animals.

Table 15. Summary of histological findings at end of dosing necropsy in rats administered oral olaparib daily for 4 weeks

	Males				Females			
	Grp 1 0 mg/kg	Grp 2 5 mg/kg	Grp 3 15 mg/kg	Grp 4 40 mg/kg	Grp 1 0 mg/kg	Grp 2 5 mg/kg	Grp 3 15 mg/kg	Grp 4 40 mg/kg
Femur and marrow								
• Increased hematopoiesis								
Minimal……..				1/10				6/10
Sternum and marrow								
• Increased hematopoiesis								
Minimal……..				3/10				7/10
Liver								
• Agonal congestion/hemorrhage								
Present……..				1/10			1/10	
• Hepatocyte pigment								
Minimal……..						1/10		4/10
Slight……..							1/10	
Spleen								
• Hematopoiesis								
Minimal……..	5/10	6/10	4/10	9/10	10/10	7/10	5/10	
Slight……..	1/10		1/10			3/10	5/10	6/10
Moderate……...								4/10
Kidney								
• Papillary mineralization								
Minimal……..				2/10				1/10
Thymus								
• Atrophy								
Minimal……..				2/10				3/10
Slight……..								5/10

Special Evaluation

- At end of dosing necropsy, myelogram data showed an increase in erythroid cell development and a corresponding decrease in myelopoietic cells primarily at the 40 mg/kg dose level. These findings were more pronounced in female animals and corresponded with microscopic findings of minimal bone marrow hematopoiesis at end of dosing necropsy.
- At end of recovery, myelogram parameters were generally comparable to controls. Although not statistically significant, a decrease in erythroid cell development was observed in recovery animals.

Table 16. Myelogram findings in rats administered oral olaparib daily for 4 weeks (% change relative to controls male/female)

	5 mg/kg/day	15 mg/kg/day	40 mg/kg/day	40 mg/kg/day (recovery)
Proerythroblasts			4.5/56.0	-55.2/-25.0
Early erythroblasts			22.4/69.4**	-16.7/-22.2
Late erythroblasts			20.2**/25.2**	4.0/7.0
Myeloblasts	-29.2/36.7*	4.2/-30.0	-16.7/-46.7*	-10.0/33.3
Promyelocytes		-27.9*/-5.4	-16.3/-8.1	28.6/0.0
Metamyelocytes			-11.0/-33.9*	7.5/-13.7
Neutrophils			-11.4/-27.7	-9.6/-10.9
Lymphocytes			-25.3/-37.3	4.4/59.8
Total erythropoietic cells			18.3***/29.6***	-5.5/-15.2
Total myelopoietic cells			-10.6*/-21.0**	5.5/1.4
M/E ratio			-22.2***/-37.5**	11.1/28.6

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Toxicokinetics

- After a single dose, C_{max} and AUC increased with increasing dose and were greater than dose proportional with ascending dose levels.
- Following repeat dosing, C_{max} and AUC increased with increasing dose. C_{max} and AUC were greater than dose proportional with each dose escalation in males. In females, C_{max} and AUC were slightly greater than dose proportional from 5 to 15 mg/kg and approximately dose proportional from 15 to 40 mg/kg.
- C_{max} and AUC were 3- to 8-fold higher in females compared to males.
- There was no apparent olaparib accumulation with repeat dosing.
- Peak plasma concentrations occurred at 0.5 to 2 h post-dose.

Table 17. Mean toxicokinetic parameters of olaparib following oral daily dosing in rats for 4 weeks

	Sex	Dose (mg/kg)	T_{max} (h)	T_{last} (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24h)}$ (h·ng/ml)	$AUC_{(0-24h)}$/D (h·ng/ml)/ (mg/kg)
Day 1	Male	5	2.0	4	66.63	13.33	207	41
		15	0.5	12	331.84	22.12	1333	89
		40	0.5	24	1851.41	46.29	7869	197
	Female	5	2.0	12	338.27	67.65	1657	331
		15	1.0	24	1646.29	109.75	7830	522
		40	0.5	24	8256.72	206.42	28372	709
Week 4	Male	5	0.5	8	69.49	13.90	239	48
		15	1.0	24	355.55	23.70	1057	70
		40	2.0	8	1291.77	32.29	5752	144
	Female	5	2.0	24	234.61	46.92	1641	328
		15	0.5	24	1951.45	130.10	6267	418
		40	0.5	24	4860.58	121.51	15551	389

Dosing Formulation Analysis

Sample concentrations were within ± 4% of the theoretical concentration on Day 1 and Day 28. Dosing formulations were considered homogenous and within the following acceptance criteria (CV of individual values ≤ 6% and ± 10% of mean). Sample concentrations were unchanged during the 24 h storage period.

Study title: AZD2281: One Month Compound-batch Comparison Oral Toxicity Study in the Rat

Study no.:	1858KR
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D Alderley, Alderley Park, Macclesfield, SK10 4TG, England
Date of study initiation:	October 1, 2007
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU0059436), lot # 060344 (previous batch, 100.1% purity), lot # C436/4 (new batch, 99.3% purity)

*This bridging toxicology study was conducted to qualify an impurity ((b) (4)) detected at (b) (4) % in a new batch of olaparib drug substance.

Key Study Findings

- Toxicokinetic parameters were comparable between the two olaparib batches with the exception of Day 1 C_{max} and AUC values in males. Males receiving the new olaparib batch had a 2 to 4-fold higher exposure on Day 1 compared to those treated with the previous batch.
- There was a high incidence of minimal to mild microscopic findings in the brain (vacuolation of cerebellar white matter) observed in animals treated with both olaparib batches (50 to 95%) compared to controls (20%). There were no associated clinical signs, and these findings were not reported in other repeat-dose toxicity studies following olaparib administration to rats or dogs for up to 26 weeks.

Methods

Doses:	0, 40 mg/kg previous batch, 40 mg/kg new batch (Group 1, 2/4, 3/5)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5.5 ml/kg
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl-β-cyclodextrin in PBS pH 7.4
Species/Strain:	AlpkHsdRccHan: WIST
Number/Sex/Group:	10/Sex/Group
Age:	~11 weeks old
Weight:	Males: 270 to 464 g Females: 181 to 257 g
Satellite groups:	6/Sex/Group 4 and 5 (TK study)
Unique study design:	None
Deviation from study protocol:	There were no reported deviations from the study protocol.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Twice daily, weekly physical examinations
Body weights	Twice weekly
Food Consumption	Weekly
Ophthalmology	Pre-test and Day 27
Hematology[1]	Day 15 and Day 29
Coagulation and Clinical chemistry[2]	Day 29
Urinalysis[3]	Day 23 (males) and Day 24 (females)
Toxicokinetics	Day 1 and Day 29 (pre-dose, 1, 2, 4, 8, 12, and 24 h)

[1]Hematology parameters: erythrocytes, hemoglobin, hematocrit, mean red cell hemoglobin, mean red cell hemoglobin concentration, mean red cell volume, red cell distribution width, reticulocytes, platelets, leukocytes, neutrophils, lymphocytes, monocytes, basophils, eosinophils, large unstained cells

[2]Coagulation and clinical chemistry parameters: prothrombin time, activated partial thromboplastin time, albumin, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, total bilirubin, calcium, cholesterol, creatinine, globulin, glucose, glutamate dehydrogenase, inorganic phosphate, potassium, sodium, total protein, triglycerides, urea, appearance

[3]Urinalysis parameters: specific gravity, pH, protein, glucose, volume, ketones, bilirubin, blood, urinary sediment, appearance

Dose Justification

A dose of 40 mg/kg/day was selected has the high dose level in a previous 28-day repeat-dose study in rats (Study No. 2229/037). The same dose level was used in this bridging toxicology study to qualify the (b) (4) impurity detected at (b) (4) % in the new olaparib drug substance batch.

Mortality

All animals survived to scheduled necropsy.

Clinical Signs

There were no test article-related clinical signs noted.

Body Weights

- There were no remarkable changes in male body weight.
- Female rats administered either batch of olaparib experienced a decrease in body weight of 8 to 10% compared to controls, starting in Week 2 until end of dosing.

Feed Consumption

There were no remarkable changes in food consumption.

Ophthalmoscopy

- Ophthalmology data was not included in the study report. According to the Applicant, there were no test article-related findings in the eye.

Hematology

- Changes in hematology parameters were generally observed with the previous olaparib batch.
    - On Day 15, male rats administered the previous batch had elevated peripheral leukocytes, neutrophils, and lymphocytes. Cell counts were comparable to controls on Day 29.
    - Females administered the previous batch had elevated platelets throughout the dosing period and increased reticulocytes and RDW on Day 29. Females also showed a significant reduction in neutrophil counts.
- For animals given the new olaparib batch, hematology parameters were comparable to control values except for a 46% decrease in female neutrophil counts at end of dosing.
- There were no macroscopic or microscopic correlates in the bone marrow or lymphoid tissues.

Table 18. Summary of hematology findings in rats administered oral olaparib daily for 4 weeks (% change relative to controls male/female)

	Parameters	40 mg/kg/day (previous batch)	40 mg/kg/day (new batch)
Day 15	Leukocytes	20.2*/0.6	8.7/-11.9
	Neutrophils	37.1/-17.9	0.7/-17.0
	Lymphocytes	16.2*/5.7	7.3/-10.9
	Platelets	10.7/38.3**	4.9/11.1
Day 29	Reticulocytes	4.9/38.0**	1.7/22.3
	RDW	0.0/20.5**	0.0/16.1
	Neutrophils	0.8/-37.5*	-12.1/-46.0
	Platelets	13.9/23.4**	11.1/18.7

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Clinical Chemistry

There were no remarkable changes in clinical chemistry parameters.

Urinalysis

No test article-related changes in urinalysis were reported.

Gross Pathology

There were no remarkable macroscopic findings.

Organ Weights

- Both olaparib batches reduced female thymus weight by ~30% compared to vehicle controls. No macroscopic or microscopic correlates were reported.

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings

- At end of dosing necropsy, microscopic findings were noted in the brain, epididymides, kidneys, and liver.
- There was a high incidence of vacuolation of cerebellar white matter reported with both batches of olaparib.
- A low incidence of minimal kidney inflammation was observed in males administered the new olaparib batch. Urinalysis and clinical chemistry were unremarkable.
- All other findings were consistent between the 2 batches.

Table 19. Summary of histopathology findings at end of dosing necropsy in rats administered oral olaparib daily for 4 weeks

	Males (mg/kg/day)			**Females** (mg/kg/day)		
	Grp1 0	Grp2 40 (prior batch)	Grp3 40 (new batch)	Grp1 0	Grp2 40 (prior batch)	Grp3 40 (new batch)
Brain						
• Cerebellar white matter vacuolation						
Minimal…….	2/10	7/10	8/10	2/10	2/10	7/10
Mild……...		1/10	1/10			3/10
Epididymides						
• Focal inflammatory cell infiltrate						
Minimal…….		1/10	2/10			
Kidneys						
• Focal unilateral mononuclear cell infiltration						
Minimal…….			2/10			
Liver						

	Males (mg/kg/day)			**Females** (mg/kg/day)		
	Grp1 0	Grp2 40 (prior batch)	Grp3 40 (new batch)	Grp1 0	Grp2 40 (prior batch)	Grp3 40 (new batch)
• Increased glycogen vacuolation Minimal……. Mild……...		1/10	1/10		1/10	

Toxicokinetics

- There were no significant differences in T_{max} and $T_{1/2}$ between the two batches of olaparib.
- After a single dose, C_{max} was 4-fold higher in males and 1.3-fold higher in females administered the new olaparib batch. AUC was also higher, approximately 2.4-fold in males and 1.5-fold in females.
- After repeat dosing, AUC values in both sexes and C_{max} in males were comparable between batches whereas females administered the new batch of olaparib had a 1.5-fold lower C_{max}.
- Consistent with other rat repeat-dose toxicity studies, systemic exposure of olaparib was markedly higher in females compared to males following single and repeat dosing.

Table 20. Mean toxicokinetic parameters of olaparib following daily oral dosing in rats for 4 weeks

	Sex	Dose (mg/kg)	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/ml)	$AUC_{(0\text{-}24h)}$ (h·ng/ml)
Day 1	Male	40 prior batch	2	2.6	367	2730
		40 new batch	2	2.7	1540	6550
	Female	40 prior batch	1	3.9	2140	13100
		40 new batch	2	4.8	2950	19000
Day 28	Male	40 prior batch	1	3.8	220	1500
		40 new batch	2	2.5	206	1860
	Female	40 prior batch	2	3.1	1530	16000
		40 new batch	4	3.1	1010	11900

Dosing Formulation Analysis

Formulation analysis was not provided in the study report. According to the Applicant, sample concentrations were within ± 3% of the theoretical concentration, and dosing formulations were considered homogenous and stable.

Study title: 26 Week Oral (Gavage) Toxicity Study in the Rat

Study no.:	TII0012
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	July 19, 2005
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436), lot # 040611, 101% purity

Key Study Findings

- Systemic exposure was significantly higher in female rats (up to 14-fold).
- There were no test article-related early mortalities.
- Female rats at the 15 mg/kg dose level experienced a 10% decrease in body weight and 21% decrease in body weight gain, consistent with higher olaparib exposure.
- The major target organ was the hematopoietic system.

Methods

Doses:	Males: 0, 5, 15, 30 mg/kg (Main study: Group 1, 2, 3, 4 and TK study: Group 9, 10, 11, 12) Females: 0, 1, 5, 15 mg/kg (Main study: Group 5, 6, 7, 8 and TK study: Group 13, 14, 15, 16)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Han Wistar rats
Number/Sex/Group:	20/Sex/Group 1-8
Age:	~4 to 5 weeks old
Weight:	Males: 125 – 179 g Females: 108 – 145 g
Satellite groups:	3/Sex for control (Group 9, 13) 9/Sex/Group 10-12, 14-16
Unique study design:	All tissues from control and high dose animals were included in the histopathology evaluation. For the low and mid dose levels, only the spleen, liver, thymus, femur, and sternum were evaluated.
Deviation from study protocol:	Deviations from the study protocol were not considered to affect the study design or interpretation of the results.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Daily, Weekly detailed examination
Body weights	Weekly
Food Consumption	Weekly
Ophthalmology	Pre-test and Week 25
Hematology[1]	From 10/Sex/Group on Week 4, 8/9, 13, 17, 21, and 26
Coagulation and Clinical chemistry[2]	From 10/Sex/Group on Week 13 and 26
Urinalysis[3]	From 10/Sex/Group on Week 12 and 26
Bone marrow smear[4]	From control and high dose animals at end of dosing necropsy
Toxicokinetics	From 3/Sex/Group on Day 1 and during Week 13 and 26 (0.25, 0.5, 1, 2, 4, 8, 12, and 24 h)

[1]Hematology parameters: hemoglobin, hemoglobin density width, red blood cells, red cell distribution width, packed cell volume, mean cell volume, mean cell hemoglobin, platelets, total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells, cell morphology, reticulocytes

[2]Coagulation and clinical chemistry parameters: prothrombin time, activated partial thromboplastin time, urea, creatinine, glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, albumin/globulin ratio, bilirubin, cholesterol, calcium, sodium, potassium

[3]Urinalysis parameters: volume, specific gravity, glucose, pH, protein, color, appearance

[4]Bone marrow smear parameters: myeloblasts, promyelocytes, neutrophilic myelocytes, neutrophilic metamyelocytes, neutrophil polymorphs, eosinophils, basophils, proerythroblasts, early normoblasts, intermediate normoblasts, late normoblasts lymphocytes, monocytes, plasma cells, megakaryoblasts/cytes, others, early myeloid cells, late myeloid cells, early erythroid cells, late erythroid cells, lymphocytes, myeloid:erythroid ratio, myeloid left shift index, erythroid left shift index

Dose Justification

According to the Applicant, the dose levels were selected based on previous findings in the 28-day repeat-dose rat study (Study No. 2229/037). Doses up to 40 mg/kg were tolerated and all animals survived to scheduled necropsy. The major target organ was the hematopoietic system with corresponding changes in hematology parameters, increased hematopoiesis in the spleen, and pigmented hepatocytes in the liver.

Mortality

Three olaparib-treated animals were found dead or euthanized moribund during the dosing period. These early deaths occurred at the 5 mg/kg/day dose level and were not considered test article-related.

Clinical Signs

- All satellite animals including controls had protruding eyes and abnormal eye color (red, dark, white, or cream) throughout the dosing period. These findings were not present in main study animals and considered to be due to blood collection via the orbital sinus.
- Scabbing and slight to marked hair loss was noted in all dose groups including controls throughout the dosing period.

- A low incidence of increased salivation in 2/20 males and 1/20 females was observed at the high dose level.

Body Weights

Females in the 15 mg/kg dose group showed a decrease in mean body weight up to 10% and a 21% decrease in body weight gain at end of dosing when compared to control values.

Feed Consumption

There were no test article-related changes in food consumption reported.

Ophthalmoscopy

No test article-related findings were reported in the eye.

Hematology

- A small, but statistically significant, decrease in red cell mass was noted primarily at doses of ≥ 15 mg/kg throughout the treatment period. Platelets were occasionally elevated up to ~25% at doses of ≥ 5 mg/kg.
- Slight changes in total leukocytes, neutrophils, and lymphocytes were noted at doses of ≥ 15 mg/kg in Week 4. These cell populations were similar to control levels during the following weeks.
- Monocyte counts were reduced during Week 13 and elevated during Week 21 in males and females, respectively. Cell counts were comparable to controls at the end of dosing.

Table 21. Summary of hematology changes in rats administered oral olaparib daily for 26 weeks (% change relative to controls male/female)

	Parameters	Low dose[#]	Mid dose[#]	High dose[#]
Week 4	Red blood cells	-4.9*/-1.1	-3.9*/2.0	-5.7*/-0.2
	Hemoglobin			-4.5**/0.0
	PCV			-4.3*/2.6
	MCV			1.5/3.0**
	MCHC			-2.0/-2.0**
	Leukocytes			23.0*/-1.9
	Neutrophils		27.0/-31.8**	10.1/-33.0**
	Lymphocytes			25.7*/4.3
Week 8/9	Hemoglobin			-3.7*/-3.2*
	MCHC			-1.5/-2.1*
	RDW			-0.8/6.2*
	HDW			-2.9/6.0*
	Platelets	-1.8/13.4*	7.4/20.5*	-3.4/16.5*
Week 13	Monocytes	-7.1/-36.4**	-14.3/-36.4**	-28.6**/-36.4**
Week 17	Hemoglobin			-2.5/-3.3*
	RDW			-3.0/5.3*
	Reticulocytes	-10.5/-13.6*	0.9/-11.4**	-4.6/-16.5**

	Parameters	Low dose[#]	Mid dose[#]	High dose[#]
Week 21	Red blood cells			0.9/-4.2*
	Hemoglobin			-1.2/-5.6***
	MCHC		-1.4/-2.7**	-3.3*/-3.0**
	Platelets		11.4/26.9***	2.2/24.6***
	Monocytes		36.4*/-9.0	36.4*/-18.2
Week 26	Red blood cells			-4.2*/-3.6
	Hemoglobin			-1.9/-2.6*
	PCV		-0.4/-3.6*	-2.8/-3.9*
	MCH	4.5*/0.5	4.5*/1.1	2.8*/1.1
	MCHC	3.2*/1.5	2.9*/3.5	2.1*/0.9
	Platelets			1.7/25.3**

[#]Male rats were given 5, 15, and 30 mg/kg/day, and females received 1, 5, and 15 mg/kg/day. Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Clinical Chemistry

- A small, but statistically significant, decrease in urea was observed in all dose groups in Week 13 and Week 26.
- Total protein levels were slightly reduced at doses of ≥ 15 mg/kg, consistent with decreases in globulin levels.
- A statistically significant decrease in calcium was noted in 15 mg/kg females with no other changes in electrolytes.

Table 22. Summary of clinical chemistry changes in rats administered oral olaparib daily for 26 weeks (% change relative to controls male/female)

	Parameters	Low dose[#]	Mid dose[#]	High dose[#]
Week 13	Urea	-0.8/-13.7*	-4.9/-9.6*	-5.2/-13.1*
	Total protein			-4.1*/-6.6***
	Globulin		-6.7*/-3.7	-6.7*/-11.1***
	A/G ratio		14.3**/0.0	7.1*/5.3**
	Calcium			0.9/-3.5**
Week 26	Urea	-11.9*/-14.1*	-17.7**/-12.7*	-13.2**/-18.4**
	Total protein			-5.6*/-8.1***
	Albumin			0.0/-6.0*
	Globulin		-6.9*/4.2	-10.3**/-12.5***
	A/G ratio		14.3**/-4.8	14.3*/4.8
	Calcium			-0.9/-2.7*

[#]Male rats were given 5, 15, and 30 mg/kg/day, and females received 1, 5, and 15 mg/kg/day. Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Urinalysis

There were no remarkable changes in urinalysis parameters.

Gross Pathology

There were no remarkable macroscopic findings reported.

Organ Weights

- Organ weight changes were noted in the thymus, heart, lungs, spleen, liver, and kidneys without macroscopic or microscopic correlates.

Table 23. Summary of organ weight changes in rats administered oral olaparib daily for 26 weeks (% change relative to controls male/female)

Organ		Low dose#	Mid dose#	High dose#
Thymus	A			-6.8/-19.0***
	R/BW			-5.2/-9.8
Lungs	A			5.1/0.8
	R/BW			7.3*/13.2***
Spleen	A	22.6*/10.0	21.0*/16.0	8.1*/-4.0
	R/BW	21.4*/5.0	21.4*/20.0**	14.3*/5.0*
Liver	A			0.3/-11.4**
	R/BW			2.7/-1.2

A – absolute weight; R/W – relative to body weight; significant finding, *p<0.05, **p<0.01, ***p<0.001

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings
There were no remarkable microscopic findings reported.

Special Evaluation

- Bone marrow smears were evaluated for control and high dose animals at end of dosing necropsy.
- In 15 mg/kg female rats, there was a statistically significant increase in late myeloid cells and myeloid/erythroid ratio and a corresponding decrease in early erythroid cells and myeloid left shift index. The hematology evaluation showed only a slight reduction in red blood cells during the dosing period, and changes in peripheral myeloid cells were variable.

Table 24. Summary of myelogram data from rats treated with oral olaparib daily for 26 weeks (% change relative to controls male/female)

Parameters	High dose
Late myeloid	-7.2/26.5***
Early erythroid	11.6/-27.6*
Myeloid LS	0.0/-20.0*
M/E ratio	0.0/28.6*

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Toxicokinetics

- Following a single dose, C_{max} and AUC increased with increasing dose. C_{max} was greater than dose proportional with each dose escalation. AUC was greater than dose proportional with increasing dose in male rats at all dose levels and from 1 to 5 mg/kg in females. AUC was approximately dose proportional in female rats from 5 to 15 mg/kg.
- After repeat dosing, C_{max} and AUC increased with increasing dose and were greater than dose proportional with each dose escalation. Systemic exposure in Week 13 and Week 26 was similar to Day 1, indicating that olaparib did not accumulate with repeat dosing at any dose level tested.
- There was a marked increase in overall exposure up to 14-fold in females compared to males during the dosing period.
- Peak plasma concentrations occurred at 1 to 4 h on Day 1 and 0.25 to 2 h after repeat dosing. The elimination half-life ranged from 1.4 to 5.5 h.

Table 25. Mean toxicokinetic parameters of olaparib following daily oral administration in rats for 26 weeks

	Dose (mg/kg)	Sex	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24h)}$ (h·ng/ml)	$AUC_{(0-24h)}$/D (h·ng/ml)/ (mg/kg)
Day 1	1	Female	2.00	3.51	38.3	38.3	334	334
	5	Male	1.00	2.54	71.7	14.3	249	49.8
		Female	4.00	2.76	358	71.6	2770	554
	15	Male	1.00	1.93	443	29.5	1350	90
		Female	1.00	2.72	2060	137.3	8840	589.3
	30	Male	1.00	1.44	1300	43.3	6210	207
Week 13	1	Female	2.00	3.48	36.7	36.7	251	251.0
	5	Male	0.25	2.75	50.1	10.0	215	43.0
		Female	1.00	3.12	303	60.6	1740	348.0
	15	Male	1.00	3.20	271	18.1	982	65.5
		Female	0.50	3.89	1670	111.3	6870	458.0
	30	Male	1.00	NC	895	29.8	4000	133.3
Week 26	1	Female	1.00	3.70	43.4	43.4	373	373
	5	Male	0.50	3.44	50.8	10.2	227	45.4
		Female	1.00	NC	396	79.2	3150	630
	15	Male	1.00	2.09	358	23.9	1640	109.3
		Female	1.00	5.52	2430	162.0	6750	450
	30	Male	0.25	2.53	1710	57.0	4230	141

NC – not calculated

Dosing Formulation Analysis

Sample concentrations were within ± 4% of the intended concentration. Dosing formulations were considered homogenous based on acceptance criteria (CV of the individual values ≤ 6% and within ± 10% of mean). The samples were considered chemically stable over a 10 day storage period at 1-10^{o}C or room temperature; however, precipitation was observed during the storage period, indicating that the solutions were not physically stable.

Study title: KU-0059436: 28 Day Oral (Gavage) Administration Toxicity Study in the Dog Followed by a 4 Week Treatment-free Period

Study no.:	2229/38
Study report location:	eCTD 4.2.3.2
Conducting laboratory and location:	(b) (4)
Date of study initiation:	October 7, 2004
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	KU-0059436 (olaparib, AZD2281, (b) (4) lot # 040502, purity 98.88%

Key Study Findings

- All animals survived to scheduled necropsy.
- The major target organ was the hematopoietic system.
- Olaparib caused a dose-dependent decrease in reticulocytes, platelets, total leukocytes, and lymphocytes at ≥ 2.5 mg/kg, corresponding to microscopic findings of bone marrow atrophy and delays in erythroid cell development.
- Minimal or slight microscopic findings were also observed in the spleen, GI tract, kidneys, urinary bladder, parathyroid, and prostate, primarily at ≥ 5 mg/kg.

Methods

Doses:	0, 2.5, 5, 15 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	1% (w/v) methylcellulose in purified water
Species/Strain:	Beagle dogs
Number/Sex/Group:	3/Sex/Group (main study) 2/Sex for Group 3 and 4 (recovery)
Age:	30 to 39 weeks old
Weight:	Male – 10.06 to 15.28 kg Female – 8.68 to 12.91 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Deviations from the study protocol were not considered to affect the study outcome or interpretation of the results.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Twice daily for routine health checks, daily physical examinations
Body weights	Weekly
Food Consumption	Daily
Ophthalmology	Pre-test and Week 4
ECG	Heart rate only at pre-test and Week 4 (2 h post-dose)
Temperature	Pre-test, Week 2 and Week 4 (2 h pre-dose and 2 h post-dose)
Hematology[1]	Pre-test, Week 4, and Week 8
Coagulation and Clinical chemistry[2]	Pre-test and Week 4
Urinalysis[3]	Pre-test and Week 4
Bone marrow smear[4]	At end of dosing necropsy for all animals
Toxicokinetics	Day 1 and during Week 4 (pre-dose, 0.5, 1, 2, 4, 8, 12, and 24 h)

[1]Hematology parameters: hemoglobin, red blood cells, packed cell volume, reticulocytes, mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration, hemoglobin distribution width, red cell distribution width, platelets, plateletcrit, mean platelet volume, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells

[2]Coagulation and clinical chemistry parameters: prothrombin time, activated partial thromboplastin time, aspartate aminotransferase, alanine aminotransferase, gamma glutamyltransferase, alkaline phosphatase, sodium, potassium, chloride, calcium, inorganic phosphorous, urea, total bilirubin, creatinine, total protein, albumin, globulin, albumin/globulin ratio, total cholesterol, glucose

[3]Urinalysis parameters: volume, specific gravity, pH, color, protein, ketones, blood, turbidity, glucose, bilirubin, urobilinogen, microscopy of sediment

[4]Bone marrow smear parameters: proerythroblasts, early erythroblasts, intermediate erythroblasts, late erythroblasts, myeloblasts, promyelocytes, myelocytes, metamyelocytes, neutrophils, eosinophils, basophils, lymphocytes, monocytes, megakaryocytes, plasma cells, reticulum cells, total erythropoietic cells, total myelopoietic cells, myeloid/erythroid ratio

Dose Justification

According to the Applicant, the dose levels were selected based on an MTD study (Study # 2229/039) and 7-day repeat-dose toxicity study (Study # 2229/046) in dogs. In the MTD study, doses of ≥ 30 mg/kg olaparib caused significant hematological toxicities. Therefore, the Applicant selected doses of 2.5, 5, and 15 mg/kg/day as the low, mid, and high dose levels for the 7- and 28-day repeat-dose studies (Study # 2229/046 and 2229/38).

Mortality

All animals survived to scheduled necropsy.

Clinical Signs

Clinical signs were unremarkable.

Body Weights

There were no test article-related effects on body weight.

Feed Consumption

There were no test article-related changes in food consumption.

Ophthalmoscopy

No test article-related effects were noted in the eye.

ECG

The ECG assessment only included heart rate measurements, and findings were unremarkable.

Temperature

There were no test article-related changes in rectal temperature.

Hematology

- A dose-dependent decrease in reticulocytes, platelets, total leukocytes and lymphocytes was observed on Day 8 and Day 15 at all dose levels.
- In Week 3 and 4, these parameters were generally comparable to control values at the 2.5 and 5 mg/kg dose levels. The 15 mg/kg dose group was still reduced in the last 2 weeks of dosing albeit with less severity. These findings were consistent with microscopic observations of slight bone marrow atrophy and a broad decrease in erythroid cell development observed in bone marrow smears at scheduled necropsy.
- At end of recovery, hematology parameters were comparable to pre-test and control values.

Table 26. Summary of hematology parameters in dogs administered oral olaparib daily for 4 weeks (% change relative to controls male/female)

	Parameters[1]	2.5 mg/kg/day	5 mg/kg/day	15 mg/kg/day
Day 8	Red blood cells			-0.6/-5.9
	Reticulocytes	-37.5/-42.9	-62.5/-28.6	-87.5/-85.7
	Platelets	-15.7/18.2	-23.3/-5.6	-36.5/-25.5
	Leukocytes	-11.2/-30.3	-11.9/-27.7	-30.6/-34.8
	Neutrophils	-7.6/-33.7	-7.6/-30.2	-31.8/-41.9
	Lymphocytes	-16.1/-27.8	-16.1/-24.1	-30.4/-24.1
	Monocytes			-25.0/-42.9
Day 15	Red blood cells			-7.5/-7.3
	Reticulocytes	-25.0/14.3	-50.0/0.0	-75.0/-71.4
	Platelets	-31.6/7.0	-39.2/-38.3	-62.0/-57.9
	Plateletcrit	-26.3/0.0	-31.6/-28.1	-47.4/-46.9
	MPV		15.5/15.5	39.2/26.2
	Leukocytes		-9.7/-21.7	-25.0/-18.8
	Neutrophils			-27.7/-16.2
	Lymphocytes	-6.4/-21.4	-8.5/-32.1	-27.7/-19.6
Day 22	Hemoglobin			-12.5/-18.9
	Red blood cells			-13.6/-17.0
	Reticulocytes	-28.6/0.0	-42.9/-33.3	-85.7/-66.7
	Platelets	-23.3/17.3	-25.1/-24.8	-57.4/-56.6
	Plateletcrit		-15.8/15.2	-36.8/-45.5
	MPV			47.4/22.1

	Parameters[1]	2.5 mg/kg/day	5 mg/kg/day	15 mg/kg/day
	PDW			23.7/13.5
	Leukocytes			-20.8/-17.5
	Neutrophils			-20.6/-11.6
	Lymphocytes		-8.5/-24.1	-25.5/-20.4
Week 4	Hemoglobin			-15.2/-24.4
	Red blood cells			-16.2/-22.8
	Packed cell volume			-17.0/-25.7
	Reticulocytes			-75.0/-50.0
	Platelets			-31.0/-16.3
	Plateletcrit			45.0/26.7
	Leukocytes			-17.1/-15.4
	Lymphocytes		2.2/-19.2	-23.9/-23.1

[1]No statistical analysis was included in the study report.

Clinical Chemistry

There were no test article-related effects on clinical chemistry.

Urinalysis

There were no remarkable changes in urinalysis.

Gross Pathology

- Macroscopic findings were noted in the pancreas (pale, 1/6 mid dose, 2/6 high dose), jejunum (red, 2/6 high dose), and urinary bladder (red, 2/6 mid dose, 1/6 high dose). Microscopic correlates of congestion and hemorrhage were noted in the GI tract and urinary bladder.
- Recovery animals were only evaluated from the mid and high dose groups, and gross pathology findings were still observed in the organs listed above.

Organ Weights

- Prostate weight was increased by 51.4% and 96.6% at 5 and 15 mg/kg, respectively, possibly correlating with inflammation observed in the histopathology evaluation. Organ weights for recovery animals were comparable to vehicle controls.

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings

- At end of dosing necropsy, a low incidence of microscopic findings was noted in the GI tract, bone marrow, spleen, kidney, urinary bladder, prostate, and parathyroid gland.
- At end of recovery necropsy, microscopic findings were still observed in the GI tract, spleen, kidney, urinary bladder, and parathyroid.

Table 27. Summary of histopathology findings at end of dosing necropsy in dogs administered oral olaparib daily for 4 weeks

	Males (mg/kg/day)				**Females** (mg/kg/day)			
	Grp1 0	Grp2 2.5	Grp3 5	Grp4 15	Grp1 0	Grp2 2.5	Grp3 5	Grp4 15
Sternum and marrow								
• Marrow atrophy								
Slight……				1/3				1/3
Spleen								
• Pigment								
Minimal……							1/3	1/3
Kidney								
• Agonal congestion/hemorrhage								
Present……								1/3
• Pyelitis								
Minimal……						1/3	1/3	
Stomach								
• Lymphocytic gastritis								
Minimal……		1/3	1/3				1/3	
Jejunum								
• Agonal congestion/hemorrhage								
Present……				1/3				1/3
Cecum								
• Dilated glands/crypt microabscesses								
Minimal……			1/3					2/3
Urinary bladder								
• Agonal congestion/hemorrhage								
Present……			1/3					
• Cystitis								
Slight……							1/3	1/3
Prostate								
• Inflammatory cell foci								
Minimal……		1/3	2/3					
Parathyroid								
• Cyst								
Present……			1/3	1/3		2/3	2/3	1/3

Special Evaluation

- Bone marrow smears were evaluated for all dose groups.
- At scheduled necropsy, doses of ≥ 2.5 mg/kg olaparib caused a delay in erythroid cell development and an increase in total myelopoietic cells, primarily in male dogs. These findings correlated with microscopic findings of bone marrow atrophy.
- At end of recovery, bone marrow activity was similar to vehicle controls.

Table 28. Summary of myelogram evaluation in dogs administered oral olaparib daily for 4 weeks (% change relative to controls male/female)

Parameters[1]	2.5 mg/kg/day	5 mg/kg/day	15 mg/kg/day
Proerythroblasts		-37.0/53.8	-59.3/15.4
Early erythroblasts		-32.6/7.5	-37.2/-32.5
Intermediate erythroblasts			-34.6/-35.6
Late erythroblasts	-36.3/-6.6	-47.6/7.4	-61.1/-51.0
Metamyelocytes	33.8/-2.0	28.1/10.2	38.1/19.3
Neutrophils	110.9/10.5	84.8/-29.8	115.2/77.2
Eosinophils	-50.0/78.3	11.5/78.3	53.8/182.6
Lymphocytes			48.4/27.8
Total erythropoietic cells	-25.3/-1.3	-34.7/9.1	-53.9/-42.9
Total myelopoietic cells	31.6/-2.2	34.0/-1.6	39.2/28.4
Myeloid/erythroid ratio	81.8/0.0	109.1/-7.7	209.1/223.1

[1]Statistical analysis was not included in the study report.

Toxicokinetics

- Following a single dose, C_{max} and AUC increased with increasing dose, and C_{max} was less than dose proportional with each dose escalation. In male dogs, AUC was dose proportional from 2.5 to 5 mg/kg and less than dose proportional from 5 to 15 mg/kg. AUC was approximately dose proportional with increasing dose in female dogs.
- Following repeat dosing, C_{max} and AUC increased with increasing dose, and C_{max} was generally less than dose proportional across the tested dose range. In male dogs, AUC was approximately dose proportional from 2.5 to 5 mg/kg and less than dose proportional from 5 to 15 mg/kg. In female dogs, AUC was less than dose proportional from 2.5 to 5 mg/kg and approximately dose proportional from 5 to 15 mg/kg.
- Peak plasma concentrations occurred at 1.0 to 5.8 h on Day 1 and 1 to 2.8 h in Week 4.
- Systemic exposure in Week 4 was comparable to Day 1, indicating little to no accumulation of olaparib after repeat dosing.
- At 15 mg/kg, C_{max} and AUC values were ~2-fold higher in female dogs compared to males after single and repeat dosing. There were no significant gender differences noted at the low and mid dose levels.

Table 29. Mean toxicokinetic parameters of oral olaparib administered to dogs daily for 28 days

	Sex	Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24h)}$ (h·ng/ml)	$AUC_{(0-24h)}$/D (h·ng/ml)/ (mg/kg)
Day 1	Male	2.5	1.0	585.68	234.27	3899.46	1559.78
		5	2.2	695.49	139.10	7292.48	1458.50
		15	4.4	1480.75	98.72	13497.91	899.86
	Female	2.5	4.7	445.45	178.18	3528.88	1411.55
		5	1.8	839.21	167.84	7539.68	1507.94
		15	5.8	1703.03	113.54	20688.46	1379.23
Week 4	Male	2.5	1.0	623.56	249.42	3426.62	1370.65
		5	1.0	815.10	163.02	5559.19	1111.84
		15	2.8	1333.92	88.93	10735.14	715.68
	Female	2.5	1.3	745.65	298.26	4921.62	1968.65
		5	1.0	1137.94	227.59	6518.71	1303.74
		15	2.6	2244.10	149.61	22231.25	1482.08

Dosing Formulation Analysis

Dosing formulations were assessed from Day 1 and were determined to be within ± 10% of the intended concentration. The Applicant did not provide an assessment of the homogeneity or stability of the test article within the study report.

Study title: 26 Week Oral (Gavage) Toxicity Study in the Dog

Study no.:	TII0011
Study report location:	eCTD 4.2.3.2.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	August 3, 2005
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436), lot # 050772, purity 101.9%

Key Study Findings

- There were no test article-related early mortalities.
- The major target organ was the hematopoietic system. Dogs given ≥ 3 mg/kg olaparib had a reduction in red cell mass, reticulocytes, platelets, and leukocyte counts.
- Minimal to slight inflammation was noted in the stomach and prostate gland at ≥ 3 mg/kg, and pigmented macrophages and Kupffer cells were observed in the liver at 1 and 10 mg/kg with no apparent effect on liver function.

Methods

Doses:	0, 1, 3, 10 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Daily
Route of administration:	Oral gavage
Dose volume:	5 ml/kg
Formulation/Vehicle:	1% (w/v) methylcellulose
Species/Strain:	Beagle dogs
Number/Sex/Group:	4/Sex/Group
Age:	4 to 6 months old
Weight:	Males – 8.8 to 11.1 kg Females – 7.7 to 11.0 kg
Satellite groups:	None
Unique study design:	None
Deviation from study protocol:	Deviations from the study protocol were not considered to affect the study design or interpretation of the results.

Observations and Schedule

Mortality	Twice daily
Clinical signs	Daily, Weekly detailed examination
Body weights	Weekly
Food Consumption	Daily
Ophthalmology	Pre-test and Week 12 and 26
ECG	Pre-test, Day 1, Week 13 and 26 (pre-dose and 2 h post-dose)
Hematology[1]	Week 4, 8, 13, 17, 21, and 26
Coagulation and Clinical chemistry[2]	Week 13 and 26
Urinalysis[3]	Week 13 and 26
Bone marrow smear[4]	At end of dosing necropsy for all groups
Toxicokinetics	Day 1 and during Week 13 and 26 (pre-dose, 0.5, 1, 2, 4, 8, 12, and 24 h)

[1]Hematology parameters: hemoglobin, hemoglobin density width, red blood cells, red cell distribution width, packed cell volume, mean cell volume, mean cell hemoglobin, platelets, total leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, large unstained cells, cell morphology, reticulocytes

[2]Coagulation and clinical chemistry parameters: prothrombin time, activated partial thromboplastin time, urea, creatinine, glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, total protein, albumin, globulin, albumin/globulin ratio, bilirubin, cholesterol, calcium, sodium, potassium

[3]Urinalysis parameters: volume, specific gravity, glucose, pH, protein, color, appearance

[4]Bone marrow smear parameters: Myeloblasts, promyelocytes, neutrophilic myelocytes, neutrophilic metamyelocytes, neutrophil polymorphs, eosinophils, basophils, proerythroblasts, early normoblasts, intermediate normoblasts, late normoblasts, lymphocytes, monocytes plasma cells, megakaryoblasts/cytes, others, total myeloid cells, total erythroid count myeloid:erythroid ratio, myeloid left shift index, erythroid left shift index

Mortality

- Male animal #33 (10 mg/kg/day) was euthanized moribund on Day 142.

- Prior to euthanasia, the animal exhibited decreased activity and cold body surface. The animal also lost 1 kg of weight overnight. The veterinary surgeon described the animal as dehydrated and lethargic with pale mucus membranes.
- The cause of death was due to herniation of a part of the intestine through the inguinal canal into the left scrotal sac.
- This early mortality was not considered test article-related.

Clinical Signs

There were no test article-related clinical signs.

Body Weights

- Male dogs exhibited a ~30% increase in body weight gain at the 1 and 3 mg/kg dose level compared to controls that was not observed in 10 mg/kg animals.
- Female dogs receiving 10 mg/kg olaparib showed a ~70% increase in body weight gain at end of dosing compared to controls.

Feed Consumption

Changes in food consumption were unremarkable.

Ophthalmoscopy

There were no test article-related findings in the eye.

ECG

There were no test article-related changes in ECG parameters or blood pressure.

Hematology

- At doses of ≥ 3 mg/kg, there was a decrease in red cell mass, platelets, and a corresponding increase in hemoglobin distribution width (HDW) throughout the dosing period. Although an increase in red cell distribution width (RDW) was observed, reticulocytes were generally decreased throughout the dosing period, indicating a lack of regenerative response and a general decrease in bone marrow activity.
- Animals given ≥ 3 mg/kg olaparib also showed a decrease in leukocyte populations, including lymphocytes, neutrophils, monocytes, and basophils compared to controls. Eosinophils were elevated up to 2-fold in the 10 mg/kg dose group throughout the dosing period.
- There were no microscopic correlates reported in the bone marrow or lymphoid organs at end of dosing necropsy.

Table 30. Summary of hematology changes in dogs administered oral olaparib daily for 26 weeks (% change relative to controls male/female)

	Parameters[1]	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Week 4	Red blood cells		-14.4/-13.5	-30.4/-32.1
	Hemoglobin			-32.3/-30.1
	PCV			-31.7/-28.8

	Parameters[1]	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
	RDW			16.0/16.5
	Platelets		-16.7/-14.1	-55.3/-37.0
	Leukocytes		-20.2/-8.8	-34.9/-49.6
	Neutrophils		-14.5/-4.1	-31.2/-53.2
	Lymphocytes		-27.4/-12.8	-39.0/-45.9
	Monocytes			-46.2/-62.1
	Basophils	-35.7/-33.3	-57.1/-33.3	-78.6/-83.3
	Large unstained cells			-70.0/-55.6
	Reticulocytes		6.0/-33.3	-47.5/-48.6

Week 8/9	Red blood cells			-33.6/-33.1
	Hemoglobin			-34.3/-28.8
	PCV			-31.1/-26.9
	RDW			53.7/33.3
	HDW			46.8/31.6
	Platelets		-14.8/-4.2	-19.9/-19.8
	Leukocytes		-20.0/-7.4	-31.1/-37.0
	Neutrophils		-25.1/0.8	-37.8/-40.3
	Lymphocytes		-12.0/-17.1	-24.5/-35.0
	Monocytes		-24.4/-3.3	-54.9/-41.0
	Eosinophils			108.6/15.4
	Basophils	-41.7/-18.2	-41.7/-36.4	-75.0/-72.7
	Large unstained cells	-35.7/9.1	-42.9/-18.2	-64.3/-54.5
	Reticulocytes		8.5/-25	8.5/-22.5

Week 13	Red blood cells			-42.2/-36.5
	Hemoglobin			-39.1/-31.4
	PCV			-34.1/-28.9
	RDW			43.4/23.0
	HDW			28.6/15.0
	Platelets			-19.2/-14.1
	Leukocytes		-16.8/7.0	-25.6/-40.3
	Neutrophils			-27.5/-50.4
	Lymphocytes			-14.5/-19.5
	Monocytes			-49.2/-51.5
	Eosinophils			121.6/0.0
	Basophils	-33.3/-46.2	-33.3/-46.2	-75.0/-76.9
	Large unstained cells			-41.7/-40.0
	Reticulocytes		-20.0/-35.0	-22.9/-57.0

Week 17	Red blood cells			-38.0/-34.0
	Hemoglobin			-35.1/-29.0
	PCV			-28.3/-25.3
	RDW			38.0/24.2
	HDW			37.0/18.0
	Platelets			-17.1/-5.6
	Leukocytes			-35.2/-28.4
	Neutrophils			-39.4/-32.3
	Lymphocytes			-37.9/-23.3
	Monocytes			-32.3/-31.3
	Eosinophils			47.9/11.1

	Parameters[1]	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
	Basophils	-10.0/-30.0	-40.0/-50.0	-70.0/-90.0
	Large unstained cells			-46.2/-50.0
	Reticulocytes		40.0/-45.3	5.5/-61.6

Week 21	Red blood cells			-36.2/-38.7
	Hemoglobin			-34.4/-34.1
	PCV			-29.4/-32.3
	RDW			35.8/24.2
	HDW			29.9/17.9
	Platelets			-11.7/-24.8
	Leukocytes			-22.3/-24.4
	Neutrophils			-12.7/-28.8
	Lymphocytes			-36.8/-17.9
	Monocytes			-33.8/-42.4
	Eosinophils			27.0/39.5
	Basophils	-33.3/-14.3	-55.6/-28.6	-77.8/-71.4
	Large unstained cells	-30.0/-12.5	-30.0/-25.0	-40.0/-37.5
	Reticulocytes		-1.5/-35.0	-40.0/-67.5

Week 26	Red blood cells			-38.6/-34.1
	Hemoglobin			-36.5/-29.1
	PCV			-29.3/-25.7
	RDW			36.6/26.4
	Leukocytes			-28.7/-23.2
	Neutrophils		-32.0/14.9	-32.5/-30.6
	Lymphocytes			-25.4/-8.7
	Monocytes			-43.2/-33.3
	Eosinophils			72.7/6.3
	Basophils	-33.3/-40.0	-60.0/-53.3	-86.7/-73.3
	Large unstained cells	-38.5/-11.1	-38.5/-11.1	-69.2/-44.4
	Reticulocytes		29.3/-47.5	15.5/-24.6

[1]No statistical analysis was included in the study report.

Clinical Chemistry

No remarkable changes in clinical chemistry parameters were reported.

Urinalysis

There were no remarkable changes in urinalysis parameters.

Gross Pathology

- At end of dosing necropsy, macroscopic findings in the mammary gland (abnormal color and shape) were reported at 3 mg/kg (3/8) and 10 mg/kg (1/7). There were no microscopic correlates.

Organ Weights

- Reduced thymus weight was reported in males given 10 mg/kg olaparib, correlating with microscopic findings of slight involution.
- Changes in spleen, thyroid, heart, and testes weights occurred without adverse correlates.

Table 31. Summary of organ weight changes in dogs administered oral olaparib daily for 26 weeks (% change relative to controls male/female)

Organ[1]		1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Spleen	A			28.6/2.3
	R/BW			33.3/-5.0
Thyroid glands	A			-36.7/10.7
	R/BW			-34.6/4.0
Thymus	A			-43.5/-7.0
	R/BW			-40.0/0.0
Heart	A			19.1/-3.2
	R/BW			23.7/-11.6
Testes	A			-23.4/
	R/BW			-18.8/

[1]No statistical analysis was included in the study report.

Histopathology

Adequate Battery: yes

Peer Review: no

Histological Findings

- At end of dosing necropsy, minimal to slight microscopic findings were observed in the liver, prostate gland, stomach, and thymus.

Table 32. Summary of histological findings at end of dosing necropsy in dogs administered oral olaparib daily for 26 weeks

	Males (mg/kg/dose)				**Females** (mg/kg/dose)			
	Grp1 0	Grp2 1	Grp3 3	Grp4 10	Grp1 0	Grp2 1	Grp3 3	Grp4 10
Liver								
• Brown pigment in Kupffer cells								
Slight		1/4		1/4				1/4
Moderate				1/4				
• Pigmented macrophages, portal tracts								
Minimal		1/4						
Slight				1/4				1/4
Prostate gland								
• Interstitial inflammatory cell infiltration								
Slight			1/4	1/3				
• Lymphoid aggregates								
Slight				1/3				
Stomach								
• Prominent mucosal associated lymphoid tissue								
Present							1/4	2/4

	Males (mg/kg/dose)				**Females** (mg/kg/dose)			
	Grp1 0	Grp2 1	Grp3 3	Grp4 10	Grp1 0	Grp2 1	Grp3 3	Grp4 10
• Focal mucosal inflammatory cells, antrum								
Minimal……								1/4
Slight……								1/4
Thymus								
• Involution								
Slight……				1/3		2/4	2/4	

Special Evaluation

- Bone marrow smears were evaluated from all dose groups.
- Male dogs given 10 mg/kg olaparib showed an increase in the M:E ratio and decrease in myeloid left shift index, reflecting a decrease in total erythroid cells and a slight increase in total myeloid cells.
- Females administered 10 mg/kg olaparib had a decrease in the M:E ratio, reflecting a slight increase in total erythroid cells and a decrease in total myeloid cells.
- These changes were minor and did not appear to correlate with the pancytopenia observed throughout the dosing period.

Table 33. Summary of myelogram evaluation in dogs given oral olaparib daily for 26 weeks (% change relative to control male/female)

Group and sex		1M	2M	3M	4M	1F	2F	3F	4F
Dose level (mg/kg/day)		0	1	3	10	0	1	3	10
Number of dogs examined		4	4	4	3	4	4	4	4
Total Myeloid Cells	Mean	**43.6**	**41.1**	**50.3**	**49.2**	**45.4**	**47.8**	**46.8**	**39.1**
	SD	8.47	10.42	3.00	11.37	6.07	6.71	8.25	9.24
Total Erythroid Cells	Mean	**38.6**	**42.3**	**36.3**	**28.5**	**37.4**	**36.6**	**36.5**	**42.5**
	SD	6.75	11.38	3.52	6.00	4.70	5.48	5.67	4.06
M:E Ratio	Mean	**1.19**	**1.10**	**1.40**	**1.83**	**1.24**	**1.35**	**1.32**	**0.94**
	SD	0.46	0.64	0.17	0.77	0.30	0.43	0.38	0.30
Myeloid Left Shift Index	Mean	**3.68**	**4.14**	**4.29**	**2.82**	**3.15**	**3.91**	**3.82**	**3.08**
	SD	1.08	1.74	1.42	1.22	1.06	0.65	1.34	0.62
Erythroid Left Shift Index	Mean	**0.26**	**0.26**	**0.23**	**0.27**	**0.28**	**0.23**	**0.26**	**0.29**
	SD	0.05	0.06	0.01	0.15	0.06	0.04	0.06	0.06

[Excerpted from Applicant's submission]

Toxicokinetics

- Following a single dose, C_{max} and AUC increased with increasing dose and were generally less than dose proportional with each dose escalation. Peak plasma concentrations occurred at 1.4 to 6 h, and $T_{1/2}$ was 2.3 to 12.4 h.

- After repeat dosing, C_{max} and AUC increased with increasing dose. C_{max} was less than dose proportional with increasing dose. AUC was less than dose proportional from 1 to 3 mg/kg and greater than dose proportional from 3 to 10 mg/kg. Systemic exposure in Week 13 and Week 26 was comparable to Day 1, indicating no apparent accumulation of olaparib after repeat dosing. In Week 13 and Week 26, peak plasma concentrations occurred at 1 to 4.25 h, and the elimination half-life was 3 to 10.6 h.
- There were no apparent gender differences noted in dogs.

Table 34. Mean toxicokinetic parameters of oral olaparib administered to dogs daily for 26 weeks

	Sex	Dose (mg/kg)	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24h)}$ (h·ng/ml)	$AUC_{(0-24h)}$/D (h·ng/ml)/ (mg/kg)
Day 1	Male	1	1.50	4.35	234	234	1470	1470
		3	1.38	4.06	559	186	3150	1050
		10	6.00	12.4	1500	150	20000	2000
	Female	1	1.50	7.47	167	167	1510	1510
		3	1.75	2.29	352	117	3030	1010
		10	2.00	4.23	1180	118	12400	1240
Week 13	Male	1	2.00	3.62	197	197	1660	1660
		3	2.50	5.30	376	125	3150	1050
		10	1.75	7.10	1070	107	14300	1430
	Female	1	1.25	8.40	188	188	1510	1510
		3	1.75	7.24	426	142	3130	1040
		10	2.00	3.68	1020	102	10800	1080
Week 26	Male	1	1.50	3.19	330	330	2180	2180
		3	1.25	3.96	597	199	3830	1280
		10	1.67	10.6	1540	154	15600	1560
	Female	1	1.00	3.82	248	248	1190	1190
		3	1.50	4.86	587	196	3600	1200
		10	4.25	4.22	1220	122	14000	1400

Dosing Formulation Analysis

Sample concentrations were within the acceptance criteria of ± 10% of the intended concentration. Formulations were also determined to be within the acceptance criteria for homogeneity (CV values ≤ 6%) except for Group 2 samples at Week 13. Group 2 samples were within the acceptance criteria at Week 17.

7 Genetic Toxicology

7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

Study title: Bacterial Reverse Mutation Test for KU-0059436 (b) (4)

Study no.:	TII0007
Study report location:	eCTD 4.2.3.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 22, 2004
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436, (b) (4) lot # 040501, 96.18% purity

Key Study Findings

- There was a slight, but statistically significant, increase in TA98 and TA100 revertants at 5000 µg/plate and WP2 *uvrA* revertants at ≥ 1000 µg/plate in the absence of S9 metabolic activation. These results were not observed in repeat experiments using the same assay conditions.
- In conclusion, olaparib was not considered mutagenic in the bacterial reverse mutation assay at concentrations up to 5000 µg/plate under the conditions tested.

Methods

Strains:	*S. typhimurium* TA1535 *S. typhimurium* TA1537 *S. typhimurium* TA98 *S. typhimurium* TA100 *E. coli* WP2 *uvrA*
Concentrations in definitive study:	8, 40, 200, 1000, 5000 µg/plate in the presence and absence of S9 metabolic activation
Basis of concentration selection:	A preliminary concentration range-finding study used the plate incorporation method to assess the cytotoxic and mutagenic effects of olaparib at concentrations of 1.6, 8, 40, 200, 1000, and 5000 µg/plate in the presence or absence of S9 metabolic activation. Plates were incubated for 24 h at 37°C before examining bacterial lawns and revertants for TA98 and WP2 *uvrA* strains. There was a slight reduction in the background bacterial lawn of the TA98

	strain at 5000 µg/plate olaparib in the absence of S9 activation. Concentrations ranging from 8 to 5000 µg/plate were selected for subsequent studies.
Negative control:	DMSO
Positive control:	See Table 35
Formulation/Vehicle:	DMSO
Incubation & sampling time:	The definitive Ames assay was conducted using both the plate incorporation and pre-incubation methods. Bacterial strains were tested with ascending concentrations of olaparib in the presence and absence of S9 mix prepared from livers of Fischer 344 rats treated with Aroclor 1254. For the plate incorporation method, plates were incubated for ~65 h at 37^{o}C before examining the number of revertants using an automated counter. For the pre-incubation method, bacterial strains were incubated with olaparib for 20 min at 37^{o}C before adding to agar plates. The plates were incubated for ~65 h at 37^{o}, and revertants were counted by an automated counter.

Table 35. Positive controls of in vitro bacterial reverse mutation assay

Strain	Without S9 mix	With S9 mix
S. typhimurium TA1535	Sodium azide 1 µg/plate	2-aminoanthracene 2 µg/plate
S. typhimurium TA1537	9-aminoacridine 50 µg/plate	2-aminoanthracene 4 µg/plate
S. typhimurium TA98 (pKM101)	2-nitrofluorene 0.5 µg/plate	2-aminoanthracene 2 µg/plate
S. typhimurium TA100 (pKM101)	Sodium azide 1 µg/plate	2-aminoanthracene 4 µg/plate
E. coli WP2 *uvrA*	4-nitroquinoline-N-oxide 1 µg/plate	2-aminoanthracene 10 µg/plate

Study Validity

The study was considered valid based on the following:

- The study included an adequate selection of bacterial strains tested in the presence and absence of S9 metabolic activation.
- The concentration selection was adequate and based on a concentration range-finding study and a top regulatory concentration level of 5000 µg/plate.
- The positive and negative controls produced the expected results.
- The S9 percentage was 10% v/v, which is within acceptable limits in the FDA/CFSAN Redbook.

Results

Concentration range-finding study

- The preliminary concentration range-finding study examined olaparib concentrations ranging from 1.6 to 5000 µg/plate in the presence and absence of S9 activation for 24 h. The study was conducted with two tester strains, TA98 and WP2 *uvrA*. The highest concentration slightly reduced the background bacterial lawn of TA98 in the absence of S9 mix, indicating some toxicity at this concentration.

- The main study tested concentrations of 8, 40, 200, 1000, and 5000 µg/plate in the presence and absence of S9 metabolic activation.

Plate incorporation assay

- According to the study report, the Ames test was considered positive if olaparib induced a concentration-related, statistically significant increase in revertants compared to solvent controls in two separate experiments. The Ames test was considered negative if the number of revertants was no higher than the number expected from normal variation in the solvent control for any tester strain in either experiment.

- In the plate incorporation assay, the positive and negative controls produced the expected results.

- In Experiment 1, a concentration of 5000 µg/plate induced a statistically significant increase (1.8-fold) in the number of TA98 revertants/plate in the absence of S9 activation compared to solvent controls (Table 36). No statistically significant differences were reported in a repeat experiment using the same assay conditions (Experiment 2).

Pre-incubation assay

- In the pre-incubation assay, the positive and negative controls produced the expected results, and precipitation was reported to have no effect on counting revertant colonies (Table 37).

- In the absence of metabolic activation, a statistically significant increase in the number of revertants/plate was observed for TA100 (1.4-fold) at 5000 µg/plate and WP2 *uvrA* (1.6 to 1.8-fold) at ≥ 1000 µg/plate (Table 37). These results were not observed in a repeat experiment using the same assay conditions (Experiment 3).

Table 36. Plate incorporation assay in the presence and absence of S9 metabolic activation (mean of 3 plates/treatment)

Table 1 - Mean Number of Revertants Per Plate - Experiment 1 - Plate Incorporation

		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	0	19.7	21.3	20.7	25.3	18.7	16.3	513.3
TA1537	0	10.0	9.7	12.7	12.0	8.7	13.0	204.7
TA98	0	23.7	31.3	18.7	25.3	33.0	43.3*	292.7
TA100	0	118.0	142.0	135.3	118.0	135.3	117.3	755.7
WP2 *uvrA*	0	22.0	33.3	36.0	35.0	33.0	26.7	1365.3
		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	10	17.3	22.7	21.7	15.3	24.0	19.7	125.3
TA1537	10	13.0	11.7	10.7	13.0	15.7	6.7	212.3
TA98	10	35.7	45.0	34.7	26.0	32.3	45.5	657.3
TA100	10	152.3	149.0	151.3	165.7	173.0	142.0	1652.0
WP2 *uvrA*	10	45.0	39.7	35.3	39.3	43.0	30.3	194.0

Table 2 - Mean Number of Revertants Per Plate - Experiment 2 - Plate Incorporation

		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA98	0	26.7	22.7	20.3	29.7	23.0	25.7	269.7

[Excerpted from Applicant's submission]

PC = positive control, *p < 0.05

Table 37. Pre-incubation method in the presence and absence of S9 metabolic activation (mean of 3 plates/treatment)

Table 3 - Mean Number of Revertants Per Plate - Experiment 2 - Pre-incubation

		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	0	19.7	20.7	20.0	28.0	16.7	23.0 ppt	611.7
TA1537	0	10.0	9.0	9.0	12.7	5.7	11.3	175.7
TA100	0	106.7	105.3	95.3	101.0	105.0	144.7*	856.0
WP2 *uvrA*	0	21.7	25.3	29.0	28.7	38.0* ppt	35.0*	1277.0
		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA1535	10	28.7	19.3	27.7	21.7	22.3	25.3 ppt	115.0
TA1537	10	15.0	10.7	12.7	12.7	12.7	12.0	213.7
TA98	10	35.0	32.3	21.0	30.3	31.7	27.3 ppt	774.0
TA100	10	119.0	110.3	128.3	111.3	124.0	139.0 ppt	1703.7
WP2 *uvrA*	10	42.0	41.0	38.3	39.3	44.0	46.7 ppt	319.3

Table 4 - Mean Number of Revertants Per Plate - Experiment 3 - Pre-incubation

		Concentration of test substance (µg/plate)						
Strain	% S-9 mix v/v	0	8	40	200	1000	5000	PC
TA98	0	21.7	21.7	30.7	24.0	23.7 ppt	31.0 ppt srl	470.7
TA100	0	113.0	131.3	124.7	119.0	121.7	116.0 ppt	271.3
WP2 *uvrA*	0	27.3	29.0	27.0	27.3	29.3	28.7	1333.0

[Excerpted from Applicant's submission]

PC = positive control, ppt = precipitation, srl = slightly reduced background bacterial lawn, *p < 0.05

7.2 *In Vitro* Assays in Mammalian Cells

Study title: *In Vitro* Mammalian Cell Cytogenetic Test: Chinese Hamster Ovary Cells

Study no.:	TII0008
Study report location:	eCTD 4.2.3.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	June 18, 2004
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436, (b) (4) , lot # 040501, purity 96%

Key Study Findings

- Olaparib was clastogenic in the chromosomal aberration assay in mammalian CHO cells under the conditions tested.

Methods

Cell line:	Chinese hamster ovary cells (12 h cell cycle time)
Concentrations in definitive study:	See Table 38 and Table 39
Basis of concentration selection:	Solubility, stability, pH, osmolality, preliminary concentration range-finding study using concentrations of 0.32, 1.6, 8, 40, 200, 1000, and 2500 µg/ml
Negative control:	DMSO
Positive control:	(-) S9 mix: 0.3 and 0.4 µg/ml mitomycin C (MMC) (+) S9 mix: 18 µg/ml cyclophosphamide (CPA) or 5 µg/ml benz(a)pyrene (BP)
Formulation/Vehicle:	DMSO
Incubation & sampling time:	Cells were incubated with ascending concentrations of olaparib or vehicle control in the presence and absence of S9 mix at 37°C and 5% CO_2 for 3 h or 1.5 cell cycles. Cells were allowed to incubate for 1.5 cell cycles before harvesting (Harvest 1) or after an additional 24 h (Harvest 2). See Table 38 and Table 39.

Table 38. Concentrations of olaparib and positive controls in cytogenetic experiment 1

Compound	Without metabolic activation		With metabolic activation	
	Dose (µg/mL)	Cytogenetic Analysis	Dose (µg/mL)	Cytogenetic Analysis
KU-0059436 (COCE42)	0	✓	0	✓
	50	✓	50	✓
	100		100	✓
	250	✓	250	✓
	500	✓	500	✓
	750		750	
	1000		1000	
MMC	0.3		-	-
	0.4	✓	-	-
BP	-	-	5	
CPA	-	-	18	✓

[Excerpted from Applicant's submission]

Cells were incubated for 3 h and harvested after 1.5 cell cycles.

Table 39. Concentrations of olaparib and positive controls in cytogenetic experiment 2

Compound	Without metabolic activation			With metabolic activation		
	Dose (µg/mL)	Cytogenetic Analysis		Dose (µg/mL)	Cytogenetic Analysis	
		Harvest 1	Harvest 2		Harvest 1	Harvest 2
KU-0059436 (COCE42)	0	✓	✓	0	✓	✓
	1	✓		50	✓	
	5	✓		100		
	10	✓		200		
	25	✓	✓	300	✓	
	50			400		
	100			500	✓	✓
	200			-		
MMC	0.3			-		
MMC	0.4	✓		-		
BP	-			5	✓	
CPA	-			18		

[Excerpted from Applicant's submission]

Activated cells were incubated for 3 h, and non-activated cells were incubated for 1.5 cell cycles. Harvest 1 occurred 1.5 cell cycles after starting olaparib treatment. Harvest 2 occurred 24 h later.

Study Validity

The study was considered valid based on the following:

- The study included an appropriate number of cells and tested olaparib concentrations in duplicate.
- The positive and negative controls provided the expected results in the presence and absence of S9 activation.
- The S9 percentage was 10% v/v, which is within acceptable limits in the FDA/CFSAN Redbook.
- The concentration selection was acceptable and based on insolubility of olaparib at 5000 µg/ml and a preliminary concentration range-finding study.
- Whenever possible, 100 cells were counted from each cell culture, and only well spread metaphases consisting of 18-22 chromosomes were scored for chromosomal damage. Cells containing more than 22 chromosomes were scored separately as numerical aberrations. If > 30% aberrations were counted excluding gaps, scoring was stopped after a minimum of 25 cells. The mitotic index was calculated from at least 1000 cells.

Results

Concentration range-finding study

- The highest concentration tested was 2500 µg/ml due to excessive precipitation of 5000 µg/ml in culture media.
- There were no differences in pH and osmolality at the tested olaparib concentrations. Precipitation was observed at concentrations of 1000 and 2500 µg/ml.
- After 3 h of incubation, both activated and non-activated cells showed over 50% cytotoxicity at concentrations of 1000 and 2500 µg/ml olaparib. After 1.5 cell cycles, cytotoxicity was over 50% at concentrations of greater than 8 µg/ml olaparib.

Cytogenetic experiment 1

- In experiment 1, CHO cells were incubated with olaparib concentrations ranging from 50 to 1000 µg/ml for 3 h in the presence and absence of S9 mix. Cells were harvested after 1.5 cell cycles.
- Precipitation was observed at concentrations of ≥ 500 µg/ml in both the activated and non-activated groups.
- Cytotoxicity was greater than 50% for activated cells incubated with ≥ 750 µg/ml olaparib and for non-activated cells treated with ≥ 500 µg/ml olaparib.
- Concentrations of 50 and 500 µg/ml olaparib induced chromosomal aberrations in activated cells. In non-activated cells, chromosomal aberrations were reported at concentrations of 50, 100, and 500 µg/ml olaparib. Chromosomal aberrations at 250 µg/ml olaparib were not considered statistically significant (Table 40).

Cytogenetic experiment 2

- In experiment 2, CHO cells were treated with olaparib concentrations of 50 to 500 µg/ml in the presence of S9 mix and concentrations of 1 to 200 µg/ml in the absence of S9 mix. Culture conditions were the same as experiment 1 except an additional set of cultures were harvested 24 h later.
- Precipitation was only observed at 500 µg/ml in the activated group.
- Cytotoxicity was greater than 50% only in non-activated cells at concentrations of ≥ 25 µg/ml olaparib.
- A statistically significant increase in chromosomal aberrations were reported for activated cells treated with ≥ 300 µg/ml olaparib and non-activated cells at ≥ 5 µg/ml olaparib. At harvest 2, increases were observed only in the activated 500 µg/ml treatment group (Table 41).

In conclusion, olaparib was positive in the in vitro chromosome aberration assay under the conditions tested. The findings were concentration-dependent, and values were outside the range of historical controls. Therefore, olaparib is considered clastogenic.

Table 40. Summary of cytogenetic analysis of olaparib in CHO cells (experiment 1)

Treatment (µg/ml)	Total cells	Aberrant cells	Mitotic index	Numerical aberrations	% cells with aberrations	
					with gaps	without gaps
Without metabolic activation (-S9), 3 h incubation						
DMSO	400	3	2.33	0	0.8	0.0
50	200	5	2.05	0	2.5	2.0**
250	200	3	1.75	0	1.5	0.5
500	125	13	1.35	0	10.4<***	5.6<***
MMC, 0.4 µg/ml	50	23	1.15	0	46.0<***	36.0<***
With metabolic activation (+S9), 3 h incubation						
DMSO	400	15	4.00	2	3.8	1.8
50	200	16	3.40	3	8.0*	2.5
100	200	16	2.35	2	8.0*	5.0*
250	200	8	3.10	3	4.0	2.0
500	50	22	2.50	0	44.0<***	40.0<***
CPA, 18 µg /ml	44	24	0.45	0	54.5<***	52.3<***

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001, <***p < 0.00005

Table 41. Summary of cytogenetic analysis of olaparib in CHO cells (experiment 2)

Treatment (µg/ml)	Total cells	Aberrant cells	Mitotic index	Numerical aberrations	% cells with aberrations	
					with gaps	without gaps
Without metabolic activation (-S9), incubation for 1.5 cell cycles (harvest 1)						
DMSO	400	9	2.98	5	2.3	1.5
1	200	9	2.55	1	4.5	1.5
5	200	29	2.4	4	14.5<***	12.0<***
10	50	17	2.55	0	34.0<***	32.0<***
25	33	13	1.00	0	39.4<***	39.4<***
MMC, 0.4 µg/ml	50	32	1.15	0	64.0<***	60.0<***
Without metabolic activation (-S9), incubation for 1.5 cell cycles (harvest 2)						
DMSO	400	11	2.63	3	2.8	1.3
25	200	8	3.05	2	4.0	3.0
With metabolic activation (+S9), 3 h incubation (harvest 1)						
DMSO	400	7	2.00	9	1.8	0.8
50	200	3	1.75	1	1.5	1.5
300	166	13	1.90	4	7.8***	6.0***
500	50	26	0.50	0	52.0<***	48.0<***
BP, 5 µg/ml	28	14	0.20	0	50.0<***	46.4<***
With metabolic activation (+S9), 3 h incubation (harvest 2)						
DMSO	400	6	2.28	2	1.5	1.0
500	50	9	0.60	2	18.0<***	16.0<***

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001, <***p < 0.00005

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title: Ku-0059436 (b) (4) Micronucleus Test in Bone Marrow Cells of CD Rats 0 h + 24 h Oral Dosing and 48 h Sampling

Study no:	775498
Study report location:	eCTD 4.2.3.3.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 3, 2004
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (Ku-0059436, (b) (4)), lot # 040502, 98.88% purity

Key Study Findings

- Oral olaparib induced micronuclei formation in the in vivo rat bone marrow micronucleus test at all dose levels tested.

Methods

Doses in definitive study:	0, 100, 200, 400 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Olaparib was administered by 3 doses given at 1 h intervals on Day 1 and Day 2. The positive control group received test article at 0 and 24 h.
Route of administration:	Oral gavage
Dose volume:	10 ml/kg
Formulation/Vehicle:	1 to 10 dilution of DMSO, 10% hydroxypropyl-B-cyclodextrin in PBS
Species/Strain:	CD rats
Number/Sex/Group:	5 Males/Group 2 and 3 5/Sex/Group 1 and 4
Satellite groups:	None
Basis of dose selection:	Olaparib was tested in two toxicity studies in rats before the definitive micronucleus test.
Negative control:	DMSO
Positive control:	50 mg/kg cyclophosphamide

Table 42. Study design of micronucleus test in rats administered oral olaparib

Dose Group	Daily Test Dose	Treatment and Number of Rats		
		Dosing (h)	Sampling (h)	No. of Rats
Vehicle Control	30 mL 10%HPBC/kg	0,1,2 + 24,25,26	48	5M + 5F
Low Dose	100 mg Ku-0059436/kg †	0,1,2 + 24,25,26	48	5M
Mid Dose	200 mg Ku-0059436/kg †	0,1,2 + 24,25,26	48	5M
High Dose	400 mg Ku-0059436/kg	0,1,2 + 24,25,26	48	10M +10F
Positive Control	50 mg Cyclophosphamide/kg	0 + 24	48	5M
Untreated	-	-	48	3M+3F

† = Animals 115 and 120 did not receive full doses on day 2

[Excerpted from Applicant's submission]

Study Validity

The study was considered valid based on the following:

- Previous PK and TK data showed systemic exposure of olaparib at doses less than or equal to the doses tested. Bone marrow toxicity was observed at the highest dose tested, indicating target tissue exposure. These results are consistent with bone marrow toxicity observed at lower doses in general toxicology studies.
- The doses appeared to be adequate based on the results of the dose range-finding study and positive micronucleus test.
- The species and number of animals were acceptable.
- Positive and negative controls induced the expected response.
- The ratio of immature erythrocytes to total erythrocytes was not less than 20% of the vehicle control value.
- The acceptance criteria required 1) uniform staining and sufficient number of polychromatic erythrocyte (PCE) cells for evaluation, 2) negative control values within the historical control ranges, and 3) an adequate response from positive controls in at least 2 animals and the total dose group.
- The micronucleus test was considered negative if the number of micronucleated (MN)-PCE were comparable to the historical control range. The test was considered positive if there was a biologically relevant and statistically significant increase of in MN-PCE (> 10%) for one or more dose groups compared to historical control values.

Results

First toxicity study
In the first toxicity study, rats (3/Sex/Group) were given 200, 300, and 400 mg/kg olaparib at 0 h and 24 h and monitored for clinical signs for 4 days. The dose formulations were suspensions, and precipitation was observed at these concentrations. No clinical signs were noted during the observation period.

Second toxicity study
To prevent precipitation, olaparib was given at lower concentrations at 0 h, 1 h, and 2 h on Day 1 and 24 h, 25 h, and 26 h on Day 2 to build up to doses of 200, 300, and 400 mg/kg. No clinical signs were noted during the observation period.

Micronucleus test

- There were no reported clinical signs during the dosing period.
- The positive and negative controls provided the expected results.
- There was an increase in the frequency of micronucleated normochromatic cells in the positive control (0.44%) and 400 mg/kg dose group (0.16%) compared to vehicle control (0.015%). These findings are indicative of bone marrow toxicity in these dose groups.
- The micronucleus test was positive for all tested doses of olaparib, consistent with findings of chromosomal aberration in mammalian CHO cells in Study No. TII0008.

Table 43. Results of micronucleus test in rats administered oral olaparib

Males	**48 hour sampling period: number of MN-PCE[1]**				
	Vehicle (mg/kg)	Olaparib (mg/kg)			Pos. control (mg/kg)
	0	100	200	400	50
	0	2	4	3	31
	0	2	4	4	33
	0	4	6	4	41
	1	5	10	5	60
	1	9	12	14	62
Median	0	4	6	4	41
Total	2	22 Φ	36 Φ	30 Φ	227 Φ
% MN-PCE	0.02	0.22	0.36	0.30	2.27
PCE/NCE Mean ± SD	0.65 ± 0.04	0.56 ± 0.10	0.48 ± 0.09	0.49 ± 0.07	0.38 ± 0.04

Females	**48 hour sampling period: number of MN-PCE[1]**				
	Vehicle (mg/kg)	Olaparib (mg/kg)			Pos. control (mg/kg)
	0	100	200	400	50
	0			5	
	0			5	
	1			6	
	1			6	
	1			8	
Median	1	N/A	N/A	6	N/A
Total	3			30 Φ	
% MN-PCE	0.03			0.3	
PCE/NCE Mean ± SD	0.67 ± 0.06			0.51 ± 0.09	

[1]No statistical analysis was included in the study report, but positive responses were denoted as Φ.
PCE = polychromatic erythrocytes, MN-PCE = micronucleated PCE, NCE = normochromatic erythrocytes, Φ = positive response in PCE

7.4 Other Genetic Toxicity Studies

None

8 Carcinogenicity

Carcinogenicity studies with olaparib have not been conducted. Consistent with recommendations in ICH S9, these studies are not essential to support an NDA for the proposed patient population.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

Study title: AZD2281: Oral Fertility and Early Embryonic Development Study in the Female Rat

Study no.:	1557GR
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D Alderley, Alderley Park, Macclesfield, SK104TG, England
Date of study initiation:	May 29, 2007
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281) lot # 060344, 100.09% purity

Key Study Findings

- Minimal maternal toxicity was noted at the 15 mg/kg dose level (↓4-6% body weight).
- A higher incidence of extended estrus (9/34 females) was observed at the 15 mg/kg dose level but had no effect on mating and fertility.
- There was a statistically significant increase in pre-implantation loss, early intrauterine deaths, and post-implantation loss in the 15 mg/kg dose group (11% of human AUC at the recommended clinical dose).
- Following a 4-week recovery period, 15 mg/kg female rats showed no treatment-related effects on mating, fertility, or embryo-fetal survival.

Methods

Doses:	0, 0.05, 0.5, 15 mg/kg (Group 1, 2, 3, 4) 0, 15 mg/kg recovery (Group 5, 6)
Frequency of dosing:	Daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Wistar Hannover sub-strain AlpkHsdRccHan-WIST
Number/Sex/Group:	22 Females/Group 1-4 (main study) 12 Female/Group 1 and 4 (recovery) 3 Females from Group 1 main study (TK) 6 Females from Group 2-4 main study (TK) 88 Males (not dosed, for mating purposes only)
Satellite groups:	None
Study design:	Females (~9 weeks of age) were dosed for at least 14 days before mating through Day 6 of pregnancy. Females were paired 1:1 with an unrelated male partner (~12 weeks of age) for a maximum of 10 nights. Control and high dose recovery animals were dosed for 19 days followed by a 4-week non-dosing period before pairing. Scheduled necropsy occurred on Day 12 post-mating or 12 days after the pairing period.
Deviation from study protocol:	There were no reported deviations from the study protocol that affected the study design or interpretation of the results.

Observations and Schedule

Fertility and reproductive parameters: corpora lutea, implantation sites, viable fetuses, intrauterine deaths, and macroscopic exam of ovaries, uterus, and cervix

Mortality	At least once daily
Clinical signs	At least once daily
Body weights	Twice weekly from Day 0 until mating and on Day 0, 3, 6, 9, and 12 post-mating (or end of pairing period)
Food Consumption	Twice weekly on body weight days from Day 0 up to Day 13 and during gestation on body weight days to Day 12 post-mating (or end of pairing period)
Estrous Cycles	Each morning from Day 0 or until mating; recovery females were assessed each morning for 14 days of dosing and then 14 days of the recovery period until mating occurred (or end of pairing period).
Pairing	After 8 pm following 14 days of dosing or 4 weeks of non-dosing for recovery females
Toxicokinetics	Group 1: Day 8 (1 and 12 h) Group 2-4 : Day 8 (0.5, 2, 12, and 24 h)

Justification of Dose

Dose levels were selected based on a 28-day repeat-dose toxicity study (Study No. 2229/037) and a dose range-finding embryo-fetal development study (Study No. 1555RR) in rats. The 15 mg/kg dose level was anticipated to cause minimal maternal toxicity, and the mid dose of 0.5 mg/kg was expected to cause a slight reduction in fetal weight without maternal toxicity or fetal loss. The low dose of 0.05 mg/kg was selected to determine a no effect level.

Mortality

All animals survived to scheduled necropsy.

Clinical Signs

No remarkable clinical signs were observed.

Body Weight

- During the 14-day dosing period, a statistically significant decrease in body weight was reported for the 15 mg/kg dose group (up to 4.5%) compared to controls. Body weight was also reduced 4-6% during the non-dosing period at this dose level.
- During gestation, a statistically significant decrease in maternal body weight (5-7%) was observed in the high dose group compared to controls.
- No remarkable differences were noted in pregnant recovery animals.

Feed Consumption

No remarkable changes in food consumption were reported.

Estrous Cycling

- A higher incidence of extended estrus was observed in the 15 mg/kg dose group (9/34) compared to controls. In the 0.05 and 0.5 mg/kg dose groups, there were 2/22 females with extended estrus (Table 44), which was comparable to recovery controls and not considered test article-related (Table 45).
- No significant differences in estrous cycling were observed in high dose females during the recovery period.

Table 44. Summary of estrous cycles in rats during olaparib dosing period

Number of females during dosing with:	Groups 1 & 5 (Control)	Group 2 (0.05 mg/kg)	Group 3 (0.5 mg/kg)	Groups 4 & 6 (15 mg/kg)
Regular pattern and duration	34	20	20[a]	25
Cycle abnormalities:				
Cycle/s with extended oestrus (lasting 2 days)	0	2	1	7
Cycle/s with extended oestrus (lasting 2 days) and extended for 6 days	0	0	0	1
Short cycle+ cycle/s with extended oestrus (lasting 2 days)	0	0	0	1
Extended cycle lasting 8 days with minor abnormalities	0	0	1	0

a= Animal 48 was pseudopregnant from 4th cycle Short cycles= cycles of 2 or 3 days
Minor abnormalities = pro-oestrus occurring before di-oestrus

[Excerpted from Applicant's submission]

Table 45. Summary of estrous cycles in rats during recovery period

Number of females during dosing with:	Group 5 (Control)	Group 6 (15 mg/kg)
Regular pattern and duration	6	11
Cycle abnormalities:		
Cycle/s with minor abnormalities	1	0
Cycle/s with extended oestrus (lasting 2 days)	0	0
Short cycle+ Cycle/s with extended oestrus (lasting 2 days)	1	0
Cycle/s with extended pro-oestrus	3	1

Minor abnormalities = pro-oestrus occurring before di-oestrus

[Excerpted from Applicant's submission]

Toxicokinetics

- After repeat dosing, C_{max} and AUC on Day 8 increased with increasing dose. C_{max} was greater than dose proportional with increasing dose, and AUC was dose proportional at 0.5 and 15 mg/kg/day.
- AUC could not be calculated for the 0.05 mg/kg dose level.
- Peak plasma concentrations occurred at 2 h post-dose for all dose levels.

Table 46. Summary of mean toxicokinetic parameters on Day 8 in female rats administered oral olaparib

Dose (mg/kg)	T_{max} (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24)}$ (ng·h/ml)	$AUC_{(0-24)}$/D (ng·h/ml)/ (mg/kg)
0.05	2	2.113	42.26	NC	NC
0.5	2	31.440	62.88	257	514
15	2	1088.873	72.59	8249	550

NC - Insufficient data to calculate.

Dosing Formulation Analysis

The sample concentrations were within ± 10% of the intended concentration. Dose formulations were considered stable and homogenous.

Necropsy

There were no test article-related macroscopic observations.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

- One female in each dose group was not pregnant at scheduled necropsy.
  - A 0.05 mg/kg female mated but did not become pregnant.
  - A 0.5 mg/kg female was pseudo-pregnant before pairing and did not resume cycling during the pairing period.
  - A 15 mg/kg female mated the night after estrus and therefore did not become pregnant.
- There were no test article-related effects on mating or fertility during the dosing and recovery periods at the doses tested.
- In the uterine examination, a statistically significant increase in early intrauterine loss corresponded to a slight increase in pre-implantation loss and a decrease in live fetuses in the 15 mg/kg dose group.
- No treatment-related effects on implantation and embryo-fetal survival were observed in the 15 mg/kg recovery group.

Table 47. Summary of mating and fertility parameters in female rats

Parameters	0 mg/kg	0.05 mg/kg	0.5 mg/kg	15 mg/kg	0 mg/kg (Recovery)	15 mg/kg (Recovery)
Mating Index	100%	100%	95.5%	100%	100%	100%
Fertility Index	100%	95%	100%	95%	92%	100%
Mean corpora lutea/litter	13	14	15	14	14	15
Mean implantation sites/litter	13	12	14	12	12	15*
Mean pre-implantation loss/litter	1 (7.1%)	1 (10.0%)	1 (6.1%)	2 (13.4%)	2 (15.9%)	1* (3.2%*)
Mean early intrauterine loss/litter	0 (3.9%)	0 (3.4%)	0 (2.8%)	1* (10.3%*)	0 (2.7%)	0 (1.7%)
Mean live fetuses/litter	12 (96.1%)	12 (96.6%)	13 (97.2%)	11 (89.7%*)	12 (97.3%)	15* (98.3%)

Parameters	0 mg/kg	0.05 mg/kg	0.5 mg/kg	15 mg/kg	0 mg/kg (Recovery)	15 mg/kg (Recovery)
Mean post-implantation loss/litter	0 (3.9%)	0 (3.4%)	0 (2.8%)	1* (10.3%*)	0 (2.7%)	0 (1.7%)

Mean per litter (% per litter); Significant finding, *p < 0.05

Reviewer note: Systemic exposure at the highest dose tested was 11% of human AUC at the recommended clinical dose. Based on lower exposures observed in female rats compared to human subjects, the submitted nonclinical data may be inadequate to assess the potential adverse effect of olaparib on fertility.

Study title: AZD2281: Oral Fertility and Early Embryonic Development Study in the Male Rat

Study no.:	1558GR
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D Alderley, Alderley Park, Macclesfield, SK10 4TG, England
Date of study initiation:	February 7, 2007
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281), lot # 060344, 100% purity

Key Study Findings

- At 40 mg/kg, males experienced reduced body weight and body weight gain as well as clinical signs of salivation and hair loss.
- There were no test article-related effects on mating and fertility rates at doses up to 40 mg/kg/day compared to controls (approximately 7% of human AUC at the recommended clinical dose based on TK analysis in 4-week repeat-dose study).

Methods

Doses:	0, 5, 15, 40 mg/kg (Group 1, 2, 3, 4)
Frequency of dosing:	Daily
Dose volume:	10 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Wistar Hannover sub-strain AlpkHsdRccHan-WIST
Number/Sex/Group:	20/Sex/Group
Satellite groups:	None
Study design:	Approximately 5 to 6 week old males were dosed for at least 70 days prior to pairing through confirmation of mating. Scheduled necropsy occurred between Day 90 and Day 98 for males and Day 12 post-mating for females. Effects of olaparib on mating, fertility, and sperm parameters were assessed. Female animals were not dosed in this study.
Deviation from study protocol:	Deviations were not considered to affect the study design or interpretation of the results.

Observations and Schedule

Fertility and reproductive parameters: sperm analysis, corpora lutea, implantation sites, viable fetuses, intrauterine deaths, microscopic exam of epididymides and testes and macroscopic exam of paired epididymides, testes, prostate, seminal vesicles, uterus, ovaries, and cervix

Mortality	At least once daily
Clinical signs	At least once daily
Body weights	Male - Twice weekly until necropsy Female – Weekly up to mating, Day 0, 3, 6, 9, and 12 post-mating
Food consumption	Male – Twice weekly up to the pairing period. Female – Day 0, 3, 6, 9, and 12 post-mating
Estrous cycles	Each morning starting 5 days before pairing until mating, (or end of pairing period)
Pairing	Late afternoon on the day of the male's last dose for a maximum of 10 nights
Hematology[1]	All males in Week 8
Sperm analysis[2]	Day 90 (8 males/group)

[1]Hematology parameters: erythrocytes, hemoglobin, hematocrit, mean red cell hemoglobin, mean red cell hemoglobin concentration, mean red cell volume, red cell distribution width, reticulocytes, platelets, leukocytes, neutrophils, lymphocytes, monocytes, basophils, eosinophils, large unstained cells

[2]Sperm parameters: count, motility, progressiveness, straightness, average path velocity, curvilinear velocity, straight line velocity

Justification of Dose Levels

Dose levels were selected based on results from 4-week and 26-week repeat-dose toxicity studies in rats (Study No. 2229/037, Study No. TII0012). The 40 mg/kg dose level was anticipated to cause minimal toxicity. The 5 and 15 mg/kg dose levels were selected to identify any dose response relationships and a no effect level for mating and fertility.

Mortality

All animals survived to scheduled necropsy.

Clinical Signs

- A dose-dependent increase in salivation was reported at the 15 mg/kg (1/20) and 40 mg/kg (6/20) dose levels.
- There was an increased incidence of hair loss in the 40 mg/kg dose group (3/20) compared to controls.

Body Weight

- Reduced body weight and body weight gain (up to ~15%) were reported in males at the 40 mg/kg dose level throughout the dosing period.
- No changes in body weight were reported for untreated females during gestation.

Feed Consumption

- A dose-dependent decrease in food consumption up to ~20% was observed primarily at the 15 and 40 mg/kg dose levels in males throughout the dosing period, corresponding to reduced body weight and body weight gain.
- There were no changes in food consumption noted for untreated females during gestation.

Hematology

- Following 7 weeks of dosing, neutrophils, monocytes, and eosinophils were slightly reduced.

Table 48. Summary of hematology changes in male rats following 7 weeks of dosing

	5 mg/kg/day	15 mg/kg/day	40 mg/kg/day
Neutrophils	-11.0	-17.5	-22.7
Monocytes			-15.4*
Eosinophils			-11.1

Significant finding, *p < 0.05

Toxicokinetics

Not conducted

Dosing Formulation Analysis

Sample concentrations were within ± 10% of the intended concentration and reported as stable and homogeneous.

Necropsy

No treatment-related macroscopic or microscopic findings were noted in males or females.

Male Reproductive Organ Weights

There were no remarkable changes in organ weights reported.

Fertility Parameters (Mating/Fertility Index, Corpora Lutea, Preimplantation Loss, etc.)

- There were no test article-related effects on sperm counts, motility, or progressiveness.
- There were no test article-related effects on male or female mating and fertility rates compared to controls.

Table 49. Summary of male rat mating and fertility parameters following administration of oral olaparib daily

Parameters	0 mg/kg	5 mg/kg	15 mg/kg	40 mg/kg
Mating Index	100%	100%	100%	100%
Fertility Index	100%	95%	100%	95%
Mean sperm count	5950	6224	6226	5779
% Motility	91	90	90	89
% Sperm progressive	66	64	62	63
% Straightness	64	64	62	64
Average straight line velocity (µm/s)	93	92	89	91
Curvilinear velocity (µm/s)	347	343	338	342
Average path velocity (µm/s)	145	144	141	143

Table 50. Summary of untreated female rat mating and fertility parameters

Parameters	0 mg/kg	5 mg/kg	15 mg/kg	40 mg/kg
Mean corpora lutea/litter	16	15	16	16
Mean implantation sites/litter	14	13	15	14
Mean pre-implantation loss/litter	2 (8.8%)	2 (9.8%)	1 (6.3%)	2 (12.8%)
Mean early intrauterine loss/litter	1 (12.2%)	1 (9.9%)	1 (6.5%)	1 (8.4%)
Mean live fetuses/litter	13 (87.8%)	12 (90.1%)	14 (93.5%)	13 (91.6%)
Mean post-implantation loss/litter	1 (12.2%)	1 (9.9%)	1 (6.5%)	1 (8.4%)

Mean per litter (% per litter)

Reviewer note: Toxicokinetic analysis was not conducted during this study. However, in a 4-week repeat-dose study in male rats, systemic exposure was 5.75 µg·h/ml at 40 mg/kg/day with no apparent accumulation with repeat dosing (approximately 7% of human AUC at the recommended clinical dose). Given the considerably low exposures observed at the highest dose tested in rats compared to exposures in humans, the nonclinical data may be inadequate to assess the potential effect of olaparib on male fertility in humans.

9.2 Embryonic Fetal Development

Study title: AZD2281: Oral Dose Range Finding Embryofetal Development Study in the Rat

Study no.:	1555RR
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	Safety Assessment UK AstraZeneca R&D Alderley, Alderley Park, Macclesfield, SK10 4TG, England
Date of study initiation:	February 12, 2007
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281, KU-0059436) lot # 060344, 100.1% purity

Key Study Findings

- Doses of ≥ 5 mg/kg/day resulted in maternal toxicity, including reduced body weight gain, food consumption, leukocytes, and reticulocytes. There was also a low incidence of gross pathology findings in the stomach.
- Olaparib was embryotoxic at doses of ≥ 5 mg/kg/day olaparib, resulting in no viable offspring (approximately 2% of human AUC at recommended clinical dose).

Methods

Doses:	0, 0.05, 0.1, 0.5, 5, 15, 40 mg/kg (Group 1, 6, 7, 2, 3, 4, 5)
Frequency of dosing:	Daily
Dose volume:	10 ml/kg for Group 1-5, 2 ml/kg for Group 6-7
Route of administration:	Oral gavage
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Wistar Hannover sub-strain AlpkHsdRccHan-WIST
Number/Sex/Group:	6 females/Group
Satellite groups:	None
Study design:	Oral olaparib was administered daily to female rats on Days 6-16 of pregnancy during the period of major organogenesis. Scheduled

	necropsy occurred on Day 21 post-mating.
Deviation from study protocol:	No deviations from the study protocol were reported.

Observations and Schedule

Reproductive parameters: corpora lutea, implantation sites, uterus weight, viable fetuses, intrauterine deaths, sex, fetal weight, external malformations/variations, and macroscopic examination of placenta

Mortality	At least once daily
Clinical signs	At least once daily
Body weights	Day 0, Day 6-16, 18, and 21 post-mating
Food Consumption	Day 6-9, 9-12, 12-16, 16-18, and 18-21 post-mating
Hematology[1]	Day 15 post-mating
Toxicokinetics	Day 16 post-mating (1 and 12 h for Group 1 and 0.5, 2, 12, 24 h for Groups 2-7)

[1]Hematology parameters: erythrocytes, hemoglobin, hematocrit, mean red cell hemoglobin, mean red cell hemoglobin concentration, mean red cell volume, red cell distribution width, reticulocytes, platelets, leukocytes, neutrophils, lymphocytes, monocytes, basophils, eosinophils

Mortality

All animals survived to scheduled necropsy on Day 21.

Clinical Signs

There were no remarkable clinical signs reported.

Body Weight

- Reduced body weight and body weight gain were observed at doses of ≥ 5 mg/kg/day, starting on Day 9 of pregnancy until scheduled necropsy on Day 21.

Table 51. Summary of changes in rat maternal body weights (% change relative to controls)

	Days post-mating				
Dose Level (mg/kg)	9	12	16	18	21
0.05					
0.1					
0.5					
5				-9.4**	-23.7***
15		-8.2*	-12.3***	-15.8***	-26.4***
40		-8.6*	-12.6***	-18.5***	-30.3***

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Table 52. Summary of changes in rat maternal body weight gain (% change relative to controls)

	Days post-mating				
Dose Level (mg/kg)	9	12	16	18	21
0.05					
0.1					
0.5				-12.1*	
5			-34.8***	-47.0***	-80.4***
15	-63.6*	-46.2***	-56.5***	-57.6***	-77.5***
40	-27.3*	-42.3***	-54.3***	-66.7***	-88.2***

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Feed Consumption

- Food consumption was reduced for the 5 mg/kg dose group from Day 16 to Day 21 and for the 15 and 40 mg/kg dose groups from the start of dosing until scheduled necropsy on Day 21.
- These findings correlated with significant decreases in maternal weight gain during the dosing period.

Table 53. Summary of changes in rat maternal food consumption (% change relative to controls)

	Day range (post-mating)				
Dose Level (mg/kg)	6 to 9	9 to 12	12 to 16	16 to 18	18 to 21
0.05					
0.1					
0.5					
5				-13.0*	-33.3***
15	-15.0*	-19.0**	-17.4**	-17.4**	-25.0***
40	-10.0*	19.0**	-21.7***	-26.1***	-41.7***

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Hematology

- A reduction in reticulocytes was observed at all dose levels with the largest decrease reported in the 15 and 40 mg/kg dose groups, consistent with hematological toxicities in repeat-dose toxicity studies.
- A slight to moderate decrease in leukocyte population was noted at doses ≥ 5 mg/kg/day.

Table 54. Summary of changes in hematology parameters in female rats administered oral olaparib (% change relative to controls)

	0.05 mg/kg	0.1 mg/kg	0.5 mg/kg	5 mg/kg	15 mg/kg	40 mg/kg
Reticulocytes	-19.2**	-17.8*	-8.7	-15.6	-34.4*	-57.6**
Leukocytes				-21.5*	-27.5*	-24.7*
Neutrophils				-40.3*	-39.8	-45.5
Lymphocytes					-27.3*	-12.9*
Monocytes				-58.3*	-60.7*	-67.9*
Basophils					-50.0*	-50.0**

Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Toxicokinetics

- Following repeat dosing on Day 6-16 post-mating, C_{max} and AUC increased with increasing dose. C_{max} was approximately dose proportional from 0.05 to 0.1 mg/kg and greater than dose proportional from 0.5 to 40 mg/kg. AUC was nearly dose proportional with increasing dose from 0.1 to 5 mg/kg and greater than dose proportional from 15 to 40 mg/kg.
- Peak plasma concentrations occurred at 2 h in dose groups of ≤ 5 mg/kg and at 0.5 h in the 15 and 40 mg/kg dose groups.

Table 55. Mean toxicokinetic parameters of oral olaparib in dams on Day 16 post-mating

Dose (mg/kg)	T_{max} (h)	$T_{1/2}$ (h)	C_{max} (ng/ml)	C_{max}/D (ng/ml)/ (mg/kg)	$AUC_{(0-24)}$ (ng·h/ml)	$AUC_{(0-24)}$/D (ng·h/ml)/ (mg/kg)
0.05	2	NC	1.614	32.28	NC	NC
0.1	2	NC	3.748	37.48	34	340
0.5	2	NC	24.65	49.30	209	418
5	2	NC	268.64	53.73	1832	366
15	0.5	3.5	1408.21	93.88	7594	506
40	0.5	2.7	4412.60	110.31	25842	646

NC - There was insufficient plasma concentration data to calculate AUC for the 0.05 mg/kg dose group and to determine half-life values for 0.05 to 5 mg/kg dose levels.

Dosing Formulation Analysis

The sample formulations were stable, homogenous and within ± 10% of the intended concentration.

Necropsy

- At scheduled necropsy, 1 to 2 animals out of 6 in the 5, 15, and 40 mg/kg dose groups had red or red/brown stomach contents resembling clotted blood. One animal at the 15 and 40 mg/kg dose levels showed red gelatinous adhesions in the stomach wall.
- Two out of 6 rats in the 15 mg/kg dose group had red fluid in the uterus, correlating with total intrauterine loss in these animals.

Histopathology

- Based on gross pathology findings, the stomach was assessed for microscopic changes, and no abnormal findings were noted.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- The number of implantations relative to the corpora lutea/rat was comparable across all dose groups.
- Total post-implantation loss and early intrauterine deaths were evident at doses of ≥ 5 mg/kg olaparib. There were no viable offspring at these dose levels.
- In the 0.5 mg/kg dose group, post-implantation loss, early intrauterine deaths, and live fetuses were comparable to the control group. However, there was a statistically significant decrease in fetal weight of 14.0% at this dose level.
- Sex ratio was not affected by olaparib in utero.

Table 56. Cesarean section observations in female rats administered oral olaparib in a dose range-finding study

Parameters	0 mg/kg	0.05 mg/kg	0.1 mg/kg	0.5 mg/kg	5 mg/kg	15 mg/kg	40 mg/kg
Number pregnant and evaluated	6	6	6	6	6	6	5
Number of litters	6	6	6	6	6	6	4
Total number of live fetuses	65	64	75	68	0	0	0
Mean corpora lutea/litter	13	12	14	14	13	13	13
Mean implantation sites/litter	12	11	14	13	12	11	12
Mean pre-implant loss/litter	1 (3.9%)	1 (7.9%)	1 (4.7%)	1 (4.6%)	1 (5.4%)	3 (18.6%)	2 (13.6%)
Mean post-implant loss/litter	1 (8.8%)	0 (3.1%)	1 (7.1%)	2 (12.3%)	12** (100%**)	11** (100%**)	12** (100%**)
Number of early intrauterine deaths/litter	1 (8.8%)	0 (3.1%)	1 (7.1%)	2 (12.3%)	12** (100%**)	11** (100%**)	12** (100%**)
Number of late intrauterine deaths/litter	0	0	0	0	0	0	0
Gravid uterus wt (g)	71.2	70.7	79.7	65.5			
Number of live fetuses/litter	11	11	13	11	0**	0**	0**
Fetal wt./litter (g)	5.0	4.9	4.9	4.3**	-	-	-
Fetal sex ratio/litter (% male)	56.4	37.0	54.1	49.1	-	-	-

Mean per litter (% per litter); Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Offspring (Malformations, Variations, etc.)

- There were no viable fetuses at doses of ≥ 5 mg/kg/day.
- No external fetal abnormalities were noted in viable offspring at ≤ 0.5 mg/kg/day.

Study title: AZD2281: Oral Embryofetal Development Study in the Rat

Study no.:	1556TR
Study report location:	eCTD 4.2.3.5.
Conducting laboratory and location:	Safety Assessment UK, AstraZeneca R&D Alderley, Alderley Park, Macclesfield, SK104TG, England
Date of study initiation:	May 25, 2007
GLP compliance:	Yes, OECD GLP principles
QA statement:	Yes
Drug, lot #, and % purity:	Olaparib (AZD2281), lot # 060344, 100.09% purity

Key Study Findings

- A slight increase in early intrauterine deaths was reported at the 0.5 mg/kg dose level, resulting in a reduced number of viable offspring compared to controls.
- Major fetal malformations (eye, vertebra/ribs, skull and diaphragm) and minor skeletal and visceral abnormalities were noted at 0.05 and 0.5 mg/kg dose levels (≤ 0.3% of human AUC at the recommended clinical dose; AUC could not be calculated for the low dose level)

Methods

Doses:	0, 0.05, 0.5 mg/kg (Group 1, 2, 3)
Frequency of dosing:	Daily
Dose volume:	2 ml/kg
Route of administration:	Oral gavage
Formulation/Vehicle:	DMSO diluted 1 in 10 by 10% hydroxypropyl β-cyclodextrin in PBS pH 7.4
Species/Strain:	Wistar Hannover sub-strain AlpkHsdRccHan-WIST
Number/Sex/Group:	22 females/Group
Satellite groups:	None
Study design:	Pregnant rats were dosed daily from Day 6 to Day 16 post-mating during the period of organogenesis. Scheduled necropsy occurred on Day 21.
Deviation from study protocol:	There were no deviations reported for this study.

Observations and Results

Parameters and endpoints evaluated: clinical signs (daily), body weight (Day 0, 6, 9, 12, 16, 18, 21 post-mating), food consumption (same days as body weight), gross pathology (Day 21), histopathology (Day 21), reproductive parameters (corpora lutea, implantations, uterus weight, intrauterine deaths, viable fetuses, sex, fetal weight,

external malformations/variations, visceral/skeletal malformations/variations, and macroscopic examination of placenta)

Justification of Dose

The doses were selected based on results from a dose range-finding study of rat embryo-fetal development (Study No.1555RR). Groups of 6 mated female rats were administered 0, 0.05, 0.1, 0.5, 5, 15, and 40 mg/kg of oral olaparib daily from Day 6 to Day 16 of pregnancy. Maternal toxicity and total early intrauterine loss were observed at doses of ≥ 5 mg/kg. Findings in the 0.5 mg/kg dose group were comparable to controls with the exception of a 14% decrease in fetal weights. For the present study, 0.05 mg/kg was selected as the low dose to determine a no effect level, and a high dose of 0.5 mg/kg was anticipated to produce fetal effects with little to no maternal toxicity or loss of fetuses.

Mortality

All animals survived to scheduled necropsy on Day 21.

Clinical Signs

There were no clinical signs reported.

Body Weight

A slight, but statistically significant, decrease in maternal body weight gain (↓19%) was reported in the 0.5 mg/kg dose group from Day 16 to Day 18 post-mating.

Feed Consumption

There were no remarkable changes in food consumption.

Toxicokinetics

Toxicokinetics were not evaluated in this embryonic fetal development study. However, toxicokinetic parameters were assessed in the dose range-finding study (Table 55, Study No. 1555RR).

Dosing Formulation Analysis

The dosing formulations were stable and within ± 10% of the intended concentration.

Necropsy

No test article-related macroscopic findings were reported.

Histopathology

No test article-related microscopic findings were reported in dams.

Cesarean Section Data (Implantation Sites, Pre- and Post-Implantation Loss, etc.)

- The number of implantation sites relative to corpora lutea/rat was comparable across all dose groups.
- No significant changes in cesarean section data were observed in the 0.05 mg/kg dose group.

- There was a statistically significant increase in post-implantation loss and early intrauterine deaths in the 0.5 mg/kg dose group compared to controls. Consequently, there was a slight decrease in the number of viable fetuses in this dose group. Olaparib also caused reduced gravid uterus and fetal weights.

Table 57. Cesarean section observations in female rats administered oral olaparib during organogenesis

Parameters	0 mg/kg	0.05 mg/kg	0.5 mg/kg
Number pregnant and evaluated	22	22	22
Number of litters	22	22	22
Total number of viable fetuses	250	244	211
Mean corpora lutea/litter	13	13	14
Mean implantation sites/litter	12	12	13
Mean pre-implant loss/litter	1	1	1
Mean post-implant loss/litter	1 (7.7%)	1 (6.5%)	3** (23.8%**)
Number of early intrauterine deaths/litter	1 (7.7%)	1 (6.1%)	3** (23.5%**)
Number of late intrauterine deaths/litter	0	0	0
Mean gravid uterus wt.(g)	73	71	56***
Number of live fetuses/litter	11 (92.3%)	11 (93.5%)	10* (76.2%**)
Fetal wt./litter (g)	4.9	4.9	4.3**
Fetal sex ratio/litter (% male)	50.7	48.8	53.8

Mean per litter (% per litter); Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

Offspring (Malformations, Variations, etc.)

- External malformations: Major eye malformations (anophthalmia, microphthalmia) were observed in the 0.5 mg/kg dose group.

- Skeletal malformations: At 0.5 mg/kg/day, fetal malformations were noted in the sternebrae (delayed ossification, bipartite, misalignment, fused), vertebrae (delayed ossification; hemicentric centra; misaligned, absent, fused, or bipartite neural arches), pelvic girdle (displaced articulation), and hindlimb (delayed ossification)

- Skeletal variations: Variants were observed in the sternbrae (delayed ossification), rib (increase in ossification centers), and hindlimb (delayed ossification of phalanges, metatarsals) at 0.5 mg/kg/day. An extra 14th rib was noted in both dose groups.

- Visceral malformations: At 0.05 and 0.5 mg/kg/day major eye malformations (anophthalmia, microphthalmia) and minor abnormalities in the ureter (kinked, dilated) were reported.

- Visceral variations: In the 0.5 mg/kg/day dose group, there were visceral variants in the abdominal cavity, including slightly dilated ureter, left sided umbilical artery, and additional liver lobe(s).

Table 58. Summary of rat fetal malformations and variations

Fetal Observations	Dose (mg/kg)		
	Group 1 0	Group 2 0.05	Group 3 0.5
External (total examined[a])	120 (22)	118 (22)	99 (22)
Head			
- Eye: anophthalmia			
Major……..			6** (3)
- Eye: microphthalmia			
Major……..			3 (3)
Oral cavity			
- Palate: non-uniform rugal pattern, slight			
Variant……	24 (15)	28 (14)	30 (15)
- Palate: non-uniform rugal pattern, severe			
Minor…….....	1 (1)	3 (2)	3 (2)
Skeletal (total examined)	130 (22)	126 (22)	112 (22)
Forelimb			
- Digits: 1 phalanx or none ossified			
Variant……	2 (1)	1 (1)	5 (5)
Sternum			
- ≥1 sternebra: misaligned			
Minor……..	1 (1)	3 (3)	11** (9)**
- Sternebrae 1 – 4: bipartite			
Minor……..			4* (3)
- Sternebrae 1 – 4: incompletely ossified			
Minor……..	1 (1)	1 (1)	5 (4)
- Sternebrae 5 & 6: bipartite			
Minor……..	2 (1)	2 (2)	6 (5)
- Sternebrae 6 & 6: incompletely ossified			
Variant…….....	8 (7)	5 (5)	25*** (13)
- Sternebrae 5 & 6: not ossified			
Variant……	1 (1)	5 (3)	6* (4)
- ≥2 sternebrae: fused			
Major……..		1 (1)	1 (1)
Rib			
- 14th: extra			
Variant…….....		5* (2)	45*** (18)***
- ≥1: ossification center			
Variant……	59 (18)	43 (17)	65* (22)
- Extra			
Major……..			1 (1)

Fetal Observations	Dose (mg/kg)		
	Group 1 0	Group 2 0.05	Group 3 0.5
- ≥1: absent Major……..			1 (1)
- ≥2: fused Major……..			1 (1)
Caudal vertebra - ≥1: not ossified Minor……..	3 (2)	3 (3)	18*** (9)*
Thoracic vertebra - ≥1 centra: asymmetrically ossified Minor……..			5* (4)
- ≥1 centra: hemicentric Minor….....			4* (3)
- ≥1 neural arches: misaligned Minor……..			3 (2)
- Hemivertebra Major……..			1 (1)
- ≥1 neural arches: absent Major……..			1 (1)
Skull - Exoccipital: fused Major……..			1 (1)
Cervical vertebra - Odontoid process: not ossified Minor……..	20 (9)	17 (8)	27* (15)
- ≥1 centra: not ossified Minor….....	40 (14)	27 (11)	46* (19)
- ≥1 neural arches: absent Major……..			2 (2)
- ≥1 neural arches: bipartite Minor……..			3 (3)
- ≥1 neural arches: incompletely ossified Minor……..	4 (2)		17*** (10)**
- ≥1 neural arches: misaligned Minor……..			5* (4)
- ≥1 neural arches: reduced in size Minor……..			2 (2)
- ≥2 neural arches: fused Major……..			4* (3)
- Ventral arch of vertebra 1: not ossified Minor……..	19 (10)	15 (8)	44*** (18)*
Pelvic girdle - Displaced articulation, 27 pre-pelvic arches Minor……..	5 (4)	6 (3)	65*** (21)***
Hindlimb - 1st metatarsal: not ossified Minor……..	16 (10)	7 (6)	53*** (19)**
- Calcaneus: ossified Variant……	26 (12)	24 (11)	1*** (1)***
- Digits: all phalanges not ossified			

Fetal Observations	Dose (mg/kg)		
	Group 1 0	Group 2 0.05	Group 3 0.5
Variant……	32 (13)	29 (14)	78*** (21)**
- ≥1 metatarsals: incompletely ossified			
Variant……	31 (17)	20 (11)	45** (20)
Body Fresh Visceral (total examined)	250 (22)	244 (22)	211 (22)
Thoracic cavity			
- Post caval lung lobe: bi-lobed			
Variant……	26 (13)	19 (11)	10* (6)*
- Thymus: partially undescended			
Variant……			5 (4)
Abdominal cavity			
- Liver: additional lobe(s)			
Variant……	6 (3)	7 (4)	15* (8)
- Umbilical artery: left of bladder			
Variant……	13 (11)	12 (8)	42*** (18)*
- Ureter: dilated, slight			
Variant……	21 (11)	26 (9)	32* (14)
- Ureter: dilated, severe			
Minor……...		5* (2)	3 (1)
- Ureter: kinked			
Minor……...	10 (8)	19 (10)	29*** (10)
- Diaphragm: herniated			
Major……...			1 (1)
Head Fresh Visceral (total examined)	130 (22)	126 (22)	112 (22)
Head			
- Eye: anophthalmia			
Major……...		1 (1)	1 (1)
- Eye: microphthalmia			
Major……...			5* (4)

[a]Number of fetuses (number of litters); Significant finding, *p < 0.05, **p < 0.01, ***p < 0.001

9.3 Prenatal and Postnatal Development

Prenatal and postnatal development studies with olaparib have not been conducted. According to the current ICH S9 Guidance, prenatal and postnatal toxicology studies are not warranted to support an NDA for the proposed patient population.

10 Special Toxicology Studies

Additional special toxicology studies were not deemed necessary to support this NDA, so they were not reviewed at this time.

11 Integrated Summary and Safety Evaluation

Pharmacology

PARP enzymes are involved in normal cellular homeostasis, such as DNA transcription, cell cycle regulation, and DNA repair. In vitro studies showed that olaparib inhibits various isoforms of PARP, including PARP1, PARP2, and PARP3. Specifically,

olaparib inhibits PARP-dependent synthesis of poly ADP-ribose (PAR), with IC_{50} values of 5 nM, 1 nM, and 4 nM, respectively. In vitro, olaparib treatment results in decreased cell viability in breast, ovarian, pancreatic, non-small cell lung and colorectal cancer, and head and neck small-cell carcinoma cell lines, as measured by colony formation assays. In vivo, tumor growth inhibitory activity was demonstrated in patient-derived mouse models of breast and lung cancers. Increased cytotoxicity and anti-tumor activity following treatment with olaparib were noted in vitro and in mouse tumor models with cell lines with deficiencies in BRCA with or without deficiencies in other genes involved in homologous recombination repair.

General Toxicology

Repeat-dose toxicity studies were conducted in rodents and dogs for up to 26 weeks. In Han Wistar rats, animal exposures at the maximum dose levels tested in 4- and 26-week repeat-dose studies were below exposures reported in humans at the recommended clinical dose (approximately 2 to 18% of human AUC). Systemic exposure was generally higher in female rats compared to males and correlated with greater toxicity. There were no test article-related mortalities, and rats experienced decreases in body weight, body weight gain, and/or food consumption. In Beagle dogs, animal exposures at the maximum dose levels tested in 4- and 26-week repeat-dose studies were approximately 12 to 26% of human AUC at the recommended clinical dose, respectively. There were no early mortalities in 4- and 26-week repeat-dose studies in dogs.

The major target organ in rodents and dogs was the hematopoietic system. Oral olaparib reduced red blood cell mass and various leukocyte populations in rats and dogs at ≥ 2 and 4% of clinical exposures, respectively. Adverse correlates were noted in the bone marrow (atrophy, reduced hematopoiesis), spleen (pigmented macrophages), liver (hemosiderin pigmented cells), and thymus (atrophy, involution). Corresponding myelogram data showed statistically significant delays in erythroid cell development at scheduled necropsy. Generally, reticulocyte and platelet counts were elevated by the end of dosing and/or recovery period, indicating a regenerative response to myelosuppression observed during the dosing period. Hematology parameters were either fully or partially recovered by the end of a 4-week non-dosing period.

Additional nonclinical findings were observed primarily in dogs and considered to be minimal in severity at the doses tested. In 4- and 26-week repeat-dose studies, dogs showed toxic effects in the GI tract (discoloration, congestion, hemorrhage, inflammation) at ≥ 2.5 mg/kg/day olaparib (approximately 4% of the human AUC at the recommended clinical dose). Toxicological findings in the liver (congestion, hemorrhage), kidney (congestion, hemorrhage, pyelitis), and urinary bladder (congestion, hemorrhage, cystitis) were minimal in severity and without adverse correlates suggesting an effect on function.

Genetic Toxicology

Olaparib was clastogenic in a chromosomal aberration assay in mammalian CHO cells in vitro and in a rat bone marrow micronucleus assay in vivo, consistent with the mechanism of action. Olaparib was not mutagenic in an in vitro bacterial reverse mutation (Ames) assay at concentrations up to 5000 µg/plate.

Carcinogenicity

The Applicant has not conducted carcinogenicity studies with olaparib. According to the current ICH S9 guidance, these studies are not warranted to support approval of an NDA in the intended patient population.

Reproductive and Developmental Toxicology

The Applicant conducted fertility and early embryonic development studies in male and female rats. Male rats were administered 5, 15, and 40 mg/kg olaparib daily for at least 70 days before mating with untreated females. There was a slight decrease in body weight, body weight gain, and food consumption at 40 mg/kg/day as well as clinical signs of increased salivation and hair loss, indicating minimal toxicity at the highest dose level tested. There were no treatment-related effects on mating/fertility indices, sperm count, or sperm motility. In addition, no abnormalities were reported in male reproductive organs at doses up to 40 mg/kg/day, consistent with findings in repeat-dose general toxicology studies. Toxicokinetic analysis was not conducted in this study. However, systemic exposure from 4-week repeat-dose toxicity studies in male rats at 40 mg/kg/day was approximately 7% of human AUC at the recommended clinical dose. At the doses tested, the studies may be inadequate to assess toxicological effects of olaparib on male fertility due to the significantly low exposures. In published studies, $Parp2^{-/-}$ male mice had reduced fertility and severely impaired spermatogenesis, suggesting the potential for adverse effects at clinically relevant exposures of olaparib (Dantzer et al. 2006). The potential for reduced male fertility that may result from olaparib treatment poses a low level of concern given that the proposed indication is to treat female patients with ovarian cancer.

Female rats received oral doses of 0.05, 0.5, and 15 mg/kg olaparib daily for at least 14 days prior to mating through the first week of pregnancy. A slight decrease in body weight was observed during the first week of dosing and during the gestation period in the 15 mg/kg/day dose group, indicating minimal maternal toxicity at the highest dose tested. There was a higher incidence of extended estrus (9/34 females) reported in the 15 mg/kg/day dose group. However, no test article-related effects were observed on mating and fertility at the highest dose tested (approximately 11% of human AUC at recommended dose). A significant decrease in embryo-fetal survival was reported at 15 mg/kg/day, reflecting a higher incidence of pre- and post-implantation loss and intrauterine deaths. Following a 4-week recovery period, no adverse effects on fertility or early embryo-fetal development were noted in the 15 mg/kg/day dose group. In

general toxicology studies, no abnormalities were noted in female reproductive organs in rats or dogs at a high dose level of 40 mg/kg/day or 15 mg/kg/day, respectively.

In a dose range-finding embryo-fetal development study, pregnant rats were administered 0.05 to 40 mg/kg/day oral olaparib during the period of organogenesis. Total implantation loss was reported for rats administered doses of ≥ 5 mg/kg/day (approximately 2% of human AUC at the recommended clinical dose). Adverse effects in pregnant rats included dose-dependent decreases in body weight, food consumption, reticulocytes, and leukocytes. The slight maternal toxicity was not considered to be the major cause of complete fetal loss at these dose levels.

Olaparib doses of 0.05 and 0.5 mg/kg/day were further evaluated during the period of organogenesis. A dose of 0.5 mg/kg/day caused major fetal malformations in the eye (anophthalmia, microphthalmia), vertebrae/ribs (extra rib or ossification center; fused or absent neural arches, ribs, and sternebrae), skull (fused exoccipital) and diaphragm (hernia). Additional abnormalities or variants included incomplete or absent ossification (vertebrae/sternebrae, ribs, limbs) and other findings in the vertebrae/sternebrae, pelvic girdle, lung, thymus, liver, ureter and umbilical artery. Some findings noted above in the eye, ribs and ureter were also reported at a dose of 0.05 mg/kg/day olaparib at lower incidence. Fetal malformations occurred in the absence of maternal toxicity and at very low maternal exposures, less than 0.3% of human AUC at the recommended clinical dose (calculated from embryo-fetal development dose range-finding study No. 1555RR). The effect on embryo-fetal survival is consistent with the pharmacology of blocking PARP activity. Double knockout of PARP1 and PARP2 causes embryonic lethality in mice, and development arrests at the onset of gastrulation (Ménissier de Murcia et al. 2003). In addition, female specific embryonic lethality was reported in the $Parp1^{+/-}Parp2^{-/-}$ background and was associated with X chromosome instability.

12 Appendix/Attachments

None

13 References

Dantzer F, Mark M, Quenet D, Scherthan H, Huber A, Liebe B, Monaco L, Chicheportiche A, Sassone-Corsi P, de Murcia G, Ménissier-de Murcia J, 2006, Poly(ADP-ribose) Polymerase-2 Contributes to the Fidelity of Male Meiosis I and Spermiogenesis, Proc Natl Acad Sci USA 103: 14854-9.

Menear KA, Adcock C, Boulter R, Cockcroft XL, Copsey L, Cranston A, Dillon KJ, Drzewiecki J, Garman S, Gomez S, Javaid H, Kerrigan F, Knights C, Lau A, Loh VM jr, Matthews IT, Moore S, O'Connor MJ, Smith GC, Martin NM, 2008, 4-[3-(4-Cyclopropanecarbonylpiperazine-1-carbonyl)-4-fluorobenzyl]-2H-phthalazin-1-one: A Novel Bioavailable Inhibitor of Poly(ADP-ribose) Polymerase-1, J Med Chem 51: 6581-6591.

Ménissier de Murcia J, Ricoul M, tartier L, Niedergang C, Huber A, Dantzer F, Schreiber V, Amé JC, Dierich A, LeMeur M, Sabatier L, Chambon P, de Murcia G, 2003, Functional Interaction Between PARP-1 and PARP-2 in Chromosome Stability and Embryonic Development in Mouse, EMBO J 22: 2255-63.

Murai J, Huang SY, Das BB, Renaud A, Zhang Y, Doroshow JH, Ji J, Takeda S, Pommier Y, 2012, Trapping of PARP1 and PARP2 by Clinical PARP Inhibitors, Cancer Res 72: 5588-5599.

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/s/

TIFFANY RICKS
11/21/2014

TODD R PALMBY on behalf of HAW-JYH CHIU
11/21/2014

TODD R PALMBY
11/21/2014

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

NDA Number: 206162 | **Applicant: AstraZeneca Pharmaceuticals, LP** | **Stamp Date: February 3, 2014**

Drug Name: Olaparib (AZD2281) | **NDA Type: 505 (b)(1); Type 1 - New Molecular Entity**

On **initial** overview of the NDA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	X		
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant submitted a rationale to justify the alternative route?	X		
7	Has the applicant submitted a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	X		

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

PHARMACOLOGY/TOXICOLOGY FILING CHECKLIST FOR NDA/BLA or Supplement

	Content Parameter	Yes	No	Comment
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m2 or comparative serum/plasma levels) and in accordance with 201.57?	X		The Applicant's proposed labeling will be reviewed during the NDA review.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Acceptability of the Applicant's proposed specifications will be determined during the NDA review.
11	Has the applicant addressed any abuse potential issues in the submission?			Not applicable.
12	If this NDA/BLA is to support a Rx to OTC switch, have all relevant studies been submitted?			Not applicable.

IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? Yes

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

None at this time.

Haw-Jyh Chiu, Ph.D. and Tiffany Ricks, Ph.D. — March 5, 2014
Reviewing Pharmacologist — Date

Todd R. Palmby, Ph.D. — March 5, 2014
Team Leader/Supervisor — Date

File name: 5_Pharmacology_Toxicology Filing Checklist for NDA_BLA or Supplement 010908

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/s/

HAW-JYH CHIU
03/05/2014

TIFFANY RICKS
03/06/2014

TODD R PALMBY
03/06/2014