PARP Activity in Peripheral Blood Lymphocytes as a Predictive Biomarker for PARP Inhibition in Tumor Tissues – A Population Pharmacokinetic/Pharmacodynamic Analysis of Rucaparib

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Abstract

Purpose: Rucaparib is a potent Poly (ADP-ribose) Polymerase (PARP) inhibitor currently under clinical development. The objectives of this analysis were to establish population PK and PK/PD models for rucaparib, and to evaluate the predictability of PARP activity in PBL for PARP activity in tumor tissues.

Experimental Design: Rucaparib concentrations and PARP activity in human PBLs and tumor issues were obtained from 32 patients with solid tumors in a Phase 1 First-in-Patient study. Simulations were conducted to evaluate different dosing regimens.

Results: A 3-compartment PK model best described the PK of rucaparib. An Emax model best described the exposure and PARP inhibition relationship. The maximum PARP inhibition (Imax) achieved in PBLs and in tumors were 90.9% and 90.0% of the baseline PARP activity, and the IC50 values were 1.05 ng/mL and 1.10 ng/mL, respectively. PAR polymer baseline value was found to be a covariate of Emin.

Conclusion: Population PK and PK/PD models have been established to describe population PK of rucaparib and the relationship between rucaparib plasma concentration and PARP inhibition in both PBLs and tumor issues. Results from this trial indicated that PARP inhibition in PBLs can be used as a substitute for PARP inhibition in melanoma tumor tissues.

Keywords

rucaparib, pharmacokinetics (PK)/pharmacodynamics (PD), poly (ADP-ribose) polymerase (PARP), mixed-effects modeling; biomarker

Many Poly (ADP-ribose) polymerase (PARP) inhibitors, such as rucaparib, olaparib, and MK-4827, have entered clinical trials in a variety of tumor types with defective homology-directed DNA repair. Unlike chemotherapy agents where maximum tolerated dose (MTD) has generally been used to guide dose selection, the selection of effective doses of targeted agents, such as PARP inhibitors, may be guided by biomarkers associated with target inhibition or modulation. PARP activity in peripheral blood lymphocytes (PBLs) or peripheral-blood mononuclear cells (PBMCs), measured by Poly (ADP-ribose) (PAR) formation, has been used as a biomarker to guide dose selection for these PARP inhibitors. However, the results of a Phase 2 study of olaparib in patients with BRCA-deficient ovarian or breast cancer showed that while PARP inhibition in PBMCs plateaued at 100 mg bid, better antitumor activity (as measured by objective response rate, median progression free survival, and objective radiologic response or 50% decline in CA125) was achieved with 400 mg bid dosing. Therefore, the question has been raised as to whether PARP inhibition in PBLs or PBMCs...
truly reflects the inhibition of PAR formation in tumor tissues? In a more recent publication, PARP inhibition in PBMC and tumor following olaparib treatment were evaluated at different doses. The result showed that PARP inhibition was greater in tumor tissue than that in PBMC across all exposure levels. However, due to the high variability and limited data, no clear relationship between olaparib dose and PARP inhibition in tumor or PBMC could be observed, and the relationship in PARP inhibitory activity between tumors and PBMCs could not be determined either.

In a Phase 1 study A4991002, PARP activity was measured in both PBLs and tumor tissues to characterize the pharmacodynamics (PD) of rucaparib (formerly known as AG-014699 and PF-01367338), a potent PARP inhibitor, following its IV administration. Plasma concentrations of rucaparib were also measured to characterize the pharmacokinetics (PK) of rucaparib. The objectives of this analysis were to: (i) characterize the population pharmacokinetics of rucaparib in cancer patients; (ii) establish a PK/PD model to describe the relationship between rucaparib pharmacokinetics and PARP inhibition in both surrogate tissue (PBLs) and tumor biopsies; (iii) evaluate the predictive ability of PARP inhibition in PBLs for that in the tumor; and (iv) evaluate different dosing regimens of rucaparib by simulation.

**Materials and Methods**

**Subjects and Study Design**

Data were collected in an open label, multicenter, dose-escalating, first in human (FIH) study A4991002 that was designed to examine the safety and pharmacokinetics (PK)/pharmacodynamics (PD) of rucaparib. A total of 32 patients participated in the study. All patients were first given a single dose of rucaparib as 30 minutes intravenous infusion between Day-4 to Day-10 to assess the pharmacokinetics of rucaparib when given alone. Then a daily 30 minutes intravenous infusion of rucaparib followed by a daily oral dose of temozolomide (TMZ, given 1 hour after the start of rucaparib infusion) was administered for five consecutive days (on Days 1–5) in each of the 28 day cycle. The study was conducted in two parts. Part 1 was open to patients with all tumor types meeting the eligibility criteria. The starting dose of rucaparib was 1 mg/m$^2$ and the doses were escalated in cohorts of patients up to the PARP Inhibitory Dose (PID), which was defined as at least 50% reduction of PARP activity in PBLs 24 hours (h) after rucaparib administration with no increase in the degree of PARP inhibition over the proceeding rucaparib dose level. The PID was determined to be 12 mg/m$^2$, and the dose range of rucaparib studied was 1–12 mg/m$^2$ with escalating steps of 2, 4, 8 mg/m$^2$ in Part 1. The TMZ dose was fixed at 100 mg/m$^2$/day (half of the clinical dose) for this part of the study. Once the PID had been identified, Part 2 of the study recruited patients with metastatic malignant melanoma. Patients received 12 mg/m$^2$ rucaparib (PID) with escalated doses of TMZ at 135, 170 mg/m$^2$, and 200 mg/m$^2$ (maximum permitted dose). The dose of rucaparib was then increased by another 50% to reach 18 for the last cohort in Part 2 with the intention of increasing the PARP inhibition in the tumor samples but this dose level was considered undeliverable due to observed toxicity. Patients entering Part 2 of this study had to consent to a pre- and post-treatment tumor biopsy to measure PARP inhibition. The study was performed in accordance with the Declaration of Helsinki (2000). The protocol was approved by a multi-center research ethics committee, as well as by Cancer Research UK and local site institutional review boards. All patients had given written informed consent prior to participation and undergoing any study-related procedures.

**PK/PD Sample Collection and Assay**

1. **PK Sampling and Assay.** Venous blood (5 mL) was collected in sodium heparinized tubes for the determination of plasma concentrations of rucaparib (the free base form) at the following nominal time points: prior to start of the infusion (0 hour), 0.25 and 0.5 hours after the start of infusion, and 0.25, 0.5, 1, 2, 4, 6, 8 and 24 hour after the end of infusion on Day -7 (single agent treatment) and Days 1 and 4 of the first cycle (combination treatment). Blood samples were centrifuged for 10 minutes immediately after the collection. Plasma was stored at -20°C before shipment on dry ice to the bioanalytical laboratory (DMPK and Bioanalysis Department, Quintiles Limited, Edinburgh, UK) for analysis of rucaparib concentrations.

After sample extraction using acetonitrile, the concentrations of rucaparib and internal standard d$_{6}$-rucaparib were determined by a validated reversed phase high-performance liquid chromatography (HPLC) with tandem mass spectrometric detection (Perkin Elmer API 365 equipped with a scieix turbo ion spray under positive ion mode) using a thermo hypersil keystone betabasic C8 5 $\mu$m column (100 $\times$ 2.1 mm i.d.). Ion spray voltage and turbo heater temperature were 4000 V and 450°C. Formic acid (0.1%) in water and formic acid (0.1%) in acetonitrile were used as mobile phase A and B respectively, and the flow rate was 200 $\mu$L. Typical retention time was 1.5 minutes for both rucaparib and d$_{6}$-rucaparib; and the dwell time was 350 ms and 150 ms for rucaparib and d$_{6}$-rucaparib, respectively. The mass transitions monitored for rucaparib and d$_{6}$-rucaparib were 324.4–293.2 and 330.3–299.1, respectively. The intra- and inter-assay variability was below 10% expressed as coefficient of variation. The limit of quantification was 2 ng/mL and the assay was validated over a range of 2–1000 ng/mL.
2. PD Sampling and Assay: Blood samples were also collected for the determination of PARP-1 activity in PBL at the following nominal time points: prior to the start of the infusion, end of infusion, 4–6 hours and 24 hours after the end of infusion on Days-7, 1, and 4 of the first cycle. An additional sample on Day 8 (3 days after last dose of rucaparib) was taken (12 mg/m²/day rucaparib only) to explore the duration of PARP-1 inhibition in PBLs.

Tumor biopsies were only required for the patients in Part 2 of the study to examine PARP-1 inhibition in target tissues. Tumor biopsies were taken at baseline, and 4–6 hours or 24 hours after treatment with rucaparib (on Day 1).

Isolation of PBLs from whole blood and preparation of tumor tissues for PARP activity assay were conducted as previously described.5 The PARP activity in both PBLs and tumor biopsies was determined by analyzing the concentrations of PAR formed as a result of PARP activity using a previously validated and published method.5 The PARP activity was expressed as pmol of PAR per 10⁶ PBLs (for PBL samples) or pmol of PAR per mg protein (for tumor biopsies).

Population PK and PK/PD Analyses
Population PK and PK/PD modeling was performed in a sequential manner using nonlinear mixed effects approach. For patients with missing PK data, their PK profiles were generated using typical population PK parameters. The method of first-order conditional estimation (FOCE) was employed in population model building and evaluation with NONMEM (Version 7.1.2, ICON).

The actual sample collection times were used in the analysis. Noncompartmental PK analysis showed that TMZ had no evident effect on rucaparib PK either after a single dose or multiple doses, and the PK parameters were available in the previous publication.5 Therefore the PK data of rucaparib with or without the co-administration of TMZ from three different sampling days (Days-7, 1, and 4) were pooled for the population PK analysis. Among all PK samples, 12% had rucaparib concentrations below quantitation limit (BQL). Considering the relative low degree of BQL data, we employed M6 as categorized by Ahn et al.11 namely replacing the first BQL with LOQ/2 and discarding the rest of the BQL data, to handle BQL values. The analysis showed that M6 provided much better model stability with similar parameter estimation as compared to the likelihood based approach (such as M2 or M3).

Log-transformed PK and PD data were used in building the population PK and PK/PD models. The inter-individual variability (IIV) of PK parameters was assumed to follow log-normal distribution as described by an exponential error model. Covariance between parameters was estimated only when strong correlation between IIVs was observed on diagnostic plots. The residual error for the log-transformed PK and PD data was assumed to follow a normal distribution and was modeled using additive structure. Inter-occasion variability (IOV) was used to describe the variability of some PK parameters in three occasions (i.e., the three different PK sampling days of Days-7, 1 and 4). Structural model selection was based on objective function values (OFV), goodness-of-fit plots, successfully convergence, plausibility, and precision of parameter estimates. Data processing, statistical, and graphical analyses were conducted using S-PLUS (7.0, Insightful).

Potential covariates were tested for significance using stepwise covariate modeling (SCM) method with statistical criteria of $P = 0.05$ for forward inclusion step and $P = 0.01$ for backward elimination step. Demographic and physiological variables evaluated included age, gender, weight, body surface area, serum creatinine, aspartate transaminase, alanine transaminase, disease stage, PAR baseline in PBL, and PAR baseline in tumor. Insignificant or poorly estimated covariates ($<6.63$ points increase of OFV upon covariate removal and/or 95% confidence interval including the null value of the parameter) were not included in the final model.

The predictability of the final PK model was evaluated through visual predictive check (VPC) by simulating data with dose of 1 mg using the final parameter estimates for 1000 virtual patients. As rucaparib exhibited linear PK over the dose range studied, observed plasma concentrations were dose-normalized and visually compared with the 5th, 50th (median), and 95th percentiles of simulated concentrations for each time point. As an alternative approach of VPC, the standardized VPC (SVPC), recently developed by our group,12 was used to evaluate the PD model for PARP activity in PBL. One thousand data set are simulated using final model based on the original data set (the data set used for model development). The percentile for each observation among the corresponding 1000 simulated values was calculated according to the equation specified in the paper.12 Distribution of percentile for each observation over the time or dose was visually compared with the uniform distribution between 0 and 1.

The stability of the final PK model and PD model for PARP activity in PBL was evaluated by nonparametric bootstrap analysis where 200 replicates were generated from the original dataset and used to conduct the model fitting. The means as well as standard errors of model parameter estimates were calculated and compared with the final model estimates. An acceptable similarity can indicate the final model is stable. Bootstrap and VPC were not conducted for the PD model for PARP activity in tumor tissues due to the small number of parents providing biopsies and sparseness of the data.
**Simulation of Rucaparib Concentrations and PARP-1 activity in PBL**

The mean values of rucaparib plasma concentrations and PARP activity in PBL under different doses and dosing regimens of rucaparib were simulated to guide future clinical studies. The final PK/PD model and typical population parameters were used for simulation.

**Results**

**Pharmacokinetic Model**

A total of 1022 rucaparib PK samples obtained from the 26 patients who had valid PK data were used for PK analysis. The PK of rucaparib following a 30 minutes infusion was best described by a three-compartment model. In addition to IIV, inclusion of IOV for total clearance (CL) and the distribution clearance (Q2) between the central and peripheral compartment further improved the fit. The effect of demographic and physiological variables on PK parameters was evaluated and none were found to be a significant covariate. Therefore, the final population PK model for rucaparib is a three-compartment model with IIV on all PK parameters and IOV on CL and Q2 for different observation days (Days-7, 1, and 4). The estimates for major parameters are listed in Table 1. In general, population typical values of the PK parameters are well estimated with the relative standard errors (RSE%) of estimates being less than 15% for all parameters. The IIV, IOV and residual error are also estimated reasonably well. Estimates for all model parameters are available in Supplementary Table 1. Examples of individual fit, observed and model predicted concentration-time profiles, at each dose level are shown in Figure 1A.

**Evaluation of PK Model**

Basic goodness-of-fit plots are shown in Supplementary Figure 1. The model described the data reasonably well with conditional weighted residual errors approximately evenly distributed over time and the predicted concentrations.

The stability and robustness of the final PK model was assessed using a bootstrap resampling technique. The mean and RSE% of the parameter estimates obtained from 200 bootstrap replicates are all similar to those obtained by fitting the original dataset (Table 1), suggesting that the model is relatively stable. The adequacy of the PK model was evaluated by VPC using dose-normalized concentrations as each patient received a different total dose. As shown in Supplementary Figure 2, the predicted 5th, 50th, and 95th percentiles matched the observed concentrations reasonably well in all three occasions (Days-7, 1, and 4), suggesting that the model adequately described the data.

**PD Model**

**PD Model for PARP Activity in PBL**

A total of 348 PARP activity data values in PBL from all 32 patients participated in the study were used in this analysis. The relationship between plasma concentrations of rucaparib and its inhibitory effect on PAR formation (PARP activity) was described by a simple direct effect model (E\textsubscript{max} model) as shown in equation 1.

$$E = E_0 - \frac{(E_0 - E_{min}) \cdot C}{IC_{50} + C}$$

(1)

where E is the PARP activity at rucaparib concentration (C); E\textsubscript{0} is the PARP activity at baseline (pre-dose of rucaparib), E\textsubscript{min} is the PARP activity at maximum PARP activity.

**Table 1. Major Population Pharmacokinetic Parameters of AG-014699 and the Stability of the Parameters Using the Bootstrap Resampling Procedure**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Original data</th>
<th>200 bootstrap replicates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean % RSE</td>
<td>Mean % RSE</td>
</tr>
<tr>
<td>Structural model</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clearance, CL (L/hr)</td>
<td>17.5 6.57</td>
<td>17.3 11.5</td>
</tr>
<tr>
<td>Volume of distribution, central compartment, V\textsubscript{1} (L)</td>
<td>15.5 11.6</td>
<td>15.5 13.4</td>
</tr>
<tr>
<td>Inter-individual variability (IIV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IIV CL (%CV)</td>
<td>51.2 32.8</td>
<td>51.1 32.0</td>
</tr>
<tr>
<td>IIV V\textsubscript{1} (%CV)</td>
<td>57.3 36.3</td>
<td>56.4 42.2</td>
</tr>
<tr>
<td>Inter-occasion variability (IOV)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IOV CL (%CV)</td>
<td>25.5 32.6</td>
<td>25.5 31.2</td>
</tr>
<tr>
<td>IOV Q2 (%CV)</td>
<td>32.9 60.9</td>
<td>33.2 61.4</td>
</tr>
<tr>
<td>Residual variability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Additive residual error, SD</td>
<td>0.208 18.1</td>
<td>0.205 16.8</td>
</tr>
</tbody>
</table>
Figure 1. (A) Observed (open symbols) and individual model predicted (lines) plasma concentration of rucaparib versus time plots in representative individual patients at each dose level; (B) Observed (open circles) and individual model predicted (solid line) PARP activity in PBL versus time plots in representative individual patients at each dose level.
inhibition, IC\textsubscript{50} is rucaparib concentration that achieves 50% PARP inhibition.

Covariate analysis suggested that the residual PARP activity at maximal inhibition (E\textsubscript{min}) is correlated with the observed baseline PARP activity (BLB), and this relationship was built into the final PD model by the following equation:

\[ E_{\text{min}} = TV(E_{\text{min}}) \left(\frac{BLB}{BLB_{\text{median}}}\right)^\alpha \]  

(2)

where TV (E\textsubscript{min}) is the population typical value of E\textsubscript{min}; BLB and BLB\textsubscript{median} represent the observed baseline PARP activity and the median value of BLB, respectively; and \( \alpha \) is the exponent of the power function for BLB effect on E\textsubscript{min}.

As shown by PARP activity versus time plots of representative individual patients at each dose level (Figure 1B) and the basic goodness-of-fit plots (Supplementary Figure 3), the model-predicted PARP activity, in general, matched the observed values reasonably well and the residual errors are also approximately evenly distributed over time and the predicted PARP activity. The parameter estimates obtained from this final PD model were presented in Table 2. In general, the population typical values of all the PD parameters were relatively well estimated, with the RSE\% of estimation being all smaller than 30% (Table 2). The population typical value of maximal PARP inhibition (I\textsubscript{max}, expressed as \% of baseline) that can be achieved by rucaparib for a patient with typical E\textsubscript{0} was calculated to be 90.9\% of the baseline by equation (3):

\[ I_{\text{max}}(\%) = \frac{E_0 - TV(E_{\text{min}})}{E_0} \times 100 \]  

(3)

The population typical value of IC\textsubscript{50} was estimated to be 1.05 ng/mL (Table 2), indicating that rucaparib is a potent PARP inhibitor in vivo. Although the estimated IC\textsubscript{50} was lower than the lower limit of quantification (LLOQ) for plasma rucaparib concentration (2 ng/mL), we believed that the IC\textsubscript{50} value was estimated reasonably well by examining the relationship between PARP inhibition and rucaparib concentration (Supplementary Figure 4). While the Pop PK model may not be able to predict the concentrations below LLOQ accurately, it is evident that the IC\textsubscript{50} estimated by the model is in the appropriate range (namely less than 2 ng/mL). In addition, the unbound IC\textsubscript{50} of 0.467–0.264 ng/mL, estimated by the PD model when plasma protein binding (54.6–74.9\%) was taken account, is consistent with the Ki value of rucaparib estimated in vitro (0.59 ng/mL for PARP-1).

Similar to those reported by Zaremba et al,\textsuperscript{13} where baseline PARP activity varied by more than 200 fold between individuals (ranged from 10 to 2,600 pmol/10\textsuperscript{6} PBMC), high inter-individual variability was observed for baseline PARP activity in this trial (ranged from 10 to 1000 pmol/10\textsuperscript{6} PBL) and estimated to be 116\%.

### Evaluation of PBL PARP Pharmacodynamic Model

The mean and RSE\% of the parameter estimates obtained from the 200 bootstrap replicates were shown to be all similar to those estimated by fitting the original dataset (Table 2), suggesting that this PD model is relatively stable. In addition, the result of SVPC (Supplementary Figure 5) showed that the percentile values of each PD observations among the corresponding 1000 simulated values are approximately evenly distributed between 0 and 1, suggesting that the PARP activity data in PBL can be adequately described by this model.

### Table 2. Population pharmacodynamic parameters of AG-014699 for inhibiting PARP activity in PBL

<table>
<thead>
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<th>Parameter</th>
<th>Original data</th>
<th>200 bootstrap replicates</th>
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<tbody>
<tr>
<td>Parameter</td>
<td>Mean</td>
<td>% RSE</td>
</tr>
<tr>
<td>Structural model</td>
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<td></td>
</tr>
<tr>
<td>TV(E\textsubscript{min}) (pmol/10\textsuperscript{6} PBL)</td>
<td>8.24</td>
<td>10.6</td>
</tr>
<tr>
<td>IC\textsubscript{50} (ng/ml)</td>
<td>1.05</td>
<td>24.0</td>
</tr>
<tr>
<td>E\textsubscript{0} (pmol/10\textsuperscript{6} PBL)</td>
<td>90.8</td>
<td>19.7</td>
</tr>
<tr>
<td>( \alpha ) (exponent)\textsuperscript{*}</td>
<td>0.620</td>
<td>14.2</td>
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<tr>
<td>Inter-individual variability (IIV)</td>
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<tr>
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<tr>
<td>IIV IC\textsubscript{50} (%CV)</td>
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<tr>
<td>IIV E\textsubscript{0} (%CV)</td>
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<td></td>
<td></td>
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<tr>
<td>Additive residual error, SD</td>
<td>0.529</td>
<td>12.5</td>
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</table>

\( E_{\text{min}} = TV(E_{\text{min}}) (\text{observed baseline PARP activity in PBL/median of observed baseline PARP activity in PBL})^{\alpha}. \)
**Pharmacodynamic Model of PARP Activity in Tumor Tissues**

Tumor biopsies were only taken from the patients who participated in Part 2 of the study (14 patients) and one patient at 4 mg/m² dose level in Part 1 of the study, and only 30 data points were available for analysis. Although limited data as described, modeling analysis of the PARP activity data in tumor tissues was still attempted in order to estimate key PD parameters such as IC₅₀ and Eₘᵢₙ. For this exercise, the same Eₘₐₓ model and the covariate relationship as the PBL PD model (different parameters values) were employed. IIV was only included to describe the variability of tumor baseline PARP activity (E₀). The basic diagnostic plots and parameter estimates are shown in Supplementary Figure 6 and Table 3, respectively. The RSE% of parameter estimation was generally less than 40% with an exception for IC₅₀ (76.0%, Table 3). Based on the estimated population typical values of the PD parameters, the population typical Iₘₐₓ value was calculated to be 90.0% of the baseline. The population typical IC₅₀ value was estimated to be around 1 ng/mL (1.10 ng/mL, Table 3), which is similar to the IC₅₀ estimated in the PBLs. Evidence that supports the accuracy of IC₅₀ estimated by the tumor PD model has been discussed in the PBL PD section. Bootstrapping and VPC were not conducted for this model due to the sparseness of the data.

**Dose/Exposure and Dose/Response Relationships**

In line with the linear PK model used for rucaparib, the exposure of rucaparib, as measured by area under the rucaparib plasma concentration curve (AUC), increased proportionally with increase of rucaparib dose. Figure 2A and 2B show observed and model predicted average percent PARP inhibition over 24 hours dosing interval and percent PARP inhibition at the end of the dosing interval (trough), respectively, in PBLs in comparison with the baseline PARP activity as a function of dose. Observed percent PARP inhibition in tumor issues are also shown in Figure 2A at the dose levels where tumor issues were collected for PARP activity assessment. The boxplot comparisons of PARP inhibition in PBL and tumor tissues are also shown in Figure 2C.

**Discussion**

PK/PD modeling and simulation has become an integral part of drug development in many therapeutic areas. However, its application has been relatively limited in oncology drug development because of the lack of measurable or predictive biomarkers for many therapeutic targets. As a result, maximum tolerated dose (MTD), which relies on toxicity rather than target modulation for its determination, has been used preferentially to optimal biologic dosing (OBD) in standard Phase 2 and 3 oncology clinical trials. Given the limitations in biomarker availability, the MTD approach has been considered as a reasonable dosing approach for chemotherapeutic agents. Importantly, as the toxicities of chemotherapeutic agents are mostly target related, their efficacy is often highly correlated with toxicity.

In contrast to the MTD approach, the use of an OBD strategy, which defines the dose at which a target is optimally inhibited or modulated, is of more importance for targeted therapies. In this setting, toxicities caused by target therapeutic agents can often result from off-target effects, and so the relationship between toxicity and anti-tumor activity is less clear. Use of an OBD may circumvent or minimize off-target effects or toxicities.

When the mechanism of action of a target therapy is well-defined and measurable through the use of biomarkers it is presumed possible to determine an OBD for clinical use. One such biomarker is PARP inhibition as measured in PBLs or PBMCs, which is an established biomarker that has been widely used in the development of PARP inhibitors. The presence of this biomarker provided an opportunity to define the optimal dosing regimen based upon an OBD approach, using modeling and simulation, instead of using the classical MTD approach alone. In order to use this information clinically, the relationship between the substitute biomarker (PARP inhibition in PBLs or PBMCs) and the biomarker at the site of action (PARP inhibition in tumor) must be well-established and understood quantitatively. In this paper, we established population PK and PK/PD models for rucaparib, and evaluated the predictability of PARP inhibition in PBLs for that in tumor, and raised points to consider in dose selection based on OBD for future trials for PARP inhibitors.

**Establishment of PK/PD Relationship**

Using the data from a FIH study of rucaparib, population PK model for rucaparib and the relationship between

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**Table 3. Population Pharmacodynamic Parameters of AG-014699 for Inhibiting PARP Activity in Tumor Tissues**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>% RSE</th>
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<tr>
<td>Structural model</td>
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<tr>
<td>TV(Eₘᵢₙ) (pmol/mg protein)</td>
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<tr>
<td>IC₅₀ (ng/ml)</td>
<td>1.10</td>
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<td>α₂ (exponent)</td>
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<td>Residual variability</td>
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<tr>
<td>Additive residual error, SD</td>
<td>0.749</td>
<td>21.7</td>
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*Eₘᵢₙ = TV(Eₘᵢₙ)(observed baseline PARP activity in tumor/median of observed baseline PARP activity in tumor)ᵃᵇ."
rucaparib plasma concentration and PARP inhibition, both in PBL and in tumor, have been established. The typical volume of distribution of rucaparib in peripheral compartment (311 L for V2 and 48 L for V3) was much larger than that in the central compartment (15.5 L), which probably was attributable to extensive tissue distribution of this small molecule. In addition, the high intercompartmental clearances (21.7 L/hr for Q2, and 52.9 L/hr for Q3) relative to central volume of distribution (15.5 L for V1) corresponded to an overall distribution half life of 8.6 minutes, which indicated a rapid distribution equilibrium post drug administration. These findings supported the tumor PK-PD model which directly linked the drug exposure in PBL to PARP inhibition in tumor.

This well-defined PK/PD relationship provided a useful tool for dosing regimen selection and evaluation. Based on this relationship, different dosing approaches could be assessed using simulation. For example, the typical PARP inhibition for the population was simulated following 5 days of daily IV dosing of rucaparib at different dose levels. To maintain the PARP inhibition above 80% throughout the 24 hours dosing interval (at steady-state [Day 5] for 80% of patients), the simulation showed a dose of at least 24 mg is required; less variability could be achieved with

![Graphs showing PARP inhibition vs dose](image)
36 mg dose. This was observed in study A4991002 as shown in Figure 2A and 2B.

**PARP Inhibition in PBL as a Predictive Biomarker for PARP Inhibition in Tumor**

Data from olaparib phase 2 study showed better antitumor activity at the dose level of 400 mg olaparib bid compared to that of 100 mg olaparib bid where maximum inhibition of PARP activity in PBMCs was achieved.9 We wanted to define whether this finding was due to the achievement of maximum inhibition in PBMCs, despite inhibition in tumor tissues not reaching a maximum inhibitory state. The availability of PARP inhibition data in both PBLs and tumor issues from study A4991002 provided an opportunity to evaluate this relationship between PARP inhibition in PBLs and tumor tissues. Although it was not possible to compare the absolute values of PARP activity between the two matrices as they were expressed in different units (see Methods), the percent PARP inhibition from baseline can be compared. Figure 2C shows the comparison plot of percent PARP inhibition from baseline in PBLs and tumor following 12 mg/m² and 18 mg/m² daily dosing of rucaparib. There was no statistically significant difference in PARP inhibition achieved in PBLs and in tumor issues (P-values > 0.05 at both dose levels). In addition, similar PD parameters were derived from PARP inhibition data in PBLs and in tumor issues by conducting PK/PD modeling. The \( t_{\text{max}} \) values were 90.9% and 90.0% of the baseline PARP activity, and the IC\(_{50}\) was 1.05 ng/mL and 1.10 ng/mL, for PARP inhibition in PBLs and in tumors respectively. Comparable PARP inhibition in PBL and tumor tissue can also be observed in Figure 2A. Similar \( t_{\text{max}} \) and IC\(_{50}\) values in PBL and tumor may in turn suggest rapid drug distribution equilibrium between these two sites, consistent with the population PK finding of rapid distribution equilibrium between central and peripheral compartments as discussed in the previous section.

**The Relationship Between PARP Inhibition and Efficacy**

The PARP inhibition and efficacy data from an olaparib Phase 2 clinical trial demonstrated a dissociation between the achievement of maximum PARP inhibition in PBMCs (PARP inhibition plateaued at 100 mg dose) and measurements of optimal anti-tumor response by objective response rate, median progression free survival, and objective radiologic response or 50% decline in CA125 (achieving at 400 mg).9 As this observed dissociation may be compound and tumor specific, further investigation is needed to determine the relationship between the level of PARP inhibition and antitumor activity for individual PARP inhibitory compound so that the concept of OBD and MTD can be appropriately used to select the optimal anti-tumor dose.

**Conclusions**

1. A population PK model has been established for rucaparib. This model can be used to predict rucaparib plasma concentration profile following any dosing regimen.
2. A predictive population PK/PD model based on PARP inhibition has been established for rucaparib. This model has provided insight into optimizing dose selection in early-phase clinical trials during the development of rucaparib.
3. The extent of PARP inhibition by rucaparib in PBLs 4–6 hours post dosing is similar to PARP inhibition in melanoma tumor tissue at the same time points. The blood assay may serve as substitute for tumor PARP activity when PARP inhibition is saturated in the PBLs.

**References**


**Supporting Information**

Additional supporting information may be found in the online version of this article at the publisher’s web-site.