

**PHARMACOKINETICS OF THE COMBINATION OF VELIPARIB AND  
TEMOZOLOMIDE IN LEUKEMIA**

by

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Ismail Ali Walbi, M.S.

University of Pittsburgh, [2014]

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**ABSTRACT:**

As part of a phase I clinical study, we assessed the pharmacokinetic properties of the combination of veliparib and temozolomide in patients with acute leukemias. Temozolomide is an oral alkylating agent that has activity in patients with acute leukemia. Veliparib is an oral Poly ADP Ribose Polymerase (PARP1 and PARP2) inhibitor that inhibits the base excision repair (BER) system, which results in increased temozolomide tumor toxicity and apoptosis.

**Methods:** Plasma samples of 22 patients were collected at the University of Maryland Greenebaum Cancer Center. Adults 18 years and older with relapsed or refractory acute myeloid leukemia (AML) or pre-B- or T-cell acute lymphocytic leukemia (ALL) were enrolled in this study. The initial starting dose of temozolomide was 150 mg/m<sup>2</sup> administered for 7 days in combination with veliparib at 20 mg twice a day. Dose escalation followed the standard 3+3 design. The temozolomide dose would be escalated to 200 mg/m<sup>2</sup>. Subsequently, only veliparib would be escalated to 40, 80, 120, and 150 mg twice daily. Veliparib administration was continued for three additional days following the last temozolomide administration. Plasma samples were collected to evaluate the PK of temozolomide alone, veliparib alone and the combination of veliparib and temozolomide. Temozolomide was quantified by HPLC-UV and veliparib by LC-MS.

**Results:** Veliparib decreased temozolomide peak concentration ( $C_{max}$ ) by 16% ( $P=0.015$ ) and increased its apparent volume of distribution ( $Vd/F$ ) by 17% ( $P=0.017$ ). On the other hand, temozolomide increased veliparib  $C_{max}$  by 33% ( $P=0.0002$ ) and decreased its  $Cl/F$  by 26% ( $P=0.001$ ). Veliparib exposure appears to be mostly linear with dose. Temozolomide clearance and volume of distribution in individual patients were not well-predicted by a published

population pharmacokinetics model (%RMSE=96%, 110.5%), but on average were similar (%MPE = 19%, 21.7%), respectively.

**Conclusion:** Veliparib has a statistically significant effect on temozolomide  $C_{\max}$  and Cl/F, and temozolomide has a statistically significant effect on veliparib  $C_{\max}$  and Cl/F. The clinical relevance of these effects is likely small.

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## 1.0 INTRODUCTION

### 1.1 Background:

Leukemia is a type of cancer that originates from bone marrow stem cells. In leukemic patients, stem cells produce abnormal white blood cells (leukemia cells), which do not die as normal cells. Leukemia can be classified into four types: chronic lymphocytic leukemia (CLL), chronic myeloid leukemia (CML), acute lymphocytic leukemia (ALL), and acute myeloid leukemia (AML).[1]

Almost 90% of leukemia cases occur in adults 20 years and older. The most common types of leukemia in this population are CLL and AML. In children and teens, ALL is the most common leukemia accounting for approximately 75% of cases.[1] From 2005 to 2009, the leukemia incidence rate increased by 0.4% per year and leukemia death rates declined by 0.8% in men and 1.4% in women. Total leukemia estimated deaths were 23,720 cases (Table 1).

Current leukemia therapy includes different chemotherapy regimens. For acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL), treatment starts with an induction followed by a consolidation stage. For AML, a widely used regimen for induction is cytarabine (ARA C) 100 to 200 mg/m<sup>2</sup> with daunorubicin (an anthracycline) 45 to 80 mg/m<sup>2</sup>/d. The combination is given as 3 days of daunorubicin and 7 days of cytarabine (3 + 7 induction regimen). Other drugs used in AML include etoposide, mitoxantrone, and fludarabine. After

induction therapy is done, consolidation therapy starts to prevent relapse in patients who are in complete remission with the same drugs used in induction therapy [2, 3]. A major cause for treatment failure in this disease is resistance to cytarabine and daunorubicin [4].

**Table 1.** 2013 estimated new leukemia cases and deaths in the United States

	Estimated new leukemia (%)	Estimated deaths (%)
Acute lymphocytic leukemia (ALL)	6,070 (12.5%)	1,430 (6%)
Chronic lymphocytic leukemia (CLL)	15,680 (32.3%)	4,580 (19.3%)
Acute myeloid leukemia (AML)	14,590 (30%)	10,370 (43.7%)
Chronic myeloid leukemia (CML)	5,920 (12.2%)	610 (2.6%)
Others	6,350 (13.1%)	6,730 (28.4%)
Total	48,610 (100%)	23,720 (100%)

Adapted from Siegel et al *Cancer statistics, 2013*

For ALL, induction therapy is done using vincristine, anthracycline, asparaginase, and a glucocorticoid. A tyrosine kinase inhibitor (imatinib) enhances the induction therapy of leukemia with the BCR-ABL fusion gene. In the consolidation stage, a commonly used regimen includes high dose methotrexate, mercaptopurine, asparaginase, and reinduction treatment [5].

Temozolomide is a 100 % bioavailable oral alkylating agent which is non-enzymatically converted at physiologic pH to its active product 5-(3-methyl)1-trizen-1-yl-imidazole-4-carboxamide (MTIC). MTIC, which can also be metabolically derived from dacarbazine, alkylates DNA by methylating the N<sup>7</sup> position of guanine (about 70%), the O<sup>6</sup> position of guanine (about 5%), and the N<sup>3</sup> position of adenine (about 9%)[6]. This methylation causes

single strand DNA breaks leading to growth arrest and apoptosis. The repair is mainly performed by base excision repair (BER) for N<sup>7</sup> guanine and N<sup>3</sup> adenine, and methyl-guanine methyl-transferase (MGMT) for O<sup>6</sup> guanine. DNA O<sup>6</sup> guanine mismatch repair (MMR) is required for the cytotoxic effect of the O<sup>6</sup> guanine lesion [7]. Temozolomide is approved for the treatment of brain tumors and it is also used in metastatic melanoma [8]. Significant activity of single agent temozolomide has been reported in patients with acute leukemia [9]. A recent study showed that temozolomide tumor toxicity was potentiated in mismatch repair deficient leukemia cells with low MGMT activity when a Poly ADP Ribose Polymerase inhibitor (PARPI) is used [10].

Poly ADP Ribose Polymerase (PARP) is a nuclear enzyme that is expressed in all cells. It is part of the base excision repair (BER) system. It recognizes DNA damage and facilitates its repair [11]. PARP adds ADP ribose units to DNA, histone and different DNA repair enzymes which affects many cellular processes[12]. PARP activity and expression is increased in human tumor cells when anticancer drugs are used, and is linked to chemotherapy resistance and prevention of apoptosis. Veliparib is an oral PARP-1 and PARP-2 inhibitor.

Veliparib inhibits the BER pathway and prevents removal of N<sup>3</sup>- and N<sup>7</sup> methyl adducts. This inhibition results in increased temozolomide tumor toxicity and apoptosis independent of MGMT or MMR status [10]. This combination is unique because it has a different molecular therapeutic approach than current methods. A phase I clinical trial was conducted to evaluate the feasibility, safety, and toxicity of administering veliparib in combination with temozolomide for the treatment of acute myeloid leukemia.

## **1.2 Purpose of the study:**

This study aims to evaluate the pharmacokinetic properties for the combination of veliparib and temozolomide. The first aim of this study is to evaluate alteration in both veliparib and temozolomide pharmacokinetics during co-administration. The second aim is to evaluate the dose-linearity of veliparib. The third aim is to compare temozolomide pharmacokinetics with historical controls.

## **2.0 METHODS:**

### **2.1 Patient selection:**

Samples were collected from 22 patients from the University of Maryland Marlene and Stewart Greenebaum Cancer Center. Patient enrolled in this study had to have:

- 1) Relapsed or refractory acute myeloid leukemia (AML).
- 2) Relapsed or refractory pre-B- or T-cell acute lymphocytic leukemia (ALL).
- 3) Chronic Myeloid Leukemia (CML) in accelerated or blastic phase.
- 4) AML arising in the setting of antecedent myelodysplasia (MDS) or myeloproliferative disorder (MPD).
- 5) Therapy-related AML.
- 6) Untreated AML or ALL in adults 60 years of age and older who are not candidates for induction chemotherapy due to poor-risk features.

Patients should be 18 years old or older because there is not enough data about using this combination in children. Patients should be able to swallow pills because veliparib cannot be given by G-tube. Patients should have normal hepatic and renal function. Pregnant women were excluded because veliparib teratogenicity has not been established yet.

## 2.2 Study design:

Veliparib was given orally as a single dose on day 1 to evaluate its pharmacokinetic properties. In all dose levels, a single dose of veliparib was given on day 1 to allow for pharmacokinetics study. Subsequent doses of veliparib were given orally twice daily on days 4 through day 12. Temozolomide was given orally on day 3 through day 9. Veliparib dose was continued 3 days beyond temozolomide to allow leukemic cells to enter S phase without single-strand break (SSB) repair.

**Table 2.** Dose Escalation Schedule

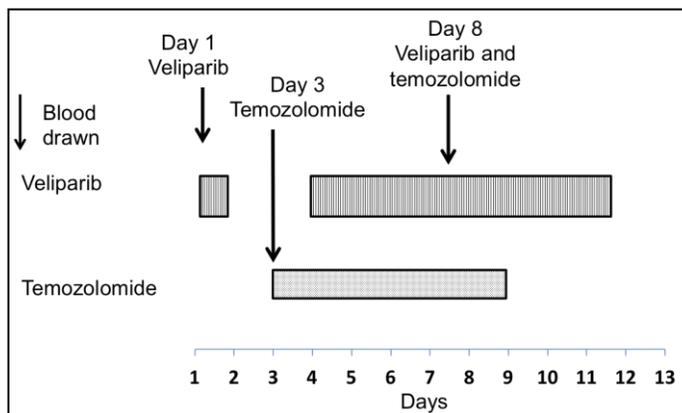
Dose Level	Dose	
	Veliparib Day 1 (half the total daily dose) Days 4 through 12	Temozolomide Days 3 through 9
1A	20 mg twice a day	150 mg/m <sup>2</sup> /day
1B	20 mg twice a day	200 mg/m <sup>2</sup> /day
2	40 mg twice a day	200 mg/m <sup>2</sup> /day
3	80 mg twice a day	200 mg/m <sup>2</sup> /day
4	120 mg twice a day	200 mg/m <sup>2</sup> /day
5	150 mg twice a day	200 mg/m <sup>2</sup> /day

Doses were escalated according to Table 2 based on the presence of dose limiting toxicities (DLT). Toxicity was evaluated based on Version 4.0 of the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE). Dose-limiting toxicity was

defined as any grade  $\geq 4$  drug-related non-hematologic toxicity, and any grade  $\geq 3$  drug-related non-hematologic toxicity that did not resolve to grade 2 within 48 hours with exception for infection, fever, febrile neutropenia, bleeding that are expected in this patient population. Also, any grade  $\geq 3$  neurotoxicity or grade  $\geq 3$  nephrotoxicity of any duration was considered a dose limiting toxicity.

If there was no DLT in three patients, the dose was escalated to the next dose level. If one out of 3 patients experienced DLT, then an additional three patients were added to the cohort. If no more patients experienced DLT, the dose was escalated to the next dose level. If more than one out of six experienced DLT, dose escalation was stopped and the dose level below this dose was expanded for consideration as the maximum tolerated dose.

Plasma samples were collected on day 1, 3, and 8 before dosing and at 0.25, 0.5, 1, 1.5, 2, 4, 6, 8, and 24 hours. Plasma samples taken on day 1 were used to evaluate the pharmacokinetic properties of veliparib when it was used alone. Plasma samples taken on day 3, after the first temozolomide dose, were used to evaluate the pharmacokinetic properties for temozolomide alone. The pharmacokinetic properties for both drugs when they were in combination were assessed by measuring their plasma concentrations from samples taken on day 8 (Figure 1).



**Figure 1.** Veliparib and temozolomide dose regimen and drawn blood samples

### **2.3 Temozolomide HPLC-UV method:**

For temozolomide quantitation in plasma, we used an HPLC-UV assay described by Kim et al. and modified in our laboratory[13]. Mobile phase used was 0.1% aqueous acetic acid-acetonitrile (90:10, v/v). A 100  $\mu$ L aliquot of plasma was placed into a 1.5 mL microcentrifuge tube containing 100  $\mu$ L internal standard aqueous ethazolastone solution. 10  $\mu$ L of 1 N HCl and 1 mL of ethyl acetate were added to each tube. Samples were vortexed for 10 minutes and then centrifuged for 5 minutes at 4500 x g at room temperature. Supernatants were transferred to 12 x 75 mm glass tubes and evaporated to dryness under a gentle stream of nitrogen. The dried residues were re-suspended in 100  $\mu$ L of mobile phase and sonicated for 2 minutes in a water bath. Finally, 20  $\mu$ L of each sample were injected into HPLC system.

### **2.4 Veliparib LC-MS method:**

For veliparib quantitation in plasma, we used an LC-MS assay developed and validated in our laboratory [14]. A gradient mobile phase was used in this assay. 0.1% (v/v) formic acid in acetonitrile was used as mobile phase solvent A, and 0.1% (v/v) formic acid in water was used as mobile phase solvent B (table 3). An aliquot of 200  $\mu$ L of plasma was placed into a 1.5 mL microcentrifuge tube. 10  $\mu$ L of internal standard, [D<sub>3</sub>]-veliparib in acetonitrile-water, and 1 mL ethyl acetate were added and mixed vigorously for 1 minute on a vortex genie set at 8. Then, the tube was centrifuged at 12,000 x g for 5 minutes at room temperature. After that, the resulting supernatants were transferred to 12 x 75 mm glass tube and evaporated to dryness under nitrogen. Dried residues were resuspended in 100  $\mu$ L mobile phase and sonicated for 2 minutes

at room temperature. Finally, it was transferred to HPLC vials and 20  $\mu\text{L}$  were injected into the LC-MS system.[14]

**Table 3.** Gradient mobile phase for veliparib assay

Time (min)	Flow rate (mL/min)	0.1% (v/v) formic acid in acetonitrile (A%)	0.1% (v/v) formic acid in water (B%)
0	0.2	2	98
10	0.2	30	70
11	0.2	80	20
14	0.3	80	20
15	0.3	2	98
25	0.3	2	98

## 2.5 Pharmacokinetic analysis:

The area under the curve (AUC) and the half-life of plasma temozolomide and veliparib concentrations were estimated by using non-compartment analysis with PK solution 2.0 (Summit Research Services). The time to reach the maximum concentration ( $T_{\max}$ ) and the maximum plasma concentration ( $C_{\max}$ ) were determined visually. We calculated the theoretical veliparib accumulation effect on day 8, based on its half-life and dosing interval using the following equation:

$$R = \frac{1}{1 - e^{-\frac{\ln 2}{t_{1/2}} \cdot \tau}} \quad (1)$$

Where R is drug accumulation index,  $\tau$  is dose interval, and  $t_{1/2}$  is half-life [15].

We used a population pharmacokinetic (POP-PK) method developed by *Osterman et al* to predict individual pharmacokinetic parameters of temozolomide clearance (Cl) and volume of distribution (Vd)[16]. Temozolomide clearance and volume of distribution are given by:

$$Cl = 10(1 + 0.2 * sex) + 4.2 * BSA \quad (2)$$

$$Vd = 30.3 + 19.9 * BSA \quad (3)$$

The predictive performance was evaluated by computation of the mean prediction error (MPE) (equation 3), which represents the bias, and the root mean square error (RMSE) (equation 4), which represents the precision.

$$MPE = \frac{\sum_{i=1}^N (PE_i)}{N} \quad (4)$$

$$RMSE = \sqrt{\frac{\sum_{i=1}^N (PE_i^2)}{N}} \quad (5)$$

Where N is the number of pairs of estimated and reference parameters (Cl and Vd), and  $PE_i$  is the relative prediction error for each pair difference between the estimated and reference value in natural logarithmic form [17].

## **2.6 Statistical analysis:**

We used the Wilcoxon signed ranks test to compare pharmacokinetic parameters when drugs were given alone and when they were combined. The dose linearity of veliparib was determined by linear regression of dose normalized peak concentration ( $C_{\max}/\text{dose}$ ) and apparent clearance ( $Cl/F$ ) versus dose using SPSS (version 21). A P-value less than 0.05 were considered statistically significant.

### 3.0 RESULTS

Intense sampling plasma samples from 22 patients were analyzed to assess pharmacokinetic properties of temozolomide and veliparib when used alone or in combination. Temozolomide dose was escalated from 150 mg/m<sup>2</sup>/day to 200 mg/m<sup>2</sup>/day and then it was fixed for the following dose levels. Veliparib dose was escalated from 20 mg to 40, 80, 120, and 150 mg twice daily. The number of patients and their sex in each dose level are listed in Table 4.

**Table 4.** Patient characteristics

	<b>Dose level</b>					
	<b>1A</b>	<b>1B</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>
<b>Number of patients</b>	2	2	1	2	10	5
<b>Sex (men/ women)</b>	1/1	1/1	0/1	1/1	7/3	2/3

On day 1, 22 patients had complete temozolomide pharmacokinetic profile. On day 3, one patient profile was excluded due to a negative elimination half-life. On day 8, 7 patient profiles were excluded for both drugs due to missing data points.

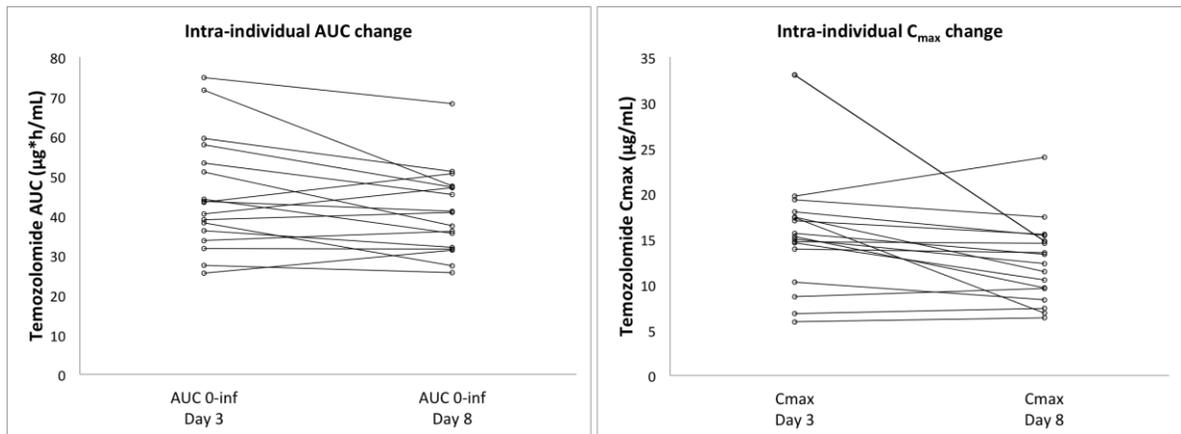
The retention time for temozolomide and its internal standard was 2.7 min and 5 min, respectively. The calibration curve was linear over the concentration range of 0.1 to 20 µg/mL. Duplicate standard curves (0.1, 0.2, 0.5, 1, 2, 5, 10, and 20 µg/mL) on three different days were used to calculate the coefficient of variation (CV%) and the accuracy. The coefficient of variation range was between 3.3 and 8.4% and the accuracy was between -3.1 and 3.7%. Precision and accuracy were also evaluated with two quality control concentrations (QCs) for each level (0.2, 3, and 15 µg/mL) on three different days. Precision and accuracy were ≤ 8.2, and -5.52%, respectively.

The retention time for veliparib and its internal standard was around 8 min. The calibration curve was linear over the concentration of 10 to 1,000 ng/mL. Duplicate standard curves (10, 30, 100, 300, 500, 750, and 1000 ng/mL) on three different days were used to calculate precision (CV%) and accuracy. The coefficient of variation range was between 2.0 and 7.3%. The accuracy ranged between -1.5 and 2.4%. Precision and accuracy were also evaluated with two QCs for each level (20, 200, 800 ng/mL) on three different days and they were ≤ 13.3, and 4.8%, respectively.

Temozolomide was taken starting day 3 through day 9. Plasma samples were collected on day 3 and day 8 to assess temozolomide pharmacokinetic alone and when combined with veliparib, respectively. Temozolomide average peak concentration ( $C_{max}$ ) for the 200 mg/m<sup>2</sup> dose was 15.3 µg/mL on day 3 and 13.07 µg/mL on day 8. Elimination half-life ( $t_{1/2}$ ) of temozolomide was 1.89 hours on day 3 and 1.95 hours on day 8. Oral apparent clearance (Cl/F) was 9.14 L/h on day 3 and 9.57 L/h on day 8. Apparent volume of distribution was 25.0 L on day 3 and 28.9 on day 8. Veliparib significantly decreased temozolomide  $C_{max}$  by 16% ( $P = 0.015$ ) and increased apparent volume of distribution by 17% ( $P = 0.017$ ) (Table 5). There was extensive intra-individual temozolomide  $C_{max}$  and AUC variability between day 3 and day 8 (Figure 2).

**Table 5.** Effect of veliparib on temozolomide pharmacokinetic properties for 200 mg/ m<sup>2</sup> temozolomide dose, mean ( $\pm$ SD)

Temozolomide parameter	Day 3 temozolomide alone	Day 8 temozolomide and veliparib	Ratio Day8/ Day3	P-value
<b>C<sub>max</sub> (<math>\mu</math>g/mL)</b>	15.3 (5.43)	13.07 (4.3)	0.84 (0.23)	0.015
<b>T<sub>max</sub> (h)</b>	0.94 (0.47)	1.25 (0.96)	1.18 (0.44)	0.398
<b>t<sub>1/2</sub> (h)</b>	1.89 (0.28)	1.95 (0.44)	1.06 (0.17)	0.636
<b>Cl/F (L/h)</b>	9.14 (2.68)	9.57 (2.3)	1.11 (0.21)	0.085
<b>Vd/F (L)</b>	25.0 (8.3)	28.9 (12.0)	1.17 (0.33)	0.017



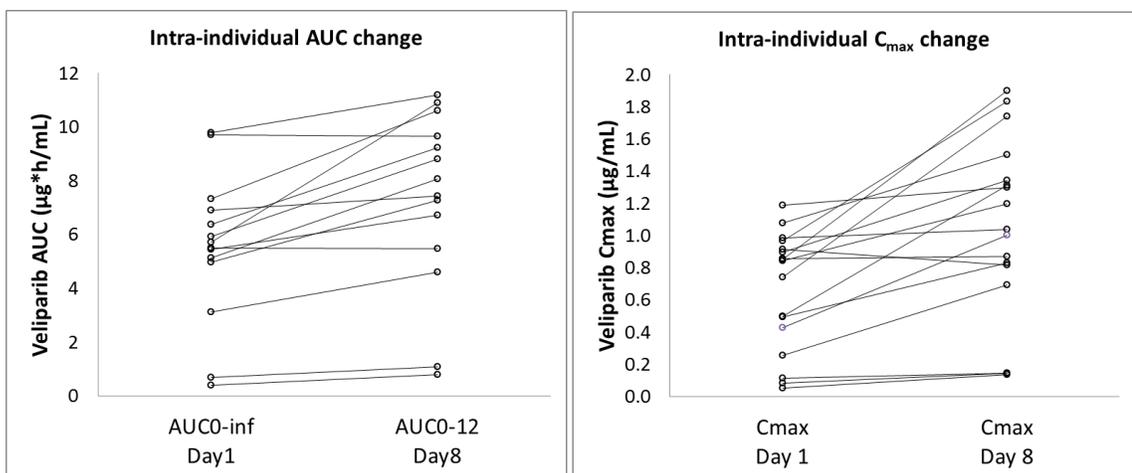
**Figure 2.** Temozolomide intra-individual variability area under the curve ( $AUC_{0-\infty}$ ) and peak plasma concentration ( $C_{max}$ ) between day 3 and day 8

**Table 6.** Effect of temozolomide on veliparib pharmacokinetic properties, mean ( $\pm$ SD)

<b>Veliparib parameter</b>	<b>Day 1 veliparib alone</b>	<b>Day 8 temozolomide and veliparib</b>	<b>Ratio Day8/ Day1</b>	<b>Corrected Ratio</b>	<b>P-value</b>
<b>C<sub>max</sub> (<math>\mu</math>g/mL)</b>	Varies by dose*	Varies by dose*	1.79(0.61)	1.33(0.38)	0.0002
<b>T<sub>max</sub> (h)</b>	1.98 (1.39)	2.24 (1.15)	1.55 (1.04)	-	0.517
<b>t<sub>1/2</sub> (h)</b>	7.79 (6.37)	7.72 (5.93)	1.24 (0.54)	-	0.296
<b>Cl/F (L/h)</b>	21.2 (9.5)	16.4 (4.7)	0.74 (0.16)	-	0.001
<b>Vd/F (L)</b>	205 (98)	175 (12)	0.91 (0.47)	-	0.217

\* See appendix for detailed parameter listings.

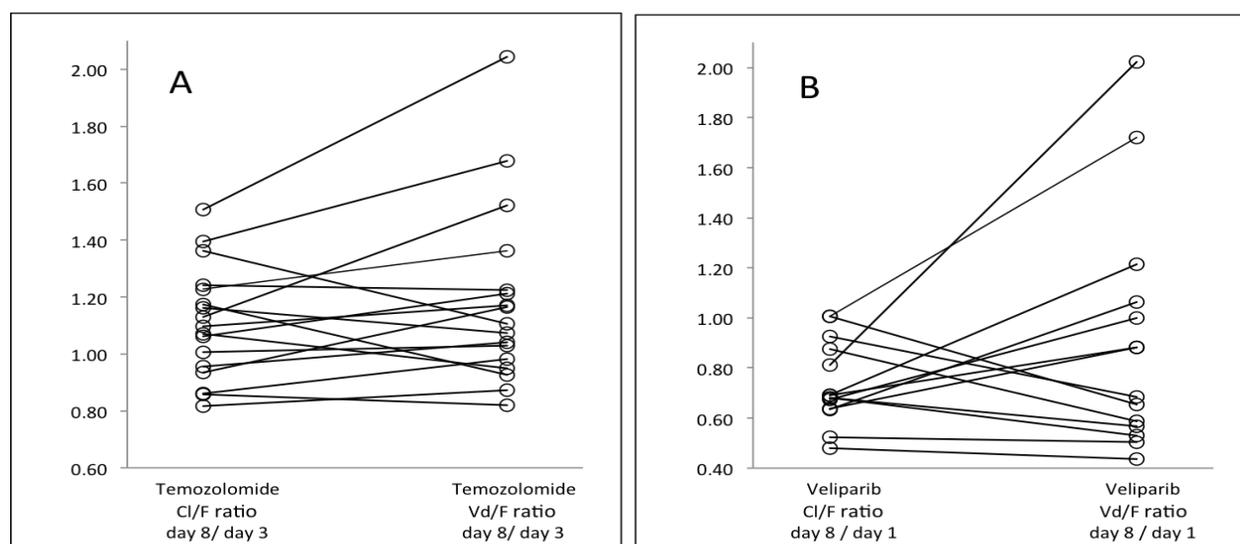
Veliparib was taken on day 1 as a single dose then starting from day 4 it was combined with temozolomide and continued for 8 days. Plasma samples were collected on day 1 and on day 8 to assess veliparib pharmacokinetics alone and when combined with temozolomide, respectively. On day 8, veliparib would have reached its steady state concentration. Veliparib C<sub>max</sub> on day 1 and day 8 varied by dose level, with the veliparib dose ranging from 20 mg to 150 mg twice daily. Average elimination half-life (t<sub>1/2</sub>) of veliparib was 7.79 hours on day 1 and 7.72 hours on day 8. Oral apparent clearance (Cl/F) was 21.2 L/h on day 1 and 16.4 L/h in day 8. Apparent volume of distribution was 205 L on day 1 and 174 on day 8. Temozolomide significantly increased veliparib C<sub>max</sub> by 33% (P = 0.0002) and decreased apparent clearance by 26% (P = 0.001) (Table 6). There was an extensive intra-individual variation for veliparib C<sub>max</sub> and AUC between day 1 and day 8. (Figure 3).



**Figure 3.** Veliparib intra-individual variability area under the curve (AUC) and peak plasma concentration ( $C_{max}$ ) between day 1 and day 8

Change in bioavailability (F) can be the reason for the observed changes in  $CL/F$  and  $Vd/F$ . We tested for concordance in the changes in  $CL/F$  and  $Vd/F$  within patients to better characterize whether these changes were caused by the impact on F. We calculated and plotted temozolomide  $CL/F$  ratio on day 8 / day 3 relative to  $Vd/F$  ratio on day 8 / day 3. Also, we calculated and plotted veliparib  $CL/F$  on day 8 / day 1 relative to  $Vd/F$  on day 8 / day 1 (figure 4). For temozolomide,  $CL/F$  and  $Vd/F$  ratios per patient moved relatively in parallel for most patients. Ratio of  $CL/F$  and  $Vd/F$ -ratio average was 0.96 with coefficient of variance (CV) 16.1%. The common factor in both parameters that we can contribute this effect to is bioavailability. Therefore, we can conclude from this result that veliparib may decrease temozolomide bioavailability which leads to changes in apparent pharmacokinetics parameters such as  $CL/F$  and  $Vd/F$ .

For veliparib, these effects were more variable. Even though the average ratio of  $CL/F$  and  $Vd/F$  ratio was 0.95, the coefficient of variance was 39.8%, which is less supportive of an effect through bioavailability for veliparib.

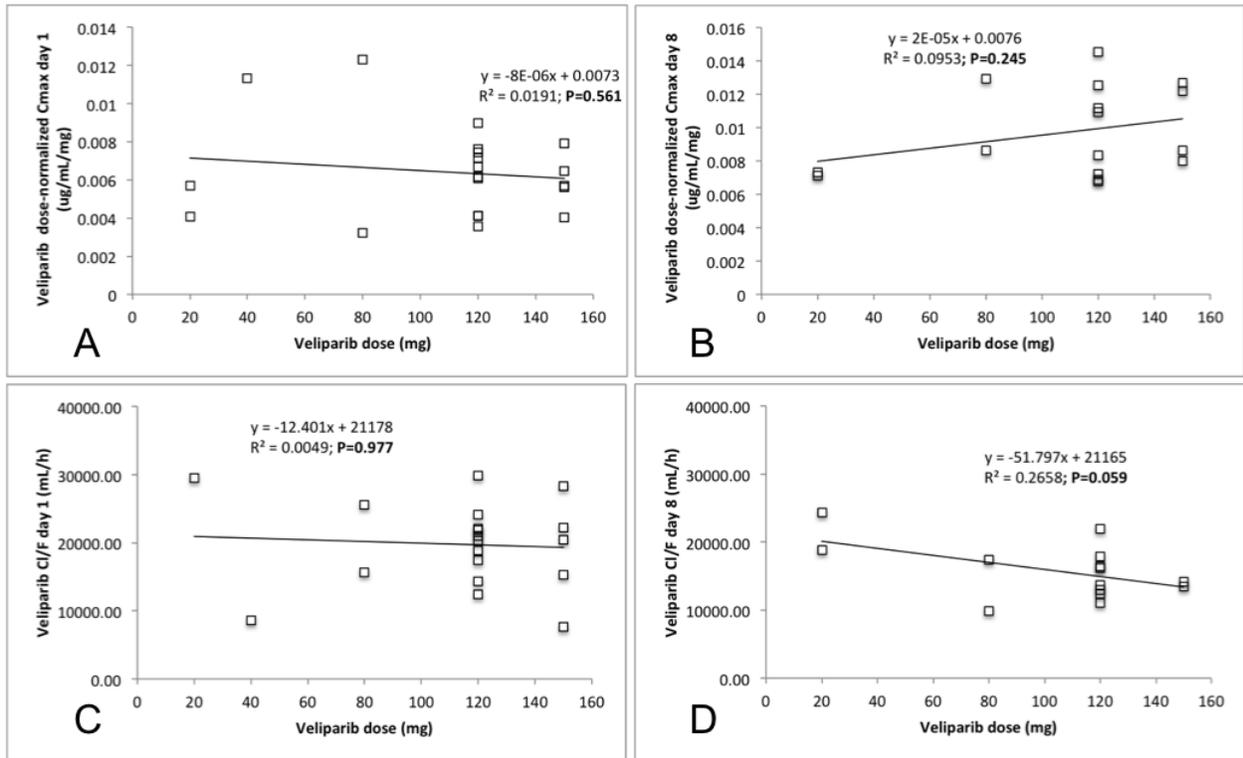


**Figure 4:** Cl/F ratio relative to Vd/F ratio for temozolomide (A), and veliparib (B)

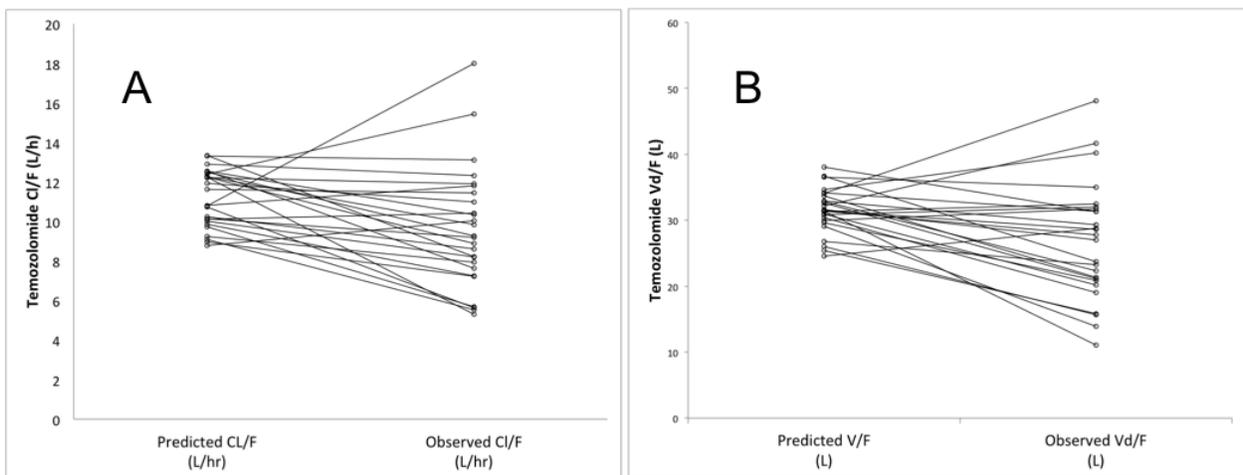
Veliparib linearity was assessed using linear regression. A linear correlation was assessed by plotting veliparib dose-normalized peak concentration and clearance vs. its dose level on day 1 and day 8. We excluded data from patients that received  $150 \text{ mg/m}^2$  temozolomide to eliminate any possible variation that could be caused by temozolomide on veliparib  $C_{\text{max}}$  or Cl/F. The slope of the dose-normalized  $C_{\text{max}}$  and Cl/F vs. dose was not statistically significant different from zero which is the expected slope for dose-normalized data under the assumption of linear pharmacokinetics ( $P = 0.561$ , and  $0.245$  for  $C_{\text{max}}$  on day 1 and 8, respectively), and ( $0.977$ , and  $0.059$  for Cl/F on day 1 and 8, respectively) (figure 5).

We used the algorithm developed by Osterman *et al.* to calculate the individual temozolomide clearance and volume of distribution values based on patient sex and body surface area (BSA). Then, we compared these values with our experimental results. The mean prediction errors (MPE) were 18.8%, 21.7% and root mean square errors (RMSE) were 95.7%, 110.5% for apparent clearance and apparent volume of distribution, respectively. Temozolomide apparent clearance and apparent volume of distribution relative to POP- PK prediction from literature are

shown in Figure 6.



**Figure 5.** Scatterplots representing veliparib PK parameters as a function of dose: day 1 dose-normalized  $C_{max}$  (A), day 8 dose-normalized  $C_{max}$  (B), day 1 clearance (C), day 8 clearance (D)



**Figure 6.** Temozolomide predicted relative to observed apparent clearance (A), and apparent volume of distribution (B)

## 4.0 DISCUSSION

Within a phase I clinical study, we assessed the pharmacokinetic properties for the combination of veliparib and temozolomide. Veliparib has a statistically significant but small effect on temozolomide  $C_{\max}$  and  $Vd/F$ . In our study, the average temozolomide peak plasma concentration was decreased by 16% when veliparib was added. However, this effect has a minor effect on temozolomide  $AUC_{0-\infty}$  with a 7% decrease on day 8 compared to day 3 ( $P=0.037$ ). It was reported that temozolomide  $C_{\max}$  is decreased by food through a 9% decrease in the extent of absorption [18]. In this context, the decrease in the extent of absorption we observed was very similar. Temozolomide volume of distribution was significantly increased by 17% ( $P= 0.017$ ) when veliparib was added. It is unlikely that the slight reduction in AUC will have any clinical significance.

Veliparib  $C_{\max}$  was significantly increased by 79 % on day 8. However, this increase was inflated by the expected veliparib accumulation effect at the dosing schedule used. After we corrected for the veliparib accumulation effect on day 8,  $C_{\max}$  on day 8 was statistically significantly increased by 33% compared to day 1 ( $P=0.0002$ ). Also, veliparib apparent clearance was statistically significant decreased by 26% on day 8. We believe that this increase in exposure will not have a critical clinical effect because veliparib is given up to 500 mg twice-daily dose, which is more than double the maximum dose in this study.

*In vitro* experiments which used recombinant human cytochrome P450 enzymes identified that the major cytochrome P450 veliparib metabolizing enzyme is CYP2D6 with minor contributions from CYP1A2, CYP2C19, and CYP3A4 [19]. Temozolomide is converted non-enzymatically at physiological pH to its active product MTIC, which is further degraded to 5-amino-imidazole-4-carbox-amide (AIC), and a highly reactive methyldiazonium ion. Temozolomide and its product are finally excreted by the kidney [20]. There is no enzymatic involvement in the conversion of temozolomide to MTIC. Therefore, pharmacologic explanations that involve competitive inhibition for metabolic pathways are unlikely to be the reason for veliparib increased apparent clearance.

Temozolomide Cl/F ratios on day 8 / day 3 relative to Vd/F ratios on day 8 / day 3 for within patients were relatively parallel for most patients. Veliparib may decrease temozolomide bioavailability which leads to changes in apparent pharmacokinetics parameters such as Cl/F and Vd/F. These effects were more variable with veliparib Cl/F and Vd/F ratios which is less supportive of an effect through bioavailability for veliparib.

Alterations of metabolic pathways without direct competitive inhibition have been reported with other chemotherapeutic drugs such as paclitaxel and cisplatin. Paclitaxel is hepatically metabolized by cytochrome P 450 enzymes (CYP2C8 and CYP3A) whereas cisplatin major route of elimination is renal excretion. Studies showed that the treatment sequence of cisplatin followed by paclitaxel decreases paclitaxel clearance by 25% which lead to increased paclitaxel pharmacologic exposure by 33% resulting in higher paclitaxel patient toxicity. Therefore, it is recommended to start with paclitaxel followed by cisplatin to overcome this effect [21]. It is not necessary to sequence veliparib before temozolomide because it is unlikely that veliparib will cause any patient toxicity at the doses explored in the current study.

Veliparib linearity was assessed using linear regression. A linear correlation was assessed by plotting veliparib dose-normalized peak concentration and clearance vs. its dose level on day 1 and day 8. Veliparib exposure appears to be linear with dose escalating.

We used the algorithms developed by Osterman *et al.* to predict temozolomide CL/F and Vd/F on day 3, and evaluated the results by calculating MPE and RMSE. Results showed that these algorithms did not predict individual values for CL/F and Vd/F well (%RMSE = 96%, 110.5%), but the population average values were similar (%MPE= 19%, 21.7%, respectively).

In conclusion, veliparib has a statistically significant effect on temozolomide  $C_{\max}$  and Vd/F. Temozolomide has a statistically significant effect on veliparib  $C_{\max}$  and Cl/F. These effects are likely not clinically relevant. This pharmacokinetic study showed that the combination of veliparib and temozolomide in leukemia patients has no relevant drug–drug interaction.

## APPENDIX A

### TEMOZOLOMIDE DATA

Patient number	BSA (m <sup>2</sup> )	Temozolomide Dose (mg/m <sup>2</sup> )	SEX	Veliparib Dose (mg)	t <sub>1/2</sub> day 3 (h)	t <sub>1/2</sub> day 8 (h)	C <sub>max</sub> day 3 (µg/mL)	C <sub>max</sub> day 8 (µg/mL)	T <sub>max</sub> day 3 (h)	T <sub>max</sub> day 8 (h)	AUC <sub>(0-inf)</sub> day 3 (µg*h/mL)	AUC <sub>(0-24)</sub> day 8 (µg*h/mL)	Real CI day 3 (L/h)	predicted CI day 3 (L/h)	Real CI day 8 (L/h)	Real Vd day 3 (L)	predicted Vd day 3 (L)	Real Vd day 8 (L)	Remark
1	2.4	150	M	20	1.8	1.6	10.2	8.3	1.0	1.0	27.4	25.6	13.1	13.3	14.1	35	36	33	-
2	1.94	150	F	20	2.3	2.9	5.9	6.3	4.0	2.0	33.8	36.0	8.6	10.3	8.1	29	31	33	not enough data on day 8
3	1.38	200	F	20	1.5	1.8	17.4	6.9	0.5	0.5	38.2	27.4	7.2	9.0	10.1	16	25	27	negative t1/2 on day 1
4	2.23	200	M	20	2.3	3.0	8.7	9.6	1.0	1.0	36.2	31.9	12.3	12.9	13.9	40	35	61	-
5	1.48	200	F	40	2.0	-	13.2	-	0.5	-	37.3	-	7.9	9.3	-	23	27	-	no data on day 8
6	1.89	200	M	80	1.9	1.9	6.8	7.4	1.0	1.5	31.8	31.6	11.9	12.2	12.0	32	31	33	-
7	1.69	200	F	80	1.7	1.6	19.7	24.0	1.0	0.5	59.5	51.2	5.7	9.7	6.6	14	29	15	-
8	1.9	200	M	120	1.4	2.0	33.0	14.8	1.0	1.5	71.6	47.5	5.3	12.2	8.0	11	31	23	-
9	1.9	200	M	120	2.2	1.6	13.6	-	0.5	-	42.8	-	8.9	12.2	-	28	31	45	not enough data on day 8
10	1.41	200	F	120	2.0	1.6	15.6	13.3	0.5	0.5	51.0	37.5	5.5	9.1	7.5	16	26	17	-
11	2.01	200	M	120	1.6	1.5	17.0	15.5	1.5	2.0	43.4	50.6	9.3	12.5	7.9	21	33	17	-
12	1.92	200	M	120	1.9	2.1	15.0	12.3	1.5	1.5	39.0	40.8	9.8	12.3	9.4	27	32	28	-
13	2.38	200	M	120	2.0	2.2	17.4	11.4	1.5	2.0	57.9	47.1	8.2	13.4	10.1	24	37	32	-
14	1.97	200	M	120	1.9	2.0	15.3	9.6	0.5	1.0	25.5	31.3	15.4	12.4	12.6	42	32	36	-
15	1.86	200	F	120	1.5	1.7	18.0	15.4	0.3	0.3	40.4	47.0	9.2	10.1	7.9	20	31	20	-
16	2.5	200	F	120	1.9	2.2	13.9	13.5	1.5	1.5	43.7	41.1	11.4	11.6	12.2	31	38	38	-
17	1.77	200	M	120	2.0	-	9.3	-	0.5	-	32.2	-	11.0	11.9	-	32	30	-	not enough data on day 8
18	2.15	200	F	150	1.8	-	13.4	-	1.0	-	36.4	-	11.8	10.8	-	31	34	-	no data on day 8
19	1.81	200	F	150	1.6	1.6	14.7	14.5	1.0	0.5	44.1	35.6	8.2	10.0	10.2	19	30	23	-
20	2.04	200	M	150	2.0	-	10.2	-	1.0	-	39.4	-	10.3	12.6	-	29	33	-	not enough data on day 8
21	2.11	200	F	150	2.6	2.8	14.6	10.5	2.0	4.0	74.9	68.0	5.6	10.7	6.2	21	34	25	not enough data to calculate day 8 t1/2
22	2.03	200	M	150	2.0	1.6	19.3	17.4	0.5	0.5	53.2	45.3	7.6	12.5	9.0	22	33	21	-

## APPENDIX B

### VELIPARIB DATA

Patient number	BSA (m <sup>2</sup> )	Temozolomide Dose (mg/m <sup>2</sup> )	SEX	Veliparib Dose (mg)	t <sub>1/2</sub> day 1 (h)	t <sub>1/2</sub> day 8 (h)	C <sub>max</sub> day 1 (µg/mL)	C <sub>max</sub> day 8 (µg/mL)	T <sub>max</sub> day 1 (h)	T <sub>max</sub> day 8 (h)	AUC <sub>(0-inf)</sub> day 1 (µg <sup>*</sup> h/mL)	AUC <sub>(0-12)</sub> day 8 (µg <sup>*</sup> h/mL)	Cl day 1 (mL/h)	Cl day 8 (mL/h)	Vd day 1 (mL)	Vd day 8 (mL)	Remark
1	2.4	150	M	20	6.3	5.7	0.05	0.14	1.5	2.0	0.4	0.8	53078	25412	478655	208350	-
2	1.94	150	F	20	5.7	-	0.12	-	4.0	-	1.2	-	16880	-	139564	-	not enough data on day 8
3	1.38	200	F	20	-	3.5	0.11	0.15	4.0	4.0	-	0.8	-	24362	-	123410	-
4	2.23	200	M	20	5.6	7.7	0.08	0.14	0.5	1.0	0.7	1.1	29483	18803	237394	209634	-
5	1.48	200	F	40	7.3	-	0.45	-	2.0	-	4.7	-	8572	-	89965	-	no data on day 8
6	1.89	200	M	80	8.9	6.9	0.26	0.69	1.5	2.0	3.1	4.6	25606	17435	327615	173414	-
7	1.69	200	F	80	3.2	5.3	0.98	1.04	1.0	2.0	5.1	8.1	15635	9928	71453	75870	-
8	1.9	200	M	120	7.1	5.3	0.86	0.87	1.0	4.0	6.9	7.4	17426	16156	179277	122534	-
9	1.9	200	M	120	4.9	-	0.80	-	0.5	-	4.0	-	29835	-	212543	-	not enough data on day 8
10	1.41	200	F	120	3.8	4.8	1.08	1.50	1.5	1.5	6.4	9.2	18817	12997	101935	89923	-
11	2.01	200	M	120	4.8	3.1	0.91	0.81	3.0	3.0	5.5	5.5	21824	21962	151669	99052	-
12	1.92	200	M	120	5.4	5.2	0.74	1.74	1.5	2.0	5.7	10.9	21047	11018	163790	82576	-
13	2.38	200	M	120	14.7	25.2	0.43	1.00	1.5	2.0	9.7	9.6	12375	12440	263085	452179	-
14	1.97	200	M	120	4.1	6.1	0.89	1.34	2.0	1.5	5.9	8.8	20277	13640	120291	120250	-
15	1.86	200	F	120	7.2	17.9	0.50	1.31	1.0	1.5	5.4	6.7	22053	17884	228719	462720	-
16	2.5	200	F	120	6.1	5.1	0.49	0.83	6.0	4.0	5.0	7.3	24187	16494	212496	120537	-
17	1.77	200	M	120	8.5	-	0.73	-	1.0	-	8.3	-	14377	-	176138	-	not enough data on day 8
18	2.15	200	F	150	8.9	-	0.61	-	4.0	-	6.8	-	22221	-	284486	-	no data on day 8
19	1.81	200	F	150	4.4	7.8	0.97	1.83	2.0	1.0	7.3	10.6	20463	14156	130701	158709	-
20	2.04	200	M	150	33.2	-	1.19	1.30	1.0	0.5	19.7	-	7607	-	364129	-	not enough data on day 8
21	2.11	200	F	150	4.3	-	0.84	1.20	1.0	4.0	5.3	-	28326	-	176014	-	not enough data to calculate day 8 t <sub>1/2</sub>
22	2.03	200	M	150	9.3	6.2	0.85	1.90	2.0	2.0	9.8	11.2	15348	13416	205183	120153	-

## BIBLIOGRAPHY

1. Siegel, R., D. Naishadham, and A. Jemal, *Cancer statistics, 2013*. CA Cancer J Clin, 2013. **63**(1): p. 11-30.
2. Robak, T. and A. Wierzbowska, *Current and emerging therapies for acute myeloid leukemia*. Clin Ther, 2009. **31 Pt 2**: p. 2349-70.
3. Burnett, A., M. Wetzler, and B. Lowenberg, *Therapeutic advances in acute myeloid leukemia*. J Clin Oncol, 2011. **29**(5): p. 487-94.
4. Xie, C., et al., *Panobinostat enhances cytarabine and daunorubicin sensitivities in AML cells through suppressing the expression of BRCA1, CHK1, and Rad51*. PLoS One, 2013. **8**(11): p. e79106.
5. Pui, C.H. and W.E. Evans, *Treatment of acute lymphoblastic leukemia*. N Engl J Med, 2006. **354**(2): p. 166-78.
6. Newlands, E.S., et al., *Temozolomide: a review of its discovery, chemical properties, pre-clinical development and clinical trials*. Cancer Treat Rev, 1997. **23**(1): p. 35-61.
7. Marchesi, F., et al., *Triazene compounds: mechanism of action and related DNA repair systems*. Pharmacol Res, 2007. **56**(4): p. 275-87.
8. Middleton, M.R., et al., *Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma*. J Clin Oncol, 2000. **18**(1): p. 158-66.
9. Seiter, K., et al., *Phase I study of temozolomide in relapsed/refractory acute leukemia*. J Clin Oncol, 2002. **20**(15): p. 3249-53.

10. Horton, T.M., et al., *Poly(ADP-ribose) polymerase inhibitor ABT-888 potentiates the cytotoxic activity of temozolomide in leukemia cells: influence of mismatch repair status and O6-methylguanine-DNA methyltransferase activity*. Mol Cancer Ther, 2009. **8**(8): p. 2232-42.
11. Schreiber, V., et al., *Poly(ADP-ribose) polymerase-2 (PARP-2) is required for efficient base excision DNA repair in association with PARP-1 and XRCC1*. J Biol Chem, 2002. **277**(25): p. 23028-36.
12. Ratnam, K. and J.A. Low, *Current development of clinical inhibitors of poly(ADP-ribose) polymerase in oncology*. Clin Cancer Res, 2007. **13**(5): p. 1383-8.
13. Kim, H., et al., *High-performance liquid chromatographic analysis and stability of anti-tumor agent temozolomide in human plasma*. J Pharm Biomed Anal, 2001. **24**(3): p. 461-8.
14. Parise, R.A., et al., *Liquid chromatography-mass spectrometric assay for the quantitation in human plasma of ABT-888, an orally available, small molecule inhibitor of poly(ADP-ribose) polymerase*. J Chromatogr B Analyt Technol Biomed Life Sci, 2008. **872**(1-2): p. 141-7.
15. Gabrielsson, J. and D. Weiner, *Pharmacokinetic--pharmacodynamic data analysis : concepts and applications*. 2nd ed. 1997, Stockholm, Sweden: Apotekarsocieteten. 770 p.
16. Ostermann, S., et al., *Plasma and cerebrospinal fluid population pharmacokinetics of temozolomide in malignant glioma patients*. Clin Cancer Res, 2004. **10**(11): p. 3728-36.
17. van Kesteren, C., et al., *A comparison of limited sampling strategies for prediction of Ecteinascidin 743 clearance when administered as a 24-h infusion*. Cancer Chemother Pharmacol, 2001. **48**(6): p. 459-66.
18. Brada, M., et al., *Phase I dose-escalation and pharmacokinetic study of temozolomide (SCH 52365) for refractory or relapsing malignancies*. Br J Cancer, 1999. **81**(6): p. 1022-30.
19. Li, X., et al., *Disposition and drug-drug interaction potential of veliparib (ABT-888), a novel and potent inhibitor of poly(ADP-ribose) polymerase*. Drug Metab Dispos, 2011. **39**(7): p. 1161-9.

20. Zhang, J., M.F. Stevens, and T.D. Bradshaw, *Temozolomide: mechanisms of action, repair and resistance*. *Curr Mol Pharmacol*, 2012. **5**(1): p. 102-14.
21. Rowinsky, E.K., et al., *Sequences of taxol and cisplatin: a phase I and pharmacologic study*. *J Clin Oncol*, 1991. **9**(9): p. 1692-703.