Phase I Study of the Poly(ADP-Ribose) Polymerase Inhibitor, AG014699, in Combination with Temozolomide in Patients with Advanced Solid Tumors

Ruth Plummer,[1] Christopher Jones,[1] Mark Middleton,[2] Richard Wilson,[4] Jeffrey Evans,[5] Anna Olsen,[3] Nicola Curtin,[1] Alan Boddy,[1] Peter McHugh,[5] David Newell,[1] Adrian Harris,[2] Patrick Johnson,[4] Heidi Steinfeldt,[6] Raz Dewji,[7] Diane Wang,[6] Lesley Robson,[8] and Hilary Calvert[1]

Abstract

Purpose: One mechanism of tumor resistance to cytotoxic therapy is repair of damaged DNA. Poly(ADP-ribose) polymerase (PARP)-1 is a nuclear enzyme involved in base excision repair, one of the five major repair pathways. PARP inhibitors are emerging as a new class of agents that can potentiate chemotherapy and radiotherapy. The article reports safety, efficacy, pharmacokinetic, and pharmacodynamic results of the first-in-class trial of a PARP inhibitor, AG014699, combined with temozolomide in adults with advanced malignancy.

Experimental Design: Initially, patients with solid tumors received escalating doses of AG014699 with 100 mg/m^2/d temozolomide × 5 every 28 days to establish the PARP inhibitory dose (PID). Subsequently, AG014699 dose was fixed at PID and temozolomide escalated to maximum tolerated dose or 200 mg/m^2 in metastatic melanoma patients whose tumors were biopsied. AG014699 and temozolomide pharmacokinetics, PARP activity, DNA strand single-strand breaks, response, and toxicity were evaluated.

Results: Thirty-three patients were enrolled. PARP inhibition was seen at all doses; PID was 12 mg/m^2 based on 74% to 97% inhibition of peripheral blood lymphocyte PARP activity. Recommended doses were 12 mg/m^2 AG014699 and 200 mg/m^2 temozolomide. Mean tumor PARP inhibition at 5 h was 92% (range, 46-97%). No toxicity attributable to AG014699 alone was observed. AG014699 showed linear pharmacokinetics with no interaction with temozolomide. All patients treated at PID showed increases in DNA single-strand breaks and encouraging evidence of activity was seen.

Conclusions: The combination of AG014699 and temozolomide is well tolerated, pharmacodynamic assessments showing proof of principle of the mode of action of this new class of agents.

Multiple pathways contribute to the repair of damaged DNA (1). Defects in these pathways are a cause of cancer susceptibility (2, 3), but, when intact, their activity is a factor in tumor resistance to widely used DNA-damaging cancer treatments (e.g., cytotoxic drugs and ionizing radiation; ref. 4). Several novel agents are being developed which target DNA repair in an attempt to improve cancer treatment (5), including agents that may exploit tumor DNA repair defects (e.g., BRCA1 and BRCA2) by inducing "synthetic lethality" (6, 7).

Base excision repair is a complex process that repairs DNA single-strand breaks caused by endogenous reactive species and anticancer agents (8). Poly(ADP-ribose) polymerase-1 (PARP) is a key enzyme in this pathway, binding to and being activated by the DNA break, effectively acting as a molecular nick sensor (9), and recruiting additional repair factors. Preclinical evidence has shown that inhibiting PARP potentiates cytotoxics, particularly alkylating agents and topoisomerase I inhibitors, and radiotherapy (10–12). Several PARP

Translational Relevance

This phase I study has potentially wide implications within cancer medicine and also in the treatment of inflammatory and ischemic conditions. To the readership of *Clinical Cancer Research*, it is highly relevant as PARP inhibitors are emerging as novel chemopotentiating and radiopotentiating agents and also drugs that may have single-agent activity in DNA repair-defective familial cancers. This trial is the first-in-class dose-defining study of these agents using target inhibition as the primary endpoint. This hypothesis testing design has subsequently also been evaluated in the phase 0 setting with another agent in the class. Therefore, this article represents importance and interest both in view of being the first full description of biological evaluation and toxicity assessment of a new class of agents and in the development of a new paradigm for dose definition, this subject being the topic of a recent special issue of *Clinical Cancer Research* (volume 14, number 12, 2008).

inhibitors are in preclinical and early clinical development (13, 14), current clinical investigation of these agents being focused in the area of cancer treatment.

AG014699 (Fig. 1), developed by a collaboration among Newcastle University, Cancer Research UK, and Agouron Pharmaceuticals (part of Pfizer GRD), is a prodrug of AG014447, a potent inhibitor of PARP, which has been shown in preclinical models to potentiate the cytotoxicity of temozolomide and irinotecan (15).

Temozolomide is an orally bioavailable monofunctional DNA-alkylating agent licensed for the treatment of gliomas and frequently used off-label for malignant melanoma (16). The predominant DNA methylation products formed by temozolomide are N^7-methylguanine (70%), N^3-methyladenine (9%), and O^6-methylguanine (5%). O^6-methylation of guanine is the primary cytotoxic lesion causing mispairing with thymine during DNA replication (17); however, high levels of the repair protein O^6-alkylguanine alkyltransferase and deficiency in the mismatch repair system can both confer resistance in tumors (18, 19). The N^7-methylguanine and N^3-methyladenine lesions formed by temozolomide do not normally contribute significantly to its cytotoxicity because they are rapidly repaired by base excision repair.

This study was designed primarily to explore (*a*) whether a PARP inhibitory dose (PID) of AG014699 was safe and tolerable and (*b*) the dose of temozolomide that could be given in combination with the PID of AG014699. A combination study was designed based on the preclinical potentiation data discussed above. At the time of design, there were no data suggesting efficacy for the single agent. The primary endpoint for (*a*) was a pharmacodynamic measure of target inhibition and for (*b*) was a conventional toxicity endpoint. A single dose of the novel agent was given before the first combination cycle to obtain pharmacokinetic and pharmacodynamic data. Inhibition of the target enzyme was the primary endpoint of the study: translational research exploring proof of principle of mechanism of action using Comet assays for DNA damage levels and pharmacogenomic samples to explore potential difference in pharmacokinetics linked to polymorphisms of the CYP2D6 gene. Therefore, this trial is the first use of this class of agents in humans, being a pharmacodynamically driven phase I study establishing the PID of this compound and showing antitumor activity.

PARP has also been shown to play a significant role in reperfusion injury and the pathogenesis of diabetes and various neurologic conditions (20), and in other disease models, inhibition of PARP is protective against ischemic or inflammatory damage (21, 22). Therefore, the study reported in this article represents the first evaluation of a novel class of drugs with potential in the management of a wide range of human diseases including cancer, diabetes, inflammatory, and ischemic conditions.

Materials and Methods

Trial design and patient recruitment. The study was done in two parts in accordance with the Declaration of Helsinki (2000). The protocol was approved by a multicenter research ethics committee as well as by Cancer Research UK and by local site institutional review boards. All patients gave written informed consent before participation and undergoing any study-related procedures. Patients were recruited over an 18-month period between 2003 and 2005.

Inclusion criteria included histologic/cytologic proof of malignancy, WHO performance status 0 to 1, age ≥18 years, and adequate bone marrow, liver, and renal function. Patients were excluded if they had prior treatment with temozolomide, nitrosoureas, dacarbazine or mitomycin C, symptomatic brain metastases, primary brain tumors, other current malignancy at a second site, or other significant comorbidity.

AG014699 was given as a 30-min daily intravenous infusion followed by oral temozolomide for 5 consecutive days repeated every 4 weeks. Disease response was assessed according to Response Evaluation Criteria in Solid Tumors every two cycles (23). Toxicity was graded according to National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0 (2006). Dose-limiting toxicity was defined as a drug-related event occurring in the first 4-week cycle of treatment as follows: neutropenia grade 4 lasting ≥5 days, fever associated (≥38.5°C) with grade ≥3 neutropenia, thrombocytopenia-platelets ≤25 × 10^9/L, anemia grade ≥3, grade 3 or 4 nonhematologic toxicity, and drug-related death. A standard three-patient cohort dose escalation design was used, but with two separate dose escalations, parts 1 and 2.
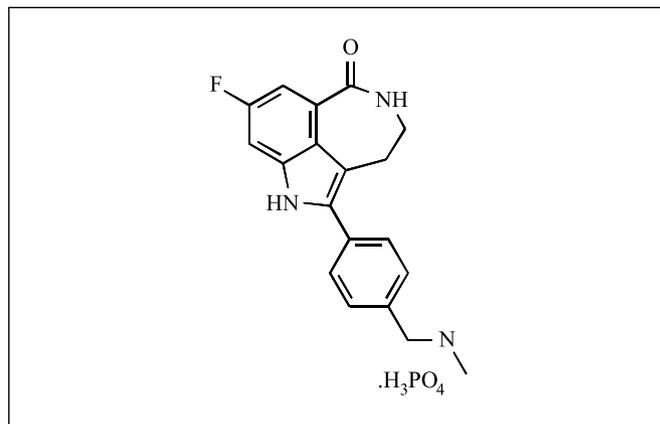
Fig. 1. Structure of AG014699, phosphate salt of tricyclic indole PARP inhibitor, with K_i ‹ 5 nmol/L.

Table 1. Patient demographics

	Part 1	Part 2
n	17	15
Male/female	13/4	8/7
Mean (range) age	56 (31-72)	48 (32-68)
Performance status 0:1:2	7:10:0	9:6:0
Tumor type	Sarcoma 3	Melanoma 15 (13 cutaneous, 1 ocular, and 1 clear cell sarcoma of soft tissue)
	Melanoma 3	
	Colorectal 3	
	Others 8	
Previous treatment	Pretreated but no DTIC/temozolomide	Chemonaive

Part 1 was open to patients of all tumor types and aimed to establish the PID of AG014699 in peripheral blood lymphocytes (PBL). Patients received a single intravenous dose of AG014699 a week before starting combination therapy (day -7) to investigate the toxicity and pharmacokinetic and pharmacodynamic profile of AG014699 alone. The temozolomide dose was fixed at 100 mg/m^2/d for this part of the study to allow for the possibility that PARP inhibition might increase the myelotoxicity of temozolomide. The PID was defined as maximal achievable (at least 50%) reduction in PARP activity 24 h after this first dose of AG014699 with no increase in the degree of PARP inhibition over the preceding AG014699 dose level.

Once the PID had been identified, patients received this dose of AG014699 and the dose of temozolomide was escalated until the maximum tolerated dose of the combination was established or the temozolomide dose reached a maximum of 200 mg/m^2 (the standard single-agent dose for this schedule). In part 2 of the study, participation was confined to patients with chemonaive melanoma with tumor deposits that were amenable to pretreatment and post-treatment biopsy.

PARP activity assay. PARP enzyme inhibition was assessed in PBL at baseline, end of infusion, and 4 to 6 and 24 h after dosing on days -7, 1, and 4 of cycle 1 during part 1 of the study to establish the PID. An additional sample on day 8 (3 days after last dose of AG014699) was taken at the PID to explore the duration of inhibition. Paired tumor biopsies were obtained from all patients in part 2 of the study to examine target tissue PARP inhibition, biopsies being taken at baseline and 4 or 24 h after treatment with AG014699. All samples were analyzed using a previously validated and published activity

assay (24) employing quantitative immunologic detection of PAR formation *ex vivo*.

AG014699 and temozolomide pharmacokinetics. Plasma samples for pharmacokinetic analysis were collected from all patients on day -7 and on days 1 and 4 of cycle 1 (combination treatment) pre-infusion, 15 min, end of infusion (T0), 15 min, 30 min and before temozolomide dose, 60 min, 2 h, 4 h, 6 h, 8 to 12 h, and 24 h post-infusion. Validated and published methods were used for the measurement of temozolomide plasma concentrations (17). Plasma samples were also analyzed for AG014447 concentrations (free base of AG014699) by high-performance liquid chromatography and tandem mass spectrometry analysis. Temozolomide and AG014699 pharmacokinetics were characterized by noncompartmental methods using WinNonlin version 3.1.

Pharmacogenomic analysis for metabolic phenotype of CYP2D6. A single 5 mL blood sample was collected at baseline in EDTA and frozen immediately at -20°C for pharmacogenomic analysis. TaqMan allelic discrimination assays were developed and validated for six of these alleles (CYP2D6 *3, *4, *6, *7, *8, and *10).

DNA strand break assessment. The method used for the alkaline Comet assay was a modified version of that described originally by Olive et al. to detect DNA strand breaks (25). An increase in Comet tail size shows an increased percentage of fragmented more mobile DNA within the cell, indicating the degree of DNA strand breaks.

Slides were stained with SYBR Gold and the percentage DNA in the tail and Olive moment was determined using KOMET5 software (Kinetic Imaging). Fifty cells from two slides were counted for each sample and the mean percentage tail DNA and Olive Tail moment was calculated.

Data analysis and compilation. Regular teleconferences were held among the four investigating sites, Cancer Research UK (study sponsor) and Pfizer GRD during the study at to discuss patient safety and study status. All data listings were made available to the investigators for preparation of this article. The patient demographics, treatment summaries, toxicities listings, and response data were extracted from this verified data set by the lead author (R.P.), an investigator at one of the clinical sites. Pharmacodynamic assays were done at the principal investigator's research site. Pharmacokinetic analyses were done by a contract research organization (Quintiles). All analyses and raw data were made available to the lead author and chief investigator for review. The first draft of this article was written by the lead author and has been reviewed and approved by all other investigators.

Results

Patient demographics and treatment. A total of 32 patients (21 males and 11 females; mean age, 52 years) were recruited and received at least one dose of AG014699: 17 patients in

Table 2. Dosing and toxicity summary

	Cohort	Dose AG014699 (mg/m^2)	Dose temozolomide (mg/m^2)	No. patients	Total no. cycles given	Dose-limiting toxicities and other grade 3/4 toxicity
Part 1	1	1	100	3	30	None
	2	2	100	4	11	None
	3	4	100	4	20	None
	4	8	100	4	5	None
	5	12	100	3	11	None
Part 2	6	12	135	3	6	None
	7	12	170	3	10	None
	8	12	200	3	16	None
	9	18	200	6	21	1/6 + 3 C2 delay after grade 3 neutropenia

Table 3. Pharmacokinetic summary of AG014699

Cohort	699 dose	Temozolomide dose		C_{max} (ng/mL)	AUC_{0-24} (ng h/mL)	AUC_{inf}* (ng h/mL)	V_d (L)	Cl* (L/h)	$t_{1/2}$ (h)
				Mean (CV%)	Mean (CV%)	Mean (CV%)	Mean (CV%)	Mean (CV%)	Mean (CV%)
1	1 mg/m^2 (n = 3)	100	day -7	25 (23)	21 (34)	24 (39)	111 [†] (34)	73 [†] (28)	1.2 [†] (55)
			day 1	27 (7)	33 (74)	38 (70)	160 [†] (78)	54 [†] (55)	3.3 [†] (119)
			day 4	27 (27)	44 (93)	— (—)	400 [†] (113)	57 [†] (55)	11.6 [†] (155)
2	2 mg/m^2 (n = 3)	100	day -7	72 (21)	137 (53)	171 (80)	218 (51)	35 (42)	6.9 (121)
			day 1	85 (15)	100 (23)	107 (14)	217 (50)	40 (3)	3.8 (17)
			day 4	74 (16)	156 (40)	— (—)	317 (71)	30 (38)	8.0 (88)
3	4 mg/m^2 (n = 3)	100	day -7	169 (61)	228 (18)	350 (42)	584 (101)	23 (32)	23.2 (123)
			day 1	134 (17)	205 (31)	254 (41)	401 (50)	32 (43)	10.6 (69)
			day 4	159 (18)	278 (24)	— (—)	403 (41)	27 (21)	10.9 (51)
4	8 mg/m^2 (n = 4)	100	day -7	456 (24)	877 (38)	1,384 (70)	281 (33)	16 (66)	19.9 (88)
			day 1	473 (33)	877 (28)	1,107 (25)	299 (33)	15 (23)	13.9 (16)
			day 4	559 (54)	1,335 (39)	— (—)	324 (26)	13 (38)	18.3 (30)
5-8	12 mg/m^2 (n = 12)	100-200	day -7	675 (71)	861 (35)	1,021 (35)	423 (50)	25 (32)	11.5 (37)
			day 1	551 (73)	893 (35)	1,178 (47)	423 (44)	24 (39)	15.0 (80)
			day 4	531 (37)	1,480 (59)	— (—)	505 (56)	21 (49)	16.8 (41)
9	18 mg/m^2 (n = 6)	200	day -7	700 (61)	1,550 (66)	1,952 (62)	410 (41)	23 (47)	13.1 (40)
			day 1	837 (58)	1,923 (74)	2,721 (82)	378 (49)	19 (57)	16.0 (46)
			day 4	863 (41)	2,882 (82)	— (—)	343 (39)	18 (50)	15.2 (37)

*AUC_{0-inf} and Cl may not be reflected accurately as the extrapolation for AUC_{0-inf} was >20% of the AUC_{0-24} for some patients.
[†] Not included in statistical analysis. This value may not be correctly estimated due to insufficient data.

part 1 and 15 in part 2 of the study (Table 1). All patients were evaluable for toxicity. Twenty-nine patients received two cycles of treatment and were evaluable for tumor response. Dose levels and the number of cycles delivered are described in Table 2.

In part 1, the dose of AG014699 was escalated through five dose levels. No dose-limiting toxicity was observed and the PID was established as 12 mg/m^2/d. In part 2 of the study, it proved possible to administer 200 mg/m^2 (the licensed dose) of temozolomide with the PID of AG014699 without dose-limiting toxicity, so the trial had reached its primary objective. One further dose level, increasing the dose of AG014699 by 50% to 18 mg/m^2/d in combination with 200 mg/m^2/d temozolomide was explored to establish whether more profound tumor PARP inhibition could be achieved. In view of the toxicities described below at this dose, 12 mg/m^2/d (the PID) AG014699 with 200 mg/m^2/d temozolomide × 5 every 28 days is recommended for future studies.

Toxicity. No toxicity of any kind attributable to AG014699 alone was observed. The combination with temozolomide was well tolerated with no toxic deaths. Myelosuppression, the dose-limiting toxicity predicted by preclinical models, was observed at the maximum dose of AG014699 evaluated (18 mg/m^2/d given with 200 mg/m^2/d temozolomide): one patient suffered grade 4 thrombocytopenia and neutropenia, with grade 3 neutropenic fever, but the patient made a full recovery by day 29. Three other patients in the cohort had cycle 2 delayed by 1, 8, and 14 days, respectively, due to grade 3 neutropenia with slow recovery of the WBC count. These 3 patients continued on treatment with a dose reduction, receiving at least two further cycles without toxicity. Three patients with tumor responses on part 1 of the trial had the dose of AG014699 increased without subsequent toxicity.

Pharmacokinetics. The pharmacokinetics of AG014447 (the free base of AG014699) is detailed in Table 3. The data are summarized giving mean values for the treated population (CV%). The drug showed linear pharmacokinetics with the C_{max} at the end of the infusion and a mean terminal half-life of 9.5 (50.4) h. The mean volume of distribution was 212 (65.9) L, indicating extensive distribution into tissues, and the mean percentage of dose recovered over 24 h in the urine after a single dose was 11%, indicating that the kidneys were not the major elimination route. Further analysis showed that $AUC_{0-24\,h}$ normalized for actual dose and $AUC_{0-24\,h}$ normalized for mg/m^2 dose did not correlate with body surface area and intersubject variability was equivalent (54% in both cases), suggesting that AG014699 can be given either as a fixed dose or based on surface area. There is no evidence that temozolomide has an effect on AG014447 pharmacokinetics either after a single dose or multiple doses.

Temozolomide pharmacokinetics was similar to those reported previously, suggesting that they were not affected by the coadministration of AG014699 (data not shown).

AG014447 was detectable in all tumor biopsy homogenates (range, 5-110 ng/g tumor protein) in samples taken 5 h (24 h in 3 patients) after the administration of the drug. Concentrations varied up to 20-fold between patients treated at the same dose level and there was no apparent correlation with the degree of PARP inhibition.

Pharmacodynamics. PARP inhibition in PBL was seen at all AG014699 dose levels studied with profound inhibition (>90%) at the end of infusion. At the lower dose levels, there was recovery of enzyme activity over 24 h; however, at the doses above 8 mg/m^2, no recovery was observed over the 24 h (Fig. 2A and B), indicating that PARP was inhibited throughout the period temozolomide exposure induces DNA strand breaks (26). Analysis of PARP inhibition on cycle 1 day 8 in patients dosed with 12 mg/m^2/d showed that enzyme activity had recovered in PBL to ~50% of baseline 72 h after the last dose of AG014699.

Based on surrogate tissue enzyme inhibition, 12 mg/m^2 was established as the PID for part 2 of the study. In part 2, paired tumor biopsies were taken in all patients and PARP inhibition of >50% was observed in all biopsies, and a trend toward AG014699 dose dependency was observed (Fig. 2C), small numbers making formal statistically comparison not feasible. Although two doses of AG014699 were investigated in part 2 (12 and 18 mg/m^2), such profound and consistent inhibition was observed in peripheral blood mononuclear cells. No correlation has been possible between the degree of inhibition in peripheral blood mononuclear cells and tumor.

Comet analysis in PBL showed evidence of DNA damage in all patients treated with AG014699 and temozolomide but not AG014699 alone. The duration of DNA damage was dependent on the dose of AG014699; at lower doses (1-8 mg/m^2), tail size was smaller at 24 h than at 4 h on day 1 (mean decrease, 8%), indicating repair of some strand breaks, whereas at PID (12-18 mg/m^2), Comet tail size was maintained or increased at the later time point (mean increase, 3%; $P = 0.03$). Additionally, there was evidence that DNA damage was retained during the treatment week with an increase, compared with baseline, in percentage DNA in the Comet tail before treatment on day 4, significantly more damage remaining at 12 mg/m^2 than for lower doses ($P = 0.002$, t test; Fig. 2D).

Pharmacogenomics. The genotype of CYP2D6 was estimated in 26 of 32 patients. In 22 patients, the homozygous wild-type for both of the CYP2D6 alleles or a heterozygous genotype containing at least one wild-type allele was observed. In the remaining 4 patients (patients 3, 5, 11, and 25), mutations in the CYP2D6 G1846A allele, designated as CYP2D6 *4, were observed. These patients were homozygous for the *4, *4 genotype and were predicted to be associated with poor
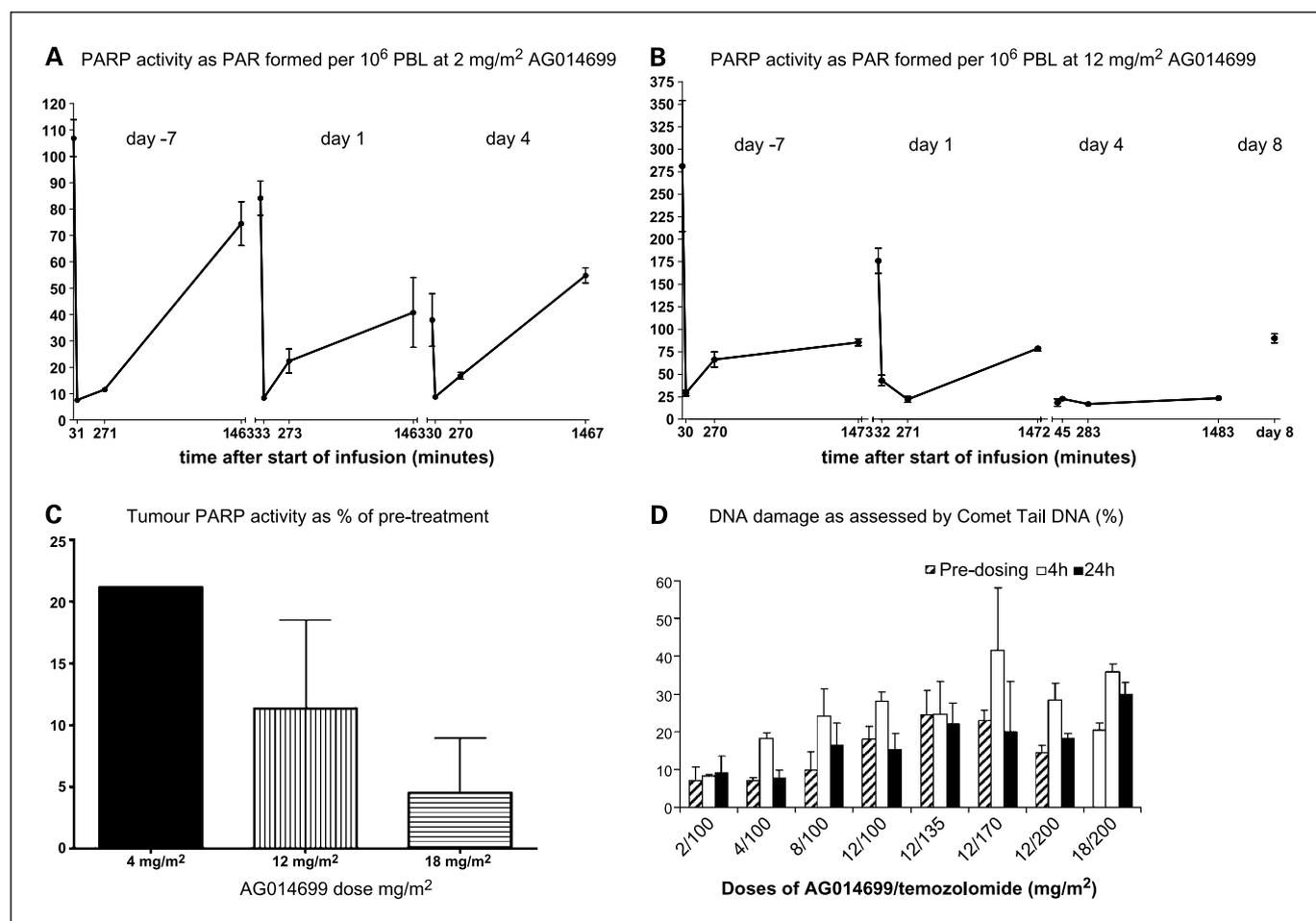
Fig. 2. Pharmacodynamic effects of AG014699. *A* to *C,* summary of PBL and tumor PARP inhibition measured using PARP activity immunoassay. *A* and *B,* representative plots from days -7, 1, and 4 of the first treatment cycle from patients treated with 2 mg/m^2 (*A*) and 12 mg/m^2 (*B*). *C,* summarized data from tumor biopsies taken 5 h after the first dose of AG014699 at the dose levels indicated. Data expressed as percentage activity compared with pretreatment biopsy in the same individual. *D,* DNA damage in peripheral blood mononuclear cells by cohort. Blood was sampled on day 4 of the first treatment cycle before (*hatched*), 4 h after (*white*), and 24 h after (*black*) temozolomide dosing. Mean of up to 6 patients.

metabolism. Three of these 4 patients were in the group who benefited from the combination, receiving 8, 8, and 16 cycles, including 2 patients with melanoma who had confirmed partial and complete responses, respectively. The fourth patient with the 4* genotype died after his test dose of a disease-related acute complication (bronchial obstruction). However, pharmacokinetic analysis showed that the exposure measured by AUC_{0-24h} was similar between patients with predicted extensive or poor metabolism.

Response. Clinical benefit was observed for several patients in parts 1 and 2 of the study. There was one documented complete response and one partial response in patients with metastatic melanoma (both patients had received no prior chemotherapy for melanoma) and a further partial response in a patient with a desmoid tumor (treated previously with extensive surgery and imatinib). Seven further patients had prolonged disease stabilization (≥6 months), 4 with melanoma and 1 each with prostate cancer, pancreatic cancer, and leiomyosarcoma.

Discussion

This study is the first to report the clinical and pharmacologic effects of PARP inhibition in humans, establishing a PID of AG014699 in a surrogate tissue (PBL) and confirming this inhibition in tumor deposits from melanoma. Dose definition in this phase I trial was established using a pharmacodynamic endpoint rather than more classic toxicity or pharmacokinetic variables. This endpoint of PARP inhibition was also used as the primary endpoint in the phase 0 study of ABT-888 done at the National Cancer Institute (27). The PID was established in combination with half-dose temozolomide due to safety concerns based on preclinical studies and previous experience of inhibiting DNA repair with MGMT inactivators, where enhancement of myelotoxicity and a significant reduction in the maximum tolerated dose of cytotoxic agents was reported with O^6-benzylguanine (28, 29) and lomeguatrib (30). The dose-toxicity relationship for temozolomide is steep, 200 mg/m²/d being well tolerated but 225 mg/m²/d causing significant myelosuppression (31). It would appear that enhanced temozolomide induced myelosuppression observed in this study when patients were dosed with 200 mg/m² temozolomide and 18 mg/m² AG014699, with 1 patient developing pancytopenia and 3 patients having delayed recovery of neutropenia, an unusual toxicity with single-agent temozolomide (32). There was no correlation between toxicity and pharmacokinetic variables, and all patients dosed with 18 mg/m² AG014699 showed similar peripheral blood mononuclear cells and tumor PARP inhibition patterns. This increase in toxicity is presumably due to persistence of unrepaired DNA strand breaks in bone marrow stem cells. However, the relative lack of toxicity observed in this study, and the ability to deliver an enzyme inhibitory dose of AG014699 in combination with full-dose temozolomide, is encouraging and in marked contrast to studies with MGMT inactivators (33–35). The complete absence of any symptomatic or laboratory toxicities as a result of PARP inhibition on its own is also encouraging for the future use of PARP inhibitors in indications when they are given as single agents.

There was no evidence of increased PARP inhibition between the dose levels of 12 and 18 mg/m² AG014699 in the surrogate pharmacodynamic tissue (peripheral blood mononuclear cells), whereas a trend to dose-dependent increase in inhibition was observed in tumor biopsies. This highlights some of the difficulties in using an easily accessible but surrogate tissue to establish a pharmacodynamically defined dose of an intravenous agent. PARP is overexpressed in malignant tissues (36, 37), overexpression of a target being frequently used as a rationale for anticancer drug development. However, there is little preclinical data into its role within the tumor and it is not known to what degree PARP must be inhibited within the tumor to prevent base excision repair. In preclinical experiments, xenograft PARP inhibition of 50% was observed at the most efficacious dose of AG014699 combined with temozolomide, where cures were seen in animals bearing SW620 tumors (15). Thus, our PID threshold was set at $\geq50\%$ inhibition, but of necessity in the surrogate tissue, then confirmed in paired tumor biopsies.

The strategy for chemopotentiation studied in this trial relies on there being a selective advantage of inhibiting PARP in the tumor compared with normal tissue. There is evidence that tumors express high levels of several DNA repair proteins, including PARP (24), resulting in chemoresistance (38–40). The majority of the DNA adducts caused by temozolomide (N^3-methyladenine and N^7-methylguanine) are rapidly repaired by base excision repair (41). Inhibition of PARP during temozolomide exposure prevents the repair of the strand breaks that are formed after base excision, thereby triggering apoptosis. A phase II study of AG014699 with temozolomide in metastatic melanoma has completed recruitment and preliminary results do suggest encouraging response rates (17% partial response and a further 17% patients having stable disease for ≥6 months) and progression-free survival (42).

Although this study was designed to establish the safety of using AG014699 in combination with a cytotoxic agent, there are emerging preclinical data suggesting that targeting DNA repair may allow exploitation of tumor-specific DNA repair defects. Specifically, PARP inhibitors are highly and selectively toxic to cells deficient in homologous recombination repair, which includes cells lacking BRCA1 and BRCA2 major causes of familial breast and ovarian cancer (6, 7, 43). These exciting new data have widened the potential cancer applications of this emerging new class of agents. In addition, there is a wealth of preclinical data showing a protective effect of PARP inhibition in the face of massive DNA damage after an ischemic insult (21, 44, 45), and once the clinical safety of these agents is established, they are likely to find very wide therapeutic application (20, 46).

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Ruth Plummer, Christopher Jones, Mark Middleton, et al.

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