Published in final edited form as:
Clin Cancer Res. 2008 December 1; 14(23): 7917–7923. doi:10.1158/1078-0432.CCR-08-1223.

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

[1]Northern Institute for Cancer Research, Newcastle University, UK

[2]Department of Medical Oncology, University of Oxford, UK

[3]Queen's University, Belfast, UK

[4]Beatson West of Scotland Cancer Centre, University of Glasgow, Glasgow, UK

[5]Weatherall Institute of Molecular Medicine, University of Oxford, UK

[6]Pfizer GRD, La Jolla, US

[7]Pfizer GRD, Sandwich, UK

[8]Cancer Research UK Drug Development Office, London, UK

Abstract

Purpose—One mechanism of tumor resistance to cytotoxic therapy is repair of damaged DNA. PARP-1 is a nuclear enzyme involved in base excision repair, one of the 5 major repair pathways. PARP inhibitors are emerging as a new class of agents which can potentiate chemo and radiotherapy. The paper reports safety, efficacy, pharmacokinetic and pharmacodynamic results of

Corresponding author details, Dr Ruth Plummer, Northern Institute for Cancer research, Paul O'Gorman Building, Newcastle University, Framlington Place, Newcastle upon Tyne, NE2 4HH, UK, ruth.plummer@ncl.ac.uk, tel +44 191 2464414, fax +44 191 2454301.
*These authors contributed equally to the reported research

Previous presentation of work
This work has been previously published in abstract form only and presented at the following major scientific meetings
ASCO 2005 (poster presentation)
NCI/EORTC/AACR Molecular Therapeutics Philadelphia meeting 2005 (oral presentation)
Plummer, R., M. Middleton, et al. (2005). "First in human phase I trial of the PARP inhibitor AG-014699 with temozolomide (TMZ) in patients (pts) with advanced solid tumors." Journal of Clinical Oncology 23(16): 208S-208S.
Plummer, R., M. Middleton, et al. (2005). "Final clinical, pharmacokinetic and pharmacodynamic results of the phase I study of the novel poly(ADP-ribose)polymerase (PARP) inhibitor, AG014699, in combination with temozolomide." Clinical Cancer Research 11(24): 9099S-9099S.

Statement of Clinical Relevance
This phase I study has potentially wide implications within cancer medicine and also in the treatment of inflammatory and ischaemic conditions. To the readership of Clinical Cancer Research it is highly relevant as PARP inhibitors are emerging as novel chemo- and radio-potentiating agents and also drugs which 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 the paper 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 also 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 N^{o} 12 2008)

Europe PMC Funders Author Manuscripts

the First-in-Class trial of a PARP inhibitor, AG-014699, combined with temozolomide in adults with advanced malignancy.

Experimental Design—Initially patients with solid tumors received escalating doses of AG-014699 with 100 mg/m^2 temozolomide daily x 5 q 28 to establish the PARP-inhibitory dose (PID). Subsequently AG-014699 dose was fixed at PID and temozolomide escalated to maximum tolerated dose or 200 mg/m^2 in metastatic melanoma patients whose tumours were biopsied. AG014699 and temozolomide pharmacokinetics, PARP activity, DNA strand single strand breaks (SSB), response and toxicity were evaluated.

Results—33 patients were enrolled. PARP inhibition was seen at all doses, PID was 12 mg/m^2 based on 74 -97% inhibition of PBL PARP activity. Recommended doses were AG014699 12 mg/m^2 and temozolomide 200 mg/m2. Mean tumor PARP inhibition at 5 hours was 92% (range 46 - 97%). No toxicity attributable to AG014699 alone was observed. AG014699 demonstrated linear pharmacokinetics with no interaction with temozolomide. All patients treated at PID showed increases in DNA SSB and encouraging evidence of activity was seen.

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

Keywords

DNA repair; PARP inhibitor; chemopotentiation; pharmacodynamics

Introduction

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 tumour resistance to widely used DNA damaging cancer treatments (e.g. cytotoxic drugs and ionizing radiation) (4). A number of novel agents are being developed which target DNA repair in an attempt to improve cancer treatment (5), including agents which may exploit tumour DNA repair defects (for example BRCA 1 and 2) by inducing “synthetic lethality” (6, 7).

Base excision repair (BER) 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), recruiting additional repair factors. Pre-clinical evidence has shown that inhibiting PARP potentiates cytotoxics, in particular alkylating agents and topoisomerase I inhibitors, and radiotherapy (10-12). A number of PARP inhibitors are in preclinical and early clinical development (13, 14), current clinical investigation of these agents being focussed in the area of cancer treatment.

AG014699 (figure 1), developed by a collaboration between 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 (TMZ) is an orally bioavailable mono-functional 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 TMZ 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 mis-pairing with thymine during DNA replication (17),

Europe PMC Funders Author Manuscripts

however high levels of the repair protein O^6-alkylguanine alkyltransferase (ATase) and deficiency in the mis-match repair system can both confer resistance in tumours (18, 19). The N^7-methylguanine and N^3-methyladenine lesions formed by TMZ do not normally contribute significantly to its cytotoxicity because they are rapidly repaired by BER.

This study was designed primarily to explore (i) whether a PARP inhibitory dose of AG014699 was safe and tolerable and (ii) the dose of TMZ that could be given in combination with the PARP inhibitory dose 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 (i) was a PD measure of target inhibition and for (ii) was a conventional toxicity endpoint. A single dose of the novel agent was given prior to the first combination cycle to obtain PK and PD 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 PK 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 PARP Inhibitory Dose (PID) of this compound and showing anti-tumour activity.

PARP has also been shown to play a significant role in reperfusion injury and the pathogenesis of diabetes and various neurological conditions (20) and in other disease models inhibition of PARP is protective against ischaemic or inflammatory damage (21, 22). Therefore, the study reported in this paper 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 ischaemic conditions.

Materials and Methods

Trial design and patient recruitment

The study was performed in two parts 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 gave written informed consent prior to participation and undergoing any study-related procedures. Patients were recruited over an eighteen month period between 2003-2005.

Inclusion criteria included histological/cytological proof of malignancy, WHO performance status of 0-1, age ≥18 years and adequate bone marrow, liver and renal function. Patients were excluded if they had prior treatment with TMZ, nitrosoureas, dacarbazine or mitomycin C, symptomatic brain metastases, primary brain tumours, other current malignancy at a second site, or other significant co-morbidity.

AG014699 was given as a 30 minute daily IV infusion followed by oral TMZ for 5 consecutive days repeated every 4 weeks. Disease response was assessed according to RECIST criteria every 2 cycles (23). Toxicity was graded according to National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 3.0 (2006). Dose limiting toxicity (DLT) 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 $\leq 25 \times 10^9$/l; anaemia ≥grade 3; grade 3 or 4 non-haematological toxicity; drug-related death. A standard three patient cohort dose escalation design was used, but with 2 separate dose escalations, part 1 and part 2.

Part 1 was open to patients of all tumour types and aimed to establish the PID of AG014699 in peripheral blood lymphocytes (PBLs). Patients received a single IV dose of AG014699

one week before starting combination therapy (day -7) to investigate the toxicity, PK and PD profile of AG014699 alone. The TMZ dose was fixed at 100mg/m^2/day for this part of the study to allow for the possibility that PARP inhibition might increase the myelotoxicity of TMZ. The PID was defined as maximal achievable (at least 50%) reduction in PARP activity 24h 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 TMZ was escalated until the MTD of the combination was established or the TMZ 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 pre- and post-treatment biopsy.

PARP activity assay

PARP enzyme inhibition was assessed in PBLs at baseline, end of infusion, 4-6 and 24 hours 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 tumour 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 hours after treatment with AG014699. All samples were analysed using a previously validated and published activity assay (24) employing quantitative immunological 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 Day 1 and 4 of cycle 1 (combination treatment) pre-infusion, 15 min, end of infusion (T0), 15 min, 30 min and before TMZ 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 analysed for AG014447 concentrations (free base of AG014699) by high-performance liquid chromatography (HPLC) and tandem mass spectrometry analysis. Temozolomide and AG014699 pharmacokinetics were characterized by non-compartmental 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 to detect DNA strand breaks (25). An increase in Comet tail size demonstrates 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 determined using KOMET5 software (Kinetic Imaging Ltd). 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 between 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 manuscript. The patient demographics, treatment summaries, toxicities listings and response data were extracted from this verified data set by the lead author (RP), an investigator at one of the clinical sites. Pharmacodynamic assays were performed at the principal investigator's research site. Pharmacokinetic analyses were performed by a contract research organisation (Quintiles), all analyses and raw data were made available to the lead author and chief investigator for review. The first draft of this manuscript 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 male, 11 female; mean age 52) were recruited and received at least one dose of AG014699: 17 patients in part 1 and 15 in part 2 of the study (table 1). All patients were evaluable for toxicity. Twenty-nine patients received 2 cycles of treatment and were evaluable for tumour response. Dose levels and the number of cycles delivered are described in table 2.

In part 1, the dose of AG014699 was escalated through 5 dose levels. No DLT was observed and the PID was established as 12 mg/m^2/day. In part 2 of the study it proved possible to administer 200 mg/m^2 (the licensed dose) of temozolomide with the PARP inhibitory dose of AG014699 without DLT, so the trial had reached its primary objective. One further dose level, increasing the dose of AG014699 by 50% to 18 mg/m^2/day in combination with 200 mg/m^2/day temozolomide was explored to establish whether more profound tumour PARP inhibition could be achieved. In view of the toxicities described below at this dose, AG014699 12 mg/m^2/day (the PID) with TMZ 200 mg/m^2/day x5 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 pre-clinical models, was observed at the maximum dose of AG014699 evaluated (18 mg/m^2/day given with TMZ 200 mg/m^2/day): one patient suffered grade 4 thrombocytopenia and neutropenia, with grade 3 neutropaenic 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 white cell count. These 3 patients continued on treatment with a dose reduction, receiving at least 2 further cycles without toxicity. Three patients with tumour responses on part 1 of the trial had the dose of AG014699 increased without subsequent toxicity.

Pharmacokinetics

Pharmacokinetic of AG014447 (the free base of AG014699) pharmacokinetics are detailed in table 3. The data are summarized below giving mean values for the treated population (CV%). The drug demonstrated linear pharmaocokinetics with the Cmax at the end of the infusion and a mean terminal half-life of 9.5 (50.4) hours. The mean volume of distribution was 212 (65.9) L, indicating extensive distribution into tissues, and the mean percentage of dose recovered over 24 hours in the urine after a single dose was 11%, indicating that the kidneys were not the major elimination route. Further analysis showed that $AUC_{0\text{-}24h}$

normalized for actual dose and $AUC_{0\text{-}24h}$ normalized for mg/m^2 dose did not correlate to BSA and inter-subjectsubject variability was equivalent (54% in both cases) suggesting that AG014699 can be given either as a fixed dose or based upon surface area. There is no evidence that TMZ has an effect on AG-014447 pharmacokinetics either after a single dose or multiple doses. Temozolomide PKs were similar to those previously reported, suggesting that they were not affected by the co-administration of AG014699 (data not shown).

AG014447 was detectable in all tumour biopsy homogenates (range 5-110 ng/g tumour protein) in samples taken 5 hours (24 hr 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 PBLs 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 hours; however, at the doses above 8 mg/m^2 no recovery was observed over the 24 hours (figure 2 a and b), indicating that PARP was inhibited throughout the period TMZ exposure induces DNA strand breaks (26). Analysis of PARP inhibition on cycle 1 day 8 in patients dosed with 12 mg/m^2/day demonstrated that enzyme activity had recovered in PBLs to ~50% of baseline 72 hours 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 tumour biopsies were taken in all patients and PARP inhibition of > 50% was observed in all biopsies, and a trend towards AG014699 dose-dependency was observed (figure 2c), small numbers making formal statistically comparison not feasible. Although 2 doses of AG014699 were investigated in part 2 (12 and 18 mg/m^2) such profound and consistent inhibition was observed in PBMC no correlation has been possible between the degree of inhibition in PBMC and tumour.

Comet analysis in PBLs showed evidence of DNA damage in all patients treated with AG014699 and TMZ 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 PARP inhibitory doses (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 to baseline, in percentage DNA in the Comet tail prior to treatment on Day 4, significantly more damage remaining at 12 mg/m^2 than for lower doses (t-test, p=0.002, figure 2d).

Pharmacogenomics

The genotype of CYP2D6 was estimated in 26/32 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 four 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 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 4th patient with the 4* genotype died after his test dose of a disease-related acute complication (bronchial obstruction). However, PK analysis showed that the exposure measured by $AUC_{0\text{-}24hr}$ were similar between patients with predicted extensive or poor metabolism..

Response

Clinical benefit was observed for a number of patients in parts 1 and 2 of the study. There was one documented complete 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 tumour (previously treated with extensive surgery and imatinib). Seven further patients had prolonged disease stabilisation (≥ 6 months), 4 with melanoma and one each with prostate cancer, pancreatic cancer and leiomyosarcoma.

Discussion

This study is the first to report the clinical and pharmacological effects of PARP inhibition in humans, establishing a PARP inhibitory dose of AG014699 in a surrogate tissue (PBLs) and confirming this inhibition in tumour deposits from melanoma. Dose definition in this phase I trial was established using a pharmacodynamic endpoint, rather than more classical toxicity or pharmacokinetic parameters. This endpoint of PARP inhibition was also used as the primary endpoint in the phase 0 study of ABT-888 performed at the NCI (27). The PID was established in combination with half-dose TMZ due to safety concerns based on pre-clinical studies and previous experience of inhibiting DNA repair with MGMT inactivators where enhancement of myelotoxicity and a significant reduction in the MTD of cytotoxic agents was reported with O^6benzyl guanine (28, 29) and lomeguatrib (30). The dose-toxicity relationship for TMZ is steep, 200 mg/m^2/day being well tolerated but 225 mg/m^2/day causing significant myelosuppression (31). It would appear that enhanced temozolomide induced myelosuppression observed in this study when patients were dosed with TMZ 200 mg/m^2 and AG014699 18 mg/m^2 with one patient developing pancytopaenia and 3 patients having delayed recovery of neutropaenia - an unusual toxicity with single agent TMZ (32). There was no correlation between toxicity and PK parameters, and all patients dosed with AG014699 18 mg/m2 showed similar PBMC and tumour 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 AG014699 12 and 18 mg/m^2 in the surrogate pharmacodynamic tissue (PBMC) whereas a trend to dose dependent increase in inhibition was observed in tumour 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 over-expressed in malignant tissues(36, 37), over-expression of a target being frequently used as a rational for anti-cancer drug development. However there is little pre-clinical data into its role within the tumour and it is not known to what degree PARP must be inhibited within the tumour to prevent BER. In preclinical experiments xenograft PARP inhibition of 50% was observed at the most efficacious dose of AG014699 combined with TMZ, where cures were seen in animals bearing SW620 tumours (15). Thus our PARP inhibitory dose threshold was set at ≥50% inhibition, but of necessity in the surrogate tissue, then confirmed in paired tumour biopsies.

The strategy for chemopotentiation studied in this trial relies upon there being a selective advantage of inhibiting PARP in the tumour compared to normal tissue. There is evidence that tumours express high levels of a number of DNA repair proteins, including PARP (24),

resulting in chemoresistance (38-40). The majority of the DNA adducts caused by TMZ (N^3methyladenine and N^7methylguanine) are rapidly repaired by BER (41). Inhibition of PARP during TMZ exposure prevents the repair of the strand breaks that are formed after base excision, thereby triggering apoptosis. A phase II study of AG014699 with TMZ 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 or more) 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 pre-clinical data suggesting that targeting DNA repair may allow exploitation of tumour 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 demonstrating a protective effect of PARP inhibition in the face of massive DNA damage after an ischaemic 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).

Acknowledgments

We gratefully acknowledge the help and support of the patients, research nurses and data coordinators involved in this study. Particular thanks go to Professor Alex Bürkle for the generous gift of the 10H PAR antibody used throughout the study for pharmacodynamic analysis.

Acknowledgement of research support

The work discussed in this research report or personnel involved have been generously supported by Cancer Research UK, Department of Health England and Pfizer GRD.

References

1. Hoeijmakers JH. Genone maintenance mechanisms for preventing cancer. Nature. 2001; 411:360–74.
2. Heinen CD, Schmutte C, Fishel R. DNA repair and tumorigenesis: lessons from hereditary cancer syndromes. Cancer Biol Ther. 2002; 1:477–85. [PubMed: 12496472]
3. Risinger MA, Groden J. Crosslinks and crosstalk: human cancer syndromes and DNA repair defects. Cancer Cell. 2004; 6:539–45. [PubMed: 15607958]
4. Gatti L, Zunino F. Overview of tumor cell chemoresistance mechanisms. Methods Mol Med. 2005; 111:127–48. [PubMed: 15911977]
5. Madhusudan S, Middleton MR. The emerging role of DNA repair proteins as predictive, prognostic and therapeutic targets in cancer. Cancer Treat Rev. 2005; 31:603–17. [PubMed: 16298073]
6. Bryant HE, Schultz N, Thomas HD, et al. Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. Nature. 2005; 434:913–7. [PubMed: 15829966]
7. Farmer H, McCabe N, Lord CJ, et al. Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. Nature. 2005; 434:917–21. [PubMed: 15829967]
8. Barnes DE, Lindahl T. Repair and genetic consequences of endogenous DNA base damage in mammalian cells. Annu Rev Genet. 2004; 38:445–76. [PubMed: 15568983]
9. de Murcia G, Menissier de Murcia J. Poly(ADP-ribose) polymerase: a molecular nick-sensor. Trends Biochem Sci. 1994; 19:172–6. [PubMed: 8016868]
10. Calabrese CR, Almassy R, Barton S, et al. Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. J Natl Cancer Inst. 2004; 96:56–67. [PubMed: 14709739]

11. Miknyoczki SJ, Jones-Bolin S, Pritchard S, et al. Chemopotentiation of temozolomide, irinotecan, and cisplatin activity by CEP-6800, a poly(ADP-ribose) polymerase inhibitor. Mol Cancer Ther. 2003; 2:371–82. [PubMed: 12700281]
12. Tentori L, Leonetti C, Scarsella M, et al. Systemic administration of GPI 15427, a novel poly(ADP-ribose) polymerase-1 inhibitor, increases the antitumor activity of temozolomide against intracranial melanoma, glioma, lymphoma. Clin Cancer Res. 2003; 9:5370–9. [PubMed: 14614022]
13. Curtin NJ. PARP inhibitors for cancer therapy. Expert Rev Mol Med. 2005; 7:1–20. [PubMed: 15836799]
14. Sheridan C. Genentech raises stakes on PARP inhibitors. Nature Biotechnology. 2006; 24:1179–80.
15. Thomas HD, Calabrese CR, Batey MA, et al. Preclinical selection of a novel poly (ADP-ribose) polymerase inhibitor for clinical trial. Mol Cancer Ther. 2007; 6:945–56. [PubMed: 17363489]
16. Danson SJ, Middleton MR. Temozolomide: a novel oral alkylating agent. Expert Rev Anticancer Ther. 2001; 1:13–9. [PubMed: 12113120]
17. Margison G, Koref MS, Povey A. Mechanisms of carcinogenicity/chemotherapy by O^6-methylguanine. Mutagenesis. 2002; 17:483–7. [PubMed: 12435845]
18. Friedman HS, McLendon RE, Kerby T, et al. DNA mismatch repair and O6-alkylguanine-DNA alkyltransferase analysis and response to Temodal in newly diagnosed malignant glioma. J Clin Oncol. 1998; 16:3851–7. [PubMed: 9850030]
19. D'Atri S, Tentori L, Lacal PM, et al. Involvement of the mismatch repair system in temozolomide-induced apoptosis. Molecular Pharmacology. 1998; 54:334–41. [PubMed: 9687575]
20. Beneke S, Diefenbach J, Burkle A. Poly(ADP-ribosyl)ation inhibitors: promising drug candidates for a wide variety of pathophysiologic conditions. Int J Cancer. 2004; 111:813–8. [PubMed: 15300792]
21. Szabo C. Pharmacological inhibition of poly(ADP-ribose) polymerase in cardiovascular disorders: future directions. Curr Vasc Pharmacol. 2005; 3:301–3. [PubMed: 16026326]
22. Graziani G, Battaini F, Zhang J. PARP-1 inhibition to treat cancer, ischemia, inflammation. Pharmacol Res. 2005; 52:1–4. [PubMed: 15911328]
23. Therasse P, Arbuck SG, Eisenhauer EA, et al. European Organization for Research and Treatment of Cancer; National Cancer Institute of the United States; National Cancer Institute of Canada. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst. 2000; 92:205–16. [PubMed: 10655437]
24. Plummer ER, Middleton MR, Jones C, et al. Temozolomide pharmacodynamics in patients with metastatic melanoma: DNA damage and activity of repair enzymes 6-alkylguanine alkyltransferase and poly(ADP-ribose) polymerase-1. Clinical Cancer Research. 2005; 11:3402–9. [PubMed: 15867241]
25. Olive PL, Chan APS, Cu CS. Comparison between the DNA precipitation and alkali unwinding assays for detecting DNA strand breaks and croos-links. Cancer Res. 1988; 48:6444–9. [PubMed: 3180060]
26. Lee SM, Thatcher N, Margison GP. O6-alkylguanine-DNA alkyltransferase depletion and regeneration in human peripheral lymphocytes following dacarbazine and fotemustine. Cancer Res. 1991; 51:619–23. [PubMed: 1824685]
27. Kummar S, Kinders R, Gutierrez M, et al. Inhibition of poly (ADP-ribose) polymerase (PARP) by ABT-888 in patients with advanced malignancies: Results of a phase 0 trial. Journal of Clinical Oncology. 2007; 25:3518. [PubMed: 17687156]
28. Schilsky RL, Dolan ME, Bertucci D, et al. Phase I clinical and pharmacological study of O6-benzylguanine followed by carmustine in patients with advanced cancer. Clin Cancer Res. 2000; 6:3025–31. [PubMed: 10955780]
29. Gajewski TF, Sosman J, Gerson SL, et al. Phase II trial of the O6-alkylguanine DNA alkyltransferase inhibitor O6-benzylguanine and 1,3-bis(2-chloroethyl)-1-nitrosourea in advanced melanoma. Clin Cancer Res. 2005; 11:7861–5. [PubMed: 16278409]
30. Ranson M, Middleton MR, Bridgewater J, et al. Lomeguatrib, a potent inhibitor of O6-alkylguanine-DNA-alkyltransferase: phase I safety, pharmacodynamic, and pharmacokinetic trial

and evaluation in combination with temozolomide in patients with advanced solid tumors. Clin Cancer Res. 2006; 12:1577–84. [PubMed: 16533784]

31. Brada M, Judson I, Beale P, et al. Phase I dose-escalation and pharmacokinetic study of temozolomide (SCH 52365) for refractory or relapsing malignancies. British Journal of Cancer. 1999; 81:1022–30. [PubMed: 10576660]
32. Middleton MR, Grob JJ, Aaronson N, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol. 2000; 18:158–66. [PubMed: 10623706]
33. Quinn JA, Pluda J, Dolan ME, et al. Phase II trial of carmustine plus O(6)-benzylguanine for patients with nitrosourea-resistant recurrent or progressive malignant glioma. J Clin Oncol. 2002; 20:2277–83. [PubMed: 11980998]
34. Ryan CW, Dolan ME, Brockstein BB, et al. A phase II trial of O6-benzylguanine and carmustine in patients with advanced soft tissue sarcoma. Cancer Chemother Pharmacol. 2006; 58:634–9. [PubMed: 16520986]
35. Ranson M, Hersey P, Thompson D, et al. A randomised trial of the combination of lomeguatrib and temozolomide alone in patients with advanced melanoma. J Clin Oncology. 2007; 25:2540–5.
36. Staibano S, Pepe S, Lo Muzio L, et al. Poly(adenosine diphosphate-ribose) polymerase 1 expression in malignant melanomas from photoexposed areas of the head and neck region. Hum Pathol. 2005; 36:724–31. [PubMed: 16084940]
37. Wharton SB, McNelis U, Bell HS, Whittle IR. Expression of poly(ADP-ribose) polymerase and distribution of poly(ADP-ribosyl)ation in glioblastoma and in a glioma multicellular tumour spheroid model. Neuropathology and Applied Neurobiology. 2000; 26:528–35. [PubMed: 11123719]
38. Bepler G, Sharma S, Cantor A, et al. RRM1 and PTEN as prognostic parameters for overall and disease-free survival in patients with non-small-cell lung cancer. J Clin Oncol. 2004; 22:1878–85. [PubMed: 15143080]
39. Bepler G, Kusmartseva I, Sharma S, et al. RRM1 modulated in vitro and in vivo efficacy of gemcitabine and platinum in non-small-cell lung cancer. J Clin Oncol. 2006; 24:4731–7. [PubMed: 16966686]
40. Bosken CH, Wei Q, Amos CI, Spitz MR. An analysis of DNA repair as a determinant of survival in patients with non-small-cell lung cancer. J Natl Cancer Inst. 2002; 94:1091–9. [PubMed: 12122100]
41. Newlands ES, Stevens MFG, Wedge SR, Wheelhouse RT, Brock C. Temozolomide: a review of its discovery, chemical properties, pre-clinical development and clinical trials. Cancer Treatment Reviews. 1997; 23:35–61. [PubMed: 9189180]
42. Plummer ER, Lorigan P, Evans J, et al. First and final report of a phase II study of the poly(ADP-ribose) polymerase (PARP) inhibitor, AG014699, in combination with temozolomide (TMZ) in patients with metastatic malignant melanoma (MM). J Clin Oncology. 2006; 24:456s.
43. Fong P, Spicer J, Reade S, et al. Phase I pharmacokinetic (PK) and pharmacodynamic (PD) evaluation of a small molecule inhibitor of Poly ADP-Ribose Polymerase (PARP), KU-0059436 (Ku) in patients (p) with advanced tumours. J Clin Oncol. 2006; 24:3022.
44. Pacher P, Liaudet L, Mabley JG, Cziraki A, Hasko G, Szabo C. Beneficial effects of a novel ultrapotent poly(ADP-ribose) polymerase inhibitor in murine models of heart failure. Int J Mol Med. 2006; 17:369–75. [PubMed: 16391839]
45. Nakajima H, Kakui N, Ohkuma K, Ishikawa M, Hasegawa T. A newly synthesized poly(ADP-ribose) polymerase inhibitor, DR2313 [2-methyl-3,5,7,8-tetrahydrothiopyrano[4,3-d]-pyrimidine-4-one]: pharmacological profiles, neuroprotective effects, and therapeutic time window in cerebral ischemia in rats. J Pharmacol Exp Ther. 2005; 312:472–81. [PubMed: 15466246]
46. Woon EC, Threadgill MD. Poly(ADP-ribose)polymerase inhibition -where now? Curr Med Chem. 2005; 12:2373–92. [PubMed: 16181138]

Europe PMC Funders Author Manuscripts

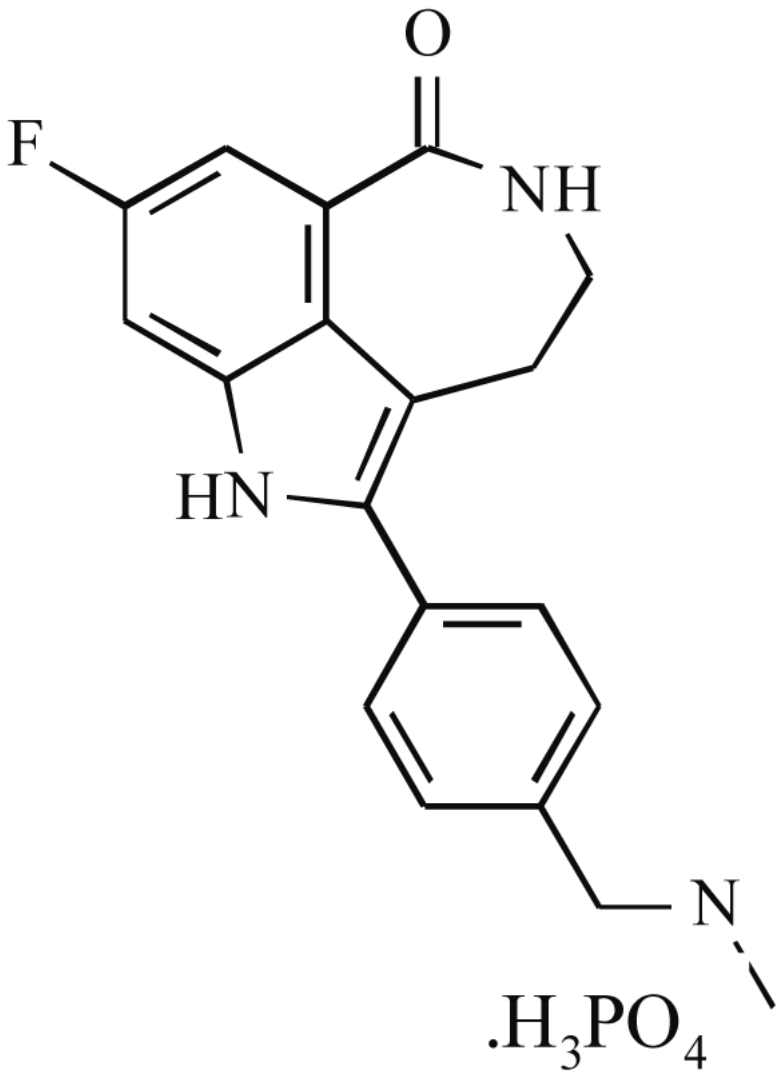

Figure 1.
Structure of AG014699, phosphate salt of tricyclic indole PARP-inhibitor with Ki < 5 nM

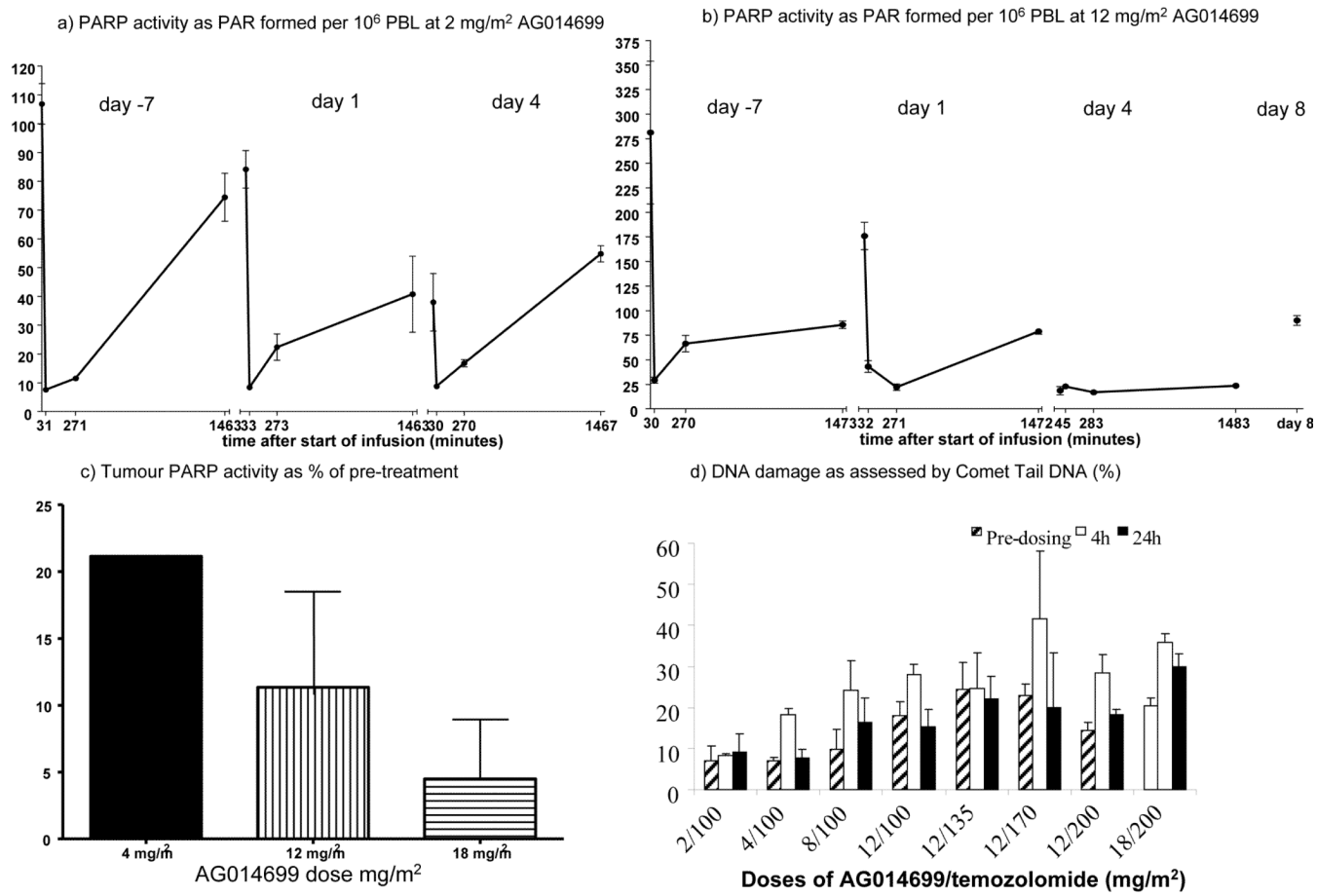

Figure 2.

Pharmacodynamic effects of AG014699.

a, b, c Summary of PBL and tumour PARP inhibition measured using PARP activity immunoassay.

a and b representative plots from day -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 Summarised data from tumour biopsies taken 5 hours after the first dose of AG014699 at the dose levels indicated. Data expressed as percentage activity compared to pre-treatment 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 (hashed), 4h after (white) and 24h after (black) temozolomide dosing. Each reading is the mean of up to 6 patients.

Europe PMC Funders Author Manuscripts

Europe PMC Funders Author Manuscripts

Table 1

Patient demographics

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

Europe PMC Funders Author Manuscripts

Table 2

Dosing and toxicity summary

	Cohort	Dose AG014699 (mg/m^2)	Dose TMZ (mg/m^2)	Number of patients	Total number of cycles given	DLTs 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 plus 3 C2 delay after Gr 3 neutropenia

Europe PMC Funders Author Manuscripts

Clin Cancer Res. Author manuscript; available in PMC 2009 June 01.

Table 3

Pharmacokinetic summary of AG014699

				C_{max} (ng/mL)		AUC_{0-24} (ng*h/mL)		AUC_{inf} [a] (ng*h/mL)		V_d (L)		Cl [a] (L/hr)		$t^{1/2}$ (hr)	
Cohort	**699 dose**	**TMZ dose**		**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** [b]	*34*	**73** [b]	*28*	**1.2** [b]	*55*
			day 1	**27**	*7*	**33**	*74*	**38**	*70*	**160** [b]	*78*	**54** [b]	*55*	**3.3** [b]	*119*
			day 4	**27**	*27*	**44**	*93*	-	-	**400** [b]	*113*	**57** [b]	*55*	**11.6** [b]	*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*	**1384**	*70*	**281**	*33*	**16**	*66*	**19.9**	*88*
			day 1	**473**	*33*	**877**	*28*	**1107**	*25*	**299**	*33*	**15**	*23*	**13.9**	*16*
			day 4	**559**	*54*	**1335**	*39*	-	-	**324**	*26*	**13**	*38*	**18.3**	*30*
5-8	**12 mg/m^2 (n=12)**	**100-200**	**day -7**	**675**	*71*	**861**	*35*	**1021**	*35*	**423**	*50*	**25**	*32*	**11.5**	*37*
			day 1	**551**	*73*	**893**	*35*	**1178**	*47*	**423**	*44*	**24**	*39*	**15.0**	*80*
			day 4	**531**	*37*	**1480**	*59*	-	-	**505**	*56*	**21**	*49*	**16.8**	*41*
9	**18 mg/m^2 (n=6)**	**200**	**day -7**	**700**	*61*	**1550**	*66*	**1952**	*62*	**410**	*41*	**23**	*47*	**13.1**	*40*
			day 1	**837**	*58*	**1923**	*74*	**2721**	*82*	**378**	*49*	**19**	*57*	**16.0**	*46*
			day 4	**863**	*41*	**2882**	*82*	-	-	**343**	*39*	**18**	*50*	**15.2**	*37*

[a] 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

[b] Not included in statistical analysis. This value may not be correctly estimated due to insufficient data