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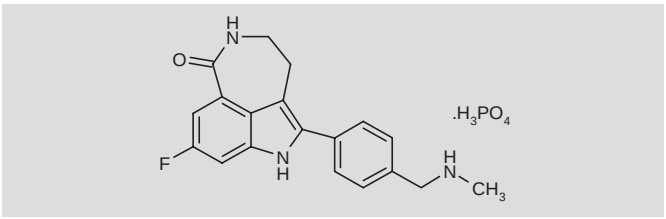
# AG-14699

*PARP Inhibitor*
*Oncolytic*

AG-014699
AG-014447 (as free base)
AG-14447 (as free base)

8-Fluoro-2-[4-(methylaminomethyl)phenyl]-3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indol-6-one phosphate
InChI=1/C19H18FN3O/c1-21-10-11-2-4-12(5-3-11)18-14-6-7-22-19(24)15-8-13(20)9-16(23-18)17(14)15/h2-5,8-9,21,23H,6-7,10H2,1H3,(H,22,24)




$C_{19}H_{21}FN_3O_5P$
Mol wt: 421.3593
CAS: 459868-92-9
CAS: 283173-50-2 (free base)
CAS: 773059-19-1 (hydrochloride)
CAS: 773059-22-6 (L-tartrate)
CAS: 773059-23-7 (acetate)
EN: 292526


## ABSTRACT

*Combination of poly(ADP-ribose)polymerase (PARP) inhibitors and conventional DNA-damaging drugs is a promising new approach to cancer treatment. In mice bearing s.c. neuroblastoma NB-1691 xenografts, coadministration of AG-14699 markedly enhanced temozolomide-induced tumor growth delay and led to complete tumor regressions. In a phase I clinical trial of AG-14699 combined with temozolomide in patients with advanced solid tumors, PARP inhibition was observed at all doses tested (46-97% at 5 h). AG-14699 alone was not toxic and combination of AG-14699 and temozolomide was well tolerated. The PARP-inhibitory dose (PID) was defined as 12 mg/m$^2$/day, and a combination of AG-14699 12 mg/m$^2$/day and temozolomide 200 mg/m$^2$/day was recommended for further clinical studies. Preliminary results of a phase II trial of AG-14699 and temozolomide in patients with metastatic melanoma indicated that the addition of AG-14699 enhanced temozolomide-associated myelosuppression, and that the response rate for combination treatment was higher than for temozolomide alone.*


## SYNTHESIS*

AG-14699 can be prepared by two related methods:

Reductive cyclization of 6-fluoro-3-(2-nitroethyl)-1*H*-indole-4-carboxylic acid methyl ester (I) by means of Zn and HCl in methanol/water gives 8-fluoro-3,4,5,6-tetrahydro-1*H*-azepino-[5,4,3-*cd*]indol-6-one (II), which is brominated by means of Pyr/HBr/$Br_2$ in THF/dichloromethane to afford the 2-bromo derivative (III). Condensation of the brominated compound (III) with 4-formylphenylboronic acid (IV) by means of $Pd(PPh_3)_4$ and $Na_2CO_3$ in refluxing $H_2O$/ethanol/toluene provides 8-fluoro-2-(4-formylphenyl)-3,4,5,6-tetrahydro-1*H*-azepino[5,4,3-*cd*]indol-6-one (V), which is finally reductocondensed with methylamine (VI) by means of $NaBH_4$ (1). Scheme 1.

Reaction of 5-fluoro-2-hydroxybenzoic acid (VII) with MeOH and $H_2SO_4$ gives the corresponding methyl ester (VIII), which is treated with tetramethylammonium nitrate and TFAA to yield 5-fluoro-2-hydroxy-3-nitrobenzoic acid methyl ester (IX). Condensation of the hydroxybenzoate (IX) with the phenylacetylene derivative (X) by means of $TF_2O$, $PdCl_2(PPh_3)_2$ and TEA affords the diphenylacetylene derivative (XI). Reduction of the $NO_2$ group of (XI) by means of Fe, HCl and $NH_4Cl$ provides the aniline derivative (XII), which is cyclized by means of CuI at 100 °C to give the indole derivative (XIII). Condensation of indole (XIII) with *N*-(2-nitrovinyl)-*N*,*N*-dimethylamine (XIV) by means of TFA yields the 3-(2-nitrovinyl)indole derivative (XV), which is reduced with $NaBH_4$ to afford the 3-(2-nitroethyl)indole derivative (XVI). Reductive cyclization of indole (XVI) by means of $H_2$ over RaNi in AcOH provides the tricyclic compound (XVII), which is finally decarboxylated by means of HBr/AcOH (2). Scheme 2.

## BACKGROUND

DNA repair plays an important role in tumor resistance to DNA-damaging cancer treatments such as cytotoxic drugs and ionizing

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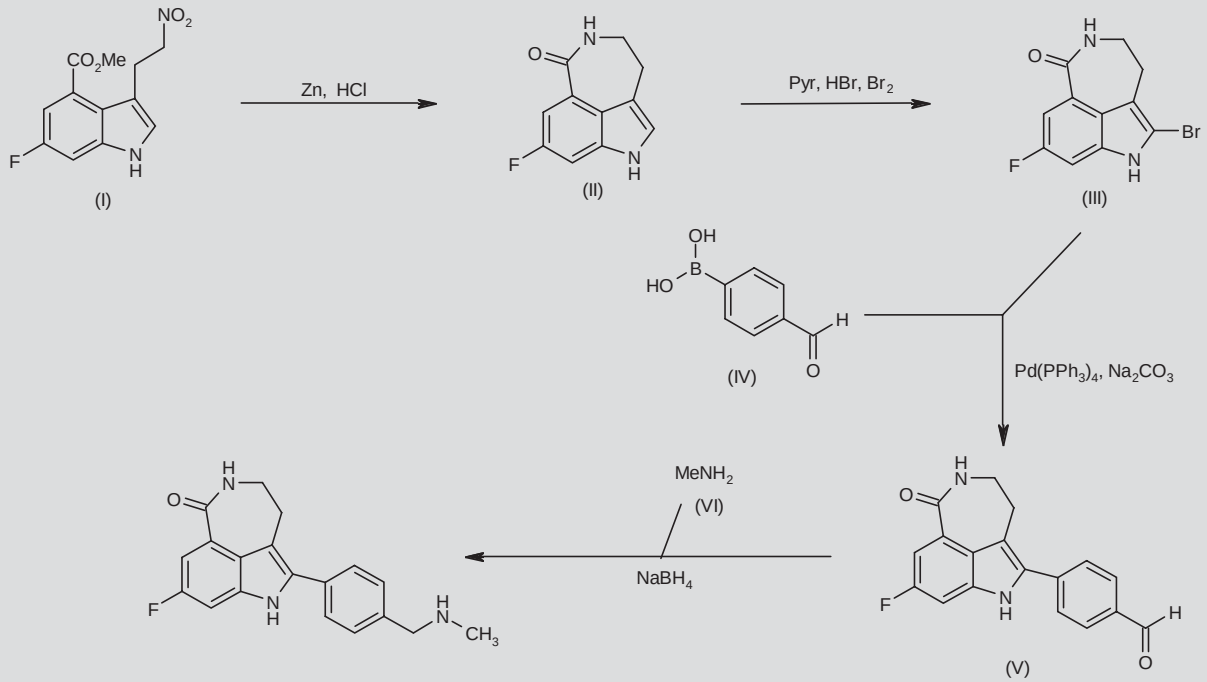
**Scheme 1.** Synthesis of AG-14699




radiation (3). The principal pathways that contribute to DNA repair include nucleotide excision repair, base excision repair, homologous recombination, nonhomologous end joining, mismatch repair and telomerase metabolism (4, 5). DNA single-strand breaks caused by anticancer agents are primarily repaired by base excision repair, whereas double-strand breaks, the most toxic of all DNA lesions, are mainly repaired by homologous recombination and nonhomologous end joining (5, 6). Targeting DNA repair mechanisms has hence been considered a promising new approach for the treatment of cancer.

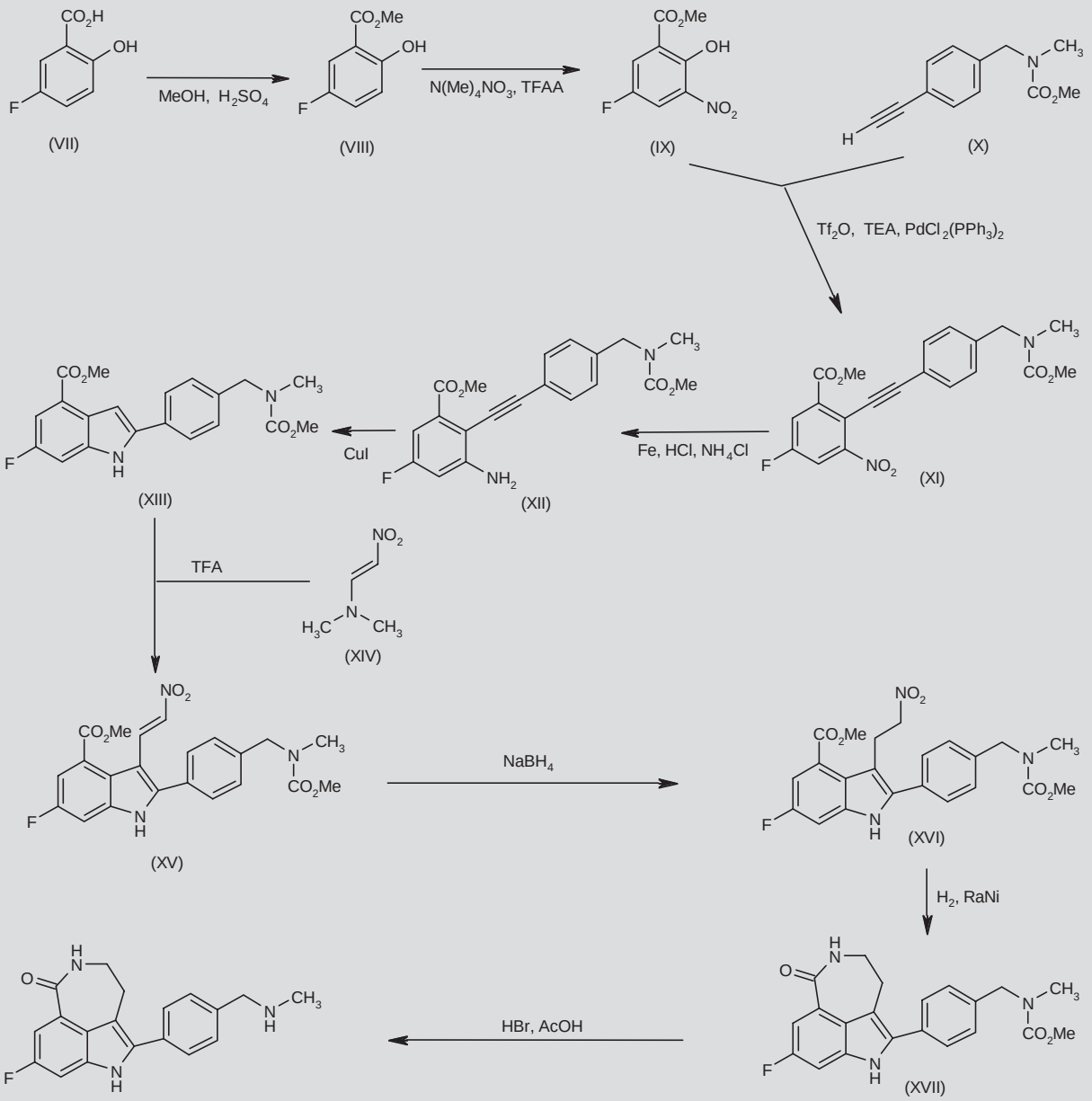
When used in combination with conventional DNA-damaging cancer treatments, DNA repair inhibitors can enhance the efficacy of cancer treatment. A number of DNA repair inhibitors have entered clinical development, including poly(ADP-ribose)polymerase (PARP) inhibitors such as olaparib (AZD-2281; AstraZeneca) and AG-14699 (Pfizer, Cancer Research UK), base excision repair inhibitors such as TRC-102 (TRACON Pharmaceuticals), 6-*O*-methylguanine-DNA methyltransferase inhibitors such as $O^6$-benzylguanine (National Cancer Institute) and inhibitors of hypermethylation of mismatch repair genes such as decitabine (MGI Pharma, a subsidiary of Eisai) (7).

PARP plays an important role in a number of cellular processes, including DNA repair. PARP-1, the best-characterized member of the PARP family, is a nuclear enzyme that promotes base excision repair by binding to and being activated by DNA breaks and recruiting additional repair factors (8). PARP has been reported to be elevated in human cancer (9, 10) and the combination of PARP inhibitors and cytotoxic agents has proved synergistic in preclinical models (5, 11, 12). BRCA1 and BRCA2 proteins are involved in the repair of double-strand DNA breaks via homologous recombination. BRCA1- and BRCA2-deficient cells have proven sensitive to PARP inhibitors. It was suggested that the accumulation of single-strand breaks following PARP inhibition led to the formation of double-strand breaks during DNA replication, which are mainly repaired via the BRCA-dependent homologous repair pathway (5, 13).

Since the 1980s, a number of PARP inhibitors have been developed based on the quinazolinone, benzimidazole, tricyclic benzimidazole, tricyclic indole and tricyclic indol-1-one core structures. In an attempt to identify a compound from different classes of PARP inhibitors for clinical use, Curtin et al. conducted a preclinical selection study. In this study, the biological properties of 42 potent PARP inhibitors with $K_i$ values ranging from 1.4 to 15.1 nmol/L were evaluated using cell-based assays. A clear trend for enhanced in vitro potency with analogues that contain a basic amine was observed. Analogues containing a basic amine also led to the highest level of chemosensitization in human colon adenocarcinoma LoVo cells. The correlation between the potency of the inhibitors and their ability to enhance temozolomide-induced growth inhibition was statistically significant ($P < 0.005$) (14, 15).

On the basis of the in vitro evaluation, the toxicity and antitumor activity of 11 PARP inhibitors alone and in combination with temozolomide were further evaluated in vivo. On the basis of the toxicity and efficacy evaluation, AG-14447, which was at least 10 times more potent than the lead compound AG-14361 in both single-dose and 5-day dosing studies, was identified as the most potent compound. At tolerable doses, AG-14447 enhanced temozolomide-induced tumor growth delay by > 100%. Although none of the 11 inhibitors

**Scheme 2.** Synthesis of AG-14699




alone was toxic, temozolomide-induced toxicity was enhanced in tumor-bearing mice receiving temozolomide combined with PARP inhibitors. Based on this study, AG-14699, a phosphate salt of AG-14447 with improved aqueous solubility, was selected for clinical development and is currently in phase II clinical trials (14-16).

## PRECLINICAL PHARMACOLOGY

Preclinical evaluation of AG-14699 in breast cancer cell lines with different *BRCA1* status demonstrated that cells with mutated *BRCA1* ($LC_{50}$ = 1.3 μM) were about 15 times more sensitive to AG-14699 than cells with wild-type *BRCA1* ($LC_{50}$ = 20 μM, $P < 0.001$). Methylated *BRCA1* cells ($LC_{50}$ = 7.6 μM) were 2.6 times more sensitive to AG-14699 than cells with wild-type *BRCA1* but less sensitive than the cells with mutant *BRCA1* ($P < 0.001$). A marked increase in DNA formation of double-strand breaks was observed in all cell lines after exposure to AG-14699 (17).

Tumor cell lines defective in homologous recombination caused by mutation and epigenetic silencing all responded well to AG-14699,

suggesting that the agent has therapeutic potential in both sporadic and hereditary cancers. Strong PARP inhibition (> 90%) was observed in all cell lines after exposure to AG-14699 10 μM (18).

The cytotoxicity of AG-14699 in ovarian surface epithelial OSEC1 (*BRCA2* heterozygote) and OSEC2 (wild-type) cell lines was evaluated by using clonogenic cell survival assays. Cell survival after treatment with AG-14699 was not affected by *BRCA2* heterozygosity (19).

The in vitro and in vivo effects of AG-14699 on the antitumor activity of temozolomide and topotecan were examined in human medulloblastoma and neuroblastoma cells. At concentrations of 0.1, 0.4 and 1 μM, AG-14699 inhibited PARP-1 activity by 92%, 98% and 98%, respectively, in NB-1691 cells and by 83%, 97% and 100%, respectively, in SH-SY5Y cells. Both topotecan and temozolomide alone inhibited the growth of all cell lines tested in a concentration-dependent manner. AG-14699 (0.4 μM) alone did not induce growth inhibition. However, the agent produced significant sensitization to both topotecan and temozolomide in all cell lines ($P \leq 0.05$). AG-14699 potentiated the activity of topotecan by 1.5- to 2.3-fold and the activity of temozolomide by 3- to 10-fold in all cell lines. Clonogenic survival assays in NB-1691 cells exposed to topotecan or temozolomide in the presence or absence of 0.4 μM AG-14699 showed that the agent enhanced both temozolomide cytotoxicity (3.2-fold at $LD_{50}$) and topotecan cytotoxicity (1.4-fold at $LD_{50}$), and that the potentiation of cell death caused by AG-14699 was not as marked as the potentiation of growth inhibition (20).

In mice bearing s.c. NB-1691 xenografts, coadministration of AG-14699 (1 mg/kg i.p. x 5 days) markedly enhanced temozolomide-induced tumor growth delay by > 300% (to 59 days) and led to complete tumor regression in 3 of the 5 mice, 2 of which persisted until the end of the experiment (100 days). AG-14699 also enhanced the topotecan-induced tumor growth delay (to 22 days) and led to 2 complete tumor regressions, one of which persisted to the end of the study. In mice bearing SH-SY5Y xenografts, AG-14699 increased the temozolomide-induced tumor growth delay by 50% (to > 89 days), and the number of mice with complete and persistent tumor regressions was increased from 2 of 5 to 4 of 5 animals. The agent did not increase topotecan-induced tumor growth delay but increased the total number of complete regressions from 3 of 5 to 4 of 5 animals (20, 21).

## PHARMACOKINETICS AND METABOLISM

In mice bearing NB-1691 tumor xenografts, following AG-14699 (1 mg/kg i.p.) administration AG-14447, the free base of AG-14699, was rapidly detected in mouse plasma and eliminated from the plasma gradually over time. Maximal levels of AG-14447 in plasma were recorded at 30 min after the first dose (mean 192 ± 13.8 nmol/L) and after the fourth dose (mean 121 ± 18.8 nmol/L). $AUC_{0\text{-}24h}$ for the first and fourth doses was 57.9 and 20.3 μmol.min/L, respectively. In NB-1691 xenografts, AG-14447 accumulated slowly, with a peak concentration recorded at 2 h after administration. The maximal concentrations of AG-14447 detected in the tumor tissue were much higher (7-12 times) than those detected in the plasma. The concentration of AG-14447 in tumor tissue remained stable after the first dose and was above 0.4 μmol/L for the entire dosing period (20).

In a phase I clinical trial of AG-14699 (1-18 mg/m$^2$/day by i.v. infusion) combined with temozolomide (100 mg/m$^2$ p.o. for 5 days every 4 weeks) in patients with advanced solid tumors, AG-14447 showed linear pharmacokinetics. The mean terminal half-life of AG-14447 was 9.5 h and the mean volume of distribution was 212 L. The low mean percentage of dose recovered over 24 h in the urine after a single dose (11%) indicated that the kidneys were not the main elimination route. $AUC_{0\text{-}24h}$ did not correlate with body surface area. In samples taken 5 h (24 h in 3 patients) after the administration of AG-14699, AG-14447 was detected in all tumor biopsy homogenates. Temozolomide did not affect the pharmacokinetics of AG-14447 after administration of single or multiple doses and the pharmacokinetic parameters of temozolomide were not affected by AG-14699 (22).

The relationship between the pharmacokinetics and pharmacodynamics of AG-14699 was evaluated using population pharmacokinetic/pharmacodynamic (PK/PD) modeling in cancer patients. The pharmacokinetic endpoint was poly(ADP-ribose) (PAR) polymer, a marker of PARP activity, in plasma and tumor. A total of 32 patients received AG-14699 1, 2, 4, 8, 12 and 18 mg/m$^2$ by 0.5-h i.v. infusion and oral temozolomide at 100, 135 or 170 mg/m$^2$ once daily x 5 days every 28 days. Nonlinear mixed-effect modeling and first-order conditional estimation with interaction were used for the PK/PD data analysis. Population pharmacokinetics of AG-14447 were described by a 3-compartment model with saturable and nonsaturable distribution to peripheral compartments. The population estimates for clearance, central volume of distribution, volume of distribution of saturable compartment and volume of distribution of nonsaturable compartment (± SE) were 17.7 L/h, 17.4 L, 28.2 L and 387 L, respectively. An indirect response PD mixture model described the inhibition of PAR formation by AG-14699 in plasma and tumor (23).

## CLINICAL STUDIES

The first-in-human phase I trial of AG-14699 in combination with temozolomide was designed to explore if a PARP-inhibitory dose (PID) of AG-14699, which was defined as the dose that leads to the maximal achievable (at least 50%) reduction in PARP activity 24 h after administration of the first dose of the agent with no increase in the degree of PARP inhibition over the preceding dose, was safe and tolerable in cancer patients, and also to determine an appropriate dose of temozolomide that could be given in combination with the PID of AG-14699. The trial was conducted in 32 patients (21 males and 11 females; mean age = 52 years) with advanced solid tumors. The primary endpoint of the study was inhibition of the target enzyme. To obtain PK and PD data, patients first received a single dose of AG-14699 before they received the first combination treatment. The patients then received escalating doses of AG-14699 (1, 2, 4, 8, 12 and 18 mg/m$^2$/day). No dose-limiting toxicity (DLT) was observed and the PID was established as 12 mg/m$^2$/day. The patients then received AG-14699 at the PID and temozolomide escalated to the licensed dose (200 mg/m$^2$) or the maximum tolerated dose (MTD). Again, no DLT was observed. The combination of AG-14699 with temozolomide was well tolerated and no toxicity attributable to AG-14699 alone was observed. No grade 3 or 4 toxicity was observed at the PID. Increased myelosuppression was observed at the maximum dose of AG-14699 (18 mg/m$^2$/day com-

bined with 200 mg/m$^2$/day temozolomide). PARP inhibition of > 90% was observed in peripheral blood lymphocytes at all AG-14699 dose levels evaluated. While recovery of PARP activity was observed at the lower dose levels (1, 2 and 4 mg/m$^2$/day), no recovery was observed over 24 h at doses above 8 mg/m$^2$. However, at 72 h after the last dose of AG-14699 (12 mg/m$^2$/day), the enzyme activity in peripheral blood lymphocytes recovered to ~50% of the baseline value. No correlation between the degree of inhibition in peripheral blood mononuclear cells and tumor was established. Comet analysis in peripheral blood lymphocytes indicated that DNA damage occurred in all patients receiving AG-14699 and temozolomide, and the duration of the DNA damage was dependent on the dose of AG-14699. AG-14699 at 12 mg/m$^2$ led to significantly more damage than lower doses ($P$ = 0.002). Several patients in this study experienced clinical benefit. One complete response and one partial response were reported in patients with metastatic melanoma who had not received chemotherapy for melanoma before the study. One partial response was reported in a patient with a desmoid tumor who previously received treatment with extensive surgery and imatinib. Seven patients (four with melanoma, one with prostate cancer, one with pancreatic cancer and one with leiomyosarcoma) experienced prolonged disease stabilization (≥ 6 months). Based on the efficacy and toxicity evaluation, a combination of 12 mg/m$^2$/day AG-14699 and 200 mg/m$^2$/day temozolomide × 5 every 28 days was recommended for future studies (22).

A phase II clinical study of AG-14699 (12 mg/m$^2$) combined with temozolomide (200 mg/m$^2$) daily x 5 every 4 weeks in 40 patients with metastatic melanoma has completed recruitment. Preliminary results indicated that temozolomide-associated myelosuppression was enhanced by the addition of AG-14699. One death due to febrile neutropenia was reported in cycle 1 of the study. Three patients were hospitalized with myelosuppression. A total of 12 patients required dose reduction of temozolomide. After dose reduction, these patients continued receiving the treatment at the reduced dose. Other adverse events reported in the study included fatigue and mild nausea. At the time data were reported, four patients achieved a partial response and four patients had prolonged stable disease. The response rate for combination treatment was higher than for temozolomide alone (24).

Another phase II trial of AG-14699 in patients with advanced breast or ovarian cancer and known mutations of *BRCA1* or *BRCA2* is currently recruiting (16).

## SOURCES

Cancer Research UK (GB); Pfizer (US).

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