

MK-4827, a PARP-1/-2 inhibitor, strongly enhances response of human lung and breast cancer xenografts to radiation

Li Wang · Kathy A. Mason · K. Kian Ang ·
Thomas Buchholz · David Valdecanas · Anjili Mathur ·
Carolyn Buser-Doepner · Carlo Toniatti · Luka Milas

Received: 19 September 2011 / Accepted: 14 November 2011 / Published online: 30 November 2011
© Springer Science+Business Media, LLC 2011

Summary The poly-(ADP-ribose) polymerase (PARP) inhibitor, MK-4827, is a novel potent, orally bioavailable PARP-1 and PARP-2 inhibitor currently in phase I clinical trials for cancer treatment. No preclinical data currently exist on the combination of MK-4827 with radiotherapy. The current study examined combined treatment efficacy of MK-4827 and fractionated radiotherapy using a variety of human tumor xenografts of differing p53 status: Calu-6 (p53 null), A549 (p53 wild-type [wt]) and H-460 (p53 wt) lung cancers and triple negative MDA-MB-231 human breast carcinoma. To mimic clinical application of radiotherapy, fractionated radiation (2 Gy per fraction) schedules given once or twice daily for 1 to 2 weeks combined with MK-4827, 50 mg/kg once daily or 25 mg/kg twice daily, were used. MK-4827 was found to be highly and similarly effective in both radiation schedules but maximum radiation enhancement was observed when MK-4827 was given at a dose of 50 mg/kg once daily ($EF=2.2$). MK-4827 radiosensitized all four tumors studied regardless of their p53 status. MK-4827 reduced PAR levels in tumors by 1 h after administration which persisted for up to 24 h. This long period of PARP inhibition potentially adds

to the flexibility of design of future clinical trials. Thus, MK-4827 shows high potential to improve the efficacy of radiotherapy.

Keywords Poly-(ADP-ribose) inhibitor · MK-4827 · Radiation · Lung and breast tumor xenografts

Introduction

Poly-(ADP-ribose) polymerases (PARPs) have been extensively studied since PARP1 was first described in 1963 [1]. PARPs are a family of enzymes that catalyze the synthesis of poly (ADP-ribose) polymers from nicotinamide adenine dinucleotide (NAD⁺) to acceptor proteins [2, 3]. PARPs are involved in the regulation of multiple cellular processes, including gene transcription, cell cycle progression, apoptosis, genomic stability, and DNA repair principally through the DNA base excision repair (BER) pathway [3–8]. Among members of the PARP family, PARP1 and PARP2 are the most abundant, accounting for greater than 80% of cellular poly-(ADP-ribosylation) capacity [9]. PARP 1 is the original and most well characterized being activated by single- and double DNA strand breaks, to which it binds, and thus opens and exposes damaged DNA to the components needed for its repair [4, 5]. Proficient DNA-repair in cancer cells constitutes a major factor responsible for tumor resistance to radiotherapy. Therefore, inhibiting DNA repair in tumor cells by interfering with PARP-1 function is a rational therapeutic strategy to enhance the effects of radiation.

Almost 30 years ago Nicotinamide was first identified as a PARP inhibitor [10]. Since then, several generations of PARP inhibitors have been developed through screening of chemical libraries leading to an improved potency and a better understanding of the structure–activity relationship [11–13].

L. Wang (✉) · K. A. Mason · D. Valdecanas · L. Milas
Department of Experimental Radiation Oncology,
The University of Texas MD Anderson Cancer Center,
Unit 066, 1515 Holcombe Blvd.,
Houston, TX 77030, USA
e-mail: Lwang6@mdanderson.org

K. K. Ang · T. Buchholz
Department of Radiation Oncology,
The University of Texas MD Anderson Cancer Center,
Houston, TX 77030, USA

A. Mathur · C. Buser-Doepner · C. Toniatti
Department of Oncology, Merck Research Labs,
33 Avenue Louis Pasteur,
Boston, MA 02115, USA

A number of these inhibitors have shown single agent antitumor activity and were able to enhance the antitumor efficacy of cytotoxic DNA-damaging agents including both chemotherapy (such as alkylating agents, topoisomerase 1 and platinum) and ionizing radiation [11, 12, 14–19]. However, many current commercially available PARP inhibitors, such as 3-aminobenzamide or nicotinamide, do not completely inhibit PARP activity and exert a variety of non-specific effects [20]. These older generation compounds, therefore, have limited utility as clinical radiosensitizers or chemosensitizers. Over the past few years, a number of novel and more specific PARP inhibitors have undergone development.

Preclinical studies have shown synergy with radiotherapy both *in vitro* and *in vivo* in a variety of human tumor types including colon carcinoma, non-small-cell lung carcinoma, head and neck squamous cell carcinoma, glioblastoma, pancreatic carcinoma, prostate carcinoma and breast cancer [4, 15, 21–30]. To improve efficacy and specificity, the current generation of PARP inhibitors is targeting both PARP-1 and PARP-2 enzymes. Among these agents is MK-4827 a potent, orally bioavailable PARP-1 and PARP-2 inhibitor recently developed by Merck & Co Inc. Preclinical studies demonstrated the antitumor efficacy of MK-4827 in BRCA-deficient xenograft models [31, 32], and currently is in phase I clinical trials in patients with advanced solid tumors (prostate, primary peritoneal, fallopian tube) and BRCA-deficient ovarian cancer [11, 14, 17, 32, 33]. However, efficacy in combination with radiotherapy has yet to be tested. The experiments undertaken in this study were designed to determine the ability of MK-4827 to enhance the effects of ionizing radiation using a number of human tumor xenografts in nude mice.

Material and methods

Drugs

MK-4827, provided by Merck Research Laboratories (Boston, MA), was used at concentrations of 25 or 50 mg/kg and given orally by gavage once or twice daily for 11 to 30 days. The agent was suspended in 0.5% Methocel (DOW Chemical Company, Houston, TX) in deionized water, mixed for 24 h, and stored at 2–8°C during each individual experiment.

Tumor cell lines and cell culture

Three human lung cancer cell lines H460 (p53 wild-type [wt]), A549 (p53 wt), Calu-6 (p53 null) and one human breast cancer cell line MDA-MB-231 (triple-negative) were obtained from the American Type Culture Collection (Manassas, VA). Cells were obtained from a batch of frozen cells previously

IMPACT-tested and maintained in liquid N₂. Seven to eleven days before inoculation, tumor cell suspensions were prepared from cells propagated as a monolayer *in vitro*. Tumor cells were maintained in RPMI 1640 medium supplemented with 10% fetal calf serum, 10,000 U/mL of penicillin–streptomycin, and 2 mM L-glutamine. Cell monolayers in 75-cm² flasks were maintained in a humidified 5% CO₂/95% air atmosphere at 37°C.

Animals

Female nude mice (Ncr Nu/Nu) were used in these studies. The mice were bred and maintained in our specific-pathogen free facility and were 3–4 months of age at the start of the experiments. Mice were housed 3–5 per cage, exposed to 12-hour light dark cycles, and given free access to sterilized pelleted food (Prolab Animal Diet, Purina Mills Inc., St. Louis, MO) and sterilized water. Animals were maintained in an American Association for Laboratory Animal Care approved facility, and in accordance with current regulations of the United States Department of Agriculture and Department of Health and Human Services. The experimental methods were approved by, and in accordance with, institutional guidelines established by the Institutional Animal Care and Use Committee. Mice were euthanized by CO₂ inhalation when tumors grew to a post-treatment tumor diameter of 14–15 mm.

Tumor implantation and treatment

Solitary tumor xenografts were produced in the muscle of the right hind leg of nude mice by inoculation of 10⁶ (H460 and Calu-6), 3×10⁶ (A549), or 7×10⁶ (MDA-MB-231) cells in a volume of 10–30 μl. Mice were randomly assigned to treatment groups consisting of 5 to 8 mice each when tumors grew to 6.0 mm in diameter at which time treatment with MK-4827 was initiated. MK-4827 was given at a dose of 25 mg/kg twice daily or 50 mg/kg once daily for either 21 days or was discontinued at 9 days from the time tumors reached 8 mm in diameter. Fractionated local tumor irradiation (XRT) was given when tumors reached 8.0 mm in diameter (7.7–8.2 mm). Radiation (2 Gy per fraction) was delivered to the tumor-bearing leg of mice once daily for 14 consecutive days or twice daily for 7 consecutive days using a small-animal irradiator (Atomic Energy of Canada, Ottawa, Canada) consisting of two parallel-opposed ¹³⁷Cs sources, at a dose rate of 5 Gy/min. During irradiation un-anesthetized mice were mechanically immobilized in a jig so that the tumor was centered within a 3.0 cm diameter radiation field and the animal's body shielded from radiation exposure. On the day when both MK-4827 and radiation were given, drug was administered 1 h before the first radiation dose of the day.

Tumor growth delay assay

Tumor growth delay was the endpoint used to determine anti-tumor efficacy of MK-4827 alone and in combination with radiation. Three mutually orthogonal diameters of tumors were measured 2–3 times/week with a vernier caliper, and the mean values were calculated. Mice were euthanized when tumors grew to 14.0–15.0 mm diameter. Regression and re-growth of tumors were expressed as the time in days for tumors in the treated groups to grow from 6.0 mm to 12.0 mm or from 8.0 mm to 12.0 mm in diameter minus the time in days for tumors in the control group to reach the same size. This was termed absolute growth delay (AGD). For groups treated with both MK-4827 and radiation, normalized growth delay (NGD) was determined as the time for tumors in the combined therapy group to grow to 12.0 mm minus time for tumors in the group treated with drug alone to grow to 12.0 mm. The radiation enhancement factor (EF) was then determined by dividing NGD for the group receiving MK-4827 plus radiotherapy by the AGD for the group given radiation only.

Tumor poly-[ADP-ribose] (PAR) level determination

The extent of PARP inhibition in MK-4827 treated mice was evaluated by measuring intratumoral levels of PAR

using an Enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions (Trevigen Inc. MD, USA). Extracts were prepared from 20 to 25 mg pieces of frozen tumor tissue. All treatment groups were normalized to the untreated control group.

Results

Mice bearing 6 mm Calu-6 lung tumor xenografts were gavaged with 25 mg/kg MK-4827 twice a day with 6 h between doses, 50 mg/kg MK-4827 once daily for 21 consecutive days. When tumors reached 8 mm in diameter, 5 to 11 days after initiation of MK-4827 treatment, tumors were locally irradiated with 2 Gy twice a day, with 6 h between doses, for 7 consecutive days. During radiation schedule, 25 mg/kg MK-4827 was given 1 h before each fraction of radiation whereas 50 mg/kg MK-4827 was given 1 h before the first daily fraction of radiation. Neither 25 mg/kg MK-4827 given twice daily nor 50 mg/kg MK-4827 given once daily caused overt toxicity to mice.

The effect of these treatments on tumor growth is shown in Table 1 and Fig. 1. MK-4827 as a single agent had no effect on tumor growth, whereas 2 Gy alone twice daily for 7 days markedly delayed tumor growth. To grow from 8 to

Table 1 Effect of MK-4827 on radioresponse of Calu-6 tumor xenografts in nude mice

Treatment ^a	No. of Mice	Time in days ^b required to grow from 8 to 12 mm	Absolute growth ^c delay	Normalized growth ^d delay	Enhancement ^e factors
Experiment 1					
Control (no treatment)	6	13.2±0.5			
XRT only (2 Gy) ×2fx for 7 days	8	30.9±2.4	17.7±2.4		
MK-4827 25 mg/kg ×2/day for 21 days	7	14.9±1.2	1.7±1.2		
MK-4827 (25 mg/kg)+XRT (2 Gy fractionated)	8	39.7±2.0	26.5±2.0	24.8±2.0	1.4
MK-4827 50 mg/kg ×1/day for 21 days	7	11.8±0.8	-1.4±0.8		
MK-4827 (50 mg/kg)+XRT (2 Gy fractionated)	5	47.8±4.0	34.6±4.0	36.0±4.0	2.0
Experiment 2					
Control (no treatment)	8	14.9±0.9			
XRT only (2 Gy) ×1fx for 14 days	8	28.8±1.4	13.9±1.4		
MK-4827 50 mg/kg ×1/day until 9 days after 8 mm	8	13.5±1.3	-1.4±1.3		
MK-4827 (50 mg/kg)+XRT (2 Gy fractionated)	8	37.0±2.4	22.1±2.4	23.5±2.4	1.7

^a MK-4827 was given by gavage 25 mg/kg twice daily (6 h in between) or 50 mg/kg once daily when tumors reached 6 mm in diameter

MK-4827 was given 21 days in Experiment 1; given until 9 days after tumor reached 8 mm (MK-4827 only) or 2 days after XRT was completed (combination) in Experiment 2. MK-4827 was given 1 h prior to each or the first XRT of the day. XRT started when tumor reached 8 mm in diameter. Twice daily fractionated XRT in Experiment 1 was given 6 h in between. Groups consisted of 6–8 mice each

^b Mean tumor diameter + SE. Time required from 8 to 12 mm

^c Absolute tumor growth delay (AGD) is the days for tumors grow from 8 to 12 mm in diameter of MK-4827 treatment groups minus the days of no treatment group

^d Normalized tumor growth delay (NGD) is defined as the time in days for tumors to reach 12 mm in the mice treated with the combination of MK-4827 and XRT minus the time in days of tumors to reach 12 mm in mice treated with MK-4827 alone.

^e Enhancement factors obtained by dividing NGD in mice treated with MK-4827 plus XRT by the AGD in mice treated with XRT alone

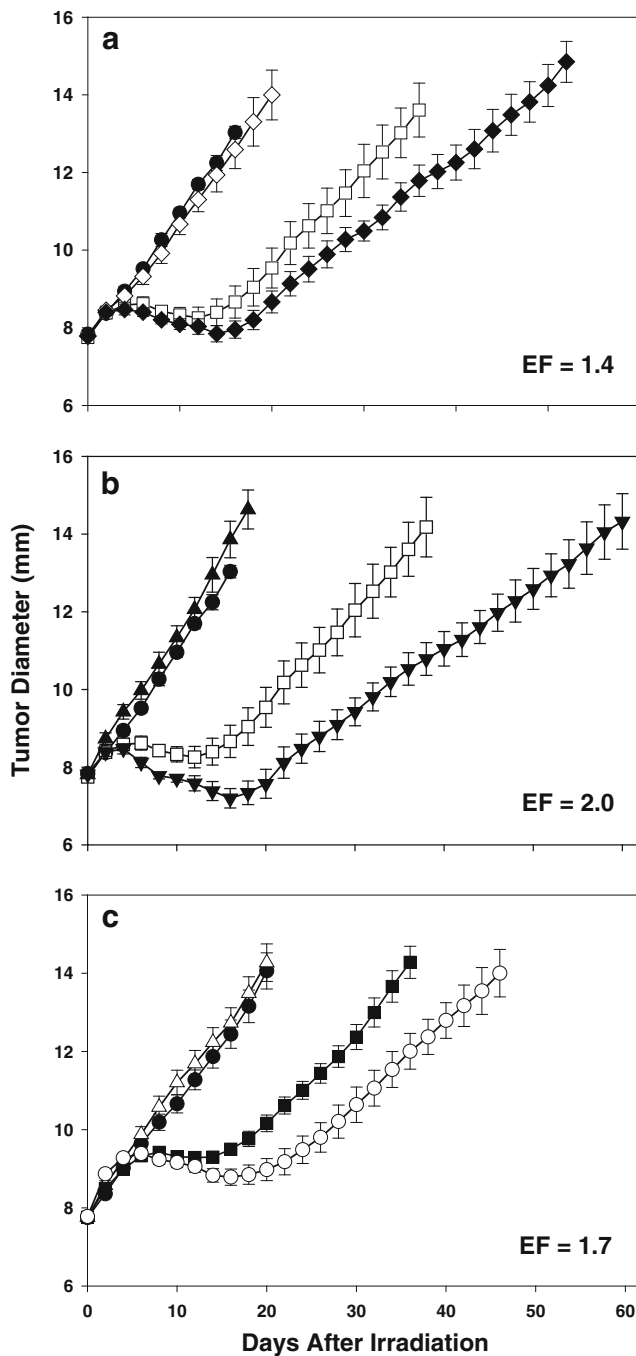


Fig. 1 Effect of MK-4827 on radiation-induced Calu-6 tumor growth delay. Panels a-c: mice bearing Calu-6 tumors 6 mm in diameter in the leg were: untreated (*solid circle*), treated with MK-4827 25 mg/kg twice daily (*open diamond*) or 50 mg/kg once daily (*solid upward triangle*) for 21 days, or MK-4827 50 mg/kg once daily (*open upward triangle*) until 9 days after tumor reached 8 mm. When tumors reached 8 mm, tumors were treated: for 7 days (*open square*) twice per day or 14 days (*solid square*) once per day with fractionated (fx) irradiation (XRT, 2 Gy per fx). On the day when both MK-4827 and radiation were given, [7 days fx for 25 mg/kg (*solid diamond*) and 50 mg/kg (*solid downward triangle*); or until 2 days after XRT for 50 mg/kg (*open circle*)], drug was administered 1 h before each or the first radiation dose of the day. Each data point represents the mean tumor size of 5 to 8 tumors; bars, SE. EF enhancement factor

12 mm in diameter, untreated tumors needed 13.2 ± 0.5 days whereas irradiated tumors needed 30.9 ± 2.4 days (Table 1). The radiation-induced tumor growth delay was further extended in mice that were also treated with MK-4827: to 39.7 ± 2.0 days after a dose of 25 mg/kg given twice daily and to 47.8 ± 4.0 days after a dose of 50 mg/kg given once daily (Table 1). Thus, MK-4827 enhanced radiation response of p53 mutant Calu-6 tumor in both cases, with the single daily dose of 50 mg/kg being more effective than 25 mg/kg given twice daily. The radiation EFs factors were 2.0 and 1.4 respectively. An additional experiment testing the effect of radiation schedule on the observed enhancement showed that similar radiation enhancement could be achieved in schedules using 50 mg/kg MK-4827 once daily in combination with the same total radiation dose delivered as 2 Gy once daily for 14 days ($EF=1.7$; Fig 1c) or 2 Gy twice daily for 7 days ($EF=1.9$).

To test whether the observed enhancing effect of MK-4827 is affected by p53 mutational status of tumors additional experiments were conducted using p53 wild type H460 and A459 human lung carcinomas. Tumors of 8 mm diameter were locally irradiated with 2 Gy twice daily for 7 consecutive days with 6 h between daily fractions. Treatment with MK-4827, 50 mg/kg, was started when tumors were 6 mm in diameter and continued 2 days after completion of radiation. When the two agents were combined, MK-4827 was given 1 h before the first daily fraction. The effect of these treatments on tumor growth is shown in Table 2 and Fig. 2. Radiotherapy alone delayed tumor growth of both H460 and A459 human lung carcinomas. To grow from 8 to 12 mm in diameter, for H460 and A459 xenografts, untreated tumors needed 7.8 ± 0.5 days and 33.2 ± 1.5 days respectively; whereas irradiated tumors needed 15.6 ± 0.5 days and 53.2 ± 3.1 days respectively (Table 2). The radiation-induced tumor growth delay was further extended in mice that were also treated with MK-4827: to 26.0 ± 3.5 days and 64.1 ± 4.2 days for H460 and A459 xenografts respectively (Table 2). Thus, MK-4827 enhanced radiation response of p53 wild type H460 and A459 tumors, the EFs were 2.2 and 1.9 respectively (Fig. 2a and b). Thus, p53 tumor status does not seem to influence the ability of MK-4827 to enhance the radiation response of these human tumor xenografts.

Similar experiments were extended to an additional histologic tumor type, the triple negative MDA-MB-231 human breast carcinoma. This tumor is characterized by the lack of protein expression of estrogen receptor (ER), progesterone receptor (PR), and the absence of HER2 protein overexpression. Results presented in Table 2 and Fig. 2c show that radiotherapy alone strongly delayed tumor growth. To grow from 8 to 12 mm in diameter, untreated tumors only needed 23.1 ± 0.9 days; whereas irradiated tumors never reached 12 mm, and were euthan-

Table 2 Effect of MK-4827 on radioresponse of H460, A549, and MDA-MB-231 tumors xenografts in nude mice

Treatment ^a	No. of Mice	Time in days ^b required to grow from 8 to 12 mm	Absolute growth ^c delay	Normalized growth ^d delay	Enhancement ^e factors
H460					
Control (no treatment)	7	7.8±0.5			
XRT only (2 Gy) ×2fx for 7 days	7	15.6±0.5	7.8±0.5		
MK-4827 50 mg/kg ×1/day until day 9 after 8 mm	7	8.4±0.5	0.6±0.5		
MK-4827 (50 mg/kg)+XRT (2 Gy fractionated)	7	26.0±3.5	18.2±3.5	17.6±3.5	2.2
A549					
Control (no treatment)	8	33.2±1.5			
XRT only (2 Gy) ×2fx for 7 days	8	53.2±3.1	20.0±3.1		
MK-4827 50 mg/kg ×1/day until day 9 after 8 mm	7	25.3±1.5	-7.9±1.5		
MK-4827 (50 mg/kg)+XRT (2 Gy fractionated)	7	64.1±4.2	30.9±4.2	38.8±4.2	1.9
MDA-MB-231					
Control (no treatment)	6	23.1±0.9			*
XRT only (2 Gy) ×2fx for 7 days	5				
MK-4827 50 mg/kg ×1/day until day 9 after 8 mm	6	24.6±1.3	-1.5±1.3		
MK-4827 (50 mg/kg)+XRT (2 Gy fractionated)	3				

^a MK-4827 was given by gavage 50 mg/kg once daily when tumors reached 6 mm in diameter. MK-4827 was given until 9 days after tumor reached 8 mm (MK-4827 only) or 2 days after XRT was completed. MK-4827 was given 1 h prior to each XRT of the day. XRT started when tumor reached 8 mm in diameter and was given 6 h in between. Groups consisted of 3–8 mice each

^b Mean tumor diameter + SE.

^c Absolute tumor growth delay (AGD) is the days for tumors grow from 8 to 12 mm in diameter of MK-4827 treatment groups minus the days of no treatment group

^d Normalized tumor growth delay (NGD) is defined as the time in days for tumors to reach 12 mm in the mice treated with the combination of MK-4827 and XRT minus the time in days of tumors to reach 12 mm in mice treated with MK-4827 alone

^e Enhancement factors obtained by dividing NGD in mice treated with MK-4827 plus XRT by the AGD in mice treated with XRT alone.

*Insufficient data. All tumors were small in all XRT groups when the mice died of abdominal metastasis (less than 8.5 mm in diameter for the 3 mice in combination group and less than 12 mm for the 5 mice in XRT only group)

ized at between 36 and 66 days posttreatment due to occurrence of abdominal metastases. The potency of radiation treatment combined with MK-4827 was greater than radiation alone showing essentially no local tumor regrowth until the occurrence of metastases. Thus, MK-4827 strongly enhanced radioresponse of the local tumor but had no effect on distant metastases.

The results of the following experiment showed that MK-4827 was highly effective in reducing the PAR levels in tumors at the time of radiation delivery. Mice bearing 8-mm Calu-6 tumors were given a single gavage of 50 mg/kg MK-4827 and tumors were assayed for PAR 10 min, 1, 2, 5, 9, 25 and 49 h later (Fig. 3). Compared with the untreated control, MK-4827 reduced PAR levels to 76.7±15.7% at 1 h (at the time of the 1st daily radiation dose) and to between 26.4±3.0% and 27.2±3.7% at 2 h to 9 h. PAR levels in tumors were still reduced by about 30% at 25 h but returned to normal levels at 2 days after treatment with MK-4827. Thus, at the time of tumor irradiation, PAR levels, and therefore PARP-activity, were strongly reduced during the 6 h interval between the first and the second radiation dose and at least for 3 h after the second daily radiation dose.

Discussion

The results of the present study add to the growing body of evidence supporting the concept of PARP inhibition as a specific molecular target for combination cytotoxic therapies in cancer treatment, including radiation. The PARP inhibitor used in this study was MK-4827, an orally bioavailable agent that inhibits both PARP-1 and PARP-2 enzymes leading to inhibition of repair of DNA damage [4, 31, 34]. Of potential clinical importance is the finding that radiation response was markedly enhanced by MK-4827 in all four tumor types investigated regardless of p53 status. This implies that the efficacy of this combined treatment modality may have generalized applicability for translation to the clinic. In addition to the significant radiation enhancement observed in three human lung cancer xenografts, it is important to note that an even more robust effect was observed when applied to therapy of a triple negative breast cancer xenograft model.

Several experimental parameters that may influence the efficacy of this agent when combined with radiotherapy were tested. To mimic clinical application of radiotherapy,

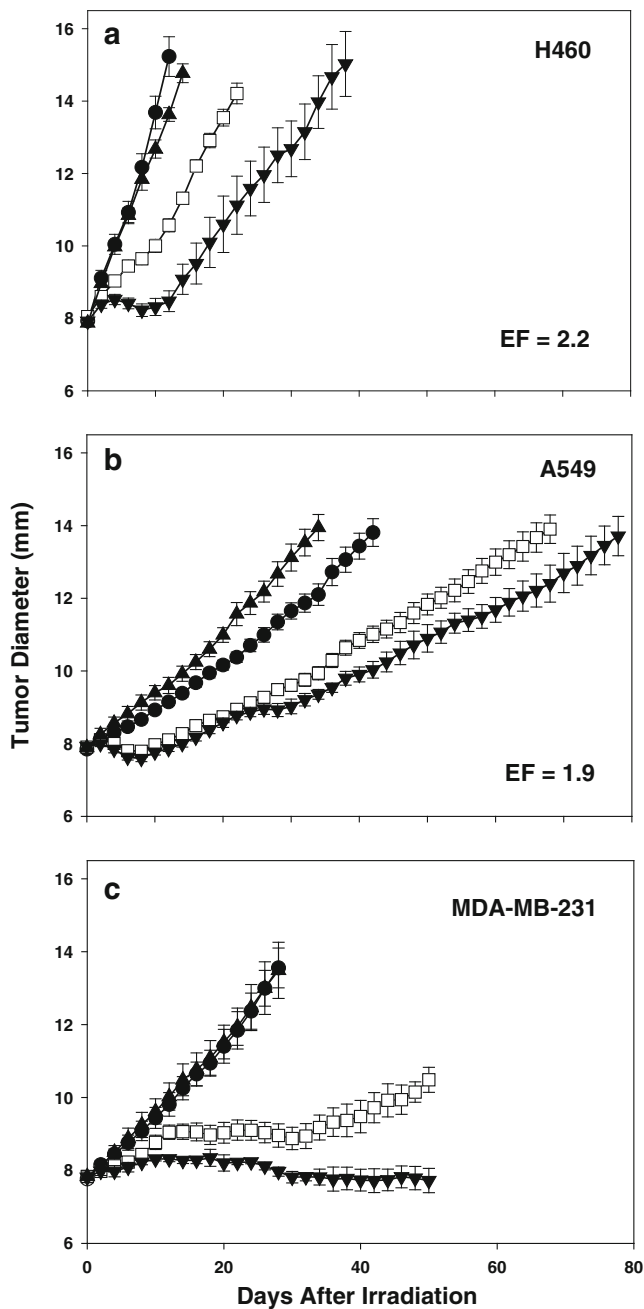


Fig. 2 Effect of MK-4827 on radiation-induced H460, A549, and MDA-MB-231 tumors growth delay. Panels a-c, mice bearing tumors 6 mm in diameter in the leg were: untreated (solid circle) or treated with MK-4827 50 mg/kg once daily (solid upward triangle) until 9 days after tumor reached 8 mm (MK-4827 only) or 2 days after irradiation (XRT) was completed (combination, solid downward triangle). When tumors reached 8 mm, tumors were treated for 7 days (open square) twice per day with fractionated (fx) irradiation (XRT, 2 Gy per fx). On the day when both MK-4827 and radiation were given (solid downward triangle), drug was administered 1 h before each radiation of the day. Each data point represents the mean tumor size of 3 to 8 tumors; bars, SE. EF enhancement factor. Panel c, EF was not calculated due to mice died before tumors reach 12 mm in diameter

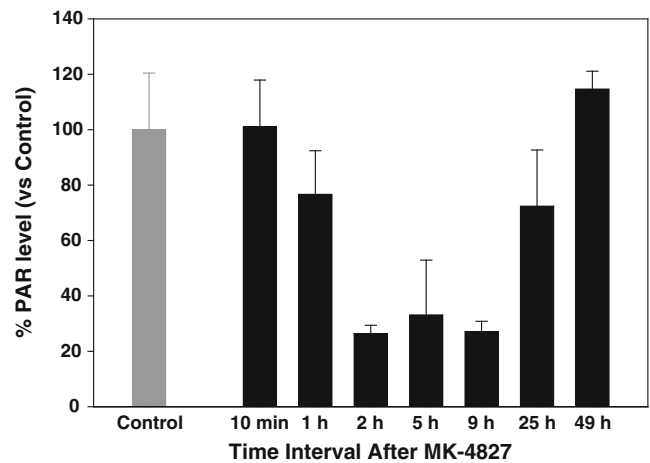


Fig. 3 Effect of MK-4827 on PAR (poly [ADP-ribose]) levels in Calu-6 tumors. Mice bearing tumor 8 mm in diameter in the leg were given a single gavage of 50 mg/kg MK-4827 and tumors were harvested and assayed for PAR level using Enzyme-linked immunosorbent assay (ELISA) at 10 min, 1 h and 2, 5, 9, 25, 49 h later. All PAR levels of the treatment groups were normalized to the untreated control group. Each data point represents the mean value of 3 tumors; bars, SD

we used fractionated radiation given either once or twice daily for a period of 1 to 2 weeks. MK-4827 was found to be highly and similarly effective in both radiation schedules. Second, we observed that the dose of MK-4827 was important in that the maximum interaction with radiation was observed when 50 mg/kg was used once daily compared to 25 mg/kg twice daily. In a Calu-6 tumor model 50 mg/kg resulted in a radiation EF of 2.0 while a much lower EF of 1.4 was achieved with the same total dose but given twice daily (Fig. 1a and b). When combined with radiation, MK-4827 was given 1 h before XRT, at which time PARP levels in tumors were reduced by about one fourth of the normal value (Fig. 3). Even more potent PARP inhibition was demonstrated 2 to 9 h after MK-4827 administration, and PARP inhibition persisted for 24 h (Fig. 3). This long period of PARP inhibition adds to the potential flexibility of administration in clinical settings.

In contrast to a relatively large number of *in vitro* studies on combination of newer generation PARP inhibitors with radiation, the *in vivo* preclinical studies on this subject are limited [22, 23, 25–27, 35]. The PARP-1 inhibitor AG14361 (15 mg/kg ip once daily for 5 days 30 min before each of 5 daily fractions of 2 Gy) enhanced radiation-induced tumor growth delay of SW620 and LoVo human colon carcinoma xenografts by two fold [23]. Another PARP inhibitor, GPI-15427 administered to mice bearing JHU006 or JHU012 human head and neck squamous cell carcinoma xenografts (10 to 300 mg/kg orally 1 h before 2 Gy radiation daily for 2 days) increased radiation-induced tumor growth delay in a supra-additive manner [25]. The effect was greater as the drug dose was

increased. Two reports dealt with ABT-888, another inhibitor of both PARP-1 and PARP-2 enzymes [22, 27]. One study reported that ABT-888 administered ip at a dose of 25 mg/kg daily for 5 consecutive days in combination with fractionated radiotherapy (2 Gy daily for 5 days 1 h after ABT-888) enhanced radiation-induced tumor growth delay of H460 lung carcinoma xenografts by EF of about 1.7 [27]. In another study, ABT-888 administered sc in an osmotic pump enhanced mouse survival and radiation-induced tumor growth delay of the HCT-116 colon carcinoma xenografts in a drug-dose dependent manner [22]. Another PARP inhibitor, E7016, was combined with temozolomide plus local tumor irradiation (1 fraction of 4 Gy 6 h after 3 mg/kg E7016 administered orally) of nude mice bearing U251 glioblastoma xenografts. The triple agent combination appeared to be only slightly more than additive [26]. Compared to the results of these reports, our data showed that the radio-enhancing efficacy of MK-4827 was generally more robust than that demonstrated by other PARP inhibitors. Our experiments showed that MK-4827 enhanced tumor radioresponses by factors of 2 and above, values rarely achieved by other PARP inhibitors.

In conclusion, the current study presented the first evidence that MK-4827, a novel, orally bioavailable, PARP-1 and PARP-2 inhibitor, strongly enhanced the effect of radiation on a variety of human tumor xenografts, both p53 wild type and p53 mutant. It is important that the enhancement of radiation response was observed in clinically relevant radiation-dose fractionation schedules. The therapeutic window during which time MK-4827 interacted with radiation lasted for several hours. These biological attributes make translation of this therapeutic combination treatment feasible for translation to the treatment of a variety of human cancers.

Acknowledgement This study was sponsored by a Laboratory Study Agreement with Merck Sharp & Dohme Corp.

Conflict of Interest Authors Anjili Mathur, Carolyn Buser-Doepner and Carlo Toniatti are current or former employees of Merck Sharp & Dohme Corp.

All other authors declare that they have no conflict of interest.

References

- Chambon P, Weill JD, Mandel P (1963) Nicotinamide mononucleotide activation of a new DNA-dependent polyadenylic acid synthesizing nuclear enzyme. *Biochem Biophys Res Commun* 11:39–43
- Jagtap P, Szabo C (2005) Poly(ADP-ribose) polymerase and the therapeutic effects of its inhibitors. *Nat Rev Drug Discov* 4:421–440
- Schreiber V, Dantzer F, Ame JC, de Murcia G (2006) Poly(ADP-ribose): novel functions for an old molecule. *Nat Rev Mol Cell Biol* 7:517–528
- Chalmers A, Johnston P, Woodcock M, Joiner M, Marples B (2004) PARP-1, PARP-2, and the cellular response to low doses of ionizing radiation. *Int J Radiat Oncol Biol Phys* 58:410–419
- Plummer ER (2006) Inhibition of poly(ADP-ribose) polymerase in cancer. *Curr Opin Pharmacol* 6:364–368
- Satoh MS, Lindahl T (1992) Role of poly(ADP-ribose) formation in DNA repair. *Nature* 356:356–358
- Gerö D, Szabó C (2008) Poly(ADP-ribose) polymerase: a new therapeutic target? *Curr Opin Anaesthesiol* 21:111–121
- Durkacz BW, Omidiji O, Gray DA, Shall S (1980) (ADP-ribose)_n participates in DNA excision repair. *Nature* 283(5747):593–596
- Shieh WM, Ame JC, Wilson MV, Wang ZQ, Koh DW, Jacobson MK, Jacobson EL (1998) Poly(ADP-ribose) polymerase null mouse cells synthesize ADP-ribose polymers. *J Biol Chem* 273:30069–30072
- Clark JB, Ferris GM, Pinder S (1971) Inhibition of nuclear NAD nucleosidase and poly ADP-ribose polymerase activity from rat liver by nicotinamide and 5'-methyl nicotinamide. *Biochim Biophys Acta* 238:82–85
- Underhill C, Toulmonde M, Bonnefoi H (2010) A review of PARP inhibitors: from bench to bedside. *Ann Oncol* 22(2):268–279
- Curtin NJ (2005) PARP inhibitors for cancer therapy. *Expert Rev Mol Med* 7(4):1–20
- Zaremba T, Curtin NJ (2007) PARP inhibitor development for systemic cancer targeting. *Anticancer Agents Med Chem* 7:515–523
- Comen EA, Robson M (2010) Inhibition of poly(ADP-ribose) polymerase as a therapeutic strategy for breast cancer. *Oncology (Williston Park)* 24(1):55–62
- Brock WA, Milas L, Bergh S, Lo R, Szabo C, Mason KA (2004) Radiosensitization of human and rodent cell lines by INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase. *Cancer Lett* 205(2):155–160
- Mason KA, Valdecanas D, Hunter NR, Milas L (2008) INO-1001, a novel inhibitor of poly(ADP-ribose) polymerase, enhances tumor response to doxorubicin. *Invest New Drugs* 26(1):1–5
- Telli ML, Ford JM (2010) PARP Inhibitors in Breast Cancer. *Clin Adv Hematol Oncol* 8(9):629–635
- Peralta-Leal A, Rodríguez-Vargas JM, Aguilar-Quesada R, Rodríguez MI, Linares JL, de Almodóvar MR, Oliver FJ (2009) PARP inhibitors: New partners in the therapy of cancer and inflammatory diseases. *Free Radic Biol Med* 47(1):13–26
- Lewis C, Low JA (2007) Clinical poly(ADP-ribose) polymerase inhibitors for the treatment of cancer. *Curr Opin Investig Drugs* 8(12):1051–1056
- Southan GJ, Szabó C (2003) Inhibitors of poly(ADP-ribose) polymerase. *Curr Med Chem* 10(4):321–340
- Noël G, Godon C, Fernet M, Giocanti N, Mégnin-Chanet F, Favaudon V (2006) Radiosensitization by the poly(ADP-ribose) polymerase inhibitor 4-amino-1,8-naphthalimide is specific of the S phase of the cell cycle and involves arrest of DNA synthesis. *Mol Cancer Ther* 5(3):564–574
- Donawho CK, Luo Y, Luo Y, Penning TD et al (2007) ABT-888, an orally active poly(ADP-Ribose) polymerase inhibitor that potentiates DNA-damaging agents in preclinical tumor models. *Clin Cancer Res* 13(9):2728–2737
- Calabrese CR, Almassy R, Barton S et al (2004) Anticancer chemosensitization and radiosensitization by the novel poly(ADP-ribose) polymerase-1 inhibitor AG14361. *J Natl Cancer Inst* 96(1):56–67
- Dungey FA, Caldecott KW, Chalmers AJ (2009) Enhanced radiosensitization of human glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition of heat shock protein 90. *Mol Cancer Ther* 8(8):2243–2254

25. Khan K, Araki K, Wang D et al (2010) Head and neck cancer radiosensitization by the novel poly (ADP-ribose) polymerase inhibitor GPI-15427. *Head Neck* 32(3):381–391
26. Russo AL, Kwon HC, Burgan WE et al (2009) In vitro and in vivo radiosensitization of glioblastoma cells by the poly (ADP-Ribose) polymerase inhibitor E7016. *Clin Cancer Res* 15(2):607–612
27. Albert JM, Cao C, Kim KW et al (2007) Inhibition of poly(ADP-ribose) polymerase enhances cell death and improves tumor growth delay in irradiated lung cancer models. *Clin Cancer Res* 13(10):3033–3042
28. Efimova EV, Mauceri HJ, Golden DW et al (2010) Poly(ADP-Ribose) polymerase inhibitor induces accelerated senescence in irradiated breast cancer cells and tumors. *Cancer Res* 70(15):6277–6282
29. Thomas HD, Calabrese CR, Batey MA et al (2007) Preclinical selection of a novel poly(ADP-ribose) polymerase inhibitor for clinical trial. *Mol Cancer Ther* 6(3):945–956
30. Dungey FA, Löser DA, Chalmers AJ (2008) Replication-dependent radiosensitization of human glioma cells by inhibition of poly(ADP-ribose) polymerase: mechanisms and therapeutic potential. *Int J Radiat Oncol Biol Phys* 72(4):1188–1197
31. Jones P, Altamura S, Boueres J et al (2009) Discovery of 2-{4-[(3S)-piperidin-3-yl]phenyl}-2H-indazole-7-carboxamide (MK-4827): a novel oral poly(ADP-ribose)polymerase (PARP) inhibitor efficacious in BRCA-1 and -2 mutant tumors. *J Med Chem* 52(22):7170–7185
32. Penning TD (2010) Small-molecule PARP modulators – current status and future therapeutic potential. *Curr Opin Drug Discov Devel* 13(5):577–578
33. Sandhu S, Wenham R, Wilding G, McFadden M, Sun L, Toniatti C, Stroh M, Carpenter C, De Bono J, Schelman W (2010) First-in-human trial of a poly(ADP-ribose) polymerase (PARP) inhibitor MK-4827 in advanced cancer patients (pts) with antitumor activity in BRCA-deficient and sporadic ovarian cancers. ASCO Annual Meeting, Chicago, IL, USA (2010):Abs 3001
34. Ménissier de Murcia J, Ricoul M, Tartier L et al (2003) Functional interaction between PARP-1 and PARP-2 in chromosome stability and embryonic development in mouse. *EMBO J* 22:2255–2263
35. Powell C, Mikropoulos C, Kaye SB, Nutting CM, Bhide SA, Newbold K, Harrington KJ (2010) Pre-clinical and clinical evaluation of PARP inhibitors as tumour-specific radiosensitisers. *Cancer Treat Rev* 36(7):566–575