

# Pharmacodynamics of Four Reported Inhibitors of Poly(ADP-ribose) Polymerase: ABT-888, AZD2281, MK-4827, and BSI-201

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## Introduction

Poly(ADP-ribose) (PAR) polymerases 1 and 2 (PARP1/2) are activated in response to DNA single-strand breaks and are pivotal to repairing damage in homologous recombination-defective and chemotherapy-damaged cells with the base excision repair pathway.<sup>1,2</sup>

ABT-888, AZD2281, MK-4827, and BSI-201 are reported PARP inhibitors currently being evaluated in phase 1-3 clinical trials.<sup>3-6</sup> Each agent has been evaluated either as a single agent in patients with BRCA-deficient malignancies or in combination with DNA-damaging chemotherapeutic agents; however, BSI-201 recently failed to meet phase 3 study objectives for survival.<sup>7</sup>

The pharmacodynamics of these 4 agents were determined in the A375 human melanoma xenograft model and several in vitro models using the validated PAR immunoassay and an immunofluorescence assay for phosphorylated histone H2AX (γH2AX), a marker of DNA damage.

To exclude the possibility that the lack of in vivo PARP inhibition by BSI-201 was due to species differences in drug metabolism, two BSI-201 metabolites, NSC D753688 (amino metabolite) and NSC D753689 (nitroso metabolite), were tested in two human breast cancer cell lines (MCF7 and BRCA1-defective MX-1) and healthy volunteer peripheral blood mononuclear cells (PBMCs).

Finally, gene expression analysis in MX-1 cells was performed after treatment with BSI-201, using ABT-888 for comparison, to provide direction for investigating the mechanism of action of BSI-201.

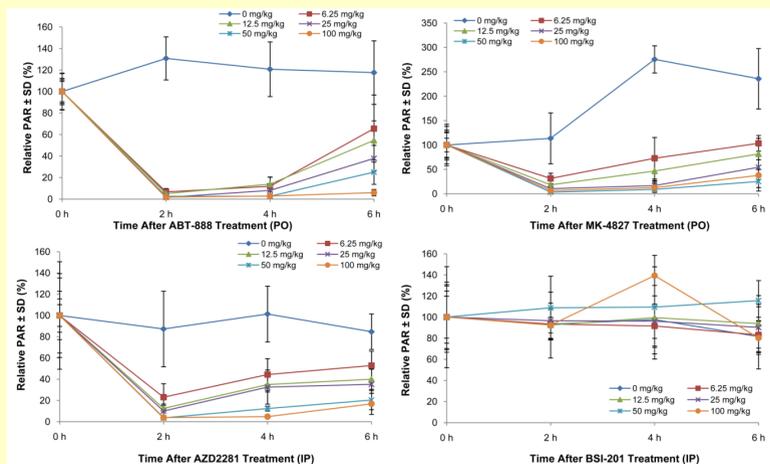
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## Acknowledgments

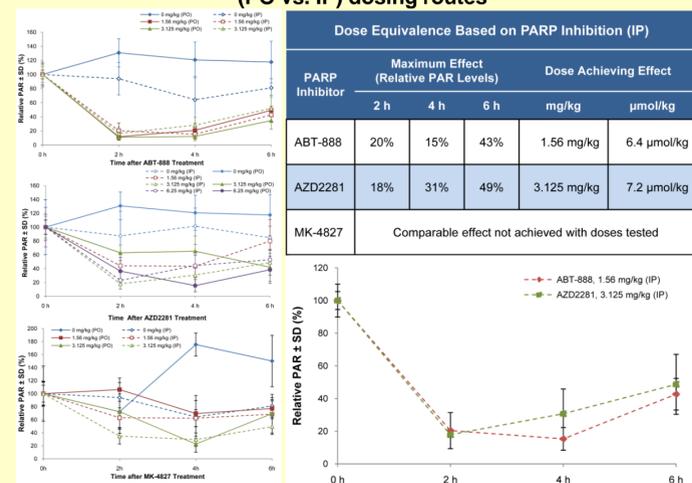
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## Treatment with ABT-888, MK-4827, and AZD2281 decreased PAR levels in A375 xenografts, but treatment with BSI-201 did not



Mean PAR levels (SD) relative to baseline (100%) in A375 tumor xenograft biopsies collected 2, 4, and 6 h after a single dose of ABT-888, MK-4827, AZD2281, or BSI-201 (n = 6 mice/dose/time point). PAR levels were significantly decreased by 2 h (P ≤ 0.001, Student's t-test) in samples from all but the BSI-201-treated animals, suggesting that BSI-201 is not a PARP1/2 inhibitor. The peak PAR levels at 4 h in the highest BSI-201 dose cohort is characteristic of response to cytotoxic chemotherapy.<sup>8</sup>

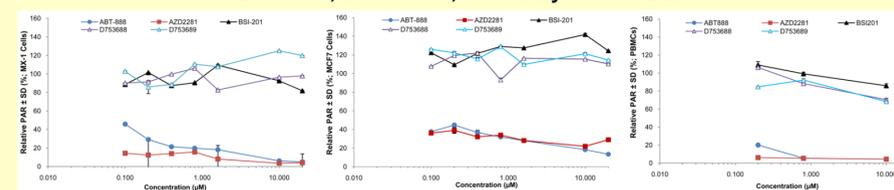
## Comparison of PAR levels when inhibitors were administered by alternate (PO vs. IP) dosing routes



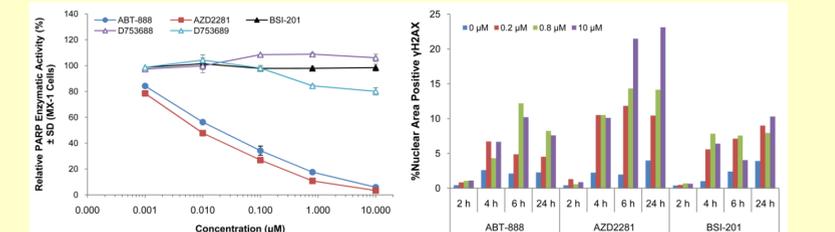
PARP inhibitors ABT-888, MK-4827, and AZD2281 were administered to mice bearing A375 tumor xenografts at a limited number of doses PO and IP, and samples were collected at 2, 4, and 6 h to compare equivalence of PARP inhibition using different dosing routes. Solid lines, PO; dashed lines, IP; color, dose level. ABT-888 given either IP or PO produced equivalent PARP inhibition. PARP inhibition by 3.125 mg/kg AZD2281 or MK-4827 was greater with IP than with PO administration, and the nadir in PAR levels after administering 6.25 mg/kg AZD2281 or 3.125 mg/kg MK-4827 IP occurred later than when administered PO (left panels). These data suggest differences in oral bioavailability between MK-4827 and AZD2281.

IP doses of ABT-888 and AZD2281 yielding similar magnitude and kinetics of PARP inhibition have been compared and found to have similar potencies when the dose was converted to μmol/kg (right panels). Higher doses of MK-4827 are being tested to achieve equivalent PARP inhibition for comparison. Mean PAR levels (SD) relative to baseline (100%) presented (n = 6 mice/dose/time point).

## Unlike ABT-888 and AZD2281, BSI-201 did not inhibit PARP1 in BRCA-deficient MX-1 cells, MCF7 cells, or healthy donor PBMCs

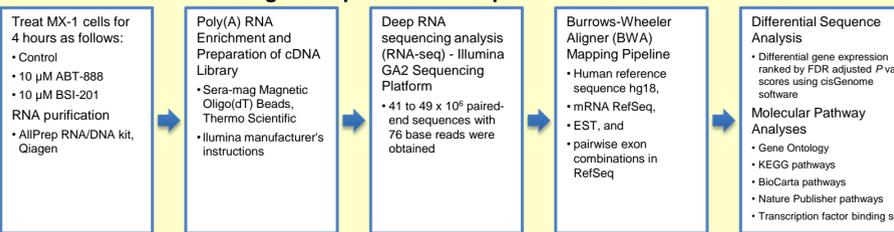


Unlike ABT-888 and AZD2281, BSI-201 and its metabolites did not inhibit PARP1 activity as measured by PAR levels in BRCA1-defective MX-1 cells, breast adenocarcinoma MCF7 cells, or healthy donor PBMCs after treatment for 2 h with drug concentrations ranging from 0.1 to 20 μM. Mean PAR levels (SD) relative to baseline (100%) presented from 2 replicates.



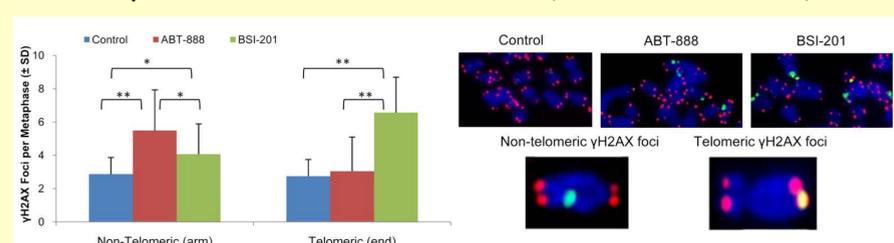
Direct inhibition of PARP1 enzymatic activity was measured using a PARP1 enzymatic assay after MX-1 cell extracts were treated for 0.5 h with PARP inhibitors; mean PARP activity (SD) relative to baseline (100%) presented from 2 replicates (left panel). PARP1 inhibition as measured by PARP1 enzymatic activity was consistent with that measured with the PAR immunoassay; however, ABT-888, AZD2281, and BSI-201 each induced dose- and time-dependent increases in γH2AX in MX-1 cells, indicating that all 3 agents cause DNA damage against a BRCA1-defective background (right panel).

## Differential gene expression in response to BSI-201 and ABT-888



Sequences with > 90% identity to the reference sequences were used for differential analyses. Gene ontology and pathway analyses of the 100 most differentially expressed genes indicated that different molecular pathways were affected by the two agents. Analysis is ongoing, but BSI-201 specifically suppressed genes in the telomere maintenance pathway, including DKC1 and PINX1.

## Telomeric γH2AX foci are induced in BSI-201-treated, but not ABT-888-treated, MX-1 cells



To explore the possible involvement of BSI-201 at the telomere, MX-1 cells were cultured for 20 h with 10 μM ABT-888 or BSI-201, and metaphase chromosome slides were prepared. Slides were stained with γH2AX antibody (green foci), analyzed for telomeres by FISH (PNA probe, pink foci), and counterstained for DNA with DAPI (blue). Representative metaphase spread images are shown; γH2AX induced by BSI-201 and ABT-888 were scored according to telomere proximity. Yellow foci indicate γH2AX and telomere signal colocalization. Mean foci/metaphase spread (SD, n = 40) are presented from 2 independent experiments. The mean number of γH2AX foci were compared between non-telomeric and telomeric groups. Bars indicate comparison of statistically different groups; \* indicate a significance level of P ≤ 0.001 and \*\* a significance level of P ≤ 0.0001 as determined by Student's t-test.

## Conclusions

- Using our validated quantitative immunoassay for PAR<sup>8</sup>, PARP1/2 inhibition was dose and time dependent in response to treatment with ABT-888, AZD2281, or MK-4827, but not BSI-201.
- Treatment of BRCA-deficient MX-1 cells, MCF7 cells, and healthy donor PBMCs confirmed lack of PARP inhibition by BSI-201, although dose- and time-dependent increases in γH2AX occurred in MX-1 cells independent of PARP inhibition.
- Location of γH2AX foci differed in BRCA1-defective cells treated with BSI-201 or ABT-888. While both BSI-201 and ABT-888 increased γH2AX at non-telomeric foci, only BSI-201 increased telomeric γH2AX foci, in agreement with differential gene expression and sequence analysis.
- ABT-888, AZD2281, and MK-4827 are PARP1/2 inhibitors, but BSI-201 is not.

## Methods

AZD2281 (NSC 753686), ABT-888 (NSC 752840), and BSI-201 (NSC 752493) were purchased from a commercial vendor; a certificate of analysis provided by the vendor confirmed > 98% purity for each compound. MK-4827 (NSC 754355) and NSCs D753688 and D753689 were synthesized by DCTD, NCI, based on publically available information. Agent purity (> 98%) and structures for each compound were confirmed by HPLC, <sup>1</sup>H NMR, and MS. A375 tumor xenograft experiments were performed as described<sup>8</sup> using doses and schedules indicated in the figure legends.

The validated PAR immunoassay and γH2AX immunofluorescence assay were performed as described.<sup>8,9</sup>

The chemiluminescent PARP activity enzyme assay (Trevigen) was performed per manufacturer's instructions; PBMCs from anonymous healthy donors were obtained at the National Cancer Institute-Frederick.

Differential gene expression methodology is outlined in the figure and figure legend. Chromosome metaphase immunocytochemistry and telomere FISH analysis were performed as described.<sup>10</sup>