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 recombinaton-defective and chemotherapy-treated cells with the base excision repair pathway.1,2 ABT-888, AZD2281, MK-4827, and BSI-201 are reported PARP inhibitors currently being evaluated in phase 1-3 clinical trials.3,4 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.5 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 757388 (amino metabolite) and NSC 757389 (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.

Methods

Pharmacodynamic and gene expression comparisons were performed after treatment with 10 µM ABT-888 or BSI-201 (NSC 752493) for 2 h in A375 xenografts, but treatment with BSI-201 did not affect γH2AX staining (data not shown). Treatment with ABT-888, MK-4827, and BSI-201 significantly decreased γH2AX foci in A375 xenografts, but treatment with BSI-201, although dose- and time-dependent increases in γH2AX occurred in MX-1 cells independent of PARP inhibition. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends.

Conclusions

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 and DAPI, analyzed for telomere signal colocalization with γH2AX foci, and scored by two independent technicians. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends. The validated PAR immunoassay and γH2AX assay were performed as described6 using doses and schedules indicated in the figure legends.

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References

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