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Proteomic profiling of FEN1 inhibition by BSM-1516 reveals chromatin-associated biomarkers for preclinical pharmacodynamic evaluation

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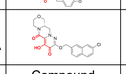
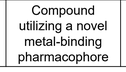
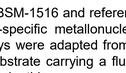
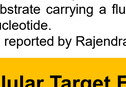
Abstract

Flap endonuclease 1 (FEN1) is a structure-specific metallonuclease essential for Okazaki fragment maturation and DNA repair. We previously reported the discovery of BSM-1516, a potent and selective small-molecule FEN1 inhibitor that synergizes with PARP-targeted and other DNA damage response therapies and exhibits favorable in vivo pharmacokinetic properties. Pharmacologic inhibition of FEN1 increases its chromatin association, induces poly(ADP-Ribosyl)ation and ssDNA gaps, and is selectively cytotoxic to cells with homologous recombination deficiency.

To characterize chromatin protein dynamics following FEN1 inhibition and identify potential pharmacodynamic (PD) biomarkers of target engagement, we employed isolation of Proteins On Nascent DNA (iPOND) coupled to mass spectrometry in proliferating cells treated with BSM-1516, alone or in combination with olaparib. FEN1 inhibition reproducibly enriched replication and DNA repair proteins, including FEN1, PARP1/2, LIG3, XRCC1, and CHD1L, reflecting PARP-dependent engagement of an alternative Okazaki fragment maturation pathway that was abrogated by co-treatment with olaparib.

Orthogonal assays for chromatin-bound proteins confirmed selective enrichment of several iPOND-identified hits, establishing tractable PD biomarker candidates. Collectively, these findings delineate a proteomic signature of FEN1 inhibition at the replication forks and lay the groundwork for ongoing in vivo studies assessing these markers as indicators of target engagement in preclinical models.

Biochemical assays: Potency and selectivity of BSM-1516 compared to historic FEN1 inhibitors

Company	Structure	ID	FEN1 IC_{50} (μM)	EXO1 IC_{50} (μM)	FEN1 vs. EXO1 selectivity (fold)
Astra Zeneca		Cmpd 4	0.21	0.72	3.4
Athersys		Cmpd 8	0.13	0.41	3.2
Ideaya		Cmpd 12	2.7	14	5.2
Artios / Merck KGaA		MSC778	0.003*	0.871*	270
Blacksmith Medicines	Compound utilizing a novel metal-binding pharmacophore	BSM-1516	0.007	0.46	65

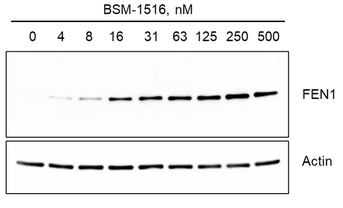
Potency of BSM-1516 and reference compounds against FEN1 and EXO1 (members of RAD2 family of structure-specific metallonucleases) was measured in enzymatic assays. These fluorescence-based assays were adapted from van Pel et al., PLOS Genetics 2013 and utilized a flap-containing synthetic substrate carrying a fluorophore on the 5' flap and a quencher at the opposite end of the DNA oligonucleotide.

* - IC_{50} were reported by Rajendra et al., 2025.

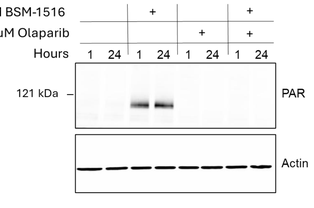
Cellular Target Engagement and Mechanism of Action

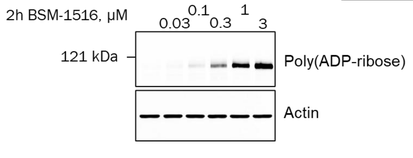
BSM-1516 stabilizes endogenous and overexpressed FEN1 and increases poly-ADP-ribosylation. **Left**: cellular thermal shift assay (CETSA™) was performed using InCELL Pulse™ target engagement assay (DiscoverX) in stably transfected HEK293 cells overexpressing FEN1 catalytic domain with C-terminal ePL tag. **Right**: cellular thermal shift performed on endogenous FEN1, detected by Western blotting, demonstrate target engagement by BSM-1516, with an EC_{50} of 21 nM calculated as the average of three independent experiments.

Company	ID	CETSA EC_{50} (μM)
Astra Zeneca	Cmpd 4	1.2
Athersys	Cmpd 8	0.21
Ideaya	Cmpd 12	>100
Blacksmith Medicines	BSM-1516	0.024



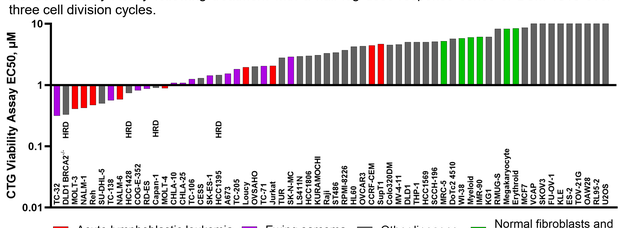
Western blot analysis of poly(ADP-ribose) (PAR) in asynchronous cells treated with a half-log dose-response series of BSM-1516 shows rapid and robust PARylation within 1 hour, with Olaparib co-treatment serving as a control that effectively blocks BSM-1516-induced PARylation.

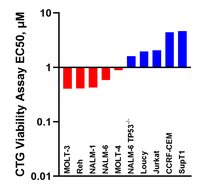




Acute lymphoblastic leukemia, Ewing Sarcoma and HRD cancer cells are hypersensitive to BSM-1516

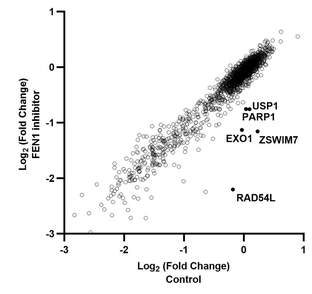
To explore BSM-1516 potential as anti cancer agent, we performed a screening assay using a panel of cancer cell lines representing diverse lineages, as well as normal fibroblasts and hematopoietic cells differentiated *in vitro* from donor cord blood. Cell viability was assessed using the CellTiter-Glo® 2.0 Cell Viability Assay following treatment with a half-log dose-response series of BSM-1516 over three cell division cycles.





ALL, EwS and HRD cell lines emerged as the most sensitive to FEN1 inhibition, consistent with prior reports (Rajendra et al., 2025; Mengwasser et al., 2019; Guo et al., 2020). Importantly, lineage-specific hematopoietic cell toxicity assays revealed limited effect of BSM-1516 on erythroid, myeloid and megakaryocyte-specific cultures derived from donors' cord blood, with calculated EC_{50} values supporting a therapeutic window of ~10 fold. Likewise, BSM-1516 showed only limited effects on normal human fibroblast (n=3) proliferation, reinforcing a favorable ~10-fold therapeutic window between tumor and normal cells. Wild-type TP53 ALL cells are more sensitive to the FEN1 inhibitor BSM-1516 than TP53-mutant cells, an observation further supported by isogenic NALM-6 $TP53^{-/-}$ cell line.

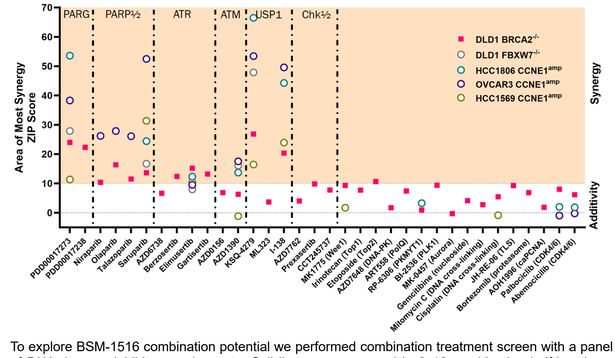
CRISPR screen identifies genes that are synthetic lethal with FEN1 inhibition



To identify genetic determinants driving FEN1 inhibitor sensitivity we performed CRISPR-Cas9 loss-of-function screen using lentiviral sgRNA sub-library that targets DNA damage response genes and metalloenzymes. Data on the graph were plotted as ratio of sgRNA abundance on day 14 versus day 0 for control and for FEN1-inhibitor (2μM) treated DLD1 cells.

Our screen confirmed several previously published FEN1 gene interactors including USP1 (Fielden et al., 2023 bioRxiv), PARP1 (Hanzlikova et al., 2018), EXO1 (Tishkoff et al., 1997) and homologous recombination repair (HRR) pathway defects (Mengwasser et al., 2019; Guo et al., 2020). PARG and WDR48 genes were not targeted by the library.

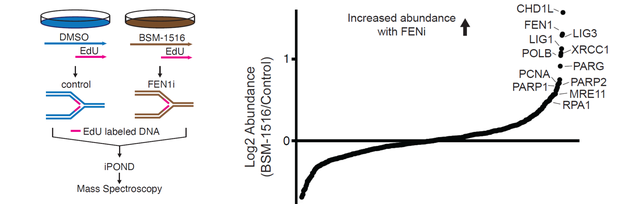
BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, and ATR in $BRCA2^{-/-}$ and $CCNE1^{amp}$ cell models

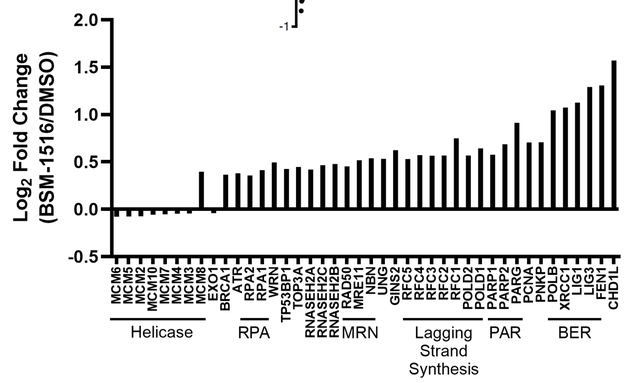


To explore BSM-1516 combination potential we performed combination treatment screen with a panel of DNA damage inhibitors and agents. Cell lines were assayed in 6x10 combination half-log dose-response matrix using CellTiter-Glo® 2.0 Cell Viability Assay with media change every 3 days. Synergy scores were calculated using Synergy Finder.

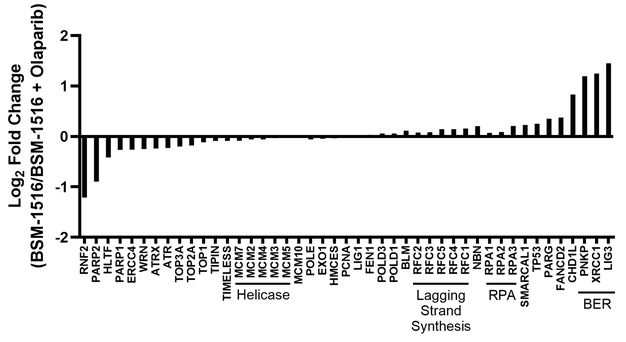
iPOND-SILAC-MS

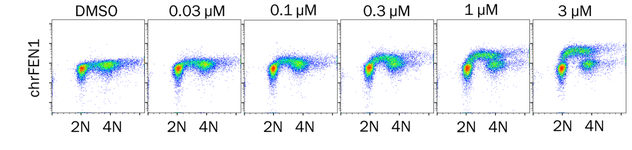
To gain a broad view of protein changes at replication forks in cells exposed to BSM-1516, we employed a proteomics technique, Isolation of Proteins On Nascent DNA (iPOND), coupled to mass spectrometry (MS). iPOND-MS data revealed that a 30-minute treatment of HEK 293T cells with 3 μM BSM-1516 led to enrichment of Okazaki fragment maturation (OFM) proteins, with FEN1 emerging as the second strongest 'hit'. PARP1/2 enzymes and the 'backup" OFM pathway repair proteins XRCC1 and LIG3 were also enriched. This result of two independent iPOND experiments aligns with FEN1's role in DNA replication and supports the on-target activity of BSM-1516. Recruitment of PARP1/2 enzymes suggests that BSM-1516 induces replication stress by causing the accumulation of unligated Okazaki fragments on the lagging DNA strand.

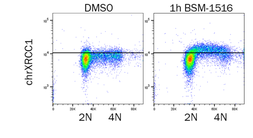




To further interrogate this response, we compared BSM-1516-treated HEK293T cells (3 μM, 30 minutes) with cells co-treated with the PARP inhibitor Olaparib (10 μM, 30 minutes), incorporating a label-swap design to control for labeling bias. PARP inhibition abolished the recruitment of PAR-binding proteins, as expected, while co-treatment enhanced PARP2 trapping without affecting FEN1 recruitment.





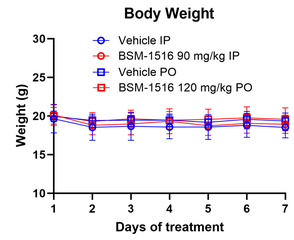


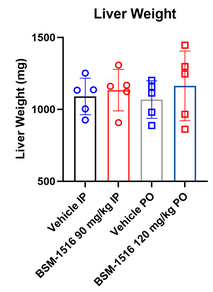
Intracellular staining of endogenous chromatin-bound FEN1 in DLD1 cells treated with a half-log dose-response series of BSM-1516 for 16h. Soluble FEN1 was extracted with detergent prior to fixation and subsequent analysis by flow cytometry. Similar analysis was performed for XRCC1 in cells treated for 1h.

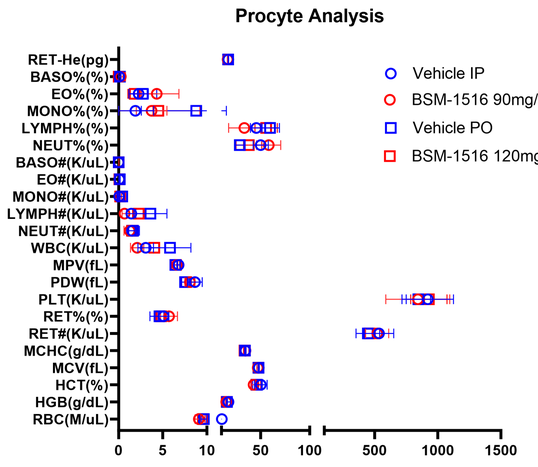
7-day repeat dose tolerability study in mice

No clinical observations in 7-day tolerability study of BSM-1516.

Biochemistry analyses showed no changes in alanine transaminase, aspartate transaminase, alkaline phosphatase, creatinine and phosphorus; bilirubin was not detected.

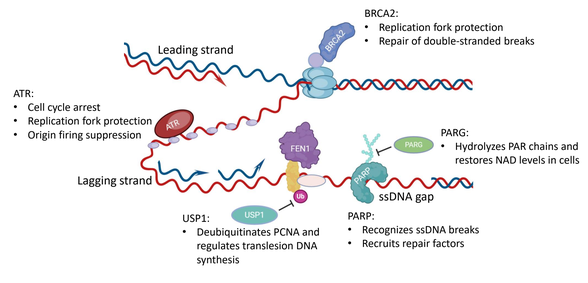






Summary

- Blacksmith Medicines FBDD approach for metalloenzymes identified a series of cell active FEN1-selective inhibitors that use a novel metal-binding pharmacophore.
- ALL and Ewing sarcoma cells as well as HRD cells are hypersensitive to pharmacologic inhibition of FEN1.
- BSM-1516 synergizes with inhibitors of USP1, PARP, PARG, ATR and Chk1.
- BSM-1516 exhibits favorable *in vivo* PK properties and is well tolerated in mice in a 7-day study.



Acknowledgements

Authors are grateful to Chris Lord, Jeff Hager, Neil Gibson, Mark Whittaker, Seth Cohen, Stephen Durant / Evotec DDR team, James Karras, Min Teng, Baskar Nammalwar, Elaine Guo for support and helpful discussions.