ASN-3186 is a potent and selective inhibitor of USP1 for the treatment of BRCA1/2 mut and HRD+ cancers

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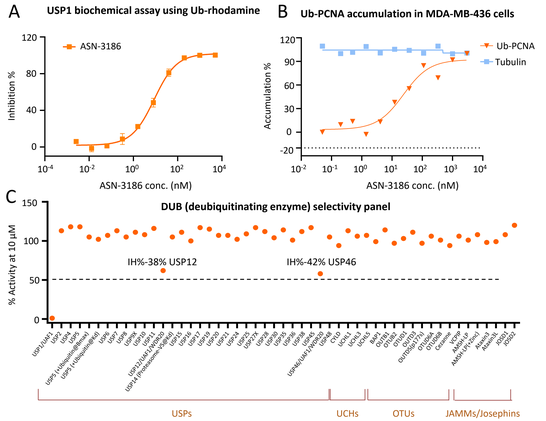
Introduction

Ubiquitin Specific Peptidase 1 (USP1) is a key regulator of DNA translesion synthesis and the Fanconi Anemia DNA repair pathway[1,2]. USP1 removes ubiquitin from a variety of substrates (PCNA, FANCI, FANCD2, PARP1, EZH2, CHK1, etc.) that are critically involved in DNA damage repair (DDR)[3]. USP1 inhibitors might treat certain cancers with DDR vulnerability.

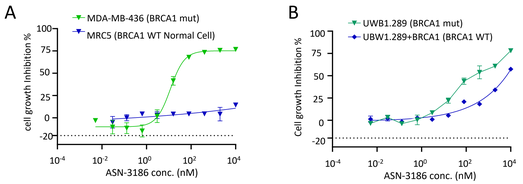
ASN-3186 is a selective and potent inhibitor of the deubiquitinating enzyme USP1. ASN-3186 treatment led to cell death in breast cancer cell lines with BRCA1/2 mutations. ASN-3186 demonstrated robust cell-killing synergy when combined with first- or second-generation PARP inhibitors (Olaparib/Saruparib). Moreover, ASN-3186 exhibited strong tumor growth inhibition in combination with PARPi in BRCA1/2mut and HRD- (homologous recombination deficiency) positive xenograft models with primary PARPi resistance. In head-to-head studies, ASN-3186 was found to be more efficacious than KSQ-4279 (a reported USP1 inhibitor)[4] as a monotherapy or in combination with Olaparib in tumor models with BRCAmut. Further development of ASN-3186 as a potential best-in-class USP1 inhibitor is being planned.

ASN-3186 is a potent and selective USP1 inhibitor



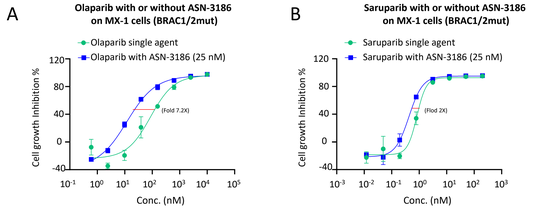
(A) In the biochemical assay, ASN-3186 exhibited potent inhibition of USP1 deubiquitinating enzymatic activity. (B) ASN-3186 dose-dependently induced Ub-PCNA accumulation by Western blot analysis of MDA-MB-436 cells treated with ASN-3186 for 6 hrs. (C) ASN-3186 showed high selectivity against a panel of DUB enzymes representing the entire human DUB family. IH%: inhibition %.

ASN-3186 suppressed tumor cell growth *in vitro*



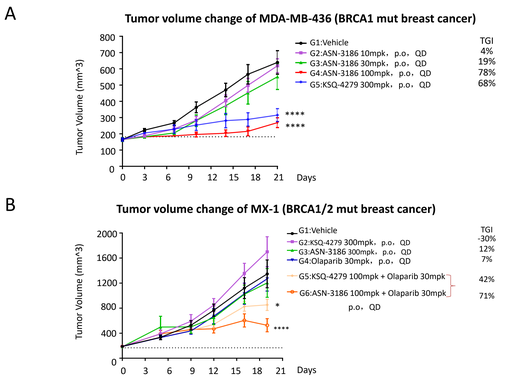
(A) ASN-3186 robustly inhibited proliferation of MDA-MB-436 breast cancer cells carrying BRCA1mut but not normal lung fibroblast cell MRC-5. (B) UBW1.289+BRCA1 cells were engineered to express WT BRCA1 on mut BRCA1 background. UWB1.289 proliferation was suppressed much more dramatically by ASN-3186 than UWB1.289+BRCA1 cells. Cell survival was determined by CellTiter Glo.

ASN-3186 synergized with PARPi *in vitro*



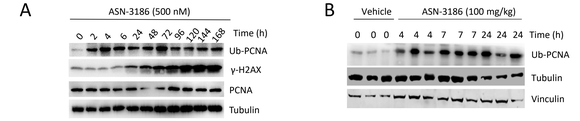
(A, B) Synergistic activity of ASN-3186 with Olaparib/Saruparib was observed in MX-1 breast cancer cells.

ASN-3186 was more efficacious than KSQ-4279 *in vivo*



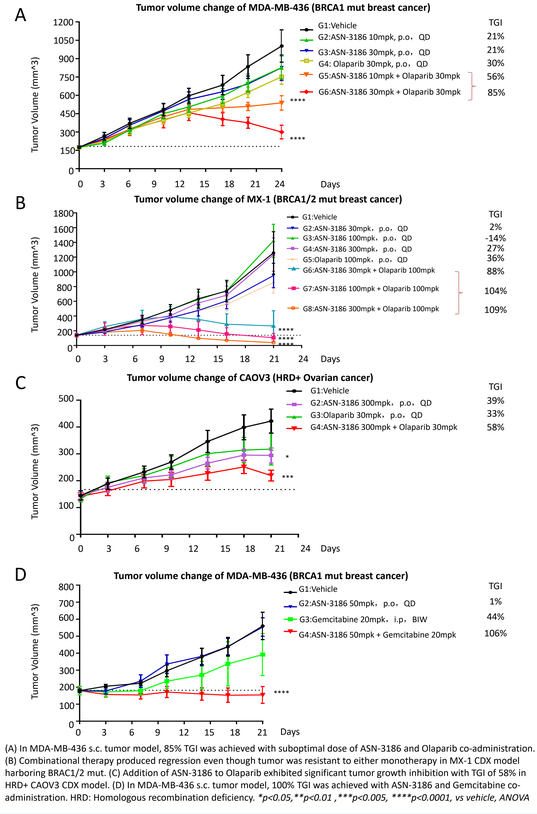
(A) 100 mg/kg of ASN-3186 produced comparable anti-tumor efficacy to KSQ-4279 at 300 mg/kg in MDA-MB-436 s.c tumor model. (B) In Olaparib-resistant MX-1 tumor model, addition of ASN-3186 to Olaparib produced significant inhibition of tumor growth, but not by KSQ-4279. TGI: tumor growth inhibition. **p<0.05,**p<0.01, ***p<0.005, ****p<0.0001, vs vehicle, ANOVA*

ASN-3186 increased Ub-PCNA level



(A) ASN-3186 strongly induced Ub-PCNA accumulation and increased γ-H2AX (DNA damage marker) in tumor cells *in vitro.* (B) Ub-PCNA increase was observed in Western blot analysis of tumor tissues after a single oral dosing of ASN-3186 in MDA-MB-436 tumor model.

ASN-3186 synergized with PARPi & Gemcitabine *in vivo*



(A) In MDA-MB-436 s.c. tumor model, 85% TGI was achieved with suboptimal dose of ASN-3186 and Olaparib co-administration. (B) Combinational therapy produced regression even though tumor was resistant to either monotherapy in MX-1 CDX model harboring BRAC1/2 mut. (C) Addition of ASN-3186 to Olaparib exhibited significant tumor growth inhibition with TGI of 58% in HRD+ CAOV3 CDX model. (D) In MDA-MB-436 s.c. tumor model, 100% TGI was achieved with ASN-3186 and Gemcitabine co-administration. HRD: Homologous recombination deficiency. **p<0.05,**p<0.01 ,***p<0.005, ****p<0.0001, vs vehicle, ANOVA*

ASN-3186 has favorable pharmacokinetic profile

Parameter	ASN-3186			
	Mouse	Rat	Dog	Monkey
CL (ml/min/kg)	11.7	23.9	3.12	3.80
Vss (L/kg)	1.29	4.18	2.39	2.35
$T_{1/2}$ (h)	1.3	2.7	10.6	8.4
F (%)	76%	105%	107%	85%

CL: clearance; Vss: steady-state volume of distribution; $T_{1/2}$: half-life; F: oral bioavailability

Conclusions

- **ASN-3186 is a highly potent USP1 inhibitor with excellent selectivities against 47 deubiquitinating enzymes.**
- **ASN-3186 selectively killed BRCAmut or HRD+ tumor cells, and synergized with PARP inhibitor to kill tumor cells.**
- **ASN-3186 was more efficacious than KSQ-4279 as monotherapy in MDA-MB-436 tumor model, as well as in combination with Olaparib in PARP inhibitor-resistant tumor model.**
- **ASN-3186 displayed high oral bioavailabilities (>75%) across species.**
- **ASN-3186 synergized with DNA synthesis inhibitor gemcitabine *in vivo*.**
- **The data above supports further development of ASN-3186 as a potential best-in-class USP1 inhibitor, as a single agent or in combination with a PARP inhibitor, for the treatment of BRCA1/2 mut and HRD+ cancers.**

References:

1. Nijman, S.M. et al., The deubiquitinating enzyme USP1 regulates the Fanconi Anemia pathway. *Mol. Cell*, 2005. **17**(3): p. 331-9.
2. Lim, K.S. et al., USP1 Is Required for Replication Fork Protection in BRCA1-Deficient Tumors. *Mol. Cell*, 2018. **72**(6): p. 925-941 e4.
3. Liang, Q. et al., A selective USP1-UAF1 inhibitor links deubiquitination to DNA damage responses. *Nat. Chem. Biol.*, 2014. **10**(4): p. 298-304.
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