Development of KSQ-4279 as a First-in-Class USP1 Inhibitor for the Treatment of BRCA-Deficient Cancers

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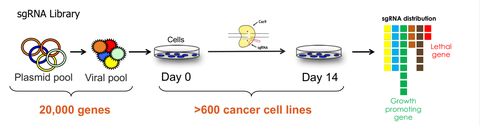
Abstract

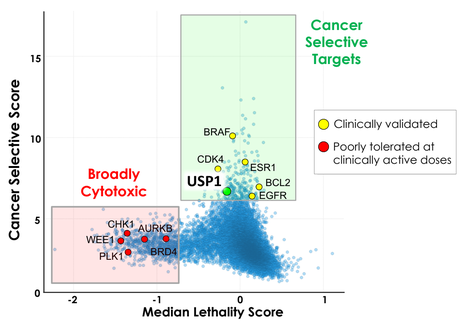
Background: Drugs targeting poly (ADP-ribose) polymerase (PARP) have provided significant clinical benefit for cancer patients with tumors harboring mutations in BRCA1/2 or other homologous recombination deficiencies (HRD). Despite their success, not all patients respond to PARP inhibitors, and those that do benefit often develop resistance. To address these challenges, we applied our proprietary CRISPRomics® technology to over 600 cancer cell lines, a subset of which contained mutations in either BRCA1/2 or other genes involved in DNA repair. The genetic dependencies that were selective for this subset of cell lines were then ranked for their suitability for therapeutic targeting.

Results: One of the top-ranked targets was the deubiquitinating enzyme USP1. USP1 has established roles in DNA damage repair processes, including Translesion synthesis and the Fanconi anemia pathway. We developed a series of small molecule inhibitors that are potent and highly selective for USP1 relative to other family members. These inhibitors were active in cells, leading to the accumulation of mono-ubiquitinated substrates of USP1 and demonstrating selective antiproliferative activity in cell lines with BRCA mutations or other HRD alterations. Evaluation of our lead compound, KSQ-4279, in both ovarian-derived and triple-negative breast cancer (TNBC)-derived tumor xenograft models, demonstrated dose-dependent tumor growth inhibition. In xenograft models with only partial sensitivity to PARP inhibitors, the combination of KSQ-4279 with a PARP inhibitor led to significantly greater and more durable tumor regressions than either agent alone. KSQ-4279 has favorable in vitro ADME properties and pharmacokinetic profile across multiple non-clinical species. Preliminary safety data indicates that KSQ-4279 is well tolerated as a single agent and in combination with PARP inhibitors, with no evidence of dose limiting heme-related toxicities.

Conclusions: Our data supports the clinical evaluation of KSQ-4279 as a potential first-in-class USP1 inhibitor in patients with tumors harboring BRCA1/2 or other HRD mutations, both as a single agent and in combination with PARP inhibitors.

Figure 1: CRISPRomics® Target Discovery Platform Identifies USP1 as Attractive Target





- Genome-scale CRISPR dependency data for 600 cancer cell lines identifies USP1 as an attractive cancer selective target

Figure 2: USP1 is a Key Regulator of DNA Damage Repair Pathways Distinct from PARP Inhibitors

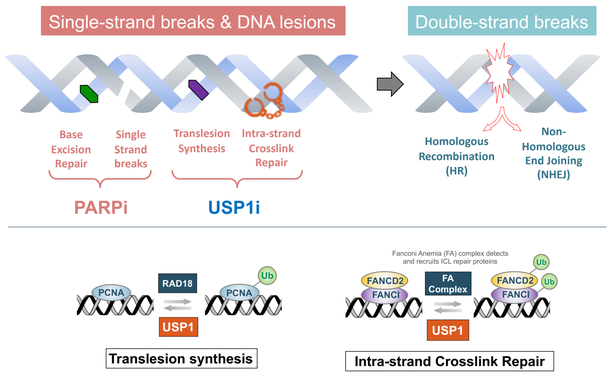
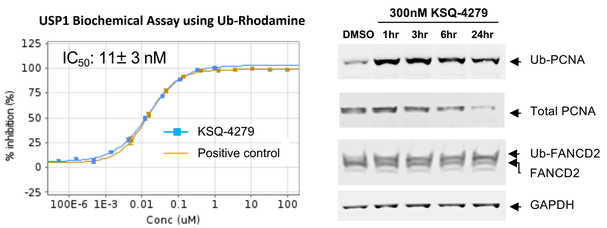
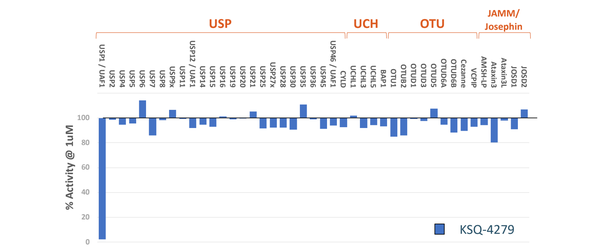


Figure 3: KSQ-4279 is a Potent and Selective USP1 Inhibitor

KSQ-4279 inhibits USP1 activity and induces accumulation of mono-ubiquitinated (Ub) substrates in MDA-MB-436 cells



DUB family profiling illustrates selectivity of KSQ-4279 for USP1



Clonogenic assays reveal USP1 inhibitors selectively target a subset of cell lines with varying molecular features including BRCA1/2 mutations

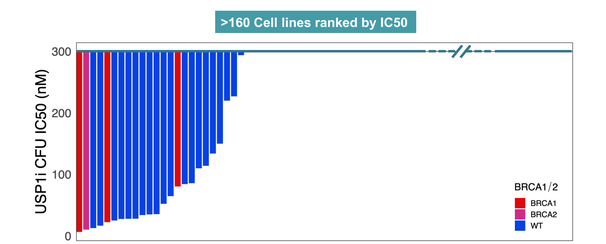
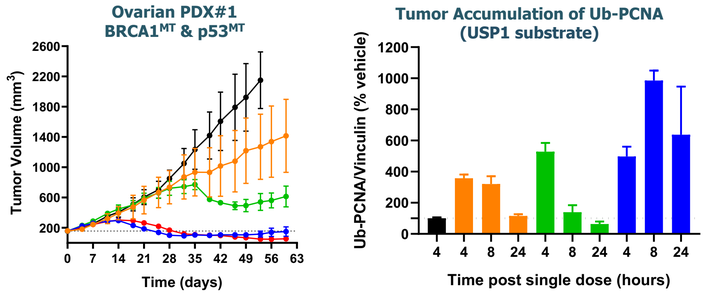
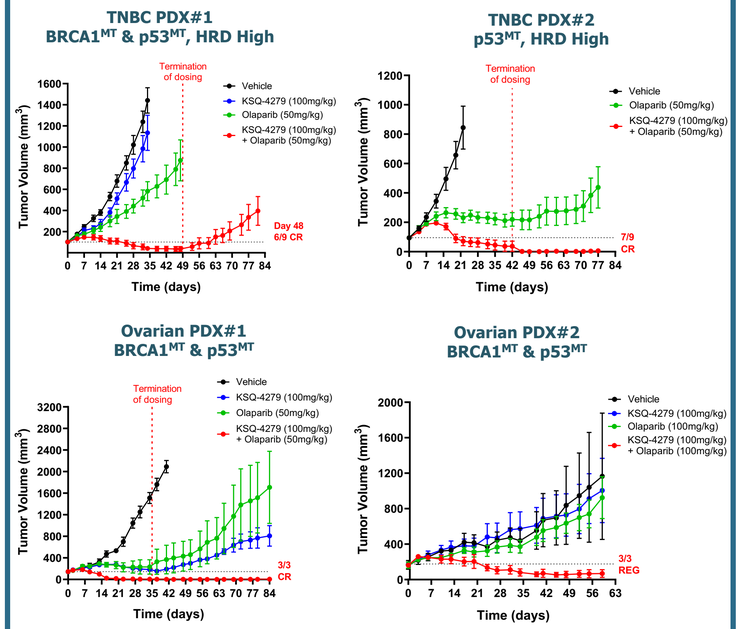


Figure 4: KSQ-4279 Demonstrates Monotherapy Activity in Human PDX Model



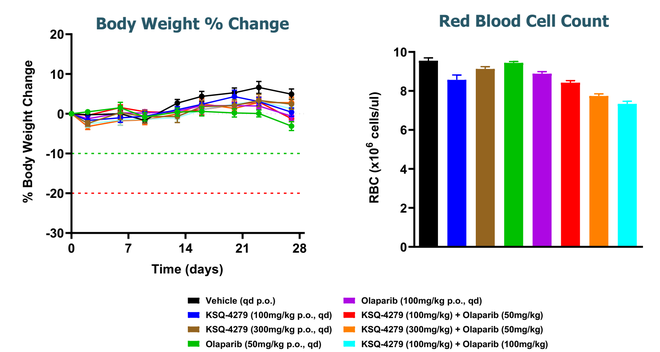
- Tumor fragments from ovarian cancer patients were injected s.c. into immunocompromised mice. Mice were randomized when tumors reached an average volume of 200mm^3 and dosed daily via oral gavage for up to 60 days with either vehicle or KSQ-4279 (n=3 mice/group)
- We observed a dose-dependent anti-tumor response in the BRCA1 mutant ovarian PDX model. Efficacy was consistent with dose-dependent increases in tumor Ub-PCNA

Figure 5: KSQ-4279 Enhances Olaparib Activity Leading to Significant and Durable Anti-tumor Response in BRCA^{MT}/high HRD PDX Models



- Human ovarian or TNBC tumor fragments were injected s.c. into immunocompromised mice. KSQ-4279 (100mg/kg) was administered in combination with the PARP inhibitor Olaparib (50 or 100mg/kg) daily via oral gavage
- Robust combination activity resulting in tumor regressions (REG) and complete responses (CR) observed across ovarian and TNBC patient derived xenograft models

Figure 6: KSQ-4279 is Well Tolerated in Combination with Olaparib at Multiple Dose Levels



- Tumor bearing NOD SCID mice were dosed daily via oral gavage with vehicle, olaparib, KSQ-4279 alone or in combination
- Body weight was monitored at least twice per week and %body weight change calculated as a measure of combination tolerability
- For hematological assessment, full clinical blood chemistry was evaluated from mice via terminal blood collected 4hrs post final dose
- Data show that combining KSQ-4279 with olaparib is well tolerated across multiple dose levels, with no evidence of dose-limiting heme-related liabilities in mice

Figure 7: KSQ-4279 Has Favorable Pharmacokinetic Profile Across Non-clinical Species

Parameter	KSQ-4279		
	Mouse	Rat	Monkey
CL (mL/min/kg)	7.34	11.9	4.34
V_{ss} (L/kg)	2.09	7.46	3.43
$t_{1/2}$ (h)	4.75	10.6	13.4
F (%)	100	114	117

- KSQ-4279 exhibits low systemic clearance, large volume of distribution and high oral bioavailability in non-clinical species
- KSQ-4279 showed a favorable in vitro ADME profile including cell permeability, efflux ratio, and metabolic stability in human liver microsomes and hepatocytes (data not shown)

Conclusions

- KSQ CRISPRomics® platform identified USP1 as an attractive cancer target
  - BRCA/HR-deficient cancers show enriched response to USP1 inhibition
  - USP1 regulates DNA repair pathways distinct from PARP inhibitors
- KSQ-4279 was discovered to be a potent and selective USP1 inhibitor
- KSQ-4279 is active as a single agent in an ovarian xenograft model and shows robust combination activity across multiple BRCA/HRD xenograft models
- KSQ-4279 is advancing towards IND filing in 2021