USP1 inhibitors show robust combination activity and a distinct resistance profile from PARP inhibitors

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Disclosure statement

• I am an employee of KSQ Therapeutics
KSQ PrecisionOne CRISPR Platform

sgRNA guides

Plasmid pool → Viral pool → Cells → Day 0 → Day 14

20,000 genes → >600 cancer cell lines

CRISPRomics® database

20,000 drop-out profiles
KSQ Knowledgebase: Identification of Cancer-Selective Targets
Database Predicts Past Successes and Failures

Cancer Selective Targets
- Clinically Validated
- Poorly tolerated at clinically active doses

Cancer Selective Potency Score

Median Lethality Score

Broadly Cytotoxic

BRAF
CDK4
HER2
ESR1
BCL2
EGFR

PSMA3
CHK1
AURKB
PLK1
BRD4
KSQ Knowledgebase Indicates that USP1 is a Cancer Selective Target

Cancer Selective Targets

Median Lethality Score

Cancer Selective Potency Score

Broadly Cytotoxic

USP1

WEE1, ATR, CHK1, RAD51

0 0.5 1

-2 -1 0 1

Median Lethality Score
USP1 is involved in a distinct set of DNA Damage Response Pathways from PARP inhibitors

- Single strand breaks & DNA lesions
  - Base Excision Repair
  - Single Strand breaks
  - Translesion Synthesis
  - Intra-strand Crosslink Repair
- Double strand breaks
  - Homologous Recombination (HR)
  - Non-Homology End Joining (NHEJ)
KSQ-4279, a Potent and Selective USP1 Inhibitor, Shows Anti-Proliferative Effects in BRCA1 Mutant Cells

**Biochemical assay**

IC$_{50}$: 11 ± 3 nM

% inhibition

Conc (uM)

% Activity

KSQ-4279, Positive control

**DUB Selectivity Panel @ 1uM**

% Activity

KSQ-4279

**Clonogenic assays reveal activity in BRCA$^{{MT}}$/HRD cells**

% Growth of DMSO

KSQ-4279 (nM)

MDAMB436  BRCA1$^{{MT}}$/HRD+

NCIH1693  BRCA2$^{{MT}}$/HRD+

CAOV3  BRCA1$^{{WT}}$/HRD+

SNGM  BRCA1$^{{WT}}$/HRD-
KSQ-4279 Induces S-Phase Arrest and DNA Damage Leading to Apoptosis and Cell Death in BRCA1 Mutant Cells

MDA-MB-436 cells (BRCA1\textsuperscript{MT}, p53\textsuperscript{MT})

**Cell Cycle**

**\(\gamma\text{H2AX}**

**Caspase-3**

- **MFI**
  - USP1i
  - DMSO
  - Day 1
  - Day 5

- **% Caspase-3**
  - USP1i
  - DMSO
  - Day 4
  - Day 5
  - Day 6
  - Day 7
Clonogenic Assay Profiling Confirms USP1 Inhibitors are Active in a Distinct Subset of Cell Lines

>160 Cell lines ranked by IC50
USP1i and PARPi Have Synergistic Activity in Clonogenic Assays
USP1i and PARPi Have Synergistic Activity in Clonogenic Assays

USP1i / PARPi combination synergy ranking across >160 cell lines

BRCA1/2 Status
- BRCA1<sup>MT</sup>
- BRCA2<sup>MT</sup>
- WT
CRISPR Screens Indicate that the Top Scoring Resistance Genes Differ between USP1i and PARPi
KSQ-4279 is Active as a Monotherapy in Ovarian PDX Model (BRCA1\textsuperscript{MT}/p53\textsuperscript{MT})
KSQ-4279 is Active as a Monotherapy in Ovarian PDX Model (BRCA1\textsuperscript{MT}/p53\textsuperscript{MT}) and in Combination With Olaparib
KSQ-4279 in Combination with Olaparib Leads to Tumor Regressions in Multiple TNBC & Ovarian PDX Models

**TNBC PDX #1**
*BRCA1<sup>MT</sup> & p53<sup>MT</sup>, HRD High*

- **Termination of dosing**
- **Vehicle (qd, po)**
- **KSQ-4279 (100mg/kg, qd, po)**
- **Olaparib (50mg/kg, qd, po)**
- **KSQ-4279 (100mg/kg)**
- **+ Olaparib (50mg/kg)**

**TNBC PDX #2**
*p53<sup>MT</sup>, HRD High*

- **Termination of dosing**
- **Vehicle (qd, po)**
- **Olaparib (50mg/kg, qd, po)**
- **KSQ-4279 (100mg/kg)**
- **+ Olaparib (50mg/kg)**

**TNBC PDX #3**
*(BRCA1 WT, p53 WT)*

- **Termination of dosing**
- **Vehicle (qd, po)**
- **KSQ-4279 (100mg/kg, qd, po)**
- **Olaparib (50mg/kg qd, po)**
- **KSQ-4279 (100mg/kg)**
- **+ Olaparib (50mg/kg)**

**Ovarian PDX #1**
*BRCA1<sup>MT</sup> & p53<sup>MT</sup>*

- **Termination of dosing**
- **Vehicle (qd, po)**
- **KSQ-4279 (100 mg/kg, qd, po)**
- **Olaparib (100mg/kg, qd, po)**
- **KSQ-4279 (100mg/kg)**
- **+ Olaparib (100mg/kg)**

**Day 48**
6/9 **CR**

**Day 48**
7/9 **CR**

**Day 48**
6/9 **CR**

**Day 48**
3/3 **REG**
KSQ-4279 – A Potential First-in-Class USP1 Inhibitor for the Treatment of Ovarian and TNBC Patients

• KSQ CRISPRomics® platform identified USP1 as an attractive cancer target
  • BRCA/HRD deficient cancers show enriched response to USP1 inhibition
  • USP1 regulates DNA damage repair pathways distinct from PARP inhibitors

• Discovered KSQ-4279, a potent and selective USP1 inhibitor

• CRISPRomics® resistance screens indicate that KSQ-4279 has a complimentary resistance profile to PARP inhibitors

• KSQ-4279 is active as a single agent in ovarian xenograft model and shows robust combination activity across multiple BRCA/HRD xenograft models

• KSQ-4279 is advancing towards IND filing in 2021
Acknowledgements