IDENTIFICATION OF DEBIO 0432 AS A POTENT AND SELECTIVE USP1 INHIBITOR FOR CANCER THERAPY

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ABSTRACT #7145

SUMMARY

Ubiquitin Specific Protease 1 (USP1) is a member of the deubiquitinating enzyme (DUB) family that plays an important role in maintaining DNA integrity. USP1 plays an important role in DNA damage repair through its deubiquitinating activities on Fanconi Anemia proteins FANCI and FANCD2, required for DNA interstrand crosslink repair, and on Proliferating Cell Nuclear Antigen (PCNA) required for translesion synthesis (TLS)[1]. USP1 is upregulated in BRCA-mutated tumors and contributes to the stabilization of the replication fork during DNA replication. In those tumors, genetic deletion of USP1 is synthetically lethal[2].

Debio 0432 is a USP1 inhibitor at preclinical stage. The molecule is predicted to bind USP1 in an allosteric pocket and is very selective among the 58 members of the DUB family. Debio 0432 biochemical activity on USP1 is below 1nM and >20x more potent than another USP1 inhibitor in development, KSQ-4279/RG-6614. Monotherapy activity has been observed in several cell lines, across several tumor types. *In vivo*, Debio 0432 shows antitumor activity in the BRCA mutant breast cancer model MDA-MB-436 and in the BRCA WT NCI-H292 lung cancer model. The downstream target Ub-PCNA, selected as pharmacodynamic marker, is modulated in a dose and plasma exposure-dependent manner. Further *in vivo* experiments in patient derived xenograft (PDX) models show anti-tumor activity in different cancer types. At active dose, the treatments in all *in vivo* studies are well tolerated.

Overall, we show that Debio 0432 is a selective and potent small molecule inhibitor of USP1, an emerging target for cancer therapy in the DDR field. It is currently undergoing IND-enabling studies.

MECHANISM OF ACTION

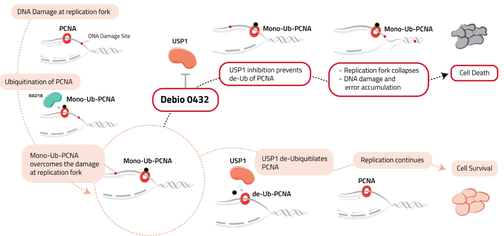


Figure 1: USP1 localizes at the replication fork and catalyzes the removal of specific monoubiquitin signals. It regulates DNA damage response (DDR) by stabilizing proteins acting in translesion synthesis (TLS), such as PCNA (Proliferating Cell Nuclear Antigen). USP1 inhibition drives accumulation of mono-Ub-PCNA (mono-ubiquitinated PCNA), leading to replication fork destabilization, DNA damage, and tumor cell death.

METHODS

The studies were conducted in accordance with institutional guidelines and NCRI Guidelines for the welfare and use of animals in cancer research[3].

DUBprofiler™ Assay was performed at Ubiquigent according to their procedures

***In vitro* colony forming assay:** briefly, cells were incubated for 14 to 21 days with serial dilution concentration of Debio 0432 or KSQ-4279. Cell growth was assessed by staining the cells with crystal violet. IC50 were calculated using GraphPad Prism software

Safety Scan: SAFETYscanE/IC50ELECT assay from DiscoverX, a Eurofins company

Western blots were performed on tumor lysate to assess expression of PCNA, Ub-PCNA and GAPDH

Mouse xenograft models (CDX or PDX): BALB/c nude or NOD/SCID mice were implanted with the respective cells and treatment started when mean tumor volumes reached 150-200mm3. Treatment was administered orally by gavage at the indicated dose and frequency (BID= twice a day, QD= once a day). Tumor volume and body weight were checked twice or tree times a week respectively.

REFERENCES

1. Garcia-Santisteban et al., Molecular Cancer (2013) 12:91
2. Lim et al., 2018, Molecular Cell 72, 925-941
3. Workman et al., British Journal of cancer. (2010) 102, 1555-1577
4. Rennie et al., Sci. Adv. 8, eabq6353 (2022)

RESULTS

In silico docking predicts binding of Debio 0432 to an allosteric pocket

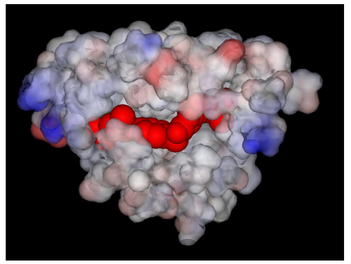
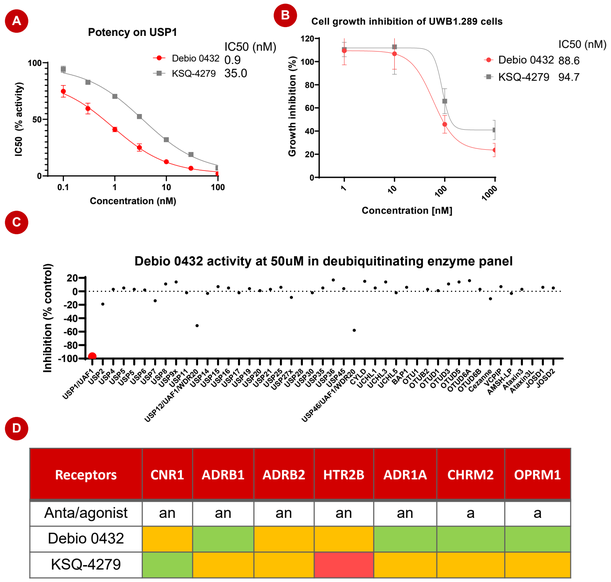


Figure 2: *in silico* docking of Debio 0432 in USP1 allosteric pocket
From the published Cryo-EM structure4 of ligand ML323 in USP1, the Swiss Institute of Bioinformatics performed docking studies of Debio 0432 in USP1.
EADock calculated binding mode of Debio 0432 (solid red Van der Waals spheres) in USP1 (transparent Van der Waals spheres) is similar to ML323 and shows that Debio 0432 occupies an allosteric pocket of the enzyme.

Debio 0432 is potent, selective and has a favorable safety profile



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Receptors	CNR1	ADRB1	ADRB2	HTR2B	ADR1A	CHRM2	OPRM1
Anta/agonist	an	an	an	an	an	a	a
Debio 0432							
KSQ-4279							

Figure 3: Debio 0432 shows good potency on USP1 and good cellular activity, is selective versus other DUB family members and has a favorable safety profile in vitro
A. Potency on USP1 was determined for Debio 0432 and KSQ-4279 using the DUBprofiler™ Assay from Ubiquigent. **B**. Colony forming assay was performed on UWB1.289 cell lines with both Debio 0432 and KSQ-4279. Relative IC50 were determined using Graphpad prism. **C**. Selectivity was assessed on a DUB enzyme panel using DUBprofiler™ Assay from Ubiquigent. Debio 0432 was tested at 50uM. **D**. A safety panel from DIscoverX was used to determine the safety profile of Debio 0432 and KSQ-4279. The compounds were tested at 13uM and a selection of receptors is shown. Color code: green: IC50>13uM, orange: IC50 between 3uM and 13uM, red :IC50 below 3uM

Debio 0432 is active as monotherapy across several tumor types, irrespective of the BRCA mutational status

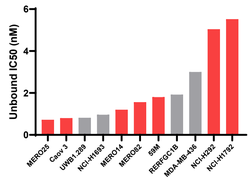

Figure 4. Debio 0432 shows activity in cell lines from different origins and, irrespective of BRCA status.
Debio 0432 was tested in the indicated cell lines by colony forming assay (CFA). The red bars represent BRCA WT cells, the grey bars BRCA mutant cells.

Debio 0432 is active in the BRCA mutant MDA-MB-436 TNBC preclinical model and pharmacodynamic marker Ub-PCNA increases with exposure, indicating target engagement

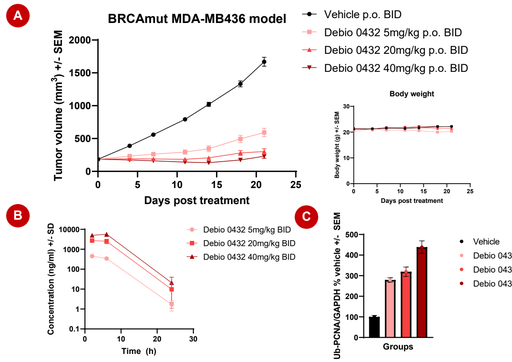

Figure 5: Debio 0432 shows dose-dependent anti-tumor activity in the TNBC MDA-MB-436 model
A. NOD/SCID mice were inoculated subcutaneously with MDA-MB-436 cells. Mice were treated with Debio 0432 orally at indicated dose or with vehicle alone, for 21 days. Body weight was assessed throughout the study as shown on the right graph. **B**. After the last dose, plasma samples were collected at 2h, 6h and 24h post-dose and Debio 0432 concentration was determined by LC-MS/MS. **C**. Tumor were collected 4h post last dose and analyzed by western blot to quantify PCNA, Ub-PCNA and GAPDH. The ratio Ub-PCNA/GAPDH was calculated and the graph indicates the % change from vehicle.

Debio 0432 is active in breast and ovarian cancer PDX models partially resistant to PARP inhibition

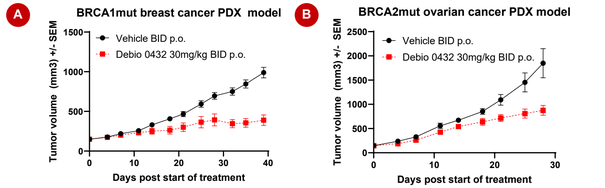

Figure 6: Debio 0432 shows monotherapy anti-tumor activity in breast and ovarian cancer patient derived xenograft (PDX) models that have been shown (historical data) to be partially resistant to PARP inhibition (TGI >30% and < 70%).
A and B. NOD/SCID mice were inoculated subcutaneously with the respective PDX cells. Mice were treated with Debio 0432 orally at 30mg/kg BID or with vehicle alone, for 38 days (breast model) and 28 days (ovarian model).

Debio 0432 shows better anti-tumor activity than KSQ-4279 in a BRCA WT lung cancer model

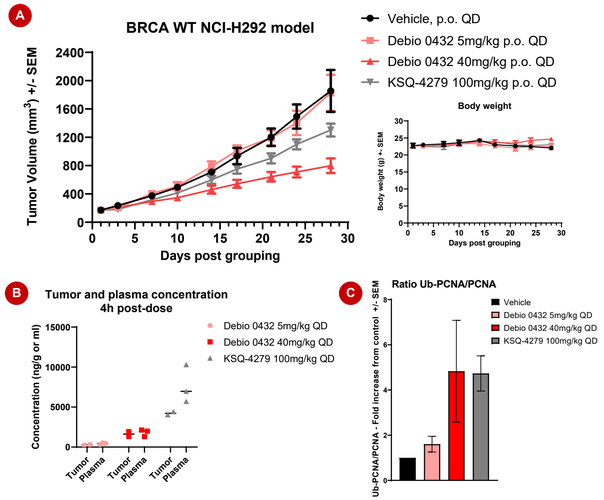

Figure 7: Debio 0432 shows better anti-tumor activity than KSQ-4279 in the lung cancer NCI-H292 model
A. BALB/c nude mice were inoculated subcutaneously with NIH-292 tumor cells. Mice were treated with compounds at indicated doses or with vehicle alone, for 28 days. Body weight was assessed throughout the study as shown on the right graph. **B**. After the last dose, plasma and tumor samples were collected 4h post-dose and Debio 0432 or KSQ-4279 concentrations were determined by LC-MS/MS. **C**. Tumors were collected 4h post last dose and analyzed by western blot to quantify PCNA, Ub-PCNA. A fold change from vehicle PCNA/Ub-PCNA ratio is shown for the 3 treated groups.

Acknowledgments

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CONCLUSIONS

- **Debio 0432 is a potent and selective USP1 inhibitor**
- **Debio 0432 shows *in vitro* and *in vivo* anti-tumor activity in several tumor types at well tolerated doses**
- **The pharmacodynamic marker Ub-PCNA is modulated and indicates good target engagement in preclinical models**
- **Regulatory submissions are underway to start clinical development**

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