33P

ANTI-TUMOR EFFECT OF DEBIO 0432, A POTENT AND SELECTIVE USP1 INHIBITOR IN COMBINATION WITH PARP INHIBITORS

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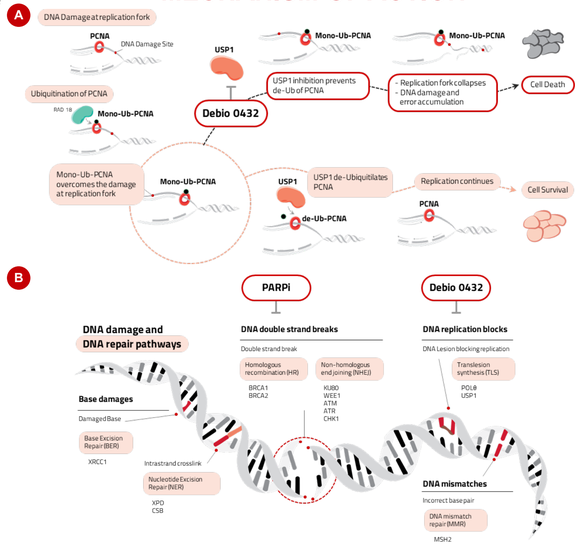
SUMMARY

Debio 0432 is a potent and selective inhibitor of Ubiquitin specific protease 1 (USP1)[1]. USP1 is key for DNA damage repair and is upregulated in some BRCA-mutated tumors, contributing to DNA replication stability[2,3]. In such tumors, genetic deletion of USP1 is synthetically lethal. Debio 0432 was previously shown to have single agent activity in models from several tumor types[1].

Here we present data showing how PARP inhibitors (PARPi) and Debio 0432 synergize in different tumor types to achieve tumor growth inhibition (TGI). In organoids derived from ovarian, breast and pancreatic cancer tissues, with a mix of homologous recombination proficient (HRP) and deficient (HRD) models, olaparib was potentiated by Debio 0432 in the majority of the cases, as defined by a positive Bliss score (>0) and lower IC50. In vivo models were used to confirm the good synergistic activity of both compounds. In a RAD51 mutant, PARPi resistant ovarian cancer PDX, the combination of both Debio 0432 and olaparib was efficient to generate a TGI suggestive of synergistic effect. Similarly, in a breast cancer model resistant to PARPi treatment, a synergism is observed between both compounds. This good combinability was also shown using the PARP1 selective inhibitor saruparib, demonstrating a class effect. Furthermore, the combination with saruparib showed no effect on white blood cell numbers, suggesting good tolerability.

Overall, in preclinical models, the combination of Debio 0432 and PARPi proves to be efficient in treatment of tumors resistant to single agent PARP therapy. Debio 0432 is currently being prepared to enter clinical development.

MECHANISM OF ACTION

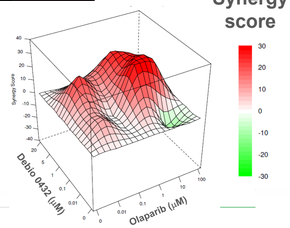


A. USP1 localizes at the replication fork and catalyzes the removal of specific monoubiquitin signals. It regulates DNA damage response (DDR) by stabilizing proteins acting in translesion synthesis (TLS), such as PCNA (Proliferating Cell Nuclear Antigen). USP1 inhibition drives accumulation of mono-Ub-PCNA (mono-ubiquitinated PCNA), leading to replication fork destabilization, DNA damage, and tumor cell death. **B.** Several DNA damage repair pathways are represented, along with actionable targets. PARP inhibitors (PARPi) target the homologous recombination pathway, while Debio 0432 targets the translesion synthesis pathway, allowing for synthetic lethality on cells relying only on these pathways.

Combination of Debio 0432 and olaparib leads to improved cytotoxicity in the majority of the organoids tested across 3 different tissue types

Organoids were treated with Debio 0432 serial dilutions or olaparib serial dilutions or a combination of both agents for 7 days. A Bliss score was established using the percentage inhibition of each condition[5,6]. A Bliss score from 0 to 10 indicates potential additive effect, > 10 indicates potential synergy (both in red), and a negative score antagonism (in green). A representative example of a 3D synergy map is provided at the lower right part of the figure. There was no correlation of presence of BRCA1/2 mutations nor HRP/HRD status with high synergy score (data not shown).

Ovarian models		Pancreatic models		Breast models	
Model Nb	Bliss score	Model Nb	Bliss score	Model Nb	Bliss score
OV01	21.98	PA01	13.35	BR01	9.73
OV02	9.54	PA02	11.72	BR02	7.47
OV03	5.90	PA03	6.19	BR03	4.51
OV04	5.53	PA04	5.15	BR04	3.83
OV05	4.84	PA05	3.93	BR05	1.80
OV06	3.86	PA06	3.93	BR06	0.62
OV07	2.72	PA07	0.04	BR07	0.07
OV08	2.65	PA08	-0.81	BR08	-0.21
OV09	2.65	PA09	-1.16	BR09	-0.60
OV10	2.56	PA10	-1.70	BR10	-1.06
OV11	2.20	PA11	-2.80	BR11	-1.22
OV12	1.87	PA12	-3.92	BR12	-1.29
OV13	1.12	PA13	-5.15	BR13	-2.73
OV14	0.71	PA14	-8.88	BR14	-3.88
OV15	0.43	PA15	-10.56	BR15	-6.33
OV16	0.35	PA16	-12.90		
OV17	0.09	PA17	-13.80		
OV18	-0.25				
OV19	-0.25				
OV20	-0.53				
OV21	-0.83				
OV22	-1.20				
OV23	-1.48				
OV24	-3.34				
OV25	-4.34				
OV26	-5.89				
OV27	-6.54				
OV28	-6.92				
OV29	-9.50				

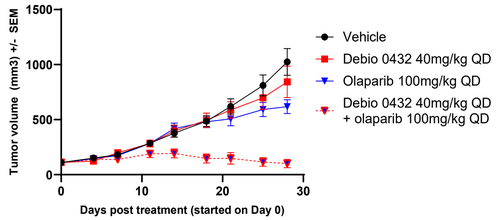


Synergy score

RESULTS

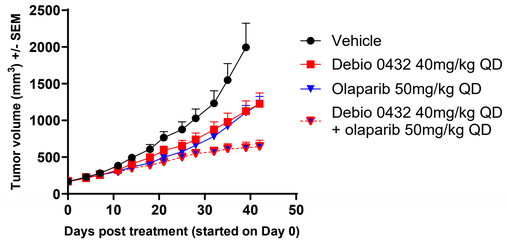
Debio 0432 and olaparib show synergistic activity in a breast cancer PDX resistant to both monotherapies

Triple negative BRCA1 mutant PDX model HBCx-8. Mice were treated once a day orally. The tumor growth inhibition of the Debio 0432 group is 20%, the olaparib group is 44% and the combination group is 101%. Analysis using the Bliss independence model[5,7] suggests a synergistic effect (CI=0.55).



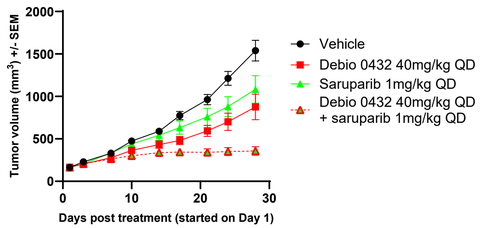
Debio 0432 and Olaparib show synergistic activity in an ovarian cancer PDX model derived from a patient resistant to olaparib

Ovarian cancer PDX LD1-2032-361588 was derived from a RAD51 mutated tumor from a patient resistant to olaparib. Mice were treated once a day orally. After 42 days of treatment, the tumor growth inhibition of the Debio 0432 group and the olaparib group are 48%, and the combination group is 75%. Analysis using the Bliss independence model[5, 7] suggests a synergistic effect (CI=0.97).



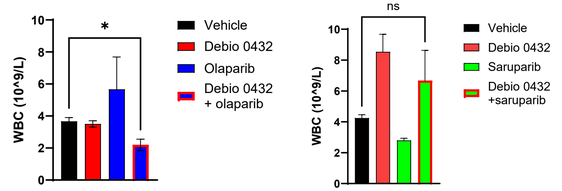
Debio 0432 and saruparib show synergistic activity in an ovarian cancer PDX

Homologous recombination proficient (HRP) ovarian cancer model OV2029. Mice were treated once a day orally. The tumor growth inhibition of the Debio 0432 group is 48%, the saruparib group is 33% and the combination group is 86%. Analysis using the Bliss independence model[5,7] suggests a synergistic effect (CI=0.76)



Combination of Debio 0432 and saruparib does not affect white blood cell numbers

After 28 days of dosing, a complete blood cell count was performed on 3-5 animals from each group (from 2 different experiments). White blood cells (WBC) were decreased by half upon combined treatment with olaparib and Debio 0432, while the combination of saruparib and Debio 0432 does not induce such decrease, indicating a potential better tolerability of the combination.



METHODS

The studies were conducted in accordance with institutional guidelines and NCRI Guidelines for the welfare and use of animals in cancer research[4].

- Organoid assay: organoids were incubated for 7 days with serial dilutions of each compound or a combination, in a matrix setting. After 7 days, growth was assessed using cell titer glow (CTG) assay. Synergy score were calculated using the synergyfinder.org website.
- In vivo PDX models: For all studies, each compound was given orally once a day (QD) throughout the experiment, as indicated on the graphs. In combination groups, Debio 0432 was given first and the 2nd drug 2h later.
  - HBCx-8 model: Athymic nude female mice were engrafted subcutaneously with approximately 20mm3 tumor fragments. When tumors reached 60-200mm^3, mice were randomized into groups of 10 and treatment started.
  - OV2029 model: BALB/c nude female mice were engrafted subcutaneously with 2-3mm3 in diameter tumor fragments. Randomization and treatment started when mean tumor size was 150-200mm^3. Ten mice per group were enrolled.
  - LD1-2032-361588 ovarian model: NU/NU female mice were engrafted subcutaneously with 50-90 mg tumor fragments. Randomization and treatment started when mean tumor volume was approximately 200mm^3. Eight mice per group were enrolled
- Combination Index (CI) calculation: $\mathbf{CI} = \frac{E_A+E_B-E_AE_B}{E_{AB}}$ where E_A, E_B, E_{AB} are $0 \leq x \leq 1$ (E=effect). Synergy is observed when CI is <1.
- Complete blood count (CBC): 400ul of blood was collected by cardiac puncture when mice were sacrificed and sent for a routine complete blood analysis, including white blood cell (WBC) count.

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ACKNOWLEDGMENTS

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CONCLUSIONS

- **Inhibition of USP1 by Debio 0432 potentiates the effect of PARP inhibitors across several tumor types *in vitro* and *in* vivo in both HR proficient and deficient models.**
- **Debio 0432 has the potential to overcome PARPi resistance, as demonstrated by sensitization of resistant models to the combination of both compounds**
- **Combination with saruparib is well tolerated and does not show sign of hematotoxicity in preclinical models**
- **Debio 0432 is foreseen to enter clinical development**

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