THE WEE1 INHIBITOR DEBIO 0123 IS SYNERGISTIC WITH PKMYT1 INHIBITOR LUNRESERTIB IN PRECLINICAL MODELS OF OVARIAN AND BREAST CANCER

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ABSTRACT #2914

SUMMARY

Debio 0123 is an investigational, orally bioavailable, highly selective and brain penetrant adenosine triphosphate (ATP)-competitive inhibitor of the WEE1 tyrosine kinase currently in phase 1 clinical trials. WEE1 is a key regulator of cell cycle progression that influences entry into mitosis by modulating activity of cyclin-dependent kinase 1 (CDK1, also referred to as cell division cycle 2 [CDC2]) through phosphorylation of Tyr15. WEE1 is an attractive opportunity as a therapeutic target in cancer therapy, either in cells relying on cell cycle checkpoints regulated by WEE1 or to potentiate DNA damaging agents. The proposed mechanism of action of Debio 0123 involves promoting uncontrolled entry into mitosis with accumulated DNA damage and, ultimately, cell death via mitotic catastrophe.

The nonclinical data suggested Debio 0123 has the potential to improve therapy outcomes of patients with cancer and is currently in clinical development as monotherapy or in combinations.

PKMYT1 is also a negative regulator of CDK1 through phosphorylation at Thr14. Thus, Debio 0123 in combination with the PKMYT1 inhibitor lunresertib induces strong phosphorylation of CDK1 at both Tyr15 and Thr14 potentially leading to premature mitotic entry, chromosome pulverization and cancer cell death.

BACKGROUND

Debio 0123 is a selective, orally available and brain penetrant ATP-competitive inhibitor of WEE1 kinase

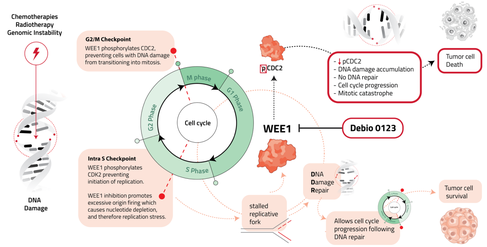


Figure 1. In cancer cells, DDR pathways are often upregulated due to genomic instability. WEE1 is a key regulator of the G2/M and S-phase checkpoints where it leads to cell cycle arrest allowing DNA damage repair. Inhibition of WEE1 reduces the phosphorylation of CDC2 (CDK1) permitting cells to proceed through the cell cycle with an accumulation of DNA damage leading to mitotic catastrophe and ultimately cell death.

WEE1 acts at both the G2/M and S-phase checkpoints making WEE1 inhibition amenable to combination with multiple agents with different mechanisms of action. Debio 0123 is a highly selective and potent WEE1 inhibitor[2]. Compared to adavosertib and azenosertib, Debio 0123 does not inhibit PLK1 or PLK2 (Figure 2).

High potency and selectivity

Target	Debio 0123 IC_{50} (nM)	adavosertib IC_{50} (nM)	azenosertib IC_{50} (nM)
WEE1	0.8	3.9	3.8

IC_{50} on WEE1 (ADP-competitive binding assay)

More selective than competition on PLK1/2

Target	Debio 0123 IC_{50} (nM)	adavosertib IC_{50} (nM)	azenosertib IC_{50} (nM)
PLK1	> 10 000	79	227
PLK2	> 10 000	40	40

IC_{50} on PLK1 and PLK2 (kinome screen)

Studies conducted using versions of adavosertib and azenosertivb synthesized by third-party contract research chemists, using publicly available information

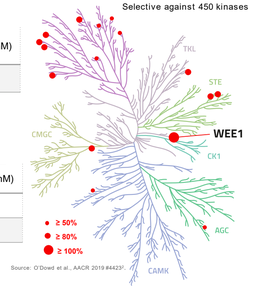


Figure 2. Cell free potency and selectivity profile screening at fixed concentration of 500nM (>100-fold IC_{50} WEE1)

METHODS

All studies were conducted in accordance with institutional and NCRI Guidelines for the welfare and use of animals in cancer research.

Concomitant knockdown of PKMYT1 and WEE1 in cancer cells by siRNA: WT or Wee1 KO A549 cells were reverse-transfected with DharmaFECT 1 and a PKMYT1 siRNA pool or a Scrambled siRNA at 50 nM for up to 96h and cell viability assessed using live/dead staining by flow cytometry.

***In vitro* combination of Debio 0123 and lunresertib:** FT282-hTERT p53^{R175H} or OVCAR3 cells were treated with different doses of Debio 0123 (0-5uM) and lunresertib (0-500nM) either alone or in combination and cell viability assessed by cell titer-GLO assay.

Mouse cell line-derived xenograft (CDX) models: Immunocompromised mice were injected subcutaneously with 4x10^6 SUM149PT or 1x10^7 OVCAR3 cells to establish tumors. Tumor size was measured using a caliper twice per week to determine tumor volume. Once tumors were established (~150mm^3), Debio 0123 was orally administered once a day either alone at 30 mg/kg (QD) or in combination with lunresertib at 10mg/kg or 20mg/kg (BID on a 3on 4off schedule).

Patient-derived xenograft (PDX) models: Immunocompromised mice were subcutaneously transplanted with 3x3x3mm fragments of patient-derived breast or ovarian tumor cells in Matrigel for tumor development. Tumor size was measured using a caliper twice per week to determine tumor volume. Once tumors were established (~150-200mm^3), Debio 0123 was orally administered once a day either alone at 30 mg/kg (QD) or in combination with lunresertib at 20mg/kg (BID on a 3d on/4d off schedule).

RESULTS

Knockdown of WEE1 and PKMYT1 is synthetic lethal

Knockdown of WEE1 or PKMYT1 in A549 WT cells resulted in a reduction of live cell counts but not a complete loss in viable cells. Knockdown of PKMYT1 in A549 WEE1^{KO} cells resulted in the abolishment of all live cells suggesting synthetic lethality of this concomitant knockdown. Collectively this data indicated a possible synergy could exist through suppressing both WEE1 and PKMYT1.

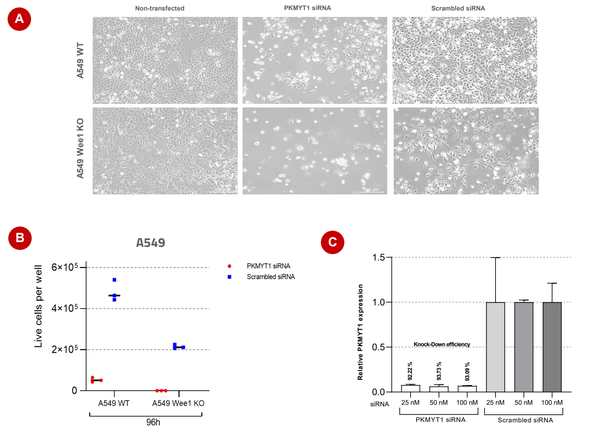


Figure 3. KO of WEE1 and PKMYT1 is synthetic lethal in A549 lung cancer cells: (A) Representative bright field images of cells 72h post-transfection and quantification of live cells per well by flow cytometry 96h post-transfection. **(B)** Live cell counts assessed by flow cytometry following WEE1 and PKMYT1 knockdown. **(C)** PKMYT1 expression as determined by flow cytometry at 96h post-transduction

REFERENCES

(1) Do K. et al., Cell Cycle. 2013 Oct 1;12(19):3159-64
(2) O'Dowd et al., Antitumor activity of the novel oral highly selective WEE1 inhibitor Debio 0123, AACR 2019 abstract #4423
(3) Gallo et al., Preclinical development of PKMYT1 and WEE1 inhibitor combinations, ENA 2023, abstract #A023

Debio 0123 demonstrates synergy in combination with lunresertib *in vitro*

WT or CCNE1-high (a key regulator of the G1 S-phase transition) FT282-hTERT p53^{R175H} or OVCAR3 cells treated with low doses of Debio 0123 or lunresertib as a monotherapy demonstrated minimal sensitivity to either drug. Similarly, WT FT282-hTERT p53^{R175H} cells demonstrated limited sensitivity to Debio 0123 in combination with lunresertib. Conversely, CCNE1-high FT282-hTERT p53^{R175H} cells or OVCAR3 cells treated with Debio 0123 in combination with lunresertib lead to significant cancer cell death, with less than 20% of cells remaining viable following treatment[3].

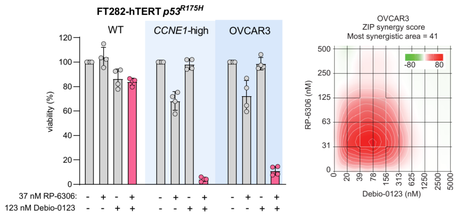
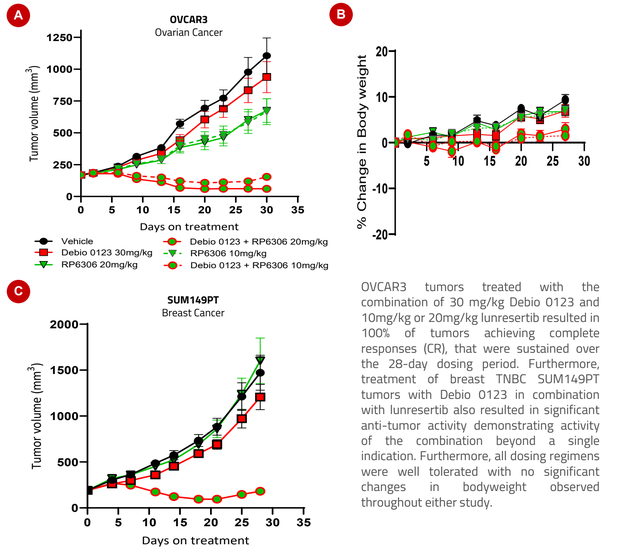


Figure 4. Debio 0123 is synergistic with lunresertib *in vitro*. FT282-hTERT p53^{R175H} or OVCAR3 cells were treated with Debio 0123 and lunresertib either alone or in combination demonstrating synergy (Bliss) at low concentrations that significantly reduce cancer cell viability in CCNe1-high FT282-hTERT p53^{R175H} or OVCAR3 cells but not WT FT282-hTERT p53^{R175H} cells.

Debio 0123 in combination with lunresertib leads to sustained complete regressions in breast and ovarian CDX models



OVCAR3 tumors treated with the combination of 30 mg/kg Debio 0123 and 10mg/kg or 20mg/kg lunresertib resulted in 100% of tumors achieving complete responses (CR), that were sustained over the 28-day dosing period. Furthermore, treatment of breast TNBC SUM149PT tumors with Debio 0123 in combination with lunresertib also resulted in significant anti-tumor activity demonstrating activity of the combination beyond a single indication. Furthermore, all dosing regimens were well tolerated with no significant changes in bodyweight observed throughout either study.

Figure 5. Combination Debio 0123 + lunresertib leads to sustained regressions of breast and ovarian cancer tumors *in vivo*. (A) Mice bearing OVCAR3 tumors were treated with Debio 0123 30mg/kg QD in combination with 10mg/kg or 20mg/kg lunresertib (BID). **(B)** Mean change in bodyweight throughout treatment in (A). **(C)** Mice bearing SUM149PT tumors were treated with Debio 0123 30mg/kg QD in combination with 20mg/kg lunresertib (BID).

Debio 0123 in combination with lunresertib leads to complete regressions in breast and ovarian PDX models

Synergy between Debio 0123 and lunresertib was further explored in PDX models of breast and ovarian cancer. Monotherapy treatment of either drug alone resulted in minor or no significant anti-tumor activity. Combination of Debio 0123 with lunresertib resulted in complete regression of all 3 PDX models tested.

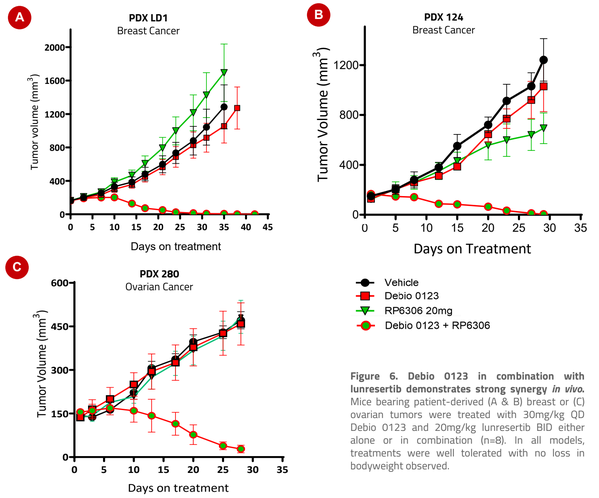


Figure 6. Debio 0123 in combination with lunresertib demonstrates strong synergy *in vivo*. Mice bearing patient-derived (A & B) breast or (C) ovarian tumors were treated with 30mg/kg QD Debio 0123 and 20mg/kg lunresertib BID either alone or in combination (n=8). In all models, treatments were well tolerated with no loss in bodyweight observed.

CLINICAL TRIALS

Debio 0123 is currently under phase I clinical investigation as a monotherapy (NCT05109975), in combination with carboplatin in patients with advanced solid tumors (NCT03968653), in combination with carboplatin and etoposide in patients with recurrent SCLC (NCT05815160), in combination with TMZ with or without RT in patients with GBM (NCT05765812), in combination with SG in patients with breast cancer (NCT06612203) and in combination with the PKMYT1 inhibitor lunresertib in patients with solid tumors (NCT04855656).

CONCLUSIONS

- Combined knockout of WEE1 and PKMYT1 in A549 cells is synthetic lethal
- Debio 0123 is synergistic with lunresertib at low concentrations in vitro
- Treatment with Debio 0123 in combination with lunresertib at sub-lethal monotherapy doses leads to deep and durable regressions in CDX models of breast and ovarian cancer
- Treatment with Debio 0123 in combination with lunresertib leads to complete regressions in PDX models of breast and ovarian cancer
- A clinical study exploring tolerability and antitumor activity of Debio 0123 in combination with lunresertib is currently ongoing (NCT04855656).

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