ANTI-TUMOR ACTIVITY OF DEBIO 0123 IN COMBINATION WITH SACITUZUMAB GOVITECAN IN PRECLINICAL MODELS OF BREAST CANCER

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ABSTRACT #3370

SUMMARY

Debio 0123 is an investigational, orally bioavailable, highly selective, adenosine triphosphate (ATP)-competitive inhibitor of the WEE1 tyrosine kinase. 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]). Inhibition of WEE1 presents an 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 entry into uncontrolled mitosis for cells with accumulated DNA damage and, ultimately, cell death via mitotic catastrophe.

The nonclinical and preliminary clinical data suggest Debio 0123 to be a good candidate for clinical development with the potential to improve therapy outcomes of patients with cancer when administered in combination with modalities that induce DNA damage, for example chemotherapies. Recently, antibody-drug-conjugates (ADCs) have shown particular promise in treating cancers through selective delivery of cytotoxic payloads to tumor cells. Sacituzumab govitecan (SG) is a TROP2 directed ADC carrying the topoisomerase 1 inhibitor (TOPO1i) payload, SN38. TROP2 expression has been observed across multiple cancer types, particularly breast, endometrial, NSCLC and CRC.

BACKGROUND

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

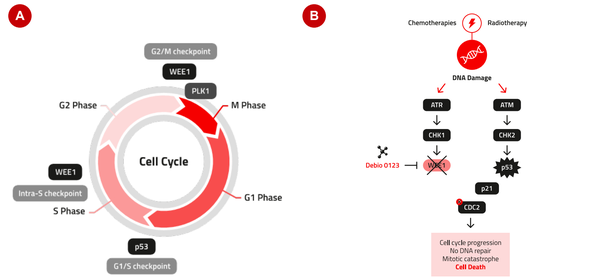


Figure 1. (A) Cell cycle checkpoints. In cancer cells, DDR pathways are often upregulated due to genomic instability. WEE1 is a key regulator of the G2/M and S-phase checkpoints where it leads to cell cycle arrest allowing DNA damage repair. **(B) WEE1 inhibition.** Inhibition of WEE1 reduces the phosphorylation 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 chemotherapies with different mechanisms of actions. Debio 0123 is a highly selective and potent WEE1 inhibitor[2]. Compared to AZD1775, Debio 0123 does not inhibit PLK1 or PLK2[3].

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	79	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

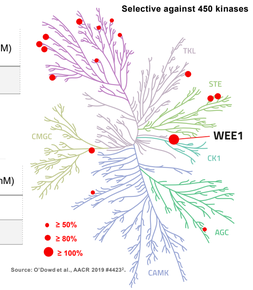


Figure 2. Cell free potency and selectivity profile screening at fixed concentration of 500nM (>100-fold IC_{50} WEE1)

METHODS

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

***In vitro* combination with SN38 and topotecan (Figure 3):** Breast (JIMT-1), colorectal (LoVo) and lung (A549) cells were seeded in monolayer conditions in 96-well plates for evaluation of treatments on proliferation by sulforhodamine B assay. Treatments were performed during exponential growth phase with Debio 0123, SN38 or topotecan as monotherapies (0-10uM) or in combination (0-10uM SN38/topotecan + 300nM Debio 0123). Cell proliferation was then measured 120hrs following treatments.

***In vitro* combination with sacituzumab govitecan (Figure 4):** Breast (MDA-MB-231, MDA-MB-468, BT20 and HCC38), colorectal (Colo205, SW480 and HCT116) and GBM (D54 and U87MG) cells were seeded in monolayer conditions in 96-well plates for evaluation of treatments on cell viability. Treatments were performed during exponential growth phase with Debio 0123 (0-8uM) and SG (0-10uM) as monotherapies or in combination in a matrix combination format. Cell viability was then measured 96hrs following treatments by CellTiter-Glo® 2.0 assay. Synergies between Debio 0123 and SG were then calculated using Bliss synergy calculation.

Mouse xenograft models (Figures 5 & 6) : NSG mice were injected subcutaneously with $5x10^6$ MDA-MB-468 or $2x10^6$ MDA-MB-231 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 (30 mg/kg QD) or in combination with SG at both 8mg/kg and 12.5mg/kg (administered on D1 and D8 only [QWx2]). For MDA-MB-231, metastatic tumor burden was assessed using In Vivo Imaging System (IVIS) once weekly, detecting tumor bioluminescence (BLI) in photons of light.

RESULTS

Debio 0123 enhances anti-proliferative effect of TOPO1i

Breast, colorectal and NSCLC cell lines were treated with increasing doses of Debio 0123, SN38 or topotecan as monotherapies or at increasing doses of topotecan or SN38 in combination with 300nM Debio 0123 for combinations. Treatment with monolayer cultures of Debio 0123 and SN38 in combination resulted in significantly reduced IC_{50} vs. Debio 0123 or SN38 monotherapy treatments, respectively. Treatment with monolayer cultures of Debio 0123 and topotecan in combination resulted in significantly reduced IC_{50} vs Debio 0123 and topotecan monotherapy treatments, respectively.

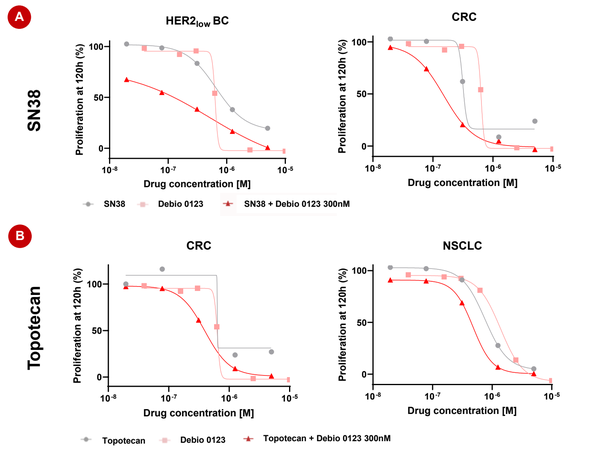


Figure 3. Debio 0123 enhances anti-proliferative effect of TOPO1 inhibitors. Cell lines were treated with Debio 0123 and SN38 (A) or topotecan (B) as monotherapies or in combination (increasing doses of SN38/topotecan + 300nM Debio 0123) and anti-proliferative effect measured at 120hours following treatment normalized to untreated control.

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) Workman et al., British Journal of cancer. (2010) 102, 1555-1577

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

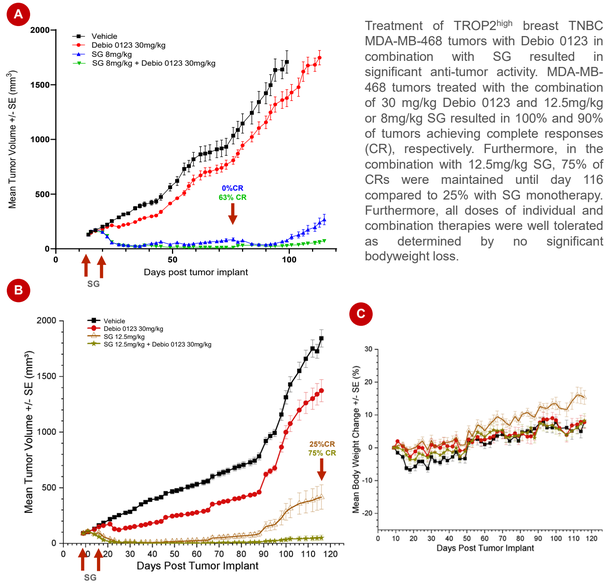
Breast, GBM and colorectal cell lines with different levels of TROP2 expression were treated with Debio 0123 and sacituzumab govitecan in combination across multiple doses. Despite the differences in TROP2 expression, a clear synergy was observed across all cell lines tested with each cell line registering a Bliss synergy score >10 (indicating synergy) at multiple low doses of both compounds in combination. Furthermore, a dose-response effect was evident through increasing doses of the combination.

Sacituzumab govitecan synergy across cell lines regardless of TROP2 expression

Cell line	Indication	TROP2	MFI	Av. Bliss
MDA-MB-231	Breast	Low	32,000	>70
MDA-MB-468	Breast	High	301,600	>30
HCC38	Breast	High	181,000	>10
BT20	Breast	Med	72,032	>40
Colo205	CRC	High	138,704	>15
HCT116	CRC	Low	10,042	>20
SW480	CRC	Med	49,837	>50
D54	GBM	Low	21,296	>10
U87MG	GBM	Low	16,100	>20

Figure 4. Debio 0123 is synergistic with SG *in vitro*. Breast, Colorectal and GBM cell lines treated with increasing doses of Debio 0123 (0-8uM) and SG (0-10uM) in a matrix format and cell viability measured by CellTiter-Glo® 2.0 assay. Bliss synergy analysis was performed to determine synergy at each dose and plotted as a surface plot (value >10 = synergy).

Debio 0123 in combination with Sacituzumab govitecan leads to sustained regressions in TROP2^{high} breast cancer *in vivo*



Treatment of TROP2^{high} breast TNBC MDA-MB-468 tumors with Debio 0123 in combination with SG resulted in significant anti-tumor activity. MDA-MB-468 tumors treated with the combination of 30 mg/kg Debio 0123 and 12.5mg/kg or 8mg/kg SG resulted in 100% and 90% of tumors achieving complete responses (CR), respectively. Furthermore, in the combination with 12.5mg/kg SG, 75% of CRs were maintained until day 116 compared to 25% with SG monotherapy. Furthermore, all doses of individual and combination therapies were well tolerated as determined by no significant bodyweight loss.

Figure 5. Combination Debio 0123 + SG leads to sustained regressions of breast cancer tumors in vivo. Mice bearing MDA-MB-468 tumors were treated with Debio 0123 30mg/kg QD in combination with **(A)** 8mg/kg SG (QW x 2) or **(B)** 12.5mg/kg SG (QW x2). **(C)** Mean change in bodyweight throughout treatment as in (B).

Debio 0123 improves response and inhibits metastasis in combination with SG in TROP2^{low} breast cancer *in vivo*

Treatment of TROP2^{low} breast TNBC MDA-MB-231 tumors with Debio 0123 in combination with SG resulted in significant anti-tumor activity. In tumors treated with 30 mg/kg Debio 0123 and 12.5mg/kg or SG resulted in 41% ($p<0.01$) tumor growth inhibition (TGI) at day 43, whereas either treatment alone resulted in minor, non-significant TGI. Interestingly, SG monotherapy significantly reduced detectable metastases by 25% compared to vehicle controls and, in combination with Debio 0123, reduced metastatic nodules in the lymph nodes and lungs by 62%. Additionally, treatment with SG and Debio 0123 in combination resulted in a significant 43.3% improvement in survival ($p<0.01$).

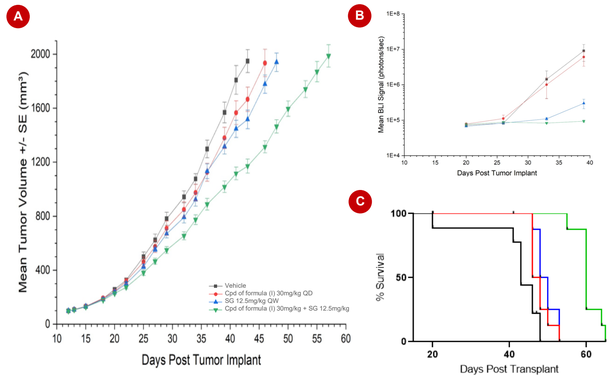


Figure 6. Debio 0123 in combination with SG demonstrates anti-tumor and anti-metastatic activity. Mice bearing MDA-MB-231-luc tumors were treated with 30mg/kg QD Debio 0123, 12.5mg/kg SG QW x2 either alone or in combination (n=8). (A) Primary tumor growth (B) Anti-metastatic activity monitored through luminescence imaging and (C) survival of animals through duration of study.

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) and in combination with the PKMYT1 inhibitor lunresertib in patients with ovarian cancer (NCT04855656).

CONCLUSIONS

- Debio 0123 improves tumor cell response to TOPO1 inhibitor treatment *in vitro*
- Debio 0123 is synergistic with sacituzumab govitecan in both TROP2^{high} and TROP2^{low} breast, colorectal and GBM cell lines *in vitro*
- Treatment with Debio 0123 in combination with sacituzumab govitecan results in anti-tumor activity *in vivo* in breast cancer leading to sustained complete regressions
- Debio 0123 significantly improves *in vivo* anti-tumor response of sacituzumab govitecan in TROP2^{low} breast cancer tumors and demonstrates anti-metastatic efficacy.
- Clinical studies exploring tolerability and antitumor activity of Debio 0123 in combination with sacituzumab govitecan will commence in 2024.

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