DEBIO 0123 IS A SELECTIVE WEE1 INHIBITOR THAT EFFECTIVELY PENETRATES THE BRAIN AND DEMONSTRATES ANTI-TUMOR ACTIVITY IN PRECLINICAL MODELS OF GLIOBLASTOMA

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ABSTRACT #6185

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 chemotherapy and radiation therapy[1]. 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 data suggest Debio 0123 to be a good candidate for clinical development with the potential to improve therapy outcomes of patients with cancer, as monotherapy or when administered in combination with modalities that induce DNA damage, for example chemotherapies and radiotherapy. Glioblastoma (GBM) is one of the most aggressive and hard-to-treat cancers with a 5-year survival rate of 6.8%, in part due to the presence of the blood brain barrier (BBB) that prevents most therapeutics from reaching the tumors at efficacious concentrations. Here we investigated the ability of Debio 0123 to cross the BBB and enhance response to standard of care (SOC) DNA damaging agent temozolomide (TMZ) *in vitro* and *in vivo*.

BACKGROUND

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

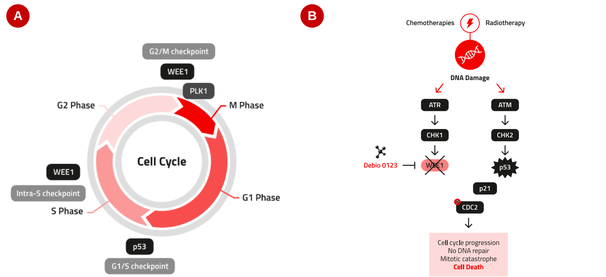


Figure 1. (A) Cell cycle checkpoints. In cancer cells, DDR pathways are often upregulated due to genomic instability. Wee1 is a key regulator of the G2/M and S-phase checkpoints where it leads to cell cycle arrest allowing DNA damage repair. **(B) WEE1 inhibition.** Inhibition of WEE1 reduces the phosphorylation 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)	Zn-C3 IC_{50} (nM)[4]
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)	Zn-C3 IC_{50} (nM)[4]
PLK1	> 10 000	79	227
PLK2	> 10 000	79	40

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

Studies conducted using versions of AZD1775 and Zn-C3 synthesized by third-party contract research chemists, using publicly available information

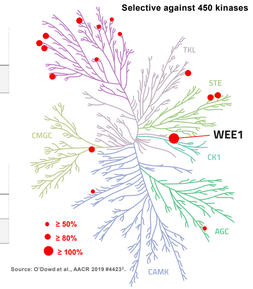


Figure 2. Cell free potency and selectivity profile screening at fixed concentration of 500nM (>100-fold IC_{50} WEE1)

METHODS

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

***In vivo* brain exposure (Figure 3):** Plasma and brain exposures of Debio 0123, AZD1775 (adavosertib), and Zn-C3 (azenosertib) were assessed in healthy male Nu/Nu mice and healthy male Wistar Han rats (Debio 0123 only). Mice were treated orally, for four consecutive days, either with 30 mg/kg Debio 0123 once daily (A), 30 mg/kg AZD1775 twice daily on days 1 to 3 and once on day 4 (C), or 80 mg/kg Zn-C3 once daily (D). Rats were treated with a single dose of 15 mg/kg Debio 0123 (B). At designated time-points, terminal plasma and brain samples were collected. Plasma and brain homogenates were analyzed by LC-MS/MS to determine the test item concentrations.

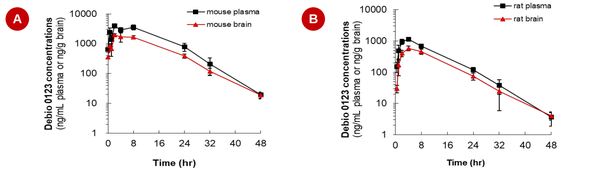
***In vitro* efficacy (Figure 4)**: T98G, LN18, SNB-19 and U251 cells were seeded in colony formation monolayer conditions in 6-well plates at 3 densities for evaluation of a proclonogenic effect. Treatments were performed at 24 hours (D1) after seeding with final concentrations between 0.1-5.0 µM of Debio 0123. Immediately following compound treatment, the plates were irradiated at 1, 2 and 4 Gy. At Day 2 and Day 7, culture medium was replaced in all plates with fresh medium without Debio 0123 and colonies counted after 14 days of incubation through staining with methylene blue for 2 hours.

Mouse xenograft models (Figures 5 & 6) : Male athymic nude mice were injected A) subcutaneously (s.c.) or B) intracranially (i.c.) with $1x10^5$ U87-MG-luc cells in Matrigel. Tumor size was measured using a caliper twice per week or using an In Vivo Imaging System (IVIS) once weekly, detecting tumor bioluminescence (BLI) in photons and tumor volume (TV) was calculated using the following formula: $TV = ab^2/2$, where a is the length of the tumor, and b is the width. Once tumors were established, Debio 0123 was orally administered once a day for 28 consecutive days either alone (30 mg/kg or 60 mg/kg QD, Figure 5) or at 10 mg/kg in combination with 3 mg/kg QD TMZ (Figure 6).

RESULTS

Debio 0123 crosses the BBB in animal species

Following oral administration of 30 mg/kg QDx4 Debio 0123 in mice or 15 mg/kg single dose, in rats, concentration data show that Debio 0123 is able to cross the BBB and distributes into the brain. The brain to plasma AUC ratios of Debio 0123 were 0.49 in mice, and 0.60 in rat. Those results based on the full kinetic profile are in good agreement with brain to plasma concentration ratios of Debio 0123 determined from single-time point measurements in mice (ratio of 0.69 at 4h post last dose), rats (ratio of 1.5 at 24h post last dose), and monkeys (ratio of 4 at 24h post last dose) (results not shown). Moreover, the brain to plasma AUC ratio in mice was over 10-fold higher for Debio 0123 than AZD1775 (ratio of 0.048) or Zn-C3 (ratio of 0.028), demonstrating Debio 0123`s favorable brain penetration properties.



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	Debio 0123	AZD1775	Zn-C3
Brain to plasma ratio	0.49	0.05	0.03

Figure 3. Debio 0123 exhibits favorable brain exposure properties in rodents compared to AZD1775 or Zn-C3. (A) Mouse plasma and brain exposures (n=3 per timepoint) of Debio 0123 on day 4, following 30 mg/kg oral, once daily administration of Debio 0123. (B) Rat plasma and brain exposures of Debio 123 following a single oral dose of 15 mg/kg Debio 0123. (C) Mouse plasma and brain exposures of AZD1775 on day 4, following 30mg/kg oral administration twice daily from day 1 to 3 and once on day 4. (D) Mouse plasma and brain exposures of Zn-C3 on day 4, following 80 mg/kg oral administration once daily. (E) Brain to plasma ratios in mice determined in A-D.

Debio 0123 improves GBM response to radiotherapy (RT) *in vitro*

GBM cell lines were treated with increasing doses of Debio 0123 and RT. The surviving fraction was calculated for each condition and plotted using the Linear-Quadratic model. Weighing of the curve was done using $1/y^2$. Statistical testing was done using an extra sum-of-squares F test and H0 = one curve accurately fits all data.

Debio 0123 significantly improved cell line response to radiotherapy in 3 out of 4 GBM cell lines tested *in vitro* (T98G, SNB-19 and LN18 cell lines p<0.0001). This was not significant in case of U251 cell line (p=0.6); however, comparison of only the control and 5 µM conditions alone leads to statistical significance.

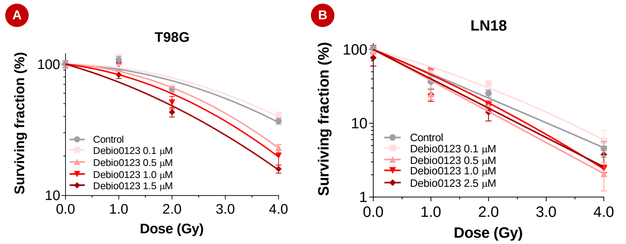

Figure 4. Debio 0123 enhances GBM cell line response to RT. (A) T98G **(B)** LN18 **(C)** SNB-19 and **(D)** U251 GBM cell lines were treated with Debio 0123 (0.1-5uM) and RT (1-4Gy) for 48 hours and colonies counted 14 days following treatment.

Debio 0123 inhibits GBM tumor growth *in vivo*

Debio 0123 treatment of U87-MG-luc tumors resulted in significant anti-tumor activity. In tumors implanted subcutaneously in male nude mice, 30 mg/kg and 60mg/kg QD treatment resulted in 50% (p<0.01) tumor growth inhibition (TGI) and 70% TGI (p<0.01) respectively compared to vehicle controls

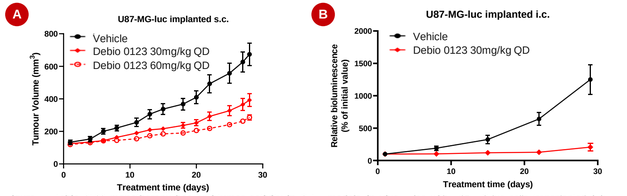

Figure 5. Debio 0123 monotherapy has anti-tumor activity in GBM models *in vivo*. Mice with U87-MG-luc cells transplanted **(A)** subcutaneously or **(B)** intracranially were treated with 30 or 60mg/kg Debio 0123 QD for 28 consecutive days (n=6).

Debio 0123 in combination with temozolomide leads to sustained regressions in an intracranial GBM model *in vivo*

The TMZ-sensitive U87-MG-luc intracranial model was used to further evaluate the efficacy and tolerability of Debio 0123 in combination with Temozolomide. SOC TMZ treatment significantly inhibited tumor growth while Debio 0123 monotherapy (10 mg/kg) resulted in a minimal anti-tumor response. However, combination of Debio 0123 and TMZ induced a significant anti-tumor response compared to vehicle (p<0.01), Debio 0123 monotherapy (p<0.01) and TMZ monotherapy (p=0.02) treatments.
Significantly, complete regressions were observed in 75% of all animals treated with Debio 0123 in combination with TMZ compared to 50% complete regressions in the TMZ-only group. Furthermore, despite all treatments ceasing on day 28, complete regressions observed in the Debio 0123 + TMZ group were sustained long-term, in some instances up to 200 days. All treatments were well tolerated with no significant body weight loss in the treated animals.

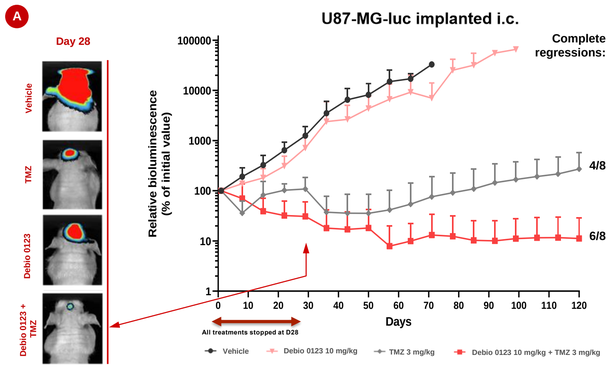

Figure 6. Debio 0123 shows potent antitumor efficacy *in vivo* in combination with Temozolomide. (A) Mice bearing U87-MG-luc tumors implanted intracranially were treated with 10mg/kg QD Debio 0123, 3mg/kg TMZ QD alone or in combination (n=8) and tumor growth monitored through luminescence imaging (representative examples of images following 28 days of each treatment group shown in left panel)

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 SCLC and in combination with TMZ with or without RT in patients with GBM (NCT05765812).

CONCLUSIONS

- Debio 0123 effectively crosses the BBB resulting in brain to plasma ratio that is 10-fold higher than other WEE1 inhibitors AZD1775 and Zn-C3.
- Debio 0123 improves response to RT effects *in vitro* in GBM cell lines.
- Debio 0123 results in significant anti-tumor effects as a monotherapy in both subcutaneous and intracranial *in vivo* models of GBM.
- Debio 0123 significantly improves response to standard of care TMZ treatment *in vivo* in an intracranial model of GBM and is well tolerated, supporting investigation of Debio 0123 in combination with TMZ +/- RT in patients with GBM.
- Debio 0123 is currently being explored in combination with TMZ with or without RT in a Phase 1 clinical trial (NCT05765812).

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
(4) Huang et al., J. Med. Chem. 2021, 64, 17, 13004

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