ANTITUMOR ACTIVITY OF THE NOVEL ORAL HIGHLY SELECTIVE WEE1 INHIBITOR DEBIO 0123

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ABSTRACT #4423

SUMMARY

The Wee1 tyrosine kinase is activated upon DNA damage and regulates the G2-M cell cycle checkpoint. Inhibition of Wee1, in conjunction with additional genetic alterations and/or addition of a DNA damaging agent, results in mitotic catastrophe and apoptosis of cancer cells, offering an attractive approach to treat cancer[1]. Only one Wee1 inhibitor, AZD1775, is currently in clinical development. The aim of the present study was to characterize the pharmacological properties of the newly discovered, orally available, and highly selective Wee1 inhibitor Debio 0123.

BACKGROUND

Wee1, a central player in cell cycle and DNA damage response

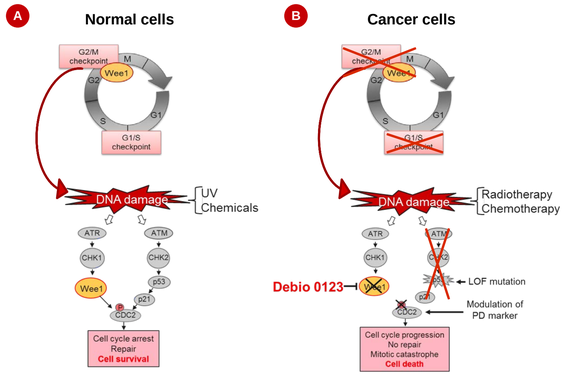


Figure 1. (A) Normal cells. Wee1 is a cell cycle checkpoint regulator and is activated upon DNA damage. Activated Wee1 interrupts cell cycle progression until repair is completed. This prevents cells to undergo apoptosis and promotes survival. Wee1 inhibition and the resulting suppression of the G2-M checkpoint would, in general, selectively impact tumor cells, with only a limited effect on healthy cells which usually display a functional G1-S checkpoint. **(B) Cancer cells.** Wee1 inhibition leads to cell-cycle progression despite unrepaired DNA damage with subsequent induction of cell death. LOF = loss of function, PD = pharmacodynamics.

METHODS

Animal studies were conducted in accordance with institutional guidelines and NCRI Guidelines for the welfare and use of animals in cancer research[2]. D1 was set as the first day of treatment.

Cell free assay: Profiling of Debio 0123 was performed on 465 selected kinases in a cell-free system.

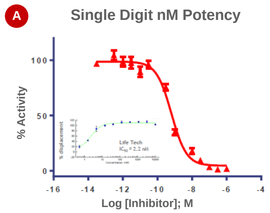
Western blot: Effects of Debio 0123 on downstream signaling were analyzed by ELISA and western blot in HT29 (colorectal adenocarcinoma) and A427 (lung carcinoma) cell lines.

In vitro proliferation assay: The in vitro growth inhibition activity of Debio 0123 was defined in a broad number of human cancer cell lines. Anti-tumor activity was assessed after 72h using a proliferation monolayer assay.

Mouse xenograft model: (Axis Bioservices) Briefly, 1.10^7 A427 tumor cells in 50% Matrigel were injected subcutaneously (sc) into the flank of male athymic nude mice. Animals were randomly assigned to treatment groups when tumors reached approximately 150 mm^3.

RESULTS

Debio 0123 is a potent and selective Wee1 Inhibitor



	IC$_{50}$ (nM)	
Assay	Debio 0123	AZD1775
Almac Discovery	0.8 ± 0.2 (Ki = 0.1 nM)	1.2 ± 0.1 (Ki = 0.2 nM)
Life Technologies	2.2	3.6

Table 2. cell free assay IC$_{50}$

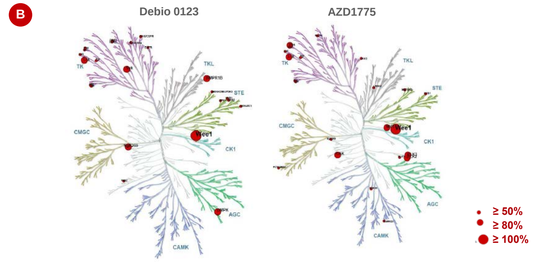


Figure 2. A) Cell free potency B) Selectivity profile Screening at fixed concentration of 0.5 µM (>100-fold IC$_{50}$ Wee1)

Debio 0123 is highly selective to Wee1. As compared to AZD1775, it does not inhibit Plk1 and Plk2, as also reported in recent publications for AZD1775[3].

Effects on downstream Wee1 signaling

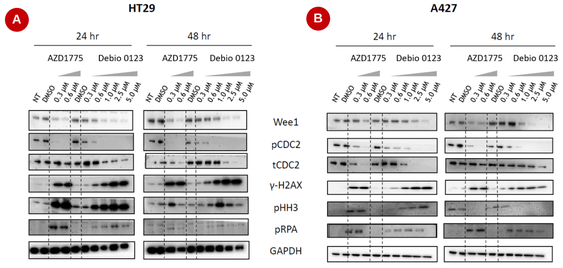


Figure 3. Effects of Debio 0123 on Wee1 downstream signaling. A) HT29 (colorectal) and **B)** A427 (lung adenocarcinoma) cells were treated with different concentrations of Debio 0123 and the Wee1 inhibitor AZD1775 for 24h and 48h. Whole cell lysates were analyzed by Western blot using the indicated antibodies. Debio 0123 reduces phosphorylation of CDC2, increases DNA damage, observed by induction of γH2AX, and augments mitosis, observed by enhanced phosphorylation of histone H3.

Debio 0123 anti-proliferative activity in a large panel of cell lines

Figure 4. Bar plot of absolute IC50 values of 159 cell lines treated with Debio 0123.

Median IC$_{50}$ value was 1.23 µM (range : 0.109 to 7.08 µM), showing a good response of cancer cells to Debio 0123 across various histotypes. While some cell lines are sensitive to Debio 0123 as monotherapy, further work is currently ongoing aiming at identification of potential predictive biomarkers and at exploring potential combinations.

Debio 0123 induced tumor regressions in a NSCLC cancer model

When administered orally once daily for 28 consecutive days, Debio 0123 induced dose-dependent anti-tumoral activity and was well tolerated at all doses tested. At 30 mg/kg, treatment with Debio 0123 resulted in tumor regression.

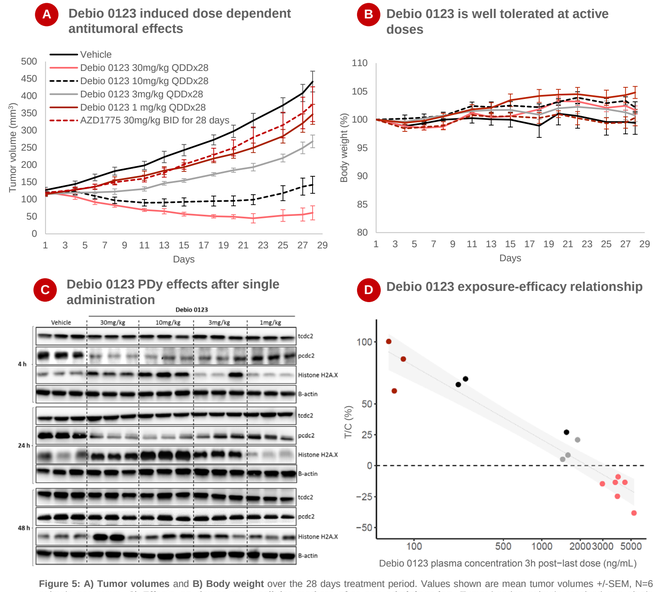


Figure 5: A) Tumor volumes and **B) Body weight** over the 28 days treatment period. Values shown are mean tumor volumes +/-SEM, N=6 animals per group. **C) Effects on downstream cellular markers after one administration**. Tumor-bearing animals received one single administration of Debio 0123. Tumors were collected at 4h, 24h, and 48h post administration. Expression of the indicated proteins was assessed by Western blot analysis. Quantification of P-CDC2/total CDC2, γH2AX levels at 4h, 24h and 48. N=3 animals per group, all 3 animals are shown. **D) Debio 0123 exposure-efficacy relationship**. Values shown are individual % T/C Value = [(individual tumor volume on Day 28 – individual starting volume) / (mean vehicle tumor volume on Day 28 – mean vehicle starting volume)] X100 as a function of Debio 0123 plasma concentration at 3h post last dose in corresponding animals, for all the tested dose levels. The black dotted line indicates the threshold for tumor regression.

CONCLUSIONS

- Debio 0123 is a highly selective and potent Wee1 inhibitor able to prevent CDC2 / Cdk1 phosphorylation and to induce apoptosis through mitotic catastrophe following cell cycle progression despite accumulation of unrepaired DNA damage both in vitro and in vivo, resulting in tumor regression.
- Debio 0123 is currently being evaluated in combination with various agents.
- The advancement of Debio 0123 into clinical studies may provide improved therapeutic outcomes for patients with cancer.

REFERENCES

(1) Do K. et al. Cell Cycle. 2013 Oct 1;12(19):3159-64.
(2) Workman et al., British Journal of Cancer (2010) 102, 1555 – 1577.
(3) Wright G. et al., 2017 ACS Chem Biol. 2017 Jul 21;12(7):1883-1892.

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