Development of Selective WEE1 inhibitors

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1. INTRODUCTION

WEE1 regulates the G2/M cell cycle checkpoint via phosphorylation of CDK1 (aka Cdc2) at Tyr^{15}, which inhibits CDK1/cyclin B kinase activity (Fig. 1; Matheson et al, 2016). Inhibition of WEE1 overrides DNA damage-induced cell cycle arrest in cells with a dysfunctional G1 checkpoint and drives TP53 mutant cancer cells into mitotic catastrophe (Fig. 2; Duda et al, 2016). It is therefore an attractive target for enhancing the effects of chemotherapeutic DNA-damaging therapies.

The potent WEE1 inhibitor AZD1775 (aka MK1775) has advanced to clinical trials in combination with DNA-damaging therapies in several cancers (https://clinicaltrials.gov). However, recent reports show that AZD1775 has single agent antiproliferative activity (Matheson et al, 2016), which is counter-intuitive considering its postulated mode of action. Other studies suggest AZD1775 exerts poor kinase selectivity, and inhibits polo-like kinase 1 (PLK1) with similar potency as WEE1 (Wright et al, 2017). PLK1 is also known to directly regulate WEE1 activity by phosphorylation of Ser^{53}, which leads to ubiquitination and subsequent proteasomal degradation of WEE1 (Kousholt et al, 2012). These findings suggest that some of the observed effects of AZD1775 may not be solely due to WEE1 inhibition. Highly selective (*in vitro* and in vivo) tool compounds are critical for biological research and further elucidation of the role of WEE1. We present here our work at Concept Life Sciences (CLS) towards the identification and development of a selective WEE1 tool compound for these studies.

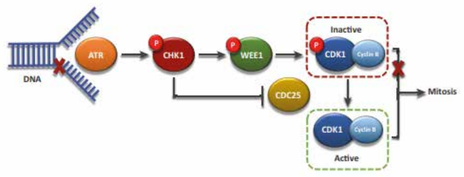


Figure 1: Schematic representation of the role pf WEE1 in the G2/M checkpoint. Taken from Matheson et al, 2016

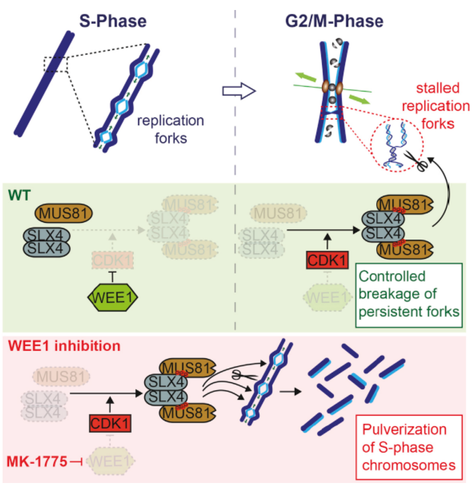
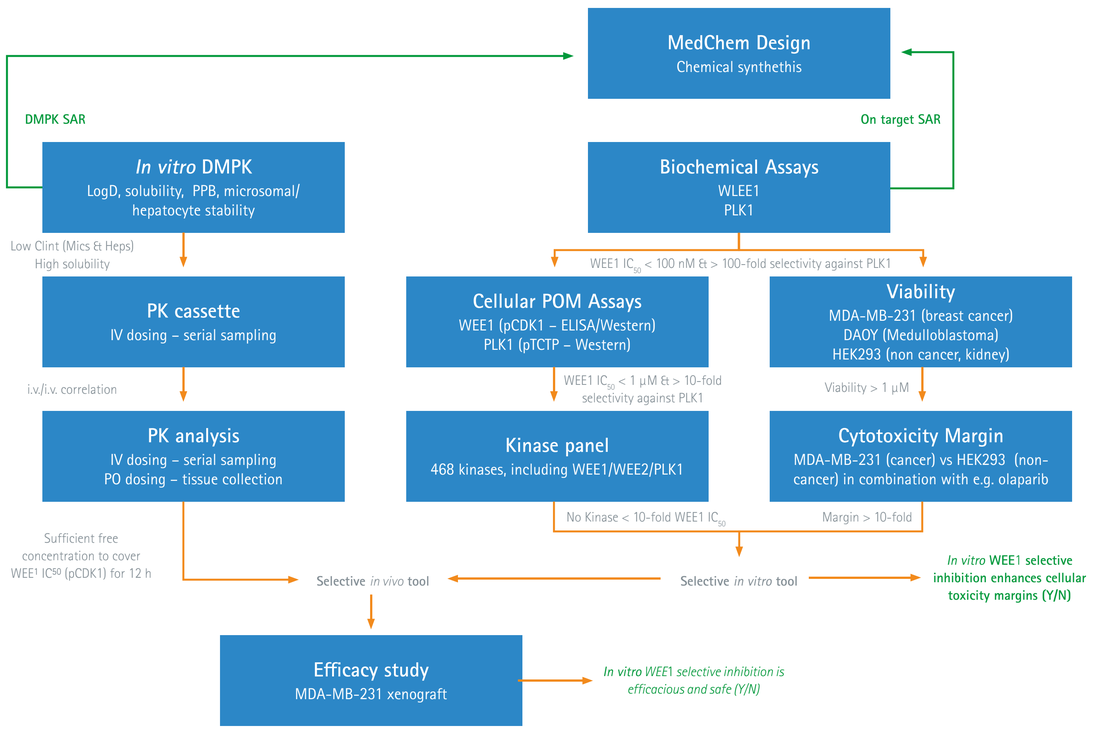


Figure 2: Proposed mechanism by which WEE1 inhibition may lead to cell death by MUS81-SLX4-dependent chromosome breakage (mitotic catastrophe). Taken from Duda et al, 2016.

2. DISCOVERY CASCADE



3. STRUCTURE BASED DESIGN OF WEE1 VS PLK1 SELECTIVITY

- Human WEE1 and human PLK1 kinases have 44% sequence similarity (BLAST alignment, kinase domain).
- Analysis of WEE1 (Zhu et al, 2017) and PLK1 (Kothe et al, 2007) Xray crystallographic data highlights key residue differences (e.g. Asn^{376}/Leu^{130}, Tyr^{378}/Leu^{132}, Glu^{303}/Arg^{47}), cavities and clashes that could be exploited within the active site to enhance kinase selectivity (Fig. 3A).
- Our Hit identification strategy aims to identify novel compounds with high WEE1 selectivity over PLK1 by exploiting these active site differences (Fig. 3B).

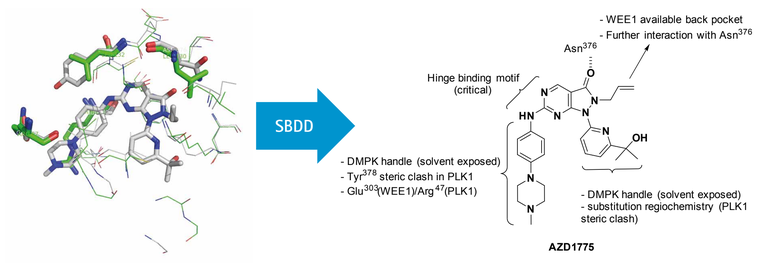


Figure 3: **(A)** Overlay of WEE1 (Zhu et al, 2017; PDB: 5v5y, grey) and PLK1 (Kothe et al 2007; PDB: 2rku, green) kinase active sites (displayed in wire). The type I WEE1 kinase inhibitor AZD1775 (in grey sticks) is co-crystallised within WEE1. Key active site differences are highlighted in grey and green sticks. Circled areas show key structural opportunities for optimisation of WEE1 selectivity over PLK1. **(B)** AZD1775 structural modification strategy aimed at the enhancement of WEE1 affinity over PLK1. Circled areas show key structural opportunities for optimisation of WEE1 selectivity over PLK1.

4. WEE1 SELECTIVITY ENHANCES CELL VIABILITY

- Multiple CLS analogues are < 10 nM inhibitors of WEE1 kinase and display a measured logD between 1 and 3 (Fig. 4).
- A range of structurally diverse analogues are >100-fold selective for WEE1 over PLK1.
- None of the tested WEE1 literature references are > 50-fold selective (AZD1775 is 22-fold selective).
- A number of CLS analogues show comparable potency to AZD1775 in the cellular mechanistic assay (pCDK1 levels; Fig. 5A); compound ranking was further confirmed by Western blotting (Fig. 5B).

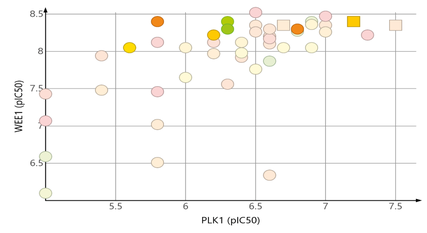


Figure 4: WEE1 biochemical IC_{50} (Eu-LanthaScreen, Thermo) vs PLK1 biochemical IC_{50} (ADP-Glo, Promega). Colours correspond to measured logD, with analogues highlighted in the presentation in brighter colouring. (Spheres are CLS analogues, squares are literature examples.)

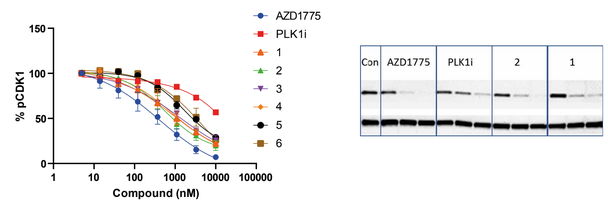


Figure 5: CLS compound (1-6) effects on cellular WEE1 activity compared to AZD1775 and selective PLK1 inhibitor (PLK1i) after 6 h treatment in non-synchronised cells by **(A)** ELISA (mean ± sd, n=2) and **(B)** Western blotting (100, 1,000 and 10,000 nM doses). Con = vehicle control.

- WEE1 active and selective compounds show reduced single agent cytotoxicity in MDA-MB-231 (Fig. 6A), HEK293 (non-cancer) and DAOY (data not shown) cells.
- There is a strong correlation between cancer (MDA-MB-231) and non-cancer (HEK293) viability IC_{50} data, suggesting that single agent cytotoxicity is not dependent on TP53 status (Fig. 6B).
- Compound ranking was further confirmed by real-time kinetic measurement of anti-proliferative effects (RealTime-Glo (Promega); Fig. 6C and IncuCyte (Essen); data not shown) in MDA-MB-231 cells.

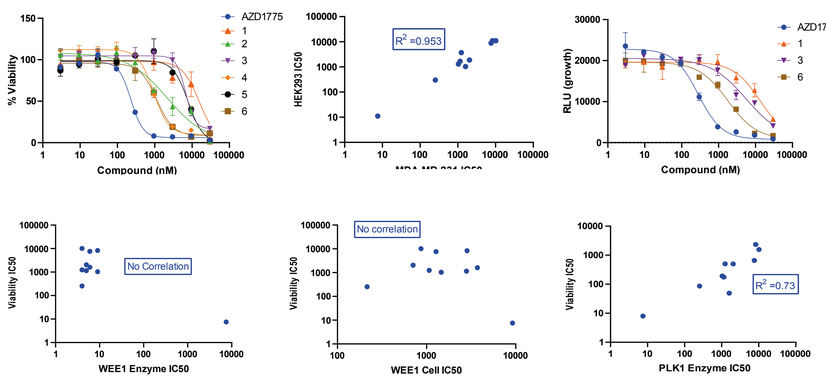


Figure 6: **(A)** Compound effects on MDA-MB-231 cell viability (CellTiter-Glo assay, Promega, mean ± sd, n=2). **(B)** Correlation of MDA-MB-231 and HEK293 viability IC_{50} values. **(C)** Compound effects on cell proliferation (RealTime-Glo assay, mean ± sd, n=2).

Figure 7 **(A)** Correlation of MDA-MB-231 and HEK293 viability IC50 values. **(B)** Correlation of MDA-MB-231 viability data with WEE1 enzyme IC_{50} **(C)** Correlation of MDA-MB-231 viability data with PLK1 enzyme IC50 Data derived from mean of 2 independent measurements.

- Single agent toxicity in MDA-MB-231 cells does not correlate with WEE1 enzyme (Fig. 7A) or cellular (Fig. 7B) activity.
- We observe a weak correlation of cytotoxicity with PLK1 enzyme activity (Fig. 7C); the significance of this finding is not yet known.

5. TOWARDS A WEE1 SELECTIVE TOOL COMPOUND

- CLS analogue 13 shows similar WEE1 activity (both biochemical, Fig. 8A and cellular Fig. 8C), increased selectivity versus PLK1 (biochemical, Fig. 8B), and reduced cytotoxicity (Fig. 8D) compared to AZD1775.

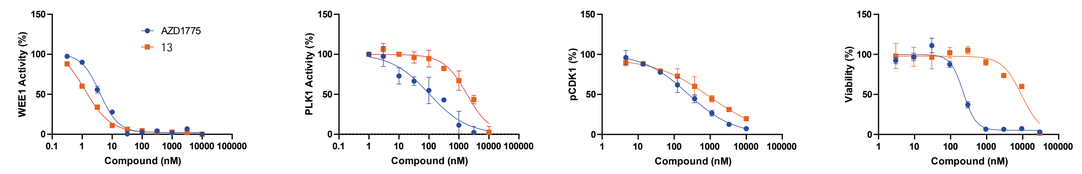


Figure 8: Profiling CLS analogue 13 versus AZD1775. Compound effects on **(A)** WEE1 biochemical inhibition, **(B)** PLK1 biochemical inhibition, **(C)** WEE1 cellular activity (pCDK1 in MDA-MB-231), **(D)** cellular viability (MDA-MB-231).

- Cross screening of a panel of 468 kinases confirmed WEE1 activity and selectivity against PLK1. Follow up IC50s against the top hits identified in the screen FLT3(D835V), MAP3K2/15, OSR1, TAOK1/2, TYK2(JH2domain-pseudokinase) is ongoing.
- Profiling of 13 shows selectivity over PLK1 can be achieved whilst retaining comparable *in vitro* DMPK properties to AZD1775 (Table 1)

CLS ID	WEE1[1]	PLK1[2]	PLK1/WEE1	pCDK1[3]	Cell viability[4]	$logD_{7.4}$	Kinetic Solubility	PPB[5]		Hepatocytes	
	IC^{50} nM	IC^{50} nM	Fold selectivity	IC^{50} µM	IC^{50} µM	N.A.	µM	Human %Fu	Mouse %Fu	Human Clint (µL/min/10^6 cells)	Mouse Clint (µL/min/10^6 cells)
AZD1775	4	86	22	0.21	0.25	2.4	8.6	25	28	3	123
13	4	1,562	391	0.86	10.2	2.9	7	12	40	17	148

Table 1: *In vitro* biological and DMPK characterisation of AZD1775 and 13. 1Eu-LanthaScreenTM (assay wall = 5 nM), 2ADP-GloTM, 3WEE1 cellular activity (MDA-MB-231), 4MDA-MB-231 cells, 5Plasma protein binding.

- i.v. bolus PK cassette (0.4 mg/kg) administration of 4 CLS analogues (including 13) and AZD1775 to CD1 male mice (3 animals using micro-sampling) carried out (Table 2)
- No i.v./i.v. correlation observed within the series
- In vivo PK profiling of 13 (and other CLS analogues) suggests free plasma level similar to AZD1775 could be achieved

Compound	AZD1775	13
In vivo PK parameters		
Dose (mg/kg)	0.4	0.4
Cmax/C0 (uM)	0.14	0.14
t1/2 (h)	0.29	0.31
Vdss	7.7	4.6
CL1 (ml/min/kg)	405	190
In vitro data		
MPPB2 (%Fu)	28	26
LogD	2.4	2.9
MLM3 (µL/min/mg)	66	282
MHep4 (µL/min/106 cells)	123	148

Table 2: In vivo PK parameters resulting from cassette dosing (0.4 mg/kg, i.v. bolus to male CD1 mice (n=3 at 0.03, 0.25, 0.5, 1, 2, 4, 8, 24 h (serial-sampling)) of 4 CLS analogues (including 1, 3 and 4) and AZD1775, and corresponding *in vitro* DMPK data. 1Clearance, 2Mouse plasma protein binding, 3Mouse liver microsomes, 4Mouse hepatocytes.

6. SUMMARY

Compared to AZD1775, 13 shows comparable cellular WEE1 activity and DMPK properties but has increased selectivity vs PLK1 (biochemical assays). This manifests in reduced single agent cytotoxicity in cancer and non cancer cell lines, consistent with the mechanistic hypothesis.

ONGOING WORK

- Further investigation of AZD1775 cellular activity to establish basis of single agent cytotoxicity.
- Follow up on kinase panel for 13; IC50 determination at FLT3(D835V), MAP3K2/15, OSR1, TAOK1/2, TYK2(JH2domain-pseudokinase).
- Organic Cation Transporter (OCT) assays - to interrogate i.v./i.v. correlation disconnect and design compounds with optimised PK parameters.

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