Development of WEE1 inhibitors with selectivity against PLK1

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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.

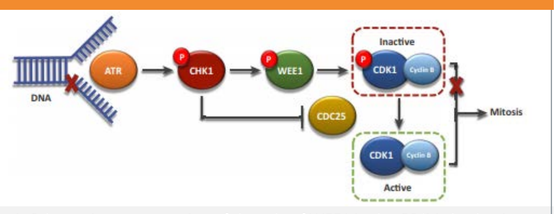


Figure 1: Schematic representation of the role pf WEE1 in the G2/M checkpoint. Taken from Matheson *et al*, 2016

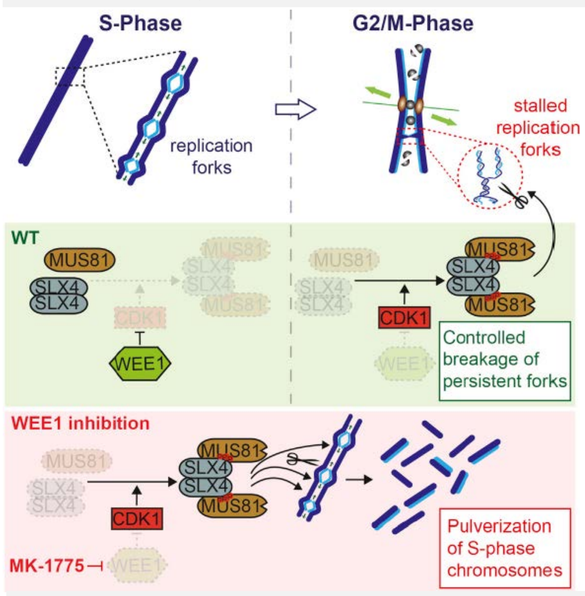
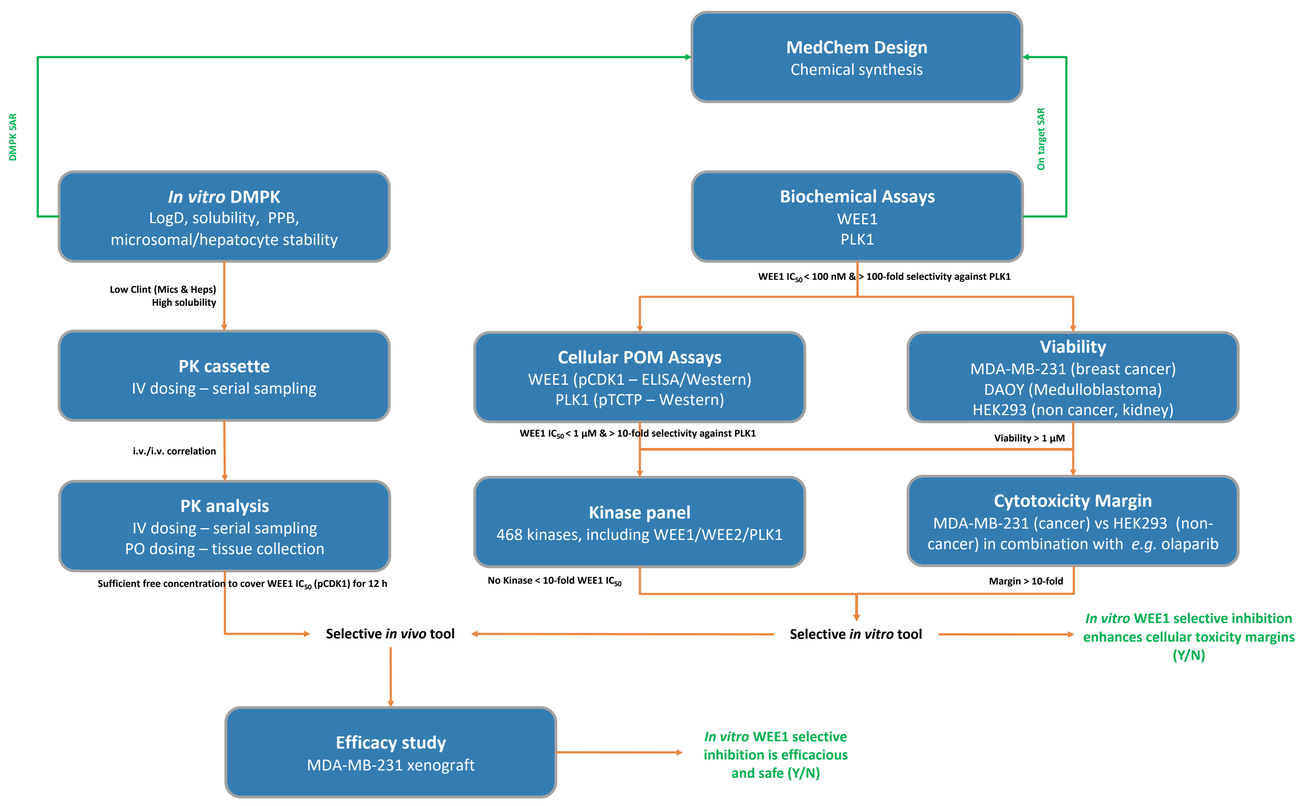


Figure 2: Proposed mechanism by which WEE1 inhibition may lead to cell death by MUS81-SLX4-dependent chromosome breakage (mitotic catastrophe). Taken from Duda *et al*, 2016.

DISCOVERY CASCADE



STRUCTURE BASED DESIGN OF WEE1 *vs* PLK1 SELECTIVITY

- Human WEE1 and human PLK1 kinases have 44.3% 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**).

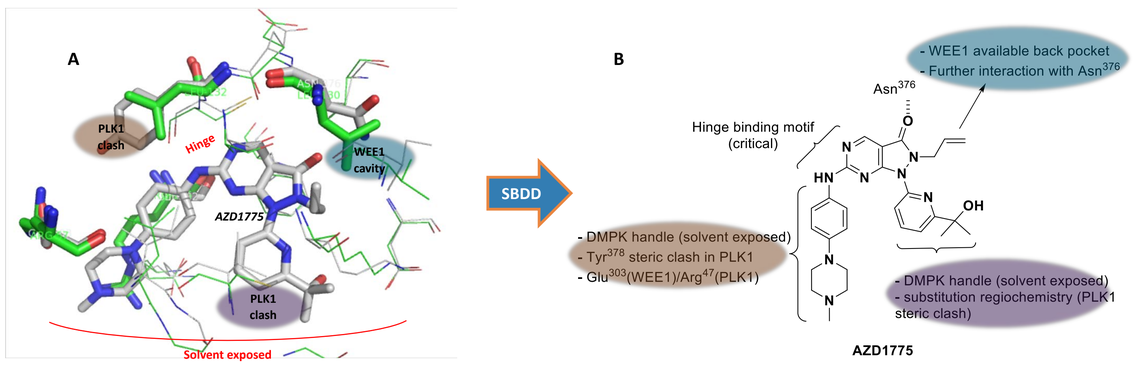


Figure 3: (**A**) Overlay of WEE1 (Zhu *et al*, 2017; PDB: 5v5y, grey) and PLK1 (Kothe *et al* 2007; PDB: 2rku, green) kinase active sites (displayed in wire). The type I WEE1 kinase inhibitor AZD1775 (in grey sticks) is co-crystallised within WEE1. Key active site differences are highlighted in grey 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.

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2. Wright G. *et al.* Dual targeting of WEE1 and PLK1 by AZD1775 elicits single agent cellular anticancer activity. ACS chem. Biol. 12: 1883-1892, 2017.
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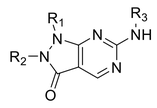
SAR of WEE1 *vs* PLK1

- Analogue SAR corroborated the SBDD working hypothesis and PLK1 *vs* WEE1 pocket analysis



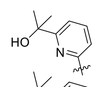
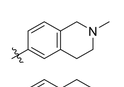
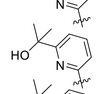
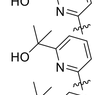
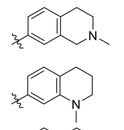
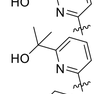
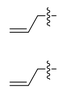
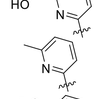
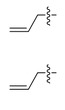
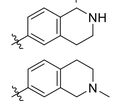
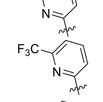
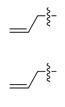
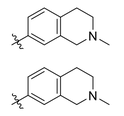
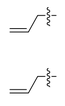
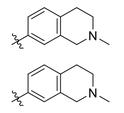
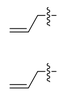
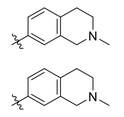
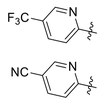
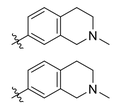
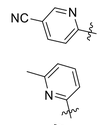
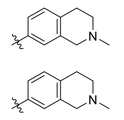
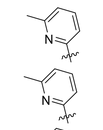
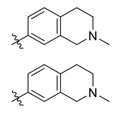
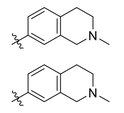
	R_1	R_2	R_3	WEE1[1] IC_{50} nM	PLK1[2] IC_{50} nM	PLK1/ WEE1	pCDK1 uM	Viability IC_{50} uM	$logD_{7.4}$
AZD1775	N.A.	N.A.	N.A.	4	86	22	0.21	0.25	2.4
1				4	128	29			2.4
2				9	191	21	1.5	1.0	2.3
3				6	50	8	3.7	1.6	4.0
4				4	129	32	-	-	1.2
5				6	658	110	1.3	7.6	2.6
6				11	587	55	-	-	2.8
7				8	1434	192	-	-	3.9
8				6	278	44	-	-	3.1
9				9	2332	245	2.8	8.3	2.3
10		H		258	>10,000	39	>10	>30	1.2
11		iPr		11	3691	324	-	-	3.3
12		cyclohexyl		84	10,000	118	-	-	4.1
13	-	-	-	4	1562	391	0.86	10.2	2.9

Table 1. SAR of selected analogues demonstrates that regions R1 and R2 can be used to tune WEE1 *vs* PLK1 selectivity.
[1]Assay wall in this assay format 5 nM, [2]Assay wall in this assay format 25 nM, [3]in MDA-MB-231 cells

WEE1 SELECTIVITY ENHANCES CELL VIABILITY

- 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. 4A)**.

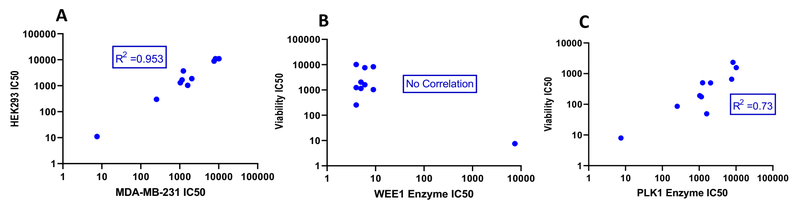


Figure 4 (**A**) Correlation of MDA-MB-231 and HEK293 viability IC_{50} values. (**B**) Correlation of MDA-MB-231 viability data with WEE1 enzyme IC_{50} **(C)** Correlation of MDA-MB-231 viability data with PLK1 enzyme IC_{50} Data derived from mean of 2 independent measurements.

- Single agent toxicity in MDA-MB-231 cells does not correlate with WEE1 enzyme (**Fig. 4B**) or cellular activity (pCDK1 data not shown).
- We observe a weak correlation of cytotoxicity with PLK1 enzyme activity (**Fig. 4C**); the significance of this finding is not yet known.

TOWARDS A WEE1 SELECTIVE TOOL COMPOUND

- 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 2**)

CLS ID	WEE1[1] IC_{50} nM	PLK1[2] IC_{50} nM	PLK1/ WEE1 Fold selectivity	pCDK1[3] IC_{50} µM	Cell viability[4] IC_{50} µM	$logD_{7.4}$ N.A.	Kinetic Solubility µM	PPB[5] Human %Fu	PPB[5] Mouse %Fu	Hepatocytes Human Clint (µL/min/10^6 cells)	Hepatocytes 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	7.0	12	40	17	148

Table 2: *In vitro* biological and DMPK characterisation of AZD1775 and **13**. [1]Eu-LanthaScreen™ (assay wall = 5 nM), [2]ADP-Glo™, [3]WEE1 cellular activity (MDA-MB-231), [4]MDA-MB-231 cells, [5]Plasma 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 3**)
- 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
CL[1] (ml/min/kg)	405	190
***In vitro* data**		
MPPB[2] (%Fu)	28	26
LogD	2.4	2.9
MLM[3] (µL/min/mg)	66	282
MHep[4] (µL/min/10^6 cells)	123	148

Table 3: *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. [1]Clearance, [2]Mouse plasma protein binding, [3]Mouse liver microsomes, [4]Mouse hepatocytes.

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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