A systems biology approach combining ProLiFiler and Cancer Data Miner for an enhanced preclinical characterization of the WEE1 inhibitor adavosertib

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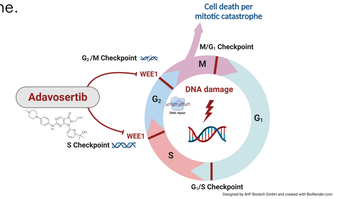
Introduction

Adavosertib (AZD1775, MK-1775), is a clinical stage inhibitor of the tyrosine kinase Wee1. It plays an important role in regulating G2/M and S checkpoints. In case of DNA damage, Wee1 activation results in cell cycle arrest and mitosis entry failure until the DNA is repaired. In cancer cells, Wee1 inhibition leads to cell death per mitotic catastrophe.

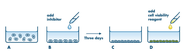


In the present study, adavosertib was investigated in a cell survival and proliferation assay with 140 human cancer cell lines (CLs) representing all major tumor types (ProLiFiler platform of Reaction Biology) followed by mechanism of action (MoA) and biomarker analyses using 4HF Biotec's Cancer Data Mining in silico platform (DataMiner™).

In vitro antitumor activity using ProLiFiler

Assay procedure of the Cell Proliferation Assay

The ProLiFiler assay is performed using a contact-free nano-dispensing system (Tecan D300E) requiring small amounts of a test compound.



Assay procedure: *Cells are seeded into 384 multi-well plates (A). The next day, compounds are added (B), and cells are incubated for 72 h (C). Subsequently, the cell viability dye CellTiter-Glo is added, and the luminescence is measured as a parameter for cell viability (D). IC_{50} values are determined from 8 concentrations in duplicates.*

Origin of cell lines

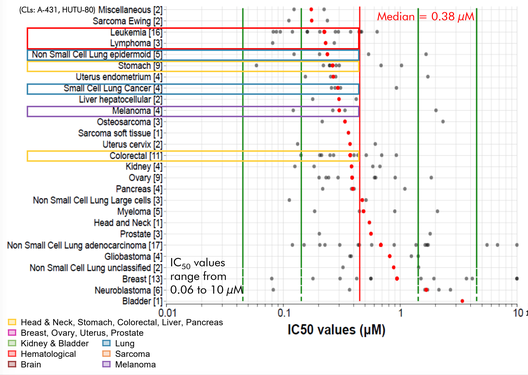
The panel currently consists of 140 human tumor cell lines derived from 21 tumor types.

Tumor type	#	Entities
Breast	13	4 ER$^+$; 5 ERBB2$^+$; 4 TNBC
Ovary	9	
Uterus	6	2 cervix; 4 endometrium
Prostate	3	1 AR$^+$; 2 AR$^-$
Kidney	4	
Colorectal	11	
Stomach	9	
Pancreas	4	
Liver	2	2 hepatocellular carcinoma
Non-Small Cell Lung	27	17 adenocarcinoma; 5 squamous; 3 large cell; 2 unclassified
Small Cell Lung	4	
Melanoma	4	
Nervous System	10	5 glioblastoma; 5 neuroblastoma
Sarcoma	6	3 osteosarcoma; 2 Ewing; 1 soft-tissue
Miscellaneous	4	1 bladder; 1 duodenum, 1 head & neck; 1 vulva (skin)
Leukemia	16	*3 ALL; 10 AML; 2 CLL; 1 CML
Myeloma	5	
Lymphoma	3	*2 DLBCL; 1 ALCL
Total	140	

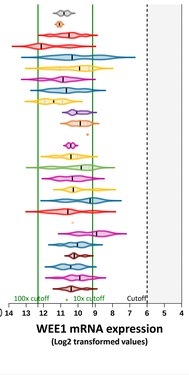
ALL: Acute Lymphoblastic Leukemia, AML: Acute Myeloid Leukemia, CLL: Chronic Lymphocytic Leukemia ,CML: Chronic Meilogenous Leukemia, DLBC: Lymphoma Diffuse Large B Cell, ALCL: Anaplastic large cell lymphoma

ProLiFiler – anti-proliferative activity of adavosertib

Potency and selectivity of adavosertib in vitro



WEE1 mRNA by cancer type



Rank ordered plot of IC_{50} data for cutoff determination

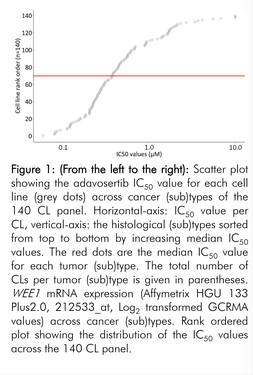


Figure 1: (From the left to the right): Scatter plot showing the adavosertib IC_{50} value for each cell line (grey dots) across cancer (sub)types of the 140 CL panel. Horizontal-axis: IC_{50} value per CL, vertical-axis: the histological (sub)types sorted from top to bottom by increasing median IC_{50} values. The red dots are the median IC_{50} value for each tumor (sub)type. The total number of CLs per tumor (sub)type is given in parentheses. *WEE1* mRNA expression (Affymetrix HGU 133 Plus2.0, 212533_at, Log_2 transformed GCRMA values) across cancer (sub)types. Rank ordered plot showing the distribution of the IC_{50} values across the 140 CL panel.

MoA Finder – drug mechanism of action of adavosertib

Drug Mechanism of Action (MoA)

The MoA Finder tool aims to identify the MoA of test compounds based on the results obtained in the ProLiFiler cell panel screening. To this end, we compare the sensitivity profile of a drug candidate to the profiles of more than 900 drugs for which the MoA is known.

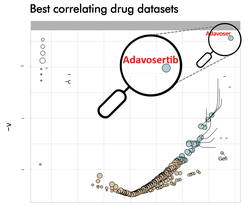
- The MoA Finder tool allows the confirmation of the mechanism of action of drugs.
- The data help to identify potential off-targets and to predict side effects.
- The MoA of compounds with unknown mechanisms, potential MoA can be predicted and suggestions for target deconvolution in a phenotypic approach can be made.

Database: *The drug sensitivity profiles, which reflect the MoAs of more than 900 anticancer agents, were collected from both the literature and/or determined in internal experiments.*

Approach: *The drug sensitivity profile (IC_{50} values from ProLiFiler screen) of a test compound will be compared to the reference compounds' drug sensitivity profiles. The correlation of the two datasets is evaluated by two statistical values, the Spearman Rho correlation value, and its p-value.*

Deliverables: *Graphical presentation of the correlation analysis. Excel table with correlation results.*

Best correlating drug datasets

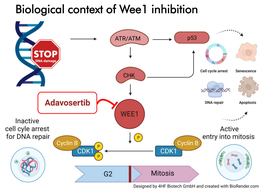


The 10 Top-ranked MoA and correlation results (> 900 tested)*;**.

Rank	Compound	Mechanism of Action	Targets	ρ (Spearman test)	BH* adjusted p-value	Cell line (n=)	Geo. Mean (µM)	Database
1	Adavosertib	Cell cycle, genome integrity	WEE1, PLK1	0.57	7.76E-07	94	1.94	GDSC2
2	YK-4-279	DNA/RNA synthesis	EWS-FLI1, DHX9, ERG, ETV1	0.46	7.81E-04	95	4.65	GDSC1
3	AZD7762	Cell cycle, genome integrity	CHEK1, CHEK2	0.44	1.17E-03	94	1.07	GDSC2
4	Wee1 Inhibitor	Cell cycle, genome integrity	WEE1, CHEK1	0.42	2.36E-03	93	8.63	GDSC2
5	AP-24534	ABL signaling	BCR-ABL, FGFR, PDGFR, VEGFR, SRC	0.4	2.13E-03	108	1.5	GDSC1
6	Pevonedistat	Cell cycle, genome integrity	NAE	0.4	5.76E-03	92	2.36	GDSC2
7	AZD6738	Cell cycle, genome integrity	ATR	0.39	6.79E-03	92	8.34	GDSC2
8	YK-4-279	DNA/RNA synthesis	EWS-FLI1, DHX9, ERG, ETV1	0.38	9.47E-03	92	10.92	GDSC2
9	Pevonedistat	Cell cycle, genome integrity	NAE	0.38	1.05E-02	86	1.24	GDSC1
10	VE821	Cell cycle, genome integrity	ATR/ATM	0.37	1.02E-02	90	57.94	GDSC2

* BH: Benjamini & Hochberg

Biological context of Wee1 inhibition



One to one comparison with other MoAs

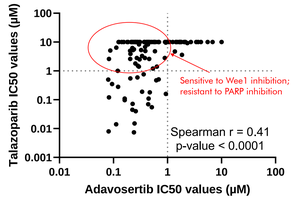


Figure 2. Top left: Volcano plot representation of MoA finder results of 471 anti-cancer drugs profiles (IC_{50} data) with that of adavosertib, which is based on the IC_{50} data from the ProLiFiler (COMPARE analysis*;**). **Top right:** Table showing the correlation results of the 10 top-ranked compounds. **Bottom left:** Graphical representation of the biological context of the proteins inhibited by TOP correlating drugs. **Bottom right:** Scatter plot showing the correlation between the sensitivity profiles of adavosertib and talazoparib (PARP inhibitor).*Huang, R., Wallqvist, A. & Covell, D.G. Anticancer metal compounds in NCI's tumor-screening database: putative mode of action. Biochem Pharmacol, (2005). Paull, K.D. et al. Display and analysis of patterns of differential activity of drugs against human tumor cell lines: development of mean graph and COMPARE algorithm. J Natl Cancer Inst, (1989). **Only drug datasets with more than 80 cell lines for comparison are shown in the table. 4HF Biotec (n=45), Sanger GDSC1 (n=251), Sanger GDSC2 (n=175).

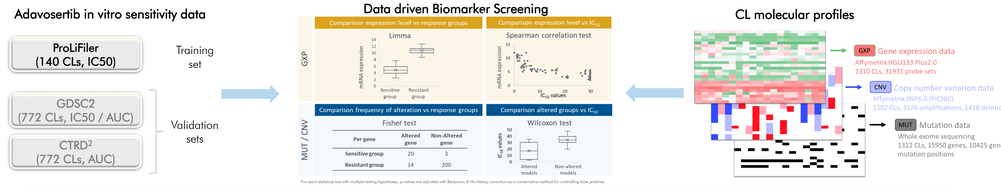
Biomarker Screening

Biomarker Analysis

The Biomarker Analysis tool reveals the specific genomic signature of tumor cell lines that are sensitive to a given test compound. To this end, we correlate the drug sensitivity profile (IC_{50} data of ProLiFiler screen) of a test drug with available gene expression and genetic alterations (mutations, deletions, amplifications) datasets of the tumor cell lines.

The Biomarker Analysis tool can be used to identify genes expression changes or genetic alterations whose presence is indicative of the efficacy of the test compound and may serve as predictive biomarkers in disease models and patient cohorts.

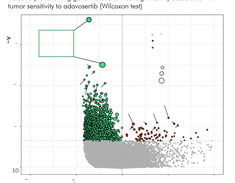
Approach: *The ProLiFiler results are correlated with the gene expression, whole-exome sequencing (mutations), and somatic copy number alteration data of the tumor cell lines. An analysis is performed for every tumor cell line to determine the correlation of its drug sensitivity to each gene with regards to the expression level, mutation status, and somatic copy number.*



Genomic alterations predicting sensitivity to adavosertib (using the GDSC2 dataset)

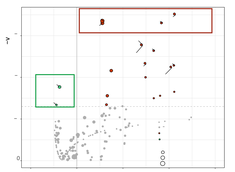
Mutations

Volcano plot showing gene mutations most significantly associated with tumor sensitivity to adavosertib (Wilcoxon test)



Amplifications

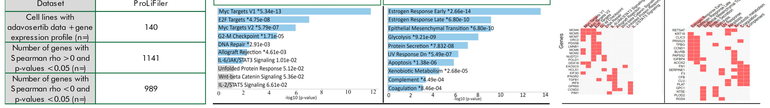
Volcano plot showing gene amplifications most significantly associated with tumor sensitivity to adavosertib (Wilcoxon test)



Transcripts and gene signatures predicting cancer sensitivity to adavosertib

Testing set

Dataset	All cancers (Hematological & Solid) ProLiFiler
Cell lines with adavosertib data + gene expression profile (n=)	140
Number of genes with Spearman rho >0 and p-values <0.05 (n=)	1141
Number of genes with Spearman rho <0 and p-values <0.05 (n=)	989



Validation sets

Dataset	Hematological cancers GDSC2	Hematological cancers CTRD2	Solid cancers GDSC2	Solid cancers CTRD2
Cell lines with adavosertib data + gene expression profile (n=)	88	137	474	611
Number of genes with Spearman rho >0 and p-values <0.05 (n=)	827	606	1194	2049
Number of genes with Spearman rho <0 and p-values <0.05 (n=)	1143	1413	2217	3891

Dataset	Shared significant genes (GDSC2 +CTD2)	Shared significant genes (GDSC2 +CTD2)
Number of genes with Spearman rho >0 and p-values <0.05 (n=)	184	962
Number of genes with Spearman rho <0 and p-values <0.05 (n=)	314	1627

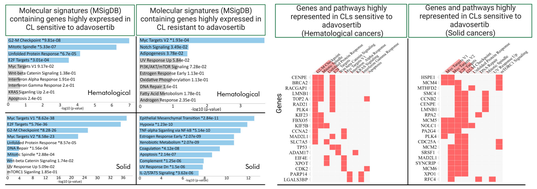


Figure 3: Left: Volcano plots showing levels of significance of individual genes for the association between adavosertib IC50 values and their gene mutation status (top plot) and gene amplification/deletions (bottom plot) (testing dataset: GDSC2; n = 761). The red and green dots show the gene alterations significantly associated with the response to adavosertib (Wilcoxon test; p-value < 0.05). **Right:** Transcripts correlating with CL response to adavosertib using ProLiFiler dataset (top) along with validation in external datasets (bottom). Tables presenting the cell line panel used for the analysis for each dataset and the number of genes / probe sets significantly positively and negatively correlated with the adavosertib IC50 values (Spearman correlation test).
The bar plots represent the results of the gene set enrichment analysis (Molecular Signatures Database Hallmark 2020) of genes correlating with adavosertib IC50 values in Profiler and external datasets. Clustergrams show the genes underlying in the 10 top-ranked gene expressions signatures of the MSigDB Hallmarks.
Xie Z, et al., Gene set knowledge discovery with Enrichr. Current Protocols (2021). Liberzon A et al., The Molecular Signatures Database Hallmark Gene Set Collection. Cell Systems (2015).

Conclusions

In conclusion, our studies demonstrate broad anticancer activity of adavosertib and confirm its proposed mechanism of action. The biomarkers we identified will facilitate the selection of pre-clinical in vivo tumor models and, if confirmed, even patient selection for clinical trials. The combined use of the ProLiFiler and Cancer Data Miner Platforms has the potential to accelerate and de-risk the development of anti-cancer agents.