ABSTRACT/ POSTER 35076

TP53 WILDTYPE STATUS CAN PREDICT SENSITIVITY TO XPO1 INHIBITORS IN PATIENT-DERIVED CANCER MODELS

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INTRODUCTION

- Selinexor (SEL) is a selective inhibitor of exportin 1 (XPO1) that leads to the nuclear accumulation and activation of key tumor suppressors (e.g., p53, p21, RB, and FOXOs), resulting in selective cancer cell death (Figure 1). Eltanexor (ELT) is an investigational compound with the same target and similar properties.

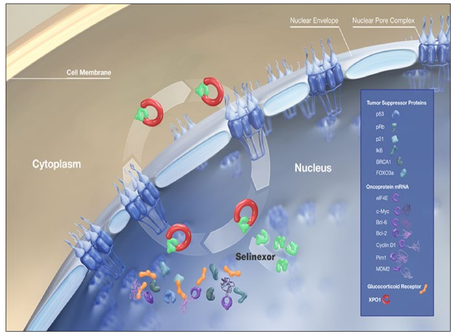


Figure 1. Selinexor mechanism of action

- In the exploratory analysis of the SIENDO trial evaluating SEL as a maintenance therapy for patients with advanced stage or relapsed endometrial cancer (EC), longer PFS was seen in the SEL arm compared to placebo for the subset of patients with *TP53* wild-type (WT) EC (Figure 2).[1]

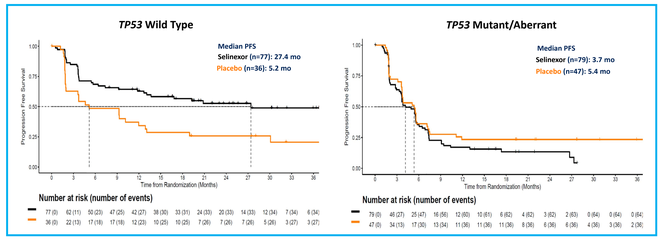


Figure 2. Patients with *TP53* wild-type EC showed longer progression-free survival in the selinexor arm compared to placebo (left), with no benefit in patients with *TP53* mutant EC (right).

- As XPO1 inhibition drives nuclear retention and functional activation of tumor suppressor proteins like p53, we investigated whether *TP53* wild type (WT) status could serve as a predictor for cancer cell sensitivity to XPO1 inhibitors.

METHODS

- Patient derived organoid (PDO) and xenograft (PDX) models were screened for *TP53* gene mutations. Models representing 18 cancer types were included. Within each cancer type, biological replicate models with *TP53* WT and biological replicate models with *TP53* mutations were selected for comparison. For *TP53* mutant models, we focused on testing point mutations within the DNA binding domain (AA95-292) that produced an amino acid change.[2]
- SEL, ELT and control agent (staurosporine for PDO or cisplatin for PDX) half maximal inhibitory concentrations (IC_{50}) after 5 days of exposure were calculated using CellTiter Glo assay. Sensitivity to SEL and ELT was considered *TP53* WT dependent for each cancer type if the IC_{50} for the *TP53* mutant models were ≥1.5-fold higher than the average IC_{50} for the *TP53* WT models.
- IC_{50} of SEL and ELT at 72h was determined for *TP53* WT HCT116 (colorectal) and SNG-M (endometrial) cell lines with syngeneic *TP53* mutant lines[3,4] using CellTiter Glo assays at 72h.
- Quantitative spatial proteomics was conducted in HCT116 cells using isobaric labelling to compare protein expressions in the nucleus of SEL treated vs. control cells, including those with *TP53* WT, *TP53* R175H and *TP53* R273H. Cells were exposed to SEL IC_{50} for 6h to induce proteomic changes without significant cell death. Protein pathway membership was assigned using WikiPathways.

RESULTS

Table 1. Cancer types with *TP53* WT-dependent XPO1 inhibitor sensitivity

Cancer Type	*TP53* Status	Selinexor IC_{50} µM	Fold Δ	Eltanexor IC_{50} µM	Fold Δ	Control IC_{50} µM
Endometrial	WT	0.061		0.067		4.042
	WT	0.031		0.019		0.933
	p.Y220C	0.333	7	0.147	3.4	2.813
	p.R213Ter	1.542	33	1.955	45	6.502
Ovarian	WT	0.089		0.3256		0.0161
	WT	0.051		0.022		0.005
	p. R273C	0.419	6	0.372	2	0.0008
	p. R175H	0.964	13.8	1.215	7	0.0336
Kidney	WT	0.0261		0.027		4.243
	WT	0.074		0.045		8.63
	p.Q167Ter	11.932	238	4.298	118	9.640
	p.R273H	0.101	2	0.091	2.5	3.936
Hepato-cellular	WT	0.119		0.057		0.0122
	WT	0.602		0.514		0.0197
	p.E271Ter	2.601	7	2.845	10	0.0062
	p.R175H	3.855	10.7	4.408	15	0.5833
Esophageal	WT	0.225		0.083		0.0054
	p.R273H	0.391	1.7	0.547	6.5	0.0289
	WT	0.083		0.073		0.9661
	p.R273H	1.448	17.4	1.098	15	6.8916
NSCLC Adeno-carcinoma	WT	0.334		1.855		0.0096
	WT	0.129		0.114		0.0009
	p.F341C	3.455	15	7.516	7.6	0.002
	p.G245S	25.399	110	13.464	13.7	0.008

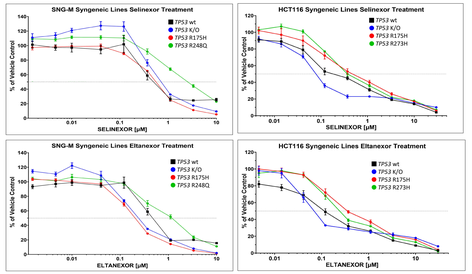
Ter=Termination PDX Control Cisplatin= Blue PDO Control Staurosporine=Green

Table 2. Cancer types with *TP53* WT-independent XPO1 inhibitor sensitivity

Cancer Type	*TP53* Status	Selinexor IC_{50} µM	Fold Δ	Eltanexor IC_{50} µM	Fold Δ	Control IC_{50} µM
Head & Neck	WT	0.124		0.129		0.0008
	p.R273H	0.368	3	0.142	1.1	0.0038
	WT	0.089		0.064		10.591
	p.R175H	0.108	1.2	0.094	1.5	1.384
Gastric	WT	1.324		2.272		0.028
	p.R248W	0.221	0.17	0.108	0.5	0.0007
	WT	0.056		0.076		3.9754
	p.R273H	0.074	1.3	0.052	0.7	1.4319
Gallbladder	WT	0.149		0.098		3.4348
	WT	0.252		0.189		8.0681
	p.R248Q	0.124	0.6	0.176	1.2	3.6319
	p.R175H	0.181	0.9	0.159	1.1	4.5145
Prostate Adeno-carcinoma	WT	0.086		0.119		9.775
	WT	0.152		0.133		7.879
	p.Y163C	0.064	0.5	0.043	0.3	9.544
	p.R280Ter	0.102	0.8	0.108	0.8	2.048
Cholangio-carcinoma	WT	0.509		0.518		0.0063
	WT	0.278		0.719		0.0232
	p.R273C	0.171	0.4	0.162	0.3	0.0032
	p.R248Q	0.371	0.9	0.242	0.4	0.0007
Cervical	WT	0.487		0.814		0.0031
	p.E285K	0.356	0.7	0.3996	0.5	0.0023
	WT	0.167		0.1484		2.37
	p.R175H	0.068	0.4	0.0442	0.3	1.53
Breast ER+/PR+	WT	29.013		24.245		0.151
	WT	0.5891		0.3201		0.0372
	p.R273H	1.0254	0.1	1.6362	0.1	0.0306
	p.H179R	0.383	0.03	0.2362	0.02	0.0452
Mixed Mullerian	WT	0.445		0.440		1.668
	WT	0.105		0.066		3.977
	p.R273H	0.099	0.4	0.073	0.3	0.167
	p.R273C	0.108	0.4	0.073	0.3	5.957
NSCLC Squamous	WT	0.274		0.388		0.0068
	WT	0.401		0.637		0.0019
	p. R273L	0.566	1.7	0.632	1.2	0.0035
	p. Y234H	3.533	10	21.249	41.5	0.0024
Pancreatic	WT	0.223		0.309		0.0037
	WT	0.464		7.178		0.0106
	p. R273H	0.635	1.8	0.738	0.2	0.0031
	p. R273H	0.554	1.6	0.611	0.2	0.0027
Bladder	WT	0.094		0.085		0.0024
	WT	0.263		0.176		0.0144
	p.V272_R273insL	6.116	34	6.209	47	0.0044
	p.W53Ter	0.252	1.4	0.267	2	0.0111
Thyroid	WT	0.262		0.117		3.938
	WT	0.107		0.131		4.709
	p.W146Ter	0.446	2.4	0.440	3.6	6.469
	p.C124Ter	0.063	0.3	0.058	0.5	2.434

- Table 1 shows cancer types where the *TP53* WT models were more sensitive to SEL and ELT than *TP53* mutant models (1.7-100-fold difference in IC_{50}). By contrast, Table 2 shows cancer types where SEL or ELT sensitivity was not associated with *TP53* status.
- *TP53* mutation status conferred resistance to cisplatin in only esophageal models, demonstrating specificity between *TP53* mutation status and XPO1 inhibition sensitivity.
- For *TP53* WT PDX models tested, SEL (IC_{50} 0.026-0.6µM) and ELT (IC_{50} 0.019-0.45µM) reduce viability with an order of magnitude lower dose than the cisplatin (IC_{50} 0.93-10.6µM).
- For PDO models tested, *TP53* WT SEL (IC_{50} 0.051-29µM) and ELT (IC_{50} 0.022-24µM) and *TP53* mutants SEL (IC_{50} 0.17-25.4µM) and ELT (IC_{50} 0.108-21.2µM) had wider sensitivity variations but showed similar overall trends with PDX models.

Figure 3. Introduction of *TP53* mutations conferred resistance to SEL and ELT

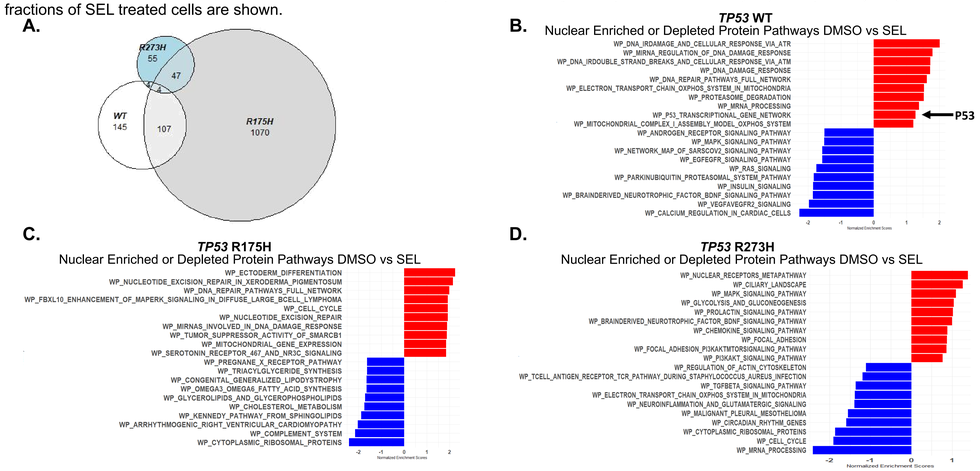


SNG-M Cell Lines	Selinexor IC_{50} µM	Fold Δ vs. *P53* WT	Eltanexor IC_{50} µM	Fold Δ vs. *P53* WT
P53 WT NEG CRISPR	0.366	--	0.371	--
P53 KO (Exon 5)	0.487	1.33	0.192	0.52
P53 R175H (Het)	0.511	1.4	0.190	0.51
P53 R248Q (Hom)	1.434	3.92	0.892	2.4

HCT-116 Cell Lines	Selinexor IC_{50} µM	Fold Δ vs. *P53* WT	Eltanexor IC_{50} µM	Fold Δ vs. *P53* WT
P53 WT NEG CRISPR[4]	0.2081	--	0.1736	--
P53 KO (Exon 2)[3]	0.057	0.27	0.0427	0.25
P53 R175H (Hom)[4]	0.343	1.65	0.316	1.83
P53 R273H(Hom)[4]	0.276	1.34	0.240	1.4

- To directly evaluate the association between *TP53* mutation and sensitivity to XPO1 inhibitors, syngeneic EC (SNG-M) and colorectal cancer (HCT-116) cell lines with introduced point mutations or knockouts were used.[3,4] An increase in IC_{50} was observed in cells *TP53* point mutations compared to *TP53* WT (Figure 3).

Figure 4. Quantitative spatial proteomics of SEL treated vs. untreated HCT116 cells with *TP53* WT, *TP53* R175H or *TP53* R273H. (A) Overlap between proteins that were significantly enriched in the nuclear fraction of each cell line after treatment with SEL vs DMSO treated cells. (B-D) Proteins set enrichment analysis of the nuclear enriched proteins. Pathways with proteins enriched (red) or depleted (blue) in the nuclear fractions of SEL treated cells are shown.



- Spatial proteomics quantified 6,893 proteins in nuclear fractions of HCT-116 cells treated with equi-toxic concentrations of SEL. Among the different cell lines, SEL treatment enhanced the nuclear expression of 260 proteins (*TP53* WT), 1,229 proteins (*TP53* R175H) and 110 proteins (*TP53* R273H).
- Minimal overlap in SEL-induced nuclear retained proteins was seen between the cell lines, consistent with context-specific mechanism of action of SEL. (Figure 4A).
- Pathway analysis was performed on nuclear retained proteins in the different cell lines. P53 pathway proteins were only significantly enriched in the nuclear fraction of the *TP53* WT cells. Other pathway differences were evident (Figure 4B-D).
- Some proteins were depleted in the nuclei of SEL treated cells compared to controls, indicating indirect effects of XPO1 inhibition, as XPO1 exclusively exports proteins from the nucleus to the cytoplasm.

CONCLUSIONS

- ***TP53* WT status can be a biomarker of sensitivity to the XPO1 inhibitors SEL and ELT in multiple cancer types tested, including endometrial (~20 fold more sensitive), ovarian, kidney, liver, esophageal, lung, pancreatic, and bladder. *TP53* WT models within these cancer types show similar sensitivity to SEL and ELT.**
- ***TP53* WT cell lines were more sensitive to SEL and ELT compared to the same lines with CRISPR engineered *TP53* point mutations.**
- **Spatial proteomics revealed differences in SEL-induced nuclear protein retention between *TP53* mutant and WT cell lines treated with equitoxic concentrations. In *TP53* WT cells, enriched nuclear proteins were members of canonical tumor suppressor pathways including the *TP53* network. In *TP53* mutant lines, proteins were members of DNA-damage, metabolic and cell cycle arrest pathways.**

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ACKNOWLEDGEMENTS: The authors thank Steve Mitchell, Trinayan Kashyap, and Liwen Zhang for editorial and scientific assistance with this work.

AACR-NCI-EORTC International Conference on Molecular Targets and Cancer Therapeutics
Oct 11-15, 2023 Boston, MA

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