**INTRODUCTION**

- Selinexor (SEL) is a selective inhibitor of the exportin 1 (XPO1) that leads to the nuclear accumulation and activation of key tumor suppressors (e.g., p53, p21, RB, and FOXO3a), resulting in selective cancer cell death (Figure 1). Ellman et al., (ELT) is an investigational compound with similar and comparable properties. In the exploratory analysis of the NCI60 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).

- As XPO1 inhibition drives nuclear retention and functional activation of tumor suppressors 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 organoids (PDO) and xenograft (PDX) models were screened for TP53 gene mutations. Models representing 18 cancer types were included. Within each cancer type, biological replicates with TP53 WT and biological replicates with TP53 mutant models were selected for comparison. For TP53 mutant models, we focused on testing point mutations within the DNA labelling to compare protein expressions in the nucleus of SEL treated vs. WT cells. Other pathway differences were evident (Figure 4B-D).

- Spatial proteomics was conducted in HCT116 cells using isotopic labelling to compare protein expression changes in the nucleus of SEL treated vs. control cells, including those with TP53 WT, TP53 R175H and TP53 R273H. Cells were exposed to SEL IC50 for 6 hours to induce protein changes without significant cell death. Protein pathway membership was assigned using WiPathways.

**RESULTS**

- Table 1 shows cancer types where the TP53 WT models were more sensitive to SEL and ELT than TP53 mutant models (1'-100-fold difference in IC50). By contrast, Table 2 shows cancer types where SEL ELT were associated with TP53 status.

- TP53 mutation status conferred resistance to cisplatin in only esophageal squamous cell carcinoma (ESCC) and colorectal cancer (HCT-116) cell lines with introduced point mutations or knockouts were used. An increase in IC50 was observed in cells with TP53 point mutations compared to TP53 WT (Figure 3).

- Figure 4A-4C shows prototypical XPO1 inhibitors (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 or 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 fraction of SEL treated cells are shown.

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

- **TP53 WT status can be a biomarker of sensitivity to 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 cell lines, 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.

**CONCLUSIONS**

- 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. An increase in IC50 was observed in cells with TP53 point mutations compared to TP53 WT (Figure 3).

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