The MDM2 degraders KTX-049 and KT-253 are highly active in wild-type TP53 Merkel cell carcinoma

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BACKGROUND

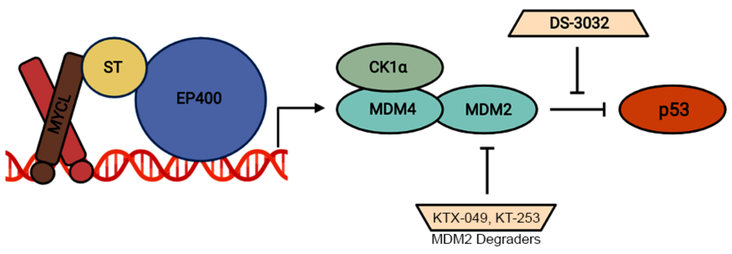
Merkel cell carcinoma (MCC) is an aggressive neuroendocrine carcinoma of the skin with a high fatality rate. Clonal integration of Merkel cell polyomavirus (MCPyV) occurs in 80% of all MCCs. Virus positive MCC (MCCP) tumors have a low tumor mutational burden with wild-type TP53 (WT p53). In contrast, virus negative MCC (MCCN) tumors present with a high mutational burden with frequent alterations in p53. MCCP tumors express MCPyV small T antigen (ST), which recruits MYCL to the EP400 complex to specifically transactivate hundreds of genes that contribute to oncogenesis, including MDM2, an E3 ubiquitin ligase of p53 that inhibits p53-mediated tumor suppression. While MDM2 inhibitors can stabilize p53 in WT p53 tumors and have shown promising activity, there are no MDM2 inhibitors approved for treatment of MCC. Moreover, MDM2 degraders have not been studied in MCC. Here, we analyze the *in vitro* and *in vivo* efficacy of MDM2 degraders in MCC tumor models and compare their efficacy to an MDM2 small molecule inhibitor, DS-3032.

MATERIALS & METHODS

Established MCCP cell lines with WT or mutant (MUT) p53 and two MCCP patient derived cell lines (PDCLs) with WT p53 were treated with KTX-049, a tool MDM2 degrader, or DS-3032. Effect on cell viability was analyzed using a luminescence-based assay and apoptosis was studied using caspase 3-7 Glo assay and annexin V/PI staining.

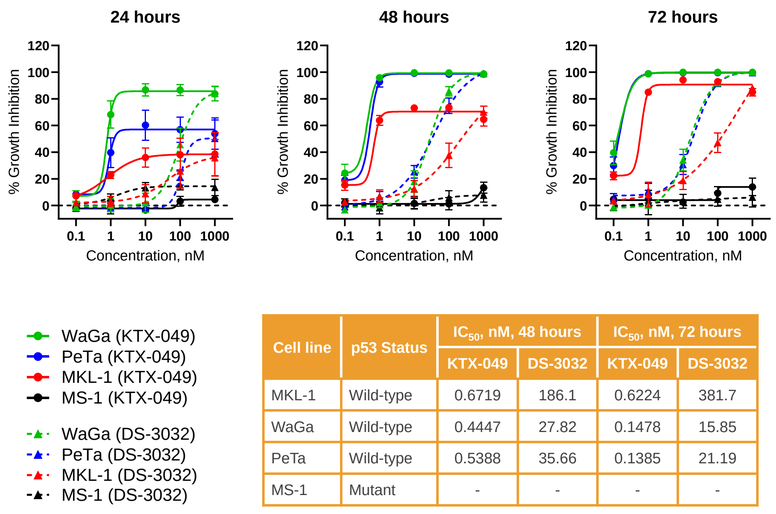
For *in vivo* testing, KT-253, a clinical stage MDM2 degrader, and DS-3032 activity were evaluated in two WT p53 patient-derived xenograft (PDX) models in NSG mice. Tumor volumes were measured twice weekly and followed until study end-point. The p53 response post *in vitro* or *in vivo* treatments were assessed by western blot (WB) and/or mass spectrometry analysis.

Reactivation of p53 by MDM2 Modulation in MCC



The ST-MYCL-EP400 (SLaP) complex drives expression of several genes, one among them is MDM2. MDM2 is a E3 ubiquitin ligase that inhibits the function of tumor suppressor p53. DS-3032 (milademetan) is a potent MDM2 inhibitor which acts by inhibiting the MDM2-p53 interaction while KTX-049 and KTX-253 are potent MDM2 degraders that degrade MDM2 protein, leading to re-activation of p53 in MCCP. Image created using Bio-Render.

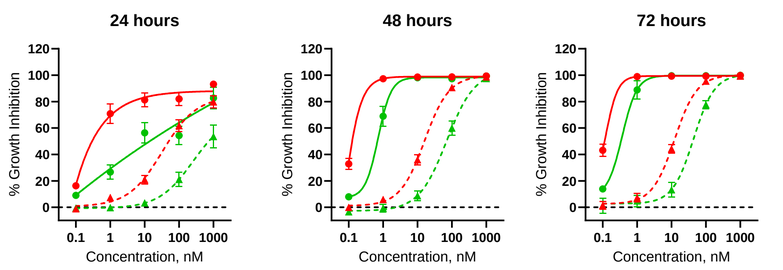
RESULTS



Cell line	p53 Status	IC_{50}, nM, 48 hours		IC_{50}, nM, 72 hours	
		KTX-049	DS-3032	KTX-049	DS-3032
MKL-1	Wild-type	0.6719	186.1	0.6224	381.7
WaGa	Wild-type	0.4447	27.82	0.1478	15.85
PeTa	Wild-type	0.5388	35.66	0.1385	21.19
MS-1	Mutant	-	-	-	-

Figure 1: KTX-049 is more potent than DS-3032 in MCC cell lines with WT p53. (A) MKL-1, WaGa, PeTa and MS-1 cell lines were treated with indicated concentrations of DS-3032 or KTX-049 (colors denoted) and cell titer Glo assay was performed at 24 hours , 48 hours or 72 hours to assess the effect on the viability of the treated cell lines. Three biological experiments were performed for each cell line and the assay was conducted in triplicate within each replicate. (B) Table denotes p53 status and IC_{50} values of KTX-049 or DS-3032 for cell lines used in A.

RESULTS continued



Cell line	p53 Status	IC_{50}, nM, 48 hours		IC_{50}, nM, 72 hours	
		KTX-049	DS-3032	KTX-049	DS-3032
MCC-301	Wild-type	0.0656	16.11	0.1227	12.32
MCC-336	Wild-type	0.7221	70.98	0.3690	43.87

Figure 2: KTX-049 > 100-fold more potent than DS-3032 in MCC PDCLs with WT p53. (A) MCC-301 and MCC-336 cells were treated with indicated concentrations of KTX-049 or DS-3032 (colors denoted) and cell titer Glo assay was performed at 24 hours, 48 hours or 72 hours to assess the effect on the viability of the treated cell lines. Three biological experiments were performed for each cell line and the assay was conducted in triplicate within each replicate. (B) Table denotes p53 status and IC_{50} values of KTX-049 or DS-3032 for cell lines used in A.

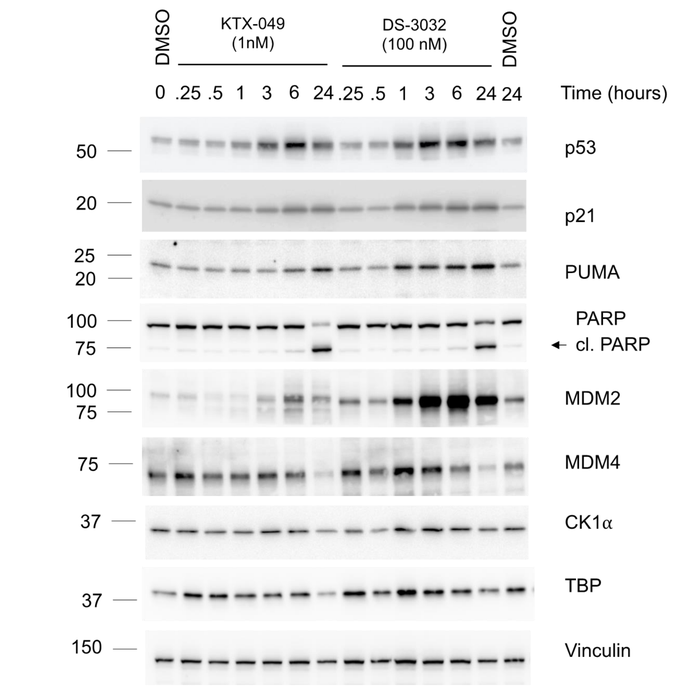
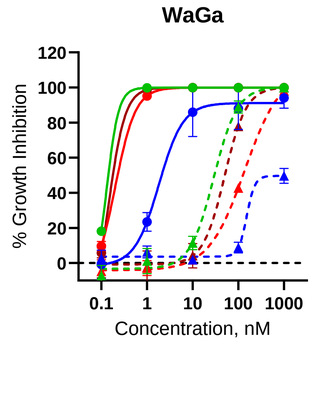


Figure 3: KTX-049 generates a rapid and sustained p53 response: MCCP WaGa cell line was treated with DMSO, 1 nM of KTX-049 or 100 nM DS-3032 for the indicated hours, followed by western blot. KTX-049 potently stabilizes p53 and leads to upregulation of p53-pathway biomarkers such as p21 and PUMA and apoptotic biomarker cl. PARP



Treatment	IC_{50} (nM)
KTX-049	0.1325
KTX-049 (4 hours + washout)	1.830
KTX-049 (24 hours + washout)	0.20174
KTX-049 (48 hours + washout)	0.1442
DS-3032	28.97
DS-3032 (4 hours + washout)	153.2
DS-3032 (24 hours + washout)	138.2
DS-3032 (48 hours + washout)	50.53

Figure 4: **MCC cells are highly sensitive to even brief exposures of KTX-049.** WaGa cells were treated with indicated concentrations of KTX-049 or DS-3032 for the indicated time followed by washout of the drug and cell titer Glo assay was performed at 72 hours. Three biological experiments were performed for each cell line and the assay was conducted in triplicate within each replicate. (A) effect on the viability of the cell treated cell lines. Error bars indicate standard deviation. (B) Table denotes IC_{50} values for conditions in A.

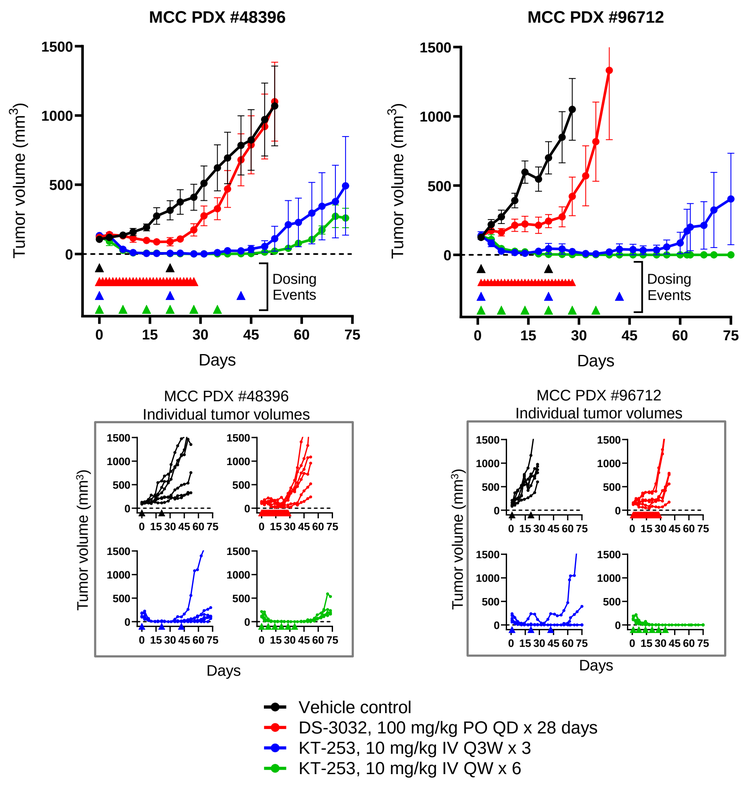


Figure 5: KT-253 is highly efficacious in MCC PDX models *in vivo*. KT-253 show robust and durable antitumor activity in MCC PDX models *in vivo*, while only transient tumor growth inhibition was observed with DS-3032. Average tumor volumes plotted when at least 4-6 mice were alive per dosing schedule. Complete responses were observed in 4/6 mice in the 10 mg/kg Q3W x 3 cohort; and 6/6 complete responses in 10 mg/kg QW x 6 cohort in the 96712 model.

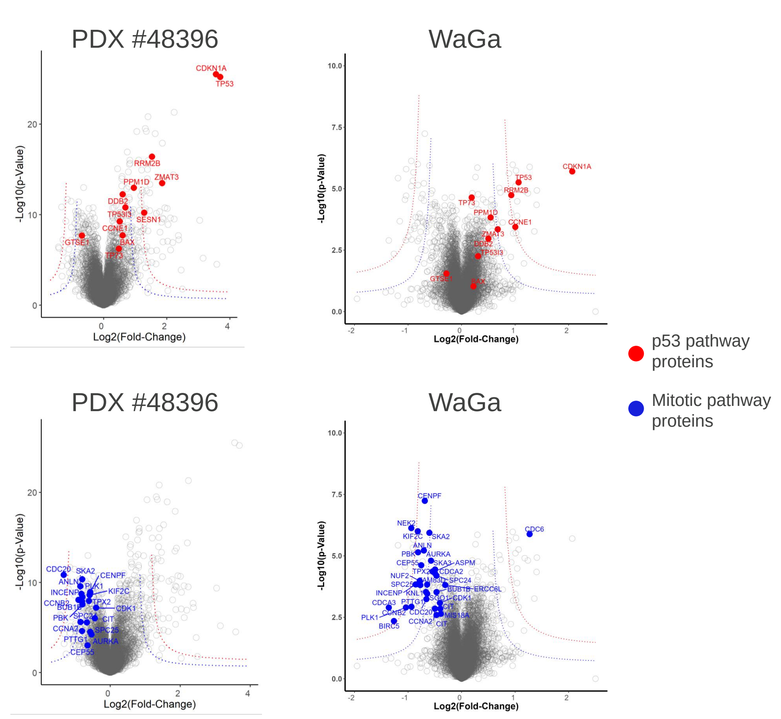


Figure 6: **MDM2 degradation in MCC models in vitro and in vivo leads to upregulation of p53 pathway and downregulation of mitotic pathway biomarkers.** Mass spectrometric analysis of 48396 tumors isolated from three different animals 24h post KT-253 treatment and WaGa cells treated with KTX-049 show significant upregulation of proteins involved in p53 signaling (shown in red). Proteins involved in mitotic pathways (shown in blue) were significantly downregulated post degrader treatment.

CONCLUSIONS

- KT-253- or KTX-049-mediated MDM2 degradation, even with brief exposures, induces rapid and up to 600-fold more potent growth inhibition than DS-3032-mediated inhibition in p53 WT MCCP cell lines
- KT-253 shows superior antitumor activity versus DS-3032 *in vivo* in WT p53 MCC PDX models
- KT-253 mediated complete responses were associated with upregulation of pharmacodynamic markers of p53 activation and inhibition of pathways involved in mitosis
- These results support exploration of KT-253 clinical activity in WT p53 MCC where degradation of MDM2 is one of the key mechanism for impeding tumor growth
- KT-253 is currently in phase I clinical trial (NCT05775406) in hematological and solid tumor malignancies including MCC

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KT-253: The safety and efficacy of this investigational agent has not been established. This agent is not approved by any regulatory body for any indication.