The MDM2 degrader KTX-049 is highly potent in TP53 wild-type (p53 WT) Merkel cell carcinoma (MCC).

Varsha Ananthapadmanabhan[1,2], Yogesh Chutake[3], Jessica Filiatrault[3], Stefanie Schalm[3], Alice McDonald[3], James A. DeCaprio[1,2]

[1]Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, MA. [2]Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA.[3]Kymera Therapeutics, Watertown, MA.

Abstract

Background: MCC is a highly aggressive neuroendocrine carcinoma of the skin with a high fatality rate. Merkel cell polyomavirus (MCPyV) integrates in about 80% of all MCCs. These virus positive MCC (MCCP) tumors have few somatic mutations and usually express wild type (WT) p53 (*TP53*). In contrast, virus negative MCC (MCCN) tumors present a high mutational burden and a predominantly UV mutational signature. MCCP tumors express MCyV small T antigen (ST) and a truncated form of large T antigen (LT). In MCCP tumors, MCV ST recruits MYCL and EP400 to form the SLaP complex that specifically trans-activates several genes. MDM2, an E3 ubiquitin ligase of p53 is a SLaP target gene and inhibits p53-mediated tumor suppression (Figure 1). The use of MDM2 inhibitors can stabilize p53 in p53 WT tumors. Currently, no MDM2 inhibitors are approved for treatment of MCC. Furthermore, MDM2 degraders have not been studied in the context of MCC.
We analyzed the efficacy of KTX-049, a potent MDM2 degrader, in MCC and compared its efficacy to the previously reported and highly potent MDM2 inhibitor, DS-3032.

Results: KTX-049 and DS-3032 reduced cell viability of p53 WT MCCP cell lines but not cell lines with non-functional p53 (Figure 2). Notably, all sensitive cell lines had ≥100-fold lower absolute IC_{50} values for KTX-049 as compared to DS-3032 (Figure 2, 3). KTX-049 also triggered a rapid and sustained p53 response in p53 WT MCC cells (Figure 4). Additionally, KTX-049 effectively reduced cell viability of the sensitive cell lines even with brief exposures (Figure 5). KTX-049 also led to apoptosis in p53 WT MCC cells (Figure 6).

Mode of action of KTX-049 and DS-3032 in MCC

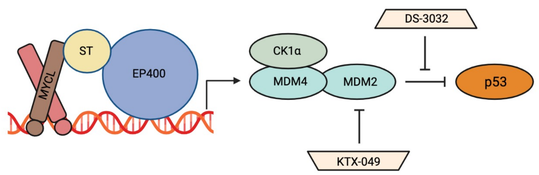
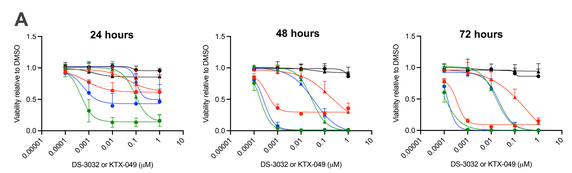


Figure 1: **Re-activation of p53 in MCC by DS-3032 or KTX-049.** The ST-MYCL-EP400 (SLaP) complex drives expression of several genes, one among them is MDM2. MDM2 is a E3 ubiquitin ligase that inhibits the function of tumor suppressor p53. DS-3032 (milademetan) is a potent MDM2 inhibitor which acts by inhibiting the MDM2-p53 interaction while KTX-049 is a potent MDM2 degrader that degrades MDM2 protein, leading to re-activation of p53 in MCCP. Schematic representation was created using Bio-Render.

Sensitivity of p53 WT MCCP cell lines to KTX-049 and DS-3032

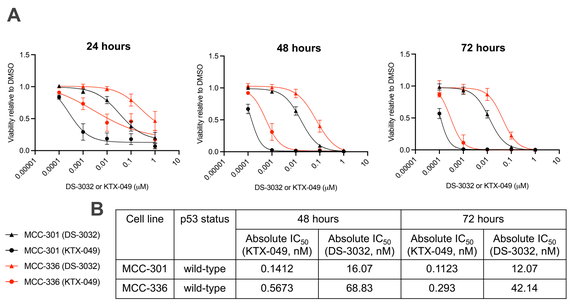


B

Cell line	p53 status	48 hours		72 hours	
		Absolute IC_{50} (KTX-049, nM)	Absolute IC_{50} (DS-3032, nM)	Absolute IC_{50} (KTX-049, nM)	Absolute IC_{50} (DS-3032, nM)
MKL-1	wild-type	0.4060	256.7	0.2205	125.1
WaGa	wild-type	0.1804	28.14	0.1222	15.96
PeTa	wild-type	0.2239	32.16	0.1416	20.07
MS-1	mutant	-	-	-	-

Figure 2: KTX-049 ≥ 100-fold 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 absolute IC_{50} values of KTX-049 or DS-3032 for cell lines used in A.

Sensitivity of p53 WT MCCP patient derived cell lines to KTX-049 or DS-3032



B

Cell line	p53 status	48 hours		72 hours	
		Absolute IC_{50} (KTX-049, nM)	Absolute IC_{50} (DS-3032, nM)	Absolute IC_{50} (KTX-049, nM)	Absolute IC_{50} (DS-3032, nM)
MCC-301	wild-type	0.1412	16.07	0.1123	12.07
MCC-336	wild-type	0.5673	68.83	0.293	42.14

Figure 3: 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 absolute IC_{50} values of KTX-049 or DS-3032 for cell lines used in A.

p53 response after treatment with KTX-049 or DS-3032

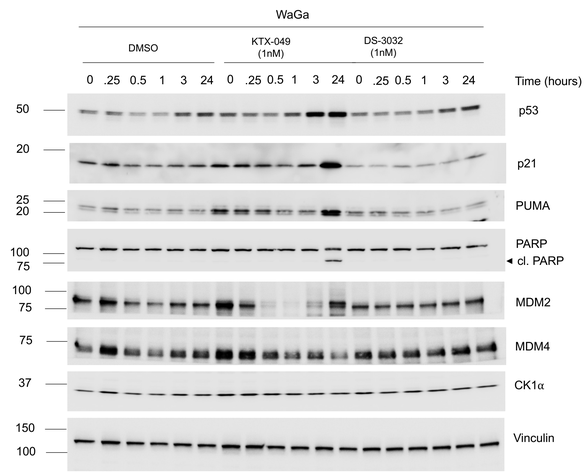
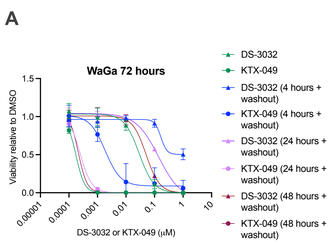


Figure 4: KTX-049 generates a rapid and sustained p53 response: MCCP WaGa cell line was treated with DMSO, or 1nM of KTX-049 or 1nM DS-3032 for the indicated hours, followed by WB analysis for the indicated proteins. Vinculin was used as a loading control.

Sensitivity of WaGa cell line to KTX-049 or DS-3032 after brief exposures



B

Treatment	Absolute IC_{50} (nM)
KTX-049	0.1533
KTX-049 (4 hours + washout)	2.083
KTX-049 (24 hours + washout)	2.683
KTX-049 (48 hours + washout)	0.248
DS-3032	29.68
DS-3032 (4 hours + washout)	2370
DS-3032 (24 hours + washout)	119.5
DS-3032 (48 hours + washout)	50.92

Figure 5: **WaGa 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 absolute IC_{50} values for conditions in A.

Induction of apoptosis in MCCP cell lines in response to KTX-049 or DS-3032

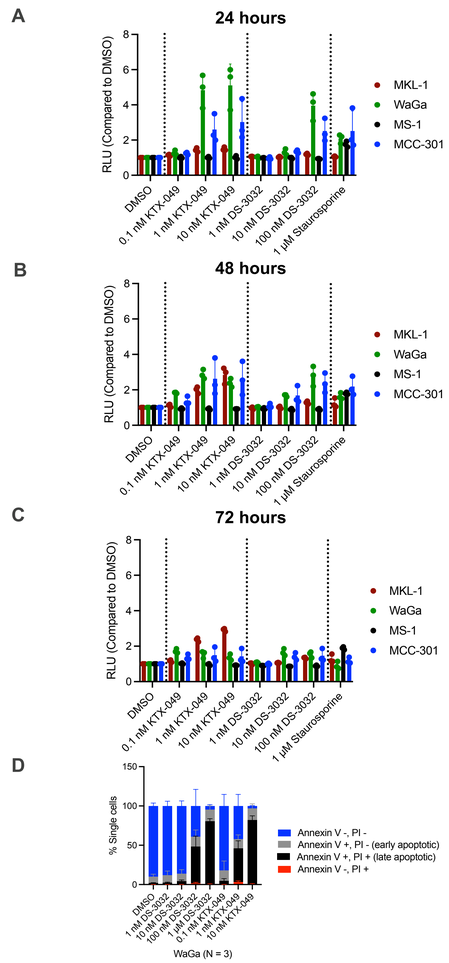


Figure 6: **KTX-049 and DS-3032 cause apoptosis in MCCP cell lines.** MKL-1, WaGa, MS-1 and MCC-301 cell lines were treated with DMSO, indicated concentrations of DS-3032 or KTX-049 and Caspase 3-7 Glo assay was performed at 24 hours (A), 48 hours (B) or 72 hours (C) to assess the effect on caspase 3-7 activation in the treated cell lines. Three biological experiments were performed for each cell line and the assay was conducted in triplicate within each replicate. Error bars indicate standard deviation. (D) WaGa cells were treated with DMSO, indicated concentrations of DS-3032 or KTX-049 and Annexin-V/PI staining was performed after 24 hours. Graph indicates mean quantifications from 3 biological replicates. Error bars indicate standard deviation.

Conclusions

KTX-049 is a promising MDM2 degrader effective against p53 WT MCC cell lines. These results provide evidence for in vivo exploration of KTX-049 in models of MCC.

References:

1. Park DE, Cheng J, Berrios C, Montero J, Cortés-Cros M, Ferretti S, et al. Dual inhibition of MDM2 and MDM4 in virus-positive Merkel cell carcinoma enhances the p53 response. Proc Natl Acad Sci U S A. 2019 Jan 15;116(3):1027–32.
2. DeCaprio JA. Molecular Pathogenesis of Merkel Cell Carcinoma. Annu Rev Pathol. 2021 Jan 24;16:69–91.
3. Houben R, Dreher C, Angermeyer S, Borst A, Utikal J, Haferkamp S, et al. Mechanisms of p53 Restriction in Merkel Cell Carcinoma Cells Are Independent of the Merkel Cell Polyoma Virus T Antigens. Journal of Investigative Dermatology. 2013 Oct 1;133(10):2453–60.
4. Cheng J, Park DE, Berrios C, White EA, Arora R, Yoon R, et al. Merkel cell polyomavirus recruits MYCL to the EP400 complex to promote oncogenesis. PLoS Pathog. 2017 Oct 13;13(10):e1006668.
5. Ishizawa J, Nakamaru K, Seki T, Tazaki K, Kojima K, Chachad D, et al. Predictive gene signatures determine tumor sensitivity to MDM2 inhibition. Cancer Res. 2018 May 15;78(10):2721–31
6. Ananthapadmanabhan V, Frost TC, Soroko KM, et al. Milademetan is a highly potent MDM2 inhibitor in Merkel cell carcinoma. *JCI Insight*. 2022;7(13):e160513. Published 2022 Jul 8. doi:10.1172/jci.insight.160513

Acknowledgments: This work was supported in part by the US Public Health Service grants R35CA232128 and P01CA203655, by the Bridge Project, a partnership between the Koch Institute for Integrative Cancer Research at MIT and the Dana-Farber/Harvard Cancer Center to J.A.D. and by Kymera Therapeutics, Inc.