Pulse Dosing of Potent and Selective Heterobifunctional MDM2 Degrader KT-253 Drives Tumor Regression and Demonstrates Differentiated Pharmacology Compared to p53/MDM2 Small Molecule Inhibitors

#P464

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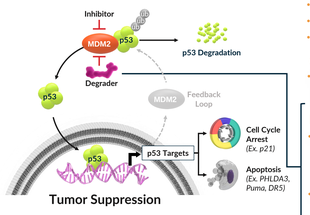
INTRODUCTION

The murine double minute 2 (MDM2) oncoprotein is a key E3 ubiquitin ligase that degrades the tumor-suppressor p53. Targeting of the MDM2/p53 interaction to stabilize p53 and induce apoptosis in wildtype (WT) p53 tumors is an emerging therapeutic approach in WT p53 hematologic and solid tumor malignancies. However, MDM2/p53 small molecule inhibitors (SMIs) have shown limited biological activity and clinical application.

KT-253 is a novel, highly potent heterobifunctional MDM2 degrader with >200-fold higher potency than MDM2 SMIs. Here, we assessed how pulse dosing versus exposure matched fractionated doses of KT-253 drives efficacy in mouse xenograft models and characterized the underlying molecular mechanisms associated with the different dosing regimens. In addition, we compared the efficacy of KT-253 to the clinical equivalent dosing schedule of a p53/MDM2 SMI.

MDM2 Degradation Effectively Induces p53 Signaling



Cancer Genetics

- p53 is **NOT mutated** in almost 50% of tumors
- MDM2 overexpression and amplification can inactivate p53
- Large opportunity in wide variety of cancers

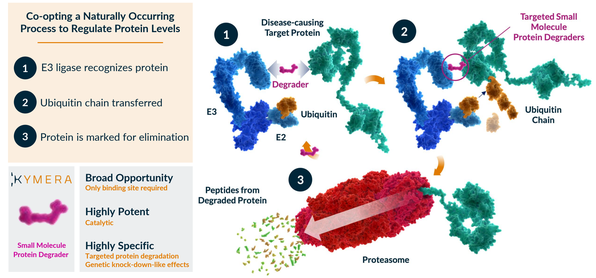
Clinical Validation

- **Small molecule inhibitors** of MDM2/p53 are active but have shown limited clinical application due to narrow therapeutic index

Degrader Advantage

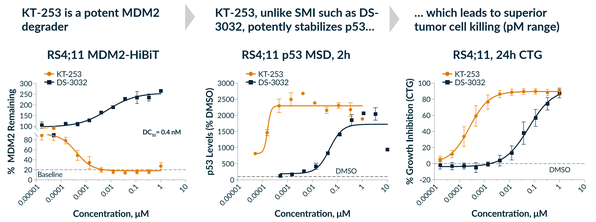
- **MDM2 degraders**, because of their catalytic and not occupancy driven pharmacology can lead to more efficient p53 stabilization and induction of an acute apoptotic response in tumor cells
- The distinct degrader pharmacology enables an intermittent dosing schedule that gives normal cells more time to recover and may increase the therapeutic index vs a SMI

Proteome Editing with Targeted Protein Degradation



RESULTS

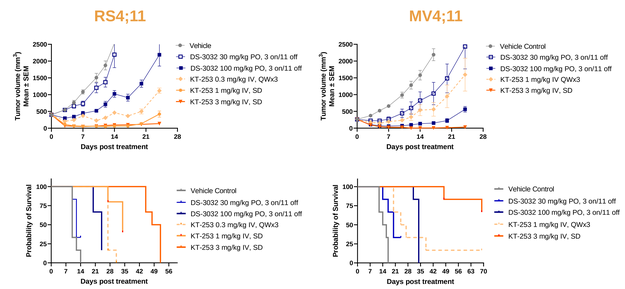
Figure 1: KT-253 is Superior to MDM2/p53 Small Molecule Inhibitors



Compound	KT-253	DS-3032	RG7388	SAR405838	HDM201	KRT-232
Company	Kymera	Sankyo/Rain	Roche	Sanofi	Novartis	Amgen/Kartos
Clinical stage	Ph I	Ph II / III	Ph II / III	Paused	Ph I / II	Multiple Ph II
RS4;11 IC_{50} (nM) (AML Cell Killing)	0.3	67	220	620	163	280
MDM2-HiBiT, DC_{50} (nM) (Degradation)	0.4	-	-	-	-	-

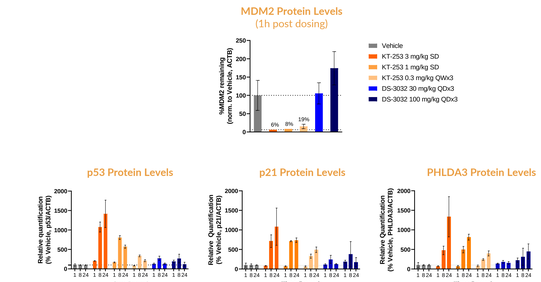
- KT-253 is **>200-fold more potent** in tumor cell killing assays than SMIs due to its mechanism of action

Figure 2: A Single Dose of KT-253 Drives Sustained Tumor Regression and is Superior to MDM2/p53 Small Molecule Inhibitors



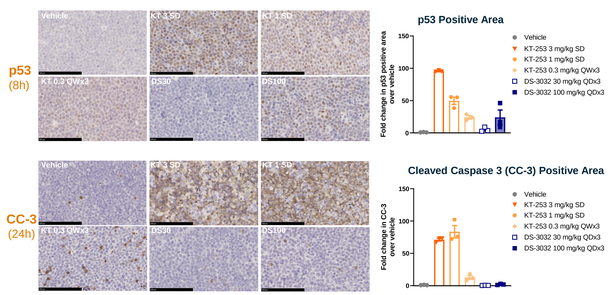
- In the RS4;11 ALL model, the median survival after a single dose of KT-253 at 3 mg/kg was 50 days vs 12 days for the clinically equivalent dosing regimen of DS-3032
- In the MV4;11 AML model, a single dose of KT-253 at 3 mg/kg led to complete responses in 5 of 6 animals, and 4 of 6 remain tumor-free on study 80 days post dosing
- No complete responses were observed following treatment with DS-3032
- n=6 animals/group

Figure 3: KT-253 Potently Degrades MDM2 and Upregulates the p53 pathway in Tumors



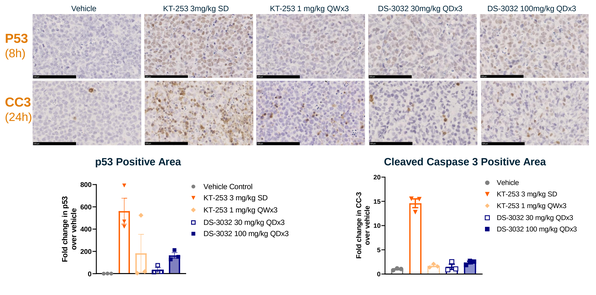
- Targeted proteomic analysis of RS4;11 tumors demonstrates robust degradation of MDM2 one hour post dosing.
- This is associated with activation of the p53 pathway as evidenced by a corresponding upregulation of proteomics biomarkers p53, p21 (cell cycle arrest marker) and PHLDA3 (apoptotic marker)

Figure 4A: A Single Dose of KT-253 Leads to Robust Activation of the p53 Pathway and Apoptosis in ALL while Exposure-Matched Weekly Dosing and SMIs Do Not



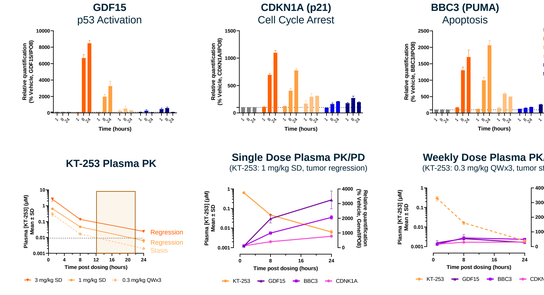
- IHC analysis of RS4;11 tumors demonstrates more robust activation of the p53 pathway and induction of cleaved caspase-3 (CC-3) following a single dose of KT-253 than following exposure-matched weekly dosing
- Induction of CC-3 was not observed following treatment with the SMI DS-3032 (n=3/group)

Figure 4B: A Single Dose of KT-253 Leads to More Robust Activation of the p53 Pathway and Apoptosis than Exposure-Matched Weekly Dosing and SMIs in AML



- IHC analysis of MV4;11 tumors demonstrates robust activation of the p53 pathway and induction of cleaved caspase-3 (CC-3) following a single dose of KT-253 but not following exposure-matched weekly dosing
- Induction of CC-3 following treatment with the SMI, DS-3032, was modest and similar to the lower weekly dosing regimen of KT-253 (n=3/group)

Figure 5: Exposures Required for Tumor Regression are Associated with Induction of Apoptotic Markers



- qPCR analysis of RS4;11 tumors demonstrates robust induction of the apoptotic gene BBC3 following single doses of KT-253 that lead to tumor regression (n=3/group)
- In contrast, exposure-matched weekly dosing of KT-253 results in only a moderate increase in apoptotic markers and correlates with tumor stasis (n=3/group)
- Similar results were observed with the apoptotic genes FAS and DR5 (data not shown)
- 12 to 22 hours of KT-253 plasma concentrations above 0.01 uM are required for tumor regression
- Plasma KT-253 concentrations above 0.02 uM are required for induction of the apoptotic gene, BBC3

METHODS

***In vitro* Assays**

All cell lines were cultured according to recommended procedures unless otherwise noted. For growth inhibition assays, cells were treated with compounds for indicated time points. Viability was assessed using Promega® CellTiter-Glo® assay, and apoptosis was assessed using Promega® Caspase-Glo® 3/7 assay.

***In vivo* Experiments**

RS4;11 and MV4;11 tumors were established subcutaneously in the hind flank of NOD/SCID mice. Mice were randomized and treated with KT-253 as either a single pulse dose or weekly for 3 weeks at doses matched for total AUC. MDM2 SMI DS-3032 (Milademetan) was dosed at its clinically equivalent dose and regimen of 3 days on/ 11 days off. Pharmacodynamic effects were assessed at mRNA level by RT-qPCR and protein level by quantitative targeted mass spectrometry and immunohistochemistry.

CONCLUSIONS

- KT-253 is a potent MDM2 degrader and a best-in-class p53 stabilizer that inhibits tumor cell growth with picomolar potency and is >200-fold more potent than clinically active MDM2 small molecule inhibitors.
- A single high dose of KT-253 leads to robust activation of the p53 pathway, apoptosis, and sustained tumor regression, while exposure-matched weekly dosing leads to cell cycle arrest and tumor stasis, as observed with MDM2 small molecule inhibitors.
- In the MV4;11 AML model, a single dose of KT-253 at 3 mg/kg led to complete responses in 5 of 6 animals, and 4 of 6 remain tumor-free on study 80 days post dosing (time of submission of this poster).
- The pulse dosing regimen of KT-253 has the potential to result in improved efficacy and safety profiles compared to the more frequent dosing of MDM2/p53 small molecule inhibitors in the clinic.

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DISCLOSURES

All authors are Kymera Therapeutics employees and equity owners.

Schalm, Mayo, Dixit, Filiatrault, Proctor are former Kymera employees

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