KT-253, a highly potent and selective heterobifunctional MDM2 degrader for the treatment of wildtype p53 tumors with superior potency and differentiated biological activity compared to small molecule inhibitors (SMI)

#3934/8

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INTRODUCTION

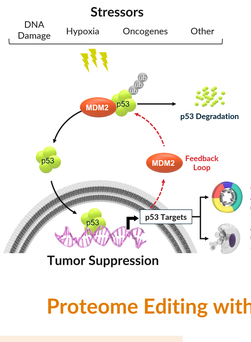
p53 is a transcription factor that regulates cellular responses to stress and guides cell fate decisions such as cell cycle arrest, DNA repair, senescence, and apoptosis. Thus, p53 is also the largest tumor suppressor. Loss of p53 function leads to inability of cells to respond to cellular stressors such as DNA damage and leads to genetic instability, a hallmark of cancer. Notably, p53 is NOT mutated in almost 50% of tumors.

The murine double minute 2 (MDM2) oncoprotein is a key E3 ubiquitin ligase that ubiquitinates and degrades p53, and overexpression or amplification of MDM2 is one of the mechanisms that inactivates p53 in wide variety of cancers.

Reversible SMIs of the MDM2/p53 interaction have been developed to stabilize p53 and to induce apoptosis in wildtype p53 tumors. However, MDM2 SMIs induce a p53/MDM2 feedback loop, resulting in upregulation of MDM2 protein levels and p53 pathway inhibition thus drastically limiting their biological activity and clinical application.

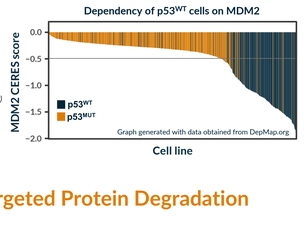
MDM2 targeted protein degradation suppresses p53-dependent MDM2 protein feedback upregulation and is therefore expected to lead to a superior response compared to SMIs.

MDM2 is the E3 Ligase that Modulates p53



Cancer Genetics

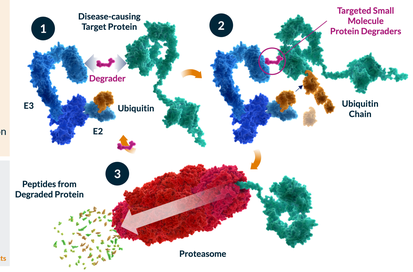
- P53 is NOT mutated in almost 50% of tumors
- Wide variety of cancers are sensitive to MDM2 modulation



Proteome Editing with Targeted Protein Degradation

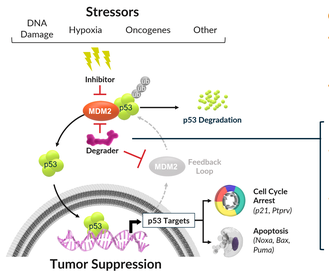
Co-opting a Naturally Occurring Process to Regulate Protein Levels

1. E3 ligase recognizes protein
2. Ubiquitin chain transferred
3. Protein is marked for elimination



KYMERA	Broad Opportunity	Highly Potent	Highly Specific
Small Molecule Protein Degrader	Only binding site required	Catalytic	Targeted protein degradation Genetic knock-down-like effects

Figure 1: MDM2 Degradation, Not inhibition, Can Overcome MDM2-p53 Autoregulatory Feedback Loop



Clinical Validation

- **MDM2 small molecule inhibitors** of MDM2/p53 interaction **show activity in the clinic...**
- **...but they induce MDM2 feedback loop** resulting in limited impact on pathway

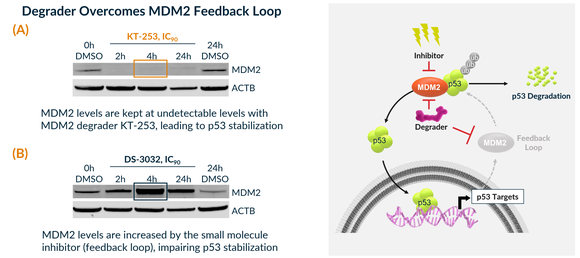
Degrader Advantage

- **MDM2 degraders**, by removing the protein, **can overcome the p53-dependent feedback loop that upregulates MDM2**
- MDM2 degrader can induce an acute apoptotic response in tumor cells, **increasing efficacy and therapeutic index vs a small molecule inhibitor**

Figure 2: KT-253 is Highly Selective MDM2 Degrader that Leads to MDM2 Degradation-Dependent Downstream Activation of p53 Targets

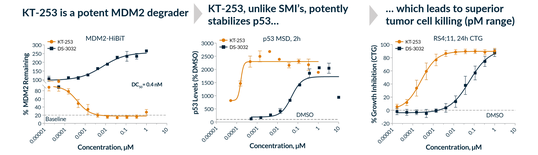
- Deep Tandem Mass Tag proteomics analysis at 8h post KT-253 treatment showed **no off-target degradation** in RS4;11 cells at 10x IC90 concentration
- ~9000 Proteins were monitored
- All upregulated proteins are **downstream targets of p53 stabilization**

Figure 3: KT-253, Unlike Small Molecule Inhibitors, Overcomes the MDM2-p53 Autoregulatory Feedback Loop



RS4;11 cells were treated with KT-253 (A) or DS-3032 (B) at IC_{90} concentrations for indicated times. Change in MDM2 levels was monitored by Western blot analysis

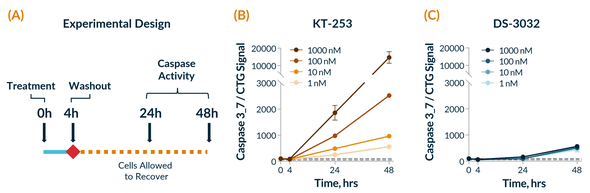
Figure 4: KT-253 is Superior to MDM2/p53 Small Molecule Inhibitors



Compound	KT-253	DS-3032	RG7388	SAR405838	HDM201	AMG-232
Company	Kymera	Sankyo/Rain	Roche	Sanofi	Novartis	Amgen/Kartos
RS4-11 IC_{50} (nM) (ALL Cell Killing)	0.3	67	220	620	163	280
MDM2-HiBiT, DC_{50} (nM) (Degradation)	0.4	-	-	-	-	-

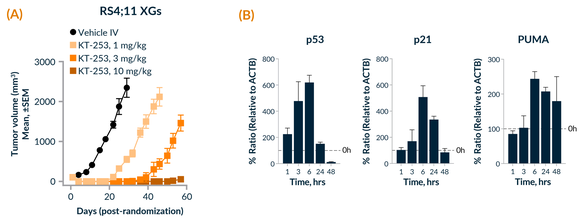
- KT-253 is **>200-fold more potent** in tumor cell killing assays than SMI's due to its mechanism of action

Figure 5: Short-term Exposure of MDM2 degrader, but not SMI, is Sufficient to Commit Cells to Undergo Apoptosis in RS4;11 (ALL) Model



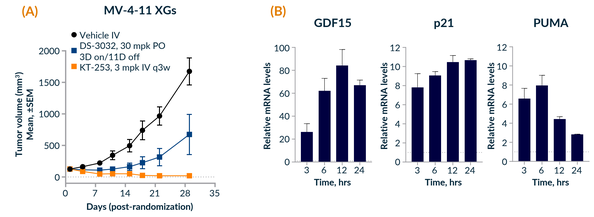
Washout experiments (A) show that 4 hr target coverage by KT-253 is sufficient to induce apoptosis (B) in RS4;11 (ALL) cells. No apoptosis observed in the same model when treated with SMI (C). These data support the hypothesis that intermittent dosing schedule of KT-253 can drive efficacy while increasing therapeutic index

Figure 6: Single dose KT-253 Leads to Sustained Tumor Regression in RS4;11 (ALL) Xenograft Model



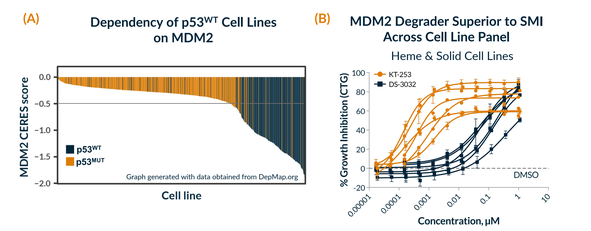
- Single dose KT-253 achieves sustained tumor regression in RS4;11 xenograft model (A)
- MDM2 degradation (KT-253, 1 mg/kg) leads to rapid increase in p53, p21, and PUMA (B)

Figure 7: KT-253 Achieves Tumor Regression in MV-4-11 (AML) Xenograft Model



- KT-253 achieves sustained tumor regression in MV-4-11 xenograft model (A)
- MDM2 degradation (KT-253, 3 mg/kg) leads to rapid upregulation of p53 downstream targets (B)

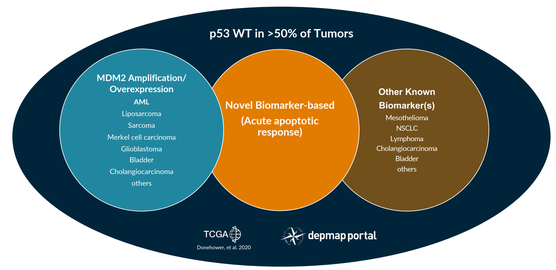
Figure 8: MDM2 Dependency Seen Across a Large Subset of Tumor Types Represents Large Franchise Potential in Liquid and Solid Tumors



Tumor Types: Uveal melanoma, Bile Duct, Bladder, Bone, Brain, Breast, Colon, Endometrial/Uterine, Gastric, Kidney, Liver, Lung, Ovarian, Pancreatic, Rhabdoid, Sarcoma, Leukemia, Lymphoma

p53WT cell lines sensitive: ALL, AML, DLBCL, Uveal Melanoma p53 mutant cell lines were not sensitive to KT-253 or DS-3032 as expected

Figure 9: Focus on Indications Where MDM2 Degradation Leads to Rapid Apoptotic Response (Biomarker of Degrader Sensitivity)



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

Subcutaneous tumors were established in immuno-compromised host strain mice using RS4;11 (ALL) or MV-4-11 (AML) cell lines. KT-253 or DS-3032 were administered IV or orally, respectively, at indicated doses.

Tumor volumes were measured by caliper and body weight was taken twice a week. PD assessment was done either by Western blot analysis or RT-qPCR analysis for downstream p53 targets as shown for both xenograft models.

Proteomics Analysis

Tandem Mass Tag discovery proteomics was performed on RS4;11 (ALL) Cells treated with KT-253 at 20 nM (equivalent to 10X IC_{90}) to a depth of ~9,000 proteins. Statistical analysis was carried out using the Limma statistical package. A weighted cutoff between statistical significance and fold-change was applied.

CONCLUSIONS

KT-253 is a Potent MDM2 Degrader and a Best-in-Class p53 Stabilizer with Potential to Treat Numerous p53 WT Tumors

- KT-253 inhibits tumor cell growth with **picomolar potency** and is **>200-fold more potent** than clinically active MDM2 small molecule inhibitors
- KT-253, unlike small molecule inhibitors, **blocks the feedback loop** which up-regulates MDM2 production and in doing so more effectively stabilizes the tumor suppressor p53
- **Short term high exposures of KT-253** are enough to induce apoptosis in cell lines and cause sustained tumor regression in xenograft models, which ensures high activity and improved therapeutic index vs SMI's
- Broad franchise opportunities available for this mechanism (p53 WT is present in >50% tumors); Kymera is focused on indications with **specific sensitivity to degrader mechanism**, such as AML, Uveal melanoma and others through a biomarker selection strategy
- Projected IND filing in **2022**

REFERENCES

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DISCLOSURES

Chutake, Mayo, Chen, Enerson, Cho, Filiatrault, Brown, Adams, Karnik, Shaw, Breitkopf, Yuan, Shi, Walther, McDonald, Qi, Growney, Sharma, Schalm, Williams, Weiss are Kymera Therapeutics employees and equity owners.

Liu, Walker are former Kymera Therapeutics employees.

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