# The MDM2/p53 axis is a therapeutic vulnerability in malignant pleural mesothelioma


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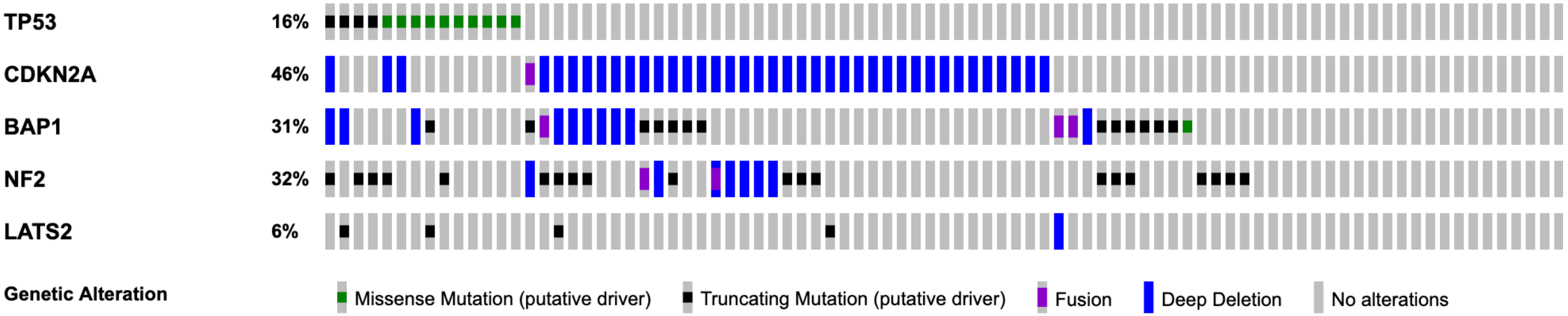
# Presenter DISCLOSURES

| Ineligible Company | Relationship(s) |
| --- | --- |
| Rain Therapeutics, Inc. | Contracted Support/Research |

# The mutation landscape across 87 MPM tumors characterized in the Cancer Genome Atlas (TCGA) reveals infrequent (16%) TP53 mutations.



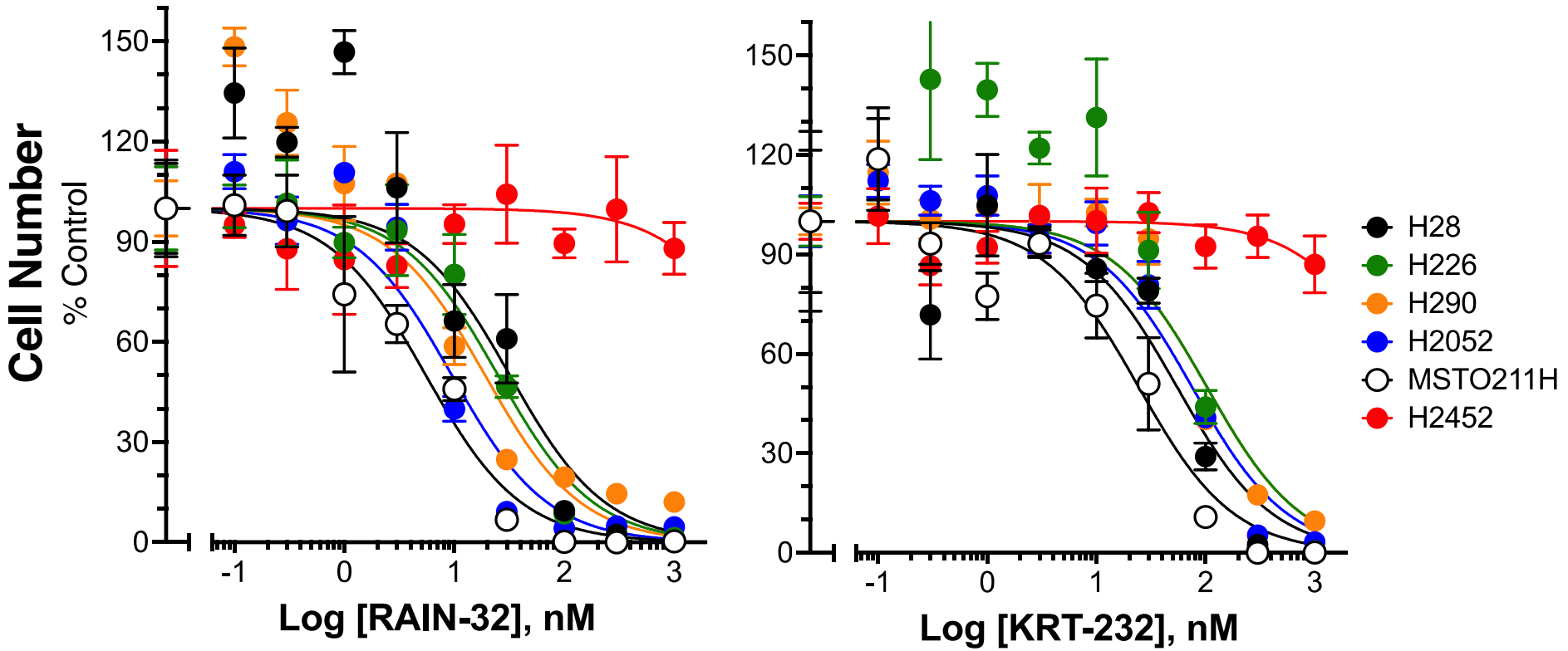

MPM samples (n=87) within the TCGA set were interrogated in cBioPortal (https://www.cbioportal.org/) and the oncoprint was generated. The key indicates the nature of the alterations (missense and truncating mutations, amplifications, deep deletions). The mutation status of BAP1, NF2 and LATS2 which have been shown to be frequent in MPM is overlayed with the TP53 mutation profile.

# Table 1: Characteristics of human MPM cell lines used for the studies

| Cell Line | H28 | H226 | H290 | H2052 | MSTO211H | H2452 |
|---|---|---|---|---|---|---|
| RAIN-32 IC50, nM | 32 | 25 | 19 | 10 | 6 | 7,448 |
| KRT-232 IC50, nM | 55 | 103 | 103 | 75 | 24 | 6,333 |
| TP53 status | WT | WT | WT | WT | WT | WT, lo mRNA |
| TP53 mRNA, rel. exp. | 0.84 | 1.05 | 1.10 | 0.55 | 0.33 | 0.00 |
| CDKN2A status | del | - | - | del | del | del |
| CDKN2A mRNA, rel. exp. | 0.0000 | 0.0033 | 0.0000 | 0.0000 | 0.0047 | 0.0002 |
| MDM2 mRNA, rel. exp. | 0.57 | 0.48 | 0.96 | 0.94 | 0.51 | 0.10 |

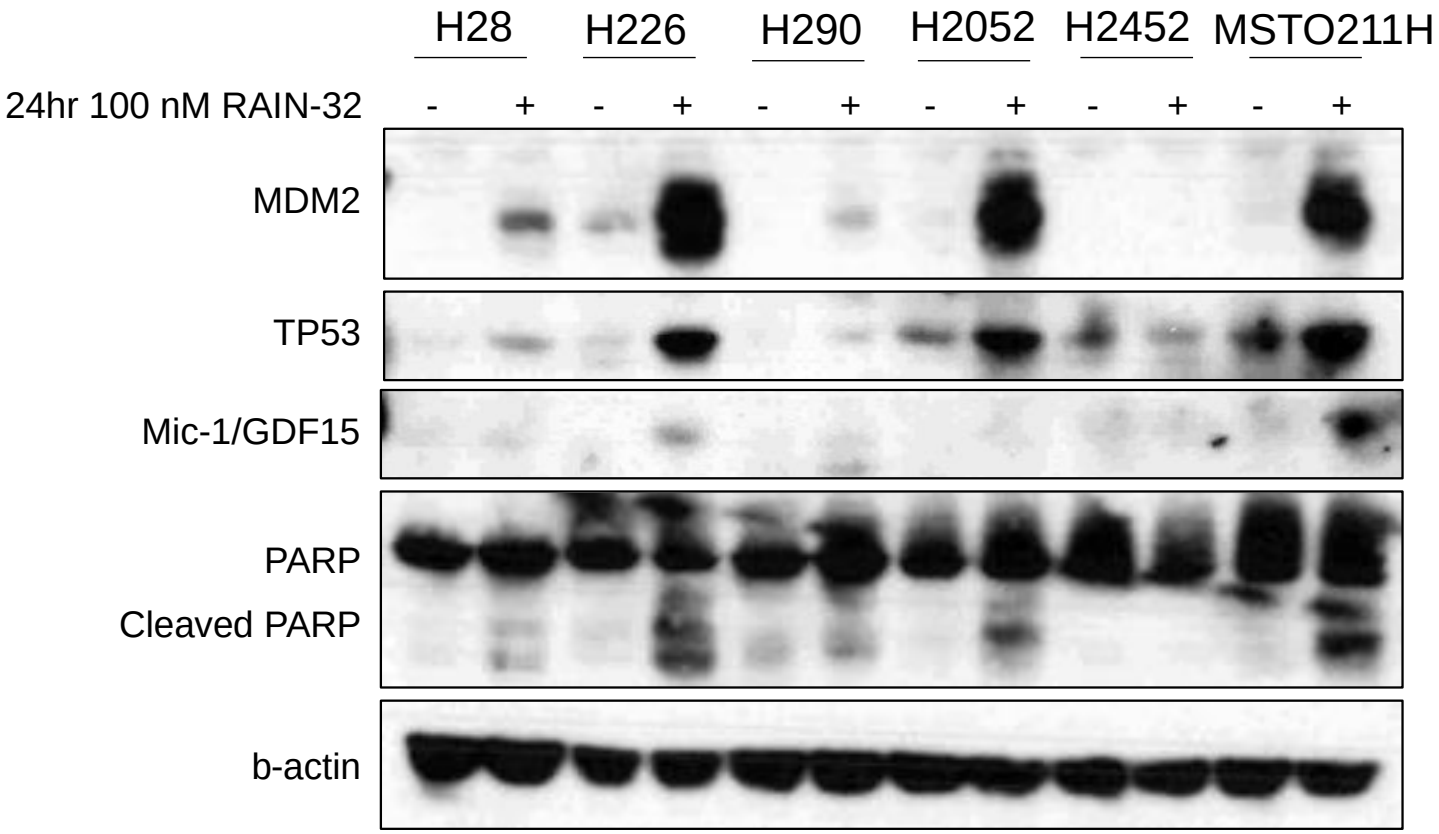
The $IC_{50}$ values for MDM2 inhibitors RAIN-32 and KRT-232 were determined from dose response experiments. The status of the *TP53* gene and *CDKN2A* locus was extracted from the CCLE. Levels of TP53, MDM2 and CDKN2A mRNAs were measured with quantitative RT-PCR. The *TP53* gene in H2452 cells is wild-type, but weakly expressed at the mRNA level, rendering this line functionally TP53 null. None of the cell lines expressed significant CDKN2A mRNA relative to positive control cell lines (not shown).

# *In vitro* sensitivity of human MPM cell lines to MDM2 inhibitors RAIN-32 and KRT-232



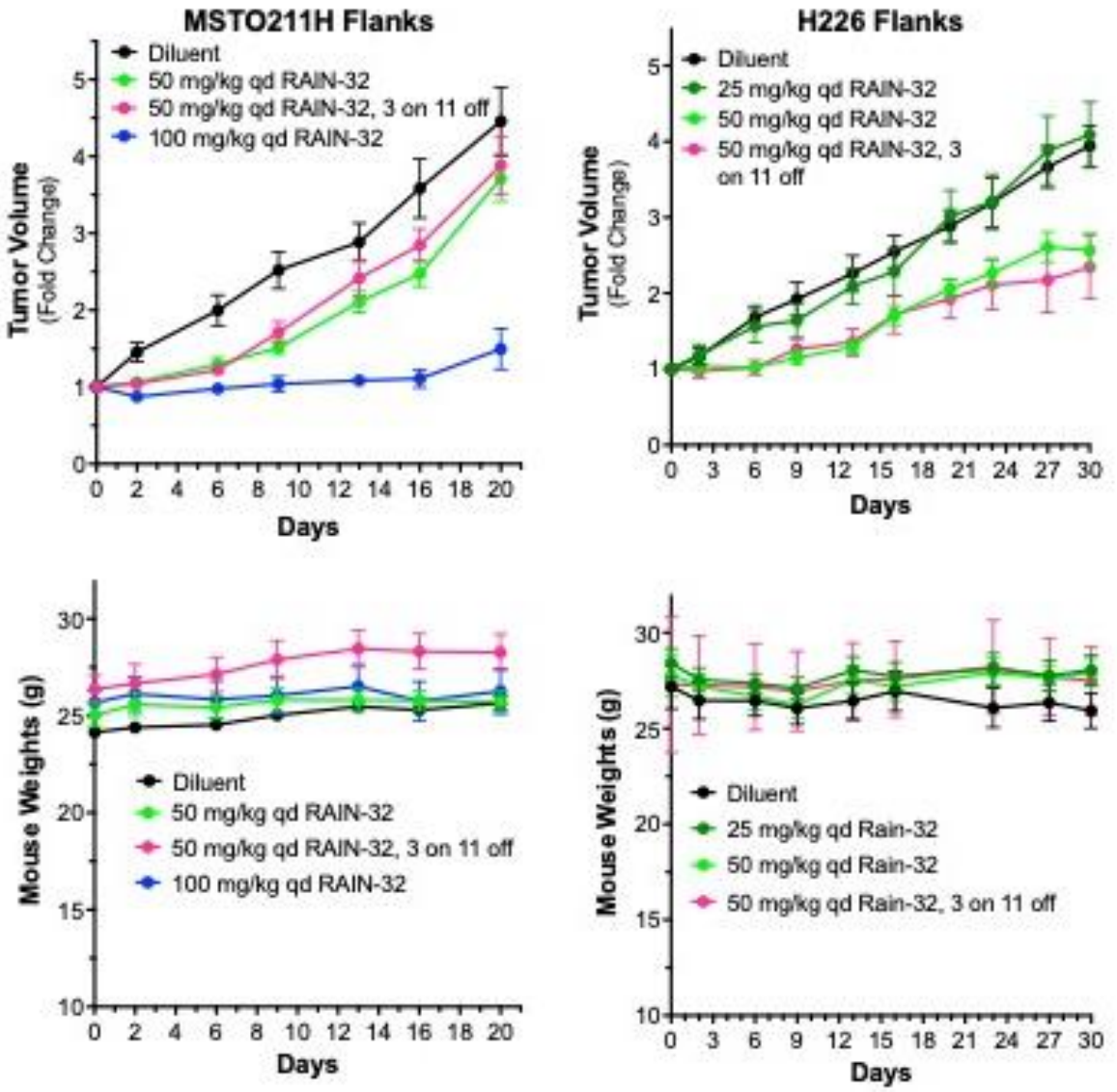

The indicated MPM cell lines were seeded in 96-well plates (200 cells/well) and after 24 hrs, the growth medium was replaced with media containing RAIN-32 or KRT-232 at the indicated concentrations (0-1 μM). After 10 days of incubation, the relative cell number was determined with CyQUANT reagent. The data are means and SEM (n=3) and presented as the percent of control wells treated with 0.1% DMSO. $IC_{50}$ values were calculated with Prism 9.0 and presented in Table 1.

# RAIN-32 stabilizes TP53 protein levels, increases target gene expression and induces PARP cleavage in MPM cell lines with wild-type *TP53*




The indicated MPM cell lines in 10 cm dishes were treated for 24 hrs with 100 nM RAIN-32. Cell extracts were prepared, submitted to SDS-PAGE and immunoblotted for p53 protein, the TP53 target genes, MDM2 and Mic-1/GDF15, PARP1 and β-actin as a loading control. The blot reveals stabilized p53 in lines bearing wild-type TP53 as well as increased expression of MDM2 and Mic-1/GDF15, two TP53 target genes. Moreover, PARP cleavage was observed in response to RAIN-32 treatment except for H2452 cells which lack TP53 mRNA expression (see Table 1).

# *In vivo* response of human MPM xenografts to RAIN-32



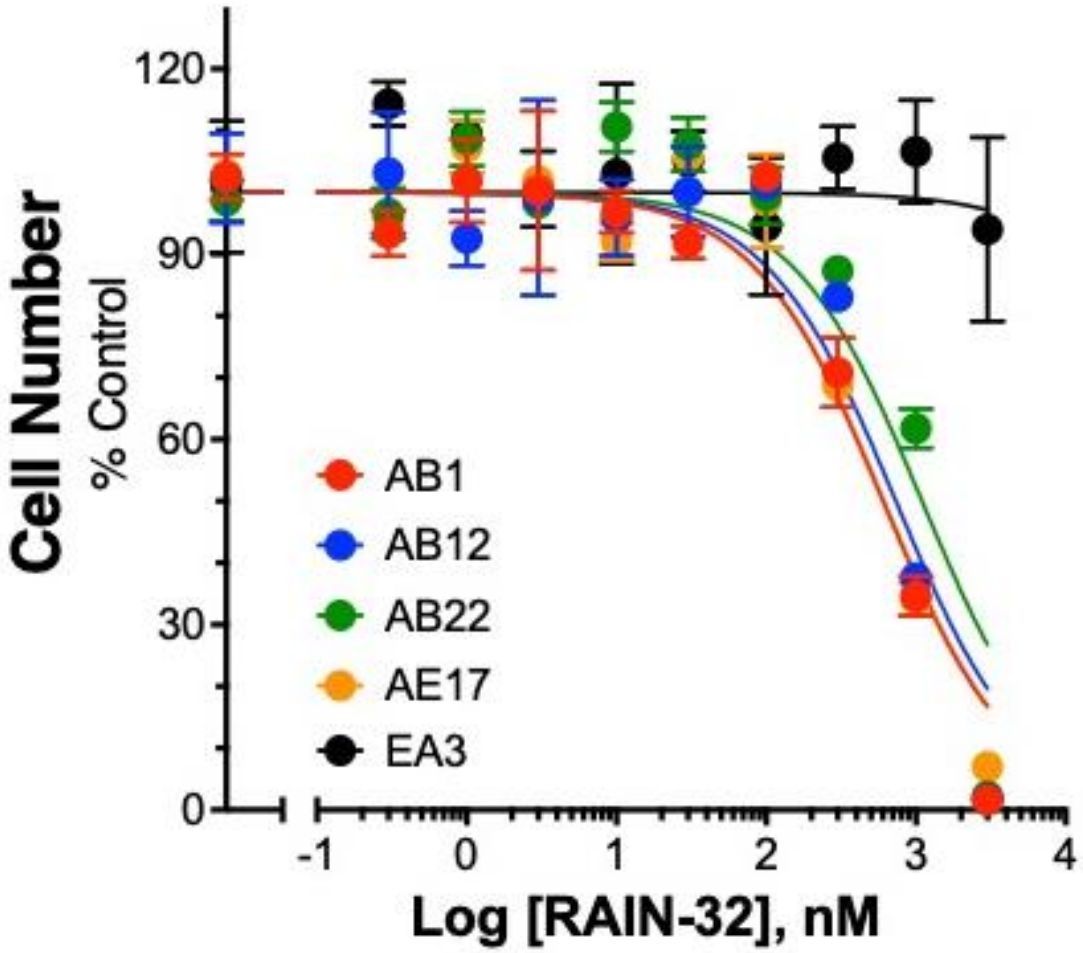

Human MPM cell lines MSTO211H and H226 cells ($2x10^6$) were implanted in the flanks of nu/nu mice. When the tumors reached ~400 $mm^3$, the mice were randomized into treatment groups (n=10) and treated by oral gavage with vehicle or RAIN-32 at the indicated doses and schedules. Tumors were measured with calipers every 3-4 days and volumes calculated. The data (means and SEM) are the tumor volumes presented as fold-change relative to each tumors pretreatment volume. Mouse weights were also measured and are presented below the tumor volume graphs.

# Next Steps: Potential combination therapy strategies with RAIN-32

**1) RAIN-32 in combination with anti-PD1-based immunotherapies.** Requires MPM models that can be propagated in immune-competent mice. Murine MPM cell lines bearing wild-type TP53 are available as transplantable models (Davis et al, Int J Cancer 6:881-6, 1992).

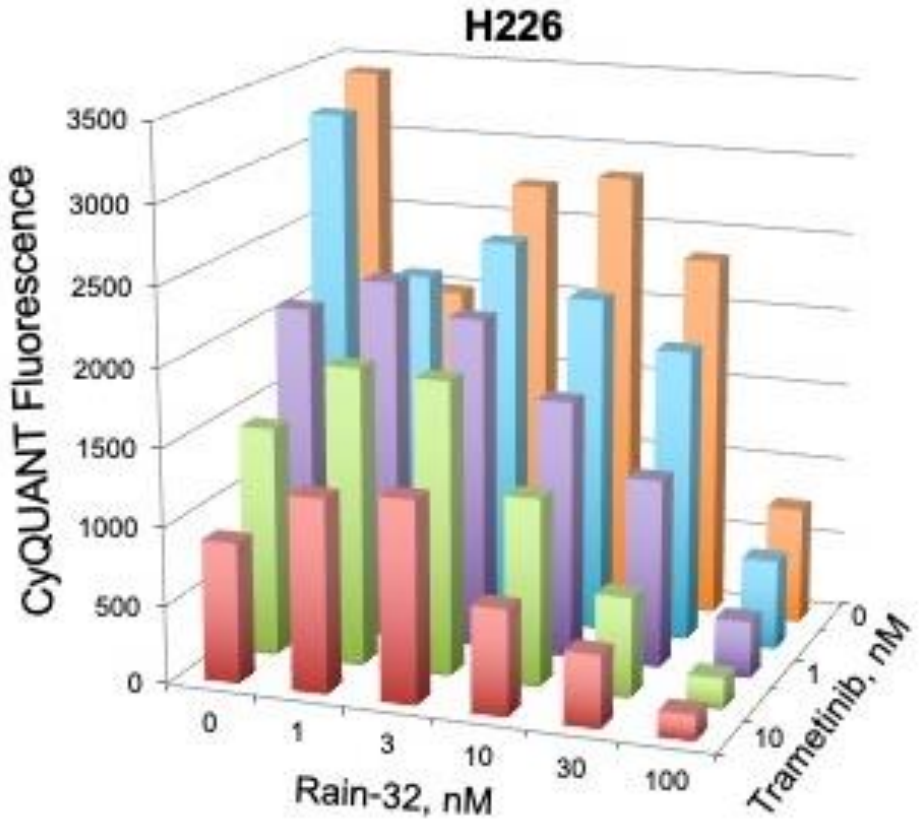
**2) RAIN-32 in combination with MAPK pathway-targeted inhibitors.** While oncogenic receptor tyrosine kinases or MAPK pathway components are not observed in MPM, the cell lines are still sensitive to MEK inhibitors.

# *In vitro* sensitivity of murine MPM cell lines to RAIN-32




The indicated murine MPM cell lines bearing wild-type TP53 and the murine EML4-ALK-positive EA3 lung cancer cell line (TP53 null as a negative control) were submitted to *in vitro* growth assays as with human MPM lines. The $IC_{50}$ values ranged from 600-1100 nM for RAIN-32 and demonstrate MDM2 inhibitor sensitivity of the murine MPM cell lines relative to a TP53 null control line.

# Synergistic *in vitro* growth inhibition in human H226 cells treated with combinations of RAIN-32 and the MEK inhibitor, trametinib




| Rain-32, nM | Trametinib, nM 0.3 | 1 | 3 | 10 |
|---|---|---|---|---|
| 100 | 0.042 | 0.053 | 0.078 | 0.218 |
| 30 | 1.178 | 0.3 | 0.28 | 0.645 |
| 10 | 1.16 | 0.494 | 0.638 | 0.989 |
| 3 | 1.315 | 0.821 | 1.401 | 2.289 |
| 1 | 0.315 | 0.888 | 1.375 | 2.143 |

| Synergism: | Very Strong | Strong | Synergism | Moderate |
|---|---|---|---|---|
| CI Values: | <0.1 | 0.1-0.3 | 0.3-0.7 | 0.7-0.85 |

H226 cells were seeded in 96-well plates (200 cells/well) and after 24 hrs, the growth medium was replaced with media containing RAIN-32 and trametinib at the indicated concentrations. After 10 days of incubation, the relative cell number was determined with CyQUANT reagent. The data were submitted to synergy analysis with the Calcusyn program and the CI values are presented. Strong to very strong synergy was observed at 30 – 100 nM RAIN-32 in combination with trametinib.

# Conclusions

- MDM2 inhibitor RAIN-32 (milademetan) selectivity inhibits *in vitro* and *in vivo* growth of MPM cell lines bearing WT *TP53*.

- In light of the fact that there are no approved therapies following MPM treatment failure with anti-PD1-based immunotherapy or cytotoxic agents, the MDM2/TP53 axis represents an attractive target for further clinical exploration in this disease.

- RAIN-32 demonstrated tumor growth inhibition as a single agent in several models and work is ongoing to evaluate combination with other MPM-active therapeutics such as anti-PD1 immunotherapies or MAPK pathway targeted inhibitors (i.e., MEK inhibitors).