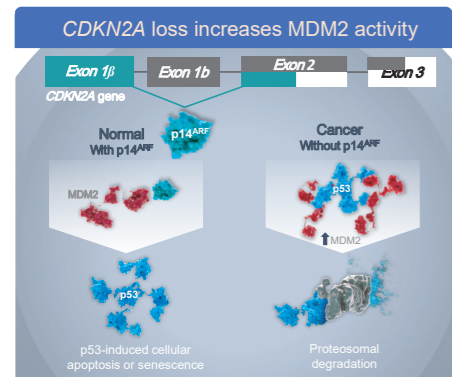


Background

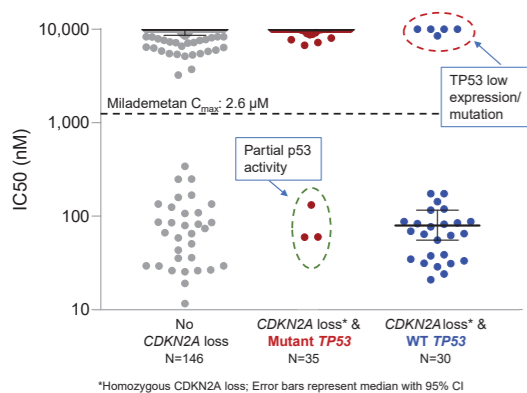
- Loss of p53 tumor suppressor function is critical for many cancers and is caused by *TP53* mutation in ~50% of tumors, but may occur through additional mechanisms:¹
 - Murine double minute 2 (MDM2) is an E3 ubiquitin ligase whose primary function is to inhibit p53 activity by impeding p53 transcriptional activity, promoting nuclear export, and inducing p53 degradation.¹
 - The use of investigational MDM2 inhibitors, such as milademetan (RAIN-32), may provide a therapeutic strategy for select tumors with wild-type (WT) *TP53* by disrupting the MDM2-p53 interaction and restoring p53 function.
- Cyclin-dependent kinase inhibitor 2A (*CDKN2A*) tumor suppressor gene is altered in more than 15% of all tumors (TCGA PanCancer Atlas) and encodes two proteins, p14^{ARF} and p16, which are inhibitors of MDM2 and cyclin-dependent kinases, respectively.
- Given the role of p14^{ARF} in regulating the MDM2-p53 pathway, we investigated the use of *CDKN2A* loss in the context of WT *TP53* as a strategy for the selection of patients who might benefit from milademetan.

Rationale for the use of an MDM2 inhibitor in tumors with *CDKN2A* loss



- p53 responds to normal cellular injury to maintain genomic integrity and protects against cancer via induction of cellular apoptosis or senescence.
- p14^{ARF} is a tumor suppressor produced by alternating splicing of exons in the *CDKN2A* gene.
- p14^{ARF} inhibits MDM2 thus activating p53.
- CDKN2A* loss abolishes p14^{ARF} inhibition of MDM2, leading to MDM2-mediated p53 degradation.
- As a disruptor of the MDM2-p53 interaction, milademetan restores / reactivates WT p53.

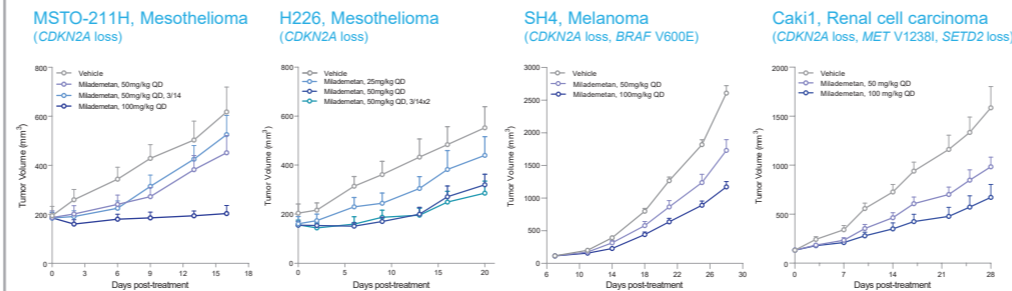
Milademetan *in vitro* activity in cell lines with *CDKN2A* loss is p53 dependent



- Milademetan, when tested in a cell line panel, displayed potent antiproliferative activity in cell lines with *CDKN2A* loss and WT *TP53*:
 - Low *TP53* expression or atypical mutations in a few insensitive cell lines is consistent with the requirement of WT *TP53* for milademetan activity.
- Milademetan had no activity in cell lines with *CDKN2A* loss and mutant *TP53*:
 - Partial activity of mutant p53 contributed to milademetan activity in 3 cell lines.

IC₅₀s of milademetan in tumor cell lines of OncoPanel, Eurofins Panlabs.² *TP53* and *CDKN2A* status of the cell lines based on Depmap CCLE data (Q2, 2020). Partial p53 activity of mutant *TP53* cell lines and low *TP53* expression of WT *TP53* labeled based on published reports.

In vivo antitumor activity of milademetan in *CDKN2A* loss models

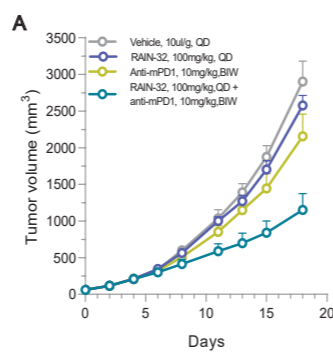


Dose-dependent antitumor activity of milademetan in *CDKN2A* loss xenograft models. Milademetan activity in nu/nu or BALB/c mice bearing xenografts. MSTO211H (1x10⁶),³ H226 (1x10⁶),³ SH4 (5x10⁶) and Caki1 (10x10⁶) cells were inoculated into both flanks of female nu/nu mice. After ~3–4 weeks for tumor establishment, groups of mice (n=10 or 8) were treated with vehicle or milademetan at the indicated doses by daily gavage. Tumor volume was measured every 3–4 days with calipers.

Synergistic antitumor activity in *CDKN2A* loss syngeneic model with milademetan plus anti-PD1 mAb combination

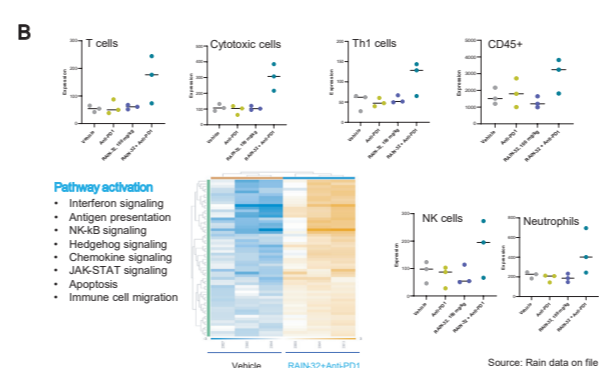
- Milademetan displayed synergistic activity in Colon-26 *CDKN2A* loss syngeneic model with an anti-programmed cell death protein 1 (PD1) antibody, with concomitant immune activation in the tumor microenvironment.

Colon-26 syngeneic model (*CDKN2A* loss, *KRAS* G12D, *FGFR1* S107F)

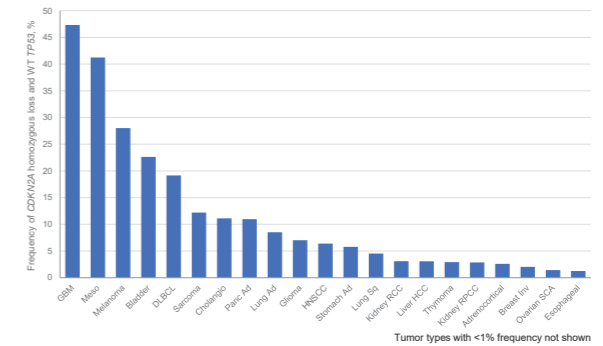


Antitumor activity of milademetan, anti-PD1 mAb, or combination in *CDKN2A* loss models. (A) Colon-26 cells (0.3 x 10⁶) were inoculated subcutaneously into BALB/c nude mice. After 6 days for tumor establishment, groups of mice (n=8) were treated with vehicle or milademetan at the indicated doses by daily gavage. Tumor volume was measured every 3–4 days with calipers. (B) Tumor FFPE sections at the end of treatment were used for RNA isolation and gene expression analysis using the nCounter[®] PanCancer IO 360[™] Panel and NanoString platform (NanoString Technologies, USA). Gene expression data was analyzed using Rosalind[®] software.

Colon-26: Immune cell and pathway alterations



CDKN2A homozygous loss and WT *TP53* frequency across tumor types



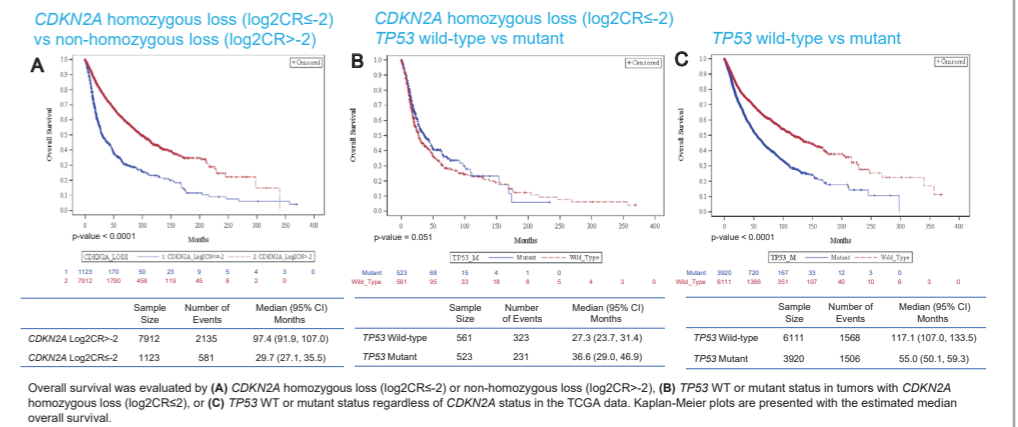
CDKN2A loss frequency across cancers. CN values of the TCGA v23.0 data were corrected for tumor purity using ABSOLUTE (Broad Institute). Frequency was calculated as the number of samples with *CDKN2A* homozygous loss (log2 copy ratios<2) and *TP53* WT / total number of samples with CN data multiplied by 100.

- Among solid tumor types, the most frequent percentage of these genotypes occurred in glioblastoma, mesothelioma, melanoma, bladder, sarcoma, pancreatic and NSCLC.
- Overall, the percentage of all tumors with *CDKN2A* homozygous loss and WT *TP53* was 6.2%.

Breast Inv = invasive breast cancer
Cholangiol = cholangiocarcinoma
DLBCL = diffuse large B-cell lymphoma
GBM = glioblastoma multiforme
HNSCC = head and neck squamous cell carcinoma
Kidney RCC = kidney renal clear cell carcinoma
Kidney RPCC = kidney renal papillary cell carcinoma
Liver HCC = hepatocellular carcinoma
Lung Ad = lung adenocarcinoma
Lung Sq = lung squamous cell carcinoma
Meso = mesothelioma
Ovarian SCA = ovarian serous cystadenocarcinoma
Panc Ad = pancreatic adenocarcinoma
Stomach Ad = stomach adenocarcinoma

Prognosis by *CDKN2A* and *TP53* genotype

- Patients with *CDKN2A* homozygous loss had significantly worse overall survival (OS) than those without *CDKN2A* homozygous loss (median OS, 29.7 vs. 97.4 months, $p < 0.0001$), and this was maintained when accounting for tumor type in a multivariate analysis ($p < 0.0001$).
- Lack of survival effect of *TP53* mutation in *CDKN2A* homozygous loss patients is presumably due to loss of p14^{ARF}.



Conclusions

- Milademetan showed potent antiproliferative activity in *CDKN2A* homozygous loss cell lines with WT *TP53*, but was inactive in cell lines with mutant *TP53*.
- Milademetan showed evidence of anti-tumor activity across multiple tumor types with *CDKN2A* homozygous loss and WT *TP53*.
- In vivo* data supported potential synergy of milademetan with anti-PD1 antibody in this genetic subset.
- Overall, the percentage of all tumors with *CDKN2A* homozygous loss and WT *TP53* was 6.2%.
- Patients with *CDKN2A* homozygous loss had a significantly worse overall survival than those without *CDKN2A* homozygous loss (median OS, 29.7 vs. 97.4 months, $p < 0.0001$), and this was maintained when accounting for tumor type in a multivariate analysis ($p < 0.0001$).
- A clinical trial evaluating the safety and efficacy of milademetan plus atezolizumab in advanced solid tumors with *CDKN2A* homozygous loss and WT *TP53* (MANTRA-4) is planned.