Combination of MDM2 inhibition with milademetan and MEK inhibition leads to improved anti-tumor activity in cancer models harboring wild-type *TP53*

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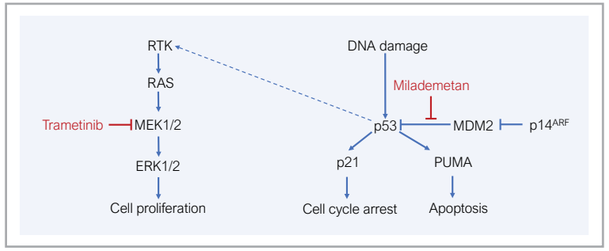
#PB050

Background

- Loss of p53 tumor suppressor function is critical for many cancers and is achieved by *TP53* mutation in ~50% of tumors, but may occur through other mechanisms in tumors with wild-type (WT) *TP53*:
  - *MDM2* gene amplification (amp).[1]
  - MDM2 overexpression.[2]
  - MDM2 regulator loss (p14^{ARF} encoded by *CDKN2A*).[3]
- As a potent disruptor of the MDM2-p53 interaction, milademetan reactivates p53.
- The MEK/ERK pathway is dysregulated in a significant number of cancers due to upregulated RTK signaling or other alterations in components of this critical pathway leading to unchecked proliferation and anti-apoptotic signaling.
- p53-dependent activation of ERK may lessen the potential anti-tumor activity achieved by p53 reactivation.[4-7]
- An MDM2 and MEK inhibitor combination was identified from a cell panel screening for synergistic effects, independent of MAPK pathway mutations.[8]
- Combining an MDM2 inhibitor and MEK inhibitor may lead to improved responses in WT *TP53* cancers that do not respond to MDM2 inhibitors alone.

Rationale for combined MDM2 and MEK inhibition



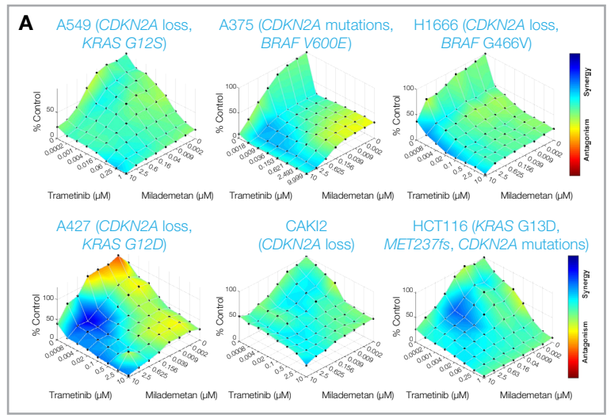
Adapted from [8]Roy et al. Cancers (Basel) 2020;12:2253.

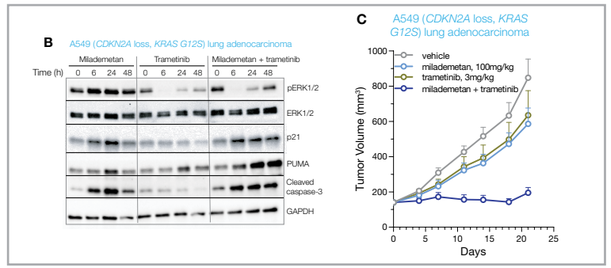
Synergistic activity of milademetan and trametinib combination in WT *TP53* cell lines

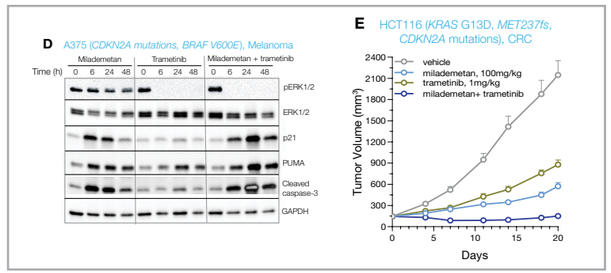
	Cell line	Tumor type	Alterations	Synergy score		
				Loewe	Bliss	HSA
MDM2 amp	SJSA1	Osteosarcoma	*MDM2* CN 44	118.1	112.6	139
	JAR	Placenta	*MDM2* CN 10.7	54.4	54.4	54.4
	94T778	LPS	*MDM2* CN 61.1	99.2	52.8	106.4
	93T449	LPS	*MDM2* CN 98.8	70.1	12.4	80.5
CDKN2A loss	A375	Melanoma	*CDKN2A* mutations, *BRAF V600E*	103.9	−31.29	109.5
	HCT116	CRC	*KRAS G13D*, *MET237fs*, *CDKN2A* mutations	158.1	69.9	175.1
	A549	Lung adenocarcinoma	*CDKN2A* loss, *KRAS G12S*	88.79	−21.98	106.87
	A427	Lung adenocarcinoma	*CDKN2A* loss, *KRAS G12D*	267.99	116.87	275.95
	H1666	Lung adenocarcinoma	*CDKN2A* loss	92.12	−2.53	93.64
	CAKI2	Renal	*CDKN2A* loss	145.9	−35.2	157.8
	SH4	Melanoma	*CDKN2A* loss, *BRAF V600E*	6.6	2.7	6.6
TP53 WT	HT1197	Bladder	*NRAS Q61R*	277.7	50.8	285.1
	HepG2	HCC	*NRAS Q61L*	106.0	23.0	113.2
	LoVo	CRC	*KRAS G13D*, *FGFR3 I533V*	75.0	−105.5	83.3
	AGS	Gastric adenocarcinoma	*KRAS G12D*	247.9	95.2	256.1
	SKCO1	CRC	*KRAS G12V*	152.9	88.8	165.5
	H1385	Lung squamous	*KRAS G12C*	348.0	228.6	354.4
	HS766T	Pancreas	*KRAS Q61H*	−22.0	−22.0	−22.0

Synergy scores for milademetan and trametinib combination (from Combenefit) in cell lines with various genetic alterations.
amp, amplification; CN, copy number; CRC, colorectal cancer; HCC, hepatocellular carcinoma; LPS, liposarcoma; WT, wild-type.

Activity of milademetan and trametinib combination in *CDKN2A* loss

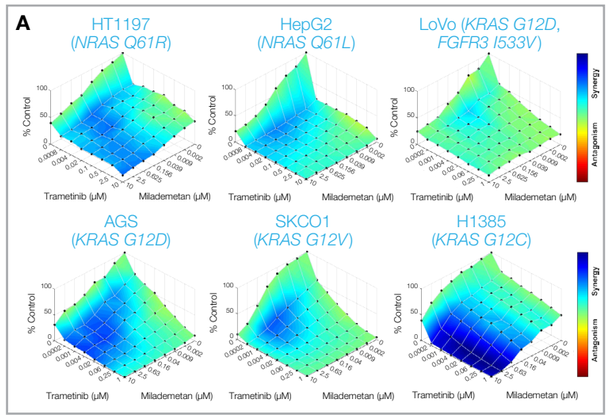


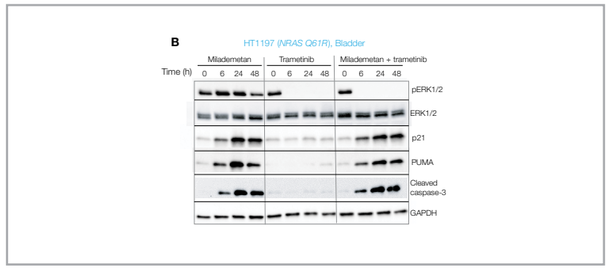


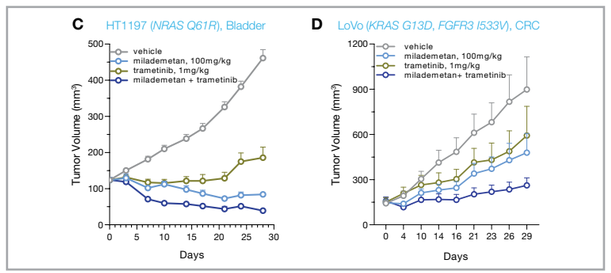


(A) Cell lines with *CDKN2A* loss were treated with milademetan and trametinib. Proliferation was assessed in a 3-day cell proliferation assay and Loewe synergy plots were generated using Combenefit software. **(B)** A549 (lung adenocarcinoma) cells and **(D)** A375 (melanoma) cells were treated with milademetan (100 nM), trametinib (50 nM), or a combination of both for indicated times. Cells were lysed and immunoblotting was performed using antibodies to identify changes in MDM2/p53 and MAPK pathways. **(C) (E)** Milademetan combined with trametinib inhibits tumor growth in A549 and HCT116 xenograft models. Immunodeficient mice bearing the indicated tumor xenografts were dosed orally with milademetan or trametinib as indicated. Tumors were measured on indicated days and mean (SEM) tumor volumes were plotted.
CN, copy number; CRC, colorectal cancer.

Activity of milademetan and trametinib combination in WT *TP53*

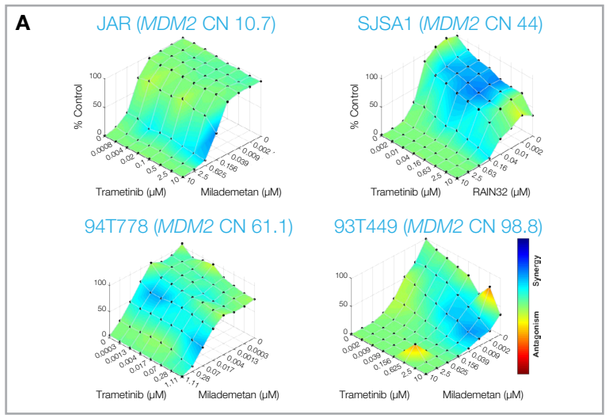


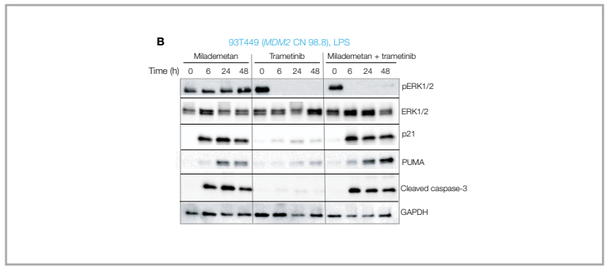




(A) Cell lines with WT *TP53* were treated with milademetan and trametinib. The proliferation was assessed in a 3-day cell proliferation assay and Loewe synergy plots were generated using Combenefit software. **(B)** HT1197 (bladder) cells were treated with milademetan (100 nM), trametinib (50 nM), or a combination of both for indicated times. Cells were lysed and immunoblotting was performed using antibodies to identify changes in MDM2/p53 and MAPK pathways. **(C) (D)** Milademetan combined with trametinib inhibits tumor growth in HT1197 and LoVo xenograft models. Immunodeficient mice bearing the indicated tumor xenografts were dosed orally with milademetan or trametinib as indicated. Tumors were measured on indicated days and mean (SEM) tumor volumes were plotted.
CN, copy number; CRC, colorectal cancer.

Activity of milademetan and trametinib combination in *MDM2* amplification





(A) *MDM2*-amplified cell lines were treated with milademetan and trametinib. Proliferation was assessed in a 3-day cell proliferation assay and Loewe synergy plots were generated using Combenefit software. **(B)** 93T449 (LPS) cells were treated with milademetan (100 nM), trametinib (50 nM), or a combination of both for indicated times. Cells were lysed and immunoblotting was performed using antibodies to identify changes in MDM2/p53 and MAPK pathways. CN, copy number; LPS, liposarcoma.

Summary

- Milademetan induced p53 activation in diverse cancer models with *MDM2* amp, *CDKN2A* loss or WT *TP53* and also harboring oncogenic drivers such as RAS and RAF alterations.
- A combination of trametinib with milademetan induced sustained MAPK inhibition.
- Synergistic anti-proliferative activity was observed using a combination of milademetan and trametinib in cell lines with *MDM2* amp, *CDKN2A* loss or WT *TP53*.
- A combination of milademetan and trametinib resulted in improved antitumor activity compared to single-agent treatment in *MDM2* amp, *CDKN2A* loss or WT *TP53* xenograft models.
- These data support clinical exploration of milademetan in combination with MAPK pathway inhibition in WT *TP53* cancers with or without *MDM2* amp or *CDKN2A* loss.

Acknowledgements

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