MDM2 inhibition in combination with MEK inhibition in pre-clinical models of lung adenocarcinomas with *MDM2* amplification

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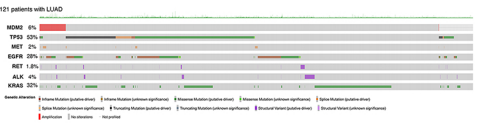
Background and Objectives

While targeted therapies have transformed the care of patients with advanced lung adenocarcinomas with genomic alterations in kinases and in *KRAS*, the development of resistance to therapy is almost universal. Novel therapies are urgently needed for patients progressing on targeted agents. We hypothesize that combination therapies aimed at a known driver and another targetable alteration in the same tumor could prolong time on targeted therapy.

*MDM2*amp are significantly enriched in patients with LUAD and a driver alteration



Genomic alteration	Sample number	Frequency of alteration (n=7,121)	Co-occurrence of MDM2 amplification		P-value (Fisher exact)
			Number	Frequency	
METex14 splice variant	147	2%	53	36%	**<0.001**
RET rearrangement	127	2%	15	12%	**0.006**
EGFR mutation	1,979	28%	162	8%	**<0.001**
ALK rearrangement	282	4%	28	10%	**0.002**
KRAS mutation	2,260	32%	104	5%	ns

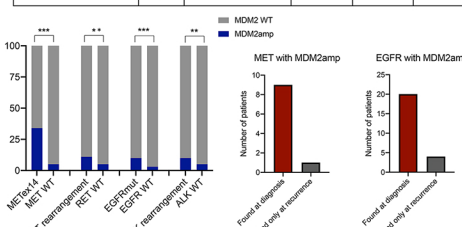


Table 1. *MDM2*amplifications are significantly enriched in patients with receptor tyrosine kinase alterations in EGFR, RET, METex14, and ALK. Analysis of 7,121 patients with lung adenocarcinoma who underwent MSK-IMPACT testing, 6% (410/7,121) harbored MDM2 amplification (MDM2amp), a known mechanism of TP53 inactivation. MDM2amp (fold-change ≥2.0) was over-represented among tumors with driver alterations **Figure 1**. Barplots are a visual representation of distribution of MDM2amp in the context of driver alteration (left). **Figure 2**. For patients that had matched samples at diagnosis and at recurrence, MDM2amp was most commonly found at diagnosis (right).

MDM2 and MEK inhibition is synergistic *in vitro*

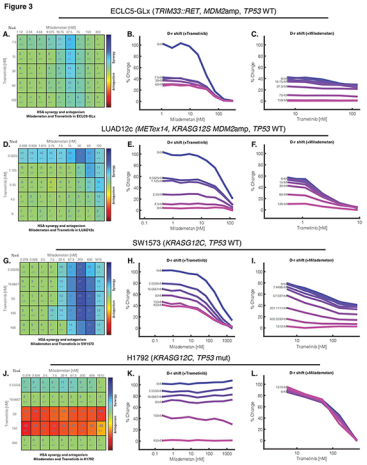


Figure 3. MDM2 inhibition with mildemetan and MEK inhibition with trametinib is synergistic in models of lung adenocarcinoma. A. Synergy matrix for ECLC5-GLx. The synergy matrices are synergy scores, calculated according to the HSA synergy model (which measures whether the expected combination effect equals to the higher effect of individual drugs). Boxes are coloured blue if the synergy score significant by t-test. **B.** Mildemetan dose-response shift (DRS) observed in the presence of increasing concentrations of trametinib **C**. DRS observed for trametinib in the presence of increasing concentrations of mildemetan. **D.** Synergy matrix and **E-F**. DRS for LUAD12c. **G**. Synergy matrix and **H-I**. DRS for SW1573. **J**. Synergy matrix and **K-L**. DRS for H1792. The number of biologic replicates (N) is indicated at the top right of the matrix. ***p<0.001, **p<0.01, *p<0.05. nM; nano molar.

MDM2 and MEK inhibition cause increased apoptosis compared to either agent alone

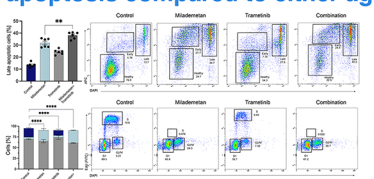


Figure 4. Annexin-V apoptosis assay by flow cytometry in ECLC5-GLx cell line (top), Click-iT cell cycle assay by flow cytometry in ECLC5-GLx cell line (bottom) after treatment with control, mildemetan (100nm), trametinib (100nm), or combination at 48h. ****p<0.0001. **p<0.01. Biological repl. shown.

MDM2 inhibition causes paradoxical phosphorylation of pERK, suppressed by MEK inhibition

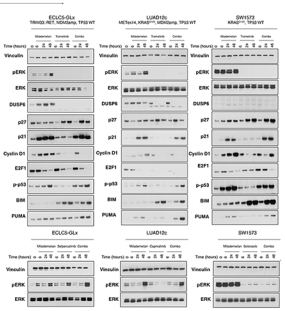


Figure 5. Western blot analysis of ECLC5-GLx (TRIM33::RET, MDM2amp) (left), LUAD12c (METex14, KRASG12S, MDM2amp) (middle), and SW1573 cell line (KRASG12C, TP53WT). ECLC5-GFP-LUCx and SW1573 cell line treated with 100nM of Mildemetan, 100nM of trametinib, or Combination for 0,6,24,48h. LUAD12c cell line treated with 100nM of Mildemetan, 20 nM of Trametinib, or Combination for 0,6,24,48h.

Transcriptomic analysis

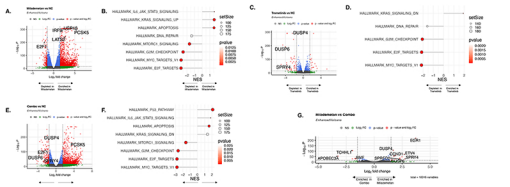


Figure 6. RNAseq of ECLC5-GLx cell line of untreated vs. 48h of treatment with Mildemean, trametinib, or combination. A-B. Differentially expressed genes (DEG) and pathway enrichment of control vs. mildemetan (100nM) C-D control vs. trametinib (100nM) E-F; control vs. combination (both 100 nM) G. DEG of mildemetan vs. combination

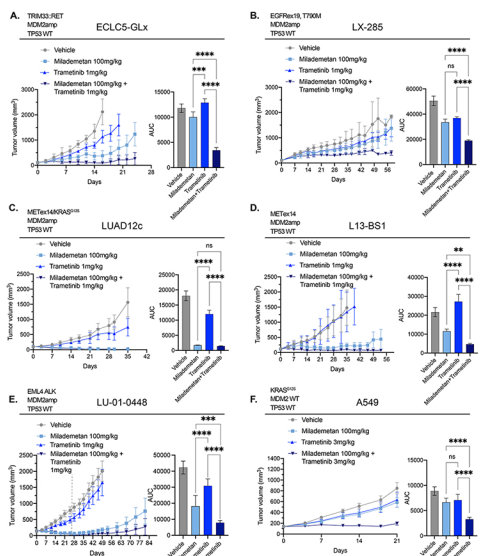


Figure 7. Efficacy of MDM2/MEK combination in vivo. 5-8 mice/group. Treatment commenced once tumors reached 150mm³. ****p<0.0001. ***p<0.001. *p<0.05 by ANOVA of area under the curve. Dashed line for Figure 7E indicates dosing cessation.

Conclusions

Combination MDM2/MEK inhibition is effective in preclinical patient-derived LUAD models harboring an oncogenic driver and *MDM2*amp. This combination strategy, which is applicable to LUADs with a wide variety of upstream driver mutations or fusions, warrants further evaluation in a clinical trial. A phase 1/2 investigator-initiated trial in patients with *MDM2*amp and a concurrent driver alteration is planned.

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