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KRAS alterations combined with *TP53* mutations as novel synthetic lethal genomic lesions for PKMYT1 inhibition

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Introduction

- Membrane-associated tyrosine- and threonine-specific Cdc2-inhibitory kinase (PKMYT1) is a cell cycle regulatory kinase that inhibits CDK1/CyclinB activity, delaying mitotic entry in tumor cells experiencing replication stress (RS).
- RS is frequently induced by genetic alterations that drive premature transition from G1 to S phase, promoting genome instability and creating a synthetic lethal (SL) relationship between these specific alterations and PKMYT1 inhibition[1]. This relationship has been demonstrated preclinically and clinically with *CCNE1* amplification and *FBXW7* mutations, common alterations in ovarian and colorectal cancers, respectively.
- Oncogenic *KRAS* gain of function (GOF) mutations are bona fide drivers of RS. Oncogenic *KRAS* promotes aberrations in the number of active replicons and replication fork progression, which leads to DNA damage and genomic instability[2]. In this genomic context, mutations in *TP53* compromise checkpoint regulation, enabling cells to proceed through the cell cycle prior to DNA damage repair.
- Here we investigate the relationship between *KRAS/TP53* alterations and PKMYT1 inhibition, mediated by the first-in-class, potent and selective PKMYT1 inhibitor lunresertib (RP-6306), alone or in combination with RS-inducing agents.

Lunresertib mechanism of action

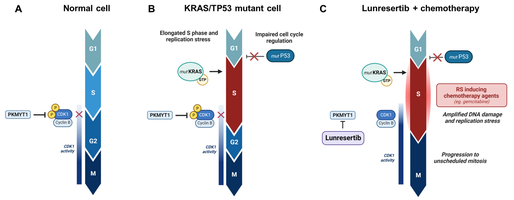

Figure 1: (A) PKMYT1 is a cell cycle regulatory kinase that inhibits CDK1/CyclinB activity, regulating entry into mitosis **(B)** Oncogenic *KRAS* GOF mutations lead to replication stress in the context of impaired cell cycle regulation due to *TP53* mutations. Tumor cells become highly dependent on PKMYT1 activity to arrest progression into mitosis with damaged DNA. **(C)** Replication stress (RS)-inducing chemotherapy agents exacerbate basal DNA damage. PKMYT1 inactivation by lunresertib promotes entering unscheduled mitosis with severely damaged DNA, eventually leading to cell death.

Results

KRAS/TP53 mutations sensitize cell lines to PKMYT1 inhibition

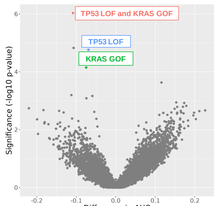

Figure 2: Cell lines with combined *KRAS/TP53* mutations display increased sensitivity to PKMYT1 inhibition. The effect of PKMYT1 inhibition was explored across >900 fully genomically annotated cell lines using PRISM (profiling relative inhibition simultaneously in mixtures) discovery platform[3,4]. Volcano plot demonstrates differences in area under the curve (AUC) for biomarker positive and negative cell lines (x-axis) and –log10 p-value of a Mann-Whitney test (y-axis).

Results

KRAS mutations lead to elongated S-phase, a key vulnerability to PKMYT1 inhibition

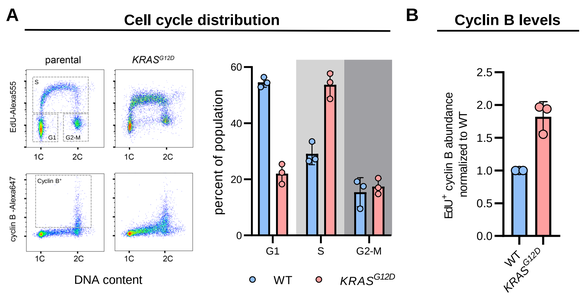

Figure 3: EBC-1 lung squamous cell carcinoma cells (*TP53* mutant) were genetically engineered to express *KRAS* G12D mutant allele. (A) Cell cycle distribution was assessed by EdU staining and flow cytometry in *KRAS* G12D mutant cells and isogenic wild type (WT) parental controls. (B) Cyclin B levels were assessed by flow cytometry on the cell lines previously described.

Lunresertib induces premature mitosis and catastrophic DNA damage in *KRAS/TP53* mutant cells

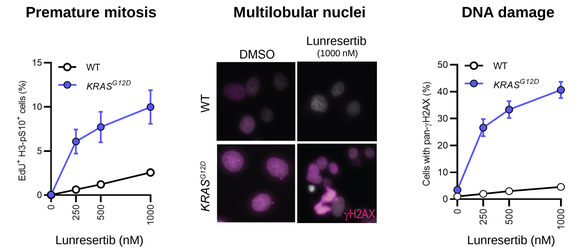

Figure 4: Lunresertib induces premature mitosis, as evidenced by expression of mitotic markers in EdU-incorporating cells. Premature cell division induces multilobular nuclei and massive DNA damage. EBC-1 cells (*TP53* mutant) were genetically engineered to express *KRAS* G12D mutant allele. Quantitation of double-positive staining for EdU and histone H3-pS10 or γH2AX by flow cytometry following lunresertib treatment with the indicated concentration for 24 h. Nuclear morphology was assessed by DAPI staining.

Sensitivity to PKMYT1 inhibition is observed in different *KRAS* activating mutation alleles

A

Cell LIne	Tissue Source	KRAS mutation	TP53 status	Fold shift in IC_{50} to WT*
BRONCH2	Human bronchial epithelial cells	**G12C**	KO	**5.2**
HPNE	Human pancreatic duct cells	**G12R**	KO	**4.9**
EBC	Lung squamous cell carcinoma	**G12D**	Mut	**4.0**
BRONCH2	Human bronchial epithelial cells	**Q61H**	KO	**6.8**
HPNE	Human pancreatic duct cells	**Q61H**	KO	**3.0**

**Data indicates median fold shift across clones*

B

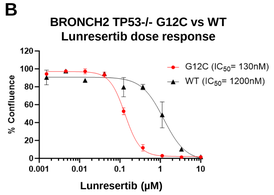

Figure 5: Sensitivity to lunresertib in observed irrespective of *KRAS* mutation codon/allele or cellular background. (A) Summary table representing median IC_{50} fold-change comparing cell lines with engineered *KRAS/TP53* mutations to WT isogenic controls. A total of 11 clones were tested, with 9 demonstrating at least a 3-fold shift in sensitivity to lunresertib (median ~5x shift). **(B)** Representative cell growth inhibition curves comparing isogenic cells with *TP53-/-* and *KRAS*-G12C mutations to WT control.

Lunresertib synergizes with replication stress inducing therapies in *KRAS/TP53* mutant cell lines

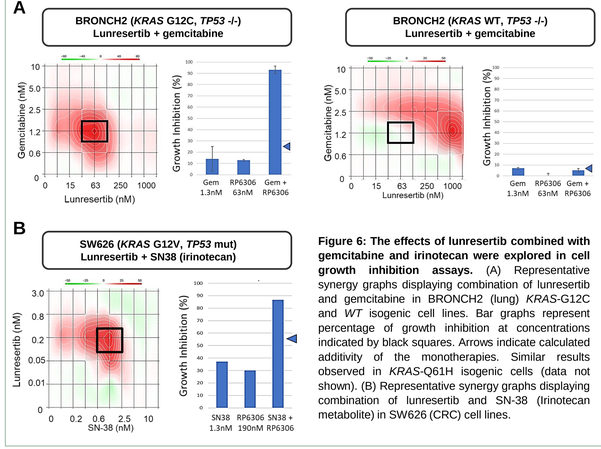

Figure 6: The effects of lunresertib combined with gemcitabine and irinotecan were explored in cell growth inhibition assays. (A) Representative synergy graphs displaying combination of lunresertib and gemcitabine in BRONCH2 (lung) *KRAS*-G12C and *WT* isogenic cell lines. Bar graphs represent percentage of growth inhibition at concentrations indicated by black squares. Arrows indicate calculated additivity of the monotherapies. Similar results observed in *KRAS*-Q61H isogenic cells (data not shown). (B) Representative synergy graphs displaying combination of lunresertib and SN-38 (Irinotecan metabolite) in SW626 (CRC) cell lines.

Lunresertib combined with RS-inducing therapies yields tumor regressions in pancreatic and CRC *TP53/KRAS* mutant PDX models

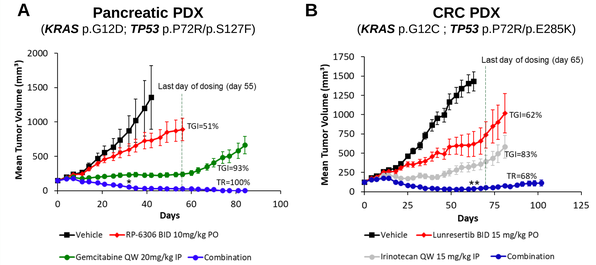

Figure 7: BALB/c nude mice bearing pancreatic or colorectal PDX xenograft tumors were treated with lunresertib combined with gemcitabine or irinotecan, respectively. (A) Tumor growth inhibition (TGI) and tumor regressions (TR) rates were calculated on day 42; combination treatment resulted in 100% complete tumor response, i.e. TR = 100%. (B) TGI's were calculated on day 63. Tumor regressions observed in the combination arm (4 of 6 tumors < 40 mm3). Treatments were well-tolerated in both models, with maximal combination mean body weight loss < 4%; n=6 animals per group. Combination arm compared to vehicle using a Browne-Forsythe ANOVA test with Welch's t-test for multiple comparisons; *p=0.0139, **p<0.0001.

Conclusions

- GOF *KRAS* mutations, combined with *TP53* alterations, show a strong SL relationship with PKMYT1 inhibition, alone and in combination with RS-inducing antitumor agents
- The SL phenotype is observed across multiple tumor indications and *KRAS* mutant codons/alleles, suggesting a potential broad scope of clinical utility beyond approved KRAS inhibitors
- *KRAS/TP53* mutations enhance G1/S transition driving replication stress and heightened dependence on PKMYT1-mediated CDK1/CyclinB inhibition
- Cell-based and PDX combination efficacy studies demonstrate robust cell growth inhibition/anti-tumor activity in pancreatic and colorectal settings, with durable tumor regressions and complete responses
- This data establishes scientific rationale to evaluate PKMYT1 inhibition in *KRAS/TP53* altered tumors, supporting further exploration in the clinic
- **Lunresertib in combination with FOLFIRI and gemcitabine is currently being evaluated in patients with advanced solid tumors (NCT05605509), including *KRAS/TP53* mutant CRC**

Disclosures

M.L.H, J.F, D.G, R.S, R.K, S.S, S.S, S.M, A.R, T.T.F.J, M.Z, C.G.M, A.V, and E.A-F are employees of Repare and may hold stock and/or stock options. **V.B, H.B and O.N** were employees of Repare at the time this work was performed.

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Bibliography

1.- Gallo D, Young JTF, Fourtounis J, Martino G, Álvarez-Quilón A, et al. **CCNE1 amplification is synthetic lethal with PKMYT1 kinase inhibition.** Nature. 2022 Apr;604(7907):749-756. PMID: 35444283.

2.- Grabocka E, Commisso C, Bar-Sagi D. **Molecular pathways: targeting the dependence of mutant RAS cancers on the DNA damage response.** (Review) Clin Cancer Res. 2015 Mar 15;21(6):1243-7. PMID: 25424849.

3.-Yu C, Mannan AM, Yvone GM, Ross KN, Zhang YL, Marton MA, Taylor BR, Crenshaw A, Gould JZ, Tamayo P, Weir BA, Tsherniak A, Wong B, Garraway LA, Shamji AF, Palmer MA, Foley MA, Winckler W, Schreiber SL, Kung AL, Golub TR. **High-throughput identification of genotype-specific cancer vulnerabilities in mixtures of barcoded tumor cell lines.** Nat Biotechnol. 2016 Apr;34(4):419-23. doi: 10.1038/nbt.3460. Epub 2016 Feb 29. PMID: 26928769; PMCID: PMC5508574.

4.- Corsello SM, Nagari RT, Spangler RD, Rossen J, Kocak M, et al. **Discovering the anti-cancer potential of non-oncology drugs by systematic viability profiling.** Nat Cancer. 2020 Feb;1(2):235-248. PMID: 32613204;