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Inhibition of ULK1/2-Mediated Autophagy Enhances Responses to RAS Inhibition and Augments Antigen-Specific T Cell Cytotoxicity

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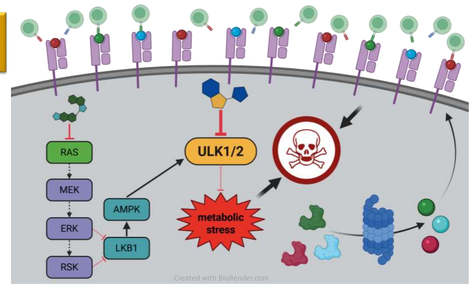
Background

Blocks a Rapid Mechanism of Resistance to RASi

Inhibition of the RAS pathway increases autophagy in cancer cells

ULK1/2 inhibition decreases RAS inhibition induced autophagy

ULK1/2 inhibition combined with RAS inhibition results in increased cancer cell death



Increases Antigen Presentation & T-cell-Mediated Killing

ULK1/2 inhibition increases antigen presentation resulting in higher levels of peptide loaded MHC I

ULK1/2 inhibitor-mediated increase in peptide-loaded MHC I results in increased antigen-specific CD8+ T cell cytotoxicity

Combining ULK1/2 inhibition with RAS inhibition will result in increased tumor cell death through both cancer cell intrinsic and immunological mechanisms

Figure 1. Combined inhibition of ULK1/2 and RAS enables a fully effective response to RAS inhibition via blocking RAS inhibitor-induced cytoprotective autophagy. Additionally, inhibition of ULK1/2 increases neoantigen presentation, eliciting greater antigen-specific CD8+ T cell activation and anti-tumor immunogenicity.

RAS Inhibition Rapidly Induces Autophagy

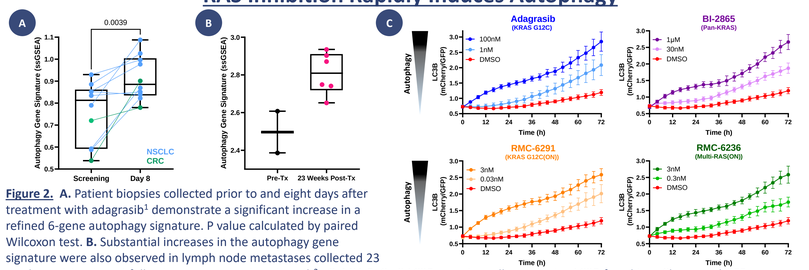


Figure 2. **A.** Patient biopsies collected prior to and eight days after treatment with adagrasib[1] demonstrate a significant increase in a refined 6-gene autophagy signature. P value calculated by paired Wilcoxon test. **B.** Substantial increases in the autophagy gene signature were also observed in lymph node metastases collected 23 weeks post-treatment following progression to sotorasib[2]. **C.** MIA PaCa-2 LC3B reporter cells expressing LC3B fused to mCherry and GFP were evaluated in the Incucyte. Cells with higher autophagy have more LC3B in the autolysosome where low pH quenches GFP resulting in an increased ratio of mCherry/GFP. Treatment with multiple RAS inhibitors demonstrated concentration-dependent increases in autophagy within 72 hours.

ULK1/2 Inhibition Prevents RAS Inhibitor-Induced Autophagy

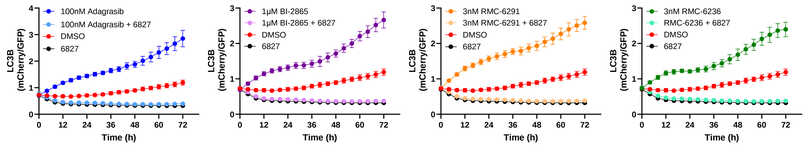


Figure 3. MIA PaCa-2 LC3B reporter cells were grown as spheroids. After two days of compaction, cells were treated with indicated concentrations of different RAS inhibitors alone or in combination with 1uM Arcus ULK1/2 inhibitor 6827. Clear reduction of RAS inhibitor-mediated autophagy with multiple modalities of RAS inhibitors were observed with ULK1/2 inhibition. Similar results observed with H358 and H1373 reporter cell lines.

Combined Inhibition of ULK1/2 and RAS Increases Cancer Cell Death

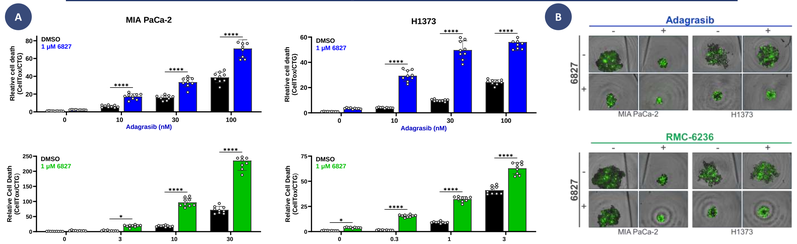


Figure 4. **A.** Cancer cell lines were grown as spheroids and treated with indicated concentrations of adagrasib or RMC-6236 alone or in combination with 0.1, 0.3 or 1 µM Arcus ULK1/2 inhibitor 6827. Relative cell death was determined by normalizing dead CellTox Green–positive cells to viable cells measured by CellTiter-Glo. Significant increases in relative cell death were observed in all three cell lines with both RAS inhibitors when combined with 6827. **B.** Representative CellTox Green images from Incucyte demonstrate reduced spheroid size and increased CellTox Green staining when RAS inhibitors are combined with 6827. Significance was determined by two-way ANOVA *<0.05, **< 0.01, ***<0.001, ****<0.0001

Combined Inhibition of ULK1/2 and RAS Increases Efficacy in Human Xenografts

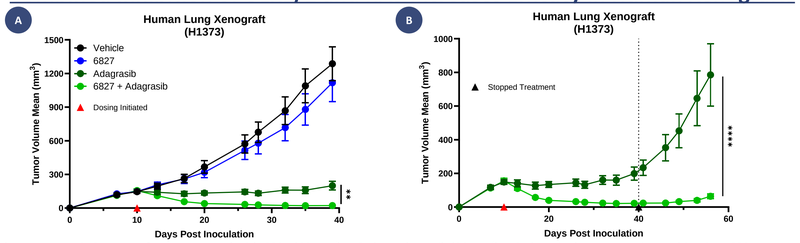


Figure 5. **A.** H1373 xenografts were treated with 6827 (60 mg/kg, QD) or adagrasib (20 mg/kg, QD) as single agents or in combination. 6827 combined with adagrasib resulted in significant tumor growth reduction. **B.** Treatment of both 6827 and adagrasib was stopped at day 40 and tumor growth continued to be monitored. In contrast to tumors treated with single agent adagrasib, those receiving a combination of 6827 and adagrasib exhibited significantly reduced tumor regrowth. Significance was determined by two-way ANOVA **< 0.01, ****<0.0001

Significantly Greater Tumor Control Achieved by Adding 6827 to a Maximally Effective Dose of Adagrasib

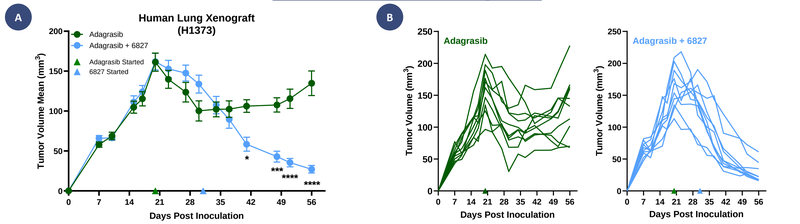


Figure 6. **A.** H1373 xenografts were treated with single-agent adagrasib for 11 days, resulting in tumor stasis as previously observed. One group was then treated with 6827 in combination with adagrasib, resulting in significant tumor growth reduction compared to adagrasib monotherapy. **B.** Individual growth curves demonstrate consistent results in each treatment group. Significance was determined by two-way ANOVA *<0.05, ***<0.001, ****<0.0001

ULK1/2 Inhibition Increases Antigen-Specific T Cell Cytotoxicity

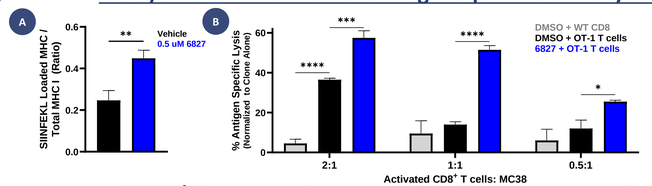


Figure 7. **A.** ULK1/2 inhibition increases SIINFEKL-loaded MHC I on mCherry MC38-OVA cells. **B.** ULK1/2 inhibition significantly enhances antigen-specific T-cell cytotoxicity. Significance was determined by a two-way ANOVA *<0.05, **< 0.01, ***<0.001, ****<0.0001

ULK1/2 Inhibition Increases CD8+ T Cell Infiltration *In Vivo*

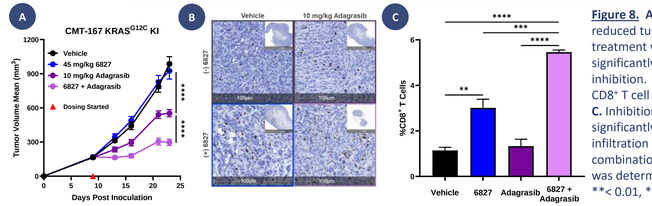


Figure 8. **A.** Adagrasib significantly reduced tumor growth while combined treatment with 6827 resulted in significantly greater tumor growth inhibition. **B.** Representative images of CD8+ T cell infiltration evaluated by IHC. **C.** Inhibition of ULK1/2 by 6827 significantly increased CD8+ T cell infiltration as a single agent and in combination with adagrasib. Significance was determined by a two-way ANOVA **< 0.01, ***<0.001, ****<0.0001

Efficacy and Survival are Significantly Improved with Combined Inhibition of ULK1/2, RAS and PD-1

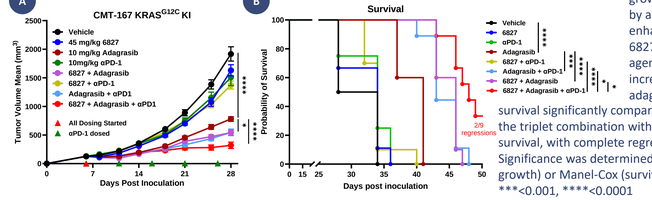
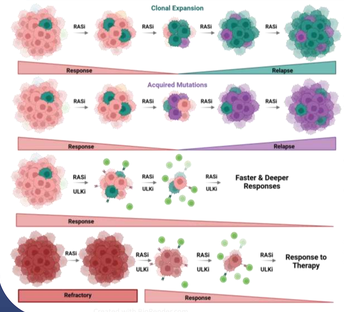


Figure 9. **A.** Significant tumor growth inhibition was mediated by adagrasib, which was further enhanced by combination with 6827 or 6827 and αPD-1. **B.** Single agent adagrasib significantly increased survival. Combining adagrasib with 6827 increased survival significantly compared to adagrasib alone, and the triplet combination with αPD-1 further increased survival, with complete regressions in 2 of 9 mice. Significance was determined by a two-way ANOVA (tumor growth) or Manel-Cox (survival) *<0.05, ,**< 0.01, ***<0.001, ****<0.0001

Conclusions



- Initial response to RASi
- Poor immune infiltration allows for expansion of existing resistant clones
- Initial response to RASi
- Adaptive resistant mutations acquired over time
- Enhanced immune cell activation eliminates clonal tumor cells
- Faster clinical responses will not allow time for acquired mutations to occur
- High basal autophagy
- Unresponsive to RASi initially
- Enhanced immune cell activation initiates anti-tumor responses

- ❖ Inhibition of ULK1/2 prevents the rapid induction of autophagy mediated by RAS inhibition
- ❖ Combined inhibition of ULK1/2 and RAS mediates significantly more cancer cell death *in vitro* and greater tumor control *in vivo* compared to RAS inhibition alone
- ❖ ULK1/2 inhibition increases antigen-specific CD8+ T cell cytotoxicity *in vitro* and infiltration of CD8+ T cells *in vivo*
- ❖ Combined inhibition of ULK1/2, RAS and PD-1 significantly increase efficacy and survival *in vivo*

References

1. Klomp J A., et al., Defining the KRAS- and ERK-dependent transcriptome in KRAS-mutant cancers. *Science* (2024)
2. Tsai Y S., et al., Rapid idiosyncratic mechanisms of clinical resistance to KRAS G12C inhibition. *Journal of Clinical Investigation* (2022)