AACR
Annual Meeting, April 2025
Presentation Number # 7242
Poster Section 37 Board # 18

Inhibition of ULK1/2-Mediated Autophagy Augments Antigen Processing and Presentation Promoting Increased Antigen-Specific CD8⁺ T Cell Cytotoxicity

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Background

- Autophagy is a cellular degradation pathway that recycles cytoplasmic contents and organelles through lysosomal degradation to adapt to changing nutrient conditions [1]
  - ULK1 and ULK2 are serine/threonine kinases that complex with ATG13, FIP200, and ATG101 to initiate autophagy [2]
  - During autophagy, cellular materials are sequestered into double-membraned vesicles called autophagosomes
  - LC3B is a marker of autophagosomes
- High levels of autophagy in cancer cells results in localization of MHC-I to autophagosomes and autolysosomes leading to its degradation, resulting in decreased antigen presentation [3]
- Tumors with elevated autophagy levels are associated with reduced antigen presentation, resulting in low immune infiltration and poor responses to therapy [4]
- Arcus has designed a potent ULK1/2 inhibitor (ULKi)

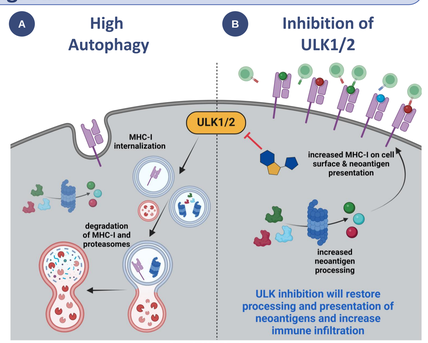

Figure 1. Schematic of the impact of autophagy on antigen processing and presentation.
A. In cells with elevated autophagy, MHC-I is internalized and degraded. **B.** Inhibition of ULK1/2 reduces autophagy, resulting in decreased MHC-I internalization and increased MHC-I on the cell surface, facilitating a protective CD8⁺ T-cell response.

Inhibition of ULK1/2 Reduces Basal Autophagy Levels

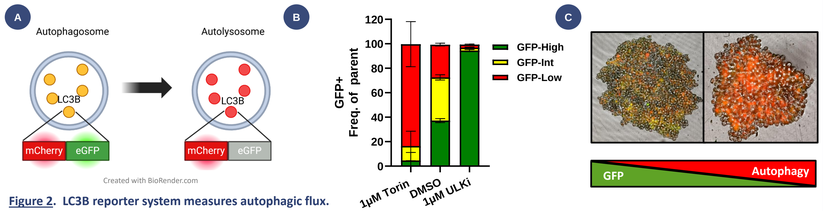

Figure 2. LC3B reporter system measures autophagic flux.
A. Schematic of the LC3B reporter system. Cancer cells were engineered to express LC3B fused to mCherry and eGFP. In cells with higher autophagy, more LC3B is present in the autolysosome where the low pH quenches GFP resulting in an increase in mCherry signal. **B.** MIA PaCa-2 LC3B reporter cells were treated with Torin-1 (mTOR inhibitor that increases autophagy), DMSO, or an Arcus ULK1/2 inhibitor. The frequency of GFP-high, intermediate or low cells were quantified by flow cytometry. **C.** Representative images of MIA PaCa-2 spheroids with low vs high autophagy.

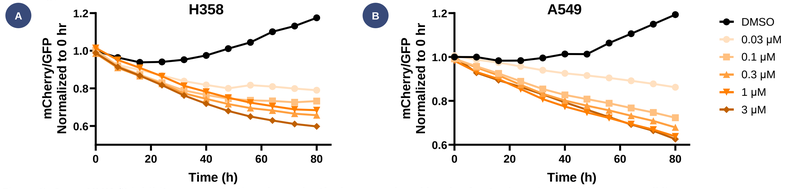

Figure 3. Arcus ULK1/2 inhibitor concentration-dependently decreases basal levels of autophagy in multiple cancer cell lines.
A-B. Cancer cell lines (H358 & A549) were grown as spheroids. After two days of compaction, cells were treated with various concentrations of an Arcus ULK1/2 inhibitor for up to 80 hrs. mCherry and GFP fluorescence were measured using the Sartorius Incucyte. Lower mCherry/GFP ratios indicate lower levels of autophagy.

ULK1/2 Inhibition Alters Subcellular Localization of MHC-I

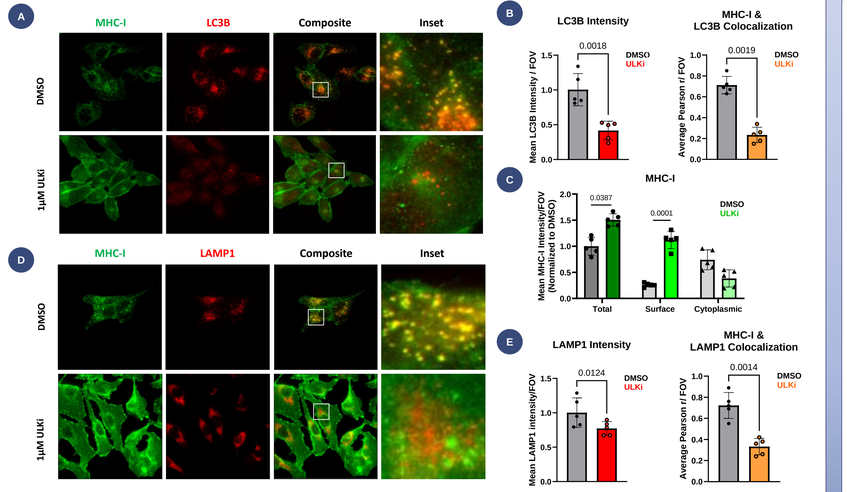

Figure 4. Inhibition of ULK1/2 decreases localization of MHC-I to autolysosomes, increasing surface MHC-I.
H358 cells were treated with DMSO or 1 μM Arcus ULK1/2 inhibitor for 72hrs. Cells were stained for MHC-I and LC3B (**A & B**) , a marker of autophagosomes, or LAMP1, a marker of autolysosomes (**D & E**) . ULK inhibition significantly increased both total and surface MHC-I levels (**C**) and decreased LC3B intensity and colocalization with MHC-I (**B**). LAMP1 intensity and colocalization with MHC-I were also decreased (**E**). Together these data show that ULK1/2 inhibition decreases autophagy resulting in increased surface expression of MHC-I. No significant change in cytoplasmic MHC-I was observed. Statistical significance was determined by paired t-tests.

ULK1/2 Inhibition Increases Levels of Peptide-Loaded MHC-I

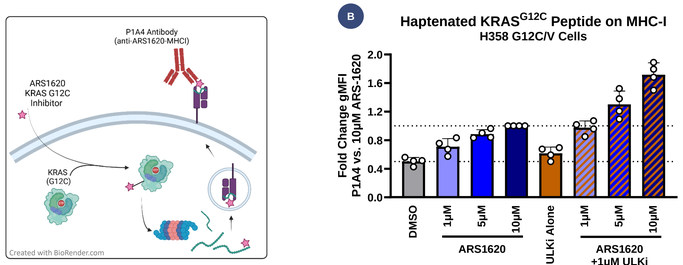

Figure 5. ULK1/2 inhibition increases KRAS neoantigen presentation.
A. Assay schematic adapted from [5]. P1A4 is an IgG1 antibody specific to the ARS1620 haptenated KRAS G12C peptide and can be used to measure KRAS^{G12C}-mutant neo-antigen presentation as previously described [5]. **B.** Inhibition of ULK1/2 increases neoantigen presentation 1.7-fold over cells treated with the highest concentration of KRAS inhibitor alone in H358 G12C/V engineered cells.

ULK1/2 Inhibition Increases Antigen Presentation on MC38-OVA Cancer Cells Enhancing CD8⁺ T Cell Cytotoxicity

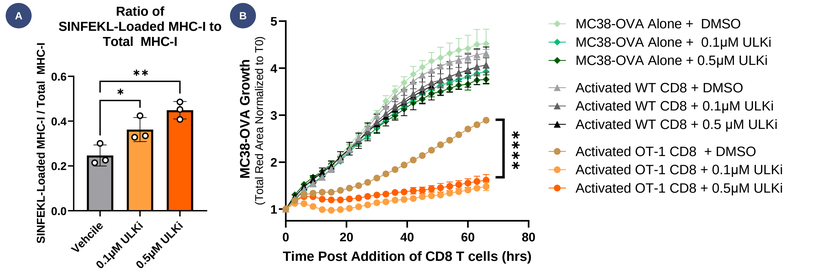

Figure 6. ULK1/2 inhibition increases antigen presentation in MC38-OVA cells, enhancing OT-1 T-cell mediated control of MC38-OVA growth.
A. ULK1/2 inhibition concentration-dependently increases the ratio of SINFEKL-loaded MHC-I to total MHC-I on mCherry MC38-OVA expressing cells. **B.** ULK1/2 inhibition has no impact on MC38-OVA growth in the absence of T cells or in the presence of T cells that do not express a TCR that recognizes the OVA antigen (WT T cells). However, ULK1/2 inhibition significantly enhances OT-1 mediated killing of mCherry expressing MC38-OVA cells compared to DMSO treated cells. Significance was determined by a one-way ANOVA *<0.05, **< 0.01, ***<0.001, ****<0.0001.

ULK1/2 Inhibition Increases NY-ESO TCR CD8⁺ T Cell Cytotoxicity

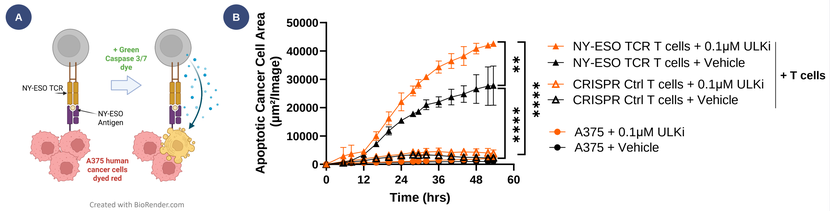

Figure 7. ULK1/2 inhibition enhances antigen-specific CD8+ T cell mediated cytotoxicity of human A375 cancer cells.
A. Assay schematic. Human CD8⁺ T cells were engineered to express the NY-ESO TCR that recognizes the NY-ESO antigen expressed by A375 cells. Cancer cell death was measured as the total area of cells that were both red (cancer cells) and green (caspase 3/7 positive) on the Sartorius Incucyte live-cell imaging system. **B.** Inhibition of ULK1/2 does not increase A375 cell death in the absence of T cells or when cultured with CRISPR control T cells. T cells expressing NY-ESO TCR treated with DMSO show significantly increased killing of A375 cancer cells compared to DMSO-treated CRISPR Control T cells. Inhibition of ULK1/2 significantly increases the ability of NY-ESO TCR T cells to kill A375 cancer cells compared to T cells expressing NY-ESO TCR treated with DMSO. Significance was determined by a one-way ANOVA, **< 0.01, ****<0.0001.

Conclusions

- Inhibition of ULK1/2 decreases basal autophagy levels in cancer cell spheroids
- Reduction of autophagy via ULK1/2 inhibition results in increased MHC-I surface expression and reduced colocalization of MHC-I with LC3B and LAMP1
- ULK1/2 inhibition increases the levels of peptide-loaded MHC-I in both human and murine cells
- ULK1/2 inhibition increases antigen-specific CD8⁺ T cell cytotoxicity in both murine and human *in vitro* systems
- These data provide a rationale for targeting ULK1/2 in tumors as inhibition of ULK1/2 enhances antigen presentation, resulting in enhanced antigen-specific T cell-mediated killing of cancer cells

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[1] Qian & Yang (2006) *Oncotarget;* [2] Shi et al. (2020) *J Cell Bio;* [3] Yamamoto et al (2020) *Nature;* [4] Herhaus et al (2024) *Cell;* [5] Zhang et al. (2022) *Cancer cell*