

# Preclinical studies with DCC-3116, an ULK kinase inhibitor designed to inhibit autophagy as a potential strategy to address mutant RAS cancers

Bryan D. Smith, Lakshminarayana Vogeti, Anu Gupta, Jarnail Singh, Gada Al-Ani, Stacie L. Bulfer, Timothy M. Caldwell, Mary J. Timson, Subha Vogeti, Yu Mi Ahn, Hikmat Al-Hashimi, Chase K. Crawley, Cale L. Heiniger, Cynthia B. Leary, Justin T. Proto, Quanrong Shen, Hanumaiah Teliikepalli, Karen Yates, Wei-Ping Lu, and Daniel L. Flynn

Abstract  
B129

Deciphera Pharmaceuticals, LLC, Waltham, MA

## INTRODUCTION

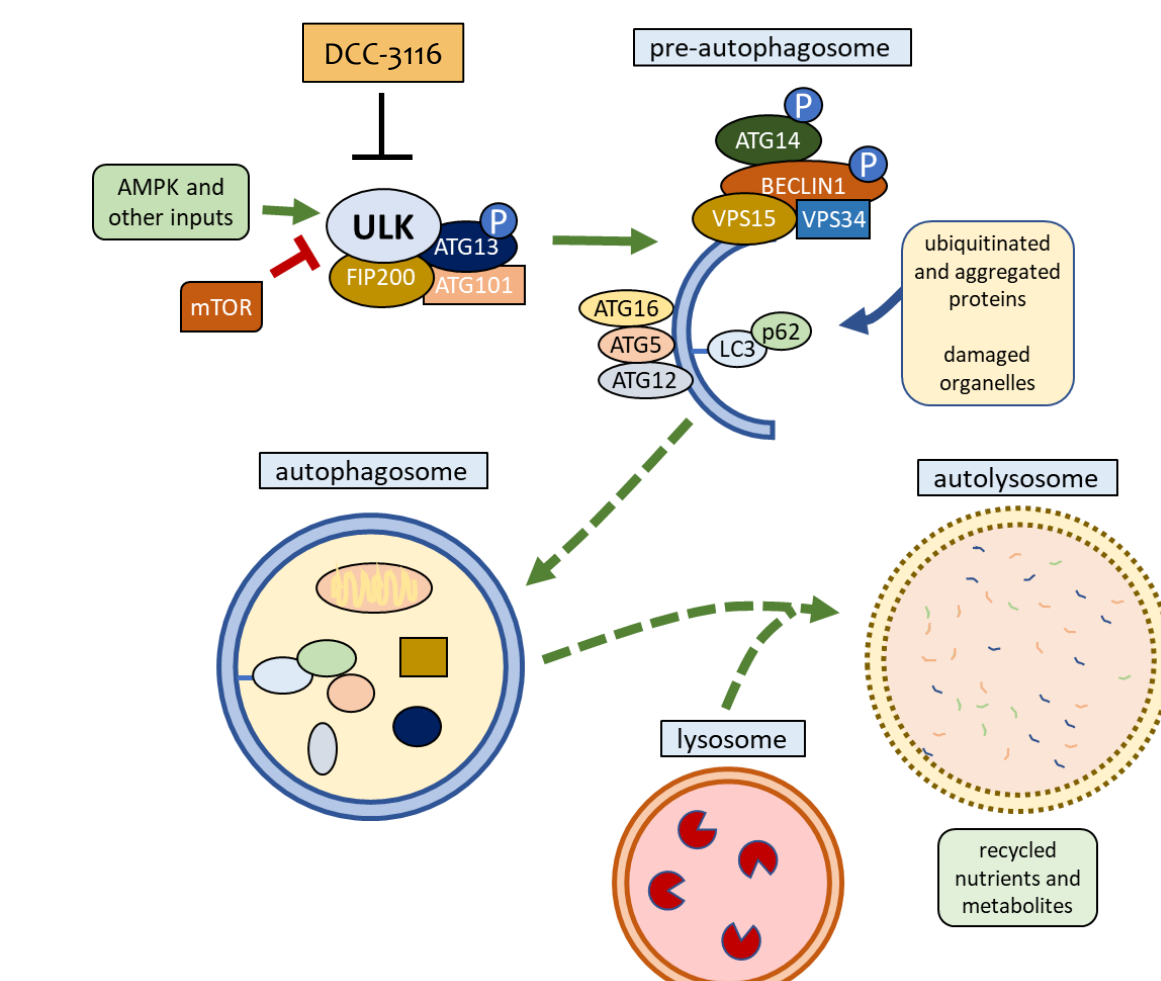
- Cancer cells activate autophagy, a catabolic process to resupply nutrients and recycle damaged organelles, in order to survive stresses such as limited nutrients and hypoxia, or chemotherapy treatments.
- RAS mutant cancers, in particular, have been found to require autophagy for tumor growth and survival.<sup>1,2</sup> Treating RAS mutant tumors with inhibitors of the downstream MAPK pathway has been largely unsuccessful, as these drugs have been shown to further stimulate autophagy, allowing for tumor cell survival.<sup>3,4</sup> Inhibiting autophagy in combination with MAPK pathway inhibition may represent a possible new treatment paradigm for RAS mutant cancers.
- Proof-of-concept for this strategy was obtained in cancer models and in a RAS mutant pancreatic cancer patient by blocking autophagy with derivatives of chloroquine, in combination with MAPK inhibitors.<sup>3,4</sup>
- ULK1/2 kinases initiate autophagy and provide the potential for a targeted approach for selectively inhibiting autophagy in RAS mutant cancers. Herein, we describe preclinical studies with the ULK kinase inhibitor DCC-3116, designed as a potential inhibitor of autophagy in RAS mutant cancers.

## METHODS

*In vitro* kinase assays were performed using cellular levels of ATP (1 mM) and a peptide substrate. In cell assays, ULK activity was assessed using an ELISA for phosphorylated ATG13 (a cellular ULK substrate). Autophagosome formation was measured using the dye, Cyto-ID. Autophagic flux was assessed using cells expressing the autophagy protein LC3 fused to luciferase. The synergy of DCC-3116 in combination with MAPK inhibitors was assessed in 2D or 3D cell growth assays. Xenograft models were used to assess pharmacokinetics (PK) and pharmacodynamics (PD), as well as efficacy *in vivo*.

## ULK KINASE: INITIATING FACTOR FOR AUTOPHAGY

Figure 1. Autophagy Pathway



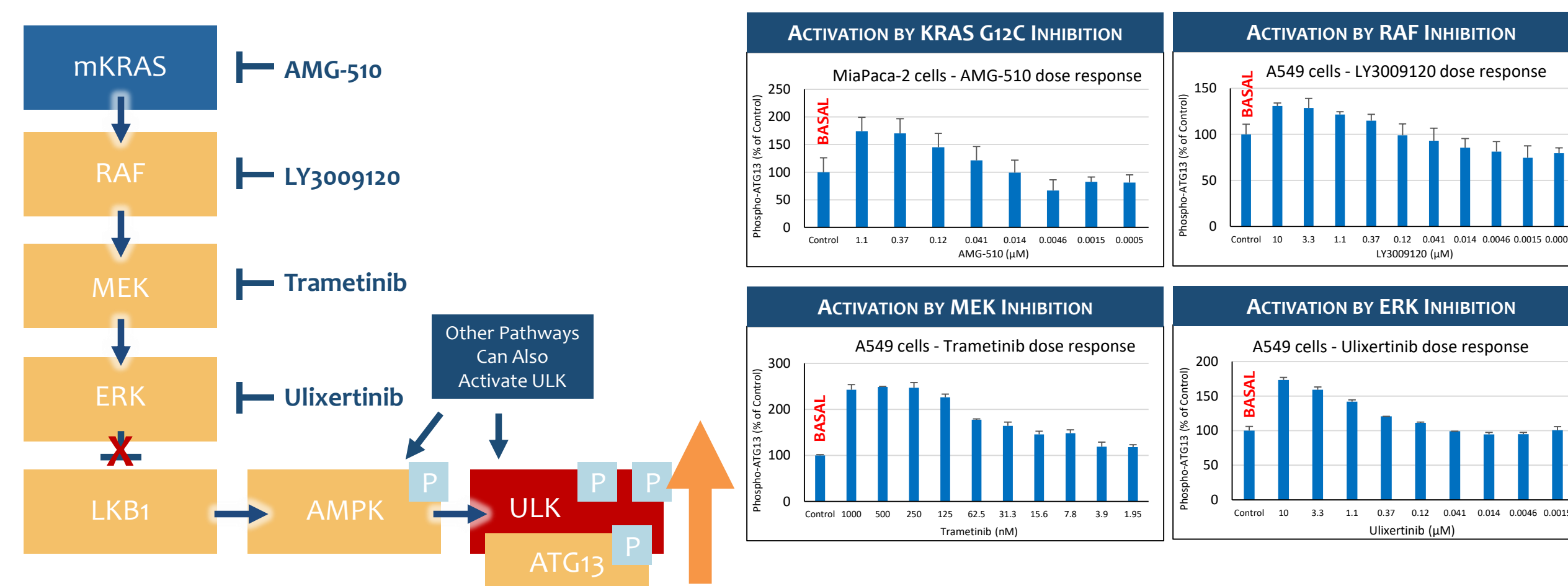
- ULK1/2 kinases initiate autophagy by phosphorylating and activating other autophagy pathway proteins (e.g. ATG13, BECLIN1, and ATG14)
- Damaged proteins, organelles, and other cargo are targeted to, and enveloped by, autophagosomes
- Fusion of autophagosomes and lysosomes allows for breakdown and recycling of metabolic precursors and nutrients

## RESULTS

### Autophagy is a Compensatory Survival Mechanism in MAPK Pathway Inhibitor-treated RAS Mutant Cancers

- Treatment of a RAS mutant cancer cell line with inhibitors of the MAPK pathway (*i.e.* RAS, RAF, MEK, or ERK inhibitors) leads to activation of ULK kinase and phosphorylation of downstream autophagy protein substrates

Figure 2. MAPK inhibition leads to increased ATG13 phosphorylation



### DCC-3116 is a Potent & Selective ULK Kinase Inhibitor Designed to Inhibit Autophagy

Potent ( $IC_{50}$  at 1 mM ATP)

- ULK1 4.7 nM; ULK2 36 nM
- Tight-binding inhibitor with residency time > 7 hours

Highly Selective

- No off-target kinases within 30-fold of ULK1
- Only 6 kinases within 100-fold, inclusive of ULK2

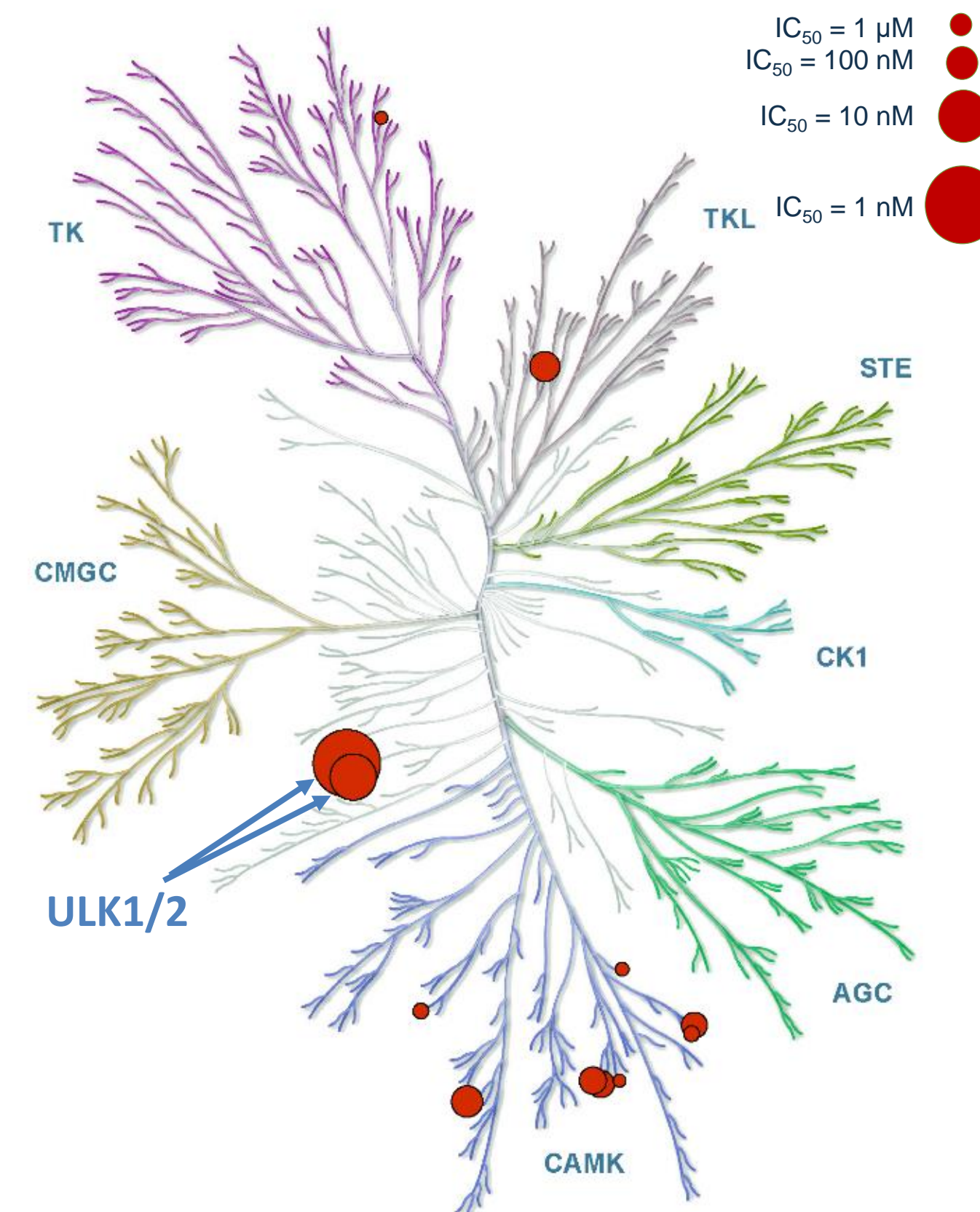
Designed to Avoid CNS Exposure

- Low Brain:Plasma ratio (4%) to avoid inhibition of CNS autophagy

Optimized Pharmaceutical Properties

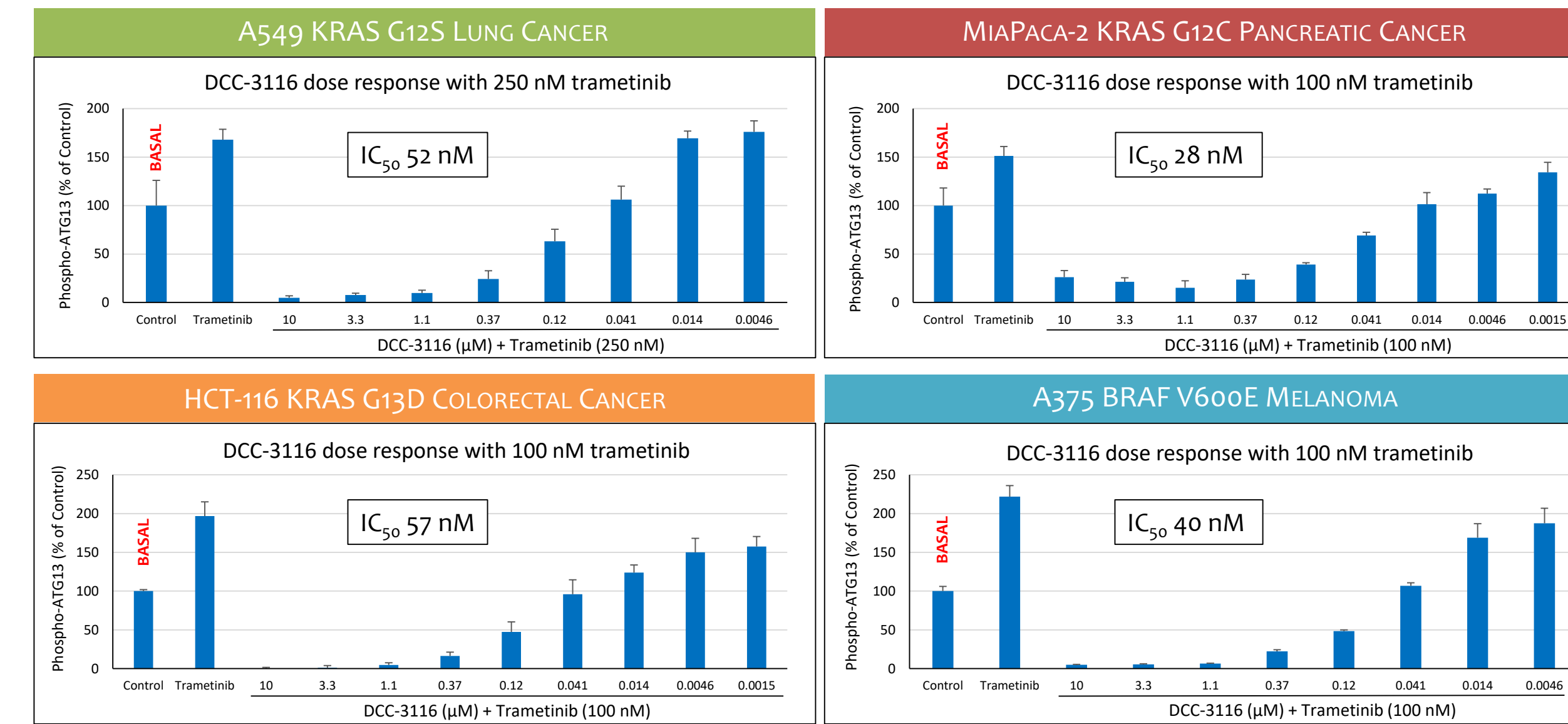
- High solubility and oral bioavailability
- Plasma Free Fraction > 10%
- CYP1A2, 2C9, 2C19, 2D6, 3A4 and hERG  $IC_{50}$  values >20  $\mu$ M

Figure 3. DCC-3116 Kinome Tree

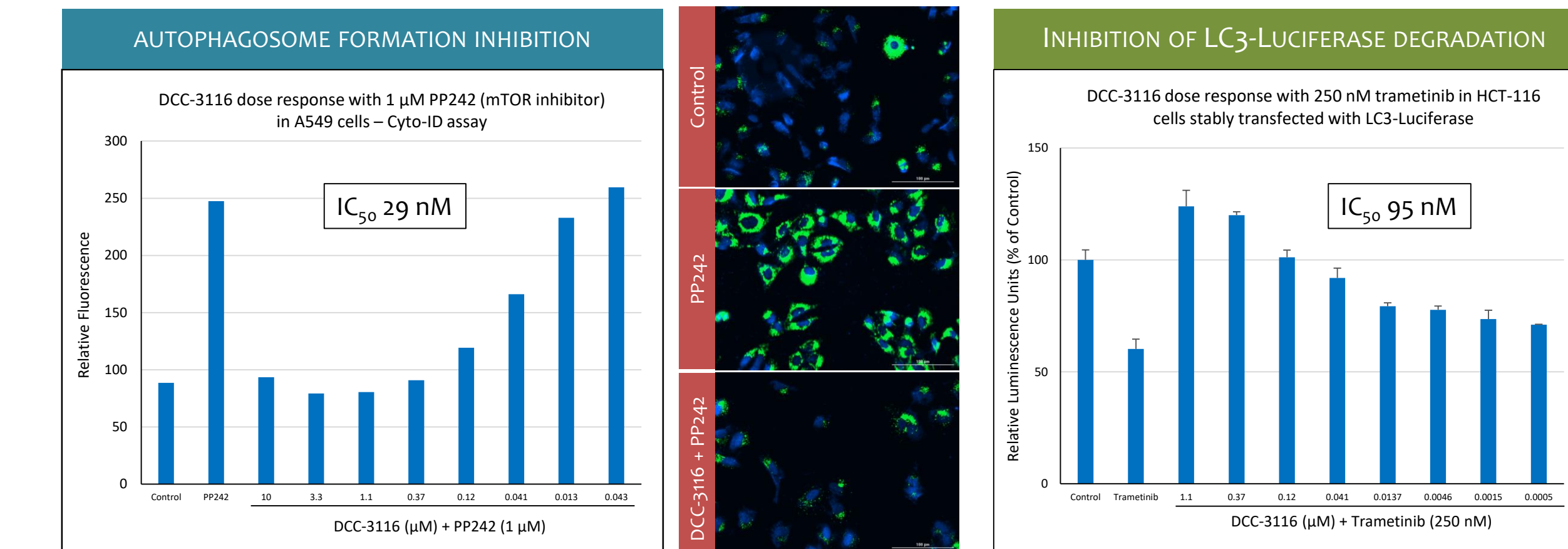


### DCC-3116 is a Potent Inhibitor of ULK Kinase and Autophagy in Cellular Assays

Figure 4. DCC-3116 Inhibits Both Basal and Trametinib-induced Phosphorylation of ULK Substrate ATG13 in RAS- and BRAF-mutant Cell Lines

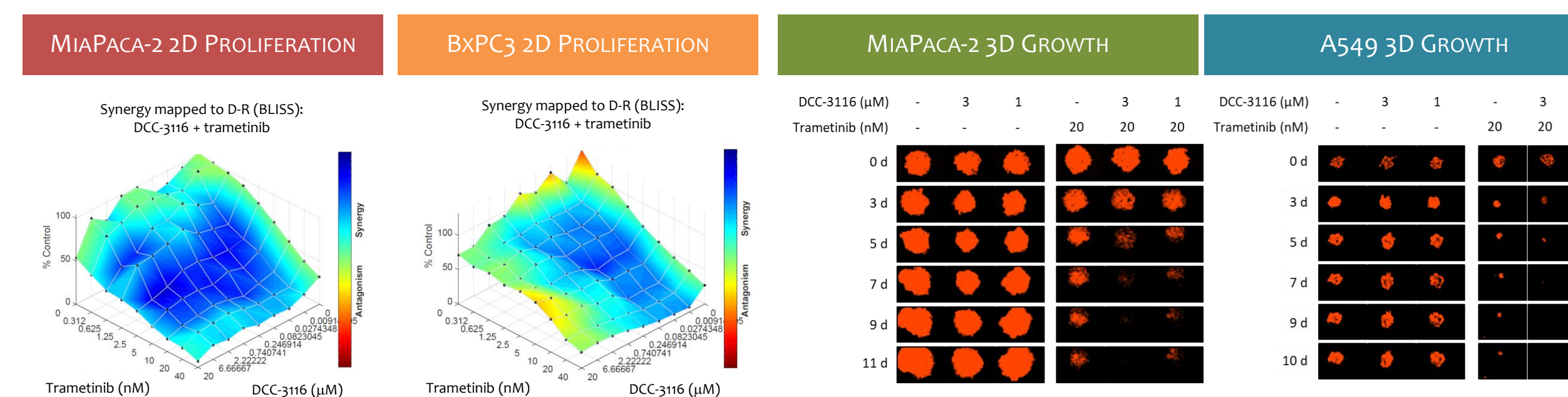


### Figure 5. DCC-3116 Inhibits Autophagosome Formation and Autophagic Flux of LC3



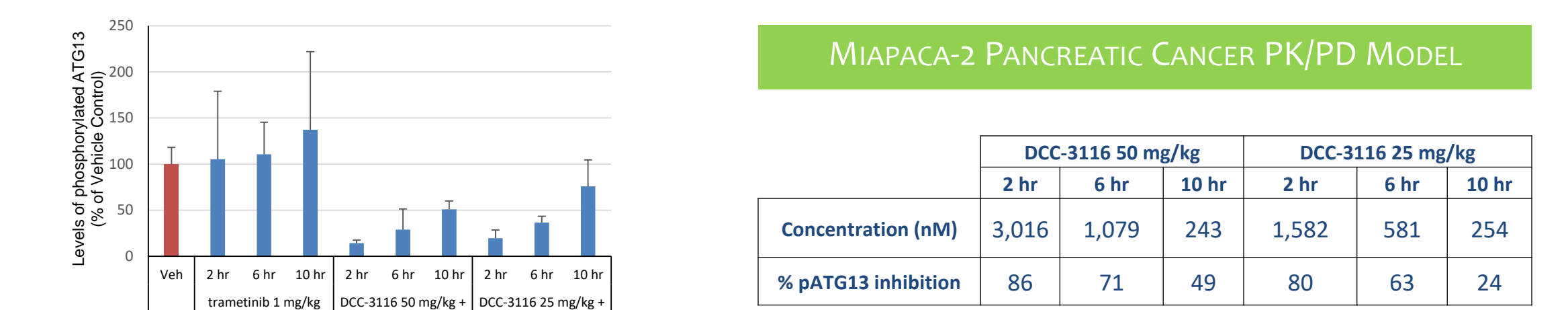
### DCC-3116 Synergizes with MAPK Inhibitors in 2D and 3D Cellular Growth Assays

Figure 6. DCC-3116 Exhibits Synergy with Trametinib in Inhibiting Cell Growth of RAS- or RAF-mutant Cancer Cells

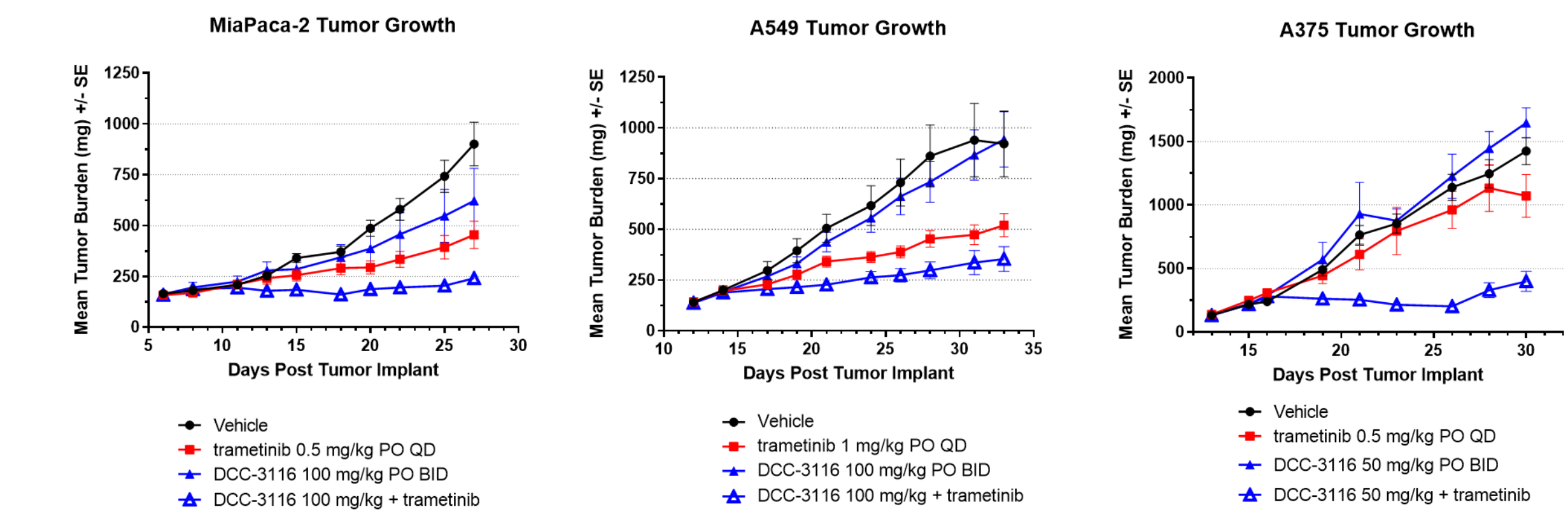


### DCC-3116 Inhibited ULK Kinase in PK/PD Models and Inhibited Tumor Growth in Combination with MAPK Inhibitors in Mouse Xenograft Models

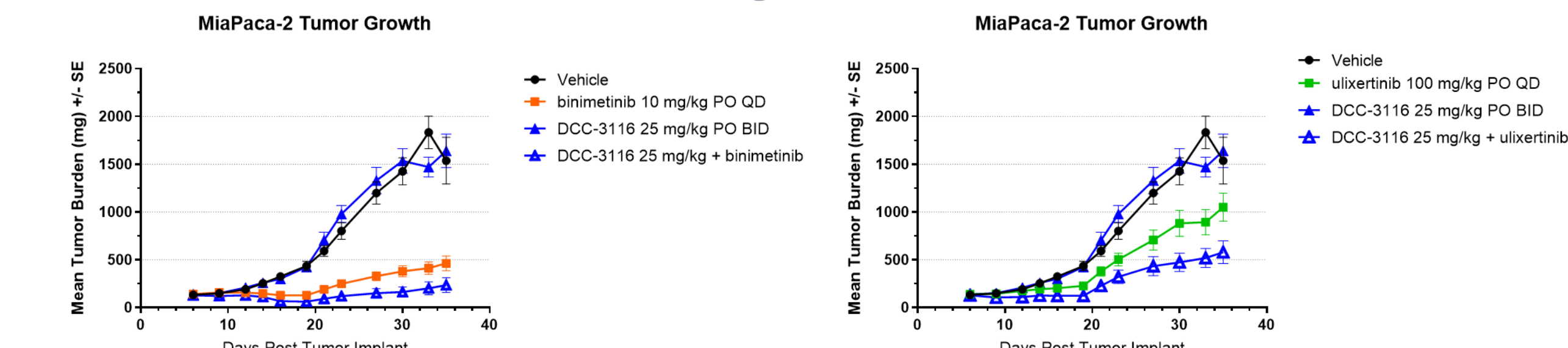
Figure 7. DCC-3116 Inhibited ATG13 Phosphorylation *in vivo* in a PK/PD Model



### Figure 8. DCC-3116, in Combination with Trametinib, Inhibited Pancreatic, Lung, and Melanoma Xenograft Tumor Growth



### Figure 9. DCC-3116, in Combination with MEK Inhibitor Binimetinib or ERK Inhibitor Ulixertinib, Decreased Pancreatic Xenograft Tumor Growth



## CONCLUSIONS

- RAS cancers have high basal autophagy, and induce greater autophagy in response to drug treatments
- ULK kinase inhibitors represent a differentiated approach to autophagy inhibition, and a first-in-class opportunity for a new therapeutic modality in RAS- and RAF-mutant cancers
- DCC-3116 is a potent, selective, and tight-binding inhibitor of ULK kinase
- DCC-3116 inhibited phosphorylation of the ULK substrate ATG13 in cancer cells, and exhibited synergy *in vitro* in combination with MAPK inhibitors in inhibiting cancer cell growth
- Oral doses of DCC-3116 led to sustained inhibition of ATG13 phosphorylation *in vivo*
- In combination with MAPK inhibitors, DCC-3116 exhibited synergy in tumor growth inhibition in mouse models
- Selectively blocking autophagy via inhibition of ULK1/2 kinases, in combination with MAPK pathway inhibition, is a promising therapeutic approach for RAS mutant cancers.
- DCC-3116 warrants further study as an inhibitor of autophagy, and has been selected as a candidate for potential clinical development in the treatment of RAS mutant cancers

## Acknowledgments

We would like to acknowledge Waheed Bhatti, Patrick Kearney, Kevin Roesch, Michael Kaufman, Arun Mandagere, Randy McCall, Steve Wilson, and Heather Vital for their work on this project and input and review of this presentation.

## References

- Guo *et al.*, Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis. *Genes and Dev.* 2011; 25: 460
- Yang *et al.*, Pancreatic cancers require autophagy for tumor growth. *Genes and Dev.* 2011; 25: 717
- Bryant *et al.*, Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer. *Nature Med.* 2019; 25: 628
- Kinsey *et al.*, Protective autophagy elicited by RAF → MEK → ERK inhibition suggests a treatment strategy for RAS-driven cancers. *Nature Med.* 2019; 25: 620