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

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INTRODUCTION

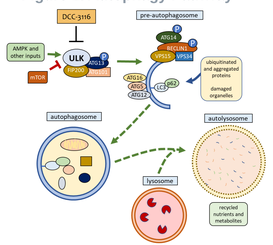
- Cancer cells activate autophagy, a catabolic process to resupply nutrients and recycle damaged organelles, in order to survive stresses such as limited nutrients and hypoxia, or chemotherapy treatments.
- RAS mutant cancers, in particular, have been found to require autophagy for tumor growth and survival.[1,2] 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.[3,4] 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.[3,4]
- 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



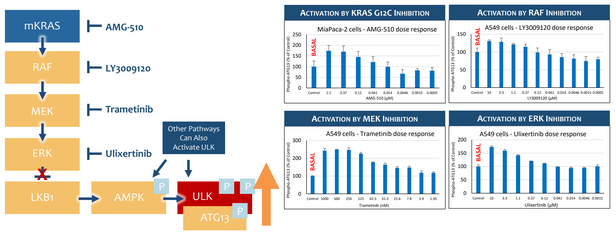
- ULK1/2 kinases initiate autophagy by phosphorylating and activating other autophagy pathway proteins (e.g. ATG13, BECLIN1, and ATG14)
- Damaged proteins, organelles, and other cargo are targeted to, and enveloped by, autophagosomes
- Fusion of autophagosomes and lysosomes allows for breakdown and recycling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

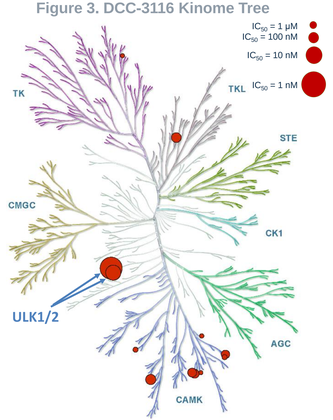
Designed to Avoid CNS Exposure

- 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 µM

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

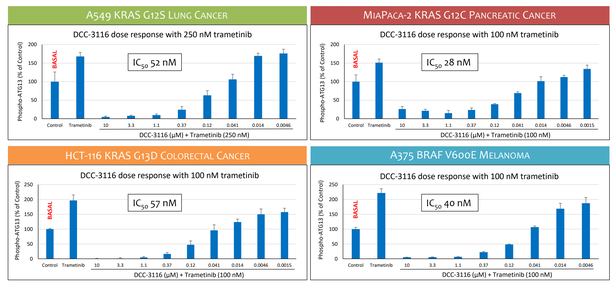
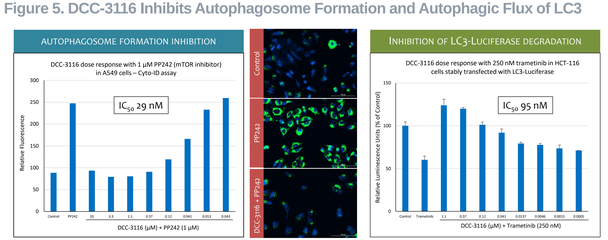
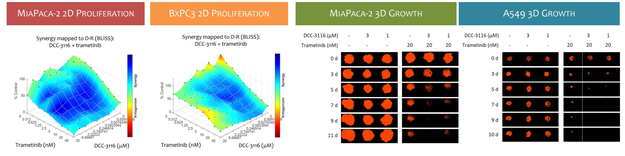


Figure 5. DCC-3116 Inhibits Autophagosome Formation and Autophagic Flux of LC3



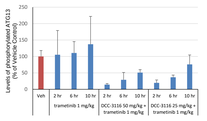
DCC-3116 Synergizes with MAPK Inhibitors in 2D and 3D Cellular Growth Assays

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



MiaPaca-2 Pancreatic Cancer PK/PD Model

	DCC-3116 50 mg/kg			DCC-3116 25 mg/kg		
	2 hr	6 hr	10 hr	2 hr	6 hr	10 hr
Concentration (nM)	3,016	1,079	243	1,582	581	254
% pATG13 inhibition	86	71	49	80	63	24

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

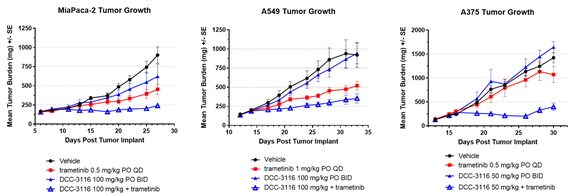
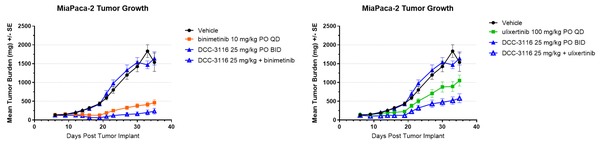


Figure 9. DCC-3116, in Combination with MEK Inhibitor Binimetinib or ERK Inhibitor Ulixertinib, Decreased Pancreatic Xenograft Tumor Growth



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**

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