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


Abstract

INTRODUCTION

• Cancer cells activate autophagy, a catabolic process to repurpose 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.1–4 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.5,6 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 kinase inhibitors initiate autophagy and provide 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 ATG3 (a ULK substrate). Autophosphorylation was measured using the DUOSet (DuoSet™ Dual-Luciferase®). Phosphorylation of downstream autophagy protein substrates 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 kinase inhibitors initiate autophagy by phosphorylating and activating other autophagy pathway proteins (e.g., ATG1, BECN1, and ATG14)
- Damaged proteins, organelles, and other cargos 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

Figure 2. MAPK inhibition leads to increased ATG13 phosphorylation

- 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.

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

- DCC-3116 is a potent, selective, and tight inhibitor of ULK kinase and autophagy.
- DCC-3116 inhibited phosphorylation of the ULK substrate ATG13 in cancer cells, and exhibited synergy with DCC-3116 dose response with 100 nM trametinib in HCT116 cells stably transfected with LC3. DCC-3116 inhibited phosphorylation of the ULK substrate ATG13 in cancer cells, and exhibited synergy with DCC-3116 dose response with 250 nM trametinib in HCT116 cells stably transfected with LC3.

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

Figure 3. DCC-3116 Kinase Tree

- Potent (IC50 = 1 nM ATP)
- ULK1: 4.7 nM, ULK2: 35 nM
- Tight-binding inhibitor with resiliency time > 7 hours

Highly Selective

- No off-target kinases within 20-fold of ULK1 and ULK2
- Only 6 kinases within 10-fold, inclusive of ULK2

Designed to Avoid CNS Exposure

- Low Brain/Plasma ratio (10-fold) to avoid inhibition of CNS autophagy

Optimized Pharmaceutical Properties

- High stability and oral bioavailability (Pharmacokinetic Fraction > 10%)
- CYP1A2 2D, CYP1B1, 3D6, 3A4, and HER2 IC50 values >20 µM

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

- DCC-3116 dose response with 100 nM trametinib in HCT116 cells stably transfected with LC3
- DCC-3116 dose response with 250 nM trametinib in HCT116 cells stably transfected with LC3

CONCLUSIONS

- RAS cancers have high basal autophagy, and lack greater autophagy in response to drug treatments
- ULK kinase inhibitors represent a differentiated approach to autophagy inhibition, as a tool in drug classes and combination strategies for RAS mutant cancers
- DCC-3116 is a potent, selective, and tight-binding inhibitor of ULK kinase
- DCC-3116 inhibited phosphorylation of several downstream autophagy protein substrates, and exhibited synergy, in combination with MAPK inhibitors in inhibiting cancer cell growth
- Combining DCC-3116 with MEK, RAF, or EGFR inhibitors increased inhibition in mouse models
- Inhibiting autophagy via inhibition of ULK kinase activity, 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

References