Acetyl-CoA carboxylase inhibition by ND646 reduces fatty acid synthesis and inhibits cell proliferation in human non-small cell lung cancer cells

**ABSTRACT**

Continuous de novo lipogenesis is a common feature of tumor cells and is required to meet the bioenergetic and biosynthetic demands of a growing tumor. The expressions and activity of the fatty acid synthetase enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase are up-regulated in many types of cancer, where lipogenesis is essential for proliferation and tumor cell survival. These enzymes are therefore attractive targets for anti-neoplastic intervention. Inhibition of ACC results in inhibition of fatty acid synthesis (FAOS) and stimulation of fatty acid oxidation in cultured cells and in animals and has been shown to reduce cancer cell growth in vitro. Our efforts to develop novel ACC inhibitors have focused on the substrate demethylation site of the thioesterase domain (TE) domain of the enzyme. This site also binds the inhibitory phosphopeptide of ACC phosphorylated by AMPK and the fungal metabolite Schroepen A, two of which suppress demethylation and inhibit mammalian ACC activity. Using state-of-the-art structure-based drug design and crystal structures of human ACC 9C3 domain, we have identified a unique series of aliphatic inhibitors with low nanomolar potency, represented by ND646, that bind to the Schroepen binding site, exhibit potent and selective activity in vitro and in vivo and show anti-neoplastic properties in cultured human non-small cell lung cancer (NSCLC) cells. The antiproliferative action of ND646 in NSCLC cell lines A458, H460, and H522 was enhanced in depleted media and abrogated by supplementation with palmitic acid, suggesting that the antiproliferative effect of ND646 is induced by depletion of cellular fatty acids. Current efforts are focused on determining if the cell growth inhibitory action of ND646 also possesses apoptotic properties and on assessing the antiproliferative action of ND646 in preclinical models of lung cancer. During the course of these studies, we also discovered that interaction of ND646 with the ACC phosphopeptide binding site markedly reduced the phosphorylation state of the enzyme in A549 cells, as determined by western blot analysis using antiproteinNphos (pACC) antibody. Furthermore, in the H460 cells, the reduction in ACC by the related analogs ND660 (EC50 = 16 μM), which closely paralleled inhibition of FASm (EC50 = 42 μM), resulted in complete loss of pACC at high doses. These observations, that were corroborated in hepatic tissue isolated from both ND646 and ND660 treated mice and in tumors of genetically engineered mouse models of lung cancer dosed with ND646, suggest that assessment of pACC could provide a sensitive biomarker of target engagement for ACC inhibitors interfering in this region of the enzyme. Additional efforts are focused on examining the genetic contexts and optimal therapeutic combinations such that ACC inhibitors may find the greatest clinical utility as anti-neoplastic agents.

**FIGURE 1:** Acetyl-CoA Carboxylase: Master regulator of de novo fatty acid synthesis and oxidation

**FIGURE 2:** ACC: Emerging Tumor Metabolism Target: Tumors Up-regulate ACC to Fuel Cell Growth

**FIGURE 3:** ND-646: Good Drug-like Profile with Significant Tumor Exposure Noted

**FIGURE 4:** ND-646 Blocks Anti-pACC Immunohistochemical Antibody Recognition in HepG2 Cells and Inhibits Fatty Acid Synthesis

**FIGURE 5:** Enzyme Occupancy Assay: ND-646 Occupies ACC Phosphorylation Site, Allowing Use of Anti-phospho ACC Antibody as Surrogate for Enzyme Inhibition

**FIGURE 6:** ND646 inhibits proliferation in multiple human non-small cell lung cancer cell lines

**FIGURE 7:** ND646 Exhibits Greater Proliferative Inhibition in LKB1/STK11 Deficient Human NSCLC Cell Lines

**FIGURE 8:** ND646 induces Apoptosis in Human NSCLC Cell Lines

**FIGURE 9:** Anti-proliferative Effects of ND646 are Enhanced in Depleted Serum

**FIGURE 10:** Anti-proliferative Effects of ND646 are Suppressed by Exogenous Palmitic Acid

**FIGURE 11:** ACC Phosphorylation State is a Potential Biomarker for In Vivo Target Engagement in Tumor Tissue

**FIGURE 12:** ND646 Inhibits Growth of A549 Human NSCLC Subcutaneous Tumors in vivo

**SUMMARY**

- Proof-of-concept tool compound ND646 is a potent and specific allosteric inhibitor of ACC1/2
- Inhibits fatty acid synthesis in HepG2 cells
- ND646 in cytotoxic against human non-small cell lung cancer cells and its effects are enhanced in depleted serum and rescued with palmitic acid
- ND646 is modestly more potent in LKB1 deficient NSCLC cell lines, in line with the LKB1 dependent regulation of ACC
- Anti-phospho ACC(P Thr408) antibody is an excellent biomarker for ACC inhibition in vitro and in vivo
- ND646 significantly suppressed the in vivo growth of A549 subcutaneous tumors

**ONGOING STUDIES**

- Further analysis of the cytostatic properties of ND646
- Optimal therapeutic combinations with ND646
- Evaluation of anti-neoplastic properties of ND646 in genetically engineered mouse models of non-small cell lung cancer

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