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

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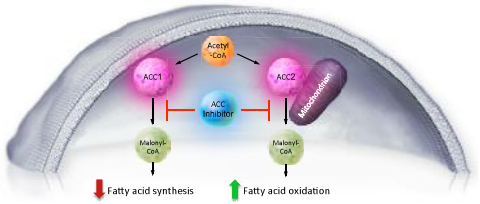
ABSTRACT

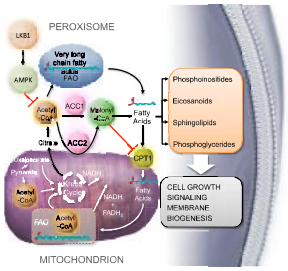
Continuous *de novo* lipogenesis is a common feature of tumor cells and is required to meet the bioenergetic and biosynthetic demands of a growing tumor. The expression and activity of the fatty acid synthetic enzymes acetyl-CoA carboxylase (ACC) and fatty acid synthase are up-regulated in many types 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 (FASyn) 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 subunit dimerization site of the biotin carboxylase (BC) domain of the enzyme. This site also binds the inhibitory phosphopeptide of ACC phosphorylated by AMPK and the fungal metabolite Soraphen A, both of which suppress dimerization and inhibit enzymatic activity. Using state-of-the-art structure-based drug design and crystal structures of human ACC2 BC domain, we have identified a unique series of allosteric inhibitors with low nanomolar potency, represented by ND646, that bind to the Soraphen 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 anti-proliferative action of ND646 in NSCLC cell lines A549, H460, and H358 was enhanced in delipidated media and attenuated by supplementation with palmitic acid, suggesting that the anti-proliferative effect of ND646 is induced by depletion of cellular fatty acids. Current efforts are focused on determining if the cell growth inhibitory actions of ND646 also possess apoptotic properties and on assessing the anti-neoplastic activity 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 anti-phosphoACC (pACC) antibody. Furthermore, in HepG2 cells, the reduction in pACC by the related analog ND630 (EC50 = 66 nM), which closely paralleled inhibition of FASyn (EC50 = 42 nM), resulted in complete loss of pACC at high doses. These observations, that were corroborated in hepatic tissue isolated from both ND646 and ND630 treated mice and in situ 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 interacting 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 co-A Carboxylase: Master regulator of de-novo fatty acid synthesis and oxidation



- Beneficial effects on denovo lipid synthesis, fibrosis and limits lipids necessary to sustain proliferation
- Nimbus: first series of small molecule **allosteric** inhibitors successfully targeting BC domain

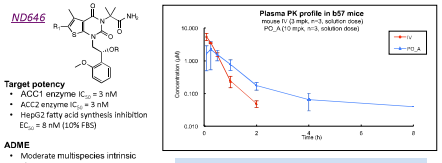
FIGURE 2: ACC: Emerging Tumor Metabolism Target: *Tumors Up-regulate ACC to Fuel Cell Growth*



ACC Oncology Validation

- **NSCLC: Targeted opportunity** via LKB1-AMPK-ACC axis
  - LKB1 inactivated in Peutz–Jeghers syndrome, NSCLC and cervical carcinoma
- **Breast cancer:** ACC1 knock down causes apoptosis in tumor cells
  - BRCA1 stabilizes inactive phosphorylated form of ACC
  - BRCA1 also implicated in prostate cancer
- **RCC: ACC overexpression**
  - associated with worse survival in clear cell Renal Cell Carcinoma

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



Target potency
- ACC1 enzyme IC_{50} = 3 nM
- ACC2 enzyme IC_{50} = 3 nM
- HepG2 fatty acid synthesis inhibition EC_{50} = 8 nM (10% FBS)

ADME
- Moderate multispecies intrinsic clearance (human, mouse, rat, dog, monkey)
- High solubility (>300 µM)
- CYP inhibition > 10 µM
- Protein binding across species (94-99%)

Mouse PK (10 mg/kg, PO)
- 29%F, Cmax 2.2 µM, Cl 35 mL/min/kg, AUC 2.5 µM.hr, T1/2 = 2.1h, Vdss 0.7 L/Kg

ND-646 Tissue Exposure in A549 Mouse Tumor Model after 50 mpk oral dose

tumor inject. route	Plasma 1hr	Plasma 8hr	Tumor tissue 1hr	Tumor tissue 8hr	Lung tissue 1hr	Lung tissue 8hr
sc	23 uM (+/-3)	0.2uM (+/-0.2)	15 uM (+/- 11)	0.9 uM (+/-0.7)	21 uM (+/-17)	0.2 uM (+/-0.1)

FIGURE 4: ND-646 Blocks Anti-pACC$^{S79/222}$ Antibody Recognition in HepG2 Cells and Inhibits Fatty Acid Synthesis

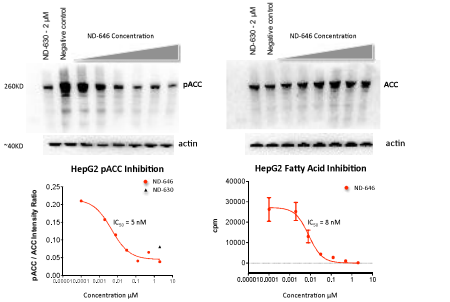
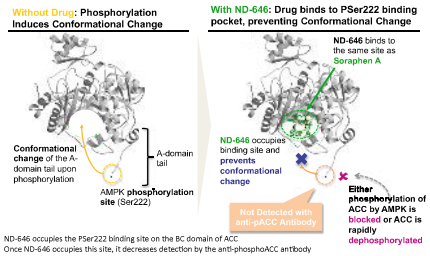


FIGURE 5: Enzyme Occupancy Assay: ND-646 Occupies ACC Phosphorylation Site, Allowing Use of Anti-Phosho ACC Antibody as Surrogate for Enzyme Inhibition



- ND-646 occupies the PSer222 binding site on the BC domain of ACC
- Once ND-646 occupies this site, it decreases detection by the anti-phosphoACC antibody
- This allows for the use of anti-phoshoACC antibody as a surrogate for enzyme inhibition in tissues

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

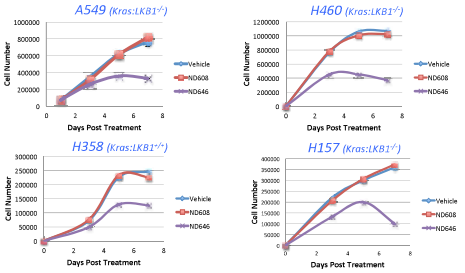
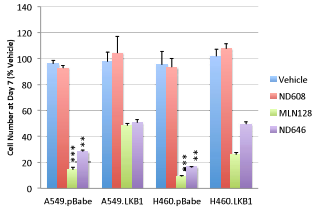
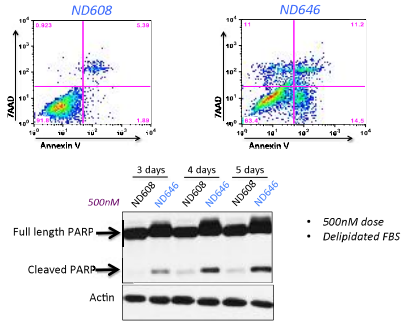


FIGURE 7: ND646 Exhibits Greater Proliferative Inhibition in LKB1/STK11 Deficient Human NSCLC Cell Lines



- A549.pBabe and H460.pBabe cells are LKB1 deficient, A549.LKB1 and H460.LKB1 cells have been reconstituted with human LKB1
- 500nM dose, cell counts taken 7 days post treatment
- ND608 is 1000x less active enantiomer (distomer)
- MLN128 is an ATP competitive mTOR kinase inhibitor

FIGURE 8: ND646 induces Apoptosis in Human NSCLC Cell Lines



- *500nM dose*
- *Delipidated FBS*

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

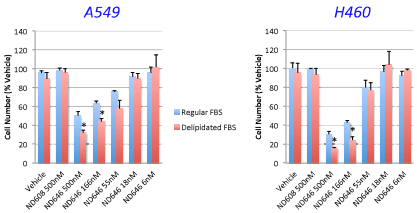
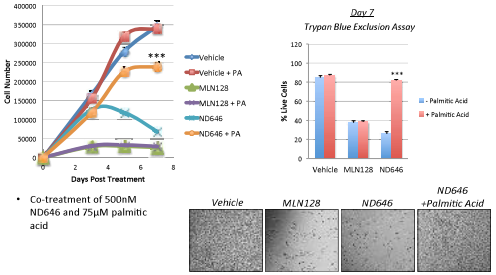
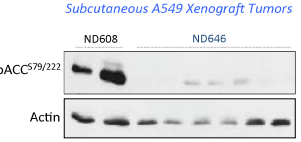


FIGURE 10: Anti-proliferative Effects of ND646 are Suppressed by Exogenous Palmitic Acid



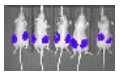
- Co-treatment of 500nM ND646 and 75µM palmitic acid

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



- *nu/nu* mice bearing A549 subcutaneous tumors were administered orally with a single dose of either 50mg/kg ND608 or ND646
- Tumors were harvested after 1hr or 8hrs post dosing and analyzed for ACC phosphorylation using anti-pACC$^{S79/222}$ antibody

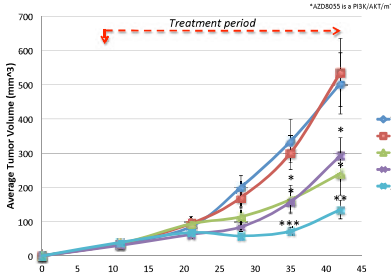
FIGURE 12: ND646 Inhibits Growth of A549 Human NSCLC Subcutaneous Tumors *in vivo*

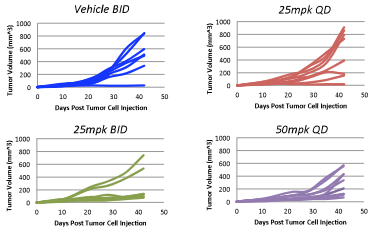


A549.Luc SubQ Tumors

PO dosing 7 days/week in *nu/nu* mice
- Vehicle BID
- ND646 *25mpk* QD
- ND646 *25mpk* BID
- ND646 *50mpk* QD
- AZD8055* *10mpk* BID

*AZD8055 is a PI3K/AKT/mTOR catalytic inhibitor





SUMMARY

- Proof-of-concept tool compound ND646 is a potent and specific allosteric inhibitor of ACC1/2
- ND646 inhibits fatty acid synthesis in HepG2 cells
- ND646 is cytotoxic against human non-small cell lung cancer cells and its effects are enhanced in delipidated 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$^{S79/222}$ 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 cytotoxic 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