Modulation of Lipid Metabolism Through Inhibition of Acetyl-CoA Carboxylase with ND-646 Leads to Potent Inhibition of Breast Cancer Cell Growth *in vitro* and *in vivo*

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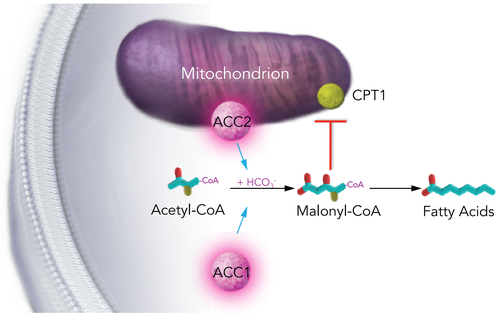
NIMBUS THERAPEUTICS

ABSTRACT

Metabolic attenuation is a promising approach to cancer therapy and rate-limiting steps in key biosynthetic pathways are particularly attractive targets. Many cancer types are dependent on fatty acid synthesis as a primary source of energy and for providing lipids for expansion of cell and nuclear membranes in rapidly proliferating cells. The rate-limiting enzyme in fatty acid synthesis, acetyl-CoA carboxylase (ACC), has been shown to be highly expressed in human breast cancer. ACC is thought to be critical for the growth and survival of cancer cells, especially within a tumor microenvironment where exogenous fatty acids might be limited. Effective therapeutic options for triple negative breast cancer are limited and identification of robust targeted agents without overt toxicity for this indication are especially needed. Dual inhibition of the ACC isozymes, ACC1 and ACC2, results in concomitant inhibition of fatty acid synthesis and stimulation of fatty acid oxidation. We have identified ND-646, a potent, selective, allosteric inhibitor of ACC with broad tissue distribution that binds to the ACC biotin carboxylase domain and potently inhibits the dimerization and enzymatic activity of both ACC1 (IC_{50} = 3.5nM) and ACC2 (IC_{50} = 4.1nM). Profiling the potency of ND-646 *in vitro* in a panel of breast cancer cell lines including triple negative and BRCA1 mutant cell lines demonstrated potent inhibition of cell proliferation with IC50s<100nM. The anti-proliferative effects were more pronounced when cells were cultured in media containing delipidated serum. Daily oral dosing of ND-646 at 25 mg/kg BID,50 mg/kg QD, and 50 mg/kg BID in mice bearing orthotopic triple negative MDA-MB-468 breast cancer xenografts led to tumor growth inhibition of 60-70% that correlated with compound exposure and target engagement in the tumor. Analysis of ND-646 treated tumors demonstrated disruption of tumor tissue architecture and induction of apoptosis and necrosis suggesting a direct effect on cell survival. These results provide further evidence that de novo lipogenesis is an important mediator of breast cancer cell growth and survival, and that selective inhibition of ACC is a viable therapeutic strategy for treatment of breast can-

1. Acetyl-CoA Carboxylase (ACC) Regulates Fatty Acid Synthesis and Oxidation



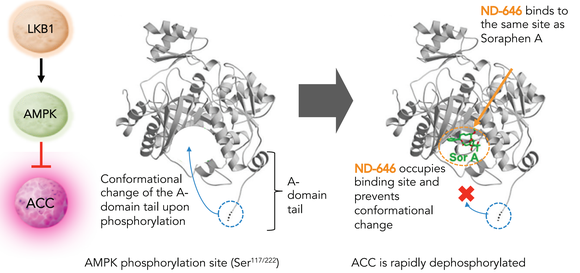
ACC Inhibition Leads to:

- Fatty acid synthesis ↓
- Tissue triglycerides ↓
- Body fat and weight ↓
- Fatty acid oxidation ↑
- Insulin sensitivity ↑

2. ND-646 Mechanism of Action

Without Drug: Phosphorylation induces conformational change

With Drug: Drug binds to pSer^{222} binding pocket, preventing conformational change



- AMPK phosphorylates the A domain tail leading to inactivation of ACC
- ND-646 occupies the pSer$^{117/222}$ binding site on the BC domain of ACC preventing the phosphopeptide from binding and allowing rapid dephosphorylation
- Allows for the use of p-ACC as a target engagement marker in cells and tissues

3. ND-646 Compound Profile

Target potency

ACC1 IC_{50}	= 3.5 nM
ACC2 IC_{50}	= 4.1 nM
HepG2 FASyn EC_{50}	= 8 nM

ADMET

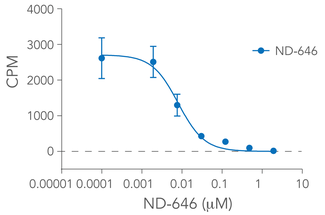
Intrinsic Clearance (human, mouse, rat, dog, cyno)	= Moderate
Aqueous Solubility	>300 µM
Cyp Inhibition	>10 µM
Multispecies Protein Binding	= 94-98%
Eurofins Panel	>1000 fold selective

Mouse PK (50 mg/kg, PO)

AUC_{last}	= 9602 hr*ng/ml
$T_{1/2}$	= 1.5 hr
C_{MAX}	= 11080 ng/ml

Inhibition of Fatty Acid Synthesis in HepG2 cells

^{14}C-acetate Incorporation Assay



4. Breast Cancer Cell Line Characteristics

MDA-MB-468
- Triple negative
- Ductal carcinoma
- Basal origin
- p53 and PTEN mutant

MCF7
- Luminal
- ER+

BT474
- Luminal
- Her2 amplified

SUM149PT
- Triple negative
- BRCA1 mutant
- p53 mutant

MDA-MB-436
- Triple negative
- BRCA1 mutant
- p53 mutant

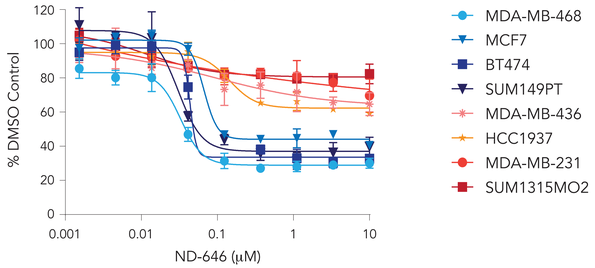
HCC1937
- Triple negative
- BRCA1 mutant
- p53 mutant

MDA-MB-231
- Triple negative
- BRCA1 mutant
- p53 mutant
- Kras mutant

SUM1315MO2
- Triple negative
- BRCA1 mutant
- Myc amplified

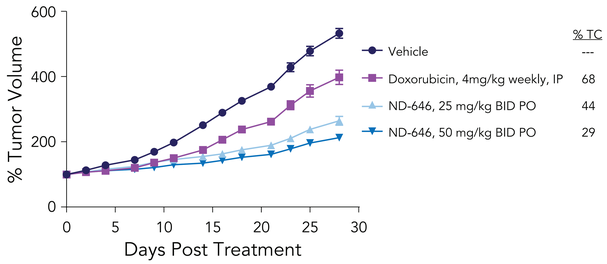
5. ND-646 Inhibits Proliferation of Breast Cancer Cell Lines *in vitro* with Differing Sensitivities

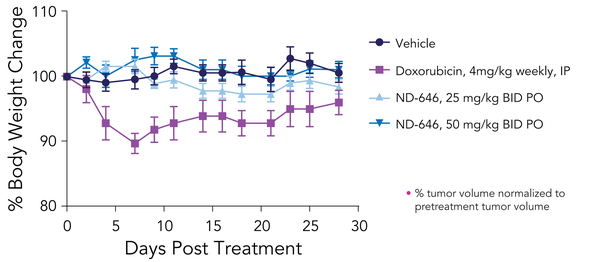
7 Day Cyquant Proliferation Assay



	MDA-MB-468	MCF7	BT474	SUM 149PT	MDA-MB-436	HCC 1937	MDA-MB-231	SUM131 5MO2
IC_{50} (absolute)	38 nM	104 nM	120 nM	44 nM	>10 µM	>10 µM	>10 µM	>10 µM

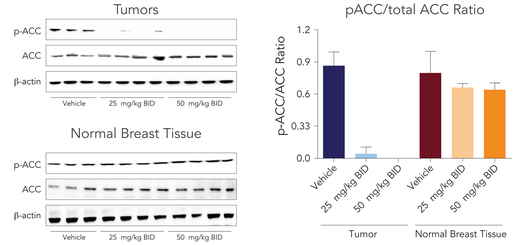
6. ND-646 Inhibits Tumor Growth of MDA-MB-468 Orthotopic Breast Cancer Xenograft Model





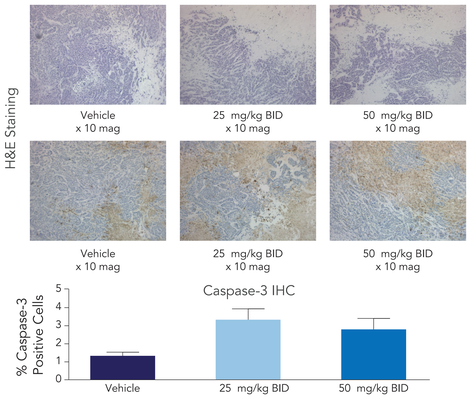
- % tumor volume normalized to pretreatment tumor volume

7. ND-646 Treatment Results in Target Engagement in MDA-MB-468 Tumors but not in Normal Breast Tissue



- Western blot analysis of tumors (human) and normal breast tissue (mouse) harvested 12hrs post last dose of ND-646

8. ND-646 Treatment Causes a Change in MDA-MB-468 Tumor Architecture and an Increase in Apoptosis



CONCLUSIONS

- ND-646 is a potent allosteric inhibitor of ACC1/2 with good drug-like properties
- Treatment of human breast cancer cell lines with ND-646 resulted in inhibition of proliferation of selected cell lines *in vitro*
- ND-646 was well tolerated and significantly inhibited growth of MDA-MB-468 orthotopic tumors
- ND-646 demonstrated prolonged target engagement in tumor tissue and induction of apoptosis in MDA-MB-468 tumors
- Profiling of ND-646 is ongoing in broad panel of breast cancer cell lines to evaluate sensitivity across diverse genetic backgrounds and identify predictors of response