ND-630, a Potent and Liver-Directed Acetyl-CoA Carboxylase Inhibitor, Reduces Hepatic Steatosis and Improves Dyslipidemia in Diet-Induced Obese and Diabetic Rat Models of Non-Alcoholic Fatty Liver Disease

Geraldine Harriman, Jeremy Greenwood*, Sathesh Bhat*, William F. Westlin, **Rosana Kapeller**, and H. James Harwood Jr.
Nimbus Therapeutics, Cambridge MA and Schrodinger*, New York NY (USA)

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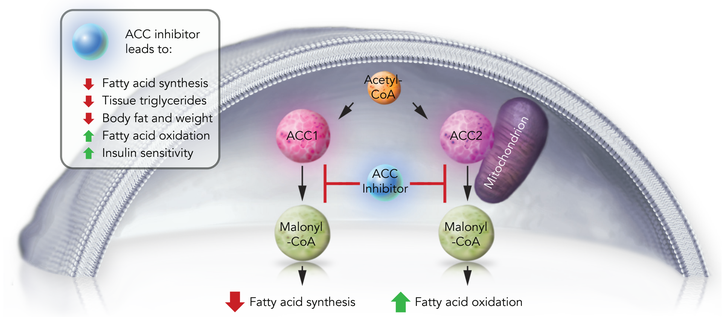
ABSTRACT

Simultaneous inhibition of the acetyl-CoA carboxylase isozymes, ACC1 and ACC2, results in concomitant inhibition of fatty acid synthesis (FASyn) and stimulation of fatty acid oxidation (FAOxn) and may favorably affect fatty liver diseases such as nonalcoholic fatty liver disease (NAFLD) and nonalcoholic steatohepatitis (NASH). Using state-of-the-art structure-based drug design technologies and crystal structures of human ACC biotin carboxylase (BC) domain, we identified a unique series of allosteric inhibitors that binds to the BC domain of the enzyme to prevent dimerization and inhibit enzymatic activity, distributes preferentially to the liver (the pharmacologic target organ), and exhibits functional activity in cultured cells and acute and chronic *in vivo* efficacy in experimental animals. The representative series analog, ND-630, inhibited human ACC1 & ACC2 (IC_{50} 2.0-7.0 nM), inhibited HepG2 cell FASyn (EC_{50} 66nM), stimulated C2C12 muscle cell FAOxn (2-fold at 200nM), distributed preferentially to the liver (135:10:1 liver:plasma:skeletal muscle at T_{max}), inhibited rat liver FASyn (ED_{50} 0.14mg/kg), and stimulated rat whole body FAOxn (MED 3mg/kg). When administered chronically by once-daily oral gavage, ND-630 demonstrated dramatic and dose-dependent reductions in hepatic steatosis in high-fat diet-induced obese (DIO) rats, high-sucrose DIO rats, and Zucker diabetic fatty (ZDF) rats. When evaluated chronically in high-sucrose DIO rats, ND-630 also markedly and dose-dependently reduced plasma triglycerides, free fatty acids, and cholesterol. These observations suggest that ND-630 and related analogs may favorably affect the hepatic steatosis, dyslipidemia, and sequalae of fatty liver diseases such as NAFLD and NASH.

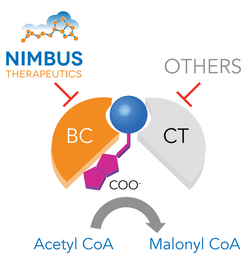
1. Acetyl CoA Carboxylase (ACC): Master Regulator of Fatty Acid Synthesis & Oxidation



- Beneficial effects on lipids, insulin sensitivity, weight, and potentially diabetes and CV risk
- Nimbus: first small molecule allosteric inhibitor successfully targeting BC domain

2. Nimbus Solves ACC Druggability Challenge by Targeting Allosteric Site in BC Domain

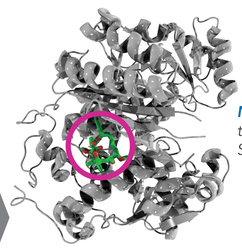
Challenges in Drugging ACC



- Only potent BC domain inhibitor: Soraphen A
- Industry efforts on analoging Soraphen proved unsuccessful
- Allosteric binding site implicated in dimerization of ACC
- Highly lipophilic binding site
- Inhibitors bind to active site
- Past programs exhibited poor drug-like properties

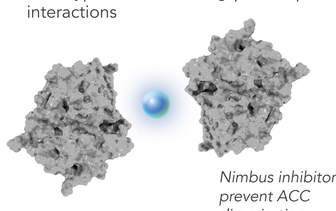
Nimbus Breakthrough

1. Novel class of ACC inhibitors



ND-630 *binds to the same site as Soraphen A*

2. Prototype for modulating protein-protein interactions



Nimbus inhibitors prevent ACC dimerization

3. ND-630: Hepatotropic ACC Inhibitor for NASH/Metabolic Disease

ND-630

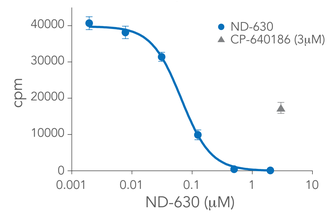
Target potency
- ACC1 IC_{50} = 2 nM
- ACC2 IC_{50} = 7 nM
- HepG2 EC_{50} = 9 nM (serum free); = 66 nM (10% serum)
- C2C12 FAOxn stim. = 2x @ 200 nM

Pharmacology
- Rat FASyn ED_{50} = 0.14 mpk PO
- Rat RQ MED = 3 mpk PO
- Rat Malonyl-CoA ED_{50} = 0.8 mg/kg (liver)
- Rat Malonyl-CoA ED_{50} = 3 mg/kg (muscle)

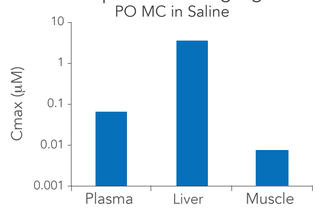
ADME
- Low multispecies intrinsic clearance (human, mouse, rat, dog, monkey)
- Eliminated predominantly as parent
- P450 inhib >50 μM
- Protein binding (98%)

Toxicology
- GLP toxicology supports clinical development

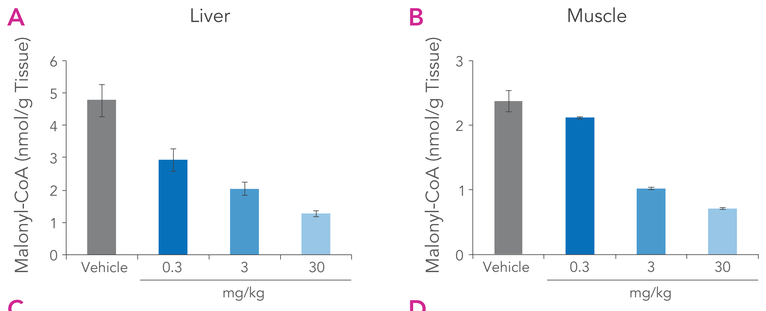
Hep-G2 Cell FASyn Inhibition
[^{14}C]acetate incorporation into FA

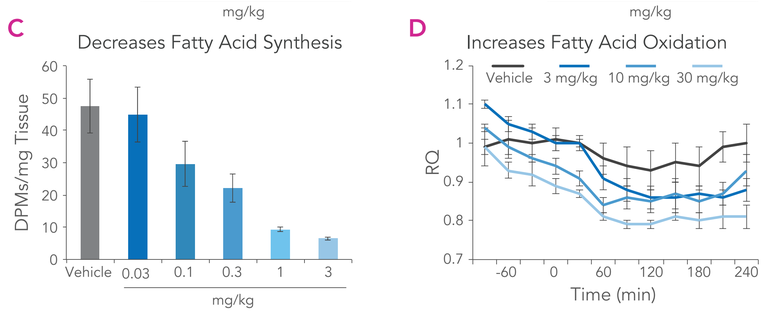


Exposure at 3 mg/Kg
PO MC in Saline



4. ACC1/2 Allosteric Inhibitor ND-630 Favorably Modulates Key Metabolic Parameters *In Vivo*





ND-630 was evaluated in three rat models of target engagement. (**A**,**B**) ND-630 shows a dose dependent reduction in the formation of the enzymatic product of acetyl coA carboxylase, malonyl coA. This reduction occurs in both the liver and muscle tissues. In alignment with the hepatoselective nature of the bio-distribution of ND-630, the ED_{50} in muscle proved to be lower. (**C**,**D**) ND-630 demonstrates its effectiveness at inhibiting the production of fatty acids in the liver (**C**, ED_{50} = 0.14 mg/kg) and modulating respiratory quotient (**D**, MED = 3 mg/kg).

5. Data Summary of ND-630 in High Fat and High Sucrose DIO Rat Studies

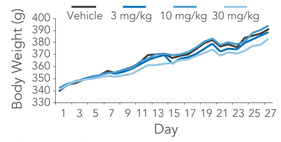
Endpoints	28-day rat DIO study high sucrose diet with 4w diet run-in	14-day rat DIO study high fat diet with 4w diet run-in
Body weight	↓	↓
Food intake	—	—
Hepatic triglycerides	↓	↓
Hepatic cholesterol	—	↓
Insulin Sensitivity (oGTT)	↑	↑
Plasma leptin	↓	↓
Plasma triglycerides	↓	—
Plasma free fatty acids	↓	nd
Plasma cholesterol	↓	—
Plasma ketone bodies	↑	nd
Plasma insulin	—	↓
Plasma glucose	—	—

- Nimbus' allosteric inhibitors show promise for diabetes, metabolic disease, and fatty liver disease

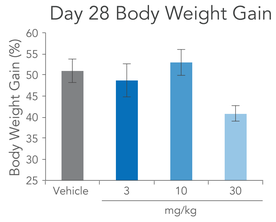
6. Weight Neutral Profile; Well Tolerated. Reduction in Weight Gain at High Dose

28-day Rat DIO Study High Sucrose Diet with 4w Diet Run-in

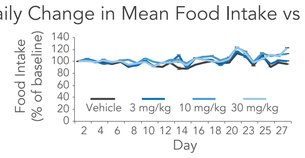
During QD Dosing of ND-630 in DIO SD Rats



Day 28 Body Weight Gain



Daily Change in Mean Food Intake vs Baseline

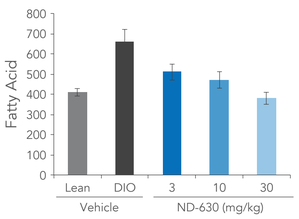


- Weight neutral, with weight gain reduction at high dose
- No change in food intake
- No differences in fed blood glucose
- Animals healthy

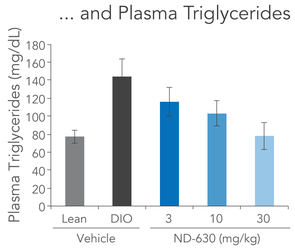
7. ND-630 Favorably Modulates Key Metabolic Parameters *In Vivo*

28-day Rat DIO Study High Sucrose Diet with 4w Diet Run-in

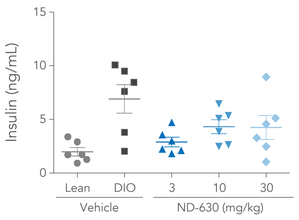
Decreases Free Fatty Acids



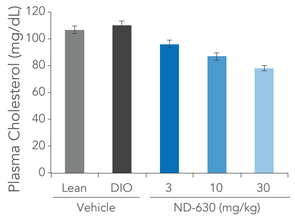
... and Plasma Triglycerides



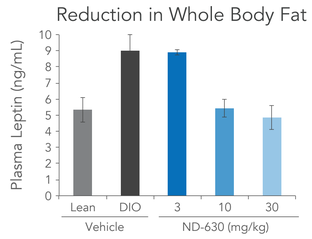
Normalizes Plasma Insulin Levels



... and Decreases Plasma Cholesterol



Reduction in Whole Body Fat



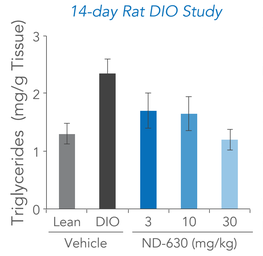
Leptin as a Surrogate Marker

(Day 20 in Rat DIO Study)

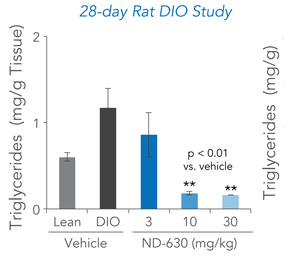
Data are mean values for n=8 animals per group ± SEM

8. Liver Triglycerides Lowered Across14-Day DIO and ZDF Studies

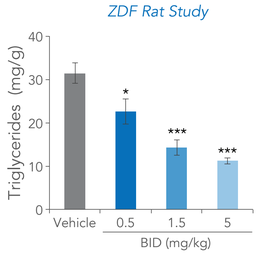
Day 14 (High Fat Diet)
14-day Rat DIO Study



Day 14 (High Sucrose Diet)
28-day Rat DIO Study



Day 37
ZDF Rat Study



- Profound hepatic triglyceride lowering in ZDF study at day 37
- Similar changes in liver cholesterol & free fatty acid

CONCLUSIONS

- ND-630 is a potent ACC1/2 inhibitor with good drug-like properties
- It is well tolerated and effective in three models of hepatic steatosis and dyslipidemia
- ND-630 was also effective at modulating several parameters of metabolic syndrome
- ND-630, designed to have hepatoselective bio-distribution, was shown to reduce hepatic steatosis and improve dyslipidemia in DIO and ZDF diabetic rat models of non-alcoholic fatty liver disease

NIMBUS THERAPEUTICS
25 First St #404, Cambridge, MA 02141, USA • www.nimbustx.com • Tel: 857.999.2009 • info@nimbustx.com