EVALUATION OF ELAFIBRANOR IN THE CHOLINE-DEFICIENT DIET MOUSE MODEL OF NASH
Sharon C Cheetham, Katie R Headland, Mike R Prow, Lucy A Pinder, Robert B Jones & Steven P Vickers
RenaSci Ltd, BioCity, Pennyfoot Street, Nottingham, NG1 1GF, UK

Introduction
The dual peroxisome proliferator activated receptor (PPAR)α/δ agonist, elafibranor, has been shown to improve non-alcoholic steatohepatitis (NASH) in man. This study examines the effects of elafibranor in mice fed a choline-deficient (CD) diet, a simple animal model of NASH. The PPARy agonist, pioglitazone, was used as a positive control.

Methods
Male C57BL/6J (JAX) mice were obtained from Charles River UK and placed on a standard maintenance diet (Teklad 2018). Two weeks after arrival, animals entered a one week baseline period during which they were dosed once daily with vehicle. Animals were then allocated into balanced groups (n=11-13). One group remained on the maintenance diet and was dosed with vehicle po bid (normal diet controls). The remaining mice were placed on a CD diet (A02082006; Research Diets Inc) from Day 1 and were dosed with vehicle po bid; elafibranor 30/20 mg/kg po qd or pioglitazone 15 mg/kg po bid for 6 weeks. The dose of elafibranor was reduced to 20 mg/kg on Day 27 due to excessive weight-loss. Mice were terminated 1 h after dosing on Day 43. Terminal plasma alanine aminotransferase levels (ALT) were determined using a Cobas c111 clinical chemistry analyser. Liver samples were assayed for triglyceride content (Cobas) or processed for histological assessment by light microscopy. Haemotoxylin and Eosin staining was used to detect steatosis, hepatocellular ballooning and lobular inflammation. Oil Red O staining was used to detect lipid deposition. Sirius Red staining was used to detect collagen deposition. Liver changes were graded using established scoring systems by a pathologist (PathCelerate) who was unaware of the treatment of the animals on CD diet received. Steatosis, ballooning and inflammation scores were combined to give a non-alcoholic fatty liver disease activity score (NAS). The vehicle was 0.5% carboxymethylcellulose and Tween 80 (98:2) and drugs were administered in a dose volume of 5 ml/kg.

Statistical analysis: Body weights, plasma ALT levels and liver triglyceride concentrations are expressed as means (adjusted using appropriate covariates) and SEM. Body weights were analysed by ANCOVA with Day 1 body weight as the covariate. Plasma ALT levels were log-transformed and analysed by ANCOVA with bleeding order as the covariate. Liver triglycerides were analysed by a general linear model with treatment and termination cohort as factors and termination order as the covariate. All comparisons were by the multiple t-test. Histology scores (means and SEM) were analysed by exact Wicoxon rank sum tests. Significant differences are denoted by *p<0.05, **p<0.01, ***p<0.001 vs. normal diet controls; †p<0.05, ††p<0.01, †††p<0.001 vs. CD diet controls.

Results

Figure 1: Body Weight
Exposure to the CD diet significantly reduced body weight compared to the normal diet control group (-12.2%, Day 43). Elafibranor reduced body weight further (-24.2%, Day 43). Pioglitazone had little effect on body weight compared to the CD diet controls. The CD diet significantly increased plasma ALT, liver triglycerides, lipid deposition, steatosis, hepatocellular ballooning, lobular inflammation, NAS and fibrosis scores compared to the normal diet controls. Elafibranor significantly inhibited all of these responses, except for the increase in plasma ALT. Pioglitazone significantly attenuated all responses, except for the increases in lipid deposition and hepatocellular ballooning.

Figure 2: Plasma ALT

Figure 3: Liver Triglycerides

Figure 4: Liver Histology

Conclusion
Elafibranor markedly reduced liver triglycerides and all of the histological features of NASH and fibrosis in mice fed a CD diet. These results support the use of the CD diet model as an initial screen for evaluating the potential of drugs to treat NASH.