ECC4703, a full thyroid hormone receptor β (THRβ) agonist, demonstrates excellent selectivity and liver targeting properties and improvement of plasma lipids, NAFLD activity score and fibrosis in NASH animal model

Jianfeng Xu[1], Yongliang Zhao[2], Jiuxiang Zhu[3], Jianfeng Xu[1], Jingye Zhou[1]

Author affiliate: 1. Eccogene (Shanghai) Co., Ltd. and affiliates. 2. Currently in CStone Pharmaceuticals. 3. Currently in TandemAI Shanghai Co., Ltd.
Contact information: jfxu@eccogene.com

EASL™ The Home of Hepatology

1 Introduction and Aim

Non-alcoholic steatohepatitis (NASH) is a metabolic liver disease featuring ectopic fat accumulation, hepatocellular damage, chronic inflammation, and progressive fibrosis. Resmetirom (MGL-3196), a THRβ agonist, has demonstrated moderate efficacy in plasma lipid lowering and liver fat reduction with potential of NASH resolution and fibrosis improvement in NASH patients[1,2]. MGL-3196 is a partial agonist compared to the natural ligand T3 in ligand binding and liver gene expression. ECC4703 is a liver targeting THRβ selective agonist with a full agonist-like profile which may provide superior efficacy for NASH and dyslipidemia.

2 Method

- *In vitro* THRα and THRβ binding measured based on recruitment of a coactivator peptide to the receptors
- *In vivo* liver targeting profile determined in PTU-induced rat hypothyroidism model with induction of THR target gene in liver and heart
- Activities in human primary hepatocyte measured with a reporter gene driven by thyroid response elements
- *In vivo* efficacy on NAS, fibrosis, and plasma lipid profile determined in DIO-CCl4 model

4 Conclusions

In summary, ECC4703 is a novel liver targeting THRβ full agonist with excellent *in vitro* and *in vivo* profile. ECC4703 has the potential to become best-in-class THRβ agonist for NASH and dyslipidemia treatment.

5 References

1 Stephen A-H *et al.* Discovery of 2-[3,5-dichloro-4-(5-isopropyl-6-oxo-1,6-dihydropyridazin-3-yloxy)phenyl]-3,5-dioxo-2,3,4,5-tetrahydro[1,2,4]triazine-6-carbonitrile (MGL-3196), a highly selective thyroid hormone receptor β agonist in clinical trials for the treatment of dyslipidemia. *J Med Chem. 2014 22, 57; 3912-23*
2 Martha J-K *et al.* Resmetirom (MGL-3196) for the treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet 2019 394; 2012-2024*

3-1 Results

1. ECC4703 is a potent and THRβ selective full agonist *in vitro*

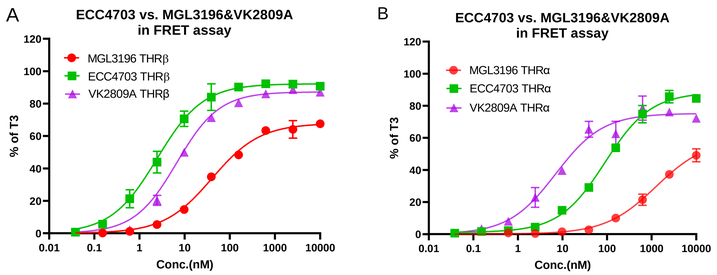


Figure 1. (A) Dose response curves in TR-FRET THRβ coactivator Assay (B) Dose response curves in TR-FRET THRα coactivator Assay

Table 1. EC_{50}, Emax and relative selectivity of ECC4703, MGL3196 and VK2809A

Profile	MGL-3196	VK2809A	ECC-4703
THRβ EC_{50} (nM), Emax	49, 64%	5.6, 91%	3.9, 92%
THRα EC_{50} (nM), Emax	799, 48%	10.6, 82%	110, 88%
Rel Selectivity β/α #	31 (n=125)	2.4 (n=4)	46 (n=10)

#: THRβ/THRα selectivity was calculated by the ratio of EC_{50} from THRα and THRβ from the assay conducted at the same day, and the relative selectivity of each compound was calculated by normalization to T3 selectivity.

3-2

2. ECC4703 is a potent and THRβ selective full agonist *in vivo* with liver targeting properties

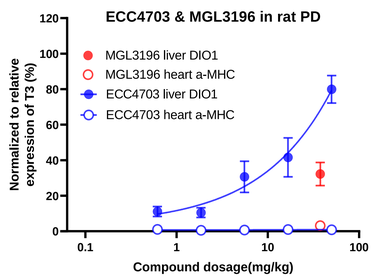


Figure 2. ECC4703 elicited robust induction of liver gene DIO1 but no effects on heart gene α-MHC at up to 50 mg/kg. Liver and heart tissue were collected 24h after single dose of T3, MGL-3196 or ECC4703 in PTU-induced rat hypothyroidism rats. Relative gene expression was normalized to the response of T3.

3-3

3. ECC4703 demonstrated potent THR agonism in primary human hepatocytes

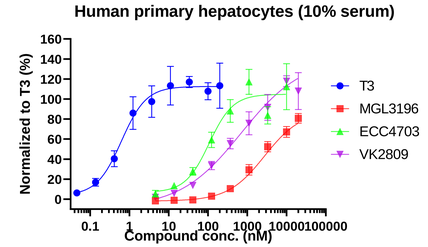
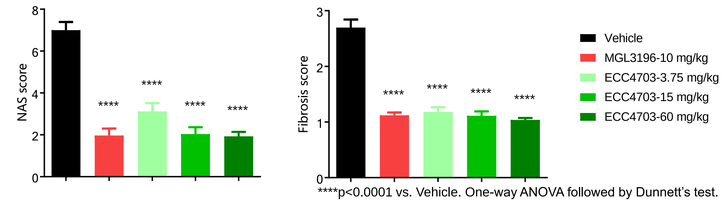


Figure 2. ECC4703 is more potent than MGL-3196 and VK2809 (91nM vs. 3842nM and 321nM, respectively) for reporter gene expression in human primary hepatocytes. Adenoviruses with Renilla luciferase driven by constitutively active promoter (EF1-Rluc) and Firefly luciferase driven by TRE-containing promoter (TRE-Fluc) were co-transduced into primary hepatocytes. The ratio of Firefly and Renilla luciferase activity reflects THR dependent transcriptional activities.

3-4

4. ECC4703 is efficacious in DIO-CCl4 NASH model



****p<0.0001 vs. Vehicle. One-way ANOVA followed by Dunnett's test.

Figure 3. DIO-CCl4 NASH model was established by 16-week high-fat diet feeding followed with 4-week CCl4 administration. Compound was orally administrated for 4 weeks from the 17th week.ECC4703 treatment resulted in significant improvement of liver NAS score and fibrosis in DIO-CCl4 NASH model.

Table 2. Plasma lipids and liver enzymes before and after 4-week treatment of MGL-3196 and ECC4703 in DIO-CCl4 NASH model.

Item	WK	Vehicle	MGL3196-10 mg/kg	ECC4703-3.75 mg/kg	ECC4703-15 mg/kg	ECC4703-60 mg/kg
TC	0	6.09±0.41	6.04±0.42	5.91±0.33	5.89±0.31	5.67±0.29
(mmol/L)	4	4.04±0.38	1.90±0.26***	1.99±0.21***	2.32±0.25**	1.86±0.34**
TG	0	1.01±0.03	0.98±0.06	0.92±0.06	0.89±0.04	0.97±0.07
(mmol/L)	4	1.54±0.22	0.80±0.07*	1.17±0.11	0.88±0.05*	1.06±0.20
HDL-c	0	2.60±0.10	2.60±0.12	2.68±0.10	2.59±0.12	2.56±0.10
(mmol/L)	4	1.70±0.14	1.04±0.14*	1.00±0.09**	1.18±0.12*	0.85±0.15**
LDL-c	0	1.21±0.05	1.20±0.07	1.18±0.04	1.14±0.04	1.13±0.05
(mmol/L)	4	1.12±0.11	0.39±0.05***	0.44±0.06***	0.46±0.06***	0.40±0.08***
ALT (U/L)	4	404.50±87.90	234.14±57.00	351.14±126.68	209.53±31.70	282.70±70.05
AST(U/L)	4	549.53±153.08	346.42±109.82	629.13±169.56	267.30±26.30	548.57±154.75

*p<0.05; **p<0.01; ***p<0.001; ****p<0.0001 vs. Vehicle. Two-way ANOVA followed by Dunnett's test.