

**First Year Progress Summary: *Bile acid activated receptors TGR5 and FXR as therapeutic targets for the treatment of PSC***

Pruritus is a common finding in PSC and remains an unmet clinical need. Bile acids have been associated with itching mostly because their dermal injection triggers pruritus; but this relationship remain poorly demonstrated in clinical settings, because the severity of pruritus does not correlate with plasma bile acids and other mediators have shown better correlation with the severity of this symptom. However, the results of clinical trials in PBC patients administered 6-ECDCA, a semi-synthetic derivative of CDCA, strongly advocate a role for bile acids and bile acid-activated receptors in the pathogenesis of pruritus. Interestingly, 6-ECDCA is a dual ligand for FXR and GP-BAR1 and GP-BAR1 has been mechanistically linked to itching caused by the intra-dermal injection of steroidal (LCA and DCA) and non-steroidal ligands of GP-BAR1 including betulinic acid, a naturally occurring triterpenoid.

In this first year we have harnessed on the CDCA and UDCA scaffolds to generate semisynthetic derivatives that are selective for FXR or GP-BAR1. The results of our investigations has led to the discovery of 7 $\alpha$ -hydroxy-5 $\beta$ -cholan-24-sulfate, a selective FXR ligand, EUDCOH, a selective GP-BAR1 ligand, and NorECDCOH, a highly preferential FXR ligand endowed with a minimal GP-BAR1 agonistic activity.

In particular NorECDCOH, a truncated side chain alcohol with both substituents on ring B in  $\alpha$ -configuration, is a potent FXR ligand with an EC<sub>50</sub> of 2  $\mu$ M, very close to that of 6-ECDCA. Indeed, both these two agents induce the expression of OST $\alpha$  with the same potency. In addition to the ability to transactivate FXR, NorECDCOH retains a certain capacity of inducing GP-BAR1 and, indeed, it increases pro-glucagon gene expression in GLUTAg cells. Because its potency in inducing FXR target genes largely overwhelms that on GP-BAR1, NorECDCOH should be considered a highly preferential FXR ligand. Supporting this view we have characterized NorECDCOH in animal models of cholestasis and itching and demonstrated that in contrast to natural and synthetic bile acids, NorECDCOH did not trigger a scratching behavior when administered to naïve mice. Moreover, we have shown that GP-BAR1 deletion exacerbates the severity of liver damage in two models of cholestasis (i.e.  $\alpha$ -naphthyl-isothiocyanate (ANIT) or 17 $\alpha$ -ethynylestradiol induced cholestasis) and that this damage is robustly attenuated by NorECDCOH in a GP-BAR1 independent manner.

In summary, results of our investigations has led to the discovery of NorECDCOH, a highly preferential FXR ligand endowed with a minimal GP-BAR1 agonistic activity. NorECDCOH attenuates liver damage in two animal models of non-obstructive cholestasis without triggering itching.