Preclinical characterization of BJT-188, a liver-targeted fatty acid synthase (FASN) inhibitor for the treatment of MASH

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Background and Aims

- **Fatty acid synthase (FASN) is an enzyme in the de novo lipogenesis (DNL) pathway that converts the metabolites of dietary sugars into the saturated fatty acid palmitate, a precursor of triglycerides and other lipotoxic lipids.**
- **The first-generation FASN inhibitor Denifanstat reduces liver steatosis, inflammation, and fibrosis in MASH patients[1]. It is also associated with adverse events, including alopecia, that may limit its clinical utility[2].**
- **Here, we aimed to develop novel targeted FASN inhibitors that retain liver-specific benefits while minimizing exposure to other tissues.**

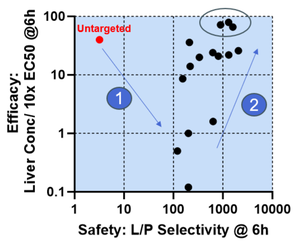
Methods

Intrinsic potency was measured using recombinant rat FASN in a fluorescent-based biochemical assay. In vitro DNL inhibition was conducted in primary hepatocytes and other cell types. Initial in vitro safety screen consisted of CYP inhibition and Cerep safety panel of 44 receptors. Predicted clearance was estimated based on metabolic stability in primary hepatocytes. Pharmacokinetic profiling in rodents was used to determine liver exposures and tissue partitioning. Single dose in vivo studies of liver DNL inhibition were conducted in rats.

Results

Figure 1: Discovery of Liver-Targeting BJT-188

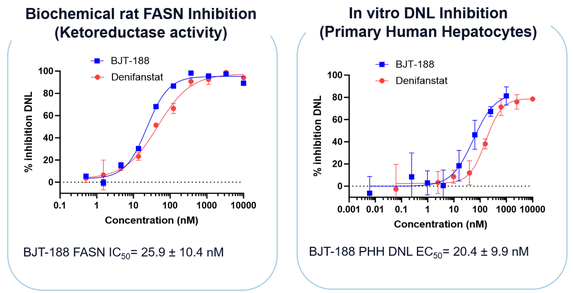
1. Optimization of L/P partitioning
2. Optimization of metabolic stability and liver exposure



Lead optimization and candidate selection. Lead optimization of liver-targeting FASN inhibitors focused first on improving predicted safety based on maximizing liver/plasma selectivity in rats. The next step was a multi-parameter optimization of intrinsic in vitro potency combined with improved stability in hepatocytes, resulting in increased rat liver concentration/EC_{50} ratio. Multiple liver-targeting lead candidates were selected for further evaluation.

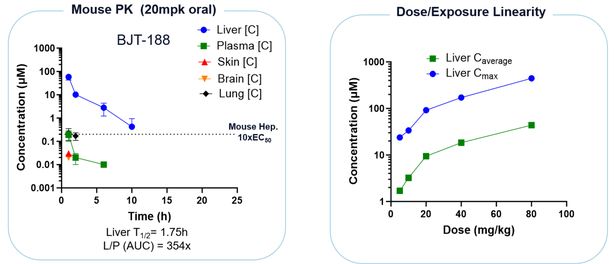
Results (Continued)

Figure 2: In Vitro Potency of BJT-188



BJT-188 is a potent FASN ketoreductase inhibitor. BJT-188 inhibits the ketoreductase function of rat FASN with an IC_{50} value of 25.9 ± 10.4 nM. In primary human hepatocytes, BJT-188 inhibits DNL with an EC_{50} value of 20.4 ± 9.9 nM. Similar inhibition potency values were obtained in primary rat and mouse hepatocytes (data not shown).

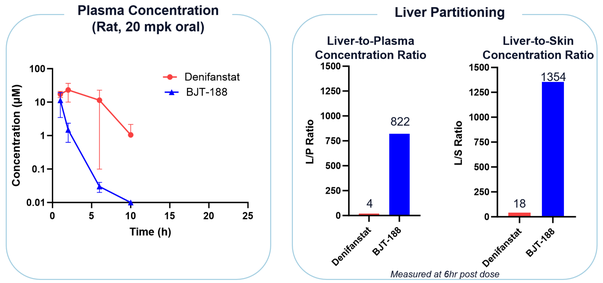
Figure 3: Mouse Pharmacokinetics



Dose (MPK)	Fold over 10xEC_{50} (mouse primary hepatocytes)	
	C_{max}	$C_{average}$
5	119x	9x
10	169x	16x
20	460x	47x
40	859x	92x
80	2225x	219x

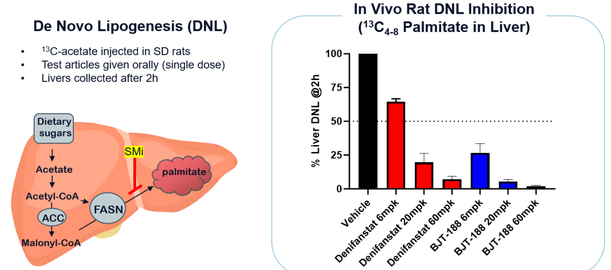
Mouse PK analysis. Analysis of plasma and tissue exposures in mouse show that, at an oral dose of 20 mpk, liver levels of BJT-188 are above the 10xEC_{50} value in mouse primary hepatocytes for the first 10h. Plasma levels are 354-fold below the liver levels. Skin levels could only be detected at the earliest time point, and exposures in other tissues were minimal to undetectable.
Pharmacokinetic analysis was also performed at doses ranging from 5 to 80 mpk. C_{max} levels increase linearly with dose from 24 to 445 µM, and $C_{average}$ from 1.7 to 44 µM. These liver concentrations are all well above the 10xEC_{50} value for DNL inhibition in mouse primary hepatocytes.

Figure 5: Rat Pharmacokinetics



Rat PK analysis. At an oral dose of 20 mpk, systemic exposures of BJT-188 in rats are generally low, with rapid plasma clearance within the first 10h. High liver uptake of BJT-188 results in a liver-to-plasma ratio of 822x at 6h post dose, and a liver-to-skin ratio of 1354x.

Figure 5: In Vivo Potency of BJT-188 in Rats and PK/PD Relationship



Inhibition of palmitate formation. FASN converts acetyl-CoA and malonyl-CoA into palmitate as part of DNL. In vivo liver DNL inhibition was determined by measuring conversion of ^{13}C-acetate to ^{13}C-palmitate after a single dose of BJT-188 in Sprague Dawley rats. All tested concentrations of BJT-188 resulted in >50% DNL inhibition. The dose response for DNL inhibition was as follows: 73% at 6 mpk, 95% at 20 mpk, and 98% at 60 mpk. We concluded that BJT-188 inhibits palmitate formation proportionally with increased liver exposure, with an ED_{50} of less than 6 mpk.

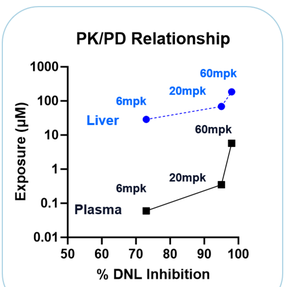


Table 1: In vitro ADME and Safety Profile of BJT-188

Species	Hepatocyte Stability ($T_{1/2}$, min)	
	BJT-188	Denifanstat
Mouse	78	9
Rat	350	107
Dog	485	133
Monkey	343	44
Human	>578	171

BJT-188 is stable in primary hepatocytes. Compared to other species, BJT-188 is most stable in human hepatocytes, with a half-life of >578 min. In human hepatocytes, 84% of the parent analyte remained at the last collection time point of 240 min.
In addition, BJT-188 tested at up to 10 µM caused no inhibition of 44 human receptors, including hERG, nor induced any activation of nuclear receptor PXR, CAR, AHR.
BJT-188 did not react with glutathione or cause any CYP inhibition at up to 10 µM. BJT-188 did not have any cytotoxic effect in primary human hepatocytes or PBMCs.

Conclusions

- **BJT-188 is a potent FASN ketoreductase inhibitor. In primary human hepatocytes, BJT-188 inhibits DNL with an EC_{50} value of 20.4 ± 9.9 nM. Similar inhibition potency values were obtained in mouse and rat hepatocytes.**
- **In mice and rats, BJT-188 exposures in plasma and skin are well below the levels obtained in the liver, suggesting a low potential for alopecia.**
- **In a rat PK/PD model, BJT-188 inhibits palmitate formation with an ED_{50} of less than 6 mpk. At 60 mpk, DNL inhibition by BJT-188 reaches about 98%.**
- **BJT-188 is currently progressing towards IND enabling studies.**

References

1. Loomba R et al., Denifanstat for the treatment of metabolic dysfunction-associated steatohepatitis: a multicentre, double-blind, randomised, placebo-controlled, phase 2b trial. Lancet Gastroenterol Hepatol. 2024 Dec;9(12):1090-1100.
2. Syed-Abdul MM et al., Fatty Acid Synthase Inhibitor TVB-2640 Reduces Hepatic de Novo Lipogenesis in Males With Metabolic Abnormalities. Hepatology. 2020 Jul;72(1):103-118.

Acknowledgements

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