Non-Invasive Pharmacodynamic Markers of SZN-043 Target Engagement and Wnt Pathway Activation

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Introduction

Wnt signaling plays a central role in hepatocyte expansion during development and tissue repair. R-spondins (RSPOs) amplify Wnt signaling via stabilization of Frizzled and LRP co-receptors. SZN-043 is a bispecific, R-spondin mimetic, fusion protein targeting hepatocytes via the asialoglycoprotein receptor 1 (ASGR1) subunit of the asialoglycoprotein receptor (ASGPR). ASGR1 is involved in clearance of circulating ALP. As a consequence of SZN-043 binding the ASGR1 receptor, ALP concentration rises and can be used as a measure of target engagement. SZN-043 has been shown to induce hepatocyte proliferation and to improve hepatic function in preclinical models of acute and chronic liver injury. In this study, we analyzed the effect of SZN-043 on non-invasive pharmacodynamic markers of target occupancy by measuring serum alkaline phosphatase (ALP) and of Wnt/β-catenin activation by measuring serum leukocyte cell-derived chemotaxin-2 (LECT2), a direct Wnt target gene.

Methods

Male C57BL/6J mice were dosed intravenously with SZN-043 at either 3, 10, 30 or 100 mg/kg on Day 0. Serum and liver samples were collected at selected time points up to 14 days after treatment (n=4 per dose per time point). A control group without SZN-043 treatment was included on Day 0 (n=4). Serum ALP was measured using a clinical analyzer and serum LECT2 was measured by ELISA. Liver samples were analyzed for the expression of the genes encoding for ALP and LECT2, *Alpl* and *Lect2* respectively, as well as other genes, including the Wnt target gene *Axin2*, and the proliferation marker *Mki67*. AUC was calculated using the trapezoidal method.

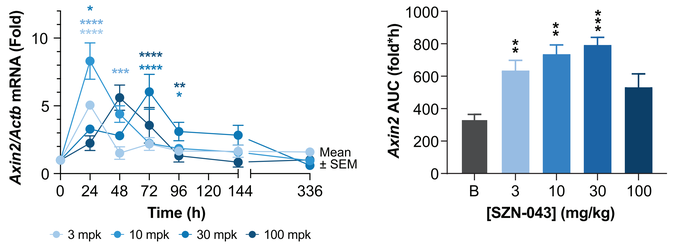
B is baseline calculated with values at t=0. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001

Conclusions

Serum ALP concentrations can be used as a non-invasive and dose-dependent pharmacodynamic marker of SZN-043 target occupancy while serum LECT2 concentrations can be used as a pharmacodynamic marker of Wnt activation.

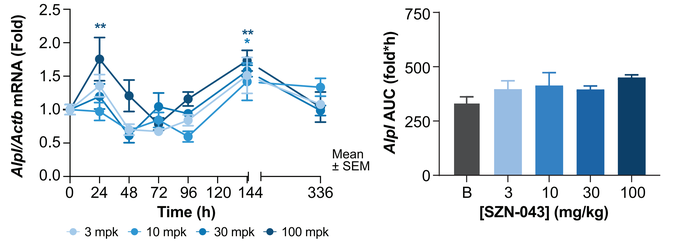
Results

Liver *Axin2*



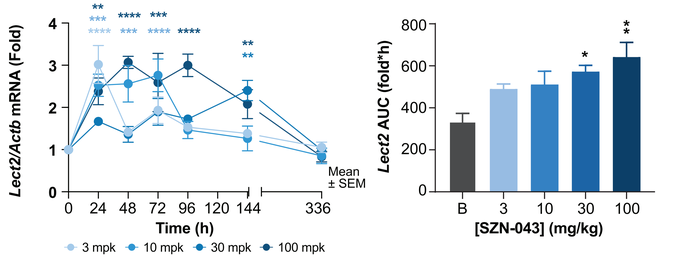
- *Axin2* expression, a direct Wnt/β-catenin target gene, was elevated in a dose dependent manner in response to SZN-043, with the exception of the highest dose. Axin2 expression peaked between 24-72 h.

Liver *Alpl*



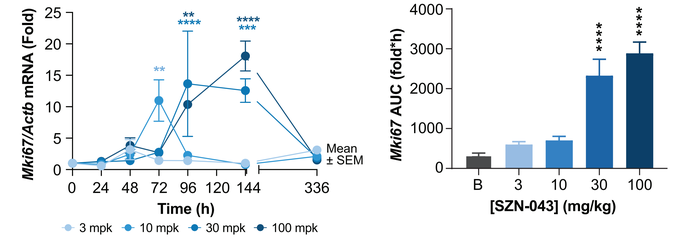
- *Alpl* the gene that encodes for Alkaline Phosphatase (ALP) was not significantly affected, except mildly (fold < 2) at the highest dose at 24h and 144h.

Liver *Lect2*



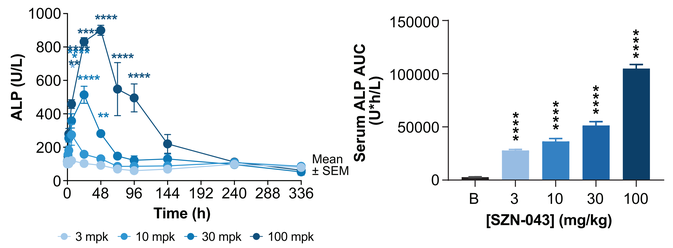
- Liver hepatokine *Lect2* was elevated in response to SZN-043 treatment in a dose-dependent manner. *Lect2* peaks between 24 and 48 h. Expression returns to baseline for all treatments by 14 d

Liver *Mki67*



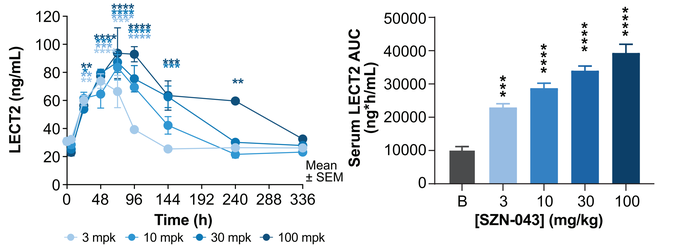
- The proliferation marker *Mki67* was elevated in response to SZN-043 treatment in a dose-dependent manner. The peak of this response was delayed in a dose-dependent manner also, with a range between 48 and 144 h.

Serum ALP



- Elimination of circulating ALP occurs through the ASGR receptor. SZN-043, which binds to the ASGR receptor increases ALP serum concentration by interfering with ALP elimination. Consistent with this observation, SZN-043 does not affect Alpl gene expression substantially.
- ALP serum concentration was elevated in a dose-dependent manner. The T_{max} of ALP induction was dose-dependent from 6 h for 3 mg/kg SZN-043 to 2 days for 100 mg/kg of SZN-043. Serum ALP concentration returns to baseline by day 10.

Serum LECT2



- Serum LECT2 concentration was elevated in a dose-dependent manner. Serum concentration peaks between 2 d and 3 d and doesn't return to baseline until 14 d for the highest concentration.

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