SZN-043, a Hepatocyte-Targeted R-spondin Mimetic, Promotes Transient Hepatocyte Proliferation and Zonal Gene Expression Changes in Mice (3525528)

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Introduction

Wnt signaling is critical for hepatocyte development and for regeneration after liver injury, and it contributes to the region-specific expression of metabolic genes. When Wnt signaling is blocked or absent, liver regeneration is impaired, and there is a delay and reduction in hepatocyte proliferation and tissue regeneration (Planas-Paz 2016). Wnt signaling is a major regulator of liver zone-specific metabolic gene expression, and active signaling promotes expression of many genes enriched in or specific to the central zone (Benhamouche 2006; Planas-Paz 2016). R-spondins enhance Wnt signaling via stabilization of Wnt receptors, and regulation of R-spondin activity robustly affects liver zonation (Planas-Paz 2016, Rocha 2015). We have built a hepatocyte-targeted R-spondin mimetic, SZN-043, that binds the E3 ubiquitin ligases, ZNRF3, RNF43, and the hepatocyte-specific receptor, ASGR1 (Zhang 2020). To gain a more comprehensive understanding of how SZN-043 impacts the liver, we tested the effect of repeated dosing in mice and interrogated how it impacts hepatocytes and all other cell types and how the liver responds upon washout.

Aims

- Characterize how SZN-043 impacts Wnt target and liver zone-specific gene expression
- Define how SZN-043 affects hepatocyte proliferation
- Determine if the effects of SZN-043 on the hepatocytes normalize over time

Method

C57Bl/6 mice were dosed with either a control antibody (anti-GFP) twice weekly or with SZN-043 at 10 mg/kg intraperitoneally (IP) daily for two weeks. Liver samples were collected at 24 hours after the first dose, 24 hours after the last dose, and at a range of washout timepoints (Day 7, 14, 27, and 56 after the last dose). We perfused livers and performed single cell RNA seq (scRNA-seq) at 24 hours after the first and last dose and 56 days after the last dose. We also examined tissue by immunohistochemistry, RNA in situ hybridization, and/or RT-qPCR at all timepoints.

Figure 1.

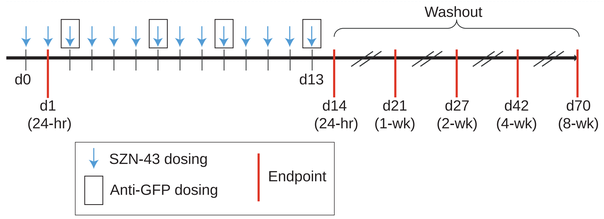
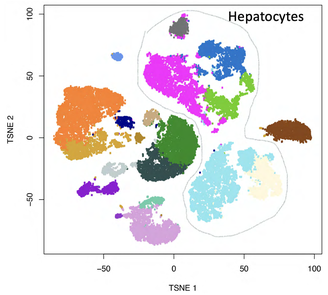


Figure 2.

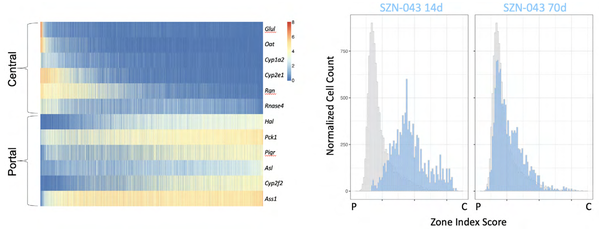
For the scRNA-seq, livers were perfused and dissociated. From each animal, hepatocytes and all non-hepatocytes (minus red blood cells) were purified in parallel, and we applied 10X Genomics 3' scRNA-seq with v3 reagents. After filtering, there are 18,916 hepatocytes and 21,212 non-hepatocytes. Zone index score was defined as C + (-P), where C is the peri-central zone gene average Z-score and P is the average peri-portal score.



Results

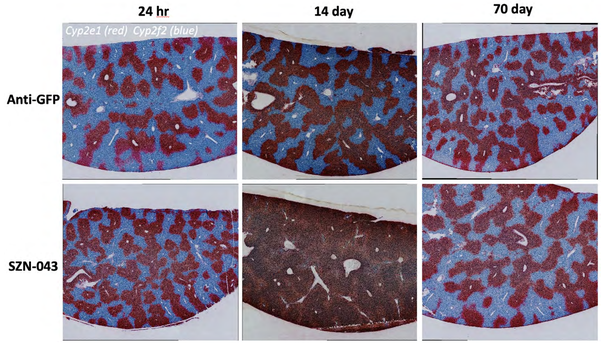
SZN-043 causes a transient expansion of Wnt target and central zone gene expression

Figure 3.



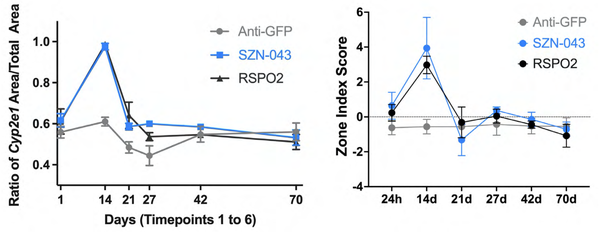
Using single cell gene expression, 18,916 hepatocytes were ordered based on their expression of peri-central and peri-portal genes (heatmap). SZN-043 treatment expands peri-central gene expression and simultaneously diminishes expression of peri-portal enriched genes, and this effect normalizes upon washout: histograms show the distribution of zone index scores for anti-GFP (gray) and SZN-043 at 14d (blue, left) and washout at 70d (blue, right). Hepatocytes with higher peri-central zone gene expression are positioned toward the right of the x-axis, while those with higher peri-portal zone gene expression are positioned toward the left.

Figure 4.



We interrogated the expression of the pericentral (and Wnt target) gene, *Cyp2e1*, and the periportal gene, *Cyp2f2* by RNA in situ hybridization at all timepoints. Above are example images for the indicated treatment at 24-hours after the first dose (left), 24-hours after the last dose at day 14 (middle), or following a two-month washout at day 70 (right). *Cyp2e1* expression increases in intensity and expands in area into the peri-portal region while *Cyp2f2* expression diminishes, and and these effects normalize upon washout.

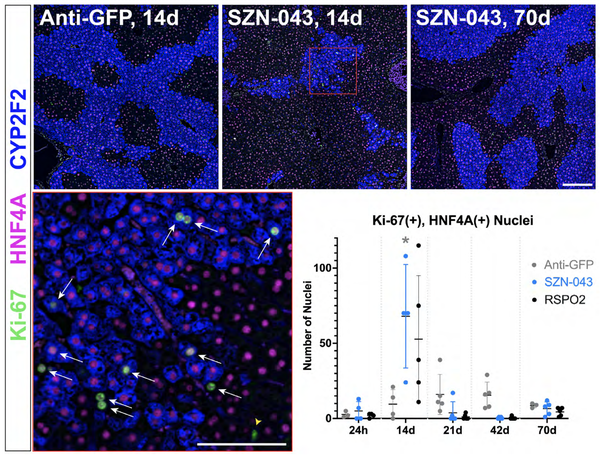
Figure 5.



We quantified the ratio of the *Cyp2e1* expression domain to total liver area at the indicated timepoints (left). We also used bulk liver RT-qPCR for *Glu1*, *Cyp2e1*, *Hal*, and *Cyp2f2* to calculate a zone index score (right) to corroborate this result.

SZN-043 promotes transient proliferation of hepatocytes

Figure 6.

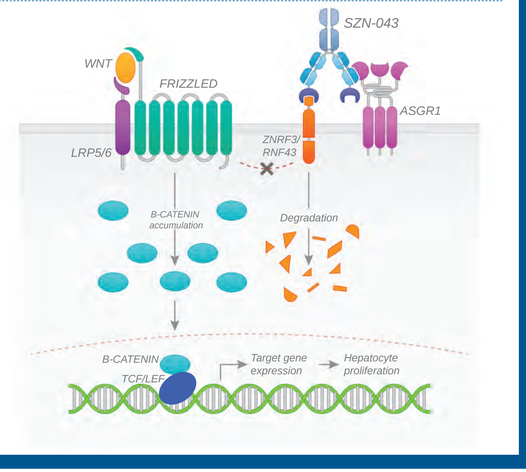


The three top image panels show immunohistochemistry for the proliferative gene, Ki-67, the hepatocyte marker, HNF4A, and the periportal enzyme, CYP2F2, at the indicated treatment and timepoint (scale bar = 200 microns) . The enlarged image panel shows a higher magnification view of the red boxed region in the upper middle panel, with HNF4A/Ki-67 double-labeled cells indicated by white arrows and an example Ki67(+)/HNF4A(-) cell indicated by the yellow arrowhead (scale bar = 100 microns). In other studies, we have noted that SZN-043 induces proliferation in the first few days after dosing. By quantifying co-labeled of HNF4A and Ki-67 double-positive nuclei, we observe a significant increase in the number of proliferating hepatocytes after two-weeks of SZN-043 dosing that normalizes upon washout (*, adj. p-value = 0.03, bottom right plot). We do not detect an increase in proliferating HNF4A(-) cells at 14d (data not shown). Note that at the protein level, the periportal gene, CYP2F2 is strongly reduced by SZN-043, but expression normalizes upon washout, mimicking the effect on mRNA levels.

Conclusions

- **SZN-043 induces a transient increase in the number of proliferating hepatocytes**
- **SZN-043 activates and expands Wnt target and central zone-associated gene expression**
- **The increase in hepatocyte proliferation and the expansion of central zone associated gene expression both normalize within 7 days of dosing cessation**
- **The ability of SZN-043 to target hepatocytes and promote their proliferation suggests its utility as a regenerative therapy for liver diseases with hepatocyte loss.**

Figure 7.



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