Selective inhibition of human β-catenin DNA transactivation activity using splice switching oligonucleotides for an improved therapeutic window in treating hepatocellular carcinoma

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BACKGROUND AND AIMS
Wnt/β-catenin plays a critical role in embryonic development, tissue homeostasis and repair after injury. Alterations in this pathway are implicated in many human diseases including cancer. Dysregulation of the Wnt/β-catenin pathway may play a key role in the pathogenesis of Hepatocellular Carcinoma (HCC). Reducing β-catenin by siRNA or ASO treatment has shown significant inhibition of liver tumor growth in an HCC mouse model.1,2 Due to the importance of Wnt/β-catenin in normal cellular function, many drugs targeting this pathway have failed due to toxicity. Splice switching oligonucleotides (SSO) have been reported to inhibit the transcriptional activation activity of β-catenin while maintaining essential functions such as binding with E-cadherin3. Our goal is to design and develop SSO with drug like properties targeting the DNA transactivation domain of β-catenin in treating HCC. This will reduce the downstream proteins responsible for HCC development, while leaving intact the domains interacting with α-catenin and E-cadherin that are important for cell adhesion.

METHODS
The HepG2 Topflash cell line was used to assay the SSO inhibition of β-catenin transcriptional activity. Anti-proliferative assays with SSO were carried out in Huh-6 and PLC/PRF/5 cell lines using CellTiterGlow. SSO effects on different regions of the β-catenin transcript were analyzed by qPCR. Effects of SSO on downstream gene expression such as c-Myc, CCND1 and AXIN2 were analyzed by qPCR. Effects of SSO on downstream proteins responsible for HCC development, while targeting this pathway have failed due to toxicity. Splice switching oligonucleotides (SSO) have been reported to inhibit the transcriptional activation activity of β-catenin while maintaining essential functions such as binding with E-cadherin3. Our goal is to design and develop SSO with drug like properties targeting the DNA transactivation domain of β-catenin in treating HCC. This will reduce the downstream proteins responsible for HCC development, while leaving intact the domains interacting with α-catenin and E-cadherin that are important for cell adhesion.

RESULTS
In the Hep3B-Luc (Axin1) orthotopic mouse model a Hep3B-luc orthotopic mouse model

CONCLUSIONS
1. Human β-catenin SSO lead ALG-135041 showed position specific effects in modifying targeted RNA: only altering splicing at the 3’ end corresponding to DNA Transactivation Domain.
2. Downstream genes were down-regulated as a result of ALG-135041 treatment and cancer cell growth inhibition was achieved in vitro and in vivo.
3. Retaining E-cadherin interaction in the truncated β-catenin could explain the promising safety results seen in the mouse study and therefore, further study is warranted.

REFERENCES

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