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

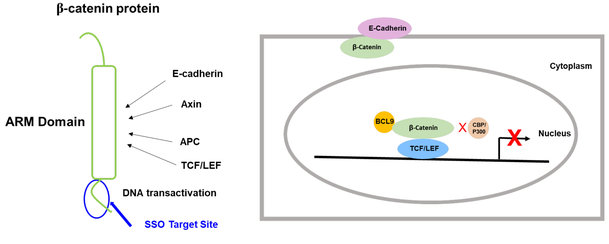
Wnt/β-catenin plays a critical role in embryonic development, tissue homeostasis and repair after injury. Aberrations in this pathway are implicated in many human diseases including cancers. Dysregulation of the Wnt/β-catenin pathway may play a key role in the pathogenesis of Hepatocellular Carcinoma (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-cadherin[3]. 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. SSO in vivo efficacy (10 x 15 mg/kg or 10 x 30 mg/kg, SC, QOD) was carried out in a Hep3B-luc orthotopic mouse model with the positive control sorafenib (20 x 60 mg/kg, PO, QD). Bioluminescence and body weight (BW) were monitored during the study and liver tumor weight was measured at the end of study.

RESULTS

Strategy in precision targeting β-catenin's nuclear function



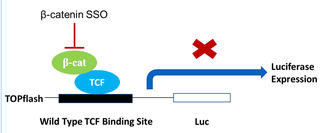
Hypothesis:

1) Truncated β-catenin cannot activate downstream gene expression due to lack of binding with other transcription factors such as CBP/P300
2) Truncated β-catenin has normal functions such as binding with E-cadherin and helps maintain cell-cell interactions

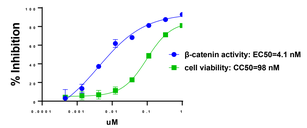
ALG-135041 is a single strand 20 mer SSO with phosphorothioate (PS) bonds

ALG-135041 showed inhibition of the β-catenin pathway and cell growth in hepatoma cell lines

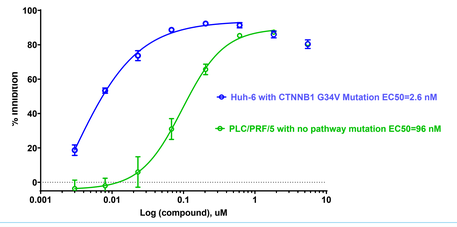
HepG2-TOPflash CTNNB1 aa 25-140 deletion



ALG-135041 transcription and cell viability Inhibitions in HepG2-TOPflash

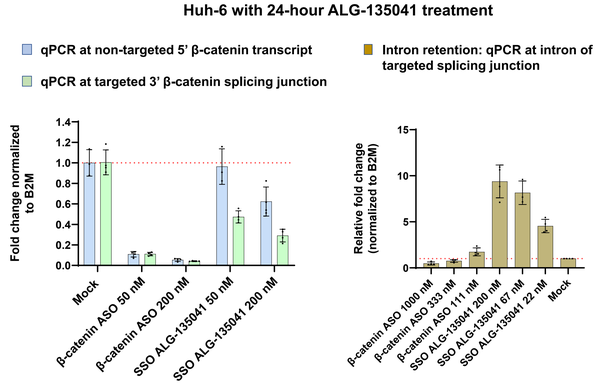


ALG-135041 inhibition of hepatoma cell line growth



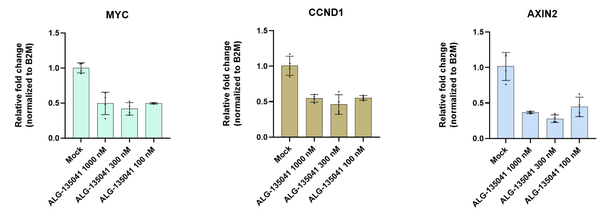
ALG-135041 showed inhibition of proper splicing via intron retention at targeted splicing junction

Huh-6 with 24-hour ALG-135041 treatment

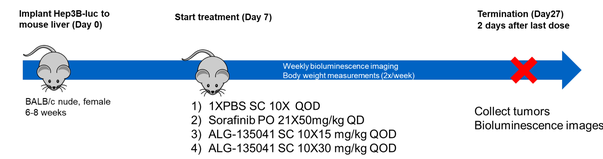


ALG-135041 reduced downstream gene expression

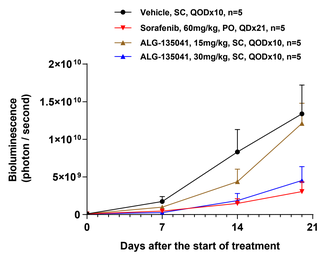
qPCR assays in Huh-6 with 72-hour ALG-135041 treatment



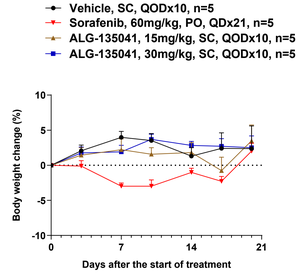
ALG-135041 exhibited a better therapeutic index than sorafenib in the Hep3B-Luc (Axin1) orthotopic mouse model



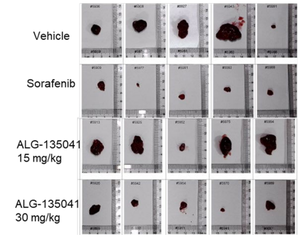
Bioluminescence: ALG-135041 10 x 30 mg/kg showed similar TGI as sorafenib



BW: ALG-135041 10 x 30 mg/kg showed better profile than sorafenib

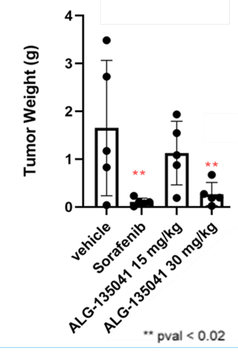


Tumor Image: ALG-135041 10 x 30 mg/kg showed similar TGI as sorafenib

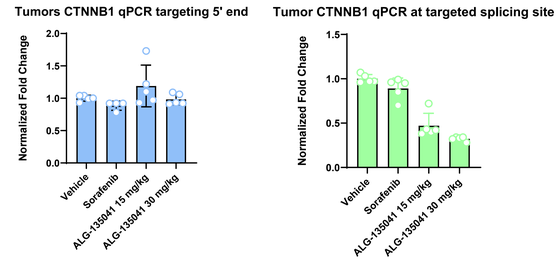


*** TGI = Tumor Growth Inhibition**

Tumor Weight: ALG-135041 10 x 30 mg/kg showed similar TGI as sorafenib



qPCR in harvested tumors demonstrated alteration of targeted CTNNB1 RNA splicing at the 3' end



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

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