Abstract 2629: Epigenetic Modulation of the MYC Oncogene as a Potential Novel Therapy for HCC

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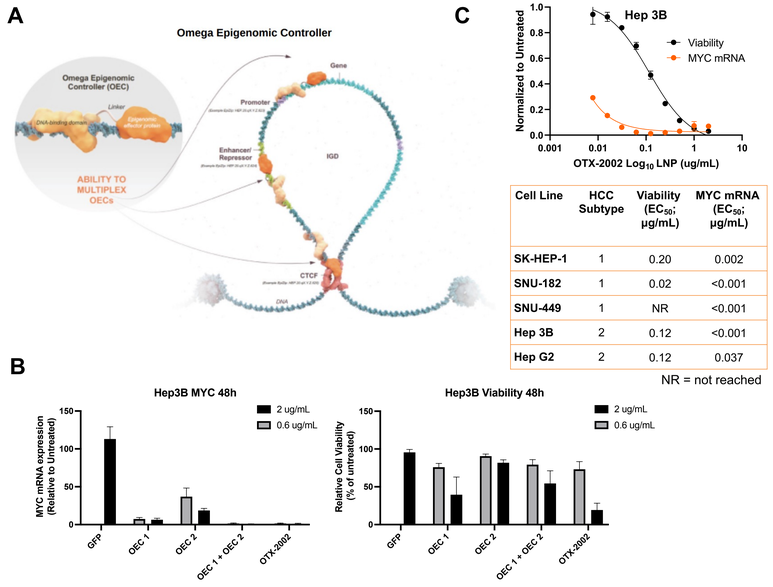
Background

Hepatocellular carcinoma (HCC), a leading cause of cancer deaths worldwide, represents an unmet clinical need with few therapeutic options. Sorafenib, a tyrosine kinase inhibitor, has been used as a systemic therapy for HCC but patients frequently develop resistance with oncogenic c-MYC (MYC) identified as a correlating prognostic factor. MYC over-expression is common in HCC (up in ~70% of viral and alcohol related HCC) and associated with aggressive disease. While MYC represents an attractive therapeutic target in many tumor types, it has historically been considered undruggable, largely because it lacks a structured binding pocket, and its expression is tightly autoregulated. The MYC gene is part of an insulated genomic domain (IGD), a DNA loop bound by CTCF (CCCTC-binding factor). Publications have shown that disruption of the MYC IGD can reduce MYC expression. Our approach is to epigenetically modulate levels of MYC expression, pre-transcriptionally, by utilizing targeted mRNA therapeutics: Omega Epigenomic Controllers (OECs). Here we present preclinical proof of concept data with our development candidate OEC, OTX-2002, which shows:

- The first systematic approach to use mRNA therapeutics delivered by lipid nano-particles as programmable epigenetic medicines
- Disruption of MYC expression pre-transcriptionally by epigenetic regulation
- Precise and specific targeting of MYC in HCC in vitro and in vivo

Figure 1. OECs Directed to the MYC IGD Decrease MYC mRNA Levels and HCC Viability

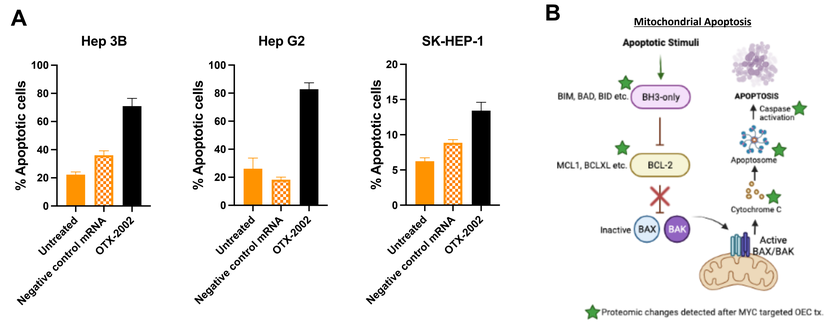


Cell Line	HCC Subtype	Viability (EC_{50}; µg/mL)	MYC mRNA (EC_{50}; µg/mL)
SK-HEP-1	1	0.20	0.002
SNU-182	1	0.02	<0.001
SNU-449	1	NR	<0.001
Hep 3B	2	0.12	<0.001
Hep G2	2	0.12	0.037

NR = not reached

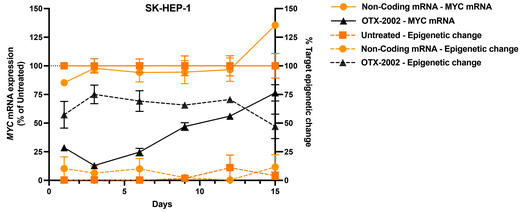
(**A**) Mechanism of action of Omega Epigenomic Controllers (OECs); OECs are mRNA therapeutics delivered in lipid nanoparticles and utilize intrinsic cellular machinery to express 2 proteins, a DNA binding domain and an epigenetic effector protein to modulate gene expression by binding regulatory regions within IGDs (**B**) MYC mRNA (left) and cell viability (right) measured in Hep 3B cells 48 h after treatment with 0.6 or 2 µg/mL of GFP mRNA, OEC1, OEC2, a combination of OEC1 + OEC2 or OTX-2002 (a bicistronic mRNA encoding both OEC1 and OEC2) (**C**) Representative dose curve in Hep 3B of OTX-2002 where MYC mRNA and cell viability were analyzed at 72 h (top) and EC_{50} analysis of OTX-2002 treatment in five HCC cell lines which represent two of the major HCC tumor subtypes (bottom). NR = not reached

Figure 2. OECs Directed to the MYC IGD Induce Apoptotic Cell Death in HCC Cell Lines



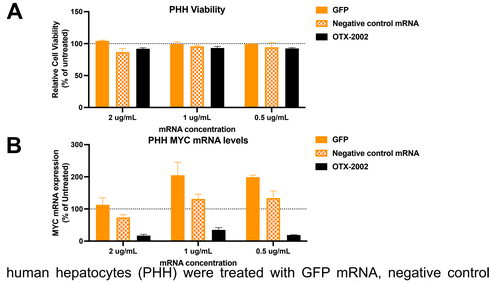
(**A**) Annexin V/PI staining of three HCC cell lines treated with either negative control mRNA or MYC targeted OEC, OTX-2002, for 72 h (**B**) Pathway representing mitochondrial apoptosis; stars indicate pathway alterations found in HCC cells treated with MYC targeted OECs through proteomic analysis (Somalogic).

Figure 3. MYC Targeted OECs Exhibit Durable MYC mRNA Repression and Epigenetic Modifications



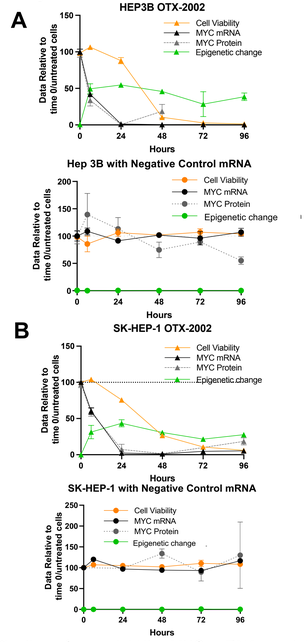
Evaluation of durability of MYC targeted OEC OTX-2002 over 15-day analysis of MYC mRNA expression and targeted epigenetic change as compared to negative control (non-coding) mRNA.

Figure 4. MYC mRNA but not Viability of Primary Human Hepatocytes is Affected by OEC Treatment In Vitro



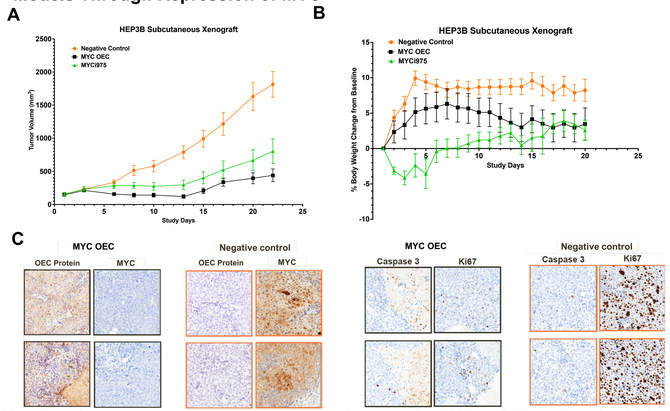
Primary human hepatocytes (PHH) were treated with GFP mRNA, negative control mRNA, or OTX-2002 at 3 doses and (**A**) cell viability and (**B**) MYC mRNA were analyzed. OTX-2002 reduced MYC mRNA expression with no effect on cell viability at 72 h post treatment.

Figure 5. OECs Repress MYC mRNA and Protein Through Epigenetic Changes Prior to Cell Viability Loss



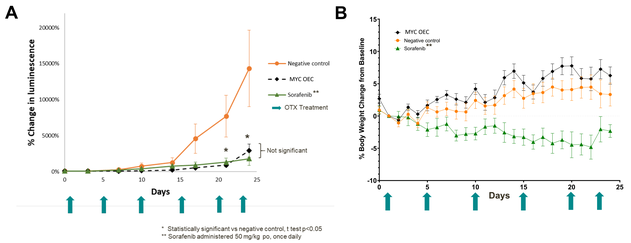
Kinetics of cell viability, MYC mRNA, protein and epigenetic modifications in (**A**) Hep 3B or (**B**) SK-HEP-1 treated with either OTX-2002 or negative control mRNA.

Figure 6. OECs Reduce Tumor Growth in HCC Subcutaneous Xenograft Models Through Repression of MYC



Hep 3B subcutaneous tumors treated with negative control mRNA or OEC at 3 mg/kg Q5D or MYCi975 (a nonclinical MYC inhibitor) at 100 mg/kg IP QDx5. OEC treated mice showed (**A**) a reduction in tumor growth with (**B**) minimal effect on body weight compared to negative control. (**C**) IHC staining from 2 extracted terminal tumors (48 h post last dose) from each group treated with negative control mRNA or MYC OEC. OEC treated tumors show expression of OEC, decrease in MYC and proliferation (Ki67) and increase in apoptosis (Caspase 3).

Figure 7. OECs Reduce Tumor Growth in HCC Orthotopic Xenograft Models



Hep 3B orthotopic tumors were treated with negative control mRNA or OEC at 3 mg/kg Q5D or sorafenib 50 mg/kg PO QD. OEC treated mice showed (**A**) a reduction in tumor burden in the mouse liver with (**B**) minimal effect on body weight.

Conclusions

- OECs tuneably regulate MYC mRNA and protein expression through precise epigenetic targeting of the MYC IGD
- Downregulation of MYC in HCC cells results in the loss of viability of MYC-addicted cancer cells while sparing normal cells
- Delivery of OECs to HCC xenograft models reduce tumor growth and is well tolerated as demonstrated by lack of animal weight change
- This epigenetic control of the MYC oncogene should be potentially applicable to other MYC-dependent tumors