Abstract P-307: Modulation of the MYC oncogene using programmable epigenetic mRNA therapeutics as a novel therapy for hepatocellular carcinoma

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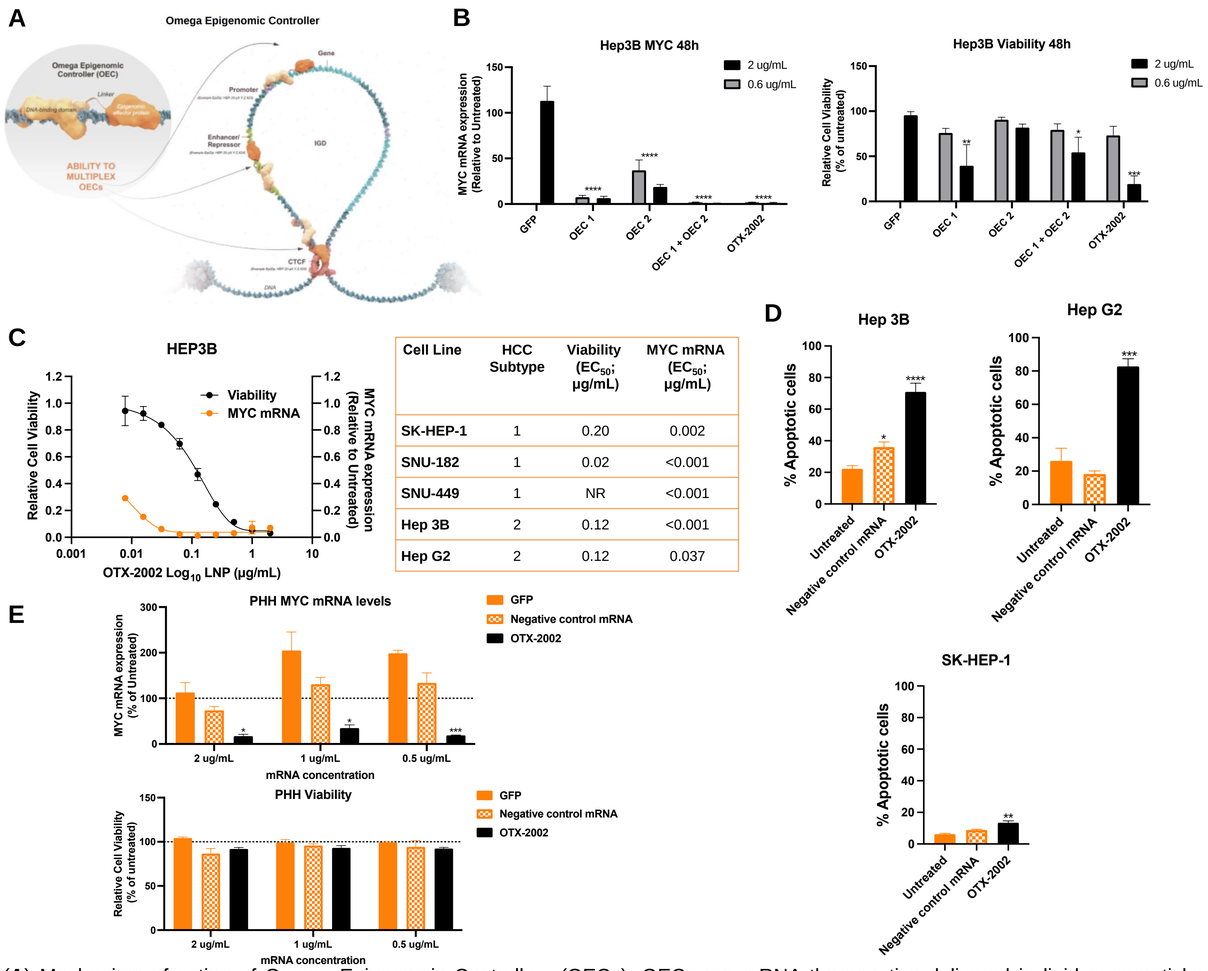
Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer deaths worldwide with a significant unmet medical need. MYC over-expression is associated with aggressive disease in up to ~70% of HCC. While MYC represents an attractive therapeutic target, it has historically been considered undruggable, due to a lack of a structured binding pocket and tightly regulated expression. The MYC gene and its regulatory elements are part of an insulated genomic domain (IGD), a chromatin looping region anchored by CTCF. At Omega, our approach is to target IGDs and epigenetically modulate gene expression pre-transcriptionally utilizing engineered, programmable, mRNA therapeutics called Omega Epigenomic Controllers (OECs). Here we present preclinical proof of concept data with our development candidate OEC, OTX-2002, which shows:

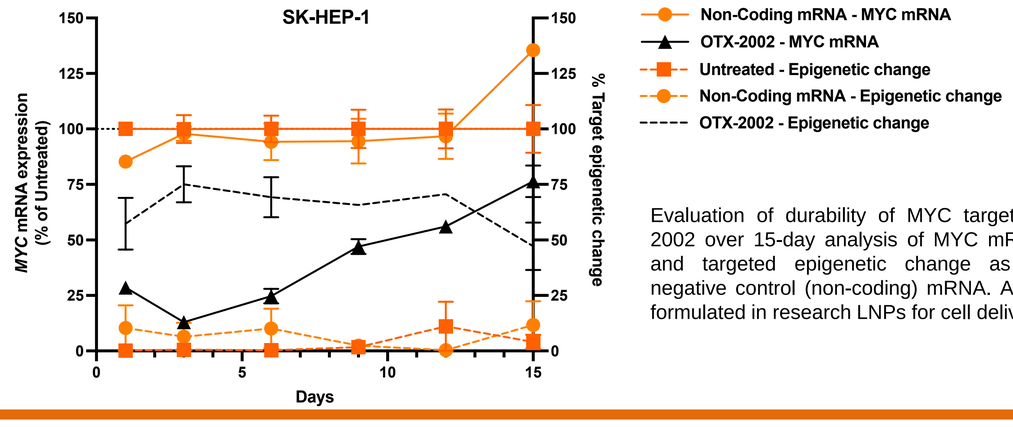
- First demonstration of preclinical proof of concept for mRNA therapeutics as programmable epigenetic medicines
- Precise targeting of MYC and potent antitumor activity in in vitro and in vivo models of HCC, including target modulation in relevant tissues in non-human primates
- OTX-2002 efficacy as a monotherapy and in combination with HCC standard of care compounds
- Evidence that OTX-2002 regulates tumor PD-L1 expression

Figure 1. OECs directed to the MYC IGD decrease MYC mRNA levels and HCC viability with no effect on normal hepatocyte viability



(**A**) Mechanism of action of Omega Epigenomic Controllers (OECs); OECs are mRNA therapeutics delivered in lipid nanoparticles (LNPs) 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); statistics indicate significance vs. GFP treated samples (**C**) Representative dose curve in Hep 3B of OTX-2002 where MYC mRNA and cell viability were analyzed at 72 h (right) and EC_{50} analysis of OTX-2002 treatment in five HCC cell lines which represent two of the major HCC tumor subtypes (left) NR = not reached (**D**) Annexin V/PI staining of three HCC cell lines treated with either negative control mRNA or MYC targeted OEC, OTX-2002, for 72 h; statistics indicate significance vs. untreated samples (**E**) Primary human hepatocytes (PHH) were treated with GFP mRNA, negative control mRNA, or OTX-2002 at 3 doses and MYC mRNA (Top) or cell viability (Bottom) was analyzed; statistics indicate significance vs. GFP treated control. All mRNAs were formulated in research LNPs for cell delivery.

Figure 2. 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. All mRNAs were formulated in research LNPs for cell delivery.

Figure 7. Proteomic analysis reveals pathways whose inhibition synergizes with OTX-2002

Proteomic analysis of SKHEP1 cells treated with MYC targeted OECs showed increase in protein levels of the pro-survival AKT protein; OTX-2002 shows combinatorial efficacy with AKT inhibitor MK-2206. All mRNAs were formulated in research LNPs for cell delivery.

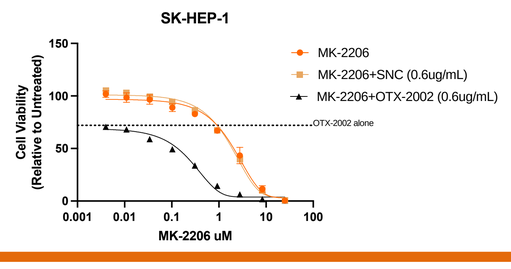
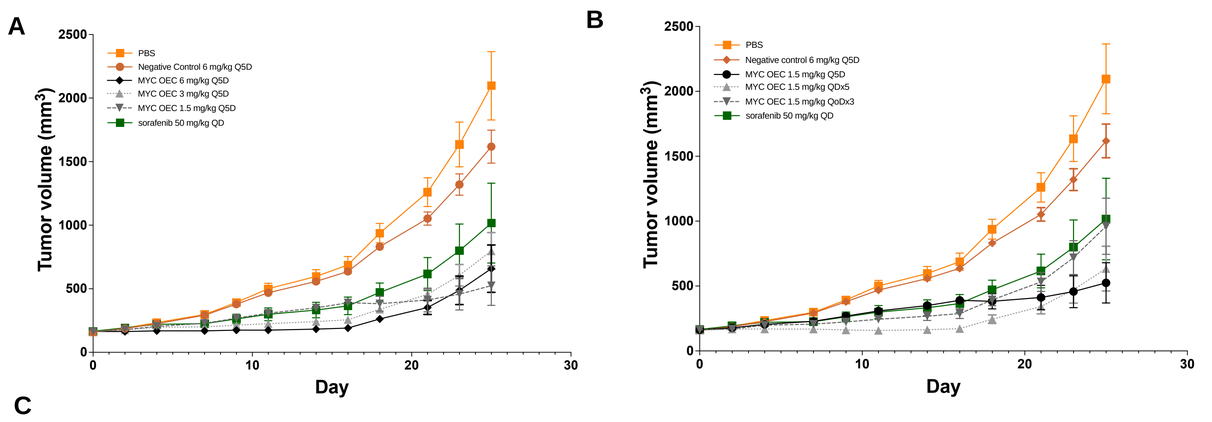
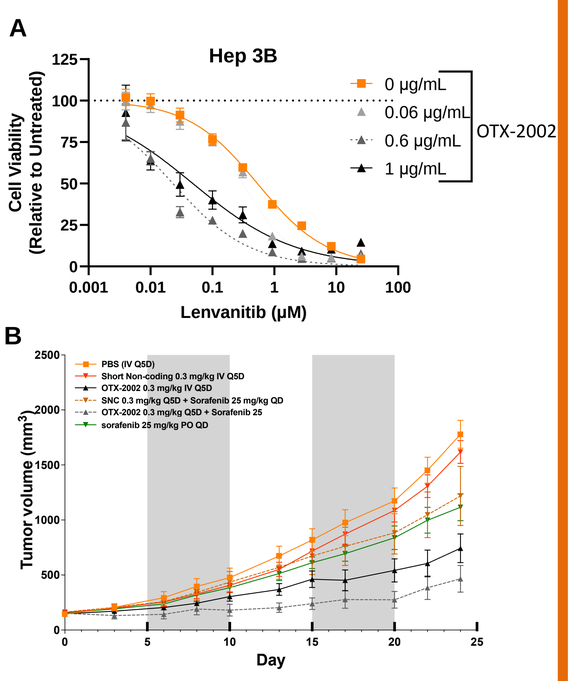


Figure 3. MYC targeted OECs reduce tumor burden in subcutaneous and orthotopic HCC xenograft models



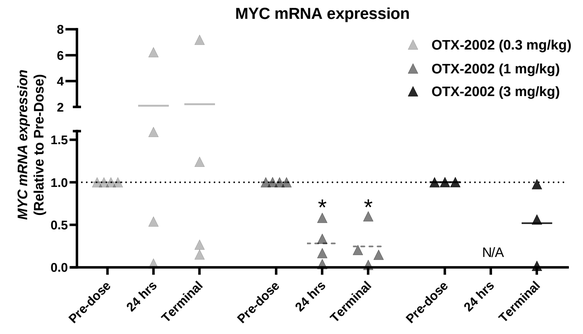
(A) MYC targeted OEC efficacy in Hep3B subcutaneous xenograft at 6 mg/kg, 3 mg/kg and 1.5 mg/kg dosed once every 5 days. mRNA was formulated in research LNPs. **(B)** MYC targeted OECs efficacy in Hep3B subcutaneous xenograft dosed at 1.5 mg/kg Q5D, QDx5, and QoDx3. mRNA was delivered in research LNPs **(C)** OTX-2002 (formulated in Acuitas LNPs for in vivo delivery) efficacy in orthotopic HCC xenograft model, generated by Hep3B implant into mouse livers.

Figure 4. OEC OTX-2002 increases potency of lenvatinib and sorafenib



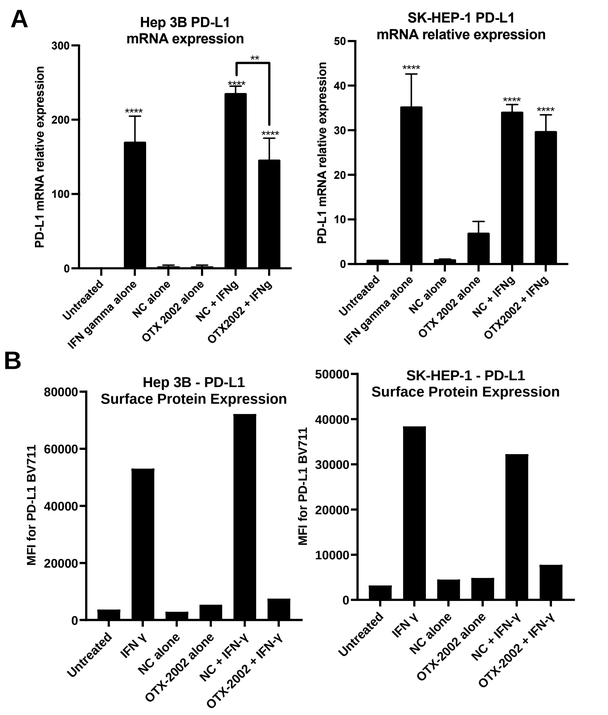
(**A**) Dose response studies of Lenvatinib in Hep 3B cells with and without increasing doses of OTX-2002 (delivered in research LNPs) **(B)** In vivo assay evaluating OTX-2002 alone or in combination with sorafenib. Animals were dosed once every 5 days at sub-optimal doses of sorafenib and OTX-2002 in order to evaluate combinatorial efficacy. mRNAs were formulated in Acuitas LNPs for in vivo delivery (In vitro synergy has also been demonstrated; data not shown).

Figure 5. OTX-2002 reduces MYC mRNA levels in NHP liver biopsies



MYC mRNA analysis by RT-qPCR from NHP liver biopsies. Animals were treated with 0.3, 1, or 3 mg/kg of OTX-2002 and biopsies were taken prior to dosing, 24 hrs post one dose, and at termination. N/A; Samples were not collected at this time point. Significant difference was calculated using multiple t-Test comparison of each timing point per dose to pre-dose samples (*p<0.05). mRNAs were formulated in Acuitas LNPs for in vivo delivery

Figure 6. OTX-2002 represses IFN-γ induced PD-L1 expression on tumor cells



(A) OTX-2002 effect on PD-L1 mRNA expression after interferon gamma treatment; statistics indicate significance vs. untreated **B)** MYC repression by OTX-2002 blocks interferon gamma induction of PD-L1 surface expression as quantified by Anti-PD-L1-BV711 MFI. All mRNAs were formulated in research LNPs for cell delivery.

Conclusions:

- OTX-2002 downregulation of MYC in HCC cells results in the loss of viability of MYC-addicted cancer cells while sparing normal cells
- OTX-2002 shows strong in vivo activity through tunable downregulation of MYC in subcutaneous and orthotopic human liver cancer models in mice
- OTX-2002 shows downregulation of MYC in serial biopsies from nonhuman primate livers
- OTX-2002 in combination with HCC SOC sorafenib and lenvatinib demonstrate synergy in vitro and in vivo suggesting the potential for clinical benefit and/or improved tolerability using lower doses
- Proteomic studies also suggest potential advantageous combinations of OTX-2002 with novel agents
- In vitro studies demonstrate a reduction in PD-L1 expression after OTX-2002 treatment, indicating possible immunomodulatory activity
- OTX-2002 effectively targets the MYC oncogene and could provide great therapeutic benefit to a variety of MYC driven cancers

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Basic Hepatocellular Cancer

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