Effect of MYC-Targeting Programmable Epigenomic mRNA Therapeutics on TME and Immunotherapy Responses

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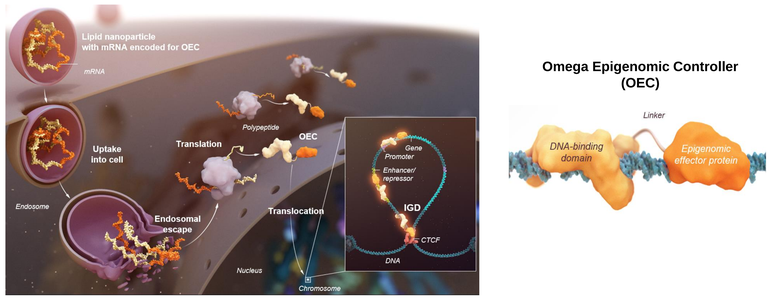
Background

- c-MYC is a master transcription factor (TF) critical for multiple cell physiologies.
- As a pleiotropic TF, MYC regulates the tumor microenvironment (TME) and impacts cancer cell initiation, growth, and survival.
- Although MYC expression is normally tightly controlled in normal cells, dysregulated MYC expression is a driver of oncogenic transformation in multiple tumor types. (e.g., HCC, NSCLC, Burkitt's lymphoma).
- A direct MYC-targeting anti-cancer agent has remained elusive, largely due the absence of a well-defined drug binding pocket and tight autoregulation.
- The MYC gene resides alone with its regulatory elements within an insulated genomic domain (IGD) and represents a potential therapeutic target for pre-transcriptional gene modulation via an epigenetic approach for the treatment of multiple cancers including HCC.
- We are developing programmable epigenomic mRNA medicines designed to controllably tune gene expression, pre-transcriptionally, with defined durability with high specificity by targeting IGDs and regulatory elements within it.
- We have rationally designed Omega Epigenomic Controllers (MYC-OEC: clinical candidate OTX-2002; development candidate MYC Lung OEC; and mouse-sequence surrogate muMYC OEC) to downregulate MYC expression, thereby selectively killing cancer cells while sparing normal cells.
- We investigated the role of MYC-OECs in the modulation of the TME and enhanced antitumor activity of checkpoint blockade inhibitors (CBI) in vivo.

Figure 1. Structure and Mechanism of Action of OECs



Mouse (mu)MYC OEC Decreases MYC mRNA and Protein and the Viability of Mouse Liver Cancer Cells

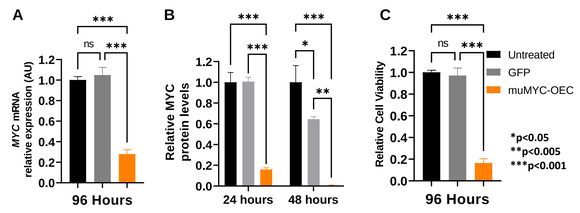


Figure 2. In vitro results in Hepa1-6 mouse liver cancer cells. Hepa1-6 mouse liver cancer cells were treated with GFP or mouse-selective MYC Omega Epigenomic Controller (muMYC OEC) in a lipid nanoparticle (LNP) then **(A)** MYC mRNA, **(B)** MYC protein and **(C)** cell viability were assessed.

MYC OECs Reduce Interferon γ-induced Surface Expression of PD-L1 in HCC and NSCLC Cell Lines

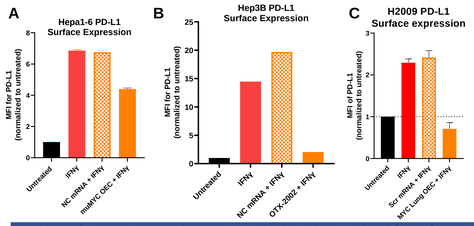


Figure 3. PD-L1 mRNA and surface protein expression in human liver cancer Hep 3B cells. Surface PD-L1 protein expression was assessed 24 hrs after Interferon gamma (IFN-γ) simulation and 48 hrs after LNP transfection with MYC OEC (OTX-2002 or MYC Lung OEC) or negative control (NC or Scr) mRNA. **(A)** Hepa1-6 (mouse liver); **(B)** Hep 3B (human HCC cells) or **(C)** H2009 (NSCLC cells) were treated.

Combination of muMYC OEC With Checkpoint Blockade Immunotherapy (CBI) Decreases Hepa1-6 Tumor Growth

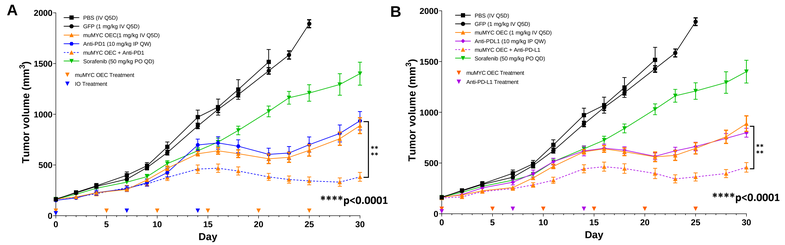


Figure 4. MuMYC OEC combination with checkpoint blockade inhibitors in Hepa1-6 syngeneic mouse model. C57BL/6 mice bearing subcutaneous Hepa1-6 tumors were divided into 9 treatment groups (10-12 mice each). Negative control GFP mRNA and muMYC OEC were evaluated by dosing at 1 mg/kg intravenous injection (IV) every 5 days (Q5D). **(A)** Anti-PD-1 or **(B)** anti-PD-L1 at 10 mg/kg intraperitoneal (IP) once per week (QW).

muMYC OEC Alone or in Combination with Anti-PD1 Treatment Represses Regulatory T-cells (Tregs) in the Hepa1-6 Mouse Tumors

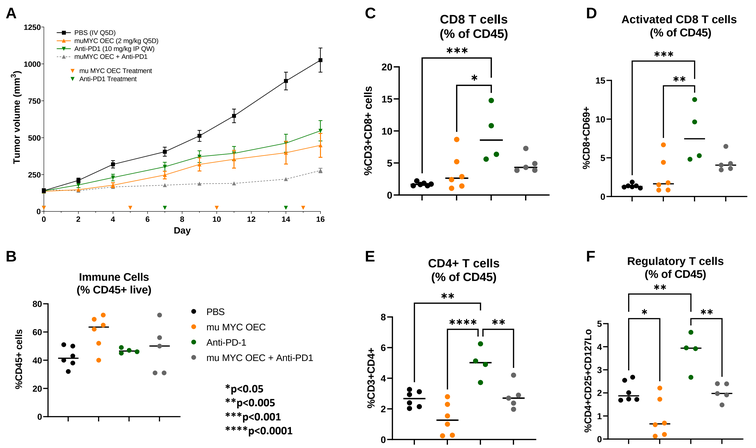


Figure 5. Combination of muMYC OEC and anti-PD-1 in Hepa1-6 syngeneic mouse model of HCC to assess infiltrating immune cells in the tumor microenvironment using flow cytometry. C57BL/6 mice bearing subcutaneous Hepa 1-6 tumors were split into 4 groups (14 mice each) and treated with PBS, muMYC OEC (2 mg/kg IV Q5Dx4), anti-PD-1 (10 mg/kg IP QWx3) or a combination of muMYC OEC + anti-PD-1. **(A)** Tumor growth; **(B)** Six tumors from each group were freshly collected to measure tumor immune cell profiling (CD45+); **(C)** total CD8+ T cells (CD45+CD3+CD8+); **(D)** activated CD8 T cells (CD45+CD3+CD8+CD69+); **(E)** CD4+ T cells (CD45+CD3+CD4+); **(F)** regulatory T cells (Tregs; CD45+CD3+CD4+CD26+CD127Lo).

muMYC OEC Alone or in Combination With CBI Confers Immune Memory

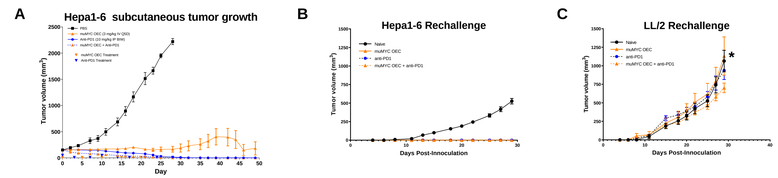


Figure 6. Assessment of immune memory of muMYC OEC using mouse syngeneic models.C57BL/6 mice bearing subcutaneous Hepa1-6 tumors were divided into 4 groups (20 mice each for treatment groups and 10 mice PBS control) and treated with PBS, muMYC OEC (3 mg/kg IV Q5Dx6), anti-PD-1 (10 mg/kg IP BIWx4), or in combination. **(A)** Anti-PD-1 and combination treatments eliminated 20/20 Hepa1-6 tumors; muMYC OEC eliminated 14/20 tumors. About 70 days after last dose, age matched mice naïve to Hepa1-6 cells and previously treated mice with no visible Hepa1-6 tumors were rechallenged; left flank with Hepa1-6 cells and right flank with LL/2 cells. **(B)** Hepa1-6 and **(C)** LL/2 subcutaneous tumors. *Two outlier mice were removed from muMYC OEC pretreatment group implanted with LL/2

Immune Cell Depletion Shows muMYC OEC Single Agent is Partially Driven Through Adaptive But Not Innate Immunity

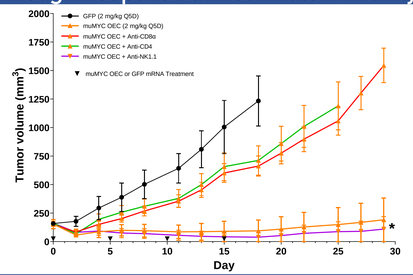


Figure 7. Immune cell depletion in combination of muMYC OEC and CBI in Hepa1-6 mouse model to assess contributions of adaptive and innate immunity to tumor growth inhibition. C57BL/6 mice bearing Hepa1-6 tumors were left untreated or pre-treated (PT) anti-CD4, anti-CD8α, or anti-NK1.1 antibodies twice (400 µg Q5D) then split into 5 subgroups of 8 mice each. Each of these subgroups were treated as follows: Untreated with muMYC OEC or GFP mRNA; anti-CD4, anti-CD8α, or anti-NK1.1 pretreatment groups with muMYC OEC. GFP mRNA or muMYC OEC dosed at 2 mg/kg IV Q5Dx4.

*One outlier mouse removed from muMYC OEC single agent analysis

Conclusions

- MYC OECs downregulate MYC expression in HCC cells resulting in the loss of viability of MYC-addicted cancer cells.
- MYC OECs downregulate expression of PD-L1 protein on the surface of tumor cells.
- MYC OECs in combination with CBI (anti-PD-1 or anti-PD-L1) significantly reduce HCC xenograft tumor growth compared to either single agent alone at well-tolerated doses.
- Antitumor activity of MYC OECs is partially driven through an adaptive immune response (T-cells).
- MYC OECs as a single agent or in combination with CBI represses inhibitory Tregs to more effectively enlist the adaptive immune system to inhibit HCC tumors.

In summary, these results support the evaluation of MYC OECs in combination with CBIs in cancer patients. OTX-2002 is currently being evaluated in a Phase 1/2 clinical trial as a monotherapy in patients with HCC and other solid tumor types known for association with the MYC Oncogene and in combination with SoC including CBIs for patients with HCC (NCT05497453).

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