Detection and quantification of site-specific DNA methylation from liquid biopsies as a pharmacodynamic biomarker of OTX-2002, a novel MYC-targeting epigenomic controller

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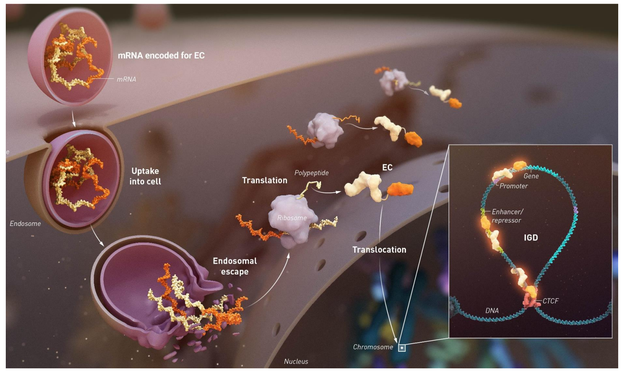
Introduction

Omega Therapeutics has developed a novel platform of programmable epigenomic mRNA medicines capable of modifying chromatin state to specifically tune gene expression at the pre-transcriptional level. OTX-2002 is a first-in-class mRNA therapeutic delivered via lipid nanoparticles (LNP) that is pioneering clinical development of epigenomic controllers (ECs). The MYCHELANGELO I trial (NCT05497453) investigates pre-transcriptional inhibition of *MYC* with OTX-2002 in patients with hepatocellular carcinoma (HCC). OTX-2002 encodes two proteins that durably modify chromatin, in part, through CpG DNA methylation at the *MYC* locus. We have previously shown that EC-directed *MYC* methylation leads to concomitant downregulation of *MYC* expression and loss of HCC cellular viability *in vitro* and inhibition of HCC xenograft growth *in vivo*.[1]

Here, we demonstrate the utility of a minimal hybridization-based target enrichment assay that enables ultra-deep DNA methylation sequencing from liquid biopsies to assess target engagement of OTX-2002.

Figure 1. EC Mechanism of Action



Methods

Preclinical studies included mice bearing subcutaneous Hep3B HCC xenografts that were intravenously dosed with OTX-2002 or control. cfDNA was extracted from plasma using the QIAmp Circulating Nucleic Acid kit.

DNA methylation was assessed using EM-conversion (NEB) followed by NGS library preparation, using Twist Bioscience's NGS Methylation Detection System for target enrichment where indicated. The *MYC* Methylation enrichment panel spanned a total of 51.5 kb, including both the *MYC* promoter and gene body as well as promoter CpG islands from control genes. Epiallele detection, measured as the variant epiallele fraction (VEF), was performed using the EpiAlleleR package[2] after Bismark mapping to identify methylated *MYC* molecules as opposed to averaging per-CpG rates over a region.

Methylation Detection and Assay Characterization

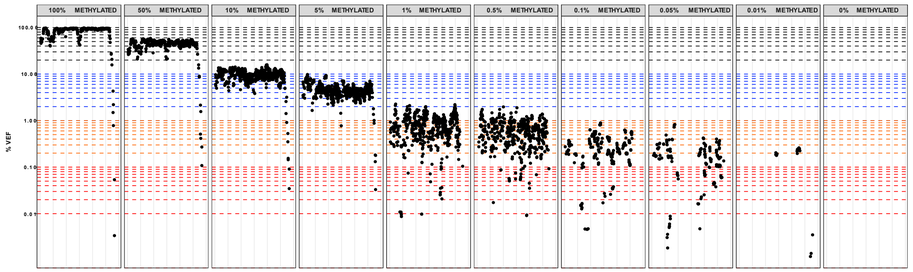
Table 1. Whole-genome methylation sequencing identifies OTX-2002-dependent methylated *MYC* from ctDNA derived from a subcutaneous HCC xenograft model

Treatment	Total Reads	Tumor Reads	Tumor Fraction	% Methylated *MYC* Reads* (# reads)
PBS	780M	190K	0.025%	0 (1)
PBS	679M	180K	0.026%	0 (0)
OTX-2002	714M	820K	0.115%	0 (0)
OTX-2002	709M	51M	7.192%	8.6% (38)

**(+/- 2kb TSS)*

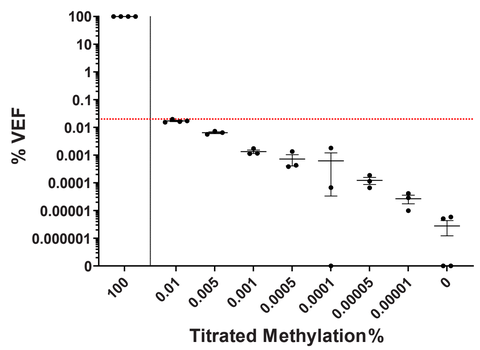
Whole-genome methylation sequencing was performed on cell-free DNA (cfDNA) extracted from animals treated with OTX-2002 or PBS control. Reads were mapped to a hybrid human+mouse genome to categorize circulating tumor DNA (ctDNA) based on reads that mapped to human sequences, and the tumor fraction was estimated against the background of mouse-mapping cfDNA. On-target DNA methylation by OTX-2002 was confirmed via detection of ctDNA reads containing methylation at the *MYC* promoter. Circulating tumor MYC fragments were rare overall, indicating the necessity for target enrichment for efficient detection.

Figure 2. The *MYC* Methylation Panel allows for methylation detection in a genomic DNA (gDNA) titration down to the number of copies assayed



To capture rare ctDNA fragments, we designed a minimal hybridization panel for target enrichment (Methods). We initially characterized the assay by performing targeted methylation sequencing on a titration series of methylated control gDNA spiked into unmethylated control gDNA (Zymo Research). Using an analysis pipeline geared towards epiallele detection at *MYC*, the fully unmethylated control gDNA sample showed no methylation signal, whereas we were able to robustly identify *MYC* methylation in samples down to the titration containing 0.05% methylated gDNA. This was at the theoretical limit of the number of copies present in the assay.

Figure 3. The *MYC* Methylation Panel detects methylation down to 0.00001% using synthetic controls



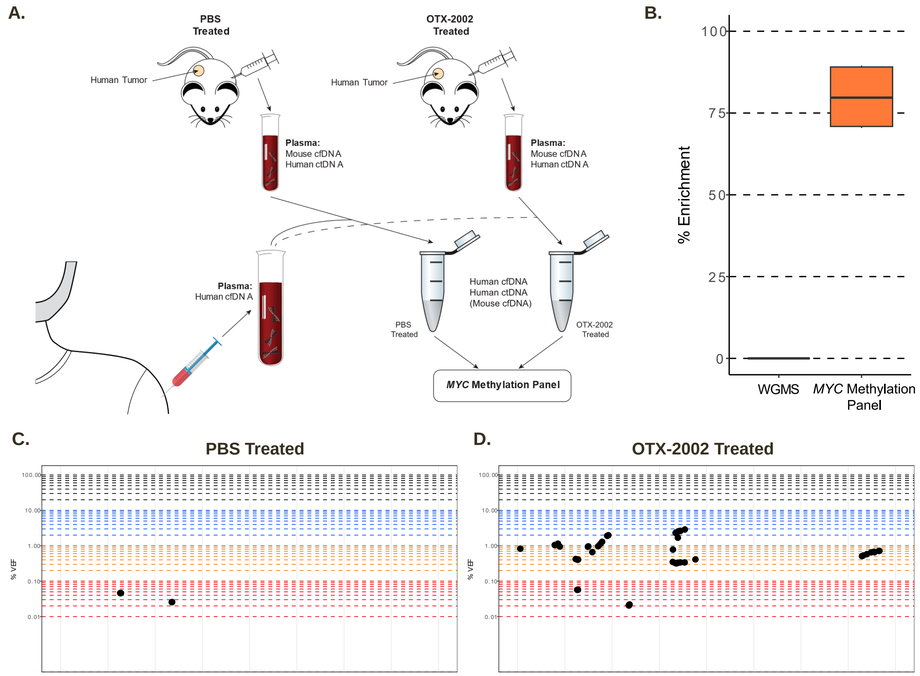
From a detection perspective, ctDNA represents a distinct challenge from gDNA, as molar ratios of various fragments become skewed.

To understand the raw performance of the hybrid capture platform in the absence of conversion methods (enzymatic- or bisulfite-based), oligonucleotides were synthesized to represent the *MYC* promoter in a CpG converted state either fully "methylated" or fully "unmethylated." These oligos were mixed at known ratios to establish an ultra-low titration series and spiked into a background of mouse gDNA prior to hybridization and library construction.

We observed reproducible epiallelic detection down to 0.00001% methylated alleles compared to a fully unmethylated control (equivalent to 1 methylated allele in 10 million unmethylated alleles). The dashed red line indicates the estimated average methylation of each group when analyzed on an averaged, per-CpG basis.

Preclinical Detection of OTX-2002-Dependent *MYC* Methylation

Figure 4. Detection of OTX-2002-dependent *MYC* methylation within cfDNA samples mimicking clinical derivation



A. Experimental schematic for cfDNA samples. Since cfDNA from the preclinical Hep3B xenograft model contains human ctDNA in a background of mouse cfDNA, we examined if the sensitivity of the assay would be sufficient to survey clinical samples. We performed a spike-in experiment where cfDNA extracted from a pool of 3 mice treated with OTX-2002 or PBS control was diluted into cfDNA extracted from healthy human plasma. Despite the excess murine cfDNA (which is excluded from the *MYC* Methylation Panel), these samples intend to mimic the human ctDNA:cfDNA dilution in potential clinically-derived samples. **B.** The *MYC* Methylation Panel provides the necessary enrichment over whole-genome sequencing to detect regions of interest. **C.** cfDNA from PBS-treated mice did not have appreciable *MYC* methylation. **D.** In comparison, *MYC* methylation was robustly detected in cfDNA from OTX-2002-treated mice even when diluted in cfDNA from healthy human donors. VEF plots include data points observed down to the limit of detection.

Conclusions

- A minimal capture-hybridization panel enables ultra-deep methylation sequencing with the technical ability to detect rare methylation alleles.
- Target engagement by OTX-2002 can be identified by *MYC* methylation signal detection in cfDNA.
- A version of this assay is being used to evaluate *MYC* methylation in plasma and tumor tissue samples in the Phase 1/2 MYCHELANGELO I trial of OTX-2002 in Hepatocellular Carcinoma and Other Solid Tumor Types Associated with the *MYC* Oncogene (NCT05497453).

References

1. Senapedis W, et al. (2022) https://doi.org/10.1158/1538-7445.AM2022-2629
2. Nikolaienko O, et al. (2023) https://doi.org/10.1093/gigascience/giad087