A PRECLINICAL ASSAY PIPELINE TO IDENTIFY AND OPTIMIZE BRACHYURY-TARGETED THERAPIES IN CHORDOMA AND OTHER SOLID TUMORS

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ABSTRACT

- Chordoma is a rare bone cancer of the skull base and axial spine that arises from remnants of the embryonic notochord.
- Disease incidence is 1 per million, with median survival from diagnosis of 8 years.
- There are no approved systemic therapies for the treatment of chordoma. Standard care is maximal surgical resection +/- radiation, which cures ~30% of patients.
- A nearly universal hallmark of chordoma is the expression of *TBXT*, which encodes the T-box transcription factor Brachyury.
- Brachyury (TBXT) is a key driver of chordoma pathogenesis
- Brachyury has been associated with tumor progression, chemotherapy resistance, and poor patient outcomes in other cancers including lung, colon, breast, and prostate
- The objectives of this study are to establish a preclinical assay pipeline that enables the identification and optimization of Brachyury-targeted therapies and assess the role of Brachyury in the progression and response to standard of care therapy of other solid tumor models.

BACKGROUND

- Brachyury expression, amplification, and a single-nucleotide polymorphism (G177D) is strongly associated with sporadic and familial chordoma
- Functional genomics identified Brachyury as the most selectively essential gene in chordoma[1]
- The Structural Genomics Consortium (SGC) recently solved the crystal structure of Brachyury bound to DNA and identified new binding pockets
- Multiple structure-guided, open-source small molecule series bind to distinct Brachyury pockets with low micromolar affinity[2]

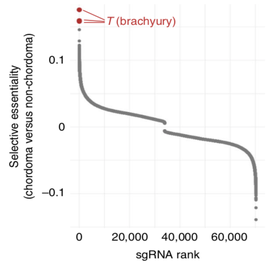

Figure 1. Brachyury promotes chordoma cell proliferation. A genome-wide CRISPR screen identified *TBXT* (Brachyury) as the most selectively essential gene in chordoma[1].

Crystal structure of Brachyury bound to DNA identified new binding pockets

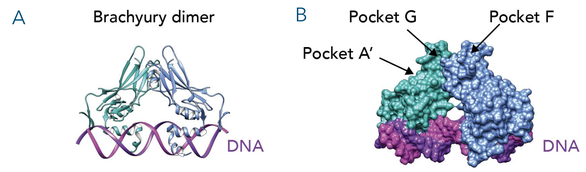

Figure 2. Structure of the Brachyury DNA-binding domain (DBD) bound to DNA[3]. (A) Ribbon diagram of the Brachyury DBD dimer in contact with DNA. (B) Surface rendering of the DBD shows three shallow pockets that are targeted by the open-source small molecules.

OPPORTUNITIES FOR DRUGGING BRACHYURY

Leveraging AI for Brachyury Drug Development

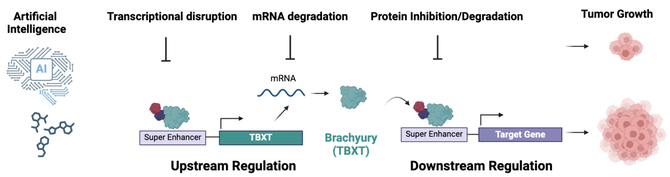

Figure 3. Targeting Brachyury with different therapeutic modalities. Leveraging the power of emerging AI, we aim to target Brachyury transcription, translation, or protein function using antisense oligonucleotides, rSMs, functional inhibitors and/or degraders to repress Brachyury function and inhibit tumor growth.

BUILDING A PRE-CLINICAL ASSAY PIPELINE

Purified full-length Brachyury binds to the Brachyury response element in vitro

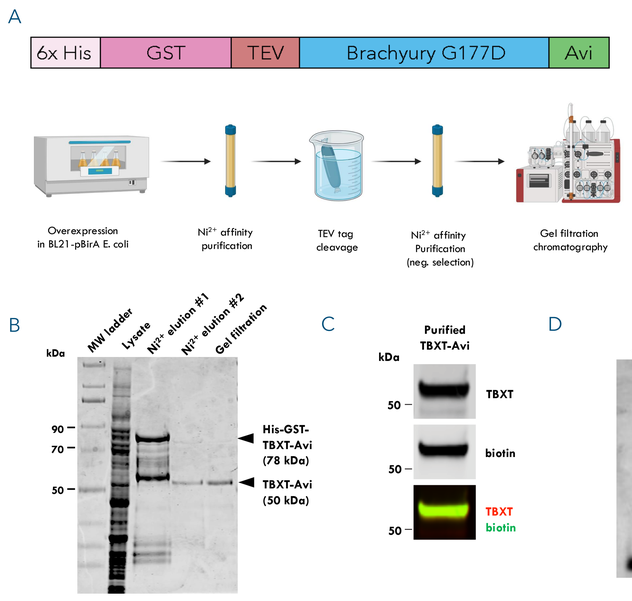

Figure 4. Workflow for Brachyury purification and functional assessment by a DNA-binding assay. (A) Schematic of recombinant Brachyury expression in *E.coli* and purification by nickel-affinity and size-exclusion chromatography. (B) Coomassie-stained protein gel analyzing different steps of the purification process. (C) Purified Brachyury-Avi was immunoblotted for Brachyury and probed with Streptavidin conjugated to an infrared dye. (D) Increasing concentrations of purified Brachyury were incubated with 5 nM of annealed DNA oligos containing the Brachyury response element, resolved by gel electrophoresis under native conditions, and stained with SYBR green.

High-throughput SPR identifies small molecule hits and measures steady-state affinity

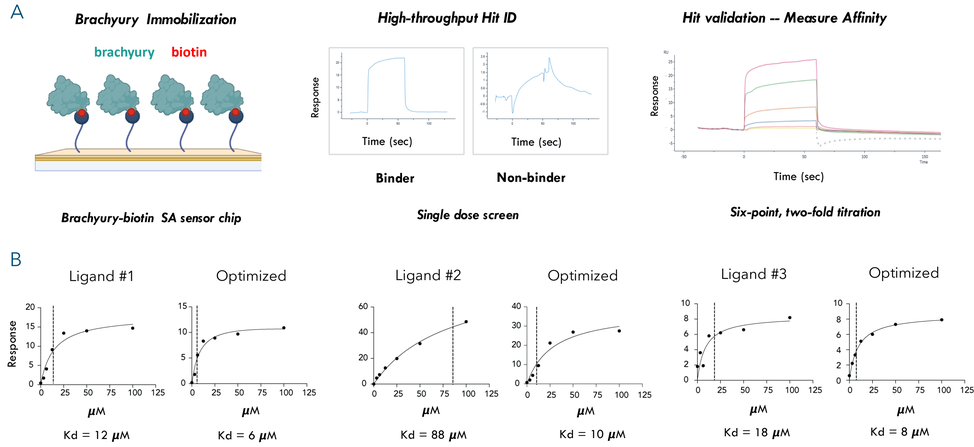

Figure 5. High-throughput Brachyury SPR and the initial optimization of Brachyury ligands. (A) Biotinylated Brachyury is immobilized on a streptavidin-coated sensor chip in a Biacore 8K SPR system. High-throughput Hit Identification using a single dose can distinguish between binders and non-binders. Hits are validated using a six-point, two-fold titration to measure their steady-state affinity. (B) Three Brachyury ligands were optimized by a single round of medicinal chemistry and show higher Brachyury affinity. Binding curves show ligand concentration vs response.

Development and validation of a Brachyury reporter assay

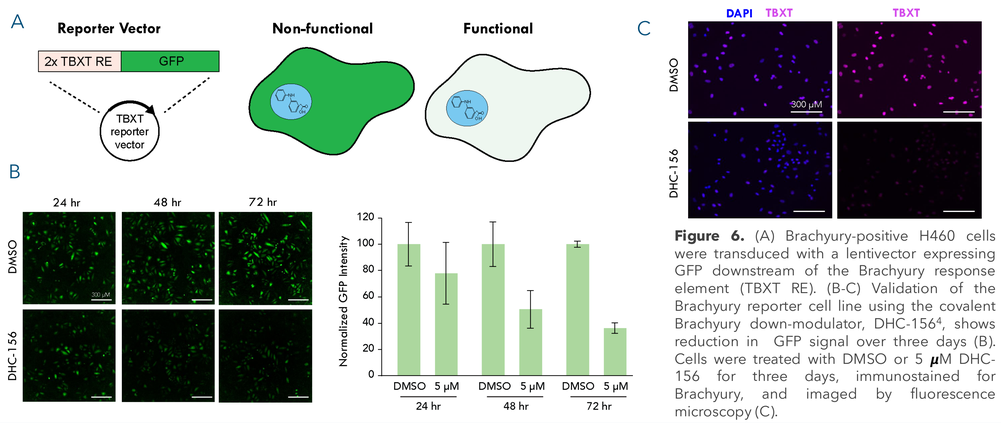

Figure 6. (A) Brachyury-positive H460 cells were transduced with a lentivector expressing GFP downstream of the Brachyury response element (TBXT RE). (B-C) Validation of the Brachyury reporter cell line using the covalent Brachyury down-modulator, DHC-156[4], shows reduction in GFP signal over three days (B). Cells were treated with DMSO or 5 μM DHC-156 for three days, immunostained for Brachyury, and imaged by fluorescence microscopy (C).

BUILDING A PRE-CLINICAL ASSAY PIPELINE

Brachyury knockdown reduces the expression of its target genes

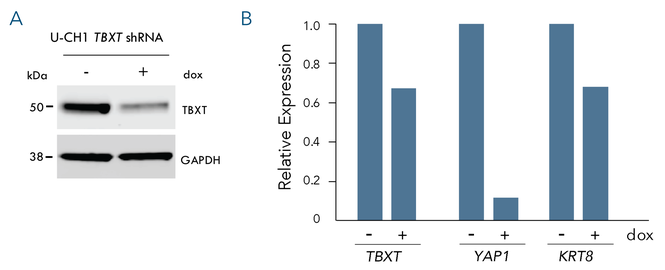

Figure 7. TBXT knockdown in U-CH1 cells reduces the expression of its gene targets. (A) Whole cell lysates from doxycycline-inducible U-CH1 *TBXT* shRNA cells treated with or without doxycycline for six days and immunoblotted for Brachyury and GAPDH. (C) U-CH1 *TBXT* shRNA cells were treated with or without doxycycline for six days and the expression of its targets *TBXT*, *YAP1*, and *KRT8* were assessed by RT-PCR.

Assessing the role of Brachyury in other solid tumors

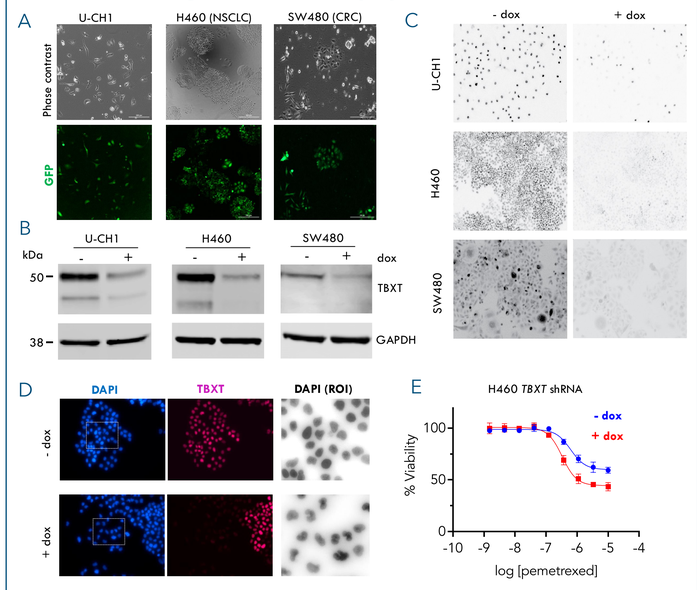

Figure 8. Development of doxycycline-inducible *TBXT* shRNA cell lines to explore the role of Brachyury in non-small cell lung (NSCLC) and colorectal cancers (CRC). (A) U-CH1, H460 (NSCLC), and SW480 (CRC) cells were transduced with a lentivector expressing a doxycycline-inducible *TBXT* shRNA that constitutively expresses GFP. (B) Whole cell lysates from cells treated with or without doxycycline were immunoblotted for TBXT and GAPDH. (C) Cells were treated with or without doxycycline, fixed and immunostained for Brachyury, and imaged by fluorescence microscopy. (D) TBXT knockdown in H460 cells alters nuclear morphology. H460 *TBXT* shRNA cells were cultured with or without doxycycline for four days, fixed and immunostained for TBXT and DAPI, and imaged by fluorescence microscopy. (E) H460 *TBXT* shRNA cells were cultured with or without doxycycline for seven days and treated with a titration of pemetrexed for three days. Cell viability was measured with CellTiter-Glo.

KEY FINDINGS

- Small molecule Brachyury ligands have been discovered and are being optimized for therapeutic development
- Our pre-clinical assay pipeline enables the identification, optimization, and validation of Brachyury-targeted molecules to catalyze drug discovery for chordoma and other Brachyury-positive tumors
- The CF labs biochemical and cell-based functional assay capabilities enable the identification, optimization, and validation of Brachyury-targeted molecules to catalyze drug discovery for chordoma and other Brachyury-positive tumors
- Brachyury inhibition sensitizes non-chordoma tumor cells to standard of care therapy. Ongoing studies will elucidate Brachyury's role in tumor growth and resistance to therapy

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For more information, visit: www.chordoma.org