CATALYZING TBXT AS A THERAPEUTIC TARGET THROUGH AN INTEGRATED DRUG DISCOVERY PIPELINE

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Abstract #: A071

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 approximately 1 per million, with a median survival of 8 years.
- Standard of care consists of maximal surgical resection with or without radiation, which cures only ~30% of patients. Systemic therapies are needed to target advanced disease.
- A nearly universal hallmark of chordoma is expression of TBXT, which encodes the T-box transcription factor Brachyury. TBXT is a key driver of chordoma pathogenesis, and its expression has been associated with tumor progression, therapy resistance, and poor patient outcomes in several other cancers, including lung, colon, and breast.
- We established an integrated preclinical assay pipeline spanning biochemical, structural, cellular, and in vivo approaches to enable TBXT-targeted drug discovery and development.
- We generated an inducible TBXT knockdown mouse model to evaluate the safety and phenotypic consequences of TBXT inhibition.
- Ongoing studies are assessing the broader therapeutic potential of TBXT inhibition in more common cancers that are TBXT-positive such as lung, colon and breast.

BACKGROUND

- Functional genomics identified *TBXT* as the most selectively essential gene in chordoma[1].
- The Structural Genomics Consortium (SGC) recently solved the crystal structure of TBXT DNA-binding domain[2].
- Multiple structure-guided, open-source small molecule series bind to distinct TBXT pockets with low micromolar affinity[2].

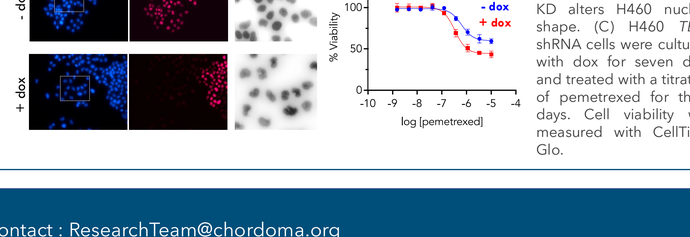


Figure 1. (A) genome-wide CRISPR screen identified *TBXT* (*T*, brachyury) as the most selectively essential gene in chordoma[1]. (B) Surface rendering of the DBD shows two shallow pockets that are targeted by open-source small molecules[2-3].

Animal health and behavior were normal after chronic TBXT knockdown

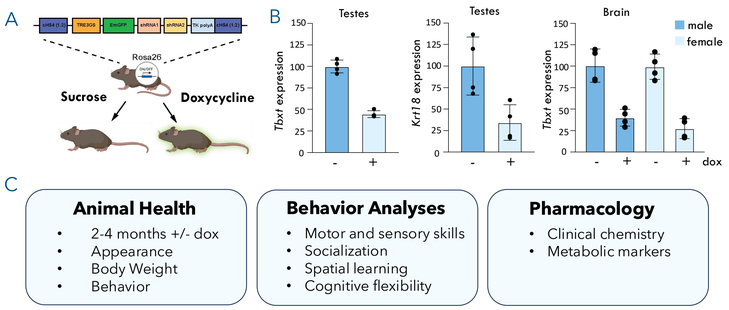


Figure 2. (A) Schematic of the doxycycline-inducible *Tbxt* shRNA mouse model. (B) Dox efficiently reduced *Tbxt* expression in the testes and brain, with decreased *Krt18* expression in the testes confirming functional target knockdown (n = 4 mice). (C) Summary of phenotypic assessments following inducible TBXT knockdown. No significant change in animal health, neurobehavioral function, or clinical chemistry.

Assessing the role of TBXT in other tumor types

Figure 3. (A) Cell lysates were blotted for TBXT and GAPDH or KU80. (B) TBXT KD alters H460 nuclear shape. (C) H460 *TBXT* shRNA cells were cultured with dox for seven days and treated with a titration of pemetrexed for three days. Cell viability was measured with CellTiter-Glo.

OPPORTUNITIES FOR DRUGGING TBXT

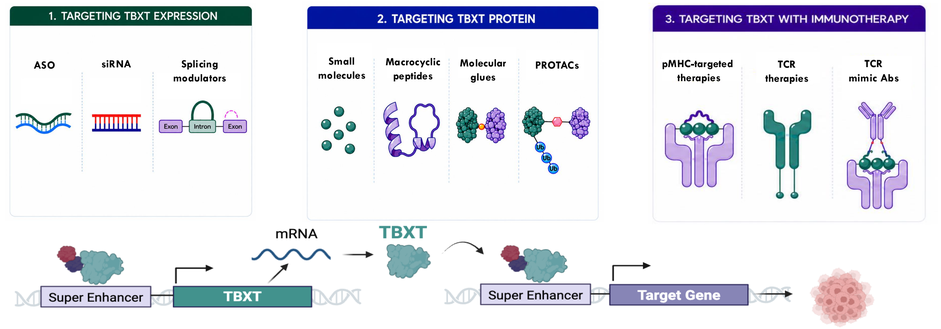


Figure 4. TBXT expression and function can be targeted using different modalities. Figures were generated by DALL-E and modified by the authors

PRECLINICAL ASSAY PIPELINE

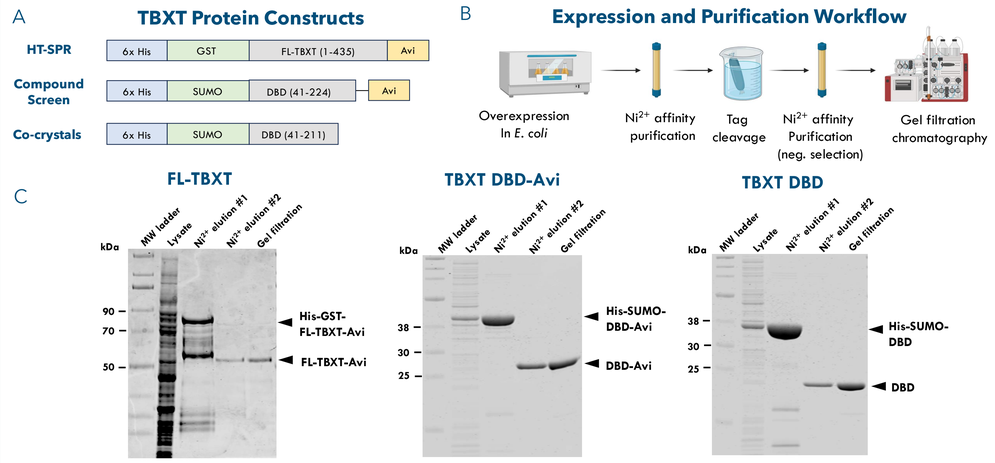


Figure 5. (A) TBXT expression constructs. (B) Schematic of recombinant TBXT expression in *E.coli* and purification by nickel-affinity and size-exclusion chromatography. (C) Coomassie-stained protein gels analyzing different steps of the purification process for FL-TBXT, TBXT DBD-Avi, and TBXT DBD

High-throughput TBXT SPR identifies and validates small molecule hits

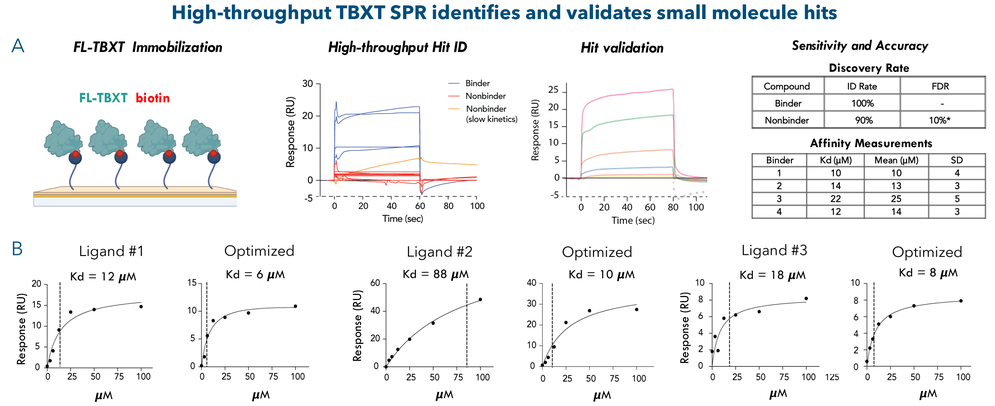


Figure 6. High-throughput FL-TBXT SPR validation and the initial optimization of TBXT ligands. (A) Biotinylated FL-TBXT is immobilized on a SA sensor chip in a Biacore 8K system. Blinded Hit ID using a single dose screen of four binders and ten non-binders accurately identified hits. Hits are validated using a six-point titration and dose-response curves are generated to measure their steady-state affinity. (B) Three open-source TBXT ligands were optimized by a single round of medicinal chemistry and show higher TBXT affinity. Binding curves show ligand concentration vs response.

Crystal structure of the TBXT DNA-binding domain

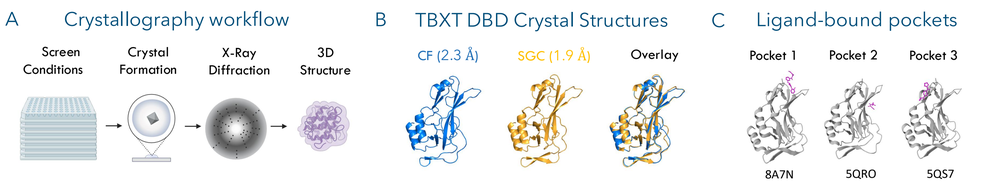
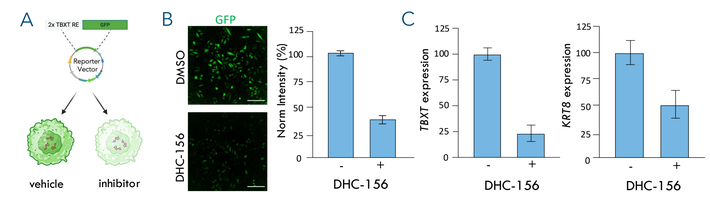


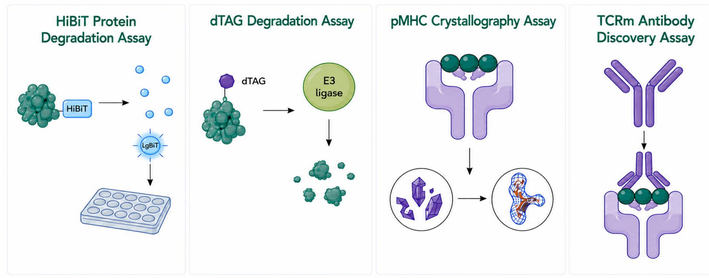
Figure 7. (A) Crystallography workflow from protein crystallization through X-ray diffraction and 3D structure determination. (B) The CF-derived TBXT DBD crystal structure (2.3 Å) closely matches the previously reported SGC structure (1.9 Å), with a CαRMSD of 0.47 Å. (C) Published SGC co-crystal structures[2] reveal three ligandable pockets on the TBXT DBD (PDB IDs: 8A7N, 5QRO, and 5QS7).[2]

PRECLINICAL ASSAY PIPELINE

Development of functional TBXT cell-based assays



D Assays under development for different therapeutic modalities



- Screen PROTACs, glues and other degraders
- Measure degradation kinetics to support hit optimization
- Enable rapid validation of TBXT degradation
- Confirm on-target effects of candidate degraders
- Solve structures of TBXT peptide-HLA complexes
- Enable structure-guided design of pMHC-targeted therapies
- Measure binding of TCR mimics by SPR
- Assess kinetics and affinity to guide optimization

Figure 8. (A) TBXT-responsive GFP reporter schematic. (B) Covalent TBXT downmodulator DHC-156[4] (5 μM, 72 hours) reduces GFP expression in H460 TBXT reporter cells. (C) RT-qPCR analysis demonstrating *TBXT* and its downstream target *KRT8* are suppressed by DHC-156 (10 μM, 24 hours) in UM-Chor1 cells. (D) New assays under development include HiBiT and dTAG protein degradation assays, peptide-MHC structural biology, SPR-based characterization of TCR-mimic and bispecific antibodies. Figure was generated by DALL-E and modified by the authors.

DERISKING TBXT DRUG DISCOVERY

The first TBXT Challenge incentivized the development of new ligands

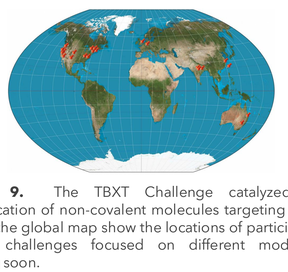


Figure 9. The TBXT Challenge catalyzed the identification of non-covalent molecules targeting TBXT. Pins in the global map show the locations of participants. Future challenges focused on different modalities coming soon.

KEY FINDINGS AND FUTURE DIRECTIONS

- Small-molecule TBXT ligands were discovered and are being optimized to improve potency.
- The CF labs biochemical and cell-based assay capabilities catalyze TBXT-targeting drug discovery for chordoma and more common TBXT-positive tumors.
- Preliminary data suggest that TBXT inhibition may sensitize non-chordoma tumor cells to standard-of-care therapy.
- Developing TBXT-HiBiT and luciferase-based TBXT reporter assays to support the high-throughput discovery of TBXT degraders and functional inhibitors.
- Establishing SPR assays to prioritize TBXT-derived peptide–HLA complexes for TCR mimic antibody discovery.
- Profiling TBXT expression across commercially available PDX models to identify additional indications for TBXT-targeted therapies.

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Presented at the American Association for Cancer Research Drug Discovery and Development Conference; July 21-24, 2026; Boston, MA

For more information, visit: www.chordoma.org