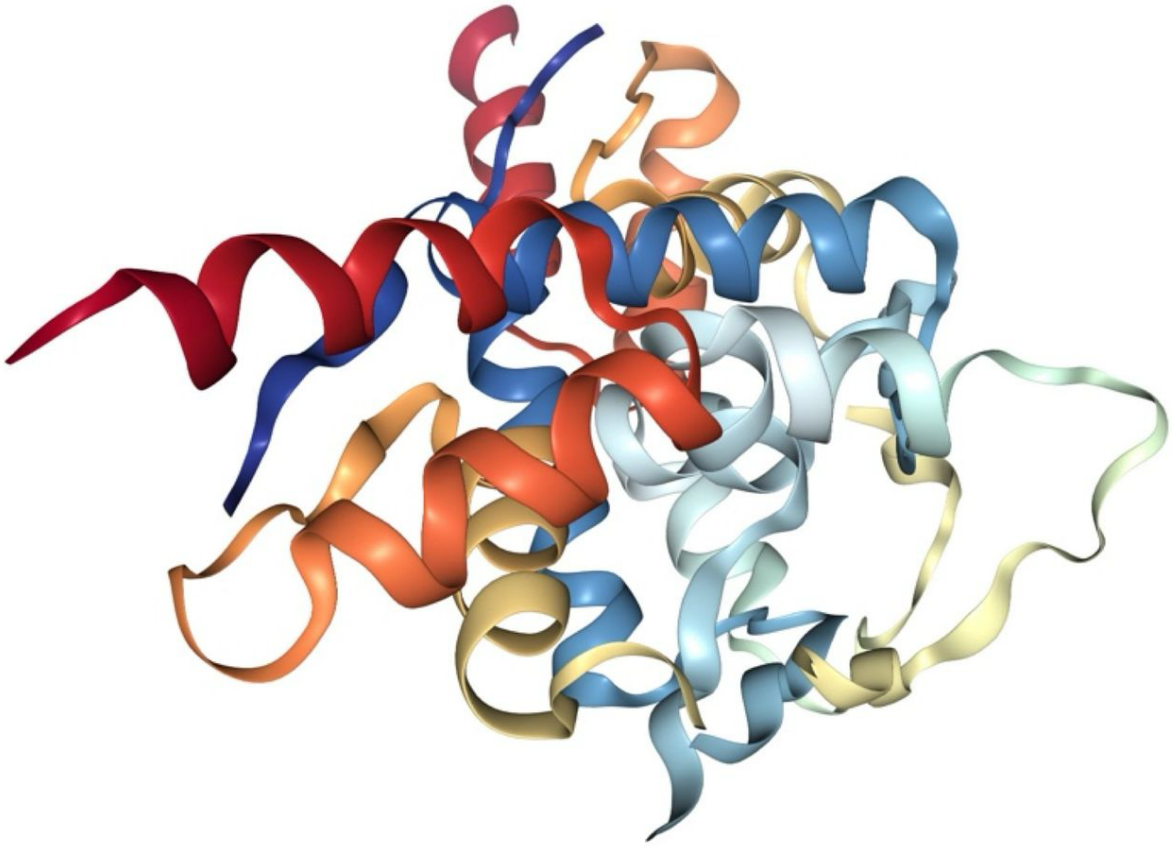
BCL6

B-cell lymphoma 6 protein (Homo sapiens)



Case study

V2.0

About BCL6

B-cell lymphoma 6 protein (BLC6) is a transcriptional repressor with an important role in the formation of the germinal center. Most B cell lymphomas arise from germinal center B cells. Some of the target genes whose expression is directly suppressed by BCL6 include DNA damage sensors (such as TP53, ATR, CHEK1, and ARF) as well as genes encoding cell cycle regulators (such as CDKN1A, CDKN1B, CDKN2A, and CDKN2B).

BCL6 has been shown to be an oncogenic driver and genetic knockdown studies reveal it to be a relevant therapeutic target. Inhibition of BCL6 is recognized as a therapeutic strategy for treatment of hematological cancers such as leukemia and B-cell lymphoma.

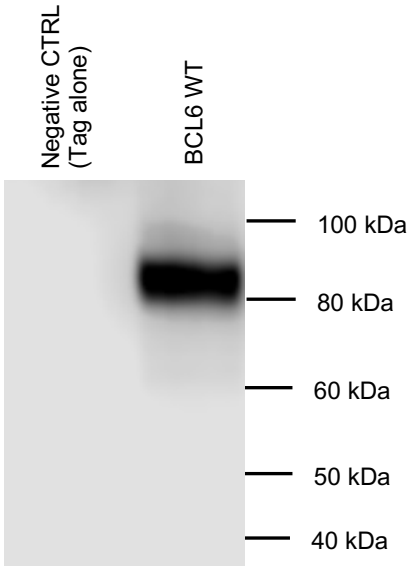
Therapeutic challenges of BCL6

BCL6 homodimerizes through its amino terminal BTB-POZ domain. The dimer interface then mediates its interaction with corepressors such as BCOR and NCOR2. Interaction with corepressors is essential for the transcriptional repression and oncogenic activity of BCL6.

Blocking the protein-protein interactions of BCL6 and its corepressors has been proposed as an effective approach for targeting BCL mediated tumorigenesis. There are several BCL6 inhibitors that directly target the interaction of corepressors and the BTB-POZ domain of BCL6.

The Boehringer Ingelheim compound BI-3812 potently inhibits the interaction of the BTB-POZ domain of BCL6 with several corepressors in vitro (IC50≤3nM). In a cellular context, BI-3812 inhibits the BCL6-corepressor complex formation with an IC50 of 40nM. However, BI-3812 and its analog BI-3802 have poor bioavailability and so are not clinical drug products.

Figure 1: Step 1A - Expression test of BCL6 in HEK293 cells.



Blotted with anti-Micro-tag antibody

GSK137 is a small molecule inhibitor of BCL6 activity that also binds the corepressor binding groove in the BTB-POZ domain to inhibit BCL6 interaction with corepressors. This inhibitor demonstrates good solubility and permeability. GSK137, administered orally, suppressed IgG responses and reduced numbers of germinal centers and germinal center B cells.

Targeting BCL6 with MICRO-TAG target engagement

BCL6 is a considered a challenging drug target due to its intrinsically disordered nature. There are now clinical drug candidates targeting BCL6. More potent and selective anti-BCL6 inhibitors are desired in order to target a variety of cancers in single-agent and combination therapies.

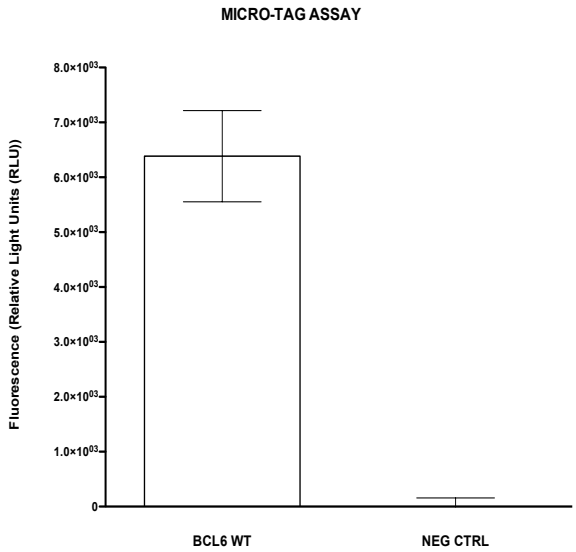
CellarisBio's MICRO-TAG cellular target engagement technology offers a unique way to approach to identify novel therapeutic candidates that directly engage with BCL6 within the context of the cell. The MICRO-TAG system designed to identify direct binders to BCL6 has 4 steps:

Step 1: Design a MICRO-TAG reporter for the target.
Step 2: Establish thermal melting profile of the target.
Step 3: Run a pilot test with a reference compound.
Step 4: Scale the system for compound discovery.

Step 1: Design MICRO-TAG reporter system specific for BCL6

DNA construct encoding for BCL6 transfected into HEK293 cells and expression of the target was detected, as shown in **Figure 1**. MICRO-TAG system works on the basis of enzyme complementation that generates fluorescence signal. Hence, following satisfactory expression of the target, enzyme complementation of the MICRO-TAG construct is tested, as shown in **Figure 2**.

Figure 2: Step 1B - Activity of MICRO-TAG target construct for BCL6.



Step 2: Establish the thermal melting profile

Once target expression and enzyme complementation steps are completed, the target in the MICRO-TAG system is tested for thermal profile. Intact HEK293 cells carrying the expression construct are subjected to short thermal gradient ranging typically from 37C to 75C.

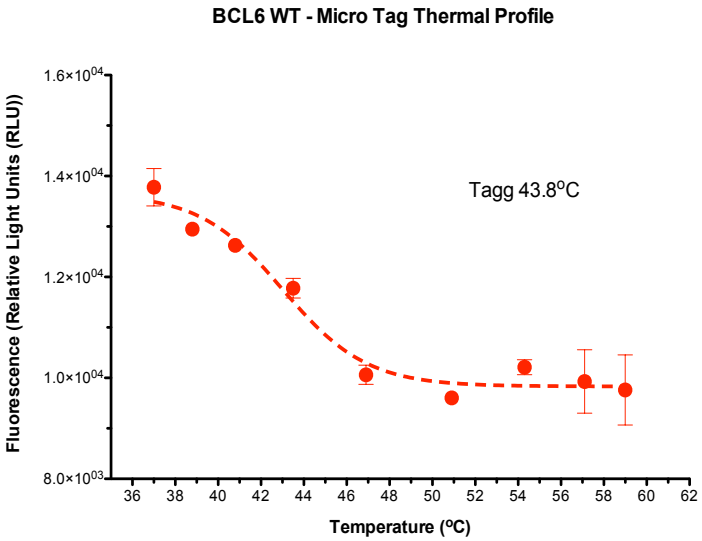
Fluorescence signal originating from BCL6 is detected at each temperature point is detected and thermal melting profile of the target is established. This step has to be performed empirically in order to establish the unique fingerprint of the target. The temperature of aggregation (T_{agg}) for BCL6 is calculated as 44C, as shown in **Figure 3**. At this temperature, the target in the cell is expected to be in half structured and half unwound state.

Step 3: Run a pilot test with a reference compound

Once the thermal melting profile of the target is empirically established, response of the target can be tested in a pilot test using a reference or a tool compound. Reference compounds specific to the target are preferred.

To test response of BCL6, we used the reference compound BI-3812. Intact cells carrying MICRO-TAG constructs for BCL6 were treated with BI-3812 in dose-dependent manner, then exposed to thermal challenge at 43.8C. The ligand stabilized the target in dose-dependent manner with EC50 of 0.3nM, as shown in **Figure 4**.

Figure 3: Step 2 – Thermal melting profile of BCL6.



The pilot phase also provides an opportunity to optimize the assay to capture more response from the cellular drug-target engagement.

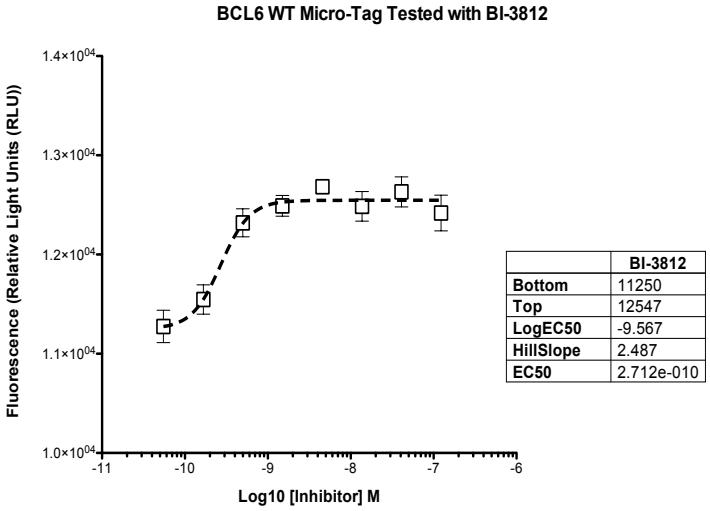
Step 4: Scale the system for compound discovery

Once the pilot step is accomplished, the reporter assay is ready for scale-up. Depending on requirements, there are several options to consider.

Transient expression of the MICRO-TAG target can be used for low-throughput screens aiming at validation of advanced drug candidates. Stable MICRO-TAG reporter assay can be generated using lentiviral knock-in or CRISPR approach. This latter approach is more tuned for high-throughput primary screens aimed at discovering of novel candidates.

In the case of BCL6, we proceeded with transient-expression system and set up a medium-throughput screen for validation of already identified small molecule candidates.

Figure 4: Step 3 – Cell target engagement of reference compound BI-3812 with BCL6.



Accelerate and De-Risk Drug Discovery with CellarisBio

CellarisBio's is a drug discovery technology company, based in San Diego, California. Our MICRO-TAG cell target engagement platform is built to tackle challenging drug targets.

We work across various therapeutic targets classes such as:

- Enzymes
- Membrane proteins
- Transcription factors
- Other challenging proteins

We work with multiple therapeutic modalities such as:

- Small molecules
- Peptide
- PROTACS
- Antibodies

Accelerate your drug discovery with CellarisBio:

- Discover drug candidates using DNA-Encoded Libraries or plated libraries,
- Validate drug candidates for potency and selectivity,
- Analyze for in-cell kinetics and live-cell imaging.

Some of the drug targets we worked with:

What drug targets are you interested in?

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