#4756

Enabling fragment-based drug discovery for direct small molecule inhibition of MYC

Mark Wade, Charlotte Hodson, Stanislava Panova, Barbara Farkaš, Davide Branduardi, George Ward, Aimee Blair, Alison Woolford, Maria Ahn, John Lyons, Juan Bueren-Calabuig
Astex Pharmaceuticals, 436 Cambridge Science Park, Cambridge, CB4 0QA, UK

INTRODUCTION

MYC overexpression or dysregulation is a hallmark of most malignancies, yet targeting of MYC-driven cancers remains a clinically unmet need[1]. Both indirect and direct modalities of MYC inhibition have been evaluated. Indirect MYC targeting via inhibition of its binding to partner proteins is challenging due to issues around redundancy and patient selection. Furthermore, antisense and other epigenetic approaches have so far failed. While direct targeting is challenging due to the intrinsically disordered nature of MYC[2], peptides/miniproteins, such as OmoMYC[3] (which essentially prevent the interaction of MYC with its obligate heterodimeric binding partner, MAX) are being tested in the clinic. Currently there are no small molecule MYC inhibitors in clinical trials. Here, we used biophysical, biochemical and functional assays to test a selection of published tool compounds that are reported to target MYC.

Figure 1

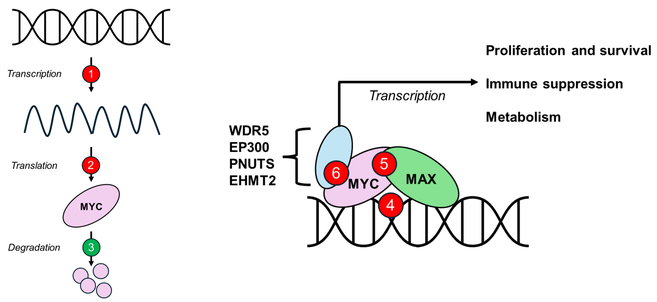

Fig. 1 Multiple entry points for inhibition of MYC. MYC can be targeted by suppressing its transcription (1) or translation (2), or by promoting its degradation (3). Alternatively, MYC's activity as a transcription factor can be blocked by preventing interaction with DNA (4) or with its cognate binding partner, MAX (5). MYC-dependent transactivation can also be inhibited by blocking the interaction with one of its transcriptional co-activators (6).

Figure 2

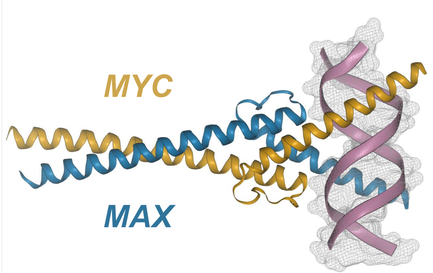

Fig 2. Astex determination of the MYC/MAX heterodimer structure. Shown are the bHLHZip domains of MYC (orange) and MAX (blue) bound to E-box DNA (purple)

Figure 3

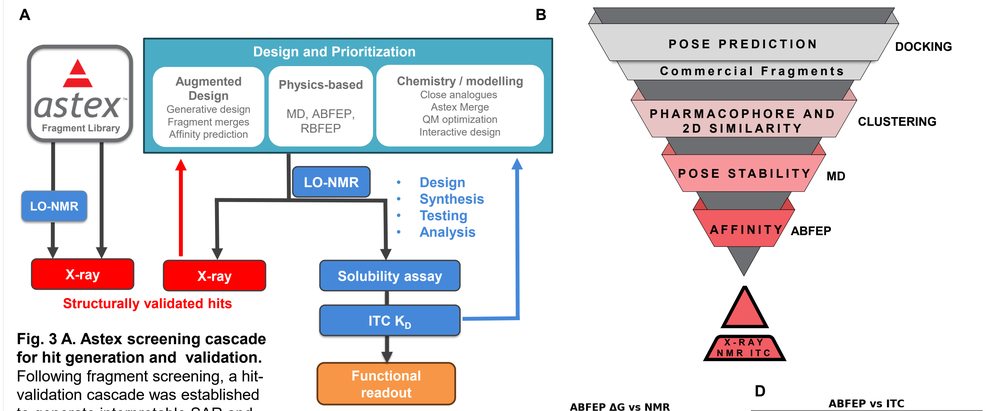

Fig. 3 A. Astex screening cascade for hit generation and validation. Following fragment screening, a hit-validation cascade was established to generate interpretable SAR and optimise fragments prior to growth into lead compounds.

B. An enhanced modelling cascade. Extensive *in silico* cascade for augmentation of Pyramid screen, including docking, short-trajectory molecular dynamics (MD), and absolute binding free energy perturbation (ABFEP); virtual hits followed up by experimental cascade translated into an enriched hit pool (**C** ABFEP ΔG vs 1D NMR binary readout **D** ABFEP ΔG vs ITC ΔG).

Figure 4

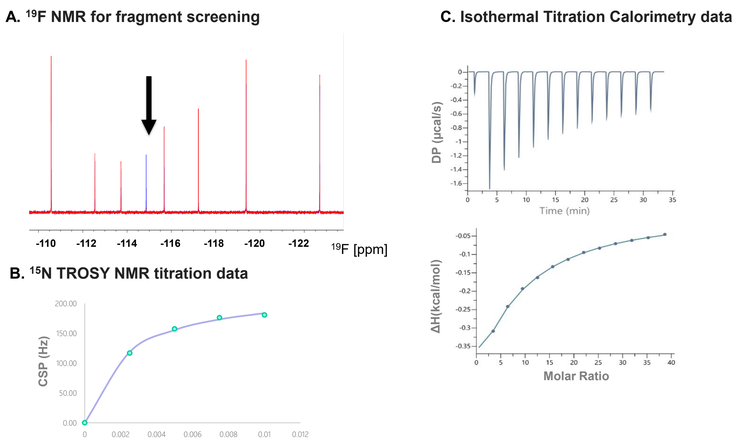

Fig 4. NMR Fragment Screening. A. ^{19}F NMR spectra for a given cocktail of the Fluorinated Fragment library from screening MYC:MAX DBD. Spectra show a fragment mixture in the absence (red) or presence (blue) of MYC:MAX heterodimer. **B.** The hit was validated using a 2D protein-observed NMR assay. **C.** Validation of the hit by Isothermal Titration Calorimetry.

Multiple fragment hits have been structurally validated in X-ray

Figure 5

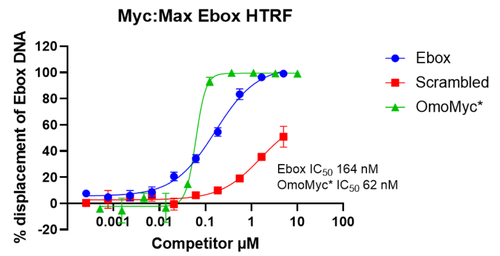

Fig 5. ***Assays developed to measure a functional effect in vitro***
Homogenous Time Resolved Fluorescence is a proximity-based assay: binding of DNA to MYC:MAX produces a FRET signal, while displacement of DNA separates donor and acceptor fluorophores, reducing the HTRF signal. Figure illustrates displacement of Ebox DNA from MYC:MAX by competitor Ebox & scrambled DNA and recombinant OmoMYC.

Figure 6

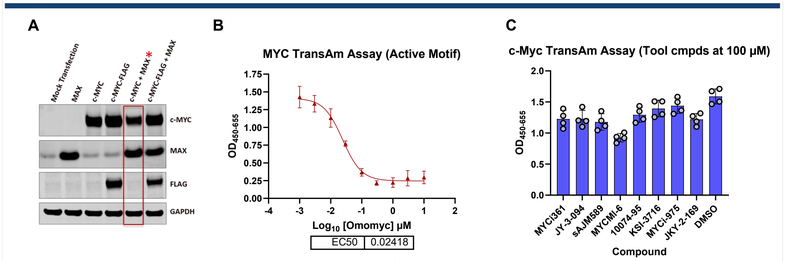

Fig 6. ***Assays developed to measure a functional effect in cell lysates***
A Generation of transfected HEK293T cell lysates for TransAm (Active Motif) analysis. Lysate from MYC & MAX-overexpressing cells (*) was used in B & C. **B** Inhibition of MYC/MAX lysate binding to immobilised E-box oligo by recombinant OmoMYC. **C** Literature compounds do not inhibit MYC/MAX binding to immobilised E-box oligo.

Summary

- Our robust biophysical screening cascade enables reliable fragment hit identification
- We developed a soakable MYC:MAX crystal system and determined the crystal structure of the MYC/MAX heterodimer bound to DNA
- Virtual screening and rigorous free energy calculations have been integrated into a design-make-test loop for fragment-based drug discovery
- We have identified structurally validated hits
- We have developed a suite of biophysical and biochemical assays to progress hit matter
- The MYC-targeting literature compounds we tested do not appear to bind the MYC/MAX complex directly

References

1) Whitfield J.R. and Soucek L., PMID 39972241
2) Madden S.K. et al., PMID 33397405
3) Demma M.J. et al., PMID 31501275

Poster presented at AACR Annual Meeting, San Diego April 17-22, 2026 (Poster available to download from Astex's website at www.astx.com)