BIOLOGY SECTION

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OVERVIEW

Targeted cancer therapies by means of small molecules or antibodies act on specific molecular targets to block cancer growth and progression. Kinases have become attractive molecular targets for the treatment of numerous cancer types; hence the U.S. Food and Drug Administration (FDA) has approved many small-molecule kinase inhibitors for clinical use, some of them irreversible inhibitors. Similarly, given the importance of epigenetic marks in tumorigenesis, modifiers of DNA or histones have become attractive therapeutic targets; currently, there are 6 epigenetic drugs clinically approved for cancer treatment by the FDA. In collaboration with Óscar Fernández-Capetillo, we recently started an early drug discovery project to develop SETD8 inhibitors, as non-advanced inhibitors have been described so far. By developing a non-radioactive assay, we have been able to perform a screening campaign and identify several molecules as starting points to obtain good SETD8 inhibitors that are both reversible and irreversible.

"We have identified MASTL PROTACs with a nanomolar degradation concentration 50 and a maximum 93% degradation of MASTL via E3 ligase and proteasome recruitment."

RESEARCH HIGHLIGHTS

Microtubule-associated serine/threonine protein kinase-like (MASTL)

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. We tested in our biochemical assay using active human full-length MASTL protein, around 250 new compounds, both MASTL-is and MASTL PROTAC-like molecules. For the most potent biochemical inhibitors and PROTACs molecules, we measured MASTL engagement in cells (BRET assay). In the case of PROTACs, we also evaluated their MASTL degradation capacity in cells in order to determine the best linker length and binding to E3 ligase ligand. We identified a nanomolar MASTL degrader (ETP-823) that will be used to study its pharmacological implications (FIGURE 1). In addition, we characterised the ADME-T properties of our most potent MASTL-is to identify the compound with the best pharmacokinetic profile.

Telomeric repeat binding factor 1 (TRF1)

This project is carried out in collaboration with the CNIO Telomeres and Telomerase Group. We are working to identify disruptors of TRF1 binding to ds telomeric DNA. After virtual screening and wet assay, only 1 compound, ETP-631, qualified as a potential hit; more orthogonal assays to validate the direct binding to hTRF1 are under study. We also performed a new screening of a collection of 1500 molecules selected from our ETP-library that bear a privileged structure to disrupt protein-DNA complexes. After analogue searching, we identified several hits from different chemical series. We are now validating these hits applying orthogonal assays against TRF1 and the TelDNA probe, such as CETSA and the fluorescent displacement assay, respectively, using freshly prepared and/or resynthesized samples. Compounds that disrupt the binding of TRF1 to ds telomeric DNA by binding to TRF1 will be tested in a TRF1 phenotypic assay.

SET domain containing lysine methyltransferase 8 (SETD8)

This project is conducted in collaboration with the CNIO Genomic Instability Group. Our main objective is to generate and optimise novel SET8 inhibitors as new therapeutic agents. In 2021, we performed a screening campaign with a commercial library of 1500 irreversible molecules, through which several hits were identified and some validated after resynthesis. Moreover, a small internal library of irreversible molecules was tested identifying possible hits with low micromolar activity. The irreversible mechanism of action of all the hits is being validated by biochemical assays and proteomics in order to gain information to improve activity.

Collaborations with other CNIO Groups

ETP-Biology provided support for *in vivo* studies of selected compounds and drugs, such as pharmacokinetics, distribution and/or antitumour efficacy, performed by the Microenvironment and Metastasis and the Brain Metastasis Groups. Furthermore, in collaboration with the DNA Replication Group, we prepared hits identified in a virtual screening campaign for the validation of PrimPol inhibitors. We also gave technical support by setting up a biochemical assay to be able to perform screening campaigns for the Topology and DNA Breaks Group and the H12O - CNIO Haematological Malignancies Clinical Research Unit. Finally, we collaborated with the Experimental Oncology Group, helping to validate RNAseq data with small molecules.

Collaborations with other institutions

Target X. ETP-Biology performed biomarker evaluation studies against target X in a previous collaboration with *VIB* (the Flanders Institute for Biotechnology).

Refoxy Pharma collaboration. ETP-Biology gave logistics and data analysis support. ■

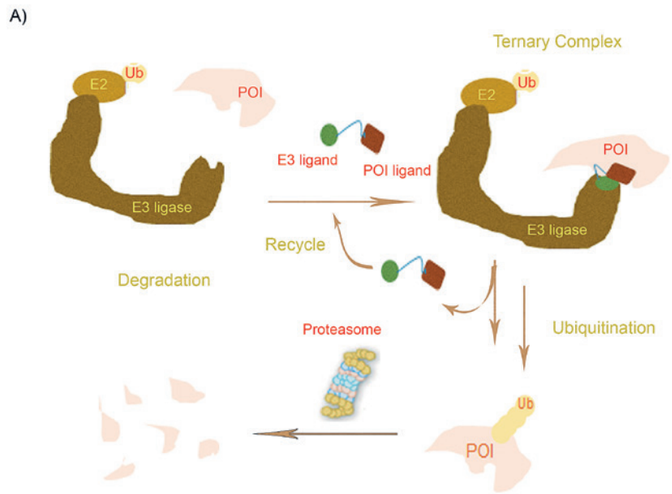

FIGURE 1 PROTAC MASTL (ETP-823) downregulates the protein levels of its target. (**A**) Model of PROTAC-induced degradation. (**B**) Dose-dependent downregulation of MASTL levels in MCF-7 after 24h treatment with ETP-823. (**C**) Degradation concentration 50, maximum degradation concentration and duration of MASTL degradation. (**D**) ETP-823's degradation is dependent on the proteasome and the presence of the linkage between both targeting ligands. MCF-7 cells were treated with the indicated compounds for 24 h and analysed by Western blotting. MLN4924: NEDD8-Activating Enzyme inhibitor; Bortezomib: proteasome inhibitor.

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