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. 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.”
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-α and MASTL PROTAC-like molecules. For the most potent biochemical inhibitors and PROTACs molecules, we measured MASTL engagement in cells (BRAT 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-α 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 BRET assay. Compounds that disrupt the binding of TRF1 to ds telomeric DNA by binding to TRF1 will be tested in a TRF1 phenotypic assay.

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 H120 - 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).

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