BIOLOGY SECTION

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* *Plan de Empleo Joven de la Comunidad de Madrid* (Youth Employment Plan, Community of Madrid)

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**Titulado Superior* (Advanced Degree)
#*Plan de Empleo Joven* (Youth Employment Plan, until January)

Students in Practice
Andrea Álvarez (Feb.-July) (Bachelor's Degree Final Project, *Univ. Autónoma de Madrid*, Spain), Jada Li (June - July) (MISTI Internship, USA), Noelia Martin (Feb.-July) (Bachelor's Degree Final Project) and María Cuerda (since Oct.) (Master's Thesis)(*Univ. Complutense de Madrid*, Spain)

Visiting Scientists
Lucía Jiménez and Wolfgang Link (until November) (*IIBm (CSIC-UAM),* Madrid, Spain)

OVERVIEW

A high-quality small-molecule probe for target validation has to be cell permeable and demonstrate target engagement and selectivity, as well as pharmacological and phenotypic response. PROTACs (PROteolysis TArgeting Chimeras) have emerged as new promising pharmacological modalities. Moreover, PROTACs represent the chemical equivalent of small interfering RNA (siRNA), albeit allowing removal of a protein at a post-translational level. Parameters such as the maximum level of target degradation (Dmax), confirmation of a proteasome dependent degradation mechanism, and kinetic parameters of POI degradation and selective degradation have to be taken into account to use PROTACs for target validation. In collaboration with Marcos Malumbres, we started an early drug discovery project to develop MASTL inhibitors and PROTACs, as non-advanced inhibitors have already been described. We have been able to develop both types of molecules, generating a set of PROTACS that meet the requirements to be used as chemical tools for target validation and to define their clinical niche.

"We identified selective and potent MASTL PROTACs with *in vivo* levels needed to perform PK/PD and proof of concept studies."

RESEARCH HIGHLIGHTS

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

This project is undertaken in collaboration with the CNIO Cell Division and Cancer Group. In 2022, we tested in our biochemical assay using active human full-length MASTL protein around 190 new compounds, both MASTL-i and MASTL PROTAC-like molecules. We measured MASTL engagement in cells (BRET assay). In the case of PROTACs, we also evaluated their MASTL degradation capacity in cells, both in a dose response and time dependent manner. We identified a set of nanomolar MASTL degraders with different linker and E3 ligase ligand that have been used to study their broad degradation capacity with proteomics. In addition, we performed pharmacokinetics studies of several MASTL inhibitors and PROTACs, identifying a MASTL inhibitor and a PROTAC that have achieved enough plasma levels to allow PK/PD studies to be performed (FIGURE 1).

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, and so far we have identified several hits from different chemical series after virtual screening and wet assays, and screening of a collection of 1500 molecules selected from our ETP-library and analogue searching. We confirmed the specific disruption of the binding of TRF1 to dsTelDNA with screen and counter screen alpha assays, and a fluorescent displacement assay to discard the binding of the compounds to dsTelDNA. Now we are validating these hits by applying orthogonal assays against TRF1 and the dsTelDNA probe, such as EMSA and thermofluor assay with freshly prepared and/or resynthesised 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. After 2 different screening campaigns, we identified both reversible and irreversible possible hits with micromolar activity. The covalent mechanism of action of the hits was validated by time dependent biochemical assays and the formation of adducts by proteomics with purified SETD8. In order to identify the reactive amino acid in SETD8, we are going to perform biochemical assays with a mutant protein and proteomics studies in cells to evaluate their selectivity. In addition, all possible hits have been tested in a cellular assay that measures monomethylation of H4K20 in order to prioritise chemical serials to improve their biochemical activity.

Collaborations with other CNIO Groups

The ETP-Biology Section performed *in vivo* studies of selected compounds and drugs such as pharmacokinetics and distribution studies in collaboration with the Microenvironment and Metastasis Group, the Brain Metastasis Group, and the Genomic Instability Group. Furthermore, we performed screening campaigns with the Topology and DNA Breaks Group and the H12O - CNIO Haematological Malignancies Clinical Research Unit, identifying several hits that are under validation. Finally, we collaborated with the Experimental Oncology Group, the Melanoma Group, the DNA Replication Group, and the Chromosome Dynamics Group giving support to perform cellular screenings.

Collaborations with other institutions

Refoxy collaboration: We gave logistics and data analysis support.

Collaboration with CRG/UIC: This project is conducted in collaboration with Dr R. Wright. We characterised, in terms of ADME-T and pharmacokinetics, a NUDIX5 inhibitor previously identified by the researcher. ■

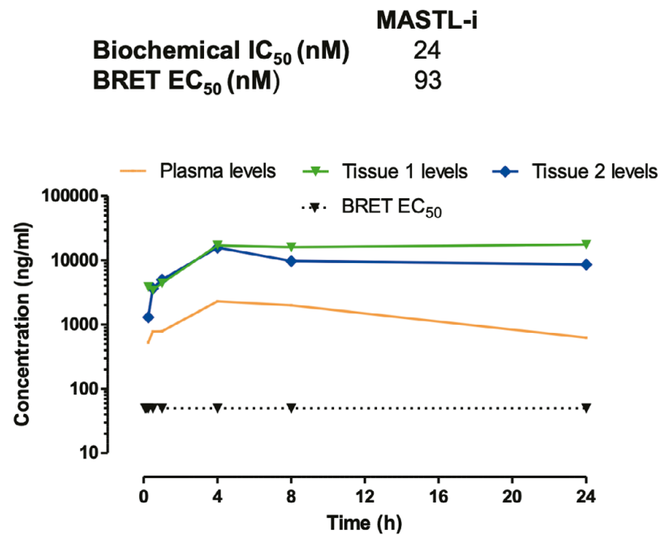

FIGURE 1 Plasma and tissue levels of MASTL inhibitor after oral administration. Nanomolar biochemical and cellular MASTL-I achieves levels in mice clearly above the EC_{50} to modulate MASTL in cells, which guarantees *in vivo* target modulation.

PUBLICATIONS

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- Baquero JM, Marchena-Perea E, Mirabet R, Torres-Ruiz R, Blanco-Aparicio C, Rodríguez-Perales S, Helleday T, Benítez-Buelga C, Benítez J, Osorio A (2022). OGG1 inhibition triggers synthetic lethality and enhances the effect of PARP inhibitor olaparib in BRCA1-deficient TNBC cells. *Front Oncol* 12, 888810.
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