

STSM grantee 7 OneHealthdrugs Call 01/02/2026- 30/04/2026: Aurora Gaza

Project Title: “Biological evaluation of small-molecule hydrophobic degraders for antiparasitic drug discovery and chemical biology applications”

Home institution: University of Bologna – Alma Mater Studiorum (Prof.ssa Maria Laura Bolognesi) **Host institution:** University of Glasgow, School of Infection and Immunity, College of Medical, Veterinary and Life Sciences, (UCL) (Prof. Harry de Koning)

The Short-Term Scientific Mission (STSM) was carried out at the University of Glasgow, School of Infection and Immunity, under the supervision of Prof. Harry de Koning. The project aimed to investigate the antiparasitic potential of a library of fifteen Hydrophobic Tag (HyT) degraders targeting trypanothione reductase (TR) in *Leishmania* and to establish their mechanism of action.

During the STSM, the biological activity of the compounds was evaluated against *Trypanosoma brucei brucei*, *Leishmania mexicana* promastigote, and *Leishmania* intracellular amastigotes using the Alamar Blue assay. Comparative analyses revealed promising stage-dependent activity, with several compounds displaying selective efficacy against the intracellular amastigote stage while showing no significant cytotoxicity toward THP-1-derived macrophages at the tested concentrations. To determine whether the observed antiparasitic activity resulted from TR degradation rather than enzyme inhibition, methods to directly monitor and quantify intracellular TR protein levels were needed. To this end, CRISPR-Cas9 technology was employed to generate genetically modified *L. mexicana* cell lines for gene detection and target validation studies. Both a TR-Myc tagged line, and a single-allele TR knockout (Blasticidin or G418) were established, providing essential tools to investigate target engagement and the biological role of TR. The TR-Myc tagged cell line will be used for future Western blot analyses to monitor TR protein levels following treatment with selected HyT degraders.

Beyond the scientific outcomes, the STSM significantly strengthened the collaboration between the University of Bologna and the University of Glasgow and as expected, provided advanced training in parasitology, molecular biology, and cell-based assays, broadening the expertise from synthetic medicinal chemistry to multidisciplinary antiparasitic drug discovery (i.e. cell culturing techniques, PCR, CRISPR-Cas9 technology, microscopy, Western Blot analysis).

This collaboration has contributed to the preparation of a book chapter currently in press, and a flash presentation at the NMMC30 Conference (Messina, September 2026) collaborative work will focus on completing the mechanistic characterization of the most promising HyT degraders and on preparing joint scientific publications.

