

MOLECULAR CYTOGENETICS UNIT

Sandra Rodríguez-Perales
Unit Head

Research Scientist
Raúl Torres

Graduate Students
Alejandro Alonso (since September),
Maria Cruz Casado (until August),
Alejandro Nieto (since September),
Pilar Puig



OVERVIEW

Recurrent chromosomal rearrangements, changes in the structure of native chromosomes, are very common and well-known hallmarks of cancer. A better understanding of these cancer-causing mechanisms will lead to novel therapeutic regimens to fight cancer. The research activity of the Molecular Cytogenetics and Genome Editing Unit focuses on increasing our knowledge about the role of chromosomal rearrangements in cancer development and progression and discovering new therapeutic targets. With the combined use of CRISPR genome editing and cytogenetic technologies, we are creating models that recapitulate chromosomal and genetic cancer alterations. The goal of the Unit is to provide CNIO and external researchers with the latest technologies used in the fields of molecular cytogenetics and genome editing. The Unit is continuously implementing and developing new technologies in those fields.

“In 2022, we applied genome engineering approaches to reproduce and eliminate chromosome rearrangements and gene alterations. We also provided access to the latest cytogenetic and CRISPR technologies.”

We also participate in collaborative projects with clinical and basic science investigators across the CNIO and other institutions.

Technicians
M. Carmen Martín, Francisco José
Moya (TS) ‘(PEJ)’ ”

“Titulado Superior (Advanced Degree)
“Plan de Empleo Joven (Youth Employment
Plan, until March)

Master’s Students
Alejandro Alonso (Jan.-Aug.)
(*Maastricht University*, The
Netherlands), Alejandro Nieto
(Jan.-Aug.) (*Univ. Autónoma de
Madrid*, Spain), Paula M. Ojeda

(Feb.-Sep.) (*Univ. Complutense de
Madrid*, Spain)

Visiting Scientists
Beatriz Álvarez and Daniel Lucena
(Sep.-Dec.) (*CIB Margarita Salas*,

Madrid, Spain), Carlos Carrasco
(July.-Dec.) (*Univ. Complutense de
Madrid*, Spain)

RESEARCH HIGHLIGHTS

Molecular cytogenetics. The “Optimization Optical Genome Mapping” (OGM, Bionano) technique, based on the analysis of ultra-high long DNA molecules, provides a genome-wide high-resolution analysis of copy number and structural cytogenetic variations. We are optimising the use of OGM technology in our Unit, comparing it with standard techniques (e.g., karyotyping, fluorescent in situ hybridisation) using hematologic and solid tumour fresh and frozen samples. We think OGM represents a promising complementary approach to existing cytogenetic techniques for the characterisation of cancer cells. OGM enables a time and cost-effective analysis allowing the identification of complex cytogenetic rearrangements, including some that are currently inaccessible to standard techniques.

Technological and translational activities. Approximately 20% of human cancers contain specific fusion oncogenes (FOs). Due to their tumour-specific expression, FOs offer unique advantages such as diagnostic and therapeutic targets.

- **FO targeted diagnosis:** In the clinic, conventional diagnostic techniques like qRT-PCR, FISH, or NGS are routine. But these methods require specialised machinery and personnel, are expensive, time-consuming, and involve multiple steps.
- **FO targeted therapy:** Many currently used treatments are non-selective, leading to severe side effects responsible for prolonged recovery and frequently resulting in relapses.

In this regard, we applied the RNA-targeting Cas13 system to selectively cleave FO transcripts. Contrary to the CRISPR/Cas9

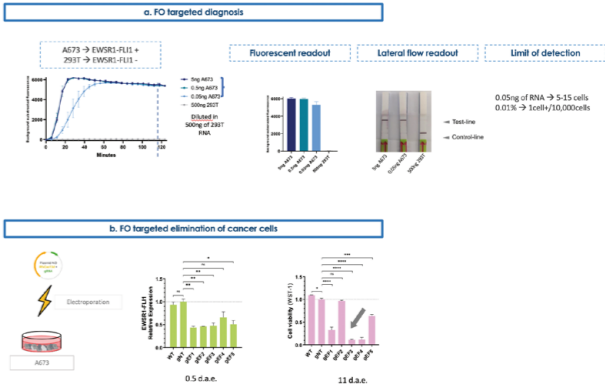


FIGURE 1 We have taken advantage of the versatile CRISPR/Cas13 system to: (a) develop a sensitive, specific, affordable, and instrument-free diagnostic test for FO detection in patient samples; and (b) silence FO RNA inducing efficient and selective elimination of cancer cells.

method, RNA targeting with Cas13 results in reversible and temporally controllable alterations without modifying the DNA. Furthermore, compared to shRNAs, the Cas13 method is associated with high knockdown efficiency and no off-target effects, offering unique advantages when used for therapeutic purposes. Diagnostic methods based on Cas13 provide rapid RNA detection with attomolar sensitivity and single-base mismatch specificity. ■

PUBLICATIONS

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- Montero-Conde C *et al.* (incl. Moya FJ, Currás-Freixes M, Gonzalez-Neira A, Megías D, Blasco MA, Caleiras E, Rodríguez-Perales S, Robledo M) (2022). Comprehensive molecular analysis of immortalization hallmarks in thyroid cancer reveals new prognostic markers. *Clin Transl Med* 12, e1001.
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PATENT

- Malats Riera N, Bork P, Kartal E, Molina Montes E, Rodríguez S, Estudillo L, Real FX, Schmidt TSB, Zeller G, Wirbel J, Mais-trenko OM. Faecal Microbiota Signature for Pancreatic Cancer. PCT application (2022). *PCT/EP2022/077087*. WO2023052486A1.

AWARDS AND RECOGNITION

- Raul Torres-Ruiz has received the 2022 ESGCT Young Investigator Award from the European Society for Gene and Cell Therapy (ESGCT).