OVERVIEW

Haematological malignancies include a myriad of heterogeneous diseases with high frequency, such as non-Hodgkin’s lymphoma, or with high mortality rates, such as multiple myeloma and acute myeloid leukaemia. In the laboratory, we dissect the biology of haematological cancers by investigating: (i) novel diagnosis and prognosis biomarkers; (ii) innovative tools and techniques to identify and monitor the disease; (iii) original therapeutic targets and treatments against haematological malignancies; and (iv) how to decipher the biological processes underlying the diseases and characterise the drivers, oncogenes and tumour suppressors.

The following main lines of research define our laboratory:

- Role of hnRNP K, a novel driver of lymphoma and leukaemia in tumorigenesis.
- Molecular fingerprint in clonal evolution, heterogeneity and drug resistance.
- Liquid biopsy and next-generation sequencing.
- Immunotherapy: NK-CAR and T-CAR in haematological and paediatric cancers.

“We have identified a novel master regulator of cancer, hnRNP K, a tumour suppressor now characterised as an oncogene. Lymphoma patients might benefit from more personalised therapies based on targeting hnRNP K.”
Uncovering the oncogenic role of hnRNP K in B-cell lymphomas

Heterogeneous nuclear ribonucleoprotein K (hnRNP K) is a RNA-binding protein that is aberrantly expressed in cancer. We, and others, have previously shown that reduced hnRNP K expression downmodulates tumor-suppressive programmes. However, overexpression of hnRNP K is the more commonly observed clinical phenomenon, yet its functional consequences and clinical significance remain unknown. hnRNP K is overexpressed in patients with diffuse large B-cell lymphoma and in mouse models without MYC genomic alterations. Clinical samples, mouse models, global screening assays, and biochemical studies have revealed that hnRNP K’s oncogenic potential stems from its ability to post-transcriptionally and translationally repress gene expression. We, and others, have previously shown that reduced hnRNP K expression downmodulates tumor-suppressive programmes. We investigated the impact of mitochondrial load and activity on the progression and relapse of MM patients. RNAseq data from 770 newly diagnosed patients with MM revealed overexpression of mitochondrial activity-related genes correlating with poor outcome. The expression of mitochondrial genes and proteins were elevated in patients who relapsed with bortezomib compared with previous stages, concomitant with an increase in mitochondrial activity. In proteasome inhibitor-relapsed MM patients, an elevation in c-Myc and CD38 expression, both involved in metabolic activation, could explain the consequent increase in mitochondrial activation triggered by proteasome inhibitor treatment. In vitro and in vivo studies with primary MM cells and the JMM-3-Luc-GFP cell line showed the efficacy of the mitochondrial inhibitor tigecycline, alone and in combination with the frontline treatment bortezomib, reversing the bortezomib resistance induced by mitochondrial activation. Our findings provide a strong rationale for investigating tigecycline and other mitochondrial inhibitors in combination with current MM therapies (work under review in Blood).

Mitochondrial activity plays a key role in multiple myeloma

Mitochondria control several key biological pathways involving cell proliferation and apoptosis. Many studies have implicated a functional role for mitochondria in tumour formation and development; however, their impact on the pathogenesis of multiple myeloma (MM) remains largely unexplored. We investigated the impact of mitochondrial load and activity on the progression and relapse of MM cancer cells, inducing the expansion and dominance of the affected clones within a patient’s bone marrow. To test this hypothesis, we established fluorescence-marked isogenic AMO-1 MM sublines with WT, mono-, and bi-allelic TP53 alterations, and co-cultivated these cells in different in vitro competition assays. In our model, we were able to observe clonal evolution and estimate competitive advantages of both mono- and bi-allelic TP53 variants. Strikingly, we demonstrated that subclones with TP53 double hits outcompete and overgrow other TP53 variants. Reflecting these results, a meta-analysis, including publicly available data sets, confirmed single- and double-hits myeloma to be significantly enriched in patients who relapsed (work published in Blood).

Figure 1 hnRNP K is a bona fide oncogene when overexpressed and represents a novel mechanism for c-Myc activation in the absence of MYC gene expression. (A) OS (hnRNP K expression expression, (B) OS (hnRNP K OE) tumorigenic in mouse models. (C) OS and (D) F/S DLD, based on hnRNP K expression, (A) OS mice spleen and liver size and (C) weight. (E) RIP assay in BAG3- (left) and murine (right) cells. (F) Fluorescent antibody binding curves.

• PUBLICATIONS

• PATENT
  - Barrón García A, Ayala Díaz RM, Martinez-Lopez J, Orellana de le Fuente ME, Rapado Martínez M. Method for determining the presence or absence of minimal residual disease (MRD) in a subject who has been treated for a disease. PCT/ES2021/000000

• AWARDS AND RECOGNITION
  - Miguel Gallego was awarded AECF’s investigator 2019 (PI). (Project: enfermedad en fase inicial sarcoma avanzado. In children, adolescents and young adults.

• PATENT
  - Barrón García A, Ayala Díaz RM, Martinez-Lopez J, Orellana de le Fuente ME, Rapado Martínez M. Method for determining the presence or absence of minimal residual disease (MRD) in a subject who has been treated for a disease. PCT/ES2021/000000

Figure 2 Clonal evolution and immunophenotypic advantages of TP53 variants. (A) Experimental Rosette chart. (B) Clonal competition assays of TP53 mono-allelic (red), TP53 bi-allelic (yellow) and TP53 mono-arelic (TP53/1 mono) cells (purple).