

# EXPERIMENTAL ONCOLOGY GROUP

Mariano Barbacid  
Group Leader

Research Scientists  
Carmen Guerra, Sara García-Alonso

Post-Doctoral Fellows  
Federico Virga (January-October)

Graduate Students  
Gonzalo M. Aizpurua, Sara Barrambana, Oksana Brehey, Laura



de La Puente, Ana María Fernández, Fernando Fernández, Jing Li (until August), Vasiliki Liaki, Lucía Morales, Marina Salmón (until November), Pian Sun, Elena Zamorano (since December; CIBERONC, Madrid)

Bioinformatician  
Ruth Álvarez (until November)

Administrative Assistant  
Patricia T. Guerra

Undergraduate Students  
Lucía Baselga (since Oct.) (Undergraduate trainee, Univ. *Politécnica de Madrid*, Spain), Irene Herruzo (until June) (BS Thesis, *UAM*, Madrid, Spain), Mirto Kostopoulou (since Nov.) (Erasmus + Fellowship, National and Kapodistrian University

of Athens, Greece), Lucía Lomba (since Oct.) (Master's Thesis, *UCM*, Madrid, Spain), Sonsoles Liria (Jan.-Apr.) (Master's Thesis, *Univ. Francisco de Vitoria*, Madrid, Spain), Laura Martínez (Mar.-June) (BS Thesis, *UCM*, Madrid, Spain), Melissa Minic (since Sept.) (Erasmus + Fellowship, *FH Campus Wien*, Austria), Blanca Rosas (since Sept.) (BS Thesis, *UCM*, Madrid, Spain)

External Associates  
Alfredo Carrato (*Hosp. Ramón y Cajal*, Madrid, Spain), Matthias Drosten (*Centro de Investigación del Cáncer*, Salamanca, Spain), Monica Musteanu (*UCM*, Madrid, Spain), Bruno Sainz (*UAM*, Madrid, Spain), Juan Velasco (*Eli Lilly*, Alcobendas, Spain)

Technicians  
Rebeca Barrero (since October), M. Carmen González (TS)', Silvia Jiménez, Marta San Román, Raquel Villar

*\*Titulado Superior* (Advanced Degree)

## OVERVIEW

The main area of interest of our Group is to identify therapeutic strategies against KRAS mutant lung and pancreatic tumours. For almost 4 decades, KRAS oncoproteins were thought to be undruggable targets. However, selective KRAS inhibitors, at least against one of the KRAS oncogenic isoforms, KRAS<sup>G12C</sup>, have been recently approved by the FDA. Yet patients develop drug resistance rather quickly indicating that successful treatment of KRAS mutant tumours will require combination with inhibitors of KRAS signalling pathways, such as the MAP kinase and the PI3 kinase pathways. Unfortunately, all inhibitors tested thus far in the clinic have failed due to excessive toxicities. A potential exception is RAF1. Ablation of this kinase induced significant levels of tumour regression with limited toxicities in experimental models. Ironically, the tumour-inducing effect of RAF1 is not mediated by its kinase activity. Hence, pharmacological targeting of RAF1 will require the use of strategies capable of degrading the protein. To identify such compounds, we have determined the tertiary structure of the full RAF1 protein using Cryo-Electron Microscopy (Cryo-EM) technologies. These results have identified structural vulnerabilities that will make it possible to design selective RAF1 degraders.

“The tertiary structure of RAF1, bound to the Hsp90 and Cdc37 chaperones, has revealed structural vulnerabilities that will make it possible to generate pharmacologically active RAF1 degraders capable of inhibiting KRAS mutant lung tumours.”



RESEARCH HIGHLIGHTS

KSR induces RAS-independent MAPK pathway activation and modulates the efficacy of KRAS inhibitors

KSR1/2 have long been considered scaffolding proteins required for optimal MAPK pathway signalling. However, recent evidence suggests that they play a more complex role within this pathway. We have demonstrated that ectopic expression of KSR1 or KSR2 is sufficient to activate the MAPK pathway and to induce cell proliferation in the absence of RAS proteins. In contrast, ectopic expression of KSR proteins is not sufficient to induce cell proliferation in the absence of either RAF or MEK proteins, indicating that they act upstream of RAF. Indeed, KSR1 requires dimerization with at least 1 member of the RAF family to stimulate proliferation, an event that results in the translocation of the heterodimerized RAF protein to the cell membrane. Mutations in the conserved DFG motif of KSR1 that affect ATP binding impair induction of cell proliferation. We have also shown that increased expression levels of KSR1 decrease the responsiveness to the KRAS<sup>G12C</sup> inhibitor sotorasib in human cancer cell lines. These results suggest that high KSR1 or KSR2 expression levels in tumours could render strategies aimed at inhibiting RAS largely ineffective. Indeed, we further show that KRAS<sup>G12C</sup> inhibitors are less effective when KSR1 expression levels are elevated. In conclusion, our data should raise awareness that KSR1 or KSR2 expression levels are direct modulators of the effectiveness of RAS inhibition.

Structure of the RAF1 kinase bound to the HSP90 and CDC37 chaperones: identification of selective RAF1 degrons

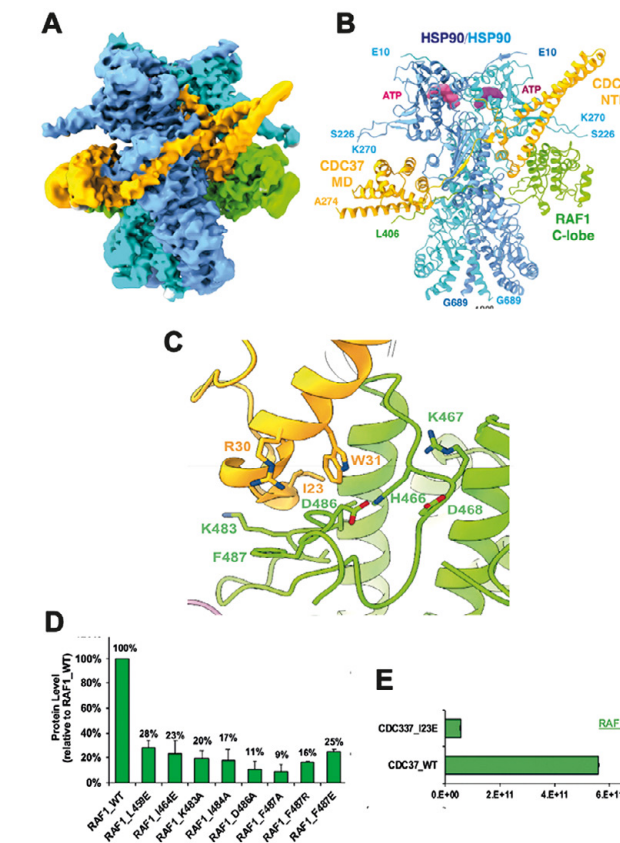
We have described the structure of the full-length RAF1 protein in complex with HSP90 and CDC37 obtained by Cryo-Electron Microscopy (FIGURE 1A and B). The reconstruction reveals a RAF1 kinase with an unfolded N-lobe separated from its C-lobe. The hydrophobic core of the N-lobe is trapped in the HSP90 dimer, while CDC37 wraps around the chaperone and interacts with the N- and C-lobes of the kinase. The structure indicates how CDC37 can discriminate between the different members of the RAF family. Our structural analysis also reveals that the folded RAF1 assembles with 14-3-3 dimers, suggesting that after folding follows a similar activation as B-RAF. Finally, disruption of the interaction between CDC37 and the DFG segment of RAF1 unveils potential vulnerabilities to attempt the pharmacological degradation of RAF1 for therapeutic purposes (FIGURE 1C).

Despite the well-conserved sequence amongst members of the RAF family, they contain substantial functional differences.

Whereas RAF1 and A-RAF are client proteins of the HSP90-CDC37 chaperone system, B-RAF is not. Therefore, the HSP90-CDC37 chaperone system adds an extra regulatory layer to this kinase family. The structure of the complex highlights the key interactions of the HSP90 chaperone and its cochaperone CDC37 with RAF1. Moreover, our combined biochemical and functional analysis of the interacting regions indicates that CDC37 can recognise segments of RAF1 that are different from their counterparts in B-RAF.

We propose a model in which RAF1 would be unstable until it becomes associated with CDC37, followed by binding to HSP90. The HSP90-CDC37 chaperone system couples the folding of the client protein with ATP hydrolysis cycles (FIGURE 1B). RAF1 is phosphorylated in residues S259 and S621, thereby, once the HSP90-CDC37 renders this protein folded, the complex is disrupted and RAF1 associates with 14-3-3 in a manner similar to B-RAF. We speculate that the interaction of RAF1 with the HSP90-CDC37 system could control the dynamics of RAF1 heterodimers formed with the 14-3-3 proteins, thus influencing the levels of homo or heterodimers of this signalling module, and thereby controlling cellular proliferation.

Our mutagenesis analysis of the interface between CDC37 and RAF1 highlights the importance of this association for RAF1 stability. Indeed, we observed a reduction in the levels of RAF1 when the mutant isoforms were co-expressed with HSP90 and CDC37 (FIGURE 1D and E). These observations raise the possibility that the interface between RAF1 and CDC37 may represent a vulnerable spot, which could be targeted to induce the degradation of RAF1, reproducing the therapeutic results obtained in experimental models of *Kras*/*Trp53*-induced lung tumours upon ablation of RAF1 expression. ■



**FIGURE 1** Cryo-EM structure of the RAF1-HSP90-CDC37 (RHC) complex. **(A)** Cryo-EM maps of the RAF1-HSP90-CDC37 complex at 3.67 Å global resolution. Blue and cyan for each monomer of the HSP90 dimer, yellow for CDC37 and green for RAF1. **(B)** Overview of the RAF1-HSP90-CDC37 assembly in complex with ATP. **(C)** Detailed view of the main interacting region between CDC37 and RAF1. **(D)** Levels of wild type and mutant RAF1 proteins co-expressed with HSP90 and CDC37. The levels of each protein were quantified by mass spectrometry, using RAF1 common peptides for all the mutants. **(E)** Mean levels of RAF1 bound to strep-tagged wild type and mutant CDC37 I23E proteins.

PUBLICATIONS

Drosten M, Barbacid M (2022). KRAS inhibitors: going non-covalent. *Mol Oncol* 16, 3911-3915.

García-Alonso S, Mesa P, Ovejero LP, Aizpurua G, Lechuga CG, Zarzuela E, Santiveri CM, Sanclemente M, Muñoz J, Musteanu M, Campos-Olivas R, Martínez-Torrecuadrada J, Barbacid M\*, Montoya G\* (2022). Structure of the RAF1-HSP90-CDC37 complex reveals the basis of RAF1 regulation. *Mol Cell* 82, 3438-3452. \*Co-senior authors.

Paniagua G, Jacob HKC, Brehey O, García-Alonso S, Lechuga CG, Pons T, Musteanu M, Guerra C, Drosten M, Barbacid M (2022). KSR induces RAS-independent MAPK pathway activation and modulates the efficacy of KRAS inhibitors. *Mol Oncol* 16, 3066-3081.

Drosten M, Barbacid M (2022). Targeting KRAS mutant lung cancer: light at the end of the tunnel. *Mol Oncol* 16, 1057-1071.

Martínez-Bosch N, Cristóbal H, Iglesias M, Gironella M, Barranco L, Visa L, Calafato D, Jiménez-Parrado S, Earl J, Carrato A, Manero-Rupérez N, Moreno M, Morales A, Guerra C, Navarro P, García de Frutos P (2022). Soluble AXL is a novel blood marker for early detection of pancreatic ductal adenocarcinoma and differential diagnosis from chronic pancreatitis. *EBioMedicine*, 75, 103797.

Lazcanoiturburu N, García-Sáez J, González-Corrales C, Roncero C, Sanz J, Martín-Rodríguez C, Valdecantos MP, Martínez-Palacián A, Almalé L, Bragado P, Calero-Pérez S, Fernández A, García-Bravo M, Guerra C, Montoliu L, Segovia JC, Valverde ÁM, Fabregat I, Herrera B, Sánchez A (2022). Lack of EGFR catalytic activity in hepatocytes improves liver regeneration following DDC-induced cholestatic injury by promoting a pro-restorative inflammatory response. *J Pathology* 258, 312-324.

Diego-González L, Fernández-Carrera A, Igea A, Martínez-Pérez A, Real Oliveira MECD, Gomes AC, Guerra C, Barbacid M, González-Fernández Á, Simón-Vázquez R (2022). Combined inhibition of FOSL-1 and YAP using siRNA-lipoplexes reduces the growth of pancreatic tumors. *Cancers (Basel)* 14, 3102-3123.

Casadevall D, Hernández-Prat A, García-Alonso S, Arpi-Llucià O, Menéndez S, Qin M, Guardia C, Moracho B, Sánchez-Martin FJ, Zazo S, Gavilán E, Sabbaghi MA, Eroles P, Cejalvo JM, Lluch A, Rojo F, Pandiella A, Rovira A, Albanell J (2022). mTOR inhibition and T-DM1 in HER2-positive breast cancer. *Mol Cancer Res* 20, 1108-1121.

París-Muñoz A, Aizpurua G, Barber DF (2022). Helios expression is downregulated on CD8+ Treg in two mouse models of lupus during disease progression (2022). *Front Immunol* 13, 922958.

AWARDS AND RECOGNITION

- M. Barbacid:
- “Santiago Ramón y Cajal” National Research Prize 2022, Spain.
  - Honorary Doctorate (“Doctor Honoris Causa”) from the *Universidad Nacional de Educación a Distancia*, Madrid, Spain.
  - “Premio a la Excelencia en la Trayectoria Científica” AstraZeneca Foundation Lifetime Achievement in Science Award, Spain.
  - Member of the *Universidad Internacional Menéndez Pelayo*’s Advisory Board, Madrid, Spain.

Publications at other institutions

- París-Muñoz A, Aizpurua G, Barber DF (2022). Helios expression is downregulated on CD8+ Treg in two mouse models