EXPERIMENTAL ONCOLOGY GROUP

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OVERVIEW

For over three decades, KRAS proteins were thought to be undruggable. This concept came to an end when, in 2013, Shokat and co-workers identified a small groove in the $KRAS^{G12C}$ oncoprotein, which allows the insertion of small inhibitory chemotypes. These results led to a frenetic race to develop a new generation of KRAS inhibitors. Unfortunately, these inhibitors have provided somewhat disappointing results in the clinic, primarily due to the rapid onset of tumour resistance. In our laboratory, we reasoned that the most efficacious way to overcome tumour resistance was to target KRAS signalling at independent nodes, thus thwarting the capacity of tumour cells to utilise alternative pathways that bypass the antitumoural effect of KRAS inhibitors. This strategy has proven to be correct. Indeed, we have recently demonstrated that the genetic ablation of three independent nodes involved in downstream (RAF1), upstream (EGFR) and orthogonal (STAT3) KRAS signalling led to the complete and permanent regression of experimental pancreatic tumours.

We have successfully translated these results to a pharmacological scenario.

“We have reported that a combination of KRAS, EGFR and STAT3 inhibitors induced the complete and durable regression of experimental and patient-derived (PDX) pancreatic tumour models. These results should guide the development of new clinical trials to benefit pancreatic cancer patients.”

RESEARCH HIGHLIGHTS

Systemic *KRAS* ablation disrupts myeloid cell homeostasis in adult mice

We have interrogated the role of *KRAS* in an experimental system to generate potentially relevant information regarding the use of panKRAS or panRAS inhibitors in the clinic. Here, we report that the systemic ablation of *Kras* expression in adult mice does not induce significant changes in overall survival, body weight, glucose levels, metabolic profiles or heart function. In contrast, flow cytometry and histopathological analyses of organs such as blood, bone marrow and spleen showed a significant increase in the myeloid lineage, leading to myelomonocytic metaplasia. In this context, replacement of the KRAS isoforms with HRAS is sufficient to maintain adult homeostasis, suggesting that the unique properties of the *KRAS* locus are primarily due to its pattern of expression rather than to the activity of its gene products.

A triple therapy induces complete and durable regression of experimental KRAS-driven pancreatic tumours

During the last few years, several RAS inhibitors have been developed, including those selective against various isoforms as well as RAS(ON) inhibitors capable of blocking all isoforms. These RAS(ON) inhibitors have led to significant tumour regression and increased survival in mouse tumour models as well as in the clinic. Regrettably, their therapeutic efficacy has been thwarted by the rapid appearance of tumour resistance, thus making it necessary to develop novel combination therapies.

We reasoned that overcoming tumour resistance could be achieved by targeting distinct nodes that control KRAS signalling pathways. Indeed, we had already reported the regression of a subset of small genetically engineered PDAC tumours by the concomitant ablation of *Raf1* and *Egfr*, two genes encoding downstream and upstream KRAS effectors, respectively. Yet, the tumour-inhibitory response faded away in larger tumours. We have now determined that tumour resistance involves the functional activation of the transcription factor STAT3 via phosphorylation on its regulatory residue, Tyr^{705}. Indeed, resistant tumours lacking RAF1/EGFR expression undergo rapid cell death upon the genetic ablation or pharmacological degradation of STAT3.

These observations led us to investigate the therapeutic effect of concomitantly ablating the three signalling nodes, R*af1*, E*gfr* and S*tat3*. This strategy led to the rapid regression of mouse pancreatic tumours. Moreover, these animals remained tumour free for over 200 days, indicating that this strategy also prevented the onset of tumour resistance. Next, we translated this genetically based strategy to a pharmacological scenario. RAF1 ablation was replaced by the panRAS(ON) inhibitor, RMC-6236, EGFR ablation by afatinib, an irreversible tyrosine kinase inhibitor, and STAT3 ablation was successfully replaced by a STAT3-selective PROTAC, SD36.

RAS(ON) inhibitors used as monotherapy have been shown to induce regression of KRAS-driven orthotopic and genetically engineered mouse tumours, doubling the survival of the treated mice. More importantly, they are also increasing survival of PDAC patients in phase II clinical trials, thus offering a new and promising alternative to current cytotoxic treatments. Unfortunately, these tumours end up progressing due to the onset of tumour resistance, ultimately leading to the death of the host. We have obtained similar results in orthotopic and GEM tumour models using RMC-6236 as monotherapy. In contrast, our triple combination therapy using RMC-6236 along with EGFR and STAT3 inhibitors not only induced complete and durable tumour regression but efficiently prevented the onset of tumour resistance (Figure 1).

Finally, our triple combination therapy was equally effective in inducing tumour regression in patient-derived xenograft (PDX) models. Moreover, these results also shed light on the mechanisms involved in tumour regression. Since PDX tumours are grown in immunodeficient NU-Foxn1^{nu} mice, the observed efficient regression implies that the triple therapy described here exerts its antitumour effects independently of the adaptive immunity provided by the tumour microenvironment.

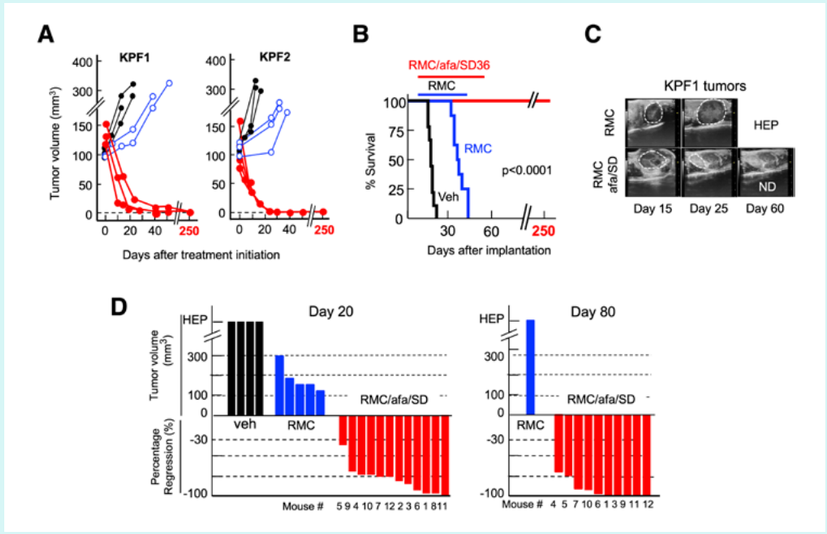

FIGURE 1 Combined inhibition of KRAS, EGFR and STAT3 in orthotopic and genetically engineered mouse tumour models induces complete and durable tumour regressions. (**A**) Tumour volume visualised by ultrasound of orthotopic tumours: two independent *Kras*$^{+/FSFG12V}$;*P53*$^{F/F}$;*Elas-tTA;TetO-Flpo* (KPeF) tumour cell lines (KPF1/KPF2) were implanted in the pancreas of C57BL/6 mice and treated with vehicle (solid lines), with the RAS(ON) inhibitor RMC-6236 (blue lines) (blue arrowheads) or with a combination of RMC-6236, afatinib and SD36 (red lines) for the indicated period of time. (**B**) Kaplan-Meier survival curve of C57BL/6 mice implanted with three independent tumour cell lines (KPF1 to KPF3) treated with vehicle (n=9) (V, solid line), RAS(ON) inhibitor RMC-6236 (n=8) (blue line) and with a combination of RMC-6236, afatinib and SD36 (n=10) (red line) for the indicated times. Mice were maintained for up to 250 days post-implantation without evidence of tumour resistance. The P value was obtained using log-rank Mantel-Cox test, p<0.0001. (**C**) Representative ultrasound images of the peritoneal cavity of C57BL/6 mice implanted with the KPF1 tumour cell line and treated with RMC-6236 or with the combination of RMC-6236, afatinib and SD36 (RMC/afa/SD) at the indicated times. Tumours are marked with dotted lines. ND, not detected. HEP, mouse sacrificed at humane endpoint due to large tumour burden. (**D**) Waterfall plot of tumour response of genetically engineered tumour-bearing mice after 20 (left) and 80 (right) days of treatment with vehicle (solid bars), RMC-6236 (blue bars) or the triple combination (red bars).

The road to optimising the triple combination therapy described here to be used in a clinical scenario will not be easy. Yet, the generation of novel, more efficacious and better tolerated EGFR inhibitors or STAT3 degraders is not going to be insurmountable. Thus, the results reported here should open the door to new therapeutic options to improve the clinical outcome of PDAC. ■

PUBLICATIONS

- Liaki V, Barrambana S, Kostopoulou M, Lechuga CG, Zamorano-Dominguez E, Acosta D, Morales-Cacho L, Álvarez R, Sun P, Rosas-Perez B, Barrero R, Jiménez-Parrado S, López-García A, San Roman M, López-Gil JC, Drosten M, Sainz B Jr, Musteanu M, Caleiras E, Dusetti N, Poli V, Sánchez-Bueno F, Guerra C, Barbacid M (2025). A targeted combination therapy achieves effective pancreatic cancer regression and prevents tumor resistance. *Proc Natl Acad Sci U S A*. PMID: 42224594.
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- Drosten M, Barbacid M (2025). The rapidly growing landscape of RAS inhibitors: from selective allele blockade to broad inhibition strategies. *Mol Oncol* 19, 2991-2995.
- Liaki V, Rosas-Perez B, Guerra C (2025). Unlocking the genetic secrets of pancreatic cancer: KRAS allelic imbalances in tumor evolution. *Cancers* 17, 1226.
- Barrambana S, Zamorano-Domínguez E, Liaki V, Guerra C (2025). Navigating the complexities of pancreatic ductal adenocarcinoma: a review on therapeutic models and RAS inhibitors. *Adv Drug Deliv Rev* 224, 115652.
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- Fernández-Pisonero I, Lorenzo-Martín LF, Drosten M, Santos E, Barbacid M, Alarcón B, Bustelo XR (2025). Active R-RAS2/TC21 prevents cell cycle arrest and morphological alterations in mouse embryonic fibroblasts lacking RAS proteins. *Oncogene* 44, 1905-1921.
- López-Merino E, Fernández-Rodrigo A, Jiang JG, Gutiérrez-Eisman S, Fernández de Sevilla D, Fernández-Medarde A, Santos E, Guerra C, Barbacid M, Esteban JA, Briz V (2025). Different RAS isoforms regulate synaptic plasticity in opposite directions. *EMBO J* 44, 2106-2133.
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PATENT APPLICATIONS

- Barbacid M, Guerra C, Liaki, V (2025). Novel Therapy. *EP25382815.6*.
- Guerra C, Liaki V. Triple combined therapy inhibiting EGFR, RAF1, and STAT3 against pancreatic ductal adenocarcinoma. *EP4658254* (European regional phase entry 2025).

AWARDS AND RECOGNITION

- M. Barbacid:
  - Invited Speaker, *Cancer Genetics: History & Consequences* Meeting, Cold Spring Harbor, NY, USA.
  - Premio Valor Añadido, Fundación Transforma, Madrid, Spain.
  - Premio a los Valores Humanos y Culturales, Fundación Siglo Futuro, Guadalajara, Spain.
  - XIII Premio Prensa y Poder Mudima, Aranda de Duero, Burgos, Spain.
- S. García-Alonso:
  - Premios 'Castilla y León' 2025 (Castile and León Awards 2025), Social Sciences and Humanities category, Valladolid, Spain.
  - Distinguished lecturer, Embassy of Spain in the United Arab Emirates and AICEAU, Dubai, United Arab Emirates.
  - Clara Campoamor Award, City Council of Madrid, Madrid, Spain.
  - II Premios Maruja Mallo in the Science category (2nd Maruja Mallo Awards for Science), Fundación Ortega-Marañón, Madrid, Spain.
  - Premios Woman 2025 in the Science category (2025 Woman Awards: Science), *Woman Madame Figaro*, Madrid, Spain.
  - Premio 'Pasión por la Ciencia' 2025 ('Passion for Science' Award 2025), University of Navarra, Pamplona, Spain.
  - Opening lecturer, XVII Research Congress in Health Sciences, Complutense University of Madrid, Madrid, Spain.
  - Invited professor, UCM Summer Courses, Complutense University of Madrid, Madrid, Spain.
  - VII Premio Corazón de Oro (7th Heart of Gold Award), NGO Believe in Art, Zaragoza, Spain.
  - Invited speaker, *Medicine 2044: Predicting the Future* Congress, Zaragoza, Spain.