



Control/Tracking Number: 2025-RA-1345-WCLC

Fumarate and ASS1 in KRAS-mutant NSCLC: the influence of cetuximab in combination with KRAS G12C inhibitors (sotorasib and fulzerasib)

D. Olmo González¹, M. Zhou², J. Codony-Servat², Y. Wang³, N. Oliveira⁴, J. Zhang³, F. Zhou³, F. Yan³, J. González², K. Valencia², M.A. Molina-Vila⁵, J. Bertran-Alamillo⁵, A. Giménez-Capitán⁵, H. Shen³, R. Rosell⁶

¹FIOR group, Dexeus University Hospital, Barcelona, Spain, ²FIOR group, FIGTP - Fundació Institut d'Investigació Germans Trias i Pujol, Badalona, Spain, ³GenFleet Therapeutics (Shanghai) Inc., Shanghai, China, ⁴University of Lisbon - Faculty of Pharmacy, Lisbon, Portugal, ⁵Pangaea Oncology, Dexeus University Institute, Barcelona, Spain, ⁶Instituto Oncológico Dr. Rosell-IOR, Dexeus University Hospital, Barcelona, Spain

Introduction

Clinical trials using KRASG12C inhibitors as monotherapies have exhibited brief responses. It has been identified that EGFR is involved in a dominant resistance mechanism to KRASG12C inhibitors in colorectal cancer. Therefore, targeting EGFR activation with cetuximab, a key component of the adaptive resistance mechanism, is of significant interest. Notably, in KRASG12C-mutant lung cancer patients, fulzerasib plus cetuximab attained an 80% response rate (KROCUS).

Beyond its role in oncogenic signalling, KRAS has been implicated in the remodelling of aspartate metabolism and the urea cycle, directly affecting the expression of argininosuccinate synthetase 1 (ASS1) and fumarate accumulation. Interestingly, in colorectal cancer, specific KRAS mutations can lead to ASS1 overexpression, facilitating cell proliferation and generating key intermediates of the TCA cycle, such as fumarate. These findings suggest that ASS1 expression and fumarate levels could serve as key prognostic biomarkers in different cancer types.

In this study, we evaluated the synergistic effects of fulzerasib or sotorasib (KRASG12C inhibitors) in combination with cetuximab in KRAS-mutant lung cancer cell lines, along with analysis of RAS signaling rebound. In addition, identification of a wide range of proteins and assessment of fumarate levels.

Methods

A panel of KRAS G12C-mutant cell lines, including H358, H23, H2122, H1792, H2030 and Mia-PaCa-2 were used. Reagents, antibodies, cetuximab and sotorasib were purchased. Fulzerasib was provided by Genfleet. Cell viability was assessed by MTT. The following primary antibodies were used for western blot analysis: EGFR Y1068, HER3 Y1289, FASN, EphA2 S897, YAP Y357, MIG6, ASS1, ERK1/2 T202/Y204, MRAS and Hsp90. Fumarate levels were quantified using a spectrophotometric kit.

Results

The IC₅₀ values for both fulzerasib and sotorasib were notably low in H358, H2122, and Mia PaCa-2 cells, whereas they were high in H2030 cells and moderately elevated in the H23 cell line. The combination with cetuximab enhanced cytotoxicity. H358 cells showed a high synergistic effect, with a combination index of 0.53 when paired with fulzerasib. Other cell lines exhibited slightly synergistic effects (values below 1), while H23 cells showed an additive response.

Western blot analysis revealed that the ERK1/2 rebound, typically induced by fulzerasib or sotorasib alone, was effectively abolished in H358 cells. Suppression of ERK1/2 resulted in attenuation of YAP Y357 phosphorylation.

ASS1 expression appeared to increase more substantially in the more resistant cell lines, H23 and H2030, following treatment with sotorasib or fulzerasib. In contrast, ASS1 expression levels remained largely unaffected in H358 by KRASG12C inhibitors treatment. Fumarate levels seem to be higher in H358 compared to more resistant cell lines. Fulzerasib and sotorasib seem to increase levels of fumarate in a similar extent.

Conclusions

Cetuximab combined with fulzerasib or sotorasib inhibits cell viability in H358 cell line, preventing ERK1/2 rebound and YAP1 activation. Levels of ASS1 in H358 cells were stable before treatment. In contrast, ASS1 levels were low and upregulated after sotorasib treatment in H23 cells. Low ASS1 expression is shown to harbour a poor prognosis. Further research could clarify the relevance of ASS1 and fumarate as a biomarker and therapeutic target.