

INTRODUCTION

Background. The RAF–MEK–ERK cascade is a central oncogenic signaling axis in non–small cell lung cancer (NSCLC). Although RAF1 rearrangements have been reported across multiple tumor types, their functional significance and optimal therapeutic strategy in NSCLC remain poorly defined.

Clinical rationale. We identified a VCL–RAF1 fusion in a patient with NSCLC who experienced a transient response to the MEK inhibitor trametinib.

Objective. We investigated whether VCL–RAF1 activates MAPK signaling, promotes migration-related and clonogenic phenotypes, and confers sensitivity to MAPK-pathway inhibitors.

METHODS

Expression and signaling analysis. 293FT and NIH/3T3 cells were transiently transfected with V5-tagged pcDNA3.1–VCL–RAF1 or empty vector. Fusion expression and RAF–MEK–ERK activation were assessed by immunoblotting for V5, p-RAF1/RAF1, p-MEK/MEK, and p-ERK/ERK. α-Tubulin served as the loading control.

Scratch-wound assay. Confluent 293FT cells expressing VCL–RAF1 or empty vector were scratched, and wound closure was monitored at the indicated time points.

Stable expression and colony formation. VCL–RAF1 was subcloned into pBABE-puro, packaged into retroviral particles, and used to transduce NIH/3T3 cells. Puromycin-selected clones were validated by immunoblotting and evaluated using colony-formation assays.

Drug-response analysis. Stable VCL–RAF1-expressing cells were treated with serial concentrations of trametinib, cobimetinib, sorafenib, ulixertinib, LY3009120, and dabrafenib. Cell growth/viability was measured using Alamar Blue assay after 72 hours, and concentration–response curves were fitted by nonlinear regression.

DISCUSSION AND CONCLUSION

Actionable Take Away Message

- VCL–RAF1 expression increased MEK and ERK phosphorylation, supporting constitutive MAPK-pathway activation. Representative scratch-assay images suggested faster wound closure, while two independent stable clones showed increased colony formation. The apparent potency of trametinib and cobimetinib is consistent with a targetable MAPK dependency.

Future Direction for Research

- Further studies will quantify signaling and wound closure across independent replicates, validate drug sensitivity in matched controls and additional models, confirm p-ERK target suppression, and evaluate antitumor efficacy in vivo when combining with immunotherapy.

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RESULTS

VCL–RAF1 activates MAPK signaling.

Transient expression of V5-tagged VCL–RAF1 in 293FT and NIH/3T3 cells increased MEK and ERK phosphorylation relative to vector controls, consistent with activation of the RAF–MEK–ERK pathway (Figure 1).

VCL–RAF1 may enhance scratch-wound closure.

Representative images suggested more rapid wound closure in VCL–RAF1-expressing 293FT cells than in vector-control cells. Quantitative analysis and control for cell proliferation are required to confirm a migration phenotype (Figure 2).

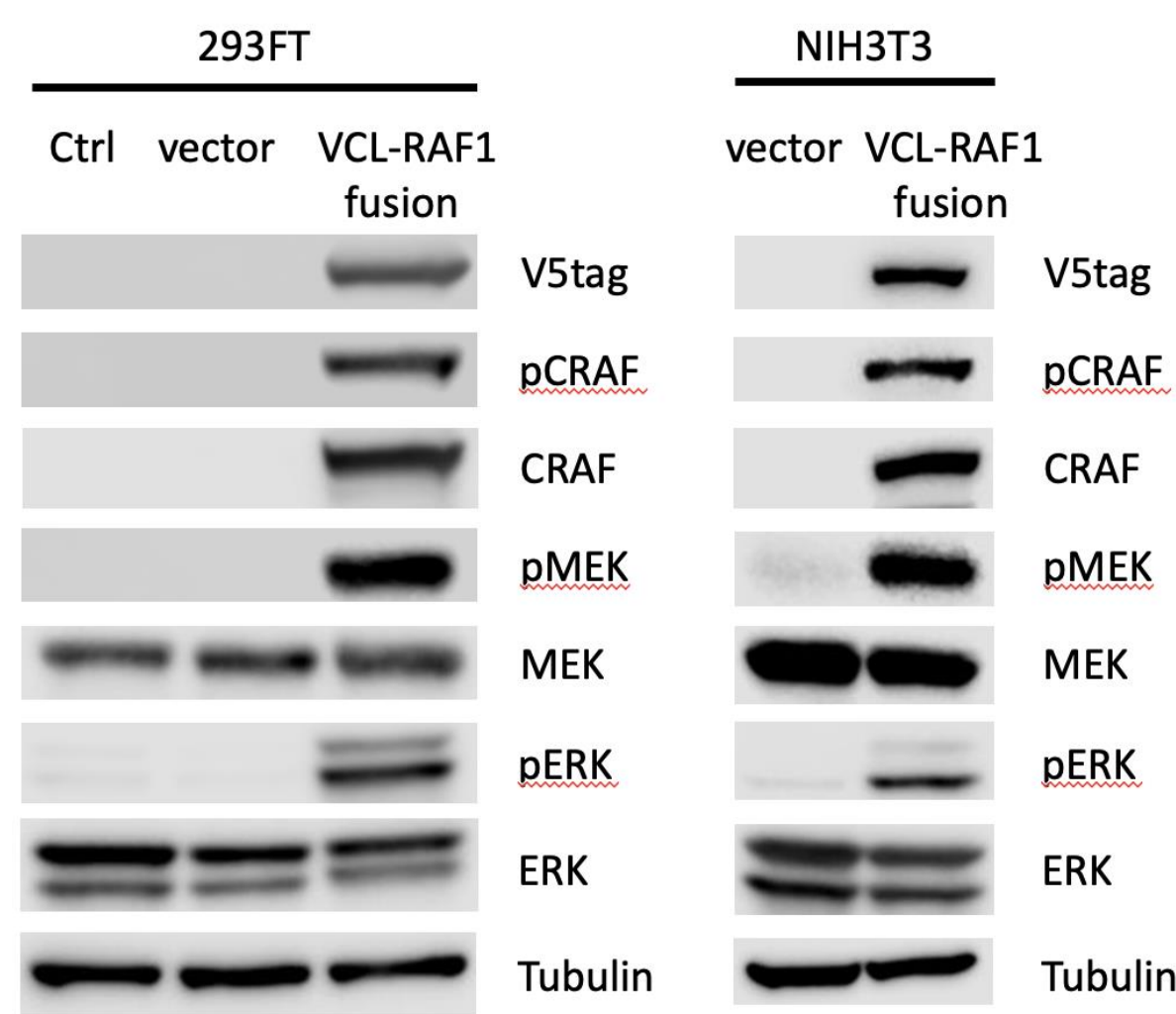


Figure 1. The western blot result from 293FT and NIH3T3 cell which transfected with pcDNA3.1-VCL-RAF1 plasmid

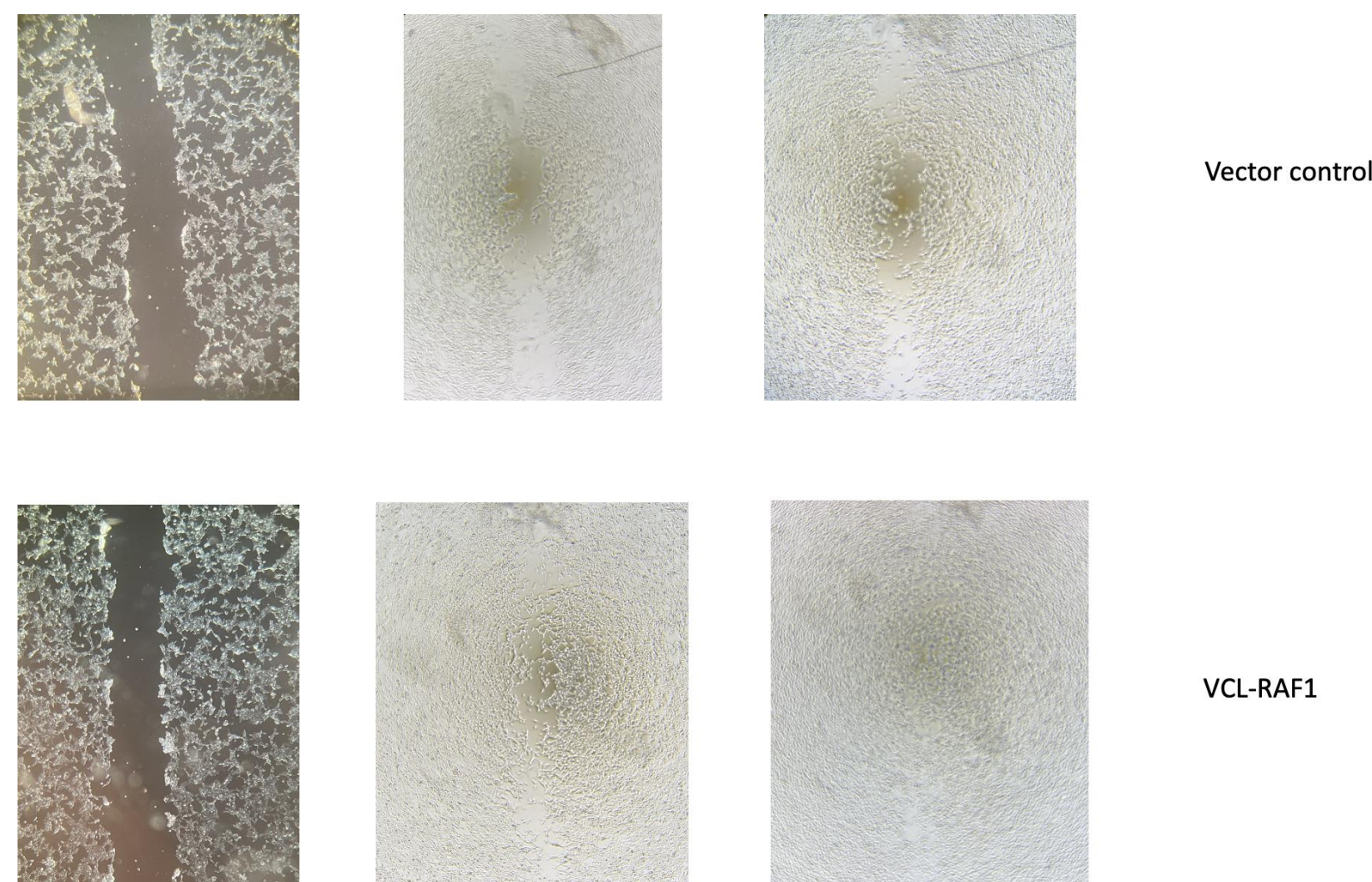


Figure 2. The scratch assay from 293FT cell which transfected with pcDNA3.1-VCL-RAF1 plasmid

Stable VCL–RAF1 expression enhances clonogenic growth.

Two puromycin-selected NIH/3T3 clones stably expressing VCL–RAF1 retained elevated p-MEK and p-ERK levels and formed more colonies than vector-control cells, supporting enhanced clonogenic growth and an in vitro transforming phenotype (Figure 3).

VCL–RAF1-expressing cells are sensitive to MEK inhibition.

Dose–response profiling demonstrated concentration-dependent growth inhibition by trametinib and cobimetinib. These findings support further evaluation of MEK inhibition as a therapeutic strategy, although matched vector-control curves are needed to establish fusion-selective sensitivity (Figure 4).

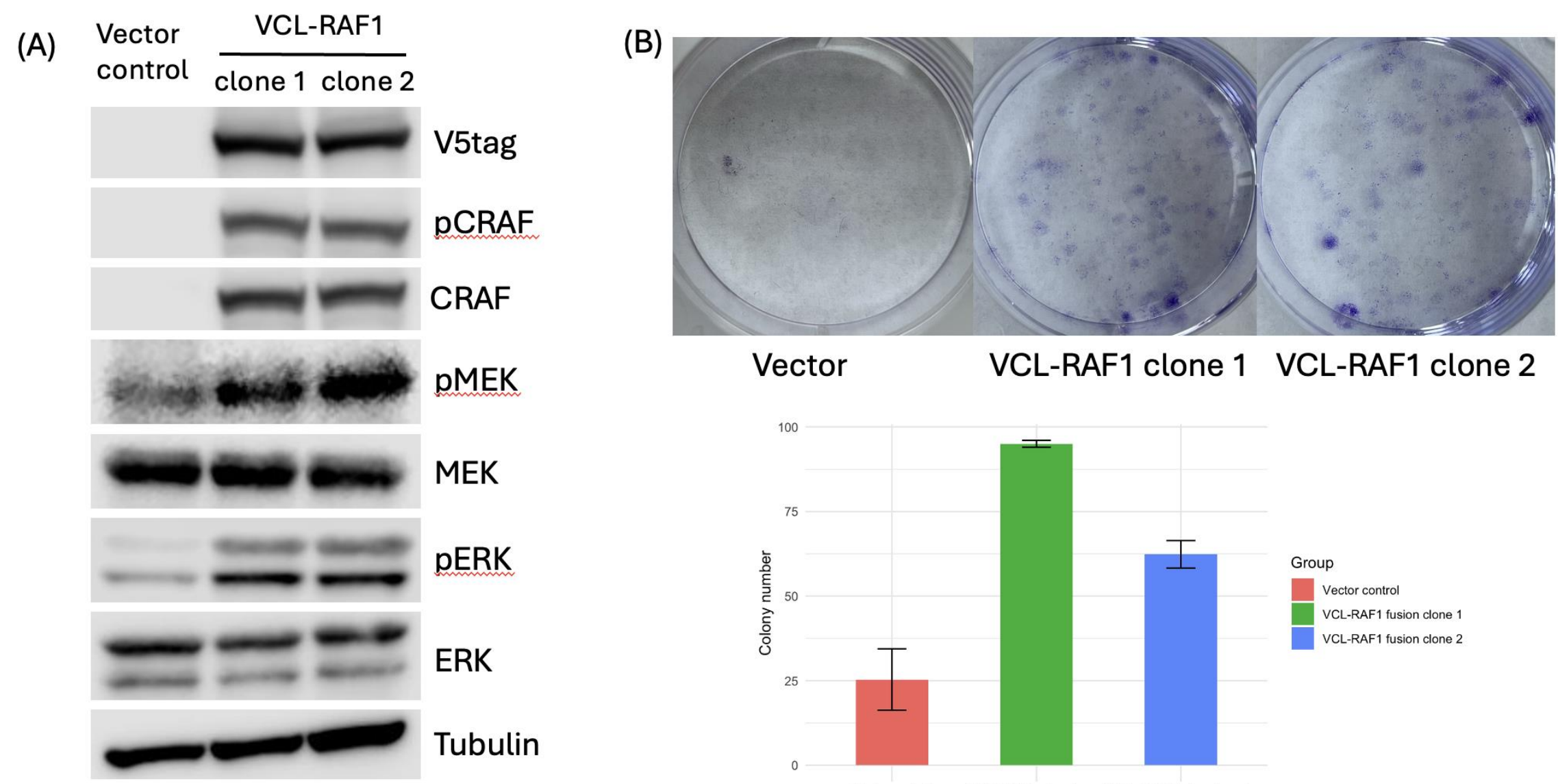


Figure 3. RAF1 fusion had oncogenicity. (A) Western blot of NIH3T3 cell with transfection of retroviral vector with pBABE-puro-VCL-RAF1 and selected by puromycin. (B) Colony formation assay of NIH3T3 cell with transfection of retroviral vector with pBABE-puro-VCL-RAF1 and selected by puromycin.

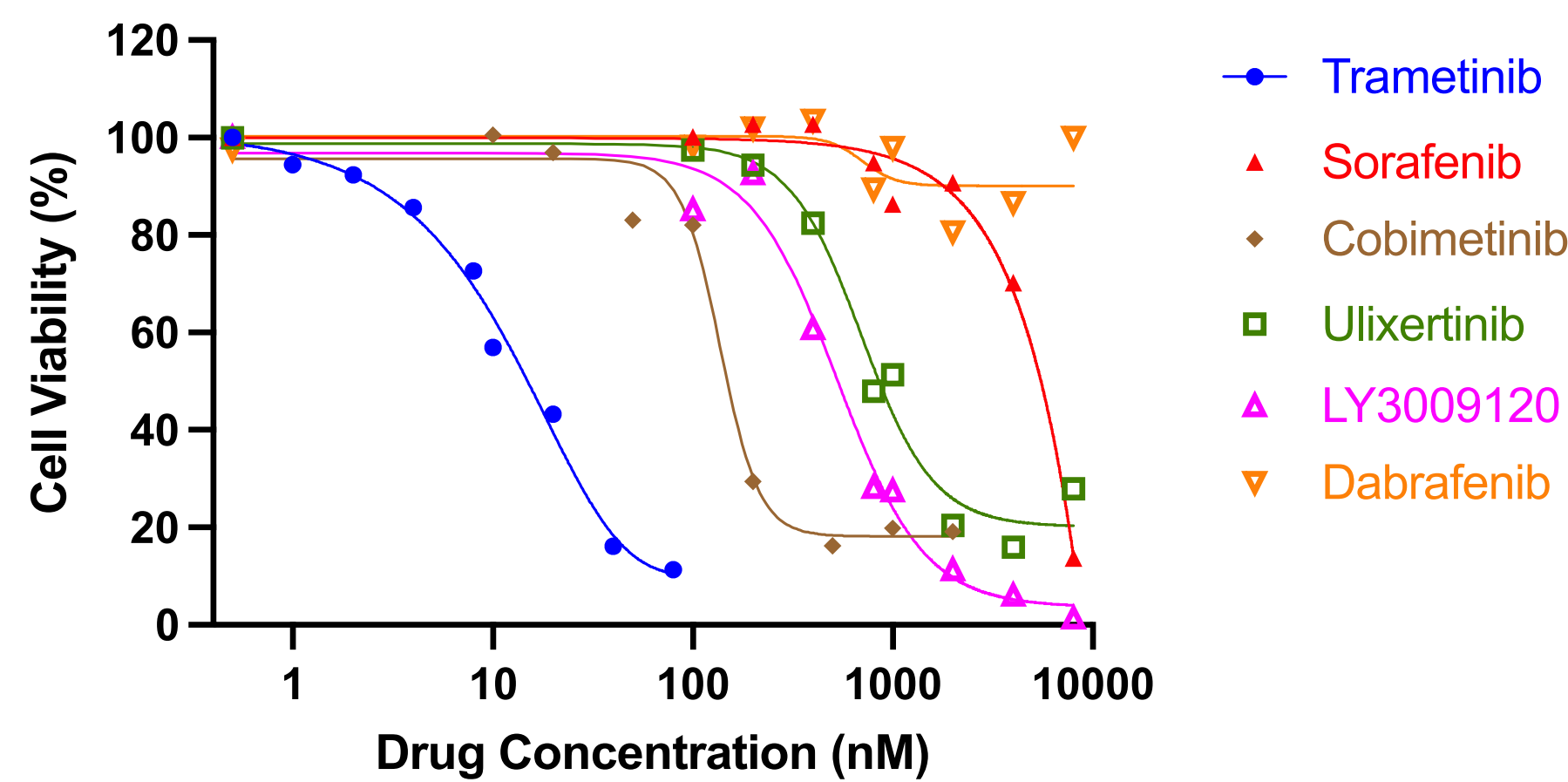


Figure 4. RAF1 fusion could be suppressed by therapeutic dose of Trametinib and Cobimetinib.