Antitumor effects of KRAS-G12C inhibitors can be improved by farnesyltransferase inhibitors in preclinical models

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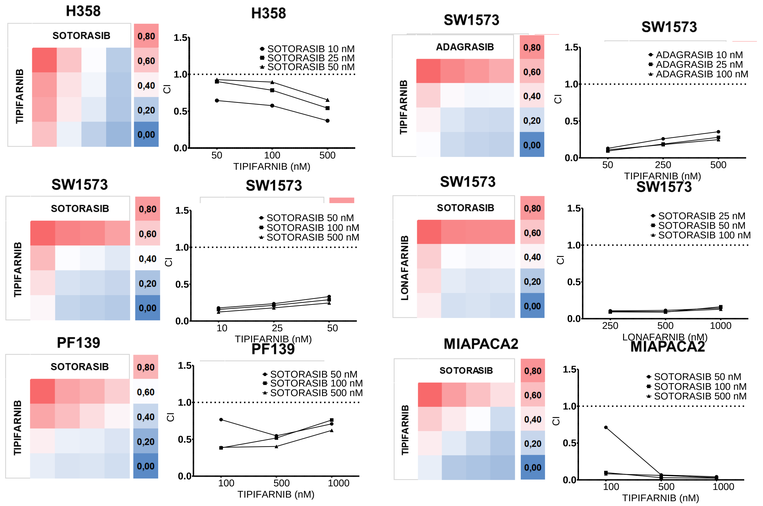

Figure 1. Combination of KRAS G12C inhibitors with farnesyle-transferase inhibitors. Various combinations of KRAS G12C inhibitors (sotorasib, adagrasib) with Ftis (tipifarnib, lonafarnib) show synergistic anticancer effects in human lung (H358, PF139, SW1573) and pancreatic (MIAPACA2) adenocarcinoma cells in 6 day long 2D combinational tests. Graphs show control-normalized heatmaps of cell viability results with the corresponding combinational index (CI) values. CIs were calculated by CompuSyn Software. CI values less than 1 indicate synergy while values equal to or more than 1 represent additive or antagonistic effect, respectively.

Introduction

Farnesyltransferase inhibitors (FTI) failed as monotherapies for various cancer types until the discovery of their potency in HRAS mutant human head and neck and bladder cancers. On the other hand, RAS mutant cancers have been a great challenge for cancer research until the discoveries and approvals of several KRAS-G12C allele specific inhibitors. However, G12C inhibitors were effective mainly in lung cancer and de novo and acquired resistance prompted the development of various combinational modalities including immunotherapy as well as SOS- and SHP2-inhibitors.

Methods and Materials

Clinically approved farnesyl-transferase inhibitors (tipifarnib and lonafarnib) were combined with, novel KRAS G12C inhibitors (sotorasib and adagrasib) using on human lung-, pancreatic and colorectal- adenocarcinoma cells in vitro in and in vivo. Antitumoral effects were evaluated for proliferation, apoptosis and migratory activity. Mechanisms of action were investigated by immunoblot analyzes of various farnesylated protein, RAS activation and signaling, videomicroscopy and also histopathological analyzes of xenograft tumors.

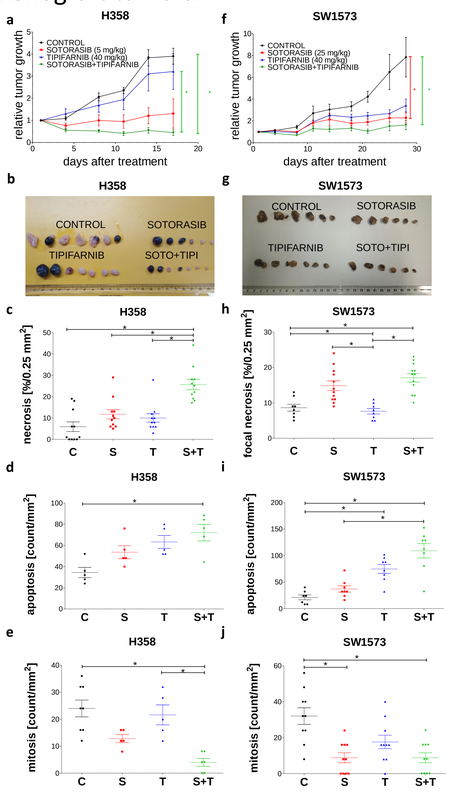

Figure 2. Combination of sotorasib and tipifarnib in H358 and SW1573 xenograft models of lung adenocarcinoma. H358 xenografts were given sotorasib (5 mg/kg i.p.) and/or tipifarnib (40 mg/kg i.p.), while SW1573 xenografts were treated with sotorasib (25 mg/kg i.p.) and/or tipifarnib (40 mg/kg i.p.) therapy. Treatment started after randomization when tumors reached 100 mm^3 (n/group=7). The weekly treatment schedule was five days on and two days off. a Tumor volume was determined twice a week using a caliper. Relative tumor volume growth was normalized with each tumor's starting volume on the day of the first treatment. Error bars represent SEM. b Pictures of the harvested tumors. c-e Histopathological analysis of necrotic areas and frequency of apoptosis and mitosis. Asterisks marks statistically significant differences with $p<0.05$. Statistical significance was tested with the Kruskal-Wallis test followed by Dunn's multiple comparison test.

Results

Synergistic anticancer effects were observed upon the combination of FTIs with G12C inhibitors showed in KRAS G12C mutant human cancer cell lines in vitro. We found that the combination interfered with the compensatory re-activation of HRAS, farnesylation of RHEB in the PI3K/mTOR pathway and lamin structure. Furthermore, we observed enhanced efficacy of sotorasib upon combination with tipifarnib in the xenograft models of lung adenocarcinoma affecting mitotic and apoptotic rates and inducing necrosis.

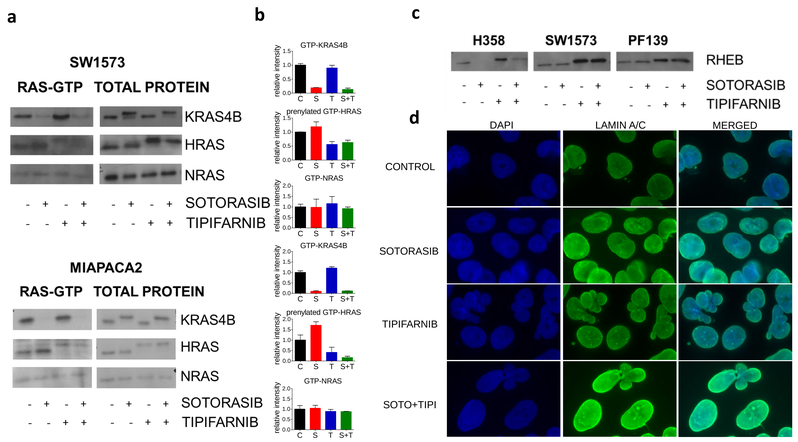

Figure 3. Mechanism of action of synergistic anticancer effects of combinational treatment. a) Representative blots of the RAS proteins in SW1573 and MIAPACA2 cell lines. b) Graphs represent normalized levels of GTP-bound RAS proteins. c) Representative blots of the RHEB protein in LUAD cell lines. Upward shift of RHEB protein in tipifarnib and combinational treatment marks successful block of its farnesylation. d) Immunofluorescence labeling of Lamin A/C proteins. DAPI (blue) staining reveals lobular cell nuclear morphology upon tipifarnib and combinational treatment. These treatments also lead to the accumulation of distinct spots of Lamin A/C (green) in the nucleus

Discussion

Our findings suggest the potential clinical applicability of the combination of KRAS-G12C inhibitors and farnesyl-transferase inhibitors.

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KINETO Lab offers various preclinical model systems for drug discovery and validation.

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