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## **Synergistic Antitumor Effect of an XPO1 Inhibitor and/or Sotorasib with V-ATPase Inhibitors Pretreatment in KRAS G12C NSCLC**

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### **Introduction**

Non-small cell lung cancer (NSCLC) bearing KRAS G12C mutation has demonstrated limited clinical efficacy to KRAS G12C selective inhibitors, such as the covalent inhibitor sotorasib, due to primary and acquired resistance mechanisms. A major focus has been placed on developing combined therapies to avoid resistance. Selinexor, a selective inhibitor of Exportin-1 (XPO1), has shown promising efficacy in KRAS-mutant lung cancer cell lines. Additionally, the V-ATPase inhibitor omeprazole, has been demonstrated to increase cytoplasmic retention of cytotoxic drugs when pretreated, increasing the sensitivity of cells to chemotherapy and some targeted therapies. This study aims to evaluate the efficacy of combining selinexor or sotorasib with omeprazole pretreatment in KRAS G12C NSCLC cell lines and to identify the mechanism of action of these drug combinations.

### **Methods**

Four KRAS G12C-mutated NSCLC cell lines (H358, H23, H358R [sotorasib-resistant], and H2030) were used in this study, ranging from more sotorasib-sensitive to resistant phenotypes, respectively. All experiments were performed administering omeprazole to cells 24 hours before treatment with selinexor or sotorasib for 72 hours. Cell viability was assessed by MTT assays using varying doses of omeprazole pretreatment. Synergy was studied using Loewe, Bliss and HSA models with Combenefit and SynergyFinder programs. Cell viability in H358 spheroids (3D conditions) was evaluated using CellTiter-Glo 3D Assays. Colony Formation Assays (CFA) were performed by treating cells for 15 days. Western blot analyses were conducted to analyze the protein levels of

XPO1, the KRAS signaling pathway, the MRAS-PP1C-SHOC2 complex, and markers such as pEphA2, RanGAP1, IRE1 $\alpha$ , DLC1, and AURKA.

## **Results**

Combined therapy of selinexor with omeprazole pretreatment and sotorasib with omeprazole pretreatment increases sensitivity to both drugs across the four cell lines used in the study. Furthermore, omeprazole pretreatment can overcome resistance to selinexor or sotorasib in the resistant cell lines, as observed in the cell viability assays. Evidence suggests synergistic effects of selinexor/sotorasib with omeprazole pretreatment. Likewise, synergy of these drug combinations was also observed in 3D conditions using H358 spheroids and in the CFA. The CFA highlighted the effectiveness of the triple combination of selinexor and sotorasib pretreated with omeprazole, as it was able to almost eliminate all colonies. Regarding the mechanism of action of these drug combinations, selinexor significantly reduced the protein levels of XPO1, and sotorasib markedly reduced pEphA2 in all lines. The combination of selinexor/sotorasib with omeprazole reduced the levels of RanGAP1 and AURKA across all cell lines, as well as IRE1 $\alpha$  in the two most sotorasib-sensitive cell lines, H358 and H23. Combination treatments increased DLC1 levels in the H23 and H2030 cell lines but were not detected in H358 or H358R.

## **Conclusions**

This study highlights the synergistic antitumor effect of selinexor or sotorasib with omeprazole pretreatment in KRAS G12C NSCLC cell lines. It should be noted that pretreatment with omeprazole has demonstrated to potentially overcome resistance to both treatments. Future *in vivo* studies are necessary along with unveiling the role of XPO1 since XPO1 expression was present in cell lines and was not decreased by sotorasib, only by selinexor.